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Note

An efficient, unambiguous synthesis of methyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside. Further studies on the specificity of antigalactopyranan monoclonal antibodies

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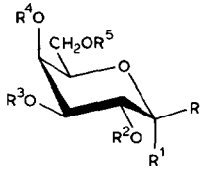
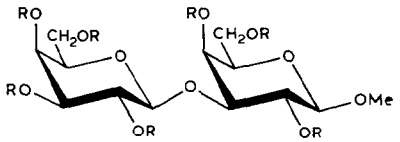
The oligosaccharide 3-*O*-β-D-galactopyranosyl-D-galactose has been isolated from a variety of sources (see ref. 1), and also synthesized[2–4]. Its methyl β-glycoside **8** was obtained by Gorin[5], in admixture with the α,1→3, β,1→2, and α,1→2 linked isomers, by a condensation of methyl 4,6-*O*-benzylidene-β-D-galactopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and subsequent removal of the blocking groups.

A prerequisite for an efficient synthesis of **8** is easy access to a 2,4,6-tri-*O*-substituted derivative of methyl β-D-galactopyranoside (**1**), bearing readily removable blocking groups, to be used as the nucleophile in the condensation reaction. Since previous routes[6,7] to 2,4,6-protected galactosides seemed tedious, we have examined the benzylation of **1** under conditions of enhanced nucleophilicity for HO-3, with the aim of converting it eventually to **4**. The use of the "direct stannylation" method[8,9] for the benzylation on a large scale yielded 64% of crystalline methyl 3-*O*-benzyl-β-D-galactopyranoside (**2**), the key intermediate in the present synthesis. The compound showed a melting point close to that reported for one of the products of the partial benzylation of **1** with benzyl bromide and sodium hydride in *N,N*-dimethylformamide, to which structure **2** was assigned on the basis of the ^{1}H-n.m.r. spectrum of its per-*O*-acetate[10]. Chromatography of the material in the mother liquor from the crystallization gave, in addition to **2**, methyl 6-*O*-benzyl-β-D-galactopyranoside (**5**). The assignment of the positions of the benzyl groups in **2** and **5** is based on comparison of the observed ^{13}C-n.m.r. data (Table I) with those reported[11] for **1**.

Conventional benzoylation of **2** afforded the fully protected, readily crystallizable benzoate **3**. The ^{13}C-n.m.r. spectrum of this product showed features in full agreement with the assigned structure, according to the generally accepted rules[12,13] of the ^{13}C-n.m.r. spectroscopy of carbohydrates. The ^{1}H-n.m.r. spectrum of **3** was



	R	R^1	R^2	R^3	R^4	R^5
1	OMe	H	H	H	H	H
2	OMe	H	H	Bn	H	H
3	OMe	H	Bz	Bn	Bz	Bz
4	OMe	H	Bz	H	Bz	Bz
5	OMe	H	H	H	H	Bn
6	H	Br	Bz	Bz	Bz	Bz



7 R = Bz
8 R = H

TABLE I

^{13}C-N.M R. CHEMICAL SHIFTS OF COMPOUNDS **2–5, 7,** AND **8**

Carbon atom	*Chemical shifts (δ, p.p.m. from Me_4Si)*					
	2[a]	**3**[b]	**4**[b]	**5**[a]	**7**[b]	**8**[a]
C-1	103.87	102.06	101.90	103.88	101.94	103.53
C-2	69.93	70.90	73.02	70.72	70.88	69.92
C-3	80.13	76.09	71.21	72.87	77.14	82.61
C-4	65.30	66.45	70.43	69.01	70.12	68.51
C-5	75.11	71.05	71.14	73.56	71.59	74.81
C-6	61.09	62.35	62.32	69.44	62.80	61.04
C-1′					101.30	104.46
C-2′					69.40	71.16
C-3′					71.34	72.61
C-4′					67.47	68.66
C-5′					71.00	75.12
C-6′					61.55	61.04
CH_2Ph	71.18	70.77		73.10		
CH_3	57.21	56.64	56.82	57.21	56.49	57.16

[a]Spectrum taken in D_2O at room temperature. [b]Spectrum taken in $CDCl_3$.

