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Note

Synthesis of 2,3-epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside*

EUGENIA FALENT-KWAST†, PAVOL KOVÁČ, AD BAX, AND CORNELIS P J GLAUDEMANS
NIADDK, National Institutes of Health, Bethesda, MD 20892 (U S A.)
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The combining site of monoclonal immunoglobulin J539 has been mapped using deoxyfluorogalactosides as ligand-probes[2,3]. The results provide a remarkably detailed picture of the interaction of antigen with antibody, but it is still desirable to prepare a series of affinity labels. These will be useful in establishing the distances between reactive amino-acid side chains of the protein and its galactosyl-binding subsites, and in the preparation of stoichiometric, covalently linked hapten-antibody complexes for future n.m.r. studies. We here report on the preparation of a potential affinity label for (1→6)-β-D-galactopyranan-binding monoclonal antibodies, and show that the compound has a high degree of affinity for monoclonal IgA J539.

This laboratory has previously reported[4] the synthesis of 2,3-epoxypropyl D-galactopyranoside by the oxidation of the corresponding allyl glycoside. Also, we reported on systematic syntheses of (1→6)-β-D-galacto-oligosaccharides and their methyl β-glycosides[5–8]. In those syntheses selective deprotection of OH at position 6 was achieved by the dehaloacetylation of otherwise fully substituted 6-*O*-chloroacetyl[5] or -bromoacetyl[6] D-galactose derivatives, and we have here used this strategy to prepare the glycoside **12** (Scheme 1).

Thus, 2,3,4-tri-*O*-acetyl-6-*O*-bromoacetyl-[7] (**3**) and -6-*O*-chloroacetyl-[5] (**4**) α-D-galactopyranosyl bromide (freshly prepared from **1** and **2**, respectively) were condensed with allyl alcohol in the presence of mercuric cyanide to give the corresponding allyl β-glycosides **5** and **6**. Compounds **5** and **6** were *O*-dehaloacetylated by treatment with thiourea to give the crystalline nucleophile **8**. This reaction was accompanied by some acetyl-group migration, the product of which, **7**, was isolated by column chromatography and identified by analysis of its ^{1}H- and ^{13}C-n.m.r. spectra. Condensation of **8** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide gave the β-linked disaccharide **10**. The α-linked disaccharide **9**, formed as

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†Visiting Fellow from Poland.

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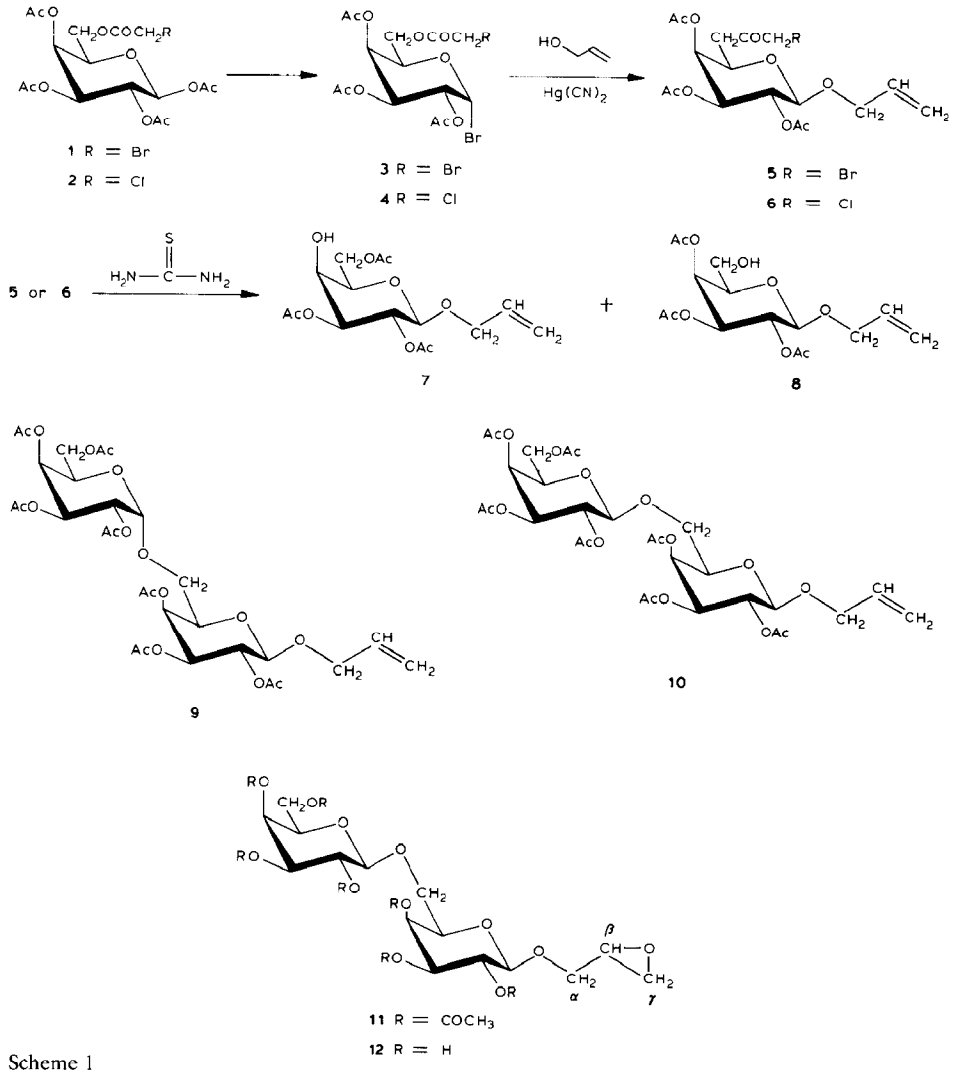

