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Synthesis of Structural Elements of the Capsular Polysaccharide of *Streptococcus pneumoniae* Type 8

Franciscus A. W. Koeman, Johannis P. Kamerling*, and Johannes F. G. Vliegenthart

Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University,
P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands

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Abstract: The synthesis is reported of propyl 4-*O*-α-D-galactopyranosyl-β-D-glucopyranosiduronic acid (**25**), 4-*O*-[4-*O*-(β-D-glucopyranosyluronic acid)-β-D-glucopyranosyl]-D-glucopyranose (**34**), and 4-*O*-(4-*O*-β-D-glucopyranosyl-α-D-glucopyranosyl)-D-galactopyranose (**38**), each representing a structural element of the repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* type 8, [→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→]$_n$. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate (**12**) was coupled to allyl 2-*O*-acetyl-3-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside (**7**) in dichloromethane-ether, using trimethylsilyl trifluoromethanesulfonate as a promoter, to give disaccharide derivative **20**. Detritylation of **20**, followed by oxidation and deprotection, afforded disaccharide propyl glycoside **25**. Coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**8**) to allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**17**) in dichloromethane, with trimethylsilyl trifluoromethanesulfonate as a promoter, resulted in trisaccharide derivative **26**. Deacetylation of **26**, followed by 6-*O*-tritylation, benzylation, detritylation, and oxidation gave a protected trisaccharide derivative (**31**), which, after deprotection, afforded **34**. Coupling of allyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**10**) to 4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-D-glucopyranosyl trichloroacetimidate (**19**) in ether, with trimethylsilyl trifluoromethanesulfonate as a promoter, gave trisaccharide derivative **35**. Deallylation of **35**, followed by hydrogenolysis afforded **38**.

INTRODUCTION

The current polysaccharide vaccine Pneumovax® 23 against pneumococcal diseases such as pneumonia, otitis media, and meningitis, contains a mixture of the purified capsular polysaccharides of 23 serotypes[1] of *Streptococcus pneumoniae*. This selection of 23 serotypes, of the 85 different serotypes known today, covers 90% of all pneumococcal infections. In view of the immunological problems[2] related to this vaccine, attention is paid to the preparation of better alternatives based on oligosaccharide-conjugates. One of the constituents of the current vaccine is the capsular polysaccharide of serotype 8, of which the structure has been characterised[3] as:

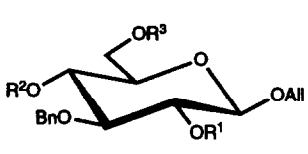
[→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*-(1→]$_n$ **(1)**

Here we report on the synthesis of three structural elements of the capsular polysaccharide of serotype 8, namely, propyl 4-*O*-α-D-galactopyranosyl-β-D-glucopyranosiduronic acid (**25**), 4-*O*-[4-*O*-(β-D-glucopyranosyluronic acid)-β-D-glucopyranosyl]-D-glucopyranose (**34**), and 4-*O*-(4-*O*-β-D-glucopyranosyl-α-D-glucopyranosyl)-D-galactopyranose (**38**). During the preparation of this manuscript the synthesis of two protected fragments of the capsular polysaccharide[4] was reported.

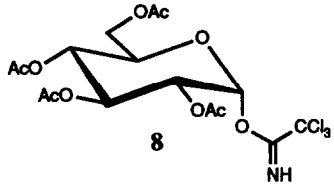
RESULTS AND DISCUSSION

The tetrasaccharide repeating unit of the capsular polysaccharide of *S. pneumoniae* serotype 8 can be divided into a glucuronic acid, a galactose, and a cellobiose element. For the synthesis of the aimed oligosaccharides **25**, **34**, and **38**, which form overlapping fragments of the tetrasaccharide repeating unit, a series of suitably protected synthons was prepared. For the glucuronic acid element allyl 2-*O*-acetyl-3-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside (**7**) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**8**) were synthesised, for the galactose element allyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**10**) and 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl trichloroacetimidate (**12**), and for the cellobiose part allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**17**) and 4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-D-glucopyranosyl trichloroacetimidate (**19**).

Complete deacetylation of allyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[5] (**2**) at strong alkaline conditions (pH 11-12) (→**3**), followed by isopropylidenation with 2,2-dimethoxypropane (→**4**, 83% from **2**), acetylation (→**5**), and de-isopropylidenation gave **6** (84% from **4**). Then **6** was tritylated with trityl chloride in pyridine to afford 'glucuronic acid acceptor' **7** (78%). 'Glucuronic acid donor' **8** was synthesised by imidation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose[6] using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene[7] as a base in dichloromethane in a yield of 76%.

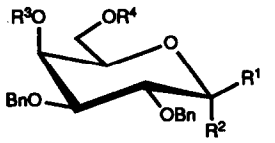


2 $R^1 = R^2 = R^3 = Ac$
3 $R^1 = R^2 = R^3 = H$
4 $R^1 = H$; $R^2, R^3 = C(CH_3)_2$
5 $R^1 = Ac$; $R^2, R^3 = C(CH_3)_2$
6 $R^1 = Ac$; $R^2 = R^3 = H$
7 $R^1 = Ac$; $R^2 = H$; $R^3 = Tr$



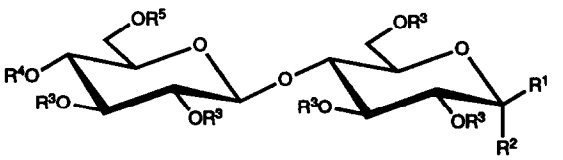
8

Both galactose synthons **10** and **12** were prepared from allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[8] (**9**), using the following sequence of reactions. Regioselective reductive ring opening of **9** with sodium cyanoborohydride and hydrogen chloride[9] afforded 'galactose acceptor' **10** (60%). The 'galactose donor' **12** was prepared by deallylation of **9** via isomerisation using potassium *tert*-butoxide and depropenylation with mercuric oxide and mercuric chloride[10] (→**11**, 74%), and subsequent imidation using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (→**12**, 96%).



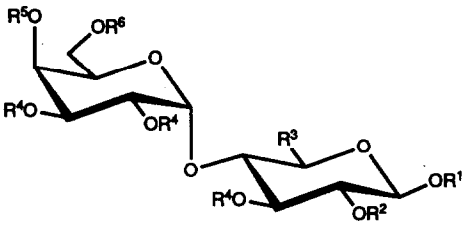
9 R^1 = OAll; R^2 = H; R^3,R^4 = CHPh
10 R^1 = OAll; R^2 = R^3 = H; R^4 = Bn
11 R^1,R^2 = H,OH; R^3,R^4 = CHPh
12 R^1 = H; R^2 = $OCNHCCl_3$; R^4,R^5 = CHPh

For the synthesis of the cellobiose synthons **17** and **19**, the same approach was followed as for the galactose synthons. Thus, allyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[11] (**13**) was deacetylated (→**14**, quantitative), 4,6-*O*-benzylidenated with benzaldehyde dimethyl acetal in *N*,*N*-dimethylformamide (→**15**), and benzylated to give **16** (77% from **14**). Regioselective reductive ring opening of **16** with sodium cyanoborohydride and hydrogen chloride afforded 'cellobiose acceptor' **17** (78%). Deallylation of **16** (→**18**, 93%), and subsequent imidation, gave 'cellobiose donor' **19** (79%).



