Probing the D-1,4,5-IP_3/D-1,3,4,5-IP_4 Functional Interface. Synthesis and Pharmacology of Novel D-3-Modified *myo*-Inositol Trisphosphate Analogues

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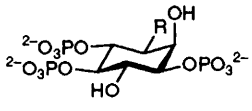
To explore the biological significance of the D-3 position phosphorylation of the second messenger D-*myo*-inositol 1,4,5-trisphosphate (IP_3) in cellular signalling, three novel D-3-substituted analogues of IP_3 have been synthesized; their binding and Ca^{2+}-release profiles at the IP_3-receptor have been studied and shown to correlate with the steric requirement of the D-3 substituent.

Phosphoinositide-based agonist stimulation of cell surface receptors and associated specific cellular responses have been the subject of intensive study in the past decade.[1] Many agonists such as neutrotransmitters, hormones, and growth factors, through specific extracellular interactions, stimulate phosphatidylinositol-specific phospholipase C (PI-PLC)-catalysed hydrolysis of a minor membrane lipid component, phosphatidylinositol 4,5-bisphosphate (PIP_2).[1] This event gives rise to two second messengers, D-*myo*-inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3, through its well-characterized receptor, mobilizes Ca^{2+} from intracellular stores eliciting specific cellular responses including, *inter alia*, secretion and cellular proliferation.[1] One of the known ways by which the IP_3 molecule is metabolized is through the IP_3-3-kinase-mediated phosphorylation at the D-3 position of the *myo*-inositol ring to form D-*myo*-inositol 1,3,4,5-tetrakisphosphate (IP_4).[2] Considerable controversy exists as to whether IP_4 possesses an independent or accessory second messenger role. Some evidence suggests that IP_4 may modulate Ca^{2+} entry across the plasma membrane.[3] Indeed, IP_4-activated Ca^{2+} channels have been detected in the plasma membrane of endothelial cells.[4] IP_4 can also directly mobilize intracellular Ca^{2+} stores, albeit less potently, in several cell types,[5] and at least in SH-SY5Y cells this appears to occur *via* the intracellular IP_3 receptor population.[5d,6] Hence, the production of IP_4 may well serve to attenuate the more potent IP_3-induced Ca^{2+} signal.

Another novel pathway of phosphoinositide metabolism is the PI-3-kinase-catalysed generation of D-3-phosphorylated PI's which may have second messenger actions and have been implicated in cellular proliferation.[7] Following these important findings, which highlight the importance of the D-3 position of *myo*-inositol in multiple signalling pathways, we have synthesized a variety of both D-3-modified *myo*-inositol analogues for cell growth inhibition studies,[8] and *myo*-inositol trisphosphates[9b,d] to explore the structure-activity relationships (SAR) of IP_3 receptor function.

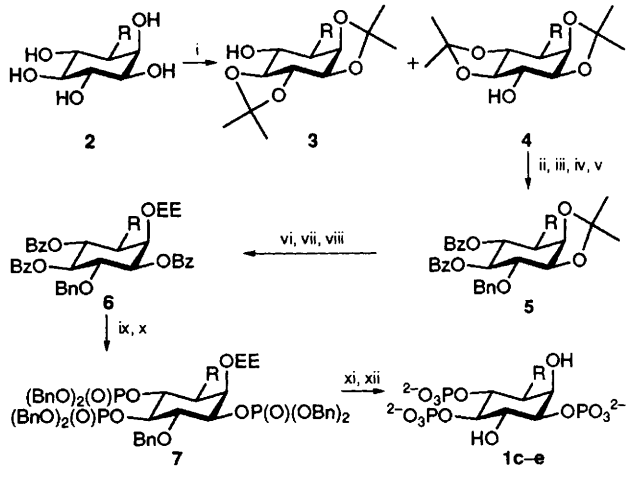
Herein, we describe the synthesis and pharmacology of D-3-chloro-3-deoxy-*myo*-inositol 1,4,5-trisphosphate (3-Cl-IP_3) **1c**, D-3-bromo-3-deoxy-*myo*-inositol 1,4,5-trisphosphate (3-Br-IP_3) **1d**, and D-3-*O*-methyl-*myo*-inositol 1,4,5-trisphosphate (3-OMe-IP_3) **1e** (Fig. 1). These enantiomerically pure analogues together with the previously reported 3-deoxy-*myo*-inositol 1,4,5-trisphosphate **1a**[9d] and 3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate **1b**[9b] were designed to systematically probe the IP_3 binding subsite proximate to the D-3 position.



