Synthesis and Ca^{2+}-Mobilizing Activity of Purine-Modified Mimics of Adenophostin A: A Model for the Adenophostin−Ins(1,4,5)P_3 Receptor Interaction

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The synthesis of a series of adenophostin A analogues modified at C-6 and C-2 of adenine is described. The target compounds were synthesized by a convergent route involving a modified Vorbrüggen condensation of either 6-chloropurine or 2,6-dichloropurine with a protected disaccharide, yielding two versatile intermediates capable of undergoing substitution with a range of nucleophiles. The new analogues showed a range of abilities to mobilize Ca^{2+} from the intracellular stores of permeabilized hepatocytes and are among the first totally synthetic compounds to approach the activity of adenophostin A. In agreement with the biological results, docking studies of adenophostin A using the recently reported X-ray crystal structure of the type 1 Ins(1,4,5)P_3 receptor binding core suggested that, in likely binding modes of adenophostin A, the area around N^6 may be relatively open, identifying this region of the adenophostin A molecule as a promising target for further elaboration. The docking results also point to specific interactions involving residues within the binding domain of the Ins(1,4,5)P_3 receptor that may be involved in the molecular recognition of the adenophostins.

Introduction

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is an intracellular messenger that mediates the release of Ca^{2+} from intracellular stores by binding to Ins(1,4,5)P_3-gated Ca^{2+} channels [Ins(1,4,5)P_3 receptors, InsP_3Rs] located on the endoplasmic reticulum.[1,2] With the synthesis of many inositol-based analogues of Ins(1,4,5)P_3, a sound understanding of structure−activity relationships at the Ins(1,4,5)P_3 receptor has been established;[3,4] although with the exception of recently reported dimeric Ins(1,4,5)P_3 analogues,[5] none of these ligands has proven to be more potent than Ins(1,4,5)P_3 itself. In 1993 adenophostins A (**2**) and its 6″-acetylated derivative adenophostin B were isolated from a culture broth of *Penicillium brevicompactum*[6] and were shown to be 10 to 100 times more potent than Ins(1,4,5)P_3 at releasing Ca^{2+} from permeabilized cells, and to also bind to InsP_3Rs with much greater affinity than Ins(1,4,5)P_3 in equilibrium competition binding assays.[6−11] Three total syntheses of adenophostin A have since been reported,[12−15] and various novel compounds related to the adenophostins have also been synthesized in efforts to clarify the structural basis for the high affinity interaction of the adenophostins with InsP_3Rs.[16−28] Synthetic disaccharide-like analogues such as **3**,[26] for example, are slightly less potent than Ins(1,4,5)P_3 in Ca^{2+} release assays, and no adenophostin analogue lacking the adenine base (or an aromatic base-substitute) has so far attained a potency greater than that of Ins(1,4,5)P_3.[9−11]

To investigate which features of the aromatic component of adenophostin A are important for high affinity interactions with InsP_3Rs, we have recently synthesized several adenophostin A analogues in which the nucleobase motif has been replaced by various surrogates.[29−31] Although this approach provided several novel compounds (e.g., **4**−**6**, Figure 1) with a range of potencies greater than that of Ins(1,4,5)P_3, none was more potent than adenophostin A.[11] To explore this base-modification approach further, and to test steric tolerability around the nucleobase, we have now turned our attention to elaboration of the adenine. Several observations make N^6 of adenophostin A an attractive target for modification. First, early results for the activity of the hypoxanthine analogue[7] of adenophostin B, prepared by deamination of natural material, indicated that it had activity similar to that of adenophostin A. Second, the most potent totally synthetic adenophostin A analogue so far identified is purinophostin (**4**)[11,30] in which the 6-amino group is simply deleted. This suggests that although a purine ring, or equivalent, is necessary for high potency, the 6-amino group does not itself contribute greatly to the activity of adenophostin A. Here we report the synthesis of a series of N^6-modified analogues (**7**−**13**, Figure 2), which are elaborated versions of adenophostin A designed to explore the introduction of a range of different sized hydrophobes. The route to analogues **7**−**13** is versatile and also provides an entry to C-2 modified ligands. Such compounds could have potential in the design of high affinity adenophostin-based tools for exploring the mechanism of action of InsP_3Rs. We also report the results of docking experiments using adenophostin A and the recently reported X-ray crystal structure of the type 1 InsP_3R binding core.

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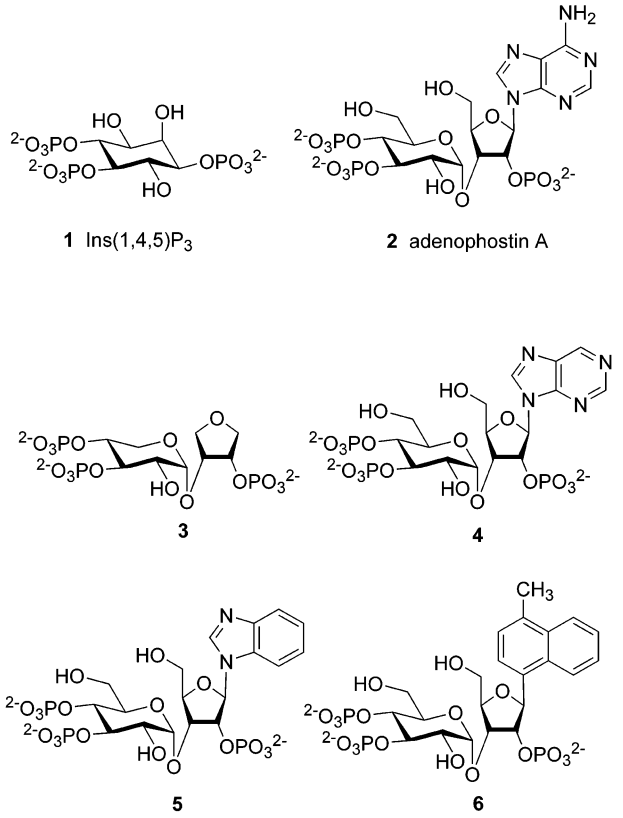


Figure 1. Structures of D-*myo*-inositol 1,4,5-trisphosphate (**1**), adenophostin A (**2**), and examples of synthetic adenophostin analogues (**3**−**6**).

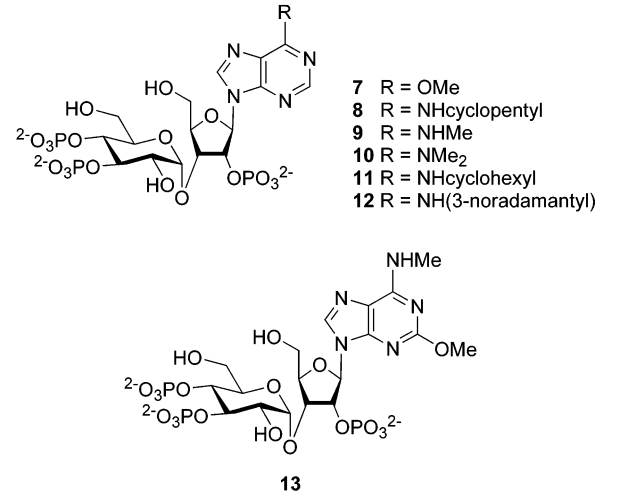
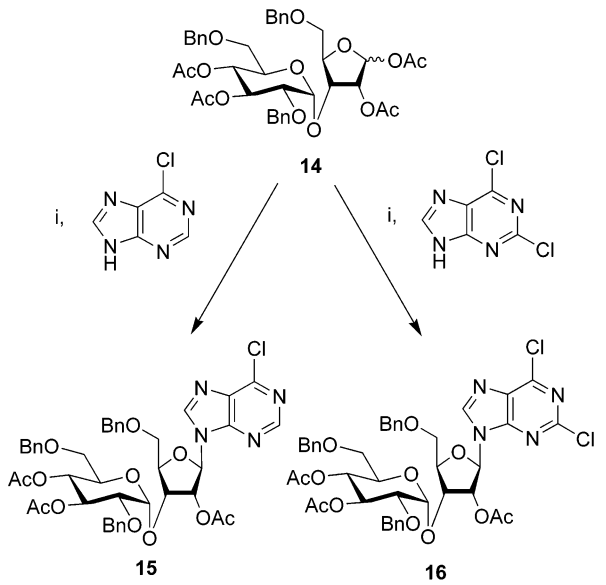


Figure 2. Structures of synthetic purine-modified adenophostin analogues synthesized in the present work.

Results and Discussion

Synthesis. The synthesis of all the adenophostin analogues **7**−**13** begins with the disaccharide precursor **14** (Scheme 1).[29−32] The target compounds were synthesized by a convergent route involving a modified Vorbrüggen condensation of either 6-chloropurine or 2,6-dichloropurine with disaccharide **14** to yield two versatile intermediates (**15** and **16**) capable of undergoing substitution with a range of nucleophiles. Treatment of **14** with 6-chloropurine, trimethylsilyl triflate (TMSOTf), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile gave the 6-chloro derivative **15** in 89% yield. A

Scheme 1[a]



[a] Reagents and conditions: (i) TMSOTf, DBU, MeCN, 0 °C to 60 °C; 89% (**15**), 67% (**16**).

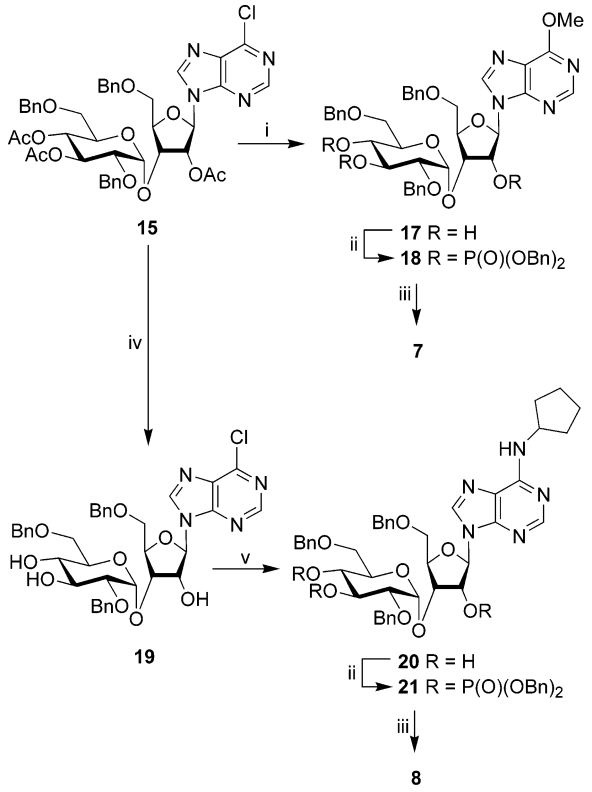
similar reaction between **14** and 2,6-dichloropurine afforded the 2,6-dichloro derivative **16** in 67% yield.

With **15** and **16** in hand, attention now turned to the reaction of these key intermediates with a range of nucleophiles. The 6-methoxy analogue of adenophostin A (**7**, Figure 2) was chosen as the first target. Reaction of **15** with 2 equiv of sodium methoxide in methanol at 65 °C gave the 6-methoxy derivative **17** (Scheme 2). Since these conditions also cleaved the acetate protecting groups, no additional deprotection step was required, and the required triol **17** was isolated in 74% yield. Phosphate groups were then introduced using the phosphoramidite method. Thus, triol **17** was reacted with a preformed complex between 1*H*-tetrazole and bis(benzyloxy)(diisopropylamino)phosphine in dichloromethane. The resulting trisphosphite was oxidized in situ with 3-chloroperoxybenzoic acid (*m*CPBA) to give the protected trisphosphate **18**. Deprotection of **18** to give the 6-methoxy analogue of adenophostin A was achieved with catalytic hydrogen transfer using $Pd(OH)_2$ as catalyst and cyclohexene as transfer reagent, providing **7** in 83% yield.

