SUPPORTING INFORMATION

Stereochemistry of Linoleic Acid Esters of Hydroxy Linoleic Acids.

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General Information

All reactions were performed in oven-dried glassware sealed with rubber septa and under nitrogen atmosphere, unless otherwise indicated. Air- and/or moisture-sensitive liquids or solutions were transferred by cannula or syringe. Organic solutions were concentrated by rotary evaporator at 30 millibar with the water bath heated to not more than 40 °C, unless specified otherwise. Tetrahydrofuran (THF), dichloromethane (DCM) were purified with a Pure-Solve MD-5 Solvent Purification System (Innovative Technology). 99.8% Extra Dry N, N-dimethylformamide (DMF) were supplied by Acros Organics. Thin-layer chromatography (TLC) was performed using 0.2 mm commercial silica gel plates (silica gel 60, F254, EMD Chemicals) and visualized with a UV lamp (short and long wave) and/or aqueous potassium permanganate (KMnO₄) stain. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian 600 MHz (^1H at 600 MHz, ^13C at 150 MHz, ^13C DEPTQ at 150 MHz). All spectra were taken in CDCl₃ with shifts reported in parts per million (ppm) referenced to protium or carbon of the solvent (7.26 or 77.16, respectively). Coupling constants are reported in Hertz (Hz). Data for ^1H-NMR are reported as follows: chemical shift (ppm, reference to protium; s = single, d = doublet, t = triplet, m = multiplet, coupling constant (Hz), and integration). High Resolution Mass Spectra (HRMS) were acquired on an Agilent 6230 High Resolution time-of-flight mass spectrometer and reported as m/z for the molecular ion [M+Na]^+, [M+H]^+ and [M-H].
General Synthetic Procedures

The synthesis of 9- and 13-LAHLL

*selenium dioxide oxidation*

To a stirred solution of selenium dioxide (1.0 g, 9.3 mmol, 1.0 eq) in CH₂Cl₂ (18 mL) was added neat methyl linoleate (9) (2.7 g, 3.0 mL 9.3 mmol, 1.0 eq) under nitrogen atmosphere at 23 °C and stirred for 24 hours. The reaction was diluted with an aqueous solution of NaCl (10% w/w, 25 mL). The resulting mixture was extracted with CH₂Cl₂ (20 mL × 3). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The crude material was purified via silica gel column chromatography using hexane:EtOAc (90:10 to 89:11) to give methyl 9-hydroxyl linoleate 6 (290 mg, 10%) the methyl 13-hydroxyl linoleate 7 (310 mg, 11%) as oils. Spectra were in agreement with those previously reported¹.

**Esterification**

To a stirred solution of methyl 9-hydroxyl linoleate 6 (63 mg, 0.2 mmol, 1.0 eq) in dry CH₂Cl₂ (5.0 mL) was added neat pyridine (82 μL, 1.0 mmol, 5.0 eq) dropwise via syringe. The reaction...
vessel was placed in an ice water bath and stirred for 5 minutes. Neat linoleoyl chloride (72 μL, 0.22 mmol, 1.1 eq) was added via syringe slowly. The reaction mixture was stirred at 23 °C for 20 h. The reaction was diluted with water (2.5 mL) and stirred for 10 minutes. The organic phase was collected, and the aqueous layer was extracted with CH₂Cl₂ (4.0 mL x 2). The combined CH₂Cl₂ layers were washed with 0.5 M aqueous hydrochloric acid (3.5 mL), saturate aqueous sodium bicarbonate (3.5 mL), and then brine (3.5 mL). The CH₂Cl₂ solution was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (97:3) to give the 9-LAHLA methyl ester 10 (91 mg).

**Selective hydrolysis of the 9-LAHLA methyl ester 10**

To a stirred solution of 9-LAHLA methyl ester 10 (91 mg, 0.16 mmol, 1.0 eq.) in THF (0.8 mL) at 23 °C was added an aqueous solution of lithium hydroxide (0.32 mL, 1 M, 0.32 mmol, 2.0 eq). The reaction mixture was stirred for 24 h and cooled to 0 °C. The reaction was diluted with 2N aqueous hydrochloric acid (6 mL) and then extracted with ether (18 mL x 3). The combined ether solution was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAC:AcOH (94:5:1) to give the 9-LAHLA 4 (82 mg, 0.146 mmol, 72% for two steps).

