1D-*myo*-Inositol 1,4,5-Trisphosphate and 1D-*myo*-Inositol 1,3,4,5-Tetrakisphosphate Analogues Modified at C-3; Synthesis of 1D-3-*C*-(Trifluoromethyl)-*myo*-inositol 1,4,5-Trisphosphate and 1L-*chiro*-Inositol 1,2,3,5-Tetrakisphosphate from L-Quebrachitol

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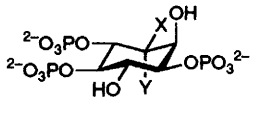
The novel 3-modified 1D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] analogues 1D-3-*C*-(trifluoromethyl)-*myo*-inositol 1,4,5-trisphosphate and 1L-*chiro*-inositol 1,2,3,5-tetrakisphosphate are synthesized from L-quebrachitol, and the preliminary results on their Ca^{2+} releasing activity suggest that the Ins(1,4,5)P_3 receptor can accommodate some steric bulk in the axial region of the 3-position of Ins(1,4,5)P_3 when the equatorial 3-hydroxy group is retained.

The intracellular second messengers 1D-*myo*-inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3] (Fig. 1) and diacylglycerol, formed by phospholipase C catalysed hydrolysis of the minor membrane lipid phosphatidylinositol 4,5-bisphosphate, play an important role in signal transduction processes.[1] Ins(1,4,5)P_3 mediates the release of Ca^{2+} from nonmitochondrial stores by interaction with a specific receptor, which has been purified,[2,3] cloned and sequenced.[4,5] Current evidence[6] suggests that there are at least three subtypes of Ins(1,4,5)P_3 receptors, and although little is known of the specificity of ligand interaction at these sites, the potential for selective manipulation needs exploration. The metabolism of Ins(1,4,5)P_3 involves two main pathways. Dephosphorylation of the 5-phosphate group of Ins(1,4,5)P_3 by Ins(1,4,5)P_3-5-phosphatase terminates the Ca^{2+} mobilizing signal,[7] while phosphorylation of the 3-hydroxy group by Ins(1,4,5)P_3-3-kinase gives rise to the putative additional second messenger 1D-*myo*-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4].[8,9]

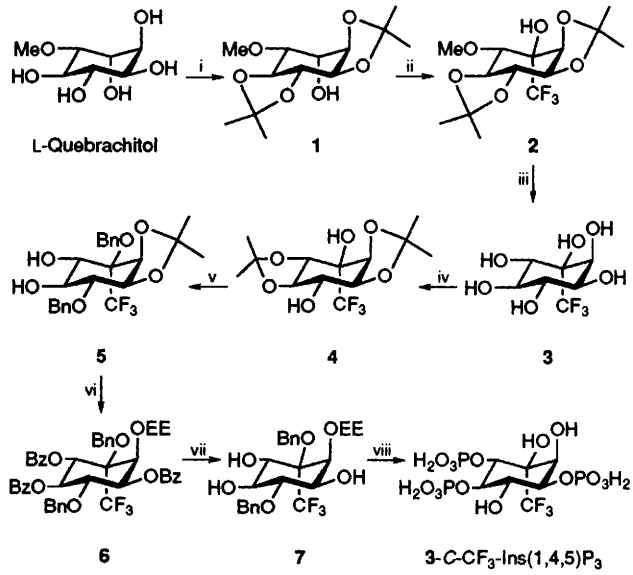
Within the last decade numerous analogues of Ins(1,4,5)P_3 have been synthesized.[10–12] Some of us have prepared previously L-*chiro*-inositol 2,3,5-trisphosphate [L-ch-Ins(2,3,5)P_3][13]† and 1D-3-deoxy-3-fluoro-*myo*-inositol 1,4,5-trisphosphate [3-F-Ins(1,4,5)P_3][14] through inversion of the 3-hydroxy group of Ins(1,4,5)P_3 or by isosteric replacement of hydroxy with a fluorine atom, respectively (Fig. 1). Both analogues possessed relatively potent ligand and agonist activity at the Ins(1,4,5)P_3 receptor, were resistant to Ins(1,4,5)P_3-3-kinase, and acted as potent inhibitors of the enzyme as expected. On the other hand, the analogues were recognised by Ins(1,4,5)P_3-5-phosphatase with high affinity. 3-F-Ins(1,4,5)P_3 was hydrolysed by the enzyme at a similar rate to Ins(1,4,5)P_3, while L-ch-Ins(2,3,5)P_3 was an inhibitor.[15] These results suggested that the environment around C-3 is of major importance for recognition not only by Ins(1,4,5)P_3-3-kinase but also by Ins(1,4,5)P_3-5-phosphatase. Therefore, novel 3-modified Ins(1,4,5)P_3 analogues could be of great utility in the search for Ins(1,4,5)P_3 receptor agonists and antagonists that are stable to one or both routes of metabolism. Herein, we describe the synthesis of the novel 1D-3-*C*-(trifluoromethyl)-*myo*-inositol 1,4,5-trisphosphate [3-*C*-CF_3-Ins(1,4,5)P_3] (Scheme 1) and 1L-*chiro*-inositol 1,2,3,5-tetrakisphosphate [L-ch-Ins(1,2,3,5)P_4] (Scheme 2) from L-quebrachitol, together with preliminary results on their binding and Ca^{2+} releasing activity.



