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Synthesis of glucopyranoside-based ligands for D-*myo*-inositol 1,4,5-trisphosphate receptors

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Dedicated to the memory of Professor Roy H. Gigg (1930–2001).

Abstract

Adenophostins A and B are naturally occurring glyconucleotides that interact potently with receptors for D-*myo*-inositol 1,4,5-trisphosphate, an important second messenger molecule in most cell types. Here we describe the design and synthesis of glucopyranoside-based analogues of adenophostin A lacking the adenine component. The key synthetic strategy involves glycosylation of selectively protected alcohols, derived from methyl β-D-ribofuranoside or 1,4-anhydroerythritol, using glycosyl donors synthesised from 2,6-di-*O*-benzyl-D-glucopyranose derivatives. Further elaboration and deprotection of the coupled products gave two trisphosphate analogues; methyl 3-*O*-α-D-glucopyranosyl-β-D-ribofuranoside 2,3′,4′-trisphosphate (''ribophostin'') and (3′*S*,4′*R*)-3′-hydroxytetrahydrofuran-4′-yl α-D-glucopyranoside 3,4,3′-trisphosphophosphate (''furanophostin''). The route to furanophostin was further modified to give (3′*S*,4′*R*)-3′-hydroxytetrahydrofuran-4′-yl α-D-glucopyranoside 3′-phosphate 3,4-bisphosphorothioate, the first phosphorothioate-containing adenophostin analogue. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

1D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] acts as a second messenger in most cells, where it binds to tetrameric intracellular receptors [IP_3Rs], resulting in the opening of an intrinsic Ca^{2+} channel through which Ca^{2+} flows from the lumen of the endoplasmic reticulum into the cytosol.[1] Since the discovery of the second messenger function of Ins(1,4,5)P_3, many synthetic analogues of Ins(1,4,5)P_3 have been prepared.[2] Structure–activity studies of these have shown that all high-affinity agonists of IP_3Rs contain groups equivalent to the 4*R*,5*R*-*trans*-diequatorial bisphosphate and adjacent 6-hydroxyl group of Ins(1,4,5)P_3, together with an appropriately positioned non-vicinal phosphate group. The most active of these analogues attained similar potencies to that of Ins(1,4,5)P_3, but none has surpassed it.

In 1993, adenophostins A and B, isolated[3] from culture broths of *Penicillium brevicompactum*, were shown to stimulate the release of Ca^{2+} from Ins(1,4,5)P_3-sensitive intracellular stores and to bind to cerebellar IP_3Rs with higher affinity than any other known ligand, including Ins(1,4,5)P_3. Adenophostins A and B were identified as 3′-*O*-α-D-glucopyranosyladenosine 2′,3″,4″-trisphosphate (**2a**) and its 6″-*O*-acetyl ester (**2b**), respectively.[4,5] Several total syntheses of **2a** have since appeared.[6–8] It was quickly realised that the glucose 3″,4″-bisphosphate/2″-hydroxyl structure of the adenophostins was able to mimic the inositol 4,5-bisphosphate/6-hydroxyl triad of Ins(1,4,5)P_3 at the binding sites of IP_3Rs,[5] although the precise role of the 2′-phosphorylated adenosine component, and how this structure could confer enhanced affinity for IP_3Rs, was not clear.

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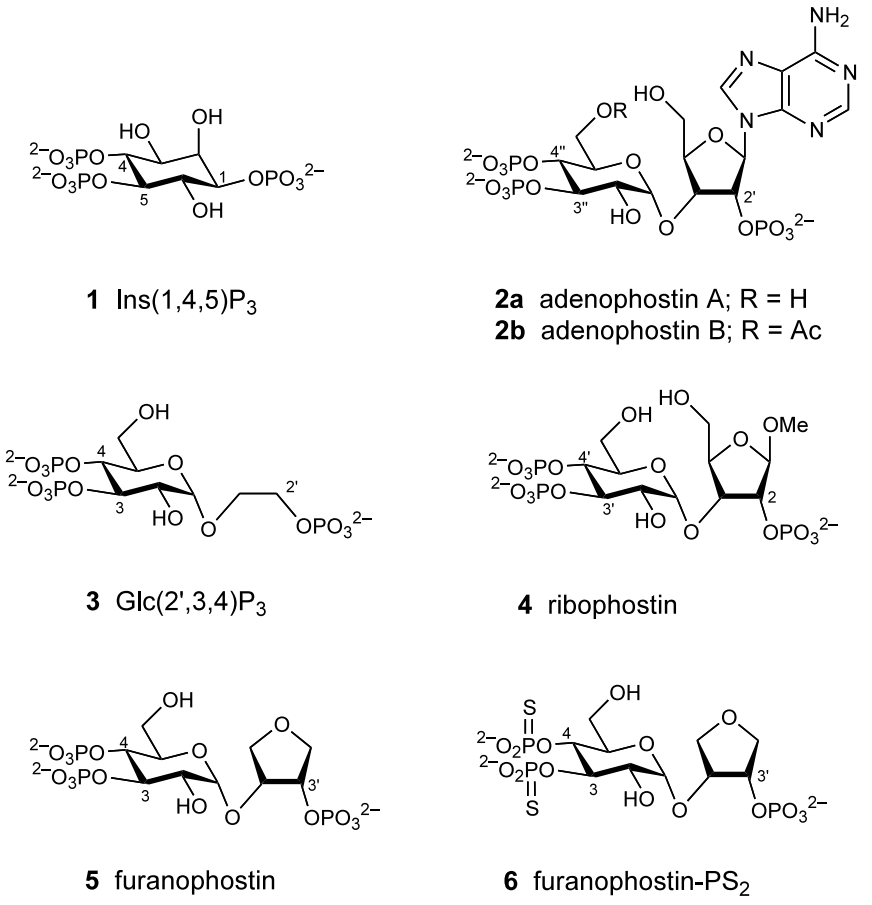


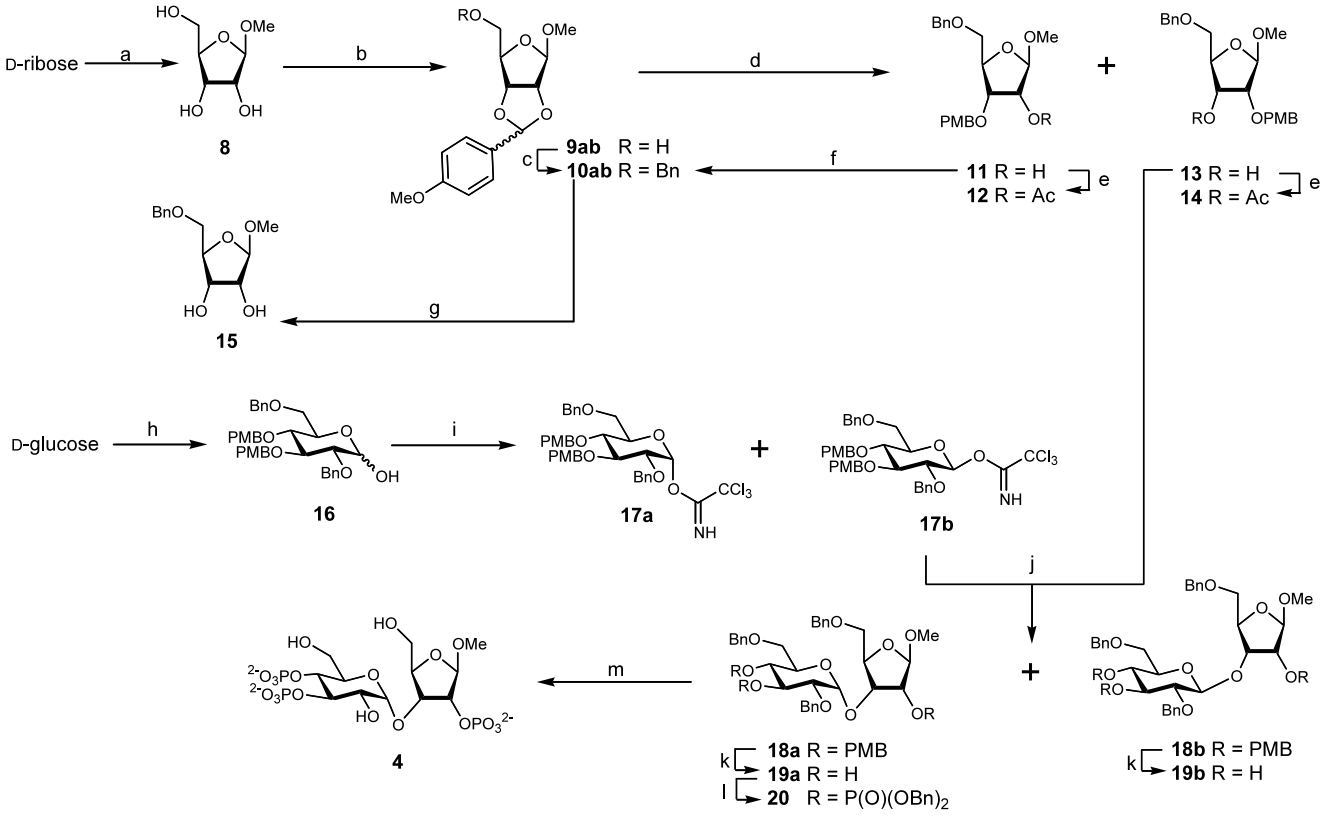
Fig. 1. Structures of Ins(1,4,5)P_3 (**1**), adenophostins A (**2a**) and B (**2b**), and synthetic analogues (**3**–**6**).

Initial attempts to elucidate the role of the adenosine led to the synthesis of 2-hydroxyethyl α-D-glucopyranoside 2′,3,4-trisphosphate[9,10] (**3**) which was found to be around tenfold less potent[9–11] than Ins(1,4,5)P_3. This suggested that, while the pyranoside 3,4-bisphosphate/2-hydroxyl component contained the essential structures responsible for binding and Ca^{2+} release, at least part of the adenosine moiety was required for the high potency of the adenophostins. Molecular modelling simulations[9] of **3** established that its flexible bimethylene chain did not allow the 2′-phosphate to mimic accurately the corresponding phosphate of either **1** or **2a**. In the present paper we describe the synthesis of methyl 3-*O*-α-D-glucopyranosyl-β-D-ribofuranoside 2,3′,4′-trisphosphate (**4**) and (3′*S*,4′*R*)-3′-hydroxytetrahydrofuran-4′-yl α-D-glucopyranoside 3,3′,4-trisphosphate (**5**),[†] in both of which the adenine ring of **2a** has effectively been deleted, but the third phosphate is held on a furanoid ring as in **2a**. In addition, the design and preparation of (3′*S*,4′*R*)-3′-hydroxytetrahydrofuran-4′-yl α-D-glucopyranoside 3′-phosphate-3,4-bisphosphorothioate (**6**) as a potential high-affinity partial agonist, is described. Preliminary accounts of the syntheses of **4**[12] and **5**[13] have appeared, and **5** has been synthesised by another group (Fig. 1).[14] A comprehensive structure–activity study incorporating these ligands has been published.[15]

2. Results and discussion

For the synthesis of **4**, the intermediate methyl 5-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-D-ribofuranoside (**13**) (Scheme 1) was required as a suitable glycosyl acceptor. D-Ribose was converted into the known[16] methyl β-D-ribofuranoside **8**. The acid-catalysed reaction of **8** with 1.05 equivalents of *p*-methoxybenzaldehyde dimethyl acetal[17] at 70 °C in DMF with continuous removal of the liberated MeOH gave the 2,3-*O*-*p*-methoxybenzylidene derivative **9ab**. Compound **9ab** has been prepared previously,[18] but NMR data, showing it to be a ca. 3:2 diastereoisomeric mixture, are reported here for the first time. Benzylation of **9ab** with NaH and benzyl bromide gave fully protected **10ab**, again as a 3:2 diastereomeric mixture. Cleavage of the *p*-methoxybenzylidene acetal

[†] In naming some compounds of this paper note that primes have been used when not strictly necessary, to facilitate understanding of structure–activity arguments and NMR spectral assignments.



