Structure−Activity Relationships of Lysophosphatidic Acid: Conformationally Restricted Backbone Mimetics

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Lysophosphatidic acid (LPA) has associated with it an intriguing cell biology that is thought to be mediated through its interaction with G-protein coupled receptor(s). In an effort to extend the structure−activity relationships of LPA, we have produced a series of LPA analogues in which the glycerol core in LPA was replaced with conformationally restricted aryl substructures. The aryl substructures encompassed aminophenol, resorcinol, dihydroxy benzophenone, and tocopherol systems. The benzophenone moiety was investigated both as a conformationally restricting substructure for LPA and as a possible photoreactive alkylating agent for the LPA receptor(s). All LPA analogues were evaluated for their potency and efficacy in mobilizing calcium ions from internal stores in MDA MB-231 cells. Ten of the 14 analogues exhibited activity in this assay at doses up to 5 μM; none of the compounds exhibited nonreceptor-mediated lytic activity at this maximal concentration. The receptor response showed surprising tolerance for manipulation in the backbone region of LPA, although none of the compounds were equipotent to LPA. This tolerance for a variety of structures has given us new leads into the realization of novel agonists and antagonists of the LPA receptor(s).

Introduction

Lysophosphatidic acid (LPA) (Figure 1) has received increasing attention due to the variety of biological responses that it evokes, including platelet aggregation, smooth-muscle contraction, cell morphology changes, and mitogenic effects.[1] There exists evidence suggesting that LPA is the endogenous ligand for one or more G-protein coupled receptors.[2−4] As part of our continued interest in the structure−activity relationships (SARs) of LPA and related lipid phosphoric acid mediators, we have been investigating the effects on receptor-mediated responses of alterations in the three substructural domains of LPA. The substructural domains of LPA—the "LPA pharmacophore"—may be postulated to consist of the phosphate "head" group, a "linker" region illustrated by glycerol in LPA, and a lipophilic "tail" illustrated by the fatty acyl chain of LPA (Figure 1). The studies reported here and our previous studies have used calcium mobilization and/or inhibition of adenylyl cyclase in cultured MDA MB-231 cells as a measure of receptor-mediated responses.

Our previous SAR studies have shown that the phosphate group is critical for activity, since all of the phosphate surrogates examined to date have resulted in sharply diminished activities in calcium mobilization and inhibition of adenylyl cyclase[5] (unpublished data). In contrast, our studies of the lipophilic "tail" have found that while oleic acid exhibits the greatest potency, considerable variation in the fatty acyl chain length and level of unsaturation can be tolerated, reminiscent of PAF (unpublished data).[6] We have also investigated a family of 2−5 carbon analogues of the glycerol "linker" subunit, and we found that most of these analogues exhibit significantly depressed activities.[7] However, the glycerol subunit can be replaced by the *N*-acyl system without loss of either potency or efficacy[5] and by *N*-acyl-L-serine with only a slight loss in activity. Interestingly, the *N*-acyl-D-serine-based analogue is significantly less active than LPA.[8]

To expand our knowledge concerning the SAR profile of the LPA receptor we wanted to investigate further the extent of structural tolerance permitted in the backbone region. We report now the replacement of the glycerol backbone of LPA with a variety of aromatic moieties (Figure 2). Further impetus for this synthetic effort was the hope that we would identify structures that might have antagonistic activity. Moreover, we wished to identify photoreactive alkylating agents for use in labeling putative LPA receptors. In our previous studies of LPA SAR, we determined that the hydroxyl at the 2 position of glycerol is not necessary for agonist activity; however, this functionality may play an important role in solubility.[5] The most successful compound was realized by the replacement of the glycerol backbone with ethanolamine. The proton of the amide functionality in the *N*-acyl ethanolamine analogue might replace a requirement that the hydroxyl proton on the glycerol backbone fulfills, since both protons are five bonds away from the phosphorus atom (Figure 3). We also determined that fatty acid chains with unsaturation are more active than saturated fatty acid chains (unpublished data). Although the activity differential between unsaturated and saturated fatty acid groups could be a consequence of binding to the receptor(s), we believe that an important contribution to this activity differential is the enhanced solubility in aqueous solutions of the unsaturated fatty acid systems relative to

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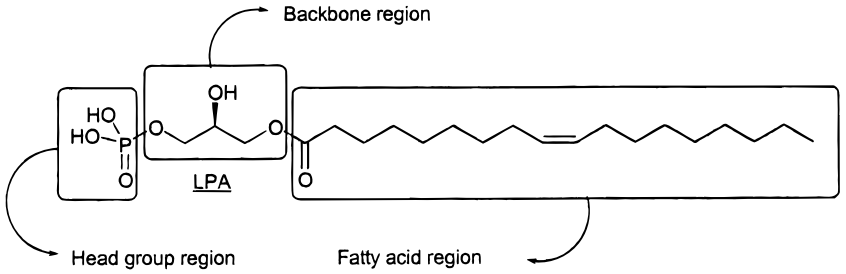


Figure 1.

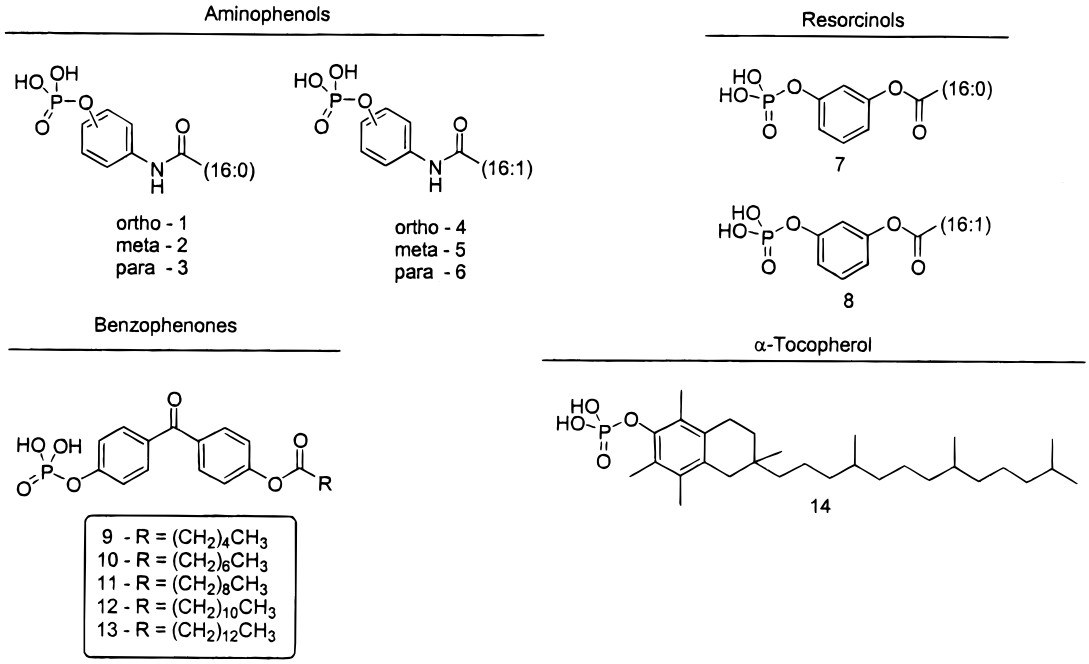


Figure 2.

