Synthesis of Racemic *myo*-Inositol 1,4,5-Trisphosphate 3-*O*-Methylenecarboxylate, a Ca^{2+}-Mobilising *myo*-Inositol 1,3,4,5-Tetrakisphosphate Analogue

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Synthesis of a biologically potent inositol polyphosphate analogue, *myo*-inositol 1,4,5-triphosphate-3-*O*-methylene carboxylate incorporating an efficient oxidative cleavage of an *O*-allyl ether in the presence of protected phosphate triesters is described.

The mobilisation of Ca^{2+} from intracellular stores by generation of D-*myo*-inositol 1,4,5-triphosphate [Ins(1,4,5)P_3] **1** (Fig. 1) is now well established,[1,2] as is the metabolism of this second messenger *via* the Ins(1,4,5)P_3 5-phosphatase and 3-kinase pathways.[3,4] The latter route affords the tetrakisphosphate *myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] **2**, the proposed role of which in mediating extracellular Ca^{2+} entry remains controversial,[5] although recent evidence has been presented that Ins(1,3,4,5)P_4 gates a plasma membrane Ca^{2+} channel in endothelial cells.[6]

There has been extensive recent chemical effort in inositol polyphosphate synthesis.[7,8] As part of an ongoing programme aimed at a study of structure–activity relationships in inositol phosphates,[4,7,9] we have developed methods for the synthesis of structurally modified inositol polyphosphates. Currently, there is much interest in the synthesis of analogues possessing groups isosteric with phosphates in attempts to produce novel biological activity. This has been primarily for inositol 1-monophosphate, where inhibitors of *myo*-inositol 1-phosphatase are being sought[10,11] and for Ins(1,4,5)P_3, where the

challenge is to produce receptor antagonists and metabolic enzyme inhibitors.[7–9] Thus, in the latter area, syntheses of hexadeoxy inositol 1,4,5-tris(methylene sulfonic)acid[12] as well as a number of analogues possessing sulfonamide, sulfate, methylphosphonate and carboxymethyl groups[13] have been reported, but none of these compounds had any Ca^{2+}-mobilising or antagonistic properties. Moreover, all of the above possessed multiple phosphate surrogate groups. We believe that a conservative approach should be more successful. Consequently, we have synthesised initially, as an Ins(1,3,4,5)P_4 analogue, the first example of an inositol polyphosphate that possesses both phosphates and a non-phosphate phosphate group surrogate, *myo*-inositol 1,4,5-trisphosphate 3-*O*-methylene carboxylate [3-CME Ins(1,4,5)P_3] **3**. The synthesis of branched-chain lipophilic phosphate isosteres based upon a 1-*C*-methylene carboxylate has recently been reported,[14] testifying to current interest in methylenecarboxylates as potential phosphate surrogates.

Treatment of *myo*-inositol **4** (Scheme 1) with 2,2-dimethoxypropane and *p*-toluene sulfonic acid produced a mixture of bis(isopropylidene) ketals from which the racemic 3,6-dibenzoate could be easily isolated and converted by saponification to the 1,2 : 4,5-diketal **5**.[15] Conversion to the 3-*O*-allyl **6**[16] and 3-*O*-allyl-6-*O*-benzyl **7**† derivatives was achieved by treatment of **5** first with allyl bromide/barium oxide and barium hydroxide (63% yield) and then **6** with benzyl chloride/sodium hydride, respectively, (100% yield). Removal of the isopropylidene groups by treatment of **7** with *p*-toluene sulfonic acid in ethyl acetate–acetone–water afforded **8** (88% yield). Regioselective introduction of a 1-*O*-*p*-methoxybenzyl ether in **8** was achieved by treatment of **8** with dibutyl tin oxide in refluxing toluene, followed by caesium fluoride/*p*-methoxybenzyl bromide to give **9** in 74% yield. After reintroduction of the 4,5-*O*-isopropylidene ketal by use of 2-methoxypropene and *p*-toluene sulfonic acid giving **10** (90% yield) the remaining 2-hydroxy group was benzylated to produce **11** (94% yield) (cf. T. Desai J. Grigg, R. Grigg, S. Payne and S. Penades, *Carbohydr. Res.*, 1992, **234**, 1). The isopropylidene group and the 1-*O*-*p*-methoxybenzyl ether were successively cleaved by treatment of **11** with refluxing hydrochloric acid to produce the key triol 3-*O*-allyl-2,6-di-*O*-benzyl-*myo*-inositol **12** in 90% yield. Phosphitylation of **12** was effected using biscyanoethoxydiisopropylaminophosphine-tetrazole in dichloromethane[17] to afford the corresponding trisphosphite which was smoothly oxidised with Bu^{t}OOH to the fully protected trisphosphate **13** in 80% overall yield from **12**. The efficient oxidative cleavage of the 3-*O*-allyl ether of **13** in the presence of the cyanoethyl protected phosphate triesters was achieved by treatment of **13** with $NaIO_4/RuCl_3$·hydrate[18] to afford **14** in 64% yield. Cyanoethyl and benzyl protecting groups of **14** were subsequently removed by treatment with sodium in liquid ammonia[19] to provide crude **14**, which was subjected to ion-exchange chromatography on Q-Sepharose eluting with a gradient of triethylammonium bicarbonate buffer, to afford pure **3** in 68% yield.

