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Synthesis of a Photoaffinity Analogue of Phosphatidylinositol 3,4-Bisphosphate, an Effector in the Phosphoinositide 3-Kinase Signaling Pathway

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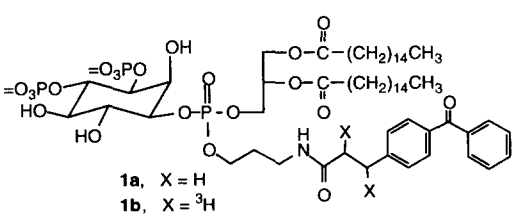
Summary. A Ferrier rearrangement strategy starting from α-D-glucose gave a protected inositol, which after coupling to a chiral diacylglycerol phosphoramidite, provided a tritium-labeled, benzophenone-containing derivative of P-1-(*O*-aminopropyl) linked dipalmitoyl PtdIns(3,4)P_2.

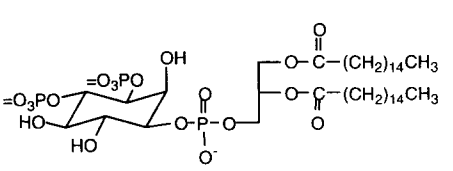
Phosphoinositide polyphosphates are key signaling molecules in cellular communication via protein kinases, in endo- and exocytosis, and in vesicular trafficking of proteins.[1] In particular, the phosphoinositide 3-kinase (PI 3-K)[2] pathway has been linked to mechanisms of oncogene transformation, cytoskeletal rearrangements, membrane association of signaling proteins, and trafficking of proteins by coated vesicles. A number of PI 3-K isozymes have been characterized, and each is a heterodimer comprised of a regulatory 85-kDa domain and a catalytic 110-kDa domain.[2a] PI 3-K catalyzes the phosphorylation of PI(4,5)P_2 to PI(3,4,5)P_3, a second messenger recognized as an effector in the phosphorylation of pleckstrin[3a], the activation of Akt/PKB kinase[3b,c], and as the ligand for centaurin[3d], a brain protein linking extracellular events to cytoskeletal changes. Dephosphorylation of PI(3,4,5)P_3 by 5-kinases leads to PI(3,4)P_2, which has recently been demonstrated to be the ultimate messenger of the PI 3-K signaling pathway in the activation of Akt kinase in platelet membranes.[4]

Recently, we have prepared a variety of affinity probes for isolation and characterization of proteins with specific binding sites for inositol polyphosphates ($InsP_n$s) and their phospholipid counterparts, the phosphoinositide polyphosphates ($PtdInsP_n$s).[5] The PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 affinity probes[6a,b] were prepared by convergent asymmetric synthesis, in which the protected D-*myo*-inositol moiety was derived from α–D-glucose via a Ferrier rearrangement[5,7], while the 3-phosphorylated1,2-*O*-diacyl-*sn*-glyceryl synthon was derived from a commercial chiral precursor. We now describe the extension of the triester approach[6b] to the synthesis of a P-1-(*O*-aminopropyl) linked photoaffinity analog (**1**) of dipalmitoyl PtdIns(3,4)P2 as well as the parent ligand (**19**).

To prepare the D-*myo*-inositol intermediate **12** (Scheme 1), the 2,3-diol of 4,6-benzylidene ketal of methyl α-D-glucopyranoside **2a** was converted to bis-3,4-PMB ether **2b**. Selective cleavage of the benzylidene



