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Synthesis of adenophostin A and congeners modified at glucose

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A convergent route is described to the super-potent 1D-*myo*-inositol 1,4,5-trisphosphate receptor agonist adenophostin A (**2**) and analogues **5** and **7**, in which the glucose bisphosphate unit is replaced by corresponding xylose bisphosphate and mannose bisphosphate units respectively. Adenosine was converted into its 2′,3′-*O*-*p*-methoxybenzylidene derivative **8ab**, which was selectively N^6-dimethoxytritylated by a transient protection method. 5′-*O*-Benzylation followed by reductive acetal cleavage gave, after separation from its 3′-*O*-*p*-methoxybenzyl isomer, the versatile glycosyl acceptor 5′-*O*-benzyl-N^6-dimethoxytrityl-2′-*O*-*p*-methoxybenzyladenosine **13**. Coupling of **13** with selectively protected glucopyranosyl, xylopyranosyl or mannopyranosyl dimethyl phosphites gave the required 3′-*O*-α-pyranosyl adenosine derivatives. Acidic hydrolysis gave corresponding N^6-unprotected triols which were phosphitylated using bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate without further N^6-protection. Deprotection gave the target trisphosphates **2**, **5** and **7**. Synthetic adenophostin A (**2**) was identical with a sample of natural material in all respects. Analogues **5** and **7** will be useful for structure–activity studies on the adenophostins.

Introduction

In 1983 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] (Fig. 1) was identified as a second messenger responsible for increasing intracellular Ca^{2+} concentration in stimulated cells and its role has now been well characterised.[1] Ins(1,4,5)P_3 interacts with a tetrameric receptor situated in the lipid bilayer of the endoplasmic reticulum, and the synthesis and biological evaluation of numerous Ins(1,4,5)P_3 analogues have led to a good understanding of structure–activity relationships at this receptor.[2]

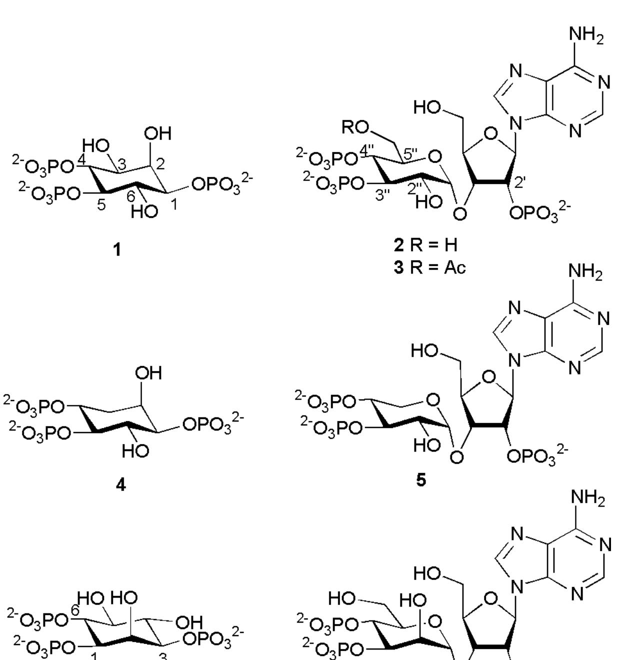
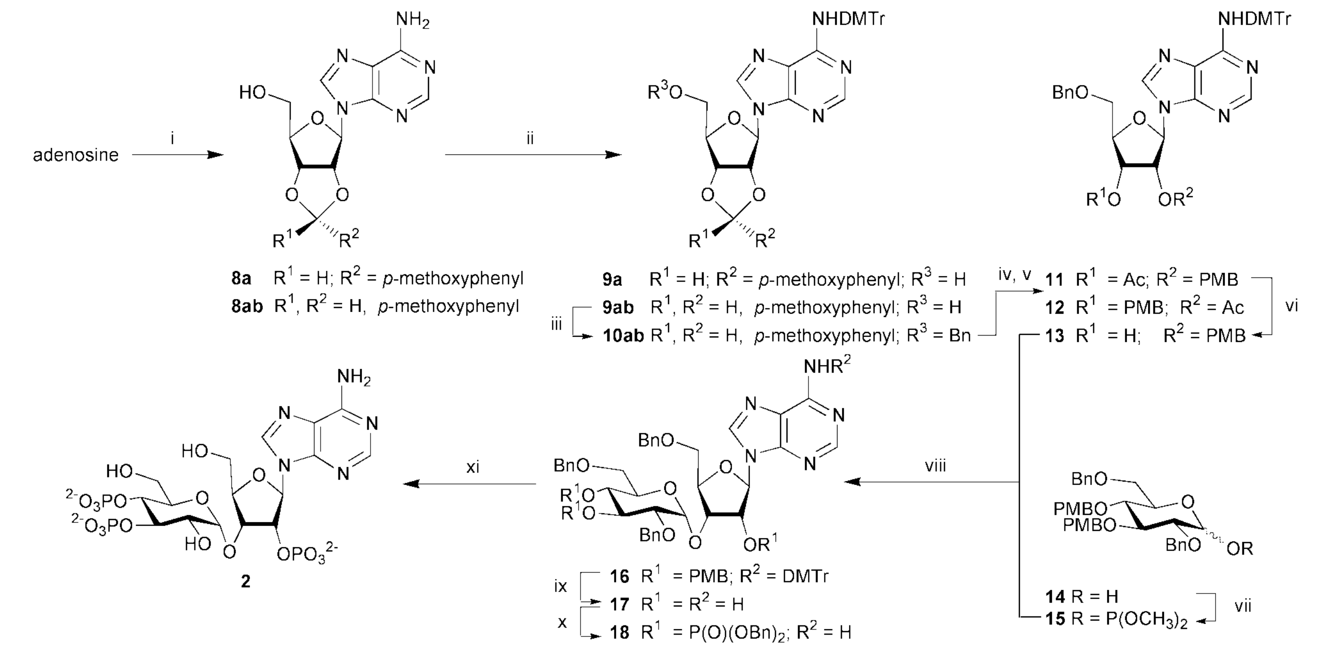


Fig. 1 Relationship of 1D-*myo*-inositol 1,4,5-trisphosphate (**1**) to adenophostins A (**2**) and B (**3**), and of inositol trisphosphates **4** and **6** to target compounds **5** and **7**.

The isolation[3] and initial characterisation[4,5] of the natural glyconucleotides adenophostins A (**2**) and B (**3**) in 1993 was an extremely important development; **2** and **3** are by far the most potent Ins(1,4,5)P_3 receptor agonists yet identified. They were reported to elicit Ca^{2+}-release from cerebellar microsomes with potencies 100-fold higher than Ins(1,4,5)P_3,[5] and subsequent studies have demonstrated potencies 10–100 fold greater than Ins(1,4,5)P_3 in other cell types.[6,7] The adenophostins are now finding widespread use as pharmacological tools for the investigation of cell signalling mechanisms,[8] and a potential application in parthenogenetic oocyte activation in the biotechnology of animal reproduction has been proposed.[9]

The extraordinary activity of the adenophostins was unexpected, as their structures bear little obvious resemblance to Ins(1,4,5)P_3. Nevertheless, it is clear that the 3″,4″-bisphosphate of **2** and **3** corresponds stereochemically to the critical 4,5-bisphosphate of Ins(1,4,5)P_3, and all three molecules contain a third phosphate, which enhances affinity for the receptor.[2,5] Several Ins(1,4,5)P_3 mimics based on the adenophostins have been prepared,[7,10–14] and some structure–activity relationships have been elucidated. Importantly, it appears that, for potency to exceed that of Ins(1,4,5)P_3, a base (or base surrogate) is necessary. We have recently synthesised base-modified adenophostin analogues[14] to explore this requirement.

As part of our continuing research programme investigating structure–activity relationships of **1** and **2**, a route was required to **2** which would also allow the synthesis of analogues **5** and **7** in which the glucopyranosyl bisphosphate moiety is replaced with xylopyranosyl and mannopyranosyl bisphosphate units respectively. Little attention has so far been directed at modifications to the glucopyranosyl component of the adenophostins, and **5** and **7** were considered suitable target molecules with which to begin studies in this area. Assuming that, at the Ins(1,4,5)P_3 receptor binding site, the 5″-hydroxymethyl and 2″-hydroxy groups of adenophostin A mimic the 3- and 6-OH groups of Ins(1,4,5)P_3 respectively, then **5** can be seen as structurally analogous to 3-deoxy-Ins(1,4,5)P_3 (**4**),[15] while **7** is related to Ins(1,3,6)P_3 (**6**),[16] in which the equivalent of the equatorial 6-OH group in Ins(1,4,5)P_3 is changed to axial. Both **4** and **6** may interact in novel ways with Ins(1,4,5)P_3 receptors as a



Scheme 1 *Reagents and conditions*: i) *p*-methoxybenzaldehyde, $(EtO)_3CH$, PTSA, 35–40 °C, 4 h (90%); ii) (*a*) Me_3SiCl, pyridine, room temp., 2 h; (*b*) DMTrCl, room temp., 16 h; (*c*) conc. aq. NH_3, room temp., 30 min (97%); iii) KOH, BnCl, benzene–dioxane (2:1), reflux, 20 min (95%); iv) DIBAL-H, CH_2Cl_2, −78 to −25 °C, 2 h; v) Ac_2O, pyridine, room temp., 16 h (93% from **10ab**); vi) conc. aq. NH_3, MeOH, $CHCl_3$, room temp., 48 h (100%); vii) $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 20 min; viii) (*a*) 4 Å sieves, dioxane–toluene (3:1), room temp., 2 h; (*b*) $ZnCl_2$, $AgClO_4$, dark, 7 h (53%); ix) TFA, CH_2Cl_2, room temp., 1.75 h (81%); x) (*a*) $(BnO)_2PNPr^i_2$, imidazolium triflate, CH_2Cl_2, room temp., 1 h; (*b*) MCPBA, −78 °C, 10 min (70%); xi) wet $Pd(OH)_2/C$, MeOH–cyclohexene–H_2O (14:7:1), reflux, 2.5 h (92%). Bn = benzyl, DMTr = dimethoxytrityl, PMB = *p*-methoxybenzyl.

result of their slight structural differences to Ins(1,4,5)P_3; **4** has been shown to behave as a partial agonist at the Ins(1,4,5)P_3 receptors of cerebellar microsomes[17] while there is evidence that **6** may show selectivity for the type 1 Ins(1,4,5)P_3 receptor subtype.[18] Both analogues, however, bind with lower affinities than Ins(1,4,5)P_3 itself, and further investigation of **6** in particular has been limited by its low potency. The glyconucleotides **5** and **7**, on the other hand, might be expected to have higher affinities for Ins(1,4,5)P_3 receptors than the corresponding inositol phosphates, due to the enhancing effect of their adenosine 2′-monophosphate (2′-AMP) component.

Two previous syntheses of adenophostin A have been reported. In the first, Hotoda *et al.*[19] glycosylated N^6,N^6-dibenzoyl-5′-*O*-monomethoxytrityl-2′-*O*-*p*-methoxybenzyladenosine with 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide. A rather different approach was reported by van Straten *et al.*,[20] who first prepared a 3-*O*-α-D-glucopyranosyl-D-ribofuranose derivative, followed by a Vorbrüggen condensation with an activated adenine derivative. A disadvantage of both of these previous routes was the large number of protection/deprotection steps between construction of the pyranosyladenosine unit and the final compound. In the present work we describe an efficient route to a suitably protected glycosyl acceptor, which allows a convergent approach to the synthesis of compounds **2**, **5** and **7**, with minimal manipulation between phosphite-mediated coupling and final trisphosphates. A preliminary report of our synthesis of **2** has appeared.[21]

Results and discussion

For consistency with selectively protected glucopyranose[11] and xylopyranose[22] units previously prepared in these laboratories, we required an adenosine derivative protected with a *p*-methoxybenzyl ether at position 2′ and a benzyl ether at position 5′ together with appropriate N^6-protection. Attempts to 5′-*O*-benzylate the known[23] N^6-benzoyl-2′-*O*-*p*-methoxybenzyladenosine selectively with benzyl bromide and sodium hydride at reduced temperature,[24] or with benzyl bromide–silver oxide,[25] or with benzyl trichloroacetimidate,[26] or *via* stannyl ethers[27] were unsuccessful and so an alternative strategy was sought.

Treatment of adenosine with zinc chloride–*p*-methoxybenzaldehyde gave the 2′,3′-*O*-*p*-methoxybenzylidene derivative[28] **8ab** (Scheme 1). Precipitation of the partially purified product from *p*-methoxybenzaldehyde gave the kinetic product (*endo* diastereoisomer) as judged by NMR spectroscopy; subsequent addition of diisopropyl ether to the mother liquor gave a second crop containing a 2:1 *endo*:*exo* diastereoisomeric mixture. The identities of diastereoisomers were ascertained by analogy to literature ^{1}H NMR data for the corresponding benzylidene derivatives which have been unambiguously assigned by NOE experiments.[29] NMR data for **8ab** and the pure *endo* diastereoisomer **8a** are reported for the first time here. Repeating the reaction at 40–45 °C gave an inseparable 3:2 *exo*:*endo* diastereomeric mixture, a ratio which, in our hands, was also obtained when benzaldehyde was substituted for *p*-methoxybenzaldehyde.[30] This latter result is at variance with that of Baggett *et al.*,[31] who reported exclusively the *exo* diastereoisomer under these conditions. Exposing the pure *endo* diastereoisomer **8a** to the higher temperature conditions also gave a 3:2 *exo*:*endo* product mixture, suggesting this to be the equilibrium ratio. An improved yield of **8ab** (as a 3:2 *exo*:*endo* mixture) was obtained using *p*-methoxybenzaldehyde, triethyl orthoformate and PTSA;[28b] direct acetal exchange with *p*-methoxybenzaldehyde dimethyl acetal in DMF in the presence of PTSA, successful in the preparation of methyl 2,3-*O*-*p*-methoxybenzylidene-β-D-ribofuranoside,[13] gave no product by TLC.

Selective protection of the N^6-position with a dimethoxytrityl group in the presence of the free primary hydroxy group was achieved by the sequential treatment of **8ab** with chlorotrimethylsilane, dimethoxytrityl chloride and concentrated aqueous ammonia, to give the required product **9ab** in 97% yield after column chromatography. The ^{1}H NMR spectrum of **9ab** revealed a D_2O-exchangeable triplet at 5.2 ppm, confirming that the 5′-hydroxy group remained unprotected. Although transient protection is well established for N^6-benzoylation,[32] to the best of our knowledge this is the first example of its application to N^6-dimethoxytritylation. Benzylation of the 5′-hydroxy group was achieved in 95% yield using benzyl chloride–potassium hydroxide,[33] a superior method in this case to sodium hydride–benzyl bromide. Substitution at position 5′ rather than N^6 was confirmed by the ^{13}C NMR spectrum of **10ab**, which indicated a methylene carbon at 73.5 ppm, typical of benzyl ether methylenes and by typical[34] deshielding of the 5′-methylene carbon to 70.1 ppm.

