SYNTHESIS OF PSEUDOTRISACCHARIDES RELATED TO RIBOSTAMYCIN

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The three protected sisamine derivatives **2i**, **2j** and **3**, with a free 5-hydroxyl group, have been synthesized. Glycosylation at the 5 position with various pentofuranose derivatives yielded after deprotection of the **6a**~**i** ribostamycin related aminoglycoside. These pseudotrisaccharides showed only low antibacterial activities with respect to the parent compounds.

The knowledge of the mechanisms by which aminoglycoside antibiotics are inactivated has permitted specific chemical modifications of these powerful drugs aimed at preventing such inactivation[1,2]. The pioneering work of H. UMEZAWA and S. UMEZAWA has shown that removal of the 3′ and 4′-hydroxyl groups yielded aminoglycoside derivatives active against resistant strains containing a 3′-phosphorylating enzyme[3,4], and that 6′-*N*-methylation protects the parent compound from enzymatic 6′-*N*-acetylation[5]. 4′,5′-Unsaturated 4,6-disubstituted 2-deoxystreptamine antibiotics, as illustrated by sisomicin or 4′-deoxy-4′,5′-dehydro tobramycin[6] have comparable or higher potencies than the saturated parent compounds (gentamicin C_{1a} and tobramycin, respectively).

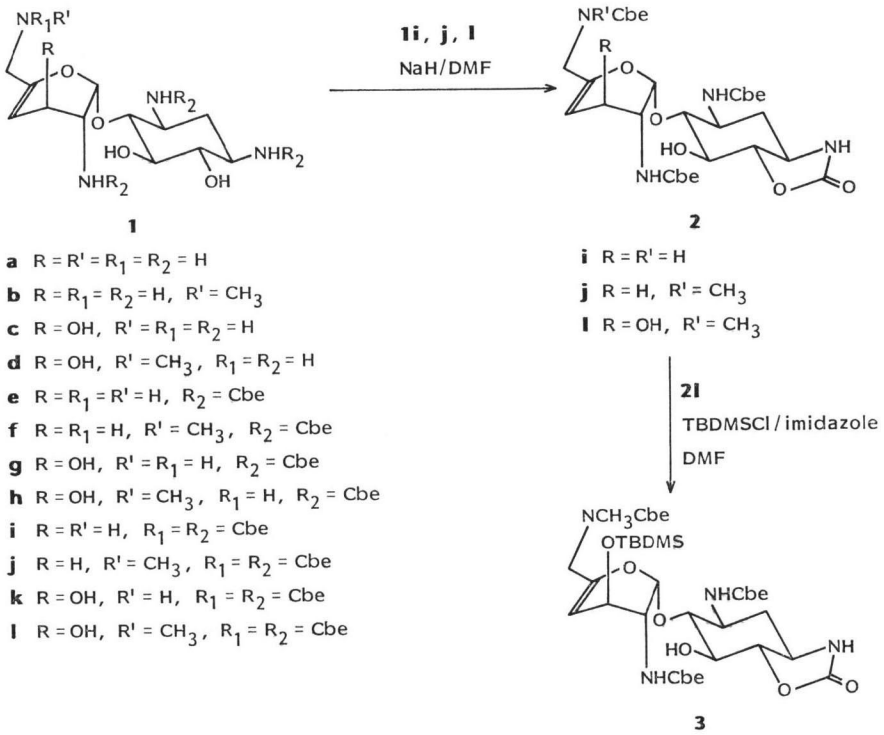
Sisamine (**1a**) and its derivatives **1b**, **1c** and **1d**, described in our previous paper[7], possess, in addition to a 4′,5′ double bond (see Scheme 1), an appropriate structure to avoid enzymatic 3′-*O*-phosphorylation (**1a**, **1b**), 6′-*N*-acetylation (**1b**, **1d**), or both (**1b**). The 5-*O*-glycosylation of these pseudodisaccharides would provide an entry into the 4,5-disubstituted 2-deoxystreptamine antibiotic family which possesses a novel structure in the C-4 sugar moiety and would provide further information on the structure-activity relationships in this series. Ribostamycin (**5a**) or xylostasin (**5b**) are probably the best prototypes of this group of antibiotics (Fig. 1).

Based on these considerations, we decided to undertake the synthesis of 4′,5′-unsaturated analogues of **5a** and **5b**, as we know that the total chemical synthesis of ribostamycin[8,9] and butirosin[10~12] derivatives by C-5 glycosylation with a protected furanosyl bromide has been proved to be quite efficient. Other analogues in these series have been obtained by mutational biosynthesis[13,14].

