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Synthesis of 2-*O*-α-D-Glucopyranosyl-β-D-galactopyranoside Derivatives Suitable for Linking to Proteins

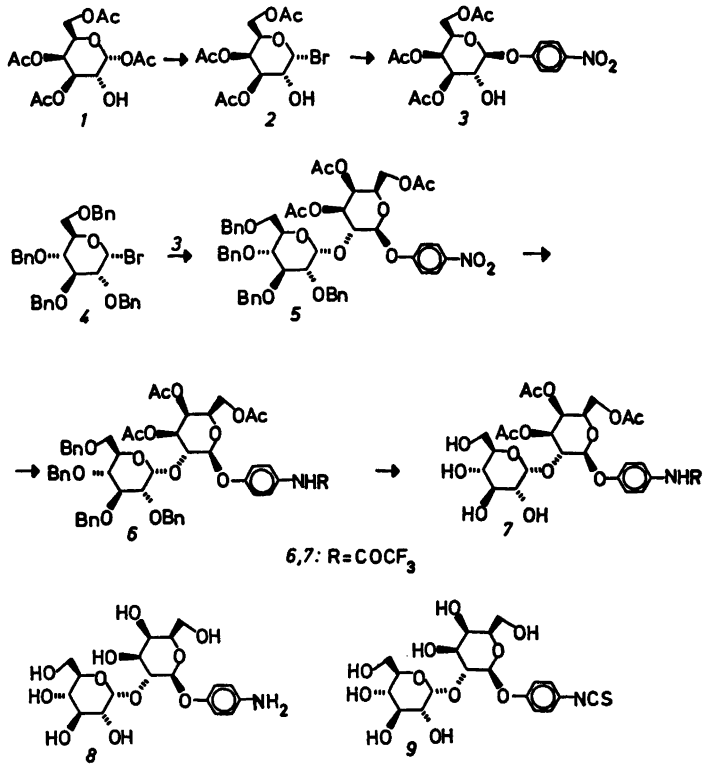
PER J. GAREGG,[a] IRWIN J. GOLDSTEIN [b] and TOMMY IVERSEN [a]

[a] Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm, Sweden. [b] The University of Michigan, Department of Biological Chemistry, Ann Arbor, Michigan 48104, USA

The synthesis of *p*-trifluoroacetamidophenyl 3,4,6-tri-*O*-acetyl-2-*O*-(α-D-glucopyranosyl)-β-D-galactopyranoside (*7*) suitable, after deacylation, for linking of 2-*O*-α-D-glucopyranosyl-β-D-galactopyranoside residues to proteins is described. The disaccharide is of interest in blood platelet agglutination studies.

In connection with studies on human platelet aggregation,[1] a disaccharide glycoside containing a 2-*O*-α-D-glucopyranosyl-β-D-galactopyranoside with an aglycone suitable for covalent linking to peptides or proteins was required. The present paper describes the synthesis of such a disaccharide.

1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose [2] (*1*), in dichloromethane, was treated with hydrogen bromide. The α-bromo sugar *2* thus obtained was dissolved in dry acetone and



condensed with *p*-nitrophenol in the presence of potassium carbonate,[3] to give the glycoside 3 with the hydroxyl group in the 2-position free, in an 81 % yield from *1*. Glycosidation of *3*, with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (*4*) in dichloromethane containing tetraethylammonium bromide and molecular sieve [4] afforded the fully protected disaccharide derivative *5*, in a 27 % yield from *3*. Hydrogenation of the nitro group in *5* and conversion of the product into the *N*-trifluoroacetyl derivative *6* was followed by hydrogenolysis to give the final product *7* in 70 % yield from *5*. The *N*-trifluoroacetyl derivative *6* is an appropriate derivative to store inasmuch as the free amine *8*, readily produced from *7* by deacylation, and the isothiocyanate *9*, produced from *8* by reaction with thiophosgene,[5] are unsuitable for storage due to their instability. The *p*-isothiocyanato derivative *9* reacts with free amino groups and acidic hydroxyls in peptides and proteins.[5] The use of the disaccharide derivative *9* in platelet aggregation studies will be published elsewhere.

