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A Ca^{2+}-mobilising carbohydrate-based polyphosphate: synthesis of 2-hydroxyethyl α-D-glucopyranoside 2′,3,4-trisphosphate

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Abstract

Two routes to a glucose-based mimic of the second messenger 1D-*myo*-inositol 1,4,5-trisphosphate related to adenophostin A are described. Fischer glycosidation of D-glucose with allyl alcohol in the presence of a strong cation-exchange resin gave a 7:3 α:β-anomeric mixture of allyl glucopyranosides (**5ab**) from which the pure α anomer **5a** was isolated by crystallisation. Treatment of **5ab** with 1.05 equiv of dibutyltin oxide followed by 2.1 equiv of benzoyl chloride gave allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside, which was converted in 3 steps into allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside (**4**). Alternatively, treatment of **5a** with 2.5 equiv of dibutyltin oxide followed by benzyl bromide gave allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**9**) which was also converted into **4**. Compound **4** was elaborated to the phosphorylation precursor 2-hydroxyethyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**12**) in a convenient one-pot reaction, and **12** was phosphorylated and deblocked to afford 2-hydroxyethyl α-D-glucopyranoside 2′,3,4-trisphosphate. The 2,6-di-*O*-benzyl derivative **9** was converted in high yield into 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranose, a useful intermediate for the synthesis of adenophostin A and related compounds. © 1996 Elsevier Science Ltd.

Keywords: Adenophostin; Inositol phosphates; Second messenger; Selective protection; Stannylene

1. Introduction

Many cell-surface receptors on stimulation activate phospholipase C, producing the second messenger 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**]. Ins(1,4,5)P_3

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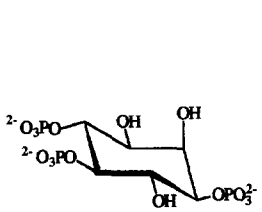
interacts with a family of intracellular receptor-operated Ca^{2+} channels to mobilise non-mitochondrial Ca^{2+} in many cell types [1,2], and much biological interest has followed the discovery of this signalling pathway in 1983 [3]. Additionally, the chemical synthesis of structurally modified analogues of **1** has improved understanding of its structure–activity profiles with respect to its receptor and metabolising enzymes [2,4]. In particular, a vicinal D-*threo* bisphosphate arrangement is essential for Ca^{2+}-releasing activity [2,5], while the third phosphate provides enhanced affinity for the receptor.

The recently discovered adenophostins [6,7] A and B (**2a** and **2b**) are full agonists at the Ins(1,4,5)P_3 receptor, with potencies 10–100 times that of Ins(1,4,5)P_3 [7,8]. The structure of **2a** has recently been confirmed by total synthesis [9]. The exceptional potency of **2a** and **2b** is intriguing, as they bear little apparent resemblance to Ins(1,4,5)P_3. However, the D-glucose 3,4-bisphosphate moiety possesses D-*threo* stereochemistry and the position-2 hydroxyl group may be regarded as analogous to position 6 of Ins(1,4,5)P_5. Indeed, molecular modelling studies [7,10] demonstrate similarity between positions 4, 3, and 2 of the glucose ring of **2a** and positions 4, 5, and 6, respectively, of Ins(1,4,5)P_3. In addition, the adenophostins possess a third phosphate which, similarly to Ins(1,4,5)P_3, is essential for high potency: **2c**, in which this third phosphate is removed, possessed a 1000-fold lower binding affinity than **2a** [7].

Although the broad basis for the activity of **2a** and **2b** is clear, a full structural rationalisation for their high potency is lacking. Consequently, we have prepared 2-hydroxyethyl α-D-glucopyranoside 2',3,4-trisphosphate (**3**) to determine the importance of the adenosine component of **2a**. A preliminary account of this work has appeared [11] and **3** has also been prepared from D-galactose by another group [10]; biological characterisation has been reported [10]. A series of related 2-hydroxyethyl and 3-hydroxypropyl xylopyranosides have been described [12].



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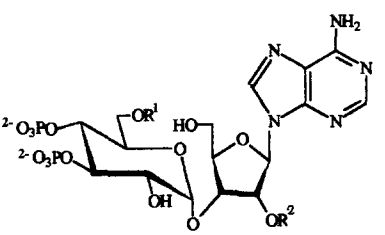
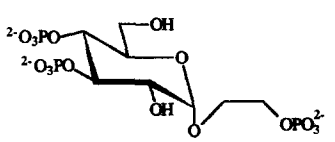


2a R^1=H; R^2=PO_3^{2-}
2b R^1=$COCH_3$; R^2=PO_3^{2-}
2c R^1=R^2=H



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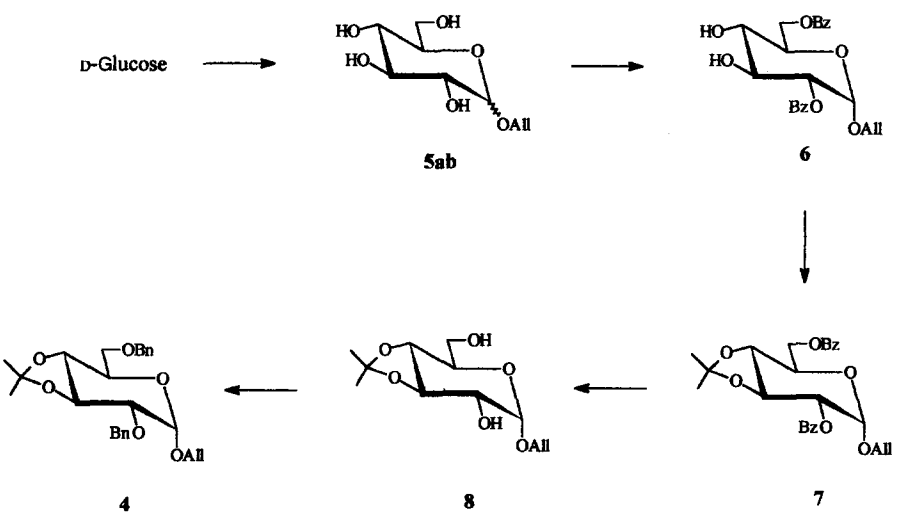
2. Results and discussion

Our route required preparation of the intermediate allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-D-glucopyranoside (**4**). Alcoholysis of D-glucose with allyl alcohol (Scheme 1) in the presence of a strong cation-exchange resin produced a syrup from which, in agreement with Lee and Lee [13], no crystalline product could be isolated. After column chromatography a 7:3 α:β-anomeric mixture **5ab** was obtained, as estimated from the integral ratio of the anomeric protons in the ^{1}H NMR spectrum; the pure α anomer **5a** was isolated only in poor yield (14%) by fractional crystallisation.

