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Studies on Aminosugars. XXV. The Synthesis of Position Isomers of α-D-Glucopyranosyl-2-deoxystreptamine*[1]

Yoshio NISHIMURA, Tsutomu TSUCHIYA and Sumio UMEZAWA

Department of Applied Chemistry, Faculty of Engineering, Keio University, Koganei-shi, Tokyo

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Racemic *O*-isopropylidene derivative of *N,N'*-diethoxycarbonyl-2-deoxystreptamine was glucosidized with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride to afford two kinds of position isomers of α-glucoside, which led to 4-*O*-(α-D-glucopyranosyl)- and 6-*O*-(α-D-glucopyranosyl)-2-deoxystreptamine. Structural assignments were made by the determination of Δ[M] of the glucosides in a tetramminecopper(II) sulfate solution.

The preparation of α-glucopyranosides in high yields remains an important problem of carbohydrate chemistry. The Koenigs-Knorr reaction provided a large number of glycopyranosides. However, the reaction leads to the formation of either α- or β-glycoside. In this reaction, the contributing factors for the ratio and the yields of α- and β-glycosides are the stabilities of the anomeric halides, the chemical and stereochemical properties of the group at C-2 in sugars, the interaction of polar substituents with the ring oxygen in sugars, the polar and steric interactions between substituents of both sugar and aglycone molecules in forming the glycosides and the solvent effects. These factors make the reaction complicated.

On the other hand, α-glycosides of 2-deoxystreptamine have received particular attention in recent years in the field of chemistry of antibiotics, since these kinds of glycosides are important components of useful antibiotics such as kanamycins, paromomycins, neomycins, gentamicins, hygromycin B and destomycin.

We have recently reported the total synthesis of kanamycins[1)] as well as the synthesis of paromamine[2)] and neamine[3)]. In relation to these syntheses, we took an interest in the glycosidation reaction between glucose and 2-deoxystreptamine with particular reference to the formation of anomeric *cis* derivatives. The glycosidation reaction involves several interesting problems, such as the difference in reactivity among the three hydroxyl groups of 2-deoxystreptamine for glycosidation, the reaction conditions to force the glucosyl halide into α-glycosidation, the procedure to separate the position isomers formed which are quite similar in their structures, and the method for determination of the position of 2-deoxystreptamine glycosidized. The present paper describes a detailed study of the modified Koenigs-Knorr reaction between 2,3,4,6-tetra-*O*-benzyl-α-D-glucosyl chloride (III) and the racemic *O*-isopropylidene derivative (II) of *N,N'*-diethoxycarbonyl-2-deoxystreptamine (I) as a basic research for the synthesis of more complex α-glycosides of 2-deoxystreptamine.

In order to make the formation of α-linkage favorable, the hydroxyl groups at C-2 and at other carbon atoms of the sugar halide were masked with benzyl groups, which are nonparticipating with the anomeric position and were successfully used in the case of the total synthesis of kanamycins.[1)] Regarding the *O*-isopropylidene derivative of 2-deoxystreptamine, we had already prepared another one, namely, *N,N'*-dicarbobenzoxy-mono-*O*-isopropylidene-2-deoxystreptamine for the synthesis of kanamycins. However, in the present study, the carbobenzoxyl groups were replaced with ethoxycarbonyl groups.

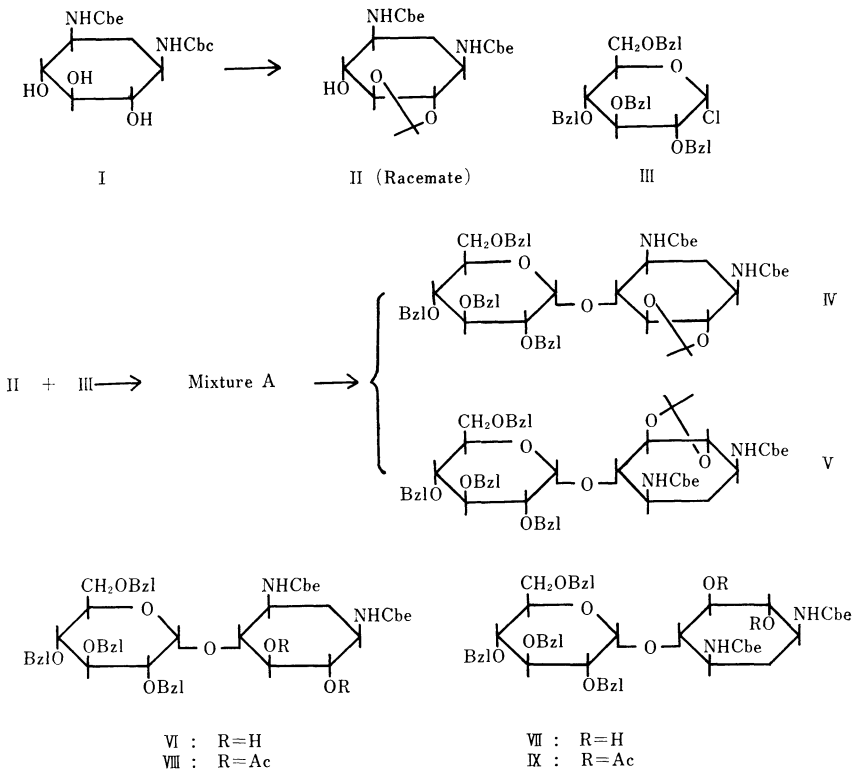
Synthesis of Glucosides. *N,N'*-Diethoxycarbonyldeoxystreptamine (I) was prepared from deoxystreptamine and ethyl chloroformate and acetonated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in dimethyl formamide (DMF) to give a racemic mixture (II) of *N,N'*-diethoxycarbonyl-4,5- and 5,6-*O*-isopropylidenedeoxystreptamine in a 71% yield.

