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SYNTHESIS OF P-5 TETHERED INOSITOL-1,2,6-TRISPHOSPHATE, AN AFFINITY REAGENT FOR α-TRINOSITOL RECEPTORS

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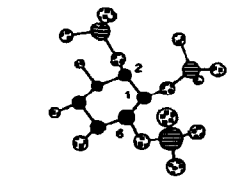
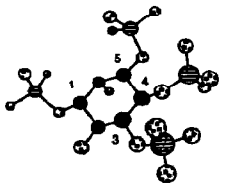
Summary: The synthesis of D-*myo*-P-5-(*O*-aminopropyl)-Ins(1,2,5,6)P_4, a phosphodiester analog of Ins(1,2,6)P_3 tethered at the C-5 position, has been achieved and a photoaffinity label has been prepared.

D-*myo*-Inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) is released from phosphatidylinositol bisphosphate by the action of phospholipase C on phosphatidylinositol 4,5-bisphosphate, and activates calcium release via a tetrameric ion channel.[1] α-Trinositol (Perstorp Pharma, Sweden) is a commercial inositol trisphosphate regioisomer, Ins(1,2,6)P_3, and is produced by partial degradation of phytic acid with phytase. Ins(1,2,6)P_3 inhibited inflammatory reactions and edema in skin burn injury following peripheral administration,[2] and is effective in treating acute abnormalities of nerve function in early experimental diabetes.[3] Although Ins(1,2,6)P_3 acts as a neuropeptide Y (NP-Y) antagonist, it does not act at NP-Y receptors.[4-7] Recent evidence suggested that high-affinity [^{3}H]Ins(1,2,6)P_3 binding can be readily displaced from rat heart membranes by Ins(1,2,5,6)P_4[8a] and by Ins(1,3,4,5)P_4 but not by Ins(1,4,5)P_3.[8b]





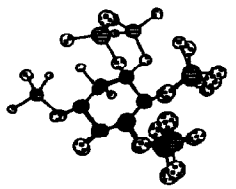
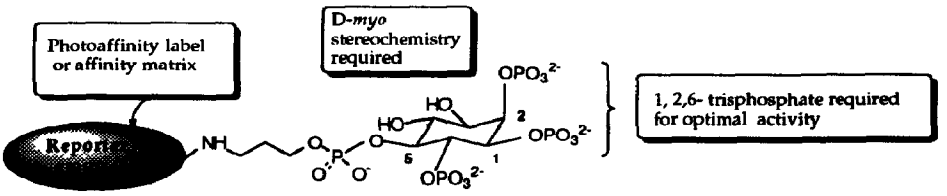


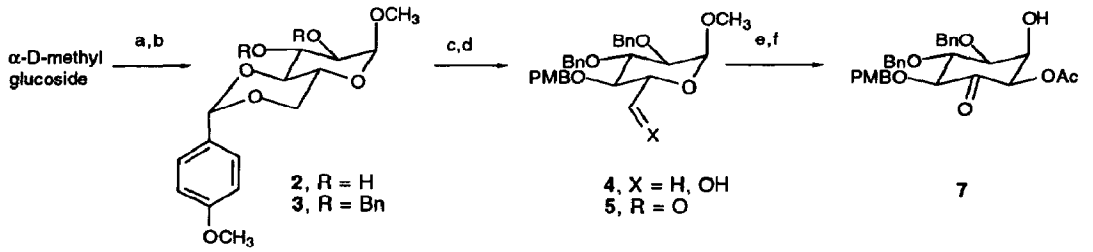
Figure 1. Chem3D representations showing superposition of Ins(1,2,6)P_3 and Ins(1,3,4,5)P_4.

In analogy to the mimicry of Ins(1,4,5)P_3 by Ins(1,2,4,5)P_4,[9] we noted that Ins(1,2,6)P_3 and Ins(1,3,4,5)P_4 can be mapped onto one another (Fig. 1),[10] but that Ins(1,2,5,6)P_4 would exhibit even better superposition. On this basis, we designed an affinity reagent (13) for Ins(1,2,6)P_3 that



