Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 762–766
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological activity of phosphatidylinositol-3,4,5-trisphosphorothioate

Honglu Zhang,[a] Yong Xu,[a] Nicolas Markadieu,[b] Renaud Beauwens,[b] Christophe Erneux[c] and Glenn D. Prestwich[a,*]

[a]*Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, UT 84108-1257, USA*
[b]*Department of Cell Physiology, Université Libre de Bruxelles, Campus Erasme, Route de Lennik 808, 1070 Bruxelles, Belgium*
[c]*Institut de Recherche Interdisciplinaire (IRIBHM), Université Libre de Bruxelles, Campus Erasme, Route de Lennik 808, 1070 Bruxelles, Belgium*

Received 28 September 2007; revised 8 November 2007; accepted 12 November 2007
Available online 17 November 2007

Abstract—Metabolically-stabilized analogs of PtdIns(3,4,5)P_3 have shown long-lived agonist activity for cellular events mediated by this phosphoinositide. We describe an efficient method for the total asymmetric synthesis of the trisphosphorothioate (PT) analog of PtdIns(3,4,5)P_3. Intracellular delivery of dipalmitoyl PtdIns(3,4,5)PT_3-mimicked insulin in activating sodium transport in A6 cells.

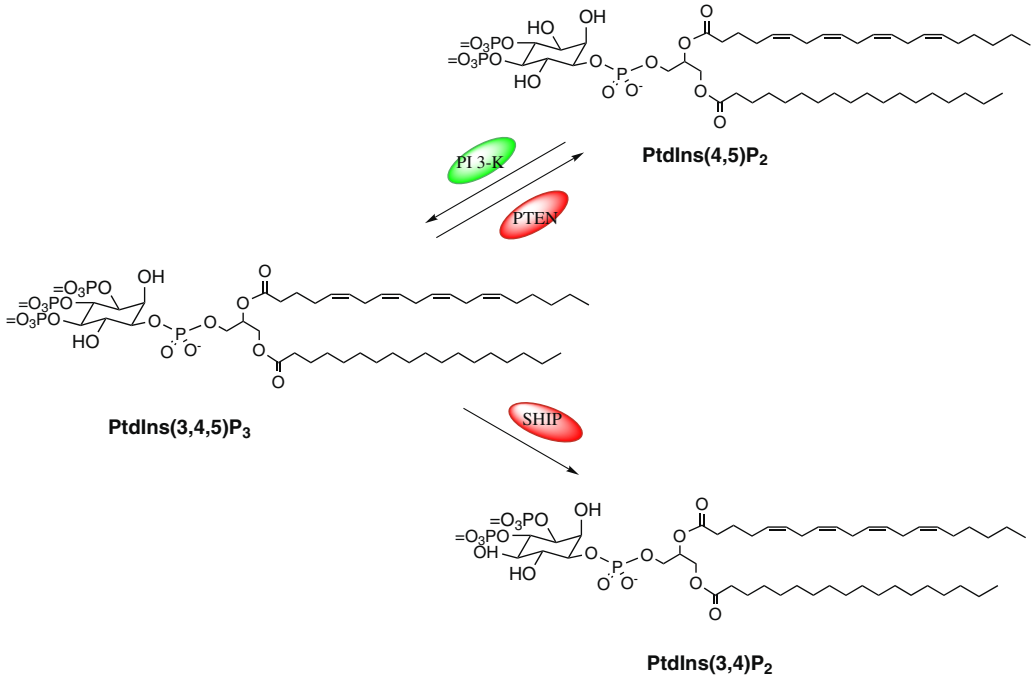
Phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P_3) is a crucially important second messenger found ubiquitously in higher eukaryotic cells.[1] The activation of Class I PI 3-K and subsequent production of PtdIns(3,4,5)P_3 is an important cell signaling event that has been causally linked to the activation of variety of downstream cellular processes.[2] These signaling events include cell proliferation and transformation,[3] cell shape and motility,[4] and the action of insulin in altering glucose transport[5] PtdIns(3,4,5)P_3-regulated signaling is governed both by its formation by PI 3-K and its conversion into phosphatidylinositol 4,5- and 3,4-bisphosphates by the 3-phosphatase PTEN[6] or the 5-phosphatase SHIP,[7] respectively. Control of PI 3-K and PtdIns(3,4,5)P_3 levels have emerged as important targets[8] in cardiovascular disease, allergy and autoimmune disease,[9] cancer, and diabetes.[8]

