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Synthesis of Some Thiophosphate Analogues (C-S-P) of Phosphatidylinositol

Maria A. Alisi, Mario Brufani, Luigi Filocamo*, Gianluca Gostoli, Sperandina Lappa

Dipartimento di Scienze Biochimiche "A. Rossi Fanelli", Università "La Sapienza", Via degli Apuli 9, 00185 Roma (Italy)

Stefano Maiorana

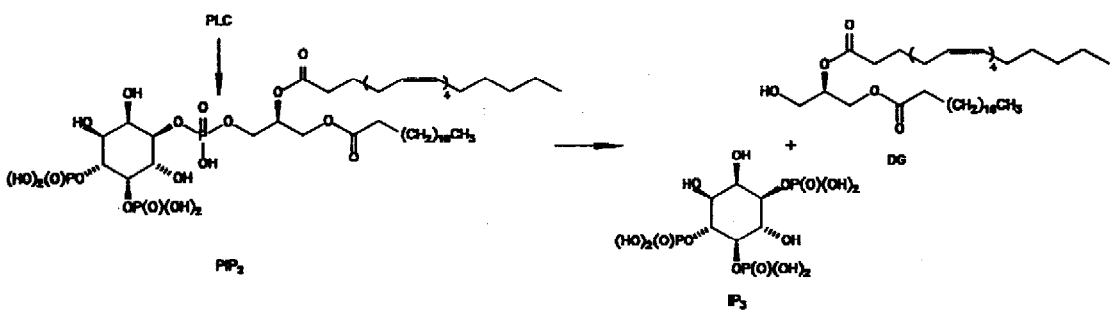
Dipartimento di Chimica Organica e Industriale dell'Università, Via Venezian 21, 20133 Milano (Italy)

Maria C. Cesta, Enrico Ferrari, and Pier G. Pagella

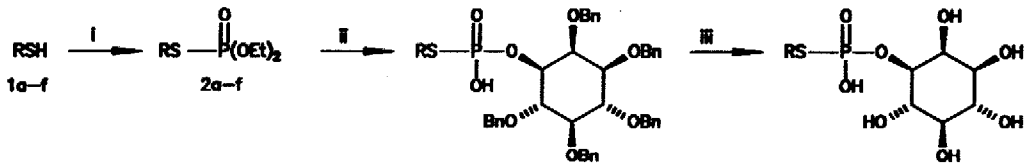
Mediolanum Farmaceutici S.p.a., Via S. G. Cottolengo 31, 20143 Milano (Italy)

Abstract: the synthesis of analogues of phosphatidylinositol (PI), designed to show a novel mode of PI-phospholipase C (PI-PLC) inhibition, is described.

Recent research on the role of inositol phospholipids in cellular signalling pathways[1] has revived interest in the syntheses of inositol phosphates and phosphatidylinositol (PI) analogues[2] that can modulate the important intracellular signal transduction system based on the metabolism of phosphatidylinositol. Inositol-specific phospholipase C (PI-PLC) is a key enzyme in this system[1] which acts on phosphatidyl-*myo*-inositol 4,5-bisphosphate (PIP_2) to yield the second messengers D-*myo*-inositol 1,4,5-trisphosphate (IP_3), which mediates the release of calcium ions from intracellular stores and diacylglycerol (DG), involved in the activation of protein kinase C. A complex pattern of events then occurs, many of which have been elucidated[3].



To date, non-specific PLC inhibitors are known[4], and only lately the syntheses of some PI and PIP_2 analogues as inhibitors of PLC have been accomplished[5]. Among them, the analogues of PIP_2 were found to inhibit PLC *in vitro* but are inactive in the assay with intact cells[6].



i) n-BuLi/$(OEt)_2POCl$/THF
ii) a. TMSBr/CH_2Cl_2 - b. (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol/TPSCl/Et_3N/Py
iii) EtSH/$BF_3{\cdot}Et_2O$/$CHCl_3$

