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## *Communications to the Editor*

### **Synthesis and Biology of 1D-3-Deoxyphosphatidylinositol: A Putative Antimetabolite of Phosphatidylinositol-3-phosphate and an Inhibitor of Cancer Cell Colony Formation**

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Ligand-activated growth factor and mitogen receptors activate phosphatidylinositol (PtdIns) phospholipase C which hydrolyzes PtdIns 4,5-bisphosphate to form inositol 1,4,5-triphosphate [Ins(1,4,5)P<sub>3</sub>] and diacylglycerol.<sup>1</sup> Ins(1,4,5)P<sub>3</sub> releases Ca<sup>2+</sup> from nonmitochondrial stores to increase the cytoplasmic-free Ca<sup>2+</sup> concentration whereas diacylglycerol is an activator of protein kinase C (PKC).<sup>2</sup> The increase in cytoplasmic-free Ca<sup>2+</sup> concentration and the increased activity of PKC lead to a sequence of events that culminate in DNA synthesis and cell proliferation.<sup>3</sup> Recently, another PtdIns signaling pathway has been identified and linked to the action of some growth factors and oncogenes. Phosphatidylinositol-3-kinase (PtdIns-3-kinase) is found associated with a number of protein tyrosine kinases, including the ligand-activated receptors for insulin, platelet-derived growth factor, epidermal growth factor, and colony-stimulating factor, as well as oncogene tyrosine kinases.<sup>4</sup> The PtdIns-3-kinase phosphorylates the D-3 position of the *myo*-inositol ring of PtdIns to yield a class of PtdIns-3-phosphates that are not substrates for hydrolysis by PtdIns phospholipase C,<sup>5</sup> and therefore

appear to exert their signaling action independently of the inositol phosphate pathway.

PtdIns-3-kinase is found in cellular complexes with ligand-activated growth factor receptor, with oncogene protein tyrosine kinases,<sup>6</sup> and with p21<sup>ras</sup>.<sup>7</sup> The PtdIns-3-kinase is a heterodimer consisting of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit.<sup>8</sup> The 85 kDa subunit acts as an adaptor protein whose *src*-homology (SH2) domains interact with specific tyrosine phosphorylated residues on the tyrosine kinase receptors and other tyrosine phosphorylated proteins.<sup>9</sup> This brings the PtdIns-3-kinase p110 kDa catalytic subunit from the cytoplasm to the plasma membrane where substrates, PtdIns and its phosphates, are located.<sup>10</sup> Recently a guanine nucleotide binding (G) protein-dependent PtdIns-3-kinase activity has been described that is inactive in the absence of added G<sub>βγ</sub> subunits.<sup>11</sup> The mechanism by which PtdIns-3-phosphates stimulate cell growth is not known. They have been suggested to act by reorganizing cytoskeletal elements,<sup>12</sup> by activating protein kinase C-ζ,<sup>13</sup> or by affecting vesicular trafficking in cells.<sup>14</sup>

