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Synthesis of difluoromethyl substituted lysophosphatidic acid analogues

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Abstract—Lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycerol 3-phosphate) displays an intriguing cell biology that is mediated via interactions both with G-protein coupled seven transmembrane receptors and with nuclear hormone receptor PPARγ. We describe a new and efficient route to enantiomerically homogeneous lysophospholipid analogues from (*S*)-1,2,4-butanetriol to give two 3-difluoromethyl substituted analogues of 2-acyl-*sn*-glycerol 3-phosphate. These compounds are migration-blocked analogues of the liable *sn*-2 LPA species. Preliminary studies were conducted on a nuclear reporter assay in which monocytic cells were transfected with a luciferase construct activated by a PPARγ nuclear receptor response element and have shown that the 3-difluoromethyl substituted analogues are fully active as natural LPA.

1. Introduction

Lysophosphatidic acid (LPA, 1- or 2-acyl-*sn*-glycerol 3-phosphate) is a naturally occurring phospholipid that shows a variety of biological activities on a wide spectrum of cell types.[1,2] LPA binds and activates four G protein-coupled receptors (GPCRs): LPA_1, LPA_2, LPA_3 and LPA_4 receptors (formerly Edg-2, Edg-4, Edg-7 and an orphan GPCR, p2y9/GPR23).[3,4] LPA plays a critical role as a general growth, survival and pro-angiogenic factor, in the regulation of physiological and pathophysiological processes in vivo and in vitro. Abnormalities in LPA metabolism and function in ovarian cancer patients may contribute to the initiation and progression of the disease.[5] Thus, LPA receptors constitute a potential target for cancer therapy.

LPA receptors exhibit characteristic responses to LPA species with different chain lengths, different instauration patterns, and different acyl positions. The tumor promoter ovarian cancer-activating factor is *sn*-2 LPA rather than the more common *sn*-1 LPA isomer.[6] *sn*-2 LPA activates LPA_2 and LPA_3 receptors,[7] but studies of positional specificity are confounded by a chemical equilibrium favoring the *sn*-1 isomer by almost 6-fold. To circumvent this intramolecular rearrangement, we synthesized *sn*-2 LPA analogues where one hydroxyl group was transformed in the isosteric difluoromethyl moiety to provide LPA analogues that could not undergo acyl migration or further acylation. In addition to GPCR receptors, LPA was recently shown to be an agonist of the nuclear transcription factor PPARγ.[8] PPARγ has long been implicated in atherogenesis.[9,10] PPAR are lipid-activated transcription factors of the nuclear receptor super family that heterodimerize with the retinoic acid X receptor (RXR). PPAR/RXR heterodimers bind to specific peroxisome proliferator response elements (PPRE) to regulate gene expression.[11] Many compounds activate PPARγ, including the anti-diabetes drug rosiglitazone, oxidized phospholipids, fatty acids, eicosanoids, and oxidized LDL. PPARγ is expressed in macrophages and monocytes, vascular smooth muscle cells, endothelial cells, and is highly expressed in atherosclerotic lesions and hypertensive vascular wall.[10,12]

The isosteric substitution of essential hydroxyl groups by fluorine has been a mainstay of analogue design when metabolic stability is desired.[13,14] It is particularly favored as a substitute when the presence of an electronegative atom is sufficient for the interaction of the ligand with the target protein. The difluoromethyl substitution can introduce unexpected biological activity, since the difluoromethyl group has been viewed as being isosteric with a hydroxyl group[15] or as a hydrogen bond donor,[16] allowing for a variety of interactions with solvents and biological molecules.[16,17] Indeed, the high electron density gives rise to the ability of the difluoromethyl substitutent to act as an acceptor in intra- and intermolecular hydrogen bonds, although the acceptor role has not been substantiated. These interactions can result in modified binding to a receptor. Therefore, we decided to test the hypothesis that LPA analogues with a difluoromethyl group in the *sn*-1

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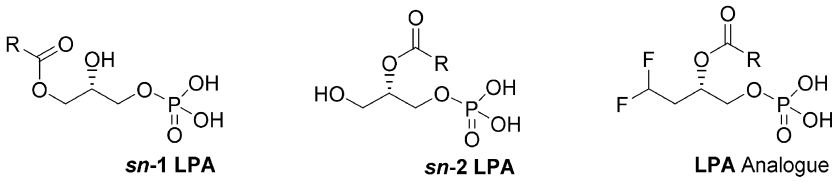


Figure 1. LPA and isosteric analogs.

position might mimic 2-acyl-*sn*-glycerol 3-phosphate as a biological ligand. Such mimics could thus be useful in defining the regiochemical selectivity of LPA receptors for the *sn*-2 acyl position.

Ligand recognition by GPCRs, as well as substrate recognition by enzymes, generally shows a preference for the naturally occurring enantiomer over the unnatural one. However, recognition of LPA by its receptors can be viewed as an exception, as both the natural L (*R*) and unnatural D (*S*) stereoisomers of LPA are equally active in some bioassays.[18] In contrast to the enantiomers of natural LPA, our preliminary biological results have demonstrated that the unnatural D (2*S*) stereoisomers of some *O*-methylated LPA analogues (OMPT)[19] are more active than naturally occurring L (2*R*) enantiomorphs.[20] On the basis of these results, we synthesized the (2*S*) enantiomers of *sn*-2 acyl LPA analogs as the target non-migrating LPA analogues (Fig. 1).

