Phosphonothioate and Fluoromethylene Phosphonate Analogues of Cyclic Phosphatidic Acid: Novel Antagonists of Lysophosphatidic Acid Receptors§

Yong Xu,† Guowei Jiang,† Ryoko Tsukahara,‡ Yuko Fujiwara,‡ Gabor Tigyi,‡ and Glenn D. Prestwich*,†

Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257, and Department of Physiology, College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee 38163

Received March 26, 2006

Isoform-selective antagonists of the lysophosphatidic acid (LPA) G-protein coupled receptors (GPCRs) have important potential uses in cell biology and clinical applications. Novel phosphonothioate and fluoromethylene phosphonate analogues of carbacyclic phosphatidic acid (ccPA) were prepared by chemical synthesis. The pK_a values of these amphilic phosphonolipids and the parent cyclic phosphonate were measured titrimetrically using the Yasuda−Shedlovsky extrapolation. The pharmacological properties of these and other ccPA analogues were characterized for LPA receptor (LPAR) subtype-specific agonist and antagonist activity using Ca^{2+}-mobilization assays in RH7777 cells expressing the individual EDG-family GPCRs. In particular, the phosphonothioate ccPA analogue inhibited Ca^{2+} release through LPA_1/LPA_3 activation and was an LPA_1/LPA_3 antagonist. The monofluoromethylene phosphonate ccPA analogue was also a potent LPA_1/LPA_3 antagonist. In contrast, the difluoromethylene phosphonate ccPA analogue was a weak LPAR agonist, while ccPA itself had neither agonist nor antagonist activity.

Introduction

The unique *Physarum* phospholipid PHYLPA (Figure 1), isolated from myxoamoebae of *Physarum polycephalum*, is an inhibitor of eukaryotic DNA polymerase.[1] PHYLPA has a cyclopropane-containing fatty acyl chain and also possesses a cyclic phosphate at *sn*-2 and *sn*-3 positions of the glycerol carbons.[1,2] PHLYPA inhibits the proliferation of human fibroblasts cultured in a chemically defined medium.[3] Although this compound is referred to as a cyclic phosphatidic acid (cPA) analogue, the absence of the second acyl group makes it more accurately a cyclic form of lysophosphatidic acid (LPA). The biological activities of synthetic analogues of PHYLPA[4] and the structure−activity relationships (SARs) indicated that the cyclic phosphate was required for antiproliferative activity.[5] The cellular effects elicited by cPA include antimitogenic regulation of the cell cycle,[3,5,6] regulation of actin stress fiber formation and rearrangement,[6] inhibition of cancer cell invasion and metastasis,[7] inhibition of platelet aggregation,[8] regulation of differentiation and viability of neuronal cells, activation of Cl^- currents,[9] and mobilization of intracellular calcium.[3] cPA is generated in blood and is bound to serum albumin.[10] Recently, a metabolically stabilized carbacyclic analogue of cPA, known as ccPA, was described as a novel inhibitor of metastatic cancer (Figure 1).[11,12]

In exploring a wide range of metabolically stabilized LPA analogues,[13] we have identified the phosphorothioate[14,15] and mono- and difluoromethylene phosphonate moieties[16,17]as important bioactive mimics of the phosphate. We thus envisaged the synthesis of the corresponding cyclic phosphonothioate and mono(di)fluoromethylene phosphonate analogues of ccPA (Figure 1). On the basis of studies of second pK_a values for acyclic phosphomonoesters,[18] these analogues were expected to have pK_a values more closely matched the parent cyclic phosphate than the cyclic methylenephosphonate ccPA. Thus, in addition to retaining the metabolic stability of the carbacylic structure, we anticipated that these ccPA analogues would have improved receptor binding properties. Since LPA receptors (LPARs) are responsible for cell proliferation and differentation, cell migration, and cell invasion, we hypothesized that these ccPA analogues could be potential LPAR antagonists.[19,20] Herein, we describe the synthesis of novel ccPA and their activities on human LPAR subtypes.[20−22]

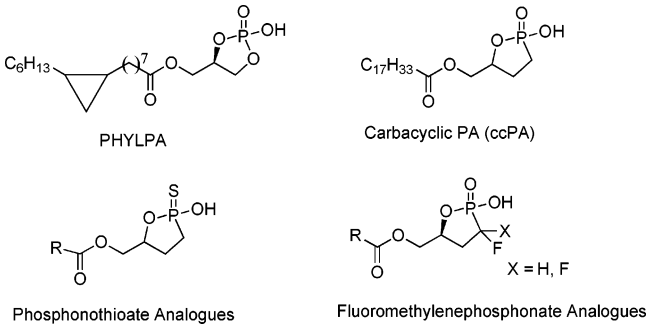


Figure 1. Cyclic phosphatidic acids and their carbacyclic analogues.

Phosphonothioate, or phosphothioic acids, and their derivatives have generated considerable interest as inhibitors of enzymes involved in metabolism of the natural phosphates. Phosphonothioates thus have potential applications for the selective manipulation of fundamental cellular responses that could validate new therapeutic approaches for human diseases.[23] One common approach for stabilization of phosphate monoesters is the use of a mono- or difluoromethylene phosphonate analogues in place of the higher pK_a methylene or methyl phosphonates. Though less well studied, recent experimental and theoretical reports have suggested that the α-fluoromethylene phosphonate groups should be able to better mimic the phosphate than either the methylene or difluoromethylene derivatives.[18,24] Several monofluoromethylene phosphonates have been studied as potential enzyme inhibitors and as probes for the elucidation of biochemical processes.[25] Indeed, we have

§ Dedicated to Professor Iwao Ojima on the occasion of his 60th birthday.

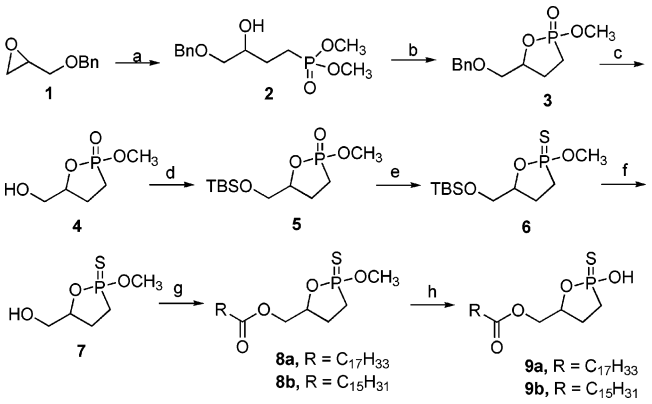
* Corresponding author. Fax: 01-801-585-9053. email: gprestwich@pharm.utah.edu.

† The University of Utah.

‡ University of Tennessee Health Science Center.

10.1021/jm060351+ CCC: $33.50
Published on Web 07/27/2006

Scheme 1. Synthesis of the Cyclic Phosphonothioates[a]



[a] Reagents and conditions: (a) $LiCH_2P(O)(OCH_3)_2$, THF, $BF_3.Et_2O$, 100%; (b) PPTS, toluene, 44%; (c) 10% Pd/C, 1 atm H_2, 78%; (d) TBSCl, imidazole, DMF, 97%; (e) Lawesson's reagent, toluene, 67%; (f) TBAF·$3H_2O$, HOAc, THF, 69%; (g) RCOOH, DCC/DMAP, CH_2Cl_2, 73−86%; (h) *t*-$BuNH_2$, 71%.

recently described the enantioselective synthesis[17,26,27] and selected biological activities of a variety of fluorinated analogues of LPA.[13,16] The biological results demonstrated that α-monofluorinated phosphonate mimics of LPA were significant phosphatase-resistant and isoform-selective agonists for LPARs.

