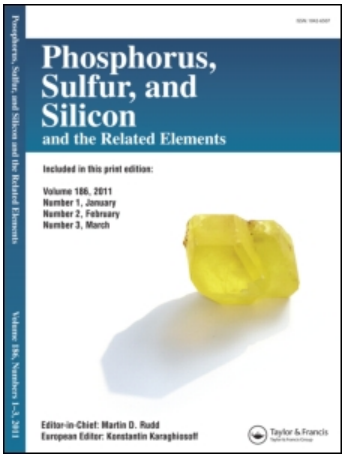

Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

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Allan M. Cooke[a]; Leroy James[b]; Stefan R. Nahorski[b]; Barry V. L. Potter[a]
[a] Departments of Chemistry, Leicester University, Leicester, U.K. [b] Departments of Pharmacology, Leicester University, Leicester, U.K.

To cite this Article Cooke, Allan M. , James, Leroy , Nahorski, Stefan R. and Potter, Barry V. L.(1990) 'Paosphorothioate Analogues of Inositol Phosphates', Phosphorus, Sulfur, and Silicon and the Related Elements, 51: 1, 19 — 22
To link to this Article: DOI: 10.1080/10426509008040672
URL: http://dx.doi.org/10.1080/10426509008040672

Phosphorus, Sulfur, and Silicon, 1990, Vols. 51/52, pp. 19-22
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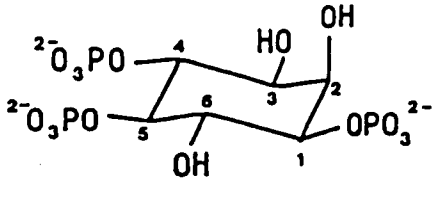
PHOSPHOROTHIOATE ANALOGUES OF INOSITOL PHOSPHATES

ALLAN M. COOKE[a], LEROY JAMES[a,*], STEFAN R. NAHORSKI[*]
& BARRY V.L. POTTER[a,§]
Departments of [a]Chemistry and [*]Pharmacology, Leicester University, Leicester LE1 7RH, U.K.

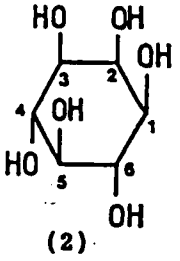
ABSTRACT: Novel analogues of the intracellular second messenger D-myo-inositol 1,4,5-trisphosphate, which possess phosphorothioate groups in place of phosphate groups have been synthesized. They exhibit unusual biological properties which will be of considerable application in understanding the phosphoinositide cycle.

INTRODUCTION:

D-myo-inositol 1,4,5-trisphosphate [IP_3 (1), based upon myo-inositol (2), Fig. 1] is an intracellular second messenger, produced by receptor-mediated, phospholipase C-catalyzed cleavage of phosphatidylinositol 4,5-bisphosphate.[1] IP_3 binds to an intracellular receptor, probably coupled to a calcium channel, with the result that calcium flows into the cytosol from intracellular stores. This rise in calcium concentration couples the external signal to the cellular response. After release, IP_3 is rapidly metabolized by two major pathways: 5-phosphatase catalyzed degradation to 1,4-IP_2[2] and 3-kinase mediated phosphorylation to 1,3,4,5-IP_4.[3] 1,4-IP_2 is clearly inactive, but the precise role of 1,3,4,5-IP_4 is not yet clear. It may play a part in gating entry of extracellular calcium into the cell, or in regulating the movement of Ca^{2+} between intracellular stores.[4]



(1)



(2)

FIGURE 1

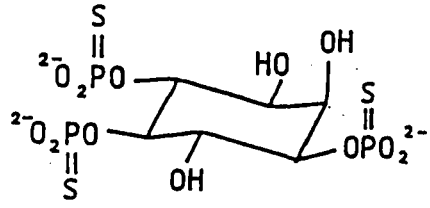
The complex metabolism of IP_3 has made precise interpretation of its biological actions and those of its metabolites difficult. We have therefore synthesized phosphatase-resistant analogues of IP_3, which are recognized by enzymes and receptor sites[5], but which are metabolically stable to degradation by phosphatases.[6]

§To whom correspondence should be addressed

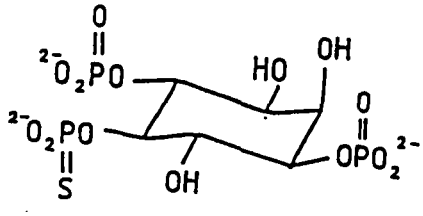
DISCUSSION:

Myo-Inositol 1,4,5-Trisphosphorothioate (IPS_3, 3)

