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SYNTHESIS AND CONFORMATIONAL PROPERTIES OF METHYL 6,6-DI-*C*-METHYL-β-D-GALACTOPYRANOSIDE. PROBES FOR THE COMBINING SITES OF D-GALACTOSYL-BINDING PROTEINS*

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ABSTRACT

The binding of D-galactopyranosyl groups by lectins and antibodies can involve the 5-hydroxymethyl group. In order to examine the nature of these binding reactions, it was of interest to synthesize 6,6-di-*C*-methyl-D-galactose which was found to exist, like D-galactose, extensively in the pyranose forms. 2,3,4,6-Tetra-*O*-acetyl-7-deoxy-6-*C*-methyl-α-D-*galacto*-heptopyranosyl bromide was prepared under standard conditions and converted into methyl 6,6-di-*C* methyl-β-D-galactopyranoside (**6**). Evidence based on ^{13}C-n.m.r. studies indicates that the favored conformer of **6** has O-4 and O-6 in *syn*-axial-like relationship. General comments are presented on the nature of the binding of oligosaccharides by proteins.

INTRODUCTION

Kabat's interpretation[1] of the observation by Springer and Williamson[2] that 3-*O*-methyl-D-fucose is as active an inhibitor of the agglutination of human O(H) red cells as are L-fucose and 3-*O*-methyl-L-fucose, whereas D-fucose is inactive, substantiated the view that a complementarity of hydrophobic surfaces can provide an important driving force for the complexing of oligosaccharides with lectins and antibodies. That this is in fact the case now seems well established[3,4].

In 1978, we proposed[5] that intramolecular hydrogen bonding between a hydroxyl group and a sterically well-disposed proton acceptor may allow otherwise complementary hydrophobic regions to form a complex. This contention has since received experimental support in two instances[3,4]. The original effort[5], involving an investigation of the binding of anti-β-D-galactosyl antibodies with a series of β-D-galactosyl-related haptens, was not conclusive. The choice of an anti-β-D-Gal*p* antibody preparation for these studies derived from the ubiquitous D-*galacto* con-

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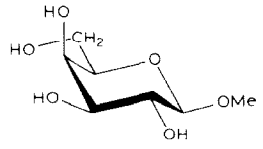
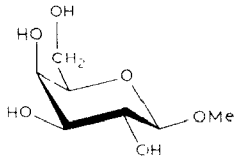
figuration for the terminal groups of the wide variety of oligosaccharides that occur in glycoproteins and glycolipids. These units include α-D-Gal*p*, β-D-Gal*p*, α-D-Gal*p*NAc, β-D-Gal*p*NAc, and α-L-Fuc*p*. The phenomenon of "clustering"[6,7] of β-D-Gal*p* groups is particularly noteworthy in these regards.

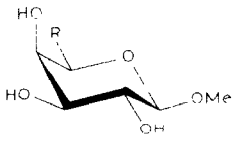
Any attempt to understand the binding potential of terminal D-Gal groups must involve an assessment of contributions by the CH_2OH-5 groups. In our earlier studies[5], the 6-deoxy derivative **2** of methyl β-D-galactopyranoside (**1**) was found to be a very poor anti-β-D-Gal*p* inhibitor ($\Delta\Delta G°$ 6.7 kJ/mol) relative to **1**. Methyl α-L-arabinopyranoside (**3**) was even less potent ($\Delta\Delta G°$ 9.2 kJ/mol). These observations clearly required an involvement of the hydroxymethyl group of **1** in the binding reaction.



