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Synthesis of Cyclic Phosphonate Analogues of (Lyso)phosphatidic Acid Using a Ring-Closing Metathesis Reaction

Honglu Zhang,[†] Ryoko Tsukuhara,[‡] Gabor Tigyi,[‡] and Glenn D. Prestwich*[,†]

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

gprestwich@pharm.utah.edu

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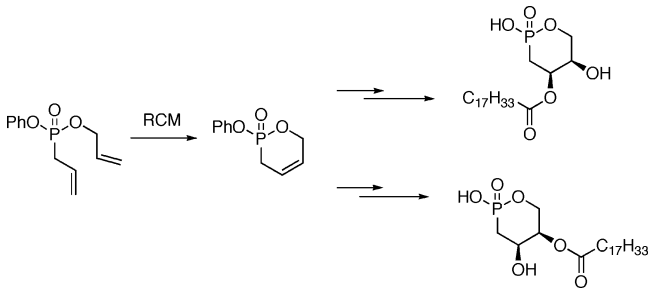

We describe a versatile and efficient method for the preparation of acyloxy-substituted six-membered cyclic phosphonates using the ring-closing metathesis. After closure, the key cyclic phosphonate intermediate was dihydroxylated and converted to a new class of conformationally constrained PA and LPA analogues. The oleoyloxy-substituted cyclic phosphonate **4** had unique receptor-selective properties as a ligand, showing partial activation of the LPA_2 GPCR and weak antagonism of the LPA_1 GPCR.

Introduction

Lysophosphatidic acid (LPA) and phosphatidic acid (PA) elicit a rich palette of biological responses in human health and pathophysiology.[1−3] The majority of these cellular events are transduced via three G-protein-coupled receptors (GPCRs): LPA_1, LPA_2, and LPA_3.[4] Recently, two additional LPA effectors, LPA_4/GPR23[5] and the nuclear transcription factor PPARγ, have been reported.[6] While the importance of LPA_4 remains unknown, PPARγ appears to mediate the action of LPA in neointimal thickening during atherosclerosis.[7,8] The importance of LPA receptors and downstream signaling events as a rich source of potential therapeutic targets has led to increased interest in the functional lipidomics of LPA and LPA binding proteins.[9,10] In addition, LPA signaling and biosynthesis via lysoPLD[11] are under active investigation for new cancer therapies.[12−14] In addition, LPA signaling regulates aspects of neural development.[15]

The pleiotropic actions of LPA can be dissected by appreciating receptor isoform-specific physiology,[16] and to this end LPA analogues with isoform-selective agonist and antagonist activities are urgently needed. LPA_1 is essential for cell invasion in cancer[17] and this isoform plays a crucial role in brain development.[18,19] The absence of LPA_1 expression results in craniofacial

* To whom correspondence should be addressed. Phone: +1-801-585-9051. Fax: +1-801-585-9053.
[†] The University of Utah.
[‡] University of Tennessee Health Science Center.

(1) Sengupta, S.; Wang, Z. N.; Tipps, R.; Xu, Y. *Semin. Cell Dev. Biol.* **2004**, *15*, 503−512.
(2) Hla, T.; Lee, M. J.; Ancellin, N.; Paik, J. H.; Kluk, M. J. *Science* **2001**, *294*, 1875−1878.
(3) Moolenaar, W. H.; Kranenburg, O.; Postma, F. R.; Zondag, G. C. *Curr. Opin. Cell. Biol.* **1997**, *9*, 168−173.
(4) Fukushima, N.; Ishii, I.; Contos, J. J.; Weiner, J. A.; Chun, J. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 507−534.
(5) Noguchi, K.; Ishii, S.; Shimizu, T. *J. Biol. Chem.* **2003**, *278*, 25600−25606.
(6) McIntyre, T. M.; Pontsler, A. V.; Silva, A. R.; StHilaire, A. S.; Xu, Y.; Hinshaw, J. C.; Zimmerman, G. A.; Hama, K.; Aoki, J.; Arai, H.; Prestwich, G. D. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 131−136.
(7) Zhang, C. X.; Baker, D. L.; Yasuda, S.; Makarova, N.; Balazs, L.; Johnson, L. R.; Marathe, G. K.; McIntyre, T. M.; Xu, Y.; Prestwich, G. D.; Byun, H. S.; Bittman, R.; Tigyi, G. *J. Exp. Med.* **2004**, *199*, 763−774.
(8) Siess, W.; Tigyi, G. *J. Cell Biochem.* **2004**, *92*, 1086−1094.
(9) vanMeer, G. *EMBO J.* **2005**, *24*, 3159−3165.
(10) Tanyi, J.; Croetzer, D.; Wolf, J.; Yu, S.; Hasegawa, Y.; Lahad, J.; Cheng, K. W.; Umezu-Goto, M.; Prestwich, G. D.; Morris, A. J.; Newman, R. A.; Felix, E. A.; Lapis, R.; Mills, G. B. In *Functional Lipidomics*; Feng, L., Prestwich, G. D., Eds.; CRC Press/Taylor & Francis: New York, 2006; p 101−123.
(11) Tokumura, A. *J. Cell Biochem.* **2004**, *92*, 869−881.