Scheme 1. (a) MeOH, H_2SO_4; (b) *p*-$MeOC_6H_4CH(OMe)_2$, PTSA, DMF, 70 °C; (c) NaH, BnBr, DMF; (d) DIBAL–H, CH_2Cl_2; (e) Ac_2O, pyridine; (f) DDQ, CH_2Cl_2, 3 Å MS; (g) 80% acetic acid, 60 °C; (h) see Ref. 10; (i) Cl_3CCN, K_2CO_3, CH_2Cl_2; (j) $Me_3SiOSO_2CF_3$, Et_2O, 4 Å MS; (k) DDQ, 12:1 CH_2Cl_2–H_2O; (l) $(BnO)_2PNPr^i_2$, CH_2Cl_2, 1*H*-tetrazole then MCPBA, −78 °C to rt; (m) H_2, Pd–C, 40 psi, 4:1 MeOH–H_2O. PMB = *p*-methoxybenzyl.

with $LiAlH_4$–$AlCl_3$ in refluxing tetrahydrofuran,[19] $NaCNBH_3$–Me_3SiCl in acetonitrile[17] or DIBAL–H in dichloromethane[20] all gave the required **13**, but also the more polar isomer **11**, in approximately equal proportions, the latter reagent giving by far the best yield. The structures of **11** and **13** were confirmed by preparation of acetates **12** and **14**, respectively, the ^{1}H NMR spectra of which, respectively, revealed a deshielded doublet corresponding to H-2, and a deshielded triplet corresponding to H-3. The enantiomer of **11** has previously been prepared as part of an anomeric mixture, but no physical data were reported.[21] Although the regioselectivity of acetal cleavage was somewhat disappointing, the unrequired isomer **11** was easily re-oxidised to **10ab** (interestingly, as a 92:8 diastereomeric mixture) using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dry dichloromethane.[22]

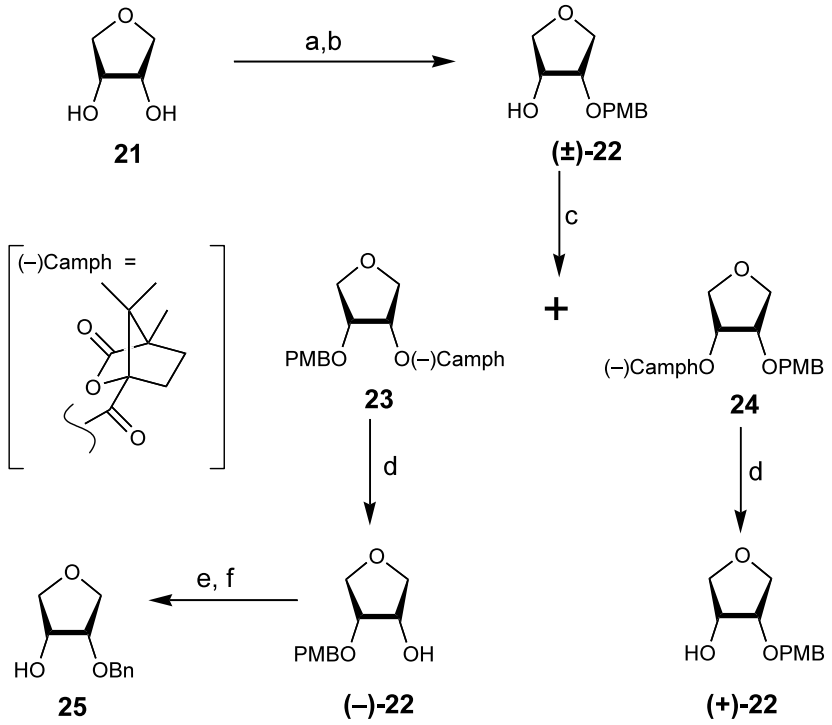
A preparation of the corresponding 2-*O*- and 3-*O*-allyl ribofuranosides by a different route has been reported by Desai et al.[23] This report described anomerisation of methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-β-D-ribofuranoside on acidic hydrolysis, to give a ca. 1:4 α:β-anomeric mixture of products. We found that the more labile *p*-methoxybenzylidene acetal of **10ab** could be removed without anomerisation by treatment with 80% (v/v) aq acetic acid to give **15**. Therefore, **10ab** appears to be a more suitable intermediate than methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside to prepare derivatives of methyl β-D-ribofuranoside substituted at C-5.

With a suitable glycosyl acceptor in hand, an appropriate donor was required. Reaction of the known[10] 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose (**16**) with trichloroacetonitrile in the presence of potassium carbonate[24] resulted in the initial formation of the required β anomer **17b**, the kinetic product, accompanied by slow conversion into the α anomer **17a**, which is the thermodynamic product. The reaction was monitored closely by TLC and quenched when the optimum conversion to the β anomer was observed, before the formation of too much of the α anomer. Flash chromatography yielded crystalline **17b** in only moderate yield (48%), accompanied by significant quantities of **17a**. Configurations of the two anomers were easily assigned on the basis of their ^{1}H NMR spectra. The axial H-1 of the β anomer exhibited a typically large coupling constant of 8.3 Hz and was upfield (δ 5.78) of the equatorial H-1 of the α anomer, (δ 6.51, $J_{1,2}$ 3.3 Hz). The trichloroacetimidate NH proton was also clear in the ^{1}H NMR spectra of both anomers.

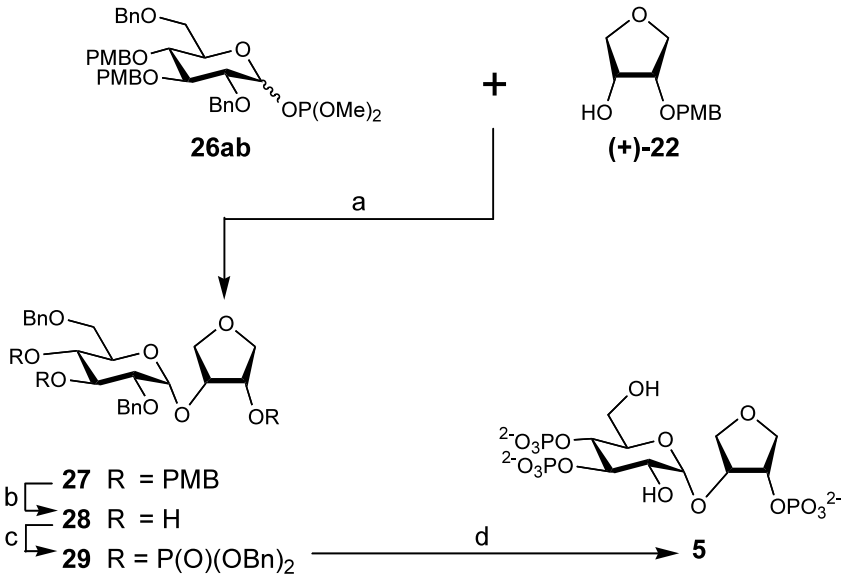
Coupling of **13** and **17b** was achieved using trimethylsilyl triflate as a promoter in ether at room temperature, conditions which generally favour α-stereoselectivity.[24] The product ran as a single spot on TLC, but the ^{1}H NMR spectrum clearly demonstrated that although the α-glucopyranosyl compound **18a** had been formed as the major product, the β-glucopyranosyl anomer **18b** was present as a ca. 20% contaminant, which could not be removed at this stage. However, on treatment of the mixture with DDQ, the required crystalline triol **19a** could be separated from the β-coupled isomer **19b** by column chromatography. Phosphitylation of **19a** with bis(benzyloxy)diisopropylaminophosphine followed by oxidation of the intermediate trisphosphite triester with *m*-chloroperoxybenzoic acid (MCPBA) gave the fully protected trisphosphate **20**. Compound **20** was deprotected by hydrogenolysis over palladium on carbon to give the required trisphosphate **4** which was purified by ion-exchange chromatography on Q Sepharose Fast Flow resin. Surprisingly, the isolated triethylammonium salt of **4** was poorly soluble in water, and it was therefore converted into the freely soluble hexapotassium salt before use.

The activity of trisphosphate **4** in displacement of [^{3}H]Ins(1,4,5)P_3 from the Ins(1,4,5)P_3 receptors of hepatic membranes and in release of Ca^{2+} from hepatocytes was found to be very similar to that of Ins(1,4,5)P_3 itself, although still about 10- to 20-fold lower than that of adenophostin A.[11,15] The lower activity of **4** relative to the adenophostins suggested that the adenine (or similar) moiety was required to engender potency greater than that of Ins(1,4,5)P_3. Analogues of **2a** in which adenine has been replaced by imidazole, purine, benzimidazole, uracil and other aromatic structures have subsequently been prepared by this group.[25–27] The demonstration of Ins(1,4,5)P_3-like potency for **4**, however, raised the question as to whether this compound represented the minimal structure for such activity in carbohydrate polyphosphates. In particular, would conformational restraint of the third phosphate using a tetrahydrofuran ring alone engender comparable potency, or did the 4-hydroxymethyl and/or 1-methoxyl groups of **4** somehow contribute to activity? Such considerations led to the design of **5**.

The glycosyl acceptor required for the synthesis of **5**, (+)-**22** (Scheme 2), was prepared from commercially available 1,4-anhydroerythritol (**21**) by the optical resolution of its racemic *p*-methoxybenzyl ether (±)-**22**. Attempts to obtain (±)-**22** by monoalkylation of **21** using NaH and *p*-methoxybenzyl chloride in DMF failed, as only dialkylated material was formed. However, preparation of the *p*-methoxybenzylidene acetal followed by reductive cleavage, similarly to reactions on **10ab**, gave (±)-**22** in excellent yield. Esterification of (±)-**22** with (−)-(*S*)-camphanic chloride gave the chromatographically separable, crystalline diastereoisomers **23** and **24**. Saponification of the esters gave enan-



Scheme 2. (a) *p*-$MeOC_6H_4CH(OMe)_2$, PTSA, DMF, 70 °C; (b) DIBAL–H, CH_2Cl_2; (c) (−)-(*S*)-camphanic chloride, pyridine, 0 °C to rt; (d) NaOH, MeOH, reflux; (e) NaH, BnBr, DMF; (f) CF_3COOH, CH_2Cl_2. PMB = *p*-methoxybenzyl.



Scheme 3. (a) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4 Å MS; (b) CF_3COOH, CH_2Cl_2; (c) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole then MCPBA, −78 °C to rt; (d) H_2, Pd–C, 40 psi, 5:1 MeOH–H_2O. PMB = *p*-methoxybenzyl.

tiomeric *p*-methoxybenzyl ethers (−)-**22** and (+)-**22**, which were highly crystalline, in contrast to the racemic mixture. The absolute configurations of (−)-**22** and (+)-**22** were established by converting (−)-**22** into monobenzyl ether **25**, identified by comparison of its optical rotation with that of its known[28] enantiomer.

Glycosylation of (+)-**22** (Scheme 3) was achieved by reaction with dimethyl phosphite **26ab**[8] in the presence of $ZnCl_2$ and $AgClO_4$.[29] Glycosyl donor **26ab** was chosen rather than trichloroacetimidate **17b** due to greater ease of preparation and because phosphite-mediated coupling tends to give a higher proportion of α-coupled products. In the present case, only the required α-glycoside **27** was isolated, in 74% yield. The three *p*-methoxybenzyl ethers were smoothly removed from **27** to give **28** using 10% trifluoroacetic acid in dichloromethane,[30] superior conditions in our hands to the DDQ-mediated cleavage used on **18ab**. Triol **28** was phosphitylated and oxidised as described for triol **19a** giving **29**, which was deprotected using catalytic hydrogenolysis to give the target compound **5**.

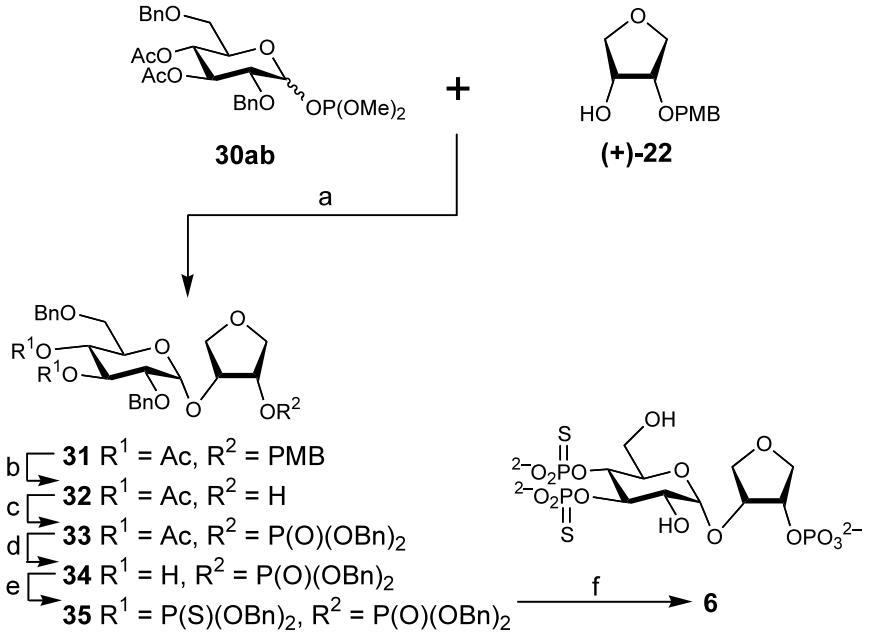
When tested for Ca^{2+} release from permeabilised hepatocytes, **5** behaved as a full agonist with a potency similar to Ins(1,4,5)P_3 and to **4**.[13,15] Several conclusions may be drawn relating to the structural basis for the activity of **3**, **4**, **5** and adenophostin A. First, the similar behaviour of **4** and **5** indicates that the 1-methoxyl group of **4** does not hinder activity, but neither does it enhance it. Subsequent studies have shown that even when this methoxyl group is replaced by imidazole, giving a compound more obviously similar to adenophostin A, the activity is not substantially increased.[26] Similarly, the 4-hydroxymethyl group of **4** is not essential for high potency at Ins(1,4,5)P_3 receptors, although in adenophostin A itself it may still be involved, as adenophostin A may interact with the receptor differently to **4** and **5**. Second, because **5** can be viewed as a conformationally restricted analogue of **3**, but is more potent than it, the view that the limited potency of **3** is related to the flexibility and/or over-extended conformation of the ethylphosphate structure[9] receives strong support; clearly conformational restriction alone can markedly enhance activity. Third, since **4** and **5** are essentially equipotent with Ins(1,4,5)P_3, but more potent than other disaccharide-based Ins(1,4,5)P_3 analogues,[11,15] it is probable that their common furanoid ring structure presents their 2- and 3′-phosphates, respectively, in an optimal position for Ins(1,4,5)P_3-like potency. Finally, it is notable that **4** and **5**, like the adenophostins, appear to behave as full agonists of hepatic Ins(1,4,5)P_3 receptors, in that maximal doses of **4**, **5** and Ins(1,4,5)P_3 all mobilise the same portion of the intracellular Ca^{2+} stores.

