Synthesis of DL-6-*O*-(2-Aminoethyl-1-phospho)-*myo*-inositol-1,3,4,5-tetrakisphosphate as a Precursor of Affinity Materials for I(1,3,4,5)P_4 Binding Proteins

Sung-Kee Chung,* Li Min Zhao, and Youngha Ryu

Department of Chemistry, Pohang University of Science and Technology, Pohang 790-784 Korea
Received May 8, 1999

D-*myo*-inositol 1,4,5-trisphoshate [I(1,4,5)P_3] plays a key role in intracelluar signal transduction as a second messenger for receptor mediated mobilization of intracellular calcium ion.[1] One of the major metabolic pathways involves a specific phosphorylation of I(1,4,5)P_3 by 3-kinase to generate D-*myo*-inositol 1,3,4,5-tetrakisphosphates [I(1,3,4,5)P_4], another second messenger that was proposed to mediate the influx of extracellular calcium.[2] The other major metabolic pathway begins with dephosphorylation of I(1,4,5)P_3 by 5-phosphatase to yield I(1,4)P_2. Thus, the interaction of inositol polyphosphates (IP_n) with their receptors and metabolic enzymes has been intensively studied by investigating the structure-activity relationship with IP_n structural analogs.[1] Affinity labels or affinity matrixes which are IP_n analogs tethered with various reporter groups such as photoaffinity label and flourophores, or with immobilizing resins have also been used for probing the active site or specific binding site of their specific cellular targets or for purifying IP_n binding proteins, respectively.[3]

Recently, the preparation of P-1-tethered I(1,3,4,5)P_4 derivatives[4] and their usage for purification and photoaffinity labeling of putative I(1,3,4,5)P_4 binding proteins from rat brain[5] were reported by Prestwich group. Our previous studies on the inhibition of I(1,3,4,5)P_4 binding proteins prepared from both porcine platelets[6] and pig cerebellum[7] have suggested the binding pocket domains as shown in Figure 1. Since some extra space may be available around the C-6 equatorial direction of I(1,3,4,5)P_4 in the proposed binding pocket model of I(1,3,4,5)P_4 receptor (P_{42}^{IP4}), compound **1**, an I(1,3,4,5)P_4 derivative containing aminoethyl tether at 6-position, has been designed and synthesized as a potential precursor of affinity probes for purification and structural studies of the receptor proteins.

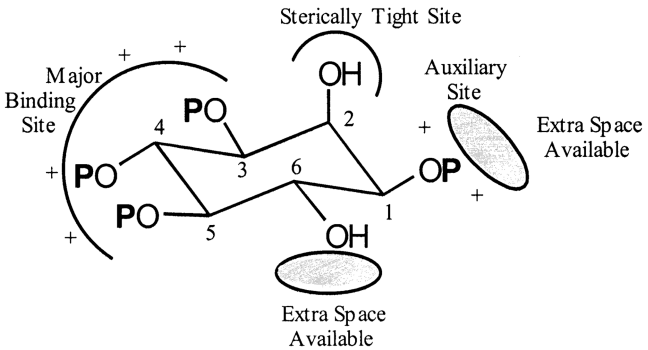
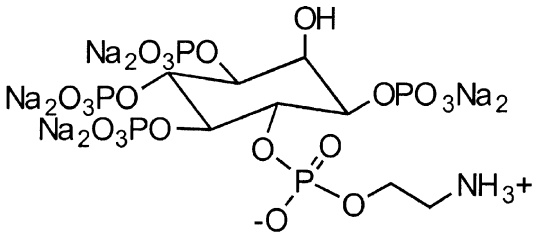


Figure 1. A Proposed binding pocket domain model for I(1,3,4,5)P_4 receptor.