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dimorphism, semilethality due to defective suckling behavior, and generation of a small fraction of pups with frontal hematoma.[18] Although LPA_2 is not essential for normal mouse development, it acts redundantly with LPA_1 to mediate LPA responses in fibroblasts.[20] LPA_3 has recently been found to be critically important for embryo implantation and spacing.[21] In addition, phosphatidic acid (PA) regulates phosphoinositide metabolism and plays key roles in cell growth and protein trafficking.[22] PA is thus an important lipid mediator of cellular physiology, and its metabolism,[23] and interactions with PA-binding proteins deserve continued attention.[24] In addition, short chain PA analogues have proven to be important bioactive ligands of the LPA GPCRs.[25]

The search for metabolically stabilized, receptor-isoform specific agonists and antagonists for LPA receptors has been an ongoing focus of our research[26−33] and that of others.[25,34−36] By targeting one of five receptors, we can separate the overlapping effects of the native ligand. Moreover, the production of metabolically stabilized analogues that can resist acyl migration and hydrolysis, as well as phosphatase and acyltransferase activities, provides more useful ligands for cellular and organismal biology.

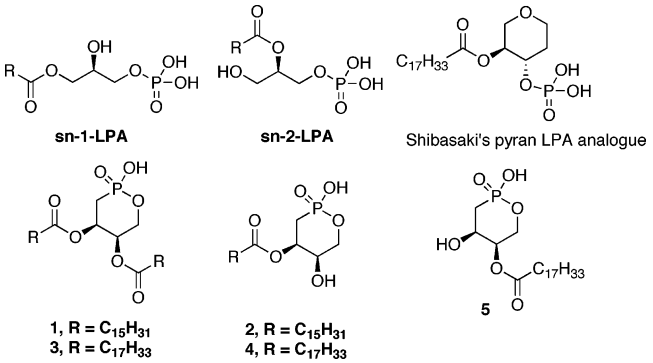


FIGURE 1. (Top) LPA and pyran analogue; (bottom) target racemic cyclic phosphonates **1**−**5**.

Recently, the Shibasaki research group reported a selective LPA_1 agonist that can distinguish between LPA_1 and LPA_2.[37] Inspired by the selectivity of these cyclic deoxysugar-like agonists for LPA_1 and LPA_2, and intrigued by the antimetastatic properties of five-membered ring cyclic phosphatidic acid[38] and its modeling into the LPA GPCRs,[25] we designed a new family of six-membered ring cyclic phosphonates (Figure 1). These molecules provide conformational constraint by converting the glyceryl backbone to a ring structure and metabolic stabilization via the phosphonate.[37] Herein we describe the synthesis of analogues **1**−**5** using a ring-closing metathesis reaction as the key step to form the cyclic phosphonate; we further demonstrate the biological activities of these new LPA and PA analogues with the three canonical LPA_1, LPA_2, and LPA_3 GPCRs.

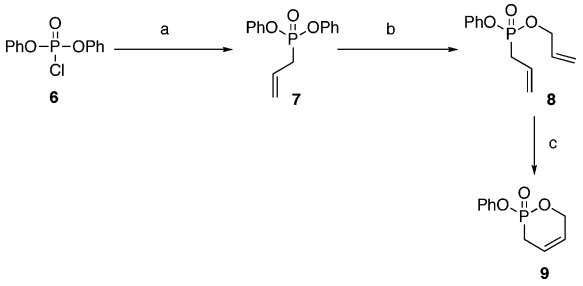
Results and Discussion

The ring-closing metathesis (RCM) is an efficient approach for the preparation of P-sugars.[39,40] Indeed, the diastereoselective RCM in the synthesis of P-stereogenic phosphinates also revealed the power of RCM reaction in generating P-heterocycles that have potential utility in drug development.[41] We envisaged the use of RCM to construct the phosphonate building block **9**. To accommodate both saturated and unsaturated acyl groups, we used two different protecting groups. The phenyl phosphonate substrate was acceptable for palmitoyl analogues, because reductive deprotection could be used.[39] For the oleoyloxy-substituted cyclic phosphate, we used a methyl phosphonate that could be deprotected with $TMSBr/CH_2Cl_2$ without reduction of the oleoyl olefinic bond.

The diphenyl allylphosphonate **7** was prepared (Scheme 1) using the reaction of diphenyl phosphoryl chloride with allylmagnesium bromide in refluxing ether.[42] One of the two phenoxy groups of the resulting diphenyl allyl phosphate **7** was subsequently displaced with lithium allyloxide in THF/HMPA (4:1)[43] to give the diene **8** as the main product in 58% yield.[39,43] The amount of diallyl side product was minimized by using

