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Note

Synthesis and characterization of methyl 2-*O*-β-D-galactopyranosyl-β-D-galactopyranoside and methyl 2-*O*-(2-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-galactopyranoside

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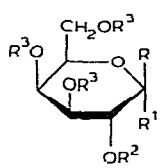
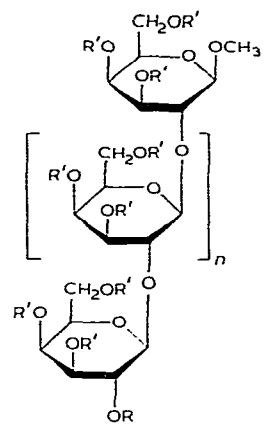
We have recently shown that 1-*O*-sulfonyl-D-galactopyranose derivatives having a 2-*O*-benzoyl group can be used to prepare β-D-galactopyranosides stereoselectively and in high yields[1]. We have recently applied this glycoside-forming reaction to the synthesis of methyl β-D-(1→6)-linked galacto-oligosaccharides[2]. In this report, we have investigated the synthesis of β-D-(1→2)-linked methyl galacto-oligopyranosides that are to be used for measuring binding constants of homogeneous myeloma proteins[3].



1 R = H, R^1 = OTs, R^2 = Bz, R^3 = Bzl
2 R = OMe, R^1 = H, R^2 = Bz, R^3 = Bzl
3 R = OMe, R^1, R^2 = H, R^3 = Bzl
4 R = OMe, R^1, R^2, R^3 = H



5 n = 0, R = Bz, R′ = Bzl
6 n = 0, R = H, R′ = Bzl
7 n = 0, R, R′ = H
8 n = 1, R = Bz, R′ = Bzl
9 n = 1, R = H, R′ = Bzl
10 n = 1, R, R′ = H

The 1-*O*-sulfonyl-D-galactopyranose derivative **1** was coupled with methanol in acetonitrile, and then debenzoylated to give **3**. Compound **3** was allowed to react with one equivalent of **1** to give the disaccharide derivative **5** as described previously[1]. Debenzoylation of **5** with sodium ethoxide in ethanol gave the disaccharide derivative **6**, generally in good yield[1].

Coupling of **6** with **1** gave the trisaccharide **8** in good yield. Debenzoylation of **8** with sodium ethoxide or methoxide in ethanol or methanol gave almost no yield of the debenzoylated trisaccharide **9**. Liquid-chromatographic analysis of the products on silica gel with 1:2 (v/v) ethyl acetate–hexanes showed the presence of **6**, **3**, and 3,4,6-tri-*O*-benzyl-D-galactopyranose.

Apparently, debenzoylation was followed immediately by glycosidic cleavage of the reducing end-group under the strongly basic conditions. In fact, it has been shown previously[4,5] that β-D-galactopyranosyl groups can be cleaved at room temperature at pH >8.9. Attempts to remove the 2-*O*-benzoyl group under less basic conditions, with ammonia in methanol, ammonium hydroxide in aqueous methanol, triethylamine in aqueous methanol, dilute sodium hydroxide in aqueous methanol, and even by reduction with lithium aluminum hydride failed to give the debenzoylated trisaccharide **9**. In most examples the benzoyl group was not cleaved.

It was found that dilute barium methoxide in methanol at room temperature for several days gave the debenzoylated product **9** in ~50% yield. The experiment also gave some unreacted **8** and the disaccharide **6**.

An attempt was made to prepare a tetrasaccharide from **9** by reaction with **1**. After 48 h, all of **9** had reacted, but several products had been formed. Attempts to debenzoylate the crude mixture and to separate the tetrasaccharide fraction failed. Numerous products were formed, including **9**, **6**, and **3**. Repeated attempts to prepare the tetrasaccharide failed to give the desired product. A similar phenomenon was found in synthesis of the β-D-(1→6)-linked methyl galacto-oligopyranoside[2].

The oligosaccharides **6** and **9** were debenzylated by hydrogenolysis with 5% palladium-on-carbon. The free oligosaccharides **7** and **10** were purified on a column of Bio-Gel P-2 with water as the eluant.

The ^{1}H-n.m.r. spectra of the oligosaccharides **5**, **6**, **8**, and **9** were used to determine the d.p. of the product from the ratio of the aromatic (δ 7.0–7.5) to the methoxyl (δ 3.38) group protons. The presence or absence of the benzoyl group (δ 8.05, two meta protons) was determined similarly.

