Total Synthesis of Aminoglycoside Antibiotics, Apramycin and Saccharocin (KA-5685)

Kuniaki TATSUTA,* Kohji AKIMOTO, Hideaki TAKAHASHI, Takao HAMATSU, Masahiko ANNAKA, and Mitsuhiro KINOSHITA

Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Kohoku-ku, Yokohama 223

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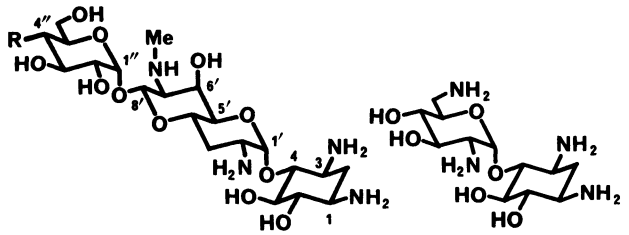
Apramycin and saccharocin (KA-5685) have been synthesized from previously synthesized neamine (**3**). Addition of allylmagnesium chloride to 4-*O*-[3′,4′-*O*-cyclohexylidene-2′-deoxy-2′-(tosylamino)-α-D-*gluco*-hexodialdo-1′,5′-pyranosyl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine which was derived from **3** through the 6′-*N*-oxide, gave 4-*O*-[3′,4′-*O*-cyclohexylidene-2′,7′,8′,9′-tetradeoxy-2′-(tosylamino)-L-*glycero*- and D-*glycero*-α-D-*gluco*-non-8′-enopyranosyl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamines which were in turn converted into 4-*O*-[(8′*S*)-7′-*N*,6′-*O*-carbonyl-2′,3′,7′-trideoxy-8′-*O*-methyl-7′-(methylamino)-2′-(tosylamino)-D-*glycero*-α-D-*allo*-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**25**), *via* azidonitration of 1-*N*-acetyl-4-*O*-[3′,6′-di-*O*-acetyl-4′,8′-anhydro-2′,7′-dideoxy-2′-(tosylamino)-L-*glycero*-α-D-*gluco*-oct-7′-enopyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine and epimerization of the 6′-hydroxyl group. Successive deprotection of **25** afforded aprosamine, from which 1,2′,3,7′-tetrakis(*N*-benzyloxycarbonyl)aprosamine (**28**) was prepared, and glycosidation of **28** with 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranosyl fluoride or 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride, followed by hydrogenolysis, completed the total synthesis.

Apramycin[1] (**1**) and saccharocin[2a] (**2**: antibiotic KA-5685 [2b]) are aminoglycoside antibiotics active against Gram-positive and Gram-negative bacteria including resistant strains of other aminoglycoside antibiotics. Apramycin (**1**) was isolated in 1967 as nebramycin component 2 from fermentation broths of *Streptomyces tenebrarius*,[1a] and saccharocin and KA-5685 (**2**), which were found to be identical with each other later on, were independently reported in 1983 as metabolites of *Saccharopolyspora sp.* by two Japanese groups.[2] Structural studies have revealed that antibiotics **1**[1b] and **2**[2] contain the unusual bicyclic aminooctodialdose and 2-deoxystreptamine units as common structures, and, in addition, 4-amino-4-deoxy-D-glucose and D-glucose units respectively. The unique structural features as well as the opportunity to develop synthetic strategy for the construction of more diverse analogs of these interesting antibiotics have prompted substantial recent synthesis efforts.[3,4] Very recently, the first total synthesis of **1** was disclosed in our laboratories.[5]

Herein we describe in detail, the first total synthesis of apramycin (**1**) and saccharocin (**2**: KA-5685).

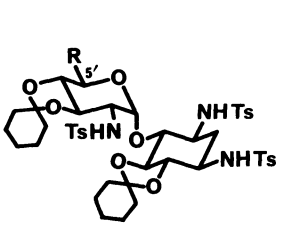
Our point of departure is the known aminoglycoside antibiotic, neamine (**3**), which has already been synthesized by one of us[6] and Umezawa.[7] Neamine (**3**) was successively converted into 6′-*N*-(benzyloxycarbonyl)-3′,4′:5,6-di-*O*-cyclohexylidene-1,2′,3-tri-*N*-tosylneamine (**4**) by preferential *N*-benzyloxycarbonylation,[8] *N*-tosylation and *O*-cyclohexylidenation[9] in a 79% overall yield. Saponification of **4** with potassium *t*-butoxide followed by treatment of the resulting 6′-amino compound with 30% aqueous formaldehyde and $NaBH_3CN$[10] afforded the 6′-dimethylamino compound, which after oxidation with *m*-chloroperbenzoic acid gave the *N*-oxide **5** in an 83% overall yield. Treatment of **5** with benzoyl chloride and *N*,*N*-diisopropylethylamine produced the aldehyde, 4-*O*-[3′,4′-*O*-cyclohexylidene-2′-deoxy-2′-(tosylamino)-α-D-*gluco*-hexodialdo-1′,5′-pyranosyl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**6**) in a 75% yield, suggesting that the procedure would be useful for the preparation of the aldehydes from the primary amines.[11] Other couples of reagents (acetic anhydride, α-toluenesulfonyl chloride, trifluoroacetic anhydride, mesyl chloride, methanesulfonic anhydride, and tosyl chloride) and bases (*N*,*N*-diisopropylethylamine, 4-dimethylaminopyridine, *N*-methylmorpholine, and pyridine, *etc.*) were investigated, but gave **6** in lower yields.

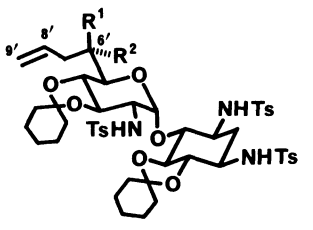
Addition of allylmagnesium chloride to **6** gave a mixture of the (6′*S*)- and (6′*R*)-alcohols,[3a,12] 4-*O*-[3′,4′-*O*-cyclohexylidene-2′,7′,8′,9′-tetradeoxy-2′-(tosylamino)-L-*glycero*- and D-*glycero*-α-D-*gluco*-non-8′-enopyranosyl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamines (**7** and **8**) in 37 and 41% yields, respectively. Reaction with allyltributyltin[13] also gave the similar ratio of the products with that by the Grignard reagent, but allyllithium[13] did not. The stereochemistry



1 (Apramycin): R = NH_2 **3** (Neamine)

2 (Saccharocin): R = OH



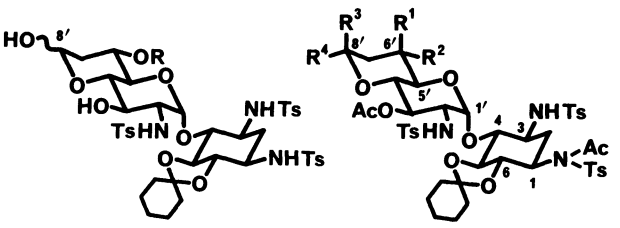


4: R = CH_2NHZ **7**: R^1 = H, R^2 = OH

5: R = $CH_2N(O)Me_2$ **8**: R^1 = OH, R^2 = H

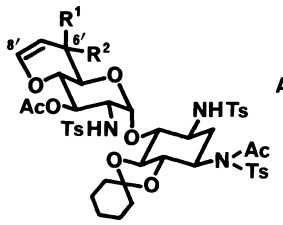
6: R = CHO **16**: R^1 = H, R^2 = OAc

Chart 1.

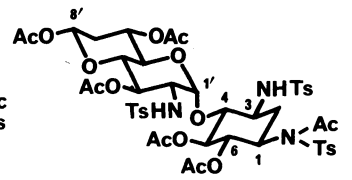


9: R = H
17: R = Ac

10a: $R^1=R^4=H$, $R^2=R^3=OAc$
10b: $R^1=R^3=H$, $R^2=R^4=OAc$
11: $R^1=R^4=OAc$, $R^2=R^3=H$



13: $R^1=H$, $R^2=OAc$
14: $R^1=OAc$, $R^2=H$

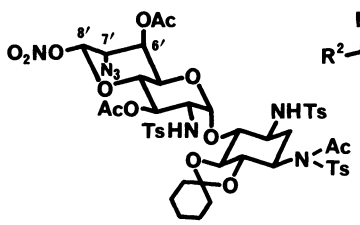


12

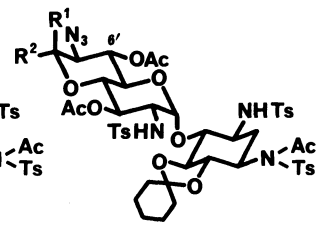
Chart 2.

at C-6′ in **7** and **8** was reasonably clarified by ^{1}H-NMR of the derivatives **10a, b** and **11** as follows. Oxidative cleavage of the olefin group of **7** with osmium tetraoxide and then sodium periodate to give the aldehyde, followed by selective removal of the cyclohexylidene group by aqueous acetic acid, afforded the octodialdose[3a] derivative **9** in a 60% yield. Acetylation of **9** with acetic anhydride and pyridine at 40 °C gave a mixture of the 1-*N*-acetyl-α- and β-acetates, 1-*N*-acetyl-4-*O*-[(8′*S*)- and (8′*R*)-3′,6′,8′-tri-*O*-acetyl-2′,7′-dideoxy-2′-(tosylamino)-L-*glycero*-α-D-*gluco*-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamines (**10a** and **10b**) in 34 and 37% yields. Similarly, **8** was transformed into a single β-acetate **11**. Their ^{1}H-NMR decoupling revealed the H-6′ methine protons in **10** and **11** to be axial ($J_{5',6'}$=10 Hz) and equatorial ($J_{5',6'}$=2.5 Hz), respectively, assigning the stereochemistry of **7** and **8**. Also, the 1-*N*-acetyl structures of **10** and **11** were deduced by the ^{1}H-NMR spectrum of the 1-*N*-acetyl-3′,5,6,6′,8′-penta-*O*-acetyl derivative **12**, which was formed by removal of the cyclic acetal of **10b** followed by acetylation. All methine protons of the 2-deoxystreptamine moiety were completely assigned in **12**, and the significant downfield shift was observed for the H-1 signal (δ 4.34) in comparison with the H-3 signal (δ 3.40).

