Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 453–458

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Simplification of adenophostin A defines a minimal structure for potent glucopyranoside-based mimics of D-*myo*-inositol 1,4,5-trisphosphate

Rachel D. Marwood[‡], Andrew M. Riley[‡], Vanessa Correa[§]

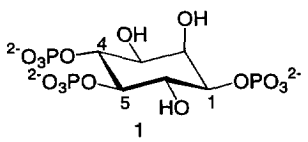
Colin W. Taylor[§] and Barry V. L. Potter[‡*]

[‡]*Wolfson Laboratory of Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK.*
[§]*Department of Pharmacology, Tennis Court Road, University of Cambridge, Cambridge, CB2 1QJ, UK.*

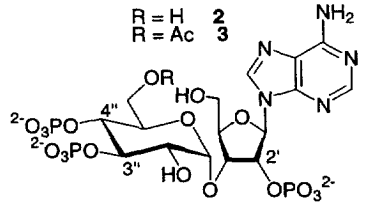
Received 29 October 1998; accepted 22 December 1998

Abstract: The synthesis of 1-*O*-[(3*S*,4*R*)-3-hydroxytetrahydrofuran-4-yl]-α-D-glucopyranoside 3,4,3'-trisphosphate (**7**), a novel Ca^{2+} mobilising agonist at the Ins(1,4,5)P_3 receptor, designed by excision of two motifs of adenophostin A is reported, defining a potential minimal structure for potent glucopyranoside-based agonists of Ins(1,4,5)P_3 receptors. © 1999 Elsevier Science Ltd. All rights reserved.

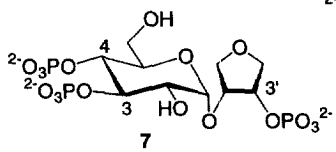
The role of the second messenger D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] in Ca^{2+} signalling has been the subject of close scrutiny for several years. Its major function is to mediate the release of Ca^{2+} from intracellular stores on binding to its receptor[1]. With the preparation of Ins(1,4,5)P_3 analogues and their biological evaluation has come a greater understanding of the structural motifs required for binding and Ca^{2+} release,[2] although synthetic analogues whose potency exceed that of Ins(1,4,5)P_3 have not yet been designed.



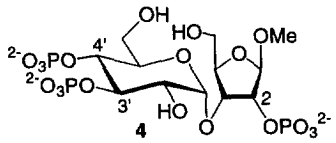
1



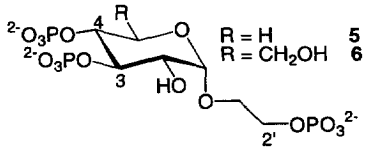
R = H **2**
R = Ac **3**



7



4



R = H **5**
R = CH_2OH **6**

*Email: B.V.L.Potter@Bath.ac.uk Fax: +44 1225 826114

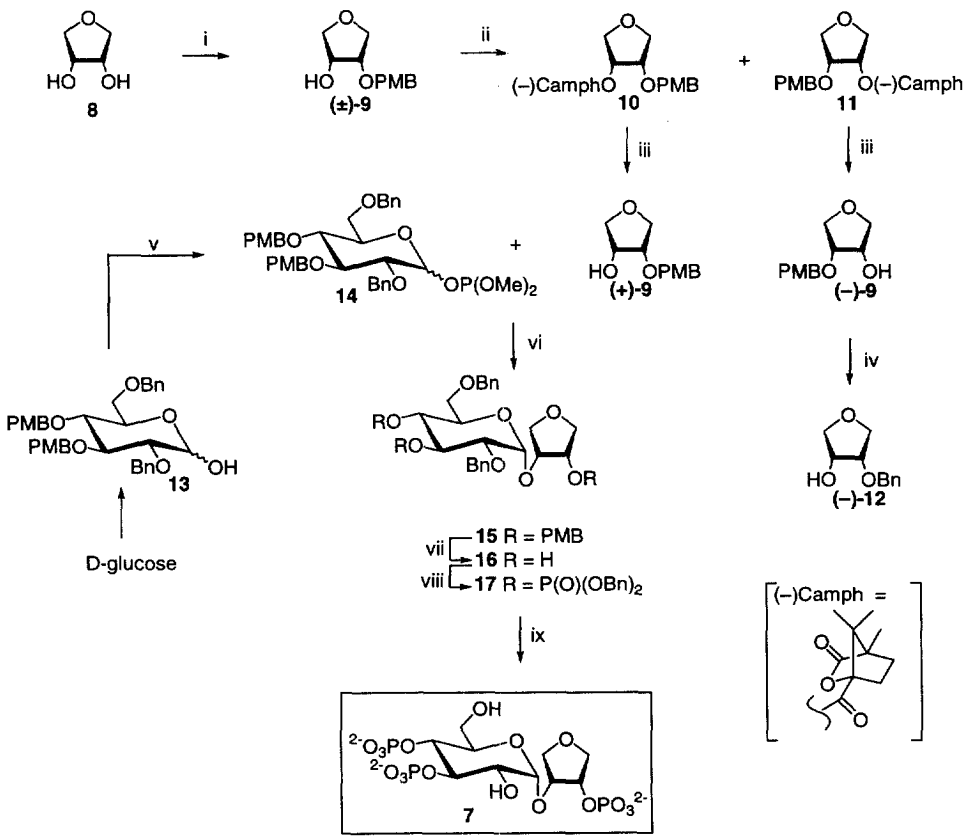
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00006-2

