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Synthesis of oligosaccharins: a chemical synthesis of propyl *O*-β-D-galactopyranosyl-(1→2)-*O*-α-D-xylopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside*

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ABSTRACT

Condensation of allyl 3,4-di-*O*-benzyl-β-D-xylopyranoside with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl chloride in dichloromethane in the presence of silver triflate gave allyl 2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-3,4-di-*O*-benzyl-β-D-xylopyranoside (**7**, 83%). Compound **7** was converted in five steps into 2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-3,4-di-*O*-benzyl-α-D-xylopyranosyl bromide (**13**), which was condensed immediately with allyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3-di-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside to give crystalline allyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)- (1→2)-*O*-(3,4-di-*O*-benzyl- α-D-xylopyranosyl)-(1→6)-*O*-(2,3-di-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**23**, 50%). *O*-Deacetylation of **23** followed by catalytic hydrogenolysis gave the title glycoside.

INTRODUCTION

Certain oligosaccharide fragments, released by enzymes from various complex polysaccharides of the plant cell wall into solution in the extracellular fluid[1], have been called[2] "oligosaccharins" and they display diverse biological functions in plants, especially a hormone-like function[3]. For example, the xyloglucan nonasaccharide **1** (XG9), generated from the primary cell wall by the action of cellulase, inhibited the 2,4-dichlorophenoxyacetic acid-induced elongation of etiolated pea stem segments at nanomolar concentration[4]. Such xyloglycan fragments could be feedback modulators of pea endo-(1→4)-β-glucanase[5], a key enzyme which produces the xyloglucan oligosaccharins. Much of the structure of **1** is unnecessary for anti-auxin activity[6,7] and it is the α-L-fucosyl-(1→2)-β-D-galactosyl-(1→2) side chain that is important for its biological activity.

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
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α-L-Fuc-(1→2)-β-D-Gal-(1→2)-α-D-Xyl
1
↓
6
α-D-Xyl-(1→6)-β-D-Glc-(1→4)-β-D-Glc-(1→4)-β-D-Glc-(1→4)-D-Glc
6
↑
1
α-D-Xyl

1

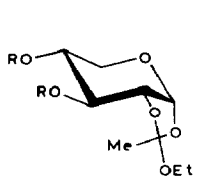
As part of a program concerned with the structure–activity relationships and mode of action of xyloglucan oligosaccharides in the plant cell, we now report the synthesis of the tetrasaccharide glycoside **26**.

RESULTS AND DISCUSSION

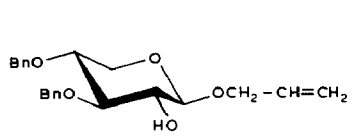
The general strategy of the synthesis of **26** was as follows: **12** (or **13**) + **22**→**23**→**26** in which the alcohol **22** and the disaccharide glycosyl donor **12** (or **13**) were the key building blocks. An allyl β-glycoside was selected in order to ensure the β configuration at the "reducing" end and also, after catalytic hydrogenation of the double bond, to mimic a potential aliphatic anchoring arm. The allyl group can be removed to provide a reducing tetrasaccharide. The synthesis of the glycosyl donors **12** and **13** was undertaken first.

The amorphous benzylated orthoester **3** (79%) was obtained conventionally from 3,4-di-*O*-acetyl-1,2-*O*-(1-ethoxyethylidene)-α-D-xylopyranose[8] (**2**). Treatment of **3** with allyl alcohol in chlorobenzene for 40 h at 85° in the presence of 2,6-dimethylpyridinium perchlorate[9], followed by *O*-deacetylation (Zemplén), gave the crystalline allyl β-D-xylopyranoside derivative **4** (74%). The ^{1}H-n.m.r. data for **4** (δ 4.38, d, $J_{1,2}$ 6.4 Hz, H-1) indicated the β configuration. Although benzylated orthoesters have been used as glycosyl donors in disaccharide synthesis[10–12], the yields and stereoselectivity have not been consistently high. Therefore, 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose[13] (**5**) was converted into the galactosyl donor **6** in four steps, namely mild selective acid-catalysed opening[14] of the orthoester ring, acetylation, selective anomeric *O*-deacetylation with benzylamine in ether[15], and reaction with the Vilsmeier reagent[16] generated *in situ*. Attempted direct conversion[17–19] of **5** into the chloride **6**, using trimethylsilyl chloride, was unsuccessful. Condensation of **4** with freshly prepared **6** in 1,2-dichloroethane in the presence of silver triflate, followed by chromatography, gave the crystalline disaccharide derivative **7** (83%). The ^{1}H-n.m.r. data for **7** (δ 4.78, d, $J_{1',2'}$ 7.9 Hz, H-1') demonstrated the β configuration of the new glycosidic bond. *O*-Deacetylation (Zemplén) of **7** gave crystalline **8**. Treatment of **8** with potassium *tert*-butoxide in methyl sulfoxide effected allyl → prop-1-anyl rearrangement and

provided the crystalline disaccharide derivative **9**. Acetylation (acetic anhydride–pyridine) of **9**, followed by treatment with mercuric chloride and yellow mercuric oxide in acetone–water[20], gave the crystalline hemiacetal **11** in good yield. Reaction of **11** with the Vilsmeier reagent generated *in situ* from either oxalyl chloride or bromide gave the required glycosyl donors **12** and **13**.



