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THE PREPARATION AND OXYAMINATION OF 4,6-DI-*O*-ACETYL-2,3-DIDEOXY-α-D-*erythro*-HEX-2-ENOPYRANOSYL 4,6-DI-*O*-ACETYL-2,3-DIDEOXY-α-D-*erythro*-HEX-2-ENOPYRANOSIDE. SYNTHESIS OF TWO NEW DIAMINO DERIVATIVES OF α-D-MANNOPYRANOSYL α-D-MANNOPYRANOSIDE

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ABSTRACT

4,6-Di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranose (4,6-di-*O*-acetyl-D-pseudoglucal), prepared from 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (tri-*O*-acetyl-D-glucal), was condensed, by catalysis with boron trifluoride, with an equivalent of the original glycal, to give crystalline 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside in 40–50% yield. The dienic disaccharide was *cis*-oxyaminated with chloramine-T in the presence of osmium tetraoxide, furnishing in 72% yield an ~2:3.5:1 mixture of the 2,2′-dideoxy-2,2′-di-(*p*-toluenesulfonamido), 2,3′-dideoxy-2,3′-di-(*p*-toluenesulfonamido), and 3,3′-dideoxy-3,3′-di-(*p*-toluenesulfonamido) derivatives of α-D-mannopyranosyl α-D-mannopyranoside 4,6,4′,6′-tetraacetate. Separation of the mixture, achieved partly before and partly after *O*-deacetylation, was followed by high-yielding, reductive *N*-desulfonylations of the individual isomers with sodium in liquid ammonia, to give two new disaccharides, namely, 2-amino-2-deoxy-α-D-mannopyranosyl 2-amino-2-deoxy-α-D-mannopyranoside and its 2,3′-diamino-2,3′-dideoxy isomer, and the known 3,3′-diamino-3,3′-dideoxy isomer. The amino sugars were isolated as their dihydrochlorides, and characterized further by preparation of their di-*N*-acetyl derivatives.

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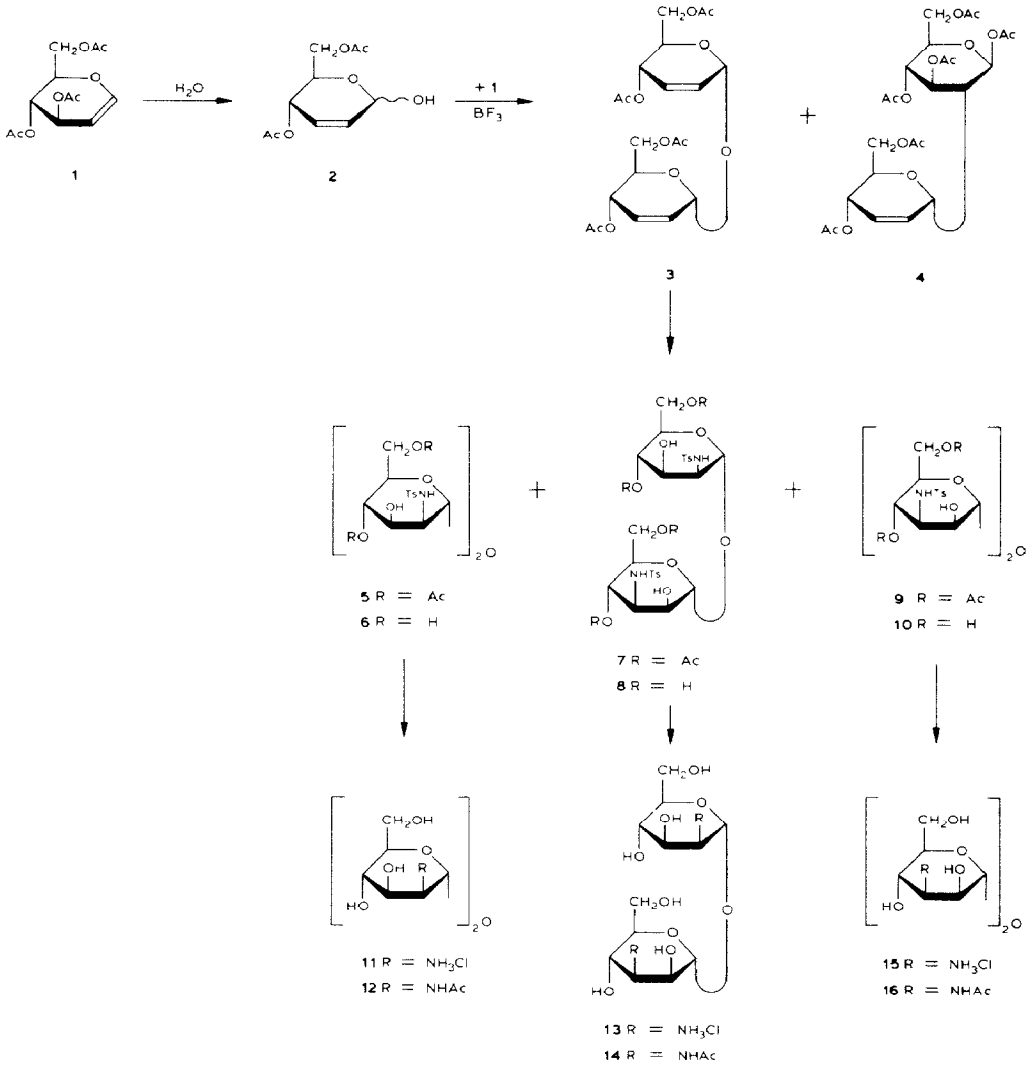
INTRODUCTION

Some aminated derivatives or analogs of α,α-trehalose are known to occur as metabolites of certain *Streptomyces* species, and to possess antibiotic activity[1,2]. They are trehalosamine (2-amino-2-deoxy-α,α-trehalose)[3] and its 4-amino-4-deoxy isomer[4], and 2-amino-2-deoxy-α-D-glucopyranosyl α-D-mannopyranoside[5]. It has been suggested[2] that the antimycobacterial activity displayed by trehalosamine may be due to a possible action of this compound as a competitive inhibitor for mycobacterial trehalase.

