

# Activation of IP<sub>3</sub> receptors by synthetic bisphosphate ligands†

Kana M. Sureshan,<sup>a</sup> Andrew M. Riley,<sup>a</sup> Ana M. Rossi,<sup>b</sup> Stephen C. Tovey,<sup>b</sup> Skarlatos G. Dedos,<sup>b</sup> Colin W. Taylor<sup>b</sup> and Barry V. L. Potter<sup>\*a</sup>

Received (in Cambridge, UK) 30th October 2008, Accepted 20th January 2009

First published as an Advance Article on the web 4th February 2009

DOI: 10.1039/b819328b

Ca<sup>2+</sup> release by D-myo-inositol 1,4,5-trisphosphate receptors (IP<sub>3</sub>R) is widely considered to require the vicinal 4,5-bisphosphate motif of IP<sub>3</sub>, with P-5 and P-4 engaging the  $\alpha$  and  $\beta$  domains of the binding site; using synthesis and mutagenesis we show that the adenine of synthetic glyconucleotides, through an interaction with Arg504, can replace the interaction of either P-1 or P-5 with the  $\alpha$ -domain producing, respectively, the most potent bisphosphate agonist yet synthesised and the first agonist of IP<sub>3</sub>R without a vicinal bisphosphate motif; this will stimulate new approaches to IP<sub>3</sub>R ligand design.

D-myo-Inositol 1,4,5-trisphosphate (IP<sub>3</sub>, **1**, Fig. 1) is an intracellular messenger that evokes Ca<sup>2+</sup> release from the intracellular stores of most animal cells by binding to IP<sub>3</sub> receptors (IP<sub>3</sub>R), which are IP<sub>3</sub>-gated Ca<sup>2+</sup> channels.<sup>1,2</sup> Extensive structure–activity studies suggest that the vicinal 4,5-bisphosphate structure of IP<sub>3</sub> is essential, while the 1-phosphate has an ancillary role that substantially increases affinity.<sup>3</sup> For more than 20 years, this enduring conclusion has guided design of new ligands for the IP<sub>3</sub>R. Other inositol phosphate regioisomers are active through different binding modes,<sup>4</sup> but they all have the vicinal bisphosphate structure. The fungal metabolite, adenophostin A (AdA, **2**, Fig. 1),<sup>5</sup> which is at least ten times more potent than IP<sub>3</sub>, provides additional opportunities to explore the mechanisms of IP<sub>3</sub>R activation. AdA is a glyconucleotide trisphosphate structurally related to IP<sub>3</sub>. It binds to a site that substantially overlaps the IP<sub>3</sub>-binding site,<sup>6</sup> and structure–activity studies with synthetic analogues of AdA have established that the adenine (or another aromatic group) is essential for its enhanced affinity.<sup>6,7</sup>

The IP<sub>3</sub>-binding core (IBC) of the IP<sub>3</sub>R forms a clam-like structure with IP<sub>3</sub> held between two domains ( $\alpha$  and  $\beta$ ) linked by a flexible hinge.<sup>8</sup> The 4-phosphate group (P-4) of IP<sub>3</sub> interacts mainly with residues in the  $\beta$ -domain, while the 1-phosphate (P-1) and 5-phosphate (P-5) interact predominantly with the  $\alpha$ -domain (Fig. 2). We therefore speculated that binding of IP<sub>3</sub> might pull the two domains together in a manner reminiscent of glutamate binding to ionotropic glutamate receptors,<sup>9</sup> and so cause the IBC to adopt a more constrained structure.<sup>10</sup> Because both P-1 and P-5 interact with the  $\alpha$ -domain, it may be possible to bridge the two domains using a ligand with only one of these groups. The

fact that the bisphosphate Ins(4,5)P<sub>2</sub> is a full agonist with low affinity<sup>11</sup> supports this suggestion. But the inability<sup>11</sup> of Ins(1,4)P<sub>2</sub> to mobilise Ca<sup>2+</sup> or bind to IP<sub>3</sub>R suggests that interaction of P-1 alone with the  $\alpha$ -domain, where it directly contacts only Arg568 (R568, Fig. 2), is too weak to stabilise binding. Ins(1,5)P<sub>2</sub> is also inactive<sup>12</sup> presumably because it has insufficient interactions with the  $\beta$ -domain. Hence, the prevailing view is that the vicinal bisphosphate moiety of IP<sub>3</sub> is essential for activity.