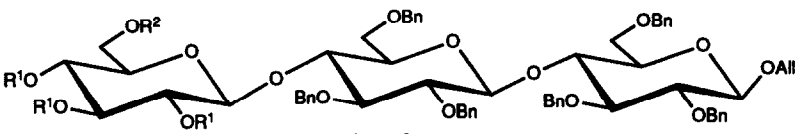
13 R^1 = OAll; R^2 = H; R^3 = R^4 = R^5 = Ac
14 R^1 = OAll; R^2 = R^3 = R^4 = R^5 = H
15 R^1 = OAll; R^2 = R^3 = H; R^4,R^5 = CHPh
16 R^1 = OAll; R^2 = H; R^3 = Bn; R^4,R^5 = CHPh
17 R^1 = OAll; R^2 = R^4 = H; R^3 = R^5 = Bn
18 R^1,R^2 = H,OH; R^3 = Bn; R^4,R^5 = CHPh
19 R^1,R^2 = H,$OCNHCCl_3$; R^3 = Bn; R^4,R^5 = CHPh

Coupling of 'galactose donor' **12** to 'glucuronic acid acceptor' **7** in dichloromethane-ether at -70°, using trimethylsilyl trifluoromethanesulfonate as a promoter, gave allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-6-*O*-trityl-β-D-glucopyranoside (**20**, 60%). After detritylation of **20** with perchloric acid[12] (→**21**, 76%), and oxidation with the Jones-reagent[13] (→**22**), a methylation was carried out with diazomethane for separation purposes (→**23**, 70%). Because complete deacetylation of **23** with lithium hydroxide in water failed, a two-step saponification was used. To this end **23** was treated with sodium methoxide in methanol, to give a deacetylated product, and then with lithium hydroxide in water-acetone to remove the methyl ester group (→**24**, 90%). Finally, hydrogenolysis of **24**, leading to the removal of the benzyl groups and the benzylidene group, as well as to the conversion of the allyl group into a propyl group, afforded propyl 4-*O*-α-D-galactopyranosyl-β-D-glucopyranosiduronic acid (**25**, 73%).

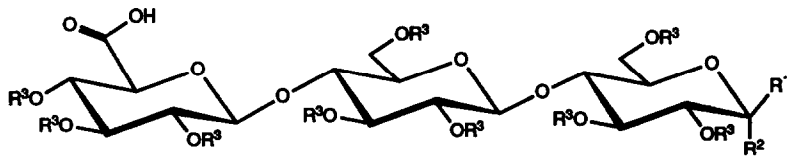


20 R^1 = All; R^2 = Ac; R^3 = CH_2OTr; R^4 = Bn; R^5,R^6 = CHPh
21 R^1 = All; R^2 = Ac; R^3 = CH_2OH; R^4 = Bn; R^5,R^6 = CHPh
22 R^1 = All; R^2 = Ac; R^3 = COOH; R^4 = Bn; R^5,R^6 = CHPh
23 R^1 = All; R^2 = Ac; R^3 = COOMe; R^4 = Bn; R^5,R^6 = CHPh
24 R^1 = All; R^2 = H; R^3 = COOH; R^4 = Bn; R^5,R^6 = CHPh
25 R^1 = Propyl; R^2 = R^4 = R^5 = R^6 = H; R^3 = COOH

Coupling of the 'glucuronic acid donor' **8** to 'cellobiose acceptor' **17** in dichloromethane at -30°, with trimethylsilyl trifluoromethanesulfonate as a promoter, gave allyl 4-*O*-[4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl]-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**26**, 74%). Deacetylation of **26** (→**27**), followed by tritylation (→**28**), benzylation (→**29**, 20% from **26**), and detritylation with perchloric acid[12] gave **30** (80%). Then oxidation with the Jones-reagent[13] (→**31**, 75%), followed by deallylation[7] with palladium (II) chloride and sodium acetate in acetic acid (→**33**, 51%), and debenzylation yielded 4-*O*-[4-*O*-(β-D-glucopyranosyluronic acid)-β-D-glucopyranosyl]-D-glucopyranose (**34**, 90%). It has to be noted that during the deallylation step, **31** is partially converted into **32**, having a saturated allyl (propyl) group.

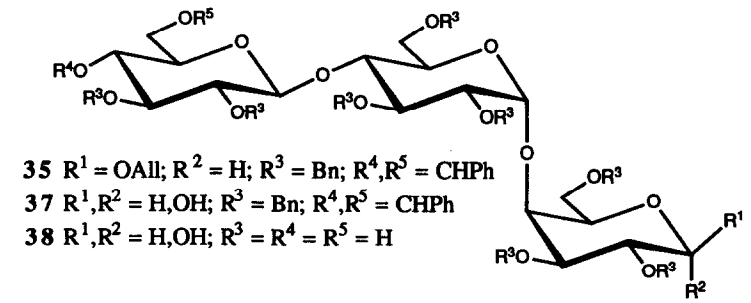


26 R^1 = R^2 = Ac
27 R^1 = R^2 = H
28 R^1 = H; R^2 = Tr
29 R^1 = Bn; R^2 = Tr
30 R^1 = Bn; R^2 = H

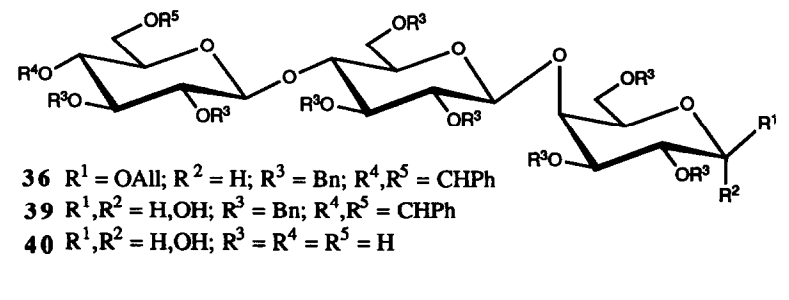


31 R^1 = OAll; R^2 = H; R^3 = Bn
32 R^1 = OPropyl; R^2 = H; R^3 = Bn
33 R^1,R^2 = H,OH; R^3 = Bn
34 R^1,R^2 = H,OH; R^3 = H

Coupling of 'cellobiose donor' **19** and 'galactose acceptor' **10** in ether at -70°, using trimethylsilyl trifluoromethanesulfonate as a promoter, gave allyl 4-*O*-[4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl]-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**35**, 55%), and its β-coupling product (**36**, 15%), which could be separated by column chromatography. Deallylation of **35** with potassium *tert*-butoxide, followed by mercuric oxide and mercuric chloride[10] (→**37**, 71%) and hydrogenation yielded 4-*O*-(4-*O*-β-D-glucopyranosyl-α-D-glucopyranosyl)-D-galactopyranose (**38**, 80%). In a similar way also the β-coupling product was deallylated (→**39**, 62%) and debenzylated/debenzylidenated (→**40**, 77%).



35 R^1 = OAll; R^2 = H; R^3 = Bn; R^4,R^5 = CHPh
37 R^1,R^2 = H,OH; R^3 = Bn; R^4,R^5 = CHPh
38 R^1,R^2 = H,OH; R^3 = R^4 = R^5 = H



36 R^1 = OAll; R^2 = H; R^3 = Bn; R^4,R^5 = CHPh
39 R^1,R^2 = H,OH; R^3 = Bn; R^4,R^5 = CHPh
40 R^1,R^2 = H,OH; R^3 = R^4 = R^5 = H

EXPERIMENTAL

General methods.— ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker AC 300, at 360 MHz with a Bruker HX 360, and at 500 MHz with a Bruker AM 500 apparatus at 25°. Two-dimensional double-quantum filtered ^{1}H-^{1}H correlation spectra (2D DQF ^{1}H-^{1}H COSY) were recorded in the phase-sensitive mode[14], and two-dimensional homonuclear Hartmann-Hahn spectra (2D HOHAHA) with a 120 ms MLEV-17 mixing sequence[15]. ^{13}C-NMR spectra (APT, 50 MHz) were recorded at 25° with a Bruker WP 200 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) or internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^{1}H, and to the signal for internal Me_4Si ($CDCl_3$; indirectly to $CDCl_3$, δ 76.9) or external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C.

Column chromatography was performed on Kieselgel 60 (Merck, <230 mesh) and fractions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by examination under UV light and by charring with aq 50 % sulfuric acid. Optical rotations were measured at 20° with a Perkin-Elmer 241 polarimeter, using a 10-cm 1-mL cell. In working-up procedures, washings were carried out three times with appropriate quantities of water or aq 10% sodium hydrogencarbonate unless indicated otherwise. The Jones reagent, used in oxidation reactions, consisted of a mixture of chromium (VI) oxide (76.7 g), conc. H_2SO_4 (28.5 mL), and water (111.5 mL). Evaporations were conducted under reduced pressure at 40°. All solvents were distilled from appropriate drying agents.