Attention then turned to selective, simultaneous protection of positions 2 and 6. Methyl 2,6-di-*O*-benzyl-α-D-glucopyranoside was produced in 30% yield when methyl α-D-glucopyranoside was treated with 1.5 equiv of bis(tributyltin) oxide, followed by neat benzyl bromide [14], presumably via the 2,6-bis(tributyltin) diether. A possible method of disubstitution of **5a** would be to treat it with 2 equiv of bis(tributyltin) oxide and to benzylate it as above. An alternative possibility was to use stannylene acetals.

Reaction of methyl α-D-glucopyranoside with 1 equiv of dibutyltin oxide results in formation of the 2,3-*O*-dibutylstannylene derivative, with selective esterification [15] or alkylation [16] occurring at position 2. In addition, benzyl 2,3-di-*O*-benzyl-α-D-glucopyranoside has been regioselectively methoxymethylated at position 6 via its 4,6-*O*-dibutylstannylene derivative [17]. Therefore, we reasoned that treating an alkyl glucopyranoside with more than 2 equiv of dibutyltin oxide ought to result first in the formation of the 2,3-*O*-dibutylstannylene derivative, followed by the 2,3:4,6-di-*O*-dibutylstannylene derivative. The latter derivative of an α anomer would be expected to direct substitution at positions 2 and 6.

Since it had been more difficult than expected to isolate the α anomer **5a** from the glycosidation reaction, an ideal situation would be the isolation of a 2,6-disubstituted



Scheme 1. All = allyl; Bn = benzyl; Bz = benzoyl.

α-anomeric derivative directly from reactions on **5ab**. Since such methodology would potentially be of general interest to carbohydrate chemists, both esterification and alkylation were attempted using a bis-stannylene approach.

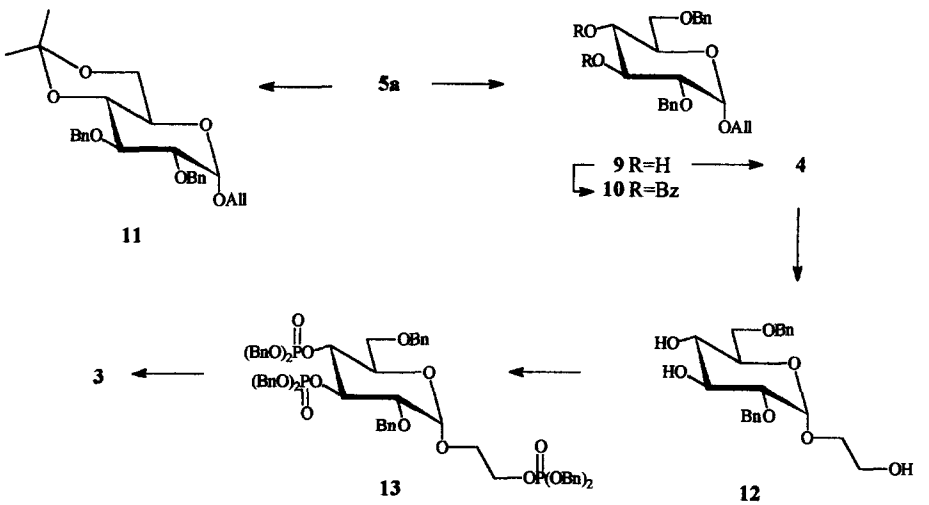
When **5ab** was treated with 2.5 equiv of dibutyltin oxide in toluene and the reaction mixture cooled, a precipitate formed which could not be redissolved (and therefore benzoylated) in toluene or dioxane. In related studies Qin and Grindley [18] noted the poor solubility of methyl 2,3:4,6-di-*O*-dibutylstannylene-α-D-glucopyranoside in chloroform and suggested oligomerisation as the reason. Reaction of **5ab** with 1.05–1.2 equiv of dibutyltin oxide did not cause precipitation, and treatment of the cooled solution with 2.1 equiv of benzoyl chloride gave a mixture of products from which the known [19] allyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside (**6**) could be isolated by a combination of column chromatography and crystallisation. This two-step preparation of **6** from D-glucose represents an improvement on the five steps of a previous report [19].

Reaction of **6** with 2-methoxypropene and *p*-toluenesulfonic acid in acetone provided **7**. It is noteworthy that the best yields of 3,4-*O*-isopropylidene derivatives were obtained with a short reaction time. Subsequently, a variation of this method has been described on the enantiomer of **6** using THF as solvent over a longer period [20]. Basic methanolysis of the benzoate esters provided allyl 3,4-*O*-isopropylidene-α-D-glucopyranoside (**8**), which was benzylated with sodium hydride and benzyl bromide in DMF to give **4**.

Having established one route to **4**, we turned our attention to potential direct dibenzylation. Alkylation is carried out at higher temperatures than esterification, and at such temperatures the bis-stannylene intermediate might be soluble. As benzylation tends to be less selective than benzoylation, the pure α anomer was used initially to test the viability of this method. Reaction of **5a** with 2.5 equiv of dibutyltin oxide followed by treatment of the product with benzyl bromide in refluxing toluene or acetonitrile (both containing quaternary ammonium salts [21]) resulted in sluggish reactions producing many products (TLC), none of which dominated and which were not completely separable by column chromatography. However, when the stannylene derivative was stirred with neat benzyl bromide at elevated temperature for 2 days, a major product and several minor products were observed. After workup and removal of benzyl bromide, the required 2,6-di-*O*-benzyl derivative **9** (Scheme 2) was obtained in 44% yield by crystallisation (two crops). The structure of **9** was confirmed by preparation of the dibenzoate **10** which showed deshielded triplets, corresponding to H-3 and H-4, in the ^{1}H NMR spectrum, and by reaction with 2-methoxypropene to give **4**. Treatment of the mother liquor from benzylation with 2-methoxypropene provided a single new product, identified as allyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (**11**) [1] on the basis of the characteristic δ_C values of the isopropylidene carbons [23], and thereby establishing that all of **9** had been isolated by crystallisation.

The benzylation technique was then applied to **5ab** on a 35 g-scale. In this case **9** could still be crystallised from the (more complicated) product mixture, but only in poor

[1] Since the submission of this manuscript the conversion of the dibutylstannylene acetal of a diol into a dialkyl derivative on heating with alkyl halides at high temperature has been reported [22].



