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Note

The synthesis of methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside and derivatives*†

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Formation of a 3,6-anhydrohexose by alkali treatment of a hexose 6-sulfate has been commonly used to ascertain the position of the sulfate group at C-6 of the D-galactose residue of algal polysaccharides[2]. More recently, this procedure has been applied to glycosaminoglycuronoglycan sulfates, such as keratan sulfate, heparin, and heparan sulfate[3], where the sulfate ester group may be located at C-6 of the 2-acetamido-2-deoxy-D-glucose residues. Numerous glycoproteins containing sulfated sugar residues have been isolated, but very little is known of their chemical structure[4]. In order to ascertain the location of the sulfate group in 2-acetamido-2-deoxy-D-glucose residues of a purified glycoprotein obtained from a bronchial-sputum mucin, the glycoprotein was treated with alkali and then methanolyzed to give mainly methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside with traces of the β-D anomer[1]. For identification and quantitative determination, methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**4**) was synthesized and characterized by crystalline derivatives, and its separation from other components studied by combined g.l.c.–m.s.

Selective tosylation[5] of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**) at −10° afforded, in 53% yield, crystalline methyl 2-acetamido-2-deoxy-6-*O*-tosyl-

*Dedicated to Dr. Horace S. Isbell, in honor of his 75th birthday.

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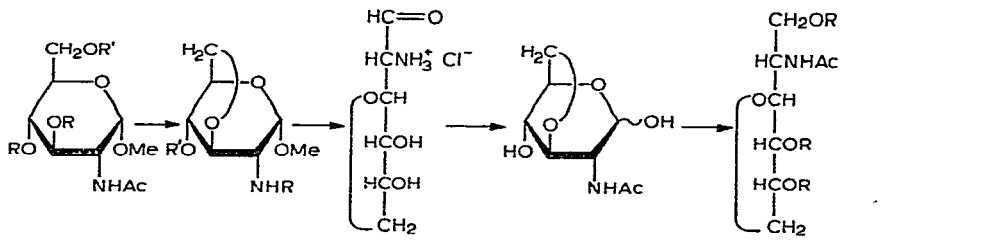

1 R=R'=H 4 R=Ac, R'=H 7 8 9 R=H
2 R=H, R'=Ts 5 R=R'=H 10 R=COC$_6$H$_4$N=NC$_6$H$_5$
3 R=Ac, R'=Ts 6 R=R'=Ac 11 R=Ac

α-D-glucopyranoside (**2**), and a small amount of the known methyl 2-acetamido-2-deoxy-3,6-di-*O*-tosyl-α-D-glucopyranoside[6]. The 6-sulfonate (**2**) was converted into crystalline methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**4**) by treatment with sodium hydroxide in ethanol, followed by selective *N*-acetylation, since the reaction mixture contained some methyl 2-amino-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**5**). Compound **5** moved much more slowly than **4** on t.l.c and gave the characteristic red color for anhydro compounds with anisaldehyde, but, in contrast to **4**, gave a positive ninhydrin reaction. Compound **4** was further characterized as the crystalline 4-acetate (**6**), and the structures assigned to **4** and **6** were supported by the mass-spectral data, which showed the fragmentation patterns characteristic of 3,6-anhydro sugar derivatives[7].

Hydrolysis of methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**4**) afforded crystalline 2-amino-3,6-anhydro-2-deoxy-D-glucose hydrochloride (**7**), the properties of which indicate the *aldehydo* form, as previously observed for other 3,6-anhydro sugars[8] when cyclization causes a strained, pyranoid ring. On the amino acid analyzer, **7** gave a single peak, located exactly between those of 2-amino-2-deoxy-D-glucose and ammonia, as observed earlier by Sampson and Meyer[3]. G.l.c.[9] of the per(trimethylsilyl) derivative (**13**) of **7** shows a major peak, probably the α-D anomer, followed by a very small peak (probably the β-D anomer) that was not investigated. The mass spectrum of **13** was consistent with the structure proposed. Since crystalline **7** decomposes within a few hours at room temperature, it was immediately converted into the *N*-acetyl derivative (**8**), which was not purified, but the structure of which was supported by m.s. of the per(trimethylsilyl) derivative (**14**). Two distinct peaks were observed on g.l.c., the first and major one corresponding to the α-D anomer of **14** and the second one probably to the β-D anomer, since the mass spectra of both compounds were almost identical. Reduction of **8** with sodium borohydride in borate buffer gave syrupy 2-acetamido-3,6-anhydro-2-deoxy-D-glucitol (**9**), a standard for the study of hexosamine-containing polymers by g.l.c.–m.s., since alditol acetates give a fragmentation pattern simpler than that of cyclic sugars[10]. The alditol **9** was characterized as crystalline tri-*O*-(*p*-phenylazobenzoyl) (**10**) and tri-*O*-acetyl (**11**) derivatives. The latter compound and the per(trimethylsilyl) derivative (**15**) of **9** show fragmentation patterns by m.s. that are in agreement with the structures proposed, and the mass