The discovery in 1993 of adenophostins A and B (**2** and **3**),[3] isolated from a culture broth of *Penicillium brevicompactum*, brought with it a new perspective on the pharmacophore required for activity at Ins(1,4,5)P_3 receptors. With potencies some 10 to100-fold greater than Ins(1,4,5)P_3,[4,5,10] this was the first time that derivatives of a structure other than inositol had exhibited Ins(1,4,5)P_3-like activity. Although similarities exist between Ins(1,4,5)P_3 and the adenophostins, i.e. the 4,5-(bisphosphate)/6-hydroxyl and the 3",4"-(bisphosphate)/2"-hydroxyl motifs respectively, the unique structures of the adenophostins and their unusual potencies have stimulated great interest in syntheses aimed at identifying the pharmacophore responsible for their enhanced activity.

As part of an investigation to determine the importance of the adenine component of the adenophostins we[6] and others[7] initially synthesised 2-hydroxyethyl α-D-glucopyranoside 2',3,4-trisphosphate (**6**), and another group synthesised the xylopyranoside equivalent **5**.[8] **6** Was found to be a full agonist, but with a potency some 10-fold lower than Ins(1,4,5)P_3 at the Ins(1,4,5)P_3 receptors of platelets,[9] SH-SY5Y neuroblastoma cells[7] and hepatocytes.[10] **6** Was, however, more potent than many inositol-based Ins(1,4,5)P_3 mimics, and showed that effective Ca^{2+}-releasing polyphosphates based on carbohydrates were a real possibility. Reasoning that activity might be improved by introducing a greater degree of rigidity into the molecule, we went on to synthesise various phosphorylated disaccharides incorporating a D-glucopyranosyl 3,4-bisphosphate moiety with an α-glycosidic linkage to a second sugar containing one or more phosphates. All proved to be full agonists at Ins(1,4,5)P_3 receptors, but with a range of activities,[9,10] the most potent being 3-*O*-(α-D-glucopyranosyl)-β-D-ribofuranoside 2,3',4' trisphosphate (ribophostin, **4**),[10,11] whose structure was closest to adenophostin A itself. The activity of **4** in displacement of [^{3}H]Ins(1,4,5)P_3 from the Ins(1,4,5)P_3 receptors of hepatic membranes and in release of Ca^{2+} from hepatocytes was found to be very similar to that of Ins(1,4,5)P_3 (which is still 10 to 100-fold less than that of adenophostin A). The lower activity of **4** relative to the adenophostins suggested that the adenine component (or a similar structure) was somehow required for potency to surpass that of Ins(1,4,5)P_3. The finding of Ins(1,4,5)P_3-like activity for **4**, however, raises the question as to whether it represents the minimal structure sufficient for such activity in carbohydrate polyphosphates. In particular, is a ribofuranose structure, with its 4-hydroxymethyl group, absolutely required or could further simplifications be made? There is also the possibility that the *O*-methyl group of **4**, which replaced the adenine of adenophostin A, might compromise activity. These considerations have now led us to develop a synthesis of 1-*O*-[(3*S*,4*R*)-3-hydroxytetrahydrofuran-4-yl]-α-D-glucopyranoside 3,4,3'-trisphosphate (**7**), an analogue that lacks both *O*-methyl and 4-hydroxymethyl moieties of **4**, but retains the rigidity of the ribofuranose ring. This also served the additional purpose of exploring the effect of a simple conformational restriction on the activity of **6**.

