Supporting Information (SI-1) for

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_3 receptor

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Predicted binding modes of compounds 2-7 in Ins(1,4,5)P$_3$R

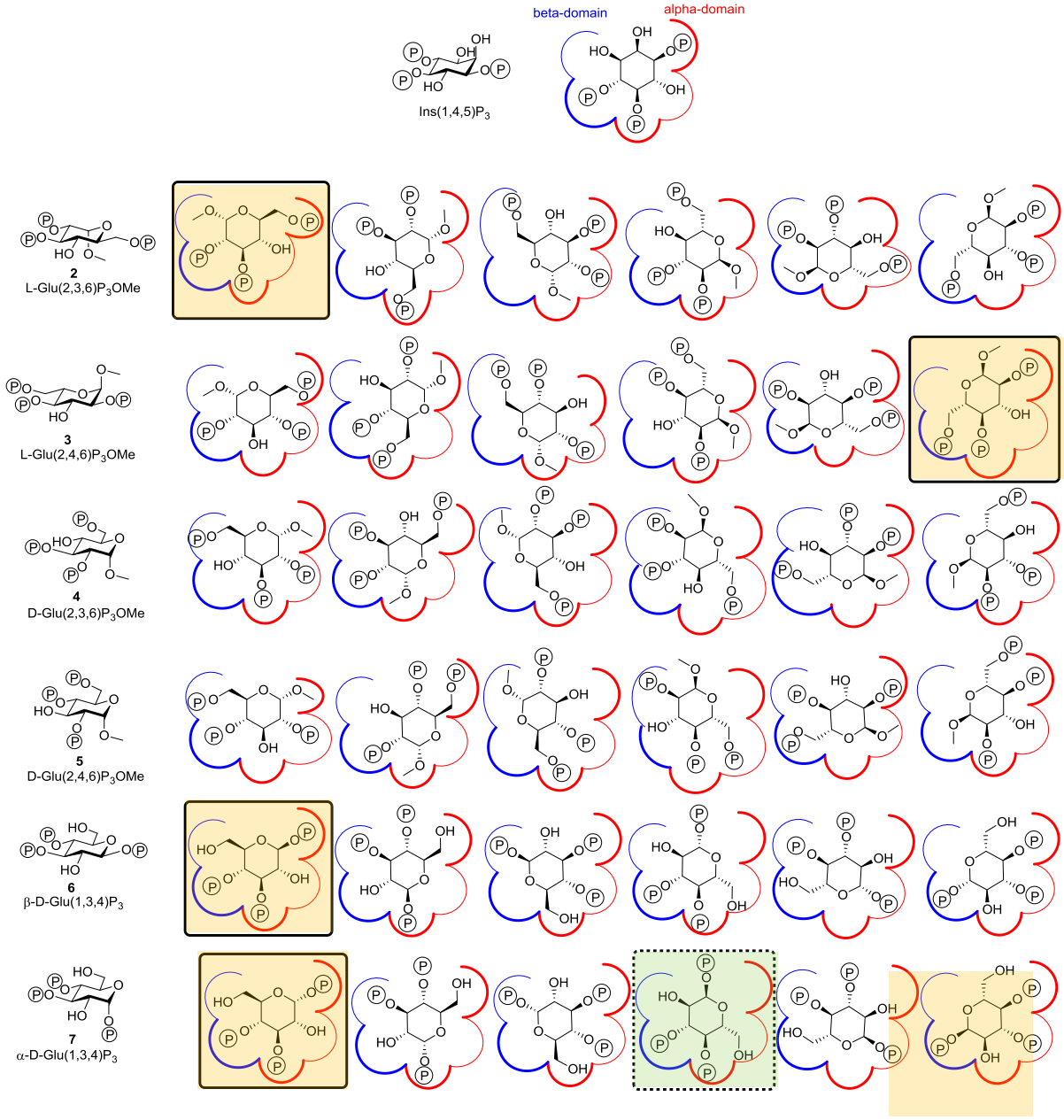
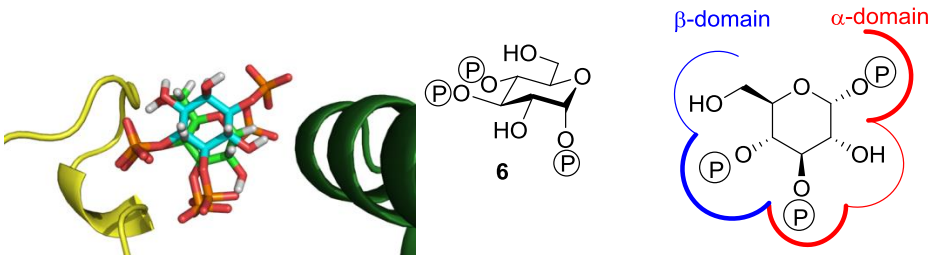