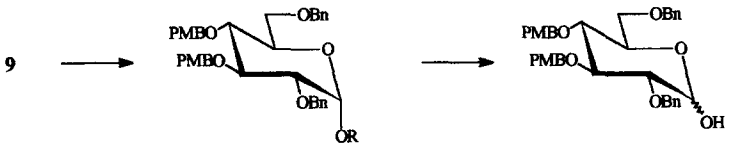
Scheme 2.

yield (ca. 15%). Reaction of the mother liquor with 2-methoxypropene in this case gave a mixture of several acetal-containing species which could not be separated further. Although the yield of crystalline **9** isolated from the anomeric mixture was rather disappointing, this method has been found to be the most convenient overall to produce **9** in multigram quantities. While this work was in progress similar selectivity was reported for the corresponding methyl α-glycoside [18], confirming the general applicability of this method.

Sequential treatment of **4** with OsO_4–$NaIO_4$ followed by excess of $NaBH_4$ [24] directly provided 2-hydroxyethyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**12**). Loss of the ketal was unexpected and presumably occurs by production of osmic acid during reduction of the intermediate aldehyde. Phosphorylation of **12** with dibenzyloxy(diisopropylamino)phosphine [25] followed by oxidation of intermediate phosphites with *m*-chloroperoxybenzoic acid (MCPBA) gave **13**. The eight benzyl groups of **13** were removed in one step using sodium in liquid ammonia [26] to provide the target trisphosphate **3** which was purified by ion-exchange chromatography. The product was quantified by total phosphate assay. It was not possible to determine accurately the stoichiometry of the triethylammonium salt.

Trisphosphate **3** was examined for Ca^{2+}-mobilising activity at the platelet Ins(1,4,5)P_3 receptor [27]. It was found to be a full agonist but with a potency some 10-fold lower than Ins(1,4,5)P_3 in agreement with another study in SH-SY5Y neuroblastoma cells [10], thereby demonstrating that at least part of the adenosine motif is important for the extreme potency of the adenophostins. Further biological characterisation of our material will be published elsewhere.

Diol **9** was easily converted in high yield into 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-D-glucopyranose (**16**) (Scheme 3), a selectively protected intermediate without the labile *trans* isopropylidene group, ideal for the preparation of **2a** and



14 R=All
15EZ R=prop-1-enyl

16

Scheme 3. PMB = *p*-methoxybenzyl.

analogues using trichloroacetimidate methodology [28]. Thus, *p*-methoxybenzylation with sodium hydride and *p*-methoxybenzyl chloride gave fully protected **14**, which was isomerised to the corresponding prop-1-enyl glycosides **15EZ** using potassium *tert*-butoxide in Me_2SO [29]. Removal of the propenyl group with no loss of *p*-methoxybenzyl ethers was achieved using acid hydrolysis [30], giving crystalline **16** as a 1:1 anomeric mixture. The use of this intermediate in adenophostin synthesis is in progress.

3. Experimental

Materials and methods.—TLC was performed on precoated plates (Merck aluminium sheets silica 60 F_{254}, Art. no. 5554). Products were visualised by spraying phosphomolybdic acid in MeOH followed by heating. Flash chromatography refers to the method of Still et al. [31] and was carried out using Sorbsil C60 silica gel.

The ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM GX-270 or GX-400 spectrometer. Unless otherwise stated, chemical shifts were measured in ppm relative to internal tetramethylsilane. The ^{31}P NMR spectra were recorded on a JEOL FX-90Q or GX-400 spectrometer and ^{31}P NMR chemical shifts were measured in ppm relative to external 85% H_3PO_4. *J* values are given in Hz. Melting points (uncorrected) were determined using a Reichert–Jung Thermo Galen Kofler block. Microanalysis was carried out at the University of Bath Microanalysis Service. Mass spectra were recorded at the University of Bath Mass Spectrometry service and at the EPSRC Mass Spectrometry Service Centre, Swansea. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter. Ion-exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using Sepharose Q Fast Flow resin and gradients of triethylammonium hydrogen carbonate (TEAB) as eluent. Compounds containing phosphates were assayed quantitatively by the Briggs phosphate test [32].

Allyl α-D-glucopyranoside (**5a**).—A mixture of D-glucose (18 g, 0.1 mole), Dowex 50-X2-100 (H^+) (10 g), and allyl alcohol (200 mL) was heated under reflux for 90 min. The suspension was cooled, then filtered, and the residue was well washed with EtOH (30 mL). The combined filtrate and washings were concentrated and the syrupy residue was subjected to flash chromatography (loading solvent and eluent, 9:1 EtOAc–MeOH). The eluate was concentrated to give a white solid **5ab** (15.6 g), which was shown by ^1H NMR (D_2O; 270 MHz; ref. int. HDO) to be a ca. 7:3 α:β-anomeric mixture (H-1_α, δ

4.92, $J_{1,2}$ 3.7 Hz; H-1$_\beta$, δ 4.46, $J_{1,2}$ 7.9 Hz). Fractional crystallisation from EtOAc–EtOH (ca. 1:1) gave the pure α anomer **5a** (3.1 g, 14%); mp 99–100 °C, lit. [33] 100.5–101.5 °C; $[\alpha]_D$ +140.6° (*c* 1.4, H_2O), lit. [33] +151.1°.

*Allyl 2,6-di-*O*-benzoyl-α-*D*-glucopyranoside* (**6**).—A mixture of **5ab** (5.0 g, 22.7 mmol) and dibutyltin oxide (5.9 g, 23.8 mmol) was heated under reflux in dry toluene (150 mL) for 2 h with continuous azeotropic removal of water (Dean–Stark trap). The solution was cooled to 0 °C and triethylamine (1 mL) and benzoyl chloride (5.5 mL, 47.7 mmol, dropwise) were added. The solution was allowed to warm to room temperature and was stirred for a further 16 h, when TLC (EtOAc) indicated a major product (R_f 0.63) and several minor products. MeOH (10 mL) was added and stirring was continued for 5 min. The solvents were evaporated and the residue was taken up in ether (250 mL). The solution was stirred with saturated aq $NaHCO_3$ (150 mL) for 30 min, and the resulting suspension was filtered through Celite. The ethereal layer was dried ($MgSO_4$), filtered, and concentrated to give an orange oil, which was subjected to flash chromatography (eluent 7:3 hexane–EtOAc). The eluate was concentrated and the residue was crystallised from EtOH to give the title compound (3.4 g, 35%); mp 135–136 °C, lit. [19] 136–137 °C; $[\alpha]_D$ +70.1° (*c* 4.3, $CHCl_3$), lit. [19] +74°.

*Allyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-α-*D*-glucopyranoside* (**7**).—A solution of **6** (1.2 g, 2.8 mmol) in acetone (30 mL) was stirred with *p*-toluenesulfonic acid (30 mg) and 2-methoxypropene (1.5 mL, 15.7 mmol) at room temperature for 5 min, when TLC (3:2 hexane–EtOAc) indicated complete conversion of starting material (R_f 0.13) into a product (R_f 0.48). The solvents were evaporated and the residue dissolved in ether (50 mL). The organic extract was washed with saturated aq $NaHCO_3$ (30 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (eluent 19:1 then 17:3 hexane–EtOAc) to give **7** as a pale-yellow oil (774 mg, 59%); $[\alpha]_D$ +69.4° (*c* 2.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 1.47, 1.50 (2 s, 6 H, CMe_2), 3.54 (t, 1 H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4), 4.03 (m, 1 H, C*H*HCH = CH_2), 4.19–4.32 (m, 3 H, CH*H*CH = CH_2, H-3, H-5), 4.48 (dd, 1 H, $J_{6a,6b}$ 12.2, $J_{6a,5}$ 6.3 Hz, H-6a), 4.68 (dd, 1 H, $J_{6a,6b}$ 12.2, $J_{6b,5}$ 2.4 Hz, H-6b), 5.10 (m, 1 H, ^3J 10.3, ^2J 1.0 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.19–5.25 (m, 2 H, $CH_2CH = CH_{cis}H_{trans}$, H-2), 5.36 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.80 (m, 1 H, $CH_2CH = CH_2$), 7.43–7.59 (m, 6 H, Ph), 8.06–8.11 (m, 4 H, Ph); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 26.56, 26.82 (CMe_2), 64.01 (C-6), 69.12 ($CH_2CH = CH_2$), 70.01, 73.20, 74.76, 75.45 (C-2–C-5), 95.74 (C-1), 111.56 (CMe_2), 117.85 ($CH_2CH = CH_2$), 128.38, 128.44 (aromatic CH), 129.48 (C-1 of Ph), 129.68 (aromatic CH), 129.94 (C-1 of Ph), 129.99 (aromatic CH), 133.16 (aromatic CH), 133.34 ($CH_2CH = CH_2$), 133.47 (aromatic CH), 165.95, 166.19 (2 × C = O); FAB^+ mass spectrum: *m/z* 469 ($[M + 1]^+$, 74%). Anal. Calcd for $C_{26}H_{28}O_8$ (468.50): C, 66.64; H, 6.03. Found: C, 66.9; H, 6.20.

*Allyl 3,4-*O*-isopropylidene-α-*D*-glucopyranoside* (**8**).—NaOH (514 mg, 12.9 mmol) was added to a solution of **7** (1.5 g, 3.2 mmol) in MeOH (100 mL) and the mixture was heated under reflux for 1 h. After cooling, CO_2 was bubbled through the cooled solution for several hours. The solvent was evaporated and the glassy residue was extracted with $CHCl_3$ (2 × 100 mL). The combined organic extracts were washed with water (100 mL), dried ($MgSO_4$), filtered, and concentrated. The resultant residue was purified by flash chromatography (eluent 7:3 then 1:1 hexane–EtOAc) to give the title compound as

a colourless oil (779 mg, 93%); R_f 0.35 (EtOAc); $[\alpha]_D$ +114.0° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 1.45, 1.46 (2 s, 6 H, CMe_2), 2.07 (br s, 1 H, exch. D_2O, 6-OH), 2.40 (d, 1 H, *J* 10.4 Hz, exch. D_2O, 2-OH), 3.30 (t, 1 H, $J_{4,3} = J_{4,5} = 9.2$ Hz, H-4), 3.73–3.92 (m, 5 H, H-2, H-3, H-5, H-6a, H-6b), 4.04–4.11 (m, 1 H, C*H*HCH = CH_2), 4.23–4.30 (m, 1 H, CH*H*CH = CH_2), 5.01 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.25 (m, 1 H, ^3J 10.3, ^2J 1.4 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.32 (m, 1 H, ^3J 17.2, ^2J 1.5 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.92 (m, 1 H, $CH_2CH = CH_2$); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 26.42, 26.79 (CMe_2), 62.40 (C-6), 69.07 ($CH_2CH = CH_2$), 71.83, 72.39, 73.98, 78.75 (C-2–C-5), 97.97 (C-1), 111.12 (CMe_2), 118.19 ($CH_2CH = CH_2$), 133.34 ($CH_2CH = CH_2$); FAB^+ mass spectrum: *m/z* 261 ($[M + 1]^+$, 78%). Anal. Calcd for $C_{12}H_{20}O_6$ (260.29): C, 55.36; H, 7.75. Found: C, 55.2; H, 8.05.

*Allyl 2,6-di-*O*-benzyl-α-*D*-glucopyranoside* (**9**).—A mixture of **5a** (2.0 g, 9.1 mmol) and dibutyltin oxide (5.7 g, 22.7 mmol) was heated under reflux in dry toluene (300 mL) for 4 h with continuous azeotropic removal of water (Dean–Stark trap). The solution was cooled and concentrated to give a white residue which was dried on a vacuum line for 2 h. Benzyl bromide (18 mL, 151.4 mmol) was added and this mixture was stirred under N_2 at 100–110 °C for 2 days. The pale-yellow solution thus obtained was cooled, diluted with ether (100 mL), and vigorously stirred with saturated aq $NaHCO_3$ (75 mL) for 30 min. The resultant suspension was filtered through Celite and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (eluent 8:2 hexane–EtOAc to remove benzyl bromide, then 1:1) gave a mixture of benzylated products (2.8 g, R_f 0.30–0.51 in EtOAc). Crystallisation from diisopropyl ether gave exclusively **9** (1.6 g, 44%); R_f 0.22 (3:2 EtOAc–hexane); mp 74–77 °C; $[\alpha]_D$ +76.4° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$; 270 MHz): δ 3.12 (br s, 2 H, exch. D_2O, 2 × OH), 3.37 (dd, 1 H, $J_{2,1}$ 3.7, $J_{2,3}$ 9.7 Hz, H-2), 3.58 (t, 1 H, $J_{4,3} = J_{4,5} = 9.2$ Hz, H-4), 3.68–3.78 (m, 3 H, H-5, H-6a, H-6b), 3.88–3.98 (m, 2 H, H-3, C*H*HCH = CH_2), 4.10–4.15 (m, 1 H, CH*H*CH = CH_2), 4.54, 4.59 (AB, 2 H, J_{AB} 12.2 Hz, PhCH_2O), 4.62, 4.67 (AB, 2 H, J_{AB} 12.1 Hz, PhCH_2O), 4.81 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.18 (m, 1 H, ^3J 10.3, ^2J 1.0 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.29 (m, 1 H, ^3J 17.2, ^2J 1.0 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.89 (m, 1 H, $CH_2CH = CH_2$), 7.25–7.33 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 68.29 ($CH_2CH = CH_2$), 69.44 (C-6), 70.03, 70.74, 72.72 (C-3–C-5), 72.75, 73.49 (2 × PhCH_2), 79.08 (C-2), 95.46 (C-1), 117.87 ($CH_2CH = CH_2$), 127.58, 127.96, 128.05, 128.31, 128.46 (Ph), 133.71 ($CH_2CH = CH_2$), 137.90, 137.96 (2 × C-1 of Ph); FAB^- mass spectrum: *m/z*: 553 ($[M + NBA]^-$, 100%). Anal. Calcd for $C_{23}H_{28}O_6$ (400.47): C, 68.97; H, 7.05. Found: C, 68.8; H, 7.08.

