Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 10, pp. 1175-1178, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00193-X

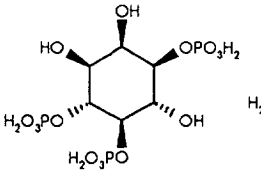
RAPID SYNTHESIS AND PROPERTIES OF (±)-6-DEOXY-6-FLUORO-*MYO*-INOSITOL-1,4,5-TRIS(PHOSPHATE) AN ANALOGUE OF *MYO*-INOSITOL-1,4,5-TRIS(PHOSPHATE).

Philippe Guédat[+], Marc Poitras[#], Bernard Spiess[+], Gaetan Guillemette[#] and Gilbert Schlewer[+]*

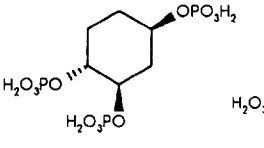
[+]Laboratoire de Pharmacochimie Moléculaire du CNRS, Faculté de Pharmacie, 74 route du Rhin 67401 Illkirch (France)
Fax: (33) 88 67 47 94 ; e-mail: schlewer@pharma.u-strasbg.fr
[#] Université de Sherbrooke, Faculté de Médecine, Département de Pharmacologie, 3001, 12e Avenue Nord, Sherbrooke, Québec, Canada J1H 5N4

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 ^{31}P-NMR studies.

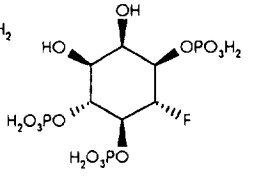
Myo-inositol 1,4,5-tris(phosphate) **1** possesses secondary messenger properties.[1,2] It has been shown that the affinity of **1** for its *Endoplasmic Reticulum* receptors (ERR) is largely pH dependent.[3] This was interpreted in terms of ionization state variations of the phosphate groups.[4-6] In the inositol-phosphate field, the behaviour of the phosphate groups is considerably influenced by the chemical environnement. 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]



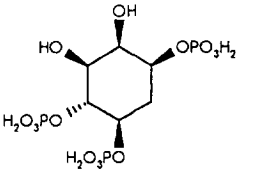
1



2



3



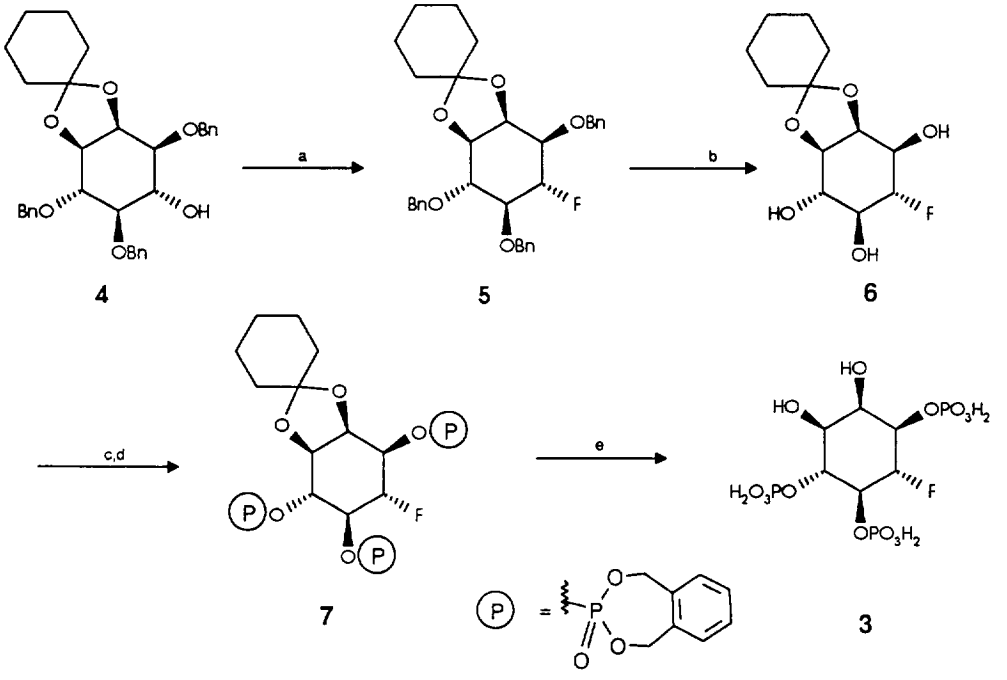
8

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 donnor effects of either the lone pairs or the hydroxyl hydrogen. Submitted to binding analyses, the results will be interpreted by making use of ^{31}P-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 derivative[11] leading to optically active **3**.