Scheme 1

a minor by-product, was also isolated. Structures **9** and **10** were assigned on the basis of ^{1}H- and ^{13}C-n.m.r. spectral data. The α stereochemistry of the intersugar linkage in **9** was inferred from the small spacing (3.2 Hz) of the doublet signal for H-1′ in the ^{1}H-n.m.r. spectrum of the compound. The lines in the ^{13}C-n.m.r. spectra of **9** and **10** were readily assigned with the aid of the unambiguously assigned spectra[8] of the corresponding methyl glycosides.

Oxidation of the double bond in **10** under the conditions used[4] in the preparation of the corresponding monosaccharide derivative gave the epoxypropyl derivative **11** as a mixture of diastereoisomers, as evidenced by the n.m.r. spectra, which showed two sets of signals for certain protons and carbon atoms (see Experimental). Deacetylation (Zemplén) afforded the crystalline title glycoside **12**, which was

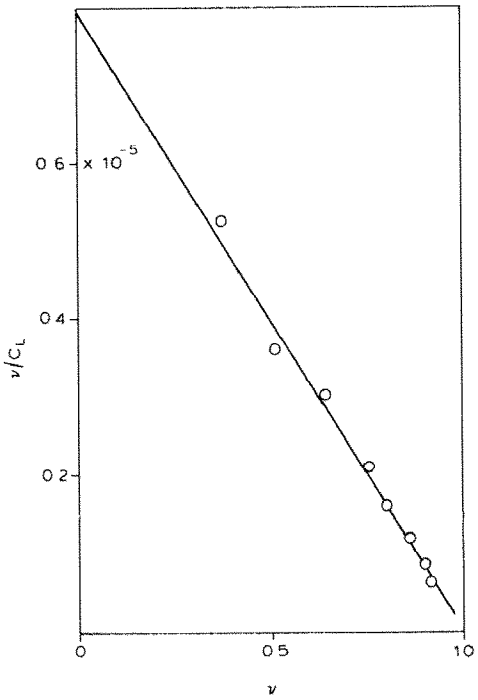

Fig. 1. Scatchard plot for the binding of **12** to the Fab' fragment of J539.

studied by n.m.r. spectroscopy. Using ligand-induced tryptophanyl fluorescence change[9] we could show (Fig. 1) that **12** had a high affinity for monoclonal anti-galactan IgA J539 ($K_a = 0.98 \times 10^5$ L/mol).

