

## *myo*-Inositol 1,4,5-Trisphosphorothioate: A Novel Analogue of a Biological Second Messenger

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DL-*myo*-inositol 1,4,5-trisphosphorothioate, a novel analogue of the biologically active second messenger D-*myo*-inositol 1,4,5-trisphosphate, has been synthesised by use of phosphite chemistry.

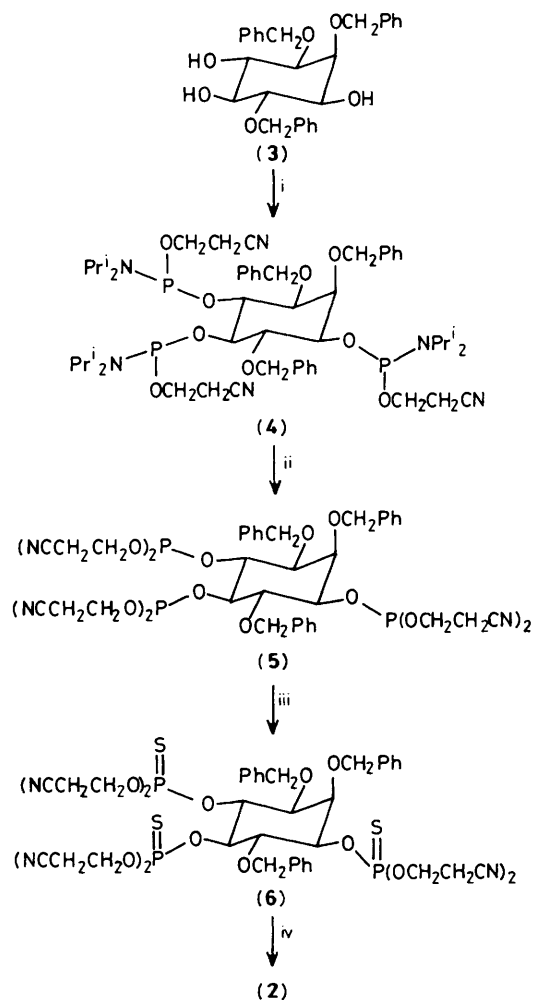
The membrane lipid phosphatidylinositol 4,5-bisphosphate is cleaved by receptor-stimulated phospholipase-C-catalysed hydrolysis to two second messengers: diacylglycerol, which is involved in the activation of protein kinase C,<sup>1</sup> and D-*myo*-inositol 1,4,5-trisphosphate (1), which activates the release of calcium in stimulated cells from intracellular stores located in the endoplasmic reticulum.<sup>2,3</sup> On account of these recent discoveries there has been a revival of interest in the biological effects of inositol phosphates.<sup>4</sup> Of special chemical interest have been efforts directed towards the synthesis of *myo*-inositol 1,4,5-trisphosphate. The first synthesis was reported by Ozaki *et al.*<sup>5†</sup> We have recently reported studies, using a phosphite chemistry approach,<sup>6</sup> which led to the synthesis of biologically active DL-*myo*-inositol 1,4,5-trisphosphate.<sup>7</sup> Syntheses of *myo*-inositol 1-phosphate and 4-phosphate have also been reported.<sup>8</sup>

D-*myo*-inositol 1,4,5-trisphosphate is rapidly deactivated after it has completed its role as a second messenger. Two deactivation pathways have been identified: the 5-phosphate group can be removed by a specific phosphatase<sup>9</sup> forming the 1,4-bisphosphate, which is further degraded to *myo*-inositol; alternatively, phosphorylation by a specific kinase forms the 1,3,4,5-tetrakisphosphate,<sup>10,11</sup> which has itself been reported to have second messenger activity,<sup>12</sup> and which is then metabolised to *myo*-inositol *via* the 1,3,4-trisphosphate.

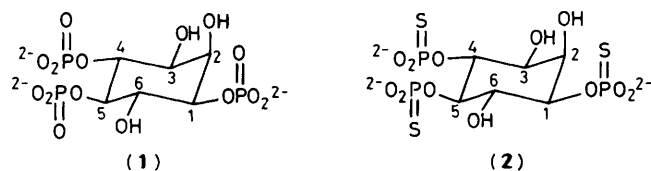
We have recently introduced *myo*-inositol phosphorothioates as novel analogues of inositol phosphates possessing phosphatase resistance.<sup>13</sup> These analogues should be of use for investigating the interaction of inositol phosphates with enzymes and receptors and the metabolism of these compounds. The replacement of phosphate groups by phosphorothioates is expected to confer resistance to degradation without greatly affecting binding properties. Moreover, the prospect of preparing radioactive <sup>35</sup>S-labelled analogues of these molecules enhances their attractiveness. Nucleoside phosphorothioates, including analogues of the other known second messengers adenosine cyclic 3',5'-phosphate and guanosine cyclic 3',5'-phosphate, have proved of enormous value in fields ranging from mechanistic enzymology to molecular biology.<sup>14</sup> We report here the first synthesis of such

an analogue for an inositol phosphate second messenger, DL-*myo*-inositol 1,4,5-trisphosphorothioate (2).