spectrum of **11** is different from that of 2-acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-D-glucitol.

The elution data in g.l.c. of the glycoside **4**, as the 4-*O*-(trimethylsilyl) derivative (**12**) or as the 4-acetate (**6**), and of the alditol acetate **11**, and those of the hydrochloride **7** in the amino acid analyzer, as well as the m.s. data of **4**, as the trimethylsilyl ether (Fig. 1) or acetate (Fig. 2), and of **11** (Fig. 3) allow an easy identification and

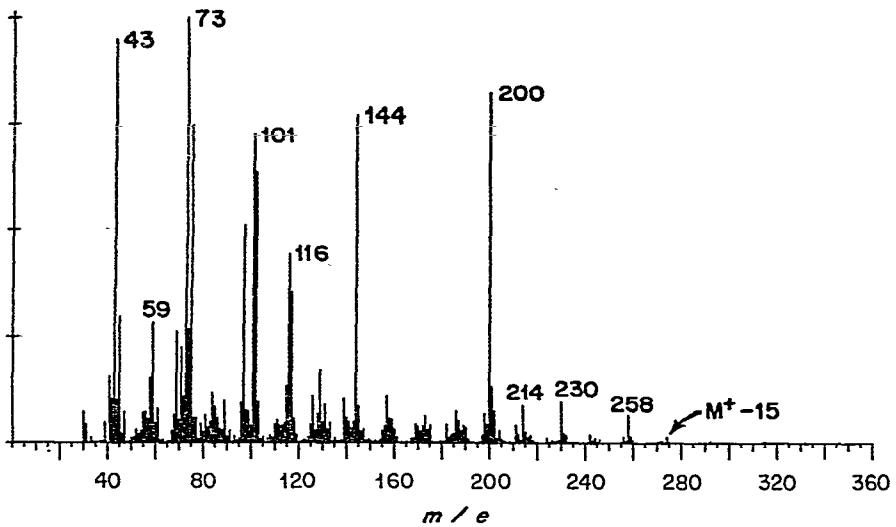

Fig. 1. Mass spectrum of methyl 2-acetamido-3,6-anhydro-2-deoxy-4-*O*-trimethylsilyl-α-D-glucopyranoside (**12**).

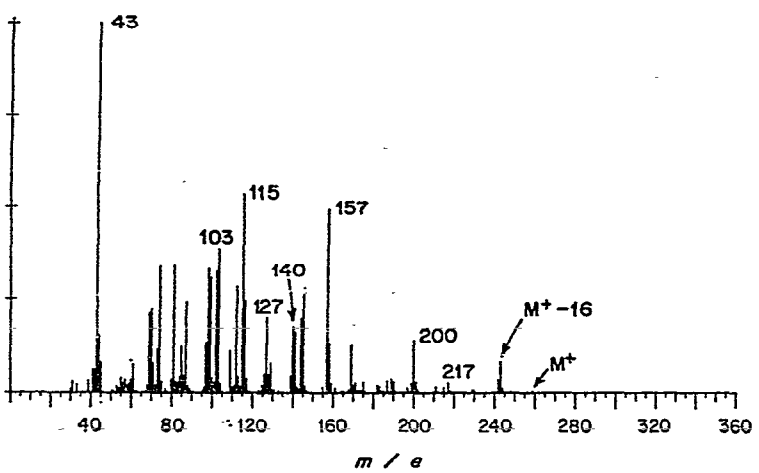

Fig. 2. Mass spectrum of methyl 2-acetamido-4-*O*-acetyl-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**6**).