Our strategies were as follows:

The free 6′-amino group of 1,3,2′-tricarboethoxysisamine[7] (**1e**) was first ethoxycarbonylated (Cbe) (Scheme 1). Treatment of the resulting *N*-protected sisamine **1i** with NaH/DMF produced the 1,6 cyclic carbamate **2i** in a good yield. Following the same procedure, the two other cyclic carbamate derivatives **2j** and **2l** were synthesized from **1j** and **1l**, respectively. The conformation and rigidity of the 4′-enopyranoside moiety of **1l** did not allow the formation of the 1,6: 2′,3′ bicyclic carbamate derivative. Although the 5-hydroxyl group is not readily accessible to derivation in the neamine-paromamine series[8,9,15], monoacetylation of **2l** led to the 5-acetate as the major compound. A more hindering pro-

Scheme 1.



1

a R = R' = R_1 = R_2 = H
b R = R_1 = R_2 = H, R' = CH_3
c R = OH, R' = R_1 = R_2 = H
d R = OH, R' = CH_3, R_1 = R_2 = H
e R = R_1 = R' = H, R_2 = Cbe
f R = R_1 = H, R' = CH_3, R_2 = Cbe
g R = OH, R' = R_1 = H, R_2 = Cbe
h R = OH, R' = CH_3, R_1 = H, R_2 = Cbe
i R = R' = H, R_1 = R_2 = Cbe
j R = H, R' = CH_3, R_1 = R_2 = Cbe
k R = OH, R' = H, R_1 = R_2 = Cbe
l R = OH, R' = CH_3, R_1 = R_2 = Cbe

2

i R = R' = H
j R = H, R' = CH_3
l R = OH, R' = CH_3

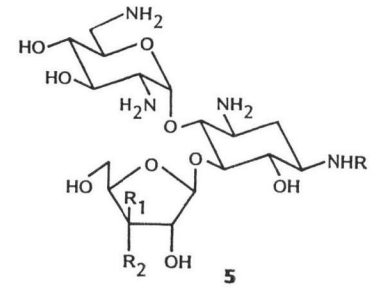
3

tective group was thus required to achieve selective 3'-*O*-protection: treatment of **2l** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in DMF, in the presence of imidazole, at room temperature[16] resulted in the expected 3'-*O*-TBDMS derivative **3** in addition to a very small amount of 3',5-di-*O*-TBDMS derivative.

From the three protected pseudodisaccharides **2i**, **2j** and **3**, several ribostamycin related aminoglycoside antibiotics were synthesized by glycosylation. The fully protected, ribostamycin analogs **4a**, **4b** and **4f** (Fig. 2) were obtained by treatment of **2i**, **2j** and **3**, respectively, with 2,3,5-tri-*O*-benzoylribofuranosylbromide[17] in the presence of mercuric cyanide. Similarily, the glycosylation of **2j** and **3** with 2,3,5-tri-*O*-benzoylxylofuranosylbromide[18] resulted in the protected xylostasin analogues **4c** and **4g**, respectively. Finally, the D-arabinofuranosyl derivatives **4d**, **4h** and the L-arabinofuranosyl derivatives **4e**, **4i** were obtained from **2j** and **3** using 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl bromide[19] and 2,3,5-tri-*O*-benzoyl-L-arabinofuranosyl bromide[20], respectively. Refluxing the glycosylation products in 1.33 N NaOH (H_2O - EtOH, 2: 1) resulted in the simultaneous removal of the carbamates, esters and the TBDMS ether protecting groups[21]. Compounds **6a**~**i** thus obtained (Fig. 1) were neutralized with 0.1 N sulfuric acid. Analyses were performed using the sulfate derivatives (see Table 1 and Experimental).

The antibacterial activities of the deprotected molecules **6a**~**i** were determined by an agar dilution method in Mueller-Hinton medium by comparison with ribostamycin. Only **6a** had a significant antibiotic activity (25% of the ribostamycin activity against *Escherichia coli* K12 and *Staphylococcus aureus* 209P).

Fig. 1.