Figure S1. Possible binding modes for the Ins(1,4,5)P$_3$R ligands. The α- and β-domains of the IBC are color coded and the regions in which phosphates are required to bind are in bold. The likely binding modes that could plausibly mimic Ins(1,4,5)P$_3$ binding are highlighted in yellow, and an additional possible binding mode for **7** has been highlighted in green.

Molecular modeling of compounds 2, 3, 6 and 7

A



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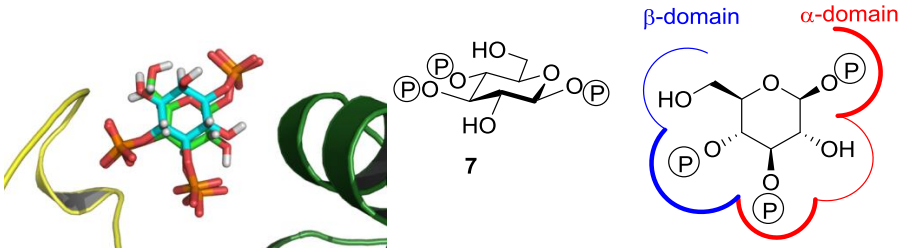


Figure S2. The highest scoring docking conformations of **6** (carbon atoms shown in green, row A) and **7** (green carbons, row B) using GOLD software and the 1N4K X-ray crystal structure[1] of the type 1 $InsP_3R$ IBC compared to the crystallographic $Ins(1,4,5)P_3$ (aqua carbons).

Molecular Modeling Methods

The X-ray crystal structure of the IBC of mouse Type 1 $Ins(1,4,5)P_3R$ in complex with $Ins(1,4,5)P_3$ (PDB ID 1N4K) was used in this work.[1] Compounds **1**-**6** were built and minimized using Chem3D version 15.1 and Mercury version 3.10. GOLD version 5.6.1 was used for docking experiments. The compounds were docked 100 times, with two water molecules in the binding site (1139 and 1198) being allowed to toggle and spin while the remaining waters molecules were removed. The lysine residues in the binding site (K412, K508 and K569) were permitted constrained movement. The highest scoring solutions were exported and figures were prepared using PyMOL (DeLano Scientific LLC).

Docking of Ins(1,4,5)P_3 itself was able to reproduce the binding conformation in the X-ray structure with the inclusion of two of the waters (assigned labels 1139 and 1198 in the crystal structure) and allowing limited movement of the lysine residue side chains.[1] The calculated binding modes from an optimised GOLD docking protocol also predicted that the axial phosphate in α-D-glucopyranosyl 1,3,4-trisphosphate (**6**) would cause the ligand to shift slightly to accommodate the axial steric bulk, disrupting it from overlapping as closely as **7** with the positioning of the bound Ins(1,4,5)P_3. In docking, the axial 1-phosphate of **6** seems to be able to interact more with the side chain of Arg504 and less with the side chain of Arg568 than the auxiliary phosphate in Ins(1,4,5)P_3 (Figure S2).