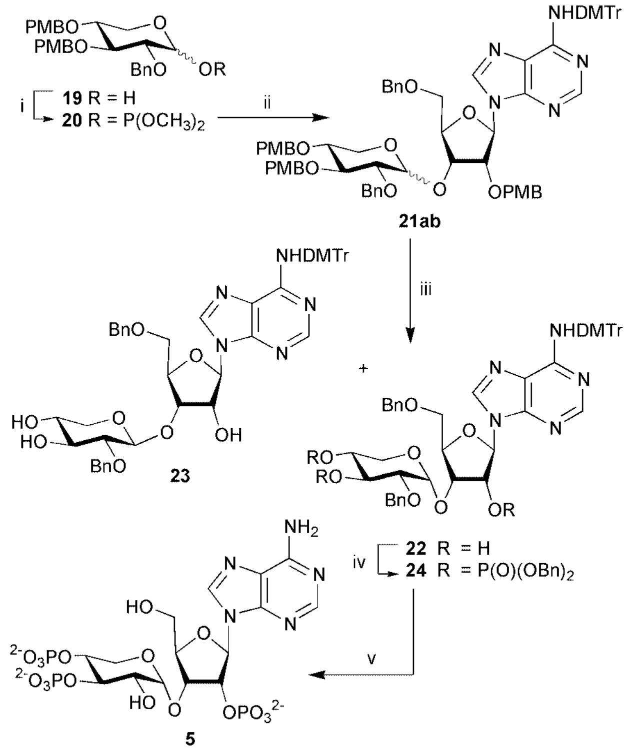
Treatment of **10ab** with DIBAL-H in dichloromethane at low temperature gave an inseparable mixture of the 2′- and 3′-*O*-*p*-methoxybenzyl ethers in a 3 : 2 ratio. The temperature of this reaction was crucial; repeating it at 0 °C gave only traces of these products. Similarly, using the established reductive cleavage reagents $LiAlH_4$–$AlCl_3$, $BH_3{\cdot}N(CH_3)_3$–$AlCl_3$, $NaBH_3CN$–$(CH_3)_3SiCl$, $NaBH_3CN$–HCl or $NaBH_3CN$–TFA also gave very poor results. Acetylation of the product mixture gave acetates **11** and **12** which could be separated by column chromatography. Introduction of an acetyl ester also facilitated identification of the regioisomers; the ^{1}H NMR spectra of both **11** and **12** exhibited a deshielded doublet of doublets corresponding to methines geminal to acetates, the positions of which were identified by 2D COSY experiments. The 3′-*O*-acetyl derivative **11** was treated with methanolic ammonia to give the required glycosyl acceptor **13**.

With **13** in hand, attention turned to coupling with an appropriate activated derivative of 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranose[11] (**14**). Phosphite glycosylation methodology was selected for three reasons. First, use of the trichloroacetimidate derivative of **14** had proved disappointing with model adenosine acceptors;[30] second, phosphites tend to give good selectivity for α-anomeric products[35,36] and third, the 3′-hydroxy of adenosine is relatively unreactive and a phosphite donor had been employed successfully to glycosylate an acid-sensitive, unreactive alcohol in a previous example.[37] Reaction of **14** with dimethoxy(diethylamino)-phosphine[36] and 1*H*-tetrazole smoothly gave dimethyl phosphites **15**, confirmed by ^{1}H and ^{31}P NMR spectroscopy to be a 1 : 1 anomeric mixture. Using a carefully controlled ratio and quantity of silver perchlorate–zinc chloride as promoter, alcohol **13** was glycosylated with **15** to give **16** in 53% yield. The ^{1}H NMR spectrum of **16** displayed a doublet at 5.2 ppm (*J* 3.4 Hz) corresponding to H-1″ and thereby confirming preparation of the α-glucopyranosyl anomer; none of the corresponding β-anomer was detected.

Deprotection of the three *p*-methoxybenzyl ethers and the *N*-dimethoxytrityl group of **16** was achieved in one step with 10% TFA in dichloromethane to give triol **17** in 81% yield. Note the short reaction time (1.75 h) compared to the 41 h required by a DDQ-mediated protection of a 2′-*O*-*p*-methoxybenzyl ether in the synthesis of **2** by Hotoda *et al.*[19] Triol **17** was phosphitylated using bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate,[38] a method which obviates the need for base protection. The intermediate trisphosphite triester was oxidised with MCPBA, then quenched at −78 °C to avoid possible oxidation of the adenine base. The ^{31}P NMR spectrum of the product **18** confirmed the presence of three phosphate triesters, while the presence of the free unphosphorylated amino group was substantiated by the presence of a broad singlet in the ^{1}H NMR spectrum at 6.1 ppm.

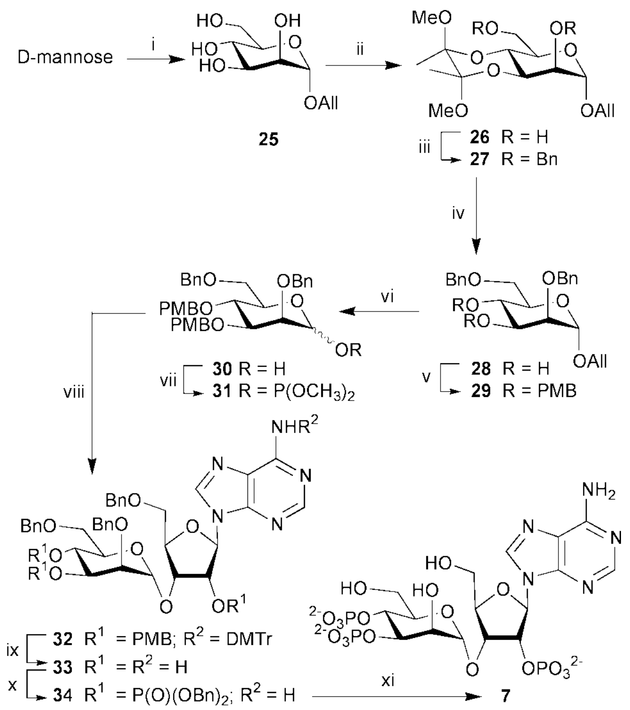
Attempts to deprotect **18** with sodium in liquid ammonia, or catalytic hydrogenation over palladium black or palladium on carbon were unsuccessful. Catalytic hydrogenation over 20% palladium hydroxide allowed isolation of the target compound **2**, but in poor yield, and after the rather long reaction time of 5 days. However, complete deprotection of **18** in high yield was readily achieved by catalytic transfer hydrogenation.[39] The crude product was purified by ion exchange chromatography, being isolated first as the free acid before being converted into the sodium salt. Both the ^{1}H and ^{31}P NMR spectra and the negative ion FAB mass spectrum of **2** were in full agreement with those reported previously for both natural[3,4] and synthetic[19,20] adenophostin A. Synthetic **2** had the same retention time as a sample of natural adenophostin A in analytical reverse phase HPLC and eluted as a single peak (using an ODS column and eluting with a gradient of 10–30% acetonitrile and 0.05 mol dm^{-3} phosphate buffer, containing 0.1% w/v of tetrabutylammonium hydrogensulfate).

The successful glycosylation, phosphorylation and deprotection strategies developed in the synthesis of **2** were now adapted to prepare the other targets **5** and **7**. 2-*O*-Benzyl-3,4-*O*-di-*O*-*p*-methoxybenzyl-D-xylopyranose[22] (**19**, Scheme 2) was phos-



Scheme 2 *Reagents and conditions*: i) $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 20 min; ii) (*a*) **13**, 4 Å sieves, dioxane–toluene (3 : 1), room temp., 2 h; (*b*) $ZnCl_2$, $AgClO_4$, dark, 9 h (46%); iii) TFA, CH_2Cl_2, room temp., 1.75 h (81%); iv) (*a*) $(BnO)_2PNPr^i_2$, imidazolium triflate, CH_2Cl_2, room temp., 1.5 h; (*b*) MCPBA, −78 °C, 10 min (68%); v) wet $Pd(OH)_2$/C, MeOH–cyclohexene–H_2O (11 : 5 : 1), reflux, 2.5 h (85%). Bn = benzyl, DMTr = dimethoxytrityl, PMB = *p*-methoxybenzyl.

phitylated in a similar fashion to **14**, giving glycosyl donor **20** as a 2 : 3 α : β anomeric mixture as indicated by ^{1}H and ^{31}P NMR spectroscopy. Glycosylation of **13** with **20** gave an inseparable 1 : 1 anomeric mixture (**21ab**) as judged by integral ratios of the H-1″ anomeric protons in the ^{1}H NMR spectrum. Treatment of this mixture with 10% TFA in dichloromethane gave the required α-coupled triol **22** and its β-anomer **23**, which were readily separated by column chromatography. The anomeric configurations of **22** and **23** were easily distinguished using ^{1}H NMR spectroscopy by comparing the coupling constants of the H-1″ protons: that of **22** resonated at 5.17 ppm with a relatively small axial–equatorial coupling constant of 3.8 Hz; that of **23** resonated at 4.46 ppm with a larger axial–axial coupling constant of 7.9 Hz. Compound **22** was phosphitylated and oxidised to give **24** without prior protection of position N^6, as described for **17**, and was smoothly deprotected similarly to **18** to give **5**. The purity of **5** (*xylo*-adenophostin) was confirmed by analytical HPLC under similar conditions to those described for **2**.



Scheme 3 *Reagents and conditions*: i) AllOH, HCl, 50–60 °C, 5 h (α-anomer by crystallisation, 61%); ii) butanedione, $(MeO)_3CH$, CSA, MeOH, reflux, 10 h (78%); iii) NaH, BnBr, DMF, 0 °C to room temp., 14 h (88%); iv) TFA, H_2O, CH_2Cl_2, 15 min (85%); v) NaH, PMBCl, DMF, room temp., 17 h (66%); vi) $PdCl_2$, MeOH, room temp., 3 h (79%); vii) $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 20 min; viii) (*a*) **13**, 4 Å sieves, dioxane–toluene (3 : 1), room temp., 2 h; (*b*) $ZnCl_2$, $AgClO_4$, dark, 8 h (61%); ix) TFA, CH_2Cl_2, room temp., 5 h (82%); x) (*a*) $(BnO)_2PNPr^i_2$, imidazolium triflate, CH_2Cl_2, room temp., 1 h; (*b*) MCPBA, −78 °C, 10 min (56%); xi) wet $Pd(OH)_2/C$, MeOH–cyclohexene–H_2O (10 : 5 : 1), reflux, 2.5 h (71%). All = allyl, Bn = benzyl, DMTr = dimethoxytrityl, PMB = *p*-methoxybenzyl.

The selectively protected intermediate (**30**, Scheme 3), required for the synthesis of **7** was not known, and a route to it was developed from allyl α-D-mannopyranoside (**25**). Although **25** was first described as a crystalline solid (mp 98–99 °C, $[a]_D$ +99, no spectroscopic data given),[40] a later report gave significantly different physical properties (mp 138–139 °C, $[a]_D$ +51.6)[41]. In other cases, crystalline **25** was not isolated.[42] In our hands, the successful preparation of **25** was found to require a careful choice of conditions. Various combinations of temperature, amount and type of acid catalyst and work-up were tried before procedures were established that gave crystalline **25** in good yield and uncontaminated by unreacted hexose, β-anomer or other by-products. In all cases the material resisted crystallisation until it had been further purified by flash chromatography on a short column of silica, and a final recrystallisation was still required to remove traces of β-anomer. In the present case, this was necessary because it was found that the presence of any impurities made purification after the next step difficult.

Selective protection of the two equatorial hydroxy groups at positions 3 and 4 in **25** was readily accomplished using the butane diacetal (BDA) protecting group.[43] Initially this was achieved by acid-catalysed reaction of **25** with 2,2,3,3-tetramethoxybutane (TMB) in methanol according to the original procedure reported by Montchamp *et al.*[43] for methyl α-mannopyranoside. However, it was later found that identical results could be achieved more conveniently by using the simplified method of Hense *et al.*,[44] which employs commercially available butanedione in place of TMB. After 1 hour, two major products were present in the reaction mixture, but after 10 hours, only the 3,4-diacetal **26** was detected by TLC. Longer reaction times led to the gradual accumulation of methyl mannopyranosides with consequent reduction in the yield of **26**. The use of $BF_3{\cdot}Et_2O$ as catalyst and lower temperatures[44] in place of CSA and reflux was not successful, at least on this scale, with equilibration being incomplete after 3 days at room temperature. Benzylation of **26** with benzyl bromide and sodium hydride in DMF gave the fully protected compound **27**.

At this stage, one strategy would be to remove the allyl group from **27** and convert the product into a glycosyl donor with the BDA protection still in place. However, it has been shown that BDA protection of positions 3 and 4 in mannose has a deactivating effect on thioglycoside glycosyl donors,[45] and it was felt that, in the present case, similar effects might lead to difficulties at the critical coupling reaction with **13**. Accordingly, the BDA group was removed from **27** using TFA, giving the 3,4-diol **28**, which was then alkylated with *p*-methoxybenzyl chloride and sodium hydride to give **29**. The allyl protection at the anomeric position of **29** was selectively cleaved using a catalytic amount of $PdCl_2$ in methanol at room temperature.[46] The reaction mixture became increasingly acidic as the reaction progressed, but it was found that this method was compatible with moderately acid-labile protecting groups (BDA or *p*-methoxybenzyl), providing that the acid was neutralised before work-up. Mannopyranose **30** was obtained as a 3 : 1 mixture of α- and β-anomers as judged by ^1H NMR spectroscopy. Phosphitylation of **30** with 1*H*-tetrazole and dimethoxy(diethylamino)phosphine in dichloromethane as for **14** and **19** then gave glycosyl donor **31**. The ^{31}P and ^1H NMR spectra of **31** indicated that the α- and β-anomers were present in a ratio of approximately 10 : 1.

Glycosylation of **13** with **31** proceeded smoothly, as expected, to give exclusively the α-coupled product **32**. Cleavage of the acid-labile protecting groups in **32** was achieved with 10% TFA in dichloromethane. The reaction was significantly slower than the equivalent deprotection of **16** and of **21ab**, but was complete after 5 hours. Monitoring of the reaction by TLC indicated that cleavage of the dimethoxytrityl group took place rapidly, suggesting that one of the *p*-methoxybenzyl groups in **32** was refractory to the reaction conditions. The triol **33** was isolated in good yield, indicating that extended exposure to the acidic reaction conditions had not been detrimental to the glycosidic or nucleosidic linkages. Phosphitylation of **33** and oxidation of phosphites as described for **17** and **22** furnished the fully protected trisphosphate **34**. However, deprotection of **34** by catalytic transfer hydrogenation for 10 hours as described for **18** and **24** gave a product contaminated by a minor impurity, clearly apparent in the ^{31}P and ^1H NMR spectra. It was thought that this impurity may have arisen from intramolecular phosphate migration, which would be favoured by the *cis*-relationship of O-2 and O-3 on the mannopyranosyl ring. The reaction was therefore repeated for a shorter time (2.5 hours) and pure **7** was isolated after ion exchange chromatography followed by conversion into the sodium salt. The purity of **7** (*manno*-adenophostin) was confirmed by analytical HPLC as described for **2** and **5**.

Synthetic adenophostin A (**2**) was shown to be equipotent with naturally occurring adenophostin A in evoking Ca^{2+} release from the intracellular stores of permeabilised cells.[21] Full biological evaluation of **2**, **5** and **7** will be reported elsewhere. The synthesis of these three related glyconucleotides by a convergent approach employing the versatile glycosyl acceptor **13** demonstrates the value of this strategy, not only as an efficient route to adenophostin A but also to the first adenophostin analogues with modified pyranosyl rings. Because the glucopyranosyl ring of the adenophostins is analogous to the inositol ring of $Ins(1,4,5)P_3$, this approach may allow the design of novel glyconucleotide counterparts to conventional inositol-based polyphosphates, potentially with enhanced affinities for $Ins(1,4,5)P_3$ receptors, for intervention in the polyphosphoinositide pathway of cellular signalling.

