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SYNTHESIS OF 2,3-EPOXYPROPYL β-GLYCOSIDES OF (1→6)-β-D-GALACTOPYRANOOLIGOSACCHARIDES AND THEIR BINDING TO MONOCLONAL ANTI-GALACTAN IgA J539 AND IgA X24*

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ABSTRACT

Reaction of 2,3,4-tri-*O*-acetyl-6-*O*-(bromoacetyl)-α-D-galactopyranosyl bromide (**2**) with allyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside gave allyl *O*-[2,3,4-tri-*O*-acetyl-6-*O*-(bromoacetyl)-α- and -β-D-galactopyranosyl]-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside, **4** (4%) and **5** (88%), respectively. Selective removal of the bromoacetyl group from **5** gave allyl *O*-(2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (**6**), which, after condensation with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) yielded both allyl *O*-(2,3,4,6-tetra-*O*-acetyl-α- and -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, **7** (10%) and **8** (70%), respectively. When **6** was condensed with **2**, allyl *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)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (75%) was obtained. This was selectively *O*-de(bromoacetyl)ated to yield the nonaacetate, which was condensed with bromide **1** to give allyl *O*-(2,3,4,6-tetra-*O*-acetyl-α- and -β-D-galactopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-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, **14** (4%) and **15** (70%). Epoxidation of the allyl group of **8** and **15** with *m*-chloroperoxybenzoic acid, and removal of the acetyl protecting groups with sodium methoxide, gave, respectively, 2,3-epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**17**) and the corresponding tetrasaccharide **19**. Sequential acetylation and *O*-debenzylation of 6-*O*-benzyl-D-galactose, followed by coupling of the product with bromide **1**, yielded *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→6)-1,2,3,4-tetra-*O*-acetyl-β-D-galactopyranose (**12**). Conversion of **12** into the bromide by treatment with bromotrimethylsilane, and condensation of the product with nucleophile **6** also gave the β-linked tetrasaccharide **15** of this series. Epoxidation of the allyl group, followed by removal of acetyl blocking groups in the latter compound, again gave 2,3-

*Affinity Labels for Anti-(1→6)-β-D-galactopyranan Antibodies, Part III. For Part II, see ref. 1.
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epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**19**). Compounds **17** and **19** showed a high degree of affinity for antigalactan monoclonal antibodies IgA J539 and IgA X24.

INTRODUCTION

This paper reports the preparation of 2,3-epoxypropyl glycosides, namely 2,3-epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**17**) and the corresponding tetrasaccharide 2,3-epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**19**). In addition, there were obtained pure allyl β-glycosides of the fully acetylated tri- and tetra-galactosides having the terminal D-galactosyl group linked α, namely the per-*O*-acetyl derivatives of, respectively, allyl *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**7**) and allyl *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**14**).

For many years, we have studied a set of monoclonal antibodies (myeloma and hybridoma) which are capable of binding β-D-(1→6)-linked D-galactopyranosyl residues[2]. We have obtained considerable insight into the mode of binding of these antigens with this set of monoclonal antibodies[3]. However the *very* precise location of the antigen-binding sites and subsites has not yet been possible, because the antibody has not crystallized with ligand bound to the combining area.

Affinity labeling using 2,3-epoxypropyl derivatives of oligo-2-deoxy-2-acetamido-β-D-glucopyranosides has been reported[4]. This prompted us to prepare a series of 2,3-epoxypropyl β-(1→6)-D-galactopyranosides. These include the mono-[5] (EPGal) and di-[1] ($EPGal_2$), and the tri- ($EPGal_3$) and tetragalactoside

TABLE I

BINDING CONSTANTS OF 2,3-EPOXYPROPYL β-D-GALACTOSIDES WITH MONOCLONAL ANTI-GALACTAN IgA J539 (Fab') AND IgA X24

Compound	K_a	
	J539 (Fab')	*X24*
β-D-Gal*p*–OCH_2–CH(O)–CH_2	[a]0.52 × 10^4	[a]0.23 × 10^4
β-D-Gal*p*-(1→6)-β-D-Gal*p*–OCH_2–CH(O)–CH_2	[b]0.78 × 10^5	[b]0.68 × 10^5
17	0.58 × 10^6	0.41 × 10^6
19	0.77 × 10^6	0.50 × 10^6

[a]K_a measured for Fab' fragment; data from ref. 5. [b]Data from ref. 1.

($EPGal_4$) reported herein. Several of these potential affinity-labels may be capable of forming covalent linkages in the general antibody-combining area. If so, subsequent localization of the affected amino acid residues by sequence analysis, together with the known spatial structure of these immunoglobulins[6], may reveal a high degree of detail of the mode of binding. Here, we report the synthesis of these oligosaccharides, and also that they show a high degree of affinity (see Table I) with two representative, anti-(1→6)-β-D-galactopyranan, monoclonal antibodies, namely, IgA J539 and IgA X24. (ref. 7).

RESULTS AND DISCUSSION

We recently reported[1] the chemical synthesis of 2,3-epoxypropyl *O*-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside using derivative 3 [obtained from the corresponding 6-*O*-(bromoacetyl) derivative by selective *O*-de(bromoacetyl)-ation][1] as a key intermediate. Such compounds have many features necessary in a good intermediate, but also possess certain drawbacks, such as the capability of migration of acetyl blocking-groups during removal of the bromoacetyl group from O-6. This migration may also occur during coupling reactions, especially when using silver triflate and collidine as promoters. With this in mind, we have made attempts to modify the synthesis of higher homologs of these epoxypropyl glycosides. We tried to apply benzyl or *tert*-butyldiphenylsilyl[8] functions as temporary protecting-groups at O-6, or benzoyl or phenylbenzoyl[8], or both, as permanent protective-groups elsewhere. None of these approaches were successful, due to our inability to remove, selectively, these temporary or permanent protective-groups in the presence of the anomeric epoxypropyl group.

