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SYNTHESIS OF RACEMIC *MYO*-INOSITOL 1,3,4-TRISPHOSPHATE *VIA* A PHOSPHITE-TRIESTER APPROACH

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ABSTRACT: Phosphitylation of (±)-2,4,5-tri-O-benzyl-*myo*-Inositol with bis(2-cyanoethyl)chlorophosphine gave, after oxidation followed by basic hydrolysis and subsequently hydrogenolysis, (±)-*myo*-Inositol 1,3,4-trisphosphate.

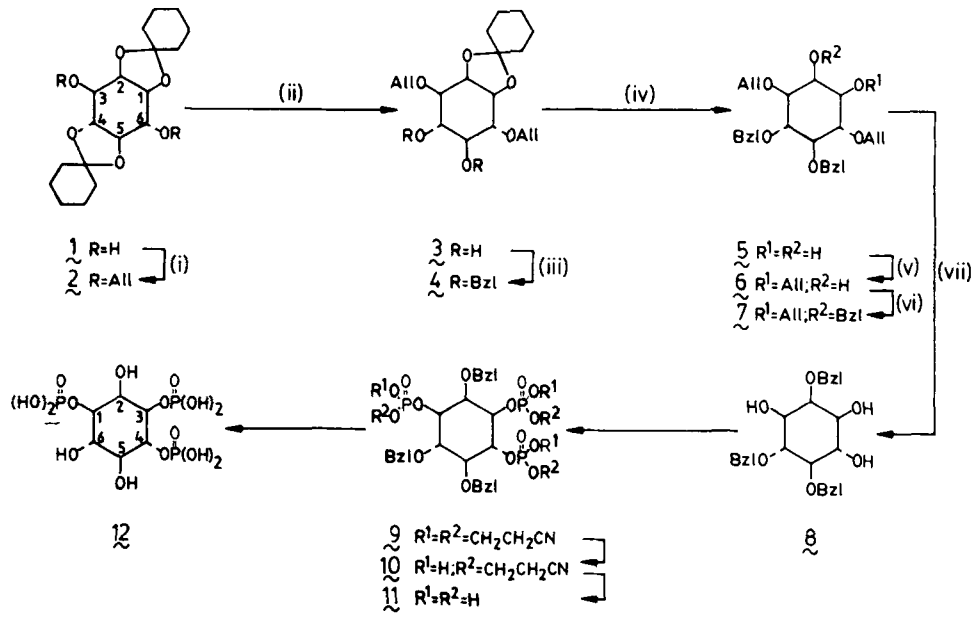
At present the biological function and the different pathways involved in the metabolism of inositol phosphates have been fairly well established. For instance, a crucial metabolic step is the conversion of D-*myo*-inositol (Ins) into the lipid phosphatidyl inositol 4,5-bisphosphate [(Ptd)Ins (4,5)P2]. The latter is then transformed by a receptor mediated enzymic hydrolysis into D-*myo*-inositol 1,4,5-trisphosphate [Ins(1,4,5)P3], which acts as a secondary messenger, within the target cell, mobilising the release of Ca^{2+} from an intracellular store[1a-e]. The action of Ins(1,4,5)P3 may then be terminated by two different mechanisms. In the major one the 5-phosphate is hydrolyzed by a specific 5-phosphatase[1f]. The released Ins(1,4)P2 is then hydrolyzed, *via* inositol 1- and 4-phosphates, to give free Ins, which is further recycled in the brain to provide more (Ptd)Ins(4,5)P2[1f,g]. In the other pathway, Ins (1,4,5)P3 is first fosforylated to Ins(1,3,4,5)P4 followed by hydrolysis of the 5-phosphate to afford Ins(1,3,4)P3[1h,i] which is presumably further degraded to D-*myo*-inositol.

As part of a programme to gain more insight into the process of transmembrane signalling, we required effective phosphorylation procedures to prepare naturally occurring and modified inositol phosphate derivatives.

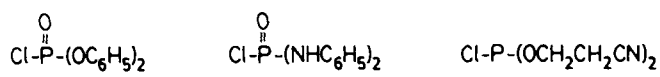
We report the synthesis of racemic *myo*-inositol 1,3,4-trisphosphate (12) *via* a phosphite-triester method.

An approach to the target molecule 12 entails, apart from the resolution of the individual enantiomers[2], two distinct stages: (a) preparation of a suitably protected *myo*-inositol derivative (i.e. 8); (b) effective phosphorylation of 8 to afford, after deblocking, Ins(1,3,4)P3. Up to now several routes have been devised[3a-f] for a regioselective protection of the five equatorially and the one axially orientated hydroxyl groups in *myo*-inositol. On the basis of this information we selected[4] (±)-1,2:4,5-di-O-cyclohexylidene *myo*-inositol[3c] (1) as the starting product for the preparation of key intermediate 2,4,5-tri-O-benzyl-*myo*-inositol (8). The conversion of 1 into crystalline 8, prepared earlier by Gigg et al.[3d] starting from (±)-1,2:4,5-di-O-isopropylidene-*myo*-inositol, could be achieved in seven steps (see Scheme; steps i→vii) in an overall yield of 42%. Apart from this, the merits of the approach illustrated in the Scheme can be briefly summarized as follows. The new and crystalline di-O-allyl derivatives 2[5] and 3[5] could be obtained in a high yield. The acid catalyzed removal (step ii) of the 4,5-O-cyclohexylidene function, in the presence of 1,2-ethane-di-ol, from 2 to afford 3 proceeds satisfactory[3e]. Further, allylation (step v) of intermediate 1,2-O-stannylated 5 to give 6 could be performed with a very high degree of regioselectivity using phase-transfer conditions[6].