*1 Part XL of "Studies on Antibiotics and Related Substances" by Sumio Umezawa. A part of this paper was read at the 23rd Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1970. (See Abstracts of Papers of the Meeting, Vol. III, p. 1902).

1) S. Umezawa, S. Koto, K. Tatsuta and T. Tsumura, *J. Antibiot.*, **21**, 162 (1968); This Bulletin, **42**, 529 (1969); S. Umezawa, K. Tatsuta and S. Koto, *J. Antibiot.*, **21**, 367 (1968); This Bulletin, **42**, 533 (1969); S. Umezawa, S. Koto, K. Tatsuta, H. Hineno, Y. Nishimura and T. Tsumura, *J. Antibiot.*, **21**, 424 (1968); This Bulletin, **42**, 537 (1969).

2) S. Umezawa and S. Koto, This Bulletin, **39**, 2014 (1966).

3) K. Tatsuta, E. Kitazawa and S. Umezawa, *J. Antibiot.*, **20**, 53 (1967); This Bulletin, **40**, 2371 (1967).



VI : R=H
VIII : R=Ac

VII : R=H
IX : R=Ac

Chart 1. Cbe: $CO_2C_2H_5$ Bzl: $CH_2C_6H_5$

2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride (III) was prepared by the method of Austin *et al.*[4] The chloride proved to be a homogeneous anomer that was characterized through its NMR spectrum.

The racemic monoisopropylidene derivative (II) was condensed with chloride (III). As in the previous studies on the synthesis of kanamycins,[1] the condensation was similarly effected with the use of mercuric cyanide and Drierite in dioxane-benzene and under an extremely anhydrous condition, which is most important for glycosidation. The crude condensed products (here named mixture A), showed four spots (R_f 0.58, 0.54, 0.46, 0.40) on thin-layer chromatography (TLC). Separation of the mixture A into its components was performed by silica gel column chromatography, and the two products separated (R_f 0.46 and 0.40) were found to be 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-5,6-*O*-isopropylidenedeoxystreptamine (IV) and 6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyl-4,5-*O*-isopropylidenedeoxystreptamine (V), respectively, by their elemental analyses, IR and NMR spectra and also by the fact that they were derivable to 4-*O*- and 6-*O*-(α-D-glucopyranosyl)deoxystreptamine (X and XI), respectively. There is a large difference between IV and V with respect to melting points (62 and 168°C), solubilities in chloroform and the NMR signals assignable to anomeric hydrogens (τ 4.51 and 4.90). This suggests that the products have fairly different conformations as a whole. Another separated, minor product (R_f 0.54) was supposed to be an isomer having β-anomeric configuration.

Compounds IV and V were deisopropylidenated with aqueous acetic acid and gave 4-*O*- and 6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyldeoxystreptamine, respectively (VI and VII).

Separation of mixture A into its components by silica gel column chromatography described above, was, however, troublesome because deisopropylidenation of the compounds took place during chromatography. For this reason, after rough purification by column chromatography with ammonia-treated silica gel, the mixture was subjected to deacetonation by hydrolysis followed by acetylation. Although the hydrolyzate of mixture A showed the presence of products of the same R_f-value and the products could not be separated by chromatography, the acetylated products gave three spots (R_f 0.78, 0.71 and 0.65) on TLC and were successfully separated by column chromato-

4) P. W. Austin, F. E. Hardy, J. G. Buchanan and J. Baddiley, *J. Chem. Soc.*, **1964**, 2128.

