Studies on Aminosugars. XXXIV. Synthesis of 4-*O*- and 6-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine[1]

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Racemic *O*-isopropylidene derivative of *N,N'*-diethoxycarbonyl-2-deoxystreptamine was glycosidated with 4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl chloride to afford two kinds of position isomers of α-glucoside, which was led to 4-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)- and 6-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine.

A series of aminosugar glycosides, namely, kanamycin A, B, C, and other related compounds have been synthesized in our laboratories. A group in them is monoglycosyl-2-deoxystreptamines, which are of interest in connection with the investigation of the relationship between chemical structure and antibacterial activity of aminoglycoside antibiotics. 4-*O*- and 6-*O*-α-D-glucopyranosyl-2-deoxystreptamine,[2] 4-*O*-,[3] 5-*O*-[4] and 6-*O*-α-D-glucosaminyl-2-deoxystreptamine,[3] 4-*O*- and 6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxy streptamine[5] and 6-*O*-(3-amino-3-deoxy-D-glucosyl)-2-deoxystreptamine[6] have been prepared, and, it has been found that, among them, 4-*O*- and 5-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine[4,7] and 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine possess antibacterial activity. As an extension of the work, we describe here the syntheses of 4-amino-4-deoxy-α-D-glucopyranosyl-2-deoxystreptamines (**9**, **10**).

4-Azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl chloride[8] (**1**) was condensed with a mixture (**2**) of racemic *N,N'*-diethoxycarbonyl-4,5- and 5,6-*O*-isopropylidene-2-deoxystreptamines[2] in dry benzene–dioxane in the presence of mercuric cyanide and Drierite (Chart 1). Separation of the products by column chromatography gave 4-*O*-(4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl)-*N,N'*-diethoxycarbonyl-5,6-*O*-isopropylidene-2-deoxystreptamine (**3**) and 6-*O*-(4-azido-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranosyl)-*N,N'*-diethoxycarbonyl-4,5-*O*-isopropylidene-2-deoxy-

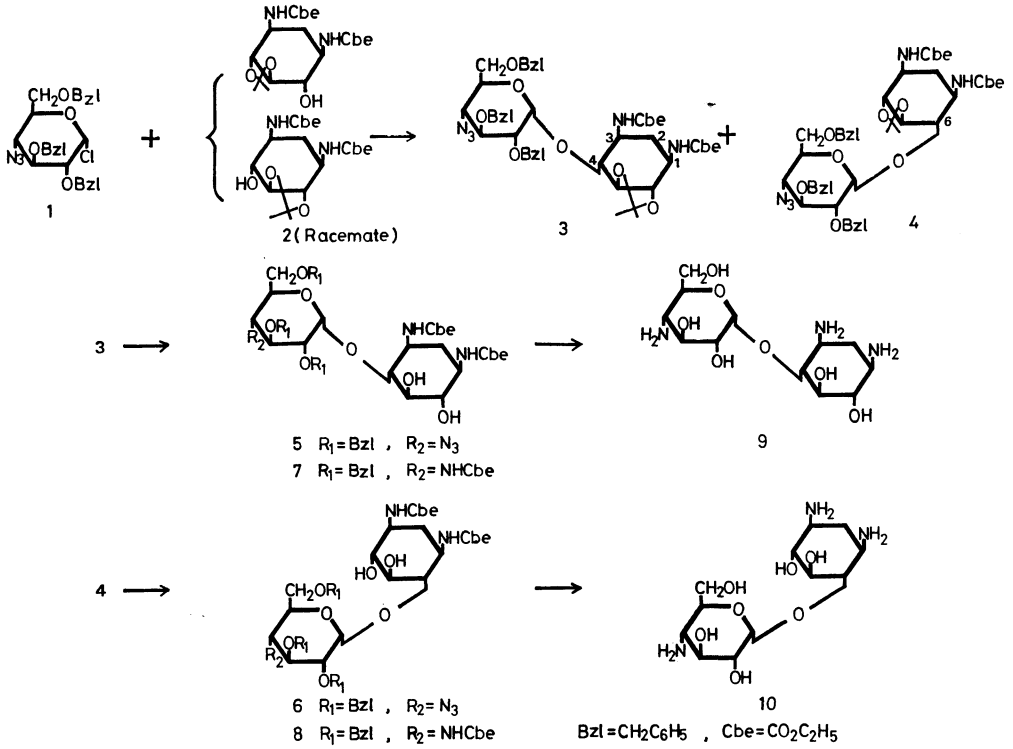

Chart 1.