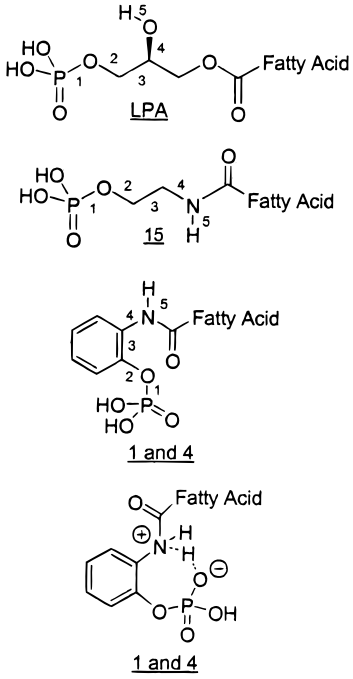


Figure 3.

their saturated counterparts, thereby enabling greater effective concentrations.

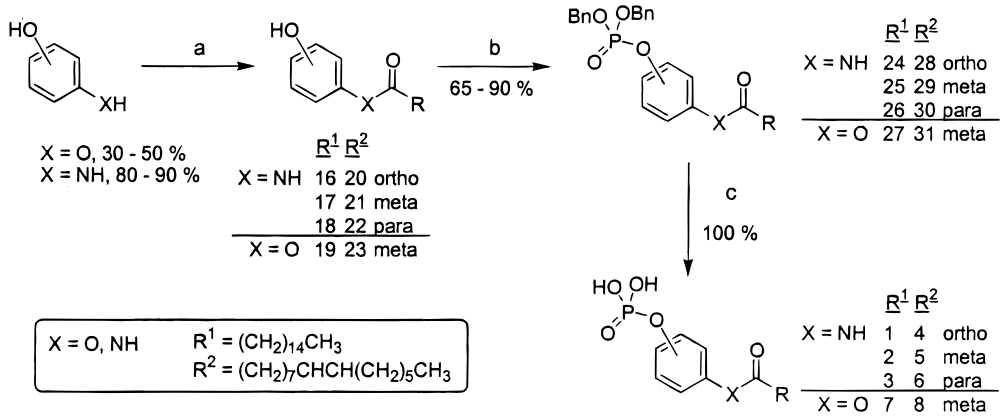
We also desired a mimetic that would be capable of alkylating the receptor. Previously, van der Bend et al.[2] produced an aryl diazirine alkylating agent that alkylated a putative LPA receptor upon photoactivation. To overcome the high reactivity and concomitant low selectivity of the carbene species generated by diazirine photodecomposition, we replaced the glycerol in the linker region of LPA with a benzophenone moiety. The benzophenone moiety has been reported to possess high selectivity for the photolabeling of proteins.[9] We hoped that these benzophenone-based systems might prove useful as photoactivated probes for the putative LPA receptor(s).

Chemistry

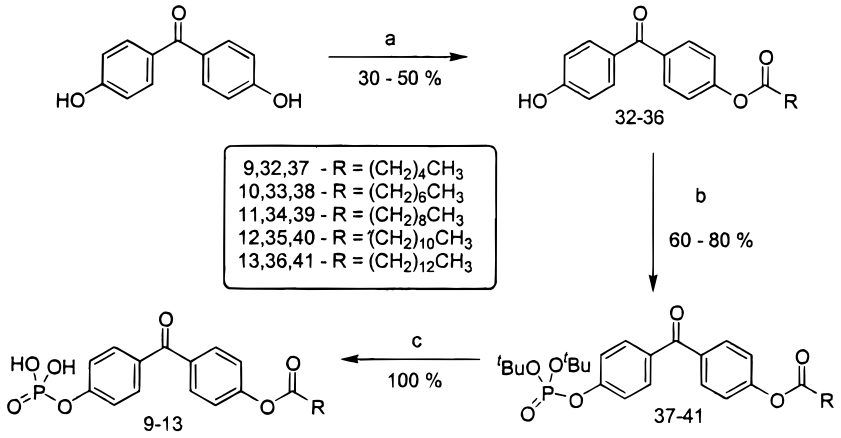
Four classes of conformationally restricted LPA analogues were synthesized. Each class possessed a different aromatic backbone in place of the traditional glycerol backbone in phospholipids. The length of the fatty acyl chains were chosen to approximate the overall length of 1-oleoyl-LPA. The synthesis of each class is described below.

The resorcinol and aminophenol backboned mimetics were synthesized by similar methods (Scheme 1). O- or N-acylation was accomplished by condensation of the appropriate acid chloride with the aryl derivatives, followed by phosphorylation of the remaining hydroxyl with dibenzyl diisopropylphosphoramidite. The benzyl protection groups on the phosphate triester were removed by hydrogenolysis to yield the desired lipid phosphate.

Scheme 1



Scheme 2



The synthesis of the benzophenone class was achieved by the monoacylation of 4,4′-dihydroxybenzophenone with the appropriate acid chloride, followed by phosphorylation of the remaining hydroxyl with di-*tert*-butyl diisopropylphosphoramidite. The removal of the protecting groups on the phosphate triester was accomplished by acid hydrolysis (Scheme 2).

The last class is the least developed of the four. α-Tocophorol was phosphorylated and deprotected as in Scheme 2. This was done to explore the further restriction of the backbone region beyond that of the previous three classes.

Results and Discussion

A series of LPA analogues **1**−**14**, in which the glycerol backbone of LPA was replaced by aminophenol, resorcinol, benzophenone, and α-tocopherol aromatic groups, were assayed for their ability to mobilize intracellular calcium in MDA MB-231 cells. Each of the aminophenol- and resorcinol-based analogues were acylated with palmitoyl or palmitoleoyl fatty acid chains to approximate the length of oleoyl-LPA, which is the most potent LPA derivative and our standard for assay. Table 1 summarizes the SAR data for calcium ion mobilization in MDA MB-231 cells, while the dose−response curves are shown in Figure 4.

