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Xylopyranoside-based agonists of D-*myo*-inositol 1,4,5-trisphosphate receptors: synthesis and effect of stereochemistry on biological activity

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Abstract

The synthesis of a series of tetrahydrofuranyl α- and β-xylopyranoside trisphosphates, designed by excision of three motifs of adenophostin A is reported. The synthetic route features improved preparations of allyl α-D-xylopyranoside and its 2-*O*-benzyl ether, and gives access to four diastereoisomeric trisphosphates, which show a range of abilities to mobilise Ca^{2+} from the intracellular stores of hepatocytes. A comparison of the potencies of the four trisphosphates provides useful information relating to the effects of stereochemical variation on the recognition of carbohydrate-based trisphosphates by D-*myo*-inositol 1,4,5-trisphosphate receptors. 1-*O*-[(3′*S*,4′*R*)-3-hydroxytetrahydrofuran-4-yl] α-D-xylopyranoside 3,4,3′-trisphosphate (**8**) is the most active member of the series with a potency close to Ins(1,4,5)P_3; a β-linked analogue, 1-*O*-[(3′*R*,4′*S*)-3-hydroxytetrahydrofuran-4-yl] β-D-xylopyranoside 3,4,3′-trisphosphate, is ca. 20-fold weaker than Ins(1,4,5)P_3, and the other compounds are much less active. While no compound attained a potency close to that of adenophostin A, we believe that **8** represents the minimal structure for potent Ca^{2+}-releasing activity in this type of carbohydrate-based analogue. © 2001 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 firmly established as a second messenger that binds to its own intracellular receptor to cause release of Ca^{2+} from intracellular stores.[1] In recent years many Ins(1,4,5)P_3 analogues have been prepared and from their biological evaluation has come an understanding of some of the structural motifs responsible for receptor binding and Ca^{2+} mobilising activity.[2,3] In 1993 Takahashi and co-workers isolated two glyconucleotides, adenophostins A and B (**2** and **3**) from *Penicillium brevicompactum*.[4] Analyses of both Ca^{2+} mobilisation by adenophostins A and B and their binding to Ins(1,4,5)P_3 receptors suggest they are full agonists with affinities 10–100 times greater than Ins(1,4,5)P_3.[4–11] Adenophostins were the first examples of agonists of Ins(1,4,5)P_3 receptors that were not based on inositol, although there are several important similari-

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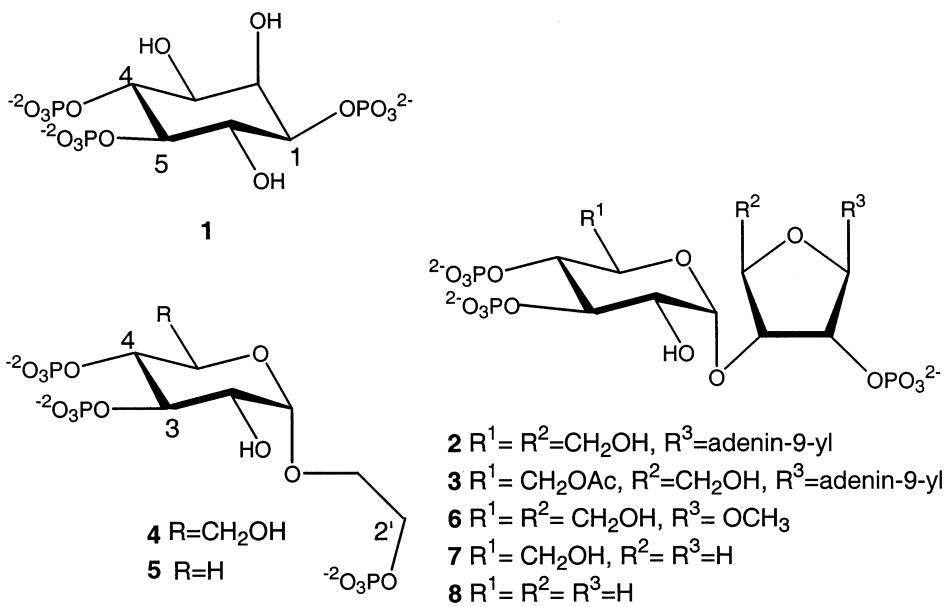
ties in structure between Ins(1,4,5)P_3 and the adenophostins. Thus, the 3″,4″-(bisphosphate)/2″-hydroxyl grouping of the adenophostins is thought to mimic the crucial 4,5-(bisphosphate)/6-hydroxyl triad of Ins(1,4,5)P_3. However, the obvious differences in the structure of the adenophostins and Ins(1,4,5)P_3 and the unusual potencies of the former have stimulated considerable interest in synthesis aimed at identifying the structural basis for the enhanced activity of adenophostins. Three total syntheses of adenophostin A have been reported.[11–14]

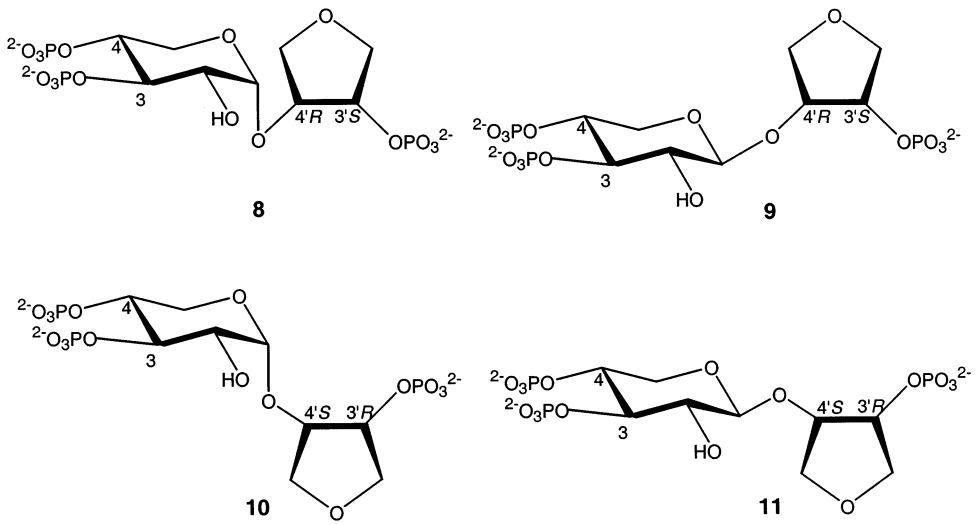
rating a D-glucopyranosyl 3,4-bisphosphate moiety with an α-glycosidic linkage to a second sugar containing one or more phosphates were designed and synthesised. They all proved to be full agonists, but with a range of activities.[7,8] The most potent was methyl 3-*O*-(α - D - glucopyranosyl) - β - D - ribofuranoside 2,3′,4′ trisphosphate (ribophostin, **6**)[8,18,19] in which the location of the 2-phosphate group in space is restricted by a ribofuranoside ring, as in the adenophostins. The potency of **6** in Ca^{2+} release assays was similar to that of Ins(1,4,5)P_3, but still about 20-fold less than



To determine whether the adenine component of the adenophostins was essential for activity, we and others designed and synthesised 2-hydroxyethyl α-D-glucopyranoside 2′,3,4-trisphosphate (**4**),[15,16] and another group synthesised the xylopyranoside equivalent (**5**)[17] as simplified analogues of the adenophostins. Both **4** and **5** were shown to be full agonists of Ins(1,4,5)P_3 receptors but with 10–20-fold lower affinity than Ins(1,4,5)P_3. They were, however, more potent than many inositol-based Ins(1,4,5)P_3 mimics and showed that the design of chiral Ca^{2+}-releasing polyphosphates based on carbohydrates is possible. The lower affinity of **4** compared to adenophostin A was thought to be due, at least in part, to the conformational flexibility of the ethylphosphate side chain,[15] and so various phosphorylated disaccharides incorporate

that of adenophostin A. The lower affinity of **6** suggests that the adenine component of the adenophostins is not essential for activity, but enhances affinity for the Ins(1,4,5)P_3 receptor. This led to further simplification and the synthesis of 1-*O*-[(3′*S*,4′*R*)-3-hydroxytetrahydrofuran-4-yl] α-D-glucopyranoside 3,4,3′-trisphosphate (furanophostin, **7**)[9,20] which lacks both the *O*-methyl and the 4-hydroxymethyl moieties but retains the rigidity of the five-membered ring. Again the activity of **7** was found to be similar to Ins(1,4,5)P_3 and to **6**. Recently we designed and synthesised α- and β-D-glucopyranosylmethanol 3,4,1′-trisphosphate analogues based on **4** but with a shorter side chain.[21] In these molecules the third phosphate group is attached to a carbon centre fixed in the α- and β-positions. However, bio-

logical evaluation showed that the α-C-analogue was only comparable to **4**, while the β-C-analogue was even weaker. This finding suggests that the second ring of **6** and **7** is required for greater affinity.



