

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

Honglu Zhang,[†] Nicolas Markadieu,[‡] Renaud Beauwens,[‡] Christophe Erneux,[§] and Glenn D. Prestwich*[†]

Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257, Department of Cell Physiology, Université Libre de Bruxelles, IRIBHM, Université Libre de Bruxelles, Campus Erasme, Route de Lennik 808, 1070 Bruxelles, Belgium

Received July 24, 2006; E-mail: gprestwich@pharm.utah.edu

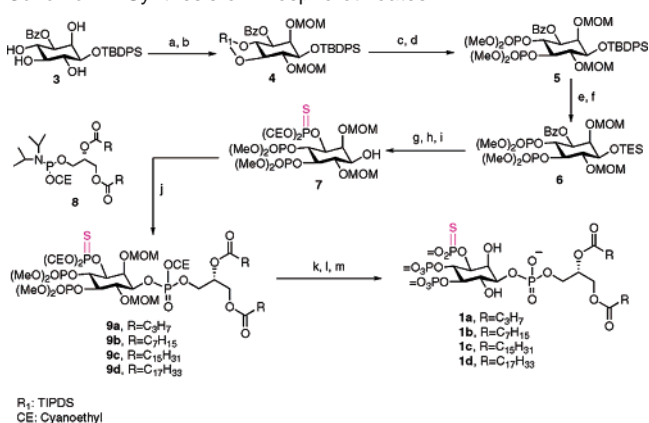
The phosphoinositide 3-kinase (PI 3-K) signaling pathway contains important therapeutic targets in human pathophysiology.^{1,2} Phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P₃) is a ubiquitous signaling lipid found in higher eukaryotic cells³ and activates a plethora of downstream cellular 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)P₃-regulated signaling is modulated by the lipid 3-phosphatase PTEN⁸ and SH2 domain-containing inositol 5-phosphatase SHIP.⁹

A metabolically stabilized (ms) analogue of PtdIns(3,4,5)P₃ that resists lipid 3- and 5-phosphatases would have numerous applications in understanding the role of PtdIns(3,4,5)P₃ in cell physiology. The ms-PtdIns(3,4,5)P₃ 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)P₃ 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)P₃.

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)P₃ 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 methylene-phosphonate 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)P₃ analogues that are resistant to the 3-phosphatase PTEN: 3-PT-PtdIns(3,4,5)P₃ and 3-MP-PtdIns(3,4,5)P₃. Further, we show both selective binding to a PtdIns(3,4,5)P₃-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)P₃ (3-PT-PtdIns(3,4,5)P₃) is illustrated in Scheme 1. Treatment of TBDPS ether **3**^{15,16} with the bulky bifunctional reagent TBDPSCl₂ 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 **1a–d**



^a Conditions: (a) TIPDSCl₂, 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·3H₂O, DMF, 91%; (f) TESCl, imidazole, CH₂Cl₂, 88%; (g) DIBAL-H, CH₂Cl₂, -78 °C, 84%; (h) bis(2-cyanoethoxy)(diisopropylamino)phosphine, 1*H*-tetrazole, phenylacetyl disulfide, 72%; (i) NH₄F, MeOH, 85%; (j) 1*H*-tetrazole, CH₂Cl₂, rt, *t*-BuOOH; (k) TEA, BSTFA, CH₃CN; (l) TMSBr/CH₂Cl₂ (2:3), rt; (m) MeOH.

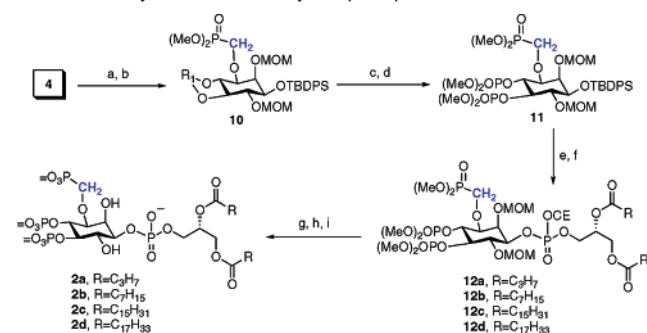
subsequent *m*-CPBA oxidation generated the protected 4,5-bis-phosphate **5** in good yield. Since attempts to remove TBDPS in the presence of the cyanoethyl phosphate protecting groups failed to give a satisfactory result, the TBDPS was replaced with TES at this stage. Reduction of the benzoyl ester **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 NH₄F 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)P₃ analogues **1a–d**.