**Selective hydrolysis of the 9-LAHLA methyl ester 10**

To a stirred solution of 9-LAHLA methyl ester 10 (91 mg, 0.16 mmol, 1.0 eq.) in THF (0.8 mL) at 23 °C was added an aqueous solution of lithium hydroxide (0.32 mL, 1 M, 0.32 mmol, 2.0 eq). The reaction mixture was stirred for 24 h and cooled to 0 °C. The reaction was diluted with 2N aqueous hydrochloric acid (6 mL) and then extracted with ether (18 mL x 3). The combined ether solution was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAC:AcOH (94:5:1) to give the 9-LAHLA 4 (82 mg, 0.146 mmol, 72% for two steps).
29.2, 29.1, 29.0, 27.8, 27.3, 25.8, 25.2, 25.0, 24.8, 22.7, 14.2; IR (film, cm\(^{-1}\)): 2925, 2854, 1708, 1734, 1463, 1170; HRMS (ESI) calc. for C\(_{36}\)H\(_{62}\)O\(_4\)Na [M+Na]\(^+\): 581.4540, obs. 581.4539.

**Esterification**

To a stirred solution of methyl 13-hydroxyl linoleate 7 (50 mg, 0.16 mmol, 1.0 eq) in dry CH\(_2\)Cl\(_2\) (2.0 mL) was added neat pyridine (65 μL, 0.80 mmol, 5.0 eq). The reaction was cooled to 0 °C and stirred for 10 min. Neat linoleoyl chloride (57 μL, 0.18 mmol, 1.1 eq) was added via syringe slowly. The reaction mixture was warmed to 23 °C and stirred overnight. The reaction was diluted with water (2.0 mL) and extracted with CH\(_2\)Cl\(_2\) (3.0 mL × 2). The combined CH\(_2\)Cl\(_2\) solution was washed with 0.5 M aqueous hydrochloric acid (3.0 mL), saturate aqueous sodium bicarbonate (3.0 mL), and then brine (3.0 mL). The CH\(_2\)Cl\(_2\) solution was dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (96:4) to give the 13-LAHLA methyl ester 11 (82 mg).

**methyl (9Z,11E)-13-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)octadeca-9,11-dienoate (13-LAHLA methyl ester, 11)**

R\(_f\) = 0.6 (silica gel, 90:10 hexanes:EtOAc); 1\(H\) NMR (600 MHz, CDCl\(_3\)) δ 6.48 (dd, \(J = 15.2, 11.1\) Hz, 1H), 5.93 (t, \(J = 11.0\) Hz, 1H), 5.55 (m, 1H), 5.47 – 5.27 (m, 6H), 3.66 (s, 3H), 2.76 (t, \(J = 6.9\) Hz, 2H), 2.29 (m, 4H), 2.15 (m, 2H), 2.06 – 2.02 (m, 4H), 1.62 (s, 4H), 1.39 – 1.23 (m, 30H), 0.88 (m, 6H); 13\(C\) NMR (150 MHz, CDCl\(_3\)) δ 174.5, 173.3, 133.7, 131.2, 130.4, 130.2, 128.2, 128.0, 127.7, 74.6, 51.6, 34.8, 34.7, 34.2, 31.7, 29.7, 29.6, 29.5, 29.3, 29.2, 27.9, 27.3, 25.8, 25.2, 25.1, 25.0, 22.7, 14.2; IR (film, cm\(^{-1}\)): 2926, 2855, 2360, 2341, 1739, 1458, 1171; MS (ESI) calc. for C\(_{37}\)H\(_{64}\)O\(_4\)Na [M+Na]\(^+\): 595.47, obs. 595.39.

**Selective hydrolysis of 13-LAHLA methyl ester 11**

To a stirred solution of 13-LAHLA methyl ester 11 (82 mg, 0.14 mmol, 1.0 eq) in THF (0.7 mL) at 23 °C was added an aqueous solution of lithium hydroxide (0.3 mL, 1 M, 0.30 mmol, 2.0
eq). The reaction mixture was stirred for 24 hours and cooled to 0 °C. The reaction was diluted with 2N aqueous hydrochloric acid (6 mL) and then extracted with ether (15 mL × 3). The combined ether solution was dried over sodium sulfate, filtered and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAC:AcOH (93:6:1) to give the 13-LAHLA 3 (68 mg, 0.12 mmol, 76% for two steps). (9Z,11E)-13-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)octadeca-9,11-dienoic acid (13-LAHLA, 3) \( R_f = 0.4 \) (silica gel, 80:20 hexanes:EtOAc); \(^1\)H-NMR (600 MHz, CDCl\(_3\)) \( \delta 6.49 \) (dd, \( J = 15.2, 11.1 \) Hz, 1H), 5.93 (t, \( J = 11.0 \) Hz, 1H), 5.55 (dd, \( J = 15.2, 7.5 \) Hz, 1H), 5.47 – 5.28 (m, 6H), 2.77 (t, \( J = 6.8 \) Hz, 2H), 2.34 (m, 2H), 2.31 – 2.27 (m, 2H), 2.16 (m, 2H), 2.04 (m, 4H), 1.62 (m, 4H), 1.38 – 1.25 (m, 31H), 0.88 (m, 6H); \(^{13}\)C NMR-DEPTQ (150 MHz, CDCl\(_3\)) \( \delta 179.2, 173.5, 133.8, 131.2, 130.4, 130.2, 128.2, 128.1, 128.0, 127.7, 74.8, 34.9, 34.7, 34.0, 31.7, 29.7, 29.5, 29.3, 29.1, 29.0, 27.8, 27.3, 25.8, 25.2, 25.0, 24.8, 22.7, 14.2; IR (film, cm\(^{-1}\)): 2927, 2854, 2362, 2341, 1735, 1708, 1460, 1174; HRMS (ESI) calc. for C\(_{36}\)H\(_{62}\)O\(_4\)Na [M+Na]\(^+\): 581.4540, obs. 581.4543.

