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C-Glycoside based mimics of D-*myo*-inositol 1,4,5-trisphosphate

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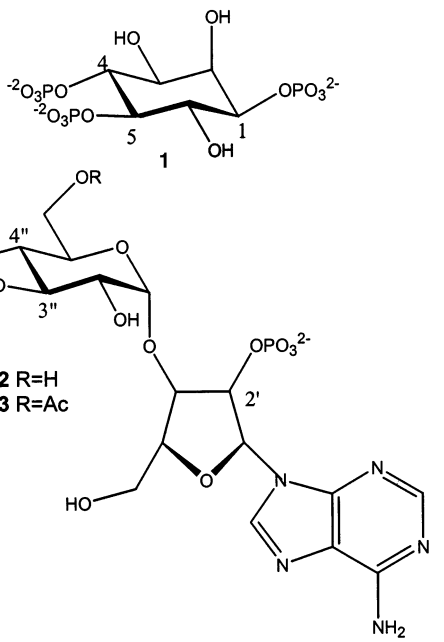
Abstract

Epimeric *C*-glycoside based polyphosphates, α- and β-D-glucopyranosylmethanol 3,4,1′-trisphosphates (**8** and **9**) were prepared from D-glucose. The key intermediate, allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside, was prepared in five steps (67% yield) from allyl α-D-glucopyranoside without the need for chromatography. Compounds **8** and **9** were shown to be full agonists at the Ins(1,4,5)P_3 receptors of permeabilised hepatocytes, but with markedly different potencies. Such *C*-glycoside analogues are worthy of further development as Ins(1,4,5)P_3 receptor ligands. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Adenophostin; *myo*-Inositol; Selective protection

1. Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is an intracellular signalling molecule that stimulates mobilisation of intracellular Ca^{2+} stores by binding to the Ins(1,4,5)P_3 receptor, an intracellular Ins(1,4,5)P_3-gated Ca^{2+} channel [1]. In 1993 the glyconucleotides adenophostins A and B (**2** and **3**) were isolated from *Penicillium brevicompactum* and found to be very potent agonists of Ins(1,4,5)P_3 receptors [2–6]. Adenophostins A and B are 10–100 times more potent than Ins(1,4,5)P_3 in both binding affinity for the receptor and the ability to mobilise Ca^{2+} in cells. Structure–activity investigations have established that high-affinity Ins(1,4,5)P_3 receptor ligands must contain the 4,5-(bisphosphate)/3″,4″-(bisphosphate) and adjacent 6-hydroxyl/2″-hydroxyl motifs of Ins(1,4,5)P_3/adenophostin, respectively. However, slight variations in the third phosphate group are tolerated [7].



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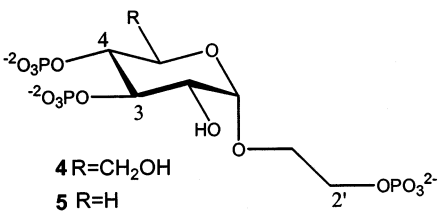
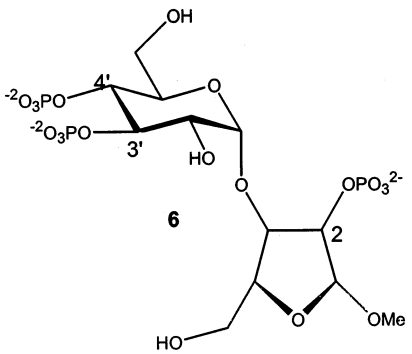
We and other groups have synthesised various carbohydrate-based ligands of Ins(1,4,5)P_3 receptors that are related to adenophostins but lack the adenine base (**4**–**7**) [8–14]. All of these compounds were similar to, or less potent than, Ins(1,4,5)P_3, suggesting that the adenine base may play an essential role in the increased potencies of the adenophostins. However, the third phosphate group in the above compounds may not be in the appropriate position for optimal binding, particularly in **4** and **5**.



4 R=CH_2OH
5 R=H



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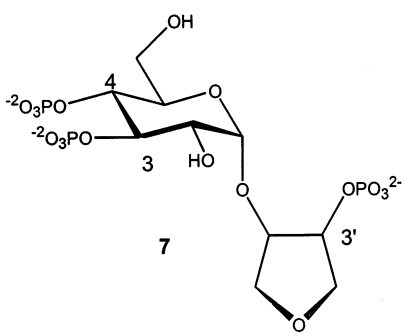

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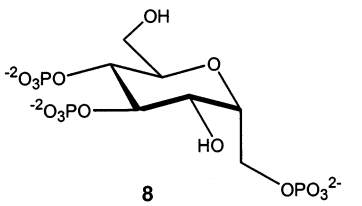
The lower affinity of **4** compared with Ins(1,4,5)P_3 was thought to be due to the conformational flexibility of the side chain perhaps preventing the third phosphate from achieving an optimal interaction with the receptor [14]. We therefore envisaged that it would be of interest to design a monosaccharide polyphosphate Ins(1,4,5)P_3 mimic, having the third phosphate closer to the ring and using a less flexible chain. It is clearly impractical to attempt synthesis of the chain-shortened version of **4**, therefore a *C*-glycoside derivative was an attractive target. On the basis of all the above considerations, we designed the syntheses of α- and β-D-glucopyranosylmethanol 3,4,1′-trisphosphate analogues (**8** and **9**) based on **4** but with a shorter side chain. In **8** and **9**, the third phosphate is attached to a carbon centre fixed in the α and β positions. Biological evaluation of these compounds, together with previous results, may help to clarify the structural requirements for the significant biological activity of adenophostins and their carbohydrate based derivatives.