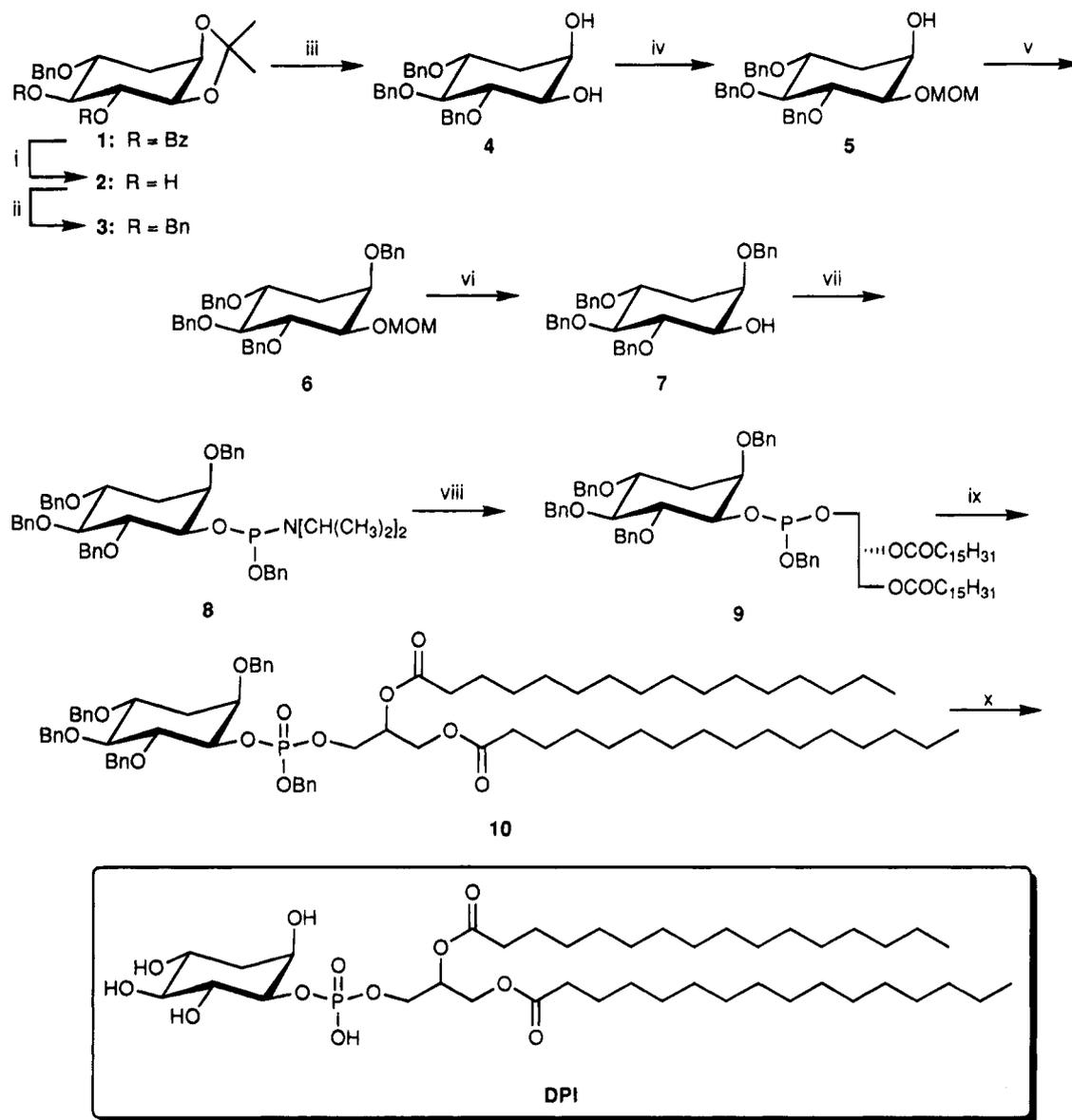
The PtdIns-3-kinase signaling pathway provides an attractive target for the development of antiproliferative drugs because it is important for both cell growth and transformation.<sup>15</sup> PtdIns-3-kinase takes on added importance in view of the recent finding that it is the direct target for the Ras proteins, key regulators of cell growth.<sup>16</sup> As part of our studies to synthesize analogues of PtdIns as potential antimetabolites<sup>17</sup> of the PtdIns-3-kinase signaling pathway, we have synthesized 1D-3-deoxyphosphatidylinositol (DPI). We report the synthesis of this compound together with its ability to inhibit cancer cell growth *in vitro*. Additionally, DPI is shown to be an important tool to measure the activity of PtdIns-3-kinase and other PtdIns kinases in cell lysates from breast cancer cells.

