Synthesis of phosphorofluoridate analogues of *myo*-inositol 1,4,5-tris(phosphate) and their biological activity

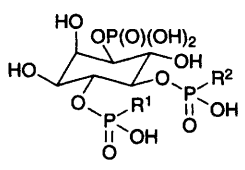
Yutaka Watanabe,*[a] Shinsuke Sofue,[a] Shoichiro Ozaki[a] and Masato Hirata[b]

[a] *Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790-77, Japan*
[b] *Department of Biochemistry, Faculty of Dentistry, Kyushu University 61, Fukuoka 812, Japan*

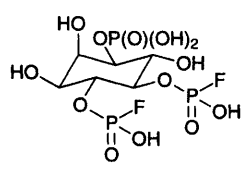
Phosphorofluoridate analogues of *myo*-inositol 1,4,5-tris(phosphate), 4- and 5-phosphorofluoridate and 4,5-bis(phosphorofluoridate), were prepared and their biological activity towards $InsP_3$ 5-phosphatase was found to be similar to or more active than that for $InsP_3$ while they proved to be less active in the binding assay than $InsP_3$.

An intracellular second messenger, D-*myo*-inositol 1,4,5-tris(phosphate) **1** ($InsP_3$) is known to mobilize Ca^{2+} ions from non-mitochondrial store sites.[1] Its physiological action commences from interaction of $InsP_3$ with a receptor on the endoplasmic reticulum, and it is metabolized by two pathways featuring $InsP_3$ 5-phosphatase and 3-kinase. In addition to the receptor protein and metabolic enzymes, two other binding proteins, $PLC_{\delta 1}$ and its analogous 130 kDa molecule, which we first isolated from rat brain cytosol by affinity chromatography, were recently found to have a high affinity towards $InsP_3$.[2] One of our aims is to show how these proteins interact with $InsP_3$ in their active domain in order to elucidate their mode of action at the molecular level, leading to potential new agonists and antagonists. To this end, we have designed phosphorofluoridate derivatives[3] of $InsP_3$ which are expected to bond covalently to binding sites in the domains of the $InsP_3$-recognizing proteins. There have been no reports on the preparation of such analogues, while a variety of regio-, stereo- and functional group-modified isomers have been prepared and evaluated biologically.[4] Here we describe the synthesis of three inositol phosphorofluoridate analogues, *myo*-inositol 1,4-bis(phosphate) 4-phosphorofluoridate **2**, its positional isomer 5-phosphorofluoridate **3**, and 1-phosphate 4,5-bis(phosphorofluoridate) **4**, and preliminary biological results.

Synthesis of phosphorofluoridates **2** and **3** was carried out using 3,6-dibenzyl-1,2-cyclohexylidene-*myo*-inositol **5** which was easily derived from *myo*-inositol in three steps. Thus, partial triethylsilylation of **5** followed by phosphorylation employing dibenzyl phosphoramidate gave **7a** and **7b**, a mixture of which was then desilylated yielding 4- and 5-free inositol derivatives **8a** and **8b** (*ca.* 1:1 ratio), which were separated by chromatography on silica gel. Isomer **8a** was then phosphorylated *via* the amidite method using benzyl 2-cyanoethyl phosphoramidite, and the product **9a** was decyanoethylated with triethylamine to yield **10a**, which was readily transformed to the corresponding phosphorofluoridate **11a** by reaction with 2-fluoro-1-methylpyridinium salt **14**.[5]