1a

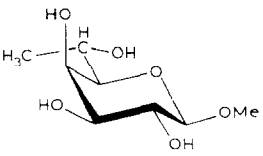


1b

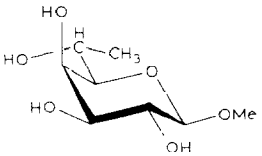


2 R = Me

3 R = H



4



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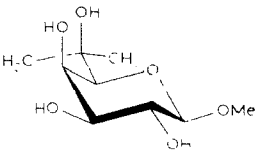


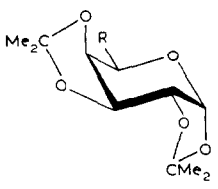
6

In order to examine the involvement of the hydroxymethyl group of **1** in more detail, the 6-*C*-methyl derivatives **4** and **5** were prepared in the anticipation that these would strongly tend to exist in the conformations shown. It proved that the L-isomer (**4**) was a substantially poorer inhibitor than the D-isomer (**5**) ($\Delta\Delta G°$ 3.3 kJ/mol). Also, the D-isomer (**5**) was somewhat more potent ($\Delta\Delta G°$ 1.3 kJ/mol) than the 6-deoxy compound (**2**). This latter observation has gained relevance since it is now established[8] that methyl β-D-galactopyranoside resides, in aqueous solution, extensively in the conformation **1a**, which has OH-6 in the same orientation as was expected for the D-isomer (**5**). It was proposed[5] that, in the event the lower potency of **5**, relative to **1**, was not due to the presence of the 6-*C*-methyl group, methyl β-D-galactopyranoside (**1**) may be accepted by the combining site in the conformation displayed in structure **1b**. Indeed, evidence has recently been obtained that the substitution of a hydrogen atom by a *C*-methyl group may not adversely influence binding[3]. On the other hand, this need not be the case should the methyl group be required to occupy a rigidly held, strongly hydrophilic region of the combining site. For these reasons, it was of interest to synthesize the 6,6-di-*C*-methyl derivative of methyl β-D-galactopyranoside in the expectation that this

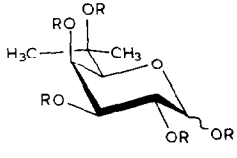
compound would, in aqueous solution, display the conformational preference depicted in **6**. This report deals with the preparation of **6** and the examination by n.m.r. spectroscopy of its conformational preference as well as the conformational preferences of the previously reported mono-*C*-methyl derivatives **4** and **5**.

RESULTS AND DISCUSSION

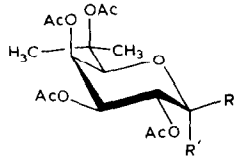
The oxidation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**7**)[9] was conveniently performed on a 50-g scale by applying a modification of the procedure of Garegg and Samuelson[10] using chromium trioxide–pyridine complex in the presence of acetic anhydride. The crude 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-1,6-hexadialdo-1,5-pyranose (**8**), obtained in 93% yield, was converted directly into the diastereomeric mixture of 7-deoxy-1,2:3,4-di-*O*-isopropylidene-D,L-*glycero*-β-D-*galacto*-heptopyranose (**9**) by alkylation with methylmagnesium iodide as previously described[11]. The product was then re-oxidized, as just described, to provide the ketone **10** which, without purification, was converted into crystalline 7-deoxy-1,2:3,4-di-*O*-isopropylidene-6-*C*-methyl-α-D-*galacto*-heptopyranose (**11**) by repetition of the Grignard reaction. The overall yield of **11** was 50% based on **7** used as starting material.



7 R = CH_2OH
8 R = CHO
9 R = CH(Me)OH
10 R = $COCH_3$
11 R = $C(Me_2)OH$



12 R = H
13 R = Ac



14 R = H, R′ = Br
15 R = OMe, R′ = H

Hydrolysis of the isopropylidene groups of **11** with trifluoroacetic acid in wet dichloromethane proceeded to completion in 2 h at 0° to provide 7-deoxy-6-*C*-methyl-D-*galacto*-heptose (**12**, 6,6-di-*C*-methyl-D-galactose). No evidence was obtained for any product derived from the elimination of the tertiary hydroxyl group. The ^{1}H-n.m.r. spectrum of **12** for a solution in deuterium oxide indicated that it existed to the extent of 90% in the pyranose form with a ratio of β to α anomer of 1.6 (based on the relative intensities of the respective anomeric protons; H-1α: δ 5.29, $J_{1,2}$ 3.8 Hz; and H-1β: δ 4.57, $J_{1,2}$ 7.5 Hz). This ratio is essentially identical to that for D-galactopyranose[12] in aqueous solution. Two additional doublets at δ 5.27 (*J* 5.0 Hz) and 5.20 (*J* 3.5 Hz), corresponding to 4% each of the total signal

in the anomeric region, were also observed in this spectrum and likely arose from the furanose forms of **12**, but this was not further investigated.