(12) Mills, G. B.; Eder, A.; Fang, X.; Hasegawa, Y.; Mao, M.; Lu, Y.; Tanyi, J.; Tabassam, F. H.; Wiener, J.; Lapushin, R.; Yu, S.; Parrott, J. A.; Compton, T.; Tribley, W.; Fishman, D.; Stack, M. S.; Gaudette, D.; Jaffe, R.; Furui, T.; Aoki, J.; Erickson, J. R. *Cancer Treat. Res.* **2002**, *107*, 259−283.
(13) Tanyi, J. L.; Morris, A. J.; Wolf, J. K.; Fang, X.; Hasegawa, Y.; Lapushin, R.; Auersperg, N.; Sigal, Y. J.; Newman, R. A.; Felix, E. A.; Atkinson, E. N.; Mills, G. B. *Cancer Res.* **2003**, *63*, 1073−1082.
(14) Raynal, P.; Montagner, A.; Dance, M.; Yart, A. *Pathol. Biol.* **2005**, *53*, 57−62.
(15) Fukushima, N. *J. Cell Biochem.* **2004**, *92*, 993−1003.
(16) Bandoh, K.; Aoki, J.; Taira, A.; Tsujimoto, M.; Arai, H.; Inoue, K. *FEBS Lett.* **2000**, *478*, 159−165.
(17) Hama, K.; Aoki, J.; Fukaya, M.; Kishi, Y.; Sakai, T.; Suzuki, R. *J. Biol. Chem.* **2004**, *279*, 17634−17639.
(18) Contos, J. J.; Ishii, I.; Chun, J. *Mol. Pharmacol.* **2000**, *58*, 1188−1196.
(19) Contos, J. J.; Chun, J. *Gene* **2001**, *267*, 243−253.
(20) Contos, J. J.; Ishii, I.; Fukushima, N.; Kingsbury, M. A.; Ye, X.; Kawamura, S.; Brown, J. H.; Chun, J. *Mol. Cell. Biol.* **2002**, *22*, 6921−6929.
(21) Ye, X. Q.; Hama, K.; Contos, J. J. A.; Anliker, B.; Inoue, A.; Skinner, M. K.; Suzuki, H.; Amano, T.; Kennedy, G.; Arai, H.; Aoki, J.; Chun, J. *Nature* **2005**, *435*, 104−108.
(22) Frohman, M. A.; Sung, T. C.; Morris, A. J. *Biochim. Biophys. Acta* **1999**, *1439*, 175−186.
(23) Exton, J. H. *J. Biol. Chem.* **1990**, *265*, 1−4.
(24) Manifava, M.; Thuring, J. W.; Lim, Z. Y.; Packman, L.; Holmes, A. B.; Ktistakis, N. T. *J. Biol. Chem.* **2001**, *276*, 8987−8994.
(25) Fujiwara, Y.; Sardar, V.; Tokumura, A.; Baker, D.; Murakami-Murofushi, K.; Parrill, A.; Tigyi, G. *J. Biol. Chem.* **2005**, *280*, 35038−35050.
(26) Xu, Y.; Prestwich, G. D. *Org. Lett.* **2002**, *4*, 4021−4024.
(27) Xu, Y.; Prestwich, G. D. *J. Org. Chem.* **2002**, *67*, 7158−7161.
(28) Xu, Y.; Qian, L.; Prestwich, G. D. *J. Org. Chem.* **2003**, *68*, 5320−5330.
(29) Xu, Y.; Qian, L.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 2267−2270.
(30) Xu, Y.; Aoki, J.; Shimizu, K.; Umezu-Goto, M.; Hama, K.; Takanezawa, Y.; Yu, S.; Mills, G. B.; Arai, H.; Qian, L.; Prestwich, G. D. *J. Med. Chem.* **2005**, *48*, 3319−3327.
(31) Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B.; Prestwich, G. D. *J. Med. Chem.* **2003**, *46*, 5575−5578.
(32) Qian, L.; Xu, Y.; Simper, T. D.; Jiang, G.; Aoki, J.; Umezu-Goto, M.; Arai, H.; Yu, S.; Mills, G. B.; Tsukahara, R.; Makarova, N.; Fujiwara, Y.; Tigyi, G.; Prestwich, G. D. *ChemMedChem* **2006**, *1*, 376−383.
(33) Prestwich, G. D.; Xu, Y.; Qian, L.; Gajewiak, J.; Jiang, G. *Biochem. Soc. Trans.* **2005**, *33*, 1357−1361.
(34) Lynch, K. R.; Macdonald, T. L. *Prostaglandins Other Lipid Mediatiators* **2001**, *64*, 33−45.
(35) Parrill, A. L. *Biochem. Soc. Trans.* **2005**, *33*, 1366−1369.
(36) Tigyi, G.; Parrill, A. L. *Prog. Lipid Res.* **2003**, *42*, 498−526.
(37) Tamaruya, Y.; Suzuki, M.; Kamura, G.; Kanai, M.; Hama, K.; Shimizu, K.; Aoki, J.; Arai, H.; Shibasaki, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 2834−2837.
(38) Ishihara, R.; Tatsuta, M.; Iishi, H.; Baba, M.; Uedo, N.; Higashino, K.; Mukai, M.; Ishiguro, S.; Kobayashi, S.; Murakami-Murofushi, K. *Int. J. Cancer* **2004**, *110*, 188−193.
(39) Stoianova, D. S.; Hanson, P. R. *Org. Lett.* **2001**, *3*, 3285−3288.
(40) Stoianova, D. S.; Whitehead, A.; Hanson, P. R. *J. Org. Chem.* **2005**, *70*, 5880−5889.
(41) Dunne, K. S.; Bisaro, F.; Odell, B.; Paris, J. M.; Gouverneur, V. *J. Org. Chem.* **2005**, *70*, 10803−10809.
(42) Pergament, I.; Srebnik, M. *Org. Lett.* **2001**, *3*, 217−219.

SCHEME 1. Construction of Key Intermediate 9 Using RCM[a]



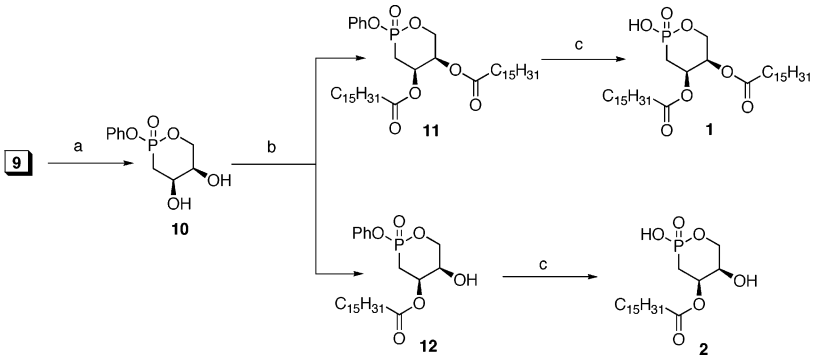
[a] Reagents and conditions: (a) allylmagnesium bromide, Et_2O, reflux, 10 h, 21%; (b) allyl alcohol, *n*-BuLi, THF/HMPA (4:1), −78 °C, 2 h, 58%; (c) Grubbs catalyst, CH_2Cl_2, reflux, 8 h, 90%.

short reaction times and by minimizing the excess of the allyloxide. Treatment of phosphonate **8** with first generation Grubbs metathesis catalyst[44] afforded the unsaturated six-membered cyclic phosphonate **9** in high yield.