TABLE II

^{1}H-N.M.R. DATA FOR **2–5**, **7** AND **8**

Proton, and coupling	*Chemical shifts (δ, p.p.m.) peak multiplicities[a] and coupling constants (Hz)*					
	2[b]	**3**[c]	**4**[c]	**5**[b]	**7**[c,d]	**8**[e,f]
H-1	4.135 d	4.543 d	4.641 d	4.120 d	4.520 d	4.236 d
H-2	3.488 dd	5.549 dd	5.368 dd	3.386 dd	5.600 dd	3.52 m
H-3	3.346 dd	3.820 dd	4.135 dd	3.468 dd	4.289 m	3.67 m
H-4	3.928 bd	5.926 dd	5.779 dd	3.727 d	6.016 dd	4.063 dd
H-5	3.419 bt	4.109 td	4.158 t	3.61 m	4.104 td	3.56 bt
H-6a	3.595 dd	4.638 dd	4.617 dd	3.613 m	4.560 m	3.64 m
H-6b	3.672 dd	4.443 dd	4.428 dd	3.613 m	4.560 m	3.62 m
H-1′					5.006 d	4.462 d
H-2′					5.576 dd	3.46 m
H-3′					5.378 dd	3 53 m
H-4′					5.869 bd	3.784 dd
H-5′					4.252 m	3.50 m
H-6′a					4.698 dd	3.64 m
H-6′b					4.313 m	3.62 m
CH_2Ph	4.602 d	4.713 d		4.486 d		
	4.493 d	4.512 d		4.430 d		
CH_3	3.440 s	3.521 s	3.551 s	3.442 s	3.436 s	3.449 s
$J_{1,2}$	7.8	8.0	7.9	7.6	7.8	7.9
$J_{2,3}$	9.8	10.0	10.0	9.9	9.8	[g]
$J_{3,4}$	3.3	3.4	3.5	3.3	3 5	3.4
$J_{4,5}$	[h]	[h]	[h]	[h]	[h]	[h]
$J_{5,6a}$	4.6	6.3	6.2	[g]	5.8	[g]
$J_{5,6b}$	7.5	6.5	6.9	[g]	[g]	[g]
$J_{6a,6b}$	11.7	11.3	11.4	[g]	[g]	[g]
$J_{1',2'}$					7.7	7.2
$J_{2',3'}$					10.4	[g]
$J_{3',4'}$					3.3	3.0
$J_{4',5'}$					[h]	[h]
$J_{5',6'a}$					5.8	[g]
$J_{5',6'b}$					[g]	[g]
$J_{6'a,6'b}$					10.6	[g]
J_{CH_2Ph}	11.8	12.7		11.9		

[a]Symbols: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. [b]Spectrum taken in D_2O at 50°. [c]Spectrum taken in $CDCl_3$. [d]Assignment confirmed by homonuclear selective decoupling. [e]Spectrum taken in D_2O at room temperature. [f]Assignment confirmed by 2-D homonuclear correlation spectroscopy. [g]Not determined due to partial overlapping of signals. [h]$J_{H,H} < 1$ Hz.

similar to that of the corresponding acetate[10], showing the doublet of doublets for H-3 as the most upfield of all the ring-proton signals. This confirms the position of the benzyl ether group at C-3. Subsequent catalytic hydrogenolysis under standard conditions gave the nucleophile **4**, isolated as an amorphous solid.

The condensation of **4** with tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**6**), promoted with silver triflate (trifluoromethanesulfonate), was carried out under base-deficient conditions. It afforded an excellent yield of the fully protected disaccharide derivative **7**, the ^{13}C- and ^{1}H-n.m.r. spectra of which fully confirmed

the assigned structure (Tables I and II). Debenzoylation of **7** with sodium methoxide in methanol then readily gave the crystalline title compound **8**, the structure of which was further confirmed by 1-D and 2-D n.m.r. spectroscopy. The proton-signal and carbon-signal assignments given in Tables I and II are definitive. For the details of the n.m.r. methodology, see the Experimental section.

The degree of specificity in monoclonal antibodies is of obvious interest. Ten years ago[14] our laboratory demonstrated the high specificity of a number of antigalactan IgA's. As part of that work, precipitin reactions between antigalactan IgA's and a (1→3)-β-D-galactopyranan (SD-galactan) were run on Ouchterlony agar gels[15] by double diffusion. The SD-galactan was obtained by Smith degradation[16] of Larch arabinogalactan[17], a polysaccharide having a core of 1→3 linked β-D-galactopyranosyl residues with branches of 1→6 linked β-D-galactopyranosyl units. Although no precipitin lines were obtained[14], it was later found by us that concentrated spotting of the agar gels *versus* the SD-galactan led to faint lines. We assumed that these lines were due to precipitation of the IgA by residual unoxidized, 1→6 linked galactosyl units in the SD-galactan. Indeed, periodate oxidation studies on the SD-galactan showed the possible presence of one 1→6 linked galactosyl residue per six galactoses (A. Roy, unpublished). Nevertheless, the assumption remained to be verified that there was no binding to the (1→3)-β-D-galactopyranan backbone.

