THE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-4-*O*-α-L-FUCOPYRANOSYL-α-D-GLUCOSE*

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(Received June 18th, 1971)

ABSTRACT

Condensation of 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide with the 2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal, in the presence of mercuric cyanide or silver carbonate, gave the 2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(tri-*O*-acetyl-α-L-fucopyranosyl)-D-glucose diethyl acetal in 32 and 13% yield, respectively. Hydrolysis of the acetyl and carbonate protective groups by alkali, and of the acetal and isopropylidene groups by acid, followed by introduction of an *N*-acetyl group, gave the title disaccharide, which was characterized by a hexa-*O*-acetyl derivative. This disaccharide is useful as a reference compound in the study of the carbohydrate fragments obtained by enzymic hydrolysis, or partial hydrolysis with acid, of glycoproteins and glycolipids, and as a starting material for their synthesis.

INTRODUCTION

2-Amino-2-deoxy-*O*-α-L-fucopyranosyl-D-glucose disaccharides have been reported to be a part of the carbohydrate determinant of blood-group glycoproteins having A, B, H, and Le^a activity[1], as well as of the carbohydrate moiety of a glycolipid isolated from erythrocytes and from cancer tissues[2]. In previous communications[3,4] from this laboratory, the synthesis of 2-acetamido-2-deoxy-3- and 6-*O*-β-L-fucopyranosyl-D-glucose had been described as part of a program of structural identification and synthesis of glycoproteins and glycolipids. In the present report, the synthesis of the disaccharide 2-acetamido-2-deoxy-4-*O*-α-L-fucopyranosyl-D-glucose is described.

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*Amino Sugars LXXVI. This is publication No. 543 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, 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-03564-12), National Institutes of Health, U. S. Public Health Service. Preliminary communication: *Abstr. Papers Amer. Chem. Soc. Meeting*, 161 (1971) C-1.

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DISCUSSION AND RESULTS

Because it had been shown that the 4-hydroxyl group of derivatives of 2-acetamido-2-deoxy-D-glucopyranosides does not react with glycosyl halides[3,5], the condensation of an open-chain compound, the 2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal[6] (**2**), with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide[7] (**1**) was performed. This condensation in the presence of mercuric cyanide gave 2-amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(tri-*O*-acetyl-α-L-fucopyranosyl)-D-glucose diethyl acetal 2,3-carbonate (**3**) in 32% yield (based on **2**).

Although the values of the molecular rotations of **3** and of the intermediates **4**–**8** lay between those expected for an α- and a β-L glycosidic linkage, respectively (see Table I), the n.m.r. spectrum of **3** (see Fig. 1) clearly indicated the α-L configura-

TABLE I

MOLECULAR ROTATIONS OF COMPOUNDS PREPARED, COMPARED TO THE SUM OF THOSE OF THEIR CONSTITUENTS

Compound	$[M]_D$ (*degrees*) × 10^{-2}
Methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside[a] (**10**) (Ref. 10) + compound **2**[a]	−751
Methyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside[a] (**11**) (Ref. 10) + compound **2**[a]	−171
Compound **3**[a]	−375
Methyl α-L-fucopyranoside (**12**)[b] (Ref. 11) + compound **2**[a]	−543
Methyl β-L-fucopyranoside (**13**)[b] (Ref. 11) + compound **2**[a]	−163
Compound **4**[c]	−391
Compound **10**[a] + 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal[b] (**14**) (Ref. 6)	−429
Compound **11**[a] + compound **14**[b]	+51
Compound **6**[a]	−106
Compound **12**[b] + 2-acetamido-2-deoxy-α-D-glucose[b] (Ref. 12)	−209
Compound **12**[b] + 2-acetamido-2-deoxy-β-D-glucose[b] (Ref. 13)	−399
Compound **8**[d], at start of mutarotation	−102
at equilibrium	−106
Compound **10**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose[a] (Ref. 14)	−102
Compound **10**[a] + 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose[a] (Ref. 15)	−455
Compound **9**[a]	−64

