Synthesis and Biological Activities of 2-Amino-2-deoxy- and 6-Amino-6-deoxy-α-D-glucopyranosyl-2,5-dideoxystreptamines

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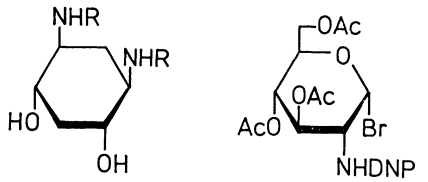
To investigate a relationship between structural features of aminocyclitols and biological activities of aminocyclitol antibiotics, 4-*O*-(2′-amino-2′-deoxy-α-D-glucopyranosyl)- and 4-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamines (**8** and **15a**) have been synthesized, and their biological activities against several microorganisms were determined.

Chemical modification of aminocyclitol antibiotics[1] has so far been extensively carried out in order to elucidate a structure-activity relationship and to synthesize new antibiotics that are active against resistant bacteria. Most investigations have however been aimed at modification of aminosugar moieties of antibiotics, but few exploitations of a relationship between structural feature of aminocyclitol and biological activities of the corresponding antibiotics have been attempted.[2]

In continuation of the preceding report,[3] a synthesis of biologically active α-aminoglycosides of 2,5-dideoxystreptamine,[4] *viz.* 4-*O*-(2′-amino-2′-deoxy-α-D-glucopyranosyl)- and 4-*O*-(6′-amino-6′-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamines (**8** and **15a**) is described in the present article.

Condensation of *N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**1a**)[4] with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-α-D-glucopyranosyl bromide (**2**)[5] was effected in the presence of mercuric cyanide and mercuric bromide in dry dioxane at reflux temperature for three days and afforded, after fractionation by a silica gel column chromatography, 4,6-di-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,4″-dinitroanilino)-D-glucopyranosyl]-*N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**5**)[6] (5.2%) and a practically homogeneous 4-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,4″-dinitroanilino)-α-D-glucopyranosyl]-*N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**6**) (13%), together with syrupy 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-(2′,4′-dinitroanilino)-D-arabino-hex-1-enopyranose (**3**)[7] (33.5% based on **2**) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-D-glucose (**4**)[5] (6.7% based on **2**). Treatment of **6** with methanolic ammonia at room temperature gave crystalline 4-*O*-[2′-deoxy-2′-(2″,4″-dinitroanilino)-α-D-glucopyranosyl]-*N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**7**) in 41% yield. Removal of the protecting groups of **7** was then effected by treatment with Dowex 1×2 (OH^-), followed by hydrogenolysis in the presence of palladium black, giving **8** in 63% yield, as a homogeneous glassy product, which was characterized as the tri-*N*-acetyl derivative (**9**).

Biological activity of **8** was determined by a paper disk method comparing with that of antibiotic neamine, and the results were shown in Table 1. No direct proof to establish the absolute configuration of **8** has been done owing to a minute sample. However, on the basis of an empirical results that only 4-*O*-α-D-aminoglycosides of 2-deoxystreptamine have antibacterial activities,[9] **8** was tentatively assigned as 4-*O*-α-D-glucosaminide of



1a, R=Cbz
1b, R=Cbe

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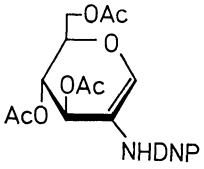

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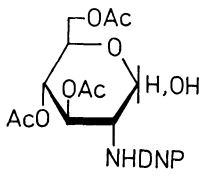

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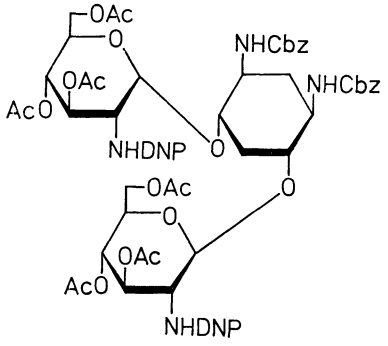

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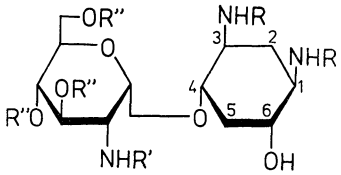
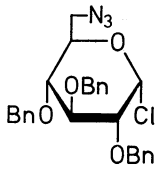