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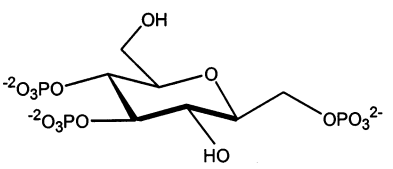

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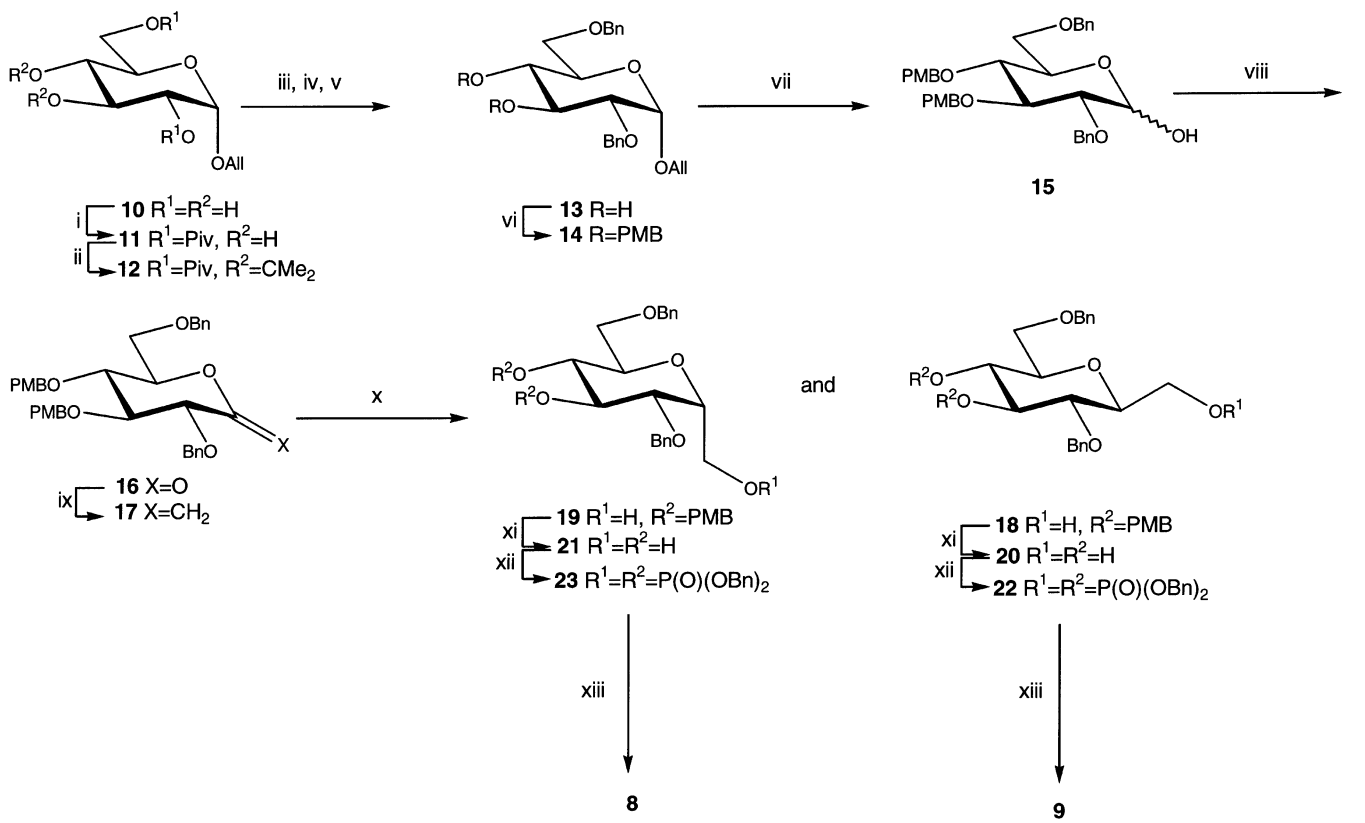
2. Results and discussion

The glycopyranose intermediate **15** [14a,b] was prepared from D-glucose (Scheme 1). Fischer glycosylation with allyl alcohol in the presence of HCl gave a mixture of pyranosides from which the α anomer **10** was isolated [14a,b]. Although stannylene mediated direct dibenzylation of positions 2 and 6 in **10** has been reported [14b], the overall yield was less than 44% on a 2 g scale and less than 15% on 35 g scale. Moreover, stannylene mediated dibenzylation requires extensive purification of the product by flash chromatography, therefore limiting the scale of the synthesis. We therefore designed a different synthetic strategy. Even though the proposed route was longer it was hoped that the overall yield would be higher and no purification by chromatography would be required.

Selective acylation of C-2 and C-6 was achieved by the treatment of **10** with trimethylacetylchloride (pivaloyl chloride) at 0 °C in pyridine [15]. One major product was formed, and the crude material obtained from this reaction was carried forward to the next step. Positions C-3 and C-4 were then protected by an isopropylidene acetal using 2-methoxypropene in THF in the presence of a catalytic amount of *p*-toluenesulphonic acid [16] and the product **12** was used immediately in the next step without purification. The trimethylacetyl groups were removed and replaced by benzyl protecting groups using sodium hydride and benzyl bromide in DMF and again the product of this reaction was used with no purification. Finally, the isopropylidene group was removed by stirring the crude intermediate with 1 M HCl for 30 min, and crystallisation from ether–hexane gave pure **13** [14b] (67% yield over five steps). This method was found to be the most convenient and efficient method to produce **13** in multigram quantities. Similar reactions on D-xylose to produce selectively allyl 2-*O*-benzyl-α-D-xylopyranoside (to be reported elsewhere), confirmed the general applicability of this method.

The diol **13** was easily converted in high yield into **14** [14b], a selectively protected intermediate without the labile trans isopropylidene group. Thus, *p*-methoxybenzylation with sodium hydride and *p*-methoxybenzyl chloride in DMF gave the fully protected product **14**. The allyl glycoside was then cleaved by stirring **14** vigorously with palladium chloride in methanol for 4 h to give **15**. The reaction mixture becomes increasingly acidic as the reaction proceeds, and it must be neutralised before workup to prevent the formation of



Scheme 1. (i) PivCl, pyridine, 0 °C, 2.5 h; (ii) 2-methoxypropene, PTSA, THF, 30 min; (iii) NaOH, MeOH, reflux, 1 h; (iv) NaH, BnBr, DMF, 0 °C, 90 min; (v) 1 M HCl–MeOH, 1:10, rt, 30 min (67%, over five steps); (vi) NaH, PMBCl, DMF, rt, 12 h (77%); (vii) $PdCl_2$, MeOH, rt, 4 h (71%); (viii) (a) oxalyl chloride, CH_2Cl_2, −78 °C. (b) Me_2SO added dropwise. (c) **15** in CH_2Cl_2 added dropwise. (d) NEt_3 (67%); (ix) pyridine, THF, Tebbe reagent (1.1 equiv) added dropwise −45 °C for 30 min 65%; (x) 9-BBN, THF 0 °C, 3 h, then H_2O_2, 5% KOH (58%) or BH_3-THF, 0 °C, 2 h, then H_2O_2, 5% KOH (74%); (xi) CF_3COOH, CH_2Cl_2 (80%); (xii) (a) $(BnO)_2PNPr^i_2$, tetrazole. (b) MCPBA, −78 °C to RT (78%); (xiii) H_2, Pd–C, 50 psi, MeOH–water, 24 h. All = allyl, Piv = $(CH_3)_3CCO$, (Pivaloyl), PMB = *p*-methoxybenzyl, Bn = benzyl.