Ins(1,4,5)P_3; X = OH, Y = H
Ins(1,3,4,5)P_4; X = OPO_3^{2-}, Y = H
3-F-Ins(1,4,5)P_3; X = F, Y = H
L-ch-Ins(2,3,5)P_3; X = H, Y = OH
L-ch-Ins(1,2,3,5)P_4; X = H, Y = OPO_3^{2-}
3-*C*-CF_3-Ins(1,4,5)P_3; X = OH, Y = CF_3

Fig. 1 Structures of D-*myo*- and L-*chiro*-inositol analogues

The naturally occurring cyclitol L-quebrachitol (1L-2-*O*-methyl-*chiro*-inositol) was converted to the diacetonide **1**[16] (Scheme 1). Swern oxidation of the remaining unprotected axial hydroxy group of **1** was carried out to give an unstable ketone[17,18] which was trifluoromethylated[19] with trifluoromethyltrimethylsilane (TMS–CF_3) to give the adduct **2** in 49% yield. The configuration at C-3 was verified by a single crystal X-ray structural analysis of **2**. We note here that similar α-face selectivity was observed when the ketone was subjected to nucleophilic addition of methylmagnesium bromide.[18] Demethylation and removal of the protecting groups in **2** by BBr_3 provided the novel 1D-3-*C*-(trifluoromethyl)-*myo*-inositol **3**‡ in 60% yield. Treatment of **3** with 2-methoxypropene

