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The First Synthesis and Iron Binding Studies of the Natural Product, *myo*-Inositol 1,2,3-Trisphosphate.

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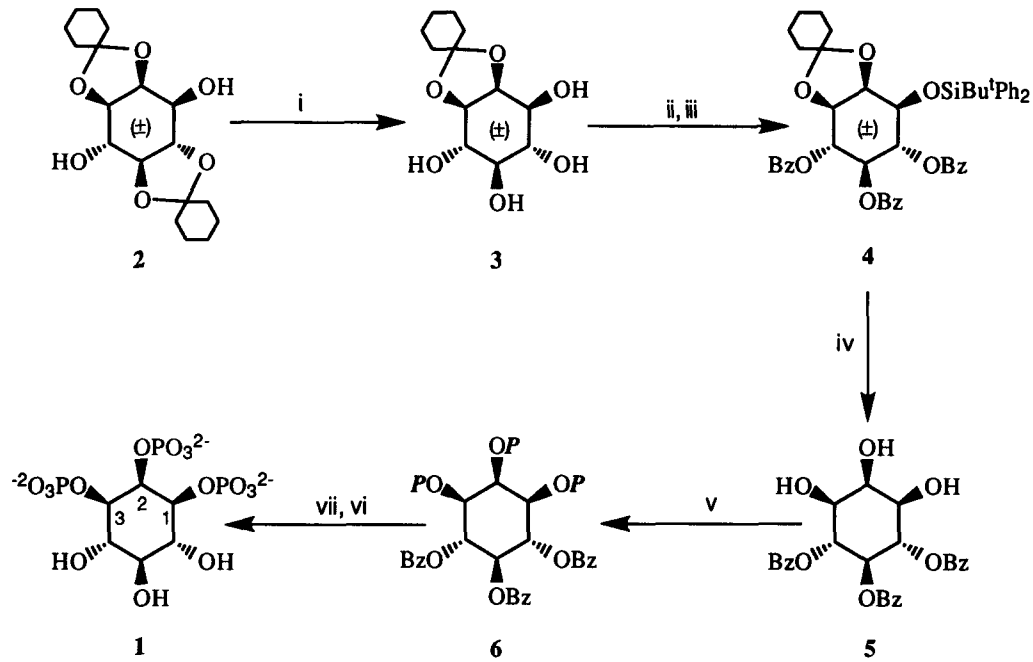
Abstract: The natural product *myo*-inositol 1,2,3-trisphosphate **1** has been prepared and shown to inhibit Fe^{3+}-catalysed hydroxyl radical formation.

myo-Inositol hexakisphosphate (phytic acid) is a widespread inositol phosphate, found in all eucaryotic cells, where it is present at concentrations 10-100 μM; however its biological role remains elusive. Phytic acid is an excellent chelator of Fe^{3+} with an affinity[1] in the region of 10^{25} and it also inhibits Fe^{3+}-catalysed $HO^{\cdot}$ production;[2] it has recently been suggested that it may act as both an intracellular, low molecular weight chelator of Fe^{3+} and antioxidant. All *myo*-inositol pentakisphosphate isomers bind Fe^{3+} with high affinity; however only those with the 1,2,3-trisphosphate groups are also antioxidant.[3] It has been proposed that *myo*-inositol 1,2,3-trisphosphate **1** represents the simplest structure able to bind Fe^{3+} and function as an antioxidant, but this has never been tested. The synthesis of this recently discovered natural product[4] is discussed along with its Fe^{3+} binding studies.

The synthesis of trisphosphate **1** is shown in Scheme 1. Diol **2** was synthesized from *myo*-inositol and 1,1-diethoxycyclohexane,[5] which was converted to **3** by the selective removal of the *trans*-cyclohexylidene ring.[6] The tetrol **3** was regioselectively silylated[7] at the 1-position, the product from which was then benzoylated to give the fully protected intermediate **4**. Both the *cis*-cyclohexylidene and silyl groups of **4** were removed by treatment with aqueous trifluoroacetic acid at 50°C to give **5**, which was crystallised from diethyl ether. The triol **5** was phosphorylated with dibenzyl *N,N*-diisopropylphosphoramidite[8] and the intermediate phosphite was oxidised[9] to yield the trisphosphate **6** as a crystalline compound, the structure of which was confirmed by X-ray diffraction analysis[10] (Figure 1). Deprotection of **6** was achieved in a quantitative yield by hydrogenolysis followed by base catalysed hydrolysis of the benzoyl esters to give the trisphosphate **1**, which was crystallised as its hexa(cyclohexylammonium) salt. All new compounds were fully characterised by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy,[11] IR and mass spectrometry, and elemental analysis.