The *o*-aminophenol analogues **1** and **4** were anticipated to be the most effective compounds, due to their structural similarity to N-fatty acyl ethanolamide phosphate **15** (Figure 3), which is almost equipotent to LPA (EC_{50} = 3 nM). A simple analysis suggests that these

Table 1. Relative Potencies of the 10 Active LPA Mimetics Synthesized[a]

compd	EC_{50}s (μM) MDA MB 231 cells
7	0.31 ± 0.11
2	0.46 ± 0.13
5	0.46 ± 0.05
8	0.85 ± 0.29*
3	1.56 ± 0.82*
6	1.83 ± 0.64
13	1.99 ± 0.65*
12	4.71 ± 0.68*
11	>500*
10	>500*
15	0.003
LPA	0.005

[a] The EC_{50} values with an asterisk denote those that did not reach a maximal response at the highest concentration used.

o-aminophenol derivatives **1** and **4** could be viewed as "conformationally restricted" analogues of ethanolamide phosphate **15**. However, these ortho-substituted analogues (**1** and **4**) proved to be completely inactive at the highest concentration tested (5 μM) (Table 1). The inactivity of **1** and **4** could reflect a structurally dictated and incorrect "syn" relationship of the 2-amido phosphate system, as compared to an "anti" relationship possibly required for activity. It is also possible that a hydrogen bond that can be formed between the amide functionality and the phosphate headgroup (Figure 3) serves to further "lock" the conformation of these analogues, thereby inhibiting minor structural adjustments of the system necessary to adopt the "active" conformation. In addition, such a H-bonding would

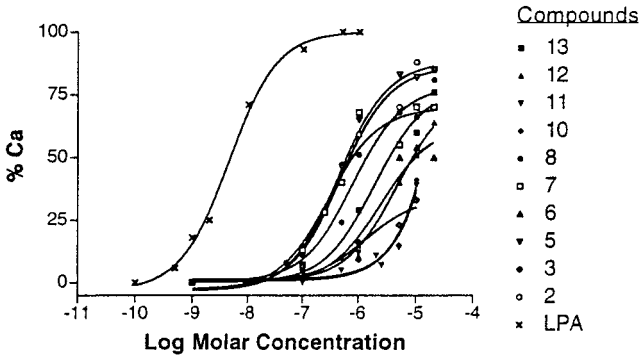


Figure 4. Dose−response curves for calcium mobilization in MDA MB-231 cells. Calcium mobilization was measured as a function of INDO-1AM fluorescence enhancement and presented as a percent of the total available calcium defined by cellular lysis with digitonin. The values for analogues were normalized to the maximal LPA response which is typically 30% of the total amount of calcium released by digitonin. All points used to calculate the EC_{50} values were done in triplicate and were curve-fitted with GraphPAD (GraphPAD software, San Diego, CA). The EC_{50} values for these curves are presented in Table 1.

depress the charge density at the phosphate headgroup along with depleting the compound of a possible hydrogen accepting site. Previous studies by other laboratories[8] and our own laboratory (unpublished data) showed that the lack of a dianionic phosphate destroys agonistic activity. We believe that the depressed activity of the ortho-substituted aminophenol derivatives relative to the aminophenol derivatives is not simply a consequence of the increased steric bulk of the "linker unit". This belief is based on the significant activity of the meta-substituted and benzophenone-based analogues discussed below.

Interestingly, the most active of the aminophenol-based compounds were the meta-substituted derivatives **2**, **5**, and **7** (Table 1). These compounds adopted a conformation that more closely mimics an "anti" relationship between the fatty acid moiety and the phosphate as viewed by molecular modeling of these analogues. In addition, compounds **2**, **5**, and **7** have a three-carbon backbone separating the phosphate and amide or ester functionalities analogous to LPA, but they lack the 2-hydroxyl moiety. The para-substituted LPA analogue **3** was less active than the meta-derivatives **2**, **5**, and **7**. This observation parallels our previous finding that as the distance between the phosphate and ester or amide functionality is increased in aliphatic backbone compounds,[5] activity is decreased.

A third set of lipid phosphate species incorporated a benzophenone moiety as a potential photoaffinity probe. The benzophenone moiety has the ability to be activated to its excited state at wavelengths not associated with protein damage (>320 nm) and is highly efficient in its ability to insert covalently into reactive C−H bonds.[9] Moreover, the benzophenone moiety can be manipulated in ambient light during experimental conditions. The myristylated benzophenone analogue **13** was the most active (Table 1), and our progress in applying this reagent as a photoaffinity probe in labeling experiments will be reported in due course. As seen in Table 1, the ability of the benzophenone analogues to mobilize calcium decreases sharply as the numbers of methylene subunits in the fatty acid chain is reduced. Only benzophenone mimic **9** exhibited no activity, which we attribute to its having an insufficiently long fatty acid chain (hexyl). Although **13** was the most active benzophenone mimic, it was considerably less potent than LPA (EC_{50}s: LPA = 5 nM, **13** = 1.99 μM). However, it is possible that the diminished potency of these benzophenone species will be counteracted by the increased efficiency of protein cross-linking by the benzophenone subunit.

The benzophenone-based analogues show that the putative LPA receptor(s) have a surprisingly large tolerance for substitution in the linker region. This led us to further explore aromatic linker mimics. Phosphorylated α-tocopherol, the fat-soluble vitamin, has the structural elements identified in these studies for activity and we hypothesized that this species might function as a LPA mimetic. However, the phosphorylated form of α-tocopherol **14** was not an agonist for calcium mobilization in MDA MB 231 cells at concentrations up to 5 μM.

The increased rigidity that these aromatic substructures confer on these analogues might result in compounds that bind to the receptor but do not enable the conformational alteration needed to express activity. Therefore, the LPA analogues that showed no detectable agonist activity (**1**, **4**, **9**, **14**) were tested to see if they exhibited antagonistic activity. To test for antagonist activity, the effects of the analogues were monitored on the Ca^{2+} response in MDA MB-231 cells to LPA. In practice, our antagonist assay examined the Ca^{2+} response to LPA at 1 and 10 nM individually. These results were compared to the Ca^{2+} response of samples that contained (1 or 10 nM) LPA and analogues **1**, **4**, **9**, or **14** at 1 μM. None of these analogues (**1**, **4**, **9**, or **14)** proved to affect the response of LPA at either concentration.

