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SYNTHESIS AND CHARACTERIZATION OF METHYL 6-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSIDE AND METHYL *O*-β-D-GALACTOPYRANOSYL-(1→6)-*O*-β-D-GALACTOPYRANOSYL-(1→6)-β-D-GALACTOPYRANOSIDE*

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ABSTRACT

6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-D-galactopyranosyl chloride (**8**) was prepared from 1,6-anhydro-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-galactopyranose *via* the corresponding 1,6-di-*O*-acetyl derivative **7**. The glycosyl chloride **8** was converted into the 1-*O*-tosyl derivative (**9**) which was allowed to react with methanol in acetonitrile to form methyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-galactopyranoside (**10**). Compound **10** was *O*-deacetylated with ammonium hydroxide in methanol to give **11**. Reaction of **9** with **11** under the same conditions of glycosidation gave the corresponding disaccharide derivative **12**. *O*-Deacetylation of **12** followed by glycosidation with **9** gave the corresponding trisaccharide derivative **16**. Appropriate deblocking sequences gave the title compounds. The structures of the glycosides were determined with the aid of both ^{1}H- and ^{13}C-n.m.r. spectroscopy. No evidence of α-D linkages was found.

INTRODUCTION

We have been engaged in improving the stereoselectivity and yield of syntheses of β-D-glycopyranosides[1–3] and oligosaccharides. A systematic study of glycoside-forming reactions of the 1 eq., 2 eq. type has been undertaken in this laboratory[3], and the best conditions found involve the reaction in acetonitrile at room temperature of 1-*O*-sulfonyl sugar derivatives having a participating Bz-2 group. We have now extended the investigation of glycoside-forming reactions of D-galactopyranose derivatives of this type by applying the system to the stereoselective synthesis of a β-D-(1→6)-linked disaccharide and trisaccharide of D-galactopyranose in the form of methyl β-glycosides. Our purpose is to prepare this series of oligosaccharides for the measurement of binding constants of homogeneous myeloma proteins[4].

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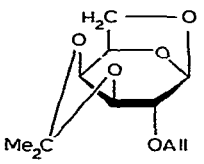
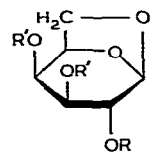
RESULTS AND DISCUSSION

2-*O*-Allyl-1,6-anhydro-β-D-galactopyranose (**2**) was prepared in crystalline form from 1,6-anhydro-3,4-di-*O*-isopropylidene-β-D-galactopyranose by allylation to give **1**, and hydrolysis of the isopropylidene group as previously described[5]. Benzylation with benzyl chloride gave a syrupy 2-*O*-allyl-1,6-anhydro-3,4-di-*O*-benzyl-β-D-galactopyranose (**3**) in 79% yield. The ^{1}H-n.m.r. spectrum showed aromatic benzyl protons at δ 7.4 and no hydroxyl protons. *O*-Deallylation was achieved in two steps. First, the allyl group was rearranged by the treatment of **3** with potassium *tert*-butoxide in toluene to the 2-propenyl derivative **4**. Its ^{1}H-n.m.r. spectrum showed two quartets, one centered at δ 5.8 characteristic of the olefinic proton adjacent to an ether oxygen atom, and one characteristic of the methyl group centered at δ 1.54. The coupling constants indicated a *cis* isomer, and no evidence of a *trans* isomer was found[6]. In the next step, the propenyl group of **4** was hydrolyzed with 5% of hydrochloric acid in 1,4-dioxane to give crystalline 1,6-anhydro-3,4-di-*O*-benzyl-β-D-galactopyranose (**5**). Compound **5** was *O*-benzoylated with benzoyl chloride in triethylamine to give the crystalline 2-*O*-benzoyl derivative **6**.

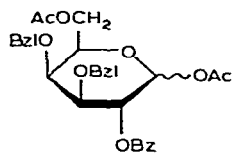
Chloroacetolysis of **6** was attempted at room temperature with chloroacetic anhydride–sulfuric acid or chloroacetic anhydride–chloroacetyl chloride–sulfuric acid, but little reaction occurred other than decomposition of **6**. Variations in reaction temperature and reaction time did not improve the results. Acetolysis of **6**



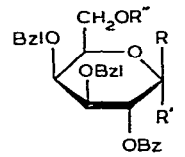
(1)



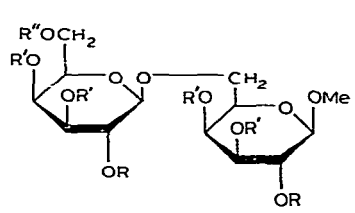
2 R = All, R' = H
3 R = All, R' = Bzl
4 R = Pre, R' = Bzl
5 R = H, R' = Bzl
6 R = Bz, R' = Bzl



