

# Both D- and L-glucose polyphosphates mimic D-*myo*-inositol 1,4,5-trisphosphate: new synthetic agonists and partial agonists at the Ins(1,4,5)P<sub>3</sub> receptor

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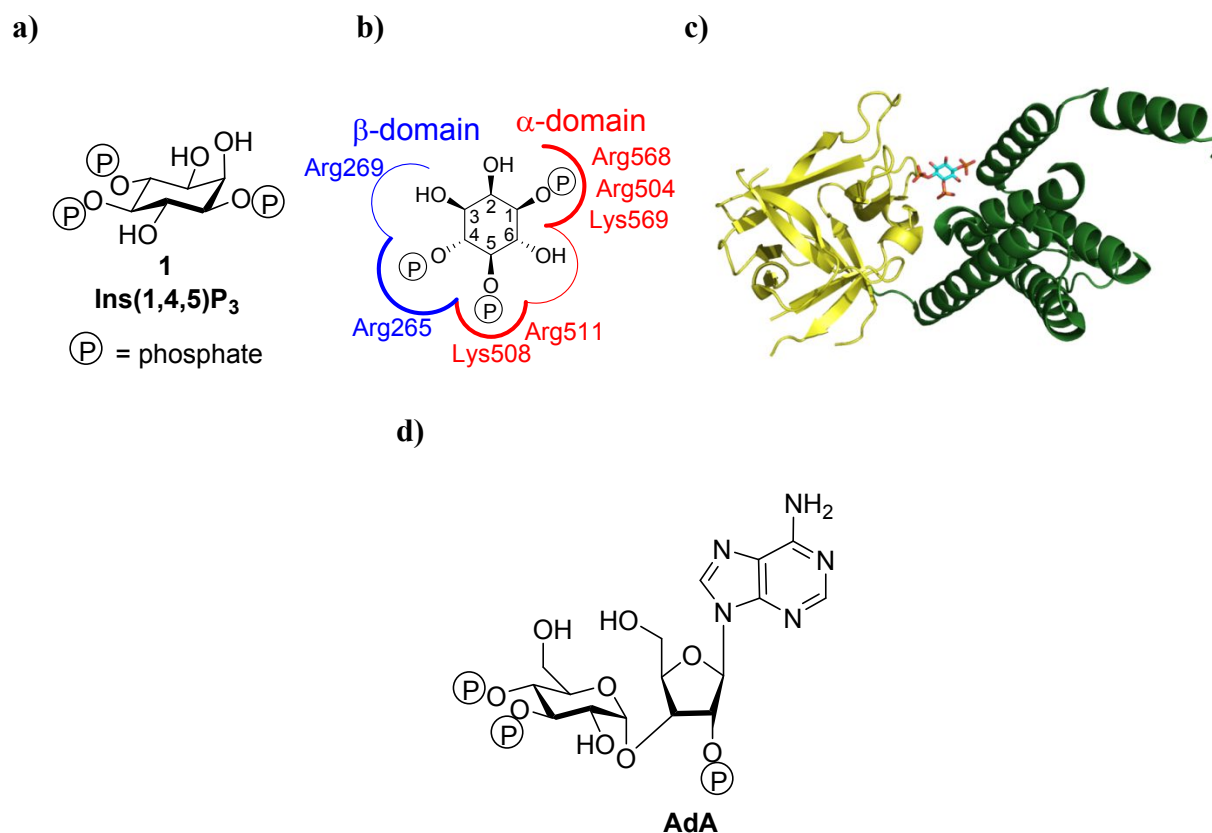
**ABSTRACT:** Chiral sugar derivatives are potential cyclitol surrogates in the Ca<sup>2+</sup>-mobilizing intracellular messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P<sub>3</sub>]. Six novel polyphosphorylated analogues derived from both D- and L-glucose were synthesized. Binding to Ins(1,4,5)P<sub>3</sub> receptors [Ins(1,4,5)P<sub>3</sub>R] and abilities to release Ca<sup>2+</sup> from intracellular stores *via* type 1 Ins(1,4,5)P<sub>3</sub>Rs were investigated. β-D-Glucopyranosyl 1,3,4-trisphosphate, with similar phosphate regiochemistry and stereochemistry to Ins(1,4,5)P<sub>3</sub> and α-D-glucopyranosyl 1,3,4-trisphosphate are full agonists, being equipotent and 23-fold less potent than Ins(1,4,5)P<sub>3</sub> respectively in Ca<sup>2+</sup>-release assays, and similar to Ins(1,4,5)P<sub>3</sub> and 15-fold weaker in binding assays. They can be viewed as truncated analogues of adenophostin A and refine structure-activity relationship understanding for this Ins(1,4,5)P<sub>3</sub>R agonist. L-Glucose-derived ligands, methyl α-L-glucopyranoside 2,3,6-trisphosphate and methyl α-L-glucopyranoside 2,4,6-trisphosphate are also active, while their corresponding D-enantiomers, methyl α-D-glucopyranoside 2,3,6-trisphosphate and methyl α-D-glucopyranoside 2,4,6-trisphosphate, are inactive. Interestingly, both L-glucose-derived ligands are partial agonists: they are amongst the least efficacious agonists of Ins(1,4,5)P<sub>3</sub>R yet identified, providing new leads for antagonist development.

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3 INTRODUCTION  
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6 *D-myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P<sub>3</sub>, **1**] is a second messenger that binds to tetrameric *D-myo*-inositol  
7 1,4,5-trisphosphate receptors [Ins(1,4,5)P<sub>3</sub>Rs] on the endoplasmic reticulum. Ins(1,4,5)P<sub>3</sub>Rs are Ca<sup>2+</sup> channels  
8 that open to release Ca<sup>2+</sup> to the cytosol.<sup>1,2</sup> The resulting local or global increases in cytosolic Ca<sup>2+</sup> concentration  
9 regulate diverse cellular processes, including mitochondrial metabolism, cell proliferation, differentiation,  
10 smooth muscle contraction, secretion, exocytosis and ion channel opening.<sup>3</sup>  
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17 Ins(1,4,5)P<sub>3</sub> (Figure 1a) binds to the Ins(1,4,5)P<sub>3</sub>-binding core (IBC; residues 224-604) close to the *N*-terminus  
18 of each of the four Ins(1,4,5)P<sub>3</sub>R subunits (Figure 1b, c). The IBC consists of an  $\alpha$ -helical domain and a  $\beta$ -trefoil  
19 domain, between which there is a cleft rich in basic amino acid residues. Ins(1,4,5)P<sub>3</sub> binding within the cleft  
20 allows phosphates at positions 1 and 5 to interact with the  $\alpha$ -domain, while the 4-phosphate interacts with the  $\beta$ -  
21 domain (Figure 1c).<sup>4</sup> As Ins(1,4,5)P<sub>3</sub> interacts with both domains, it pulls the two sides of the clam-like IBC  
22 together.<sup>5,6</sup> The clam closure leads to channel opening, possibly by re-arranging Ca<sup>2+</sup>-binding sites such that  
23 Ca<sup>2+</sup> can bind to the Ins(1,4,5)P<sub>3</sub>R and trigger conformational changes that lead to opening of the Ca<sup>2+</sup>-permeable  
24 pore.<sup>1,5,6</sup>  
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**Figure 1.** Structures of Ins(1,4,5)P<sub>3</sub>R and its agonists (a) Structure of Ins(1,4,5)P<sub>3</sub> (**1**). (b) Binding mode of **1** to the IBC of Ins(1,4,5)P<sub>3</sub>R with key amino acid residues involved in binding labelled. (c) Crystal structure of the IBC of type 1 InsP<sub>3</sub>R with Ins(1,4,5)P<sub>3</sub> (**1**) bound (PDB: 1N4K). The α-domain is shown in green and the β-domain in yellow. (d) Structure of the Ins(1,4,5)P<sub>3</sub>R agonist adenophostin A (AdA). panel B indicates schematically how **1** binds to the IBC of Ins(1,4,5)P<sub>3</sub>R with the key amino acid residues involved in binding labeled, panel C shows the structure of the Ins(1,4,5)P<sub>3</sub>R agonist adenophostin A (AdA) and panel D shows a crystal structure of the IBC of type 1 InsP<sub>3</sub>R with Ins(1,4,5)P<sub>3</sub> (**1**) bound (1N4K). The α-domain is shown in green and the β-domain in yellow.

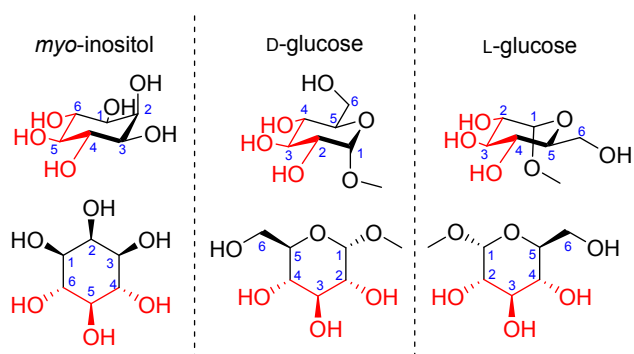
Modulators of Ins(1,4,5)P<sub>3</sub>R activity are highly sought after, and many studies have examined structure-activity-relationship (SARs) of ligands binding to Ins(1,4,5)P<sub>3</sub>R attempting to identify partial agonists or antagonists.<sup>7-9</sup> Synthetic analogues have played a key role in this process, including the potent glyconucleotide adenophostins<sup>4,10</sup> (Figure 1d), with a recent synthetic study reporting the effects of replacing the glucose moiety of adenophostin A (AdA) with a *chiro*-inositol core.<sup>11</sup> This highlights developing interest in examining less explored isomers of inositol than the *myo*-form.<sup>12</sup>

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3 The phosphates attached to the 4- and 5-positions of Ins(1,4,5)P<sub>3</sub> (Figure 1a) are thought to be essential to agonist  
4 activity as each interacts with a different domain of the IBC.<sup>13,14</sup> The 1-phosphate increases affinity, but it is not  
5 essential for receptor activation.<sup>4</sup> The hydroxyl group attached to the 6-position of Ins(1,4,5)P<sub>3</sub> appears to be  
6 important for optimal activity but it is not essential,<sup>15</sup> while hydroxyl groups attached to the 2- and 3-positions  
7 are less involved in ligand binding.<sup>16</sup>  
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14 A few Ins(1,4,5)P<sub>3</sub>R antagonists have been identified, but these suffer major drawbacks including poor target  
15 selectivity, cell impermeability (heparin),<sup>17</sup> inconsistent effectiveness in assays (2-aminoethoxydiphenylborane,  
16 2-APB),<sup>17</sup> low potency (caffeine),<sup>17,18</sup> and disputed activity (xestospongins).<sup>17</sup> For decades, analogues of  
17 Ins(1,4,5)P<sub>3</sub> have been synthesized and investigated in attempts to discover a selective antagonist for  
18 Ins(1,4,5)P<sub>3</sub>R that could be rendered, at least temporarily, cell-permeable with enzymatic- or photo-labile  
19 protecting groups.<sup>19,20</sup> To date, however, only a small number of analogues of Ins(1,4,5)P<sub>3</sub> with minor structural  
20 modifications have been identified as partial agonists or antagonists at Ins(1,4,5)P<sub>3</sub>R. Most of these compounds  
21 demonstrate that conservative modifications to the phosphates attached to positions 4 and 5 and the hydroxyl  
22 group attached to position 3 can lead to degrees of antagonist activity.<sup>21-24</sup> However, many analogues of  
23 Ins(1,4,5)P<sub>3</sub> with modifications to the same regions are inactive.<sup>25</sup> Attaching a bulky substituent to the axial 2-  
24 position hydroxyl can also lead to partial agonist activity<sup>26</sup> and interestingly even using a simple benzene ring  
25 as a surrogate for inositol in a benzene polyphosphate approach, as a dimer or biphenyl, can provide low-affinity  
26 antagonists.<sup>27,28</sup>  
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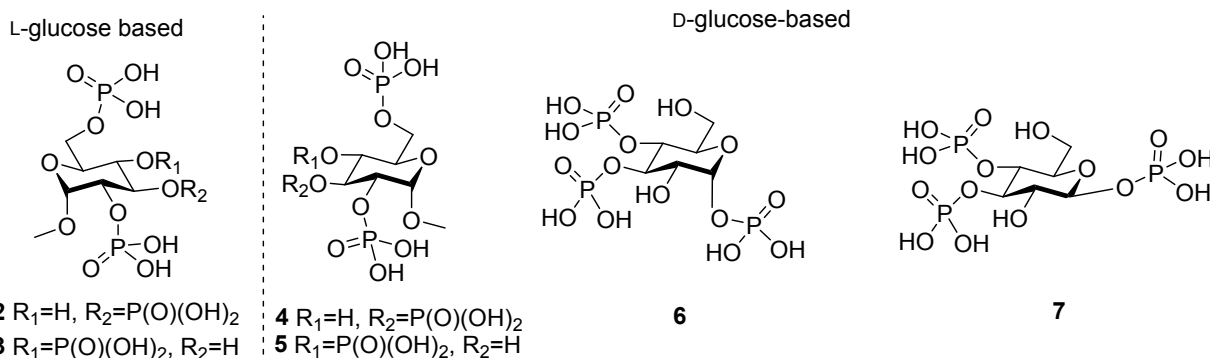
41 Stimulated in part by the discovery of the adenophostins,<sup>4,9,29</sup> there have been a number of studies to investigate  
42 polyphosphates of D-glucose and of other sugars,<sup>29-33</sup> as inositol phosphate analogues (see below).<sup>34</sup> By using  
43 such carbohydrates, the need for optical resolution of protected cyclitol precursors or resulting phosphate  
44 regioisomers is bypassed, as chiral starting materials are readily available. Also, the structural features of  
45 carbohydrates offer additional opportunities for synthetic versatility. In this study, we return to and expand upon,  
46 the use of D-glucose to try to identify novel Ins(1,4,5)P<sub>3</sub>R ligands. We also investigate, perhaps counter-  
47 intuitively, the use of L-glucose as a starting material.  
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3 Although several studies have generated D-glucose-based ligands of Ins(1,4,5)P<sub>3</sub>R,<sup>29,30,33</sup> no ligands based on  
4 the L-glucose enantiomer have been synthesized and nor is it known whether ligands with this scaffold would  
5 bind to Ins(1,4,5)P<sub>3</sub>R. In a previous study,<sup>4</sup> Ins(4,5)P<sub>2</sub> [albeit a low-affinity Ins(1,4,5)P<sub>3</sub>R ligand] was effectively  
6 mimicked by a D-Gluc(3,4)P<sub>2</sub> surrogate. We noted that both D- and L-glucose offer three hydroxyl groups of the  
7 requisite relative configuration that could in principle be used to mimic the 4,5,6-hydroxyl groups in *myo*-  
8 inositol. Thus, we anticipated that we could use this similarity (Figure 2, highlighted in red), alongside intrinsic  
9 structural differences of L- and D-glucose to prepare diverse chiral ligands with appropriately located phosphates  
10 These would present different structural motifs to the Ins(1,4,5)P<sub>3</sub>R and allow further investigation of the binding  
11 site and perhaps identify novel activity. With this in mind we designed and synthesized six novel ligands based  
12 on L-glucose and D-glucose (Figure 3) and evaluated their activity at Ins(1,4,5)P<sub>3</sub>R.



