

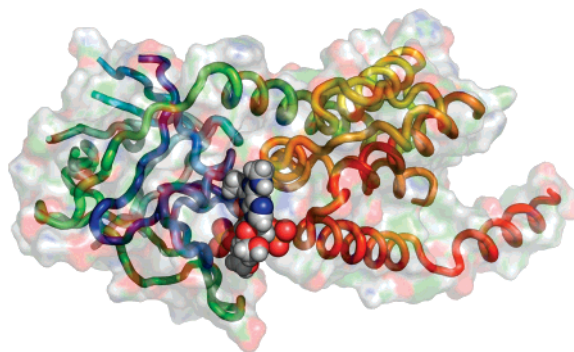
## 2-Position Base-Modified Analogues of Adenophostin A as High-Affinity Agonists of the D-*myo*-Inositol Trisphosphate Receptor: In Vitro Evaluation and Molecular Modeling

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Adenophostin A (AdA) is a potent agonist of the D-*myo*-inositol 1,4,5-trisphosphate receptor (Ins(1,4,5)P<sub>3</sub>R). Various 2-aminopurine analogues of AdA were synthesized, all of which (guanophostin **5**, 2,6-diaminopurinophostin **6**, 2-aminopurinophostin **7**, and chlorophostin **8**) are more potent than 2-methoxy-*N*<sup>6</sup>-methyl AdA, the only benchmark of this class. The 2-amino-6-chloropurine nucleoside **11**, from Vorbrüggen condensation of 2-amino-6-chloropurine with appropriately protected disaccharide, served as the advanced common precursor for all the analogues. Alcoholysis provided the precursor for **5**, ammonolysis at high temperature the precursor for **6**, and ammonolysis under mild conditions the precursor for synthesis of **7** and **8**. For **8**, the debenzoylation of precursor leaving the chlorine untouched was achieved by judicious use of BCl<sub>3</sub>. The reduced potency of chlorophostin **8** and higher potency of guanophostin **5** in assays of Ca<sup>2+</sup> release via recombinant Ins(1,4,5)P<sub>3</sub>R are in agreement with our model suggesting a cation- $\pi$  interaction between AdA and Ins(1,4,5)P<sub>3</sub>R. The similar potencies of 2,6-diaminopurinophostin (**6**) and 2-aminopurinophostin (**7**) concur with previous reports that the 6-NH<sub>2</sub> moiety contributes negligibly to the potency of AdA. Molecular modeling of the 2-amino derivatives suggests an interaction between the carboxylate side chain of Glu505 of the receptor and the 2-NH<sub>2</sub> of the ligand, but for 2-methoxy-*N*<sup>6</sup>-methyl AdA the carboxylate group of Glu505 is deflected away from the methoxy group. A helix-dipole interaction between the 1-phosphate of Ins(1,4,5)P<sub>3</sub> and the 2'-phosphate of AdA with  $\alpha$ -helix 6 of Ins(1,4,5)P<sub>3</sub>R is postulated. The results support a proposed model for high-affinity binding of AdA to Ins(1,4,5)P<sub>3</sub>R.

### Introduction

In almost every animal cell, diverse extracellular stimuli lead to activation of phospholipase C and thereby to formation of

inositol 1,4,5-trisphosphate (Ins(1,4,5)P<sub>3</sub>, **1**) from phosphatidylinositol 4,5-bisphosphate.<sup>1</sup> The biological effects of Ins(1,4,5)P<sub>3</sub> are mediated by Ins(1,4,5)P<sub>3</sub> receptors (Ins(1,4,5)P<sub>3</sub>R). These

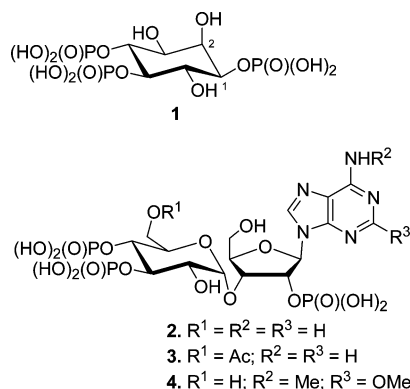
<sup>†</sup> University of Bath.

<sup>‡</sup> University of Cambridge.

(1) Berridge, M. J.; Lipp, P.; Bootman, M. D. *Nature Rev. Mol. Cell Biol.* **2000**, *1*, 11–21.

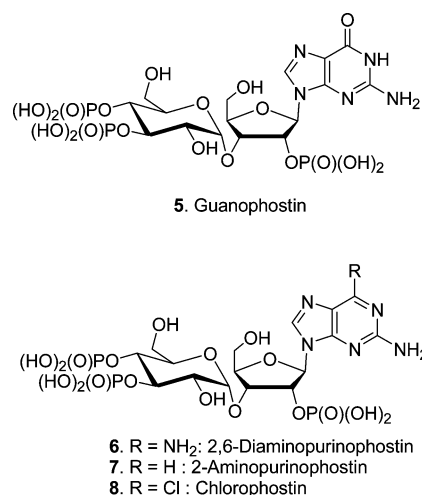
are  $\text{Ca}^{2+}$ -permeable channels expressed predominantly in the membranes of the endoplasmic reticulum, from which they allow rapid release of  $\text{Ca}^{2+}$  into the cytosol.<sup>2</sup> The ensuing increase in cytosolic  $\text{Ca}^{2+}$  concentration, which often takes the form of complex  $\text{Ca}^{2+}$  waves, spikes, and local  $\text{Ca}^{2+}$  signals, controls many different aspects of cellular activity. Many analogues of  $\text{Ins}(1,4,5)\text{P}_3$  have been synthesized,<sup>3</sup> but with the exception of dimeric  $\text{Ins}(1,4,5)\text{P}_3$ ,<sup>4</sup> none is more potent than  $\text{Ins}(1,4,5)\text{P}_3$ .

In 1993, Takahashi et al. isolated adenophostins A (2) and B (3) from culture broths of *Penicillium brevicompactum*:<sup>5</sup> both are full agonists of  $\text{Ins}(1,4,5)\text{P}_3\text{R}$  with potencies at least 10 times greater than  $\text{Ins}(1,4,5)\text{P}_3$ .<sup>6</sup> This has stimulated much interest to synthesize analogues of adenophostin A (AdA). These analogues have been useful in defining structure-activity relationships (SAR),<sup>7</sup> but none of them has matched the potency of AdA itself.



The adenine ring (or a surrogate) is essential for the enhanced affinity of AdA,<sup>8</sup> but the details of its interactions with the  $\text{Ins}(1,4,5)\text{P}_3\text{R}$  are not defined. The adenosine moiety may orient the 2'-phosphate in a position that strengthens its interactions with the amino acid residues that interact with the 1-phosphate of  $\text{Ins}(1,4,5)\text{P}_3$ , in effect allowing the 2'-phosphate of AdA to super-optimally mimic the 1-phosphate of  $\text{Ins}(1,4,5)\text{P}_3$ .<sup>9</sup> Alternatively, the adenine moiety may itself make supplementary interactions with the  $\text{Ins}(1,4,5)\text{P}_3\text{R}$ .<sup>10</sup> Purine-modified analogues of AdA are likely to discriminate between these alternatives.

Purine-modified AdA analogues (predominantly at the C-6 position) have been synthesized, but the 2-position of this base has not been explored, with the exception of 2-methoxy-*N*<sup>6</sup>-methyladenophostin (4),<sup>7a</sup> which is ca. 6-fold less potent than AdA and only 2-fold more potent than  $\text{Ins}(1,4,5)\text{P}_3$ . This suggests less steric tolerance of 2-modified analogues. However, our recently proposed model<sup>7a</sup> of AdA-docked into the  $\text{Ins}(1,4,5)\text{P}_3$ -binding core of the  $\text{Ins}(1,4,5)\text{P}_3\text{R}$  shows the carboxylate anion of Glu505 near the 2-position of the adenine. This prompted us to consider that an H-bond donor or cationic moiety (e.g.,  $\text{NH}_2$ ) at the 2-position may interact constructively with Glu505. To explore this possibility further, we report the synthesis and biological evaluation of various 2-amino purine analogues (5–8) of AdA. A preliminary report for one analogue has appeared.<sup>11</sup>



## Results and Discussion

Vorbrüggen condensation of a silylated purine with a properly protected disaccharide is an ideal method for the synthesis of disaccharide nucleosides.<sup>12</sup> We<sup>13</sup> and others<sup>14</sup> have used this strategy successfully for the synthesis of various AdA analogues via Vorbrüggen condensation of an appropriate disaccharide with silylated nucleobase. However, Vorbrüggen condensation of guanine (or its derivatives) with acylated sugars is known<sup>15</sup> to form a mixture of N-7 and N-9 regioisomers, even under thermodynamic control, and hence is not suitable for synthetic

(2) Taylor, C. W.; da Fonseca, P. C. A.; Morris, E. P. *Trends Biochem. Sci.* **2004**, 29, 210–219.

(3) (a) Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed. Engl.* **1995**, 34, 1933–1972. (b) Potter, B. V. L.; Nahorski, S. R. In *Drug Design, Molecular Modeling and the Neurosciences*; Kozikowski, A. P., Ed.; Raven Press: New York, 1993; pp 383–416. (c) Billington, D. C. *The Inositol Phosphates: Chemical Synthesis and Biological Significance*; VCH: Weinheim, 1993; pp 1–153.

(4) Riley, A. M.; Morris, S. A.; Nerou, E. P.; Correa, V.; Potter, B. V. L.; Taylor, C. W. *J. Biol. Chem.* **2002**, 277, 40290–40295.

(5) Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J. Antibiot.* **1993**, 46, 1643–1647.

(6) Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, 269, 369–372.

(7) (a) Rosenberg, H. J.; Riley, A. M.; Laude, A. J.; Taylor, C. W.; Potter, B. V. L. *J. Med. Chem.* **2003**, 46, 4860–4871. (b) Chrétien, F.; Moitessier, N.; Roussel, F.; Mauger, J.-P.; Chapleur, Y. *Current Org. Chem.* **2000**, 4, 513–534. (c) Marwood, R. D.; Shuto, S.; Jenkins, D. J.; Potter, B. V. L. *Chem. Commun.* **2000**, 219–220. (d) Marwood, R. D.; Jenkins, D. J.; Correa, V. A.; Taylor, C. W.; Potter, B. V. L. *J. Med. Chem.* **2000**, 43, 4278–4287. (e) Shuto, S.; Horne, G.; Marwood, R. D.; Potter, B. V. L. *Chem. Eur. J.* **2001**, 7, 4937–4946.

(8) Correa, V. A.; Riley, A. M.; Shuto, S.; Horne, G.; Nerou, E. P.; Marwood, R. D.; Potter, B. V. L.; Taylor, C. W. *Mol. Pharmacol.* **2001**, 59, 1206–1215.

(9) Wilcox, R. A.; Erneux, C.; Primrose, W. U.; Gigg, R.; Nahorski, S. R. *Mol. Pharmacol.* **1995**, 47, 1204–1211.

(10) Riley, A. M.; Correa, V.; Mahon, F.; Taylor, C. W.; Potter, B. V. L. *J. Med. Chem.* **2001**, 44, 2108–2117.

(11) Sureshan, K. M.; Trusselle, M.; Tovey, S. C.; Taylor, C. W.; Potter, B. V. L. *Chem. Commun.* **2006**, 2015–2017.