Thus, the synthesis of trigalactoside **17** was approached essentially along the lines previously reported[1], except for some modifications of the coupling and oxidation procedures. All coupling reactions were carried out in the presence of mercuric cyanide and mercuric bromide, in order to minimize the migration of acetyl protecting-groups. We also used a larger excess of promoters, and increased the polarity of the reaction solvent by going from benzene to dichloromethane. In that way, a reasonably good balance between high yield, limited time, and the prevention of side reactions could be achieved.

Previously described[1] allyl galactoside **3** was coupled with the known[9] bromoacetyl bromide **2**, to give the disaccharide **5**, as well as some (4%) α-linked product **4**. Debromoacetylation of **5** at room temperature with thiourea yielded the key nucleophile **6** (76% from **3**).

Condensation of bromide **1** with **6** gave the protected trisaccharide **8** (70%), as well as 10% of α-linked product **7**. Oxidation of the allyl group of **8** with *m*-chloroperoxybenzoic acid occurred satisfactorily in the presence of radical inhibitor[10] and led to the epoxypropyl trigalactoside **16**, which is diastereoisomeric at the 2-epoxypropyl position, as evidenced from n.m.r. spectra (see the Experimental section). *O*-Deacetylation of **16** with sodium methoxide in methanol yielded the

target epoxypropyl trigalactoside **17** (77% from **8**), as the same mixture of diastereoisomers at the aglycon position.

The synthesis of the epoxypropyl tetragalactoside **15** was accomplished by either a stepwise (route *a*, 43% from **6**) or a blockwise (route *b*, 68% from **6**) approach. The latter route was investigated in order to overcome possible complications, such as increasing acetyl migrations inherent in repetitive coupling-reactions. The strategy *a* is essentially the same as that just described for the synthesis of trisaccharide **17**. Condensation of **6** with bromide **2** (to give **10**), *O*-de(bromoacetyl)ation (to give **11**), and coupling with bromide **1** gave the tetrasaccharide **15**, as well as the α-linked product **14** (4%).

Condensation of **6** with the digalactosyl bromide **13** (route *b*) gave the same tetrasaccharide **15** (68%) in higher yield. However, this method requires the separate preparation of bromide **13**: Easily available 6-*O*-benzyl-D-galactose* was first acetylated with acetic anhydride and sodium acetate, debenzylated by catalytic transfer-hydrogenolysis[12], and coupled with bromide **1**, to give the digalactose derivative **12**. Treatment of **12** with bromotrimethylsilane then yielded bromide **13** (60%).

Although routes *a* and *b* both yielded identical **15**, route *b* did so in higher yield and with greater facility. It could, however, be argued that route *a* is still preferable, even though we were unable to detect in **12** any furanoside arising in the original acetylation of 6-*O*-benzyl-D-galactose.

Oxidation of the allyl group of **15** (to give **18**), followed by *O*-deacetylation, gave the target tetrasaccharide **19** in good yield (76% from **15**), again as a mixture of diastereoisomers at the 2-epoxypropyl position.

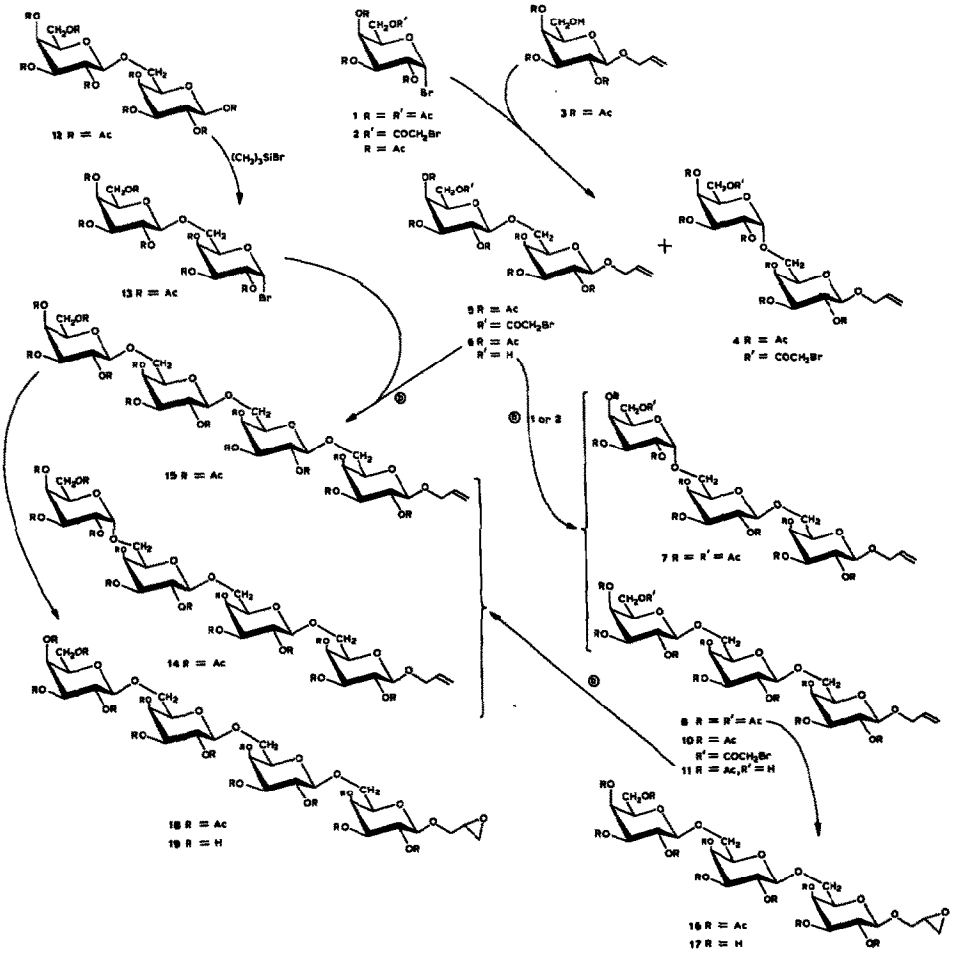
Structures of all the aforementioned compounds were assigned on the basis of ^{1}H- and ^{13}C-n.m.r.-spectral data. These were interpreted with the aid of the previously unambiguously assigned spectra[1] of similar compounds, and by comparison of signals in the series of mono-, di-, tri-, and tetra-saccharides.

