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## Synthesis of Fluorescent Phosphatidylinositols Using a Novel Inositol H-Phosphonate

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Abstract: Coupling of 1,2-diradyl-sn-glycerol 5 with the novel inositol *H*-phosphonate derivative, 6-O-benzyl-2,3:4,5-di-O-isopropylidene-myo-inositol *H*-phosphonate (3), gave fluorescent analogs of phosphatidylinositol (PtdIns, 1) and PtdIns(4,5)-bisphosphate (PtdIns(4,5)P<sub>2</sub>, 2). Unlike the corresponding phosphoramidate, 3 was stable at -20 °C for several months, making it a useful intermediate for the synthesis of myo-inositol phospholipids. © 1998 Elsevier Science Ltd. All rights reserved.

Various phosphoramidites have been used to phosphitylate protected inositols.<sup>1</sup> However, the application of the *H*-phosphonate methodology, which is based on the  $PCl_3$ /imidazole/triethylamine system and is used widely in nucleic acid chemistry,<sup>2</sup> to the preparation of inositide phospholipids has not been demonstrated. We report here a convenient and rapid route to fluorescent phosphatidylinositols 1 and 2. These can be used in lieu of radioactively labeled lipids in enzymatic assays and biological probes in signaling pathways, avoiding the generation of hazardous waste products. To enhance the stability of 1 and 2, we inserted an ether linkage at the *sn*-1 position of the glycerol backbone, and to render these compounds cell permeant we placed a short-chain ester at the *sn*-2 position.



As outlined in Scheme 1, the fluorescent diradyl glycerol 5 was obtained by using 4-methoxyphenylsn-glycerol<sup>3</sup> 6 as a starting material. Monoalkylation of diol 6 via a di-n-butylstannylene derivative with 4 equiv of 1,12-dibromododecane in the presence of 2 equiv of cesium fluoride in DMF at rt was accomplished in 75% yield. 1-O-( $\omega$ -Bromododecyl)-3-O-(4'-methoxyphenyl)-sn-glycerol 7 was easily separated from its 2-O-(12'-bromododecyl) regioisomer, which was obtained in 25% yield, by column chromatography. Since attempts at removal of the 4-methoxyphenyl group of 7 with ceric ammonium nitrate (CAN) to give diol 8 resulted in a yield of only 25%, we converted alcohol 7 to acetate 9, and then removed the aryl ether with CAN, affording 10 in 86% yield. Basic methanolysis of the acetate group gave 8 in 80% yield. After diol 8 was protected with 2,2-dimethoxypropane, the 2-naphthyl group was introduced in an ether linkage by  $S_N 2$  reaction of bromide 11 with pre-formed 2-naphthoxide ion in DMF at rt, affording 12 in 83% yield. Acid hydrolysis of acetonide 12 (*p*-TsOH, CH<sub>2</sub>Cl<sub>2</sub>) gave diol 13, which reacted with *tert*-butyldimethylsilyl (TBDMS) chloride in DMF in a regioselective manner to give 3-O-silyl ether 14 as the major product. Acylation with caproic anhydride in pyridine/CH<sub>2</sub>Cl<sub>2</sub> in the presence of catalytic DMAP, followed by hydrolysis of silyl ether 15 (10% aq. HCl, EtOH, rt, 1 h), gave 5 without acyl migration, as judged by the <sup>1</sup>H NMR signal of the *sn*-2 proton (CDCl<sub>3</sub>,  $\delta$  4.98 (m, 1H)).

Scheme 1: Synthesis of 1-O-(12'-(2''-naphthyldodecyl))-2-caproyl-sn-glycerol (5)



(i) (a) di-*n*-butyltin oxide, MeOH; (b) CsF, 1,12-dibromododecane, DMF, 75%; (ii) Ac<sub>2</sub>O, DMAP, py, 90%; (iii) CAN, MeCN:H<sub>2</sub>O (3:1), 86%; (iv) K<sub>2</sub>CO<sub>3</sub>, MeOH, 80%; (v) 2,2-dimethoxypropane, *p*-TsOH, 100%; (vi) 2-naphthol, K<sub>2</sub>CO<sub>3</sub>, DMF, 83%; (vii) *p*-TsOH, CH<sub>2</sub>Cl<sub>2</sub>, 82%; (viii) TBDMSCl, imidazole, DMF, rt, 86%; (ix) caproic anhydride, DMAP, py, CH<sub>2</sub>Cl<sub>2</sub>, rt, 100%; (x) 10% aq. HCl, EtOH, rt, 1 h, 96%.