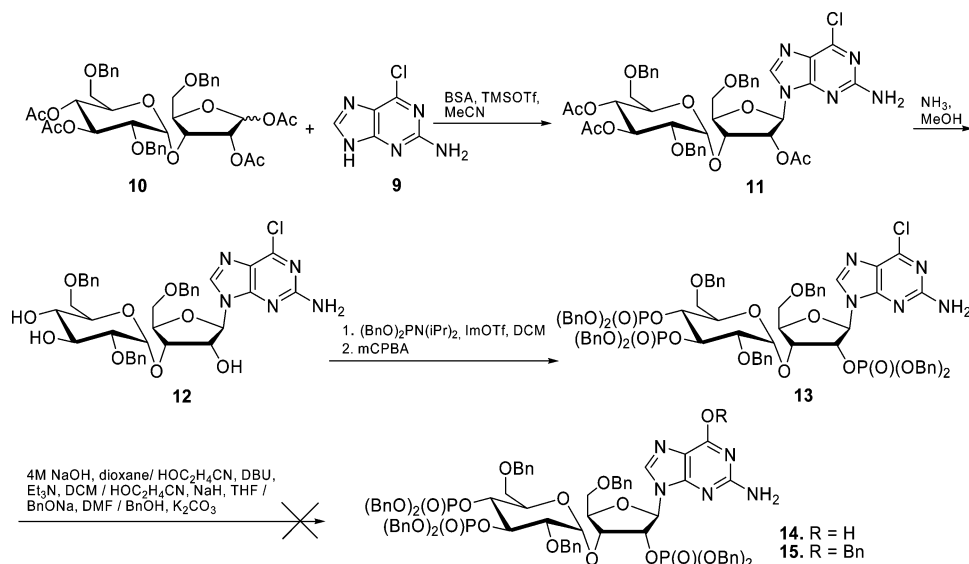
(12) Efimtseva, E. V.; Mikhailov, S. N. *Russ. Chem. Rev.* **2004**, 73, 401–414.

(13) Borissov, C. N.; Black, S. J.; Paul, M.; Tovey, S. C.; Dedos, S. G.; Taylor, C. W.; Potter, B. V. L. *Org. Biomol. Chem.* **2005**, 3, 245–252.

(14) de Kort, M.; Luijendijk, J.; van der Marel, G.; van Boom, J. H. *Eur. J. Org. Chem.* **2000**, 3085–3092. (b) van Straten, N. C. R.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1997**, 53, 6509–6522. (c) de Kort, M.; Regenbogen, A. D.; Overkleeft, H. S.; Challiss, R. A. J.; Iwata, Y.; Miyamoto, S.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **2000**, 56, 5915–5928. (d) de Kort, M.; Correa, V.; Valentijn, A. R. P. M.; van der Marel, G. A.; Potter, B. V. L.; Taylor, C. W.; van Boom, J. H. *J. Med. Chem.* **2000**, 43, 3295–3303. (e) Mochizuki, T.; Kondo, Y.; Abe, H.; Taylor, C. W.; Potter, B. V. L.; Matsuda, A.; Shuto, S. *Org. Lett.* **2006**, 8, 1455–1458.

(15) (a) Koshkin, A. A.; Fensholdt, J.; Pfundheller, H. M.; Lomholt, C. *J. Org. Chem.* **2001**, 66, 8504–8512. (b) Robins, M. J.; Zou, R.; Guo, Z.; Wnuk, S. F. *J. Org. Chem.* **1996**, 61, 9207–9212. (c) Zhong, M.; Robins, M. J. *Tetrahedron Lett.* **2003**, 44, 9327–9330.

## SCHEME 1. Attempted Synthesis of Guanophostin (5)



purposes. Although we have synthesized a variety of 6-modified AdA analogues by substitution of the chlorine of 6-chloropurine nucleosides with various amines, the resistance of 2-chloro derivatives to ammonolysis prevented the use of 2-chlorinated purines for the synthesis of 2-amino purine analogues. However, 2-amino-6-chloropurine (**9**) is reported<sup>16</sup> to undergo regiospecific condensation under Vorbrüggen conditions and, hence, we decided to use **9** for glycosylation with an aim to synthesize various 6-substituted 2-aminopurine nucleoside analogues by manipulating the 6-chlorine motif. Vorbrüggen condensation of 2-amino-6-chloropurine (**9**) and the known<sup>7a</sup> disaccharide **10** using TMSOTf as the Lewis acid was initially investigated. The yields of the condensation were found to depend largely on the silylating agent used for the generation of persilylated purine. When TMSOTf was used, a mixture of the expected nucleoside **11** (27%) and a dimeric compound (23%, presumably glycosylated at N-9 and C2-NH<sub>2</sub>) was obtained. Variation of the solvent or relative stoichiometry of the reactants did not show any improvement in the yield or product distribution. Similarly, when TMSCl-HMDS was used as the silylating agent, **11** was obtained in very low yield. In addition, we have observed the formation of two isomeric nucleosides under these conditions previously.<sup>7d</sup> However, the yield of **11** could be improved (90–100% in various trials) when *N,O*-bis-trimethylsilyl-acetamide (BSA) was used as the silylating agent and TMSOTf as the catalyst for the condensation. Interference of the liberated acids (TfOH or HCl) during the silylation in the subsequent condensation reaction could be a plausible reason for the low yield when TMSOTf or TMS-Cl was used as the silylating agent. Based on this argument, the high yield of **11** when BSA was used for silylation could be attributed to the neutral nature of liberated acetamide.

Having achieved the synthesis of the versatile intermediate **11**, we attempted to synthesize 3'- $\alpha$ -D-glucopyranosylguanosine 2',3',4"-trisphosphate (**5**), "guanophostin", the guanine analogue of AdA (Scheme 1). It is known that 2-amino-6-chloropurine nucleosides can be converted to guanine nucleosides by treatment with oxygen nucleophiles. Since the guanine oxygen can potentially interfere during further synthetic steps, we decided

to generate the guanine moiety toward the end of the synthetic plan. For this purpose, methodologies to deprotect the acetate groups in **11** without affecting the chlorine were investigated. Aminolysis with *n*-butylamine in methanol gave a mixture of C-6-substituted and unsubstituted triols along with partially deacylated derivatives. However, use of the comparatively less nucleophilic ammonia<sup>17</sup> gave more promising results. Thus, the triacetate, **11** on ammonolysis in methanol at room temperature yielded the expected triol **12** in 90% yield. Chemoselective phosphitylation<sup>18</sup> followed by in situ oxidation provided the protected trisphosphate **13** in very good yield. However, several attempts to transform **13** to the guanosine derivative **14** met with failure. Attempts to hydrolyze the chlorine using 4 M NaOH<sup>19</sup> failed, and **13** could be recovered unchanged.

Since it is well-known that the 6-Cl can be substituted with alkoxides, we attempted to substitute the chlorine with the alkoxide generated from 3-hydroxypropionitrile as the resulting alkoxy derivative will undergo  $\beta$ -elimination to generate the guanine moiety. When DBU<sup>20</sup> was used as the base (for the generation of alkoxide), the reaction was sluggish, and most of the starting material remained unchanged even after 5 days. However, when a stronger base, NaH,<sup>16</sup> was used, the generated alkoxide displaced the benzyl protecting groups on phosphorus leaving the chlorine intact. Because generation of the guanine moiety in unprotected form failed, we attempted to generate the oxo-functionality in guanine in protected form. Since the sugar hydroxyl and phosphate groups were protected as benzyl ethers and benzyl esters, respectively, in **13**, we chose to generate the benzyl protected guanine **15** from **13**. Chen et al. reported the displacement of chlorine in 6-chloropurine derivatives with an alcohol in the presence of  $\text{K}_2\text{CO}_3$ .<sup>21</sup> Analogous use of benzyl alcohol in the presence of  $\text{K}_2\text{CO}_3$  (or NaH) in

(16) Rosenbohm, C.; Pedersen, D. S.; Frieden, M.; Jensen, F. R.; Arent, S.; Larsen, S.; Koch, T. *Bioorg. Med. Chem.* **2004**, *12*, 2385–2396.

(17) Brotzel, F.; Chu, Y. C.; Mayr, H. *J. Org. Chem.* **2007**, *72*, 3679–3688.

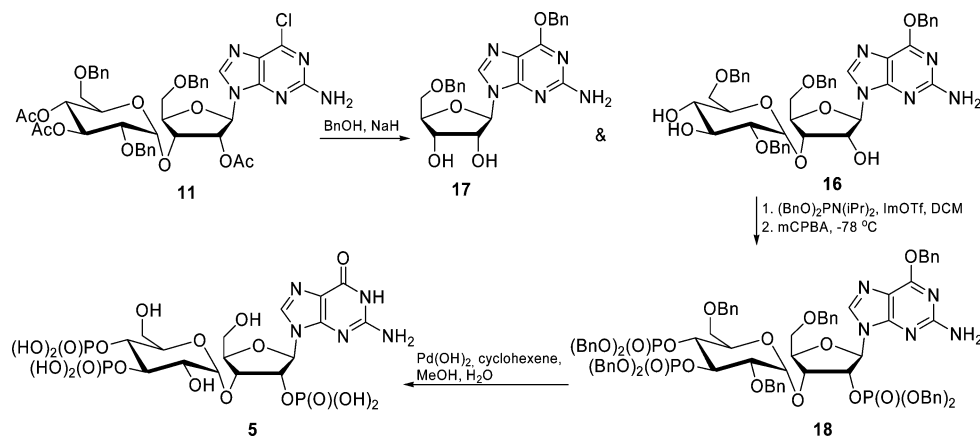
(18) Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1998**, *120*, 12395–12401.

(19) Moon, H. R.; Kim, H. O.; Chun, M. W.; Jeong, L. S.; Marquez, V. E. *J. Org. Chem.* **1999**, *64*, 4733–4741.

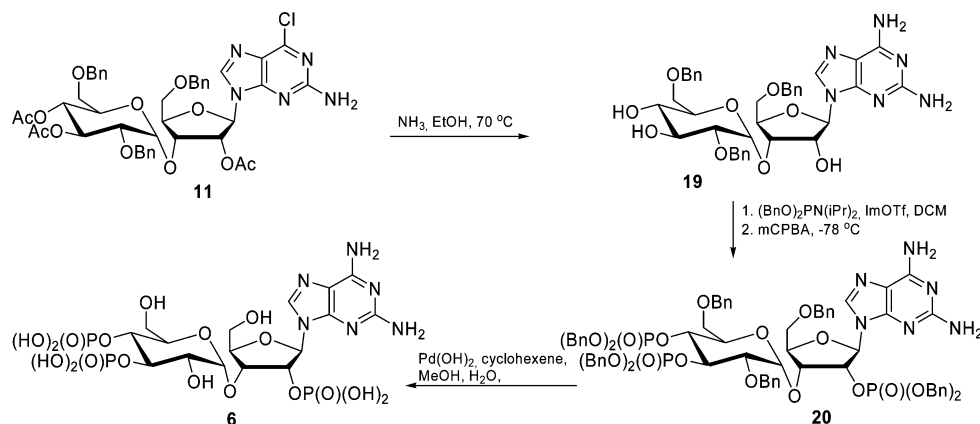
(20) Ashwell, M.; Bleasdale, C.; Golding, B. T.; O'Neill, I. K. *J. Chem. Soc. Chem. Commun.* **1990**, 955–956.

(21) Chen, X.; Kern, E. R.; Drach, J. C.; Gullen, E.; Cheng, Y.-C.; Zemlicka, J. *J. Med. Chem.* **2003**, *46*, 1531–1537.

## SCHEME 2. Synthesis of Guanophostin (5)



## SCHEME 3. Synthesis of 2,6-Diaminopurinophostin (6)



our case, unfortunately, resulted in the loss of a phosphate triester from the molecule.