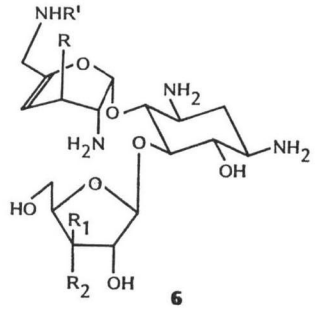


5

Ribostamycin (**a**) R_1 = H, R_2 = OH

Xylostasin (**b**) R_1 = OH, R_2 = H

Butirosin (**c**) R_1 = H, R_2 = OH,
R = L-$COCH(OH)(CH_2)_2NH_2$



6

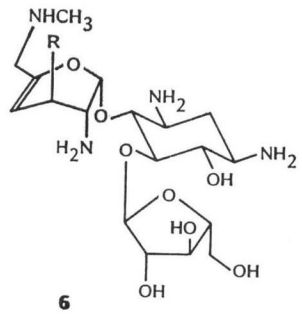
a R = R' = R_1 = H, R_2 = OH

b R = R_1 = H, R' = CH_3, R_2 = OH

c R = R_2 = H, R' = CH_3, R_1 = OH

f R = R_2 = OH, R' = CH_3, R_1 = H

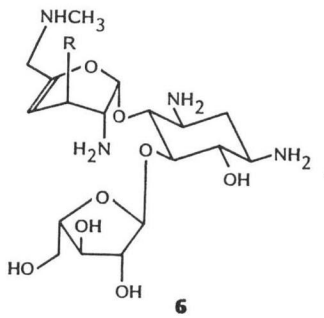
g R = R_1 = OH, R' = CH_3, R_2 = H



6

d R = H

h R = OH

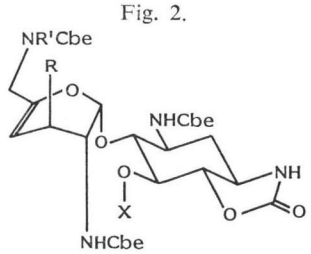


6

e R = H

i R = OH

Fig. 2.



4

a R=R'=H, X=2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl

b R=H, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl

c R=H, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-β-D-xylofuranosyl

d R=H, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl

e R=H, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl

f R=*O*-TBDMS, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl

g R=*O*-TBDMS, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-β-D-xylofuranosyl

h R=*O*-TBDMS, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl

i R=*O*-TBDMS, R'=CH_3, X=2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl

Table 1. ^{13}C NMR chemical shifts[a] of the pseudotrisaccharides.[b]

C	5a	6a	6b	6c	6d	6e	6f	6g	6h	6i
1	51.0	50.9	50.8	50.8	50.8	51.0	50.9	50.8	50.9	50.7
2	30.3	29.2	29.0	28.8	29.0	30.8	28.9	29.2	29.1	29.1
3	49.6	49.2	29.2	49.4	49.2	49.5	49.3	49.3	49.2	49.4
4	77.4	79.0	79.1	78.5	80.1	79.5	79.3	79.0	80.3	78.8
5	85.8	84.5	84.5	82.4	83.9	83.7	82.9	81.5	82.8	83.4
6	73.7	72.9	72.7	73.3	71.8	74.4	72.9	73.5	71.9	73.4
1′	96.0	97.5	97.6	98.2	98.5	98.2	97.2	97.8	98.3	97.6
2′	54.7	46.7	46.6	46.7	46.7	46.6	52.5	52.6	52.7	52.5
3′	71.9	24.1	24.3	24.8	25.4	25.4	63.3	64.3	64.6	64.0
4′	69.3	101.3	103.4	103.1	103.4	103.3	104.6	104.3	104.8	104.7
5′	70.0	144.3	142.8	143.2	143.3	142.9	145.7	146.2	146.3	146.0
6′	41.3	41.4	50.3	50.4	50.4	50.5	50.2	50.2	50.2	50.1
1″	111.1	111.2	111.2	104.8	109.2	111.0	110.2	104.5	110.0	110.5
2″	76.1	75.7	75.6	70.2	82.1	81.9	75.6	70.1	81.9	81.7
3″	70.0	70.6	70.6	74.6	76.6	76.7	70.6	74.5	77.4	76.0
4″	83.3	83.4	83.4	76.6	82.5	86.0	83.6	76.5	85.3	84.5
5″	61.9	63.0	62.9	66.1	61.8	62.6	62.8	65.9	62.2	62.2
NCH_3	—	—	32.9	33.2	33.1	32.9	33.3	33.3	33.2	33.2

[a] The assignments were made according to references 7, 22 and 23. Close values may be exchanged.
[b] H_2SO_4 salts.

Experimental

Evaporations were performed with a rotary evaporator, under reduced pressure. The NMR spectra were recorded with a Varian T60 spectrometer (^1H) and a Brucker WP80 spectrometer (^1H and ^{13}C). The chemical shifts are reported in ppm down field from internal TMS, dioxane (67.4 ppm) being used as internal reference for ^{13}C spectra in D_2O. The optical rotations were measured with a Perkin Elmer 141 polarimeter. The melting points were observed with a Reichert Köffler melting point apparatus and were not corrected. The microanalyses were performed with a Perkin Elmer 240 elemental analyzer. The solvents were dried by distillation over an appropriate dessicating agent just prior to use. The reactions were followed by TLC monitoring (Merck, Silica gel 60F254). The organic extracts were dried over $MgSO_4$ or Na_2SO_4. The analytical results are given only when they agree with the calculated values within $\pm 0.4\%$. The homogeneity of the compounds was demonstrated by TLC and their structure confirmed by ^{13}C NMR.