The acetylation of **12** proceeded with difficulty, even in the presence of 4-(dimethylamino)pyridine, undoubtedly owing to the severe steric crowding experienced by the OH-4 and -6. The peracetylated compound **13** could be isolated in 42% yield after a reasonable reaction time, and underacetylated material could be recycled. Treatment of **13** with hydrogen bromide, in the usual manner, provided the glycosyl bromide **14**, which could be glycosylated under standard Koenigs–Knorr conditions, using silver carbonate as promoter, to provide the peracetylated methyl β-D-glycoside **15** in 80% yield. Transesterification of **15** with sodium methoxide in methanol provided the target compound **6**.

The ^{1}H-n.m.r. spectral parameters recorded for compounds **1**, **4**, **5**, and **6** are presented in Table I. Examination of the vicinal-coupling constants for the pyranose ring protons showed that the compounds all reside in essentially the $^4C_1$ (D) chair conformation. Indeed, the chemical shifts of H-1, -2, and -3 are almost invariant. In the monomethyl compounds **4** and **5**, the resonances for H-4 could be seen to be shifted downfield by 0.038 and 0.183 p.p.m., respectively, relative to the corresponding resonance of **1**, and in the dimethyl compound **6**, this proton was de-

TABLE I

^{1}H-N.M.R. DATA FOR COMPOUNDS **1**, **4**, **5**, AND **6**[a]

Atoms	*Compound*			
	1	**4**[b]	**5**[b]	**6**
H-1	4.315	4.282	4.312	4.311
($J_{1,2}$)	(7.9)	(7.9)	(7.9)	(7.6)
H-2	3.512	3.498	3.495	3.517
($J_{2,3}$)	(9.9)	(9.9)	(9.9)	(9.9)
H-3	3.644	3.620	3.626	3.584
($J_{3,4}$)	(3.5)	(3.4)	(3.3)	(3.3)
H-4	3.919	3.957	4.102	4.161
($J_{4,5}$)	(0.8)	(<1)	(<1)	(0.9)
H-5	3.690	3.356	3.302	3.364
($J_{5,6}$)	(7.6)	(8.1)	(8.4)	
H-6	3.761	4.029	3.962	
($J_{6,7}$)	(4.4)	(6.5)	(6.3)	
H-6	3.804			
($J_{6,7}$)	(7.6)			
H-7[c]		1.221	1.303	1.310 1.326
OCH_3	3.573	3.581	3.551	3.585

[a]All spectra were recorded at 360 MHz with 32k data points and a sweep width of 2100 Hz. The concentration of the samples was 30mM in deuterium oxide and chemical shifts (δ) are reported relative to the signal of acetone (0.1%) as internal standard set at δ 2.225 at 294 K. Vicinal coupling constants are in Hz. [b]The previously reported spectra[5] used an external standard and the chemical shifts for H-3 and -4 of **4** were inadvertently reported as τ values. [c]H-7 refers to the protons of the 6-*C*-methyl group(s).

shielded by 0.242 p.p.m. Deshielding of this magnitude may be expected[13] to arise owing to the steric compression of H-4 by bulky substituents at C-6, but their interpretation, in terms of achieving a decision regarding the preferred side-chain conformation in **6**, is hazardous since even minor pyranose ring distortions may be expected to cause shifts of similar magnitude[14]. All the *C*-methylations of C-6 caused a shielding of H-5 by more than 0.3 p.p.m.