Having failed in various attempts to generate the guanine or protected guanine moiety in the presence of phosphate triesters, we chose to generate the guanine moiety before phosphorylation. To prevent the possible interference of guanine oxygen (O-6) during the phosphorylation (guanine oxygen can potentially compete with sugar hydroxyls for phosphorylation), we decided to generate the *O*-protected guanine moiety. Since the protecting groups used elsewhere in the molecule are benzyl ethers (or esters), we envisioned that generation of *O*-benzyl guanine derivative is an economical synthetic strategy, as it will reduce the number of steps for deprotection. Treatment of **11** with sodium benzoxide generated in situ from benzyl alcohol (6 equiv) and sodium hydride (5 equiv) in DMF at 80 °C provided a mixture of **16** (43%) and **17** (41%). However, surprisingly, the use of only 2 equiv of sodium hydride and BnOH as solvent resulted in the exclusive formation of **16** (88%, Scheme 2). During the treatment of sodium benzoxide with 2-amino-6-chloropurine, in addition to the expected formation 6-*O*-benzylguanine, the formation of the 2-*N*-benzylguanine analogue was reported.<sup>22</sup> In our case, the formation of 2-*N*-benzylated product was not observed. Triol **16** on chemoselective phosphitylation using imidazolium triflate as catalyst followed by in situ oxidation provided the fully protected trisphosphate **18** in 82% yield. Removal of benzyl protecting groups by transfer hydrogenolysis followed by purification by ion-exchange column chromatography provided guanophostin (**5**) in pure form.<sup>11</sup>

To compare the effect of an additional NH<sub>2</sub> group at the 2-position of AdA, we synthesized 2,6-diaminopurinophostin (**6**, Scheme 3). For the synthesis of **6**, unlike that of guanophostin, the chlorine has to be substituted with an amino group. It is well-known that chlorine in 6-chloropurine nucleosides can be substituted with various amines at higher temperature, and we have used this methodology for the synthesis of various 6-substituted AdA analogues.<sup>7a</sup> By analogy, ammonolysis of the nucleoside **11** at higher temperature provided the 2,6-diaminopurine derivative **19** in very good (98%) yield. The *O*-selective phosphitylation followed by in situ oxidation provided the protected trisphosphate **20** in 84% yield. Hydrogenolytic removal of benzyl protecting groups provided the 2,6-diaminopurinophostin (**6**).

The 6-NH<sub>2</sub> is not essential for the enhanced biological activity of AdA,<sup>7d</sup> and it can be substituted with alkylamines without much loss of potency.<sup>7a</sup> Hence, we decided to synthesize 6-modified 2-aminopurine analogues of AdA by manipulating the 6-Cl group. 2-Aminopurine nucleosides are fluorescent, and this makes them attractive bioanalytical probes.<sup>23</sup> For this reason, synthesis of 2-aminopurine nucleosides has attracted much attention.<sup>24</sup> Active, fluorescent analogues of AdA might offer additional opportunities to examine receptor-ligand interactions. Hence, we undertook the synthesis of 2-aminopurinophostin (**7**) (Scheme 4). First, to test our theory that a cation- $\pi$  interaction contributes to the increased affinity of AdA, we required

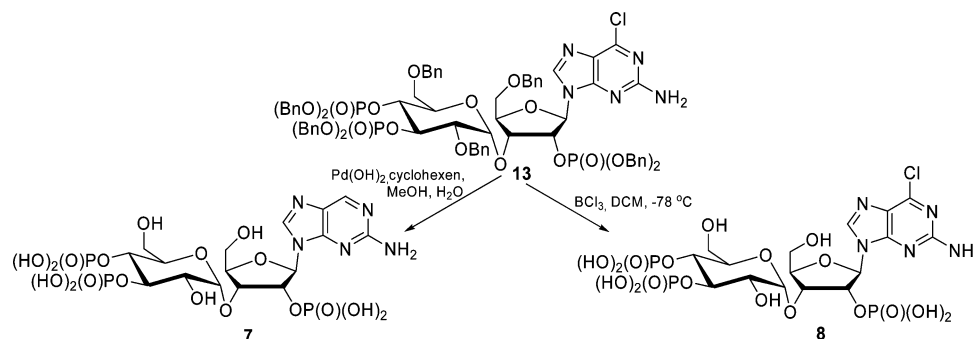
(23) Guest, C. R.; Hochstrasser, R. A.; Sowers, L. C.; Millar, D. P. *Biochemistry* **1991**, 30, 3271–3279.

(24) Garner, P.; Yoo, J. U.; Sarabu, R. *Tetrahedron* **1992**, 48, 4259–4270.

(22) Frihart, C. R.; Leonard, N. J. *J. Am. Chem. Soc.* **1973**, 95, 7174–7175.



## SCHEME 4. Syntheses of 2-Aminopurinophostin (7) and Chlorophostin (8)



halogen-substituted AdA analogues; halogen-substituted aromatics have weaker cation- $\pi$  interactions with a cationic residue.<sup>25</sup> Chlorine is the ideal replacement because, being similar in size to a methyl group, it will not impose any steric hindrance. In addition, halogen bonding, the attractive interaction between halogen and oxygen (or nitrogen), is important in enzyme-substrate recognition,<sup>26</sup> and halogen-substituted analogues of AdA may reveal whether such interactions contribute to their binding to InsP<sub>3</sub>R. Finally, AdA analogues with chlorine at the 6-position could be of future use for further conjugation, such as immobilization through a spacer, etc.

For the synthesis of both “chlorophostin” and “2-aminopurinophostin”, the highly advanced intermediate **13** was used. Thus, hydrogenolysis of **13** with Pd(OH)<sub>2</sub> and cyclohexene resulted in the formation of 2-aminopurinophostin (**7**) in a single step. The concomitant displacement of chlorine with hydrogen under hydrogenolytic debenzylation conditions is advantageous. Because chlorine undergoes reduction under the hydrogenolytic conditions used to remove the benzyl protecting groups, such methods cannot be used to synthesize chlorophostin. However, Hanna et al. deprotected the benzyl protecting groups in 2-amino-6-chloro-9-(2,3,5-tri-*O*-benzyl- $\alpha$ -D-arabinofuranosyl)purine without affecting the chlorine using BCl<sub>3</sub>,<sup>27</sup> and BCl<sub>3</sub> has been used to deprotect the benzyl esters of phosphate triesters.<sup>28</sup> Based on these reports, we attempted to deprotect all the benzyl groups in **13** using BCl<sub>3</sub>. Thus, treatment of **13** with BCl<sub>3</sub> provided chlorophostin (**8**) in good yield.

The ability of these 2-aminopurine analogues (**5–8**) of AdA to stimulate Ca<sup>2+</sup> release via Ins(1,4,5)P<sub>3</sub>R was measured by using a low-affinity Ca<sup>2+</sup> indicator trapped within the endoplasmic reticulum of DT40 cells expressing only recombinant rat type I Ins(1,4,5)P<sub>3</sub>R as previously reported.<sup>29</sup> The results demonstrate that each of these analogues (**5–8**) is a full agonist with a potency comparable or slightly lower than that of AdA (Table 1).

Guanophostin is the most potent of the analogues: it is essentially equipotent with AdA and (18  $\pm$  5)-fold more potent than Ins(1,4,5)P<sub>3</sub>. The remaining analogues (**6–8**) are only

TABLE 1. Stimulation of Ca<sup>2+</sup> Release via Recombinant Rat Type 1 Ins(1,4,5)P<sub>3</sub> Receptor<sup>a</sup>

	EC <sub>50</sub> (nM)	Hill coefficient	% release
Ins(1,4,5)P <sub>3</sub> , <b>1</b>	23.7 $\pm$ 3.7	1.30 $\pm$ 0.14	81 $\pm$ 1
adenophostin A, <b>2</b>	0.9 $\pm$ 0.1	1.20 $\pm$ 0.06	81 $\pm$ 2
2,6-diaminopurinophostin, <b>6</b>	2.2 $\pm$ 0.5	1.32 $\pm$ 0.10	80 $\pm$ 2
2-aminopurinophostin, <b>7</b>	2.4 $\pm$ 0.8	1.00 $\pm$ 0.06	80 $\pm$ 4
chlorophostin, <b>8</b>	3.2 $\pm$ 1.0	1.01 $\pm$ 0.02	85 $\pm$ 1
guanophostin, <b>5</b>	1.3 $\pm$ 0.3	0.99 $\pm$ 0.08	83 $\pm$ 1

<sup>a</sup> The half-maximally effective concentration (EC<sub>50</sub>), the Hill coefficient, and the percentage of the Ca<sup>2+</sup> stores released by a maximally effective concentration of each ligand are shown. Results (means  $\pm$  SEM) are from six independent experiments for Ins(1,4,5)P<sub>3</sub>, and three independent experiments for the other ligands, with each experiment comprising a concentration–effect relationship determined with three replicates.

slightly less potent than guanophostin. All of the analogues are more potent than the 2-methoxy analogue **4**, which is (5.9  $\pm$  0.5)-fold less potent than AdA. The latter data were determined in a different biological assay where the type 2 InsP<sub>3</sub>R predominates<sup>7a</sup> and showed EC<sub>50</sub> values of 13 nM, 14 nM, and 82 nM for *N*<sup>6</sup>-methyl-AdA, AdA, and 2-methoxy-*N*<sup>6</sup>-methyl-AdA **4**, respectively. That **6** and **7** are equipotent agrees with previous suggestions that the contribution of the 6-NH<sub>2</sub> of AdA toward Ins(1,4,5)P<sub>3</sub>R recognition is negligible.<sup>7d,30</sup>

The weaker activity of the 2-methoxy derivative **4** suggested that the receptor close to the 2-position is sterically intolerant, but the relatively higher potencies of the newly synthesized 2-amino derivatives show that the 2-position can accommodate an NH<sub>2</sub> group. This suggests that, although the 2-position may be sterically sensitive, a hydrogen bond donor/acceptor there improves binding to the Ins(1,4,5)P<sub>3</sub>R. This is consistent with our model of AdA docked into the Ins(1,4,5)P<sub>3</sub>-binding core, which shows the carboxylate side chain of Glu505 close to the purine 2-position.<sup>7a</sup> Hydrogen bonding or electrostatic attraction between the NH<sub>2</sub> and ionized COOH could be responsible for the higher potency of the new 2-aminopurine analogues (**5–8**) relative to the methoxy derivative **4**; such an interaction is not possible for **4**, that could only accept a hydrogen bond. Potency thus seems to reflect the competing effects of unfavorable steric interactions and favorable electrostatic or hydrogen bonding interactions with Glu505. However, it is interesting to note that this proposed interaction does not improve the potency of **6** over **7** (compared to **2**), that might potentially be supposed could be more potent than AdA **2** itself, if viewed as AdA with an additional 2-amino group. Accommodation of the 2-amino group by the protein may modulate the overall binding in this region

(25) Mecozzi, S.; West, A. P. Jr.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10566–10571.

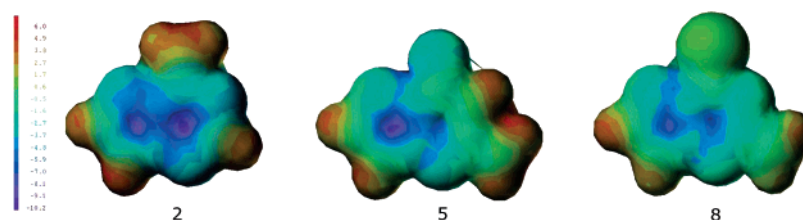
(26) (a) Auffinger, P.; Hays, F. A.; Westhof, E.; Ho, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16789–16794. (b) Metrangola, P.; Neukirch, H.; Pilati, T.; Resnati, G. *Acc. Chem. Res.* **2005**, *38*, 386–395.