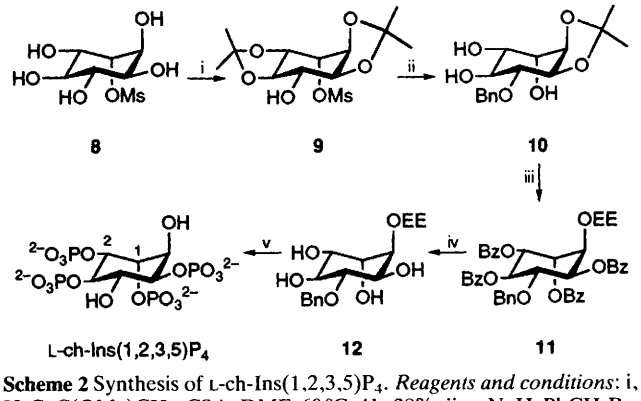


Scheme 1 Synthesis of 3-*C*-CF_3-Ins(1,4,5)P_3. *Reagents and conditions*: i, H_2C=C(OMe)Me, camphorsulfonic acid (CSA), DMF, 60 °C, 4 h, 81%; ii, a, $(COCl)_2$, Me_2SO, CH_2Cl_2; then diisopropylethylamine, −73 to −30 °C, 1 h, 77%; b, TMS–CF_3, Bu^n_4NF (equimolar), THF, 1 h, 49%; iii, BBr_3, CH_2Cl_2, −43 to 0 °C, 12 h, 60%; iv, as for i, 30%; v, a, NaH, $PhCH_2Br$, DMF, 0 °C, 1 h, 79%; b, AcCl (catalyst), MeOH–CH_2Cl_2 (1 : 2), 1 h, 78%; vi, a, PhCOCl, pyridine (py), DMAP (catalyst), 25 °C, 16 h, 88%; b, conc. HCl (catalyst), MeOH–THF 4 : 1, reflux, 2 h, 38%; c, PhCOCl, py, DMAP (catalyst), 25 °C, 16 h, 85%; d, H_2C=CHOEt, pyridinium toluene-*p*-sulfonate (catalyst), CH_2Cl_2, 36 h, 50%; vii, K_2CO_3, MeOH, 25 °C, 1 h, 83%; viii, a, NaH, tetrabenzyl pyrophosphate, DMF, 0 to 25 °C, 16 h, 69%; b, H_2 (50 psi), 10% Pd/C, 90% EtOH. (EE = EtOMeCH).

afforded a mixture of regioisomeric bisacetonides, from which **4** was isolated and fully characterised by ^{1}H NMR decoupling experiments, ^{13}C NMR, ^{19}F NMR and mass spectrometry. Dibenzylation of the suitably protected diol **4** was followed by chemoselective cleavage of the *trans*-acetonide to give the diol **5**. Next, benzoylation of **5** and acetonide cleavage was followed by selective benzoylation of the equatorial hydroxy group and ethoxyethyl (EE) protection of the axial hydroxy group to furnish the fully protected **6**. Finally, removal of the benzoate groups of **6** gave the triol **7**, which after phosphorylation with tetrabenzyl pyrophosphate,[20,21] followed by debenzylation afforded the desired 3-*C*-CF_3-Ins(1,4,5)P_3 as the free acid.§

L-ch-Ins(1,2,3,5)P_4 was synthesized in a straightforward fashion from the known mesylated *chiro*-inositol **8**, which was prepared from L-quebrachitol in three steps as described previously[22] (Scheme 2). Treatment of **8** with 2-methoxypropene gave a 1:1 mixture of regioisomeric bisacetonides, from which **9** was easily separated by crystallisation from EtOAc–hexanes. Benzylation of the alcohol **9**, followed by treatment with $LiAlH_4$ and chemoselective cleavage of the *trans*-acetonide protective group furnished the triol **10** in 73% overall yield. Next, benzoylation of **10** and acetonide cleavage was followed by selective benzoylation of the equatorial hydroxy group and ethoxyethyl (EE) protection of the remaining axial hydroxy group to give **11** in 69% overall yield. Removal of the benzoate groups of **11** gave the tetrol **12**, which after phosphitylation with *O*,*O*′-dibenzyl-*N*,*N*′-diisopropylphosphoramidite [$(BnO)_2PN(Pr^i_2)$],[23] followed by oxidation of the phosphite to the corresponding phosphate and debenzylation[13] afforded the desired L-ch-Ins(1,2,3,5)P_4 isolated as an octasodium salt¶ (Scheme 2).

The preliminary results on the binding and Ca^{2+} releasing activity of the new analogues revealed that 3-*C*-CF_3-Ins(1,4,5)P_3 is *ca.* threefold less potent than Ins(1,4,5)P_3 in binding assays, while L-ch-Ins(1,2,3,5)P_4 is more than sevenhundredfold less potent. In agreement with the binding studies, 3-*C*-CF_3-Ins(1,4,5)P_3 and Ins(1,4,5)P_3 appeared equipontent in Ca^{2+} releasing activity, while L-ch-Ins(1,2,3,5)P_4 was of much lower potency. Since 3-*C*-CF_3-Ins(1,4,5)P_3 directly mimics Ins(1,4,5)P_3, this finding would suggest that the Ins(1,4,5)P_3 receptor can accommodate some steric bulk in the axial region of the 3-position. On the other hand, the observation that the presence of the bulkier axially oriented phosphate group in the 1-position of L-ch-Ins(1,2,3,5)P_4 leads to a substantial decrease in the Ca^{2+} releasing activity, suggests that this steric pocket is for steric and/or electronic