The synthesis of **7** required the coupling of a suitably protected glucopyranosyl donor to a tetrahydrofuran-based glycosyl acceptor. The acceptor **(+)-9** was prepared from commercially available 1,4-anhydroerythritol (**8**) by the optical resolution of the racemic *p*-methoxybenzyl ether (±)-**9**. Initial attempts to obtain (±)-**9** by straightforward monoalkylation of **8** using *p*-methoxybenzyl chloride/NaH/DMF failed, as only

dialkylated material was formed. (±)-**9** Was successfully prepared in high yield by reaction of **8** with *p*-methoxybenzylidene dimethyl acetal[12] followed by reduction of the crude mixture of epimeric acetal products with DIBAL-H. Reaction of (±)-**9** with (–)-(*S*)-camphanic chloride gave diastereoisomers **10** and **11** which were separated by flash chromatography and then recrystallised, yielding pure **10** and **11** each on a 5 g scale.[13] Saponification of the esters gave enantiomeric *p*-methoxybenzyl ethers (+)-**9** and (–)-**9**, which proved to be highly crystalline[14] [(±)-**9** was a liquid]. The absolute configurations of (+)-**9** and (–)-**9** were determined by converting (–)-**9** into a monobenzyl ether, which was identified as (–)-**12** by comparison of its optical rotation with that reported for the enantiomer.[15]



Reagents and conditions: i) a) *p*-methoxybenzylidene dimethyl acetal (1.05 equiv.), PTSA, DMF, 70 °C; b) DIBAL-H (2.5 equiv.), CH_2Cl_2, –78 °C; 90 % yield for two steps; ii) (–)-(*S*)-camphanic chloride, pyridine, 0 °C to rt, **10** (80 % yield), **11** (82 % yield); iii) NaOH, MeOH, reflux, 94–97 %; iv) a) NaH, BnBr, DMF; b) CF_3COOH, CH_2Cl_2; 87 % yield for two steps; v) $(MeO)_2PNEt_2$, tetrazole; vi) $AgClO_4$, $ZnCl_2$, dioxane, toluene, 4 Å sieves, 76 %; vii) CF_3COOH, CH_2Cl_2, 84 %; viii) a) $(BnO)_2PNPr^i_2$, tetrazole; b) MCPBA, –78 °C to rt; 82 % yield over two steps; ix) H_2, Pd–C, 40 psi, MeOH–H_2O, 93 %. PMB = *p*-methoxybenzyl.

The glycosyl intermediate **13** was prepared from D-glucose. Fischer glycosylation with allyl alcohol followed by tin-mediated benzylation furnished allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside. Subsequent alkylation with *p*-methoxybenzyl chloride/NaH/DMF followed by cleavage of the allyl glycoside gave **13**. Reaction with dimethyl *N,N*-diethylphosphoramidite in the presence of tetrazole resulted in the di-*O*Me phosphite (**14**) in an anomeric ratio of 52:48 α:β. This glycosyl phosphite was used without further purification. Zinc chloride and silver perchlorate promoted glycosylation[16] of **(+)-9** with **14** yielded solely the α-coupled product (H-1, δ 5.18, *J* 3.4 Hz) in good yield. Deprotection of the PMB protecting groups proceeded smoothly with 10 % trifluoroacetic acid in dichloromethane to give triol **16**. Phosphitylation and oxidation with MCPBA furnished the phosphate precursor **17**, which on deprotection by means of hydrogenation yielded the trisphosphate **7**, which we designate 'furanophostin'. This final product was purified on Q Sepharose resin eluting with a 0–1 mol dm^{-3} gradient of triethylammonium hydrogen carbonate, pH 7.5; **7** eluted at 0.50–0.55 mol dm^{-3} to give the triethylammonium salt[17] which was quantified by total phosphate assay before biological evaluation.

