



D-*myo*-Inositol-1,4,5-trisphosphate and Adenophostin Mimics: Importance of the Spatial Orientation of a Phosphate Group on the Biological Activity

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Abstract—Three different routes for the synthesis of heterocyclic analogues of the second messenger D-*myo*-inositol-1,4,5-trisphosphate (InsP₃) and the natural adenophostins, starting from allyl D-xyloside are described. The two diastereoisomers at C-2 of new compounds, which we named xylophostins, were obtained. The preliminary biological studies shows that the presence of the adenine residue has a beneficial effect on the affinity for the receptor. The low potency of one of the two diastereoisomeric compounds shows that the configuration of the carbon bearing the non-vicinal phosphate group is an important requirement for a high affinity to the receptor. These results provide evidence for the existence of a binding pocket for the adenine ring nearby the InsP₃ binding site. The consequence of these stabilizing interactions should be to place the phosphate group in a suitable position to perfectly mimic InsP₃ in the more active diastereoisomer. Obviously, in the other diastereoisomer, the phosphate cannot accommodate the same orientation, thus explaining the low affinity. The existence of such a binding pocket for adenine is in line with the high potency of adenophostins. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

The understanding and the control of cell signaling pathways is now the subject of intense interest. One of the most important pathways is the phosphoinositide one in which D-*myo*-inositol-1,4,5-trisphosphate (InsP₃) plays a pivotal role. This second messenger is released from phosphatidyl-inositol-4,5-bisphosphate (PIP₂), a phospholipid anchored in the internal layer of cell membrane. This release is mediated by a specific phospholipase PLC activated by a G-protein coupled to an external receptor which binds the first messenger such as hormones, neurotransmitters etc. Hydrolysis of PIP₂ produces InsP₃ and another lipidic second messenger diacylglycerol (DAG). InsP₃ binds to a specific receptor (InsP₃R) located on the endoplasmic reticulum and liberates calcium from internal stores.^{1,2} This calcium release is responsible for the cell response by activation of calcium sensitive proteins such as calmodulin. DAG has its own action on cell response and both actions are often difficult to separate and to study independently. It is obvious that agonists and antagonists of InsP₃R are

of importance to study the receptor pharmacology, and compounds derived from these studies would be of interest as therapeutics to stimulate or block cells responses. In this context, the search for new analogues of InsP₃ has become a growing field (Fig. 1).^{3–7} Until recently, most of the analogues were based on modification of inositol derivatives. Some years ago, we introduced new sugar derivatives as mimics of InsP₃. Our analogues were based on D-xylose hypothesizing that the pyranose ring would mimic the inositol ring.⁸ Given the very unimportant role, if any, of the 2- and 3-hydroxyl groups in the biological activity of InsP₃, it was reasonable to take into account the three phosphates and the 6-OH. A good target was then the trisphosphate **8** derived from D-xylose. This compound proved unstable and we decided to move the phosphate group away from the anomeric position. This led to the preparation of trisphosphate **9**.⁸ In the mean time, an important discovery was made during screening of micro-organism metabolites by Japanese industry.^{9–11} A series of highly potent agonists of InsP₃R, the adenophostins was then available.^{12,13}

These compounds were immediately chosen as targets for total synthesis^{14–16} and as models for developing

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other analogues. Thus, the gluco analogue **4** was prepared by Potter^{17,18} and Gigg¹⁹ and exhibited almost the same biological activity as **9** in terms of affinity and ability to release Ca^{++} .^{19,20} Very recently more elaborated compounds including the ribose ring of adenophostin like **6**^{21,22} and **7**²³ have been reported together with an open chain derivatives **5** having the adenine ring.^{24,25} In continuation with our own program, we tried to enhance the affinity of our lead compound **9** by taking into account structural informations provided by the adenophostins.

We report here our efforts in the synthesis of analogues **10** and **11**, which differ only by the stereochemistry at C-2 of the propyl tether between the xylose ring and the adenine but show striking different biological properties.

Results and Discussion

Most of the potency of InsP_3 analogues is related to the presence of the *trans* bisphosphate moiety and the hydroxyl group at C-6 (inositol numbering). The third phosphate only enhances the affinity for the receptor. In the adenophostins, the ribose ring should play the role of a scaffold, presenting the adenine moiety and the phosphate group in a proper spatial arrangement. In this context, the stereochemistry at C-2, which bears the phosphate group should be important.

First approach

Our first synthesis began with the allylglycoside **12** protected at position 2 (carbohydrate numbering) with a benzyl group. Conventional epoxidation of **12** with mCPBA gave the expected epoxide as a 7:3 diastereomeric mixture

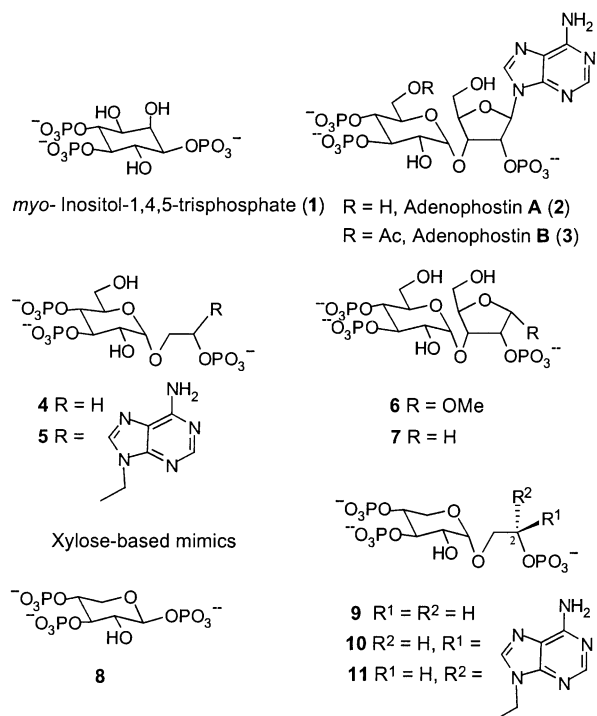
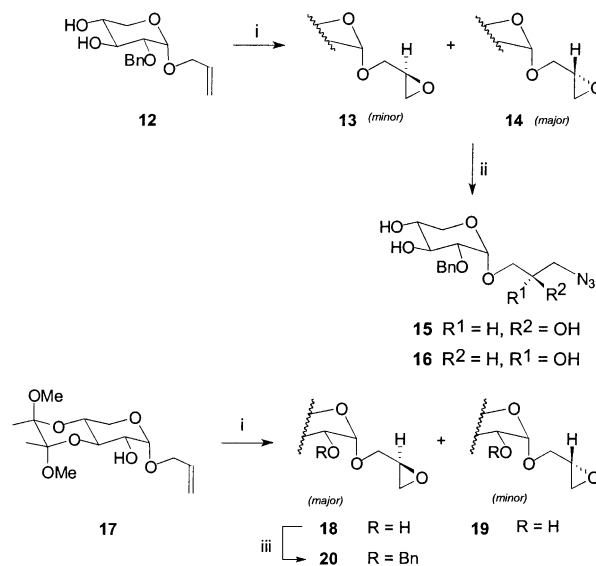


Figure 1. InsP_3 and some analogues.

of **14** and **13**. Although some diastereoselectivity was observed, epoxides **13** and **14** could not be efficiently separated using preparative high-pressure chromatography. In order to modify the diastereoselectivity of the epoxidation step, we changed the allylglycoside precursor and compound **17** bearing a free hydroxyl group at position 2 was used. Treatment of **17** with mCPBA afforded epoxide **21** as a 7:3 mixture of diastereoisomers. Further investigation on this mixture led to the establishment of the (*S*) configuration of the major epoxide, which corresponds to the one of adenophostin (see structural determination). This prompted us to use it as follows (Scheme 1).

The mixture of epoxides **21** was benzylated and the resulting mixture was treated with the sodio derivative of adenine to give a mixture of *N*-9 and *N*-7 regioisomers (purine numbering) in a 2:1 ratio from which the *N*-9 substituted derivative **23** was separated by column chromatography. The remainder of the synthesis consisted in the removal of the cyclic acetal group and phosphorylation of the hydroxyl functions using 2-(*N,N*-diisopropylamino)-5,6-benzo-1,3,2-dioxaphosphepane (**41**) followed by oxidation of the corresponding phosphites with *t*-butyl hydroperoxide giving the protected derivative **25**. Deprotection of all benzyl-protecting groups was cleanly achieved by catalytic hydrogenation over Pd (10%) on charcoal under 20 bars to give phosphates **26** isolated as the sodium salt. This compound was actually a 3:7 mixture of **10** and **11**. This mixture was tested for its affinity towards InsP_3R and gave encouraging results, which prompted us to find more stereospecific routes to each compounds (*R* and *S* isomers) (Scheme 2).