Allyl 3-O-benzyl-4,6-O-isopropylidene-β-D-glucopyranoside (**4**).— To a solution of allyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-β-D-glucopyranoside[5] (**2**, 8.5 g, 19.5 mmol) in CH_2Cl_2 (25 mL) and MeOH (60 mL) was added sodium methoxide (pH 11-12). After stirring overnight the solution was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated (→**3**, quantitative). To a solution of the residue in acetone (30 mL) were added 2,2-dimethoxypropane (70 mL) and a catalytic amount of *p*-toluenesulfonic acid, and the mixture was stirred for 3 h to give a complete reaction (TLC 9:1 CH_2Cl_2-acetone; **4** R_F 0.37). The mixture was neutralised with solid sodium hydrogencarbonate, filtered, and concentrated. A solution of the residue in CH_2Cl_2 (50 mL) was washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2-acetone) of the residue gave **4**, isolated as a white solid (5.7 g, 83%), $[\alpha]_D$ -17° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 133.5 ($OCH_2CH=CH_2$), 128.3-127.6 (Ph), 118.1 ($OCH_2CH=CH_2$), 102.2 (C-1), 99.3 ($C(CH_3)_2$), 80.7, 74.0 (2 C), and 67.2 (C-2,3,4,5), 74.2 (OCH_2Ph), 70.4 ($OCH_2CH=CH_2$), 61.1 (C-6), 29.1 and 19.0 ($C(CH_3)_2$); ^{1}H, δ 7.37-7.28 (m, 5 H, Ph), 5.930 (m, 1 H, $OCH_2CH=CH_2$), 5.34-5.20 (m, 2 H, $OCH_2CH=CH_2$), 4.896 and 4.759 (2 d, each 1 H, OCH_2Ph), 4.403 (d, 1 H, H-1), 4.355 and 4.134 (2 m, each 1 H, $OCH_2CH=CH_2$), 3.992 (dd, 1 H, H-6a), 3.797 (t, 1 H, H-6b), 3.721 ('t', 1 H, H-4), 3.538 (m, 1 H, H-2), 3.492 (t, 1 H, H-3), 3.264 (m, 1 H, H-5), 2.397 (d, 1 H, HO-2), 1.478 and 1.425 (2 s, each 3 H, $C(CH_3)_2$); $J_{1,2}$ 7.3, $J_{2,3}$ 8.8, $J_{3,4}$ 8.9, $J_{4,5}$ 9.8, $J_{5,6a}$ 5.4, $J_{5,6b}$ 10.5, $J_{6a,6b}$ -10.8, $J_{2,OH}$ 1.9 Hz.

Anal. Calc. for $C_{19}H_{26}O_6$: C, 65.13; H, 7.48. Found: C, 64.98; H, 7.69.

Allyl 2-O-acetyl-3-O-benzyl-β-D-glucopyranoside (**6**).— A solution of **4** (5.7 g, 16.2 mmol) in pyridine (55 mL) and acetic anhydride (30 mL) was stirred for 20 h, when TLC showed the disappearance of starting material and the formation of a new spot (R_F 0.52, 9:1 CH_2Cl_2-acetone). The solution was concentrated and co-concentrated with toluene (2 x 30 mL), EtOH (2 x 30 mL), and CH_2Cl_2 (2 x 30 mL) (→**5**, quantitative). NMR data ($CDCl_3$): ^{13}C, δ 169.2 ($COCH_3$), 138.4 and 128.1-127.4 (Ph), 133.4 ($OCH_2CH=CH_2$), 117.1 ($OCH_2CH=CH_2$), 100.3 (C-1), 99.2 ($C(CH_3)_2$), 78.7, 74.1, 72.5, and 66.9 (C-2,3,4,5), 73.6 (OCH_2Ph),

69.7 ($OCH_2CH=CH_2$), 62.0 (C-6), 29.0 and 18.9 ($C(CH_3)_2$), 20.7 ($COCH_3$); ^{1}H, δ 7.34-7.27 (m, 5 H, Ph), 5.819 (m, 1 H, $OCH_2CH=CH_2$), 5.27-5.15 (m, 2 H, $OCH_2CH=CH_2$), 4.987 ('t', 1 H, H-2), 4.811 and 4.625 (2 d, each 1 H, OCH_2Ph), 4.452 (d, 1 H, H-1), 4.290 and 4.049 (2 m, each 1 H, $OCH_2CH=CH_2$), 3.932 (dd, 1 H, H-6a), 3.807 (t, 1 H, H-6b), 3.799 (t, 1 H, H-4), 3.547 (t, 1 H, H-3), 3.250 (m, 1 H, H-5), 2.001 (s, 3 H, Ac), 1.493 and 1.436 (2 s, each 3 H, $C(CH_3)_2$); $J_{1,2}$ 8.0, $J_{2,3}$ 9.2, $J_{3,4}$ 9.3, $J_{4,5}$ 9.5, $J_{5,6a}$ 5.4, $J_{5,6b}$ 10.5, $J_{6a,6b}$ -10.8 Hz.

To a solution of **5** in MeOH (100 mL), Dowex-50 (H^+) resin (25 g) was added, and the mixture was stirred for 2 h at 65°, when TLC showed the de-*O*-isopropylidenation to be complete (R_F 0.45; 4:1 CH_2Cl_2-MeOH). After filtration, the solution was evaporated to dryness, and column chromatography (9:1 CH_2Cl_2-MeOH) of the residue gave **6**, isolated as a white foam (4.8 g, 84%), $[\alpha]_D$ -5° (*c* 1, CH_2Cl_2). ^{1}H-NMR data ($CDCl_3$): δ 7.34-7.29 (m, 5 H, Ph), 5.844 (m, 1 H, $OCH_2CH=CH_2$), 5.29-5.16 (m, 2 H, $OCH_2CH=CH_2$), 4.994 (dd, 1 H, H-2), 4.746 and 4.675 (2 d, each 1 H, OCH_2Ph), 4.462 (d, 1 H, H-1), 4.308 and 4.072 (2 m, each 1 H, $OCH_2CH=CH_2$), 3.701 (m, 1 H, H-4), 3.532 (t, 1 H, H-3), 3.357 (m, 1 H, H-5), 2.721 (d, 1 H, HO-4), 2.027 (s, 3 H, Ac); $J_{1,2}$ 8.0, $J_{2,3}$ 9.5, $J_{3,4}$ 9.2, $J_{4,5}$ 9.5, $J_{5,6a}$ 3.8, $J_{5,6b}$ 4.6, $J_{4,OH}$ 3.3 Hz.

Anal. Calc. for $C_{18}H_{24}O_7 \cdot 0.5\ H_2O$: C, 59.82; H, 6.97. Found: C, 59.97; H, 6.96.

Allyl 2-O-acetyl-3-O-benzyl-6-O-trityl-β-D-glucopyranoside (**7**).— A mixture of **6** (2.27 g, 6.44 mmol) and trityl chloride (2.70 g, 9.7 mmol) in pyridine (60 mL) was stirred for 20 h at 90°. Then TLC showed the disappearance of the starting compound, and the formation of a new spot (R_F 0.27, 95:5 CH_2Cl_2-acetone). After concentration a solution of the residue in CH_2Cl_2 (30 mL) was washed with water, 0.5 M sulfuric acid, water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2-acetone) of the residue afforded **7**, isolated as a white solid (3.00 g, 78%), $[\alpha]_D$ -12° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 169.4 ($COCH_3$), 143.5, 138.1, and 128.4-126.9 (Ph), 133.6 ($OCH_2CH=CH_2$), 116.9 ($OCH_2CH=CH_2$), 99.6 (C-1), 86.7 ($OCPh_3$), 82.3, 74.0, 72.6, and 71.6 (C-2,3,4,5), 74.2 (OCH_2Ph), 69.2 ($OCH_2CH=CH_2$), 63.6 (C-6), 20.7 ($COCH_3$); ^{1}H, δ 7.47-7.23 (m, 20 H, 4 Ph), 5.875 (m, 1 H, $OCH_2CH=CH_2$), 5.30-5.16 (m, 2 H, $OCH_2CH=CH_2$), 5.023 (dd, 1 H, H-2), 4.746 and 4.699 (2 d, each 1 H, OCH_2Ph), 4.436 (d, 1 H, H-1), 4.37-4.30 (m, 2 H, $OCH_2CH=CH_2$), 3.784 (m, 1 H, H-4), 3.494 (t, 1 H, H-3), 2.483 (d, 1 H, HO-4), 2.024 (s, 3 H, Ac); $J_{1,2}$ 8.0, $J_{2,3}$ 9.5, $J_{3,4}$ 9.2, $J_{4,OH}$ 2.7 Hz.

