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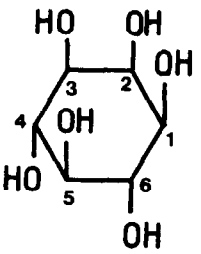
SYNTHESIS OF DL-MYO-INOSITOL 1,4,5-TRISPHOSPHATE

Allan M. COOKE and Barry V.L. POTTER*
Department of Chemistry, Leicester University, Leicester LE1 7RH, UK

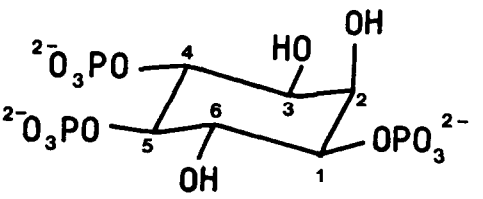
Roy GIGG
Laboratory of Lipid and General Chemistry, National Institute for Medical Research,
Mill Hill, London NW7 1AA

ABSTRACT: DL-Myo-inositol 1,4,5-trisphosphate has been synthesised from (±) 1,2,4-tri-O-benzyl-myo-inositol using a phosphite chemistry approach.

The identification of a phosphate ester of myo-inositol (1), D-myo inositol 1,4,5-trisphosphate (2), as an intracellular second messenger for calcium mobilisation[1] has regenerated a great biological interest in the inositol phosphates.[2] Isolation of (2) from natural sources is not practicable for large quantities of material and there is consequently a need for chemically synthesised material as well as analogues of (2) and radioactive derivatives.



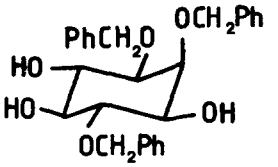
(1)



(2)

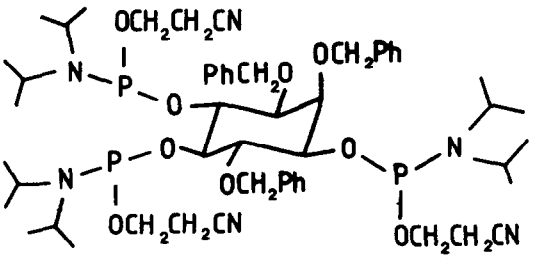
A synthesis of (2) has recently been reported,[3] but the phosphorylation and deblocking methods were not considered satisfactory. We report here a synthesis of racemic (2) starting from (±) 1,2,4-tri-O-benzyl-myo-inositol[4] using a phosphite chemistry approach[5] based upon diisopropylamino(2-cyanoethyl)chlorophosphine (3),[6] a reagent developed for DNA synthesis.

(±)-1,2,4-Tri-O-benzyl-myo-inositol (0.20 mmole) (4) was treated with (3) (0.66 mmole) and diisopropylethylamine (0.60 mmole) in dichloromethane (3 ml) to yield the trisphosphoroamidite



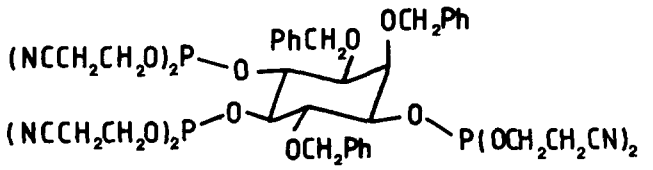
(4)

(i)



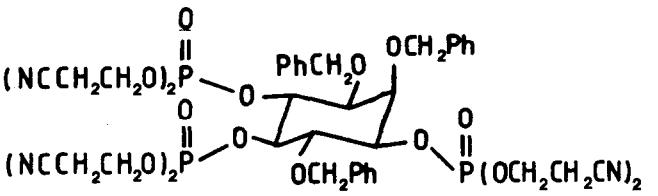
(5)

(ii)



(6)

(iii)



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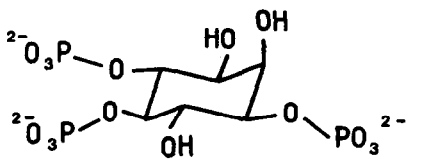
(iv)

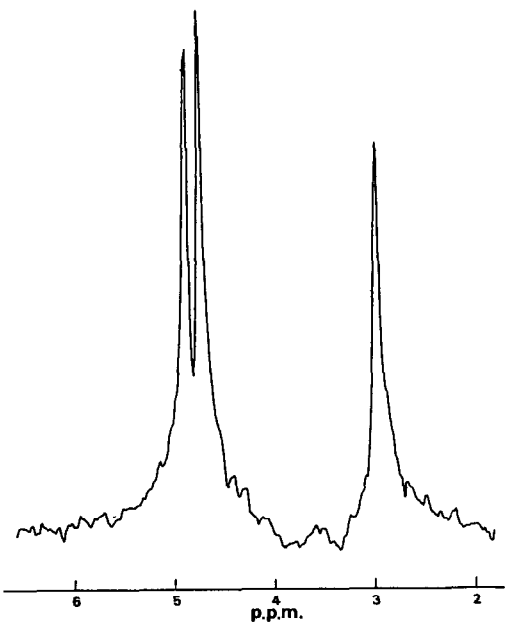


(2)