2 R = Ac
3 R = Bn



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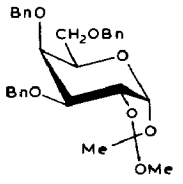

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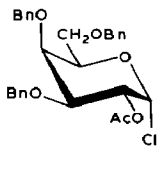

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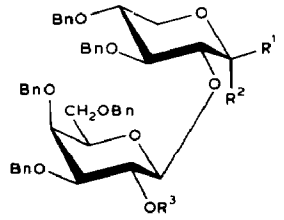
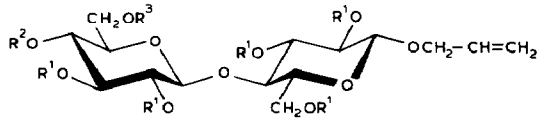


7 R^1 = $OCH_2CH{=}CH_2$, R^2 = H, R^3 = Ac
8 R^1 = $OCH_2CH{=}CH_2$, R^2 = R^3 = H
9 R^1 = $OCH{=}CH{-}CH_3$, R^2 = R^3 = H
10 R^1 = $OCH{=}CH{-}CH_3$, R^2 = H, R^3 = Ac
11 R^1, R^2 = H, OH
12 R^1 = H, R^2 = Cl, R^3 = Ac
13 R^1 = H, R^2 = Br, R^3 = Ac



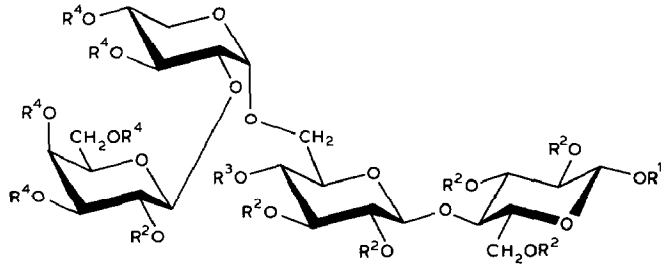
14 R^1 = R^2 = R^3 = Ac
15 R^1 = H, R^2, R^3 = PhCH
16 R^1 = Bn, R^2, R^3 = PhCH
17 R^1 = R^2 = Bn, R^3 = H
18 R^1 = R^2 = Bn, R^3 = Ac
19 R^1 = R^3 = Bn, R^2 = H
20 R^1 = R^2 = Bn, R^3 = Ac
21 R^1 = Bn, R^2 = R^3 = H
22 R^1 = Ac, R^2 = R^3 = H

The alcohol **17** was selected as an appropriate glycosyl acceptor and was synthesised as follows. Crystalline allyl hepta-*O*-acetyl-*β*-cellobioside[21] was *O*-deacetylated (Zemplén) and then treated with α,α-dimethoxytoluene in *N*,*N*-dimethylformamide in the presence of toluene-*p*-sulfonic acid to give 75% of the crystalline 4′,6′-*O*-benzylidene derivative **15**. Treatment of **15** with benzyl bromide in *N*,*N*-dimethylformamide in the presence of sodium hydride gave crystalline **16**. Reductive cleavage[22] of the benzylidene ring with $LiAlH_4$–$AlCl_3$ then gave crystalline **17** (60%) and **19** (29%), the structures of which were confirmed by the ^{1}H-n.m.r. data for the respective *O*-acetyl derivatives **18** and **20**. The poor regioselectivity of this reaction may reflect steric hindrance around O-6′.

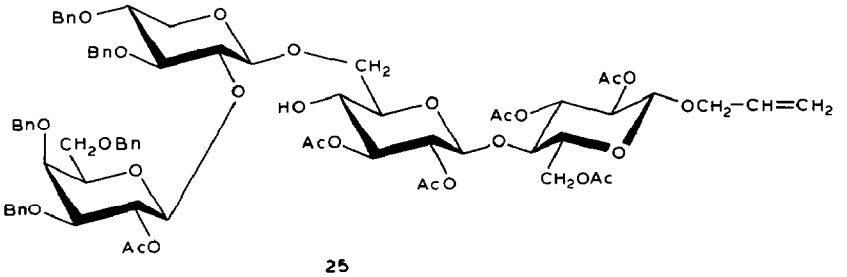
Attempted condensation of the chloride **12** or the bromide **13** with the primary alcohol **17**, using either the silver triflate procedure or the halide ion-catalysed reaction[23], gave only ~10% of the unwanted *β*-xyloside, and no α-linked product derivative was detected. This low reactivity of **17** is probably due to steric hindrance around O-6′

by the benzyl groups. In agreement with this observation, reaction of the crystalline diol **21** with the chloride **13**, in the presence of silver triflate, resulted in exclusive glycosylation at O-4″. In marked contrast, condensation of allyl penta-*O*-acetyl-*β*-cellobioside[21] (**22**) with the freshly prepared bromide **13**, in 1,2-dichloroethane in the presence of mercuric bromide, gave 50% of the crystalline α-product **23** and 9% of the amorphous *β*-product **25**. The use of the freshly prepared chloride **12**, in the presence of silver triflate, gave similar results (50% of **23** and 16% of **25**). The ^{1}H-n.m.r. data for **23** (δ 4.93, d, $J_{1,2}$ 3.5 Hz, Xyl H-1) demonstrated that the new glycosidic bond was α, and the $[\alpha]_D$ values (chloroform) (+4° and −30°, respectively) of **23** and **25** confirmed the assigned configurations.

