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SYNTHESIS OF METHYL *O*-(3-DEOXY-3-FLUORO-β-D-GALACTOPYRANOSYL)-(1→6)-β-D-GALACTOPYRANOSIDE AND METHYL *O*-(3-DEOXY-3-FLUORO-β-D-GALACTOPYRANOSYL)-(1→6)-*O*-β-D-GALACTOPYRANOSYL-(1→6)-β-D-GALACTOPYRANOSIDE*

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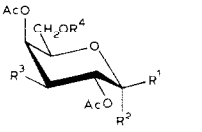
ABSTRACT

Condensation of 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-α-D-galactopyranosyl bromide (**3**) with methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (**4**) gave a fully acetylated (1→6)-β-D-galactobiose fluorinated at the 3′-position which was deacetylated to give the title disaccharide. The corresponding trisaccharide was obtained by reaction of **4** with 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-D-galactopyranosyl bromide (**5**), dechloroacetylation of the formed methyl *O*-(2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside to give methyl *O*-(2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (**14**), condensation with **3**, and deacetylation. Dechloroacetylation of methyl *O*-(2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside, obtained by condensation of disaccharide **14** with bromide **5**, was accompanied by extensive acetyl migration giving a mixture of products. These were deacetylated to give, crystalline for the first time, the methyl β-glycoside of (1→6)-β-D-galactotriose in high yield. The structures of the target compounds were confirmed by 500-MHz, 2D, ^{1}H- and conventional ^{13}C- and ^{19}F-n.m.r. spectroscopy.

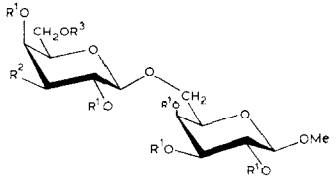
INTRODUCTION

This laboratory has extensively investigated the modes of binding of saccharides to monoclonal immunoglobulins. Hydrophobic, hydrophylic, and electrostatic forces may, *a priori*, play a role in these interactions. In order to elucidate the possible role of hydrogen bonding in the binding process, we have in the past[2,3] measured the binding constants of several deoxyfluoro-β-D-galactopyranosides with monoclonal anti-β-galactan IgA. The results of these studies

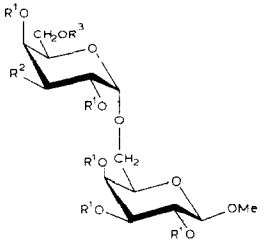
*Synthesis of specifically fluorinated methyl β-glycosides of (1→6)-β-D-galactooligosaccharides, Part III. For Part II, see ref. 1.



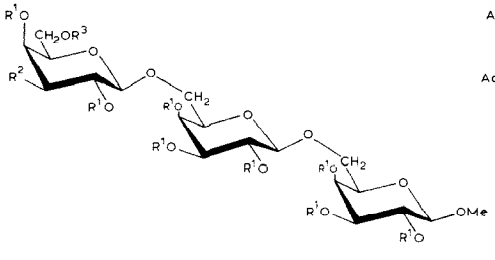
	R^1	R^2	R^3	R^4
1	H	OAc	F	Ac
2	OAc	H	F	Ac
3	H	Br	F	Ac
4	OMe	H	OAc	H
5	H	Br	OAc	$COCH_2Cl$
6	OAc	H	OAc	$COCH_2Cl$
7	OAc	H	OAc	H
8	OMe	H	OAc	Ac



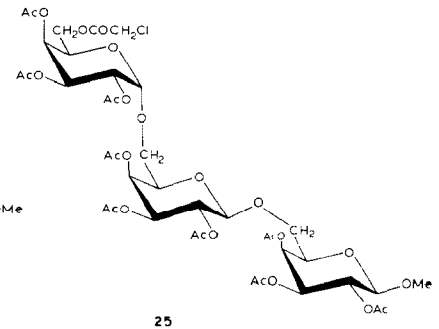
	R^1	R^2	R^3
9	Ac	F	Ac
10	H	F	H
11	Ac	OAc	$COCH_2Cl$
12	H	OH	H
13	Ac	OAc	Ac
14	Ac	OAc	H



	R^1	R^2	R^3
15	Ac	F	Ac
16	Ac	OAc	$COCH_2Cl$
17	Ac	OAc	Ac
18	H	OH	H



	R^1	R^2	R^3
19	Ac	F	Ac
20	H	F	H
21	Ac	OAc	$COCH_2Cl$
22	H	OH	H
23	Ac	OAc	Ac
24	Ac	OAc	H



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indicate that the binding involves hydrogen bonding, hydrogen donation coming from the OH-2 and -3 of the D-galactosyl residue binding to the antibody subsite having the highest affinity for such a residue. In order to explore this problem further, we have initiated the synthesis of carbohydrate derivatives related to (1→6)-β-D-galactopyranan in which certain hydroxyl groups are replaced by a fluorine atom. Recently, we have reported[4] the high yielding synthesis of 3-deoxy-3-fluoro-D-galactose and its conversion to the corresponding methyl β-D-glycoside. We describe herein the synthesis of ligands that are more closely related to (1→6)-β-D-galactopyranan than are the simple monosaccharides, namely the methyl β-glycosides of (1→6)-β-D-galactooligosaccharides in which a 3-deoxy-3-fluoro-D-galactosyl group is linked to the galactopyranosyl end-group.