In order to provide additional, new substrates for studies addressing trehalase specificity, which one of us (J.D.) was pursuing[6], and to evaluate any an-



timycobacterial activities of synthetic analogs, we undertook some years ago the chemical synthesis of 3-amino-3-deoxy-α,α-trehalose[7] and[8] of the 3,3′-diamino-3,3′-dideoxy analogs having the D-*gluco*,D-*gluco*, D-*gluco*,D-*manno*, and D-*manno*,D-*manno* configurations. The 3-monoamino compound proved to be a substrate[7] for cockchafer trehalase, whereas the diamino derivatives showed[6] no affinity for this enzyme. However, the D-*manno*,D-*manno* diamine (**15**) was found to exhibit, *in vitro,* a remarkable inhibitory activity (at 10 μg/mL) against *Mycobacterium tuberculosis* (human strain H37RV) and *Mycobacterium avium**. Prompted by this observation, we have initiated the syntheses of further, related amino disaccharides, and we report here the preparation of two positional isomers of **15**, namely, the 2,2′-diamino analog **11** and the unsymmetrically substituted, 2,3′-diamino analog **13**.

RESULTS

One successful approach to 2- and 3-aminodeoxy mannopyranosides has been the application[9a–c] of the osmium tetraoxide-catalyzed *cis*-oxyamination of alkenes[10] to alkyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosides. The reaction furnished the corresponding 2- and 3-(*p*-toluenesulfonamido)-α-D-mannopyranoside derivatives in acceptable yields, and the products were subsequently *N*-desulfonylated (and simultaneously *O*-deacetylated) by sodium in liquid ammonia, to give the desired aminodeoxyglycosides. For use of this approach in the trehalose series, the symmetrical diene **3**, namely 4,6-di-*O*-acetyl-2,3-di-deoxy-α-D-*erythro*-hex-2-enopyranosyl 4,6-di-*O*-acetyl-α-D-*erythro*-hex-2-enopyranoside, was needed. In 1966, Lundt and Pedersen[11] had reported that action of hydrogen fluoride upon 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-*arabino*-hex-1-enitol (**1**, tri-*O*-acetyl-D-glucal) produced an unstable glycosyl fluoride which, on treatment with aqueous acetone, gave, among other products, a disaccharide (m.p. 68–72°, $[\alpha]_D$ +78°) in 30% crude yield, and they had assigned to it the constitution of **3**, but without determination of the interglycosidic configuration. We have now achieved a practical synthesis for **3**, and established the α,α configuration by catalytic hydrogenation that led to the known[12], crystalline 2,3,2′,3′-tetradeoxy-α,α-trehalose.

The disaccharide **3** arises as a major product, and can be isolated crystalline in 40–50% yield, when the glycal **1** and 4,6-di-*O*-acetyl-2,3-dideoxy-D-*erythro*-hex-2-enopyranose (**2**, di-*O*-acetyl-D-pseudoglucal) are allowed to interact, in the presence of boron trifluoride in benzene solution, under carefully controlled conditions. The pseudoglucal **2** to be used is first generated (in a preceding, separate operation) by treating half of the starting glucal **1** with water[13] at 80°, isolated, and then subjected to the Ferrier reaction[14a] with the second half of **1**. In this process,

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the known[14b] 2-deoxy-2-*C*-glycosyl-D-glucose **4** (m.p. 206°) is formed, by dimerization of **1**, as a second major product. Fortunately, **3** and **4** (and minor by-products) are readily separable by column chromatography, and we routinely perform the procedure on a 20-g scale for the preparation of **3**, despite the complexity* of the reactions involved.

Oxyamination of **3** afforded a mixture of *p*-toluenesulfonamido disaccharides in 82% yield. Column chromatography of the mixture permitted the removal of a small proportion (10%) of monoaminated, unsaturated material that resulted from incomplete reaction, and of other fast-moving contaminants. The main product (R_F 0.4) was isolated crystalline in 55% yield. Although it appeared chromatographically homogeneous, and showed invariant physical properties in several experiments, it proved to be a 1:2 mixture of positional isomers, namely, the 2,2′-dideoxy-2,2′-ditosylamido and 2,3′-dideoxy-2,3′-ditosylamido α-D-mannopyranosyl α-D-mannopyranoside 4,6,4′,6′-tetraacetates **5** and **7**, which we were unable to separate at this stage. From fractions containing slow-moving product (R_F 0.25), the crystalline 3,3′-dideoxy-3,3′-ditosylamido isomer **9** was isolated pure in 9–10% yield. Mixed fractions amounted to an additional 7% of diaminated products.