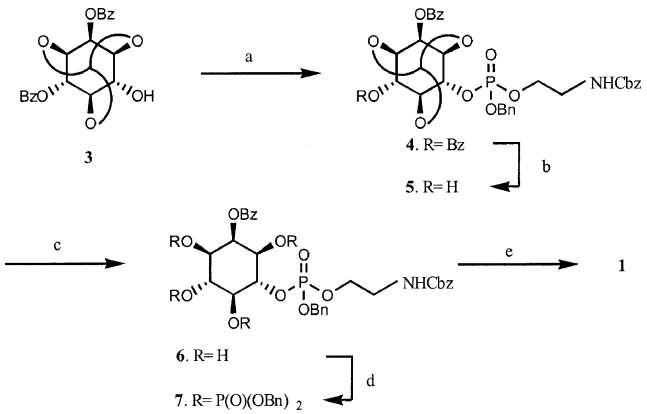
1

Results and Discussion

The selectively protected inositol, DL-2,4-dibenzoyl *myo*-inositol monoorthoformate (**3**) was prepared from *myo*-inositol according to the literature procedure[8]. The phosphoramidite reagent **2** bearing a protected aminoethylgroup was prepared from (benzyloxy)dichlorophosphine in 60% yield by a sequential reaction with diisopropylamine and then *N*-Cbz-2-amino-1-propanol.[9] The reaction of **3** with the phosphoramidite **2** in the presence of 1*H*-tetrazole in CH_2Cl_2 followed by oxidation with mCPBA gave the phosphate diester **4** in 88% yield (Scheme 1). The 4-benzoate group of **4** was selectively hydrolyzed by treatment with NaOMe in MeOH at room temperature to afford **5** in 66% yield after chromatography. Removal of the orthoformate in **5** by heating with p-toluenesulfonic acid in a mixture of MeOH and CH_2Cl_2 gave **6** in 82% yield. A complete phosphitylation of the hydroxyl groups in **6** by dibenzyl *N*,*N*-diisopropylphosphoramidite in the presence of 1*H*-tetrazole in CH_2Cl_2 followed by mCPBA oxidation gave the fully protected 6-*O*-(2-aminopropyl) ester of I(1,3,4,5,6)P_5 derivative **7** in 64% yield. All benzyl and Cbz protecting groups of **7** were removed by hydrogenolysis (50psi H_2) over 10% Pd/C catalyst in ethanol. Subseqent hydrolysis of the remaining benzoate with KOH in hot aqueous methanol, ion exchange on Dowex 50X8-100(H^+) resin, and titration of the resulting protonated phosphates with 1N-NaOH solution to pH 10 afforded the sodium salt of DL-6-*O*-(2-aminoethyl-1-phospho)-*myo*-inositol-1,3,4,5-tetrakisphophate (**1**) in 84% yield. The ^{31}P NMR of **1** clearly showed five resonances at δ 2.10, 5.70, 6.30, 6.78, and 6.84 ppm relative to the external reference of 85% phosphoric acid. This compound is now being used for the purification and the affinity probe of the I(1,3,4,5)P_4 receptor.

Experimental

General. All commercial chemicals were used as



Scheme 1. *Reagents and conditions*: (a) (i) ($CbzNHCH_2CH_2O$)(BnO)PN(i-Pr)$_2$ (**2**), 1*H*-tetrazole, CH_2Cl_2; (ii) mCPBA, -42 °C - r.t., 88%; (b) NaOMe, MeOH, 0 °C - r.t., 66%; (c) p-TsOH, CH_2Cl_2/MeOH, 70 °C, 82%; (d) (i) (BnO)$_2$PN(iPr)$_2$,1*H*-tetrazole, CH_2Cl_2; (ii) mCPBA, -42 °C - r.t., 64%; (e) (i) H_2 (50 psi), 10% Pd/C, EtOH; (ii) KOH, aq-MeOH, 70 °C; (iii) Dowex 50WX8(H^+); (iv) 1N-NaOH, pH = 10, 84%.

obtained without further purification, except for solvents which were purified and dried by standard methods prior to use. Analytical TLC was carried out on Merck 60 F254 silica gel plate (0.25 mm thickness), and visualization was done with UV light, and/or by spraying with a 5% solution of phosphomolybdic acid followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (70-230 mesh or 230-400 mesh). NMR spectra were recorded on a Bruker AM 300 or DPX 300 spectrometer. Chemical shifts are reported in ppm, and tetramethylsilane and phosphoric acid (85%) were used as internal and external standard for ^{1}H NMR and ^{31}P NMR, respectively. Mass spectra(FAB) were determined on a micromass PLATFORM II.