Attention now turned to the introduction of substituted amines at the N^6-position. To avoid possible complications from competing aminolysis of acetate esters, the acetate groups of **15** were first removed (Scheme 2) to give triol **19**. Reaction of **19** with cyclopentylamine at 80 °C for 3 h proceeded smoothly to produce the N^6-cyclopentyl derivative **20**, isolated in 54% yield. Phosphate units were introduced as previously described for **18**, and the removal of all benzyl protecting groups was achieved by catalytic transfer hydrogenation as before to give N^6-cyclopentyl adenophostin A (**8**).

A similar strategy was now used synthesize a series of N^6-substituted adenophostins with groups of varying size and hydrophobicity (Scheme 3). In each case the nucleophilic substitution at C-6 was carried out directly on the acetate-protected 6-chloro dervative **15**, whose higher solubility in most organic solvents made it easier to purify and to use than triol **19**. Reaction of **15** with

Scheme 2[a]

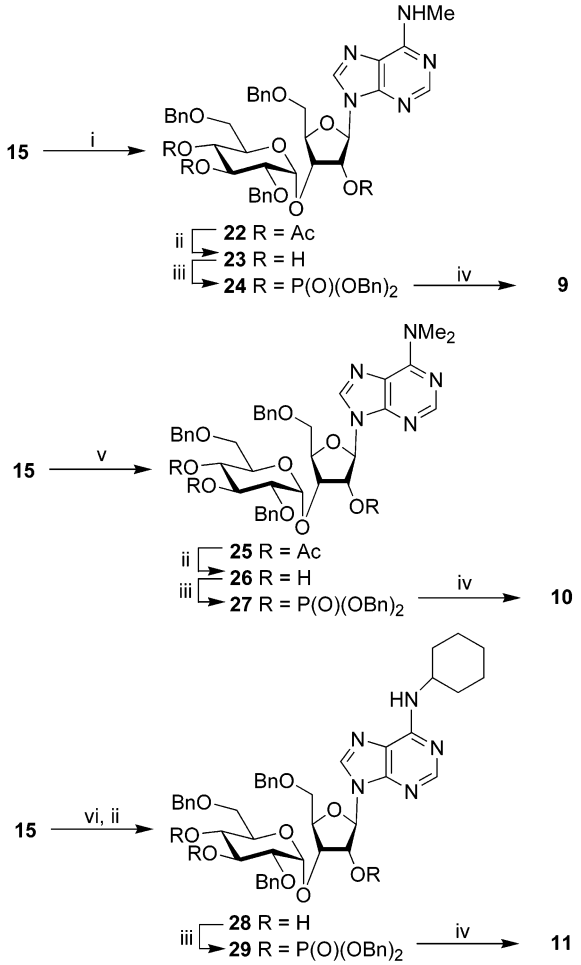


[a] Reagents and conditions: (i) NaOMe, MeOH, reflux, 30 min, 74%; (ii) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2; (b) *m*CPBA, −78 °C, 69% (**18**), 25% (**21**); (iii) $Pd(OH)_2/C$, MeOH/cyclohexene/H_2O, reflux, 83% (**7**), 46% (**8**); (iv) NaOMe, MeOH, rt, 55%; (v) cyclopentylamine, EtOH, 80 °C, 3 h, 54%.

methylamine or dimethylamine proceeded smoothly in each case to give N^6-alkyl compounds **22** and **25**, respectively. The acetyl groups of **22** and **25** were then removed using sodium methoxide in methanol to give crystalline triols **23** and **26**. When the reaction of **15** with cyclohexylamine was carried out, longer reaction times were required, resulting in some acetate deprotection. The mixture of products was therefore deacetylated using sodium methoxide in methanol before purification, giving triol **28**. Phosphitylation/oxidation of triols **23**, **26**, and **28** as described for **18**, followed by removal of all benzyl protecting groups by catalytic hydrogen transfer as before gave the corresponding trisphosphates **9**−**11**, respectively. Attempts to apply the same synthetic strategy to the synthesis of an N^6-noradamantyl analogue of adenophostin A, an attractive target with a classical bulky hydrophobe, met with problems. Reaction of **15** with 3-aminonoradamantane was extremely sluggish, and none of the desired product was isolated. These difficulties were eventually overcome by using a modified approach (see below).

To explore further elaboration of the adenine, a route to a 2,6-disubstituted adenophostin A from **16** was now explored (Scheme 4). Prolonged reaction of **16** with a large excess of methylamine hydrochloride and triethylamine in dichloromethane and ethanol at reflux gave rise to several inseparable products, however, and FAB mass spectrometry of the crude product showed no peak corresponding to the required disubstituted product. It is known that C-2 in 2,6-dichloroadenosine is less reactive to nucleophilic attack than C-6,[33] and this

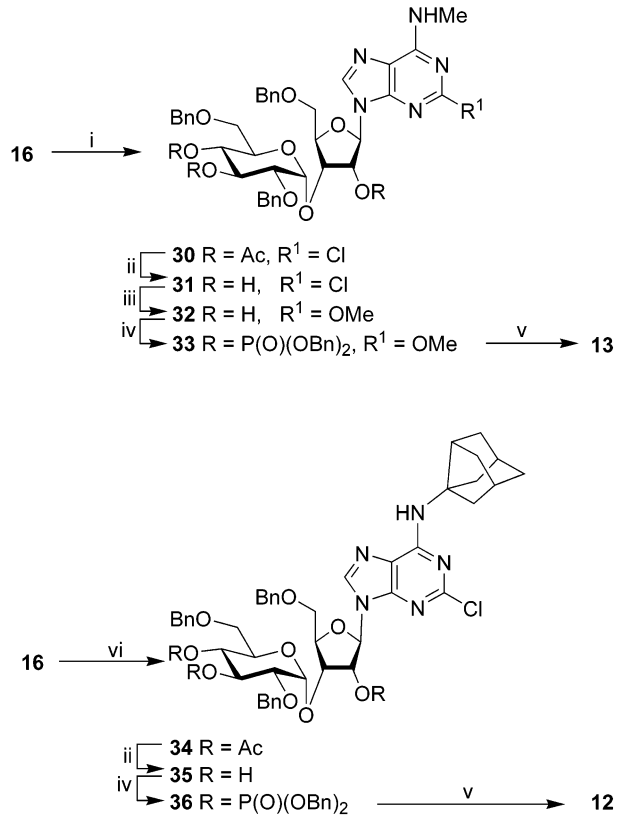
Scheme 3[a]



[a] Reagents and conditions: (i) methylamine hydrochloride, CH_2Cl_2/EtOH, Et_3N, 60 °C, 67%; (ii) NaOMe, MeOH, rt, 94% (**23**), 57% (**26**); (iii) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2; (b) *m*CPBA, −78 °C, 36% (**24**), 54% (**27**), 68% (**29**); (iv) $Pd(OH)_2/C$, MeOH/cyclohexene/H_2O, reflux, 80% (**9**), 31% (**10**), 46% (**11**); (v) dimethylamine hydrochloride, CH_2Cl_2/EtOH, Et_3N, 60 °C, 2 h, 96%; (vi) cyclohexylamine, CH_2Cl_2/EtOH, Et_3N, 60 °C, 16 h, 50%.

difference in reactivity suggested that an initial reaction of **16** with a nucleophile under controlled conditions should yield a 6-substituted product. This should then be capable of undergoing further substitution at C-2 with a stronger nucleophile. Accordingly, the reaction time was reduced to 4 h, and one major product was isolated and identified as the N^6-methyl-2-chloro derivative **30** (Scheme 4) on the basis of its ^{1}H NMR and FAB mass spectra. Substitution of the 2-chlorine atom with a methoxy group required forcing conditions. When **30** was heated at 65 °C with 2 equiv of sodium methoxide in methanol for 16 h and the product was isolated, the lack of any *O*-methyl signal in the ^{1}H NMR spectrum and the characteristic isotope pattern for chlorine in the FAB mass spectrum confirmed that only acetate deprotection had occurred, with no substitution at C-2. However, when the product (triol **31**) was refluxed with a large excess of sodium methoxide in methanol for a further 16 h, the required triol **32** was successfully isolated in 71% yield after purification by flash chromatography and crystallization. Phosphitylation/oxidation of **32**, as described for **17** gave fully protected **33** which, after deprotection, yielded trisphosphate **13**.

Scheme 4[a]

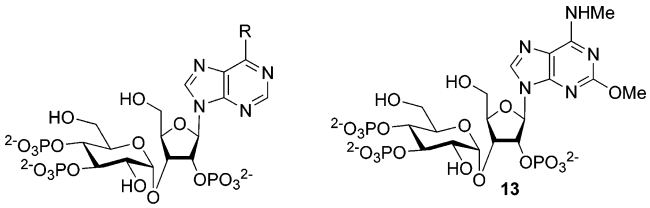


[a] Reagents and conditions: (i) methylamine hydrochloride, CH_2Cl_2/EtOH, Et_3N, 60 °C, 4h, 67%; (ii) NaOMe, MeOH, rt 57% (**31**), 84% (**35**); (iii) NaOMe, MeOH, reflux; 16 h, 71%; (iv) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2 (b) *m*CPBA, −78 °C, 57% (**33**), 46% (**36**); (v) $Pd(OH)_2$/C, MeOH/cyclohexene/H_2O, reflux, 59% (**12**), 47% (**13**); vi) 3-aminonoradamantane hydrochloride, CH_2Cl_2/EtOH, Et_3N, 60 °C, 48 h, 80%.

Finally, we returned to the synthesis of N^6-noradamantyl adenophostin (**12**). Results from earlier experiments had indicated that C-6 in **16** was considerably more reactive than the corresponding position in **15**, prompting us to make a second attempt at the synthesis of the N^6-noradamantyl analogue via **16**. When **16** was reacted with 3-aminonoradamantane hydrochloride and triethylamine in dichloromethane and ethanol at 60 °C overnight (Scheme 6), the N^6-monosubstituted compound (**34**) was isolated in good yield (80%). The acetate esters of **34** were removed using sodium methoxide in methanol, and subsequent phosphitylation followed by oxidation yielded fully protected **36**. Catalytic transfer hydrogenation as before successfully removed all benzyl protecting groups with concomitant reduction at C-2 to yield N^6-noradamantyl adenophostin A (**12**).

Ca^{2+} Flux Experiments. The ability of compounds **7** to **13** to mobilize Ca^{2+} from the intracellular stores of permeabilized rat hepatocytes was examined. The results were compared with those obtained for adenophostin A (**2**) and Ins(1,4,5)P_3. Because the analogues were tested in experiments spanning a considerable period, during which there was some variation in the absolute sensitivity to Ins(1,4,5)IP_3 and **2**, the EC_{50} value (i.e., the concentration causing half-maximal Ca^{2+} release) for each analogue is expressed relative to that for adenophostin A measured in parallel experiments. Table 1 reports both these relative potencies (which provide the most reliable comparison with adenophostin A) and the absolute EC_{50} values. The N^6-cyclopentyl (**8**), N^6-methyl (**9**), and N^6-dimethyl (**10**) analogues were the most active, being almost as potent as adenophostin A: each had an EC_{50} ratio of about 0.8 (<1 is less active than adenophostin A). The N^6-cyclohexyl analogue (**11**), N^6-noradamantyl derivative (**12**), and 6-methoxy analogue (**7**) were weaker (EC_{50} ratios of about 0.5). All these compounds are nevertheless considerably more potent than Ins(1,4,5)P_3, and the first three have activities very close to that of adenophostin A. Interestingly, the N^6-methyl-2-methoxy analogue (**13**) was significantly less potent than all the other compounds (0.18) and only some 2-fold more potent than Ins(1,4,5)P_3.

