Tools for Cell Signaling: Synthesis of the 3-Phosphatase-Resistant 1,3,4,5-InsP$_4$ Mimic, 1D-*myo*-Inositol 1,4,5-Trisphosphate 3-Phosphorothioate

Alan P. Kozikowski,*,† Abdul H. Fauq,† Robert A. Wilcox,‡ and Stefan R. Nahorski‡

Neurochemistry Research, Mayo Foundation for Medical Education and Research, 4500 San Pablo Road, Jacksonville, Florida 32224, and Department of Cell Physiology and Pharmacology, University of Leicester, P.O. Box 138, MSB, University Road, Leicester LE1 9HN, U.K.

Received January 19, 1994•

Summary: The synthesis of 1D-*myo*-inositol 1,4,5-trisphosphate 3-phosphorothioate, a 3-phosphatase-resistant analogue of 1,3,4,5-InsP$_4$ is reported, and this compound is shown to elicit $^{45}Ca^{2+}$ release with an EC$_{50}$ which is comparable to that of the natural metabolite, thus supporting the idea that 1,3,4,5-InsP$_4$ can interact with the 1,4,5-InsP$_3$ receptors.

The intracellular second messenger molecule, 1D-*myo*-inositol 1,4,5-trisphosphate (1,4,5-InsP$_3$), has been the subject of intense interest among both chemists and biologists.[1] This messenger molecule which is formed upon degradation of the minor membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, plays a key role in the release of intracellular calcium pools.[1,2] One mechanism by which the 1,4,5-InsP$_3$ calcium-mobilizing signal is terminated is through the action of a 5-phosphatase which yields in turn 1D-*myo*-inositol 1,4-bisphosphate, a compound that fails to mobilize intracellular calcium. A second route of metabolism of 1,4,5-InsP$_3$ involves the action of an ATP-dependent 3-kinase that converts it to 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-InsP$_4$)[1] (Figure 1). Considerable controversy exists as to whether this particular metabolite plays an independent or accessory second messenger role.[1,3] Some evidence exists that 1,3,4,5-InsP$_4$ may stimulate the entry of calcium across the plasma membrane.[4] Additionally, 1,3,4,5-InsP$_4$ has also been found to mobilize intracellular calcium stores in several cell types,[5] albeit less potently than 1,4,5-InsP$_3$, and at least in SH-SY5Y cells this action appears to occur via the intracellular 1,4,5-InsP$_3$ receptor population.[5] However, many of these latter results have been viewed with skepticism, since exogenous 1,3,4,5-InsP$_4$ may itself be converted to 1,4,5-InsP$_3$ through the action of a 3-phosphatase (Figure 1), thus explaining its putative activity at the 1,4,5-InsP$_3$ receptors.[6a] Contamination of some commercially available samples of 1,3,4,5-InsP$_4$ with 1,4,5-InsP$_3$ has also plagued some of these studies.[6b]

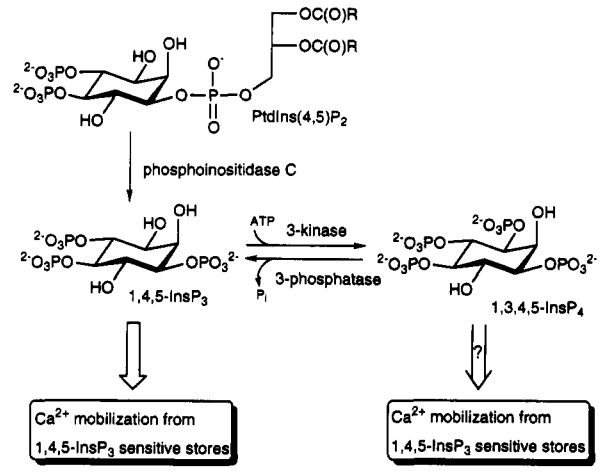


Figure 1. Some aspects of the phosphatidylinositol cell signaling pathway.