Using the unambiguously synthesized methyl 3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside described here, we have observed that the tryptophanyl fluorescence[18] of monoclonal IgA J539 remains unchanged upon the addition of this compound. Thus, we conclude that this monoclonal antibody does not show affinity for 1→3 linked β-D-galactopyranosyl residues.

EXPERIMENTAL

Melting points were determined with a Buchi melting point apparatus. Optical rotations were measured at 25° with a Perkin–Elmer automatic polarimeter, Model 241 MC. Thin-layer chromatography (t.l.c.) on precoated slides of Silica Gel G (Analtech) was performed with A, 5:1 (v/v) dichloromethane–methanol; B, 10:1 carbon tetrachloride–acetone; C, 10:1 toluene–acetone; and D, 3:1 dichloromethane–methanol. Detection was accomplished by charring after spraying with 5% (v/v) sulfuric acid in ethanol or, where applicable, by prior examination under u.v. light. Preparative chromatography was performed by gradient elution from slurry-packed columns of Silica Gel 60 (Merck, Prod. No. 9385 or 5111).

Toluene was dried with sodium hydride and distilled. Nitromethane was redistilled from urea, dried with P_2O_5, and distilled. Solutions in organic solvents were dried with sodium sulfate, and concentrated at 40°/2 kPa.

Proton and ^{13}C-n.m.r. spectra were recorded with a Nicolet NT-300 spectrometer operating at 300.053 MHz for ^1H and 75.456 MHz for ^{13}C. Either $CDCl_3$

(δ *vs.* Me_4Si, 77.0 p.p.m.) or MeOH (δ *vs.* Me_4Si, 49.0 p.p.m.) were used as spectral references for ^{13}C-n.m.r. measurements. Internal Me_4Si or DOH (δ *vs.* Me_4Si, 4.7 p.p.m.) were used as spectral references for ^1H spectroscopy. ^1H Chemical shifts could in most cases be measured with an accuracy of ± 0.005 p.p.m. ^{13}C-^1H Chemical shift correlation mapping (CSCM) experiments were performed utilizing a new protocol[19] incorporating selective spin-flip pulses which provided homonuclear decoupling in the ^1H dimension. After acquisition, the 128 × 2K or 64 × 4K data sets were zero filled to 2K in the ^1H dimension. For ^1H-^1H chemical shift correlation, either the standard 2-D ^1H homonuclear *J* correlation spectroscopy (COSY)[20] experiment was used or homonuclear selective decoupling was performed.

One-dimensional ^1H- and ^{13}C-n.m.r. experiments along with 2-D ^{13}C-^1H chemical shift correlation mapping experiments were performed on each compound. Proton and ^{13}C assignments were first routinely made from the respective 1-D spectra. ^1H-^1H Coupling constants were determined from first order analyses of the 1-D ^1H-n.m.r. spectra where possible. Consideration of the C-H connectivities obtained from the CSCM experiments allowed unambiguous confirmation of the ^1H and ^{13}C assignments for each of the monosaccharides.

Assignment of the spectra of the disaccharides was more difficult due to overlapping of some of the ^1H resonances, which obscured the ^1H-^1H coupling patterns. In these cases 2-D, ^1H-^1H homonuclear correlation experiments were required. For instance, in the ^1H-n.m.r. spectrum of **8** a total of 13 protons were observed to resonate between 3.44 and 3.67 p.p.m. However, the use of the 2-D COSY experiment allowed the unambiguous assignment of all the coupled proton pairs. Since only neighbouring protons are significantly coupled in these systems, carbon signals could be assigned on the basis that carbons bound to coupled proton pairs must be bound to each other. Therefore, the spectrum of each ring could be unambiguously mapped by starting from any carbon or proton and working in both directions.

