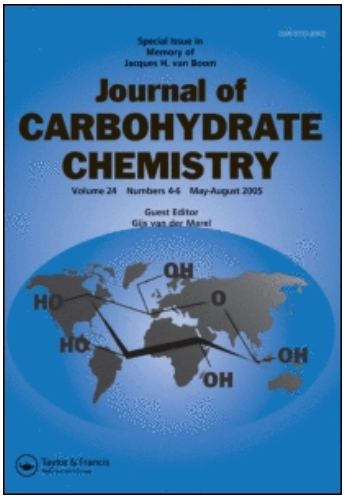

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SYNTHESIS OF METHYL O-β-D-GALACTOPYRANOSYL-(1→6)-(3-DEOXY-3-FLUORO-β-D-GALACTOPYRANOSYL)-(1→6)-β-D-GALACTOPYRANOSIDE. CONFIRMATION OF THE LOCATION OF SUBSITE D IN THE MONOCLONAL IgA J539*

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ABSTRACT

Bromoacetylation of methyl 2,4-di-O-benzoyl-3-deoxy-3-fluoro-β-D-galactopyranoside, followed by the cleavage of the methoxy group from the resulting 6-O-bromoacetyl derivative 2 with 1,1-dichloromethyl methyl ether gave 2,4-di-O-benzoyl-6-O-bromoacetyl-3-deoxy-3-fluoro-α-D-galactopyranosyl chloride (3). Reaction of 3 with methyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside promoted by silver trifluoromethanesulfonate afforded methyl O-(2,4-di-O-benzoyl-6-O-bromoacetyl-3-deoxy-3-fluoro-β-D-galacto-pyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (5). O-Debromoacetylation of 5 with thiourea gave the disaccharide nucleophile 6 which was condensed with 2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl bromide to afford the expected β-(trans)-linked trisaccharide derivative 7. Debenzoylation of 7 gave the methyl β-glycoside 8 of the (1→6)-linked D-galactotriose having the HO-3 of the internal residue replaced by a fluorine atom. Compound 8 was used to further delineate the subsites in the combining area of the monoclonal anti-(1→6)-β-D-galactan-specific immunoglobulin IgA J539.

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*Synthesis of specifically fluorinated methyl β-glycosides of (1→6)-β-D-galactooligosaccharides. VI.[1]

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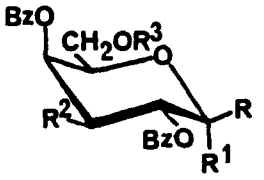
INTRODUCTION

This laboratory has been extensively involved in studies of the mode of binding of carbohydrates with immunoglobulins. We have already synthesized a large number of deoxyfluoro sugars related to (1→6)-β-D-galactopyranans and used them to evaluate hydrogen bonding in binding of (1→6)-β-D-galactan-specific monoclonal antibodies to their homologous saccharides (c.f. previous papers in this series, and papers cited therein). The results obtained made it possible[2] to map, in great detail, the combining area of the monoclonal anti-galactan immunoglobulin A J539. In order to further delineate the individual subsites[2,3] in the combining area of this (1→6)-β-D-galactan-specific immunoglobulin we have prepared the title trisaccharide 8, and determined its affinity for the above antibody. The syntheses of trisaccharides isomeric to 8, in that they contained the fluorine atom at positions 3 of either the non-reducing β-D-galactopyranosyl end-group or the β-D-galactopyranoside residue, has been described.[5,6]

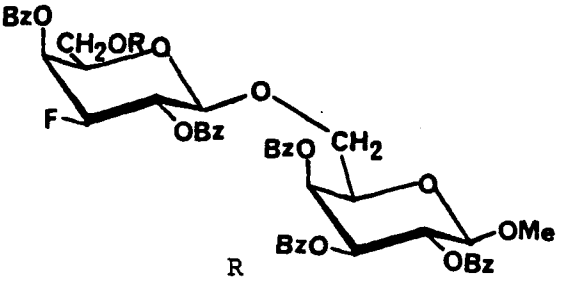
RESULTS AND DISCUSSION

The synthesis of the trisaccharide 8 required a glycosyl donor derived from 3-deoxy-3-fluoro-D-galactose bearing at O-6 a selectively removable blocking group. Coupling of such an intermediate to O-6 of an appropriately protected derivative of methyl β-D-galactopyranoside gives a disaccharide derivative amenable to deprotection at O-6'. The resulting disaccharide nucleophile then has that position open for further extension. Accordingly, we prepared the glycosyl halide 3 to become the internal unit in the trisaccharide 8.