(Benzyloxy)dichlorophophine[9] was prepared from phosphorous trichloride and benzyl alcohol in 61% yield. The reagent was unstable even at -20 °C and used immediately.

Chloro(*N*,*N*-diisopropylamino)(benzyloxy)phophine.[9] To a solution of (benzyloxy)dichlorophosphine (3,9 g, 0.018 mol) in CH_2Cl_2 (10 mL) at -42 °C was added dropwise a solution of diisopropylamine (3.64 g, 0.036 mol) in CH_2Cl_2 (5 mL). The mixture was allowed to warm up to rt over 1h and stirred for 30 min. The salt precipitated was removed by filtration and washed with CH_2Cl_2 (2 × 3 mL), and combined filtrates were evaporated. The residue was dissolved in diethyl ether (25 mL), and the additional ammonium salt precipated was removed by filtration. Evaporation of the ether filtrate yielded the salt free phophine product (3.8 g, 81%): ^{31}P NMR ($CDCl_3$) δ 184.16; ^{1}H NMR ($CDCl_3$) δ 7.2-7.4 (m, 5H), 4.93 (d, *J* = 8.1 Hz), 3.85 (m, 2H), 1.28 (bs, 6H), 1.26 (bs, 6H). The crude product was used without further purification.

(Benzyloxy)[(*N*-Cbz-2-aminoethyl)oxy](*N*,*N*-diisoproylamino)phosphine (2).[9] To a solution of *N*-Cbz-2-amino-1-ethanol (0.67 g, 3.4 mmol) and diisopropylethylamine (0.88 g, 6.8 mmol) in CH_2Cl_2 (12 mL) at 0 °C was added dropwise a solution of chloro(*N*,*N*-diisopropylamino)(benzyloxy) phosphine (2.25 g, 7.2 mmol) in CH_2Cl_2 (7.5 mL). The solution was stirred at 0 °C for 10 min and at rt for 75 min, diluted with CH_2Cl_2 (30 mL), washed with 10% Na_2CO_3 solution and water, dried over $MgSO_4$, and evaporated to give an oil, which was chromatographed on silica gel. Elution with ethyl acetate-hexane-triethylamine (10 : 20 : 1) afforded the phosphoramidite **2** (1.09 g, 74%) as a colorless oil: ^{31}P NMR ($CDCl_3$) δ 153.2; ^{1}H NMR ($CDCl_3$) δ 7.2-7.4 (m, 10H), 5.19 (bs, 1H), 5.08 (s, 2H), 4.69 (m, 2H), 3.5-3.8 (m, 4H), 3.39 (m, 2H), 1.19 (bs, 6H), 1.16 (bs, 6H).

Benzyl *N*-Cbz-2-amino-1-ethyl 6-(2,4-dibenzoyl-*myo*-inositol-1,3,5-orthoformate) phosphate (4). To a solution of 2,4-dibenzoyl-*myo*-inositol-1,3,5-orthoformate **3**[8] (216 mg, 0.54 mmol) and 1*H*-tetrazole (150 mg, 2.16 mmol) in CH_2Cl_2 (15 mL) was added a solution of **2** (350 mg, 0.81 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at rt for 5 h, cooled to -42 °C, and treated with 3-chloroperoxybezoic acid (300 mg). The mixture was stirred at 0 °C for 30 min and at rt for 30 min. After dilution with CH_2Cl_2 (50 mL) the mixture was washed with 10% Na_2SO_3 solution and saturated $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated to give a crude oil, which was purified by chromatography on silica gel. Elution with ethyl acetate-hexane-triethylamine (50 : 50 : 1) gave the phosphodiester **4** (358 mg, 88%): ^{31}P NMR ($CDCl_3$) δ 1.31, 1.28 (ratio 1 : 1, two diasteromers); ^{1}H NMR ($CDCl_3$) δ 7.1-8.2 (m, 20H), 5.76 (m, 1H, H-4), 5.63 (d, *J* = 1.2 Hz, 1H), 5.53 (m, 1H, H-2), 5.51 (bs, 1H), 5.05 (s, 2H), 5.03 (m, 2H), 4.57-4.45 (m, 3H), 3.98 (m, 2H), 3.27 (m, 2H); MS (FAB) m/z 746 (M^++1).

