Supporting Information

Synthesis, Pharmacology and Cell Biology of \textit{sn}-2-Aminoxy Analogues of Lysophosphatidic Acid

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Table of Contents

I. Chemical Synthesis S2-S11
II. Pharmacology and Cell Biology Protocols S11-S13
III. Table 2. Inhibition of ATX activity S13
IV. Photomontages of cell migration in scratch wound assay S14-S15
V. $^1\text{H}$, $^{13}\text{C}$ and $^{31}\text{P}$ NMR spectra of compounds 3-12 S16-S46

I. Chemical Synthesis

**General Procedures.** Chemicals were obtained from Aldrich and Acros and were used without further purification. Solvents were purchased anhydrous (Et$_2$O and THF) or reagent-grade and distilled before use: CH$_2$Cl$_2$ was distilled from CaH$_2$. Reactions requiring anhydrous conditions were carried out in oven-dried glassware (2 h, 120 °C) under inert atmosphere (N$_2$ or Ar) unless otherwise indicated. Concentration *in vacuo* refers to the use of rotary evaporator for solvent removal, and purification on SiO$_2$ refers to flash chromatography (FC) on silica gel. NMR spectra were recorded at 400 MHz ($^1\text{H}$), 101 MHz ($^{13}\text{C}$) or 162 MHz ($^{31}\text{P}$) at ambient temperature. Chemical shifts are reported relative to those of internal CDCl$_3$ peaks ($\delta$$_H$ 7.24), and ($\delta$$_C$ 77.0) and to CD$_3$OD peaks ($\delta$$_H$ 4.78) and ($\delta$$_C$ 49). Optical rotations were obtained at ambient temperature. Symbols: s, singlet; bm, broad multiple; bs, broad singlet; dd, doublet of doublets; m, multiple; p-quintuplet; q, quartet; t, triplet. Coupling constants ($J$) are all reported in Hz.
(S)-1-Benzyloxy-3-(tert-butylidimethylsilanyloxy)-propan-2-ol (3). To the solution of 2 (892 mg, 4.9 mmol) and imidazole (741 mg, 10.9 mmol) in DMF (20mL), TBDMSOCl (817 mg, 4.9 mmol) solution in DMF (5 mL) was added dropwise at 0°C. The reaction was conducted overnight at 0-3°C then concentrated and purified on SiO₂ using hexanes/acetone 95:5 to produce pure 3 in form of sticky oil (1.3 g, 4.4 mmol) in 85% yield. Rf 0.44 (hexanes/acetone, 8.5:1.5); [α]²⁰_D +1.54 (c 1.42, CHCl₃); ¹H NMR (CDCl₃) δ: 7.30-7.25 (m, 5H), 4.49 (s, 2H), 3.79 (p, 1H, J = 5.6), 3.63-3.55 (m, 2H), 3.49-3.42 (m, 2H), 2.40 (s, 1H), 0.82 (s, 9H), 0.00 (s, 6H); ¹³C NMR (CDCl₃) δ: 138.0, 128.4, 127.7, 127.7, 73.4, 70.9, 70.6, 63.9, 25.8, 18.2, -5.5; LRMS (ESI) m/z 297.2 (M + H). HRMS (ESI) for C₁₆H₂₉O₃Si found: 297.1881, calcd: 297.1886.

(R)-2-[1-Benzyloxymethyl-2-(tert-butylidimethylsilanyloxy)-ethoxy]isoindole-1,3-dione (4). The solution of 3 (1.02 g, 3.45 mmol), Ph₃P (2.26 g, 8.63 mmol) and PhOH (1.41 g, 8.63 mmol) in THF (90 mL) was cooled to 0°C in ice bath and DEAD (1.42 mL, 8.63 mmol) was introduced dropwise. The reaction was allowed to warm up to rt for 2h and then it was concentrated in vacuo and purified using FC with the mixture of hexanes/ethyl acetate 9:1 to yield compound 4 as colorless oil (1.42 g, 93%). Rf 0.44 (hexanes/ethyl acetate 8:2); [α]²⁰_D -0.72 (c 1.95, CHCl₃);
\(^1\)H NMR (CDCl\(_3\)) \(\delta\): 7.80-7.76 (m, 2H), 7.72-7.68 (m, 2H), 7.27-7.21 (m, 5H), 4.53-4.49 (m, 3H), 4.01-3.92 (m, 2H), 3.88-3.81 (m, 2H), 0.82 (s, 9H), 0.24 (d, 6H, \(J = 6.0\)); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\): 163.6, 137.8, 134.2, 128.9, 128.2, 127.6, 127.4, 123.3, 86.6, 73.5, 69.0, 61.9, 25.7, 18.1, -5.6, -5.6; LRMS (MALDI) \(m/z\) 464.21 (M + Na). HRMS (MALDI) for C\(_{24}\)H\(_{31}\)NNaO\(_5\)Si found: 464.1886, calcd: 464.1869.