Treatment of **23** with benzoyl chloride in pyridine gave the amorphous benzoate **24**. Comparison of the 300-MHz ^{1}H-n.m.r. spectra of **24** and **23** in $CDCl_3$ showed a 1.43 p.p.m. downfield displacement of the signal of H-4′ upon benzyolation, which demonstrated that **23** was a tetrasaccharide derivative with the reported structure. *O*-Deacetylation (Zemplén) of **23** followed by catalytic hydrogenolysis (Pd–C) then gave 88% of the title tetrasaccharide glycoside **26**.



23 $R^1 = CH_2{-}CH{=}CH_2, R^2 = Ac, R^3 = H, R^4 = Bn$
24 $R^1 = CH_2{-}CH{=}CH_2, R^2 = Ac, R^3 = Bz, R^4 = Bn$
26 $R^1 = CH_2CH_2CH_3, R^2 = R^3 = R^4 = H$



25

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–25° with a

Perkin–Elmer Model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Perkin–Elmer R-32 (90 MHz) or a Bruker AM-300 (300 MHz) instrument for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated. Unprimed numbers refer to the "reducing" unit and primed numbers to the non-reducing unit. The purity of products was determined by t.l.c. on Silica Gel 60 F_{154} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 63–200 μm). Elemental analyses were performed by the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique (Vernaison, France).

3,4-Di-O-benzyl-1,2-O-(1-ethoxyethylidene)-α-D-xylopyranose (**3**). — To a solution of **2** (13.24 g) in methanol (200 mL) was added methanolic 2M sodium methoxide (4 mL). After overnight storage at room temperature, the solution was concentrated. A solution of the residue in dry *N,N*-dimethylformamide (200 mL) was stirred for 2 h at room temperature in the presence of sodium hydride (6 g of a 60% suspension in oil), and freshly distilled benzyl bromide (14 mL) was added dropwise. After 12 h, the excess of benzyl bromide was destroyed by the slow addition of methanol (50 mL) and stirring for 2 h. The mixture was diluted with dichloromethane (500 mL), washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (200 g) with 5:1 hexane–ethyl acetate (containing 0.5% of triethylamine) to give **3** (13.75 g, 79%), which was used immediately in the next reaction. ^{1}H-N.m.r. data (90 MHz): δ 7.31 and 7.30 (2 m, 10 H, 2 Ph), 5.59 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 4.33 (dd, 1 H, $J_{2,3}$ 3 Hz, H-2), 3.57 (q, 2 H, OCH_2CH_3), 1.68 (s, 3 H, CH_3), 1.21 (t, 3 H, OCH_2CH_3).

Allyl 3,4-di-O-benzyl-β-D-xylopyranoside (**4**). — A 0.1M solution of 2,6-dimethylpyridinium perchlorate in 1,2-dichlorethane (1 mL) was added to a solution of orthoester **3** (3.779 g) and freshly distilled allyl alcohol (6.4 mL) in chlorobenzene (75 mL). The mixture was heated for 40 h at 85°, cooled, diluted with dichloromethane (250 mL), washed with aqueous 5% sodium hydrogen carbonate and with water, dried (Na_2SO_4), and concentrated. To a solution of the residue in methanol (40 mL) was added methanolic 2M sodium methoxide (0.5 mL). After 1 h at room temperature, the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to give **4** (2.601 g, 74%), m.p. 60–60.5° (from hexane–ether), $[\alpha]_D$ −37° (*c* 1.15, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.33 (m, 10 H, 2 Ph), 4.38 (d, 1 H, $J_{1,2}$ 6.4 Hz, H-1), 3.99 (dd, 1 H, $J_{4,5e}$ 4.2, $J_{5a,5e}$ 11.6 Hz, H-5*e*), 3.30 (dd, 1 H, $J_{4,5a}$ 8.0, H-5*a*), 2.60 (d, 1 H, OH).

Anal. Calc. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.07. Found: C, 71.20; H, 6.82.

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-galactopyranosyl chloride (**6**). — A solution of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactopyranose (**5**, 1.03 g) in 95% aqueous acetic acid (20 mL) was stirred for 20 min at room temperature and then concentrated, and toluene was evaporated several times from the residue. To a solution of the residue in pyridine (20 mL) was added acetic anhydride (10 mL). The mixture was stirred for 1 h at room temperature, then concentrated, and toluene was evaporated several times from the residue. ^{1}H-N.m.r. data (90 MHz): δ 6.36 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.54 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 2.09 and 2.00 (2 s, 6 H, 2 Ac).