Table 1
$^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3, **8** and **9** [a]

	EC_{50} (nM)	*h*	% release with 10 μM	*n*
Ins(1,4,5)P_3	144 ± 6	1.68 ± 0.26	47 ± 2	5
8	2414 ± 173	4.99 ± 1.84	42 ± 5	3
9	nd	nd	19 ± 3	5
4 [b]	1867 ± 64	2.76 ± 0.10	47 ± 3	3

[a] The EC_{50} values and Hill coefficients (*h*) were separately determined for *n* independent experiments by fitting results to logistic equations. Results are shown as means ± S.E.M.

[b] Data for **4** obtained in an independent study [9] when Ins(1,4,5)P_3 had an EC_{50} value and Hill coefficient (*h*) of 153 ± 11 and 2.25 ± 0.20 nM, respectively.

Table 2
$^{45}Ca^{2+}$ release data for combined stimulation with Ins(1,4,5)P_3 and **9** [a,b]

	% Ca^{2+} release
Ins(1,4,5)P_3 (150 nM)	22 ± 4
9 (10 μM)	21 ± 2
Ins(1,4,5)P_3 (150 nM) with **9** (10 μM)	38 ± 1

[a] The percentage of the intracellular Ca^{2+} stores released by a submaximal concentration of Ins(1,4,5)P_3 alone or in combination with **9** are shown.

[b] Results are shown as means ± S.E.M. for three independent experiments.

side products if acid-labile groups such as *p*-methoxybenzyl ethers are present. The protected glycopyranose intermediate **15** was oxidised under Swern conditions to give the lactone **16**, which was purified by flash chromatography with a yield of 67%. Elaboration to the *exo*-methylene derivative **17** was carried out by the Tebbe reaction [17–19]. The product **17** was obtained in 65% yield after purification by flash chromatography.

The α and β epimers of the *C*-glucosylmethanol were then synthesised by hydroboration reactions [18]. Hydroboration of **17** using 9-BBN produced exclusively 2,6-di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-β-D-glucopyranosylmethanol (**18**), while hydroboration of **17** with a borane–THF complex produced a mixture of α and β products **19** and **18** in an approximate ratio of 1:2. The two products could not be separated by flash chromatography at this stage.

The PMB protecting groups were removed with 10% trifluoroacetic acid in dichloromethane. The products of this reaction were then easily separated by flash chromatography to give the corresponding triols **20** and **21** in good yields. These were phosphitylated with tetrazole-activated bis(benzyloxy)diisopropylaminophosphine in dichloromethane [20] and oxidation of the intermediate phosphites with *m*-chloroperbenzoic acid (MCPBA) gave the fully protected compounds **22** and **23**, which were obtained in high yields after purification by flash chromatography. Deprotection by hydrogenation over Pd/C yielded trisphosphates **8** and **9**. The final products were purified on Q Sepharose Fast Flow resin eluting with a 0–1 mol dm^{-3} gradient of triethylammonium hydrogen carbonate (pH ~ 7.5), to give the triethylammonium salts of **8** and **9**, which were quantified by total phosphate assay [21] before biological evaluation.

The biological activities of **8** and **9** were assessed by measuring $^{45}Ca^{2+}$ release from permeabilised rat hepatocytes. Maximally effective concentrations (10 μM) of Ins(1,4,5)P_3 and **8** released the same fraction of the intracellular Ca^{2+} stores, 47 ± 2% ($n = 5$) and 42 ± 5% ($n = 3$), respectively; half-maximal effects (EC_{50}) occurred with 144 ± 6 nM and 2.41 ± 0.17 μM, respectively (Table 1). At a concentration of 10 μM, **9** released only 19 ± 3% ($n = 5$) of the stores. Higher concentrations of **9** were not examined, but its inability to antagonise the response to a submaximal concentration of Ins(1,4,5)P_3 (Table 2) suggests that it is not a partial agonist. The results are therefore consistent with **9** being a full agonist with an EC_{50} of about 10 μM. Thus, trisphosphate **8** was similar in potency to **4** and **5**, while **9** was considerably weaker.

Previous studies [9,13] have clearly demonstrated that sugar-based polyphosphates (e.g. **6** and **7**) are able to approach the potency of Ins(1,4,5)P_3 but not of adenophostin. Such molecules possess a glucopyranosyl 3,4-bisphosphate with an auxiliary phosphate, which is accommodated in an optimal position for binding using a second ring as in adenophostin. A challenge therefore is to explore the possibility of optimal positioning of

the third phosphate without the need for the second ring. This would also be useful in the design of related molecules since it would avoid problems of coupling strategies (e.g. in the synthesis of **6** and **7**).

While this work was in progress a report appeared [8b] on a synthesis of the 2′-deoxy derivative of **5**. Not surprisingly, this molecule was some 2000-fold less potent than Ins(1,4,5)P_3 in mobilising intracellular Ca^{2+}, showing the expected parallel with deletion of the 6-hydroxyl group of Ins(1,4,5)P_3 [7]. It is encouraging to see that the *C*-glycosides mimics prepared here (**8** and **9**) do possess Ins(1,4,5)P_3-like Ca^{2+}-mobilising activity, although neither has attained the activity of **6** or **7**. The differential potencies of **8** and **9** are of interest and indicate that **8** presents the auxiliary phosphate group in the more favourable position. However, a comparison of the activity of **8** with that of **4** (Table 1) indicates that both molecules possess similar potency, implying no significant advantage of **8** over **4** in binding to the Ins(1,4,5)P_3 receptor.