(27) Hanna, N. B.; Ramasamy, K.; Robins, R. K.; Revankar, G. R. *J. Heterocycl. Chem.* **1988**, *25*, 1899–1903.

(28) (a) Kim, H. S.; Barak, D.; Harden, T. K.; Boyer, J. L.; Jacobson, K. A. *J. Med. Chem.* **2001**, *44*, 3092–3108. (b) Kim, H. S.; Ohno, M.; Xu, B.; Kim, H. O.; Choi, Y.; Ji, X. D.; Maddileti, S.; Marquez, V. E.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **2003**, *46*, 4974–4987.

(29) Laude, A. J.; Tovey, S. C.; Dedos, S. G.; Potter, B. V. L.; Lummis, S. C. R.; Taylor, C. W. *Cell Calcium* **2005**, *38*, 45–51.

(30) Takahashi, S.; Kinoshita, T.; Takahashi, M. *J. Antibiot.* **1994**, *47*, 95–100.



**FIGURE 1.** AM1 electrostatic potential energy surfaces of the purine rings of **2**, **5**, and **8**. Red corresponds to most positive and purple to most negative. The EP surfaces were generated using the MOLCAD program (Tripos Associates).

relative to **2** and the potencies observed in general do not vary greatly within the set, thus making it very hard to draw rigorous SAR conclusions related to additivity of motifs in the absence of any desirable structural biology information. Purinophostin, the parent for **6** and **7** was studied earlier<sup>7d</sup> and found to be only *ca* 1.3-fold weaker than AdA, but in a different assay system, using permeabilized hepatocytes. While the sequential addition of amino groups at C-2 (**7**), and at C-2 and C-6 (**6**) leads to apparent further decreases in activity relative to AdA, the decreases are rather small for structural interpretation. The ( $3.5 \pm 1.2$ )-fold lesser potency of chlorophostin relative to AdA, however, is interesting because introduction of an  $\text{NH}_2$  at the 2-position (in analogues **5**–**7**) increases or retains the activity of the parent analogue: the chlorine at the 6-position must therefore be responsible for the reduced potency of chlorophostin. Chlorine is the size of a methyl group, and the 6-position is sterically tolerant;<sup>7a</sup> the reduced potency of chlorophostin should not, therefore, be attributable to a steric effect.

Our model of AdA binding places the adenine ring parallel to the guanidinium side chain of Arg504, and we have suggested that a cation- $\pi$  interaction between them may contribute to the high affinity of AdA.<sup>7a</sup> Such interactions are known to make important contributions to the binding of many small ligands to proteins. The most frequently observed interactions between proteins and nucleobases are H bonds, hydrophobic contacts or  $\pi$ - $\pi$  stacking.<sup>31</sup> However, a systematic analysis of protein structures with side chains bound to nucleobase ligands revealed that cation- $\pi$  interactions are more common than  $\pi$ - $\pi$  stacking interactions. Arg-adenine is the most frequent cation- $\pi$  pair.<sup>32</sup> A recent analysis of the structures of proteins bound to adenine-based ligands suggests the coexistence of cation- $\pi$  and  $\pi$ - $\pi$  stacking interactions.<sup>33</sup> The strength of a cation- $\pi$  interaction is very sensitive to the electron density and polarization<sup>34</sup> on the face of the aromatic ring,<sup>35</sup> with theoretical<sup>25</sup> and experimental studies<sup>36</sup> showing that introduction of electron-withdrawing groups<sup>37</sup> in the aromatic partner reduces the strength of the interaction and electron-donating groups enhance the interaction. Recent computational research<sup>35</sup> found a correlation between the binding energies of cation- $\pi$  complexes calculated at a high

level of theory and the corresponding electrostatic potential energy (EP) surfaces calculated using the AM1 semiempirical method. To rationalize the effects of substituents on potential cation- $\pi$  interactions between the purine rings of **2**, **5**, and **8** with Arg504, the EP surfaces of the rings were generated (Figure 1 and Supporting Information). Adenine was found to have the most negative EP surface, enhanced by the 6-amino group. Like adenine, guanine also has an amino group; however, the inductive/mesomeric effects of this group appear to be outweighed by the effects of the oxygen at the 6-position causing the EP surface of guanine to be a little less negative than that of adenine. The 6-Cl-substituted ring exhibited the least negative EP surface due to the deactivating halogen. This indicates that a cation- $\pi$  interaction involving chlorophostin would be the least energetically favorable (Figure 1) and, other things being equal, might thereby provide an explanation for the lesser potency of chlorophostin that is consistent with our model for the interaction of AdA with Ins(1,4,5) $\text{P}_3\text{R}$ . However, the reduction in basicity of 2- $\text{NH}_2$  group, due to the electron-withdrawing effect of the chlorine atom, and the resultant reduction in the strength of the potential interaction between 2- $\text{NH}_2$  and Glu505 being responsible for the reduced potency of chlorophostin cannot be ruled out.

The higher potency of guanophostin compared to **6** and **7** suggests that it forms generally more favorable interactions in addition to those suggested for the 2- $\text{NH}_2$  group. Only in guanophostin could the  $\text{N}^1\text{H}$  motif potentially be either a H-bond donor or acceptor; in the other ligands, it could only be an acceptor. The hypoxanthine analogue of adenophostin B is almost equipotent with AdA (measured in a different assay system).<sup>30</sup> While not a perfect comparison, the H-bond donor ability of  $\text{N}^1\text{H}$  is unlikely to be responsible for the increased potency of guanophostin (*vide infra*). An *ab initio* study of cation- $\pi$  interactions in protein–DNA complexes revealed that those involving Arg and guanine are generally more stable than Arg-adenine pairs.<sup>32,38</sup> This might account for the increased potency of guanophostin and would fit also with our suggestion that a cation- $\pi$  interaction is responsible for the increased affinity of AdA-like ligands.<sup>7a</sup>

Molecular models of guanophostin and other analogues with the 2'-endo conformation of the ribose ring were built in Sybyl 7.1 (Tripos Associates) and docked into the Ins(1,4,5) $\text{P}_3\text{R}$ -binding core of the Ins(1,4,5) $\text{P}_3\text{R}$  (PDB file no. 1N4K) using GOLD<sup>39</sup> (Version 2.2). Details of the molecular modeling methods are described elsewhere.<sup>7a</sup> The highest scored binding mode of each of these novel ligands closely resembled binding mode B of AdA (described in ref 7a). The known interactions of the 4,5-

(31) McDonald, I. K.; Thornton, J. M. *J. Mol. Biol.* **1994**, 238, 777–793.

(32) Biot, C.; Buisine, E.; Kwasigroch, J.-M.; Wintjens, R.; Rooman, M. *J. Biol. Chem.* **2002**, 277, 40816–40822.

(33) (a) Mao, L.; Wang, Y.; Liu, Y.; Hu, X. *J. Am. Chem. Soc.* **2003**, 125, 14216–14247. (b) Mao, L.; Wang, Y.; Liu, Y.; Hu, X. *J. Mol. Biol.* **2004**, 336, 787–807.

(34) Cubero, E.; Luque, F. J.; Orozco, M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, 95, 5976–5980.

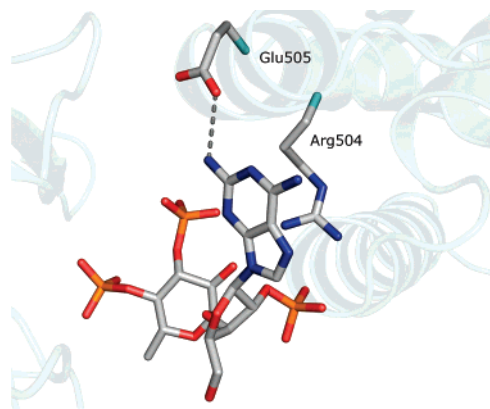
(35) Hunter, C. A.; Low, C. M. R.; Rotger, C.; Vinter, J. G.; Zonta, C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, 99, 4873–4876.

(36) Sorme, P.; Arnoux, P.; Kahl-Knutsson, B.; Leffler, H.; Rini, J. M.; Nilsson, U. J. *J. Am. Chem. Soc.* **2005**, 127, 1737–1743.

(37) Zhong, W.; Gallivan, J. P.; Zhang, Y.; Li, L.; Lester, H. A.; Dougherty, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, 95, 12088–12093.

(38) Wintjens, R.; Lievin, J.; Rooman, M.; Buisine, E. *J. Mol. Biol.* **2000**, 302, 395–410.

(39) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, 267, 727–748.

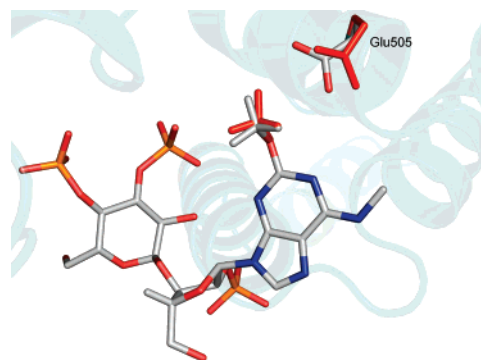


**FIGURE 2.** Highest scoring docked conformation of 2,6-diaminopurinophostin forms a hydrogen bond with the carboxylate of Glu505 (hydrogen atoms are not shown).

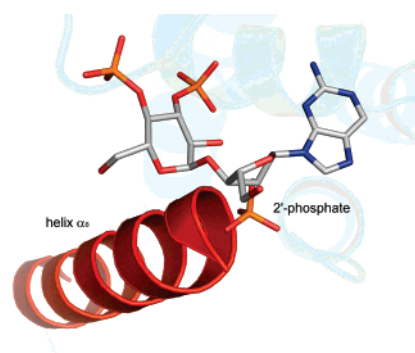
bisphosphate of Ins(1,4,5)P<sub>3</sub> with the Ins(1,4,5)P<sub>3</sub>-binding core were reproduced by the glucose 3'',4''-bisphosphate group of the newly synthesized 2-aminopurine derivatives, and a cation- $\pi$  interaction between the guanidinium side chain of Arg504 and the purine was present. In addition to the interactions observed in binding mode B of AdA, hydrogen bonding interactions were observed between the 2-amino group of the analogues and residue Glu505 (Figure 2). This could contribute to the potencies of these analogues (see also the Supporting Information). We can see no possibility for any interaction between N<sup>1</sup>H of **5** and the receptor (and by implication also hypoxanthine) in our model. The EP surface of chlorophostin **8** also shows the 2-NH<sub>2</sub> region to be relatively less positive than that in **5**, which could also destabilize any potential Glu505 interaction. For similar docks of **7** and **8**, see the Supporting Information.

With the aim of examining the reduced potency of 2-methoxy-*N*<sup>6</sup>-methyl-adenophostin (**4**) in comparison to the newly synthesized 2-amino derivatives **5–8**, a docking study was performed on 2-methoxy-*N*<sup>6</sup>-methyl-AdA using the methods described previously.<sup>7a</sup> As observed in the studies on **5–8**, the highest scored binding mode of 2-methoxy-*N*<sup>6</sup>-methyl-AdA (**4**) reproduced the ligand-binding core interactions of AdA (mode B). The highest scoring binding modes of both guanophostin and 2-methoxy-*N*<sup>6</sup>-methyl-AdA were subjected to energy minimization using the Tripos force field and the resulting structures compared. The only significant difference between the two minimized structures was the positions of the purine rings and Glu505, which were at a greater distance apart in the 2-methoxy-*N*<sup>6</sup>-methyl-AdA structure (Figure 3). The side chain of Glu505 was observed to have moved away from the 2-position methoxy group with an rms deviation of 0.70 Å between the two energy-minimized structures. This suggests that a methoxy group at the 2-position of the adenine ring may be sterically unfavorable, and this may account for the reduced potency of **4**.

