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Studies of Aminosugars. XIV. Syntheses of 6, 6′-Diamino-6, 6′-dideoxymaltosylamine, 1′, 6, 6′-Triamino-1′, 6, 6′-trideoxysucrose and 6, 6′-Diamino-6, 6′-dideoxytrehalose[1]

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Several aminodisaccharides have been synthesized for the investigation of the relationship between the structural and biochemical characteristics of aminoglycosides. The preferential tosylation of maltose, followed by acetylation and bromination, yielded 2, 2′, 3, 3′, 4′-penta-*O*-acetyl-6, 6′-di-*O*-tosylmaltosyl bromide, of which the bromine atom and the two tosyloxy groups were replaced by azido groups. The removal of acetyl groups and the catalytic hydrogenation of the azido groups gave 6, 6′-diamino-6, 6′-dideoxymaltosylamine. By an analogous procedure, 1′, 6, 6′-triamino-1′, 6, 6′-trideoxysucrose was prepared from sucrose *via* 2, 3, 3′, 4, 4′-penta-*O*-acetyl-1′, 6, 6′-triazido-1′, 6, 6′-trideoxysucrose. 6, 6′-Diamino-6, 6′-dideoxytrehalose was obtained by the ammonolysis of 2, 2′, 3, 3′, 4, 4′-hexa-*O*-acetyl-6, 6′-di-*O*-tosyltrehalose.

There are a number of antibiotics which contain aminosugars in their molecules, for example, streptomycins, neomycins, paromomycins, and kanamycins.[2] With reference to the chemical structures of these antibiotics, the authors attempted to find some new aminosugar derivatives which show antibacterial or other biochemical activities. Our first approach to this goal was the syntheses of deoxystreptamine derivatives. 4, 6-Di-*O*-(β-D-glucopyranosyl)-deoxystreptamine[3] and 4, 6-di-*O*-[3-(or 6-)amino-3-(or 6-)deoxy-β-D-glucopyranosyl]-deoxystreptamine[4] were thus synthesized, but these compounds were not endowed with antibacterial activities. A degradation product of kanamycin, 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-deoxystreptamine[5](6AD), however, had been found to exhibit antibacterial activities. A comparison of the chemical structure of 6AD with that of the deoxystreptamine derivatives mentioned above led to the assumption that at least an α-anomeric configuration linking an aminosugar moiety to deoxystreptamine (aglycon) would be necessary to give the compounds in question the antibacterial activities. The first successful compound, which had a strong antitubercular activity, was produced during the search in this direction by the condensation of 2-amino-2-deoxy-D-glucose and deoxystreptamine by acid reversion[6] and DNP methods;[7] this compound was proved to be 5-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-deoxystreptamine.[8]

As can be seen, the production of an aminosugar derivative with the α-anomeric configuration seems to be more promising than that of a derivative with the β-configuration. Generally speaking, the synthesis of a compound with the α-anomeric configuration is more difficult than that of the corresponding β-anomer, and no successful method such as the Koenigs-Knorr reaction producing the β-configuration has ever been discovered. For this reason, the authors utilized some natural disaccharides with the α-anomeric configuration in order to synthesize aminodisaccharides for the investigation of the relationship between the structural and biochemical characteristics of aminoglycosides.

The present paper will describe the preparation of the titled compounds from the natural disaccharides, maltose, sucrose, and trehalose *via* their tosylated intermediates.

Maltose (I) contains eight hydroxyl groups, one an anomeric hydroxyl group, two primary, and the others, secondary hydroxyl groups. On the preferential tosylation of I with *p*-toluenesulfonyl chloride, followed by acetylation, 1, 2, 2′, 3, 3′, 4′-hexa-*O*-acetyl-6, 6′-di-*O*-tosylmaltose (II) was obtained in a 45% yield.

In the next step, the anomeric acetyloxy group

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[1] This article constitutes Part XXVII of a series entitled "Studies of Antibiotics and Related Substances," primarily by Sumio Umezawa. A part of this paper was presented at the 19th Annual Meeting of the Chemical Society of Japan, Tokyo, March, 1966.

[2] S. Umezawa, Y. Ito and S. Fukatsu, *J. Antibiotics*, **A11**, 120, 162 (1958); This Bulletin, **32**, 81 (1959).

[3] S. Umezawa and Y. Ito, This Bulletin, **34**, 1540 (1961).

[4] S. Koto, Y. Ito and S. Umezawa, *ibid.*, **38**, 1447 (1965).

[5] S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962).

[6] T. Tsuchiya, H. Fujita and S. Umezawa, *ibid.*, **A17**, 181 (1964).

[7] S. Umezawa and S. Koto, *ibid.*, **A17**, 186 (1964).

[8] S. Umezawa, T. Tsuchiya and H. Fujita, *ibid.*, **A19**, 222 (1966).