The mixture of **5** and **7** was quantitatively *O*-deacetylated with sodium methoxide, whereafter, separation of isomers became possible owing to the very low solubility, in acetone, of the minor component **6**, which was obtained in 34% yield. The preponderant isomer **8** was isolated in 57% yield. *O*-Deacetylation of **9** similarly gave **10**. The three deacetylated bis-sulfonamides were then reductively desulfonylated[15] with sodium in liquid ammonia. Compound **6** furnished the hitherto unknown 2-amino-2-deoxy-α-D-mannopyranosyl 2-amino-2-deoxy-α-D-mannopyranoside, isolated in 90% yield as its dihydrochloride **11**, and compound **8** gave, in 78% yield, the unsymmetrical 2,3′-diamino-2,3′-dideoxy isomer **13**, also not previously described. The known[8] 3,3′-diamino-3,3′-dideoxy isomer **15** was obtained from **10** in 76% yield. The three diamino sugars were further characterized by preparation of their bis-*N*-acetyl derivatives **12**, **14**, and **16**.

Structural assignment of the two new disaccharides rests firmly on spectroscopic and hydrolytic comparison with the known isomer. Thus, the ^{13}C-n.m.r. spectra of **11** and **15** were clearly distinct, each displaying a set of 6 signals, in conformity with the symmetrical disaccharide structures, whereas **13** gave 11 signals, one of which had double intensity and was due to C-6 and C-6′ (the only triplet present in the partially proton-decoupled spectrum). The spectrum of **13** represented a close superposition of the spectra of **11** and **15** (see Table I). The

*The presence, in the reaction mixture, of small proportions of one or both anomers of **3** (α,β and β,β) cannot be precluded, and other transformation products appear to be present as well. According to Ferrier and Prasad[14b], **1** is isomerized by boron trifluoride to give 1,4,6-tri-*O*-acetylpseudoglucal which, in the absence of alcohols, dimerizes to **4** (which was isolated) and the corresponding, anomeric 1-acetate (for which indirect evidence was obtained), moreover, 3,4,6-tri-*O*-acetyl-D-allal was presumed to be a minor product. We have now observed that **3**, too, is produced, besides **4** and other products, when **1** *alone* is treated with boron trifluoride.

TABLE I

^{13}C-N M R CHEMICAL SHIFTS[a] (p.p.m.) OF DIAMINO DISACCHARIDE DIHYDROCHLORIDES[b]

Compound	*C_a-1*	*C_b-1*	*C_a-2*	*C_b-2*	*C_a-3*	*C_b-3*	*C_a-4*	*C_b-4*	*C_a-5*	*C_b-5*	*$C_{a,b}$-6*
11	94.1		55.8		69.2		68.2		75.6		62.7
13	94.0	96.6	55.9	68.8	69.2	55.4	68.2	65.4	75.5	75.7	62.7
15		96.6		69.1		55.4		65.6		75.7	62.7

[a]From 20-MHz spectra, obtained with a Varian FT-80 instrument, of aqueous solutions standardized with reference to sodium 4,4-dimethyl-4-silapentane-1-sulfonate. [b]The C_a series refers to the 2-amino-2-deoxy moiety, and the C_b series, to the 3-amino-3-deoxy moiety.

TABLE II

^{1}H-N M R DATA FOR BIS-ACETAMIDO DISACCHARIDES[a]

Compound	*Chemical shifts (δ)*							*Coupling constants (Hz)*			
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6,6′*	*NAc*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
12	5.05d	4.375dd	4.09dd	3.65t	3.72dt[b]	3.835d[c]	2.04s	1.7	4.8	9.3	9.9
14[d,e]	5.065d	4.41dd	4.14dd	3.63t	←3.75–3.90m→		2.05s	1.7	4.7	9.4	9.2
	5.11d	3.92dd	4.20dd	3.71t			2.06s	1.5	3.2	9.9	9.9
16	5.13d	3.96dd	4.24dd	3.69t	←3.73–3.90m→		2.06s	1.8	3.0	9.7	9.7

[a]From 300-MHz spectra of solutions in D_2O containing sodium 4,4-dimethyl-4-silapentane-1-sulfonate as the internal standard. [b]A doublet (spaced 9.9 Hz) of two triplets (spaced 3 Hz). [c]Two equal, closely spaced singlets integrating for 2 protons. [d]Upper line, 2-acetamido moiety; lower line, 3-acetamido moiety. [e]In addition to the signals listed, **14** gave a 6-proton doublet (*J* 6.2 Hz) at δ 1.16, and a septet (*J* 6.2 Hz) centered at δ 4.00, for one molecule of 2-propanol of crystallization.

same was true for the ^{1}H-n.m.r. spectra of the acetamido derivatives, where all individual features for **12** and **16** occurred jointly for **14**, although the unequal, molecular moieties in the latter compound mutually caused minor differences in the signals, relative to the corresponding ones for **12** and **16** (see Table II). For example, the ring protons situated at the acetamidated positions were found to resonate at lowest field (next to the anomeric protons), and to give narrow doublets of doublets, at δ 4.375 (H-2,2′) and 4.41 (H-2), for **12** and **14**, respectively, and doublets of doublets, with one small and one large splitting, at δ 4.24 (H-3,3′) and 4.20 (H-3′), for **16** and **14**, respectively. Hydrolysis of the three acetamido disaccharides with hydrochloric acid gave 2-amino-2-deoxymannose (from **12** and **14**) and 3-amino-3-deoxymannose (from **14** and **16**), chromatographically identified by comparison with authentic samples.