The identification of proton coupling networks in **12** was accomplished from a two-dimensional COSY spectrum[10–12], which provided the connectivity information for all geminal and vicinal couplings except H-4–H-5 and H-4'–H-5'. The anomeric protons (H-1,H-1') were distinguished by a selective INEPT experiment[13,14]. In that experiment magnetization is transferred from a preselected ^1H resonance to any ^{13}C nucleus having a significant long range coupling (2–20 Hz) with the designated proton. The decoupler frequency is set at the center of the preselected ^1H signal, and a set of low power (~20 Hz rf field strength) decoupler pulses interleaved by delays of 20–30 ms is applied synchronously with non-selective ^{13}C pulses to accomplish the transfer of spin polarization. During ^{13}C data acquisition conventional broad-band proton decoupling is employed. Fig. 2a shows the anomeric region of the regular ^{13}C spectrum, and Fig. 2b shows the selective INEPT spectrum that was obtained by transfer from the Cα proton of the aglycon at 4.17 p.p.m.; this distinguishes C-1 from C-1'. The selective INEPT method detects three-bond ^1H–^{13}C connectivity, and since C-1 has a vicinal coupling with the Cα proton, the C-1 resonance appears in the ^{13}C selective INEPT spectrum. A two-dimensional heteronuclear shift-correlation experiment[14,15,12] then correlated C-1 with H-1 and C-1' with H-1'. This assignment procedure confirmed the

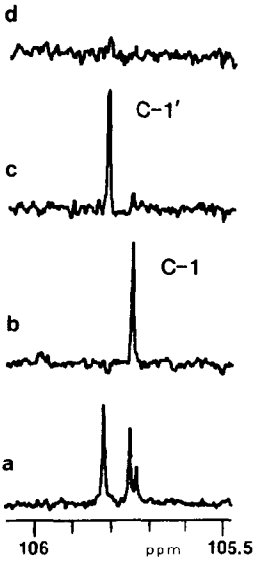

Fig. 2. Anomeric region of the ^{13}C spectrum of **12**. Key: *a*, regular ^{1}H-decoupled ^{13}C spectrum; *b–d*, selective INEPT spectra obtained by transfer from *b*, the Cα proton at 4.17 p.p.m., *c*, H-6 at 4.11 p.p.m., and *d*, H-6′ at 3.83 p.p.m. Measuring times were 4 min for spectrum *a* and 30 min each for spectra *b–d*.

expectation that the chemical shift differences between the H-1 and C-1 signals for the two diastereomers would be larger than those between H-1′ and C-1′. A selective INEPT experiment involving the pulsing of H-6 at 4.11 p.p.m. was used to show the presence of vicinal, $^{3}J_{HCOC}$ coupling across the glycosidic linkage. The resulting spectrum (Fig. 2c) shows the C-1′ resonance and confirms the ^{1}H resonance at 4.11 p.p.m. as belonging to H-6 rather than H-6′ or H-5. As expected, a selective INEPT transfer from H-6′ (Fig. 2d) did not result in any magnetization transfer to either of the anomeric carbons. After complete ^{1}H assignments had been made, a two-dimensional ^{1}H–^{13}C shift-correlation spectrum was used to assign all ^{13}C-resonances. The most crowded region of this spectrum is shown in Fig. 3, which displays the excellent resolution obtainable with this method when the spectrum is recorded in the pure absorption mode[15]. The ^{1}H and ^{13}C chemical shifts and ^{1}H–^{1}H couplings, taken from the one-dimensional ^{1}H spectrum, are presented in Table I.

EXPERIMENTAL

Melting points are uncorrected. N.m.r. spectra (^{1}H- and ^{13}C-) were recorded with Jeol FX 100, Varian HR 220, and Nicolet 500 spectrometers. The spectra were taken on solutions in $CDCl_3$, with Me_4Si as the internal standard, unless indicated otherwise. Optical rotations were measured with a Perkin–Elmer 241 MC automatic polarimeter. T.l.c. was carried out on Silica Gel HLF (Analtech), and