Anal. Calc. for $C_{37}H_{38}O_7.H_2O$: C, 72.53; H, 6.58. Found: C, 72.42; H, 6.38.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (**8**).— A solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (1.58 g, 4.05 mmol) in *N,N*-dimethylformamide (5 mL) was heated to 60°. To this solution was added hydrazine acetate[6] (445 mg, 4.8 mmol) and the mixture was stirred for 3 h at 60°. Then TLC (5:1 CH_2Cl_2-acetone) showed the complete disappearance of the starting compound and the formation of a new spot. The mixture was diluted with EtOAc (10 mL) and washed with aq 5% sodium chloride and water, dried ($MgSO_4$), filtered, and concentrated. To a solution of the residue in CH_2Cl_2 (20 mL) at 0°, were added trichloroacetonitrile (4 mL, 40 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.75 mL, 5 mmol). After 2 h the starting material had disappeared (TLC; 5:1 CH_2Cl_2-acetone) and the solution was concentrated and purified by flash chromatography to yield **8** (1.51 g, 76%), $[\alpha]_D$ +118° (*c* 1, CH_2Cl_2), lit[16] $[\alpha]_{578}^{20}$ +103° (*c* 1.2, $CHCl_3$), R_F 0.70 (9:1 CH_2Cl_2-acetone). NMR data ($CDCl_3$): ^{13}C, δ 169.9-169.0 ($COCH_3$), 160.1 ($OCNHCCl_3$), 92.5 (C-1), 90.3 ($OCNHCCl_3$), 69.6, 69.4, 69.3, and 67.4 (C-2,3,4,5), 61.0 (C-6), 20.2-19.9 ($COCH_3$); ^{1}H, δ 8.699 (s, 1 H, $OCNHCCl_3$), 6.566 (d, 1 H, H-1), 5.138 (dd, 1 H, H-2), 2.081, 2.053, 2.037, and 2.021 (4 s, each 3 H, 4 Ac); $J_{1,2}$ 3.7, $J_{2,3}$ 10.2 Hz.

Allyl 2,3,6-tri-O-benzyl-β-D-galactopyranoside (**10**).— To a mixture of allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[8] (**9**, 11.4 g, 23.3 mmol) and sodium cyanoborohydride (19.0 g, 302 mmol) in dry tetrahydrofuran (250 mL) was added a saturated solution of hydrogen chloride in ether until the evolution of gas ceased. Then the mixture was diluted with CH_2Cl_2 (250 mL) and water (100 mL), filtered through Celite, and the organic layer was washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2-acetone) of the residue afforded **10**, isolated as a syrup (6.82 g, 60%), $[\alpha]_D$ +7° (*c* 1, CH_2Cl_2), R_F 0.17 (85:15 CH_2Cl_2-acetone). ^{1}H-NMR data ($CDCl_3$): δ 7.36-7.25 (m, 15 H, 3 Ph), 5.951 (m, 1 H, $OCH_2CH{=}CH_2$), 5.37-5.16 (m, 2 H, $OCH_2CH{=}CH_2$), 4.928 and 4.732 (2 d, each 1 H, OCH_2Ph), 4.717 and 4.586 (2 s, each 2 H, 2 OCH_2Ph), 4.429 and 4.136 (2 m, each 1 H, $OCH_2CH{=}CH_2$), 4.413 (d, 1 H, H-1), 4.018 (m, 1 H, H-4), 3.804 (dd, 1 H, H-6a), 3.723 (dd, 1 H, H-6b), 3.680 (dd, 1 H, H-2), 3.553 (t, 1 H, H-5), 3.494 (dd, 1 H, H-3), 2.517 (bs, 1 H, HO-4); $J_{1,2}$ 7.8, $J_{2,3}$ 9.4, $J_{3,4}$ 3.4, $J_{4,5}$≈0, $J_{5,6a}$ 5.9, $J_{5,6b}$ 6.0, $J_{6a,6b}$ -9.9 Hz.

Anal. Calc. for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99. Found: C, 73.31; H, 6.86.

2,3-Di-O-benzyl-4,6-O-benzylidene-D-galactopyranose (**11**).— To a solution of allyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[8] (**9**, 1.72 g, 3.52 mmol) in *N,N*-dimethylformamide (50 mL) at 80°, was added potassium *tert*-butoxide until the solution turned black. After stirring for 2 h at 80°, the mixture was cooled to room temperature, diluted with CH_2Cl_2 (40 mL), washed with water, and concentrated. The residue was dissolved in 9:1 acetone-water (30 mL), mercuric oxide (14 mg) and mercuric chloride (4.0 g) were added, and the mixture was stirred for 2 h at room temperature, when TLC (7:3 hexane-EtOAc) showed the disappearance of starting material. After concentration, a solution of the residue in CH_2Cl_2 (40 mL) was washed with water, aq 10% potassium iodide, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 hexane-EtOAc) of the residue afforded **11**, isolated as a syrup (1.18 g, 74%), $[\alpha]_D$ +70° (*c* 1, CH_2Cl_2), lit[10] +78° (*c* 0.5, $CHCl_3$), R_F 0.32 (7:3 hexane-EtOAc). NMR data ($CDCl_3$): ^{13}C, δ 138.4-137.7 and 128.9-125.6 (Ph), 100.7 (OCHPh), 97.2 (C-1β), 92.0 (C-1α); ^{1}H, δ 7.37-7.26 (m, 15 H, 3 Ph), 5.503 and 5.491 (2 s, 1 H, OC*H*Ph), 5.373 (d, 0.5 H, H-1α); $J_{1\alpha,2}$ 3.4 Hz.

2,3-Di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl trichloroacetimidate (**12**).— To a solution of **11** (623 mg, 1.39 mmol) and trichloroacetonitrile (1.4 mL, 14 mmol) in CH_2Cl_2 (15 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (250 μL, 1.67 mmol). After stirring for 2 h, TLC (85:15 CH_2Cl_2-acetone) showed the disappearance of starting compound. The solution was concentrated and purified by flash chromatography to yield **12** as a glass (793 mg, 96%), R_F 0.27 (95:5 toluene-acetone). ^{1}H-NMR data ($CDCl_3$): δ 8.561 (s, 1 H, OCN*H*CCl_3), 7.52-7.26 (m, 15 H, 3 Ph), 6.635 (d, 1 H, H-1), 5.511 (s, 1 H, OC*H*Ph); $J_{1,2}$ 3.4 Hz.

