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

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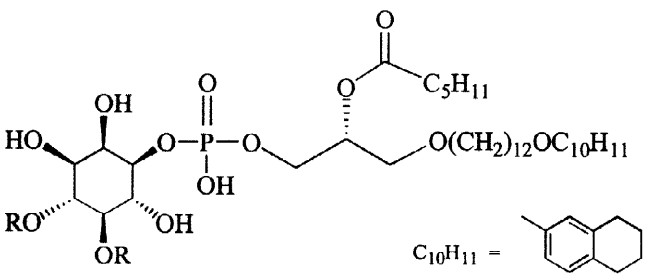
Abstract: Coupling of 1,2-diradyl-*sn*-glycerol **5** with the novel inositol *H*-phosphonate derivative, 6-*O*-benzyl-2,3:4,5-di-*O*-isopropylidene-*myo*-inositol *H*-phosphonate (**3**), gave fluorescent analogs of phosphatidylinositol (PtdIns, **1**) and PtdIns(4,5)-bisphosphate (PtdIns(4,5)P_2, **2**). 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.

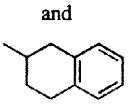
Various phosphoramidites have been used to phosphitylate protected inositols.[1] However, the application of the *H*-phosphonate methodology, which is based on the PCl_3/imidazole/triethylamine system and is used widely in nucleic acid chemistry,[2] to the preparation of inositide phospholipids has not 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.



1, R = H
2, R = PO_3H_2

$C_{10}H_{11}$ =

and



As outlined in Scheme 1, the fluorescent diradyl glycerol **5** was obtained by using 4-methoxyphenyl-*sn*-glycerol[3] **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*-(ω-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

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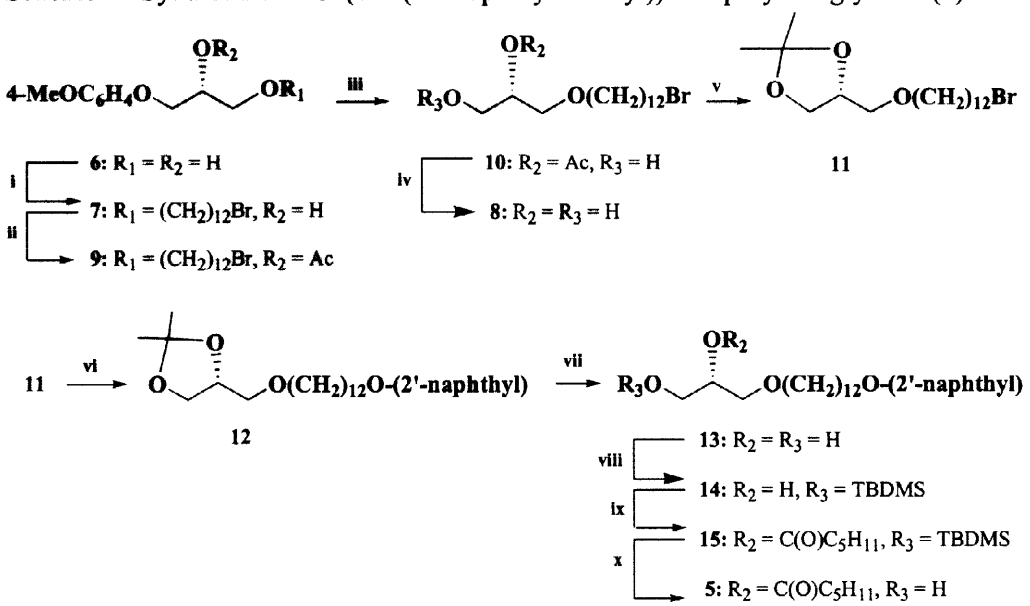
CAN, affording **10** in 86% yield. Basic methanolysis of the acetate group gave **8** in 80% yield. After diol **8** was protected with 2,2-dimethoxypropane, the 2-naphthyl group was introduced in an ether linkage by S_N2 reaction of bromide **11** with pre-formed 2-naphthoxide ion in DMF at rt, affording **12** in 83% yield. Acid hydrolysis of acetonide **12** (*p*-TsOH, CH_2Cl_2) 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_2Cl_2 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 ^1H NMR signal of the *sn*-2 proton ($CDCl_3$, δ 4.98 (m, 1H)).

