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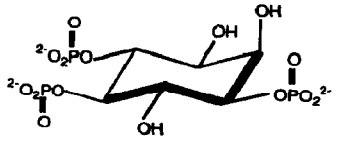
# Synthesis of (±)-3-*O*-Alkylated *Myo*-Inositol 1,4,5-Trisphosphate Analogues as Potent Receptor Ligands and Enzyme Inhibitors

**Changsheng Liu and Barry V L Potter***

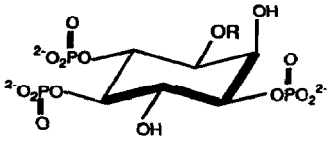
**Department of Medicinal Chemistry, School of Pharmacy and Pharmacology**
**University of Bath, Claverton Down, Bath BA2 7AY, UK.**

**Abstract:** *The synthesis of novel analogues of* myo-*inositol 1,4,5-trisphosphate alkylated at the 3-position from* myo-*inositol is described using a protection/deprotection sequence employing allyl, benzyl and* p-*methoxybenzyl groups.*

Receptor-mediated phospholipase C-catalysed cleavage of phosphatidylinositol 4,5-bisphosphate releases D-*myo*-inositol 1,4,5-trisphosphate Ins(1,4,5)$P_3$ **(1)** (Figure), as a second messenger linking the spatially separated events of cell surface receptor stimulation and release of intracellular calcium from intracellular stores[1,2]. Ins(1,4,5)$P_3$ acts through an endoplasmic reticular receptor which has been isolated[3], cloned and sequenced[4,5] and reconstituted[6]; Ins(1,4,5)$P_3$ is metabolised primarily *via* two pathways[7]: deactivation by a 5-phosphatase to Ins(1,4)$P_2$ or phosphorylation by a 3-kinase to the tetrakisphosphate Ins(1,3,4,5)$P_4$ **(2)**. The function of the latter remains controversial[8] and Ins(1,3,4,5)$P_4$ may gate a plasma membrane $Ca^{2+}$ channel[9].



(1)



R = $PO_3^{2-}$ (2)
R = Me (3)
R = Et (4)
R = $Pr^n$ (5)

