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SYNTHESIS OF METHYL GLYCOSIDES OF β-(1→6)-LINKED D-GALACTOBIOSE, GALACTOTRIOSE, AND GALACTOTETRAOSE HAVING A 3-DEOXY-3-FLUORO-β-D-GALACTOPYRANOSIDE END-RESIDUE*

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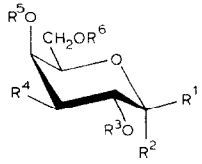
ABSTRACT

Methyl 2,4-di-*O*-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside was synthesized by sequential tritylation, acetylation, and detritylation of methyl 3-deoxy-3-fluoro-β-D-galactopyranoside, and used as the initial nucleophile in the synthesis of methyl β-glycosides of (1→6)-β-D-galacto-biose, -triose (**20**), and -tetraose (**22**) having a 3-deoxy-3-fluoro-β-D-galactopyranoside end-residue. The extension of the oligosaccharide chains, to form the internal units in **20** and **22**, was achieved by use of 2,3,4-tri-*O*-acetyl-6-*O*-bromoacetyl-α-D-galactopyranosyl bromide as a glycosyl donor, and mercuric cyanide or silver triflate as the promotor. While fewer by-products were formed in the reactions involving mercuric cyanide, the reactions catalyzed by silver triflate were stereospecific and yielded only the desired β (trans) products.

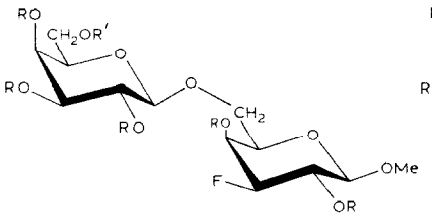
INTRODUCTION

To evaluate the importance of hydrogen bonding between ligands and immunoglobulins specific for (1→6)-β-D-galactopyranan, this laboratory has previously synthesized several deoxyfluorogalactosides[2–4] and reported[2] on the binding of some of these. More recently[5], we have studied the binding affinities of *inter alia* a series of synthetic[1,6] methyl β-glycosides of (1→6)-β-D-galactooligosaccharides, some containing a 3-deoxy-3-fluoro-D-galactopyranosyl end-group. Although important conclusions regarding the details of the binding mode of the related homologous polysaccharide to IgA J539 could be made, further model compounds need to be studied to confirm suggested mechanisms. The present work describes the synthesis of methyl glycosides **12, 20,** and **22**. Compounds **12** and **20** are isomeric to those described previously[1,6], but having a methyl 3-deoxy-3-fluoro-β-D-galactopyranoside end-residue.

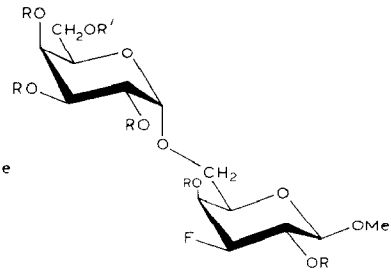
*Synthesis of specifically fluorinated methyl β-glycosides of (1→6)-β-D-galactooligosaccharides. Part IV. For Part III, see ref. 1.



	R^1	R^2	R^3	R^4	R^5	R^6
1	OMe	H	H	F	H	H
2	OMe	H	H	F	H	Tr
3	OMe	H	Ac	F	Ac	Tr
4	OMe	H	Ac	F	H	Tr
5	OMe	H	Ac	F	Ac	H
6	OMe	H	Ac	F	H	Ac
7	H	Br	Ac	OAc	Ac	Ac
8	OAc	H	Ac	OAc	Ac	$COCH_2Br$
9	H	Br	Ac	OAc	Ac	$COCH_2Br$
10	OMe	H	Ac	F	Ac	Ac



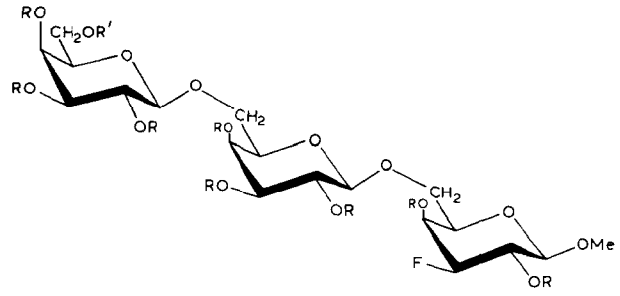
11 R = Ac, R′ = Ac
12 R = H, R′ = H
13 R = Ac, R′ = $COCH_2Br$
14 R = Ac, R′ = H