It is interesting, finally, to note the regioselectivity observed in the oxyamination of the disaccharidic enoside **3**. The preparative ratios of 4:7:2 for the products **5, 7,** and **9** implies a preference by a factor of ~1.4 for amination at C-2. This constitutes a reversal of regioselectivity from monosaccharidic analogs previously *cis*-oxyaminated by the same method. Although the methyl glycoside analog of **3** was reported[9a] to give regioisomers in the ratio of 1:1, the ethyl glycoside was

preferentially aminated[9c] at C-3 (by a factor of 2.3), as were[9d] several methyl 2,3,4-trideoxy-α-DL-hex-2-enopyranosides variedly substituted at C-6 (by factors of 2.5–4.5).

EXPERIMENTAL

General methods. — Optical rotations were measured at ~25° with a Perkin–Elmer 241 polarimeter. Infrared spectra were recorded for Nujol mulls, unless otherwise indicated, with a Unicam SP 1100 or a Perkin–Elmer 783 spectrometer. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck AG, Germany), and the medium-pressure technique was usually employed for separations on a multigram scale. For t.l.c., glass plates or aluminum sheets precoated with silica gel (Macherey-Nagel & Co., or E. Merck AG) were used, and spots were made visible by spraying the plates with 5% sulfuric acid in ethanol, and heating them briefly. Unless otherwise stated, the following solvent combinations (v/v) were used for chromatography: (*A*) 3:1 ether–hexane, (*B*) 1:3 ethyl acetate–hexane, (*C*) the same solvents, but 3:2, (*D*) the same, but 2:1, (*E*) the same, but 3:1, (*F*) the same, but 4:1, (*G*) 1:4 methanol–chloroform, and (*H*) 2:1 methanol–ether.

*Preparation of 4,6-di-O-acetyl-2,3-dideoxy-α-*D-erythro-*hex-2-enopyranosyl 4,6-di-O-acetyl-2,3-dideoxy-α-*D-erythro-*hex-2-enopyranoside* (**3**). — *A. 4,6-Di-O-acetyl-2,3-dideoxy-*D-erythro-*hex-2-enopyranose* (**2**) *from 3,4,6-tri-O-acetyl-1,5-anhydro-2-deoxy-*D-arabino-*hex-1-enitol* (**1**). It was revealed by t.l.c. (solvent *A*) that the conversion of the glycal **1** (R_F 0.6) into the pseudoglucal **2** (R_F 0.4) by[13] boiling water during 15 min is accompanied by the formation of considerable proportions of more-slowly moving products. Their occurrence can be retarded, if not completely suppressed, by performing the reaction at lower temperature, although some of the glycal then remains unreacted. After several pilot experiments, the following procedure was adopted.

Crystalline **1** (9.0 g) was introduced into vigorously stirred water (450 mL) at 80 ±1°. The crystals melted at once, to give an oil that dissolved within minutes as the reaction progressed. After exactly 30 min, the flask was rapidly cooled under running tap-water. T.l.c. showed chiefly **2**, remnant **1** (~10%, visually estimated), and only traces of the slow-moving by-products. The cooled solution was freeze-dried, in 3 batches, when an efficient apparatus was available; otherwise, it was evaporated in a rotary evaporator at 30° (bath temperature), preferably also in 2 or 3 batches. The latter procedure tended to cause a slight increase in the proportion of by-products. The colorless oil obtained was dried by evaporation with several portions of added benzene, and the slow-moving contaminants, if present in larger than traces, were removed by passing the material through a short column (3.5 × 9 cm) of silica gel by means of 1:1 ether–benzene. For the present purposes, it was unnecessary to remove unconsumed **1**, although this can be done by chromatography.

Thus, a small-scale experiment with **1** (1.0 g) gave **1** (120 mg, recovered crystalline), **2** (720 mg, 85%), and mixed fractions (~40 mg) containing mainly slow-moving products. Chromatographically pure **2** had $[\alpha]_D$ +107.2° (*c* 2.2, chloroform); ν_{max} 3450 (broad, OH) and 1740 cm^{-1} (CO), with the sharp band at 1650 cm^{-1} that is characteristic for **1** being absent; ^{1}H-n.m.r. data (in $CDCl_3$): δ 5.90 (m, H-2,3), 5.45 (nm, H-1), 5.30 (m, H-4), 4.25–4.15 (m, H-5,6,6'), and 2.10 (s, OAc).