Experimental

Materials and methods

Chemicals were purchased from Aldrich, Fluka, Lancaster and Sigma chemical companies. Dichloromethane was dried over calcium hydride, distilled and stored over 4 Å molecular sieves. *N*,*N*-Dimethylformamide (DMF) was dried over barium oxide, distilled under reduced pressure and stored over 4 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled and stored over potassium hydroxide pellets. Toluene, dioxane and triethylamine were purchased in anhydrous form.

TLC was performed on precoated plates (Merck aluminium sheets silica 60 F_{254}, Art. No. 5554). Products were visualised by UV light (254 nm) or by dipping in a solution of phosphomolybdic acid in methanol followed by heating. Flash chromatography was carried out using Merck-Kieselgel 60 (0.040–0.063 mm) under pressure.

^{1}H and ^{13}C NMR spectra were recorded on either JEOL GX270 or EX 400 or Varian Mercury 400 spectrometers. Chemical shifts were measured in ppm relative to internal tetramethylsilane or HDO. ^{31}P NMR spectra were recorded on JEOL GX270 or EX400 spectrometers and ^{31}P NMR chemical shifts were measured in ppm and denoted positive downfield from external 85% H_3PO_4. *J* values are given in Hz. Proton assignments were established with 2D COSY experiments, and the number of protons attached to carbon atoms was established by DEPT experiments. Mps (uncorrected) were determined using a Reichert-Jung Thermo Galen Kofler block. Microanalysis was carried out at the University of Bath Microanalysis Service. Low resolution mass spectra were recorded at the University of Bath Mass Spectrometry Service using +ve and −ve fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol (NBA) as the matrix. High resolution accurate mass spectra were recorded at the University of Bath Mass Spectrometry Service. Optical rotations were measured at ambient temperature using an Optical Activity Ltd. AA-10 polarimeter in a cell volume of 1 cm^3 or 5 cm^3, and $[a]_D$ 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 either Q Sepharose Fast Flow resin and gradients of triethylammonium bicarbonate as eluent, or MP1 AG ion exchange resin and a gradient of 150 mM aqueous TFA as eluent. Synthetic phosphates were quantified by total phosphate assay.[47]

2′,3′-*O*-*p*-Methoxybenzylideneadenosine (8ab)

Method A. A suspension of zinc chloride (70 g, 0.50 mol) in *p*-methoxybenzaldehyde (350 cm^3) was stirred for 30 min under N_2, whereupon adenosine (25.0 g, 0.09 mol) was added and the mixture was stirred for 18 h. The resulting viscous cream-coloured suspension was divided into two. Each half was poured into 400 cm^3 of chilled water and this mixture was extracted with chloroform (400 cm^3, 300 cm^3, 200 cm^3). The combined organic extracts were washed with water (300 cm^3), dried ($MgSO_4$), filtered and concentrated. The suspensions from each half, containing product and *p*-methoxybenzaldehyde, were recombined and refrigerated at 4 °C overnight. Precipitated product was recovered by filtration (13.8 g, *endo* isomer by ^{1}H NMR), and remaining product left in the filtrate was precipitated by addition of diisopropyl ether (300 cm^3) and refrigeration (10.0 g mixture of *endo* and *exo* isomers, 2:1 *endo*:*exo*, as indicated by ^{1}H NMR integral ratio of *p*-methoxybenzylidene C*H*). Combined yield (23.80 g, 66%); R_f 0.26 (ethyl acetate–ethanol, 9:1).

Data for *endo* diastereoisomer **8a**: mp 215–217 °C (from ethanol) (Found: C, 55.9; H, 4.9; N, 18.1. Calcd for $C_{18}H_{19}N_5O_5$: C, 56.1; H, 5.0; N, 18.2%); δ_H (d$_6$-DMSO; 400 MHz) 3.63–3.65 (2 H, m, simplifies on D_2O exch., 5′-H$_A$, 5′-H$_B$), 3.77 (3 H, s, OCH_3), 4.35–4.39 (1 H, m, 4′-H), 5.06 (1 H, dd, *J* 2.2, 6.6, 3′-H), 5.28 (1 H, t, *J* 5.4, D_2O exch., 5′-OH), 5.47 (1 H, dd, *J* 2.9, 6.3, 2′-H), 5.96 (1 H, s, *p*-methoxybenzylidene C*H*), 6.30 (1 H, d, *J* 2.9, 1′-H), 6.99 (2 H, m, *meta*-H of *p*-methoxybenzylidene ring), 7.38 (2 H, s, D_2O exch., NH_2), 7.50 (2 H, m, *ortho*-H of *p*-methoxybenzylidene ring) and 8.17 and 8.38 (2 H, 2 s, 2-H and 8-H); δ_C (d$_6$-DMSO; 100 MHz) 55.83 (OCH_3), 62.05 (C-5′), 83.12 (C-3′), 84.32 (C-2′), 86.79 (C-4′), 90.30 (C-1′), 107.29 (*p*-methoxybenzylidene *C*H), 114.44 (*meta*-C of *p*-methoxybenzylidene ring), 119.38 (C-5), 128.44 (*ipso*-C of *p*-methoxybenzylidene ring), 129.24 (*ortho*-C of *p*-methoxybenzylidene ring), 140.67 (C-8), 149.34 (C-4), 153.36 (C-2), 156.36 (C-6) and 161.02 (*para*-C of *p*-methoxybenzylidene ring); *m*/*z* (FAB$^+$) 386 [(M + 1)$^+$, 100%].

Method B. A mixture of adenosine (20.0 g, 0.07 mol), *p*-methoxybenzaldehyde (400 cm^3), triethyl orthoformate (100 cm^3) and dry PTSA (40.3 g, 0.26 mol) was stirred at 35–40 °C for 4 h under N_2, whereupon the resulting purple solution was poured into 0.3 mol dm^{-3} aqueous $NaHCO_3$ solution (1000 cm^3) and stirred vigorously for 15 min. Ethyl acetate (300 cm^3) was added and the resulting organic layer was set aside. The aqueous layer was extracted with more ethyl acetate (300 cm^3) and the combined organic layers were washed with saturated aq. NaCl (200 cm^3), dried ($MgSO_4$), filtered and concentrated. Diisopropyl ether (400 cm^3) was added and the suspension was refrigerated at 4 °C for 48 h. The product was isolated as a white powder (26.0 g as a mixture of *endo* and *exo* isomers, 2:3 *endo*:*exo*, by ^{1}H NMR, 90%).

Data for mixture of diastereoisomers **8ab**: δ_H (d$_6$-DMSO; 400 MHz) 3.57–3.62 (2 H, m, 5′-H$_A$, 5′-H$_B$), 3.77 (1.8 H, s, OCH_{3exo}), 3.79 (1.2 H, s, OCH_{3endo}), 4.26–4.31 (0.6 H, m, 4′-H$_{exo}$), 4.37–4.40 (0.4 H, m, 4′-H$_{endo}$), 5.05–5.10 (1 H, m, 3′-H), 5.16 (0.6 H, t, *J* 5.6, D_2O exch., 5′-OH$_{exo}$), 5.29 (0.4 H, t, *J* 5.6, D_2O exch., 5′-OH$_{endo}$), 5.45–5.49 (1 H, m, 2′-H), 5.97 (0.4 H, s, *p*-methoxybenzylidene C*H*$_{endo}$), 6.19 (0.6 H, s, *p*-methoxybenzylidene C*H*$_{exo}$), 6.28 (0.6 H, d, *J* 2.9, 1′-H$_{exo}$), 6.30 (0.4 H, d, *J* 2.9, 1′-H$_{endo}$), 6.95–7.02 (2 H, m, *meta*-H of *p*-methoxybenzylidene ring), 7.39–7.52 (4 H, m, simplifies to 2 H, m on D_2O exch., *ortho*-H of *p*-methoxybenzylidene ring, NH_2) and 8.18 and 8.39 (2 H, 2 s, 2-H, 8-H); δ_C (d$_6$-DMSO; 100 MHz) 55.21 (OCH_3), 61.57 (C-5′), 80.47, 82.81, 84.45 (C-4′, C-3′, C-2′ all *exo*), 82.59, 83.67, 86.30 (C-4′, C-3′, C-2′ all *endo*), 87.98 (C-1′$_{exo}$), 89.55 (C-1′$_{endo}$), 102.87 (*p*-methoxybenzylidene *C*H$_{exo}$), 106.56 (*p*-methoxybenzylidene *C*H$_{endo}$), 113.66 (*ortho*-C of *p*-methoxybenzylidene ring$_{exo}$), 113.79 (*ortho*-C of *p*-methoxybenzylidene ring$_{endo}$), 119.14 (C-5), 128.07 (*ipso*-C of *p*-methoxybenzylidene ring), 128.51, (*meta*-C of *p*-methoxybenzylidene ring), 139.72 (C-8$_{exo}$), 139.88 (C-8$_{endo}$), 148.81 (C-4), 152.68 (C-2), 156.18 (C-6), 160.26 (*para*-C of *p*-methoxybenzylidene ring$_{exo}$) and 160.38 (*para*-C of *p*-methoxybenzylidene ring$_{endo}$).

N^6-Dimethoxytrityl-2′,3′-*O*-*p*-methoxybenzylideneadenosine (9ab)

To a suspension of **8ab** (3.83 g, 8.78 mmol, concentrated from 3 × 20 cm^3 dry pyridine) in dry pyridine (50 cm^3) under N_2 was added chlorotrimethylsilane (2.79 cm^3, 22.0 mmol), whereupon the starting material dissolved. The solution was stirred for 2 h, dimethoxytrityl chloride (7.83 g, 22.0 mmol) was added and the resultant orange mixture was stirred overnight. The mixture was cooled to 0 °C and the reaction was quenched by addition of water (5 cm^3). The cooling bath was removed and after 10 min concentrated aq. NH_3 (20 cm^3) was added. The solution was stirred for a further 30 min, then was partitioned between 5% (w/v) aq. $NaHCO_3$ (150 cm^3) and CH_2Cl_2 (150 cm^3). The aqueous layer was back-extracted with CH_2Cl_2 (2 × 50 cm^3) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The dark orange oil thus obtained was subjected to flash chromatography (eluent ethyl acetate–hexane, 3:2) to give the *title compound* as a pale cream foam (6.60 g, 97%); R_f 0.29 (ethyl acetate–hexane, 3 : 2).

Data for *endo* diastereoisomer **9a**: (Found: M^+, 687.267. Calcd for $C_{39}H_{37}N_5O_7$ M^+: 687.269); δ_H (d_6-DMSO; 400 MHz) 3.49–3.61 (2 H, m, 5′-H_A, 5′-H_B), 3.71 (6 H, s, 2 × OCH_3 of DMTr), 3.78 (3 H, s, OCH_3 of *p*-methoxybenzylidene), 4.35–4.39 (1 H, m, 4′-H), 5.03 (1 H, dd, *J* 2.3, 6.5, 3′-H), 5.22 (1 H, t, *J* 6.6, D_2O exch., 5′-OH), 5.48 (1 H, dd, *J* 2.8, 6.5, 2′-H), 5.95 (1 H, s, *p*-methoxybenzylidene C*H*), 6.30 (1 H, d, *J* 2.8, 1′-H), 6.82–6.86 (4 H, m, ArCH), 6.98–7.01 (2 H, m, ArCH), 7.19–7.29 (9 H, m, ArCH), 7.48 (1 H, br s, D_2O exch., NH), 7.51–7.96 (2 H, m, ArCH) and 8.32 and 8.48 (2 H, 2 s, 2-H, 8-H); δ_C (d_6-DMSO; 100 MHz) 55.36 (2 × OCH_3), 55.58 (OCH_3), 61.76 (C-5′), 69.93 (DMTr Cq), 82.84, 84.01, 86.70 (C-4′, C-3′, C-2′), 90.01 (C-1′), 106.95 (*p*-methoxybenzylidene *C*H), 113.35, 114.15 (*meta*-C of *p*-methoxyphenyl rings), 120.97 (C-5), 126.86, 128.07, 128.34, 128.71, 128.91, 130.12 (ArCH, *ipso*-C of *p*-methoxybenzylidene ring), 137.52 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 140.85 (C-8), 145.57 (*ipso*-C of DMTr phenyl ring), 148.31 (C-4), 151.79 (C-2), 153.97 (C-6), 158.04 (*para*-C of DMTr *p*-methoxyphenyl rings) and 160.75 (*para*-C of *p*-methoxybenzylidene ring); *m*/*z* (FAB^+) 688 [$(M + H)^+$, 26%] and 303 (100).

Data for mixture of diastereoisomers **9ab**, *endo* : *exo* 2 : 3: δ_H ($CDCl_3$; 270 MHz) 3.77–3.84 (1 H, m, 5′-H_{Aexo}, 5′-H_{Aendo}), 3.77 (6 H, s, 2 × OCH_3 of DMTr), 3.80 (3 H, s, OCH_3 of *p*-methoxybenzylidene), 3.94–4.00 (1 H, m, 5′-H_B), 4.53 (0.4 H, m, 4′-H_{exo}), 4.68 (0.6 H, m, 4′-H_{endo}), 5.16–5.18 (1 H, m, 3′-H), 5.26–5.35 (1 H, m, 2′-H), 5.98 (0.4 H, d, *J* 4.95, 1′-H_{endo}), 6.00–6.02 (1 H, m, *p*-methoxybenzylidene CH_{endo}, 1′-H_{exo}), 6.25 (0.6 H, s, *p*-methoxybenzylidene CH_{exo}), 6.77–7.02 (7 H, m, ArCH), 7.21–7.50 (10 H, m, ArCH), 7.76, 7.83 (1 H, 2 s, 2-H or 8-H) and 8.01 and 8.02 (1 H, 2 s, 2-H or 8-H); δ_C ($CDCl_3$; 100 MHz) 55.59 (OCH_3), 63.41 (C-5′$_{exo}$), 63.72 (C-5$_{endo}$), 71.12 (DMTr Cq), 80.52, 84.21, 86.43 (C-2′, C-3′, C-4′ all *endo*), 83.07, 84.01, 85.91 (C-2′, C-3′, C-4′ all *exo*), 92.26 (C-1′$_{exo}$), 94.47 (C-1′$_{endo}$), 104.93 (*p*-methoxybenzylidene *C*H_{exo}), 107.85 (*p*-methoxybenzylidene *C*H_{endo}), 113.41, 114.15 (*meta*-C of *p*-methoxyphenyl rings), 122.43 (C-5$_{exo}$), 122.56 (C-5$_{endo}$), 127.12, 127.97, 128.14, 128.28, 128.83, 128.93, 130.27 (ArCH, and *ipso*-C of *p*-methoxybenzylidene ring), 137.30 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 139.49, (C-8$_{exo}$), 139.81 (C-8$_{endo}$), 145.28 (*ipso*-C of DMTr phenyl ring), 147.31 (C-4$_{endo}$), 147.45 (C-4$_{exo}$), 152.02 (C-2$_{endo}$), 152.20 (C-2$_{exo}$), 154.73 (C-6$_{exo}$), 154.76 (C-6$_{endo}$), 158.46 (*para*-C of DMTr *p*-methoxyphenyl rings), 160.79 (*para*-C_{endo} of *p*-methoxybenzylidene ring) and 161.00 (*para*-C_{exo} of *p*-methoxybenzylidene ring).