Benzyl *N*-Cbz-2-amino-1-ethyl 6-(2-benzoyl-*myo*-inostol-1,3,5-orthoformate) phosphate (5). A solution of **4** (1.13 g, 1.6 mmol) and NaOMe (8.6 mg, 1.6 mmol) in MeOH (30 mL) was stirred at 0 °C for 3h, and at rt for 2.5h. Several drops of diluted HCl were added to the solution to adjust its pH to 7. The solution was diluted with water (80 mL) and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on silica gel and elution with ethyl acetate-hexane-triethylamine (20 : 20 : 1) afforded the 4-debenzoylated product **5** (640 mg, 66%): ^{31}P NMR ($CDCl_3$) δ 0.56, 0.27 (ratio 1 : 2; two diasteromers); ^{1}H NMR ($CDCl_3$) δ 7.2-8.2 (m, 15H), 5.52 (m, 1H), 5.50 (m, 1H, H-2), 5.07 (s, 2H), 5.00 (m, 1H), 4.58 (bs, 1H), 4.3-4.4 (m, 3H), 4.10-4.15 (m, 2H), 3.15-3.35 (m, 2H).

Benzyl *N*-Cbz-2-amino-1-ethyl 4-(2-benzoyl-*myo*-inositol) phosphate (6). A mixture of **5** (260 mg, 0.035 mmol) and *p*-toluenesulfonic acid monohydrate (100 mg) in MeOH (8 mL) and CH_2Cl_2 (2 mL) was stirred at 70 °C for 4.5h. The solution was cooled down to ambient temperature and evaporated. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated $NaHCO_3$ solution and water. The organic layer was dried over $MgSO_4$, filtered and evaporated to give **6** (210 mg, 82%) as a colorless oil: ^{31}P NMR (Acetone-d_6) δ 2.26, 2.24 (1 : 1); ^{1}H NMR (Acetone-d_6) δ 8.05-7.28 (m, 15H), 5.77 (t, *J* = 2.8 Hz, H-2), 5.05-5.19 (m, 2H), 5.05 (s, 2H), 5.03 (m, 1H), 4.84 (m, 1H), 4.63 (bs, 1H), 4.28

(d, 1H), 4.13-4.18 (m, 2H), 3.98-4.02 (m, 1H), 3.82-3.90 (m, 2H), 3.58 (m, 1H), 3.39 (m, 2H); MS (FAB) m/z 632 (M^++1).