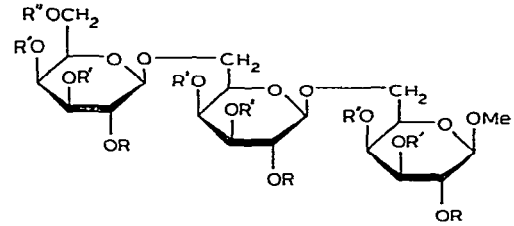
7



8 R = H, R' = Cl, R'' = Ac
9 R = H, R' = OTs, R'' = Ac
10 R = OMe, R' = H, R'' = Ac
11 R = OMe, R' = H, R'' = H



12 R = Bz, R' = Bzl, R'' = Ac
13 R = Bz, R' = Bzl, R'' = H
14 R = H, R' = Bzl, R'' = H
15 R = H, R' = H, R'' = H



16 R = Bz, R' = Bzl, R'' = Ac
17 R = Bz, R' = Bzl, R'' = H
18 R = H, R' = Bzl, R'' = H
19 R = H, R = H, R'' = H

All = CH_2=CH—CH_2
Pre = CH_3—CH=CH

was successful, however, when a relatively high ratio of acetic acid was used in an acetolysis mixture of 60:50:1 (v/v) acetic acid–acetic anhydride–sulfuric acid. It has been observed previously[7] that these relatively mild conditions provide pure products and avoid epimerization in the acetolysis of a number of aldo-hexose and -pentose derivatives. The product, 1,6-di-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-D-galactopyranose (**7**), was formed as an α,β mixture (39:11). The two anomers crystallized together and were not separated. Two anomeric proton-peaks were observed in the ^{1}H-n.m.r. spectrum at δ 6.56 (0.78 H, α-D anomer) and 5.84 (0.22 H, β-D anomer). The D-galactosyl chloride **8** was prepared by reaction of the diacetate **7** with hydrogen chloride in benzene or diethyl ether. Treatment of chloride **8** on a high-vacuum rack with silver *p*-toluenesulfonate in acetonitrile afforded the 1-sulfonate **9**, as described previously[8,9]. Compound **9** was not isolated but was allowed to react directly with 1 equiv. of methanol for 18 h at room temperature in the dark. The reaction afforded methyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-galactopyranoside (**10**) in 90% yield after liquid chromatography on silica gel. No α-D anomer could be detected. The ^{1}H-n.m.r. spectrum of **10** showed H-2 at δ 5.75 ($J_{2,3}$ 10.2 Hz), H-1β at 4.50 ($J_{1,2}$ 8.0 Hz), and OMe-β at 3.50. A signal for an α methoxyl group appears at δ ~3.33 in the spectra of similarly substituted compounds[3]. In the ^{13}C-n.m.r. spectrum of **10**, the signal for C-1 appeared at 102.38 p.p.m. Signals for C-1 atoms with β-D anomeric substituents have been observed at 102–103 p.p.m., whereas signals for C-1α carbons have been found at 98–100 p.p.m. in related compounds[3].

O-Deacetylation of **10** with ammonium hydroxide in methanol gave crystalline methyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-galactopyranoside (**11**). Disaccharide **12** was prepared by glycosidation of **9** with **11** in acetonitrile as described earlier. In this case also, no α-D linkage could be detected. *O*-Deacetylation was carried out with ammonium hydroxide to afford crystalline **13**. *O*-Debenzylation with 5% palladium-on-charcoal afforded crystalline methyl 6-*O*-β-D-galactopyranosyl-β-D-galactopyranoside (**15**).

Trisaccharide **16** was prepared in the same way as described for disaccharide **12**, and a similar sequence of *O*-deacetylation, *O*-debenzoylation, and *O*-debenzylation afforded **17**, **18**, and methyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**19**), respectively. Attempts to prepare a tetrasaccharide by a similar sequence of reactions failed presumably owing to steric factors.

The formation of the disaccharide and trisaccharide derivatives **12** and **16** was established by the presence, in their ^{1}H-n.m.r. spectra, of signals corresponding to an acetyl group (δ 1.98–1.99) and a methoxyl group (δ 3.19). The d.p. of the various oligomers was determined from the ratio of the aromatic to methoxyl or acetyl group protons. The signal of anomeric protons coincided with the signals of the benzyl and other ring protons and could not be used to identify the configuration of the D-galactosyl linkages.

The chemical shift of the OCH_3-1 protons shows some interesting differences in this series (Table I). In the monomeric compounds **10** and **11**, the methoxyl signal is at δ 3.50 and 3.46, respectively. These values are close to that of methyl

TABLE I

DATA FOR TRISACCHARIDES **16–19**

Data	*Compounds*			
	16	**17**	**18**	**19**
M.p. (degrees)	84–86	93–95	79–82	Foam
$[\alpha]_D^{20-25}$ (degrees)	+30.1	+25.2	−4.9	+38.1
(concentration)	1.30[a]	1.01[a]	0.80[a]	1.20[b]
Mol. formula	$C_{81}H_{84}O_{20}$	$C_{82}H_{82}O_{19}$	$C_{61}H_{70}O_{16}$	$C_{19}H_{34}O_{16}$
Anal. Calc.: C; H	71.37; 5.99	71.81; 6.03	69.17; 6.66	44.02; 6.61
Found: C; H	70.75; 5.98	71.76; 6.47	69.80[c]; 6.00[c]	43.94; 7.26[c]
Yield (%)[d]	84	90	89	77
^{1}H-n.m.r. (δ) (OMeβ)	3.19	3.20	3.50	3.44

[a]In chloroform. [b]In water. [c]Accurate elemental analysis could not be obtained because of difficult crystallization. [d]Of pure compound.