The conformational properties of compounds **4, 5,** and **6** were examined by nuclear Overhauser enhancement[15] (nOe) studies. Saturation of the 6-*C*-methyl group of **4** caused an nOe of 20% for H-4, 7% for H-5, and 28% for H-6. In the case of the D-isomer (**5**), irradiation of the 6-*C*-methyl group caused no enhancement of the signal for H-4, but still providing an nOe of 7% for H-5 and 22% for

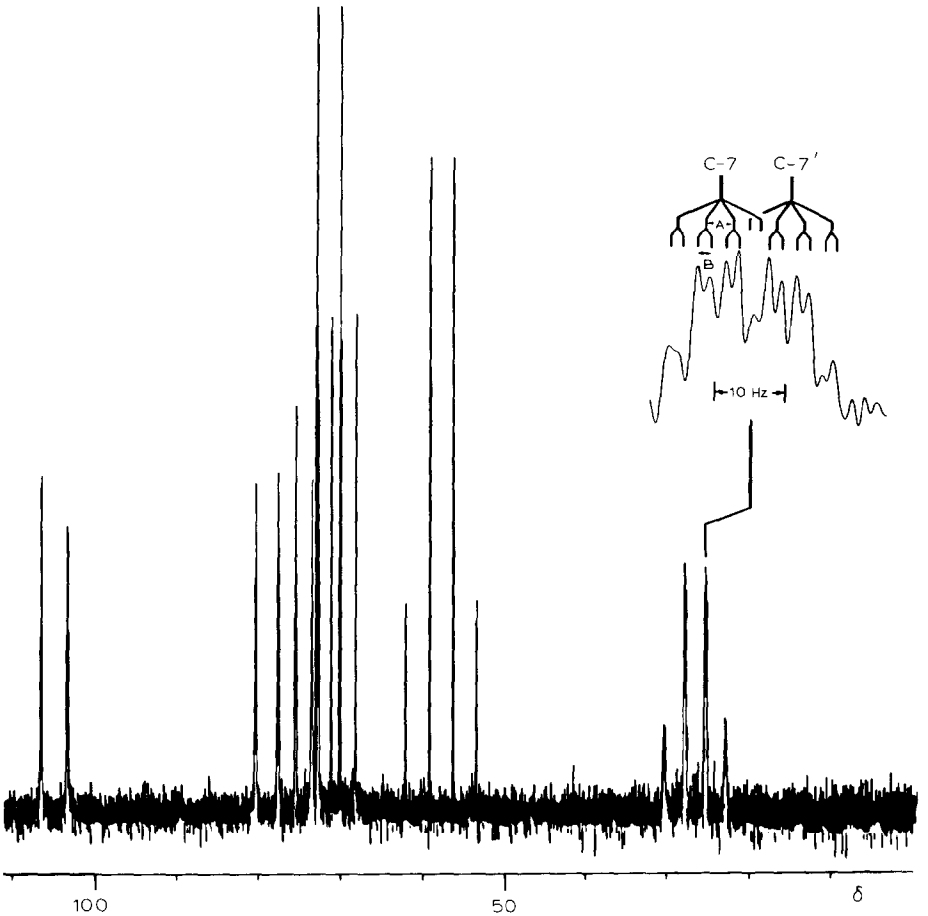


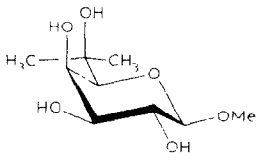
Fig. 1. 50-MHz, ^{1}H-coupled ^{13}C-n.m.r. spectrum of compound **6** in deuterium oxide solution. The insert shows an expansion of one of the central signals of the two overlapping quartets arising from the *C*-methyl groups. Signal assignments are provided in the Experimental section. A = 4.3 ±0.1, B = 1.8 ±0.1 Hz.

H-6. These results confirm the configurational assignments and require the compounds to exist extensively in the conformations shown.

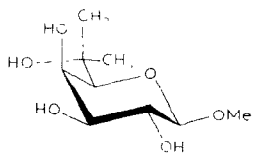
Attempts to use such nOe experiments to establish the side-chain conformation in **6** were complicated because their selective irradiation was not possible as the methyl group resonances were separated by only 6 Hz at 360 MHz. Irradiation of either H-4 or -5 of **6** produced no enhancement of either methyl group signal, which was not unexpected since methyl-group relaxation need not be dominated by intramolecular dipole–dipole relaxation.