\(\text{(R)-2-aminoxy-3-benzyloxy-1-(tert-butylidimethylsilanyloxy)-propane (5).}\) Compound 4 (1.42 g, 3.22 mmol) was dissolved in anhydrous CH\(_2\)Cl\(_2\) (20 mL) and hydrazine monohydrate was added (0.63 mL, 12.9 mmol) at 0°C. After 2 h the reaction was accomplished by filtration over the pad of Celit and concentration. The crude product was dissolved in ether and white precipitate was filtered of using Whatman PTFE syringe filter. After concentration resulted colorless oil was dried under high vacuum to yield almost quantitatively desired compound (1 g, 3.21 mmol). \(R_f\) 0.23 (hexanes/ethyl acetate 8:2); [\(\alpha\)]\(_{20}^D\) +8.47 (c 1.83, CHCl\(_3\)); \(^1\)H NMR (CDCl\(_3\)) \(\delta\): 7.28 (d, 5H, \(J = 4.4\)), 5.2 (bs, 2H), 4.53-4.46 (m, 4H), 3.77-3.65 (overlapping signals, 3H), 3.59-3.50 (m, 2H), 0.83 (s; 9H), 0.00 (d, 6H, \(J = 1.6\)); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\): 138.2, 128.3, 127.7, 127.6, 83.7, 73.4, 68.8, 61.7, 25.9, 18.3, -5.4, -5.4; LRMS (ESI) \(m/z\) 312.2 (M + H). HRMS (ESI) for C\(_{16}\)H\(_{30}\)NO\(_3\)Si found: 312.1990, calcd: 312.1995.

\(\text{(R)-2-N-Boc-aminoxy-3-benzyloxy-1-(tert-butylidimethylsilanyloxy)-propane (6).}\)
Compound 5 (1 g, 3.21 mmol) was dissolved in CH$_2$Cl$_2$ (20ml) and di-tert-butyl dicarbonate (2.06 mL, 9.66 mmol) was added at rt. The reaction was conducted overnight, concentrated and purified on SiO$_2$ using hexanes/ethyl acetate 95:5. Pure compound 6 was isolated as colorless sticky oil in 92% yield (1.22 g, 2.97 mmol). R$_f$ 0.59 (hexanes/ethyl acetate 8:2); $[\alpha]_{D}^{20}$ +6.89 (c 2.12, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$: 7.44 (s, 1H), 7.32-7.31 (m, 5H), 4.54 (s, 2H), 3.97 (p, 1H, $J$ = 5.6), 3.85-3.81 (m, 1H) 3.78-3.74 (m, 1H), 3.70-3.62 (m, 2H), 1.45 (s, 9H), 0.86 (s, 9H), 0.04 (d, 6H, $J$ = 1.2); $^{13}$C NMR (CDCl$_3$) $\delta$: 156.8, 138.0, 128.3, 127.7, 127.6, 84.5, 81.5, 77.2, 73.4, 68.6, 61.7, 28.1, 25.8, 18.2, -5.4, -5.4; LRMS (MALDI) m/z 434.25 (M + Na). HRMS (MALDI) for C$_{21}$H$_{37}$NNaO$_5$Si found: 434.2311, calcd: 434.2339.

