THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-3-*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 August 14th, 1970; accepted September 11th, 1970)

ABSTRACT

Condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside gave crystalline benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside. Removal of the benzylidene group gave a crystalline compound which was de-*O*-acetylated to crystalline benzyl 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucopyranoside, characterized as the hexaacetate. The same products were obtained by de-*O*-acetylation of the condensation product, followed by hydrolysis of the benzylidene group and acetylation. Catalytic hydrogenolysis of the benzyl group gave crystalline 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucose, characterized by a crystalline heptaacetate. This disaccharide may be used as a reference compound in the study of glycoproteins by partial hydrolysis with acid, and as the starting material for the synthesis of glycopeptides.

INTRODUCTION

Most glycoproteins present in such animal fluids as blood and pancreatic juice contain a carbohydrate moiety linked to a protein chain through a glycosylamine linkage[1]. The carbohydrate core in the vicinity of the carbohydrate–protein bond is composed of 2-acetamido-2-deoxy-β-D-glucopyranosyl and α-D-mannosyl residues[2], but very little information on the precise structure of this core is available. Recently, a compound consisting of an α-D-mannopyranosyl group linked to *N*-(L-aspart-4-oyl)chitobiosylamine has been isolated from ribonuclease[3].

As part of a program of synthesis of (*a*) fragments of carbohydrate chains of glycoproteins for identification by gas–liquid chromatography combined with mass spectrometry[4,5], and (*b*) antigenic glycoproteins[6], we have accomplished the synthesis of 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose.

---

*Amino sugars. LXVIII. This is publication No. 524 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.

DISCUSSION

Condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[7] (**1**) with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[8] (**2**) in the presence of mercuric cyanide gave crystalline benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**3**) in 35% yield. Koenigs–Knorr condensation of **1** with various D-hexoses in the presence of silver oxide is known[9] to give mainly the α-D-glycosides. Similarly, treatment of the benzoate analog of **1** with methanol in the absence of an acid acceptor gave the α-D-glycoside[10]. As shown by the optical rotation of compound **3**, condensation in the presence of mercuric cyanide in benzene–nitromethane gave mainly the α-D anomer. Removal of the benzylidene group from **3** gave **5**, which was acetylated to the hexaacetate **6**, or saponified to give benzyl 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucopyranoside (**7**). Compound **7** was also obtained by de-*O*-acetylation of **3** to give crystalline **4**, followed by removal of the benzylidene group. All of these transformations proceeded with excellent yields and, with the exception of **6**, the products were crystalline, thus showing that **3** was not composed of a mixture of anomers. Hydrogenolysis of the benzyl aglycon from **7** gave, in 92% yield, crystalline 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucose (**8**), further characterized by a crystalline heptaacetate (**9**).

The α-D configuration of the glycosidic linkage of disaccharides **3** to **9** is strongly suggested by a comparison of their optical rotations with those of the parent mono-