5′-*O*-Benzyl-N^6-dimethoxytrityl-2′,3′-*O*-*p*-methoxybenzylidene-adenosine (10ab)

A solution of **9ab** (6.06 g, 8.83 mmol) in benzene (85 cm^3) and dioxane (41 cm^3) under N_2 was sequentially treated with KOH powder (85%, 14.6 g, 221 mmol) and benzyl chloride (3.05 cm^3, 26.5 mmol) and the resultant mixture was heated under reflux for 20 min, then was cooled and partitioned between diethyl ether (200 cm^3) and iced water (150 cm^3). The ethereal layer was washed with water (2 × 75 cm^3), and the combined aqueous layers were extracted with ether (100 cm^3). The combined ethereal layers were dried ($MgSO_4$), filtered and concentrated to give a yellow oil which was subjected to flash chromatography (eluent ethyl acetate–hexane, 1 : 1) to yield the *title compound* as a pale cream foam (6.53 g, 95%).

Data for *endo* diastereoisomer **10a**: (Found: C, 70.8; H, 5.5; N, 8.85. Calcd for $C_{46}H_{43}N_5O_7$: C, 71.0; H, 5.6; N, 9.0%); δ_H ($CDCl_3$; 400 MHz) 3.68–3.72 (2 H, m, 5′-H_A, 5′-H_B), 3.77 (6 H, s, 2 × OCH_3 of DMTr), 3.82 (3 H, s, OCH_3 of *p*-methoxybenzylidene), 4.46, 4.49 (2 H, AB, J_{AB} 12.2, OCH_2Ar), 4.65 (1 H, q, *J* 2.4, 4′-H), 5.04 (1 H, dd, *J* 2.4, 6.4, 3′-H), 5.51 (1 H, dd, *J* 2.2, 6.6, 2′-H), 5.95 (1 H, s, *p*-methoxybenzylidene C*H*), 6.27 (1 H, d, *J* 2.5, 1′-H), 6.78–6.94 (7 H, m, ArCH), 7.20–7.48 (15 H, m, ArCH) and 7.96 and 8.05 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100.4 MHz) 55.21 (2 × OCH_3), 55.34 (OCH_3), 70.10 (C-5′), 70.64 (DMTr Cq), 73.45 (O*C*H_2Ar), 82.79, 84.86, 85.57 (C-2′, C-3′, C-4′), 90.89 (C-1′), 107.66, (*p*-methoxybenzylidene *C*H), 113.13, 113.89 (*meta*-C of *p*-methoxyphenyl rings), 121.32 (C-5), 126.81, 127.72, 127.78, 127.89, 128.14, 128.31, 128.47, 128.78, 130.10 (ArCH, *ipso*-C of *p*-methoxybenzylidene ring), 137.31 (*ipso*-C of Bn ring), 137.45 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 138.81 (C-8), 145.43 (*ipso*-C of DMTr phenyl ring), 148.39 (C-4), 152.47 (C-2), 154.15 (C-6), 158.26 (*para*-C of DMTr *p*-methoxyphenyl rings) and 160.88 (*para*-C of *p*-methoxybenzylidene ring); *m*/*z* (FAB^+) 688 [$(M + H)^+$, 26%] and 303 (100).

Data for mixture of diastereoisomers **10ab**, *endo* : *exo* 2 : 3: δ_H ($CDCl_3$; 400 MHz) 3.67 (0.4 H, 0.5 ABX, $^2J_{AB}$ 13.9, $^3J_{AX}$ 4.2, 5′-H_{Aendo}), 3.68–3.72 (1.6 H, m, 5′-H_{Aexo}, 5′-H_B), 3.77, 3.81 and 3.82 (6 H, s, 2 × OCH_3 of DMTr), 3.81 and 3.82 (3 H, 2 s, OCH_3 of *p*-methoxybenzylidene), 4.46 (0.4 H, AB, J_{AB} 12.2, OC*H*HAr$_{endo}$), 4.48–4.54 (2.2 H, m, OCH_2Ar_{exo}, OCH*H*Ar$_{endo}$, 4′-H_{exo}), 4.65 (0.4 H, q, *J* 2.4, 4′-H_{endo}), 5.04 (0.4 H, dd, *J* 2.4, 6.4, 3′-H_{endo}), 5.13 (0.6 H, dd, *J* 3.7, 6.1, 3′-H_{exo}), 5.45 (0.6 H, dd, *J* 2.9, 6.4, 2′-H_{exo}), 5.51 (0.4 H, dd, *J* 2.2, 6.6, 2′-H_{endo}), 5.95 (0.4 H, s, *p*-methoxybenzylidene CH_{endo}), 6.13 (0.6 H, s, *p*-methoxybenzylidene CH_{exo}), 6.21 (0.6 H, d, *J* 2.4, 1′-H_{exo}), 6.27 (0.4 H, d, *J* 2.5, 1′-H_{endo}), 6.78–6.94 (7 H, m, ArCH), 7.20–7.48 (15 H, m, ArCH), 7.03 and 7.95 (1 H, 2 s, 2-H or 8-H) and 7.96 and 8.05 (1 H, 2 s, 2-H or 8-H); δ_C ($CDCl_3$; 100.4 MHz) 55.21 (2 × OCH_3), 55.34 (OCH_3), 70.10 (C-5′), 70.64 (DMTr Cq), 73.45 (O*C*H_2Ar), 81.33, 84.00 and 84.27 (C-2′, C-3′, C-4′ all *exo*), 82.79, 84.86, 85.57 (C-2′, C-3′, C-4′ all *endo*), 89.98 (C-1′$_{exo}$), 90.89 (C-1′$_{endo}$), 104.26 (*p*-methoxybenzylidene *C*H_{exo}), 107.66, (*p*-methoxybenzylidene *C*H_{endo}), 113.13, 113.89 (*meta*-C of *p*-methoxyphenyl rings), 121.32 (C-5), 126.81, 127.72, 127.78, 127.89, 128.14, 128.31, 128.47, 128.78 and 130.10 (ArCH, and *ipso*-C of *p*-methoxybenzylidene ring), 137.31 (*ipso*-C of Bn ring), 137.45 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 138.81 (C-8), 145.43 (*ipso*-C of DMTr phenyl ring), 148.39 (C-4), 152.47 (C-2), 154.15 (C-6), 158.26 (*ipso*-C of DMTr *p*-methoxyphenyl rings) and 160.88 (*para*-C of *p*-methoxybenzylidene ring).

3′-*O*-Acetyl-5′-*O*-benzyl-N^6-dimethoxytrityl-2′-*O*-*p*-methoxybenzyladenosine (11) and 2′-*O*-acetyl-5′-*O*-benzyl-N^6-dimethoxytrityl-3′-*O*-*p*-methoxybenzyladenosine (12)

To a solution of **10ab** (1.00 g, 1.29 mmol) in CH_2Cl_2 at −78 °C under N_2 was added a solution of DIBAL-H in CH_2Cl_2 (1 mol dm^{-3}; 6.5 cm^3, 6.50 mmol) dropwise. The reaction mixture was allowed to warm slowly to −25 °C over 2 h, after which time TLC (ethyl acetate–pentane, 1 : 1) indicated conversion into two products (R_f 0.51 and 0.39). The reaction mixture was cooled to −78 °C and quenched by addition of ethyl acetate (5 cm^3). Diethyl ether (100 cm^3) was added and the cooling bath was removed. Ice-cold NaOH solution (1 mol dm^{-3}) was added until there were two clear layers; the resulting aqueous layer was discarded and the organic layer was washed with water (75 cm^3), dried ($MgSO_4$), filtered and concentrated to a yellow oil which was dissolved in pyridine (10 cm^3) and acetic anhydride (5 cm^3) and stirred overnight. The reaction mixture was concentrated, and then concentrated repeatedly from toluene. The residue was subjected to flash chromatography (eluent ethyl acetate–hexane, 2 : 3, then 1 : 1, then 3 : 2). Initial fractions contained pure **11**, while further fractions contained a mixture of the two regioisomers. The material in these fractions was repeatedly re-chromatographed to completely separate the two regioisomers. Yield of **11** (0.58 g, 55% from **10ab**); R_f 0.35 ($CHCl_3$–acetone, 19 : 1) (Found: C, 70.0; H, 5.8; N, 8.5. Calcd for $C_{48}H_{47}N_5O_8$: C, 70.1; H, 5.8; N, 8.5%); δ_H ($CDCl_3$; 400

MHz) 2.14 (3 H, s, CH_3CO), 3.67 (1 H, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.1, 5′-H_A), 3.74–3.79 (1 H, m, 5′-H_B, obscured by OCH_3), 3.75 (3 H, s, OCH_3), 3.76 (6 H, s, 2 × OCH_3), 4.34–4.39 (2 H, AB, J_{AB} 12.0, OC*H*HAr, overlapping with 4′-H), 4.50 (1 H, AB, J_{AB} 12.0, OCH*H*Ar), 4.54 and 4.58 (2 H, AB, J_{AB} 11.7, OCH_2Ar), 4.68 (1 H, dd, *J* 5.3, 6.4, 2′-H), 5.43 (1 H, dd, *J* 2.8, 5.1, 3′-H), 6.12 (1 H, d, *J* 6.7, 1′-H), 6.66–6.69 (2 H, m, ArCH), 6.79–6.83 (4 H, m, ArCH), 6.88 (1 H, br s, D_2O exch., NH), 6.95–6.99 (2 H, m, ArCH), 7.22–7.38 (14 H, m, ArCH) and 7.77 and 8.06 (2 H, s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 21.36 (CH_3CO), 55.59 (3 × OCH_3), 69.96 (C-5′), 70.96 (DMTr Cq), 72.14, 79.01 and 82.44 (C-2′, C-3′, C-4′), 72.86 and 74.06 (2 × OCH_2Ar), 86.38 (C-1′), 113.36 and 113.95 (*meta*-C of *p*-methoxyphenyl rings), 121.20 (C-5), 127.00, 128.01, 128.08, 128.23, 128.82, 129.02, 129.73 and 130.32 (ArCH, *ipso*-C of PMB ring), 137.49 (*ipso*-C of Bn ring), 137.68 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 138.23 (C-8), 145.65 (*ipso*-C of DMTr phenyl ring), 149.05 (C-4), 152.60 (C-2), 154.19 (C-6), 158.38 (*para*-C of DMTr *p*-methoxyphenyl rings), 159.60 (*para*-C of PMB ring) and 170.39 (CH_3*C*O); *m*/*z* (FAB^+) 822 [(M + H)$^+$, 30%], 303 (100) and 91 (17).

Yield of **12** (0.39 g, 38% from **10ab**); R_f 0.30 ($CHCl_3$–acetone, 19:1); δ_H ($CDCl_3$; 400 MHz) 3.54 (1 H, 0.5 ABX, $^2J_{AB}$ 10.7, $^3J_{AX}$ 3.9, 5′-H_A), 3.77–3.79 (1 H, m, 5′-H_B, obscured by OCH_3), 3.77 (3 H, s, OCH_3), 3.78 (6 H, s, 2 × OCH_3), 4.22–4.24 (1 H, m, 4′-H), 4.34 (1 H, AB, J_{AB} 10.7, OC*H*HAr), 4.46–4.58 (4 H, m, 3 × OCH*H*Ar, 3′-H), 5.72 (1 H, dd, *J* 2.9, 4.9, 2′-H), 6.17 (1 H, d, *J* 2.9, 1′-H), 6.78–6.87 (9 H, m, simplifies to 8 H, m on D_2O exch., ArCH, NH), 7.17–7.35 (14 H, m, ArCH) and 8.05 and 8.07 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 20.81 (CH_3CO), 55.23 (3 × OCH_3), 68.40 (C-5′), 70.61 (DMTr Cq), 72.89 and 73.39 (2 × OCH_2Ar), 74.41, 75.33 and 81.55 (C-2′, C-3′, C-4′), 87.09 (C-1′), 113.15 and 113.84 (*meta*-C of *p*-methoxyphenyl rings), 126.81 (C-5), 127.72, 127.87, 128.51, 128.78, 129.30, 129.86 and 130.10 (ArCH, *ipso*-C of PMB ring), 137.43 (*ipso*-C of Bn ring), 137.53 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 138.57 (C-8), 145.11 (*ipso*-C of DMTr phenyl ring), 148.00 (C-4), 152.49 (C-2), 153.66 (C-6), 158.26 (*para*-C of DMTr *p*-methoxyphenyl rings), 159.66 (*para*-C of PMB ring) and 169.91 (CH_3*C*O).

5′-*O*-Benzyl-*N*^{6}-dimethoxytrityl-2′-*O*-*p*-methoxybenzyladenosine (13)

A solution of **11** (3.85 g, 4.69 mmol), in methanol (60 cm^3) chloroform (30 cm^3) and concentrated aq. NH_3 (15 cm^3) was stirred in a sealed flask for 48 h. The solvents were evaporated and the residue was repeatedly concentrated from chloroform and then subjected to flash chromatography (eluent ethyl acetate–hexane, 3:2) to yield the *title compound* as a white foam in quantitative yield; R_f 0.39 (ethyl acetate–hexane, 1:1) (Found: C, 70.6; H, 5.9; N, 8.9. Calcd for $C_{46}H_{45}N_5O_7$: C, 70.8; H, 5.8; N, 9.0%); δ_H ($CDCl_3$; 400 MHz) 3.66 (1 H, 0.5 ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 3.5, 5′-H_A), 3.77 (3 H, s, OCH_3), 3.78–3.82 (1 H, 0.5 ABX obscured by OCH_3, $^3J_{BX}$ 2.6, 5′-H_B), 3.78 (6 H, s, 2 × OCH_3), 4.18–4.21 (1 H, m, 4′-H), 4.34 (1 H, t, *J* 5.0, 3′-H), 4.47 (1 H, t, *J* 4.7, 2′-H), 4.56 and 4.60 (2 H, AB, J_{AB} 12.0, OCH_2Ar), 4.57 and 4.66 (2 H, AB, J_{AB} 11.7, OCH_2Ar), 6.16 (1 H, d, *J* 4.1, 1′-H), 6.71–6.84 (7 H, m, ArCH), 6.93 (1 H, br s, D_2O exch., NH), 7.11–7.38 (15 H, m, ArCH) and 7.97 and 8.07 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 55.59 (3 × OCH_3), 69.69 (C-5′), 70.33, 81.24 and 84.20 (C-2′, C-3′, C-4′), 70.96 (DMTr Cq), 72.97 and 73.90 (2 × OCH_2Ar), 87.03 (C-1′), 113.35 and 114.18 (*meta*-C of *p*-methoxyphenyl rings), 121.30 (C-5), 127.00, 127.94, 128.06, 128.13, 128.77, 128.82, 129.02 and 130.33 (ArCH and *ipso*-C of PMB ring), 137.67 (*ipso*-C of DMTr *p*-methoxyphenyl rings), 137.72 (*ipso*-C of Bn ring), 138.53 (C-8), 145.63 (*ipso*-C of DMTr phenyl ring), 148.62 (C-4), 152.49 (C-2), 154.19 (C-6), 158.38 (C-4 of DMTr *p*-methoxyphenyl rings) and 159.82 (*para*-C of PMB ring); *m*/*z* (FAB^+) 780 [(M + H)$^+$, 32%], 303 (100) and 91 (21).