The knowledge gained from this SAR study illustrated considerable tolerance of the LPA receptor(s) for structural alterations in the backbone region. Our studies significantly enhance our knowledge concerning the structural requirements for activity at the LPA receptor presented in previously reported studies. Although we did not produce an agonist with equal activity to LPA, these novel LPA analogues provide a variety of new lead structures that can used for further development of LPA-based agonists.

Experimental Section

All melting points were taken on a Thomas-Hoover UNI-MELT melting point apparatus and are uncorrected. All solvents were filtered through appropriate desiccant under nitrogen immediately prior to use. All nuclear magnetic resonance spectra were obtained with a General Electric QE300 spectrometer at 300 MHz, and chemical shifts are reported in ppm. Elemental analysis were performed either by Atlantic Microlab. Inc., Norcross, GA, or at the University of Virginia on a Perkin-Elmer PE 2400 C, H, N analyzer. Thin-layer chromatography was performed on Merck silica gel 60 F-254 precoated plates, and visualization was effected with phosphomolybdic acid in ethanol. The thin-layer chromatography R_f values reported were obtained using the chromatographic solvent system described for large scale chromatographic purification using 2-hydroxyethyl-(9*Z*)-octadec-9-enoate as an internal standard. The R_f values for 2-hydroxyethyl-(9*Z*)-octadec-9-enoate obtained under the following conditions were as follows: 20% ethyl acetate in hexanes, R_f = 0.14; 30% ethyl acetate in hexanes, R_f = 0.29; 5% acetone in

chloroform, $R_f = 0.45$; 15% acetone in chloroform, $R_f = 0.55$. Column chromatography was performed on E. Merck silica 60 (230−400 mesh). Radial chromatography was executed on a Chromatotron 7924 from Harrison Research System.

General Procedure A: Acylation of Dihydroxybenzophenones. Hexanoyl chloride (0.36 mL, 2.6 mmol) was added to a solution of 4-4′-dihydroxybenzophenone (1.00 g, 5.2 mmol) and pyridine (0.21 mL, 2.6 mmol) in 50 mL of THF and stirred at room-temperature overnight. The reaction mixture was diluted with ethyl acetate (75 mL) and extracted with saturated aqueous NH_4Cl (3 × 75 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash chromatography using 5% acetone in chloroform.

General Procedure B: Acylation of the Aminophenols. Pamitoyl chloride (1.26 g, 4.6 mmol) was added to a solution of 2-aminophenol (1.00 g, 9.16 mmol) in 100 mL of THF and stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (150 mL) and extracted with saturated aqueous NH_4Cl (3 × 100 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash chromatography using 15% acetone in chloroform.

General Procedure C: Acylation of Resorcinol. Pamitoyl chloride (1.25 g, 4.5 mmol) was added to a solution of resorcinol (1.00 g, 9.1 mmol) and pyridine (0.36 mL, 4.5 mmol) in 100 mL of THF and stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (150 mL) and extracted with saturated aqueous NH_4Cl (3 × 100 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash chromatography using 5% acetone in chloroform.

General Procedure D: Hydroxyl Phosphorylation of the Aminophenol Analogues. Dibenzyl diisopropylphosphoramidite (0.31 mL, 0.93 mmol) was added to a solution of *N*-1-(2-hydroxyphenyl)hexadecanamide (**16**) (200 mg, 0.57 mmol) and tetrazole (65 mg, 0.93 mmol) in 50 mL of 1:1 THF/CH_2Cl_2. The reaction was stirred at room temperature overnight, and 30% aqueous H_2O_2 (0.2 mL) was added and stirred for an additional 2 h. The reaction was then brought to 0 °C, and excess H_2O_2 was quenched with saturated aqueous sodium metabisulfite. The resulting reaction mixture was diluted with ethyl acetate (50 mL) and extracted with saturated aqueous sodium metabisulfite (2 × 50 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by radial chromatography using 20% ethyl acetate in hexanes.

General Procedure E: Hydroxyl Phosphorylation of the Benzophenone Analogues. Di-*tert*-butyl diisopropylphosphoramidite (0.31 g, 1.13 mmol) was added to a solution of 4-(4-hydroxybenzoyl)phenyl octanoate (**33**) (0.24 g, 0.70 mmol) and tetrazole (0.80 g, 1.13 mmol) in 50 mL of 1:1 THF/CH_2Cl_2. The reaction was stirred at room temperature overnight, and 30% aqueous H_2O_2 (0.2 mL) was added and stirred for an additional 2 h. The reaction was then brought to 0 °C, and excess H_2O_2 was quenched with saturated aqueous sodium metabisulfite. The resulting reaction mixture was diluted with ethyl acetate (50 mL) and extracted with saturated aqueous sodium metabisulfite (2 × 50 mL). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by radial chromatography using 20% ethyl acetate in hexanes.

General Procedure F: Deprotection of Lipid Phosphotriesters with Saturated Fatty Acid Chains. Dibenzyl (2-(palmitoylamino)phenyl) phosphate (**24**) (0.14 g, 0.16 mmol) was dissolved in 2 mL of THF. To this solution was added 10% Pd/C (catalytic), and the resulting mixture was placed under H_2 atmosphere for 2 h. The reaction mixture was then filtered through a layer of Celite using a 1:1 solution of methanol and chloroform. The organic layer was removed under reduced pressure to give the desired lipid phosphate analogue.

General Procedure G: Deprotection of Lipid Phosphotriesters with Unsaturated Fatty Acid Chains. Dibenzyl (2-((*Z*)-9-hexadecenoylamino)phenyl) phosphate (**28**) (0.11 g, 0.18 mmol) was dissolved in 2 mL of a 1:1 mixture of THF/cyclohexene. To this solution was added 10% Pd/C, and the resulting mixture was stirred for 4 h at 78 °C and under a nitrogen atmosphere. The reaction mixture was brought to room temperature and then filtered through a layer of Celite using a 1:1 methanol/chloroform mixture. The organic layer was removed under reduced pressure to give the desired lipid phosphate analogue.

General Procedure H: Deprotection of *tert*-Butyl Protected Phosphotriester Species. 4-(4-((Di(*tert*-butoxy)-phosphoryl)oxy)benzoyl)phenyl hexanoate (**32**) (0.11 g, 0.17 mmol) was dissolved in 1.5 mL of ethyl acetate to which 2 drops of concentrated HCl was added. The reaction mixture was stirred for 6 h at room temperature followed by the removal of the solvents under reduced pressure to give the desired lipid phosphate analogue.