RESULTS AND DISCUSSION

The stepwise synthesis of a homologous series of oligosaccharides such as **20** requires three intermediates: (a) a 2,3,4-tri-*O*-substituted methyl β-D-galactopyranoside as the initial nucleophile, (b) a 2,3,4-tri-*O*-substituted D-galactosyl halide bearing a 6-*O*-blocking group that can be selectively removed (to form the

internal unit of the oligosaccharide), and (c) a glycosyl halide derived from 3-deoxy-3-fluoro-D-galactose (to form the D-galactosyl end-group of the oligosaccharide). For the internal unit of the oligosaccharide, we selected previously reported[5] 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-D-galactopyranosyl bromide (**5**). For the preparation of the other two intermediates needed, **2** and **4**, we followed recently described procedures[4,6] but, this time, by use of a superior quality of a solution of hydrobromic acid in acetic acid (see Experimental section), the desired halide **3** was obtained in crystalline form in 85% yield.

Condensation of **3** and **4** gave the disaccharide derivative **9** in 71% yield, together with a small amount of the α-linked product **15**, as shown by ^{13}C-n.m.r. spectroscopy. In the spectra of **9** and **15**, signals at δ 102.1 and 102.2, respectively, were assigned to C-1 atoms. The doublets centered at δ 100.1 and 96.9, respectively, were assigned to C-1′ atoms on the basis of the characteristic three-bond coupling to F-3′. The presence of that fluorine atom in both **9** and **15** is further evident from the strong downfield shift of the signals for C-3′, which appeared as doublets in both cases at δ 88.9 and 85.6, respectively, and showed typical $^{1}J_{C,F}$ values of 192.9 and 191.7 Hz.

The reaction of **4** with bromide **5** gave **11** and **16** whose structures were confirmed by ^{13}C-n.m.r. spectroscopy. The presence of the $ClCH_2CO$-6 in **11** and **16** was evident from the line due to $ClCH_2$ appearing in the ^{13}C-n.m.r. spectra at δ 40.5. Also, **11** and **16** could be converted into the fully acetylated analogs **13** and **17** by catalytic dechlorination.

In order to optimize the yield of **14**, the key intermediate in the synthesis of oligosaccharides of these series, *O*-dechloroacetylation (not described in the Experimental section) of 1,2,3,4-tetra-*O*-acetyl-6-*O*-chloroacetyl-β-D-galactopyranose[5](**6**) and **11** with thiourea[7] or hydrazine dithiocarbonate[8] was investigated as a model. In contrast to the previous report[8], we observed the formation by the latter reagent of many by-products. Also due to the presence of a large excess of reagent, and of acetic acid and 2,6-dimethylpyridine as the solvents, the reaction was difficult to monitor by t.l.c. We cannot recommend this reagent where acyl migration to a primary hydroxyl group can be anticipated. Unfortunately, no such case was studied in the original work[8]. *O*-Dechloroacetylation of **6** and **11** with thiourea[7] gave a better result. Formation of some by-products, as well as the observed debenzylidenation[7], appears to be due to the acidic nature of the pseudohydantoin hydrochloride[7,9] formed in the reaction mixture, and this could be minimized by conducting the reaction in the presence of an *equimolar* amount of 2,6-dimethylpyridine. Thus, **14** was obtained in 96% yield. The absence of acetyl migration was evident from a comparison (Fig. 1) of the ^{13}C-n.m.r. spectrum of **14** with those of its synthetic precursor **11**, the fully acetylated analog[6] **13**, and methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside[6] (**4**). Thus, the conversion of **11** to **14** is evident from the absence in the spectrum of **14** of the line at δ 40.5 (CH_2Cl), and from the downfield shift of the line for C-5′ (δ 70.6 to 73.7), as a result of the removal of the negative (upfield) β-shift-effect of *O*-acyl-6′. Also an upfield shift of