Allyl 4-O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**16**).— A solution of allyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside[11] (**13**, 10.8 g, 16 mmol) in CH_2Cl_2 (25 mL) and MeOH (80 mL) was adjusted to pH 10 by the addition of sodium methoxide, stirred overnight, and then neutralised with Dowex-50 (H^+) resin, filtered, and concentrated (→**14**, quantitative). To a solution of the residue in *N,N*-dimethylformamide (35 mL) were added benzaldehyde dimethyl acetal (3.6 mL, 24 mmol) and a catalytic amount of *p*-toluenesulfonic acid, and the mixture was stirred under reduced pressure for 2 h at 50°, neutralised with solid sodium hydrogencarbonate, filtered, and concentrated (→**15**). A solution of the crude residue and benzyl bromide (14.5 mL, 122 mmol) in *N,N*-dimethylformamide (75 mL) was added to a suspension of sodium hydride (3.2 g, 133 mmol) in *N,N*-dimethylformamide (80 mL) at 0°. After stirring overnight, MeOH (25 mL) was added, and the solution was diluted with CH_2Cl_2 (150 mL), washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chro-

matography (93:7 toluene-acetone) of the residue afforded **16**, isolated as a white solid (11.4 g, 77%), $[\alpha]_D$ +4° (*c* 1, CH_2Cl_2), R_F 0.43 (9:1 toluene-acetone). ^{1}H-NMR data ($CDCl_3$): δ 7.39-7.24 (m, 30 H, 6 Ph), 5.954 (m, 1 H, OCH_2CH=CH_2), 5.486 (s, 1 H, OC*H*Ph), 5.335 and 5.290 (2 m, each 1 H, OCH_2CH=CH_2).

*Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**17**).— To a suspension of **16** (9.9 g, 10.7 mmol) and sodium cyanoborohydride (7.4 g, 118 mmol) in freshly distilled tetrahydrofuran (150 mL) was added a saturated solution of hydrogen chloride in ether until the evolution of gas ceased (pH 3). Then the mixture was diluted with CH_2Cl_2 (200 mL), and the solution was filtered through silica, washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue gave **17**, isolated as a syrup (7.74 g, 78%), $[\alpha]_D$ +17° (*c* 1, CH_2Cl_2), R_F 0.36 (9:1 toluene-acetone). NMR data ($CDCl_3$): ^{13}C, δ 138.9-137.8 and 127.9-126.7 (Ph), 133.8 (OCH_2CH=CH_2), 116.6 (OCH_2CH=CH_2), 102.3 and 102.0 (C-1,1'), 84.1, 82.4, 81.8, 81.3, 76.2, 74.7, 73.5, and 72.4 (C-2,3,4,5,2',3',4',5'), 74.8, 74.6, 74.5 (2 C), 73.1, and 72.8 (6 OCH_2Ph), 70.4 (OCH_2CH=CH_2), 69.7 and 67.8 (C-6,6'); ^{1}H, δ 7.34-7.20 (m, 30 H, 6 Ph), 5.949 (m, 1 H, OCH_2CH=CH_2), 5.326 and 5.192 (2 m, each 1 H, OCH_2CH=CH_2), 4.119 (m, 1 H, OCH*H*CH=CH_2), 2.878 (d, 1 H, HO-4').

Anal. Calc. for $C_{57}H_{62}O_{11}$: C, 74.2; H, 6.8. Found: C, 73.9; H, 6.8.

*4-*O*-(2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-2,3,6-tri-*O*-benzyl-*D*-glucopyranosyl trichloroacetimidate* (**19**).— To a solution of **16** (1.41 g, 1.53 mmol) in *N,N*-dimethylformamide (50 mL) at 80° was added potassium *tert*-butoxide. After 2 h the mixture was cooled, diluted with CH_2Cl_2 (50 mL), and the solution was washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in 9:1 acetone-water (100 mL), and mercuric oxide (17 mg) and mercuric chloride (2.4 g) were added. After stirring overnight, the mixture was concentrated, and the residue dissolved in CH_2Cl_2 (75 mL). The solution was washed with water, aq 5% potassium iodide, water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated, affording **18** (1.25 g, 93%). To a solution of **18** (0.8 g, 0.91 mmol) in CH_2Cl_2 (25 mL) were added trichloroacetonitrile (0.9 mL, 9.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (160 μL, 1.1 mmol). After stirring for 2 h, TLC (7:3 hexane-EtOAc) showed a complete conversion of **18**, and the mixture was concentrated and purified by flash chromatography (7:3 hexane-EtOAc) to yield **19**, isolated as a syrup (736 mg, 79 %), $[\alpha]_D$ +112° (*c* 1, CH_2Cl_2), R_F 0.54 (7:3 hexane-EtOAc). ^{1}H-NMR data ($CDCl_3$): δ 8.699 (s, 0.7 H, α-OCN*H*CCl_3), 8.590 (s, 0.3 H, β-OCN*H*CCl_3), 6.439 (d, 0.3 H, H-1α), 5.781 (d, 0.7 H, H-1β), 5.499 (s, 0.3 H, α-OC*H*Ph), 5.480 (s, 0.7 H, β-OC*H*Ph); $J_{1\alpha,2}$ 3.3, $J_{1\beta,2}$ 7.5 Hz.

*Allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-6-*O*-trityl-β-*D*-glucopyranoside* (**20**).— A mixture of **7** (320 mg, 538 μmol), **12** (360 mg, 607 μmol) and powdered molecular sieves (4 Å, 1 g) in CH_2Cl_2 (2 mL) and ether (15 mL) was stirred and cooled to -70°. Then a solution of trimethylsilyl trifluoromethanesulfonate (10 μL, 55 μmol) in ether (100 μL) was added and stirring was continued for 16 h, when TLC showed the disappearance of **7** and the formation of a new spot (R_F 0.43, 95:5 toluene-acetone). Triethyl amine was added, and the mixture was filtered through Celite, washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue afforded **20**, isolated as an amorphous powder (314 mg, 60%), $[\alpha]_D$ +128° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 169.3 ($COCH_3$), 143.5, 138.5-137.6, and 128.6-126.0 (Ph), 133.5 (OCH_2CH=CH_2), 117.0 (OCH_2CH=CH_2), 100.4 (O*C*HPh), 99.3 (C-1), 96.4 (C-1'), 86.3 (O*C*Ph_3), 83.8, 76.0, 74.5, 74.1, 73.6, 72.7, 70.2, and 62.5 (C-2,3,4,5,2',3',4',5'), 73.8 and 71.4 (2 C) (3 OCH_2Ph), 69.0 and 68.9 (C-6' and OCH_2CH=CH_2), 63.3 (C-6), 20.6 ($COCH_3$); ^{1}H, δ 7.47-7.13 (m, 35 H, 7 Ph), 5.929 (m, 1 H, OCH_2CH=CH_2), 5.719 (d, 1 H, H-1'), 5.431 (s, 1 H, OC*H*Ph), 5.321 and 5.224 (2

m, each 1 H, $OCH_2CH{=}CH_2$), 5.167 (dd, 1 H, H-2), 4.76-4.53 (m, 6 H, 3 OCH_2Ph), 4.532 (d, 1 H, H-1), 4.458 and 4.208 (2 m, each 1 H, $OCH_2CH{=}CH_2$), 4.100 (dd, 1 H, H-4), 3.951 (dd, 1 H, H-2'), 3.941 (dd, 1 H, H-6b'), 3.824 (t, 1 H, H-3), 3.802 (d, 1 H, H-4'), 3.676 (m, 1 H, H-5), 3.516 (dd, 1 H, H-6a'), 3.501 (dd, 1 H, H-3'), 3.417 (dd, 1 H, H-6b), 3.286 (dd, 1 H, H-6a), 3.010 (bs, 1 H, H-5'), 1.918 (s, 3 H, Ac); $J_{1,2}$ 7.8, $J_{2,3}$ 9.3, $J_{3,4}$ 8.5, $J_{4,5}$ 9.3, $J_{5,6a}$ 2.1, $J_{5,6b}$ 5.3, $J_{6a,6b}$ -9.7, $J_{1',2'}$ 3.7, $J_{2',3'}$ 10.2, $J_{3',4'}$ 3.3, $J_{4',5'}{=}J_{5',6a'}{=}J_{5',6b'}{\approx}1.0$, $J_{6a',6b'}$ -12.8 Hz.

*Allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**21**).— To a solution of **20** (84 mg, 82 μmol) in CH_2Cl_2 (5 mL) were added a few drops of aq 70% perchloric acid, whereby a deep yellow colour appeared. The mixture was neutralised with solid sodium hydrogencarbonate, filtered through Celite, washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue gave **21**, isolated as a syrup (49 mg, 76%), $[\alpha]_D$ +104° (*c* 1, CH_2Cl_2), R_F 0.23 (95:5 toluene-acetone). ^{1}H-NMR data ($CDCl_3$): δ 7.45-7.15 (m, 20 H, 4 Ph), 5.839 (m, 1 H, $OCH_2CH{=}CH_2$), 5.661 (d, 1 H, H-1'), 5.467 (s, 1 H, OCHPh), 5.249 and 5.174 (2 m, each 1 H, $OCH_2CH{=}CH_2$), 5.026 (dd, 1 H, H-2), 4.83-4.53 (m, 6 H, 3 OCH_2Ph), 4.459 (d, 1 H, H-1), 4.294 (m, 1 H, $OCHCH{=}CH_2$), 3.493 (m, 1 H, H-5), 1.866 (s, 3 H, Ac); $J_{1,2}$ 7.8, $J_{2,3}$ 9.2, $J_{4,5}$ 9.5, $J_{5,6a}$ 2.5, $J_{5,6b}$ 4.4, $J_{1',2'}$ 3.7 Hz.

*Methyl [allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-β-*D*-glucopyranosid] uronate* (**23**).— To a solution of **21** (59 mg, 75.4 μmol) in acetone (5 mL) at 0° was added Jones reagent (100 μL), and the mixture was stirred for 5 h while the temperature was allowed to rise to room temperature. The mixture was poured onto ice and extracted with CH_2Cl_2 (3 x 5 mL). The combined extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated (→**22**). To a solution of the crude residue in CH_2Cl_2 (5 mL) was added dropwise a solution of diazomethane in ether until the yellow colour persisted. The excess of diazomethane was destroyed with acetic acid and the mixture was poured in water (5 mL). The layers were separated and the organic layer was washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (98:2 toluene-acetone) of the residue afforded **23**, isolated as a syrup (43 mg, 70%), $[\alpha]_D$ +126° (*c* 1, CH_2Cl_2), R_F 0.31 (95:5 toluene-acetone). NMR data ($CDCl_3$): ^{13}C, δ 169.2 and 168.3 (C-6 and $COCH_3$), 138.5-137.7 and 128.8-126.2 (Ph), 133.2 ($OCH_2CH{=}CH_2$), 117.3 ($OCH_2CH{=}CH_2$), 100.8 (OCHPh), 99.7 and 99.5 (C-1,1'), 81.1, 78.1, 75.5, 75.2, 75.1, 74.4, 72.2, and 63.3 (C-2,3,4,5,2',3',4',5'), 74.3, 73.6, 71.6, 69.6, and 69.1 (3 OCH_2Ph, $OCH_2CH{=}CH_2$, and C-6'), 52.6 (OCH_3), 20.6 ($COCH_3$); ^{1}H, δ 7.42-7.07 (m, 20 H, 4 Ph), 5.723 (m, 1 H, $OCH_2CH{=}CH_2$), 5.394 (s, 1 H, OCHPh), 5.253 (d, 1 H, H-1'), 5.156 and 5.084 (2 m, each 1 H, $OCH_2CH{=}CH_2$), 4.953 (dd, 1 H, H-2), 4.80-4.44 (m, 6 H, 3 OCH_2Ph), 4.378 (d, 1 H, H-1), 4.216 (m, 1 H, $OCHCH{=}CH_2$), 3.648 (s, 3 H, OCH_3), 3.592 (t, 1 H, H-3), 3.441 (bs, 1 H, H-5'), 1.782 (s, 3 H, Ac); $J_{1,2}$ 7.5, $J_{2,3}$ 9.0, $J_{3,4}$ 8.7, $J_{1',2'}$ 3.5 Hz.

*Allyl 3-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-galactopyranosyl)-β-*D*-glucopyranosiduronic acid* (**24**).— To a solution of **23** (42 mg, 51.8 μmol) in CH_2Cl_2 (1 mL) and MeOH (5 mL) was added sodium methoxide (pH 11). After stirring for 20 h, the solution was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated. A solution of the residue in acetone (3 mL) and 1 M lithium hydroxide (1 mL) was stirred for 3 h, when TLC showed the reaction to be complete. Then the solution was filtered through a column of Dowex-50 (H^+) resin, concentrated, and co-concentrated with toluene (3 x 3 mL), EtOH (3 x 3 mL), and CH_2Cl_2 (3 x 3 mL) to afford **24** as a foam (35 mg, 90%), $[\alpha]_D$ +106° (*c* 1, CH_2Cl_2), R_F 0.22 (95:5 toluene-acetone). ^{1}H-NMR data ($CDCl_3$): δ 7.36-7.21 (m, 20 H, 4 Ph), 5.907 (m, 1 H, $OCH_2CH{=}CH_2$), 5.453 (s, 1 H, OCHPh), 5.37-5.19 (m, 2 H, $OCH_2CH{=}CH_2$), 5.178 (d, 1 H, H-1'); $J_{1',2'}$ 3.6 Hz.

Propyl 4-O-α-D-galactopyranosyl-β-D-glucopyranosiduronic acid (**25**).— A solution of **24** (35 mg, 46.4 μmol) in EtOAc (2 mL) and EtOH (5 mL), containing 10% Pd-C (20 mg), was hydrogenolysed at 1 kg/cm^2 for 8 h, when TLC (4:1 CH_2Cl_2-MeOH) showed the absence of UV-positive spots. The mixture was filtered through Celite, and concentrated. Purification on a Sep-Pak C-18 cartridge (H_2O) and lyophilisation gave **25**, isolated as a white powder (13.5 mg, 73%), $[\alpha]_D$ +98° (*c* 1, water), R_F 0.34 (1:1 CH_2Cl_2-MeOH). NMR data (D_2O): ^{13}C, δ 174.5 (C-6), 104.6 (C-1), 101.3 (C-1'), 78.8, 78.3, 76.4, 75.1, 73.3, 71.5, 71.1, and 70.6 (C-2,3,4,5,2',3',4',5'), 74.0 ($OCH_2CH_2CH_3$), 62.6 (C-6'), 24.5 ($OCH_2CH_2CH_3$), 11.9 ($OCH_2CH_2CH_3$); ^1H (COSY, HOHAHA), δ 5.482 (d, 1 H, H-1'), 4.539 (d, 1 H, H-1), 4.097 (d, 1 H, H-5), 3.991 (H-4'), 3.852 and 3.614 ($OCH_2CH_2CH_3$), 3.811 (H-4), 3.802 (H-2'), 3.764 (H-3), 3.345 (H-3'), 3.329 (H-2), 1.614 (m, 2 H, $OCH_2CH_2CH_3$), 0.898 (t, 3 H, $OCH_2CH_2CH_3$); $J_{1,2}$ 8.0, $J_{4,5}$ 9.2, $J_{1',2'}$ 3.2 Hz.

Allyl 4-O-[4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**26**).— A mixture of **17** (693 mg, 0.92 mmol), powdered molecular sieves (4 Å, 1.5 g), and trimethylsilyl trifluoromethanesulfonate (200 μL, 1.2 mmol) in CH_2Cl_2 (5 mL) was cooled to -30° and stirred for 20 min. Then a solution of **8** (600 mg, 1.22 mmol) in CH_2Cl_2 (5 mL) was added dropwise. After stirring for 16 h, TLC showed the disappearance of **17** and the formation of a new product (85:15 CH_2Cl_2-acetone; **26** R_F 0.42), and triethyl amine (1.5 mL) was added. The mixture was filtered and concentrated, and a solution of the residue in CH_2Cl_2 (10 mL) was washed with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 toluene-acetone) of the residue afforded **26**, isolated as a syrup (695 mg, 74%), $[\alpha]_D$ -2° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 170.5, 170.1, 169.2, and 168.9 (4 $COCH_3$), 139.2-137.9 and 128.4-126.9 (Ph), 133.9 ($OCH_2CH=CH_2$), 117.1 ($OCH_2CH=CH_2$), 102.5, 102.3, and 99.7 (C-1,1',1"), 82.8 (2 C), 81.8, 81.5, 76.8 (2 C), 74.7, 74.5, 72.9, 71.6, 71.3, and 68.0 (C-2,3,4,5,2',3',4',5',2",3",4",5"), 75.0, 74.8 (3 C), 73.1, and 73.0 (6 OCH_2Ph), 70.1 ($OCH_2CH=CH_2$), 67.9 and 67.5 (C-6,6'), 61.5 (C-6"), 20.5 ($COCH_3$); ^1H, δ 7.32-7.20 (m, 30 H, 6 Ph), 5.937 (m, 1 H, $OCH_2CH=CH_2$), 5.319 and 5.211 (2 m, each 1 H, $OCH_2CH=CH_2$), 1.988, 1.981, 1.948, and 1.924 (4 s, each 3 H, 4 Ac).

