



RAPID SYNTHESIS AND PROPERTIES OF (±)-6-DEOXY-6-FLUORO-MYO-INOSITOL-1,4,5-TRIS(PHOSPHATE) AN ANALOGUE OF MYO-INOSITOL-1,4,5-TRIS(PHOSPHATE).

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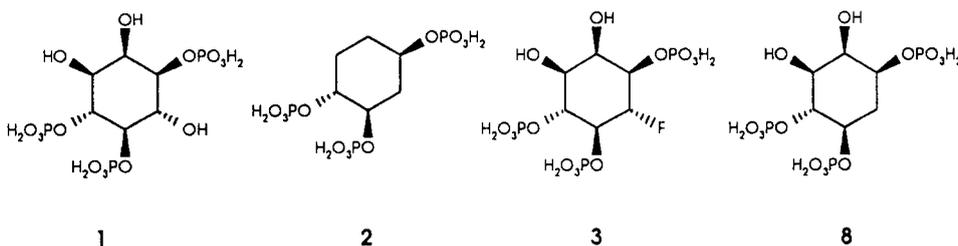
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Abstract. (±)-6-Deoxy-6-fluoro-*myo*-inositol-1,4,5-tris(phosphate) was prepared from *myo*-inositol derivatives. Its affinity for the *Endoplasmic Reticulum* receptors was interpreted using ³¹P-NMR studies. Copyright © 1996 Elsevier Science Ltd

Myo-inositol 1,4,5-tris(phosphate) **1** possesses secondary messenger properties.^{1,2} It has been shown that the affinity of **1** for its *Endoplasmic Reticulum* receptors (ERR) is largely pH dependent.³ This was interpreted in terms of ionization state variations of the phosphate groups.⁴⁻⁶ In the inositol-phosphate field, the behaviour of the phosphate groups is considerably influenced by the chemical environment. Especially, the proximity of hydroxyl functions which participates in the stabilization of the protons of the phosphates.⁵ The presence of these OH groups also dramatically influences the biological properties of the molecules.^{4,7} Notably, removal of all of the non phosphorylated hydroxyls on the inositol ring, leading to **2**, resulted in a 400 fold drop in affinity towards the ERR^{7,8} and removal of only the hydroxyl in position 6 considerably reduced the activity.^{9,10}