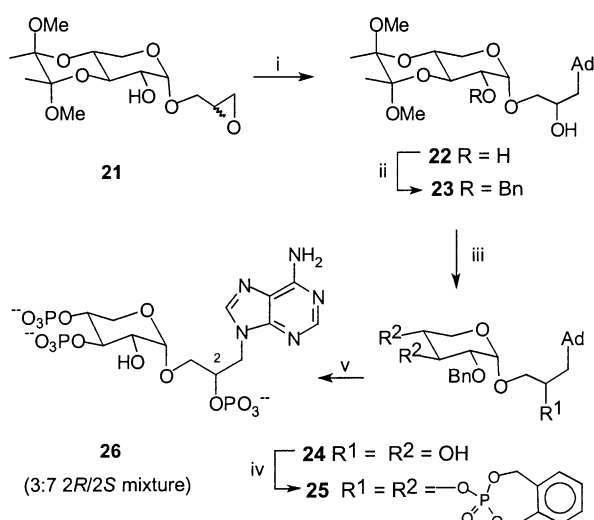


Scheme 1. Reagents: (i) mCPBA, CH_2Cl_2 ; (ii) NaN_3 , $\text{CH}_3\text{OCH}_2\text{CH}_2\text{OH}/\text{H}_2\text{O}$, NH_4Cl ; (iii) NaH, BnBr, DMF.

Second approach

In a second approach, we started from allyl ether **12** with the hope to introduce the diol function in a diastereoselective manner by taking advantage of the

asymmetric dihydroxylation (AD). If dihydroxylation of olefin **12** with AD-mix β led to almost 1:1 mixture of the diastereoisomeric diols, it was found that the protection of the two hydroxyl groups of **12** was beneficial for the diastereoselectivity.²⁶ Using modified Sharpless conditions²⁷ it was possible to carry out the AD of the bisbenzoate **27**²⁶ with an 8.1:1 diastereoisomeric ratio. The resulting diol mixture was selectively tosylated at the primary alcohol to yield compound **29** that was treated as before with the sodio derivative of adenine. Again a mixture of *N*-9 and *N*-7 derivatives in a 3.5:1 ratio was obtained. Column chromatography on silica gel allowed nice separation of the two positional isomers and gave the *N*-9 derivative **30**, which was almost diastereomerically pure. Less than 5% of the other diastereoisomer at C-2 was detectable on ¹H and ¹³C NMR spectra. Reaction of **30** with sodium methoxide gave the



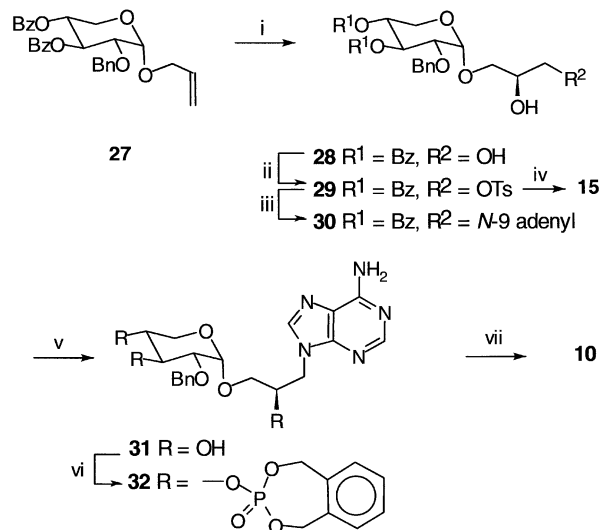
Scheme 2. Reagents: (i) adenine, NaH, DMF, 90 °C; (ii) BnBr, NaH, DMF; (iii) CH₂Cl₂/CF₃COOH/H₂O 20:9.5:0.5; (iv) **41**, 1*H*-tetrazole, CH₂Cl₂ then *t*BuOOH, 0 °C; (v) H₂, Pd/C (10%), 20 bars, MeOH.

triol **31** and phosphitylation of **31** using (**41**) followed by in situ oxidation of the corresponding phosphites gave the protected phosphate derivative **32**. Deprotection of all the benzyl protecting groups gave compound **10** isolated as its hexasodium salt (Scheme 3).

Structure determination

In order to determine the diastereoselection of each epoxidation reaction, the mixture of epoxides **14** and **13** was opened with sodium azide giving a mixture of the expected azido triols from which pure major isomer **15** was obtained by column chromatography. Epoxides **18** and **19** cannot be completely separated by preparative high-pressure chromatography, however small amounts of the major compound **18** were obtained. Compound **20** was obtained by protection at position 2 of **18** with a benzyl group. As before, treatment of **18** with sodium azide followed by removal of the cyclic acetal group gave the azido triol **16**. ¹H and ¹³C NMR analyses showed that compounds **15** and **16** were diastereoisomers. Not unexpectedly, the presence of the hydroxyl

group at C2 directs the epoxidation from one face of the olefinic bond, by formation of an hydrogen bond with the peracid.^{28,29} The presence of a benzyl group at O-2 reverts this diastereoselectivity. The benzyl group then shielded one face of the double bond so that epoxidation should take place from the other face. In each case the formation of a 7:3 mixture should result from two different conformations of the *O*-allyl group around the C–O bond. We have undisputedly established the stereochemical assignment by chemical correlation as follows. The 2(*R*) configuration of the major product **28** of the AD reaction has been established by degradation of the glycerol aglycon and comparison with literature data.²⁶ This configuration allowed us to establish the stereochemistry of the above described compounds. Tosylate **29** was treated with sodium azide to provide the expected azido derivative, which was debenzoylated to provide the corresponding triol of 2(*R*) configuration **15**. This compound was identical to the major product **15** resulting from the first epoxide route. It was concluded that the diastereomeric mixture of epoxide **21** obtained in our first approach contained about 70% of the 2(*S*) diastereoisomer.



Scheme 3. Reagents: (i) AD-mix β , PYR(DHQD)₂, *t*BuOH/H₂O; (ii) TsCl, pyridine; (iii) adenine, NaH, DMF, 90 °C; (iv) NaN₃, DMF, 90 °C; (v) MeONa, MeOH; (vi) **41**, 1*H*-tetrazole, CH₂Cl₂ then *t*BuOOH, 0 °C; (vii) H₂, Pd/C (10%), 20 bars, MeOH.

Third approach

The second approach allowed us to obtain compound **10** contaminated with less than 5% of the other diastereoisomer (NMR determination). Given the lower affinity

Table 1. Inhibition of [³H]InsP₃ binding to cerebellar microsomes by the different compounds^a

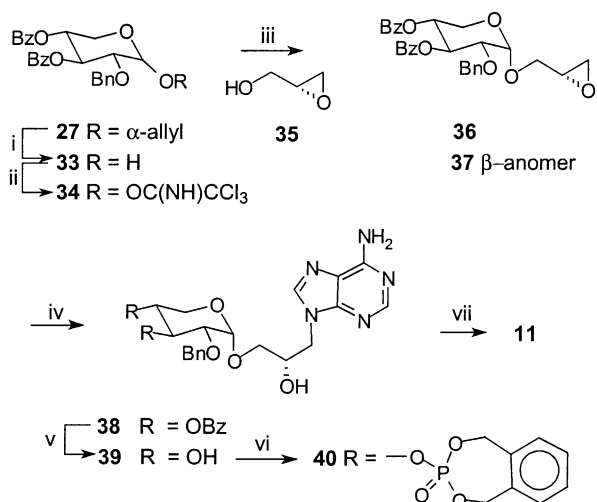
Compounds	IC ₅₀ (μM)
InsP ₃	0.018
9	0.43
10	3
26	0.087
11	0.066

^aData are means of triplicate determinations in two different experiments.

of the 2(*R*) isomer **10** for the receptor (see Table 1), it was clear that the 2(*S*) isomer having the absolute configuration of the ribose moiety of the adenophostin models should have a good affinity for the receptor. Thus we searched for a new route to this 2(*S*) isomer starting from the allyl glycoside **27** which was deallylated using the catalytic procedure developed by Liu³⁰ to provide the free hemiacetal **33** in 68% yield. Activation of the hemiacetalic hydroxyl via the Schmidt procedure³¹ gave the trichloroacetimidate **34** as an anomeric mixture in 86% yield, the kinetically favored β anomer being formed preferentially under these conditions β/α 3/1. No attempts were made to separate these imidates, which were reacted at -20°C with the enantiomerically pure (*R*) (+) glycidol **35** using trimethylsilyl triflate as the promoter. A mixture of anomeric glycosides **36** and **37** was obtained in a 1.1:1 ratio in favor of the α anomer (Scheme 4).

The remainder of the synthesis from these epoxides was identical to that described above: opening of the epoxide ring with adenine anion, which gave the expected alcohols in 57% yield. Careful chromatography allowed the separation of the *N*-9 and *N*-7 isomers formed in a 3:1 ratio. Zemplén debenzoylation of the pure α anomer **38** gave compound **39** as a pure stereoisomer. Phosphorylation of this compound gave the protected trisphosphate **40** in 74% yield and removal of the benzyl protecting groups proceeded uneventfully to yield pure **11**, which was isolated as its hexasodium salt.