Our molecular docking experiments<sup>13</sup> have suggested that the 2'-AMP motif of AdA interacts with the  $\alpha$ -domain of the IBC, perhaps more strongly than P-1 of IP<sub>3</sub>. We therefore reasoned that it may be possible for 3''-dephospho-AdA (**3**, Scheme 1) to bridge the domains effectively and release Ca<sup>2+</sup>. Alternatively, if the 2'-AMP interacts with the  $\beta$ -domain, it may allow 4''-dephospho-AdA (**4**) to activate IP<sub>3</sub>R. We therefore developed methods to synthesise **3** and **4** (Scheme 1).<sup>‡</sup> Briefly, thioglycosides **5a** and **5b** were used to glycosylate a ribofuranose acceptor leading to disaccharides **6a** and **6b**, which were individually subjected to Vorbrüggen condensations with silylated 6-chloropurine to give **7a** and **7b**, the precursors for **3** and **4**, respectively. Ammonolysis of each installed the N-6 amino group and exposed the required pairs of hydroxyl groups for phosphorylation. Phosphitylation in the presence of imidazolium triflate and *in situ* oxidation followed by removal of benzyl protecting groups by transfer hydrogenolysis gave **3** and **4**, which were purified to homogeneity.

Using methods reported previously,<sup>14</sup> we show that **4** does not evoke Ca<sup>2+</sup> release *via* recombinant IP<sub>3</sub>R1, but **3** is effective, albeit at high concentrations (Fig. 3). These results support the idea that P-3'' and P-4'' of AdA mimic P-5 and P-4 of IP<sub>3</sub>, respectively, and that the 2'-AMP motif of AdA is able to bind the  $\alpha$ -domain of the IBC more effectively than P-1 of IP<sub>3</sub>. This allows bisphosphate **3** to pull the two domains together and activate IP<sub>3</sub>R1, even though it lacks a vicinal bisphosphate. Bisphosphate **3**, the AdA equivalent of Ins(1,4)P<sub>2</sub>, is the first agonist of IP<sub>3</sub>R without a vicinal bisphosphate motif.

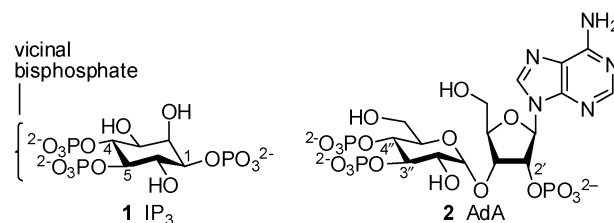
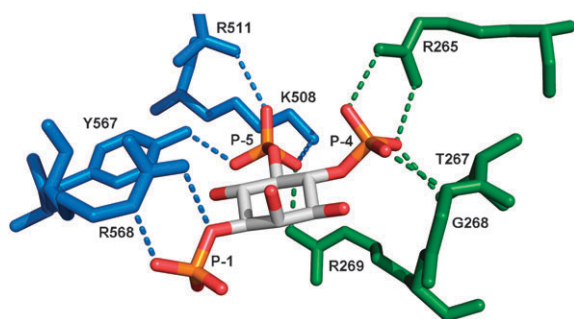


Fig. 1 IP<sub>3</sub> receptor ligands; IP<sub>3</sub> (**1**) and adenophostin A (**2**).

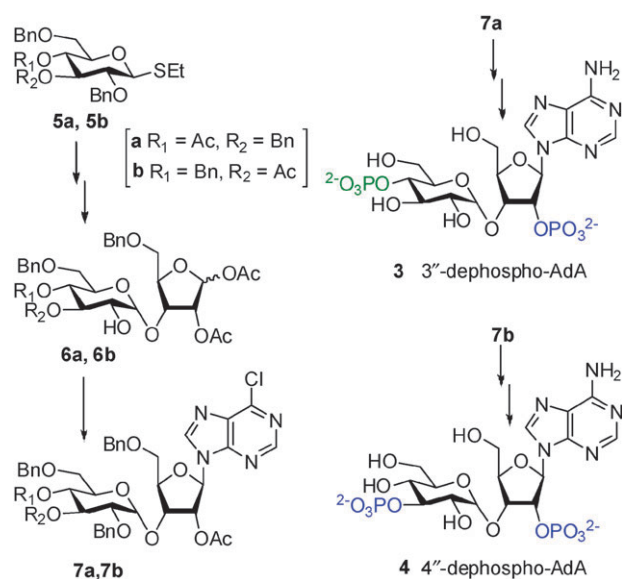
<sup>a</sup> Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, UK Bath BA2 7AY. E-mail: B.V.L.Potter@bath.ac.uk; Fax: +44 (0)1225386114; Tel: +44 (0)1225386639