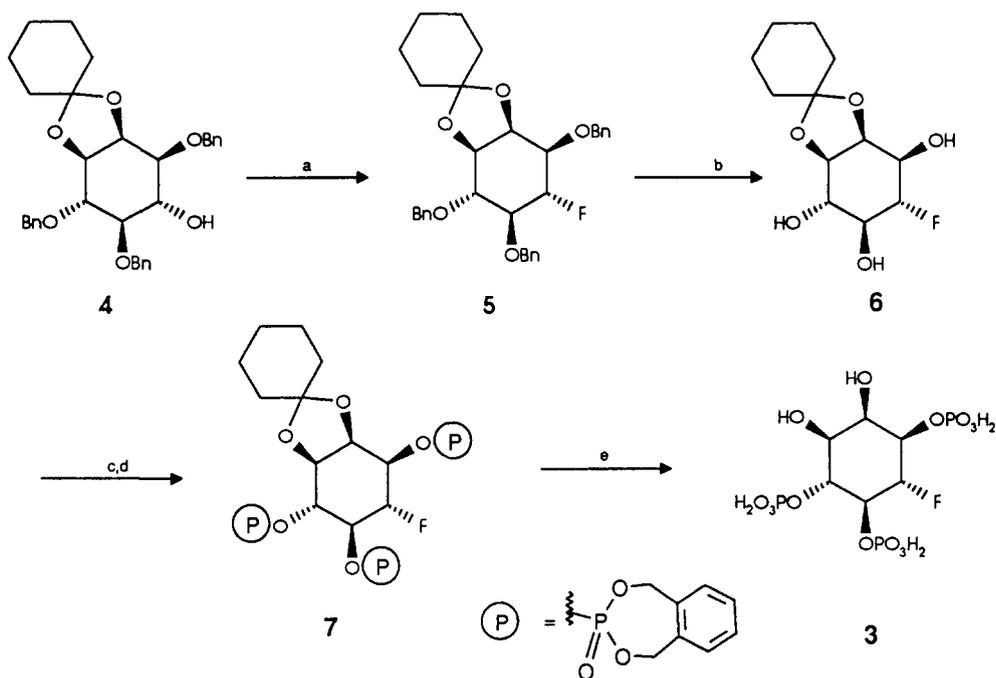


To investigate the type of interaction associated with the hydroxyl in position 6 it seemed interesting to us to operate an isosteric replacement of this hydroxyl by an isopolar fluorine atom. Such a replacement could give information concerning the relative acceptor or donor effects of either the lone pairs or the hydroxyl hydrogen. Submitted to binding analyses, the results will be interpreted by making use of ³¹P-NMR titrations.

We report a rapid synthesis of racemic 6-deoxy-6-fluoro-*myo*-inositol-1,4,5-tris(phosphate) **3**, capable of providing the large quantities of compound required for the physico-chemical analyses, in addition to the binding assays. The synthesis of **3** was previously reported by Ley and Co.¹¹ This synthesis used an enzymatic digestion of benzene and the fluorine atom was introduced by a stereoselective opening of an epoxide obtained from the benzene derivative¹¹ leading to optically active **3**.

Synthesis

The starting material of the synthesis was (\pm)-1,2-*O*-cyclohexylidene-3,5,6-tri-*O*-benzyl *myo*-inositol (**4**) prepared using known methodologies.^{12,13} Treatment of the (\pm)-3,5,6 tribenzyl derivative **4** with DAST^{14,15} afforded (\pm)-4-deoxy-4-fluoro-3,5,6-tri-*O*-benzyl-1,2-*O*-cyclohexylidene *myo*-inositol **5** identified by proton and fluorine NMR¹⁶, it is interesting to note that in that case the use of DAST led to retention of configuration.



Scheme : Synthesis of 6-deoxy-6-fluoro *myo* inositol-1,4,5-tris(phosphate) a: DAST; b: $H_2, Pd/C$; c: *N,N*-diethyl -*O*-Xyllylene phosphoramidite, tetrazole, d: *mCPBA*, e: $MeOH, H_2O$

Such retentions of configuration have been previously observed and require the assistance of neighbouring groups, which, in our case was a benzyl group.¹⁷⁻¹⁹ Removal of the protective groups by hydrogenolysis gave the fluoro derivative **6**. The free hydroxyl groups were then phosphorylated by means of *N,N* diethyl-*O*-xyllylene phosphoramidite followed by a treatment with *mCPBA* according to Perich.²⁰ The last step concerns

the hydrogenolysis of the protected hydroxyl functions leading to the expected (\pm) -6-deoxy-6-fluoro-*myo*-inositol-1,4,5-tris(phosphate) (**3**) which was stabilized as a cyclohexylammonium salt to avoid any migration of the phosphate or the formation of a cyclic phosphate.

Binding properties.

Binding of the fluoro derivative **3** was tested on adrenal cortex microsomes.²¹ Compound **3** showed low affinity ($K_d = 1.6 \mu\text{M}$) compared to the parent compound **1** ($K_d = 0.01 \mu\text{M}$). A similar decrease in potency was observed for the 2,3,6-trideoxy compound **2** ($K_d = 4 \mu\text{M}$)⁷ as well as for the 6-deoxy *myo*-inositol-1,4,5-tris(phosphate) (**8**) which was found to be around 500 times less active^{9,10}. As for **8** differing from **3** only in the lack of the hydroxyl group in position 6; this low affinity confirms the importance of this functional group.