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

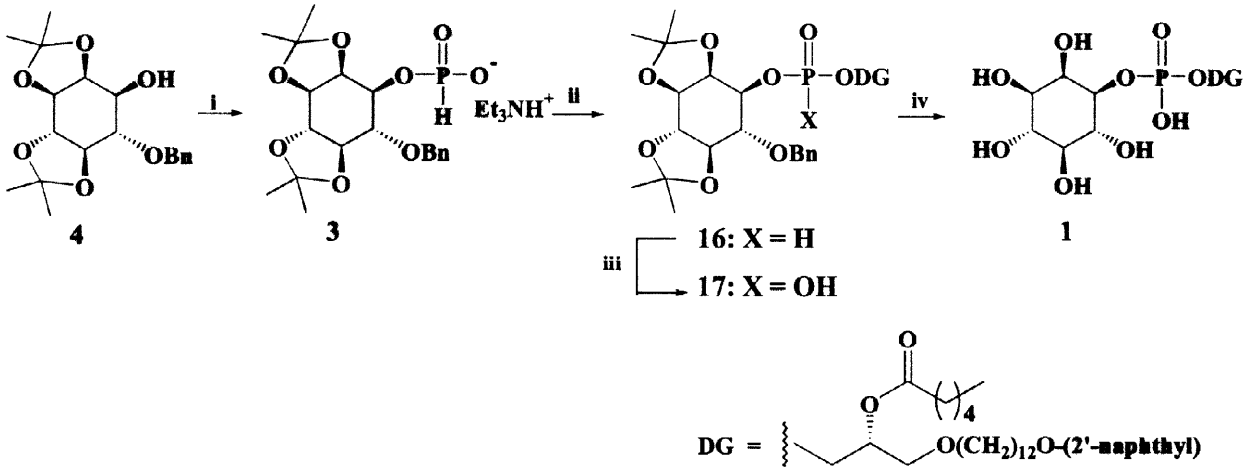


(i) (a) di-*n*-butyltin oxide, MeOH; (b) CsF, 1,12-dibromododecane, DMF, 75%; (ii) Ac_2O, DMAP, py, 90%; (iii) CAN, MeCN:H_2O (3:1), 86%; (iv) K_2CO_3, MeOH, 80%; (v) 2,2-dimethoxypropane, *p*-TsOH, 100%; (vi) 2-naphthol, K_2CO_3, DMF, 83%; (vii) *p*-TsOH, CH_2Cl_2, 82%; (viii) TBDMSCl, imidazole, DMF, rt, 86%; (ix) caproic anhydride, DMAP, py, CH_2Cl_2, 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**)[4] is easily converted into the corresponding *H*-phosphonate **3** with PCl_3 and imidazole in the presence of triethylamine in 67% yield (Scheme 2). Activation of the triethylammonium salt of **3** with 5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxophosphorinane (NPCl)[5] 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 $CHCl_3$/MeOH gradient).[6]

H-Phosphonate **16** was also converted into PtdIns(4,5)P_2 analog **2**, as outlined in Scheme 3. Selective deprotection[4] of the *trans*-isopropylidene group of **16** afforded diol **18**, which on reaction with dibenzyl *N,N*-

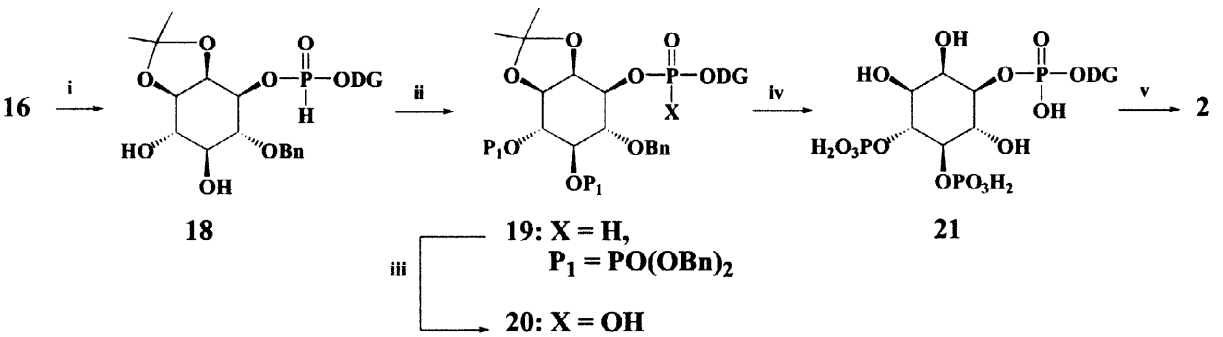
Scheme 2: Synthesis of fluorescent PtdIns analog 1



(i) (a) PCl_3, imidazole, Et_3N, -15 °C, 80 min; (b) $Et_3NH^+ HCO_3^-$, 67% overall; (ii) **5**, NPCl (1.08 equiv), py, 15 min, rt; (iii) I_2 (2 equiv), py/H_2O (98:2), 5 min, rt, 85%; (iv) (a) PPTS (0.5 equiv), ethylene glycol, $CHCl_3$, reflux, 14 h; (b) 10% Pd/C, H_2, 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) $(BnO)_2PN(Pr\text{-}i)_2$, (3 equiv), 1*H*-tetrazole (5.9 equiv), rt, 2 h, CH_2Cl_2; (b) mCPBA, -40 °C to rt, 80%; (iii) I_2 (1.1 equiv), py/H_2O (98:2), rt, 20 min, 88%; (iv) 0.1 M HCl, CH_2Cl_2, 55%; (v) 10% Pd/C, H_2, EtOH/H_2O (4:1), 24 h, 83% (see footnote 8 for conditions to avoid overhydrogenation).