1a, X = H
1b, X = ^{3}H



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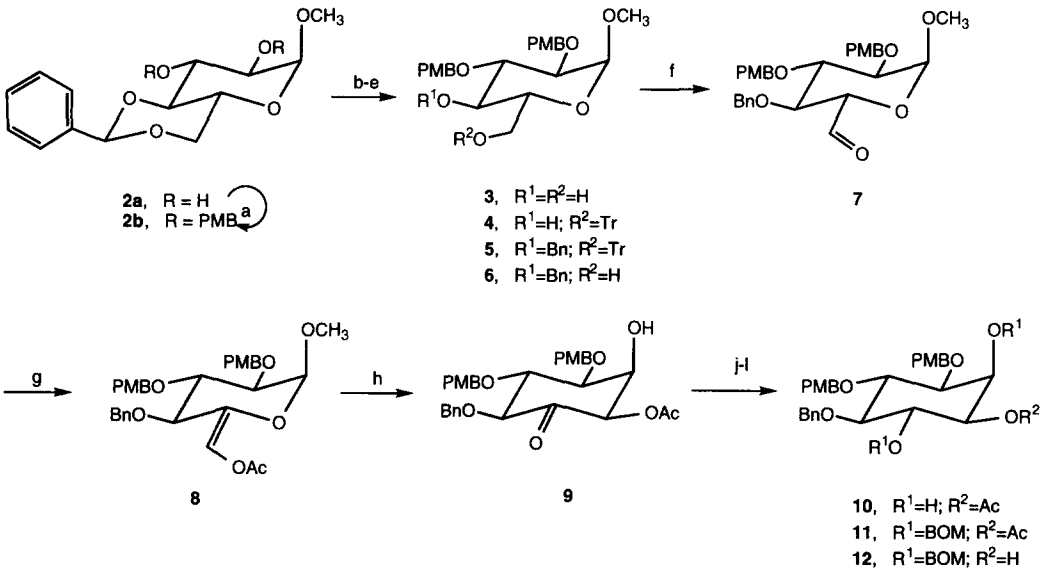
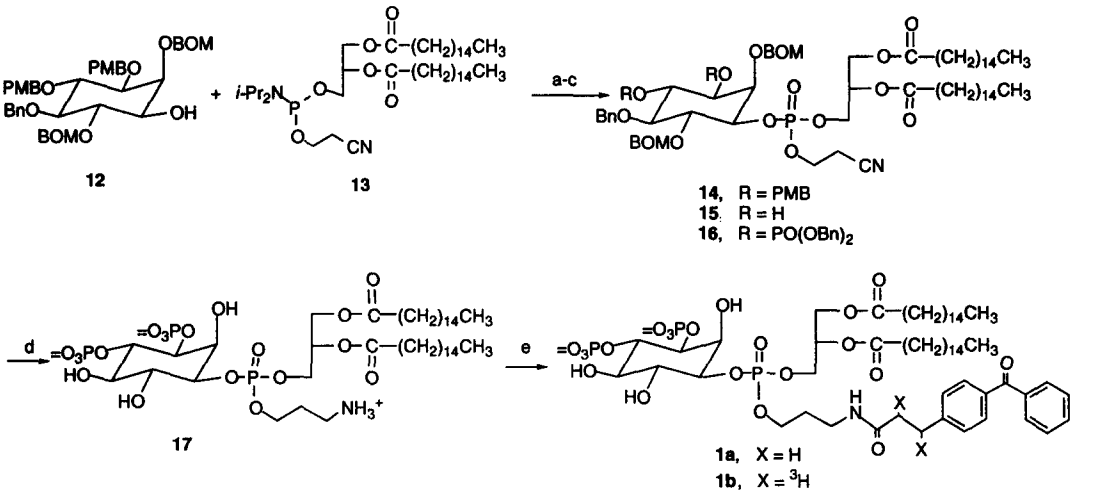
acetal with DIBAL-H[8] gave product **6** only in 25% yield. A better yield of **6** was obtained (40% in four steps) by hydrolysis of the acetal, selective tritylation of the primary hydroxy group, protection of the remaining free hydroxyl group as benzyl ether, and finally detritylation.[9] Enol acetate **8** was synthesized by Swern oxidation, using DMSO/oxalyl chloride[10], followed by treatment with anhydrous potassium carbonate and acetic anhydride. Ferrier rearrangement[7], using 10 equiv. of mercury(II) acetate and sat'd sodium chloride solution gave stereoselective inosose **9** in 63% yield. Selective reduction of the carbonyl with sodium triacetoxyborohydride[11] provided the semi-protected D-*myo*-inositol skeleton **10**. The final intermediate **12** was obtained by protection of



