Carbohydrate Research, 77 (1979) 255–261

Note

Syntheses of 2-acetamido-2-deoxy-3-*O*-, -4-*O*-, and -6-*O*-α-D-mannopyranosyl-D-glucoses

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(Received October 18th, 1978; accepted for publication in revised form, March 15th, 1979)

This work describes synthesis of 2-acetamido-2-deoxy-3-*O*-, -4-*O*-, and -6-*O*-α-D-mannopyranosyl-D-glucoses by condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide with benzyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside, benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside, and 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose, respectively. The title disaccharides were then obtained by the following deprotection methods: *p*-methoxybenzylidene by 80% acetic acid at 100°; *O*-acetyl by methanolic triethylamine or ammonia; and *O*-benzyl by hydrogenolysis in the presence of palladium-on-carbon. The anomeric configuration of the α-D-mannopyranosyl linkage in the disaccharides was confirmed on the basis of their susceptibility to hydrolysis by the α-D-mannosidase from *Aspergillus niger*.

Variously linked 2-acetamido-2-deoxy-*O*-α-D-mannopyranosyl-D-glucoses are desirable as reference compounds in the study of glycoprotein structure, and also as potential substrates in the isolation and characterization of α-D-mannosidases[1–6] and glycosyltransferases. D-Mannose and 2-acetamido-2-deoxy-D-glucose constitute the core of the asparagine-linked oligosaccharides of many glycoproteins in which a D-mannosyl residue is β-D-(1→4)-linked to the distal (from asparagine) 2-acetamido-2-deoxy-D-glucosyl residue of the *N*,*N*′-diacetylchitobiose constituent[7]; depending upon the complexity of the carbohydrate structure[7], to the core-mannose residue are attached chains comprised of (proceeding towards the nonreducing end) α-D-mannopyranosyl, 2-acetamido-2-deoxy-β-D-glucopyranosyl, β-D-galactopyranosyl, and sialic acid residues. L-Fucopyranose may also be present, joined by an α linkage to the innermost or the outermost 2-acetamido-2-deoxy-D-glucopyranosyl residue[8]. In this connection, Shaban and Jeanloz have reported syntheses of 2-acetamido-2-deoxy-3-*O*-α-[9] (**1**) and -β-[10], -4-*O*-α-[11] (**2**), and -β-[12], and -6-*O*-α-D-mannopyrano-

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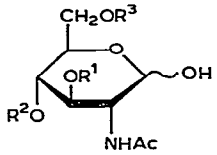
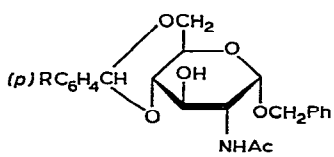
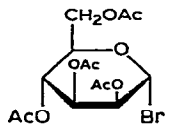


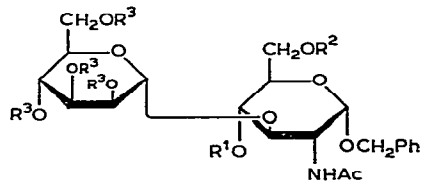
1 R^1 = α-D-mannopyranosyl R^2 = R^3 = H
2 R^2 = α-D-mannopyranosyl R^1 = R^3 = H
3 R^3 = α-D-mannopyranosyl R^1 = R^2 = H





5 R = H
6 R = OMe

4



7 (R^1 R^2) = >CHC$_6$H$_4$OMe(*p*) R^3 = Ac

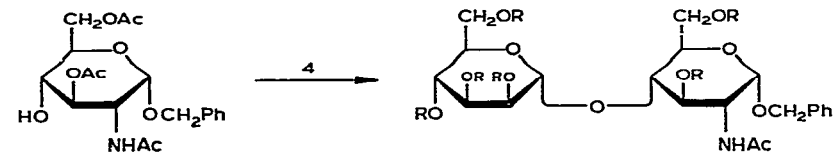
8 R^1 = R^2 = H R^3 = Ac
9 R^1 = R^2 = R^3 = H