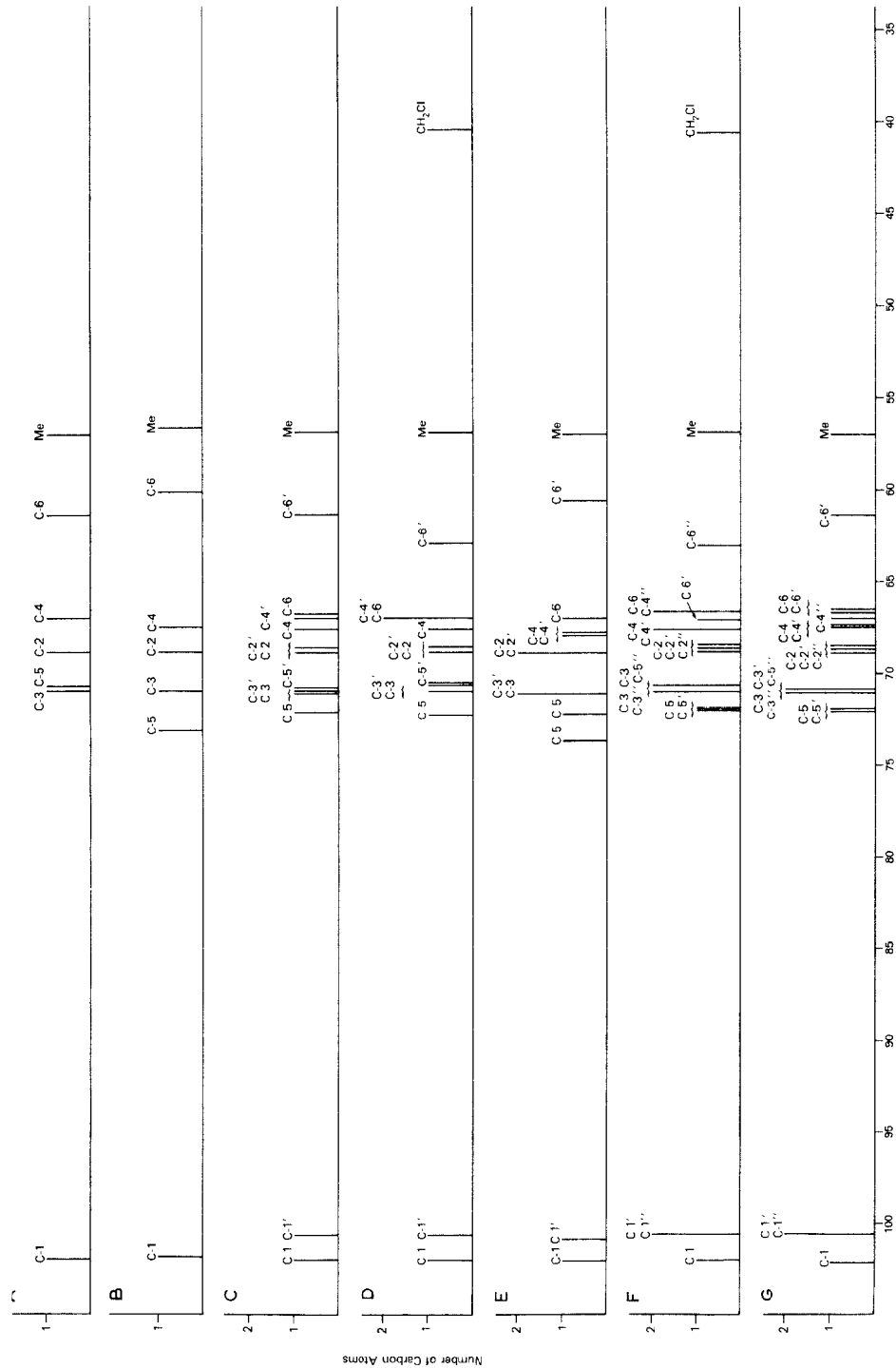

Fig. 1. Comparison of diagnostically significant shifts of lines in the ^{13}C-n.m.r. spectra of (A) **8**, (B) **4**, (C) **13**, (D) **11**, (E) **14**, (F) **21**, and (G) **23**.

the line due to C-6′ to a δ value close to that noted for the C-6 line of **4** (δ 62.9 to 60.6) was observed.

Reaction mixtures resulting from condensation of the disaccharide nucleophile **14** with bromides **3** and **5** contained by-products arising from acetyl migration but gave mainly **19** and **21** in 67 and 58% yield, respectively. The ^{13}C-n.m.r. spectrum of **19** showed signals for anomeric carbons at δ 102.1, 100.7, and 100.1 (doublet $^3J_{C,F}$ 11 Hz). The first and the last were also present in the spectrum of **9**, and therefore the line at δ 100.7 could be assigned to the anomeric carbon of the internal D-galactosyl residue. Other features of the spectrum confirmed that **9** and **19** belong to the same homologous series. Thus, identical or very similar shifts were observed for carbon atoms occupying the same positions relative to the glycosidic linkage. Similar regularities have been observed in homologous series of cello-[12] and xylo-oligosaccharides[12–14].

By-products due to acetyl migration were not observed in the preparation of

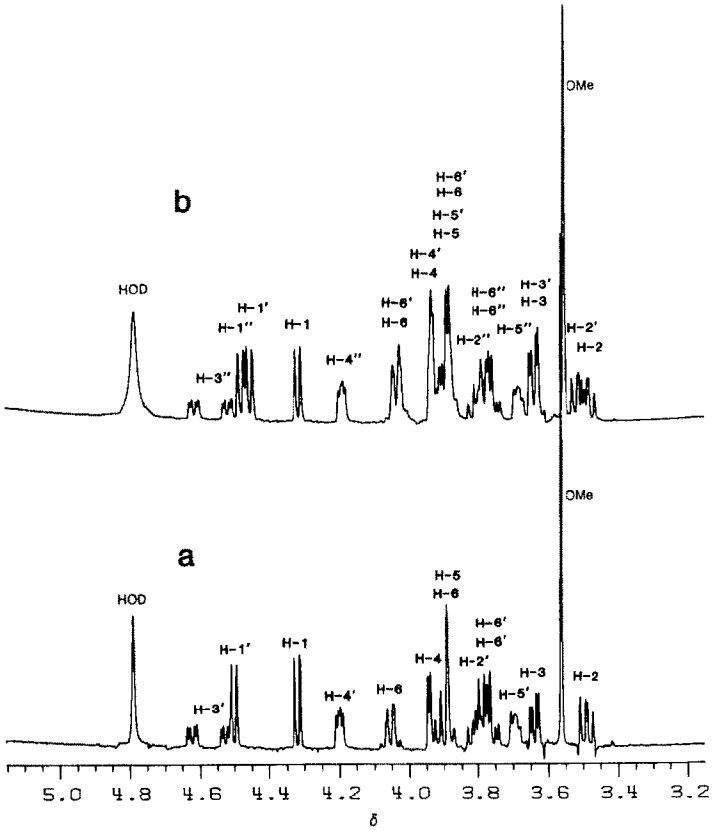

Fig. 2. 500-MHz F.t.–n.m.r. spectra of **10** (a) and **20** (b) for solutions in D_2O at 22°; spectral width, 1000 Hz; pulse width, 60°; data points, 16K; number of accumulated FID, 16; and double-exponential multiplication apodization (DM 8) for resolution enhancement.