Table 1. $^{45}Ca^{2+}$ Release Data for **2**, **7**−**13** from Permeabilized Hepatocytes



2 R = NH_2 **10** R = NMe_2
7 R = OMe **11** R = NHcyclohexyl
8 R = NHcyclopentyl **12** R = NH(3-noradamantyl)
9 R = NHMe

	EC_{50}, nM[a]	h[a]	max. response, %[a]	EC_{50} with respect to **2**[b]	n[a]
Ins(1,4,5)P_3	185 ± 18	2.3 ± 0.3	35 ± 1	0.08 ± 0.01	11
2	14 ± 1.0	2.4 ± 0.3	35 ± 1	1	13
7	19 ± 1.9	3.8 ± 1.5	34 ± 3	0.57 ± 0.07	5
8	13 ± 1.6	2.2 ± 0.2	35 ± 2	0.77 ± 0.06	5
9	13 ± 2.3	2.1 ± 0.4	37 ± 4	0.80 ± 0.08	5
10	19 ± 2.1	2.1 ± 0.1	39 ± 2	0.82 ± 0.08	4
11	25 ± 7.0	2.3 ± 0.1	45 ± 1	0.56 ± 0.16	4
12	34 ± 2.0	2.1 ± 0.2	49 ± 2	0.47 ± 0.06	6
13	82 ± 3.5	8.8 ± 1.6	34 ± 1	0.17 ± 0.01	5

[a] The EC_{50} values and Hill coefficients (h) were separately determined for n independent experiments by fitting results to four-parameter logistic equations. The maximum response denotes the % of actively accumulated Ca^{2+} released by a maximally effective concentration of the analogue. [b] The EC_{50} for each agonist is expressed relative to the EC_{50} for **2** in paired comparisons. Results are shown as means ± SEM.

Molecular Docking of Ins(1,4,5)P_3 and Adenophostin A. While this work was nearing completion, the X-ray crystal structure of the binding core of the mouse type 1 receptor ($InsP_3R1$) in complex with Ins(1,4,5)P_3 was published.[34] Hepatocytes, which were used in the Ca^{2+} flux experiments described above, express more $InsP_3R2$ than $InsP_3R1$, but the residues forming the active site are conserved with the exception that Gly268 in $InsP_3R1$ is replaced by Leu in $InsP_3R2$. We have previously demonstrated that full length $InsP_3R1$

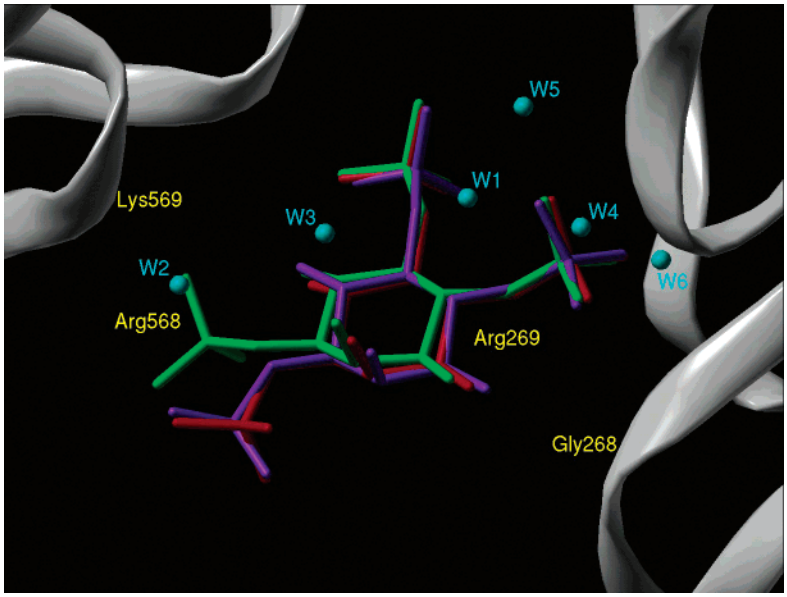


Figure 3. Crystallographically observed binding mode of Ins(1,4,5)P_3 (purple) at the receptor binding core[34] and GOLD[38] docked binding mode of Ins(1,4,5)P_3 (mode A, red) in docking experiments carried out with six molecules of water (W1 to W6) placed in the binding site. When W2 was omitted, similar results were obtained for the docked position of Ins(1,4,5)P_3 in the site, but the crystallographically observed conformation of the 1-phosphate group was not reproduced (mode B, green). For clarity, hydrogen atoms are not shown.

and InsP_3R2 both bind adenophostin A with seven to 8-fold greater affinity than Ins(1,4,5)P_3.[35] The receptor binding core includes only residues 224−604 of the full length (2700 residue) receptor, and it is possible that residues outside the binding core domain could influence the access of Ins(1,4,5)P_3 and adenophostin A to the binding site.[35] However, studies agree that the 224−604 InsP_3R1 binding core does bind adenophostin A with higher affinity than Ins(1,4,5)P_3.[35−37] We therefore reasoned that docking experiments using the InsP_3R1 binding core structure might yield some clues to the origin of the enhanced affinity of adenophostin A for InsP_3Rs.

The docking studies were carried out using GOLD,[38] an automated docking program that uses a genetic algorithm to explore possible bound conformations of flexible ligands to protein binding sites. It should be noted that this and other docking approaches take only limited account of protein flexibility, which may be an important factor in receptor−ligand interactions.[39] The X-ray crystal structure showed that six water molecules were directly involved in the formation of hydrogen bonds that bridge Ins(1,4,5)P_3 and the receptor,[34] and it was necessary to include some of these water molecules in the docking experiments in order to reproduce the observed binding mode of Ins(1,4,5)P_3 (see Experimental Section for details). Figure 3 compares the crystallographically observed position of Ins(1,4,5)P_3 in the binding site with docking results for Ins(1,4,5)P_3 using either six or five molecules of water, while Figure 4 shows the highest scored binding modes of adenophostin A when docked using the same conditions. While the inclusion of water molecule W2 in the docking experiments has a relatively minor effect on the results for Ins(1,4,5)P_3, the effect is greatly enhanced for adenophostin A. In binding mode A (Figure 4), which was highly scored in dockings using six water molecules, the adenophostin molecule has the 3′*endo* conformation. In contrast, when dockings were performed with five water molecules in the site, solutions of type B, with the 2′*endo* conformation were more highly scored.

The sensitivity of the docking results to the placing of a single water molecule illustrates the difficulty of establishing a definitive binding mode for adenophostin A to this highly hydrophilic site by molecular docking. However, some useful conclusions can be drawn. First, it is clear that, as previously suggested,[7,40] the glucose 3″,4″-bisphosphate of adenophostin A can mimic the 4,5-bisphosphate of Ins(1,4,5)P_3 at the Ins(1,4,5)P_3 binding site. The crystallographically observed interactions of the 4,5-bisphosphate of Ins(1,4,5)P_3 with Arg265, Thr267, Gly268, Arg269, Arg508, Arg511, and Tyr567 and with the water molecules are all reproduced by the glucose 3″,4″-bisphosphate of adenophostin A in the binding modes scored most highly by GOLD. Second, and also as previously suggested[7,40] it appears that the 2′-phosphate group of adenophostin A could interact with the binding site in a different way to the 1-phosphate group of Ins(1,4,5)P_3. In the X-ray structure, the 1-phosphate of Ins(1,4,5)P_3 has two contacts with Arg568, but only an indirect interaction with the amide NH of Lys569 via a molecule of water (W2). In many highly scored docked conformations of adenophostin A, however, its 2′-phosphate group shows modified interactions with the binding site. The particular combination of residues involved is highly sensitive to the docking procedure, but it appears that the 2′-phosphate of adenophostin A can, at least potentially, interact with the amide NH of Lys569 and the side chain of Arg504 in addition to, or instead of, Arg568. Third, none of the highly scored docked conformations of adenophostin A shows any interaction of N^6 with the site, consistent with the potent activity of purinophostin (**4**, Figure 1).[11] The space around N^6 is relatively open, consistent with the tolerance for even quite large N^6-substituents found in the present work. In binding mode A (Figure 4) the adenine simply projects outward into solvent and has no interactions with the binding core, while in mode B

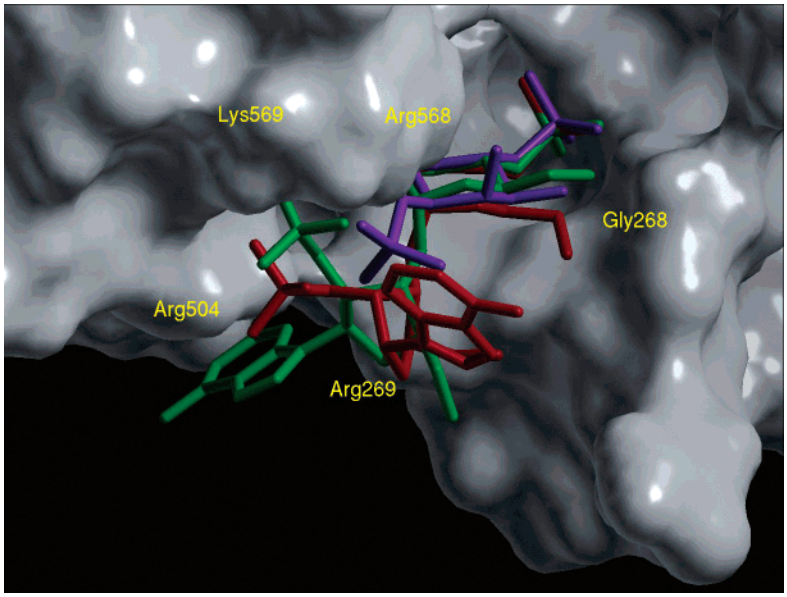


Figure 4. Possible binding modes for adenophostin A identified by GOLD[38] docking experiments. Mode A (red) was highly scored by GOLD in docking experiments with six molecules of water (W1 to W6 in Figure 3) in the site, while mode B (green) was more highly scored when W2 was omitted. In mode B, the side chain of Arg504 shows a potential cation−π interaction with the adenine of adenophostin A. The crystallographically observed[34] binding mode of Ins(1,4,5)P_3 (purple) is shown for comparison. For clarity, water molecules and hydrogen atoms are not shown.

the N^3 atom of adenine accepts a hydrogen bond from Arg269. A hydrogen bonding interaction involving N^3 could contribute to the greater potency of adenophostin A and purinophostin (**4**, Figure 1) relative to the benzimidazole analogue (**5**). Interestingly, in mode B there is also a potential cation−π interaction between the guanidinium side chain of Arg504 and the adenine of adenophostin A. This kind of interaction would not be sensed by GOLD, and is therefore not optimized in the docking experiments. However, planar stacking of arginine and aromatic side-chains within proteins has previously been observed,[41] and such cation−π interactions are increasingly recognized as being important in molecular recognition.[42] Comparable interactions between arginine and adenine were readily identifiable in RNA binding proteins such as poly A binding protein (1CVJ) using the program ENTANGLE,[43] and a similar cation−π interaction between an arginine side chain and the adenine of $NADP^+$ in the X-ray crystal structure of aldehyde dehydrogenase in complex with $NADP^+$ has been reported.[44] A cation−π interaction could also explain why the naphthalenyl analogue **6** (Figure 1) is more potent than Ins(1,4,5)P_3, even though its aromatic component has no groups that can engage in hydrogen bonding with the binding site. On the basis of these considerations regarding a possible role for the adenine, we propose mode B (Figure 4) as a working model for the interaction of adenophostin A with InsP_3Rs.