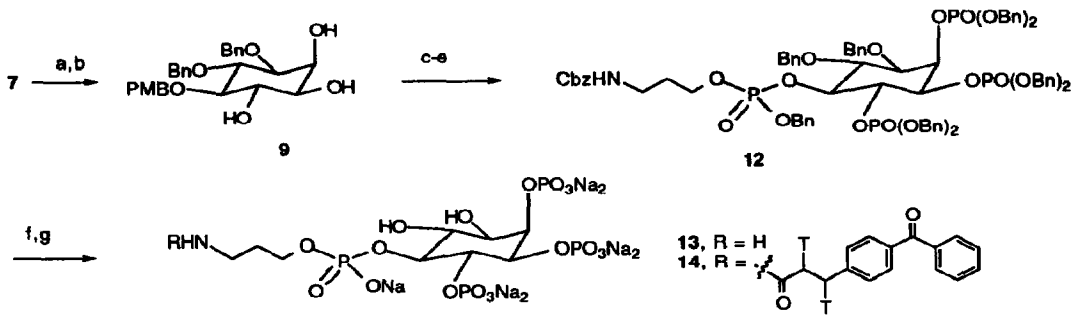
incorporates a P-5-(*O*-aminopropyl) tether. We report herein the synthesis of this probe and its conversion to an affinity resin and a photoaffinity label for isolation and characterization[11-13] of protein targets of α-Trinositol.

The synthesis employed a modified version of the Ferrier rearrangement route initially developed[14] for P-1-tethered Ins(1,3,4,5)P_4, in which a different protecting group pattern was required for regioselective modification at the 5-position. Thus, selective protection of C-4 and C-6 hydroxyls of the methyl-α-D-glucopyranoside as the *O*-(4-methoxy benzylidene) acetal using 4-methoxybenzaldehyde dimethyl acetal **1**, provided methyl 4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside **2**.[15] The C-2 and the C-3 hydroxyls were then benzylated to give acetal **3**, and a regioselective reductive cleavage of the benzylidene acetal was achieved using sodium cyanoborohydride and trimethylsilyl chloride. The resulting methyl 2,3-di-*O*-benzyl-4-*O*-(4-methoxybenzyl)-α-D-glucopyranoside **4**[16,17] was oxidized under Swern conditions[18] to aldehyde **5** (> 90% as hydrate), which was converted (Ac_2O, CH_3CN, K_2CO_3)[19] without purification to the Z-enol acetate **6**. Ferrier rearrangement of the enol acetate in acetone using mercuric acetate and aqueous NaCl[20] furnished the inosose **7**[21] with the desired axial hydroxyl at C-2.



Scheme 1. Reagents: (**a**) **1**, PMB acetal, *p*-TsOH, DMF, 2.5 h, RT, 78%; (**b**) BnBr, NaH, DMF, 2 h, RT, 97%; (**c**) TMSCl, $NaCNBH_3$, CH_3CN, overnight RT, 73%; (**d**) $(COCl)_2$, DMSO, Et_3N, 30 min, -78 °C, 98%; (**e**) Ac_2O, anhyd. K_2CO_3, 80 °C, 8 h, 83%; (**f**) $Hg(OAc)_2$, Me_2CO-H_2O (3:2), NaCl, RT, 22 h, 61%.

Stereoselective reduction of **7** with sodium triacetoxyborohydride[22] to diol **8** followed by methanolysis of the acetate provided the triol **9** with the C-5 position differentially protected from the C-1, C-2, and the C-6 hydroxyls. Phosphitylation of **9** with (dibenzyloxy)diisopropylaminophosphine,[23] followed by *m*CPBA oxidation gave the protected tris(phosphotriester) **10**. After removal of the PMB group with ceric ammonium nitrate[24] and



Scheme 2. Reagents: (a) $NaBH(OAc)_3$, HOAc, Me_3CN, 25 min, RT, 78%; (b) 0.35 M NaOH/MeOH, 80 °C, 90 min, 71%; (c) $iPr_2NP(OBn)_2$, tetrazole, CH_2Cl_2, overnight, RT; then, *m*CPBA, CH_2Cl_2, -40 °C, 2 h, 74%; (d) $(NH_4)_2Ce(NO_3)_2$, $MeCN-H_2O$ (9:1), 80 min, RT, 53%; (e) (N-Cbz-aminopropyloxy)P(OBn)N(iPr_2), tetrazole, 3 h, RT; then *m*CPBA, -40 °C, 15 min, 67%; (f) 10% Pd-C, H_2, 12 h; then Chelex, Na^+ form; (g) 0.25 M TEAB, BZDC-NHS, overnight, RT; then DEAE-cellulose (HCO_3^- form).