Vicinal $^{13}C-^{1}H$ coupling is known[16] to be torsion-angle dependent. In the ^{1}H-coupled ^{13}C-n.m.r. spectrum of **6** (see Fig. 1), the signals for the *C*-methyl carbons (C-7 and -7′) were found at δ 26.51 and 26.29, respectively, and were separated by 11 Hz at 50 MHz. As seen in Fig. 1, each of these resonances is split into a quartet by a one-bond $^{13}C-^{1}H$ coupling of 128 Hz, with the result that these resonances appear as a superimposition of two quartets, separated by 11 Hz, between δ 20 and 30. The insert in Fig. 1 shows the expansion of one of the central signals of this pattern, where it can be seen that the lines corresponding to each of the carbon resonances is split further by coupling constants of 4.3 ±0.1 Hz (A) and 1.8 ±0.1 Hz (B). The other central signal for the methyl quartet was virtually identical.

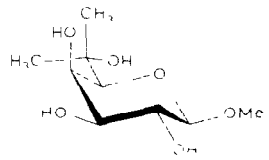
A $^{3}J_{CCCH}$ coupling of ~4.3 Hz is expected[16] for the coupling between geminal methyl groups, thus the 1.8-Hz splitting that was observed could be assigned to the coupling with H-5. $^{3}J_{CH}$ Values of sp^3 hybridized carbon atoms are sensitive to both the electronegativity and the steric requirements of directly bonded substituents. For this reason and also because the conformers were not likely in exactly staggered orientations, an interpretation of the $^{3}J_{CH}$ coupling constant, in terms of a precise, average dihedral angle, should not be attempted. It is noteworthy, however, that the Karplus equation ($^{3}J_{CH} = 3.09 - 0.38\cos(\phi - 5.53) + 2.57\cos2(\phi - 5.53)$) derived by Spoormaker and de Bie[17] for substituted *tert*-butyl fragments, as well as the generally observed dihedral-angle dependence of $^{3}J_{CH}$ in pyranose and related structures[18,19] require a coupling constant ~2 Hz to correspond to a dihedral angle of ~60° (or 120°). What is factual is that both of the methyl-group carbon atoms show the *same* small coupling with H-5, a situation that requires the *average* conformer to be well represented by **6a**.



6a



6b



6c

Since hard-sphere calculations[20] do not serve well for estimating conformational preferences that involve nonbonded interactions with hydroxyl groups, the interaction-free energies developed by Angyal[12] to rationalize the conformational

properties of aldopyranoses in aqueous solution were used to estimate the conformational equilibrium for compound **6**. On this basis, the conformational, free-energy differences between the three staggered conformers about the C-5–C-6 bond are −4.2 (**6a**), 0 (**6b**), and −1.90 (**6c**) kJ/mol. It would follow that, at equilibrium, the mole fractions of conformers **6a, 6b,** and **6c** are 0.64, 0.11, and 0.25, respectively. Although this situation could be accommodated by the ^{13}C-n.m.r. data, as mentioned earlier, such attempts would include too many assumptions. The point of importance is that the introduction of the two *C*-methyl groups to form compounds of type **6** can be expected to make readily available the orientation of the 6-hydroxyl group that is displayed in conformer **6a**.

