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Concise Synthesis of Acyl Migration-Blocked 1,1-Difluorinated Analogues of Lysophosphatidic Acid

Yong Xu and Glenn D. Prestwich*

Department of Medicinal Chemistry and Center for Cell Signaling, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257

gprestwich@pharm.utah.edu

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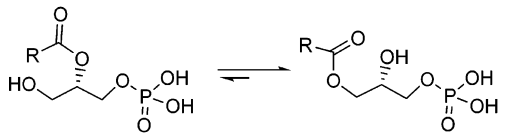
Abstract: Lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycerol 3-phosphate) is an important phospholipid mediator produced by activated platelets and by ovarian cancer cells. Efforts to understand LPA signaling through G-protein-coupled receptors are hampered by the facile acyl migration that results in equilibration to a mixture of the 1- or 2-acyl species under physiological conditions. We describe a new and efficient route to enantiomerically homogeneous lysophospholipid analogues from D-mannitol 1,2:5,6-bis-acetonide to give two 1,1-difluorodeoxy analogues of (2*R*)-acyl-*sn*-glycerol 3-phosphate. These compounds are migration-blocked analogues of the labile *sn*-2 LPA species. The ^{19}F NMR of diastereotopic fluorines of the difluoromethyl group shows an unexpected solvent dependence.

Lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycerol 3-phosphate) is an important lysophospholipid mediator produced by activated platelets.[1] The activity of LPA in eliciting a variety of biological effects, including platelet aggregation, smooth muscle contraction, changes in cell morphology, and stimulation of cell growth and proliferation,[2] have recently heightened scientific interest in this lysolipid. In particular, LPA is present in elevated levels in ascites and plasma of ovarian cancer patients and may thus contribute to the progression of this and other forms of cancer.[3]

LPA elicits numerous cellular functions via interaction with specific G-protein coupled receptors belonging to the *e*ndothelial *d*ifferentiation *g*ene (edg) family.[4a] These receptors have recently been reclassified as LPA and sphingosine 1-phosphate receptors; specifically, edg-2, edg-4, and edg-7 are now known as LPA_1, LPA_2, and LPA_3, respectively.[4b] Pharmacological concentrations of LPA must be produced extracellularly to induce receptor-dependent biological responses.[4c,d] LPA receptors exhibit characteristic responses to LPA species with different chain lengths, different unsaturation patterns, and different acyl positions.[4e] For example, LPA produced by stimulated platelets is distinct from the LPA found in ascites of ovarian cancer patients. In platelets, *sn*-1 LPA is preferentially produced, but ascites contains an elevated amount of *sn*-2 LPA.[5] However, 2-acyl-*sn*-glycerol 3-phosphate species are very labile under physiological conditions. Intramolecular acyl chain migration, which is facilitated by both acidic and basic conditions, affords an equilibrium mixture of 1-acyl- and 2-acyl-*sn*-glycerol 3-phosphates that favors the 1-acyl isomer[6] (Scheme 1). The instability of 2-acyl-*sn*-glycerol 3-phosphate thus seriously compromises both isolation of naturally occurring species and determination of the activating ligand in structure−activity studies.

SCHEME 1. Acyl Migration in LPA



Replacement of hydroxyl by fluorine in bioactive compounds has provided many valuable analogues,[7] resulting from the similarity of the C−F and C−O bond lengths and the ability of fluorine to function as a weak hydrogen-bond acceptor. Moreover, the strategic substitution of hydroxyl by fluorine can enhance biological activity by altering pharmacokinetics and metabolism, and the resulting fluorinated analogues are valuable probes for determining mechanism of action.[8a] Difluoromethyl substitution can introduce unexpected biological activity, as the difluoromethyl group can be viewed as being isosteric with a hydroxyl group.[8b,c] We decided to test the hypothesis that fluorinated analogues of LPA, particularly with fluorine in the *sn*-1-position, might mimic 2-acyl-*sn*-glycerol 3-phosphate as a biological ligand but would lack the propensity to undergo intramolecular acyl migration. To this end, we describe herein the synthesis of the 1,1-difluoro analogue of (2*R*)-acyl-*sn*-glycerol 3-phosphate using a flexible modular strategy with two key features. First, the installation of fluorine in the 1-position was accomplished prior to acylation, thus avoiding acyl chain