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

tion. The spectrum showed, in addition to the multiplet signal for five methyl groups at τ 8.77, signals for three acetoxyl groups, two axial and one equatorial, at τ 7.90, 7.95, and 8.15, respectively, indicating that compound **3** exists in the *C1* (L) conformation. The anomeric proton of this glycoside appeared as a one-proton doublet having $J_{1,2}$ 4.5 Hz, characteristic of axial–equatorial interaction[8], and this indicated the

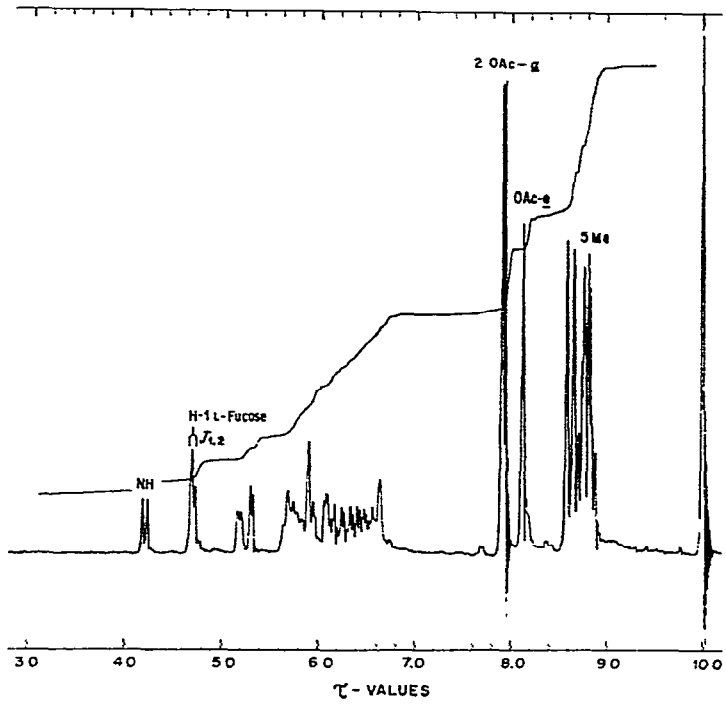
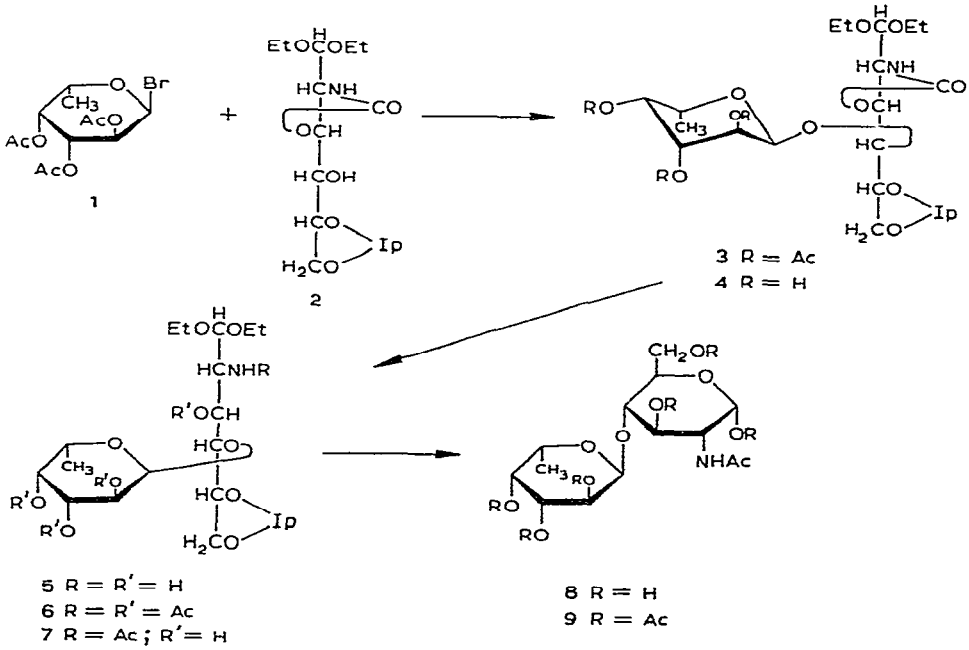