Allyl 2,3,6-tri-O-benzyl-4-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4-tri-O-benzyl-6-O-trityl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (**29**).— To a solution of **26** (695 mg, 0.55 mmol) in CH_2Cl_2 (1 mL) and MeOH (5 mL) was added sodium methoxide (pH 10). After stirring for 2 h, the mixture was neutralised with Dowex-50 (H^+) resin, filtered, and concentrated (→**27**). To a solution of the residue in pyridine (50 mL) at 60° was added trityl chloride (180 mg, 0.65 mmol), and the mixture was stirred for 20 h at 60°. Then TLC showed the disappearance of starting material and the formation of a new compound (95:9 CH_2Cl_2-MeOH; **28** R_F 0.37). ^1H-NMR data ($CDCl_3$) of acetylated **28**: δ 7.45-7.20 (m, 45 H, 9 Ph), 5.937 (m, 1 H, $OCH_2CH=CH_2$), 5.319 and 5.217 (2 m, each 1 H, $OCH_2CH=CH_2$), 1.986, 1.979, and 1.947 (3 s, each 3 H, 3 Ac). After concentration, a solution of the residue in *N,N*-dimethylformamide (10 mL) was added to a suspension of sodium hydride (70 mg, 2.9 mmol) in *N,N*-dimethylformamide (8 mL), and benzyl bromide (0.25 mL, 2.0 mmol) was added dropwise at 0°. After 2 h TLC (9:1 toluene-acetone) showed the disappearance of the starting compound (R_F 0.29) and one new compound (R_F 0.40). Methanol was added, and the solution was diluted with CH_2Cl_2 (30 mL), washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue gave **29**, isolated as a syrup (175 mg, 20% from **26**), $[\alpha]_D$ +3° (*c* 1, CH_2Cl_2). ^1H-NMR data ($CDCl_3$): δ 7.47-7.18 (m, 60 H, 12 Ph), 5.949 (m, 1 H, $OCH_2CH=CH_2$), 5.324 and 5.209 (2 m, each 1 H, $OCH_2CH=CH_2$).

Allyl 2,3,6-tri-O-benzyl-4-O-[2,3,6-tri-O-benzyl-4-O-(2,3,4-tri-O-benzyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (**30**).— To a solution of **29** (175 mg, 110 μmol) in CH_2Cl_2 (3 mL) were

added a few drops of aq 70% perchloric acid, and after stirring for 10 min, solid sodium hydrogencarbonate was added. Then the mixture was diluted with CH_2Cl_2 (3 mL), filtered, washed twice with water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue afforded **30**, isolated as a syrup (119 mg, 80%), $[\alpha]_D$ +12° (*c* 1, CH_2Cl_2), R_F 0.39 (95:5 toluene-acetone). NMR data ($CDCl_3$): ^{13}C, δ 139.2-138.0 and 128.2-127.0 (Ph), 134.0 ($OCH_2CH=CH_2$), 117.0 ($OCH_2CH=CH_2$), 102.5, 102.4, and 102.2 (C-1,1',1"), 84.5, 82.9, 82.8, 82.6, 81.8, 81.6, 77.8 (2 C), 77.0, 76.4, 76.0, and 74.7 (C-2,3,4,5,2',3',4',5',2",3",4",5"), 75.5 (2 C), 75.0, 74.8 (2 C), 74.6, 73.0 (2 C), and 72.9 (9 OCH_2Ph), 70.1 ($OCH_2CH=CH_2$), 68.1 and 67.7 (C-6,6'), 61.7 (C-6"); ^1H, δ 7.33-7.14 (m, 45 H, 9 Ph), 5.946 (m, 1 H, $OCH_2CH=CH_2$), 5.323 and 5.185 (2 m, each 1 H, $OCH_2CH=CH_2$).

Allyl 4-O-[4-O-(2,3,4-tri-O-benzyl-β-D-glucopyranosyluronic acid)-2,3,6-tri-O-benzyl-β-D-glucopyranosyl]-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**31**).— To a solution of **30** (69 mg, 51 μmol) in acetone (3 mL) at 0° was added Jones reagent (150 μL). After stirring for 3 h at 0°, when TLC showed the reaction to be complete (95:5 toluene-acetone; **31** R_F 0.27), the mixture was diluted with water (5 mL), and the solvent was concentrated for 50%. The solution was extracted with ether (3 x 5 mL), and the combined extracts were washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 toluene-acetone) of the residue gave **31**, isolated as a syrup (53 mg, 75%), $[\alpha]_D$ +25° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 170.1 (C-6"), 139.2-137.5 and 128.2-126.9 (Ph), 134.0 ($OCH_2CH=CH_2$), 117.0 ($OCH_2CH=CH_2$), 102.5, 102.4, and 101.6 (C-1,1',1"), 83.3, 82.2, 82.6, 82.3, 82.1, 81.5 (3 C), 78.9, 75.8, 74.6, and 73.1 (C-2,3,4,5,2',3',4',5',2",3",4",5"), 75.4, 74.9, 74.8, 74.7, 73.0 (2 C), and 72.9 (3 C) (9 OCH_2Ph), 70.1 ($OCH_2CH=CH_2$), 68.0 and 67.6 (C-6,6'); ^1H, δ 7.33-7.14 (m, 45 H, 9 Ph), 5.915 (m, 1 H, $OCH_2CH=CH_2$), 5.321 and 5.186 (2 m, each 1 H, $OCH_2CH=CH_2$).

4-O-[4-O-(β-D-glucopyranosyluronic acid)-β-D-glucopyranosyl]-D-glucopyranose (**34**).— A mixture of **31** (52 mg, 38 μmol), palladium (II) chloride (45 mg, 254 μmol) and sodium acetate trihydrate (36 mg, 265 μmol) in aq 96% acetic acid (10 mL) was sonicated in an ultrasonic cleaner for 72 h. Then TLC (9:1 CH_2Cl_2-MeOH) showed the disappearance of starting material (**31**, R_F 0.44) and the formation of two new spots (R_F 0.40 and 0.29). The mixture was diluted with CH_2Cl_2 (10 mL), filtered, washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2-MeOH) of the residue gave **32** (19 mg) and **33** (26 mg, 51%), both isolated as a syrup. **33**: $[\alpha]_D$ +40° (*c* 1, CH_2Cl_2). ^{13}C-NMR data ($CDCl_3$): δ 169.3 (C-6"), 139.3-137.6 and 129.8-127.0 (Ph), 102.6 and 101.5 (C-1',1"), 97.2 (C-1β), 91.2 (C-1α), 83.3, 82.5, 82.2, 81.5, 79.9, 78.9 (2 C), 76.7, 75.8, 74.6, 72.9, and 70.2 (C-2,3,4,5,2',3',4',5',2",3",4",5"), 75.5 (3 C), 75.1, 74.8, 74.7, 73.5, 73.2, and 73.0 (9 OCH_2Ph), 67.7 (2 C) (C-6,6').