Synthesis

The starting material of the synthesis was (±)-1,2-*O*-cyclohexylidene-3,5,6-tri-*O*-benzyl *myo*-inositol (**4**) prepared using known methodologies.[12,13] Treatment of the (±)-3,5,6 tribenzyl derivative **4** with DAST[14,15] afforded (±)-4-deoxy-4-fluoro-3,5,6-tri-*O*-benzyl-1,2-*O*-cyclohexylidene *myo*-inositol **5** identified by proton and fluorine NMR[16], 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-Xylylene 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*-xylylene phosphoramidite followed by a treatment with *m*CPBA according to Perich.[20] The last step concerns

the hydrogenolysis of the protected hydroxyl functions leading to the expected (±)-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 (Kd = 1.6 μM) compared to the parent compound **1** (Kd = 0.01 μM). A similar decrease in potency was observed for the 2,3,6-trideoxy compound **2** (Kd = 4 μM)[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.

^{31}P-NMR titrations

To establish how the isosteric replacement could influence the behaviour of the phosphate we have performed some ^{31}P-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]

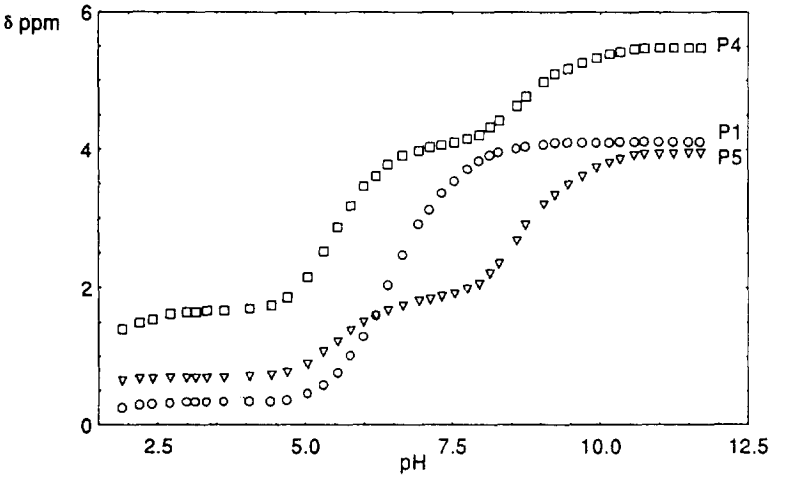


Figure 1: ^{31}P-NMR (H_2O containing 10% D_2O) titration curves of 3 performed at 37°C, medium: .2M KCl.

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 probabily not by its lone pairs but more likely by its hydroxyl hydrogen.

Aknowledgements.

We thank, the Medical Research Council of Canada for financial help for the biochemical studies.