The phosphorylation of free hydroxyl groups in properly protected *myo*-inositol derivatives is not as well advanced as the so far developed protective-group strategy. For instance, phosphorylation of 8 with reagent 13 would afford 9 (R^1=R^2=C_6H_5). However, removal of all protective groups by hydrogenolysis will be accompanied by migration of the phosphate groups via an intermediate cyclic phenyl phosphate ester[3f]. The latter could be overcome by using reagent 14, the anilide groups of which can be removed prior to the hydrogenolysis of the benzyl groups. Unfortunately, however, the low reactivity of 14 makes this reagent less generally applicable[3a,b,e]. We reasoned that the monofunctional phosphitylating reagent 15[7] carrying solely base-labile 2-cyanoethyl groups would be more effective. In a typical experiment we treated 8 (1 mmol) in acetonitrile (10 ml) containing N,N-di-isopropylethylamine (9 mmol) with 15 (6 mmol). Work-up, after 20 min at 20°C, and analysis of the crude mixture by ^{31}P-NMR spectroscopy revealed the presence of mainly three phosphite resonances (δp-values: 139.45, 140.34 and 141.91 ppm). Oxidation of the phosphite functions with t-butylhydroperoxide[8] gave, after work-up, the trisphosphate triester 9 (yield 90%, based on 8, δp-values: -1.87, -2.54 and -2.72 ppm). Ammonolysis of 9 afforded the trisphosphate diester 10 (δp-values: -0.97, -1.45 and -1.93 ppm) in a quantitative yield. The remaining 2-cyanoethyl P(V)-protective groups in 10 were then removed[9] with NaOH(0.2 N) in dioxane/MeOH/H_2O at 50°C to yield, after neutralization (Dowex 50W, H^+-form) and purification (Sephadex LH 20), pure 11 (δp-values: 5.17, 3.14 and 2.09 ppm). Finally, hydrogenolysis of 11 over Pd(C) gave 12 in an overall yield of 80% (based on 8). The identity and homo-



Scheme: i(1→2) AllBr/NaH/DMF (yield 94%, mp 63.5°C); ii (2→3) $HOCH_2CH_2OH$ (1 eq)/TsOH(0.04 eq)/CH_2Cl_2(0°C →R.T.), (yield 71%, mp 127°C); iii (3→4)BzlBr/NaH/DMF; iv(4→5) HOAc/H_2O(4:1)/2h, 95°C [yield(iii+ iv) 90%, mp 72°C]; v(5→6) n-Bu_2SnO (1 eq)/MeOH reflux (1,5h); AllBr (1.25 eq)/n-Bu_4NI (1.25 eq) toluene 95°C, 2.5h (yield 85%, mp 61°C); vi(6→7)BzlBr/NaH/DMF (yield 98%); vii (7→8) Pd(C)/TsOH (cat.)/MeOH-H_2O(5:1) reflux 16h (yield 83%, mp 134°C).



13 14 15

geneity of Ins(1,3,4)P3 thus obtained was unambiguously ascertained by elemental analysis, ^1H- and ^{31}P-NMR spectroscopy[10].

The results presented in this paper indicate that a phosphite triester approach, or modifications thereof, promises to be a valuable tool for the introduction[11] of naturally occurring or modified phosphate functions in *myo*-inositol.

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2. It should be noted that the formulae 1-12 only refer to racemates of these compounds. See: R. Parthasarathy and F. Eisenberg (Biochem. J. 235, 313 (1986) for a discussion of the stereochemistry and nomenclature of *myo*-inositol derivatives.
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4. Acid catalyzed acetalation of *myo*-inositol with 1,1-diethoxycyclohexane in DMF (2h, 95°C) gives, after neutralization and work-up, the 1,2:3,4-, 1,2:5,6- and 1,2:4,5-di-O-cyclohexylidene derivatives. Fractionate crystallisation then affords pure 1 (23%). Acid (p-TsOH) treatment (2h, 95°C) of the mother liquid in DMF, results after crystallisation, in another portion of 1 (19%). Repetition of the acetal-exchange process gives 1 in a total yield of 60%.
5. Satisfactory elemental and spectroscopic (^1H- and ^{13}C-NMR) data were obtained.
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7. Reagent 15 was prepared by adding 2-cyanoethanol (1 eq.) to a cooled (0°C) soln. of Et_2O containing equimolar amounts of 2-cyanoethyldichlorophosphine [N.D. Sinha et al., Nucl. Acids Res. 12, 4539 (1984)] and triethylamine. After 1 h at 20°C, filtration of $N(Et)_3$.HCl and evaporation of solvent afforded crude 15 (δp-value: 166.48 ppm).
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10. Anal. Calc. for $C_6H_9O_{15}P_3Na_6$: P, 16.83; Found:P, 16.93. ^{31}P-NMR (D_2O, pH 8) data (δ-values in ppm): 4.71 (2P), 3.05 (1P). ^1H-NMR (D_2O) data (δ-values in ppm):3.52 (t, 1H, H5, J4-5=J5-6= 9 Hz), 3.81 (t, 1H, H6, J6-1= 9 Hz), 3.89-4.10 (m, 2H, H1 and H3), 4.22 (m, 1H, H4), 4.36 (broad signal, 1H, H2).
11. We also prepared (±)-Ins(1,4,5)P3 using the above described phosphite-triester method.

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