Next, catalytic dihydroxylation of cyclic olefin in **9** (OsO_4/NMO/acetone/H_2O) provided diol **10** in modest yield, but TLC indicated complete consumption of starting material. Simply adding citric acid to the reaction mixture resulted in hydrolysis of the intermediate osmate ester to afford diol **10** in high yield but with essentially no diastereoselectivity (ds = 1:1).[39,40] Acylation of diol **10** with palmitic acid in CH_2Cl_2 using the water-soluble carbodiimide EDCI as condensation reagent afforded two desired products, the bisacylated PA analogue and monoacylated LPA analogue. ^{1}H NMR spectroscopy, including ^{1}H-^{1}H COSY, allowed identification of the products as the dipalmitoyloxy-substituted cyclic phosphonate **11** and 4-palmitoyloxy-cyclic phosphonate **12**. For example, palmitoylation of the 4-OH caused the H_4 resonance to shift downfield to ca. 5.2 ppm; from the ^{1}H-^{1}H COSY spectrum, a strong correlation was observed between H_3 (α-H of phosphonate) and H_4 but not between H_3 and H_5. The free cyclic phosphonic acids were obtained in excellent yields by reductive cleavage of the phenyl phosphonates with 1 atm H_2/PtO_2 in MeOH.

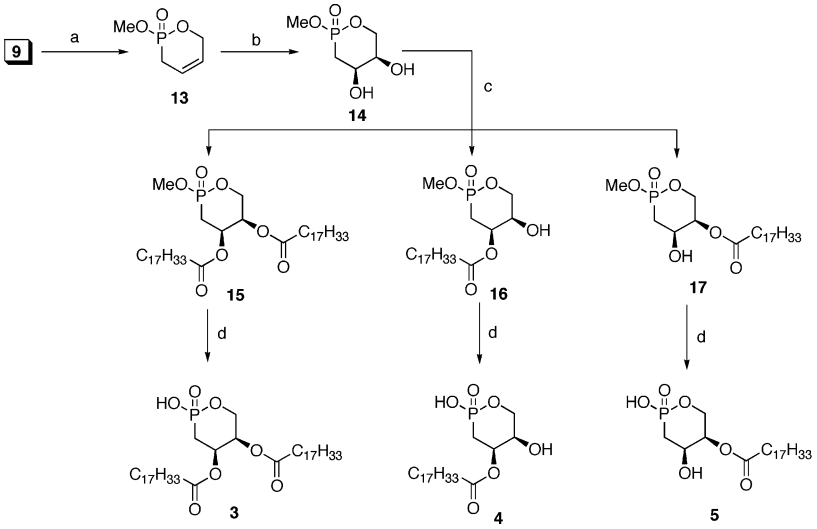
Scheme 3 shows the modified route employed to obtain the unsaturated oleoyloxy analogues. Reaction of **9** with lithium methoxide in methanol at −5 °C replaced the phenoxy group with a methoxy protecting group to give desired cyclic phosphonate **13** in high yield. Dihydroxylation as shown in Scheme 2 afforded diol **14**. Acylation of diol **14** with oleic acid was conducted in CH_2Cl_2/DMF instead of CH_2Cl_2, because of the poor solubility of diol **14** in CH_2Cl_2. Three separable products

SCHEME 2. Synthesis of Racemic Palmitoyloxy Analogues 1 and 2[a]



[a] Reagents and conditions: (a) OsO_4, NMO, citric acid, acetone/*t*-BuOH, 75%; (b) palmitic acid, EDCI, DMAP, CH_2Cl_2, 28% of **11**, 48% of **12**; (c) PtO_2, MeOH, H_2, 74% of **1**, 82% of **2**.

SCHEME 3. Synthesis of Oleoyloxy Analogues 3, 4, and 5[a]



[a] Reagents and conditions: (a) MeOH, *n*-BuLi, 89%; (b) QsO_4, NMO, citric acid, acetone/*t*-BuOH, 78%; (c) oleic acid, EDCI, DMAP, CH_2Cl_2/DMF (3:1), 18% of **15**, 38% of **16**, 12% of **17**; (d) TMSBr, CH_2Cl_2.

TABLE 1. Activity of 2, 4, and 5 on LPA Receptors

	LPA_1		LPA_2		LPA_3		
cmpd	EC_{50} (E_{max})[a] nM	IC_{50} (inhib %)[c] nM	EC_{50} (E_{max}) nM	IC_{50} (inhib %) nM	EC_{50} (E_{max}) nM	IC_{50} (inhib %)[c] nM	PPARγ
2	NE[b]	NE	NE	NE	NE	NE	NE
4	NE	>307 (33.6 at 30 μM)	>6380 (39.2 at 30 μM)	NE	NE	>1200 (45.5 at 30 μM)	NE
5	NE	97 (41.6)	>11300 (56.8 at 30 μM)	NE	>7510 (10.8 at 30 μM)	NE	NE

[a] E_{max} = maximal efficacy of compound/maximal efficacy of LPA 18:1 × 100. [b] NE = no effect was shown at the highest concentration (30 μM) tested. [c] Inhib % = % maximal inhibition of the response to 200 nM of LPA 18:1.

were obtained and were identified with ^{1}H NMR and ^{1}H-^{1}H COSY as dioleoyloxy-substituted cyclic phosphonate **15** and both the 4- and 5-oleoyloxy cyclic phosphonates **16** and **17**. Reaction of these intermediates with TMSBr in dry CH_2Cl_2 provided the three cyclic phosphonic acids **3**−**5** in high yield.