In addition to regulation of PtdIns(3,4,5)P_3 production, catabolism by the phosphatases PTEN and SHIP have emerged as crucial processes to control PtdIns(3,4,5)P_3-dependent signaling (Scheme 1). The availability of affinity probes for isolation and characterization of PtdInsP_n binding proteins, and the availability of tools for real-time localization of PtdInsP_ns in cells are providing a continuous influx of new information on the roles of phosphoinositides in cellular processes[10] However, many questions regarding the specific effects of PtdIns(3,4,5)P_3 remain unanswered due to its transient nature in the cell.

We recently described the preparation and activity of 3-phosphatase-resistant, metabolically-stabilized (ms) analogs of PtdIns(3,4,5)P_3 that was both stable to degradation by PTEN and acted as an inhibitor of PTEN[11] One of these analogs incorporated a single phosphorothioate (PT) substituent. Phosphorothioates are important phosphomimetics that show greatly reduced rates of enzyme-mediated hydrolysis[12] However, the replacement of P=O by P=S also affects the pK_a of the phosphate and removes a H-bond acceptor. [13,14] Indeed, we demonstrated that PtdIns(3)PT had reduced binding activity for cognate PtdIns(3)P-selective FYVE and PX domain binding proteins, which was attributable to reduced H-bonding.[15] We hypothesized that a 3,4,5-tris(PT) analog of PtdIns(3,4,5)P_3 could be either an antagonist or a long-lived agonist in the related signaling pathway, because it would be more slowly dephosphorylated by both PTEN and SHIP, and could potentially block the normal receptor-mediated signaling involving PtdIns(3,4,5)P_3. We also observed that a

Keywords: Metabolically-stabilized; PtdIns(3,4,5)P_3; Asymmetric synthesis; Activating sodium transport.
* Corresponding author. Tel.: +1 801 585 9051; fax: +1 801 585 9053; e-mail: gprestwich@pharm.utah.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.11.041



Scheme 1. Pathways for PtdIns(3,4,5)P_3 biosynthesis and catabolism.