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**Figure 2:** Structures of methyl  $\alpha$ -D-glucopyranoside and methyl  $\alpha$ -L-glucopyranoside relative to *myo*-inositol with the shared stereochemistry of the hydroxyl groups highlighted in red.



14 **Figure 3.** Structures of  $Ins(1,4,5)P_3$  (**1**) analogues: methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) and methyl  $\alpha$ -L-glucopyranoside  
15 2,4,6-trisphosphate (**3**), methyl  $\alpha$ -D-glucopyranoside 2,3,6-trisphosphate (**4**) and methyl  $\alpha$ -D-glucopyranoside 2,4,6-trisphosphate (**5**),  $\alpha$ -D-  
16 glucopyranosyl 1,3,4-trisphosphate (**6**),  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**).  
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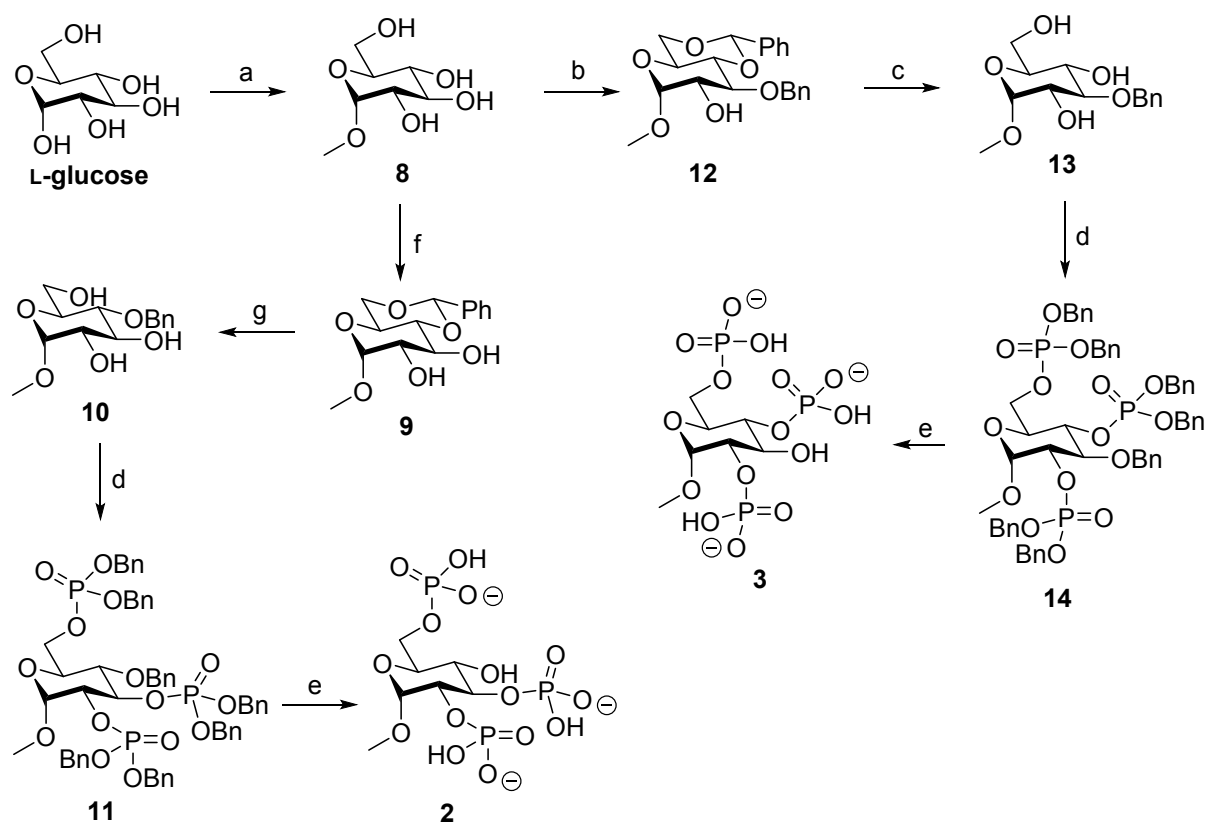
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23 We ensured that the designed ligands retained structures equivalent to the critical 4,5-bisphosphate motif of  $Ins(1,4,5)P_3$   
24 and had no major structural modifications in regions believed to be necessary for ligand binding; modifications in regions  
25 equivalent to the 2-O position were permitted, as these were expected to remain outside the binding pocket. We  
26 hypothesized that the L-glucose-based ligands [methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) and methyl  $\alpha$ -L-  
27 glucopyranoside 2,4,6-trisphosphate (**3**)] might have sufficiently conservative structural changes relative to  $Ins(1,4,5)P_3$   
28 that they could still bind to  $Ins(1,4,5)P_3R$ , possibly with novel activity. The D-glucose-based ligands [methyl  $\alpha$ -D-  
29 glucopyranoside 2,3,6-trisphosphate (**4**) and methyl  $\alpha$ -D-glucopyranoside 2,4,6-trisphosphate (**5**)] were not expected to  
30 adopt orientations that position the phosphate groups in appropriate regions of the IBC (see Supporting Information for  
31 details of all possible predicted binding modes). Their bioassay was designed to enable confirmation of  
32 enantioselectivity. From a practical standpoint, the synthesis of ligands **4** and **5** was optimized first with D-glucose before  
33 the commercially available, but considerably more costly, L-glucose was used to make the respective L-enantiomers, **2**  
34 and **3**. We anticipated that the two ligands with phosphates at the anomeric carbon,  $\alpha$ -D-glucopyranosyl 1,3,4-  
35 trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**), would help to elucidate the basis for the high affinity  
36 of AdA.<sup>4,11,35</sup> We expected both novel truncated analogues to be agonists due to their structural similarity to  $Ins(1,4,5)P_3$ ,  
37 but were interested to compare their activities with AdA and the previously analysed truncated analogue,  $Glu(3,4)P_2$ .<sup>4</sup>  
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## RESULTS

## Chemistry

Methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) was synthesized in a five-step route from readily available L-glucose (Scheme 1). Refluxing L-glucose in an acidic methanol solution resulted in protection of the anomeric hydroxyl with a methyl group. Multiple recrystallizations from ethanol afforded methyl  $\alpha$ -L-glucopyranoside **8** in 45% yield. The 4- and 6-position hydroxyls were protected with a benzylidene group to form **9** in 91% yield. This benzylidene group was reduced regioselectively, opening to form methyl 4-*O*-benzyl- $\alpha$ -D-glucopyranoside (**10**). The benzylidene reduction was first attempted with borane-THF and  $\text{AlCl}_3$ , but this was found to be insufficiently regioselective and produced inseparable regioisomers. The reaction was successfully carried out with borane-THF and  $\text{La}(\text{Tf})_3$  following the method of Shie et al.<sup>36</sup> to yield **10** in 31% yield following purification. The hydroxyl groups in triol **10** were then phosphitylated with dibenzyl diisopropylphosphoramidite and subsequent oxidation with mCPBA formed **11** in 86% yield. The benzyl protecting groups on phosphates and *O*-4 were then removed by stirring a solution of **11** with Pearlman's catalyst under hydrogen overnight. After filtration to remove catalyst and evaporation of the solvent, **2** was collected as the triethylammonium salt in 53% yield.

**Scheme 1.** Synthesis of L-glucose-derived ligands: methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) and methyl  $\alpha$ -L-glucopyranoside 2,4,6-trisphosphate (**3**)<sup>a</sup>

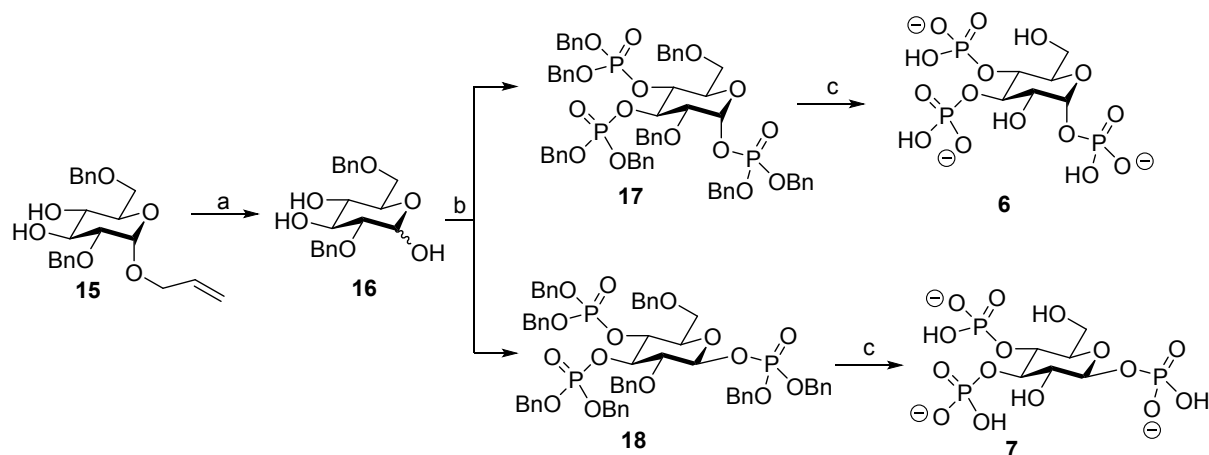


<sup>a</sup>Reagents and conditions: (a) MeOH, reflux, 5d (b) (1) TMSCl, py, 22h (2) DCM, benzaldehyde, FeCl<sub>2</sub>·6H<sub>2</sub>O, MeCN, triethylsilane, 0°C - room temperature, 1.5h (c) MeOH, H<sub>2</sub>O, 1M HCl<sub>(aq)</sub>, reflux, 3h (d) (1) DCM, 5-phenyl-1*H*-tetrazole, (BnO)<sub>2</sub>PN(*i*Pr)<sub>2</sub>, 20h (2) mCPBA, -78°C to room temperature (e) MeOH:H<sub>2</sub>O (10:1 v/v), cat. Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 24h (f) MeCN, benzaldehyde dimethyl acetal, cat. CSA, 24h (g) BH<sub>3</sub>-THF, La(Tf)<sub>3</sub>, 7d.

Methyl  $\alpha$ -L-glucopyranoside 2,4,6-trisphosphate (**3**) was also synthesized in five steps from L-glucose, diverging from the synthesis of **2** after the first methylation step. Methyl  $\alpha$ -L-glucopyranoside (**8**) was protected in a regioselective one-pot reaction that involved persilylation followed by FeCl<sub>2</sub>-catalyzed benzylidene protection as described by Bourdreux et al.<sup>37</sup> to yield **12** in 79% yield. The acid-labile benzylidene group was removed through reflux with HCl<sub>(aq)</sub> to form **13** in 93% yield. Phosphorylation using standard phosphoramidite methodology<sup>38</sup> was then employed to give **14** in 40% yield. After debenzylation with hydrogen and Pearlman's catalyst and filtration and evaporation of the solvent, the final product, **3**, was collected as its triethylammonium salt in 90% yield.



**Scheme 2.** Synthesis of ligands with phosphate groups at the anomeric carbon:  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**)<sup>a</sup>

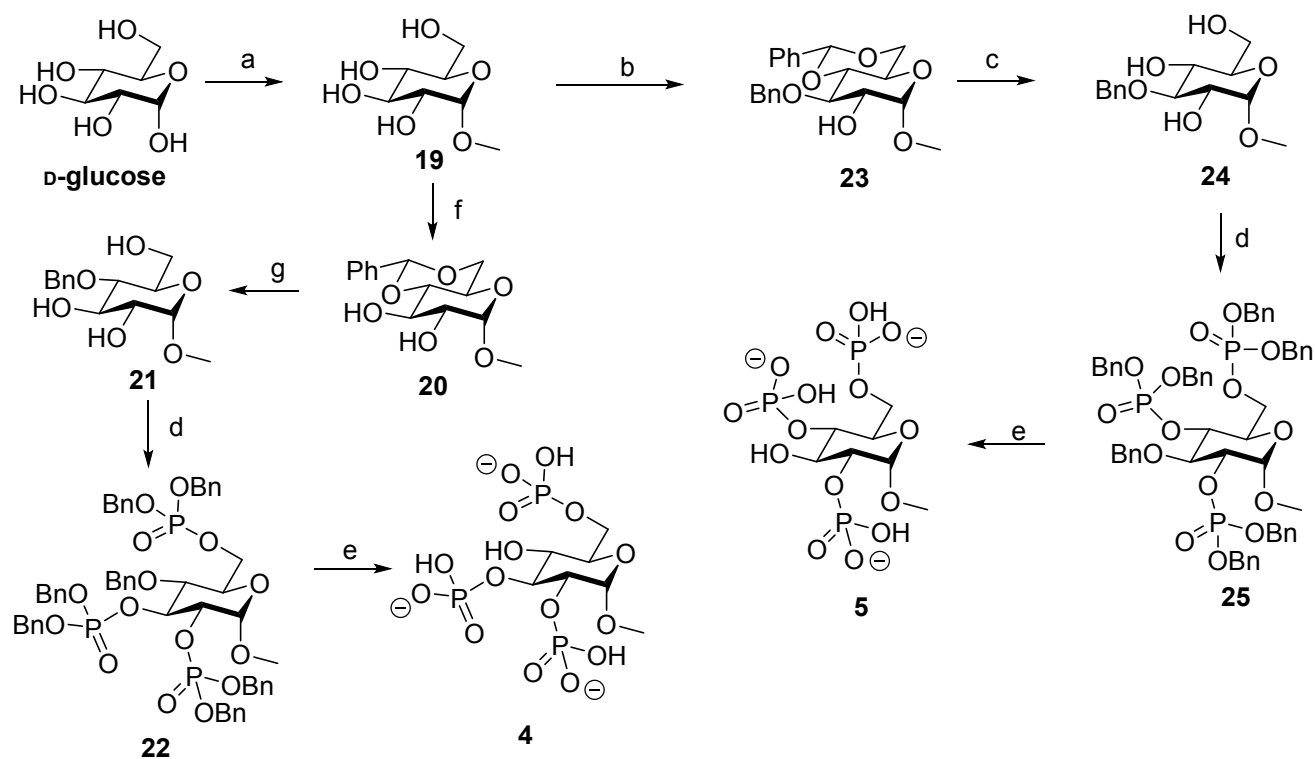


<sup>a</sup>Reagents and conditions: (a) MeOH, cat. PdCl<sub>2</sub>, 6h (b) (1) DCM, 5-phenyl-1*H*-tetrazole, (BnO)<sub>2</sub>PN(*i*Pr)<sub>2</sub>, 20h (2) mCPBA, -78°C to room temperature (c) MeOH:H<sub>2</sub>O (10:1 v/v), cat. Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, NaHCO<sub>3</sub>, 24h.