Conclusions

We have described a versatile strategy for the synthesis of C-6 modified analogues of adenophostin A and illustrated its application to the synthesis of 6-methoxy adenophostin A (**7**) and a series of N^6-alkyl analogues (**8**−**11**) having a range of different sized hydrophobes. The strategy was further developed to give the first 2,6-modified adenophostin analogue (**13**) and also N^6-noradamantyl adenophostin A (**12**). A comparison of the abilities of **7** to **13** to mobilize Ca^{2+} from the intracellular stores of permeabilized hepatocytes showed that the introduction of hydrophobes at N^6 was well tolerated by the receptor, giving a series of new compounds with greater potency than the natural ligand, Ins(1,4,5)P_3. N^6-methyl, N^6-dimethyl, and N^6-cyclopentyl analogues showed potencies approaching that of adenophostin A itself while the introduction of larger hydrophobes (N^6-cyclohexyl and N^6-noradamantyl) or a methoxy group at C-6 appeared to have a greater impact on activity. Notably, addition of a methoxy group to C-2 of **9**, as in the 2,6-disubstituted analogue **13**, led to a marked reduction in potency. These results identify N^6 as a promising target for further modifications to adenophostin A. Docking experiments with adenophostin A pointed to specific interactions involving residues within the binding domain of the Ins(1,4,5)P_3 receptor that may be involved in the molecular recognition of the adenophostins. The docking results broadly support the biological findings with respect to **7**−**13** and are consistent with the activities of other nucleobase-modified analogues. A model is proposed for the binding of adenophostin A to Ins(1,4,5)P_3 receptors, featuring a cation−π interaction between an arginine residue and the adenine of adenophostin A.

Experimental Section

Chemicals were purchased from Acros, Aldrich, Sigma, and Fluka. Dimethylformamide (DMF) was distilled from barium oxide under reduced pressure and then stored over 4 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled, and then stored over potassium hydroxide pellets. Dichloromethane and acetonitrile were dried over calcium hydride, distilled, and then stored over 4 Å molecular sieves. 1,4-Dioxane, dimethyl sulfoxide (DMSO), ethanol, diethyl ether, THF, and toluene were purchased in anhydrous form. Ins(1,4,5)P_3 was purchased from American Radiolabeled Chemicals. TLC was performed on precoated plates (Merck aluminum sheets silica 60 F_{254}, Art. No. 5554). Products were visualized under UV light and by dipping into phosphomolybdic acid in MeOH, followed by heating or by dipping into anisaldehyde in ethanol followed by heating. Flash chroma-

tography was carried out on silica gel (particle size 40−63 μm). ^{1}H and ^{13}C NMR spectra were recorded on JEOL JMN GX-270 or EX-400 NMR spectrometers. Unless otherwise stated, chemical shifts were measured in ppm relative to internal tetramethylsilane. ^{31}P NMR chemical shifts were measured in ppm and denoted positive downfield from external 85% H_3PO_4. Melting points were determined using a Reichert−Jung hot stage microscope apparatus and are uncorrected. 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 ion fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol 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 mL or 5 mL, and specific rotation are given in 10^{-1} deg mL g.$^{-1}$ HPLC analysis was carried out on a Dynamax model SD-200 with reverse phase column: APEX ODS II 5 μm S/N 7121103. A gradient of 0.05 M triethylammonium acetate buffer containing 0.1% tetrabutylammoniumhydrogen sulfate and acetonitrile was used as eluent at 0.9 mL/min, with a UV detector set at 259 nm. HPLC purification was carried out on a Hewlett-Packard series chromatograph with a strong anion-exchange resin (AGMP1, column size 3 mm × 150 mm). A gradient of 150 mM trifluoroacetic acid was used as eluent at 1 mL/min, with the UV detector set at 259 nm. Synthetic phosphates were assayed by an adaptation of the Briggs phosphate test.[45]

$^{45}Ca^{2+}$ Release from Permeabilized Rat Hepatocytes. The methods were similar to those reported previously.[10] Briefly, permeabilized hepatocytes were loaded to steady state (5 min at 37 °C) with $^{45}Ca^{2+}$ in a cytosol-like medium (CLM: KCl, 140 mM, NaCl, 20 mM, 2 mM $MgCl_2$, 1 mM EGTA, 300 μM $CaCl_2$, free [Ca^{2+}] = 200 nM, 20 mM Pipes, pH7.0) containing ATP (1.5 mM), creatine phosphate (5 mM) creatine phosphokinase (5 units/mL), and FCCP (10 μM). After 5 min, thapsigargin (1.25 μM) was added to the cells to inhibit further Ca^{2+} uptake, 30 s later the cells were added to appropriate concentrations of the agonists, and after a further 60 s the $^{45}Ca^{2+}$ contents of the stores were determined by rapid filtration. Concentration−response relationships were fitted to a four-parameter logistic equation using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}) and Hill slope (*h*) were determined. All results are expressed as means ± SEM. Ins(1,4,5)P_3 was from American Radiolabeled Chemicals.

Molecular Docking Experiments. Modeling was carried out on a Silicon Graphics Octane2 workstation using Sybyl 6.8 (Tripos Associates) molecular modeling software. The crystal structure of the Ins(1,4,5)P_3 binding core of mouse InsP_3R1 in complex with Ins(1,4,5)P_3 (1N4K[34]) was retrieved from the Brookhaven Protein Database and prepared for docking experiments using Sybyl 6.8 (Tripos Associates) molecular modeling software as follows. Atom types for the phosphate oxygen atoms in the bound molecule were first corrected to the O.co2 type, and hydrogen atoms were then added to Ins(1,4,5)P_3, water molecules, and protein. Atomic charges for Ins(1,4,5)P_3 and for the 140 crystallographically determined water molecules were calculated by the Gasteiger Hückel method, and dictionary charges (Kollmann all atom) were assigned to the protein. All heavy atoms were then fixed by designating them as a single aggregate within Sybyl, and hydrogen atoms were minimized using the Tripos force field and a constant dielectric of 1, terminating at a gradient of 0.01 kcal mol^{-1}A^{-1}. The bound Ins(1,4,5)P_3 molecule and 134 water molecules were then removed, leaving in the active site only the six water molecules (W1−W6)[34] that interact directly with Ins(1,4,5)P_3. In other experiments, the orientations of the 140 water molecules were first optimized by cycles of molecular dynamics and minimizations, but this approach gave almost identical orientations for W1−W6 as the simple minimization strategy described.

A molecular model of Ins(1,4,5)P_3 with fully ionized phosphate groups was built in Sybyl and charges were calculated by the Gasteiger Hückel method. The structure was then minimized using the Tripos force field and a distance-dependent dielectric of 1. Docking studies were then carried out using GOLD[38] (Version 2.0) running under Microsoft Windows XP on a 2.0 GHz Pentium 4 PC. The model of Ins(1,4,5)P_3 was docked 50 times to the protein using GOLD. An active site radius of 15 Å and automatic cavity detection were used, with six molecules of water (W1 to W6)[34] in the binding site, as described above. The GoldScore[38] fitness function was employed. The three highest scoring ("fittest") solutions found by Gold were almost identical, and each reproduced the crystallographically determined position and conformation of Ins(1,4,5)P_3 in the binding site with <0.6 Å rms deviation (Figure 3, mode A). To determine the minimum number and combination of water molecules required, water molecules were then systematically removed, giving active sites with various combinations of 5, 4, 3, 2, 1, and zero water molecules in place, and Ins(1,4,5)P_3 was redocked each time. These experiments showed that the position of Ins(1,4,5)P_3 in the active site could be reliably reproduced by docking to a protein with five water molecules (W1, W3, W4, W5, and W6)[34] in the site (Figure 3, mode B). These five water molecules participate in a network of hydrogen bonds involving the 4,5-bisphosphate of Ins(1,4,5)P_3. The fact that an analogous structure (the glucose 3″,4″-bisphosphate) exists in adenophostin A justified the inclusion of these water molecules in subsequent docking experiments with adenophostin A. Similar results could be obtained using as few as three deeply buried water molecules in the site (W3, W4, and W5) but many more cycles of docking were required before the cluster of fittest solutions was identified. In both cases an additional water molecule (W2) was required to consistently reproduce the crystallographically determined orientation of the 1-phosphate group and its interaction with Arg568. Dockings using less than three water molecules were much less successful, giving rise to solutions in which the Ins(1,4,5)P_3 was docked in various inverted, rotated, and distorted conformations. In general, the more water molecules (up to the maximum of 6) that were placed in the site, the fewer cycles of docking were required to give reproducible and concordant results.

Models of adenophostin A were built within Sybyl 6.8 with either 2′*endo* or 3′*endo* conformations of the ribose and then charged and minimized as for Ins(1,4,5)P_3. The resulting structures were used in GOLD docking experiments exactly as described for Ins(1,4,5)P_3 above using five (W1, W3, W4, W5, and W6) or six (W1 to W6) water molecules in the active site. The fittest solutions identified fell into two clusters, represented by structures A and B in Figure 4. Whether type A (3′*endo*) or type B (2′*endo*) solutions were more highly scored by GOLD depended on whether the sixth water molecule (W2) was included in the docking experiments. Docking with as few as three deeply buried water molecules (W3, W4, and W5) in the site gave the same results as for five water molecules, but many more cycles of docking were required before the cluster of fittest solutions was identified. The fittest solution obtained when adenophostin A was docked with either three or five water molecules in the site (mode B, Figure 4) showed a potential cation−π interaction between a surface-exposed residue, Arg504, and the adenine of adenophostin A.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-chloro-9-β-D-ribofuranosylpurine (15). To a stirred solution of **14** (500 mg, 0.66 mmol), 6-chloropurine (113 mg, 0.73 mmol), and DBU (0.3 mL, 2.00 mmol) in acetonitrile (5 mL) at 0 °C was added TMSOTf (0.48 mL, 2.65 mmol) dropwise. The mixture was heated at 60 °C for 1 h, after which time it was cooled and quenched by careful addition of saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to leave a yellow oil. Purification by flash chromatography on silica using CH_2Cl_2−acetone (40:1) as eluent gave the title compound (500 mg, 89%) as a colorless oil; $[\alpha]^{20}_D$ +71.0

