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Synthesis and Biological Activity of PTEN-Resistant Analogues of Phosphatidylinositol 3,4,5-Trisphosphate

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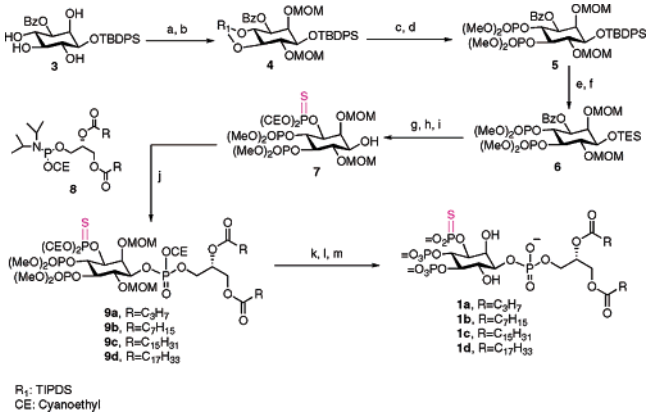
The phosphoinositide 3-kinase (PI 3-K) signaling pathway contains important therapeutic targets in human pathophysiology.[1,2] Phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P_3) is a ubiquitous signaling lipid found in higher eukaryotic cells[3] and activates a plethora of downstream cellular processes.[4] These signaling events include cell proliferation and transformation,[5] cell shape and motility,[6] and insulin action and alteration of glucose transport.[7] PtdIns(3,4,5)P_3-regulated signaling is modulated by the lipid 3-phosphatase PTEN[8] and SH2 domain-containing inositol 5-phosphatase SHIP.[9]

A metabolically stabilized (ms) analogue of PtdIns(3,4,5)P_3 that resists lipid 3- and 5-phosphatases would have numerous applications in understanding the role of PtdIns(3,4,5)P_3 in cell physiology. The ms−PtdIns(3,4,5)P_3 analogues could separate the activation of signal transduction from the degradation of the signal by phosphatase action in cells. This chemical biology approach to dissection of the PI 3-K pathway is complementary to the use of siRNA knockdowns or genetic knockouts for PTEN and SHIP. We focused first on a 3-stabilized PtdIns(3,4,5)P_3 analogue, that is, one resistant to hydrolysis by PTEN, and we selected two stabilized phosphomimetic isosteres to replace the 3-phosphate of PtdIns-(3,4,5)P_3.

Phosphorothioates are phosphomimetics that show reduced rates of enzyme-mediated hydrolysis.[10] However, the replacement of P═O by P═S also affects the pK_a of the phosphate and removes a H-bond acceptor.[11,12] For example, the phosphorothioate analogue of PtdIns(3)P had reduced binding activity for cognate binding proteins, due in part to reduced H-bonding.[13] We hypothesized that a 3-phosphorothioate of PtdIns(3,4,5)P_3 could be either an antagonist or a long-lived agonist in the PI 3-K signaling pathway because of reduced dephosphorylation by PTEN. Moreover, the methylenephosphonate analogue of PtdIns(3)P bound selectively to one of two cognate binding proteins.[14] We now describe the first asymmetric total syntheses of two PtdIns(3,4,5)P_3 analogues that are resistant to the 3-phosphatase PTEN: 3-PT−PtdIns(3,4,5)P_3 and 3-MP−PtdIns(3,4,5)P_3. Further, we show both selective binding to a PtdIns(3,4,5)P_3-binding protein and the ability of these analogues to increase sodium transport in A6 cell monolayers.

