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TOTAL SYNTHESIS OF L-2,2-DIFLUORO-2-DEOXY-*MYO*-INOSITOL 1,4,5-TRISPHOSPHATE, A POTENT INHIBITOR OF THE ENZYMES OF D-*MYO*-INOSITOL 1,4,5-TRISPHOSPHATE METABOLISM

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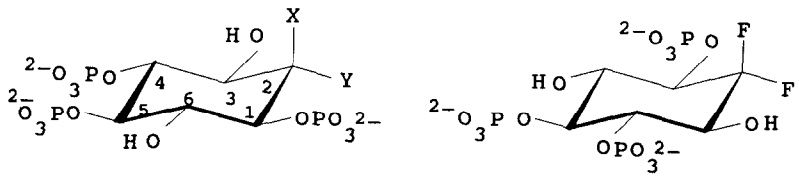
Abstract: The synthesis of the enantiomers of 2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate is reported. L-2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate is a potent inhibitor of 3-kinase and 5-phosphatase.

It is now generally accepted that the cyclitol polyphosphate, D-*myo*-inositol 1,4,5-trisphosphate Ins(1,4,5)P_3 [(1), Fig. 1], is a second messenger which releases Ca^{2+} from intracellular stores[1,2] via a receptor which has now been isolated[3], cloned and sequenced[4,5] and which, when reconstituted, is gated in response to Ins(1,4,5)P_3[6]. A major challenge is now the elucidation of the structural basis for interaction of Ins(1,4,5)P_3 both with its receptor and with the metabolic enzymes, Ins(1,4,5)P_3 3-kinase and 5-phosphatase. This will facilitate the rational design of agonists, antagonists and enzyme inhibitors which may be of therapeutic value. Recent progress in inositol phosphate chemistry[7,8] and the molecular recognition of inositol phosphates by proteins has been reviewed[9].



X=OH; Y=H (1) X=Y=H (2)
X=H; Y=F (3) X=Y=F D-(4a) L-(4b)

Fig. 1: **Structures of D-Ins(1,4,5)P_3 (1), related synthetic analogues and the fluorinated D- and L- enantiomers of 2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate (4a) and (4b).**

Ring-modified and phosphate-modified inositol analogues have been synthesized[7,8] in attempts to understand the role of three phosphate and hydroxyl groups of Ins(1,4,5)P_3 in receptor binding specificity and stimulation. Isosteric replacement of a hydroxyl group with fluorine[10] has led to fluorinated inositol analogues[11-14] and inositol polyphosphate analogues[15-19]. D-3-fluoro-3-deoxy-*myo*-inositol was found to inhibit cell growth in NIH 3T3 cells[11] and 5-fluoro-5-deoxy-*myo*-inositol is taken up by L1210 cells and incorporated into cellular phospholipid by phosphatidylinositol synthase[20], although 5,5-difluoro-5-deoxy-*myo*-inositol is a much poorer substrate[13]. Reports of biological activity for non-fluorinated ring-modified analogues of Ins(1,4,5)P_3, including 2-deoxy-Ins(1,4,5)P_3 [(**2**), Fig. 1), have appeared[21-26]. We recently reported the first biological evaluation of some fluorinated inositol phosphate analogues[18] and studied the interaction of the two synthetic analogues DL-2-deoxy-2-fluoro-*scyllo*-inositol 1,4,5-trisphosphate [DL-2-F-Ins(1,4,5)P_3] (**3**) and DL-2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate [DL-2,2-F_2-Ins(1,4,5)P_3] DL-(**4ab**) with the Ca^{2+}-releasing Ins(1,4,5)P_3 receptor and the metabolic enzymes Ins(1,4,5)P_3 5-phosphatase and 3-kinase. Racemic 2,2-F_2-Ins(1,4,5)P_3, although in other respects an excellent Ins(1,4,5)P_3 analogue, was apparently and unexpectedly not a substrate for 5-phosphatase. Since 2-deoxy-Ins(1,4,5)P_3 (**2**) is known to be a substrate for this enzyme[22], we suspected that L-2,2-difluoro-2-deoxy-*myo*-inositol trisphosphate L-(**4b**) might be acting as a potent 5-phosphatase inhibitor. We have now resolved (**4ab**) into individual D- and L- enantiomers. We report here the total synthesis of these enantiomers.

