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Synthesis of Inositol Phospholipids With Thiophosphoester Bonds

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Abstract: the synthesis of phosphatidylinositol (PI) analogues (±)1-*O*-(1-*O*-octadecanoyl-2-*O*-acetyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (7), (±)1-*O*-(1,2-di-*O*-octadecyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14a**), (±)1-*O*-(1,2-di-*O*-octyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14b**) and (±)1-*O*-(1-*O*-octadecyl-2-*O*-methyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14c**) designed to show a novel mode of PI-phospholipase C (PI-PLC) inhibition, is described.

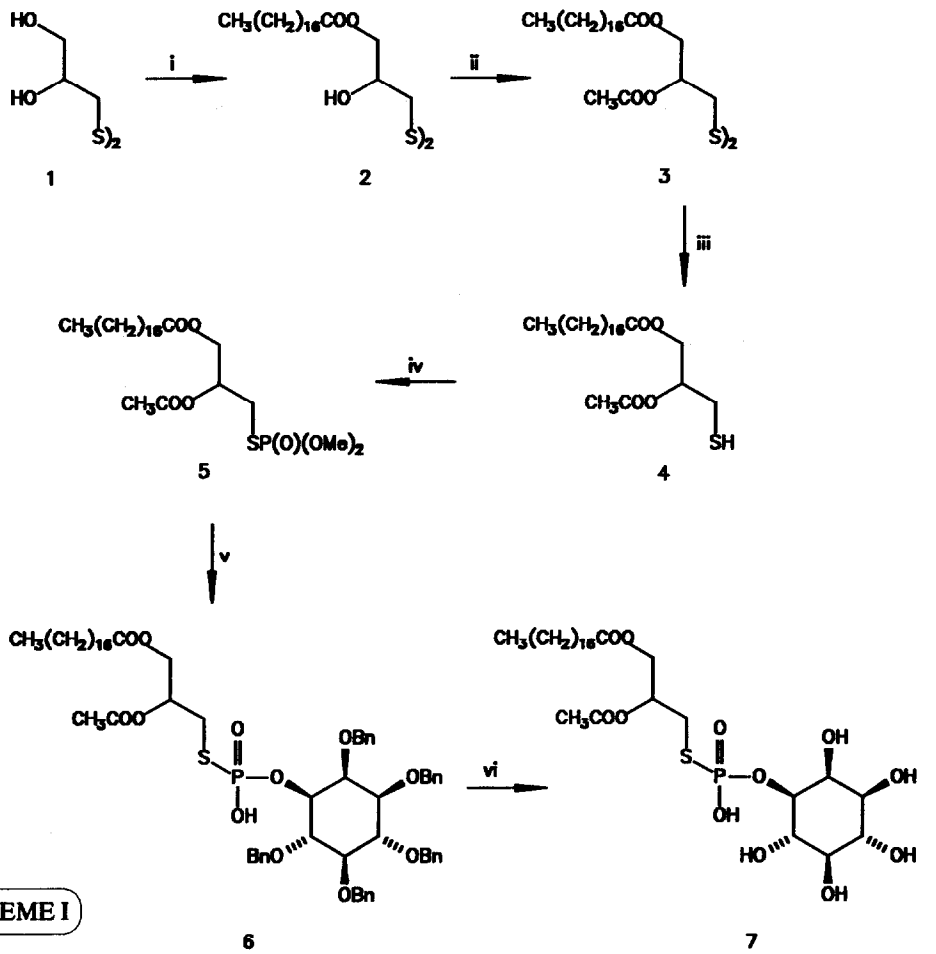
The inositol phospholipids are precursors of second messengers in receptor-mediated intracellular Ca^{2+} mobilisation and protein phosphorylation[1].

Inositol-specific phospholipase C (PI-PLC) is a key enzyme in the signal transduction system; in fact it 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.

Phosphatidylinositol also appears in glycosylated forms to provide an anchorage for hydrophilic proteins in cell membranes and because of their susceptibility to hydrolysis by PI-PLC to enable rapid release of these proteins from the membranes[2].

To date the syntheses of some PI and PIP_2 analogues as inhibitors of PLC have been accomplished, but some of the synthesised compounds show only a slight degree of inhibition of PLC from human platelets others are not effective in the test for PLC inhibitions on intact cells[3].