In the present work we have taken the simplification of **7** a step further by replacing the glucopyranosyl 3,4-bisphosphate structure in **7** with the xylopyranosyl equivalent to give **8**, a new and potent xylopyranoside-based agonist of Ins(1,4,5)P_3 receptors. We suggest that **8** represents the minimal structure in carbohydrate derived polyphosphates sufficient for high affinity agonist activity of Ins(1,4,5)P_3 receptors. In order to explore the effect of stereochemical variations on the biological activity of **8**, and in particular the positioning of the non-vicinal phosphate group for potent activity, we have also synthesised three of its diastereoisomers **9**, **10** and **11**. The series of trisphosphates **8**–**11** may be regarded as conformationally restricted analogues of the original xylopyranoside-based analogue **5** and its β-linked equivalent.[17] A comparison of the abilities of the four stereoisomers to release Ca^{2+} from permeabilised hepatocytes provides insights into the influence of stereochemistry on biological activity in analogues of this type.

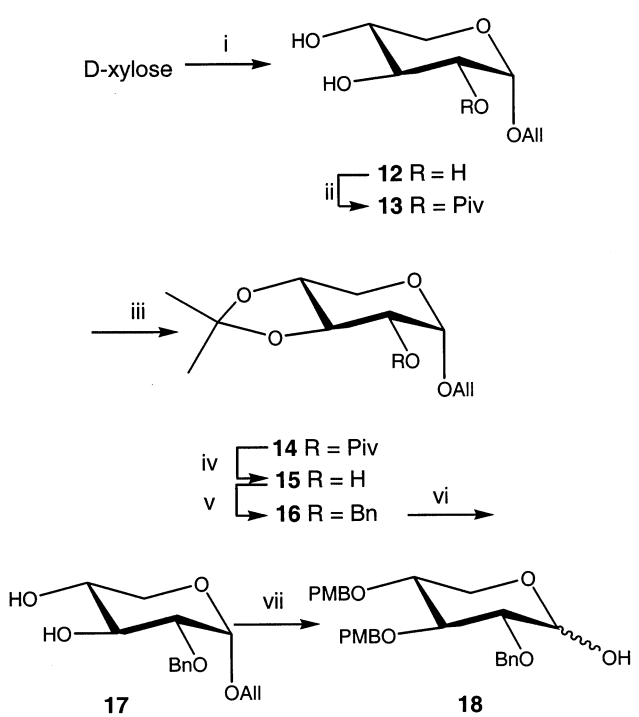
2. Results and discussion

Although the required regioselectively protected D-xylopyranose **18** (Scheme 1) had already been prepared and reported,[22] the overall yield from allyl α-D-xylopyranoside (**12**) was restricted by the unavoidable formation of two butane diacetal protected regioisomers at the first step of the synthetic route. A different route was therefore investigated, with the aim of increasing the overall yield of **12** while reducing the need for time-consuming purifications by column chromatography. Thus Fischer glycosidation of xylose by a modification (see Section 4) of the previously described method[22] gave the allyl glycoside (**12**) as colourless crystals in 41% yield over three crops. The selective acylation of position 2 of **12** was achieved using trimethylacetyl chloride (pivaloyl chloride).[23] After several systematic changes relating to equivalents of trimethylacetyl chloride, temperature and reaction time, the best conditions were established (see Section 4). The required 2-*O*-trimethylacetyl ester **13** could be isolated by crystallisation in 53% yield, without the need for purification by chromatography.

Positions 3 and 4 in **13** were then protected with an isopropylidene acetal by reaction of **13** with 2-methoxypropene in THF in the presence of a catalytic amount of *p*-toluenesulphonic acid.[24] The product **14** of this reaction was used immediately in the next step without purification. The trimethylacetyl group was removed and replaced by benzyl protecting groups using sodium hydride and benzyl bromide in DMF and again the product of these reactions **16** was used with

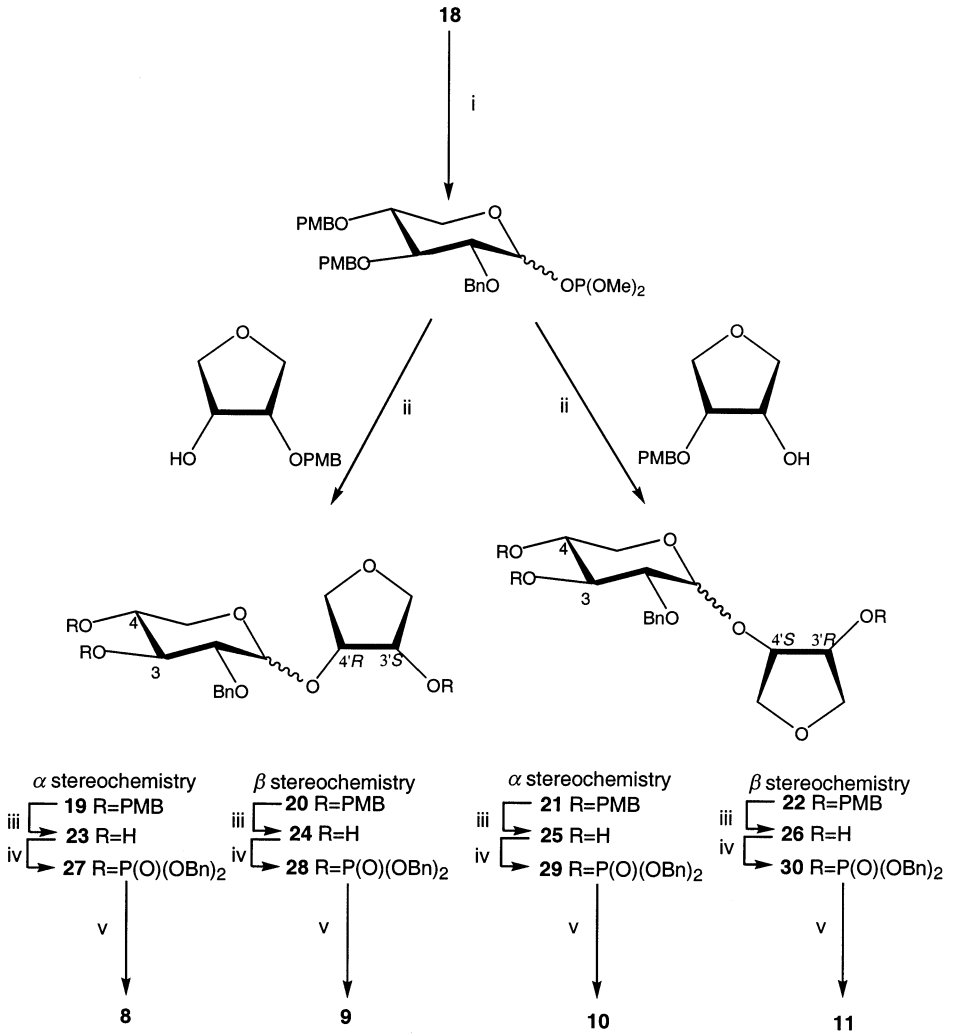
no purification. Finally the isopropylidene group was removed by stirring **16** with HCl in MeOH for 30 min to give allyl 2-*O*-benzyl-α-D-xylopyranoside (**17**) in high yield (76% over the four steps from **13**) after purification by flash chromatography followed by crystallisation. Compound **17** has been prepared previously but was reported to be an oil.[22] The diol **17** was easily converted in high yield into 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α,β-D-xylopyranose (**18**), a selectively protected intermediate without the labile trans isopropylidene group; alkylation of **17** with sodium hydride and *p*-methoxybenzyl chloride in DMF gave the fully protected product, from which the allyl protection at the anomeric position was then removed using a catalytic amount of $PdCl_2$ in MeOH to give xylopyranose **18**.

Activation of **18** with dimethyl *N*,*N*-diethylphosphoramidite in the presence of 1*H*-tetrazole gave a glycosyl phosphite (Scheme 2), which was used without further purification to glycosylate each of two enantiomeric alcohols. Thus, zinc chloride and silver perchlorate promoted glycosylation[25] of (+)-(3*R*,4*S*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol[20] and of (−)-(3*S*,4*R*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol[20] with the glycosyl phosphite gave a total of four diastereoisomeric products **19**–**22**. It is interesting to note that, in each case, a mixture of α- and β-coupled products was formed using this xylopyranosyl phosphite glycosyl donor, whereas only the α-coupled product was detected when the same conditions were applied to the analogous glucopyranosyl phosphite in the synthesis of furanophostin (**7**).[20] The α and β epimers could not be separated at this stage. However, after removal of the PMB ethers using 10% trifluoroacetic acid in CH_2Cl_2, the resulting α- and β-linked products could, in each case, be separated then by flash chromatography followed by crystallisation to give the diastereosiomeric triols **23**–**26**. Phosphitylation of each triol with 1*H*-tetrazole-activated bis(benzyloxy)diisopropylaminophosphine in CH_2Cl_2 and oxidation of the intermediate phosphites with *m*-chloroperbenzoic acid (MCPBA) then gave the fully protected trisphosphates **27**–**30**, respectively. Deprotection of each by hydrogenation over palladium on carbon yielded the target trisphosphates **8**–**11**, respectively, which were purified by ion-exchange chromatography on Q Sepharose Fast Flow resin and isolated as their triethylammonium salts. Finally, each trisphosphate was accurately quantified by total phosphate assay[26] before biological evaluation in permeabilised rat hepatocytes.