The replacement of phosphate groups in certain inositol phosphates with phosphorothioates can give partial agonists, which bind to Ins(1,4,5)P_3 receptors, but release only a fraction of the Ins(1,4,5)P_3-releasable Ca^{2+} pool.[2,31,32] Such compounds could provide a step towards the development of Ins(1,4,5)P_3 receptor antagonists. In a recent example, replacement of the 4- and 5-phosphate groups of 3-deoxy-3-fluoro-Ins(1,4,5)P_3 with phosphorothioates produced a partial agonist with an affinity only tenfold less than Ins(1,4,5)P_3 for type 1 Ins(1,4,5)P_3 receptors.[33] We reasoned that adenophostin analogues, such as **5**, might be promising candidates for this approach in that they are related to C-3 modified Ins(1,4,5)P_3 analogues [hydroxymethyl of glucose replacing 3-hydroxyl of Ins(1,4,5)P_3)] and retain

high affinity for the receptor. Furthermore, their structure (non-vicinal phosphate located on a separate ring) has advantages in terms of convergent synthetic strategy for creating novel ligands with the 2′-phosphate and 3,4-bisphosphorothioate pattern. In **6**, the two vicinal phosphate groups of furanophostin (**5**) have been replaced by phosphorothioates to give the first phosphorothioate-containing adenophostin analogue, furanophostin-PS_2.

The synthetic route to **6** (Scheme 4) required a glycosyl donor in which the 3- and 4-hydroxyl groups of glucose could selectively be exposed in the final stages of the synthesis, allowing late introduction of the sensitive vicinal bisphosphorothioate structure. This was conveniently achieved using the glycosyl donor **30ab**,[26] in which O-3 and O-4 of glucose are protected as acetate esters. Thus, $ZnCl_2/AgClO_4$-mediated glycosylation of (+)-**22** with **30ab** was employed, giving solely the α-coupled product **31**. The two acetate groups have a deactivating effect on the glycosyl donor,[34] and the reaction was considerably slower than the corresponding glycosylation of (+)-**22** with **26ab**. The single *p*-methoxybenzyl ether of **31** was smoothly cleaved using 10% trifluoroacetic acid in dichloromethane[30] without anomerisation to give the crystalline alcohol **32**, and the exposed hydroxyl group was subjected to phosphitylation followed by oxidation as before giving **33**. Cleavage of the two acetate esters on glucose using ammonia-saturated MeOH at room temperature required overnight reaction, conditions which resulted in the formation of more polar products, presumably from unwanted attack on the dibenzylphosphate ester. However, the required diol **34** was isolated in moderate (64%) yield. Phosphitylation of **34** with bis(benzyloxy)diisopropylaminophosphine as before now gave an intermediate bisphosphite, which was reacted with elemental sulphur in pyridine–DMF[35] to give fully-protected **35**. Total deprotection using sodium in liquid ammonia, and final purification by ion-exchange chromatography as before gave the target compound **6**. The ^{31}P NMR spectrum of **6** was distinctive, showing a single peak corresponding to the 3′-phosphate group at high field (0.68 ppm) with the phosphorus atoms of the 3- and 4-phosphorothioate groups resonating much further downfield at 51.42 and 50.01 ppm.

In displacement of [^{3}H]Ins(1,4,5)P_3 from the Ins(1,4,5)P_3 receptors of hepatic membranes,[15] **6** was found to be around fivefold weaker than the corresponding trisphosphate **5**, demonstrating that, as expected from studies with inositol polyphosphates, phosphorothioate substitution of the vicinal bisphosphate leads to a decrease in affinity for Ins(1,4,5)P_3 receptors. However, maximal concentrations of Ins(1,4,5)P_3 and **6** each caused similar amounts of Ca^{2+} to be released from the intracellular stores of permeabilised hepatocytes.[15] These preliminary results suggest that **6** behaves as a full agonist of hepatic Ins(1,4,5)P_3 receptors, although rapid measurements of rates of Ca^{2+} release will be required to establish the efficacy of **6** unequivocally. A possible explanation for the apparent high efficacy of **6** may be that its hydroxymethyl group closely mimics the 3-hydroxyl of



Scheme 4. (a) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4 Å MS; (b) CF_3COOH, CH_2Cl_2; (c) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, then MCPBA, −78 °C to rt; (d) NH_3–satd MeOH; (e) $(BnO)_2PNPr^i_2$, CH_2Cl_2, 1*H*-tetrazole then S_8, DMF, pyridine; (f) Na, liquid NH_3, −78 °C. PMB = *p*-methoxybenzyl.

$Ins(1,4,5)P_3$ and therefore does not provide sufficient structural perturbation[2,33] around the pseudo-3 position to significantly reduce efficacy. Future attempts to develop phosphorothioate-containing adenophostin analogues as partial agonists or antagonists may therefore require more substantial modification to C-5 of the pyranoside ring.

In summary, we have described the synthesis of three glucopyranoside-based $Ins(1,4,5)P_3$ receptor ligands **4**, **5** and **6** related to the adenophostins but lacking the adenine. In **6**, the important vicinal bisphosphate structure common to the adenophostins and $Ins(1,4,5)P_3$ has been replaced with a bisphosphorothioate to give the first phosphorothioate-containing adenophostin analogue. Compounds **4** and **5** (ribophostin and furanophostin) interact potently with $Ins(1,4,5)P_3$ receptors, although with equilibrium binding affinities lower than that of adenophostin A itself, and similar to that of the natural ligand $Ins(1,4,5)P_3$. The 3,4-bisphosphorothioate analogue of **5** (furanophostin-PS_2, **6**) has lower affinity for hepatic $Ins(1,4,5)P_3$ receptors and, at least in initial experiments, appears to behave as a full agonist.

Evidence is now emerging that, under some conditions, adenophostin analogues such as **4** and **5** may not behave simply as mimics of $Ins(1,4,5)P_3$, but are more similar to the adenophostins. A recent electrophysiological study of the effects of **4** and **5** in *Xenopus* oocytes[36] showed that in the absence of ATP, **4** and **5** activated $Ins(1,4,5)P_3$ receptor channels with gating properties similar to those of adenophostin A, and unlike those of $Ins(1,4,5)P_3$. Furthermore, electrophysiological studies in rat basophilic leukaemia (RBL-1) cells have now shown that under physiological conditions of low intracellular Ca^{2+} buffering, ribophostin (**4**) behaves similarly to adenophostin A in activating the store-operated Ca^{2+} current (I_{CRAC}) while $Ins(1,4,5)P_3$ is largely inactive.[37] Further studies using **5**, **6** and other analogues are in progress. Thus, it appears that in some cases, adenophostin A has qualitatively different effects on Ca^{2+} signalling to $Ins(1,4,5)P_3$ and that, surprisingly, adenophostin analogues such as **4** and **5** may sometimes behave as adenophostin mimics rather than as mimics of $Ins(1,4,5)P_3$. These compounds may therefore be useful tools for the investigation of intracellular Ca^{2+} signalling.

3. Experimental

General methods.—Thin-layer chromatography (TLC) was performed on pre-coated plates (E. Merck aluminium sheets silica 60 F_{254}). Products were visualised by spraying with phosphomolybdic acid in MeOH followed by heating. Flash chromatography was carried out using Sorbsil C60 Silica Gel. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM EX270 or EX400 NMR spectrometers. ^{31}P NMR spectra were recorded on JEOL FX90Q or EX400 NMR spectrometers and ^{31}P NMR chemical shifts were measured in ppm relative to external 85% H_3PO_4. 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 using *m*-nitrobenzyl alcohol (NBA) as the matrix. Optical rotations were measured at rt using an Optical Activity Ltd. AA-10 polarimeter. 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 quantitatively using the Ames phosphate assay[38] or by a modification of the Briggs phosphate assay.[39]

*Methyl 2,3-*O-p-*methoxybenzylidene-β-*D-*ribofuranoside* (**9ab**).—A 100 mL flask containing methyl β-D-ribofuranoside **8**[16] (4.23 g, 25.8 mmol), dry DMF (50 mL), *p*-methoxybenzaldehyde dimethyl acetal[17] (4.9 g, 27 mmol) and *p*-toluenesulfonic acid (50 mg) was fitted with an air condenser, attached to a water pump and evacuated. The solution was stirred at 70 °C until MeOH ceased to condense (4 h), then $NaHCO_3$ (0.5 g) was added and the suspension was allowed to cool to rt. The solvents were evaporated and the colourless oil was extracted with ether (2 × 150 mL). The combined organic extracts were washed with water (150 mL), dried ($MgSO_4$), filtered and concentrated to give the title compound as a colourless oil, which was shown by ^1H NMR to be a ca. 3:2 diastereoisomeric mixture (6.80 g, 93%); $[\alpha]_D$ −43° (*c* 3.1, $CHCl_3$), lit.[18] −61°; ^1H NMR ($CDCl_3$; 400 MHz):δ 7.43–7.37 (m, 2 H, *ortho*-H of *p*-methoxyphenyl), 6.92–6.89 (m, 2 H, *meta*-H of *p*-methoxyphenyl), 5.92 (s, 0.4 H, ArC*H*O_2^{min}), 5.72 (s, 0.6 H, ArC*H*O_2^{maj}), 5.12 (s, 0.6 H, H-1^{maj}), 5.09 (s, 0.4 H, H-1^{min}), 4.96, 4.70 (AB, 0.8 H, $J_{2,3}$ 5.6 Hz, H-2^{min} and H-3^{min}), 4.88, 4.66 (AB, 1.2 H, $J_{2,3}$ 6.3 Hz, H-2^{maj} and H-3^{maj}), 4.60 (t, 0.6 H, *J* 2.9 Hz, H-4^{maj}), 4.52 (t, 0.4 H, *J* 2.9 Hz, H-4^{min}), 3.80 (s, 3 H, ArO*Me*), 3.74–3.65 (m, 2 H, H-5a, H-5b), 3.46 (s, 3 H, OMe), 3.32 (dd, 0.6 H, *J* 10.5, 3.4 Hz, exch D_2O, OH^{maj}), 3.16 (dd, 0.4 H, *J* 9.8, 3.4 Hz, exch D_2O, OH^{min}); ^{13}C NMR ($CDCl_3$; 100.4 MHz): δ 160.84, 160.68 (2 × *para*-C), 132.00 (*ipso*-C), 128.35 (*ortho*-C^{maj}), 128.07 (*ortho*-C^{min}), 113.83 (*meta*-C), 109.64 (C-1^{maj}), 105.74 (Ar*C*HO_2^{maj}), 104.08 (Ar*C*HO_2^{min}), 88.11, 86.16, 84.98, 82.33, 80.96 (C-2–C-4), 64.04 (C-5^{min}), 63.95 (C-5^{maj}), 55.30, 55.56 (2 × OMe). The "min" and "maj" superscripts denote signals arising from the minor and major diastereoisomers, respectively.

*Methyl 5-*O-*benzyl-2,3-*O-p-*methoxybenzylidene-β-*D-*ribofuranoside* (**10ab**).—A solution of **9ab** (6.1 g, 21.6 mmol) in dry DMF (250 mL) was stirred at rt with

NaH (1.08 g of a 60% w/w dispersion in mineral oil, 27.0 mmol) and benzyl bromide (2.8 mL, 23.8 mmol) for 2.5 h, when TLC (EtOAc) indicated consumption of the starting material (R_f 0.35) to give a product (R_f 0.7). MeOH (20 mL) was added and stirring was continued for 30 min. The solvents were evaporated and the mixture was extracted with ether (2 × 200 mL). The combined organic layer was washed with water (200 mL), dried, filtered and concentrated. The syrup thus obtained was purified by flash chromatography (eluent 9:1 then 7:3 hexane–EtOAc) to give the title compound as a pale yellow oil, which was shown by ^{1}H NMR to be a ca. 3:2 diastereoisomeric mixture (6.5 g, 81%); $[\alpha]_D$ −22.6° (*c* 3.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 7.44–7.24 (m, 7 H, Ar), 6.92–6.85 (m, 2 H, *meta*-H of *p*-methoxyphenyl), 5.92 (s, 0.4 H, ArC*H*O$_2^{min}$), 5.73 (s, 0.6 H, ArC*H*O$_2^{maj}$), 5.11 (s, 0.6 H, H-1^{maj}), 5.07 (s, 0.4 H, H-1^{min}), 4.89–4.42 (m, 5 H, H-2, H-3, H-4, PhCH_2O), 3.79 (s, 3 H, ArO*Me*), 3.57–3.50 (m, 2 H, H-5a, H-5b), 3.32 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 160.77 (*para*-C^{maj} of *p*-methoxyphenyl), 160.61 (*para*-C^{min} of *p*-methoxyphenyl), 137.98 (*ipso*-C^{maj} of Bn), 137.91 (*ipso*-C^{min} of Bn), 128.38, 128.35, 128.16, 128.09, 127.69, 127.63 (Ar), 113.77 (*meta*-C of *p*-methoxyphenyl), 108.94 (C-1^{maj}), 108.81 (C-1^{min}), 106.02 (Ar*C*HO$_2^{maj}$), 104.08 (Ar*C*HO$_2^{min}$), 85.72, 84.91, 84.51, 84.18, 82.64, 81.86 (C-2–C-4), 73.24 (Ph*C*H$_2$O^{min}), 73.19 (Ph*C*H$_2$O^{maj}), 71.05 (C-5^{min}), 70.94 (C-5^{maj}), 55.26, 54.77, (2 × OMe). The "min" and "maj" superscripts denote signals arising from the minor and major diastereoisomers, respectively. FABMS (positive ion): *m*/*z* 373 ([M + 1]$^+$, 40%), 91 (100). Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.71; H, 6.50. Found: C, 67.6; H, 6.45.