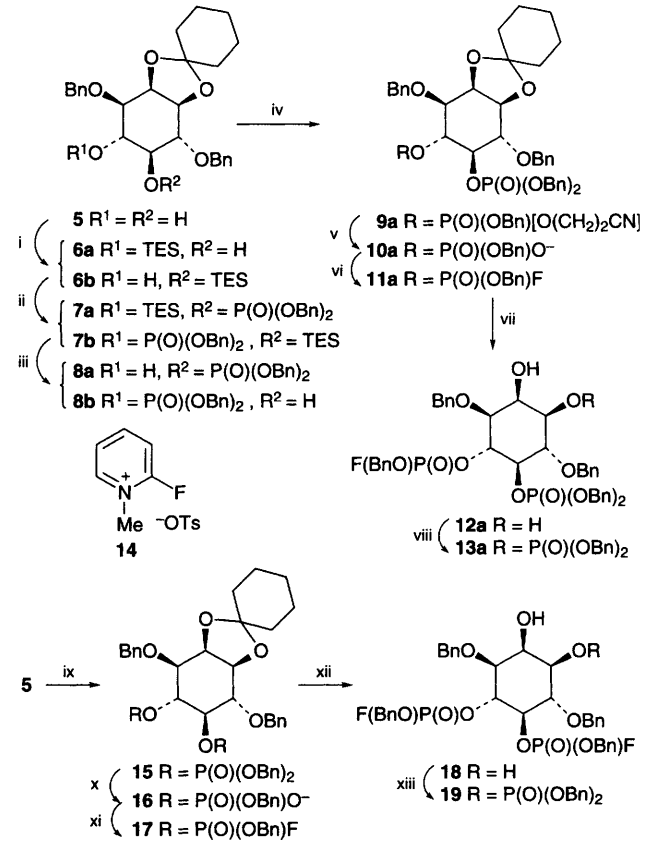
1 $R^1 = R^2 = OH$
2 $R^1 = F$, $R^2 = OH$
3 $R^1 = OH$, $R^2 = F$



4

Further reactions involving decyclohexylidenation, regioselective phosphorylation using tribenzyl phosphite and pyridinium hydrogen tribromide,[6] and deprotection under hydrogenolysis conditions gave the final product **2**.† In a manner similar to that for **8a**, 4-dibenzyl phosphate **8b** was converted into 5-phosphorofluoridate analogue **3** *via* the positional isomers **9b** and **13b**.

For the synthesis of 4,5-bis(phosphorofluoridate) **4**, compound **5** was phosphorylated and, after partial debenzylation of the phosphorylation product **15**, the resultant bis(phosphoric diester) **16** was transformed into bis(phosphorofluoridate) **17** in



Scheme 1 *Reagents and conditions*: i, Et_3SiCl (1.1 equiv.), Py, 0 °C, 2 h (90%, *ca.* 1:1 ratio of **6a** and **6b**); ii, $(BnO)_2PnPr^i_2$, tetrazole then MCPBA (91%); iii, $Bu_4NF{\cdot}3H_2O$, $PhCO_2H$, THF, 0 °C, 3 h (46% for **8a** and 46% for **8b**); iv, (**8** to **9**) $NC(CH_2)_2O(BnO)PNPr^i_2$, tetrazole then MCPBA (97 and 96%); v, Et_3N–MeCN (1:1), room temp., 2 h (1.5 h for **9b**) then 40 °C, 2.5 h (97 and 90%); vi, **14**, Et_3N, room temp., 2 h (55 and 87%); vii, (**11** to **12**) CF_3CO_2H, commercial CH_2Cl_2, −15 °C, 6 h (75% based on recovered **11a** and 77% for **11b**); viii, $(BnO)_3P$, $PyHBr_3$, Et_3N, 0 °C, 1 h (56 and 67%); (deprotection of **13a** and **13b** to **2** and **3**) H_2, Pd–C, AcOEt–MeOH–H_2O (1:3:1), room temp., 6 h (100% each); ix, $(BnO)_2PNPr^i_2$, tetrazole then MCPBA (97%); x, PhSH, Et_3N, room temp., 2 h; xi, **14**, Et_3N, room temp., 4 h (43%); xii, (**17** to **18**) CF_3CO_2H, CH_2Cl_2, −20 °C, 4 h (68%); xiii, $(BnO)_3P$, $PyHBr_3$, Et_3N, 0 °C, 0.5 h (68%); (**19** to **4**) H_2, Pd–C, MeOH–H_2O (2:1), room temp., 9 h (100%). TES = triethylsilyl.