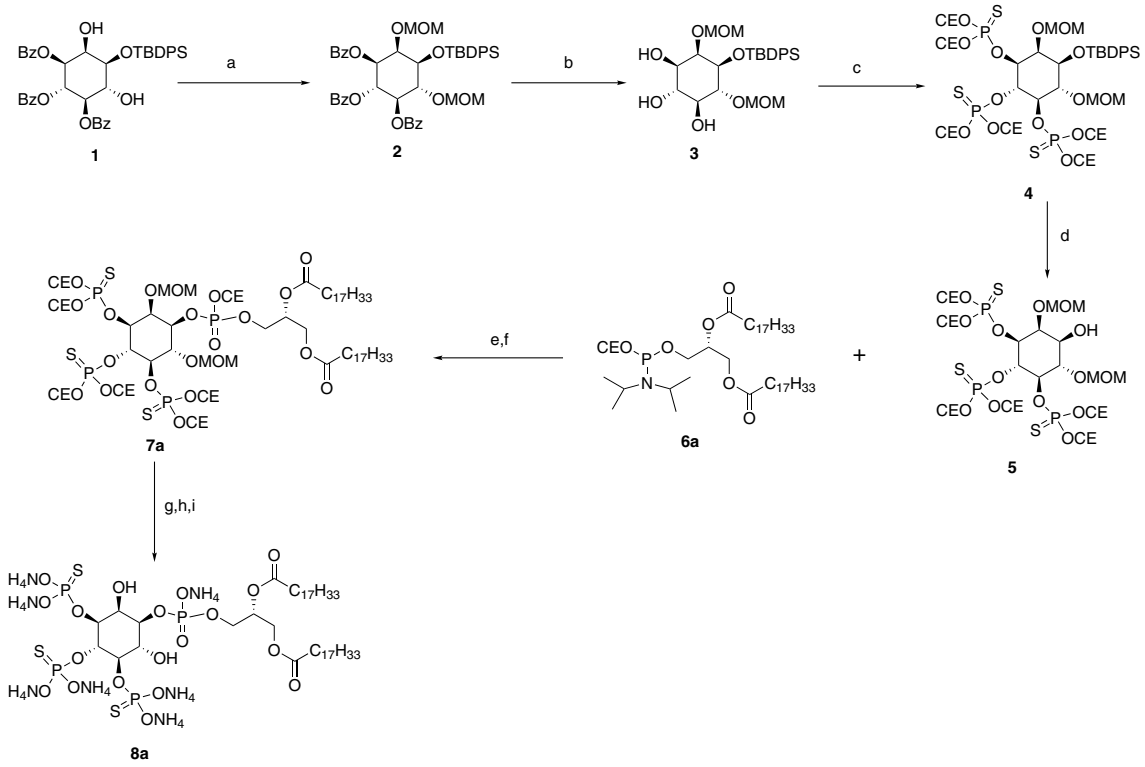
PT analog of PtdIns(5)P[16] and a tris(PT) cyclopentyl analog[17] of Ins(1,4,5)P_3 had agonistic activities and long biological half-lives. We now describe the first asymmetric total synthesis of a PtdIns(3,4,5)PT_3, a trisphosphorothioate analog of PtdIns(3,4,5)P_3 that is resistant to hydrolysis by both PTEN and SHIP. In addition, we document the ability of these analogs to effect in sodium transport in A6 cell monolayers, an amphibian renal epithelium model expressing the epithelial sodium channels.[18]

Starting from 3,4,5-benzoyl inositol **1**,[19,20] the fully protected inositol intermediate **2** was obtained by reaction of diol **1** with MOMCl and DIPEA at 50 °C (Scheme 2). Treatment of compound **2** with 1.0 M NaOMe in methanol–THF gave the desired triol **3** in 85% yield, which was then phosphorylated and oxidized with elemental sulfur to yield the corresponding phosphorothioate **4** in 28% yield.[15] Use of the HF·Py complex in THF–pyridine solution selectively removed the TBDPS ether. This deprotection reaction was very slow, and required supplemental addition of HF·Py after 3 days reaction, and 3 weeks to give key intermediate **5** in low yield.[15]

The phosphoramidite **6a**[15] was then coupled with inositol **5** in the presence of 1*H*-tetrazole to yield the phosphite intermediate, which was oxidized utilizing *t*-BuOOH to give protected phosphorothioate **7a** as shown in Scheme 2. The cyanoethyl groups (CE) on the phosphorothioate were removed by using triethylamine (TEA) plus bis(trimethylsilyl)trifluoroacetamide (BSTFA) in anhydrous acetonitrile. BSTFA was added to prevent the phosphorothioate anion from undergoing re-alkylation. The cleavage of the *O*-silyl derivatives was achieved by aqueous hydrolysis at neutral pH to give the MOM ether-protected intermediate. The MOM groups were removed using ethanethiol and $BF_3{\cdot}Et_2O$ at rt to give the final product **8a**.[15,21]

Although this synthetic route afforded the desired tris(PT) **8a**, a more efficient synthetic approach was deemed necessary. The disadvantages included the low yield of oxidation with elemental sulfur, and the low yield resulting from a TBDPS deprotection protocol that required several weeks. The sulfur–pyridine reaction conditions to convert the phosphite esters to the phosphorthioates[15] resulted in decomposition of the six cyanoethyl groups (CE) under basic conditions. We thus chose to replace the TBDPS group with TES, because TES is still sterically bulky but can be easily removed under milder conditions. Also, phenylacetyl disulfide[11,22] was selected as an alternative to elemental sulfur, and this reagent can be employed under neutral conditions to minimize destruction of the CE protecting groups.