*Methyl 5-*O*-benzyl-2-*O*-*p*-methoxybenzyl-β-*D*-ribofuranoside* (**13**) *and methyl 5-*O*-benzyl-3-*O*-*p*-methoxybenzyl-β-*D*-ribofuranoside* (**11**).—A solution of DIBAL–H (1.0 M in CH_2Cl_2; 15 mL, 15.0 mmol) was added dropwise to a solution of acetal **10ab** (1.12 g, 3.0 mmol) in dry CH_2Cl_2 (10 mL) at −78 °C. The solution was stirred at −78 °C for 10 min, then was warmed to 0 °C over 90 min. MeOH (10 mL) was added dropwise and the system solidified to a gel. 10% w/v aq KOH (30 mL) was added to dissolve the gel and the resultant biphasic solution was diluted with CH_2Cl_2 (100 mL) and water (100 mL). The organic layer was washed with water (100 mL) and the combined aqueous layers were re-extracted with CH_2Cl_2 (100 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue thus obtained was subjected to flash chromatography (eluent 15:1 $CHCl_3$–Me_2CO) to give **13** (480 mg, 43%); R_f 0.45 (10:1 $CHCl_3$–Me_2CO); mp 42–43 °C (*i*-PrOH); $[\alpha]_D$ +34.6° (*c* 2.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 7.34–7.28 (m, 5 H, Ph), 7.27–7.24 (m, 2 H, *ortho*-H of PMB), 6.92–6.86 (m, 2 H, *meta*-H of PMB), 4.88 (d, 1 H, $J_{1,2}$ 0.5 Hz, H-1), 4.67–4.50 (m, 4 H, 2 overlapping ArCH_2O AB systems), 4.17–4.07 (br m, 2 H, sharpens on D_2O exch, H-3, H-4), 3.83 (dd, 1 H, $J_{2,3}$ 3.7 Hz, H-2), 3.79 (s, 3 H, ArO*Me*), 3.63 (dd, 1 H, $J_{5a,5b}$ 10.4, $J_{5a,4}$ 3.5 Hz, H-5a), 3.54 (dd, 1 H, $J_{5b,4}$ 6.1 Hz, H-5b), 3.32 (s, 3 H, OMe), 2.70–2.50 (br s, 1 H, exch D_2O, OH); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 159.56 (*para*-C of PMB), 138.22 (*ipso*-C of Bn), 129.64 (Ar), 129.21 (*ipso*-C of PMB), 128.33, 127.61,127.54 (Ar), 113.97 (*meta*-C of PMB), 105.85 (C-1), 83.17, 81.62 (C-2, C-4), 73.28, 72.53 (2 × Ar*C*H$_2$O), 71.71 (C-3), 71.61 (C-5), 55.25, 55.16 (2 × OMe); FABMS (positive ion): *m*/*z* 374 ([M$^+$], 10%), 121 (100). Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.35; H, 7.00. Found: C, 67.4; H, 7.05.

A sample of **13** was converted into its acetate **14** with Ac_2O in pyridine; R_f 0.55 (10:1 $CHCl_3$–Me_2CO); $[\alpha]_D$ +17.5° (*c* 2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 7.34–7.26 (m, 5 H, Ph), 7.25–7.20 (m, 2 H, *ortho*-H of PMB), 6.90–6.82 (d, 2 H, *meta*-H of PMB), 5.13 (t, 1 H, $J_{3,2} = J_{3,4} = 5.3$ Hz, H-3), 4.89 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.62–4.48 (m, 4 H, 2 × overlapping ArCH_2O AB systems), 4.32 (q, 1 H, $J_{4,5a} = J_{4,5b} = 5.3$ Hz, H-4), 4.06 (dd, 1 H, H-2), 3.79 (s, 3 H, ArO*Me*), 3.58–3.56 (m, 2 H, H-5a, H-5b), 3.34 (s, 3 H, OMe), 2.07 (s, 3 H, $MeCO_2$); FABMS (positive ion): *m*/*z* 416 ([M$^+$], 25%), 121 (100). Anal. Calcd for $C_{23}H_{28}O_7$: C, 66.32; H, 6.78. Found: C, 66.6; H, 6.69.

Further elution gave **11** as a pale yellow syrup (371 mg, 33%); R_f 0.3 (10:1 $CHCl_3$–Me_2CO); $[\alpha]_D$ −28.8° (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.35–7.19 (7 H, m, Ar); 6.88–6.82 (m, 2 H, *meta*-H of PMB), 4.85 (s, 1 H, H-1), 4.56 (s, 2 H, ArCH_2O), 4.49 (s, 2 H, ArCH_2O), 4.20 (q, 1 H, $J_{4,3} = J_{4,5a} = J_{4,5b} = 5.8$ Hz, H-4), 4.04 (dd, 1 H, $J_{3,2}$ 4.9 Hz H-3), 3.99 (d, 1 H, H-2), 3.78 (s, 3 H, ArO*Me*), 3.54–3.49 (m, 2 H, H-5a, H-5b), 3.31 (s, 3 H, OMe), 2.80–2.26 (br s, 1 H, exch D_2O, OH); ^{13}C NMR ($CDCl_3$; 67.8 MHz): δ 159.58 (*para*-C of PMB), 138.15 (*ipso*-C of Bn), 129.66 (Ar), 129.17 (*ipso*-C of PMB), 128.33, 127.61 (Ar), 113.95 (*meta*-C of PMB), 108.52 (C-1), 80.54, 79.15 (C-2–C-4), 73.24, 72.46, 71.62 (C-5, 2 × Ar*C*H$_2$O), 55.25, 54.99 (2 × OMe); FABMS (positive ion): *m*/*z* 374 ([M$^+$], 20%), 121 (100). Anal. Calcd for $C_{21}H_{26}O_6$: C, 67.35; H, 7.00. Found: C, 67.5; H, 7.07.

A sample of **11** was converted into its acetate **12** with Ac_2O in pyridine; R_f 0.5 (10:1 $CHCl_3$–Me_2CO); $[\alpha]_D$ +21.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 7.34–7.25 (m, 5 H, Ph), 7.22–7.14 (m, *ortho*-H of PMB), 6.85–6.79 (m, 2 H, *meta*-H of PMB), 5.18 (d, 1 H, $J_{2,3}$ 4.4 Hz, H-2), 4.87 (s, 1 H, H-1), 4.57, 4.54 (AB, 2 H, J_{AB} 12.3 Hz, ArCH_2O), 4.51, 4.34 (AB, 2 H, J_{AB} 11.1 Hz, ArCH_2O), 4.20 (1 H, ddd, $J_{4,3}$ 7.7, $J_{4,5b}$ 5.7, $J_{4,5a}$ 3.6 Hz, H-4), 4.11 (dd, 1 H, H-3), 3.77 (s, 3 H, ArO*Me*), 3.59 (dd, 1 H, $J_{5a,5b}$ 10.7 Hz, H-5a), 3.48 (dd, 1 H, H-5b), 3.33 (s, 3 H, OMe), 2.12 (s, 3 H, $MeCO_2$); FABMS (positive ion): *m*/*z* 416 ([M$^+$], 30%), 121 (100).

Anal. Calcd for $C_{23}H_{28}O_7$: C, 66.32; H, 6.78. Found: C, 66.1; H, 6.82.

Regeneration of **10ab**.—DDQ (591 mg, 2.6 mmol) was added to a solution of **11** (811 mg, 2.2 mmol) in dry CH_2Cl_2 (5 mL) containing freshly activated 3 Å molecular sieves. The mixture, which rapidly turned black, was stirred at rt for 3 h, when TLC (10:1 $CHCl_3$–Me_2CO) indicated consumption of starting material (R_f 0.3) to give a product (R_f 0.6). 10% w/v aq Na_2SO_3 (50 mL) and CH_2Cl_2 (100 mL) were added and the mixture was stirred vigorously for 10 min. The organic layer was collected and washed with satd aq $NaHCO_3$ (2 × 100 mL) and satd aq NaCl (100 mL), dried ($MgSO_4$), filtered and concentrated. Flash chromatography (eluent 19:1 hexane–EtOAc then 7:3) gave acetal **10ab**, which was shown by ^{1}H NMR to be a 92:8 diastereoisomeric mixture (569 mg, 71%); $[\alpha]_D$ −26.4° (*c* 3.3, $CHCl_3$).

*Methyl 5-*O*-benzyl-β-*D*-ribofuranoside* (**15**).—A solution of acetal **10** (216 mg, 0.6 mmol) in 80% v/v aq AcOH (preheated to 60 °C, 23 mL) was stirred at 60 °C for 25 min, then rapidly cooled (ice-bath). Toluene (50 mL) was added and the mixture was evaporated to dryness. The orange syrup thus obtained was subjected to flash chromatography (eluent 9:1 hexane–EtOAc to remove *p*-methoxybenzaldehyde then 1:4) to give the title compound as a pale yellow syrup, which TLC (cf. 23) and ^{1}H NMR showed to be exclusively the β anomer (125 mg, 85%); $[\alpha]_D$ −42.0° (*c* 4.9, $CHCl_3$) lit.[23] −47.7°.

*2,6-Di-*O*-benzyl-3,4-di-*O*-p-methoxybenzyl-β-*D*-glucopyranosyl trichloroacetimidate* (**17b**) *and 2,6-di-*O*-benzyl-3,4-di-*O*-p-methoxybenzyl-α-*D*-glucopyranosyl trichloroacetimidate* (**17a**).—To a solution of **16**[10] (1.00 g, 1.67mmol) in freshly distilled, dry CH_2Cl_2 (10 mL) was added freshly flame-dried K_2CO_3 (1.00 g, 7.25 mmol), followed by freshly distilled trichloroacetonitrile (1.00 mL). The mixture was left to stir at rt under an atmosphere of nitrogen for 140 min, after which time TLC (30:1 $CHCl_3$–Me_2CO) indicated a small amount of starting material (R_f 0.14), a major product (R_f 0.47) and a minor product (R_f 0.58). The reaction mixture was filtered through a pad of Celite and concentrated to a colourless oil. This oil was purified by flash chromatography (eluent 50:1 $CHCl_3$–Me_2CO), to give the β anomer **17b** (0.59 g, 48%), which was crystallised from a minimum volume of 1:1 ether–petroleum ether at −4 °C; mp 80–81 °C; $[\alpha]_D$ +16.5° (*c* 4.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 8.69 (s, 1 H, C=NH), 7.31–7.20 (m, 12 H, Ar), 7.09 (m, 2 H, *ortho*-H of PMB), 6.85–6.79 (m, 4 H, *meta*-H of PMB), 5.78 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.93 (AB, 1 H, J_{AB} 11.0 Hz, OCH*H*Ar), 4.82 (AB, 1 H, J_{AB} 10.4 Hz, OCH*H*Ar), 4.78–4.47 (m, 6 H, 3 × OC*H*$_2$Ar), 3.80, 3.79 (2 s, 6 H, 2 × OCH_3), 3.73–3.56 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b); ^{13}C NMR ($CDCl_3$; 100.4 MHz): δ 161.23 (C=NH), 159.20 (*para*-C of PMB), 138.11, 138.02 (*ipso*-C of Bn), 130.63, 130.19 (*ipso*-C of PMB), 129.64, 129.46, 128.47, 128.36, 127.93, 127.89, 127.76, 127.61 (ArCH), 113.79, (*meta*-C of PMB), 98.35 (C-1), 75.90, 77.03, 81.00, 84.29 (C-2–C-5), 75.32, 74.91, 74.60, 73.35 (4 × O*C*H$_2$Ar), 68.21 (C-6), 55.27 (2 × OCH_3); FABMS (negative ion): *m*/*z* 744 ([M − H]$^-$, 34%), 311 (73), 188 (100). Anal. Calcd for $C_{38}H_{40}N_1O_8Cl_3$: C, 61.26; H, 5.41; N, 1.88. Found: C, 61.4; H, 5.41; N 1.86.