	R	R′	R″
6	Cbz	DNP	Ac
7	Cbz	DNP	H
8	H	H	H
9	Ac	Ac	H

Abbreviation:
Bn=benzyl, Cbe=ethoxycarbonyl,
Cbz=benzyloxycarbonyl,
DNP=2,4-dinitrophenyl



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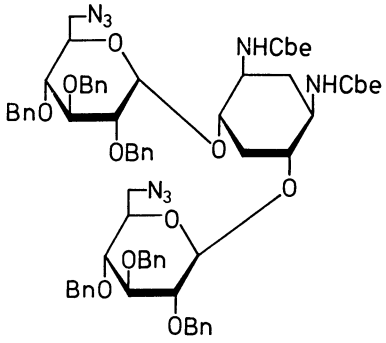

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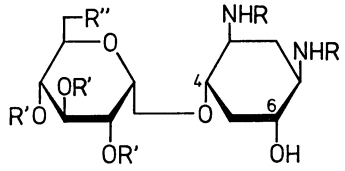
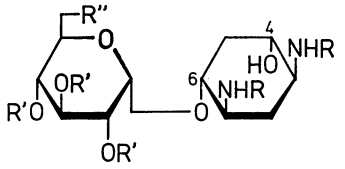
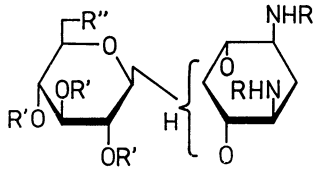


12a, 14a, 15a, 16a



12b, 14b, 15b, 16b



13, 17, 18

	R	R'	R''
12a,b,13	Cbe	Bn	N_3
14a,b,17	Cbe	Bn	NHCbe
15a,b,18	H	H	NH_2
16a,b	Ac	H	NHAc

2,5-dideoxystreptamine, *viz.* 5-deoxyparomamine.

In the case of a preparation of 6-amino-6-deoxy-D-glucopyranosyl-2,5-dideoxystreptamine, *N,N'*-diethoxycarbonyl-2,5-dideoxystreptamine (**1b**) was used instead of **1a** in order to improve solubilities of the products. Condensation of **1b** with 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-glucopyranosyl chloride (**10**)[10] was conducted in the presence of mercuric cyanide in a mixture of benzene and dioxane for two weeks at reflux temperature. A mixture of crude products thus obtained could successfully be separated by a silica gel column chromatography to give a crystalline pseudo trisaccharide derivative (**11**)[6] (25%), 6-*O*-(6'-azido-2',3',4'-tri-*O*-benzyl-6'-deoxy-α-D-glucopyranosyl)-*N,N'*-diethoxycarbonyl-2,5-dideoxystreptamine (**12b**) (15%), 4- or 6-*O*-β-D-glycoside (**13**) (3%), and an amorphous 4-*O*-α-D-glycoside (**12a**) being contaminated with a trace of **12b** and **13**. Catalytic reduction of the azido group of **12a** by Raney nickel T-4[11] followed by treatment with ethyl chloroformate gave a crystalline 4-*O*-(2',3',4'-tri-*O*-benzyl-6'-deoxy-6'-ethoxycarbonylamino-α-D-glucopyranosyl)-*N, N'*-diethoxycarbonyl-2,5-dideoxystreptamine (**14a**) in 5% yield based on **1b** used. The similar treatment of **12b** and **13** gave the corresponding tri-*N*-ethoxycarbonyl derivatives (**14b** and **17**) in 61 and 74% yields, respectively. Hydrogenolysis of **14a** and **14b** in the presence of palladium black and subsequent treatment with aqueous barium hydroxide at 120 °C gave crude free aminoglycosides, which were purified by Amberlite CG-50 (NH_4^+) column chromatography using aqueous ammonia as an eluent to afford 4- and 6-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamines (**15a** and **15b**) as a homogeneous glass in 74 and 68% yields, respectively. They were

Table 1. Biological activities of synthesized aminoglycosides (**8** and **15a**)[a]