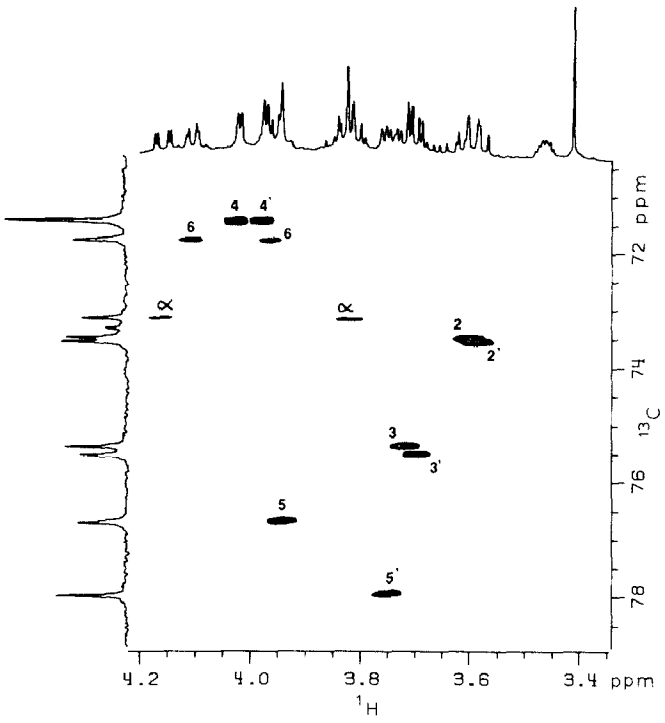

Fig. 3. Most crowded region of the two-dimensional, absorption-mode, ^{1}H–^{13}C chemical shift relation spectrum of **12**, recorded at 500 MHz. The absorption mode spectrum was obtained from a data set consisting of blocks of 128 1024 data points, with acquisition times of 64 and 122 ms in the t_1 and t_2 dimension, respectively. The spectrum was acquired in the amplitude modulated mode[15], with the ^{1}H decoupler frequency positioned at the low field side of the spectrum (4.70 p.p.m.). Total measuring time was 1.5 h using 50 mg of sample in a 10 mm sample tube.

flash chromatography was performed on columns of Silica Gel 60 (Merck, 230–400 mesh), with A, 3:1; B, 4:1; C, 5:1; or D, 6:1 (v/v) carbon tetrachloride–acetone; or E, 9:1 toluene–acetone; or F, 1:1 chloroform–methanol as eluting solvents.

All reactions were performed in dry solvents, under argon. Non-aqueous solutions obtained during workup procedures were dried over magnesium sulfate, and concentrated under reduced pressure at ≤40°.

*Allyl 2,3,4-tri-*O*-acetyl-6-*O*-bromoacetyl-* (**5**) *and -chloroacetyl-* (**6**) *β-*D*-galactopyranoside.* — A solution of **1** or **2** (12 mmol) in dichloromethane (25 mL) was treated with a solution of hydrogen bromide in acetic acid (33%, 35 mL). The mixture was concentrated after 1.5 h at room temperature and then co-evaporated with toluene to remove acetic acid. A solution of the residue in toluene was added to a stirred suspension of mercuric cyanide (1.5 g, 6 mmol), mercuric bromide (0.15 g), and Drierite (12 g) in allyl alcohol (24 mL). After being stirred for 18 h (t.l.c., solvent B) at room temperature the mixture was concentrated, diluted with dichloromethane, and filtered. The filtrate was washed twice with an aqueous solution of potassium bromide, dried, and concentrated.

TABLE I

^{1}H- AND ^{13}C-N M R DATA[a] FOR **12** IN D_2O

Position	*^{13}C Chemical shift[b] (δ, p.p.m.)*	*^{1}H Chemical shift[b] (δ, p.p m.)*	*^{1}H–^{1}H Coupling constants[b] (Hz)*
Cα	73.20 (73 38)	4.17, 3.82 (4 31, 3.67)	$J_{\alpha,\beta}$ = 2 5 (5.5)
Cβ	54.38 (54.36)	3.51	$J_{\beta,\gamma}$ = 4.4 (3.06)
Cγ	48.00 (47.88)	3.03, 2.90 (3.025, 2 87)	$J_{\gamma,\gamma}$ = 4.4
1	105 74 (105.72)	4.508 (4.53)	$J_{1,2}$ = 7.9 (7.9)
2	73.53	3.60	$J_{2,3}$ = 9.5
3	75.41	3.72	$J_{3,4}$ = 3.4
4	71 49	4 02	$J_{4,5}$ <1
5	76.74	3.94	$J_{5,6}$ = 3 2, 7.0[c]
6	71.83	3.96, 4.11	$J_{6,6}$ = −10[c]
1′	105.82	4.512	$J_{1',2'}$ = 7.9
2′	73.61	3.58	$J_{1',3'}$ = 9
3′	75.56	3.70	$J_{3',4'}$ = 3.4
4′	71.49	3.98	$J_{4',5'}$ <1
5′	78 00	3 75	$J_{5',6'}$ = 8, 5.5[c]
6′	63.84	3.83	

