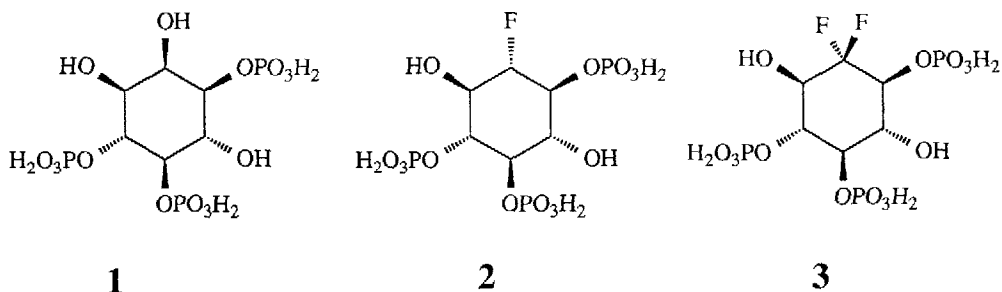


FLUORINATED ANALOGS OF INS(1,4,5)P₃

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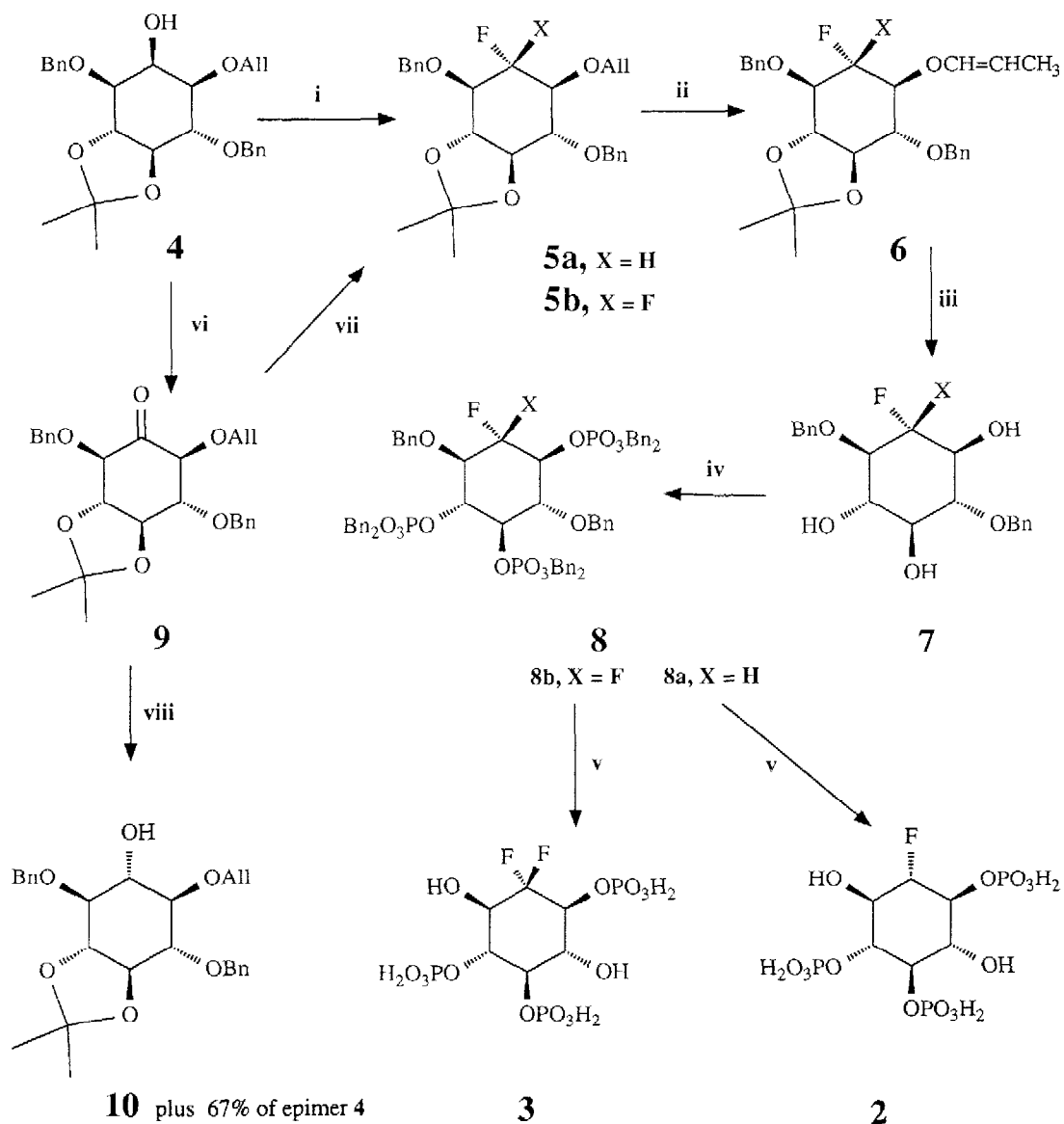
Summary: 2-Fluoro-2-deoxy-Ins(1,4,5)P₃ (**2**) and 2,2-difluoro-2-deoxy-Ins(1,4,5)P₃ (**3**) were synthesized from protected inositol precursors. The monofluoro compound with free 3,6-hydroxyl groups underwent slow defluorination at pH > 13, as determined by ¹⁹F-NMR, while the difluoro compound was inert.