References and notes

1. Berridge, M.J.; Irvine, R.F. *Nature*, **1984**, *312*, 315-321.
2. Berridge, M.J. *Mol. Cell. Endocrinol.* **1994**, *98*, 119-124.
3. Worley, P.F.; Baraban, J.M.; Supattapone, S.; Wilson, V.S.; Snyder, S.H. *J. Biol. Chem.* **1987**, *262*, 12132-12136.
4. Schmitt, L.; Bortmann, P.; Schlewer, G.; Spiess, B. *J. Chem. Soc. ,Perkin Trans. 2*, **1993**, 2257-2263.
5. Schmitt, L.; Schlewer, G.; Spiess, B. *Biochim. Biophys. Acta*, **1991**, *1075*, 139-140.
6. Schmitt, L.; Spiess, B.; Schlewer, G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1225-1230.
7. Kozikowski, A.P.; Ognyanov, V.I.; Fauq, A.H.; Nahorski, S.R.; Wilcox, R.A. *J. Am. Chem. Soc.* **1993**, *115*, 4429-4434.
8. Schmitt, L.; Spiess, B.; Schlewer, G. *Tetrahedron Lett.* **1992**, *33*, 2013-2016.
9. Bonis, D.; Sezan, A.; Mauduit, P.; Cleophax, J.; Gero, S.D.; Rossignol, B. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 894-900.
10. Potter, B.V.L. *Phosphorus Sulfur and Silicon*, **1993**, *73*, 143-146.
11. Ley, S.V.; Parra, M.; Redgrave, J.L.; Sternfeld, F. *Tetrahedron*, **1990**, *46*, 4995-5026.
12. Massy, D.J.; Wyss, P. *Helv. Chim. Acta*, **1990**, *73*, 1037-1040.
13. Gigg, J.; Gigg, R.; Martin-Zamora, E. *Tetrahedron Lett.* **1993**, *34*, 2827-2830.
14. Offer, J.L.; Voorheis, H.P.; Metclafe, J.C.; Smith, G.A. *J. Chem. Soc. ,Perkin Trans. 2*, **1992**, 953-960.
15. Jiang, C.; Schedler, D.J.A.; Morris, P.E.; Zayed, A.H.A.; Baker, D.C. *Carbohydr. Res.* **1990**, *207*, 277-285.
16. Compound **5**: ^{1}H-RMN ($CDCl_3$ / C_6D_6 ; 25 : 75, 300 MHz): 7,5-7,2 (*m*, 15H, -(C_6H_5)$_3$); 5,0 (*dt*,1H, $^2J_{H4F}$ = 51,2, $^3J_{H4H3}$ = $^3J_{H4H5}$ = 8,1); 4,9-4,6 (*m*, 6H, -(CH_2-C_6H_5)$_3$); 4,18 (*ddd*, $^3J_{H2H1}$ = 5,6, $^3J_{H2H3}$ = $^3J_{H2F}$ = 4,0, 1H, *H2*); 3,97 (*t*, H, $^3J_{H1H2}$ = $^3J_{H1H6}$ = 6,2, *H1*); 3,77 (*dd*, $^3J_{H6H5}$ = 8,6, $^3J_{H6H1}$ = 6,4, 1H, *H6*); 3,62 (*ddd*, $^3J_{H3Fcis}$ = 11,3, $^3J_{H3H4}$ = 8,7, $^3J_{H3H2}$ = 3,8, 1H, *H3*); 3,46 (d*t*, $^3J_{H5Fcis}$ = 16,2, $^3J_{H5H4}$ = 8,1, $^3J_{H5H6}$ = 8,1, 1H, *H5*); 1,8-0,7 (*m*, 10H, -(C_6H_{10})). ^{19}F-RMN ($CDCl_3$): -195.72 (*dddd*, $^2J_{H4F}$ = 51,2, $^3J_{H5Fcis}$ = 16,2, $^3J_{H3Fcis}$ = 11,3, $^3J_{H2F}$ = 4,0, 1F, *F4*)..
17. Yang, S.S.; Chiang, Y.C.P.; Beattie, T.R. *Carbohydr. Res.* **1993**, *249*, 259-263.
18. Yang, S.S.; Beattie, T.R.; Shen, T.Y. *Synthetic Comm.* **1986**, *16*, 131-138.
19. Ballereau, S.; Guédat, P.; Spiess, B.; Rehnberg, N.; Schlewer, G. *Tetrahedron Lett.* **1995**, *36*, 7449-7450.
20. Perich, J.W.; Johns, R.B. *Tetrahedron Lett.* **1987**, *28*, 101-102.
21. Poitras, M.; Bernier, S.; Richard, D.; Servant, M.; Guillemette, G. *J. Biol. Chem.* **1993**, *268*, 24078-24082.
22. Cozzone, P.J.; Jardetzky, O. *Biochemistry*, **1976**, *15 (22)*, 4853-4859.
23. Mernissi Arifi, K.; Schmitt, L.; Schlewer, G.; Spiess, B. *Anal. Chem.* **1995**, *67*, 2567-2574.

(*Received in Belgium* 18 *March* 1996; *accepted* 19 *April* 1996)