syl-D-glucoses[13] (**3**). We report here the synthesis of disaccharides **2** and **3** by routes different from those followed by Shaban and Jeanloz[11,13]. 2-Acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucopyranose (**1**) was also synthesized by way of the Koenigs–Knorr reaction of tetra-*O*-acetyl-α-D-mannopyranosyl bromide[14] (**4**) with benzyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside (**6**); Shaban and Jeanloz[9] employed condensation of **4** with the 4,6-*O*-benzylidene analog (**5**) of **6**.

RESULTS AND DISCUSSION

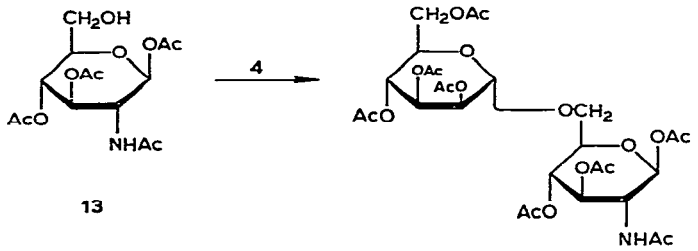
Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**4**) was condensed with benzyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside[15] (**6**) under conditions similar to those used by Shaban and Jeanloz[9] for condensation of **4** with **5**. The 4,6-substituent was removed from **7** by hydrolysis with 80% acetic acid at 100°, and benzyl 2-acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**8**) was obtained in 40% overall yield from **6**. Deacetylation of **6** with aqueous triethylamine at 4° afforded benzyl 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucopyranoside (**9**) in 93% yield; hydrogenolysis in the presence of palladium-on-carbon catalyst gave **1** in 82% yield.

For synthesis of the 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-D-glucose



10

11 R = Ac
12 R = H



13

14

derivative **2**, Shaban and Jeanloz[11] resorted to the reaction of **4** with an open-chain derivative, 2-amino-2-*N*,3-*O*-carbonyl-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal, as the 4-OH group of benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside failed[13] to react with **4**. The inability of the 4-hydroxyl group of other derivatives of 2-acetamido-2-deoxy-D-glucopyranose to undergo reaction with acetylated glycosyl halides has also been reported[13,16–18] by others. However,

TABLE I

MOLECULAR ROTATIONS OF 2-ACETAMIDO-1,3,4-TRI-*O*-ACETYL-2-DEOXY-6-*O*-(2,3,4,6-TETRA-*O*-ACETYL-α-D-MANNOPYRANOSYL)-α- AND -β-D-GLUCOPYRANOSE (**14**) COMPARED WITH THE SUM OF THOSE OF THE CONSTITUENTS

Compound	$[\alpha]_D^a$ *(degrees)*	$[M]_D^b$ *(degrees)*	*Reference*
2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-glucopyranose			
α Anomer (**15**)	+91.6	+317.9	21
β Anomer (**13**)	+5.5	+19.1	20
Methyl 2,3,4,6-tetra-*O*-acetyl-D-mannopyranoside			
α Anomer (**16**)	+49.0	+177.4	22
β Anomer (**17**)	−47.0	−170.1	22
14	+36.1	+244.4	This work
α Anomer of **14**	+68.0	+460.4	13
15 + **16**		+495.3	
15 + **17**		+147.8	
13 + **16**		+196.5	
13 + **17**		−151.0	

[a]In chloroform. [b]$[M]_D = [\alpha]_D \times$ mol. wt./100.

condensation of **4** with benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside[19] (**10**) in acetonitrile in the presence of mercuric cyanide and mercuric bromide, followed by *O*-deacetylation of the resultant **11** without isolation, afforded **12** in 17% overall yield from **10**. Palladium-catalyzed hydrogenolysis of **12** yielded a monohydrate of **2** in 71% yield.