The affinities of all saccharides were measured[13] with two D-galactan-specific, monoclonal immunoglobulins[7], namely, J539 (Fab') and X24. It can be seen in Table I that their affinities are somewhat higher than those of the methyl glycosides previously reported[3]. The binding appears quite sufficient to permit expectation that the 2,3-epoxypropyl di-, tri-, and tetra-saccharide ($EPGal_2$, $EPGal_3$, and $EPGal_4$) would be capable of *selectively* labeling the combining area of these proteins.

EXPERIMENTAL

Optical rotations were measured with a Perkin–Elmer 241 MC automatic polarimeter. T.l.c. was carried out on Silica Gel GHLF (Analtech), and flash

*6-*O*-Benzyl-D-galactose was obtained from 1,2,3,4-di-*O*-isopropylidene-α-D-galactose by benzylation[11] at O-6, followed by hydrolysis with 55% acetic acid.



chromatography was performed using columns of Silica Gel 60 (Merck, 230–400 or >400 mesh), with *A*, 2:1; *B*, 3:1; *C*, 4:1; *D*, 5:1; or *E*, 9:1 (v/v) carbon tetrachloride–acetone; or *F*, 1:3 chloroform–methanol as eluting solvents. N.m.r. spectra (^{1}H- and ^{13}C-) were recorded with a Varian FX-300 spectrometer for $CDCl_3$ solutions, with Me_4Si as the internal standard, or, in D_2O, with TSP (sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate) as the internal standard.

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

Allyl O-*(2,3,4-tri-*O*-acetyl-6-*O*-(bromoacetyl)-α-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**4**) *and allyl* O-*(2,3,4-tri-*O*-acetyl-6-*O*-(bromoacetyl)-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*- *galactopyranoside* (**5**). — Bromide **2** (0.882 g, 1.8 mmol) was added to a mixture of nucleophile **3** (0.416 g, 1.2 mmol), mercuric cyanide (0.253 g, 1 mmol), mercuric bromide (0.025 g), and Drierite (1 g) in benzene (5 mL) at room temperature, and the suspension was stirred overnight (t.l.c., solvent *B*). The mixture was diluted with dichloromethane and filtered. The filtrate was washed twice with an aqueous solution of potassium bromide, dried, and concentrated. Chromatography (solvent *D*) gave **4** (0.04 g, amorphous solid, 4%); $[\alpha]_D$ +60.6° (*c* 0.5, $CHCl_3$); ^{1}H-n.m.r.: δ 5.80–5.93 (m, 1 H, $OCH_2CH{=}CH_2$), 5.46 (bd, 1 H, $J_{3',4'}$ 3.0 Hz, H-4'), 5.43 (bd, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.21–5.34 (m, 4 H, H-2,3', and $OCH_2CH{=}CH_2$), 5.12 (dd, 1 H, $J_{2',3'}$ 10.7, $J_{1',2'}$ 3.4 Hz, H-2'), 5.02 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.4 Hz, H-3), 4.97 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1'), 4.54 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.35 and 4.10 (2 m, 2 × 1 H, $O{-}CH_2CH{=}CH_2$), 4.17–4.28 (m, 3 H, H-5,6',6a'), 3.85 (2 H, $COCH_2Br$), 3.81–3.90 (m, 2 H, H-5',6), 3.46 (m, 1 H, H-6a), and 1.98–2.18 (18 H, 6 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.5 (C=C), 100.2 (C-1), 96.6 (C-1'), 71.4 and 71.0 (C-3 and 5), 70.0 ($OCH_2CH{=}CH_2$), 68.8 (C-2), 67.9 (C-3'), 67.4 and 67.3 (C-2',4,4'), 66.4 (C-5'), 65.7 (C-6), 63.3 (C-6'), 25.1 (CH_2Br), and 20.5–20.7 ($COCH_3$).

Anal. Calc. for $C_{29}H_{39}BrO_{18}$: C, 46.10; H, 5.20. Found: C, 45.91; H, 5.32.

Eluted next was **5** (0.8 g, 88%); $[\alpha]_D$ −4.7° (*c* 0.9, $CHCl_3$): ^{1}H-n.m.r.: δ 5.81–5.90 (m, 1 H, $OCH_2CH{=}CH_2$), 5.39 and 5.37 (2 bd, 2 × 1 H, $J_{3,4} = J_{3',4'}$ 3.4 Hz, H-4,4'), 5.15–5.34 (m, 4 H, H-2,2', and $OCH_2CH{=}CH_2$), 5.02 and 4.98 (2 dd, 2 × 1 H, H-3,3'), 4.54 and 4.51 (2 d, 2 × 1 H, $J_{1,2} = J_{1',2'} = 7.7$ Hz, H-1,1'), 4.37 and 4.11 (2 m, 2 × 1 H, $OCH_2CH{=}CH_2$), 4.25 (m, 2 H, H-6',6a'), 3.95 (m, 1 H, H-5'), 3.84 (2 H, CH_2Br), 3.74–3.89 (m, 3 H, H-5,6,6a), and 1.98–2.16 (18 H, 6 OAc); ^{13}C-n.m.r.: δ 133.2 and 117.6 (C=C), 100.7 (C-1'), 99.9 (C-1), 72.2 (C-5), 70.5–71.0 (C-3,3',5'), 69.7 ($OCH_2CH{=}CH_2$), 68.8 and 68.5 (C-2,2'), 67.6 (C-4), 67.1 and 66.9 (C-4',6), 62.8 (C-6'), 25.1 (CH_2Br), and 20.5–20.7 ($COCH_3$).