EXPERIMENTAL

General methods. Concentrations were performed at reduced pressure. Optical rotations (*c* 0.5 to 2.0) were determined at room temperature (23 – 25 °C) using a Perkin-Elmer 141 polarimeter. NMR spectra were recorded using a Varian XL-100 instrument, in deuteriochloroform unless otherwise stated. Only pertinent parts of spectra for key compounds are given below. The remaining portion of all spectra was invariably in accordance with the presumed structures. Analytical TLC was performed on "Merck DC-Fertigplatten, Kieselgel F 254" and preparative TLC on 2 mm "Merck PSC-Fertigplatten, Kieselgel F 254". The absorbent for silica gel column chromatography was "Merck, Kieselgel 0.040 – 0.063 mm".

3,4,6-Tri-O-acetyl-α-D-galactopyranosyl bromide (2). A solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (*1*)[3] (4.0 g) in dichloromethane (20 ml) at 0 °C was added dropwise with stirring to a saturated solution of hydrogen bromide in dichloromethane (150 ml) also at 0 °C. The reaction was monitored by TLC (toluene – ethyl acetate, 1:1). After 1 h at 0 °C when no starting material remained, the solution was concentrated to a syrup which was used directly in the next step, $[\alpha]_D$ +188° (chloroform). NMR: δ 2.05 – 2.20 (9 H, OAc), 3.96 (1 H, dd, $J_{1,2}$ 4 Hz, $J_{2,3}$ 10 Hz, H-2), 4.10 – 4.19 (2 H, m, H-6, H-6′), 4.50 (1 H, t, H-5), 5.22 (1 H, dd, $J_{3,4}$ 3 Hz, H-3), 5.48 (1 H, d, H-4), 5.70 (1 H, OH), 6.62 (1 H, d, H-1).

p-*Nitrophenyl 3,4,6-tri-O-acetyl-β-D-galactopyranoside* (3). *p*-Nitrophenol (4 g) and potassium carbonate (4 g) were added to acetone (100 ml) (dried over potassium carbonate) and the glycosyl halide *2* (prepared from the tetraacetate (4 g) and used immediately) dissolved in dry acetone (20 ml) was added. The mixture was stirred at room temperature for 30 min and then refluxed for 30 min.[3] The solution was diluted with 250 ml chloroform and shaken with several portions of ice-cold saturated aqueous sodium carbonate until no further *p*-nitrophenol was removed (absence of yellow coloration). The solution was dried over sodium sulfate, filtered and concentrated to a syrup (4 g). Column chromatography on silica gel (toluene – ethyl acetate, 1:1) yielded chromatographically homogeneous *3* (3.8 g), $[\alpha]_D$ −12° (chloroform), R_F 0.46 (TLC same solvent). (Found: C 51.1, H 5.21, N 2.86. $C_{18}H_{21}NO_{11}$ requires: C 50.6, H 4.95, N 3.28). The NMR assignments were corroborated by spin decoupling experiments. Decoupling irradiation at the broad multiplet at δ 4.15 (4 H, H-2, H-5, H-6 and H-6′) caused the H-1 (d) to collapse into a singlet and the H-3 (dd) to collapse into a doublet. Irradiation at δ 5.45 (H-4) caused the H-3 signal to collapse into a doublet. From chemical shift considerations H-2 could not be acetylated. The following assignments were made: δ 2.08 – 2.16 (9 H, OAc), 2.88 (1 H, OH), 4.15 (4 H, H-2, H-5, H-6 and H-6′), 5.00 (1 H, dd, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3), 5.08 (1 H, d, $J_{1,2}$ 8 Hz, H-1), 5.45 (1 H, d, H-4), 7.12 and 8.18 (2 H each, both d, both $J_{H,H}$ 9 Hz, *p*-$NO_2C_6H_4O$ protons).