Test organisms	Zone of inhibition,[b] mm					
	8			**15a**		
	A	B	C	A	B	C
Bacillus subtilis ATCC 6633	27 (36)	24 (34)	21 (31)	30 (31)	28 (29)	24 (25)
Staphylococcus aureus 6538 p	15 (21)	11 (19)	0 (17)	22 (24)	18 (21)	15 (19)
Escherichia coli K-12	28 (35)	23 (32)	20 (29)	35 (33)	31 (30)	27 (28)
Klebsiella pneumoniae 7	—	—	—[c]	17 (0)	13 (0)	10 (0)
Mycobacterium smegmatis ATCC 607	—	—	—[c]	37 (30)	30 (24)	24 (18)

a) Determined by paper disk method (Hole method). Concentrations: A, 1000 μg/ml; B, 500 μg/ml; C, 250 μg/ml. b) Data in the parentheses were those of antibiotic neamine determined as the reference compound under the same conditions. c) Not determined.

further characterized by converting into the corresponding tri-*N*-acetyl derivatives (**16a** and **16b**). Compound **17** was also converted into the free aminoglycoside (**18**).

In the PMR spectra of **15a** and **15b** in deuterium oxide, signals of the anomeric proton appeared at δ 5.14 and 5.10 as a doublet with 3 and 3.5 Hz spacings, respectively. Empirical rule[12] on difference in the chemical shift of anomeric protons of α-D-glucopyranosyl 2-deoxystreptamine between 4- and 6-glycosides might not be applied to our compounds which lacked the 5-hydroxyl group. But it seems to support the above configurational assignment that the anomeric proton of 4-*O*-α-D-glycoside resonated at relatively lower field than that of 6-*O*-α-D-glycoside. Examination on biological activities of **15a** and **15b** indicated that the

former had a remarkable antibacterial activity almost comparable to that of neamine, but the latter had no activity, confirming the absolute configuration deduced from the PMR data.

According to the present results, it has been presumed that aminocyclitol antibiotics which contain 2,5-dideoxystreptamine possess their antibacterial activities.

Experimental[13]

Modified Königs-Knorr Reaction of N,N′-*Dibenzyloxycarbonyl-2,5-dideoxystreptamine (**1a**) and 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-α-D-glucopyranosyl Bromide (**2**).*[5] A mixture of **1a** (2.0 g), Drierite (6.0 g) and dry dioxane (150 ml) was heated at reflux to distil out some of the solvent. Then **2** (7.7 g), mercuric cyanide (7.7 g), and mercuric bromide (6.0 g) were added to this mixture in three portions every 24 h under gentle reflux and stirring. Three days after the last addition of the reagents, TLC (1: 4 acetone–benzene) showed that all **1a** had been consumed and four main components (R_f 0.73, 0.50, 0.31, and 0.18) formed. An insoluble material was filtered off and the filtrate was evaporated to dryness. The residue was taken up in chloroform (300 ml), washed with a saline water, aqueous sodium hydrogencarbonate, and water, successively, dried over anhydrous sodium sulfate, and evaporated to give a crystalline residue (*ca.* 12 g). TLC indicated the presence of four main components, along with several minor components. The products were chromatographed on a silica gel column (300 g, Wako gel C-200) with 1: 4 acetone–benzene as an eluant. Fractions were separated according to the results of TLC.

The first fractions (R_f 0.73) were evaporated to give 3,4,6-tri-*O*-acetyl-1,2-dideoxy-2-(2′,4′-dinitroanilino)-D-arabino-hex-1-enopyranose (**3**, 2.2 g, 33.5% yield based on **2** used) as a yellowish glass; $[\alpha]_D^{20}$ −115° (*c* 1, acetone); IR, 3340, 1750, 1670 cm^{-1}; PMR ($CDCl_3$) δ 2.02 (s, 3), 2.17 (s, 3), 2.21 (s, 3) (OAc), 5.44 (t, 1, $J_{3,4}=J_{4,5}=4.5$ Hz, H-4), 5.61 (dd, 1, $J_{1,3}=0.8$ Hz, H-3), 7.01 (d, 1, H-1).

Found: C, 47.57; H, 4.36; N, 8.86%. Calcd for $C_{18}H_{19}N_3O_{11}$: C, 47.69; H, 4.22; N, 9.27%.

The second fractions (R_f 0.50) were evaporated and crystallized from acetone–petroleum ether to give crystalline 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-D-glucose (**4**, 0.45 g, 6.7% yield based on **2** used); mp 159—162 °C. This compound was identified with an authentic sample[5] by IR spectra and TLC.