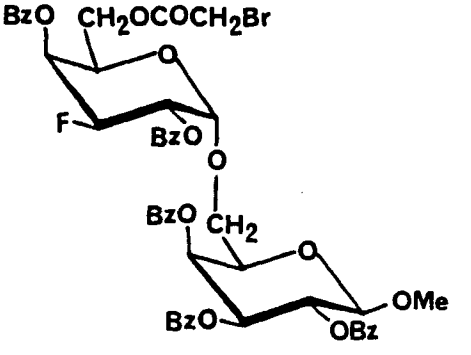
Halide 3 can be prepared in two ways starting from 3-deoxy-3-fluoro-D-galactose. In the first one, the starting fluoro sugar would be treated in a manner similar to the treatment of D-galactose in our earlier work.[1,6,7] Since furanose structures may then result,[1,6,7] we chose an approach assuring the pyranose structure. This offered a further demonstration of the effectiveness of the



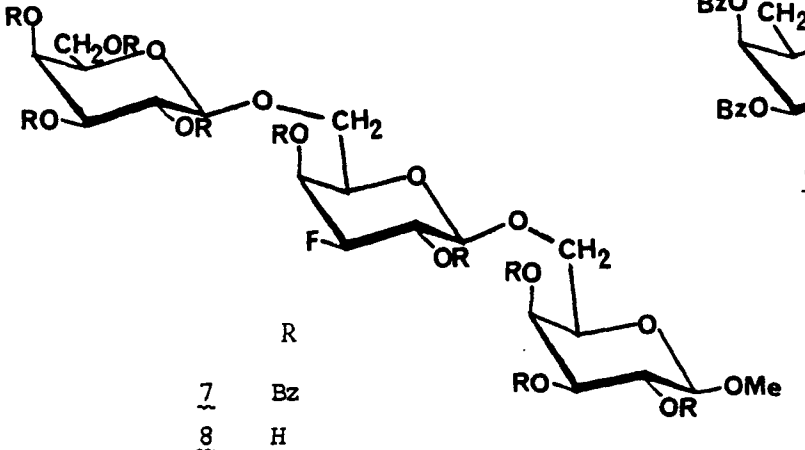
	R	R^1	R^2	R^3
1	OMe	H	F	H
2	OMe	H	F	$COCH_2Br$
3	H	Cl	F	$COCH_2Br$
4	H	OMe	F	$COCH_2Br$
10	OMe	H	OBz	H
11	H	Br	OBz	Bz



	R
5	$COCH_2Br$
6	H



9



	R
7	Bz
8	H

preparation of a complex glycosyl donor from a glycoside. The conversion of glycosides into glycosyl halides using 1,1-dihalogenomethyl methyl ethers[8] is generally applicable. Since selective blocking strategies are often easier to apply to glycosides than to reducing sugars, this approach is the one of choice to synthesize complex glycosyl donors (c.f. ref. 9-11), especially if one can start from commercially available alkyl or aryl glycosides. Accordingly, the starting benzoate 1[1] was bromoacetylated and the resulting compound 2 was treated with 1,1-dichloromethyl methyl ether (DCMME) in the presence of $ZnCl_2$. The desired, crystalline glycosyl chloride 3 was isolated in 71% yield, in addition to the α-glycoside 4 (~11%), resulting from anomerisation[11] of 2.

To assure efficient utilization of the precious glycosyl chloride 3, it was treated with an equimolar amount of 10. The desired disaccharide 5 was isolated crystalline in 73% yield. Unlike analogous reactions, this particular glycosylation was not stereospecific, and a small amount (2%) of the α-(cis)-product 9 was also obtained. The structure of 9 followed clearly from the analysis of its ^1H and ^{13}C NMR spectral data (see Table 1 and Experimental). Thus, in the ^1H NMR spectrum of 9 the signal of H-1' ($J_{1,2}$ 3.9 Hz), which appeared as a triplet due to stronger coupling of H-1' to the fluorine atom at C-3 ($J_{H\text{-}1',F\text{-}3'}$ 3.9 Hz), was diagnostically most important (c.f. ^1H NMR data for 5 in the Experimental). In the ^{13}C NMR spectrum of 9 the signal of C-1' appeared upfield, as expected, at δ 96.87 (c.f. δ 100.61 for the C-1' in the β-linked disaccharide 5).