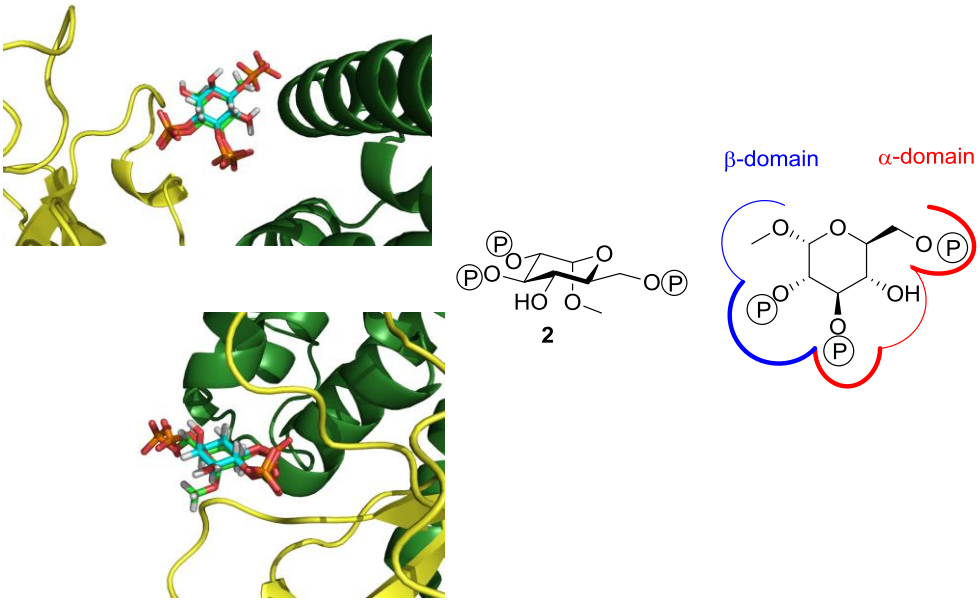


Figure S3. Two views showing the most favorable docking conformation of methyl α-L-glucopyranosyl 2,3,6-trisphosphate (**2,** green carbon atoms) to the 1N4K crystal structure[1] of InsP_3R as predicted by the molecular docking experiments. Crystallographic Ins(1,4,5)P_3 (**1**, aqua carbons) is depicted for comparison, α-domain shown in green; β-domain shown in yellow.

In docking studies, there was found to be sufficiently significant overlap of the vicinal phosphates of **2** with those of Ins(1,4,5)P_3 such that the interactions with the residues in the binding site do not appear to deviate from those with Ins(1,4,5)P_3. The auxiliary phosphate (extended in **2**) appears still to interact with the side chain of Arg568, although the residue is predicted to bend from its usual position to accommodate the increased steric bulk of the extended phosphate in that region (Figure S3).