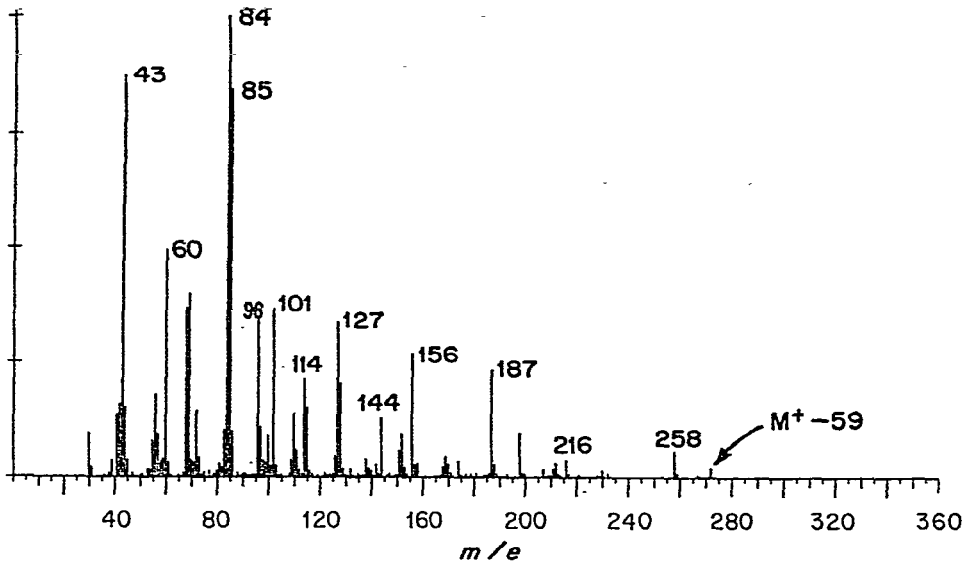

Fig. 3. Mass spectrum of 2-acetamido-1,4,5-tri-*O*-acetyl-3,6-anhydro-2-deoxy-D-glucitol (**11**).

estimation of these derivatives in the complex mixture of sugars obtained after alkaline degradation of sulfated glycoproteins.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting-points". Optical rotations were determined in 1-dm semimicrotubes, with a Perkin–Elmer Model 141 polarimeter; the chloroform used was analytical-reagent grade and contained ~0.75% of ethanol. I.r. spectra were recorded with a Perkin–Elmer Model 237 spectrophotometer.

G.l.c. of the trimethylsilyl ether **12** and of the acetates **6** and **11** was performed with a Perkin–Elmer Model 900 gas chromatograph equipped with a flame-ionization detector, with nitrogen as a carrier gas, on a stainless-steel column (0.07 $cm^2 \times 270$ cm) of GasChrom Q (100/120 mesh) coated with 3% OV-225 (Applied Science Laboratories Inc., P.O. Box 440, State College, Pa. 16801), programmed for a rise of 10°/min from 80–250° for the trimethylsilyl ether and from 125–250° for the acetates. The retention time (t_R) is given relative to that of hexa-*O*-trimethylsilyl-*myo*-inositol for **12**, and to that of hexa-*O*-acetyl-*myo*-inositol for **6** and **11**. The correction factor (corr. fact.) was obtained by the formula: area of standard × area of sample^{-1} × mol. wt. of standard × mol. wt. of sample^{-1}, the amounts of standard and sample being identical.