The third fractions (R_f 0.31) were evaporated and crystallized from acetone–petroleum ether to give a crystalline 4,6-di-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,4″-dinitroanilino)-D-glucopyranosyl-*N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**5**, 330 mg, 5.2% yield based on **1a** used); mp 291—294 °C; $[\alpha]_D^{21}$ +63° (*c* 0.3, pyridine); IR, 3340, 1750, 1520 cm^{-1}; PMR (DMSO-d_6) δ 1.76 (s, 3), 1.78 (s, 3), 1.98 (s, 3), 2.08 (s, 3), 2.10 (s, 3) (OAc), 5.18 (m, 4, C$\underline{H}_2$ of Cbz), 7.52 (s, 10, aromatic protons of Cbz).

Found: C, 52.73; H, 4.83; N, 8.48%. Calcd for $C_{58}H_{64}N_8O_{28}$: C, 52.73; H, 4.97; N, 8.29%.

The third fractions (R_f 0.18) were evaporated and crystal lized from acetone–petroleum ether to give crystalline product (821 mg). Recrystallization from acetone gave 4-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,4″-dinitroanilino)-α-D-glucopyranosyl]-*N*,*N*′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**6**, 529 mg, 12.5% yield based on **1a** used) as homogeneous crystals; mp 286.5 °C; $[\alpha]_D^{20}$ +61.3° (*c* 0.75, pyridine); IR, 3340, 1740, 1690, 1520 cm^{-1}; PMR (DMSO-d_6), δ 1.77 (s, 3), 1.95 (s, 3), 2.10 (s, 3) (OAc), 5.16 (s, 4, C$\underline{H}_2$ of Cbz), 7.48 (s, 10, aromatic protons of Cbz).

Found: C, 54.98; H, 5.28; N, 7.71%. Calcd for $C_{40}H_{45}N_5O_{17}$: C, 55.36; H, 5.27; N, 8.07%.

*4-O-[2′-deoxy-2′-(2″,4″-dinitroanilino)-α-D-glucopyranosyl]-N,N′-dibenzyloxycarbonyl-2,5-dideoxystreptamine (**7**).* Compound **6** (567 mg) was treated with methanolic ammonia (100 ml) under stirring overnight at room temperature. The reaction mixture was evaporated to give a crystalline residue. Recrystallization from dioxane–methanol gave **7** (198 mg, 41%) as pale yellowish crystals; mp 277—279 °C; $[\alpha]_D^{16}$ +90° (*c* 0.3, *N*,*N*-dimethylformamide); IR, 3300, 1690, 1530 cm^{-1}.

Found: C, 54.51; H, 5.25; N, 9.17%. Calcd for $C_{34}H_{39}N_5O_{14}\cdot 1/2H_2O$: C, 54.39; H, 5.33; N, 9.33%.

*4-O-(2′-Amino-2′-deoxy-α-D-glucopyranosyl-2,5-dideoxystreptamine (**8**).* A solution of **7** (190 mg) in dioxane (30 ml) and water (6 ml) was treated with Dowex 1×2 (OH^-) (8 ml) under stirring overnight at room temperature. The resin was removed by filtration and the filtrate was evaporated to give a powder. The product was directly hydrogenolyzed in the presence of palladium black (30 mg) in a mixture of dioxane (10 ml) and water (10 ml) overnight at room temperature (the initial hydrogen pressure of 3.4 kg/cm^2). The crude product was crystallized from ethanol–ether to give **8** (50 mg, 63%) as a white powder; mp 151—155 °C (bubbling); $[\alpha]_D^{18}$+162° (c 0.8, H_2O); R_f Glucosamine Hydrochloride 0.31, R_f 2,5-Dideoxystreptamine Dihydrochloride 0.37.

Compound **8** (20 mg) was treated with two drops of acetic anhydride in methanol (2 ml) overnight at room temperature. The product was crystallized from ethanol–ether to give the tri-*N*-acetyl derivative (**9**, 20 mg, 71%) as crystals; mp 320—322 °C; $[\alpha]_D^{21}$ +140° (*c* 1.1, H_2O); IR, 1640, 1550 cm^{-1}.

Found: C, 48.58; H, 7.11; N, 9.13%. Calcd for $C_{18}H_{31}N_3O_9\cdot 1/2H_2O$: C, 48.86; H, 7.29; N, 9.49%.