***N*-1-(2-Hydroxyphenyl) Hexadecanamide (16).** This aminophenol analogue was prepared as described in procedure B (80% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 79−80 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.42 (s, 1H), 7.26−6.83 (m, 4H), 2.45 (t, $J = 7.32$ Hz, 2H), 1.72 (q, $J = 6.93$ Hz, 2H), 1.26 (s, 24H), 0.88 (t, $J = 6.93$ Hz, 3H).

***N*-1-(3-Hydroxyphenyl) Hexadecanamide (17).** This aminophenol analogue was prepared as described in procedure B (82% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 112−114 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75−6.61 (m, 4H), 2.37 (t, $J = 6.93$ Hz, 2H), 1.73 (q, $J = 6.93$ Hz), 1.26 (s, 24H), 0.88 (t, $J = 6.93$ Hz, 3H).

***N*-1-(4-Hydroxyphenyl) Hexadecanamide (18).** This aminophenol analogue was prepared as described in procedure B (84% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 133 °C (dec); $R_f = 0.25$; ^{1}H NMR (300 MHz, CD_3OD) δ 7.34 (d, $J = 8.86$, 2H), 6.60 (d, $J = 8.86$, 2H), 2.15 (t, $J = 7.7$ Hz, 2H), 1.45 (q, $J = 6.94$ Hz, 2H), 1.09 (s, 24H), 0.68 (t, $J = 6.93$ Hz, 3H).

3-Hydroxyphenyl Palmitate (19). This resorcinol analogue was prepared as described in procedure C (36% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 66−68 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20−6.32 (m, 4H), 2.54 (t, $J = 7.7$ Hz, 2H), 1.75 (q, $J = 7.7$ Hz, 2H), 1.26 (s, 24H), 0.89 (t, $J = 6.94$ Hz, 3H).

***N*-1-(2-Hydroxyphenyl) (*Z*)-9-Hexadecenamide (20).** This aminophenol analogue was prepared as described in procedure B (70% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 36−38 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.42 (s, 1H), 7.12−6.83 (m, 4H), 5.35 (m, 2H), 2.43 (t, $J = 7.32$ Hz, 2H), 2.02 (m, 4H), 1.72 (q, $J = 7.31$ Hz, 2H), 1.32 (m, 16H), 0.88 (t, $J = 7.32$ Hz, 3H).

***N*-1-(3-Hydroxyphenyl) (*Z*)-9-Hexadecenamide (21).** This aminophenol analogue was prepared as described in procedure B (67% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 62−64 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12−6.54 (m, 4H), 5.35 (m, 2H), 2.34 (t, $J = 7.32$ Hz, 2H), 2.02 (m, 4H), 1.72 (q, $J = 7.31$ Hz, 2H), 1.32 (m, 16H), 0.88 (t, $J = 7.32$ Hz, 3H).

***N*-1-(4-Hydroxyphenyl) (*Z*)-9-Hexadecenamide (22).** This aminophenol analogue was prepared as described in procedure B (84% yield) and purified by flash chromatography using 15% acetone in chloroform to afford a white solid: mp 101−103 °C; $R_f = 0.25$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, $J = 8.45$ Hz, 2H), 6.75 (d, $J = 8.60$ Hz, 2H), 5.35 (m, 2H), 2.33 (t, $J = 7.32$ Hz, 2H), 2.02 (m, 4H), 1.72 (q, $J = 7.31$ Hz, 2H), 1.32 (m, 16H), 0.88 (t, $J = 7.32$ Hz, 3H).

3-Hydroxyphenyl (*Z*)-9-Hexadecenoate (23). This resorcinol analogue was prepared as described in procedure C (38% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a clear oil: $R_f = 0.5$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20−6.32 (m, 4H), 5.36 (m, 2H), 2.54 (t,

J = 7.7 Hz, 2H), 2.03 (m, 4H), 1.75 (q, J = 7.7 Hz, 2H), 1.32 (m, 16H), 0.89 (t, J = 6.94 Hz, 3H).

4-(4-Hydroxybenzoyl) Phenyl Hexanoate (32). This benzophenone analogue was prepared as described in procedure A (30% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 85−87 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.48 Hz, 2H), 7.73 (d, J = 8.85 Hz, 2H), 7.20 (d, J = 8.86 Hz, 2H), 6.90 (d, J = 8.47 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.78 (q, J = 7.32 Hz, 2H), 1.38 (m, 4H), 0.93 (t, J = 7.31 Hz, 3H).

4-(4-Hydroxybenzoyl) Phenyl Octanoate (33). This benzophenone analogue was prepared as described in procedure A (35% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 86−87 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.48 Hz, 2H), 7.73 (d, J = 8.85 Hz, 2H), 7.20 (d, J = 8.86 Hz, 2H), 6.90 (d, J = 8.47 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.78 (q, J = 7.32 Hz, 2H), 1.38 (m, 4H), 0.93 (t, J = 7.31 Hz, 3H).

4-(4-Hydroxybenzoyl) Phenyl Decanoate (34). This benzophenone analogue was prepared as described in procedure A (33% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 87−88 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.86 Hz, 2H), 7.73 (d, J = 8.85 Hz, 2H), 7.20 (d, J = 8.86 Hz, 2H), 6.90 (d, J = 8.86 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.78 (q, J = 7.32 Hz, 2H), 1.27 (m, 12H), 0.88 (t, J = 7.31 Hz, 3H).

4-(4-Hydroxybenzoyl) Phenyl Laurate (35). This benzophenone analogue was prepared as described in procedure A (33% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 89−91 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.48 Hz, 2H), 7.73 (d, J = 8.47 Hz, 2H), 7.20 (d, J = 8.86 Hz, 2H), 6.90 (d, J = 8.86 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.78 (q, J = 7.32 Hz, 2H), 1.27 (m, 16H), 0.88 (t, J = 7.31 Hz, 3H).

4-(4-Hydroxybenzoyl) Phenyl Myristate (36). This benzophenone analogue was prepared as described in procedure A (36% yield) and purified by flash chromatography using 5% acetone in chloroform to afford a white solid: mp 94−96 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, J = 8.48 Hz, 2H), 7.73 (d, J = 8.86 Hz, 2H), 7.20 (d, J = 8.47 Hz, 2H), 6.90 (d, J = 8.86 Hz, 2H), 2.60 (t, J = 7.7 Hz, 2H), 1.78 (q, J = 7.7 Hz, 2H), 1.27 (m, 20H), 0.88 (t, J = 6.93 Hz, 3H).