Both  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**) were synthesized via a divergent route, starting with allyl 2,6-di-O-Bn- $\alpha$ -D-glucopyranoside (**15**). Palladium chloride-catalyzed deallylation yielded **16**, although purification of this compound was found to be very difficult at this step and purification after phosphorylation proved to be much more effective. Thus, slightly impure **16**, was phosphorylated to yield a pure, partially separable mixture of the epimeric phosphates **17** and **18** (56% yield total: 14% **17**, 19% **18**, 23% mixed epimers). It was unclear how stable **17** and especially the phosphorylated  $\beta$ -epimer **18** would be, as there are reports of compounds with phosphate groups at the anomeric carbon atom being unable to survive purification by silica column chromatography in some cases and not in others.<sup>39-41</sup>

We found in this case that both compounds survived silica gel chromatography, although **18** showed slight degradation by <sup>31</sup>P NMR over time (approx. 30% after three weeks at 4°C). Catalytic hydrogenolysis of both compounds **18** and **19** was carried out in the presence of sodium bicarbonate to prevent acidic hydrolysis of the potentially labile C-1 phosphates. The final products **6** and **7** were purified by reverse phase ion pair chromatography and collected as their triethylammonium salts.

**Scheme 3:** Synthesis of D-glucose-derived ligands: methyl  $\alpha$ -D-glucopyranoside 2,3,6-trisphosphate (**5**) and methyl  $\alpha$ -D-glucopyranoside 2,4,6-trisphosphate (**4**)<sup>a</sup>



<sup>a</sup>Reagents and conditions: (a) MeOH, reflux, 5d (b) (1) TMSCl, py, 22h (2) DCM, benzaldehyde, Fe(II)Cl<sub>2</sub>·6H<sub>2</sub>O, MeCN, triethylsilane, 0°C - Room temperature, 1.5h (c) MeOH, H<sub>2</sub>O, 1M HCl (aq), reflux, 3h (d) (1) DCM, 5-phenyl-1*H*-tetrazole, (BnO)<sub>2</sub>PN(*i*Pr)<sub>2</sub>, 20h (2) mCPBA, -78°C -Room temperature (e) MeOH:H<sub>2</sub>O (10:1 v/v), cat. Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, 24h (f) MeCN, benzaldehyde dimethyl acetal, cat. CSA, 24h (g) BH<sub>3</sub>-THF, La(Tf)<sub>3</sub>, 7d.

Methyl  $\alpha$ -D-glucopyranoside 2,3,6-trisphosphate (**4**) and methyl  $\alpha$ -D-glucopyranoside 2,4,6-trisphosphate (**5**) were synthesized using the same methods as described for their enantiomers (**1** and **2**) starting the route with D-glucose (Scheme 3).

The relative stabilities of  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**) were first investigated by allowing each compound (as triethylammonium salts) to remain in a solution of D<sub>2</sub>O at room temperature at pH 7. Over the course of two months, neither isomer showed any sign of degradation. Following this, a mixture of the compounds in a known starting ratio was exposed to increasingly harsh conditions and relative isomer degradation was monitored through <sup>1</sup>H NMR spectroscopy (see Supporting Information). From the results of the hydrolysis study, we determined that the  $\beta$ -epimer (**7**) degraded more readily than the corresponding  $\alpha$ -epimer (**6**). Both

1 compounds were surprisingly durable though and required strongly acidic conditions to be fully hydrolyzed while  
2 strongly basic conditions only produced limited degradation. We are therefore confident that both compounds remained  
3 intact during the near neutral conditions of the biological assays. All final compounds were assessed by HPLC (see  
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## 11 Biology

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15 Permeabilized HEK-Ins(1,4,5)P<sub>3</sub>R1 cells were used to determine the ability of compounds **2-7** (with Ins(1,4,5)P<sub>3</sub> and  
16 AdA as controls) to evoke Ca<sup>2+</sup> release from intracellular stores (Figure 4 and Table). Maximally effective concentrations  
17 of Ins(1,4,5)P<sub>3</sub>, α-D-glucopyranosyl 1,3,4-trisphosphate (**6**) or β-D-glucopyranosyl 1,3,4-trisphosphate (**7**) released the  
18 same fraction (ca. 80%) of the intracellular Ca<sup>2+</sup> stores, suggesting that these two epimeric compounds are both full  
19 agonists (Figure 4). Compound **7** was equipotent with Ins(1,4,5)P<sub>3</sub> and **6** was ca. 20-times less potent than Ins(1,4,5)P<sub>3</sub>.  
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27 The L-glucose-based ligands methyl α-L-glucopyranoside 2,3,6-trisphosphate (**2**) and methyl α-L-glucopyranoside 2,4,6-  
28 trisphosphate (**3**) were much less potent than Ins(1,4,5)P<sub>3</sub> (Figure 4), while their enantiomers methyl α-D-  
29 glucopyranoside 2,3,6-trisphosphate (**4**) and methyl α-D-glucopyranoside 2,4,6-trisphosphate (**5**) were, as predicted,  
30 inactive. The Ca<sup>2+</sup> release evoked by maximally effective concentrations of **2** or **3** was only ca. 70% of that evoked by  
31 Ins(1,4,5)P<sub>3</sub>, suggesting that **2** and **3** are partial agonists. Since partial agonists bind to Ins(1,4,5)P<sub>3</sub>Rs, but activate them  
32 less effectively than full agonists, a partial agonist must bind to more Ins(1,4,5)P<sub>3</sub>Rs than a full agonist to evoke  
33 comparable Ca<sup>2+</sup> release. We performed equilibrium competition binding assays using [<sup>3</sup>H]-Ins(1,4,5)P<sub>3</sub> and the active  
34 ligands to examine relationships between ligand binding and functional responses. The affinities of **6** and **7** for  
35 Ins(1,4,5)P<sub>3</sub>R aligned with their potencies in functional assays, with **7** having an affinity indistinguishable from that of  
36 Ins(1,4,5)P<sub>3</sub>, while **6** had ca. 15-fold lower affinity (Figure 5, Table). The EC<sub>50</sub>/K<sub>d</sub> values for Ins(1,4,5)P<sub>3</sub>, AdA, **6** and **7**  
37 were similar, consistent with each being a full agonist (Table). Comparison of the concentrations of **2** and **3** required to  
38 occupy 50% of binding sites (K<sub>d</sub>) and to evoke release of 39% of the Ca<sup>2+</sup> stores (EC<sub>39</sub>, i.e. the Ca<sup>2+</sup> release evoked by a  
39 half-maximally effective Ins(1,4,5)P<sub>3</sub> concentration) confirmed that **2** and **3** are weak partial agonists: their EC<sub>39</sub>/K<sub>d</sub>  
40 values (132 and 462, respectively) were much greater than that of Ins(1,4,5)P<sub>3</sub> (17). HPLC was used to confirm the purity  
41 of the compounds used in the biological assays (details in the Supporting Information).  
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**Table 1. Receptor binding and Ca<sup>2+</sup> Release by Ins(1,4,5)P<sub>3</sub>R mediated by Ins(1,4,5)P<sub>3</sub>, AdA and compounds 2-7<sup>a</sup>**

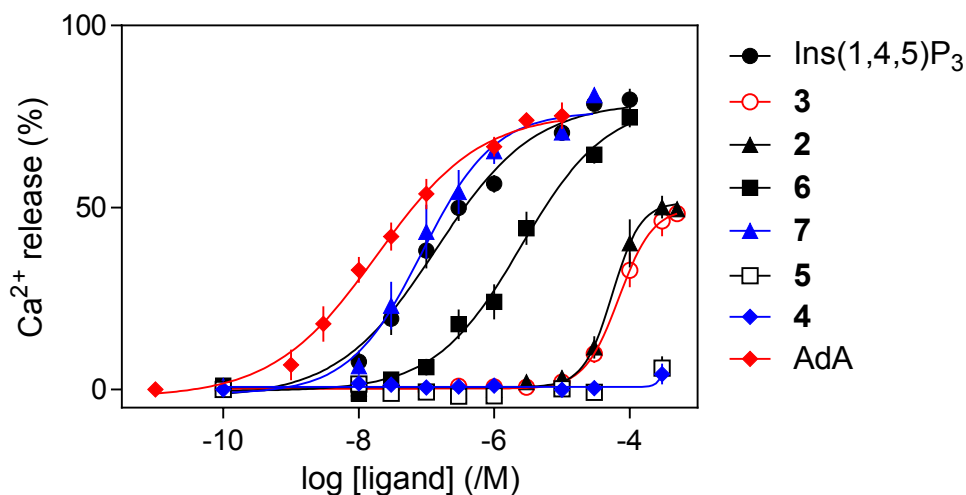
	Ca <sup>2+</sup> release			Binding			
	pEC <sub>50</sub> EC <sub>50</sub>	Release (%)	<i>h</i>	pK <sub>d</sub> K <sub>d</sub> (nM)	<i>h</i>	EC <sub>50</sub> /K <sub>d</sub>	<sup>h</sup> EC <sub>39</sub> /K <sub>d</sub>
Ins(1,4,5)P <sub>3</sub>	6.90 ± 0.12	78.8 ± 1.3		8.06 ± 0.03		<b>14</b>	
	126nM		0.7 ± 0.1	8.7	1.1 ± 0.2	(5 - 46)	<b>17</b> (5 - 63)
<b>2</b>	4.06 ± 0.09*	56.2 ± 2.6*	1.4 ± 0.2	5.91 ± 0.03*	0.9 ± 0.1	<b>71</b>	<b>132</b>
	87.7μM			1230		(34 - 148)	(49 - 355)
<b>3</b>	3.98 ± 0.04*	53.1 ± 5.0*	1.3 ± 0.2	6.26 ± 0.07*	0.9 ± 0.1	<b>191*</b>	<b>462*</b>
	104μM			549		(123 - 295)	(128 - 1667)
<b>4</b>	ND	4.3 ± 2.1 <sup>#</sup>	ND	48 ± 12 <sup>§</sup>	ND	ND	
<b>5</b>	ND	5.9 ± 2.1 <sup>#</sup>	ND	53 ± 3 <sup>§</sup>	ND	ND	
<b>6</b>	5.53 ± 0.20*	78.8 ± 3.0	0.9 ± 0.2	6.89 ± 0.09*	0.7 ± 0.1	<b>23</b>	
	2.96μM			129		(5 - 105)	
<b>7</b>	7.09 ± 0.18	75.2 ± 1.4		7.95 ± 0.05		<b>7</b>	
	80nM		1.0 ± 0.1	11.2	1.1 ± 0.2	(2 - 29)	
AdA	7.62 ± 0.12*	77.8 ± 4.5	0.8 ± 0.1	8.86 ± 0.14*	1.2 ± 0.2	<b>17</b>	
	24nM			1.4		(5 - 51)	

<sup>a</sup>Effects of ligands on Ca<sup>2+</sup> release from the intracellular stores of permeabilized HEK-Ins(1,4,5)P<sub>3</sub>R1 cells and on [<sup>3</sup>H]-Ins(1,4,5)P<sub>3</sub> binding to cerebellar membranes are summarized. Results from functional assays are means ± SEM (pEC<sub>50</sub> (-log of the half-maximally effective concentration), Ca<sup>2+</sup> release (%) and Hill coefficient, *h*) and means (EC<sub>50</sub>) from 5-11 independent experiments, each performed in duplicate. Results from binding experiments are means ± SEM (pK<sub>d</sub> (-log of the equilibrium dissociation constant) and *h*) and means (K<sub>d</sub>) from 3 independent experiments. The pK<sub>d</sub> values for Ins(1,4,5)P<sub>3</sub> and AdA have been published (Mills et al.)<sup>42</sup> and are reproduced with permission. Final columns show EC<sub>50</sub>/K<sub>d</sub> or (for partial agonists and Ins(1,4,5)P<sub>3</sub>) EC<sub>39</sub>/K<sub>d</sub> (mean and 95% CI). ND, not determined. \**P* < 0.05 relative to Ins(1,4,5)P<sub>3</sub>.

