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A NOVEL REAGENT FOR THE SYNTHESIS OF MYO-INOSITOL PHOSPHATES: N,N-DIISOPROPYL DIBENZYL PHOSPHORAMIDITE

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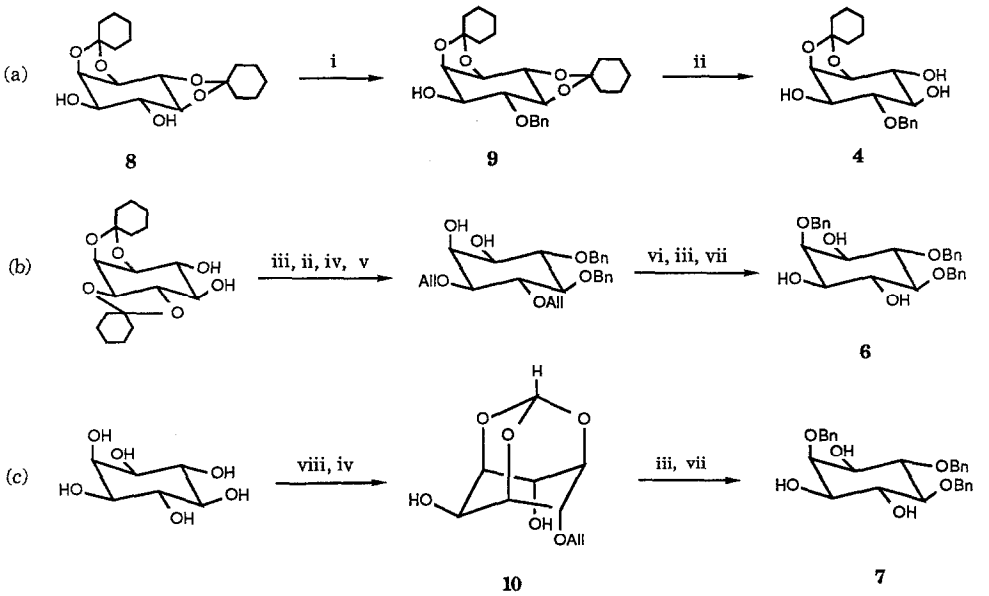
Summary: It has been found that partially protected inositols are phosphorylated in near quantitative yields by use of N,N-diisopropyl dibenzyl phosphoramidite, 1-*H*-tetrazole and MCPBA in CH_2Cl_2. Subsequent hydrogenolysis of the resulting perbenzylated products gives the corresponding free inositol phosphates in quantitative yields without tedious ion exchange chromatography.

Convincing evidence has recently been accumulated which indicates that the metabolism of inositol phospholipids of the cell membrane regulates the mobilization of calcium ion and triggers enzyme activity within a stimulated cell.[1] Thus, D-myo-inositol-1,4,5-triphosphate (**1**) has been shown to function as a second messenger to mobilize intracellular calcium ion from an intracellular store. In addition, Irvine and Moor have demonstrated that D-myo-inositol-1,3,4,5-tetraphosphate, **3** (a metabolite of **1**) might control the influx of calcium ion across the plasma membrane.[2] In order to explore the biochemical processes involved a simple, general, and efficient methodology is required for chemical syntheses of inositol phosphates. Recent accomplishments utilizing benzyl pyrophosphate[3-5] and phosphites[6-8] are the most promising strategies currently available. Each has advantages and disadvantages. The former protects the phosphates as benzyl esters, which can be removed easily by hydrogenolysis; however, phosphorylation of the inositol precursor needs strongly basic conditions. Although the reaction conditions of the latter approach are milder, the purification after esterification requires tedious ion exchange chromatography. Here we report a new strategy which overcomes the disadvantages of both methods.

Four partially protected myo-inositol derivatives (**4-7**) were chosen for this study and were synthesized by standard or literature procedures. For the triol **4**, the key reaction was the tin-mediated benzylation[9] of **8** which proceeded with 97% chemoselectivity.[10] Selective hydrolysis of the *trans*-cyclohexylidine of **9** then afforded compound **4**. 1,2,4-Tri-O-benzyl-myo-inositol **5** was prepared according to

Scheme 1



i) n-Bu_2SnO; BnBr, CsF; ii) CSA; iii) NaH, BnBr; iv) NaH, AllBr; v) 80% AcOH; vi) n-Bu_2SnO; All I, CsF; vii) Pd/C, TsOH; viii) $HC(OMe)_3$, TsOH, DMF.

the published work of Gigg and co-workers.[11] 2,4,5-Tri-O-benzyl-myo-inositol **6** was synthesized from 1,2:3,4-di-O-cyclohexylidine-myo-inositol, as shown in Scheme 1b. The tetrol **7** was prepared by a modification of the elegant strategy of Billington and Baker (Scheme 1c) involving the chemoselective O-allylation leading to the diol **10**.[4]