Further elution gave the syrupy α-anomer **17a** (0.25 g, 20%); $[\alpha]_D$ +13.6° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz): δ 8.57 (s, 1 H, C=NH), 7.32–7.23 (m, 12 H, ArCH), 7.08–7.05 (m, 2 H, *ortho*-H of PMB), 6.85–6.79 (m, 4 H, *meta*-H of PMB), 6.51 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.87, 4.76 (AB, 2 H, J_{AB} 10.4 Hz, OC*H*$_2$Ar), 4.74, 4.69 (AB, 2 H, J_{AB} 11.7 Hz, OC*H*$_2$Ar), 4.60, 4.47 (AB, 2 H, J_{AB} 12.1 Hz, OC*H*$_2$Ar), 4.77, 4.45 (AB, 2 H, J_{AB} 10.3 Hz, OC*H*$_2$Ar), 4.06–3.94 (m, 2 H, H-3, H-5), 3.79, 3.78 (2 s, 6 H, 2 × OCH_3), 3.80–3.71 (m, 3 H, H-2, H-4, H-6a), 3.65 (dd, 1 H, $J_{6b,6a}$ 10.9, $J_{6b,5}$ 1.9 Hz, H-6b); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 161.32 (s, C=NH), 159.31, 159.12 (*para*-C of PMB), 138.04, 137.91 (*ipso*-C of Bn), 130.85, 130.28 (*ipso*-C of PMB), 129.74, 129.68, 128.36, 127.94, 127.69, 127.59 (ArCH), 113.83, 113.77 (*meta*-C of PMB), 94.42 (C-1), 81.07, 79.43, 76.50, 73.17 (C-2–C-5), 75.31, 74.94, 73.46, 72.88 (O*C*H$_2$Ar), 68.03 (C-6), 55.27 (2 × OCH_3); FABMS (negative ion): *m*/*z* 744 ([M − H]$^-$, 1%), 121 (100). A satisfactory elemental analysis could not be obtained for this compound.

*Methyl 2′,5,6′-tri-*O*-benzyl-3-*O*-*D*-glucopyranosyl-2,3′,4′-tri-*O*-p-methoxybenzyl-β-*D*-ribofuranosides* (**18ab**).—A mixture of **17b** (188 mg, 0.25 mmol), and **13** (86 mg, 0.23 mmol) was stirred in dry ether (2.5 mL) at rt with 4 Å molecular sieves (90 mg) for 30 min, whereupon trimethylsilyl triflate (0.01 mL of a 0.2 M solution in ether, 2 nmol) was added. After 5 min TLC (30:1 $CHCl_3$–Me_2CO) showed formation of a product (R_f 0.26) from the imidate (R_f 0.32), and the glycosyl acceptor (R_f 0.23). The reaction was quenched with Et_3N (6 drops), and the mixture was concentrated. The resulting clear oil was subjected to flash chromatography (eluent 30:1 $CHCl_3$–Me_2CO), and again (eluent 35:1 $CHCl_3$–Me_2CO), to give an inseparable glucopyranosyl anomeric mixture of the title compound in a 4:1 α:β anomeric ratio, as calculated from ^{1}H NMR integral ratios (120 mg, 54%); Selected ^{1}H NMR data for α-coupled product: ^{1}H NMR (400 MHz; $CDCl_3$): δ 5.09 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.93 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1); FABMS (negative ion): *m*/*z* 1109 ([M + NBA]$^-$, 100%). Anal Calcd for $C_{57}H_{64}O_{13}$: C, 71.53; H, 6.74. Found: C, 71.5; H, 6.72.

*Methyl 2′,5,6′-tri-*O*-benzyl-3-*O*-β-*D*-glucopyranosyl-β-*D*-ribofuranoside* (**19b**) *and methyl 2′,5,6′-tri-*O*-benzyl-3-*O*-α-*D*-glucopyranosyl-β-*D*-ribofuranoside* (**19a**).

—A solution of **18ab** (379 mg, 0.40 mmol) in CH_2Cl_2 (12 mL) and water (1 mL) was stirred for 20 min, whereupon DDQ (551 mg, 2.38 mmol) was added. After 60 min TLC (30:1 $CHCl_3$–Me_2CO) showed consumption of starting material (R_f 0.26). The reaction mixture was diluted with CH_2Cl_2 (60 mL), and the organic layer washed with aq 10% w/v Na_2SO_3 (3 × 50 mL), followed by 50 mL each of satd aq $NaHCO_3$ and satd aq NaCl. The organic layer was dried ($MgSO_4$) filtered and concentrated to give a clear oil, which was subjected to flash chromatography (eluent 7:3 EtOAc–hexane), to give the β anomer **19b** (27 mg, 11%); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.35–7.23 (m, 15 H, ArCH), 4.90 (s, 1 H, H-1), 4.81, 4.58 (AB, 2 H, J_{AB} 11.5 Hz, OCH_2Ar), 4.54–4.50 (m, 4 H, 2 × OCH_2Ar), 4.45 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.33–4.28 (m, 1 H, H-4), 4.18 (dd, 1 H, $J_{3,4}$ 6.8, $J_{3,2}$ 4.4 Hz, H-3), 4.11 (d, 1 H, H-2), 3.70 (dd, 1 H, $J_{6'a,6'b}$ 10.5, $J_{6'a,5}$ 3.2 Hz, H-6′a), 3.63–3.59 (m, 2 H, H-5a, H-6′b), 3.56 (dd, 1 H, $J_{5b,5a}$ 10.5, $J_{5b,4}$ 5.6 Hz, H-5b), 3.49–3.40 (m, 3 H, H-3′, H-4′, H-5′), 3.33 (s, 3 H, OCH_3), 3.27–3.23 (m, 1 H, H-2′), 3.06, 2.86, 2.30 (3 × br. s, 3 H, 3 × OH).

Further elution gave the α anomer **19a** (135 mg, 57%), which crystallised spontaneously on standing; mp 103–105 °C; $[\alpha]_D$ +28.3° (*c* 3.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.36–7.23 (m, 15 H, ArCH), 4.88 (s, 1 H, H-1), 4.74, 4.69 (AB, 2 H, *J* 11.7 Hz, OCH_2Ar), 4.69 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.51 (s, 2 H, OCH_2Ar), 4.52, 4.44 (AB, 2 H, J_{AB} 12.2 Hz, OCH_2Ar), 4.22 (m, 2 H, H-2, H-4), 4.01 (m, 1 H, H-3), 3.92 (t, 1 H, $J_{3'2'} = J_{3',4'} = 9.3$ Hz, H-3′), 3.74 (dt, 1 H, $J_{5',4'}$ 9.8, $J_{5',6'a}$ 3.9, $J_{5',6'b}$ 3.9 Hz, H-5′), 3.57–3.45 (m, 5 H, H-4′, H-5a, H-5b, H-6′a, H-6′b), 3.38 (dd, 1 H, H-2′), 3.32 (s, 3 H, OCH_3), 2.87, 2.74 (2 br s, 2 H, D_2O exch, OH), 1.69 (br s, 1 H, D_2O exch, OH); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 138.06, 137.86, 137.18 (*ipso-C* of Bn), 128.75, 128.51, 128.42, 128.33, 127.74, 127.69, 127.63, 127.57 (ArCH), 108.34 (C-1), 97.79 (C-1′), 80.27 (C-2 or C-4), 79.11 (C-2 or C-4), 78.35 (C-2′), 74.14, 73.55, 73.32 (O*C*H_2Ar), 73.28 (C-3), 73.24 (C-3′), 71.69 (C-5 or C-6′), 70.84 (C-5′), 70.74 (C-4′), 69.00 (C-5 or C-6′), 55.03 (OCH_3); FABMS (positive ion): *m*/*z* 597 ([M + 1]$^+$, 12%), 565 (48), 343 (3), 255 (2). HRFABMS (positive ion): Calcd for $C_{33}H_{40}O_{10}$ [M$^+$]: 596.262. Found: 596.259.

Methyl 2′,5,6′-tri-O-*benzyl-3*-O-α-D-*glucopyranosyl-2,3′,4′-tris*-O-*(dibenzyloxyphosphoryl)*-β-D-*ribofuranoside* (**20**).—A mixture of bis(benzyloxy)-(diisopropylamino)phosphine (372 mg, 1.08 mmol), dry CH_2Cl_2 (3 mL) and 1*H*-tetrazole (113 mg, 1.62 mmol) was stirred at rt for 30 min, whereupon a solution of **19a** (107 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) was added and stirring was continued for a further 30 min. TLC (7:3 EtOAc–hexane) indicated complete conversion of starting material (R_f 0.14) into a product (R_f 0.49), and ^{31}P NMR spectroscopy showed phosphite triester signals. The system was cooled to −78 °C, MCPBA (432 mg, 2.15 mmol) was added, the cooling bath was removed, and the mixture was allowed to warm to rt. The mixture was diluted with CH_2Cl_2 (100 mL) and the organic extract was separated and washed with 10% aq Na_2SO_3 (50 mL), satd aq $NaHCO_3$ (2 × 50 mL), and satd aq NaCl (50 mL). The organic solution was dried ($MgSO_4$), filtered and concentrated to give a white solid which was purified by flash chromatography (eluent 20:1 $CHCl_3$–Me_2CO then 10:1, then 4:1) to give the title compound as a colourless oil (220 mg, 89%); $[\alpha]_D$ +34.7° (*c* 8.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz): δ 7.35–7.01 (m, 45 H, ArCH), 5.09 (d, 1 H, $J_{1',2'}$ 3.4 Hz, H-1′), 5.03–4.80 (m, 13 H, H-1, H-3′, 11 × OCH_2Ar), 4.77–4.67 (m, 3 H, 2 × OCH_2Ar, H-2), 4.60 (q, 1 H, $J_{4',3'} \approx J_{4',5'} \approx J_{HP} \approx 9.5$ Hz, H-4′), 4.53–4.34 (m, 5 H, 4 × OCH_2Ar, H-3), 4.31–4.28 (AB, 2 H, J_{AB} 11.7 Hz, OCH*H*Ar overlapping with H-4), 3.84–3.82 (m, 1 H, H-5′), 3.65 (ABX, 2 H, J_{AB} 10.4, $J_{6'a,5'} = J_{6'b,5'} = 3.9$ Hz, H-6′a and H-6′b), 3.60–3.55 (m, 2 H, H-2′, H-5a), 3.52 (dd, 1 H, $J_{5b,5a}$ 10.5, $J_{5b,4}$ 5.1 Hz, H-5b), 3.25 (s, 3 H, OCH_3); ^{31}P NMR ($CDCl_3$; 162 MHz; ^{1}H decoupled) δ −1.22, −1.94, −2.25 (3 s); FABMS (positive ion): *m*/*z* 1377 ([M$^+$], 4%), 91 (100). HRFABMS (positive ion): Calcd for $C_{75}H_{80}O_{19}P_3$ [M$^+$]: 1377.450. Found: 1377.451.

Methyl 3-O-α-D-*glucopyranosyl*-β-D-*ribofuranoside 2,3′,4′-trisphosphate* (**4**).—10% palladium on activated charcoal (200 mg), was added to a solution of **20** in MeOH (40 mL) and water (10 mL). This mixture was shaken under 40 psi pressure in an atmosphere of H_2 for 18 h, after which it was filtered through Celite. The filtrate was concentrated to a glassy clear solid. The residue was dissolved in de-ionised water (300 mL) and purified by ion-exchange chromatography on Q Sepharose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 7.5. The triethylammonium salt of the title compound eluted over 800–850 mM buffer. After concentration of the appropriate fractions, the triethylammonium salt of **4** was found to have very low solubility in water. Therefore **4** was converted into its potassium salt with 0.1 M aq KOH (2 mL), and subsequently quantified by a modification of the Briggs total phosphate assay[39] (60 μmol, 78%); $[\alpha]_D$ +79.1° (*c* 1.70 calculated for free acid, MeOH); ^{1}H NMR (triethylammonium salt, CD_3OD; 400 MHz): δ 5.13 (d, 1 H, $J_{1',2'}$ 3.7 Hz, H-1′), 4.94 (s, 1 H, H-1), 4.58 (dd, 1 H, J_{HP} 9.5, $J_{2,3}$ 4.3 Hz, H-2), 4.45 (q, 1 H, $J_{3',2'} \approx J_{3',4'} \approx J_{HP} \approx 9$ Hz, H-3′), 4.44 (dd, 1 H, $J_{3,4}$ 7.3 Hz, H-3), 4.11–4.04 (m, 2 H, H-4, H-4′), 3.93 (ABX, 1 H, $J_{6'a,6'b}$ 13.0, $J_{6'a,5}$ 3.5 Hz, H-6′a), 3.73–3.69 (m, 3 H, H-5a, H-5′, H-6′b), 3.62 (dd, 1 H, H-2′), 3.54 (ABX, 1 H, $J_{5b,5a}$ 11.9, $J_{5b,4}$ 6.4, Hz, H-5b), 3.35 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 108.96 ($^3J_{CP}$ 3.7, C-1), 98.93 (C-1′), 82.71 (C-4), 78.83, 76.46, 76.29, 73.78, 73.52, 73.32 (C-2, C-3, C-2′–C-5′), 64.87, 61.98 (C-5,