In all our modeling studies with AdA analogues, the 2'-phosphate occupies a position proximal to the axis of  $\alpha$ -helix-6 of the Ins(1,4,5)P<sub>3</sub>-binding core (Figure 4). The formation of a macrodipole in an  $\alpha$ -helix, due to the coaxial alignment of the peptide groups, results in the development of a partial net charge at the helix termini,<sup>40</sup> and this dipolar charge distribution may be important in, for example, protein folding,<sup>41</sup> binding of charged species at the helical termini,<sup>42–45</sup> and concentrating



**FIGURE 3.** 2-Methoxy analogue **4** was docked into the Ins(1,4,5)-P<sub>3</sub>-binding core and subjected to energy minimization during which Glu505 rotated away from the methoxy group at the 2-position. Atoms shown in red represent the positions of the 2-OMe group and Glu505 after minimization (hydrogen atoms are not shown).



**FIGURE 4.** 2'-Phosphate of the adenophostin analogues situated in close proximity to the N terminus of  $\alpha$ -helix-6 when docked into the Ins(1,4,5)P<sub>3</sub> receptor binding core. This potentially favorable interaction is demonstrated above using the docked conformation of 2-aminopurinophostin (hydrogen atoms not shown); see also the Supporting Information.

cations within ion channels.<sup>46</sup> The favorable interaction of a charged species with an oppositely charged helix dipole has been exploited in protein engineering.<sup>47</sup> From the structure of Ins(1,4,5)P<sub>3</sub> bound to the binding core of the Ins(1,4,5)P<sub>3</sub>R, it is clear, although never previously remarked upon, that the 1-phosphate of Ins(1,4,5)P<sub>3</sub> is also directed toward  $\alpha$ -helix-6. This suggests the possibility of a helix-dipole interaction between the 1-P of Ins(1,4,5)P<sub>3</sub> and  $\alpha$ -6 helix of Ins(1,4,5)P<sub>3</sub>R.<sup>11</sup> However, a more general analysis of the locations of charged species at the termini of the  $\alpha$ -helices of protein structures has questioned the existence of such electrostatic interaction,<sup>48</sup> and

(41) Hol, H. G. J.; Halie, L. M.; Sander, C. *Nature* **1981**, 294, 532–536.

(42) (a) Hol, W. G. J. *Progr. Biophys. Mol. Biol.* **1985**, 45, 149–195. (b) Miller, J. S.; Kennedy, R. J.; Kemp, D. S. *J. Am. Chem. Soc.* **2002**, 124, 945–962.

(43) Xu, L.; Chong, Y. H.; Hwang, I. Y.; D'Onofrio, A.; Amore, K.; Beardsley, G. P.; Li, C. L.; Olson, A. J.; Boger, D. L.; Wilson, I. A. *J. Biol. Chem.* **2007**, 282, 13033–13046.

(44) van Straaten, K. E.; Barends, T. R. M.; Dijkstra, B. W.; Thunnissen, A. *J. Biol. Chem.* **2007**, 282, 21197–21205.

(45) Fukunaga, R.; Yokoyama, S. *Nature Struct. Mol. Biol.* **2007**, 14, 272–279.

(46) Doyle, D. A.; Cabral, J. M.; Pfuertner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. *Science* **1998**, 280, 69–77.

(47) (a) Nicholson, H.; Becktel, W. J.; Matthews, B. W. *Nature* **1988**, 336, 651–656. (b) Serrano, L.; Fersht, A. R. *Nature* **1989**, 342, 296–299. (c) Nicholson, H.; Anderson, D. E.; Dao-Pin, S.; Matthews, B. W. *Biochemistry* **1991**, 30, 9816–9828.

(40) Hol, W. G. J.; van Duijnen, P. T.; Berendsen, H. J. C. *Nature* **1978**, 273, 443–446.



another study noted that the strength of the interaction between helix dipole and charged species depends on whether the helical termini are accessible to water.<sup>49</sup> In an aqueous environment, the dipole is counteracted by an electrostatic reaction field generated by the solvent which drastically reduces the strength of the helix dipole. A recent review<sup>50</sup> suggested that the importance of macrodipoles has sometimes been overestimated due to the use of inadequately low values of the relevant dielectric constant. Hence, the presence of the 1-P of Ins(1,4,5)P<sub>3</sub> (2'-phosphate in AdA analogues) near the  $\alpha$ -6 helix, while notable, does not unambiguously establish the potential role of a helix-dipole interaction in Ins(1,4,5)P<sub>3</sub> binding. Nevertheless, our models of AdA and other analogues position the 2'-phosphate closer to the  $\alpha$ -6 helix than the corresponding crystallographic distance for the 1-P of Ins(1,4,5)P<sub>3</sub>, so this interaction could be of greater significance. However, even if the helix dipole does play a role in Ins(1,4,5)P<sub>3</sub> recognition, the slightly closer position of the 2'-phosphate of AdA is insufficient explanation for the increased potency of AdA and its analogues. Further experiments are necessary to unravel the role of helix-dipole interactions in ligand recognition by Ins(1,4,5)P<sub>3</sub> receptors.

In conclusion, we have synthesized four 2-position-modified analogues of AdA and established their biological activity. In contrast to the previous benchmark compound of this class, the 2-methoxy purine derivative **4**, 2-amino base-modified AdA derivatives are potent agonists of Ins(1,4,5)P<sub>3</sub>R. This suggests that the 2-position of a purine base deserves further exploration for design of high affinity ligands of Ins(1,4,5)P<sub>3</sub>R. Among the 2-aminopurine analogues, the substituent at the 6-position slightly influenced potency. The similar potencies of 2-amino and 2,6-diamino analogues agree with previous results showing that the 6-NH<sub>2</sub> does not contribute to potency.<sup>7d,30</sup> The slightly lower potency of chlorophostin and slightly higher potency of guanophostin support our original model of the interaction of AdA with the Ins(1,4,5)P<sub>3</sub>R involving a cation- $\pi$  interaction.<sup>7a</sup>

Molecular modeling was used to gain further insight into the interactions of these novel ligands with the Ins(1,4,5)P<sub>3</sub>R. The relative difference in potency of 2-OMe and 2-NH<sub>2</sub> derivatives is mirrored by the modeling studies. The carboxylate side chain of Glu505 approaches the NH<sub>2</sub> group in 2-aminopurine derivatives as expected, while it is deflected in the 2-methoxy derivative. Although the receptor region close to the 2-position is sterically intolerant, introduction of a smaller group that can interact with the carboxylate side chain Glu505 may compensate. However, modification of the 2-position with a bulkier group may lead to loss of potency. It is noteworthy that both the models of these AdA analogues and the actual crystal structure of Ins(1,4,5)P<sub>3</sub> bound to its binding core suggest the possibility of a helix-dipole interaction in ligand recognition. The significance of this putative interaction awaits further investigation.

## Experimental Section

**Measurement of Ca<sup>2+</sup> Release from Permeabilized Cells.** The effects of Ins(1,4,5)P<sub>3</sub> or AdA analogues on intracellular Ca<sup>2+</sup> stores were measured using a low-affinity Ca<sup>2+</sup>-indicator trapped within the intracellular stores of permeabilized cells.<sup>29,51</sup> DT40 cells stably

expressing only rat type 1 Ins(1,4,5)P<sub>3</sub>R (DT40-Ins(1,4,5)P<sub>3</sub>R1) were harvested by centrifugation (650  $\times$  g; 2 min) and resuspended ((2–3)  $\times$  10<sup>7</sup> cells/mL) in Hepes-buffered saline (HBS: 135 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl<sub>2</sub>, 1.5 mM CaCl<sub>2</sub>, 11.6 mM Hepes, 11.5 mM D-glucose, pH 7.3) supplemented with Mag-fluo-4AM (20  $\mu$ M), Pluronic F-127 (0.05%), and bovine serum albumin (1 mg/mL). After 1 h at 20 °C in the dark, the Mag-fluo-4-loaded cells were harvested (650  $\times$  g; 2 min) and resuspended ( $\sim$ 2  $\times$  10<sup>6</sup> cells/mL) in Ca<sup>2+</sup>-free cytosolic-like medium (CLM: 140 mM KCl, 20 mM NaCl, 2 mM MgCl<sub>2</sub>, 1 mM EGTA, 20 mM Pipes, pH 7.0). The cells were permeabilized by incubation with saponin (10  $\mu$ g/mL, 4 min at 37 °C), harvested (650  $\times$  g; 2 min), and resuspended in Mg<sup>2+</sup>-free CLM (140 mM KCl, 20 mM NaCl, 1 mM EGTA, 375  $\mu$ M CaCl<sub>2</sub> ( $\sim$ 200 nM free [Ca<sup>2+</sup>]), 20 mM Pipes, pH 7.0). The permeabilized cells (with Mag-fluo-4 trapped within the lumen of the ER) were then attached to 96-well plates ( $\sim$ 8  $\times$  10<sup>5</sup> cells/well) coated with poly-L-lysine (0.01%) and centrifuged onto the plate (300  $\times$  g; 2 min). Immediately before an experiment, the cells were washed twice in Mg<sup>2+</sup>-free CLM to remove cytosolic Mag-fluo-4, and the plates were then mounted in a Flexstation fluorescence plate reader (Molecular Devices, Sunnyvale, CA), which allows automated additions to the sample wells while recording fluorescence. Mag-fluo-4 fluorescence was monitored by excitation at 485 nm with emission detected at 520 nm. Active Ca<sup>2+</sup> uptake into the ER was initiated by addition of Mg<sup>2+</sup>-ATP (1.5 mM), and after 150 s, when the stores had loaded to a steady-state Ca<sup>2+</sup> content, Ins(1,4,5)P<sub>3</sub> was added. The amount of Ca<sup>2+</sup> released by Ins(1,4,5)P<sub>3</sub> was expressed as a fraction of the total Ca<sup>2+</sup> content of the ER as assessed by addition of 1  $\mu$ M ionomycin. Data are presented as means  $\pm$  SEM means from at least three independent experiments, each performed in triplicate. Concentration-effect relationships were fitted to four-parameter logistic equations using nonlinear curve-fitting procedures (GraphPad Prism, San Diego, CA).