Compound **4** was chosen for initial examination because its triphosphate derivative **12** is a known substance.[5a] The reagents N,N-diethyl dibenzyl phosphoramidite (**11a**)[12] and N,N-diisopropyl dibenzyl phosphoramidite (**11b**)[13] were investigated, and the latter proved preferable because it is more stable and

$(BnO)_2 P NR_2$

11

a R = Et

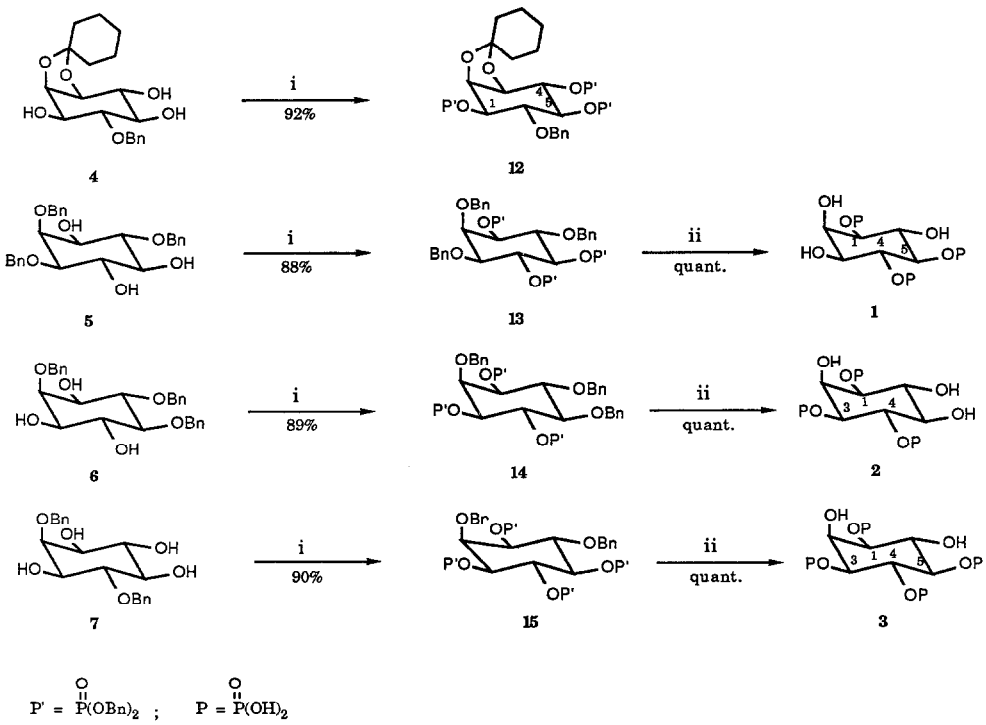
b R = *i*-Pr

therefore allows purification by column chromatography. 5-(p-Nitrophenyl)-tetrazole[14] and 1-*H*-tetrazole were used as catalysts. Oxidation of the phosphite esters with *tert*-BuOOH[15] in CH_3CN was plagued by the formation of side products which made purification very difficult. On the other hand, with MCPBA,[12] the reaction went smoothly. Eventually, conditions were

developed[16] utilizing **12b**, 1-*H*-tetrazole, and MCPBA[12] in CH_2Cl_2, which gave the benzylated phosphates in excellent yields.

Accordingly, the polyols **4**, **5**, **6**, and **7** afforded the benzyl esters **12**, **13**, **14**, and **15**, respectively in 88-92% yields (Scheme 2). Subsequent hydrogenolysis of the last three substances resulted in myo-inositol triphosphates **1**, **2**, and **3**, respectively in quantitative yields without any indication of phosphono-migration based on their ^1H and ^{31}P- NMR.

Scheme 2



i) see footnote 16
ii) H_2/Pd, EtOH-H_2O

In conclusion, the procedure using N,N-diisopropyl dibenzyl phosphor-amidite **11b**, 1-*H*-tetrazole, and MCPBA in CH_2Cl_2 is an efficient method for the phosphorylation of inositol derivatives. Hydrogenolyses of the resulting benzyl-protected phosphate esters give the corresponding free inositol phosphates in quantitative yields without tedious ion exchange chromatography. The method therefore provides a very mild and convenient protocol for syntheses of inositol phosphates.

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16. A standard procedure is the following: To a mixture of tetrol **7** (100 mg, 0.277 mmol) and 1-*H*-tetrazole (233 mg, 3.33 mmol) in 10 mL of CH_2Cl_2 was added N,N-diisopropyl dibenzyl phosphoramidite **11b** (575 mg, 1.66 mmol). The reaction mixture was stirred at room temperature for 2 h and then cooled to -40°C in dry ice-CH_3CN bath, and MCPBA (80-85% purity, 478 mg, 2.22 mmol) in 5 mL of CH_2Cl_2 was added. The resulting solution was stirred at 0°C for 45 min. The reaction mixture was diluted with 40 mL of CH_2Cl_2, washed with Na_2SO_3 (10%, 20 mL X 2), $NaHCO_3$ (15 mL X 2), water (15 mL), sat. NaCl aq. solution. The crude product obtained was purified by flash chromatography.

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