The key differentially protected *myo*-D-inositol derivative (-)-6-O-benzyl-2,3:4,5-di-O-isopropylidene*myo*-inositol (4)<sup>4</sup> is easily converted into the corresponding *H*-phosphonate 3 with PCl<sub>3</sub> and imidazole in the presence of triethylamine in 67% yield (Scheme 2). Activation of the triethylammonium salt of 3 with 5,5dimethyl-2-oxo-2-chloro-1,3,2-dioxophosphorinane (NPCl)<sup>5</sup> and coupling with fluorescent diradyl-*sn*-glycerol 5 gave phosphite diester 16 in 85% yield. Oxidation of 16 to 17, then deprotection of the acetal and benzyl groups, gave 1, which was purified by chromatography on a carboxymethyl cellulose (CM52) column (elution with a CHCl<sub>3</sub>/MeOH gradient).<sup>6</sup>

*H*-Phosphonate 16 was also converted into PtdIns(4,5)P<sub>2</sub> analog 2, as outlined in Scheme 3. Selective deprotection<sup>4</sup> of the *trans*-isopropylidene group of 16 afforded diol 18, which on reaction with dibenzyl N,N-

Scheme 2: Synthesis of fluorescent PtdIns analog 1



(i) (a) PCl<sub>3</sub>, imidazole, Et<sub>3</sub>N, -15 °C, 80 min; (b) Et<sub>3</sub>NH<sup>+</sup> HCO<sub>3</sub><sup>-</sup>, 67% overall; (ii) 5, NPCl (1.08 equiv), py, 15 min, rt; (iii) I<sub>2</sub> (2 equiv), py/H<sub>2</sub>O (98:2), 5 min, rt, 85%; (iv) (a) PPTS (0.5 equiv), ethylene glycol, CHCl<sub>3</sub>, reflux, 14 h; (b) 10% Pd/C, H<sub>2</sub>, EtOH, 100%.

diisopropylphosphoramidate and 1*H*-tetrazole, followed by *in situ* oxidation with mCPBA, gave the corresponding 4,5-bis-dibenzyl phosphate 19 in 80% yield (Scheme 3).

Scheme 3: Synthesis of fluorescent phosphatidylinositol 4,5-bis-phosphate analog 2



(i) PPTS, ethylene glycol,  $CH_2Cl_2$ , 67%; (ii) (a)  $(BnO)_2PN(Pr-i)_2$ , (3 equiv), 1*H*-tetrazole (5.9 equiv), rt, 2 h,  $CH_2Cl_2$ ; (b) mCPBA, -40 °C to rt, 80%; (iii) I<sub>2</sub> (1.1 equiv), py/H<sub>2</sub>O (98:2), rt, 20 min, 88%; (iv) 0.1 M HCl,  $CH_2Cl_2$ , 55%; (v) 10% Pd/C,  $H_2$ , EtOH/H<sub>2</sub>O (4:1), 24 h, 83% (see footnote 8 for conditions to avoid overhydrogenation).

Surprisingly, the *H*-phosphonate group was not oxidized under these conditions.<sup>7a</sup> A second oxidation with  $I_2$ /water/pyridine was required to obtain phosphodiester **20**.<sup>7b</sup> Acid hydrolysis (0.1 M HCl, CH<sub>2</sub>Cl<sub>2</sub>) of the remaining isopropylidene group in **20** and catalytic hydrogenolysis of the benzyl functions<sup>8</sup> gave the final product **2**.<sup>9</sup> HPLC analysis<sup>10</sup> showed similar elution profiles for **2** and for a radiolabeled PtdIns(4,5)P<sub>2</sub>.<sup>11</sup>

In conclusion, fluorescent PtdIns derivatives have been prepared using stable *H*-phosphonate 3 as an intermediate. The fluorophore was inserted via an ether linkage at the end of the *sn*-1 chain of the diradyl glycerol moiety by displacement of Br<sup>-</sup> from  $\omega$ -bromoalkyl ether 11 using  $\beta$ -naphthoxide ion in DMF.

