THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-6-*O*-α-D-MANNOPYRANOSYL-D-GLUCOSE*

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(Received October 31st, 1970)

ABSTRACT

Condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide with either benzyl 2-acetamido-3-*O*-acetyl- or 3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (obtained via the 6-*O*-trityl- and 3,4-di-*O*-acetyl-6-*O*-trityl derivatives) gave benzyl 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-α-D-glucopyranoside penta- and hexaacetate in 42% and 65% yield, respectively. Removal of the protective *O*-acetyl and *O*-benzyl groups gave the title compound, which was characterized by a hepta-*O*-acetyl derivative. All intermediates were obtained in crystalline form. The title compound is useful as a reference standard for determination of the structure of the carbohydrate core of glycoproteins.

INTRODUCTION

The present paper describes the synthesis and characterization of 2-acetamido-2-deoxy-6-*O*-α-D-mannopyranosyl-D-glucose (**9**) as part of a program to synthesize fragments of carbohydrate chains of glycoproteins and glycolipids[1–7].

DISCUSSION

In order to obtain the disaccharide **9**, two routes were studied. In the first, the known benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside[8] (**5**) was condensed with tetra-*O*-acetyl-α-D-mannopyranosyl bromide[9] (**1**), to give benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**7**) in 42% yield; other possible condensation products, such as the β anomer condensed with the hydroxyl group on C-4 of **5**, or the two possible β-D anomers, were not observed.

*Amino Sugars LXX. This is publication No. 529 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. This work was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases (AM-03564), National Institutes of Health, U. S. Public Health Service.

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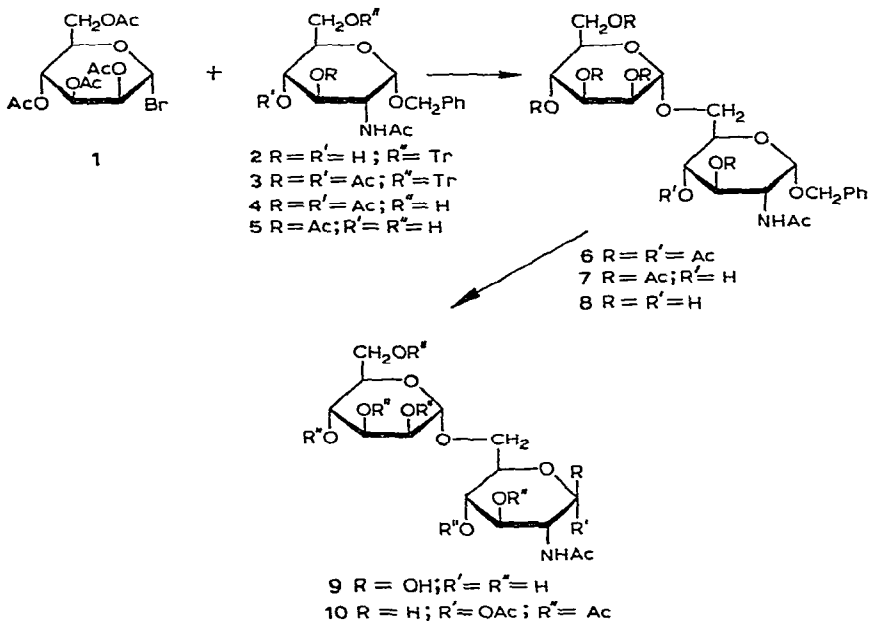
In a second study, tritylation of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[10] gave the 6-*O*-trityl derivative **2**, which was acetylated to give **3**. Compound **3** was detritylated to give the 3,4-di-*O*-acetyl derivative **4**, and condensation of **4** with bromide **1** gave the fully acetylated disaccharide **6** in 65% yield. Saponification of the *O*-acetyl groups of both **6** and **7** gave **8**, which was hydrogenolyzed to afford the crystalline disaccharide **9**. All intermediates were obtained in crystalline form, and all reactions, except the condensation to form the disaccharides, resulted in excellent yields (over 80%).

The α-D configuration of the (1→6)-linkage is clearly indicated by the comparison of the optical rotation of **6**, **7**, and **9** with that of various α-D-(1→3) or -(1→4) analogs[6,7] or with the sum of the optical rotations of the constituents, as described earlier[2–7] (see Table I).

The great difference between the optical rotation of the fully acetylated disaccharide **6** and that of the analog **7** (having a free hydroxyl group at C-4 of the 2-acetamido-2-deoxy-D-glucose moiety) is of interest for an evaluation of the contribution of each asymmetric center to the total optical activity (see Lemieux and Martin[11], Brewster[12], and Whiffen[13]). Very little change in the optical rotation was observed when the disaccharide **9** was dissolved in aqueous methanol. Comparison of its molecular rotation with the sum of those of the constituents (see Table I) suggests a β-D configuration for the hexosamine moiety of **9**, but an α-D configuration of this moiety in the crystalline peracetate **10**.