Myo-inositol 1,4,5-trisphosphorothioate (3) has been synthesized from a protected precursor, 1,2,4-tri-O-benzyl-myo-inositol, using a phosphite approach.[7] Deblocking of benzyl and cyanoethyl protecting groups of the fully protected phosphorylated precursor using sodium in liquid ammonia gave either IP_3[8] (1) or IPS_3[7] (3) respectively, depending upon whether t-BuOOH or sulphur had been used in the phosphite oxidation step. Synthetic IP_3 was found to be active at binding to a receptor in cerebellum, specific for D-IP_3[9], and at mobilizing intracellular Ca^{2+} in permeabilized Swiss 3T3 cells[10,11], GH_3 cells[11] and hepatocytes.[12] IPS_3 was found to be a full agonist and only some 3 fold less potent than IP_3. However, IPS_3 was completely resistant to the action of 5-phosphatase[13] and was found to be a potent competitive inhibitor of this enzyme with a K_i of 6μM.[14] IPS_3 did not compete with D-[^{3}H] IP_3 for the 3-kinase[12], even up to 100μM[15], and is therefore unlikely to be a substrate for this enzyme. This route of synthesis offers the possibility of introducing ^{35}S-radiolabel and it is clear that this, coupled with the novel properties of IPS_3, will lead its considerable application in the phosphoinositide field. IPS_3 has already been employed to demonstrate that oscillations in intracellular Ca^{2+} concentration are probably not caused by fluctuating levels of IP_3.[16]



(3)



(4)

FIGURE 2

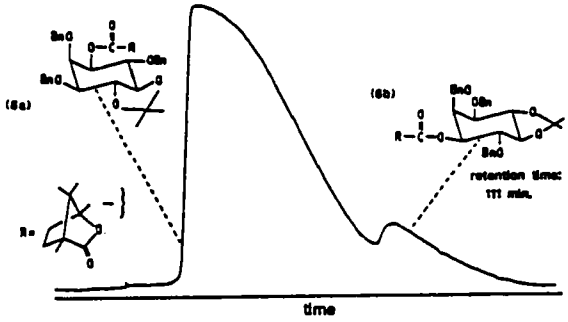
Myo-Inositol 1,4-Bisphosphate-5-Phosphorothioate (IP_3-5S, 4)

Despite the obvious advantages of IPS_3 as an IP_3 analogue it would be preferable to have an analogue nearer in structure to IP_3, yet enjoying the advantages of phosphatase stability. Such requirements are fulfilled by myo-inositol 1,4-bisphosphate-5-phosphorothioate (IP_3-5S, 4), in which only the 5-phosphate group has been modified by a phosphorothioate. This compound has been synthesized by a novel route[17] involving mixed P(III) and P(V) chemistry, which can also be used for IP_3. IP_3 prepared via this route was as active in binding and Ca^{2+} release assays as the previous material.[18] IP_3-5S exhibited comparable activity to IPS_3, yet was resistant to 5-phosphatase and was an inhibitor of this enzyme.[19] It is not yet known whether IP_3-5S is a substrate for the 3-kinase, but preliminary data suggest that it may behave as a potent inhibitor of this enzyme.[15]

D-Myo-Inositol 3-Phosphorothioate (IP_1-1S, 8b)

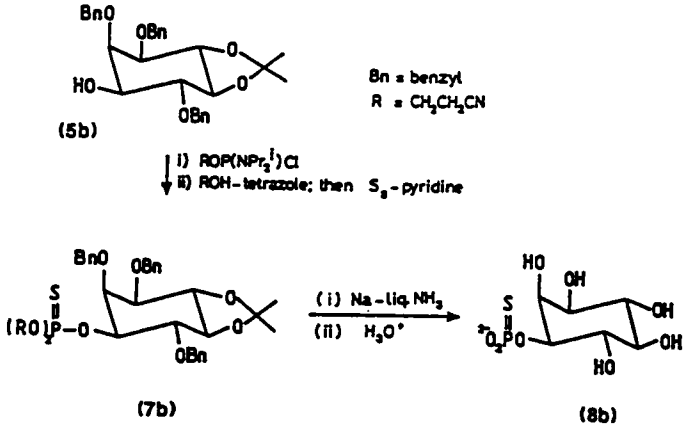
The enzyme myo-inositol 1-phosphatase dephosphorylates both enantiomers of myo-inositol 1-phosphate and is potently inhibited by Li^+ in an uncompetitive fashion.[20] This Li^+ sensitivity is at present under extensive scrutiny on account of its link to treatment of manic depression. Consequently, the synthesis of non-hydrolysable analogues of myo-inositol 1-phosphate is of interest. A synthesis of racemic inositol 1-phosphorothioate has been reported.[21] We present here the synthesis of D-myo-inositol-3-phosphorothioate.

FIGURE 3



The required protected inositol DL-1,2,4-tri-O-benzyl-5,6-isopropylidene-myo-inositol (5) was resolved using a combination of crystallisation and HPLC. When racemic (5) is reacted with 1S-(-)-camphanic acid chloride, two diastereoisomeric camphanates are formed (6a and 6b, Fig. 3), of which (6b) crystallizes out.[22] After removal of camphanate from (6b), the resulting (5b) was converted to the protected phosphorothioate (7b) by the phosphitylation route employed for IPS_3.[7] Reductive deblocking of the benzyl groups and removal of the ketal with acid gave (8b) (Fig. 4). We also demonstrate here that the other camphanate (6a) can be readily separated from the resulting unequal mixture of diastereoisomers by HPLC on a Prepsil column. Removal of the camphanate moiety from (6a) gives L-1,2,4-tri-O-benzyl-5,6-isopropylidene-myo-inositol and deketalisation gives L-1,2,4-tri-O-benzyl-myo-inositol, the precursor for the synthesis of D-IP_3.

FIGURE 4



ACKNOWLEGEMENTS: This work was supported by SERC, The Research Corporation Trust and in part by Merck Sharp & Dohme. B.V.L.P. is a Lister Institute Research Fellow.

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