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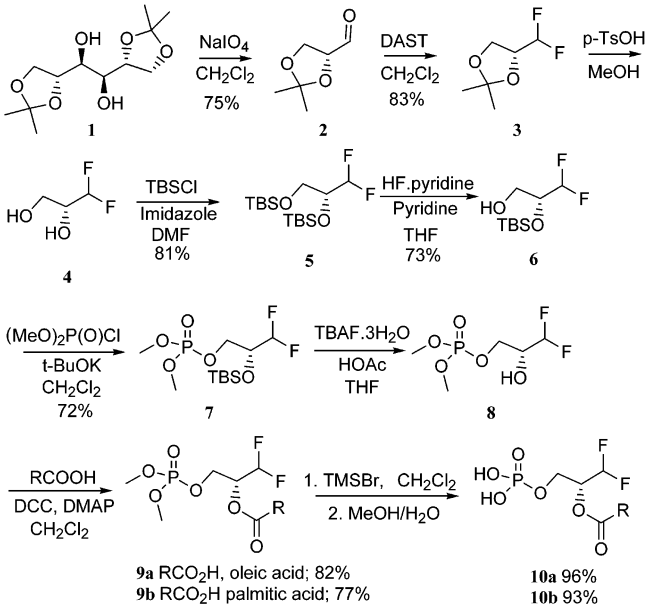
SCHEME 2. Synthesis of Difluoromethylene Analogues of LPA



migration during the synthesis. Second, phosphorylation was postponed until the end of the synthesis to simplify purification of synthetic intermediates.

Synthesis of the target LPA analogues **10a** and **10b** (Scheme 2) involved nonreductive deprotection of the penultimate dimethyl phosphates **9** with trimethylsilane bromide to permit incorporation of unsaturated acyl chains. The key step for the synthesis was the introduction of the difluoromethyl group by the 1,1-difluorination of a C-1 aldehyde.[9] Thus, commercially available D-mannitol 1,2:5,6-bis-acetonide was oxidatively cleaved with $NaIO_4$ to afford the acetonide-protected D-glyceraldehyde **2**.[10] Addition of (diethylamino)sulfur trifluoride (DAST) to a solution of the aldehyde **2** in CH_2Cl_2 afforded the difluorinated compounds in high yield after purification by distillation under reduced pressure.

Next, acidic cleavage of the acetonide-protecting group provided the diol intermediate **4**. The crude diol obtained after removal of the acetonide was immediately converted to the bis-silyl ether **5**, and the more labile TBDMS ether of the primary alcohol was cleaved selectively by treatment with a solution of pyridinium hydrofluoride in a mixture of pyridine and THF at rt.[11] Initial attempts to obtain the primary alcohol **6** from bis-TBDMS ether **5**, utilizing 4.0 equiv of pyridinium hydrofluoride, resulted in disappointing yields (17%) after 48 h at rt. However, an increase to 6.0 equiv gave the primary alcohol in good yield (73%) after 20 h at rt. The primary alcohol **6** was then phosphorylated with dimethylphosphoryl chloride in the presence of *t*-BuOK to give a good yield of phosphate **7**.[12] The 2-TBDMS ether was further deprotected with tetra(*n*-butyl)ammonium fluoride (TBAF) in THF to give alcohol **8** in 72% yield; neutralization of TBAF with acetic acid permitted desilyation of the secondary alcohol without the migration of phosphate.[13] DCC-promoted esterification of alcohol **8** with oleic acid or palmitic acid provided good yields of esters **9a** and **9b**, respectively. Importantly, the introduction of the acyl groups at this stage circumvents problems with acyl group migration during other synthetic operations. Finally, treatment of protected phosphates **9** with bromotrimethylsilane and subsequent addition of 5% aq methanol provided the desired difluorinated LPA analogues **10** in essentially quantitative yield.[14]

Since intermediate **2** has the potential for loss of stereochemical integrity at C-2, the ee of alcohol **8** was determined by preparation of the Mosher ester. Thus, the (+)-MTPA ester of **8** showed >97% ee by ^{19}F NMR.[15] Considering the optical purity of the starting compounds, D-mannitol 1,2:5,6-bis-acetonide (98% ee) and (*R*)-Mosher's acid chloride (99% ee), no apparent loss of optical purity was observed.