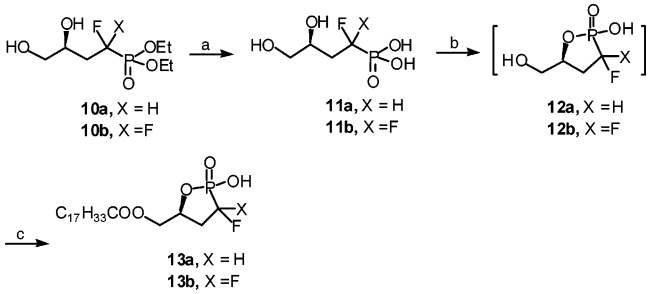
Chemical Synthesis

Current methods for the preparation of phosphonothioates were developed for specific compounds and employed conditions that would preclude application to the synthesis of labile LPA analogues. For example, methyl and ethyl esters have often been used as protecting groups during syntheses of phosphatidic and phosphonatidic acids. However, deprotection with trimethylsilyl iodide or trimethylsilyl bromide gave low yields of phosphonothioate or phosphorothioate derivatives.[28] Deprotection of the dibenzyl esters of phosphonothioic acids with sodium in liquid ammonia[29] and deprotection of the cyanoethyl esters under mild basic conditions[14] are presently the most frequently used methods for the synthesis of phosphonothioates and phosphothioates.

We sought a convenient and versatile synthetic route to phosphonothioate ccPA. Regiospecific ring opening of the commercially available benzyl glycidol ether with a carbon nucleophile, derived from dimethyl methylphosphonate in the presence of BF_3·OEt_2, furnished the corresponding racemic γ-hydroxy phosphonate **2** (Scheme 1).[30] An intramolecular transesterification reaction of 4-(benzyloxy)-3-hydroxybutanephosphonate to the corresponding cyclic compound **3** was achieved with pyridinium *p*-toluenesulfonate (PPTS) in toluene. With the cyclic intermediate in hand, it was important to conduct the hydrogenolysis of the benzyl protecting group prior to thionation of phosphonate.

Thus, hydrogenolysis (Pd−C, 1 atm H_2) proceeded readily at room temperature (rt). The primary alcohol **4** was protected as a *tert*-butyldimethylsilyl (TBS) ether **5**, which can survive thionation and yet still be removed under mild conditions. Introduction of the P═S group is the key transformation for the synthesis of phosphonothioates and phosphorothioates. We chose Lawesson's reagent for this transformation, as improved yields of thiophosphoryl products and milder reaction conditions were reported [31] in comparison to P_2S_5.[32] Thus, thionation of cyclic phosphonate **5** with Lawesson's reagent was accomplished by refluxing in toluene for 8 h to give the desired cyclic phosphonothioate **6** in good yield. The TBS ether **6** was removed with TBAF that had been neutralized with acetic acid, affording the primary alcohol **7**. Esterification (DCC, DMAP, and either oleic acid or palmitic acid, rt) provided the racemic esters **8a** and **8b**, respectively.

Scheme 2. Synthesis of the Cyclic Mono- and Difluorophosphonates[a]



[a] Reagents and conditions: (a) TMSBr, CH_2Cl_2; CH_3OH/H_2O, 75−93%; (b) DCC, DMF; (c) RCOCl, pyridine, DMF, 51%.

Deprotection of the methyl ester of the cyclic phosphonothioate required a careful choice of reagent. While the commonly used electrophiles TMSBr or TMSI fail to deprotect phosphonothioates, *tert*-butylamine,[33] trimethylamine,[34] and lithium in liquid ammonia[34] have been used for similar reactions. We selected *tert*-butylamine, because it was reported to deprotect methyl esters of either phosphonothioates or phosphorothioates under mild conditions. In the event, each of the charged phosphonothioates **9** was obtained by refluxing the protected esters **8** in *tert*-butylamine for 3 days.

α-Monofluoroalkylphosphonates can be prepared in modest yields by electrophilic fluorination of α-carbanions of alkyl phosphonate esters with NFSI or Selectfluor.[35] Consequently, we reasoned that electrophilic fluorination might be a viable method for the preparation of α-monofluoroalkylphosphonic acids. However, attempts at electrophilic fluorination of the cyclic phosphonate anion with NFSI and Selectfluor at low temperatures were unsuccessful using a variety of bases (NaH, LDA, KDA and *n*-BuLi) and solvents (DMF, THF). An alternate approach was thus required.

We previously described the addition of diethyl iodomonofluoro- and iododifluoromethylene phosphonates to allyl alcohol catalyzed by tetrakis(triphenylphosphine)palladium in hexane to give the corresponding iodohydrins in 72% yield.[17,27] The epoxides were formed by treatment with K_2CO_3−MeOH for 5 min at room temperature and then subjected to a hydrolytic kinetic resolution (HKR) reaction with 0.45 equiv of H_2O in a minimum volume of THF in the presence of 1.0 mol % of (*R*,*R*)-Salen-Co-OAc.[17,27] The diols **10a** and **10b** were obtained in 99% ee. When neither *p*-TsOH or PPTS could cyclize the phosphonate, we turned to dicyclohexylcarbondiimide (DCC), which had been reported to promote efficient intramolecular, high-dilution cyclization of phosphates or phosphonates, which generated the corresponding cyclic analogues.[36−38] When the formation of either five- and six-membered cyclic phosphates is possible, the five-membered ring was favored for both the phosphate or phosphonate products. For example, α-glycerophosphate formed a five-membered ring preferentially upon treatment with DCC.[37] The diols **11a** and **11b** were prepared readily by treatment with TMSBr (Scheme 2).[39,40] Thus, reaction of diol **11a** with DCC was followed immediately by esterification of the remaining hydroxy group. NMR data established the cyclization product **13** indeed possessed the desired five-membered ring. Compound **13a** had a double doublet resonance at 31−32 ppm in the ^{31}P NMR, while the typical acyclic

Table 1. Aqueous pK_a Values of ccPA and Its Analogues **9a**, **9b**, **13a**, and **13b** Measured in Water−Methanol Mixtures and Extrapolated to Zero Methanol Content[a]

compound	pK_a value
ccPA	6.58 ± 0.07
9a	6.76 ± 0.05
9b	6.48 ± 0.01
13a	5.60 ± 0.01
13b	4.62 ± 0.09

[a] See text for experimental details.

monofluoromethylene phosphonate would have resonances at ca. 20 ppm. ^{1}H NMR spectra showed a 3-H resonance at 4.49−4.38 ppm; the chemical shift of 3-H in the six-membered ring would be expected to occur above 5.2 ppm.[26]

pK_a Determination of ccPA and Its Analogues in Methanol−Water Mixture. Acid dissociation constants (pK_a values) are important in pharmaceutical drug design and discovery, where knowledge of the ionization state of a particular functional group is often vital in order to understand the pharmacokinetic and pharmacodynamic properties of new entities. Ionizable groups in aqueous solution can be determined readily by pH-metric titration, but poorly water-soluble compounds are generally unsuitable for pH-metric titration. However, if the compound is sufficiently soluble in a water-miscible organic solvent, it is possible to determine the apparent pK_a (p_sK_a) pH-metrically in cosolvent mixtures. Aqueous pK_a values can be then determined by extrapolation of the p_sK_a values to zero organic solvent content.[41,42]

The apparent acid dissociation constants (p_sK_a) of ccPA and analogues **9a**, **9b**, **13a**, and **13b** were determined pH-metrically in methanol−water mixtures. A glass electrode calibration procedure based on a four-parameter equation ($pH = \alpha + Sp_cH + j_H[H^+] + j_{OH}[OH^-]$) was used to obtain pH readings based on the concentration scale (p_cH). The Yasuda-Shedlovsky extrapolation ($p_sK_a + \log [H_2O] = A/\epsilon + B$) was used to derive acid dissociation constants in aqueous solution (pK_a).[41,42] The measured pK_a values are shown in Table 1. Interestingly, ccPA and the two phosphonothioates **9** had very similar pK_a values in the range of 6.46−6.78. The monofluoromethylenephosphonate **13a** was one pK_a unit more acidic at 5.60, while difluoromethylenephosphonate analogue **13b** showed the lowest pK_a value (4.62) as expected from the strong electron-withdrawing ability of the difluoromethylene group.