2. Results and discussion

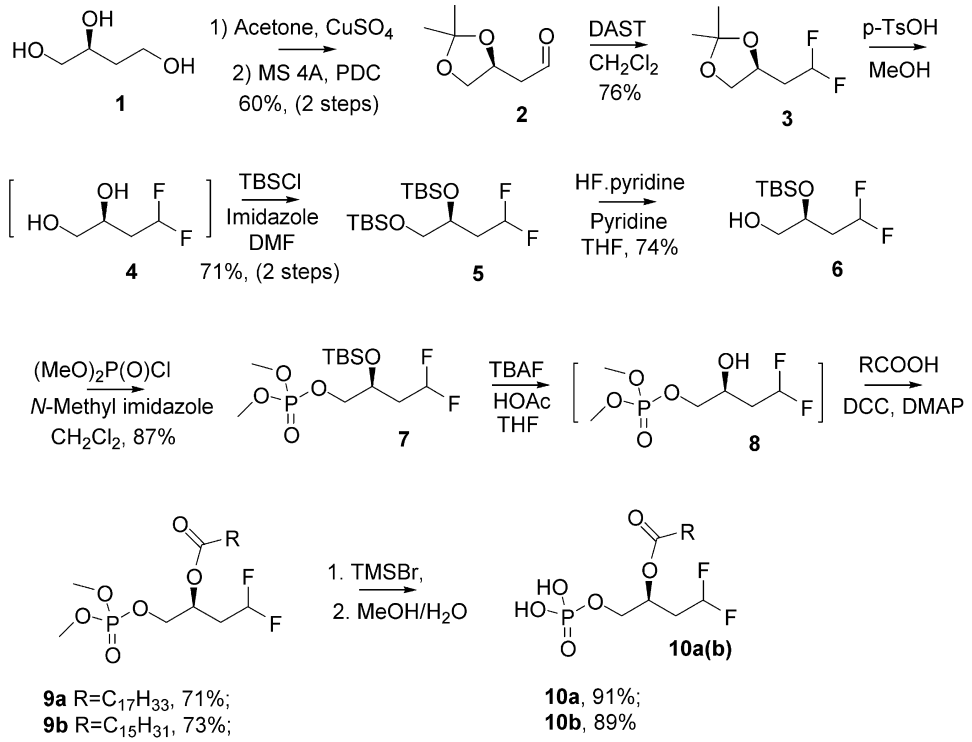
The most general strategy for preparation of difluoromethylated compounds has been conversion of an aldehyde[21] into the corresponding *gem*-difluoride with reagents such as SF_4,[22] aminosulfur trifluorides (DAST,[23] Deoxo-Fluor[24]), and SeF_4.[25] Recently, we synthesized 1,1-difluoro substituted 1-deoxy-(2*S*)-acyl-*sn*-glycerol-3-phosphates as migration-blocked *sn*-2 LPA analogues.[26] However, these analogues failed to show either agonist or antagonist activity when tested in cells expressing LPA_1, LPA_2, or LPA_3 receptors.[8] Since difluoromethyl group can be viewed as isosteric with a hydroxyl group, it could be argued that those analogues had suffered a truncation of the three-carbon glycerol backbone, known to be important for LPA–receptor interactions. Thus, to remedy this unintentional truncation, we designed the four-carbon chain, 1-difluoromethyl-deoxy-(2*S*)-acyl-*sn*-glycerol-3-phosphate **10**, which retains the three-carbon glycerol backbone plus the difluoromethyl group mimicking the C-1 hydroxyl group.

The synthetic routes to the target difluoromethyl compounds 1-difluorodeoxy-2-acyl-*sn*-glycerol-3-phosphate **10** were based on the following design considerations. First, it was necessary to install the fluorine prior to acylation to avoid acyl chain migration during the synthesis. Second, for ease of synthetic manipulations, the deprotection of the penultimate dimethyl phosphate with TMSBr was selected to permit incorporation of unsaturated acyl chains, as well as to reveal the charged phosphate at the final step of the synthesis. Thus, the commercial chiral synthon, (*S*)-1,2,4-butanetriol **1** was chosen as the starting compound. The triol was protected to form the isopropylidene ketal by using $CuSO_4$ as dehydrating agent, and the resulting primary alcohol was oxidized with PDC to provide (*S*)-3,4-dihydroxybutanal acetonide **2**.[27] Nucleophilic fluorination of the aldehyde to difluoromethyl group with DAST gave difluoromethyl intermediate **3** in 76% yield after purification by vacuum distillation.