Moitessier et al. [8a] synthesised 2′,3,4-trisphosphates of (2-hydroxyethyl) α- and β-D-xylopyranosides and 3′,3,4-trisphosphates of (3-hydroxypropyl) α- and β-D-xylopyranosides. It was found that three of the mimics were comparable and released approximately the same amount of intracellular Ca^{2+}, roughly with tenfold lower potency than Ins(1,4,5)P_3, with only the larger and more flexible β-hydroxypropyl mimic having a much lower potency. On the basis of these results, it might be reasoned that the *C*-glycosides with a less flexible chain should both behave similarly, while based upon the structure of Ins(1,4,5)P_3, one might expect the β modified *C*-glycoside to have higher potency. However, on examination of molecular models this is clearly not the case. The differential activity of **8** and **9** can be qualitatively explained by the fact that the α-*C*-glycoside phosphate group could in **8** readily access some of the conformational space described by the 1-phosphate of Ins(1,4,5)P_3. While this is not impossible for the β-*C*-glycoside phosphate group in **9**, it does seem to be more difficult and we also cannot exclude potentially disfavourable steric or missing electronic interaction of **9** with the receptor protein due to the extra CH_2 group. The results show that the anomeric oxygen atom in **4** is not essential for biological activity and confirm that the three-dimensional location of the third phosphate group plays an important role in strong binding to the receptor.

These *C*-glycoside based polyphosphate analogues represent steps in designing high potency ligands using insights gained from the adenophostins and are worthy of further development.

3. Experimental

General methods.—Chemicals were purchased from Aldrich, Sigma and Fluka. DMF was distilled from barium oxide under reduced pressure and then stored over 4 Å molecular sieves. Pyridine, Me_2SO, CH_2Cl_2, THF, toluene and dioxane were purchased in anhydrous forms. Ins(1,4,5)P_3 was from American Radiolabeled Chemicals.

Thin-layer chromatography (TLC) was performed on precoated plates (E. Merck aluminum sheets Silica 60 F_{254}, Art. No. 5554). Products were visualised by dipping plates into phosphomolybdic acid in MeOH, followed by heating. Flash chromatography was carried out on silica gel (particle size 40–63 μm).

^1H and ^{13}C NMR spectra were recorded on JEOL JMN GX-270 or EX-400 NMR spectrometers. Unless otherwise stated, chemical shifts were measured in ppm relative to internal tetramethylsilane. ^{31}P NMR chemical shifts were measured in ppm and denoted positive downfield from 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. FAB Mass spectra [*m*-nitrobenzyl alcohol (mNBA)] were recorded at the University of Bath Mass Spectrometry Service using a VG Analytical Autospec Mass Spectrometer. Optical rotations were measured at ambient temperature using an Optical Activity Ltd. AA-10 polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2

g^{-1}. Ion-exchange chromatography was performed on an LKB-Pharmacia Medium Pressure Ion-Exchange Chromatograph using Q Sepharose Fast Flow resin and gradients of triethylammonium hydrogen carbonate (TEAB) as eluent. Compounds containing phosphates were assayed by a modification of the Briggs phosphate test [21].

$^{45}Ca^{2+}$ release from permeabilised rat hepatocytes.—Permeabilized hepatocytes were loaded to steady state (5 min at 37 °C) with $^{45}Ca^{2+}$ in a cytosol-like medium (CLM: KCl, 140 mM, NaCl, 20 mM, 2 mM $MgCl_2$, 1 mM EGTA, 300 μM $CaCl_2$, 20 mM Pipes, pH 7.0) containing ATP (1.5 mM), creatine phosphate (5 mM) creatine phosphokinase (5 units/mL) and FCCP (10 μM). After 5 min, thapsigargin (1.25 μM) was added to the cells (still at 37 °C) to inhibit further Ca^{2+} uptake, 30 s later the cells were added to appropriate concentrations of the agonists and after a further 60 s the $^{45}Ca^{2+}$ contents of the stores were determined by rapid filtration.

Concentration–response relationships were fitted to a logistic equation using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}) and Hill slope (*h*) were determined. All results are expressed as means ± SEM. Cytosol-like medium (CLM); ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA); piperazine - *N*,*N*′ - bis[2 - ethanesulfonic acid] (PIPES); adenosine 5′-triphosphate (ATP); carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP); concentration causing half the maximal effect (EC_{50}); Hill coefficient (*h*).

Allyl 2,6-di-O-trimethylacetyl-α-D-glucopyranoside (**11**).—Compound **10** (5.0 g, 22 mmol) was dissolved in dry pyridine (50 mL) under N_2. The solution was cooled to 0 °C and trimethylacetyl chloride (5.6 mL, 45 mmol) was added dropwise over 1 h. Stirring was continued for a further hour. The mixture was poured into ice water (200 mL) and extracted with Et_2O (200 mL). The organic soln was washed successively with 1 M HCl (200 mL) and satd $NaHCO_3$ (200 mL), dried ($MgSO_4$) and concentrated to give crude **11**, which was used in the next step without further purification. A small sample was subjected to flash chromatography (3:1 eluent Et_2O–hexane) to give the title compound as an oil.

$[\alpha]_D^{20}$ +90.6° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 5.79–5.89 (m, 1 H, $CH_2CH{=}CH_2$), 5.28 (dd, 1 H, ^2J 1.5, ^3J 17.1 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.17 (dd, 1 H, ^2J 0.9, ^3J 10.3 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.02 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 4.61 (dd, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 4.42 (dd, 1 H, $J_{6a,5}$ 4.9 Hz, H-6a), 4.33 (dd, 1 H, $J_{6b,5}$ 1.9, $J_{6b,6a}$ 12.2 Hz, H-6b), 4.14–4.19 (m, 1 H, $CH_2CH{=}CH_2$), 3.92–4.09 (m, 2 H, H-3, $CH_2CH{=}CH_2$), 3.80–3.85 (m, 1 H, H-5), 3.38 (t, 1 H, $J_{4,3}$ 9.3, Hz, H-4) and 1.22 (s, 18 H, 2 × CMe_3); ^{13}C NMR ($CDCl_3$; 67 MHz): δ 179.4 and 178.5 (2 × C=O), 133.4 ($CH_2CH{=}CH_2$), 117.6 ($CH_2CH{=}CH_2$), 95.1 (C-1), 69.8, 70.7, 71.5, 72.9 (4 × CH), 68.4 ($CH_2CH{=}CH_2$), 63.2 (C-6), 38.9 (CMe_3), 27.2 (3 × Me) and 27.0 (3 × Me); Anal. Calcd for $C_{19}H_{32}O_8$: C, 58.75; H, 8.3. Found: C, 58.8; H, 8.32%.