³¹P-NMR titrations

To establish how the isosteric replacement could influence the behaviour of the phosphate we have performed some ³¹P-NMR analyses on **3**. Figure 1 shows the chemical shift variations for the three phosphorus atoms versus the pH. It is well known that these chemical shift variations are directly correlated with the ionization state of the phosphate.^{22,23} It is interesting to note that, for compound **3** two types of curve shapes are observed. The phosphate in position 1 behaves like an isolated monophosphate giving a classical phosphate titration curve. The phosphates in position 4 and 5 show biphasic curves characteristic for vicinal bis(phosphates).²³

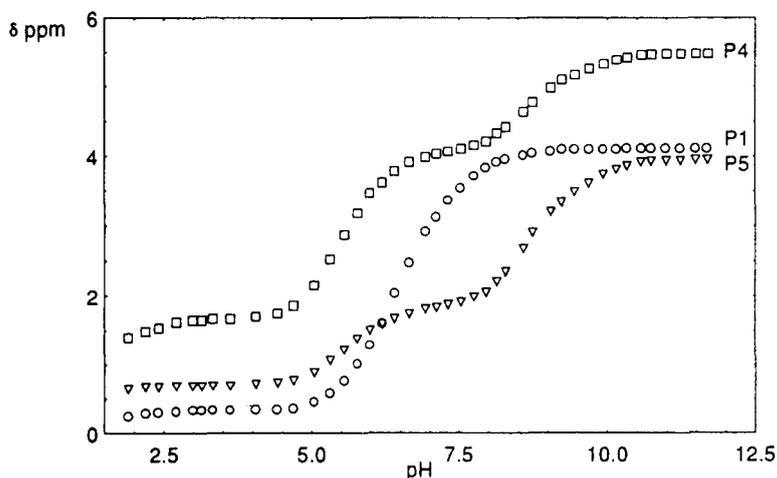


Figure 1: ³¹P-NMR (H_2O containing 10% D_2O) titration curves of **3** performed at 37°C, medium: .2M KCl.

Figure 1 shows the superimposition of a mono and a bis(phosphate). This situation is comparable with that of the 2,3,6-trideoxy derivative **2**⁸; but largely differs from the curves obtained with the parent compound **1** where the three phosphates appear to be engaged in complexe cooperative processes.²³ Removal or isosteric replacement by a fluorine atom seems to cancel the interactions between the phosphate in position 1 and the vicinal phosphates in position 4 and 5. These results enlighten the fact that the OH group in position 6 mediates the interactions probably not by its lone pairs but more likely by its hydroxyl hydrogen.

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- Compound **5**: ¹H-RMN (CDCl₃ / C₆D₆; 25 : 75, 300 MHz): 7,5-7,2 (*m*, 15H, -(C₆H₅)₃); 5,0 (*dt*, 1H, ²J_{H₄F} = 51,2, ³J_{H₄H₃} = ³J_{H₄H₅} = 8,1); 4,9-4,6 (*m*, 6H, -(CH₂-C₆H₅)₃); 4,18 (*ddd*, ³J_{H₂H₁} = 5,6, ³J_{H₂H₃} = ³J_{H₂F} = 4,0, 1H, *H*₂); 3,97 (*t*, H, ³J_{H₁H₂} = ³J_{H₁H₆} = 6,2, *H*₁); 3,77 (*dd*, ³J_{H₆H₅} = 8,6, ³J_{H₆H₁} = 6,4, 1H, *H*₆); 3,62 (*ddd*, ³J_{H₃F_{cis}} = 11,3, ³J_{H₃H₄} = 8,7, ³J_{H₃H₂} = 3,8, 1H, *H*₃); 3,46 (*dt*, ³J_{H₅F_{cis}} = 16,2, ³J_{H₅H₄} = 8,1, ³J_{H₅H₆} = 8,1, 1H, *H*₅); 1,8-0,7 (*m*, 10H, -(C₆H₁₀)). ¹⁹F-RMN (CDCl₃): -195.72 (*dddd*, ²J_{H₄F} = 51,2, ³J_{H₅F_{cis}} = 16,2, ³J_{H₃F_{cis}} = 11,3, ³J_{H₂F} = 4,0, 1F, *F*₄).
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