(R)-2-N-Boc-aminoxy-3-(tert-butyldimethylsilanyloxy)-propan-1-ol (7). Pd/C (180 mg) was added to a solution of 6 (600 mg, 1.46 mmol) in the mixture of CH$_2$Cl$_2$/CH$_3$OH 5:2 (30 mL) and balloon with H$_2$ was attached. The reaction was conducted for 4 h and after TLC showed consumption of the starting material it was finished by filtration over the pad of Celite and evaporation of the solvents. Pure compound 7 was obtained in 95% (445 mg, 1.39 mmol). R$_f$ 0.38 (hexanes/ethyl acetate 8:2); $[\alpha]_{D}^{20}$ +11.75 (c 0.8, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$: 7.36 (s, 1H), 3.85-3.80 (m, 1H), 3.76-3.66 (m, 3H), 3.60-3.55 (m, 1H), 1.45 (s, 9H), 0.87 (s, 9H), 0.04 (s, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$: 158.3, 87.7, 82.6, 61.8, 60.2, 28.1, 25.8, 18.2, -5.4, -5.4; LRMS (MALDI) m/z 344.20 (M + Na). HRMS (MALDI) for C$_{14}$H$_{31}$NNaO$_5$Si found: 344.1858, calcd: 344.1869.
Palmitic acid (R)-2-N-Boc-aminooxy-3-(tert-butyldimethylsilyloxy) propyl ester (8a). To the mixture of 7 (150 mg, 0.467 mmol) and palmitic acid (119 mg, 0.467 mmol) in anhydrous CH$_2$Cl$_2$ (3 ml) solution of EDC (124 mg, 0.65 mmol) and DMAP (34 mg, 0.28 mmol) was added at rt under argon atmosphere. The reaction was conducted overnight, then diluted with CH$_2$Cl$_2$ and H$_2$O was added. The water layer was extracted 3 times with CH$_2$Cl$_2$, the combined organic phases were washed with sat. NaCl, dried with Na$_2$SO$_4$ and concentrated in vacuo. Purification on SiO$_2$ using hexanes/ethyl acetate 95:5 led to white solid product in 81% yield (212 mg, 0.38 mmol). R$_f$ 0.42 (hexanes/ethyl acetate 9:1); [α]$^\text{D}$ +8.52 (c 1.55, CHCl$_3$); $^1$H NMR (CDCl$_3$) δ: 7.41 (s, 1H), 4.46 (dd, 1H, J = 3.5, 12.0), 4.15 (dd, J = 4.8, 12.0), 3.98-3.93 (m, 1H), 3.80 (dd, 1H, J = 4.8, 10.0), 3.66 (dd, 1H, J = 7.2, 10.4), 2.31 (t, 2H, J = 7.6), 1.62-1.58 (m, 2H), 1.44 (s, 9H), 1.32-1.18 (m, 24H), 0.88-0.83 (m, 12H), 0.04 (s, 6H); $^{13}$C NMR (CDCl$_3$) δ: 174.1, 156.6, 83.2, 81.7, 61.2, 60.5, 34.2, 31.9, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.3, 29.1, 28.1, 25.8, 24.9, 22.7, 18.2, 14.1, -5.5, -5.5; LRMS (MALDI) m/z 582.42 (M + Na). HRMS (MALDI) for C$_{30}$H$_{61}$NNaO$_6$Si found: 582.4139, calcd: 582.4166.

Oleic acid (R)-2-N-Boc-aminooxy-3-(tert-butyldimethylsilyloxy) propyl ester (8b).
Following the procedure described for compound 8a, colorless oily product 8b was obtained in 80% yield (220 mg, 0.37 mmol). Rf 0.42 (hexanes/ethyl acetate 9:1); $[\alpha]_{D}^{20} +8.54$ (c 1.37, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$: 7.41 (s, 1H), 5.34-5.28 (m, 2H), 4.46 (dd, 1H, $J = 3.5, 12.0$), 4.15 (dd, $J = 4.8, 12.0$), 3.98-3.94 (m, 1H), 3.81 (dd, 1H, $J = 4.8, 10.0$), 3.67 (dd, 1H, $J = 7.2, 10.4$), 2.31 (t, 2H, $J = 7.6$), 2.00-1.95 (m, 4H), 1.62-1.58 (m, 2H), 1.44 (s, 9H), 1.28-1.23 (m, 20H), 0.86-0.83 (m, 12H), 0.04 (s, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$: 174.1, 156.6, 128.9, 129.7, 83.2, 81.7, 61.2, 60.6, 34.2, 31.9, 29.7, 29.7, 29.5, 29.3, 29.2, 29.1, 28.2, 27.2, 27.1, 25.8, 24.9, 22.7, 18.2, 14.1, -5.5; -5.5; LRMS (MALDI) m/z 608.42 (M + Na). HRMS (MALDI) for C$_{30}$H$_{61}$NNaO$_6$Si found: 608.4299, calcd: 608.4322.