9 and **19**, or in the preparation of their fully acetylated analogs[6] under the conditions used for the reaction of **14** with either **3** or **5**. It appears that the acetyl group shows a greater tendency to migrate with the lengthening of the oligosaccharide. Thus, dechloroacetylation of **21** resulted in the formation of two by-products even before 50% of the starting material had been used and these by-products eventually amounted to some 40% of the material. Consequently, the product of dechloroacetylation of **21** was deacetylated to give the crystalline methyl β-glycoside of (1→6)-β-D-galactotriose (**22**) in 85% yield. The observed optical rotation value was close to that found[6,10] for the methyl β-glycoside of the corresponding disaccharide **12**, but different from that reported by Srivastava *et al.*[11]. The ^{13}C- and ^{1}H-n.m.r. data supported structure **22**.

Deacetylation of **9** and **19** gave the title oligosaccharides **10** and **20** in crystalline form and excellent yields. The ^{13}C-n.m.r. spectra interpreted by comparison with those of the corresponding nonfluorinated derivatives[6,11] **12** and **22**, and of

TABLE I

^{1}H-N M R DATA OF COMPOUNDS **10** AND **20**

Proton	*Compound*			
	10		**20**	
	Chemical shift (δ)	*Coupling constant[a] (Hz)*	*Chemical shift (δ)*	*Coupling constant[a] (Hz)*
H-1	4.319	$^{3}J_{1,2}$ 7.9	4.319	$^{3}J_{1,2}$ 7.9
H-2	3.488	$^{3}J_{2,3}$ 9.8	3.486	$^{3}J_{2,3}$ 9.4
H-3	3.637	$^{3}J_{3,4}$ 3.5	3.641	$^{3}J_{3,4}$ 3.3
H-4	3.942	$^{3}J_{4,5} \leq 1$	3.935	$^{3}J_{4,5} \leq 1$
H-5	3.890	$^{3}J_{5,6}$ [b]	3.900	$^{3}J_{5,6}$ [b]
H-6	3.9–4.06	$^{2}J_{6a,6b}$ [b]	3.89–4.04	$^{2}J_{6a,6b}$ [b]
OCH_3	3.561		3.557	
H-1′	4.499	$^{3}J_{1',2'}$ 8.0	4.457	$^{3}J_{1',2}$ 8.0
H-2′	3.813	$^{3}J_{2',3'}$ 9.6; $^{3}J_{2',F}$ (13.4)	3.513	$^{3}J_{2',3'}$ 9.4
H-3′	4 473	$^{2}J_{3',F}$ 48.1(47.9); $^{3}J_{3',4'}$ 3.5	3.641	$^{3}J_{3',4'}$ 3.3
H-4′	4.199	$^{3}J_{4',F}$ 6.3(6.5); $^{3}J_{4',5'} \leq 1$	3.935	$^{3}J_{4',5'} \leq 1$
H-5′	3.695	$^{3}J_{5',6'}$ [b]	3.900	$^{3}J_{5',6}$ [b]
H-6′	3 75–3.80	$^{2}J_{6'a,6'b}$ [b]	3.89–4.04	$^{2}J_{6'a,6b}$ [b]
H-1″			4 482	$^{3}J_{1'',2''}$ 7.9
H-2″			3.814	$^{3}J_{2'',3''}$ 9.5; $^{3}J_{2'',F}$ (13.3)
H-3″			4.567	$^{2}J_{3'',F}$ 48.1(47.9); $^{3}J_{3'',4''}$ 3.4
H-4″			4.193	$^{3}J_{4'',F}$ 6.0(6.4); $^{3}J_{4'',5''} < 1$
H-5″			3.686	$^{3}J_{5'',6''}$ [b]
H-6″			3.79–3.76	$^{2}J_{6''a,6b''}$ [b]

[a]Values in parentheses were determined from ^{19}F-n.m.r. spectra taken at 93.66 MHz with a JEOL FX-100 n.m.r. spectrometer. [b]These values were not determined due to overlapping of signals of the coupled protons.