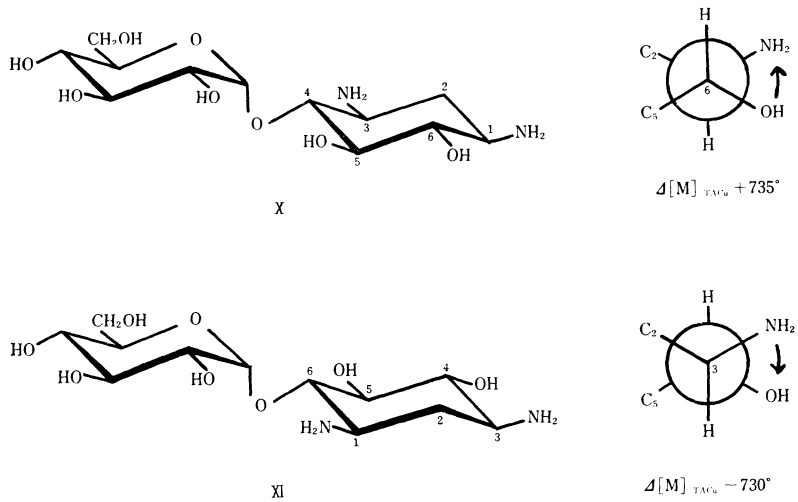


Chart 2.

graphy. The resulting diacetyl derivatives VIII (R_f 0.65) and IX (R_f 0.78) were identical with those derived from the above mentioned pure deisopropylidenation products (VI and VII) by acetylation, and the result indicated that VIII is 5,6-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyldeoxystreptamine and IX is 4,5-di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*′-diethoxycarbonyldeoxystreptamine. The overall yields of VIII and IX from II were 17 and 30%, respectively. It is noteworthy that the hydroxyl group at C-6 in 2-deoxystreptamine is more favorably glycosidated than the hydroxyl group at C-4. This indicates that the unsymmetrical structure of the sugar component greatly influences its approach to the 2-deoxystreptamine component.

VIII and IX were then hydrogenolyzed with palladium black and hydrogen to give debenzylated products, which were then deacetylated and de-*N*-ethoxycarbonylated in a hot barium hydroxide solution to give the final products 4-*O*- and 6-*O*-(α-D-glucopyranosyl)deoxystreptamine (X and XI) in 42 and 57% yield, respectively.

Photolysis of VII was attempted with the aid of bromine according to BeMiller, Meyers and Wing.[5] Removal of benzyl groups proceeded with ease. However, it was found that photolysis was always accompanied by hydrolysis of glycosidic linkage. Addition of powdered potassium hydroxide improved the situation. However, the yield of the product was still variable (95—30%).

Structural Assignment. The problem to differentiate the positional isomers was solved by the determination of Δ[M] in a tetramminecopper (II) sulfate solution (TACu). This reagent was first introduced by Umezawa, Tsuchiya and Tatsuta.[6] Unlike Cupra B[7] or ammoniacal cuprous chloride solution[6] (CuAm), this reagent forms a copper complex with vicinally situated amino and hydroxyl groups without being disturbed by the presence of any other vicinally situated hydroxyl groups. In a six-membered chair-form, a pair of vicinally situated *trans*-diequatorial amino and hydroxyl groups gives a value $\Delta[M]_{TACu}$*2 −900 or +900° according to whether the projection angle*3 between the pair groups (∼60°) is clockwise or counterclockwise. X gave $\Delta[M]_{TACu}$ +735°. This value indicates that the copper complex is formed between 1-amino and 6-hydroxyl groups of 2-deoxystreptamine moiety, showing X to be 4-*O*-(D-glucosyl)deoxystreptamine. XI gave $\Delta[M]_{TACu}$ −730°, indicating XI to be 6-*O*-(D-glucosyl)deoxystreptamine.

Experimental

The NMR spectra were measured with a Varian A-60D spectrometer unless otherwise stated. Thin-layer chromatography was carried out on microscope slides coated with silica gel.

***N*,*N*′-Diethoxycarbonyl-2-deoxystreptamine (I).** To a solution of deoxystreptamine dihydrochloride (6 g) and anhydrous sodium carbonate (27 g) in water (150 ml) ethyl chloroformate (10.2 ml) was added with

5) *C. f.*, *Chem. & Eng. News*, April 24, 51 (1967).

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

7) R. E. Reeves, "Advances in Carbohydrate Chemistry," Vol. 6, Academic Press, New York (1951), p. 107.

*2 $\Delta[M]_{TACu} = ([\alpha]_{436}TACu - [\alpha]_{436} \text{ in water}) \times \frac{\text{Mol. wt.}}{100}$

*3 See footnote of Reference 6 on page 1235.

vigorous stirring. The mixture was then stirred at room temperature for 4 hr and allowed to stand overnight. Inorganic precipitate was filtered and washed with methanol. The filtrate and the washings were combined and evaporated to give a residue, which was dissolved in hot dioxane, filtered, and the filtrate was evaporated. The resulting solid was recrystallized from methanol; yield 7.38 g (94%), mp 231—232°C, $[\alpha]_D^{19}$ ~0° (*c* 0.75, water); IR (KBr): 3450 (broad, OH), 3350 (amide, *ν* NH), 1695 (NHCOO), 1545 (amide II), 1470 (w), 1375 (w), 1305, 1240, 1045, 857, 780 cm^{-1}.