The aforesaid intermediate **9** was converted by our method[14] using tosyl chloride and triethylamine, and acetylation into the acetyl glycal, 1-*N*-acetyl-4-*O*-[3′,6′-di-*O*-acetyl-4′,8′-anhydro-2′,7′-dideoxy-2′-(tosylamino)-L-*glycero*-α-D-*gluco*-oct-7′-enopyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**13**) in a 52% overall yield. The glycal **13** was treated with 0.5 M† hydrochloric acid, followed by acetylation to give **12** as one of the anomeric acetates, supporting the 1-*N*-acetyl structure. Though **13** possessed an acetoxyl group at C-6′ having the (*S*)-configuration which was incompatible with the natural stereochemistry, it was clear that **13** could be a useful intermediate provided that a cis cyclic carbamate formation could be achieved with the appropriate S_N2 inversion at the later stage (for an example: **24**→**25**). On the other hand, the glycal **14**, which was similarly derived from the other alcohol **8**, possessed the natural (*R*)-configuration at C-6′, but, on azidonitration[15] with sodium azide and ammonium ceric nitrate, gave a single (7′*R*,8′*R*)-product **15** with the undesired axial azido group owing to the sizeable steric interactions of thc C-6′ axial acetoxyl group. Then, **8** was efficiently transformed into the glycal **13** through the inversion of the 6′-hydroxyl group. Mesylation of **8** followed by displacement reaction with potassium acetate gave the corresponding acetate **16** in an 85% yield, which was identical with the acetate of **7**. As described above, oxidative cleavage of the olefin group and selective hydrolysis of **16** afforded the octodialdose derivative **17**, which was in turn led to the key glycal **13** in a 55% yield.



15



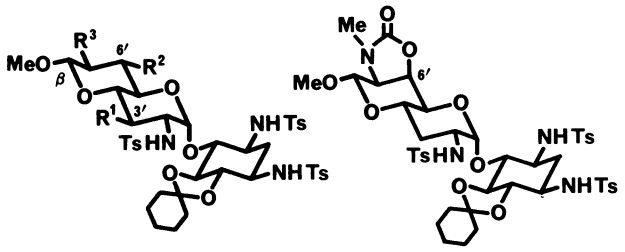
18: $R^1=ONO_2$, $R^2=H$
19: $R^1=H$, $R^2=ONO_2$

Chart 3.

Azidonitration[15] of **13** gave two major azidoglycosyl nitrates **18** and **19** having the (7′*S*,8′*S*)- and (7′*S*,8′*R*)-configurations in 63 and 14% yields. The stereochemistry was defined by their ^{1}H-NMR spectra. Remarkably, the addition was effectively stereoselective, generating only one of the two possible configurations at C-7′. Treatment of either **18** or **19** with barium hydroxide in methanol afforded the corresponding deacetylated methyl β-glycoside, 4-*O*-[(8′*S*)-7′-azido-2′,7′-dideoxy-8′-*O*-methyl-2′-(tosylamino)-D-*thereo*-α-D-*gluco*-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**20**) in 71% or 40% yield, along with less than 7% yield of the α-glycoside. In a straightforward fashion **20** was transformed in a 61% overall yield into the 7′-[*N*-(benzyloxycarbonyl)-methylamino] derivative **22** through **21** *via* a four-step process: catalytic reduction, *N*-benzyloxycarbonylation, hydride reduction and again *N*-benzyloxycarbonylation.

Mesylation of **22** afforded quantitatively the labile dimesylate **23**, the C-3′ position of which was selectively chlorinated to form the 3′-chloro compound, by analogy with the results obtained by Umezawa and co-workers.[16] Dechlorination of the intermediate with tributylstannane gave the 3′-deoxy compound, 4-*O*-{(8′*S*)-7′-[*N*-(benzyloxycarbonyl)methylamino]-2′,3′,7′-trideoxy-6′-*O*-mesyl-8′-*O*-methyl-2′-(tosylamino)-D-*glycero*-β-L-*talo*-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl}-5,6-

† 1 M=1 mol dm^{-3}.



20: $R^1=R^2=OH$, $R^3=N_3$

21: $R^1=R^2=OH$, $R^3=NHZ$

22: $R^1=R^2=OH$, $R^3=N<^{Z}_{Me}$

23: $R^1=R^2=OMs$, $R^3=N<^{Z}_{Me}$

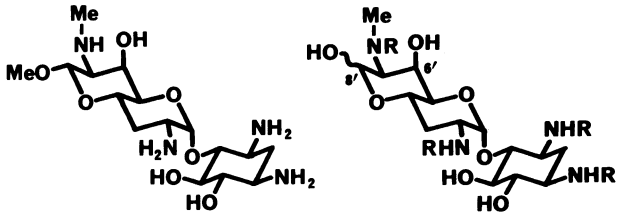
24: $R^1=H$, $R^2=OMs$, $R^3=N<^{Z}_{Me}$

25

Chart 4.

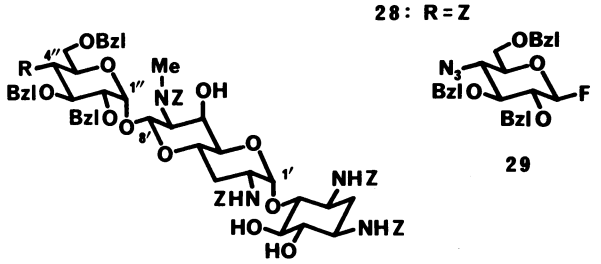
O-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**24**) in an 81% yield from **23**. All attempts[17] to form a cis cyclic carbamate failed, except for epimerization[18] of the 6′-hydroxyl group by heating **24** with sodium acetate trihydrate in 2-methoxyethanol, which yielded only 4-*O*-[(8′*S*)-7′-*N*,6′-*O*-carbonyl-2′,3′,7′-trideoxy-8′-*O*-methyl-7′-(methylamino)-2′-(tosylamino)-D-*glycero*-α-D-*allo*-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-*O*-cyclohexylidene-1,3-di-*N*-tosyl-2-deoxystreptamine (**25**) needed for the apramycin skeleton. The stereochemistry was also confirmed by the ^{1}H-NMR and IR spectra.[17,18]

De-*N*-tosylation of **25** with sodium metal in liquid ammonia followed by alkaline hydrolysis to remove the *N*,*O*-carbonyl group and subsequent acidic hydrolysis to remove the cyclohexlidene group afforded the aminooctodialdose derivative, methyl β-aprosaminide[1b] (**26**) in a 65% overall yield, which was further hydrolyzed with 4M hydrochloric acid to give aprosamine[1b,4] (**27**). Both **26** and **27** were identical in all respects with the naturally derived products, thus setting the stage for introduction of the 4-amino-4-deoxy-D-glucose or D-glucose unit.

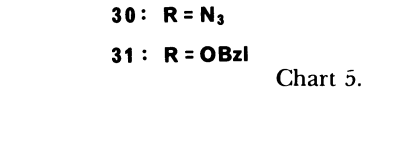


26 (Methyl β-aprosaminide) 27 (Aprosamine): R=H

28: R=Z



29



30: $R=N_3$

31: R=OBzl

Chart 5.

N-Benzyloxycarbonylation of **27** generated the key intermediate for the following glycosidation, 1,2′,3,7′-tetrakis(*N*-benzyloxycarbonyl)aprosamine[4] (**28**) in a 77% yield.

Methanolysis of **28** with a methanolic hydrogen chloride solution or methylation with methyl iodide and silver oxide, to give the corresponding methyl glycoside, followed by catalytic reduction gave the aforesaid methyl β-aprosaminide (**26**) in a fairly good yield (81% or 70%). The findings suggested that the 1,3-diaxial interaction between C-6′- and C-8′- substituents would then lead to the less sterically encumbered transition state and thus to the exclusive formation of the β-glycoside even under acidic conditions, where the anomeric effect could be predominant.[19] Further, reaction of *O*-benzylglycosyl halides with alcohols are known to yield exclusively the corresponding α-glycosides owing to the anomeric effect as commonly seen in carbohydrates.[19-22] Consequently, it was expected that reaction of the appropriate *O*-benzylglycosyl halides with **28** would produce the desired α-glycosyl-β-aprosaminides in reasonable yields. In the glycosidation studies on **28**, a large number of variables including catalyst and temperature were assayed.[20-22] The best result was realized under modified Mukaiyama conditions[20] by using 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-β-D-glucopyranosyl fluoride (**29**), which was prepared from the corresponding α-D-glycosyl chloride,[22] to give two major products having R_f 0.48 and 0.44 on thin-layer chromatography. The R_f 0.44-substance, which was found to be the desired glycoside **30** by the following chemical and physical evidence, could be isolated by silica-gel column chromatography in a modest yield (40% yield based on unrecovered alcohol **28**). It should be noted, however, that the glycosidation was achieved without protection of the C-5, 6, and 6′ hydroxyl groups. Hydrogenolysis of **30** furnished apramycin (**1**) in a 74% yield, which was identical with the natural antibiotic in all respects including antibacterial activity, but the R_f 0.48-substance did not give **1**.

Similarly, glycosidation of the key alcohol **28** employing 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride[20] provided the expected glycoside **31** in a 24% yield (42% yield based on unrecovered **28**) after column chromatography. Hydrogenolysis of **31** completed the synthesis, giving saccharocin (**2**: KA-5685) identical with the natural antibiotic in all respects.

The antibacterial activities of **1**, **2**, **26**, and **27**, which are shown in Table 1, were identical with those of the natural products and their derivatives.

The application of this synthetic strategy for the construction of other related antibiotics as well as some of their potentially therapeutically interesting analogs is an exciting prospect.

Experimental

Melting points were determined on a micro hot-stage Yanaco MP-S3 and were uncorrected. IR and Mass spectra were recorded on Hitachi Perkin-Elmer 225 and Hitachi M-80H (SIMS) spectromters, respectively, and ^{1}H-NMR spectra with TMS as internal standard on a Varian EM-390 (90 MHz) or a Bruker WM 250 spectromter (250 MHz). Optical rotations were measured on a Carl Zeiss photoelectric polar-

TABLE 1. MINIMAL INHIBITORY CONCENTRATION (mcg/ml) OF **1**, **2**, **26**, AND **27**

	1	**2**	**26**	**27**
Staphylococcus aureus FDA 209P	1.56	6.25	12.5	12.5
Klebsiella pneumoniae PCI 602	3.12	6.25	12.5	25
Salmonella typhi T-63	1.56	3.12	6.25	12.5
Escherichia coli K-12 ML 1629	1.56	12.5	12.5	25
Escherichia coli K-12 C600 R135	3.12	6.25	6.25	12.5
Escherichia coli JR66/W677	3.12	12.5	6.25	25
Pseudomonas aeruginosa A3	0.78	1.56	3.12	6.25
Pseudomonas aeruginosa NO. 12	12.5	25	100	50

imeter. Silica-gel TLC and column chromatography were performed on Merck TLC 60F-254 and Wakogel C-200 or Kieselgel 60. In general, organic solvents were purified and dried by the appropriate procedures, and evaporation and concentration were carried out under reduced pressure below 30 °C.