A sample of **9** was converted into its 3,4-dibenzoate **10** with benzoyl chloride in pyridine in the usual way; R_f 0.72 (3:2 EtOAc–hexane); $[\alpha]_D$ −10.0° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 3.57–3.61 (m, 2 H, H-6a, H-6b), 3.80 (dd, 1 H, $J_{2,1}$ 3.9, $J_{2,3}$ 10.1 Hz, H-2), 4.08 (m, 1 H, C*H*HCH = CH_2), 4.19 (ddd, 1 H, $J_{5,6a}$ 5.7, $J_{5,6b}$ 9.3, $J_{5,4}$ 9.8 Hz, H-5), 4.25 (m, 1 H, CH*H*CH = CH_2), 4.45–4.60 (m, 4 H, 2 × PhCH_2O AB systems), 5.00 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.25 (m, 1 H, ^3J 10.3, ^2J 1.5 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.38 (m, 1 H, ^3J 17.6, ^2J 1.5 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.52 (t, 1 H, $J_{4,3} = J_{4,5} = 10.3$ Hz, H-4), 5.95–6.00 (m, 2 H, t of H-3 overlapping with $CH_2CH = CH_2$), 7.14–8.13 (m, 20 H, Ph).

The mother liquor of the benzylation mixture was treated with 2-methoxypropene and

p-toluenesulfonic acid in acetone as described for **7**. A single new product was formed (TLC: R_f 0.71 in 3:2 EtOAc–hexane). Purification and flash chromatography as for **7** gave allyl 2,3-di-*O*-benzyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (**11**) as a pale-yellow oil (480 mg, 12% from **5a**); $[\alpha]_D$ +26.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ 1.43, 1.48 (2 s, 6 H, CMe_2), 3.50 (dd, 1 H, $J_{2,1}$ 3.9, $J_{2,3}$ 9.3 Hz, H-2), 3.61–3.72 (m, 3 H, H-4, H-5, H-6ax), 3.83 (dd, 1 H, $J_{6eq,5}$ 3.4, $J_{6eq,6ax}$ 8.3 Hz, H-6eq), 3.90 (t, 1 H, $J_{3,2} = J_{3,4} = 9.3$ Hz, H-3), 3.97–4.03 (m, 1 H, C*H*HCH = CH_2), 4.14–4.18 (m, 1 H, CH*H*CH = CH_2), 4.65, 4.81 (AB, 2 H, J_{AB} 12.2 Hz, PhCH_2O), 4.75 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.80, 4.85 (AB, 2 H, J_{AB} 11.7 Hz, PhCH_2O), 5.23 (m, 1 H, ^3J 10.3, ^2J 1.0 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.32 (m, 1 H, ^3J 17.1, ^2J 1.0 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.93 (m, 1 H, $CH_2CH = CH_2$), 7.24–7.39 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 19.18 (axial C*Me*), 29.22 (equatorial C*Me*), 62.58 (C-6), 63.37 (CH), 68.36 ($CH_2CH = CH_2$), 73.57 (PhCH_2O), 74.91 (CH), 75.07 (PhCH_2O), 78.99, 79.11 (C-2, C-3), 96.78 (C-1), 99.34 (CMe_2), 118.23 ($CH_2CH = CH_2$), 127.43, 127.78, 127.85, 128.02, 128.22, 128.36 (Ph), 133.69 ($CH_2CH = CH_2$), 138.29, 139.08 (2 × C-1 of Ph); FAB$^+$ mass spectrum: *m/z* 441 ([M + 1]$^+$, 40%). Anal. Calcd for $C_{26}H_{32}O_6$ (440.54): C, 70.89; H, 7.32. Found: C, 71.1; H, 7.40.

*Allyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-α-*D*-glucopyranoside* (**4**).—*(a)* A solution of **8** (717 mg, 2.8 mmol) in dry DMF (5 mL) was stirred with NaH (215 mg of an 80% w/w dispersion in mineral oil, 6.9 mmol) and benzyl bromide (0.7 mL, 6.1 mmol) at room temperature for 3 h. MeOH (5 mL) was added and stirring was continued for 15 min. The solvents were evaporated and the residue was dissolved in ether (100 mL). The ethereal extract was washed with water (50 mL), dried ($MgSO_4$), filtered, and concentrated. The residual oil was purified by flash chromatography (eluent 19:1 hexane–EtOAc) to give the title compound as a pale-yellow oil (922 mg, 76%); R_f 0.68 (2:3 hexane–EtOAc); $[\alpha]_D$ +27.8° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 1.45, 1.47 (2 s, 6 H, CMe_2), 3.34 (t, 1 H, $J_{4,3} = J_{4,5} = 9.8$ Hz, H-4), 3.61 (dd, 1 H, $J_{6a,6b}$ 10.7, $J_{6a,5}$ 5.4 Hz, H-6a), 3.70 (dd, 1 H, $J_{2,1}$ 2.4, $J_{2,3}$ 10.3 Hz, H-2), 3.74 (dd, 1 H, $J_{6a,6b}$ 10.7, $J_{6b,5}$ 2.4 Hz, H-6b), 3.97–4.03 (m, 3 H, H-3, H-5, C*H*HCH = CH_2), 4.13–4.21 (m, 1 H, CH*H*CH = CH_2), 4.59, 4.60 (AB, 2 H, J_{AB} 12.7 Hz, PhCH_2O), 4.63, 4.84 (AB, 2 H, J_{AB} 12.2 Hz, PhCH_2O), 4.92 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.19 (m, 1 H, ^3J 10.7, ^2J 1.4 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.33 (m, 1 H, ^3J 17.1, ^2J 1.5 Hz, $CH_2CH = CH_{cis}H_{trans}$), 5.92 (m, 1 H, $CH_2CH = CH_2$), 7.25–7.58 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 26.54, 26.92 (C*Me*$_2$), 68.65 ($CH_2CH = CH_2$), 69.42 (C-6), 71.01 (CH), 71.96, 73.41 (2 × PhCH_2O), 74.32, 77.60, 77.83 (3 × CH), 96.80 (C-1), 110.75 (CMe_2), 117.90 ($CH_2CH = CH_2$), 127.47, 127.56, 127.74, 128.02, 128.35 (Ph), 133.83 ($CH_2CH = CH_2$), 138.11 (C-1 of Ph); FAB$^+$ mass spectrum: *m/z* 441 ([M + 1]$^+$, 12%). Anal. Calcd for $C_{26}H_{32}O_6$ (440.54): C, 70.89; H, 7.32. Found: C, 70.9; H, 7.38.

(b) Using the method for the preparation of **7** from **6**, the title compound was prepared in 71% yield from **9**.

*2-Hydroxyethyl 2,6-di-*O*-benzyl-α-*D*-glucopyranoside* (**12**).—A saturated aqueous solution of $NaIO_4$ (20 mL) containing OsO_4 (80 mg, 0.3 mmol) was added to a solution of **4** (392 mg, 0.9 mmol) in ether (20 mL) and the mixture was vigorously stirred at room temperature for 5 h, when TLC (2:3 EtOAc–hexane) indicated consumption of starting

material (R_f 0.56) to give a product (R_f 0.08–0.18). The mixture was diluted with ether (100 mL) and water (50 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated to give a dark-grey residue which was dissolved in MeOH (30 mL) and cooled to 0 °C. Sodium borohydride (480 mg, 12.7 mmol) was added and the suspension was stirred for 1 h. The mixture was concentrated and the residue partitioned between ether (100 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (eluent 1:1 hexane–EtOAc then EtOAc) gave **12** as a colourless oil (203 mg, 56%); R_f 0.52 (4:1 EtOAc–MeOH); $[\alpha]_D$ +48.2° (*c* 2.6, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 3.35 (dd, 1 H, $J_{2,1}$ 3.4, $J_{2,3}$ 9.8 Hz, H-2), 3.38–3.43 (m, 1 H, -C*H*HCH$_2$-), 3.45 (t, 1 H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4), 3.58–3.69 (m, 6 H, simplifies to 5 H, m, on D_2O exch., OH, H-6a, H-6b, -CH*H*C*H*$_2$-), 3.74 (ddd, 1 H, $J_{5,6a}$ 2.4, $J_{5,6b}$ 4.9, $J_{5,4}$ 9.8 Hz, H-5), 3.83 (br s, 2 H, exch. D_2O, 2 × OH), 3.97 (t, 1 H, $J_{3,2} = J_{3,4} = 9.8$ Hz, H-3), 4.50, 4.55 (AB, 2 H, J_{AB} 12.2 Hz, PhC*H*$_2$O), 4.56 (AB, 1 H, J_{AB} 12.2 Hz, PhC*H*HO), 4.66 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.73 (AB, 1 H, J_{AB} 12.2 Hz, PhCH*H*O), 7.23–7.36 (m, 10 H, Ph); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 61.47 (C-2′), 69.37 (C-6), 70.28 (C-1′), 70.50, 70.63, 72.88 (C-3, C-4, C-5), 73.55 (2 × PhC*H*$_2$O), 79.52 (C-2), 97.15 (C-1), 127.67, 127.71, 128.33, 128.38, 128.44, 128.66 (Ph), 137.54, 138.00 (2 × C-1 of Ph); FAB$^-$ mass spectrum: *m/z* 403 [M − 1]$^-$, 50%; 557 [M + NBA]$^-$, 100. Anal. Calcd for $C_{22}H_{28}O_7$ (404.46): C, 65.32; H, 6.98. Found: C, 65.6; H, 7.12.

*2-Dibenzyloxyphosphoryloxyethyl 2,6-di-O-benzyl-3,4-bis-O-(dibenzyloxyphosphoryl)-α-*D*-glucopyranoside* (**13**).—A mixture of dibenzyloxy(diisopropylamino)phosphine (565 mg, 1.6 mmol), dry CH_2Cl_2 (3 mL), and 1*H*-tetrazole (220 mg, 3.1 mmol) was stirred at room temperature for 15 min, whereupon a solution of **12** (106 mg, 0.26 mmol) in dry CH_2Cl_2 (2 mL) was added and stirring was continued for a further 30 min. TLC (EtOAc) indicated complete conversion of starting material (R_f 0.17) into a product (R_f 0.76 with streaking) and ^{31}P NMR spectroscopy showed phosphite triester signals at 140.72, 141.31 (AB, J_{AB} 4.9 Hz), and 139.05 ppm. The system was cooled to −78 °C, MCPBA (900 mg) was added, and the system was warmed to room temperature. After 10 min TLC showed conversion of the trisphosphite into a new product (R_f 0.46). The mixture was extracted with EtOAc (100 mL) and the organic extract was washed with 50 mL each of aq 10% Na_2SO_3, 1 M HCl, saturated aq $NaHCO_3$, and saturated aq NaCl. The organic solution was dried ($MgSO_4$), filtered, and concentrated to give a pale-yellow solid, which was purified by flash chromatography (eluent 20:1 then 10:1 $CHCl_3$–acetone) to give the title compound as a colourless oil (247 mg, 80%); $[\alpha]_D$ +12.5° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 3.45–3.51 (m, 1 H, OC*H*HCH$_2$OP[O][OBn]$_2$), 3.56 (dd, 1 H, $J_{2,1}$ 3.4, $J_{2,3}$ 9.3 Hz, H-2), 3.65–3.70 (m, 2 H, H-6a, OCH*H*CH$_2$OP[O][OBn]$_2$), 3.73–3.77 (dd, 1 H, $J_{6b,6a}$ 10.7, $J_{6b,5}$ 3.9 Hz, H-6b), 3.89 (ddd, 1 H, $J_{5,6b}$ 3.9, $J_{5,6a}$ 9.3, $J_{5,4}$ 9.8 Hz, H-5), 4.09–4.17 (m, 2 H, OCH$_2$C*H*$_2$OP[O][OBn]$_2$), 4.32, 4.45 (AB, 2 H, J_{AB} 11.7 Hz, PhC*H*$_2$O), 4.43–4.48 (AB, 1 H, PhC*H*HO), 4.61–4.68 (m, 3 H, PhCH*H*O, H-3, H-4), 4.86–5.07 (m, 13 H, 6 × PhC*H*$_2$O AB systems, H-1), 7.12–7.32 (m, 40 H, Ph); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 66.10, 66.95 [C-2′ (with C–P coupling)], 68.00 (C-6 or C-1′), 66.94, 69.15 [POC*H*$_2$Ph (with C–P coupling)], 69.35, 69.40, 69.73 (6 × POC*H*$_2$Ph), 73.08, 73.21 (2 × PhC*H*$_2$O), 74.40, 77.91, 78.42, 78.46 (C-2–C-5), 96.69