Found: C, 47.18; H, 7.26; N, 8.99%. Calcd for $C_{12}H_{22}N_2O_7$: C, 47.02; H, 7.24; N, 9.15%.

Racemic Mixture (II), *N,N'*-Diethoxycarbonyl-4,5 and 5,6-*O*-isopropylidene-2-deoxystreptamine. A solution of I (3.98 g), 2,2-dimethoxypropane (7 m*l*) and fuse-dried *p*-toluenesulfonic acid (82 mg) in DMF (46 m*l*) was heated on a bath (110°C) for 4 hr, cooled, and neutralized with ethanol-washed Amberlite IRA-400 (OH form). A thin-layer chromatogram (benzene-ethanol 5 : 1) of this solution showed that the starting material (I, R_f 0.16) disappeared and II (R_f 0.53) appeared as a single spot. The solution was evaporated *in vacuo* and further coevaporated with water and with a mixture of toluene and ethyl acetate to give a solid, which was recrystallized from ethyl acetate; yield 3.18 g (71%), mp 199—200°C, $[\alpha]_D^{21}$ ~0° (*c* 1, methanol); IR(KBr): ~3400 (broad), 3330; 1725, 1710, 1690 (amide I); 1560 (sh.), 1550, 1540 (amide II); 1450 (w); 1382, 1372 (isopropylidene); 1310, 1220, 1045, 840, 780 cm^{-1}; NMR(methanol-d_4): *τ*: 8.75 (6-p. t., *J* 7.1 Hz, $CH_2C\underline{H}_3$), 8.57 (6-p. s., isopropylidene), 8.2—9.0 (1-p. H_{ax}-2, overlapped with methyl and isopropylidene signals), 7.8 (1-p. doublet of triplets, *J* ~13 and ~3.5 Hz), 6.2—6.7 (5-p. m., H-1,3,4,5,6), 5.87 (4-p. q., $C\underline{H}_2CH_3$). NMR(DMSO-d_6) : *τ*: 8.81 (6-p. t., $CH_2C\underline{H}_3$), 8.62 (6-p. s., isopropylidene), 8.4—9.0 (H_{ax}-2 N), 8.2 (1-p. broad doublet, H_{eq}-2), 6.3—6.8 (5-p. m.), 5.95 (4-p. q., $C\underline{H}_2CH_3$), 4.9 (1-p.), 3.05 (1-p. d.), 2.83 (1-p. d.). NMR(pyridine-d_5): *τ*: 8.87 (6-p. t., CH_2-$C\underline{H}_3$), 8.52 (6-p. s.), 8.05 (1-p. q., *J* ~12 Hz, H_{ax}-2), 7.22 (1-p. doublet of triplets, *J* ~13 and ~3.5 Hz, H_{eq}-2), 5.6—6.3 (5-p. m.), 5.82 (4-p. q., $C\underline{H}_2CH_3$), 3.5 (1-p.), 2.18 (1-p. d., *J* 8 Hz), 1.98 (1-p. d., *J* 8 Hz).

In pyridine-d_5, the signals assignable to H_{ax}-2 appeared without being overlapped with other signals, and H_{ax}-2, H_{eq}-2 and skeleton hydrogens resonated at lower fields than that measured in methanol-d_4 or dimethyl sulfoxide-d_6.

Found: C, 51.83; H, 7.72; N, 8.03%. Calcd for $C_{15}H_{26}N_2O_7$: C, 52.01; H, 7.57; N, 8.09%.

2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl Chloride (III). This compound was prepared by the method of Austin *et al*[4]. except that, in the final step, the product was purified by passing a short column of silica gel with dry benzene-methyl ethyl ketone (10 : 1). Syrup, $[\alpha]_D^{20}$ +116° (*c* 1, benzene)(lit,[4] +95° in benzene), R_f 0.89 (TLC, benzene - methyl ethyl ketone 10 : 1). NMR($CDCl_3$): *τ*: 5.6—6.6 (6-p. skeleton hydrogens), 4.8—5.6 (8-p. m., $C\underline{H}_2C_6H_5$), 3.85 (1-p. d., *J* 3.5 Hz, H-1), 2.5—2.8 (20-p. m., phenyl).