<sup>b</sup> Department of Pharmacology, University of Cambridge, UK CB2 1PD

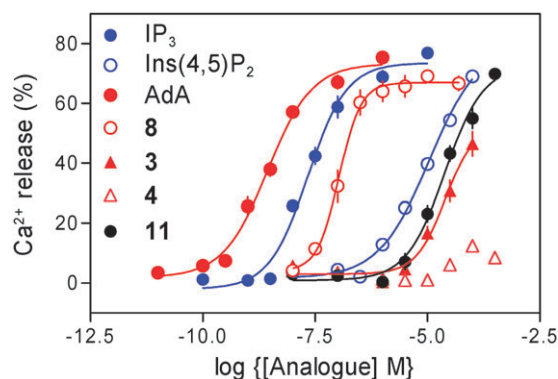
† Dedicated to Dr Melanie N. Trusselle (1973–2008).



**Fig. 2** Interactions of IP<sub>3</sub> with the  $\alpha$  (blue) and  $\beta$  (green) domains of the IBC of IP<sub>3</sub>R1 (PDB code 1n4k). Water molecules are omitted and only direct interactions between IP<sub>3</sub> and residues in the IBC are shown.

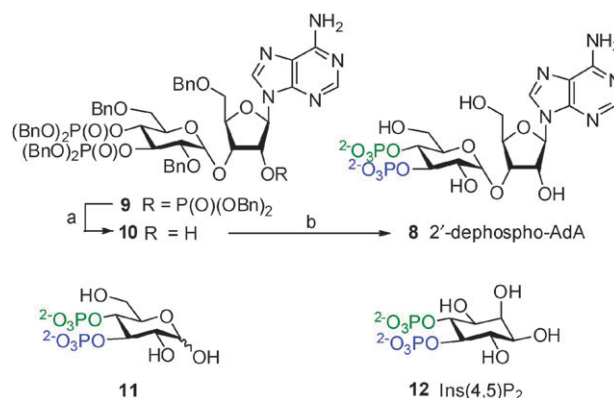


**Scheme 1** Structures and syntheses of 3''- and 4''-dephospho-AdA. Phosphate groups are coloured according to putative interactions with the  $\alpha$  (blue) or  $\beta$  (green) domains of the IBC. Bn = benzyl.



**Fig. 3** Concentration-dependent effects of ligands on Ca<sup>2+</sup> release via IP<sub>3</sub>R1 stably expressed in DT40 cells. Results show means  $\pm$  SEM;  $n = 8$ .

The 2'-AMP motif of AdA may allow P-2' to bind more effectively than P-1 of IP<sub>3</sub>, or the adenosine itself may have



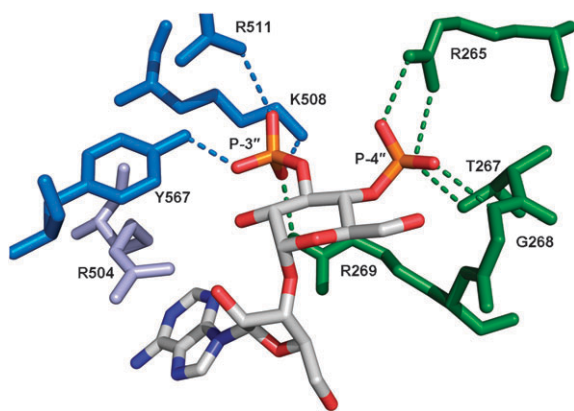
**Scheme 2** Synthesis of 2'-dephospho-AdA and structures of glucose 3,4-bisphosphate and Ins(4,5)P<sub>2</sub>: (a) K<sub>2</sub>CO<sub>3</sub>, BnOH, 70 °C, 93%; (b) Pd(OH)<sub>2</sub>/C, cyclohexene, MeOH, H<sub>2</sub>O, 80 °C, 92%. Bn = benzyl. Phosphate groups are coloured according to putative interactions with the  $\alpha$  (blue) or  $\beta$  (green) domains of the IBC.

interactions with the IBC. To distinguish between these two possible roles, we synthesised 2'-dephospho-AdA, the adenosine equivalent of Ins(4,5)P<sub>2</sub> (**8**, Scheme 2). Interestingly, treatment of a benzyl-protected version of AdA (**9**)<sup>15</sup> with sodium benzoxide in BnOH led to removal of the 2'-dibenzylphosphate group giving bisphosphate **10** as the major product with minor amounts of other by-products. Use of the milder base potassium carbonate resulted in clean and highly selective conversion into **10**. Deprotection and purification as before gave **8** in excellent yield. The 2'-phosphate triester in **9** is comparatively less crowded and hence more prone to nucleophilic attack than those in the 3'',4'' vicinal positions and this is likely to be the reason for this interesting high selectivity. We also synthesised the related glucose 3,4-bisphosphate (**11**, Scheme 2). Because **11** can be viewed as AdA without the 2'-AMP motif, any difference in the activities of **8** and **11** illustrates the role of the adenosine moiety. In functional assays, **8** was 100 times more potent than **11** (Fig. 3), confirming that the adenosine motif directly contributes to AdA binding even when it lacks the 2'-P group. Compound **8** is the first potent synthetic bisphosphate agonist of the IP<sub>3</sub>R; it is >30-fold more potent than Ins(4,5)P<sub>2</sub> (Fig. 3, Table 1).