In conclusion, we would like to comment briefly on the picture that is emerging for the binding of oligosaccharides by lectins and antibodies. Evidence is accumulating[3,4] that hydrophobic bonding provides an important driving force. To date, the structure of the combining site is not known, but the binding of the complementary hydrophobic surfaces appears to leave most of the hydroxyl groups exposed to the aqueous phase. It is well known, however, that the surfaces of immunoglobulins are rich in polar residues of amino acids that form the protein molecule. Consequently, when such polar groups reside at the periphery or within the combining site, the disposition of hydroxyl groups of the oligosaccharide next to those polar groups will be energetically much more favorable than would be the case, should these polar groups be brought into contact with a hydrophobic region of the oligosaccharide. This does not, however, necessarily mean that a compatibility for the penetration of a rigidly held hydrophilic grouping into a complementary, rigidly held, hydrophilic acceptor site will add importantly to the driving force of the reaction. Thus, for example, should the anti-β-D-Gal antibodies[5] have accepted methyl β-D-galactopyranoside in conformation **1a**, the relatively poor inhibition ($\Delta\Delta G°$ 5.4 kJ/mol) exhibited by the D-6-*C*-methyl derivative (**5**) may have arisen because the methyl group encounters a rigidly held, hydrated hydrophilic region of the combining site. This situation could also be the cause of the poor inhibition ($\Delta\Delta G°$ 6.7 kJ/mol) displayed by the 6-deoxy compound **2**. If so, the small (1.3 kJ/mol) difference between the binding reactions of compounds **2** and **5** would suggest that the contribution to the stability of the complex made by the OH-6 groups of **1a** and **5** is rather weak. Evidently, therefore, such matters as interactions between polar groups may have an important bearing on the specificity of a reaction, but may not necessarily be of major importance to the driving force (ΔH–$T\Delta S$) of the binding. Indeed, the stabilization gained by the bringing together of complementary hydrophobic regions from aqueous solution may be sufficient[5] to cause hydrophilic groups, in certain instances, to leave the aqueous phase and become engaged with an appropriate polar acceptor, in the combining site; and the next change in energy resulting from this specific interaction actually making a destabilizing contribution to the formation of the complex. To dissect these matters is a challenge for the future in which synthetic carbohydrate chemistry will undoubtedly play a role of key importance.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 polarimeter. Solvent evaporations were performed on a rotary evaporator under the vacuum of a water aspirator at a bath temperature <40°. Thin-layer chromatograms were developed on Silica Gel H (E. Merck, Darmstadt) and the spots detected with 5% sulfuric acid in ethanol after heating at 100°. Column chromatography was performed on Silica Gel 50 (230–400 mesh, E. Merck) unless otherwise stated. ^{1}H-N.m.r. spectra were recorded at 100 MHz (Varian HA-100) or 200 MHz (Bruker WH-200) with either (^{2}H)chloroform as solvent and tetramethylsilane as internal standard (signal set at δ 0), or deuterium oxide as solvent and acetone as internal standard (signal set at δ 2.225).

*1,2:3,4-Di-O-isopropylidene-α-*D-galacto-*1,6-hexodialdo-1,5-pyranose* (**8**). — Chromium trioxide (76.8 g, 0.768 mol) was added to a stirred solution of dichloromethane (2 L) and anhydrous pyridine (121 g, 1.53 mol) over a period of 30 min. The mixture was stirred for an additional 15 min. 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (**7**) (50 g, 0.192 mol) in dichloromethane (200 mL) was then added, followed by acetic anhydride (29.5 g, 0.288 mol), and the reaction was monitored by t.l.c. (3:2, v/v, toluene–ethyl acetate). After ~2 h, the reaction was completed and the mixture was poured into ethyl acetate (1 L). A tarry precipitate formed which was extracted with ethyl acetate. The combined solutions were filtered through a pad of Silica Gel G. The filter cake was washed with ethyl acetate and the combined filtrates were evaporated *in vacuo* to give a syrup, which was freed of pyridine and acetic anhydride by coevaporations with toluene. Solvent removal in a high vacuum left 46 g of a pale-yellow oil whose ^{1}H-n.m.r. spectrum (which was in accord with that published by Horton *et al.*[7]) indicated ~90% purity.

*7-Deoxy-1,2:3,4-di-O-isopropylidene-*D,L-glycero-*β-*D-galacto-*heptopyranose* (**9**). — The crude aldehyde **8** (50 g, 193 mmol) was converted into the diastereomeric mixture **9** (48.5 g of crude product) by the procedure previously described[11], and used without purification to prepare the ketone **10**.

