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Synthesis and conformational studies of carrabiose and its 4′-sulphate and 2,4′-disulphate

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ABSTRACT

Methyl α-carrabioside (**13**), and its 4′-sulphate (**19**) and 2,4′-disulphate (**20**) have been synthesised *via* glycosylation of methyl 3,6-anhydro-2-*O*-benzyl-α-D-galactopyranoside with 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-D-galactopyranosyl bromide and subsequent partial or complete debenzylation, sulphation, and deprotection of the resulting disaccharide derivatives. Conformational studies have been carried out on **13**, **19**, and **20** on the basis of 1D and 2D ^{1}H-n.m.r. spectroscopy and molecular mechanics calculations.

INTRODUCTION

Carrageenans, extracted from red marine algae (*Rhodophyceae*), are sulphated polysaccharides that are well known for their gel-forming ability, particularly iota- and kappa-carrageenan which are used extensively in food, cosmetic, and pharmaceutical technology[1]. The repeating units are 3-linked β-D-galactopyranose 4-sulphate alternating with 4-linked 3,6-anhydro-α-D-galactopyranose (**1**, kappa) or 3,6-anhydro-α-D-galactopyranose 2-sulphate (**2**, iota).

Gelation by carrageenan has been proposed to involve helical structures and aggregates of helices[2–4]. The occurrence of right-handed double helices in the solid state has been established for iota-carrageenans by X-ray fibre diffraction studies[5] and similar structures may be present in kappa-carrageenan[6]. In addition, there is strong support for the double helix being the ordered tertiary form in solution at low temperature[2,7,8]. The transition from random coil to helix is temperature dependent and correlates with gel formation[2,8]. However, the factors connected with the conformational order–disorder thermal transitions are not well understood.

An approach to the understanding of the structural factors that determine the behavior of carrageenans is to study the repeating units. The structures of hepta-*O*-acetylcarrabiose dimethyl acetal[9] and neocarrabiose[10] have been published, and we now report syntheses and conformational studies of methyl α-D-carrabioside (**13**) and its 4′-sulphate (**19**) and 2,4′-disulphate (**20**).

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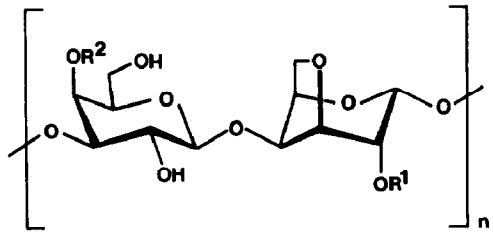
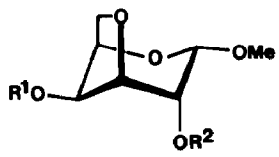


1 $R^1 = R^2 = SO_3^{\ominus}$ (iota-carrageenan)

2 $R^1 = H$, $R^2 = SO_3^{\ominus}$ (kappa-carrageenan)



3 $R^1 = R^2 = H$

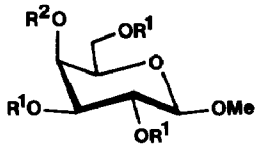
4 $R^1 = Ac$, $R^2 = H$

5 $R^1 = R^2 = Ac$

6 $R^1 = R^2 = Bn$

7 $R^1 = H$, $R^2 = Bn$

8 $R^1 = H$, $R^2 = SO_3^{\ominus}$

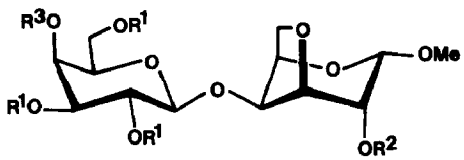


9 $R^1 = R^2 = H$

10 $R^1 = Ac$, $R^2 = Bn$

11 $R^1 = Ac$, $R^2 = H$

12 $R^1 = H$, $R^2 = SO_3^{\ominus}$



13 $R^1 = R^2 = R^3 = H$

14 $R^1 = Ac$, $R^2 = H$, $R^3 = Bn$

15 $R^1 = Ac$, $R^2 = R^3 = Bn$

16 $R^1 = R^2 = Ac$, $R^3 = Bn$

17 $R^1 = R^2 = Ac$, $R^3 = H$

18 $R^1 = Ac$, $R^2 = R^3 = H$

19 $R^1 = R^2 = H$, $R^3 = SO_3^{\ominus}$

20 $R^1 = H$, $R^2 = R^3 = SO_3^{\ominus}$

RESULTS AND DISCUSSION

Synthesis. — Regioselective acetylation of methyl 3,6-anhydro-α-D-galactopyranoside (**3**) was achieved by treatment with acetic anhydride in pyridine at 0° to give mainly the 4-acetate **4** (52%). Treatment of **4** with the sulphur trioxide–pyridine complex and saponification of the product gave methyl 3,6-anhydro-α-D-galactopyranoside 2-sulphate (**8**, 78%).

Methyl 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-D-galactopyranoside (**10**), prepared conventionally from 1,2,3,6-tetra-*O*-acetyl-4-*O*-benzyl-α-D-galactopyranose[11] *via* the α-bromide, was converted into **12** by catalytic hydrogenolysis, followed by sulphation and hydrolysis, as described for **8**.