Tetra-*N*-ethoxycarbonylsisamine (**1i**)

Crude 1,3,2′-tri-*N*-ethoxycarbonylsisamine[7] (**1e**) (10.3 g, 19.8 mmol) was dissolved in 70% aqueous methanol (140 ml) containing sodium carbonate (4.2 g, 40 mmol). The solution was cooled in an ice bath and ethyl chloroformate (3.7 ml, 40 mmol) added. A solid formed which was filtered and washed with water and dried to give pure hydrated **1i** (4.5 g). Concentration of the solution gave a white, solid compound, which was dissolved in water. Extraction with methylene chloride gave after drying and evaporation of the combined organic solutions, 3.1 g of a product which was chromatographed on silica gel (EtOAc) to give **1i** (1.5 g). Yield 6 g (53%), mp 210° C, ref[24] 213~215°C, $[\alpha]_D^{20}$ +104° (*c* 1, MeOH), ref[24] $[\alpha]_D^{23}$ +107° (*c* 1, MeOH). ^{13}C NMR (DMSO) 156.1, 155.9, 155.6 carbonyls (Cbe); 147.1 C-5′; 97.2, 94.1 C-1′,4′; 81.9, 76.5, 74.1 C-4,5,6; 59.6, 59.4 CH_2O (Cbe); 51.2, 49.8, 47.8 C-1,3,2′; 41.9 C-6′; 34.8 C-2; 22.1 C-3′; 14.6 CH_3 (Cbe).

Anal Calcd for $C_{24}H_{40}N_4O_{12}\cdot\frac{1}{2}H_2O$: C 49.39, H 7.29, N 9.58
Found: C 49.22, H 7.06, N 9.57

Tetra-*N*-ethoxycarbonyl-6′-*N*-methylsisamine (**1j**) and Tetra-*N*-ethoxycarbonyl-3′-hydroxy-6′-*N*-methylsisamine (**1e**)

1j and **1e** were synthesized following the procedure described above.

1j (5.8 g, 79%) was obtained from crude **1f**[7] (6.48 g, 12.5 mmol). mp 180°C, $[\alpha]_D^{20}$ +111.5° (*c* 0.66, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 157.3, 156.6 carbonyls (Cbe); 145.4 C-5′; 97.8, 97.2 C-1,4′; 80.6, 77.0, 76.0 C-4,5,6; 61.7, 61.2, 60.8 CH_2O (Cbe); 51.6, 50.5, 50.2, 47.7 C-1,6′,3,2′; 34.7 C-2′; 34.2 NCH_3; 23.2 C-3′; 14.7 CH_3 (Cbe).

Anal Calcd for $C_{25}H_{43}N_4O_{12}\cdot\frac{1}{2}H_2O$: C 50.07, H 7.22, N 9.35
Found: C 50.20, H 7.03, N 9.21

1l (4.8 g, 77%) was obtained from crude **1h**[7] (5.5 g, 10 mmol). mp 115°C, $[\alpha]_D^{20}$ +101.5° (*c* 0.2, $CHCl_3$). ^{13}C NMR (DMSO) 156.6, 156.25, 156.1, 156.0 carbonyls (Cbe); 146.9 C-5′; 101.7 C-4′; 99.0 C-1′; 81.9, 76.8, 74.4 C-4,5,6; 63.5 C-3′; 61.1, 60.2, 59.8 CH_2O (Cbe); 55.1, 51.6, 50.1, 49.7 C-2′,1,3,6′; 35.1 C-2; 34.6 NCH_3; 14.9 CH_3 (Cbe).

Anal Calcd for $C_{25}H_{42}N_4O_{13}\cdot 2H_2O$: C 46.72, H 7.21, N 8.72
Found: C 46.63, H 6.80, N 9.12

1,6-*N*,*O*-Carbonyl-3,2′,6′-tri-*N*-ethoxycarbonylsisamine (**2i**)

1,3,2′,6′-Tetra-*N*-ethoxycarbonylsisamine (**1i**) (6g, 10.4 mmol) was dissolved in DMF (400 ml) and treated with sodium hydride dispersed in oil (52 mmol) at 0°C. The reaction mixture was vigorously stirred for 1 hour at 0°C and for 3 hours at room temperature. Acetic acid (8 ml) was added carefully, followed by water (20 ml). The reaction mixture was evaporated to dryness, then triturated in water. The solid compound was removed by filtration and the solution was extracted with ethyl acetate. The organic layers were dried and evaporated to yield a residue which was mixed with the solid compound and dissolved in ethyl acetate. Upon cooling, the starting material crystallized was collected (1.4 g). Evaporation of the solution resulted in the formation of a solid compound, which was chromatographed to give **2i**, (3.1 g, 57%). mp 100°C (softening), $[\alpha]_D^{20}$ +101° (*c* 0.66, $CHCl_3$). ^{13}C NMR ($(CD_3)_2CO$) 160.8 carbonyl (cyclic carbamate); 157.5, 156.9 carbonyls (Cbe); 148.2 C-5′; 98.3, 96.1 C-1′,4′; 84.5, 82.8, 74.5 C-4,6,5; 61.0 CH_2O (Cbe); 54.9, 52.5, 48.5 C-1,3,2′; 43.3 C-6′; 33.4 C-2; 23.6 C-3′; 15.0 CH_3 (Cbe).