Anal. Calc. for $C_{29}H_{39}BrO_{18}$: C, 46.10; H, 5.20. Found: C, 45.94; H, 5.25.

Allyl O-*(2,3,4-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-*D*-galactopyranoside* (**6**). — Thiourea (0.15 g, 2 mmol) in methanol (5 mL) and then 2,6-lutidine (0.12 mL, 1 mmol) were added to a solution of **5** (0.7 g, 0.9 mmol) in dichloromethane (10 mL), and the mixture was stirred at room temperature until the reaction was complete (20 min; t.l.c., solvent *A*). The mixture was filtered, and the filtrate was concentrated *in vacuo* in the presence of toluene, diluted with dichloromethane, washed with aqueous sodium chloride, dried, and concentrated. Chromatography (solvent *B*) gave **6** (0.49 g, 86%); $[\alpha]_D$ −8.8° (*c* 1.1, $CHCl_3$); ^{1}H-n.m.r.: δ 5.79–5.90 (m, 1 H, $OCH_2{-}CH{=}CH_2$), 5.41 and 5.37 (2 bd, 2 × 1 H, $J_{3,4} = J_{3',4'} = 3.3$ Hz, H-4,4'), 5.16–5.34 (m, 4 H, H-2,2', and $OCH_2CH{-}CH_2$), 5.03 and 5.00 (2 dd, 2 × 1 H, H-3,3'), 4.53 and 4.51 (2 d, 2 × 1 H, $J_{1,2} = J_{1',2'} = 7.8$ Hz, H-1,1'), 4.37 and 4.11 (2 m, 2 × 1 H, $OCH_2CH{=}CH_2$), 3.52–3.89 (m, 6 H, H-5,6,6a,5',6',6a'), and 1.98–2.18 (18 H, 6 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.6

(C=C), 101.0 (C-1), 100.1 (C-1′), 73.8 (C-5′), 72.2 (C-5), 71.1 and 70.9 (C-3, 3′), 69.9 ($OCH_2CH=CH_2$), 69.0 and 68.9 (C-2,2′), 67.9 and 67.7 (C-4,4′), 67.3 (C-6), 60.8 (C-6′), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{27}H_{38}O_{17}$: C, 51.10; H, 6.04. Found: C, 51.08; H, 6.05.

Allyl O-*(2,3,4,6-tetra*-O-*acetyl-α*-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* (**7**) *and allyl* 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)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**8**). — Bromide **1** (0.9 g, 2.2 mmol) was added to a mixture of nucleophile **6** (0.9 g, 1.4 mmol), mercuric cyanide (0.42 g, 1.5 mmol), mercuric bromide (0.04 g), and Drierite (2 g) in benzene (10 mL) at room temperature, and the suspension was stirred for 2 days (t.l.c., solvent *B*). The mixture was worked up as described for the preparation of **5**. Chromatography (solvent *C*) gave **7** (0.14 g, 10%); $[\alpha]_D$ +44.0° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 5.74–5.88 (m, 1 H, OCH_2–CH=CH_2), 5.41, 5.37, and 5.34 (3 bd, 3 H, 3 × H-4), 4.92–5.29 (m, 6 H, 3 × H-2 and 3 × H-3), 4.91 (d, 1 H, $J_{1'',2''}$ 3.3 Hz, H-1″), 4.50 and 4.47 (2 d, 2 × 1 H, $J_{1,2} = J_{1',2'}$ = 7.9 Hz, H-1,1′), 4.32 (m, 1 H, $OCH_2CH=CH_2$), 4.10–4.21 and 3.40–3.90 (2 m, 10 H, 3 × H-5, 3 × H-6, 3 × H-6a, and $OCH_2CH=CH_2$), and 1.95–2.20 (30 H, 10 OAc); ^{13}C-n.m.r.: δ 133.1 and 117.4 (C=C), 100.8 (C-1′), 99.8 (C-1), 96.6 (C-1″), 72.2 (C-5), 71.3 (C-5′), 70.9 (C-3,3′), 69.6 ($OCH_2CH=CH_2$), 68.8 and 68.6 (C-2,2′), 67.8 (C-3″), 67.1–67.6 (C-2,″,5″,6, and 3 × C-4), 66.5 (C-6′), 61.4 (C-6″), and 20.4–20.6 ($COCH_3$).

Anal. Calc. for $C_{41}H_{56}O_{26}$: C, 51.03; H, 5.85. Found: C, 50.79; H, 5.90.