Receptor Activation Studies. The ligand properties of the compounds were evaluated on the activation of LPARs designed (by convention) LPA_1, LPA_2, and LPA_3 by Ca^{2+} mobilization in RH7777 cells expressing each individual human LPAR subtype. Table 2 illustrates calcium responses elicited through the activation of human LPA_1, LPA_2, and LPA_3 receptors. In these experiments, RH7777 cells, which are intrinsically unresponsive to LPA, were transfected with human LPA_1, LPA_2, and LPA_3, respectively.[43,44] While ccPA itself had no detectable LPA receptor agonist or antagonist effects, the difluoromethylene phosphonate ccPA **13b** was a weak LPA receptor agonist at 10 μM, compared to an EC_{50} value for natural *sn*-1-oleoyl LPA of 50−100 nM for the different LPA receptor isoforms. Thus, **13b** was >100-fold less potent than natural LPA.

In contrast, the monofluoromethylene phosphonate ccPA **13a** was an LPA_1/LPA_3 antagonist. For LPA_1, **13a** was a partial antagonist, with a maximal inhibition of 46% of the response induced by 200 nM *sn*-1-oleoyl LPA. Interestingly, both the phosphonothioate ccPA analogues **9a** and **9b** were also LPA_1/LPA_3 selective antagonists. The oleyl analogue **9a** was a partial LPA_1 antagonist, blocking 64% of the response to 200 nM *sn*-1-oleoyl LPA. Importantly, the phosphonothioate ccPA analogues did not show any agonist-related activitation of the three LPA GPCRs tested.

Our observation that ccPA was not an antagonist on LPA GPCRs suggested that the inhibition of metastasis was not due to a direct effect on LPARs,[12] and that ccPA must have additional targets mediating anti-metastatic effect. Intriguingly, the simple substitution of a P═O bond with a P═S bond in the phosphonothioate resulted in a potent LPA_1/LPA_3 antagonist. This effect cannot be due solely to matching the pK_a values, which were very similar for ccPA and the phosphonothioate analogues **9**. In addition, the monofluoromethylene phosphonate ccPA analogue **13a**, with a lower pK_a value of 5.60, was observed to be a potent LPA_1/LPA_3 antagonist. Surprisingly, this antagonist activity was abrogated upon introduction of a second fluorine atom in analogue **13b**, which lowered the pK_a value to 4.62. The difluoromethylenephosphonate **13b** was now a weak LPAR agonist. Clearly, the number of fluorine atoms at the α-position of the cyclic phosphonate controls both the pK_a and the nature of the ligand−receptor interaction.

The ccPA structure appears to offer a promising scaffold for the design of new LPAR antagonists with selective interactions with receptors. Selective and potent LPAR antagonists are highly desirable for dissecting the importance of LPAR function in cell biology,[13,19,20] and recent computational studies now offer avenues to incorporate selective design strategies.[19,45] In addition, LPAR antagonists may have therapeutic potential to control abnormal cell growth, differentiation, and inflammation in cancer and vascular pathophysiology.[20−22]

Recent evidence shows that LPA is produced extracellularly from lysophosphatidylcholine by the lysophospholipase D activity of autotaxin (ATX/lysoPLD).[46] This enzyme is a ubiquitous exo-phosphodiesterase that was originally identified as an autocrine motility factor for melanoma cells and has been implicated in tumor progression. The local production of LPA by ATX/lysoPLD could support an invasive microenvironment for tumor cells, exacerbating metastasis.[22] Thus, LPAR antagonists and ATX inhibitors may have therapeutic potential in cancer therapy by blocking the growth-supporting and antiapoptotic effects of LPA and reducing its titer. The mechanisms of enhanced tumor cell invasion by LPA include two important molecular mechanisms. First, LPAR-mediated activation of the Rho and Rac GTPase pathways are essential for the regulation of the actin cytoskeleton and cell motility.[47] Second, LPA has been shown to regulate the activity of matrix metalloproteinases, which are also intricately involved in metastasis as well as in the LPA-induced transphosphorylation of the epidermal growth factor (EGF) receptor.[48]

Currently available LPAR antagonists represent a promising start to the development of useful chemical tools, although none can be considered definitive in determining receptor selectivity or biological functions, especially for studies in vivo. Several LPAR antagonists have been reported. A list of compounds with reported LPAR selectivity includes the ethanolamide phosphate (VPC12249, LPA_1/LPA_3 antagonist),[49,50] 3-(4-[4-([1-(2-chlorophenyl)-ethoxy]carbonylamino)-3-methyl-5-isoxazolyl]benzylsulfanyl)propanoic acid (Ki16425, LPA_1/LPA_3 antagonist),[51] decyl fatty alcohol phosphates (FAP-10, LPA_1/LPA_3 antagonist and LPA_2 agonist),[44] diacylglyceryl pyrophosphate (DGPP, an LPA_1/LPA_3 antagonist).[43] Appropriately validated compounds are essential to advance in vivo studies, particularly in view of potential off-target effects. The development of more selective, more stable, more potent, and more drug-like antagonists is

Table 2. Effects of ccPA Analogues **9a**, **9b**, **13a**, and **13b** on RH7777 Cells Expressing LPA_1−LPA_3

	LPA_1				LPA_2				LPA_3			
compd	EC_{50} (nM)	E_{max}[b] (%)	IC_{50} (nM)	K_i (nM)	EC_{50} (nM)	E_{max} (%)	IC_{50} (nM)	K_i (nM)	EC_{50} (nM)	E_{max} (%)	IC_{50} (nM)	K_i
ccPA	NE	NA	NE[c]	NA[d]	NE	NA	NE	NA	NE	NA	NE	NA
9a	NE	NA	941 ± 197[e]	403 ± 84.5	NE	NA	NE	NA	NE	NA	1270 ± 160	636 ± 80
9b	NE	NA	799 ± 197	314 ± 77.5	NE	NA	NE	NA	NE	NA	2340 ± 150	1150 ± 73.3
13a	NE	NA	106 ± 58[f]	60.7 ± 33.5	NE	NA	NE	NA	NE	NA	7720 ± 1740	3170 ± 713
13b	>1940	13.0 ± 0.66 at 30 μM	NE	NA	>9460	82.0 ± 2.27 at 30 μM	NE	NA	>7030	65.4 ± 5.12 at 30 μM	NE	NA

[a] Data represent the average of four independent measurements (mean ± SD). [b] E_{max}= maximal efficacy of the drug/maximal efficacy of LPA 18:1 at saturation. [c] NE = no effect at the highest concentration (30 μM) tested. [d] NA = not applicable. [e] Partial antagonist with 64% inhibition of the 200 nM at 30 μM LPA response. [f] Partial antagonist with 46% inhibition of the 200 nM LPA response at 30 μM.

eagerly awaited. The findings presented herein provide a useful platform for further optimization of ccPA analogues as LPAR antagonists.

In conclusion, we have demonstrated efficient enantioselective syntheses of phosphonothioate and fluoromethylene phosphonate ccPA analogues and their biological activities as LPA_1/LPA_3 antagonists. These versatile synthetic routes are currently being employed for the synthesis of other phosphonothioate and fluoromethylene phosphonate analogues and will be reported in due course.