We examined the potency of monoacyloxy cyclic phosphates **2**, **4**, and **5** for activation of three LPA GPCRs (LPA_1, LPA_2, and LPA_3) and with the nuclear receptor PPARγ. None of the analogues activated PPARγ. Among the three GPCRs, LPA_2 was found to be selectively activated by the cyclic phosphate **4**. This is a noteworthy result, as it has been difficult to prepare selective agonists capable of distinguishing LPA_1 and LPA_2. This experiment also suggested that the new LPA scaffold can be recognized by the GPCRs and indicates that additional modeling[25,35] could be instructive. LPA analogues with oleoyl chains are generally more potent ligands for LPA_3 than those with palmitoyl chains.[30,31] This attribute was born out also in these studies (Table 1), since oleoyloxy-substituted cyclic phosphonates **4** and **5** can be recognized by the three LPA receptors, while the palmitoyl-substituted cyclic phosphonate **2** has little or no interaction with these receptors. Moreover, the position of the oleoyloxy group in the cyclic phosphonates is also important. Analogue **4** is a selective agonist of LPA_2 and an antagonist of LPA_1 and LPA_3, while phosphonate **5** is an antagonist of LPA_1 and an agonist of LPA_2 and LPA_3. On the basis of these structure−activity relationships, stereoselective synthesis or introducing another functional group to change the three-dimensional arrangement of cyclic phosphonate **4** and **5** to selectively meet certain receptors may enhance the selective activation with LPA receptors.

Conclusion

We describe a facile RCM/dihydroxylation strategy to generate several novel cyclic phosphonate analogues of PA and LPA. This method permits the preparation selective agonists and antagonists with a novel phosphatase-resistant and conformationally constrained scaffold. The interaction experiment of LPA analogues **2**, **4**, and **5** with LPA receptors showed that the oleoyloxy-substituted cyclic phosphonate **4** is a potential selective LPA_2 agonist that can distinguish LPA_1 and LPA_2.

Experimental Section

Diphenyl Allylphosphonate (7). Mg (2.4 g, 10 mmol) was placed in a dry, three-necked flask equipped with condenser and additional funnel. Dry ether (75 mL) followed by a solution of allylbromide (8.64 mL, 10 mmol) in ether (50 mL) was added. The reaction mixture was heated for 1 h, and then a solution of $ClPO(OPh)_2$ (10 mmol) in ether (50 mL) was added. The reaction was refluxed overnight. Then it was cooled, and water (50 mL) was added. The organic layer was dried on Na_2SO_4, ether was removed by evaporating, and residue was distilled with yield of 21%. ^{1}HNMR(400 MHz, $CDCl_3$) δ 7.33−7.29 (m, 4H), 7.20−7.13 (m, 6H), 5.99−5.87 (m, 1H), 5.36−5.29 (m, 2H), 2.98 (dt, J = 7.6, 1.2 Hz, 1H), 2.92 (dt, J = 7.6, 1.2 Hz, 1H).

Allylphosphonic Acid Phenyl Ester Allyl Ester (8). To a solution of the allyl alcohol (0.42 mL, 6.06 mmol) in 15 mL of dry THF was added *n*-BuLi (2.40 mL, 2.5 M in hexanes) at −45 °C, and the solution was warmed to room temperature over 2 h. This solution was added to the diphenyl allylphosphonate (1.00 g, 3.64 mmol) in 16 mL of THF and 4 mL of HMPA at −78 °C over 2 h. After an additional hour at −78 °C, the reaction mixture was quenched with saturated NH_4Cl, extracted with EtOAc, and dried with Na_2SO_4. Removal of the solvent and flash chromatography (hexanes/EtOAc 3:1) afforded **8** (503 mg, 58.0%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32−7.10 (m, 5H), 5.92−5.77 (m, 2H), 5.33−5.18 (m, 4H), 4.66−4.51(m, 2H), 2.79 (d, J = 7.6 Hz, 1H), 2.74 (d, J = 7.6 Hz, 1H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 25.33. ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.7, 150.6, 132.8, 132.8, 129.9, 126.9, 126.8, 125.2, 121.0, 120.9, 120.8, 120.7, 118.4, 67.2, 67.2, 32.6, 31.3. MS (CI): 239.1 $[M + H]^+$. CI-HRMS: $[M + H]^+$ calcd for $C_{12}H_{16}O_3P$, 239.0837; found, 239.0822.

2-Phenoxy-3,6-dihydro-1,2-oxaphosphinine-2-oxide (9). To a solution of **8** (300 mg, 1.26 mmol) in 80 mL of dry CH_2Cl_2 was added catalyst $(PCy_3)_2Cl_2Ru{=}CHPh$ (45 mg, 3% mol), and the mixture was refluxed for 8 h, diluted with CH_2Cl_2, flushed with air, and stirred overnight at room temperature. Removal of the solvent and flash chromatography (hexanes/EtOAc 2:1) afforded **9** (240 mg, 90%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26−7.05 (m, 5H), 5.74−5.60 (m, 2H), 4.84−4.72 (m, 2H), 2.61−2.42 (m, 2H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 16.90. ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.3, 150.3, 130.0, 125.5, 125.4, 125.2, 120.3, 120.3, 120.2, 70.1, 70.0, 22.7, 21.4. MS (CI): 211.0 $[M + H]^+$. CI-HRMS: $[M + H]^+$ calcd for $C_{10}H_{12}O_3P$, 211.0524; found, 211.0517.

