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Communications to the Editor

3-Deoxy-D-*myo*-inositol 1-Phosphate, 1-Phosphonate, and Ether Lipid Analogues as Inhibitors of Phosphatidylinositol-3-kinase Signaling and Cancer Cell Growth

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Growth factors and certain oncogenes activate a range of phospholipid-mediated signal transduction pathways resulting in cell proliferation. Phosphatidyl *myo*-inositol (PI) occupies a unique position in that it can undergo reversible phosphorylation at multiple sites to generate five different phosphoinositides,[1] while its metabolites regulate two pathways important for cell proliferation: the inositol phosphate/diacylglycerol signaling pathway[2,3] and the phosphatidylinositol 3-phosphate (PI-3-kinase) pathway.[4,5] In the first pathway, PI-specific phospholipase C (PI-PLC) hydrolyses a minor membrane phospholipid, PI(4,5)P_2, to give the water-soluble Ins(1,4,5)P_3 and a lipophilic diacylglycerol (DAG). Ins(1,4,5)P_3 interacts specifically with membrane receptors to release Ca^{2+},[6] a key event in cellular signal transduction, while DAG is an endogenous activator of protein kinase C (PKC).[7] Ins(1,4,5)P_3 is metabolized by either hydrolysis of the phosphate at position 5 giving Ins(1,4)P_2 or phosphorylation at position 3 giving Ins(1,3,4,5)P_4. Ins(1,4)P_2 is not active as a Ca^{2+}-mobilizing agent and is subsequently degraded by other phosphatases. However, it has been suggested that Ins(1,3,4,5)P_4 may play a role in refilling the intracellular Ca^{2+} stores with extracellular Ca^{2+}.[8] Together, the increase in [Ca^{2+}] and the increased activity of PKC lead to a sequence of events that culminate in DNA synthesis and cell proliferation.

In the second pathway, PI-3-kinase has been found associated with almost every growth factor receptor or oncogene transformation.[9] PI-3-kinase phosphorylates PI at position 3 of the *myo*-inositol ring to give a class of PIs that are poor substrates for hydrolysis by PI-PLC, e.g., PI(3,4)P_2 and PI(3,4,5)P_3. The exact mechanism by which 3-phosphorylated PIs modulate cell growth is not known, but they appear to be important modulators of protein interaction and enzyme activity through binding to specific sites on proteins. For example, binding of PI(3,4)P_2, PI(4,5)P_2, or PI(3,4,5)P_3 to pleckstrin-homology (PH) domains on enzymes such as AKT (protein kinase B) leads to enzyme activation, whereas the Src-homology-2 (SH2) domain that mediates protein tyrosine phosphate binding binds specifically PI(3,4,5)P_3.[10] Some studies have also provided evidence that PKC is activated by PI(3,4)P_2 and PI(3,4,5)P_3,[11−13] while adapter protein-2 (AP-2) is the only protein with high affinity and isomer-specific binding to PI(3)P.[14] Thus, inhibition of the production of such lipids produced by PI-3-kinase can result in inhibition of many acute cellular responses. The fungal metabolite wortmannin, an inhibitor of PI-3-kinase,[15] has shown antitumor activity in animal models but is relatively toxic and nonspecific and inhibits a variety of other related kinases.

Our studies have been directed toward the synthesis of 3-substituted *myo*-inositol derivatives to selectively block the effects of *myo*-inositol-derived second messengers on cell proliferation and transformation while leaving other aspects of *myo*-inositol signaling unaffected. This strategy may offer a basis for the selective control of cancer cell growth without disrupting the function of normal cells. Our approach has been to synthesize PI analogues modified on the inositol ring and then to improve the antiproliferative activity of the most promising analogues by making additional changes in the diacylglycerol moiety. We have found that 1D-3-deoxyphosphatidyl-*myo*-inositol (**1**)[16] (Chart 1) and its 3-fluoro derivative **2**[17] at relatively high concentrations

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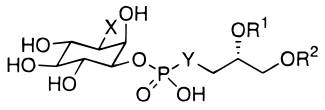
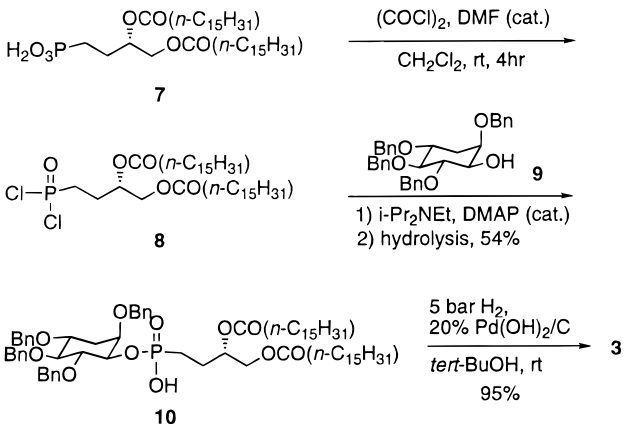
Chart 1