Eluted next was **8** (0.95 g, 70%); $[\alpha]_D$ −18.2° (*c* 0.9, $CHCl_3$); ^{1}H-n.m.r.: δ 5.79–5.92 (m, 1 H, $OCH_2CH=CH_2$), 5.37–5.41 (m, 3 H, 3 × H-4), 5.13–5.35 (m, 5 H, 3 × H-2 and $OCH_2CH=CH_2$), 4.95–5.03 (m, 3 H, 3 × H-3), 4.48–4.54 (m, 3 H, 3 × H-1), 4.37 and 4.10 (2 m, 2 × 1 H, $OCH_2CH=CH_2$), 4.13–4.18 and 3.70–3.94 (2 m, 9 H, 3 × H-5, 3 × H-6, and 3 × H-6a), and 1.99–2.17 (30 H, 10 OAc); ^{13}C-n.m.r.: δ 133.2 and 117.6 (C=C), 100.7 and 100.6 (C-1,1′), 100.0 (C-1), 72.2 and 72.0 (C-5,5′), 70.8–71.0 (3 × C-3 and C-5″), 69.7 ($OCH_2CH=CH_2$), 68.9, 68.7 and 68.4 (3 × C-2), 67.6 and 67.3 (C-4,4′), 66.4–66.9 (C-6,6′,4″), 61.2 (C-6″), and 20.5–20.6 ($COCH_3$).

Anal. Calc. for $C_{41}H_{56}O_{26}$: C, 51.03; H, 5.85. Found: C, 50.90; H, 5.86.

Allyl 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)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**10**). — Bromide **2** (0.88 g, 1.8 mmol) was added to a mixture of nucleophile **6** (0.76 g, 1.2 mmol), mercuric cyanide (0.25 g, 1 mmol), mercuric bromide (0.03 g), and Drierite (2 g) in 3:1 benzene–dichloromethane (8 mL) at room temperature, and the suspension was stirred for 2 days (t.l.c., solvent *B*). The mixture was worked up as described for the preparation of **5**. Chromatography (solvent *D*) gave two fractions. Eluted first (0.15 g, 12%) was a mixture of α and β isomer in the ratio α:β ~3:2 (^{1}H-n.m.r.). Eluted next was **10** (0.94 g, 75%); $[\alpha]_D$ −13.5° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 5.79–5.92 (m, 1 H, $OCH_2CH=CH_2$), 5.35–5.42 (m, 3 H, 3 × H-4), 5.13–5.34 (m, 5 H, 3 × H-2 and $OCH_2CH=CH_2$), 4.95–5.03

(m, 3 H, 3 × H-3), 4.54–4.89 (m, 3 H, H-1,1′,1″), 4.37 and 4.11 (2 m, 2 × 1 H, $OCH_2CH=CH_2$), 4.23 (m, 2 H, H-6″,6a″), 3.96 (m, 1 H, H-5″), 3.85 (2 H, CH_2Br), 3.70–3.89 (m, 6 H, H-5,6,6a,5′,6′,6a′), and 1.97–2.19 (27 H, 9 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.6 (C=C), 100.7 and 100.6 (C-1,1′), 100.0 (C-1″), 72.2 and 72.1 (C-5,5′), 70.7–71.1 (3 × C-3 and C-5″), 69.8 ($OCH_2CH=CH_2$), 68.9, 68.7, and 68.4 (3 × C-2), 67.6 and 67.4 (C-4,4′), 67.0, 66.9, and 66.6 (C-6,6′,4″), 63.0 (C-6″), 25.2 (CH_2Br), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{41}H_{55}BrO_{26}$: C, 47.18; H, 5.31. Found: C, 47.12; H, 5.37.

Allyl O-(2,3,4-tri-O-acetyl-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 (**11**). — A solution of thiourea (0.114 g, 1.5 mmol) in methanol (2 mL), followed by 2,6-lutidine (0.06 mL, 0.5 mmol) were added to a solution of **10** (0.52 g, 0.5 mmol) in dichloromethane (5 mL), and the mixture was stirred at room temperature for ~0.5 h (t.l.c., solvent *A*). The mixture was worked up as described for the preparation of **6**. Chromatography (solvent *B*) gave **11** (0.38 g, 82%); $[\alpha]_D$ −14.4° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 5.80–5.88 (m, 1 H, $OCH_2CH=CH_2$), 5.35–5.42 (m, 3 H, 3 × H-4), 5.13–5.34 (m, 5 H, 3 × H-2 and $OCH_2CH=CH_2$), 4.95–5.04 (m, 3 H, 3 × H-3), 4.46–4.52 (m, 3 H, 3 × H-1), 4.37 and 4.11 (2 m, 2 × 1 H, $OCH_2CH=CH_2$), 3.50–3.88 (m, 9 H, 3 × H-5, 3 × H-6, and 3 × H-6a), and 1.95–2.20 (27 H, 9 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.6 (C=C), 100.8 (C-1,1′), 100.0 (C-1″), 73.9 (C-5″), 72.3 and 71.9 (C-5,5′), 70.9–71.1 (3 × C-3), 69.8 ($OCH_2CH=CH_2$), 68.8–68.9 (3 × C-2), 66.6–67.9 (3 × C-4, and C-6,6′), 60.8 (C-6″), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{39}H_{54}O_{25}$: C, 50.76; H, 5.90. Found: C, 50.49; H, 5.96.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-1,2,3,4-tetra-O-acetyl-β-D-galactopyranose (**12**). — Crystalline 6-*O*-benzyl-D-galactose (m.p. 92–93°; 1.35 g, 5 mmol) was added in portions to a stirred suspension of fused sodium acetate (2 g) in acetic anhydride (10 mL) at room temperature. After being stirred for 3 h (t.l.c., solvent *E*) at ~100°, the mixture was processed conventionally and evaporated to dryness. The crude product was dissolved in methanol (50 mL) containing 10% of formic acid, and this solution was added to a stirred suspension of 10% palladium-on-charcoal (4 g, Aldrich) in the same solvent mixture (150 mL). After 1 h at room temperature (t.l.c., solvent *D*), the catalyst was removed by filtration and washed with methanol. The filtrates were combined, and co-evaporated with toluene to dryness. The crude product was dissolved in benzene (10 mL), and mercuric cyanide (0.76 g, 3 mmol), mercuric bromide (0.08 g), and Drierite (4 g), followed by tetra-*O*-acetyl-α-D-galactosyl bromide **1**; (2.47 g, 6 mmol) were added to this solution at room temperature. After being stirred for 1 day (t.l.c., solvent *B*), the mixture was worked up as described for the preparation of **5**. Chromatography (solvent *C*) gave **12** (1.98 g, 58%); $[\alpha]_D$ −1.5° (*c* 0.9, $CHCl_3$); ^{1}H-n.m.r.: δ 5.69 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.41 (bd, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.37 (bd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 5.32 (dd, 1 H, $J_{1,2}$ 8.3, $J_{2,3}$ 10.1 Hz, H-2), 5.15 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.1 Hz, H-2′), 5.07 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.5 Hz, H-3), 4.97 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.5 Hz, H-3′), 4.52 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.13 and 3.78