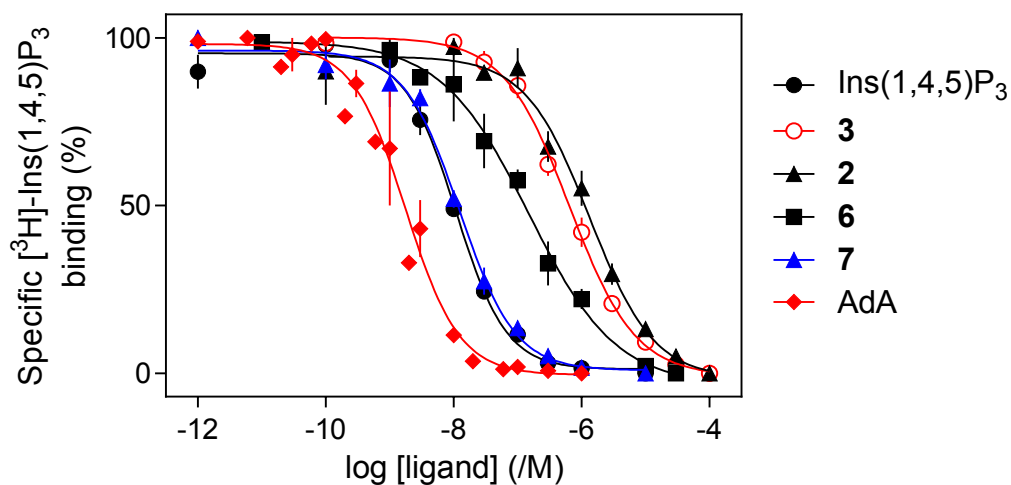
<sup>#</sup>Ca<sup>2+</sup> release evoked by 300 μM of the ligand

<sup>§</sup>Specific binding of [<sup>3</sup>H]-Ins(1,4,5)P<sub>3</sub> in presence of 30μM of competing ligand

$^1EC_{39}$  reports the concentration of ligand required to evoke the same  $Ca^{2+}$  release (39% of the intracellular stores) as evoked by a half-maximally effective concentration of  $Ins(1,4,5)P_3$ .



**Figure 4.** Concentration-dependent effects of  $Ins(1,4,5)P_3$  and related ligands on  $Ca^{2+}$  release from intracellular stores of permeabilized HEK- $Ins(1,4,5)P_3R1$  cells. Results are mean  $\pm$  SEM from 5-11 independent experiments, each with duplicate determinations.

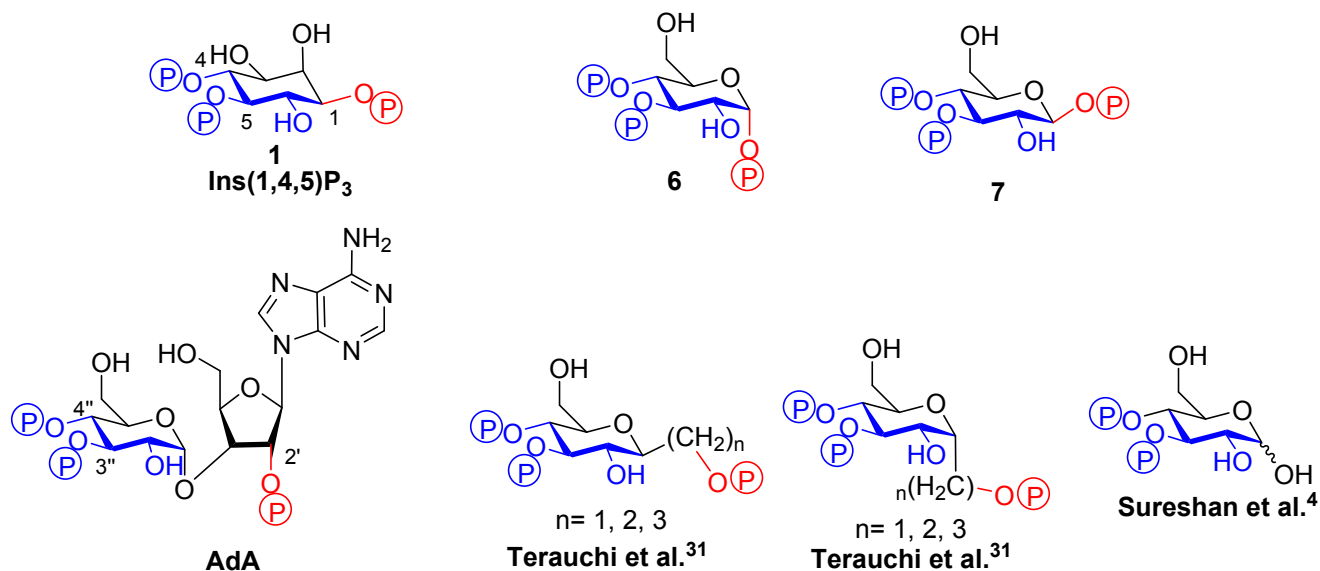


**Figure 5.** Equilibrium-competition binding to cerebellar membranes using  $[^3H]$ - $Ins(1,4,5)P_3$  (1.5nM) and the indicated concentrations of competing ligands. Results are means  $\pm$  SEM from 3 independent experiments. The results for  $Ins(1,4,5)P_3$  and AdA have been published (Mills et al.)<sup>42</sup>.

## DISCUSSION

Of the glucose polyphosphates considered in this study, the two that bound to Ins(1,4,5)P<sub>3</sub>R with highest affinity were  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**). Both compounds, which can be considered as truncated analogues of adenophostin A (AdA, Figure 6),<sup>43</sup> were found to be full agonists of Ins(1,4,5)P<sub>3</sub>R, and the  $\beta$ -epimer (**7**) was equipotent with Ins(1,4,5)P<sub>3</sub>.

Compounds containing a phosphate group attached to the anomeric carbon atom, as featured in **6** and **7**, have not been investigated as Ins(1,4,5)P<sub>3</sub>R ligands, presumably due to concerns over their stability, at least in the case of the  $\beta$ -epimer.<sup>30</sup> Nevertheless, we found that both  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**) were surprisingly durable as their triethylammonium salts and neither compound showed signs of degradation after two months in neutral aqueous solution at room temperature (see Supporting Information for details). Both **6** and **7** were eventually degraded under strongly acidic conditions, and HPLC traces were taken to confirm their hydrolysis to glucose 3,4-bisphosphate (details in the Supporting Information).



**Figure 6.** Structural comparison of Ins(1,4,5)P<sub>3</sub> (**1**), AdA and some of its truncated analogues including  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**) and  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**7**). The conserved regions of the structures involved in binding are drawn in blue while the differing auxiliary phosphate is shown in red.

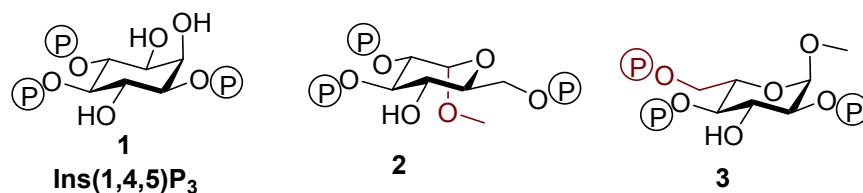
Previous studies using synthetic analogues of AdA<sup>29–33,44</sup> demonstrated that the adenine moiety significantly increases potency of the agonist, the vicinal phosphates are crucial to activity, and minor adjustments to the placement of the auxiliary phosphate can be tolerated.<sup>4,33</sup> The general consensus for AdA binding has been that the ligand interacts with the binding site of Ins(1,4,5)P<sub>3</sub>R with the 3'', 4'' and 2' phosphates mimicking the 4, 5 and 1 phosphates of Ins(1,4,5)P<sub>3</sub> respectively.<sup>26,45</sup> However, these previous studies employed analogues which differed from Ins(1,4,5)P<sub>3</sub> in several ways and it has therefore been difficult to isolate the specific impact of replacing the *myo*-inositol ring with D-glucopyranose.

This has implications for the possible mode of action of AdA and related compounds. Indeed, a cryo-EM study<sup>46</sup> of tetrameric Ins(1,4,5)P<sub>3</sub>R1 has recently proposed that AdA interacts with the IBC in a completely different way to Ins(1,4,5)P<sub>3</sub>, with the two domains of the IBC being pulled together by the 3''- and 4''-phosphate groups of AdA interacting with one domain and the adenine moiety interacting with the other.<sup>46</sup> In this model of AdA binding to Ins(1,4,5)P<sub>3</sub>R, the glucose bisphosphate structure of AdA only coincidentally resembles the *myo*-inositol 4,5-bisphosphate of Ins(1,4,5)P<sub>3</sub> and there is no structural correspondence between the glucose ring of AdA and the inositol ring of Ins(1,4,5)P<sub>3</sub>. However, this conclusion does not support the observed activities of the compounds in this study and other AdA analogues; it should therefore be viewed with caution.<sup>11,46</sup>

In the present study, we found that the closest possible glucose-containing analogue of Ins(1,4,5)P<sub>3</sub>, namely compound **7**, is effectively indistinguishable from Ins(1,4,5)P<sub>3</sub> in our assays of Ins(1,4,5)P<sub>3</sub>R binding and Ca<sup>2+</sup> release. This is entirely consistent with the idea that, in the Ins(1,4,5)P<sub>3</sub>-binding site, the glucopyranoside ring of **7** is closely analogous to the *myo*-inositol ring of Ins(1,4,5)P<sub>3</sub> (Figure S2). In turn, this establishes that the flexible Ins(1,4,5)P<sub>3</sub>-binding site can accommodate the equatorial glucopyranoside hydroxymethyl (CH<sub>2</sub>OH) group and pyranoside ring oxygen in place of the *myo*-inositol 3-OH group and C-2, respectively, with no impact on activity. The  $\alpha$ -epimer **6** is approximately 27-fold less potent than  $\beta$ -epimer **7** in Ca<sup>2+</sup> release. This shows that the axial phosphate group in **6** can still contribute to binding [Gluc(3,4)P<sub>2</sub> is much less potent] and is also consistent with an earlier report that *D-chiro*-Ins(1,3,4)P<sub>3</sub>, the C-1 epimer of Ins(1,4,5)P<sub>3</sub> having an axial 1-phosphate group, had 25-fold lower potency than Ins(1,4,5)P<sub>3</sub>.<sup>34,47</sup>

Thus, the effects of trisphosphates **6** and **7** provide strong support for the argument that compounds containing the D-glucopyranosyl 3,4-bisphosphate structure mimic  $\text{Ins}(1,4,5)\text{P}_3$  due to the direct structural analogy between glucopyranosyl and *myo*-inositol rings depicted in Figures 2 and 6. In such compounds, it is highly likely that the glucose 3,4-bisphosphate structure simply pulls together the two domains of the IBC in the manner proposed for the inositol 4,5-bisphosphate motif of  $\text{Ins}(1,4,5)\text{P}_3$ . We recently reported studies in which the glucose ring of AdA<sup>11</sup> and ribophostin<sup>42</sup> was replaced by D-*chiro*-inositol, leading to both modest and significant increases in biological activity respectively. It therefore remains to be conclusively established whether the additional components present in the AdA molecule can, counter-intuitively, induce a completely unrelated role for the glucose bisphosphate component of AdA itself, as suggested in the cryo-EM study.<sup>46</sup>

Previous studies of C-glycosidic truncated analogues of AdA with different chain lengths tethering the third, auxiliary phosphate group (Figure 6) have demonstrated that positioning of this phosphate has a significant effect on the affinity of the agonist.<sup>29–33</sup> It has been observed that the  $\text{Ca}^{2+}$ -releasing potency of these analogues at  $\text{Ins}(1,4,5)\text{P}_3\text{R}$  decreases as the length and flexibility of the linkage to the auxiliary phosphate increases.<sup>33</sup> The orientation of the linkage also plays a role, with axial linkages usually resulting in more potent compounds. However, even the most potent of these compounds [Figure 6, Terauchi et al.<sup>30</sup> axial linkage,  $n = 2$ ] is still weaker than  $\text{Ins}(1,4,5)\text{P}_3$  and compound **7**.



**Figure 7.**  $\text{Ins}(1,4,5)\text{P}_3$  (**1**) and analogues methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) and methyl  $\alpha$ -L-glucopyranoside 2,4,6-trisphosphate (**3**) with their structural differences contribute to their  $\text{Ins}(1,4,5)\text{P}_3\text{R}$  partial agonist activity in dark red.

Methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) was found to be a partial agonist of  $\text{Ins}(1,4,5)\text{P}_3\text{R}_1$ , while its D-glucose-based enantiomer, compound **4**, was inactive. This supports the structural alignment of trisphosphate **2** with  $\text{Ins}(1,4,5)\text{P}_3$  shown in Figure 7 and in our molecular modeling in Figure S3. No such alignment is possible for compound **4** because it does not possess a vicinal bisphosphate motif whose stereochemistry matches that of  $\text{Ins}(1,4,5)\text{P}_3$ .

In the predicted binding conformation of methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate (**2**) (Figure S3), the axial methyl group is positioned in a region of the binding site normally occupied by the 3-hydroxyl of  $\text{Ins}(1,4,5)\text{P}_3$ . In the