**Preparation of 9-HLA (13) and 13-HLA (14)**

9-HLA methyl ester 6 (50 mg, 0.16 mmol, 1.0 eq) was dissolved into methanol (5 mL). To the reaction was added 1 M aqueous solution of NaOH (5 mL) at 23 °C. The reaction mixture was warmed to 50 °C and stirred for 1 h. The reaction was cooled to 0 °C, diluted with 1N aqueous hydrochloric acid (5 mL), and then extracted with ethyl acetate (10 mL × 3). The combined ethyl acetate solution was dried over sodium sulfate, filtered and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAC:AcOH (50:49:1) to give the 9-HLA 12 (38 mg, 0.13 mmol, 80%). Hydrolysis of 13-HLA methyl ester 7 (50 mg, 0.16 mmol, 1.0 eq) was carried out under same procedure to obtain 13-HLA 13 (40 mg, 0.14 mmol, 84%). Spectra were in agreement with those previously reported.\(^1\)
The synthesis of 15-LAHLA and 15-HLA

Preparation of 2-(prop-2-yn-1-yloxy)tetrahydro-2H-pyran (15)

![Chemical structure of 15]

To a solution of 3-butyn-1-ol (4.2 mL, 71.4 mmol, 1.0 eq) in CH$_2$Cl$_2$ (100 mL) at 0 °C was added solid TsOH·H$_2$O (150 mg, 0.7 mmol, 0.01 eq) followed by adding neat 3,4- dihydro-2H-pyran (7.2 mL, 78.5 mmol, 1.1 eq). After stirring at 0 °C for 10 min, the reaction was warmed to 23 °C and stirred for 1 h. The reaction was diluted with saturated sodium bicarbonate (35 mL) and extracted with CH$_2$Cl$_2$ (50 mL × 2). The combined CH$_2$Cl$_2$ solution was dried over sodium sulfate, filtered and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAC (10:1) to give the acetal 15 (9.5 g, 95% yield, R$_f$ = 0.3 in 10:1 hexanes/EtOAc) as an oil. NMR and mass spectrometry were in complete agreement with literature values.$^2$

Regioselective ring opening of epoxide 14

![Chemical structure of 14 and 15]

Under an atmosphere of nitrogen, n-BuLi (2.5 M in hexane, 14.4 mL, 36 mmol, 1.0 eq) was added to the solution of 15 (5.0 g, 36 mmol) in anhydrous THF (200 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 15 min. To the reaction was added BF$_3$·Et$_2$O (4.5 mL, 36 mmol, 1.0 eq) and stirred at −78 °C for 30 min. Then the reaction mixture was warmed up to 0 °C and stirred for 1 h. After that, the reaction was cooled to −78 °C again and a solution of epoxide 14 (3.0 g, 36 mmol, 1.0 eq) in anhydrous THF (20 mL) was added. The reaction mixture was stirred at −78 °C for another 3 h. The reaction was diluted with saturated aqueous ammonium chloride (50 mL) and extracted with ethyl acetate (50 mL × 3). The combined ethyl acetate solution was washed...
with brine (30 mL), dried over sodium sulfate, filtered and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (3:1 to 1:1) to yield 16 (7.3 g, 90%) as an oil. NMR and mass spectrometry were in complete agreement with literature values.³

**Removal of the THP protecting group**

Alcohol 16 (1.3 g, 5.6 mmol, 1.0 eq) was dissolved in methanol (56 mL). Stirring was initiated, giving a clear solution. The reaction was cooled to 0 °C and stirred for 15 min. Solid 4-methylbenzenesulfonic acid (215 mg, 1.1 mmol, 0.2 eq) was added in one portion. The reaction mixture was warmed to 23 °C and stirred for 2 h. The resulting mixture was evaporated under reduced pressure to remove methanol, diluted with ethyl acetate (50 mL). The ethyl acetate solution was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL × 2), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (4:1 to 2:1) to yield 17 (724 mg, 90%) as an oil.