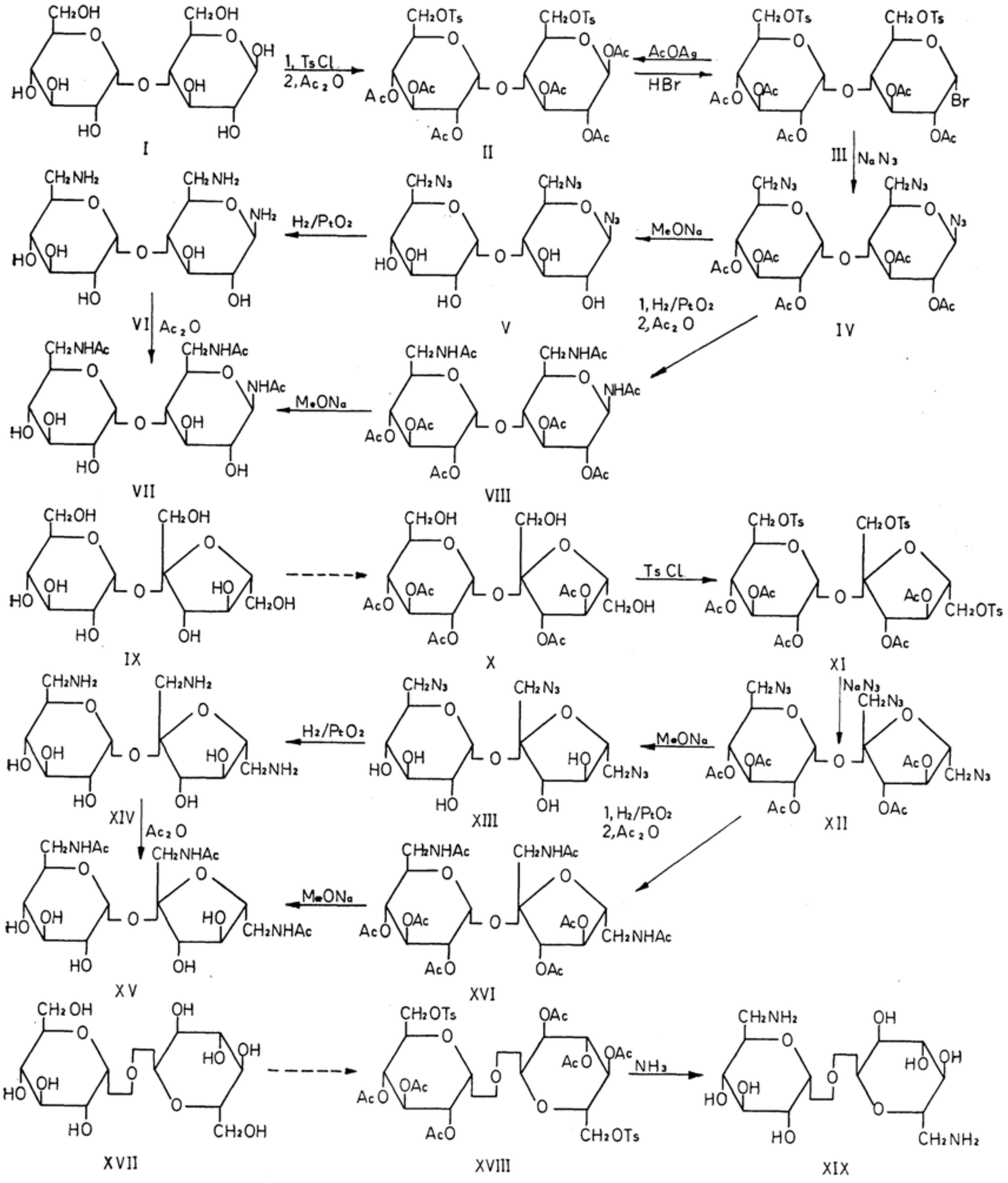

Chart 1

of II was easily replaced by a bromine atom by the action of hydrogen bromide in acetic acid, and 2, 2′, 3, 3′, 4′-penta-*O*-acetyl-6, 6′-di-*O*-tosylmaltosyl bromide (III) was produced nearly quantitatively.

The anomeric acetyloxy group of II and the bromine atom of III probably had the β- and α-configurations respectively, since there was an increase (+77300) in the molecular rotation of III (+151300) from II (+74000). The molecular rotations of octa-*O*-acetylmaltose, penta-*O*-acetyl-D-glucopyranose, and tetra-*O*-acetyl-6-*O*-tosyl-D-glucopyranose, each with a β-anomeric acetyloxy group, are +42700,[9] +1600,[10] and +11300[11] respectively, while those of the corresponding compounds, each with an α-anomeric bromine

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[9] W. Korytnyk, *J. Chem. Soc.*, **1959**, 650.

[10] E. Fischer, *Ber.*, **49**, 584 (1916).

[11] M. Akagi, S. Tejima and M. Haga, *Chem. Pharm. Bull. (Japan)*, **11**, 559 (1963).

atom instead of a β-anomeric acetyloxy group, are +125900,[12] +81400,[13] and +86900[14] respectively. Therefore, the increases in the molecular rotations of the latter compounds from the corresponding former compounds are +83200, +79800, and +75600 respectively.

As these increases are, by a modification of Hudson's rule, deemed to reflect the contribution of the anomeric center when the β-anomeric acetyloxy group in each of the above-mentioned compounds is replaced by the α-anomeric bromine atom, the above-mentioned assignments for II and III are reasonable.