Next, acidic cleavage of the acetonide with methanolic *p*-TsOH gave the diol intermediate, which was converted to the bis-silyl ether using excess *tert*-butyldimethylsilyl (TBDMS) chloride and imidazole in anhydrous DMF. The more labile primary TBDMS was then cleaved selectively using pyridinium–HF in pyridine–THF at ambient temperature.[28] Phosphorylation of the primary alcohol with dimethylphosphoryl chloride gave phosphate **7**. Initial attempts using potassium *tert*-butoxide as base provided modest yields (56%) of phosphate **7**.[29] Exploration of several organic bases revealed that *N*-methylimidazole was optimal, providing 87% yield after 24 h reaction time.[30] The secondary TBDMS ether then removed with tetrabutylammonium fluoride (TBAF) in THF to give the secondary alcohol;[31] neutralization of TBAF with acetic acid permitted this desilylation to occur without phosphate migration (as monitored by ^{31}P NMR). DCC-promoted esterification of **8** with either oleic acid or palmitic acid provided good yields of esters **9a** and **9b**. Finally, treatment of each ester **9** with bromotrimethylsilane (TMSBr) and subsequent addition of 5% aq. methanol provided the desired fluorinated LPA analogues **10a** and **10b** in nearly quantitative yield (Scheme 1).[32,33]

Since the two fluorines of the difluoromethyl group are diastereotopic, in the aprotic solvent $CDCl_3$, the ^{19}F resonance of compounds **6** and **7** exhibited chemical shifts in the ^{19}F NMR spectra that differed by as much as ~1.0 ppm. In addition, each ^{19}F resonance was split into a dddd peak by the two smaller vicinal $^3J_{FF}$, the intermediate geminal $^2J_{HF}$ and the larger geminal $^2J_{FF}$ couplings. However, in the hydrogen-bonding solvent CD_3OD, the pattern was strikingly different, showing an apparent doublet of triplets and the absence of chemical shift difference between the two CHF_2 fluorines. The spectra changes can be visualized clearly by titration of CD_3OD into $CDCl_3$. As the ratio of CD_3OD increased, the chemical shift difference between the diastereotopic fluorines gradually deceased. One plausible reason is the formation of $C-F\cdots D-O-CD_3$ hydrogen bonds diminished the differences in the chemical environments of the diastereotopic difluoromethyl fluorine atoms.[16]

We have found that the phosphoric acid forms of LPA analogues can be labile during storage or when made as stock solutions for biological evaluation. Thus, we have adopted a standard protocol to obtain a stable sodium salt



Scheme 1.

form of each LPA analogue. For example, **10a** was dissolved in 1.0 M triethylammonium bicarbonate (TEAB) buffer (pH 8.0) to give a slightly cloudy solution, which was absorbed onto a sodium ion-exchange column (Dowex 50WX8-200 resin, neutral Na^+ form). The desired mixed neutral sodium salt of **1a** was eluted with Nanopure water. The product solution was lyophilized to give an amorphous white powder, which was stored in solid form at −80 °C under nitrogen atmosphere. Aqueous or DMSO solutions of LPA analogues were prepared and used

