Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 12, pp. 1295-1300, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00210-3

CHEMICAL SYNTHESIS AND BIOLOGICAL EVALUATION OF 1D-1,2,4,5-InsP4 and ITS 3-FLUORINATED COUNTERPART 1D-3-F-1,2,4,5-InsP4 — POTENT 1D-1,4,5-InsP3-LIKE CALCIUM MOBILIZING ANALOGUES

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ABSTRACT. Syntheses of optically pure 1D-3-F-2,4,5-InsP$_3$, 1D-3-F-1,2,4,5-InsP$_4$, and 1D-1,2,4,5-InsP$_4$ are reported starting from L-quebrachitol. The latter two compounds are shown to be nearly equipotent to 1D-1,4,5-InsP3 in both binding and calcium release experiments.

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$_4$).[1,3] 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] Moreover, in recent studies using whole patch-clamp recording from CA1 pyramidal neurons in hippocampal slices, 1,3,4,5-InsP$_4$ was shown to play a fundamental role in the calcium accumulation that leads to neuronal death.[6] The effects of other inositol tetrakisphosphates on cellular homeostasis would thus appear to be an important area for further exploration.

Accordingly, we felt that it would be of interest to explore the synthesis, binding, and calcium release studies of *non-racemic* InsP$_4$ analogues possessing phosphate groups at positions 1, 2, 4, and 5. The 2-position of the 1,4,5-InsP$_3$ molecule has been

shown previously to be capable of tolerating steric bulk.[7] During the course of the preparation of this manuscript, a report appeared by Hirata *et al.* describing the synthesis and biological characterization of DL-1,2,4,5-$InsP_4$.[8a] Pror to Hirata's report, several other articles have appeared describing syntheses of racemic 1,2,4,5-$InsP_4$.[8b] In contrast to these prior efforts we now describe the synthesis and biological evaluation of the D-isomer of 1,2,4,5-$InsP_4$ and its 3-fluorinated counterpart 3-F-1,2,4,5-$InsP_4$. Additionally, for comparison purposes we describe the biology of 1D-3-F-2,4,5-$InsP_3$ together with its method of synthesis. The structures of the inositol phosphates which are pertinent to this article are depicted in Figure 1.

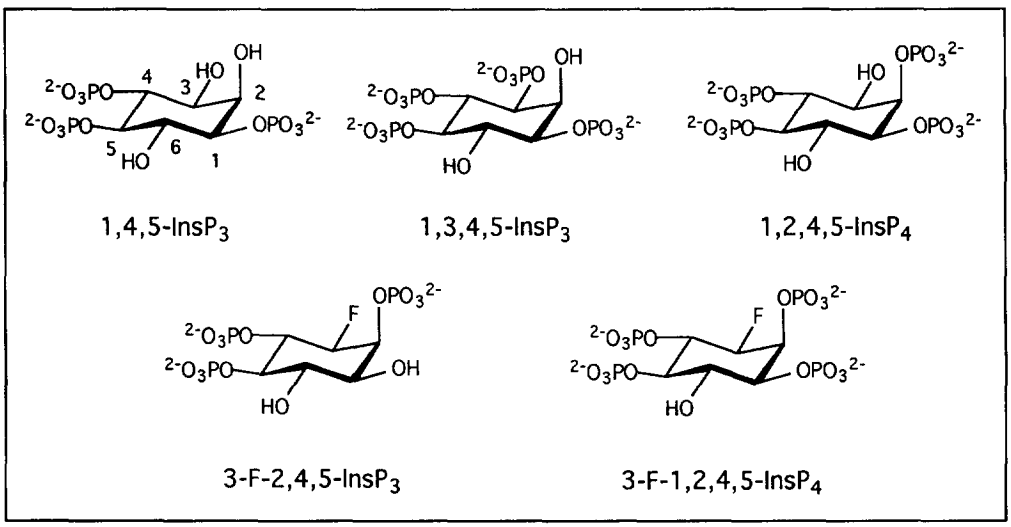
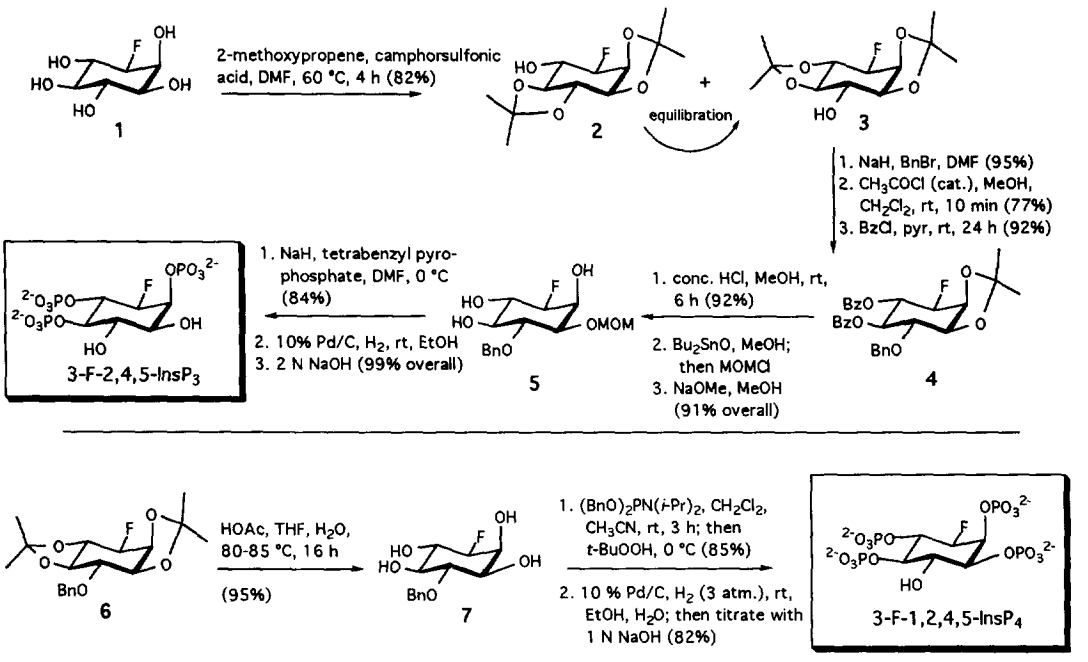