**Figure**

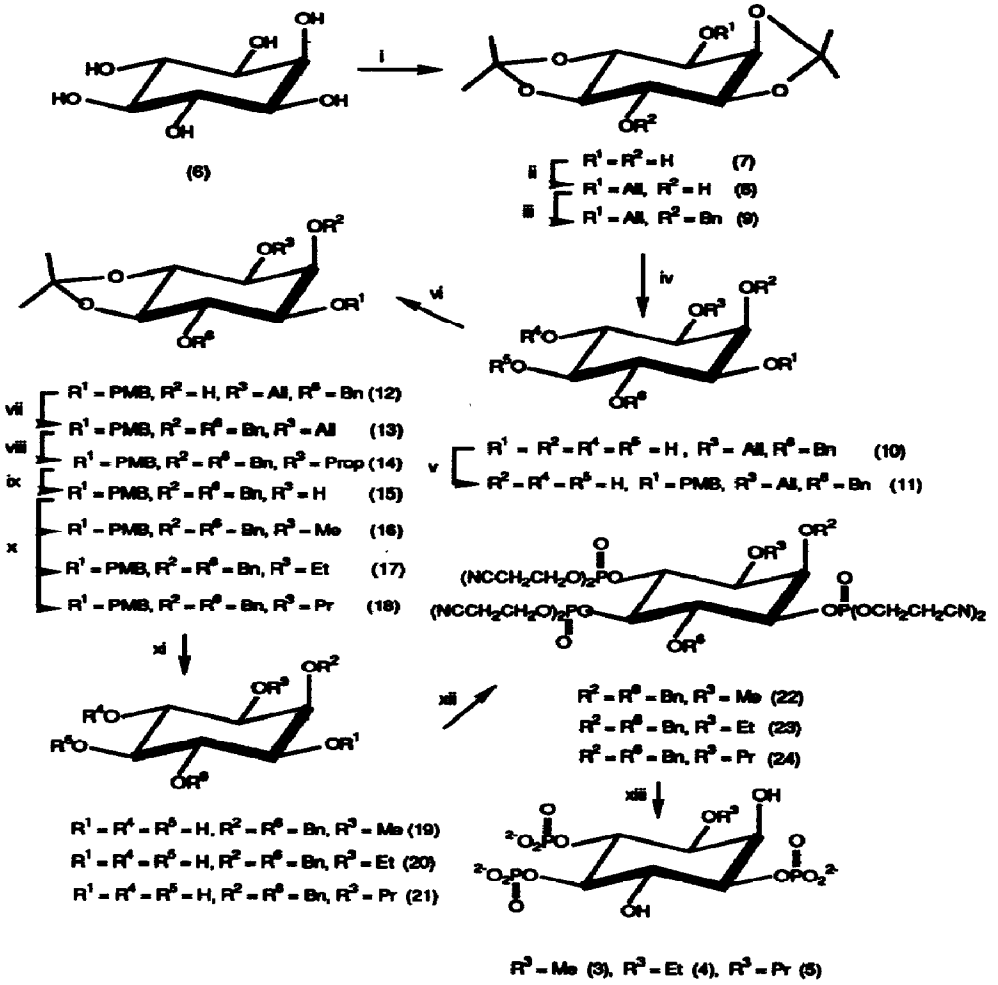
In order to study structure-activity relationships in inositol tris- and tetrakisphosphates[7,10] we have synthesized *myo*-inositol polyphosphates and their analogues as potential enzyme inhibitors and receptor antagonists[11]. In particular, analogues modified at the crucial 3-position, the site of phosphorylation by 3-kinase, are of particular interest and may lead to novel modulators of Ins(1,4,5)$P_3$/Ins(1,3,4,5)$P_4$ function. For example, we have synthesised L-*chiro*-inositol 2,3,5-trisphosphate[12] and evaluated 3-fluoro-Ins(1,4,5)$P_3$[13],

both potent and useful receptor ligands and enzyme inhibitors. We now report the synthesis of three racemic 3-*O*-alkylated analogues of Ins(1,4,5)$P_3$, designed to explore steric tolerance of the Ins(1,4,5)$P_3$ receptor and metabolic enzymes at the 3-position.

Conversion of the inositol 1,2:4,5 diketal[14] (**7**), prepared from *myo*-inositol (**6**), to the 3-*O*-allyl (**8**)[15] and 3-*O*-allyl-6-*O*-benzyl (**9**) derivatives was achieved by treatment of (**7**) first with allyl bromide/barium oxide and barium hydroxide to give (**8**) (63% yield) which was treated with sodium hydride/benzyl bromide to give the fully protected (**9**) (m.p. 122 - 123°C) (Scheme). Removal of the isopropylidene groups by treatment of (**9**) with *p*-toluene sulfonic acid in ethyl acetate/acetone/water afforded (**10**) (m.p. 152 - 153°C, 88% yield). Regioselective introduction of a 1-*O*-*p*-methoxybenzyl ether in (**10**) was achieved by treatment first with dibutyltin oxide in refluxing toluene, followed by caesium fluoride/*p*-methoxybenzyl bromide to give (**11**) (m.p. 132 - 133°C, 74% yield). After reintroduction of the 4,5-*O*-isopropylidene ketal by use of 2-methoxypropene and *p*-toluene sulfonic acid giving (**12**) (m.p. 88.5°C, 90% yield) the remaining 2-hydroxyl group was benzylated to produce (**13**)[16] (m.p. 78°C, 94% yield). The allyl group of (**13**) was isomerized to propenyl using rhodium complex $[(Ph_3)P]_3RhCl$ in the presence of diazabicyclo (2,2,2)octane to give (**14**) (m.p. 96 - 98°C, 93% yield). Removal of the propenyl group by treatment of (**14**) with mercuric chloride and mercuric oxide in acetone/water afforded the key intermediate (**15**) (m.p. 99°C). The 3-*O*-methyl ether (**16**) (as an oil), 3-*O*-ethyl ether (**17**) (m.p. 83°C) and 3-*O*-*n*-propyl ether (**18**) (as an oil) derivatives were synthesised by treatment of the anion of (**14**) with methyl iodide, ethyl iodide or *n*-propyl iodide respectively. The isopropylidene group and the 1-*O*-*p*-methoxybenzyl ether were successively cleaved by treatment of (**16**), (**17**) or (**18**) with refluxing hydrochloric acid to produce the respective triols 3-*O*-methyl-(**19**) (m.p. 116°C), 3-*O*-ethyl-(**20**) (m.p. 115°C) or 3-*O*-propyl-2,6-di-*O*-benzyl-*myo*-inositol (**21**) (m.p. 112°C). Phosphitylation of (**19**), (**20**) or (**21**) was effected using bis(2-cyanoethyl)N,N-diisopropylphosphoramidite/tetrazole in dichloromethane[17] to afford the corresponding trisphosphites which were smoothly oxidised with *tert*-BuOOH to the fully protected trisphosphates (**22**), (**23**) or (**24**) in *ca* 70% overall yield respectively. Treatment of (**22**), (**23**) or (**24**) each with sodium in liquid ammonia[16] yielded (**3**), (**4**) or (**5**) respectively, which were purified by ion-exchange chromatography on DEAE-Sephadex, eluting with a gradient of triethylammonium bicarbonate buffer and quantified by the Briggs phosphate assay.