Scheme 1. Preparation of inositol precursor. Reagents and conditions: (**a**) PMBCl, NaH, DMF, 50 °C, overnight; (**b**) *p*-TsOH, MeOH, 2 h, rt; (**c**) TrCl, DMAP, Et_3N, DMF, 11 h, rt.; (**d**) BnBr, NaH, *n*-Bu_4NI, DMF, 2 h, rt; (**e**) 5% H_2SO_4/MeOH, acetone, rt, 50 min; (**f**) $(COCl)_2$, DMSO, CH_2Cl_2, -78 °C, 45 min, Et_3N; then -78 °C to rt, 1 h; (**g**) K_2CO_3, Ac_2O, CH_3CN, reflux, overnight; (**h**) (i) $Hg(OAc)_2$, acetone/water (3:2) rt, 40 min; (ii) sat'd NaCl, rt, 21 h; (**j**) $NaBH(OAc)_3$, AcOH, CH_3CN, 40 min; (**k**) BOMCl, Proton-sponge®, *n*-Bu_4NI, 40 °C, 48 h; (**l**) 0.35 M NaOH/MeOH, reflux, 1.5 h.

the remaining hydroxyl groups as benzyloxymethyl ether (BOM)[6,7a] and basic methanolysis of the acetate. The PMB-ethers mask the hydroxy groups that are destined for phosphorylation, whereas the benzyl and BOM ethers mask the hydroxyls remaining in the final compound.

The optically-pure coupling reagent **13** was obtained from (+)-1,2-*O*-isopropylidene-*sn*-glycerol.[6] The protected PIP_2-triester **16** was obtained from protected inositol **12** and the phosphoramidite **13** as described previously for the $PI(4,5)P_2$ triester.[6b] Thus, coupling of **12** and **13** using 1*H*-tetrazole as catalyst in CH_2Cl_2 was followed by oxidation with *m*-CPBA, deprotection of the PMB-ether with DDQ in wet CH_2Cl_2, phosphorylation of the free hydroxy groups with dibenzyl N,N-di*iso*propylphosphoramidite, and finally low-temperature oxidation with *m*-CPBA.



Scheme 2. Coupling and conversion to affinity probe. Reagents and conditions: (**a**) (i) 1*H*-tetrazole, CH_2Cl_2, rt, 1 h; (ii) *m*-CPBA, CH_2Cl_2, -40 °C to rt, 30 min; (**b**) DDQ, wet CH_2Cl_2, rt, 5 h; (**c**) (i) 1*H*-tetrazole, $(BnO)_2PN(i\text{-}Pr)_2$, CH_2Cl_2, rt, 1 h; (ii) *m*-CPBA, CH_2Cl_2, -40 °C to rt, 1 h; (**d**) H_2, Pd/C (10%), $NaHCO_3$, *t*-BuOH/water, 22 h; (**e**) BZDC-NHS, 0.25 M TEAB, DMF, rt, 18 h. The symbol "=" in the phosphates denotes the presence of a counterion (H^+, Na^+, Et_3N^+) appropriate to each solution or stage of purification.

The final BZDC-tethered PtdIns(3,4)P_2 triesters **1a** and **1b** were obtained by first hydrogenation (50 psi H_2,*t*-BuOH/water (6:1), 10% Pd/C, $NaHCO_3$) and then coupling with [^{1}H] or [^{3}H]BZDC-NHS ester[12] in 0.25 M TEAB buffer and DMF.[6] Alternatively, removal of the cyanoethyl group of **16** by β–elimination (di*iso*propylethylamine, methanol, 1.5 h, rt)[6b] followed by hydrogenation as above gave the PtdIns(3,4)P_2-diester **19**. Biochemical experiments employing these materials will be described in due course.

