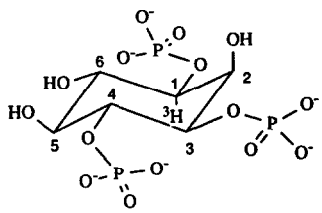
Tetrahedron Letters,Vol.29,No.41,pp 5217-5220,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

Fluorinated Analogs and Tritiated Enantiomers of Inositol (1,3,4)-Trisphosphate

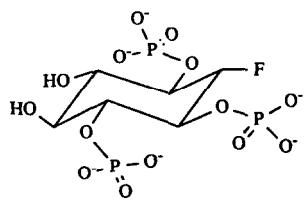
Marcus F. Boehm and Glenn D. Prestwich*
Department of Chemistry
State University of New York
Stony Brook, New York 11794-3400

Summary: The total syntheses of 2-fluoro- and 2,2-difluoro-2-deoxy analogs of DL-*myo*-Ins (1,3,4)P_3 are described. Resolution of a key intermediate followed by borotritide reduction and phosphorylation provided both D- and L-[1-^{3}H]-Ins(1,3,4)P_3 enantiomers with specific activities 15 Ci/mmol.

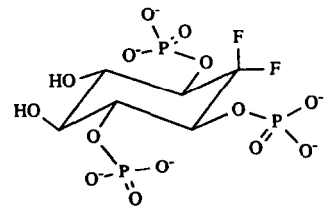
Phosphoinositides are precursors for intracellular second messengers which mediate a variety of biochemical and physiological processes, including muscle contraction, carcinogenesis and protein synthesis. These events are activated by external messengers, e.g., hormones, growth factors, and neurotransmitters, which bind to external receptors on the target cells.[1] Information is then transmitted through the cell membrane by a series of membrane-bound G-proteins which activate a specific phospholipase C, which then cleaves phosphatidyl inositol 4,5-bisphosphate to Ins(1,4,5)P_3 and a diacylglycerol.[2] The second messenger Ins(1,4,5)P_3, which activates release of intracellular Ca^{++}, is a substrate for a specific 3-kinase, yielding Ins(1,3,4,5)P_4, followed by a 5-phosphatase to give Ins(1,3,4)P_3;[3] phosphatases and kinases also operate on Ins(1,3,4)P_3.[4] Recently, specific high-affinity receptors for Ins(1,4,5)P_3 have been purified from brain tissue[5], but no receptors for the putative second messenger Ins(1,3,4)P_3 have been characterized. Indeed, the lack of a commercial source of high specific activity [^{3}H]-Ins(1,3,4)P_3 is the primary reason that this has not been examined in detail.[6] We describe herein the preparation of both enantiomers of radiolabeled [1-^{3}H]-Ins(1,3,4)P_3 *via* reduction of a suitably protected 1-keto intermediate. A related 2-keto intermediate was also employed for the production of 2-fluorinated 2-deoxy analogs[7] of racemic Ins(1,3,4)P_3 as probes for binding site characterization.



L-1(or D-1)