B. Condensation of **2** *with* **1**. The syrupy **2** obtained as just described from 9.0 g of **1** (and still containing a small proportion thereof) was dissolved in reagent-grade benzene (60 mL), and the solution was dried with molecular sieve 4A, filtered, and chilled in an ice–water bath to ~5°. A separate solution of **1** (8.0 g) in dry benzene (80 mL) was similarly cooled, and freshly distilled boron trifluoride etherate (2 mL) dissolved in dry, chilled benzene (30 mL) was introduced, with swirling, through a paper filter. *Immediately* after the addition of the catalyst to the solution of **1**, the solution of **2** was added dropwise, but rapidly, during the course of 3 min, and with magnetic stirring. After a further 15 min, the flask was taken from the ice bath and swirled in lukewarm water for 5 min, in order to warm the contents to 20°. (Observance of the stated time periods and temperatures is crucial.) The reaction was then immediately quenched by stirring the dark-violet solution with a fairly large amount of anhydrous potassium carbonate (finely pulverized in a mortar). The violet color was abruptly discharged after ~10 min of efficient stirring, giving way to an orange coloration. Stirring was continued for 5 min or more, until moist indicator paper proved the supernatant solution to be neutral. The salt was filtered off, and successively washed exhaustively with benzene and ether. The filtrate and washings were combined, treated with activated charcoal, the suspension filtered, and the filtrate evaporated, to give a honey-colored syrup. T.l.c. (solvent *A*) showed a weak spot for **1** (R_F 0.6), accompanied by another weak spot, immediately below it (possibly due to tri-*O*-acetylpseudoglucal or tri-*O*-acetyl-D-allal), a strong spot for **3** (R_F 0.4; compound **2** has the same mobility), a medium-strong spot for **4** (R_F 0.25), and a weak spot at R_F 0.2 (possibly the anomer of **4**). It was feasible to isolate **3** from this crude mixture of products by direct crystallization from ether–hexane, with the aid of seed crystals. In one experiment, for instance, impure crystals (7.8 g) were deposited after 3 days at 0°, but the necessary recrystallizations inordinately decreased the yield of pure **3**. It proved more advantageous to subject the entire material to column chromatography on silica gel (230 g), with solvent *B* as the eluant. The fast-moving material emerging first amounted to ~250 mg. Fractions containing exclusively, or mainly, the disaccharide **3** gave crystalline material on evaporation. One or two recrystallizations of individual batches from 2-propanol, sometimes with the addition of a little hexane, or from aqueous ethanol, gave pure **3** totalling 6.05 g (43.8%); m.p. 68–69°, 69–70°, or 73.5–74° (depending on the mode of recrystallization) (lit.[11] m.p. 68–72°), $[\alpha]_D$ +79.4° (*c* 2.2, chloroform), lit.[11] $[\alpha]_D$ +78°; ^{1}H-n.m.r. data (250 MHz, $CDCl_3$): δ 5.94 (dd, $J_{2,3}$ 10.2, $J_{3,4}$ 1.6 Hz, H-3), 5.81 (ddd, $J_{1,2}$ 2.7, $J_{2,4}$ −2

Hz, H-2), 5.44 (nm, H-1), 5.33 (ddd, $J_{4,5}$ 9.7 Hz), 4.21 (m, H-6,6'), 4.10 (m, H-5), and 2.08 (s, OAc).

Anal. Calc. for $C_{20}H_{26}O_{11}$ (442.4): C, 54.29; H, 5.92. Found: C, 54.44; H, 6.03.

A similar experiment, using **2** (prepared from 2.5 g of **1**) and **1** (2.5 g) in a fully analogous manner, but with proportionately less catalyst (0.2 mL of boron trifluoride etherate), yielded 1.76 g of pure **3** (43.3%). In this instance, a relatively significant amount of unconsumed **1** (828 mg) was recovered crystalline by chromatography. Based on the consumed **1**, the yield of **3** was, therefore, 51.8%.

In some cases, chromatographic fractions of **3** showed a marginally slower-moving, satellite spot, which may have been due to an anomer of **3**. This contaminant was removable by recrystallization.

Continued elution of the columns produced the slow-moving material; it usually showed a double spot in t.l.c. (R_F 0.2–0.25). The dimer **4** crystallized directly, as long needles, from the eluates on standing, or was isolated by evaporation, followed by trituration of the residue with methanol; double m.p. 194–196°, 206–207°, as reported[14b]. The i.r. spectra of **3** and **4** (for Nujol mulls) show characteristic differences in band positions and intensities at 1150–1050, 1000–900, and 750 cm^{-1}. The second, slow-moving component present in the mother liquors of **4** was not obtained crystalline.

C. Hydrogenation of **3**. Pure **3** (221 mg, 0.5 mmol) in methanol (10 mL) was hydrogenated in the presence of 10% palladium-on-carbon (100 mg). Hydrogen uptake (26 mL) was complete within 15 min. The tetrahydro derivative showed the same mobility as **3** in t.l.c. (solvent *A*), but the spots had different colors (yellowish brown *vs.* black). The catalyst was filtered off, and washed well with methanol, and, by evaporation, the product was obtained as a colorless syrup (214 mg, 96%) that was subsequently *O*-deacetylated with 0.1M sodium methoxide solution at 25°. De-ionization, filtration through a layer of Celite, and evaporation of the filtrate gave 2,3,2',3'-tetradeoxy-α,α-trehalose, which crystallized on trituration with 2-propanol; yield, 100 mg (72%); m.p. 163–165°, $[\alpha]_D$ +199.8° (*c* 3.3, ethanol); lit.[12] m.p. 165–166°, $[\alpha]_D$ +201°. From *moist* 2-propanol, the compound crystallized as a hydrate, double m.p. 151°, 163–165°; ^{13}C-n.m.r. data (H_2O): δ 93.7 (C-1), 76.3 (C-4), 67.7 (C-5), 63.4 (C-6), and 30.4 and 28.4 (C-2,3).