All compounds are racemic

Reagents: (i) $ClP(OCH_2CH_2CN)NPr^i_2$, 3.3 equiv., $C_2H_5N(Pr^i)_2$, 3 equiv./CH_2Cl_2;
(ii) tetrazole-$HOCH_2CH_2CN$, 3 equiv./CH_2Cl_2; (iii) t-BuOOH;
(iv) Na-liq. NH_3.

(5) [δ_p, 150.46] in ca. 90% yield. A small peak at δ 146.0 due to the sole other product was tentatively assigned to material possessing a 4,5-cyclic phosphoroamidite system arising from a small amount of mono-phosphitylation of the vic diol system. Reaction of (5) with tetrazole (0.66 mmole) and 2-cyanoethanol (0.66 mmole) yielded the trisphosphite triester (6) [δ_p, 139.6, P-1; 140.2, 141.0, P-4, P-5 (not assigned), $^5J_{pp}$ = 3.4 Hz]. ^{31}P NMR spectroscopy showed $^5J_{pp}$ spin-spin coupling in the form of an AB system for two of the phosphite triesters, confirming bis-phosphitylation of the vicinal 4,5-diol system. Oxidation of (6) with anhydrous t-butyl hydroperoxide (3 ml)[7] afforded quantitatively the trisphosphate triesters (7) [δ_p, -3.4] and finally complete deblocking with sodium in liquid ammonia[8] effected reductive removal of the benzyl groups and removal of the 2-cyanoethyl groups by β-elimination to give crude (2), which was purified by ion exchange chromatography.[9] Since the resulting material still contained minor amounts of inositol phosphate impurities, a small sample was subjected to final purification by HPLC.[10] We also found that (2) could be purified in quantity by gradient elution using a DEAE Sephadex ion-exchanger.[11] DL-myo-inositol 1,4,5-trisphosphate was obtained in ca. 50% yield, as determined by quantitative phosphate analysis. The ^{31}P NMR spectrum of (2)[12] was in accord with that reported recently for material of natural origin.[13]



24.15 MHz ^{31}P NMR spectrum of synthetic DL-myo-inositol 1,4,5-trisphosphate (ca. 240 mM in D_2O, pH 12 with added EDTA); NMR parameters were: sweep width, 1K Hz; pulse width, 8 μs; collected in 4K; no. of transients, 100; broad band proton decoupling; determined using a Jeol FX 60 NMR spectrometer; reference, external 85% H_3PO_4.

We have demonstrated our synthetic route using racemic (4), however, methods have been recently reported whereby resolved material can be obtained[3,14] and the present synthetic method can therefore be used to prepare optically active (2). Synthesis of other inositol phosphates using this methodology as well as biological testing[15] are in progress.

We thank the S.E.R.C. for a Research Studentship (to A.M.C.), Dr M.R. Hamblin for valuable advice and assistance, and Dr I. Batty and Professor S.R. Nahorski for preliminary biological testing and assistance with the HPLC separation.

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10. HPLC purification was performed on a Partisil SAX 10 column using ammonium formate-phosphoric acid buffer as reported: I.R. Batty, S_3R. Nahorski, and R. Irvine, Biochem. J. 232, 211 (1985). A small sample of [^{3}H]-D-myo-inositol 1,4,5-trisphosphate was added to permit detection.

11. Ca. 300 μmole of (2) were purified on a column of DEAE Sephadex A-25 anion exchanger equilibrated in 100 mM triethylammonium bicarbonate (TEAB). When this column was run using a linear gradient of TEAB (0.1-1.0 M) clean separation of (2) from other phosphate-containing impurities was observed. (2) Was eluted at a buffer concentration of ca. 700 mM. TEAB was removed by evaporation in vacuo to yield the triethylammonium salt of (2) as a colourless glass.

12. ^{31}P NMR (D_2O, pH 12) ammonium salt: δp, 3.38, J_{PH} = 5.83; 5.11, J_{PH} = 6.93 Hz; 5.21, J_{PH} = 7.60 Hz; relative to H_3PO_4; determined on a Bruker WH400 at 162 MHz.

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