D-1,4,5-IP_3	R
1a	H
1b	F
1c	Cl
1d	Br
1e	OMe

Synthesis of all the D-3-modified inositol trisphosphate analogues utilized L-quebrachitol (a naturally occurring cyclitol) as the starting material. Efficient syntheses of the D-3-deoxy-3-halogeno-*myo*-inositols (**2**, R = Cl, Br) were reported from these laboratories.[8] D-3-*O*-Methyl-*myo*-inositol (**2**, R = OMe) (Scheme 1) was prepared by saponification and methylation of the known D-4-*O*-benzyl-3-Ocamphanoyl-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol,[10] followed by debenzylation and acidic hydrolysis of the cyclohexylidene groups. Acid-catalysed acetalization of each of the cyclitols **2** gave the bis-acetonides **3** and **4** in ratios ranging between 1:3 to 1:5.† The undesired regioisomer **3** could be recycled to the desired isomer **4** by acid-catalysed equilibration.

Partial acidic hydrolysis of the *trans*-acetonide, followed by benzoylation provided the dibenzoate **5**. After removal of the remaining *cis*-acetonide, the equatorial 1-OH group was selectively benzoylated.[9] The 2-OH group was blocked by treatment with ethyl vinyl ether and camphorsulfonic acid. The resulting tribenzoate **6** was saponified to furnish the 1,4,5-triol which could be trisphosphorylated to provide compound



Scheme 1 Synthesis of the analogues **1c–e**, R =Cl, Br, or OMe. Yields indicated in parentheses are in the same order. (EE = 1-ethoxyethyl.)

Reagents and conditions: i, 2-methoxypropene, camphorsulfonic acid, DMF, 70 °C, 4–5 h, (79, 89, 76%); ii, separation; iii, NaH, benzyl bromide (BnBr), DMF, 0 °C, 2–16 h, (96, 95, 98%); iv, AcCl (cat), MeOH, CH_2Cl_2, 1 h, (86, 67, 73%); v, BzCl, Py, room temp., 2 h, (93, 98, 98%); vi, AcCl, MeOH, 4–16 h, (100, 98, 98%); vii, BzCl, Py, room temp. 16–40 h, (93, 77, 72%); viii, ethyl vinyl ether, pyridium toluene-*p*-sulfonate, CH_2Cl_2, 3–16 h, (95, 91, 98%); ix, K_2CO_3, MeOH, room temp. (16–24 h), (100, 67, 76%); x, dibenzyl *N,N'*-diisopropylphosphoramidite, 1*H*-tetrazole, CH_2Cl_2, room temp. 4–16 h, then Bu^tOOH, CH_2Cl_2, (69%, 67%) (R = Cl, Br); NaH, tetrabenzyl pyrophosphate, DMF, 16 h, (76%), (R = OMe); xi, H_2, 10% Pd–C, EtOH, 40 psi, 12–16 h, (97, 90, 92%); xii, titrate with 1 mol dm^{-3} NaOH.

7 either by treatment with dibenzyl *N,N'*-diisopropylphosphoramidite followed by *tert*-butyl hydroperoxide, or by treatment of its derived trialkoxide with tetrabenzyl pyrophosphate.[11] Hydrogenolysis of **7** occurred uneventfully concurrent with *in situ* hydrolysis of the ethoxyethyl group and provided 3-Cl-IP_3 **1c**, 3-Br-IP_3 **1d**, and 3-OMe-IP_3 **1e** in reasonable overall yields.‡