Preparation of Mixture A. To a solution of III (2.4 g) in dry benzene (18 m*l*) and dry dioxane (6 m*l*) II (1.2 g), freshly prepared powder Drierite (2.1 g), and well dried powder mercuric cyanide (2.2 g) were added, and the mixture was refluxed for 4 hr with vigorous stirring. The reaction mixture was filtered with the aid of Celite and the residue was washed with chloroform. The filtrate and the washings were combined and evaporated to give a viscous solid, which was dissolved in chloroform, filtered, and the solution was washed with 2% sodium bicarbonate solution, then with water, dried over sodium sulfate and evaporated to give a solid (3.2 g). On TLC with benzene - methyl ethyl ketone (5 : 1), the solid (mixture A) showed five spots of R_f 0.40 (V), 0.46 (IV), 0.54, 0.58 and 0.71, the last being 2,3,4,6-tetra-*O*-benzyl-D-glucose.

Separation of Mixture A into 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*N,N'*-diethoxycarbonyl-5,6-*O*-isopropylidene-2-deoxystreptamine (IV) and 6-*O*-(2,3,4,6-Tetra-*O*-benzyl-*α*-D-glucopyranosyl)-*N,N'*-diethoxycarbonyl-4,5-*O*-isopropylidene-2-deoxystreptamine (V). The chromatographic separation of the crude mixture A into its components with the use of silica gel (Mallinckrodt, or most kinds of silica gel purchased from Kanto-Kagaku, with or without treating with ammonia[*4]) was unsuccessful in most cases owing to de-*O*-isopropylidenation of the components during the procedure; a kind of silica gel purchased from Kanto-Kagaku successfully achieved the separation: The mixture A (3 g) was chromatographed on a silica gel column (Kanto-Kagaku, 300 g, 45×480 mm) with dry benzene - methyl ethyl ketone (9 : 2) and the portions containing pure IV and V, respectively, were separated from overlapping portions; the result of a typical run was as follows: the portion containing the substances of R_f 0.58 and 0.71 weighed 0.13 g; R_f 0.54, 0.25 g (part A); R_f 0.54 and 0.46, 0.32 g; R_f 0.46 (IV), 0.35 g (part B); R_f 0.46 and 0.40, 0.59 g; R_f 0.40 (V), 0.53 g (part C).

Part A was recrystallized from methanol to give crystals; mp 232—234°C, $[\alpha]_D^{19}$ +13° (*c* 1, chloroform); IR (Nujol): 3340 (*ν*NH), 1690, 1550, 1300, 1290, 1145, 1080, 1040, 775; 730, 712, 693 cm^{-1} (phenyl).

Found: C, 68.02; H, 6.96; N, 3.05%. Calcd for $C_{49}H_{60}N_2O_{12}$: C, 67.73; H, 6.96; N, 3.22%.

The substance was supposed to be an isomer having *β*-anomeric configuration.

Part B could not be crystallized from any solvent tested; amorphous solid (IV); mp 60—62°C $[\alpha]_D^{18}$ +31.7° (*c* 1, chloroform); IR(Nujol): 3330; 1725, 1710, 1695 (amide I); 1550 (sh.), 1530 (amide II); 1255, 1220, 1155, 1135, 1065, 1040, 1025, 840, 775; 730, 695 cm^{-1} (phenyl); NMR($CDCl_3$) : *τ* : 8.83 and 8.76 (3-p. t. each, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 8.57 (6-p. s., isopropylidene), 5.9—6.8 (15-p. skeleton hydrogens and $C\underline{H}_2CH_3$), 4.95—5.6 (8-p, $C\underline{H}_2C_6H_5$), 4.51 (1-p. d., *J*~3.5 Hz, H-1′), 2.6—2.9 (20-p. m., phenyl).

Found: C, 67.94; H, 6.90; N, 3.11%. Calcd for $C_{49}H_{60}N_2O_{12}$: C, 67.73; H, 6.96; N, 3.22%.

Part C was recrystallized from methanol to give V; mp 167—168°C, $[\alpha]_D^{19}$ +53° (*c* 1, methanol). This compound, unlike the above compound (IV), is scarcely soluble in cold chloroform. IR(Nujol): 3340; 1690 (amide I); 1550 (sh.), 1530 (amide II); 1275, 1220, 1160, 1065, 1040, 840, 775; 730, 695 cm^{-1} (phenyl); NMR (in warm $CDCl_3$) : *τ* : 8.83 and 8.78 (3-p. t. each, *J* 7.1 Hz, $CH_2C\underline{H}_3$), 8.62 (6-p. s., isopropylidene), 5.7—6.8 (15-p., skeleton hydrogens and ($CH_2C\underline{H}_3$),

[*4] See Experimental for the preparation of X and XI.

5.1—5.6 (8-p.) $C\underline{H}_2C_6H_5$), 4.90 (1-p. d., J ~3.5 Hz, H-1'), 2.6—2.9 (20-p. m., phenyl).

Found: C, 67.81; H, 7.16; N, 3.21%. Calcd for $C_{49}H_{60}N_2O_{12}$: C, 67.73; H, 6.96; N, 3.22%.