1 design of **2**, we further anticipated that the phosphate group at C-6 of L-glucose would mimic the auxiliary 1-phosphate  
2 of Ins(1,4,5)P<sub>3</sub> to some extent as there is evidence from previous studies showing that the Ins(1,4,5)P<sub>3</sub>R can accommodate  
3 more sterically demanding groups in this region of the binding site.<sup>4,48–50</sup> A very recent example of this is that replacement  
4 of the Ins(1,4,5)P<sub>3</sub> 1-phosphate by a pyrophosphate, which increases both charge and steric bulk, does not affect  
5 activity.<sup>37</sup> In addition, trisphosphate **2** contains an hydroxyl group appropriately placed to mimic the important 6-OH  
6 group of Ins(1,4,5)P<sub>3</sub>.  
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13 In studies of Ins(1,4,5)P<sub>3</sub> analogues as partial agonists, it has been shown that perturbations in the equivalent of the 3-  
14 hydroxyl of Ins(1,4,5)P<sub>3</sub> can result in partial agonist activity,<sup>51</sup> especially when this disruption occurs in conjunction with  
15 a modification to the vicinal phosphate pair or other region of the ligand.<sup>18,21,24,51</sup> It has been observed in multiple studies  
16 that limited, equatorial extension of substituents from the 3 position equivalent can be tolerated,<sup>52–54</sup> but larger groups  
17 hinder binding<sup>55,56</sup> and inversion of the 3-hydroxyl to axial results in a slight decrease in ligand activity.<sup>21,57–59</sup> Figure 7  
18 therefore suggests that the axial *O*-methyl group is the most likely component of **2** that causes it to display partial agonist  
19 activity, perhaps by interfering with the ligand binding to the β-domain of the IBC or by reducing the extent of domain  
20 closure.  
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30 Methyl α-L-glucopyranoside 2,4,6-trisphosphate (**3**) was designed to bind to the Ins(1,4,5)P<sub>3</sub>R in a manner that would  
31 potentially satisfy the essential binding requirements by positioning the pyranoside ring oxygen in place of the non-  
32 essential 3-OH group of Ins(1,4,5)P<sub>3</sub> while the axial 1-methoxy group occupied the place of the unimportant 2-OH group  
33 of Ins(1,4,5)P<sub>3</sub> (Figure 7 and S4). In this binding mode, the L-glucose 6-phosphate group would enter the region of the  
34 binding site usually occupied by the 4-phosphate of Ins(1,4,5)P<sub>3</sub>.  
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41 In previous studies, it has been shown that conservative modifications to the phosphates attached to the 4 and 5 equivalent  
42 positions (and sometimes in conjunction with a modification to the 3 position equivalent) can produce partial agonists  
43 and even low-affinity antagonists.<sup>21–24,51,55,60</sup> Bello et al.<sup>25</sup> hypothesized that if a ligand could bind to only one side of the  
44 IBC (through disruption of the interactions of either the 4 or 5 equivalent phosphates), it would be unable to pull the  
45 clam-like structure of the binding site closed and would therefore be unable to activate the receptor. Thus, a suitable  
46 modification to the 4-phosphate of Ins(1,4,5)P<sub>3</sub> might weaken the important interaction with the β-domain of the clam-  
47 shell structure and induce sub-optimal activation of Ins(1,4,5)P<sub>3</sub>R. However previous studies attempting to generate  
48 partial agonists with modifications solely to the 4-phosphate have failed to identify any active Ins(1,4,5)P<sub>3</sub> analogues.<sup>25</sup>  
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1 Pleasingly, our assays show that L-glucose trisphosphate **3** also behaves as a partial agonist of Ins(1,4,5)P<sub>3</sub>R1, with  
2 improved binding affinity and higher EC<sub>50</sub>/K<sub>d</sub> ratio than partial agonist **2**. The fact that the D-enantiomer **5** is inactive  
3 supports the structural alignment of **3** with Ins(1,4,5)P<sub>3</sub> depicted in Figure 7. The "extended" 4-phosphate group  
4 equivalent in **3** may thus disrupt the interaction of the ligand with the β-domain of the IBC as theorized.<sup>25</sup> It is likely that  
5 the absence in **3** of an equivalent to the 3-OH group in Ins(1,4,5)P<sub>3</sub> also contributes to a decreased interaction between  
6 the β-domain of the IBC and the ligand. Indeed, activity for 3-deoxy-Ins(1,4,5)P<sub>3</sub> at Ins(1,4,5)P<sub>3</sub>R has been reported to  
7 drop to up to 40 fold.<sup>9</sup>

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15 The two partial agonists, α-L-glucopyranoside 2,3,6-trisphosphate (**2**) and α-L-glucopyranoside 2,4,6-trisphosphate (**3**),  
16 indicate that perturbations of the ring structure of the ligand are sufficient to induce partial agonism. Both ligands suffer  
17 from low affinity and as a result, structurally related compounds are currently being developed that will incorporate  
18 similar structural differences to Ins(1,4,5)P<sub>3</sub> and hopefully maintain the desired decreased efficacy while increasing  
19 affinity. The most promising avenue seems to be adapting the structure of **3** by generating other ligands with extended  
20 4-position phosphate equivalents. This could hypothetically be continued with L-glucose, but inositol could also prove  
21 to be a useful starting material as ligands could be synthesized with a similar extension of the 4-position hydroxyl without  
22 loss of an equivalent hydroxyl to position 3 in Ins(1,4,5)P<sub>3</sub>, perhaps thereby improving ligand affinity while retaining  
23 partial agonist activity. Such work is in progress.

## 34 35 36 37 CONCLUSIONS

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40 We have synthesized four novel active ligands for the Ins(1,4,5)P<sub>3</sub>R based on both D-glucose and L-glucose templates as  
41 inositol surrogates. The two ligands based on L-glucose, namely methyl α-L-glucopyranoside 2,3,6-trisphosphate (**2**) and  
42 methyl α-L-glucopyranoside 2,4,6-trisphosphate (**3**), are low-affinity, low-efficacy partial agonists of Ins(1,4,5)P<sub>3</sub>R,  
43 while their respective D-glucose-based enantiomers **4** and **5** are inactive. Two further synthetic D-glucose-based  
44 trisphosphates, α-D-glucopyranosyl 1,3,4-trisphosphate (**6**) and β-D-glucopyranosyl 1,3,4-trisphosphate (**7**) can be  
45 regarded as close analogues of Ins(1,4,5)P<sub>3</sub> but they are also related structurally to the naturally-occurring  
46 glyconucleotide Ins(1,4,5)P<sub>3</sub>R agonist adenophostin A (AdA). They can therefore further our understanding of how AdA  
47 binds to Ins(1,4,5)P<sub>3</sub>R. Both **6** and **7** were found to be full agonists of Ins(1,4,5)P<sub>3</sub>R, with the perhaps surprisingly stable  
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1  $\beta$ -epimer **7** being equipotent to Ins(1,4,5)P<sub>3</sub> itself and potentially useful as a chemical biology tool under physiological  
2 conditions (with degradation induced under extremes of pH). The potency of **7** demonstrates that the structural  
3 differences between *myo*-inositol and D-glucose need not result in any decrease in ligand activity. This is consistent with  
4 the D-glucopyranosyl 3,4-bisphosphate moiety of AdA directly mimicking the D-*myo*-inositol 4,5-bisphosphate  
5 structure of Ins(1,4,5)P<sub>3</sub> at the binding site of Ins(1,4,5)P<sub>3</sub>Rs.  
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11 Partial agonists **2** and **3** are the first L-glucose-derived ligands that have been synthesized for Ins(1,4,5)P<sub>3</sub>R. Both  
12 compounds provide evidence for the viability of generating partial agonists and potential antagonists of Ins(1,4,5)P<sub>3</sub>R by  
13 deliberately disrupting the crucial moieties involved in binding to the IBC clam shell and pulling the domains together  
14 upon ligand binding. We hypothesize that the axial *O*-methyl group of compound **2** and the extended phosphate in the  
15 equivalent of the 4-position phosphate in Ins(1,4,5)P<sub>3</sub> of compound **3** cause the partial agonist activity of these  
16 compounds by either disrupting the interactions of the ligand with the  $\beta$  domain of the IBC or by preventing complete  
17 closure of the IBC upon binding. These partial agonists could prove to be interesting starting points to generate  
18 structurally similar compounds with even lower efficacy and higher affinity that could result in the generation of  
19 improved partial agonists or antagonists.  
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## 33 EXPERIMENTAL

### 34 General Synthesis

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39 Chemicals were purchased from Sigma-Aldrich, Acros, or Alfa Aesar. Anhydrous solvents were purchased from Sigma-  
40 Aldrich. TLC was performed on precoated plates (Merck Aluminum sheets silica 60 F254, art No. 5554). Chromatograms  
41 were visualised under UV light and by dipping plates into phosphomolybdic acid in EtOH, followed by heating. Flash  
42 column chromatography was performed using RediSep Rf disposable flash columns on an ISCO CombiFlash Rf  
43 automated flash chromatography machine. Reverse phase chromatography was performed on LiChroprep RP-18 (25-40  
44  $\mu$ m, Merck) using a BioLogic LP system (BioRad), eluting at 5 mL/min with a gradient of 0–10% MeCN in 0.05 M  
45 triethylammonium bicarbonate (TEAB) buffer, collecting 7 mL fractions. Fractions containing the target polyphosphate  
46 were identified using a modification of the Briggs phosphate assay.<sup>61</sup> The purity of all of the final compounds used in  
47 biological assays were assessed by HPLC and found to be >95% pure (vide infra and HPLC data in Supporting  
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Information). Proton  $^1\text{H}$  NMR and COSY spectra were recorded on a Bruker Avance III (400 MHz) spectrometer. Proton chemical shifts are reported in ppm ( $\delta$ ) relative to internal tetramethylsilane (TMS,  $\delta$  0.0 ppm) or with the solvent reference relative to TMS employed as the internal standard ( $\text{CDCl}_3$ ,  $\delta$  7.26 ppm;  $\text{CD}_3\text{OD}$ ,  $\delta$  3.31 ppm). The following abbreviations are used to describe the multiplicity of the chemical shifts: br, broad; s, singlet; d, doublet; dd, double doublet; q, quartet; m, multiplet; t, triplet.  $^{13}\text{C}$  and HSQC spectra were recorded on a Bruker Avance III (100 MHz) spectrometer with complete proton decoupling. Carbon chemical shifts are reported in ppm ( $\delta$ ) relative to TMS with the respective solvent resonance as the internal standard ( $\text{CDCl}_3$ ,  $\delta$  77.0 ppm,  $d_4$ -MeOH,  $\delta$  49.0 ppm).  $^{31}\text{P}$  NMR spectra were recorded on a Bruker Avance III (162 MHz) spectrometer with complete proton decoupling. Phosphorus chemical shifts are reported in ppm ( $\delta$ ) relative to an 85%  $\text{H}_3\text{PO}_4$  external standard ( $\text{H}_3\text{PO}_4$ ,  $\delta$  0.0 ppm). All NMR data were collected at 25 °C. Optical rotations were measured at ambient temperature using an Optical Activity Ltd. AA-10 polarimeter in a cell volume of 5  $\text{cm}^3$ , and specific rotation is given in  $10^{-1}$  deg  $\text{cm}^3$   $\text{g}^{-1}$ . Melting points were determined using a Stanford Research Systems Optimelt MPA100 automated melting point system and are uncorrected. Mass spectra were recorded on a Thermo Orbitrap Exactive Mass Spectrometer. All reactions were carried out under an argon atmosphere employing oven-dried glassware unless stated otherwise.

**Methyl 3-O-benzyl-4,6-O-benzylidene- $\alpha$ -L-glucopyranoside (12)** Methyl  $\alpha$ -L-glucopyranoside (**8**) (93 mg, 0.480 mmol) was dissolved in dry pyridine (1 mL) and put under argon. To this solution, trimethylsilyl chloride (0.30 mL, 2.39 mmol, 5 equiv) was added dropwise. The solution was allowed to stir for 22 h at room temperature. The reaction was then diluted with EtOAc and washed with water (2 x 30 mL). The organic phase was dried over  $\text{MgSO}_4$  and concentrated *in vacuo* to yield the per-silylated glucopyranoside crude product. The per-silylated product was not purified, but NMR was used to confirm that the reaction had proceeded to completion. The per-silylated product was dissolved and co-evaporated twice with toluene before being dissolved in dry DCM (0.7 mL) and put under argon. To this solution, benzaldehyde (0.15 mL, 1.42 mmol, 3 equiv) was added and the reaction was cooled in an ice bath. To this chilled solution, iron (III) chloride hexahydrate (3.4 mg, 0.013 mg, 0.026 equiv) dissolved in MeCN (0.12 mL) was added dropwise. Triethylsilane (0.08 mL, 0.528 mmol, 1.1 equiv) was then added dropwise and the reaction was allowed to warm to room temperature and stir for 1.5 h. After this time, the solution was diluted with EtOAc (50 mL), washed with sat.  $\text{NaHCO}_3$  solution (50 mL), and extracted from the aqueous phase twice more with EtOAc (2 x 30 mL). The combined organic phases were dried over  $\text{MgSO}_4$  and concentrated *in vacuo* to yield the crude product. The product was purified

with flash chromatography (petroleum ether/EtOAc, 0-100%) to yield the pure product as a white solid (140.7 mg, 0.378 mmol, 79% yield); mp 184.0-185.3 °C;  $[\alpha]_D^{21}$  -87.5 ( $c=1.00$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.51-7.27 (m, 10H, Ar), 5.57 (s, 1H, H-7), 4.97 (d,  $J=11.6$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.82 (d,  $J=3.9$  Hz, 1H, H-1), 4.79 (d,  $J=11.6$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.30 (dd,  $J=4.3, 9.8$  Hz, 1H, H-6), 3.87-3.71 (m, 4H, H-2, H-3, H-5, H-6), 3.65 (t,  $J=9.2$  Hz, 1H, H-4), 3.45 (s, 3H, OMe), 2.29 (d,  $J=7.2$  Hz, 1H, OH);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  138.6 (Ar), 137.5 (Ar), 129.1 (Ar), 128.6 (Ar), 128.4 (Ar), 128.2 (Ar), 127.9 (Ar), 126.2 (Ar), 101.4 (C-7), 100.0 (C-1), 82.1 (C-4), 79.0 (C-2 or 3 or 5), 75.0 ( $\text{CH}_2\text{Ph}$ ), 72.6 (C-2 or 3 or 5), 69.2 (C-6), 62.7 (C-2 or 3 or 5), 55.6 (OMe); HRMS (ESI):  $m/z$  calcd  $[\text{M}+\text{Na}]^+$  for  $\text{C}_{21}\text{H}_{24}\text{O}_6$ : 395.14651, found: 395.14658.

**Methyl 3-*O*-benzyl- $\alpha$ -L-glucopyranoside (13)** Methyl 3-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -L-glucopyranoside (**12**) (135.7 mg, 0.364 mmol) was dissolved in MeOH (3 mL). To this solution, water (0.15 mL) and 1 M HCl (aq) (0.3 mL) were added. The reaction was heated to reflux for 3 h. After this time, the reaction was quenched with the addition of NaHCO<sub>3</sub> (aq) (25.2 mg in 5 mL water). The solution was concentrated *in vacuo* and the residue was dissolved and co-evaporated with toluene twice to yield the crude product. The product was purified with flash chromatography (pet ether/EtOAc, 0-100%) to yield the pure product as a white solid (96.3 mg, 0.339 mmol, 93% yield). mp 91.2- 93.8 °C;  $[\alpha]_D^{22}$  -89.5 ( $c=0.93$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.39-7.29 (m, 5H, Ar), 5.03 (d,  $J=11.5$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.76 (d,  $J=3.9$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.73 (d,  $J=11.5$  Hz, 1H, H-1), 3.88-3.75 (m, 2H, H-6 x2), 3.70-3.52 (m, 4H, H-2, H-3, H-4, H-5), 3.44 (s, 3H, OMe), 2.30 (d,  $J=2.4$  Hz, 1H, OH), 2.14 (d,  $J=9.2$  Hz, 1H, OH), 1.93 (dd,  $J=5.7, 7.2$  Hz, 1H, OH);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  138.6 (Ar), 128.8 (Ar), 128.1 (Ar), 99.7 (C-1), 82.8 (C-3), 75.1 ( $\text{CH}_2\text{Ph}$ ), 72.9 (C-2), 71.1 (C-5), 70.2 (C-4), 62.5 (C-6), 55.5 (OMe); HRMS (ESI):  $m/z$  calcd  $[\text{M}+\text{Na}]^+$  for  $\text{C}_{14}\text{H}_{20}\text{O}_6$ : 307.11521, found: 307.11515.