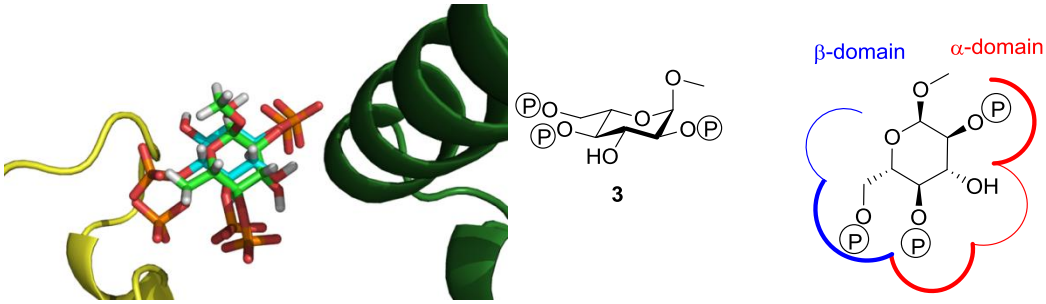
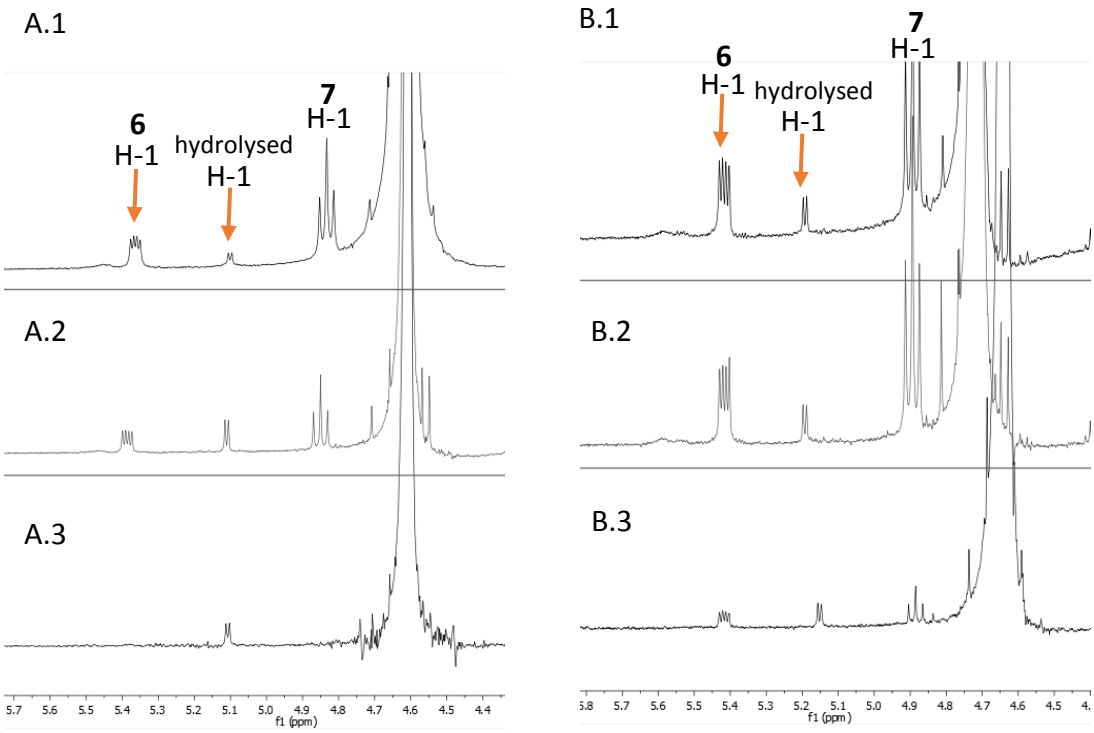
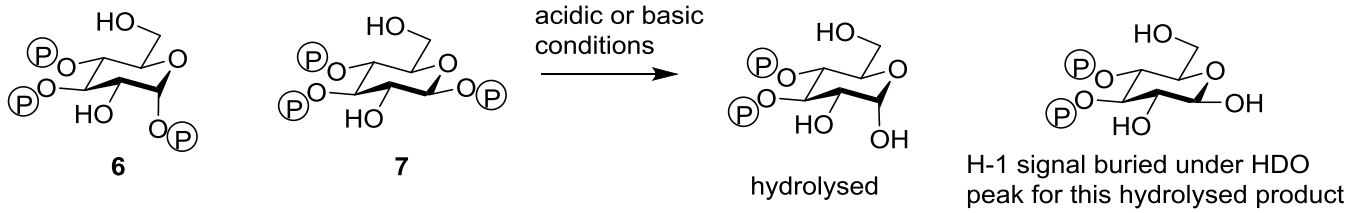


Figure S4. Docking prediction for methyl α-L-glucopyranosyl 2,4,6-trisphosphate (**3,** green) to the 1N4K crystal structure[1] of $InsP_3R$ as predicted by the molecular docking experiments. Crystallographic Ins(1,4,5)P_3 (**1**, aqua) is depicted for comparison, α-domain shown in green; β-domain shown in yellow.

The extended phosphate of compound **3** is expected to be accommodated in the region of the binding site normally occupied by the 4-phosphate of Ins(1,4,5)P_3 and to interact with the side chain of Arg265, resulting in the central ring of the ligand being slightly shifted from the position of Ins(1,4,5)P_3 (Figure S4). The axial *O*-methyl of **3** is then able to point out of the binding site and into solvent.