Dibenzyl (2-(Palmitoylamino)phenyl) Phosphate (24). This aminophenol phosphate analogue was prepared as described in procedure D (79% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a white solid: mp 48−50 °C; R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−6.94 (m, 4H), 7.31 (s, 10H), 5.1 (d, J = 8.85 Hz, 4H), 2.24 (t, J = 7.71 Hz, 2H), 1.66 (q, J = 7.31 Hz, 2H), 1.27 (s, 24H), 0.88 (t, J = 6.94 Hz, 3H).

Dibenzyl (3-(Palmitoylamino)phenyl) Phosphate (25). This aminophenol phosphate analogue was prepared as described in procedure D (88% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a white solid: mp 46−48 °C; R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−6.82 (m, 4H), 7.30 (s, 10H), 5.1 (d, J = 8.47 Hz, 4H), 2.3 (t, J = 7.31 Hz, 2H), 1.66 (m, 2H), 1.27 (s, 24H), 0.88 (t, J = 6.74 Hz, 3H).

Dibenzyl (4-(Palmitoylamino)phenyl) Phosphate (26). This aminophenol phosphate analogue was prepared as described in procedure D (61% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a white solid: mp 65−67 °C; R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 8.85 Hz, 2H), 7.32 (s, 10H), 6.99 (d, J = 8.09 Hz, 2H), 5.1 (d, J = 8.47 Hz, 4H), 2.33 (t, J = 7.7 Hz, 2H), 1.66 (q, J = 7.7 Hz, 2H), 1.27 (s, 24H), 0.88 (t, J = 6.55 Hz, 3H).

3-((Di(benzyloxy)phosphoryl)oxy) Phenyl Palmitate (27). This resorcinol phosphate analogue was prepared as described in procedure D (65% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a white solid: mp 45−46 °C; R_f = 0.32; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 10H), 7.30−6.91 (m, 4H), 5.12 (d, J = 8.47 Hz, 4H), 2.53 (t, J = 7.7 Hz, 2H), 1.74 (q, J = 7.31 Hz, 2H), 1.28 (s, 24H), 0.89 (t, J = 6.55 Hz, 3H).

Dibenzyl (2-((*Z*)-9-Hexadecenoylamino)phenyl) Phosphate (28). This aminophenol phosphate analogue was prepared as described in procedure D (75% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a clear oil: R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−6.94 (m, 4H), 7.31 (s, 10H), 5.35 (m, 2H), 5.1 (d, J = 8.85 Hz, 4H), 2.24 (t, J = 7.71 Hz, 2H), 2.0 (m, 4H), 1.66 (q, J = 7.31 Hz, 2H), 1.29 (m, 24H), 0.88 (t, J = 6.94 Hz, 3H).

Dibenzyl (3-((*Z*)-9-Hexadecenoylamino)phenyl) Phosphate (29). This aminophenol phosphate analogue was prepared as described in procedure D (78% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a clear oil: R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44−6.84 (m, 4H), 7.31 (s, 10H), 5.35 (m, 2H), 5.12 (d, J = 8.47 Hz, 4H), 2.31 (t, J = 7.71 Hz, 2H), 2.0 (m, 4H), 1.68 (q, J = 7.31 Hz, 2H), 1.31 (m, 24H), 0.88 (t, J = 6.94 Hz, 3H).

Dibenzyl (4-((*Z*)-9-Hexadecenoylamino)phenyl) Phosphate (30). This aminophenol phosphate analogue was prepared as described in procedure D (79% yield) and purified by radial chromatography using 30% ethyl acetate in hexanes to afford a clear oil: R_f = 0.26; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, J = 8.85 Hz, 2H), 7.33 (s, 10H), 7.0 (d, J = 8.85 Hz, 2H), 5.35 (m, 2H), 5.1 (d, J = 8.47 Hz, 4H), 2.33 (t, J = 7.7 Hz, 2H), 2.0 (m, 4H), 1.69 (m, 2H), 1.27 (m, 24H), 0.88 (t, J = 6.69 Hz, 3H).

3-((Di)benzyloxy)phosphoryl)oxy) Phenyl (*Z*)-9-Hexadecenoate (31). This resorcinol phosphate analogue was prepared as described in procedure D (65% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a clear oil: R_f = 0.32; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 10H), 7.30−6.91 (m, 4H), 5.37 (m, 2H), 5.12 (d, J = 8.47 Hz, 4H), 2.53 (t, J = 7.7 Hz, 2H), 2.05 (m, 2H), 1.75 (q, J = 7.31 Hz, 2H), 1.32 (m, 24H), 0.89 (t, J = 6.93 Hz, 3H).

4-(4-((Di(*tert*-butoxy)phosphoryl)oxy)benzoyl)phenyl Hexanoate (37). This benzophenone phosphate analogue was prepared as described in procedure E (76% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a clear oil: R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−7.77 (m, 4H), 7.29 (d, J = 8.86 Hz, 2H), 7.18 (d, J = 8.47 Hz, 2H), 2.57 (t, J = 7.7 Hz, 2H), 1.73 (q, J = 7.7 Hz, 2H), 1.52 (s, 18H), 1.38 (m, 4H), 0.91 (t, J = 7.31 Hz, 3H).

4-(4-((Di(*tert*-butoxy)phosphoryl)oxy)benzoyl)phenyl Octanoate (38). This benzophenone phosphate analogue was prepared as described in procedure E (78% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a clear oil: R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−7.74 (m, 4H), 7.31 (d, J = 8.09 Hz, 2H), 7.19 (d, J = 8.45 Hz, 2H), 2.57 (t, J = 7.321 Hz, 2H), 1.76 (m, 2H), 1.53 (s, 18H), 1.31 (m, 8H), 0.89 (t, J = 6.16 Hz, 3H).

4-(4-((Di(*tert*-butoxy)phosphoryl)oxy)benzoyl)phenyl Decanoate (39). This benzophenone phosphate analogue was prepared as described in procedure E (70% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a white solid: mp 43−44 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−7.77 (m, 4H), 7.29 (d, J = 8.09 Hz, 2H), 7.19 (d, J = 8.47 Hz, 2H), 2.56 (t, J = 7.7 Hz, 2H), 1.71 (m, 2H), 1.51 (s, 18H), 1.27 (m, 12H), 0.85 (t, J = 7.31 Hz, 3H).