O-Debromoacetylation of 5 yielded the disaccharide nucleophile 6 which, when treated with glycosyl bromide 11 under the standard conditions of silver trifluoromethanesulfonate (triflate)-catalyzed glycosylation, gave the D-galactotriose derivative 7. Subsequent debenzoylation of 7 (sodium methoxide in toluene-methanol) gave the target trisaccharide 8 as a crystalline monohydrate. Its ^{13}C NMR spectrum was in full accord with the expected structure.

The spectrum (Table 1) was interpreted by comparison with those[4,5] of the isomeric trisaccharides, and the signals for the carbon atoms of the internal D-galactosyl residue were readily recognized as those appearing as doublets showing characteristic coupling constants, $^{1-4}J_{CF}$.

The affinity of 8 for monoclonal IgA J539 Fab' (ref. 12) was measured using the ligand-induced tryptophanyl fluorescence change[13] and found to be 4.2×10^4 LM^{-1}. If the four subsites - each capable of binding one D-galactosyl residue - in IgA J539 are called D, B, A, and C in going from[2,3] the light (L) to the heavy (H) chain, it has been proposed that their relative affinity for a D-galactosyl residue decreases in the order A > B > C > D. Previous measurements[2,3] delineated subsites A, B and C but subsite D was placed near the L-chain out of stereochemical considerations only. It is known that subsite A requires interaction of HO-3 and HO-2 with the surface of the protein. Hence, 8 will be incapable of having its internal D-galactosyl residue engage subsite A and this trisaccharide can, therefore, not bind to BAC (Fig. 1). Assuming the positioning[2] of subsite D to be correct, the staggered conformation of the trisaccharide[2] will allow 8 to bind either to subsites AC or to ABD, as indicated in Fig. 1. Methyl 6-O-(3-deoxy-3-fluoro-β-D-galactopyranosyl)-β-D-galactopyranoside[3] (12) binds to subsites A and C and is reported[2] to have a K_a of 0.82×10^4. Therefore 8, with a K_a five times larger must involve other contacts, i.e. subsites ABD (Fig. 1). Trisaccharide 8 would also have the possibility to bind to three subsites if site D were on the H-chain side of C but in that case the maximal fluorescence change[2] caused by 8 would be expected around 20%, as in the case[3] of compound 12, as it would not engage the protein tryptophanyl residue at position 91L. However, 8 shows a F_{max} of 40.6% and engages, therefore, TRP 91L. Hence, subsite D must be on the L-chain side of B. This agrees also with the finding that the K_a found for 8 (4.2×10^4) is only slightly higher than that previously reported

TABLE 1

^{13}C NMR Chemical Shifts (δ) and J_{CF} Coupling Constants (Hz, in parentheses) for 2 - 9

Carbon atom	Compound							
	2[a]	3[a]	4[b]	5[a]	6[b]	7[a]	8[c]	9[a]
C-1	101.71 (9.5)	91.12 (9.1)	97.7 (8.6)	102.06	102.1	102.17	103.87	102.39
C-2	70.56 (18.9)	69.32 (18.9)	70.0 (18.3)	69.71	69.8	69.83[e]	70.80[g]	69.66
C-3	88.97 (195.8)	85.51 (193.1)	85.9 (192.9)	71.63	71.7	71.70[f]	72.80[f]	71.75
C-4	67.79 (17.6)	67.88 (17.1)	68.8 (17.1)	68.65	68.7	68.64	68.72[h]	68.49
C-5	70.18 (6.2)	69.72 (4.7)	66.3 (6.1)	72.98	72.9[d,e]	72.82	73.84	72.12
C-6	63.39 (3.1)	62.95 (4.0)	63.8[d]	68.33	68.2	67.94[e]	69.31	66.98
C-1'				100.61 (11.3)	100.8 (11.0)	100.58 (11.3)	102.64 (12.1)	96.87 (8.9)
C-2'				70.51 (18.8)	70.8 (18.9)	70.76 (19.8)	69.43 (17.1)	69.66 (19.7)
C-3'				88.74 (194.0)	89.0 (194.7)	89.04 (194.9)	92.84 (182.9)	85.80 (191.7)