**oct-2-yne-1,5-diol (17)**

\[ \text{R} \text{f} = 0.20 \text{ (silica gel, 2:1 hexanes:EtOAc, KMnO} \text{4)} ; \ \text{^1H-NMR} \text{ (600 MHz, CDCl}_3\text{): } \delta 4.24 \text{ (t, } J = 2.3 \text{ Hz, 2H), 3.75 \text{ (m, 1H), 3.35 \text{ (s, OH), 3.00 \text{ (s, OH), 2.45 \text{ (m, 1H), 2.31 \text{ (m, 1H), 1.58 – 1.41 \text{ (m, 3H), 1.41 – 1.28 \text{ (m, 1H), 0.92 \text{ (t, } J = 7.1 \text{ Hz, 3H). ^13C-NMR} \text{ (150 MHz, CDCl}_3\text{): } \delta 82.8, 80.9, 69.9, 51.1, 38.5, 27.6, 19.0, 14.1; IR \text{ (film, cm}^{-1}\text{): 2958, 2872, 1423, 1008; HRMS (ESI) calc. for C}_8\text{H}_{14}\text{O}_2\text{Na [M+Na]}^+ : 165.0886, obs. 165.0887.} \]

**Selective bromination**

S9
To a solution of oct-2-yne-1,5-diol 17 (724 mg, 5.1 mmol, 1.0 eq) in anhydrous CH$_2$Cl$_2$ (51 mL) was added solid triphenylphosphine (1.5 g, 5.6 mmol, 1.1 eq) and carbon tetrabromide (1.8 g, 5.6 mmol, 1.1 eq) at 0 °C. The reaction mixture was warmed to 23 °C and stirred overnight. The solvent was removed under reduced pressure to obtain the crude material, which was purified via flash column chromatography using hexanes:EtOAc (8:1 to 4:1) to yield 18 (946 mg, 91%) as an oil.

8-bromooct-6-yn-4-ol (18)

R$_f$ = 0.80 (silica gel, 2:1 hexanes:EtOAc, KMnO$_4$); $^1$H-NMR (600 MHz, CDCl$_3$): δ 3.92 (t, $J$ = 2.4 Hz, 2H), 3.75 (m, 1H), 2.46 (m, 1H), 2.36 (m, 1H), 2.03 (s, OH), 1.55 – 1.46 (m, 2H), 1.46 – 1.40 (m, 1H), 1.40 – 1.28 (m, 1H), 0.92 (t, $J$ = 7.2 Hz, 3H). $^{13}$C-NMR (150 MHz, CDCl$_3$): δ 84.5, 77.8, 69.8, 38.5, 28.0, 18.9, 15.4, 14.1. IR (film, cm$^{-1}$): 2958, 2931, 2233, 1424, 1209, 1013. HRMS (ESI) calc. for C$_8$H$_{13}$BrONa [M+Na]$^+$: 227.0045, obs. 227.0047.

Preparation of known alkyne 19

Under an atmosphere of nitrogen, to a solution (trimethylsilyl)acethylene (3.4 mL, 24.0 mmol, 1.8 eq) in anhydrous THF (20 mL) was added n-BuLi (2.5M in hexanes, 11.6 mL, 29.0 mmol, 1.2 eq) at −78 °C and stirred for 20 min. To the reaction mixture was added a solution of 8-bromooctanoic acid (3.0 g, 13.5 mmol, 1.0 eq) in anhydrous THF (60 mL) and neat HMPA (40 mL) via syringe at −78 °C, and then stirred for 2 h. The reaction was diluted with saturated aqueous ammonium chloride (20 mL) and extracted with ethyl acetate (50 mL $\times$ 3). The combined ethyl acetate solution was washed with brine (30 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via flash silica gel column chromatography using hexane:EtOAc (5:1) to give S-1 (2.7 g, 85%) as a colorless oil.

A 250 mL round-bottom flask was equipped with stir bar and charged with S-1 (2.7 g, 11.2 mmol, 1.0 eq) in MeOH (150 mL). Concentrated sulfuric acid (3 mL) was added at 23 °C. The
reaction was warmed to reflux and stirred for 2 h. The solvent was then removed under reduced pressure to obtain the crude material, which was dissolved into ethyl acetate (200 mL). The ethyl acetate was washed with saturated aqueous sodium bicarbonate (30 mL) and brine (30 mL), dried over sodium sulfate, filtered, and concentrated in vacuo to obtain the crude product S-2, which was then used for the next step without any purification.