6′-N-(Benzyloxycarbonyl)-3′,4′:5,6-di-O-cyclohexylidene-1,2′,3-tri-N-tosylneamine (4). To a solution of neamine (**3**: 50 g) in 50% aqueous methanol (500 ml) containing triethylamine (23.8 ml) was added *N*-(benzyloxycarbonyloxy)-5-norbornene-2,3-dicarboximide[8] (53.4 g), and the solution was stirred at room temperature for 5 h. After addition of 28% aqueous ammonia (110 ml), the solution was evaporated to a residue of the 6′-*N*-benzyloxycarbonyl derivative. A solution of the residue in 70% aqueous 1,4-dioxane (1 l) was stirred with tosyl chloride (106.4 g) and Na_2CO_3 (61.1 g) at room temperature for 5 h. After filtration, the filtrate was concentrated to give gummy precipitates, which were washed with water and then dried to give a solid of the tri-*N*-tosyl derivative (166 g). To a solution of the solid in DMF (1.66 *l*) were added 1,1-dimethoxycyclohexane (187 ml) and anhydrous TsOH (2.67 g), and the solution was stirred at 50 °C for 1 d under reduced pressure (≃25 Torr††). After further addition of 1,1-dimethoxycyclohexane (187 ml), the reaction was continued at the same conditions for another 2 d. After addition of triethylamine (2.16 ml) and $NaHCO_3$ (6.51 g), the mixture was evaporated to a residue, which was chromatographed on silica gel (2 kg) with 2:1 toluene-ethyl acetate to give a solid of **4** (132.2 g, 79% from **3**): R_f 0.64 (20:1 $CHCl_3$-MeOH); mp 139—142 °C; $[\alpha]_D^{23}$ −38° (*c* 1.0, $CHCl_3$); IR (KBr): 1700 (NH-CO), and 1160 cm^{-1} (SO_2); ^{1}H-NMR (acetone-d_6): δ=2.39, 2.40, and 2.43 (each 3H, s, Ts), 5.06 (2H, s, CH_2 of Z), and 5.30 (1H, d, H-1′, $J_{1',2'}$=3.5 Hz).

Found: C, 58.76; H, 6.19; N, 5.34; S, 8.59% Calcd for $C_{53}H_{66}N_4O_{14}S_3$: C, 58.98; H, 6.16; N, 5.19; S, 8.91%.

3′,4′:5,6-Di-O-cyclohexylidene-6′,6′-di-N-methyl-1,2′,3-tri-N-tosylneamine 6′-N-oxide (5). A solution of **4** (60.8 g) in *t*-butyl alcohol (1830 ml) containing water (3.10 ml) and potassium *t*-butoxide (63.2 g) was stirred at 70 °C for 1 h. The reaction solution diluted with ethyl acetate (1000 ml) was neutralized with 0.1 M HCl (2100 ml), and the organic layer was washed with a saturated aqueous NaCl solution, dried, and then evaporated to give a solid of the 6′-amino compound (56 g).

To a stirred and ice-cooled solution of the solid in acetonitrile (1030 ml) were added 30% aqueous formaldehyde (52.0 ml), $NaBH_3CN$ (10.6 g), and acetic acid (32.0 ml), and stirring was continued at room temperature for 1 h. The reaction mixture neutralized with a saturated aqueous $NaHCO_3$ solution was extracted with ethyl acetate (400 ml×3). The combined extracts were washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue of the dimethylamino compound (60 g): R_f 0.47 (10:1 $CHCl_3$-MeOH).

A solution of the residue in chloroform (1120 ml) was stirred with *m*-chloroperbenzoic acid (9.80 g) at room temperature for 1 h. The solution diluted with chloroform (500 ml) was washed successively with saturated aqueous $NaHCO_3$ and NaCl solutions, dried, and then evaporated to a residue, which was chromatographed on silica gel (500 g) with 10:1→8:1 chloroform-methanol to give a solid of the monohydrate of **5** (47.1 g, 83% from **4**): R_f 0.36 (10:1 $CHCl_3$-MeOH); mp 175—179 °C; $[\alpha]_D^{23}$ −23° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ=2.42 (9H, s, Ts), 3.24 and 3.31 (each 3H, s, NMe_2), and 5.45 (1H, d, H-1′, $J_{1',2'}$=3.0 Hz).

Found: C, 55.78; H, 6.42; N, 5.40%. Calcd for $C_{47}H_{64}N_4O_{13}S_3 \cdot H_2O$: C, 56.05; H, 6.60; N, 5.56%.

4-O-[3′,4′-O-Cyclohexylidene-2′-deoxy-2′-(tosylamino)-α-D-gluco-hexodialdo-1′,5′-pyranosyl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (6). To a stirred and ice-cooled solution of **5** (11.5 g) in acetone (115 ml) were added *N,N*-diisopropylethylamine (3.99 ml) and benzoyl chloride (2.66 ml), and stirring was continued at room temperature for 3 min and then at 40 °C for 10 min. The resulting solution diluted with chloroform (250 ml) was washed successively with saturated aqueous $NaHCO_3$ and NaCl solutions, dried, and evaporated to a residue, which was chromatographed on silica gel (600 g) with 3:1→2:1 chloroform-acetone to give a solid of the monohydrate of **6** (8.24 g, 75%): R_f 0.38 (20:1 $CHCl_3$-MeOH); mp 144—148 °C; $[\alpha]_D^{23}$ −15° (*c* 1.0, $CHCl_3$); IR (KBr): 1735 (CHO), and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$+D_2O): δ=2.41 (9H, s, Ts×3), and 9.67 (1H, s, CHO).

Found: C, 56.41; H, 6.18; N, 4.21%. Calcd for $C_{45}H_{57}N_3O_{13}S_3 \cdot H_2O$: C, 56.18; H, 6.18; N, 4.37%.

4-O-[3′,4′-O-Cyclohexylidene-2′,7′,8′,9′-tetradeoxy-2′-(tosylamino)-L-glycero- and D-glycero-α-D-gluco-non-8′-enopyranosyl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamines (7 and 8). To an allylmagnesium chloride solution prepared *in situ* from magnesium (2.39 g), allyl chloride (3.21 ml), and iodine (0.05 g) in THF (120 ml) was added dropwise with stirring at 5 °C under argon, a solution of **6** (6.20 g) and allyl chloride (4.82 ml) in THF (186 ml), and stirring was continued at room temperature for 15 min and then at 60 °C for 30 min. After the reaction was quenched with a saturated aqueous NH_4Cl solution, the reaction mixture was extracted with ethyl acetate (200 ml×3). The combined extracts were washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue, which was chromatographed on silica gel (600 g) with 40:1:1 chloroform-acetone-methanol to give **7** (2.35 g, 37%) and **8** (2.61 g, 41%) having the R_f-values of 0.48 and 0.43 (20:1 $CHCl_3$-MeOH) respectively.

7: Mp 134—137 °C; $[\alpha]_D^{23}$ −20° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$+D_2O): δ=3.97 (1H, dd, H-5′, $J_{4',5'}$=9.5 Hz, $J_{5',6'}$=7.0 Hz), 5.11 (1H, dd, H-9′a, $J_{7',9'a}$=2.0 Hz, $J_{8',9'a}$=10 Hz), 5.14 (1H, dd, H-9′b, $J_{7',9'b}$=2.0 Hz, $J_{8',9'b}$=17.5 Hz), 5.34 (1H, d, H-1′, $J_{1',2'}$= 3.0 Hz), and 5.90 (1H, ddt, H-8′, $J_{7',8'}$=7 Hz).

Found: C, 58.67; H, 6.58; N, 4.14; S, 9.49%. Calcd for $C_{48}H_{63}N_3O_{13}S_3$: C, 58.46; H, 6.44; N, 4.26; S, 9.75%.

8: Mp 131—133 °C; $[\alpha]_D^{23}$ −5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$+D_2O): δ=3.94 (1H, m, H-6′), 4.12 (1H, dd, H-5′, $J_{4',5'}$=10 Hz, $J_{5',6'}$=3.5 Hz), 5.09 (1H, dd, H-9′a, $J_{7',9'a}$≈1.5 Hz, $J_{8',9'a}$=10 Hz), 5.14 (1H, dd, H-9′b, $J_{7',9'b}$≈2.0 Hz, $J_{8',9'b}$=16.5 Hz), 5.31 (1H, d, H-1′, $J_{1',2'}$=2.5 Hz), and 5.88 (1H, ddt, H-8′, $J_{7',8'}$=7 Hz).

††1 Torr≈133.322 Pa.

Found: C, 58.57; H, 6.58; N, 4.02; S, 9.48%. Calcd for $C_{48}H_{63}N_3O_{13}S_3$: C, 58.46; H, 6.44; N, 4.26; S. 9.75%.

5,6-O-Cyclohexylidene-4-O-[(8'RS)-2',7'-dideoxy-2'-(tosylamino)-L-glycero-α-D-gluco-*octodialdo-1',5':8',4'-dipyranos-1'-yl]-1,3-di-N-tosyl-2-deoxystreptamine (9).* A solution of **7** (3.2g) in 75% aqueous 1,4-dioxane (128ml) was stirred with OsO_4 (41mg) at room temperature. After 30min, to the stirred solution was added portionwise $NaIO_4$ (2.94g), and stirring was continued at room temperature for 1h. The resulting solution diluted with ethyl acetate was washed with a saturated aqueous NaCl solution (50ml) containing $NaHSO_3$ (17mg), dried, and evaporated to a residue of the aldehyde (3.2g): R_f 0.44 (15:1 $CHCl_3$-MeOH); ^{1}H-NMR ($CDCl_3$+D_2O): δ=2.40 (9H, broad s, Ts×3), and 9.79 (1H, broad s, CHO).

A solution of the residue in a 2:1:1 mixture (64ml) of acetic acid, 1,4-dioxane and water was stirred at room temperature for 1.5h. The mixture was poured into a saturated aqueous $NaHCO_3$ solution, and extracted with ethyl acetate (100ml×3). The combined extracts were washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue, which was chromatographed on silica gel (150g) with 3:1→2:1 chloroform-acetone to give a solid of the monohydrate of **9** (1.80g, 60% from **7**): R_f 0.17 (15:1 $CHCl_3$-MeOH); mp 180—183°C; $[\alpha]_D^{23}$ −13° (*c* 1.0, MeOH); ^{1}H-NMR (CD_3CN+D_2O): δ=3.88 (1H, ddd, H-6' $J_{5',6'}$=9.5 Hz, $J_{6',7'ax}$=11.5 Hz, $J_{6',7'eq}$=5.0 Hz), 4.69 (0.5H, dd, H-8'ax, $J_{7'ax,8'ax}$=10 Hz, $J_{7'eq,8'ax}$=2.0 Hz), 5.08 and 5.09 (1H in total, each d, H-1', $J_{1',2'}$=4.0 Hz and 3.5 Hz respectively), and 5.21 (0.5H, dull d, H-8'eq, $J_{7',8'eq}$≈3 Hz).

