Carbohydrate Research, 195 (1989) 145–149
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

An economical access to [6-^{3}H]-labelled L-galactose and L-fucose

HELGA LAY, JOCHEN LEHMANN, LOTHAR ZISER,

Institut für Organische Chemie und Biochemie der Universität Freiburg i. Br., Albertstr. 21, D-7800 Freiburg i. Br. (F.R.G.)

AND WERNER REUTTER

Institut für Molekularbiologie und Biochemie, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33 (F.R.G.)

(Received April 17th, 1989; accepted for publication, June 16th, 1989)

In tissues of eukaryotic organisms, L-fucose occurs in small amounts at the terminal positions of *N*-glycans. The substantial increase of L-fucose in hepatoma[1], colon carcinoma[2], and neuroblastoma cells[3] indicates its potential significance in malignancy. In glycoproteins of the liver plasma membrane, L-fucose turns over several times faster than the protein backbone and the core sugars[4]. These involvements of L-fucose warrant further studies using the radiolabelled sugar.

Whole-animal studies, where large amounts of labelled material are needed, are expensive and an inexpensive procedure for the synthesis of labelled L-fucose is desirable. Galactose can be converted into its enantiomer by interchanging its functionalities at C-1 and C-6. This principle was used in the synthesis of L-galactose from D-galactose, starting from D-galacturonic acid[5].

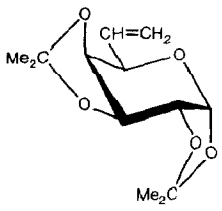
We now describe an alternative route for small-scale preparations of L-[6-^{3}H]galactose and, thereby, of L-[6-^{3}H]fucose and introduce the methylene group for protecting carbonyl functions.

1,2:3,4-Di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose[6], easily accessible by methyl sulfoxide (Me_2SO)-mediated oxidation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactose[7], can be converted in high yield into 6,7-dideoxy-1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hept-6-enose[8] (**1**), using the "instant ylid" methyltriphenylphosphonium bromide plus sodium amide. The commercially available "instant ylid" (Fluka) and a standardised procedure greatly facilitate the formerly tedious preparation that gave moderate yields[8,9]. Crude **1** was deprotected by aqueous acetic acid to give 6,7-dideoxy-D-*galacto*-hept-6-enose[9] (**2**) which, without purification, was reduced with sodium borohydride to give crystalline 1,2-dideoxy-L-*galacto*-hept-1-enitol (**4**). For the preparation of **4a***, sodium borotritide

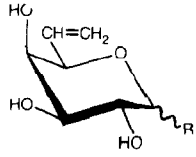
*The suffix "a" indicates [^{3}H]-labelling.

0008-6215/89/$ 03.50

was used with an excess of chromatographically pure **2**. The excess of **2** was converted almost quantitatively into *N*-*p*-nitrophenyl-6,7-dideoxy-L-*galacto*-hept-6-enosylamine (**3**) and separated from **4a** by chromatography on silica gel.

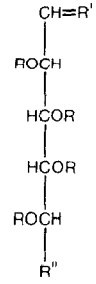


1



2 R = OH

3 R = $NHC_6H_4NO_2$-p



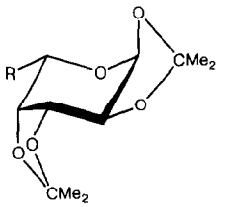
	R	R'	R''
4	H	CH_2	CH_2OH
4a	H	CH_2	C^3H_2OH
5	Ac	CH_2	CH_2OAc
6	Ac	O	CH_2OAc