Allyl 2,6-di-O-trimethylacetyl-3,4-O-isopropylidene-α-D-glucopyranoside (**12**).—A soln of crude **11** (8.82 g, 22.0 mmol), 2-methoxypropene (5.5 mL, 56 mmol) and *p*-toluenesulphonic acid (100 mg) in THF (100 mL) was stirred for 2 h at rt under N_2. TLC (1:1 Et_2O–hexane) indicated consumption of the starting material. Diethyl ether (250 mL) was added and the mixture was washed with satd $NaHCO_3$ (250 mL). The organic layer was dried ($MgSO_4$) and filtered, and a few drops of Et_3N were added before the organic layer was concentrated. The product of this reaction was used directly in the next step without purification. A small sample was purified by flash chromatography for analysis.

$[\alpha]_D^{20}$ +114° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 5.78–5.92 (m, 1 H, $CH_2CH{=}CH_2$), 5.28 (dd, 1 H, ^2J 1.7, ^3J 17.2 Hz, $CH_2CH{=}CH_{cis}H_{trans}$), 5.17–5.20 (m, 2 H, H-1, $CH_2CH{=}CH_{cis}H_{trans}$), 4.83 (dd, 1 H, $J_{2,1}$ 3.7, $J_{2,3}$ 10.6 Hz, H-2), 4.37 (dd, 1 H, $J_{6b,5}$ 2.2, $J_{6b,6a}$ 11.9 Hz, H-6b), 4.10–4.20 (m, 1 H, H-6a), 3.91–4.18 (m, 4 H, H-3, H-5, $CH_2CH{=}CH_2$), 3.30–3.37 (m, 1 H, H-4), 1.44 (s, 3 H, CMe_2), 1.42 (s, 3 H, CMe_2), and 1.23 (s, 9 H, CMe_3), 1.22 (s, 9 H, CMe_3); ^{13}C NMR ($CDCl_3$; 67 MHz): δ 177.9 (2 × C=O), 133.4

($CH_2CH{=}CH_2$), 117.8 ($CH_2CH{=}CH_2$), 111.0 (CMe_2), 95.3 (C-1), 69.8, 70.7, 71.5, 72.9 (4 × CH), 69.8 ($CH_2CH{=}CH_2$), 63.5 (C-6) 38.8 (2 × CMe_3), 27.0 and 27.1 (CMe_3) and 26.4 and 26.7 (2 × Me); Anal. Calcd for $C_{22}H_{36}O_8$: C, 61.66; H, 8.47. Found: C, 61.9; H, 8.35.

Allyl 2,6-di-O-*benzyl*-α-D-*glucopyranoside* (**13**).—A soln of crude **12** (9.77 g, 22 mmol) and NaOH (3.6 g, 90 mmol) in MeOH (50 mL) was heated under reflux for 1 h. The mixture was cooled to rt and the pH was adjusted to 8 by careful addition of solid CO_2. The solvents were evaporated off and the residue was partitioned between Et_2O (100 mL) and water (50 mL). The aq layer was back-extracted with Et_2O (10 mL) and the combined organic fractions were dried ($MgSO_4$), filtered and evaporated. The oil obtained was dissolved in dry DMF (40 mL) and was stirred at 0 °C with NaH (2.25 g of an 60% w/w dispersion in mineral oil, 56 mmol) and benzyl bromide (6.1 mL, 56 mmol) was added slowly. The mixture was stirred at rt for 90 min. Water (50 mL) was added and stirring continued for 60 min. The solvents were evaporated and the residue was dissolved in Et_2O (200 mL). The extract was washed with water (500 mL), dried ($MgSO_4$), filtered and evaporated. The oil was dissolved in MeOH (50 mL) and stirred with 1 M HCl (5 mL) for 30 min. TLC (1:2 Et_2O–hexane) indicated consumption of the starting material. Solid $NaHCO_3$ was added until the mixture was neutral. The solvents were evaporated off and the residue was partitioned between CH_2Cl_2 (100 mL) and water (50 mL). The aq layer was back-extracted with CH_2Cl_2 (200 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The product was crystallised from Et_2O–hexane to give the title compound (6.1 g, 67% over the previous five steps from **10**).

$[\alpha]_D^{20}$ + 76.3° (*c* 0.8, $CHCl_3$); Lit. $[\alpha]_D^{20}$ + 76.4° (*c* 0.8, $CHCl_3$) [15]; mp 75–77 °C; Lit. mp 74–77 °C [14b]. ^{1}H NMR identical to Ref. [14b].

2,6-Di-O-*benzyl-3,4-di*-O-*(*p-*methoxybenzyl)*-α-D-*glucopyranoside* (**15**).—Compound **14** was prepared from **13** in 77% yield as described by Jenkins et al. [14b]. Compound **14** (5.5 g, 8.6 mmol) in methanol (50 mL) was stirred vigorously with $PdCl_2$ (304 mg, 1.7 mmol) for 3 h. TLC (10:1 CH_2Cl_2–acetone) showed consumption of the starting material. Triethylamine (1 mL) was added to neutralize the reaction. It was filtered through Celite, the solvents were evaporated off and the residue was subjected to flash chromatography (eluent 10:1 CH_2Cl_2–acetone) to give a pale-yellow oil, which was crystallised from Et_2O to give the title compound as a white solid (3.59 g, 70.5 %).