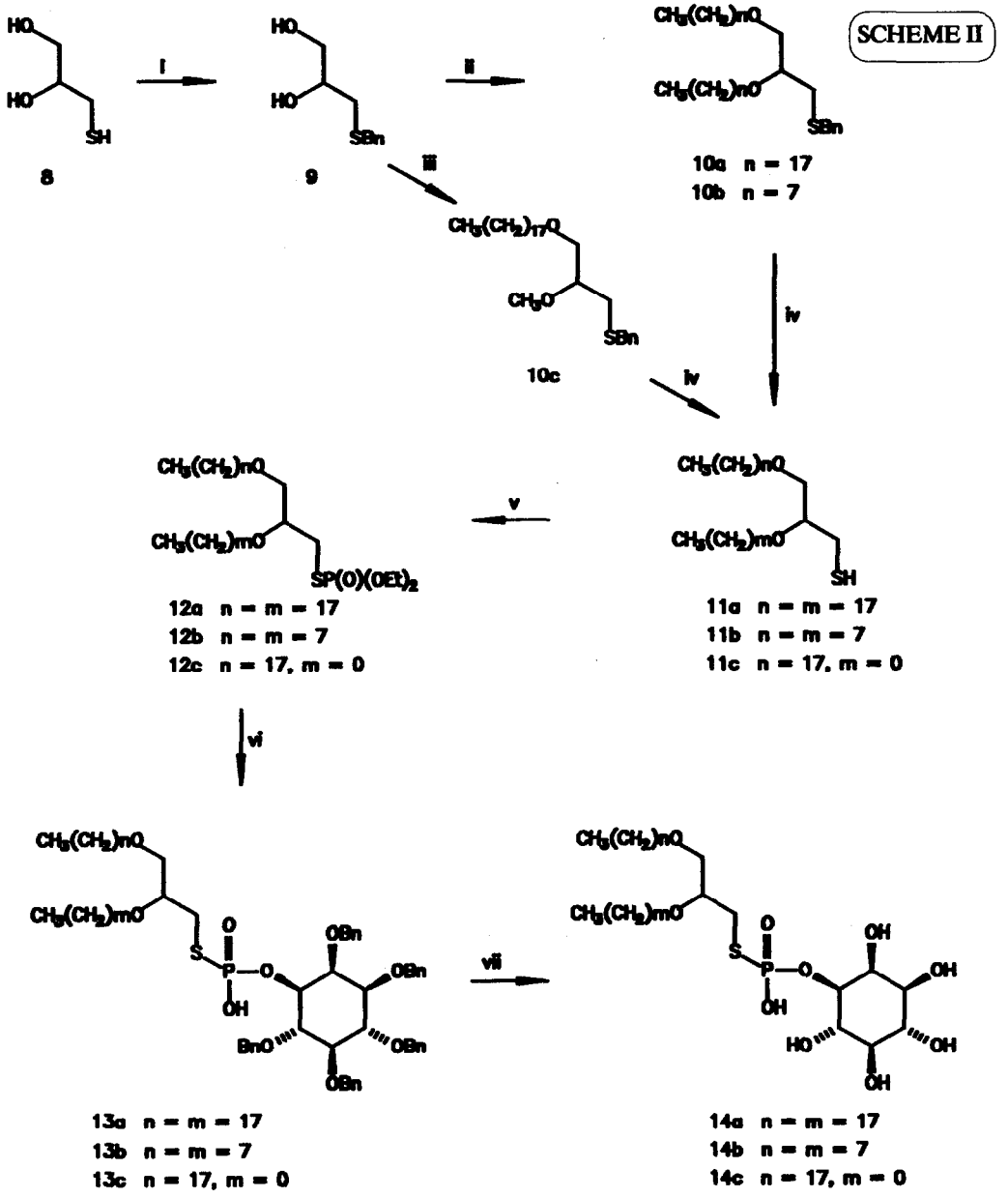
Along this line, we recently reported on the synthesis of some phosphothiolate analogues of phosphatidylinositol in which the diacylglycerol moiety was replaced by alkylthiols or diacylthioglycerols[4]. As part of an ongoing programme directed towards the preparation of inhibitors of PI-PLC, we here describe the synthesis of the racemic compounds (±)1-*O*-(1-*O*-octadecanoyl-2-*O*-acetyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (7), (±)1-*O*-(1,2-di-*O*-octadecyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14a**), (±)1-*O*-(1,2-di-*O*-octyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14b**) and (±)1-*O*-(1-*O*-octadecyl-2-*O*-methyl-*rac*-3-thioglycerylphosphoryl)-*myo*-inositol (**14c**).



SCHEME I

i)$CH_3(CH_2)_{16}COCl$, Py, CH_2Cl_2; ii) Ac_2O, DMAP, Toluene; iii) Dithiothreitol, EtOH/NH_4OH; iv) a. N-Chlorosuccinimide, Benzene - b. $(MeO)_3P$; v) a. TMSBr, CH_2Cl_2 - b. (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol, TPSCl, Et_3N, Py; vi) EtSH, $BF_3{\cdot}Et_2O$,$CHCl_3$.