Scheme 1. (i) AllOH, HCl, reflux, 16 h; (ii) $(CH_3)_3COCl$, pyridine, −40 °C, 2.5 h; (53%); (iii) 2-methoxypropene, PTSA, THF, 30 min; (iv) NaOH, MeOH, reflux, 1 h; (v) NaH, BnBr, DMF, 0 °C, 90 min; (vi) 1 M HCl:MeOH 1:10, rt, 30 min (76%, over four steps); (vii) (a) NaH, PMBCl, DMF, rt, 12 h (84%); (b) $PdCl_2$, MeOH, 0 °C to rt, 4 h (90%). All, allyl; Piv, $(CH_3)_3CCO$, (pivaloyl); PMB, *p*-methoxybenzyl; Bn, benzyl.

Maximally effective concentrations of $Ins(1,4,5)P_3$, **8** or **11** released the same fraction of the intracellular Ca^{2+} stores (47 ± 2, 51 ± 5 and 46 ± 2%, respectively, Fig. 1 and Table 1). Furthermore, combined application of 10 μM $Ins(1,4,5)P_3$ with 10 μM of either **8** or **11** released no more Ca^{2+} than either of the agonists applied alone. The concentration of **8** required to cause half-maximal Ca^{2+} release (EC_{50}) was only three- to fourfold higher than that for $Ins(1,4,5)P_3$, while **11** was considerably weaker. The responses to both agonists were positively co-operative. Interestingly, there was no significant interaction between **9**



Scheme 2. (i) $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2; (ii) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4 Å sieves, glycosyl acceptor, (70–76%); (iii) CF_3COOH, CH_2Cl_2 (18–34%); (iv) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2; (b) MCPBA, −78 °C to rt (73–99%); (v) H_2, Pd–C, 50 psi, MeOH–H_2O, 24 h (65–85%).

(the β epimer of **8**) and Ins(1,4,5)P_3 receptors; even at 10 μM **9** failed to cause significant Ca^{2+} mobilisation and nor did it affect the response to a submaximal concentration of Ins(1,4,5)P_3 (Table 2). Finally, the results for **10** are consistent with it being a very low affinity full agonist ($EC_{50} > 10$ μM); alone at 10 μM it released less than 50% of the Ins(1,4,5)P_3-sensitive Ca^{2+} stores (Table 1) and its effects were approximately additive with a submaximal concentration of Ins(1,4,5)P_3 when added in combination with it (Table 2).

These results demonstrate that both **8** and **11** behave as full agonists in this assay and that diastereoisomer **8** has a potency in Ca^{2+} release assays similar to that of Ins(1,4,5)P_3, ribophostin (**6**) and furanophostin (**7**). This shows that the 5-hydroxymethyl moiety of the glucose ring in **6** and **7** is not essential for

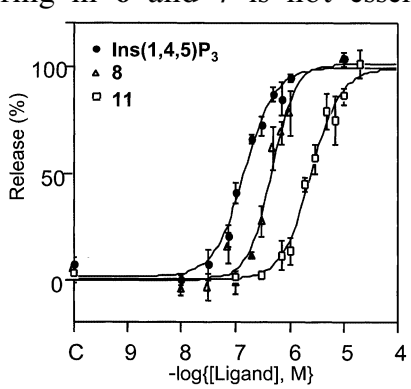


Fig. 1. Effects of Ins(1,4,5)P_3 and xylopyranosides **8** and **11** on $^{45}Ca^{2+}$ release from the Ins(1,4,5)P_3-sensitive Ca^{2+} stores of permeabilised hepatocytes.

Table 1
$^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3, **8**, **9**, **10** and **11** [a]

	Stereochemistry	EC_{50} (nM)	*h*	*n*	% release at 10 μM
Ins(1,4,5)P_3		144 ± 6	1.68 ± 0.26	5	47 ± 2
8	α,3′*S*,4′*R*	487 ± 58	2.51 ± 1.00	3	51 ± 5
9	β,3′*S*,4′*R*	nd	nd	5	3 ± 3
10	α,3′*R*,4′*S*	nd	nd	5	12 ± 2
11	β,3′*R*,4′*S*	2694 ± 276	1.78 ± 0.43	3	46 ± 2

[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.

potent activity at Ins(1,4,5)P_3 receptors, although the very gradually decreasing potency in the series **6** > **7** > **8** suggests that each hydroxymethyl group in **6** may have a slight enhancing effect on activity. The results also confirm that, at the receptor binding site, the orientation of **6** and **7** relative to that of Ins(1,4,5)P_3 is such that the 5-hydroxymethyl group of the glucose moiety mimics the 3-hydroxyl of Ins(1,4,5)P_3. Had an inverted binding orientation been involved (in which case the 5-hydroxymethyl group would be equivalent to the 6-OH of Ins(1,4,5)P_3), then a dramatic difference in activity between the glucopyranoside-based analogues (**6**, **7**) and their xylopyranoside-equivalent (**8**) would have been expected, analogous to the large difference between Ins(1,4,5)P_3 and 6-deoxy-Ins(1,4,5)P_3. The fact that **8** (similar to **6** and **7**) is still 10–100-fold less potent than the adenophostins supports previous arguments that the adenine component of the adenophostins plays a pivotal role in their activity. The similar activities of **1**, **6** and **7** suggest that **8**, with its single hydroxyl group, is the minimal structure for potent agonism in a simple carbohydrate-derived agonist of Ins(1,4,5)P_3 receptors. Because this remaining OH group is equivalent to the crucial 6-OH of Ins(1,4,5)P_3, its removal would undoubtedly result in a dramatic decrease in potency. Indeed, while this work was in progress a report appeared[27] on a synthesis of the 2-deoxy derivative of **5**. This molecule, having no remaining hydroxyl group, 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-OH group of Ins(1,4,5)P_3.[2]

Clearly, the stereochemical differences between analogues **8**, **9**, **10** and **11** have a significant effect on the recognition of these molecules by Ins(1,4,5)P_3 receptors. The structural simplicity of **8**–**11** suggested that a molecular modelling approach (see Section 4) might be used to account for the impact of stereochemical variation on their potencies. It is important to appreciate, however, that modelling of adenophostin A[10,15] and even of the simpler analogues **8**–**11** presents many difficulties, including those of simulating the combined influence of charged phosphate groups, solvent, counter-ions, pseudorotational equilibria in five-membered rings, and anomeric effects associated with the glycosidic linkage. It is also likely that the conformation of these molecules is sensitive to the ionisation state[28,29] of the three phosphate groups under physiological conditions. With these reservations in mind, a consideration of predicted low-energy conformations of **8**–**11** can be used to suggest qualitative explanations for their differing potencies.

Table 2
$^{45}Ca^{2+}$ release data for combined stimulation with Ins(1,4,5)P_3, **9** and **10** [a]

	% Ca^{2+} release	*n*
Ins(1,4,5)P_3 (150 nM)	22 ± 4	3
9 (10 μM)	4 ± 4	3
Ins(1,4,5)P_3 (150 nM) with **9** (10 μM)	30 ± 3	3
10 (10 μM)	13 ± 3	3
Ins(1,4,5)P_3 (150 nM) with **10** (10 μM)	34 ± 1	3

[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** and **10** are shown. Results are shown as means ± S.E.M for three independent experiments.

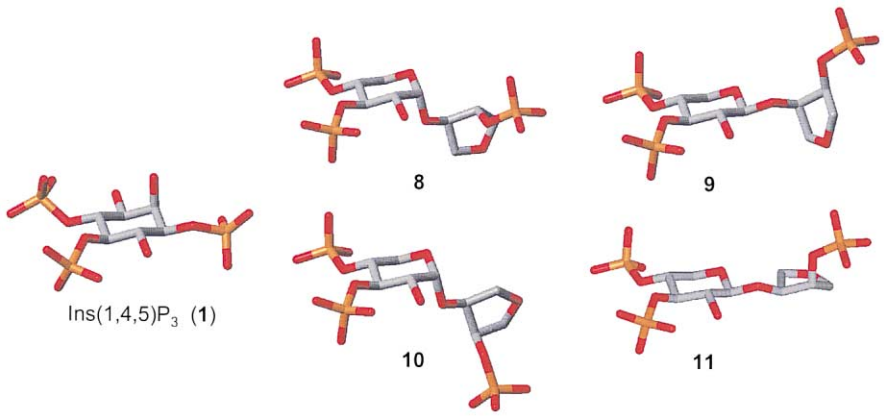


Fig. 2. X-ray crystal structure of Ins(1,4,5)P_3 derived from its complex with the PH domain of phospholipase C-δ_1 compared with low energy conformers of diastereoisomers **8**–**11** as determined by molecular modelling experiments (see Section 4).