methyl 3-deoxy-3-fluoro-β-D-galactopyranoside[4,15], were fully consistent with the prepared structures. The spectra of **10** and **20** shows that the oligosaccharides belong to the same homologous series, and the line-pattern of the spectrum of **22** shows also a close relation. Thus, the spectrum of **20** shows signals for anomeric carbons at δ 103.9, 103.3, and 102.7, the latter appearing as a doublet with $J_{C,F}$ 12.1 Hz. A line at δ 103.9, and the doublet at 102.7 ($J_{C,F}$ 12.2 Hz), is present also in the spectrum of **12**, whereas the spectrum of the nonfluorinated trisaccharide **22** shows a line for the anomeric carbon atom of the internal β-D-galactopyranosyl residue at δ 103.4. The spectrum of **22** shows a three-carbon line at δ 72.7 assigned to the three C-3 atoms of the three β-D-galactosyl residues which are magnetically nearly equivalent. The spectrum of **20** shows the expected, two-carbon line at δ 72.7, and the spectrum of **10** only a one-carbon signal at that frequency. In agreement with the strong downfield shift-effect due to fluorine substitution, the signals of C-3′ and -3″ of **10** and **20**, respectively, appears at δ 93.1 ($^1J_{C,F}$ 183.1 Hz). The proposed structures for these two disaccharides were confirmed by the 500 MHz ^{1}H-spectra (Fig. 2). The doublet of quartets centered at δ 4.573 (Fig. 2a) was assigned to H-3′ of **10**. The large splitting of 48.1 Hz between the two quartets in **10** is consistent with the typical geminal coupling between fluorine and hydrogen atoms when linked to the same carbon atom, and thus confirms the presence of F at C-3′. The quartet showing *J* 9.6 and 3.5 Hz arose from the vicinal coupling between H-3′ and the neighboring protons H-2′ and -4′. The doublets centered at δ 4.319 (*J* 7.9 Hz) and 4.499 (*J* 8 Hz) were assigned to the anomeric H-1 and -1′, respectively. The singlet at δ 3.561 was assigned to OCH_3-1. The remaining proton-signal assignments in the spectrum of **10** were based on the contour map of 2D shift-correlated spectroscopy (COSY). Proton signals in the spectrum of **20** were assigned similarly (see Table I). These data agree with those obtained by ^{13}C-n.m.r. spectroscopy.

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi melting point apparatus. Optical rotations were determined with a Perkin–Elmer automatic polarimeter, Model 241 MC. Natural abundance, noise-decoupled ^{13}C-n.m.r. spectra (25°) were recorded, for solutions in $CDCl_3$ (internal standard tetramethylsilane) or D_2O (internal standard MeOH; MeOH − Me_4Si signal = 49.0 p.p.m.), with a JEOL FX-100 spectrometer operating at 25 MHz. Two to five thousand transients were accumulated for each spectrum by use of 8k data points over a spectral width of 5000 Hz, with a repetition rate of 1 s, and a pulse angle of about 50°. ^{19}F-N.m.r. spectra were recorded, in the FT mode at 93.66 MHz, with the same JEOL instrument. ^{1}H-N.m.r. spectra were recorded with a Nicolet 500 spectrometer, under the experimental conditions as described in the legend to Fig. 2. Preparative chromatography was performed by gradient elution from columns of slurry-packed Silica gel 60 (Merck, Product No. 9385). T.l.c. was performed on

Silica gel G-coated glass slides (250 μm, Analtech) in (*A*) 8:1 toluene–acetone; (*B*) 7:2 toluene–acetone; (*C*) 1:1 toluene–ether; (*D*) 5:1 dichloromethane–methanol; (*E*) 5:2 toluene–acetone; and (*F*) 35:1 dichloromethane–methanol. Detection was by charring with 5% (v/v) H_2SO_4 in ethanol or spraying with anilinium hydrogen phthalate in acetone (1.66 g phthalic acid and 1 mL aniline in 100 mL acetone) and heating; this reagent reveals the presence of hydrolyzed glycosyl halides. Unless otherwise stated, all condensation reactions were performed overnight at room temperature in benzene as the solvent, with $Hg(CN)_2$ and $HgBr_2$ as HBr scavenger and catalyst. When the reaction appeared to be complete, the mixture was filtered through Celite, the solids were washed with dichloromethane, and the filtrate was washed with aqueous KBr solution, dried, and evaporated. The resulting residue was acetylated with 1:1 (v/v) pyridine–acetic anhydride to convert unreacted nucleophile and other products that would otherwise move closely to the desired material on t.l.c. A standard solution of hydrazine dithiocarbonate was prepared as described[8]. Glycosyl halide **5**, obtained as an almost chromatographically pure oil by treatment of **6** as described for the preparation of **3**, was used without further purification[5]. The solution of 33% hydrogen bromide in acetic acid was purchased from Fluka A.G.

*2,4,6-Tri-*O*-acetyl-3-deoxy-3-fluoro-α-*D*-galactopyranosyl bromide* (**3**). — A solution of 3-deoxy-3-fluoro-D-galactose[4] (0.55 g) in dry pyridine (6 mL) was frozen, acetic anhydride (30 mL) added, and, with occasional shaking, the mixture allowed to warm up to room temperature. After 16 h, t.l.c. showed complete reaction and the mixture was evaporated (133 Pa). A solution in dichloromethane (6 mL) of the residue (**1** and **2**) was treated for 1 h with 33% HBr in acetic acid (9 mL), and the mixture processed as described[4]. The residue obtained on evaporation was crystallized from ether–petroleum ether to give chromatographically homogeneous **3**. Part of this was recrystallized, m.p. 101–102°, $[\alpha]_D^{25}$ +251.6° (*c* 1.25, chloroform); ^{13}C-n.m.r.: δ 87.7 (d, $^3J_{F,C}$ 8.5 Hz, C-1), 86.1 (d, $^1J_{F,C}$ 191.7 Hz, C-3), 71.4 (d, $^3J_{F,C}$ 4.9 Hz, C-5), 68.8 (d, $^2J_{F,C}$ 19.5 Hz, C-2), 66.9 (d, $^2J_{F,C}$ 17.1 Hz, C-4), and 60.9 (d, $^4J_{F,C}$ 2.4 Hz, C-6). The compound was stored at −20° under dry conditions for several months without noticable change.

Anal. Calc. for $C_{12}H_{16}BrFO_7$: C, 38.83; H, 4.43; Br, 21.52. Found: C, 39.02; H, 4.49; Br, 21.94.