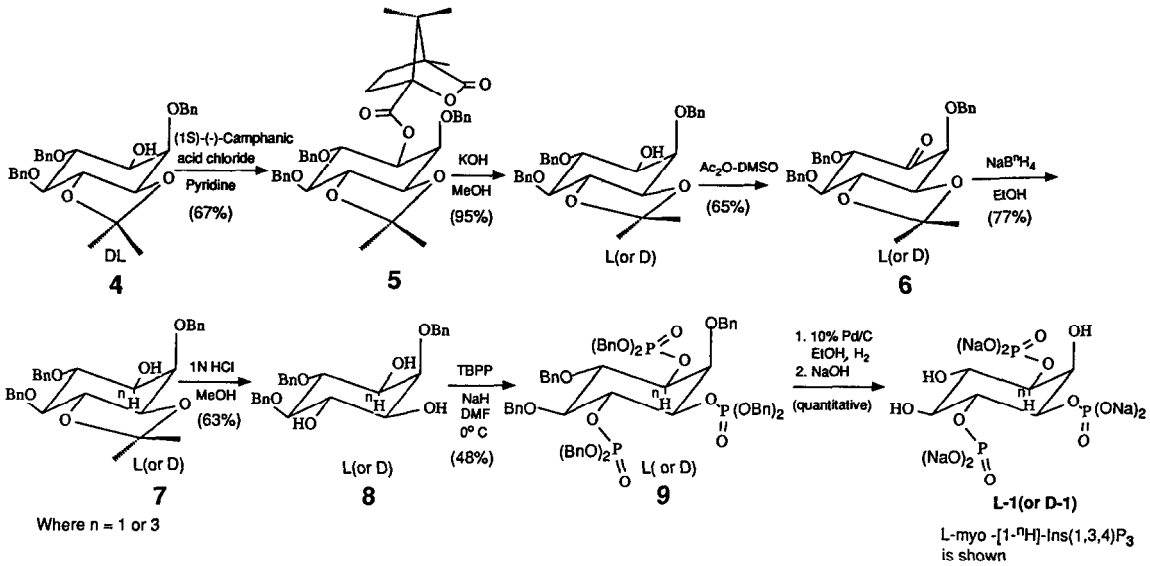


2



3

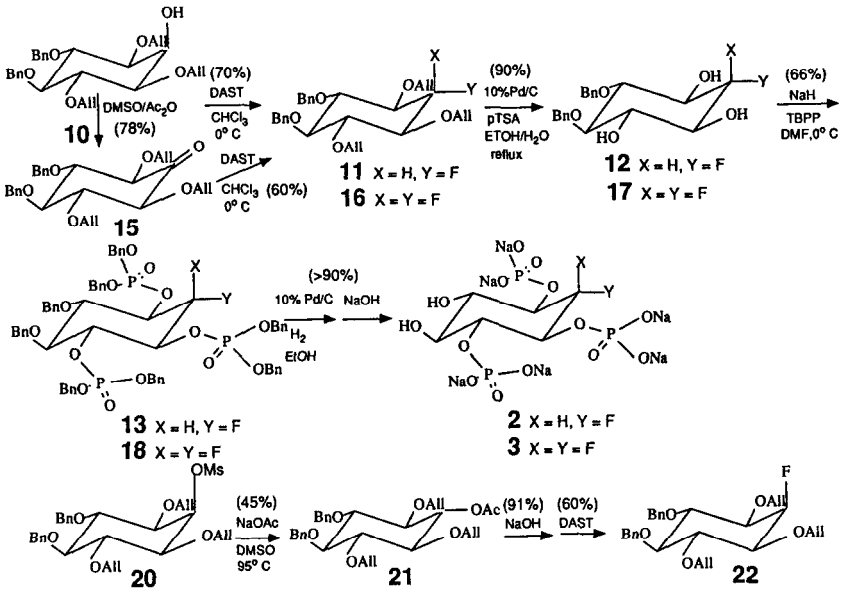
Scheme 1



Scheme 1 summarizes the preparation of the radiolabeled enantiomers of Ins(1,3,4)P_3 (L-*myo* enantiomer is shown). The known 2,4,5-tri-O-benzyl inositol[8] was converted to 2,5,6-tri-O-benzyl-3,4-O-isopropylidene inositol **4** and resolved by separation of the diastereomeric camphanate esters **5** by a combination of HPLC and crystallization.[8] Hydrolysis followed by oxidation (DMSO-Ac_2O) afforded ketone **L-6** (or **D-6**). Reduction with sodium borotritide (65.4 Ci/mmol) in ethanol (20 °C, 2 h) proceeded to give the [1-^{3}H]-alcohol **L-7** (or **D-7**) in a 3:1 eq:ax ratio[9], and the desired equatorial alcohol was deketalized to give the triol **L-8** (or **D-8**). The trianion (NaH, DMF, 0 °C) of triol **8** was treated with tetrabenzylpyrophosphate to give the perbenzylated species **L-9** (or **D-9**), and hydrogenation followed by titration to pH 9 with NaOH afforded the hexasodium salt **L-1** (or **D-1**). ^{31}P-NMR of the unlabeled compound **L-1** (or **D-1**) showed three peaks at the reported chemical shifts[6] for the three nonequivalent phosphates. Optical rotations[6] of the Ins(1,3,4)P_3 sodium salts (c (g/100 mL) = 3.4, H_2O) were $[\alpha]_D^{22}$ +7.5° (L-*myo*) and $[\alpha]_D^{22}$ -7.2° (D-*myo*). Using the assay procedures[5,10a] which demonstrated specific binding of [^{3}H]-Ins(1,4,5)P_3 and [^{3}H]-Ins(1,3,4,5)P_4 to membrane receptor proteins in rat brain, no specific binding (i.e., displaceable by Ins(1,3,4,)P_3 or Ins(1,3,4,5)P_4) of either [^{3}H]-**D-1** or [^{3}H]-**L-1** to rat forebrain or cerebellum proteins could be detected.[10b]