TABLE II

^{13}C-N.M.R. DATA FOR DISACCHARIDES **12–15** AND TRISACCHARIDES **16–19**[a]

Atoms	*Chemical shifts (δ)*							
	12	**13**	**14**	**15**	**16**	**17**	**18**	**19**
C-1	102.27	102.26	104.44	104.38	102.72	102.32	104.38	104.26
C-3	80.09	80.11	82.15	73.19	79.92	79.98	82.06	73.04
C-2	72.52	72.38	72.97	71.23	72.12	72.41	72.57	71.09
	72.73	72.77		71.14				
C-4		73.98	74.27	69.34	to	to	to	69.53
	74.14	74.48	74.51	69.13				69.06
C-5	74.56	75.15	75.23	74.23	74.54	75.25	75.20	74.18
C-6	68.55	68.43	68.54	69.13	67.50	67.70	68.35	69.05
C-1′	101.83	101.85	103.82	103.82	101.63	101.69	103.63	103.78
						101.76		
C-3′	80.20	80.20	82.15	73.19	79.68	79.81	82.06	73.04
C-2′			72.97					71.09
C-4′								
C-5′		75.15	75.23	75.60				74.18
C-6′	63.22	62.02	62.12	61.41	67.23	67.17	68.07	69.05
C-1″					101.63		103.63	103.78
C-3″					80.13	80.22	82.37	73.13
C-2″								71.09
C-4″								
C-5″								75.55
C-6″					63.17	61.99	61.99	61.40
OMe	56.20	56.19	57.16	57.72	56.21	56.26	57.10	57.71
Ac	20.80				20.73			

[a]These assignments are tentative based on analogies, discussed in the experimental section. Solvent $CDCl_3$, except D_2O for **15** and **19**. Additional assignments: CH_3CO, 170.74–170.80; C_6H_5CO, 165.54–165.72; C_6H_5CO and $C_6H_5CH_2$, 138.5–126.90; and $C_6H_5CH_2$, 72.97–71.47 p.p.m. Compare values with those for methyl β-D-galactopyranoside[10]. [b]Indistinguishable; may be rearranged; overlapping, primed, and unprimed numbers; see methyl glycoside unit. [c]Overlapping, indistinguishable primed and double primed numbers.

3,4-di-*O*-benzyl-β-D-galactopyranoside (δ 3.50). Glycosidation at C-6 of **11** gave **12**, and *O*-deacetylation **13**, both compounds having OCH_3-1 resonances at δ 3.19. *O*-Debenzoylation to **14** resulted in a return of the absorption to δ 3.50, the same value range observed for the benzoylated monomers **10** and **11**. Apparently, the conformation of OBz-2 has little or no influence on the chemical shift of the OCH_3-1 protons located on the same sugar unit, but the OBz-2′ of the adjacent D-galactose residue is in a conformation that allows it to influence the methoxyl group magnetic environment. The same differences were observed for the benzoylated and debenzoylated trisaccharide derivatives **16**, **17**, and **18**.

The β-D-linked structures of the di- and tri-saccharide derivatives are consistent with their ^{13}C-n.m.r. data (Table II). The atoms C-1 bearing the β-methoxyl group, and C-1′ and C-1″, the β-D-galactosyl interunit linkages, resonate at 101.63–102.56 p.p.m. in the 2-*O*-benzoylgalactoside derivatives **10**–**13**, **16**, and **17**. After *O*-debenzoylation, the same anomeric carbons in **14**, **15**, **18**, and **19** resonate at 103.63–104.44 p.p.m. No signals were observed between 98.0 and 100 p.p.m., which is the region where C-1 atoms of 2-*O*-benzoyl-α-D-galactosides absorb[3]. The C-1 resonances of 2-*O*-substituted β-D-galactose derivatives have generally been found up-field of those of the corresponding 2-*O*-unsubstituted compounds[10–12].

The β-methoxyl carbon resonates at 56.19–56.55 p.p.m. in fully substituted (**12**, **16**) and 6-*O*-deacetylated derivatives (**13**, **17**), close to the range (57.10–57.30 p.p.m.) observed[10] for methyl 2,6-di-*O*-methyl- and methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranosides. In fully deblocked di- (**15**) and trisaccharide (**19**), the glycosidic methoxyl carbon resonates at 57.72 and 57.71 p.p.m., close to the value[13] for methyl β-D-galactopyranoside (58.3 p.p.m.). In contrast, the α-methoxyl carbon resonates at 55.05–55.15 p.p.m. for several substituted α-D-galactopyranoside derivatives[9], and at 55.93 p.p.m. for methyl α-D-galactopyranoside[14]. The methoxyl chemical shift changes observed on deblocking the 2-*O*-substituted derivatives (**13**→**14**; **17**→**18**) are in the expected down-field direction.