The syntheses of the disaccharide glycosides **13**, **19**, and **20** required selective

protection of HO-2 in the acceptor **3**. Regioselective benzylation of **3** with benzyl bromide in *N,N*-dimethylformamide at 0° in the presence of barium oxide–barium hydroxide, followed by column chromatography, gave the 2,4-di-*O*-benzyl derivative (**6**, 30%) and the 2-*O*-benzyl compound (**7**, 63%). Mercuric salt-promoted glycosylation of **7** with 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-α-D-galactopyranosyl bromide in benzene at 60° yielded the methyl α-carrabioside derivative **15** (68%). Catalytic hydrogenolysis (10% Pd–C) of **15** with 1 mol of hydrogen followed by flash-column chromatography gave the key compounds **14** (27%) and **18** (60%).

The conversion of **14** into the 4′-sulphate **19**, related to kappa-carrageenan (**2**), was accomplished by acetylation (→ **16**) and then hydrogenolysis to furnish **17** almost quantitatively. Treatment of **17** with the sulphur trioxide–pyridine complex, followed by saponification, afforded **19** (81%), isolated as the potassium salt.

Compound **18** was used to synthesise both methyl α-carrabioside (**13**) and the 2,4′-disulphate **20**, related to iota-carrageenan (**1**). Treatment of **18** with the sulphur trioxide–pyridine complex and subsequent saponification yielded **20** (89%), isolated as the dipotassium salt.

N.m.r. spectroscopy. — The δ and J values (Table I) obtained by first-order analysis of the ^{1}H-n.m.r. spectra of monosaccharide derivatives **3**, **8**, **9**, and **12** and the

TABLE I

^{1}H-N.m.r. data (δ in p.p.m., J in Hz)

Atom	**3**	**8**	**9**	**12**	**13**	**19**	**20**
H-1	4.861	5.003			4.855	4.854	5.062
H-2	3.995	4.557			4.057	4.056	4.635
H-3	4.325	4.598			4.513	4.519	4.818
H-4	4.448	4.504			4.564	4.578	4.668
H-5	4.385	4.458			4.664	4.639	4.698
H-6*endo*	4.202	4.267			4.249	4.249	4.334
H-6*exo*	4.014	4.078			4.069	4.067	4.150
H-1′			4.306	4.386	4.565	4.612	4.668
H-2′			3.494	3.526	3.510	3.520	3.550
H-3′			3.634	3.811	3.670	3.811	3.859
H-4′			3.914	4.690	3.940	4.685	4.735
H-5′			3.686	~3.85	~3.80	3.870	~3.92
$J_{1,2}$	2.6	2.7			2.6	2.6	2.6
$J_{2,3}$	5.4	5.4			5.4	5.5	5.5
$J_{3,4}$	~0	~0			~0	~0	~0
$J_{3,5}$	1.1	1.0			0.8	1.1	0.8
$J_{4,5}$	2.1	2.1			1.9	2.0	1.9
$J_{5,6endo}$	~0	~0			~0	~0	~0
$J_{5,6exo}$	3.0	3.1			3.0	2.9	3.0
$J_{6exo,6endo}$	−10.6	−10.7			−10.9	−10.8	−10.7
$J_{1',2'}$			7.9	7.9	7.9	7.9	7.9
$J_{2',3'}$			9.9	10.1	10.0	10.1	10.1
$J_{3',4'}$			3.5	3.3	3.5	3.3	3.2
$J_{4',5'}$			1.0	<0.5	<0.5	0.7	<0.5

disaccharide derivatives **13**, **19**, and **20** were used as input parameters for the calculation of the theoretical values, using the PANIC program (Bruker software). As expected, the *J* values were in agreement with the galactopyranose ring being in the $^4C_1$ conformation ($J_{1',2'} \sim 8$, $J_{2',3'} \sim 10$, $J_{4',5'} \leqslant 1$ Hz) and the 3,6-anhydrogalactopyranose moiety being in the $^1C_4$ conformation ($J_{1,2} \sim 2.6$, $J_{2,3} \sim 5.5$ Hz) in each of the compounds.

On the other hand, the δ values for the resonances of protons in the interglycosidic region in **13**, **19**, and **20** did not show any significant differences other than those due to the presence of the sulphate groups, as deduced from the corresponding data for **3**, **8**, **9**, and **12**.

2D-NOESY experiments on **13**, **19**, and **20** showed the expected connections for *syn*-vicinal protons and cross-peaks for H-1/H-6*endo*, H-1′/H-5′, and H-1′/H-3′ for the monosaccharide residues. Interglycosidic cross-peaks H-1′/H-3 and H-1′/H-4 were detected only in the NOESY spectrum of compound **19**. The NOESY spectra for **13** and **20** were not conclusive, due to overlapping of the resonances of H-1′ and H-4.

Thus, the disaccharide derivatives **13**, **19**, and **20** adopt similar conformations.

Molecular mechanics calculations. — Hard-sphere energy calculations were performed for compound **13**, using the PFOS program[12]. The local minima of the energy map were optimised using the MM2(85) program[13] (see Experimental). The relative steric energy for the local minima (conformations C1–C6) and the relevant geometrical features are given in Table II. A view of the global minimum (conformation C2) is shown in Fig. 1.