*6-Azido-2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl Chloride (**10**).* This compound was prepared from crystalline methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside[14] by the direction of Umezawa and his coworkers;[9] syrup, $[\alpha]_D^{22}$ +117° (*c* 2.54, chloroform) (lit,[9] $[\alpha]_D^{24}$ +119° (*c* 0.94, chloroform)).

*N,N′-Diethoxycarbonyl-2,5-dideoxystreptamine (**1b**).* To a solution of 2,5-dideoxystreptamine dihydrochloride[4] (303 mg) in water (20 ml) was added sodium carbonate (1.1 g) and ethyl chloroformate (0.50 ml), and the mixture was stirred for 3 h at room temperature. The mixture was evaporated to dryness and the residue was extracted with hot dioxane (4×20 ml). The extracts were evaporated to give a syrup, which was crystallized from 2-propanol to give **1b** (250 mg, 62%) as crystals; mp 200—201 °C; IR, 3300, 1690, 1540 cm^{-1}; PMR (D_2O) δ 1.24 (t, 6, J=7 Hz, 2C$\underline{H}_3$ of Cbe), 4.15 (q, 4, 2C$\underline{H}_2$ of Cbe).

Found: C, 49.88; H, 7.55; N, 9.60%. Calcd for $C_{12}H_{22}N_2O_6$: C, 49.64; H, 7.64; N, 9.65%.

*Modified Königs-Knorr Reaction of **1b** and **10**.* A mixture of syrupy **10** (4.86 g) and Drierite (6 g) in dry benzene (160 ml) and dry dioxane (54 ml) was magnetically stirred at 50—60 °C for 30 min. Then **1b** (1.98 g) and mercuric cyanide (4.92 g) were added to this mixture, and it was gently refluxed under vigorous agitation. After two days, a 5-g portion of mercuric cyanide was added to the mixture, and, after 9 days, **10** (1.32 g) and mercuric cyanide (1.3 g) were added. Twelve days after the last addition of the reagents, TLC (1: 12 acetone–benzene) indicated a disappearance of **1b**. The reaction mixture was cooled to room temperature and an insoluble matter was removed by filtration. The filtrate was evaporated to give a syrup which was taken up in chloroform (180 ml) and washed with aqueous sodium hydrogencarbonate and water, successively. The chloroform solution was dried over

anhydrous sodium sulfate and evaporated to give an amorphous solid (*ca.* 12 g). TLC showed that it contained three main components (R_f 0.31, 0.06, 0.03), together with several minor components. The products were throughly mixed with silica gel (11 g, Wako gel C-300) and the mixture was transfered to a top of a silica gel column (90 g, packed with benzene). The column was eluted with benzene, 10: 1 benzene–ethyl acetate, 5: 1 benzene–ethyl acetate, 1: 5 acetone–benzene, and 1: 3 acetone–benzene, successively. The fractions were separated according to the results of TLC.

The first fractions (R_f 0.31) were evaporated and crystallized from 2-propanol to give crystalline 4,6-di-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**11**, 2.02 g, 24.6%). Recrystallized sample melted at 150—156 °C; $[\alpha]_D^{32}$ +57.5° (*c* 0.37, chloroform); IR, 3320, 2130, 1630, 1540 cm^{-1}; PMR ($CDCl_3$) δ 1.14 (t, 6, J=7 Hz, 2C$\underline{H}_3$ of Cbe), 4.07 (q, 4, 2C$\underline{H}_2$ of Cbe), 7.30 (s, 30, aromatic protons of Bn).

Found: C, 65.52; H, 6.41; N, 9.18%. Calcd for $C_{66}H_{76}N_8O_{14}$: C, 65.76; H, 6.36; N, 9.30%.

The second fractions (R_f 0.06) were evaporated and crystallized from ethanol to give crystalline 6-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**12b**, 776 mg, 15%). Recrystallized sample melted at 204—205 °C; $[\alpha]_D^{32}$ +45.9° (*c* 0.47, chloroform); IR, 3320, 2120, 1680, 1540 cm^{-1}; PMR ($CDCl_3$) δ 1.16 (t, 3), 1.23 (t, 3) (J=7 Hz, C$\underline{H}_3$ of Cbe), 4.08 (q, 2), 4.24 (q, 2), (C$\underline{H}_2$ of Cbe), 7.30 (s, 5), 7.32 (s, 5), 7.35 (s, 5) (aromatic protons of Bn).