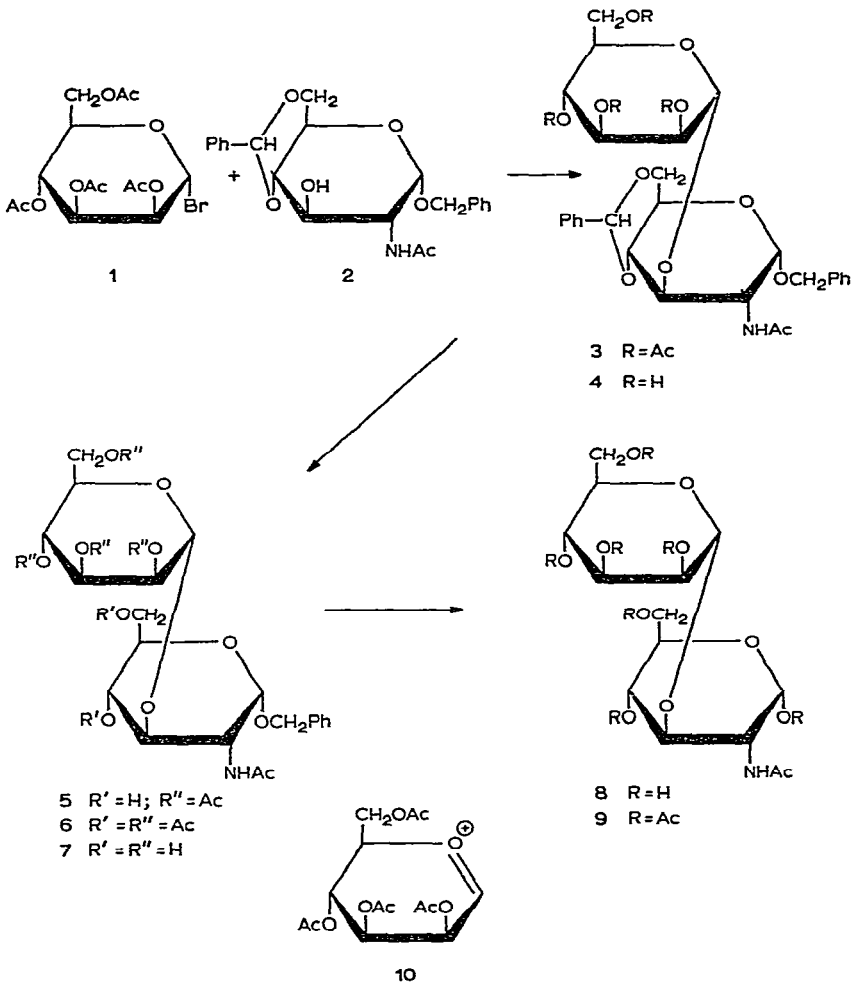
TABLE I

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

Compound	$[M]_D$ (*degrees*) $\times 10^{-2}$
Methyl tetra-*O*-acetyl-α-D-mannopyranoside[a] (**11**) + benzyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[b] (**12**) (Ref. 8)	+539
Methyl tetra-*O*-acetyl-β-D-mannopyranoside[a] (**13**) + compound **12**[b]	+181
Compound **3**[a]	+496
Compound **11**[a] + benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside[a] (**14**) (Ref. 8)	+628
Compound **13** + compound **14**	+270
Compound **7**[a]	+574
Methyl α-D-mannopyranoside[c] + 2-acetamido-2-deoxy-α-D-glucose[c]	+295
Methyl α-D-mannopyranoside[c] + 2-acetamido-2-deoxy-β-D-glucose[c]	+93
Compound **8**[d], at start of mutarotation	+234
Compound **8**[d], at equilibrium	+222
Compound **11**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[a]	+543
Compound **11**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[a]	+181
Compound **9**[a]	+440

[a]Optical rotation determined in chloroform; [b]in pyridine; [c]in water; [d]in 2:3 water–methanol.

saccharides[5,11–13] (see Table I). The α-D configuration can be assigned to C-1 of the 2-acetamido-2-deoxy-D-glucose residue of **8** on the basis both of mutarotation and initial optical rotation; the equilibrium mixture consists preponderantly of the α-D anomer. Similarly, the fully acetylated derivative **9** possesses the α-D configuration at both of its anomeric carbon atoms.



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), 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), 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); all compounds studied showed only one spot. Evaporations were conducted *in vacuo* with the bath temperature below 40°. Solutions in volatile solvents of less than 5 ml were evaporated under a stream of nitrogen. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-(tetra-O-acetyl-α-D-manno-pyranosyl)-α-D-glucopyranoside (**3**). — A mixture of dry benzyl 2-acetamido-4,6-*O*-benzylidene-α-D-glucopyranoside[8] (**2**, 1.5 g) and mercuric cyanide (0.8 g) in dry 1:1 benzene–nitromethane (180 ml) was concentrated to 130 ml under atmospheric pressure, and cooled to room temperature. A solution of tetra-*O*-acetyl-α-D-manno-pyranosyl bromide[7] (**1**, 2.5 g) in 1,2-dichloroethane (25 ml) was added, and the mixture was stirred for 48 h, and diluted with 1,2-dichloroethane (100 ml). The mixture was washed successively with a cold, saturated solution of sodium hydrogen carbonate and water, dried (anhydrous sodium sulfate), and evaporated to a syrup (3.5 g), which was chromatographed on silica gel with 19:1 chloroform–ethanol to give a fraction enriched in compound **3**. Crystallization from abs. ethanol gave prismatic needles, 1.3 g (35%), m.p. 171°; $[\alpha]_D^{20}$ +68° (*c* 1.4, chloroform), $[\alpha]_D^{20}$ +69° (*c* 1.1, abs. ethanol); i.r. data: ν_{max}^{KBr} 1665 (CONH), 1750 (OAc), and 3425 cm^{-1} (NH); n.m.r. data (chloroform-*d*): τ 2.63 (10 H, 2 Ph), 3.27 (deuteratable doublet, *J* 9.0 Hz, NH), 8.02 (3 OAc+NHAc); the mass spectrum showed peaks at *m/e* 91 ($PhCH_2$), 331 (**10**), 532 ($M^+ - CH_2Ph$, PhCHO); 638 ($M^+ - CH_2Ph$); t.l.c. in 19:1 chloroform–ethanol: R_F 0.48.