The synthesis of fluorodeoxy analogs is illustrated in Scheme 2. Reaction of protected alcohol **10** with DAST in $CHCl_3$ at 0 °C resulted in mono-fluorination to give **11**. Removal of allyl groups of **11** with 10% Pd/C and *p*-TsOH in EtOH gave the triol **12** as a white solid; spectral data was consistent with the expected equatorial fluorine.[11] The trianion of **12** (NaH, DMF, 0 °C) was treated with tetrabenzyl pyrophosphate to give the perbenzylated precursor **13**.[12] Debenzylation (H_2, 10% Pd/C, EtOH) and

Scheme 2



titration of the filtrate with 1N NaOH gave the deprotected mono-fluorinated phosphate as the hexasodium salt **2** in quantitative yield.[13] ^{19}F-NMR and ^{31}P-NMR indicated the presence of the 2-fluorine and three nonequivalent phosphates. The geminal coupling ($^{2}J_{FH}$ = 52 Hz) and small vicinal coupling ($^{3}J_{FH}$ = 13 Hz) supports the assignment of the fluorine to the expected equatorial position.

In order to confirm the configuration of the fluorine atom, we prepared the axial C-2 fluorine analog by the S_N2 reaction of the 2-mesylate **20** with NaOAc/DMSO followed by basic hydrolysis.[14] Fluorination with DAST led to the protected compound **22**, which could not be selectively deprotected using 10% Pd/C, *p*-TsOH. Isomerization of the allyl ether with $RhCl(Ph_3P)_3$ followed by HCl hydrolysis also failed to provide a homogeneous product. Nonetheless, the ^{19}F-NMR of **22** showed $^{2}J_{FH}$ = 52 Hz and a vicinal coupling of $^{3}J_{FH}$ = 28 Hz, allowing assignment of the axial fluorine substituent.

The 2,2-difluoro-2-deoxy-inositol triphosphate analog was synthesized by oxidation of the alcohol **10** with DMSO/Ac_2O, followed by reaction of ketone **15** with 2 equivalents of DAST at ambient temperature for one day, which gave the difluoro compound **16**. Deprotection of the allyl groups with 10% Pd/C and *p*-TsOH in refluxing EtOH afforded triol **17**; phosphorylation (to **18**), reductive debenzylation, and titration with NaOH gave the 2,2-difluoro-2-deoxy-Ins(1,3,4)P_3 as the hexasodium salt **3**. ^{19}F-NMR and ^{31}P-NMR confirmed the presence of two fluorines with geminal coupling of 257 Hz and three nonequivalent phosphates.[13]

Acknowledgments. We thank the New York State Center for Science and Technology and the Center for Biotechnology for support of this work. The generous advice and guidance of Dr. J.F. Marecek (State University of New York at Stony Brook) in synthetic design and the encouragement and biological assays provided by Mr. S. Supattapone and Dr. S.H. Snyder (Johns Hopkins University Medical School) are greatly appreciated.[15]

References

1. (a) Berridge, M., *Ann. Rev. Biochem.*, **1987** *56*, 159-193; (b) Berridge, M., Nature, **1984** *312*, 315-312.

2. Majerus, P.W., Connolly, T.M., Bansal, V.S., Inhorn, R.C., Ross, T.S., Lips, D.L., *J. Biol. Chem.* **1988**, *263*, 3051-3054.

3. Shears, S.B., Storey, D.J., Morris, A.J., Cubitt, A.B., Parry, J.B., Michell, R.H., and Kirk, C.J. *Biochem. J.* **1987**, *242*, 393-402.

4. (a) Balla, T., Guillemette, G., Baukal, A.J., and Catt, K.J., *J. Biol. Chem.* **1987**, *262*, 9952-9955; (b) Bansal, V.S., Inhorn, R.C., and Majerus, P.W. *J. Biol. Chem.* **1987**, *262*, 9444-9447.

5. (a) Willcocks, A.L., Cooke, A.M., Potter, B.V.L., and Nahorski, S.R. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 1071-1078; (b) Supattapone, S., Worley, P.F., Baraban, J.M., and Snyder, S.H. *J. Biol Chem.* **1988**, *263*, 1530-1534.

6. (a) Synthesis of unlabeled racemic Ins(1,3,4)P_3: deSolms, S.J., Vacca, J.P., and Huff, J.R. *Tetrahedron Lett.* **1987**, *28*, 4503-4506; (b) optically active forms, Ozaki, S., Kohno, M., Nakahira, H., Bunya, M., and Watanabe, Y. *Chemistry Lett.* **1988**, 77-80. Absolute stereochemistry assignments were made based upon assignments of Ozaki *et al.*, who reported $[\alpha]_D^{22}$ -6° (c = 0.5, H_2O) for D-*myo* Ins(1,3,4)P_3.