The stereoselectivity of the glycoside-forming reaction was determined by ^{13}C-n.m.r. spectroscopy. In all of the compounds, the C-1 carbon atoms, whether bearing the methoxyl group or a galactopyranose residue, resonate at 101.6–106.0 p.p.m., indicating a β-D-galactopyranosyl structure. No resonances could be observed between 98 and 100 p.p.m., which is the region for C-1 carbon atoms of α-D-galactopyranosides[1,2]. Furthermore, the methoxyl-group carbon atom resonates between 56.6 and 58.3 p.p.m., indicative of a β linkage. The lack of any detectable peaks for C-1α carbons or for an α-methoxyl group indicate that the coupling reactions are essentially 100% β-stereoselective.

TABLE I

PROPOSED ^{13}C-N.M.R. CHEMICAL SHIFTS[a]

Carbon atom	*Compound*								
	2	**3**	**4**[b]	**5**	**6**	**7**[b]	**8**	**9**	**10**[b]
C-1	102.6	104.3	105.0	104.2	104.3	103.2	103.3	103.0	103.4[c]
C-2	72.2	71.4	72.1	79.8	80.5	79.3	76.2	76.2	81.1[c]
C-3	80.4	82.1	74.2	81.4[c]	81.3[c]	73.6	82.4[c]	82.1[c]	73.4
C-4	73.0	73.0	70.0			69.6			69.5
C-5	73.9	73.8	76.2			75.9			75.9
C-6	69.0	68.8	62.3	68.9	69.0[c]	61.7	69.4	69.0	61.6[c]
C-1′				102.2	106.0	104.1	101.6	101.8	103.3[c]
C-2′				71.8		73.8	79.0	80.2	81.0[c]
C-3′				80.6[c]	81.6[c]	73.6	83.3[c]	83.4[c]	73.4
C-4′						69.5			69.5
C-5′						76.1			75.9
C-6′				68.9	68.7[c]	61.7	69.4	69.0	61.7[c]
C-1″							101.6	105.9	104.9
C-2″							71.9		72.5
C-3″							80.8	81.7	73.8
C-4″									69.3
C-5″									76.5
C-6″							68.8	68.6	61.9[c]
OMe	56.6	56.9	58.3	57.1	57.5	57.7	56.1	58.3	57.9
C=O	165.5			165.5			165.5		

[a]In chloroform-*d*, p.p.m. values relative to Me_4Si = 0 except in *b*. [b]In D_2O, p.p.m. values relative to Me_4Si = 0 using 1,4-dioxane as internal reference at 67.4 p.p.m. [c]Assignments not unequivocal.

The assignments of the other carbon resonances in the ^{13}C-n.m.r. spectra of the oligosaccharides (Table I) were based on comparison to the chemical shifts shown by methyl β-D-galactopyranoside[6] **4** and the blocked monosaccharides[1] **2** and **3**. As general rules, it was assumed that benzylation or glycosylation of an equatorial or primary hydroxyl group should shift the attached carbon atom downfield ~7 p.p.m. and adjacent carbons upfield ~1–2 p.p.m. Benzylation of an axial hydroxyl group (O-4) should cause C-4 to shift downfield by only ~3 p.p.m.[6,7]. Benzoylation should cause a slight downfield shift (~1–2 p.p.m.) on a carbon atom. The benzylic methylene groups in compounds **2** and **3** were assigned at 72.5, 73.6, and 74.6 p.p.m. by off-resonance spectroscopy. The C-2′,-2″,-4′,-4″,-4,-5′, and -5″ atoms in compounds **5**, **6**, **8**, and **9** that could not be distinguished from one another or from the benzyl methylene atoms were not assigned.

The ^{13}C chemical shifts of compounds **5**, **6**, **8**, and **9** show that the glycosidic linkages are β-(1→2) and that the oligosaccharides **5** and **8** show large downfield shifts of the anomeric carbon atom of the non-reducing end upon debenzoylation. A downfield shift of 1.7 p.p.m. is observed for C-1 when compound **2** is debenzoylated to **3**. The shifts in C-1′ and C-1″ of **5** and **8** when debenzoylated are 3.8 and 4.3 p.p.m.

downfield, respectively. This large chemical shift may indicate a change in the conformation of the pyranoid ring of the non-reducing end or a change in the conformation of the non-reducing end relative to the rest of the molecule. A change in conformation may account for the increased base-lability of the β-galactopyranosyl–O bond and for the failure to form the tetrasaccharide. A similar conformational change was postulated for the β-(1→6)-linked galacto-oligosaccharides[2].

The ^{13}C spectra of **7** and **10** also indicate, from the chemical shift of the anomeric and C-2 carbon atoms, a β-(1→2)-linked oligosaccharide. The assignments of C-5, C-5′, and C-5″ were based on a deuterium-induced, differential isotope-shift experiment (DIS) as described by Pfeffer *et al.*[8]. The free oligosaccharides **7** and **10** showed none of the chemical-shift anomalies that occurred in the benzylated di- and tri-saccharides.