The most stable conformers found for **8**–**11** are shown in Fig. 2, together with the conformation of Ins(1,4,5)P_3 identified in an X-ray crystallographic study of the PH domain of phospholipase C-δ_1 in complex with Ins(1,4,5)P_3.[30] For diastereoisomer **8**, a particularly well-defined global minimum energy conformation was identified, similar to that reported for adenophostin A.[†] In this conformer, the tetrahydrofuranyl ring is positioned below the plane of the xylopyranosyl ring, presumably in an orientation that avoids steric clashes with the receptor binding pocket and does not disrupt binding of the 3′-phosphate group. This phosphate group is located in a similar region of space to the 1-phosphate group of Ins(1,4,5)P_3, although in a slightly more extended position. Thus, as has been suggested for adenophostin A,[10,15] the non-vicinal phosphate in **8** may be held close to some ideal position for optimal interaction with the region of the receptor binding site that normally accommodates the 1-phosphate group of Ins(1,4,5)P_3. As in the case of adenophostin A,[10] a local minimum conformer of **8** (corresponding to a 2′-endo conformation of adenophostin A) with slightly higher energy and a different puckering of the five-membered ring was also found.

In low energy conformations of the other diastereoisomers **9**–**11**, the 3′-phosphate group is held in different regions of space relative to the xylopyranosyl ring. Presumably the 3′-phosphate groups in the less active isomers **9**–**11** are, to different degrees, less able to interact effectively with the Ins(1,4,5)P_3 receptor binding pocket, and can only approach the ideal position and orientation at greater energetic costs than can the 3′-phosphate group in **8**. The almost negligible activity of **9** is striking, particularly when it is compared to that of the relatively potent β-linked analogue **11**. A possible explanation for this difference, in addition to the factors already discussed, is that in low energy conformations of **9**, such as that shown in Fig. 2, regions of the hydrophobic tetrahydrofuranyl ring may interfere with binding to the receptor.

3. Conclusions

We have described the synthesis of a series of novel tetrahydrofuranyl xylopyranoside-based trisphosphates related to the adenophostins and to Ins(1,4,5)P_3. The most active member of the series (**8**) is of comparable potency to Ins(1,4,5)P_3 and to the previously described ribophostin (**6**) and furanophostin (**7**), from which **8** is a logical development. Further simplification would inevitably result in a substantial decrease in potency, and analogue **8** is therefore likely to represent the simplest possible structure for

[†] It should noted, however, that this conformation of **8** resembles a 3′-endo conformation of adenophostin A whereas, on the basis of ^{1}H NMR evidence, the 2′-endo form of adenophostin A is thought to predominate in solution. It has been suggested that the 2′-endo conformation might be essential to the high potency of adenophostin A.[10]

potent Ca^{2+}-releasing activity in this type of carbohydrate-based analogue. The weaker activities of the other three diastereoisomers **9**, **10** and **11** may be related to the effect of differing stereochemistry at three stereogenic centres on the spatial positioning of the non-vicinal phosphate group and tetrahydrofuranyl ring.

4. 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, CH_2Cl_2, THF, toluene and dioxane were purchased in anhydrous form. Ins(1,4,5)P_3 was from American Radiolabeled Chemicals. TLC was performed on precoated plates (E. Merck aluminium 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. Mps (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.[26]

Modelling was carried out using the SYBYL software package (v6.3, Tripos Associates) on a Silicon Graphics workstation. The TRIPOS force field was used, with added parameters for carbohydrates.[31] Charges were calculated using a semi-empirical method (MNDO) for structures with fully ionised phosphate groups, and the resulting charged structures were energy minimised, terminating at a gradient of 0.05 kcal mol^{-1} $Å^{-1}$, and using a distance-dependent dielectric of 4*r*. Each structure was then subjected to 100 cycles of simulated annealing, with heating at 300 K for 2 ps followed by exponential cooling to 0 K over 10 ps during each cycle, generating low-energy conformations for each analogue. These conformers were then further investigated by systematic searching about the torsions ϕ and ψ of the glycosidic linkage to create relaxed maps, allowing local and global minimum energy conformers for each analogue **8**–**11** to be identified.

$^{45}Ca^{2+}$ release from permeabilised rat hepatocytes.—Permeabilised 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 (five units per 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 four parameter logistic equation using KALEIDAGRAPH 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. CLM, cytosol-like medium; EGTA, ethylene glycol-bis(β-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]; ATP, adenosine 5′-triphosphate; FCCP, carbonyl cyanide *p*-trifuoromethoxyphenylhydrazone; EC_{50}, concentration causing half the maximal effect; *h*, Hill coefficient.

Preparation of allyl α-D-xylopyranoside (**12**)[22].—Allyl alcohol (1000 mL) and acetyl chloride (6.0 mL) were stirred together for 30 min, after which time D-xylose (100.00 g, 0.67 mol) was added. The mixture was heated at reflux for 16 h and then cooled to rt. Solid $NaHCO_3$ (7.00 g) was slowly added, and stirring continued for 30 min. The mixture was then filtered and the filtrate was concentrated by evaporation under reduced pressure, leaving an off-white solid. The solid was dissolved in a minimum of EtOH (700 mL) and the solution was kept at −20 °C for 24 h. Diisopropyl ether (ca 150 mL) was added immediately before the collection of the title compound over three crops as white fluffy crystals (52.25 g, 41%); mp 100–103 °C; Lit mp 101–103 °C;[22] $[\alpha]_D^{20}$ +140° (*c* 3.19, $CHCl_3$); Lit $[\alpha]_D^{20}$ +149° (*c* 3.2, $CHCl_3$).[22]

Allyl 2-O-trimethylacetyl-α-D-xylopyranoside (**13**).—A solution of **12** (10.0 g, 52.6 mmol) in dry pyridine (200 mL) was stirred at −40 °C under N_2. Trimethylacetyl chloride (6.8 mL, 55.2 mmol) was added dropwise over 1 h. The mixture was stirred at −40 °C for a further 90 min. The reaction was quenched by adding water (100 mL) and then allowed to reach rt and extracted with Et_2O (100 mL). The organic layer was washed with 1 M HCl (100 mL) and satd aq $NaHCO_3$ solution (100 mL), dried ($MgSO_4$), filtered and concentrated to give an oil which was dissolved in hexane and refrigerated at −20 °C. The title compound was collected as crystals over three crops (7.6 g, 53%); mp 76–77 °C; $[\alpha]_D^{20}$ +154° (*c* 1.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 270 MHz) δ_H 5.77–5.89 (m, 1 H, CH_2CH=CH_2), 5.29 (dd, 1 H, ^2J 1.6, ^3J 17.0 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.18 (dd, 1 H, ^2J 1.5, ^3J 11.7 Hz, CH_2CH=$CH_{cis}H_{trans}$), 4.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.62 (dd, 1 H, $J_{2,1}$ 3.7, $J_{2,3}$ 9.9 Hz, H-2), 4.13–4.20 (m, 2 H, CH_2CH=CH_2), 3.44–3.76 (m, 4 H, H-3, H-4, H-5$_2$) and 1.22 (s, 9 H, C(O)C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 178.60 (*C*(O)C(CH_3)$_3$), 133.57 (CH_2CH=CH_2), 117.51 (CH_2CH=CH_2), 95.28 (C-1), 73.07, 72.14, 70.44 (C-2, C-3, C-4), 68.39 (CH_2CH=CH_2), 61.42 (C-5), 38.89 (C(O)*C*(CH_3)$_3$) and 27.08 (C(O)C(CH_3)$_3$); Anal. Calcd for $C_{13}H_{22}O_6$: C, 56.92; H, 8.08. Found: C, 56.9; H, 8.08.