(S)-1-palmitoyl-2-(R)-N-Boc-aminoxypropan-3-ol (9a). Solution of compound 8a (183 mg, 0.31 mmol) in anhydrous THF was treated with 1M THF solution of Bu$_4$NF (0.62 mL, 0.62 mmol) at rt. The reaction was stirred for 1 h, concentrated in vacuo and purified on SiO$_2$ using hexanes/ethyl acetate 7:3 as an eluting mixture of the solvents. White solid product was obtained in 95% yield (135 mg, 0.29 mmol). Rf 0.38 (hexanes/ethyl acetate 7:3); $[\alpha]_{D}^{20} +7.40$ (c 2.42, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$: 7.41 (s, 1H), 4.34 (dd, 1H, $J = 7.6, 12.0$), 4.05-3.96 (m, 2H), 3.62-3.60 (m, 2H), 2.31 (t, 2H, $J = 7.6$), 1.63-1.54 (m, 2H), 1.45 (s, 9H), 1.32-1.20 (m, 24H), 0.85 (t, 3H, $J = 7.2$); $^{13}$C NMR (CDCl$_3$) $\delta$: 174.1, 84.6, 82.9, 61.2, 59.5, 34.2, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 28.2, 28.1, 24.9, 22.7, 14.1; LRMS (CI) m/z 446.1 (M + 1). HRMS (CI) for C$_{24}$H$_{48}$NO$_6$ found: 446.3476 calcd: 446.3482.
(S)-1-oleoyl-2-(R)-N-Boc-aminoxypropan-3-ol (9b). Following the procedure described above compound 9b was synthesized in 93% yield (143 mg, 0.3 mmol) as transparent oil. R_f 0.38 (hexanes/ethyl acetate 7:3); [α]_D^20 +6.10 (c 1.23, CHCl_3); ^1^H NMR (CDCl_3) δ: 7.41 (s, 1H), 5.34-5.28 (m, 2H) 4.34 (dd, 1H, J = 4.4, 8), 4.15 (bs, 1H), 4.08-3.95 (m, 2H), 3.62-3.58 (m, 2H), 2.31 (t, 2H, J = 7.6), 2.01-1.95 (m, 4H), 1.61-1.58 (m, 2H), 1.45 (s, 9H), 1.34-1.21 (m, 20H), 0.85 (t, 3H, J = 6.8); ^13^C NMR (CDCl_3) δ: 174.1, 158.5, 129.9, 129.7, 84.5, 82.9, 61.2, 59.4, 34.1, 31.9, 29.7, 29.6, 29.5, 29.3, 29.1, 29.0, 28.1, 27.2, 27.1, 24.6, 22.6, 14.1; LRMS (MALDI) m/z 494.3 (M + Na). HRMS (MALDI) for C_{26}H_{49}NNaO_6 found: 494.3455, calcd: 494.3452.

Dimethyl 1-palmitoyl-2-(R)-N-Boc-aminoxypropane-3-phosphate ester (11a). Compound 9a (100 mg, 0.22 mmol) was dissolved in anhydrous CH_2Cl_2 (1.5 mmol) and N-methylimidazole (47 µL, 0.59 mmol) was added followed by dimethyl chlorophosphate (47 µL, 0.44 mmol) at rt. The reaction was stirred overnight at rt, concentrated in vacuo and subjected to flash chromatography (hexanes/ethyl acetate 7:3, 5:5). The pure oily product was obtained in 80% yield (97 mg, 0.176 mmol). R_f 0.44 (hexanes/ethyl acetate 7:3); [α]_D^20 +2.8 (c 0.52, CHCl_3); ^1^H NMR (CDCl_3) δ: 7.97 (s, 1H), 4.39-4.33 (m, 2H), 4.23-4.21 (m, 1H), 4.8-4.11 (m, 2H), 4.79-4.74 (m, 6H), 2.33 (t, 2H, J = 7.2), 1.64-1.56 (m, 2H), 0.44 (s, 9H), 1.32-1.20 (m, 24H), 0.85 (t, 3H, J =
7.2); $^{13}$C NMR (CDCl$_3$) $\delta$: 173.6, 156.5, 81.2, 81.1, 81.0, 64.6, 64.6, 60.9, 54.6, 54.6, 54.5, 54.4, 34.1, 31.9, 29.7 29.6, 29.6, 29.4, 29.3, 29.3, 29.1, 28.2, 24.8, 22.7, 14.1; $^{31}$P NMR (CDCl$_3$) $\delta$: 3.26; LRMS (MALDI) m/z 576.3 (M + Na$^+$). HRMS (MALDI) for C$_{26}$H$_{52}$NNaO$_9$P found: 576.3251, calcd: 576.3272.