Anal Calcd for $C_{22}H_{34}N_4O_{11}$: C 49.8, H 6.46, N 10.56
Found: C 49.85, H 6.73, N 9.21

1,6-*N*,*O*-Carbonyl-3,2,6′-tri-*N*-ethoxycarbonyl-6′-*N*-methylsisamine (**2j**) and 1,6-*N*,*O*-Carbonyl-3,2′,6′-tri-*N*-ethoxycarbonyl-3′-hydroxy-6′-*N*-methylsisamine (**2l**)

2j and **2l** were synthesized following the procedure described above.

2j (1.13 g, 49%) was obtained from **1j** (2.5 g, 4.2 mmol) and NaH (21 mmol) in 100 ml DMF, $[\alpha]_D^{20}$ +120° (*c* 0.26, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 160.8 carbonyl (cyclic carbamate); 156.8, 156.4, 156.2 carbonyls (Cbe); 145.3 C-5′; 97.5, 96.6 C-4′,1′; 83.6, 81.15, 73.4 C-4,6,5; 61.3, 60.6 CH_2O (Cbe); 54.2, 51.3, 50.2, 47.3 C-1,3,6′,2′; 34.0 NCH_3; 32.1 C-2; 23.0 C-3′; 14.3 CH_3 (Cbe).

Anal Calcd for $C_{23}H_{36}N_4O_{11}\cdot\frac{1}{2}H_2O$: C 49.9, H 6.73, N 10.12
Found: C 49.71, H 6.63, N 9.84

2l (7 g, 66.5%) was obtained from **1l** (11.45 g, 19 mmol) and NaH (94.5 mmol) in 400 ml DMF, mp 135°C (softening), $[\alpha]_D^{20}$ +114° (*c* 1, $CHCl_3$). ^{13}C NMR (CD_3OD) 162.8 carbonyl (cyclic carbamate); 158.8, 158.4 carbonyls (Cbe); 148.4 C-5′; 102.9 C-4′; 99.8 C-1′; 85.4, 82.8, 74.7 C-4,6,5; 65.4 C-3′; 62.8, 62.0, 61.9 CH_2O (Cbe); 55.8, 55.4, 52.5, 51.1 C-1,2′,3,6′; 34.7 C-2; 33.2 NCH_3; 15.0 CH_3 (Cbe).

Anal Calcd for $C_{23}H_{36}N_4O_{12}\cdot\frac{1}{2}H_2O$: C 48.50, H 6.54, N 9.83
Found: C 48.66, H 6.48, N 9.59

1, 6-*N*,*O*-Carbonyl-3, 2′, 6′-tri-*N*-ethoxycarbonyl-3′-*tert*-butyldimethylsilyloxy-6′-*N*-methylsisamine (**3**)

2l (14.1 g, 26.2 mmol) was dissolved in DMF (300 ml) containing imidazole (7.14 g, 0.1 mol). Tertiobutyldimethylsilyl chloride (7.9 g, 52 mmol) was added to the solution at room temperature. The reaction mixture was stirred for 15 hours at room temperature, water (10 ml) was added, and the solution was evaporated to dryness. Chromatography on silica gel (EtOAc) yielded **3** (13.5 g, 75%). mp 128°C, $[\alpha]_D^{20}$ +147° (*c* 0.8, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 160.8 carbonyl (cyclic carbamate); 157.2, 156.7 carbonyls (Cbe); 147.3 C-5′; 101.5 C-4′; 98.7 C-1′; 83.9, 81.8, 73.2 C-4,6,5; 65.7 C-3′; 61.8, 60.9, 60.4 CH_2O (Cbe); 54.4, 54.4, 51.5, 50.1 C-1,2′,3,6′; 34.4 NCH_3; 32.3 C-2; 25.8, 18.0, −4.5 TBMDS;

14.7 CH_3 (Cbe).

Anal Calcd for $C_{29}H_{30}N_4O_{12}Si$: C 51.61, H 7.47, N 8.30
Found: C 51.48, H 7.60, N 8.11

Preparation of the Tribenzoylfuranosyl Bromides

2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl bromide was prepared from commercial 1,2,3,5-tetra-*O*-benzoyl-D-ribofuranose, and the tribenzoyl-D-xylo, D-arabino and L-arabinofuranosyl bromides from the corresponding methyl tribenzoyl furanosides[18,19]. The precursor (5 g, 10 mmol) was dissolved in methylene chloride (25 ml), and commercial HBr/acetic acid (10 ml) was added at room temperature. After 15 hours, the reaction mixture was evaporated to dryness. It was redissolved in benzene, and evaporated. The latter procedure was repeated. The product was then dissolved in benzene and lyophilized three times, controlled by ^1H NMR and used, without further purification, in the glycosylation reactions.