2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-glucopyranosyl dimethyl phosphite (15)

To a mixture of glucopyranose **14** [11] (2.00 g, 3.33 mmol) and 1*H*-tetrazole (0.35 g, 5.00 mmol) in CH_2Cl_2 (20 cm^3) under N_2 was added dimethoxy(diethylamino)phosphine [36] (0.72 cm^3, 4.33 mmol). The mixture was stirred at room temperature for 20 min, when TLC (ethyl acetate–hexane, 1:3) indicated complete conversion into product (R_f 0.66). The reaction mixture was partitioned between diethyl ether (150 cm^3) and water (100 cm^3). The resulting ethereal layer was washed with saturated aq. NaCl (100 cm^3), dried ($MgSO_4$), filtered and concentrated to give a colourless, mobile oil which was shown by ^1H NMR spectroscopy to be a 1:1 anomeric mixture, and which was used for the next step without further purification; δ_H ($CDCl_3$; 400 MHz) 3.48–3.74 (11 H, m, 2 × $POCH_{3\alpha}$, 2 × $POCH_{3\beta}$, 2-H, 3-H, 5-H, 6-H_A, 6-H_B), 3.77, 3.78, 3.78 and 3.79 (6 H, 4 s, 4 × 1.5 OCH_3), 3.94–3.99 (1 H, m, 4-H), 3.94–3.99 (3 H, m, 3 × OCH*H*Ar), 4.70–4.91 (5 H, m, 5 × OCH*H*Ar), 4.94 (0.5 H, t, *J* 8.07, 1-H_β), 5.54 (0.5H, dd, *J* 3.18, $J_{H\text{-}P}$ 8.55, 1-H_α), 6.80–6.85 (4 H, m, 2 × *meta*-H of PMB rings), 7.05–7.12 (2 H, m, *ortho*-H of PMB rings) and 7.20–7.38 (12 H, m, ArCH); α and β subscripts denote signals arising from α- and β-anomers respectively; δ_P ($CDCl_3$; 36 MHz; ^1H decoupled) 141.14 ($OP_\beta(OCH_3)_2$) and 142.31 ($OP_\alpha(OCH_3)_2$).

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-2′,3″,4″-tri-*O*-*p*-methoxybenzyl-*N*^{6}-dimethoxytrityladenosine (16)

A mixture of dimethyl phosphite **15** (2.42 g, 3.50 mmol) and acceptor **13** (1.36 g, 1.75 mmol) in dioxane (18 cm^3) and toluene (6 cm^3) under N_2 was stirred with 4 Å molecular sieves (approx. 1.8 g) for 2 h, when dry zinc chloride (0.57 g, 4.20 mmol) and silver perchlorate (1.74 g, 8.40 mmol) were added. The flask was wrapped in foil to exclude light, and stirring was continued for 7 h. Solid $NaHCO_3$ (1.50 g) and water (60 cm^3) were added and the reaction mixture was diluted with ethyl acetate (80 cm^3) and was stirred for a further 30 min, then was filtered through a Celite pad, and the residue was well washed with ethyl acetate. Water (50 cm^3) was added to the filtrate and the resulting aqueous layer was discarded. The organic layer was washed with saturated aq. NaCl (70 cm^3), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate–hexane, 3:7, then 1:1) to yield the *title compound* as a colourless oil (1.26 g, 53%); R_f 0.33 (ethyl acetate–hexane, 2:3); $[\alpha]_D^{21}$ +10.0 (*c* 1.5, $CHCl_3$) (Found: C, 71.9; H, 6.2; N, 5.0. Calcd for $C_{82}H_{83}N_5O_{14}$: C, 72.3; H, 6.2; N, 5.1%); δ_H ($CDCl_3$; 400 MHz) 3.43–3.79 (7 H, m, 2″-H, 4″-H, 5′-H_A, 5′-H_B, 5″-H, 6″-H_A, 6″-H_B), 3.69, 3.77, 3.78 and 3.79 (15 H, 4 s, 5 × OCH_3), 3.96 (1 H, t, *J* 9.3, 3″-H), 4.34–4.76 (14 H, m, 9 × OCH*H*Ar, 2′-H, 3′-H, 4′-H), 4.87 (1 H, AB, J_{AB} 10.3, OCH*H*Ar), 5.22 (1 H, d, *J* 3.4, 1″-H), 6.21 (1 H, d, *J* 4.9, 1′-H), 6.65–6.67 (2 H, m, ArCH), 6.79–6.86 (10 H, m, ArCH), 7.01–7.05 (4 H, m, ArCH), 7.21–7.36 (24 H, m, ArCH), and 7.89 and 8.04 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 55.44, 55.49 (2 × OCH_3), 68.48 (C-6″), 69.52 (C-5′), 70.87 (DMTr Cq), 71.09 (C-5″), 72.36 and 72.50 (2 × OCH_2Ar), 73.01 (C-3′), 73.67, 73.75, 74.97 and 75.59 (4 × OCH_2Ar), 77.31 (C-4″), 79.65 (C-2′), 79.83 (C-2″), 81.66 (C-3″), 82.32 (C-4′), 87.10 (C-1′), 96.35 (C-1″), 113.38, 113.94 and 114.00 (*meta*-C of *p*-methoxyphenyl rings), 121.60 (C-5), 127.04, 127.90, 127.99, 128.04, 128.12, 128.19, 128.28, 128.52, 128.61, 128.74, 129.07, 129.80 and 130.36 (ArCH), 129.34, 130.66 and 131.30 (3 × *ipso*-C of PMB ring), 137.75, 137.88, 138.08 and 138.39 (*ipso*-C of DMTr *p*-methoxyphenyl rings, 3 × *ipso*-C of benzyl rings), 139.07 (C-8), 145.76 (*ipso*-C of DMTr phenyl ring), 148.83 (C-4), 152.51 (C-2), 158.49 (*para*-C of DMTr *p*-methoxyphenyl rings) and 159.37, 159.46 and 159.61

(3 × *para*-C of PMB ring); *m/z* (FAB^+) 1362 [(M + H)$^+$, 8%], 303 (100) and 121 (75).

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyladenosine (17)

TFA (7 cm^3) was added to a solution of **16** (1.28 g, 0.94 mmol) in CH_2Cl_2 (63 cm^3) and the resulting bright orange solution was stirred at room temperature under N_2 for 1.75 h before being poured into saturated aq. $NaHCO_3$ (500 cm^3). CH_2Cl_2 (150 cm^3) was added and the colourless mixture was stirred vigorously for 15 min. The organic layer was collected and the aqueous layer was back-extracted with CH_2Cl_2 (2 × 150 cm^3). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate–ethanol, 14:1) to yield the *title compound* as a white solid (530 mg, 81%); R_f 0.28 (ethyl acetate–ethanol, 14:1); mp 95–115 °C (from ethanol); $[\alpha]_D^{25}$ −2.3 (*c* 2.6, $CHCl_3$) (Found C, 63.3; H, 5.9; N, 9.8. Calcd for $C_{37}H_{41}N_5O_9$: C, 63.5; H, 5.9; N, 10.0%); δ_H ($(CD_3)_2CO$; 400 MHz) 3.45–3.51 (2 H, m, 2″-H, 4″-H), 3.66–3.81 (4 H, m, 5′-H_A, 5′-H_B, 6″-H_A, 6″-H_B), 3.93–3.96 (1 H, m, 5″-H), 4.04 (1 H, t, *J* 9.0, 3″-H), 4.38–4.39 (1 H, m, 4′-H), 4.50–4.56 (4 H, m, 2 × OCH_2Ar), 4.61–4.64 (2 H, m, 3′-H, OH), 4.81–4.87 (5 H, m, OCH_2Ar, 2′-H, 2 × OH), 5.22 (1 H, d, *J* 3.9, 1″-H), 6.12 (1 H, d, *J* 5.4, 1′-H), 6.91 (2 H, br s, NH_2), 7.20–7.42 (15 H, m, ArCH), 8.21 and 8.25 (2 H, 2 s, 2-H, 8-H); δ_C ($(CD_3)_2CO$; 100 MHz) 70.68 (C-6″), 70.74 (C-5′), 71.51 (C-4″), 73.21 (C-5″), 73.75, 73.88 and 73.96 (3 × OCH_2Ar), 74.30 (C-3″), 75.11 (C-2′), 78.49 (C-3′), 80.23 (C-2″), 83.05 (C-4′), 89.14 (C-1′), 99.10 (C-1″), 120.35 (C-5), 128.11, 128.25, 128.35, 128.46, 129.02, 129.08 and 129.15 (ArCH), 139.19 and 139.78 (3 × *ipso*-C of Bn rings), 140.03 (C-8), 150.73 (C-4), 153.77 (C-2) and 157.04 (C-6); *m/z* (FAB^+) 700 [(M + H)$^+$, 100%] and 91 (99).

2″,5′,6″-Tris-*O*-[bis(benzyloxy)phosphoryl]-3′-*O*-α-D-glucopyranosyl adenosine (18)

A mixture of **17** (100 mg, 0.14 mmol), bis(benzyloxy)(diisopropylamino)phosphine (0.16 cm^3, 0.47 mmol) and imidazolium triflate [38] (100 mg, 0.46 mmol) in CH_2Cl_2 (3 cm^3) under N_2 was stirred for 30 min, after which time TLC (ethyl acetate–hexane, 7:3) indicated some starting material remaining; therefore a further 1.0 equiv. each of bis(benzyloxy)(diisopropylamino)phosphine and imidazolium triflate was added. TLC after a further 30 min indicated complete conversion to the trisphosphite (R_f 0.68). Water (1 drop) was added and the solution was cooled to −78 °C. MCPBA (139 mg, 0.49 mmol) was added and stirring was continued for 10 min. 10% (w/v) Aq. Na_2SO_3 (15 cm^3) and ethyl acetate (20 cm^3) were added and the mixture was allowed to warm to room temperature. The resulting organic layer was washed with 15 cm^3 each of saturated aq. $NaHCO_3$ and saturated aq. NaCl and then dried ($MgSO_4$), filtered and concentrated to give an oil which was subjected to flash chromatography (eluent chloroform–acetone, 4:1, then 7:3) to yield the *title compound* as a colourless oil (148 mg, 70%); R_f 0.11 (chloroform–acetone 9:1); $[\alpha]_D^{20}$ +11.7 (*c* 3.0, in $CHCl_3$) (Found C, 63.9; H, 5.7; N, 4.6. Calcd for $C_{79}H_{80}N_5O_{18}P_3$: C, 64.1; H, 5.45; N, 4.7%); δ_H ($CDCl_3$; 400 MHz) 3.54–3.69 (4 H, m, 2″-H, 5′-H_A, 5′-H_B, 6″-H_A, 6″-H_B), 3.83–3.86 (1 H, m, 5″-H), 4.30 (1 H, AB, J_{AB} 11.7, OC*H*HAr), 4.36–4.81 (14 H, m, 4′-H, 3′-H, 4″-H, 11 × OC*H*HAr), 4.88–5.07 (8 H, m, 3″-H, 7 × OC*H*HAr), 5.33 (1 H, d, *J* 3.4, 1″-H), 5.62–5.65 (1 H, m, 2″-H), 6.09 (2 H, br s, NH_2), 6.35 (1 H, d, *J* 6.3, 1′-H), 6.95–7.40 (45 H, m, ArCH), 7.92 and 8.24 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 68.51 (C-6″), 68.28–70.07 (C-5′, 6 × POCH_2Ar with C-P coupling), 70.27 (C-5′), 71.77, 73.53 and 73.76 (3 × OCH_2Ar), 73.87 and 74.54 (C-3′, C-4′ with C-P coupling), 77.00 (C-2″), 77.40 (C-2′ with C-P coupling), 78.28 (C-3″ with C-P coupling), 82.60 (C-4′), 85.78 (C-1′), 95.60 (C-1″), 119.98 (C-5), 127.80, 127.93, 128.02, 128.17, 128.26, 128.48, 128.61, 128.65, 128.68, 128.74, 128.79, 129.03 and 129.10 (ArCH), 135.16–135.32 (2 × *ipso*-C of benzylphospho ring with C-P coupling), 135.88, 136.03 and 136.38 (4 × *ipso*-C of benzylphospho ring with C-P coupling), 137.55, 137.81 and 138.21 (3 × *ipso*-C of Bn rings), 139.60 (C-8), 150.26 (C-4), 153.06 (C-2) and 155.57 (C-6); δ_P ($CDCl_3$; 162 MHz, ^1H decoupled) −1.31, −2.02 and −2.19 (3 s); *m/z* (FAB^+) 1478 [(M + H)$^+$, 6%] and 91 (100).

3-*O*-α-D-Glucopyranosyladenosine 2′,3″,4″-trisphosphate (adenophostin A) (2)

A mixture of **18** (59 mg, 0.04 mmol) and wet 20% palladium hydroxide on carbon (177 mg), in methanol (7 cm^3), cyclohexene (3.5 cm^3) and water (0.5 cm^3) was heated under reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed copiously with methanol and water. Concentration of the filtrate afforded a residue which was applied to an MP1 AG ion exchange resin column and eluted with a gradient of 0–100% 150 mmol dm^{-3} aq. TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20 °C) gave the *title compound* as the free acid (24 mg, 92%), which was dissolved in water and eluted through a short column of Na^+ Diaion WK-40 ion exchange resin to give, after concentration, the sodium salt (Found: M^-, 668.039. Calcd for $C_{16}H_{25}N_5O_{18}P_3$ $(M - H)^-$: 668.040); δ_H (D_2O; 400 MHz) 3.60–3.73 (6 H, m, 2″-H, 5′-H_A, 5′-H_B, 5″-H, 6″-H_A, 6″-H_B), 3.97 (1 H, q, *J* 8.9, 4″-H), 4.28 (1 H, m, 4′-H), 4.37 (1 H, q, *J* 9.3, 3″-H), 4.48 (1 H, m, 3′-H), 5.10–5.14 (2 H, m, 1″-H, 2′-H), 6.18 (1 H, d, *J* 6.4, 1′-H) and 8.24 and 8.34 (2 H, 2 s, 2-H, 8-H); δ_P (D_2O; 162 MHz; ^1H decoupled) 0.32, 0.87 and 1.22 (3 s); λ_{max} (H_2O) 259 nm, ε 15 400, pH 7.5; *m/z* (FAB^-) 668 [(M-H)$^-$, 100%], 266 (34) and 113 (44).

2-*O*-Benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-xylopyranosyl dimethyl phosphite (20)

To a mixture of xylopyranose **19** [22] (1.23 g, 2.57 mmol) and 1*H*-tetrazole (0.27 g, 3.85 mmol) in CH_2Cl_2 (12 cm^3) under N_2 was added dimethoxy(diethylamino)phosphine [36] (0.55 cm^3, 3.34 mmol). The mixture was stirred for 20 min, when TLC (ethyl acetate–toluene, 1:4) indicated complete conversion into product (R_f 0.63). The reaction mixture was partitioned between diethyl ether (100 cm^3) and water (75 cm^3). The resulting ethereal layer was washed with saturated aq. NaCl (75 cm^3), dried ($MgSO_4$), filtered and concentrated to give a clear runny oil, which was used without further purification; δ_H ($CDCl_3$; 400 MHz) 3.23 (0.6 H, q, *J* 9.8, 4-H_β), 3.40–3.95 (11.4 H, m, 2-H, 3-H, 4-H_α, 5-H_A, 5-H_B, $P(OCH_3)_2$ with C-P coupling), 3.77 (3.6 H, s, $OCH_{3\alpha}$), 3.77 (2.4 H, s, $OCH_{3\beta}$), 4.54–4.90 (6.6 H, 1-H_β, 3 × OCH_2Ar), 5.42 (0.4 H, dd, *J* 3.4, $J_{H\text{-}P}$ 8.6, 1-H_α), 6.81–6.89 (4 H, m, ArCH) and 7.22–7.37 (9 H, m, ArCH); δ_P ($CDCl_3$; 161.7 MHz; ^1H decoupled) 140.88 $OP_\beta(OMe)_2$ and 142.05 $OP_\alpha(OMe)_2$; α and β subscripts denote signals arising from α- and β-anomers respectively.