The chemical shifts observed for the primary carbon atoms in this series of compounds suggests their most probable conformation in solution. The expected up-field shift of the signal of the terminal C-6′ atom in the disaccharide series is observed on 6-*O*-deacetylation (**12**→**13**; 63.22→62.02 p.p.m.), and 2-*O*-debenzoylation to **14** results in little change. The same changes are observed for the trisaccharide series (**16**→**17**→**18**). However, the interunit C-6 and C-6′ of trisaccharide **16** resonate at 67.50 and 67.23, and for **17** at 67.70 and 67.17 p.p.m. These values are significantly below the corresponding value of the signal of C-6 for disaccharides **12** and **13** (68.55 and 68.43 p.p.m., respectively). On 2-*O*-debenzoylation of trisaccharide **17** to give **18**, the shift position of the signals for C-6 and C-6′ returns in each case, to a value close to that observed for disaccharide **14** (*i.e.*, 68.35 and 68.07, respectively, *vs.* 68.54). It is obvious that the addition of a third sugar unit influences the magnetic environment of the two interunit C-6 carbons (C-6 and C-6′). Removal of the benzoate group apparently removes this influence. This change parallels the changes in C-1 methoxyl-proton resonances on glycosidation of **11** to **12** and on *O*-debenzoylation

of **13**. Since the methoxyl group in the disaccharide series corresponds, in steric relationship, to C-6 of the reducing end group in the trisaccharide series, the change in chemical shift of C-6 is not surprising. The identical shift in C-6′, however, has no parallel in the disaccharide series and suggests an additional steric restraint in the trisaccharide. It is not feasible to analyze the conformation of these saccharides in detail, however. Even if the ϕ angle is determined by the exo-anomeric effect[15,16], the ψ angle and rotation around the C-5–C-6 bond allow too many possible conformations.

The success of this synthesis depends on several factors. The 1-*p*-toluenesulfonate is preformed, and the reaction is, therefore, between two species in homogeneous solution. Since *p*-toluenesulfonate is a good leaving- and a poor nucleophile-group, the steric course of the reaction is dominated by the participation of the 2-*O*-benzoyl group. The fact that an exactly analogous reaction leading to the tetrasaccharide failed suggests that, occasionally, special steric factors may come into play at specific steps in the synthesis of a homologous series.

EXPERIMENTAL

General. — ^{1}H-N.m.r. spectra were determined with a Varian A-60-A spectrometer for solutions in chloroform-*d* with tetramethylsilane (Me_4Si) as an internal reference or for solutions in deuterium oxide with acetone (δ 2.07) as a reference for deblocked glycosides. ^{13}C-N.m.r. spectra were determined with a Varian XL-100-15 spectrometer in pulsed Fourier-transform, proton-noise-decoupled mode for similar solutions. The spectra are reported with chemical shifts downfield from Me_4Si, assuming the acetone methyl peak to be located at 30.6 p.p.m. in deblocked glycosides. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter in jacketed 1-dm cells. Melting points were determined on a "Meltemp" apparatus with a 76-mm immersion thermometer. T.l.c. was performed on "Baker-Flex" silica gel 1B-F (2.5 × 7.5 cm) plates. High-pressure, liquid chromatography (l.c.) was performed with a Valvco septumless injector (1.0 mL), a Glenco pump model HPLPS-1, a Waters differential refractometer R-401, a stainless-steel column (25 × 1 cm, internal diameter) containing silica gel (Whatman, Partisil M 9 10/25), and a flow rate of 8.0 mL/min. L.c. for water solutions was performed with the same injector and pump, a Micromeritics 771 refractive-index detector, a stainless-steel column (73 × 1.9 cm, external diameter, jacketed with circulating 60° water) containing Bio-Gel P-2, and a flow rate of 3.0 mL/min. Preparative l.c. (p.l.c.) was performed with a Waters Prep-500 instrument equipped with a silica gel column. Spectrograde acetonitrile was dried with calcium hydride. Silver *p*-toluenesulfonate (Eastman Organic Chemicals, Rochester, NY 14650) was recrystallized from acetonitrile and dried under high vacuum before use.

2-O-Allyl-1,6-anhydro-β-D-galactopyranose (**2**). — This compound was prepared from 2-*O*-allyl-1,6-anhydro-3,4-di-*O*-isopropylidene-β-D-galactopyranose (**1**)

as described previously[5]. This syrup was crystallized from benzene, m.p. 54–55°, $[\alpha]_D^{23}$ −28.4° (*c* 1, chloroform), lit.[5] $[\alpha]_D$ −27.5° (*c* 1, chloroform).