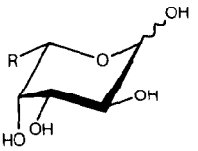
Ozonolysis[10] of **4** or **4a** gave L-galactose (**11**) or (**11a**) in quantitative yield. Alternatively, the penta-acetate (**5**) of **4** could be degraded[11] by OsO_4/IO_4 to give 2,3,4,5,6-penta-*O*-acetyl-L-galactose (**6**), which, without purification, also yielded L-galactose (**11**) upon deacetylation. The intermediates for the preparation of L-[6-^{3}H]fucose (**12a**) from **11a** were not isolated, since the identity of **12a** was proved unequivocally by comparison with L-fucose†. 1,2:3,4-Di-*O*-isopropylidene-β-L-[6-^{3}H]galactose[7] (**7a**), 1,2:3,4-di-*O*-isopropylidene-6-*O*-tosyl-β-L-[6-^{3}H]galactose[12] (**8a**), 6-*S*-benzoyl-1,2:3,4-di-*O*-isopropylidene-6-thio-β-L-[6-^{3}H]galactose[13] (**9a**), and finally 1,2:3,4-di-*O*-isopropylidene-β-L-[6-^{3}H]fucose[13] (**10a**) were prepared according to methods described in the literature. Compound **9a** was isolated by chromatography on silica gel and the other intermediates were used without purification. Hydrolysis of **10a** with aqueous acetic acid yielded L-[6-^{3}H]fucose (**12a**) which co-chromatographed in various solvent systems with L-fucose. The L-[6-^{3}H]fucose can be stored on Whatman No. 3 paper and its yield from **4a**, determined on the basis of radioactivity, was ~90%.

EXPERIMENTAL

Methods. — All reactions were monitored by t.l.c. on Silica Gel F_{254} (Merck), using *A*, EtOAc–MeOH–H_2O (7:2:1); and *B*, EtOAc–cyclohexane (1:1). Column

†The intermediates **7a**–**10a** were identified by t.l.c. comparison with authentic samples.





7a R = C^3H_2OH **11a** R = C^3H_2OH

8a R = C^3H_2OTs **12a** R = C^3H_3

9a R = C^3H_2SCOPh

10a R = C^3H_3

chromatography was performed on Silica Gel 32–63, 60 A (ICN). Radioactive material was detected with a Berthold Automatic TLC-Linear Analyzer, or by autoradiography using X-ray film "Curix" (Agfa-Gevaert), and assayed with a Berthold BF 815 liquid scintillation counter, using Quickszint 501 (Zinsser) for solutions in organic solvents, and Quickszint 1 (Zinsser) for aqueous solutions. P.c. was performed on Whatman No. 3 paper with 1-butanol–C_5H_5N–H_2O (6:4:3). Solutions were concentrated *in vacuo*. ^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Bruker WM 250 (250 MHz) spectrometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points are uncorrected. Ozonolysis was carried out with a Fischer ozone generator 500 M.

1,2-Dideoxy-L-galacto-*hept-1-enitol* (**4**). — Methyltriphenylphosphonium bromide plus sodium amide (8.46 g, 20.3 mmol) in dry Et_2O (40 mL) was stirred for 15 min and a solution of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose[6] (5.25 g, 20.3 mmol) in Et_2O (5 mL) was added. After stirring for another 15 min, the mixture was concentrated, a solution of the residue in water (50 mL) was extracted with CH_2Cl_2 (3 × 50 mL), and the combined extracts were dried ($MgSO_4$) and concentrated. Column chromatography (1:5 EtOAc–cyclohexane) of the residue yielded **1** (3.3 g, 60%), R_F 0.58 (solvent *B*).

A mixture of **1** (3.3 g, 12.9 mmol) and aq. 60% acetic acid (100 mL) was boiled for 90 min under reflux. The solution was concentrated with repeated addition of water to yield syrupy **2**, R_F 0.43 (solvent *A*).

A solution of **2** (230 mg, 1.3 mmol) in water (5 mL) was treated with sodium borohydride (120 mg, 3.17 mmol) overnight. After adding acetic acid to decompose excess of reductant, the sodium ions were removed by Amberlite IR-120 (H^+) resin. The combined eluate and washings were concentrated, and boric acid was removed by repeated distillation with MeOH from the residue. Crystallization from

MeOH–Et_2O then gave **4** (202 mg, 87%), m.p. 146°, $[\alpha]_D^{23}$ +37° (*c* 1, chloroform), R_F 0.36 (solvent *A*).

