THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-4-*O*-α-D-MANNOPYRANOSYL-D-GLUCOSE*†

MOHAMMED SHABAN** AND ROGER W. JEANLOZ***

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (U. S. A.)

(Received October 22nd, 1970)

ABSTRACT

Condensation of the 2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal with tetra-*O*-acetyl-α-D-mannopyranosyl bromide in the presence of mercuric cyanide gave the 2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-D-glucose diethyl acetal in 33% yield. Successive removal of the *O*-acetyl, carbonate, diethyl acetal, and isopropylidene protective groups proceeded with good yields, and gave, in crystalline form, the title compound, which was characterized by a crystalline octaacetyl derivative. The title compound is useful as a reference compound for the determination of the structure of the carbohydrate core of glycoproteins.

INTRODUCTION

In a previous paper[1], we have described the synthesis of 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose as part of a program[2] designed for the synthesis of fragments of carbohydrate chains of glycoproteins, and for that of antigenic glycoproteins and glycolipids. The present paper describes the synthesis and characterization of the disaccharide 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose (**8**) as part of the same program.

DISCUSSION

Condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[3] (**1**) with benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside had shown that the hydroxyl group at C-4 of the hexosamine moiety is not reactive and that no, or very little,

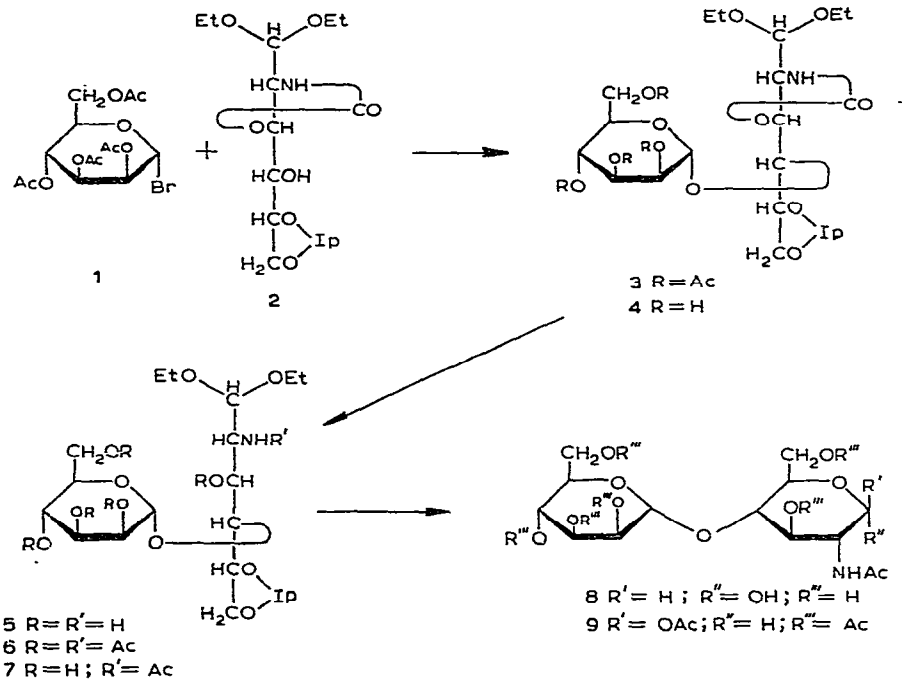
*Dedicated to Dr. Nelson K. Richtmyer in honor of his 70th birthday.

†Amino Sugars LXIX. This is publication No. 536 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 03564-11), National Institutes of Health, U. S. Public Health Service.

**On leave of absence from the Faculty of Science, Alexandria University, Alexandria, Egypt.

***To whom enquiries should be sent.

condensation had taken place at this position[4]. In order to obtain the disaccharide **8**, recourse was had to the open-chain intermediate, 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose 2,3-carbonate diethyl acetal (**2**) which Heyns *et al.*[5] had successfully condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(diphenylphosphoramido)-α-D-glucopyranosyl bromide to give derivatives of chitobiose and of its α isomer. Condensation of **1** with **2** in the presence of mercuric cyanide gave the disaccharide **3** in a yield (33%) similar to that of the preparation of the (1→3)-linked disaccharide[1]. Removal of the various protective groups proceeded without difficulty, and the various intermediates **4**–**7** were obtained in excellent yields, and, except **5**, in crystalline form. The crystalline 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose (**8**) was obtained in 60% yield from **7**, and characterized by formation of the crystalline octaacetyl derivative **9**; the latter compound was also obtained directly from **6**.