The synthetic sequence to 3-phosphorothioate−PtdIns(3,4,5)P_3 (3-PT−PtdIns(3,4,5)P_3) is illustrated in Scheme 1. Treatment of TBDPS ether **3**[15,16] with the bulky bifunctional reagent TBDPSCl_2 in the presence of imidazole selectively afforded the diol 4,5-bis-silyl ether in 88% yield as a single product; the diols were then protected to give compound **4**. Next, TIPDS deprotection, bisphosphorylation with dimethyl *N*,*N*-diisopropylphosphoramidite, and

Scheme 1. Synthesis of Phosphorothioates **1**[a]



[a] Conditions: (a) TIPDSCl_2, imidazole, Py, 88%; (b) MOMCl, DIPEA, DMF, 65 °C, 63%; (c) TBAF, THF, 77%; (d) *N*,*N*-dimethylphosphoramidite, 1*H*-tetrazole, *m*-CPBA, 81%; (e) TBAF·3H_2O, DMF, 91%; (f) TESCl, imidazole, CH_2Cl_2, 88%; (g) DIBAL-H, CH_2Cl_2, −78 °C, 84%; (h) bis(2-cyanoethoxy)(diisopropylamino)phosphine, 1*H*-tetrazole, phenylacetyl disulfide, 72%; (i) NH_4F, MeOH, 85%; (j) 1*H*-tetrazole, CH_2Cl_2, rt, *t*-BuOOH; (k) TEA, BSTFA, CH_3CN; (l) TMSBr/CH_2Cl_2 (2:3), rt; (m) MeOH.

subsequent *m*-CPBA oxidation generated the protected 4,5-bis-phosphate **5** in good yield. Since attempts to remove TBDPS in the presence of the cyanoethyl phosphate protecting groups failed to give a satisfactory result, the TBDPS was replaced with TES at this stage. Reduction of the benzoyl ester **6** with DIBAL-H at −78 °C followed by thiophosphorylation with bis(2-cyanoethoxy)-(diisopropylamino)phosphine in the presence of 1*H*-tetrazole and phenylacetyl disulfide provided the desired TES ether.[17] Deprotection of TES with the weakly acidic reagent NH_4F in methanol gave the key advanced intermediate **7** in 80% yield. Condensation of **7** with each of four different freshly prepared 1,2-di-*O*-acyl-*sn*-glycero cyanoethyl (*N*,*N*-diisopropylamino) phosphoramidites **8a**−**d** in the presence of 1*H*-tetrazole, followed by *t*-BuOOH oxidation, gave the fully protected lipids **9a**−**d**.[13] Removal of the cyanoethyl groups with triethylamine and bis(trimethylsilyl)trifluoroacetamide (BSTFA) followed by removal of the MOM and methyl ester groups with TMSBr afforded the 3-PT−PtdIns(3,4,5)P_3 analogues **1a**−**d**.

Scheme 2 summarizes the preparation of the 3-methylenephosphonate−PtdIns(3,4,5)P_3 (3-MP−PtdIns(3,4,5)P_3, **2**), in which reduction of **4** with DIBAL-H was followed by alkylation with dimethyl phosphonomethyltriflate (*n*-BuLi/HMPA) to give methylenephosphonate **10** in 80% yield. Use of excess HMPA to chelate the Li^+ cation and enhance the nucleophilicity of the alkoxide was the key to obtaining a high yield. Selective desilylation of **10** with 1 M TBAF in THF provided the 4,5-diol, which was bisphosphorylated to give TBDPS ether **11**. Removal of the TBDPS group

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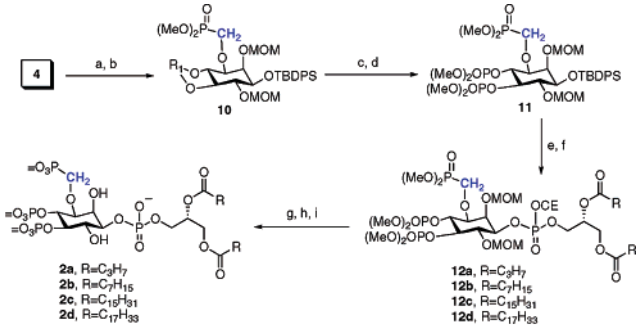
Scheme 2. Synthesis of Methylenephosphonates **2**[a]



[a] Conditions: (a) DIBAL-H, CH_2Cl_2, −78 °C, 88%; (b) *n*-BuLi, HMPA, dimethyl phosphonomethyltriflate, THF, −78 °C to rt, 80%; (c) TBAF, THF, 90%; (d) *N*,*N*-dimethylphosphoramidite, 1*H*-tetrazole, *m*-CPBA, 95%; (e) TBAF·$3H_2O$, DMF, 75%; (f) 1*H*-tetrazole, **8a**−**d**, CH_2Cl_2, rt, *t*-BuOOH; (g) TEA, BSTFA, CH_3CN; (h) TMSBr/CH_2Cl_2 (2:3), rt; (i) MeOH.