Allyl 3,4-O-isopropylidene-2-O-trimethylacetyl-α-D-xylopyranoside (**14**).—A solution of **13** (5.0 g, 18 mmol) in THF (50 mL) was stirred with *p*-toluenesulphonic acid (20 mg) and 2-methoxypropene (3.49 mL, 36 mmol) at rt for 30 min under N_2. TLC (EtOAc) indicated consumption of the starting material and showed the presence of one product. The mixture was diluted with Et_2O (50 mL) and washed with satd aq $NaHCO_3$ (30 mL). The organic layer was dried ($MgSO_4$) and filtered, a few drops of Et_3N were added before the organic layer was concentrated. The residue was used directly in the next step without purification. A small sample was purified for analysis by flash chromatography (1:7 eluent Et_2O–hexane); $[\alpha]_D^{20}$ +143° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 5.80–5.90 (m, 1 H, CH_2CH=CH_2), 5.29 (dd, 1 H, ^3J 17.3 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.19 (dd, 1 H, ^3J 10.2 Hz, CH_2CH=$CH_{cis}H_{trans}$), 5.16 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.82 (dd, 1 H, H-2), 4.16–4.21 (m, 1 H, $CHCH$=CH_2), 3.90–3.96 (m, 3 H, H-3, H-5a, $CHCH$=CH_2), 3.76 (dd, 1 H, ^3J 10.2 Hz, H-5b), 3.48–3.54 (m, 1 H, H-4), 1.46 (s, 6 H, 2 × isopropylidene CH_3) and 1.23 (s, 9 H, C(O)C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 177.96 (*C*(O)C(CH_3)$_3$), 133.50 (CH_2CH=CH_2), 117.83 (CH_2CH=CH_2), 110.86 (isopropylidene *C*(CH_3)$_2$), 95.10 (C-1), 75.75 (C-2), 74.12 (C-3), 72.88 (C-4), 68.96 (CH_2CH=CH_2), 61.27 (C-5), 38.77 (C(O)*C*(CH_3)$_3$) and 26.54, 26.81, 27.10 (2 × isopropylidene CH_3, C(O)C(CH_3)$_3$); Anal. Calcd for $C_{16}H_{26}O_6$: C, 61.13; H, 8.34. Found: C, 61.00; H, 8.29.

Allyl 3,4-O-isopropylidene-α-D-xylopyranoside (**15**).—A solution of crude **14** (5.72 g, 18.0 mmol), NaOH (1.46 g, 36 mmol) in MeOH (100 mL) was heated under reflux for 1 h. The mixture was cooled to rt and neutralised 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 aqueous layer was back-extracted with Et_2O (100 mL) and the combined organic fractions were dried ($MgSO_4$). A few drops of Et_3N were added before the organic layer was concentrated. The residue was used directly in the next step without purification. For analytical purposes, a small

sample was purified by flash chromatography (1:1 eluent Et_2O–hexane); $[\alpha]_D^{20}$ + 133° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 5.87–5.97 (m, 1 H, CH_2CH=CH_2), 5.32 (dd, 1 H, ^3J 17.3 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 5.24 (dd, 1 H, ^3J 10.2 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 4.95 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.23–4.28 (m, 1 H, CH_2CH=CH_2), 4.02–4.07 (m, 1 H, CH_2CH=CH_2), 3.94 (t, 1 H, $J_{3,4}$ 9.7 Hz, H-3), 3.83 (dd, 1 H, H-2), 3.66–3.74 (m, 2 H, H-5), 3.38–3.44 (m, 1 H, H-4), 2.43 (d, 1 H, *J* 10.8 Hz, 2-OH) and 1.45, 1.46 (2s, 6 H, 2 × isopropylidene CH_3); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 133.35 (CH_2CH=CH_2), 118.07 (CH_2CH=CH_2), 110.70 (isopropylidene $C(CH_3)_2$), 97.50 (C-1), 79.3 (C-2), 73.6 and 71.9 (C-4, C-3, C-2), 68.84 (CH_2CH=CH_2), 61.57 (C-5) and 26.40, 26.73 (2 × isopropylidene CH_3); Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.94. Found C, 57.10; H, 7.94.

Allyl 2-O-*benzyl-3,4*-O-*isopropylidene*-α-D-*xylopyranoside* (**16**).—A solution of crude **15** (4.18 g, 18 mmol) in dry DMF (25 mL) was stirred at 0 °C with NaH (760 mg of an 60% w/w dispersion in mineral oil, 19 mmol) and BnBr (2.17 mL, 19 mmol) was added slowly under N_2. The mixture was stirred at rt for 90 min, after which time TLC (1:2 Et_2O–hexane) showed complete consumption of the starting material. Water (25 mL) was added carefully and stirring continued for 60 min. The solvents were evaporated and the residue was dissolved in Et_2O (50 mL). The extract was washed with water (25 mL), dried ($MgSO_4$), filtered and concentrated. The product of this reaction was used directly in the next step without purification. A small sample was purified for analysis by flash chromatography (1:9 eluent Et_2O–hexane); $[\alpha]_D^{20}$ + 30.6° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.24–7.39 (m, 5 H, ArCH), 5.86–5.96 (m, 1 H, CH_2CH=CH_2), 5.34 (dd, 1 H, ^3J 17.3 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 5.21 (dd, 1 H, ^3J 10.2 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 4.83–4.86 (m, 2 H, H-1, OCH_2Ar), 4.64 (AB, 1 H, J_{AB} 12.3 Hz, OCH_2Ar), 4.15–4.20 (m, 1 H, CH_2CH=CH_2), 3.91–4.00 (m, 2 H, H-3, CH_2CH=CH_2), 3.86 (dd, 1 H, ^2J 9.7, ^3J 4.7 Hz, H-5a), 3.73 (dd, 1 H, *J* 10.2 Hz, H-5b), 3.65 (dd, 1 H, $J_{2,1}$ 3.5, $J_{2,3}$ 10.2 Hz, H-2), 3.35–3.41 (m, 1 H, H-4) and 1.46, 1.47 (2s, 6 H, 2 × isopropylidene CH_3); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 138.05 (C-1 of benzyl ether ring), 133.69 (CH_2CH=CH_2), 128.3, 127.9, 127.6 (ArCH), 117.80 (CH_2CH=CH_2), 110.54 (isopropylidene $C(CH_3)_2$), 96.24 (C-1), 77.9, 77.91, 74.1 (C-4, C-3, C-2), 71.8 (OCH_2Ar), 68.4 (CH_2CH=CH_2), 61.1 (C-5) and 26.8, 26.4 (2 × isopropylidene CH_3); Anal. Calcd for $C_{18}H_{24}O_5$: C, 67.48; H, 7.55. Found C, 67.40%; H, 7.53.

Preparation of allyl 2-O-*benzyl*-α-D-*xylopyranoside* (**17**)[22].—A solution of **16** (5.86 g, 18 mmol) in MeOH (25 mL) was stirred with 1 M HCl (2.5 mL) for 30 min. TLC (4:1 Et_2O–hexane) indicated consumption of 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 (50 mL) and water (25 mL). The aqueous layer was back-extracted with CH_2Cl_2 (100 mL) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The syrup obtained was subjected to flash chromatography (4:1 eluent Et_2O–hexane then Et_2O) to give the title compound as a colourless syrup (3.87 g, 76% over the previous four steps) which slowly crystallised; mp 46–47 °C (from isopropyl ether); Lit.[22] oil. $[\alpha]_D^{20}$ + 115° (*c* 2.3, $CHCl_3$); Lit $[\alpha]_D^{20}$ + 113° (*c* 2.3, $CHCl_3$).[22]

Preparation of 2-O-*benzyl-3,4-bis*-O-*(p-methoxybenzyl)*-D-*xylopyranose* (**18**)[22].—A solution of allyl 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-α-D-xylopyranoside (6.87 g, 13.2 mmol), obtained from **17** as previously described,[22] in MeOH (60 mL) was cooled to 0 °C and $PdCl_2$ (0.47 g, 2.64 mmol) was added. The flask was fitted with a drying tube, the cooling bath removed and the reaction mixture was stirred vigorously for 4 h, after which time TLC (3:2 ether–hexane) indicated almost complete conversion of starting material into a product (R_f 0.24). The reaction mixture was quenched with Et_3N and filtered through a Celite pad. The filtrate was concentrated and the dark brown residue subjected to flash chromatography (3:2 eluent ether–hexane) to yield the title compound (mixture of α and β anomers) as a white solid (5.72 g, 90%).

1-O-*[(3′*S*,4′*R*)-3-(*p*-Methoxybenzyloxy)-tetrahydrofuran-4-yl] 2*-O-*benzyl 3,4-di*-O-*(p-methoxybenzyl)-α and β*-D-*xylopyranosides* (**19** and **20**).—To a stirred mixture of **18** (2.0 g, 4.6 mmol) and 1*H*-tetrazole (440 mg, 6.25 mmol) in CH_2Cl_2 (10 mL) was added bis(-methoxy)(diethylamino)phosphine (0.9 mL, 5.4 mmol). After 20 min, TLC (1:4 EtOAc–toluene) indicated complete conversion into product (R_f 0.63). The reaction mixture was partitioned between Et_2O (100 mL) and water (75 mL). The resulting ethereal layer was washed with brine (75 mL), dried ($MgSO_4$), filtered and concentrated to give crude 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-D-xylopyranosyl dimethyl phosphite[14] as a colourless oil (2.38 g), which was used without further purification. A solution of the dimethyl phosphite (2.38 g), (+)-(3*R*,4*S*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol[20] (747 mg, 3.3 mmol) and 4 Å molecular sieves (3 g) in (21:7 mL) dioxane–toluene was stirred for 2 h under N_2. Zinc chloride (679 mg, 4.99 mmol) and silver perchlorate (2.04 g, 9.88 mmol) were added to the reaction mixture which was stirred in the dark for a further 2 h, TLC (4:1 toluene–EtOAc) showed consumption of the starting material. Sodium bicarbonate (3 g), EtOAc (100 mL) and water (75 mL) were added to the mixture and it was stirred vigorously for a further 30 min. The mixture was filtered through a Celite pad and the organic layer was washed with brine (100 mL), dried ($MgSO_4$), filtered and concentrated. The oil obtained was subjected to flash chromatography (7:3 eluent EtOAc–hexane) to give a ~2:1 (judged by ^{1}H NMR) anomeric mixture of the title compounds (1.60 g, 70%).

