Rapid and practical synthesis of D-*myo*-inositol 1,4,5-trisphosphate

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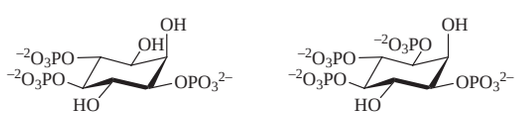
COMMUNICATION

A concise synthetic sequence to biologically active D-*myo*-inositol 1,4,5-trisphosphate is described involving just five steps from *myo*-inositol and minimal chromatography with a key transformation of orthoacetate into acetate protection.

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] is a cellular second messenger first identified in 1983.[1] Phospholipase C catalyses hydrolysis of phosphatidylinositol 4,5-bisphosphate to Ins(1,4,5)P_3 and diacylglycerol. Ins(1,4,5)P_3 interacts specifically with a tetrameric receptor-operated Ca^{2+} channel on the endoplasmic reticulum to mobilise Ca^{2+} stores in stimulated cells.[2] Ins(1,4,5)P_3 mediates the agonist-induced response *via* this rise in intracellular Ca^{2+} concentration. There is continuing interest in the biology of Ins(1,4,5)P_3 and the many other related inositol polyphosphates, and in the synthesis of analogues that may offer the prospect of pharmacological intervention in this signalling pathway.[3]



Structures of D-Ins(1,4,5)P_3 **1** and D-Ins(1,3,4,5)P_4 **2**

Since Ozaki and co-workers first prepared optically active Ins(1,4,5)P_3 in 1986,[4] many routes have been described for the synthesis of enantiomerically pure Ins(1,4,5)P_3 from diverse starting materials.[3] They are generally time-consuming long linear sequences involving extensive chromatography. The critical strategic points in most routes to chiral inositol phosphates are desymmetrisation of *myo*-inositol (a *meso* compound) and resolution of an intermediate. The most rapid route to chiral **1** hitherto described is probably that of Salamonczyk and Pietrusiewicz.[5] This route, however, involves a difficult precipitation-driven equilibrium and is not readily modified to provide materials leading to other inositol phosphate derivatives. Here, we describe a shorter synthetic sequence which obviates the need for tedious chromatographic separations and long sequences of protecting group manipulations and which, by modification of reaction conditions and/or isolation procedures, can provide material for alternative targets. The route is also applicable to relatively large scale preparations.

We have recently published[6] a rapid synthesis of D-*myo*-inositol 1,3,4,5-tetrakisphosphate [D-Ins(1,3,4,5)P_4, **2**] and its enantiomer L-Ins(1,3,4,5)P_4 by a chiral desymmetrisation approach. In acid catalysed deprotection of intermediates utilising orthoformate protection[7] we often observed small quantities of formate esters as products of partial deprotection. While these esters were not stable enough to be useful synthetically, this finding did suggest that a different orthoester such as an orthoacetate might be useful as an intermediate protecting group that could be later transformed by partial hydrolysis into an acetate ester of defined regiochemistry such as **8**‡ (Scheme 1). Building upon the above we have now developed a synthesis of chiral Ins(1,4,5)P_3 involving only five steps from *myo*-inositol. Furthermore, the methodology developed has potential application in the synthesis of other inositol phosphates. *myo*-Inositol orthoacetate **4**§ was prepared by modifying methodology established for the synthesis of the orthoformate orthoester of *myo*-inositol.[8] *myo*-Inositol was treated with triethyl orthoacetate and PTSA in DMF. The acid was removed by precipitation as a salt. After filtration and evaporation *in vacuo* the residue was dissolved in hot methanol and filtered to remove any unreacted *myo*-inositol. Recrystallisation from methanol–chloroform–light petroleum followed by further recrystallisation from methanol gave *myo*-inositol orthoacetate **4** of sufficient purity for use in the next step, in 60–65% yield. Orthoacetate **4** was treated with 2.1 equivalents of 1*S*-(−)-camphanoyl chloride and a catalytic amount of DMAP in dichloromethane to yield the two diastereoisomeric dicamphanates **5**¶ and **6**. In our synthesis of the enantiomers of *myo*-inositol 1,3,4,5-tetrakisphosphate,[6] careful chromatography was required to separate the analogous 2,4- and 2,6-diastereoisomeric dicamphanate esters of *myo*-inositol orthoformate. As a consequence of the orthoacetate functionally, the corresponding diesters **5** and **6** were easier to crystallise and thus were separable without chromatography. Crystallisation from ethyl acetate gave the required 1D-2,6-diester **5** in 37% yield. Control over the selectivity of the desymmetrisation could be achieved by changing the reaction conditions. An excess