(*c* 1.14, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.73 (s, 1 H, H-8), 8.48 (s, 1 H, H-2), 7.37−7.23 (m, 15 H, ArC*H*), 6.41 (d, 1 H, $J_{1',2}$ 5.5 Hz, H-1′), 5.69 (t, 1 H, $J_{2',1'} = J_{2',3}$ 5.5 Hz, H-2′), 5.44 (t, 1 H, $J_{3'',4''} = J_{3'',2''}$ 9.8 Hz, H-3″), 5.02 (t, 1 H, $J_{4'',3''} = J_{4'',5''}$ 9.8 Hz, H-4″), 4.96 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.71 (dd, 1 H, $J_{3',4'}$ 3.9 Hz, $J_{3',2}$ 5.3 Hz, H-3′), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.56−4.48 (m, 5 H, 2 × OCH_2Ar, H-4′), 4.34 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.00−3.96 (m, 1 H, H-5″), 3.70 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'a,5'}$ 10.9 Hz, H-5′a), 3.60 (dd, 1 H, $J_{5'b,4}$ 2.7 Hz, $J_{5'b,5'}$ 10.9 Hz, H-5′b), 3.56 (dd, 1 H, $J_{2'',1''}$ 3.5 Hz, $J_{2'',3}$ 9.8 Hz, H-2″), 3.40−3.36 (m, 2 H, H-6″a, H-6″b), 1.99 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3CO) and 1.87 (s, 3 H, CH_3CO); MS: (FAB) *m*/*z* 845.3 [(M), 65%], *m*/*z* calcd for $C_{43}H_{44}N_4O_{12}Cl$ [M + H]$^+$ ^{37}Cl, 847.2771 found *m*/*z* 847.2738, [M + H]$^+$ ^{35}Cl, 846.2834 found *m*/*z* 846.2820; Anal. $C_{43}H_{44}N_4O_{12}$ C, H, N.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-2,6-dichloro-9-*β*-D-ribofuranosylpurine (16). To a stirred solution of **14** (500 mg, 0.66 mmol), 2,6-dichloropurine (138 mg, 0.73 mmol), and DBU (0.4 mL, 2.67 mmol) in acetonitrile (5 mL) at 0 °C was added TMSOTf (0.48 mL, 2.65 mmol), dropwise. The mixture was heated at 60 °C for 1 h, after which time it was cooled and quenched by careful addition of saturated aqueous $NaHCO_3$ solution (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 30 mL), and the combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to leave a yellow oil. Purification by flash chromatography on silica using EtOAc−hexane (3:7) as eluent gave the title compound (390 mg, 67%) as a colorless oil. $[\alpha]^{20}_D$ +63.3 (*c* 0.44, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.45 (s, 1 H, H-8), 7.37−7.16 (m, 15 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2}$ 5.6 Hz, H-1′), 5.61 (t, 1 H, $J_{2',1'} = J_{2',3}$ 5.6 Hz, H-2′), 5.45 (t, 1 H, $J_{3'',4''} = J_{3'',2''}$ 9.8 Hz, H-3″), 5.02 (t, 1 H, $J_{4'',3''} = J_{4'',5''}$ 9.8 Hz, H-4″), 4.97 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″, 4.69 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, $J_{3',2}$ 5.6 Hz, H-3′), 4.63 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.56−4.42 (m, 5 H, 2 × OCH_2Ar, H-4′), 4.36 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.02−3.98 (m, 1 H, H-5″), 3.70 (dd, 1 H, $J_{5'a,4}$ 2.4 Hz, $J_{5'a,5'}$ 10.9 Hz, H-5′a), 3.60−3.55 (m, 2 H, H-2″, H-5′b), 3.45−3.38 (m, 2 H, H-6″a, H-6″b), 1.99 (s, 3 H, CH_3CO), 1.96 (s, 3 H, CH_3CO) and 1.89 (s, 3 H, CH_3CO); MS: (FAB) *m*/*z* 879.1 [(M + H)$^+$, 83%], *m*/*z* calcd for $C_{43}H_{43}N_4O_{12}Cl_2$ [M + H]$^+$ $^{37}Cl_2$ 883.2352 found *m*/*z* 883.2394, [M + H]$^+$ ^{35}Cl,^{37}Cl 881.2381 found *m*/*z* 881.2391, [M + H]$^+$ $^{35}Cl_2$ 879.2411 found *m*/*z* 879.2400; Anal. Calcd for $C_{43}H_{43}N_4O_{12}Cl_2$ C, H, N.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-methoxy-9-*β*-D-ribofuranosylpurine (17). A solution of 1 M NaOMe (0.8 mL, 0.8 mmol) in MeOH was added to a stirred solution of **15** (400 mg, 0.4 mmol) in MeOH. The mixture was heated at reflux for 30 min after which it was cooled, neutralized with Dowex 50WX4−50 ion-exchange resin, and filtered. The filtrate was concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using EtOAc−hexane (4:1) as eluent to give the title compound as a colorless oil (250 mg, 74%); $[\alpha]^{20}_D$ +16.7 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.44 (s, 1 H, H-8), 8.22 (s, 1 H, H-2), 7.37−7.24 (m, 15 H, ArC*H*), 6.27 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 4.79 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.69 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.62−4.56 (m, 1 H, H-2′), 4.53−4.41 (m, 6 H, 2.5 × OCH_2Ar, H-4′), 4.21−4.19 (m, 4 H, H-3′, OCH_3), 4.10 (t, 1 H, $J_{3'',2''} = J_{3'',4''}$ 9.4 Hz, H-3″), 3.96−3.91 (m, 1 H, H-5″), 3.71−3.65 (m, 3 H, H-4″, H-6″a, H-6″b), 3.60 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'a,5'}$ 10.5 Hz, H-5′a), 3.54 (dd, 1 H, $J_{5'b,4}$ 2.7 Hz, $J_{5'b,5'}$ 10.5 Hz, H-5′b) and 3.43 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.4 Hz, H-2″); MS: (FAB) *m*/*z* 715.3 [(M + H)$^+$, 11%], *m*/*z* calcd for $C_{38}H_{42}N_4O_{10}$ [M + H]$^+$, 715.2979 found *m*/*z* 715.2983.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″-tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-6-methoxy-9-*β*-D-ribofuranosylpurine (18). Bis(benzyloxy)(diisopropylamino)phosphine (405 mg, 1.17 mmol) and 1*H*-tetrazole (123 mg, 1.76 mmol) were stirred together in CH_2Cl_2 (3 mL) for 30 min, and the mixture thus obtained was then added to triol **17**. After a further 20 min, TLC (EtOAc:MeOH, 95:5) indicated conversion of **17** into a single trisphosphite. The reaction mixture was then cooled to −78 °C, and *m*CPBA (360 mg, 1.25 mmol) was added. After 10 min, 10% w/v aq Na_2SO_3 solution (15 mL) and EtOAc (20 mL) were added, and the mixture was allowed to warm to room temperature. The organic layer was separated and washed with saturated aqueous $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using EtOAc−hexane (1:1 then 4:1) to give the title compound (200 mg, 69%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.40 (s, 1 H, H-8), 8.07 (s, 1 H, H-2), 7.40−6.94 (m, 45 H, ArC*H*), 6.40 (d, 1 H, $J_{1',2}$ 6.2 Hz, H-1′), 5.62−5.57 (m, 1 H, H-2′), 5.33 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.06−4.87 (m, 8 H, 3.5 × OCH_2Ar, H-3″), 4.80−4.35 (m, 13 H, 5 × OCH_2Ar, H-3′, H-4′, H-4), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.14 (s, 3 H, OCH_3), 3.86−3.83 (m, 1 H, H-5″), 3.71−3.57 (m, 4 H, H-5′a, H-5′b, H-6″a, H-6″b) and 3.43 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.7 Hz, H-2″); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P −0.37, −0.97, −1.14; MS: (FAB) *m*/*z* 1496.0 [(M + H)$^+$, 78%], *m*/*z* calcd for $C_{80}H_{81}N_4O_{19}P_3$ [M + H]$^+$, 1495.4786 found *m*/*z* 1495.4815.

3′*O*-α-D-Glucopyranosyl-6-methoxy-9-*β*-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (Na$^+$ salt) (7). A mixture of **18** (35 mg, 0.023 mmol) and $Pd(OH)_2$ on carbon (10%, 110 mg) in cyclohexene (3 mL), MeOH (6 mL) and water (0.5 mL) was heated at 65 °C for 31 h. The catalyst was filtered off and washed well with deionized water and MeOH. The filtrate and washings were concentrated under reduced pressure to leave a glassy solid. The residue was dissolved in deionized water (1 mL), applied to a Diaion WK-20 resin column (Na$^+$ form), and eluted with deionized water. The eluent was concentrated under reduced pressure to give the title trisphosphate (0.019 mmol, 83%) as its sodium salt; ^{1}H NMR (400 MHz; D_2O) δ_H 8.32 and 8.28 (2 s, 2 H, H-2, H-8), 6.14 (d, 1 H, $J_{1',2}$ 6.2 Hz, H-1′), 5.19 (d, 1 H, $J_{1'',2}$ 3.9 Hz, H-1″), 5.10−5.04 (m, 1 H, H-2′), 4.55−4.48 (1 H, m, H-3′), 4.38−4.23 (m, 2 H, H-4′, H-4″), 3.97 (s, 3 H, OCH_3) and 3.83−3.52 (m, 7 H, H-5′a, H-5′b, H-2″, H-3″, H-5″, H-6″a, H-6″b); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 5.64, 4.93 and 4.87; MS: (FAB) *m*/*z* 684.1 [(M − H)$^-$, 28%], *m*/*z* calcd for $C_{17}H_{27}N_4O_{19}P_3$ [M − H]$^-$, 684.0437 found *m*/*z* 684.0447.

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-6-chloro-9-*β*-D-ribofuranosylpurine (19). To a stirred solution of **15** (300 mg, 0.35 mmol) in MeOH was added NaOMe (30 mg, 0.56 mmol). The mixture was stirred for 1.5 h at room temperature, after which time it was neutralized with Dowex 50WX4−50 ion-exchange resin and filtered. The filtrate was concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using EtOAc−hexane (7:3) as eluent to give the title compound as a colorless oil (140 mg, 55%); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.65 (s, 1 H, H-8), 8.44 (s, 1 H, H-2), 7.37−7.23 (m, 15 H, ArC*H*), 6.23 (d, 1 H, $J_{1',2}$ 6.2 Hz, H-1′), 4.81 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.70 (AB, 1 H, 0.5 × OCH_2Ar), 4.68−4.64 (m, 1 H, H-2′), 4.56−4.40 (m, 7 H, 2.5 × OCH_2Ar, H-2′, H-4′, O*H*), 4.26 (br s, 1 H, O*H*), 4.22 (dd, 1 H, $J_{3',4}$ 2.7 Hz, $J_{3',2}$ 5.5 Hz, H-3′), 4.06 (t covered by s, $J_{3'',2} = J_{3'',4''}$ 9.7 Hz, H-3″, O*H*), 3.92−3.88 (m, 1 H, H-5″), 3.68−3.65 (m, 3 H, H-4″, H-6″a, H-6″b), 3.62 (dd, 1 H, $J_{5'a,4'}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5′a), 3.55 (dd, 1 H, $J_{5'b,4'}$ 2.3 Hz, $J_{5'b,5'a}$ 10.9 Hz, H-5′b) and 3.43 (dd, 1 H, $J_{2'',1''}$ 3.5 Hz, $J_{2'',3''}$ 9.7 Hz, H-2″); MS: (FAB) *m*/*z* 719.1 [(M + H)$^+$, 32%], *m*/*z* calcd for $C_{37}H_{39}N_4O_9Cl$ [M + H]$^+$ ^{37}Cl, 721.2454 found *m*/*z* 721.2481, [M + H]$^+$ ^{35}Cl, 719.2483 found *m*/*z* 719.2478.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-cyclopentylamino-9-*β*-D-ribofuranosylpurine (20). To a stirred solution of **19** (140 mg, 0.19 mmol) in ethanol (10 mL) were added cyclopentylamine (0.04 mL, 0.38 mmol) and Et_3N (0.03 mL, 0.23 mmol). The mixture was heated at 80 °C for 3 h and then cooled and concentrated under reduced pressure. Water (20 mL) was added to the residue, and the resulting solution was extracted with EtOAc (3 × 25 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica using EtOAc−hexane (8:2) and then EtOAc−

MeOH (10:1) to give the title compound (80 mg, 54%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.44 (s, 1 H, H-8), 8.03 (s, 1 H, H-2), 7.37−7.21 (m, 15 H, ArC*H*), 6.28 (d, 1 H, $J_{1',2}$ 5.9 Hz, H-1′), 6.18 (broad s, 1 H, N*H*), 5.92 (broad s, 1 H, O*H*), 4.77 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.66 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.58−4.42 (m, 7 H, 2.5 × OCH_2Ar, H-2′, H-3′), 4.19−4.06 (m, 2 H, H-4′, H-3″), 3.99−3.96 (m, 1 H, H-5″), 3.78−3.63 (m, 3 H, H-4″, H-6″a, H-6″b), 3.61−3.50 (m, 2 H, H-5′a, H-5′b), 3.40 (dd, 1 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 9.8 Hz, H-2″), 2.3 (broad s, 2 H, O*H*), 2.17−2.09 (m, 2 H, cyclopentyl ring), 1.79−1.63 (m, 4 H, cyclopentyl ring) and 1.59−1.25 (m, 3 H, cyclopentyl ring); MS: (FAB) *m*/*z* 768.2 [(M + H)$^+$, 88%], *m*/*z* calcd for $C_{42}H_{49}N_5O_9$ [M + H]$^+$, 768.3608 found *m*/*z* 768.3612.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-6-cyclopentylamino-9-β-D-ribofuranosylpurine (21). Phosphorylation of **20** (80 mg, 0.10 mmol) was performed as described for the synthesis of **18**. Purification was achieved by flash chromatography on silica using EtOAc−hexane (1:1 then 4:1 then 1:0) to give the title compound (40 mg, 25%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.48 (s, 1 H, H-8), 8.00 (s, 1 H, H-2), 7.63 (d, 1 H, *J* 8.9 Hz, N*H*), 7.38−6.95 (m, 45 H, ArC*H*), 6.29 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.57−5.52 (m, 1 H, H-2′) 5.30 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.00−4.85 (m, 8 H, H-3″, 3.5 × OCH_2Ar), 4.76−4.26 (m, 14 H, H-3′, H-4′, H-4″, 5.5 × OCH_2Ar) 3.82−3.80 (m, 1 H, H-5″), 3.68−3.48 (m, 5 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b) and 2.19−1.20 (m, 9 H, cyclopentyl ring); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 0.15, −0.66, −0.79; MS: (FAB) *m*/*z* 1564.8 [(M + H)$^+$, 44%], *m*/*z* calcd for $C_{84}H_{89}N_5O_{18}P_3$ [M + H]$^+$ 1564.5364, found *m*/*z* 1564.5345.