7. Card, P.J. *J. Carbohydrate Chem.*, **1985**, *4*, 451; Welch, J. *Tetrahedron* **1987**, *43*, 2123-3197.

8. (a) Gigg, J., Gigg, R., Payne S., Conant, R., *J. Chem. Soc. Perkin Trans. I*, **1987**, 1757-1762; (b) *Ibid.*, 2411-2414; (c) *Ibid.*, 423-429.

9. The epimeric alcohols are readily separated by SiO_2 chromatography: R_f (1:1 Et_2O-hexanes) = 0.25 (eq), 0.38 (ax).

10. (a) Thiebert, A.B., Supattapone, S., Worley, P.F., Barbaran, J.M., Meek, J.L., and Snyder, S.H., *Biochem. Biophys. Res. Commun.* **1987**, *148*, 1283-1289; (b) Supattapone, S., and Snyder, S.H., personal communication.

11. Compound **12**: ^{19}F-NMR ($CDCl_3$) δ -192.7 (ddd, J = 52 Hz, *J* = 13 Hz, *J* = 13 Hz, F_{eq}); ^{1}H-NMR ($CDCl_3$) δ 2.49 (d, *J* = 2.4 Hz, -OH), 2.57 (bs, -OH), 2.67 (d, *J* = 2.1 Hz, -OH), 3.43-3.83 (m, -CH-), 4.29 (ddd, *J* = 51.9 Hz, *J* = 9.3 Hz, *J* = 9.3 Hz, -CH_{ax}F-), 4.79-4.97 (m, Bz-CH_2-), 4.78-4.97 (m, Bz-H); mp = 161-163 °C. R_f = 0.25 (ether). Compound **17**: ^{19}F-NMR ($CDCl_3$) δ -113.6 (d, *J* = 244 Hz, F_{eq}), -126.5 (ddd, *J* = 244 Hz, *J* = 22 Hz, *J* = 22 Hz, F_{ax}), ^{1}H-NMR ($CDCl_3$) δ 2.32 (d, *J* = 4.2 Hz, -OH), 2.45 (d, *J* = 2.4 Hz, -OH), 2.59 (bs, -OH), 3.43-3.86 (m, -CH-), 4.80-4.98 (m, Bz-CH_2-), 7.36 (bs, Bz-H); mp = 175-177 °C; R_f = 0.23 (ether). ^{31}P chemical shifts are referenced to external 85% H_3PO_4 (δ = 0 ppm) and ^{19}F chemical shifts are referenced to $CFCl_3$ (δ = 0 ppm). Downfield shifts are positive.

12. Compound **13**: ^{19}F-NMR ($CDCl_3$) δ -198.1 (ddd, *J* = 48.7 Hz, F_{eq}); ^{31}P-NMR ($CDCl_3$) δ -0.87, -0.95, -1.53; R_f = 0.29 (ether). Compound **18**: ^{19}F-NMR ($CDCl_3$) δ -110.4 (d, *J* = 248 Hz, F_{eq}), -125.1 (ddd, *J* = 248 Hz, *J* = 20.3 Hz, *J* = 20.3 Hz, F_{ax}); ^{31}P-NMR ($CDCl_3$) δ 5.59, 6.71, 6.99; R_f = 0.30 (ether).

13. Compound **2**: ^{19}F-NMR (D_2O) δ -196.8 (ddd, *J* = 50.5 Hz, *J* = 13.1 Hz, *J* = 13.1 Hz, F_{eq}); ^{31}P-NMR (D_2O) δ 4.15, 5.28, 5.54. LRFAB-MS, 554.2 ± 0.5; calculated for $C_6FH_8Na_6O_{14}P_3$ 553.85. Compound **3**: ^{19}F-NMR (D_2O) δ -108.6 (d, *J* = 247 Hz, F_{eq}), -125.0 (ddd, *J* = 247 Hz, *J* = 21.5 Hz, *J* = 21.5 Hz, F_{ax}), ^{31}P-NMR (D_2O) δ -0.46, -0.52, -1.12. LRFAB-MS, 572.2 ± 0.5; calculated for $C_6F_2H_7Na_6O_{14}P_3$ 571.84.

14. The Mitsunobu reaction (DIAD, Ph_3P, $MeCO_2H$) failed. The S_N2 reaction described is also extremely sluggish, affording 45% yield of acetate **21** in 48 h.

15. The preparation and biological activity of the tritiated and fluorodeoxy analogs of the Ins(1,4,5)P_3 isomers will be described elsewhere: G.D. Prestwich, J.F. Marecek, S. Supattapone, and S.H. Snyder, in preparation.

(Received in USA 28 July 1988)