The anomeric form of the disaccharide linkage was ascertained by comparison of the molecular rotation of the intermediates **3** and **7** and of the final compound **8** with those of various derivatives, as previously described[1,2,6–8]. The molecular rotation of **3** was compared with the sum of the molecular rotations of the starting material **2** and of methyl tetra-*O*-acetyl-α- (**10**) and β-D-mannopyranoside (**11**), respectively. Similarly, the molecular rotation of the intermediate compound **6** was compared with the sum of the molecular rotations of 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-3,4-di-*O*-methyl-D-glucose diethyl acetal[5] and of **10** and **11**, respectively. Finally, the molecular rotation of **8** at equilibrium was compared with the sum of the molecular rotations of methyl α-D-mannopyranoside and of 2-acetamido-2-

deoxy-α- and -β-D-glucose, respectively (see Table I). All of these comparisons established that the disaccharide linkage was α-D.

TABLE I

MOLECULAR ROTATION OF SELECTED DISACCHARIDES COMPARED TO THE SUM OF THE CONSTITUENTS

Compound	$[M_D]$ (*degrees*) × 10^{-2}
Methyl tetra-*O*-acetyl-α-D-mannopyranoside[a] (**10**) + compound **2**[a]	−21
Methyl tetra-*O*-acetyl-β-D-mannopyranoside[a] (**11**) + compound **2**[a]	−371
Compound **3**[a]	−49
Compound **10**[a] + 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal (**12**)[b]	+205
Compound **11**[a] + compound **12**[b]	−150
Compound **6**[a]	+202
Methyl α-D-mannopyranoside[b] + 2-acetamido-2-deoxy-α-D-glucose[b]	+295
Methyl α-D-mannopyranoside[b] + 2-acetamido-2-deoxy-β-D-glucose[b]	+93
Compound **8**, at start of mutarotation[c]	+295
at equilibrium[c]	+253
Compound **10**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[a]	+543
Compound **10**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[a]	+181
Compound **9**[a]	+10

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

Similar comparisons suggested the β-D configuration for the hexosamine moiety of the crystalline, fully acetylated disccharide **9**, and the direction of the mutarotation and the sum of rotations indicated that the hexosamine moiety of the crystalline disaccharide **8** had the α-D configuration (see Table I).

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-1 (Supleco Inc., Bellefonte, Pa. 16823), programmed for a rise of 5° per min from 200 to 320°; 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) that was used without pretreatment. The ratio of weight of substance to weight of adsorbent was 1:80 to 1:120. The volume of the fractions

eluted was 3–4 ml per gram of the substance to be chromatographed. The ratio of diameter of the column to its length was 1:25. T.l.c. was performed on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany): each compound 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.

*2-Amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)*D*-glucose diethyl acetal 2,3-carbonate* (**3**). — A mixture of dry 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal 2,3-carbonate[5] (**2**, 2.6 g) and mercuric cyanide (1 g) in dry 1:1 benzene–nitromethane (200 ml) was concentrated to 150 ml under atmospheric pressure, and then cooled to room temperature. A solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[3] (**1**, 3.0 g) in benzene (30 ml) was added, and the mixture was stirred for 4 days at room temperature. Additional amounts of bromide **1** (1.0 g) and mercuric cyanide (0.5 g) were added, and the mixture was stirred for a further 3 days. The mixture was diluted with benzene (100 ml), washed successively with a cold, saturated solution of sodium hydrogen carbonate (5 × 25 ml) and water, dried (anhydrous sodium sulfate), and evaporated. The residue was chromatographed on a column of silica gel with 2:5 acetone–chloroform to give a crystalline fraction. Crystallization from acetone–ether–pentane gave 1.7 g (33%) of needles, m.p. 147–148°; $[\alpha]_D^{20}$ −7.6° (*c* 1.7, chloroform); i.r. data: ν_{max}^{KBr} 1775 (oxazolidine CONH), 1750 (OAc), and 3300 cm^{-1} (NH); n.m.r. data (chloroform-*d*): τ 3.84 (NH); 7.85, 7.90, 7.95, 8.00 (4 OAc); 8.56 (12 H, 4 Me); t.l.c. in 2:5 acetone–chloroform: R_F 0.49.