EXPERIMENTAL

General methods. — ^1H-N.m.r. spectra were determined with a Varian A-60-A spectrometer on solutions in chloroform-*d* with tetramethylsilane (Me_4Si) as the internal standard. ^{13}C-N.m.r. spectra were determined with a Varian XL-100-15 spectrometer in pulsed Fourier-transform, proton-noise-decoupled mode on solutions in chloroform-*d* with Me_4Si as the internal standard, or in D_2O with 1,4-dioxane (67.4 p.p.m.) as the internal standard. The spectra are reported with chemical shifts downfield from Me_4Si. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter in jacketed 1-dm cells at 25°. Thin-layer chromatography was performed on "Baker-Flex" silica gel 1B-F (2.5 × 7.5 cm) plates with 1:2 (v/v) ethyl acetate–hexanes as eluent. High-pressure liquid chromatography (l.c.) was performed with a Glenco pump, model HPLS-1, a Valco septumless injector (1.0 mL loop), a Waters differential refractometer R-401, and a stainless-steel column (25 cm × 10 mm inside diameter) containing silica gel (Whatman Partisil-10). Deblocked oligosaccharides were purified on a jacketed column (0.75 × 29.0 in.) at 60° packed with Biogel P-2 (Biorad Lab. >400 mesh).

Materials. — Spectrograde acetonitrile was dried with phosphorus pentaoxide, distilled and stored over calcium hydride. Silver *p*-toluenesulfonate (Eastman Organic Chemicals, Rochester, NY 14650) was recrystallized from acetonitrile and dried under high vacuum before use. 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1-*O*-*p*-tolylsulfonyl-D-galactopyranose (**1**), methyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**2**), methyl 3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**3**), methyl 2-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**4**), and methyl 2-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**6**) were synthesized as described previously[1].

Methyl 2-O-[*2-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4,6-tri-O-benzyl-β-D-galactopyranosyl*]-*3,4,6-tri-O-benzyl-β-D-galactopyranoside* (**9**). — The disaccharide **6** (0.5 g, 0.56 mmol) was coupled with the 1-*O*-tosyl derivative **1** (0.44, 0.62 mmol) in dry acetonitrile (3.0 mL) as described previously. Separation by l.c. on a

column of silica gel (1:2 EtOAc:hexane) gave the trisaccharide derivative **8**, 0.7 g, $[\alpha]_D^{25}$ +2.2° (*c* 1, chloroform). The trisaccharide derivative **8** was debenzoylated with barium oxide (50 mg) in methanol (50 mL) for 3 days at room temperature. Carbon dioxide was bubbled into the solution to precipitate the barium ions. The mixture was filtered, evaporated to a syrup, and chromatographed on a column of silica gel (1:2 EtOAc–hexane) to give the debenzoylated trisaccharide **9**, 0.3 g, $[\alpha]_D^{25}$ −15.8° (*c* 1, chloroform); ^{1}H-n.m.r. data: δ 7.5–7.0 (45 H, aromatics), 5.1–3.5 (39 H, ring and benzylic protons) 3.38 (3 H, methoxyl), 2.3 (1 H, broad, exchangeable with D_2O, hydroxyl).

Anal. Calc. for $C_{82}H_{88}O_{16}$: C, 74.07; H, 6.67. Found: C, 73.75; H, 6.60.

Methyl 2-O-(β-D-galactopyranosyl)-β-D-galactopyranoside (**6**). — The benzylated disaccharide **6** (240 mg) was dissolved in 1:1:1 1,4-dioxane–methanol–water (30 mL) and 5% palladium-on-carbon (100 mg) was added. The mixture was stirred under hydrogen at slightly over 1 atm pressure for 48 h. Filtration of the catalyst followed by evaporation of the solvent gave a glass that was fractionated on a P-2 column of Bio-Gel. The disaccharide **7** was collected and freeze-dried from water, yield 0.08 g (84%); $[\alpha]_D^{25}$ +4.3° (*c* 1, water).

Anal. Calc. for $C_{13}H_{24}O_{11} \cdot H_2O$: C, 41.71; H, 7.00. Found: C, 41.24; H, 7.21.

Methyl 2-O-[2-O-(β-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside (**10**). — The benzylated trisaccharide **9** (0.18 g) was debenzylated as already described. The deprotected trisaccharide was obtained as a freeze-dried solid after chromatography on a column of Bio-Gel P-2; yield 60 mg (85%), $[\alpha]_D^{25}$ +19.3° (*c* 1, water).

Anal. Calc. for $C_{19}H_{34}O_{16} \cdot 3\ H_2O$: C, 39.85; H, 7.04. Found: C, 39.73; H, 6.69.

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