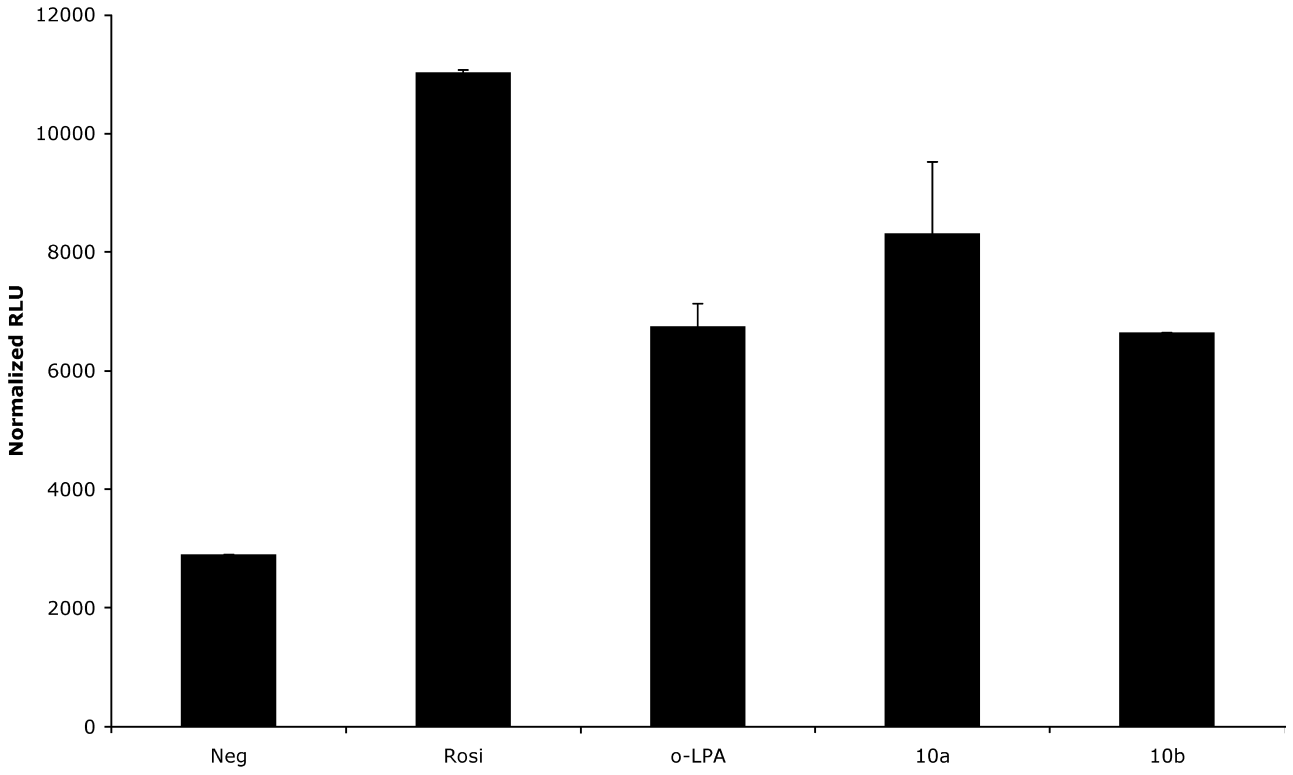


Figure 2. Lipid activation of a peroxisome proliferator responsive element-reporter. Key: RLU, relative light units; Neg, negative control; Rosi, rosiglitazone; o-LPA, *sn*-1-oleoyl lysophosphatidic acid).

within several days to minimize hydrolysis or other decomposition.

2.1. Synthetic LPA analogs activate PPRE function

We found that the fluorodeoxy LPA analogues induced luciferase expression from the acyl-CoAoxidase PPRE reporter (Fig. 2). The effective concentrations for reporter activation by **10a**, **10b**, and LPA were equivalent, so substitution of difluoromethyl for the hydroxyl of LPA was indeed an effective strategy to create stabilized LPA mimetic for this receptor. **10a** has oleyl chain and **10b** has palmitoyl chain, so there was no specificity for the acyl residue. Importantly, the trissulfonamid translocase enhanced the effect of **10a** and **10b** in stimulating the PPRE reporter, as anticipated from the free phosphoryl group of **10a** and **10b**.[8] Since **10a** and **10b** cannot be acylated, intracellular expression of LPA acyltransferase did not change the response to either of these two stable LPA analogues. However, these analogues failed to show either agonist or antagonist activity when tested in insect cells expressing LPA_1, LPA_2, or LPA_3 receptors.[34] The GPCR system is more stereoselective in the requirements for LPA mimetics, and thus the *sn*-3 difluoromethyl substituted LPA analogues elicits 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 backbones and acyl chains in order to create a panel of LPA analogues that differentiate among the nuclear transcription factor PPARγ and the LPA receptor subtypes.

3. Experimental

3.1. 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 distillated from sodium wire. Methylene chloride was distillated from CaH_2. Reactions were performed under an inert atmosphere (N_2 or Ar) unless otherwise indicated. ^1H and ^{13}C spectra were recorded at 400 MHz (^1H), 101 MHz (^{13}C), 162 MHz (^{31}P) and 376 MHz (^{19}F), temp. 25 °C. Chemical shifts are given in ppm with TMS as internal standard (δ=0.00); ^{31}P, 85% H_3PO_4 (δ=0.00); ^{19}F, $CFCl_3$ (δ=0.00).

3.1.1. (*S*)-3,4-Dihydroxybutanal acetonide (2). To a solution of (*S*)-1,2,4-butanetriol (20.1 g, 200 mmol) in 380 mL of anhydrous acetone at room temperature were added anhydrous $CuSO_4$ (23 g) and a catalytic amount of *p*-toluenesulfonic acid (0.23 g, 1.211 mmol). The reaction mixture was stirred at room temperature for 3 days and then quenched with solid potassium carbonate (0.33 g, 2.422 mmol). Inorganic salts were filtered and washed with ethyl acetate. All organic solvents were combined and concentrated to give a colorless oil which was purified by flash chromatograph (2% methanol/chloroform) to afford the protected triol, (*S*)-1,2,4-Butanetriol-1,2-acetonide (26.5 g, 182 mmol, 91% yield). The protected triol (8.16 g, 55.9 mmol) was dissolved in 120 mL of anhydrous dichloromethane followed by slow addition of pyridinium dichromate (25.2 g, 67.1 mmol) and powdered 4Å molecule sieves (12.4 g). The suspension was stirred vigorously overnight at room temperature. Hexane/ethyl acetate (1:1, 100 mL) was added to the reaction mixture that was then stirred for 30 min. The black suspension was filtered through a short flash silica gel column to remove excess PDC and its reduced forms. The organic solvents were removed and the residue was distillated under vacuum (22 mm Hg) to give 5.20 g (36.1 mmol, 66% yield) of acetonide **2** as a colorless oil (bp 90 °C/22 mm Hg).

^1H NMR ($CDCl_3$): 9.80 (s, 1H), 4.52 (m, 1H), 4.17 (m, 1H), 3.57 (m, 1H), 2.80 (m, 1H), 1.40 (s, 3H), 1.34 (s, 3H). ^{13}C NMR ($CDCl_3$): 199.9 (s), 109.5 (s), 70.7 (s), 69.1 (s), 47.8 (s), 26.8 (s), 25.4 (s). $[\alpha]_D^{20}$=+16.1° (2.12, $CHCl_3$); lit.[27] +16.5° (5.32, $CHCl_3$).