C-4'				67.73 (17.3)	68.6 (~14)	67.49 (16.0)	66.86 (15.9)	68.77 (15.2)
C-5'				70.14 (4.5)	72.9[d,e]	71.60[d,e]	72.52 (5.4)	67.07 (5.0)
C-6'				63.13 (4.0)	60.0[d]	66.19[d]	68.69 (4.0)	63.83
C-1''						100.84	103.44	
C-2''						69.83[e]	70.80[g]	
C-3''						71.60[d,e,f]	72.75[f]	
C-4''						67.94[e]	68.67[h]	
C-5''						71.25	75.17	
C-6''						61.65	61.04	
Me	57.20		55.8	56.70	56.8	56.79	57.40	57.14
CH_2Br	25.22	25.10	25.2	25.08				25.25

a. At 75 MHz in $CDCl_3$; b. At 25 MHz in $CDCl_3$; c. At 75 MHz in D_2O; d. Broad singlet; e. Two-carbon signal; f. The assignments may be reversed; g. The assignments may be reversed; h. The assignments may be reversed.

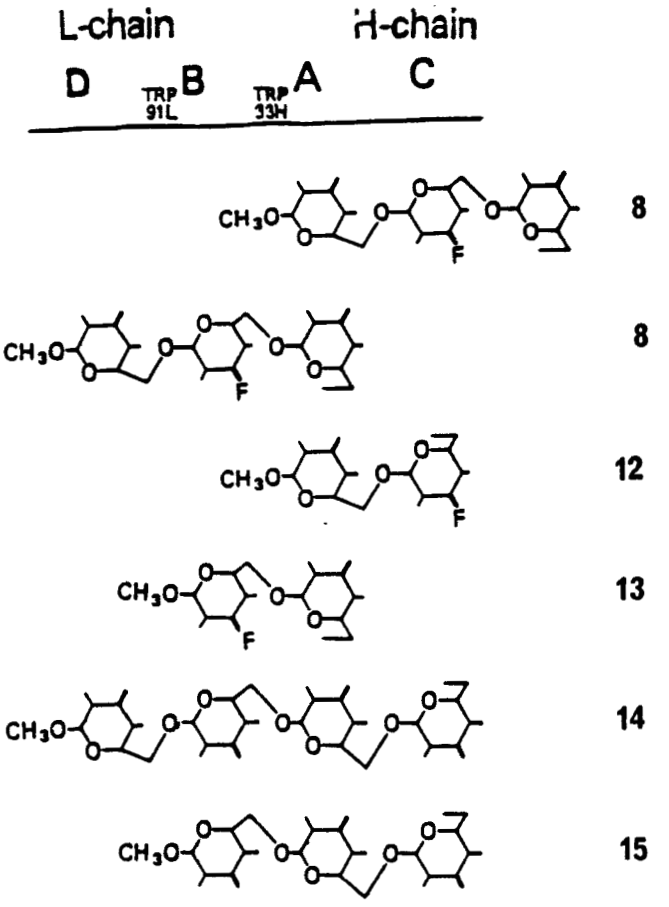


Figure 1. Schematic representation of the binding of compound 8 (two possibilities), and comparison with the binding of other oligosaccharides[3] to subsites A, B, C, and D of monoclonal IgA J539. The approximate locations of the two solvent-exposed tryptophanyl residues (TRP) of the antibody are indicated.

for methyl 6-O-(β-D-galactopyranosyl)-3-deoxy-3-fluoro-β-D-galactopyranoside[5] (13) (3.6×10^4). This reflects only a small additional binding due to engagement of subsite D. It is known that the maximally binding methyl β-glycoside of (1→6)-β-linked D-galactotetraose (14) binds[15] with a K_a of 5.9×10^5, while the corresponding trioside [15] binds with a K_a of 4.9×10^5.

EXPERIMENTAL

General methods.– These have been reported.[1] Unless otherwise stated, optical rotations were measured for solutions in chloroform. Thin-layer chromatography (TLC) on precoated plates of silica gel (Analtech) was performed with mixtures of appropriately adjusted polarity consisting of A, toluene-acetone; B, carbon tetrachloride-acetone; C, dichloromethane-acetone; and D, toluene-ethyl acetate. Except for the purification of the glycosyl chloride 3, the silica gel was deactivated by addition of 5-10% of water.