A solution of the residue (1.04 g, 96% from **5**) in ether (25 mL) and benzylamine (5.4 mL) was stirred for 5 h at room temperature, then concentrated. The residue was

dissolved in dichloromethane (100 mL), and the solution was washed with M hydrochloric acid, saturated aqueous ammonium chloride, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (50 g) with 2:1 hexane–ethyl acetate to give amorphous 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-galactopyranose (680 mg, 71% from the corresponding diacetate).

A mixture of this residue with *N,N*-dimethylformamide (0.6 mL), oxalyl chloride (1.5 mL), and dichloromethane (10 mL) was stirred for 3 h at room temperature, diluted with dichloromethane (30 mL), washed with saturated aqueous ammonium chloride, saturated aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and evaporated. The residue was eluted from a column of silica gel (20 g) with 5:2 hexane–ethyl acetate to give **6** (0.61 g, 57% from **5**). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (m, 15 H, 3 Ph), 6.37 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 5.41 (dd, 1 H, $J_{2,3}$ 10 Hz, H-2), 2.09 (s, 3 H, Ac).

This unstable chloride was not submitted to elemental analysis but was used immediately in the next condensation step.

*Allyl 2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-3,4-di-*O*-benzyl-β-*D*-xylopyranoside* (**7**). — A solution of freshly prepared chloride **6** (460 mg) and alcohol **4** (249 mg) in anhydrous dichloromethane (8 mL) was stirred for 15 min at −15° under dry argon in the presence of activated powdered 4 Å molecular sieves (300 mg). Silver triflate (456 mg) was added, and the mixture was stirred overnight in the dark at room temperature, diluted with dichloromethane (50 mL), filtered, washed with ice-cold dilute hydrochloric acid, saturated aqueous ammonium chloride, satured aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (60 g) with 22:1 dichloromethane–ethyl acetate to give **7** (474 mg, 83%), m.p. 76–77° (from ether–hexane), $[\alpha]_D$ −18° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.30 (m, 25 H, 5 Ph), 5.82 (m, 1 H, $CH_2CH{=}CH_2$), 5.39 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.1 Hz, H-2'), 4.78 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.41 (d, 1 H, $J_{1,2}$ 6.2 Hz, H-1),. 3.95 (dd, 1 H, $J_{3',4'}$ 2.8, $J_{4',5'}$ 1 Hz, H-4'), 3.86 (dd, 1 H, $J_{4,5e}$ 4.1, $J_{5a,5e}$ 12 Hz, H-5*e*), 3.45 (dd, 1 H, H-3'), 3.17 (dd, 1 H, $J_{4,5a}$ 8.9, H-5*a*), 1.77 (s, 3 H, Ac).

Anal. Calc. for $C_{51}H_{56}O_{11}$: C, 72.49; H, 6.68. Found: C, 72.57; H, 6.82.

*Prop-1-enyl 3,4-di-*O*-benzyl-2-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-β-*D*-xylopyranoside* (**9**). — The disaccharide **7** was *O*-deacetylated in the usual manner (MeOH–MeONa) to give a quantitative yield of alcohol **8**, m.p. 95–96.5° (from ether). A solution of **8** (167 mg) and potassium *tert*-butoxide (200 mg) in dry methyl sulfoxide (5 mL) was stirred for 2 h at 60° under argon, then cooled, diluted with saturated aqueous ammonium chloride, and extracted with dichloromethane (30 mL). The organic extract was washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (5 g) with 3:1 hexane–ethyl acetate (containing 0.5% of triethylamine) to give **9** (133 mg, 80%), m.p. 115–117° (from ether–hexane), $[\alpha]_D$ −18° (*c* 0.6, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (m, 25 H, 5 Ph), 6.07 (dd, 1 H, $OCH{=}CHCH_3$), 1.56 (dd, 3 H, $OCH{=}CHCH_3$).

Anal. Calc. for $C_{49}H_{54}O_{10}$: C, 73.30; H, 6.78. Found: C, 73.25; H, 6.63.

*Prop-1-enyl 2-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-*D*-galactopyranosyl)-3,4-di-*O*-benzyl-β-*D*-xylopyranoside* (**10**). — The disaccharide **9** (173 mg) was stirred with an-

hydrous pyridine (5 mL) and acetic anhydride (2 mL) for 10 h at room temperature, then concentrated, and toluene was evaporated from the residue. The crude product was eluted from a column of silica gel (5 g) with 2:1 hexane–ethyl acetate to give **10** (156 mg, 86%), m.p. 80–81.5° (from ether–hexane), $[\alpha]_D$ −9° (*c* 1.3, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.30 (m, 25 H, 5 Ph), 6.02 (dd, 1 H, OC*H*=CHCH$_3$), 5.39 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10.2 Hz, H-2'), 4.69 (d, 1 H, H-1'), 4.62 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 3.99 (dd, 1 H, $J_{3',4'}$ 2.7, $J_{4',5'}$ 1 Hz, H-4'), 3.86 (dd, 1 H, $J_{4,5e}$ 4.3, $J_{5a,5e}$ 11.7 Hz, H-5*e*), 3.46 (dd, 1 H, H-3'), 3.21 (dd, 1 H, $J_{4,5a}$ 8.5 Hz, H-5*a*), 1.76 (s, 3 H, Ac), 1.53 (dd, 1 H, OCH=CHC*H*$_3$).