Benzyl *N*-Cbz-2-amino-1-ethyl 6-[2-benzyl-1,3,4,5-tetrakis(dibenzylphospho)-*myo*-inositol] phosphate (7). To a solution of **6** (180 mg, 0.29 mmol) in CH_2Cl_2 were added dibenzyloxy-(*N*,*N*-diisopropylamino)-phosphine (860 mg, 2.5 mmol) and 1*H*-tetrazole (217 mg, 3.1 mmol). The mixture was stirred at rt for 8 h, cooled to 42 °C, and treated with 3-chloroperoxybenzoic acid (1.0 g). The mixture was stirred at -42 °C for 30 min, 0 °C for 1h, and at rt for 30 min. After addition of CH_2Cl_2 (20 mL), the mixture was washed with 10% Na_2SO_3 solution (2×15 mL), saturated $NaHCO_3$ solution (2×15 mL) and water (20 mL), dried over $MgSO_4$ and evaporated to give a crude oil, which was chromatographed on silica gel to afford the product **7** (314 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 3.25 (m, 2H), 3.89 (m, 2H), 4.32-4.94 (m, 5H), 4.90-5.11 (m, 18H), 6.19 (bs, 1H), 7.10-8.05 (m, 55H); ^{31}P NMR ($CDCl_3$) δ 0.47, 0.70, 0.81, 0.85, 0.99, 1.07, 1.15, 1.18, 1.30, 1.43 (two diasteromers of about equal amount); MS (FAB) m/z 1672 (M^++1)

DL-6-O-(2-aminoethyl-1-phospho)-*myo*-inositol-1,3,4,5-tetrakisphosphate (1). The mixture of **7** (100 mg, 0.06 mmol) and 10% Pd/C (61 mg) in ethanol (50 mL) was shaken on a Parr apparatus under H_2 (50 psi) for 15 h. The mixture was filtered and the precipitate was washed with 50% aqueous ethanol. The combined filtrate and washing were adjust to pH 8.0 with a few drops of concentrated ammonium hydroxide and evaporated. The residue was dissolved in 50% aqueous MeOH (4 mL), and 1N-KOH solution (2 mL) was added. The resulting solution was heated at 60 °C for 4.5 h, cooled to the ambient temperature. The aqueous solution was loaded on Dowex 50WX8-100(H^+) and eluted with water. The strongly acidic fractions were combined, washed with CH_2Cl_2 (2×15 mL), lyophilized to give an oil (32 mg, 84%). The residue was dissolved in water (2 mL), adjusted to pH 10 with aqueous 1N-NaOH solution, and lyophilized to give the sodium salt of **1**: ^{1}H NMR (D_2O) δ 2.92 (t, J = 5.1 Hz, 2H), 4.02 (m, 2H), 4.31 (bs, 1H), 4.33 (d, J = 12.9 Hz), 4.49-4.72 (m, 3H), 4.76 (m, 1H); ^{13}C NMR (D_2O) δ 41.16, 64.63, 67.22, 71.58, 73.77, 74.08, 74.17, 75.81; ^{31}P NMR (D_2O) δ 2.10, 5.70, 6.30, 6.78, 6.84 (equal intensities).

Acknowledgment. This work was supported by Korea Science and Engineering Foundation/Center for Biofunctional Molecules and the Ministry of Education/Basic Science Institute Fund (98-3437).

References

1. (a) Berridge, M. J. *Nature* **1993**, *361*, 315. (b) Potter, B. V. L.; Lampe, D. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1933, and references therein.
2. Irvine, R. F.; Cullen, P. J. *Current Biology* **1993**, *3*, 540.
3. Prestwich, G. D. *Acc. Chem. Res.* **1996**, *29*, 503.
4. Estevez, V. A.; Prestwich, G. D. *J. Am. Chem. Soc.* **1991**,*113*, 9885.
5. Theibert, A. B.; Estevez, V. A.; Ferris, C. D.; Danoff, S. K.; Barrow, R. K.; Prestiwich, G. D.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 3165.
6. Cullen, P. J.; Chung, S. K.; Chang, Y. T.; Dawson, A. P.; Irvine, R. F. *FEBS Letters* **1995**, *358*, 240
7. Stricker, R.; Chang, Y. T.; Chung, S. K.; Reiser, G. *Biochem. Biophys. Res. Commun.* **1996**, *228*, 596.
8. (a) Chung, S. K.; Chang, Y. T.; Lee, J. W.; Ji, Y. K. *Korean J. Med. Chem.* **1997**, *7*, 82. (b) Banerjee, T.; Srikantiah, S. M. *Tetrahedron Lett.* **1994**, *35*, 8053.
9. 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.