Condensation of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose[20] (**13**) with **4** in acetonitrile in the presence of mercuric cyanide afforded crystalline **14** in 45% yield. The molecular rotation (+244°) of **14** was in reasonable agreement with that (+197°) calculated from the molecular rotations of its component monosaccharides (Table I). For 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranose (the α anomer of **14**), a molecular rotation of +460°, in good agreement with the calculated value (+495°, see Table I), has been reported[13]. *O*-Deacetylation of **14** was achieved with 2% methanolic ammonia, and the crystalline disaccharide **3** was isolated in 71% yield. The physical properties of **3** thus prepared were in agreement with those of **3** obtained by Shaban and Jeanloz[13] through condensation of **4** with both benzyl 2-acetamido-3-*O*-acetyl- and -3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranosides.

The α-D-mannosidase[5] from *Aspergillus niger* failed to act on the (1→3)-linked disaccharide (**1**), as this enzyme is specific only for (1→4)- and (1→6)-linked α-D-mannosides; accordingly, both **2** and **3** were hydrolyzed by this enzyme. None of the three disaccharides (**1**–**3**) were hydrolyzed[4] by either the β-D-mannosidase or the linkage-specific (1→2)-α-D-mannosidase from *A. niger*.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at room temperature with a Perkin–Elmer Model 141 automatic polarimeter. I.r. spectra were recorded with a Perkin–Elmer Model 457 spectrophotometer. Thin-layer chromatography was performed on silica gel G (layer-thickness, 0.5 mm) with the following solvents: (*A*) 4:5:1 1-butanol–acetone–water, (*B*) 7:5:2 1-propanol–ethyl acetate–water. Solutions were evaporated under diminished pressure at <35°. Elementary analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

*Benzyl 2-acetamido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-glucopyranoside* (**8**). — A solution of benzyl 2-acetamido-2-deoxy-4,6-*O*-(*p*-methoxybenzylidene)-α-D-glucopyranoside[15] (**6**, 2.6 g, 6.1 mmol) in 1:1 benzene–nitromethane (350 mL) was dried by azeotropic distillation of ~60 mL of the solvent. The temperature of the solution was lowered to 40–50°, and mercuric cyanide (1.51 g, 6.0 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide[14] (**4**, 2.5 g, 6.1 mmol) were added. The mixture was stirred for 20 h at 45°, at which time the same amounts of mercuric cyanide and **4** as previously used were added. Stirring was continued for another 36 h, whereupon the mixture was cooled to room temperature, diluted with benzene (300 mL), and washed successively with saturated aqueous

sodium hydrogencarbonate and water. The dried (sodium sulfate) organic layer was evaporated to a syrup, which was crystallized from ~10:1 pentane–benzene. Recrystallization from benzene–ether–pentane afforded 3.2 g of the fully protected, essentially homogeneous (t.l.c.), disaccharide **7**.

A solution of the foregoing derivative **7** (2.5 g) in acetic acid (100 mL) was diluted with water (25 mL). The clear solution was stirred for 10 min at 100°, and then evaporated to dryness, traces of acetic acid and water being removed by repeated evaporation of toluene from the residue. *p*-Methoxybenzaldehyde was removed from the residue by extraction with ether (200 mL), and the ether-insoluble residue was crystallized from ethanol–ether–pentane to afford 1.2 g (40% from **6**) of compound **8**, m.p. 170–171°, $[\alpha]_D$ +102.5° (*c* 1.0, chloroform); ν_{max}^{KBr} 3500 (OH), 3320 (NH), 1735 (ester carbonyl), 1645 (amide I), and 1550 cm^{-1} (amide II).

Shaban and Jeanloz[9] reported, for **8** prepared *via* condensation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**5**) with **4**, the values m.p. 174–175°, $[\alpha]_D^{20}$ +121° (*c* 0.8, methanol).