General Procedure for the Glycosylation Reactions

The 5-hydroxy-free sisamine derivative (1 mmol), mercuric cyanide (3 mmol) and 3Å molecular sieves (2 g) were weighed in a 250-ml two necked round bottomed flask containing a magnetic stirring bar. The flask was dried under high vacuum, at 80°C, for 15 hours, then fitted with a rubber septum and a reflux condenser connected to dry argon pressure. Methylene chloride (30 ml) and thefuranosyl bromide derivative (3~5 mmol) dissolved in benzene (30 ml), were introduced through the septum.

The mixture was stirred and refluxed for 15 hours. Triethylamine (1 ml) in methanol (5 ml) was added at room temperature and the reaction mixture was stirred for 15 minutes, then filtered and evaporated to dryness. The residue was chromatographed on silica gel (ethyl acetate - heptane) to give the homogeneous protected pseudotrisaccharide.

4a: 213 mg (22%). $[\alpha]_D^{20}$ +39° (*c* 0.76, $CHCl_3$). ^{13}C NMR ($(CD_3)_2CO$) 166.4, 165.7, 160.1, 157.4, 156.9, 156.6 carbonyls; 147.9 C-5′; 134.1, 133.7, 130.4, 129.3 *C*-aromatic; 10.6 C-1′; 97.7, 96.3 C-4′,1′; 83.5, 80.9, 80.4, 80.4, 76.0, 72.5 C-4,5,6,2″,3″,4″; 65.2 C-5″; 61.0 CH_2O(Cbe); 54.5, 52.6, 48.2 C-1,3,2′; 43.5 C-6′; 33.3 C-2; 23.8 C-3′; 14.9 CH_3 (Cbe).

4b: 700 mg (70%). $[\alpha]_D^{20}$ +44° (*c* 0.98, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 166.0, 165.2, 159.9, 157.1, 156.5, 156.0 carbonyls; 145.1 C-5′; 133.3, 133.1, 129.6, 129.6, 128.7, 128.2 *C*-aromatic; 105.1 C-1″; 97.1, 97.1 C-1′,4′; 82.9, 79.6, 78.7, 78.7, 75.1, 71.7 C-4,5,6,2″,3″,4″; 64.6 C-5″; 61.6, 60.7 CH_2O (Cbe); 54.1, 51.6, 50.1, 47.1 C-1,3,6′,2′; 34.1 NCH_3; 32.2 C-2; 23.5 C-3′; 14.4 CH_3 (Cbe).

4c: 450 mg (45%). $[\alpha]_D^{20}$ +55° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 165.6, 165.4, 159.9. 157.3, 156.4, 156.3 carbonyls; 144.9 C-5′; 133.3, 129.8, 129.1, 128.5 *C*-aromatic; 100.7 C-1″; 98.3, 97.8 C-1′,4′; 83.1, 78.7, 78.7, 72.9, 71.6, 69.75 C-4,5,6,2″,3″,4″; 63.4 C-5″; 61.6, 61.2, 60.9 CH_2O (Cbe); 54.3, 52.0, 50.2, 47.9 C-1,3,6′,2′; 33.8 NCH_3; 32.0 C-2; 22.7 C-3′; 14.9, 14.6 CH_3 (Cbe).

4d: 630 mg (64%). ^{13}C NMR ($CDCl_3$) 166.3, 165.7, 165.5, 159.9, 157.3, 156.5, 155.8 carbonyls; 145.0 C-5′; 133.5, 133.1, 130.1, 129.8, 129.0, 128.4 *C*-aromatic; 105.1 C-1″; 97.0, 96.2 C-1′,4′; 83.8, 82.2, 82.2, 78.3, 77.3, 76.9 C-4,5,6,2″,3″,4″; 63.7 C-5″; 61.8, 61.8, 60.8 CH_2O (Cbe); 54.4, 51.6, 50.3, 47.0 C-1,3,6′, 2′; 34.5 NCH_3; 32.6 C-2; 23.2 C-3′; 14.8, 14.6, 14.5 CH_3(Cbe).