(2 m, 2 × 2 H, H-6,6a,6′,6a′), 4.01 and 3.89 (2 bt, 2 × 1 H, H-5,5′), and 1.97–2.23 (24 H, 8 OAc); ^{13}C-n.m.r.: δ 100.3 (C-1′), 92.2 (C-1), 72.9 (C-5), 70.8–70.9 (C-3,3′,5′), 68.3 and 67.9 (C-2,2′), 67.1 and 67.0 (C-4,4′), 65.6 (C-6), 61.3 (C-6′), and 20.5–20.7 ($COCH_3$).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.67; H, 5.69.

O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-α-D-galactopyranosyl bromide (**13**). — Bromotrimethylsilane (1.2 mL, 9.0 mmol) was added dropwise to a solution of **12** (0.34 g, 0.5 mmol) in chloroform (15 mL) at −10°. The mixture was allowed to warm to +10°, and was kept for 3 h (t.l.c., solvent *A*). Co-evaporation with toluene, and purification of the residue by chromatography on a short column of silica gel (solvent *C*) gave **13** (0.21 g, 60%); ^{1}H-n.m.r.: δ 6.66 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 5.31–5.46 (m, 3 H, 2 × H-4 and one H-3), 5.13 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{1',2'}$ 7.8 Hz, H-2′), 4.93–5.01 (m, 2 H, H-2, and H-3 or 3′), 4.48 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.41 and 3.87 (2 m, 2 × 1 H, 2 × H-5), 4.10 and 3.74 (2 m, 2 × 2 H, 2 × H-6 and 2 × H-6a), and 1.96–2.14 (21 H, 7 OAc); ^{13}C-n.m.r.: δ 100.9 (C-1′), 88.3 (C-1), 72.4 (C-5), 70.8 and 70.7 (C-3′,5′), 66.9–68.4 (C-3, 2 × C-2, and 2 × C-4), 66.1 (C-6), 61.2 (C-6′), and 20.6–20.7 ($COCH_3$).

Allyl O-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-(1→6)-O-(2,3,4-tri-O-acetyl-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 (**14**) *and allyl 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)-2,3,4-tri-O-acetyl-β-D-galactopyranoside* (**15**). — (*a*) Bromide **1** (0.124 g, 0.3 mmol) was added to a mixture of nucleophile **11** (0.185 g, 0.2 mmol), mercuric cyanide (0.045 g, 0.18 mmol), mercuric bromide (0.005 g), and Drierite (0.3 g) in 1:1 benzene–dichloromethane (2 mL). The suspension was stirred for 27 h (t.l.c., solvent *A*) at room temperature, and worked up as described for the preparation of **5**. Chromatography (solvent *C*) gave **14** (0.01 g, 4%); $[\alpha]_D$ +26.4° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 5.80–5.88 (m, 1 H, $OCH_2CH{=}CH_2$), 5.35–5.47 (4 bd, 4 H, 4 × H-4), 5.26 (m, 1 H, $OCH_2CH{=}CH_2$), 4.94–5.28 (m, 10 H, $OCH_2CH{=}CH_2$, 4 × H-2, 4 × H-3, and H-1‴), 4.46–4.53 (3 d, $J_{1,2} = J_{1'',2''} = J_{1''',2'''}$ = 8.1 Hz, H-1,1′,1″), 4.37 (m, 1 H, $OCH_2CH{=}CH_2$), 4.04–4.22 (m, 4 H, H-5″,6‴,6a‴, and $OCH_2CH{=}CH_2$), 3.65–3.87 (m, 8 H, H-5,5′,5‴ and H-6,6′,6a′,6″,6a″), 3.44 (m, 1 H, H-6a), and 1.95–2.20 (39 H, 13 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.6 (C=C), 100.9 and 100.7 (C-1′,1″), 100.0 (C-1), 96.8 (C-1‴), 72.2 (C-5,5′), 71.5 (C-5″), 71.1 and 71.0 (C-3,3′,3″), 69.8 ($OCH_2CH{=}CH_2$), 68.6–68.9 (C-2,2′,2″), 67.9 (C-3‴), 67.2–67.6 (C-2‴ and 4 × C-4), 66.7–66.8 (C-6,6′,5‴), 65.6 (C-6″), 61.5 (C-6‴), and 20.6–20.8 ($COCH_3$).

Anal. Calc. for $C_{53}H_{72}O_{34}$: C, 50.80; H, 5.79. Found: C, 50.81; H, 5.83.