The NOESY spectrum of compound **19**, which indicated the close proximity of H-1′ to both H-3 and H-4, strongly supported the stable conformation in solution being in the neighborhood of C2–C3, where the distances H-1′–H-3 and H-1′–H-4 are <2.5 Å (see Table II).

On the other hand, the interglycosidic angles of the global minimum ($-93°$, $173°$) are near to those ($-91.7°$, $-172.6°$) found[9] for the hexa-*O*-acetylcarrabiose dimethyl acetal in the solid state.

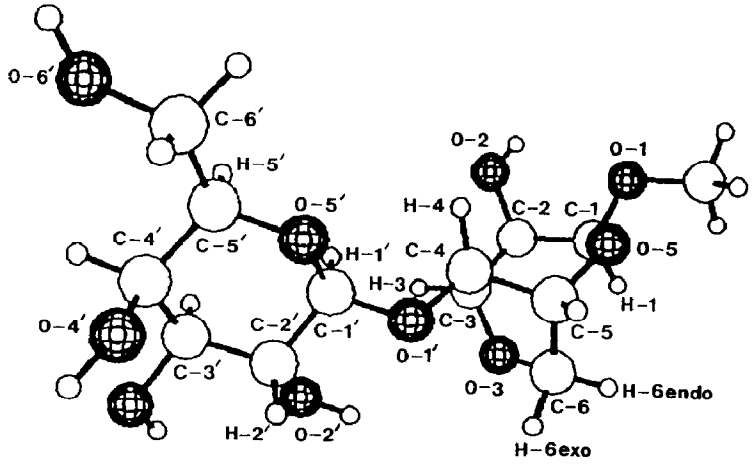


Fig. 1. A view of the global minima for **13** (conformation C2), showing the atomic numbering.

TABLE II

Relative energies (kcal.mol^{-1}) and relevant geometrical features of stable conformers (C1–C6) for compound **11**: only non-bonding distances <4 Å are given for each conformer

	C1	*C2*	*C3*	*C4*	*C5*	*C6*
Φ	−53	−93	−105	−106	61	84
Ψ	−162	173	179	72	111	−168
H-1′–H-3	2.16	2.27	2.25			3.94
H-1′–H-4	3.33	2.49	2.44	2.27	3.55	3.61
H-1′–H-5				2.53		
H-2′–H-3					2.39	
H-2′–H-4					2.36	2.30
H-2′–H-5					2.41	
H-5′–H-3	3.23					
O-2′–H-3		3.18	2.84			
O-2′–H-4				3.36	3.88	2.82
O-2′–H-5					2.87	
O-2–H-1′		3.74	3.70			
O-2–H-2′						3.05
O-4′–H-4					3.47	
O-4′–H-3						2.92
E	2.16	0.00	0.13	8.92	5.16	5.14

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. T.l.c. was performed on Silica Gel GF_{254} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on silica gel (Merck 70–230). Flash-column chromatography was carried out on silica gel 40–70 μm (MN). ^{1}H-N.m.r. spectra were recorded with a Varian XL-300 or Bruker AM-200 spectrometer, and ^{13}C-n.m.r. spectra (50 MHz) with a Bruker AM-200 spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polarimeter.

N.m.r. spectroscopy. — For the conformational analysis, samples (10–20 mg) of **3**, **8**, **9**, **12**, **13**, **19**, and **20**, previously deuterated with D_2O, were dissolved in 0.5 mL of 99.98% D_2O and degassed in the n.m.r. tube under argon. All the experiments were performed at 30°. Chemical shifts are given in p.p.m. downfield from that of sodium 3-(trimethylsilyl)-1-propanesulfonate.

2D-NOESY experiments were effected in the phase-sensitive mode by using the pulse sequence 90°–t_1–t_m–90°–acq., with a mixing time of 2.0 s for **13**, **19**, and **20**.

Molecular mechanics calculations. — Hard-sphere energy calculations were performed for **13** using the PFOS program[12]. The constituent monosaccharides were assumed to be rigid entities with a value of 117° for the glycosidic bond angle, leaving as variables the torsion angles Φ (O-5′–C-1′–O-1′–C-4) and Ψ (C-1′–O-1′–C-4–C-5). The coordinates for constructing the galactose and 3,6-anhydrogalactose moieties were taken from a data bank with optimised MM2 structures. The MeO-1 was oriented with Φ 60°, according to previous findings[14], and left free during the minimisation process.

Only the *trans–gauche* rotamer was considered for the conformation around the C-5–C-6 bond of the D-galactopyranosyl residue[15]. The energy diagrams for the Φ/Ψ conformational map were obtained using a 10° grid. The local minima of this map were optimised through molecular mechanics calculations by applying the MM2(85) program, which partially accounts for hydrogen bonding. Prior to these calculations, lone pairs were added to the oxygen atoms. Only the *trans–gauche* rotamer of the hydroxymethyl group was used as starting point in the geometry optimisation through MM2.