3.1.2. 1,2(*S*)-Acetonide-4,4-difluoro-1,2-butanediol (3). To 0.748 g (5.194 mmol) of (*S*)-3,4-dihydroxybutanal acetonide dissolved in dry CH_2Cl_2 (10 mL) was slowly added, with good stirring, (0.824 mL, 6.233 mmol) of diethylaminosulfur trifluoride (DAST). After being stirred at room temperature for 24 h, the reaction mixture was quenched with 10% $NaHCO_3$ solution (20 mL). The aqueous layer was extracted with CH_2Cl_2 (20 mL×2), then combined the organic layer and dried with anhydrous Na_2SO_4. The solvent was removed by fractional distillation until the head temperature reached 40 °C. The residue was then distillated at reduced pressure (ca. 20 mm Hg) collecting the fraction distilling at 59–60 °C to give 0.655 g (3.947 mmol, 76%) of acetonide **3** as a colorless liquid.

^1H NMR ($CDCl_3$): 5.93 (tdd, *J*=56.4, 6.4, 2.8 Hz, 1H), 4.23 (m, 1H), 4.08 (dd, *J*=8.0, 6.4 Hz, 1H), 3.57 (dd, *J*=8.4, 7.2 Hz, 1H), 2.15–1.99 (m, 2H), 1.38 (s, 3H), 1.32 (s, 3H). ^{13}C NMR ($CDCl_3$): 115.5 (t, *J*=238.53 Hz), 109.3 (s), 70.4 (dd, *J*=8.5, 3.8 Hz), 69.1 (s), 38.4 (t, *J*=20.6 Hz), 26.8 (s), 25.5 (s). ^{19}F NMR ($CDCl_3$): −115.71 (1F, dddd, *J*=286.8, 56.1, 25.2, 16.2 Hz), −118.23 (1F, dddd, *J*=286.8, 56.1, 25.2, 16.2 Hz). MS (CI) *m*/*z* 167.1 (M^++1, 100.00), 151.1 (M^+-CH_3, 3.07). HRMS, M^++1. Found: 167.0878. Calcd for $C_7H_{13}O_2F_2$, 167.0884. $[\alpha]_D^{20}$=−7.8° (1.17, MeOH).

3.1.3. 1,2(*S*)-Di[1-(*tert*-butyl)-1,1-dimethylsilyl]-4,4-difluoro-butane-1,2-diol (5). TsOH (34 mg, 0.205 mmol, 0.10 equiv.) was added to a solution of (2*R*)-3,3-difluoro-1,2-propanediol 1,2-acetonide (0.341 g, 2.054 mmol) in MeOH (8 mL), and the solution was stirred at room temperature for 24 h. After addition of NEt_3 (0.2 mL), the solvent was removed under reduced pressure. After addition of anhydrous DMF (3 mL), imidazole (0.420 g, 6.16 mmol, 3.0 equiv.) and *tert*-butyldimethylsilyl chloride (TBDMSCl) (0.87 g, 5.75 mmol, 2.8 equiv.), the reaction mixture was stirred at room temperature for another 24 h. The solution was diluted with water (10 mL) and ethyl acetate (10 mL), and the aqueous layer was separated and extracted with ethyl acetate (20 mL×3). The combined

organic layers were dried over anhydrous Na_2SO_4, concentrated and the residue was purified by chromatography (*n*-hexane/ethyl acetate 100:1, R_f=0.22) to afford 0.52 g (0.145 mmol, 71%) of diol **5** as a colorless liquid.

^{1}H NMR ($CDCl_3$): 5.93 (tdd, *J*=56.8, 6.0, 3.2 Hz, 1H), 3.86 (m, 1H), 3.57 (dd, *J*=10.4, 5.2 Hz, 1H), 3.40 (dd, *J*=10.4, 7.2 Hz, 1H), 2.13–1.90 (m, 2H), 0.87 (s, 9H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.035 (s, 3H). ^{13}C NMR ($CDCl_3$): 116.4 (t, *J*=237.7 Hz), 68.5 (m), 67.1 (s), 39.2 (t, *J*=21.5 Hz), 25.9 (s), 25.7 (s), 18.3 (s), 18.0 (s), −4.4 (s), −5.2 (s), −5.4 (s), −5.5 (s). ^{19}F NMR ($CDCl_3$): −116.96 (2F, ddd, *J*=56.8, 18.4, 14.7 Hz). MS (CI) *m/z* 355.2 (M^++1, 100.00), 297.1 ($M^+-C_4H_9$, 13.12). HRMS, M^++1. Found: 355.2300. Calcd for $C_{16}H_{37}O_2F_2Si_2$, 355.2300 $[\alpha]_D^{20}=-23.6°$ (1.33, MeOH).