Our molecular modelling suggests a key role for arginine 504 (R504) in recognising the adenine of AdA, perhaps *via* a cation- $\pi$  interaction.<sup>13</sup> Using targeted mutagenesis, we established a cell line expressing only IP<sub>3</sub>R1 with R504 mutated to glutamine, to determine whether R504 selectively contributes to the activity of AdA analogues. For the mutant IP<sub>3</sub>R, the EC<sub>50</sub> for IP<sub>3</sub> and Ins(4,5)P<sub>2</sub> was reduced by 57- and 8.8-fold, respectively. For AdA and **8**, the reductions were 434- and

**Table 1** Ca<sup>2+</sup> release *via* IP<sub>3</sub>R1. Results (means  $\pm$  SEM,  $n = 3-8$ ) show the EC<sub>50</sub> values for each agonist in DT40 cells stably expressing IP<sub>3</sub>R1

Agonist	EC <sub>50</sub>
IP <sub>3</sub>	35 $\pm$ 5 nM
Ins(4,5)P <sub>2</sub>	7.59 $\pm$ 2.63 $\mu$ M
AdA	2.9 $\pm$ 0.9 nM
<b>8</b>	240 $\pm$ 5 nM



**Fig. 4** Suggested interactions of **8** with the IBC of IP<sub>3</sub>R1, based on molecular docking experiments using AdA.<sup>13</sup> The side chain of R504 is shown in pale blue.

107-fold, and the response to **3** ( $\leq 3$  mM) was abolished. The greater effect of this mutation on the potency of AdA and **8** is consistent with the proposed interaction between adenine and R504 (Fig. 4). Indeed, the potency of AdA and **8** at IP<sub>3</sub>R1 both depend largely on R504 because, at the mutated receptor, AdA is only equipotent with IP<sub>3</sub>, and **8** is less than 3-fold more potent than Ins(4,5)P<sub>2</sub>.

We conclude that, while the three phosphate groups and adenine of AdA most likely make incremental contributions to IP<sub>3</sub>R binding, a vicinal bisphosphate moiety is not essential for IP<sub>3</sub>R activation. P-4 of IP<sub>3</sub>, which contacts the  $\beta$ -domain of the IBC, is required, but P-5, which contacts the  $\alpha$ -domain, can (as in **3**) make an interaction that can be substantially compensated by a cation– $\pi$  interaction between the adenine of AdA and R504 in the  $\alpha$ -domain. The same interaction can also serve to mitigate the loss of P-1 to provide a potent agonist (**8**) with only two phosphate groups. Aside from challenging the long-standing dogma that a vicinal bisphosphate is essential for agonists of IP<sub>3</sub>R, these results illustrate the potential to design less polar IP<sub>3</sub>R ligands. At their simplest, these might comprise two motifs that interact with the IBC domains linked by a suitable spacer.

Since inositol polyphosphates often bind to sites rich in Arg and Lys residues, replacing interactions with a polar phosphate by cation– $\pi$  interactions should have more general applications in the chemical biology of inositol phosphate signalling and probably elsewhere.

We acknowledge the support by Programme Grants from the Wellcome Trust [082837 to AMR (Bath) and BVLP, 072084 to CWT].

## Notes and references

‡ All new compounds were thoroughly characterised and exhibited satisfactory parameters using standard spectroscopic techniques. A typical example follows.