The effects of synthetic Ins(1,4,5)P_3 and **7** on unidirectional $^{45}Ca^{2+}$ efflux from the intracellular stores of permeabilised rat hepatocytes were determined using methods previously described.[10] Briefly, permeabilised hepatocytes (10^7 cells/mL) loaded to a steady state with $^{45}Ca^{2+}$ in a cytosol-like medium (CLM)[10] at 37 °C were diluted 5-fold into the same medium but supplemented with thapsigargin (1 μM) to prevent further $^{45}Ca^{2+}$ uptake. After 15 s, appropriate concentrations of either Ins(1,4,5)P_3 or **7** were added and after a further 60 s the $^{45}Ca^{2+}$ contents of the stores were determined using a rapid filtration method.[10]

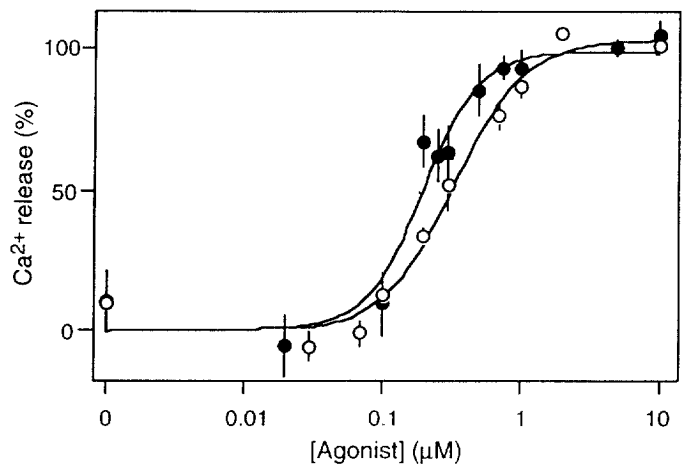


Figure. $^{45}Ca^{2+}$ release from permeabilised hepatocytes by Ins(1,4,5)P_3 (●) and **7** (○). For each experiment, the $^{45}Ca^{2+}$ released in response to each concentration of agonist was determined and the concentration-effect relationship was then fitted to a logistic equation[10] from which the maximal response was determined. The results (means ± S.E.M. of 3–5 independent determinations) are shown as percentages of that maximal response.

Maximally effective concentrations (10 μM) of either Ins(1,4,5)P_3 or **7** released the same fraction of the intracellular Ca^{2+} stores, 29 ± 6 % and 29 ± 1 % respectively. A similar response, release of 28 ± 2 % of the actively sequestered Ca^{2+}, was evoked by the simultaneous addition of maximal concentration of both agonists. The concentration of **7** required to cause half-maximal Ca^{2+} release (EC_{50}) was only 1.7-fold higher than that for Ins(1,4,5)P_3 (Figure), and the responses to both agonists were positively co-operative (Table).

	EC_{50}	*h*	n
Ins(1,4,5)P_3	194 ± 30 nM	2.08 ± 0.22	3
7	329 ± 48 nM	2.18 ± 0.46	5

Table. $^{45}Ca^{2+}$ release data for Ins(1,4,5)P_3 and **7**. The EC_{50} values and Hill coefficients (*h*) were separately determined for n independent experiments by fitting results to a logistic equation.[10] Results are shown as means ± S.E.M.