**2-Amino-6-chloro-9-[2'-O-acetyl-5'-O-benzyl-3'-O-(3'',4''-di-O-acetyl-2'',6''-di-O-benzyl- $\alpha$ -D-glucopyranosyl)- $\beta$ -D-ribofuranosyl]purine (**11**).** A solution of disaccharide **10** (626 mg, 0.833 mmol), 2-amino-6-chloropurine (272 mg, 1.60 mmol), and *N,O*-bis-trimethylsilyl-acetamide (BSA, 1.15 mL, 4.7 mmol) in acetonitrile (25 mL) was heated to reflux (100 °C) for 30 min. When the solution became clear, the reaction mixture was cooled to room temperature, and then TMSOTf (325  $\mu$ L, 1.78 mmol) was added dropwise. Then the reaction mixture was heated to 70 °C and stirring continued at this temperature overnight. TLC showed disappearance of starting material and the formation of a single new spot. The solution was cooled, diluted with ethyl acetate, washed successively with satd aq NaHCO<sub>3</sub> solution, water, and brine, and dried over anhyd MgSO<sub>4</sub>. Column chromatography with 46% ethyl acetate–54% hexane provided pure **11** (645 mg, 90%) as a white solid: mp 81 °C; [ $\alpha$ ]<sub>D</sub> +78.5 (*c* 1, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.90 (s, 3H, COCH<sub>3</sub>), 1.96 (s, 3H, COCH<sub>3</sub>), 2.02 (s, 3H, COCH<sub>3</sub>), 3.41 (m, 2H, H<sub>A</sub>-6'' and H<sub>B</sub>-6''), 3.60 (dd, 1H, 10.3, 3.7 Hz, H-2''), 3.63 (dd, 1H, 11.0, 2.9 Hz, H<sub>A</sub>-5'), 3.71 (dd, 1H, 10.83, 2.64 Hz, H<sub>B</sub>-5'), 4.01 (dt, 1H, 10.3, 3.59 Hz, H-5''), 4.35–4.68 (m, 7H, 3  $\times$  PhCH<sub>2</sub> and H-4'), 4.85 (dd, 1H, 5.29, 4.23 Hz, H-3'), 5.03 (d, 1H, 3.44 Hz, H-1''), 5.06 (dd, 1H, 10.3, 9.51 Hz, H-4''), 5.19 (s, 2H, NH<sub>2</sub>), 5.48 (t, 1H, 9.78 Hz, H-3''), 5.72 (t, 1H, 5.29 Hz, H-2'), 6.20 (d, 1H, 5.55 Hz, H-1'), 7.26–7.40 (m, 15H, 3  $\times$  Ph), 8.08 (s, 1H, H-8); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  20.4 (COCH<sub>3</sub>), 20.7 (COCH<sub>3</sub>), 20.9 (COCH<sub>3</sub>), 67.9 (C-6''), 69.0 (C-4''), 69.1 (C-5''), 69.2 (C-5''), 71.8 (C-3''), 73.3 (PhCH<sub>2</sub>), 73.5 (PhCH<sub>2</sub>), 73.7 (PhCH<sub>2</sub>), 74.1 (C-2'), 76.5 (C-3'), 76.6 (C-2''), 82.6 (C-4'), 86.0 (C-1'), 98.0 (C-1''), 125.5, 127.7, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 128.7, 137.2, 137.4, 137.6, 140.8, 151.4, 153.5, 159.1, 169.8 (O-COCH<sub>3</sub>), 170.3 (O-COCH<sub>3</sub>), 170.4 (O-COCH<sub>3</sub>); *m/z* (ES) = 860.3 [(M)<sup>+</sup>, 100], 861.3 [(M + H)<sup>+</sup>, 50], 882.3 [(M + Na)<sup>+</sup>, 100]; HRMS mass calcd for C<sub>43</sub>H<sub>46</sub><sup>35</sup>ClN<sub>5</sub>O<sub>12</sub> [M]<sup>+</sup>

(48) Chakrabarti, P. *Protein Eng.* **1994**, *7*, 471–474.

(49) Sengupta, D.; Behera, R. N.; Smith, J. C.; Ullmann, G. M. *Structure* **2005**, *13*, 849–855.

(50) Warshel, A.; Sharma, P. K.; Kato, M.; Parson, W. W. *Biochim. Biophys. Acta Proteins Proteomics* **2006**, *1764*, 1647–1676.

(51) Tovey, S. C.; Sun, Y.; Taylor, C. W. *Nature Protocols* **2006**, *1*, 259–263.



860.2904, found 860.2910. Anal. Calcd for  $C_{43}H_{46}ClN_5O_{12} \cdot 1/4 C_7H_8$ : C, 60.86; H, 5.48; N, 7.93. Found: C, 60.60; H, 5.56; N, 7.58.

**2-Amino-6-chloro-9-{5'-O-benzyl-3'-O-[2'',6''-di-O-benzyl- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribose]purine (12).** A solution of triacetate **11** (30 mg, 0.035 mmol) in methanol (2 mL) was saturated with ammonia (bubbled at rt for 5 min), and the solution was stirred at rt. When TLC showed complete disappearance of the starting material (12 h), the solvent was evaporated and the residue was dissolved in DCM, washed with water (3 times) to remove the acetamide and then with brine, and the solution dried over anhyd  $MgSO_4$ . The gummy residue obtained after evaporation was chromatographed using ethyl acetate as eluent to afford **12** (21 mg, 82%) as a colorless solid. Scale up using **11** (380 mg) and methanol (15 mL) provided 293 mg of **12** (90.4%): mp 97 °C;  $[\alpha]_D -73.6$  (c 1.06,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.19 (br s, 4''-OH), 3.45–3.66 (m, 6H, H-2'', H-5A'', H-5B'', H-4'', H-6A'', H-6B''), 3.80 (ddd, 1H, 9.78, 4.90, 2.16 Hz, H-5''), 4.11–4.18 (m, 2H, H-3' and H-3''), 4.21 (m, 1H, H-4'), 4.35–4.50 (m, 6H, H-2', 2''-OH and 2  $\times$   $PhCH_2$ ), 4.83 (ABq, 2 H, 20.94, 11.55 Hz,  $PhCH_2$ ), 4.86 (d, 1H, 3.52 Hz, H-1''), 5.76 (dd, 1H, 5.09, 2.74 Hz, H-1'), 6.01 (d, 4.70 Hz, 3''-OH), 6.88 (br s, 2H,  $NH_2$ ), 7.13–7.33 (m, 15H, 3  $\times$  Ph), 7.96 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  69.02 (C-6''), 69.60 (C-4''), 70.04 (C-5'), 72.37 (C-3''), 72.51 (C-5''), 73.65 ( $PhCH_2$ ), 74.01 ( $PhCH_2$ ), 75.30 ( $PhCH_2$ ), 77.42 (C-2'), 79.36 (C-2''), 81.44 (C-3'), 83.28 (C-4'), 86.43 (C-1'), 100.45 (C-1''), 124.86 (C-5), 127.67, 127.77, 127.84, 127.95, 128.22, 128.33, 128.41, 128.76, 128.83, 128.89, 129.21 (aromatic carbons), 135.80, 137.02, 137.99 (ipso carbons), 140.29 (C-8), 151.48 (C-4 or C-6), 154.33 (C-6 or C-4), 159.84 (C-2);  $m/z$  (FAB+) = 734.2 [M + H] $^+$ , 100; HRMS mass calcd for  $C_{37}H_{41}^{35}ClN_5O_9$  [M + H] $^+$  734.25928, found 734.26378. Anal. Calcd for  $C_{37}H_{40}ClN_5O_9$ : C, 60.53; H, 5.49; N, 9.54. Found: C, 60.30; H, 5.63; N, 9.43.

**2-Amino-6-chloro-9-{5'-O-benzyl-2'-O-(dibenzylxyphosphoryl)-3'-O-[2'',6''-di-O-benzyl-3'',4''-bis(dibenzylxyphosphoryl)- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribose]purine (13).** A solution of the triol **12** (297 mg, 0.405 mmol), bis(benzylxy)(diisopropylamino)phosphine (602 mg, 1.744 mmol), and imidazolium triflate (480 mg, 2.202 mmol) in anhydrous DCM (10 mL) was stirred at room temperature under  $N_2$  for 30 min. The reaction mixture was cooled to  $-78$  °C, and then *m*-CPBA (685 mg, 60–70%) was added and the mixture stirred for 45 min at this temperature and then at rt for an additional 30 min. Usual workup in ethyl acetate followed by column chromatography using ethyl acetate–hexane– $Et_3N$  (55:43:2; v/v/v) provided pure **13** (575 mg, 94%) as a gum:  $[\alpha]_D +31.7$  (c 1.4,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.48 (dd, 1H, 10.47, 2.56 Hz, H-5A''), 3.56–3.70 (m, 4H, H-2'', H-5B'', H-6A'', H-6B''), 3.81–3.85 (m, 1H, H-5''), 4.24–4.80 (m, 11H, H-3', H-4', H-4'' and 4  $\times$   $PhCH_2$ ), 4.70–4.79 (m, 2 H,  $PhCH_2$ ), 4.80–5.02 (m, 9H, H-3'' and 4  $\times$   $PhCH_2$ ), 5.34 (d, 1H, 3.72 Hz, H-1''), 5.50 (ddd, 1H, 8.61, 4.70, 6.26 Hz, H-2'), 6.17 (d, 1H, 6.51 Hz, H-1'), 6.97–7.40 (m, 45H, 9  $\times$  Ph), 7.84 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  66.30 (C-6''), 68.93 (C-5'), 68.97, 69.03, 69.19, 69.25, 69.38, 69.43, 69.54, 69.59, 69.67, 69.73, 69.80 (6  $\times$   $PhCH_2OP$ ,  $^{31}P$  coupled), 69.92 (C-5''), 71.37 ( $PhCH_2OC$ ), 73.19 ( $PhCH_2OC$ ), 73.46 ( $PhCH_2OC$  and C-3'), 74.25 (C-4''), 76.69 (C-2''), 76.76 (C-2'), 77.93 (C-3''), 82.20 (C-4'), 85.07 (C-1'), 95.15 (C-1''), 127.41, 127.53, 127.56, 127.64, 127.70, 127.78, 127.85, 127.88, 127.94, 127.97, 128.10, 128.17, 128.19, 128.22, 128.25, 128.32, 128.39, 128.47, 128.55 (aromatic carbons), 134.68, 134.75, 134.83, 134.89, 135.47, 135.54, 135.61, 135.67, 135.94, 135.97, 136.02, 136.05 (6  $\times$   $POCH_2C$ ,  $^{31}P$  coupled), 137.07, 137.57, 137.82 (3  $\times$   $COCH_2C$ ), 140.57 (C-8), 151.03 (C-4 or C-6), 153.58 (C-6 or C-4), 158.89 (C-2);  $^{31}P$  NMR (161.94 MHz,  $CDCl_3$ )  $\delta$   $-2.04$ ,  $-1.93$ ,  $-1.217$ ;  $m/z$  (FAB+) = 1514.6 [(M + H) $^+$ , 10]; HRMS mass calcd for  $C_{79}H_{80}^{35}ClN_5O_{18}P_3$  [M + H] $^+$  1514.4394, found 1514.4396.

**2-Amino-6-O-benzyl-9-{5'-O-benzyl-3'-O-[2'',6''-di-O-benzyl- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribose]purine (16).** Method A. To a solution of nucleoside **11** (50 mg, 0.058 mmol) and benzyl alcohol (36.5  $\mu$ L, 0.35 mmol) in DMF (0.5 mL) was added NaH (12 mg,

0.3 mmol) at rt and the mixture stirred overnight and then at 80 °C for 3 h. When the TLC showed transformation of the starting material into a single new spot, the mixture was diluted with dichloromethane, washed with water and brine, dried over  $MgSO_4$ , and evaporated. Benzyl alcohol was removed by chromatography using 35% ethyl acetate–hexane as eluent. Further elution with ethyl acetate–methanol (95:5, v/v) gave an inseparable mixture of two products. Further purification of this mixture by reversed-phase column chromatography (in RP-18) using water–acetonitrile as eluent provided the expected **16** (20 mg, 43%) and the deglycosylated product **17** (11 mg, 41%).