DL-2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate (**4ab**) was synthesized by diethylaminosulphur trifluoride fluorination of 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-2-inosose[28] (**6**) [prepared from (**5**)] to give (**7**), removal of non-benzylic protecting groups to give the racemic triol (**9ab**); followed by bis(2-cyanoethyl)-N,N-diisopropylaminophosphine phosphitylation, oxidation of the resulting trisphosphite with *t*-butyl hydroperoxide and deprotection using sodium in liquid ammonia, as described for Ins(1,4,5)P_3[29]. Racemic (**4ab**) was purified by ion-exchange chromatography. The individual enantiomers were synthesized by preparation of the diastereoisomeric mixed 1-*O*-[S-(-)-camphanyl] esters (**11**) and (**12**) of DL-2,2-difluoro-2-deoxy-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol (**10ab**) followed by their separation by flash chromatography on silica gel (Fig. 2). The absolute configuration of the diastereoisomeric camphanate of the D-enantiomer, D-(**4a**), was determined by single crystal X-ray crystallography[30] (Fig. 3) and, after removal of the chiral auxiliary and the isopropylidene group, the pure enantiomers of 2,2-difluoro-3,6-dibenzyl-*myo*-inositol (**9a**) and (**9b**) were individually polyphosphorylated, deblocked and purified as for the racemic modification to give D-(**4a**) and L-(**4b**) respectively. The enantiomers were purified on an ion-exchange column of DEAE Sephadex A-25 using a gradient of triethylammonium bicarbonate buffers, pH 8.0,

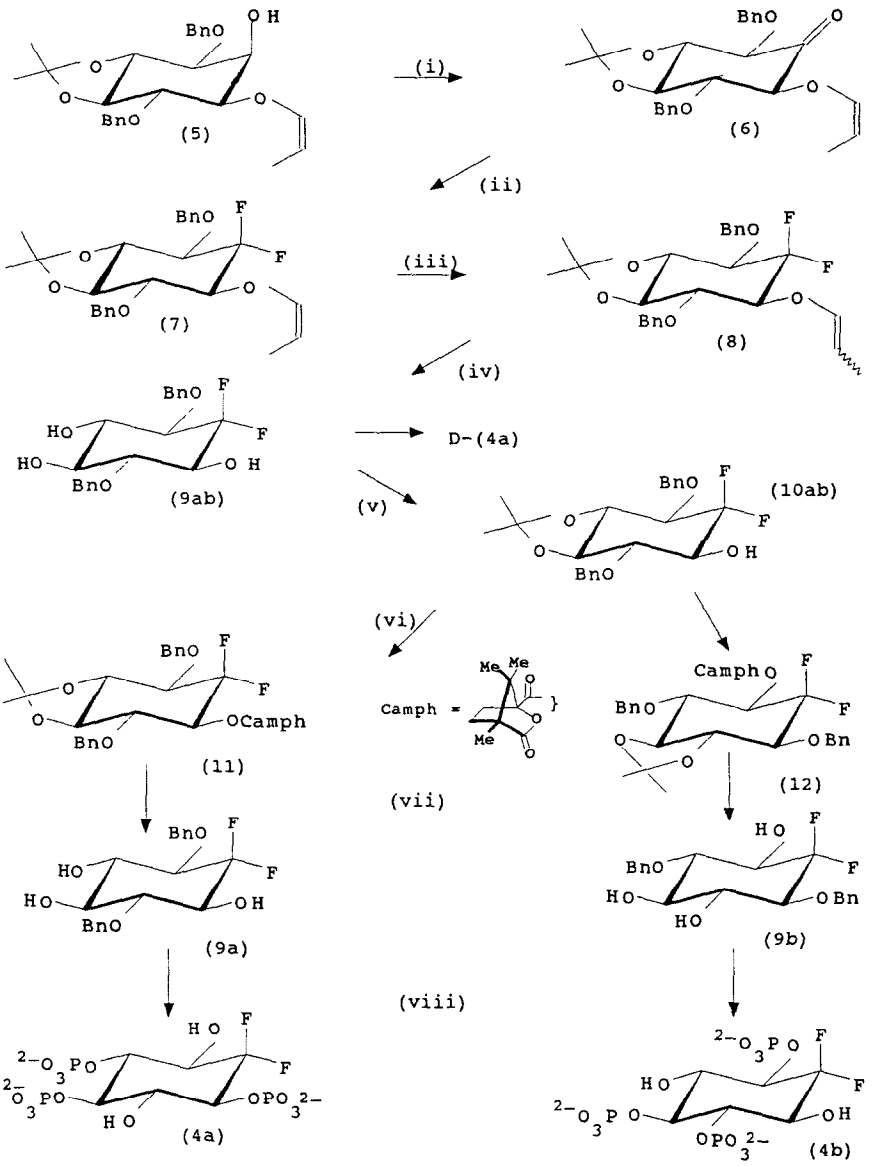


Fig. 2: Synthesis of fluorinated inositol phosphate analogues and resolution of triol (9ab) via the diastereomeric camphanates (11) and (12)

