Synthesis of D-3-Deoxy-*myo*-Inositol 1,4,5-Trisphosphate and its Effect on Ca^{2+} Release in NIH 3T3 Cells

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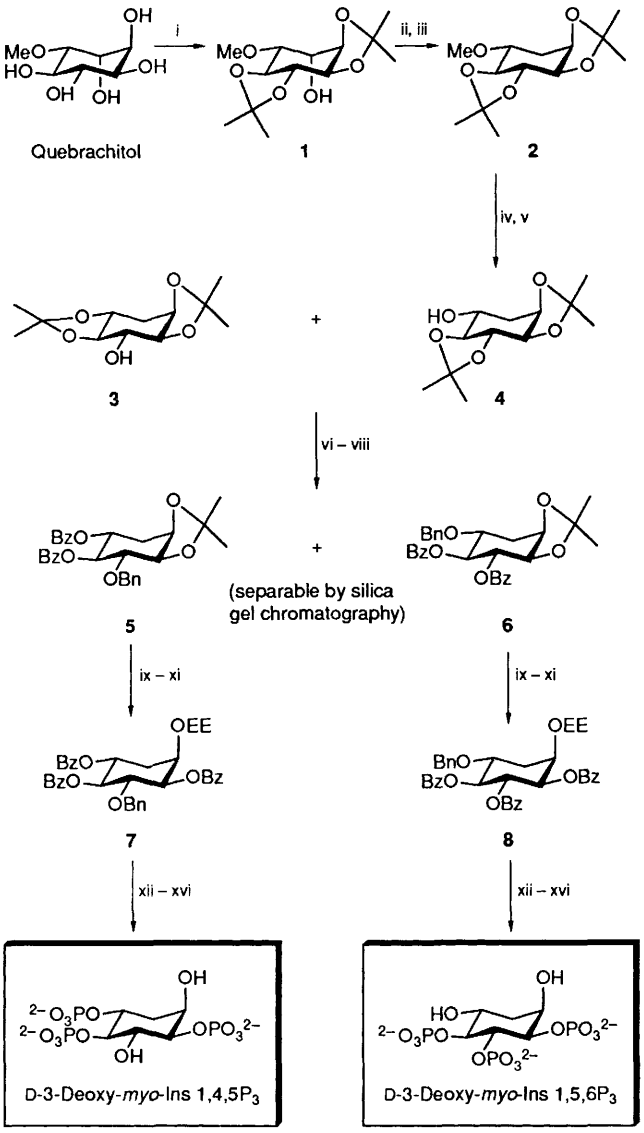
The synthesis of D-3-deoxy-*myo*-inositol 1,4,5-trisphosphate is reported together with its effect on Ca^{2+} release in permeabilized NIH 3T3 cells.

The importance of inositol phosphates for intracellular signalling is now well appreciated.[1,2] Stimulation of cell surface receptors by a variety of ligands initiates the hydrolysis of the membrane-located phosphatidylinositol 4,5-bisphosphate to give initially D-*myo*-inositol 1,4,5-trisphosphate (Ins 1,4,5P_3) and diacylglycerol. Ins 1,4,5P_3 binds to specific recognition sites on the endoplasmic reticulum resulting in the mobilization of intracellular Ca^{2+} stores.[3] The further metabolism of Ins 1,4,5P_3 is quite complex. The action of various kinases and phosphatases results in the conversion of Ins 1,4,5P_3 to many different inositol phosphates whose biochemical roles as yet remain to be elucidated.[4–6] A potentially important transformation is through the action of a *myo*-inositol 3-kinase to give *myo*-inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5P_4).[1] Ins 1,3,4,5P_4 may control the refilling of the Ins 1,4,5P_3 regulated intracellular Ca^{2+} pools.[7] The extent to which the conversion of Ins 1,4,5P_3 to Ins 1,3,4,5P_4 occurs in different cell preparations used for studying inositol phosphate second messenger action is not clear, but agents capable of exhibiting Ins 1,4,5P_3-like agonist effects on Ca^{2+} release without being subject to metabolism by the 3-kinase pathway should serve as useful probes of inositol phosphate function. Accordingly, we elected to prepare D-3-deoxy Ins 1,4,5P_3 and to evaluate its ability to induce intracellular Ca^{2+} release from permeabilized NIH 3T3 cells.[8]

As shown in Scheme 1, quebrachitol was converted to its diacetonide **1**, and the remaining free hydroxyl group of **1** was removed by the Barton deoxygenation procedure.[9] Next, BBr_3 was used to remove all protecting groups, and the resulting compound, viburnitol, was converted to a 1:1.3 mixture of bis-acetonides **3** and **4**, respectively. This mixture was benzylated, the *trans*-acetonide cleaved selectively, and the free hydroxy groups benzoylated to provide **5** and **6**. At this stage, separation of the regioisomers could be accomplished readily by silica gel chromatography. Compound **5** was converted in turn to its tribenzoate **7** by acetonide cleavage followed by mono-benzoylation. The axial hydroxyl was protected as its ethoxyethyl ether, and the benzoate groups at positions 1, 4 and 5 were removed by base hydrolysis. Phosphorylation by use of sodium hydride and tetrabenzylpyrophosphate, followed by hydrogenolysis over PtO_2, exposure to water at 23 °C, and titration to a pH of 10 with 1 M NaOH gave the desired D-3-deoxy Ins 1,4,5P_3.