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*'-diethoxycarbonyl-2-deoxystreptamine (VII). The solution of V (0.30 g) in 80% aqueous acetic acid (4 ml) was heated at 80°C for 5 min. Product (VII) appeared as a single spot (TLC, R_f 0.36 with benzene-ethanol 10 : 1). The solution was poured into water and the resulting precipitates were filtered and washed throughly with water. The soild was recrystallized from acetone; yield 0.23 g (80%); mp 212—213°C, $[\alpha]_D^{18}$ +15° (*c* 1, chloroform); IR(KBr): 3580 (sh.), 3520, 3360, 1700, 1550, 1457 (w), 1355, 1305, 1270, 1250, 1230, 1110, 1080 (sh), 1065, 1030, 915 (w), 865 (w), 780, 748, 740, 697 cm^{-1}; NMR($CDCl_3$): τ : 8.84 and 8.78 (3-p. t. each, J 7.1 Hz, $CH_2C\underline{H}_3$), 5.92 and 5.87 (2-p. q. each, J 7.1 Hz, $C\underline{H}_2CH_3$), 5.05—5.65 (9-p. $C\underline{H}_2C_6H_5$ and an anomeric hydrogen(?)), 2.6—2.8 (20-p. m., phenyl).

Found: C, 66.81; H, 6.88; N, 3.40%. Calcd for $C_{16}H_{56}N_2O_{12}$: C, 66.65; H, 6.81; N, 3.38%.

4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*'-diethoxycarbonyl-2-deoxystreptamine (VI). Compound IV was treated likewise as above and the product was recrystallized from acetone; yield 75%; mp 185—186°C, $[\alpha]_D^{20}$ +28° (*c* 1, chloroform); IR (KBr): 3580 (sh.), 3450 (broad), 3360, 1695, 1550 (sh.), 1540, 1457 (w), 1362, 1315, 1295, 1285, 1250, 1250, 1225, 1085, 1065, 1040, 1030 (sh.), 865 (w), 780, 745, 735, 697 cm^{-1}; NMR($CDCl_3$): τ : 8.84 and 8.78 (3-p. t. each, J 7.1 Hz, $CH_2C\underline{H}_3$), 5.95 and 5.88 (2-p. q. each, J 7.1 Hz, $C\underline{H}_2CH_3$), 5.05—5.6 (9-p., $C\underline{H}_2$-C_6H_5 and an anomeric hydrogen(?)), 2.6—2.8 (20-p. m., phenyl).

Found: C, 66.76; H, 7.19; N, 3.40%. Calcd for $C_{46}H_{56}N_2O_{12}$: C, 66.65; H, 6.81; N, 3.38%.

5,6-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*'-diethoxycarbonyl-2-deoxystreptamine (VIII) and 4,5-Di-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-*N*,*N*'-diethoxycarbonyl-2-deoxystreptamine (IX). *a*) As the chromatographic separation of mixture A into pure IV and V was troublesome, a mixture mainly consisting of IV and V was first obtained as follows: Mixture A (3.2 g) was chromatographed on a silica gel column (260 g, 33×730 mm) with chloroform ethyl acetate (3 : 1) and the portion (1.5 g) containing mainly IV and V was separated. The mass was again chromatographed by the same procedure to give a solid (1.26 g) which showed three spots (R_f 0.40 (V), 0.46 (IV) and 0.54) on TLC with benzene-methyl ethyl ketone (5 : 1). The silica gel used in this experiment was prepared[8] as follows: Mallinckrodt silica gel in 6N aqueous ammonia was allowed to stand overnight, filtered and activated at 150°C for 3 hr on a flat plate.

b) *Deisopropylidenation.* A solution of the solid (1.26 g) in 50% aqueous acetic acid (20 ml) was heated at 80°C for 1 hr. The products showed the same R_f-value (0.36, TLC with benzene-ethanol 10 : 1) The solution was poured into water and the precipitates were filtered to give a soild, 1.16 g (97%).

c) *Acetylation and Chromatography.* A solution of the solid (1.14 g) in pyridine (5 ml) and acetic anhydride (5 ml) was allowed to stand at 50°C overnight. The reaction mixture showed three spots (R_f 0.78, 0.71 and 0.65) on TLC with chloroform-ethyl acetate (3 : 1). The mixture was poured into water and the resulting syrup was dissolved in chloroform. The solution was washed throughly with water, dried over sodium sulfate and evaporated to give a pale yellow solid. This was chromatographed on a silica gel column (Mallinckrodt, 300 g, 33×750 mm) with chloroform-ethyl acetate (3 : 1), and the eluates containg the substance of R_f 0.78, 0.71 and 0.65 (TLC with the above solvent system) were separated respectively and evaporated to give amorphous solids 0.48 g (IX), 0.11 g and 0.26 g (VIII).

