Total Synthesis from L-Quebrachitol of the D-*myo*-Inositol 1,4,5-Trisphosphate Analogue, L-*chiro*-Inositol 2,3,5-Trisphosphate, a Potent Inositol 1,4,5-Trisphosphate 5-Phosphatase and 3-Kinase Inhibitor

Changsheng Liu,[a] Stefan R. Nahorski[b] and Barry V. L. Potter*[a]

[a] *Department of Chemistry, University of Leicester, Leicester LE1 7RH, UK and School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK*
[b] *Department of Pharmacology and Therapeutics, University of Leicester, Leicester LE1 9HN, UK*

A concise synthesis of L-*chiro*-inositol 2,3,5-trisphosphate, a novel receptor agonist and a potent inhibitor of the metabolic enzymes which act upon D-*myo*-inositol 1,4,5-trisphosphate, is reported using methodology which accomplishes the required regio-protection of L-*chiro*-inositol for phosphorylation in one step.

D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] **1** (Fig. 1) is a second messenger, generated by agonist-stimulated, receptor mediated, phospholipase *C*-catalysed cleavage of the minor membrane lipid phosphatidylinositol 4,5-bisphosphate.[1] Ins(1,4,5)P_3 releases sequestered Ca^{2+} from an endoplasmic reticular intracellular store and its generation is a key event in signal transduction for a wide range of extracellular agonists. Ins(1,4,5)P_3 acts through a receptor which has now been isolated,[2] cloned,[3] sequenced[4] and reconstituted.[5]

Intensive recent biological interest has centred upon Ins(1,4,5)P_3. Additionally, there is significant potential for the design and chemical synthesis of novel receptor ligands and inhibitors for the metabolic enzymes Ins(1,4,5)P_3 5-phosphatase, which deactivates Ins(1,4,5)P_3 by dephosphorylation, and 3-kinase which phosphorylates the equatorial 3-hydroxy group.[6] Such compounds may have potential therapeutic value. Indeed, there is an urgent need for chemically-modified analogues of Ins(1,4,5)P_3 to aid investigations of structure activity relationships in relation to all three binding proteins.[6,7] Few such analogues of great utility have yet been reported. We have prepared potent analogues of Ins(1,4,5)P_3 including non-hydrolysable phosphorothioates[6–8] and fluoro-analogues.[9]

Although some success in the development of 5-phospha-

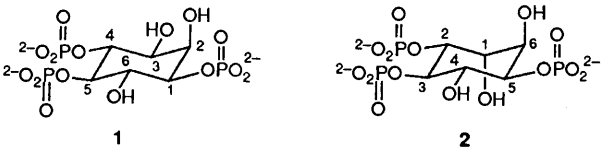


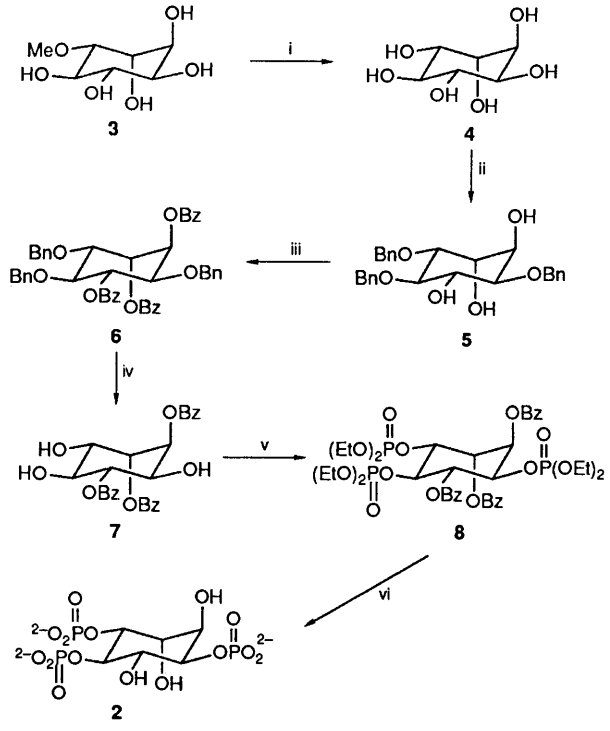
Fig. 1 Structures of D-*myo*-inositol 1,4,5-trisphosphate **1** and L-*chiro*-inositol 2,3,4,5-trisphosphate **2**

tase inhibitors has already been achieved,[10–13] it is a particular challenge to explore the design of 3-kinase inhibitors, for in contrast to 5-phosphatase which is relatively non-specific in its binding of inositol phosphates, 3-kinase appears to be more specific in its recognition of such ligands than the receptor itself. We envisaged that L-*chiro*-inositol 2,3,5-trisphosphate **2**,† which may be visualized as Ins(1,4,5)P_3 with an inverted 3-hydroxy group, is of obvious interest as a potential 3-kinase inhibitor and we were consequently led to develop a synthetic route to optically active **2**. While this present work was in progress a synthesis of racemic **2** from benzene *via* a photooxidation procedure was published.[14] However, no biological data on **2** have yet been reported.