Scheme 2 Synthesis of L-ch-Ins(1,2,3,5)P_4. *Reagents and conditions*: i, H_2C=C(OMe)CH_3, CSA, DMF, 60 °C, 4 h, 38%; ii, a, NaH, $PhCH_2Br$, DMF, 0 °C, 1 h, 99%; b, $LiAlH_4$, THF, 25 °C, 82%; c, AcCl (catalyst), MeOH–CH_2Cl_2 (1:2), 3 min; iii, a, PhCOCl, py, DMAP (catalyst), 25 °C, 16 h, 91% overall; b, conc. HCl (catalyst), MeOH, 25 °C, 99%; c, PhCOCl, py, DMAP (catalyst), 25 °C, 16 h, 75%; d, H_2C=CHOEt, pyridinium toluene-*p*-sulfonate (catalyst), CH_2Cl_2, 25 °C, 99%; iv, K_2CO_3, MeOH, 25 °C, 85%; v, a, $(BnO)_2PN(Pr^i_2)$, 1*H*-tetrazole, CH_2Cl_2; then 70% Bu^tO_2H, CH_2Cl_2, 25 °C, 85%; b, H_2 (1 atm), 10% Pd/C, MeOH; c, 1 mol dm^{-3} NaOH, 86%

reasons not all encompassing. Detailed data on the binding, Ca^{2+} release activity, and interaction of the analogues with the metabolic enzymes will be published in full elsewhere.

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Footnotes

† Note the different conventional numbering of the *myo*- and *chiro*-inositol systems.

‡ $[\alpha]_D^{25}$ −3.8 (*c* 0.6, H_2O); ^{1}H NMR ([$^2H_6$]DMSO, 300 MHz) δ 5.57 (s, 1 OH), 5.22 (d, *J* 4.5 Hz, 1 OH), 4.94 (d, *J* 4.7 Hz, 1 OH), 4.84 (d, *J* 4.7 Hz, 1 OH), 4.69 (d, *J* 4.7 Hz, 1 OH), 4.64 (d, *J* 5.9 Hz, 1 OH), 3.74 (br s, 1 H), 3.62 (br s, 1 H), 3.49–3.16 (m, 3 H); ^{19}F NMR ([$^2H_6$] DMSO, 282 MHz, ^{1}H-decoupled) δ −64.76; The corresponding isostere 1D-3-*C*-methyl-*myo*-inositol was prepared previously by some of us.[18]

§ $[\alpha]_D^{25}$ −16.9 (*c* 1.4, H_2O); ^{1}H NMR (D_2O, 300 MHz, free acid) δ 4.42 (d, *J* 9.6 Hz, 1 H), 4.30 (t, *J* 8.8 Hz, 1 H), 4.19 (br s, 1 H), 4.06 (t, *J* 9.1 Hz, 1 H), 3.90 (t, *J* 9.0 Hz, 1 H); ^{31}P NMR (D_2O, 121 MHz, free acid, ^{1}H-decoupled) δ 4.22, 2.81, 2.40; ^{19}F NMR (D_2O, 282 MHz, free acid, ^{1}H-decoupled) δ −68.98.

¶ $[\alpha]_D^{25}$ −18.0 (*c* 1.2, H_2O); ^{1}H NMR (D_2O, 300 MHz, free acid) δ 4.54 (dt, *J* 9.6, 3.2 Hz, 1 H), 4.54 (t, *J* 3.6 Hz, 1 H), 4.43 (tt, *J* 9.7, 2.3 Hz, 1 H), 4.32–4.21 (m, 3 H), 3.81 (t, *J* 9.3 Hz, 1 H); ^{31}P NMR (D_2O, 121 MHz, octasodium salt, ^{1}H-decoupled) δ 8.48, 7.81, 6.90, 6.24.

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