1) A part of this paper was read at the 26th Annual Meeting of the Chemical Society of Japan, Kanagawa, April, 1972 (See Abstracts of papers of the Meeting Vol. VI, p. 1269).

2) Y. Nishimura, T. Tsuchiya, and S. Umezawa, This Bulletin, **43**, 2960 (1970).

3) S. Umezawa, T. Miyazawa, and T. Tsuchiya, *J. Antibiotics*, **25**, 530 (1972).

4) S. Umezawa, T. Tsuchiya, and H. Fujita, *ibid.*, **19**, 222 (1966).

5) Y. Nishimura, T. Tsuchiya, and S. Umezawa, This Bulletin, **44**, 2521 (1971).

6) S. Koto, K. Tatsuta, E. Kitazawa, and S. Umezawa, *ibid.*, **41**, 2769 (1968).

7) T. Tsuchiya, H. Fujita, and S. Umezawa, *J. Antibiotics*, **17**, 181 (1964).

8) Y. Takagi, T. Tsuchiya, and S. Umezawa, This Bulletin, **46**, 1261 (1970).

streptamine (**4**) in yields of 9.6 and 23%, respectively. Higher yield of 6-*O*-isomer was again here observed.[2,5]

After hydrolysis of **3** and **4** with aqueous acetic acid, the deisopropylidenated products (**5** and **6**) were reduced with Raney nickel and hydrogen to the corresponding amino-derivatives, which were then ethoxycarbonylated to give 4-*O*- and 6-*O*-(2,3,6-tri-*O*-benzyl-4-deoxy-4-ethoxycarbonylamino-α-D-glucopyranosyl)-*N*, *N'*-diethoxycarbonyl-2-deoxystreptamine (**7** and **8**), respectively. Debenzylation of **7** and **8** with palladium black and hydrogen followed by hydrolysis with hot barium hydroxide gave 4-*O*- and 6-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (**9** and **10**), respectively. Simultaneous reduction of the azido group and debenzylation of **5** and **6** by use of palladium and hydrogen were not adopted by the same reason described in a previous paper.[5]

Respective assignment of 4-*O*- and 6-*O*-glycosidic linkage to **9** and **10** were established by the determination of the values of Δ[M] in tetraminecopper (II) sulfate solutions (TACu).[9] The Δ[M] values of **9** and **10** were +1540° and +165° respectively. The sign and the magnitude of the Δ[M] of **9** indicated that TACu formed complexes between C-1 NH_2 and C-6 OH in 2-deoxystreptamine moiety and between C-3 OH and C-4 NH_2 in the sugar moiety. On the other hand, in **10**, formations of complexes occurred between C-3 NH_2 and C-4 OH in 2-deoxystreptamine moiety and between C-3 OH and C-4 NH_2 in the sugar moiety giving the low Δ[M] value by counteraction of both complexes.

Both compounds, **9** and **10**, showed no antibacterial activity against organisms tested, showing that the position of the amino group in aminoglycosidic deoxystreptamines is one of the important factors in exibiting the antibacterial activity. Other biological activities of **9** and **10** are now under study.

Experimental

The NMR spectra were measured with a Varian A-60D spectrometer. Tetramethylsilane (for the solution other than deuterium oxide) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (for the solution of deuterium oxide) were used as the internal standards. Thin-layer chromatography (tlc)was carried out on microscope slides coated with silica gel, and the spots were visualized with sulfuric acid. Paper chromatography (ppc) was carried out on Toyo Roshi No. 50 paper.