Methyl O-*(2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**9**). — The glycosyl halide **3** (556 mg, 1.5 mmol) was added to a suspension of **4** (320 mg, 1 mmol), $Hg(CN)_2$ (190 mg, 0.75 mmol), $HgBr_2$ (100 mg), and Drierite (1 g) in benzene (5 mL) that had been stirred for 2 h, and the mixture stirred overnight with the exclusion of moisture. After processing, t.l.c. showed the presence of one major component, R_F 0.40. Column chromatography first gave a small amount of the α-linked isomer **15**, R_F 0.45; ^{13}C-n.m.r.: δ 102.2 (C-1), 96.9 (d, $^3J_{F,C}$ 9.8 Hz, C-1′), 85.6 (d, $^1J_{C,F}$ 191.7 Hz, C-3′), 71.4 (C-5), 71.2 (C-3), 68.9, 67.8 (2 d, $^2J_{F,C}$ 18.3 and 17.1 Hz, C-2′,4′), 68.8 (C-2), 67.3 (C-4), 65.9 (C-6), 61.6 (C-6), and 57.0 (Me).

The β-linked disaccharide **9** (435 mg, 71%) was subsequently eluted, m.p. 181–181.5° (ethanol), $[\alpha]_D^{25}$ −0.8° (*c* 0.63, chloroform); ^{13}C-n.m.r.: δ 102.1 (C-1), 100.1 (d, $^3F_{F,C}$ 11 Hz, C-1′), 88.8 (d, $^1J_{F,C}$ 192.9 Hz, C-3′), 72.3 (C-5), 71.0 (C-3), 70.0 (d, $^3J_{F,C}$ 4.9 Hz, C-5′), 69.5 (d, $^2J_{F,C}$ 19.5, C-2′), 68.9 (C-2), 67.7 (C-4), 67.1 (d, $^2J_{F,C}$ 14.7 Hz, C-4′), 66.9 (C-6), 61.4 (C-6′), and 57.0 (Me); c.i.–m.s. (NH_3): *m/z* 628 (M + 18).

Anal. Calc. for $C_{25}H_{35}FO_{16}$: C, 49.17; H, 5.77; F, 3.10. Found: C, 48.92; H, 5.68; F, 2.84.

Methyl O-*(2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-* (**16**) *and -β-*D*-galacto-pyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**11**). — A solution of **5**, prepared from **6** (1.91 g, 4.5 mmol), in dry benzene (5 mL) was added to a mixture of **4** (0.96 g, 3 mmol), $Hg(CN)_2$ (0.57 g, 2.25 mmol), $HgBr_2$ (250 mg), and Drierite (3 g) in benzene (10 mL) that had been stirred for 2 h. After being stirred for an additional 16 h, the mixture was processed as just described and chromatography afforded **15** (0.165 g, 8%), m.p. 188–189° (ethanol), $[\alpha]_D^{25}$ +81.5° (*c* 1.2, chloroform); ^{13}C-n.m.r.: δ 102.2 (C-1), 96.5 (C-1′), 71.4 (C-5), 71.1 (C-3), 68.9 (C-2), 68.0 (C-3′), 67.5 (C-2′), 67.4 (2 C, C-4,4′), 66.5 (C-5′), 65.7 (C-6), 63.3 (C-6′), and 57.0 (Me).

Anal. Calc. for $C_{27}H_{37}ClO_{18}$: C, 47.33; H, 5.44; Cl, 5.17. Found: C, 47.38; H, 5.29; Cl, 5.38.

Subsequent elution gave **11** (1.60 g, 78%), colorless foam, $[\alpha]_D^{25}$ −8.1° (*c* 1.3, chloroform); ^{13}C-n.m.r.: δ 102.0 (C-1), 100.7 (C-1′), 72.3 (C-5′), 71.0, 70.7 (C-3,3′), 70.6 (C-5′), 68.8, 68.5 (C-2,2′), 67.6 (C-4), 67.0 (2 C, C-4′,6), 62.9 (C-6′), and 56.9 (Me).

Anal. Calc. for $C_{27}H_{37}ClO_{18}$: C, 47.33; H, 5.44; Cl, 5.17. Found: C, 47.10; H, 5.51; Cl, 5.25.

Compounds **11** and **16** both showed a peak at *m/z* 702 (M + 18 based on ^{35}Cl) in c.i.–m.s. (NH_3).

Methyl O-*(2,3,4,6-tetra-*O*-acetyl-α-* (**17**) *and -β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**13**). — A 1% solution of **16** in 1:1 ethanol–acetone was hydrogenolyzed in the presence of solid $NaHCO_3$ with Pd–C as the catalyst. When the gas uptake had ceased (~3 h), t.l.c. (*C*) showed only a single product (R_F 0.3). Conventional processing gave crystalline **17** from ethanol, m.p. 164–166 (lit.[6] m.p. 165–165.5°). When the β-linked disaccharide **11** was hydrogenolyzed identically, the product formed was indistinguishable by t.l.c. and ^{13}C-n.m.r. spectroscopy from the fully acetylated compound[6] **13**.

Methyl O-α- (**18**) *and -β-*D*-galactopyranosyl-(1→6)-β-*D*-galactopyranoside* (**12**). — A suspension of the chloroacetylated disaccharide **16** (100 mg) in dry methanol (5 mL) was treated with a few drops of M sodium methoxide in methanol. When a clear solution had formed, t.l.c. (*D*) showed the presence of a single product, R_F 0.3. Conventional processing gave crystalline **18** (100%), m.p. 161–163° (methanol) (lit.[6] m.p. 161–162°). Identical treatment of **11** yielded **12**, m.p. 218–220° (lit.[6,10] m.p. 218–220°).