Methyl 3-O-benzyl-β-D-galactopyranoside (**2**). — A mixture of **1** (fine powder, 19.4 g, 100 mmol) and dibutyltin oxide (25 g, 100 mmol) in dry benzene (300 mL) was refluxed overnight in a Soxhlet apparatus containing molecular sieves 3A. Tetrabutylammonium iodide (37 g, 100 mmol) was then added, followed by benzyl bromide (25 mL, 100 mmol), and the mixture was stirred for 2.5 h at reflux temperature. T.l.c. (solvent A) showed the presence of a small amount of unchanged **1**, together with several faster moving products, the predominant one having R_F 0.6. The clear yellow solution was concentrated to dryness, the residue was dissolved in dichloromethane (250 mL), and the solution kept for 2 h at 5°. The separated, crystalline product **2** (7 g) was sufficiently pure (t.l.c.) for use in the next step. The material remaining in the mother liquor was chromatographed to give first a further crop of the major reaction product **2** (11.3 g, total yield 65%). A portion was recrystallized from 2-propanol to give material melting at 138–139° (lit.[8] m.p. 135–137°).

Subsequently eluted was methyl 6-*O*-benzyl-β-D-galactopyranoside (2.2 g, 7.7%), m.p. 104–105° (lit.[9] 100–102°).

The yield of **2** from a four-fold scaleup of the reaction was 62%.

*Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-β-*D*-galactopyranoside* (**3**). — A solution of the foregoing compound (9.95 g) in pyridine (100 mL) was treated with benzoyl chloride (30 mL) for 2 h, when t.l.c. showed that the reaction was complete. The product (R_F 0.6, solvent B), isolated in the usual manner, readily crystallized from ethanol (19.9 g, 96%). Recrystallization of a portion gave a pure sample of **3**, m.p. 142–143°, $[\alpha]_D$ +94° (*c* 1.6, chloroform).

Anal. Calc. for $C_{35}H_{32}O_9$: C, 70.45; H, 5.40. Found: C, 70.53; H, 5.56.

*Methyl 2,4,6-tri-*O*-benzoyl-β-*D*-galactopyranoside* (**4**). — A solution of the benzyl derivative **3** (12 g) in 2-methoxyethanol (250 mL) was stirred in a hydrogen atmosphere in the presence of 5% palladium-on-charcoal catalyst (4 g). When t.l.c. showed the reaction to be complete, the single, slower moving product was isolated conventionally and collected as a solid, colorless foam, $[\alpha]_D$ +8.3° (*c* 1.5, chloroform).

Anal. Calc. for $C_{28}H_{26}O_9$: C, 66.39; H, 5.17. Found: C, 66.69; H, 5.45.

*Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-*D*-galactopyranoside* (**7**). — A solution of the bromide **6** (1.58 g, 2.4 mmol) and the nucleophile **4** (1.01 g, 2 mmol) in 1:1 nitromethane–toluene (10 mL) was concentrated to approximately one half of the original volume. The solution was cooled to −25° and stirred while a solution of silver trifluoromethanesulfonate (0.67 g, 2.6 mmol) and *sym*-collidine (0.225 mL, 1.7 mmol) in the same solvent was added dropwise under anhydrous conditions. After 20 min at −20°, examination by t.l.c. (solvent C) showed that **6** was no longer present, and that only traces of **4** remained. In addition to small amounts of fast moving byproducts, the reaction mixture contained one major component of R_F 0.4. To isolate the product, *sym*-collidine (0.1 mL) was added, the mixture was diluted with dichloromethane (20 mL) and filtered, the filtrate was washed with aqueous sodium thiosulfate, then dried and concentrated. Chromatography of the residue yielded **7** (1.84 g, 85%) as a colorless foam, $[\alpha]_D$ +91° (*c* 1.26, chloroform).

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.62; H, 4.83. Found: C, 68.92; H, 5.01.

*Methyl 3-*O*-β-*D*-galactopyranosyl-β-*D*-galactopyranoside* (**8**). — A solution of sodium methoxide in methanol (M, 2 mL) was added to a solution of **7** (1.4 g). After 6 h at room temperature the solution was neutralized with Dowex 50 W (H^+) resin, filtered, and concentrated to a dry, solid residue. Crystallization from methanol gave material (480 mg, 91.5%) melting at 200–201°. Recrystallization of a portion gave pure **8**, which showed m.p. 201–202°, $[\alpha]_D$ +24.5° (*c* 1, water).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.81; H, 6.78. Found: C, 43.74; H, 6.61.

Effect of **8** *on the fluorescence of Ig J539 Fab'*. A solution of J539 Fab' (1500 μL, 0.05 OD_{200}; 0.7×10^{-7}M) in phosphate buffered saline, pH 7.4, was irradiated in a fluorometer at 295 nm. The fluorescence of the protein at 340 nm was unaffected by the addition of a 0.1M solution (5 μL) of **8** in water.

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