4-O-(4-Azido-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranosyl)-N, N'-diethoxycarbonyl-5,6-O-isopropylidene-2-deoxystreptamine (3) and 6-O-(4-Azido-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranosyl)-N, N'-diethoxycarbonyl-4,5-O-isopropylidene-2-deoxystreptamine (4). A mixture of **1** (1.99 g, 2 mmol) and freshly prepared Drierite (1.75 g) in anhydrous benzene–dioxane (2:1, 27 ml) was heated at 60°C under stirring for 20 min and to the mixture, **2** (1.11 g, 3.2 mmol) and well dried mercuric cyanide (2.39 g) were added, and the mixture was refluxed for 10 hr under stirring. Mercuric cyanide (2.39 g) was added and the reaction was continued for another 10 hr. The mixture was filtered and the residue was washed with chloroform. The filtrate and the washings combined were evaporated to give a syrup which was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate and evaporated to give a thick syrup (2.89 g). On tlc with chloroform–ethyl acetate (3:1), the syrup showed six spots of R_f 0.97 (starting material, **1**), 0.71, 0.65, 0.57, 0.49, 0.05 (starting material, **2**). The syrup was chromatographed on a column (48×850 mm) of silica gel (Wako Gel, 850 g) with chloroform–ethyl acetate (3:1), and the fractions of 3050—3200 ml and 3260—3490 ml containing the products of R_f 0.57 and R_f 0.49, respectively, were evaporated to give solids, **3** (0.31 g, 9.6% based on **2**) and **4** (0.74 g, 23% based on **2**). **4** was recrystallized from ether to give colorless crystals (0.73 g) but **3** could not be crystallized.

Compound **3**: mp 60—64°C, $[\alpha]_D^{17}$ +76° (*c* 1, chloroform); IR (KBr): 2100 (N_3), 1700 (amide I), 1535 (amide II); NMR ($CDCl_3$): τ 8.78 (6H, t, *J* 7 Hz, $CO_2CH_2C\underline{H}_3$), 8.62 and 8.58 (3H, s, each, isopropylidene), 5.89 (4H, q, *J* 7 Hz, $CO_2C\underline{H}_2CH_3$), 4.85—5.65 (6H, m, $OC\underline{H}_2C_6H_5$), 4.49 (1H, d, *J* ~3 Hz, H-1), 2.65 (15H, s, $OCH_2C_6\underline{H}_5$).

Found: C, 63.01; H, 7.00; N, 8.65%. Calcd for $C_{42}H_{53}N_5O_{11}$: C, 62.75; H, 6.64; N, 8.71%.

Compound **4**: mp 171.5—172.5°C; $[\alpha]_D^{17}$ +82.2° (*c* 1.8, chloroform); IR (KBr): 2100 (N_3), 1690 (amide I), 1535 (amide II); NMR ($CDCl_3$): τ 8.85 and 8.82 (3H, t, each, *J* 7 Hz, $CO_2CH_2C\underline{H}_3$), 8.66 (6H, s, isopropylidene), 5.94 and 5.91 (2H, q, each, *J* 7 Hz, $CO_2C\underline{H}_2CH_3$), 4.9—5.6 (6H, m, $OC\underline{H}_2C_6H_5$), 4.83 (1H, d, *J* ~3 Hz, H-1), 2.68 (15H, s, $OCH_2C_6\underline{H}_5$).

Found: C, 62.54; H, 6.45; N, 9.05%. Calcd for $C_{42}H_{53}N_5O_{11}$: C, 62.75; H, 6.64; N, 8.71%.

4-O-(4-Azido-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranosyl)-N, N'-diethoxycarbonyl-2-deoxystreptamine (5). A solution of **3** (290 mg) in 50% aqueous acetic acid (6.5 ml) was heated at 90°C for 30 min. The solution was poured into water and the resulting precipitate was filtered and washed thoroughly with water. The solid was recrystallized from aqueous acetone (5:1), 230 mg; mp 183—184°C; $[\alpha]_D^{17}$ +122° (*c* 1.5, pyridine); IR (KBr): 3400, 3300, 2100 (N_3), 1690 (amide I), 1540 (amide II); NMR (in pyridine-d_5 containing a small amount of D_2O): τ 8.88 and 8.75 (3H, t, each, *J* 7.3 Hz, $CO_2CH_2C\underline{H}_3$), 5.5—6.5 (15H, skelton protons and $CO_2C\underline{H}_2CH_3$), 4.8—5.6 (6H, m, $OC\underline{H}_2C_6H_5$), 3.80 (1H, d, *J* ~3 Hz, H-1), 2.3—2.9 (15H, m, $OCH_2C_6\underline{H}_5$).

Found: C, 61.02; H, 6.42; N, 8.88%. Calcd for $C_{39}H_{49}N_5O_{11}$: C, 61.33; H, 6.47; N, 9.17%.