2,6-Di-O-*benzyl-3,4-di*-O-*(*p-*methoxybenzyl)*-D-*glucono-1,5-lactone* (**16**).—Dry CH_2Cl_2 (1 mL) was placed into a 100 mL flask under an atmosphere of N_2. A soln of oxalyl chloride in dry CH_2Cl_2 (1.7 mL, 3.4 mmol of 2 M soln) was injected and the flask was cooled to − 78 °C. Anhyd Me_2SO (0.5 mL, 0.7 mmol) dissolved in dry CH_2Cl_2 was added dropwise (care evolution of gas) and stirring was continued for 5 min. A soln of **15** (2.0 g, 3.3 mmol in 2 mL dry CH_2Cl_2) was added dropwise and stirring was continued for 20 min. Triethylamine (1.8 mL, 13 mmol) was added dropwise and the reaction was stirred for a further 5 min, then the mixture was allowed to heat up to rt. The mixture was stirred with water (40 mL) for 10 min, then CH_2Cl_2 (80 mL) was added. The organic layer was separated and the aq layer was re-extracted with CH_2Cl_2 (80 mL). The combined organic layers were washed successively with satd NaCl (80 mL), 1% HCl (80 mL) and 10% aq $NaHCO_3$ (80 mL), dried and concentrated. The residue was subjected to flash chromatography (eluent 1:1 Et_2O–hexane) to give the title compound as an oil (1.33 g, 67%).

$[\alpha]_D^{20}$ + 64.7° (*c* 0.5, $CHCl_3$); IR υ 1755 cm^{-1} (carbonyl); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.07–7.39 (m, 14 H, Ph, 4 × ortho H of PMB rings), 6.80–6.86 (m, 4 H, 4 × meta H of PMB rings), 4.98 (0.5 × AB, 1 H, J_{AB} 11.42 Hz, $ArCH_2O$), 4.40–4.67 (m, 8 H, H-5, 3.5 × $ArCH_2O$), 4.09 (d, 1 H, $J_{2,3}$ 6.2, H-2), 3.85–3.95 (m, 2 H, H-3, H-4), 3.79 (s, 3 H, OMe), 3.78 (s, 3 H, OMe), 3.69 (dd, 1 H, $J_{6a,6b}$ 10.8, $J_{6b,5}$ 2.3 Hz, H-6b) and 3.63 (dd, 1 H, $J_{6a,5}$ 3.5 Hz, H-6a); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.5, 159.5 (2 × para C of PMB ring), 137.2, 137.8 (2 × ipso C of Ph), 129.9, 129.8, 128.6, 128.5, 128.3, 128.0 (aromatic *C*H), 114.1

(meta C of PMB ring), 98.7 (C-1), 80.9, 78.5, 77.7 and 77.7 (4 × CH), 75.9, 74.0, 73.8 and 73.7 (4 × Ar*C*H_2O), 68.5 (C-6) and 55.6 (OMe); FABMS *m*/*z* calcd for $C_{36}H_{38}O_8$ 598.2566. Found *m*/*z* 598.2518.

*2,6-Anhydro-3,7-di-*O*-benzyl-4,5-di-*O*-(*p*-methoxybenzyl)-1-deoxy-*D*-glucohept-1-enitol* (**17**).—To a soln of **16** (1.07 g, 1.8 mmol) in dry toluene (3.5 mL) and dry THF (1.5 mL), pyridine (26 μL) was added. The mixture was cooled to −45 °C and Tebbe Reagent (3.9 mL, 2 mmol) was added slowly. The mixture was stirred at −40 °C to −45 °C for 1 h and then at 0 °C for a further 30 min. It was then cooled to −10 °C to −15 °C and NaOH (1 mL, 15% w/v) was added. The cooling bath was removed and the reaction mixture was diluted with Et_2O (50 mL). The inorganic residue was removed by filtration through Celite and the filter cake was washed with excess Et_2O. The solvents were evaporated off and the residue was subjected to flash chromatography (eluent 1:3 Et_2O–hexane) to give the title compound, which was recrystallised from hexane (691 mg, 65.1%).

Mp 52–54 °C; $[\alpha]_D^{20}$ +42.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.03–7.36 (m, 14 H, ArH, 4 × ortho H of PMB rings), 6.77–6.86 (m, 4 H, 2 × meta H of PMB rings), 4.39–4.81 (m, 11 H, H-6, H_2-1, 4 × ArCH_2O), 3.93 (d, 1 H, $J_{3,4}$ 7.2 Hz, H-3), 3.79 (s, 3 H, OMe), 3.77 (s, 3 H, OMe) and 3.71–3.74 (m, 4 H, H-4, H-5, H_2-7); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 159.2 and 156.3 (2 × para C of PMB ring and C-2), 137.9 (ipso C of Ph), 130.5, 130.3 (2 × ipso C of PMB), 129.6, 128.4, 128.4, 127.9, 127.8, 127.6 (aromatic *C*H), 113.8, 113.7 (2 × meta C of PMB ring), 94.6 (C-1), 84.4, 78.9, 78.6 and 77.2 (4 × CH), 74.1, 73.5 and 72.8 (4 × Ar*C*H_2O), 68.7 (C-7) and 55.3 (OMe); FABMS: *m*/*z* calcd for $C_{37}H_{40}O_7$ $[M+H]^+$ 597.2852. Found *m*/*z* 597.2833; Anal. Calcd for $C_{37}H_{40}O_7$: C, 74.47; H, 6.75. Found: C, 74.3; H, 6.75.

*2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl)-β-*D*-glucopyranosylmethanol* (**18**).—9-BBN (0.5 M in THF, 1.68 mL, 0.8 mmol) was added to **17** (100 mg, 0.2 mmol) at 0 °C. The mixture was stirred for 3 h. H_2O_2 (3 mL, 30%) and 5% aq KOH were added. The product was extracted into Et_2O and washed with satd NaCl, dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent 2:3 EtOAc–hexane) to give the title compound (60 mg, 58%).