We[9,12] and others[13] have reported recently some structure-activity relationship (SAR) data concerning the importance of the phosphate and hydroxy groups of the IP_3 molecule. It is now known that: (*i*) by and large, the vicinal 4,5-bisphosphate moiety is essential for Ca^{2+} mobilization, (*ii*) the 1-phosphate group enhances potency, and (*iii*) the 6-OH group is crucial to activity. The removal [9d,e] or modification[14] of the 2-OH group does not seem to significantly attenuate the Ca^{2+} release response. However, the role of the 3-OH has been less well understood. Whereas deoxygenation[9d] or substitution of OH by fluorine[9b] at the D-3 position of IP_3 does not affect Ca^{2+} releasing ability significantly, L-*chiro*-2,3,5-IP_3, in which an axial OH group is located at what would be the D-3 position of IP_3, is much less potent.[15] As noted previously, phosphorylation of the D-3 position may act to attenuate the Ca^{2+} releasing signal, a possibility that could be rigorously explored by studying the IP_3 analogues **1a–e**. This structural series embodies a gradation in the steric bulk of the 3-substituent. The pharmacological evaluation of the analogues **1a–e** for competitive IP_3 binding and Ca^{2+} release in permeabilized SH-SY5Y neuroblastoma cells, are presented in Table 1 in the form of IC_{50} and EC_{50} values, respectively. A good correlation was found between binding (IC_{50}) and functional (EC_{50}) data. Analogues **1a** and **1b** are similar to IP_3 in binding and Ca^{2+} releasing activity, but as the size of the D-3-substituent increases from H and F to Cl, Br, and OCH_3,§ the binding and the Ca^{2+}-releasing activity of the analogues sharply declines in that order (Table 1). A 12-, 21-, and 148-fold difference between the EC_{50} data of IP_3, and 3-Cl-IP_3 **1c**, 3-Br-IP_3 **1d**, and 3-OMe-IP_3 **1e**, respectively, strongly suggests that IP_3 receptor function is highly sensitive to the steric bulk of the D-3 substituent. More importantly, with respect to IP_3 receptor binding and function, the analogues, with increasing bulk at position 3, *smoothly* switched from being IP_3-like (as in **1a** and **1b**) to being IP_4-like (as in **1d** and **1e**). In addition, these data lead us to postulate that, at least for the purpose of termination or attenuation of the signal, it may be the steric bulk rather than the charge on the 3-phosphate moiety which determines this specific role of 1,3,4,5-IP_4. 3-Cl-IP_3 **1c**, having an intermediate-sized substituent, represents an intermediate stage between the IP_3- and IP_4-like pharmacology at the IP_3 receptor.

Table 1 The IP_3 receptor binding and Ca^{2+} release profiles of IP_3 and analogues **1a–e**[a]

Analogue	IC_{50}/ nmol dm^{-3}	EC_{50}/ nmol dm^{-3}
1,4,5-IP_3	4.4 ± 0.1	52.1 ± 2.3
1a	30.3 ± 2.0	155.7 ± 20.1
1b	12.6 ± 0.1	120.2 ± 10.9
1c	32.0 ± 1.4	639.8 ± 77.9
1d	69.5 ± 5.2	1100 ± 100
1e	271.1 ± 25.6	7700 ± 1100

[a] Displacement of specific IP_3 receptor [^{3}H]-IP_3 binding from bovine adrenal cortex membranes and Ca^{2+} release *via* the intracellular IP_3 receptor of SH-SY5Y cells were used to determine IC_{50} and EC_{50} values, respectively. Results represent the average of at least four experiments.

In conclusion, our current findings, specifically directed at the IP_3/IP_4 interface, suggest that phosphorylation at the D-3 position by IP_3-3-kinase may be the physiological mechanism which dissects the biological function of these two important polyphosphates at the IP_3 receptor. Further studies of the interaction of these compounds with the putative membrane 1,3,4,5-IP_4 binding sites are in progress and will be reported in due course.

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Footnotes

† The diacetonides **3** and **4** were separated by silica-gel chromatography, or crystallization except for the corresponding 3-OMe analogues which could be separated only after benzylation.

‡ The structures of all the reported compounds were established by a combination of ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy.