Since the two fluorines of the difluoromethyl group are diastereotopic, a typical AB pattern should be evident in the ^{19}F NMR spectra, with each peak split into a doublet of doublets by the smaller vicinal $^3J_{HF}$ and intermediate geminal $^2J_{HF}$ couplings. Fluorine chemical shifts have been known to be strongly solvent-dependent.[16] Figure 1 illustrates the proton-coupled ^{19}F AB quartet for compound **9** in $CDCl_3$, with $^2J_{FF} = 296.2$ Hz, $^2J_{HF} = 55.3$ Hz, and $^3J_{HF} = 12.0$ Hz. Similar ^{19}F NMR spectra were obtained in the polar aprotic solvents acetone-d_6 and DMSO-d_6, indicating that solvent polarity alone did not substantially affect the ^{19}F resonances. However, in the hydrogen-bonding solvent CD_3OD, the pattern was strikingly different, showing an apparent doublet of triplets. The spectral changes can be visualized by titration of CD_3OD into $CDCl_3$; as the ratio of CD_3OD increased, the chemical shift difference ($\Delta\delta$) between the diastereotopic fluorines gradually decreased (Figure 1): $CDCl_3$, $\Delta\delta F = \delta F_a - \delta F_b = 0.89$ ppm; $CDCl_3/CD_3OD = 3:1$, $\Delta\delta F = 0.30$ ppm; $CDCl_3/CD_3OD$ 1:3, $\Delta\delta F = 0.03$ ppm; CD_3OD, $\Delta\delta F = 0.03$ ppm). It is clear that the formation of C−F···D−O−CD_3 hydrogen bonds diminished the differences in the chemical environments of the diastereotopic difluoromethyl fluorine atoms.

The preparation of receptor-specific agonists and antagonists for LPA receptors is an active area of ligand design. Structure−activity studies have demonstrated that analogues lacking the 2-hydroxyl moiety retain activity in selected in vitro assays.[17a] Even more dramatically, different analogues, such as the *N*-palmitoylserine phosphoric acids,[17b] are potent competitive inhibitors of LPA receptors in *Xenopus* oocytes. Degradation-resistant α-hydroxyphosphonate and methylenehydroxyethanola-

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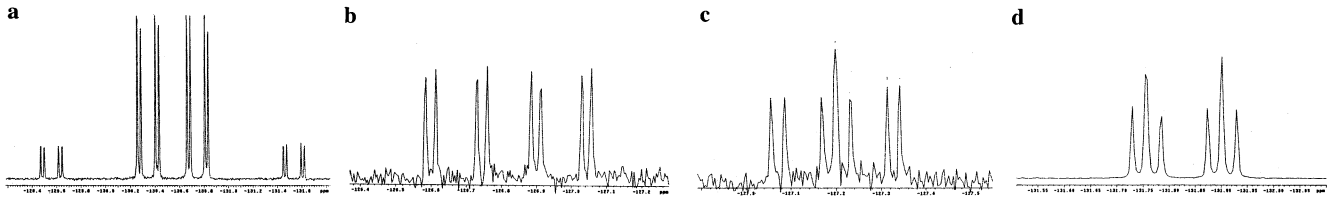

FIGURE 1. ^{19}F NMR (376 MHz) spectra of compound **9** in (a) $CDCl_3$, (b) CD_3Cl/CD_3OD 3:1 (v/v), (c) CD_3Cl/CD_3OD 1:1 (v/v), and (d) CD_3OD.

mide phosphoric acid derivatives can differentiate between ligand selectivity of the platelet aggregation and mitogenic responses from the edg receptor responses.[17c] However, to date, a comprehensive analysis of fluorinated LPA analogues as selective agonists or antagonists for individual LP receptors has not yet been reported. Preliminary results indicate that both compounds **10a** and **10b** activate platelets (A. J. Morris, personal communication); however, these analogues failed to show either agonist or antagonist activity when tested in cells expressing LPA_1, LPA_2, or LPA_3 receptors (K. Hama, J. Aoki, personal communication). Importantly, however, both difluoro analogues **10a** and **10b** are essentially equipotent with *sn*-1-oleoyl-LPA for the activation of the PPARγ nuclear receptor.[18] These results demonstrate that the difluoro LPA analogues elicit different structure−activity profiles with intracellular versus extracellular receptors.