**Chemistry.** As shown in Scheme 1, the synthesis of DPI began with the previously prepared intermediate **1** available from the synthesis of 1D-3-deoxy-*myo*-inositol 1,4,5-triphosphate.<sup>18</sup> As illustrated, **1** was transformed into **4** by a sequence of conventional steps. The diol **4** was protected preferentially at the 1-position via a cyclic stannylene<sup>19</sup> and subsequently benzylated to give the

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**Scheme 1.**<sup>a</sup> Synthesis of 1D-3-Deoxyphosphatidylinositol

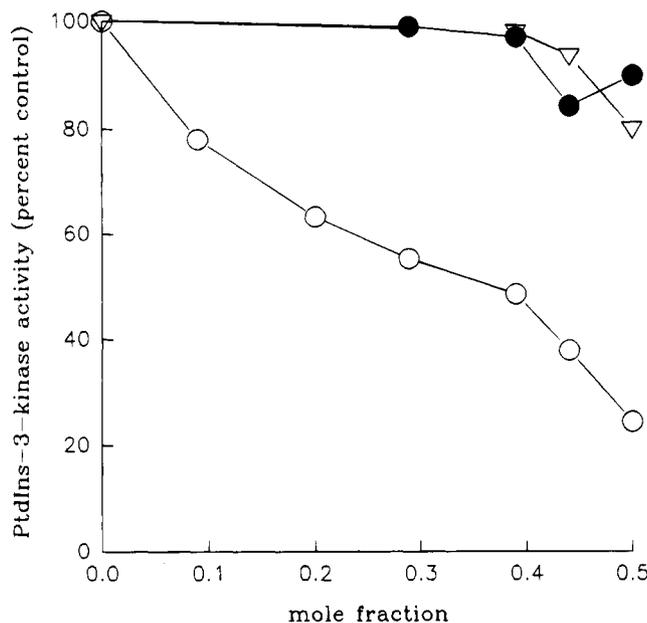
<sup>a</sup> Reagents and conditions: (i)  $K_2CO_3$ , MeOH (83%); (ii) NaH, BnBr, DMF (99%); (iii) HCl, MeOH (95%); (iv) (a)  $n-Bu_2SnO$ , MeOH, reflux; (b) MOMCl, DMF/toluene (59% overall); (v) NaH, BnBr, DMF (76%); (vi) HCl, MeOH/H<sub>2</sub>O (98%); (vii) BnOP[N(CH(CH<sub>3</sub>)<sub>2</sub>)<sub>2</sub>]<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub> (82%); (viii) 1,2-dipalmitoyl-*sn*-glycerol, tetrazole, CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>CN (31%); (ix) *t*-BuOOH, CH<sub>2</sub>Cl<sub>2</sub> (88%); (x) 20% Pd(OH)<sub>2</sub>/C, H<sub>2</sub>, *t*-BuOH (89%).

fully protected intermediate **6**. The MOM protecting group was removed, and the 1-position was phosphitylated with (benzyloxy)bis(diisopropylamino)phosphorus to afford **8**. Next, **8** was coupled with 1,2-dipalmitoyl-*sn*-glycerol, and the phosphite was oxidized to the fully protected phosphate (**10**). Finally, all benzyl groups were removed by hydrogenolysis over Pd(OH)<sub>2</sub>/C in *t*-BuOH. *t*-BuOH was chosen as the solvent in order to minimize the possibility of transesterification of the palmitoyl groups of the diacylglycerol moiety. Of all of the steps executed in the synthesis of DPI, the hydrogenolysis reaction proved to be the most difficult, and the conditions in this step were arrived at only after considerable experimentation.

**Biology. Cell Growth Inhibition.** To assay the compound for cell growth inhibition, HT-29 human colon carcinoma cells were exposed to various concentrations of DPI over a 7 day period.<sup>20</sup> DPI was found to inhibit colony formation by these carcinoma cells with an IC<sub>50</sub> of 35  $\mu$ M. The PtdIns analogue bearing a fluorine atom

in place of the 3-hydroxy group exhibited an IC<sub>50</sub> of 37  $\mu$ M in the same assay.<sup>21</sup>