The ability of *myo*-inositol 1,2,3-trisphosphate **1** to inhibit Fe^{3+}-catalysed hydroxyl radical formation was studied in a hypoxanthine/xanthine oxidase system. This generates $HO^{\cdot}$, which in turn generates formaldehyde from dimethylsulphoxide (present in the assay).[3] Both **1** and phytic acid bind to Fe^{3+} with high affinities and both completely inhibited Fe^{3+}-catalysed hydroxyl radical formation at >100μM (Figure 2). We conclude that the 1,2,3 (equatorial-axial-equatorial) trisphosphate grouping in phytic acid is the orientation needed to inhibit Fe^{3+}-catalysed hydroxyl radical formation. This may allow phytic acid to function as a 'safe' carrier of Fe^{3+} in the cell.



Scheme 1: Synthesis of *myo*-inositol 1,2,3-trisphosphate **1**.
i. *p*-TsOH, toluene, hexane, EtOH; ii. Bu^{t}Ph$_2$SiCl, imidazole, pyridine (58%); iii. benzoyl chloride, DMAP, pyridine (73%); iv. aqueous CF_3CO_2H (49%); v. $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, then *m*-CPBA (56%); vi. H_2, Pd/C (10%), EtOH, room temperature, overnight; vii. NaOH (0.5M), quantitative. *Abbreviations*: ***P*** = $(BnO)_2P(O)$; Bn = benzyl; Bz = benzoyl.

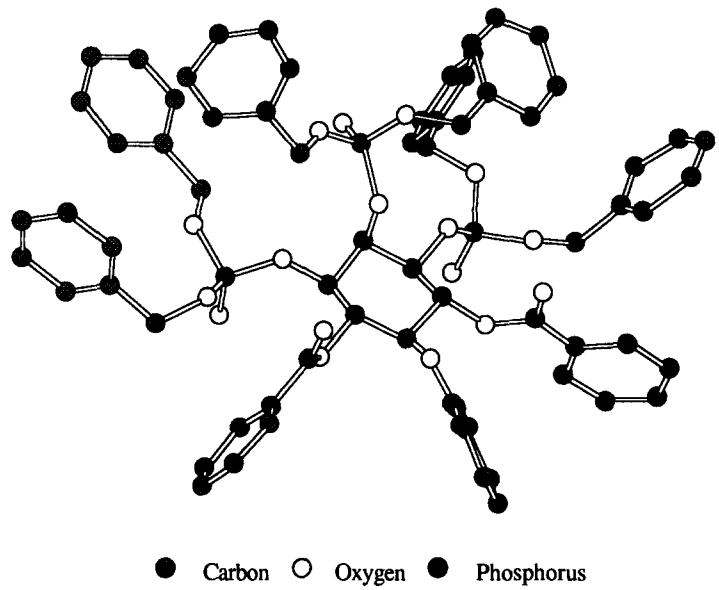


Figure 1: Crystal structure of 1,2,3-tris(dibenzylphosphoryl)-4,5,6-tribenzoyl *myo*-inositol **6**.[11]

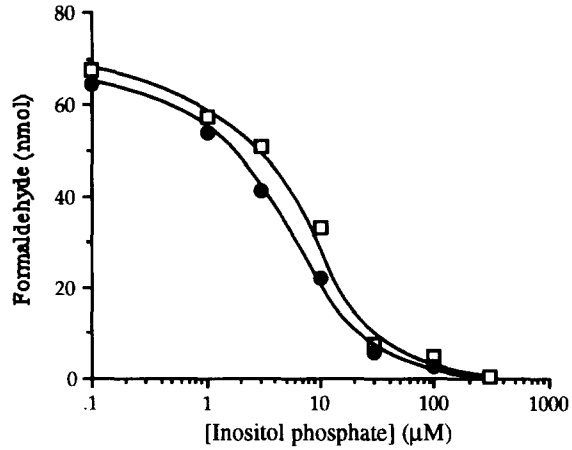


Figure 2: Effects of phytic acid (●) and *myo*-inositol 1,2,3-trisphosphate (**1**, ❑) on HO$^{\bullet}$ generation. This result is typical of three independent experiments.