3′*O*-α-D-Glucopyranosyl-6-cyclopentylamino-9-β-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (Na$^+$ salt) (8). Deprotection of **21** (40 mg, 0.025 mmol) and purification were performed as described for the synthesis of **7** to give the target compound **8** (0.012 mmol, 46%) as a highly hygroscopic solid; ^{1}H NMR (400 MHz; D_2O) δ_H 8.02 and 8.00 (2 s, 2 H, H-2, H-8), 6.04 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 5.15 (d, 1 H, $J_{1'',2}$ 3.9 Hz, H-1″), 5.08−5.03 (m, 1 H, H-2′), 4.55−4.41 (m, 1 H, H-3′), 4.32−4.21 (m, 3 H, H-4′, H-4″, N*H*), 3.83−3.44 (m, 7 H, H-5′a, H-5′b, H-2″, H-3″, H-5″, H-6″a, H-6″b) and 1.99−1.42 (m, 9 H, cyclopentyl ring); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.28, 2.56 and 1.59; MS: (FAB) *m*/*z* 736.2 [(M + H)$^+$, 44%], *m*/*z* calcd for $C_{21}H_{34}N_5O_{18}P_3$ [M − H]$^-$ 736.1033, found *m*/*z* 736.1019.

General Procedure for the Amination of 15. To a solution of **15** in CH_2Cl_2 (5 mL) and EtOH (1 mL) were added the corresponding amine/amine salt (7 equiv) and dry Et_3N (14 equiv). The mixture was heated at 60 °C for the required time, then cooled and concentrated under reduced pressure. The residue was taken up in EtOAc (30 mL) and washed with water (20 mL). The extract was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to leave an oil, which was purified by flash chromatography on silica.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6-tri-*O*-benzyl-3′*O*-α-d-glucopyranosyl-6-methylamino-9-β-D-ribofuranosylpurine (22). The amination was carried out with methylamine hydrochloride (218 mg, 3.2 mmol) as described above, and the product was purified by flash chromatography on silica using CH_2Cl_2−acetone (40:1) as eluent to give **22** (260 mg, 67%) as a colorless oil; $[\alpha]^{20}_D$ +40.0 (*c* 0.60, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.41 (s, 1 H, H-8), 8.04 (s, 1 H, H-2), 7.40−7.23 (m, 15 H, ArC*H*), 6.30 (d, 1 H, $J_{1',2'}$ 5.1 Hz, H-1′), 5.87 (broad s, 1 H, N*H*), 5.71 (t, 1 H, $J_{2',1'} = J_{2',3'}$ 5.1 Hz, H-2′), 5.42 (t, 1 H, $J_{3'',2} = J_{3'',4}$ 9.8 Hz, H-3″), 5.03 (t, 1 H, $J_{4'',3} = J_{4'',5''}$ 9.8 Hz, H-4″), 4.97 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.75 (t, 1 H, $J_{3',2} = J_{3',4}$ 5.1 Hz, H-3′), 4.61 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.56−4.44 (m, 5 H, H-4′, 2 × OCH_2Ar), 4.41 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 3.99−3.95 (m, 1 H, H-5″), 3.74 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5′a), 3.64 (dd, 1 H, $J_{5'b,4}$ 3.1 Hz, $J_{5'b,5'}$ 10.9 Hz, H-5′b), 3.54 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.8 Hz, H-2″), 3.39−3.33 (m, 2 H, H-6″a, H-6″b), 3.19 (broad s, 3 H, NCH_3), 1.97 (s, 3 H, CH_3CO), 1.93 (s, 3 H, CH_3CO) and 1.88 (s, 3 H, CH_3CO); MS: (FAB) *m*/*z* 840.3 [(M + H)$^+$, 42%], *m*/*z* calcd for $C_{44}H_{49}N_5O_{12}$ [M + H] 840.3455 found *m*/*z* 840.3469.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-methylamino-9-β-D-ribofuranosylpurine (23). To a solution of **22** (100 mg, 0.12 mmol) was added NaOMe (5 mg, 0.09 mmol), and the mixture was stirred for 30 min. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with $CHCl_3$ (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an oil (80 mg, 94%) which was crystallized from MeOH; mp 174−175 °C; $[\alpha]^{20}_D$ 0 ± 1 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.31 (s, 1 H, H-8), 8.02 (s, 1 H, H-2), 7.49−7.22 (m, 15 H, ArC*H*), 6.25 (d, 1 H, $J_{1',2}$ 5.9 Hz, H-1′), 6.09 (broad s, 1 H, N*H*), 5.67 (broad s, 1 H, O*H*), 4.80 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.70 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.68−4.38 (m, 7 H, H-2′, H-3′, 2.5 × OCH_2Ar), 4.19−4.18 (m, 1 H, H-4′), 4.11 (t, 1 H, $J_{3'',2''} = J_{3'',4''}$ 9.7 Hz, H-3″), 3.96−3.90 (m, 1 H, H-5″), 3.78−3.60 (m, 3 H, H-4″, H-6″a, H-6″b), 3.58−3.47 (m, 2 H, H-5′a, H-5′b), 3.42 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.7 Hz, H-2″) and 3.18 (broad s, 3 H, NCH_3); MS: (FAB) *m*/*z* calcd for $C_{38}H_{43}N_5O_9$ [M + H]$^+$ 714.3139 found *m*/*z* 714.3139. Anal. Calcd for $C_{38}H_{43}N_5O_9$ C, H, N.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-6-methylamino-9-β-D-ribofuranosylpurine (24). Phosphorylation of **23** (80 mg, 0.10 mmol) was performed as described for the synthesis of **18**. Purification by flash chromatography on silica using EtOAc−hexane (1:1 then 4:1) gave the title compound (60 mg, 36%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.31 (s, 1 H, H-8), 7.83 (s, 1 H, H-2), 7.52−6.95 (m, 45 H, ArC*H*), 6.32 (d, 1 H, $J_{1',2}$ 6.2 Hz, H-1′), 5.73 (br s, 1 H, NH), 5.65−5.60 (m, 1 H, H-2′), 5.32 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.05−4.86 (m, 8 H, H-3″, 3.5 × OCH_2Ar), 4.76−4.36 (m, 13 H, H-3′, H-4′, H-4″, 5 × OCH_2Ar), 4.30 (AB, 1 H, 0.5 × OCH_2Ar), 3.84−3.82 (m, 1 H, H-5″), 3.66−3.53 (m, 5 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b) and 3.16 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P −0.24, −0.91, −1.05; MS: (FAB) *m*/*z* 1494.8 [(M + H)$^+$, 84%], *m*/*z* calcd for $C_{80}H_{82}N_5O_{18}P_3$ [M + H]$^+$ 1494.4946 found *m*/*z* 1494.4942.

3′*O*-α-D-Glucopyranosyl-6-methylamino-9-β-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (Na$^+$ salt) (9). Deprotection of **24** (28 mg, 0.018 mmol) and purification were performed as described for the synthesis of **7** to give the target compound **9** (0.016 mmol, 80%) as a highly hygroscopic solid; ^{1}H NMR (400 MHz; D_2O) δ_H 8.09 (s, 2 H, H-2, H-8), 6.12 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 5.20 (d, 1 H, $J_{1'',2}$ 3.9 Hz, H-1″), 5.17−5.11 (m, 1 H, H-2′), 4.49−4.48 (m, 1 H, H-3′), 4.38−4.30 (m, 3 H, H-4′, H-3″, N*H*), 3.87 (m, 1 H, H-4″), 3.78−3.59 (m, 6 H, H-5′a, H-5′b, H-2″, H-5″, H-6″a, H-6″b) and 2.92 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 2.45, 2.10 and 0.86; MS: (FAB) *m*/*z* 682.1 [(M − H)−, 84%], *m*/*z* calcd for $C_{17}H_{28}N_5O_{18}P_3$ [M − H]$^-$ 682.0564 found *m*/*z* 682.0567.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-(dimethylamino)-9-β-D-ribofuranosylpurine (25). The reaction was carried out with dimethylamine hydrochloride (180 mg, 2.20 mmol) for 2 h as described for **22**, and purification by flash chromatography on silica using CH_2Cl_2−acetone (50:1 then 9:1) as eluent gave the title compound (290 mg, 96%) as a colorless oil; $[\alpha]^{20}_D$ +52.9 (*c* 0.55, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.46 (s, 1 H, H-8), 8.17 (s, 1 H, H-2), 7.47−7.17 (m, 15 H, ArC*H*), 6.44 (d, 1 H, $J_{1',2}$ 5.1 Hz, H-1′), 5.82 (t, 1 H, $J_{2',1'} = J_{2',3'}$ 5.1 Hz, H-2′), 5.53 (t, 1 H, $J_{3'',2''} = J_{3'',4''}$ 9.8 Hz, H-3″), 5.16 (t, 1 H, $J_{4'',3} = J_{4'',5''}$ 9.8 Hz, H-4″), 5.08 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.85 (t, 1 H, $J_{3',2'} = J_{3',4}$ 5.1 Hz, H-3′), 4.74 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.70−4.57 (m, 5 H, H-4′, 2 × OCH_2Ar), 4.43 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.11−4.06 (m, 1 H, H-5″), 3.85 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5′a), 3.75 (dd, 1 H, $J_{5'b,4}$ 3.1 Hz, $J_{5'b,5'}$ 10.9 Hz, H-5′b), 3.67−3.64 (m, 7 H, H-2″, N(CH_3)$_2$), 3.52−3.44 (m, 2 H, H-6″a, H-6″b), 2.08 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO) and 1.99 (s, 3 H, CH_3CO); MS: (FAB) *m*/*z* 854.2 [(M + H)$^+$, 48%], *m*/*z* calcd for $C_{45}H_{50}N_5O_{12}$ [M + H] 854.3567, found *m*/*z* 854.3619; Anal. Calcd for $C_{45}H_{50}N_5O_{12}$ C, 63.30; H, 5.90; N 8.27%. Found: C, 62.80; H, 5.30; N 7.97%.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-(dimethylamino)-9-β-D-ribofuranosylpurine (26). Deacetylation of **25** (290 mg, 0.34 mmol) was performed as described for the synthesis of **23**. Crystallization from MeOH, gave pure **26** (140 mg, 57%); mp 153−155 °C; $[\alpha]^{20}_D$ +2.2 ± 1 (*c* 0.45, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 8.02 (s, 1 H, H-2), 7.35−7.24 (m, 15 H, ArC*H*), 6.20 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 4.85 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.76−4.38 (m, 8 H, H-2′, H-3′, 3 × OCH_2Ar), 4.25−4.23 (m, 1 H, H-4′), 4.08 (t, 1 H, $J_{3'',2}$ = $J_{3'',4''}$ 9.8 Hz, H-3″), 3.91−3.88 (m, 1 H, H-5″), 3.69−3.49 (m, 11 H, H-5′a, H-5′b, H-4″, H-6″a, H-6″b, N(CH_3)$_2$) and 3.41 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.8 Hz, H-2″); MS: (FAB) *m*/*z* calcd for $C_{39}H_{45}N_5O_9$ [M + H]$^+$ 728.3250 found *m*/*z* 728.3280.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4-tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-6-(dimethylamino)-9-β-D-ribofuranosylpurine (27). Phosphorylation of **26** (40 mg, 0.05 mmol) was performed as described for the synthesis of **18**. Purification was achieved by flash chromatography on silica using EtOAc−hexane (1:1 then 4:1) to give the title compound (45 mg, 54%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 7.89 (s, 1 H, H-2), 7.39−6.95 (m, 45 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.69−5.64 (m, 1 H, H-2′), 5.32 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.09 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 5.06−4.85 (m, 10 H, H-3″, 4.5 × OCH_2Ar), 4.47−4.36 (m, 10 H, H-3′, H-4′, H-4″, 3.5 × OCH_2-Ar), 4.29 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.84−3.82 (m, 1 H, H-5″) and 3.65−3.43 (m, 11 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b, N(CH_3)$_2$); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P −0.05, −0.66, −0.82; MS: (FAB) *m*/*z* 1508.4 [(M + H)$^+$, 79%], *m*/*z* calcd for $C_{81}H_{84}N_5O_{18}P_3$ [M + H]$^+$ 1508.5102 found *m*/*z* 1508.5068.