[a]Chemical shifts are relative to internal TSP (4,4-dimethyl-4-silapentanoate). [b]Values in parentheses refer to the less abundant diastereoisomer [c]Couplings obtained from a computer simulation for the X part of an ABX spin system.

Pure compound **5** (4.7 g, 84%), m.p. 67–69°, $[\alpha]_D$ −11.7° (*c* 1, $CHCl_3$), was obtained by chromatography of the crude product (solvent D). ^{1}H-N.m.r. (220 MHz): δ 5.77–5.98 (m, 1 H, $OCH_2CH{=}CH_2$), 5.41 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ <1 Hz, H-4), 5.18–5.34 (m, 3 H, H-2 and $OCH_2CH{=}CH_2$), 5.05 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 4.57 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.93–4.43 (m, 5 H, H-5, 6, 6a, and $OCH_2CH{=}CH_2$), 3.86 (2 H, $COCH_2Br$), 2.02, 2.06, and 2.21 (9 H, 3 OAc). ^{13}C-N.m.r. (25.16 MHz): δ 133.3 and 117.7 (*C*=*C*), 100.0 (C-1), 70.8 (C-3), 70.4 (C-5), 70.0 ($OCH_2CH{=}CH_2$), 68.8 (C-2), 67.1 (C-4), 63.0 (C-6), 25.2 (CH_2Br), and 20.7–20.8 ($COCH_3$).

Anal. Calc. for $C_{17}H_{23}BrO_{10}$: C, 43.70; H, 4.96. Found: C, 43.98; H, 4.67.

Compound **6** (3.9 g, 76%) crystallized directly from isopropyl ether. Recrystallization from the same solvent gave material having m.p. 66–67° and $[\alpha]_D$ −12.2° (*c* 1.5, $CHCl_3$). ^{1}H-N.m.r. (220 MHz): δ 5.75–5.96 (m, 1 H, $OCH_2CH{=}CH_2$), 5.40 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ <1 Hz, H-4), 5.16–5.34 (m, 3 H, H-2 and $OCH_2CH{=}CH_2$), 5.05 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 4.56 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.95–4.41 (m, 5 H, H-5, 6, 6a, and $OCH_2CH{=}CH_2$), 4.09 (2 H, CH_2Cl), 2.0, 2.09, and 2.18 (9 H, 3 OAc). ^{13}C-N.m.r. (25.16 MHz): δ 133.3 and 117.7 (*C*=*C*), 100.1 (C-1), 70.8 (C-3), 70.4 (C-5), 70.0 ($OCH_2CH{=}CH_2$), 68.7 (C-2), 67.0 (C-4), 62.9 (C-6), 40.5 (CH_2Cl), and 20.6–20.8 ($COCH_3$).

Anal. Calc. for $C_{17}H_{23}ClO_{10}$: C, 48.29; H, 5.48; Cl, 8.39. Found: C, 48.24; H, 5.40; Cl, 8.12.