Racemic (**3**) - (**5**) were evaluated as $Ca^{2+}$-mobilising agonists in permeabilised SH SY5Y cells. Compound (**3**) was essentially equipotent to Ins(1,3,4,5)$P_4$ but relative $EC_{50}$'s increased markedly in the order of increasing 3-position chain length ie R = Me > Et > $Pr^n$. All three 3-*O*-alkylated analogues were potent 5-phosphatase inhibitors with 3-*O*-methyl Ins(1,4,5)$P_3$ having a $K_i$ some 5-fold lower than the apparent $K_i$ for Ins(1,4,5)$P_3$. Compound (**3**) also had a $K_i$ for 3-kinase inhibition some 7-fold higher than the apparent $K_i$ for Ins(1,3,4,5)$P_4$. Clearly, if the L- isomers of these mixtures are inactive, as expected[7,11], then the true potencies of these compounds as receptor ligands and enzyme inhibitors are even more marked. Full biological details will be published elsewhere.

Previous reports focusing upon analogues derived from inversion[12], deletion[18] and fluorination[13,19] of the crucial 3-hydroxyl group have demonstrated little loss of $Ca^{2+}$-mobilising activity relative to Ins(1,4,5)$P_3$, especially in the latter two cases. The present work is the first report to demonstrate that hydrophobic bulk at the 3-position is reasonably well tolerated when R = Me, but not when R > Me. The situation is alleviated, however, in the case of substitution by an *O*-methylenecarboxylate[16] which, though bulky, will bear a negative charge at physiological pH.




**Reagents and conditions:**

(i) (a) $CH_3C(CH_3O)_2CH_3$, PTSA, reflux, (b) BzCl, pyridine, (c) NaOH, reflux; (ii) allyl bromide, BaO,$Ba(OH)_2$; (iii) BnCl, NaH; (iv) PTSA, ethyl acetate/acetone/water; (v) (a) dibutyltin oxide, reflux, (b) CsF, (*p*-MeO)BnCl; (vi) 2-methoxypropene, PTSA; (vii) BnCl, NaH; (viii) diazabicyclo[2,2,2]octane, $[(Ph_3)P]_3RhCl$, ethanol/benzene/water; (ix) $HgCl_2$, HgO, acetone/water; (x) MeI or EtI or $Pr^nI$, NaH, in DMF; (xi) M HCl, reflux; (xii) (a) $Pr^i_2NP(OBn)_2$, tetrazole in $CH_2Cl_2$, (b) 70% *tert*-BuOOH; (xiii) (a) Na/liq, $NH_3$, (b) $H_2O$

Scheme

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