The TES ether **9** was thus obtained in high yield in two steps: first, deprotection of the TBDPS ether **2** with solid TBAF in DMF for 3 h, followed by reaction with TESCl and imidazole at room temperature.[11] Next, removal of the three benzoyl groups of TES–ether **9** sodium methoxide gave only a low yield of triol **10**, accompanied by partial TES cleavage. As an alternative, DIBAL-H/−78 °C was employed for benzoyl removal,[11] because the TES and MOM groups were stable to these conditions; compound **10** was obtained in 81% yield. Then, triol **10** was converted to the protected tris(PT) in a two-step, one-pot reaction. Phosphitylation with 1*H*-tetrazole and bis(2-cyanoethoxy) (diisopropylamino)phosphine was followed by oxidation of the resulting phosphite ester with phenylacetyl disulfide in 30 min, giving the desired phosphorothioate **11** in 72% yield.[11] Since the cyanoethyl

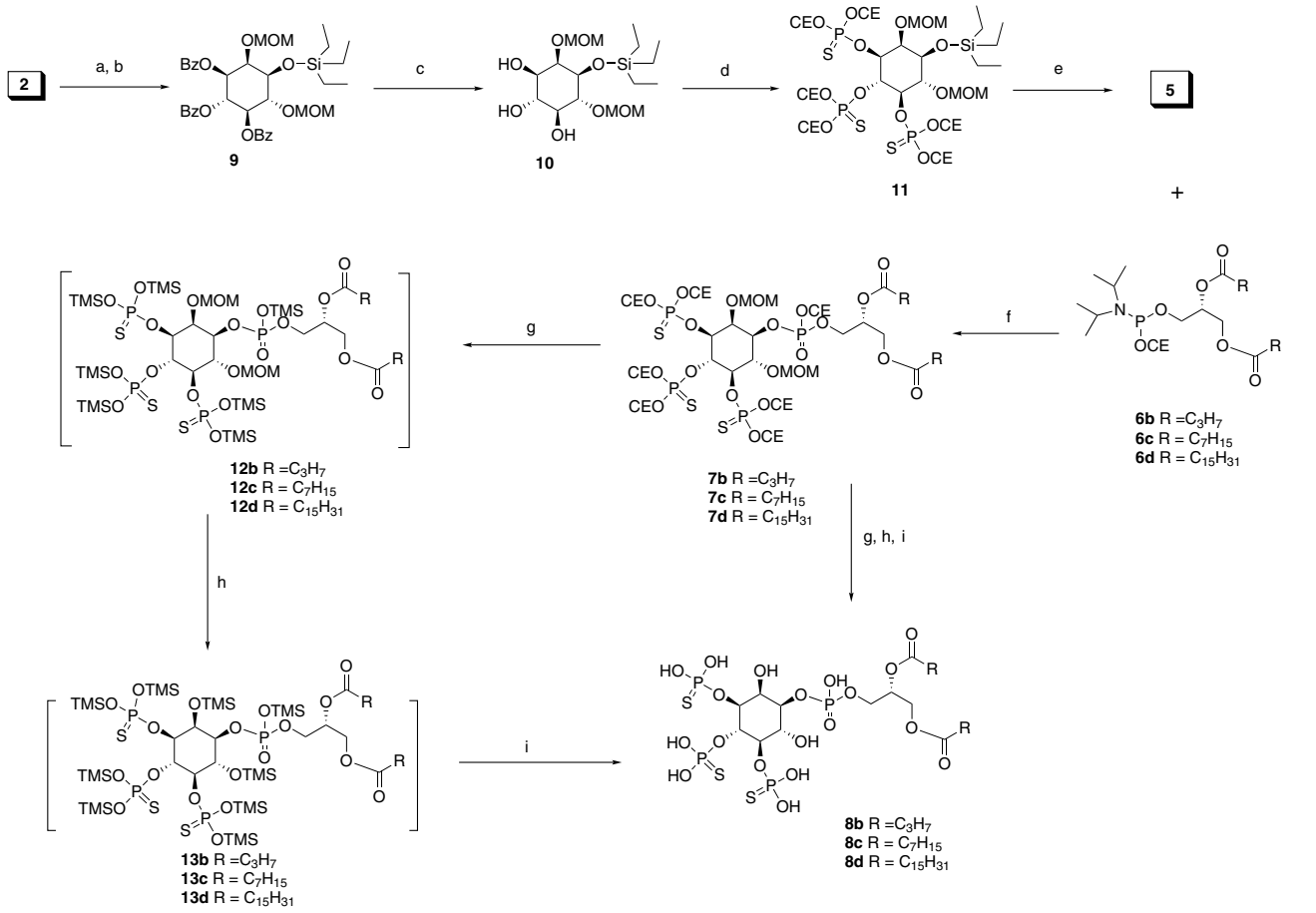


Scheme 2. Reagents and conditions: (a) MOMCl, DIPEA, DMF, 50 °C, 75%; (b) NaOMe, CH_3OH/THF, 85%; (c) Bis(2-cyanoethoxy)(diisopropylamino)phosphine, 1*H*-tetrazole; S, CS_2/Py, 28%; (d) HF·Py, THF/Py, 3 weeks, 53%; (e) 1*H*-tetrazole, CH_2Cl_2; (f) *t*-BuOOH, 48%; (g) TEA, BSTFA, CH_3CN; (h) NH_4OAc, H_2O; (i) EtSH, $BF_3{\cdot}Et_2O$, 88%.

groups were labile under basic conditions, we chose the weakly acidic reagent NH_4F for deprotection of the TES. After reaction with NH_4F in MeOH for 3 h the key intermediate **5** was obtained in 90% yield as a single product (Scheme 3)[11] The modifications introduced herein gave the protected inositol intermediate more efficiently and in higher overall yield.

Condensation of **5** with each of the freshly prepared phosphoramidites **6b–d** in the presence of 1*H*-tetrazole and *t*-BuOOH oxidation gave rise to the fully protected phosphatidylinositide analogs **7b–d**.[11] The cyanoethyl groups in intermediates **7b–d** were removed by using triethylamine and BSTFA in anhydrous acetonitrile. After 24 h, we removed the solvents to afford a residue, of which the ^1H NMR showed that the cyanoethyl groups were eliminated and the hydroxy groups were protected with TMS. Thus, BSTFA effectively prevents the phosphorothioate anions from undergoing realkylation. The TMS protected intermediates also provided us a method to remove the methyl and MOM group; that is, TMSBr could be added directly to the residue, and this led to complete removal of the methyl and MOM groups to give products **13b–d**.