p-*Nitrophenyl 3,4,6-tri-O-acetyl-2-O-(tetra-O-benzyl-α-D-glucopyranosyl)-β-D-galactopyranoside* (5). The *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-β-D-galactopyranoside *3* (2.2 g) was dissolved in purified dichloromethane (50 ml) which contained tetraethylammonium bromide (2.1 g) and molecular sieve 4 Å (5 g). Tetra-*O*-benzyl-α-D-glucopyranosyl bromide *4* freshly prepared from tetra-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-α-D-glucopyranose (3.5 g) was added and the mixture was stirred at room temperature under nitrogen for 5 days.[4] The solids were removed by filtration through a bed of Celite, and the filtrate was diluted with dichloromethane and washed with water, saturated aqueous sodium hydrogen carbonate and finally water. The solution was dried over anhydrous sodium sulfate, filtered and concentrated to a syrup (4.7 g). Column chromatography on silica gel (toluene – ethyl acetate, 2:1) yielded chromatographically homogeneous *5* (1.3 g), $[\alpha]_D$ +13° (chloroform), R_F 0.64 (TLC same solvent). (Found: C 65.7, H 5.93, N 1.83. $C_{52}H_{55}NO_{16}$ requires: C 65.8, H 5.84, N 1.47). The ^1H NMR spectrum was in accordance with that expected for *5*.

p-*Trifluoroacetamidophenyl 3,4,6-tri-O-acetyl-2-O-(tetra-O-benzyl-α-D-glucopyranosyl)-β-D-ga-*

lactopyranoside (6). The foregoing compound *5* (500 mg) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (25 ml) using Adams catalyst (100 mg). When sufficient hydrogen to account for the conversion of a nitro to an amino group had been consumed, trifluoroacetic anhydride (0.8 ml) and pyridine (1.9 ml) were added and the mixture was kept at 60 °C for 30 min.[6] The catalyst was removed by filtration and the filtrate concentrated. The residue was dissolved in toluene and shaken with water. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to a syrup (545 mg). Column chromatography on silica gel (toluene–ethyl acetate, 2:1) yielded chromatographically homogeneous *6* (400 mg), $[\alpha]_D$ +27° (chloroform), R_F 0.51 (TLC, same solvent). The ^{1}H NMR spectrum was in accordance with that expected for *6*.

p-*Trifluoroacetamidophenyl 3,4,6-tri*-O-*acetyl-2*-O-*(α*-D-*glucopyranosyl)-β*-D-*galactopyranoside* (7). The syrupy product *6* (290 mg) from the above reaction was hydrogenated, in a Parr apparatus, in ethanol (25 ml) using 10 % palladium on charcoal (150 mg) as catalyst. When hydrogen consumption had ceased, the catalyst was removed by filtration and the filtrate was concentrated to yield *7* as a chromatographically homogeneous syrup (175 mg), $[\alpha]_D$ +34° (acetone). NMR: (acetone-d_6): δ 2.03–2.15 (9 H, OAc), 5.17 (1 H, dd, $J_{2,3}$ 10 Hz, $J_{3,4}$ 3 Hz, H-3 galactose residue), 5.33 (1 H, d, $J_{1,2}$ 8 Hz, H-1 galactose residue), 5.44 (2 H, d, $J_{1,2}$ 4 Hz, H-1 glucose residue, H-4 galactose residue), 7.18 and 7.64 (2 H each, both d, both $J_{H,H'}$ 9 Hz, *p*-$CF_3CONHC_5H_4O$–).

An aliquot of *7* was hydrolysed with 0.25 M aqueous sulfuric acid for 24 h at 100 °C. The product was reduced with sodium borohydride and acetylated.[7] The glucitol hexaacetate and galactitol hexaacetate thus obtained were indistinguishable from authentic standards on GLC.

Another aliquot of *7* was methylated,[8] hydrolyzed, reduced with sodium borohydride and acetylated.[9] The two *O*-methylalditol acetates thus obtained were indistinguishable from authentic 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl-D-galactitol, respectively, on GLC and MS.

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