Anal. Calc. for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.10; H, 7.68.

Treatment of **4** (30 mg) conventionally with 1:1 Ac_2O–C_5H_5N (2 mL) gave 3,4,5,6,7-penta-*O*-acetyl-1,2-dideoxy-L-*galacto*-hept-1-enitol (**5**; 58 mg, 89%), m.p. 109° (from MeOH–H_2O), $[\alpha]_D^{23}$ −2.0° (*c* 1, chloroform), R_F 0.40 (solvent *B*). ^{1}H-N.m.r. data ($CDCl_3$, 250 MHz): δ 5.19–5.47 (m, 6 H, $J_{1a,2}$ 12.0, $J_{1b,2}$ 17.4, $J_{6,7a}$ 7.7, $J_{6,7b}$ 4.7 Hz, H-1,3,4,5,6,6), 5.68 (ddd, 1 H, $J_{2,3}$ 4.8 Hz, H-2), 3.86 (dd, 1 H, $J_{7a,7b}$ 11.7 Hz, H-7a), 4.28 (dd, 1 H, H-7b), 2.03, 2.06, 2.09, 2.12, and 2.13 (5 s, 15 H, 5 OAc).

Anal. Calc. for $C_{17}H_{24}O_{10}$: C, 52.58; H, 6.23. Found: C, 52.35; H, 6.05.

L-*Galactose* (**11**). — Using a capillary tube, oxygen and ozone (O_2 10 min, O_3 15 min = 2 mmol, O_2 10 min) were passed into a solution of **4** (170 mg, 0.96 mmol) in dry MeOH (40 mL) at −78°. 10% Methyl sulfide in dry MeOH (1 mL) was then added, the solution was allowed to reach room temperature and concentrated, and the residue was crystallized from PrⁱOH–MeOH to yield **11** (160 mg, 92.8%), m.p. 158–160°, $[\alpha]_D^{23}$ −79° (*c* 0.5, water).

Preparation of the radiolabelled compounds. — A solution of **2** (78 mg, 0.44 mmol) in 0.1M Na_2CO_3 (0.3 mL) was added to NaB^3H_4 (1000 mCi, 64.5 Ci. $mmol^{-1}$). The mixture was kept for 24 h at room temperature, then transferred to a solution[14] of *p*-nitroaniline (900 mg) in methanol (2.4 mL) and acetic acid (0.9 mL). The mixture was heated for 10 min at 110°, then allowed to reach room temperature, diluted with water (100 mL), and extracted with Et_2O (4 × 50 mL). The aqueous solution, which contained **4a** (R_F 0.49, solvent *A*), *N*-*p*-nitrophenyl-6,7-dideoxy-L-*galacto*-hept-6-enosylamine (**3**; R_F 0.62, solvent *A*), and traces of *p*-nitroaniline (R_F 0.81, solvent *A*), was concentrated to dryness. The residue was dissolved in the minimum amount of solvent *A* and eluted from a column (2 × 25 cm) of silica gel with the same solvent. All three components were separated completely and **3** had m.p. 216° (dec., from ethanol).

Anal. Calc. for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.45. Found: C, 53.06; H, 5.24; N, 9.27.

The fractions that contained **4a** were combined, dissolved in MeOH, and ozonolysed to give **11a** (R_F 0.23, solvent *A*) quantitatively. Compound **11a** was stirred with anhydrous copper sulfate (1 g), sulfuric acid (0.1 mL), and acetone (15 mL) for 6 h at room temperature. Conc. ammonia was added dropwise until the mixture turned bright blue, the inorganic material was collected and washed with acetone (4 × 10 mL), and the combined filtrate and washings were concentrated. A solution of the residue (**7a**; R_F 0.23, solvent *B*) in dry C_5H_5N (20 mL) was treated with tosyl chloride (300 mg) for 12 h at room temperature. Crushed ice (~1 g) was added, and the solution was diluted with Et_2O (250 mL), washed with ice-cold water (4 × 50 mL), dried ($CaCl_2$), and concentrated to dryness. The radioactivity was associated with **8a** (R_F 0.56, solvent *B*).