The computer-assisted, g.l.c.–m.s. analyses of **6** and **11–15** were performed on an analytical system consisting of an IBM-1800 computer, which was fed raw data generated by a single-focusing, magnetic scanning Hitachi–Perkin–Elmer RMU-6 mass spectrometer, interfaced with a Perkin–Elmer Model 990 gas chromatograph

equipped with a stainless-steel column (0.07 $cm^2 \times 300$ cm) of Chromosorb W (HP) (80/100-mesh) coated with 3% OV-1 for the per(trimethylsilyl)ated derivatives, and with 3.8% SE-30 (Supelco Inc., Bellefonte, Pa. 16823) for the alditol derivatives. The columns were conditioned overnight at 300°, and equilibrated at 150° for the per-(trimethylsilyl) and 130° for the alditol derivatives. After application of the sample, the temperature of the column was programmed for a rise of 12°/min from 150–320° for the per(trimethylsilyl) derivatives and from 130–210° for the alditol derivatives, with nitrogen as a carrier gas and a flow rate of 30 ml/min. Per(trimethylsilylation) was performed with bis(trimethylsilyl)trifluoroacetamide, as previously described[11].

Column chromatography was performed on Silica Gel Davison (60–200 mesh, grade 950, Davison Chemical, Baltimore, Md. 21226) used without pretreatment. The proportion of weight of substance to weight of silica gel was 1:60 to 1:100. The ratio of diameter of the column to its length was 1:8 to 1:12. The volume of the fractions eluted was 2–3 ml/g of substance to be chromatographed. T.l.c. of nonpolar compounds was performed on precoated, silica gel plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) and, for polar compounds, on precoated cellulose plates (layer thickness, 0.10 mm; E. Merck); distance of solvent travel, ~6 cm. The zones were detected by spraying the chromatogram with (*A*) 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol, (*B*) 1:10 conc. sulfuric acid–ethanol, (*C*) 1% (v/v) ninhydrin in 1:1 (v/v) ethanol–acetone, (*D*) aniline hydrogen phthalate solution[12], followed by heating on a hot plate for a few min, or by examination of the chromatogram under (*E*) normal or (*F*) u.v. light.

Evaporations were conducted *in vacuo*, with a bath temperature below 45°. Solutions (< 5 ml) in volatile solvents were concentrated under a stream of nitrogen. Microanalyses were performed by Dr. M. Manser, Zurich, Switzerland.

*Methyl 2-acetamido-2-deoxy-6-*O*-tosyl-α-*D*-glucopyranoside* (**2**). — To a stirred solution of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside[13] (**1**) (1.00 g) in anhydrous pyridine (10 ml) was slowly added a solution of *p*-toluene-sulphonyl chloride (930 mg, 1.15 mol. equiv.) in anhydrous pyridine (3 ml), both solutions having previously been cooled at −10°. The mixture was stirred at −10° for 3 h, then at ~5° overnight, and at room temperature for one day. The excess chloride was decomposed by addition of ice, and the solution was stirred for 30 min, poured onto ice, and extracted with chloroform (4 × 70 ml). The chloroform layers were washed three times each with ice-cold water (30 ml), ice-cold M sulfuric acid (30 ml), saturated, aqueous sodium hydrogen carbonate (30 ml), and water, dried (sodium sulfate), and evaporated. Several additions of toluene, followed by evaporation, gave a yellow syrup, which was dried *in vacuo* overnight (1.35 g), and then purified by chromatography on a column of silica gel in 9:1 (v/v) chloroform–ethanol solution; a trace amount of a component faster moving on t.l.c. was eluted first, followed by methyl 2-acetamido-2-deoxy-3,6-di-*O*-tosyl-α-D-glucopyranoside[6] (10 mg), a trace of an unidentified product, and, finally, homogeneous **2**. Crystallization and recrystallization from chloroform–hexane–toluene gave **2** as needles (881 mg, 53%), m.p. 70–73°; $[\alpha]_D^{23}$ +68° (*c* 2.0, chloroform); i.r. data: ν_{max}^{KBr} 3400–3300 (broad, OH, NH),

1650 (Amide I), 1600 (Ar), 1545 (Amide II), 1450, 1200, 1190, 1185 (S=O), 810, 765, and 685 cm^{-1} (Ar); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A,B*): R_F 0.2; in 4:1 (v/v) chloroform–ethanol (*A,B*): R_F 0.4.

Anal. Calc. for $C_{16}H_{23}NO_8S$: C, 49.35; H, 5.95; N, 3.59; O, 32.87. Found: C, 49.41; H, 5.94; N, 3.49; O, 32.82.