1-O-*[(3′*R*,4′*S*)-3-(*p*-Methoxybenzyloxy)-tetrahydrofuran-4-yl] 2*-O-*benzyl 3,4-bis*-O-*(p-methoxybenzyl)-α and β*-D-*xylopyranosides* (**21** and **22**).—The title compounds (1.75 g, 76%, ~1:1 anomeric mixture judged by ^{1}H NMR) were obtained from 2-*O*-benzyl-3,4-bis-*O*-(*p*-methoxybenzyl)-D-xylopyranosyl dimethyl phosphite (2.38 g, 4.16 mmol) and (−)-(3*S*,4*R*)-4-*p*-methoxybenzyloxy-tetrahydrofuran-3-ol[20] (747 mg, 3.3 mmol) as described for the synthesis of **19** and **20**.

1-O-*[(3′*S*,4′*R*)-3-Hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-α*-D-*xylopyranoside* (**23**) *and 1*-O-*[(3′*S*,4′*R*)-3-hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-β*-D-*xylopyranoside* (**24**).—A solution of the mixture of **19** and **20** (1.60 g, 2.32 mmol) and TFA (2 mL) in CH_2Cl_2 (20 mL) was stirred for 20 min, TLC (10:1 CH_2Cl_2–acetone) showed consumption of the starting material. Satd aq $NaHCO_3$ was added to neutralise the reaction. The mixture was extracted with EtOAc (4 × 100 mL) and the combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The oil obtained was subjected to flash chromatography (20:1 eluent EtOAc–MeOH) to give the title compound **23** which was crystallised from EtOAc–hexane (261 mg, 34%); mp 119–120 °C (EtOAc–hexane); $[\alpha]_D^{20}$ +87.2° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.26–7.37 (m, 5 H, Ar), 4.77 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1), 4.75, 4.71 (AB, 2 H, J_{AB} 11.7 Hz, ArCH_2O), 4.25–4.26 (m, 1 H, H-3′), 4.07–4.11 (m, 1 H, H-4′), 3.96 (dd, 1 H, $J_{5'b,4'}$ 5.9, $J_{5'b,5'a}$ 9.4 Hz, H-5′b), 3.88–3.91 (m, 2 H, H-3, H-2′b), 3.80 (dd, 1 H, $J_{5'a,4'}$ 4.4 Hz, H-5′a), 3.68–3.72 (m, 1 H, H-2′a), 3.51–3.65 (m, 3 H, H-4, H-5) and 3.35 (dd, 1 H, $J_{2,3}$ 8.8 Hz, H-2); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.34 (C-1 of benzyl ether ring), 128.92, 128.73, 128.68 (3 × Ar), 98.82 (C-1), 79.40, 79.00 (2 × CH), 74.59 (CH_2), 73.78 (CH), 73.13 (CH_2), 71.11 (CH), 71.11 (Ar*C*H_2O), 70.28 (CH) and 62.41 (C-5); Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.79. Found C, 58.80; H, 6.76.

Further elution gave compound **24** as an oil (150 mg, 20%); $[\alpha]_D^{20}$ −8.33° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.26–7.34 (m, 5 H, Ar), 4.85, 4.65 (AB, 2 H, J_{AB} 11.4 Hz, ArCH_2O), 4.45 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.19–4.25 (m, 2 H, H-4′, H-3′), 3.94–3.98 (m, 3 H, H-5b, H-2′b, H-5′b), 3.77–3.80 (m, 2 H, H-2′a, H-5′a), 3.62–3.63 (m, 1 H, H-4), 3.45–3.52 (m, 1 H, H-3), 3.23–3.30 (m, 4 H, H-2, H-5a, 2 × OH) and 3.18 (broad s, 1 H, OH); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.96 (C-1 of benzyl ether ring), 128.84, 128.36, 128.22 (Ar), 104.02 (C-1), 81.43, 80.63 and 75.75 (3 × CH), 74.96, 73.29 (CH_2), 71.04 (CH), 70.19 (Ar*C*H_2O), 69.51 (CH) and 65.60 (C-5); MS: (FAB) *m*/*z* Calcd for $C_{16}H_{22}O_7$, $[M+H]^+$327.1443. Found 327.1455.

1-O-*[(3′*R,*4′*S*)*-3-Hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-α*-D-*xylopyranoside* (**25**) *and 1*-O-*[(3′*R,*4′*S*)*-3-hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-β*-D-*xylopyranoside* (**26**).—Compounds **25** (145 mg, 18%) and **26** (155 mg, 19%) were obtained from the mixture of **21** and **22** (1.75 g, 2.50 mmol) as described for the synthesis of **23** and **24**.

25: mp 144–145 °C; $[\alpha]_D^{20}$ +132° (*c* 0.2, $CDCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.25–7.35 (m, 5 H, Ar), 4.74 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.66, 4.57 (AB, 2 H, J_{AB} 11.4 Hz, ArCH_2O), 4.22–4.25 (m, 1 H, H-3′), 4.12–4.16 (m, 1 H, H-4′), 3.89–3.99 (m, 2 H, H-3, H-5′b), 3.85 (dd, 1 H, $J_{2'b,3'}$ 5.6, $J_{2'b,2'a}$ 9.4 Hz, H-2′b), 3.72–3.76 (m, 2 H, H-2′a, H-5′a), 3.46–3.63 (m, 3 H, H-4, H-5) and 3.33 (dd, 1 H, $J_{2,3}$ 9.4 Hz, H-2); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 137.28 (C-1 of benzyl ether ring), 128.63, 128.43 (ArCH), 95.08 (C-1), 79.44, 76.53 (2 × CH), 74.79 (CH_2), 73.49 (CH), 72.57 (CH_2), 71.40, 69.63 (2 × CH), 68.89 (Ar*C*H_2O) and 61.85 (C-5); Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.79. Found: C, 58.70; H, 6.79.

Further elution gave compound **26** which was crystallised from EtOAc–hexane; mp 166–167 °C; $[\alpha]_D^{20}$ −28.3° (*c* 0.4, MeOH); ^1H NMR (CD_3OD; 400 MHz) δ_H 7.27–7.43 (m, 5 H, Ar), 4.95, 4.81 (AB, 2 H, J_{AB} 11.1 Hz, ArCH_2O), 4.55 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.23–4.31 (m, 2 H, H-2′b and H-5′b), 3.94–3.98 (m, 1 H, H-3′ or H-4′), 3.86–3.9 (m, 2 H, H-3, H-2′a or H-5′a), 3.76–3.80 (m, 1 H, H-3′ or H-4′), 3.63 (dd, 1 H, *J* 4.1, J_{gem} 9.1 Hz, H-2′a or H-5′a), 3.46–3.57 (m, 2 H, H-4, H-5b) and 3.21–3.3 (m, 2 H, H-2, H-5a); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 140.21 (C-1 of benzyl ether ring), 129.70, 129.54 and 128.96 (Ar), 105.10 (C-1), 83.02, 80.18, 78.02 (3 × CH), 75.98, 73.66 (2 × CH_2), 72.23 (Ar*C*H_2O), 72.13, 71.60 (2 × CH) and 67.17 (C-5); Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.88; H, 6.79. Found: C, 58.50; H, 6.80.