3.1.4. 2(*S*)-[1-(*tert*-Butyl)-1,1-dimethylsilyl]-4,4-difluorobutane-1,2-diol (6). The HF·pyridine complex (70%, 6.02 mmol fluoride, 0.175 mL) was added to a mixture of pyridine (0.53 mL) and a solution of DiTBS ether **5** (0.355 g, 1.003 mmol) in THF (5 mL). The reaction mixture was stirred for 20 h. After completion of the reaction (TLC control), the solution was diluted with ethyl acetate (30 mL), washed with 0.5 M HCl (6 mL×2) and saturated $CuSO_4$ solution (6 mL) and dried over anhydrous Na_2SO_4. After removal of the solvents, the residue was purified by chromatography (*n*-hexane/ethyl acetate 5:1, R_f=0.30) to give 0.178 g (0.742 mmol, 74%) of diol **6** as a colorless liquid.

^{1}H NMR ($CDCl_3$): 5.89 (tdd, *J*=56.8, 6.0, 3.6 Hz, 1H), 3.96 (m, 1H), 3.60 (m, 1H), 2.14–1.99 (m, 2H), 1.93 (br, 1H), 0.85 (s, 9H), 0.073 (s, 3H), 0.069 (s, 3H). ^{13}C NMR ($CDCl_3$): 116.1 (t, *J*=237.3 Hz), 68.0 (dd, *J*=7.0, 4.0 Hz), 66.3 (s), 38.4 (t, *J*=20.7 Hz), 25.7 (s), 17.9 (s), −4.6 (s), −5.1 (s). ^{19}F NMR ($CDCl_3$): −116.42 (1F, dddd, *J*=286.8, 56.8, 23.7, 14.3 Hz), −117.77 (1F, dddd, *J*=286.8, 56.8, 23.7, 14.3 Hz). MS (CI) *m/z* 241.1 (M^++1, 100.00), 183.0 ($M^+-C_4H_9$, 8.93). HRMS, M^++1. Found: 241.1429. Calcd for $C_{10}H_{23}O_2F_2Si$, 241.1435. $[\alpha]_D^{20}=-16.3°$ (1.79, MeOH).

3.1.5. 1-Phospho-2(*S*)-[1-(*tert*-butyl)-1,1-dimethylsilyl]-4,4-difluoro-butane-1,2-diol dimethyl ester (7). *N*-methylimidazole (26 mg, 0.321 mmol, 1.4 equiv.) was added to a stirred solution of (55 mg, 0.229 mmol) (2*R*)-3,3-difluorine-2-di[[1-(*tert*-butyl)-1,1-dimethylsilyl]oxyl]-1-propanol and dimethyl chlorophosphate (40 mg, 0.275 mmol, 1.2 equiv.) in CH_2Cl_2 (10 mL) at room temperature. After stirring for 24 h, the reaction was complete. A saturated aqueous solution of NH_4Cl (5 mL) was added to the reaction mixture and stirred for 10 min. The aqueous phase was extracted with CH_2Cl_2 (5 mL×3), the organic solution was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by chromatography (*n*-hexane/ethyl acetate 3:2, R_f=0.25) to give 69 mg (0.199 mmol, 87%) of phosphotriester **7** as a colorless oil.

^{1}H NMR ($CDCl_3$): 5.98 (tdd, *J*=56.8, 6.0, 4.4 Hz, 1H), 4.80 (m, 1H), 3.73 (d, *J*=2.8 Hz, 3H), 3.70 (d, *J*=2.8 Hz, 3H), 3.72–3.69 (m, 2H), 2.43–2.13 (m, 2H), 0.84 (s, 9H), 0.024 (s, 3H), 0.021 (s, 3H). ^{13}C NMR ($CDCl_3$): 115.3 (t, *J*=239.2 Hz), 67.8 (dd, *J*=12.3, 5.4 Hz), 64.6 (s), 64.6 (s), 54.3 (d, *J*=5.4 Hz), 36.9 (td, *J*=22.2, 5.3 Hz), 25.7 (s), 18.1 (s), −5.6 (s). ^{19}F NMR ($CDCl_3$): −116.13 (1F, ddd, *J*=286.8, 26.3, 15.8 Hz), −117.16 (1F, ddd, *J*=286.8, 26.3, 15.8 Hz). ^{31}P NMR ($CDCl_3$): 1.473 (s). MS (CI) *m/z* 349.0 (M^++1, 100.00), 241.1 (M^++2-$C_2H_6PO_3$, 14.04). HRMS, M^++1. Found: 349.1419. Calcd for $C_{12}H_{28}F_2O_2PSi$, 349.1412. $[\alpha]_D^{20}=-21.0°$ (0.92, MeOH).