[11] In our previous synthesis[15] and in work by the Bruzik laboratory,[20] the TMS-esters **12** were generally converted to the ammonium salts. Then, reaction of the ammonium salts with TMSBr frequently gave only partial cleavage of the MOM ethers. However, we observed that the MOM groups were completely removed by direct exposure of the TMS-esters **12** to TMSBr in CH_2Cl_2.[23,24] This is very convenient for the purification of the final product, because all the reaction reagents are readily removed under vacuum. Finally, methanol hydrolysis of the fully TMS protected intermediates **13b–d** gave the final PtdIns(3,4,5)PT_3 analogs **8b–d** in their acid forms (Scheme 3).

To test the function of these analogs, we used A6 cell monolayers, a renal epithelium model that expresses epithelial sodium channels (ENaC), in which carrier-mediated intracellular delivery[25] of PtdIns(3,4,5)P_3 activates GLUT4 translocation to the plasma membrane[5] and sodium transport[26] ENaC activity is the rate-limiting step of the sodium transport and is stimulated by insulin[27] DiC_{16}-PtdIns(3,4,5)P_3 is an early mediator of the insulin-stimulated sodium transport in A6 cells. Thus, we compared the effect of the unmodified diC_{16}-PtdIns(3,4,5)P_3 with diC_{16}-PtdIns(3,4,5)PT_3 **8d** on sodium transport across confluent monolayers of A6 cells. As shown in Fig. 1, apical addition of analog **8d** increased sodium transport.[28] The 3,4,5-tris(PT) analog **8d** also mimicked the activity of unstabilized PtdIns(3,4,5)P_3. The lag time observed between PtdIns(3,4,5)PT_3 addition and the final effect on sodium transport revealed that the rate of intracellular delivery of this analog is faster than that of unstabilized PtdIns(3,4,5)P_3. We propose that these PtdIns(3,4,5)PT_3 analogs may have other applications as pharmacological tools to probe role of PtdIns(3,4,5)P_3 in a cellular context.