**Methyl 3-*O*-benzyl- $\alpha$ -L-glucopyranoside 2,4,6-tris(dibenzyl phosphate) (14)** Methyl 3-*O*-benzyl- $\alpha$ -L-glucopyranoside (**13**) (96.3 mg, 0.339 mmol) was dissolved in dry DCM (4 mL) and the solution was put under argon. 5-Phenyl-1*H*-tetrazole (297 mg, 2.03 mmol, 6 equiv) was added to the solution, followed by dibenzyl diisopropylphosphoramidite (0.55 mL, 1.52 mmol, 4.5 equiv). The reaction was allowed to stir at room temperature overnight. The next day, after the confirmation of successful phosphorylation with  $^{31}\text{P NMR}$ , the reaction flask was cooled to -78°C and mCPBA (502 mg, 70% purity, 2.03 mmol, 6 equiv.) was added. The reaction was allowed to stir at room temperature for 10 min before the solution was diluted with EtOAc (50 mL), washed with 10% Na<sub>2</sub>SO<sub>3</sub> solution (2 x 30 mL), dried over MgSO<sub>4</sub> and concentrated to yield the crude product. The crude product was purified with flash chromatography (pet ether/EtOAc, 0-100%) to yield the pure product as a colorless oil (144.2 mg, 0.135 mmol, 40%

1 yield).  $[\alpha]_D^{21}$  -38.8 ( $c=1.01$ ,  $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.34-7.08 (m, 35H, Ar), 5.03 (s, 2H,  $\text{CH}_2\text{Ph}$  x2),  
2 5.01 (s, 2H,  $\text{CH}_2\text{Ph}$  x2), 4.98 (d,  $J=3.2$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.96 (d,  $J=3.2$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.94-4.85 (m, 6H, H-1,  $\text{CH}_2\text{Ph}$   
3 x5), 4.80-4.75 (m, 3H,  $\text{CH}_2\text{Ph}$  x3), 4.42-4.18 (m, 4H, H-2, H-3, H-6 x2), 3.98 (t,  $J=9.4$  Hz, 1H, H-4), 3.86 (dd,  $J=10.0$ ,  
4 5.2 Hz, 1H, H-5), 3.28 (s, 3H, OMe);  $^{31}\text{P NMR}$  ( $\text{CDCl}_3$ , 162 MHz):  $\delta$  -0.99, -1.74, -1.86;  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz):  
5  $\delta$  138.1 (Ar), 136.0 (Ar), 135.9 (Ar), 135.8-135.6 (m, Ar), 128.7-128.5 (m, Ar), 128.3 (Ar), 128.0 (m, Ar), 127.9 (Ar),  
6 127.7 (Ar), 127.5 (Ar), 97.6 (C-1), 78.4-78.3 (m, C-4), 76.8-76.7 (m, C-2 or 3), 75.1 (C-2 or 3,  $\text{CH}_2\text{Ph}$ ), 69.8-69.4 (m,  
7  $\text{CH}_2\text{Ph}$ ), 69.0-68.9 (m, C-5), 66.1 (d,  $J=5.1$  Hz, C-6), 55.7 (OMe); HRMS (ESI):  $m/z$  calcd  $[\text{M}+\text{H}]^+$  for  $\text{C}_{56}\text{H}_{59}\text{O}_{15}\text{P}_3$ :  
8 1065.31396, found: 1065.31280.

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17 **Methyl  $\alpha$ -L-glucopyranoside 2,4,6-trisphosphate triethylammonium salt (3)** Methyl 3-*O*-benzyl- $\alpha$ -L-  
18 glucopyranoside 2,4,6-tris(dibenzyl phosphate) (14) (141.9 mg, 0.133 mmol) was dissolved in MeOH (7.1 mL).  
19 Ultrapure water (0.71 mL) was added dropwise to the solution, ensuring that the precipitate formed upon addition was  
20 able to dissolve back into solution.  $\text{Pd}(\text{OH})_2/\text{C}$  (20% ,  $\geq 50\%$  wet, 71.0 mg) was added to the solution and the reaction  
21 flask was flushed with hydrogen. The reaction was allowed to stir at room temperature for 24 h, after which the catalyst  
22 was filtered off and the collected filtrate was evaporated to yield the product as a free acid. No purification steps were  
23 deemed to be necessary, but triethylamine was added to sharpen the phosphorus NMR signals and to convert the product  
24 from the free acid into the triethylammonium salt. The product was concentrated *in vacuo*, lyophilised and collected as  
25 a colorless glass (96.1 mg, 0.120 mmol, 90% yield).  $[\alpha]_D^{22}$  -46.5 ( $c=0.88$ , MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  4.90  
26 (d,  $J=3.0$  Hz, 1H, H-1), 4.29-4.18 (m, 2H, H-3, H-6), 4.05-3.97 (m, 3H, H-2, H-4, H-6), 3.68 (d,  $J=10.0$  Hz, 1H, H-5),  
27 3.38 (s, 3H, OMe), 3.14 (q,  $J=7.3$  Hz, approx. 18H, TEA  $\text{CH}_2\text{CH}_3$ ), 1.30 (t, 7.3 Hz, approx. 27H, TEA  $\text{CH}_2\text{CH}_3$ );  $^{31}\text{P}$   
28 NMR ( $\text{CD}_3\text{OD}$ , 162 MHz):  $\delta$  1.90, 1.71, 1.28;  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta$  100.2 (d,  $J=4.0$  Hz, C-1), 76.3 (d,  $J=$   
29 5.1 Hz, C-2 or 4), 74.6 (d,  $J=4.0$  Hz, C-2 or 4), 73.5 (d,  $J=4.9$  Hz, C-3), 71.0-71.2 (m, C-5), 63.9 (d,  $J=4.7$  Hz, C-6),  
30 55.6 (OMe), 46.9 (TEA  $\text{CH}_2\text{CH}_3$ ), 9.4 (TEA  $\text{CH}_2\text{CH}_3$ ). HRMS (ESI):  $m/z$  calcd  $[\text{M}-\text{H}]^-$  for  $\text{C}_7\text{H}_{17}\text{O}_{15}\text{P}_3$ : 432.97075,  
31 found: 432.97065.

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48 **Methyl 4-*O*-benzyl- $\alpha$ -L-glucopyranoside 2,3,6-tris(dibenzyl phosphate) (11)** Methyl 4-*O*-benzyl- $\alpha$ -L-  
49 glucopyranoside (10) (65.5 mg, 0.230 mmol) was dissolved in dry DCM (2 mL) and put under argon. 5-Phenyl-1*H*-  
50 tetrazole (202 mg, 1.38 mmol, 6 equiv) was added to the solution followed by dibenzyl diisopropylphosphoramidite  
51 (0.36 mL, 1.04 mmol, 4.5 equiv). The reaction was allowed to stir at room temperature overnight. The following day,  
52 the reaction was cooled to  $-78^\circ\text{C}$  and mCPBA (70% pure, 342 mg, 1.38 mmol, 6 equiv) was added. The reaction was  
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1 allowed to stir for 10 min at room temperature before it was diluted with EtOAc (50 mL) and washed twice with 10%  
2 Na<sub>2</sub>SO<sub>3</sub> solution (2 x 30 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated to yield the crude product. The  
3 residue was purified with flash chromatography (petroleum ether/EtOAc, 0-100%) to yield the pure product as a colorless  
4 oil (210.5 mg, 0.198 mmol, 86% yield). [ $\alpha$ ]<sub>D</sub><sup>21</sup> -42.3 (*c*= 1.06, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.33-7.09 (m,  
5 35H, Ar), 5.05-4.81 (m, 15H, H-1, H-3, CH<sub>2</sub>Ph x13), 4.46 (d, *J*= 10.7 Hz, 1H, CH<sub>2</sub>Ph), 4.25 (ddd, *J*=13.3, 6.2, 3.6 Hz,  
6 1H, H-2), 4.19-4.16 (m, 2H, H-6), 3.75 (dq, *J*= 10.0, 2.3 Hz, 1H, H-5), 3.50 (t, *J*= 9.5 Hz, 1H, H-4), 3.24 (s, 3H, OMe);  
7 <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz):  $\delta$  -0.76, -1.32, -2.01; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  137.6 (Ar), 136.1-135.7 (m, Ar),  
8 128.7-127.8 (m, Ar), 97.6 (C-1), 78.4 (dd, *J*= 8.7, 6.5 Hz, C-3), 76.2 (C-4), 75.3 (dd, *J*= 4.7, 3.2 Hz, C-2), 74.6 (CH<sub>2</sub>Ph),  
9 69.8 (d, *J*= 5.6 Hz, CH<sub>2</sub>Ph), 69.6-69.4 (m, CH<sub>2</sub>Ph), 69.1 (d, *J*= 8.3 Hz, C-5), 65.8 (d, *J*= 5.6 Hz, C-6), 55.5 (OMe);  
10 HRMS (ESI): *m/z* calcd [M+H]<sup>+</sup> for C<sub>56</sub>H<sub>59</sub>O<sub>15</sub>P<sub>3</sub>: 1065.31396, found: 1065.31297.  
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21 **Methyl  $\alpha$ -L-glucopyranoside 2,3,6-trisphosphate triethylammonium salt (2)** Methyl 4-O-benzyl- $\alpha$ -L-  
22 glucopyranoside 2,3,6-tris(dibenzyl phosphate) (**11**) (60 mg, 0.056 mmol) was dissolved in MeOH (3 mL). To this  
23 solution, ultrapure water (0.3 mL) was added dropwise, ensuring that the white precipitate that formed returned to  
24 solution. Pd(OH)<sub>2</sub>/C (30 mg, 20% wt) was added to the solution and the flask was flushed with hydrogen. The reaction  
25 was left to stir under hydrogen at room temperature overnight. The palladium catalyst was filtered off with a PTFE filter  
26 and the solution was concentrated to yield the product as a free acid. No purification was deemed necessary. The free  
27 acid was converted to the triethylammonium salt through the addition of triethylamine to the free acid followed by  
28 concentration *in vacuo*. The product was lyophilised and collected as a colourless glass (44.5 mg, 0.056 mmol, 100%  
29 yield). [ $\alpha$ ]<sub>D</sub><sup>21</sup> -37.6 (*c*= 1.00, MeOH); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  4.92 (d, *J*= 3.6 Hz, 1H, H-1), 4.35 (q, *J*= 8.7 Hz,  
30 1H, H-3), 4.17 (ddd, *J*= 11.1, 5.2, 2.0 Hz, 1H, H-6), 4.07-3.98 (m, 2H, H-2, H-6), 3.73-3.68 (m, 1H, H-5), 3.55 (dd, *J*=  
31 9.8, 8.7 Hz, 1H, H-4), 3.39 (s, 3H, OMe), 3.09 (q, *J*= 7.3 Hz, approx. 18H, TEA CH<sub>2</sub>CH<sub>3</sub>), 1.27 (t, *J*= 7.3 Hz, approx.  
32 27H, TEA CH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz):  $\delta$  2.50, 1.53, 1.15; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  100.5 (C-1), 78.5  
33 (C-3), 75.5 (C-2), 72.4 (d, *J*= 8.7 Hz, C-5), 72.0 (C-4), 65.7 (d, *J*= 4.9 Hz, C-6), 55.4 (OMe), 47.0 (TEA CH<sub>2</sub>CH<sub>3</sub>), 9.4  
34 (TEA CH<sub>2</sub>CH<sub>3</sub>). HRMS (ESI): *m/z* calcd [M-H]<sup>-</sup> for C<sub>7</sub>H<sub>17</sub>O<sub>15</sub>P<sub>3</sub>: 432.97075, found: 432.97067.  
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50 **2,6-Di-O-benzyl-D-glucopyranose (16)** Allyl 2,6-di-O-benzyl-D-glucopyranoside (**15**) (60 mg, 0.133 mmol) as  
51 synthesized in the method outlined by Jenkins et al.<sup>29</sup> was dissolved in dry MeOH (1.54 mL). To this solution, PdCl<sub>2</sub>  
52 (6.2 mg, 0.03 mmol, 0.25 equiv) was added and the reaction was allowed to stir at room temperature for 6 hours with a  
53 drying tube affixed to the flask. After this time, the reaction was quenched with the addition of excess NaHCO<sub>3</sub> and  
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1 allowed to stir for 5 minutes before being filtered through celite and concentrated to yield the crude product. The product  
2 of this reaction could not be successfully purified, although following phosphorylation (see below), the products could  
3 be successfully isolated.  
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7 2,6-Di-*O*-benzyl- $\alpha$ -D-glucopyranosyl 1,3,4-tris(dibenzylphosphate) (17) and 2,6-di-*O*-benzyl- $\beta$ -D-glucopyranosyl 1,3,4-  
8 tris(dibenzylphosphate) (18) 2,6-di-*O*-benzyl-D-glucopyranose (16) (154.7 mg, 0.429 mmol) was added to dry DCM (4.5  
9 mL). To this suspension, 5-phenyl-1*H*-tetrazole (376 mg, 2.58 mmol, 6 equiv) was added, followed by dibenzyl  
10 diisopropylphosphoramidite (0.67 mL, 1.93 mmol, 4.5 equiv). The reaction was allowed to stir under argon at room  
11 temperature overnight, after which it was cooled to -78°C and mCPBA (70% purity, 636 mg, 2.58 mmol, 6 equiv) was  
12 added. The reaction was then diluted with EtOAc (100 mL) and washed twice with 10% Na<sub>2</sub>SO<sub>3</sub> solution (2 x 30 mL).  
13 The organic phase was dried over MgSO<sub>4</sub> and concentrated to yield the crude product. The product was purified using  
14 flash chromatography (petroleum ether/EtOAc, 0-100%). The stereoisomers of the product were partially isolated and  
15 collected as colorless oils (total: 276.6 mg, 0.242 mmol, 56% yield;  $\alpha$ -epimer: 70.4 mg, 0.062 mmol, 14% yield;  $\beta$ -  
16 epimer: 92.4 mg, 0.081 mmol, 19% yield; remaining unseparated mix of epimers: 113.8 mg, 0.100 mmol, 23% yield).  
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20 **2,6-Di-*O*-benzyl- $\alpha$ -D-glucopyranosyl 1,3,4-tris(dibenzylphosphate) (17):** *R<sub>f</sub>* (EtOAc) 0.63; [ $\alpha$ ]<sup>20</sup><sub>D</sub> 2.68 (*c*= 1.01,  
21 chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.35-7.09 (m, 40H, Ar), 5.91 (dd, *J*= 3.3, 7.0 Hz, 1H, H-1 $\alpha$ ), 5.07-4.81 (m,  
22 13H, H-3, CH<sub>2</sub>Ph x12), 4.72-4.61 (m, 3H, H-4, CH<sub>2</sub>Ph x2), 4.44 (d, *J*= 12.0 Hz, 1H, CH<sub>2</sub>Ph), 4.31 (d, *J*= 12.0 Hz, 1H,  
23 CH<sub>2</sub>Ph), 3.96 (dq, *J*= 10.0, 1.6 Hz, 1H, H-5), 3.70 (dd, *J*= 11.2, 3.9 Hz, 1H, H-6), 3.65 (dt, *J*= 9.7, 3.1 Hz, 1H, H-2), 3.54  
24 (dd, *J*= 11.2, 1.8 Hz, 1H, H-6); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz):  $\delta$  -1.66, -2.31, -2.50; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  138.0  
25 (Ar), 137.0 (Ar), 136.2-135.6 (m, Ar), 128.6-127.6 (m, Ar), 94.8 (d, *J*= 5.6 Hz, C-1), 77.9-77.8 (m, C-3), 77.0 (m, C-2),  
26 73.7-73.6 (m, C-4), 73.3 (CH<sub>2</sub>Ph), 73.0 (CH<sub>2</sub>Ph), 71.6 (C-5), 70.0-69.9 (m, CH<sub>2</sub>Ph), 69.7-69.6 (m, CH<sub>2</sub>Ph), 69.5-69.4  
27 (m, CH<sub>2</sub>Ph), 67.5 (C-6); HRMS (ESI): *m/z* calcd [M+Na]<sup>+</sup> for C<sub>62</sub>H<sub>63</sub>O<sub>15</sub>P<sub>3</sub>: 1163.32720, found: 1163.32495.  
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31 **2,6-Di-*O*-benzyl- $\beta$ -D-glucopyranosyl 1,3,4-tris(dibenzylphosphate) (18):** *R<sub>f</sub>* (EtOAc) 0.57; [ $\alpha$ ]<sup>21</sup><sub>D</sub> 35.7 (*c*= 0.71,  
32 chloroform); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.31-7.03 (m, 40H, Ar), 5.31 (t, *J*= 7.2 Hz, 1H, H-1 $\beta$ ), 5.04-4.87 (m, 11H,  
33 CH<sub>2</sub>Ph x11), 4.81-4.60 (m, 5H, C-3, C-4, CH<sub>2</sub>Ph x3), 4.42 (d, *J*= 12.0 Hz, 1H, CH<sub>2</sub>Ph), 4.31 (d, *J*= 12.0 Hz, 1H, CH<sub>2</sub>Ph),  
34 3.77-3.55 (m, 4H, C-2, C-5, C-6 x2); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz):  $\delta$  -1.90, -2.22, -2.59; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  
35  $\delta$  138.0 (Ar), 137.7 (Ar), 136.1-135.4 (m, Ar), 128.6-127.6 (m, Ar), 98.6 (C-1), 80.1 (m, C-3 or 4), 79.7-79.6 (m, C-2 or  
36 5), 74.5 (d, *J*= 3.5 Hz, C-3 or 4), 74.1 (CH<sub>2</sub>Ph), 74.0-73.9 (m, C-2 or 5), 73.3 (CH<sub>2</sub>Ph), 70.0 (d, *J*= 5.8 Hz, CH<sub>2</sub>Ph), 69.8-  
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69.7 (m, CH<sub>2</sub>Ph), 69.6-69.5 (m, CH<sub>2</sub>Ph), 68.1 (C-6); HRMS (ESI): *m/z* calcd [M+Na]<sup>+</sup> for C<sub>62</sub>H<sub>63</sub>O<sub>15</sub>P<sub>3</sub>: 1163.32720, found: 1163.32505.