Methyl 4-O-acetyl-3,6-anhydro-α-D-galactopyranoside (**4**). — To a solution of **3** (ref. 16) (1 g, 5.7 mmol) in dry pyridine (5 mL) at 0° was added dropwise a solution of acetic anhydride (1.3 mL) in pyridine (5 mL) during 30 min. The mixture was kept for 48 h at 4°, then concentrated. Column chromatography (1:1 hexane–EtOAc) of the residue yielded **4** (0.65 g, 52%) and **5** (0.29 g, 19%). Compound **4** had m.p. 109–110° (from ether), $[\alpha]_D$ +66° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^1H (200 MHz), δ 5.52 (d, 1 H, $J_{4,5}$ 1.9 Hz, H-4), 4.79 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1), 4.51 (d, 1 H, $J_{3,4}$ 0, $J_{2,3}$ 5.3 Hz, H-3), 4.49 (m, 1 H, H-5), 4.26 (d, 1 H, $J_{5,6endo}$ 0 Hz, $J_{6endo,6exo}$ −10.1 Hz, H-6), 4.02 (dd, 1 H, H-2), 4.00 (dd, 1 H, $J_{5,6exo}$ 3.0 Hz, H-6), 3.59 (s, 3 H, OMe), 2.12 (s, 3 H, OAc).

Anal. Calc. for $C_9H_{14}O_6$: C, 49.34; H, 6.42. Found: C, 49.05; H, 6.42.

Methyl 3,6-anhydro-2-O-benzyl-α-D-galactopyranoside (**7**). — To a solution of **3** (5 g, 0.03 mol) in dry *N,N*-dimethylformamide (30 mL) at 0° was added a mixture of barium oxide (3.2 g, 0.02 mol) and barium hydroxide hydrate (7.6 g, 0.04 mol). The suspension was stirred for 30 min, then benzyl bromide (4 mL) in *N,N*-dimethylformamide (20 mL) was added slowly during 1 h. The mixture was kept for 48 h at 4°, then concentrated *in vacuo*, the residue was extracted with boiling chloroform (2 × 100 mL), and the combined extracts were concentrated. Column chromatography (1:1 hexane–EtOAc) of the residue afforded **7** (4.8 g, 63%) and **6** (3.0 g, 30%). Compound **7** had m.p. 82–83° (from ether), $[\alpha]_D$ +66° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^1H (200 MHz), δ 7.33 (m, 5 H, Ph), 4.88 (d, 1 H, PhC*H*), 4.74 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 4.60 (d, 1 H, $J_{4,5}$ 1.8 Hz, H-4), 4.53 (d, 1 H, PhC*H*), 4.30 (m, 1 H, $J_{5,6exo}$ 2.5 Hz, H-5), 4.15 (d, 1 H, $J_{2,3}$ 5.3 Hz, H-3), 4.02 (m, 2 H, H-6*exo*,6*endo*), 3.72 (dd, 1 H, H-2), 3.54 (s, 3 H, OMe); ^{13}C (50 MHz), δ 98.7 (C-1), 80.62, 77.31, 76.68, 73.94, 71.21, 69.13, 57.63 (OMe).

Anal. Calc. for $C_{14}H_{18}O_5$: C, 63.16; H, 6.77. Found: C, 63.12; H, 6.80.

Methyl 3,6-anhydro-α-D-galactopyranoside 2-sulphate (**8**). — To a stirred solution of **4** (0.5 g, 2.3 mmol) in dry pyridine (5 mL) was added portionwise sulfur trioxide–pyridine complex (1.1 g). The mixture was kept overnight at room temperature, the pH was adjusted to 9 with M KOH, and the mixture was stirred for 2 h. The solvent was evaporated and a solution of the residue in distilled water (20 mL) was boiled for 2 h under reflux, neutralised with M HCl, and concentrated. The residue was extracted with hot methanol, and the extract was treated with activated charcoal and concentrated. The residue was eluted from a column (5 × 20 cm) of Sephadex G-10 (water), and the fractions were monitored by t.l.c. (1:2 MeOH–EtOAc). The appropriate fractions were combined and concentrated. The residue was passed through a column (1.5 × 5 cm) of Sephadex SP-25 (K^+ form) to give, after concentration, **8** (0.52 g, 78%) as the hygroscopic potassium salt, m.p. 238–240° (dec.) (from MeOH–H_2O), $[\alpha]_D$ +42° (*c* 5, water).

N.m.r. data (D_2O): ^1H (300 MHz), see Table I; ^{13}C (50 MHz), δ 97.5 (C-1), 80.1 (C-3), 78.3 (C-5), 75.1 (C-2), 71.1 (C-4), 70.2 (C-6), 58.8 (OMe).

Anal. Calc. for $C_7H_{11}KO_8S$: C, 28.57; H, 3.74. Found: C, 28.12; H, 3.58.