To a solution of crude S-2 (2.7 g) in THF (100 mL) was added TBAF (1.0 M in THF, 15.5 mL, 15.5 mmol, 1.5 eq) at 23 °C and stirred overnight. The reaction was diluted with brine (30 mL) and extracted with ethyl acetate (50 mL × 3). The combined ethyl acetate solution was dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified via flash silica gel column chromatography using hexane/EtOAc (50:1 to 30:1) to give alkyne 19 (1.7 g, 83% over two steps) as an oil. NMR and mass spectrometry were in complete agreement with literature values.4

copper-catalyzed cross coupling reaction

Under an atmosphere of nitrogen, the known alkyne 19 (556 mg, 3 mmol, 1.5 eq) was dissolved in anhydrous N, N-dimethylformaide (20 mL). Stirring was initiated, giving a clear solution. The reaction was cooled to 0 °C and stirred for 15 min. To the reaction was added NaI (457 mg, 3 mmol, 1.5 eq), CuI (580 mg, 3.0 mmol, 1.5 eq) and Cs₂CO₃ (994 mg, 3.0 mmol, 1.5 eq). The reaction mixture was stirred at 0 °C for 20 minutes and the color changed to green. A solution of 8-bromooc-6-yn-4-ol 18 (415 mg, 2.0 mmol, 1.0 eq) in anhydrous N, N-dimethylformaide (10 mL) was added by syringe and the reaction was stirred at 0 °C for 10 minutes. The reaction was warmed to 23 °C and stirred overnight. The reaction was diluted with saturated aqueous ammonium chloride (10 mL). The reaction mixture was stirred vigorously until the color changed to blue. The resulting mixture was extracted with ethyl acetate (15 mL × 3). The combined ethyl acetate organics were washed with brine (10 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography
using hexanes:EtOAc (8:1 to 4:1) to yield 20 (530 mg, 85%) as an oil, which was dissolved in ethyl acetate, stored at 0 °C, and used for the next step as soon as possible.

**methyl 15-hydroxyoctadeca-9,12-diynoate (20)**

R\(_f\) = 0.40 (silica gel, 4:1 hexanes:EtOAc, KMnO\(_4\)); \(^1\)H-NMR (600 MHz, CDCl\(_3\)): δ 3.72 (m, 1H), 3.65 (s, 3H), 3.13 (m, 2H), 2.39 (m, 1H), 2.32 – 2.25 (m, 3H), 2.13 (m, 2H), 1.98 (br s, OH), 1.64 – 1.57 (m, 2H), 1.51 – 1.43 (m, 4H), 1.39 – 1.30 (m, 8H), 0.95 – 0.89 (m, 3H). \(^{13}\)C-NMR (150 MHz, CDCl\(_3\)): δ 174.5, 80.8, 77.5, 76.8, 74.2, 69.9, 51.6, 34.5, 34.2, 29.1, 28.9, 28.8, 28.7, 27.9, 25.0, 19.0, 18.9, 14.1, 9.9; IR (film, cm\(^{-1}\)): 2933, 2858, 1736, 1439, 1172. HRMS (ESI) calc. for C\(_{19}\)H\(_{30}\)O\(_3\)Na [M+Na]\(^+\): 329.2091, obs. 329.2093.

**Semihydrogenation of the skipped diyne**

A flask was charged with nickel acetate tetrahydrate (431 mg, 1.7 mmol, 1.0 eq) and ethanol (25 mL). The solution was purged with H\(_2\) gas and a suspension of NaBH\(_4\) (65 mg, 1.7 mmol, 1.0 eq) in ethanol (5 mL) was added. The color of reaction mixture immediately turned to black. The reaction was stirred at 23 °C for 20 min. Neat ethylenediamine (0.35 mL, 5.1 mmol, 3.0 eq) was added, followed by adding a solution of the skipped diyne 20 (530 mg, 1.7 mmol, 1.0 eq) in ethanol (5 mL). The reaction was stirred vigorously at 23 °C for 30 min. The reaction was filtered through celite and washed with ethyl acetate (100 mL). The filtrate was concentrated in vacuo. The concentrate was diluted in hexanes:EtOAc (4:1, 50 mL), washed with brine (10 mL × 2), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (12:1 to 8:1) to yield 8 (455 mg, 85%) as an oil.