Methyl O-*(2,3,4-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**14**). — To a solution of the chloroacetylated disaccharide **11** (2.6 g, 3.8 mmol) in warm (50°) 1:5 dichloromethane–methanol (50 mL) was added a solution of thiourea (0.87 g, 11.4 mmol) and 2,6-dimethylpyridine (0.43 mL, 3.7 mmol) in methanol (50 mL) over a period of 2 h. The mixture was stirred at 40–50° until t.l.c. (*B*) showed that the reaction was complete (~3 h). After concentration at 40°/133 Pa, the residue was partitioned between water and dichloromethane. The organic phase was evaporated and the residue resolved on a column of silica gel to give **14** (2.2 g, 96%) as a colorless foam, $[\alpha]_D^{25}$ −11° (*c* 1.2, chloroform); ^{13}C-n.m.r.: δ 102.1 (C-1), 100.9 (C-1′), 73.7 (C-5′), 72.2 (C-5), 71.1 (2 C, C-3,3′), 68.9 (2 C, C-2,2′), 67.8, 67.9 (C-4,4′), 67.0 (C-6), 60.6 (C-6′), and 57.0 (Me).

Anal. Calc. for $C_{25}H_{36}O_{17}$: C, 49.34; H, 5.96. Found: C, 49.59; H, 6.10.

Methyl O-*(2,4,6-tri*-O-*acetyl-3-deoxy-3-fluoro-β*-D-*galactopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**19**). — The glycosyl halide **3** (0.55 g, 1.5 mmol) was added to a stirred mixture of **14** (0.61 g, 1 mmol), $Hg(CN)_2$ (0.19 g, 0.75 mmol), $HgBr_2$ (0.1 g), and Drierite (1 g) in benzene (5 mL), and treated as described for the preparation of **9** and **15**. T.l.c. showed the presence of a major product (R_F 0.3) together with the product of hydrolysis of **3** (R_F 0.35), and a small proportion of unreacted **14** (R_F 0.2). After the usual processing, chromatography showed, in the trisaccharide zone, minor by-products that were unchanged by acetylation. The major product **19** was obtained pure as a colorless foam (0.6 g, 67%), $[\alpha]_D^{25}$ −80.3° (*c* 1.2, chloroform); ^{13}C-n.m.r.: δ 102.1 (C-1), 100.7 (C-1′), 100.1 (d, $^3J_{C,F}$ 11 Hz, C-1″), 88.8 (d, $^1J_{C,F}$ 194.1 Hz, C-3″), 72.3 (2 C, C-5,5′), 71.0 (2 C, C-3,3′), 70.1 (d, $^3J_{C,F}$ 4.9 Hz, C-5″), 69.6 (d, $^2J_{C,F}$ 19.5 Hz, C-2″), 68.7, 68.6 (C-2,2′), 67.6, 67.5 (C-4,4′), 67.0 (d, $^2J_{C,F}$ 14.6 Hz, C-4″), 66.8, 66.7 (C-6,6′), 61.4 (C-6″), and 57.0 (Me); c.i.–m.s. (NH_3): *m/z* 916 (M + 18).

Anal. Calc. for $C_{37}H_{51}FO_{24}$: C, 49.44; H, 5.71; F, 2.11. Found: C, 49.18; H, 5.77; F, 1.85.

Methyl O-*β*-D-*galactopyranosyl-(1→6)*-O-*β*-D-*galactopyranosyl-(1→6)-β*-D-*galactopyranoside* (**22**) *and peracetate* (**23**). — Disaccharide **14** (1.65 g, 2.71 mmol), bromide **5** (prepared from **6**; 2.3 g, 5.42 mmol), $Hg(CN)_2$ (0.68 g, 2.7 mmol), and $HgBr_2$ (350 mg) were allowed to react in benzene (10 mL) in the presence of Drierite (3 g). After processing, chromatography revealed the presence of three minor by-products (R_F 0.35, 0.32, and 0.25; *E*) resistant to acetylation, in addition to the major product (R_F 0.3). The ^{13}C-n.m.r. spectrum of the minor product having R_F 0.35 showed it to be the α-linked trisaccharide **25**: δ 102.0 (C-1), 100.9 (C-1′), 96.6 (C-1″), 72.5 (C-5), 71.5 (C-5′), 71.0 (2 C, C-3,3′), 68.9, 68.7 (C-2,2′), 68.0 (C-3″), 67.8, 67.6, 67.3 (2 × 1 C, 1 × 3 C, C-2″,4,4′,4″,6), 66.6 (C-5″), 65.7 (C-6′), 63.3 (C-6″), 56.9 (Me), and 40.6 (CH_2Cl). Spectra of the other by-products showed that these substances were due to acetyl migration and subsequent glycosylation.

The major product **21** (1.55 g, 58.7%), obtained as a colorless foam, had $[\alpha]_D^{25}$ −9° (*c* 0.8, chloroform); ^{13}C-N.m.r.: δ 102.0 (C-1), 100.6 (2 C, C-1,1″), 72.0, 71.9 (C-5,5′), 71.0, 70.7 (2 × 2 C, C-3,3′,3″,5″), 68.8, 68.6, 68.4 (C-2,2′,2″), 67.6 (2 C, C-4,4′), 67.1 (C-4″), 66.7, 66.5 (C-6,6′), 63.0 (C-6″), 56.9 (Me), and 40.6 (CH_2Cl).

