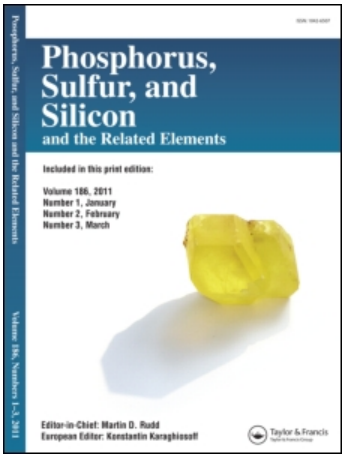

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Phosphoranylation of Myo-Inositol : A New Route for a Simultaneous Synthesis of Several Myo-Inositol Phosphates

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PHOSPHORANYLATION OF MYO-INOSITOL : A NEW ROUTE FOR A SIMULTANEOUS SYNTHESIS OF SEVERAL MYO-INOSITOL PHOSPHATES

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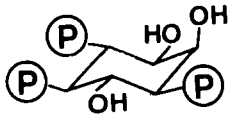
The recent discovery of the role of D-myo-inositol-1,4,5 trisphosphate Ins $(1,4,5)P_3$ 1 as the second messenger for receptor-mediated Ca^{2+} mobilization (1) has generated a great interest in the chemical preparation of myo-inositol phosphates 2 which were only available by enzymatic extraction from bovine brains (°).



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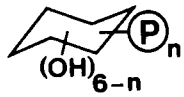

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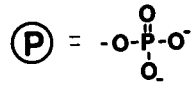
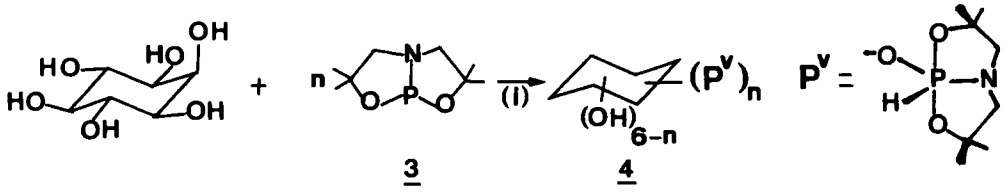


Since june 1986 there are, at least, 20 papers dealing with the synthesis of this type of compounds. All these preparations require, as it was done in 1959 by Pizer and Ballou (2) for the preparation of myo-inositol - 1 phosphate, the protection and, at the end of the reaction, the deprotection of the suitable OH functions of myo-inositol (3). The phosphorylation of the remaining OH functions is accomplished either by means of a tetracoordinated phosphorus compound $X_3P(O)$ (Khorana's technique (4)) or by means of a tricoordinated phosphorus X_3P followed by oxidation of the obtained phosphite (Letsinger et al. Caruthers) (5).) All these syntheses are always long (from 10 to 20 steps) and lead to the desired product with a poor yield relatively to myo-inositol.

In this communication we describe an original preparation of myo-inositol phosphates which is distinguishable from all the syntheses mentionned above by the fact that the reaction occurs with unprotected myo-inositol and leads to several products at the same time. The synthetic scheme is the following :

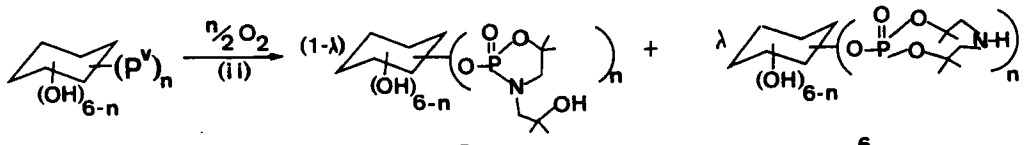
(i) partial or total phosphoranylation of myo-inositol by means of the 3,3,7,7 - tetramethyl - 2,8 dioxa - 5 - aza - 1 - phospha ^{III} bicyclo (3,3,0) octane "bicyclophosphane" 3



3 4

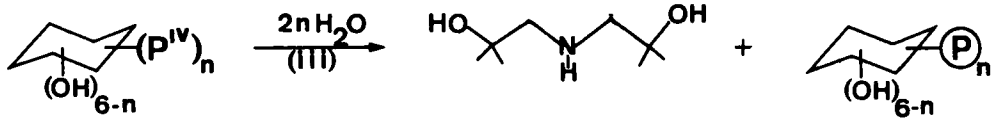
(°) This tedious procedure explains the very expensive price of the commercially available derivatives.

(ii) oxidation of the "bicyclophosphoranes" 4 leading to the neutral esters 5 and 6.



5 6

(iii) hydrolysis of the mixture of 5 and 6 to obtain the myo-inositol phosphates 2 which could be separated by ion exchange chromatography.