*Benzyl 2-acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-α-D-glucopyranoside* (**9**). — To a solution of **8** (800 mg) in methanol (20 mL) was added triethylamine (10 mL) and water (8 mL). After 16 h at 4°, the volatile components were removed with the aid of repeated addition and evaporation of toluene. The residue thus obtained was crystallized from methanol–ether to give 0.55 g (93%) of **9**, m.p. 248–250°, $[\alpha]_D$ +161° (*c* 0.5, 95% ethanol) [lit.[9] m.p. 252°, $[\alpha]_D^{20}$ +168° (*c* 1.1, methanol)]; R_F 0.79 (solvent *A*), 0.73 (solvent *B*); ν_{max}^{KBr} 3460–3280 (OH, NH), 1645 (amide I), 1545 (amide II), and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{21}H_{31}NO_{11}$: C, 53.27; H, 6.60; N, 2.96. Found: C, 53.12; H, 6.65; N, 3.17.

*2-Acetamido-2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose* (**1**). — Compound **9** (300 mg) was dissolved in 95% ethanol (250 mL) containing acetic acid (1 mL), and hydrogenolyzed under pressure (45 $lb.in^{-2}$) in the presence of 5% palladium-on-carbon catalyst (600 mg) for 36 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated to dryness. The residue was crystallized from ethanol to yield 200 mg (82%) of **1**, m.p. 130–132°, $[\alpha]_D$ +56.5° (1 h, *c* 1.0, water). Shaban and Jeanloz[9] reported for the α anomer of **1** m.p. 129–130° (dec.), $[\alpha]_D^{20}$ +61 → +58° (equil.; *c* 1.5, 60% methanol).

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.65. Found: C, 43.60; H, 6.71; N, 3.31.

*Benzyl 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-α-D-glucopyranoside* (**12**). — A solution of benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside[19] (**10**, 2.0 g, 5.1 mmol) in dry acetonitrile (20 mL) containing mercuric cyanide (0.62 g, 2.5 mmol) and mercuric bromide (0.90 g, 2.5 mmol) was stirred with compound[14] **4** (2.8 g, 6.8 mmol) for 8 h at room temperature. The solvent was evaporated off, and the residual syrup was dissolved in chloroform (150 mL). The chloroform solution was washed successively with M aqueous potassium bromide (3 × 100 mL), saturated aqueous sodium hydrogencarbonate (2 × 100 mL), and water (2 × 100 mL). The

solvent was evaporated from the dried (sodium sulfate) chloroform solution, and the residual syrup was acetylated with pyridine (20 mL) and acetic anhydride (12 mL). Removal of solvent from the mixture (toluene), afforded crude **11**, which was deacetylated without further purification.

Crude **11** thus obtained, in methanol (36 mL), was mixed with triethylamine (18 mL) and water (18 mL). After 20 h at 4°, the mixture was evaporated. The residue was chromatographed on a column (2.5 × 60 cm) of silica gel with 13:6:1 chloroform–methanol–water as solvent. Fractions containing **12** (t.l.c.) were pooled and evaporated to yield 0.60 g of residue, which from ethanol–ether afforded 0.40 g (17% from **10**) of **12** as a hygroscopic, amorphous solid. For analyses, **12** (20 mg) was dissolved in methanol, and the solution was stirred with Dowex-50 X-4 (H^+) resin, which was filtered off after 20 min. The filtrate was decolorized with Norit A and evaporated to dryness. Amorphous **12** was precipitated by ether from a solution of the residue in ethanol; $[\alpha]_D$ +155° (*c* 1.0, 50% methanol); R_F 0.75 (solvent *A*), 0.71 (solvent *B*); ν_{max}^{KBr} 3420–3380 (broad, OH and NH), 1650 (amide I), 1540 (amide II), and 700 cm^{-1} (phenyl).

Anal. Calc. for $C_{21}H_{31}NO_{11} \cdot H_2O$: C, 51.32; H, 6.77; N, 2.85. Found: C, 51.24; H, 6.70; N, 2.85.