A solution of **33** (17 mg, 13 μmol) in 4:1 EtOH-EtOAc (8 mL), containing 10% Pd-C (15 mg), was hydrogenolysed at 1 kg/cm^2 for 8 h, when TLC (1:1 CH_2Cl_2-MeOH) showed the absence of starting material, and the mixture was filtered through Celite, concentrated, and co-concentrated twice with water (4 mL). The residue was lyophilised to yield **34**, isolated as a white powder (6 mg, 90%), $[\alpha]_D$ +29° (*c* 1, H_2O). ^1H-NMR (COSY, HOHAHA) data (D_2O): δ 5.224 (d, 0.3 H, H-1α), 4.662 (d, 0.7 H, H-1β), 4.548 (d, 1 H, H-1'), 4.538 (d, 1 H, H-1"), 3.972 (H-6a'), 3.958 (H-6aβ), 3.823 (H-6b'), 3.812 (H-6bβ), 3.768 (H-5"), 3.581 (H-2α), 3.371 (H-2'), 3.352 (H-2"), 3.283 (t, 0.7 H, H-2β); $J_{1\alpha,2}$ 3.8, $J_{1\beta,2}$ 8.0, $J_{1',2'}$ 7.9, $J_{1"2"}$ 8.0 Hz.

Allyl 4-O-[4-O-(2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-α-D-glucopyranosyl]-2,3,6-tri-O-benzyl-β-D-galactopyranoside (**35**).— A mixture of **19** (418 mg, 0.41 mmol), **10** (350 mg, 0.71 mmol), and powdered molecular sieves (4 Å, 1.5 g) in dry ether (20 mL) was cooled to -70°. After stirring for 20 min, trimethylsilyl trifluoromethanesulfonate (100 μL, 0.83 mmol) was added, and the mixture

was stirred for 4 h at -30°. Then TLC showed the disappearance of **19** and the formation of a new spot (R_F 0.28, 65:35 hexane-EtOAc). The mixture was neutralised with aq 25% ammonia, diluted with CH_2Cl_2 (15 mL), filtered, and concentrated. A solution of the residue in CH_2Cl_2 (10 mL) was washed with water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:2 hexane-EtOAc) of the residue gave **35** (302 mg, 55%) and **36** (84 mg, 15%), both isolated as syrups. **35**: $[\alpha]_D$ +75° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 139.3-137.3 and 128.7-125.8 (Ph), 133.9 ($OCH_2CH=CH_2$), 117.3 ($OCH_2CH=CH_2$), 102.9 and 102.7 (C-1,1"), 100.8 and 100.3 (C-1' and OCHPh); ^1H, δ 7.37-7.15 (m, 45 H, 9 Ph), 5.953 (m, 1 H, $OCH_2CH=CH_2$), 5.449 (s, 1 H, OCHPh), 5.327 and 5.184 (2 m, each 1 H, $OCH_2CH=CH_2$), 5.046 (d, 1 H, H-1'); $J_{1',2'}$ 3.5 Hz. **36**: $[\alpha]_D$ +12° (*c* 1, CH_2Cl_2). NMR data ($CDCl_3$): ^{13}C, δ 139.3-137.5 and 129.0-126.1 (Ph), 134.3 ($OCH_2CH=CH_2$), 117.1 ($OCH_2CH=CH_2$), 103.1, 102.9, and 102.7 (C-1,1',1"), 101.2 (OCHPh); ^1H, δ 7.37-7.20 (m, 45 H, 9 Ph), 5.994 (m, 1 H, $OCH_2CH=CH_2$), 5.497 (s, 1 H, OCHPh), 5.320 and 5.174 (2 m, each 1 H, $OCH_2CH=CH_2$).

4-O-(4-O-β-D-Glucopyranosyl-α-D-glucopyranosyl)-D-galactopyranose (**38**).— To a solution of **35** (97 mg, 72 μmol) in dry *N,N*-dimethylformamide (6 mL) at 60° was added potassium *tert*-butoxide until the solution turned black. After stirring for 20 h the solution was diluted with CH_2Cl_2 (10 mL), washed twice with water, and concentrated. To a solution of the residue in 9:1 acetone-water (20 mL) were added mercuric oxide (12 mg) and mercuric chloride (840 mg), and the mixture was stirred overnight, when TLC (95:5 toluene-acetone) showed the absence of the starting compound, diluted with CH_2Cl_2 (25 mL), and concentrated. A solution of the residue in CH_2Cl_2 (15 mL) was washed with water, aq 10% potassium iodide, water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (97:3 toluene-acetone) of the residue afforded **37**, isolated as a syrup (67 mg, 71%). A solution of **37** (67 mg, 51 μmol) in 1:1 EtOAc-EtOH (15 mL), containing 10% Pd-C (50 mg), was hydrogenolysed at 1 kg/cm^2 for 12 h, when TLC (4:1 CH_2Cl_2-MeOH) showed the absence of starting material. Then the mixture was filtered through Celite, concentrated, and lyophilised to yield **38**, isolated as a white powder (20 mg, 80%), $[\alpha]_D$ +156° (*c* 1, H_2O). ^1H-NMR (COSY, HOHAHA) data (D_2O): δ 5.300 (d, 0.25 H, H-1α), 4.935 and 4.927 (2 d, 1 H, H-1'), 4.647 (d, 0.75 H, H-1β), 4.530 (d, 1 H, H-1"), 4.253 (H-6'), 4.072 (H-4α), 4.011 (H-4β), 3.946 (H-3α), 3.913 (H-6a"), 3.875 (H-3'), 3.854 (H-2α), 3.733 (H-6b"), 3.712 (H-3β), 3.682 (H-4',5'), 3.587 (H-2'), 3.528 (H-2β), 3.504 (H-3"), 3.484 (H-5"), 3.416 (H-4"), 3.319 (H-2"); $J_{1\alpha,2}$ 3.9, $J_{1\beta,2}$ 7.8, $J_{1',2'}$ 3.8, $J_{1",2"}$ 8.0 Hz.

Anal. Calc. for $C_{18}H_{32}O_{16}\cdot H_2O$: C, 41.38; H, 6.56. Found: C, 41.47; H, 6.53.

4-O-(4-O-β-D-Glucopyranosyl-β-D-glucopyranosyl)-D-galactopyranose (**40**).— To a solution of **36** (48 mg, 35 μmol) in dry *N,N*-dimethylformamide (4 mL) at 60° was added potassium *tert*-butoxide until the solution turned black. After stirring for 20 h the solution was diluted with CH_2Cl_2 (8 mL), washed twice with water, and concentrated. To a solution of the residue in 9:1 acetone-water (12 mL) were added mercuric oxide (5 mg) and mercuric chloride (960 mg), and the mixture was stirred overnight, when TLC (95:5 toluene-acetone) showed the absence of the starting compound, diluted with CH_2Cl_2 (15 mL), and concentrated. A solution of the residue in CH_2Cl_2 (10 mL) was washed with water, aq 10% potassium iodide, water, aq 10% sodium hydrogencarbonate, and water, dried ($MgSO_4$), filtered, and concentrated. Column chromatography (97:3 toluene-acetone) of the residue afforded **39**, isolated as a syrup (29 mg, 62%). A solution of **39** (29 mg, 22 μmol) in 1:1 EtOAc-EtOH (15 mL), containing 10% Pd-C (40 mg), was hydrogenolysed at 1 kg/cm^2 for 12 h, when TLC (4:1 CH_2Cl_2-MeOH) showed the absence of starting material. Then the mixture was filtered through Celite, concentrated, and lyophilised to yield **40**, isolated as a white powder (8.5 mg, 77%), $[\alpha]_D$ +26° (*c* 1, H_2O). ^1H-NMR data (D_2O): δ 5.270 (d, 0.2 H, H-1α), 4.686 (d, 1 H, H-1'), 4.606 (d, 0.8 H, H-1β), 4.507 (d, 1 H, H-1"); $J_{1\alpha,2}$ 3.7, $J_{1',2'}$ 7.9, $J_{1\beta,2}$ 7.8, $J_{1",2"}$ 7.9 Hz.

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