*Allyl 2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**8**). — (*a*) A solution of thiourea (2.2 g, 29 mmol) in methanol (20 mL) was added to a solution of **6** (4 g, 9.5 mmol) in dichloromethane (100 mL), and the mixture was stirred overnight at room temperature. T.l.c. (solvent E) then showed that ~50% of the starting material was consumed. *sym*-Collidine (0.63 mL, 4.75 mmol) was added, and the mixture was refluxed until t.l.c. showed that the reaction was complete (~3–4 h). The solution was diluted with dichloromethane, washed with saturated aqueous sodium chloride, dried, and concentrated. The residue was chromatographed (solvent C) to give first **7** (0.32 g, 10%, amorphous); ^{1}H-n.m.r. (220 MHz): δ 5.75–5.96 (m, 1 H, $OCH_2CH{=}CH_2$), 5.16–5.40 (m, 3 H, H-2 and $OCH_2CH{=}CH_2$), 4.96 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3 Hz, H-3), 4.51 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.73–4.43 (m, 6 H, H-4, 5, 6, 6a, and $OCH_2CH{=}CH_2$), 2.61 (m, 1 H, disappears on deuteration, O*H*), 2.07, 2.10, and 2.13 (9 H, 3 OAc); ^{13}C-n.m.r. (25.16 MHz): δ 133.6 and 117.4 (*C*=*C*), 100.0 (C-1), 73.4 (C-5), 72.1 (C-3), 69.7 ($OCH_2CH{=}CH_2$), 69.2 (C-2), 67.0 (C-4), 62.6 (C-6), and 20.8 ($COCH_3$).

Eluted next was **8** (2.4 g, 74%), m.p. 96–97°, $[\alpha]_D$ +1.5° (*c* 1.5, $CHCl_3$); ^{1}H-n.m.r. (220 MHz): δ 5.77–5.98 (m, 1 H, $OCH_2CH{=}CH_2$), 5.40 (bd, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 5.18–5.34 (m, 3 H, H-2 and $OCH_2CH{=}CH_2$), 5.08 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.2 Hz, H-3), 4.57 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.38 and 4.14 (2 × m, 2 × 1 H, $OCH_2CH{=}CH_2$), 3.50–3.84 (m, 3 H, H-5, 6, and 6a), 2.39 (m, 1 H, disappears on deuteration, O*H*), 2.03, 2.09, and 2.20 (9 H, 3 OAc); ^{13}C-n.m.r. (25.16 MHz): δ 133.6 and 117.2 (*C*=*C*), 100.1 (C-1), 73.5 (C-5), 71.2 (C-3), 69.9 ($OCH_2CH{=}CH_2$), 69.2 (C-2), 67.8 (C-4), 60.4 (C-6), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{15}H_{22}O_9$: C, 52.02; H, 6.40. Found: C, 52.37; H, 6.64.

(*b*) A solution of thiourea (1.6 g, 20 mmol) in methanol (15 mL) was added to a solution of **5** (3.4 g, 7.2 mmol) in dichloromethane (60 mL), and the mixture was stirred at room temperature. The reaction was monitored by t.l.c. (solvent E) and at 10 minutes intervals 3 portions of *sym*-collidine (0.48 g, 0.24, and 0.24 mL) were added. When the reaction was complete (~0.5 h) the mixture was worked up as described in (*a*). Chromatography (solvent C) gave **8** (1.95 g, 78%, m.p. 96–97°).

Allyl O-*(2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**9**) *and allyl* O-*(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**10**)*. — Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.851 g, 4.5 mmol) was added to a mixture of nucleophile **8** (1.039 g, 3 mmol), mercuric cyanide (0.568 g, 2.25 mmol), mercuric bromide (0.06 g) and Drierite (3 g) in benzene (20 mL) at room temperature, and the suspension was stirred overnight (t.l.c., solvent A). The mixture was worked up as described above for the preparation of **6**. Recrystallization of the

*Compound **10** can be obtained also by the condensation of **8** (1 mmol) with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1.1 mmol) in nitromethane–toluene (1:1) at −25°, using silver triflate (1.1 mmol) and *sym*-collidine (0.85 mmol) as promoters. The overall yield of **10** is somewhat lower but no α isomer (**9**) is formed