In summary, we report the first synthesis of the natural product *myo*-inositol 1,2,3-trisphosphate **1**. The crystal structure of the key intermediate 1,2,3-tris(dibenzylphosphoryl)-4,5,6-tribenzoyl *myo*-inositol **6** is presented. The 1,2,3 (equatorial-axial-equatorial) trisphosphate grouping present in both **1** and phytic acid inhibits Fe^{3+}-catalysed hydroxyl radical formation. Further biological investigations and full experimental details for the synthesis of **1** will be reported in a forthcoming full paper.

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References and notes

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10. Crystallographic data for **6**: Monoclinic $P2_1$, a = 15.564(3), b = 18.835(2), c = 22.738(6)Å, β = 95.04(2)°, V = 6640(2)Å^3, Z = 4, ρ_{calc} = 1.292 g/cm^3, μ = 0.162 mm^{-1}, R = 0.0566, wR2 = 0.1429 for 7032 observed data [Fo≥4.0σ(Fo)] from 12807 collected data. The diffraction data were collected on an Enraf-Nonius CAD4 diffractometer at 293(2) K in the ω-2θ scan mode using Mo-Kα radiation to a maximum 2θ value of 50°. The intensity data were corrected for Lorentz-polarization and intensity decay using DATRED (Brookhaven Natl. Lab. & University of Birmingham). The structure was solved by direct methods (SHELXS-86, Sheldrick 1990) and refined by the full-matrix least-squares method (SHELXL-93, Sheldrick 1993). All non-hydrogen positions were found including two molecules of water.
The atomic coordinates and data for the X-ray structure are available from the Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge, CB2 1EU, United Kingdom.
11. Melting points and selected NMR data:

4. M.p. 173.5 - 175 °C; ^{1}H-NMR (250.1 MHz, $CDCl_3$): δ 0.95 (s, 9H, But), 1.19-2.06 (m, 10H, CH_2 of cyclohexylidene), 4.09-4.16 (m, 2H), 4.30 (dd, 1H, J_{HH} = 3.9 Hz, J_{HH} = 9.3 Hz), 5.36 (t, 1H, J_{HH} = 9.8 Hz), 5.79 (dd, 1H, J_{HH} = 10.3 Hz, J_{HH} = 7.0 Hz), 6.03 (t, 1H, J_{HH} = 9.4 Hz), 7.16-7.86 (m, 25H, Ph).

5. M.p. 100.5 - 103 °C; ^{1}H-NMR (250.1 MHz, CD_3OD): δ 4.02 (dd, 2H, H-1/3, J_{HH} = 2.7 Hz, J_{HH} = 9.7 Hz), 4.19 (t, 1H, H-2, J_{HH} = 2.7 Hz), 5.67 (t, 1H, H-5, J_{HH} = 9.9 Hz), 5.85 (t, 2H, H-4/6, J_{HH} = 9.9 Hz), 7.19-7.50 (m, 9H, Ph), 7.69-7.72 (m, 2H, Ph), 7.88-7.92 (m, 4H, Ph). ^{1}H-NMR (250.1 MHz, DMSO-d_6): peaks include δ 5.27 (d, OH-1/3, J_{HH} = 6.1 Hz), 5.50 (d, OH-2, J_{HH} = 2.9 Hz), exchangeable in D_2O.

6. M.p. 131.5 - 132.5 °C.

1. ^{31}P-NMR (101.3 MHz, D_2O, ^{1}H decoupled, referenced to 85% H_3PO_4): δ 1.78 (s, P-2), 4.08 (s, P-1/3). ^{1}H-NMR (250.1 MHz, D_2O, referenced to acetone at 2.05 ppm): δ [0.97-1.25 (m, 30H), 1.47 (br d, 6H, J_{HH} = 11.9 Hz), 1.62 (br d, 12H, J_{HH} = 3.8 Hz), 1.80 (br s, 12H), 2.85-3.07 (m, 6H), (6 x cyclohexylammonium)], 3.22 (t, 1H, H-5, J_{HH} = 9.2 Hz), 3.66 (t, 2H, H-4/6, J_{HH} = 9.5 Hz), 3.81 (br t, 2H, H-1/3, $J_{HH} \approx J_{PH} \approx$ 8.9 Hz), 4.55 (br d, 1H, H-2, J_{PH} = 9.8 Hz).

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