It is of interest that no formation of a β-D-linked disaccharide was observed in the synthesis of the (1→3)- (ref. 6), (1→4)- (ref. 7), and (1→6)-linked 2-acetamido-2-

deoxy-*O*-α-D-mannopyranosyl-D-glucose, despite the use of mercuric cyanide as the catalyst and acid acceptor known to favor the formation of both the α-D- and β-D-linked disaccharides (see Staněk *et al.*[14] and Evans *et al.*[15]).

TABLE I

MOLECULAR ROTATIONS OF SELECTED DISACCHARIDES, COMPARED TO THE SUM OF THOSE OF THE CONSTITUENTS

Compound	$[M]_D$ (*degrees* × 10^{-2})
Methyl tetra-*O*-acetyl-α-D-mannopyranoside[a] (**11**) + benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside[a] (**12**)	+622
Methyl tetra-*O*-acetyl-β-D-mannopyranoside[a] (**13**) + compound **12**	+277
Compound **6**[a]	+1108
Compound **7**[a]	+615
Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-3-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside[a] (ref. 6)	+574
Methyl α-D-mannopyranoside[b] (**14**) + 2-acetamido-2-deoxy-α-D-glucose[b]	+295
Compound **14** + 2-acetamido-2-deoxy-β-D-glucose[b]	+105
Compound **9**[c] at equilibrium	+134
2-Acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose[c] at equilibrium (ref. 6)	+222
2-Acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose[c] at equilibrium (ref. 7)	+253
Compound **11** + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucose[a]	+529
Compound **11** + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucose[a]	+176
Compound **10**[a]	+461

[a]Optical rotation determined in chloroform; [b]in water; [c]in 1:1 water–methanol.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Optical rotations were determined, in semimicrotubes, with a Perkin–Elmer Model 141 polarimeter. The chloroform used was analytical-reagent grade and contained about 0.75% of ethanol. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer Model 237 spectrophotometer. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer, for solutions in chloroform-*d* with tetramethylsilane as the internal standard. G.l.c. of the per-*O*-(trimethylsilyl) derivatives was performed with a Perkin–Elmer Model 900 gas chromatograph by use of a column of Chromosorb GHP coated with 3% OV-11 (Supleco Inc., Bellefonte, Pa. 16823, U. S. A.), programmed for a rise of 5°/min from 200 to 232°; t'_R is given relative to that of hexakis-*O*-(trimethylsilyl)-*myo*-inositol as unity. Column chromatography was performed on Silica Gel Merck (70–325 mesh; E. Merck, Darmstadt, Germany), used without pretreatment. The ratio of weight of substance to weight of adsorbent was 1:80 to 1:120. The volume of the fraction eluted was 3–4 ml per gram of the substance to be chromatographed. The ratio of diameter to length of the columns was 1:25. T.l.c. was performed on precoated

Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany); all compounds showed only one spot. Evaporations were conducted *in vacuo* with the bath temperature below 40°. Solutions in less than 5 ml of volatile solvents were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

Benzyl 2-acetamido-2-deoxy-6-O-trityl-α-D-glucopyranoside (**2**). — A solution of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[10] (1.33 g) in dry pyridine (15 ml) was treated with chlorotriphenylmethane (1.33 g) for 48 h at room temperature, and then poured into ice–water. The syrup that separated was dissolved in chloroform, and the solution was washed with water, dried (sodium sulfate), and evaporated; repeated addition and distillation of toluene gave a residue that showed two spots on t.l.c. in 4:1 benzene–methanol, one spot corresponding to triphenylmethanol. Six recrystallizations from ether–hexane or ether–pentane gave 2.1 g (84%) of **2** as fine needles, m.p. 101–102°, $[\alpha]_D^{20}$ +39° (*c* 1.3, chloroform); i.r. data: ν_{max}^{KBr} 1655 (CONH) and 3350 cm^{-1} (broad; OH); n.m.r. data (chloroform-*d*): τ 2.66 (20 H, 4 Ph), 3.66 (deuteratable doublet, *J* 9.0 Hz, NH), 6.20 and 7.13 (deuteratable, two OH), and 8.13 (N-Ac); t.l.c. in 4.1 benzene–methanol: R_F 0.52.

Anal. Calc. for $C_{34}H_{35}NO_6$: C, 73.76; H, 6.37; N, 2.53; Found: C, 73.78; H, 6.41; N, 2.60.

Benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (**3**). — A solution of **2** (1.0 g) in dry pyridine (10 ml) was cooled to 0° and treated with acetic anhydride (15 ml). After 48 h at room temperature, the solution was poured into ice–water, and the mixture was extracted with chloroform. The extract was dried (sodium sulfate) and evaporated, and toluene was repeatedly added to and distilled from the residue. Crystallization from chloroform–hexane gave 1.0 g (91%) of fine needles, m.p. 80–81°, $[\alpha]_D^{20}$ +87° (*c* 1.2 chloroform); i.r. data: ν_{max}^{KBr} 1655 (CONH) and 1745 cm^{-1} (OAc); n.m.r. data (chloroform-*d*): τ 2.63 (20 H, 4 Ph), 4.18 (deuteratable doublet, *J* 9.5 Hz, NH), 8.02, 8.10 (two OAc), and 8.28 (N-Ac); t.l.c. in 19:1 chloroform–ethanol: R_F 0.56.

Anal. Calc. for $C_{38}H_{39}NO_8$: C, 71.57; H, 6.16; N, 2.20. Found: C, 71.48; H, 6.15; N, 2.25.

Benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-α-D-glucopyranoside (**4**). — A solution of **3** (1.0 g) in glacial acetic acid (10 ml) was cooled to 10° and treated with a 32% solution of hydrogen bromide in glacial acetic acid (1 ml). The mixture was shaken for 75 sec, and then filtered rapidly on a sintered-glass funnel, into ice–water. The filtrate was extracted with chloroform, and the extract was successively washed with saturated sodium hydrogen carbonate solution and water, dried (sodium sulfate), and evaporated, to give a residue which was crystallized from benzene–hexane, affording 0.4 g (82%) of **3** as long needles, m.p. 166–167°, $[\alpha]_D^{20}$ +128° (*c* 1.2, chloroform); i.r. data: ν_{max}^{KBr} 1655 (CONH), 1750 (OAc), and 3350 cm^{-1} (OH); n.m.r. data (chloroform-*d*): τ 2.63 (5 H, Ph), 4.20 (doublet, *J* 9.5 Hz, NH), 7.58 (deuteratable, OH), 7.95 (6 H, two OAc), and 8.10 (N-Ac); t.l.c. in 4:1 benzene–methanol: R_F 0.45.

Anal. Calc. for $C_{19}H_{25}NO_8$: C, 57.71; H, 6.37; N, 3.54. Found: C, 57.86; H, 6.29; N, 3.52.

Benzyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (**5**). — This compound was prepared as previously described[8]. When the product of the debenzylidenation was crystallized from acetone–ether–pentane, hygroscopic microcrystals were obtained, m.p. 55–56°, $[\alpha]_D^{20}$ +106° (*c* 1.4, chloroform); i.r. data: ν_{max}^{KBr} 1660 (CONH), 1750 (OAc), and 3400 cm^{-1} (broad; OH); n.m.r. data (chloroform-*d*): τ 2.70 (5 H, Ph), 3.59 (doublet, *J* 9.0 Hz, NH), 8.0 (OAc), and 8.20 (NAc); t.l.c. in 7:3 benzene–methanol: R_F 0.55.

Anal. Calc. for $C_{17}H_{32}NO_7$: C, 57.78; H, 6.56; N, 3.96; O, 31.69. Found: C, 57.87; H, 6.55; N, 3.88; O, 31.70.

Recrystallization from ethyl acetate–2-isopropoxypropane, as previously described [8], gave needles having m.p. 121°, $[\alpha]_D^{20}$ + 111° (*c* 0.9 pyridine), $[\alpha]_D^{20}$ + 106° (*c* 1.4, chloroform), identical with the product previously described {lit.[8] m.p. 119–121°; $[\alpha]_D^{25}$ +132° (*c* 1, pyridine)*}.

Benzyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-6-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**6**). — A mixture of dry **4** (1.1 g) and mercuric cyanide (1 g) in dry 1:1 benzene–nitromethane (180 ml) was concentrated to 130 ml under atmospheric pressure and then cooled to room temperature. A solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[9] (**1**, 1.5 g) in dry 1,2-dichloroethane (15 ml) was added, and the mixture was stirred for 3 days at room temperature. Additional amounts of the bromide (0.5 g) and mercuric cyanide (0.5 g) were added, and the mixture was stirred for a further 24 h. The mixture was diluted with 1,2-dichloroethane (100 ml), washed successively with a cold, saturated solution of sodium hydrogen carbonate and water, dried (sodium sulfate), and evaporated to a syrup (3 g) which was chromatographed on silica gel with 19:1 chloroform–ethanol, to give a fraction enriched in compound **6**. Chromatography of this fraction on another column of silica gel, with 1:1 ethyl acetate–ether, gave 1.3 g (65%) of **6**, which crystallized from ether–hexane as microcrystals having m.p. 76–78°, $[\alpha]_D^{20}$ +143° (*c* 1.2, chloroform); i.r. data: ν_{max}^{KBr} 1675 (CONH) and 1750 cm^{-1} (OAc); n.m.r. data (chloroform-*d*): τ 2.60 (5 H, Ph), 4.20 (doublet, *J* 9.5 Hz, NH), and 7.67 (21 H, 6 OAc+NAc); t.l.c. in 1:1 ethyl acetate–ether: R_F 0.41.