1,6-Anhydro-3,4-di-O-benzyl-β-D-galactopyranose (**5**). — Crystalline **2** (13.0 g) was dissolved in dry tetrahydrofuran (60 mL) and added to a suspension of sodium hydride (14.0 g) in tetrahydrofuran (50 mL). Benzyl chloride (33 mL) was added slowly while the reaction mixture was maintained at reflux. After 24 h, t.l.c. (chloroform) showed some unreacted **2**. Additional benzyl chloride (10 mL) was added slowly and the mixture boiled under reflux for additional 12 h. After this period, t.l.c. showed completion of the reaction. Excess sodium hydride was eliminated with ethanol, and tetrahydrofuran was evaporated under diminished pressure. The residue was partitioned between chloroform and water, and the organic phase was washed with water, dried, and evaporated to dryness. The crude 2-*O*-allyl-1,6-anhydro-3,4-di-*O*-benzyl-β-D-galactopyranose (**3**) was obtained as a syrup and was pure enough for the next step (yield 19.8 g, 81%). A small amount of this crude material was purified by analytical t.l.c. (1:2, v/v, ethyl acetate–hexane) to afford pure **3**; $[\alpha]_D^{23}$ −26.5° (*c* 2.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.37 (10 H, 2 $C_6H_5CH_2$), 6.11–5.59 (m, 1 H, -C*H*=CH_2), 5.42 (bs, 1 H, H-1), 4.69–4.50 (6 H, H-3, H-4, and 2 $C_6H_5CH_2$), 4.00–3.89 (m, 4 H, H-2, H-5, and -OCH_2-CH=), 3.70 (d, 1 H, $J_{5,6}$ 5.8 Hz, H-6), and 3.51 (s, 1 H, H-6′).

Syrupy **3** (11.8 g) was dissolved in toluene (200 mL) containing potassium *tert*-butoxide (6.0 g), and boiled under reflux for 17 h. After this period, ^{1}H-n.m.r. showed signals for the propenyl methyl group at δ 1.54 as a quartet, and for the olefinic proton adjacent to the ether oxygen atom at δ 5.80 as a quartet, and no resonances for allyl methine group at δ 5.30–5.08. The reaction mixture was cooled to room temperature and washed with water until neutral. The organic phase was dried and concentrated to afford 1,6-anhydro-3,4-di-*O*-benzyl-2-*O*-(1-propenyl)-β-D-galactopyranose (**4**) as syrup (yield 11.0 g, 93%), $[\alpha]_D^{23}$ −33.8° (*c* 0.7, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.30 (10 H, aromatic), 5.80 (q, 1 H, $J_{H,H}$ 6.0, J_{H,CH_3} 1.5 Hz, -O-C*H*=CH-CH_3), 5.41 (m, 1 H, H-1), 4.64–4.39 (7 H, H-3, -4, -O-CH=C*H*-CH_3, and 2 $C_6H_5CH_2$), 3.86 (m, 2 H, H-2, -5), 3.71–3.51 (2 H, H-6, -6′), and 1.54 (q, 3 H, *J* 6.5 and 1.5 Hz, CH_3).

Syrupy **4** (7.0 g) was dissolved in 1,4-dioxane (70 mL) containing 5% hydrogen chloride (3 mL) and heated on a steam bath, and the reaction was monitored by t.l.c. After 1 h, t.l.c. (2:3, v/v, ethyl acetate–hexane) showed completion of reaction. The reaction mixture was neutralized with sodium hydrogencarbonate and evaporated to dryness, and the residue extracted with chloroform, washed with water, dried, and concentrated to a syrup. This syrup was crystallized from cyclohexane to afford **5** (yield 3.5 g, 56%), m.p. 70–71°; $[\alpha]_D^{23}$ −36.9° (*c* 1.4, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 7.31 (10 H, aromatic), 5.36 (s, 1 H, H-1), 4.70–4.36 (6 H, H-3, -4, and 2 $C_6H_5CH_2$), 3.87–3.57 (4 H, H-2, -5, -6, and -6′), and 2.15 (bs, 1 H, OH D_2O exchangeable).

Anal. Calc. for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found: C, 70.64; H, 6.53.

1,6-Anhydro-2-O-benzoyl-3,4-di-O-benzyl-β-D-galactopyranose (**6**). — Crystal-

line **5** (15.6 g) was dissolved in triethylamine (100 mL) and benzoyl chloride (22 mL) was added dropwise for 1 h. The reaction mixture was kept for an additional 1 h at room temperature, and t.l.c. (2:3, v/v, ethyl acetate–hexane) after this period showed completion of reaction. The excess of benzoyl chloride was decomposed by addition of water (2 mL), and the mixture stirred for 2 h. Triethylamine was removed by vacuum distillation, the residue was dissolved in chloroform, washed with water, saturated sodium hydrogencarbonate, and water, dried, and concentrated to a thick syrup (22.0 g). This syrup was crystallized from 95% ethanol to afford **6** (yield 17.2 g, 85%), m.p. 89–90°, $[\alpha]_D^{23}$ +0.8° (*c* 2.5, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.05 (m, 2 H, *m*-Bz), 7.47–7.17 (13 H, *o*- and *p*-Bz, and 2 $C_6H_5CH_2$), 5.40 (s, 1 H, H-1), 5.14 (bs, 1 H, H-2), 4.84–4.49 (4 H, 2 $C_6H_5CH_2$), 4.39 (m, 2 H, H-3, -4), and 3.85–3.50 (3 H, H-5, -6, and -6′).