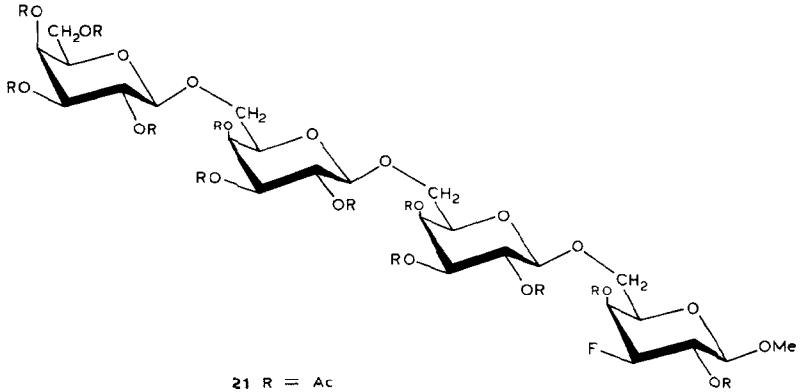
15 R = Ac, R′ = Ac
16 R = Ac, R′ = $COCH_2Br$

RESULTS AND DISCUSSION

Methyl β-D-glycosides of (1→6)-β-D-galactooligosaccharides having 3-deoxy-3-fluoro-D-galactosyl end-groups were previously prepared[1,6] by use of methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside as the initial nucleophile. To form the internal galactosyl residue(s) and galactosyl end-group of the oligosaccharides, 2,3,4-tri-*O*-acetyl-6-*O*-chloroacetyl-α-D-galactopyranosyl bromide and 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-α-D-galactopyranosyl bromide, respectively, were glycosyl donors. Although the di- and the tri-saccharide of those series were obtained in good yields, difficulties that prevented the synthesis of related higher oligosaccharides by that approach were experienced during the preparation of some of the intermediates. The difficulties arose from acetyl migration during the glycosylation step and, primarily, during *O*-dechloroacetylation. Since a bromoacetylated hydroxyl group can be regenerated under conditions milder[7,8] than those required



17 R = Ac, R′ = Ac
18 R = Ac, R′ = $COCH_2Br$
19 R = Ac, R′ = H
20 R = H, R′ = H



21 R = Ac
22 R = H

by a chloroacetylated hydroxyl group, the syntheses described herein used 2,3,4-tri-*O*-acetyl-6-*O*-bromoacetyl-α-D-galactopyranosyl bromide (**9**) to form the internal units of the oligosaccharides. Minor difficulties experienced in previous syntheses were due to the necessity to separate, *inter alia*, the α and β products formed during coupling reactions catalyzed by mercuric cyanide. Srivastava, Schuerch, and assoc.[9,10] have described successful, stereospecific β-glycosylations using 1-*O*-sulfonyl-D-galactopyranose derivatives as glycosyl donors. Particularly when higher oligosaccharides are to be obtained, it is sometimes more advantageous to perform the glycosylation stereospecifically, even though the conditions chosen may lead to a lower yield, so as to be able to avoid difficult separations. At the outset of the present work, we have, therefore, compared the stereoselectivity of the D-galactosylation when catalyzed by mercuric cyanide and by silver triflate.

The initial nucleophile used (**5**) was obtained in excellent yield from previously described[3] compound **1** by conventional tritylation and acetylation, followed by detritylation[8,11] with iodotrimethylsilane. A very minor by-product (3%),

isolated by chromatography of the material remaining in the mother liquor after crystallization of **5**, was identified by ^{13}C-n.m.r. spectroscopy as the 2,6-diacetate **6**, resulting from acetyl migration. The spectra of **5** and **6** were interpreted by comparison with those of the corresponding peracetate[4] and the nonfluorinated analogs[12], taking into account the generally accepted rules of ^{13}C-n.m.r. spectroscopy of carbohydrates[13,14].

Glycosylations leading to the disaccharides **11** or **13**, promoted by either mercuric cyanide or silver trifluoromethanesulfonate, gave good yields but no α (cis) products were formed in the triflate-promoted reactions. Therefore, in the subsequent syntheses of the tri- and the tetra-saccharide, only that method was applied. The structure of **11** and **12**, assigned on the basis of specific optical rotation, was confirmed by ^{13}C-n.m.r. spectroscopy. The spectra were interpreted with the aid of the analyzed spectrum of **5**, and those of the corresponding, isomeric fluorinated[6] and nonfluorinated analogs[12]. Thus, signals for C-1 and -1′ were easily recognized as doublets ($^3J_{C,F}$) and singlets, respectively, in the anomeric region. Signals for C-1′ of the α-linked products appeared at δ 96.5 (*c.f.* δ 96.6 for the corresponding nonfluorinated analogs[12]), and those for the β-linked counterparts at δ 101.0 (*c.f.* δ 100.7 for the nonfluorinated analogs[12]). The presence of fluorine was reflected by a doublet of C-3 which, due to the strong fluorine-induced shift-effect, appeared downfield at δ ~90 and showed a typical large $^1J_{C,F}$ coupling constant. The presence of the bromoacetyl group at C-6′ in **13** manifested itself by the signal of the CH_2Br group at δ 25.2. The interpretation of the spectra of the higher mol.-wt. oligosaccharides was done in a similar manner.