Anal. Calc. for $C_{33}H_{43}NO_{17}$: C, 54.61; H, 5.97; N, 1.92; O, 37.41. Found: C, 54.46; H, 5.94; N, 2.11; O, 37.27.

Benzyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**7**). — Compounds **5** (537 mg) and **1** (1.2 g) were condensed in the way just described for compound **6**. The residue obtained after processing of the reation mixture was chromatographed on a column of silica gel with 1:1 ethyl acetate–ether. A fraction was obtained that crystallized from acetone–pentane to give 520 mg (42%) of **7** as microcrystals, m.p. 82–83°, $[\alpha]_D^{20}$ +90° (*c* 0.8,

*A re-examination of the sample previously described[8] showed $[\alpha]_D^{20}$ +113° (*c* 0.9, pyridine).

chloroform); i.r. data: ν_{max}^{KBr} 1660 (CONH), 1745 (OAc), and 3350 cm^{-1} (OH); t.l.c. in 1:1 ethyl acetate–ether: R_F 0.28.

Anal. Calc. for $C_{31}H_{41}NO_{16}$: C, 54.48; H, 6.05; N, 2.05; O, 37.44. Found: C, 54.37; H, 6.03; N, 2.15; O, 37.50.

Benzyl 2-acetamido-2-deoxy-6-O-α-D-mannopyranosyl-α-D-glucopyranoside (**8**). — *From* **6**. A solution of **6** (200 mg) in methanol (5 ml) was treated with 0.1M sodium methoxide in methanol (0.5 ml) for 24 h at 4°. The solution was deionized with Dowex-50 (H^+) ion-exchange resin (1 ml) and then evaporated. The residue was crystallized from methanol–ethyl acetate to give 119 mg (91%) of **8** as microcrystals containing 0.5 molecule of water per molecule, m.p. 117–118°, $[\alpha]_D^{20}$ +167° (*c* 0.7. methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH) and 3370 cm^{-1} (broad; OH); g.l.c. data: peak at t'_R 31.60; t.l.c. in 1:1 benzene–methanol: R_F 0.33.

Anal. Calc. for $C_{21}H_{31}NO_{11}\cdot 0.5\,H_2O$: C, 52.26; H, 6.69; N, 2.90. Found: C, 52.67; H, 6.59; N, 2.84.

From **7**. De-*O*-acetylation of **7** by the procedure just described gave **8**, having m.p., mixed m.p. (116–118°), i.r. spectrum, and R_F value identical with those of the compound obtained from **6**.

2-Acetamido-2-deoxy-6-O-α-D-mannopyranosyl-β-D-glucose (**9**). — A solution of **8** (292 mg) in a mixture of 95% ethanol (49 ml) and acetic acid (1 ml) was hydrogenolyzed with hydrogen under pressure (3.4 atm.) in the presence of 10% palladium-on-charcoal (300 mg) for 72 h, the catalyst was filtered off, and the solution was evaporated. Crystallization of the product from methanol–acetone gave 222 mg (94%) of **9** as hygroscopic needles containing 0.5 molecule of water per molecule, softening at 136°, and melting at 142–144°; $[\alpha]_D^{20}$ +38 → +35° (equilibrium after 48 h; *c* 1.2, 50% methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH) and 3350 cm^{-1} (broad; OH); g.l.c. data: double peak at t'_R 15.4 and 17.1.

Anal. Calc. for $C_{14}N_{25}NO_{11}\cdot 0.5\,H_2O$: C, 42.85; H, 6.68; N, 3.57. Found: C, 42.97; H, 6.73; N, 3.43.

2-Acetamido-1,3,4-tri-O-acetyl-2-deoxy-6-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-glucose (**10**). — A solution of compound **9** (100 mg) in pyridine (2 ml) was treated with acetic anhydride (3 ml) for 24 h at room temperature. Evaporation gave a residue that crystallized from benzene–pentane to give **10** as microcrystals (133 mg, 86%), m.p. 75–77°, $[\alpha]_D^{20}$ +68° (*c* 0.6, chloroform); i.r. data: ν_{max}^{KBr} 1665 (CONH) and 1750 cm^{-1} (OAc); t.l.c. in 4:1 benzene–methanol: R_F 0.49.

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 49.52; H, 5.75; N, 2.16; O, 42.54.

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