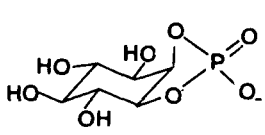
5,6 2

The first experiment was performed with n = 3. In DMSO as solvent at room temperature, the phosphoranylation of myo-inositol was very rapid. The ^{31}P NMR control showed the disappearance of the peak at δ = 163 ppm corresponding to the "bicyclophosphane" 3 and the formation of at least 10 doublets : $\delta\ ^{31}P$ = -40 ppm $^{1}J_{P-H}$ = 815 Hz accompanied by 3 minor signals (10%) at δ = 134 ppm. These latters represents the phosphite tautomer form which has been detected in many bicyclophosphoranes of polyols (6). The number of "phosphorane" peaks can be explained by the following reasons : the trisphosphoranylation of myo-inositol gives rise to 12 diastereisomers which possess 32 different ^{31}P nuclei. The presence of the phosphit tautomer form increases this number and finally, the formation of species corresponding to the fixation, on the myo-inositol, of a number of "bicyclophosphoranes" different from 3 (1.2.4.5.6) could not be excluded.

The oxidation of this mixture was achieved with a 20% excess of t.Bu.OOH at room temperature. We have noted the total disappearance of the phosphoranes and phosphites peaks and the formation of two sets of peaks : a major one corresponding to 5 (n = 3) and a minor one corresponding to 6 (n = 3). We have also showed that elementar sulfur, in DMSO as solvent, transforms, very rapidly, the bicyclophosphoranes 4 into two types of compounds 7 and 8.

7 8

The hydrolysis of the mixture of 5 and 6 (n = 3) was performed at room temperature. It was instantaneous in acidic medium pH = 2. The resulting reaction mixture was submitted to HPLC separation on PARTISIL SAX 10 Column in the same experimental conditions as those used by Irvine et al (7). Among the collected fractions we have identified the myo-inositol phosphates 9 - 12 : (8).



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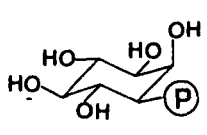

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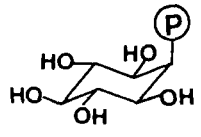

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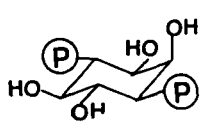

12

The same synthetic scheme was applied to the myo-inositol per-phosphorane 4 (n = 6) obtained with (U-^{14}C) myo-inositol as starting material.

Several types of chromatographic separation have been carried out :(i) Liquid chromatography of the final reaction mixture on ACCELL QMA Column in the same experimental conditions as those used by Wreggett and Irvine (9) for the separation of myo-inositol derivatives extracted from rat parotid-gland slices. The comparison of the obtained chromatogram with that of Wreggett andIrvine indicates the presence of 4 groups of myo-inositol phosphates i.e. myo-inositol monophosphates Ins P (39%), myo-inositol bisphosphates Ins P_2 (13%), myo-inositol trisphosphates Ins P_3 (30%) and myo-inositol tetrakisphosphates Ins P_4 (3%).

(ii) HPLC analysis on PARTISIL SAX 10 anion exchange column (Water-Ammonium formate linear gradient 0 - 2 M full scale). It shows also the formation of several groups of myo-inositol phosphates. We have performed the ^{31}P, ^{13}C and ^{1}H NMR analysis of the fractions corresponding to 0.5.M ammonium formate concentration. This study indicates the presence in this fraction of several myo-inositol polyphospates.

(iii) HPLC analysis of this last fraction on NUCLEOSIL SB 5 anion exchange column (Water-Ammonuim sulfate gradient : 0 - 0,4 M during 18 minutes ; 0.4 - 1.5 M during 12 minutes (Waters Gradient n° 4). Total duration 40 minutes). The obtained chromatogram indicates the presence of one major derivative and 6 other minor myo-inositol phosphates. We continue our inverstigation in order to separate and identify these coumponds.

In other words, this preparation could be considered as a synthetic analogue of the chemical or enzymatic hydrolysis of phytic acid, recently achieved by Phillippy et al (10) which affords also several myo-inositol phosphates. However, it differs from the latter, by the fact (i) that it leads directly to radio-labelled derivatives (ii) that it could be applied to any inositol, for instance chiro inositol. This possibility is of some interest since this unusual inositol isomer has been found in the glycosyl-phosphatidylinositol derived from the H 35 hepatoma cell as well as in the glycosyl-phosphatidylinositol anchored proteins (11).

(iii) that it affords the possibility to obtain modified myo-inositol phosphates for instance the myo-inositol thiophosphates.

In conclusion, this simple synthetic scheme represents a convenient route to obtain simultaneously several myo-inositol phosphates. Indeed, it needs further improvement, particularly in the separation and identification of the obtained coupounds. This is what we intend to do in the near future.

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