2-Phenoxy-1,2-oxaphosphorinane-4,5-diol-2-oxide (10). To a solution of **9** (200 mg, 0.95 mmol) in 10 mL of acetone and 4 mL of *t*-BuOH were added citric acid (225 mg, 1.17 mmol), NMO hydrate (140 mg, 1.03 mmol), and OsO_4 (2 drops 2.5 wt % solution in 2-propanol). After 48 h, removal of the solvent and flash chromatography (EtOAc/MeOH 9:1) afforded **10** (175 mg, 75%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26−7.04 (m, 5H), 4.32−3.99 (m, 5H), 3.80−3.77 (m, 1H), 2.33−2.21 (m, 1.2H), 2.11−2.01 (m, 0.8H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 23.57, 23.55. ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.0, 149.9, 149.8, 149.7, 130.3, 130.1, 125.7, 125.7, 120.8, 120.8, 120.2, 120.2, 69.9, 69.8, 68.5, 68.2, 67.9, 67.9, 67.8, 67.7, 67.5, 67.5, 27.9, 27.7, 26.7, 26.5. MS (CI): 245.0 $[M + H]^+$. CI-HRMS: $[M + H]^+$ calcd for $C_{10}H_{14}O_5P$, 245.0579; found, 245.0577.

2-Phenoxy-1,2-oxaphosphorinane-4,5-*O*-dipalmitoyl-2-oxide (11) and 2-Phenoxy-1,2-oxaphosphorinane-4-*O*-palmitoyl-5-hydroxy-2-oxide (12). A solution of diol **10** (50 mg, 0.21 mmol), palmitic acid (52 mg, 0.22 mmol), EDCI (88 mg, 0.46 mmol), and

(43) Moriarty, R. M.; Enache, L. A.; Zhao, L.; Gilardi, R.; Mattson, M. V.; Prakash, O. *J. Med. Chem.* **1998**, *41*, 468−477.
(44) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100−110.

DMAP (28 mg, 0.23 mmol) in 4 mL of dry CH_2Cl_2 was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water, and dried with Na_2SO_4. Concentration of the solvent gave a crude residue, which was purified by column chromatography (EtOAc/hexanes 1:2 to 5:1) to afford **11** (42 mg, 28%) and **12** (47 mg, 48%) as white solids.

Compound 11. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31−7.11 (m, 5H), 5.45 (m, 1H), 5.22 (d, br, J = 2.8 Hz, 1H), 4.41−4.28 (m, 2H), 2.45−2.28 (m, 2H), 2.25 (q, J = 8.8 Hz, 4H), 1.53 (t, J = 6.8 Hz, 4H), 1.18 (s, 48H), 0.81 (t, J = 6.8 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 19.70. ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.6, 172.5, 150.1, 150.0, 130.1, 125.7, 120.5, 120.5, 67.2, 67.1, 67.1, 65.9, 65.8, 34.3, 34.2, 32.1, 29.9, 29.88, 29.85, 29.7, 29.69, 29.6, 29.5, 29.3, 29.3, 26.4, 25.2, 25.1, 24.9, 22.9, 14.3. MS (MALDI): 743.5 $[M + Na]^+$. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{42}H_{73}NaO_7P$, 743.5275; found, 743.5278.

Compound 12. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36−7.17 (m, 5H), 5.42 (m, 1H), 4.45−4.37 (m, 1H), 4.31 (dt, J = 12.4, 2.8 Hz, 1H), 4.11 (m, 1H), 2.53 (m, 1H), 2.35 (m, 3H), 1.62 (t, J = 7.2 Hz, 2H), 1.24 (s, 24H), 0.87 (t, J = 6.8 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 19.91. ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.2, 150.1, 149.99, 130.1, 125.6, 120.4, 120.4, 69.6, 69.6, 67.7, 67.6, 66.7, 66.6, 34.5, 32.1, 29.9, 29.9, 29.8, 29.8, 29.6, 29.59, 29.46, 29.3, 26.0, 25.0, 24.8, 22.9, 14.3. MS (MALDI): 483.33 $[M + H]^+$. MALDI-HRMS: $[M + H]^+$ calcd for $C_{26}H_{44}O_6P$, 483.3116; found, 483.3112.

2-Hydroxy-1,2-oxaphosphorinane-4,5-*O*-dipalmitoyl-2-oxide (1). A suspension of Adams catalyst (PtO_2, 40 mg, 0.18 mmol) in 3 mL of MeOH was flushed with hydrogen, and a solution of **11** (42 mg, 0.058 mmol) in 2 mL of MeOH was added. After it was stirred for 3 h at room temperature, the reaction mixture was diluted with MeOH and filtered through Celite, Removal of the solvent afforded **1** (27.8 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 1:1): δ 5.20 (m, 2H), 4.15 (t, J = 12.8 Hz, 1H), 4.06 (t, J = 11.4 Hz. 1H), 2.32 (t, J = 7.2 Hz, 2H), 2.18 (t, J = 7.2 Hz, 2H), 2.12−1.98 (m, 2H), 1.57 (t, J = 6.8 Hz, 2H), 1.50 (t, J = 6.8 Hz, 2H), 1.18 (s, 48H), 0.79 (t, J = 6.8 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ 1:1): δ 20.20. ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ 1:1): δ 173.5, 173.2, 69.5, 67.8, 65.6, 34.3, 32.0, 29.8, 29.7, 29.66, 29.59, 29.5, 29.4, 29.2, 25.1, 24.8, 22.7, 13.9. MS (MALDI): 667.50 $[M + Na]^+$. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{36}H_{69}NaO_7P$, 667.4609; found, 667.4601.