4e: 600 mg (61%). $[\alpha]_D^{20}$ +34° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 166.3, 166.0, 165.9, 160.2, 157.3, 156.8, 156.2 carbonyls; 145.8 C-5′; 133.6, 133.1, 129.9, 129.2, 128.5, 128.4 *C*-aromatic; 106.1 C-1″; 97.7, 97.5 C-1′,4′; 82.9, 82.4, 80.8, 79.7, 77.6, 77.6 C-4,5,6,2″, 3″,4″; 63.4 C-5″; 61.7, 61.0, 60.7 CH_2O (Cbe); 54.6, 51.7, 50.5, 47.0 C-1,3,6′,2′; 34.2 NCH_3; 32.5 C-2; 24.8 C-3′; 14.8, 14.7, 14.3 CH_3 (Cbe).

4f: 330 mg (31%). $[\alpha]_D^{20}$ +78.5° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 166.0, 165.2, 159.9, 157.4, 156.7, 156.3 carbonyls; 146.8 C-5′; 133.3, 133.1, 129.9, 129.1, 128.4 *C*-aromatic; 105.4 C-1″; 102.2 C-4′; 98.0 C-1′, 83.1, 80.2, 79.4, 77.7, 75.6, 72.4 C-4,5,6,2″,3″,4″; 65.7, 64.8 C-3′,5″; 61.8, 60.9 CH_2O (Cbe); 54.4, 54.1, 51.6, 50.2 C-1,2′,3,6′; 34.4 NCH_3; 32.5 C-2; 25.8, 18.0, −4.6 TBDMS; 14.6 CH_3 (Cbe).

4g: 290 mg (27%). $[\alpha]_D^{20}$ +76° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 165.6, 165.2, 160.0, 158.7, 156.5 carbonyls; 143.6 C-5′; 133.3, 129.8, 129.2, 128.4 *C*-aromatic: 102.7, 100.8, 100.8, C-4′,1′,1″; 83.2, 78.7, 78.7, 72.9, 71.6, 69.7 C-4,5,6,2″,3″,4″; 66.0 C-3′; 63.2 C-5″; 61.6, 61.2, 60.9 CH_2O (Cbe); 54.8, 54.8, 52.1, 49.9 C-1,2′,3,6′; 34.3 NCH_3; 31.8 C-2; 25.8, 18.0, −4.6 TBDMS; 14.9, 14.6 CH_3 (Cbe).

4h: 666 mg (60%). ^{13}C NMR ($CDCl_3$) 166.2, 165.9, 165.6, 159.7, 157.5, 156.5, 156.0 carbonyls;

146.0 C-5′; 133.4, 133.0, 130.0, 129.8, 129.8, 129.1, 128.5 *C*-aromatic; 105.4 C-1″; 102.5 C-4′; 98.2 C-1′; 83.5, 82.1, 78.7, 77.7, 77.2 C-4,5,6,2″,3″,4″; 66.0 C-3′; 63.6 C-5″; 61.9, 61.2, 60.8 CH_2O (Cbe); 54.4, 54.0, 51.7, 50.0 C-1,2′,3,6′; 34.6 NCH_3; 32.6 C-2; 25.8, 18.0, −4.6 TBDMS; 14.8, 14.6 CH_3 (Cbe).

4i: 310 mg (37%). $[\alpha]_D^{20}$ +76° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$) 166.1, 165.6, 165.4, 160.0, 157.3, 156.5, 156.0 carbonyls; 146.5 C-5′, 133.3, 132.8, 129.9, 129.8, 129.1, 128.9, 128.3, 128.2 *C*-aromatic; 106.1 C-1″; 102.2 C-4′; 97.9 C-1′; 82.5, 82.5, 81.2, 79.8, 78.0, 77.5 C-4,5,6,2″,3″,4″; 65.6 C-3′; 63.5 C-5″; 61.7, 60.7 CH_2O (Cbe); 54.5, 53.5, 51.4, 50.0 C-1,2′,3,6′; 34.1 NCH_3; 32.5 C-2; 25.6, 17.8, −4.7 TBDMS; 14.7, 14.6, 14.3 CH_3 (Cbe).

Deprotection: General Procedure

The protected pseudotrisaccharide was dissolved in ethanol (40 ml), and 2 N aqueous NaOH (80 ml) was added. The solution was refluxed for 12~24 hours and evaporated to dryness. The syrupy residue was chromatographed on silica gel (ethanol - conc NH_4OH, 4: 1). After evaporation of the fractions containing the aminoglycoside and two lyophilizations of aqueous solutions, the product was dissolved in water (10 mg/ml) and the solution was neutralized with 0.1 N H_2SO_4 (final pH 6.1). Lyophilization of the latter solution yielded the pseudotrisaccharide sulfate as a white hygroscopic amorphous powder.