^{1}H NMR and ^{13}C NMR spectra for solutions in $CDCl_3$ (internal standard MeOH, δ_{MeOH} *vs.* Me_4Si, 49.0 p.p.m.) were recorded at room temperature with Varian FX-100, Varian HR-220 and Varian FX-300 spectrometers. The frequencies of measurements are listed below, as required. Proton-signal assignments were done by first-order analysis of the spectra, their comparison with the spectra of related compounds and, where feasible, by homonuclear selective decoupling. Carbon-signal assignments were done by mutual comparison of the spectra of related substances described here and elsewhere.[1,4,5]

Silver trifluoromethanesulfonate was obtained from Aldrich Chemical Co., and dried at 100°/133 Pa for 8 h. Dichloromethane of HPLC purity was dried before use with phosphorus pentoxide, distilled, and stored over anhydrous calcium sulfate. Chloroform was washed consecutively with concentrated sulfuric acid (twice), water, dried with phosphorus pentoxide, distilled, and stored over anhydrous calcium sulfate in the dark at -25°C. Anhydrous condi-

tions were maintained using argon and common laboratory glassware equipped with rubber septa; reagents and solvents were handled with Hamilton, Series 1000 gas-tight syringes. Solutions in organic solvents were dried with anhydrous sodium sulfate and concentrated at 40°C/2 kPa.

Methyl 2,4-Di-O-benzoyl-6-O-bromoacetyl-3-deoxy-3-fluoro-β-D-galactopyranoside (2).– 2,6-Lutidine (0.49 mL, 4.2 mmol) followed by bromoacetyl bromide (0.34 mL, 3.9 mmol) was added with stirring at -30° to a solution of 1 (1.15 g, 2.8 mmol) in dichloromethane (10 mL). Cooling was removed and the solution was stirred until TLC (solvent A) showed that the reaction was complete (~20 min). After addition of methanol (1 mL), the mixture was partitioned between dichloromethane and aqueous sodium hydrogen carbonate solution. The organic phase was decolorized by passing it through a short column of silica gel, to give pure, amorphous 2 (1.5 g, 98%), $[\alpha]_D$ + 63° (c 0.9). ^{1}H NMR (220 MHz): δ 5.87 (bt, 1 H, $J_{4,5}$ < 1 Hz, $J_{H\text{-}4,F\text{-}3}$ ~5 Hz, H-4), 5.67 (ddd, 1 H, $J_{2,3}$ 10 Hz, $J_{H\text{-}2,F\text{-}3}$ 12 Hz, H-2), 4.93 (ddd, 1H, $J_{3,4}$ 4 Hz, $J_{H\text{-}3,F\text{-}3}$ 47 Hz, H-3), 4.60 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.36 (d, 2H, H-6a, H-6b), 4.07, (bt, 1 H, $J_{5,6a}$ = $J_{5,6b}$ ~6 Hz, H-5), 3.79 (s, 2 H, CH_2Br), 3.52 (s, 3 H, OCH_3).

Anal. Calcd for $C_{23}H_{22}BrFO_8$: C, 52.58; H, 4.22; F, 3.61. Found: C, 52.27; H, 4.25; F, 3.48.

2,4-Di-O-benzoyl-6-O-bromoacetyl-3-deoxy-3-fluoro-α-D-galactopyranosyl chloride (3).– Freshly fused zinc chloride (30 mg) was added to a solution of 2 (1.33 g) in DCME (4 mL) and the mixture was stirred with the exclusion of atmospheric moisture at 80°C. After 1.5 h, when almost all reagent vaporized, TLC (solvent A) showed that the starting material was consumed and that two products were formed. DCME (2 mL) was added and after 30 min at 80° when the faster of the two products strongly predominated, as shown by TLC, the mixture was diluted with toluene, and concentrated to dryness. The residue was chromatographed (solvent B) to give first 3 (945 mg, 71%), mp 120-120.5°C (from dichloromethane-ether), $[\alpha]_D$ +146° (c 1.2). ^{1}H NMR (220 MHz): δ 6.55 (t, 1 H,

$J_{1,2} = J_{H\text{-}1,F\text{-}3}$ ~5 Hz, H-1), 5.99 (bdd, 1 H, $J_{4,5}$ < 1 Hz, $J_{H\text{-}4,F\text{-}3}$ 5.5 Hz, H-4), 5.69 (sx, 1 H, $J_{2,3} = J_{H\text{-}2,F\text{-}3}$ 10 Hz, H-2), 5.29 (ddd, 1 H, $J_{3,4}$ 4 Hz, $J_{H\text{-}3,F\text{-}3}$ 47 Hz, H-3), 4.65 (bt, 1 H, $J_{5,6}$ ~6 Hz, H-5), 4.43-4.25 (m, 2 H, H-6a, H-6b), 3.77 (s, 2 H, CH_2Br).