crude product from isopropyl ether–ethanol gave **10** (1.2 g, 59%). The material in the mother liquors was chromatographed (solvent C) to give **9** (0.2 g, 10%, solid foam), $[\alpha]_D$ +76.5° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. (220 MHz): δ 5.77–5.98 (m, 1 H, $OCH_2CH{=}CH_2$), 5.44 (m, 2 H, H-4 and 4′), 5.18–5.36 (m, 4 H, H-2,2′, and $OCH_2CH{=}CH_2$), 5.13 and 5.01 (2 × dd, 2 H, $J_{2,3}$ 10.5, $J_{2',3'}$ 10.5, $J_{3,4}$ 3.0, $J_{3',4'}$ 3.0 Hz, H-3 and 3′), 4.96 (d, 1 H, $J_{1',2'}$ 3.2 Hz, H-1′), 4.56 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.41–4.43 (m, 8 H, H-5,5′, 6,6′, 6a,6a′, and $OCH_2CH{=}CH_2$), and 2.02–2.18 (7 OAc); ^{13}C-n.m.r. (25.16 MHz): δ 133.4 and 117.5 (*C*=*C*), 100.2 (C-1), 96.6 (C-1′), 71.5 (C-5), 71.1 (C-3), 70.0 ($OCH_2CH{=}CH_2$), 68.9 (C-2), 67.9 (C-3′), 67.5, 67.4 with double intensity (C-2′, 4, and 4′), 66.5 (C-5′), 65.7 (C-6), 61.6 (C-6′), 20.6 ($COCH_3$).

Eluted next was **10** (0.39 g, total yield 78%, m.p. 140–141°, $[\alpha]_D$ −16° (*c* 1.6, $CHCl_3$); ^{1}H-n.m.r. (220 MHz): δ 5.77–5.98 (m, 1 H, $OCH_2CH{=}CH_2$), 5.38 (m, 2 H, H-4 and 4′), 5.16–5.30 (m, 4 H, H-2, 2′, and $OCH_2CH{=}CH_2$), 5.02 (m, 2 H, H-3 and 3′), 4.55 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.52 (d, $J_{1',2'}$ 7.8 Hz, H-1′), 3.75–4.43 (m, 8 H, H-5, 5′, 6, 6′, 6a, 6a′, and $OCH_2CH{=}CH_2$), and 2.01–2.18 (7 OAc); ^{13}C-n.m.r. (75 MHz): 133.3 and 117.5 (*C*=*C*), 100.7 (C-1), 100.0 (C-1′), 72.2 (C-5), 71.0, 70.8, 70.7 (C-3, 3′, and 5′), 69.7 ($OCH_2CH{=}CH_2$), 68.9, 68.6 (C-2 and 2′), 67.7 (C-4), 67.0, 66.9 (C-4′ and 6), 61.3 (C-6′), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{29}H_{40}O_{18}$: C, 51.48; H, 5.96. Found: C, 51.31; H, 5.58.

2,3-Epoxypropyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-O-acetyl-β-D-galactopyranoside (**11**). — A stirred mixture of **10** (1 g, 1.5 mmol) and *m*-chloroperbenzoic acid (0.320 g, 1.9 mmol) in dichloromethane (10 mL) was heated overnight under reflux. A fresh portion of *m*-chloroperbenzoic acid (0.32 g) was added, and after a further 8 h at reflux temperature starting material (t.l.c., solvent A) was no longer present. The mixture was cooled, diluted with dichlormethane, and washed, first with dilute solutions of sodium bisulfite (twice) and sodium bicarbonate, then water, then dried and concentrated. Recrystallization from carbon tetrachloride gave **11** (0.86 g, 84%), m.p. 114–116°, $[\alpha]_D$ −15.3° (*c* 1.3, $CDCl_3$); ^{1}H-n.m.r. (500 MHz): δ 5.37 (m, 2 H, H-4 and 4′), 5.18 (m, 2 H, H-2 and 2′), 5.0 (m, 2 H, H-3 and 3′), 4.48–4.61 (4 x d, 2 H, H-1, and 1′ of two isomers), 4.14 (m, 2 H, H-5 and 5′), 3.80–3.95 (m, 4 H, H-6, 6′, 6a, and 6′a), 3.50, 3.77, and 4.08 (3 × m, 2 H, Hγ of two isomers), 3.15 (m, 1 H, Hβ), 2.58, 2.69, and 2.80 (3 × m, 2 H, Hα of two isomers), and 1.96–2.16 (7 OAc); ^{13}C-n.m.r. (300 MHz): δ 101.4 and 101.0 (C-1 of two isomers), 100.6 (C-1′), 72.4 (C-5), 67.0–70.9 (C-2, 2′, 3, 3′, 4, 4′, 5′, and 6′), 66.74 and 66.66 (Cα of two isomers), 61.3 (C-6′), 50.6 and 50.2 (Cβ of two isomers), 44.0 and 43.9 (Cγ of two isomers), and 20.5–20.7 ($COCH_3$).