Experimental Section

General Procedures. Chemicals were obtained from Aldrich and Arcos Chemical Corporation and were used without prior purification. Solvents used were of reagent grade and were distilled before use. THF was distilled from sodium wire, and methylene chloride was distilled from CaH_2. Reactions were performed under an inert atmosphere (N_2 or Ar) unless otherwise indicated. Flash chromatography (FC) employed Whatman 230−400 mesh ASTM silica gel. ^{1}H and ^{13}C spectra were recorded at 400 MHz (^{1}H), 101 MHz (^{13}C), 162 MHz (^{31}P) and 376 MHz (^{19}F) at 25 °C. Chemical shifts are reported in ppm with TMS as internal standard for ^{13}C and ^{1}H (δ = 0.00); for ^{31}P, 85% H_3PO_4 was assigned δ = 0.00; for ^{19}F, $CFCl_3$ (δ=0.00).

Dimethyl 4-(Benzyloxy)-3-hydroxybutanephosphonate (2). A 2.5 M solution of *n*-BuLi (60 mL, 150 mmol) in hexane was added dropwise to a stirred solution of dimethyl methylphosphonate (18.6 g, 16.25 mL, 150 mmol) in dry THF (150 mL) at −78 °C under a nitrogen atmosphere. After 15 min of stirring, a solution of the benzyl glycidol ether (8.21 g, 7.65 mL, 50 mmol) in THF (25 mL) was added dropwise, followed by BF_3·OEt_2 (25.35 mL, 200 mmol), which was slowly introduced, while maintaining the temperature below −70 °C. After stirring for 2 h, the reaction was quenched with saturated NH_4Cl (150 mL) and was allowed to warm to room temperature. The residue obtained after concentration in vacuo was extracted with four portions of EtOAc (200 mL × 4). The organic extracts were washed with brine, dried with Na_2SO_4, and concentrated, and the residue was purified by FC (acetone/hexane: 1:1, R_f = 0.30) to yield 14.4 g of the pure hydroxy phosphonate ester (50 mmol, 100%). ^{1}H NMR ($CDCl_3$): δ 7.30−7.22 (m, 5H), 4.48 (s, 2H), 4.34 (m, 1H), 3.76 (d, J = 10.8 Hz, 6H), 3.39 (m, 2H), 2.23 (m, 1H), 2.09 (m, 1H), 1.96 (m, 1H), 1.85 (m, 1H). ^{31}P NMR ($CDCl_3$): δ 36.60 (s). MS (CI) *m*/*z* 289.1 (M^++1, 100.00). HRMS, M^++1, Found: 289.1211. Calcd for $C_{13}H_{22}O_5P$, 289.1217.

Methyl 3-Hydroxyl-4-benzyloxybutane 1,3-Cyclic Phosphonate (3). To dimethyl 4-(benzyloxy)-3-hydroxybutanephosphonate (20.2 g, 70.18 mmol) dissolved in anhydrous toluene (450 mL) was added PPTS (pyridinium *p*-toluene sulfonate, 34.0 g, 140 mmol). The mixture was heated to 80 °C for 20 h. After cooling to room temperature, H_2O (200 mL) was added, and the mixture was extracted with ethyl acetate. The organic phase was dried with Na_2SO_4 and concentrated, and the residue was purified by FC (acetone/hexane: 1:1, R_f = 0.48) to yield the pure hydroxy phosphonate ester. (7.97 g, 31.13 mmol, 44%). ^{1}H NMR ($CDCl_3$): δ 7.33−7.26 (m, 5H), 4.57 (s, 2H), 4.34 (m, 1H), 3.76 (d, J = 10.8 Hz, 3H), 3.56 (m, 2H), 2.23 (m, 1H), 2.09 (m, 1H), 1.96 (m, 1H), 1.85 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 137.6 (s), 128.4 (s), 127.7 (s), 127.7 (s), 127.5 (s), 77.3 (d, J = 10.0 Hz), 73.5 (s), 72.1 (d, J = 6.1 Hz), 52.4 (d, J = 6.9 Hz), 25.8 (s), 18.3 (d, J = 121.2 Hz). ^{31}P NMR ($CDCl_3$): δ 51.04 (s). MS (CI) *m*/*z* 257.1 (M^++1, 100.00). HRMS, M^++1, Found: 257.0980. Calcd for $C_{12}H_{18}O_4P$, 257.1017.

Methyl 3,4-Dihydroxybutane 1,3-Cyclic Phosphonate (4). A solution of **3** (2.1 g, 8.20 mmol) in absolute methanol (100 mL) containing 10% Pd−C catalyst (0.83 g) was stirred at ambient temperature under hydrogen (1 atm) until gas uptake ceased (18 h). Filtration and evaporation under reduced pressure gave compound **4,** which was purified flash chromatography (acetone−hexane 2:1, R_f = 0.18) to give 1.06 g of homogeneous product (6.40 mmol, 78% yield).^{1}H NMR ($CDCl_3$): δ 4.27 (m, 1H), 3.68−3.76 (m, 2H), 3.72 (d, J = 12.0 Hz, 3H), 3.60 (m, 1H), 2.10−2.22 (m, 2H), 2.00 (m, 1H), 1.80 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 79.3 (d, J = 10.0 Hz), 64.5 (d, J = 6.1 Hz), 52.5 (d, J = 6.9 Hz), 24.9 (s), 18.5 (d, J = 120.7 Hz). ^{31}P NMR ($CDCl_3$): δ 52.11 (s). MS (CI) *m*/*z* 167.0 (M^++1, 100.00). HRMS, M^++1, Found: 167.0474. Calcd for $C_5H_{12}O_4P$, 167.0475.

Methyl 3-Hydroxyl-4-*tert*-Butyldimethylsilyloxybutane 1,3-Cyclic Phosphonate (5). Alcohol **4** (0.420 g, 2.53 mmol) was dissolved in anhydrous DMF (10 mL) and stirred with imidazole (0.206 g, 3.04 mmol, 1.2 equiv) and *tert*-butyldimethylsilyl chloride (TBSCl) (0.420 g, 2.78 mmol, 1.1 equiv) for 24 h at room temperature. The solution was diluted with water (5 mL) and ethyl acetate (20 mL), and the aqueous layer was separated and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried with Na_2SO_4 and concentrated in vacuo, and the residue was purified by FC (hexanes−ethyl acetate 2:1, R_f = 0.40) to afford 0.392 g of the TBDMS ether **5** as a colorless liquid (1.40 mmol, 55%). ^{1}H NMR (CD_3Cl): δ 4.22 (m, 1H), 3.76−3.71 (m, 5H), 2.24−2.06 (m, 2H), 1.97−1.74 (m, 2H), 0.84 (s, 9H), 0.02 (m, 6H). ^{13}C NMR (CD_3Cl): δ 78.2 (d, J = 9.2 Hz), 67.2 (d, J = 6.1 Hz), 52.1 (d, J = 6.9 Hz), 25.6 (s), 25.5 (d, J = 23.0 Hz), 18.7 (d, J = 122.7 Hz), 18.1 (s), −5.6 (s), −5.7 (s). ^{31}P NMR ($CDCl_3$): δ 113.43 (s). MS (CI) *m*/*z* 281.2 (M^++1, 100.00). HRMS, M^++1, Found: 281.1342. Calcd for $C_{11}H_{26}O_4PSi$, 281.1346.

Methyl 3-Hydroxyl-4-*tert*-butyldimethylsilyloxybutane 1,3-Cyclic Phosphonothioate (6). A solution of **5** (0.553 g, 1.98 mmol) and Lawesson's Reagent (2,4-bis(*p*-methoxyphenyl)-1,3-dithiadiphosphetane-2,4-disulfide) (0.44 g, 1.09 mmol) in toluene (3 mL) was stirred and heated at reflux for 4 h. The reaction mixture was washed by water (3 mL) and extracted with toluene (3 × 3 mL). The combined extracts were dried over anhydrous Na_2SO_4 and filtered, the solvent was removed in a vacuum, and the residue was purified by FC (EtOAc:hexane, 1:10, R_f = 0.30) to give 0.39 g of **6** (1.320 mmol, 67% yield) as a colorless liquid.^{1}H NMR (CD_3Cl): δ 4.37 (m, 1H), 3.67−3.71 (m, 5H), 2.12−2.32 (m, 4H), 0.85 (s, 9H), −0.05 (s, 6H). ^{13}C NMR (CD_3Cl): δ 81.5 (d, J = 3.9 Hz), 65.4 (d, J = 6.8 Hz), 52.4 (d, J = 6.9 Hz), 29.2 (d, J = 84.5 Hz), 25.7 (s), −0.1 (s), −5.4 (s), −5.6 (s). ^{31}P NMR (CD_3Cl): δ 113.43 (s). MS (CI) *m*/*z* 297.1 (M^++1, 100.00). HRMS, M^++1, Found: 297.1128. Calcd for $C_{11}H_{26}O_3PSSi$, 297.1146.