Stability study





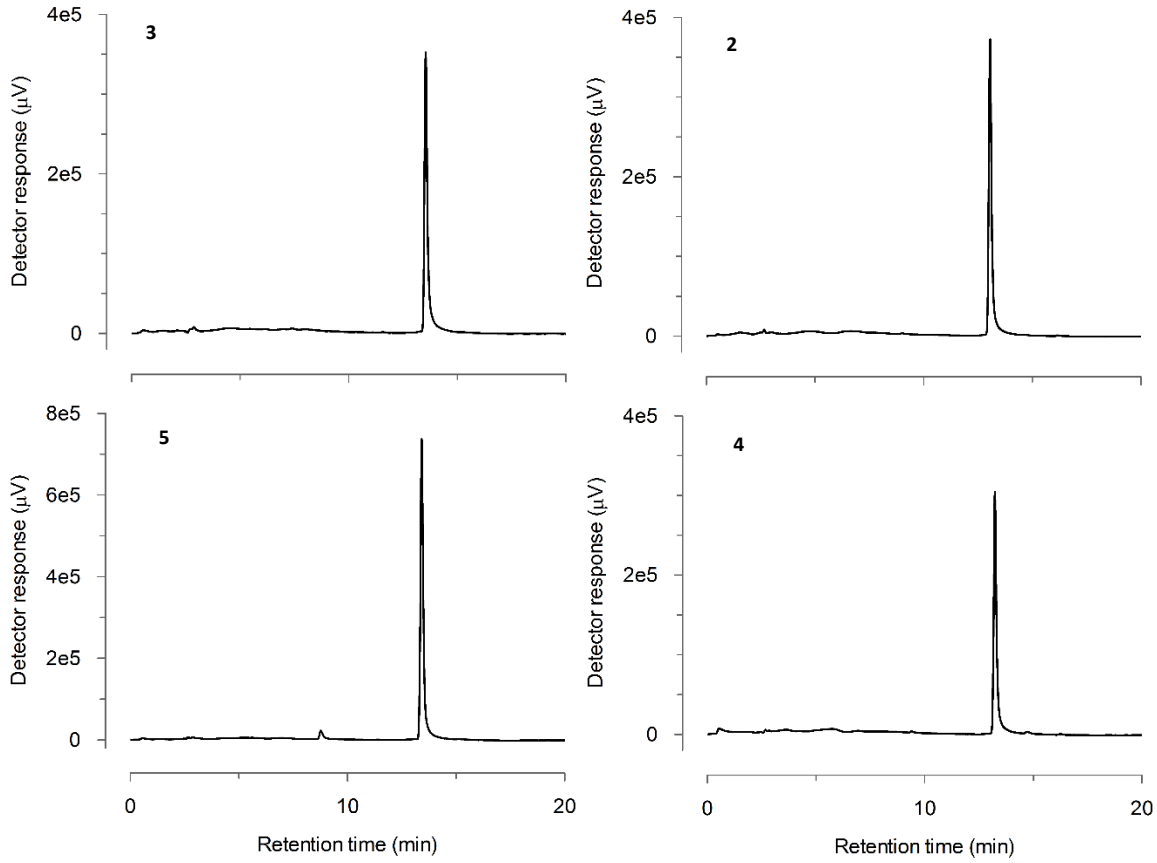
NMR TRACE	CONDITIONS	RELATIVE INTEGRATION OF 7 COMPARED TO NORMALISED 6 PEAK
A.1	Neutral pH at RT	2.3
A.2	pH 3 for a week at RT	1.6
A.3	pH 1 for 1 day at RT	complete hydrolysis
B.1	Neutral pH at RT	2.3
B.2	pH 10 for a week at RT	2.3
B.3	pH 14 for 1 day at RT	1.7