Anal. Calcd for $C_{22}H_{19}ClFO_7$: C, 49.87; H, 3.61. Found: C, 49.97; H, 3.70.

The material eluted next was shown by spectral evidence to be 4 (154 mg, 11.5%), the product of anomerisation of 2. ^1H NMR (220 MHz): δ 5.91 (bdd, 1 H, $J_{4,5}$ < 1 Hz, $J_{H\text{-}4,F\text{-}3}$ 5.5 Hz, H-4), 5.54 (sx, 1 H, $J_{2,3} = J_{H\text{-}2,F\text{-}3}$ 10 Hz, H-2), 5.26 (bt, 1 H, $J_{1,2} = J_{H\text{-}1,F\text{-}3}$ ~4 Hz, H-1), 5.22 (ddd, 1 H, $J_{3,4}$ 4 Hz, $J_{H\text{-}3,F\text{-}3}$ 48.5 Hz, H-3), 4.43-4.25 (m, 3 H, H-5, H-6a, H-6b), 3.79 (s, 2 H, CH_2Br), 3.43 (s, 3 H, OCH_3).

Methyl O-(2,4-Di-O-benzoyl-6-O-bromoacetyl-3-deoxy-3-fluoro-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (5).— A solution of the glycosyl chloride 3 (650 mg, 1.22 mmol), the nucleophile 10 (621 mg, 1.22 mmol) and 2,4,6-collidine (0.152 mL, 1.15 mmol) in dichloromethane (5 mL) was added at room temperature to a stirred suspension of silver triflate (321 mg, 1.25 mmol) in dichloromethane (5 mL). After 30 min all chloride 3 was consumed and only traces of unchanged 10 remained (TLC, solvent A). Essentially two products were formed, one largely predominating, both moving faster on TLC than the starting nucleophile 10. After conventional processing, the crude product was chromatographed to give first the minor, α-linked product 9, (25 mg, 2%), mp 120-122°C (from dichloromethane-ethanol), $[\alpha]_D$ +173° (c 0.5). ^1H NMR (300 MHz): δ 5.93 (bt, 1 H, $J_{4',5'}$ < 1 Hz, $J_{H\text{-}4',F\text{-}3'}$ ~4.5 Hz, H-4'), 5.86 (bd, 1 H, $J_{4,5}$ < 1 Hz, H-4), 5.75 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 5.62-5.51 (m, 2 H, H-2, H-3), 5.37 (t, 1 H, $J_{1',2'} = J_{H\text{-}1',F\text{-}3'}$ 3.9 Hz, H-1'), 5.21 (ddd, 1 H, $J_{3',4'}$ 4 Hz, $J_{H\text{-}3',F\text{-}3'}$ 48 Hz, H-3'), 4.69 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.48-3.77 (m, 6 H, H-5, H-5', H-6a, H-6b, H-6'a, H-6'b), 3.79 (s, 2 H, CH_2Br), 3.49 (s, 1 H, OCH_3).

Continued elution gave the desired disaccharide 5 (0.9 g, 73%), mp 120-122°C (from carbon tetrachloride), $[\alpha]_D$ +107° (c 0.7). ^{1}H NMR (300 MHz): δ 5.89 (bd, 1 H, $J_{4,5}$ < 1 Hz, H-4), 5.86 (bt, 1 H, $J_{H\text{-}4',F\text{-}3'}$ 5.5 Hz, H-4'), 5.71 (dd, partially overlapped with the multiplet of H-2', $J_{2,3}$ 11 Hz, H-2), 5.76-5.66 (m, partially overlapped with the doublet of doublets of H-2, H-2'), 5.55 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3), 4.92 (ddd, 1 H, $J_{H\text{-}3',F\text{-}3'}$ 46.5 Hz, $J_{3',4'}$ 3.2 Hz, H-3'), 4.75 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.59 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.29-4.10 (m, 4 H, H-5, H-6a, H-6b, H-6'b), 4.02 (bt, 1 H, H-5'), 3.84 (dd, 1 H, H-6'a), 3.71 (s, 2 H, CH_2Br), 3.24 (s, 3 H, OCH_3).