Anal. Calc. for $C_{29}H_{40}O_{19}$: C, 50.29; H, 5.82. Found: C, 50.48; H, 5.87.

2,3-Epoxypropyl O-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**12**). — Methanolic sodium methoxide (0.7 mL, 1M) was added to a stirred solution of **11** (0.715 g, 1.03 mmol) in methanol (50 mL). After 0.5 h at room temperature no starting material was present (t.l.c., solvent F). The solution was neutralized

(Amberlite IR-120, H^+), filtered, and concentrated to dryness. Recrystallization from methanol–water gave **12** (0.25 g, 62%), m.p. 145–150°, $[\alpha]_D$ −16.3° (*c* 0.9, $CHCl_3$). N.m.r. data (^1H- and ^{13}C-) are given in Table I.

Anal. Calc. for $C_{15}H_{26}O_{12} \cdot H_2O$: C, 43.26; H, 6.78. Found: C, 43.49; H, 6.91.

Measurement of the binding affinity of **12** *for monoclonal IgA J539 Fab'.* Affinity purified[16] monoclonal IgA J539 was treated with pepsin and the Fab' fragment was isolated as described[17]. Solutions of IgA J539 Fab' in phosphate-buffered saline (0.01M phosphate; 0.714×10^{-6}M in protein) were titrated with **12** while the fluorescence enhancement at 330 nm was monitored as a function of free ligand concentration (c_l). A Scatchard plot of the fraction of bound sites (ν over c_l *versus* ν) showed a straight line with an extrapolated value of $K_a = 0.98 \times 10^5$ L/mol (Fig. 1).

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REFERENCES

1 G. Ekborg, B. Vranešic, A. K. Bhattacharjee, P. Kovač and C. P. J. Glaudemans, *Carbohydr. Res.*, 142 (1985) 203–211
2 C. P. J. Glaudemans, P. Kovač, K. Rasmussen, *Biochemistry*, 23 (1984) 6732–6736.
3 C. P. J. Glaudemans and P. Kovač, *Mol. Immunol.*, 22 (1985) 651–655
4 M. E. Jolley, C. P. J. Glaudemans, S. Rudikoff and M. Potter, *Biochemistry*, 13 (1974) 3179–3184.
5 A. K. Bhattacharjee, E. Zissis, and C. P. J. Glaudemans, *Carbohydr. Res.*, 89 (1981) 249–254.
6 P. Kovač and C. P. J. Glaudemans, *Carbohydr. Res.*, 140 (1985) 289–298
7 P. Kovač and C. P. J. Glaudemans, *Carbohydr. Res.*, 140 (1985) 313–318
8 P. Kovač, E. A. Sokolosky, and C. P. J. Glaudemans, *Carbohydr. Res.*, 128 (1984) 101–109.
9 M. E. Jolley and C. P. J. Glaudemans, *Carbohydr. Res.*, 33 (1974) 377–382
10 W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 64 (1976) 2226–2246
11 A. Bax and R. Freeman, *J. Magn. Reson.*, 44 (1981) 542–561
12 A. Bax, W. Egan and P. Kovač, *J. Carbohydr. Chem.*, 3 (1984) 593–611
13 A. Bax, *J. Magn. Reson.*, 57 (1984) 314–318
14 A. A. Maudsley, L. Muller, and R. R. Ernst, *J. Magn. Reson.*, 28 (1977) 463–470
15 A. Bax and S. K. Sarkar, *J. Magn. Reson.*, 60 (1984) 170–176
16 M. Potter and C. P. J. Glaudemans, *Methods Enzymol.*, 28 (1972) 388–393
17 S. Rudikoff, M. Potter, D. M. Segal, E. A. Padlan and D. R. Davies, *Proc. Natl. Acad. Sci. U.S.A.*, 69 (1972) 3689–3692