1 X = H; Y = O; $R^1 = R^2$ = palmitoyl
2 X = F; Y = O; $R^1 = R^2$ = palmitoyl
3 X = H; Y = CH_2; $R^1 = R^2$ = palmitoyl
4 X = H; Y = O; R^1 = Me; $R^2 = C_{18}H_{37}$-*n*
5 X = H; Y = CH_2; R^1 = Me; $R^2 = C_{18}H_{37}$-*n*
6 X = OH; Y = O; R^1 = Me; $R^2 = C_{18}H_{37}$-*n*

Scheme 1

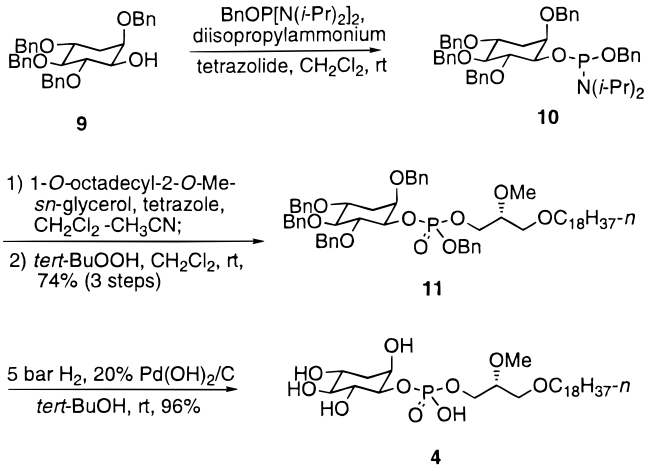


inhibit colony formation by HT-29 human colon carcinoma cells, with IC_{50} values of 35 and 37 μM, respectively. We hypothesize that the relatively low potency of these compounds may be due to their hydrolysis by phospholipases including PI-PLC, and the DAG produced by hydrolysis can activate PKC and may lead to tumor cell proliferation.

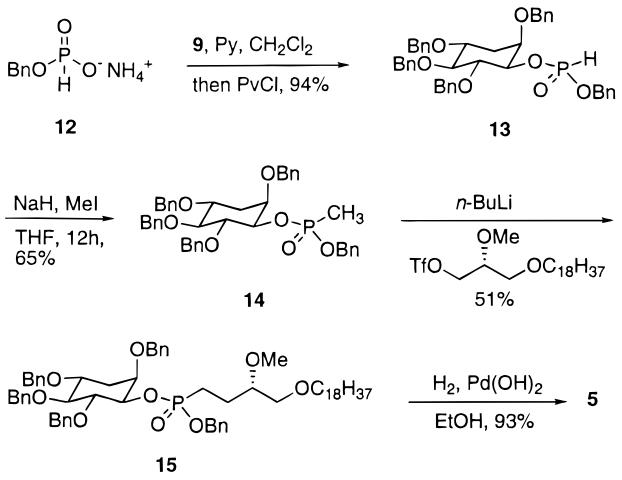
To decrease the susceptibility of the 3-deoxy-PI analogues to phospholipase hydrolysis, we adopted two separate synthetic strategies. First, we synthesized a phosphonate derivative (**3**), in which the *sn*-3 oxygen of the DAG is replaced by methylene group, rendering this compound resistant to hydrolysis by PI-PLC and maintaining relatively high concentration as an antimetabolite in the PI-3-kinase signaling pathway (Scheme 1). Second, we synthesized ether lipid analogues of 3-deoxy-PI. The ether lipid analogue **4** is of synthetic interest not only because of its greater stability to phospholipases but also due to the known antitumor activity of some members of its family.[18] Ether lipids such as 1-*O*-octadecyl-2-*O*-methylglycerophosphocholine (edelfosine) are inhibitors of PI-PLC with IC_{50}'s in the low micromolar range.[19] Thus we expected that the 3-deoxy-PI ether lipid would not be a substrate for PI-PLC. A further advantage of the ether lipids is that they have shown intrinsic antitumor activity against a variety of tumor types. Some ether lipid analogues that have undergone clinical trial as antitumor agents are inhibitors of PI-3-kinase.[20] They affect several aspects of lipid intracellular signaling, and their antitumor activity may arise from a combination of effects on the signaling pathway. 1-*O*-Octadecyl-2-*O*-methylphosphatidylinositol (**6**), however, has not shown good antitumor activity.[21]

For the synthesis of the phosphonate analogue **3** (Scheme 1), the dichloride **8** was prepared from (*S*)-3,4-

Scheme 2



Scheme 3



bis(palmitoyloxy)butylphosphonic acid (**7**)[22] with oxalyl chloride in the presence of a catalytic amount of DMF at room temperature. The inositol component, 1D-2,4,5,6-tetra-*O*-benzyl-3-deoxy-*myo*-inositol (**9**), was obtained as reported before.[23] Phosphorylation of **9** with **8** in the presence of a base afforded monoester chloride intermediate which was transformed into **10** by hydrolysis, a reaction which proceeded in a surprisingly sluggish manner. After purification by preparative TLC, catalytic hydrogenation of **10** using $Pd(OH)_2$/C in *tert*-butyl alcohol[24] provided the target phosphonate **3** in good yield.