Scheme 2 summarizes the preparation of the 3-methylenephosphonate-PtdIns(3,4,5)P₃ (3-MP-PtdIns(3,4,5)P₃, **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

[†] The University of Utah.

[‡] Department of Cell Physiology, Université Libre de Bruxelles.

[§] IRIBHM, Université Libre de Bruxelles.

Scheme 2. Synthesis of Methyleneephosphonates 2^a

^a Conditions: (a) DIBAL-H, CH₂Cl₂, -78 °C, 88%; (b) *n*-BuLi, HMPA, dimethyl phosphonemethyltriflate, THF, -78 °C to rt, 80%; (c) TBAF, THF, 90%; (d) *N,N*-dimethylphosphoramidite, 1*H*-tetrazole, *m*-CPBA, 95%; (e) TBAF·3H₂O, DMF, 75%; (f) 1*H*-tetrazole, **8a–d**, CH₂Cl₂, rt, *t*-BuOOH; (g) TEA, BSTFA, CH₃CN; (h) TMSBr/CH₂Cl₂ (2:3), rt; (i) MeOH.

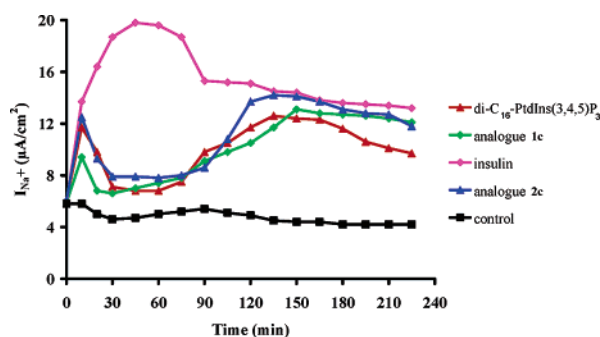


Figure 1. Stimulation of A6 cell monolayers. Experimental details for triplicate measurements of sodium transport (I_{Na^+} , $\mu A/cm^2$)¹⁹ are in the Supporting Information. A representative result is illustrated.

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)P₃ analogues **2a–d**.

To test the function of these analogues, we used carrier-mediated intracellular delivery¹⁸ of PtdIns(3,4,5)P₃, which is known to activate GLUT4 translocation to the plasma membrane⁷ and sodium transport.¹⁹ The physiological function of the 3-PT– and 3-MP–PtdIns(3,4,5)P₃ 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 DiC₁₆–PtdIns(3,4,5)P₃ is an early mediator of the insulin-stimulated sodium transport in A6 cells.¹⁹ Thus, we compared the effect of the unmodified diC₁₆–PtdIns(3,4,5)P₃ with diC₁₆–3-PT–PtdIns(3,4,5)P₃ **1c** and diC₁₆–3-MP–PtdIns(3,4,5)P₃ **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)P₃. The lag time observed between PtdIns(3,4,5)P₃ analogue addition and the final effect on sodium transport was due to intracellular delivery. The spatiotem-

poral coordination of lipid production and removal are likely required for normal physiology, and thus PtdIns(3,4,5)P₃ 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)P₃-binding protein Grp1 (Supporting Information Figure 2). DiC₈–3-PT–PtdIns(3,4,5)P₃ **1b** bound to Grp1 with 5-fold reduced affinity relative to that of diC₈–PtdIns(3,4,5)P₃, but the diC₈–3-MP analogue **2b** showed no binding at all. Moreover, while PTEN rapidly hydrolyzed diC₈–PtdIns(3,4,5)P₃, no hydrolysis was observed with either **1b** or **2b** (Supporting Information Figure 3). Interestingly, diC₈–3-PT analogue **1b** showed >90% inhibition of PTEN activity at 0.4 μM , while the diC₈–3-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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