The replacements of the bromine atom and the two tosyloxy groups of III with azido groups were carried out by heating III in dimethylformamide with sodium azide, thus affording 2, 2′, 3, 3′, 4′-penta-*O*-acetyl-6, 6′-diazido-6, 6′-dideoxymaltosyl azide (IV) in a good yield (60%). The anomeric azido group was also supposed to be in the β-configuration from a consideration of its value of molecular rotation (+50800). In the next step, the azide compound (IV) was converted to 6, 6′-diamino-6, 6′-dideoxymaltosylamine (VI), mp 138—140°C (decomp.), $[\alpha]^{12}_{D}$ +113°, by de-*O*-acetylation with sodium methoxide in methanol, followed by catalytic hydrogenation with platinum oxide, in a yield of 65%. The structural evidence of VI was obtained by means of the periodate oxidation of the tri-*N*-acetylated derivative (VII) of VI, where three molecular proportions of periodate were consumed for one molecular proportion of VII; this result indicates that the three acetamido groups are located at the C-1-, 6-, and 6′-positions of maltose, and precludes any other possible locations for these three acetamido groups.

The β-anomeric glycosylamine structure of VI was presumed from the values of the molecular rotations of VI and VII (+40300 and +36700 respectively) and from the nuclear magnetic resonance spectrum of VII, which provided another proof of this structure. The signals at τ 8.04 (singlet), 8.02 (singlet), and 7.95 (singlet) with the relative intensities of 3 : 3 : 3 may be ascribed to nine *N*-acetyl protons, the unresolved multiplet at τ 6—7 (12 protons) may be assigned to *H*-2, 2′, 3, 3′, 4, 4′, 5, 5′, 6, 6′, and the doublets at τ 5.09 (J=7.5 cps) and 4.71 (J=3.0 cps), each with a relative intensity of 1, may be assigned to the anomeric hydrogen of C-1 and C-1′ respectively.

The sucrose derivative, 1′, 6, 6′-triamino-1′, 6, 6′-trideoxysucrose (XIV), was prepared likewise from sucrose (IX). As the starting material, 2, 3, 3′, 4, 4′-penta-*O*-acetylsucrose (X) was prepared from IX by the method of McKeown, Serenius, and Hayward;[15] X was then tosylated in the usual manner to give 2, 3, 3′, 4, 4′-penta-*O*-acetyl-1′, 6, 6′-tri-*O*-tosylsucrose (XI). The three tosyloxy groups of XI were then replaced by azido groups, in the same way as has been described in the preparation of the corresponding maltose derivative, to give 2, 3, 3′, 4, 4′-penta-*O*-acetyl-1′, 6, 6′-triazido-1′, 6, 6′-trideoxysucrose (XII). After the acetyl groups of XII had been removed by sodium methoxide in methanol, the de-*O*-acetylated product (XIII) was hydrogenated with platinum oxide to give 1′, 6, 6′-triamino-1′, 6, 6′-trideoxysucrose (XIV).

Structural proof was also obtained by the periodate oxidation of the tri-*N*-acetylated derivative, XV, of XIV.

The totally-acetylated compounds VIII and XVI were also prepared from IV and XII respectively by catalytic hydrogenation, followed by acetylation; these compounds were then converted to VII and XV respectively by de-*O*-acetylation.

The trehalose derivative, 6, 6′-diamino-6, 6′-dideoxytrehalose (XIX), was prepared by the usual ammonolysis of the starting material, 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6,6′-di-*O*-tosyltrehalose (XVIII), which had been derived from trehalose (XVII) by the method of Bredereck.[16] The structure of XIX was confirmed by the fact that the hydrolysis of XIX with hydrochloric acid gave only 6-amino-6-deoxy-D-glucose on paper chromatography.

Experimental

Thin-layer Chromatography, Silica-gel Column Chromatography, and Paper Chromatography. Thin-layer chromatography was conducted by the ues of silica gel (Daiichi Pure Chemicals Co.); the prepared plate was activated at 110°C and then stored in a desiccator. The spray reagent used was concentrated sulfuric acid. Solvent systems used: benzene - ethyl acetate (9 : 4) (Solvent A); chloroform - ethanol (9 : 1) (Solvent B); chloroform - ethanol (1 : 1) (Solvent C); ethanol - chloroform (5 : 2) (Solvent D), and the upper layer of chloroform - methanol - 17% aqueous ammonia (2 : 1 : 1) (Solvent E). Silica-gel column chromatography was carried out by the use of silica gel (Kanto Chemical Co.) activated at 110°C before use. Paper chromatography was conducted by the descending technique on Toyo filter paper No. 50, and the substances were detected by the use of ninhydrin spray (0.25% in pyridine). Solvent system used: *n*-butanol-pyridine - water - acetic acid (6 : 4 : 3 : 1) (Solvent F).

General Procedure for Nuclear Magnetic Resonance Spectrometry. The NMR spectrum was determined at a frequency of 60 Mcps with a Japan Electron Optics JNM-C-60 spectrometer in deuterium oxide. Sodium 2, 2-dimethyl-2-silapentane-5-sulfonate was used as an internal reference in the sample. Peak

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[12] D. H. Brauns, *J. Am. Chem. Soc.*, **51**, 1800 (1929).