Found: C, 52.98; H, 5.75; N, 4.75%. Calcd for $C_{41}H_{53}N_3O_{14}S_3\cdot H_2O$: C, 53.17; H, 5.99; N, 4.54%.

1-N-Acetyl-4-O-[(8'S)- and (8'R)-3',6',8'-tri-O-acetyl-2',7'-dideoxy-2'-(tosylamino)-L-glycero-α-D-gluco-*octodialdo-1',5':8',4'-dipyranos-1'-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamines (10a and 10b).* A solution of **9** (5.6mg) and acetic anhydride (0.06ml) in pyridine (0.11ml) was stirred at 40°C for 16h. The mixture diluted with ethyl acetate was washed with a saturated aqueous $NaHCO_3$ solution, dried, and evaporated to a residue, which was chromatographed on silica gel (1g) with 4:1 benzene-ethyl acetate to give **10a** (2.4mg, 37%) and **10b** (2.2mg, 34%) having the R_f-values of 0.33 and 0.38 (2:1 benzene-ethyl acetate) respectively.

10a: Mp 160—162°C; $[\alpha]_D^{23}$ +18° (*c*1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ=1.61, 2.12, 2.16, and 2.23 (each 3H, s, Ac), 1.80 (1H, ddd, H-7'ax, $J_{6',7'ax}$=11.5 Hz, J_{gem}=13.5 Hz, $J_{7'ax,8'}$=3.8 Hz) , 3.48 (1H, dt, H-2', $J_{1',2'}$=3.8 Hz, $J_{2',NH}$=$J_{2',3'}$=10 Hz), 3.62 (1H, t, H-4', $J_{3',4'}$=$J_{4',5'}$=10 Hz), 3.95 (1H, t, H-5', $J_{5',6'}$=10 Hz), 4.38 (1H, dd, H-6, $J_{1,6}$=10.5 Hz, $J_{5,6}$=9 Hz), 4.53 (1H, dt, H-1, $J_{1,2ax}$=10.5 Hz, $J_{1,2eq}$=4.5 Hz), 4.94 (1H, t, H-3'), and 6.07 (1H, dull d, H-8').

Found: C, 54.54; H, 5.89; N, 3.86%. Calcd for $C_{49}H_{61}N_3O_{18}S_3$: C, 54.69; H, 5.71; N, 3.90%.

10b: Mp 164—168°C; $[\alpha]_D^{23}$ +60° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$+D_2O): δ=1.55, 2.07, 2.15, and 2.22 (each 3H, s, Ac), 3.22 (1H, t, H-4', $J_{3',4'}$=$J_{4',5'}$=10 Hz), 3.42 (1H, dd, H-2', $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=10 Hz), 3.48 (1H, t, H-5, $J_{4,5}$=$J_{5,6}$=9.5 Hz), 3.71 (1H, t, H-4, $J_{3,4}$=9.5 Hz), 3.83 (1H, t, H-5', $J_{5',6'}$=10 Hz), 4.38 (1H, dd, H-6, $J_{1,6}$=10.5 Hz, $J_{5,6}$=9.5 Hz), 4.54 (1H, dt, H-1, $J_{1,2ax}$=$J_{1,6}$= 10.5 Hz, $J_{1,2eq}$=4 Hz), 4.92 (1H, t, H-3'), 4.95 (1H, ddd, H-6', $J_{6',7'ax}$= 10.5 Hz, $J_{6',7'eq}$=5 Hz), 5.28 (1H, d, H-1'), and 5.60 (1H, dd, H-8', $J_{7'ax,8'}$=10 Hz, $J_{7'eq,8'}$=2.0 Hz).

Found: C, 54.78; H, 5.82; N, 3.65%. Calcd for $C_{49}H_{61}N_3O_{18}S_3$: C, 54.69; H, 5.71; N, 3.90%.

1-N-Acetyl-4-O-[(8'R)-3',6',8'-tri-O-acetyl-2',7'-dideoxy-2'-(tosylamino)-D-glycero-α-D-gluco-*octodialdo-1',5':8',4'-dipyranos-1'-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (11).* A sample of **8** (315mg) was treated with OsO_4 and $NaIO_4$ followed by hydrolysis with acetic acid by the procedure described in the preparation of **9**, and then acetylated as described for **10a,b** to give a solid of **11** (154mg, 45%): R_f 0.43 (2:1 benzene-ethyl acetate); mp 164—169°C; $[\alpha]_D^{23}$ +30° (*c* 1.0, $CHCl_3$); IR (KBr): 1750 (ester), and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$): δ=1.60, 2.05, 2.14, and 2.18 (each 3H, s, Ac), 3.79 (1H, t, H-4', $J_{3',4'}$=$J_{4',5'}$=10 Hz), 4.36 (1H, dd, H-5', $J_{5',6'}$=2.5 Hz), 5.12 (1H, d, H-1', $J_{1',2'}$=3.5 Hz), 5.59 (1H, m, H-6'), and 5.85 (1H, dd, H-8', $J_{7'ax,8'}$=9.5 Hz, $J_{7'eq,8'}$=3.0 Hz).

Found: C, 54.57; H, 5.78; N, 3.68; S, 8.90%. Calcd for $C_{49}H_{61}N_3O_{18}S_3$: C, 54.69; H, 5.71; N, 3.90; S, 8.94%.

1-N-Acetyl-5,6-di-O-acetyl-4-O-[(8'R)-3',6',8'-tri-O-acetyl-2',7'-dideoxy-2'-(tosylamino)-L-glycero-α-D-gluco-*octodialdo-1',5':8'4'-dipyranos-1'-yl]-1,3-di-N-tosyl-2-deoxystreptamine (12).*

A) From 10b: A solution of **10b** (23mg) in a 2:1:1 mixture (0.5ml) of acetic acid, 1,4-dioxane and water was warmed at 50°C for 1d. The resulting solution diluted with ethyl acetate was washed with a saturated aqueous $NaHCO_3$ solution, dried, and evaporated to give a residue: R_f 0.28 (1:1 benzene-ethyl acetate).

A solution of the decyclohexylidenated product in pyridine (0.5ml) was stirred with acetic anhydride (0.3ml) at 40°C overnight. After addition of a few drops of ethanol, the solution was evaporated to a residue, which was partitioned between ethyl acetate and a saturated aqueous $NaHCO_3$ solution. The organic layer was dried and evaporated to a residue, which was chromatographed on silica gel (2g) with 5:2 benzene-ethyl acetate to give a solid of **12** (13mg, 56% from **10b**): R_f 0.50 (1:1 benzene-ethyl acetate); mp 161—164°C; $[\alpha]_D^{23}$ +58° (*c* 1.0, $CHCl_3$); IR (KBr): 1750 (ester), and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$+D_2O): δ=1.34, 1.80, 2.06, 2.11, 2.15, and 2.25 (each 3H, s, Ac), 2.40, 2.41, and 2.47 (each 3H, s, Ts), 3.19 (1H, t, H-4', $J_{3',4'}$=$J_{4',5'}$=9.8 Hz), 3.40 (1H, m, H-3), 3.46 (1H, dd, H-2', $J_{1',2'}$=3.8 Hz, $J_{2',3'}$=10.8 Hz), 3.80 (1H, t, H-4, $J_{3,4}$=$J_{4,5}$= 9.5 Hz), 4.13 (1H, t, H-5', $J_{5',6'}$≈9.5 Hz), 4.34 (1H, m, H-1), 4.80 (1H, dd, H-3'), 4.94 (1H, dt, H-6', $J_{6',7'ax}$=9.5 Hz, $J_{6',7'eq}$=5.5 Hz), 5.18 (1H, d, H-1'), 5.22 (1H, t, H-5, $J_{5,6}$=9.5 Hz), 5.56 (1H, dd, H-8', $J_{7'ax,8'}$=10.5 Hz, $J_{7'eq,8'}$=2.5 Hz), and 5.65 (1H, dull t, H-6, $J_{1,6}$=10 Hz).

Found: C, 52.21; H, 5.40; N, 3.88; S, 8.65%. Calcd for $C_{47}H_{57}N_3O_{20}S_3$: C, 52.26; H, 5.32; N, 3.89; S, 8.90%.

B) From 13: A solution of **13** (7.7mg) in a 1:1 mixture (0.15ml) of 0.5M HCl and 1,4-dioxane was warmed at 50°C for 2d, and then evaporated to a residue of the octodialdose derivative. The residue was acetylated with acetic anhydride (0.2ml) in pyridine (0.4ml) at 40°C for 13h, and worked up as described above to give two products having R_f-values of 0.50 and 0.45 (1:1 benzene-ethyl acetate). The R_f 0.50-substance (2.5mg, 31%) was identical with **12** in all respects. The R_f 0.45-substance (2.8mg) seemed to be the corresponding (8'*S*)-isomer, but was not further investigated.

1-N-Acetyl-4-O-[3',6'-di-O-acetyl-4',8'-anhydro-2',7'-dideoxy-2'-(tosylamino)-L-glycero-α-D-gluco-*oct-7'-enopyranos-1'-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (13).* *A)*[14] *From 9*: To a stirred and ice-cooled solution of **9** (1.27g) in acetonitrile (25.4ml) were added triethylamine (1.17ml) and tosyl chloride (0.8g), and stirring was continued at room temperature for 12h. After addition of ethanol (0.16ml) and a saturated aqueous $NaHCO_3$ solution (0.3ml), the mixture was evaporated to a residue. The residue was partitioned between chloroform and a saturated aqueous $NaHCO_3$ solution, and the combined organic layers were evaporated to give a solid of the glycal (1.4g): R_f 0.39 (15:1 $CHCl_3$-MeOH).

A solution of the solid in pyridine (28ml) was stirred with acetic anhydride (14ml) at 40°C for 19h. After addition of ethanol (8.6 ml), the resulting solution was neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with

ethyl acetate. The combined extracts were washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue, which was chromatographed on silica gel (60g) with 2:1→3:2 hexane-ethyl acetate to give a solid of **13** (0.73g, 53%): R_f 0.86 (1:1 benzene-EtOAc); R_f 0.40 (3:1 benzene-EtOAc); mp 161—164°C;$[\alpha]_D^{23}$ +98° (*c* 1.0, $CHCl_3$); IR (KBr): 1745 cm^{-1} (ester), ^{1}H-NMR ($CDCl_3$): δ=1.58, 2.20, and 2.23 (each 3H, s, Ac), 3.59 (1H, dd, H-4′, $J_{3',4'}$=9.0 Hz, $J_{4',5'}$=10.5 Hz), 3.91 (1H, dd, H-5′ $J_{5',6'}$=8.0 Hz), 4.74 (1H, dd, H-3′, $J_{2',3'}$=10.5 Hz), 4.84 (1H, dd, H-7′, $J_{6',7'}$=2.3 Hz, $J_{7',8'}$=6.0 Hz), 5.20 (1H, d, H-1′, $J_{1',2'}$=4.0 Hz), 5.32 (1H, m, H-6′), and 6.29 (1H, dd, H-8′, $J_{6',8'}$=1.5 Hz).