In conclusion, we have demonstrated a concise and efficient synthesis of two acyl-migration blocked 2-acyl LPA analogues. This versatile synthetic route is currently being employed for the synthesis of other migration-blocked and hydrolysis-blocked LPA analogues with different acyl chains in order to create a panel of LPA analogues that differentiate among the LPA receptor subtypes. The detailed biological evaluation of these analogues will be reported in due course.

Experimental Section

General Procedures. Chemicals were obtained from Aldrich and Acros and used without prior purification. Solvents were reagent grade and distilled before use: THF was distilled from sodium wire, and CH_2Cl_2 was distilled from CaH_2. Reactions were performed under an inert atmosphere (N_2 or Ar) unless otherwise indicated. NMR spectra were recorded at 25 °C at 400 MHz (^1H), 101 MHz (^{13}C), 162 MHz (^{31}P), and 376 MHz (^{19}F). Chemical shifts are given in ppm relative to tetramethylsilane as the internal standard for ^1H and ^{13}C spectra ($\delta = 0.00$); external standards were used for ^{31}P (85% H_3PO_4, $\delta = 0.00$) and ^{19}F ($CFCl_3$, $\delta = 0.00$).

(*R*)-Glyceraldehyde acetonide (2) was prepared from D-mannitol-1,2:5,6-bis-acetonide as described[10] to give aldehyde **2** as a clear liquid. $[\alpha]^{20}_D$: +64.4 (lit.[19] $[\alpha]^{20}_D$ +64.9).

(2*R*)-3,3-Difluoro-1,2-propanediol 1,2-Acetonide (3). To a well-stirred solution of 8.10 g (62.3 mmol) of aldehyde **2** in dry CH_2Cl_2 (100 mL) was slowly added 10.2 mL (74.8 mmol) of DAST. After being stirred for 24 h at rt, the reaction mixture was quenched with 10% $NaHCO_3$ solution (80 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined organics were dried (Na_2SO_4). The solvent was removed by fractional distillation until the head temperature reached 40 °C. The residue was then distilled at reduced pressure (ca. 24 mmHg), collecting the fraction distilling at 65−66 °C to give 6.5 g (51.2 mmol, 83%) of difluoride **3** as a clear liquid. ^1H NMR ($CDCl_3$): δ 5.68 (td, J = 56.0, 4.8 Hz, 1H), 4.23 (m, 1H), 4.10 (m, 2H), 1.45 (s, 3H), 1.37 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 114.83 (t, J = 243.9 Hz), 111.19 (s), 74.83 (t, J = 27.6 Hz), 64.19 (dd, J = 5.3, 2.0 Hz), 26.50 (s), 25.11 (s). ^{19}F NMR ($CDCl_3$): δ −127.02 (1F, ddd, $^2J_{FF}$ = 292.0, $^2J_{FH}$ = 54.0, $^3J_{FH}$ = 10.5 Hz), −129.82 (1F, ddd, $^2J_{FF}$ = 292.0, $^2J_{FH}$ = 54.0, $^3J_{FH}$ = 10.5 Hz). MS (CI): m/z 153.0 (M^++1, 100.00), 137.0 ($M^+ - CH_3$, 6.56). HRMS: $M^+ + 1$, found 153.0739, calcd for $C_6H_{11}O_2F_2$ 153.0727. $[\alpha]^{20}_D$: −3.1 (1.09, MeOH).