2-Hydroxy-1,2-oxaphosphorinane-4-*O*-palmitoyl-5-hydroxyl-2-oxide (2) was obtained from **12** in 82% yield analogously as described for compound **1**. ^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$ 2:1): δ 5.09−4.92 (m, 1H), 4.15−3.96 (m, 2H), 3.85 (s, 1H), 2.32 (dd, J = 7.2, 3.6 Hz, 1H), 2.27 (t, J = 7.6 Hz, 1H), 2.07(dt, J = 17.2, 12.8 Hz, 1H), 1.93 (m, 1H), 1.54 (t, J = 7.2 Hz, 2H), 1.18 (s, 24H), 0.80 (t, J = 6.8 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ 2:1): δ 20.96, 20.09. ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ 2:1): δ 173.8, 72.0, 67.4, 66.9, 34.4, 32.1, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 24.9, 22.8, 14.0. MS (MALDI): 429.24 $[M + Na]^+$. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{20}H_{39}NaO_6P$, 429.2382; found, 429.2381.

2-Methoxy-3,6-dihydro-[1,2]oxaphosphinine-2-oxide (13). To 7 mL of MeOH was added *n*-BuLi (0.36 mL, 2.5 M in hexanes, 0.91 mmol) at −78 °C, and the solution was warmed to 0 °C over 2 h. Compound **9** (160 mg, 0.76 mmol) was added dropwise to this solution in 7 mL of MeOH, and after the addition was completed, the mixture was stirred for an hour at −5 °C. The reaction mixture was quenched with a small amount of saturated NH_4Cl solution, MeOH and water were removed under reduced pressure, and the residue was extracted with EtOAc and dried with Na_2SO_4. Removal of the solvent and flash chromatography (EtOAc/hexanes 2:1) afforded **13** (100 mg, 89%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.74−5.60 (m, 2H), 4.80−4.66 (m, 2H), 3.73 and 3.70 (s, 3H), 2.54−2.32 (m, 2H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 21.84. ^{13}C NMR (101 MHz, $CDCl_3$): δ 125.5, 125.3, 120.7, 120.6, 69.2, 69.1, 51.9, 51.88, 22.7, 21.4. MS (CI): 149.0 $[M + H]^+$. CI-HRMS: $[M + H]^+$ calcd for $C_5H_{10}O_3P$, 149.0358; found, 149.0353.

2-Methoxy-1,2-oxaphosphorinane-4,5-diol-2-oxide (14). To a solution of **13** (26 mg, 0.18 mmol) in 2 mL of acetone and 0.8 mL of *t*-BuOH were added citric acid (45 mg, 0.24 mmol), NMO hydrate (27 mg, 0.20 mmol), and OsO_4 (3 drops of a 2.5% solution in 2-propanol). After 48 h, the solvent was removed under reduced pressure. The residue was flash chromatographed (EtOAc/MeOH 4:1) to afford **14** (25 mg, 78%) as an oil.^{1}H NMR (400 MHz, $CDCl_3$): δ 4.443 (br, −OH, 2H), 4.31−4.13 (m, 2H), 4.02 (m, 1H), 3.91 (s, 1H), 3.73 (dd, J = 24.8, 11.2 Hz, 3H), 2.30−2.06 (m, 2H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 28.39, 28.14. ^{13}C NMR (101 MHz, $CDCl_3$): δ 69.3, 69.2, 68.6, 68.3, 68.2, 68.17, 67.7, 53.2, 53.1, 51.97, 51.9, 27.3, 26.1, 26.1. MS (CI): 183.0 $[M + H]^+$. CI-HRMS: $[M + H]^+$ calcd for $C_5H_{12}O_5P$, 183.0422; found, 183.0432.

2-Methoxy-1,2-oxaphosphorinane-4,5-*O*-dioleoyl-2-oxide (15). A solution of diol **14** (80 mg, 0.44 mmol) and oleic acid (108 mg, 0.38 mmol), EDCI (176 mg, 0.92 mmol), and DMAP (56 mg, 0.44 mmol) in 9 mL of dry CH_2Cl_2 and 3 mL of DMF was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water, and dried with Na_2SO_4. Concentration of the solvent gave a crude residue, which was purified by column chromatography (EtOAc /hexanes 1:1 to EtOAc/MeOH 20:1) to afford **15** (55 mg, 18%), **16** (75 mg, 38%), and **17** (23 mg, 12%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.41−5.34 (m, 1H), 5.27 (q, J = 5.6 Hz, 4H), 5.20 (m, 1H), 4.31−4.05 (m, 2H), 3.77 (dd, J = 16.8, 10.8 Hz, 3H), 2.30 (m, 2H), 2.21 (m, 4H), 1.94 (d, J = 5.2 Hz, 8H), 1.55 (m, 4H), 1.23 (m, 40H), 0.81 (t, J = 6.8 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 25.35, 24.05. ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.7, 172.4, 130.3, 130.27, 129.9, 129.8, 67.5, 67.3, 67.27, 65.6, 53.1, 51.2, 34.4, 34.3, 34.2, 32.1, 30.0, 29.9, 29.7, 29.5, 29.4, 29.3, 29.2, 27.4, 27.4, 26.1, 25.2, 25.1, 24.9, 22.9, 14.3. MS (MALDI): 733.56 $[M + Na]^+$. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{41}H_{75}NaO_7P$, 733.5240; found, 733.5231.

2-Methoxy-1,2-oxaphosphorinane-4-*O*-oleoyl-5-hydroxy-2-oxide (16) was obtained from **14** in 38% yield analogously as described for compound **15**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.27 (t, J = 5.6 Hz, 2H), 5.04 (d, J = 10.4 Hz, 1H), 4.24 (dt, J = 26.0, 12.0 Hz, 1H), 4.02 (d, J = 12.0 Hz, 1H), 3.94 (s, 1H), 3.74 (s, 1H), 3.71 and 3.69 (s, 3H), 2.38 (m, 1H), 2.29 (t, J = 7.6 Hz, 2H), 2.15 (m, 1H), 1.94 (d, J = 5.2 Hz, 4H), 1.55 (t, J = 6.4 Hz, 2H), 1.20 (s, 20H), 0.81 (t, J = 6.8 Hz,3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 25.22. ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.0, 130.2, 129.9, 70.5, 69.2, 69.2, 66.4, 66.38, 52.0, 51.9, 34.5, 34.4, 32.1, 29.9, 29.88, 29.7, 29.5, 29.4, 29.3, 29.3, 27.4, 27.3, 25.0, 24.7, 23.5, 22.9, 14.3. MS (MALDI): 447.31 $[M + H]^+$. MALDI-HRMS: $[M + H]^+$ calcd for $C_{23}H_{44}O_6P$, 447.2876; found, 447.2876.