Found: C, 55.63; H, 5.68; N, 3.86; S, 9.48%. Calcd for $C_{47}H_{57}N_3O_{16}S_3$: C, 55.55; H, 5.65; N, 4.14; S, 9.47%.

B) From 17: **17** (0.6g) was treated with triethylamine and tosyl chloride in acetonitrile, followed by acetylation, as described above, to give a solid of **13** (0.35g, 55%).

*1-N-Acetyl-4-O-[3′,6′-di-O-acetyl-4′,8′-anhydro-2′,7′-dideoxy-2′-(tosylamino)-*D*-glycero-α-*D*-gluco-oct-7′-enopyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (14).* A sample of **8** (400mg) was treated by the procedure described in the preparation of **13** through **9**, and then worked up to give, after reprecipitation from benzene-hexane, a solid of **14** (95mg, 23%): R_f 0.33 (3:1 benzene-EtOAc); mp 142—145°C; $[\alpha]_D^{23}$ −35° (*c* 1.0, $CHCl_3$): IR (KBr): 1745 cm^{-1} (ester); ^{1}H-NMR ($CDCl_3+D_2O$): δ=1.69, 2.09, and 2.17 (each 3H, s, Ac), 3.83 (1H, dd, H-4′, $J_{3',4'}$=9.5 Hz, $J_{4',5'}$=11 Hz), 4.46 (1H, dd, H-5′, $J_{5',6'}$=4.0 Hz), 4.98 (1H, t, H-7′, $J_{6',7'}=J_{7',8'}$=6.0 HZ), 5.13 (1H, d, H-1′, $J_{1',2'}$=3.5 Hz), 5.24 (1H, dd, H-3′, $J_{2',3'}$=11 Hz), 5.49 (1H, dd, H-6′), and 6.43 (1H, d, H-8′).

Found: C, 55.59; H, 5.62; N, 4.10%. Calcd for $C_{47}H_{57}N_3O_{16}S_3$: C, 55.55; H, 5.65; N, 4.14%.

*1-N-Acetyl-4-O-[(8′*R*)-3′,6′-di-O-acetyl-7′-azido-2′,7′-dideoxy-8′-O-nitro-2′-(tosylamino)-*L*-threo-α-*D*-gluco-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (15).* To a solution of **14** (246mg) in acetonitrile (4.9ml) were added sodium azide (24mg) and ammonium ceric nitrate (400mg) at −10°C under argon, and the mixture was stirred at this temperature for 3h. After further addition of ammonium ceric nitrate (270mg), stirring was continued for another 3h. The reaction mixture diluted with ice-cooled chloroform was washed sequentially with saturated aqueous NaCl and $NaHCO_3$ solutions under ice-cooling, dried, and evaporated to a residue, which was chromatographed on silica gel (30g) with 6:1→5:1 benzene-ethyl acetate to give a solid of **15** (163mg, 60%): R_f 0.45 (3:1 benzene-EtOAc); mp 160—163°C; $[\alpha]_D^{23}$ −7.5°(*c* 1.0, $CHCl_3$); IR (KBr): 2120 (azide), 1655 and 1290 cm^{-1} (ONO_2), ^{1}H-NMR ($CDCl_3+D_2O$): δ=1.68 (3H, s, Ac), 2.18 (6H, s, Ac×2), 2.43 (6H, s, Ts×2), 2.45 (3H, s, Ts), 4.08 (1H, t, H-4′, $J_{3',4'}=J_{4',5'}$=10.5 Hz), 4.09 (1H, dd, H-7′, $J_{6',7'}$=3.0 Hz, $J_{7',8'}\simeq$0.5 Hz), 4.50 (1H, dd, H-5′, $J_{5',6'}$=3.0 Hz), 5.07 (1H, d, H-1′, $J_{1',2'}$=3.5 Hz), 5.21 (1H, t, H-3′, $J_{2',3'}\simeq$10 Hz), 5.41 (1H, t, H-6′), and 6.04 (1H, apparently s, H-8′).

Found: C, 50.54; H, 5.09; N, 8.52%. Calcd for $C_{47}H_{57}N_7O_{19}S_3$: C, 50.40; H, 5.13; N, 8.75%.

*4-O-[6′-O-Acetyl-3′,4′-O-cyclohexylidene-2′,7′,8′,9′-tetradeoxy-2′-(tosylamino)-*L*-glycero-α-*D*-gluco-non-8′-enopyranosyl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (16).* *A) From 8*: To a stirred and ice-cooled solution of **8** (1.64g) in pyridine was added mesyl chloride (0.26ml), and stirring was continued at room temperature for 2.5h. After addition of ethanol (0.39ml), the resulting solution was neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The extracts were washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue, which was chromatographed on silica gel (60g) with 3:1 benzene-ethyl acetate to give a solid of the mesylate (1.70g): R_f 0.21 (3:1 benzene-EtOAc).

A solution of the solid (2.29g) in DMSO (22.9ml) was stirred with potassium acetate (1.26g) at 78°C for 18h. The solution diluted with ethyl acetate was washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue,which was chromatographed on silica gel (100g) with 4:1→3:1 benzene-ethyl acetate to give a solid of **16** (1.96g, 85% from **8**): R_f 0.35 (3:1 benzene-EtOAc); R_f 0.69 (15:1 $CHCl_3$-MeOH); mp 136—138°C; $[\alpha]_D^{23}$ −33° (*c* 1.0, $CHCl_3$); IR (KBr): 1735, 1720 (ester), and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3+D_2O$): δ=2.07 (3H, s, Ac), 4.16 (1H, dd, H-2′, $J_{1',2'}$=3.5 Hz, $J_{2',3'}$=9.5 Hz), 5.02 (1H, m, H-6′), 5.07 (1H, dd, H-9′a, $J_{7',9'a}$=2.0 Hz, $J_{8',9'a}$=10 Hz), 5.12 (1H, dd, H-9′b, $J_{7',9'b}$=2.0 Hz, $J_{8',9'b}$=16 Hz`, 5.47 (1H, d, H-1′), and 5.79 (1H, m, H-8′).

Found: C, 58.26; H, 6.39; N, 3 Calcd for $C_{50}H_{65}N_3O_{14}S_3$: C, 58.40; H, 6.37; N, [illegible] %.

B) From 7: A solution of **7** (10mg) in pyridine (0.25ml) was stirred with acetic anhydride (0.10ml) at room temperature for 3h. After addition of a few drops of ethanol, the resulting solution was evaporated to a residue, which was chromatographed on silica gel (1g) with 3:1 benzene-ethyl acetate to give a solid of **16** (9.8mg, 94%).

*4-O-[(8′RS)-6′-O-Acetyl-2′,7′-dideoxy-2′-(tosylamino)-*L*-glycero-α-*D*-gluco-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (17).* A sample of **16** (1.24g) was treated by the procedure described in the preparation of **9** and then worked up to give, through the aldehyde: R_f 0.67 (1:1 benzene-EtOAc); ^{1}H-NMR ($CDCl_3+D_2O$): δ=2.09 (3H, s, Ac) and 9.77 (1H, broad s, CHO), a solid of **17** (0.94g, 82%): R_f 0.15 (1:1 benzene-EtOAc); R_f 0.28 (15:1 $CHCl_3$-MeOH); mp 164—166°C; $[\alpha]_D^{23}$ +2.5° (*c* 1.0, $CHCl_3$); IR (KBr): 1730 (ester), and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3+D_2O$): δ=1.98 (3H, s, Ac), 2.35 (3H, s, Ts), and 2.41 (6H, s, Ts×2).

Found: C, 54.15; H, 5.88; N, 4.15%. Calcd for $C_{43}H_{55}N_3O_{15}S_3$: C, 54.40; H, 5.83; N, 4.42%.

*1-N-Acetyl-4-O-[(8′S)- and (8′*R*)-3′,6′-di-O-acetyl-7′-azido-2′,7′-dideoxy-8′-O-nitro-2′-(tosylamino)-*D*-threo-α-*D*-gluco-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamines (18 and 19).* To a solution of **13** (262mg) in acetonitrile (5.2ml) were added sodium azide (25mg) and ammonium ceric nitrate (424mg) at −17°C under argon, and the mixture was stirred at this temperature for 1.5h. After further addition of ammonium ceric nitrate (140mg), stirring was continued for another 1h. The reaction mixture was worked up as described for **15** to give **18** (182mg, 63%) and **19** (41mg, 14%) having the R_f-values of 0.44 and 0.50 (3:1 benzene-ethyl acetate) respectively.

18: Mp 158—161°C; $[\alpha]_D^{23}$ −13° (*c* 1.0, $CHCl_3$); IR (KBr): 2115 (azide), 1750 (ester), 1650 and 1280 cm^{-1} (ONO_2); ^{1}H-NMR ($CDCl_3$): δ=1.57, 2.19, and 2.20 (each 3H, s, Ac), 3.76 (1H, t, H-4′, $J_{3',4'}=J_{4',5'}$=10 Hz), 3.94 (1H, dd, H-7′, $J_{6',7'}$=10.5 Hz, $J_{7',8'}$=4 Hz), 4.44 (1H, t, H-5′, $J_{5',6'}$=10 Hz), 5.40 (1H, dull t, H-6′), and 6.20 (1H, d, H-8′).

Found: C, 50.63; H, 5.25; N, 8.55; S, 8.28%. Calcd for $C_{47}H_{57}N_7O_{19}S_3$: C, 50.40; H, 5.13; N, 8.75; S, 8.59%.

19: Mp 153—157°C; $[\alpha]_D^{23}$ +25° (*c* 1.0, $CHCl_3$); IR (KBr): 2115 (azide), 1750 (ester), 1660 and 1285 cm^{-1} (ONO_2); ^{1}H-NMR ($CDCl_3+D_2O$): δ=1.62, 2.22, and 2.24 (each 3H, s, Ac), 3.34 (1H, t, H-4′, $J_{3',4'}=J_{4',5'}$=10 Hz), 3.43 (1H, dd, H-2′,$J_{1',2'}$= 3.5 Hz, $J_{2',3'}$=10.5 Hz), 3.50 (1H, dd, H-7′, $J_{6',7'}$=10 Hz, $J_{7',8'}$= 9.0 Hz), 4.12 (1H, t, H-5′, $J_{5',6'}$=10 Hz), 4.48 (1H, m, H-1), 5.13 (1H, t, H-6′), 5.14 (1H, dd, H-3′), 5.32 (1H, d, H-1′), and 5.43 (1H, d, H-8′).