One of the by-products, formed consistently in the coupling reactions catalyzed by silver trifluoromethanesulfonate, was the 6-(6′ or 6″)acetate of the nucleophile used. Thus, in addition to compounds **11**, **17**, and **21**, compounds **10**, **11**, and **17** were, respectively, formed in reactions where **5**, **14**, and **19** were used as nucleophiles. The formation of such products has previously been explained[15]. More than 20% of the nucleophile may be consumed in such an undesirable reaction which, in part, accounts for the moderate yield (~40%) of the tri- and tetra-saccharide isolated from the coupling reactions. This indicates that the previous failure to synthesize a (1→6)-β-D-galactotetrasaccharide[10] was due to various side reactions, rather than the supposed "steric factors"[9,10]. During the triflate-catalyzed glycosidations described herein, the reaction mixtures were deliberately kept deficient in base and, consequently, the formation[16] of orthoesters as end products was not observed. Ways to improve the yields of the glycosidation step in these and related syntheses would require more stable blocking groups, in both the halide and the nucleophile, as suggested[16].

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi melting-point apparatus. Optical rotations were measured, at 25°, with a Perkin–Elmer

automatic polarimeter, Model 241 MC. T.l.c. on precoated plates of Silica gel GF (Analtech) was performed with: (*A*) 10:1 dichloromethane–methanol, (*B*) 15:1 toluene–acetone, (*C*) 4:1 toluene–acetone, (*D*), 3:1 toluene–acetone, (*E*) 3:1 carbon tetrachloride–acetone, and (*F*) 5:2 toluene–acetone. Detection was effected by charring with 5% (v/v) H_2SO_4 in ethanol. Preparative chromatography was performed by gradient elution from columns of slurry-packed Silica gel 60 (Merck, Prod. No. 9385). Natural abundance, noise-decoupled ^{13}C-n.m.r. spectra (at 25°) were recorded for solutions in $CDCl_3$ (blocked compounds, internal standard Me_4Si) or D_2O (deblocked oligosaccharides, internal standard MeOH, δ_{MeOH} *vs.* δ_{Me_4Si} 49.0), with a Jeol FX-100 spectrometer operating at 25 MHz. Solutions in organic solvents were dried with anhydrous Na_2SO_4, filtered, and concentrated at 40°/2 kPA.

*Methyl 3-deoxy-3-fluoro-6-*O*-trityl-β-*D*-galactopyranoside* (**2**). — Chlorotriphenylmethane (3 g, 11 mmol) was added to a solution of methyl 3-deoxy-3-fluoro-β-D-galactopyranoside[3] (**1**; 2 g, 10 mmol) in dry pyridine (10 mL). The solution was stirred at room temperature for 5 h and at 50° for an additional 16 h. T.l.c. showed that the reaction was practically complete. The mixture was processed conventionally and crystallization from dichloromethane–isopropyl ether (twice) yielded **2** (3.4 g, 76%), m.p. 105–109°, $[\alpha]_D^{25}$ −35.5° (*c* 1.1, chloroform); ^{13}C-n.m.r.: δ 103.5 (d, $^3J_{C,F}$ 11 Hz, C-1), 93.3 (d, $^1J_{C,F}$ 185.5 Hz, C-3), 86.9 (Ph_3C), 72.3 (d, $^3J_{C,F}$ 7.3 Hz, C-5), 69.8 (d, $^2J_{C,F}$ 18.3 Hz, C-2), 67.6 (d, $^2J_{C,F}$ 17.1 Hz, C-4), 62.3 (C-6), and 57.0 (Me).

Anal. Calc. for $C_{26}H_{27}FO_5$: C, 71.21; H, 6.20. Found: C, 71.25; H, 6.15.

*Methyl 2,4-di-*O*-acetyl-3-deoxy-3-fluoro-6-trityl-β-*D*-galactopyranoside* (**3**). — A solution of trityl derivative **2** (1.7 g, 3.87 mmol) in pyridine (5 mL) was treated with acetic anhydride (2.3 mL, 22.5 mmol). After 2 h at room temperature, t.l.c. (*B*) showed that no starting material (R_F 0.2) was present, and that two products were formed in approximately equal amounts (R_F 0.4 and 0.5). More acetic anhydride (~5 mL) was added and, after a further 4 h at 50°, t.l.c. showed that the slower moving of the two products (presumably **4**, resulting from acetylation of only the equatorial hydroxyl group at C-2 in **2**) was no longer present. The product was isolated in the usual manner and crystallization from dichloromethane–ethanol gave **3** (1.85 g, 91%). Recrystallization of a portion yielded material showing m.p. 234–234.5°, $[\alpha]_D^{25}$ −58.3° (*c* 1.9, chloroform); ^{13}C-n.m.r.: δ 101.3 (d, $^3J_{C,F}$ 11 Hz, C-1), 89.4 (d, $^1J_{C,F}$ 192.9 Hz, C-3), 86.9 (Ph_3C), 71.1 (d, $^3J_{C,F}$ 6.1 Hz, C-5), 70.1 (d, $^2J_{C,F}$ 19.5 Hz, C-2), 67.1 (d, $^2J_{C,F}$ 15.9, C-4), 60.6 (C-6), and 56.9 (Me).