3′*O*-α-D-Glucopyranosyl-6-(dimethylamino)-9-β-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (10). Deprotection of **27** (20 mg, 0.013 mmol) was performed as described for the synthesis of **7** to give the target compound **10**, which was purified by HPLC (see Methods) to give **10** (0.004 mmol, 31%) as the free acid; ^{1}H NMR (400 MHz; D_2O) δ_H 8.29 and 8.17 (2 s, 2 H, H-2, H-8), 6.19 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 5.12 (d, 1 H, $J_{1'',2}$ 3.9 Hz, H-1″), 5.10−5.05 (m, 1 H, H-2′), 4.46−4.44 (m, 1 H, H-3′), 4.34−4.27 (m, 2 H, H-4′, H-3″), 3.93−3.86 (m, 1 H, H-4″) and 3.71−3.56 (m, 12 H, H-5′a, H-5′b, H-2″, H-5″, H-6″a, H-6″b, N(CH_3)$_2$); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 4.61 (2 × P) and 4.52 (1 P); MS: (FAB) *m*/*z* 698.0 [(M + H)$^+$, 40%], *m*/*z* calcd for $C_{18}H_{30}N_5O_{18}P_3$ [M + H]$^+$ 698.0877 found *m*/*z* 698.0894.

2′,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-6-cyclohexylamino-9-β-D-ribofuranosylpurine (28). The reaction of **15** with cyclohexylamine (0.08 mL, 1.1 mmol) was carried out overnight as described for the synthesis of **22**. The oil obtained was dissolved in MeOH and NaOMe (30 mg, 0.56 mmol) was added, and the mixture was stirred for 30 min. It was then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with $CHCl_3$ (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an oil which was crystallized from MeOH (70 mg, 50%); mp 103−106 °C; $[\alpha]^{20}_D$ +0.01 ± 1 (*c* 0.38, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.26 (s, 1 H, H-8), 8.01 (s, 1 H, H-2), 7.37−7.22 (m, 15 H, ArC*H*), 6.25 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.85 (broad s, 2 H, N*H*, O*H*), 4.78 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.65 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.59−4.38 (m, 7 H, H-2′, H-3′, 2.5 × OCH_2Ar), 4.17−4.09 (m, 3 H, H-4′, H-3″, O*H*), 3.97−3.94 (m, 1 H, H-5″), 3.76−3.64 (m, 3 H, H-4″, H-6″a, H-6″b), 3.58−3.51 (m, 2 H, H-5′a, H-5′b), 3.47 (d, 1 H, *J* 5.1 Hz, cyclohexyl), 3.41 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.9 Hz, H-2″), 2.11−1.91 (m, 3 H, cyclohexyl), 1.91−1.77 (m, 2 H, cyclohexyl), 1.68−1.66 (m, 1 H, cyclohexyl), 1.52−1.42 (m, 2 H, cyclohexyl) and 1.34−1.22 (m, 2 H, cyclohexyl); MS: (FAB) *m*/*z* 782.3 [(M + H)$^+$, 60%], *m*/*z* calcd for $C_{43}H_{51}N_5O_9$ [M + H]$^+$, 782.3765 found *m*/*z* 782.3768. Anal. Calcd for $C_{43}H_{51}N_5O_9$ C, H, N.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-6-cyclohexylamino-9-β-D-ribofuranosylpurine (29). Phosphorylation of **28** (40 mg, 0.05 mmol) was performed as described for the synthesis of **18**. Purification by flash chromatography on silica using EtOAc−hexane (2:3 then 1:1 then 4:1) gave the title compound (55 mg, 68%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 8.28 (s, 1 H, H-8), 7.82 (s, 1 H, H-2), 7.39−6.94 (m, 45 H, ArC*H*), 6.32 (d, 1 H, $J_{1',2}$ 6.6 Hz, H-1′), 5.69 (br s, 1 H, NH), 5.64−5.59 (m, 1 H, H-2′), 5.31 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.08−4.30 (m, 21 H, H-3′, H-4′, H-3″, H-4″, 8.5 × OCH_2Ar,), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.89−3.81 (m, 1 H, H-5″), 3.66−3.23 (m, 5 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b) and 2.18−1.18 (m, 11 H, cyclohexyl); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P −0.23, −0.89, −1.05; MS: (FAB) *m*/*z* 1563.6 [(M + H)$^+$, 64%], *m*/*z* calcd for $C_{85}H_{90}N_5O_{18}P_3$ [M + H]$^+$ 1563.5605 found *m*/*z* 1563.5602.

3′*O*-α-D-Glucopyranosyl-6-cyclohexylamino-1-β-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (Na$^+$ salt) (11). Deprotection of **29** (25 mg, 0.016 mmol) and purification were performed as described for the synthesis of **7** to give the target compound **11** (15.27 μmol, 46%) as a highly hygroscopic solid; ^{1}H NMR (400 MHz; D_2O) δ_H 8.07 and 8.03 (2 s, 2 H, H-2, H-8), 6.09 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.19 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.14−5.08 (m, 1 H, H-2′), 4.59−4.49 (m, 1 H, H-3′), 4.35−4.26 (m, 3 H, H-4′, H-3″, H-4″) and 3.89−3.58 (m, 6 H, H-5′a, H-5′b, H-2″, H-5″, H-6″a, H-6″b), 1.89−1.82 (m, 2 H, cyclohexyl), 1.73−1.59 (m, 2 H, cyclohexyl), 1.49−1.45 (m, 1 H, cyclohexyl) and 1.35−1.08 (m, 6 H, cyclohexyl); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.31, 2.66 and 1.49; MS: (FAB) *m*/*z* 752.1 [(M + H)$^+$, 60%], *m*/*z* calcd for $C_{22}H_{36}N_5O_{18}P_3$ [M + H]$^+$ 752.1346 found *m*/*z* 752.1361.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-2-chloro-6-methylamino-9-β-D-ribofuranosylpurine (30). The reaction was carried out with methylamine hydrochloride (179 mg, 2.60 mmol) and **16** (390 mg, 0.44 mmol), for 4 h as described for **22**, and purification by flash chromatography on silica using CH_2Cl_2−acetone (40:1) as eluent gave the title compound (260 mg, 67%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.99 (s, 1 H, H-8), 7.36−7.24 (m, 15 H, ArC*H*), 6.27 (d, 1 H, $J_{1',2}$ 5.5 Hz, H-1′), 6.16 (broad s, 1 H, N*H*), 5.61 (t, 1 H, $J_{2',1'}$ = $J_{2',3'}$ 5.5 Hz, H-2′), 5.43 (dd, 1 H, $J_{3'',2''}$ 10.1 Hz, $J_{3'',4''}$ 9.4 Hz, H-3″), 5.05 (t, 1 H, $J_{4'',3}$ = $J_{4'',5''}$ 9.4 Hz, H-4″), 4.97 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.71 (t, 1 H, $J_{3',2}$ = $J_{3',4}$ 5.5 Hz, H-3′), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.55−4.47 (m, 5 H, H-4′, 2 × OCH_2Ar), 4.34 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.00−3.96 (m, 1 H, H-5″), 3.63 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'a,5'b}$ 10.9 Hz, H-5′a), 3.55 (dd, 1 H, $J_{5'b,4}$ 3.1 Hz, $J_{5'b,5'}$ 10.5 Hz, H-5′b), 3.40 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 10.1 Hz, H-2″), 3.36−3.26 (m, 2 H, H-6″a, H-6″b), 3.16 (broad s, 3 H, NCH_3), 1.98 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3-CO) and 1.89 (s, 3 H, CH_3CO); MS: (FAB) *m*/*z* 874.1 [(M + H)$^+$, 90%], *m*/*z* calcd for $C_{44}H_{47}N_5O_{12}Cl$ [M + H]$^+$ ^{37}Cl 876.2992 found *m*/*z* 876.3042, [M + H]$^+$ ^{35}Cl 874.3021 found *m*/*z* 874.3043.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-2-chloro-6-methylamino-9-β-D-ribofuranosylpurine (31). Deacetylation of **30** (70 mg, 0.08 mmol) was performed as described for the synthesis of **23** to yield the title compound (50 mg, 84%) as an oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.97 (s, 1 H, H-8), 7.37−7.21 (m, 15 H, ArC*H*), 6.28 (br s, 1 H, N*H*), 6.22 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 4.73 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.65 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar) 4.50−4.38 (m, 7 H, H-2′, H-4′, 2.5 × OCH_2Ar), 4.16−4.11 (m, 3 H, H-3′, H-3″, H-5″), 3.92−3.87 (m, 1 H, H-6″a), 3.74 (t, 1 H, $J_{3'',2}$ = $J_{3'',4''}$ 10.0 Hz, H-4″), 3.66 (dd, 1 H, $J_{6''b,5}$ 3.1 Hz, $J_{6''b,6''}$ 10.5 Hz, H-6″b), 3.53−3.48 (m, 2 H, H-5′a, H-5′b), 3.41 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 10.0 Hz, H-2″) and 3.14 (s, 3 H, NCH_3); MS: (FAB) *m*/*z* 748.2 [(M + H)$^+$, 36%], *m*/*z* calcd for $C_{38}H_{42}N_5O_9Cl$ [M + H]$^+$ ^{37}Cl 750.2719 found *m*/*z* 750.2754, [M + H]$^+$ ^{35}Cl 748.2749 found *m*/*z* 748.2759.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-2-methoxy-6-methylamino-9-β-D-ribofuranosylpurine (32). NaOMe (61 mg, 1.13 mmol) was added to a solution of **31** (100 mg, 0.11 mmol) in MeOH (10 mL), and the mixture was heated at reflux overnight. The mixture was allowed to cool and then concentrated under reduced pressure to leave a white solid, which was washed with water (10 mL) and extracted with