Methyl 3,4-Dihydroxyl-butane 1,3-Cyclic Phosphonothioate (7). A solution of **6** (143 mg, 0.48 mmol) in THF (8 mL) was treated consecutively with acetic acid (83 μL, 1.45 mmol) and tetrabutylammonium fluoride trihydrate (457 mg, 1.45 mmol) at room

temperature. After the solution was stirred for 18 h, the reaction was complete (TLC). The solvent was then evaporated under reduced pressure, and the crude product was purified by FC on a short column (acetone:hexane, 3:2, $R_f = 0.45$) to afford 61 mg of a colorless liquid (0.33 mmol, 69% yield). ^{1}H NMR (CD_3Cl): δ 4.30 (m, 1H), 3.55−3.76 (m, 5H), 2.92 (m, 1H), 2.02−2.31 (m, 5H). ^{13}C NMR (CD_3Cl): δ 82.2 (d, $J = 3.8$ Hz), 64.5 (d, $J = 6.1$ Hz), 52.5 (d, $J = 6.8$ Hz), 29.5 (d, $J = 92.5$ Hz), 25.4 (s). ^{31}P NMR (CD_3Cl): δ 113.99 (s). MS (CI) *m*/*z* 183.0 (M^++1, 100.00). HRMS, M^++1, Found: 183.0245. Calcd for $C_5H_{12}O_3PS$, 183.0246.

Methyl 3-Hydroxyl-4-oleoyloxylbutane 1,3-Cyclic Phosphonothioate (8a). To a solution of alcohol **7** (14 mg, 0.077 mmol) and oleic acid (24 mg, 0.133 mmol) in dry CH_2Cl_2 (2 mL) at room temperature was added dropwise a solution of DCC (19 mg, 0.093 mmol) and DMAP (4 mg, 0.038 mmol) in dry CH_2Cl_2 (2 mL). The solution was stirred at room temperature for 16 h and filtered, the solvent was removed, and the residue was purified by FC (*n*-hexanes−EtOAc 2:1, $R_f = 0.40$) to afford 26 mg (0.059 mmol, 77%) of **8a** as a colorless liquid. ^{1}H NMR (CD_3Cl): δ 5.29 (m, 2H), 4.42 (m, 1H), 4.26 (m, 1H), 4.07 (m, 1H), 3.72 (d, $J = 12.0$ Hz, 3H), 1.60−2.35 (m, 10H), 1.23−1.27 (m, 22H), 0.85 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (CD_3Cl): δ 173.5 (s), 130.0 (s), 129.7 (s), 78.7 (d, $J = 10.7$ Hz), 65.4 (d, $J = 6.9$ Hz), 52.6 (d, $J = 6.9$ Hz), 34.0 (s), 33.4 (s), 31.9 (s), 29.7 (s), 29.7 (s), 29.5 (s), 29.3 (s), 29.1 (s), 29.1 (s), 27.2 (s), 27.1 (s), 25.7 (s), 24.8 (s), 22.7 (s), 19.1 (s), 17.9 (s), 14.1 (s). ^{31}P NMR (CD_3Cl): δ 112.96 (s). MS (CI) *m*/*z* 447.1 (M^+, 100.00). HRMS, M^+, Found: 447.2632. Calcd for $C_{23}H_{43}O_4PS$, 447.2648.

3-Hydroxyl-4-oleoyloxylbutane 1,3-Cyclic Phosphonothioate (9a). A solution of **8a** (18 mg, 0.04 mmol) in 3 mL of *tert*-butylamine was refluxed for 48 h. The excess *tert*-butylamine was removed by evaporation, and the resulting residue was purified by FC (CH_2Cl_2:CH_3OH:H_2O, 8:1:0.05, $R_f = 0.14$). Further purification was performed by open column on Dowex 50WX8 100−200 (H^+ form, $CHCl_3$) and afforded 14 mg of a colorless liquid (0.03 mmol, 75% yield.) ^{1}H NMR (CD_3OD): δ 5.34 (m, 2H), 4.44 (m, 1H), 4.20 (dd, $J = 12.0$, 3.2 Hz, 1H), 4.09 (dd, $J = 11.6$, 6.0 Hz, 1H), 2.35 (t, $J = 8.0$ Hz, 2H), 2.10−2.20 (m, 2H), 2.02 (m, 4H), 1.61 (m, 2H), 1.31 (m, 22H), 0.89 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.2 (s), 128.9 (s), 128.8 (s), 76.0 (s), 65.6 (s), 33.0 (s), 31.1 (s), 28.9 (s), 28.8 (s), 28.6 (s), 28.5 (s), 28.3 (s), 28.2 (s), 26.1 (s), 24.0 (s), 21.8 (s), 12.5 (s). ^{31}P NMR (CD_3OD): δ 93.88 (s). MS (CI) *m*/*z* 433.3 (M^++1, 100.00). HRMS, M^++1, Found: 433.2544. Calcd for $C_{22}H_{41}O_4PS$, 433.2547.

Methyl 3-Hydroxyl-4-palmitoyloxylbutane 1,3-Cyclic Phosphonothioate (8b). To a solution of alcohol **7** (22 mg, 0.12 mmol) with palmatic acid (34 mg, 0.13 mmol) in dry CH_2Cl_2 (2 mL) at room temperature was added dropwise a solution of DCC (30 mg, 0.15 mmol) and DMAP (7 mg, 0.06 mmol) in dry CH_2Cl_2 (2 mL). The solution was stirred at room temperature for 16 h and filtered, the solvent was removed, and the residue was purified by FC (*n*-hexanes−EtOAc 5:1, $R_f = 0.21$) to afford 41 mg (0.10 mmol, 73%) of **8b** as a white solid. ^{1}H NMR (CD_3Cl): δ 4.53 (m, 1H), 4.28 (dd, $J = 12.4$, 3.6 Hz, 1H), 4.09 (dd, $J = 11.6$, 5.2 Hz, 1H), 3.73 (d, $J = 12.0$ Hz, 3H), 2.10−2.20 (m, 2H), 2.02 (m, 2H), 1.58 (m, 2H), 1.23−1.27 (m, 24H), 0.85 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (CD_3Cl): δ 173.5 (s), 78.9 (d, $J = 5.3$ Hz), 65.5 (d, $J = 6.8$ Hz), 52.7 (d, $J = 6.1$ Hz), 34.1 (s), 31.9 (s), 29.8 (s), 29.7 (s), 29.6 (s), 29.4 (s), 29.3 (s), 29.2 (s), 29.1 (s), 28.9 (s), 26.4 (s), 24.8 (s), 24.7 (s), 14.1 (s). ^{31}P NMR (CD_3Cl): δ 112.98 (s). MS (CI) *m*/*z* 421.2 (M^++1, 100.00). HRMS, M^++1, Found: 421.2544. Calcd for $C_{22}H_{41}O_4PS$, 421.2564.