[13] D. H. Brauns, *ibid.*, **47**, 1280 (1925).

[14] H. Ohle and L. von Vargha, *Ber.*, **61**, 1203 (1928).

[15] G. G. McKeown, R. S. E. Serenius and L. D. Hayward, *Can. J. Chem.*, **35**, 28 (1957).

[16] H. Bredereck, *Ber.*, **63**, 959 (1930).

positions are given in τ-values.

1,2,2′,3,3′,4′-Hexa-*O*-acetyl-6,6′-di-*O*-tosylmaltose (II). To a solution of maltose monohydrate (1.20 g, 3.33 mmol) in dry pyridine, Drierite (anhydrous calcium sulfate, 1.2 g) was added; the mixture was then allowed to stand overnight. After the mixture had been cooled to −15°C, *p*-toluenesulfonyl chloride (1.47 g, 7.71 mmol) was added in one portion, and the mixture was kept at −15°C for 1 hr, at 0°C for 20 hr and then at 5°C for 48 hr. Acetic anhydride (5.6 m*l*) was added to the resultant cold solution, and the solution was again set aside overnight at room temperature. After the addition of a small volume of water, the solution was left for 1 hr. The reaction mixture was then poured into a large volume of ice and water.

The precipitate which separated was collected by a centrifuge and washed with water, yielding 3.6 g of a crude product. Thin-layer chromatography with Solvent A proved the product to be composed of three components, with R_f values of 0.20 (minor spot), 0.44 (main spot), and 0.54 (minor spot). This product was dissolved in a solvent mixture of benzene - ethyl acetate (8 : 5) and chromatographed on a silica-gel column (51×330 mm) with the same solvent. After 320 m*l* of eluate had been passed through, every fraction (15 g) was tested by thin-layer chromatography. The substance with an R_f value of 0.44 appeared in the fractions of tube Nos. 21—27; these fractions were combined and evaporated under reduced pressure, and the residue was recrystallized from ethanol, thus affording needles of II [1.35 g. (45%)], mp 190—192°C, $[\alpha]_D^{18}$ +82° (*c* 1.9, chloroform); IR spectrum (KBr disk): 2960, 1765 (ester), 1600, 1370, 1240, 1225, 1190, 1175 ($\nu_s SO_2$) cm^{-1}.

Found: C, 50.74; H, 5.15; O, 36.89; S, 6.91; CH_3CO, 29.18%. Calcd for $C_{38}H_{46}O_{21}S_2$: C, 50.55; H, 5.14; O, 37.21; S, 7.10; CH_3CO, 28.60%.

2, 2′, 3, 3′, 4′ - Penta-*O*-acetyl-6, 6′-di-*O*-tosylmaltosyl Bromide (III). A sample (200 mg) of II was dissolved in 1 m*l* of glacial acetic acid saturated with hydrogen bromide at 0°C, after which the solution was allowed to stand for 30 min at room temperature. The solution was then poured into a large volume of ice and water, and the mixture was extracted several times with chloroform. The combined extracts were washed thrice with ice-cold water, dried over sodium sulfate, and concentrated to a solid, which was then recrystallized from ligroin (boiling point range, 80—110°C); yield, 191 mg (93%); mp 141—142°C, $[\alpha]_D^{17}$ +164° (*c* 0.5, chloroform).

On thin-layer chromatography with Solvents A and B, the product showed single spots with R_f values of 0.60 and 0.80 respectively. The product also gave a strong positive Beilstein test for halogen. IR spectrum (KBr disk): 2950, 1760 (ester), 1600, 1370, 1240, 1220, 1190, 1175 ($\nu_s SO_2$) cm^{-1}.

Found: C, 46.92; H, 4.85; S, 7.01; Br, 8.48%. Calcd for $C_{36}H_{43}O_{19}S_2Br$: C, 46.81; H, 4.69; S, 6.94; Br, 8.65%.

1, 2, 2′, 3, 3′, 4′ - Hexa - *O* - acetyl - 6, 6′-di-*O*-tosylmaltose (II) from 2, 2′, 3, 3′, 4′-Penta-*O*-acetyl-6, 6′-di-*O*-tosylmaltosyl Bromide (III). To a sample (100 mg) of III dissolved in dry benzene (5 m*l*), silver acetate (300 mg) was added, and the mixture was refluxed for 5 hr in the dark. The resultant mixture was filtered, and the filtrate was evaporated to a solid which was recrystallized from ethanol; yield, 75.6 mg; mp 190—191°C, $[\alpha]_D^{17}$ +80° (*c* 1.0, chloroform). The product was proved to be identical with II by thin-layer chromatography and by a study of its IR spectrum. The Beilstein test of the product was negative.