Found: C, 50.50; H, 5.23; N, 8.62%. Calcd for $C_{47}H_{57}N_7O_{19}S_3$: C, 50.40; H, 5.13; N, 8.75%.

*4-O-[(8′S)-7′-Azido-2′,7′-dideoxy-8′-O-methyl-2′-(tosylamino)-*D*-threo-α-*D*-gluco-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di-N-tosyl-2-deoxystreptamine (20).* *A)*

From 18: A solution of **18** (177.5 mg) in methanol (5.4 ml) was stirred with $Ba(OH)_2 \cdot 8H_2O$ (100 mg) at 60 °C for 25 min. The mixture diluted with methanol was neutralized with Dry Ice, and then evaporated to a residue, which was chromatographed on silica gel (17 g) with 3:1 chloroform-acetone to give a solid of **20** (108 mg, 71%): R_f 0.44 (15:1 $CHCl_3$-MeOH); mp 157—160 °C ; $[\alpha]_D^{23}$ +5° (*c* 1.0, MeOH); IR (KBr): 2115 (azide), and 1160 cm^{-1} (SO_2); ^{1}H-NMR (acetone-d_6+D_2O): δ=2.41, 2.43, and 2.44 (each 3H, s, Ts), 2.98 (1H, dd, H-3′, $J_{2',3'}$=9.8 Hz, $J_{3',4'}$=9.5 Hz), 3.23 (1H, dd, H-2′, $J_{1',2'}$=3.5 Hz), 3.47 (1H, t, H-6′, $J_{5',6'}$=$J_{6',7'}$=9.5 Hz), 3.53 (3H, s, OMe), 3.79 and 3.80 (each 1H, t, H-4′, and H-5′, $J_{4',5'}$=9.5 Hz), 4.21 (1H, d, H-8′, $J_{7',8'}$=8.0 Hz), and 5.34 (1H, d, H-1′).

Found: C, 52.20; H, 5.62; N, 8.45%. Calcd for $C_{42}H_{54}N_6O_{14}S_3$: C, 52.38; H, 5.65; N, 8.73%.

B) From 19: A solution of **19** (177 mg) was treated with $Ba(OH)_2 \cdot 8H_2O$ in methanol as described above to give a solid of **20** (61 mg, 40%) having R_f 0.44 (15:1 $CHCl_3$-MeOH) and another solid (11 mg) having R_f 0.47. The latter solid, probably the (8′*R*)-isomer, was not further investigated.

4-O-[(8′S)-7′-(Benzyloxycarbonylamino)-2′,7′-dideoxy-8′-O-methyl-2′-(tosylamino)-D-threo-α-D-gluco-*octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-O-cyclohexylidene-1,3-di*-N-*tosyl-2-deoxystreptamine (21).* A solution of **20** (45.6 mg) in methanol (0.91 ml) was shaken with platinum black and 3-atm hydrogen at room temperature for 2 h, filtered and evaporated to give a solid of the amino compound (46 mg): R_f 0.07 (10:1 $CHCl_3$-MeOH).

To a stirred and ice-cooled solution of the solid in 75% aqueous acetone (1.9 ml) were added Na_2CO_3 (6.3 mg) and benzyloxycarbonyl chloride (0.0085 ml), and stirring was continued at room temperature for 10 min. The reaction mixture diluted with ethyl acetate was washed with a saturated aqueous NaCl solution, dried, and evaporated to a residue, which was chromatographed on silica gel (4 g) with 5:2 chloroform-acetone to give a solid of **21** (38.7 mg, 76.3%): R_f 0.49 (10:1 $CHCl_3$-MeOH); mp 160—164 °C ; $[\alpha]_D^{28}$ −10° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$+D_2O): δ=3.47 (3H, s, OMe), 4.94 and 5.18 (each 1H, d, J_{AB}=12.5 Hz, CH_2Ph), and 5.05 (1H, d, $J_{1',2'}$=4.0 Hz, H-1′).

Found: C, 55.87; H, 5.83; N, 5.14%. Calcd for $C_{50}H_{62}N_4O_{16}S_3$: C, 56.06; H, 5.83; N, 5.23%.

4-O-{(8′S)-7′-[N-(Benzyloxycarbonyl)methylamino-2′,-7′-dideoxy-8′-O-methyl-2′-(tosylamino)-D-threo-α-D-gluco-*octodialdo-1′,5′:8′,4′-dipyranos-1′-yl}-5,6-O-cyclohexylidene-1,3-di*-N-*tosyl-2-deoxystreptamine (22).* A solution of **21** (38.1 mg) in THF (1.2 ml) was stirred with $LiAlH_4$ (13.5 mg) at 80 °C for 3 h. After quenching with $Na_2SO_4 \cdot 10H_2O$, the reaction mixture diluted with ethyl methyl ketone was washed with a saturated aqueous NaCl solution, dried and evaporated to a residue of the methylamino compound (40 mg): R_f 0.09 (10:1 $CHCl_3$-MeOH).

The residue was again *N*-benzyloxycarbonylated as described above to give, after column chromatography on silica gel (4 g) with 5:2 chloroform-acetone, a solid of **22** (31.3 mg, 81%): R_f 0.51 (10:1 $CHCl_3$-MeOH); mp 163—167 °C ; $[\alpha]_D^{23}$ −44° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1675 (NH-CO), and 1155 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$+D_2O): δ=2.98 (1H, t, H-4′, $J_{3',4'}$=$J_{4',5'}$=9.5 Hz), 3.03 (1H, t, H-7′, $J_{6',7'}$=$J_{7',8'}$=9.5 Hz), 3.13 (1H, dd, H-2′, $J_{1',2'}$=4 Hz, $J_{2',3'}$=9.5 Hz), 3.22 (3H, s, NMe), 3.32 (1H, t, H-6′, $J_{5',6'}$=9.5 Hz), 3.50 (3H, s, OMe), 3.91 (1H, t, H-3′), 3.98 (1H, t, H-5′), 4.93 (1H, d, H-1′), and 4.93 (1H, d, H-8′).

Found: C, 56.25; H, 6.11; N, 5.02; S, 8.69%. Calcd for $C_{51}H_{64}N_4O_{16}S_3$: C, 56.44; H, 5.94; N, 5.16; S, 8.86%.

*4-O-{(8′S)-7′-[N-(Benzyloxycarbonyl)methylamino]-2′,7′-dideoxy-3′,6′-di-*O-*mesyl-8′-*O-*methyl-2′-(tosylamino)*-D-threo-α-D-gluco-*octodialdo-1′,5′:8′,4′-dipyranos-1′-yl}-5,6-O-cyclohexylidene-1,3-di*-N-*tosyl-2-deoxystreptamine (23).* A solution of **22** (14.3 mg) in pyridine (0.29 ml) was stirred with mesyl chloride (0.004 ml) at 8 °C for 11 h. After further addition of mesyl chloride (0.002 ml), stirring was continued at 8 °C for 15 h and then at room temperature for 5 h. After addition of a few drops of water, the resulting solution was evaporated to a residue, which was partitioned between ethyl acetate and a saturated aqueous $NaHCO_3$ solution. The combined organic layers were evaporated to a residue, which was chromatographed on silica gel (1.4 g) with 2:1 benzene-ethyl acetate to give quantitatively a solid of **23** (16.3 mg): R_f 0.56 (4:3 benzene-ethyl acetate); mp 161—164 °C ; $[\alpha]_D^{23}$ +2.5° (*c* 1.0, $CHCl_3$), IR ($CHCl_3$): 1695 (NH-CO), 1175 and 1160 cm^{-1} (SO_2); ^{1}H-NMR ($CDCl_3$): δ=2.42, 2.44, and 2.48 (each 3H, s, Ts), 2.80 and 2.96 (each 3H, s, Ms), 3.00 (3H, s, NMe), and 3.45 (3H, s, OMe).

Found: C, 51.22; H, 5.46; N, 4.40; S, 12.67%. Calcd for $C_{53}H_{68}N_4O_{20}S_5$: C, 51.28; H, 5.52; N, 4.51; S, 12.91%.

*4-O-{(8′S)-7′-[N-(Benzyloxycarbonyl)methylamino]-2′,3′,7′-trideoxy-6′-*O-*mesyl-8′-*O-*methyl-2′-(tosylamino)*-D-glycero-β-L-talo-*octodialdo-1′,5′:8′,4′-dipyranos-1′-yl}-5,6-*O-*cyclohexylidene-1,3-di*-N-*tosyl-2-deoxystreptamine (24).* A solution of **23** (9.1 mg) in DMF (0.27 ml) was stirred with LiCl (6.2 mg) at 100 °C for 2 h under argon. The solution diluted with ethyl acetate was washed with a saturated aqueous $NaHCO_3$ solution, dried, and evaporated to a residue, which was chromatographed on silica gel (1 g) with 3:1 benzene-ethyl acetate to give a solid of the chloro compound (7.8 mg, 90%): R_f 0.64 (4:3 benzene-ethyl acetate); positive Beilstein test.

To a solution of the solid (6.9 mg) in 1,4-dioxane (0.21 ml) were added tributylstannane (0.0138 ml) and α,α′-azobis-(isobutyronitrile) (0.7 mg), and the reaction mixture was heated at 80 °C for 1.5 h under argon. The resulting solution was partitioned between acetonitrile (1 ml) and hexane (0.2 ml×3). The acetonitrile layer was evaporated to a residue, which was chromatographed on silica gel (1 g) with 2:1 benzene-ethyl acetate to give a solid of **24** (6.0 mg, 90%): R_f 0.58 (4:3 benzene-EtOAc); mp 162—168 °C ; $[\alpha]_D^{23}$ +13° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (50 °C, $CDCl_3$+D_2O): δ=2.44 (9H, s, Ts×3), 2.76 (3H, s, OMs), 3.00 (3H, s, NMe), and 3.44 (3H, s, OMe).

Found: C, 54.47; H, 5.90; N, 4.64; S, 10.97%. Calcd for $C_{52}H_{66}N_4O_{17}S_4$: C, 54.44; H, 5.80; N, 4.88; S, 11.18%.