Anal. Calc. for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found: C, 72.44; H, 5.84.

*1,6-Di-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-*D*-galactopyranose* (**7**). — Benzoate **6** (3.0 g) was dissolved in 3:2 (v/v) glacial acetic acid–acetic anhydride (50 mL) and conc. sulfuric acid (0.5 mL) in acetic anhydride (5 mL) was added at 10°. T.l.c. (1:2, v/v, ethyl acetate–hexane) after 1 h showed a slow-moving product and no starting material **7**. The reaction mixture was poured over crushed ice and stirred for 8 h to give a crude crystalline compound. This crude diacetate **7** was filtered off and washed several times with cold water and dried (yield 3.47 g, 97%), m.p. 81–82°; recrystallized from ethanol-water as fine needles, m.p. 84–85°, $[\alpha]_D^{22}$ +90.1° (*c* 1.14, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.01 (m, 2 H, *m*-Bz), 7.51–7.20 (13 H, *o*- and *p*-Bz, and 2 $C_6H_5CH_2$), 6.56 (d, 0.78 H, $J_{1,2}$ 3.2 Hz, H-1α), 5.85 (q, 1.22 H, H-2 and -1β), 5.15–4.57 (4 H, 2 $C_6H_5CH_2$), 4.29–3.95 (5 H, H-3, -4, -5, -6, -6′), 2.03, and 1.95 (s, 3 H each, 2 Ac).

Anal. Calc. for $C_{31}H_{32}O_9$: C, 67.87; H, 5.88. Found: C, 68.19; H, 5.92.

*6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-α-*D*-galactopyranosyl chloride* (**8**). — The di-*O*-acetyl derivative **7** (7.0 g) was dissolved in dry diethyl ether (250 mL) and cooled to 0°. The solution was saturated with dry hydrogen chloride, the flask was tightly stoppered, and the solution allowed to come to room temperature. After 48 h, the reaction was complete (t.l.c.). Dry nitrogen was bubbled through the solution to remove the excess of hydrogen chloride and the solution was evaporated under reduced pressure at a temperature <20° to give a clear, colorless syrup. The syrup was dissolved in dichloromethane and the solution was washed with cold sodium hydrogencarbonate solution, cold water, dried ($MgSO_4$), and evaporated to a clear syrup. This syrup was crystallized from carbon tetrachloride–hexane to afford **8** (yield 5.1 g, 76%), m.p. 94.5–95°, $[\alpha]_D^{23}$ +152.9° (*c* 0.73, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.05 (m, 2 H, *m*-Bz), 7.50–7.23 (13 H, *o*- and *p*-Bz, and 2 $C_6H_5CH_2$), 6.49 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 5.74 (q, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 5.10–4.49 (4 H, 2 $C_6H_5CH_2$), 4.26–3.95 (5 H, H-3, -4, -5, -6, -6′), and 1.87 (s, 3 H, Ac).

Anal. Calc. for $C_{29}H_{29}ClO_7$: C, 66.35; H, 5.57; Cl, 6.75. Found: C, 66.38; H, 5.85; Cl, 6.65.

*Methyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-*D*-galactopyranoside* (**10**). —

The following reaction was performed on a high-vacuum rack in a reaction vessel that had two chambers separated by a fritted-glass filter. In one chamber was added absolute methanol (1 equiv., 0.17 g) in a sealed capillary, in the other chamber silver *p*-toluenesulfonate (1.56 g) and chloride **8** (2.7 g). The reaction vessel was evacuated for several hours to dry the reagents. Dry acetonitrile was added by distillation (5.0 mL) under dry nitrogen. The formation of the 1-*O*-tosyl-D-galactopyranose derivative **9** took place immediately as shown by the rapid precipitation of silver chloride. After 10 min, the acetonitrile solution of **9** was introduced through a filter into the chamber containing the methanol. Suction was provided by cooling the receiver. The capillary containing methanol was broken, the content mixed with **9** and then placed in the dark for 17 h at room temperature. Chloroform (20 mL) was added and the solution washed with saturated sodium thiosulfate (1 × 20 mL), sodium hydrogencarbonate (1 × 20 mL), and water. The organic phase was dried ($MgSO_4$) and evaporated to a syrup (2.5 g). The syrup was purified by l.c. on a column of silicic acid with 1:2 (v/v) ethyl acetate–hexane as solvent. The monosaccharide fraction (17 counts) was evaporated to give 2.3 g (86%) of a syrup, $[\alpha]_D^{23}$ +18.5° (*c* 0.22, chloroform), that was homogeneous by t.l.c. (2:3, v/v, ethyl acetate–hexane). The product was crystallized from ethyl acetate–hexane to give 2.18 g (81.3%) of **10**, m.p. 136°, $[\alpha]_D^{24}$ +18.7° (*c* 0.76, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.04 (m, 2 H, *m*-Bz), 7.52–7.27 (13 H, *o*- and *p*-Bz and 2 $C_6H_5CH_2$), 5.75 (q, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.2 Hz, H-2), 5.21–4.61 (4 H, 2 $C_6H_5CH_2$), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1β), 4.37–3.65 (5 H, H-3, -4, -5, -6, -6′), 3.50 (s, 3 H, OMeβ), and 2.01 (s, 3 H, Ac); ^{13}C-n.m.r. ($CDCl_3$): 102.38 (C-1), 80.33 (C-3), 74.37 (C-4), 72.58 and 72.39 (C-2, -5), 63.23 (C-6), 56.41 (OMe), and 20.80 (Ac).