Compound VIII, mp 71—73°C, $[\alpha]_D^{19}$ +25° (*c* 1, methanol); IR(KBr): 3400; 1745, 1725 (ester); 1705 (amide I); 1540 (amide II); 1455, 1375 (sh.), 1365, 1230, 1150, 1090, 1070, 1035, 910, 850, 775, 735, 695 cm^{-1}; NMR (in $CDCl_3$ with a Japan Electron Optics 4H-100 spectrometer at a frequency of 100 MHz): τ: 8.86 and 8.78 (3-p. t. each, J 7.1 Hz, $CH_2C\underline{H}_3$), 8.18 and 7.98 (3-p. s. each, OAc), 6.1—6.8 (9-p. unresolved m.), 5.93 and 5.88 (2-p. q. each, J 7.1 Hz, $C\underline{H}_2CH_3$), 5.46, 5.30 and 5.15 (2-p. s. each, $C\underline{H}_2C_6H_5$), at τ 5.64, 5.46, 5.25 and 5.07 (2-p. AB quartet, $C\underline{H}_2C_6H_5$, J 11 Hz), 5.0—5.7 (2-p., overlapped with $C\underline{H}_2C_6H_5$; H-5,6), 4.92 (1-p. d., H-1', J~3 Hz), 2.6—2.8 (20-p. m., phenyl).

Found: C, 65.85; H, 6.47; N, 3.19%. Calcd for $C_{50}H_{60}N_2O_{14}$: C, 66.09; H, 6.58; N, 3.39%.

Compound IX, mp 61—63°C, $[\alpha]_D^{19}$ +61° (*c* 1, methanol); IR(KBr): 3340; 1750, 1725 (ester); 1705 (amide I); 1530 (amide II); 1455, 1375 (sh.), 1365, 1230, 1150, 1070, 1040, 910, 850, 775, 735, 695 cm^{-1}; NMR (100 MHz, in $CDCl_3$): τ : 8.86 and 8.78 (3-p. t. each, J 7.1 Hz, CH_2CH_3), 8.03 and 7.98 (3-p. s. each, OAc), 6.9—7.4 (1-p. m., H_{eq}-2), 6.1—6.8 (9-p. unresolved m., H-1,3,6,2',3',4',5',6',6''), 5.93 and 5.88 (2-p. q. each, J 7.1 Hz, CH_2CH_3), 5.50, 5.23 and 5.10 (2-p. s. each, $C\underline{H}_2C_6H_5$), at τ 5.60, 5.42, 5.18 and 5.00 (2-p. AB quartet, $C\underline{H}_2C_6H_5$, J 11 Hz), 5.0—5.7 (2-p., overlapped with $C\underline{H}_2C_6H_5$; H-4,5), 4.90 (1-p. d., H-1', J ~3.5 Hz), 2.6—2.8 (20-p. m., phenyl).

Found: C, 66.31; H, 6.88; N, 3.13%. Calcd for $C_{50}H_{60}N_2O_{14}$: C, 66.09; H, 6.58; N, 3.39%.

The signals which differentiate VIII and IX are those assignable to one of the acetyls (the one lying in the higher field), the benzyls and the phenyls (VIII has one prominent peak and IX has three prominent peaks), and the feature suggests that VIII and IX have conformations differing as a whole from each other. The difference in chemical shifts assignable to the anomeric hydrogens in VIII and IX was unexpectedly small.

Preparation of VII from IX, and VI from VIII. To a solution of IX (444 mg) in methanol (8 ml) 2.4N sodium methylate in methanol (0.2 ml) was added and the solution was allowed to stand for 1 hr, whereupon some crystals deposited. Chloroform was added to the mixture and the resulting solution was treated with Dowex 50W×8 (H^+ form). The resulting solution was evaporated to give a residue, which was recrystallized from acetone; colorless crystals (350 mg, 87%), mp 212—213°C, $[\alpha]_D^{20}$ +15° (*c* 1, chloroform). Its IR and NMR spectra were identical with those of the specimen obtained from V.

8) J. G. Buchanan and J. C. P. Schwarz, *J. Chem. Soc.*, **1962**, 4770.

VI was obtained similarly from VIII in 91% yield.