$[\alpha]_D^{20}$ +4.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.04–7.72 (m, 14 H, 10 × Ph, 4 × ortho H of PMB rings), 6.80–6.90 (m, 4 H, 2 × H-3 and 2 × H-5, PMB ring), 4.84–4.88 (1.5 × AB, 3 H, PhCH_2O), 4.75, 4.66 (AB, 2 H, J_{AB} 12.0 Hz, PhCH_2O), 4.59, 4.54 (AB, 2 H, J_{AB} 10.5 Hz, PhCH_2O), 4.48 (AB, 1 H, PhCH_2O), 3.86 (dd, 1 H, *J* 2.3, *J* 12.0 Hz, H-1′b), 3.79 (2 × s, 6 H, 2 × OMe), 3.60–3.74 (m, 4 H, H-1′a, H-3, H-6a, H-6b), 3.51–3.57 (2 × t, 2 H, H-2, H-4), 3.45 (ddd, 1 H, H-5) and 3.34 (ddd, 1 H, H-1); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 159.4 and 159.3 (2 × para C of PMB rings), 138.2, 138.0 (2 × ipso C of Ph rings), 130.9, 130.4 (2 × ipso C of PMB ring), 129.8, 129.6, 128.7, 128.6, 128.2, 128.1 and 127.9 (aromatic *C*H), 114.1, 114.0 (2 × meta C of PMB ring), 87.0 (C-3), 79.5 (C-1), 78.9, 78.4 and 78.3 (C-5, C-4, C-2), 75.6, 75.4, 75.0 and 73.77 (4 × Ph*C*H_2O), 69.44 (C-6), 62.4 (C-1′) and 55.6 (OMe); Anal. Calcd for $C_{37}H_{42}O_8$: C, 72.29; H, 6.89. Found: C, 72.1; H, 6.89.

*2,6-Di-*O*-benzyl-3,4-di-*O*-(*p*-methoxybenzyl-β and α-*D*-glucopyranosylmethanol* (**18** *and* **19**).—To compound **17** (570 mg, 0.9 mmol) was added a 1.0 M soln of BH_3–THF in THF (2.9 mL, 2.9 mmol) at 0 °C. It was stirred for 2 h and allowed to heat up to rt. H_2O_2 (0.9 mL, 30%) was added and stirring continued for 30 min. Water (10 mL) and Et_2O (20 mL) were added. The organic layer was washed with satd NaCl (10 mL), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography (eluent 2:1 Et_2O–hexane) to give the title compounds as a mixture in a ratio approximately 1:2 α:β (587 mg, 73.5%).

*2,6-Di-*O*-benzyl-β and α-*D*-glucopyranosylmethanol* (**20** *and* **21**).—The mixture of **18** and **19** (87 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (10 mL), TFA (1 mL) was added and the mixture was stirred for 20 min. TLC (7:3 Et_2O–hexane) showed that the reaction had gone to completion. Saturated aq $NaHCO_3$ (50 mL) was added to neutralize the reaction. The products were extracted with CH_2Cl_2

(2 × 50 mL), the organic layer was dried ($MgSO_4$), filtered and concentrated to give a white solid. It was purified by flash chromatography (eluent 4:1 EtOAc–hexane) to give **20** (20.5 mg, 39%).

Mp 68–70 °C; $[\alpha]_D^{20}$ +6.3° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.25–7.38 (m, 10 H, Ph), 4.78, 4.72 (AB, 2 H, J_{AB} 11.4 Hz, PhCH_2O), 4.57, 4.54 (AB, 2 H, J_{AB} 12.0 Hz, PhCH_2O), 3.87 (dd, 1 H, *J* 2.3 Hz, H–1′b), 3.67–3.7 (m, 3 H, H-6a, H-6b, H-1′a), 3.4–3.64 (m, 4 H, H-2, H-3, H-4, H-5) and 3.32–3.39 (m, 1 H, H-1); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 138.2 and 137.6 (2 × ipso C of Ph ring). 128.8, 128.7, 128.3, 128.2, 128.1 and 128.03 (Ph), 77.62, 78.7 and 79.28 (3 × CH), 77.0, 75.0 (2 × Ph*C*H_2O), 73.9 (CH), 72.2 (CH), 70.5 (C-6) and 62.4 (C-1′); FABMS: *m*/*z* calcd for $C_{21}H_{26}O_6$ $[M+H]^+$ 375.1807. Found: *m*/*z* 375.1805.

Further elution gave **21** (22 mg, 41.2%).

$[\alpha]_D^{20}$ +5.6° (*c* 0.17, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.20–7.37 (m, 10 H, Ph), 4.64, 4.63 (AB, 2 H, J_{AB} 11.7 Hz, PhCH_2O), 4.58, 4.54 (AB, 2 H, J_{AB} 12.0 Hz, PhCH_2O), 4.17 (ddd, 1 H, $J_{1,2}$ 5.9 Hz, $J_{1,1'a}$ 4.1, $J_{1,1'b}$ Hz, 9.1 Hz, H-1), 3.90 (dd, 1 H, $J_{1'b,1'a}$ 12.3 Hz, H-1′b), 3.77 (dd, 1 H, H-1′a), 3.63–3.73 (m, 5 H, H-3, H-4, H-5, H-6a, H-6b) and 3.60 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 137.6 and 137.5 (2 × ipso C of Ph ring). 128.8, 128.7, 128.4, 128.1 and 128.0 (Ph), 78.5 and 77.0 (2 × CH), 74.6, 74.0 (2 × Ph*C*H_2O), 73.5 (CH), 72.1 (C-6), 71.8 (CH), 70.8 (CH) and 58.8 (C-1′); FABMS: *m*/*z* calcd for $C_{21}H_{26}O_6$ $[M+H]^+$ 375.1807. Found: *m*/*z* 375.1810.

*2,6-Di-*O*-benzyl-β-*D*-glucopyranosylmethanol 3,4,1′-tris(dibenzylphosphate)* (**22**).—A mixture of bis(benzyloxy)(diisopropylamino)-phosphine (276 mg, 0.8 mmol), tetrazole (84 mg, 1.2 mmol) and dry CH_2Cl_2 (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon **20** (50 mg, 0.1 mmol) was added and stirring was continued for 30 min. The mixture was cooled to 0 °C and MCPBA (460 mg, 1.6 mmol) was added. The mixture was stirred at rt for 10 min, and was then diluted with CH_2Cl_2 (50 mL). The soln was washed successively with 10% (w/v) aq Na_2SO_3, satd aq $NaHCO_3$ (25 mL) and satd aq NaCl (25 mL), dried ($MgSO_4$), filtered and concentrated. The concentrate was purified by flash chromatography (eluent 3:2 EtOAc–hexane) to give the title compound as an oil (137 mg, 99.1%).