3.1.6. 1-Phospho-2(*S*)-oleoyl-4,4-difluoro-butane-1,2-diol dimethyl ester (9a). A solution of **7** (48 mg, 0.138 mmol) in THF (5 mL) was treated successively with acetic acid (32 μL, 0.552 mmol) and *tetra*-butylammoniumfluoride trihydrate (174 mg, 0.552 mmol) at room temperature. After stirring for 18 h, the reaction was completed (TLC control), then the solvent was evaporated under reduced pressure and the crude product was purified by pass through a short column to afford a colorless liquid. To the alcohol solution and (43 mg, 48 μL, 0.152 mmol) of oleic acid in dry CH_2Cl_2 (1 mL) at room temperature were added dropwise a solution of DCC (43 mg, 0.207 mmol) and DMAP (10 mg, 0.083 mmol) in dry CH_2Cl_2 (2 mL). The solution was stirred at room temperature for 16 h and filtered, the solvent removed, and the residue was purified by chromatography (*n*-hexane/ethyl acetate 1:1, R_f=0.24) to give 49 mg, (0.098 mmol, 71%) of protected analogue **9a** as a waxy solid.

^{1}H NMR ($CDCl_3$): 5.89 (tdd, *J*=56.8, 6.0, 4.4 Hz, 1H), 5.34 (m, 2H), 5.20 (m, 1H), 4.16 (m, 1H), 4.06 (m, 1H), 3.77 (d, *J*=2.8 Hz, 3H), 3.40 (d, *J*=2.8 Hz, 3H), 2.29 (t, *J*=8.0 Hz, 2H), 2.30–2.10 (m, 2H), 1.98 (m, 4H), 1.59 (m, 2H), 1.24 (20H, m), 0.85 (t, *J*=8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): 172.8 (s), 130.0 (s), 129.7 (s), 114.9 (t, *J*=239.3 Hz), 67.6 (s), 67.5 (s), 66.7 (d, *J*=6.9 Hz), 35.3 (t, *J*=22.3 Hz), 34.1 (s), 33.9 (s), 31.9 (s), 29.7 (s), 29.7 (s), 29.5 (s), 29.3 (s), 29.1 (s), 29.1 (s), 29.0 (s), 27.2 (s), 27.1 (s), 24.9 (s), 24.7 (s), 22.7 (s), 14.1 (s). ^{19}F NMR (CDOD): −117.91 (dt, *J*=56.5, 16.9 Hz). ^{31}P NMR ($CDCl_3$): 2.225 (s). MS (CI) *m/z* 499.1 (M^++1, 100.00). HRMS, M^++1. Found: 499.3004. Calcd for $C_{24}H_{46}F_2O_6P$, 499.3000. $[\alpha]_D^{20}=-10.3°$ (0.30, MeOH).

3.1.7. 1-Phospho-2(*S*)-oleoyl-4,4-difluoro-butane-1,2-diol (10a). Thoroughly dried **9a** (15 mg, 0.030 mmol, 5 h under high vacuum) was dissolved in anhydrous methylene chloride (1 mL) at room temperature. Bromotrimethylsilane (14 μL, 0.105 mmol) was added with a dry syringe and stirred for 4 h. TLC indicated that all of the reactant had disappeared, then the solvent removed under reduced pressure and dried under vacuum. The residue was dissolved in 95% methanol (1 mL) for 1 h, then the solvent removed under reduced pressure and dried under vacuum, got final product (13 mg, 0.027 mmol, 91% yield.). The labile acid forms of these analogues were then converted to neutral sodium salts. Thus, product **10a** was dissolved in 2 mL of 1.0 M triethylammonium bicarbonate (TEAB) buffer (pH 8.0) to give a slightly cloudy solution, which was absorbed to a sodium ion-exchange column (Dowex 50WX8-200 resin, neutral Na^+ form). The desired mixed neutral sodium salt of **10a** was eluted with Nanopure water. The product solution was lyophilized to give sodium salt as white amorphous solid, which was stored in solid form at −80 °C under nitrogen atmosphere.

^{1}H NMR (CD_3OD): 5.99 (tt, J=56.0, 4.0 Hz, 1H), 5.34 (m, 2H), 5.22 (m, 1H), 4.02 (m, 2H), 2.35 (t, J=7.2 Hz, 2H), 2.26–2.03 (m, 2H), 2.03 (m, 4H), 1.58 (m, 2H), 1.32 (m, 20H), 0.89 (t, J=7.2 Hz, 3H). ^{13}C NMR (CD_3OD): 172.5 (s), 128.9 (s), 128.8 (s), 114.9 (t, J=237.7 Hz), 66.5 (d, J=7.6 Hz), 65.8 (d, J=4.6 Hz), 34.3 (t, J=22.2 Hz), 33.0 (s), 32.5 (s), 31.06 (s), 28.8 (s), 28.8 (s), 28.6 (s), 28.5 (s), 28.3 (s), 28.3 (s), 28.2 (s), 28.1 (s), 26.1 (s), 24.0 (s), 23.9 (s), 21.7 (s), 12.5 (s). ^{19}F NMR (CD_3OD): −117.85 (dt, J=58.0, 4.9 Hz). ^{31}P NMR ($CDCl_3$): 0.870 (s). MS (CI) m/z 471.3 (M^++1, 32.34), 421.3 (M^+-CF_2H, 100.00). HRMS, M^++1. Found: 471.2657. Calcd for $C_{22}H_{42}F_2O_6P$, 471.2687. $[\alpha]_D^{20}$=−17.5° (0.04, MeOH).