6-O-(4-Azido-2,3,6-tri-O-benzyl-4-deoxy-α-D-glucopyranosyl)-N, N'-diethoxycarbonyl-2-deoxystreptamine (6). Compound **4** (720 mg) was treated likewise as described in the preparation of **5**. Crude solid obtained was crystallized from aqueous acetone (5:1), 630 mg (93%); mp 185.5—186°C; $[\alpha]_D^{18}$ +114° (*c* 1.5, pyridine); IR (KBr): 3440, 3300, 2100 (N_3), 1690 (amide I), 1540 (amide II); NMR (in pyridine-d_5 containing a small amount of D_2O): τ 8.88 and 8.85 (3H, t, each, *J* 7.0 Hz, $CO_2CH_2C\underline{H}_3$), 5.6—6.4 (15H, skeleton protons and $CO_2C\underline{H}_2CH_3$), 4.8—5.6 (6H, m, $OC\underline{H}_2C_6H_5$), 4.06 (1H, d, *J* ~3 Hz, H-1), 2.3—2.9 (15H, m, $OCH_2C_6\underline{H}_5$).

Found: C, 61.60; H, 6.48; N, 8.98%. Calcd for $C_{39}H_{49}N_5O_{11}$: C, 61.33; H, 6.47; N, 9.17%.

4-O-(2,3,6-Tri-O-benzyl-4-deoxy-4-ethoxycarbonylamino-α-D-glucopyranosyl)-N,N'-diethoxycarbonyl-2-deoxystreptamine (7). Compound **5** (230 mg) was dissolved in aqueous dioxane (1:15, 3.2 ml) and the solution was hydrogenated with Raney nickel (T-4) and hydrogen under 50 p.s.i. at 42°C for 1 hr. On tlc with benzene–acetone (2:1), **5** (R_f 0.47) disappeared and a product (R_f 0.20) appeared. Filtration and evaporation of the solution gave a syrup (0.22 g), which was dissolved

9) S. Umezawa, T. Tsuchiya, and K. Tatsuta, This Bulletin, **39**, 1235 (1966).

in acetone (3.2 ml). To the solution, water (3.2 ml) was added under vigorous stirring. Anhydrous sodium carbonate (0.15 g) was added to the resulting suspension, and, after agitation for a while, ethoxycarbonyl chloride (0.063 g) was added slowly. Agitation was continued for 30 min. On tlc with the above-mentioned solvent system, the reaction mixture showed a major spot at R_f 0.40. The mixture was evaporated and the residue was dissolved in chloroform. The solution was washed with water, dried over sodium sulfate and evaporated to give a solid (0.23 g). Recrystallization from aqueous ethanol (1:2) gave a colorless solid, 199 mg (84%); mp 142—143°C, $[\alpha]_D^{17}$ +29.8° (*c* 1.4, chloroform); IR (KBr): 3400, 3300, 1695 (amide I), 1540 (amide II); NMR (in $CDCl_3$): τ 8.87, 8.81, and 8.78 (3H, t, each, *J* 7.2 Hz, $CO_2CH_2C\underline{H}_3$), 2.70 (15H, s, $OCH_2C_6\underline{H}_5$).

Found: C, 62.51; H, 7.23; N, 4.94%. Calcd for $C_{42}H_{55}N_3O_{13}$: C, 62.29; H, 6.85; N, 5.19%.

6-O-(2,3,6-Tri-O-benzyl-4-deoxy-4-ethoxycarbonylamino-α-D-glucopyranosyl)-N,N′-diethoxycarbonyl-2-deoxystreptamine (8). Compound **6** (629 mg) was dissolved in aqueous dioxane (1:8, 9 ml) and treated likewise as described above. The chloroform solution obtained after treatment with ethoxycarbonyl chloride was washed with water, dried over sodium sulfate and evaporated to give a residue (0.59 g), which was chromatographed on a column (14×280 mm) of silica gel (Wako Gel, 15 g) with benzene–acetone (2:1). The portion (36—46 ml) containing the major product (R_f 0.40 with benzene–acetone 2:1) was evaporated to give a solid (0.38 g). Recrystallization from aqueous ethanol gave a colorless solid, 315 mg (51%); mp 194—195.5°C, $[\alpha]_D^{17}$ +9.8° (*c* 1.5, chloroform); IR (KBr): 3400, 3300, 1690 (amide I), 1545 (amide II); NMR (in $CDCl_3$): τ 8.87, 8.83, and 8.81 (3H, t, each, *J* 7.0 Hz, $CO_2CH_2C\underline{H}_3$), 2.70 (15H, s, $OCH_2C_6\underline{H}_5$).