*7-Deoxy-1,2:3,4-di-O-isopropylidene-α-*D-galacto-*heptopyranose-6-ulose* (**10**). — The mixture **9** was oxidized on a 50-g (182-mmol) scale with the same procedure as that described for the preparation of **8**. The product was a yellow oil (90% yield) whose ^{1}H-n.m.r. spectrum indicated a high level of purity; ^{1}H-n.m.r. ($CDCl_3$): δ 5.56 (d, 1 H, H-1), 2.25 (s, 3 H, $COCH_3$), 1.51, 1.45, 1.37, and 1.32 (4 s, 12 H, 2 Me_2C).

*7-Deoxy-1,2:3,4-di-O-isopropylidene-6-C-methyl-α-*D-galacto-*heptopyranose* (**11**). — Methylmagnesium iodide was prepared under standard conditions using magnesium (8.52 g, 350 mmol) and methyl iodide (49.8 g, 350 mmol) in ether (350 mL). A solution of the ketone **10** (50 g, 183 mmol) in ether (250 mL) was added dropwise with stirring. After the addition was complete, t.l.c. indicated complete disappearance of the starting material. The reaction mixture was cooled in ice–

water and a solution of 10% ammonium chloride in water (300 mL) was added. Work-up in the usual manner using diethyl ether as extractant, drying (magnesium sulfate), and solvent removal left a solid residue that was crystallized from hot Skelly Solve B. On recrystallization from the same solvent, pure material (35 g, 66%) was obtained, m.p. 83–85°, $[\alpha]_D^{20}$ −58.5° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.65 (d, 1 H, $J_{1,2}$ 5 Hz, H-1), 4.68–4.24 (m, 3 H, H-2, -3, -4), 3.48 (d, 1 H, $J_{4,5}$ 1.0 Hz, H-5), 3.30 (s, 1 H, OH), and 1.50–1.22 (18 H, 6 CH_3); ^{13}C-n.m.r. ($CDCl_3$): δ 24.11, 25.01, 25.86, 26.07, 26.53, 27.10 (6 × CH_3), 70.59, 71.23, 71.95 (C-2, -3, -4, -5, -6), 96.97 (C-1), 108.54, and 109.58 (Me_2CO_2).

Anal. Calc. for $C_{14}H_{24}O_6$: C, 58.31; H, 8.39. Found: C, 58.13; H, 8.50.

*7-Deoxy-6-*C*-methyl-α,β-*D*-galacto-heptopyranose* (**12**). — Trifluoroacetic acid (90% aqueous, 75 mL) was added to a solution of **11** (15 g, 52 mmol) in dichloromethane at 0°. The solution was stirred, at 0°, until t.l.c. (2:2:1, v/v, ethyl acetate–1,4-dioxane–water, upper phase) indicated complete disappearance of the starting material (~2 h). The solution was evaporated *in vacuo* and the resulting syrup dried in an oil-pump vacuum to give a white foam (10.5 g, 97%), $[\alpha]_D^{20}$ +49.1° (*c* 1.0, water); ^{1}H-n.m.r. (D_2O) showed the product to contain ~90% of the pyranose form with a ratio of β to α anomer of 8:5: δ 5.29 (d, $J_{1,2}$ 3.8 Hz, H-1α), 4.57 (d, $J_{1,2}$ 7.5 Hz, H-1β), 4.21 (d, $J_{3,4}$ 2.9 Hz, H-4α), 4.14 (d, $J_{3,4}$ 3.3 Hz, H-4β), 3.83 (dd, $J_{2,3}$ 10.5 Hz, H-2α), 3.77 (dd, $J_{3,4}$ 3.0 Hz, H-3α), 3.76 (s, H-5α), 3.58 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.3 Hz, H-3β), 3.50 (dd, H-2β), 3.37 (s, H-5β), 1.32 and 1.29 (2 s, $Me_2\beta$), 1.32 and 1.27 (2 s, $Me_2\alpha$).