2, 2′, 3, 3′, 4′ - Penta - *O* - acetyl-6, 6′- diazido-6, 6′-dideoxymaltosyl Azide (IV). To a solution of III (2.0 g) in dry *N*, *N*-dimethylformamide (20 m*l*) sodium azide (1.5 g) was added; the suspension was then vigorously agitated at 100°C for 6 hr. The resultant dark solution was evaporated, and the residue was dried by coevaporation with toluene. The dark solid obtained was extracted with acetone, and the extract was concentrated to a syrup, which, on thin-layer chromatography with Solvent A, showed virtually only one spot (R_f 0.59). The syrup was then dissolved in the same solvent system and chromatographed on a silica-gel column (48×400 mm) with the same solvent. An eluate between 660—765 m*l* was evaporated to dryness, and the colorless residue was recrystallized from ethanol - water; yield, 816 mg (60%); mp 78—79°C (decomp.), $[\alpha]_D^{15}$ +81° (*c* 1, chloroform); Beilstein test negative; IR spectrum (KBr disk): 2930, 2115 (azide), 1760 (ester), 1370, 1230, 1075—1025 cm^{-1}.

Found: C, 42.46; H, 4.89; N, 20.33%. Calcd for $C_{22}H_{29}O_{13}N_9$: C, 42.11; H, 4.66; N, 20.09%.

6, 6′-Diazido-6, 6′-dideoxymaltosyl Azide (V). A solution of IV (500 mg) in dry methanol (10 m*l*) was treated with 0.75 N methanolic sodium methoxide (0.5 m*l*) at room temperature overnight. After neutralization to pH 7 with Amberlite IRC 50 (H form) (previously washed with methanol), the solution was evaporated *in vacuo* and the residue, which showed a single spot (R_f 0.78) on thin-layer chromatography with Solvent C, was recrystallized from methyl ethyl ketone - petroleum ether (boiling point range, 30—60°C); yield, 300 mg (90%); mp 65—67°C (decomp.), $[\alpha]_D^{12}$ +98° (*c* 0.5, water); IR spectrum (KBr disk): 3400, 2920, 2120 (azide), 1285, 1245 cm^{-1}.

Found: C, 34.68; H, 4.82; N, 29.97%. Calcd for $C_{12}H_{19}O_8N_9$: C, 34.53; H, 4.59; N, 30.21%.

6, 6′-Diamino-6, 6′-dideoxymaltosylamine (VI). A solution of V (150 mg) in 50% aqueous ethanol (15 m*l*) was hydrogenated with Adams' platinum oxide (60 mg) in a Paar shaker-type apparatus at 35—40°C for 3 hr under an initial pressure of hydrogen of 50 p. s. i. g. After the catalyst had been removed by filtration, the filtrate was concentrated to a small volume; then to it was added Dowex 1×2 (OH form) (0.5 m*l*), and it was immediately filtered again. The alkaline filtrate obtained was evaporated to dryness under reduced pressure to give 90 mg of the crude product as a colorless solid. The solid showed a single spot (R_f 0.62) on thin-layer chromatography with Solvent E and showed no azide absorption at 2120 cm^{-1} in the infrared spectrum. This compound was recrystallized from 2.5% aqueous ammonia - methanol - ethanol to yield 85 mg (70%) of VI monohydrate; mp 138—140°C (decomp.), $[\alpha]_D^{12}$ +113° (*c* 0.5, water); ninhydrin test positive, Fehling's test positive; IR spectrum (KBr disk): ∼3300, 2900, 1600, 1450—1350, 1140—1025 cm^{-1}.

Found: C, 40.19; H, 7.68; N, 11.41%. Calcd for $C_{12}H_{25}O_8N_3 \cdot H_2O$: C, 40.33; H, 7.62; N, 11.76%.

A small quantity of VI was heated with 3 N hydrochloric acid for 3 hr in a boiling water bath, and the hydrolyzate was submitted to paper chromatography with Solvent F. Detection by ninhydrin coloration showed a spot corresponding to 6-amino-6-deoxy-D-glucose[*1] (6AG), in addition to another spot (R_{f6AG} 4.5).

1, 6, 6′ - Triacetamido - 1, 6, 6′ - trideoxymaltose (VII) from 6, 6′-Diamino-6, 6′-dideoxymaltosylamine (VI). To a suspension of VI (280 mg) in dry methanol (2.8 m*l*) acetic anhydride (0.8 m*l*) was added; the mixture was stirred for 2 hr at room temperature, and evaporated, and the solution of the residue in methanol was treated with active charcoal and evaporated to give a solid, which was then recrystallized from methanol by adding acetone; yield, 263 mg (72%); mp 124—126°C (decomp.); $[\alpha]^{12}_{D}$ +79° (*c* 0.5, water).

On thin-layer chromatography with Solvent D, the product showed a single spot with an R_f value of 0.34. Ninhydrin test negative. NMR spectrum data: τ 8.04, 8.02 and 7.95 (singlets, 3 protons each, *NAc*), 6—7 (multiplet, 12 protons, *H*-2, 2′, 3, 3′, 4, 4′, 5, 5′, 6, 6′), 5.09 (doublet, 1 proton, *H*-1′, J=7.5 cps), 4.71 (doublet, 1 proton, *H′*-1′, J=3.0 cps); IR spectrum (KBr disk): 3400, 3300, 2920, 1650 (Amide I), 1560 (Amide II), 1430, 1375, 1290 cm^{-1}.

Found: C, 46.38; H, 6.90; N, 8.74%. Calcd for $C_{18}H_{31}O_{11}N_3$: C, 46.45; H, 6.71; N, 9.03%.