C-6′), 55.18 (OCH_3); ^{31}P NMR (CD_3OD; 162 MHz; ^1H decoupled) δ_P 1.10, 1.05, −0.38, (3 s); FABMS (negative ion): m/z 565 ($[M-H]^-$ 100%); HRFABMS (negative ion): Calcd for $C_{12}H_{24}O_{19}P_3$ $[M-H]^-$: 565.012. Found: 565.012.

cis-4-(p-Methoxybenzyloxy)-tetrahydrofuran-3-ol [(±)-22].—To a solution of 1,4-anhydroerythritol (**21**) (10.4 g, 100 mmol) in dry DMF (100 mL) in a 250 mL round bottom flask was added *p*-methoxbenzaldehyde dimethyl acetal (19.1 g, 105 mmol) and a catalytic amount of PTSA (100 mg). The flask was fitted with a 250 mm air condenser connected to a filter pump and the solution was stirred at 70 °C under reduced pressure for 3 h, after which time TLC (Et_2O) showed the reaction to be complete, with complete conversion of starting material into two products (R_f 0.52 and 0.58). Excess $NaHCO_3$ (500 mg) was added and the mixture was allowed to cool. The solvents were removed by evaporation under reduced pressure and the oily residue was taken up in Et_2O (500 mL), washed with water (300 mL), and dried ($MgSO_4$). A few drops of Et_3N were added, and the solution was concentrated by evaporation under reduced pressure to give the crude mixture of epimeric acetals as a yellow oil (23.0 g). A portion of this product (8.10 g) was dissolved in dry CH_2Cl_2 (100 mL) and stirred at −78 °C under N_2. A solution of DIBAL–H (90 mL of a 1.0 M solution in CH_2Cl_2, 90 mmol) was added dropwise over 15 min. After 4 h, TLC (1:1 EtOAc–hexane) showed the reaction to be almost complete with conversion of the two epimeric acetals into a single, more polar product (R_f 0.26). The cooling bath was removed, and after a further 45 min the mixture was poured into a rapidly stirring mixture of EtOH (20 mL) and CH_2Cl_2 (200 mL) at 0 °C [CARE! FIZZING!]. NaOH (0.5 M, 300 mL) was added and stirring continued to dissolve the gelatinous material that had formed. The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (2 × 300 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated by evaporation under reduced pressure to give an oil. Purification by flash chromatography (2:3 EtOAc–hexane) gave the racemic alcohol (±)-**22** as a colourless oil (7.12 g, 31.7 mmol, 90% yield from **21**); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28–7.25 (m, 2 H, *ortho*-H of PMB), 6.92–6.88 (m, 2 H, *meta*-H of PMB), 4.55, 4.53 (AB, 2 H, J_{AB} 11.3 Hz, OCH_2Ar), 4.25–4.20 (m, 1 H, H-3), 4.05 (q, 1 H, $J_{4,3} \approx J_{4,5a} \approx J_{4,5b} \approx 5.5$ Hz, H-4), 3.90–3.86 (m, 2 H, H-2a and H-5a), 3.81 (s, 3 H. $ArOCH_3$), 3.77–3.72 (m, 2 H, H-2b and H-5b), 2.79 (d, 1 H, J 5.5 Hz, D_2O exch, 3-OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.62 (*para*-C of PMB), 129.63 (*ortho*-C of PMB), 129.24 (*ipso*-C of PMB), 114.03 (*meta*-C of PMB), 78.00 (C-4), 73.48 (CH_2), 72.33 (CH_2), 70.28 (C-3), 70.03 (CH_2), 55.30 ($ArOCH_3$); FABMS (positive ion): m/z 224 ($[M^+]$, 20%); 121 (100).

(3S,4R)-3-[(−)-Camphanoyloxy]-4-(p-methoxybenzyloxy)-tetrahydrofuran (**23**) *and (3R,4S)-3-[(−)-camphanoyloxy]-4-(p-methoxybenzyloxy)-tetrahydrofuran* (**24**).—To a stirred solution of the racemic alcohol (±)-**22** (7.0 g, 31.2 mmol) in anhyd pyridine (60 mL) at 0 °C was added (−)-(*S*)-camphanic chloride (8.0 g, 36.9 mmol). After 5 min, the cooling bath was removed and the mixture was stirred for a further 1 h, after which time TLC (ether) showed complete conversion of starting material (R_f 0.32) into two products (R_f 0.40 and 0.48). Excess camphanic chloride was destroyed by addition of water (10 mL) and the solvents were removed by evaporation under reduced pressure. The residue was taken up in Et_2O (200 mL) and water (200 mL). The organic layer was separated and washed sequentially with 1 M HCl and satd aq $NaHCO_3$ (200 mL of each), dried ($MgSO_4$) and concentrated by evaporation under reduced pressure to give an oil. The diastereoisomers were separated by flash chromatography (2:1 Et_2O–pentane) followed by crystallisation.

The less polar diastereoisomer **24** was crystallised from Et_2O (5.09 g, 12.6 mmol, 80% of this diastereoisomer); mp 67–68 °C; R_f 0.24 (2:1 Et_2O–pentane); $[\alpha]_D$ −41° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.26–7.21 (m, 2 H, *ortho*-H of PMB), 6.90–6.85 (m, 2 H, *meta*-H of PMB), 5.47 (dt, 1 H, $J_{3,4}$ 4.8, $J_{3,2a}$ 4.8, $J_{3,2b}$ 2.6 Hz, H-3), 4.54, 4.43 (AB, 2 H, J_{AB} 11.2 Hz, OCH_2Ar), 4.20 (dt, 1 H, $J_{4,5b}$ 7.5, $J_{4,5a}$ 4.9 Hz, H-4), 4.09 (dd, 1 H, $J_{2a,2b}$ 10.8 Hz, H-2a), 3.98–3.92 (m, 2 H, H-2b and H-5a), 3.80 (s, 3 H, $ArOCH_3$), 3.66 (dd, 1 H, $J_{5b,5a}$ 8.3 Hz, H-5b), 2.48–2.37 (m, 1 H, camph CH_2), 2.02–1.84 (m, 2 H, camph CH_2), 1.72–1.60 (m, 1 H, camph CH_2), 1.10 (s, 3 H, camph CH_3), 0.99 (s, 3 H, camph CH_3), 0.96 (s, 3 H, camph CH_3); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 178.15 (C=O), 166.94 (C=O), 159.44 (*para*-C of PMB), 129.56 (*ortho*-C of PMB), 129.40 (*ipso*-C of PMB), 113.82 (*meta*-C of PMB), 91.18 (camph quaternary C), 77.20 (C-4), 72.74 (C-3), 72.62 (OCH_2Ar), 71.05 (CH_2), 69.31 (CH_2), 55.29 ($ArOCH_3$), 54.91 (camph quaternary C), 54.19 (camph quaternary C), 30.77 (camph CH_2), 28.85 (camph CH_2), 16.70 (camph CH_3), 16.59 (camph CH_3), 9.70 (camph CH_3); FABMS (positive ion): m/z 426 ($[M+Na]^+$, 40%), 404 ($[M^+]$, 100%); FABMS (negative ion): 197 [(camphO)$^-$, 100%]; (camph = camphanoyl); Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H 6.98, Found C, 65.5; H, 7.09.

The more polar diastereoisomer **23** was crystallised from diisopropyl ether; (5.21 g, 12.9 mmol, 82% of this diastereoisomer) mp 81–83 °C; R_f 0.18 (2:1 Et_2O–pentane); $[\alpha]_D$ +27° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 270 MHz) δ 7.28–7.22 (m, 2 H, *ortho*-H of PMB), 6.89–6.84 (m, *meta*-H of PMB), 5.50 (dt, 1 H, $J_{3,4}$ 4.8, $J_{3,2a}$ 4.8, $J_{3,2b}$ 2.6 Hz, H-3), 4.57, 4.44 (AB, 2 H, J_{AB} 11.5 Hz, OCH_2Ar), 4.22–4.15 (m, 1 H, H-4), 4.09 (dd, 1 H, $J_{2a,2b}$ 10.6 Hz, H-2a), 3.97–3.90 (m, 2 H, H-2b and

H-5a), 3.80 (s, 3 H, $ArOCH_3$), 3.66 (dd, 1 H, $J_{5b,5a}$ 8.4, $J_{5b,4}$ 8.1 Hz, H-5b), 2.46–2.35 (m, 1 H, camph CH_2), 2.07–1.86 (m, 2 H, camph CH_2), 1.74–1.64 (m, 1 H, camph CH_2), 1.10 (s, 3 H, camph CH_3), 1.05 (s, 3 H, camph CH_3), 0.89 (s, 3 H, camph CH_3); ^{13}C NMR ($CDCl_3$, 68 MHz) δ 178.04 (C=O), 166.75 (C=O), 159.44 (*para*-C of PMB), 129.58 (*ortho*-C of PMB), 129.37 (*ipso*-C of PMB), 113.80 (*meta*-C of PMB), 91.09 (camph quaternary C), 76.99 (C-4), 72.64 (C-3), 72.59 (CH_2), 71.02 (CH_2), 69.23 (CH_2), 55.29 ($ArOCH_3$), 54.85 (camph quaternary C), 54.20 (camph quaternary C), 30.73 (camph CH_2), 28.95 (camph CH_2), 16.59 (camph CH_3), 16.36 (camph CH_3), 9.68 (camph CH_3); FABMS (positive ion): m/z 426 ($[M+Na]^+$, 55%), 404 ($[M^+]$, 100%); (camph = camphanoyl); Anal. Calcd for $C_{22}H_{28}O_7$: C, 65.33; H 6.98, Found C, 65.5; H, 7.00.

*(3*R*,4*S*)-4-(*p*-Methoxybenzyloxy)-tetrahydrofuran-3-ol [(+)-**22**].*—To a solution of the camphanate ester **24** (3.10 g, 7.66 mmol) in MeOH (100 mL) were added NaOH pellets (2.0 g, 50 mmol). The mixture was heated at reflux for 1 h and then allowed to cool. The NaOH was neutralised by careful addition of solid CO_2, and water (100 mL) was added. The MeOH was removed by evaporation under reduced pressure and the remaining aqueous solution was extracted with CH_2Cl_2 (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and concentrated by evaporation under reduced pressure to give an oil which slowly crystallised. Crystallisation from Et_2O at −20 °C gave pure (+)-**22** as colourless needles (1.62 g, 7.22 mmol, 94% yield); mp 53–54.5 °C; $[\alpha]_D$ +13° (*c* 1, $CHCl_3$); Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.29; H 7.19, Found C, 64.6; H, 7.21. Spectroscopic data were identical to those for (±)-**2**.

*(3*S*,4*R*)-4-(*p*-Methoxybenzyloxy)-tetrahydrofuran-3-ol [(−)-**22**].*—Saponification of **23** (2.20 g, 5.43 mmol) and crystallisation as described for **24** gave (−)-**22** as colourless needles (1.18 g, 5.26 mmol, 97% yield); mp 53–54.5 °C; $[\alpha]_D$ −13° (*c* 1, $CHCl_3$); Anal. Calcd for $C_{12}H_{16}O_4$: C, 64.29; H 7.19, Found C, 64.4; H, 7.17. Spectroscopic data were identical to those for (±)-**22**.

*Determination of absolute configuration of (−)-**22**.*—To a solution of the alcohol (−)-**22** (449 mg, 2.00 mmol) in dry DMF (10 mL) was added NaH (80 mg of a 60% dispersion in mineral oil, 4.0 mmol) followed by benzyl bromide (0.30 mL, 2.5 mmol). The mixture was stirred overnight at rt and then water (2 mL) was carefully added to destroy excess NaH. The solvents were removed by evaporation under reduced pressure and the residue was taken up in Et_2O (50 mL), washed with water (50 mL), dried ($MgSO_4$) and concentrated by evaporation under reduced pressure to give an oil. Dry CH_2Cl_2 (9 mL) was added followed by TFA (1 mL), and after 1 h at rt, TLC (ether) showed total conversion into a single product (R_f 0.26). The solvents were removed by evaporation under reduced pressure and the residue was taken up in CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$ (50 mL), dried ($MgSO_4$) and concentrated to give a yellow oil which was purified by flash chromatography (eluent 4:1 Et_2O–hexane) yielding **25** as a colourless liquid [336 mg, 1.73 mmol, 87% yield from (−)-**22**]; $[\alpha]_D$ −26.5° (*c* 1, MeOH); Lit.[28] +27.52° for the enantiomer. Spectroscopic data were identical to those reported for the enantiomer.[28]