When the conversion of **1** into **3** was carried out without isolation of the intermediate **2** the diacetate **3** was isolated in 89% yield.

*Methyl 2,4-di-*O*-acetyl-* (**5**) *and 2,6-di-*O*-acetyl-3-deoxy-3-fluoro-β-*D*-galactopyranoside* (**6**). — A suspension of the trityl derivative **3** (2.1 g, 4 mmol) and NaI (1.8 g, 12 mmol) in dry acetonitrile (40 mL) protected from atmospheric moisture was stirred until a clear solution was formed. The solution was cooled in ice and chlorotrimethylsilane (1.53 mL, 12 mmol) was added to the stirred solution under

anhydrous conditions. A dark-brown color developed immediately due to evolved I_2. After 2 min, ice–water (100 mL) was added and the mixture stirred for 15 min. The precipitate (triphenylmethanol) was removed by filtration, washed with cold water (3×), and the filtrate and washings, after having been stirred with an aqueous $Na_2S_2O_3$ solution, were partitioned between dichloromethane and water. The colorless dichloromethane solution was dried and concentrated to a small volume. Addition of ether gave the crystalline, chromatographically homogeneous 2,4-diacetate **5** (940 mg). Two recrystallizations of a portion from ether gave **5**, m.p. 118–119° $[\alpha]_D^{25}$ +27° (*c* 1, chloroform); ^{13}C-n.m.r.: δ 101.6 (d, $^3J_{C,F}$ 9.8 Hz, C-1), 89.0 (d, $^1J_{C,F}$ 194.1 Hz, C-3), 72.6 (d, $^3J_{C,F}$ 5 Hz, C-5), 70.1 (d, $^2J_{C,F}$ 18.3 Hz, C-2), 68.8 (d, $^2J_{C,F}$ 15.9 Hz, C-4), 60.1 (d, $^4J_{C,F}$ 2.4 Hz, C-6), and 57.1 (Me).

Anal. Calc. for $C_{11}H_{17}FO_7$: C, 47.14; H, 6.11; F, 6.77. Found: C, 46.93; H, 6.39; F, 6.80.

Material in the mother liquor was chromatographed and grave first methyl 2,6-di-*O*-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside (**6**) resulting from acetyl migration (35 mg, 3%), m.p. 110–111° (from ether), $[\alpha]_D^{25}$ +4.6° (*c* 1.2, chloroform); ^{13}C-n.m.r.: δ 101.3 (d, $^3J_{C,F}$ 12.2 Hz, C-1), 91.4 (d, $^1J_{C,F}$ 188 Hz, C-3), 71.1 (d, $^3J_{C,F}$ 6.1 Hz, C-5), 69.7 (d, $^2J_{C,F}$ 18.3 Hz, C-2), 67.2 (d, $^2J_{C,F}$ 17.1 Hz, C-4), 62.6 (d, $^4J_{C,F}$ 3.7 Hz, C-6), and 56.7 (Me).

Anal. Calc. for $C_{11}H_{17}FO_7$: C, 47.14; H, 6.11; F, 6.77. Found: C, 46.88; H, 6.11; F, 6.84.

Eluted next was more of the 2,4-diacetate **5** (55 mg, total yield 89.5%).

Methyl O-*(2,3,4,6-tetra-*O*-acetyl-α-* (**15**) *and -β-*D*-galactopyranosyl)-(1→6)-2,4-di-*O*-acetyl-3-deoxy-3-fluoro-β-*D*-galactopyranoside* (**11**). — (*a*) 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**7**) (0.61 g, 1.5 mmol) was added to a stirred mixture of the nucleophile **5** (0.28 g, 1 mmol), $Hg(CN)_2$ (190 mg, 0.75 mmol), $HgBr_2$ (10 mg), and Drierite (1 g) in dry benzene (5 mL). Stirring was continued for 20 h at room temperature, with the exclusion of atmospheric moisture. T.l.c. (*D* and *E*; in *D* separation of **11** from the slightly-slower moving product of hydrolysis of **7** could be achieved, but separation of **15** from **11** was very poor; these could be separated in *E* in which the product of hydrolysis of **7** and **11** showed almost the same mobility) showed that almost no **5** was present, and that one major product (R_F 0.3, *E*) was formed. The minor products showed R_F 0.35, 0.25, and 0.2 (*c.f.*, 0.15 for **5**). Dichloromethane (30 mL) was added, the mixture was filtered, the solids were washed with dichloromethane, and filtrate and washings were shaken with aqueous KBr solution. The organic phase was dried, filtered, and concentrated. The residual material was acetylated with acetic anhydride and pyridine, converting hydrolysis- and decomposition-products of **7** to faster-moving materials. The crude mixture was chromatographed to give first the material having R_F 0.35, shown to be the α-linked disaccharide **15** (25 mg, 4%), m.p. 184–185° (from ethanol, twice), $[\alpha]_D^{25}$ +94° (*c* 0.7, chloroform); ^{13}C-n.m.r.: δ 101.6 (d, $^3J_{C,F}$ 11 Hz, C-1), 96.5 (C-1′), 89.0 (d, $^1J_{C,F}$ 194.1 Hz, C-3), 70.8 (d, $^3J_{C,F}$ 4.9 Hz, C-5), 69.9 (d, $^2J_{C,F}$ 19.5 Hz, C-2), 67.9, 67.6, 67.4 (C-2′,3′,4′), 67.3 (d, $^2J_{C,F}$ 15.9 Hz, C-4), 66.6 (C-5′), 65.6 (d, $^4J_{C,F}$ 2.5 Hz, C-6), 61.7 (C-6′), and 57.1 (Me).