(2*R*)-3,3-Difluoro-1,2-bis[(1-*tert*-butyl-1,1-dimethylsilyl)-oxy]propane (5). To a solution of acetonide **3** (2.20 g, 14.47 mmol) in MeOH (30 mL) was added *p*-TsOH (0.412 g, 2.17 mmol, 0.15 equiv), and the solution was stirred for 24 h at rt. After addition of NEt_3 (1 mL), the solvent was removed under reduced pressure. Next, crude diol **4** was dissolved in anhydrous DMF (16 mL) and stirred with imidazole (2.96 g, 43.41 mmol, 2.9 equiv) and *tert*-butyldimethylsilyl chloride (TBSCl) (6.11 g, 40.52 mmol, 2.8 equiv) for 24 h at rt. The solution was diluted with water (60 mL) and ethyl acetate (100 mL), and the aqueous layer was separated and extracted with ethyl acetate (3 × 80 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified on silica gel (*n*-hexanes−ethyl acetate 60:1, R_f = 0.36) to afford bis-TBDMS ether **5** as a colorless liquid 3.97 g (11.68 mmol, 81%). ^1H NMR ($CDCl_3$): δ 5.67 (td, J = 55.6, 4.0 Hz, 1H), 3.72 (m, 2H), 3.62 (m, 1H), 0.84 (s, 9H), 0.83 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H), 0.003 (s, 3H), 0.000 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 120.79 (t, J = 243.5 Hz), 78.26 (dd, J = 23.7, 21.4 Hz), 68.83 (t, J = 4.5 Hz), 31.40 (s), 31.24 (s), 23.86 (s), 23.73 (s), 0.76 (s), 0.58 (s), 0.03 (s), 0.00 (s). ^{19}F NMR ($CDCl_3$): δ −130.58 (1F, ddd, J (as above) = 284.1, 55.3, 5.3 Hz), −134.05 (1F, ddd, J = 284.1, 55.3, 5.3 Hz). MS (CI): m/z 314.2 ($M^+ + 1$, 100.00), 283.1 ($M^+ - C_4H_9$, 10.42). HRMS: $M^+ + 1$, found 341.2134, calcd for $C_{15}H_{35}O_2F_2Si_2$ 341.2143. $[\alpha]^{20}_D$: −10.1 (0.61, MeOH).

(2*R*)-3,3-Difluoro-2-bis[(1-*tert*-butyl-1,1-dimethylsilyl)-oxy]-1-propanol (6). The HF·pyridine complex (70%, 30 mmol fluoride) was added to a mixture of pyridine (2.62 mL), and then a solution of bis-ether **5** (1.70 g, 5.00 mmol) in THF (25 mL) was added. The reaction mixture was stirred for 20 h at rt. After completion of the reaction (monitored by TLC), the solution was diluted with ethyl acetate (100 mL), washed with 0.5 M HCl (2 × 20 mL) and then with saturated $CuSO_4$ solution (20 mL), and dried (Na_2SO_4). After concentration in vacuo, the residue was purified on silica gel (*n*-hexanes−ethyl acetate 5:1, R_f = 0.31) to afford 0.82 g of monoether **6** as a colorless liquid (3.63 mmol, 73%). ^1H NMR ($CDCl_3$): δ 5.58 (td, J = 53.6, 6.0 Hz, 1H), 3.68 (m, 2H), 3.59 (m, 1H), 1.79 (br, 1H), 0.79 (s, 9H), 0.00 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 120.05 (t, J = 234.5 Hz), 77.37 (dd, J = 27.6, 22.3 Hz), 67.21 (dd, J = 6.5, 3.0 Hz), 30.62 (s), 23.06 (s), 0.11 (s), 0.00 (s). ^{19}F NMR ($CDCl_3$): δ −128.55 (1F, ddd, J = 289.4, 55.3, 6.4 Hz), −130.25 (1F, ddd, J = 289.4, 55.3, 6.4 Hz). MS (CI) m/z 227.1 ($M^+ + 1$, 100.00), 169.0 ($M^+ - C_4H_9$, 8.11). HRMS: $M^+ + 1$, found 227.1264, calcd for $C_9H_{21}O_2F_2Si$ 227.1279. $[\alpha]^{20}_D$: −11.3 (0.79, MeOH).

(2*R*)-3,3-Difluoro-2-bis[(1-*tert*-butyl-1,1-dimethylsilyl)-oxy]-1-phosphopropane Dimethyl Ester (7). To a stirred solution of 128 mg (0.566 mmol) of ether **6** and dimethyl chlorophosphate (98 mg, 0.679 mmol, 1.2 equiv) in CH_2Cl_2 (10