Found: C, 62.38; H, 6.53; N, 9.11%. Calcd for $C_{39}H_{49}N_5O_{10}$: C, 62.63; H, 6.60; N, 9.37%.

The third fractions (R_f 0.03) were evaporated and crystallized from ethanol to give crystalline 4- or 6-*O*-(6′-azido-2′,3′,4′-tri-*O*-benzyl-6′-deoxy-β-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-2,5-dideoxystreptamine (**13**, 162 mg, 3.2%). Recrystallized sample melted at 221—223 °C; $[\alpha]_D^{20}$ +43.5° (*c* 0.58, chloroform); IR, 3310, 2110, 1680, 1540 cm^{-1}; PMR ($CDCl_3$) δ 1.12 (t, 3), 1.23 (t, 3) (J=7 Hz, C$\underline{H}_3$ of Cbe), 4.13 (q, 4, 2C$\underline{H}_2$ of Cbe), 7.26 (s, 15, aromatic protons of Bn).

Found: C, 61.80; H, 6.52; N, 8.88%. Calcd for $C_{39}H_{49}N_5O_{10}\cdot H_2O$: C, 61.60; H, 6.71; N, 9.14%.

An unresolved mixture (*ca.* 2 g) was rechromatographed on a silica gel column (80 g) and eluted with the same solvents mixture to afford **12b** (86 mg, total yield 16.7%), **13** (67 mg, total yield 4.5%), and a mixture (964 mg) containing the 4-*O*-α-glycoside (**12a**) mainly, along with **12b** and **13**.

*6-O-(2′,3′,4′-Tri-O-benzyl-6′-deoxy-6′-ethoxycarbonylamino-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**14b**).* A solution of **12b** (258 mg) in a mixture of dioxane (6 ml) and ethanol (6 ml) was hydrogenated in the presence of Raney nickel T-4[11] at room temperature for 24 h (the initial hydrogen pressure of 3.4 kg/cm^2). The catalyst was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in a mixture of 1: 1: 1 acetone–methanol–water (30 ml) and treated with an excess of ethyl chloroformate in the presence of sodium carbonate overnight at room temperature. An insoluble matter was filtered off and the filtrate was evaporated to dryness. The residue was taken up in chloroform (30 ml), and the solution was washed with aqueous sodium hydrogencarbonate and water, successively, dried over anhydrous sodium sulfate, and evaporated to give a product, which was crystallized from methanol to give crystalline **14b** (166 mg, 60.6%), mp 250—252 °C. Recrystallization from chloroform–ethanol gave an analytical sample, mp 256—258 °C; $[\alpha]_D^{22}$ +13.4° (*c* 0.84, chloroform); IR, no absorption due to an azido group was observed; PMR ($CDCl_3$) δ 1.15 (t, 3), 1.24) (t, 6) (J=7 Hz, C$\underline{H}_3$ of Cbe), 4.06 (q, 2), 4.12 (q, 4) (C$\underline{H}_2$ of Cbe), 5.67 (d, 1, $J_{1',2'}$=3.5 Hz, H-1′), 7.31 (s, 10), 7.34 (s, 5) (aromatic protons of Bn).

Found: C, 63.58; H, 7.01; N, 5.14%. Calcd for $C_{42}H_{55}N_3O_{12}$: C, 63.55; H, 6.98; N, 5.29%.

*4- or 6-O-(2′,3′,4′-Tri-O-benzyl-6′-deoxy-6′-ethoxycarbonylamino-β-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**17**).* Compound **13** (136 mg) was hydrogenated in a mixture of dioxane (15 ml), ethanol (5 ml), and ethyl acetate (10 ml) similarly as described in the preparation of **14b**. The product was treated with ethyl chloroformate and sodium carbonate in 75% aqueous dioxane to give crude **17**, which was crystallized from ethanol to give crystalline **17** (108 mg, 74%), mp 254—256 °C; $[\alpha]_D^{21}$ +11.1° (*c* 0.39, chloroform); IR, no absorption due to an azido group was observed; PMR (DMSO-d_6) δ 1.00 (t, 3), 1.16 (t, 6) (J=7 Hz, C$\underline{H}_3$ of Cbe), 3.97 (q, 2), 4.01 (q, 4) (C$\underline{H}_2$ of Cbe), 7.26 (s, 5), 7.30 (s, 10) (aromatic protons of Bn).