*Methyl 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-*D*-galactopyranoside* (**10**). — To a stirred solution of 1,2,3,6-tetra-*O*-acetyl-4-*O*-benzyl-α-D-galactopyranose[11] (0.747 g, 1.7 mmol) in dry CH_2Cl_2 (25 mL) was added a solution of $TiBr_4$ in EtOAc (18 mL, 10%) at room temperature. After 8 h (t.l.c., 3:2 hexane–EtOAc), toluene (40 mL) and NaOAc (3.7 g) were added, and the mixture was stirred until it became colorless (1.5 h), then filtered through Celite, and concentrated *in vacuo* at room temperature. A solution of the resulting syrup in MeOH (20 mL) was stirred with 4 Å molecular sieves (1 g) for 10 min, $Hg(CN)_2$ (0.343 g, 1.36 mmol) and $HgBr_2$ (0.673 g, 1.86 mmol) were added, and the solution was stirred overnight in the dark, diluted with CH_2Cl_2 (40 mL), filtered (Celite), washed with saturated aqueous sodium hydrogen carbonate (20 mL), aqueous 10% potassium iodide (20 mL), and water (30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (3:2 hexane–EtOAc) of the residue gave syrupy **10** (0.6 g, 86%), $[\alpha]_D$ +8.6° (*c* 0.7, methanol). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 7.32 (m, 5 H, Ph), 5.36 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.4 Hz, H-2), 4.96 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.75 (d, 1 H, PhC*H*), 4.56 (d, 1 H, PhC*H*), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.29 (dd, 1 H, $J_{5,6exo}$ 6.3, $J_{6endo,6exo}$ −10.8 Hz, H-6*exo*), 4.10 (dd, 1 H, $J_{5,6endo}$ 6.8 Hz, H-6*endo*), 3.99 (dd, 1 H, $J_{4,5}$ 0.9 Hz, H-4), 3.75 (ddd, 1 H, $J_{5,6endo}$ 6.7, H-5), 3.48 (s, 3 H, OMe), 2.05, 2.01, 2.00 (s, 9 H, 3 OAc).

Anal. Calc. for $C_{20}H_{26}O_9$: C, 58.34; H, 6.34. Found: C, 58.60; H, 6.29.

*Methyl 2,3,6-tri-*O*-acetyl-β-*D*-galactopyranoside* (**11**). — A solution of **10** (0.2 g, 0.63 mmol) in EtOAc (15 mL) was treated with Pd–C (10%, 0.1 g) and H_2 at atmospheric pressure until 15 mL of H_2 had been consumed. The mixture was then filtered through Celite and concentrated. Column chromatography (1:1 hexane–EtOAc) of the residue gave **11** (150 mg, 96%), m.p. 105–107° (from ether–hexane), $[\alpha]_D$ +3.3° (*c* 0.7, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 5.23 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.2 Hz, H-2), 4.95 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 4.39 (d, 1 H, H-1), 4.35 (dd, 1 H, $J_{5,6endo}$ 6.6 Hz, $J_{6endo,6exo}$ −11.5 Hz, H-6*endo*), 4.31 (dd, 1 H, $J_{5,6exo}$ 6.2 Hz, H-6*exo*), 4.05 (m, 1 H, H-4), 3.75 (ddd, 1 H, $J_{5,6exo}$ 6.5 Hz, $J_{5,4}$ 1.1 Hz, H-5), 3.50 (s, 3 H, OMe), 2.39 (d, 1 H, $J_{4,OH}$ 6.3 Hz, HO-4), 2.06, 2.09, 2.1 (s, 9 H, 3 OAc).

Anal. Calc. for $C_{13}H_{20}O_9$: C, 48.75; H, 6.25. Found: C, 48.77; H, 6.34.

*Methyl β-*D*-galactopyranoside 4-sulphate* (**12**). — To a stirred solution of **11** (0.65 g, 2 mmol) in dry pyridine (5 mL) was added sulphur trioxide–pyridine complex (1 g) portionwise. The mixture was kept overnight at room temperature, then worked-up as described for **8**, to give **12** as the syrupy potassium salt (0.65 g, 73%), $[\alpha]_D$ +1.9° (*c* 1.2, water). N.m.r. data[17]: ^1H see Table I; ^{13}C (D_2O), δ 105.0 (C-1), 77.90 (C-4), 75.61 (C-5), 73.07 (C-3), 72.20 (C-2), 62.23 (C-6), 58.58 (OMe).

Anal. Calc. for $C_7H_{13}KO_9S$: C, 26.92; H, 4.17. Found: C, 26.67; H, 3.82.

*Methyl 3,6-anhydro-2-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**15**). — To a stirred mixture of **7** (0.62 g, 2.33 mmol), mercuric cyanide (1 g), and 4 Å molecular sieves (1 g) in benzene (30 mL) under argon