These results show that **7** behaves as a full agonist in this assay, with a potency in Ca^{2+} release assays similar to those of Ins(1,4,5)P_3 and to ribophostin (**4**). This finding enables us to draw several conclusions relating to the structural basis for the activity of **4**, **6**, **7** and adenophostin A. First, the similar behaviour of **4** and **7** indicates that the *O*-methyl group in **7** does not hinder activity, but neither does it enhance it. In a similar way, the 4-hydroxymethyl group of **4** is not essential for potent activity at Ins(1,4,5)P_3 receptors. The equivalent motif may yet play a role in the activity of adenophostin A, because the interaction of adenophostin A with the receptor binding site must in some way be different to that of **4** or **7**. Secondly, because **7**, which can be viewed as a conformationally restricted analogue of **6**, was more potent than **6**, the hypothesis that the limited activity of **6** was in part related to the flexibility of the ethylphosphate structure[6,7] is given strong support; it is now clear that conformational restriction alone can give rise to a marked enhancement of activity. Thirdly, since **4** and **7** are essentially equipotent with Ins(1,4,5)P_3 and more potent than other disaccharide-based Ins(1,4,5)P_3 analogues, it is probable that their common rigid five-membered ring structure delivers their 2- and 3'-phosphates respectively to an optimal position for Ins(1,4,5)P_3-like potency. Thus, the similar activities of **4** and **7** suggest that **7** may approach the minimal structure for potent agonism[18] in a simple carbohydrate-based polyphosphate mimic of Ins(1,4,5)P_3. Finally, furanophostin is still 10 to 100-fold less potent than the adenophostins. This finding supports previous arguments[10] that the adenine component of the adenophostins plays a pivotal role in their activity, by engaging with a nearby region of the Ins(1,4,5)P_3 receptor and stabilising binding interactions, and/or optimising the position of the 2'-phosphate at the binding site.

ACKNOWLEDGEMENTS:

We thank the Wellcome Trust for a Prize Studentship (RDM) and Programme Grant Support (045491 to BVLP and 039662 to CWT), and the BBSRC (CWT).