1, 6, 6′ - Triacetamido - 2, 2′, 3, 3′, 4′-penta-*O*-acetyl-1, 6, 6′-trideoxymaltose (VIII). A solution of IV (250 mg) in ethanol (25 m*l*) was hydrogenated for 4 hr in the way used in the synthesis of VI. The crude product obtained was immediately acetylated with acetic anhydride (1 m*l*) and dry pyridine (5 m*l*) overnight. The resultant mixture was evaporated in vacuo to dryness to give 220 mg of a syrup. On thin-layer chromatography with Solvent B, the syrup was proved to be composed of three components, with R_f values of 0.49 (minor spot), 0.39 (minor spot), and 0.30 (main spot). The syrup was dissolved in a solvent mixture chloroform - ethanol (9 : 1) and chromatographed on a silica-gel column (28×110 mm) with the same solvent. Every fraction (5 g) was tested by thin-layer chromatography. The substance with an R_f value of 0.30 was eluted in the fractions of tube Nos. 21—35. These fractions were then combined and evaporated to give a solid, which was recrystallized from chloroform - ether; yield, 156 mg (58%); mp 146—-147°C, $[\alpha]^{15}_{D}$ +81° (*c* 0.5, chloroform); IR spectrum (KBr disk): 3320 (νNH), 2930, 1760 (ester), 1665 (Amide I), 1540 (Amide II), 1430, 1370, 1235 cm^{-1}.

Found: C, 49.74; H, 6.18; N, 6.07%. Calcd for $C_{28}H_{41}O_{16}N_3$: C, 49.77; H, 6.12; N, 6.22%.

1, 6, 6′ - Triacetamido - 1, 6, 6′ - trideoxymaltose (VII) from 1, 6, 6′ - Triacetamido - 2, 2′, 3, 3′, 4′ - penta-*O*-acetyl-1, 6, 6′-trideoxymaltose (VIII). A solution of VIII (90 mg) in dry methanol (2 m*l*) was treated with 0.75 N methanolic sodium methoxide (0.1 m*l*) at room temperature overnight, and then neutralized with Amberlite IRC 50 (H form) as has been described in the synthesis of V. The product obtained showed a single spot (R_f 0.34) on thin-layer chromatography with Solvent D, proving it to be identical with the sample obtained from VI. Recrystallization from methanol and acetone gave 43 mg (69%) of a pure product; mp 123—125°C (decomp.), $[\alpha]^{12}_{D}$ +79° (*c* 1.0, water).

2, 3, 3′, 4, 4′ - Penta - *O* - acetyl-1′, 6, 6′-tri-*O*-tosylsucrose (XI). The starting material, 2, 3, 3′, 4, 4′-penta-*O*-acetylsucrose (X), was prepared from sucrose *via* the 2, 3, 3′, 4, 4′-penta-*O*-acetyl-1′, 6, 6′-tri-*O*-trityl intermediate by the method of McKeown, Serenius and Hayward15); mp 154—156°C, $[\alpha]^{18}_{D}$ +28° (*c* 0.5, chloroform) [lit., dihydrate; mp 155—156°C $[\alpha]^{22}_{D}$ +22° (*c* 3.1, chloroform)]. To a cold (−15°C) solution of X (2.0 g, 3.58 mmol) in dry pyridine (50 m*l*), *p*-toluenesulfonyl chloride (3.48 g, 18.3 mmol) was added; the mixture was kept at the same temperature for 1 hr, and then at room temperature for 3 days. After a small volume of water had been added and the mixture had been set aside for 1 hr, the reaction mixture was poured into a large volume of ice and water. The resultant precipitate was collected by a centrifuge and washed with water, yielding 4.5 g of a crude product. On thin-layer chromatography with Solvent A, the crude product showed three spots, with R_f values of 0.60 (main spot), 0.43 (minor spot), and 0.13 (minor spot). The product was dissolved in a solvent mixture of benzene - ethyl acetate (9 : 4) and chromatographed on a silica-gel column (53×490 mm) with the same solvent. After 765 m*l* of the eluate had been mixed in, every fraction (15 g) was tested by thin-layer chromatography. The substance with an R_f value of 0.60 was eluted in the fractions of tube Nos. 16—29. These fractions were then combined and evaporated to give a residue, which was recrystallized from ethanol; yield 2.46 g (67%); mp 72°C (lit.,17) mp 58.5—61°C), $[\alpha]^{12}_{D}$ +44° (*c* 0.5, chloroform); IR spectrum (KBr disk) 2960, 1760 (ester), 1600, 1370, 1225, 1190, 1175 (ν_s SO_2) cm^{-1}.

Found: C, 50.64; H, 5.02; S, 9.21%. Calcd for $C_{43}H_{50}O_{22}S_3$: C, 50.88; H, 4.97; S, 9.48%.