In order to help resolve this controversy, we felt it would be valuable to prepare the 3-phosphorothioate analogue of 1,3,4,5-InsP$_4$, for a number of studies have demonstrated that the phosphorothioate group is resistant to the action of intracellular phosphatases.[7] To prepare this compound in the enantiomerically correct form, we chose to start our synthesis from the natural product L-quebrachitol, a byproduct of latex production. As shown in Scheme 1, this compound was converted through a sequence of steps involving acetonide formation, mesylation, demethylation, and reformation of acetonides to a 1:1 mixture of **2** and **3**. The regiochemistry of these diacetonides was established by double resonance experiments. The mesylate group served as the only viable protecting group for the axial 3-hydroxyl in **1**, since it was capable of surviving the harsh demethylation conditions employing boron tribromide. Crystallization afforded pure **3**, and the undesired regioisomer **2** was recycled through an acid-catalyzed equilibration process to provide additional quantities of **3**. The free hydroxyl group of **3** was benzylated, the mesylate group removed by LAH reduction without any evidence of competing deoxygenation, and the newly freed hydroxyl inverted by an oxidation/reduction protocol. After protection of the C-3 hydroxyl of **4** as its PMB ether, the more strained *trans*-acetonide was cleaved selectively, the resulting diol benzoylated, and then the *cis*-acetonide hydrolyzed to afford **5**. The equatorial hydroxyl group of **5** was selectively benzoylated, and the lone axial alcohol was protected at its (benzyloxy)methyl ether to give **6**. The benzoate groups were removed by treatment with

† Mayo Foundation for Medical Education and Research.
‡ University of Leicester.
• Abstract published in *Advance ACS Abstracts*, April 15, 1994.

(1) Reviews: Berridge, M. J. *Nature (London)* **1993**, *361*, 315–325. (b) Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197–205. (c) Shears, S. B. In *Advances in Second Messenger and Phosphoprotein Research*; Putney, J. W., Jr., Ed.; Raven: New York, 1992; Vol. 26; pp 63–92. (d) Potter, B. V. L.; Nahorski, S. R. In *Drug Design for Neuroscience*; Kozikowski, A. P., Ed.; Raven: New York, 1993; pp 383-416.

(2) (a) Berridge, M. J.; Irvine, R. F. *Annu. Rev. Biochem.* **1987**, *56*, 159–193. (b) Streb, H.; Irvine, R. F.; Berridge, M. J.; Sculz, I. *Nature* **1983**, *306*, 67–69.

(3) (a) Irvine, R. F. *FEBS Lett.* **1990**, *263*, 5–9. (b) Irvine, R. F. *BioEssays* **1991**, *13*, 419–423.

(4) Irvine, R. F.; Moor, R. M.; Pollock, W. K.; Smith, P. M.; Wreggett, K. A. *Proc. R. Soc. Lond. Biol.* **1988**, *320*, 281–298.