3.1.8. 1-Phospho-2(*S*)-palmitoyl-4,4-difluoro-butane-1,2-diol dimethyl ester (9b). A solution of **7** (25 mg, 0.064 mmol) in THF (3 mL) was treated successively with acetic acid (15 μL, 0.256 mmol) and *tetra*-butyl-ammoniumfluoride trihydrate (81 mg, 0.256 mmol) at room temperature. After stirring for 4 h, the reaction was completed (TLC control), then the solvent was evaporated under reduced pressure and the crude product was purified by pass through a short column to afford a colorless liquid. To the alcohol and (18 mg, 0.071 mmol) of palmitic acid in dry CH_2Cl_2 (1 mL) at room temperature was added dropwise a solution of DCC (20 mg, 0.096 mmol) and DMAP (5 mg, 0.038 mmol) in dry CH_2Cl_2 (1 mL). The solution was stirred at room temperature for 16 h and filtered, the solvent removed, and the residue was purified by chromatography (*n*-hexane/ethyl acetate 1:1, R_f=0.26) to give 24 mg (0.052 mmol, 73%) of the protected analogue **9b** as a waxy solid.

^{1}H NMR (CD_3OD): 5.89 (tdd, J=56.8, 6.0, 4.4 Hz, 1H), 5.21 (m, 1H), 4.17 (m, 1H), 4.09 (m, 1H), 3.76 (d, J=2.8 Hz, 3H), 3.73 (d, J=2.8 Hz, 3H), 2.30 (t, J=8.0 Hz, 2H), 2.26–2.12 (m, 2H), 1.57 (m, 2H), 1.22 (m, 26H), 0.85 (t, J=6.8 Hz, 3H). ^{13}C NMR (CD_3OD): 172.8 (s), 114.9 (t, J=239.2 Hz), 67.6 (s), 67.5 (s), 66.7 (m), 35.3 (t, J=22.2 Hz), 34.1 (s), 33.9 (s), 31.9 (s), 29.7 (s), 29.6 (s), 29.6 (s), 29.4 (s), 29.3 (s), 29.2 (s), 29.0 (s), 24.8 (s), 22.7 (s), 14.1 (s). ^{19}F NMR (CD_3OD): −117.51 (dt, J=56.5, 15.6 Hz). ^{31}P NMR (CD_3OD): 2.218 (s). MS (CI) m/z 473.1 (M^++1, 54.60), 225.1 ($M^+-C_5H_{11}-CF_2H$, 100.00). HRMS, M^++1. Found: 473.2835. Calcd for $C_{22}H_{42}F_2O_6P$, 473.2844. $[\alpha]_D^{20}$=−14.6° (0.28, MeOH).

3.1.9. 1-Phospho-2(*S*)-palmitoyl-4,4-difluoro-butane-1,2-diol (10b). Thoroughly dried **9b** (14 mg, 0.030 mmol, 5 h under high vacuum) was dissolved in anhydrous methylene chloride (1 mL) at room temperature. Bromotrimethylsilane (14 μL, 0.104 mmol) was added with a dry syringe and stirred for 4 h. TLC indicated that all of the reactant had disappeared, then the solvent removed under reduced pressure and dried under vacuum. The residue was dissolved in 95% methanol (1 mL) for 1 h and got final product (12 mg, 0.027 mmol, 89%). The LPA analogue **10b** was converted to the corresponding sodium salt using the same procedure as for **10a**. ^{1}H NMR (CD_3OD): 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): 173.7 (s), 114.4 (t, J=242.3 Hz), 71.2 (td, J=23.7, 8.5 Hz), 63.9 (d, J=4.6 Hz), 34.7 (s), 33.1 (s), 30.8 (s), 30.8 (s), 30.7 (s), 30.6 (s), 30.5 (s), 30.4 (s), 30.1 (s), 25.9 (s), 23.7 (s), 14.5 (s). ^{19}F NMR (CD_3OD): −116.65 (dt, J=58.0, 4.9 Hz). ^{31}P NMR ($CDCl_3$): 0.709 (s). MS (CI) m/z 445.3 (M^++1, 46.30), 323.2 ($M^+-C_5H_{11}-CF_2H$, 100.00). HRMS, M^++1. Found: 445.2510. Calcd for $C_{20}H_{42}F_2O_6P$, 445.2531. $[\alpha]_D^{20}$=−18.8° (0.04, MeOH).

3.2. Synthetic LPA analogs activate PPRE function

CV-1 cells were transiently transfected with a luciferase reporter under the control of the PPAR responsive element of rat acyl-CoA oxidase and SV40 β-galactosidase to normalize transfection efficiency. These cells were stimulated for 18 h with 5 μM of the stated lipid (rosi, rosiglitazone; o-LPA, oleoyl lysophosphatidic acid) before luciferase and β-galactosidase were determined as described in 'Methods'. Data are the range of two determinations and represent the result of two experiments. *Methods*: CV-1 cells were transiently transfected with the PPAR responsive element of rat acyl-CoA oxidase and SV40 β-galactosidase and stimulated production of luciferase was determined as previously described.[35]

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