Figure 1. Structures of the inositol phosphates described in this article.

Chemistry

All three new inositol phosphates described in this paper were prepared from quebrachitol, a by-product of latex production.[9] Accordingly, the fluorinated inositol **1** prepared from quebrachitol[10] as described previously was converted to a mixture of diacetonides **2** and **3**, **2** was equilibrated to **3**, and **3** then benzylated (Scheme I). Next, the cis-acetonide group was removed selectively, and the resulting diol reprotected by benzoylation to afford **4**. The remaining trans-acetonide was cleaved, and then a MOM protecting group was introduced at the 1-position through the derived stannylene intermediate. Phosphorylation of **5** was brought about employing tetrabenzyl pyrophosphate and sodium hydride, and the benzyl and MOM groups removed through hydrogenolysis. Lastly, titration with sodium hydroxide furnished the hexasodium salt of 3-F-2,4,5-$InsP_3$.

Scheme I. Synthesis of the Fluorinated Inositol Phosphates 3-F-2,4,5-InsP$_3$ and 3-F-1,2,4,5-InsP$_4$.



To prepare 3-F-1,2,4,5-InsP$_4$, intermediate **6** obtained from the benzylation of **3** was converted to the tetraol **7**. Phosphorylation was then carried out with dibenzyl *N,N*-diisopropylphosphoramidite followed by oxidation with *t*-butyl hydroperoxide. Hydrogenolysis effected removal of the benzyl groups, and again titration with 1 N NaOH afforded the sodium salt of the desired tetrakisphosphate.