The binding efficiency of the compounds was assayed in equilibrium competition binding experiments to cerebellar membranes with [³H]InsP₃. The analogues completely inhibited specific InsP₃ binding (Fig. 2). Concentrations that inhibited 50% of [³H]InsP₃ binding (IC₅₀) are given in Table 1. Data indicate that the order of potency is **11** > **26**. InsP₃ is about 4 times more efficient than **11**. The compound **10** also binds InsP₃R but with a much lower affinity.



We also tested the ability of the different compounds to release ⁴⁵Ca²⁺ from permeabilized hepatocytes (Fig. 3). Compounds **11** released almost 100% of ⁴⁵Ca²⁺ at a 10^{-5}M concentration. Compound **10** was unable to empty calcium stores at the maximal tested concentration (10^{-4}M). The half maximal response for the **11** (EC₅₀) was $465 \times 10^{-9} \times \text{M}$ and those for **26** was $858 \times 10^{-9} \times \text{M}$. This is to be compared with the EC₅₀ for InsP₃ that was $89 \times 10^{-9} \text{M}$. The rank order of potency of the ligands (InsP₃ > **11** > **26** > **10**) was therefore the same as that found with radioligand binding. The most striking feature of these results is the high activity of the (*S*) isomer **11** as compared to the (*R*) one **10**. This absolute configuration is identical to that of the natural product adenophostin A. It is well known that the two phosphates at C-4 and C-5 in a *trans* arrangement are needed for the biological activity.³² The results described here clearly show the importance of the spatial orientation of the third phosphate group and show that the presence of the adenine group strongly enhances the potency of compound **11** as compared to our previously described analogue **9**.⁸ The low potency of one of the two diastereoisomeric compounds shows that the configuration of the carbon bearing the non-vicinal phosphate group is an important requirement for a high affinity to the receptor and provides evidences for interactions of the purine base with a binding pocket nearby the InsP₃ binding site. These interactions should place the phosphate group in a suitable position to perfectly mimic InsP₃. In the other diastereoisomer, for several reasons, the phosphate cannot adopt the same orientation thus explaining the low affinity. One reason should be that stabilizing interactions of the adenine ring with a

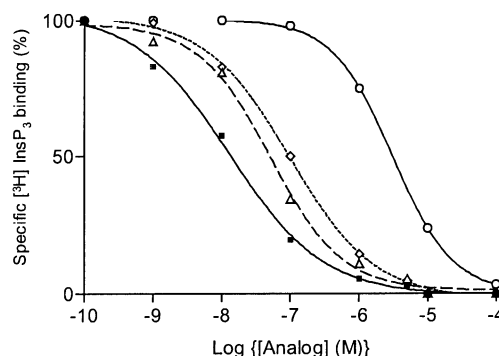


Figure 2. Inhibition of specific [³H] InsP₃ binding to rat cerebellar microsomes by increasing concentrations of inositol phosphates analogues: ■ for InsP₃ (1); △ for **11**; ○ for **10** and ◇ for **26**.

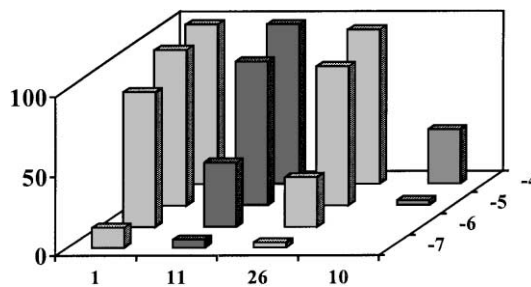


Figure 3. Analogue-induced [⁴⁵Ca²⁺] release from permeabilized hepatocytes. Each data point represents the mean of at least three determinations.

possible binding pocket takes place and directs the phosphate far from its receptor site. The existence of such a binding pocket for adenine is in line with the high potency of adenosine phosphates and all analogues having the adenine ring.³³

Conclusion

One can conclude that the very high affinity of adenosine phosphates for InsP₃R should be not only the consequence of favorable interactions of the adenine ring at the binding site. It is likely that the rigid adenosine ring system largely contributes to a fine adjustment of the phosphate position, which mimics even better that of the 1-phosphate of InsP₃. The exact nature of adenine interactions, likely hydrophobic ones,^{13,34,35} at the binding site should be clarified by synthesis of adenosine phosphates analogues modified on the purine base.³⁸

Experimental

General procedures

Optical rotations were determined at 20 °C with a Perkin-Elmer model 141 automatic. NMR spectra were recorded at 25 °C with a Bruker AC250 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal tetramethylsilane for ¹H NMR and indirectly to the central line of CDCl₃, δ 77.03 for ¹³C NMR spectra; those in deuterium oxide are reported relative to external 2,3 dimethyl-2-silapentane-5 sulfate (DSS). Infrared spectra were recorded on a Perkin-Elmer IRFT Spectrum 1000 spectrometer. All reactions were monitored by thin-layer chromatography on Kieselgel 60F₂₅₄ (Merck) with detection by UV light and/or by charring with 15% sulfuric acid in ethanol. Elemental analyses and mass spectra were performed by the Service Central d'Analyse du CNRS at Vernaison (France). [³H]InsP₃ (17–21 Ci/mmol) was obtained from Du Pont New England Nuclear and InsP₃ from Calbiochem.

(3-Oxypropyl) 3,4-O-[(2S,3S) (2,3-dimethoxy butane-2,3-dyl)]- α -D-xylopyranoside (21). Compound **17** (12 g, 39.5 mmol) and 50% *m*CPBA (20.44 g, 59.2 mmol) in CH₂Cl₂ (300 mL) was stirred at room temperature for 24 h. The reaction mixture was diluted with CH₂Cl₂ (400 mL) and then washed with H₂O, saturated aqueous NaHCO₃ solution and H₂O. The organic layer was dried (MgSO₄) and evaporated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 3:4) to give compound **21** (9.85 g, 78%) as a 3/7 diastereoisomeric mixture of **19** and **18**.

(3-Oxypropyl) 2-O-benzyl-3,4-O-[(2S,3S) (2,3-dimethoxybutane-2,3-dyl)]- α -D-xylopyranoside (22). A solution of **21** (8 g, 25 mmol) in dry DMF (50 mL) was added dropwise to a NaH (1.1 g of a 60% dispersion in oil, 27.5 mmol) suspension in dry DMF (50 mL). The mixture was stirred at room temperature for 15 min, and

then benzyl bromide (3 mL) was added. After 1 h, excess NaH was destroyed by addition of MeOH, and the solvents were removed by evaporation under reduced pressure. The residue was diluted with CH₂Cl₂ (500 mL) and washed with water (2 $\times$ 50 mL). The organic layer was dried over MgSO₄ and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 3:2) to give **22** (8.6 g, 86%).

[2-Hydroxy-3-(6-amino-9H-purin-9-yl)]-propyl 2-O-benzyl-3,4-O-[(2S,3S) 2,3-dimethoxybutane-2,3-dyl]- α -D-xylopyranoside (23). To a solution of adenine (2.025 g, 15 mmol) in DMF (70 mL) was added sodium hydride (60% dispersion in mineral oil, 600 mg, 15 mmol). The mixture was stirred at 80 °C under inert atmosphere for 90 min then a solution of **22** (2.05 g, 5 mmol) in DMF (15 mL) was added dropwise. After 3 h of heating, the reaction was cooled to room temperature and filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was diluted with CH₂Cl₂ (500 mL). The organic layer was washed twice with water and dried over MgSO₄ and concentrated. The residue was purified by column chromatography (CH₂Cl₂/MeOH 9:1) to give compound **23** (2.043 g, 75%).

[3-(6-Amino-9H-purin-9-yl)-2-(phosphonooxy)]propyl 3,4-bis-O-(3,4-dihydro-3-oxido-2,4,3-benzodioxaphosphin-3-yl)- α -D-xylopyranoside (25). To a solution of **23** (2 g, 3.7 mmol) in CH₂Cl₂ (20 mL) was added 95% aqueous TFA (10 mL). The mixture was stirred at room temperature for 10 min and then concentrated under reduced pressure to give the desired triol **24** in 82% yield. The crude residue (1.3 g, 3 mmol) was dissolved in CH₂Cl₂ (40 mL) then 1*H*-tetrazole (2.52 g, 36 mmol) and 2(*N,N*-diisopropylamino)-5,6-benzo-1,3,2 dioxaphosphane (**41**) (6 g, 22.5 mmol) were added to the solution. The reaction mixture was stirred under argon at room temperature for 4 h then cooled to 0 °C and *t*BuOOH (70% solution in water, 10 mL) was added. The solution was allowed to warm to room temperature and was then stirred for 15 min. The clear solution was diluted with CH₂Cl₂ (250 mL) and this solution was washed successively with water (10 mL), saturated aqueous NaHCO₃ solution (2 $\times$ 10 mL) and water (2 $\times$ 10 mL), dried over MgSO₄ and concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (CH₂Cl₂/MeOH 99:1 then 94:6) to afford **25** (2.230 g, 76%) as a white solid.