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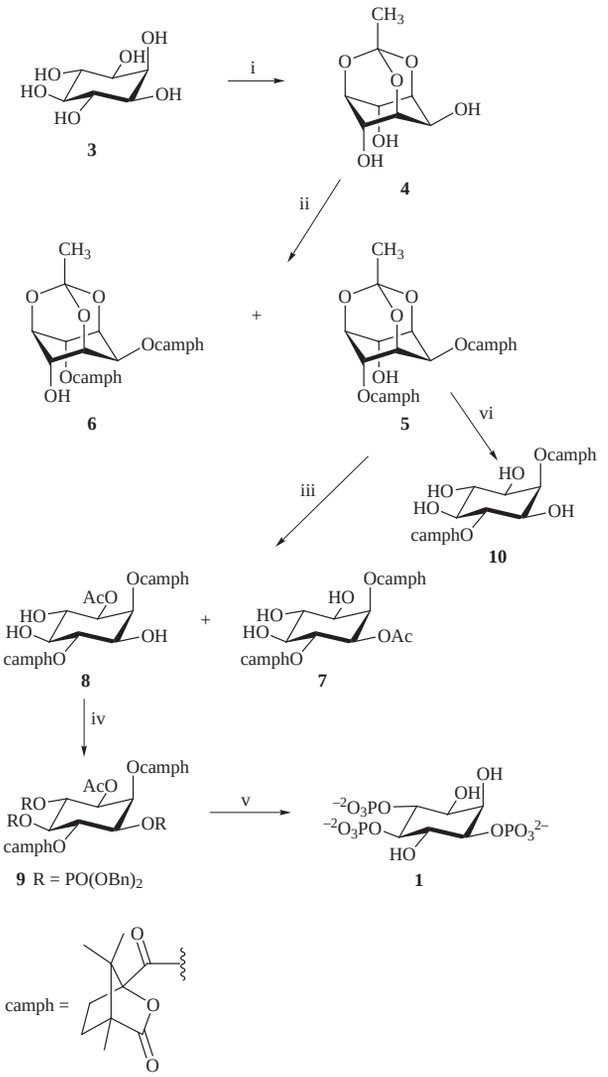
‡ Data for compound **8**: mp 226–229 °C; $[a]_D$ +5.2 (*c* 1.9, DMF) (Found: C, 57.5; H, 6.5. Calc. for $C_{28}H_{38}O_{13}$: C, 57.63; H, 6.74%); δ_H(400 MHz; $[^2H]_7$DMF; TMS) 0.96, 1.02, 1.05, 1.10, 1.12, 1.17 (18H, 6 × s, camph CH_3), 1.55–1.67 (2H, m, camph CH_2), 1.94–2.14 (4H, m, camph CH_2), 2.02 (3H, s, $COCH_3$), 2.49–2.61 (2H, m, camph CH_2), 3.61 (1H, dt, H-5, *J* 5.9, 9.3 Hz D_2O, exchange gives t, *J* 9.8 Hz) 3.86 (1H, dt, H-4, *J* 4.9, 9.8 Hz, D_2O exchange gives t, *J* 9.8 Hz), 4.15–4.17 (1H, m, H-1, D_2O exchange gives dd, *J* 2.8, 10.4 Hz), 4.96 (1H, dd, H-3, *J* 2.8, 10.1 Hz), 5.29 (1H, t, H-6, *J* 10.1 Hz), 5.58 (1H, d, 5-OH, *J* 5.4 Hz), 5.60 (1H, m, 4-OH), 5.62–5.63 (1H, m, H-2, D_2O exchange gives t, *J* 2.8 Hz), 5.75 (1H, d, 1-OH, *J* 5.9 Hz); *m*/*z* (FAB+) [Found: $(M + H)^+$, 583.2394. $C_{28}H_{39}O_{13}$ requires 583.2391].

§ Data for compound **4**: mp 185–187 °C (with softening at 165 °C) (Found: C, 47.0; H, 6.0. Calc. for $C_8H_{12}O_6$: C, 47.06; H, 5.92%); δ_H(400 MHz; $[^2H]_6$DMSO) 1.28 (3H, s, CH_3), 3.94–4.0 (4H, m, Ins-H), 4.21–4.27 (2H, m, Ins-H), 5.19 (1H, s, 2-OH), 5.38 (2H, d, 4-OH and 6-OH, *J* 5.2 Hz); δ_C(100 MHz; $[^2H]_6$DMSO) 24.30 (CH_3), 57.70, 67.24, 69.24, 74.98 (Ins C), 107.66 (CCH_3); *m*/*z* (FAB+) 205 [$(M + H)^+$, 100%].