Acetylation of **2** (80 mg) with anhydrous pyridine–acetic anhydride in the usual way gave, after chromatography on silica gel (19:1 chloroform–ethanol), a crystalline residue (79 mg), which was crystallized from 2-isopropoxypropane at −20° to give prisms (53 mg, 55%), which were rapidly filtered off at *ca.* −10°, and became amorphous at room temperature and liquid at 60°; $[\alpha]_D^{22}$ +86° (*c* 0.6, chloroform); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*): R_F 0.5.

Anal. Calc. for $C_{20}H_{27}NO_{10}S$: C, 50.73; H, 5.75; N, 2.96; O, 33.78. Found: C, 50.55; H, 5.76; N, 2.84; O, 33.63.

Methyl 2-acetamido-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**4**). — To a solution of **2** (400 mg) in ethanol (4 ml) was added M sodium hydroxide (2 ml), and the mixture was heated at reflux for 30 min. T.l.c. then showed two components giving a red color (anisaldehyde), the major corresponding to **4**, and the minor (positive ninhydrin reaction, R_F ~0.1 in 4:1 chloroform–ethanol) to methyl 2-amino-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**5**). The solution was cooled to room temperature, neutralized with carbon dioxide, and concentrated. The residue was dissolved in 4:1 methanol–water (10 ml), acetic anhydride (1 ml) was added, and the solution was kept at room temperature overnight. After evaporation, the last traces of acetic anhydride were removed from the residue by repeated additions and evaporations of toluene. Water (15 ml) was added and the solution was passed successively through columns of Amberlite IR-45 (OH^-, 20–50 mesh) resin, and of Amberlite CG-50 (H^+, 200–400 mesh) resin, and both columns were washed with water (50 ml). After evaporation of water, the colorless residue was purified by chromatography on silica gel with 19:1 chloroform–ethanol. Crystallization from ethyl acetate gave **4** as plates (103 mg, 46%), m.p. 150–152°, $[\alpha]_D^{23}$ +130° (*c* 0.5, chloroform); i.r. data: ν_{max}^{KBr} 3400 (shoulder, OH), 3330 (NH), 1635 (Amide I), and 1545 cm^{-1} (Amide II); t.l.c. in 9:1 chloroform–ethanol (*A*): R_F 0.3; in 4:1 chloroform–ethanol (*A*): R_F 0.45; and in 60:25:4 chloroform–methanol–water (*A*): R_F 0.3.

Anal. Calc. for $C_9H_{15}NO_5$: C, 49.76; H, 6.96; N, 6.45; O, 36.83. Found: C, 49.76; H, 6.87; N, 6.37; O, 36.68.

G.l.c. of the 4-trimethylsilyl ether (**12**) of **4**, obtained as described under "*General*": t_R 1.55, corr. fact. 3.7; m.s. data of **12** (see Fig. 1): *m/e* 289 (M^+), 274 ($M^+ - \cdot Me$), 258 ($M^+ - \cdot OMe$), 230 ($M^+ - NHAc$), 214 ($M^+ - \cdot CH_3 - HOAc$), 200 ($M^+ - \cdot OSiMe_3$), 144 ($Me_3SiO{-}CH{=}CH{-}\overset{+}{C}H{-}NH_2$), 116 ($Me_3Si\overset{+}{O}{-}\dot{C}H{=}CH_2$), 101 (116 − ·Me), 73, 59, and 43.

Identical treatment of **3** gave **4** showing properties identical with those of the compound just described.

Acetylation of **4** with pyridine and acetic anhydride, in the usual way, gave

methyl 2-acetamido-4-*O*-acetyl-3,6-anhydro-2-deoxy-α-D-glucopyranoside (**6**), as needles (62% yield), m.p. 120–122.5° (from ethanol–ether), $[\alpha]_D^{23}$ +20° (*c* 0.1, chloroform); i.r. data: ν_{max}^{KBr} 3390 (NH), 1750 (OAc), 1660 (Amide I), and 1530 cm^{-1} (Amide II); m.s. data (see Fig. 2): *m/e* 259 (M^+), 243 ($M^{\ddagger}\cdot - H -$ ·Me), 217 ($M^{\ddagger}\cdot -$ CH_2=C=O), 200, 169 (200 – ·OMe), 157, 140, 127 (169 – CH_2=C=O), 115 (157 – CH_2= C=O), 103 and 43; t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*): R_F 0.5; g.l.c.: t_R 1.12, corr. fact. 1.7 (2-acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-D-glucitol: t_R 1.75, corr. fact. 1.6).