Found: C, 63.05; H, 6.29; N, 5.11%. Calcd for $C_{42}H_{55}N_3O_{12}$: C, 63.55; H, 6.98; N, 5.29%, and, for hemihydrate: C, 62.83; H, 7.03; N, 5.23%.

*4-O-(2′,3′,4′-Tri-O-benzyl-6′-deoxy-6′-ethoxycarbonylamino-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2,5-dideoxystreptamine (**14a**).* A mixture (964 mg) of **12a**, **12b**, and **13** obtained in the condensation reaction of **1b** and **10** was again fractionated by silica gel chromatography to give a fraction (492 mg) containing **12a** mainly. This product was hydrogenated in dioxane similarly as described in the preparation of **17**, and further converted into the tri-*N*-carboethoxy derivative in the usual manner. TLC (30:1 chloroform–ethanol) indicated that it consisted of a main component, along with two minor components identical with **14b** and **17**. The mixture was separated by the silica gel column (40 g) with 40: 1 chloroform–ethanol as an eluent to give a homogeneous syrup, which was crystallized from ethanol–hexane to give **14a** (268 mg, 5.0% yield based on **2b** used) as crystals, mp 189—190 °C; Recrystallized sample melted at 193—196 °C; $[\alpha]_D^{21}$ +31.7° (*c* 0.38, chloroform); PMR ($CDCl_3$) δ 1.14 (t, 3), 1.24 (t, 3), 1.26 (t, 3) (J=7 Hz, C$\underline{H}_3$ of Cbe), 4.07 (q, 2), 4.12 (q, 2), 4.15 (q, 2) (C$\underline{H}_2$ of Cbe), 7.29 (s, 5), 7.31 (s, 5), 7.33 (s, 5) (aromatic protons of Bn); (DMSO-d_6, 100 MHz) δ 0.91 (t, 3), 1.16 (t, 6) (J=7 Hz, C$\underline{H}_3$ of Cbe), 3.18 (q, 2), 4.01 (q, 4) (C$\underline{H}_2$ of Cbe), 5.06 (d, 1, $J_{1',2'}$=4 Hz, H-1′), 7.28 (s, 5), 7.30 (s, 5), 7.33 (s, 5) (aromatic protons of Bn).

Found: C, 63.75; H, 6.98; N, 5.23%. Calcd for $C_{42}H_{55}N_3O_{12}$: C, 63.55; H, 6.98; N, 5.29%.

*4-O-(6′-Amino-6′-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (**15a**).* A solution of **14a** (244 mg) in ethanol (25 ml) containing a few drop of acetic acid was hydrogenated in the presence of freshly prepared palladium black at 50—60 °C for 24 h (the initial hydrogen pressure of 3.4 kg/cm^2). The catalyst was removed by filtration and the filtrate was evaporated to give a syrup (165 mg), which was taken up in a small amount of methanol. The solution was added dropwise to aqueous barium hydroxide (5 ml, containing 1.3 g of $Ba(OH)_2\cdot 8H_2O$) at 60—70 °C, and the mixture was heated at 120 °C for 6 h. After having been neutralized with cabon dioxide, the reaction mixture was filtered centrifugally to remove barium carbonate and the filtrate was evaporated to give a syrup, which was chromatographed on Amberlite CG-50 (NH_4^+) column (4 ml). After having been washed with water, 0.05 M, and 0.1 M aqueous ammonia, successively, the column was then eluted with 0.2 M aqueous ammonia to give **15a** (69 mg, 74%) as a homogeneous glass; mp 153 °C; $[\alpha]_D^{21}$ +104.6° (*c* 1.39, H_2O); IR, 3350, 1570 cm^{-1}; PMR (D_2O) 5.14 (d, 1, $J_{1',2'}$=3 Hz, H-1′); R_f Glucosamine Hydrochloride 0.19, R_f 2-Deoxystreptamine Dihydrochloride 0.53.

Found: C, 45.15; H, 7.50; N, 12.51%. Calcd for $C_{12}H_{25}N_3$-

$O_6 \cdot 1/3H_2CO_3$: C, 45.16; H, 7.89; N, 12.81%.

Treatment of **15a** (18 mg) with acetic anhydride (1 ml) in methanol (1 ml) at room temperature for 3 h gave the tri-*N*-acetyl derivative (**16a**, 21 mg, 82%) as a glass, mp 235 °C (decomp); $[\alpha]_D^{22}$ +62° (*c* 1.05, H_2O); PMR (D_2O) δ 2.01 (s, 6), 2.05 (s, 3) (NAc), 5.08 (d, 1, $J_{1',2'}$=3 Hz, H-1').