One of the major problems in inositol polyphosphate synthesis concerns the difficulty of multiple regiospecific protection of the various ring hydroxy groups in free inositol to afford an intermediate suitable for polyphosphorylation.[7] This invariably involves extensive manipulation of the molecule using a variety of permanent and temporary protecting groups. Clearly a simplified procedure would be of great utility. We reasoned that if the strategy of tin-mediated alkylation of vicinal hydroxy groups,[15] where regiospecific alkylation of an equatorial hydroxy group occurs in a vicinal axial–equatorial pair, were employed on totally unprotected L-*chiro*-inositol, the unique symmetry of the molecule would lead to multiple protection at the 2,3 and 5 positions in one step. We thus report here the synthesis of **2** *via* a route centred upon a one-step regiospecific tribenzylation of L-*chiro*-inositol (Scheme 1).

L-Quebrachitol **3** was demethylated with HI to give L-*chiro*-inositol **4**.[16] Treatment of **4** under reflux with a four-fold molar excess of dibutyltin oxide and tetrabutylammonium iodide in acetonitrile, followed by a five-fold excess of benzyl chloride afforded as the major product (32% yield) the crystalline L-2,3,5-tri-*O*-benzyl-*chiro*-inositol **5**‡ {m.p. 96–97 °C; $[\alpha]_D^{20}$ −46.7° (*c* 2.3, EtOH)}, which was benzoylated with an excess of benzoyl chloride to generate the syrupy L-1,4,6-tri-*O*-benzoyl-2,3,5-tri-*O*-benzyl-*chiro*-inositol **6** in quantitative yield. The structure of **5** was unambiguously assigned by ^{1}H-COSY NMR spectroscopy on both **5** and its triacetate. Compound **6** was debenzylated by catalytic hydrogenation over freshly-prepared palladium chloride on charcoal to give the crystalline L-1,4,6-tri-*O*-benzoyl-*chiro*-inositol **7** {m.p. 189–190 °C; $[\alpha]_D^{20}$ −36.6° (*c* 0.99, EtOH)} in 94% yield.

We also demonstrate for the first time the application of the commercially available phosphitylating agent diethoxychlorophosphine in inositol chemistry. Thus, phosphitylation of **7** using diethoxychlorophosphine generated the trisphosphite hexaethylester, which was not isolated but oxidised to the syrupy trisphosphate **8** with Bu^{t}OOH (overall yield 73%). The ethyl groups were removed first using bromotrimethylsilane and then the benzoyl groups were deblocked using sodium hydroxide solution to give fully deprotected trisphosphate **2**,



Bn = benzyl, Bz = benzoyl

Scheme 1 Synthesis of L-*chiro*-inositol 2,3,5-trisphosphate. *Reagents and conditions*: i, 47% aq. HI; ii, (*a*) Bu_2SnO, Bu_4NI–MeCN, (*b*) BnCl, reflux; iii, BzCl–pyridine; iv, H_2–$PdCl_2$/C (5%) in EtOH; v, (*a*) $(EtO)_2PCl$–MeCN, (*b*) 70% aq. Bu^{t}OOH; vi, (*a*) Me_3SiBr–CH_2Cl_2 (*b*) OH^-–H_2O

which was purified by anion-exchange chromatography on a column of diethylaminoethyl (DEAE) Sephadex A-25 using a linear gradient of triethylammonium hydrogencarbonate. Pure L-*chiro*-inositol 2,3,5-triphosphate was eluted at an ionic strength of *ca.* 800 mmol dm^{-3} and, after evaporation of fractions containing product, **2** was quantified as a glass using a quantitative Briggs phosphate assay (yield 87%).

Compound **2** was found to be a potent agonist for the release of intracellular Ca^{2+} from permeabilised human neuroblastoma cells being only some 5–10 fold less potent than Ins(1,4,5)P_3. It released Ca^{2+} in a sustained fashion similar to inositol 1,4,5-trisphosphorothioate.[6] K_i values for the inhibition of human erythrocyte membrane Ins(1,4,5)P_3 5-phosphatase-catalysed dephosphorylation of [5-^{32}P]Ins(1,4,5)P_3 and crude rat brain 3-kinase-catalysed phosphorylation of Ins(1,4,5)P_3 were performed as we previously described for other analogues.[9,13] The K_i values for these enzymes were 7.7 and 7.1 μmol dm^{-3}, respectively. As expected, **2** was not a substrate for 3-kinase, but surprisingly it was also not dephosphorylated by 5-phosphatase. The biological results will be presented in full elsewhere.

We have already noted that deletion of the 6-hydroxy group of Ins(1,4,5)P_3, adjacent to the site of action of 5-phosphatase, generates a moderately potent 5-phosphatase inhibitor in 6-deoxy-Ins(1,4,5)P_3.[13] However, the finding that **2** is a potent inhibitor was entirely unexpected and poses the intriguing question as to how a change in orientation of a hydroxy group remote from the site of action of the enzyme can have such a radical effect. Whether this is the result of subtle conformational changes induced in the molecule relative to Ins(1,4,5)P_3, or of non-productive binding of **2** to the enzyme offer intriguing prospects for further investigation.

† Note the different conventional numbering of the *myo*- **1** and *chiro*- **2** inositol systems.

‡ All new compounds gave satisfactory spectroscopic data and elemental analysis where appropriate.

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