The compound **7** was synthesised as shown in Scheme I. *rac*-1,1'-Dithiobis(2,3-propanediol)[5] **1** was selectively acylated on the primary alcoholic groups with equimolar amounts of stearoyl chloride and pyridine in methylene chloride at -15 °C for 2 h obtaining the acylated disulfide **2** after chromatography on silica gel eluting with $CHCl_3$/MeOH 97:3 (yield 20%). Using a large excess of acetic anhydride (4 equiv) in presence of DMAP (5 equiv) in toluene at r.t. for 30 min, acetylation of the secondary alcoholic groups of the disulfide **2** was accomplished. After workup, the crude derivative was reduced with dithiothreitol in EtOH/NH_4OH (pH 9.6) at r.t. for 1 h to give thiol **4** (yield 83% from **2**). Chlorination of the mercapto group with N-chlorosuccinimide in dry benzene and subsequent reaction with trimethylphosphite[6] afforded phosphothiolate **5** after chromatography on silica gel eluting with light petroleum/diethyl ether 15:85 (yield 61%). This one was transesterified with trimethylsilylbromide in dry CH_2Cl_2 at r.t. for 6 h and, after evaporation of the solvent at reduced pressure, coupled under the influence of triisopropylbenzenesulfonyl chloride (TPSCl, 4 equiv) with (±)-1,2,4,5,6-penta-*O*- benzyl-*myo*-inositol[7] in pyridine and triethylamine



i) BnCl, EtOH/NaOH 2 M 1:1; ii) $CH_3(CH_2)nCl$, $Bu_4N^+Br^-$, Benzene/NaOH 50%; iii) a. $CH_3(CH_2)_{17}Cl$, $Bu_4N^+Br^-$, Benzene/NaOH 50% - b. CH_3I, NaH, THF; iv) Na, n-BuOH; v) n-BuLi, $(EtO)_2POCl$, THF; vi) a. TMSBr, CH_2Cl_2 - b. (±)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol, TPSCl, Et_3N, Py; vii) EtSH, $BF_3{\cdot}Et_2O$,$CHCl_3$.

(6 equiv) at r.t. for 24 h. The product **6** was 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 mercaptan[8] (yield 20% from **5**).

The synthesis of racemic 1,2-di-*O*-alkyl-3-thioglycerylphosphoryl derivatives of *myo*-inositol **14a-c** was performed as shown in Scheme II. *rac*-1-Thioglycerol **8** was *S*-benzylated with equimolar amounts of benzyl chloride in EtOH/NaOH 2 M 1:1 at r.t. in quantitative yield. Dialkylation of the diol **9** was performed using phase transfer catalysis conditions: 50% aqueous NaOH/benzene 1:1, an excess of alkyl chloride (10 equiv) and 5 mole % of tetrabutylammonium bromide as catalyst. The reaction was stirred at r.t. for 3 days and the product was purified by elution (hexane/diethyl ether 96:4) on a chromatographic column of silica gel (yields 20-40%) and debenzylated by refluxing the dialkylethers **10a-b** in 1-butanol with sodium (yields 84-92%). The 1-*O*-octadecyl-2-*O*-methyl-3-thioglyceryl derivative **10c** was obtained using these phase transfer catalysis conditions: 50% aqueous NaOH/benzene 1:1, an excess of alkyl chloride (6 equiv) and 2 mole % of tetrabutylammonium bromide as catalyst. The reaction was stirred at r.t. for 2 days and the product was purified by elution (hexane/diethyl ether 70:30) on a chromatographic column of silica gel (yield 35%), methylated with NaH and methyl iodide in THF at r.t. for 1 h and debenzylated like the dialkylethers **10a-b** (yield 89%). Thiols **11a-c** were reacted with n-BuLi in THF at 0°C for 30 min to generate the anion and then with diethyl chlorophosphate at r.t. for 1 h to give the diethyl phosphothiolates **12a-c** (yields 47- 54%). The last two steps were performed as for the dimethylphosphothiolate **5** achieving **13a-c** after condensation with pentabenzylinositol and **14a-c** after debenzylation (yields 33-42% from **12a-c**)[9].

These synthetic routes provide versatile and convenient pathways to these stable analogues which possess interesting biological activity[10]. Adaption of these routes to a synthesis of homochiral analogues of these compounds is now in progress.

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9. Compounds were characterised by 200 MHz ^{1}H-NMR, FT-IR and C,H,P,S analysis.
10. To be reported elsewhere.

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