### Synthesis of 3'-O-( $\alpha$ -D-glucopyranosyl)-adenosine-3'',4''-bisphosphate (**8**).

A suspension of bisphosphate **10** (45 mg, 0.037 mmol) and 20% Pd(OH)<sub>2</sub> on carbon (120 mg) in a mixture of cyclohexene (1.6 mL), MeOH (3 mL) and H<sub>2</sub>O (0.22 mL) was heated at 80 °C overnight. The mixture was filtered through a membrane filter and solvents evaporated *in vacuo*. Purification on an AG ion-exchange column (0–100% gradient elution, with 150 mM TFA and water) afforded pure bisphosphate **8** (20 mg, 92%). <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): 3.74–3.90 (m, 6H, H-2'', H-5'', H-5A', H-5B' H-6A'', H-6B''), 4.10 (ddd, like a q, 1H, 9.66 Hz, 9.66 Hz, 9.66 Hz, H-4''), 4.42 (dd, 1H, 7.24 Hz, 3.86 Hz, H-4'), 4.47–4.55 (m, 2H, H-3', H-3''), 4.87 (dd, like a t, 1H, 5.80 Hz, 5.31 Hz, H-2'), 5.19 (d, 1H, 3.38 Hz, H-1''), 6.19 (d, 1H, 5.80 Hz, H-1'), 8.40 (s, 1H, H-2), 8.50 (s, 1H, H-8). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): 60.08 (C-6''), 60.79 (C-5'), 70.48 (C-2'', <sup>31</sup>P coupled), 71.60 (d, 3.83 Hz, C-5'', <sup>31</sup>P coupled), 72.83 (C-4'', <sup>31</sup>P coupled), 73.48 (C-2'), 76.37 (C-3'), 77.71 (C-3'', <sup>31</sup>P coupled), 83.95 (C-4'), 88.23 (C-1'), 99.02 (C-1''), 118.91 (C-5), 142.81 (C-8), 144.43 (C-2), 148.16 (C-4), 149.91 (C-6). <sup>31</sup>P NMR (161.94 MHz, D<sub>2</sub>O with excess of TEA): 4.50, 3.62. *m/z* (ES+) = 590.2 [(M + H)<sup>+</sup>, 100%]; 612.1 [(M + Na)<sup>+</sup>, 100%]; *m/z* (ES–) 588.2 [(M – H)<sup>+</sup>, 100%]; HRMS: mass calcd for C<sub>16</sub>H<sub>26</sub>O<sub>15</sub>N<sub>5</sub>P<sub>2</sub> [(M + H)<sup>+</sup>, 590.0895; found, 590.0895.

- M. J. Berridge, *Nature*, 1993, **361**, 315.
- J. K. Foskett, C. White, K. H. Cheung and D.-O. D. Mak, *Physiol. Rev.*, 2007, **87**, 593.
- B. V. L. Potter and D. Lampe, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1933.
- A. M. Riley, R. Payne and B. V. L. Potter, *J. Med. Chem.*, 1994, **37**, 3918.
- M. Takahashi, K. Tanzawa and S. Takahashi, *J. Biol. Chem.*, 1994, **269**, 369.
- V. A. Correa, A. M. Riley, S. Shuto, G. Horne, E. P. Nerou, R. D. Marwood, B. V. L. Potter and C. W. Taylor, *Mol. Pharmacol.*, 2001, **59**, 1206.
- H. Hotoda, K. Murayama, S. Miyamoto, Y. Iwata, M. Takahashi, Y. Kawase, K. Tanzawa and M. Kaneko, *Biochemistry*, 1999, **38**, 9234.
- I. Bosanac, J.-R. Alattia, T. K. Mal, J. Chan, S. Talarico, F. K. Tong, K. I. Tong, F. Yoshikawa, T. Furuichi, M. Iwai, T. Michikawa, K. Mikoshiba and M. Ikura, *Nature*, 2002, **420**, 696.
- C. W. Taylor, P. C. A. da Fonseca and E. P. Morris, *Trends Biochem. Sci.*, 2004, **29**, 210.
- J. Chan, A. E. Whitten, C. M. Jeffries, I. Bosanac, T. K. Mal, J. Ito, H. Porumb, T. Michikawa, K. Mikoshiba, J. Trehwella and M. Ikura, *J. Mol. Biol.*, 2007, **373**, 1269.
- P. J. Lu, D. M. Gou, W. R. Shieh and C. S. Chen, *Biochemistry*, 1994, **33**, 11586.
- D. Bello, T. Aslam, G. Bultynck, A. M. Z. Slawin, H. L. Roderick, M. D. Bootman and S. J. Conway, *J. Org. Chem.*, 2007, **72**, 5647.
- H. J. Rosenberg, A. M. Riley, A. J. Laude, C. W. Taylor and B. V. L. Potter, *J. Med. Chem.*, 2003, **46**, 4860.
- A. J. Laude, S. C. Tovey, S. G. Dedos, B. V. L. Potter, S. C. R. Lummis and C. W. Taylor, *Cell Calcium*, 2005, **38**, 45.
- C. N. Borissow, S. J. Black, M. Paul, S. C. Tovey, S. G. Dedos, C. W. Taylor and B. V. L. Potter, *Org. Biomol. Chem.*, 2005, **3**, 245.