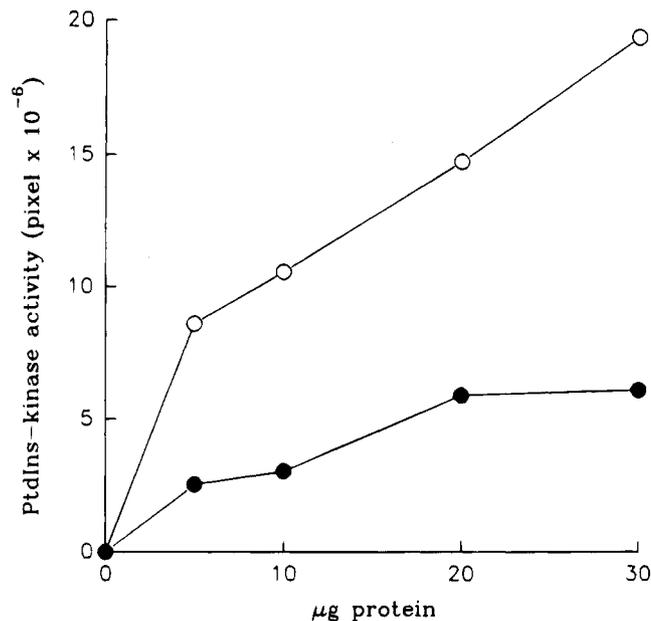
**PtdIns-3-Kinase Activity.** Phosphatidylinositol-3-kinase was prepared as phosphotyrosine immunoprecipitates from lysates of platelet-derived growth factor stimulated Swiss mouse 3T3 fibroblasts as previously described.<sup>22</sup> PtdIns-3-kinase activity was assayed as described<sup>22</sup> by the [ $\gamma$ -<sup>32</sup>P]ATP-dependent phosphorylation of PtdIns. In marked contrast to 1D-3-deoxy-3-fluoro-PtdIns (FTI) which inhibits this enzyme with an IC<sub>50</sub> of 30  $\mu$ M, DPI was found to have no effect on PtdIns-3-kinase activity at concentrations up to 250  $\mu$ M; additionally it was not a substrate for the kinase, as no phosphorylated product was detected by TLC analysis (Figure 1). Apparently, the electronic nature of the group at the 3-position of these PtdIns analogues is one element essential for recognition by the enzyme. The steric volume of this substituent also plays a role, for 1D-3-chloro-3-deoxy-PtdIns, like DPI, fails to inhibit the PtdIns-3-kinase.



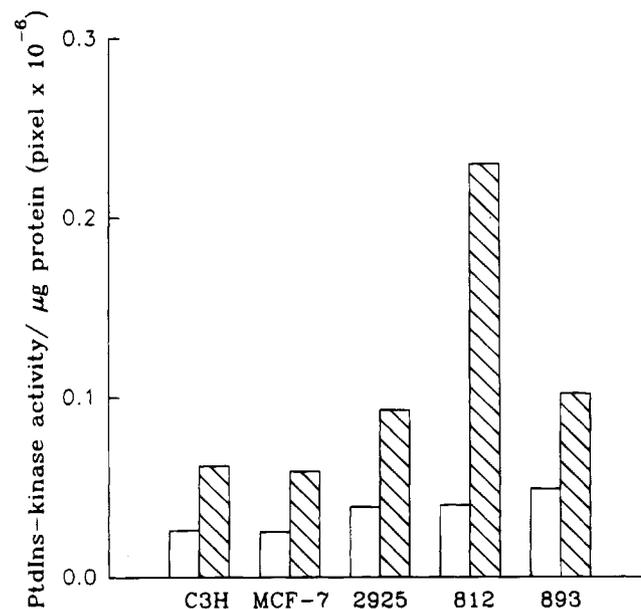
**Figure 1.** Effects of 1D-3-substituted-PtdIns on PtdIns-3-kinase activity. The ability of (●) 1D-3-deoxy-PtdIns; (○) 1D-3-deoxy-3-F-PtdIns; and (△) 1D-3-deoxy-3-Cl-PtdIns when prepared as mixed vesicles with PtdIns to inhibit the ATP-dependent phosphorylation of PtdIns by immunoprecipitated PtdIns-3-kinase was measured as described in the text. The concentration of PtdIns in the assay was 100  $\mu$ M, and the mole fraction of 1D-3-substituted-PtdIns was varied as shown.