Anal. Calc. for $C_{25}H_{35}FO_{16}$: C, 49.17; H, 5.77; F, 3.11. Found: C, 49.17; H, 5.79; F, 3.33.

Eluted next was the β-linked, amorphous **11** (518 mg, 85%), $[\alpha]_D^{25}$ −1.3° (*c* 0.9, chloroform); ^{13}C-n.m.r.: δ 101.4 (d, $^3J_{C,F}$ 11 Hz, C-1), 101.0 (C-1′), 89.0 (d, $^1J_{C,F}$ 192.9 Hz, C-3), 72.0 (d, $^3J_{C,F}$ 6.1 Hz, C-5), 70.9 (2 C, C-3′,5′), 70.0 (d, $^2J_{C,F}$ 19.5 Hz, C-2), 68.7 (C-2′), 67.8 (d, $^2J_{C,F}$ 15.9 Hz, C-4), 67.5 (br.s., C-6), 67.1 (C-4′), 61.4 (C-6′), and 57.0 (Me).

Anal. Calc. for $C_{25}H_{35}FO_{16}$: C, 49.17; H, 5.77; F, 3.11. Found: C, 49.62; H, 5.74; F, 3.11.

(*b*) An anhydrous solution of silver trifluoromethanesulfonate (282 mg, 1.1 mmol) and 2,4,6-trimethylpyridine (112 μL, 0.85 mmol) in 1:1 toluene–nitromethane (2 mL) was added dropwise to a cold (−25°), stirred, anhydrous solution of the bromide **7** (452 mg, 1.1 mmol) and the nucleophile **5** (280 mg, mmol) in the same solvent mixture (3 mL). AgBr precipitated immediately and, after 15 min at −25°, t.l.c. (*E*) showed that the reaction was practically complete. The major product cochromatographed with **11** as obtained under (*a*). Three minor by-products were also present, but no material was detected in the area of mobility of **15**. 2,4,6-Trimethylpyridine (100 μL) was added to neutralize the excess of triflic acid, followed by dichloromethane (30 mL), and the mixture was filtered. The filtrate was washed with a $Na_2S_2O_3$ solution and the dichloromethane solution was dried and concentrated. Elution of the crude product from a column of silica gel gave, first, material having R_F 0.7, which cochromatographed with, and produced a ^{13}C-n.m.r. spectrum superimposable with that of methyl 2,3,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside (**10**) (45 mg, 16%), m.p. 81–82°; lit.[3] m.p. 81.5–82.5°.

Eluted next was the disaccharide derivative **11** (396 mg, 65%), identical (m.p. ^{13}C-n.m.r.) with the substance obtained under (*a*).

Methyl O-β-D-galactopyranosyl-(1→6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**12**). — A 2% solution of **11** in methanol was treated with a methanolic M solution of sodium methoxide until the solution was strongly alkaline to litmus. After 16 h at room temperature, the solution was made neutral with Dowex 50 W (H^+) ion-exchange resin and evaporated to yield pure **12** (100%). Crystallization from methanol–ethanol (twice) gave material showing m.p. 175–176°, $[\alpha]_D^{25}$ −13.3° (*c* 0.7, water); ^{13}C-n.m.r.: δ 103.4 (C-1′), 103.1 (d, $^3J_{C,F}$ 13.4 Hz, C-1), 93.0 (d, $^1J_{C,F}$ 183.1 Hz, C-3), 75.2 (C-5′), 72.8 (C-′), 72.6 (d, $^3J_{C,F}$ 8.5 Hz, C-5), 70.8 (C-2′), 69.4 (d, $^2J_{C,F}$ 18.3, C-2), 68.7 (2 C, C-6,4′), 66.9 (d, $^2J_{C,F}$ 17.1 Hz, C-4), 61.1 (C-6′), and 57.6 (Me).

Anal. Calc. for $C_{13}H_{23}FO_{10}$: C, 43.57; H, 6.47; F, 5.30. Found: C, 43.34; H, 6.64; F, 5.16.