[3-(6-Amino-9H-purin-9-yl)-2-(phosphonooxy) propyl]- α -D-xylopyranoside 3,4-bisphosphate hexasodium salt (26). Compound **25** (230 mg, 0.23 mmol) was dissolved in MeOH (25 mL) and Pd/C (10%, 200 mg) was added. The mixture was stirred under hydrogen atmosphere (20 bars) during 12 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness. The residue was dissolved in water (3 mL) and applied to a column of Bio-Rad Chelex 100 resin (Na⁺ form). The column was eluted with water, fraction containing compound **26** were combined and freeze dried (157 mg, 94%).

[(2S) 3-Oxypropyl] 3,4-O-[(2S,3S) (2,3-dimethoxybutane-2,3-dyl)]- α -D-xylopyranoside (18). Small amounts of **18** was obtained from **21** by column chromatography. R_f 0.44 (EtOAc/hexane, 1:9); $[\alpha]_D + 261.4$ (c 0.96 in chloroform); ^1H NMR (250 MHz, CDCl_3) δ 4.88 (d, $^3J_{(1,2)} = 3$ Hz, 1H; H-1), 4.68 (s, 2H; CH_2Ph), 3.52–3.91 (m, 7H; H-2, H-3, H-4, H-5b, H-5a, H-1'a, H-1'b), 3.30 (s, 3H; OCH_3), 3.26 (s, 3H; OCH_3), 3.20 (m, 1H; H-2'), 2.84 (dd, $^3J_{(2',3'a)} = 4.4$ Hz, $^3J_{(3'a,3'b)} = 5.1$ Hz, 1H; H-3'a), 2.66 (dd, $^3J_{(2',3'b)} = 2.2$ Hz, 1H; H-3'b), 2.14 (d, $^3J_{(\text{H},\text{OH})} = 9.5$ Hz 1H; OH), 1.35 (s, 3H; CH_3), 1.30 (s, 3H; CH_3). ^{13}C NMR (62.9 MHz, CDCl_3) δ 99.6, 99.2 (CH_3COCH_3), 98.9 (C-1), 70.4, 69.6, 65.7 (C-2, C-3, C-4), 68.4 (C-1'), 59.8 (C-5), 49.8 (C-2'), 47.7 (OCH_3), 44.7 (C-3'), 17.5 and 17.1 (CH_3). $\text{C}_{14}\text{H}_{24}\text{O}_8$ (320.3): calcd C 52.5, H 7.6, Found C 52.62, H, 7.42.

[(2S) (3-Oxypropyl)] 2-O-benzyl-3,4-O-[(2S,3S) (2,3-dimethoxybutane-2,3-dyl)]- α -D-xylopyranoside (20). To a solution of **18** (176 mg, 0.55 mmol) in dry THF (3 mL) was added NaH (22 mg of a 60% dispersion in oil, 0.6 mmol). The mixture was stirred at room temperature for 15 min, and then benzyl bromide (70 μL) was added. After 1 h, excess NaH was destroyed by addition of MeOH, and the solvents were removed by evaporation under reduced pressure. The residue was diluted with CH_2Cl_2 (50 mL) and washed with water (2×5 mL). The organic layer was dried over MgSO_4 and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc 3:2) to give **20** (205 mg, 91%). R_f 0.39 (hexane/EtOAc, 3:2); $[\alpha]_D + 166.4$ (c 0.93 in chloroform); ^1H NMR (250 MHz, CDCl_3) δ 7.40–7.19 (m, 5H, Ar), 4.85 (d, $^2J_{(\text{H},\text{H})} = 12.4$ Hz; 1H; CH_2Ph), 4.72 (d, $^3J_{(1,2)} = 3.6$ Hz, 1H; H-1), 4.65 (d, 1H; CH_2Ph), 4.09 (dd, $^3J_{(3,4)} = 9.4$ Hz, H-3), 3.82–3.63 (m, 3H, H-4, H-5a, H-1'a), 3.62–3.44 (m, 3H, H-2, H-5b, H-1'b), 3.30 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.16 (m, 1H, H-2'), 2.75 (dd, $^3J_{(2',3'a)} = 4.4$ Hz, $^3J_{(3'a,3'b)} = 4.7$ Hz, 1H, H-3'a), 2.65 (dd, $^3J_{(2',3'b)} = 2.9$ Hz, 1H, H-3'b), 1.36 (s, 3H, CH_3), 1.31 (s, 3H, CH_3). ^{13}C NMR (62.9 MHz, CDCl_3) δ 138.5 (Ar ipso), 128.1, 127.4 (Ar), 99.5, 99.3 (CH_3COCH_3), 98.0 (C-1), 76.4 (C-2), 73.2 (CH_2Ph), 69.8 (C-3), 68.0 (C-1'), 66.3 (C-4), 59.3 (C-2'), 47.8 (OCH_3), 44.5 (C-3'), 17.7 and 17.4 (CH_3). $\text{C}_{21}\text{H}_{30}\text{O}_8$ (410.46): calcd C 61.5, H 7.4, found C 61.38, H, 7.32

[(2R) (2-Hydroxy-3-tosyloxy)propyl] 2-O-benzyl-3,4-di-O-benzoyl- α -D-xylopyranoside (29). Compound **AU**²⁶ (750 mg, 1.43 mmol) was dissolved in dry pyridine (20 mL) and tosyl chloride (300 mg, 1.57 mmol) was added. The mixture was stirred for 2 h at room temperature then concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (200 mL) and washed with aqueous HCl (3 N) and H_2O . The organic layer was dried over MgSO_4 and concentrated. The residue was purified by column chromatography (hexane/EtOAc 4:1 then 1:1) to give **29** (712 mg, 73%). R_f 0.57 (H / A 1:1); ^1H NMR (250 MHz, CDCl_3) δ 7.97 (m, 4H; Ar), 7.70 (m, 2H; Ar), 7.50 (m, 4H; Ar), 7.35 (m, 5H; Ar), 7.20 (m, 4H; Ar), 5.84 (dd, 1H; H-3), 5.19 (ddd, $^3J_{(3,4)} = 10$ Hz, $^3J_{(4,5a)} = 5.5$ Hz, $^3J_{(4,5b)} = 5.5$ Hz, 1H; H-4), 4.87 (d, 1H; H-1), 4.57 (s, 2H; CH_2Ph), 4.10 (m, 1H; H-1'a), 3.93 (dd, 1H; H-5a), 3.85 (m, 2H; H-2', H-3'a),

3.73 (dd, $^2J_{(5a,5b)} = 10$ Hz, 1H; H-5b), 3.63 (dd, $^3J_{(1,2)} = 3.5$ Hz, $^3J_{(2,3)} = 10$ Hz, 1H; H-2), 3.69 (m, 1H; H-3'b), 3.46 (dd, $^2J_{(1'a,1'b)} = 10$ Hz, $^3J_{(1'a,2')} = 7$ Hz, 1H; H-1'b), 2.41 (s, 3H; CH_3); ^{13}C NMR (62.9 MHz, CDCl_3) δ 165.5, 165.4 (C=O), 132.4, 144.8 (Ar ipso), 128.8, 129.4 (Ar ipso), 127.7, 127.8, 128.2, 128.2, 129.5, 129.7, 132.4, 132.9 (Ar), 97.5 (C-1), 69.2, 70.0 (C-1', C-3'), 67.8, 69.9, 71.2, 76.7 (C-2, C-3, C-4, C-2'), 58.6 (C-5), 21.3 (CH_3); $\text{C}_{36}\text{H}_{36}\text{O}_{11}\text{S}$ (676.20): calcd C 63.89, H 5.37, S 4.73; found C 63.98, H 5.35, S 4.75.