Anal. Calc. for $C_{36}H_{43}NO_{15}$: C, 59.24; H, 5.95; N, 1.91; O, 32.89. Found: C, 59.37; H, 6.02; N, 1.97; O, 32.59.

Attempts to hydrolyze **3** (3 mg) with 2M hydrochloric acid (1.5 ml) for 1 h at 100° gave unchanged starting material (as indicated by t.l.c.). After 6 h of hydrolysis, however, t.l.c. on a cellulose plate in 5:5:3:1 pyridine–ethyl acetate–water–acetic acid showed two zones corresponding to mannose and 2-amino-2-deoxyglucose hydrochloride.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-α-D-mannopyranosyl-α-D-glucopyranoside (**4**). — A solution of **3** (440 mg) in methanol (50 ml) was treated with 0.2M sodium methoxide (1.5 ml) for 24 h at 0°. The crystalline product was filtered off, washed with methanol and water, and dried. Recrystallization from

p-dioxane–methanol gave 280 mg (83%) of **4** as needles, m.p. 326–327°; $[\alpha]_D^{20}$ +121° (*c* 0.5, pyridine); i.r. data: ν_{max}^{KBr} 1655 (CONH) and 3350 cm^{-1} (OH); t.l.c. in 1:1 ethyl acetate–ether: R_F 0.15.

Anal. Calc. for $C_{28}H_{35}NO_{11}$: C, 59.88; H, 6.28; N, 2.45; O, 31.33. Found: C, 59.81; H, 6.25; N, 2.45; O, 31.09.

Reacetylation of **4** with pyridine and acetic anhydride for 24 h at room temperature gave **3**, as indicated by m.p., mixed m.p., t.l.c., and i.r. spectral data.

*Benzyl 2-acetamido-2-deoxy-3-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*D*-glucopyranoside* (**5**). — A solution of **3** (200 mg) in 60% acetic acid (5 ml) was heated for 30 min at 100°, and evaporated. The residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from acetone–ether gave 150 mg (76%) of **5** as long needles, m.p. 174–175°; $[\alpha]_D^{20}$ +121° (*c* 0.8, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1735 (OAc), and 3500 cm^{-1} (OH); t.l.c. in 4:1 chloroform–ethanol: R_F 0.34.

Anal. Calc. for $C_{29}H_{39}NO_{15}$: C, 54.28; H, 6.12; N, 2.18; O, 37.40. Found: C, 54.21; H, 6.13; N, 2.12; O, 37.23.

*Benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-3-*O*-(tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*D*-glucopyranoside* (**6**). — *From* **3**. Compound **3** (200 mg) was heated with 60% acetic acid (5 ml) for 30 min at 100°. The solution was evaporated, and the residue was dried in the presence of phosphorus pentaoxide, and then treated with pyridine (2 ml) and acetic anhydride (3 ml) for 24 h at room temperature. Evaporation gave an amorphous product which was purified by chromatography on silica gel with 1:1 ethyl acetate–ether to afford 222 mg (92%) of **6**; $[\alpha]_D^{20}$ +80° (*c* 0.7, chloroform); i.r. data: ν_{max}^{KBr} 1655 (CONH) and 1750 cm^{-1} (OAc); t.l.c. in 1:1 ethyl acetate–ether: R_F 0.54.

Anal. Calc. for $C_{33}H_{34}NO_{17}$: C, 54.61; H, 5.97; N, 1.92; O, 37.41. Found: C, 54.71; H, 6.00; N, 1.85; O, 37.00.

From **5** *and* **7**. Acetylation of either **5** or **7** with pyridine and acetic anhydride in the usual way gave **6**, as shown by t.l.c., i.r. spectrum, and optical rotation.

*Benzyl 2-acetamido-2-deoxy-3-*O*-α-*D*-mannopyranosyl-α-*D*-glucopyranoside* (**7**). — *From* **5**. A solution of **5** (430 mg) in methanol (5 ml) was treated with 0.2M sodium methoxide solution (0.5 ml) for 24 h at 5°. The solution was deionized by passage through Dowex-50 (H^+) ion-exchange resin (1 ml) and then evaporated. The residue was crystallized from methanol to give 242 mg (74%) of **7** as needles, m.p. 252°; $[\alpha]_D^{20}$ +168° (*c* 1.1, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH) and 3300 cm^{-1} (OH); g.l.c. data: peak at t'_R 26.75; t.l.c. in 2:1 benzene–methanol: R_F 0.28.