*4-O-[(8′S)-7′-N,6′-O-Carbonyl-2′,3′,7′-trideoxy-8′-*O-*methyl-7′-(methylamino)-2′-(tosylamino)*-D-glycero-α-D-allo-*octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-5,6-*O-*cyclohexylidene-1,3-di*-N-*tosyl-2-deoxystreptamine (25).* A solution of **24** (5.4 mg) in 2-methoxyethanol (0.22 ml) was heated with sodium acetate trihydrate (5.4 mg) at 130 °C for 2 d. The reaction mixture was partitioned between ethyl acetate and a saturated aqueous NaCl solution, and the organic layer dried and then evaporated to a residue, which was chromatographed on silica gel (0.5 g) with 2:3 benzene-ethyl acetate to give a solid of **25** (3.6 mg, 78%): R_f 0.12 (4:3 benzene-ethyl acetate); mp 159—162 °C ; $[\alpha]_D^{23}$ −25° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$): 1750—1745 (cis cyclic carbamate),[17,18] and 1160 cm^{-1} (SO_2); ^{1}H-NMR (CD_3CN+D_2O): δ=2.83 (3H, s, NMe), 3.42 (3H, s, OMe), 3.52 (1H, dd, H-7′, $J_{6',7'}$=7.0 Hz, $J_{7',8'}$=6.0 Hz), 4.22 (1H, dd, H-5′, $J_{4',5'}$=10.5 Hz, $J_{5',6'}$=3.8 Hz), 4.42 (1H, d, H-8′), 4.71 (1H, dd, H-6′), and 4.93 (1H, d, H-1′, $J_{1',2'}$=3.5 Hz).

Found: C, 54.97; H, 5.74; N, 5.64%. Calcd for $C_{44}H_{56}N_4O_{14}S_3$: C, 54.99; H, 5.87; N, 5.83%.

Methyl β-Aprosaminide (26). *A) From 25*: To a solution of **25** (19.8 mg) in liquid ammonia (≃6 ml) at −35 °C was added sodium metal (25 mg×3) with stirring, and the resulting deep-blue solution was kept at −35 °C for 15 min. After evaporation of ammonia at room temperature, methanol (0.2 ml) and water (1.8 ml) were added, and the

resulting solution of the detosylated intermediate was heated at 100 °C for 5 h to hydrolyze the cyclic carbamate. To the solution was added Dowex 50WX2 resin (H form, 200—400 mesh, 6.0 ml), and the mixture was allowed to stand overnight at room temperature in order to remove the cyclohexylidene group. The resin was packed in a column, washed with water, and then treated with 1 M aqueous ammonia. The ninhydrin-positive fractions were collected and evaporated to give a solid, which was purified by chromatography over CG-50 resin (NH_4 form, 0→0.2 M aqueous ammonia) to yield a solid of the monohydrate of **26** (5.3 mg, 65%): R_f 0.62 (1 : 3 : 2 $CHCl_3$-MeOH-17% aqueous NH_3); mp 141—143 °C ; $[\alpha]_D^{23}$ +108° (*c* 0.6, water)[lit,[1b] $[\alpha]_D^{27}$ +104° (*c* 1.1, water)]; MS (SIMS): *m/z* 393 (M^++1); ^{1}H-NMR (5% ND_3 in D_2O): δ=1.20 (1H, q, H-2ax, *J*=13 Hz), 1.65 (1H, q, H-3′ax, *J*=12 Hz), 1.97 (1H, dt, H-2eq, $J_{1,2eq}=J_{2eq,3}$=3.5 HZ, J_{gem}=13 Hz), 2.12 (1H, m, H-3′eq), 2.35 (3H, s, NMe), 2.46 (1H, dd, H-7′, $J_{6',7'}$=3.0 Hz, $J_{7',8'}$=8.5 Hz), 2.70 and 2.86 (each 1H, m, H-1 and H-3, *vice versa*), 3.01 (1H, dt, H-2′, $J_{1',2'}=J_{2',3'eq}$= 3.5 Hz, $J_{2',3'ax}$=12.5 Hz), 3.14, 3.30, and 3.49 (each 1H, t, H-4, H-5, or H-6, *J*≃9.5 Hz), 3.64 (1H, dd, H-5′, $J_{4',5'}$=10 Hz, $J_{5',6'}$=2.5 Hz), 3.78 (1H, m, H-4′), 4.26 (1H, t, H-6′, *J*≃3 Hz), 4.52 (1H, d, H-8′), 5.14 (1H, d, H-1′). This spectrum was superimposable on that of the naturally derived **26** and the reported spectrum.[1b]

Found: C, 46.59; H, 8.10; N, 13.47%. Calcd for $C_{16}H_{32}N_4O_7 \cdot H_2O$: C, 46.82; H, 8.35; N, 13.65%.

B) From 28 by Methanolysis: A solution of **28** (5.0 mg) in 1.2% methanolic hydrogen chloride solution (0.33 ml) was heated in a sealed tube at 73 °C for 5 h, and evaporated to a residue, which was chromatographed on silica gel (1.5 g) with 3 : 2 chloroform-acetone to give a solid of the methyl glycoside (4.5 mg): R_f 0.44 (12 : 1 $CHCl_3$-MeOH); ^{1}H-NMR (50 °C, $CDCl_3$): δ=2.98 (3H, s, NMe), 3.46 (3H, s, OMe).

A solution of the solid in 67% aqueous 1,4-dioxane (0.35 ml) was shaken with palladium black and 3-atm hydrogen at room temperature for 4.5 h, filtered and evaporated to a residue. The residue was purified by chromatography over CG-50 resin (NH_4 form, 0→0.2 M aqueous ammonia) to give a solid of the monohydrate of **26** (1.8 mg, 81% from **28**).

C) From 28 by Methylation: To a stirred solution of **28** (4.5 mg) in acetonitrile (0.011 ml) were added methyl iodide (0.045 ml) and Ag_2O (9.0 mg), and stirring was continued at room temperature for 1 h. After filtration, the filtrate was evaporated to give a solid of the methyl glycoside. The solid was hydrogenated as described above to give a solid of the monohydrate of **26** (1.4 mg, 70% from **28**).

Aprosamine (27). *A) From 26*: A solution of the monohydrate of **26** (20.5 mg) in 4 M HCl (1.3 ml) was heated in a sealed tube at 95 °C for 3 d. The brown solution was evaporated and co-evaporated with toluene to give a residue which was purified by chromatography over CG-50 resin (NH_4 form, 0→0.2 M aqueous ammonia) to give a solid of the free base of **27**. The solid was dissolved in 0.5 M HCl and evaporated to a residue, which was reprecipitated from water-acetone to give a solid of the tetrahydrochloride trihydrate of **27** (20 mg, 70%): R_f 0.46 (1 : 3 : 2 $CHCl_3$-MeOH-17% aqueous NH_3); mp 176—180 °C (decomp); $[\alpha]_D^{25}$ +44° (*c* 1.0, water) [lit, $[\alpha]_D^{25}$ +21.6° (*c* 1.27, water);[1b] $[\alpha]_D^{22}$ +53° (*c* 1.075, water)[4]]; MS (SIMS): *m/z* 379 (M^++1); ^{1}H-NMR (D_2O): δ=1.92 (1H, q, H-2ax, *J*=12.5 Hz), 2.04 (1H, m, H-3′ax), 2.35 (1H, m, H-2eq), 2.55 (1H, dt, H-3′eq, J_{gem}=12.5 Hz, $J_{2',3'eq}=J_{3'eq,4'}$=4 Hz,), 2.81 and 2.82 (3H in total, each s, NMe), 4.51 and 4.58 (1H in total, each t, H-6′, *J*≃3), 5.20 and 5.47 (1H in total, each d, $J_{7',8'ax}$=9.0 Hz, $J_{7',8'eq}$=4.0 Hz, H-8′ax and H-8′eq respectively), 5.75 and 5.77 (1H in total, each d, H-1′, $J_{1',2'}$=3.0 Hz and 3.5 Hz, respectively). This ^{1}H-NMR spectrum was found to be superimposable on that of the naturally derived aprosamine and the reported spectrum.[1b]

Found: C, 30.92; H, 6.87; N, 9.47%. Calcd for $C_{15}H_{30}N_4O_7 \cdot 4HCl \cdot 3H_2O$: C, 31.15; H, 6.97; N, 9.69%.

B) From Natural Apramycin (1): A solution of apramycin (**1** : 9.9 mg) in 4 M HCl (0.1 ml) was heated at 95 °C for 7 h. The solution was evaporated and co-evaporated with toluene to a residue, which was purified as described above to give a solid of the tetrahydrochloride trihydrate of **27** (8.6 mg, 81%). The solid was identical with the aforeside synthetic aprosamine in all respects.

1,2′,3,7′-Tetrakis(N-benzyloxycarbonyl) aprosamine (28). To a stirred and ice-cooled solution of the free base of **27** (12.8 mg) in 80% aqueous acetone (0.48 ml) were added Na_2CO_3 (18 mg), 1 M NaOH (0.04 ml) and benzyl chloroformate (0.023 ml), and stirring was continued at room temperature for 2 h. After filtration, the filtrate was agitated with CG-50 resin (H-form) and again filtrated. The filtrate was neutralized with triethylamine (0.023 ml) and then evaporated to a residue, which was chromatographed on silica gel (1 g) with 15 : 1 chloroform-methanol to give a solid: mp 196—198 °C (decomp). Reprecipitation from ethanol-ether yielded the monohydrate of **28** (24 mg, 77%): R_f 0.38 (15 : 1 $CHCl_3$-MeOH); mp 188—190 °C (decomp); $[\alpha]_D^{23}$ +43° (*c* 1.0, MeOH) [lit,[4] prisms of **28**: mp 233—234.5 °C; $[\alpha]_D^{22}$ +48.8° (*c* 1.005, DMF)]; IR (KBr): 1710—1670 (NH-CO); ^{1}H-NMR (CD_3CN+D_2O): δ=3.00 (3H, s, NMe), and 4.21 (1H, broad s, H-6′).

Found: C, 60.44; H, 5.95; N, 5.94%. Calcd for $C_{47}H_{54}N_4O_{15} \cdot H_2O$: C, 60.51; H, 6.05; N, 6.01%.

4-Azido-2,3,6-tri-O-benzyl-4-deoxy-β-D-glucopyranosyl Fluoride (29). To a stirred solution of 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl chloride[22] (130 mg) in acetonitrile (0.65 ml) was added AgF (130 mg), and stirring was continued at room temperature for 22 h under protection from light. The reaction mixture was filtered and a saturated aqueous NaCl solution was added to precipitate any silver ions from solution. The mixture was again filtered and evaporated to a residue, which was partitioned between chloroform and water. The organic layer was dried and evaporated to a residue, which was chromatographed on silica gel (4 g) with 4 : 1 benzene-ethyl acetate to give a syrup of **29** (122 mg, 97%): R_f 0.41 (benzene); $[\alpha]_D^{23}$ +108° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$): 2120 cm^{-1} (azide); ^{1}H-NMR ($CDCl_3$): δ=5.19 (1H, dd, H-1, $J_{1,2}$=6.4 Hz, $J_{1,F}$=52 Hz, apparently two doublets at δ=5.06 and 5.32).