**Method B.** To a solution of nucleoside **11** (50 mg, 0.058 mmol) in benzyl alcohol (0.5 mL) was added NaH (4.8 mg, 0.12 mmol) at rt and the mixture stirred at 120 °C overnight. Usual workup in ethyl acetate followed by column chromatography (elution with 35% EtOAc–hexane to remove the benzyl alcohol and then with 5% MeOH in EtOAc) provided pure **16** (41 mg, 88%) as a colorless gum:  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.48 (dd, 1H, 9.79, 3.43 Hz, H-2''), 3.51–3.62 (m, 3H, H-5A'', H-5B'', H-6A''), 3.62–3.71 (m, 2H, H-4'', H-6B''), 3.80–3.87 (m, 1H, H-5''), 4.08–4.19 (m, 2H, H-3' and H-3''), 4.28–4.32 (m, 1H, H-4'), 4.42–4.58 (m, 5H, H-2', 2  $\times$   $PhCH_2$ ), 4.73 (ABq, 2 H, 38.64, 11.69 Hz,  $PhCH_2$ ), 4.86 (d, 1H, 3.56 Hz, H-1''), 5.60 (AB q, 19.45, 2.33 Hz, c-6- $OCH_2Ph$ ), 5.90 (br s, 2H,  $NH_2$ ), 6.07 (d, 1H, 6.10 Hz, H-1'), 7.20–7.50 (m, 20H, 4  $\times$  Ph), 7.89 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  68.68 (C-6- $OCH_2Ph$ ), 68.81 (C-6''), 69.35 (C-4''), 69.96 (C-5'), 71.92 (C-3''), 72.41 (C-5''), 73.75 ( $PhCH_2$ ), 73.82 ( $PhCH_2$ ), 74.41 ( $PhCH_2$ ), 77.20 (C-2'), 79.36 (C-2''), 80.85 (C-3'), 82.84 (C-4'), 86.40 (C-1'), 99.82 (C-1''), 115.17 (C-5), 127.75, 127.78, 128.02, 128.05, 128.37, 128.40, 128.64, 128.67, 129.07 (aromatic carbons), 136.12, 136.26 (ipso carbons), 137.06 (C-8), 137.22, 137.71 (ipso carbons), 154.09 (C-4), 159.47 (C-2), 161.25 (C-6);  $m/z$  (ES+) = 806.4 [(M + H) $^+$ , 100], 828.4 [(M + Na) $^+$ , 80]; HRMS mass calcd for  $C_{44}H_{47}N_5O_{10}$  [M + H] $^+$  806.3396, found 806.3404.

**2-Amino-6-O-benzyl-9-{5'-O-benzyl-2'-O-(dibenzylxyphosphoryl)-3'-O-[2'',6''-di-O-benzyl-3'',4''-bis(dibenzylxyphosphoryl)- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribose]purine (18).** A solution of the triol **16** (40 mg, 0.05 mmol), bis(benzylxy)(diisopropylamino)phosphine (82 mg, 0.24 mmol), and imidazolium triflate (68 mg, 0.31 mmol) in anhydrous DCM (3 mL) was stirred at room temperature under a nitrogen atmosphere for 45 min. The reaction mixture was cooled to  $-78$  °C, *m*-CPBA (84 mg) was added, and the mixture stirred for 1.5 h, gradually allowing the temperature to attain rt. Usual workup in ethyl acetate followed by chromatography using ethyl acetate–hexane– $Et_3N$  (55:43:2; v/v/v) afforded pure **18** (65 mg, 82%) as a colorless gum:  $[\alpha]_D +28.8$  (c 1.4,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.50 (dd, 1H, 10.37, 3.02 Hz, H-5A''), 3.56–3.68 (m, 4H, H-5B'', H-2'', H-6A'', H-6B''), 3.80–3.86 (m, 1H, H-5''), 4.32 (dd, 1H, 6.05, 3.02 Hz, H-4'), 4.32 (AB q, 2H, 48.17, 11.88 Hz,  $CH_2Ph$ ), 4.35 (AB q, 2H, 14.04, 11.88 Hz,  $CH_2Ph$ ), 4.43–4.66 (m, H, H-4'',  $CH_2Ph$ ), 4.68 (dd, 1H, 4.75, 3.02 Hz, H-3'), 4.71–4.79 (m, H,  $CH_2Ph$ ), 4.84–5.04 (m, H-3'',  $CH_2Ph$ ), 5.36 (d, 1H, 3.46 Hz, H-1''), 5.50 (s, 2H,  $CH_2Ph$ ), 5.56 (ddd, 1H, 8.64, 4.75, 6.05 Hz, H-2'), 6.18 (d, 1H, 6.05 Hz, H-1'), 6.96–7.50 (m, 50H, 10  $\times$  Ph), 7.68 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  67.80 (C-6- $OCH_2Ph$ ), 68.18 (C-6''), 68.97 (C-5'), 68.91, 69.06, 69.18, 69.23, 69.33, 69.39, 69.43, 69.49, 69.63, 69.69, 71.37, 73.15, 73.42 ( $CH_2Ph$ ,  $^{31}P$  coupled), 69.87 (C-5'',  $^{31}P$  coupled), 73.33 (C-3',  $^{31}P$  coupled), 74.24 (C-4'',  $^{31}P$  coupled), 76.60–76.71 (m, C-2', C-2'',  $^{31}P$  coupled), 77.98 (C-3'',  $^{31}P$  coupled), 81.91 (C-4'), 85.00 (C-1',  $^{31}P$  coupled), 95.10 (C-1''), 115.52 (C-5), 127.40, 127.49, 127.54, 127.63, 127.71, 127.74, 127.81, 127.91, 127.93, 127.94, 128.07, 128.16, 128.17, 128.20, 128.24, 128.30, 128.33, 128.37, 128.40 (aromatic carbons), 134.87, 134.95, 135.02, 135.50, 135.58, 135.64, 135.71, 135.99, 136.08, 136.30, 137.24, 137.56, 137.88 (ipso carbons,  $^{31}P$  coupled), 137.78 (C-8), 153.97 (C-4), 159.03 (C-2), 160.74 (C-6);  $^{31}P$  NMR (161.94 MHz,  $CDCl_3$ )  $\delta$   $-1.40$ ,  $-1.967$ ,  $-2.101$ ;  $m/z$  (ES+) = 1587.8 [(M + H) $^+$ , 100]; HRMS

mass calcd for  $C_{86}H_{87}N_5O_{19}P_3$  [ $M + H$ ], 1586.52081, found 1586.52098.

**3'- $\alpha$ -D-Glucopyranosylguanosine 2',3',4''-Trisphosphate (Guanophostin A) (5).** To a solution of protected phosphate **18** (65 mg, 0.041 mmol) and cyclohexene (5 mL) in a mixture of methanol (10 mL) and water (0.75 mL) was added  $Pd(OH)_2$  (20% on carbon, 160 mg), and the mixture was stirred at 80 °C overnight. After cooling, the mixture was filtered through a membrane filter washing well with water and methanol. The combined filtrate and washings was evaporated under diminished pressure. Purification was done by ion-exchange column chromatography using AG resin column and 150 mM TFA (0–100% gradient elution) as eluent to obtain very pure **5** (22.2 mg, 79%):  $^1H$  NMR (400 MHz,  $D_2O$ )  $\delta$  3.67–3.85 (m, 6H, H-5'', H-6\_A'', H-6\_B'', H-5\_A', H-5\_B', H-2''), 4.04 (dd, 1H, 16.0, 9.11 Hz, H-4''), 4.37 (m, 1H, H-4'), 4.40–4.45 (m, 1H, H-3''), 4.52 (m, 1H, H-3'), 5.16 (d, 1H, 3.73 Hz, H-1''), 5.20–5.26 (m, 1H, H-2'), 6.17 (d, 1H, 6.18 Hz, H-1'), 9.00 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $D_2O$ )  $\delta$  60.03 (C-5'), 60.47 (C-6''), 70.21 (C-2'',  $^{31}P$  coupled), 71.36 (C-5'',  $^{31}P$  coupled), 72.95 (C-4'',  $^{31}P$  coupled), 73.42 (C-3',  $^{31}P$  coupled), 75.39 (C-2',  $^{31}P$  coupled), 77.90 (C-3'',  $^{31}P$  coupled), 84.46 (C-4'), 88.36 (C-1',  $^{31}P$  coupled), 98.04 (C-1''), 108.31 (C-5), 136.53 (C-8), 149.38 (C-4), 154.84 (C-6), 155.18 (C-2);  $^{31}P$  NMR (161.94 MHz,  $D_2O$  with excess of TEA)  $\delta$  3.278, 3.54, 4.297;  $m/z$  (ES) = 684.1 [( $M - H$ ), 100]; HRMS mass calcd for  $C_{16}H_{25}N_5O_{19}P_3$  [ $M - H$ ] 684.0362, found 684.0379.

**2,6-Diamino-9-[5'-O-benzyl-3'-O-(2'',6'')-di-O-benzyl- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribofuranosyl]purine (19).** A solution of **11** (300 mg, 0.349 mmol) in ethanol in a pressure tube was bubbled with ammonia at 0 °C for 0.5 h and then heated at 70 °C for 5 days. The solvents were evaporated, and the residue thus obtained on chromatographic purification using ethyl acetate–methanol– $Et_3N$  (93:6:1; v/v/v) provided the required **19** (244 mg, 98%) as a colorless solid: mp 185 °C; [ $\alpha$ ]<sub>D</sub> –26.2 (c 1.3,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  3.41–3.45 (m, 2H, H-5\_A' and H-2''), 3.50 (dd, 1H, 10.3, 2.01 Hz, H-5\_B'), 3.55 (t, 1H, 9.55 Hz, H-4''), 3.62 (dd, 1H, 10.55, 4.77 Hz, H-6\_A''), 3.67 (dd, 1H, 10.8, 2.26 Hz, H-6\_B''), 3.77–3.80 (m, 1H, H-5''), 3.97 (br s, 1H, H-4'), 4.09 (m, 1H, H-3'), 4.24 (t, 1H, 9.55 Hz, H-3''), 4.32 (dd, 1H, 8.04, 4.27 Hz, H-2'), 4.42 (ABq, 2 H, 19.48, 11.69 Hz,  $PhCH_2$ ), 4.47 (s, 2H,  $PhCH_2$ ), 4.78 (ABq, 2 H, 27.89, 12.31 Hz,  $PhCH_2$ ), 4.80 (d, 1H, 3.52 Hz, H-1''), 5.66 (d, 1H, 8.04 Hz, H-1'), 7.13–7.32 (m, 15H, 3  $\times$  Ph), 7.70 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  69.22 (C-6''), 69.74 (C-4''), 70.05 (C-5'), 71.68 (C-3''), 72.83 (C-5''), 73.56 ( $PhCH_2$ ), 73.80 ( $PhCH_2$ ), 73.85 ( $PhCH_2$ ), 77.26 (C-2'), 78.89 (C-2''), 81.58 (C-3'), 82.72 (C-4'), 86.0 (C-1'), 100.51 (C-1''), 113.22 (C-5), 127.7, 127.85, 128.15, 128.40, 128.62, 128.68, 128.72, 129.10 (aromatic carbons), 135.34 (C-8), 136.13, 137.34, 138.01 (ipso carbons), 152.45 (C-4 or C-6), 155.55 (C-6 or C-4), 160.16 (C-2);  $m/z$  (FAB+) = 715.1 [( $M + H$ )<sup>+</sup>, 100]; HRMS mass calcd for  $C_{37}H_{43}N_6O_9$  [ $M + H$ ]<sup>+</sup> 715.30915, found 715.30995.