2-Methoxy-1,2-oxaphosphorinane-5-*O*-oleoyl-4-hydroxy-2-oxide (17). Compound **17** was obtained from **14** in 12% yield analogously as described for compound **15**. ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.27 (s, 2H), 5.04 (s, 1H), 4.36 (m, 1H), 4.30−4.16 (m, 2H), 3.78 and 3.75 (s, 3H), 2.88 (br, 1H), 2.33 (t, J = 7.6 Hz, 2H), 2.22−2.07 (m, 2H), 1.94 (d, J = 4.4 Hz, 4H), 1.57 (s, 2H), 1.23 (s, 20H), 0.81 (t, J = 6.8 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 26.76. ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.5, 130.3, 129.9, 70.1, 70.0, 66.8, 65.1, 65.0, 53.0, 52.9, 34.4, 32.1, 30.0, 29.9, 29.7, 29.5, 29.4, 29.3, 29.2, 28.5, 27.4, 27.4, 27.3, 25.1, 22.9, 14.3. MS (MALDI): 447.30 $[M + H]^+$. MALDI-HRMS: $[M + H]^+$ calcd for $C_{23}H_{44}O_6P$, 447.2876; found, 447.2870.

2-Hydroxy-1,2-oxaphosphorinane-4,5-*O*-dioleoyl-2-oxide (3). To a solution of **15** (16 mg, 0.0225 mmol) in 1 mL of dry CH_2Cl_2 was added 0.2 mL of TMSBr. The mixture was stirred for 4 h, and then the solvent was removed completely. To the residue was added 2 mL of MeOH and 2 drops of water, and after the mixture was stirred for 1 h, the solvent was concentrated to afford product **3** (14 mg, 89%). ^{1}HNMR (400 MHz, $CDCl_3$): δ 5.32−5.20 (m, 6H), 4.23−4.14 (m, 2H), 2.32 (t, J = 7.2 Hz, 2H), 2.23−2.17 (m, 4H), 1.94 (m, 8H), 1.55 (m, 4H), 1.23 (s, 40H), 0.81 (t, J = 6.8 Hz,

6H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 26.03. ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.0, 172.6, 130.3, 130.2, 129.9, 34.4, 34.2, 32.1, 30.0, 29.95, 29.8, 29.6, 29.4, 29.4, 29.3, 29.27, 27.4, 27.4, 25.2, 24.9, 14.3. MS (MALDI): 719.50 $[M + Na]^+$. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{40}H_{73}NaO_7P$, 719.4992; found, 719.4986.

2-Hydroxy-1,2-oxaphosphorinane-4-*O*-oleoyl-5-hydroxyl-2-oxide (4). Compound **4** was obtained from **16** in 77% yield analogously as described for compound **3**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (br, 1H), 5.38−5.32 (m, 2H), 5.30−5.24 (m, 0.4H), 5.12−5.07 (m, 0.6H), 4.53−4.22 (m, 2H), 4.06−3.99 (m, 1H), 2.81−2.56 (m, 1H), 2.47−2.39 (m, 1H), 2.39−2.33 (m, 2H), 1.99 (m, 4H), 1.63 (t, J = 7.2 Hz, 2H), 1.30 (m, 20H), 0.87 (t, J = 6.8 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 24.94, 22.42. ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.4, 130.3, 129.9, 71.1, 70.7, 65.8, 45.6, 43.9, 34.4, 34.2, 33.6, 32.1, 30.0, 29.9, 29.7, 29.5, 29.3, 29.29, 29.2, 27.4, 27.37, 25.0, 22.9, 14.4. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{22}H_{41}O_6P$, 455.2533; found, 455.2536.

2-Hydroxy-1,2-oxaphosphorinane-5-*O*-oleoyl-4-hydroxyl-2-oxide (5) Compound **5** was obtained from **17** in 61% yield analogously as described for compound **3**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38−5.32 (m, 2H), 5.30−5.27 (m, 0.4H), 5.12−5.07 (m, 0.6H), 4.51−4.22 (m, 2H), 4.04−3.99 (m, 1H), 2.81−2.56 (m, 1H), 2.47−2.38 (m, 1H), 2.37−2.33 (m, 2H), 1.99 (m, 4H), 1.64 (m, 2H), 1.29 (m, 20H), 0.87 (t, J = 6.8 Hz, 3H). ^{31}P NMR (162 MHz, $CDCl_3$): δ 24.53, 22.03. ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.4, 130.3, 129.9, 70.8, 65.8, 45.8, 44.1, 34.4, 34.2, 32.1, 30.0, 29.9, 29.8, 29.6, 29.4, 29.3, 29.26, 27.5, 27.4, 25.0, 22.9, 14.4. MALDI-HRMS: $[M + Na]^+$ calcd for $C_{22}H_{41}O_6P$, 455.2533; found, 455.2528.

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Supporting Information Available: General procedure and selected ^{1}H NMR, ^{31}P NMR, and ^{13}C NMR spectra for new compounds in pdf format. This material is available free of charge via the Internet at http://pubs.acs.org.

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