1-O-*[(3′*S,*4′*R*)*-3-Hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-α*-D-*xylopyranoside 3,4,3′-tris(dibenzylphosphate)* (**27**).—A mixture of bis(benzyloxy)(diisopropylamino)phosphine (518 mg, 1.50 mmol), 1*H*-tetrazole (158 mg, 2.25 mmol) and dry CH_2Cl_2 (5 mL) was vigorously stirred at rt for 30 min under N_2, whereupon triol **23** (83 mg, 0.25 mmol) was added and stirring was continued for 30 min. The mixture was cooled to −78 °C and MCPBA (863 mg, 60%, 3.0 mmol) was added. The mixture was stirred at rt for 10 min, then was diluted with CH_2Cl_2 (50 mL). The solution 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 residue was purified by flash chromatography (4:1 eluent EtOAc–hexane) to give the title compound as an oil (279 mg, 99%); $[\alpha]_D^{20}$ +0.3° (*c* 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.04–7.39 (m, 35 H, Ar), 4.66–5.06 (m, 16 H, H-1, H-3, H-3′, 6.5 × ArCH_2O), 4.31–4.47 (m, 2 H, H-4, 0.5 × ArCH_2O), 4.08–4.12 (m, 1 H, H-4′), 3.84–3.96 (m, 4 H, H-5a, H-5b, H-2′b, H-5′b), 3.79 (dd, 1 H, $J_{5'a,4'}$ 5.3, $J_{5'a,5'b}$ 9.4 Hz, H-5′a) and 3.43–3.49 (m, 2 H, H-2, H-2′a); ^{13}C NMR ($CDCl_3$; 67 MHz) δ_C 137.589 (C-1 of benzyl ether ring), 136.02–135.50 (C-1 of benzyl ester rings), 128.50, 128.50, 128.48, 128.41, 128.61, 128.22, 128.13, 128.06, 127.94, 127.88, 127.71, 127.60, 127.49 (ArCH), 95.68 (C-1), 77.09, 75.35, 75.26, 74.03 and 73.93 (5 × CH), 71.97, 69.99 (2 × CH_2), 68.91–69.71 (Ar*C*H_2O) and 59.69 (C-5); ^{31}P NMR ($CDCl_3$; 162 MHz) δ_p −0.70, −0.62 and 0.11; MS: (FAB) *m*/*z* Calcd for $C_{58}H_{61}O_{16}P_3$ $[M+H]^+$ 1107.3250. Found 1107.3233.

1-O-*[(3′*S,*4′*R*)*-3-Hydroxytetrahydrofuran-4-yl] 2*-O-*benzyl-β*-D-*xylopyranoside 3,4,3′-tris(dibenzylphosphate)* (**28**).—Compound **28** (89 mg, 87%) was obtained from **24** (30 mg, 0.09 mmol) as described above for the synthesis of **27**.

$[\alpha]_D^{20}$ −10.4° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$; 400 MHz) δ_H 7.02–7.34 (m, 35 H, Ar), 4.75–5.14 (m, 14 H, H-3′, ArCH_2O), 4.49–4.58 (m, 2 H, H-3, ArCH_2O), 4.39 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.31–4.35 (m, 1 H, H-4′), 4.23–4.30 (m, 1 H, H-4), 4.19 (m, 1 H, H-5b), 3.85–3.93 (m, 3 H, H-5′b, H-2′a, H-2′b), 3.74 (dd, 1 H, $J_{5'a,4'}$ 6.5 Hz, H-5′a) and 3.23–3.28 (m, 2 H, H-2, H-5a); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.89 (C-1 of benzyl ether ring), 136.12–135.64 (C-1 of benzyl ester ring), 128.83, 128.80, 128.75, 128.61, 128.59, 128.46, 128.23, 128.21, 128.12, 128.03, 127.97, 127.91,

127.81 (Ar), 103.09 (C-1), 79.55, 79.26, (2 × CH), 76.40 (CH_2), 74.58, 73.86, 71.36 (3 × CH), 70.10 (CH_2), 68.87–70.04 (7 × Ar*C*H_2O) and 63.42 (C-5); ^{31}P NMR ($CDCl_3$; 162 MHz) δ_p −0.76, −0.60 and −0.43; MS: (FAB) *m/z* Calcd for $C_{58}H_{61}O_{16}P_3$ $[M+H]^+$ 1107.3238. Found 1107.3238.

1-O-[(3′R,4′S)-3-Hydroxytetrahydrofuran-4-yl] 2-O-benzyl-α-D-xylopyranoside 3,4,3′-tris(dibenzylphosphate) (**29**).—Compound **29** (210 mg, 73%) was obtained from **25** (85 mg, 0.23 mmol) as described above for the synthesis of **27**.

$[\alpha]_D^{20}$ +9.4° (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.13–7.40 (m, 35 H, Ar), 4.87–5.16 (m, 13 H, H-1, 6 × ArCH_2O), 4.77–4.82 (m, 1 H, H-3′), 4.66–4.69 (m, 2 H, H-3, Ar*C*H_2O), 4.4–4.49 (m, 2 H, H-4, Ar*C*H_2O), 4.10–4.18 (m, 1 H, H-2′a), 3.92–4.03 (m, 2 H, H-5a, H-5b), 3.85–3.91 (m, 3 H, H-2′b, H-4′, H-5′b), 3.74 (dd, 1 H, $J_{5'a,4'}$ 6.3, $J_{5'a,5'b}$ 9.1 Hz, H-5′a) and 3.51 (dd, 1 H, $J_{2,1}$ 3.5, $J_{2,3}$ 9.7 Hz, H-2); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 137.80 (C-1 of benzyl ether ring), 136.13–134.49 (6 × C-1 of benzyl ester rings), 132.95, 130.17, 128.72, 128.70, 128.63, 128.55, 128.43, 128.36, 128.29, 128.22, 128.16, 128.01, 127.83 (Ar), 96.90 (C-1), 78.53, 77.99, 76.32, 76.26, 75.92 (5 × CH), 74.14, 73.87 (CH_2) 70.79–70.79 (O*C*H_2Ar) and 60.33 (C-5); ^{31}P NMR ($CDCl_3$; 109 MHz)δ_p −0.55, −0.80 and −0.95; MS: (FAB) *m/z* Calcd for $C_{58}H_{61}O_{16}P_3$ $(M+1)^+$ 1107.3250. Found 1107.3252.

1-O-[(3′R,4′S)-3-Hydroxytetrahydrofuran-4-yl] 2-O-benzyl-β-D-xylopyranoside 3,4,3′-tris(dibenzylphosphate) (**30**).—Compound **30** (107 mg, 79%) was obtained from **26** (30 mg, 0.09 mmol) as described above for the synthesis of **27**.

$[\alpha]_D^{20}$ −10.8° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$; 400 MHz) δ_H 7.04–7.37 (m, 35 H, Ar), 4.71–5.02 (m, 14 H, H-3′, 6.5 × Ar*C*H_2O), 4.52–4.61 (m, 3 H, H-1, H-3, 0.5 × Ar*C*H_2O), 4.29–4.37 (m, 1 H, H-4), 4.23 (dd, 1 H, *J* 5.3, *J* 10.2, H-2′a), 4.10 (dd, 1 H, *J* 5.3, *J* 12.0, H-5b), 3.89–3.97 (m, 2 H, H-4′, H-5′a), 3.78–3.82 (m, 2 H, H-2′b, H-5′b), 3.36–3.40 (m, 1 H, H-2) and 3.14 (dd, 1 H, *J* 9.4, *J* 12.0, H-5a); ^{13}C NMR ($CDCl_3$; 100.4 MHz) δ_C 138.11 (C-1 of benzyl ether ring), 136.13–135.71 (6 × C-1 of benzyl ester ring), 128.88, 128.82, 128.80, 128.75, 128.61, 128.56, 128.44, 128.41, 128.34, 128.32, 128.25, 128.22, 128.20, 128.05, 128.00, 127.89, 127.59 (ArCH), 102.86 (C-1), 79.20, 79.10 (2 × CH_2), 76.32, 76.26, 75.58, 75.54 and 74.29 (5 × CH), 73.79–69.43 (7 × Ar*C*H_2O) and 62.89 (C-5); ^{31}P NMR ($CDCl_3$; 162 MHz) δ_p −0.25, −0.83 and −1.07; MS: (FAB) *m/z* Calcd for $C_{58}H_{61}O_{16}P_3$ $[M+H]^+$ 1107.3250. Found 1107.3232.

1-O-[(3′S,4′R)-3-Hydroxytetrahydrofuran-4-yl] α-D-xylopyranoside 3,4,3′-trisphosphate (**8**).—10% palladium on activated charcoal (200 mg) was added to a solution of compound **27** (94 mg, 0.084 mmol) in MeOH (20 mL) and water (5 mL) and the mixture was shaken under an atmosphere of H_2 at 50 psi at rt for 24 h. The suspension was filtered and washed well with de-ionised water. The combined filtrate was concentrated to a glassy 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 8. The triethylammonium salt of **8** eluted between 68 and 82%. Fractions containing the title compound, as judged by phosphate assay, were combined and evaporated to give a residue from which MeOH (3 × 100 mL) was evaporated to give the title trisphosphate as its triethylammonium salt (0.071 mmol, 85%); $[\alpha]_D^{20}$ +30.2° (*c* 1.3, MeOH); ^{1}H NMR (D_2O; 400 MHz) δ_H 4.99 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.58–4.61 (m, 1 H, H-3′), 4.25–4.29 (m, 1 H, H-4′), 4.14–4.22 (m, 1 H, H-3), 3.91–3.97 (m, 1 H, H-4), 3.82–3.90 (m, 2 H, H-2′a, H-5′a), 3.67–3.75 (m, 3 H, H-2′b, H-5′b, H-5a), 3.58 (dd, 1 H, $J_{2,3}$ 8.8 Hz, H-2) and 3.45–3.52 (m, 1 H, H-5b); ^{31}P NMR (D_2O; 162 MHz) δ_P 1.05 and 0.89 (2P); MS: (FAB) *m/z* Calcd for $C_9H_{18}O_{16}P_3$ $[M-H]^-$ 474.9807. Found 474.9801.