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mL) at 0 °C was added *t*-BuOK (89 mg, 0.792 mmol, 1.4 equiv). The mixture was stirred for 2 h at rt, and the reaction was complete as determined by TLC. The reaction was quenched by addition of saturated aq NH_4Cl (5 mL), the mixture was stirred for 10 min, and the aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The organics were dried (Na_2SO_4), concentrated, and purified on silica gel (*n*-hexanes−ethyl acetate 3:2, $R_f = 0.41$) to afford 136 mg of phosphotriester **7** as a colorless liquid (0.407 mmol, 72%). ^{1}H NMR ($CDCl_3$): δ 5.88 (td, $J = 53.2$, 3.2 Hz, 1H), 4.38 (m, 2H), 3.83 (m, 1H), 3.73 (d, $J = 0.8$ Hz, 3H), 3.70 (d, $J = 0.8$ Hz, 3H), 0.81 (s, 9H), 0.002 (s, 3H), 0.000 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 118.80 (td, $J = 234.2$, 6.9 Hz), 81.75 (t, $J = 22.2$ Hz), 66.65 (dd, $J = 8.5$, 3.1 Hz), 60.21 (t, $J = 6.54$ Hz), 31.35 (s), 23.85 (s), 0.00 (s), −0.03 (s). ^{19}F NMR ($CDCl_3$): δ −131.75 (1F, ddd, $J = 292.4$, 54.6, 7.9 Hz), −134.1 (1F, ddd, $J = 292.4$, 54.6, 7.9 Hz). ^{31}P NMR ($CDCl_3$): δ 1.467 (s). MS (CI): *m*/*z* 335.0 ($M^+ + 1$, 100.00), 276.9 ($M^+ - C_4H_{10}$, 13.15). HRMS: $M^+ + 1$, found 335.1258, calcd for $C_{11}H_{26}F_2O_2PSi$ 335.1255. $[\alpha]^{20}_D$: −75.7 (0.504, MeOH).

(2*R*)-3,3-Difluoro-2-oleoyl-1-phosphopropane Dimethyl Ester (9a). A solution of TBDMS ether **7** (59 mg, 0.178 mmol) in THF (5 mL) was treated successively with acetic acid (41 μL, 0.706 mmol) and tetrabutylammonium fluoride trihydrate (223 mg, 0.706 mmol) at rt. After being stirred for 4 h, the reaction was complete (TLC). The solvent was removed in vacuo, and the crude product was purified only by passing through a short silica gel bed (ethyl acetate, $R_f = 0.48$) and concentrated in vacuo to afford the alcohol **8** as a colorless liquid. To the crude alcohol **8** was added 55 mg (62 μL, 0.194 mmol) of oleic acid in dry CH_2Cl_2 (2 mL) followed by dropwise addition of a solution of DCC (55 mg, 0.266 mmol) and DMAP (13 mg, 0.106 mmol) in dry CH_2Cl_2 (3 mL). The solution was stirred for 16 h at rt, filtered, concentrated in vacuo, and purified on silica gel (*n*-hexanes−ethyl acetate 1:1, $R_f = 0.26$) to afford 71 mg of oleate **9a** as a waxy solid (0.146 mmol, 82%). ^{1}H NMR ($CDCl_3$): δ 5.86 (td, $J = 54.8$, 4.0 Hz, 1H), 5.28 (m, 2H), 5.15 (m, 1H), 4.20 (m, 2H), 3.73 (d, $J = 4.4$ Hz, 3H), 3.70 (d, $J = 4.4$ Hz, 3H), 2.34 (t, $J = 7.6$ Hz, 2H), 1.93 (m, 4H), 1.58 (m, 2H), 1.22 (m, 20H), 0.81 (t, $J = 6.4$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 172.52 (s), 130.25 (s), 129.90 (s), 112.72 (t, $J = 244.6$ Hz), 70.04 (td, $J = 25.24$, 7.64 Hz), 63.91 (d, $J = 4.6$ Hz), 54.76 (d, $J = 6.1$ Hz), 34.18 (s), 34.09 (s), 32.11 (s), 29.97 (s), 29.88 (s), 29.73 (s), 29.53 (s), 29.33 (s), 29.27 (s), 29.18 (s), 27.43 (s), 27.36 (s), 25.16 (s), 24.92 (s), 22.88 (s), 14.31 (s). ^{19}F NMR ($CDCl_3$): δ −130.101 (1F, ddd, $J = 294.7$, 53.8, 10.5 Hz), −131.0 (1F, ddd, $J = 294.7$, 53.8, 10.5 Hz). ^{31}P NMR ($CDCl_3$): δ 2.111 (s). MS (CI): *m*/*z* 485.3 ($M^+ + 1$, 64.53), 359.2 ($M^+ - C_2H_6PO_4$, 100.00). HRMS: $M^+ + 1$, found 485.2867, calcd for $C_{23}H_{44}F_2O_6P$ 485.2844. $[\alpha]^{20}_D$: −8.6 (1.08, MeOH).