We also tested the hypothesis that an ether lipid analogue would be a more potent inhibitor of cell growth. Compound **4** was synthesized with 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycerol instead of dipalmitoylglycerol through similar steps as in the synthesis of **1**[23] (Scheme 2). Phosphonate **5** was also synthesized to prevent PI-PLC hydrolysis. Synthesis of compound **5** was effected through *H*-phosphonate methodology[25] using ammonium *O*-benzyl-*H*-phosphonate (**12**) (Scheme 3), which is readily available by hydrolysis of dibenzyl phosphite with ammonium hydroxide.[26] The phosphorylation of **9** was complete within 10 min after addition of pivaloyl chloride into a dichloromethane solution of **9**, **12**, and pyridine at room temperature. Subsequent alkylation of *H*-phosphonate **13** with MeI provided methyl phosphonate **14**,[27] the anion of which was then

Table 1. Effects of Compounds **1**−**5** on PI-PLC and PI-3-kinase Activity and Growth Inhibition of HT-29 in Vitro

	IC_{50} (μM)		
compd	PI-PLC	PI-3-K	growth inhibition HT-29
1	N/A[b]	>250[a]	35[a]
2	8	30	37
3	N/A[b]	N/A[b]	10
4	19.9	2.5	2.1
5	10	5.3	45

[a] Reported in ref 16. [b] N/A, not active, with <20% inhibition at 100 μM.

Table 2. Antitumor Activity of **1** and **4** against HT-29 Human Colon in Scid Mice

compd dose (mg/kg)	schedule[a]	tumor vol[b] on day 10 (cm^3)	T/C (%)	*P*[c]
control		0.27 ± 0.04		
1				
500	ip, qd 4−5	lethal		
250	ip, qd 4−7	0.30 ± 0.06	111.1	NS
4				
150	ip, qd 4−7	0.09 ± 0.07	33.3	<0.05
100	ip, qd 4−7	0.32 ± 0.09	118.0	NS
50	ip, qd 4−7	0.28 ± 0.05	103.7	NS

[a] e.g., 250 mg/kg ip, qd 4−7 means that the 250 mg/kg dose was given as an intraperitoneal injection each day from days 4 to 7 (4 daily injections) after the tumors were implanted. [b] Tumor volume values are the mean for 8 mice per group with SE. [c] The *P* column is the significance value for a Student's test comparing the tumor volumes in the treated group to the tumor volumes in the control group; 0.05 is usually the maximum value for significance. NS, not significant, meaning that these studies were not repeated.

coupled with the triflate of 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycerol to provide the fully protected precursor **15**.[28] Final hydrogenation furnished the desired phosphonate **5**.

Biological Activity. In vitro inhibition of bovine PI-PLC[19] and of bovine brain p110/p85 PI-3-kinase[16] was measured as previously described.[16] Inhibition of colony formation in soft agarose of HT-29 colon carcinoma cells with continuous 7-day drug exposure was measured as described.[16]

The most important result is the finding that replacement of the diacylglycerol moiety with an ether lipid group results in an over 15-fold increase in growth inhibition activity (compare **1** and **4**). Although replacement of phosphate by phosphonate increases the growth inhibiting activity of 3-deoxy-PI by almost 3-fold (compare compounds **1** and **3**), it decreases the growth inhibiting activity of the 3-deoxy ether lipid analogue (compare **4** and **5**). These compounds are only weak inhibitors of PI-PLC compared to 1-*O*-octadecyl-2-*O*-methylglycerophosphocholine which has an IC_{50} under the same assay conditions of around 1 μM.[29] The 3-deoxy ether lipid PIs were relatively potent inhibitors of PI-3-kinase with IC_{50} values of 2−5 μM. *O*-Octadecyl-2-*O*-methylglycerophosphocholine has previously been found to be an inhibitor of PI-3-kinase with an IC_{50} of 35 μM, while *myo*-inositol-containing analogue **6** is a much weaker inhibitor with an IC_{50} of 90 μM.[30] Thus, the presence of a 3-deoxy-*myo*-inositol moiety appears to impart PI-3-kinase inhibiting activity to the compounds.

Preliminary studies of in vivo antitumor activity were conducted in *scid* (severe combined immunodeficient) mice implanted subcutaneously with 10^7 HT-29 human colon adenocarcinoma cells. Injection of compounds **1** and **4** was begun 4 days after tumor inoculation in groups of 8 mice as 4 daily intraperitoneal injections of the compounds suspended in 3% EtOH, 3% Tween 20, 0.9% NaCl. Tumor volume was measured with calipers on day 10. As shown in Table 2, compound **1** was lethal at a daily dose of 500 mg/kg and exhibited no antitumor activity at one-half this dose. Compound **4** was not toxic at the highest dose tested of 150 mg/kg/day and inhibited tumor growth by 67%. There was no antitumor activity at doses of 100 and 50 mg/kg/day.

In summary, we present the synthesis and the bioactivity of several rationally designed phosphatidylinositol analogues. Further studies of these compounds in animals using other tumor xenografts will be reported in due course.

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