1-O-[(3′S,4′R)-3-Hydroxytetrahydrofuran-4-yl] β-D-xylopyranoside 3,4,3′-trisphosphate (**9**).—The title compound (0.049 mmol, 77%) as its triethylammonium salt was obtained from compound **28** (72 mg, 0.064 mmol) as described above for the synthesis of **8**.

$[\alpha]_D^{20}$ −17.7° (*c* 1.2, MeOH); ^{1}H NMR (CD_3OD; 400 MHz) δ_H 4.54–4.60 (m, 1 H,

H-3′), 4.37–4.42 (m, 2 H, H-1, H-4′), 3.92–3.97 (m, 3 H, H-5a, H-5b, H-3), 3.83–3.88 (m, 2 H, H-2′b, H-5′b), 3.69–3.73 (m, 2 H, H-$2'_a$, H-$5'_a$), 3.34–3.38 (m, 1 H, H-2) and 3.23–3.31 (m, 1 H, H-4); ^{31}P NMR (CD_3OD; 162 MHz) δ_p 0.81, 1.05, 1.20; MS: (FAB) m/z Calcd for $C_9H_{18}O_{16}P_3$ $[M-H]^-$ 474.9807. Found 474.9801.

1-O-[(3′R,4′S)-3-Hydroxytetrahydrofuran-4-yl] α-D-xylopyranoside 3,4,3′-trisphosphate (**10**).—The title compound as its triethylammonium salt (0.064 mmol, 65%) was obtained from compound **29** (110 mg, 0.099 mmol) as described above for the synthesis of **8**. $[\alpha]_D^{20}$ +40.8° (*c* 1.5, MeOH); ^{1}H NMR (D_2O; 270 MHz) δ_H 4.79 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.53–4.57 (m, 1 H, H-3′), 4.10–4.23 (m, 2 H, H-3, H-4′), 3.63–3.95 (m, 7 H, H-5a, H-5b, H-5′a, H-5′b, H-2′a, H-2′b) and 3.50 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2); ^{31}P NMR (D_2O; 109 MHz) δ_p 0.34 and 0.80 (2P); MS: (FAB) m/z Calcd for $C_9H_{18}O_{16}P_3$ $[M-H]^-$ 474.9807. Found 474.9802.

1-O-[(3′R,4′S)-3-Hydroxytetrahydrofuran-4-yl] β-D-xylopyranoside 3,4,3′-trisphosphate (**11**).—The title compound (0.071 mmol, 85%) as its triethylammonium salt was obtained from compound **30** (94 mg, 0.084 mmol) as described above for the synthesis of **8**. $[\alpha]_D^{20}$ −21.1° (*c* 0.52, MeOH); ^{1}H NMR (CD_3OD; 400 MHz) δ_H 4.78–4.81 (m, 1 H, H-3′), 4.54 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.40–4.45 (m, 1 H, H-4′), 4.19–4.3 (m, 1 H, H-2′b), 4.06–4.08 (m, 1 H, H-3), 3.92–3.98 (m, 1 H, H-5′b), 3.86 (dd, 1 H, $J_{2'a,3'}$ 3.5, $J_{2'a,2'b}$ 9.6, H-2′a), 3.73 (dd, 1 H, $J_{5'a,4'}$ 6.2, $J_{5'a,5'b}$ 8.8, H-5′a), 3.34–3.44 (m, 2 H, H-2, H-4) and 3.01–3.08 (m, 3 H, H-5a, H-5b, OH); ^{31}P NMR (CD_3OD; 162 MHz) δ_p 0.71, 1.32 and 1.47; MS: (FAB) m/z Calcd for $C_9H_{18}O_{16}P_3$ $[M-H]^-$ 474.9807. Found 474.9815.

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References

[1] Berridge, M. J. *Nature* **1993**, *361*, 315–325.
[2] Potter, B. V. L.; Lampe, D. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1933–1972.
[3] Wilcox, R. A.; Primrose, W. U.; Nahorski, S. R.; Challiss, R. A. J. *Trends Pharmacol. Sci.* **1998**, *19*, 467–475.
[4] Takahashi, S.; Kinoshita, T.; Takahashi, M. *J. Antibiot.* **1994**, *47*, 95–100.
[5] Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, *269*, 369–372.
[6] Hirota, J.; Michikawa, T.; Miyawaki, A.; Takahashi, M.; Tanzawa, K.; Okura, I.; Furuichi, T.; Mikoshiba, K. *FEBS Lett.* **1995**, *368*, 248–252.
[7] Murphy, C. T.; Bullock, A. J.; Lindley, C. J.; Mills, S. J.; Riley, A. M.; Potter, B. V. L.; Westwick, J. *Mol. Pharmacol.* **1996**, *50*, 1223–1230.
[8] Marchant, J. S.; Beecroft, M. D.; Riley, A. M.; Jenkins, D. J.; Marwood, R. D.; Taylor, C. W.; Potter, B. V. L. *Biochemistry* **1997**, *36*, 12780–12790.
[9] Shuto, S.; Tatani, K.; Ueno, Y.; Matsuda, A. *J. Org. Chem.* **1998**, *63*, 8815–8824.
[10] Hotoda, H.; Murayama, K.; Iwata, Y.; Takahashi, M.; Kawase, Y.; Tanzawa, K.; Keneko, M. *Biochemistry* **1999**, *38*, 9234–9241.
[11] Marwood, R. D.; Correa, V.; Taylor, C. W.; Potter, B. V. L. *Tetrahedron: Asymmetry* **2000**, *11*, 397–403.
[12] Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Kaneko, M. *Tetrahedron Lett.* **1995**, *36*, 5037–5040.
[13] van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1997**, *53*, 6509–6522.
[14] Marwood, R. D.; Riley, A. M.; Jenkins, D. J.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1935–1947.
[15] Wilcox, R. A.; Erneux, C.; Primrose, W. U.; Gigg, R.; Nahorski, S. R. *Mol. Pharmacol.* **1995**, *47*, 1204–1211.
[16] Jenkins, D. J.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1995**, 1169–1170.
[17] Moitessier, N.; Chrétien, F.; Chapleur, Y.; Humeau, C. *Tetrahedron Lett.* **1995**, *36*, 8023–8026.
[18] Desai, T.; Gigg, J.; Gigg, R. *Carbohydr. Res.* **1996**, *280*, 209–221.
[19] Jenkins, D. J.; Marwood, R. D.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1997**, 449–450.
[20] Marwood, R. D.; Riley, A. M.; Correa, V.; Taylor, C. W.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 453–458.
[21] Rosenberg, H. J.; Riley, A. M.; Correa, V.; Taylor, C. W.; Potter, B. V. L. *Carbohydr. Res.* **2000**, *329*, 7–16.
[22] Jenkins, D. J.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans.1* **1998**, 41–49.
[23] Jiang, L.; Chan, T.-H. *J. Org. Chem.* **1998**, *63*, 6035–6038.
[24] Cai, J.; Davison, B. E.; Ganellin, R. C.; Thaisrivongs, S. *Tetrahedron Lett.* **1995**, *36*, 6535–6536.
[25] Ogawa, T.; Takahashi, Y.; Matsui, M. *Carbohydr. Res.* **1982**, *102*, 207–215.
[26] Sawyer, D. A.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans.1* **1992**, 923–932.
[27] Roussel, F.; Hilly, M.; Chrétien, F.; Mauger, Y.; Chapleur, Y. *J. Carbohydr. Chem.* **1999**, 697–707.
[28] Felemez, M.; Marwood, R. D.; Potter, B. V. L.; Spiess, B. *Biochem. Biophys. Res. Commun.* **1999**, *266*, 334–340.
[29] Felemez, M.; Bernard, P.; Schlewer, G.; Spiess, B. *J. Am. Chem. Soc.* **2000**, *122*, 3156–3165.
[30] Ferguson, K. M.; Lemmon, M. A.; Schlessinger, J.; Sigler, P. B. *Cell* **1995**, *83*, 1037–1046.
[31] Pérez, S.; Meyer, C.; Imberty, A. In *Modelling of Biomolecular Structures and Mechanisms*; Pullman, A.; Jortner, J.; Pullman, B., Eds.; Kluwer: Dordrecht, 1995; pp. 425–454.