Anal. Calcd for $C_{50}H_{44}BrFO_{16}$: C, 60.06; H, 4.43; Br, 7.99; F, 1.90. Found: C, 60.07; H, 4.60; Br, 7.81; F, 1.94.C,

Methyl O-(2,4-di-O-benzoyl-3-deoxy-3-fluoro-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (6).— A solution of thiourea (231 mg, 3.04 mmol) in a minimum amount of methanol was added slowly with stirring to a solution of the disaccharide 5 (0.76 g, 0.76 mmol) in chloroform (20 mL). When the reaction was almost complete (TLC, solvent C, ~1 h), the mixture was washed with aqueous sodium chloride solution, the organic phase was dried, concentrated, and the residue was chromatographed, to give pure 6 (500 mg, 75%), mp 142-145°C (from dichloromethane-ether), $[\alpha]_D$ +137° (c 0.9).

Anal. Calcd for $C_{48}H_{43}FO_{15}$: C, 65.59; H, 4.93; F, 2.16. Found: C, 65.45; H, 5.14; F, 2.11.

Methyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1→6)-(2,4-di-O-benzoyl-3-deoxy-3-fluoro-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (7).— A solution of the nucleophile 6 (390 mg, 0.44 mmol), the glycosyl donor 11 (435 mg, 0.66 mmol) and 2,4,6-collidine (0.078 mL, 0.5 mmol) in dichloromethane (5 mL) was added at -25° to a stirred suspension of silver triflate (186 mg, o.72 mmol) in dichloromethane (5 mL). Silver

bromide slowly separated and TLC (solvent D) showed that the reaction was complete after 30 min. After conventional processing, isolation of the main product by chromatography gave the trisaccharide 7 (0.6 g, 92%), mp 179-182°C (unchanged after 3 recrystallizations from dichloromethane-ether), $[\alpha]_D$ +99° (c 0.75). ^{1}H NMR (300 MHz): δ 5.96-5.84 (m, 3 H, H-4, H-4', H-4''), 5,78-5.50 (m, 5 H, H-2, H-2', H-2'', H-3, H-3''), 4.49 (ddd, partially overlapped with one of the signals of the anomeric protons, $J_{3',4'}$ 3.2 Hz, $J_{H\text{-}3',F\text{-}3'}$ 47 Hz, H-3'), 4.72, 4.62, 4.58 (3xd, $J_{1,2} \sim J_{1',2'} \sim J_{1'',2''} \sim$7.8 Hz, H-1, H-1', H-1''), 4,26-3.58 (m, 9 H, H-5, H-5', H-5'', H-6a, H-6b, H-6'a, H-6'b, H-6''a, H-6''b), 3.24 (s, 3 H, OCH_3).

Anal. Calcd for $C_{82}H_{69}FO_{24}$: C, 67.57; H, 4.84; F, 1.30. Found: C, 67.54; H, 4.84; F, 1.31.

Methyl O-β-D-galactopyranosyl-(1→6)-(3-deoxy-3-fluoro-β-D-galactopyranosyl)-(1→6)-β-D-galactopyranoside (8).— Methanolic sodium methoxide (1 M) was added to a suspension of 7 (0.55 g) in methanol (100 mL) until the supernatant solution was strongly alkaline. The mixture was heated with stirring at 50-60°C until all starting material dissolved and then kept at 50°C overnight. After cooling (0°C), the solution was neutralized with Dowex 50 W (H^+-form) resin, concentrated, and the solid residue (195 mg, 100%) was recrystallized from methanol (twice) to give pure 8 (150 mg, 76%). The thus obtained material softened at ∿147°C and melted unsharply above this temperature, $[\alpha]_D$ -3.6° (c 1.25, water).

Anal. Calcd for $C_{19}H_{33}FO_{15} \times H_2O$: C, 42.37; H, 6.55; F, 3.52. Found: C, 42.75; H, 6.64; F, 3.35.

Affinity constant measurements.— Ligand-induced fluorescence change of the IgA Fab' (c.f. ref. 12) was measured as previously described.[13] All affinity constants reported here have been calculated taking into account corrections for bound ligand.

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