**2,6-Diamino-9-[5'-O-benzyl-2'-O-(dibenzylloxyporphoryl)-3'-O-[2'',6'')-di-O-benzyl-3',4''-bis-(dibenzylloxyporphoryl)- $\alpha$ -D-glucopyranosyl]- $\beta$ -D-ribosyl]purine (20).** A solution of the triol **19** (70 mg, 0.098 mmol), bis(benzylloxy)(diisopropylamino)phosphine (135 mg, 0.39 mmol), and imidazolium triflate (85 mg, 0.39 mmol) in anhydrous DCM (6 mL) was stirred at room temperature under  $N_2$  for 1 h. Then the mixture was then cooled to –78 °C, and *m*-CPBA (34 mg) was added and stirred for 30 min at this temperature and then at rt for an additional 1 h. The mixture was then diluted with DCM and washed successively with  $NaHCO_3$  solution, water, and brine. The concentrated organic layer was chromatographed to give pure phosphate **20** (123 mg, 84%) as a colorless gum: [ $\alpha$ ]<sub>D</sub> +30.0 (c 1,  $CHCl_3$ );  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  2.33 (br s, 2H, exchangeable,  $NH_2$ ), 3.52 (dd, 1H, 10.77, 3.01 Hz, H-5\_A'), 3.56–3.66 (m, 4H, H-6\_A'', H-6\_B'', H-5\_B' and H-2''), 3.83 (ddd, 1H, 10.02, 4.01, 2.51 Hz, H-5''), 4.25–4.47 (m, 6H, H-4' and 2.5  $\times$   $PhCH_2$ ), 4.51–4.77 (m, 9H, H-3', H-4'' and 3.5  $\times$   $PhCH_2$ ), 4.86–5.04 (m, 7H, H-3'' and 3  $\times$   $PhCH_2$ ), 5.34 (d, 3.51 Hz, H-1''), 5.49 (br s, 2H, exchangeable,  $NH_2$ ), 5.58 (ddd, 1H, 8.77,

6.01, 4.76 Hz, H-2'), 6.17 (d, 1H, 6.30 Hz, H-1'), 6.98–7.36 (m, 45H, 9  $\times$   $C_6H_5$ ), 7.60 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$  68.2 (C-6''), 69.0 (C-5'), 69.1, 69.2, 69.26, 69.32, 69.4, 69.46, 69.49, 69.55, 69.71, 69.74, 69.77, 69.8, 69.9 ( $^{31}P$  coupled  $PhCH_2OP$ ), 69.93 (C-5''), 71.5, 73.2, 73.5 (3  $\times$   $PhCH_2OC$ ), 73.4 (C-3'), 74.3 (C-4'',  $^{31}P$  coupled, d, 5.37 Hz), 76.9 (C-2',  $^{31}P$  coupled, d, 5.37 Hz), 76.96 (C-2''), 78.02 (C-3'',  $^{31}P$  coupled), 81.9 (C-4'), 85.04 (C-1',  $^{31}P$  coupled, d, 5.37 Hz), 95.2 (C-1''), 114.3 (C-5), 127.49, 127.56, 127.59, 127.63, 127.7, 127.8, 127.95, 128.0, 128.02, 128.2, 128.23, 128.26, 128.37, 128.41, 128.45, 128.47, 128.52 (aromatic carbons), 135.01, 135.08, 135.15, 135.58, 135.66, 135.73, 135.8, 136.07, 136.14, 136.15 ( $^{31}P$  coupled, ipso carbons of  $POCH_2Ph$ ), 136.6 (C-8), 137.4, 137.6, 138.0 (ipso carbons of  $COCH_2Ph$ ), 152.1 (C-4), 155.7 (C-6), 159.8 (C-2);  $^{31}P$  NMR (161.9 MHz,  $CDCl_3$ )  $\delta$  –1.265, –1.936, –2.101;  $m/z$  (ES) = 1495.8 [( $M + H$ )<sup>+</sup>, 100]; HRMS mass calcd for  $C_{79}H_{81}N_6O_{18}P_3$  [ $M$ ]<sup>+</sup> 1494.4795, found 1494.4795.

**2,6-Diamino-9-[3'-O-( $\alpha$ -D-glucopyranosyl)- $\beta$ -D-ribosyl]-purine 2',3',4''-Trisphosphate [2,6-Diaminopurinophostin (6)].** To a solution of phosphate **20** (100 mg, 0.067 mmol) and cyclohexene (3 mL) in a mixture of methanol (5.25 mL) and water (0.5 mL) was added 20%  $Pd(OH)_2$  on carbon (150 mg) and the mixture stirred at 80 °C overnight. After cooling, the mixture was filtered through a membrane filter, and the filter was washed with water and methanol. The combined filtrate and washings was concentrated, and the crude product thus obtained was purified by ion-exchange column chromatography, using AG resin column and 150 mM TFA as eluent as described above, to afford 2-aminoadenophostin **6** (37 mg, 81%):  $^1H$  NMR (400 MHz,  $D_2O$ )  $\delta$  3.62–3.75 (m, 6H, H-5'', H-6\_A'', H-6\_B'', H-5\_A', H-5\_B', H-2''), 4.00 (dd, 1H, 18.78, 9.39 Hz, H-4''), 4.27 (m, 1H, H-4'), 4.40 (dd, 1H, 18.39, 9.0 Hz, H-3''), 4.47 (dd, 1H, 4.70, 3.13 Hz, H-3'), 5.12–5.17 (m, 2H, H-1'' and H-2'), 6.02 (d, 1H, 6.26 Hz, H-1'), 8.04 (s, 1H, H-8);  $^{13}C$  NMR (100 MHz,  $D_2O$ )  $\delta$  60.07 (C-6''), 60.93 (C-5'), 70.32 (C-2'',  $^{31}P$  coupled), 71.43 (C-5'',  $^{31}P$  coupled), 73.0 (C-4'',  $^{31}P$  coupled), 73.86 (C-3',  $^{31}P$  coupled), 75.14 (C-2',  $^{31}P$  coupled), 77.92 (C-3'',  $^{31}P$  coupled), 84.10 (C-4'), 86.75 (C-1',  $^{31}P$  coupled), 98.11 (C-1''), 111.19 (C-5), 140.66 (C-8), 149.85 (C-6), 150.84 (C-4), 152.16 (C-2);  $^{31}P$  NMR (161.94 MHz,  $D_2O$ )  $\delta$  0.101, –0.302, –0.808;  $m/z$  (ES) = 683.1 [( $M - H$ ), 100]; HRMS mass calcd for  $C_{16}H_{26}N_6O_{18}P_3$  [ $M - H$ ] 683.05164, found 683.05179.

**2-Amino-9-[3'-O-( $\alpha$ -D-glucopyranosyl)- $\beta$ -D-ribosyl]purine 2',3',4''-Trisphosphate [2-Aminopurinophostin (7)].** To a solution of protected phosphate **13** (80 mg, 0.0528 mmol) and cyclohexene (2.1 mL) in a mixture of MeOH (4 mL) and water (0.3 mL) was added  $Pd(OH)_2$  (20% on carbon, 200 mg), and the mixture was stirred at 80 °C overnight. The mixture was filtered through a membrane filter and washed with water and methanol. The combined filtrate was purified by chromatography on a reversed-phase column (RP-18) using 0.05 M TEAB–MeCN eluent system to get pure 2-aminopurinophostin **7** (32 mg, 90%):  $^1H$  NMR (400 MHz,  $D_2O$ )  $\delta$  1.22 (t, 7.35 Hz,  $CH_3$  of TEA), 3.14 (q, 7.35 Hz,  $CH_2$  of TEA), 3.71–3.89 (m, 6H, H-5'', H-6\_A'', H-6\_B'', H-5\_A', H-5\_B', H-2''), 4.00 (dd, 1H, 19.02, 9.64 Hz, H-4''), 4.42 (dd, 1H, 6.34, 3.30 Hz, H-4'), 4.46 (dd, 1H, 17.75, 9.13 Hz, H-3''), 4.63 (dd, 1H, 5.07, 3.04 Hz, H-3'), 5.31 (d, 3.80 Hz, H-1''), 5.31–5.35 (m, 1H, H-2'), 6.20 (d, 1H, 6.59 Hz, H-1'), 8.25 (s, 1H, H-8 or H-6), 8.58 (s, 1H, H-6 or H-8);  $^{13}C$  NMR (100 MHz,  $D_2O$ )  $\delta$  8.11 ( $CH_3$  of TEA), 46.5 ( $CH_2$  of TEA), 60.22 (C-5'), 61.28 (C-6''), 70.98 (C-2'',  $^{31}P$  coupled), 71.85 (C-5'',  $^{31}P$  coupled), 72.28 (C-4'',  $^{31}P$  coupled), 73.67 (C-3',  $^{31}P$  coupled), 74.44 (C-2',  $^{31}P$  coupled), 77.08 (C-3'',  $^{31}P$  coupled), 84.16 (C-4'), 86.89 (C-1',  $^{31}P$  coupled), 97.96 (C-1''), 127.37 (C-5), 143.58 (C-8), 148.86 (C-6), 152.78 (C-4), 159.33 (C-2);  $^{31}P$  NMR (161.94 MHz,  $D_2O$ )  $\delta$  1.156, 0.875, –0.235;  $m/z$  (ES) = 668.1 [( $M - H$ ), 100]; HRMS mass calcd for  $C_{16}H_{26}N_5O_{18}P_3$  [ $M - H$ ] 668.0413, found 668.0412.

**2-Amino-6-chloro-9-[3'-O-( $\alpha$ -D-glucopyranosyl)- $\beta$ -D-ribosyl]-purine 2',3',4''-Trisphosphate [Chlorophostin (8)].** To a solution of protected phosphate **13** (60 mg, 0.04 mmol) in DCM (4 mL)

was added  $\text{BCl}_3$  (400  $\mu\text{L}$ ) at  $-78^\circ\text{C}$ , and the mixture was stirred at this temperature for 2 h. The reaction mixture was then neutralized with aqueous ammonia, and then the solvents were evaporated. The residue thus obtained was purified by ion-exchange chromatography using AG resin column and 150 mM TFA as eluent (0–100% gradient elution) to provide pure chlorophostin **8** (20.6 mg, 74%):  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  1.22 (t, 7.39 Hz,  $\text{CH}_3$  of TEA), 3.14 (q, 7.39 Hz,  $\text{CH}_2$  of TEA), 3.74–3.90 (m, 6H, H-5'', H-6A'', H-6B'', H-5A', H-5B', H-2''), 4.08 (m, 1H, H-4''), 4.42 (m, 1H, H-4'), 4.51 (m, 1H, H-3''), 4.61–4.69 (m, 1H, H-3'), 5.29 (d, 3.45 Hz, H-1''), 5.28–5.32 (m, 1H, H-2'), 6.20 (d, 1H, 5.91 Hz, H-1'), 8.41 (s, 1H, H-8);  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ )  $\delta$  8.11 ( $\text{CH}_3$  of TEA), 46.54 ( $\text{CH}_2$  of TEA), 60.17 (C-6''), 61.07 (C-5'), 70.53 (C-2'',  $^{31}\text{P}$  coupled), 71.58 (C-5'',  $^{31}\text{P}$  coupled), 72.77 (C-4'',  $^{31}\text{P}$  coupled), 73.87 (C-3',  $^{31}\text{P}$  coupled), 74.73 (C-2',  $^{31}\text{P}$  coupled), 77.71 (C-3'',  $^{31}\text{P}$  coupled), 84.15 (C-4'), 87.32 (C-1',  $^{31}\text{P}$  coupled), 98.06 (C-1''), 123.58 (C-5), 143.10 (C-8), 150.52 (C-6), 153.10 (C-4),

159.48 (C-2);  $^{31}\text{P}$  NMR (161.94 MHz,  $\text{D}_2\text{O}$ )  $\delta$  TEA salt  $-2.10$ ,  $-1.60$ ,  $-1.25$ ;  $m/z$  (ES) = 702.1 [(M – H), 100]; HRMS mass calcd for  $\text{C}_{16}\text{H}_{25}\text{ClN}_5\text{O}_{18}\text{P}_3$  [M – H], 702.0023, found 702.0036.

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**Supporting Information Available:**  $^{13}\text{C}$  NMR data for intermediates and target compounds **5–8**; computational modeling of **7** and **8** docked to the Ins(1,4,5) $\text{P}_3\text{R}$  showing various interactions between the ligand and the receptor; electrostatic potential energy surfaces of the purine rings; NMR spectra of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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