(C-1), 127.50, 127.61, 127.74, 127.85, 127.91, 128.00, 128.03, 128.24, 128.31, 128.40, 128.47, 128.58 (Ph CH), 135.72, 135.79, 135.86, 135.92, 136.15, 136.23 (6 × C-1 of benzyl ester rings), 137.95, 138.07 (2 × C-1 of benzyl ether rings); ^{31}P NMR ($CDCl_3$; 162 MHz): δ −2.29 (sextet, 1 P, J_{HP} 8.0 Hz), −1.85 (sextet, 1 P, J_{HP} 7.6 Hz), −0.89 (septet, 1 P, J_{HP} 7.9 Hz, $OCH_2CH_2OP[O][OBn]_2$); FAB$^+$ mass spectrum: m/z 1186 ([M + 1]$^+$, 60%). Anal. Calcd for $C_{64}H_{67}O_{16}P_3$ (1185.15): C, 64.84; H, 5.70. Found: C, 65.1; H, 5.62.

2-Hydroxyethyl α-D-glucopyranoside 2',3,4-trisphosphate (**3**).—Ammonia was condensed into a three-necked flask at −78 °C. An excess of Na was added to dry the liquid NH_3, about 30 mL of which was then distilled into a second three-necked flask and kept at −78 °C. Sodium was added until the solution remained blue for 10 min. A solution of **13** (106 mg, 89 μmol) in dry dioxane (2 mL) was added. The mixture was stirred for ca. 90 s, then the reaction was quenched with MeOH (1 mL), followed by water (1 mL). The solvents were evaporated. The residue was dissolved in de-ionised water (300 mL) to give an opalescent solution and purified by ion-exchange chromatography on Q Sepharose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8.0. The triethylammonium salt of **3** was eluted between 470–550 mM buffer. Fractions containing **3**, as judged by the Briggs phosphate assay [32], were combined and concentrated to give a residue from which MeOH was evaporated three times to give glassy **3** as its triethylammonium salt (31 μmol, 35%); $[\alpha]_D$ +90.5° (*c* 0.8 calcd for free acid, TEAB, pH 8.6); ^{1}H NMR (D_2O, pH ~4; 400 MHz): δ 3.54–3.60 (m, 1 H, H-5), 3.55 (dd, 1 H, *J* 3.9, 9.8 Hz, H-2), 3.67–3.74 (m, 4 H, $OCH_2CH_2OPO_3^{2-}$, H-6a, H-6b), 3.82–3.91 (m, 3 H, H-3 or H-4, $OCH_2CH_2OPO_3^{2-}$), 4.26 (ddd, 1 H, *J* 8.8, 9.3 Hz, H-3 or H-4), 4.85 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1); ^{31}P NMR (D_2O, pH ~4; 36 MHz): δ 0.32, 0.45, 0.52 (3 s); FAB$^-$ mass spectrum: m/z 463 ([M − 1]$^-$, 100%). Mass Calcd for $C_8H_{18}O_{16}P_3$ [M-H]$^-$: 462.981. Found: 462.983.

Allyl 2,6-di-O-benzyl-3,4-di-O-(p-methoxybenzyl)-α-D-glucopyranoside (**14**).—A solution of **9** (1.9 g, 4.7 mmol) in dry DMF (10 mL) was stirred with NaH (326 mg of an 80% w/w dispersion in mineral oil, 10.4 mmol) and *p*-methoxybenzyl (PMB) chloride (1.4 mL, 10.0 mmol) at room temperature for 3 h, when TLC (3:2 EtOAc–hexane) indicated consumption of starting material (R_f 0.27) to give a product (R_f 0.71). MeOH (5 mL) was added and stirring was continued for 15 min. The solvents were concentrated and the residue was dissolved in $CHCl_3$ (200 mL). The organic solution was washed with water (100 mL), dried ($MgSO_4$), filtered, and concentrated. The residual oil was purified by flash chromatography (eluent 19:1 hexane–EtOAc) to give the title compound as a pale-yellow oil (2.2 g, 72%); $[\alpha]_D$ +13.8° (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 3.55 (dd, 1 H, $J_{2,1}$ 3.7, $J_{2,3}$ 9.7 Hz, H-2), 3.57–3.64 (m, 2 H, H-4, H-6a), 3.68–3.80 (m, 2 H, H-5, H-6b), 3.77, 3.80 (2 s, 6 H, 2 × OMe), 3.95–4.18 (m, 3 H, H-3, $CH_2CH=CH_2$), 4.36–4.94 (m, 9 H, 4 × $ArCH_2O$ AB systems, H-1), 5.19 (m, 1 H, ^3J 10.4, ^2J 0.9 Hz, $CH_2CH=CH_{cis}H_{trans}$), 5.29 (m, 1 H, ^3J 17.2, ^2J 1.0 Hz, $CH_2CH=CH_{cis}H_{trans}$), 5.90 (m, 1 H, $CH_2CH=CH_2$), 6.78–6.88 (m, 4 H, 2 × H-3 and H-5 of PMB rings), 7.04 (d, 2 H, *J* 8.5 Hz, H-2 and H-6 of PMB ring), 7.24–7.38 (m, 12 H, aromatic CH); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 55.24 (OMe), 68.15, 68.45 (C-6 and $CH_2CH=CH_2$), 70.27 (C-5), 73.17, 73.40, 74.63, 75.36 (4 × $ArCH_2O$), 77.39, 79.94, 81.88 (C-2, C-3, C-4), 95.72 (C-1), 113.75, 113.78 (C-3

and C-5 of PMB rings), 118.06 ($CH_2CH=CH_2$), 127.60, 127.78, 127.83, 128.02, 128.31, 128.36, 129.48, 129.53 (Ar), 131.13 [C-1 of PMB ring(s)], 133.79 ($CH_2CH=CH_2$), 137.99, 138.27 (2 × C-1 of benzyl rings), 159.19, 159.14 (2 × C-4 of PMB rings); FAB^+ mass spectrum: m/z 641 ($[M+1]^+$, 10%). Anal. Calcd for $C_{39}H_{44}O_8$ (640.77): C, 73.09; H, 6.93. Found: C, 73.4; H, 6.88.