Racemic 3-CME Ins(1,4,5)P_3 was found to be a calcium mobilising agonist in permeabilised neuroblastoma cells with a potency similar to Ins(1,3,4,5)P_4 [EC_{50} = 3.50; cf 2.5 μmol dm^{-3} for D-Ins(1,3,4,5)P_4]. It had a six fold higher K_i for Ins(1,4,5)P_3 3-kinase than Ins(1,3,4,5)P_4 but was twice as potent at binding to Ins(1,4,5)P_3 5-phosphatase and was an inhibitor of this enzyme. Presumably only the D-enantiomer is recognised by these proteins. Full biological details for **3** will be described elsewhere.

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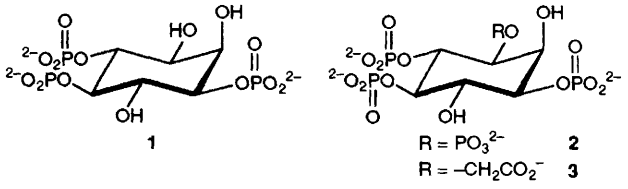

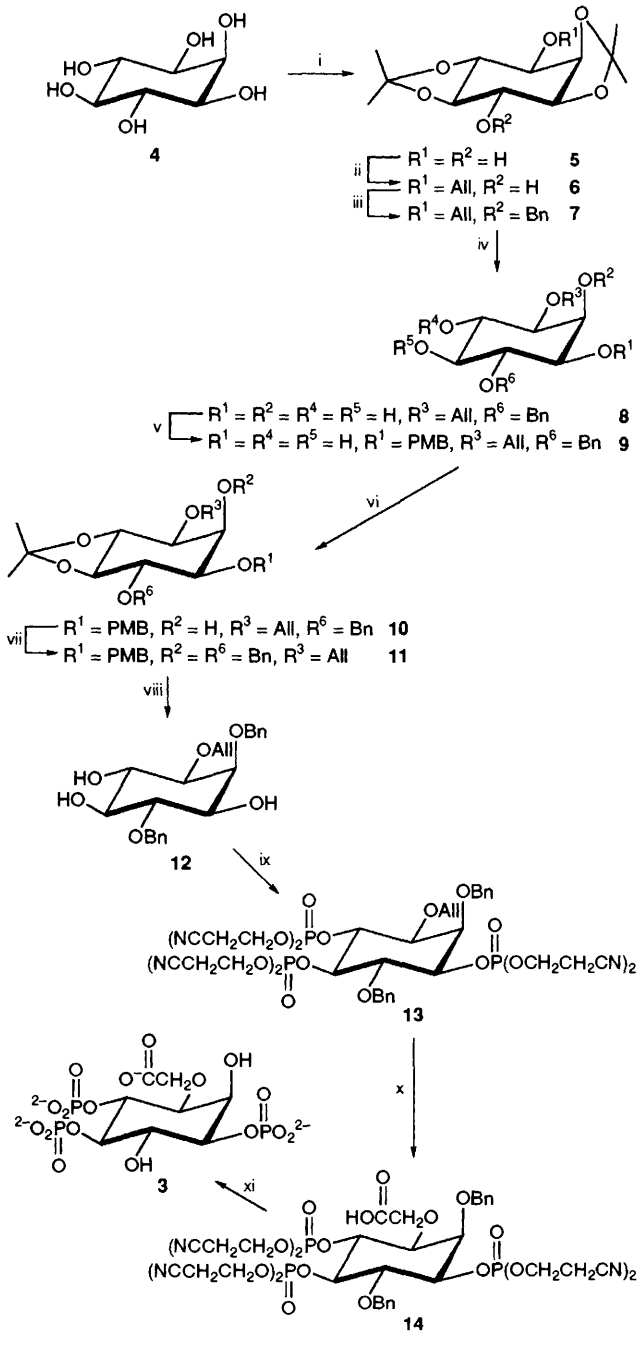

Scheme 1 *Reagents and conditions*: i, (*a*) MeC(MeO)$_2$Me, PTSA, reflux, (*b*) BzCl, pyridine, (*c*) NaOH, reflux; ii, allyl bromide, BaO/Ba(OH)$_2$; iii, BnCl, NaH; iv, PTSA, ethyl acetate–acetone–water (9 : 9 : 1 v/v); v, (*a*) dibutylin oxide, reflux, (*b*) CsF, (*p*-MeO)BnCl; vi, 2-methoxypropene, PTSA; vii, BnCl, NaH; viii, M HCl, reflux; ix, $Pr^i_2NP(OCH_2CH_2CN)_2$, tetrazole in CH_2Cl_2, (*b*) 70% Bu^{t}OOH; x, $NaIO_4$, $RuCl_3$·xH_2O; xi, (*a*) Na/liq. NH_3, (*b*) H_2O. All compounds except **4** are racemic. All = allyl, PMB = *p*-methoxybenzyl, Bn = benzyl, PTSA = *p*-toluene sulfonic acid.

† All new compounds exhibited satisfactory spectroscopic and analytical data.

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