Anal. Calc. for $C_{11}H_{17}NO_6$: C, 50.96; H, 6.61; N, 5.40; O, 37.03. Found: C, 50.63; H, 6.55; N, 5.31; O, 37.20.

2-Amino-3,6-anhydro-2-deoxy-D-glucose hydrochloride (**7**). — Compound **4** (50 mg) was hydrolyzed with 2M hydrochloric acid (3.5 ml) for 2 h at 100°. The solution was cooled to room temperature, ethanol (10 ml) was added, and the solvent was evaporated at room temperature. Several additions of ethanol to the residue, followed by evaporation, gave a brown syrup (37 mg), which was dried first under a stream of nitrogen and then in a desiccator in the presence of soda lime. Crystallization from methanol gave white prisms (20 mg, 44%), which sintered at 110°, and decomposed at 145°. The compound was unstable and neither an optical rotation nor a correct elementary analysis could be obtained; i.r. data: ν_{max}^{KBr} 3400–3150 (broad OH, NH_3^+), 1715 (C=O), 1600, 1510, 1500, 1410 (NH_3^+), and 1050–1030 cm^{-1} (C–O–C); t.l.c. on cellulose in 4:1:5 1-butanol–acetic acid–water (*C,D,F*): R_F 0.1; in 5:5:1:3 ethyl acetate–pyridine–water–acetic acid (*C,D,F*): R_F 0.45; in 5:5:1:3 ethyl acetate–pyridine–acetic acid–formic acid (*C,D,F*): R_F 0.35; the corresponding values for 2-amino-2-deoxy-D-glucose hydrochloride are, respectively, R_F ~0.05, 0.4, and 0.2.

Compound **7** gave a single peak, when examined on a Technicon amino acid AutoAnalyzer according to the methods of (A) Miller and Piez[14] and (B) Degand *et al.*[15]: t_R 35.5 min (A), 152 min (B) [t_R of 2-amino-2-deoxy-D-glucose 0 min (A, B), t_R of ammonia 71 min (A), 304 min (B)]; m.s. data of trimethylsilyl 2-amino-3,6-anhydro-2-deoxy-4-*O*-trimethylsilyl-α-D-glucopyranoside (**13**): *m/e* 305 ($M^{\ddagger}$), 290 ($M^{\ddagger}$ – ·Me), 215 ($M^{\ddagger}$ – $HOSiMe_3$), 157, 144 (Me_3SiO–CH=CH–$\overset{+}{C}$H–NH_2), 131, 129, 116 ($Me_3Si\overset{+}{O}$–CH=CH_2), 101 (Me_2Si=$\overset{+}{O}$–CH=CH_2), 75, and 73.

2-Acetamido-3,6-anhydro-2-deoxy-D-glucitol (**9**). — A solution of crude **7** (38 mg) in water (1 ml) was rapidly passed through a small column of Amberlite IR-45 (AcO^-, 20–50 mesh) resin. The column was washed with water (20 ml), and the combined eluates were concentrated (bath temperature below 30°) to give a yellow syrup, which was dissolved in methanol (2 ml); water (50 μl) was added and then acetic anhydride (0.5 ml), and the solution was kept at room temperature for 3 h. Methanol (8 ml) was added under cooling and the solvents were evaporated (bath temperature below 30°). Several distillations of toluene and two of methanol from the residue gave 2-acetamido-3,6-anhydro-2-deoxy-D-glucose (**8**) as a yellow residue (36 mg) that was not further purified; i.r. data: ν_{max}^{KBr} 3400–3300 (broad, OH, NH), 1655 (Amide I), and 1550 cm^{-1} (Amide II); t.l.c. in 4:1 (v/v) chloroform–ethanol (*A*):