Scheme 3. Reagents and conditions: (a) TBAF·3H_2O, DMF, 86%; (b) TESCl, imidazole, CH_2Cl_2, 87%; (c) DIBAL-H, CH_2Cl_2, −78 °C, 81%; (d) Bis(2-cyanoethoxy)(diisopropylamino)phosphine, 1*H*-tetrazole; phenylacetyl disulfide, 72%; (e) NH_4F, MeOH, 90%; (f) 1*H*-tetrazole, CH_2Cl_2, rt; *t*-BuOOH; (g) TEA, BSTFA, CH_3CN; (h) TMSBr/CH_2Cl_2 (2:3), rt; (i) MeOH.

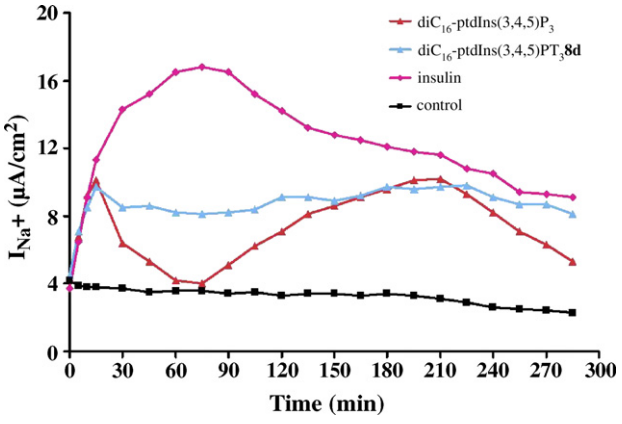


Figure 1. Stimulation of A6 cell monolayers. Experiment and evaluation of sodium transport (I_{Na^+}, μA/cm^2) was carried out as described[26] Briefly, A6 cells were subcultured onto 24-mm Millicell inserts (Millipore, Bedford, MA) for 10 days and the day before the experiment, incubated overnight in a serum-free 260 mosmol/kg H_2O amphibian Ringer solution. DiC_{16}-PtdIns(3,4,5)P_3, diC_{16}-PtdIns(3,4,5)PT_3 **8d** (50 μM) was complexed by histone H1 carrier (50 μM) and then added to the apical side of the monolayer. Results were compared with insulin basolateral stimulation (100 nM) and control (histone H1 alone).

Acknowledgments

We thank the NIH (Grant NS 29632 to G.D.P.) for financial support of work at Utah and the 'Fonds de la Recherche Scientifique Médicale' for support of work in Bruxelles. We thank P. Neilsen (Echelon Biosciences, Salt Lake City) for providing PtdIns(3,4,5)P_3 and histone.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.041.

References and notes

1. Traynor-Kaplan, A. E.; Harris, A. L.; Thompson, B. L.; Taylor, P.; Sklar, L. A. *Nature* **1988**, *334*, 353.
2. Toker, A.; Cantley, L. C. *Nature* **1997**, *387*, 673.
3. Cantley, L. C.; Auger, K. R.; Carpenter, C.; Duckworth, B.; Graziani, A.; Kapeller, R.; Soltoff, S. *Cell* **1991**, *64*, 281.