Dimethyl 1-oleoyl-2-(R)-N-Boc-aminoxypropane-3-phosphate ester (11b). Following the procedure described above, compound 11b was obtained as colorless oil in 83% yield (98 mg, 0.169 mmol). $R_f$ 0.44 (hexanes/ethyl acetate 7:3); $[\alpha]^{20}_D$ +1.9 (c 0.84, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$: 7.99 (s, 1H), 5.31-5.28 (m, 2H), 4.37-4.30 (m, 2H), 4.23-4.19 (m, 1H), 4.15-4.09 (m, 2H), 3.76-3.72 (m, 6H), 2.3 (t, 2H, $J$ = 7.6), 2.00-11.94 (m, 4H), 1.60-1.55 (m, 2H), 1.42 (s, 9H), 1.32-1.80 (m, 20H), 0.83 (t, 3H, $J$ = 6.8); $^{13}$C NMR (CDCl$_3$) $\delta$: 173.6, 156.5, 129.9, 129.7, 81.7, 81.0, 80.9, 64.6, 64.5, 54.6, 54.6, 54.5, 54.4, 34.0, 31.8, 29.7, 29.6, 29.4, 29.2, 29.1, 29.0, 28.1, 27.1, 27.1, 24.7, 22.6, 14.0; $^{31}$P NMR (CDCl$_3$) $\delta$: 3.22; LRMS (MALDI) m/z 602.3 (M + Na$^+$). HRMS (MALDI) for C$_{26}$H$_{52}$NNaO$_9$P found: 602.3400, calcd: 602.3428.

1-palmitoyl-2-(R)-aminoxypropane-3-phosphate ester (12a). Compound 11a (20 mg, 0.036 mmol) was dried overnight under high vacuo prior to the reaction. Then it was dissolved in anhydrous CH$_2$Cl$_2$ (0.5 mL) and treated with BSTFA (67 µl, 0.25 mmol) and TMSBr (29 µl, 0.22 mmol) under Argon atmosphere. After 1h wet CH$_2$Cl$_2$ (5% of water, 2 mL) was introduced
and the reaction was stirred for another 45 min. After that it was concentrated in vacuo, re-
dissolved in 90% methanol (1 mL) and stirred for another 30 min. The white precipitate was
filtered off using syringe filter, and the organic filtrate was concentrated in vacuo to yield in 86%
final compound in form of white solid (13 mg, 95% pure). $^1$H NMR (CD$_3$OD) δ: 4.42-4.37 (m,
1H), 4.33 (dd, 1H, $J = 3.6, 12.8$), 4.19-4.11 (m, 2H), 4.08-4.01 (m, 1H), 2.28 (t, 2H, $J = 7.6$),
1.53-1.47 (m, 2H), 1.26-1.10 (m, 24H), 0.78 (t, 3H, $J = 7.2$); $^{13}$C NMR (CD$_3$OD) δ: 173.6, 111.2,
83.0, 65.0, 62.4, 34.6, 33.1, 30.8, 30.6, 30.5, 30.3, 30.2, 30.2, 28.1, 25.9, 23.7, 14.5; $^{31}$P NMR (CD$_3$OD) δ: 1.17; LRMS (MALDI) m/z 448.3 (M + Na$^+\text{)}$. HRMS (MALDI) for C$_{19}$H$_{40}$NNaO$_7$P
found: 448.2440, calcd: 448.2435.