**methyl (9Z,12Z)-15-hydroxyoctadeca-9,12-dienoate (8)**

R\(_f\) = 0.50 (silica gel, 4:1 hexanes:EtOAc, KMnO\(_4\)); \(^1\)H-NMR (600 MHz, CDCl\(_3\)): δ 5.57 – 5.51 (m, 1H), 5.47 – 5.29 (m, 3H), 3.66 (s, 3H), 3.64 (s, 1H), 2.84 – 2.76 (m, 2H), 2.30 (t, \(J = 7.6\) Hz, 2H), 2.24 (m, 2H), 2.04 (m, 2H), 1.65 – 1.23 (m, 14H), 0.97 – 0.90 (m, 3H). \(^{13}\)C-NMR (150 MHz, CDCl\(_3\)): δ 174.5, 131.6, 130.6, 127.6, 125.7, 71.3, 51.6, 39.2, 35.5, 34.2, 29.7, 29.3, 29.2, 27.4,

_Esterification and selective hydrolysis of the methyl ester_

Under an atmosphere of nitrogen, neat linoleic acid (30 µL, 0.1 mmol, 2.0 eq) was dissolved in anhydrous CH₂Cl₂ (3 mL). Stirring was initiated, giving a clear solution. The reaction was cooled to 0 °C and stirred for 15 min. To the reaction was added solid EDCI (19 mg, 0.1 mmol, 2.0 eq) and DMAP (12 mg, 0.1 mmol, 2.0 eq). The reaction mixture was stirred at 0 °C for 20 min. A solution of 8 (15 mg, 0.05 mmol, 1.0 eq) in anhydrous CH₂Cl₂ (1 mL) was added. The reaction was warmed to reflux and stirred overnight. The solvent was then removed under reduced pressure and the crude material was purified via flash column chromatography using hexanes:EtOAc (10:1 to 6:1) to get the intermediate (22 mg).

The intermediate (22 mg) was dissolved in THF:H₂O (3 mL : 3 mL), cooled to 0 °C, and stirred for 15 min. To the reaction was added LiOH·H₂O (10 mg, 0.23 mmol, 6.0 eq) in one portion. The reaction was warmed to 23 °C and stirred overnight. The reaction was diluted with 1N aqueous hydrochloric acid (2 mL) and extracted with ethyl acetate (8 mL × 3). The combined ethyl acetate solution was washed with brine (5 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc:acetic acid (75:23:2) to yield 15-LAHLA (20 mg, 76% over two steps) as an oil.

(9Z,12Z)-15-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)octadeca-9,12-dienoic acid (15-LAHLA, 5) \( R_f = 0.20 \) (silica gel, 3:1 hexanes:EtOAc, KMnO₄); **¹H-NMR** (600 MHz, CDCl₃): δ 5.44 (m, 1H), 5.34 (m, 7H), 4.91 (m, 1H), 2.77 (t, \( J = 6.8 \) Hz, 4H), 2.37 – 2.24 (m, 6H), 2.04 (m, 6H), 1.62 (m, 4H), 1.52 (m, 2H), 1.32 (m, 24H), 0.89 (m, 6H). **¹³C-NMR** (150 MHz, CDCl₃) δ 179.8, 173.8, S1.

**Preparation of 15-HLA**

The 15-HLA methyl ester 8 (40 mg, 0.13 mmol, 1.0 eq) was dissolved in THF:H₂O (4 mL : 4 mL), cooled to 0 °C, and stirred for 15 min. To the reaction was added solid LiOH·H₂O (33 mg, 0.77 mmol, 6.0 eq) in one portion. The reaction was warmed to 23 °C and stirred overnight. The reaction was diluted with 1N aqueous hydrochloric acid (3 mL) and extracted with ethyl acetate (10 mL × 3). The combined ethyl acetate solution was washed with brine (5 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc:acetic acid (66:32:2) to yield 15-HLA 21 (34 mg, 90%) as an oil.

*(9Z,12Z)-15-hydroxyoctadeca-9,12-dienoic acid (15-HLA, 21)*

Rf = 0.20 (silica gel, 2:1 hexanes:EtOAc, KMnO₄); ¹H NMR (600 MHz, Chloroform-d) δ 5.52 (m, 1H), 5.45 – 5.28 (m, 3H), 3.65 (m, 1H), 2.79 (t, J = 7.8 Hz, 2H), 2.32 (m, 2H), 2.23 (m, 2H), 2.02 (m, 2H), 1.62 (m, 2H), 1.49 – 1.23 (m, 12H), 0.91 (t, J = 6.9 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 179.6, 131.4, 130.5, 127.6, 125.5, 71.4, 39.0, 35.3, 34.2, 29.6, 29.2, 29.1, 27.3, 25.9, 24.8, 19.0, 14.2. IR (film, cm⁻¹): 3340, 3010, 2856, 1710, 1465. HRMS (ESI) calc. for C₁₈H₃₁O₃ [M-H]⁻: 295.2279, obs. 295.2278.