To a solution of the product in dry *N*,*N*-dimethylformamide (3 mL) was

added potassium thiobenzoate[13] (200 mg), and the mixture was heated for exactly 30 min at 150°, cooled, diluted with Et_2O (250 mL), washed, dried, and concentrated as described for **8a**. The radioactivity was associated with **9a** (R_F 0.64, solvent *B*). Elution of the product from a column (2 × 25 cm) of silica gel with solvent *B* gave fractions that contained **9a** (small amounts of **8a** can be recycled), which were combined and concentrated to dryness.

The radioactive residue was boiled under reflux in MeOH (10 mL) with Raney nickel (1 mL, settled); after 1 h, the nickel was removed by centrifugation and washed with MeOH (4 × 10 mL). No tritium exchange took place during the reductive desulfuration. The combined methanol solutions, in which the radioactivity was associated exclusively with **10a**, were concentrated almost to dryness, and a solution of the residue in aq. 60% acetic acid (100 mL) was heated at 110° for 90 min, then co-concentrated repeatedly with water. The resulting aqueous solution, which contained almost all the radioactivity as **12a**, was concentrated to dryness, and a solution of the residue in MeOH (~2 mL) was applied to Whatman No. 3 paper (2–4 sheets) and chromatographed (1-butanol–C_5H_5N–H_2O, 6:4:3). Compound **12a**, located by autoradiography and eluted with water as required, co-chromatographed with L-fucose in p.c. (R_F 0.40, 6:4:3 1-butanol–C_5H_5N–H_2O) and t.l.c. (R_F 0.26, solvent *A*; 0.57, 1:1 benzene–MeOH). The radioactivity was associated exclusively with the spot for L-fucose. The total yield of radioactivity as pure **12a** was 365 mCi, corresponding to 91%, calculated from radioactivity originally incorporated into **4a**.

ACKNOWLEDGMENT

This work was funded by the Bundesminister für Forschung und Technologie (0319050 A8).

REFERENCES

1 P. Vischer and W. Reutter, *Eur. J. Biochem.*, 84 (1978) 363–368.
2 Y. Fukushi, E. Nudelman, S. B. Levery, S. Hakomori, and H. Rauvalla, *J. Biol. Chem.*, 259 (1984) 10511–10517.
3 U. V. Santer and M. C. Glick, *Cancer Res.*, 43 (1983) 4159–4166.
4 W. Kreisel, B. Volk, and W. Reutter, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 1828–1831.
5 H. L. Frush and H. S. Isbell, *Methods Carbohydr. Chem.*, 1 (1962) 127–130.
6 G. B. Howarth, D. G. Lance, W. A. Szarek, and J. K. N. Jones, *Can. J. Chem.*, 47 (1969) 75–79.
7 O. T. Schmidt, *Methods Carbohydr. Chem.*, 2 (1963) 318–325.
8 J. Lehmann and H. Schäfer, *Chem. Ber.*, 105 (1972) 969–974.
9 H. H. Lee, P. G. Hodgson, R. J. Bernacki, W. Korytnyk, and M. Sharma, *Carbohydr. Res.*, 176 (1988) 59–72.
10 M. A. Bernstein and L. D. Hall, *Carbohydr. Res.*, 78 (1980) c1–c3.
11 R. U. Lemieux, R. Pappo, and D. S. Allen, *J. Org. Chem.*, 21 (1956) 478–479.
12 O. T. Schmidt, *Methods Carbohydr. Chem.*, 1 (1962) 191–194.
13 M. Brockhaus, E.-F. Fuchs, and J. Lehmann, *Chem. Ber.*, 111 (1978) 811–813.
14 J. Honeyman, *Methods Carbohydr. Chem.*, 2 (1963) 95–99.