Oxyamination of **3**. — To a solution of **3** (7.00 g, 15.8 mmol) in *tert*-butyl alcohol (200 mL) at 50° was added freshly prepared chloramine-T (13.4 g, 47.7 mmol) followed by silver nitrate (10.0 g), and the mixture was stirred for 30 min at 40°. A 0.08M solution of osmium tetraoxide in alkene-free hexane (4.5 mL) was then added, and stirring was continued for 22 h at 40°; compound **3** (R_F 0.8) was then absent, and several, slower-moving products were present, with the strongest spot having R_F ~0.4 (t.l.c., solvent *E*). Sodium hydrogensulfite (3.5 g) in water (115 mL) was added, and the mixture was stirred for 1.5 h at 40°, and then filtered through a layer of Celite. The greyish residue on the filter was successively washed with *tert*-butyl alcohol and ethyl acetate, and further extracted by repeated suspen-

sion in fresh ethyl acetate. The organic filtrates were combined, and evaporated, to give a pale-yellow foam. The foam was dissolved in ethyl acetate (250 mL), and the solution was washed once with water and twice with brine, dried (Na_2SO_4), treated with activated charcoal, the suspension filtered, and the filtrate evaporated. The beige-colored, solid residue (~15 g) was chromatographed under medium pressure on a column of silica gel (250 g), by means of solvent *C* as the eluant. Early fractions (A) contained *p*-toluenesulfonamide, identified after crystallization. These were followed by fractions B (combined dry-weight, 1.4 g) containing more of the amide, and two carbohydrate derivatives, R_F 0.6 and 0.5 (t.l.c. with solvent *E*), as well as traces of faster-moving impurities. Subsequent, chromatographically homogeneous fractions (C, R_F 0.4) furnished the bulk (5.92 g) of reaction product, which consisted of the two inseparable, isomeric bis-tosylamido disaccharide tetraacetates **5** and **7**. Fractions (D) that followed contained mixtures of **5, 7,** and the isomer **9** (R_F 0.25). Final elution, with pure ethyl acetate, produced fractions (E, 0.38 g) that contained traces of **9** only, and consisted mainly of slower-moving by-products lacking a tosylamido group (n.m.r.).

Fraction D was rechromatographed on the same column, yielding a second crop (1.04 g) of pure (**5** + **7**), and a mixture (F) of **5, 7,** and **9**. Trituration of F with a small amount of ethyl acetate caused separation of pure, crystalline **9** (1.15 g). The mother liquor there from was chromatographed on a small column of silica gel (40 g) with solvent *D*, giving pure crops of (**5** + **7**) (0.16 g) and **9** (0.10 g), and mixtures of the three compounds (0.90 g, 7%) that were not processed further. Isolated yields were, therefore, 7.12 g (55%) of pure (**5** + **7**), and 1.25 g (9.7%) of pure **9**.

Both (**5** + **7**) and **9** showed 60-MHz, ^{1}H-n.m.r. signals at δ 7.4 (AB-q, arom.), 2.4 (Me of Ts), and 2.0 (OAc) in the intensity ratios of 4:3:6, and broad absorption at δ ~6.0 (N–H) which disappeared on deuterium exchange. The i.r. spectra, very well resolved for **9**, and less so for (**5** + **7**), were, overall, quite similar; characteristic patterns were seen in the OH, NH stretch region [for (**5** + **7**): two broad bands, at 3470 and 3260 cm^{-1}; for **9**: three bands, at 3470 (with a pronounced shoulder at 3570), 3300, and 3220 cm^{-1}], and at 910 cm^{-1}, where **9** had a distinctive band that was not given by the isomers.

The crystalline mixtures (**5** + **7**) obtained from several experiments melted in the range 105–115° and had $[\alpha]_D$ +40.8° ±0.5° (*c* 1.2, acetone). Compound **9** showed slight sintering at 246°, and m.p. 250–253° (dec.), $[\alpha]_D$ +107° (*c* 0.7, acetone).

Anal. Calc. for $C_{34}H_{44}N_2O_{17}S_2$ (816.8): C, 49.99; H, 5.43; N, 3.43; S, 7.85. Found for (**5** + **7**): C, 49.88; H, 5.55; N, 3.29; S, 7.69. Found for **9**: C, 49.87; H, 5.25; N, 3.24; S, 7.66.

The foregoing fraction B crystallized, in part, on standing. Recrystallization from 1,2-dichloroethane–carbon tetrachloride gave *p*-toluenesulfonamide (300 mg). The material remaining in the mother liquor could not be crystallized. Inspec-

tion by n.m.r. and i.r. spectroscopy, and a positive Baeyer test, suggested the presence of unsaturated, mono-oxyaminated products (1.0 g, 10%).

*2-Deoxy-2-(*p*-toluenesulfonamido)-α-*D*-mannopyranosyl 2-deoxy-2-(*p*-toluenesulfonamido-α-*D*-mannopyranoside* (**6**) *and 2-deoxy-2-(*p*-toluenesulfonamido)-α-*D*-mannopyranosyl 3-deoxy-3-(*p*-toluenesulfonamido)-α-*D*-mannopyranoside* (**8**). — Addition of sodium methoxide (950 mg) to a solution of the crystalline mixture (**5** + **7**) (6.50 g) in absolute methanol (65 mL) effected complete *O*-deacetylation within 15 min, as indicated by t.l.c. (solvent *F*), which showed a single, virtually immobile spot. De-ionization with a cation-exchange resin, filtration, and evaporation of the filtrate, gave a white solid (5.16 g after drying, 100%). Brief trituration of the solid with boiling acetone (20 mL) caused partial dissolution. After storage of the suspension overnight at 0°, the white crystals (**6**) were isolated, washed with cold acetone, and dried in a desiccator; yield, 1.77 g (34.3%); m.p. 238–240° (dec.); R_F ~0.45 (solvent *G*). A faint trace of a more-mobile impurity was removed from an analytical sample by recrystallization from a large proportion of hot methanol (in which **6** is sparingly soluble) or, better, from 2-propanol–water, which raised the m.p. to 242° (dec.); $[\alpha]_D$ +40° (*c* 0.4, methanol).

