Bisphosphorylation of a *vic*-Diol using a Phosphite Approach: Synthesis of *myo*-Inositol 4,5-Bisphosphate

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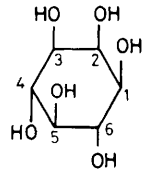
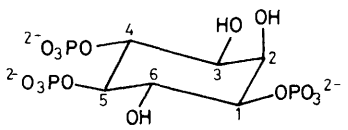
(±)-*myo*-Inositol 4,5-bisphosphate was synthesized using a phosphite chemistry approach; bisphosphorylation was confirmed by the observation of the first $^5J_{PP}$ coupling in a bisphosphite triester intermediate.

The recent discovery that a phosphate ester of *myo*-inositol (**1**), D-*myo*-inositol 1,4,5-trisphosphate (**2**),[1,2] generated by receptor-stimulated phosphodiesteratic hydrolysis of phosphatidylinositol 4,5-bisphosphate, acts as the probable intracellular second messenger for calcium mobilisation, has created a dramatic upsurge of interest in inositol phosphates. D-*myo*-Inositol 1,3,4-trisphosphate[3] and 1,3,4,5-tetrakisphosphate[4,5] have also been discovered, but their biological roles are as yet unclear. Isolation of such biophosphates from natural sources is difficult and does not yield large quantities of material.[6] Moreover, the need for radioactive derivatives and analogues of these molecules must be addressed by chemical synthesis.

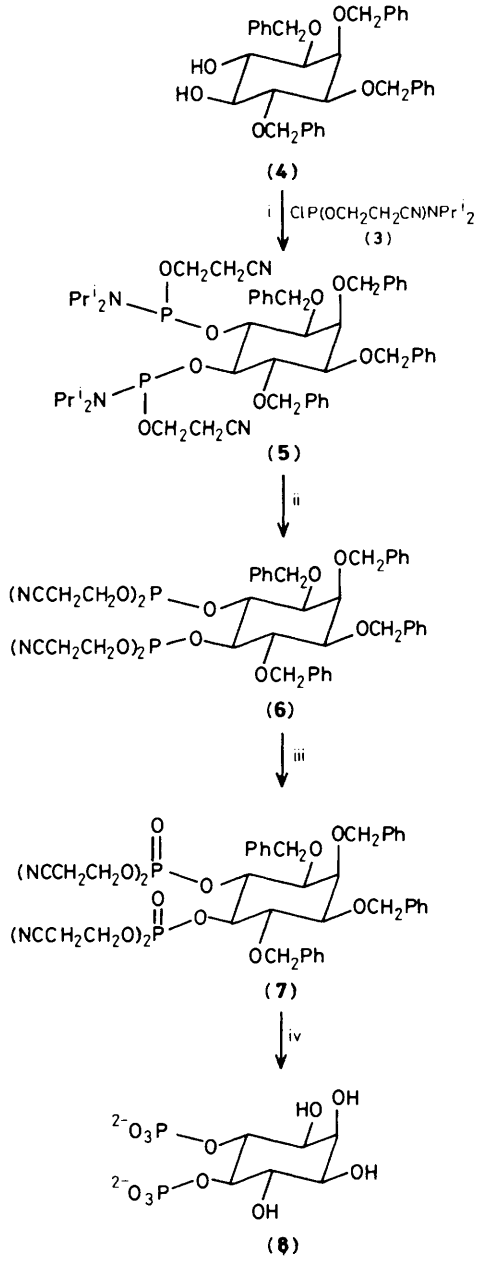
Recently, the first chemical synthesis of (**2**) has been reported,[7] but the authors state that their phosphorylation and deblocking reactions are unsatisfactory. One of the problems concerning the synthesis of such molecules is that phosphorylation of *vic*-diol systems using conventional P^V phosphorylating agents often leads to formation of undesired cyclic 5-membered phosphates. We suggest an efficient phosphorylation procedure for *myo*-inositol derivatives based upon phosphite chemistry using the reagent developed by Sinha *et al.*[8] for DNA synthesis, 2-cyanoethyl *N*,*N*-di-isopropylphosphoramidochloridite (**3**), recently used for the synthesis of α-L-fucopyranosyl phosphates[9] and branched ribonucleotides.[10] Bisphosphitylation of protected adenosine using a different reagent has also been observed recently.[11] Our preliminary experiments revealed that a simple *vic*-diol such as propane-1,2-diol could be phosphitylated with (**3**) and subsequently converted into the bisphosphate in *ca.* 80% yield. We now report a synthesis of (±)-*myo*-inositol 4,5-bisphosphate[12] (Scheme 1) using this approach. We chose this system since *trans*-vicinal 4,5-bisphosphate substitution is known to be a requisite structural feature for calcium mobilising activity in an inositol phosphate.[2]