Eluted next was **15** (0.175 g, 70%); $[\alpha]_D$ −22.0° (*c* 0.4, $CHCl_3$); ^{1}H-n.m.r.: δ 5.79–5.93 (m, 1 H, $OCH_2CH{=}CH_2$), 5.33–5.39 (m, 4 H, 4 × H-4), 5.12–4.94 (m, 10 H, 4 × H-2, 4 × H-3, and $OCH_2CH{=}CH_2$), 4.45–4.54 (4 d, 4 H, 4 × H-1), 4.37 and 4.09 (2 m, 2 × 1 H, $OCH_2CH{=}CH_2$), 4.15–4.21 (m,2 H, H-6‴,6a‴), 3.69–3.94 (m, 10 H, 4 × H-5, and 6,6a,6′,6a′,6″,6a″), and 1.97–2.19 (39 H, 13 OAc); ^{13}C-n.m.r.: δ 133.3 and 117.5 (C=C), 100.5–100.6 (C-1,1′,1″), 100.0 (C-1‴), 72.2, 72.0,

and 71.8 (C-5,5′,5″), 70.7–71.0 (4 × C-3 and C-5‴), 69.7 ($OCH_2CH=CH_2$), 68.5–68.8 (4 × C-2), 67.3–67.6 (C-4,4′,4″), 66.2–67.0 (C-6,6′,6″,4‴), 61.2 (C-6‴), and 20.5–20.7 ($COCH_3$).

Anal. Calc. for $C_{53}H_{72}O_{34}$: C, 50.80; H, 5.79. Found: C, 50.67; H, 5.83.

(*b*) Bromide **13** (0.11 g, 0.16 mmol) was added to a suspension of nucleophile **6** (0.085 g, 0.13 mmol), mercuric cyanide (0.023 g, 0.09 mmol), mercuric bromide (0.003 g), and Drierite (0.15 g) in dichloromethane (1.5 mL) at room temperature. After monitoring for 1 day (t.l.c., solvent *A*), the mixture was worked up as described for the preparation of **5**. Chromatography (solvent *C*) gave **15** (0.111 g, 68%).

2,3-Epoxypropyl 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)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**16**). — *m*-Chloroperoxybenzoic acid (0.192 g, 1 mmol, 90% pure) was added to a stirred solution of **8** (0.65 g, 0.67 mmol) and 4,4′-thiobis(6-*tert*-butyl-3-methylphenol) (0.016 g) in dichloromethane (5 mL), and the mixture was heated under reflux overnight in an oil bath at 48° (t.l.c., solvent *A*). After being cooled, the mixture was diluted with dichloromethane, washed with dilute solutions of sodium hydrogensulfite and sodium hydrogencarbonate (twice), dried, and concentrated. Chromatography (solvent *B*) gave **16** as an amorphous solid (0.6 g, 91%); $[\alpha]_D$ −20.7° (*c* 0.6, $CHCl_3$); ^{1}H-n.m.r.: δ 5.36–5.39 (m, 3 H, 3 × H-4), 5.13–5.26 and 4.95–5.04 (2 m, 6 H, 3 × H-2 and 3 × H-3), 4.48–4.62 (6 d, 3 H, 3 × H-1 of two isomers), 4.13–4.22 (m, 2 H, H-6″,6a″), 3.80–3.94 (m, 7 H, 3 × H-5, H-6,6a,6′,6a′), 4.10, 3.73, and 3.51 (3 m, 2 Hγ of two isomers), 3.16 (m, 1 H, Hβ), 2.81, 2.70, and 2.59 (3 m, 2 Hα of two isomers), and 1.98–2.20 (30 H, 10 OAc); ^{13}C-n.m.r.: δ 101.4 and 101.0 (C-1 of two isomers), 100.7 (C-1′,1″), 72.4 (C-5), 72.0 (C-5′), 66.5–71.0 (3 × C-2, 3 × C-3, 3 × C-4, C-6,6′,5″), 66.64 and 66.58 (Cα of two isomers), 61.2 (C-6″), 50.6 and 50.3 (Cβ of two isomers), 44.1 and 43.9 (Cγ of two isomers), and 20.6–20.7 ($COCH_3$).

Anal. Calc. for $C_{41}H_{56}O_{27}$: C, 50.20; H, 5.76. Found: C, 49.88; H, 5.67.

2,3-Epoxypropyl 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)*-O-*(2,3,4-tri*-O-*acetyl-β*-D-*galactopyranosyl-(1→6)-2,3,4-tri*-O-*acetyl-β*-D-*galactopyranoside* (**18**). — *m*-Chloroperoxybenzoic acid (0.035 g, 0.18 mmol; 90% pure) was added to a stirred solution of **15** (0.15 g, 0.12 mmol) and 4,4′-thiobis(6-*tert*-butyl-3-methylphenol) (0.003 g) in dichloromethane (1.5 mL). The mixture was heated overnight under reflux (t.l.c., solvent *A*), and then processed as described for the preparation of **16**. Chromatography (solvent *B*) gave **18** (0.137 g, 90%); $[\alpha]_D$ −20.2° (*c* 0.9, $CHCl_3$); ^{1}H-n.m.r.: δ 5.36–5.38 (m, 4 H, 4 × H-4), 5.11–5.24 and 4.95–5.04 (2 m, 8 H, 4 × H-2 and 4 × H-3), 4.47–4.62 (m, 4 H, 4 × H-1), 4.12–4.20 (m, 2 H, H-6‴,6a‴), 3.75–3.94 (m, 10 H, 4 × H-5, 3 × H-6, and 3 × H-6a), 4.10, 3.71, and 3.51 (3 m, 2 Hγ of two isomers), 3.16 (m, 1 H, Hβ), 2.81, 2.69, and 2.59 (3 m, 2 Hα of two isomers), and 1.96–2.18 (13 H, 9 OAc); ^{13}C-n.m.r.: δ 101.4 and 101.0 (C-1 of two isomers), 100.6 and 100.7 (C-1′,1″,1‴), 72.4 (C-5), 71.9 and 72.0 (C-5′,C-5″), 66.3–71.0 (4 × C-2,

4 × C-3, 4 × C-4, C-6,6′,6″,5‴ and Cα), 61.2 (C-6‴), 50.6 and 50.2 (Cβ of two isomers), 44.0 and 43.9 (Cγ of two isomers), and 20.5–20.6 ($COCH_3$).