Reagents and conditions: i $(CH_3CO)_2O$ (21 equiv)/DMSO, RT 16h, (b) aq $NaHCO_3$, ii(a) DAST (4 equiv)/CH_2Cl_2, RT 5h, (b) aq $NaHCO_3$, iii(a) DABCO (0.2 equiv)/$(Ph_3P)_3RhCl$ (0.08 equiv)/EtOH:H_2O (9:1), reflux 2h, (b) H_2O, iv(a) M HCl-MeOH (1:5), reflux 30 min, (b) excess solid $NaHCO_3$, v(a) $(MeO)_2CMe_2$ (40 equiv), *p*-TsOH/Me_2CO, RT 2h, (b) Et_3N, solid $NaHCO_3$; vi(a) S-(-)-camphanic acid chloride (2 equiv)/C_5H_5N, RT 12h, (b) H_2O; vii(a) NaOH/MeOH, reflux 1h, (b) M HCl-methanol (1:5), reflux 30 min (c) excess solid $NaHCO_3$; viii(a) $(NCCH_2CH_2O)_2P\text{-}NPr^i_2$ (6 equiv), tetrazole (10 equiv)/CH_2Cl_2, RT 1h, (b) 70% aq Bu^tOOH, -78°C 1h, (c) Na-liq NH_3, -78°C 15 min, (d) H^+-Dowex.

and were isolated as their triethylammonium salts and assayed by quantitative phosphate analysis.

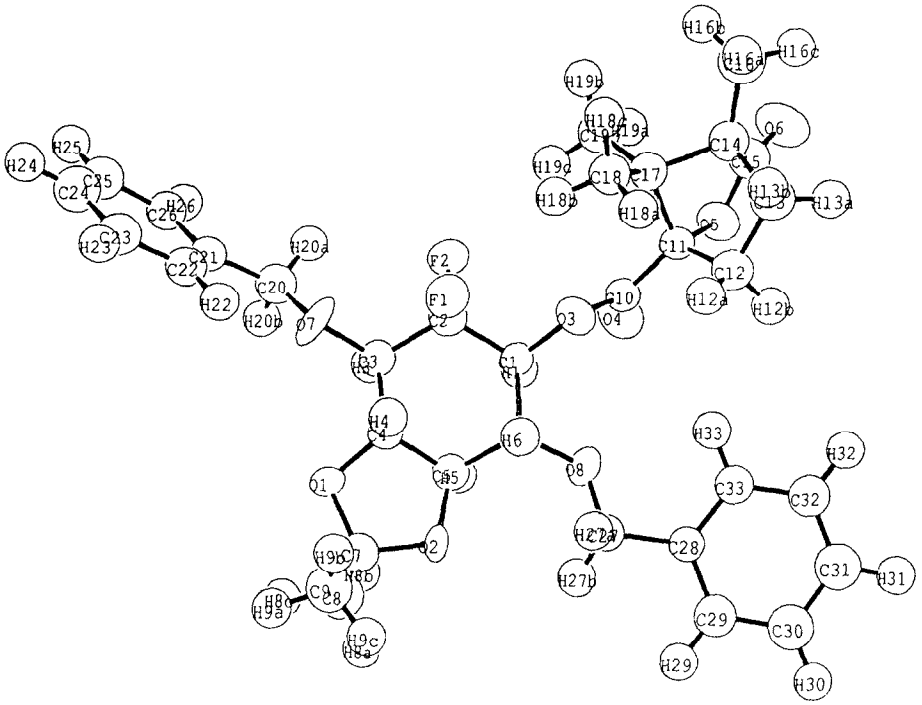


Fig. 3: Absolute configuration of 1-*O*-S-(-)-camphanyl-D-2,2-difluoro-2-deoxy-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol (11) determined by X-ray analysis.

Using fluorinated Ins(1,4,5)P_3 analogues we attempted to probe the role of the unique axial 2-hydroxyl group in determining the affinity and specificity of Ins(1,4,5)P_3 for its receptor and metabolic enzymes[18]. We previously determined the EC_{50} of DL-(**4ab**) to be 0.41μM[18] [EC_{50} for D-Ins(1,4,5)P_3, 0.13μM] D-(**4a**) and L-(**4b**) had EC_{50} values of 0.21μM and 53μM respectively. D-(**4a**) was an excellent analogue of Ins(1,4,5)P_3, being a full agonist in Ca^{2+} release, a substrate for erythrocyte membrane 5-phosphatase and rat brain 3-kinase, and bound with high affinity by the enzymes. By contrast, L-(**4b**) did not release Ca^{2+} effectively and was not a substrate for 5-phosphatase, but was a relatively potent inhibitor (K_i = 26μM). Moreover, it inhibited the phosphorylation of Ins(1,4,5)P_3 by 3-kinase potently (K_i = 11.5μM) [NB K_i for L-Ins(1,4,5)P_3 = 292μM[21]].

Modification of the 2-position of L-Ins(1,4,5)P_3 by fluorination has led to the production of a moderately potent 5-phosphatase inhibitor and, most interestingly, of the first small molecule 3-kinase inhibitor. This inhibitor has the advantage that it is a very poor agonist for Ca^{2+} release. Although the precise reasons for these inhibitory activities are not yet known, it is clear that L-**(4b)** represents a novel lead in the design of further molecular entities for pharmacological intervention in the polyphosphoinositide signalling pathway.

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