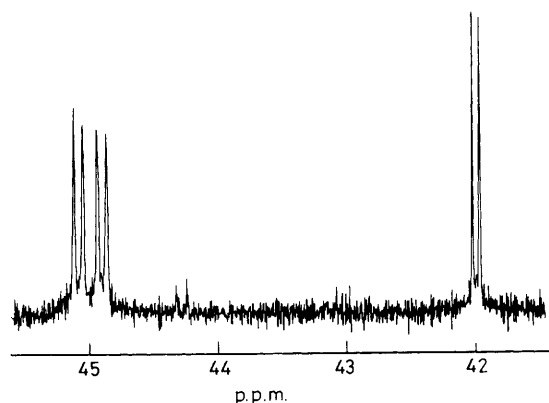
The route (Scheme 1) is adapted from our synthesis of the 1,4,5-trisphosphate.<sup>7</sup> (±)-1,2,4-Tri-*O*-benzyl-*myo*-inositol<sup>15</sup> (3) is converted into the corresponding tris[di-(2-cyanoethyl)] phosphite (5) *via* the trisphosphoramidite (4) as previously described,<sup>7</sup> and this is oxidised to the tris[di-(2-cyanoethyl)] phosphorothioate (6) [ $\delta_p$  (MeCN) 66.6(s)] using sulphur in pyridine.<sup>16</sup> Complete removal of all protecting groups is accomplished using sodium in liquid ammonia,<sup>7</sup> the 2-cyanoethyl groups being removed by  $\beta$ -elimination and the benzyl



**Scheme 1.** Reagents and conditions: i, CIP(OCH<sub>2</sub>CH<sub>2</sub>CN)NPr<sub>2</sub>, EtNPr<sub>2</sub> (5 equiv. of each) in CH<sub>2</sub>Cl<sub>2</sub>; ii, tetrazole, HOCH<sub>2</sub>CH<sub>2</sub>CN (5 equiv.) in CH<sub>2</sub>Cl<sub>2</sub>; iii, sulphur in pyridine; iv, Na liq. NH<sub>3</sub> (all compounds shown are racemic).



† Added in proof: two other important synthetic routes have been reported recently: C. B. Reese and J. G. Ward, *Tetrahedron Lett.*, 1987, **28**, 2309; J. P. Vacca, S. J. de Solms, and J. R. Huff, *J. Am. Chem. Soc.*, 1987, **109**, 3478.



**Figure 1.** Proton-coupled  $^{31}\text{P}$  n.m.r. spectrum (121.5 MHz) of DL-*myo*-inositol 1,4,5-trisphosphorothioate (ca. 80mM in 80%  $\text{D}_2\text{O}$ , pH 9, containing 200mM-triethylammonium hydrogen carbonate buffer and EDTA); sweep width 1824 Hz, pulse width 8  $\mu\text{s}$ , acquisition time 2.25 s; no. of transients 56, collected in 8 K, line broadening  $-1.5$  Hz, gaussian broadening 0.22 Hz.

groups by reductive cleavage to yield crude (2). Purification on a column of DEAE Sephadex A-25 using a linear gradient of triethylammonium hydrogen carbonate gave DL-*myo*-inositol 1,4,5-trisphosphorothioate (2) (eluted at ca. 800mM-buffer) in ca. 25% yield. [ $^{31}\text{P}$  n.m.r. ( $\text{D}_2\text{O}$ ; pH 9) proton-coupled  $\delta_{\text{P}}$  42.0 (d,  $J_{\text{PH}}$  8.0 Hz), 44.9 (d,  $J_{\text{PH}}$  9.3 Hz), and 45.1 (d,  $J_{\text{PH}}$  9.3 Hz) (Figure 1)]. The proton-coupled  $^{31}\text{P}$  n.m.r. spectrum of (2) was similar to that reported for authentic D-(2)<sup>17</sup> and synthetic DL-(2)<sup>7</sup> except that the resonances were shifted ca. 40 p.p.m. downfield as expected for phosphorothioates (see ref. 14 and refs. therein).

In preliminary biological evaluation of (2), using a rat cerebellum membrane receptor-binding assay specific for D-*myo*-inositol 1,4,5-trisphosphate,<sup>18</sup> DL-*myo*-inositol 1,4,5-trisphosphorothioate was found to have a high affinity for such receptors, indicating the potential of such analogues in future studies on receptor-linked phosphoinositide metabolism. Biological data on this compound will be reported elsewhere.

We thank S.E.R.C. for a research studentship (to A.M.C.), Dr. M. R. Hamblin for discussions, The Research Corporation Trust for partial financial support, Dr. W. Dukat for assistance in obtaining  $^{31}\text{P}$  n.m.r. spectra, and Professor S. R. Nahorski and Mr. A. Willcocks for preliminary biological testing.

Received, 22nd May 1987; Com. 698

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