Anal. Calc. for $C_{53}H_{72}O_{35}$: C, 50.16; H, 5.72. Found: C, 49.96; H, 5.75.

2,3-Epoxypropyl O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**17**). — Methanolic sodium methoxide (M) was added dropwise to a stirred solution of **16** (0.15 g, 0.15 mmol) in methanol (5 mL) until t.l.c. (solvent *F*) revealed a change in the substrate (pH >8.5). The mixture was stirred for an additional 1 h at room temperature, and the precipitate was removed by filtration, and recrystallized from methanol–water, to give **17** (0.042 g, 49%). The filtrates were combined and the base neutralized (Amberlite ICR-50 C.P., Mallinckrodt), and, after filtration and evaporation to dryness, gave additional **17** (0.029 g; total yield 84%); $[\alpha]_D$ −17.4° (*c* 0.4, H_2O); ^{1}H-n.m.r. (D_2O): δ 3.46–3.55 (m, 3 H, H-1,1′,1″), 4.32 (m, part of Hα of the less abundant isomer), 4.17 (m, part of Hα of the more abundant isomer), 3.47 (m, 1 H, Hβ), and 3.01–3.05 and 2.86–2.92 (2 m, 2 H, 2 Hγ of two isomers); ^{13}C-n.m.r. (D_2O): δ 105.6–105.8 (3 × C-1), 77.8 (C-5″), 76.5 (C-5,5′), 75.5 (C-3″), 75.3 (C-3,3′), 73.4 (3 × C-2), 73.2 and 73.0 (Cα of two isomers), 71.8 (C-6,6′), 71.4 (3 × C-4), 63.7 (C-6″), 54.4 and 54.2 (Cβ of two isomers), and 47.8 and 47.7 (Cγ of two isomers).

Anal. Calc. for $C_{21}H_{36}O_{17} \cdot H_2O$: C, 43.60; H, 6.62. Found: C, 43.48; H, 6.70.

2,3-Epoxypropyl O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-O-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**19**). — Methanolic sodium methoxide (M) was added dropwise to a stirred solution of **18** (0.08 g, 0.06 mmol) in methanol (2 mL) until t.l.c. (solvent *F*) revealed a change in the substrate (pH >8.5). The mixture was now stirred for 40 min at room temperature, and (following the procedure described for the preparation of **17**) yielded **19** (0.035 g, 81%); $[\alpha]_D$ −11.6° (*c* 0.4, H_2O); ^{1}H-n.m.r. (D_2O): δ 4.48–4.57 (m, 4 H, H-1,1′,1″,1‴), 4.33 (m, part of Hα of the less abundant isomer), 4.17 (m, part of Hα of the more abundant isomer), 3.47 (m, 1 H, Hβ), and 3.01–3.05 and 2.86–2.92 (2 m, 2 H, 2 Hγ of two isomers); ^{13}C-n.m.r. (D_2O): δ 105.6–105.8 (4 × C-1), 77.9 (C-5‴), 76.6 (C-5,5′,5″), 75.6 (C-3‴), 75.4 (C-3,3′,3″), 73.5 (4 × C-2), 73.3 and 73.1 (Cα of two isomers), 71.8 (C-6,6′,6″), 71.5 (4 × C-4), 63.7 (C-6‴), 54.4 and 54.3 (Cβ of two isomers), and 47.9 and 47.8 (Cγ of two isomers).

Anal. Calc. for $C_{27}H_{46}O_{22} \cdot 2\ H_2O$: C, 42.74; H, 6.64. Found: C, 42.94; H, 6.56.

Binding studies. — Monoclonal antibodies were purified by affinity chromatography[7]. The preparation of the Fab′ fragment of IgA J539 has been described[5]. Binding constants were determined on the antibody solutions (714nM) in phosphate-buffered saline at pH 7.4 by measuring the ligand-induced concentration[13]. The binding constants reported here are corrected for bound ligand.

REFERENCES

1 E. FALENT-KWAST, P. KOVÁČ, A. BAX, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 145 (1986) 332–340.

2 C. P. J. GLAUDEMANS, P. KOVÁČ, AND K. RASMUSSEN, *Biochemistry,* 23 (1984) 6732–6736, and references cited therein.
3 C. P. J. GLAUDEMANS, *Mol. Immun.*, in press, and references cited therein.
4 Y. ESHDAT, J. F. MCKELVY, AND N. SHARON, *J. Biol. Chem.*, 248 (1973) 5892–5898.
5 M. E. JOLLEY, C. P. J. GLAUDEMANS, S. RUDIKOFF, AND M. POTTER, *Biochemistry,* 13 (1974) 3179–3184.
6 S. W. SUH, T. N. BHAT, M. A. NAVIA, G. H. COHEN, D. N. RAO, S. RUDIKOFF, AND D. R. DAVIES, *Proteins: Struct., Funct., and Genetics,* 1 (1986) 74–80.
7 M. POTTER AND C. P. J. GLAUDEMANS, *Methods Enzymol.*, 47 (1972) 388–394.
8 E. M. NASHED AND C. P. J. GLAUDEMANS, in preparation.
9 P. KOVÁČS AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 140 (1985) 313–318.
10 Y. KISHI, M. ARATANI, H. TANINO, T. FUKUYAMA, AND T. GOTO, *J. Chem. Soc., Chem. Commun.*, (1972) 64–65.
11 C. E. BALLOU AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 76 (1954) 3188–3193.
12 V. S. RAO AND A. S. PERLIN, *Carbohydr. Res.*, 83 (1980) 175–177.
13 M. E. JOLLEY AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 33 (1974) 377–382.