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9. Structures and compositions of all intermediates were confirmed by ^{1}H-, ^{13}C- and ^{31}P-NMR and by elemental analysis and/or HRMS in the FAB, EI, or CI mode. Final phosphoinositides were confirmed by ^{1}H- and ^{31}P-NMR and MALDI-MS. Selected experimental procedures and data follow; detailed procedures may be found in Refs. 6a and 6b (for analogous compounds) or may be obtained from G.D.P.

Glucosyl derivative **6** (19.5 g) was obtained from SiO_2 as a white solid: TLC (SiO_2) ethyl acetate/hexane (4:1), R_f ~0.65; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.31-7.25 (m, 9H); 6.91-6.83 (m, 4H); 4.92-4.86 (dd, J = 10.4 Hz, J_2 = 3 Hz; 2H); 4.77-4.60 (m, 4H); 4.51-4.49 (d, J = 3.5 Hz; 1H); 4.01-3.92 (t, J = 9.2 Hz; 1H); 3.79 (s, 6H); 3.74-3.59 (m, 2H); 3.52-3.42 (m, 2H); 3.35 (s, 3H); 1.70 (s, br, 1H) ppm, ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 159.3; 159.2; 138.2; 131.0; 130.2; 129.7; 129.6; 128.5; 128.0; 127.8; 113.8; 113.8; 98.2; 81.7; 79.6; 77.9;77.4; 75.4; 75.0; 73.0; 70.7; 61.8; 55.2 (2) ppm, calc. for $C_{30}H_{36}O_8$: C: 68.69% H: 6.92% found: C: 69.02% H: 6.86%. HRMS (DCI, NH_3): calc. for $C_{30}H_{39}NO_8$ (MNH_4^+): 542.2754; found: 542.2744.

Protected inositol **12** (860 mg) was obtained as a colorless oil: TLC (SiO_2) ethyl acetate/hexane (1:1), R_f ~0.60; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.37-7.14 (m, 19H); 6.84-6.81 (m, 4H); 5.09-4.55 (m, 14H); 4.18-4.08 (m, 1H); 4.02-3.90 (m, 2H); 3.79 (s, 6H); 3.52-3.36 (m, 3H) ppm, ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 159.2; 159.1; 138.9; 137.7; 137.4; 131.2; 130.4; 129.7; 129.4; 128.5; 128.4; 128.1; 127.9; 127.7; 127.0; 113.8; 113.7; 96.6; 95.9; 83.0; 82.9; 79.7; 75.8; 75.5; 72.3; 70.1; 69.8; 55.3 ppm. HRMS (FAB): calc. for $C_{45}H_{50}NaO_{10}$ (MNa^+): 773.3302; found: 773.3267.