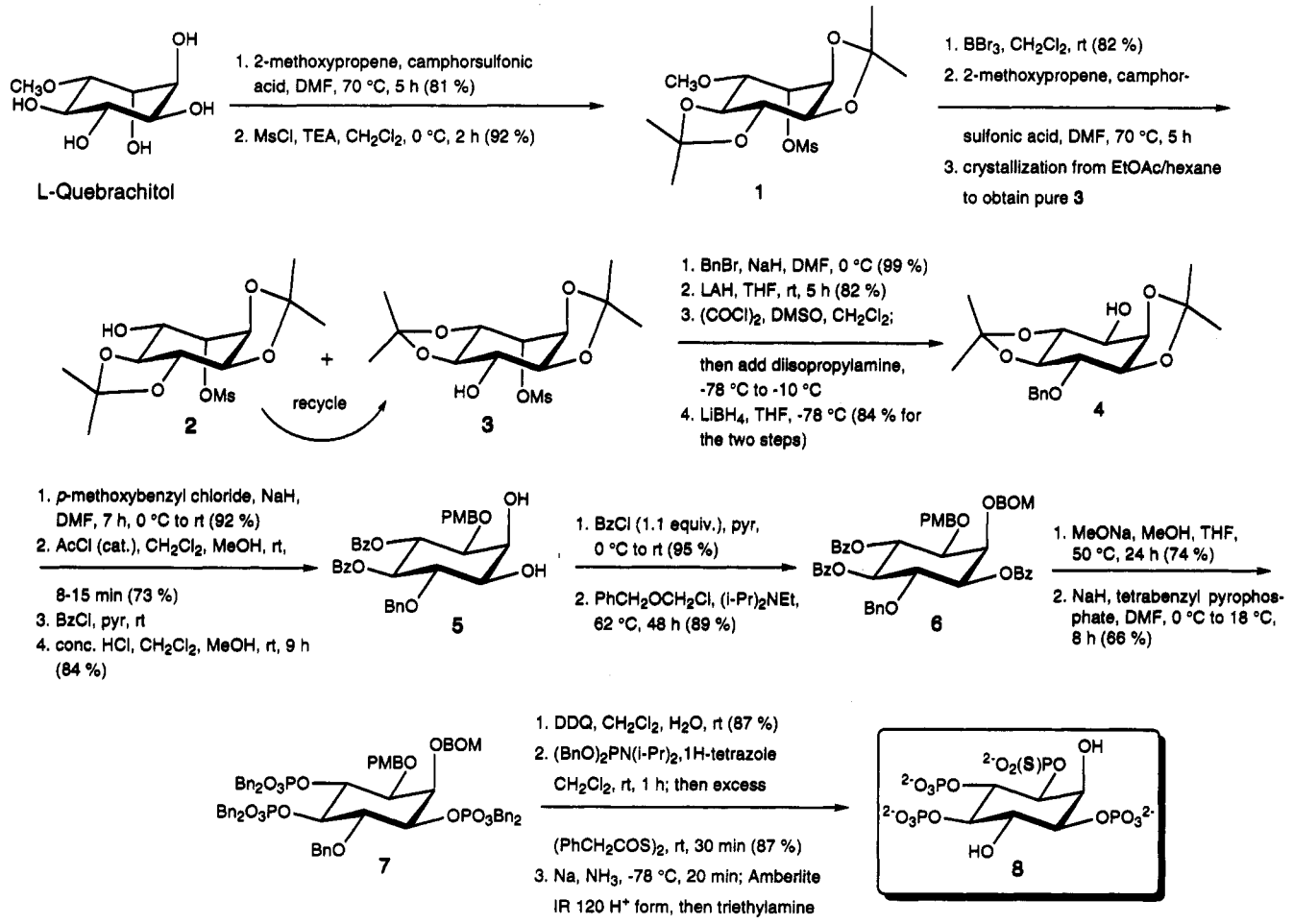
(5) Wilcox, R. A.; Challiss, R. A. J.; Baudin, G.; Vasella, A.; Potter, B. V. L.; Nahorski, S. R. *Biochem. J.* **1993**, *294*, 191–194 and references cited therein.

(6) (a) Cullen, P. J.; Irvine, R. F.; Drøback, B. K.; Dawson, A. P. *Biochem. J.* **1989**, *259*, 931–933. (b) Irvine, R. F. In *Advances in Second Messenger and Phosphoprotein Research*; Putney, J. W., Jr., Ed.; Raven: New York, 1992; pp 161–185.

(7) For the synthesis of other phosphorothioate-containing inositol analogs, see: (a) Lampe, D.; Mills, S. J.; Potter, B. V. L. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2899–2906. (b) Dreef, C. E.; Mayr, G. W.; Jansze, J.-P.; Roelen, H. C. P. F.; Van der Marel, G. A.; Van Boom, J. H. *BioMed. Chem. Lett.* **1991**, *1*, 239–242.

0022-3263/94/1959-2279$04.50/0

Scheme 1. Synthesis of 1D-*myo*-Inositol 1,4,5-Trisphosphate 3-Phosphorothioate



sodium methoxide in methanol, and the resulting triol was directly phosphorylated with NaH/tetrabenzyl pyrophosphate[8] in DMF to provide the fully protected derivative 7. Selective removal of the PMB group from the trisphosphate 7 by use of wet DDQ now allowed for phosphitylation at the 3-position. Exposure of the phosphite to phenylacetyl disulfide[7b,9] followed by deprotection with sodium in ammonia gave the target compound 8. Since the sodium salt of 8 was found to be unstable, this $InsP_4$ analogue was best isolated and stored as its triethylammonium salt. The ^1H NMR of the product was similar to the published spectrum of natural 1,3,4,5-$InsP_4$.[10,11]