Anal. Calc. for $C_{28}H_{43}NO_{16}$: C, 51.76; H, 6.67; N, 2.16; O, 39.41. Found: C, 51.86; H, 6.60; N, 2.32; O, 39.31.

*2-Amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-α-*D*-mannopyranosyl-*D*-glucose diethyl acetal 2,3-carbonate* (**4**). — A solution of **3** (600 mg) in methanol (20 ml) was treated with 0.1M sodium methoxide solution (2 ml) for 12 h at 4°. The solution was deionized by passage through Dowex-50 (H^+) ion-exchange resin (1.5 ml), and then evaporated. The residue obtained was crystallized from methanol–acetone–benzene to give 400 mg (93%) of **4** as needles, m.p. 188–189°; $[\alpha]_D^{20}$ −2.7° (*c* 1.3, methanol); i.r. data: ν_{max}^{KBr} 1737 (oxazolidine CONH), and 3350 cm^{-1} (broad; OH and NH); g.l.c. datum: peak at t'_R 14.00; t.l.c. in 4:1 benzene–methanol: R_F 0.25; n.m.r. datum (D_2O): τ 8.73 (12 H, 4 Me).

Anal. Calc. for $C_{20}H_{35}NO_{12}$: C, 49.90; H, 7.33; N, 2.91; O, 39.82. Found: C, 49.86; H, 7.24; N, 2.92; O, 39.74.

*2-Amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-α-*D*-mannopyranosyl-*D*-glucose diethyl acetal* (**5**). — A solution of **4** (330 mg) and finely powdered barium hydroxide octahydrate (600 mg) in water (10 ml) was heated for 4 h at 80° under a stream of nitrogen, and cooled. Carbon dioxide was bubbled into the solution, the barium carbonate was filtered off and washed with methanol, and the filtrate and washings were combined and evaporated to dryness. The residue was mixed with methanol, the

suspension was filtered, and the filtrate was evaporated. The glassy residue (275 mg, 82%) showed only one spot on t.l.c. with 3:2 benzene–methanol (R_F 0.50), but has not yet been crystallized; $[\alpha]_D^{20}$ +53° (*c* 1.0, methanol); i.r. datum: ν_{max}^{KBr} 3400 cm^{-1} (broad; OH and NH).

2-Acetamido-3-O-acetyl-5,6-O-isopropylidene-4-O-(tetra-O-acetyl-α-D-mannopyranosyl)-D-glucose diethyl acetal (**6**). — Compound **5** (275 mg) was dissolved in pyridine (3 ml), acetic anhydride (5 ml) was added, and the solution was kept for 24 h at room temperature. The solution was evaporated, and the residue was freed of solvents by repeated addition and distillation of toluene. Crystallization of the residue from benzene–pentane gave 400 mg (81%) of **6** as prisms, m.p. 138–139°; $[\alpha]_D^{20}$ +29° (*c* 1.3, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1760 (OAc), and 3400 cm^{-1} (NH); t.l.c. in 19:1 chloroform–ethanol: R_F 0.34.

Anal. Calc. for $C_{31}H_{49}NO_{17}$: C, 52.62; H, 6.98; N, 1.98; O, 38.44. Found: C, 52.82; H, 6.84; N, 2.02; O, 38.46.