(2*R*)-3,3-Difluoro-2-oleoyl-1-phosphopropane (10a). An aliquot of protected ester **9a** (55 mg, 0.114 mmol) was thoroughly dried (5 h, 1 μm Hg) and dissolved in dry CH_2Cl_2 (2 mL) at rt, and then bromotrimethylsilane (53 μL, 0.398 mmol) was added dropwise with a dry syringe and the mixture was stirred for 4 h at rt. When TLC indicated that all of the reactant had disappeared, solvents were removed in vacuo, and the residue was dissolved in 95% methanol (1 mL) for 1 h and then reconcentrated in vacuo to give 50 mg of LPA 2-oleate analogue **10a** as a colorless oil (0.110 mmol, 96%) that was homogeneous by TLC ($CH_2Cl_2/CH_3OH/H_2O$, 20:10:1, $R_f = 0.58$). ^{1}H NMR (CD_3OD): δ 6.03 (t, $J = 54.4$ Hz, 1H), 5.53 (m, 2H), 5.24 (m, 1H), 4.18 (m, 2H), 2.41 (t, $J = 7.2$ Hz, 2H), 2.02 (m, 4H), 1.63 (m, 2H), 1.30 (m, 20H), 0.89 (t, $J = 6.4$ Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.70 (s), 130.88 (s), 130.78 (s), 114.43 (t, $J = 242.4$ Hz), 71.22 (td, $J = 23.73$, 8.45 Hz), 63.89 (d, $J = 4.6$ Hz), 34.67 (s), 33.06 (s), 30.84 (s), 30.78 (s), 30.61 (s), 30.44 (s), 30.34 (s), 30.26 (s), 30.16 (s), 30.04 (s), 28.12 (s), 25.84 (s), 23.73 (s), 14.15 (s). ^{19}F NMR (CD_3OD): δ −130.10 (1F, ddd, $J = 295.8$, 55.3, 9.4 Hz), −131.7 (1F, ddd, $J = 295.8$, 55.3, 9.4 Hz). ^{31}P NMR ($CDCl_3$): δ 0.742 (s). MS (CI): *m*/*z* 457.2 ($M^+ + 1$, 13.75), 377.2 ($M^+ + 2 - H_2PO_3$, 100.00). HRMS: $M^+ + 1$, found 457.2535, calcd for $C_{21}H_{40}F_2O_6P$ 457.2531. $[\alpha]^{20}_D$: −9.3 (1.02, MeOH).

(2*R*)-3,3-Difluoro-2-palmitoyl-1-phosphopropane Dimethyl Ester (9b). A solution of TBDMS ether **7** (59 mg, 0.178 mmol) in THF (5 mL) was treated successively with acetic acid (41 μL, 0.706 mmol) and tetrabutylammonium fluoride trihydrate (223 mg, 0.706 mmol) and processed as described for **9a** to give crude alcohol **8**. The crude alcohol was directly esterified with 50 mg (0.194 mmol) of palmitic acid in dry CH_2Cl_2 (2 mL) at rt by dropwise addition of a solution of DCC (55 mg, 0.266 mmol) and DMAP (13 mg, 0.106 mmol) in dry CH_2Cl_2 (3 mL). The solution was stirred for 16 h at rt, filtered, and concentrated in vacuo, and the residue was purified on silica gel (*n*-hexane/ethyl acetate 1:1, $R_f = 0.36$) to afford 62 mg of ester **9b** as a waxy solid (0.136 mmol, 77%). ^{1}H NMR (CD_3OD): δ 6.05 (td, $J = 54.8$, 4.4 Hz, 1H), 5.30 (m, 1H), 4.29 (m, 2H), 3.80 (d, $J = 5.2$ Hz, 3H), 3.77 (d, $J = 4.8$ Hz, 3H), 2.42 (t, $J = 7.6$ Hz, 2H), 1.64 (m, 2H), 1.28 (m, 24H), 0.89 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.59 (s), 114.34 (t, $J = 244.0$ Hz), 71.11 (td, $J = 25.34$, 6.94 Hz), 65.39 (d, $J = 5.3$ Hz), 54.42 (d, $J = 6.1$ Hz), 34.76 (s), 34.65 (s), 33.08 (s), 30.78 (s), 30.69 (s), 30.57 (s), 30.48 (s), 30.37 (s), 30.04 (s), 26.76 (s), 26.05 (s), 25.86 (s), 23.73 (s), 14.44 (s). ^{19}F NMR (CD_3OD): δ −131.7 (1F, dt, $J = 55.3$, 10.5 Hz), −131.9 (1F, dt, $J = 55.3$, 10.5 Hz). ^{19}F NMR ($CDCl_3$): δ −130.1 (1F, ddd, $J = 296.2$, 55.3, 12.0 Hz), −131.0 (1F, ddd, $J = 296.2$, 55.3, 12.0 Hz). ^{31}P NMR (CD_3OD): δ 1.816 (s). MS (CI): *m*/*z* 459.3 ($M^+ + 1$, 83.09), 333.2 ($M^+ - C_2H_6PO_4$, 100.00). HRMS: $M^+ + 1$, found 459.2708, calcd for $C_{21}H_{42}F_2O_6P$ 459.2687. $[\alpha]^{20}_D$: −10.3 (0.80, MeOH).