3-Hydroxyl-4-palmitoyloxylbutane 1,3-Cyclic Phosphonothioate (9b). A solution of **8b** (38 mg, 0.090 mmol) in 3 mL of *tert*-butylamine was refluxed for 72 h. The excess *tert*-butylamine was removed by evaporation, and the resulting residue was purified on silica gel (CH_2Cl_2/CH_3OH, 4:1, $R_f = 0.49$). Further purification was performed by open column on Dowex 50WX8 100−200 (H^+ form, $CHCl_3$) and afforded 23 mg of a colorless liquid (0.056 mmol, 62% yield). ^{1}H NMR ($CDCl_3$): δ 7.96 (br, 1H), 4.52 (m, 1H), 4.32 (dd, $J = 12.0$, 3.2 Hz, 1H), 4.08 (dd, $J = 11.6$, 6.0 Hz, 1H), 2.32 (t, $J = 8.0$ Hz, 2H), 2.10−2.20 (m, 2H), 2.02 (m, 2H), 1.58 (m, 2H), 1.22 (m, 24H), 0.86 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.0 (s), 66.1 (s), 65.5 (s), 34.3 (s), 33.4 (s), 31.9 (s), 29.7 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 26.9 (s), 14.1 (s). ^{31}P NMR ($CDCl_3$): δ 94.32 (s). MS (CI) *m*/*z* 407.1 (M^++1, 100.00). HRMS, M^++1, Found: 407.2397. Calcd for $C_{20}H_{40}O_4PS$, 407.2409.

Diethyl 1- and 1,1-Difluoro-(3*S*)-4-hydroxybutylphosphonate (10a and 10b). These compounds were prepared as previously described.[17]

1-Fluoro-(3*S*),4-dihydroxybutylphosphonate (11a). The phosphonate **10a** (46 mg, 0.189 mmol) in 5 mL flask was dried in vacuo. Anhydrous TMS bromide (0.25 mL, 1.89 mmol) and CH_2Cl_2 (0.5 mL) were added into the flask. The solution was stirred at room temperature for 5 h. TMS bromide and volatile products were evaporated under high vacuum during 6 h. The residue was dissolved in MeOH/H_2O (95%, 1.0 mL) and stirred for 30 min at room temperature, and then thoroughly dried to provide 33 mg of an oily product (0.176 mmol, 93%). ^{1}H NMR ($CDCl_3$): δ 4.90 (m, 1H), 3.85 (m, 1H), 3.50 (m, 2H), 2.05 (m, 2H), 2.18−2.03 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 87.7 (m), 70.1 (d, $J = 8.3$ Hz), 68.4 (d, $J = 13.1$ Hz), 67.5 (s), 66.6 (s), 35.3 (m). ^{19}F NMR ($CDCl_3$): δ −207.39 (1F, m), −212.58 (1F, m). ^{31}P NMR ($CDCl_3$): δ 17.57 (d, $J = 76.10$ Hz), 18.00 (d, $J = 75.1$ Hz). MS (CI) *m*/*z* 188.0 (M^++1, 79.05). HRMS, M^+-H_2O, Found: 171.0223. Calcd for $C_4H_9FO_5P$, 171.0223.

1-Fluoro-(3*S*)-hydroxyl-4-oleoyloxylbutane 1,3-Cyclic Phosphonate (13a). The phosphonate **11a** (29 mg, 0.154 mmol) in 100 mL flask was dried in vacuo. Anhydrous DMF (40 mL) was added into the flask, followed by addition of dicyclohexylcarbodiimide (DCC, 1.0 M CH_2Cl_2 solution, 1.4 equiv., 0.216 mL). The solution was stirred at room temperature for 16 h at which point water (0.2 mL) was added and stirred for additional 10 min, and then all solvent was removed under high vacuum. The residue was dissolved in anhydrous pyridine (2 mL), and oleyl chloride (0.084 mL, 1.4 equiv) was added. After stirred for 16 h at room temperature, the solvent was removed under vacuum. The resulting residue was purified by FC ($CHCl_3$:CH_3OH:H_2O, 65:25:4, $R_f = 0.30$). Further purification was performed by open column on Sephedex LH-20 ($CHCl_3$:CH_3OH, 7:3) and afforded 43 mg of a colorless liquid (0.100 mmol, 65% yield). ^{1}H NMR (CD_3OD): δ 5.23 (m, 2H), 4.67 (m, 1H), 4.30 (m, 1H), 4.03 (m, 2H), 2.25 (m, 2H), 1.92 (m, 4H), 1.50 (m, 2H), 1.20 (m, 22H), 0.77 (m, 3H). ^{19}F NMR (CD_3OD): δ -198.86 (1F, m), −203.94 (1F, m). ^{31}P NMR (CD_3OD): δ 29.50 (d, $J = 72.9$ Hz), 30.10 (d, $J = 75.1$ Hz). MS HRMS (MALDI), M^++Na, Found: *m*/*z* 457.2537. Calcd for $C_{22}H_{39}FO_5PNa$, 457.2490.

1,1-Difluoro-(3*S*)-4-dihydroxybutylphosphonate (11b). The phosphonate **10b** (34 mg, 0.130 mmol) in 5 mL flask was dried in vacuo. Anhydrous TMS bromide (0.23 mL, 1.65 mmol) and CH_2Cl_2 (0.2 mL) were added into the flask. The solution was stirred at room temperature for 5 h. TMS bromide and volatile products were evaporated under high vacuum during 6 h. The residue was dissolved in MeOH/H_2O (95%, 1.0 mL) and stirred for 30 min at room temperature and then thoroughly dried to provide the oily product (20 mg, 0.097 mmol, 75%). ^{1}H NMR ($CDCl_3$): δ 4.08 (m, 1H), 3.50 (m, 2H), 2.20 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 65.4 (s), 65.1 (s), 36.7 (m). ^{19}F NMR ($CDCl_3$): δ −113.90 (2F, m). ^{31}P NMR ($CDCl_3$): δ 6.93 (t, $J = 245.0$ Hz). MS (CI) *m*/*z* 207.0 (M^++1, 68.00). HRMS, M^++1, Found: 207.0245. Calcd for $C_4H_{10}F_2O_5P$, 207.0234.

1,1-Difluoro-3(*S*)-hydroxyl-4-oleoyloxylbutane 1,3-Cyclic Phosphonate (13b). The same procedure was used as for the preparation of **13a**. The resulting residue was purified by FC ($CHCl_3$:CH_3OH:H_2O, 65:25:4, $R_f = 0.50$). Further purification was performed by open column on Sephadex LH-20 ($CHCl_3$:CH_3OH, 7:3) and to give a colorless liquid(49% yield). ^{1}H NMR (CD_3OD): δ 5.35 (m, 2H), 4.38 (m, 1H), 2.55 (m, 2H), 2.35 (*t*, $J = 7.6$ Hz, 2H), 2.02 (m, 4H), 1.61 (m, 2H), 1.29 (m, 22H), 0.90 (*t*, $J = 7.2$ Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.8 (s), 129.7 (s), 129.6 (s), 68.7 (s), 65.7 (s), 37.0 (m), 33.6 (s), 31.9 (s), 29.7 (s), 29.6 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 29.1 (s), 29.0 (s), 26.9 (s), 24.7 (s), 22.6 (s), 13.3

(s). ^{19}F NMR (CD_3OD): δ −115.0 (2F, m). ^{31}P NMR (CD_3OD): δ 12.97 (s). MS HRMS (MALDI), M^++Na, Found: 475.2505. Calcd for $C_{22}H_{39}F_2O_5PNa$, 475.2401.

pK_a Determination of ccPA and Its Analogues in Methanol−Water Mixture. All titrations were performed by using AR-15 pH meter (Fisher Scientific) in solutions of 0.10 M NaCl under argon atmosphere at 25 ± 0.5 °C using standardized 0.5 M HCl or 0.05 M KOH titrants. The electrode calibrations was followed the procedure.[41,42] Sample solutions were prepared from 2 to 3.5 mM. For the methanol−water experiments, 10−40 wt % methanol were utilized. Sample solutions were preacidified to below pH 3.0 using 0.5 M HCl and titrated alkalimetrically to between 9.5 and 11.0.