Anal. Calc. for $C_{51}H_{56}O_{11}$: C, 72.49; H, 6.68. Found: C, 72.67; H, 6.66.

2-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4-di-O-benzyl-D-xylopyranose (**11**). — A solution of mercuric chloride (70 mg) in acetone (1 mL) was added dropwise to a stirred suspension of **10** (143 mg) and yellow mercuric oxide (104 mg) in 9:1 acetone–water (5 mL). The mixture was stirred for 15 min at room temperature, diluted with dichloromethane (40 mL), filtered, washed with 10% aqueous potassium iodide, saturated aqueous ammonium chloride, and water, dried (Na_2SO_4), and concentrated. The residue was crystallized from ethyl acetate–hexane to give **11** (119 mg, 88%), m.p. 156–157°, $[\alpha]_D$ +22° (*c* 0.2, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.32 (m, 25 H, 5 Ph), 5.44 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.1 Hz, H-2'β), 5.40 (dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.1 Hz, H-2'α), 5.21 (dd, $J_{1,2}$ 3.4, $J_{1,OH}$ 2.0 Hz, H-1α), 4.63 (d $J_{1',2'}$ 8.0 Hz, H-1'α), 3.93 (dd, $J_{3',4'}$ 3.0, $J_{4',5'}$ 1 Hz, H-4'α), 3.87 (dd, $J_{3',4'}$ 3.0, $J_{4',5'}$ 1 Hz, H-4'β), 3.60 (dd, $J_{2,3}$ 9.1 Hz, H-2α), 3.46 (dd, H-3'α), 3.13 (d, OH), 1.76 (s, 3 H, Ac), α:β ratio ~8:1 (integrated H-2').

Anal. Calc. for $C_{48}H_{52}O_{11}\cdot 0.5H_2O$: C, 70.83; H, 6.56. Found: C, 70.85; H, 6.48.

2-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4-di-O-benzyl-α-D-xylopyranosyl chloride (**12**). — A M solution of oxalyl chloride in 1,2-dichloroethane (1.25 mL) was added dropwise to a solution of **11** (103 mg) in 1,2-dichloroethane (2 mL) and *N,N*-dimethylformamide (0.25 mL). The mixture was stirred for 2 h at room temperature, diluted with ether (20 mL), washed with ice-cold saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. The residue was eluted from a short column of silica gel with 5:2 hexane–ethyl acetate to give **12** (97 mg, 92%), $[\alpha]_D$ +50° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (m, 25 H, 5 Ph), 6.08 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.44 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 1.88 (s, 3 H, OAc).

This unstable chloride was not submitted to elemental analysis but was used immediately in the next key condensation step.

2-O-(2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-3,4-di-O-benzyl-α-D-xylopyranosyl bromide (**13**). — Compound **11** (95 mg) was converted into the unstable bromide **13** (91 mg, 89%) as described above, but using oxalyl bromide instead of oxalyl chloride; **13** had $[\alpha]_D$ +58° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz): δ 7.30 (m, 25 H, 5 Ph), 6.41 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.44 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 1.89 (s, 3 H, Ac).

Allyl 4-O-(4,6-O-benzylidene-β-D-glucopyranosyl)-β-D-glucopyranoside (**15**). — Compound **14** (5.182 g) was *O*-deacetylated in the usual manner (MeOH–MeONa). A solution of the dry residue in *N,N*-dimethylformamide (15 mL) was stirred under water-pump vacuum for 2 h at 100° in the presence of α,α-dimethoxytoluene (1.2 mL)

and toluene-*p*-sulfonic acid (181 mg), and then concentrated. The residue was triturated in 5:1 ether–hexane, and the precipitate was separated and dissolved in methanol (50 mL). The solution was neutralized in the presence of Dowex 1-X8 (OH^-) resin, filtered, and concentrated. The residue was crystallized from 2-propanol–methanol to give **15** (1.752 g, 49%), m.p. 205–210° (dec.), $[\alpha]_D$ −44° (*c* 1, *N,N*-dimethylformamide).

Anal. Calc. for $C_{22}H_{30}O_{11}\cdot 0.75H_2O$: C, 54.60; H, 6.56. Found: C, 54.52; H, 6.60.

More **15** (923 mg, 26%) was obtained from the mother liquors, after elution from a column of silica gel (50 g) with 11:1 dichloromethane–methanol.

*Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**16**). — A solution of **15** (2.344 g) in dry *N,N*-dimethylformamide (20 mL) was stirred for 30 min at room tempeature in the presence of sodium hydride (1.186 g) and, after cooling to 0°, freshly distilled benzyl bromide (4.4 mL) was added dropwise. The mixture was stirred for 4 h at room temperature, and the excess of benzyl bromide was destroyed by slow addition of methanol (5 mL) and stirring for 1 h. The mixture was diluted with dichloromethane (100 mL), washed with saturated aqueous ammonium chloride, water, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was crystallized from ether–hexane to give **16** (3.410 g, 74%), m.p. 151–152°, $[\alpha]_D$ −3° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.37 (m, 25 H, 5 Ph), 5.95 (m, 1 H, $OCH_2CH{=}CH_2$), 5.48 (s, 1 H, PhC*H*), 5.26 (m, 2 H, $OCH_2CH{=}CH_2$), 4.53 and 4.42 (2 d, 2 H, *J* 8 and 8.2 Hz, H-1, 1′), 3.14 (m, 1 H, H-5′).

Anal. Calc. for $C_{57}H_{60}O_{11}\cdot 0.5H_2O$: C, 73.61; H, 6.61. Found: C, 73.77; H, 6.69.

*Allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**17**) and *allyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**19**). — A solution of aluminium chloride (625 mg) in ether (5 mL) was added dropwise to a refluxing solution of **16** (479 mg) and lithium aluminium hydride (200 mg) in 1:1 dichloromethane–ether (10 mL). The mixture was boiled under reflux for 2.5 h, and the excess of reagents was destroyed by the addition of ethyl acetate (3 mL), then water (5 mL). The mixture was diluted with ether (50 mL), and the organic layer was washed with water, dried ($CaCl_2$), and concentrated. The residue was eluted from a column of silica gel (25 g) with 5:2 hexane–ethyl acetate to give, first, **17** (284 mg, 60%), m.p. 113.5–114° (from hexane–ethyl acetate), $[\alpha]_D$ +19° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.30 (m, 30 H, 6 Ph), 3.92 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3′), 3.79 (dd, 1 H, $J_{5,6a}$ 3.7, $J_{6a,6b}$ 11 Hz, H-6a), 3.67 (dd, 1 H, $J_{5,6b}$ 1.6 Hz, H-6b), 3.13 (m, 1 H, H-5′), 1.49 (t, $J_{6',OH}$ 7 Hz, HO-6′).

Anal. Calc. for $C_{57}H_{62}O_{11}$: C, 74.17; H, 6.77. Found: C, 74.28; H, 6.81.

Next eluted was **19** (136 mg, 29%), m.p. 112.5–113.5 (from hexane–ethyl acetate), $[\alpha]_D$ +17° (*c* 0.3, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.32 (m, 30 H, 6 Ph), 4.43 and 4.38 (2 d, 2 H, $J_{1,2} = J_{1',2'} = 7.8$ Hz, H-1,1′). Mass spectrum: m/z 940 ($M^+ + 18$).

Anal. Calc. for $C_{57}H_{62}O_{11}\cdot 2H_2O$: C, 71.38; H, 6.94. Found: C, 71.35; H, 6.87.

*Allyl 4-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranosyl)-2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**18**). — Compound **17** (10 mg) was acetylated with anhydrous pyridine (0.3 mL) and acetic anhydride (0.1 mL) overnight at room temperature, then

concentrated, and toluene was evaporated from the residue. The crude product was eluted from a column of silica gel (1 g) with 7:2 hexane–ethyl acetate to give **18** (10 mg, 95%). ^{1}H-N.m.r. data (300 MHz): δ 7.23 (m, 30 H, 6 Ph), 3.96 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.1$ Hz, H-3'), 3.79 (dd, 1 H, H-6a), 3.63 (dd, 1 H, H-6b), 1.80 (s, 3 H, Ac).

Allyl 4-O-(4-O-acetyl-2,3,6-tri-O-benzyl-β-D-glucopyranosyl)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**20**). — Acetylation of **18** (10 mg), as described for the preparation of **17**, gave **20** (10 mg, 95%). ^{1}H-N.m.r. data (300 MHz): δ 7.23 (m, 30 H, 6 Ph), 5.06 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9.5$ Hz, H-4'), 4.42 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1'), 4.24 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.77 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.5$ Hz, H-3'), 1.92 (s, 3 H, Ac).

Allyl 2,3,6-tri-O-benzyl-4-O-(2,3-di-O-benzyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**21**). — A solution of **16** (200 mg) in acetic acid (7 mL) and water (3 mL) was stirred for 15 min at 100°, cooled, and concentrated, and toluene was evaporated from the residue. The crude product was eluted from a short column of silica gel with 1:1 hexane–ethyl acetate to give **21** (142 mg, 79%), m.p. 128–129° (from hexane–ethyl acetate), $[\alpha]_D$ +5.5° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.30 (m, 25 H, 5 Ph), 3.79 (dd, 1 H, $J_{5,6a}$ 4.0, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.69 (dd, 1 H, $J_{5,6b}$ 2.0, $J_{6a,6b}$ 10.8 Hz, H-6b), 3.63 (dd, 1 H, $J_{5',6'a}$ 3.0, $J_{6'a,6'b}$ 11.9 Hz, H-6'a), 3.08 (m, 1 H, $J_{4',5'}$ 9.2, $J_{5',6'b}$ 6.0 Hz, H-5').