(**1**)



(**2**)



Scheme 1. *Reagents and conditions:* i, (**3**), $EtNPr^i_2$ in CH_2Cl_2; ii, tetrazole, $HOCH_2CH_2CN$–CH_2Cl_2; iii, Bu^tOOH; iv, NH_4OH, 60 °C, then Na–liq. NH_3.

Thus, (±)-1,2,3,4-tetra-*O*-benzyl *myo*-inositol (**4**)[13] was treated with (**3**) (2 equiv.) in dichloromethane to give the bisphosphoramidite (**5**). Treatment of (**5**) with tetrazole and 2-cyanoethanol gave the phosphite triester (**6**). ^{31}P N.m.r. spectroscopy showed that about 90% of the phosphorus could be accounted for by an AB system assignable to the 4,5-bisphosphite triester system (**6**) (δ 140.45, 140.75 p.p.m.; $^5J_{PP}$ 2.93 Hz) (Figure 1). To the best of our knowledge, apart from the exceptional case of interbenzylic long range phosphorus–phosphorus coupling through a π-system,[14] no examples of such a coupling through five bonds in an aliphatic system have previously been reported,[15] and the observation of this effect is a key indication that bisphosphitylation has occurred. This effect disappears when the bisphosphite (**6**) is oxidised to the bisphosphate (**7**).

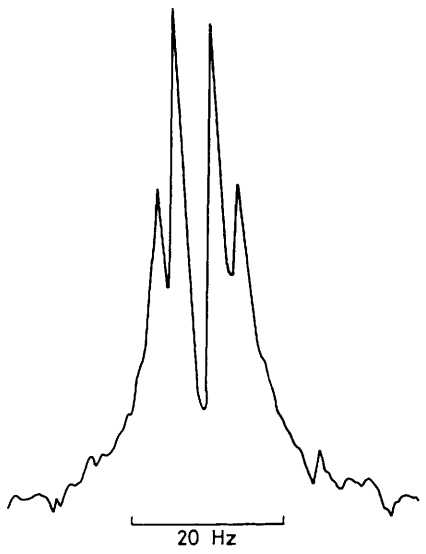


Figure 1. 24.15 MHz ^{31}P n.m.r. spectrum of (±)-1,2,3,4-tetra-*O*-benzyl-*myo*-inositol-5,6-bisphosphite tetra-2-cyanoethyl ester (**6**) in CH_2Cl_2. ^{31}P N.m.r. parameters were: sweep width 1 kHz; pulse width, 8 μs; recorded in 4K.

Oxidation of (**6**) with t-butyl hydroperoxide[16] gave the phosphate triester (**7**). Ammonolysis removed the 2-cyanoethyl groups (heating to 60 °C for 3 h was necessary; mild ammonolysis removed only one 2-cyanoethyl group from each phosphotriester) and treatment with sodium in liquid ammonia[17] quantitatively removed the benzyl groups to give (±)-*myo*-inositol 4,5-bisphosphate (**8**) [δ (pH 12) 4.68, 4.86 p.p.m.; $^3J_{PH}$ 6.82, 7.30 Hz, respectively] in *ca.* 50% yield.

We thank the S.E.R.C. for a postdoctoral fellowship (to M. R. H.)

Received, 20th November 1986; Com. 1654

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