§ Molecular volumes, as calculated with the SYBYL program (Version 5.41), for F, Cl, Br, and OMe substituents are 10.1, 19.4, 25.2, and 30.1 Å^3, respectively. SYBYL (Version 5.41), Tripos Associates Inc., 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144, 1991. We are thankful to Terry Hashey for calculating these values.

References

1 Reviews: M. J. Berridge, *Nature*, 1993, **361**, 315; M. J. Berridge and R. F. Irvine, *Nature*, 1989, **341**, 197.
2 G. R. Batty, S. R. Nahorski and R. F. Irvine, *Biochem. J.*, 1985, **232**, 211.
3 R. F. Irvine, in *Inositol Phosphates and Calcium Signalling*, ed. J. W. Putney, Raven Press, New York, 1992, p. 161; R. F. Irvine, *FEBS Lett.*, 1990, **263**, 5; (*c*) R. F. Irvine, *Bioessays*, 1991, **13**, 419.
4 A. Lückhoff and D. E. Clapham, *Nature*, 1992, **355**, 356.
5 (*a*) S. K. Joseph, C. A. Hansen and J. R. Williamson, *Mol. Pharmacol.*, 1989, **36**, 391; (*b*) J. A. Ely, L. Hunyardy, A. J. Baukal and K. J. Catt, *Biochem. J.*, 1990, **268**, 333; (*c*) I. Parker and I. Ivorra, *J. Physiol.*, 1991, **433**, 207; (*d*) D. J. Gawler, B. V. L. Potter, R. Gigg and S. R. Nahorski, *Biochem. J.*, 1991, **276**, 163.
6 R. A. Wilcox, R. A. J. Challiss, C. Liu, B. V. L. Potter and S. R. Nahorski, *Mol. Pharmacol.*, 1993 in the press.
7 D. R. Kaplan, M. Whitman, B. Schaffhausen, D. C. Pallas, M. White, L. Cantley and T. M. Roberts, *Cell*, 1987, **50**, 1021; C. P. Downes and A. N. Cartner, *Cell. Signalling*, 1991, **3**, 501.
8 G. Powis, I. A. Aksoy, D. C. Melder, S. Aksoy, H. Eichinger, A. H. Fauq and A. P. Kozikowski, *Cancer Chemother. Pharmacol.*, 1991, **29**, 95.
9 (*a*) A. P. Kozikowski, A. H. Fauq and J. M. Rusnak, *Tetrahedron Lett.*, 1989, 3365; (*b*) A. P. Kozikowski, A. H. Fauq, G. A. Aksoy, M. J. Seewald and G. Powis, *J. Am. Chem. Soc.*, 1990, **112**, 7403; (*c*) A. P. Kozikowski, A. H. Fauq, G. Powis and D. C. Melder, *Med. Chem. Res.*, 1991, **1**, 277; (*d*) A. P. Kozikowski and A. H. Fauq, *J. Chem. Soc., Chem. Commun.*, 1990, 1638; (*e*) A. P. Kozikowski, V. I. Ognyanov, A. H. Fauq, S. R. Nahorski and R. A. Wilcox, *J. Am. Chem. Soc.*, 1993, **115**, 4429.
10 J. P. Vacca, S. J. deSolms and J. R. Huff, *J. Am. Chem. Soc.*, 1987, **109**, 3478.
11 H. Khorana and A. R. Todd, *J. Chem. Soc.*, 1953, 2257.
12 A. P. Kozikowski, V. I. Ognyanov, C. Chen, A. H. Fauq, S. T. Safrany, R. A. Wilcox and S. R. Nahorski, *J. Med. Chem.*, 1993, **36**, 3035 and references cited therein.
13 M. Hirata, Y. Watanabe, M. Yoshida, T. Koga and S. Ozaki, *J. Biol. Chem.* 1993, **268**, 19260.
14 M. Hirata, F. Yanaga, T. Koga, T. Ogasawara, Y. Watanabe and S. Ozaki, *J. Biol. Chem.*, 1990, **265**, 8404.
15 S. T. Safrany, R. A. Wilcox, C. Liu, B. V. L. Potter and S. R. Nahorski, *Eur. J. Pharmacol.*, 1992, **226**, 265.