[(2R) [2-Hydroxy-3-(6-amino-9H-purin-9-yl)]-propyl] 3,4-di-O-benzoyl-2-O-benzyl- α -D-xylo-pyranoside (30). To a solution of adenine (398 mg, 2.94 mmol) in DMF (6 mL) was added sodium hydride (60% dispersion in mineral oil, 113 mg, 2.94 mmol). The mixture was stirred at 80 °C under inert atmosphere for 90 min then a solution of **29** (710 mg, 0.98 mmol) in DMF (5 mL) was added dropwise. After 3 h of heating, the reaction was cooled to room temperature and filtered through Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1) to give compound **30** (420 mg, 63%). R_f 0.55 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9:1); ^1H NMR (250 MHz, CDCl_3) δ 8.29 (s, 1H; H-2''), 7.96 (m, 4H; Ar), 7.89 (s, 1H; H-8''), 7.51 (m, 2H; Ar), 7.38 (m, 5H; Ar), 7.20 (m, 4H; Ar), 5.93 (dd, $^3J_{(2,3)} = 10$ Hz, 1H; H-3), 5.84 (m, 2H; NH_2), 5.23 (ddd, $^3J_{(3,4)} = ^3J_{(4,5)} = 10$ Hz, 1H; H-4), 4.90 (d, $^3J_{(1,2)} = 3$ Hz, 1H; H-1), 4.60 (s, 2H; CH_2Ph), 4.41 (m, 1H; H-3'a), 4.30 (m, 2H; H-2', H-3'b), 3.96 (dd, $^3J_{(4,5a)} = 6$ Hz, $^2J_{(5a,5b)} = 11$ Hz, 1H; H-5a), 3.82 (dd, $^3J_{(1'a,2')} = 4$ Hz, $^2J_{(1'a,1'b)} = 11$ Hz, 1H; H-1'a), 3.74 (m, 2H; H-2, H-5b), 3.41 (dd, $^3J_{(1'b,2')} = 6.5$ Hz, 1H; H-1'b); ^{13}C NMR (62.9 MHz, CDCl_3) δ 166.2, 165.9 (C=O), 156.0 (C-6''), 153.0 (C-2''), 150.1 (C-4''), 142.0 (C-8''), 137.2 (Ar ipso), 129.2, 128.9 (Ar ipso), 133.6, 133.5, 130.0, 128.6, 128.3, 128.2 (Ar), 119.2 (C-5), 97.8 (C-1), 73.3 (CH_2Ph), 70.6 (C-1'), 77.3, 71.7, 70.3, 68.8 (C-2, C-3, C-4, C-2'), 59.1 (C-5''), 47.1 (C-3'); $\text{C}_{34}\text{H}_{33}\text{N}_5\text{O}_8$ (639.3): calcd C 63.83, H 5.20, N 10.95; found C 63.95, H 5.18, N 10.92.

[(2R) [3-(6-Amino-9H-purin-9-yl)-2-hydroxy]propyl] 2-O-benzyl- α -D-xylopyranoside (31). Compound **30** (370 mg, 0.58 mmol) was dissolved in MeOH (50 mL) and a catalytic amount of sodium was added. After completion of the reaction, TLC monitoring, the mixture was neutralized using Amberlite IRA 120 H^+ . The resin was filtered off and the filtrate was concentrated. The residue was purified by column chromatography (EtOAc/MeOH 9:1) to afford the triol derivative **31** (165 mg, 66%). R_f 0.12 (EtOAc/MeOH, 9:1); ^1H NMR (250 MHz, CD_3OD) δ 8.46 (s, 1H; H-2''), 8.10 (s, 1H, H-8''), 7.37 (m, 5H, Ar), 4.70 (d, $^2J_{(\text{H},\text{H})} = 12$ Hz, 1H, CH_2Ph), 4.64 (d, $^3J_{(1,2)} = 3.5$ Hz, 1H, H-1), 4.57 (d, 1H, CH_2Ph), 4.30 (dd, $^3J_{(2',3'a)} = 3$ Hz, $^3J_{(3'a,3'b)} = 12$ Hz, 1H, H-3'a), 4.18 (dd, $^3J_{(2',3'b)} = 7$ Hz, 1H, H-3'b), 4.06 (m, 1H, H-2'), 3.70 (dd, $^3J_{(2,3)} = ^3J_{(3,4)} = 9$ Hz, 1H, H-3), 3.62 (dd, $^3J_{(1'a,1'b)} = 11$ Hz, $^3J_{(1'a,2')} = 4$ Hz, 1H, H-1'a), 3.42 (m, 2H, H-4, H-5a), 3.24 (dd, 2H, H-2, H-1'b), 3.18 (dd, $^3J_{(4,5b)} = 5.5$ Hz, $^3J_{(5a,5b)} = 10$ Hz, 1H, H-5b); ^{13}C NMR (62.9 MHz, CD_3OD) δ 157.2 (C-6''), 154.6 (C-2''), 150.8 (C-4''), 143.6 (C-8''), 139.6 (Ar ipso), 128.9 128.4,

128.2 (Ar), 119.8 (C-5), 98.9 (C-1), 74.2 (CH₂Ph), 70.7 (C-1'), 81.2, 74.2, 71.5, 69.5 (C-2, C-3, C-4, C-2'), 62.9 (C-5'), 47.6 (C-3'); C₂₀H₂₅N₅O₆ (431.20): calcd C 55.7, H 5.8, N 16.2; found C 55.95, H 5.81, N 16.04.

[(2R) [3-(6-Amino-9H-purin-9-yl)-2-(phosphonooxy)propyl] 3,4 bis-O-(3,4-dihydro-3-oxido-2,4,3-benzodi-oxa-phosphopin-3-yl)-α-D-xylopyranoside (32). The triol derivative **31** (160 mg, 0.37 mmol) was dissolved in CH₂Cl₂ (12 mL) then 1*H*-tetrazole (308 mg, 4.44 mmol) and 2(*N,N*-diisopropylamino)-5,6-benzo-1,3,2 di-oxa-phosphepane (**41**) (832 mg, 3.08 mmol) were added to the solution. The reaction mixture was stirred under argon at room temperature for 4 h then cooled to 0 °C and *t*BuOOH (70% solution in water, 1.5 mL) was added. The solution was allowed to warm to room temperature and was then stirred for 15 min. The clear solution was diluted with CH₂Cl₂ (100 mL) and this solution was washed successively with water (10 mL), saturated aqueous NaHCO₃ solution (2 × 10 mL) and water (2 × 10 mL), dried over MgSO₄ and concentrated by evaporation under reduced pressure. The residue was purified by column chromatography (CH₂Cl₂/MeOH 99:1 then 94:6) to afford **32** (252 mg, 70%) as a white solid. *R*_f 0.52 (CH₂Cl₂/MeOH, 9:1); ¹H NMR (250 MHz, CDCl₃) δ 8.42 (s, 1H; H-2''), 8.12 (s, 1H; H-8''), 7.26 (m, 17H; Ar), 5.42 (m, 4H; CH₂PhCH₂), 5.20 (m, 8H; CH₂PhCH₂), 4.75 (m, 1H; H-3), 4.68 (d, 1H; H-1), 4.63 (m, 4H; H-4, H-2', H-3'a, H-3'b), 4.59 (d, 1H; CH₂Ph), 4.46 (d, ²*J*_(H,H) = 12 Hz 1H; CH₂Ph), 4.08 (dd, ³*J*_(4,5a) = 6, ²*J*_(5a,5b) = 11 Hz, 1H; H-5a), 4.01 (dd, ³*J*_(1'b,2) = 1.5 Hz, 1H; H-1'b), 3.76 (dd, ³*J*_(4,5b) = 11 Hz, 1H; H-5b), 3.51 (dd, ³*J*_(1,2) = 4 Hz, ³*J*_(2,3) = 10 Hz, 1H; H-2), 3.25 (dd, ²*J*_(1'a,1'b) = 11 Hz, ³*J*_(1'a,2') = 6.5 Hz, 1H; H-1'a); ³¹P NMR (100.13 MHz, CDCl₃) δ -1.58, -1.49, 0.94; C₄₄H₄₆N₅O₁₅P₃ (977.80): calcd C 54.05, H 4.74, N 7.16; found C 54.22, H 4.72, N 7.18.

[(2R) 3-(6-Amino-9H-purin-9-yl)-2-(phosphonooxy)-propyl] α-D-xylopyranoside 3,4-bisphosphate hexasodium salt (10). Compounds **32** (80 mg, 0.08 mmol) was dissolved in MeOH (5 mL) and Pd/C (10%, 80 mg) was added. The mixture was stirred under hydrogen atmosphere (20 bars) for 12 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness. The residue was dissolved in water (3 mL) and applied to a column of Bio-Rad Chelex 100 resin (Na⁺ form). The column was eluted with water, fraction containing compound **10** were combined and freeze dried (55 mg, 98%). ¹H NMR (250 MHz, D₂O) δ 8.44 (s, 1H; H-2''), 8.36 (s, 1H; H-8''), 4.88 (d, ³*J*_(1,2) = 2.8 Hz, 1H; H-1), 4.60 (m, 3H, H-3'a, H-3'b, H-2'), 4.34 (ddd, ³*J*_(2,3) = 7.3 Hz, ³*J*_(3,4) = 7.7 Hz, ³*J*_(H,P) = 7.6 Hz, 1H; H-3), 4.08–3.57 (m, 5H; H-2, H-4, H-5a, H-5b, H-1'a), 3.52 (dd, ³*J*_(1'b,2') = 3.4 Hz, ³*J*_(1'a,1'b) = 11 Hz, 1H; H-1'b); ³¹P NMR (100.13 MHz, D₂O) δ 4.70, 4.17, 4.05. C₁₃H₁₆N₅O₁₅P₃Na₆ (713.15): calcd C 21.89, H 2.26, N 9.82; found C 21.95, H 2.25, N 9.79.

[(2R) 3-Azido-2-hydroxypropyl] 2-O-benzyl-α-D-xylopyranoside (15). To a solution of **29** (475 mg, 0.7 mmol) in DMF (10 mL) was added NaN₃ (91 mg, 1.4 mmol). The mixture was heated at 80 °C and stirred overnight.