$[\alpha]_D^{20}$ −5.5° (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.04–7.46 (m, 40 H, Ph), 4.72–5.09 (m, 13 H, PhCH_2O), 4.63–4.70 (m, 1 H, H-3), 4.48–4.55 (m, 1 H, H-4), 4.44 (0.5 × AB, 1 H, J_{AB} 11.1 Hz, PhCH_2O), 4.40 (0.5 × AB, 1 H, J_{AB} 12.3 Hz, PhCH_2O), 4.25–4.3 (m, 2 H, H-1′b, PhCH_2O), 4.07–4.12 (m, 1 H, H-1′a), 3.79 (dd, 1 H, $J_{6b,5}$ 1.5, $J_{6b,6a}$ 11.1 Hz, H-6b), 3.66 (dd, 1 H, $J_{6a,5}$ 5.3 Hz, H-6a), 3.51–3.65 (m, 2 H, H-2, H-5) and 3.43–3.46 (m, 1 H, H-1); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 138.2 and 137.6 (ipso C of Ph rings), 136.2, 135.9, 135.8, 128.7 and 128.7 (Ph) and 74.6, 73.5, 70.2, 70.1, 69.8, 69.7, 69.0 and 66.2 (CH_2); ^{31}P NMR ($CDCl_3$; 109 MHz): δ_p 0.02, −0.19, −0.79; FABMS: *m*/*z* calcd for $C_{63}H_{65}O_{15}P_3$ $[M+H]^+$1155.3614: Found: *m*/*z* 1155.3628.

*2,6-Di-*O*-benzyl-α-*D*-glucopyranosylmethanol 3,4,1′-tris(dibenzylphosphate)* (**23**).—The title compound (90.5 mg, 78%) was obtained from compound **21** (42 mg, 0.112 mmol) as described above for **22**.

$[\alpha]_D^{20}$ +7.7° (*c* 1.81, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.16–7.35 (m, 40 H, Ph), 4.86–5.11 (m, 12 H, PhCH_2O), 4.76–4.81 (m, 1 H, H-3), 4.61–4.67 (m, 1 H, H-4), 4.57 (AB, 1 H, J_{AB} 11.7 Hz, PhCH_2O), 4.29–4.44 (m, 4 H, H-1′b, PhCH_2O), 4.04–4.14 (m, 2 H, H-1, H-1′a), 3.94–3.98 (m, 1 H, H-5), 3.64–3.70 (m, 2 H, H-6a, H-2) and 3.58 (dd, 1 H, $J_{6b,5}$ 2.9, $J_{6b,6a}$ 10.8 Hz, H-6b); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 138.1 and 137.3 (ipso C of PH ring), 135.9, 135.8, 128.7 and 128.7 (Ph) 77.7 and 77.6 (2 × CH), 73.8, 73.8 (2 × CH_2), 73.4, 71.2, 71.8 (3 × CH) and 69.9, 69.8, 69.7, 69.6, 68.1 and 64.1 (CH_2); ^{31}P NMR ($CDCl_3$; 162 MHz): δ_p 0.21, −0.594, −1.07; FABMS: *m*/*z* calcd for $C_{63}H_{65}O_{15}P_3$ $[M+H]^+$ 1155.3614. Found: *m*/*z* 1155.3648.

*α-*D*-Glucopyranosylmethanol 3,4,1′-trisphosphate* (**8**).—10% palladium on activated charcoal (50%, 200 mg) was added to a soln of compound **23** (90 mg, 0.08 mmol) in MeOH (20 mL) and water (5 mL) and the mixture was hydrogenated at 50 psi at rt for 24 h, after

which it was filtered. The filtrate was concentrated to give a glassy clear solid. The residue was dissolved in de-ionised water and purified by ion-exchange chromatography on Q Sepharose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8. The triethylammonium salt of **8** eluted between 80 and 92% TEAB. Fractions containing compound **8**, as judged by phosphate assay, were combined and concentrated to give a residue from which MeOH (3 × 100 mL) was evaporated to give the title trisphosphate as its triethylammonium salt (58 μmol, 73%).

$[\alpha]_D^{20}$ +19.8° (*c* 0.9, MeOH); ^{1}H NMR (CD_3OD; 400 MHz): δ 4.35 (q, 1 H, *J* 8.8 Hz, H-3), 4.12–4.26 (m, 3 H, H-1, H-1′a, H-1′b), 4.05–4.10 (q, 1 H, H-4), 3.90 (dd, 1 H, $J_{6a,5}$ 4.1, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.79–3.86 (m, 2 H, H-2, H-5) and 3.72 (d, 1 H, H-6b); ^{31}P NMR (CD_3OD; 162 MHz): δ 2.70, 2.11, 2.10; S; FABMS: *m*/*z* calcd for $C_7H_{16}O_{15}P_3$ $[M-H]^-$ 432.9657. Found: *m*/*z* 432.9694.

β-D-*Glucopyranosylmethanol 3,4,1′-trisphosphate* (**9**).—The title compound (69 μmol, 86%) as its triethylammonium salt was obtained from compound **22** (90 mg, 0.08 mmol), as described above for the synthesis of **8**.

$[\alpha]_D^{20}$ −3.7° (*c* 1.3, MeOH); ^{1}H NMR (CD_3OD; 400 MHz): δ 4.15–4.22 (m, 2 H, H-3, H-1′b), 3.96–4.07 (m, 2 H, H-4, H-1′a), 3.86 (dd, 1 H, $J_{6a,5}$ 3.8, $J_{6a,6b}$ 12.6 Hz, H-6a), 3.77 (dd, 1 H, $J_{6b,5}$ 1.8 Hz, H-6b), 3.50 (t, 1 H, *J* 8.8 Hz, H-2), 3.39–3.43 (m, 1 H, H-5) and 3.29–3.36 (m, 1 H, H-1); ^{31}P NMR (CD_3OD; 162 MHz): δ −5.90, −5.85, −5.38; FABMS: *m*/*z* calcd for $C_7H_{16}O_{15}P_3$ $[M-H]^+$ 432.9657. Found: *m*/*z* 432.9684.

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