Cells can communicate with one another in a number of ways.¹ For example, external messengers (hormones, growth factors, etc.) can bind to external receptors on a target cell, activating a second messenger system.² A recently discovered second messenger is *myo*-inositol-1,4,5-trisphosphate³ (**1**) which arises from cleavage of a cell membrane component, phosphatidylinositol-4,5-bisphosphate, by a G protein-activated phospholipase C.⁴ Once released, the Ins(1,4,5)P₃ binds to specific receptors on the endoplasmic reticulum and stimulates the release of calcium from intracellular storage sites.



Fluorodeoxy sugars are molecules in which a C-OH is replaced by a C-F. Bond lengths and polarization are similar in both groups; however, the C-F bond can only accept but not donate a hydrogen bond.⁵ Such compounds are potentially useful as probes for studies of the active site of enzymes and for membrane transport studies. Several fluorodeoxy inositols⁶ and a 2-deoxy-2-fluoro-1-phosphatidyl-*scyllo*-inositol^{6d} have been reported. Recently, we reported the synthesis of 2-fluoro and 2,2-difluoro-2-deoxy

analogs of racemic $\text{Ins}(1,3,4)\text{P}_3$, suspected to be either an alternative agonist or a by-product of $\text{Ins}(1,4,5)\text{P}_3$ metabolism.⁷ We now describe the synthesis and chemical stability of two fluorideoxy analogs (**2** and **3**) of the second messenger $\text{Ins}(1,4,5)\text{P}_3$.



Scheme 1. Synthesis of fluorideoxy inositol phosphates. Reagents: (i) DAST, CH_2Cl_2 , 0°C ; (ii) $(\text{Ph}_3\text{P})_3\text{RhCl}$, DABCO, EtOH; (iii) MeOH, H_3O^+ ; (iv) $((\text{BnO})_2\text{PO})_2\text{O}$, NaH, DMF; (v) 10% Pd/C, H_2 , EtOH; (vi) DMSO- Ac_2O ; (vii) DAST, CH_2Cl_2 , 25°C ; (viii) NaBH_4 , EtOH.

The common intermediate for each synthesis was 1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene-*myo*-inositol 4.⁸ Scheme I summarizes the preparation of the fluorinated analogs. Reaction of protected cyclohexitol 4 with DAST in CH₂Cl₂ at 0 °C resulted in fluorination at the 2-position with inversion of configuration, yielding the corresponding 2-deoxy-2-fluoro-*scyllo*-inositol 5a (68%). The allyl group was isomerized to the 1-propenyl ether 6a (as a mixture of *E* and *Z* isomers) using Wilkinson's catalyst (91%), and then ether and isopropylidene groups were removed by mild acid hydrolysis (83%). The resulting 2-deoxy-2-fluoro-1,4-di-O-benzyl-*scyllo*-inositol 7a was phosphorylated using tetrabenzyl pyrophosphate⁹ after generation of the trisanion with NaH in DMF at 0 °C, affording the perbenzylated species 8a in 44% yield. The eight benzyl groups were simultaneously removed by catalytic hydrogenolysis with Pd/C to yield 2-deoxy-2-fluoro-*scyllo*-inositol-1,4,5-trisphosphate (2) which was isolated as the ammonium salt. For analysis and further studies it was converted to the hexasodium salt by ion-exchange chromatography.¹⁰

Oxidation of precursor 4 using acetic anhydride-DMSO afforded the unstable 2-inosose 9 in 66% yield.⁸ This was fluorinated using an excess of DAST in CH₂Cl₂ at 25 °C to give a 60% yield of 2,2-difluoro-1-O-allyl-3,6-di-O-benzyl-4,5-O-isopropylidene *myo*-inositol (5b). The same sequence of reactions (5b → 6b → 7b → 8b → 3) used for the monofluoro derivative produced the desired 2,2-difluoro-*myo*-Ins(1,4,5)P₃ (3)¹¹ via perbenzylated intermediate 8b.