The solvent was evaporated, the residue was diluted with CH₂Cl₂ (100 mL). The organic layer was washed with water, dried and concentrated under reduced pressure. The crude residue was debenzoylated using the Zemplen procedure to give after column chromatography (hexane/EtOAc 2:3) compound **15** (170 mg, 50%). *R*_f 0.50 (EtOAc); [α]_D +74.0 (*c* 0.36 in chloroform); ¹H NMR (250 MHz, CDCl₃) δ 7.35 (m, 5H; Ar), 4.72 (d, ³*J*_(1,2) = 3.5 Hz, 1H; H-1), 4.71 (d, ²*J*_(H,H) = 11.5 Hz, 1H; CH₂Ph), 4.64 (d, 1H; CH₂Ph), 3.99 (m, 1H; H-2'), 3.88 (dd, ³*J*_(2,3) = ³*J*_(3,4) = 9 Hz, 1H; H-3), 3.75 (dd, ²*J*_(1'a,1'b) = ³*J*_(1'b,2') = 3.5 Hz, 1H; H-1'a), 3.56 (m, 5H; H-4, H-5a, H-5b, 2 × OH), 3.31 (m, 5H; H-2, H-1'b, H-3'a, H-3'b, OH); ¹³C NMR (62.9 MHz, CDCl₃) δ 137.1 (Ar ipso), 128.6, 128.4 (Ar), 97.2 (C-1), 79.6 (C-2), 73.5 (CH₂Ph), 72.7 (C-3), 69.9 (C-5), 69.7 (C-2'), 69.4 (C-4), 61.4 (C-1'), 52.7 (C-3'), IR (neat): ν = 3379 (OH), 2101 (N₃)cm⁻¹; C₁₅H₂₁N₃O₆ (339.4): calcd C 53.07, H 6.24, N 12.39; found C 53.17, H 6.26, N 12.35.

[(2S) 3-Azido-2-hydroxypropyl] 2-O-benzyl-α-D-xylopyranoside (16). *R*_f 0.46 (EtOAc); [α]_D +76.5 (*c* 0.5 in chloroform); ¹H NMR (250 MHz, CDCl₃) δ 7.34 (m, 5H; Ar), 4.72 (d, ²*J*_(H,H) = 11.6 Hz, 1H; CH₂Ph), 4.65 (d, ³*J*_(1,2) = 3.6 Hz, 1H; H-1), 4.60 (d, 1H; CH₂Ph), 4.08–3.77 (m, 2H; H-3, H-2'), 3.71–3.43 (m, 4H, H-4, H-5a, H-5b, H-1'a), 3.42–3.18 (m, 4H; H-2, H-1'b, H-3'a, H-3'b), 1.72 (s, 3H, OH); ¹³C NMR (62.9 MHz, CDCl₃) δ 137.0 (Ar ipso), 128.4, 128.1 (Ar), 96.8 (C-1), 79.3 (C-2), 73.3 (CH₂Ph), 72.8 (C-3), 69.5 (C-4), 69.4 (C-1'), 69.1 (C-2'), 61.2 (C-5), 52.9 (C-3'), IR (neat): ν = 3390 (OH), 2101 (N₃)cm⁻¹; C₁₅H₂₁N₃O₆ (339.4): calcd C 53.07, H 6.24, N 12.39; found C 52.95, H 6.08, N 12.24.

3,4-Di-O-benzoyl-2-O-benzyl-D-xylopyranose (33). To a solution of **27** (1.22 g, 2.5 mmol) in dioxane/water (8:2, 23 mL) was added an aqueous TFA solution (0.15 M, 0.07 mL, 0.9 mmol) and 10% Pd/C (1.2 g). The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 7:3) to give an anomeric mixture of **33** (760 mg, 68%) as a white powder. Data for α anomer: *R*_f 0.35 (Hexane/EtOAc, 7:3); ¹H NMR (250 MHz, CDCl₃) δ 7.96 (dd, 4H; Ar), 7.60–7.46 (m, 2H; Ar), 7.45–7.31 (m, 5H; Ar), 7.28–7.05 (m, 4H; Ar), 5.91 (dd, ³*J*_(2,3) = 8.0 Hz, ³*J*_(3,4) = 8.8 Hz, 1H; H-3), 5.33–5.14 (m, 2H; H-1, H-4), 4.67 (s, 2H; CH₂Ph), 4.12–3.96 (m, 2H; H-5a, H-5b), 3.74 (dd, ³*J*_(1,2) = 3.6 Hz, ³*J*_(2,3) = 8.4 Hz, 1H; H-2), 3.41 (s, 1H; OH). ¹³C NMR (62.9 MHz, CDCl₃) δ 165.2, 165.1 (C=O), 137.1 (Ar ipso), 132.9 (Ar), 129.5, 128.2, 128.1, 127.8 (Ar), 91.0 (C-1), 76.3 (C-2), 72.7 (CH₂Ph), 70.4 (C-3), 69.2 (C-4), 59.4 (C-5). Data for β anomer: ¹H NMR (250 MHz, CDCl₃) δ 7.93 (m, 4H; Ar), 7.57–7.44 (m, 2H; Ar), 7.44–7.29 (m, 5H; Ar), 7.24–7.05 (m, 4H; Ar), 5.68 (dd, ³*J*_(2,3) = 8.8 Hz, ³*J*_(3,4) = 9.5 Hz, 1H; H-3), 5.29–5.14 (ddd, ³*J*_(4,5a) = 5.1 Hz, 1H; H-4), 4.93 (d, ³*J*_(1,2) = 6.6 Hz, 1H; H-1), 4.84 (d, ²*J*_(H,H) = 11.7 Hz, 1H; CH₂Ph), 4.72 (d, 1H; CH₂Ph), 4.31 (dd, ²*J*_(5a,5b) = 11.7 Hz, 1H; H-5a), 3.63–3.46 (m, 2H; H-2, H-5b), 1.67 (s, 1H; OH); ¹³C NMR (62.9 MHz, CDCl₃) δ 165.3, 165.2 (C=O), 137.3 (Ar ipso), 133.3, 133.1 (Ar), 129.5, 128.3, 128.1, 127.7 (Ar), 97.6 (C-1), 78.8 (C-2),

73.7 (CH₂Ph), 69.7 (C-3), 69.2 (C-4), 62.3 (C-5); IR (KBr): ν 3445 (OH), 1726 (C=O) cm⁻¹. C₂₆H₂₄O₇ (448.15): calcd C 69.62, H 5.40; found C 69.49, H 5.39.

O-(3,4-Di-O-benzoyl-2-O-benzyl-D-xylopyranosyl)trichloroacetimidate (34). To a solution of compound **33** (722 mg, 1.61 mmol) and Cl₃CCN (0.48 mL, 4.83 mmol) in dry CH₂Cl₂ (8 mL) was added freshly dried and powdered K₂CO₃ (222 mg, 1.61 mmol). The resulting suspension was stirred under argon at room temperature for 12 h. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc 85:15) to give a β/α mixture of **34** (820 mg, 86%) as a white powder. Data for β isomer: R_f = 0.25 (hexane/EtOAc, 85:15); $[\alpha]_D$ -52.6 (c 0.76 in chloroform), mp = 133–134 °C; ¹H NMR (250 MHz, CDCl₃) δ 8.78 (s, 1H; NH), 7.43–7.28 (m, 5H; Ar), 6.23 (d, ³ $J_{(1,2)}$ = 5.1 Hz, 1H; H-1), 5.70 (dd, ³ $J_{(2,3)}$ = 5.8 Hz, ³ $J_{(3,4)}$ = 6.6 Hz, 1H; H-3), 5.37–5.23 (ddd, ³ $J_{(4,5a)}$ = 3.6 Hz, 1H; H-4), 4.84 (d, ² $J_{(H,H)}$ = 11.7 Hz, 1H; CH₂Ph), 4.75 (d, 1H; CH₂Ph), 4.47 (dd, ² $J_{(5a,5b)}$ = 12.4 Hz, 1H; H-5a), 4.01–3.80 (m, 2H; H-2, H-5 b); ¹³C NMR (62.9 MHz, CDCl₃) δ 165.3 (C=O), 160.3 (C=NH), 136.8 (Ar ipso), 133.2, 133.1 (Ar), 128.3, 128.0, 127.8 (Ar), 97.0 (C-1), 90.6 (CCl₃), 74.7 (C-2), 73.4 (CH₂Ph), 69.6 (C-3), 68.3 (C-4), 61.7 (C-5); IR (KBr): ν 1673 (C=N), 1728 (C=O) cm⁻¹. MS (IC, CH₄): m/z : 592.4 [M⁺]; C₂₈H₂₄O₇NCl₃ (591.06): calcd C 56.85, H 4.09, N 2.37; found C 57.01, H 4.10, N 2.39.