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## **References and Notes**

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- All compounds gave satisfactory analytical and spectroscopic data. 1: R<sub>f</sub> 0.19 (CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 65:25:4); <sup>1</sup>H NMR (CDCl<sub>3</sub>/CD<sub>3</sub>OD/D<sub>2</sub>O 100:15:1) δ 0.85 (t, J = 6.6 Hz, ω-CH<sub>3</sub>), 1.25 (br s, (CH<sub>2</sub>)<sub>12</sub>), 1.40-2.00 (m, CH<sub>2</sub>CH<sub>2</sub>O, CH<sub>2</sub> tetrahydronaphthyl), 2.11 (t, J = 7.4 Hz, C(O)CH<sub>2</sub>), 3.29-3.33 (m, CH tetrahydronaphthyl), 3.35-3.40 (m, CH<sub>2</sub>O), 3.68-3.85 (m, H-1, H-3, H-5, H-6 inositol), 3.94-3.97 (m, CH<sub>2</sub>O), 4.14-4.37 (m, H-2 inositol, CH<sub>2</sub>O), 4.93 (m, CHOC(O)), 7.08-7.43 (m, arom. tetrahydronaphthyl), 7.61-7.73 (m, arom. tetrahydronaphthyl); <sup>31</sup>P NMR δ -0.948; HR-FABMS [M+H]<sup>+</sup>: Calcd. for C<sub>37</sub>H<sub>58</sub>O<sub>13</sub>P: 741.3615. Found: 741.3612; HR-FABMS [M+H]<sup>-</sup>: Calcd. for C<sub>37</sub>H<sub>62</sub>O<sub>13</sub>P: 745.39. Found: 745.32; Calcd. for C<sub>37</sub>H<sub>58</sub>O<sub>13</sub>P: 741.36. Found: 741.27. 2: <sup>1</sup>H NMR (CD<sub>3</sub>OD/D<sub>2</sub>O 12/1) δ 0.86 (t, J = 6.4 Hz, ω-CH<sub>3</sub>), 1.25 (br s, (CH<sub>2</sub>)<sub>12</sub>), 1.40-1.72 (m, CH<sub>2</sub>CH<sub>2</sub>O, CH<sub>2</sub> tetrahydronaphthyl), 2.32 (t, J = 7.2 Hz, C(O)CH<sub>2</sub>), 2.62-2.64 (m, CH<sub>2</sub> tetrahydronaphthyl), 3.36-3.60 (m, H-3, CH<sub>2</sub>O, CH tetrahydronaphthyl), 3.85-4.03 (m, H-1, H-5, H-6 inositol, CH<sub>2</sub>O), 4.14-4.20 (m, H-2 inositol, CH<sub>2</sub>O), 5.14 (m, CHOC(O)), 6.52-6.87 (m, arom. tetrahydronaphthyl), 7.33-7.93 (m, arom. tetrahydronaphthyl); <sup>31</sup>P NMR δ 0.759, 4.19, 4.77; HR-FABMS [M+Na]<sup>+</sup>: Calcd. for C<sub>37</sub>H<sub>65</sub>O<sub>19</sub>P<sub>3</sub>Na: 929.3231. Found: 929.3198.
- 10. 2: HPLC: Alltech Econosphere NH<sub>2</sub> column (5  $\mu$ m) (4.6 x 250 mm); solvent A: CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O (20:9:1, v/v/v); solvent B: solvent A with 0.6 M NH<sub>4</sub>OAc; flow rate: 1.8 mL/min. Elution: (1) 100% A for 2.5 min; (2) 100% A to 100% B, linear gradient for 13 min; (3) 100% B for 11 min;  $R_t$  22 min; detection 280 nm.
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