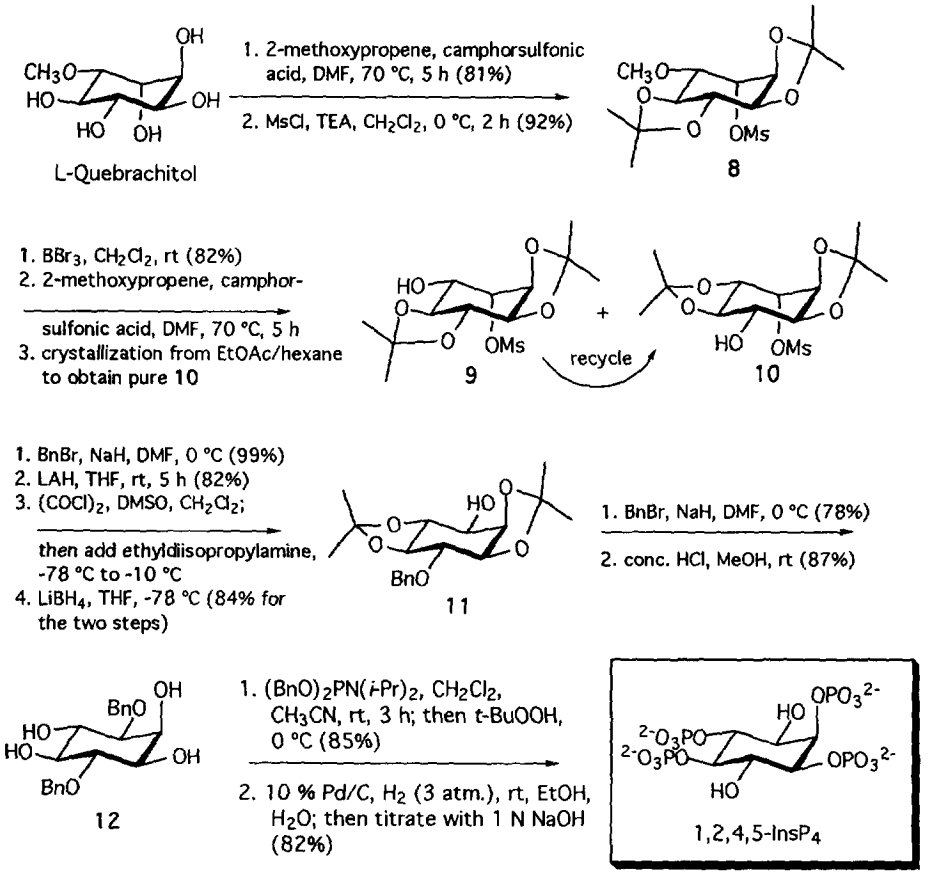
As shown in Scheme II, 1,2,4,5-InsP$_4$ was synthesized from L-quebrachitol through the mesylate **8**. After demethylation and reprotection, the diacetonide **10** was benzylated, the mesylate group removed, and an oxidation-reduction process employed to invert the stereochemistry of the C-3 hydroxyl. Further benzylation, acetonide cleavage, and phosphorylation then completed the synthesis of 1,2,4,5-InsP$_4$.

Biology

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$_3$, and the three new analogues. The data from these experiments are listed in Table I. As is evident, 1,4,5-InsP$_3$, 1,2,4,5-InsP$_4$, and 3-F-1,2,4,5-InsP$_4$ are of nearly comparable activity. The presence of the additional phosphate group at the 2-position of 1 and 2 leads to only a slight diminution in binding (about 2-fold) and calcium release activity (about 2-fold) compared to the natural metabolite 1,4,5-InsP$_3$. On the other hand, the deletion

of the phosphate group from position 1, as in 3-F-2,4,5-InsP$_3$ causes a marked reduction in both binding (about 50-fold) and calcium release activity (about 100-fold).

Scheme II. Synthesis of 1,2,4,5-InsP$_4$ from L-Quebrachitol.



Discussion

The present work reveals that the introduction of a phosphate group into the 2-position of 1,4,5-InsP$_3$ or 3-F-1,4,5-InsP$_3$ has little effect on the ability of the resulting tetrakisphosphates either to bind to the InsP$_3$ receptor or to mobilize calcium from intracellular stores. These results are consistent with those reported by the groups of Hirata[8a] and Potter[8b] for DL-1,2,4,5-InsP$_4$; the tetrakisphosphate was three-fold less potent than D-1,4,5-InsP$_3$ in calcium release with comparable potency being observed in the binding experiments. In contrast, removal of the phosphate group from the 1-position, as in 3-F-2,4,5-InsP$_3$ leads to a sharp reduction in calcium release activity.

This result is consistent with the observation that racemic 2,4,5-$InsP_3$ is about 30-fold less potent than 1,4,5-$InsP_3$ in its calcium release activity.[7]

Table I. Binding and Calcium Release Data for 1,4,5-$InsP_3$, 3-F-2,4,5-$InsP_3$, 1,2,4,5-$InsP_4$, and 3-F-1,2,4,5-$InsP_4$.[a]

Compound	Binding (IC_{50}, nM)	Calcium Release (EC_{50}, nM)
1,4,5-$InsP_3$	5.5 ± 0.3	51.6 ± 1.0
3-F-2,4,5-$InsP_3$	268.8 ± 8.6	5422.3 ± 347.6
1,2,4,5-$InsP_4$	8.7 ± 0.4	106.0 ± 3.5
3-F-1,2,4,5-$InsP_4$	9.7 ± 0.4	100.2 ± 6.8