2, 3, 3′, 4, 4′ - Penta - *O* - acetyl - 1′, 6, 6′ - triazido-1′, 6, 6′-trideoxysucrose (XII). A suspension of sodium azide (600 mg) in dry *N*, *N*-dimethylformamide (6 m*l*) was stirred with XI (600 mg) at 130°C for 6 hr. The solvent was then removed from the dark solution by distillation and codistillation with toluene under reduced pressure. As part of the acetyl groups of XI had been found to be removed by the above procedure, the dark residue obtained was acetylated overnight with acetic anhydride (9.0 m*l*) and dry pyridine (33 m*l*), and the mixture was evaporated to give a syrup. This syrup, which, on thin-layer chromatography with Solvent A, showed two spots with R_f value of 0.58 (main spot) and 0.08 (minor spot), was dissolved in a solvent mixture of benzene - ethyl acetate (9 : 4) and chromatographed on a silica-gel column (48×235 mm) with the same solvent as the eluant. An eluate between 485—605 m*l* was evaporated to dryness, and the colorless residue was recrystallized from ethanol-water, affording XII; yield, 140 mg (38%); mp 55—57°C (decomp.), $[\alpha]^{12}_{D}$ +37° (*c* 0.5, chloroform); IR spectrum (KBr disk): 2930, 2090 (azide), 1750 (ester), 1378, 1230, 1065 cm^{-1}.

Found: C, 42.23; H, 4.84; N, 19.82%. Calcd for $C_{22}H_{29}O_{13}N_9$: C, 42.11; H, 4.66; N, 20.09%.

1′, 6, 6′-Triazido-1′, 6, 6′-trideoxysucrose (XIII).

[*1] 6-Amino-6-deoxy-D-glucose was obtained from the hydrolyzate of kanamycin.

17) R. C. Hockett and M. Zief, *J. Am. Chem. Soc.*, **72**, 1839 (1950).

A sample (139 mg) of XII was treated with methanolic sodium methoxide in the way used in the synthesis of V. On thin-layer chromatography with Solvents B and C, the product obtained gave single spots with R_f values of 0.05 and 0.89 respectively. The product was recrystallized from methyl ethyl ketone - petroleum ether (boiling point range, 30—60°C), affording XIII; yield, 106 mg (98%); mp 64—65°C (decomp.), $[\alpha]_D^{15}$ +39° (*c* 0.5, water); IR spectrum (KBr disk): 3440, 2920, 2100 (azide), 1280, 1125 cm^{-1}.

Found: C, 34.82; H, 4.78; N, 29.88%. Calcd for $C_{12}H_{19}O_8N_9$: C, 34.53; H, 4.59; N, 30.21%.

1′, 6, 6′-Triamino-1′, 6, 6′-trideoxysucrose (XIV). A sample (106 mg) of XIII was hydrogenated with Adams' platinum oxide as has been described in the synthesis of VI. On paper chromatography, the crude product obtained showed virtually only one spot (R_f 0.04 or R_{f6AD}*2 1.2) with Solvent F. An aqueous solution of the product (85 mg) was then placed on a column (7×97 mm) of Dowex 1×2 (OH form) and developed with water at the rate of 0.2 m*l*/min. The ninhydrin-positive fractions were collected, neutralized with hydrochloric acid to pH 2, and evaporated to dryness. The residue was recrystallized from aqueous ethanol to afford crystalline trihydrochloride of XIV; yield, 54 mg (75%); mp 134°C (decomp.), $[\alpha]_D^{12}$ +61° (*c* 0.5, water); IR spectrum (KBr disk): 3400, 3260, 2900, 1610 ($\delta_{as}NH_3^+$), 1500 ($\delta_s NH_3^+$), 1125—965 cm^{-1}.

Found: C, 32.18; H, 6.05; N, 9.15%. Calcd for $C_{12}H_{25}O_8N_3 \cdot 3HCl$: C, 32.12; H, 6.29; N. 9.36%.

1′, 6, 6′ - Triacetamido - 1′, 6, 6′ - trideoxysucrose (XV) from 1′, 6, 6′-Triamino-1′, 6, 6′-trideoxysucrose (XIV). A sample (41 mg) of a XIV base was *N*-acetylated with acetic anhydride in dry methanol as has been described in the synthesis of VII. On thin-layer chromatography with Solvent D, the product obtained showed a single spot (R_f 0.30). Recrystallization from methanol - acetone gave crystals; yield, 44 mg (77%); mp 84—86°C (decomp.), $[\alpha]_D^{12}$ +73° (*c* 0.5, water); IR spectrum (KBr disk): 3360, 2920, 1650 (Amide I), 1550 (Amide II), 1425, 1375 cm^{-1}; NMR spectrum data: τ 7.99, 7.97 and 7.93 (singlets, 3 protons each, *NAc*); 5.5—6.6 (multiplet, 13 protons, *H*-1′ 2, 3, 3′, 4, 4′, 5, 5′, 6, 6′), and 4.54 (doublet, 1 proton, *H*-1, *J*=4.0 cps).

Found: C, 46.65; H, 6.69; N, 9.09%. Calcd for $C_{18}H_{31}O_{11}N_3$: C, 46.45; H, 6.71; N, 9.03%.