The observation that DPI does not inhibit PtdIns-3-kinase would suggest that it can be used in whole cell lysates to measure PtdIns-3-kinase activity as the difference between [ $\gamma^{32}$ P]ATP-dependent phosphorylation of PtdIns and DPI. Previous studies to measure cell PtdIns-3-kinase activity have typically employed immunoprecipitation of the kinase from cell lysates using phosphotyrosine or p85 antibodies.<sup>23</sup> With the recent reports on G protein-dependent forms of PtdIns-3-kinase,<sup>11</sup> the question of whether all the PtdIns-3-kinase activity is being measured can be raised. An alternative method is to measure the formation of PtdIns-3-phosphates in intact cells.<sup>24</sup> TLC separation of PtdIns-3-phosphate and PtdIns-4-phosphate in cell lysates gives relatively poor separation.<sup>25</sup> A specific HPLC method is available but requires high specific activity radiolabeling and derivatization prior to HPLC in order to measure the small amounts of PtdIns-3-phosphates.

Accordingly, DPI and PtdIns were used as substrates to measure PtdIns-3-kinase and other PtdIns kinase activities in whole cell lysates of MCF-7 human breast carcinoma cells (Figure 2). An approximately linear relationship was found between the amount of cell protein in the assay and the amounts of PtdIns and DPI phosphorylation. Since DPI cannot be phosphorylated at the 3-position, its phosphorylation represents other positions on the *myo*-inositol ring. The known biologically relevant positions for phosphorylation of PtdIns are 3, 4, and 5.<sup>26</sup> Additionally, in the presence of 50 nM wortmannin, a specific inhibitor of PtdIns-3-kinase, DPI was shown to be as good a substrate as PtdIns for the other PtdIns kinases in MCF-7 cell lysates.<sup>27</sup> Thus phosphorylation of DPI most likely reflects PtdIns-4- and PtdIns-5-kinase activities. In no case was formation of PtdIns bis- or trisphosphates observed, as these



**Figure 2.** Phosphorylation of PtdIns and 1D-3-deoxy-PtdIns by MCF-7 cell lysate. The [ $\gamma^{32}$ P]ATP-dependent phosphorylation of (○) PtdIns and (●) 1D-3-deoxy-PtdIns by whole cell lysates of MCF-7 human breast carcinoma cells was measured as described in the text. The concentration of the lipids in the assay was 100  $\mu$ M. There was no detectable phosphorylation of any endogenous lipids in the MCF-7 cell lysates under the assay conditions employed. The results are typical of three separate experiments.



**Figure 3.** PtdIns-3-kinase and other PtdIns-kinase activities in breast cancer cells. PtdIns-3-kinase (hatched boxes) and other PtdIns-kinase activities (open boxes) were measured in cell lysates from C3H mouse mammary carcinoma, and MCF-7, ACC-2925, ACC-812, and ACC-893 human breast carcinoma cell lines as described in the text. Each value is a mean derived from protein concentration/PtdIns and 1D-3-deoxy-PtdIns kinase activity curves similar to that shown in Figure 2. The results are typical of two separate experiments.

are readily separated from the PtdIns monophosphates by the TLC system used.

DPI was used to measure PtdIns-kinase activities in a panel of mouse and human breast cancer cell lines (Figure 3). PtdIns-3-kinase activity is approximately 2-fold higher than that of other PtdIns-kinases. How-

ever, in the ACC-812 human breast cancer cell line the PtdIns-3-kinase activity was almost 4-fold that seen in the other breast cancer cell lines, while other PtdIns-kinase activities remained the same. The significance, if any, of the elevated PtdIns-3-kinase activity in the breast cancer cell line remains to be established.

In summary, the colony-inhibiting activity found for DPI in this study is encouraging and suggestive that further structural alterations of PtdIns should be made in the quest for novel anticancer agents. Additionally, DPI is shown to serve as a possible pharmacological tool for characterizing the level of PtdIns-3-kinase activity in diverse cell lines, a feature which should gain added importance as our understanding of the role of the PtdIns-3-kinase signaling pathway in abnormal cell growth continues to evolve.

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**Supplementary Material Available:** Spectroscopic and analytical data for all new compounds (2 pages). Ordering information is given on any current masthead page.

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