(2*R*)-3,3-Difluoro-2-oleoyl-1-phosphopropane (10b). As described for **10a**, thoroughly dried ester **9b** (38 mg, 0.083 mmol) was dissolved in dry CH_2Cl_2 (1 mL) and deprotected with bromotrimethylsilane (38 μL, 0.290 mmol). The crude product was dissolved in 95% methanol (1 mL) for 1 h and reconcentrated and thoroughly dried in vacuo to give 33 mg of LPA palmitate analogue **10b** (0.077 mmol, 93%). ^{1}H NMR (CD_3OD): δ 5.81 (td, $J = 55.2$, 4.4 Hz, 1H), 5.03 (m, 1H), 3.96 (m, 2H), 2.20 (t, $J = 6.8$ Hz, 2H), 1.41 (m, 2H), 1.07 (s, 24H), 0.68 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (CD_3OD): δ 173.72 (s), 114.43 (t, $J = 242.3$ Hz), 71.22 (td, $J = 23.73$, 8.45 Hz), 63.92 (d, $J = 4.6$ Hz), 34.68 (s), 33.08 (s), 30.79 (s), 30.77 (s), 30.72 (s), 30.58 (s), 30.48 (s), 30.39 (s), 30.07 (s), 25.86 (s), 23.74 (s), 14.46 (s). ^{19}F NMR (CD_3OD): δ −132.08 (1F, ddd, $J = 295.4$, 54.2, 9.4 Hz), −132.7 (1F, ddd, $J = 295.4$, 54.2, 9.4 Hz). ^{31}P NMR (CD_3OD): δ 0.709 (s). MS (CI): *m*/*z* 431.1 ($M^+ + 1$, 3.39), 333.1 ($M^+ - H_2PO_4$, 100.00). HRMS: $M^+ + 1$, found 431.2369, calcd for $C_{19}H_{38}F_2O_6P$ 431.2375. $[\alpha]^{20}_D$: −2.1 (0.90, MeOH).

(2*R*)-3,3-Difluoro-2-*O*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-1-phosphopropane Dimethyl Ester (11). A solution of alcohol **8** and (*R*)-methoxy(trifluoromethyl)phenylacetic acid chloride in pyridine was stirred for 20 h at rt. The mixture was diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$, dried, filtered, and concentrated. Flash chromatography on silica gel gave the corresponding MTPA ester as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.52 (m, 2H), 7.40 (m, 3H), 5.87 (td, $J = 54.4$, 4.0 Hz, 1H), 5.47 (m, 1H), 4.40 (m, 1H), 4.28 (m, 1H), 3.72 (d, $J = 8.0$ Hz, 3H), 3.75 (d, $J = 8.0$ Hz, 3H), 3.55 (m, 3H). ^{19}F NMR ($CDCl_3$): δ −72.36 (s), −129.37 (1F, ddd, $J = 296.2$, 55.3, 11.0 Hz), −130.27 (1F, ddd, $J = 296.2$, 55.3, 11.0 Hz), −72.17 (1.59), −72.36 (98.41), >97% ee. ^{31}P NMR ($CDCl_3$): δ 1.728 (s).

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Supporting Information Available: NMR spectra of compounds **3**, **5**−**7**, **9a**,**b**, **10a**,**b**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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