6-*O*-(α-D-Glucopyranosyl)-2-deoxystreptamine (XI). A solution of VII (286 mg) in a mixture of methanol (15 m*l*), water (7 m*l*) and acetic acid (3 m*l*) was hydrogenated with freshly prepared palladium balck and hydrogen at 45°C under 50 lb/inch pressure overnight. The starting material (R_f 1.0, TLC with benzene-ethanol 1 : 1) disappeared and a product having R_f-value of 0.4 appeared. The solution was filtered, the filtrate was evaporated to give a colorless solid which was dissolved in 1 N barium hydroxide (30 m*l*) and the solution was heated at 90°C for 2 hr. After neutralizing the mixture with a slight excess of 2 N sulfuric acid followed by centrifugation, the supernatant layer was treated with a small volume of Dowex 1×2 (OH form), and evaporated to give a solid (100 mg) which gave a single spot on paper chromatography ($R_{f\text{deoxystreptamine}}$ 0.70, with *n*-butanol-pyridine-water-acetic acid 6 : 4 : 3 : 1). An aqueous solution of the solid was then passed through a column of Dowex 1×2 (OH form, 8 m*l*) with water, and ninhydrin-positive fraction (with 0.5% ninhydrin in pyridine) was evaporated to give a colorless solid of XI (75 mg, 66%); mp 231—232°C (decomp.), $[\alpha]_D^{15}$ +102° (*c* 0.1, water), $[\alpha]_{436}^{15}$ +186° (*c* 0.1, water), $[\alpha]_{436}^{15}$ −40° (*c* 0.1, TACu), therefore, $\Delta[M]_{TACu} = -730°$ (Mol. wt. 324.3): IR(KBr): 3400 (broad), 1625 (NH_2), 1145, 1115, 1035, 940, 915 (w), 840, 770 cm^{-1}.

Found: C, 44.21; H, 7.45; N, 8.46%. Calcd for $C_{12}H_{24}N_2O_8$: C, 44.43; H, 7.15; N, 8.64%.

On hydrolysis of XI with 6 N hydrochloric acid at 90°C for 5 hr, paper chromatography (with *n*-butanol-pyridine-water-acetic acid 6 : 4 : 3 : 1) of the hydrolyzate showed two spots corresponding to deoxystreptamine and glucose ($R_{f\text{deoxystreptamine}}$ 3.4).

4-*O*-(α-D-Glucopyranosyl)-2-deoxystreptamine (X). A solution of VIII (150 mg) in a mixture of methanol (10 m*l*), water (5 m*l*) and acetic acid (2.5 m*l*) was hydrogenated likewise as in the preparation of XI. However, in this case, the hydrogenation had to be repeated with fresh catalyst in order to complete the reaction. The resulting product was hydrolyzed with barium hydroxide. The crude product (58 mg) was chromatographed on a cellulose column (10×150 mm) with *n*-butanol-water-ethanol (5 : 2 : 1) and the portion (36 mg) containing X ($R_{f\text{deoxystreptamine}}$ 0.81) was further purified by passing Dowex 1×2 (OH form, 5 m*l*) column to give a hygroscopic solid (27 mg, 46%); $[\alpha]_D^{15}$ +127° (*c* 0.15, water), $[\alpha]_{436}^{15}$ +238° (*c* 0.15, water), $[\alpha]_{436}^{15}$ +465° (*c* 0.15, TACu), $\Delta[M]_{TACu}$ = +735° (Mol. wt. 324.3); IR(KBr): 3300—3400, 1635, 1600, 1145, 1035, 920, 915 (w), 840, 770 cm^{-1}.

Found: C, 44.28; H, 6.90; N, 8.51%. Calcd for $C_{12}H_{24}N_2O_8$: C, 44.43; H, 7.15; N, 8.64%.

On hydrolysis with 6 N hydrochloric acid, X gave glucose and deoxystreptamine.

Preparation of XI by the Photolysis of VII. To a solution of VII (264 mg) in chloroform (15 m*l*) bromine (200 mg) and powered potassium hydroxide (70 mg) were added and the mixture was irradiated with a 60 W lamp from a close distance for 30 min at 0°C under vigorous stirring. More bromine (200 mg) was added and the irradiation was continued for 30 min. The supernatant solution was decanted and the insoluble matter was washed with chloroform. The combined solution and the washings were evaporated to give a solid which was dissolved in 1 N barium hydroxide solution (80 m*l*) and heated at 90°C for 2 hr. After neutralization with a slight excess of 2 N sulfuric acid followed by centrifugation, the supernatant layer was treated with a small volume of Dowex 1×2 (OH form) and the solution was evaporated to give a colorless solid, which showed two spots of $R_{f\text{deoxystreptamine}}$ 0.70 (XI, major) and 1.0 (deoxystreptamine, minor) on paper chromatography with *n*-butanol-pyridine-water-acetic acid (6 : 4 : 3 : 1). An aqueous solution of the solid was chromatographed on a column (10 m*l*) of Dowex 1×2 (OH form) with water and the eluate containing XI was evaporated to give a solid (95 mg, 92%), which was recrystallized from a mixture of ethanol-water-acetone; mp 230°C (decomp.) $[\alpha]_D^{15}$ +102° (*c* 0.3, water). $\Delta[M]_{TACu}$ and IR spectrum were identical with those of the specimen obtained from IX.

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