Fig. 1. Partial n.m.r. spectrum at 100 MHz of 2-amino-2-deoxy-5,6-*O*-isopropylidene-4-*O*-(tri-*O*-acetyl-α-L-fucopyranosyl)-D-glucose diethyl acetal 2,3-carbonate (3) in chloroform-*d*.



α-L configuration of the glycosidic linkage as a β-L-linkage, would give equatorial–equatorial interaction. The α-L configuration, in contrast to the β-L configuration obtained in the synthesis of the (1→3) and (1→6) disaccharides[3,4], indicates that the glycosidic linkages formed by a glycosyl halide reacting under the same conditions are dependent upon the position and, hence, upon the reactivity, of the hydroxyl group with which it reacts. It had been observed that 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide forms an α-L-(1→2)-linkage with galactose[7,9]. De-*O*-acetylation of **3** with a catalytic amount of sodium methoxide gave **4** as an amorphous solid having the elementary analysis expected. Saponification of the cyclic carbonate group of **4** with aqueous barium hydroxide yielded the unstable amine **5**, and this was acetylated to give crystalline **6**. De-*O*-acetylation of **6**, followed by removal of both the diethyl acetal and isopropylidene groups by dilute acetic acid gave the crystalline, free disaccharide **8**; this was characterized by a crystalline heptaacetate (**9**).

The molecular rotations of the disaccharide **8** at the start of mutarotation and at equilibrium indicated an α-D configuration for the 2-acetamido-2-deoxy-D-glucose residue, and the preponderance of the α-D anomer in the equilibrium mixture. The hexosamine residue of the heptaacetate **9** also has the α-D configuration.

EXPERIMENTAL

General. — Melting points were determined with a Mettler FP-2 apparatus, and correspond to "corrected melting point". 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. The n.m.r. spectrum was recorded with a JEOL MH-100 n.m.r. spectrometer for a solution 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 having a 5-foot column of Chromosorb GCQ (60–80 mesh, coated with 3% of OV-1; Applied Science Laboratories, State College, Pa.), programmed for a rise of 10° per min from 130 to 280°; 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), which was used without pretreatment. The ratio of the weight of substance to the weight of adsorbent was 1:80–1:120. The volume of the fractions collected was 3–4 ml per g 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°. Volumes of <5 ml of solutions in 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*-(tri-*O*-acetyl-α-L-fucopyranosyl)-D-glucose diethyl acetal 2,3-carbonate* (**3**). — (*A*) *Mercuric cyanide procedure.* A mixture of dry **2** (Ref. 6) (2.25 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 **1** (1.5 g) in dry benzene (15 ml) was added, and the mixture was stirred for 4 days at room temperature. Additional amounts of bromide **1** (0.5 g) and mercuric cyanide (0.5 g) were added, and the mixture was stirred for an additional 3 days. The mixture was diluted with benzene (100 ml), washed successively with a cold, saturated solution of sodium hydrogen carbonate (5 × 20 ml) and water, dried (sodium sulfate), and evaporated. The residue (4.3 g) was chromatographed on a column of silica gel; elution was made in the following order: (*a*) with 1 liter of 1:1 benzene–ether, to give 1.58 g of **1**; (*b*) with 2 liters of 2:5 acetone–chloroform, to give 2.4 g of a syrup enriched in **3**; and (*c*) with 500 ml of methanol, to give 300 mg of **2**. Fraction (*b*) was rechromatographed on silica gel with chloroform (4 liters) to give 1.3 g (32.5%) of pure **3**, which crystallized from chloroform–ether–pentane as needles, m.p. 210–212°; $[\alpha]_D^{20} -63°$ (*c* 1.2, chloroform); i.r. data: ν_{max}^{KBr} 1750 (oxazolidine CONH and OAc) and 3350 cm^{-1} (NH); n.m.r. data (chloroform-*d*): τ 4.39 (one-proton doublet, *J* 4.0 Hz, NH), 4.68 (one-proton doublet, H-1 of fucose, $J_{1,2}$ 4.5 Hz), 7.90, 7.95 (2 axial OAc), 8.15 (equatorial OAc), and 8.77 (15-proton multiplet, 5 CH_3) (see Fig. 1); t.l.c. in 19:1 chloroform–ethanol: R_F 0.41, in 4:1 benzene–methanol: R_F 0.49.