High Throughput Ca^{2+} Measurements. RH7777 cells stably expressing either LPA_1, LPA_2, or LPA_3 were plated on poly-D-lysine-coated culture wells at a density of 50000 cells/well, and cultured overnight. The culture medium was then replaced with modified Krebs solution (120 mM NaCl, 5 mM KCl, 0.62 mM $MgSO_4$, 1.8 mM $CaCl_2$, 10 mM HEPES, 6 mM glucose, pH 7.4), and the cells were serum starved for 6 h. Cells were loaded with Fura-2 AM for 35 min in modified Krebs medium. Changes in intracellular Ca^{2+} concentration were monitored as described[52] by measuring the ratio of emitted light intensity at 520 nm in response to excitation by 340 and 380 nm wavelength lights, respectively. Each well was monitored for 80 s. 50 μL of the test compound (3X stock solution in modified Krebs) was added automatically to each well 15 s after the start of the measurement. Time courses were recorded using the SoftMax Pro software (Molecular Devices, Sunnyvale, CA). Ca^{2+} transients were quantified automatically by calculating the difference between maximum and baseline ratio values for each well.

Acknowledgment. We thank the Human Frontier Science Program (RG0073-2000B) and the NIH (HL070231 and NS29632) for support to G.D.P., and the NIH for CA921160 to G.T.

References

(1) Murakami-Murofushi, K.; Shioda, M.; Kaji, K.; Yoshida, S.; Murofushi, H. Inhibition of eukaryotic DNA polymerase a with a novel lysophosphatidic acid (PHYLPA) isolated from myxoamebae of *Physarum* polycephalum. *J. Biol. Chem.* **1992**, *267*, 21512−21517.

(2) Takahashi, Y.; Shimada, Y.; Shioda, M.; Yoshida, S.; Murofushi, H. et al. Isolation of a new species of Physarum lysophosphatidic acid, PHYLPA, and its effect on DNA polymerase activity. *Cell Struct. Funct.* **1993**, *18*, 135−138.

(3) Murakami-Murofushi, K.; Kaji, K.; Kano, K.; Fukuda, M.; Shioda, M. et al. Inhibition of cell proliferation by a unique lysophosphatidic acid, PHYLPA, isolated from Physarum polycephalum: Signaling events of antiproliferative action by PHYLPA. *Cell Struct. Funct.* **1993**, *18*, 363−370.

(4) Kobayashi, S.; Tokunoh, R.; Shibasaki, M.; Shinagawa, R.; Murakami-Murofushi, K. Synthesis of 1-O-acylglycerol 2,3-cyclic phosphate: determination of the absolute structure of PHYLPA, a specific inhibitor of DNA polymerase a. *Tetrahedron Lett.* **1993**, *34*, 4047−4050.

(5) Murakami-Murofushi, K.; Kobayashi, S.; Onimura, K.; Maysumoto, M.; Shioda, M. et al. Selective inhibition of DNA polymerase-α family with chemically synthesized derivatives of PHYLPA, a unique Physarum lysophosphatidic acid. *Biochim. Biophys. Acta* **1995**, *1258*, 57−60.

(6) Fischer, D. J.; Liliom, K.; Guo, H.; Nusser, N.; Virag, T. et al. Naturally occurring analogs of lysophosphatidic acid elicit different cellular responses through selective activation of multiple receptor subtypes. *Mol. Pharmacol.* **1998**, *54*, 979−988.

(7) Mukai, M.; Imamura, F.; Ayaki, M.; Shinkai, K.; Iwasaki, T. et al. Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA). *Int. J. Cancer* **1999**, *81*, 918−922.

(8) Gueguen, G.; Gaige, B.; Grevy, J.; Rogalle, P.; Bellan, J. et al. Structure−activity analysis of the effects of lysophosphatidic acid on platelet aggregation. *Biochemistry* **1999**, *38*, 8440−8450.

(9) Liliom, K.; Murakami-Murofushi, K.; Kobayashi, S.; Murofushi, H.; Tigyi, G. Xenopus oocytes express multiple receptors for LPA-like lipid mediators. *Am. J. Physiol.* **1996**, *270*, C772−C777.

(10) Kobayashi, T.; Tanaka-Ishii, R.; Taguchi, R.; Ikezawa, H.; Murakami-Murofushi, K. Existence of a bioactive lipid, cyclic phosphatidic acid, bound to human serum albumin. *Life Sci.* **1999**, *65*, 2185−2191.

(11) Ishihara, R.; Tatsuta, M.; Iishi, H.; Baba, M.; Uedo, N. et al. Attenuation by cyclic phosphatidic acid of peritoneal metastasis of azoxymethane-induced intestinal cancers in Wistar rats. *Int. J. Cancer* **2004**, *110*, 188−193.

(12) Mukai, M.; Kobayashi, S.; Murofushi, H.; Murofushi, K. Cancerous metastasis inhibitors containing carbacyclic phosphatidic acid derivatives. PCT Int., 2002; pp WO 0294286.

(13) Prestwich, G. D.; Xu, Y.; Qian, L.; Gajewiak, J.; Jiang, G. New metabolically stabilized analogues of lysophosphatidic acid: Agonists, antagonists, and enzyme inhibitors. *Biochem. Soc. Trans.* **2005**, *33*, 1357−1361.

(14) Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B. et al. Enantioselective responses to OMPT, a phosphorothioate analogue of lysophosphatidic acid with LPA3 receptor-selective agonist activity. *J. Med. Chem.* **2003**, *46*, 5575−5578.

(15) Qian, L.; Xu, Y.; Simper, T. D.; Jiang, G.; Aoki, J. et al. Synthesis of novel alkyl thiophosphate LPA analogues as potential antagonists and agonists for lysophosphatidic acid (LPA) receptors. *ChemMedChem.* **2006**, *1*, 376−383.

(16) Xu, Y.; Aoki, J.; Shimizu, K.; Umezu-Goto, M.; Hama, K. et al. Structure−activity relationships of fluorinated lysophosphatidic acid analogues. *J. Med. Chem.* **2005**, *48*, 3319−3327.

(17) Xu, Y.; Qian, L.; Prestwich, G. D. Synthesis of monofluorinated analogues of lysophosphatidic acid. *J. Org. Chem.* **2003**, *68*, 5320−5330.

(18) Berkowitz, D. B.; Bose, M. (α-Monofluoroalkyl)phosphonates: a class of isoacidic and tunable mimics of biological phosphates. *J. Fluorine Chem.* **2001**, *112*, 13−33.

(19) Parrill, A. L. Structural characteristics of lysophosphatidic acid biological targets. *Biochem. Soc. Trans.* **2005**, *33*, 1366−1369.

(20) Tigyi, G.; Parrill, A. L. Molecular mechanisms of lysophosphatidic acid action. *Prog. Lipid. Res.* **2003**, *42*, 498−526.

(21) Feng, L.; Mills, G. B.; Prestwich, G. D. Modulators of lysophosphatidic acid signaling. *Expert Opin. Ther. Pat.* **2003**, *13*, 1619−1634.

(22) Mills, G. B.; Moolenaar, W. H. The emerging role of lysophosphatidic acid in cancer. *Nat. Rev. Cancer* **2003**, *3*, 582−591.

(23) Lu, H.; Berkman, C. E. Stereoselective inhibition of glutamate carboxypeptidase by chiral phosphonothioic acids. *Bioorg. Med. Chem.* **2001**, *9*, 395−402.

(24) Berkowitz, D. B.; Bose, M.; Asher, N. G. A convenient triflate displacement approach to (α-monofluoroalkyl)phosphonates. *Org. Lett.* **2001**, *3*, 2009−2012.