Anal. Calc. for $C_{26}H_{36}N_2O_{13}S_2$ (648.7): C, 48.14; H, 5.59; S, 9.89. Found: C, 47.97; H, 5.45; S, 9.77.

The acetone mother-liquor from the foregoing isolation of **6** was evaporated, to give a crystalline residue consisting of crude **8**, R_F ~0.45 (like **6**), contaminated by small proportions of faster-moving by-products. The material was readily soluble in small volumes of cold acetone or methanol, and no additional **6** could be induced to crystallize on seeding and refrigeration. Compound **8** was freed of the accompanying, faster-moving impurities by passage through a column of silica gel (100 g), with solvent *G* as the eluant, and was so obtained as a colorless solid (3.05 g, 56.7%); $[\alpha]_D$ +59° (*c* 0.9, methanol). It did not show a distinct melting-point, but turned into a glassy foam at 140–150°, probably because of the presence of water of crystallization, even though the sample had been dried *in vacuo* at 80°.

Anal. Calc. for $C_{26}H_{36}N_2O_{13}S_2 \cdot 1.5\ H_2O$ (675.7): C, 46.21; H, 5.82; S, 9.49. Found: C, 46.35; H, 5.75; S, 9.36.

Whereas **6** showed distinct i.r. bands at 3440, 3350, 3290 (weak), and 3250 cm^{-1}, compound **8** had broad absorption in that region. The fingerprint region for **6** was also characterized by sharp peaks, whereas broadened bands occurred for the unsymmetrical isomer **8**.

*3-Deoxy-3-(*p*-toluenesulfonamido)-α-*D*-mannopyranosyl 3-deoxy-3-(*p*-toluenesulfonamido)-α-*D*-mannopyranoside* (**10**). — Sodium methoxide (70 mg) was added to a suspension of tetraacetate **9** (490 mg) in methanol (10 mL), whereupon **9** dissolved almost instantly. Processing after 15 min, as described for **6** and **8**, furnished **10**, which crystallized completely on trituration with ether that contained a little ethanol; yield, 381 mg (98%); $[\alpha]_D$ +89° (*c* 0.5, methanol). Judging from the i.r. spectrum, which resembled that of **8**, and from the melting behavior (sintering, with foaming, at 145–155°), the compound appears to have contained

some water of crystallization. The analysis afforded no clear distinction between anhydrous compound and hemihydrate.

Anal. Calc. for $C_{26}H_{36}N_2O_{13}S_2$ (648.7): C, 48.14; H, 5.59; S, 9.89. Calc. for hemihydrate (657.7): C, 47.46; H, 5.67; S, 9.75. Found: C, 47.89; H, 5.78; S, 9.71.

2-Amino-2-deoxy-α-D-mannopyranosyl 2-amino-2-deoxy-α-D-mannopyranoside dihydrochloride (**11**). — Compound **6** (1.25 g) was dissolved in liquid ammonia (~80 mL), and sodium (500 mg) was added in small pieces, with efficient magnetic stirring, the cooling-bath temperature being kept at −50°. The blue color of the mixture tended to change, through green to yellowish, after ~15 min, and was restored by the addition of sodium (100 mg). The reaction was quenched after 40 min by the introduction of ammonium chloride (800 mg), with which the mixture was stirred until it had turned completely white (~15 min). The bath was removed, and the ammonia was allowed to evaporate in a stream of nitrogen; the last traces were removed in a rotary evaporator at 25°. The dry, powdery residue was eluted with several portions of warm methanol, and salt (0.5 g) that remained undissolved was discarded. The extract was diluted with water, and stirred with a weak-acid, cation-exchange resin [Bio Rex-70 (H^+) or Amberlite IRC-50 (H^+)] until it showed pH ~6. The resin was filtered off, and washed exhaustively with water. The filtrate was stirred with a strong-base, anion-exchange resin [Dowex-1 X8 (OH^-)], which rendered the solution alkaline again. The filtrate therefrom was treated with a second batch of the acidic resin until neutral, and then subjected to a further, sequential treatment with the two types of resin. At the end, a test for chloride ion ($AgNO_3$) was negative, or only very faintly positive, and practically no amino sugar remained in the solution, as was verified by t.l.c. (methanol; check for spot near origin). The combined batches of weak-acid resin, after exhaustive washing, were successively eluted with three portions of 15% aqueous ammonia, and the eluates were evaporated under diminished pressure, to give a glassy, slightly brownish residue. The product was dissolved in water, clarified with activated charcoal, the suspension filtered through a layer of Celite, and the filtrate carefully acidified to pH 4 (indicator paper) by titration with M hydrochloric acid. Concentration of the solution to a small volume, addition of 2-propanol, and careful trituration of the resulting precipitate, followed by evaporation of the alcohol, gave **11** as an off-white powder that was dried over potassium hydroxide in a desiccator. The product (750 mg, 94%) was purified by treatment, in aqueous solution, with a small amount of activated charcoal, and recovered by evaporation with added 2-propanol; yield, 717 mg (90%); $[\alpha]_D$ +69.5° (*c* 1, water); decomposition, with gradual darkening, above 190°, and extensive charring near 235°; ν_{max} 1600 cm^{-1} (NH_3^+); for ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{12}H_{26}Cl_2N_2O_9$ (413.3): C, 34.87; H, 6.34; Cl, 17.15. Found: C, 34.68; H, 6.60; Cl, 16.90.