was added a solution of 2,3,6-tri-*O*-acetyl-4-*O*-benzyl-α-D-galactopyranosyl bromide (1.7 g, 3.7 mmol) in benzene (20 mL) during 30 min at 60°. The mixture was stirred overnight at 60°, cooled to room temperature, washed with saturated aqueous sodium hydrogen carbonate (20 mL) and aqueous 10% potassium bromide (20 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Column chromatography (1:1 hexane–EtOAc) of the residue gave syrupy **15** (1.0 g, 68%), $[\alpha]_D$ +48° (*c* 1.05, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 7.3 (m, 5 H, Ph), 5.3 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.6 Hz, H-2'), 4.93 (dd, 1 H, $J_{3',4'}$ 3.0 Hz, H-3), 4.91 (d, 1 H, PhC*H*), 4.73 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.73 (d, 1 H, PhC*H*), 4.56 (bs, 1 H, H-4), 4.54 (d, 1 H, PhC*H*), 4.52 (d, 1 H, PhC*H*), 4.46 (d, 1 H, $J_{4,5}$ 1.4 Hz, H-5), 4.38 (d, 1 H, H-1'), 4.22 (dd, 1 H, $J_{5',6'endo}$ 6.6 Hz, H-6'*endo*), 4.10 (dd, 1 H, $J_{2,3}$ 5.3, $J_{3,4}$ 1.6 Hz, H-3), 4.07 (d, 1 H, $J_{5',6'exo}$ 5.4, $J_{6'endo,6'exo}$ −11.1 Hz, H-6'*exo*), 3.96 (bs, 1 H, H-6*endo*), 3.93 (m, 1 H, H-6*exo*), 3.90 (dd, 1 H, $J_{3',4'}$ 3.0, $J_{4',5'}$ 0.9 Hz, H-4'), 3.73–3.67 (m, 2 H, H-2',5'), 3.52 (s, 3 H, OMe), 2.01 (s, 6 H, 2 OAc), 1.99 (s, 3 H, OAc); ^{13}C, δ 170.5, 170.4, 169.4 (CO), 138.5, 137.3, 128.4, 128.1, 127.9 (aromatic), 100.22 (C-1'), 98.75 (C-1), 78.34, 78.22, 76.19, 75.07, 74.08, 73.61, 73.5, 72.47, 69.6, 69.2, 62.4, 57.6 (OMe), 20.8, 20.76, 20.70 (OAc).

Anal. Calc. for $C_{33}H_{40}O_{13}$: C, 61.49; H, 6.21. Found: C, 61.50; H, 6.50.

*Methyl 3,6-anhydro-4-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**18**) *and methyl 3,6-anhydro-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-benzyl-β-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**14**). — A solution of **15** (0.8 g, 1.24 mmol) in EtOAc (5 mL) was treated with Pd–C (10%, 0.4 g) and H_2 at atmospheric pressure until 30 mL of H_2 had been consumed, then filtered through Celite, and concentrated. Column chromatography (1:4 hexane–EtOAc) of the residue yielded **18** (310 mg, 60%) and **14** (180 mg, 27%) as a syrup. Compound **18** had m.p. 131–132° (from ether), $[\alpha]_D$ +45° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 5.21 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.4 Hz, H-2'), 4.92 (dd, 1 H, $J_{3',4'}$ 3.2 Hz, H-3'), 4.70 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.58 (d, 1 H, H-1'), 4.55 (d, 1 H, $J_{3,4}$ 2.2, $J_{4,5}$ ~0 Hz, H-4), 4.49 (m, 1 H, H-5), 4.30 (m, 2 H, H-6'*exo*, H-6'*endo*), 4.30 (d, 1 H, $J_{2,3}$ 4.9 Hz, H-3), 4.05 (dd, 1 H, $J_{4',5'}$ 0.6 Hz, H-4'), 3.97 (m, 2 H, H-6*endo*, H-6*exo*), 3.93 (dd, 1 H, H-2), 3.77 (m, 1 H, $J_{5',6'}$ 6.2 Hz, H-5'), 3.54 (s, 3 H, OMe), 2.10, 2.09, 2.05 (3 s, 9 H, 3 OAc); ^{13}C, 170.8 (CO), 170.1 (CO), 169.5 (CO), 100.3 (C-1'), 96.8 (C-1), 78.3, 78.1, 76.1, 73.0, 72.3, 70.2, 69.6, 69.4, 68.8, 67.0, 62.3, 57.09 (OMe), 20.77 (2 C, OAc), 20.63 (OAc).

Anal. Calc. for $C_{19}H_{28}O_{13}$: C, 49.13; H, 6.03. Found: C, 48.70; H, 5.97.

Compound **14** had $[\alpha]_D$ +51° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 7.26 (m, 5 H, Ph), 5.26 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 4.87 (dd, 1 H, $J_{3',4'}$ 3.0 Hz, H-3'), 4.66 (d, 1 H, PhC*H*), 4.61 (d, 1 H, $J_{1,2}$ 2.6 Hz, H-1), 4.47 (d, 1 H, H-1'), 4.47 (d, 1 H, $J_{3,4}$ 2.2, $J_{4,5}$ ~0 Hz, H-4), 4.46 (d, 1 H, PhC*H*), 4.40 (bs, 1 H, H-5), 4.26 (d, 1 H, $J_{2,3}$ 5.3 Hz, H-3), 4.16 (dd, 1 H, $J_{5',6'exo}$ −11.2 Hz, H-6'*exo*), 3.97 (dd, 1 H, $J_{5',6'endo}$ 6.5 Hz, H-6'*endo*), 3.94 (d, 1 H, $J_{5,6endo}$ ~0, $J_{6endo,6exo}$ −10.0 Hz, H-6*endo*), 3.88 (dd, 1 H, $J_{5,6exo}$ 2.7 Hz, H-6*exo*), 3.87 (dd, 1 H, H-2), 3.84 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4'), 3.65 (ddd, 1 H, H-5'), 3.45 (s, 3 H, OMe), 1.98, 1.95, 1.94 (3 s, 9 H, 3 OAc).