Anal. Calc. for $C_{39}H_{53}ClO_{26}$: C, 48.12; H, 5.48; Cl, 3.64. Found: C, 48.17; H, 5.86; Cl, 3.72.

Compound **21** (1.3 g, 1.33 mmol) was treated with 2,6-dimethylpyridine (150 μL, 1.29 mmol) and thiourea (300 mg, 3.9 mmol) as described for the preparation of **14**. One major (R_F 0.30) and two minor products (R_F 0.32 and 0.35; *F* after double development) were present and isolation of pure **24** by chromatography was unsuccessful. The unresolved mixture was deacetylated and, after processing, gave crystalline **22** from aqueous ethanol (0.45 g, 85%), m.p. 203–205°, $[\alpha]_D^{25}$ −10° (*c* 0.8, water) (lit.[11] $[\alpha]_D$ +38° for amorphous **22**); ^{13}C-n.m.r.: δ 103.9 (C-1), 103.4 (2 C, C-1′,2″), 75.2 (C-5″), 73.9 (2 C, C-5,5′), 72.7 (3 C, C-3,3′,3″), 70.7 (3 C, C-2,2′,2″), 69.2 (2 C, C-6,6′), 68.7 (3 C, C-4,4′,4″), 61.1 (C-6″), and 57.4 (Me).

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.61. Found: C, 43.72; H, 6.70.

Direct deacetylation of **21** readily yielded the same crystalline trisaccharide when seeded with **22**, m.p. 202–204°. Acetylation of **22** with acetic anhydride–pyridine gave the peracetate **23** as a glass, $[\alpha]_D^{25}$ −19.7° (*c* 0.7, chloroform); ^{13}C-n.m.r.: δ 102.1 (C-1), 100.6 (2 C, C-1′,2″), 72.1, 71.9 (C-5,5′), 71.0, 70.8 (2 × 2 C, C-5″,3,3′,3″), 68.8, 68.6, 68.4 (C-2,2′,2″), 67.5, 67.4 (C-4,4′), 67.0 (C-4″), 66.7, 66.5 (C-6,6′), 61.3 (C-6″), and 57.0 (Me).

Anal. Calc. for $C_{39}H_{54}O_{26}$: C, 49.89; H, 5.80. Found: C, 49.99; H, 6.14.

*Methyl O-(3-deoxy-3-fluoro-β-*D*-galactopyranosyl)-(1→6)-β-*D*-galactopyranoside* (**10**). — Sodium methoxide in methanol was added to a solution of **9** (220 mg) until strong alkaline reaction. After 2 h at room temperature, t.l.c. (*D*) showed that the reaction was complete. The solution was made neutral with Dowex 50W (H^+) cation-exchange resin and evaporated. Crystallization from methanol–acetone gave **10** (115 mg, 90%), m.p. 200–201° (ethanol), $[\alpha]_D^{25}$ −18° (*c* 0.8, water); ^{13}C-n.m.r.: δ 103.9 (C-1), 102.7 (d, $^3J_{C,F}$ 12.2 Hz, C-1′), 93.1 (d, $^1J_{C,F}$ 183.1 Hz, C-3′), 74.0 (d, $^3J_{C,F}$ 7.3 Hz, C-5′), 73.9 (C-5), 72.7 (C-3), 70.7 (C-2), 69.6 (d, $^2J_{C,F}$ 18.3 Hz, C-2′), 69.2 (C-6), 68.8 (C-4), 66.9 (d, $^2J_{C,F}$ 17.1 Hz, C-4′), 60.7 (d, $^4J_{C,F}$ 2.4 Hz, C-6′), and 57.4 (Me).

Anal. Calc. for $C_{13}H_{23}FO_{10}$: C, 43.57; H, 6.46; F, 5.30. Found: C, 43.21; H, 6.74; F, 5.03.

*Methyl O-(3-deoxy-3-fluoro-β-*D*-galactopyranosyl)-(1→6)-O-β-*D*-galactopyranosyl-(1→6)-β-*D*-galactopyranoside* (**20**). — Compound **19** was treated as described for the preparation of **10** to give crystalline (from aqueous ethanol) **20** in nearly theoretical yield, m.p. 191–193° (sint. 188°), $[\alpha]_D^{25}$ −14° (*c* 1, water); ^{13}C-n.m.r.: δ 103.9 (C-1), 103.3 (C-1′), 102.7 (d, $^3J_{C,F}$ 12.2 Hz, C-1″), 93.1 (d, $^1J_{C,F}$ 183.1 Hz, C-3″), 73.9 (d, $^3J_{C,F}$ 4.9 Hz, C-5″), 73.8 (2 C, C-5,5′), 72.7 (2 C, C-3,3′), 70.7 (2 C, C-2,2′), 69.5 (d, $^2J_{C,F}$ 17.1 Hz, C-2″), 69.4, 69.2 (C-6,6′), 68.7 (2 C, C-4,4′), 66.8 (d, $^2J_{C,F}$ 17.1 Hz, C-4″), 60.6 (C-6″), and 57.4 (Me).

Anal. Calc. for $C_{19}H_{33}FO_{15}$: C, 43.84; H, 6.39; F, 3.65. Found: C, 43.50; H, 6.66; F, 3.45.

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