*(cis-Prop-1-enyl) 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-α-*D*-glucopyranoside* (**15Z**).—A solution of **14** (2.0 g, 3.1 mmol), and freshly sublimed potassium *tert*-butoxide (1.8 g, 15.6 mmol) in dry Me_2SO (20 mL) was stirred at 50 °C under N_2 for 3 h, when TLC (10:1 $CHCl_3$–acetone) indicated total consumption of starting material (R_f 0.66) to give a product (R_f 0.71). The dark-brown solution was cooled, water (20 mL) was added, and the system was extracted with ether (3 × 100 mL). The combined organic extracts were washed with saturated aq KCl (2 × 150 mL), dried ($MgSO_4$), filtered, and concentrated. The pale-yellow oil thus obtained was purified by flash chromatography (eluent 4:1 hexane–EtOAc) to give a mixture of *trans*- and *cis*-prop-1-enyl isomers **15EZ** (1.7 g, 85%). A portion was recolumned twice with the same solvent system to provide an analytical sample of the pure *cis* isomer **15Z** as a pale-yellow oil; $[\alpha]_D$ +2.9° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 270 MHz): δ 1.63 (dd, 3 H, ^4J 1.5, ^3J 6.8 Hz, CH = CH*Me*), 3.56–3.80 (m, 5 H, H-2, H-4, H-5, H-6a, H-6b), 3.77, 3.80 (2 s, 6 H, 2 × OMe), 4.02 (t, 1 H, $J_{3,2}=J_{3,4}=9.2$ Hz, H-3), 4.39–4.94 (m, 10 H, 4 × ArCH_2O AB systems, H-1, CH = C*H*Me), 6.00 (dd, 1 H, ^4J 1.5, ^3J 3.6 Hz, C*H* = CHMe), 6.78–6.89 (m, 4 H, 2 × H-3 and H-5 of PMB rings), 7.05 (d, 2 H, *J* 8.6 Hz, H-2 and H-6 of PMB ring), 7.24–7.34 (m, 12 H, Ar); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 9.62 (CH = CH*Me*), 55.27 (OMe), 68.16 (C-6), 70.75 (C-5), 73.23, 73.37, 74.78, 75.40 (4 × Ar*C*H_2O), 77.12, 79.61, 81.73 (C-2, C-3, C-4), 97.46 (C-1), 104.46 (CH = *C*HMe), 113.81 (C-3 and C-5 of PMB ring[s]), 127.69, 127.87, 127.96, 128.35, 128.46, 129.52, 129.59, 129.63 (aromatic CH), 130.39, 131.03 (2 × C-1 of PMB rings), 137.89, 138.24 (2 × C-1 of benzyl rings), 142.21 (*C*H = CHMe), 159.18, 159.25 (2 × C-4 of PMB rings); FAB^+ mass spectrum: m/z 641 ($[M+1]^+$, 20%). Anal. Calcd for $C_{39}H_{44}O_8$ (640.77): C, 73.09; H, 6.93. Found: C, 73.2; H, 6.89.

*2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-*D*-glucopyranose* (**16**).—A solution of **15EZ** (1.5 g, 2.3 mmol) in acetone (50 mL) was heated to 50 °C (oil bath). Aq 1 M HCl (5 mL) was added and stirring was continued for 20 min, when TLC (10:1 $CHCl_3$–acetone) indicated total consumption of starting material (R_f 0.70) to give a product (R_f 0.28). Solid $NaHCO_3$ (2 g) was added and stirring was continued as the suspension was allowed to cool to room temperature. The solvents were evaporated and the residue was extracted with ether (3 × 100 mL). The combined organic extracts were washed with saturated aq $NaHCO_3$ (150 mL) and water (150 mL), dried ($MgSO_4$), filtered, and concentrated. Recrystallisation from a minimum of ether gave the title compound (1.1 g, 77%), which reduced Fehling's solution; mp 120–131 °C; $[\alpha]_D$ +5.1° (*c* 1.4, $CHCl_3$, 2 h); ^1H NMR ($CDCl_3$; 270 MHz): δ 1.64 (s, 0.5 H, exch. D_2O, OH_β), 3.09 (d, 0.5 H, *J* 2.6 Hz, exch. D_2O, OH_α), 3.37 (dd, 0.5 H, $J_{2,1}$ 7.6, $J_{2,3}$ 9.9 Hz, H-2_β), 3.48–3.80 (m, 4.5 H, H-2_α, H-3_α or H-3_β, H-5_α or H-5_β, H-4, H-6a, H-6b), 3.78, 3.80 (2 s, 6 H, 2 × OMe), 3.94 (t, 0.5 H, $J_{3,2}=J_{3,4}=9.2$ Hz, H-3_α or H-3_β), 4.01 (ddd overlapping with t at 3.94 ppm, 0.5 H, H-5_α or H-5_β), 4.39–4.93 (m, 8.5 H, H-1_β, 4 × ArCH_2O AB systems), 5.21 (t, 0.5 H, *J* 2.6 Hz, simplifies to d on D_2O exch., H-1_α), 6.79–6.88 (m, 4

H, 2 × H-3 and H-5 of PMB rings), 7.02–7.07 (m, 2 H, H-2 and H-6 of PMB ring), 7.21–7.34 (m, 12 H, Ar). The α and β subscripts denote signals arising from the α and β anomers respectively; FAB^- mass spectrum: m/z 753 ($[M + NBA]^-$, 100%). Anal. Calcd for $C_{36}H_{40}O_8$: C, 71.97; H, 6.72. Found: C, 71.8; H, 6.66.

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