**$\alpha$ -D-Glucopyranosyl 1,3,4-trisphosphate triethylammonium salt (6)**  $\alpha$ -D-glucopyranosyl 1,3,4-trisphosphate was prepared with 2,6-di-O-benzyl- $\alpha$ -D-glucopyranosyl 1,3,4-tris(dibenzylphosphate) (**17**) (31.9 mg, 0.028 mmol) using the same hydrogenation method as described for the synthesis of  $\beta$ -D-glucopyranosyl 1,3,4-trisphosphate (**6**). The crude sodium salt of the product was purified by ion pair column chromatography on RP18 and lyophilised to yield the triethylamine salt of the product as a colorless glass (8.0 mg, 0.011 mmol, 39% yield). [ $\alpha$ ]<sup>20</sup><sub>D</sub> 33.9 (*c*=0.97, methanol); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  5.58 (dd, *J*= 7.0, 3.6 Hz, 1H, H-1), 4.45 (q, *J*= 9.0 Hz, 1H, H-3), 4.12 (q, *J*= 9.8 Hz, 1H, H-4), 3.98-3.91 (m, 2H, H-5, H-6), 3.76-3.72 (m, 1H, H-6), 3.61 (ddd, *J*= 9.5, 3.5, 2.4 Hz, 1H, H-2), 3.16 (q, *J*= 7.3 Hz, approx. 18H, TEA CH<sub>2</sub>CH<sub>3</sub>), 1.31 (t, *J*= 7.3 Hz, approx. 27H, TEA CH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  2.06, 2.06, -0.62; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz):  $\delta$  96.3 (d, *J*= 5.4 Hz, C-1), 79.2-79.1 (m, C-3), 73.8-73.6 (m, C-2, C-4, C-5), 62.1 (C-6), 47.3 (TEA CH<sub>2</sub>CH<sub>3</sub>), 9.2 (TEA CH<sub>2</sub>CH<sub>3</sub>). HRMS (ESI): *m/z* calcd [M-H]<sup>-</sup> for C<sub>6</sub>H<sub>15</sub>O<sub>15</sub>P<sub>3</sub>: 418.9551, found: 418.95422.

**$\beta$ -D-Glucopyranosyl 1,3,4-trisphosphate triethylammonium salt (7)** 2,6-di-O-benzyl- $\beta$ -D-glucopyranosyl 1,3,4-tris(dibenzylphosphate) (**18**) (23.3 mg, 0.020 mmol) was dissolved in MeOH (1.5 mL). To this solution, ultrapure water (0.15 mL) was added dropwise, ensuring that the precipitate that formed upon addition returned to solution. NaHCO<sub>3</sub> (5.15 mg, 0.061 mmol, 3 equiv) was then added, followed by 20% Pd(OH)<sub>2</sub>/C ( $\geq$ 50% wet, 11.7 mg). The reaction flask was flushed with hydrogen and left to stir at room temperature for 24 h. The catalyst was then filtered off and the collected filtrate was evaporated to yield the crude product as a sodium salt. The product was purified by ion pair column chromatography on RP18 and lyophilised to yield the triethylamine salt of the product as a colorless glass (6.7 mg, 0.008 mmol, 40% yield). [ $\alpha$ ]<sup>21</sup><sub>D</sub> 3.76 (*c*=0.61, methanol); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta$  4.98 (t, *J*= 7.6 Hz, 1H, H-1), 4.23 (q, *J*= 8.8 Hz, 1H, H-3), 4.08 (q, *J*= 9.8 Hz, 1H, H-4), 3.89 (dd, *J*= 12.7, 4.4 Hz, 1H, H-6), 3.84 (dd, *J*= 12.7, 2.1 Hz, 1H, H-6), 3.43-3.38 (m, 2H, H-2, H-5), 3.13 (q, *J*= 7.0 Hz, approx. 18H, TEA CH<sub>2</sub>CH<sub>3</sub>), 1.29 (t, *J*= 7.2 Hz, approx. 27H, TEA CH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  1.38, 1.06, -0.77; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz):  $\delta$  99.3 (C-1), 81.4 (C-3), 77.9 (C-2 or 5), 76.2 (C-2 or 5), 73.8 (C-4), 62.4 (C-6), 47.2 (TEA CH<sub>2</sub>CH<sub>3</sub>), 9.3 (TEA CH<sub>2</sub>CH<sub>3</sub>). HRMS (ESI): *m/z* calcd [M-H]<sup>-</sup> for C<sub>6</sub>H<sub>15</sub>O<sub>15</sub>P<sub>3</sub>: 418.9551, found: 418.95425.

**Methyl 4-*O*-benzyl- $\alpha$ -D-glucopyranoside 2,3,6-tris(dibenzyl phosphate) (22)** Methyl 4-*O*-benzyl- $\alpha$ -D-glucopyranoside (21) (50 mg, 0.176 mmol) was dissolved in dry DCM (2 mL) and the solution was put under argon. 5-Phenyl-1*H*-tetrazole (154 mg, 1.06 mmol, 6 equiv) was added to the solution followed by dibenzyl diisopropylphosphoramidite (0.27 mL, 0.792 mmol, 4.5 equiv). The reaction was allowed to stir at room temperature overnight. The following day, the reaction was cooled to -78°C and mCPBA (70% pure, 261 mg, 1.06 mmol, 6 equiv) was added. The reaction was allowed to stir for 10 min at room temperature before it was diluted with EtOAc (50 mL) and washed twice with 10% Na<sub>2</sub>SO<sub>3</sub> solution (2 x 30 mL). The organic layer was dried over MgSO<sub>4</sub> and concentrated to yield the crude product. The product was purified with flash chromatography (1. petroleum ether/EtOAc, 0-100% and 2. DCM/EtOAc, 0-100%). The pure product was collected as a colourless oil (81.9 mg, 0.077 mmol, 44% yield). [ $\alpha$ ]<sup>22</sup><sub>D</sub> 35.7 (*c*= 1.00, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.33-7.09 (m, 35H, Ar), 5.05-4.82 (m, 15H, H-1, H-3, CH<sub>2</sub>Ph x13), 4.47 (d, *J*= 10.7 Hz, 1H, CH<sub>2</sub>Ph) 4.26 (ddd, *J*= 9.6, 6.2, 3.6 Hz, 1H, H-2), 4.19-4.16 (m, 2H, H-6 x2), 3.76 (dq, *J*= 9.6, 2.7 Hz, 1H, H-5), 3.50 (t, *J*= 9.4 Hz, 1H, H-4), 3.25 (s, 3H, OMe); <sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz):  $\delta$  -0.76, -1.32, -2.01; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  137.6 (Ar), 136.1-135.7 (m, Ar), 128.7-128.0 (m, Ar), 97.6 (C-1), 78.5-78.4 (m, C-3), 76.2 (C-4), 75.4-75.3 (m, C-5), 74.6 (CH<sub>2</sub>Ph), 69.8 (d, *J*= 5.6 Hz, CH<sub>2</sub>Ph), 69.6-69.4 (m, CH<sub>2</sub>Ph), 69.1 (d, *J*= 8.1 Hz, C-5), 65.8 (d, *J*= 5.5 Hz, C-6), 55.5 (OMe); HRMS (ESI): *m/z* calcd [M+H]<sup>+</sup> for C<sub>56</sub>H<sub>59</sub>O<sub>15</sub>P<sub>3</sub>: 1065.31396, found: 1065.31364.

**Methyl  $\alpha$ -D-glucopyranoside 2,3,6-trisphosphate triethylammonium salt (4)** Methyl 4-*O*-benzyl- $\alpha$ -D-glucopyranoside 2,3,6-tris(dibenzyl phosphate) (22) (55.5 mg, 0.052 mmol) was dissolved in MeOH (2.8 mL). To this solution, ultrapure water (0.28 mL) was added dropwise, ensuring that the white precipitate that formed returned to solution. 20% Pd(OH)<sub>2</sub>/C ( $\geq$ 50% wet, 27.8 mg) was added to the solution and the flask was flushed with hydrogen. The reaction was left to stir under hydrogen at room temperature overnight. The palladium catalyst was filtered off with a PTFE filter and the solution was concentrated to yield the product as a free acid. No purification was deemed necessary. The free acid was converted to the triethylammonium salt through the addition of triethylamine to the free acid followed by concentration *in vacuo*. The product was lyophilised and collected as a colourless glass (24.8 mg, 0.034 mmol, 65% yield). [ $\alpha$ ]<sup>22</sup><sub>D</sub> 38.2 (*c*= 1.00, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  4.91 (d, *J*= 3.6 Hz, 1H, H-1), 4.35 (q, *J*= 8.7 Hz, 1H, H-3), 4.17 (ddd, *J*= 11.0, 5.2, 2.0 Hz, 1H, H-6), 4.07-3.98 (m, 2H, H-2, H-6), 3.72-3.68 (m, 1H, H-5), 3.54 (dd, *J*= 9.8, 8.8 Hz, 1H, H-4), 3.39 (s, 3H, OMe), 3.11 (q, *J*= 7.3 Hz, approx. 18H, TEA CH<sub>2</sub>CH<sub>3</sub>), 1.28 (t, *J*= 7.3 Hz, approx. 27H, TEA CH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (CD<sub>3</sub>OD, 162 MHz):  $\delta$  2.21, 1.25, 0.94; <sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz):  $\delta$  100.2 (C-1),

78.5 (t,  $J = 5.7$  Hz, C-3), 75.3 (t,  $J = 4.9$  Hz, C-2), 72.2 (d,  $J = 8.3$  Hz, C-5), 71.7 (C-4), 65.6 (d,  $J = 5.2$  Hz, C-6), 55.5 (OMe), 47.2 (TEA,  $\text{CH}_2\text{CH}_3$ ), 9.1 (TEA,  $\text{CH}_2\text{CH}_3$ ). HRMS (ESI):  $m/z$  calcd  $[\text{M-H}]^-$  for  $\text{C}_7\text{H}_{17}\text{O}_{15}\text{P}_3$ : 432.97075, found: 432.97076.