(25) Wnuk, S. F.; Robins, M. J. Stannyl radical mediated ceavage of p-deficient heterocyclic sulfones. Synthesis of α-fluoro esters and the first homonucleoside a-fluoromethylene phosphonate. *J. Am. Chem. Soc.* **1996**, *118*, 2519−2520.

(26) Xu, Y.; Qian, L.; Prestwich, G. D. Synthesis of α-fluorinated phosphonates from α-fluorovinylphosphonates: a new route to analogues of lysophosphatidic acid. *Org. Lett.* **2003**, *5*, 2267−2270.

(27) Xu, Y.; Prestwich, G. D. Synthesis of chiral (α, α-difluoroalkyl)-phosphonate analogues of (lyso)phosphatidic acid via hydrolytic kinetic resolution. *Org. Lett.* **2002**, *4*, 4021−4024.

(28) Borecka, B.; Chojnowski, J.; Cypryk, M.; Michalski, J.; Zielinska, J. Synthetic and mechanistic aspects of the reaction of trialkylsilyl halides with thio and seleno esters of phosphorus. *J. Organomet. Chem.* **1979**, *171*, 17−34.

(29) Piettre, S. R.; Raboisson, P. Easy and general access to α, α-difluoromethylenephosphonothioic acids. A new class of compounds. *Tetrahedron Lett.* **1996**, *37*, 2229−2232.

(30) Li, Z.; Racha, S.; Dan, L.; El-Subbagh, H.; Abushanab, E. A general and facile synthesis of β- and γ-hydroxy phosphonates. *J. Org. Chem.* **1993**, *58*, 5779−5783.

(31) Polozov, A. M.; Cremer, S. E.; Fanwick, P. E. Stereospecific thionation of 2-ethoxy-1,2-oxaphosphorinane 2-oxide and its derivatives with Lawesson's reagent. *Can. J. Chem.* **1999**, *77*, 1274−1280.

(32) Reiff, L. P.; H. S., A. Stereospecific synthesis and reactions of optically active isopropyl methylphosphinate. *J. Am. Chem. Soc.* **1970**, *92*, 6391−6392.

(33) Hermans, R. J. M.; Buck, H. M. Influence of phosphorous derivatization on the conformational behavior of model compounds for 3′,5′-xylo-cAMP studied by ^1H NMR spectroscopy. *J. Org. Chem.* **1988**, *53*, 2077−2083.

(34) Lin, G.; Tsai, M. D. Phospholipids chiral at phosphorus. 18. Stereochemistry of phosphatidylinositide-specific phospholipase C. *J. Am. Chem. Soc.* **1989**, *111*, 3099−3101.

(35) Lal, G. S.; Pez, G. P.; Syvret, R. G. Electrophilic NF fluorinating agents. *Chem. Rev.* **1996**, *96*, 1737−1755.

(36) Viztiu, D.; Kriste, A. G.; Campbell, A. S.; Thatcher, G. R. J. Inhibition of phosphatidylinositol-specific phospholipase C: studies on synthetic substrates, inhibitors and a synthetic enzyme. *J. Mol. Recogn.* **1996**, *9*, 197−209.

(37) Ukita, T.; Bates, N. A.; Carter, H. E. Studies on the alkaline hydrolysis of lecithin: synthesis of cyclic 1,2-glycerophosphate. *J. Biol. Chem.* **1955**, *216*, 867−874.
(38) Khorana, H. G.; Tener, G. M.; Wright, R. S.; Moffatt, J. G. Cyclic phosphates. III. Some general observations on the formation and properties of five-, six- and seven-membered cyclic phosphate esters. *J. Am. Chem. Soc.* **1957**, *79*, 430−436.
(39) Chambers, R. D.; Jaouhar, R.; O′Hagan, D. Fluorine in enzyme chemistry. Part 2. The preparation of difluoromethylenephosphonate analogues of glycolytic phosphates approaching an isosteric and isoelectronic phosphate mimic. *Tetrahedron* **1989**, *45*, 5101−5108.
(40) Nieschalk, J.; Batsanov, A. S.; O'Hagan, D.; Howard, J. A. K. Synthesis of monofluor- and difluoro-methylenephosphonate analogues of sn-glycero-3-phosphate as substrates for glycerol-3-phosphate dehydrogenase and the X-ray structure of the fluoromethylenephosphonate moiety. *Tetrahedron* **1996**, *52*, 165−176.
(41) Avdeef, A.; Box, K. J.; Comer, J. E. A.; Gilges, M.; Hadley, M. et al. pH-metric log P 11. pK_a determination of water-insoluble drugs in organic solvent−water mixtures. *J. Pharm. Biomed. Anal.* **1999**, *20*, 631−641.
(42) Avdeef, A.; Comer, J. E. A.; Thomson, S. J. pH-Metric log P. 3. glass electrode calibration in methanol−water, applied to pK_a determination of water-insoluble substances. *Anal. Chem.* **1993**, *65*, 42−49.
(43) Fischer, D. J.; Nusser, N.; Virag, T.; Yokoyama, K.; Wang, D. A. et al. Short-chain phosphatidates are subtype-selective antagonists of lysophosphatidic acid receptors. *Mol. Pharmacol.* **2001**, *60*, 776−784.
(44) Virag, T.; Elrod, D. B.; Liliom, K.; Sardar, V. M.; Parrill, A. L. et al. Fatty alcohol phosphates are subtype-selective agonists and antagonists of lysophosphatidic acid receptors. *Mol. Pharmacol.* **2003**, *63*, 1032−1042.
(45) Fujiwara, Y.; Sardar, V.; Tokumura, A.; Baker, D.; MurakamiMurofushi, K. et al. Identification of residues responsible for ligand recognition and regioisomeric selectivity of lysophosphatidic acid receptors expressed in mammalian cells. *J. Biol. Chem.* **2005**, *280*, 35038−35050.
(46) Aoki, J.; Taira, A.; Takanezawa, Y.; Kishi, Y.; Hama, K. et al. Serum lysophosphatidic acid is produced through diverse phospholipase pathways. *J. Biol. Chem.* **2002**, *277*, 48737−48744.
(47) Fleming, I. N.; Elliott, C. M.; Collard, J. G.; Exton, J. H. Lysophosphatidic acid induces threonine phosphorylation of Tiam1 in Swiss 3T3 fibroblasts via activation of protein kinase C. *J. Biol. Chem.* **1997**, *272*, 33105−33110.
(48) Gschwind, A.; Prenzel, N.; Ullrich, A. Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation. *Cancer Res.* **2002**, *62*, 6329−6336.
(49) Heise, C. E.; Santos, W. L.; Schreihofer, A. M.; Heasley, B. H.; Mukhin, Y. V. et al. Activity of 2-substituted lysophosphatidic acid (LPA) analogues at LPA receptors: discovery of a LPA1/LPA3 receptor antagonist. *Mol. Pharmacol.* **2001**, *60*, 1173−1180.
(50) Santos, W. L.; Heasley, B. H.; Jarosz, R.; Carter, K. M.; Lynch, K. R. et al. Synthesis and biological evaluation of phosphonic and thiophosphoric acid derivatives of lysophosphatidic acid. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3473−3476.
(51) Ohta, H.; Sato, K.; Murata, N.; Damirin, A.; Malchinkhuu, E. et al. Ki16425, a subtype-selective antagonist for EDG-family lysophosphatidic acid receptors. *Mol. Pharmacol.* **2003**, *64*, 994−1005.
(52) Durgam, G. G.; Virag, T.; Walker, M.; Tsukahara, R.; Yasuda, S. et al. Synthesis, structure−activity relationships, and biological evaluation of fatty alcohol phosphates as lysophosphatidic acid receptor ligands, activators of PPARγ, and inhibitors of autotaxin. *J. Med. Chem.* **2005**, *48*, 4919−4930.

JM060351+