4-(4-((Di(*tert*-butoxy)phosphoryl)oxy)benzoyl)phenyl Laurate (40). This benzophenone phosphate analogue was prepared as described in procedure E (63% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a white solid: mp 44−45 °C; R_f = 0.55; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83−7.79 (m, 4H), 7.31 (d, J = 8.86 Hz, 2H), 7.21 (d, J = 8.47 Hz, 2H), 2.59 (t, J = 7.32 Hz, 2H), 1.77 (q, J = 7.32 Hz, 2H), 1.53 (s, 18H), 1.27 (m, 16H), 0.88 (t, J = 6.54 Hz, 3H).

4-(4-((Di(*tert*-butoxy)phosphoryl)oxy)benzoyl)phenyl Myristate (41). This benzophenone phosphate analogue was prepared as described in procedure E (72% yield) and purified by radial chromatography using 20% ethyl acetate in hexanes to afford a white solid: mp 54−55 °C; R_f = 0.55; ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.83−7.79 (m, 4H), 7.31 (d, J = 8.86 Hz, 2H), 7.20 (d, J = 8.47 Hz, 2H), 2.59 (t, J = 7.7 Hz, 2H), 1.77 (q, J = 7.32 Hz, 2H), 1.54 (s, 18H), 1.26 (m, 20H), 0.88 (t, J = 6.93 Hz, 3H).

2-(Palmitoylamino)phenyl Dihydrogen Phosphate (1). This aminophenol phosphate analogue was deprotected as described in procedure F (100% yield) affording a white solid: mp 85−88 °C; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 8.1−6.95 (m, 4H), 2.28 (m, 2H), 1.62 (m, 2H), 1.22 (m, 24H), 0.82 (m, 3H).

3-(Palmitoylamino)phenyl Dihydrogen Phosphate (2). This aminophenol phosphate analogue was deprotected as described in procedure F (100% yield) affording a white solid: mp 150 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.36−6.85 (m, 4H), 2.24 (m, 2H), 1.6 (m, 2H), 1.22 (m, 24H), 0.82 (m, 3H).

4-(Palmitoylamino)phenyl Dihydrogen Phosphate (3). This aminophenol phosphate analogue was deprotected as described in procedure F (100% yield) affording a white solid: mp 120 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.5−7.05 (m, 4H), 2.28 (m, 2H), 1.62 (m, 2H), 1.25 (m, 24H), 0.82 (m, 3H).

3-(Phosphonooxy)phenyl Palmitate (7). This resorcinol phosphate analogue was deprotected as described in procedure F (100% yield) affording a white solid: mp 79−82 °C; ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.21−6.75 (m, 4H), 2.24 (m, 2H), 1.6 (m, 2H), 1.22 (m, 24H), 0.8 (m, 3H).

2-((*Z*)-9-Hecadecenoylamino)phenyl Dihydrogen Phosphate (4). This aminophenol phosphate analogue was deprotected as described in procedure G (100% yield) affording a clear oil: ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.31−6.98 (m, 4H), 5.36 (m, 2H), 2.31 (m, 2H), 1.94 (m, 4H), 1.62 (m, 2H), 1.26 (m, 18H), 0.88 (m, 3H).

3-((*Z*)-9-Hecadecenoylamino)phenyl Dihydrogen Phosphate (5). This aminophenol phosphate analogue was deprotected as described in procedure G (100% yield) affording a white solid: mp 120 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.36−6.85 (m, 4H), 5.35 (m, 2H), 2.24 (m, 2H), 1.9 (m, 4H), 1.6 (m, 2H), 1.22 (m, 18H), 0.8 (m, 3H).

4-((*Z*)-9-Hecadecenoylamino)phenyl Dihydrogen Phosphate (6). This aminophenol phosphate analogue was deprotected as described in procedure G (100% yield) affording a white solid: mp 140 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.5−7.05 (m, 4H), 5.35 (m, 2H), 2.3 (m, 2H), 1.9 (m, 4H), 1.65 (m, 2H), 1.22 (m, 18H), 0.82 (m, 3H).

3-(Phosphonooxy)phenyl (*Z*)-9-Hexadecenoate (8). This resorcinol phosphate analogue was deprotected as described in procedure G (100% yield) affording a clear oil: ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$ (1:1)) δ 7.3−6.75 (m, 4H), 5.39 (m, 2H), 2.45 (m, 2H), 2.05 (m, 4H), 1.62 (m, 2H), 1.22 (m, 18H), 0.85 (m, 3H).

4-(4-(Phosphonooxy)benzoyl)phenyl Hexanoate (9). This benzophenone phosphate analogue was deprotected as described in procedure H (100% yield) affording a white solid: mp 99−100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81−6.95 (m, 4H), 2.57 (m, 2H), 1.83 (m, 2H), 1.38 (m, 4H), 0.89 (m, 3H).

4-(4-(Phosphonooxy)benzoyl)phenyl Octanoate (10). This benzophenone phosphate analogue was deprotected as described in procedure H (100% yield) affording a white solid: mp 101−103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72−7.12 (m, 4H), 2.55 (m, 2H), 1.75 (m, 2H), 1.34 (m, 8H), 0.89 (m, 3H).

4-(4-(Phosphonooxy)benzoyl)phenyl Decanoate (11). This benzophenone phosphate analogue was deprotected as described in procedure H (100% yield) affording a white solid: mp 105−106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76−7.15 (m, 4H), 2.55 (m, 2H), 1.71 (m, 2H), 1.27 (m, 12H), 0.88 (m, 3H).

4-(4-(Phosphonooxy)benzoyl)phenyl Laurate (12). This benzophenone phosphate analogue was deprotected as described in procedure H (100% yield) affording a white solid: mp 106−108 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77−7.19 (m, 4H), 2.56 (m, 2H), 1.69 (m, 2H), 1.24 (m, 16H), 0.84 (m, 3H).

4-(4-(Phosphonooxy)benzoyl)phenyl Myristate (13). This benzophenone phosphate analogue was deprotected as described in procedure H (100% yield) affording a white solid: mp 113−115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77−7.19 (m, 4H), 2.56 (m, 2H), 1.69 (m, 2H), 1.24 (m, 20H), 0.84 (m, 3H).