Anal. Calc. for $C_{26}H_{41}NO_{14}$: C, 52.79; H, 6.99; N, 2.37; O, 37.86. Found: C, 52.69; H, 6.92; N, 2.34; O, 37.72.

(*b*) *Silver carbonate procedure.* The condensation just described was repeated in dichloromethane, with silver carbonate as the acid acceptor, in the presence of iodine and Drierite. Stirring was continued for 72 h, and then the mixture was processed as described, to give 575 mg (13%) of needles, m.p. and mixed m.p. 210–212°, $[\alpha]_D^{20} -63°$ (*c* 1.325, chloroform), showing the same i.r. spectrum as the compound just described.

Anal. Calc. for $C_{26}H_{41}NO_{14}$: C, 52.79; H, 6.99; N, 2.37; O, 37.86. Found: C, 52.59; H, 6.90; N, 2.40; O, 37.92.

*2-Amino-2-deoxy-4-*O*-α-*L*-fucopyranosyl-5,6-*O*-isopropylidene-*D*-glucose diethyl acetal 2,3-carbonate* (**4**). — A solution of **3** (200 mg) in methanol (20 ml) was treated with two drops of M sodium methoxide in methanol and kept for 4 h at room temperature. The solution was de-ionized by passage through Dowex 50 (H^+) (2 ml), and then evaporated. The residue was chromatographed on a column of silica gel, first with 19:1 chloroform–methanol, and then with 7:3 benzene–methanol. Evaporation of the benzene–methanol fractions gave 130 mg (84%) of **4** as an amorphous solid, further purified by twice dissolving in ethyl acetate and precipitating with pentane; $[\alpha]_D^{20} -84°$ (*c* 1.7; methanol); i.r. data: ν_{max}^{KBr} 1750 (oxazolidine CONH), 3400 (broad) cm^{-1} (OH and NH); t.l.c. in 19:1 benzene–ethanol: R_F 0.56; in 4:1 benzene–methanol: R_F 0.30.

Anal. Calc. for $C_{20}H_{35}NO_{11}$: C, 51.59; H, 7.58; N, 3.01; O, 37.81. Found: C, 51.52; H, 7.54; N, 3.01; O, 37.78.

*2-Amino-2-deoxy-4-*O*-α-*L*-fucopyranosyl-5,6-*O*-isopropylidene-*D*-glucose diethyl acetal* (**5**). — A solution of **4** (675 mg) in water (8 ml) was treated with finely powdered

barium hydroxide octahydrate (1.2 g), and kept for 4 h at 80° under a stream of nitrogen. After the solution had been cooled, carbon dioxide was bubbled through it, and the barium carbonate was filtered off and washed with methanol. The filtrate and washings were combined, and evaporated to dryness. The residue was treated with methanol, the suspension filtered, and the filtrate evaporated, to give an unstable, amorphous solid (567 mg; 89%) which could not be crystallized; i.r. datum: ν_{max}^{KBr} 3400 (broad) cm^{-1} (NH and OH).