Methyl O-(2,3,4-tri-O-acetyl-6-O-bromoacetyl-β-D-galactopyranosyl)-(1→6)-2,4-di-O-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside (**13**). — (*a*) The nucleophile **5** (0.56 g, 2 mmol) and the bromide **9** [prepared from **8** (1.41 g, 3 mmol) as described[8]] were treated as described for the preparation of **11** and **15** (*a*). The

crude product was chromatographed to give first the α-linked product **16** (32 mg, 2.3%); ^{13}C-n.m.r.: δ 101.6 (d, $^{3}J_{C,F}$ 11 Hz, C-1), 96.6 (C-1′), 89.0 (d, $^{1}J_{C,F}$ 194.1 Hz, C-3), 70.7 (d, $^{3}J_{C,F}$ 6.1 Hz, C-5), 69.9 (d, $^{2}J_{C,F}$ 19.5 Hz, C-2), 68.0 (C-3′), 67.5, 67.3 (C-2′,4′), 67.2 (d, $^{2}J_{C,F}$ 14.7 Hz, C-4), 66.5 (C-5′), 65.5 (br.s., C-6), 63.5 (C-6′), 57.3 (Me), and 25.2 (CH_2Br).

Subsequently eluted was the desired disaccharide derivative **13** (0.8 g, 58%), m.p. 129–130° (from methanol–ethanol, twice), $[\alpha]_D^{25}$ +2.7° (*c* 0.9, chloroform); ^{13}C-n.m.r.: δ 101.4 (d, $^{3}J_{C,F}$ 11 Hz, C-1), 101.0 (C-1′), 89.0 (d, $^{1}J_{C,F}$ 192 Hz, C-3), 71.9 (d, $^{3}J_{C,F}$ 6.1 Hz, C-5), 70.7 (2 C, C-3′,5′), 69.9 (d, $^{2}J_{C,F}$ 19.5 Hz, C-2), 68.6 (C-2′), 67.7 (d, $^{2}J_{C,F}$ 15.9 Hz, C-4), 67.6 (d, $^{4}J_{C,F}$ ~2.5 Hz, C-6), 67.1 (C-4′), 63.1 (C-6′), 57.1 (Me), and 25.2 (CH_2Br).

Anal. Calc. for $C_{25}H_{34}BrFO_{16}$: C, 43.55; H, 4.97. Found: C, 43.44; H, 4.91.

(*b*) The nucleophile **5** (1.7 g, 6 mmol) and the bromide **9** [prepared from **8** (3.42 g, 7.3 mmol)] were treated with silver trifluoromethanesulfonate as described for the preparation of **11** and **15** (*b*). After processing, the crude product was chromatographed to give, first, the 6-*O*-acetyl derivative (**10**) of the nucleophile **5** (0.2 g, identified by t.l.c., m.p., and ^{13}C-n.m.r.), and then the major reaction product **13** (2.53 g, 61%), identical with the substance described under (*a*). T.l.c. of the mixture showed the absence of **16**.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→6)-2,4-di*-O-*acetyl-3-deoxy-3-fluoro-β*-D-*galactopyranoside* (**17**). — To a solution of **13** (1.67 g, 2.42 mmol) in dichloromethane (25 mL) was added a solution (17 mL) of thiourea (0.55 g, 23 mmol) and 2,6-dimethylpyridine (0.268 mL, 23 mmol) in methanol (50 mL); the rest of the solution was added during 4–5 min and the course of the reaction was monitored by t.l.c. (*C*). When only traces of unchanged starting material were present (10–15 min), the mixture was evaporated at room temperature, and the residue was partitioned between dichloromethane and saturated, aqueous NaCl solution. The dichloromethane solution was dried and evaporated. The residue was chromatographed to give **14** (R_F 0.25, 1.3 g, 95%), sufficiently pure for the next step; ^{13}C-n.m.r.: δ 101.4 (d, $^{3}J_{C,F}$ 11 Hz, C-1), 100.9 (C-1′), 88.9 (d, $^{1}J_{C,F}$ 192.9 Hz, C-3), 73.6 (C-5′), 71.7 (d, $^{3}J_{C,F}$ 6.1 Hz, C-5), 71.0 (C-3′), 69.9 (d, $^{2}J_{C,F}$ 19.5 Hz, C-2), 69.0 (C-2′), 67.7 (2 C, C-4,6), 67.4 (C-4′), 60.6 (C-6′), and 56.9 (Me).

A solution of silver trifluoromethanesulfonate (334 mg, 1.3 mmol) and 2,4,6-trimethylpyridine (112 μL, 0.85 mmol) in 1:1 toluene–nitromethane (2 mL) was added to a stirred anhydrous solution of the nucleophile **14** (586 mg, 1 mmol) and the bromide **7** (493 mg, 1.2 mmol) kept at −25°. T.l.c. (*F*) showed that after 10 min all of **7** had been consumed, and that no **14** (R_F 0.2) remained. One major (R_F 0.4) and three minor products (R_F 0.5, 0.45, and 0.25) were formed. The mixture was processed as described for the preparation of **11** and **15** (*b*), and chromatographed to give first material having R_F 0.5, identical (t.l.c., ^{13}C-n.m.r.) with **11** (170 mg, 27%).