4. Pinal, N.; Goberdhan, D. C.; Collinson, L.; Fujita, Y.; Cox, I. M.; Wilson, C.; Pichaud, F. *Curr. Biol.* **2006**, *16*, 140.
5. Sweeney, G.; Garg, R. R.; Ceddia, R. B.; Li, D.; Ishiki, M.; Somwar, R.; Foster, L. J.; Neilsen, P. O.; Prestwich, G. D.; Rudich, A.; Klip, A. *J. Biol. Chem.* **2004**, *279*, 32233.
6. Maehama, T.; Dixon, J. E. *Trends Cell. Biol.* **1999**, *9*, 125.
7. Pesesse, X.; Deleu, S.; De Smedt, F.; Drayer, L.; Erneux, C. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 697.
8. Drees, B. E.; Mills, G. B.; Rommel, C.; Prestwich, G. D. *Expert Opin. Ther. Patents* **2004**, *14*, 703.
9. Wymann, M. P.; Zvelebil, M.; Laffargue, M. *Trends Pharmacol. Sci.* **2003**, *24*, 366.
10. Prestwich, G. D. *Chem. Biol.* **2004**, *11*, 619.
11. Zhang, H.; Markadieu, N.; Beauwens, R.; Erneux, C.; Prestwich, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 16464.
12. Lampe, D.; Liu, C.; Potter, B. V. *J. Med. Chem.* **1994**, *37*, 907.
13. Hampton, A.; Brox, L. W.; Bayer, M. *Biochemistry* **1969**, *8*, 2303.
14. Murray, A. W.; Atkinson, M. R. *Biochemistry* **1968**, *7*, 4023.
15. Xu, Y.; Lee, S. A.; Kutateladze, T. G.; Sbrissa, D.; Shisheva, A.; Prestwich, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 885.
16. Huang, W.; Zhang, H.; Davrazou, F.; Kutateladze, T. G.; Shi, X.; Gozani, O.; Prestwich, G. D. *J. Am. Chem. Soc.* **2007**, *129*, 6498.
17. Zhang, L.; Huang, W.; Morita, T.; Tanimura, A.; Harihar, S.; DeWald, D. B.; Prestwich, G. D. *Chem. Med. Chem.*, 2, 1281.
18. Handler, J. S.; Perkins, F. M.; Johnson, J. P. *Am. J. Physiol.* **1981**, *240*, C103.
19. Bruzik, K. S.; Tsai, M.-D. *J. Am. Chem. Soc.* **1992**, *114*, 6361.
20. Kubiak, R. J.; Bruzik, K. S. *J. Org. Chem.* **2003**, *68*, 960.
21. Kubiak, R. J.; Bruzik, K. S. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1231.
22. Dreef, C. E.; Mayr, G. W.; Jansze, J.-P.; Roelen, H. C. P. F.; Van deer Marel, G. A.; van Boom, J. H. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 239.
23. Zhang, H.; Shibuya, K.; Hemmi, H.; Nishino, T.; Prestwich, G. D. *Org. Lett.* **2006**, *8*, 943.
24. Zhang, H.; Tsukuhara, R.; Tigyi, G.; Prestwich, G. D. *J. Org. Chem.* **2006**, *71*, 6061.
25. Ozaki, S.; DeWald, D. B.; Shope, J. C.; Chen, J.; Prestwich, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11286.
26. Markadieu, N.; Blero, D.; Boom, A.; Erneux, C.; Beauwens, R. *Am. J. Physiol. Renal Physiol.* **2004**, *287*, F319.
27. Rossier, B. C.; Canessa, C. M.; Schild, L.; Horisberger, J. D. *Curr. Opin. Nephrol. Hypertens.* **1994**, *3*, 487.
28. Experimental determination and evaluation of sodium transport (I_{Na^+}, $\mu A/cm^2$). Briefly, A6 cells were subcultured onto 24-mm Millicell inserts (Millipore, Bedford, MA) for 10 days and the day before the experiment, incubated overnight in a serum-free 260 mosmol/kg H_2O amphibian Ringer solution. DiC_{16}-PtdIns(3,4,5)P_3, analog **8d** was complexed by histone H1 carrier (50 μM) and then added to the apical side of the monolayer. Results were compared with insulin basolateral stimulation (100 nM) and control (histone H1 alone). This experiment is representative of three independent experiments.