condensation of the resulting alcohol **11** with benzyloxy-(*N*-Cbz-3-aminopropyloxy)-diisopropylamino phosphine,[23] oxidation with *m*CPBA furnished the fully protected aminopropyl tethered inositol **12**. Hydrogenolysis removed all of the benzyl groups to provide the optically-active P-5 aminopropyl tethered D-*myo*-Ins-1,2,5,6-P_4 (shown as the heptasodium salt, **13**) in quantitative yield after ion-exchange chromatography (Chelex, sodium form). The photophore was then attached to the free amine by reaction of **13** with *p*-benzoyldihydrocinnamyl (BZDC) *N*-hydroxysuccinimido ester[25] in aqueous DMF furnishing the enantiomerically pure unlabeled (T = ^1H) or tritium labeled (T = ^3H) BZDC-Ins(1,2,5,6)P_4 photolabel **14**. This benzophenone-containing photoaffinity label[26] will be employed for biochemical studies analogous to those which characterized specific Ins(1,3,4,5)P_4 receptors in rat cerebellar membranes.[27,28]

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References

1. Berridge, M.J. *Nature* **1993**, *361*, 315-325.
2. Siren, M.; Linne, L.; Persson, L. In *Inositol Phosphates and Derivatives-Synthesis, Biochemistry and Therapeutic Potential*; A.B. Reitz, Ed.; ACS Series: Washington, DC, 1991; Vol. 463; pp 103-110.
3. Carrington, A.L.; Calcutt, N.A.; Ettlinger, C.B.; Gustafsson, T.; Tomlinson, D.R. *Eur. J. Pharmacol.* **1993**, *237*, 257-263.
4. Donoso, M.V.; Boric, M.; Prado, M.; Fournier, A.; St. Pierre, S.; Edvinsson, L.; Huidobrotoro, J. P. *Eur. J. Pharmacol.* **1993**, *240*, 93-97.
5. Feth, F.; Erdbrugger, W.; Rascher, W.; Michel, M.C. *Life Sci.* **1993**, *52*, 1835-1844.