Anal. Calc. for $C_{21}H_{31}NO_{11}$: C, 53.26; H, 6.59; N, 2.95; O, 37.21. Found: C, 52.97; H, 6.53; N, 2.96; O, 36.58.

From **6**. De-*O*-acetylation of **6** (100 mg) with 0.2M sodium methoxide (0.5 ml) by the procedure just described gave 54 mg (89%) of **7**, m.p. and mixed m.p. with the compound already described: 251–252°.

From **4**. Treatment of **4** with 60% acetic acid for 30 min at 100° gave **7**, m.p. and mixed m.p. with the compound already described: 251–252°.

2-Acetamido-2-deoxy-3-O-α-D-mannopyranosyl-α-D-glucose (**8**). — A solution of **7** (150 mg) in abs. ethanol (47 ml), water (2 ml), and acetic acid (1 ml) was hydrol genolyzed under pressure (3.4 atm.) in the presence of 10% palladium-on-charcoal (250 mg) for 72 h. The catalyst was filtered off, and the solution evaporated. Crystallization of the product from methanol–acetone gave 120 mg (92%) of **8** as very hygroscopic needles, m.p. 129–130° (dec.); $[\alpha]_D^{20}$ +61→+58° (equilibrium, *c* 1.5, 60% methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), 2950 (NH), and 3375 cm^{-1} (broad, OH); g.l.c. data: double peak, at t'_R 14.6, 14.8.

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.85; H, 6.57; N, 3.65; O, 45.90. Found: C, 43.65; H, 6.64; N, 3.54; O, 45.73.

2-Acetamido-1,4,6-tri-O-acetyl-2-deoxy-3-O-(tetra-O-acetyl-α-D-mannopyranosyl)-α-D-glucose (**9**). — Compound **8** (250 mg) was treated with pyridine (3 ml) and acetic anhydride (5 ml) for 24 h at room temperature. Evaporation gave a residue which crystallized from benzene in plates (350 mg, 82%), m.p. 188–190°; $[\alpha]_D^{20}$ +65° (*c* 1.3, chloroform); i.r. data: ν_{max}^{KBr} 1650 (CONH), 1750 (OAc), and 2975 cm^{-1} (NH); t.l.c. in 4:1 benzene–methanol: R_F 0.48.

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.55; H, 5.73; N, 2.12.

REFERENCES

1 A. NEUBERGER, A. GOTTSCHALK, AND R. D. MARSHALL, in A. GOTTSCHALK (Ed.), *Glycoproteins*, Elsevier, Amsterdam, 1966, p. 273.
2 For examples, see A. NEUBERGER AND R. D. MARSHALL, ref. 1, p. 390; M. D. MELAMED, *ibid.*, p. 326; E. R. B. GRAHAM,, *ibid.*, p. 359; AND R. W. JEANLOZ, *ibid.*, p. 391.
3 A. L. TARENTINO, T. H. PLUMMER, JR., AND F. MALEY, *Fed. Proc.*, 28 (1969) 606; *J. Biol. Chem.*, 245 (1970) 4150.
4 H. M. FLOWERS AND R. W. JEANLOZ, *J. Org. Chem.*, 28 (1963) 1377.
5 E. S. RACHAMAN AND R. W. JEANLOZ, *Carbohyd. Res.*, 10 (1969) 429, 435.
6 M. SPINOLA AND R. W. JEANLOZ, *J. Biol. Chem.*, 245 (1970) 4158; *Carbohyd. Res.*, 15 (1970) 361.
7 E. A. TALLEY, D. D. REYNOLDS, AND W. L. EVANS, *J. Amer. Chem. Soc.*, 65 (1943) 575.
8 P. H. GROSS AND R. W. JEANLOZ, *J. Org. Chem.*, 32 (1967) 2759.
9 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 39 (1961) 2474.
10 R K. NESS, H. G. FLETCHER, JR., AND C. S. HUDSON, *J. Amer. Chem. Soc.*, 72 (1950) 2200.
11 M. L. WOLFROM AND F. SHAFIZADEH, *J. Org. Chem.*, 21 (1956) 89.
12 J. STANĚK, *Nature*, 179 (1957) 97.
13 S. ENGLARD, G. AVIGAD, AND I. LISTOWSKY, *Carbohyd. Res.*, 2 (1966) 380.