¶ Data for compound **5**: mp 228–231 °C; $[a]_D^{24}$ +17 (*c* = 1, CH_2Cl_2) (Found: C, 59.6; H, 6.45. Calc. for $C_{28}H_{36}O_{12}$: C, 59.59; H, 6.43%); δ_H(400 MHz; $CDCl_3$; TMS) 0.98, 0.99, 1.08, 1.09, 1.10, 1.11 (18H, 6 × s, camph CH_3), 1.44 (3H, s, O_3CCH_3), 1.67–1.76 (2H, m, camph CH_2), 1.91–2.10 (4H, m, camph CH_2), 2.39–2.53 (2H, m, camph CH_2), 3.23 (1H, d, 2-OH, *J* 6.4 Hz), 4.33–4.36 (1H, m, H-3), 4.39–4.43 (1H, m, H-1), 4.45–4.50 (1H, m, H-5), 4.57–4.62 (1H, m, H-4), 5.21–5.25 (1H, m, H-2), 5.52–5.57 (1H, m, H-6); δ_C(100 MHz; $CDCl_3$) 9.59, 9.66, 16.47, 16.54, 16.65 (camph CH_3), 23.94 (O_3CCH_3), 28.76, 28.90, 30.44, 30.86 (camph CH_2), 54.41, 54.51, 54.80, 54.89, 90.86, 91.03 (camph C), 63.72, 66.78, 68.15, 69.14, 69.30, 71.86 (ins C), 108.8 (O_3C), 166.15, 166.81, 177.73, 178.02 (camph CO); *m*/*z* (FAB+) 565 [$(M + H)^+$, 100%].



Scheme 1 *Reagents and conditions*: i, Triethyl orthoacetate, PTSA, DMF, 90–100 °C, 4 h; ii, 1*S*-(−)-camphanoyl chloride, DMAP, dichloromethane, 0 °C then RT, 2 h; iii, 80% aqueous trifluoroacetic acid, 7 days; iv (*a*), $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, dichloromethane, RT, 1 h; (*b*), MCPBA, dichloromethane, −78 °C to RT; v (*a*), H_2, 10% Pd on C, MeOH–H_2O, RT, 12 h; (*b*), conc. aqueous NH_3, 60 °C, 4 h; vi, 5 M HCl–MeOH 1 : 11, reflux, 14 h

of the 2,4-diester **6** was gained by omitting the DMAP, and crystallisation from methanol gave **6** in 40–45% yield. The regiochemistry of the required 2,6-dicamphanate **5** was determined by removing the orthoacetate completely by refluxing in methanol–HCl to give tetrol **10**. The $[a]_D$ value of **10** was found to be identical to that of authentic material {$[a]_D^{25}$ −8 (*c* 1, DMF)} synthesised from the analogous 2,6-dicamphanate with orthoformate protection, a compound whose structure had been unambiguously determined previously by X-ray crystallography.[6]

Selective transformation of **5** to **8** was required to produce the appropriately protected intermediate for the synthesis of **1**. Whereas acidic hydrolysis of the analogous orthoformate, as described in our synthesis of **2**, resulted in complete loss of the protection afforded by this functionality, treatment of **5** with 80% aqueous trifluoroacetic acid at room temperature for one week yielded the acetate esters **7** and **8**. Regioisomer **8**, required to make Ins(1,4,5)P_3, could be crystallised directly from the mixture in 25% yield using methanol–light petroleum (total yield 31%). Pure **7** could be obtained in 40% yield by chromatography of the mother liquor (R_F **8** 0.32, **7** 0.47, [EtOAc]). Structure determination of these acetate esters was straightforward by ^1H-^1H COSY NMR experiments. No derivative with a 5-acetate was obtained. Phosphitylation of **8** using dibenzyl *N*,*N*-diisopropyl phosphoramidite[9] followed by oxidation of the intermediate phosphite triester with MCPBA gave the protected phosphate **9** in 72% yield. One-pot deprotection by hydrogenolysis at atmospheric pressure over 10% Pd/C, then ester hydrolysis with ammonia at 60 °C gave Ins(1,4,5)P_3. Deprotection in this manner ensured that any risk of phosphate migration was minimised. For biological evaluation, the Ins(1,4,5)P_3 **1** was further purified by ion exchange chromatography on Q-Sepharose Fast Flow resin eluting with a gradient of triethylammonium hydrogen carbonate buffer (0–100% 1 M) to give the triethylammonium salt in 70% yield (quantified by Briggs phosphate assay[10]). A typical preparation generated 60 mg of this highly active target compound. Clearly the 2,4-diester should provide the now well-established biological control L-Ins(1,4,5)P_3[11] *via* an analogous synthetic route. The other readily available regioisomeric acetate **7** could find use in the synthesis of further inositol polyphosphates or phospholipids.

Biological evaluation using saponin-permeabilised hepatocytes showed that synthetic **1** prepared *via* this route was indistinguishable in its intracellular Ca^{2+} release profile (EC_{50} = 121 ± 2 nM) from a sample of commercially available Ins(1,4,5)P_3 (EC_{50} = 122 ± 8 nM).

In summary, we have described a novel route to this highly potent second messenger in chiral form with considerable advantages over currently established routes in terms of facility, speed and potential for large scale synthesis.

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