a manner similar to the procedure mentioned above. The possible formation of the cyclic pyrophosphate was not observed, even though the 2-fluoropyridinium salt **14** is known as a condensing agent.[7] The fluoro derivative **17** was subjected successively to decyclohexylidenation, regioselective phosphorylation and deprotection under hydrogenolysis conditions, giving rise to the desired final product **4**.

The three phosphorofluoridates thus prepared had potencies for inhibiting [^{3}H]InsP_3 binding to purified InsP_3 receptor that were less than that for InsP_3. Two anlogues, 4- and 4,5-fluoro derivatives **2** and **4** were found to inhibit the dephosphorylation of [^{3}H]InsP_3 by the 5-phosphatase present in erythrocyte ghosts, with potencies similar to that for InsP_3. Surprisingly, the inhibitory potency of 5-phosphorofluoridate **3** toward 5-phosphatase was higher (about 20 fold) than those of InsP_3 and the another fluoridates **2** and **4**. These results suggest that in the recognition of InsP_3 by the receptor, second dissociation of 4- and 5-phosphoric monoester functions is important and in the case of InsP_3 5-phosphatase, the undissociated OH form, which is electronically and structurally similar to fluorine atom, is necessary for the recognition. Furthermore, a functional group at C-5 which was more electronegative than the monodissociated phosphoric monoester function might increase the affinity with InsP_3 5-phosphatase.

This work was financially supported in part by a Grant-in-Aid for Scientific Research on Priority areas No. 06240105 from the Ministry of Education, Science and Culture, Japan.

Footnote

† Each product was characterized by spectroscopic experiments (NMR and IR) and elemental analysis (or MS). ^{31}P NMR spectroscopic data (109 MHz, D_2O) for **2** (10 mg in 4 cm^3, pD 2.5) −4.22 (1P, d, $J_{P,F}$ 939 Hz), 1.30 (2P, s). For **3** (11 mg in 4 cm^3, pD 3) −4.15 (1P, d, $J_{P,F}$ 982 Hz), 1.20 (2P, br s). For **4** (8 mg in 4 cm^3, pD 3) −4.1 (2P, br d, $J_{P,F}$ *ca.* 970 Hz), 2.0 (1P, br s).

References

1 M. J. Berridge, *Nature*, 1993, **361**, 315.
2 T. Kanematsu, H. Takeya, Y. Watanabe, S. Ozaki, M. Yoshida, T. Koga, S. Iwanaga and M. Hirata, *J. Biol. Chem.*, 1992, **267**, 6518; T. Kanematsu, Y. Misumi, Y. Watanabe, S. Ozaki, T. Koga, S. Iwanaga, Y. Ikehara and M. Hirata, *Biochem. J.*, 1996, **313**, 319.
3 A. K. Balls and E. F. Jansen, *Adv. Enzymol.*, 1952, **13**, 321; R. A. Oosterbaan, P. Kunst, J. van Rotterdam and J. A. Cohen, *Biochim. Biophys. Acta*, 1958, **27**, 549.
4 B. V. L. Potter and S. R. Nahorski, *Biochem. Soc. Trans.*, 1992, **20**, 434; D. C. Billington, *The Inositol Phosphates, Chemical Synthesis and Biological Significance*, VCH, Weinheim, 1993.
5 Y. Watanabe, N. Hyodo and S. Ozaki, *Tetrahedron Lett.*, 1988, **29**, 5763.
6 Y. Watanabe, E. Inada, M. Jinno and S. Ozaki, *Tetrahedron Lett.*, 1993, **34**, 497.
7 T. Mukaiyama, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 707.

Received, 7th May 1996; Com. 6/03136F