*(3′*S*,4′*R*)-3′-(*p*-Methoxybenzyloxy)tetrahydrofuran-4′-yl 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-*D*-glucopyranoside* (**28**).*—A mixture of **26ab**[8] (1.85 g, 2.68 mmol), (+)-**22** (0.30 g, 1.34 mmol) and 4 Å molecular sieves (1.50 g) in toluene (5 mL) and dioxane (15 mL) was stirred for 2 h under an atmosphere of N_2. $ZnCl_2$ (0.44 g) and $AgClO_4$ (1.33 g) were added and the flask was wrapped in foil. After a further 2 h TLC (1:4 EtOAc–toluene) indicated the formation of one major product (R_f 0.44). The foil was removed, water (25 mL) was added, the resulting pale pink suspension was filtered through Celite and the residue was well washed with EtOAc. The filtrate and washings were transferred to a separating funnel containing EtOAc (150 mL) and water (100 mL). The organic layer was washed with satd aq NaCl (100 mL), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography (eluent 1:9 EtOAc–toluene) yielding the title compound as a colourless oil which solidified on standing (0.82 g, 76%); mp 80–83 °C (Et_2O–hexane); $[\alpha]_D$ +19.0° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 7.32–7.21 (m, 14 H, ArCH), 7.05–7.02 (m, 2 H, *ortho*-H of PMB), 6.83–6.77 (m, 6 H, *meta*-H of PMB), 5.18 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.89–4.36 (m, 10 H, 5 × OCH_2Ar), 4.20 (q, 1 H, $J_{4',3'} \approx J_{4',5'a} \approx J_{4',5'b} \approx$ 5.5 Hz, H-4′), 4.07 (q, 1 H, $J_{3',2'a} \approx J_{3,2'b} \approx 5.5$ Hz, H-3′), 4.02–3.93 (m, 3 H, H-2′a, H-3, H-5′a), 3.87–3.68 (m, 13 H, 3 × OCH_3, H-2′b, H-5, H-5′b, H-6a), 3.62–3.54 (m, 3 H, H-2, H-4, H-6b); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 159.17, 159.13, 159.06 (*para*-C of PMB), 138.34, 137.83 (*ipso*-C of Bn), 130.99, 130.36, 130.09 (*ipso*-C of PMB) 129.54, 129.47,128.32, 128.23, 127.84, 127.73, 127.64, 127.51 (ArCH), 113.69 (*meta*-C of PMB), 96.90 (C-1), 81.33 (C-3), 79.74 (C-2), 77.33 (C-3′), 77.17 (C-4), 75.94 (C-4′), 75.24, 74.64, 73.38, 72.30, 72.01 (OCH_2Ar), 70.67 (C-5), 70.45 (C-2′, or C-5′), 70.23 (C-2′, or C-5′), 68.33 (C-6), 55.18, 55.17 (2 × OCH_3); FABMS (positive ion): m/z 829 ($[M+Na]^+$, 3%); 121 (100). Anal Calcd for $C_{48}H_{54}O_{11}$: C, 71.45; H, 6.74. Found: C, 71.3; H, 6.75.

*(3′*S*,4′*R*)-3′-Hydroxytetrahydrofuran-4′-yl 2,6-di-*O*-benzyl-α-*D*-glucopyranoside* (**28**).*—Trifluoroacetic acid (2 mL) was added to a solution of **27** (500 mg, 0.62 mmol) in CH_2Cl_2 (18 mL). The resulting purple solution was stirred at rt for 10 min and then poured slowly into satd aq $NaHCO_3$ (300 mL). The aqueous layer was

extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic layer was dried ($MgSO_4$), filtered and concentrated, leaving a yellow oil which was subjected to flash chromatography (eluent 7:3 EtOAc–hexane), to give the title compound as a white solid (234 mg, 84%); mp 83–85 °C (Et_2O); $[\alpha]_D$ +70.0° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 7.36–7.22 (m, 10 H, Ph), 4.75 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.76, 4.63 (AB, 2 H, J_{AB} 11.7 Hz, OCH_2Ph), 4.53, 4.49, (AB, 2 H, J_{AB} 12.2 Hz, OCH_2Ph), 4.44 (br s, 1 H, D_2O exch, 3-OH), 4.23–4.17 (m, 1 H, H-3′), 4.11–4.07 (m, 2 H, D_2O exch, 3′-OH, 4-OH), 3.99 (q, 1 H, $J_{4',3'} \approx J_{4',5'a} \approx J_{4',5'b} \approx 5.4$ Hz, H-4′), 3.93 (dt, 1 H, $J_{3,4}$ 9.0, $J_{3,2}$ 9.0, $J_{3,OH}$ 3.9 Hz, H-3), 3.90–3.82 (m, 2 H, H-2′a, H-5′a), 3.79–3.74 (m, 2 H, H-5, H-5′b), 3.69 (dd, 1 H, $J_{2'b,2'a}$ 9.8, $J_{2'b,3'}$ 4.4 Hz, H-2′b), 3.65–3.60 (m, 2 H, H-6a, H-6b), 3.47 (dt, 1 H, $J_{4,5}$ 9.3, $J_{4,OH}$ 3.9 Hz, H-4), 3.37 (dd, 1 H, $J_{2,3}$ 9.5, H-2); ^{13}C NMR ($CDCl_3$; 100.4 MHz): δ 137.75, 137.11 (*ipso*-C of Bn), 128.48, 128.33, 128.19, 127.46, 127.42 (Ph), 98.4 (C-1), 78.90 (C-4′), 78.81 (C-2), 73.93, 73.32 (OCH_2Ar), 72.92 (C-3), 72.48 (C-5′), 71.08 (C-5), 70.60 (C-3′), 70.31 (C-2′), 70.23 (C-4), 68.94 (C-6); FABMS (positive ion): *m*/*z* 829 ($[M+Na]^+$, 3%), 121 (100); Anal Calcd for $C_{24}H_{30}O_8$: C, 64.56; H, 6.77. Found: C 64.4; H 6.78.

*(3′*S*,4′*R*) - 3′ - Dibenzyloxyphosphoryloxytetrahydrofuran-4′-yl 2,6-di*-O-*benzyl-3,4-bis*-O-*(dibenzyloxyphosphoryl)-α-*D*-glucopyranoside* (**29**).—A mixture of bis(benzyloxy)(diisopropylamino)phosphine (325 mg, 0.94 mmol) and 1*H*-tetrazole (99 mg, 1.41 mmol) in dry CH_2Cl_2 (5 mL) was stirred at rt for 30 min whereupon triol **28** (70 mg, 0.16 mmol) was added. The mixture stirred for a further 30 min, then cooled to −78 °C, and MCPBA (406 mg, 57%, 1.34 mmol) was added. The cooling bath was removed and the mixture was allowed to warm to rt. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and was washed with 25 mL each of 10% aq Na_2SO_3, satd aq $NaHCO_3$ and satd aq NaCl. The organic layer was dried ($MgSO_4$), filtered and concentrated to give an off-white solid. Flash chromatography (eluent 3:2 EtOAc–hexane) gave the title compound as a colourless oil (188 mg, 98%); $[\alpha]_D$ +30.0° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ 7.35–7.03 (m, 40 H, Ph), 5.05–4.80 (m, 14 H, 5.5 × OCH_2Ph, H-1, H-3, H-3′), 4.73 (AB, 1 H, J_{AB} 11.9, $J_{H,P}$ 8.2 Hz, POCH_2Ph), 4.63–4.50 (m, 3 H, H-4, OCH_2Ph), 4.48, 4.36 (AB, 2 H, J_{AB} 12.1 Hz, OCH_2Ph), 4.08 (q, 1 H, $J_{4',3'} \approx J_{4',5'a} \approx J_{4',5'b} \approx 5.5$ Hz, H-4′), 3.92 (dd, 1 H, $J_{5'a,5'b}$ 9.2, H-5′a), 3.90–3.85 (m, 2 H, H-2′a, H-2′b), 3.82–3.77 (m, 2 H, H-5, H-5′b), 3.75–3.69 (m, 2 H, H-6a, H-6b), 3.56 (dd, 1 H, $J_{2,3}$ 9.8, $J_{2,1}$ 3.7 Hz, H-2); ^{31}P NMR ($CDCl_3$; 161.7 MHz; ^1H decoupled): δ −1.10, −1.94, −2.25, (3 s); FABMS (positive ion): *m*/*z* 1227 ($[M+1]^+$, 9%), 91 (100); HRFABMS (positive ion): Calcd for $C_{66}H_{70}O_{17}P_3$ $[M+1]^+$: 1227.382. Found: 1227.382.

*(3′*S*,4′*R*)-3′-Hydroxytetrahydrofuran-4′-yl α-*D*-glucopyranoside 3,3′,4-trisphosphate* (**5**).—Moist palladium on carbon 10% (200 mg) was added to a solution of **29** (75 mg, 61 μmol) in MeOH (50 mL) and water (10 mL). This mixture was shaken in an atmosphere of H_2 at 50 psi for 25 h. The catalyst was removed by filtration through a PTFE membrane filter, and the filtrate was concentrated to a clear residue. The crude product was purified by ion exchange chromatography on Q Separose Fast Flow resin, eluting with a gradient of TEAB buffer (0–1 M), pH 8.0. The triethylammonium salt of the title compound eluted between 560–620 mM buffer (57 μmol, 93%); $[\alpha]_D$ +74.8° (*c* 0.6 calculated for free acid, MeOH); ^1H NMR (D_2O; 400 MHz) δ 5.25 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 4.89–4.80 (m, 1 H, H-3′), 4.50–4.43 (m, 2 H, H-3, H-4′), 4.09–4.01 (m, 3 H, H-2′a, H-4, H-5′a), 3.94–3.78 (m, 5 H, H-2′b, H-5, H-5′b, H-6a, H-6b), 3.74 (dd, 1 H, $J_{2,3}$ 9.0, H-2); ^{31}P NMR (CD_3OD; 162 MHz; ^1H-coupled): δ 1.08 (d, J_{HP} 9.8 Hz), 0.82 (d, J_{HP} 7.6 Hz), 0.04 (d, J_{HP} 7.3 Hz); FABMS (negative ion): *m*/*z* 505($[M-H]^-$, 100%); HRFABMS (negative ion): Calcd for $C_{10}H_{20}O_{17}P_3$ $[M-H]^-$: 504.9913. Found: 504.9890.

*(3′*S*,4′*R*)-3′-(*p*-Methoxybenzyloxy)tetrahydrofuran-4′-yl 3,4-di*-O-*acetyl-2,6-di*-O-*benzyl-α-*D*-glucopyranoside* (**31**).—A mixture of **30ab**[26] (1.81 g, 3.37 mmol), (+)-**22** (567 mg, 2.53 mmol) and 4 Å molecular sieves (1.50 g) in toluene (5 mL) and dioxane (15 mL) was stirred for 2 h under an atmosphere of N_2. $ZnCl_2$ (506 mg) and $AgClO_4$ (1.54 g) were added and the flask was wrapped in foil. After stirring for a further 18 h TLC (20:1 CH_2Cl_2–Me_2CO) indicated the formation of one major product (R_f 0.44). The foil was removed, toluene (75 mL) was added, the resulting suspension was filtered through Celite and the residue was well washed with toluene. The filtrate and washings were transferred to a separating funnel and washed with water, satd aq $NaHCO_3$ and satd aq NaCl (100 mL of each). The organic layer was dried ($MgSO_4$) and concentrated to give a colourless residue. Purification by flash chromatography (eluent 30:1 CH_2Cl_2–Me_2CO) gave the title compound as a colourless glass (1.04 g, 1.60 mmol, 63%); $[\alpha]_D$ +84.3° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 7.34–7.15 (m, 12 H, Ar), 6.85–6.81 (m, 2 H, *meta*-H of PMB), 5.48 (t, 1 H, $J_{3,2} = J_{3,4} = 9.8$ Hz, H-3), 5.21 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.07 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.60–4.41 (m, 6 H, 3 × AB systems of OCH_2Ar), 4.20 (q, 1 H, $J \approx 5.4$ Hz, H-3′ or H-4′), 4.06 (q, 1 H, $J \approx 5.9$ Hz, H-3′ or H-4′), 3.99–3.79 (m, 5 H, H-5, H-2′a, H-2′b, H-5′a and H-5′b), 3.76 (s, 3 H, $ArOCH_3$), 3.58 (dd, 1 H, H-2), 3.50– 3.42 (m, 2 H, H-6a and H-6b), 2.00 (s, 3 H, $MeCO_2$), 1.89 (s, 3 H, $MeCO_2$); ^{13}C NMR ($CDCl_3$; 68 MHz): δ 170.12, 169.73 (2 × C=O), 159.27 (*para*-C of PMB), 137.87, 137.48 (*ipso*-C of Bn), 129.97 (*ipso*-C of PMB), 129.61, 128.31, 128.26, 127.88, 127.68, 127.55 (ArCH), 113.77 (*meta*-C

of PMB), 96.61 (C-1), 77.60, 76.34 (2 × CH), 73.43, 72.35, 72.05 (OCH_2Ar), 71.91 (CH), 70.45, 70.04 (C-2′ and C-5′), 69.04, 68.70 (2 × CH), 67.76 (C-6), 55.19 (OCH_3), 20.84, 20.63 (CH_3CO_2); FABMS (positive ion): m/z 673 ($[M + Na]^+$, 90%); 121 (100), 91 (65); FABMS (negative ion): m/z 803 ($[M + NBA]^-$, 100%); Anal Calcd for $C_{36}H_{42}O_{11}$: C, 66.45; H, 6.51. Found: C, 66.3; H, 6.60.