**Synthesis of enantiopure S-15-LAHLA (5-S) and R-15-LAHLA (5-R)**

*regioselective ring opening and epoxide formation*

(R)-(-)-Epichlorohydrin + THPO

\[
\text{a) } n\text{-BuLi, BF}_3\text{Et}_2\text{O, THF, } -78 ^\circ \text{C} \\
\text{b) NaOH, Et}_2\text{O, 23 } ^\circ \text{C}
\]

THPO\(\text{O}\)\(\equiv\)\(\text{O}\)

72% over 2 steps
Under an atmosphere of nitrogen, \( n\)-BuLi (2.5 M in hexane, 8.5 mL, 21 mmol, 1.0 eq) was added to a solution of 15 (3.0 g, 21 mmol, 1.0 eq) in anhydrous THF (120 mL) at \(-78^\circ C\) and then stirred for 15 min. To the reaction was added BF\(_3\)·Et\(_2\)O (2.7 mL, 21 mmol, 1.0 eq) and stirred at \(-78^\circ C\) for 30 min. Then the reaction was warmed to 0 °C and stirred for 1 h. After that, the reaction was cooled to \(-78^\circ C\) again and a solution of (R)-(−)-epichlorohydrin (2.0 g, 21 mmol, 1.0 eq) in anhydrous THF (20 mL) was added. The reaction was stirred at \(-78^\circ C\) for another 3 h. The reaction was diluted with saturated aqueous ammonium chloride (40 mL) and extracted with ethyl acetate (40 mL × 3). The combined ethyl acetate solution was washed with brine (30 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via flash silica gel column chromatography to afford the chlorohydrin intermediate (4.4 g), which was used for the next step directly.

Under an atmosphere of nitrogen, the chlorohydrin intermediate (4.4 g, 19.0 mmol, 1.0 eq) was dissolved in anhydrous ether (55 mL). Stirring was initiated, giving a clear solution. Solid sodium hydroxide (4.6 g, 144 mmol, 6.0 eq) was added in one portion at 23 °C and stirred vigorously for 24 h. The reaction mixture was vacuum filtered into a dry flask containing 4Å mole sieves, rinsing the filter cake with anhydrous ether (25 mL × 3). The combined ether solution was then removed under reduced pressure. The crude material was purified via flash column chromatography using hexanes:EtOAc (10:1 to 6:1) to yield epoxide 22 (3.0 g, 72% over two steps) as an oil. Spectra were in agreement with those previously reported.\(^5\)

\(2-((4-( (R) -oxiran-2-yl)but-2-yn-1-yl)oxy)tetrahydro-2H-pyran (22)\)

\(R_f = 0.5\) (silica gel, 4:1 hexanes:EtOAc, K\(\text{MnO}_4\)); \(^1\)H NMR (600 MHz, Chloroform-\(d\)) \(\delta\) 4.75 (t, \(J = 3.5\) Hz, 1H), 4.21 (m, 2H), 3.79 (m, 1H), 3.49 (m, 1H), 3.07 (m, 1H), 2.75 (t, \(J = 4.4\) Hz, 1H), 2.62 (m, 2H), 2.47 (m, 1H), 1.79 (m, 1H), 1.70 (m, 1H), 1.57 (m, 2H), 1.50 (m, 2H). \(^13\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 96.8, 80.6, 78.3, 62.0, 54.5, 49.9, 46.5, 30.3, 25.4, 22.7, 19.1.