*2-Acetamido-2-deoxy-4-*O*-α-*D*-mannopyranosyl-*D*-glucopyranose* (**2**). — Compound **12** (350 mg), dissolved in a mixture of 95% ethanol (150 mL) and acetic acid (2 mL), was hydrogenolyzed, as already described for **1**, in the presence of 5% palladium-on-carbon catalyst (800 mg) for 48 h at a pressure of 45 $lb.in^{-2}$. The catalyst was removed by filtration through Celite and washed with 95% ethanol. The combined filtrates were evaporated, and crystallization of the residue from methanol–ether–acetone afforded the disaccharide **2** (210 mg, 71%) as a monohydrate, m.p. 146–150° (softened at ~100°), $[\alpha]_D$ +71° (1 h, *c* 1.0, 50% methanol) [lit.[11] m.p. 154–156° (dec.), $[\alpha]_D^{20}$ +77 → +66° (equil., *c* 0.655, 50% methanol)]; R_F 0.49 (solvent *A*), 0.39 (solvent *B*); ν_{max}^{KBr} 3400–3300 (broad, OH, NH), 1640 (amide I), and 1545 cm^{-1} (amide II).

Anal. Calc. for $C_{14}H_{25}NO_{11} \cdot H_2O$: C, 41.89; H, 6.78; N, 3.49. Found: C, 41.91; H, 6.64; N, 3.20.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-β-*D*-glucopyranose* (**14**). — A solution of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose[20] (**13**, 3.1 g, 8.9 mmol) in dry acetonitrile (30 mL) containing mercuric cyanide (2.25 g, 8.9 mmol) was stirred with tetra-*O*-acetyl-α-D-mannopyranosyl bromide[14] (**4**, 5.7 g, 13.9 mmol) for 4 h at room temperature. The mixture was evaporated and the residue dissolved in chloroform (200 mL). The solution was washed with M aqueous potassium bromide (3 × 150 mL), saturated aqueous sodium hydrogencarbonate (2 × 150 mL), and water (2 × 150 mL), dried (sodium sulfate), and evaporated to dryness. The residual syrup was dissolved in chloroform (20 mL), the solution was diluted with ether (30 mL), and petroleum ether (low-boiling) was then added with stirring until turbidity was permanent. After 5 h, the crystalline product (3.52 g) was collected by filtration and recrystallized from

acetone–hexane–ether to afford 2.70 g (45%) of **14**, m.p. 132–134°, $[\alpha]_D$ +36.1° (*c* 1.0, chloroform); ν_{max}^{KBr} 3350 (NH), 1750 (ester carbonyl), 1660 (amide I), and 1540 cm^{-1} (amide II).

Anal. Calc. for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.37; H, 5.87; N, 1.96.

*2-Acetamido-2-deoxy-6-*O*-α-*D*-mannopyranosyl-*D*-glucose* (**3**). — A solution of **14** (400 mg) in 2% methanolic ammonia (15 mL) was kept for 20 h at 4°. It was then evaporated and the resulting residue crystallized from methanol–acetone to give 160 mg (71%) of the disaccharide **3**, m.p. 136–138°, $[\alpha]_D$ +50.5° (1 h, *c* 1.0, water) [lit.[13] m.p. 142–144° (softening at 136°), $[\alpha]_D^{20}$ +38 → +35° (equil. after 48 h, *c* 1.2, 50% methanol)]: R_F 0.45 (solvent *A*), 0.35 (solvent *B*); ν_{max}^{KBr} 3400–3300 (broad, OH and NH), 1645 (amide I), and 1550 cm^{-1} (amide II).

Anal. Calc. for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57; N, 3.65. Found: C, 43.64; H, 6.77; N, 3.77.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of this work by a grant (AM-17441) from the National Institutes of Health, United States Public Health Service.

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