[(2R) 3-Oxypropyl] 3,4-di-O-benzoyl-2-O-benzyl-D-xylopyranoside (36 and 37). A mixture of **34** (788 mg, 1.33 mmol) and (R)-(+)-glycidol **35** (0.1 mL 1.5 mmol) in CH₂Cl₂ (3.6 mL) was stirred for 45 min under an argon atmosphere in the presence of activated molecular sieves (4 Å, 200 mg). The reaction mixture was then cooled to -20 °C and a solution of TMSOTf (0.02 M in CH₂Cl₂, 1.3 mL) was added dropwise for 15 min. After 45 min, the reaction mixture was quenched with saturated aqueous NaHCO₃ solution. The mixture was filtered through Celite and the filtrate concentrated under reduced pressure. Purification by column chromatography (hexane/EtOAc 75:25) afforded compound **36** and **37** as an anomeric mixture (362 mg, 54% α/β ratio was 55:45 on the basis of its NMR spectrum). Data for **36**: ¹H NMR (250 MHz, CDCl₃) δ 7.94 (dd, 4H; Ar), 7.58–7.45 (m, 2H; Ar), 7.43–7.28 (m, 5H; Ar), 7.27–7.03 (m, 4H; Ar), 5.95 (dd, ³ $J_{(2,3)}$ = 9.5 Hz, ³ $J_{(3,4)}$ = 10.2 Hz, 1H; H-3), 5.22 (ddd, ³ $J_{(4,5a)}$ = 5.8 Hz, ³ $J_{(4,5b)}$ = 10 Hz, 1H; H-4), 4.93 (d, ³ $J_{(1,2)}$ = 3.7 Hz, 1H; H-1), 4.64 (2H; CH₂Ph), 3.98 (dd, ² $J_{(5a,5b)}$ = 11.0 Hz, 1H; H-5a), 3.92–3.80 (m, 2H; H-1'a, H-5b), 3.77–3.62 (m, 2H; H-1'b, H-2), 3.27 (dddd, 1H; H-2'), 2.88–2.77 (m, 2H; H-3'a, H-3'b); ¹³C NMR (62.9 MHz, CDCl₃) δ 165.3, 165.2 (C=O), 137.2 (Ar ipso), 133.2, 133.1, 129.8, 129.7, 128.5, 128.3, 128.2, 128.1, 127.9 (Ar), 96.8 (C-1), 76.5 (C-2), 72.4 (CH₂Ph), 70.9 (C-3), 70.0 (C-4), 67.5 (C-1'), 58.4 (C-5), 50.2 (C-2'), 44.0 (C-3'); IR (KBr): ν 1727 (C=O) cm⁻¹; MS (IC, CH₄): m/z : 503.6 (M-1). Data for **37**: ¹H NMR (250 MHz, CDCl₃) δ 7.94 (m, 4H; Ar), 7.58–7.45 (m, 2H; Ar), 7.44–7.29 (m, 5H; Ar), 7.23–7.05 (m, 4H; Ar), 5.65 (dd, ³ $J_{(2,3)}$ = 8.7 Hz, ³ $J_{(3,4)}$ = 8.8 Hz, 1; H-3), 5.20 (ddd, ³ $J_{(4,5a)}$ = 5.1 Hz, ³ $J_{(4,5b)}$ = 8.8 Hz, 1H;

H-4), 4.87 (d, ² $J_{(H,H)}$ = 11.7 Hz, 1H; CH₂Ph), 4.73–4.64 (m, 2H; H-1; CH₂Ph), 4.30 (dd, ² $J_{(5a,5b)}$ = 11.7 Hz, 1H; H-5a), 4.14 (dd, ³ $J_{(1'a,2')}$ = 2.9 Hz, ² $J_{(1'a,1'b)}$ = 11.7 Hz, 1H; H-1'a), 3.68–3.56 (m, 2H; H-1'b, H-2), 3.51 (dd, 1H; H-5b), 3.25 (dddd, ³ $J_{(2',3'a)}$ = 4.4 Hz, ³ $J_{(2',3'b)}$ = 2.9 Hz, 1H; H-2'), 2.84 (dd, ² $J_{(3'a,3'b)}$ = 5.1 Hz, 1H; H-3'a), 2.65 (dd, 1H; H-3'b); ¹³C NMR (62.9 MHz, CDCl₃) δ 165.3, 165.2 (C=O), 137.2 (Ar ipso), 133.3, 133.2, 129.8, 129.7, 128.4, 128.3, 128.2, 128.1, 128.0 (Ar), 103.1 (C-1), 77.4 (C-2), 73.6 (CH₂Ph), 72.1 (C-3), 70.1 (C-1'), 69.6 (C-4), 62.0 (C-5), 50.3 (C-2'), 44.0 (C-3'). C₂₉H₂₈O₈ (504.18): calcd C 69.02, H 5.60; found C 69.19, H 5.58.

[(2S) 2-Hydroxy-3-(6-amino-9H-purin-9-yl)-propyl] 3,4-di-O-benzoyl-2-O-benzyl- α -D-xylopyranoside (38). Compound **38** was obtained from **36** as described for the synthesis of **23** R_f 0.54 (CH₂Cl₂/MeOH, 9:1); $[\alpha]_D$ -5.8 (c 0.93 in chloroform); mp 93–95 °C; ¹H NMR (250 MHz, CDCl₃) δ 8.25 (s, 1H; H-2''), 7.95 (m, 4H; Ar), 7.73 (s, 1H; H-8''), 7.58–7.45 (m, 2H; Ar), 7.44–7.28 (m, 5H; Ar), 7.07–7.24 (m, 4H; Ar), 6.10 (m, 2H; NH₂), 5.95 (dd, ³ $J_{(2,3)}$ = 9.5 Hz, ³ $J_{(3,4)}$ = 10.2 Hz, 1H; H-3), 5.24 (ddd, ³ $J_{(4,5a)}$ = 5.8 Hz, ³ $J_{(4,5b)}$ = 10 Hz, 1H; H-4), 4.91 (d, ³ $J_{(1,2)}$ = 3.6 Hz, 1H; H-1), 4.63 (m, 2H; CH₂Ph), 4.52–4.15 (m, 3H; H-2', H-3'a, H-3'b), 3.98 (dd, ² $J_{(5a,5b)}$ = 11.0 Hz, 1H; H-5a), 3.86 (dd, ³ $J_{(1'a,2')}$ = 5.1 Hz, ² $J_{(1'a,1'b)}$ = 10.9 Hz, 1H; H-1'a), 3.81–3.68 (m, 2H; H-2, H-5b), 3.40 (dd, ³ $J_{(1'b,2')}$ = 5.8 Hz, 1H; H-1'b), 1.78 (s, 1H; OH); ¹³C NMR (62.9 MHz, CDCl₃) δ 165.9, 165.7 (C=O), 155.6 (C-6''), 152.7 (C-2''), 150.1 (C-4''), 142.0 (C-8''), 137.0 (Ar ipso), 133.4, 133.3, 129.8, 129.5, 128.5, 128.4, 128.2, 128.1 (Ar), 119.4 (C-5''), 97.7 (C-1), 76.7 (C-2), 73.4 (CH₂Ph), 71.6 (C-3), 70.0 (C-4), 69.8 (C-1'), 68.8 (C-2'), 59.0 (C-5), 47.8 (C-3'); IR (KBr): ν 1725 cm⁻¹ (C=O); C₃₄H₃₃N₅O₈ (639.23): calcd C 63.83, H 5.20, N 10.95; found C 63.72, H 5.18, N 10.97.

[(2S) 3-(6-Amino-9H-purin-9-yl)-2-hydroxypropyl] 2-O-benzyl- α -D-xylopyranoside (39). Compound **39** (42 mg, 95%) was obtained as white crystal from **38** (66 mg, 0.1 mmol) using the Zemplén procedure. R_f = 0.13 (CH₂Cl₂/MeOH, 88:12); $[\alpha]_D$ +40.1 (c 0.53 in methanol); ¹H NMR (250 MHz, [D₄] methanol) δ 8.21 (s, 1H; H-2''), 8.03 (s, 1H; H-8''), 7.43–7.27 (m, 5H; Ar), 4.94–4.82 (m, 3H; H-1, NH₂), 4.80 (d, ² $J_{(H,H)}$ = 11.7 Hz, 1H; CH₂Ph), 4.71 (d, 1H; CH₂Ph), 4.39 (dd, ³ $J_{(2',3'a)}$ = 3.0 Hz, ² $J_{(3'a,3'b)}$ = 13.6 Hz, 1H; H-3'a), 4.30–4.08 (m, 2H; H-2', H-3'b), 3.82 (dd, ³ $J_{(2,3)}$ = 8.9 Hz, ³ $J_{(3,4)}$ = 9.2 Hz, 1H; H-3), 3.71 (dd, ³ $J_{(1'a,2')}$ = 4.7 Hz, ² $J_{(1'a,1'b)}$ = 10.3 Hz, 1H; H-1'a), 3.62–3.48 (m, 2H; H-4, H-5a), 3.47–3.28 (m, 3H; H-2, H-5b, H-1'b); ¹³C NMR (62.9 MHz, CD₃OD) δ 157.2 (C-6''), 153.7 (C-2''), 151.1 (C-4''), 143.7 (C-8''), 139.7 (Ar ipso), 129.6, 129.5, 129.0 (Ar), 119.9 (C-5''), 99.0 (C-1), 81.4 (C-2), 74.5 (CH₂Ph), 74.4 (C-3), 71.6 (C-4), 70.7 (C-1'), 69.8 (C-2'), 63.0 (C-5), 48.1 (C-3'); IR (KBr): ν 3442 cm⁻¹ (OH); C₂₀H₂₅N₅O₆ (431.18): calcd C 55.66, H 5.84, N 16.24; found C 55.53, H 5.85, N 16.18.