1-oleoyl-2-(R)-aminoxypropane-3-phosphate ester (12b). Following the procedure described
above for compound 12a compound 12b was obtained in form of a colorless oil in 85%, (16 mg,
95% pure). $^1$H NMR (CD$_3$OD) δ: 5.20-5.18 (m, 2H), 4.38-4.34 (m, 1H), 4.28 (dd, 1H, $J = 3.6$,
18.8), 4.15-4.08 (m, 2H), 4.04-3.99 (m, 1H), 2.24 (t, 2H, $J = 7.2$), 1.89-1.85 (m, 4H), 1.51-1.42
(m, 2H), 1.18-1.13 (m, 20H), 0.74 (t, 3H, $J = 7.2$); $^{13}$C NMR (CD$_3$OD) δ: 173.6, 130.9, 130.8,
111.2, 83.0, 65.0, 62.4, 34.6, 33.1, 30.8, 30.6, 30.5, 30.3, 30.2, 30.2, 28.1, 25.9, 23.7, 14.5; $^{31}$P NMR (CDCl$_3$) δ: 1.22; LRMS (MALDI) m/z 474.2 (M + Na$^+$). HRMS (MALDI) for C$_{21}$H$_{42}$NNaO$_7$P found: 474.2599, calcd: 474.2591.

II. Pharmacology and Cell Biology Protocols
Pharmacology of AO-LPA analogues on LPA receptors. The ligand properties of the compounds at the LPA<sub>1-4</sub> receptor subtypes were determined in cell lines heterologously expressing each individual receptor. RH7777 cells stably expressing either LPA<sub>1</sub>, LPA<sub>2</sub>, or LPA<sub>3</sub> and CHO cells stably expressing LPA<sub>4</sub> were plated on poly-L-lysine (0.1 mg/ml)-coated black-wall clear-bottom 96 well plates (Corning Life Sciences, Acton, MA) at a density of 5 x 10<sup>4</sup> cells/well or 4 x 10<sup>4</sup> cells/well (for CHO cells) and cultured overnight. The culture medium (DMEM containing 10% FBS for RH7777 cells, Ham’s F-12 containing 10% FBS for CHO cells) was then replaced with modified Krebs solution (120 mM NaCl, 5 mM KCl, 0.62 mM MgSO<sub>4</sub>, 1.8 mM CaCl<sub>2</sub>, 10 mM HEPES, 6 mM glucose, pH 7.4), and the cells were serum starved for 6 h (no starvation was applied to the CHO cells). Cells were loaded with Fura-2 AM for 35 min (1 h for CHO cells) in modified Krebs medium containing 2% (v/v) pluronic acid. The cells were rinsed with Krebs buffer and monitored in a FLEX station II instrument (Molecular Devices, Sunnyvale, CA) at excitation wavelengths of 340/380 nm and an emission wavelength of 510 nm for 2 min after the addition of the compound. The inhibitory activity was determined using a near EC<sub>50</sub> concentration of LPA for each receptor subtype (20 nM – 200 nM) mixed with varying concentration of the enantiomers. Each test was performed in quadruplicate, and the mean ± standard deviation (s.d.) were calculated.

Inhibition of autotaxin by AO-LPA analogues. This assay employed lysophosphatidylcholine analogue FS-3 (Echelon Biosciences, Inc. Salt Lake City, UT) as substrate and recombinant ATX-HA (generously provided by Dr. Russel Bandle (NCI)). For analysis, 50 µl of ATX-HA (0.25 µg) in assay buffer (Tris 50 mM, NaCl 140 mM, KCl 5mM, CaCl<sub>2</sub> 1 mM, MgCl<sub>2</sub> 1 mM, pH 8.0) was mixed with 25 µl of FS-3 (1 µM final concentration in assay buffer) and 25 µl of a
solution of each compound dissolved in assay buffer containing 1:1.5 bovine serum albumin in 96-well plate. FS-3 fluorescence was monitored using FLEXstation fluorescence plate reader at a time zero and after 2 h of incubation at 37 °C at excitation and emission wavelengths of 485 nm and 538 nm, respectively. Data was normalized to the corresponding vehicle control and the mean ± standard deviation of triplicate wells were expressed as percent ATX inhibition. IC₅₀, and Kᵢ values were calculated as described (Baker, D.; Fujiwara, Y.; Pigg, K. R.; Tsukahara, R.; Kobayashi, S.; Murofushi, H.; Uchiyama, A.; Murakami-Murofushi, K.; Koh, E.; Bandle, R. W.; Byun, H. S.; Bittman, R.; Fan, D.; Murph, M.; Mills, G. B.; Tigy, G., J. Biol. Chem. 2006, 281, 22786-22793).