Anal. Calc. for $C_{26}H_{34}O_{13}$: C, 56.32; H, 6.14. Found: C, 56.66; H, 5.89.

*Methyl 3,6-anhydro-4-*O*-β-*D*-galactopyranosyl-α-*D*-galactopyranoside* (**13**). — A

solution of **18** (250 mg, 0.54 mmol) in dry methanol (10 mL) was treated with sodium methoxide (2 mg). After 15 min, the solution was neutralised (M HCl) and concentrated. Column chromatography (1:1 benzene–EtOH) of the residue yielded **13** (180 mg, 98%), m.p. 207° (from 1:1 MeOH–H_2O), $[\alpha]_D$ +42° (*c* 1, methanol); lit.[18] m.p. 203–204°, $[\alpha]_D$ +44° (methanol). N.m.r. data (D_2O): ^1H (300 MHz), see Table I; ^{13}C, δ 103.58 (C-1′), 99.16 (C-1), 80.16 (C-3), 78.99 (C-4), 77.40 (C-5), 76.44 (C-5′), 73.84 (C-3′), 71.82 (C-2′), 70.18 (C-2), 70.03 (C-4′), 69.73 (C-6), 62.11 (C-6′), 58.59 (OMe).

Anal. Calc. for $C_{13}H_{22}O_{10}$: C, 46.15; H, 6.51. Found: C, 46.08; H, 6.57.

Methyl 3,6-anhydro-4-O-(β-D-galactopyranosyl 4-sulphate)-α-D-galactopyranoside 2-sulphate (**20**). — Compound **18** (0.5 g, 1.08 mmol) was treated with sulphur trioxide–pyridine complex (1.06 g) in dry pyridine (40 mL). After 24 h, the mixture was treated as described for **8**, to yield **20** (0.54 g, 89%), m.p. 256–257° (dec.) (from MeOH–H_2O), $[\alpha]_D$ +13° (*c* 0.6, water). N.m.r. data (D_2O): ^1H (300 MHz) see Table I; ^{13}C, 103.64 (C-1′), 97.97 (C-1), 79.46 (C-3), 78.74 (C-2), 77.91 (C-4′ or C-4), 77.76 (C-4 or C-4′), 75.92 (C-5′), 75.46 (C-5), 73.02 (C-3′), 72.10 (C-2′), 70.84 (C-6), 62.21 (C-6′), 59.01 (OMe).

Anal. Calc. for $C_{13}H_{20}K_2O_{16}S_2$: C, 27.18; H, 3.48. Found: C, 26.57; H, 3.82.

Methyl 2-O-acetyl-3,6-anhydro-4-O-(2,3,6-tri-O-acetyl-4-O-benzyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**16**). — Compound **14** (140 mg, 0.25 mmol) was acetylated (Ac_2O–pyridine) conventionally to yield syrupy **16** (149 mg, 99%), $[\alpha]_D$ +52° (*c* 0.75, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 7.33 (m, 5 H, Ph), 5.34 (dd, 1 H, $J_{1',2'}$ 10.5 Hz, H-2′), 5.14 (dd, 1 H, $J_{1,2}$ 2.9, $J_{2,3}$ 5.4 Hz, H-2), 4.95 (dd, 1 H, $J_{3',4'}$ 3.1 Hz, H-3′), 4.8 (d, 1 H, H-1), 4.74 (d, 1 H, *J* 11.6 Hz, PhC*H*), 4.54 (d, 1 H, PhC*H*), 4.51 (d, 1 H, H-1′), 4.50 (m, 1 H, H-5), 4.46 (d, 1 H, $J_{3,4}$ 1.9, $J_{4,5}$ ~0 Hz, H-4), 4.30 (d, 1 H, H-3), 4.24 (dd, 1 H, $J_{6'exo,6'endo}$ −11.2, $J_{5',6'endo}$ 6.4 Hz, H-6′*endo*), 4.06 (dd, 1 H, $J_{5',6'exo}$ 6.0 Hz, H-6′*exo*), 4.04 (d, 1 H, $J_{6exo,6endo}$ −10.2 Hz, H-6*endo*), 3.98 (dd, 1 H, $J_{5,6exo}$ 2.6 Hz, H-6*exo*), 3.92 (dd, 1 H, $J_{4',5'}$ 0.8 Hz, H-4′), 3.72 (m, 1 H, H-5′), 3.47 (s, 3 H, OMe), 2.13, 2.05, 2.03, 2.01 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{28}H_{36}O_{14}$: C, 56.37; H, 6.04. Found: C, 56.43; H, 6.13.