R_F 0.2; m.s. data of trimethylsilyl 2-acetamido-3,6-anhydro-2-deoxy-4-*O*-trimethylsilyl-α-D-glucopyranoside (**14**): *m/e* 347 ($M^{\ddagger}$), 332 ($M^{\ddagger}-{\cdot}Me$), 288 ($M^{\ddagger}-NH_2Ac$), 258 ($M^{\ddagger}-{\cdot}OSiMe_3$), 243 ($332-{\cdot}OSiMe_3$), 214 ($M^{\ddagger}-{\cdot}OSiMe_3-{\cdot}Ac-H$), 186 ($Me_3SiO-CH=CH-\overset{+}{C}H-NHAc$), 158, 116 ($Me_3Si\overset{+}{O}-CH=CH_2$), 101 ($116-{\cdot}Me$), 75, and 73.

A solution of **8** (30 mg) in borate buffer[16] (pH 8.0, 1 ml) was cooled to 0°, and a cold solution of sodium borohydride (50 mg) in water (1 ml) was added slowly with stirring. The solution was further stirred at 0° for 2 h and the excess of sodium borohydride decomposed by the addition of a few drops of 2M acetic acid. Water (5 ml) was added, and the solution was passed through a small column of Rexyn-300 (H^+, OH^-) resin. The resin was washed with water, and the eluates were concentrated to a syrup. Several additions of methanol, followed by evaporation, gave a semicrystalline residue (190 mg) that was dried *in vacuo*. Crystallization from methanol–ether and recrystallization from ether gave a borate complex of **9** (178 mg), as plates, decomposing at ~285° without melting; $[\alpha]_D^{20}$ +1° (*c* 0.8, water); i.r. data: ν_{max}^{KBr} 3410 (broad, OH), 1650 (shoulder, Amide I), 1575 (Amide II), 1445–1425 (B–O and C–O–H), 1050, 1015, and 925 cm^{-1} (B–O–B and B–O–C); t.l.c. on cellulose in 5:5:1:3 ethyl acetate–pyridine–acetic acid–formic acid (*D*,*E*): R_F 0.4. The elementary analysis of this compound did not give consistent values.

A solution of the borate complex (60 mg) in water (2 ml) was passed through a column of Dowex-50 (H^+, 20–50 mesh) resin; the column was washed with water (30 ml), and the eluates were concentrated to give **9** as a colorless syrup (14 mg, 88% calculated from **4**) that failed to crystallize; i.r. data: ν_{max}^{KBr} 3450–3410 (broad, OH, NH), 1635 (Amide I), 1575 (Amide II), and 1435 cm^{-1} (C–O–H); t.l.c. on silica gel in 60:25:4 chloroform–methanol–water (*A*): R_F 0.35; in 4:1:5 (v/v) 1-butanol–ethanol–water, organic phase (*A*): R_F 0.3; in 4:1:5 1-butanol–acetic acid–water (*A*): R_F 0.3*; on cellulose in 4:1:5 1-butanol–acetic acid–water (*D*); R_F 0.3*; the corresponding values for 2-acetamido-2-deoxy-D-glucitol are R_F 0.1, ~0.05, ~0.07, and ~0.07; m.s. data of 2-acetamido-3,6-anhydro-2-deoxy-1,4,5-tris-*O*-trimethylsilyl-D-glucitol (**15**): *m/e* 421 ($M^{\ddagger}$), 406 ($M^{\ddagger}-{\cdot}Me$), 318 ($M^{\ddagger}-103$), 228 ($M^{\ddagger}-103-HOSiMe_3$), 217, 186, 174 ($M^{\ddagger}-247$), 147 ($Me_2\overset{+}{S}i-OSiMe_3$), 132 ($174-CH_2=C=O$), 116, and 73.

Acetylation of the borate complex of **9** (50 mg), in the usual way, gave an acetyl borate complex (113 mg) of **9** as silky needles that softened at 102–103° without melting; the i.r. spectrum was similar to that of the borate complex of **9** just described, but with an additional band at 1720 cm^{-1} (OAc).