2″,5′-Di-*O*-benzyl-2′,3″,4″-tri-*O*-*p*-methoxybenzyl-N^6-dimethoxytrityl-3′-*O*-D-xylopyranosyladenosine (21ab)

A mixture of dimethyl phosphite **20** (1.47 g, 2.57 mmol) and **13** (1.00 g, 1.28 mmol) in dioxane (15 cm^3) and toluene (5 cm^3) under N_2 was stirred with 4 Å molecular sieves (approx. 1.50 g) for 2 h, and then dry zinc chloride (0.42 g, 3.08 mmol) and silver perchlorate (1.26 g, 6.16 mmol) were added. The flask was wrapped in foil to exclude light and stirring was continued for 9 h. Solid $NaHCO_3$ (1.00 g) and water (30 cm^3) were added and the reaction mixture was diluted with ethyl acetate (40 cm^3). After stirring for a further 30 min the mixture was filtered through a Celite pad, and the residue was well washed with ethyl acetate. Water (30 cm^3) was added to the filtrate and the resulting aqueous layer was discarded. The organic layer was washed with saturated aq. NaCl (75 cm^3), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate–hexane, 3:7, then 2:3, then 1:1) to yield the *title compound* as an inseparable 1:1 anomeric mixture (0.76 g, 46%); R_f 0.38 (ethyl acetate–toluene, 1:4); selected δ_H ($CDCl_3$; 400 MHz) 6.13 (0.5 H, d, *J* 1.5, 1′-H$_\alpha$) and 6.22 (0.5 H, d, *J* 4.4, 1′-H$_\beta$); α and β subscripts denote signals arising from α- and β-anomers respectively; *m*/*z* (FAB^+) 1246 [$(M + H)^+$, 14%], 303 (100) and 121 (72).

2″,5′-Di-*O*-benzyl-3′-*O*-α-D-xylopyranosyladenosine (22) and 2″,5′-di-*O*-benzyl-3′-*O*-β-D-xylopyranosyladenosine (23)

A solution of **21ab** (745 mg, 0.60 mmol) in CH_2Cl_2 (27 cm^3) and TFA (3 cm^3) was stirred for 1 h under N_2 before being poured into saturated aq. $NaHCO_3$ (150 cm^3). CH_2Cl_2 (75 cm^3) was added and mixture was stirred vigorously for 30 min. The resulting aqueous layer was extracted with CH_2Cl_2 (2 × 75 cm^3) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate–methanol, 14:1) to yield the α-anomer **22** (138 mg, 40%); R_f 0.51 (chloroform–methanol, 9:1); $[\alpha]_D^{18}$ +22.1 (*c* 2.0, acetone) (Found M^+, 580.240. Calcd for $C_{29}H_{34}N_5O_8$ $(M + H)^+$: 580.240); δ_H ($(CD_3)_2CO$; 400 MHz) 3.12 (1 H, br s, OH), 3.44 (1 H, dd, *J* 3.7, 9.5, 2″-H), 3.60–3.65 (3 H, m, 4″-H, 5″-H$_{ax}$, 5″-H$_{eq}$), 3.74 and 3.83 (2 H, ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 3.8, $^3J_{BX}$ 3.4, 5′-H$_A$, 5′-H$_B$), 3.95–3.99 (1 H, m, 3″-H), 4.36 (1 H, q, *J* 4.1, 4′-H), 4.57–4.63 (4 H, m, OC*H*$_2$Ar), 4.71 (1 H, br s, OH), 4.84–4.88 (1 H, m, 2′-H obscured by HDO peak), 5.03 (1 H, br s, OH), 5.17 (1 H, d, *J* 3.8, 1″-H), 6.12 (1 H, d, *J* 4.7, 1′-H), 6.98 (2 H, br s, NH_2), 7.19–7.43 (10 H, m, ArCH) and 8.22 and 8.26 (2 H, 2 s, 2-H, 8-H); δ_C ($(CD_3)_2CO$; 100 MHz) 62.82 (C-5″), 69.91 (C-5′), 70.59 (C-4″), 73.34 and 73.66 (2 × O*C*H$_2$Ar), 73.96 (C-3″), 74.44 (C-2′), 77.47 (C-3′), 79.70 (C-2″), 82.29 (C-4′), 88.66 (C-1′), 98.58 (C-1″), 119.65 (C-5), 127.72, 127.81, 128.33, 128.40 and 128.51 (ArCH), 138.40 and 138.43 (2 × *ipso*-C of Bn rings), 139.39 (C-8), 149.89 (C-4), 153.04 (C-2) and 156.28 (C-6); *m*/*z* (FAB^+) 580 [$(M + H)^+$, 100%] and 91 (79).

Further elution gave the β-anomer **23** (143 mg, 41%); R_f 0.43 (chloroform–methanol, 9:1) (Found M^+, 580.241. Calcd for $C_{29}H_{34}N_5O_8$ $(M + H)^+$: 580.240); δ_H (CD_3OD; 400 MHz) 3.18 (1 H, dd, $^2J = {}^3J$ 10.8, 5″-H$_{ax}$), 3.29 (1 H, dd, *J* 7.6, 9.1, 2″-H), 3.46 (1 H, t, *J* 8.9, 3″-H), 3.53–3.58 (1 H, m, 4″-H), 3.61 and 3.77 (2 H, ABX, $^2J_{AB}$ 11.0, $^3J_{AX}$ 3.1, $^3J_{BX}$ 2.8, 5′-H$_A$, 5′-H$_B$), 3.85 (1 H, dd, ^2J 11.4, ^3J 5.3, 5″-H$_{eq}$), 4.34 (1 H, ddd, *J* 2.9, 2.9, 5.6, 4′-H), 4.43 and 4.48 (2 H, AB, J_{AB} 12.0, OC*H*$_2$Ar), 4.46 (1 H, d, *J* 7.9, 1″-H), 4.53 (1 H, t, *J* 5.1, 3′-H), 4.66 (1 H, t, *J* 4.2, 2′-H), 4.78 and 4.86 (2 H, AB, J_{AB} 11.1, OC*H*$_2$Ar), 6.11 (1 H, d, *J* 3.8, 1′-H), 7.19–7.39 (10 H, m, ArCH) and 8.18 and 8.30 (2 H, 2 s, 2-H, 8-H); δ_C (CD_3OD; 100 MHz) 67.17 (C-5″), 70.30 (C-5′), 71.22 (C-4″), 74.63 and 75.97 (O*C*H$_2$Ar), 76.11 (C-2′), 77.62 (C-3″), 79.79 (C-3′), 82.81 (C-2″), 83.12 (C-4′), 90.27 (C-1′), 105.19 (C-1″), 120.31 (C-5), 128.62, 128.97, 128.01, 129.33 and 129.61 (ArCH), 139.14 and 140.13 (2 × *ipso*-C of Bn rings), 140.63 (C-8), 150.36 (C-4), 153.91 (C-2) and 157.11 (C-6); *m*/*z* (FAB^+) 580 [$(M + H)^+$, 100%] and 91 (67).

2″,5′-Di-*O*-benzyl-2′,3″,4″-tris-*O*-[bis(benzyloxy)phosphoryl]-3′-*O*-α-D-xylopyranosyladenosine (24)

A solution of triol **22** (101 mg, 0.17 mmol), bis(benzyloxy)-(diisopropylamino)phosphine (0.19 cm^3, 0.58 mmol) and imidazolium triflate[38] (125 mg, 0.58 mmol) in CH_2Cl_2 (3.5 cm^3) under N_2 was stirred for 1 h, after which time TLC (ethyl acetate–hexane, 7:3) indicated some starting material remaining; therefore a further 1.0 equivalent each of bis(benzyloxy)-(diisopropylamino)phosphine and imidazolium triflate was added. TLC after a further 30 min indicated conversion into the trisphosphite (R_f 0.63). Water (1 drop) was added, the solution was cooled to −78 °C and MCPBA (170 mg, 0.59 mmol) was added. After 10 min 10% (w/v) aq. Na_2SO_3 (15 cm^3) and ethyl acetate (20 cm^3) were added and the mixture was allowed to warm to room temperature. The resulting organic layer was washed with 15 cm^3 each of saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried ($MgSO_4$), filtered and concentrated to give a clear oil which was subjected to flash chromatography (eluent chloroform–acetone, 9:1, then 7:1, then 6:1, then 4:1, then 3:2) to give the *title compound* as a clear oil (161 mg, 68%); R_f 0.11 (ethyl acetate–hexane, 7:3); $[\alpha]_D^{20}$ +6.5 (*c* 1.7, $CHCl_3$) (Found M^+, 1360.425. Calcd for $C_{71}H_{73}N_5O_{17}P_3$ $(M + H)^+$: 1360.421); δ_H ($CDCl_3$; 400 MHz) 3.49 (1 H, dd, *J* 3.5, 9.4, 2″-H), 3.52 (1 H, dd, $^2J = {}^3J$ 11.0, 5″-H$_{ax}$), 3.62 and 3.76 (2 H, ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 3.2, $^3J_{BX}$ 3.4, 5′-H$_A$, 5′-H$_B$), 3.95 (1 H, dd, ^2J 11.1, ^3J 5.9, 5″-H$_{eq}$), 4.33–5.02 (14 H, m, 3′-H, 3″-H, 4′-H, 4″-H, 6 × POC*H*$_2$Ar, 2 × OC*H*$_2$Ar), 5.29 (1 H, d, *J* 3.2, 1″-H), 5.61–5.66 (1 H, m, 2″-H), 5.98 (2 H, br s, NH_2), 6.34 (1 H, d, *J* 5.9, 1′-H), 6.98–7.39 (40 H, m, ArCH) and 7.93 and 8.25 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100.4 MHz) 60.25 (C-5″), 69.40–70.23 (C-5′, 6 × PO*C*H$_2$Ar with C-P coupling), 72.12 (O*C*H$_2$Ar), 73.59 (C-4″ with C-P coupling), 73.97 (O*C*H$_2$Ar and C-3′), 77.07 (C-2″), 77.71 (C-2′, C-3″ with C-P coupling), 82.55 (C-4′), 86.01 (C-1′), 95.81 (C-1″), 120.05 (C-5), 127.78, 127.91, 127.98, 128.15, 128.19, 128.22, 128.26, 128.34, 128.48, 128.53, 128.68, 128.72 and 128.79 (ArCH), 135.14–136.23 (6 × *ipso*-C of benzylphospho rings), 137.14 and 137.64 (2 × *ipso*-C of Bn rings), 139.54 (C-8), 150.14 (C-4), 153.14 (C-2) and 155.54 (C-6); δ_P ($CDCl_3$; 162 MHz; ^1H decoupled) −0.63 (2 P, s) and −0.20 (1 P, s); *m*/*z* (FAB^+) 1360 [$(M + H)^+$, 5%] and 91 (100).

3′-*O*-α-D-Xylopyranosyladenosine 2′,3″,4″-trisphosphate (*xylo*-adenophostin) (5)

A mixture of **24** (84 mg, 0.06 mmol) and wet 20% palladium hydroxide on carbon (252 mg), in methanol (11 cm^3), cyclohexene (5 cm^3) and water (1 cm^3) was heated under reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed copiously with methanol and water. Concentration of the filtrate afforded a clear residue which was applied to an MP1 AG ion exchange resin column and eluted with a gradient of 0–100% 150 mmol dm^{-3} TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20 °C) gave the desired product as the free acid (33 mg, 85%), which was dissolved in water and eluted through a short column of Na^+ Diaion WK-40 ion exchange resin to give, after concentration, the sodium salt; (Found: M^-, 638.030. Calcd for $C_{15}H_{23}N_5O_{17}P_3$ $(M - H)^-$: 638.030); δ_H (D_2O; 400 MHz) 3.50 (1 H, dd, $^2J = {}^3J$ 10.8, 5″-H$_{ax}$), 3.61 (1 H, dd, *J* 3.4, 9.2, 2″-H), 3.65–3.71 (2 H, m, 5′-H$_A$, 5′-H$_B$), 3.76 (1 H, dd, *J* 5.6, 11.4, 5″-H$_{eq}$), 4.02–4.10 (1 H, m, 4″-H), 4.25–4.32 (2 H, m, 3″-H, 4″-H), 4.34–4.56 (1 H, m, 3′-H), 5.07 (1 H, d, *J* 3.5, 1″-H), 5.08–5.14 (1 H, m, 2′-H), 6.16 (1 H, d, *J* 6.2, 1′-H), 8.23 and 8.33 (2 H, 2 s, 2-H,8-H); δ_P (D_2O; 162 MHz; ^1H decoupled) 0.23, 0.67 and 0.78 (3 s); λ_{max} (H_2O) 259 nm, ε 15 400, pH 7.5; *m*/*z* (FAB^-) 638 [$(M - 1)^-$, 100%].

Allyl α-D-mannopyranoside (25)

Allyl alcohol (160 cm^3) and acetyl chloride (5 cm^3) were stirred together for 1 hour after which time D-mannose (20.0 g, 111 mmol) was added. The mixture was heated at 50 to 60 °C under N_2 with vigorous stirring for 5 h. TLC (ethyl acetate–propan-2-ol–water, 9:4:2) showed a major product at R_f 0.49. The clear solution was allowed to cool, triethylamine (20 cm^3) was added and the solvents were removed by evaporation *in vacuo* at 50 °C to leave an orange oil (≈30 g). Purification by flash chromatography on silica (300 g) eluting with dichloromethane–acetone 1:2 to 1:4 gave a pale yellow oil (18.4 g) which crystallised on standing. Recrystallisation from hot acetone (200 cm^3) gave the *title compound* as colourless needles (15.0 g, 61%); R_f 0.49 (ethyl acetate–propan-2-ol–water, 9:4:2); mp 100–101.5 °C (from acetone) (lit.,[40] 98–99 °C; lit.,[41] 138–139 °C); $[\alpha]_D^{20}$ +84.5 (*c* 2.0, in water) [lit.,[40] +99 (in water); lit.,[41] +51.6 (*c* 0.23, in water)]

(Found: C, 49.1; H, 7.3. Calcd for $C_9H_{16}O_6$: C, 49.1; H, 7.3%); ^{1}H NMR data were identical to those previously reported;[41] δ_C (D_2O; 100 MHz) 61.14 (C-6), 66.98 (C-4), 68.34 (O*C*H$_2$-CH=CH$_2$), 70.24 and 70.78 (C-2 and C-3), 73.00 (C-5), 99.15 (C-1), 118.58 (OCH$_2$CH=*C*H$_2$) and 133.33 (OCH$_2$*C*H=CH$_2$); *m*/*z* (FAB$^+$) 243 [(M + Na)$^+$, 100%], 221 [(M + 1)$^+$, 14] and 163 [(M − C_3H_5O)$^+$, 70]; *m*/*z* (FAB$^-$) 373 [(M + NBA)$^-$, 100%] and 219 [(M − 1)$^-$, 62].