Methyl 2-O-acetyl-3,6-anhydro-4-O-(2,3,6-tri-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**17**). — Compound **16** (0.1 g, 0.17 mmol) was treated with Pd–C (10%, 0.1 g) and H_2 in EtOAc to yield syrupy **17** (82 mg, 99%), $[\alpha]_D$ +52° (*c* 1.1, chloroform). N.m.r. data ($CDCl_3$): ^1H (300 MHz), δ 5.23 (dd, 1 H, $J_{1',2'}$ 8.2, $J_{2',3'}$ 10.5 Hz, H-2′), 5.16 (dd, 1 H, $J_{1,2}$ 2.5, $J_{2,3}$ 5.3 Hz, H-2), 4.94 (dd, 1 H, $J_{3',4'}$ 3.1 Hz, H-3′), 4.81 (d, 1 H, H-1), 4.55 (d, 1 H, H-1′), 4.55 (m, 1 H, H-5), 4.47 (d, 1 H, $J_{4,5}$ 1.8 Hz, H-4), 4.32 (d, 1 H, $J_{3,4}$ ~0 Hz, H-3), 4.31 (m, 2 H, H-6′*exo*,6′*endo*), 4.07 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4′), 4.07 (d, 1 H, $J_{6exo,6endo}$ −10.1 Hz, H-6*endo*), 3.98 (dd, 1 H, $J_{5,6exo}$ 2.8 Hz, H-6*exo*), 3.78 (m, 1 H, H-5′), 3.48 (s, 3 H, OMe), 2.16, 2.11, 2.10, 2.05 (4 s, 12 H, 4 OAc).

Anal. Calc. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.93. Found: C, 49.67; H, 5.93.

Methyl 3,6-anhydro-4-O-(β-D-galactopyranosyl 4-sulphate)-α-D-galactopyranoside, potassium salt (**19**). — Compound **17** (70 mg, 0.12 mmol) was treated with sulphur trioxide–pyridine complex (71 mg) in dry pyridine (5 mL). After 24 h, the mixture was treated as described for **8**, to yield **19** (51 mg, 81%), m.p. 221° (from methanol–water),

$[\alpha]_D$ +25° (*c* 0.95, water). N.m.r. data (D_2O): ^1H (300 MHz) see Table I; ^{13}C, δ 103.59 (C-1'), 99.34 (C-1), 80.23 (C-3), 79.30 (C-4), 77.82 (C-4'), 77.46 (C-5), 75.88 (C-5'), 72.93 (C-3'), 71.97 (C-2'), 70.35 (C-2), 70.14 (C-6), 62.21 (C-6'), 58.79 (OMe).

Anal. Calc. for $C_{13}H_{21}KO_{13}S \cdot 6H_2O$: C, 22.87; H, 5.49. Found: C, 22.82; H, 4.21.

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REFERENCES

1 H. J. Witt, in R. R. Colwell, E. R. Pariser, and A. J. Sinskey (Eds.), *Biotechnology of Marine Polysaccharides*, McGraw-Hill, 1985, pp. 346–360.
2 E. R. Morris, D. A. Rees, and G. Robinson, *J. Mol. Biol.*, 138 (1980) 349–362.
3 S. Arnott, A. Fulmer, W. E. Scott, I. C. M. Dea, R. Moorhouse, and D. A. Rees, *J. Mol. Biol.*, 90 (1974) 269–284.
4 I. T. Norton, D. M. Goodall, K. R. Austen, E. R. Morris, and D. A. Rees, *Biopolymers*, 25 (1986) 1009–1029.
5 S. Arnott, W. E. Scott, D. A. Rees, and C. G. A. McNab, *J. Mol. Biol.*, 90 (1974) 256–267.
6 N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, and J. W. B. Samuel, *J. Mol. Biol.*, 45 (1969) 85–88.
7 R. A. Jones, E. J. Staples, and A. Penman, *J. Chem. Soc., Perkin Trans. 2*, (1973) 1608–1612.
8 I. T. Norton, D. M. Goodall, E. R. Norris, and D. A. Rees, *J. Chem. Soc., Faraday Trans. 1*, 79 (1983) 2485–2486, 2489–2500, 2501–2515.
9 D. Lamba, C. Burden, W. Mackie, and B. Sheldrick, *Carbohydr. Res.*, 155 (1986) 11–17.
10 D. Lamba, A. L. Segre, S. S. B. Glover, W. Mackie, B. Sheldrick, and S. Perez, in *Recent Developments in Industrial Polysaccharides: Biomedical and Biotechnological Advances*, in press.
11 C. Subero, E. Correa, and M. Martin-Lomas, *An. Quim.*, 81 C (1985) 71–73.
12 I. Tvaroska, and S. Perez, *Carbohydr. Res.*, 149 (1986) 349–410; A. D. French, *ibid.*, 188 (1989) 206–211; S. Perez, Thèse de Doctorat d'Etat, 1978, Université de Grenoble, France.
13 U. Burkert and N. L. Allinger, *ACS Symp. Ser.*, 177 (1982) 1–339.
14 J. W. Campbell and M. M. Harding, *J. Chem. Soc., Perkin Trans. 2*, (1972) 1721–1723.
15 N. K. de Vries and H. M. Buck, *Carbohydr. Res.*, 165 (1987) 1–16.
16 W. N. Haworth, J. Jackson, and F. Smith, *J. Chem. Soc.*, (1940) 620–632.
17 M. J. Harris and J. R. Turvey, *Carbohydr. Res.*, 15 (1970) 57–63.
18 S. Hirase and C. Araki, *Bull. Chem. Soc. Jpn.*, 40 (1967) 2627–2629.