SCHEME I

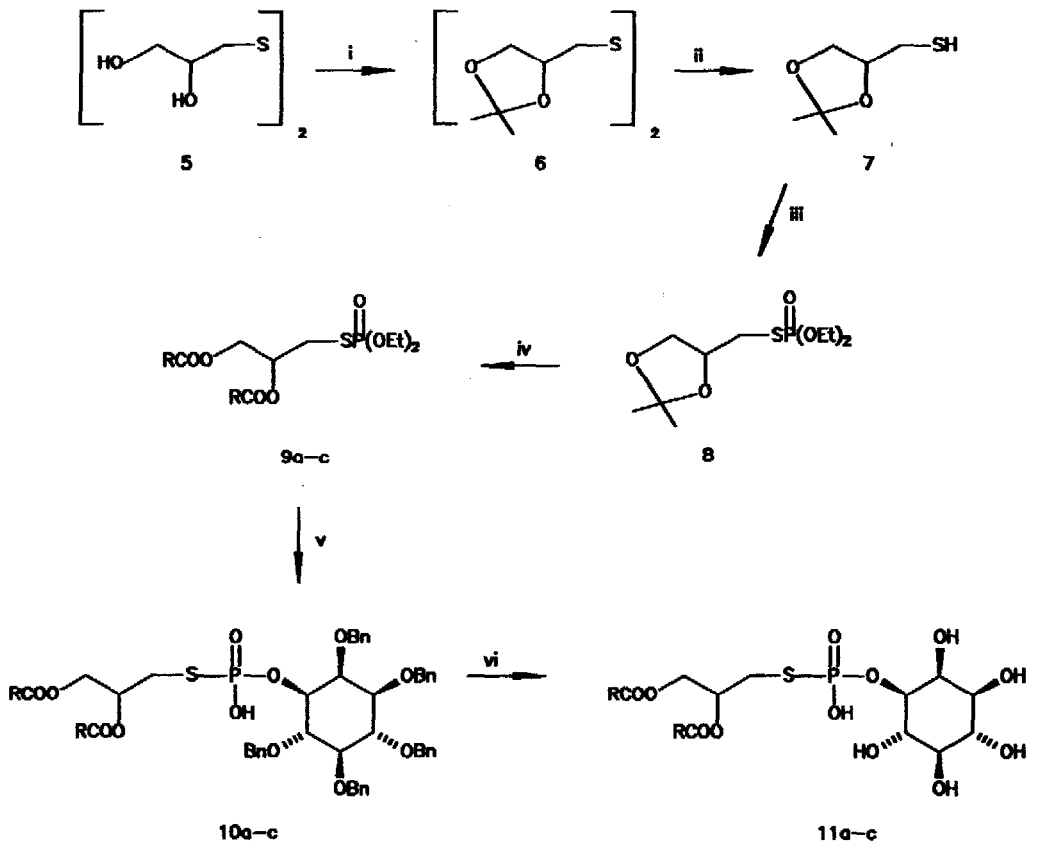
Following our Italian patent[7], we report here the synthesis of some thiophosphates analogues (C-S-P bond) of phosphatidylinositol as inhibitors of PI-PLC.

The racemic alkylphosphothiolyl-1-*myo*-inositol derivatives (**4a-f**) were synthesised as shown in Scheme I[8]. Alkylthiols **1a-f** were reacted with n-BuLi in THF at 0°C for 30 min to generate the anion and then with diethyl phosphochloride at r.t. for 1 h, giving the diethyl alkylphosphothiolates **2a-f**. These phosphothiolates were transesterified with trimethylsilylbromide in dry CH_2Cl_2 at r.t. for 6 h and, after evaporation of the solvent at reduced pressure, copuled under the influence of triisopropylbenzensulfonyl chloride (TPSCl, 4 eqiv) with (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol[9] in pyridine and triethylamine (6 eqiv) at r.t. for 24 h. The products **3a-f** were purified by gradient elution ($CHCl_3$, $CHCl_3$/MeOH 95:5 and then 9:1) on chromatographic column of silica gel and debenzylated using BF_3-etherate in ethyl mercaptane[10] (yields: see TABLE I).

TABLE I

R	Entry	Yield (from 1a-f)	Entry	Yield (from 2a-f)	Entry	Yield (from 3a-f)
$CH_3(CH_2)_{17}$–	2a	95%	3a	70%	4a	98%
$CH_3(CH_2)_{15}$–	2b	93%	3b	70%	4b	98%
$CH_3(CH_2)_{13}$–	2c	89%	3c	68%	4c	97%
$CH_3(CH_2)_{11}$–	2d	85%	3d	67%	4d	97%
$CH_3(CH_2)_7$–	2e	87%	3e	65%	4e	93%
$CH_3(CH_2)_3$–	2f	85%	3f	62%	4f	92%

The synthesis of PI thioanalogues was performed as shown in Scheme II. *rac*-1,1'-Dithiobis(2,3-propandiol)[11] (**5**) was acetalated with 2,2-dimethoxypropane in the presence of pTsOH at r.t. for 3 h to obtain, after workup, the bis-acetal **6** (yield 95%). Reduction of disulfide **6** with dithiothreitol in EtOH/NH_4OH (pH 9.6) at r.t. for 1h gave thiol **7** after extraction with $CHCl_3$ and evaporation at reduced pressure of the solvent (yield 75%). The mercapto group was esterified like the n-alkyl derivatives (yield 95%) and the resulting phosphothiolate **8** was reacted with dry $ZnCl_2$ and acyl chloride in dry ether at r.t. for 3 h affording the diacyl derivatives **9a-c** after chromatography on silica gel



i) $(CH_3)_2C(OCH_3)_2$/DMF/p-TosOH
ii) Dithiothreitol/EtOH/NH_4OH
iii) n-BuLi/$(EtO)_2POCl$/THF
iv) $ZnCl_2$/RCOCl/Et_2O
v) a. TMSBr/CH_2Cl_2 - b. (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol /TPSCl/Et_3N/Py
vi) EtSH/BF_3·Et_2O/$CHCl_3$

SCHEME II

eluting with light petroleum/diethyl ether 1:1. The last two steps were performed like for the alkylphosphothiolates achieving **10a-c** after condensation with pentabenzylinositol and **11a-c** after debenzylation (yields: see TABLE II)[12].

TABLE II

R	Entry	Yield (from 8a-c)	Entry	Yield (from 9a-c)	Entry	Yield (from 10a-c)
$CH_3(CH_2)_{14}$–	9a	64%	10a	35%	11a	91%
$CH_3(CH_2)_8$–	9b	60%	10b	33%	11b	90%
$CH_3(CH_2)_6$–	9c	55%	10c	31%	11c	87%

These synthetic routes provide versatile and convenient pathways to these stable analogues which possess interesting biological activity[13]. Adaption of these routes to a synthesis of chiral compounds of these analogues is now in progress.

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13. To be reported elsewhere.

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