5-*O*-Benzyl-2,6-di-*O*-benzyloxymethyl-3,4-di-(*O*-*p*-methoxybenzyl)-D-*myo*-inosityl 1,2-*O*-dipalmitoyl-*sn*-glyceryl 2-cyanoethyl phosphate (**14**). To a mixture of phosphoramidite **13** (530 mg, 0.69 mmol) and 1*H*-tetrazole (63 mg, 0.9 mmol) in 10 mL of dry CH_2Cl_2 was added a solution of **12** (200 mg, 0.26 mmol) in 5 ml of dry CH_2Cl_2. The mixture was stirred under N_2 (1 h, rt) cooled to -40 °C, and a solution of *m*-CPBA (190 mg, 1.1 mmol) in 5 ml of dry CH_2Cl_2 was added. It was then warmed to rt and stirred 0.5 h, diluted to 100 mL, washed (2 × 50 mL 10% aq. $NaHCO_3$, 50 mL brine), dried ($MgSO_4$), concentrated, and purified on SiO_2 (1:2 ethyl acetate/hexane) to give 290 mg (78%) of product **14** as a colorless oil: TLC (SiO_2) ethyl acetate/hexane (1:2), R_f ~0.25; ^{1}H-NMR ($CDCl_3$, 250 MHz) δ 7.39-7.15 (m, 19H); 6.84-6.77 (m, 4H); 5.25-5.20 (m, 1H); 5.14-3.94 (m, 20H); 3.79 (s, 3H); 3.77 (s, 3H); 3.55-3.44 (m, 2H) 2.54-2.41 (m, 2H); 2.38-2.24 (m, 6H); 1.40-1.20 (m, 48H); 0.91-0.86 (t, J = 6.3 Hz; 6H) ppm; ^{31}P-NMR ($CDCl_3$, 101 MHz) δ -0.57 ppm; ^{13}C-NMR ($CDCl_3$, 63 MHz) δ 173.2; 172.8; 159.6; 159.5; 138.5; 137.5 (2); 130.8; 130.1; 129.6; 129.4; 129.0; 128.8; 128.3; 127.9; 127.8; 127.6; 127.5; 127.4; 116.0; 113.8; 113.7; 96.4; 95.5 82.8; 81.1; 79.6; 75.7; 75.5; 74.1; 72.5; 70.3; 70.1; 69.8; 69.6; 69.3; 65.9; 62.0; 61.5; 55.2; 34.1; 34.0; 31.9; 29.7; 29.5; 29.4; 29.1; 24.9; 22.7; 19.4; 14.1 ppm. HRMS FAB): calc. for $C_{83}H_{120}NNaO_{17}P$ (MNa^+): 1456.8170; found: 1456.8170.

Removal of the PMB group gave **15** as a colorless oil: HRMS (FAB): calc. for $C_{67}H_{104}NNaO_{15}P$ (MNa^+): 1216.7041; found: 1216.7019. Phosphorylation followed by hydrogenolysis gave aminopropyl triester phosphoinositide **17:** ^{31}P-NMR (D_2O, 101 MHz) δ 6.80 (1); 5.65-5.17 (d, 0.5:0.5); 1.97 (1) ppm; MALDI TOF: calc. for : 1027 (M^+); found: 1026 (M^+-H), 1070 (M^+-3H+2Na). Unlabeled BZDC derivative **1a:** ^{31}P-NMR (D_2O, 101 MHz) δ 6.4 ; 7.0; 4.0 ppm; MALDI TOF: (M^+) 1279.

Radiolabeled compound **1b** was prepared starting with **17** (Na^+ form; 15 μL of 1 mg in 1 mL of 0.25 M TEAB buffer stock solution) added to [^{3}H]BZDC-NHS ester (2 mCi) in 15 μL of DMF. The mixture was stirred (18 h, rt), concentrated *in vacuo*, and the residue reconcentrated with 0.1 mL of CH_3OH. The residue was dissolved in 1 mL of H_2O, applied to a 50 × 5 mm column of DEAE cellulose, and eluted with 2 × 1 mL of 0.1 M TEAB buffer, 2 × 1 mL of 0.2 M TEAB buffer, 2 × 1 mL of 0.3 M TEAB buffer, 2 × 1 mL of 0.4 M TEAB buffer, 1 mL of 0.5 M TEAB buffer, 1 mL of 0.6 M TEAB buffer, 1 mL of 0.8 M TEAB buffer, 1 mL of 1.0 M TEAB buffer and 4 × 1 mL of 1.28 M TEAB buffer. The product eluted in 0.4-0.6 M solution to give 176 μCi (9% radiochemical yield) of product **1b**.

Unmodified di-C_{16} PtdIns(3,4)P_2 **19** was obtained as a solid: ^{31}P-NMR (D_2O, 101 MHz) δ 8.90; 7.60; 3.70 ppm; MALDI-TOF: calc. for $C_{41}H_{81}O_{19}P_3$: 970; found: 969 (M^+-H), 991 (M^+-2H+Na).
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