The major product was **17** (380 mg, 42%), $[\alpha]_D^{25}$ −9.1° (*c* 0.6, chloroform),

collected as a glassy solid; ^{13}C-n.m.r.: δ 101.2 (d, $^{3}J_{C,F}$ ~13 Hz, C-1), 100.8 (C-1′), 100.5 (C-1″), 88.8 (d, $^{1}J_{C,F}$ 192.9 Hz, C-3), 71.8 ($^{3}J_{C,F}$ not determined due to overlap of signals, 2 C, C-5,5′), 70.7 (3 C, C-3′,3″,5″), 69.8 (d, $^{2}J_{C,F}$ 18.3 Hz, C-2), 68.7, 68.3 (C-2′,2″), 67.6 (d, $^{2}J_{C,F}$ 15.9 Hz, C-4), 67.4 (C-6), 67.3 (C-6′), 66.9 (C-4′), 66.5 (C-4″), 61.3 (C-6″), and 56.9 (Me).

Anal. Calc. for $C_{37}H_{51}FO_{24}$: C, 49.44; H, 5.71. Found: C, 49.36; H, 5.53.

Methyl O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**20**). — M Sodium methoxide in methanol was added to a solution of **17** (280 mg) in methanol (10 mL), and the strongly alkaline solution was kept overnight at room temperature. It was made neutral with Dowex 50 W (H^+) cation-exchange resin, and concentrated to give a solid residue. Crystallization from aqueous ethanol (twice) gave material having m.p. 215–217°, $[\alpha]_D^{25}$ −12.3° (*c* 0.8, water); ^{13}C-n.m.r.: δ 103.5 (2 C, C-1′,1″), 103.2 (d, $^{3}J_{C,F}$ ~13 Hz, C-1), 93.0 (d, $^{1}J_{C,F}$ 183.1 Hz, C-3), 75.3 (C-5″), 73.9 (C-5′), 72.9, 72.7 (C-3′,3″), 72.6 (d, $^{3}J_{C,F}$ 7.3 Hz, C-5), 70.9 (2 C, C-2′,2″), 69.5 (d, $^{2}J_{C,F}$ 17.1 Hz, C-2), 69.1, 68.8 (1 C, 3 C, C-6,6′,4,4′), 67.0 (d, $^{2}J_{C,F}$ 15.9, C-4), 61.1 (C-6″), and 57.5 (Me).

Anal. Calc. for $C_{19}H_{33}FO_{15}$: C, 43.84; H, 6.39. Found: C, 43.89; H, 6.22.

Methyl O-(2,3,4-tri-O-acetyl-6-O-bromoacetyl-β-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→6)-2,4-di-O-acetyl-3-deoxy-3-fluoro-β-D-galactopyranoside (**18**). — The nucleophile **14** (1.35 g, 2.37 mmol) and the bromide **9**, prepared from **8** (1.33 g, 2.84 mmol), were condensed as described for the preparation of **17**. After processing, chromatography gave the major reaction product **18** (0.96 g, 41.3%), m.p. 128–130° (from chloroform–ethanol), $[\alpha]_D^{25}$ −6.6° (*c* 0.7, chloroform); ^{13}C-n.m.r.: δ 101.4 (d, $^{3}J_{C,F}$ 12.2 Hz, C-1), 101.0 (C-1′), 100.6 (C-1″), 89.0 (d, $^{1}J_{C,F}$ 192.9, C-3), 72.1 (C-5′), 72.0 (d, $^{3}J_{C,F}$ 6.1 Hz, C-5), 71.0, 70.9 (1 C, 2 C, C-3,3′,5″), 69.9 (d, $^{2}J_{C,F}$ 19.5 Hz, C-2), 68.7, 68.4 (C-2′,2″), 67.7 (d, $^{2}J_{C,F}$ 15.9 Hz, C-4), 67.2 (C-6), 67.1 (C-4′), 66.7 (C-4″), 63.1 (C-6″), 67.0 (Me), and 25.3 (CH_2Br).

Anal. Calc. for $C_{37}H_{50}BrFO_{24}$: C, 45.45; H, 5.15. Found: C, 45.29; H, 5.02.

Methyl O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-3-deoxy-3-fluoro-β-D-galactopyranoside (**22**). — A solution of the bromoacetyl derivative **18** (600 mg, 0.61 mmol) in dichloromethane (6 mL) was treated with a solution of thiourea (140 mg, 1.84 mmol) and 2,6-dimethylpyridine (68 μL, 0.58 mmol) as described for the preparation of **14**. The crude product was chromatographed to give **19** (470 mg, 90%), which was sufficiently pure for the next step; ^{13}C-n.m.r.: δ 101.3 (d, $^{3}J_{C,F}$ ~13 Hz, C-1), 101.0 (C-1′), 100.7 (C-1″), 89.0 (d, $^{1}J_{C,F}$ 192.9 Hz, C-3), 73.8 (C-5″), 71.9 (2 C, C-5,5′), 71.0 (2 C, C-3′,3″), 70.0 (d, $^{2}J_{C,F}$ 18.3 Hz, C-2), 68.8 (2 C, C-2′,2″), 67.7, 67.5 (2 × 2 C, $^{2}J_{C,F}$ not determined due to overlapping of signals, C-4,4′,6,6′), 66.7 (C-4″), 60.6 (C-6″), and 57.0 (Me).