Activity of AO-LPA Analogues on Cell migration in scratch wound assay. Non-transformed rat intestinal epithelial cells IEC-6 cells were plated at 0.5×10⁶ cells/well onto 6-well plate in DMEM supplemented with 5% FBS, 10 µg insulin, and 50 µg gentamicin sulfate/ml. The cells were grown to confluency for 4 days at 37 °C in a humidified atmosphere of 90% air-10% CO₂. They were fed on day 2 and serum starved on day 3, and the assay was performed on day 4. Plates containing confluent monolayers were marked in the center by drawing a line along the diameter of a plate with a black marker. Wounding of the monolayer was performed perpendicular to the marked line by using a gel-loading microtip. Plates were washed with HBSS and incubated with vehicle (0.1% BSA), LPA (100 nM), the test compound applied at 300 nM, 1 µM, 3 µM, and 10 µM concentration. The area of the wound was digitally photographed with the use of NIH Image software (version 1.55) at 0 h and 8 h. Image J software (version 1.37) was used to measure the area of the wound to calculate the percentage of healing, i.e. covered by migrating cells. The results represent the mean of 8 individual measurements.
Table 2. Inhibition of recombinant ATX by LPA and compounds 12a and 12b. Comparisons with same acyl chains: LPA 16:0 with 12a, and LPA 18:1 with 12b.

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<th>Cmpd</th>
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<th>Inhibition [%]</th>
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<td>LPA 16:0</td>
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<td>38.1 ± 3.4</td>
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<tr>
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<tr>
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Supplementary Figure 2. Photographic montages for scratch wound assays for compound 12a and compound 12b.

(Figures on following pages S14, S15)
Vehicle (0.1% BSA)

100nM LPA18:1

300nM

1μM

3μM

10μM

Compound 12b
### Table of Contents: $^1$H, $^{13}$C and $^{31}$P NMR spectra of compounds 3-12

1. $^1$H and $^{13}$C NMR of compound 3
2. $^1$H and $^{13}$C NMR of compound 4
3. $^1$H and $^{13}$C NMR of compound 5
4. $^1$H and $^{13}$C NMR of compound 6
5. $^1$H and $^{13}$C NMR of compound 7
6. $^1$H and $^{13}$C NMR of compound 8a
7. $^1$H and $^{13}$C NMR of compound 8b
8. $^1$H and $^{13}$C NMR of compound 9a
9. $^1$H and $^{13}$C NMR of compound 9b
10. $^1$H, $^{13}$C and $^{31}$P NMR of compound 11a
11. $^1$H, $^{13}$C and $^{31}$P NMR of compound 11b
12. $^1$H, $^{13}$C and $^{31}$P NMR of compound 12a
13. $^1$H, $^{13}$C and $^{31}$P NMR of compound 12b
$^{1}H$ NMR, CDCl$_3$, 3
$\begin{array}{c}
\text{Si} \quad \text{O} \\
\text{Si} \quad \text{O} \quad \text{OH} \\
\text{O} \quad \text{Bn}
\end{array}$

$^{13}C$ NMR, CDCl$_3$, 3
$\text{BnO-Si}$

$^1\text{H NMR, CDCl}_3, 4$
$\text{RnO}_{\cdot}\text{ONH}_{\cdot}\text{ON} \text{Si}$

$^1\text{H NMR, CDCl}_3, 5$

ppm
$\text{BrO}_2\text{ONH}_2\text{O}_2\text{Si}$

$^{13}C\text{ NMR, CDCl}_3, 5$

S22
$^1$H NMR, CDCl$_3$, \( \delta \)
$^{13}$C NMR, CDCl$_3$, 6
^13C NMR, CDCl₃, 7
$^{13}$C NMR, CDCl$_3$, 8a
$^{1}H$ NMR, CDCl$_3$, 8b
$^{13}C$ NMR, CDCl$_3$, 8b
$^{13}$C NMR, CDCl$_3$, 9a
$^1\text{H NMR, CDCl}_3, 11a$
$^{31}$P NMR, CDCl$_3$, 11a
$^{31}$P NMR, CDCl$_3$, 11b
$\text{C}_{12}\text{H}_{21}\text{O}_{3}\text{N}_{2}\text{P}_{1}$

$^{13}$C NMR, CD$_3$OD, 12a
$^{13}$C NMR, CD$_3$OD, 12b
C_{17}H_{33}O_7N_1P_1

$^{31}$P NMR, CD$_3$OD, 12b