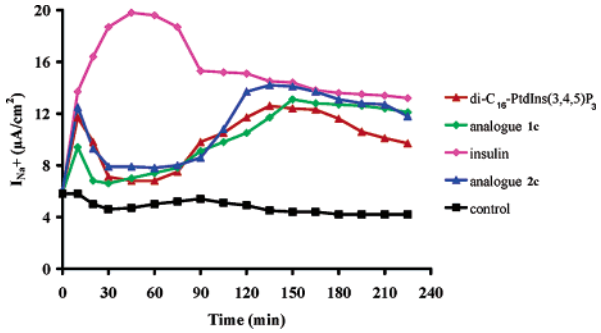


Figure 1. Stimulation of A6 cell monolayers. Experimental details for triplicate measurements of sodium transport (I_{Na^+}, $\mu A/cm^2$)[19] are in the Supporting Information. A representative result is illustrated.

followed by coupling with the phosphoramidites **8a**−**d** gave protected lipids **12a**−**d**. Removal of the protective groups gave the 3-MP−PtdIns(3,4,5)P_3 analogues **2a**−**d**.

To test the function of these analogues, we used carrier-mediated intracellular delivery[18] of PtdIns(3,4,5)P_3, which is known to activate GLUT4 translocation to the plasma membrane[7] and sodium transport.[19] The physiological function of the 3-PT− and 3-MP−PtdIns(3,4,5)P_3 analogues was examined in A6 cell monolayers, a renal epithelium model that expresses epithelial sodium channels (ENaC).[20] ENaC activity is the rate-limiting step of the sodium transport and is stimulated by insulin.[21] diC_{16}−PtdIns(3,4,5)P_3 is an early mediator of the insulin-stimulated sodium transport in A6 cells.[19] Thus, we compared the effect of the unmodified diC_{16}−PtdIns(3,4,5)P_3 with diC_{16}−3-PT−PtdIns(3,4,5)P_3 **1c** and diC_{16}−3-MP−PtdIns(3,4,5)P_3 **2c** on sodium transport across confluent monolayers of A6 cells. As shown in Figure 1, apical addition of either **1c** or **2c** increased sodium transport. Moreover, the 3-MP analogue **2c** was the most potent and long-lived mediator of sodium transport, and the 3-PT analogue **1c** also extended sodium transport compared to unstabilized PtdIns(3,4,5)P_3. The lag time observed between PtdIns(3,4,5)P_3 analogue addition and the final effect on sodium transport was due to intracellular delivery. The spatiotemporal coordination of lipid production and removal are likely required for normal physiology, and thus PtdIns(3,4,5)P_3 is necessary but not sufficient to fully mimic the action of insulin.

We tested the binding of the 3-PT and 3-MP analogues to the specific PtdIns(3,4,5)P_3-binding protein Grp1 (Supporting Information Figure 2). diC_8−3-PT−PtdIns(3,4,5)P_3 **1b** bound to Grp1 with 5-fold reduced affinity relative to that of diC_8−PtdIns(3,4,5)P_3, but the diC_8−3-MP analogue **2b** showed no binding at all. Moreover, while PTEN rapidly hydrolyzed diC_8−PtdIns(3,4,5)P_3, no hydrolysis was observed with either **1b** or **2b** (Supporting Information Figure 3). Interestingly, diC_8−3-PT analogue **1b** showed >90% inhibition of PTEN activity at 0.4 μM, while the diC_8−3-MP analogue **2b** required 40 μM for >90% inhibition (A. Branch, P. Neilsen, personal communication). Thus, analogues **1** and **2** have potential as protein-selective biological tools in the PI 3-K signaling pathway. Additional functional assays and interactions with PTEN will be reported in due course.

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Supporting Information Available: Experimental details for synthesis, characterization of new compounds, binding data, and PTEN assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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