The inosose 9 was reduced with sodium borohydride which furnished (95% yield) a 2:1 mixture of the *myo*- and *scyllo*-inositols derivatives (4 and 10) which were readily separated by column chromatography on silica gel.¹² When the *scyllo* epimer was treated with DAST in CH₂Cl₂ at 0 °C, fluorination occurred with *retention* of configuration and yielded the same 2-deoxy-2-fluoro-*scyllo*-inositol derivative 5a that was obtained from the *myo*-derivative. Although unusual, fluorination with retention of configuration when using DAST has been observed in a number of instances.¹³

Although Ins(1,4,5)P₃ (1) is reasonably stable in alkaline solution at 25 °C, the monofluoro derivative (2) was found to undergo a slow defluorination reaction on prolonged (> 4 weeks) storage under identical conditions. Fluoride was produced (peak at -124 ppm in the ¹⁹F NMR) and a mixture of phosphates was formed. The difluoro analog 3, on the other hand, was stable under these conditions. The defluorination of 2 did not occur at pH 8; this reaction occurred at a reasonable rate only above pH 12. By following the reaction in a sealed NMR tube by ¹⁹F-NMR, the half life of monofluoro 2 at pH 13 was estimated to be 2 weeks at 50 °C.

Since the ionization state of the phosphates is the same at both pH 8 and pH 13, i.e., both **2** and **3** are hexaanions), we hypothesized that the neighboring hydroxyl group was involved in the defluorination. To test this, the two free hydroxyls were blocked as methyl ethers. The resulting 2-deoxy-2-fluoro-3,6-di-O-methyl-*scyllo*-inositol-1,4,5-trisphosphate¹⁴ showed no evidence of defluorination at pH 13, 50 °C during several weeks, consistent with this hypothesis.

Both monofluoro compound **2** and difluoro compound **3** show high affinity for the rat brain $\text{Ins}(1,4,5)\text{P}_3$ receptor, and both activate calcium release from permeabilized cells *in vitro*.¹⁵ These results will be described elsewhere.

Acknowledgements. We thank the Center for Biotechnology and the New York State Foundation for Science and Technology for financial support. Encouragement and biochemical results from Dr. S.H. Snyder and Mr. R. Mourey (Johns Hopkins University) are gratefully acknowledged.

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10. Compound **2**: ¹⁹F-NMR (D₂O) δ -199.7 (dt, $J = 51$ Hz, $J = 12.6$ Hz, F_{eq}); ³¹P-NMR (D₂O) δ 5.4, 6.9, 7.1. ³¹P chemical shifts are referenced to external 85% H₃PO₄ ($\delta = 0$ ppm) and ¹⁹F chemical shifts are referenced to CFC₃ ($\delta = 0$ ppm). Downfield shifts are positive. All inositol trisphosphates are in the form of the hexasodium salt at pH 12.
11. Compound **3**: ¹⁹F-NMR (D₂O) δ -117.9 (d, $J = 246$ Hz, F_{eq}); δ -132.2 (dt, $J = 246$ Hz, $J = 22$ Hz, F_{ax}); ³¹P-NMR (D₂O) δ 5.4, 6.9, 7.2.
12. The epimeric alcohols are readily separated by SiO₂ chromatography using 3:2 hexane-ether as eluent: R_f (1:1 hexane-ether) = 0.40 (eq), 0.22 (ax).
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14. ¹⁹F-NMR (D₂O) δ -193.8 (ddd, $J = 52.9$ Hz, $J = 22.3$ Hz, $J = 12.0$ Hz, F_{eq}); ³¹P-NMR (D₂O) δ 2.14, 2.40, 2.43; ¹H-NMR (D₂O) δ 3.50 (s, OCH₃), 3.52 (s, OCH₃).
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(Received in USA 17 July 1989)