Surprisingly, the *H*-phosphonate group was not oxidized under these conditions.[7a] A second oxidation with I_2/water/pyridine was required to obtain phosphodiester **20**.[7b] Acid hydrolysis (0.1 M HCl, CH_2Cl_2) of the remaining isopropylidene group in **20** and catalytic hydrogenolysis of the benzyl functions[8] gave the final product **2**.[9] HPLC analysis[10] showed similar elution profiles for **2** and for a radiolabeled PtdIns(4,5)P_2.[11]

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^- from ω-bromoalkyl ether **11** using β-naphthoxide ion in DMF.

Acknowledgments

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References and Notes

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7. (a) This observation, which was noted previously [Brown, D. M.; Hammond, P. R. *J. Chem. Soc.* **1960,** 4229-4232], opens the possibility of introducing a modified phosphodiester (*i.e.*, oxidation with S_8 to produce a phosphorothioate) into this molecule without altering the inositol 4- and 5-phosphate groups. (b) Garegg, P.; Regberg, T.; Stawinski, J.; Strömberg, R. *Nucleosides Nucleotides* **1987,** *6*, 429-432.
8. Unfortunately, catalytic hydrogenolysis of the benzyl group using 10% Pd/C (Aldrich) and H_2 (1 atm) at rt resulted in the unanticipated partial reduction of the naphthyl ring, as evidenced by 4 additional mass units in the HR-FABMS. Catalytic hydrogenation of naphthalene ethers using Pd/C in acidic medium has been reported previously: Jung, A. Ph.D. Dissertation, The City University of New York, 1975. Reduction can be avoided by conducting the debenzylation reaction in the presence of excess $NaHCO_3$ in aq. *tert*-BuOH: Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996,** *61*, 6305-6312; Thum, O.; Chen, J.; Prestwich, G. D. *Tetrahedron Lett.* **1996,** *37*, 9017-9020. Alternatively, $Pd(OH)_2/C$ in *t*-BuOH can be used: Kosikowski, A. P.; Tückmantel, W.; Powis, G. *Angew. Chem. Intl. Ed., Engl.* **1992,** *31*, 1379-1381.
9. All compounds gave satisfactory analytical and spectroscopic data. **1**: R_f 0.19 ($CHCl_3$/MeOH/H_2O, 65:25:4); ^{1}H NMR ($CDCl_3/CD_3OD/D_2O$ 100:15:1) δ 0.85 (t, J = 6.6 Hz, ω-CH_3), 1.25 (br s, $(CH_2)_{12}$), 1.40-2.00 (m, $C\underline{H}_2CH_2O$, CH_2 tetrahydronaphthyl), 2.11 (t, J = 7.4 Hz, $C(O)CH_2$), 3.29-3.33 (m, CH tetrahydronaphthyl), 3.35-3.40 (m, CH_2O), 3.68-3.85 (m, H-1, H-3, H-5, H-6 inositol), 3.94-3.97 (m, CH_2O), 4.14-4.37 (m, H-2 inositol, CH_2O), 4.93 (m, CHOC(O)), 7.08-7.43 (m, arom. tetrahydronaphthyl), 7.61-7.73 (m, arom. tetrahydronaphthyl); ^{31}P NMR δ -0.948; HR-FABMS $[M+H]^+$: Calcd. for $C_{37}H_{58}O_{13}P$: 741.3615. Found: 741.3612; HR-FABMS $[M+H]^-$: Calcd. for $C_{37}H_{62}O_{13}P$: 745.39. Found: 745.32; Calcd. for $C_{37}H_{58}O_{13}P$: 741.36. Found: 741.27. **2**: ^{1}H NMR (CD_3OD/D_2O 12/1) δ 0.86 (t, J = 6.4 Hz, ω-CH_3), 1.25 (br s, $(CH_2)_{12}$), 1.40-1.72 (m, $C\underline{H}_2CH_2O$, CH_2 tetrahydronaphthyl), 2.32 (t, J = 7.2 Hz, $C(O)CH_2$), 2.62-2.64 (m, CH_2 tetrahydronaphthyl), 3.36-3.60 (m, H-3, CH_2O, CH tetrahydronaphthyl), 3.85-4.03 (m, H-1, H-5, H-6 inositol, CH_2O), 4.14-4.20 (m, H-2 inositol, CH_2O), 5.14 (m, CHOC(O)), 6.52-6.87 (m, arom. tetrahydronaphthyl), 7.33-7.93 (m, arom. tetrahydronaphthyl); ^{31}P NMR δ 0.759, 4.19, 4.77; HR-FABMS $[M+Na]^+$: Calcd. for $C_{37}H_{65}O_{19}P_3Na$: 929.3231. Found: 929.3198.
10. **2**: HPLC: Alltech Econosphere NH_2 column (5 μm) (4.6 x 250 mm); solvent **A**: $CHCl_3$/MeOH/H_2O (20:9:1, v/v/v); solvent **B**: solvent A with 0.6 M NH_4OAc; 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.
11. Low, M. G. In *Methods in Inositide Research;* Irvine, R. F., Ed.; Raven Press: New York, 1990, p 145-158.