(3′S,4′R)-3′-Hydroxytetrahydrofuran-4′-yl 3,4-di-O-acetyl-2,6-di-O-benzyl-α-D-glucopyranoside (**32**).—Trifluoroacetic acid (2 mL) was added to a solution of **31** (750 mg, 1.15 mmol) in CH_2Cl_2 (18 mL). The resulting purple solution was stirred at rt for 30 min, and then poured slowly, with vigorous stirring, into satd aq $NaHCO_3$ (50 mL). The organic layer was removed, dried ($MgSO_4$), and concentrated to give an oil. Purification by flash chromatography (eluent 10:1 CH_2Cl_2–Me_2CO) gave the title compound as a colourless oil (493 mg, 0.929 mmol, 81%) which crystallised after one week at rt; mp 64–65 °C (diisopropyl ether); $[\alpha]_D$ + 104° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz): δ 7.38–7.26 (m, 10 H, ArH); 5.44 (t, 1 H, $J_{3,4} = J_{3,2} = 9.9$ Hz, H-3), 5.08 (t, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.90 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.68, 4.61 (AB, 2 H, J_{AB} 11.9 Hz, OCH_2Ph), 4.58, 4.43 (AB, 2 H, J_{AB} 12.2 Hz, OCH_2Ph), 4.26 (q, 1 H, $J \approx 5.5$ Hz, H-3′ or H-4′), 4.16 (q, 1 H, $J \approx 5.5$ Hz, H-3′ or H-4′), 4.01–3.91 [m, 3 H, H-2, H-5 and (H-2′a or H-5′a)], 3.81 (dd, 1 H, *J* 9.8, 4.3 Hz, H-2′b or H-5′b), 3.67–3.63 [m, 2 H, (H-2′a and H-2′b) or (H-5′a and H-5′b)], 3.48 (ABX, 2 H, $J_{6a,6b}$ 10.8, $J_{6a,5}$ 2.7, $J_{6b,5}$ 3.7 Hz, H-6a and H-6b), 1.93 (s, 3 H, CH_3CO_2), 1.89 (s, 3 H, CH_3CO_2); ^{13}C NMR ($CDCl_3$; 100 MHz): δ 170.16, 169.67 (C=O), 137.33, 136.77 (*ipso*-C of Bn), 128.61, 128.35, 128.32, 128.19, 127.90, 127.75 (Ph), 98.23 (C-1), 78.79, 76.29 (2 × CH), 74.11, 73.42, 72.45 (3 × CH_2), 72.39, 70.82 (2 × CH), 70.81 (CH_2), 69.05, 68.75 (2 × CH), 67.49 (C-6), 20.80, 20.55 (CH_3CO_2); FABMS (positive ion): m/z 553 ($[M + Na]^+$, 20%), 181 (30), 91 (100); FABMS (negative ion): m/z 683 ($[M + NBA]^-$, 100%]; Anal Calcd for $C_{28}H_{34}O_{10}$: C, 63.39; H, 6.46. Found: C 63.1; H 6.49.

(3′S,4′R) - 3′ - Dibenzyloxyphosphoryloxytetrahydrofuran-4′-yl 3,4-di-O-acetyl-2,6-di-O-benzyl-α-D-glucopyranoside (**33**).—To a solution of the alcohol (335 mg, 0.631 mmol) in dry CH_2Cl_2 (3 mL) was added 1*H*-tetrazole (88 mg, 1.3 mmol) followed by bis(benzyloxy)(diisopropylamino)phosphine (327 mg, 0.947 mmol). The mixture was stirred under N_2 at rt for 1 h and then cooled to − 78 °C. MCPBA (380 mg, 57%, 1.26 mmol) was added, the cooling bath was removed, and the mixture was allowed to warm to rt. The mixture was diluted with CH_2Cl_2 (50 mL) and was washed with 50 mL each of 10% w/v aq Na_2SO_3, satd aq $NaHCO_3$ and satd aq NaCl. The organic layer was dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography (eluent 15:1 then 10:1 CH_2Cl_2–Me_2CO) gave the title compound as a colourless oil (407 mg, 0.515 mmol, 82%); $[\alpha]_D$ + 89.7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$; 270 MHz) δ 7.31–7.22 (m, 20 H, Ph); 5.42 (t, 1 H, *J* 9.9 Hz, H-3), 5.10–5.00 (m, 6 H, 2 x OCH_2Ph, H-1 and H-4), 4.91–4.83 (m, 1H, H-3′), 4.63–4.41 (two overlapping AB_q, 4 H, OCH_2Ph), 4.21–4.13 (m, 1 H, H-4′), 4.00–3.79 (m, 5 H, H-5, H-2′a H-2′b, H-5a′ and H-5b′), 3.55 (dd, 1 H, *J* 10.1, 3.7 Hz, H-2), 3.52–3.41 (m, 2 H, H-6a and H-6b), 1.94 (s, 3 H, CH_3CO_2), 1.87 (s, 3 H, CH_3CO_2); ^{31}P NMR ($CDCl_3$; 162 MHz, proton coupled) δ −1.34 (apparent sextet, 1P, $^3J_{HP} \sim 8$ Hz); FABMS (positive ion): m/z 813.4 ($[M + Na]^+$, 50%), 791.4 ($[M + H]^+$, 80%), 91 (100); FABMS (negative ion): m/z 943.3 ($[M + NBA]^-$, 90%], 699.2 ($[M - C_7H_7]^-$, 90%], 277.1 (40); Anal Calcd for $C_{42}H_{47}O_{13}P$: C, 63.79; H, 5.99. Found: C 63.9; H 6.00.

(3′S,4′R) - 3′ - Dibenzyloxyphosphoryloxytetrahydrofuran-4′-yl 2,6-di-O-benzyl-α-D-glucopyranoside (**34**).—A solution of **33** (360 mg, 0.455 mmol) in NH_3-saturated anhyd MeOH (20 mL) was stirred at rt in a sealed Pyrex autoclavable bottle for 18 h. TLC (EtOAc) indicated complete consumption of starting material (R_f 0.48) and the appearance of a more polar product (R_f 0.30). The solution was concentrated and the residue was purified by flash chromatography (eluent EtOAc–hexane 5:1) to give diol **34** as a colourless oil (204 mg, 0.289 mmol, 64%); $[\alpha]_D$ + 71.8° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$; 270 MHz) δ 7.36–7.25 (m, 20 H, Ph), 5.11–4.95 (m, 5 H, H-1 and 2 × OCH_2Ph), 4.91–4.84 (m, 1 H, J_{HP} 7.1 Hz, H-3′), 4.60, 4.55 (AB_q, 2 H, J_{AB} 12.3 Hz, OCH_2Ph), 4.69, 4.51 (AB_q, 2 H, J_{AB} 11.9 Hz, OCH_2Ph), 4.00–3.73 (m, 5 H, H-3, H-2′a, H-2′b, H-5′a, H-5′b), 3.70–3.62 (m, 3 H, H-5, H-6a, H-6b), 3.59–3.50 (m, 1 H, H-4), 3.33 (dd, 1 H, $J_{2,3}$ 9.7, $J_{2,1}$ 3.7 Hz, H-2), 2.97 (br s, 1 H, OH), 2.82 (br s, 1 H, OH); ^{31}P NMR ($CDCl_3$; 109 MHz, proton coupled) δ − 0.37 (apparent sextet, 1 P, $^3J_H \sim 7$ Hz); FABMS (positive ion): m/z 729.3 ($[M + Na]^+$, 30%), 707.3 ($[M + H]^+$, 90%), 91 (100);%); HRFABMS (positive ion): Calcd for $[C_{38}H_{43}O_{11}P + H^+]^+$ 707.2621. Found: 707.2622.

(3′S,4′R) - 3′ - Dibenzyloxyphosphoryloxytetrahydrofuran-4′-yl 2,6-di-O-benzyl-3,4-bis-O-(dibenzyloxythiophosphoryl)-α-D-glucopyranoside (**35**).—To a solution of diol **34** (122 mg, 0.173 mmol) in dry CH_2Cl_2 (2 mL) was added 1*H*-tetrazole (48 mg, 0.69 mmol) followed by bis(benzyloxy)(diisopropylamino)phosphine (179 mg, 0.519 mmol). The mixture was stirred under N_2 at rt for 1 h and then concentrated by evaporation under reduced pressure (no heat). The residue was taken up in dry DMF (2 mL), and dry pyridine (1 mL) was added followed by sulphur (22 mg, 0.69 mmol). The mixture was stirred under N_2 at rt for 1 h and then concentrated by evaporation in vacuo. The residue was taken up in CH_2Cl_2 (30 mL) and the solution was washed with brine (30 mL), dried ($MgSO_4$) and concentrated to give

a yellow oil. Purification by flash chromatography (eluent 1:1 EtOAc–hexane) gave the title compound as a colourless oil (182 mg, 0.145 mmol, 84%); $[\alpha]_D$ +28.3° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 270 MHz) δ 7.36–7.12 (m, 38 H, Ph), 7.05–7.01 (m, 2 H, Ph), 5.26–4.80 (m, 15 H, 5.5 × OC*H*$_2$Ph, H-1, H-3, H-4, and H-3′), 4.62 (dd, 1 H, J_{AB} 11.9, $J_{H,P}$ 10.9 Hz, 0.5 POC*H*$_2$Ph), 4.50 (br s, 2 H, OC*H*$_2$Ph), 4.37, 4.26 (AB, 2 H, J_{AB} 11.9 Hz, OC*H*$_2$Ph), 4.00–3.85 (m, 5 H, H-5, H-2′a, H-2′b, H-4′ and H-5′a), 3.79 (dd, 1 H, *J* 7.9, 5.9 Hz, H-5′b), 3.65–3.70 (m, 2 H, H-6a, H-6b), 3.61 (dd, 1 H, $J_{2,3}$ 9.2, $J_{2,1}$ 3.6 Hz, H-2); ^{31}P NMR ($CDCl_3$; 109 MHz; ^1H-decoupled): δ 69.09 and 68.38 (P-3 and P-4), −0.49 (P-3′); FABMS (positive ion): *m*/*z* 1259.3 ($[M+1]^+$, 40%), 271(50), 91 (100); FABMS (negative ion): *m*/*z* 1167.3 ($[M-C_7H_7]^-$, 50%), 293.1 ($[(BnO)_2P(S)O_2]^-$, 100%); Anal Calcd for $C_{66}H_{69}O_{15}P_3S_2$: C, 62.95; H, 5.52. Found: C 62.8; H 5.56.

*(3′*S*,4′*R*)-3′-Hydroxytetrahydrofuran-4′-yl* α-D-*glucopyranoside 3′-phosphate 3,4-bisphosphorothioate* (**6**).—Ammonia (100 mL) was condensed into a 250 mL three-neck flask at −78 °C. Excess sodium metal was added in small pieces, with stirring, until the liquid remained a deep blue–black colour. The cooling bath was removed, and 30 mL of NH_3 was then allowed to distil from the first flask into a 100-mL three-neck flask kept at −78 C under N_2. To this pure dry liquid NH_3 was now added freshly-cut sodium metal (two slivers, each approx 5 × 5 × 2 mm). After 10 min of stirring at −78 °C (the blue–black colour should be retained) a solution of **35** (94 mg, 75 μmol) in anhyd dioxane (2 mL) was added. The mixture was stirred at −78 °C for 60–90 s and then quenched by addition of EtOH in small portions (CARE!) until all the colour had disappeared. De-ionised water (30 mL) was added, and the cooling bath was removed. Most of the NH_3 was allowed to evaporate off under a stream of N_2, and the remaining liquid was then carefully transferred to a 500-mL round-bottom flask. The solvents and remaining NH_3 were then removed by evaporation under reduced pressure (no heat). The residue was taken up in de-ionised water (500 mL) and loaded onto a column of Q Sepharose Fast Flow resin. The column was eluted with a gradient of triethylammonium bicarbonate buffer (0–1 M, pH 7.5). The triethylammonium salt of **6** eluted over 850–900 mM buffer. Fractions containing **6** were combined and concentrated by evaporation under reduced pressure (no heat). Methanol was added to the residue and repeatedly evaporated to destroy remaining triethylammonium bicarbonate. Eventually, the pure triethylammonium salt of **6** remained as a glassy residue (53 mg, 57 μmol, subsequently quantified by Ames total phosphate assay,[38] 76%); $[\alpha]_D$ +38.7° (*c* 1.0, MeOH); ^1H NMR (D_2O; 270 MHz) δ 5.21 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1); 4.80–4.71 (m, 1 H, partially buried by HDO signal, H-3′), 4.64 (q, 1 H, $J_{3,2} \approx J_{3,4} \approx J_{3,P}$ 10 Hz, H-3), 4.44 (q, $J_{4',3'} \approx J_{4',5'a} \approx J_{4',5'b}$ 5 Hz, H-4′), 4.20 (dt, 1 H, $J_{4,P}$ 11.3, $J_{4,5}$ 9.0 Hz, H-4), 4.06–3.67 (m, 8 H, H-2, H-5, H-6a, H-6b, H-2′a, H-2′b, H-5′a and H-5′b); ^{31}P NMR (D_2O; 109 MHz, Et_3N added) δ 51.42 and 50.01 (P-3 and P-4), 0.675 (P-3′); FABMS (negative ion): *m*/*z* 537($[M-H]^-$, 100%); HRFABMS (negative ion): Calcd for $C_{10}H_{20}O_{15}P_3S_2$ $[M-H]^-$ 536.9457, Found: 536.9457.

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