2-Acetamido-3-O-acetyl-2-deoxy-5,6-O-isopropylidene-4-O-(tri-O-acetyl-α-L-fucopyranosyl)-D-glucose diethyl acetal (**6**). — A solution of compound **5** (567 mg) in pyridine (5 ml) was treated with acetic anhydride (7 ml) for 24 h at room temperature, and evaporated, and the residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from ether–pentane gave 830 mg (96%) of plates, m.p. 153–155°; $[\alpha]_D^{20}$ −16° (*c* 1.8, chloroform); i.r. data: ν_{max}^{KBr} 1665 (CONH), 1745 (OAc), and 3420 cm^{-1} (NH); t.l.c. in 19:1 chloroform–ethanol: R_F 0.43.

Anal. Calc. for $C_{29}H_{47}NO_{15}$: C, 53.62; H, 7.29; N, 2.16; O, 36.94. Found: C, 53.53; H, 7.33; N, 2.06; O, 36.65.

2-Acetamido-2-deoxy-4-O-α-L-fucopyranosyl-5,6-O-isopropylidene-D-glucose diethyl acetal (**7**). — A solution of **6** (650 mg) in methanol (50 ml) was treated with 0.1M sodium methoxide in methanol (3 ml) for 16 h at 4°. The solution was de-ionized by passage through Dowex 50 (H^+) (2 ml) and then evaporated. The residue was purified by three times being dissolved in ethyl acetate and precipitated with pentane, to give 410 mg (85%) of **7** as an amorphous solid, $[\alpha]_D^{20}$ −56° (*c* 1.4, methanol); i.r. data: ν_{max}^{KBr} 1650 (CONH), and 3400 (broad) cm^{-1} (NH and OH); g.l.c. datum: peak at t'_R 5.90; t.l.c. in 13:7 benzene–methanol: R_F 0.40.

Anal. Calc. for $C_{21}H_{39}NO_{11}$: C, 52.37; H, 8.16; N, 2.91; O, 36.56. Found: C, 52.21; H, 8.09; N, 2.86; O, 36.50.

2-Acetamido-2-deoxy-4-O-α-L-fucopyranosyl-α-D-glucose (**8**). — A solution of compound **7** (360 mg) in 60% acetic acid (10 ml) was heated for 1 h at 80°, cooled, diluted to 300 ml, and freeze-dried (to obviate partial acetylation during distillation). Crystallization of the residue from methanol–acetone gave 175 mg (88%) of **8** as microcrystals containing one molecule of acetone of crystallization per molecule, m.p. 128–129°: $[\alpha]_D^{20}$ −24→−25° (*c* 0.8, 50% methanol); i.r. data: ν_{max}^{KBr} 1640 (CONH), and 3350 (broad) cm^{-1} (NH and OH); g.l.c. data: two peaks at t'_R 5.60 and 6.75.

Anal. Calc. for $C_{14}H_{25}NO_{10}\cdot(CH_3)_2CO$: C, 47.99; H, 7.35; N, 3.29; O, 41.37. Found: C, 48.19; H, 7.40; N, 3.47; O, 40.89.

2-Acetamido-1,3,6-tri-O-acetyl-2-deoxy-4-O-(tri-O-acetyl-α-L-fucopyranosyl)-α-D-glucose (**9**). — Compound **8** (100 mg) in pyridine (2 ml) was treated with acetic anhydride (3 ml) for 24 h at room temperature. The solution was evaporated, and the residue was dried by repeated addition and distillation of toluene. Crystallization of the residue from acetone–ether–pentane gave 48 mg (88%) of microcrystals, m.p. 94–96°; $[\alpha]_D^{20}$ −10° (*c* 0.99, chloroform); i.r. data: ν_{max}^{KBr} 1670 (CONH), 1740 (OAc), and 3370 cm^{-1} (NH); t.l.c. in 1:1 ethyl acetate–ether: R_F 0.18.

Anal. Calc. for $C_{26}H_{37}NO_{16}$: C, 50.41; H, 6.02; N, 2.26. Found: C, 50.39; H, 6.22; N, 2.26.

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