[a]Displacement of specific $InsP_3$ receptor [^{3}H]-$InsP_3$ 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 regard to routes of metabolism, 3-F-1,2,4,5-$InsP_4$ will, of course, not serve as a substrate for the 3-kinase due to the presence of the fluorine atom. Additionally, Hirata[8a] has reported that DL-1,2,4,5-$InsP_4$ is not recognized by the 3-kinase of rat brain cytosol, presumably due to the charged nature rather than the steric bulk of the substituent at position 2. Moreover, these same workers reported that DL-1,2,4,5-$InsP_4$ was an inhibitor of the hydrolysis of D-1,4,5-$InsP_3$ to D-1,4-$InsP_2$, but was not a substrate for the same enzyme. A determination could not be made as to whether the phosphatase inhibitory activity of DL-1,2,4,5-$InsP_4$ was due solely to the presence of the L-isomer, since previously the L-isomer of the 2-benzoyl analogue of 1,4,5-$InsP_3$ was shown to be a potent inhibitor. This point can, of course, now be properly addressed using the optically pure material available from this study.[11]

Due to the ability of 1D-3-F-1,2,4,5-$InsP_4$ and 1D-1,2,4,5-$InsP_4$ to bind to the 1,4,5-IP_3 receptor and to promote calcium release with potencies nearly comparable to that of 1,4,5-$InsP_3$, these molecules may serve as useful tools in elucidating further aspects of intracellular signalling. The present work also further underscores the importance of L-quebrachitol as a building block in the construction of non-racemic inositol phosphates.

Acknowledgement. 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.

References

1. Reviews: Berridge, M. J. *Nature (London)* **1993**, *361*, 315. (b) Berridge, M. J.; Irvine, R. F. *Nature* **1989**, *341*, 197. (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. (b) Streb, H.; Irvine, R. F.; Berridge, M. J.; Sculz, I. *Nature* **1983**, *306*, 67.
3. (a) Irvine, R. F. *FEBS Lett.* **1990**, 263, 5. (b) Irvine, R. F. *BioEssays* **1991**, *13*, 419.
4. Irvine, R. F.; Moor, R. M.; Pollock, W. K.; Smith, P. M.; Wreggett, K. A. *Proc. R. Soc. Lond. Biol.* **1988**, *320*, 281.
5. Wilcox, R. A.; Challiss, R. A. J.; Baudin, G.; Vasella, A.; Potter, B. V. L.; Nahorski, S. R. *Biochem. J.* **1993**, *294*, 191, and references cited therein. Wilcox, R. A.; Challiss, R. A. J.; Liu, C.; Potter, B. V. L.; Nahorski, S. R. *Mol. Pharmacol.* **1993**, *44*, 810.
6. Tsubokawa, H.; Oguro, K.; Robinson, H. P. C.; Masuzawa, T.; Rhee, T. S. G.; Takenawa, T.; Kawai, N. *Neuroscience* **1994**, *59*, 291.
7. Hirata, M.; Watanabe, Y.; Ishimatsu, T.; Ikebe, T.; Kimura, Y.; Yamaguchi, K.; Ozaki, S.; Koga, T. *J. Biol. Chem.* **1989**, *264*, 20303.
8. (a) Hirata, M.; Narumoto, N.; Watanabe, Y.; Kanematsu, T.; Koga, T.; Ozaki, S. *Mol. Pharmacol.* **1994**, *45*, 271. (b) Mills, S. J.; Safrany, S. T.; Wilcox, R. A.; Nahorski, S. R.; Potter, B. V. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1505 and references cited therein.
9. Kozikowski, A. P.; Fauq, A. H.; Aksoy, G. A.; Seewald, M. J.; Powis, G. *J. Am. Chem. Soc.* **1990**, *112*, 7403; Kozikowski, A. P.; Fauq, A. H. *J. Chem. Soc., Chem. Commun.* **1990**, 163; Kozikowski, A. P.; Fauq, A. H.; Wilcox, R. A.; Nahorski, S. R. *J. Org. Chem.* **1994**, *59*, 2279.
10. Kozikowski, A. P.; Powis, G.; Fauq, A. H.; Tückmantel, W.; Gallegos, A. *J. Org. Chem.* **1994**, *59*, 963.
11. In preliminary experiments, 1D-3-F-1,2,4,5-InsP_4 was found to be rapidly metabolized by permeabilized SH-SY5Y cells, thus suggesting that it is the L-isomer of 1,2,4,5-InsP_4 that inhibits the phosphatase.

(*Received in USA* 31 *March* 1995; *accepted* 11 *May* 1995)