Anal. Calc. for $C_{50}H_{56}O_{11}$: C, 72.10; H, 6.78. Found: C, 72.13; H, 6.80.

Allyl 2,3,6-tri-O-acetyl-4-O-(2,3-di-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (**22**). — A solution of **15** (270 mg) in pyridine (5 mL) and acetic anhydride (3 mL) was stirred overnight at room temperature, then concentrated, and toluene was evaporated several times from the residue. A solution of the residue in 60% aqueous acetic acid (20 mL) was stirred for 20 min at 100°, cooled, and concentrated. Water and then toluene were evaporated several times from the residue, which was crystallized from ethyl acetate–hexane to give **22** (288 mg, 85%), m.p. 195–196°, lit.[21] m.p. 194–196°. ^{1}H-N.m.r. data (300 MHz): δ 5.84 (m, 1 H, $OCH_2CH{=}CH_2$), 5.20 (m, 2 H, $OCH_2CH{=}CH_2$), 5.17 (t, 1 H, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 4.97 (t, 1 H, $J_{2',3'} = J_{3',4'} = 9.3$ Hz, H-3'), 4.92 (dd, 1 H, $J_{1,2}$ 7.8 Hz, H-2), 4.83 (dd, 1 H, $J_{1',2'}$ 8 Hz, H-2'), 4.57 (d, 1 H, H-1), 4.52 (d, 1 H, H-1'), 4.49 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 12 Hz, H-6a), 4.30 and 4.07 (2 m, 2 H, $OCH_2CH{=}CH_2$), 4.08 (dd, 1 H, $J_{5,6b}$ 5.5, H-6b), 3.88 (dd, 1 H, $J_{5',6'a}$ 3.3, $J_{6'a,6'b}$ 12.2 Hz, H-6'a), 3.83 (t, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.78 (dd, 1 H, $J_{5',6'b}$ 4.7 Hz, H-6'b), 3.72 (t, 1 H, $J_{4',5'}$ 9.3 Hz, H-4'), 3.60 (m, 1 H, H-5), 3.41 (m, 1 H, H-5'), 2.84 (d, 1 H, $J_{4',OH}$ 4 Hz, HO-4'), 2.40 (t, 1 H, $J_{6',OH}$ 6.5 Hz, HO-6'), 2.12, 2.08, 2.07, 2.05, 2.04 (5 s, 15 H, 5 Ac).

Allyl O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→2)-O-(3,4-di-O-benzyl-α-D-xylopyranosyl)-(1→6)-O-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**23**). — (*a*) A solution of freshly prepared **13** (40 mg) and alcohol **22** (36 mg) in dry 1,2-dichloroethane (1 mL) was stirred at 0° under dry argon in the presence of activated powdered 4 Å molecular sieves (100 mg). Finely powdered mercuric bromide (15 mg) was added, and the mixture was stirred for 2 h at 0° and then overnight at room temperature, diluted with dichloromethane, filtered, washed with brine and then water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (8 g) with 1:1 ethyl acetate–hexane to give, first, the α-linked tetrasaccharide **23** (32 mg, 50%), m.p. 111–113° (from ether–hexane), $[\alpha]_D$ +4°

(*c* 0.7, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.30 (m, 25 H, 5 Ph), 5.83 (m, 1 H, $OCH_2CH=CH_2$), 5.37 (dd, 1 H, $J_{1''',2'''}$ 8, $J_{2''',3'''}$ 10.2 Hz, H-2'''), 5.17 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.08 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.5 Hz, H-3'), 4.93 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.92 (dd, 1 H, $J_{1,2}$ 8 Hz, H-2), 4.85 (dd, 1 H, $J_{1',2'}$ 8 Hz, H-2'), 4.67 (d, 1 H, H-1'''), 4.55 (dd, 1 H, $J_{5,6a}$ 3, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.54 (d, 1 H, H-1), 4.51 (d, 1 H, H-1'), 4.13 (dd, 1 H, $J_{5,6b}$ 5 Hz, H-6b), 3.89 (dd, 1 H, $J_{3''',4'''}$ 3, $J_{4''',5'''}$ 1 Hz, H-4'''), 3.84 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.73 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4'), 3.63 (dd, 1 H, $J_{2'',3''}$ 9.8 Hz, H-2''), 3.42 (dd, 1 H, H-3'''), 2.10, 2.05, 2.03, 2.02, 2.01, 1.82 (6 s, 10 H, 6 Ac).

Anal. Calc. for $C_{37}H_{86}O_{26}$: C, 63.56; H, 6.28. Found: C, 63.54; H, 6.35.