$CHCl_3$ (3 × 20 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an oil which was purified by flash chromatography on silica using CH_2Cl_2−acetone (40:1) as eluent to give the title compound, which was crystallized from MeOH (60 mg, 71%); mp 192−193 °C; $[\alpha]^{20}_D$ 0 ± 1 (*c* 0.40, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.79 (s, 1 H, H-8), 7.37−7.22 (m, 15 H, ArC*H*), 6.10 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.93 (br s, 1 H, N*H*), 5.06 (br s, 1 H, OH), 4.80 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.70 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 4.60−4.42 (m, 6 H, H-2′, H-3′, 2 × OCH_2Ar), 4.70 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.31−4.29 (m, 1 H, H-4′), 3.74 (t, 1 H, $J_{3'',2} = J_{3'',4''}$ 9.8 Hz, H-3″), 3.97−3.94 (m, 2 H, H-5″, OH), 3.79 (dd, 1 H, $J_{5'a,4}$ 2.7 Hz, $J_{5'b,5'}$ 10.5 Hz, H-5′), 3.68−3.61 (m, 4 H, H-5′b, H-4″, H-6″a, H-6″b), 3.86 (s, 3 H, OCH_3), 3.42 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.8 Hz, H-2″) and 3.14 (s, 3 H, NCH_3); MS: (FAB) *m*/*z* 744.2 [(M + H)$^+$, 61%], *m*/*z* calcd for $C_{39}H_{45}N_5O_{18}$ [M + H]$^+$ ^{37}Cl 744.3244 found *m*/*z* 744.3246; Anal. Calcd for $C_4H_4N_5O_{12}$ C, 62.98; H, 6.10; N 9.42%. Found: C, 62.50; H, 6.10; N 9.25%.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-2-methoxy-6-methylamino-9-*β*-D-ribofuranosylpurine (33). Phosphorylation of **32** (30 mg, 0.040 mmol) was performed as described for the synthesis of **18**. Purification was achieved by flash chromatography on silica using EtOAc−hexane (2:3 then 1:1 then 4:1) to give the title compound (35 mg, 57%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.63 (s, 1 H, H-8), 7.37−6.96 (m, 45 H, ArC*H*), 6.37 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 5.82 (broad s, 1 H, N*H*), 5.71−5.61 (m, 1 H, H-2′), 5.33 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.02−4.87 (m, 8 H, H-3″, 3.5 × OCH_2Ar), 4.77−4.27 (m, 14 H, H-3′, H-4′, H-4″, 5.5 × OCH_2Ar), 3.86−3.80 (m, 1 H, H-5″), 3.70 (s, 3 H, OCH_3), 3.69−3.53 (m, 5 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b) and 3.11 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P 0.04, −0.58, −0.79; MS: (FAB) *m*/*z* 1524.1 [(M), 89%], *m*/*z* calcd for $C_{81}H_{84}N_5O_{18}P_3$ [M + H]$^+$ 1525.5085 found *m*/*z* 1525.5159.

3′*O*-α-D-Glucopyranosyl-2-methoxy-6-methylamino-9-*β*-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (13). Deprotection of **33** (20 mg, 0.013 mmol) and purification were performed as described for the synthesis of **7** to give the target compound **13** (0.012 mmol, 92%) which was further purified by HPLC to give the title trisphosphate (6.1 *μ*mol, 47%) as the free acid; ^{1}H NMR (400 MHz; D_2O) δ_H 7.82 (s, 1 H, H-8), 5.99 (d, 1 H, $J_{1',2}$ 5.1 Hz, H-1′), 5.28−5.23 (m, 1 H, H-2′), 5.11 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.30−4.18 (m, 3 H, H-3′, H-4′, H-3″), 3.83−3.80 (m, 1 H, H-4″), 3.77 (s, 3 H, OCH_3), 3.74−3.31 (m, 6 H, H-5′a, H-5′b, H-2″, H-5″, H-6″a, H-6″b) and 3.12 (s, 3 H, NCH_3); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.15, 2.28 and 1.33; MS: (FAB) *m*/*z* 714.0 [(M + H)$^+$, 69%], *m*/*z* calcd for $C_{18}H_{30}N_5O_{18}P_3$ [M + H]$^+$ 714.0826 found *m*/*z* 714.0860.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′*O*-*a*-*d*-glucopyranosyl-2-chloro-6-(3-noradamantylamino)-9-*β*-D-ribofuranosylpurine (34). The reaction was carried out with 3-aminonoradamantane hydrochloride (212 mg, 1.2 mmol) and **16** (179 mg, 0.2 mmol), for 48 h as described for **22**, and the product was purified by flash chromatography on silica using EtOAc−hexane (4:1) as eluent to give the title compound (160 mg, 80%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.97 (s, 1 H, H-8), 7.36−7.24 (m, 15 H, ArC*H*), 6.26 (d, 1 H, $J_{1',2}$ 5.5 Hz, H-1′), 6.16 (br s, 1 H, N*H*), 5.61 (dd, 1 H, $J_{2',1'}$ 5.5 Hz, $J_{2',3'}$ 5.0 Hz, H-2′), 5.43 (t, 1 H, $J_{3'',2} = J_{3'',4''}$ 9.9 Hz, H-3″), 5.04 (t, 1 H, $J_{4'',3} = J_{4'',5''}$ 9.9 Hz, H-4″), 4.97 (d, 1 H, $J_{1'',2}$ 3.3 Hz, H-1″), 4.72 (t, 1 H, $J_{3',2} = J_{3',4}$ 5.0 Hz, H-3′), 4.62 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.55−4.46 (m, 5 H, H-4′, 2 × OCH_2Ar), 4.36 (AB, 1 H, J_{AB} 12.1 Hz, 0.5 × OCH_2Ar), 4.10−3.97 (m, 1 H, H-5″), 3.71 (dd, 1 H, $J_{5'a,4}$ 2.9 Hz, $J_{5'a,5'b}$ 10.6 Hz, H-5′a), 3.63 (dd, 1 H, $J_{5'b,4}$ 3.3 Hz, $J_{5'b,5'}$ 10.6 Hz, H-5′b), 3.54 (dd, 1 H, $J_{2'',1}$ 3.3 Hz, $J_{2'',3''}$ 9.9 Hz, H-2″), 3.43−3.35 (m, 2 H, H-6″a, H-6″b), 2.65−2.61 (m, 1 H, noradamantane), 2.35−2.19 (m, 7 H, noradamantane), 2.05−1.94 (m, 1 H, noradamantane), 1.98 (s, 3 H, CH_3CO), 1.94 (s, 3 H, CH_3CO), 1.89 (s, 3 H, CH_3CO) and 1.69−1.57 (m, 4 H, noradamantane); MS: (FAB) *m*/*z* 980.3 [(M + H)$^+$, 80%], *m*/*z* calcd for $C_{52}H_{58}N_5O_{12}Cl$ [M + H]$^+$ ^{37}Cl 982.3819 found *m*/*z* 982.3854, [M + H]$^+$ ^{35}Cl 980.3848 found *m*/*z* 980.3848.

2″,5′,6″-Tri-*O*-benzyl-3′*O*-α-D-glucopyranosyl-2-chloro-6-(3-noradamantylamino)-9-*β*-D-ribofuranosylpurine (35). Deacetylation of **34** (90 mg, 0.092 mmol) was performed as described for the synthesis of **23**. Purification was achieved by flash chromatography on silica using CH_2Cl_2−MeOH (20:1) as eluent to give the title compound (65 mg, 84%) as a colorless oil; $[\alpha]^{20}_D$ +6.8 ± 1.0 (*c* 0.59, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.98 (s, 1 H, H-8), 7.37−7.21 (m, 15 H, ArC*H*), 6.24 (d, 1 H, $J_{1',2}$ 5.9 Hz, H-1′), 5.84 (br s, 1 H, N*H*), 4.69 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 4.63 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2-Ar), 4.59−4.36 (m, 7 H, H-2′, H-3′, 2.5 × OCH_2Ar), 4.15 (t, 1 H, $J_{3'',2} = J_{3'',4''}$ 9.8 Hz, H-3″), 4.06−4.01 (m, 2 H, H-4′, H-5″), 3.95 (dd, 1 H, $J_{6''a,5}$ 2.1 Hz, $J_{6''a,6''b}$ 10.5 Hz, H-6″a), 3.76 (t, 1 H, $J_{4'',3} = J_{4'',5}$ 9.4 Hz, H-4″,) 3.66 (dd, 1 H, $J_{6''b,5}$ 7.4 Hz, $J_{6''b,6''a}$ 10.5 Hz H-6″b), 3.51−3.44 (m, 2 H, H-5′a, H-5′b), 3.41 (dd, 1 H, $J_{2'',1}$ 3.5 Hz, $J_{2'',3''}$ 9.8 Hz, H-2″), 2.64−2.61 (m, 1 H, noradamantane), 2.35−2.18 (m, 6 H, noradamantane), 2.02−1.94 (m, 2 H, noradamantane) and 1.69−1.56 (m, 4 H, noradamantane); MS: (FAB) *m*/*z* 854.2 [(M + H)$^+$, 52%], *m*/*z* calcd for $C_{46}H_{52}N_5O_9Cl$ [M + H]$^+$ ^{37}Cl 857.3535 found *m*/*z* 857.3583, [M + H]$^+$ ^{35}Cl 854.3531 found *m*/*z* 854.3502.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4-tris-*O*-[di(benzyloxy)phosphoryl]-3′*O*-α-D-glucopyranosyl-2-chloro-6-(3-noradamantylamino)-9-*β*-d-ribofuranosylpurine (36). Phosphorylation of **35** (40 mg, 0.04 mmol) was performed as described for the synthesis of **18**. Purification was achieved by flash chromatography on silica using EtOAc−hexane (2:3 then 1:1 then 4:1) to give the title compound (35 mg, 46%) as a colorless oil; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H 7.72 (s, 1 H, H-8), 7.51−6.99 (m, 45 H, ArC*H*), 6.24 (d, 1 H, $J_{1',2}$ 6.3 Hz, H-1′), 6.06 (br s, 1 H, N*H*), 5.51−5.46 (m, 1 H, H-2′), 5.30 (d, 1 H, $J_{1'',2}$ 4.3 Hz, H-1″), 5.04−4.33 (m, 21 H, H-3′, H-4′, H-3″, H-4″, 8.5 × OCH_2Ar), 4.30 (AB, 1 H, J_{AB} 11.7 Hz, 0.5 × OCH_2Ar), 3.82−3.78 (m, 1 H, H-5″), 3.72−3.52 (m, 5 H, H-5′a, H-5′b, H-2″, H-6″a, H-6″b), 2.63−2.59 (m, 1 H, noradamantane), 2.34−2.17 (m, 6 H, noradamantane), 2.09−1.98 (m, 2 H, noradamantane) and 1.65−1.56 (m, 4 H, noradamantane);^{31}P NMR (162 MHz; $CDCl_3$; ^{1}H decoupled) δ_P −0.23, −0.91, −0.11; MS: (FAB) *m*/*z* calcd for $C_{88}H_{91}N_5O_{18}P_3Cl$ [M + H]$^+$ 1636.5309 found *m*/*z* 1636.5334.

3′*O*-α-D-Glucopyranosyl-6-(3-noradamantylamino)-9-*β*-D-ribofuranosylpurine 2′,3″,4″-Trisphosphate (Na$^+$ salt) (12). Deprotection of **36** (10 mg, 0.0061 mmol)) and purification were performed as described for the synthesis of **7** to give the target compound **12** (3.6 *μ*mol, 59%) as a highly hygroscopic solid; ^{1}H NMR (400 MHz; D_2O) δ_H 8.04 and 8.03 (2 s, 2 H, H-2, H-8), 6.05 (d, 1 H, $J_{1',2}$ 5.5 Hz, H-1′), 5.19 (d, 1 H, $J_{1'',2}$ 3.5 Hz, H-1″), 5.06−5.02 (m, 1 H, H-2′), 4.57−4.22 (m, 3 H, H-3′, H-4′, H-3″), 3.83−3.55 (m, 7 H, H-5′a, H-5′b, H-2″, H-4″ H-5″, H-6″a, H-6″b), 2.37−2.35 (m, 1 H, noradamantane), 2.18−2.01 (m, 6 H, noradamantane) 1.88−1.86 (m, 2 H, noradamantane) and 1.45−1.42 (m, 4 H, noradamantane); ^{31}P NMR (162 MHz; D_2O; ^{1}H decoupled) δ_P 3.44, 2.49 and 2.49; MS: (FAB) *m*/*z* 788.0 [(M − H)$^-$, 100%], *m*/*z* calcd for $C_{25}H_{37}N_5O_{18}P_3$ [M − H]$^-$ 788.1301 found *m*/*z* 788.1336.

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Supporting Information Available: ^{13}C NMR data for selected compounds and ^{1}H NMR spectra for compounds **7**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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