2-Acetamido-2-deoxy-3,6-anhydro-1,4,5-tri-O-(p-*phenylazobenzoyl*)-D-*glucitol* (**10**). — The borate complex of **9** (18 mg) was dissolved in water (3 ml), and Dowex-50 (H^+, 20–50 mesh) resin was added until the pH was 3. The mixture was stirred for a few min, and filtered. The resin was washed with water, and the filtrate concentrated.

*In this solvent system, a second, faster-moving spot appears both on silica gel and on cellulose; it is probably a partially acetylated material formed during the development.

After several additions of methanol, followed by evaporation, the residue was dissolved in methanol (2 ml); the solution was filtered through charcoal–Celite, and the filtrate was concentrated to give a colorless syrup (4 mg) that was dried *in vacuo*. It was dissolved in anhydrous pyridine (1 ml), *p*-phenylazobenzoyl chloride (20 mg) was added, and the solution was stirred at room temperature for 72 h. The solvent was evaporated under a stream of nitrogen, and the orange, semicrystalline residue (30 mg) was dried *in vacuo*. Chloroform (3 ml) was added, and the suspension was applied to a column of silica gel (2 g). Chloroform eluted firstly *p*-phenylazobenzoic acid, and then unreacted *p*-phenylazobenzoyl chloride. 9:1 Chloroform–ethanol eluted an additional amount of *p*-phenylazobenzoyl chloride, followed by **10**, which was recrystallized from hot chloroform to give vermilion prisms (7 mg, 43%), m.p. 225–227°; $[\alpha]_D^{24}$ +3.5° (*c* 0.2, ethanol); $[\alpha]_{546}^{24}$ +4° (*c* 0.2, ethanol); i.r. data: ν_{max}^{KBr} 1675 (Amide I), 1600, 1580 (Ar), 1550–1530 (N=N), 1500, 1450, and 775 cm^{-1} (Ar); t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*,*B*,*E*): R_F 0.5.

Anal. Calc. for $C_{47}H_{39}N_7O_8$: C, 68.02; H, 4.74; N, 11.82. Found: C, 67.85; H, 4.49; N, 12.57.

*2-Acetamido-1,4,5-tri-*O*-acetyl-3,6-anhydro-2-deoxy-*D*-glucitol* (**11**). — The sodium borate complex (52 mg) of **9** was treated with Dowex-50 resin as described for the preparation of **10**, and the resulting syrup (**9**, 17 mg) was dissolved in anhydrous pyridine (3 ml). Acetic anhydride (2 ml) was added, and the solution was kept overnight at room temperature and then cooled to 0°. Methanol (10 ml) was added and the solvent was evaporated; several additions of toluene, followed by evaporation, gave a brown syrup (15 mg) that was dried *in vacuo*. It was chromatographed on a column of silica gel (3 g) in 19:1 chloroform–ethanol to give a colorless syrup (12 mg). Crystallization from ethanol–hexane gave **11** as prisms (8 mg, 30%), m.p. 96–100°; $[\alpha]_D^{23}$ +6° (*c* 0.1, chloroform); i.r. data: ν_{max}^{KBr} 3250 (NH), 1750, 1730 (OAc), 1660 (Amide I), and 1540 cm^{-1} (Amide II); m.s. data (see Fig. 3): *m/e* 331 ($M^{\ddagger}$), 272 ($M^{\ddagger}-59$), 258 ($M^{\ddagger}-73$), 216 ($M^{\ddagger}-73-CH_2{=}C{=}O$), 187 ($M^{\ddagger}-144$), 156 (216−HOAc), 144, 127 (187−HOAc), 114 (156−CH_2=C=O), 101 (144−·Ac), 96 (156−HOAc), 85 (127−CH_2=C=O), 84 (144−HOAc), 60, and 43; t.l.c. in 9:1 (v/v) chloroform–ethanol (*A*,*B*): R_F 0.5 (2-acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-D-glucitol: R_F 0.7); g.l.c.: t_R 1.36, corr. fact. 2.0 (2-acetamido-1,3,4,5,6-penta-*O*-acetyl-2-deoxy-D-glucitol: t_R 1.75, corr. fact. 1.6).

Anal. Calc. for $C_{14}H_{21}NO_8$: C, 50.75; H, 6.39; N, 4.23. Found: C, 50.86; H, 6.46; N, 4.15.

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