*1,2,3,4,6-Penta-*O*-acetyl-7-deoxy-6-*C*-methyl-α,β-*D*-galacto-heptopyranose* (**13**). — Compound **12** (5 g, 24 mmol) was dissolved in anhydrous pyridine (75 mL). Acetic anhydride (75 mL) and 4-(dimethylamino)pyridine (0.5 g) were added, and the mixture was heated with stirring at 50° for 36 h, at which time t.l.c. indicated the presence of one major component. The mixture was poured into saturated aqueous sodium hydrogencarbonate (300 mL), and processed in the usual way with chloroform as extractant and drying over magnesium sulfate. Solvent removal left a syrup that was purified by chromatography. Elution with 3:1 (v/v) hexane–ethyl acetate gave a solid (4.2 g, 42%) which was shown by ^{1}H-n.m.r. to consist of a mixture of the α and β anomers.

*2,3,4,6-Tetra-*O*-acetyl-7-deoxy-6-*C*-methyl-α-*D*-galacto-heptopyranosyl bromide* (**14**). — A solution of 45% hydrogen bromide in acetic acid (10 mL) was added, at 0°, to compound **13** (1.0 g) dissolved in dichloromethane (10 mL). After 30 min at 0°, the mixture was diluted with dichloromethane and poured into ice-cold, saturated sodium hydrogencarbonate solution. Successive washings with aqueous sodium hydrogencarbonate and water, followed by drying (sodium sulfate) and solvent removal left a yellowish oil (82%) that could be crystallized from hexane to give colorless needles, m.p. 98–103°; ^{1}H-n.m.r. ($CDCl_3$): δ 6.75 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1).

*Methyl 2,3,4,6-tetra-*O*-acetyl-7-deoxy-6-*C*-methyl-β-*D*-galacto-heptopyranoside* (**15**). — A solution of the bromide **14** (950 mg) in dichloromethane (5 mL) was

added to a vigorously stirred mixture of methanol (10 mL), silver carbonate (1.3 g), and Drierite (1.3 g). After 2 h, t.l.c. indicated the presence of one major component (R_F 0.5 in 3:2, v/v, toluene–ethyl acetate). The mixture was diluted with dichloromethane, the solids were removed by filtration, and the filtrate was evaporated to dryness leaving a yellow oil (780 mg) that was purified by column chromatography on silica gel. Evaporation of the main fraction yielded chromatographically pure **14** (680 mg, 81%) which crystallized from toluene–hexane, m.p. 108–110°, $[\alpha]_D^{20}$ −9.3° (*c* 1.0, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 5.55 (d, $J_{4,5}$ 0.8 Hz, H-4), 5.19 (dd, $J_{2,3}$ 10.3 Hz, H-2), 5.00 (dd, $J_{3,4}$ 3.4 Hz, H-3), 4.37 (d, $J_{1,2}$ 7.9 Hz, H-1), 3.99 (d, H-5), 3.52 (s, OCH_3), 2.13, 2.06, 1.98 and 1.97 (4 s, 4 $COCH_3$), 1.55 and 1.48 (2 s, CCH_3).

Anal. Calc. for $C_{17}H_{26}O_{16}$: C, 52.30; H, 6.71. Found: C, 52.36; H, 6.73.

*Methyl 7-deoxy-6-C-methyl-β-*D-galacto-*heptopyranoside* (**6**). — Compound **15** (100 mg) was *O*-deacetylated by treatment with methanol containing a catalytic quantity of sodium methoxide. After the reaction was complete, as evidenced by t.l.c. in 5:1 (v/v) 2-propanol–water, the mixture was made neutral with IR-120 (H^+) resin, and the resin removed by filtration. The solution was evaporated to dryness, the residue dissolved in water, and the solution lyophilyzed to give a white solid (55 mg, 97%), $[\alpha]_D^{20}$ −14.2° (*c* 1.0, water); ^{1}H-n.m.r., see Table I; ^{13}C-n.m.r. (D_2O; internal 1,4-dioxane, δ 67.4): δ 104.75 ($J_{C-1,H-1}$ 160.6 Hz, C-1), 79.13 (C-5), 74.20 (C-3), 73.62 (C-6), 71.59 (C-2), 69.72 (C-4), 57.81 (OCH_3), 26.51, and 26.29 (2 CCH_3) (these resonances were assigned by selective proton-irradiation.)

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