(2′*S*,3′*S*)-Allyl 3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-α-D-mannopyranoside (26)

To a solution of **25** (11.0 g, 50.0 mmol), trimethyl orthoformate (20 cm^3) and CSA (500 mg) in methanol (200 cm^3) was added butanedione (5.0 cm^3, 57 mmol). The mixture was heated at reflux under N_2. TLC (ethyl acetate) after 1 h showed two major products (R_f 0.36 and 0.40), but after 10 h only the more polar product remained. The mixture was allowed to cool, triethylamine (1 cm^3) was added, and stirring was continued at room temperature for a further 1 h. The solvents were removed by evaporation under reduced pressure, leaving an orange oil. Purification by flash chromatography on silica eluting with ethyl acetate–hexane 2:1 gave the *diacetal* **26** as a hygroscopic foam (13.1 g, 78%) (Found C, 53.5; H, 8.1. Calcd for $C_{15}H_{26}O_8$: C, 53.9; H, 7.8%); $[\alpha]_D^{23}$ +232 (*c* 1.0, in $CHCl_3$); δ_H ($CDCl_3$; 270 MHz) 1.29 (3 H, s, Me), 1.33 (3 H, s, Me), 2.49 (1 H, t, *J* 5.1, D_2O exch., 6-OH), 3.11 (1 H, d, *J* 2.4, D_2O exch., 2-OH), 3.27 (3 H, s, OMe), 3.28 (3 H, s, OMe), 3.70–3.90 (3 H, m, 5-H and 6-H$_2$,), 3.92–4.22 (5 H, m, 2-, 3- and 4-H and *CH*$_2$CH=CH), 4.89 (1 H, d, *J* 1.3, 1-H), 5.18–5.23 (1 H, m, CH$_2$CH=C*H*$_{cis}$CH$_{trans}$), 5.24–5.34 (1 H, m, CH$_2$CH=CH$_{cis}$C*H*$_{trans}$) and 5.81–5.90 (1 H, m, CH$_2$C*H*=CH$_2$); δ_C ($CDCl_3$; Me$_4$Si; 100 MHz) 17.70 and 17.79 (2 × Me), 47.92 and 48.12 (2 × OMe), 61.17 (C-6), 62.86 (C-4), 68.16, 69.71 and 70.78 (C-2, C-3 and C-5), 68.16 (*C*H$_2$CH=CH$_2$), 99.29 (C-1), 99.82 and 100.35 (C-2′ and C-3′), 117.70 (CH$_2$CH=*C*H$_2$) and 133.69 (CH$_2$*C*H=CH$_2$); *m*/*z* (FAB$^+$) 691 [(2M + Na)$^+$, 84%], 357 [(M + Na)$^+$, 60], 303 [(M − OMe)$^+$, 62] and 101 (100); *m*/*z* (FAB$^-$) 333.1 [(M − 1)$^-$, 100%].

(2′*S*,3′*S*)-Allyl 2,6-di-*O*-benzyl-3,4-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-α-D-mannopyranoside (27)

To a solution of **26** (7.10 g, 21.2 mmol) in dry DMF (100 cm^3) at 0 °C was added NaH (2.55 g of a 60% dispersion in mineral oil, 63.7 mmol). The mixture was stirred at 0 °C for 1 h and then benzyl bromide (5.6 cm^3, 47 mmol) was added gradually over 1 min. After a further 1 h at 0 °C the mixture was allowed to reach room temperature and stirred for 14 h. Excess NaH was destroyed by careful addition of water and solvents were removed by evaporation under reduced pressure. The residue was partitioned between ether and water (100 cm^3 of each) and the organic layer was washed sequentially with 0.1 mol dm^{-3} HCl and saturated NaHCO$_3$ solution (100 cm^3 of each), dried over MgSO$_4$ and concentrated by evaporation under reduced pressure to give a yellow oil. Purification by flash column chromatography using ether–hexane 1:3 as eluent gave the *title compound* (9.62 g, 88%) as a colourless oil, which slowly crystallised; mp 56–58 °C (from hexane); $[\alpha]_D^{23}$ +155 (*c* 1.1, in $CHCl_3$) (Found C, 67.6; H, 7.3. Calcd for $C_{29}H_{38}O_8$: C, 67.7; H, 7.4%); δ_H ($CDCl_3$; 400 MHz) 1.27 (3 H, s, Me), 1.33 (3 H, s, Me), 3.19 and 3.27 (6 H, 2 s, 2 × OMe), 3.72 (1 H, dd, *J* 1.4, 3.0, 2-H), 3.75–3.78 (2 H, m, 6-H$_2$), 3.91–3.96 (2 H, m, 5-H and C*H*H-CH=CH$_2$), 4.10 (1 H, dd, *J* 10.3, 3.0, 3-H), 4.13–4.18 (1 H, m, CH*H*CH=CH$_2$), 4.21 (1 H, t, *J* 10.3, 4-H), 4.57 and 4.63 (2 H, AB, J_{AB} 12.2, CH$_2$Ph), 4.67 and 4.93 (2 H, AB, J_{AB} 12.2, CH$_2$Ph), 4.86 (1 H, d, *J* 1.4, 1-H), 5.12–5.16 (1 H, m, CH$_2$-CH=C*H*$_{cis}$CH$_{trans}$), 5.18–5.24 (1 H, m, CH$_2$CH=CH$_{cis}$C*H*$_{trans}$), 5.80–5.90 (1 H, m, CH$_2$C*H*=CH$_2$), 7.22–7.33 (8 H, m, Ph), and 7.43 (2 H, d, *J* 7.3, Ph); δ_C ($CDCl_3$; 100 MHz) 17.83 (2 × Me), 47.86 and 47.97 (2 × OMe), 63.81 (C-4), 69.13 (C-3), 70.94 (C-5), 75.79 (C-2), 67.83 (*C*H$_2$CH=CH$_2$), 68.84 (C-6), 73.04 and 73.35 (2 × CH$_2$Ph), 98.46 (C-1), 99.54 and 99.82 (C-2′ and C-3′), 117.34 (CH$_2$CH=*C*H$_2$), 127.27, 127.34, 127.41, 127.52, 127.58, 127.91, 128.16 and 128.49 (Ph *C*H), 133.85 (CH$_2$-*C*H=CH$_2$) and 138.59 and 138.82 (2 × *ipso*-C of Ph); *m*/*z* (FAB$^+$) 537 [(M + Na)$^+$, 36%], 513 [(M − 1)$^+$, 14], 483 [(M − OMe)$^+$, 90], 101 (28) and 91 [(C_7H_7)$^+$, 100].

Allyl 2,6-di-*O*-benzyl-α-D-mannopyranoside (28)

To a solution of **27** (8.00 g, 15.5 mmol) in CH$_2$Cl$_2$ (40 cm^3) was added 95% (v/v) TFA in water (40 cm^3). TLC (ether–hexane, 1:1) showed complete conversion of **27** into a single product (R_f 0.24) within 15 minutes. The solvents were removed by evaporation under reduced pressure to leave an oily residue, which was taken up in ether (100 cm^3), washed with saturated NaHCO$_3$ (100 cm^3) and dried (MgSO$_4$). Concentration by evaporation under reduced pressure gave a yellow oil which was purified by flash chromatography using acetate–hexane 1:1 as eluent to give the *diol* **28** (5.28 g, 85%) as a colourless oil (Found: C, 68.6; H, 7.1. Calcd for $C_{23}H_{28}O_6$: C, 69.0; H, 7.05%); $[\alpha]_D^{23}$ +7.2 (*c* 2.1, in $CHCl_3$); δ_H ($CDCl_3$; 270 MHz) 2.64 (1 H, d, *J* 8.6, D_2O exch, OH), 3.07 (1 H, br s, D_2O exch., OH), 3.70–3.85 (6 H, m, 2-, 3-, 4- and 5-H and 6-H$_2$), 3.92–4.00 (1 H, m, C*H*H-CH=CH$_2$), 4.13–4.22 (1 H, m, CH*H*CH=CH$_2$), 4.52–4.72 (4 H, m, 2 × C*H*$_2$Ph), 4.93 (1 H, br s, 1-H), 5.14–5.20 (1 H, m, CH$_2$CH=C*H*$_{cis}$CH$_{trans}$), 5.20–5.29 (1 H, m, CH$_2$CH=CH$_{cis}$-C*H*$_{trans}$), 5.78–5.94 (1 H, m, CH$_2$C*H*=CH$_2$), and 7.25–7.34 (10 H, m, Ph); δ_C ($CDCl_3$; 68 MHz) 69.56, 70.79 and 71.45 (C-3, C-4 and C-5), 67.81 (*C*H$_2$CH=CH$_2$), 70.11 (C-6), 72.87 and 73.43 (2 × *C*H$_2$Ph), 77.76 (C-2), 96.14 (C-1), 117.29 (CH$_2$CH=*C*H$_2$), 127.50, 127.76, 127.86, 128.26 (Ph *C*H), 133.58 (CH$_2$*C*H=CH$_2$) and 137.61 and 138.09 (2 × *ipso*-C of Ph); *m*/*z* (FAB$^+$) 423 [(M + Na)$^+$, 77%], 399 [(M − 1)$^+$, 14], 343 [(M − C_3H_5O)$^+$, 15], 181 (16) and 91 [(C_7H_7)$^+$, 100]; *m*/*z* (FAB$^-$) 553 [(M + NBA)$^-$, 100%] and 399 [(M − 1)$^-$, 56].

Allyl 2,6-di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (29)

To a solution of **28** (5.00 g, 12.5 mmol) in dry DMF (100 cm^3) at 0 °C was added NaH (1.50 g of a 60% dispersion in mineral oil, 37.5 mmol). The mixture was stirred at 0 °C for 30 min and then *p*-methoxybenzyl chloride (4.0 cm^3, 30 mmol) was added dropwise over 2 min. The mixture was allowed to reach room temperature and stirred for 3 h. TLC (ether–hexane, 1:1) showed conversion into a major product (R_f 0.36), but the reaction was not complete, and so more *p*-methoxybenzyl chloride (0.6 cm^3, 4 mmol) was added and stirring was continued at room temperature for a further 14 h. Excess NaH was destroyed by careful addition of water and solvents were removed by evaporation under reduced pressure. The residue was partitioned between ether and water (100 cm^3 of each) and the organic layer was washed sequentially with 0.1 M HCl, saturated NaHCO$_3$ solution and brine (100 cm^3 of each), dried over MgSO$_4$ and concentrated by evaporation under reduced pressure to give a yellow oil. Purification by flash column chromatography using ether–hexane 1:2 as eluent gave the *title compound* **29** (5.31 g, 66%) as a colourless oil (Found: C, 73.3; H, 6.9. Calcd for $C_{39}H_{44}O_8$: C, 73.1; H, 6.9%); $[\alpha]_D^{20}$ +30 (*c* 1.4, in $CHCl_3$); δ_H ($CDCl_3$; 270 MHz) 3.68–3.80 (4 H, m, 2-H, 4-H and 6-H$_2$), 3.77 and 3.80 (6 H, 2 s, 2 × OMe), 3.86–3.98 (3 H, m, 3-H, 5-H and C*H*HCH=CH$_2$), 4.10–4.19 (1 H, m, CH*H*-CH=CH$_2$), 4.38–4.82 (8 H, m, 4 × C*H*$_2$Ar), 4.91 (1 H, d, *J* 1.6, 1-H), 5.11–5.15 (1 H, m, CH$_2$CH=C*H*$_{cis}$CH$_{trans}$), 5.16–5.24 (1 H, m, CH$_2$CH=CH$_{cis}$C*H*$_{trans}$), 5.76–5.92 (1 H, m, CH$_2$C*H*=CH$_2$), 6.76–6.82 (2 H, m, *meta*-H of PMB ring), 6.84–6.89 (2 H, m, *meta*-H of PMB ring), 7.04–7.10 (2 H, m, *ortho*-H of PMB ring) and 7.24–7.41 (12 H, m, ArH); δ_C ($CDCl_3$; 68 MHz) 55.22 (OMe), 67.69 (*C*H$_2$CH=CH$_2$), 69.23 (C-6), 71.84, 74.57 and 74.68 (C-3, C-4 and C-5), 71.83, 72.52, 73.27 and 74.73 (4 × CH$_2$Ar), 79.95 (C-2), 97.08 (C-1), 113.68 (2 × *meta*-C of

PMB rings), 117.06 ($CH_2CH=CH_2$), 127.39, 127.50, 127.70, 127.76, 128.23, 128.26, 129.21, 129.61 (Ar *C*H), 130.71 (2 × *ipso*-C of PMB rings), 133.78 ($CH_2CH=CH_2$), 138.40 and 138.43 (2 × *ipso*-C of Ph) and 159.09 (2 × *para*-C of PMB rings); *m*/*z* (FAB^+) 663 [$(M + Na)^+$, 40%], 639 [$(M - 1)^+$, 42], 519 [$(M - PMB)^+$, 88], 121 (100) and 91 [$(C_7H_7)^+$, 24].

2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-mannopyranose (30)

A solution of **29** (2.60 g, 4.06 mmol) in dry methanol (20 cm^3) was stirred vigorously with $PdCl_2$ (100 mg) for 3 hours, after which time TLC (ethyl acetate–hexane, 1:2) showed the reaction to be essentially complete with conversion of **29** (R_f 0.40) into a major product with R_f 0.14. Triethylamine (0.5 cm^3) was added and the suspension was filtered through Celite. The filtrate was concentrated by evaporation under reduced pressure and the residue was purified by flash chromatography (eluent ethyl acetate–hexane, 2:3) to give the *mannopyranose* **30** (ratio of α- to β-anomers 3:1) as a colourless glass (1.92 g, 79%) (Found: C, 71.7; H, 6.7. Calcd for $C_{39}H_{44}O_8$: C, 72.0; H, 6.7%); δ_H ($CDCl_3$; 400 MHz) 3.18 (0.75 H, br s, D_2O exch., 1-OH_α), 3.41 (0.25 H, ddd, *J* 9.3, 4.4, 2.9, 5-H_β), 3.56 (0.25 H, dd, *J* 9.3, 2.4, 3-H_β), 3.60–3.71 (2 H, m, 6-$H_{2\alpha}$ and 6-$H_{2\beta}$), 3.75–3.83 (7.75 H, 2-H_α, 2-H_β, 4-H_α and 2 × OMe), 3.88 (0.25 H, t, *J* 9.3, 4-H_β), 3.92 (0.75 H, dd, *J* 9.8, 3.4, 3-H_α), 3.97–4.01 (0.75 H, m, 5-H_α), 4.40–4.80 (8 H, m, 4 × CH_2Ar in α-anomer, 3.5 × CH_2Ar in β-anomer and H-1_β), 5.07 (0.25 H, d, *J* 11.7, CHC*H*Ar in β-anomer), 5.23 (0.75 H, br s, D_2O exch. gives d, *J* 1.6, 1-H_α), 6.78–6.81 (2 H, m, *meta*-H of PMB ring), 6.84–6.88 (2 H, m, *meta*-H of PMB ring), 7.06–7.09 (2 H, m, *ortho*-H of PMB ring) and 7.25–7.37 (12 H, m, ArH); δ_C ($CDCl_3$; 68 MHz, data for α-anomer only) 55.25 (OMe), 69.59 (C-6), 71.84, 72.62, 73.22 and 74.65 (4 × CH_2Ar), 71.37, 74.81 and 74.89 (C-3, C-4 and C-5), 79.48 (C-2), 92.68 (C-1), 113.70 and 113.75 (*meta*-C of PMB rings), 127.57, 127.83, 128.01, 128.31, 129.25 and 129.64 (Ar CH), 130.60 and 130.67 (2 × *ipso*-C of PMB rings), 137.98 and 138.40 (2 × *ipso*-C of Ph) and 159.11 (2 × *para*-C of PMB rings); *m*/*z* (FAB^+) 623 [$(M + Na)^+$, 74%], 599 [$(M - 1)^+$, 24], 121 (100); *m*/*z* (FAB^-) 753 [$(M + NBA)^-$, 100%] and 311(52).