Binding and calcium release experiments were carried out at the 1,4,5-$InsP_3$ receptor to compare the respective IC_{50} and EC_{50} values of 1,4,5-$InsP_3$, 1,3,4,5-$InsP_4$, and 8. Statistical analysis of the $\log_{10}(IC_{50})$ and $\log_{10}(EC_{50})$ values reveal that while 8 and 1,3,4,5-$InsP_4$ were equipotent, both were significantly less potent ligands and agonists than 1,4,5-$InsP_3$. Moreover, the concentration–response curve for 8, in contrast to that of 1,3,4,5-$InsP_4$, was not shifted significantly in the presence of 10 μM $InsP_6$, a potent inhibitor of 3-phosphatase,[12] thus indicating that this analogue is metabolically resistant to 1,3,4,5-$InsP_4$ 3-phosphatase activity. These findings thus provide confirmatory evidence that 1,3,4,5-$InsP_4$ can elicit calcium release at the 1,4,5-$InsP_3$ receptor in SH-SY5Y neuroblastoma cell line independent of its conversion to 1,4,5-$InsP_3$ (Table 1). Complete details of the biological experiments will be reported elsewhere.

Table 1.[a] Binding and Calcium Release Data for 1,4,5-$InsP_3$, 1,3,4,5-$InsP_4$, and 8

compd	binding (IC_{50}, nM)	^{45}Ca release (EC_{50}, nM)
1,4,5-$InsP_3$	4.4 ± 0.1	52 ± 2
1,3,4,5-$InsP_4$	152.3 ± 4.4	2436 ± 303
8	279.9 ± 18.9	2530 ± 260

[a] Displacement of specific $InsP_3$ receptor [^3H]$InsP_3$ binding from bovine adrenal cortex membranes and Ca^{2+} release via the intracellular $InsP_3$ receptor of SH-SY5Y cells were used to determine IC_{50} and EC_{50} values, respectively. Results represent the average ±SEM of at least three experiments.

In summary, a route to an optically pure 3-phosphatase-resistant analogue of 1,3,4,5-$InsP_4$ is disclosed starting from L-quebrachitol. The synthesis takes on added significance in view of the fact that the late introduction of sulfur will allow for the preparation of ^{35}S-labeled material. The

(8) (a) Vacca, J. P.; DeSolms, S. J.; Huff, J. R.; Billington, D. C.; Baker, R.; Kulagowski, J. J.; Mawer, I. M. *Tetrahedron* **1989**, *45*, 5679–5702. (b) Kozikowski, A. P.; Fauq, A. H.; Aksoy, G. A.; Seewald, M. J.; Powis, G. *J. Am. Chem. Soc.* **1990**, *112*, 7403–7404. (c) Kozikowski, A. P.; Fauq, A. H. *J. Chem. Soc., Chem. Commun.* **1990**, 163.

(9) Karmer, P. C. J.; Roelen, H. C. P. F.; Van der Eist, H.; Van der Marel, G. A.; Van Boom, J. H. *Tetrahedron Lett.* **1989**, *30*, 6757.

(10) Cerdan, S.; Hansen, C. A.; Johanson, R.; Inubushi, T.; Williamson, J. R. *J. Biol. Chem.* **1986**, *26*, 14676–14680.

(11) $[\alpha]^{23}_D$ −4.3° (c = 1.4 mg/mL, H_2O); ^1H NMR (300 MHz, D_2O, pH 6, tetrakistriethylammonium salt) δ 4.45 (1 H, t, J = 2.6 Hz), 4.38 (1 H, q, J = 9.4 Hz), 4.23 (1 H, td, J = 10, 2.6 Hz), 4.18–3.97 (2 H, m), 3.87 (1 H, t, J = 9.5 Hz), 3.14 (24 H, q, J = 7 Hz), 1.20 (36 H, t, J = 7 Hz); ^{31}P NMR (121.48 MHz, D_2O) δ 49.66 (d, J = 11.9 Hz), 4.18 (d, J = 9.0 Hz), 3.74 (d, J = 8.4 Hz), 3.40 (d, J = 6.7 Hz); MS (LSIMS, negative ion mode, aminoglycerol matrix) m/z 515 (M^+ − 1).

(12) Wilcox, R. A.; Challiss, R. A. J.; Liu, C., Potter, B. V. L.; Nahorski, S. R. *Mol. Pharmacol.* **1993**, *44*, 810–817.

ready availability of 8 will aid in furthering our understanding of the phosphatidylinositol signaling cascade.

Acknowledgment. We are indebted to the National Institute on Aging (A.P.K.) and the Wellcome Trust (R.A.W. and S.R.N.) for their support of these studies.

Supplementary Material Available: Spectroscopic and analytical data for new compounds (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead for ordering information.