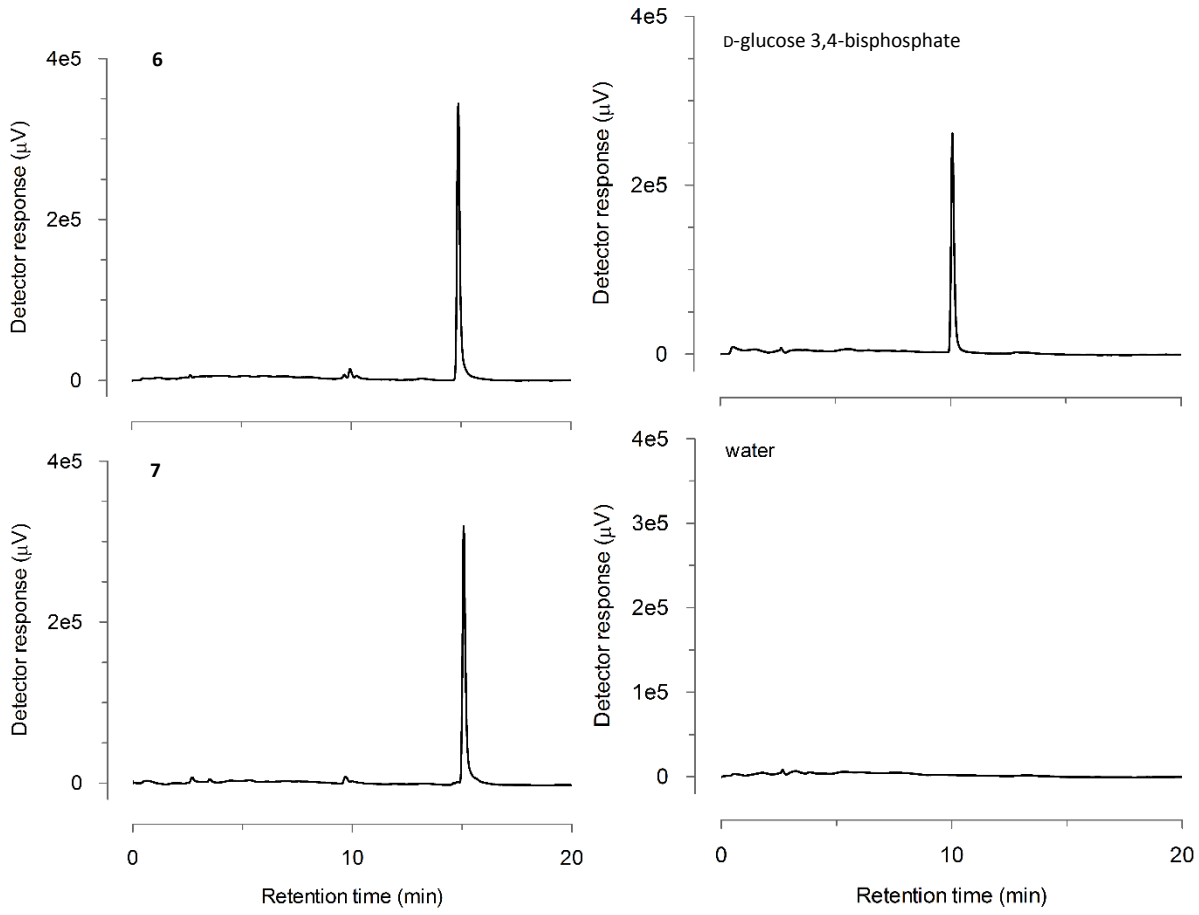
Figure S5. Stability test for α-D-glucopyranosyl 1,4,5-trisphosphate (**6**) and β-D-glucopyranosyl 1,4,5-trisphosphate (**7**). The **A.** column on the left shows a portion of the ^{1}H NMR of the compounds under increasingly acidic conditions and the **B.** column on the right shows a portion of the ^{1}H NMR of the compounds under increasingly basic conditions.

In Figure S5, a portion of the ^{1}H NMR spectrum showing the relevant peaks of interest can be seen from experiments performed under acidic and basic conditions. The differences between the integration under the isomer peaks were tracked (data in the accompanying table). By monitoring this ratio, we are able to determine the relative hydrolysis of the two epimers when exposed to different conditions.