2-Acetamido-2-deoxy-5,6-O-isopropylidene-4-O-α-D-mannopranosyl-D-glucose diethyl acetal (**7**). — A solution of **6** (555 mg) in methanol (15 ml) was treated with 0.1M sodium methoxide solution (2 ml) for 12 h at 4°. The solution was deionized by passage through Dowex-50 (H^+) ion-exchange resin (1.5 ml), and then evaporated. The residue was crystallized from ethyl acetate–pentane to give 340 mg (88%) of **7** as very hygroscopic, prismatic needles, m.p. 82–84° $[\alpha]_D^{20}$ +61° (*c* 0.6, methanol); i.r. data: ν_{max}^{KBr} 1625 (CONH) and 3400 cm^{-1} (OH and NH); g.l.c. datum: peak at t'_R 11.78; t.l.c. in 3:2 benzene–methanol: R_F 0.45.

Anal. Calc. for $C_{21}H_{39}NO_{12}$: C, 50.69; H, 7.92; N, 2.82; O, 38.59. Found: C, 50.63; H, 7.52; N, 2.92; O, 38.65.

2-Acetamido-2-deoxy-4-O-α-D-mannopyranosyl-α-D-glucose (**8**). — Compound **7** (340 mg) in 60% acetic acid (10 ml) was heated for 1 h at 80°. The solution was evaporated, and the residue was freed of solvents by repeated addition and distillation of methanol and toluene. Crystallization of the resulting syrup from methanol–acetone gave 155 mg (60%) of **8** as hygroscopic microcrystals, m.p. 154–156° (dec.); $[\alpha]_D^{20}$ +77 → +66° (equilibrium, *c* 0.655, 50% methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH) and 3370 cm^{-1} (OH); g.l.c. datum: peak at t'_R 11.70.

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.65; O, 45.90. Found: C, 43.76; H, 6.51; N, 3.50; O, 45.75.

2-Acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(tetra-O-acetyl-α-D-mannopyranosyl)-β-D-glucose (**9**). — *From* **8**. Compound **8** (280 mg) in pyridine (2 ml) was treated with acetic anhydride (4 ml) for 24 h at room temperature. Evaporation gave a residue which was dried by repeated addition and distillation of toluene. Crystallization from acetone–ether–pentane gave 430 mg (85%) as needles, m.p. 113–114°; $[\alpha]_D^{20}$ +1.5° (*c* 1.4, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1745 (OAc), and 3400 cm^{-1} (NH); t.l.c. in 4:1 benzene–methanol: R_F 0.41.

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07; O, 42.50. Found: C, 50.21; H, 6.12; N, 1.97; O, 41.98.

From **6**. A solution of **6** (220 mg) in 60% acetic acid (5 ml) was heated for 1 h at

80°, and then evaporated. The residue, which showed two spots on t.l.c. (one of which corresponded to **9**), was acetylated by treatment with pyridine and acetic anhydride, as just described, to give 170 mg (80%) of **9**, m.p. and mixed m.p. with compound obtained from **8**, 112–113°.

REFERENCES

1 M. SHABAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 17 (1971) 193.
2 H. M. FLOWERS AND R. W. JEANLOZ, *J. Org. Chem.*, 28 (1963) 1377; E. S. RACHAMAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 10 (1969) 429, 435; M. SPINOLA AND R. W. JEANLOZ, *J. Biol. Chem.*, 245 (1970) 4158; *Carbohyd. Res.*, 15 (1970) 361.
3 E. A. TALLEY, D. D. REYNOLDS, AND W. L. EVANS, *J. Amer. Chem. Soc.*, 65 (1943) 575.
4 M. SHABAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 17 (1971) 411.
5 K. HEYNS, R. HARRISON, K. PROPP, AND H. PAULSEN, *Chem. Commun.*, (1966) 671; K. HEYNS, K. PROPP, R. HARRISON, AND H. PAULSEN, *Chem. Ber.*, 100 (1967) 2655.
6 M. L. WOLFROM AND F. SHAFIZADEH, *J. Org. Chem.*, 21 (1956) 89.
7 J. STANĚK, *Nature*, 179 (1957) 97.
8 S. ENGLARD, G. AVIGAD, AND I. LISTOWSKY, *Carbohyd. Res.*, 2 (1966) 380.