6. Potter, E. K.; Edvinsson, L.; Gustafsson, T. *Eur. J. Pharmacol.* **1992**, *221*, 307-314.
7. Wahlestedt, C.; Reis, D. J.; Yoo, H.; Adamsson, M.; Andersson, D.; Edvinsson, L. *Neurosci. Lett.* **1992**, *143*, 123-126.
8. (a) C. Wahlestedt, personal communication; (b) Yoo, H.; Fallgren, B.; Lindahl, A.; Wahlestedt, C. *Eur. J. Pharmacol.* **1994**, *268*, 55-63.
9. Hirata, M.; Narumoto, N.; Watanabe, Y.; Kanematsu, T.; Koga, T.; Ozaki, S. *Molec. Pharmacol.* **1994**, *45*, 271-276.
10. Structures were minimized in SYBYL using the TRIPOS force field with manual parameterization of the pentacovalent phosphorus atoms. Minimized structures were displayed using Chem 3D. Superposition of the 3-D structures showed a slight misalignment of the adjacent phosphates resulting from the change of the axial P-2 phosphate of Ins(1,2,6)P_3 to an equatorial P-5 phosphate in Ins(1,3,4,5)P_4.
11. Prestwich, G.D.; Marecek, J.F.; Mourey, R.J.; Theibert, A.B.; Ferris, C.D.; Danoff, S.K.; Snyder, S.H. *J. Am. Chem. Soc.* **1991**, *113*, 1822-1825.
12. Marecek, J.F.; Estevez, V.A.; Prestwich, G.D. *Carbohydr. Res.* **1992**, *234*, 65-73.
13. Ozaki, S.; Watanabe, Y.; Hirata, M.; Ogasawara, T.; Kanematsu, T.; Yoshida, M. In *Drug Design for Neuroscience*; A. P. Kozikowski, Ed.; Raven Press: New York, 1993; pp 417-434.
14. Estevez, V.A.; Prestwich, G.D. *J. Am. Chem. Soc.* **1991**, *113*, 9885-9887.
15. Johansson, R.; Samuelsson, B. *J. Chem. Soc. Perkin Trans. I* **1984**, 2371-2374.
16. Mikami, T.; Asano, H.; Mitsunobu, O. *Chem. Letters* **1987**, 2033-2036.
17. Stick, R.V.; Tilbrook, D.M.G. *Aust. J. Chem.* **1990**, *43*, 1643-1655.
18. Mancuso, A.J.; Swern, D. *Synthesis* **1981**, 165.
19. Cook, S.L.; Secrist III, J.A. *J. Am. Chem. Soc.* **1979**, *101*, 1554-1564.
20. Ferrier, R.J. *J. Chem. Soc. Perkin Trans. I* **1985**, 1455-1458.
21. Satisfactory spectroscopic and analytical data were obtained for all the compounds. ^{31}P shifts are reported in ppm from 85% phosphoric acid as an external standard. Key data are reported for pivotal intermediates. Compound **4**: ^1H δ ppm (300 MHz in $CDCl_3$): 7.6-7.3 (10H, Ar*H*, m), 7.2 (2H, Ar*H*, d, *J* = 8.5 Hz), 6.9 (2H, Ar*H*, d, *J* = 8.2 Hz), 5.1-4.6 (9H, m), 4.1 (1H, C*H*-OPMB, t), 3.8 (3H, OMe, s), 3.7 (2H), 3.5 (2H), 3.4 (3H, OMe, s). Compound **7**: ^1H δ ppm (250 MHz in $CDCl_3$): 7.4-7.2 (12H, Ar*H*, m), 6.9 (2H, Ar*H*, br d), 4.95-4.7 (4H, ArCH_2, m),4.65 (2H, PMBCH_2, d, *J* = 11.0 Hz), 4.6-4.2 (5H, inositol ring, m), 4.0 (1H, C*H*-OPMB, br t), 3.8 (3H, OMe, s), 2.5 (3H, Ac, s): ^{13}C δ ppm (63 MHz in $CDCl_3$): 198, 170, 159.8, 139.8, 130.0, 129.8, 128.4, 128.1, 127.9, 113.8, 83.1, 77.5, 77.0, 76.5, 76.1, 73.2, 55.2, 42.4, 20.6. Compound **11**: ^1H δ ppm: 7.4-7.2 (40H, Ar*H*, br m), 5.1-4.8 (16H, ArCH_2, m), 4.6-4.2 (6H, ring, m), 2.4, exchangeable OH, *s*), 1.8 (1H, d); ^{31}P δ ppm (101 MHz in $CDCl_3$) 2.0, 1.6, 0.41. Compound **14** (T=^1H): ^1H δ ppm (D_2O): 7.68-7.56 (5H, m), 7.44 (2H, t, *J* = 7.5 Hz), 7.27 (2H, d, *J* = 8.1 Hz), 4.0 (2H, d), 3.6-3.2 (6H, ring, m), 2.8 (2H, t, *J* = 7.5 Hz), 2.4 (2H, t, *J* = 7.5 Hz), 2.0-1.6 (6H); ^{31}P δ ppm (D_2O): 6.0, 4.7, 4.6, 2.8.
22. Evans, D.A.; Chapman, K.T.; Carreira, E.M. *J. Am. Chem. Soc.* **1988**, *110*, 3560-3578.
23. Prestwich, G.D.; Marecek, J.F.; Mourey, R.J.; Theibert, A.B.; Ferris, C.D.; Danoff, S.K.; Snyder, S.H. *J. Am. Chem. Soc.* **1991**, *113*, 1822-1825.
24. Lindh, I.; Hindsgaul, O. *J. Am. Chem. Soc.* **1991**, *113*, 216-223.
25. Olszewski, J. D.; Dormán, G.; Elliott, J.; Prestwich, G. D. *J. Org. Chem.* **1994**, submitted.
26. Dormán, G.; Prestwich, G.D. *Biochemistry* **1994**, *33*, 5661-5673.
27. Theibert, A.B.; Estevez, V.A.; Mourey, R.J.; Marecek, J.F.; Barrow, R.K.; Prestwich, G.D.; Snyder, S.H. *J. Biol. Chem.* **1992**, *267*, 9071-9079.
28. Hammonds-Odie, L.; Burga, C.; Profit, A.; Prestwich, G.D.; Theibert, A.B. *Soc. Neurosci.* (Abstract), in press (1994).

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