A solution of nucleophile **19** (630 mg, 0.73 mmol) and bromide **7** (362 mg, 0.88 mmol) in 1:1 toluene–nitromethane (2 mL) was treated with a solution of

silver trifluoromethanesulfonate (263 mg, 1.02 mmol) and 2,4,6-trimethylpyridine (82 μL, 0.62 mmol) as described for the preparation of **17**. After being processed, the mixture was chromatographed to give first one of the faster moving, minor by-products that co-chromatographed, and whose ^{13}C-n.m.r. spectrum was superimposable with that of the trisaccharide derivative **17** (150 mg, 22.8%). The major reaction product **21** (380 mg, 44%) was collected as a glassy solid; ^{13}C-n.m.r.: δ 101.2 (d, $^{3}J_{C,F}$ 13.4 Hz, C-1), 100.9 (2 C, C-1′,1″), 100.4 (C-1‴), 89.0 (d, $^{1}J_{C,F}$ 191.7 Hz, C-3), 72.1, 71.9, 71.7 (C-5,5′,5″), 71.0 (4 C, C-3,3′,3″,5‴), 69.8 (d, $^{2}J_{C,F}$ ~15 Hz, C-2), 68.5 (3 C, C-2′,2″,2‴), 67.3 (6 C, C-4,4′,4″,6,6′,6″), 61.4 (C-6‴), and 56.9 (Me).

A solution of **21** (200 mg) in methanol (10 mL) was treated as described for the preparation of **20**. After neutralization with Dowex 50 W (H^+) resin, concentration gave a solid residue. Recrystallization from aqueous ethanol gave **22** in practically theoretical yield, m.p. 254–256°, $[\alpha]_D^{25}$ −10.6° (*c* 0.6, water); ^{13}C-n.m.r.: δ 103.5 (3 C, C-1′,1″,1‴), 103.2 (d, $^{3}J_{C,F}$ ~14.6 Hz, C-1), 92.9 (d, $^{1}J_{C,F}$ 181.1, C-3), 75.2 (C-5‴), 73.8 (2 C, C-5′,5″), 72.7 (4 C, C-3′,3″,3‴, 5, $^{2}J_{C,F}$ not determined due to overlapping of the signals), 70.8 (3 C, C-2′,2″,2‴), ~69.4 (d, $^{2}J_{C,F}$ not determined due to partial overlapping of signals, C-2), 69.3, 68.6 (2 C, 4 C, C-4′,4″,4‴, 6,6′,6″), 66.9 (d, $^{2}J_{C,F}$ 15.9 Hz, C-4), 61.1 (C-6‴), and 67.6 (Me).

Anal. Calc. for $C_{25}H_{43}FO_{20}$: C, 43.99; H, 6.35. Found: C, 43.84; H, 6.23.

REFERENCES

1 P. KOVÁČ, H. Y. C. YEH, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 140 (1985) 277–288.
2 Y. ITTAH AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 95 (1981) 189–194.
3 P. KOVÁČ AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 123 (1983) 317–331.
4 P. KOVÁČ AND C. P. J. GLAUDEMANS, *J. Carbohydr. Chem.*, 2 (1983) 317–327.
5 C. P. J. GLAUDEMANS, P KOVÁČ, AND K. RASMUSSEN, *Biochemistry*, 23 (1984) 6732–6736.
6 P. KOVÁČ AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 123 (1983) c29–c30.
7 C. A. A. VAN BOECKEL AND T. BEETZ, *Tetrahedron Lett.*, (1983) 3775–3778.
8 P. KOVÁČ AND C P. J. GLAUDEMANS, *Carbohydr. Res.*, 140 (1985) 313–318.
9 V. K. SRIVASTAVA AND C. SCHUERCH, *Carbohydr. Res.*, 106 (1982) 217–224.
10 V. K. SRIVASTAVA, S. J. SONDHEIMER, AND C. SCHUERCH, *Carbohydr. Res.*, 86 (1980) 203–214.
11 A. KLEMER, M. BIEBER, AND H. WILBERS, *Justus Liebigs Ann. Chem*, (1983) 1416–1421.
12 P. KOVÁČ, E. A. SOKOLOSKI, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 128 (1974) 101–109.
13 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
14 A. S. SHASHKOV AND O. S. CHIZHOV, *Bioorg. Khim.*, 2 (1976) 437–497.
15 P. J. GAREGG AND I. KVARNSTROM, *Acta Chem. Scand., Ser., B*, 30 (1976) 655–658
16 P. J. GAREGG AND T. NORBERG, *Acta Chem. Scand., Ser. B*, 33 (1979) 116–118