Anal. Calc. for $C_{30}H_{32}O_8$: C, 69.22; H, 6.20. Found: C, 69.58; H, 6.46.

Methyl 2-O-benzoyl-3,4-di-O-benzyl-β-D-galactopyranoside (**11**). — To a solution of **10** (1.5 g) in methanol (20 mL) was added 32% ammonium hydroxide (5.0 mL). The solution was kept for 8 h at room temperature. The solvent was distilled off under diminished pressure and the product was extracted with chloroform. The solution was washed with water, dried (Na_2SO_4), and evaporated to a syrup that crystallized from ether–hexane to give 1.37 g (99%) of **11**, m.p. 91–93°, $[\alpha]_D^{24}$ +16.5° (*c* 0.83, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 8.03 (m, 2 H, *m*-Bz), 7.51–7.23 (13 H, *o*- and *p*-Bz, and 2 $C_6H_5CH_2$), 5.74 (q, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.2 Hz, H-2), 5.17–4.59 (4 H, 2 $C_6H_5CH_2$), 4.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1β), 4.30–3.52 (5 H, H-3, -4, -5, -6, -6′), 3.46 (s, 3 H, OMeβ), and 2.35 (bs, 1 H, OH); ^{13}C-n.m.r. ($CDCl_3$): δ 102.56 (C-1), 80.35 (C-3), 75.20 (C-5), 74.38 (C-4), 72.46 (C-2), 62.00 (C-6), and 56.55 (OMe).

Anal. Calc. for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32. Found: C, 70.53; H, 6.55.

Methyl 6-O-(6-O-acetyl-2-O-benzyl-3,4-di-O-benzoyl-β-D-galactopyranosyl)-2-O-benzoyl-3,4-di-O-benzyl-β-D-galactopyranoside (**12**). — The glycosidation was carried out as described for **10**. The chloride **8** (0.8 g) and alcohol **11** (0.7 g) gave 1.1 g (85%) of **12** as crystalline compound (methanol), m.p. 89–91°, $[\alpha]_D^{25}$ +24.3° (*c* 1.27, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 3.19 (OMeβ); ^{13}C-n.m.r.: see Table II.

Anal. Calc. for $C_{57}H_{58}O_{14}$: C, 70.75; H, 6.04. Found: C, 70.36; H, 5.99.

Methyl 2-O-benzoyl-6-O-(2-O-benzoyl-3,4-di-O-benzyl-β-D-galactopyranosyl)-3,4-di-O-benzyl-β-galactopyranoside (**13**). — The acetyl group of disaccharide **12** was removed with ammonium hydroxide as described for **10** to afford the crystalline disaccharide **13** in 84% yield, m.p. 118–120° (from ethanol), $[\alpha]_D^{25}$ +22.1° (*c* 0.8, chloroform); ^{1}H-n.m.r.: δ 3.19 (OMeβ); ^{13}C-n.m.r., see Table II.

Anal. Calc. for $C_{55}H_{56}O_{13}$: C, 71.41; H, 6.10. Found: C, 71.03; H, 5.94.

Methyl 3,4-di-O-benzyl-6-O-(3,4-di-O-benzyl-β-D-galactopyranosyl)-β-D-galactopyranoside (**14**). — To a solution of **13** (0.4 g) in methanol (20 mL) was added a solution of 0.2M sodium methoxide in methanol (5.0 mL). The solution was kept for 48 h at room temperature, and then neutralized with glacial acetic acid. The solvent was distilled off and the product extracted with chloroform. The solution was washed with water, dried ($MgSO_4$), and evaporated to a syrup (0.3 g, 97%). This syrup crystallized from ether–hexane, m.p. 136–138°, $[\alpha]_D^{23}$ −6.8° (*c* 0.74, chloroform): ^{1}H-n.m.r. ($CDCl_3$): δ 3.50 (OMeβ); ^{13}C-n.m.r., see Table II.