Found: C, 48.56; H, 7.16; N, 9.70%. Calcd for $C_{18}H_{31}N_3O_9 \cdot 1/2H_2O$: C, 48.86; H, 7.29; N, 9.50%.

6-O-(6'-Amino-6'-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine (***15b***). A solution of **14b** (414 mg) in 90% aqueous 2-methoxyethanol (40 ml) containing a few drop of acetic acid was hydrogenated similarly as described in the preparation of **15a**. The product was digested with ethanol and the precipitates were collected by filtration to give a solid (182 mg). The mother liquor was shown to contain **15b** by TLC, and it was again hydrogenated similarly to give another crop of the solid (86 mg). The product was hydrolyzed with aqueous barium hydroxide as described above and purified by the resin column to give **15b** (106 mg, 67.5%) as a homogeneous glass, mp 140 °C (decomp); $[\alpha]_D^{21}$ +75.1° (*c* 0.9, H_2O); IR, 3350, 1570 cm^{-1}; PMR (D_2O) δ 5.10 (d, 1, $J_{1',2'}$ =3.5 Hz, H-1'); $R_{f\ \text{Glucosamine Hydrochloride}}$ 0.23, $R_{f\ \text{2-Deoxystreptamine Dihydrochloride}}$ 0.56.

Found: C, 41.20; H, 7.16; N, 10.77%. Calcd for $C_{12}H_{25}N_3O_6 \cdot 3/2H_2CO_3$: C, 40.49; H, 7.05; N, 10.50%.

Compound **15b** (16 mg) was converted into the tri-*N*-acetyl derivative (**16b**, 11 mg, 50%) as a white powder, mp 276 °C (decomp); $[\alpha]_D^{22}$ +57.9° (*c* 0.57, H_2O); PMR (D_2O) δ 1.98 (s, 3), 2.01 (s, 3), 2.04 (s, 3) (NAc), 5.03 (d, 1, $J_{1',2'}$=3.5 Hz, H-1').

Found: C, 47.83; H, 6.88; N, 8.68%. Calcd for $C_{18}H_{31}N_3O_9 \cdot H_2O$: C, 47.88; H, 7.37; N, 9.31%.

4- or 6-O-(6'-Amino-6'-deoxy-β-D-glucopyranosyl)-2,5-dideoxystreptamine (***18***). Compound **17** (105 mg) was hydrogenated and hydrolyzed similarly as described in the preparation of **15a**, and the product was purified by the resin column to give **18** (27 mg, 66%) as a glass, mp 144 °C (decomp); $[\alpha]_D^{21}$ +12.8° (*c* 1.33, H_2O); PMR (D_2O) δ 4.59 (d, 1, $J_{1',2'}$=7.5 Hz, H-1'); $R_{f\ \text{Glucosamine Hydrochloride}}$ 0.21, $R_{f\ \text{2-Deoxystreptamine Dihydrochloride}}$ 0.55.

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13) Melting points were measured on a Mitamura Riken micro hot stage and are uncorrected. Decomposition points were determined in a capillary in a liquid bath and are uncorrected. IR spectra were measured on a Jasco IR-E spectrophotometer in KBr disks. PMR spectra were taken on a Varian A-60D (60 MHz) or HA-100D (100 MHz) in deuteriochloroform ($CDCl_3$), dimethyl sulfoxide-d_6 (DMSO-d_6), or deuterium oxide (D_2O) with tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate, respectively, as an internal standard. Chemical shifts are given in terms of δ-values, signals being denoted by s (singlet), d (doublet), t (triplet), q (quartet), or m (complex multiplet). Values given for coupling constants are of first-order. TLC was performed on silica gel (Wako gel B-10, Wako Pure Chemical Industries, Ltd.) and the spots were visualized by heating above 150 °C with 30% sulfuric acid. Paper chromatography used Toyo Roshi No. 52 paper, with the solvent system 6: 4: 3: 1 1-butanol–pyridine–acetic acid–water and with the reference compound D-glucosamine hydrochloride and appropriate aminocyclitol hydrochlorides. Elementary analyses were done by Mr. Saburo Nakada, to whom our thanks are due.

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