**Methyl 3-*O*-benzyl- $\alpha$ -D-glucopyranoside 2,4,6-tris(dibenzyl phosphate) (25)** Methyl 3-*O*-benzyl- $\alpha$ -D-glucopyranoside (**24**) (51.9 mg, 0.183 mmol) was dissolved in dry DCM (2 mL) and the solution was put under argon. 5-Phenyl-1*H*-tetrazole (160 mg, 1.10 mmol, 6 equiv) was added to the solution, followed by dibenzyl diisopropylphosphoramidite (0.30 mL, 0.82 mmol, 4.5 equiv). The reaction was allowed to stir at room temperature overnight. The next day, after the confirmation of successful phosphorylation with  $^{31}\text{P}$ NMR, the reaction flask was cooled to  $-78^\circ\text{C}$  and mCPBA (270.7 mg, 70% purity, 1.10 mmol, 6 equiv.) was added. The reaction was allowed to stir at room temperature for 10 min before the solution was diluted with EtOAc (50 mL), washed with 10%  $\text{Na}_2\text{SO}_3$  solution (2 x 30 mL), dried over  $\text{MgSO}_4$  and concentrated to yield the crude product. The product was purified with flash chromatography (petroleum ether/EtOAc, 0-100%) to yield the pure product as a colourless oil (138.1 mg, 0.130 mmol, 71% yield).  $[\alpha]_D^{25}$  41.4 ( $c = 0.61$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.35-7.08 (m, 35H, Ar), 5.03 (s, 2H,  $\text{CH}_2\text{Ph}$  x2), 5.01 (s, 2H,  $\text{CH}_2\text{Ph}$  x2), 4.98 (d,  $J = 3.2$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.97 (d,  $J = 3.2$  Hz, 1H,  $\text{CH}_2\text{Ph}$ ), 4.94-4.85 (m, 6H, H-1,  $\text{CH}_2\text{Ph}$  x5), 4.81-4.75 (m, 3H,  $\text{CH}_2\text{Ph}$  x3), 4.43-4.18 (m, 4H, H-2, H-3, H-6 x2), 3.98 (t,  $J = 9.3$  Hz, 1H, H-4), 3.86 (dd,  $J = 10.0, 5.2$  Hz, 1H, H-5), 3.28 (s, 3H, OMe);  $^{31}\text{P}$ NMR ( $\text{CDCl}_3$ , 162 MHz):  $\delta$  -0.98, -1.73, -1.85;  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  138.1 (Ar), 136.0 (Ar), 135.9 (Ar), 135.8-132.6 (m, Ar), 128.7-128.5 (m, Ar), 128.3 (Ar), 128.0 (m, Ar), 127.8 (Ar), 127.7 (Ar), 127.5 (Ar), 97.6 (C-1), 78.3 (m, C-4), 76.7 (m, C-2 or 3), 75.0 (C-2 or 3,  $\text{CH}_2\text{Ph}$ ), 69.6 (m,  $\text{CH}_2\text{Ph}$ ), 69.4 (m,  $\text{CH}_2\text{Ph}$ ), 68.9 (m, C-5), 66.1 (m, C-6), 55.7 (OMe); HRMS (ESI):  $m/z$  calcd  $[\text{M+H}]^+$  for  $\text{C}_{56}\text{H}_{59}\text{O}_{15}\text{P}_3$ : 1065.31396, found: 1065.31336.

**Methyl  $\alpha$ -D-glucopyranoside 2,4,6-trisphosphate triethylammonium salt (5)** Methyl 3-*O*-benzyl- $\alpha$ -D-glucopyranoside 2,4,6-tris(dibenzyl phosphate) (**25**) (132.4 mg, 0.124 mmol) was dissolved in MeOH (6.6 mL). Ultrapure water (0.66 mL) was added dropwise to the solution, ensuring that the precipitate formed upon addition was able to dissolve back into solution. 20%  $\text{Pd}(\text{OH})_2/\text{C}$  ( $\geq 50\%$  wet, 66.2 mg) was added to the solution and the reaction flask was flushed with hydrogen. The reaction was allowed to stir at room temperature for 24 h, after which the catalyst was filtered off and the collected filtrate was evaporated to yield the product as a free acid. No purification steps were deemed to be necessary, but triethylamine was added to sharpen the phosphorus NMR signals and to convert the product from the free acid into the triethylammonium salt. The product was concentrated *in vacuo* before being lyophilised and

collected as a colourless glass (86 mg, 0.10 mmol, 94% yield).  $[\alpha]_D^{22}$  39.3 ( $c=0.43$ , MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  4.91 (d,  $J=2.8$  Hz, 1H, H-1), 4.26-4.16 (m, 2H, H-3, H-6), 4.05-3.98 (m, 3H, H-2, H-4, H-6), 3.70 (brd,  $J=9.8$  Hz, 1H, H-5), 3.39 (s, 3H, OMe), 3.12 (q,  $J=7.5$  Hz, approx. 18H, TEA  $\text{CH}_2\text{CH}_3$ ), 1.29 (t,  $J=7.5$  Hz, approx. 27H, TEA  $\text{CH}_2\text{CH}_3$ );  $^{31}\text{PNMR}$  ( $\text{CD}_3\text{OD}$ , 162 MHz):  $\delta$  1.99, 1.75, 1.32;  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta$  100.2 (C-1), 76.3 (d, 5.6 Hz, C-2 or 4), 74.6 (d, 3.4 Hz, C-2 or 4), 73.5 (d, 5.5 Hz, C-3), 71.1 (m, C-5), 63.9 (C-6), 55.6 (OMe), 47.0 (TEA  $\text{CH}_2\text{CH}_3$ ), (TEA,  $\text{CH}_2\text{CH}_3$ ) 9.3. HRMS (ESI):  $m/z$  calcd  $[\text{M}-\text{H}]^-$  for  $\text{C}_7\text{H}_{17}\text{O}_{15}\text{P}_3$ : 432.97075, found: 432.97063.

**HPLC.** For analysis and stability experiments, the sugar phosphates were resolved by anion exchange HPLC on a 3x250 mm CarboPac PA200 column (Dionex) fitted with 3x50 mm guard cartridge of the same material. Compounds were eluted with a gradient derived from buffer reservoirs containing water (A) and 0.6 M methanesulfonic acid (B) delivered at a flow rate of  $0.4 \text{ mL min}^{-1}$  according to the following schedule: time (min), % B; 0, 0; 20, 80; 21, 0; 31, 0. Compounds were detected with the phosphate detection reagent of Phillippy and Bland, 1988.<sup>62</sup> For this purpose, the column eluate was mixed in a mixing Tee with a solution of 0.1% (w/v) ferric nitrate nonahydrate in 2% (w/v) perchloric acid delivered at a flow rate of  $0.2 \text{ mL min}^{-1}$  and passed through a 0.192 mL internal volume knitted reaction coil before transfer to a UV detector set at 290 nm. Typically, samples of  $40 \mu\text{L}$  of  $500 \mu\text{M}$  solutions in water were injected. Data were exported from the ChromNav2 software as x,y data files and redrawn in GraFit.v7.<sup>63</sup>

## Biology methods

**Materials.** HEK-293 cells with all three Ins(1,4,5) $\text{P}_3$ R subtypes disrupted using CRISPR/Cas9 technology (HEK-3KO)<sup>64</sup> were from Kerfast (Boston, USA). Dulbecco's modified Eagle's medium/nutrient mixture F-12 with GlutaMAX (DMEM/F-12 GlutaMAX) and Mag-fluo-4 AM were from ThermoFisher. TransIT-LT1 transfection reagent was from GeneFlow (Elmhurst, Lichfield, UK). Most chemicals and foetal bovine serum (FBS) were from Sigma-Aldrich (Gillingham, UK). Cyclopiazonic acid (CPA) was from Tocris (Bristol, UK). G418 was from Formedium (Norfolk, UK). Half-area 96-well black-walled plates were from Greiner. Ins(1,4,5) $\text{P}_3$  was from Enzo (Exeter, UK). [ $^3\text{H}$ ]-Ins(1,4,5) $\text{P}_3$  was from Perkin Elmer.

**Cell Culture and Transfection.** HEK cells were cultured in DMEM/F-12 GlutaMAX medium supplemented with 10% FBS ( $37^\circ\text{C}$  in 95% air and 5%  $\text{CO}_2$ ). Cells were either passaged or used for experiments when they reached confluence.

HEK cells expressing only Ins(1,4,5)P<sub>3</sub>R1 (HEK-Ins(1,4,5)P<sub>3</sub>R1) were generated by transfecting HEK-3KO cells with the gene encoding rat Ins(1,4,5)P<sub>3</sub>R1 (lacking the S1 splice site)<sup>26</sup> cloned into pcDNA3.1(-)/Myc-His B plasmid<sup>65</sup> using TransIT-LT1 reagent following the manufacturer's instructions. To generate stable cell lines, cells were passaged 48 h after transfection in medium with G418 (1 mg/mL). Selection was maintained for 2 weeks, and medium was changed every 3 days. Monoclonal cell lines were selected by plating cells (~1 cell/well) into 96-well plates in medium containing G418 (1 mg/mL). After 4 days, wells with only one cell were identified and the cells were allowed to reach confluence. These cell lines were then expanded and their expression of Ins(1,4,5)P<sub>3</sub>R1 was confirmed by western blot using an anti-Ins(1,4,5)P<sub>3</sub>R1 antibody.<sup>26</sup>

**Ca<sup>2+</sup> Release Assays.** The free [Ca<sup>2+</sup>] within the lumen of the endoplasmic reticulum (ER) was measured using the low-affinity Ca<sup>2+</sup> indicator Mag-fluo-4.<sup>66,67</sup> The ER of HEK-Ins(1,4,5)P<sub>3</sub>R1 cells was loaded with the Ca<sup>2+</sup> indicator by incubating cells (2.4 x 10<sup>7</sup> cells/mL, 1 h, 22 °C) in HEPES-buffered saline (HBS; 135 mM NaCl, 5.9 mM KCl, 11.6 mM HEPES, 1.5 mM CaCl<sub>2</sub>, 11.5 mM glucose, 1.2 mM MgCl<sub>2</sub>, pH 7.3) supplemented with BSA (1 mg/mL), Pluronic F127 (0.4 mg/mL) and Mag-fluo-4 AM (20 μM). Cells were then suspended in Ca<sup>2+</sup>-free cytosol-like medium (CLM: 20 mM NaCl, 140 mM KCl, 1 mM EGTA, 20 mM Pipes, 2 mM MgCl<sub>2</sub>, pH 7.0) and permeabilized with saponin (10 μg/mL, 3 min, 37 °C). Permeabilized cells were centrifuged (650 xg, 3 min), and incubated in CLM (7 min, 37 °C) to allow the Ca<sup>2+</sup> stores to empty. Cells were then centrifuged (650 xg, 3 min) and re-suspended in CLM without Mg<sup>2+</sup>, but supplemented with 375 μM CaCl<sub>2</sub> to give a final free [Ca<sup>2+</sup>] of 220 nM after addition of 1.5 mM MgATP. Cells (~4 x 10<sup>5</sup>/well) were added to poly-L-lysine-coated half-area 96-well black-walled plates. Fluorescence was recorded at 20 °C at intervals of 1.44 s using a FlexStation III plate-reader (Molecular Devices, Sunnyvale, CA, USA) with excitation and emission wavelengths of 485 nm and 520 nm, respectively. MgATP (1.5 mM) was added to initiate Ca<sup>2+</sup> uptake, and when the ER had loaded to steady-state (~2.5 min), cyclopiazonic acid (CPA, 10 μM) was added to inhibit the ER Ca<sup>2+</sup> pump. Ins(1,4,5)P<sub>3</sub> or other ligands were added after a further 60 s. The amount of Ca<sup>2+</sup> released was calculated as a percentage of the fluorescence signal from fully loaded stores (F<sub>full</sub>) minus the signal from non-loaded stores (F<sub>full</sub> - F<sub>empty</sub>).

**[<sup>3</sup>H]-Ins(1,4,5)P<sub>3</sub> binding to cerebellar membranes.** Cerebellar membranes, rich in Ins(1,4,5)P<sub>3</sub>R1 were obtained from cerebella of adult Wistar rats as previously described.<sup>26</sup> Equilibrium-competition binding assays (4°C, 5 min) were performed in medium comprising 50 mM Tris, 1 mM EDTA, pH 8.3 with [<sup>3</sup>H]-Ins(1,4,5)P<sub>3</sub> (19.3 Ci/mmol, 1.5 nM),

1 cerebellar membranes, and competing ligands.<sup>26</sup> Bound and free ligand were separated by centrifugation. Non-specific  
2 binding, determined by addition of 10  $\mu$ M Ins(1,4,5)P<sub>3</sub> was always <10% of total binding.  
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5 Biological data analysis is present in Supporting Information.  
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## 10 ASSOCIATED CONTENT

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## 17 SUPPORTING INFORMATION

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20 Full synthetic details for known compounds **8-10**, **21**, **23**, **24**; overlays, molecular docking and ligand interaction diagrams for **2**, **3**, **6**, **7**;  
21 HPLC data for compounds **2-7**; stability studies of compounds **6** and **7**; biological data analysis details (SI-1); and NMR spectral data for all  
22 of the compounds (SI-2). This material is available free of charge via the Internet at <http://pubs.acs.org>.  
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## 28 AUTHOR INFORMATION

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## 50 ACKNOWLEDGMENT

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53 BVL (grant 101010) and CWT (grant 101844) are Wellcome Trust Senior Investigators.  
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## ABBREVIATIONS

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3 AdA, Adenophostin A; ATP, adenosine triphosphate; COSY, correlation spectroscopy; mCPBA, *meta*-chloroperoxybenzoic acid; ; CPA,  
4 cyclopiazonic acid; cryo-EM, cryogenic electron microscopy; CSA, camphorsulfonic acid; DCM, dichloromethane; EDTA,  
5 ethylenediaminetetraacetic acid; ER, endoplasmic reticulum; FBS, fetal bovine serum; HBS, HEPES buffered saline; HEK, human  
6 embryonic kidney; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high performance liquid chromatography; HRMS,  
7 high resolution mass spectrometry; HSQC, heteronuclear single quantum coherence; IBC, Ins(1,4,5)P<sub>3</sub> binding core; Ins(1,4,5)P<sub>3</sub>, *D*-*myo*-  
8 inositol 1,4,5-trisphosphate; Ins(1,4,5)P<sub>3</sub>R, *D*-*myo*-inositol 1,4,5-trisphosphate receptor; NMR, nuclear magnetic resonance; SAR, structure-  
9 activity relationship; THF, tetrahydrofuran; TMS, tetramethylsilane.  
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