2-Acetamido-2-deoxy-α-D-mannopyranosyl 2-acetamido-2-deoxy-α-D-mannopyranoside (**12**). — Dihydrochloride **11** (100 mg) in methanol (4 mL) was treated with acetic anhydride (0.15 mL) at room temperature, in the presence of Dowex-1

X8 (OH^-) resin. After 15 min, t.l.c. with solvent *H* showed **12** as a single spot, R_F 0.5. The suspension was filtered, the filtrate treated with a small amount of Dowex-50 (H^+) resin, and evaporated with added portions of 2-propanol, to give crystalline **12** (90 mg, 88%); $[\alpha]_D$ +59.5° (*c* 0.5, water); decomposition without melting, above 230°; ν_{max} 1650 and 1550 cm^{-1} (amide I and II); for ^{1}H-n.m.r. data, see Table II.

Anal. Calc. for $C_{16}H_{28}N_2O_{11}$ (424.4): C, 45.28; H, 6.65. Found: C, 44.93; H, 7.20.

*2-Amino-2-deoxy-α-*D*-mannopyranosyl 3-amino-3-deoxy-α-*D*-mannopyranoside dihydrochloride* (**13**). — Compound **8** (3.00 g) in liquid ammonia (200 mL) was *N*-desulfonylated with sodium (1.6 g) during 45 min at −50°. Processing of the reaction mixture (with 1.5 g of ammonium chloride), and isolation of the product, were performed exactly as detailed for the preparation of **11**, except that the aqueous solution of the isolated, crude amino sugar was, after its acidification to pH 3.5, extracted twice with ether in order to remove a u.v.-active, non-carbohydrate impurity (presumably *p*-toluenesulfinic acid). Continued processing as for **11** then furnished **13** as a white powder (1.50 g, 78%). Dried *in vacuo* over potassium hydroxide, it had an analysis corresponding to that for a monohydrate; $[\alpha]_D$ +81° (*c* 1.7, water) corresponding to +84.5° for anhydrous **13**; ν_{max} 1600 cm^{-1} (NH_3^+); for ^{13}C-n.m.r. data, see Table I. Heating of **13** caused foaming (~150°), and gradual decomposition.

Anal. Calc. for $C_{12}H_{26}Cl_2N_2O_9 \cdot H_2O$ (431.3): C, 33.42; H, 6.56; Cl, 16.44. Found: C, 33.56; H, 6.35; Cl, 16.53.

*2-Acetamido-2-deoxy-α-*D*-mannopyranosyl 3-acetamido-3-deoxy-α-*D*-mannopyranoside* (**14**). — A sample of **13** (90 mg) was *N*-acetylated as described for **11**, yielding **14**, which crystallized from 2-propanol as a monosolvate (82 mg, 81%); R_F 0.55 (solvent *H*); ν_{max} 1650 and 1540 cm^{-1} (amide I and II); $[\alpha]_D$ +66° (*c* 0.8, water, for monosolvate) and +75.4° (*c* 0.9, water, for unsolvated sample); for ^{1}H-n.m.r. data, see Table II. Heating of **14** caused foaming (130–160°), followed by gradual browning.

Anal. Calc. for $C_{16}H_{28}N_2O_{11} \cdot C_3H_8O$ (484.5): C, 47.10; H, 7.49; N, 5.78. Found: C, 47.00; H, 7.59; N, 5.85.

*3-Amino-3-deoxy-α-*D*-mannopyranosyl 3-amino-3-deoxy-α-*D*-mannopyranoside dihydrochloride* (**15**). — Compound **10** (330 mg) was *N*-desulfonylated with sodium (160 mg) in liquid ammonia (50 mL) at −50°, as described for the preparation of **11**, and processing with ion-exchange resins was performed analogously. The aqueous, ammoniacal eluate from the weak-acid resin was evaporated, to give a syrup, which was dissolved in a small amount of methanol. After freeing the solution of a small quantity of insoluble material, it was evaporated with several portions of added ethanol, to give the free diamino sugar as a white powder (155 mg). For conversion into the dihydrochloride, the base was suspended in methanol at 0°, and chilled ether that contained anhydrous hydrogen chloride was added dropwise until acidity (toward indicator paper) persisted. At this point, all of the solid dissolved. Slow addition of an excess of pure ether then caused precipitation of **15**,

which was immediately isolated, washed with ether, and dried over potassium hydroxide in a desiccator; yield, 172 mg (76.4%, calc. as **15**-dihydrate from **10**-hemihydrate); decomposition range, 190–200°; $[\alpha]_D$ +82.4° (*c* 1, water); lit.[8] for dihydrate, dec. 200–202° and $[\alpha]_D$ +84.3°. The ^{13}C-n.m.r. data (see Table I) and the i.r. spectrum indicated identity of **15** with an authentic sample thereof.

3-Acetamido-3-deoxy-α-D-mannopyranosyl 3-acetamido-3-deoxy-α-D-mannopyranoside (**16**). — A sample of **15** (75 mg) was *N*-acetylated as described for **11**, yielding **16** (58 mg, 82%), which readily crystallized upon evaporation of its aqueous solution; R_F ~0.5, marginally lower than for **12** (solvent *H*); $[\alpha]_D$ +73° (*c* 0.9, water); ν_{max} 1660, 1640 and 1580, 1560 cm^{-1} (2 doublets, amide I and II); decomposition without melting, 210–250°. For ^1H-n.m.r. data, see Table II.

Anal. Calc. for $C_{16}H_{28}N_2O_{11}$ (424.4): C, 45.28; H, 6.65; N, 6.60. Found: C, 45.24; H, 6.56; N, 6.07.

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