Anal. Calc. for $C_{41}H_{48}O_{11}$: C, 68.70; H, 6.75. Found: C, 69.12; H, 6.34.

Methyl 6-O-β-D-galactopyranosyl-β-D-galactopyranoside (**15**). — The disaccharide **14** (100 mg) was dissolved in methanol (5 mL) and water (1 mL) was added. Hydrogenolysis was performed for 36 h in the presence of palladium-on-charcoal (100 mg) at room temperature under a hydrogen atmosphere. Filtration of the catalyst gave a thick syrup that was purified by l.c. (Bio-Gel) with water as solvent. A disaccharide peak at 13.5 count was collected and evaporated to a syrup (40 mg, 80%) that crystallized from ethanol–water to give **15**, m.p. 160–165° (dec.), $[\alpha]_D^{25}$ +18.7° (*c* 1.02, water); ^{1}H-n.m.r. (D_2O): δ 3.45 (OMeβ); ^{13}C-n.m.r.: see Table II.

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 43.57; H, 7.21.

Preparation of trisaccharides **16–19**. — The trisaccharides were synthesized in the same manner as the disaccharides. Physical constants and ^{1}H-n.m.r. data reported in Table I and ^{13}C-n.m.r. in Table II. Trisaccharide **19** was isolated as a foam after several co-evaporations of the residual solvents with ethanol.

Evidence for ^{13}C-n.m.r. assignments. — The ^{13}C chemical shifts of the mono-, di-, and tri-saccharides shown in Table II are derived largely from correlations of the observed chemical shift differences with those that would be expected from changes in linkage, deblocking, and conformation:

Compound **10**. The assignments were based on the expected shifts on substitution of methyl β-D-galactopyranoside[10]. The C-1 and C-6 signals were assigned to the low- and high-field resonances. The C-3 resonance at 72.85 p.p.m. in parent compound would shift down-field on benzylation ~7 p.p.m. to 80.33 p.p.m. (7–10 p.p.m. down-field shift on etherification[13,17]). The C-2 resonance would be close to the parent value since a small down-field shift of 1–2 p.p.m. was expected on acylation[18,19], and C-3 etherification would reduce this shift to 72.58 or 72.39 p.p.m. The C-4 signal would shift down-field less than that of C-3 on benzylation (~4.5 p.p.m.[10]) since the substituent is axial, and an axial oxygen atom is associated with increased shielding of the ^{13}C nucleus to which it is bonded[20]. The C-5 signal would be at higher field than in the parent compound owing[17,19] to substitution at C-6 and C-4.

Compound **11**. On deacetylation of **10**, only the C-5 and C-6 resonances would change markedly; that of C-5 downfield and that of C-6 upfield.[18] The observed change supports the assignment of the C-4 signal at 74.37 p.p.m. in **10**.

Compound **12**. The C-1 resonances would have approximately the same position as that in **10**, whereas the inter-unit C-1′ signal was expected to be down-field but somewhat different from those of C-1 of **10** and **11**. However, the signal C-3′ was expected to be more similar to that of C-3 of **10** than of that of C-3 of **12** since the acetyl group is at C-6 in **10** and at C-6′ in **12**. The signal of C-3 of **12** would be somewhat up-field of that of C-3 of **11** owing to substitution at C-6. The resonances for C-2, -2′, -4, -4′, -5, and -5′ could not be differentiated. The substituent for C-6′ is located at the same place as that for C-6 in **10**. The C-6 resonance in **12** has an up-field shift (~7 p.p.m.) relative to that of C-6 in **11** owing to glycosidation[18,21–24].

Compound **13**. The C-1, -1′, -3, -3′, and -6 resonances are expected to be the same as for **12**. The C-6′ signal would move upfield on *O*-deacetylation of **12**. A double peak at 75.15 p.p.m. undoubtedly includes C-5′, since a down-field shift was expected on *O*-deacetylation. However, the assignment of the C-5 signal to the same frequence is in doubt.

Compound **14**. As *O*-debenzoylation at O-2 and O-2′ of **13** resulted in approximately the same down-field shift of C-1 and C-1′, and identical effect may be assigned for **14**. A similar down-field shift occurred for the C-3 and -3′ signal to assign both resonances at 82.15 p.p.m. There appears to be little change in the other signals for C-2, -2′, -4, -4′, -5, -5′, -6, and -6′. The down-field shift of the methoxyl signal is as expected.

Compound **15**. These assignments were made by analogy to the methyl β-D-galactopyranoside, with a substantial down-field shift for the C-6 signal and some up-field shift assumed for the C-1′ and -5 signals.

Compound **16**. The signals of the trisaccharide series followed the same pattern, except for the unexpected down-field shift of the C-6 and -6′ signals after *O*-debenzoylation, which is related to steric effects. The chemical shift of ^{13}C atoms is very sensitive to change in chemical bonding and steric interactions[25].

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