By carrying isomer **6** through an identical sequence of reactions, D-3-deoxy Ins 1,5,6P_3 was also obtained in similar overall yield.

Both compounds were evaluated for their effect on Ca^{2+} release using NIH 3T3 cells which were made permeable with medium containing 0.005% saponin as described previously.[10] Preliminary studies showed that the uptake of $^{45}Ca^{2+}$ by the



Scheme 1 Synthesis of D-3-Deoxy-*myo*-Inositol 1,4,5-Trisphosphate *Reagents and conditions*: i, $H_2C{=}C(OMe)CH_3$, camphorsulphonic acid (CSA), DMF, 60 °C, 4 h (80–85%); ii, NaH, CS_2, THF, 23 °C, then MeI, 23 °C; iii, $Bu^n{}_3SnH$, toluene, reflux, 1.5 h (89% overall yield); iv, BBr_3, CH_2Cl_2, 23 °C, 12 h (80%); v, $H_2C{=}C(OMe)CH_3$, CSA, DMF, 60 °C (88%); vi, NaH, $PhCH_2Br$, THF, 23 °C, 6 h (88%); vii, AcCl (cat.), MeOH/CH_2Cl_2 (1:2), 23 °C, 15 min (90%); viii, PhCOCl, pyr, 23 °C, 12 h (95%), separate by silica gel chromatography; ix, conc. HCl (cat.), MeOH, 23 °C, 3 h (95%); x, PhCOCl, pyr, 0 °C, 24 h (91%); xi, $H_2C{=}CHOEt$, pyridinium *p*-toluenesulphonate (cat.), CH_2Cl_2, 0–23 °C, 4 h (95%); xii, K_2CO_3, MeOH, 23 °C, 14 h (90%); xiii, NaH, tetrabenzylpyrophosphate, DMF, 0 °C, 8 h (50%); xiv, H_2 (1 atm), PtO_2, EtOH, 23 °C, 4 h; xv, H_2O, 23 °C, 3 h; xvi, titrate to pH = 10 with 1 M NaOH (56% overall yield for steps xiv–xvi)

cells reached a plateau by 6 min. Ins 1,4,5P_3 (Molecular Probes), 3-deoxy Ins 1,4,5P_3 or 3-deoxy Ins 1,5,6P_3 was added at 6.25 min, and the $^{45}Ca^{2+}$ remaining in the cells was measured at 7 min.

As is apparent from the dose response curve presented in Fig. 1, D-3-deoxy Ins 1,4,5P_3 acts as a full agonist in releasing $^{45}Ca^{2+}$ from the 3T3 cells, while the 1,5,6-trisphosphate is

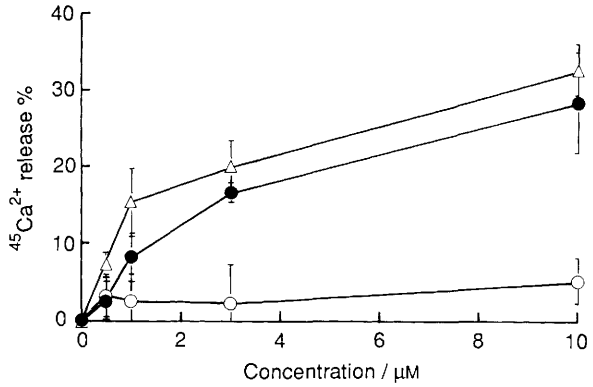


Fig. 1 Concentration-response curve for the release of $^{45}Ca^{2+}$ from non-mitochondrial stores of saponin-permeabilized NIH 3T3 cells by (●) D-3-deoxy Ins 1,4,5P_3, (○) D-3-deoxy Ins 1,5,6P_3, and (△) Ins 1,4,5P_3. $^{45}Ca^{2+}$ release is expressed as a percent of the total $^{45}Ca^{2+}$ in the cells at 6 min. Values are the mean of 5 determinations and bars represent s.d. The protocols are as reported previously (Seewald *et al.*[10])

inactive. Ins 1,3,4,5P_4 did not release $^{45}Ca^{2+}$ in this system (results not shown). From the results of these studies we can, thus, conclude that a hydroxy group is not required at the 3-position of an inositol 1,4,5P_3 in order to mobilize Ca^{2+} release from the endoplasmic reticulum. Since no second messenger role has been identified for Ins 1,5,6P_3, it is not surprising that D-3-deoxy Ins 1,5,6P_3 is inactive.

D-3-Deoxy Ins 1,4,5P_3 exhibits the same agonist effects as Ins 1,4,5P_3 on Ca^{2+} release, although its role is not further complicated by a possible simultaneous action of 3-kinase(s); this implies that the former compound may be preferred in place of Ins 1,4,5P_3 in studying intracellular Ca^{2+} release in cells.†

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† Satisfactory ^1H and ^{13}C NMR, IR, and high resolution mass spectral data were obtained for all new compounds.