Next eluted was the amorphous β-linked tetrasaccharide **25** (6 mg, 9%), $[\alpha]_D$ −30° (*c* 0.9, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.29 (m, 25 H, 5 Ph), 5.83 (m, 1 H, $OCH_2CH=CH_2$), 5.33 (dd, 1 H, $J_{1''',2'''}$ 8.5, $J_{2''',3'''}$ 10.5 Hz, H-2'''), 4.23 (d, 1 H, $J_{1'',2''}$ 7.5 Hz, H-1''), 3.16 (dd, 1 H, $J_{4'',5''a}$ 9, $J_{5''a,5''e}$ 11.7, H-5''*a*), 2.11, 2.04, 2.03, 2.02, 1.98, 1.93 (6 s, 18 H, 6 Ac).

Anal. Calc. for $C_{73}H_{86}O_{26}$: C, 63.56; H, 6.28. Found: C, 63.78; H, 6.11.

(*b*) A solution of freshly prepared **12** (38 mg) and alcohol **22** (45 mg) in dry 1,2-dichloroethane (1.5 mL) was stirred for 15 min at −15° under dry argon in the presence of activated powdered 4 Å molecular sieves (100 mg). Silver triflate (27 mg) was added, and the mixture was stirred for 3 h at −15° and then overnight at room temperature, diluted with dichloromethane, filtered, washed with ice-cold dilute hydrochloric acid, dilute aqueous ammonium chloride, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (9 g) with 10:1 dichloromethane-acetone to give, first, the tetrasaccharide **23** (32 mg, 50%), and then the tetrasaccharide **25** (10 mg, 16%).

Allyl O-*(2*-O-*acetyl-3,4,6-tri*-O-*benzyl-β*-D-*galactopyranosyl)-(1→2)*-O-*(3,4-di*-O-*benzyl-α*-D-*xylopyranosyl)-(1→6)*-O-*(2,3-di*-O-*acetyl-4*-O-*benzoyl-β*-D-*glucopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**24**). — Benzoyl chloride (0.1 mL) was added at 0° to a solution of **23** (32 mg) in dry pyridine (1.5 mL). The mixture was stirred overnight at room temperature and methanol (0.5 mL) was then added. After 1 h, the mixture was concentrated, and toluene was evaporated several times from the residue, which was eluted from a column of silica gel (3 g) with 12:1 dichloromethane–acetone to give amorphous **24** (31 mg, 91%), $[\alpha]_D$ −7° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz): δ 7.95 and 7.30 (2 m, 30 H, 6 Ph), 5.83 (m, 1 H, $OCH_2CH=CH_2$), 5.37 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.4 Hz, H-3'), 5.34 (dd, 1 H, $J_{1''',2'''}$ 8, $J_{2''',3'''}$ 10 Hz, H-2'''), 5.23 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.4 Hz, H-3), 5.16 (t, 1 H, $J_{4',5'}$ 9.4 Hz, H-4'), 5.04 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, H-2'), 5.01 (dd, 1 H, $J_{1,2}$ 7.8 Hz, H-2), 4.77 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 4.68 (d, 1 H, H-1'''), 4.64 (d, 1 H, H-1), 4.62 (dd, 1 H, $J_{5,6a}$ 2, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.54 (d, 1 H, H-1'), 4.17 (dd, 1 H, $J_{5,6b}$ 5.5 Hz, H-6b), 4.01 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.92 (m, 1 H, H-5), 3.55 (dd, 1 H, $J_{2'',3''}$ 9.5 Hz, H-2''), 3.39 (dd, 1 H, $J_{3''',4'''}$ 3.2 Hz, H-3'''), 2.12, 2.06, 2.04, 2.02, 1.88, 1.87 (6 s, 18 H, 6 Ac).

Anal. Calc. for $C_{80}H_{90}O_{27}$: C, 64.77; H, 6.11. Found: C, 64.90; H, 6.17.

Propyl O-*β*-D-*galactopyranosyl-(1→2)*-O-*α*-D-*xylopyranosyl-(1→6)*-O-*β*-D-*glucopyranosyl-(1→4)-β*-D-*glucopyranoside* (**26**). — The tetrasaccharide **23** (49 mg) was

O-deacetylated in the usual manner (MeOH–MeONa). A solution of the residue in 4:1 methanol-water (5 mL) was hydrogenated in the presence of 10% Pd–C (40 mg) for 24 h, then filtered, and concentrated. The residue was eluted from a column (2.2 × 116 cm) of Sephadex G-10 with water to give amorphous **26** (20 mg, 88%), $[\alpha]_D$ +32° (*c* 0.8, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 5.16 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.57 (d, 1 H, $J_{1''',2'''}$ 7.7 Hz, H-1'''), 4.54 and 4.49 (2 d, 2 H, *J* 7.9 Hz, H-1,1'), 3.98 (dd, 1 H, $J_{5',6'a}$ 2, $J_{6'a,6'b}$ 12 Hz, H-6' a), 3.34 and 3.32 (2 d, 2 H, *J* 7.9 and 9.4 Hz, H-2,2'), 1.64 (m, 2 H, $OCH_2CH_2CH_3$), 0.93 (t, 3 H, $OCH_2CH_2CH_3$).

Anal. Calc. for $C_{26}H_{46}O_{20}\cdot 2H_2O$: C, 43.70; H, 7.05. Found: C, 43.56; H, 7.13.

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