It should be noted that the hydrolysis observed under neutral conditions at the beginning of the stability study is the result of degradation of the mixture of the less stable protected precursors of **6** and **7** (**17** and **18**). Both **6** and **7** required more extreme pH values than initially expected to degrade. As a result, we are confident that both compounds **6** and **7** remained intact throughout the biological assays.

HPLC data


3
2
5
4
Detector response (μV)
4e5
2e5
0
8e5
6e5
0
10
20
Retention time (min)



Compound	Mean integration %	Standard deviation %
methyl α-L-glucopyranosyl 2,3,6-trisphosphate (**2**)	99.15	0.06
methyl α-L-glucopyranosyl 2,4,6-trisphosphate (**3**)	99.83	0.08
methyl α-D-glucopyranoside 2,3,6-trisphosphate (**4**)	97.21	0.71
methyl α-D-glucopyranoside 2,4,6-trisphosphate (**5**)	96.87	0.23
α-D-glucopyranosyl 1,3,4-trisphosphate (**6**)	94.53	0.10
β-D-glucopyranosyl 1,3,4-trisphosphate (**7**)	95.43	0.58
D-glucose 3,4-bisphosphate	97.20	1.76

Figure S6. HPLC traces confirming the purity for compounds **2**, **3**, **4**, **5**, **6** and **7**. The table gives values of the integrated peak area as a % of total peaks in each chromatogram.

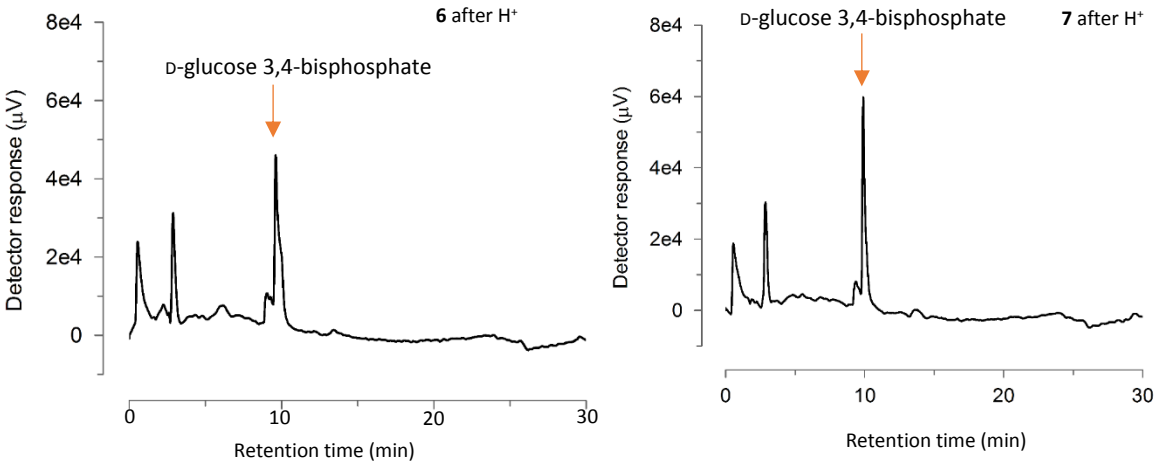


Figure S7. HPLC traces of **6** and **7** after they have been exposed to strongly acidic conditions showing the products of hydrolysis.

Inspection of Figure S6 shows elution of D-glucose 3,4-bisphosphate at 10.2 min. Acid treatment of compounds **6** and **7** generated a peak with similar retention time (Figure S7), rendering D-glucose 3,4-bisphosphate the probable hydrolysis product of both compounds as expected. Both peaks have an earlier-eluting shoulder that may be D-glucose 2,4-bisphosphate arising from acid-catalysed phosphate migration.

References

(1) Bosanac, I.; Alattia, J.-R.; Mal, T. K.; Chan, J.; Talarico, S.; Tong, F. K.; Tong, K. I.; Yoshikawa, F.; Furuichi, T.; Iwai, M.; et al. Structure of the Inositol 1, 4, 5-Trisphosphate Receptor Binding Core in Complex with Its Ligand. *Nature* **2002**, *420* (December), 3–7. https://doi.org/10.1038/nature01268.