2,6-Di-*O*-benzyl-3,4-di-*O*-*p*-methoxybenzyl-D-mannopyranosyl dimethyl phosphite (31)

To a mixture of mannopyranose **30** (1.29 g, 2.15 mmol) and 1*H*-tetrazole (0.23 g, 3.23 mmol) in CH_2Cl_2 (15 cm^3) under N_2 was added dimethoxy(diethylamino)phosphine[36] (0.46 cm^3, 2.80 mmol) and the mixture was stirred at room temperature for 20 min, whereupon TLC (ethyl acetate–toluene, 1:4) indicated complete conversion into product (R_f 0.69). The reaction mixture was partitioned between diethyl ether (80 cm^3) and water (60 cm^3) and the resulting ethereal layer was washed with saturated aq. NaCl (60 cm^3), dried ($MgSO_4$), filtered and concentrated to give a clear runny oil, which was used without further purification. δ_H ($CDCl_3$; 400 MHz) (α-anomer) 3.39–3.44 (6 H, m, $OP(OMe)_2$ with C-P coupling), 3.65–3.71 (2 H, m, 2-H, 6-H_A), 3.76–3.80 (1 H, m, 6-H_B overlapping with 2 × OCH_3), 3.76, 3.78 (6 H, 2 s, 2 × OCH_3), 3.90–4.03 (3 H, m, 3-H, 4-H, 5-H), 4.44–4.82 (8 H, m, 4 × OC*H*$_2$Ar), 5.52 (1 H, dd, *J* 1.8, J_{H-P} 8.2, 1-H), 6.78–6.86 (4 H, m, *meta*-H of PMB rings), 7.08–7.11 (2 H, m, *ortho*-H of PMB rings) and 7.21–7.39 (12 H, m, ArCH); δ_P ($CDCl_3$; 161.7 MHz; ^1H decoupled) 140.83 ($OP_\beta(OCH_3)_2$) and 141.14 ($OP_\alpha(OCH_3)_2$).

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-mannopyranosyl-2′,3″,4″-tri-*O*-*p*-methoxybenzyl-N^6-dimethoxytrityladenosine (32)

A mixture of dimethyl phosphite **31** (1.48 g, 2.15 mmol), **13** (0.84 g, 1.08 mmol) and 4 Å molecular sieves (approx. 1.2 g) in dioxane (12 cm^3) and toluene (4 cm^3) under N_2 was stirred for 2 h at room temperature, and then dry zinc chloride (0.35 g, 2.58 mmol) and silver perchlorate (1.07 g, 5.16 mmol) were added. The flask was wrapped in foil to exclude light, and stirring was continued for 8 h. Solid $NaHCO_3$ (1.00 g) and water (30 cm^3) were added and the reaction mixture was diluted with ethyl acetate (40 cm^3). After stirring for a further 30 min the mixture was filtered through a Celite pad, and the residue was well washed with ethyl acetate. Water (20 cm^3) was added to the filtrate, and the resulting aqueous layer was discarded. The organic layer was washed with saturated aq. NaCl (50 cm^3), dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent ethyl acetate–hexane, 3:7, then 1:1) to yield the *title compound* as a clear oil (0.89 g, 61%); R_f 0.53 (ethyl acetate–toluene, 1:4); $[\alpha]_D^{18}$ −1.0 (*c* 1.0, in $CHCl_3$) (Found C, 72.1; H, 6.2; N, 5.1. Calcd for $C_{82}H_{83}N_5O_{14}$: C, 72.3; H, 6.1; N, 5.1%); δ_H ($CDCl_3$; 400 MHz) 3.55 (1 H, ABX, $^2J_{AB}$10.7, $^3J_{AX}$ 2.9, 5′-H_A), 3.59–3.73 (4 H, m, 3″-H or 4″-H, 5′-H_B, 6″-H_A, 6″-H_B), 3.66 (3 H, s, OCH_3), 3.77 (9 H, s, 3 × OCH_3), 3.79 (3 H, s, OCH_3), 3.83–3.91 (3 H, m, 2″-H, 3″-H or 4″-H, 5″-H), 4.28 (1 H, t, *J* 2.9, 4′-H), 4.37–4.60 (13 H, m, 2′-H, 3′-H, 11 × OCH*H*Ar), 4.77 (1 H, AB, J_{AB} 10.3, OCH*H*Ar), 5.05 (1 H, s, 1″-H), 6.16 (1 H, d, *J* 5.4, 1′-H), 6.63–6.67 (2 H, m, ArCH), 6.78–6.81 (4 H, m, ArCH), 6.86–6.90 (2 H, m, ArCH), 6.96–6.99 (2 H, m, ArCH), 7.06–7.08 (2 H, m, ArCH), 7.20–7.35 (28 H, m, ArCH) and 7.90 and 8.07 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100 MHz) 55.07, 55.15 and 55.20 (5 × OCH_3), 69.08 (C-6″), 69.52 (C-5′), 70.58 (DMTr Cq), 71.75, 72,03, 72.52, 73.29, 73.53 and 74.70 (6 × O*C*H$_2$Ar), 72.37, 74.28 and 74.59 (C-3″, C-4″, C-5″), 73.21 (C-3′), 79.62 (C-2′, C-2″), 82.56 (C-4′), 86.18 (C-1′), 97.81 (C-1″), 113.06, 113.65 and 113.70 (3 × *meta*-C of *p*-methoxyphenyl rings), 121.00 (C-5), 126.73, 127.40, 127.48, 127.55, 127.60, 127.73, 127.17, 128.24, 128.48, 128.76, 129.16, 129.49, 129.63, 130.05 and 130.49 (ArCH), 128.81, 130.51 and 130.57 (3 × *ipso*-C of PMB rings), 137.42 and 138.17 (*ipso*-C of DMTr *p*-methoxyphenyl rings, 3 × *ipso*-C of Bn rings), 138.30 (C-8), 145.43 (*ipso*-C of DMTr phenyl ring), 148.62 (C-4), 152.29 (C-2), 153.99 (C-6), 158.20 (2 × *para*-C of DMTr *p*-methoxyphenyl rings) and 159.06, 159.11 and 159.41 (*para*-C of PMB ring); *m*/*z* (FAB^+) 1362 (M^+, 7%), 436 (6), 303 (100) and 121 (88).

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-mannopyranosyl adenosine (33)

A solution of **32** (528 mg, 0.39 mmol) in CH_2Cl_2 (27 cm^3) and TFA (3 cm^3) was stirred for 5 h under N_2 before being poured into saturated aq. $NaHCO_3$ (200 cm^3). CH_2Cl_2 (100 cm^3) was added and the mixture was stirred vigorously for 30 min. The resulting aqueous layer was extracted with CH_2Cl_2 (2 × 100 cm^3) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting crude product was purified by flash chromatography (eluent ethyl acetate–ethanol, 14:1, then 9:1) to yield the *title compound* as a white solid (223 mg, 82%); mp 175–178 °C (from ethanol); R_f 0.22 (ethyl acetate–ethanol, 14:1) (Found M^+, 700.296. Calcd for $C_{37}H_{42}N_5O_9$ $(M + H)^+$: 700.298); δ_H (d_6-DMF; 400 MHz) 3.68–3.86 (6 H, m, 4″-H, 5″-H, 5′-H_A, 5′-H_B, 6″-H_A, 6″-H_B), 3.93–3.96 (1 H, m, 3″-H), 3.99–4.00 (1 H, m, 2″-H), 4.39 (1 H, q, *J* 3.2, 4′-H), 4.34–4.62 (5 H, m, 2 × OC*H*$_2$Ar, 3′-H), 4.71 and 4.80 (2 H, AB, J_{AB} 12.2, OC*H*$_2$Ar), 4.97–5.03 (2 H, m, 2′-H, 3″-OH), 5.13 (1 H, d, *J* 4.7, 4″-OH), 5.37 (1 H, s, 1″-H), 5.95 (1 H, d, *J* 6.7, 2′-OH), 6.11 (1 H, d, *J* 6.4, 1′-H), 7.24–7.44 (15 H, m, ArCH) and 8.22 and 8.34 (2 H, 2 s, 2-H, 8-H); δ_C (d_6-DMF; 100.4 MHz) 68.83 (C-4″ or C-5″), 71.12 and 71.24 (C-5′, C-6″), 72.49 (C-3″), 73.47, 73.63 and 73.73 (3 × O*C*H$_2$Ar), 74.53 (C-4″ or C-5″), 74.72 (C-2′), 76.59 (C-3′), 79.62 (C-2″), 83.35 (C-4′), 88.10 (C-1′), 99.20 (C-1″), 120.18 (C-5), 127.86, 127.96, 128.00, 128.11, 128.24, 128.77, 128.91 and 129.03 (ArCH), 139.21, 139.21 and 139.86 (3 × *ipso*-C of Bn rings), 140.05 (C-8), 150.85 (C-4), 153.62 (C-2) and 157.14 (C-6); *m*/*z* (FAB^+) 700 [$(M + H)^+$, 70%] and 91 (100).

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″-tris-*O*-[bis(benzyloxy)phosphoryl]-3′-*O*-α-D-mannopyranosyladenosine (34)

A solution of **33** (100 mg, 0.14 mmol), bis(benzyloxy)(diisopropylamino)phosphine (0.16 cm^3, 0.49 mmol) and imidazolium triflate[38] (103 mg, 0.47 mmol) in CH_2Cl_2 (3 cm^3) under N_2 was stirred for 1 h, after which time TLC (ethyl acetate–hexane, 7:3) indicated conversion to the trisphosphite (R_f 0.67). Water (1 drop) was added and the solution was cooled to −78 °C, whereupon MCPBA (144 mg, 0.50 mmol) was added. After 10 min 10% (w/v) aq. Na_2SO_3 (15 cm^3) and ethyl acetate (20 cm^3) were added and the mixture was allowed to warm to room temperature. The resulting organic layer was washed with 15 cm^3 each of saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried ($MgSO_4$), filtered and concentrated to give a clear oil which was subjected to flash chromatography (eluent chloroform–acetone, 9:1, then 4:1, then 3:2) to give the *title compound* as a colourless oil (119 mg, 56%); R_f 0.29 (ethyl acetate–hexane, 7:3); $[\alpha]_D^{20}$ −1.9 (*c* 1.1, in $CHCl_3$) (Found M^+, 1480.483. Calcd for $C_{79}H_{81}N_5O_{18}P_3$ $(M - H)^-$: 1480.478); δ_H ($CDCl_3$; 400 MHz) 3.53, 3.66 (2 H, ABX, $^2J_{AB}$ 10.9, $^3J_{AX}$ 3.2, $^3J_{BX}$ 2.5, 5′-H_A, 5′-H_B), 3.70 (1 H, ABX, $^2J_{AB}$ 10.8, $^3J_{AX}$ 5.9, 6″-H_A), 3.75–3.78 (1 H, m, 6″-H_B), 3.82–3.86 (1 H, m, 5″-H), 4.26–4.29 (1 H, m, 4′-H), 4.37–4.44 (5 H, m, 2″-H, 2 × OCH_2Ar), 4.54 and 4.64 (2 H, AB, J_{AB} 11.7, OCH_2Ar), 4.68 (1 H, t, *J* 4.7, 3′-H), 4.79–5.03 (14 H, m, 6 × OCH_2Ar, 3″-H, 4″-H), 5.30 (1 H, d, *J* 1.5, 1″-H), 5.44 (1 H, ddd, *J* 3.5, 3.5, $J_{H\text{-}P}$ 8.5, 2′-H), 5.86 (2 H, br s, NH_2), 6.22 (1 H, d, *J* 5.0, 1′-H), 7.11–7.32 (45 H, m, ArCH) and 8.00 and 8.23 (2 H, 2 s, 2-H, 8-H); δ_C ($CDCl_3$; 100.4 MHz) 69.24–70.34 (C-5′, C-6″, 6 × $POCH_2Ar$ with C-P coupling), 72.05 (C-5″ with C-P coupling), 72.87 (C-4″ with C-P coupling), 72.96, 73.60 and 73.88 (3 × OCH_2Ar), 74.49 (C-4″), 76.28 (C-3″ with C-P coupling), 76.77 (C-2″), 77.67 (C-2′), 82.27 (C-4′), 86.84 (C-1′ with C-P coupling), 97.75 (C-1″), 119.94 (C-5), 127.50, 127.59, 127.72, 127.87, 127.96, 127.97, 128.05, 128.08, 128.13, 128.20, 128.32, 128.39, 128.49, 128.61, 128.64, 128.69 and 128.73 (ArCH), 135.31–135.95 (6 × *ipso*-C of benzylphospho ring with C-P coupling), 137.42, 138.14 and 138.39 (3 × *ipso*-C of Bn rings), 139.15 (C-8), 149.92 (C-4), 153.08 (C-2) and 155.38 (C-6); δ_P ($CDCl_3$; 162 MHz; ^1H decoupled) −1.30, −0.80 and −0.35 (3 s); *m*/*z* (FAB^+) 1480 [$(M + H)^+$, 3%] and 91 (100).

3-*O*-α-D-Mannopyranosyladenosine 2′,3″,4″-trisphosphate (*manno*-adenophostin) (7)

A mixture of **32** (61 mg, 0.04 mmol) and wet 20% palladium hydroxide on carbon (180 mg), in methanol (7.2 cm^3), cyclohexene (3.6 cm^3) and water (0.7 cm^3) was heated under reflux for 2.5 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was washed copiously with methanol and water. Concentration of the filtrate afforded a clear residue which was applied to an MP1 AG ion exchange resin column and eluted with a gradient of 0–100% 150 mmol dm^{-3} aq. TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20 °C) gave the desired product as the free acid (19 mg, 71%), which was dissolved in water and eluted through a short column of Na^+ Diaion WK-40 ion exchange resin to give, after concentration, the sodium salt (Found: M^-, 668.039. Calcd for $C_{16}H_{25}N_5O_{18}P_3$ $(M - H)^-$: 668.040); δ_H (D_2O; 400 MHz) 3.58–3.72 (5 H, m, 5′-H_A, 5′-H_B, 5″-H, 6″-H_A, 6″-H_B), 4.15–4.24 (3 H, m, 2′-H, 4′-H, 4″-H), 4.39–4.48 (2 H, m, 3′-H, 3″-H), 4.98 (1 H, s, 1″-H), 5.08–5.14 (1 H, m, 2′-H), 6.11 (1 H, d, *J* 6.4, 1′-H) and 8.24 and 8.33 (2 H, 2 s, 2-H, 8-H); δ_P (D_2O; 162 MHz; ^1H decoupled) 0.12, 0.49 and 0.86 (3 s); λ_{max} (H_2O) 259 nm, ε 15 400, pH 7.5; *m*/*z* (FAB^-) 668 [$(M - H)^-$, 100%].

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