References and notes

1. Berridge, M.J. *Nature (London)* **1993**, *361*, 315–325.
2. Potter, B.V.L.; Lampe, D. *Angew. Chem. Int. Ed. Engl.* **1995** *34*, 1972–1993.
3. Takahashi, M.; Kagasaki, T.; Hosoya, T.; Takahashi, S. *J. Antibiot.* **1993**, *46*, 1643–1647.
4. Takahashi, S.; Kinoshita, T.; Takahashi, M. *J. Antibiot.* **1994**, *47*, 95–100; Takahashi, M.; Tanzawa, K.; Takahashi, S. *J. Biol. Chem.* **1994**, *269*, 369–372.
5. Hirota, J.; Michikawa, T.; Miyawaki, A.; Takahashi, M.; Tanzawa, K.; Okura, I.; Furuichi, T.; Miksoshiba, K. *FEBS Letters* **1995**, 248–252.
6. Jenkins, D.J.; Potter, B.V.L. *J. Chem. Soc. Chem. Commun.* **1995**, 1169–1170; Jenkins, D.J.; Potter, B.V.L. *Carbohydr. Res.* **1996**, 169–182.
7. Wilcox, R.A.; Erneux, C.; Primrose, W.U.; Gigg, R., Nahorski, S.R. *Mol. Pharmacol.* **1995**, *47*, 1204–1211.
8. Moitessier, N.; Chrétien, F.; Chapleur, Y.; Humeau, C. *Tetrahedron. Lett.* **1995**, *36*, 8023–8026. These authors reported **5** to be 10-fold less potent than Ins(1,4,5)P_3 in Ca^{2+} release from permeabilised hepatocytes.
9. Murphy, C.T.; Riley, A.M.; Lindley, C.J.; Jenkins, D.J.; Westwick, J.; Potter, B.V.L. *Mol. Pharmacol.* **1997**, *52*, 741–748.
10. Marchant, J.S.; Beecroft, M.D.; Riley, A.M.; Jenkins, D.J.; Marwood, R.D.; Taylor, C.W.; Potter, B.V.L. *Biochemistry* **1997**, *36*, 12780–12790.
11. Jenkins, D.J.; Marwood, R.D.; Potter, B.V.L. *J. Chem. Soc. Chem. Commun.* **1997**, 449–450.
12. Johansson, R.; Samuelsson, B. *J. Chem. Soc. Perkin Trans 1* **1984**, 2371–2374.
13. The diastereoisomeric purity of **10** and **11** was judged by examination of the camphanate methyl resonances in their respective ^{1}H NMR spectra after recrystallisation. In neither case was contamination with the other diastereoisomer seen, and the diastereoisomeric excess of each was accordingly estimated to be > 98 %. Data for **10:** Crystals from ether; mp 67–68 °C; R_f 0.24 (ether / pentane 2:1); $[\alpha]_D^{20}$ = –41 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.96 (3 H, s), 0.99 (3 H, s), 1.10 (3 H, s), 1.60–1.72 (1 H, m), 1.84–2.02 (2 H, m), 2.37–2.48 (1 H, m), 3.66 (1 H, t, *J* 8.3 Hz), 3.80 (3 H, s), 3.92–3.98 (2 H, m), 4.09 (1 H, dd, *J* 10.8 Hz, 4.6 Hz), 4.20 (1 H, dt, *J* 7.5 Hz, 4.9 Hz), 4.43, 4.54 (2 H, AB_q, J_{AB} 11.2 Hz), 5.47 (1 H, td, *J* 4.8 Hz, 2.6 Hz), 6.85–6.90 (2 H, m), 7.21–7.26 (2 H, m).
    Data for **11**: Crystals from diisopropyl ether; mp 81–83 °C; R_f 0.18 (ether / pentane 2:1); $[\alpha]_D^{22}$ = +27 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.89 (3 H, s), 1.05 (3 H, s), 1.10 (3 H, s), 1.64–1.74 (1 H, m), 1.86–2.07 (2 H, m), 2.35–2.46 (1 H, m), 3.66 (1 H, dd, *J* 8.4 Hz, 8.1 Hz), 3.80 (3 H, s), 3.90-3.97 (2 H, m), 4.09 (1 H, dd, *J* 10.6 Hz, 4.8 Hz), 4.15–4.22 (1 H, m), 4.44, 4.57 (2 H, AB_q, J_{AB} 11.5 Hz), 5.50 (1 H, td, *J* 4.8 Hz, 2.6 Hz), 6.84–6.89 (2 H, m), 7.22–7.28 (2 H, m).
14. (+)-**9**: mp 53–54.5 °C; $[\alpha]_D^{19}$ = +13 (*c* 1, $CHCl_3$); (–)-**9**: mp 53–54.5 °C; $[\alpha]_D^{19}$ = –13 (*c* 1, $CHCl_3$).
15. ^{1}H NMR spectral data for (–)-**12** agreed with those published for (+)-(3*S*,4*R*)-4-benzyloxytetrahydrofuran-3-ol (Altenbach, H.-J.; Wolf, E. *Tetrahedron: Asymmetry* **1993**, *4*, 2155–2158). However, these authors reported $[\alpha]_D^{23}$ +27.52 (*c* 1, MeOH) for this material, while (–)-**12** had $[\alpha]_D^{23}$ –26.5 (*c* 1, MeOH). This identifies (–)-**12** as (–)-(3*R*,4*S*)-4-benzyloxytetrahydrofuran-3-ol.
16. Watanabe, Y.; Nakamoto, C.; Yamamoto, T.; Ozaki, S. *Tetrahedron* **1994**, *50*, 6523–6536.
17. Data for **7**: $[\alpha]_D^{21}$ = +15 (*c* 0.57, MeOH); ^{1}H NMR (D_2O, 400 MHz ^{1}H-^{1}H COSY) δ_H 3.74 (1 H, dd, *J* 9.0 Hz, 3.2 Hz, H-2), 3.78–3.94 (5 H, m, H-5, H-6_A, H-6_B, H-2'$_A$, H-5'$_A$), 4.01–4.09 (3 H, m, H-4, H-2'$_B$, H-5'$_B$), 4.43–4.50 (2 H, m, H-3, H-4'), 4.80–4.89 (1H, m, H-3'), 5.25 (1 H, d, *J* 2.93 Hz, H-1). ^{31}P NMR (CD_3OD, 162 MHz) (^{1}H-coupled) δ_P 0.04 (d, J_{HP} 7.3 Hz), 0.82 (d, J_{HP} 7.6 Hz), 1.08 (d, J_{HP} 9.8 Hz).
18. While the biological evaluation of **7** was in progress, another group (Tatani, K.; Shuto, S.; Ueno, Y.; Matsuda, A. *Tetrahedron Lett.* **1998**, *39*, 5065–5068) reported a synthesis of **7**, together with preliminary biological data. Binding studies in porcine cerebella found **7** to be similar to Ins(1,4,5)P_3 in its affinity for Ins(1,4,5)P_3 receptors, although no information on Ca^{2+} release was given, and **7** could therefore not be designated as an agonist or antagonist.