Dibenzyl (2,5,7,8-Tetramethyl-2-(4,8,12-trimethyldecyl)-3,4-dihydro-2*H*-6-chromenyl) Phosphate (42). This α-tocopherol analogue was phosphorylated as described in procedure D (84% yield) and purified by flash chromatography using 5% acetone in chloroform, affording a clear oil: R_f = 0.48; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 10H), 5.1 (m, 4H), 2.54 (t, J = 6.2 Hz, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 2.06 (s, 3H), 1.76 (m, 1H), 1.57−1.07 (m, 22H), 0.88−0.83 (m, 15H).

2,5,7,8-Tetramethyl-2-(4,8,12-trimentyldecyl)-3,4-dihydro-2*H*-6-cromenyl Dihydrogen Phosphate (14). This α-tocopherol phosphate analogue was deprotected as described in procedure F (100% yield) affording a clear oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.54 (m, 2H), 2.18−2.09 (m, 9H), 1.76 (m, 1H), 1.57−1.07 (m, 22H), 0.88−0.83 (m, 15H).

Compound Preparation. Phospholipids, which were stored dissolved in organic solvent (usually 1:1 chloroform/methanol) at −20° C under argon, were separated (0.1−2.0 μmol, 1−50 μL) into microcentrifuge tubes, dried in vacuo, and resuspended in water with 0.1% (w/v) fatty acid free BSA. After brief vortexing, the mixture was treated for 5 min in a bath sonicator. The solution was stored at room temperature for no more than 2 h before use. Subsequent cycles of freezing/thawing did not affect activity. In some cases, phospholipids were suspended in buffer (HKRB, calcium measurements, HEPES-buffered Dulbeco's modified Eagle's culture medium, cAMP determinations).

Cell Culture. The LPA mimetics were tested on the human breast cancer cell line MDA MB-231. The cells were grown in Dubelco's modified essential medium (DMEM) supplemented in 10% FBS and were passaged following trypsinization every 5−7 days. Their growth medium was changed every third day.

Calcium Assay. Cell monolayers grown in 150 cm^2 dishes (>80% confluent) were overlaid with 10 mL of HKRB buffer (20 mM HEPES, 103 mM NaCl, 4.8 mM KCl, 0.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 15 mM glucose, pH 7.4) containing 5 μg/mL INDO-1AM. After 45 min at 37° C, the monolayer was washed briefly with warm buffered saline, and the cells were freed from the substratum by incubation with trypsin/EDTA. After brief centrifugation, the cell pellet was resuspended in HKRB at approximately 1.5 × 10^6 cells/mL and kept at 37° C for no more than 90 min before use. Records of free intracellular calcium were made on 2 mL aliquots of cells suspended in a quartz cuvette in a temperature-controlled fluorimeter (Aminco SLM 800; SLM Instruments, Urbana, IL). Excitation was with light at 332 nm (slit width 4 nm), and emission wavelengths were 400 and 485 nm (slit widths 4 nm). Calcium responses recorded were peak responses in traces of the ratio of the emitted light. Maximum and minimum fluorescence ratios were calculated by the sequential addition of digitonin (to 75 μM) and EDTA (to 5 μM). Calcium mobilization was presented as a percent of the total available calcium (defined by subsequent treatment with digitonin) normalized to maximal LPA response, which is typically 30% of the total amount of calcium mobilized by digitonin. All points used to calculate the EC_{50} values were done in triplicate and were curved-fitted with GraphPAD (GraphPAD software, San Diego, CA).

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References

(1) Moolenaar, W. H.; Kranenburg, O.; Postma, F. R.; Zondag, G. CM. Lysophosphatidic Acid: G-Protein signaling and Cellular Responses. *Curr. Opin. Cell Biol.* **1997**, *9*, 168−173.

(2) Van der Bond, R. L.; Brunner, J.; Jalink, K.; van Corven, J.; Moolenaar, W. H.; van Blitterswijk, W. J. Identification of a Putative Membrane Receptor for the Bioactive Phospholipid, Lysophosphatidic Acid. *EMBO J.* **1992**, *11*, 249−2501.

(3) Hecht, J. H.; Weiner, J. A.; Post, S. R.; Chun, J. Ventricular Zone Gene-1 (vzg-1) Encodes a Lysophosphatidic Acid Receptor Expressed in Neurogenic Regions of the Developing Cerebral Cortex. *J. Cell Biol.* **1996**, *135*, 1071−1083.
(4) Guo, Z.; Liliom, K.; Fischer, D. J.; Bathurst, I. C.; Tomei, L. D.; Kiefer, M. C.; Tigyi, G. Molecular Cloning of a High-Affinity Receptor for the Growth factor-Like Lipid Mediator Lysophosphatidic Acid from Xenopus Oocytes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14367−14372.
(5) Jalink, K.; Hengeveld, T.; Mulder, S.; Postma, F. R.; Simon, M. F.; Chap, H.; van der Marel, G. A.; van Boom, J. H.; van Blitterswijk, W. J.; Moolenaar, W. H. Lysophophatidic Acid-Induced Ca^{2+} Mobilization in Human A431 Cells: Structure−activity Analysis. *Biochem. J.* **1995**, *307*, 609−616.
(6) Venable, M. E.; Zimmerman, G. A.; McIntyre, T. M.; Prescott, S. M. Platelet-Activating Factor: A Phospholipid Autacoid With Diverse Actions. *J. Lipid Res.* **1993**, *34*, 691−702.
(7) Lynch, K. R.; Hopper, D. W.; Carlisle, S. J.; Catalano, J. G.; Zhang, M.; Macdonald, T. L. Structure/Activity Relationships in Lysophosphatidic Acid: the 2-Hydroxyl Moiety. *Mol. Pharmacol.* **1997**, *52*, 75−81.
(8) Hooks, S. B.; Ragan, S. P.; Hopper, D. W.; Honemann, C.; Durieux, M. E.; Macdonald, T. L.; Lynch, K. R. Characterization of a Receptor Subtype-Selective Lysophosphatidic Acid Mimetic. *Mol. Pharmacol.* **1998**, *53*, 188−194.
(9) Olszewski, J. D.; Dorman, G.; Elliot, J. T.; Hong, Y.; Ahern, D. G.; Prestwich, G. D. Tethered Benzophenone Reagents for the Synthesis of Photoactivatable Ligands. *Bioconjugate Chem.* **1995**, *6*, 395−400.
(10) Kingsley, P. B.; Feigenson, G. W. The Synthesis of a Predeuterated Phospholipid: 1,2-Dimyristoyl-sn-glycero-3-phosphocholine-d_{72}. *Chem. Phys. Lipids* **1979**, *24*, 135−147.

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