[(2S) 3-(6-Amino-9H-purin-9-yl)-2-(phosphonoxy) propyl] 3,4 bis-O-(3,4-dihydro-3-oxido-2,4,3-benzodioxaphosphopin-3-yl)- α -D-xylopyranoside (40). Compound **39** (40 mg, 0.09 mmol), phosphoramidite **41** (208 mg,

0.77 mmol) and 1*H*-tetrazole (77 mg, 1.11 mmol) was dissolved in CH₂Cl₂ (2 mL). The reaction mixture was stirred at room temperature for 4 h then cooled to 0 °C and *t*BuOOH (0.5 mL) was added. Purification by column chromatography (CH₂Cl₂/MeOH 99:1 then 94:6) afforded compound **40** (67 mg, 74%) as a white powder. *R*_f 0.29 (CH₂Cl₂/MeOH, 94:6); [α]_D −1.4 (*c* 0.68 in chloroform); mp 140–142 °C; ¹H NMR (250 MHz, CDCl₃) δ 8.39 (s, 1H; H-2''), 7.86 (s, 1H; H-8''), 7.43–7.03 (m, 17H; Ar), 5.69–4.88 (m, 15H; H-1, H-3, H-2', PhCH₂Ph), 4.76 (m, 2H; CH₂Ph), 4.65–4.46 (m, 3H; H-4, H-3'a, H-3'b), 4.08 (dd, ³*J*_(4,5a) = 5.8 Hz, ²*J*_(5a,5b) = 11.0 Hz, 1H; H-5a), 3.85–3.65 (m, 3H; H-5b, H-1'a, H-1'b), 3.58 (dd, ³*J*_(1,2) = 2.9 Hz, ³*J*_(2,3) = 9.5 Hz, 1H; H-2); ¹³C NMR (62.9 MHz, CDCl₃) δ 157.0 (C-6''), 151.0 (C-2''), 150.4 (C-4''), 143.2 (C-8''), 137.2, 134.9, 134.7, 134.6 (Ar ipso), 128.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9 (Ar), 120.1 (C-5''), 96.8 (C-1), 77.6 (²*J*_(C,P) = 7.6 Hz, ³*J*_(C,P) = 5.7 Hz, C-3), 77.2 (C-2), 69.8 (²*J*_(C,P) = 5.7 Hz, C-2'), 73.6 (³*J*_(C,P) = 4.3 Hz, ²*J*_(C,P) = 3.3 Hz, C-4), 72.7 (CH₂Ph), 68.1–68.6 (6C, PhCH₂Ph), 67.1 (³*J*_(C,P) = 4.3 Hz, C-1'), 59.4 (C-5), 44.7 (³*J*_(C,P) = 5.2 Hz, C-3'); ³¹P NMR (100.13 MHz, CDCl₃) δ 0.56, −0.15 (P-3, P-4), −1.80 (P-2'); IR (KBr): ν 1284 cm^{−1} (P=O); C₄₄H₄₆N₅O₁₅P₃ (977.22): calcd C 54.03, H 4.74, N 7.16; found C 54.18, H 4.75, N 7.14.

[(2*S*) 3-(6-Amino-9*H*-purin-9-yl)-2-(phosphonoxy) propyl] α-D-xylopyranoside 3,4-bisphosphate hexasodium salt (11**). Compound **40** (46 mg, 0.05 mmol) was dissolved in MeOH (5 mL) and Pd/C (10%, 60 mg) was added. The mixture was stirred under hydrogen atmosphere (20 bars) for 12 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated to dryness. The residue was dissolved in water (3 mL) and applied to a column of Bio-Rad Chelex 100 resin (Na⁺ form). The column was eluted with water, fraction containing compound **11** were combined and freeze dried to give **11** (33 mg, 98%). [α]_D +11.5 (*c* 0.5 in water); ¹H NMR (250 MHz, D₂O) δ 8.42 (s, 1H; H-2''), 8.33 (s, 1H; H-8''), 4.95 (d, ³*J*_(1,2) = 2.8 Hz, 1H; H-1), 4.69–4.50 (m, 3H, H-3'a, H-3'b, H-2'), 4.34 (ddd, ³*J*_(2,3) = 7.3 Hz, ³*J*_(3,4) = 7.7 Hz, ³*J*_(H,P) = 7.6 Hz, 1H; H-3), 4.13–3.92 (m, 2H; H-4, H-5a), 3.90–3.53 (m, 4H; H-2, H-5b, H-1'a, H-1'b); ¹³C NMR (62.9 MHz, D₂O) δ 157.0 (C-6''), 155.3 (C-2''), 151.8 (C-4''), 146.3 (C-8''), 122.2 (C-5''), 101.38 (C-1), 76.8 (²*J*_(C,P) = 7.3 Hz, C-3), 74.2 (C-2), 73.1 (C-2', C-4), 70.4 (³*J*_(C,P) = 2.4 Hz, C-1'), 65.1 (C-5), 47.7 (C-3'); ³¹P NMR (100.13 MHz, D₂O) δ 7.76, 7.22, 7.10 (P-3, P-4, P-2'); MS (ES[−]): *m/z*: 645 [(M−4H+3Na)[−]], 623 [(M−3H+2Na)[−]], 601 [(M−2H+Na)[−]], 579 [(M−H)[−]]; C₁₃H₁₆N₅O₁₅P₃Na₆ (712.92): calcd C 21.88, H 2.26, N 9.82; found C 21.79, H 2.27, N 9.85.**

Preparation of cerebellar microsomes

Rat cerebella were homogenised with an Ultraturrax blender in an ice-cold homogenisation medium containing 250 mM sucrose, 5 mM Hepes/KOH, pH 7.4, 1 mM EGTA and supplemented with 1 mM dithiothreitol, 0.2 mM phenylmethane-sulphonyl fluoride and 10 μg mL^{−1} leupeptin, 10 μM pepstatin, 2 μM benzami-

din, 5 μg mL^{−1} aprotinin, 50 μg mL^{−1} trypsin inhibitor and 1 μg mL^{−1} orthophenanthroline, as previously described.³⁶ The homogenate was centrifuged for 5 min at 1500g, and the resulting supernatant was centrifuged for 30 min at 50,000g. The pellet was washed and resuspended at 4 mg protein/mL in washing medium containing 250 mM sucrose, 25 mM Hepes/KOH pH 7.4, supplemented with 1 mM dithiothreitol and the protease inhibitors cocktail.

Equilibrium [³H]InsP₃-binding studies

Microsomes (50–100 μg) were incubated on ice in 500 μL of the binding medium mimicking the ionic composition of the cytosol and containing 110 mM KCl, 20 mM NaCl, 25 mM HEPES pH 7.4, 1 mM NaH₂PO₄, 1 mM EDTA, supplemented with 1 mM dithiothreitol, the protease inhibitor cocktail, 1 mg/mL BSA and supplemented with 1 nM [³H]InsP₃. Non radio-active InsP₃ and the adenophostin analogues were added at the indicated concentrations. After a 6 min incubation period, 400 μL of the sample was layered on to a Whatman GF/C glass-fibre filter and washed with 1 mL of ice-cold medium consisting of 250 mM sucrose, 10 mM Na₂HPO₄ and adjusted to pH 8 to minimise the dissociation of the ligand–receptor complex. Radioactivity retained on the filter was counted by scintillation spectrometry.

⁴⁵Ca²⁺ fluxes measurements

Hepatocytes were prepared as previously described.³⁷ They were washed and resuspended at 4 × 10⁶ cells mL^{−1} in the cytosol-like medium buffered at pH 7.1 at 37 °C and containing 1 mM EGTA and 0.55 mM CaCl₂. The cells were permeabilized at 4 °C by the addition of 80 μg mL^{−1} saponin. The medium was supplemented with 5 μM CCCP, 5 μM oligomycin, 5 mM creatine phosphate and 5 u mL^{−1} creatine phosphokinase. Permeabilized cells were loaded with ⁴⁵Ca²⁺ by incubating the cell suspension for 5 min at 37 °C in the presence of 1 mM ATP, 1 mM MgCl₂ and 2–3 × 10⁵ cpm ⁴⁵Ca²⁺. The ⁴⁵Ca²⁺ release was measured at 37 °C, 15 s after the addition of the indicated concentrations of InsP₃ or adenophostin analogues. Incubations were terminated by filtration through a Whatman GF/C glass fiber filter and washing with ice-cold washing medium (4 × 3 mL).

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