1′, 6, 6′ - Triacetamido - 2, 3, 3′, 4, 4′ - penta - *O* - acetyl-1′, 6, 6′-trideoxysucrose (XVI). A sample (100 mg) of XII was hydrogenated and then acetylated in the way used in the synthesis of VIII above. On thin-layer chromatography with Solvent B, the crude product obtained showed three spots with R_f values of 0.66 (minor spot), 0.55 (minor spot), and 0.43 (main spot). The product was then dissolved in a solvent mixture of chloroform - ethanol (9 : 1) and chromatographed on a silica-gel column (28×95 mm) with the same solvent. Every fraction (5 g) was tested by thin-layer chromatography. The substance with an R_f value of 0.43 was eluted in the fractions of tube Nos. 39—51. These fractions were combined and evaporated to give a solid, which was then recrystallized from chloroform - ether to afford XVI; yield, 71 mg (66%); mp 142— 143°C, $[\alpha]_D^{12}$ +45° (*c* 0.5, chloroform); IR spectrum (KBr disk): 3300 (νNH), 1755 (ester), 1665 (Amide I), 1550 (Amide II), 1230 cm^{-1}.

Found: C, 49.57; H, 6.09; N, 5.97%. Calcd for $C_{28}H_{41}O_{16}N_3$: C, 49.77; H, 6.12; N, 6.22%.

1′, 6, 6′ - Triacetamido - 1′, 6, 6′ - trideoxysucrose (XV) from 1′, 6, 6′-Triacetamido-2, 3, 3′, 4, 4′-penta-*O*-acetyl-1′, 6, 6′-trideoxysucrose (XVI). A sample (50 mg) of XVI was de-*O*-acetylated with methanolic sodium methoxide as has been described in the synthesis of V. On thin-layer chromatography with Solvent D, the product obtained showed a single spot with an R_f value of 0.30, proving it to be identical with the sample obtained from XIV. Recrystallization from methanol-acetone yielded 30 mg (87%) of a pure product; mp 84—86°C (decomp.), $[\alpha]_D^{12}$ +73° (*c* 1.0, water).

The Periodate Oxidation of VII and XV. An accurately-weighed sample (25 mg) of VII or XV was dissolved in a mixture of a 0.1 N sodium acetate buffer solution (pH 4.7, 10 m*l*) and a 0.1 M sodium metaperiodate solution (3.21 m*l*); the oxidation was then carried out at 28°C in the dark. Iodometric titrations with sodium arsenite18) showed that both VII and XV consumed about 3 mol of periodate per mole in 24 hr (Table 1).

TABLE 1. PERIODATE OXIDATION OF VII AND XV

Reaction time hr	Moles periodate per mole	
	VII	XV
0.5	1.20	1.25
3.0	2.00	2.05
5.0	2.35	2.60
6.5	2.65	—
7.5	—	3.00
9.0	2.80	3.10
24.0	2.93	3.20

6, 6′-Diamino-6, 6′-dideoxytrehalose (XIX). The starting material, 2, 2′, 3, 3′, 4, 4′-hexa-*O*-acetyl-6, 6′-di-*O*-tosyltrehalose (XVIII), was prepared from trehalose *via* the 2, 2′, 3, 3′, 4, 4′-hexa-*O*-acetyl-6, 6′ - di - *O* - trityl intermediate by the method of Bredereck16); mp 169—172°C, $[\alpha]_D^{20}$ +134.6° (*c* 2, chloroform) [lit., mp 170—172°C, $[\alpha]_D^{20}$ +136.1° (*c* 2.4, chloroform)]. A solution of XVIII (760 mg) in dry methanol (35 m*l*) was saturated with dry ammonia at 0°C and then heated in an autoclave for 16 hr at 120°C. After cooling, the dark liquid was heated with carbon and filtered, and the filtrate was evaporated. On paper chromatography with Solvent F, the crude product obtained showed virtually only one spot with an R_f value of 0.10. An aqueous solution of the product was then passed through a column (10×300 mm) of Dowex 1×2 (OH form). The ninhydrin-positive eluate was neutralized with hydrochloric acid to pH 2 and evaporated to give a solid, which was recrystallized from methanol-acetone to afford a hygroscopic crystalline dihydrochloride monohydrate of XIX; yield, 190 mg. (58%); mp 82—83°C

[*2] R_{f6AD} is an R_f value relative to the R_f value of 6AD,5) taken as 1.0.

18) J. M. Bobbitt, "Advances in Carbohydrate Chemistry," Vol. 11, Academic Press, New York (1956), pp. 1—14.

(decomp.), $[\alpha]_D^{12}$ +167° (c 1, water); IR spectrum (KBr disk): 3400, 3260, 2900, 1600 ($\delta_{as}NH_3^+$), 1500 (δ_s NH_3^+), 1125—960 cm^{-1}.

Found: C, 35.52; H, 6.68; N, 6.31%. Calcd for $C_{12}H_{24}O_9N_2\cdot 2HCl\cdot H_2O$: C, 33.42; H, 6.54; N, 6.50%.

A small quantity of XIX was heated with 3 N hydrochloric acid in the way used in the hydrolysis of VI. On paper chromatography with Solvent F, the hydrolyzate showed only a single spot corresponding to 6-amino-6-deoxy-D-glucose (R_f 0.14).

Preliminary Bioassay. Compounds VI, XIV, and XIX did not show any antibacterial activity against *M. pyogenes var. aureus* 209p, *E. coli* and *Mycobacterium tuberculosis* 607 in a dilution of 1 : 1000. The structural similarity of VI and 6AD[5] did not reflect any biochemical similarities. This finding suggests that the unique structure of the deoxystreptamine moiety plays an important role in the antibacterial activity.

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