Found: C, 62.25; H, 6.73; N, 5.21%. Calcd for $C_{42}H_{55}N_3O_{13}$: C, 62.29; H, 6.85; N, 5.19%.

4-O-(4-Amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (9). Compound **7** (200 mg) was dissolved in aqueous dioxane (3:20, 2.3 ml) by heating. A few drops of acetic acid were added and the solution was hydrogenated with freshly prepared palladium black and hydrogen under 50 p.s.i. at 40—45°C for 5 hr. On tlc with benzene–ethanol (4:1), **7** (R_f 0.8) disappeared and a product (R_f 0.2) appeared. Filtration and evaporation of the solution gave a solid (0.13 g), which was dissolved in 1N barium hydroxide (2 ml) and heated at 90°C for 8 hr. On paper chromatography with *n*-butyl-alcohol–pyridine–water–acetic acid (6:4:3:1), the solution showed a ninhydrin positive product ($R_{f\ 2\text{-deoxystreptamine}}$ 0.52). The solution was neutralized with carbon dioxide, and the resulting suspension was boiled for a while. After centrifuging, the upper layer was evaporated. After repeated extraction and evaporation of the residue with boiling water, the final residue was charged on a column (5×44 mm) of Amberlite IRC 50 (NH_4 form) and after washing the column with water, developed with 0.03N ammonia. The portion (70—180 ml) containing **9** was evaporated to give a solid (44 mg). An aqueous solution of the product was acidified with hydrochloric acid to pH 2 and evaporated to give a solid of trihydrochloride dihydrate of **9**, 61.4 mg (53%); $[\alpha]_D^{16}$ +67° (*c* 0.3, water), $[\alpha]_{436}^{16}$ +131° (*c* 0.3, water), $[\alpha]_{436TACu}^{16}$ +487° (*c* 0.3, TACu), $\Delta[M]_{TACu}^{16}$ +1540°; IR (KBr): 3400, 2920, 1600, 1500, 1100, 1050, 870, 770 cm^{-1}; NMR (in D_2O): τ 8.75 (1H, q, *J* ~13 Hz, H_{ax}-2), 7.87 (1H, double triplets, *J* ~4 Hz and ~13 Hz, H_{eq}-2), 4.67 (1H, d, *J* ~3 Hz, H-1).

Found: C, 30.55; H, 6.59; N, 9.09; Cl, 22.50%. Calcd for $C_{12}H_{25}N_3O_7 \cdot 3HCl \cdot 2H_2O$: C, 30.74; H, 6.88; N, 8.96; Cl, 22.69%.

6-O-(4-Amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine (10). Compound **8** (0.3 g) was treated similarly as described above. The base (71 mg) obtained was treated with hydrochloric acid to give trihydrochloride dihydrate of **10**, 88.6 mg (51%); $[\alpha]_D^{16}$ +69° (*c* 0.4, water), $[\alpha]_{436}^{16}$ +141° (*c* 0.4, water), $[\alpha]_{436TACu}^{16}$ +176° (*c* 0.4, TACu), $\Delta[M]_{TACu}^{16}$ +165°; $R_{f\ 2\text{-deoxystreptamine}}$ 0.48 (ppc with the same solvent system described above). The IR spectrum of the hydrochloride was quite similar to that of **9**. NMR (in D_2O): τ 8.74 (1H, q, *J* ~13 Hz, H_{ax}-2), 7.90 (1H, double triplets, *J* ~4 Hz and ~13 Hz, H_{eq}-2), 4.83 (1H, d, *J* ~3 Hz, H-1).

Found: C, 30.65; H, 6.80; N, 9.12; Cl, 22.41%. Calcd for $C_{12}H_{25}N_3O_7 \cdot 3HCl \cdot 2H_2O$: C, 30.74; H, 6.88; N, 8.96; Cl, 22.69%.

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