4-*O*-(2,6-Diamino-2,3,4,6-tetradeoxy-α-D-glycero-hex-4-enopyranosyl)-5-*O*-β-D-ribofuranosyl-2-deoxystreptamine (**6a**)

6a (159 mg, 33%) was obtained from **4a** (800 mg, 0.82 mmol), $[\alpha]_D^{20}$ +14° (*c* 0.65, H_2O).

4-*O*-(2,6-Diamino-2,3,4,6-tetradeoxy-6-*N*-methyl-α-D-glycero-hex-4-enopyranosyl)-5-*O*-β-D-ribofuranosyl-2-deoxystreptamine (**6b**)

6b (145 mg, 28%) was obtained from **4b** (810 mg, 0.815 mmol); $[\alpha]_D^{20}$ +26° (*c* 0.5, H_2O).

4-*O*-(2,6-Diamino-2,3,4,6-tetradeoxy-6-*N*-methyl-α-D-glycero-hex-4-enopyranosyl)-5-*O*-β-D-xylofuranosyl-2-deoxystreptamine (**6c**)

6c (65 mg, 23%) was obtained from **4c** (450 mg, 0.45 mmol); $[\alpha]_D^{20}$ +18° (*c* 0.5, H_2O).

4-*O*-(2,6-Diamino-2,3,4,6-tetradeoxy-6-*N*-methyl-α-D-glycero-hex-4-enopyranosyl)-5-*O*-α-D-arabinofuranosyl-2-deoxystreptamine (**6d**)

6d (256 mg, 39%) was obtained from **4d** (1.1 g, 1.11 mmol); $[\alpha]_D^{20}$ +53° (*c* 1, H_2O).

Anal Calcd for $C_{18}H_{34}N_4O_8 \cdot 2H_2SO_4 \cdot 5H_2O$: C 29.99, H 6.71, N 7.74
Found: C 30.2, H 6.35, N 7.72

4-*O*-(2,6-Diamino-2,3,4,6-tetradeoxy-6-*N*-methyl-α-D-glycero-hex-4-enopyranosyl)-5-*O*-α-L-arabinofuranosyl-2-deoxystreptamine (**6e**)

6e (87 mg, 33%) was obtained from **4e** (600 mg, 0.6 mmol); $[\alpha]_D^{20}$ −12° (*c* 1, H_2O).

4-*O*-(2,6-Diamino-2,4,6-trideoxy-6-*N*-methyl-α-D-*threo*-hex-4-enopyranosyl)-5-*O*-β-D-ribofuranosyl-2-deoxystreptamine (**6f**)

6f (107 mg, 27%) was obtained from **4f** (650 mg, 0.58 mmol); $[\alpha]_D^{20}$ +38° (*c* 1, H_2O).

4-*O*-(2,6-Diamino-2,4,6-trideoxy-6-*N*-methyl-α-D-*threo*-hex-4-enopyranosyl)-5-*O*-β-D-xylofuranosyl-2-deoxystreptamine (**6g**)

6g (23.5 mg, 4.5%) was obtained from **4g** (750 mg, 0.67 mmol); $[\alpha]_D^{20}$ +51° (*c* 1, H_2O).

Anal Calcd for $C_{18}H_{34}N_4O_9 \cdot 2H_2SO_4 \cdot 8H_2O$: C 27.34, H 6.88, N 7.08
Found: C 27.23, H 6.58, N 7.01

4-*O*-(2,6-Diamino-2,4,6-trideoxy-6-*N*-methyl-α-D-*threo*-hex-4-enopyranosyl)-5-*O*-α-D-arabinofuranosyl-2-deoxystreptamine (**6h**)

6h (100 mg, 25%) was obtained from **4h** (585 mg, 0.522 mmol); $[\alpha]_D^{20}$ +85.5° (*c* 1, H_2O).

Anal Calcd for $C_{18}H_{34}N_4O_9 \cdot 2H_2SO_4 \cdot 7.5H_2O$: C 27.65, H 6.83, N 7.17
Found: C 27.56, H 6.68, N 7.43

4-*O*-(2,6-Diamino-2,4,6-trideoxy-6-*N*-methyl-α-D-*threo*-hex-4-enopyranosyl)-5-*O*-α-L-arabinofuranosyl-2-deoxystreptamine (**6i**)

6i (310 mg, 47%) was obtained from **4i** (1 g, 0.89 mmol); $[\alpha]_D^{20}$ +27° (*c* 1, H_2O).

Anal Calcd for $C_{18}H_{34}N_4O_9 \cdot 2H_2SO_4 \cdot 5H_2O$: C 29.34, H 6.56, N 7.60
Found: C 29.13, H 6.04, N 7.56

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