Found: C, 67.69; H, 5.98; N, 8.87%. Calcd for $C_{27}H_{28}N_3O_4F$: C, 67.91; H, 5.91; N, 8.80%.

4-O-[(8′R)-2′-Amino-8′-O-(4″-azido-2″,3″,6″-tri-O-benzyl-4″-deoxy-α-D-glucopyranosyl)-2′,7′-bis(N-benzyloxycarbonyl)-2′,3′,7′-trideoxy-7′-(methylamino)-D-glycero-α-D-allo-octodialdo-1′,5′ : 8′,4′-dipyranos-1′-yl]-1,3-bis(N-benzyloxycarbonyl)-2-deoxystreptamine (30). Before coupling, **28** was dried overnight at 50 °C under reduced pressure. To a mixture of **28** (12.6 mg, 0.0137 mmol), **29** (7.9 mg, 0.0165 mmol), $SnCl_2$ (5.5 mg, 0.029 mmol), $AgClO_4$ (5.7 mg, 0.027 mmol), and Molecular Sieves 4A (12.6 mg) was added a 4 : 1 mixture (0.189 ml) of 1,4-dioxane-benzene at 0 °C, and the mixture was stirred at this temperature for 24 h. After further addition of the same amounts of **29**, $SnCl_2$, $AgClO_4$ and Molecular Sieves 4A as described above, stirring was continued at 0 °C for another 24 h. The reaction mixture was filtered and the filtrate evaporated to give a residue. The residue was chromatographed on silica gel (1.5 g) with 20 : 1 chloroform-methanol to give two products having R_f-values of 0.44 and 0.48 (15 : 1 chloroform-methanol) with recovery of the unreacted **28** (5.4 mg) having R_f 0.38. The R_f 0.44-substance was the desired glycoside **30** as described below.

30 (R_f 0.44-substance): 4.3 mg (23% from **28**; 40% yield based on the unrecovered **28**); mp 89—92 °C ; $[\alpha]_D^{23}$ +63° (*c* 0.5, $CHCl_3$); ^{1}H-NMR (50 °C, CD_3CN+D_2O): δ=3.00

(3H, s, NMe), 3.73 (1H, dt, H-2′, $J_{1',2'}=J_{2',3'eq}=4.0$ Hz, $J_{2',3'ax}=13$ Hz), 4.13 (1H, m, H-7′), 4.27 (1H, dull t, H-6′), 5.24 (1H, d, H-1′), 5.27 (1H, d, H-8′, $J_{7',8'}=9.5$ Hz), and 5.41 (1H, d, H-1″, $J_{1'',2''}=3.0$ Hz).

Found: C, 64.57; H, 6.19; N, 6.96%. Calcd for $C_{74}H_{81}N_7O_{19}$: C, 64.76; H, 5.95; N, 7.14%.

R_f 0.48-substance: 2.1 mg; mp 89—92 °C ; $[\alpha]_D^{28}$ +62° (*c* 0.85, $CHCl_3$); IR ($CHCl_3$): 2115 cm^{-1} (azide); ^{1}H-NMR (50 °C, $CDCl_3+D_2O$): $\delta=2.98$ and 3.02 (3H in total, each s, NMe), and 7.1—7.4 (35H, m, Ph×7).

By catalytic reduction, the R_f 0.44-substance (**30**) yielded apramycin (**1**) as described below, while the R_f 0.48-substance did not give apramycin but gave another product (R_f 0.43 on TLC with 1:3:2 $CHCl_3$-MeOH-17% aqueous NH_3; apramycin:R_f 0.35). Therefore, the R_f 0.48-substance was not further investigated.

Apramycin (1). A solution of **30** (10 mg) in a 3:1:1 mixture (0.5 ml) of 1,4-dioxane, acetic acid and water was shaken with palladium black and 3-atm hydrogen at room temperature for 2 h, filtered and evaporated to give a residue. The residue was purified by chromatography over CG-50 resin (NH_4 form, 0→0.1 M aqueous ammonia) to give a solid of the monocarbonate monohydrate of **1** (3.4 mg, 74%): R_f 0.35 (1:3:2 $CHCl_3$-MeOH-17% aqueous NH_3); mp 237—240 °C (decomp); $[\alpha]_D^{23}$ +162° (*c* 0.94, water) [lit,[1] monohydrate: mp 245—247 °C; $[\alpha]_D^{25}$ +162.5° (water)]; MS (SIMS): *m/z* 540 (M^++1); ^{1}H-NMR (5% ND_3 in D_2O): $\delta=1.18$ (1H, m, H-2ax), 1.68 (1H, q, H-3′ax, $J=12$ Hz), 2.03 (1H, m, H-2eq), 2.14 (1H, m, H-3′eq), 2.39 (3H, s, NMe), 2.69 (1H,dd, H-7′, $J_{6',7'}=2.5$ Hz, $J_{7',8'}=8.5$ Hz), 3.03 (1H, dull dt, H-2′, $J_{2',3'ax}=12.5$ Hz), 4.29 (1H, broad s, H-6′), 4.92 (1H, d, H-8′), 5.15 (1H, d, H-1′, $J_{1',2'}=3.5$ Hz), and 5.37 (1H,d, H-1″, $J_{1'',2''}=3.5$ Hz). This spectrum was superimposable on that of the authentic sample of apramycin and the reported spctrum.[1b]

Found: C, 42.57; H, 7.29; N, 11.45%. Calcd for $C_{21}H_{41}N_5O_{11}\cdot H_2CO_3\cdot H_2O$: C, 42.65; H, 7.32; N, 11.30%.

4-O-[(8′R)-2′-Amino-8′-O-(2″,3″,4″,6″-tetra-O-benzyl-α-D-glucopyranosyl)-2′,7′-bis(N-benzyloxycarbonyl)-2′,3′,7′-trideoxy-7′-(methylamino)-D-glycero-α-D-allo-octodialdo-1′,5′:8′,4′-dipyranos-1′-yl]-1,3-bis(N-benzyloxycarbonyl)-2-deoxystreptamine (31). Following the procedure described for **30**, a sample of **28** (12.6 mg) was treated with 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride[20] (9.0 mg×2), $SnCl_2$ (5.5 mg×2), $AgClO_4$ (5.7 mg×2), and Molecular Sieves 4A (12.6 mg×2) in a 4:1 mixture (0.189 ml) of 1,4-dioxane and benzene, and then worked up. Column chromatography on silica gel (1.5 g) with 20:1 chloroform–methanol gave two products (4.7 mg and 1.7 mg) having R_f-values of 0.43 and 0.48 (15:1 chloroform–methanol) with recovery of the unreacted **28** (5.5 mg). The R_f 0.43-substance was the desired glycoside **31** as described below.

31 (R_f 0.43-substance): 4.7 mg (24% from **28**; 42% yield based on the unrecovered **28**); mp 94—97 °C; $[\alpha]_D^{23}$ +48° (*c* 0.80, $CHCl_3$); ^{1}H-NMR (50 °C, $CDCl_3+D_2O$): $\delta=2.97$ (3H, s, NMe), 3.52 (1H, dd, H-2″, $J_{1'',2''}=4.0$ Hz, $J_{2'',3''}=9.5$ Hz), and 5.28 (1H, d, H-1″).

Found: C, 67.44; H, 6.40; N, 3.81%. Calcd for $C_{81}H_{88}N_4O_{20}$: C, 67.67; H, 6.17; N, 3.90%.

R_f 0.48-substance: 1.7 mg; mp 97—101 °C ; $[\alpha]_D^{23}$ +57° (*c* 0.41, $CHCl_3$): IR ($CHCl_3$): 2110 cm^{-1} (azide); ^{1}H-NMR (50 °C, $CDCl_3+D_2O$): $\delta=3.02$ and 3.05 (3H in total, each broad s, NMe), and 7.1—7.4 (40H, m, Ph×8).

By catalytic reduction, the R_f 0.43-substance (**31**) yielded saccharocin (**2**) as described below, while the R_f 0.48-substance gave another product (R_f 0.46 on TLC with 1:3:2 $CHCl_3$-MeOH-17% aqueous NH_3; saccharocin: R_f 0.38). Therefore, the R_f 0.48-substance was not further investigated.

Saccharocin (2: KA-5685). A sample of **31** (8 mg) was hydrogenated and then purified as described for apramycin (**1**) to give a solid of the monocarbonate monohydrate of **2** (2.7 mg, 78%): R_f 0.38 (1:3:2 $CHCl_3$-MeOH-17% aqueous NH_3); mp 187—190 °C (decomp); $[\alpha]_D^{23}$ +175° (*c* 0.5, water) [lit, dihydrate:[2a] mp 188—190 °C; $[\alpha]_D^{24}$ +163.5° (*c* 1.0, water); KA-5685:[2b] $[\alpha]_D^{25}$ +124° (*c* 0.5, H_2O)]; MS (SIMS): *m/z* 541 (M^++1); ^{1}H-NMR (5% ND_3 in D_2O): $\delta=1.21$ (1H, q, H-2ax, $J=12.5$ Hz), 1.67 (1H, q, H-3′ax, $J=11.5$ Hz), 1.98 (1H, dt, H-2eq, $J_{1,2eq}=J_{2eq,3}=4.0$ Hz, $J_{gem}=13$ Hz), 2.12 (1H, dt, H-3′eq, $J_{2',3'eq}=J_{3'eq,4'}=4.0$ Hz, $J_{gem}=11.5$ Hz), 2.39 (3H, s, NMe), 2.68 (1H, dd, H-7′, $J_{6',7'}=2.5$ Hz, $J_{7',8'}=8.5$ Hz), 2.70 and 2.87 (each 1H, m, H-1 and H-3, *vice versa*), 3.01 (1H, m, H-2′), 3.61 (1H, dd, H-2″, $J_{1'',2''}\simeq4$ Hz, $J_{2'',3''}=10$ Hz), 4.29 (1H, t, H-6′), 4.93 (1H, d, H-8′), 5.15 (1H, d, H-1′, $J_{1',2'}=3.5$ Hz), and 5.35 (1H, d, H-1″). This spectrum was superimposable on that of the authentic antibiotic **2** and the reported spectra.[2]

Found: C, 42.73; H, 7.36; N, 9.29%. Calcd for $C_{21}H_{40}N_4O_{12}\cdot H_2CO_3\cdot H_2O$: C, 42.58; H, 7.15; N, 9.03%.

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