**Regioselective ring opening**

```
\[
\text{THPO} \quad \text{EtMgBr, Cul} \quad \text{THF, -78 °C to rt} \quad \text{THPO} \quad \text{OH} \\
\text{22} \quad \text{(R)} \quad \text{16-S} \quad \text{(S)} \quad \text{CH}_3 \\
\]```

S1
Under an atmosphere of nitrogen, the epoxide 22 (3.0 g, 15.3 mmol, 1.0 eq) and solid copper (Ⅰ) iodide (292 mg, 1.5 mmol, 0.1 eq) were dissolved in anhydrous THF (100 mL). The reaction mixture was cooled to −78 °C and stirred for 20 min. Ethylmagnesium bromide (1.0 M in THF, 23 mL, 1.5 eq) was added dropwise over 20 min and stirred at −78 °C for 1 h. The reaction mixture was warmed to 23 °C and stirred for 5 h. The reaction was diluted with saturated aqueous ammonium chloride (30 mL). The reaction mixture was stirred vigorously until the color changed to a brilliant, royal blue. The reaction mixture was extracted with ethyl acetate (50 mL x 3). The combined ethyl acetate solution was washed with brine (30 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (3:1 to 1:1) to yield 16-S (3.0 g, 87%) as an oil. Spectra were in complete agreement with literature values.³

(4S)-8-((tetrahydro-2H-pyran-2-yl)oxy)oct-6-yn-4-ol (16-S)

Rᶠ = 0.2 (silica gel, 4:1 hexanes:EtOAc, KMnO₄); ¹H NMR (600 MHz, Chloroform-d) δ 4.78 (t, J = 3.6 Hz, 1H), 4.31 – 4.16 (m, 2H), 3.82 (m, 1H), 3.72 (m, 1H), 3.50 (m, 1H), 2.47 – 2.39 (m, 1H), 2.32 (m, 1H), 2.20 (s, 1H), 1.81 (m, 1H), 1.71 (m, 1H), 1.63 – 1.39 (m, 7H), 1.34 (m, 1H), 0.90 (t, J = 7.2 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 97.0, 83.1, 78.5, 69.8, 62.1, 54.8, 38.5, 30.4, 27.9, 25.4, 18.9, 14.1. HRMS (ESI) calc. for C₁₃H₂₂O₃Na [M+Na]⁺: 249.1461, obs. 249.1460.

**Removal of the THP protecting group and Selective bromination**

The alcohol 16-S (1.0 g, 4.4 mmol, 1.0 eq) was dissolved in methanol (40 mL). Stirring was initiated, giving a clear solution. The reaction was cooled to 0 °C and stirred for 15 min. Solid 4-methylbenzenesulfonic acid (17 mg, 0.9 mmol, 0.2 eq) was added in one portion. The reaction was warmed to 23 °C and stirred for 2 h. The reaction mixture was evaporated under reduced pressure to remove methanol, diluted with ethyl acetate (30 mL). The ethyl acetate solution was washed with saturated aqueous sodium bicarbonate (10 mL) and brine (10 mL × 2), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel
column chromatography using hexanes:EtOAc (4:1 to 2:1) to yield oct-2-yne-1,5-diol \(17-S\) (553 mg, 88%) as an oil.

\(\text{(S)}\)-oct-2-yne-1,5-diol (17-S)

\(R_f = 0.20\) (silica gel, 2:1 hexanes:EtOAc, KMnO\(_4\)); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \(\delta\) 4.20 (t, \(J = 2.4\) Hz, 2H), 4.20 (s, OH), 3.72 (m, 1H), 3.67 (brs, OH), 2.41 (m, 1H), 2.27 (m, 1H), 1.54 – 1.36 (m, 3H), 1.31 (m, 1H), 0.89 (t, \(J = 7.2\) Hz, 3H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 82.7, 80.7, 69.8, 50.8, 38.4, 27.5, 18.9, 14.0. IR (film, cm\(^{-1}\)): 2958, 2872, 1423, 1008; HRMS (ESI) calc. for C\(_8\)H\(_{14}\)O\(_2\)Na [M+Na]\(^+\): 165.0886, obs. 165.0887.

To a solution of oct-2-yne-1,5-diol \(17-S\) (553 mg, 3.9 mmol, 1.0 eq) in anhydrous CH\(_2\)Cl\(_2\) (35 mL) was added triphenylphosphine (1.1 g, 4.3 mmol, 1.1 eq) and carbon tetrabromide (1.4 g, 4.1 mmol, 1.1 eq) at 0 °C. The reaction was warmed to 23 °C and stirred overnight. The solvent was removed under reduced pressure and the crude material was purified via column chromatography using hexanes:EtOAc (8:1 to 4:1) to yield \(18-S\) (691 mg, 87%) as an oil.

\(\text{(S)}\)-8-bromooct-6-yn-4-ol (18-S)

\(R_f = 0.80\) (silica gel, 2:1 hexanes:EtOAc, KMnO\(_4\)); \(^1\)H NMR (600 MHz, Chloroform-\(d\)) \(\delta\) 3.92 (t, \(J = 2.4\) Hz, 2H), 3.76 (m, 1H), 2.47 (m, 1H), 2.37 (m, 1H), 1.93 (d, \(J = 3.8\) Hz, OH), 1.54 – 1.40 (m, 3H), 1.40 – 1.31 (m, 1H), 0.93 (t, \(J = 7.2\) Hz, 3H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\)) \(\delta\) 84.5, 77.8, 69.8, 38.6, 28.0, 18.9, 15.3, 14.1. IR (film, cm\(^{-1}\)): 2960, 2930, 2871, 2232, 1425, 1208, 1011. HRMS (ESI) calc. for C\(_8\)H\(_{13}\)BrONa [M+Na]\(^+\): 227.0046, obs. 227.0047.

**Copper-catalyzed cross coupling reaction and Semihydrogenation of the skipped diynes**

Under an atmosphere of nitrogen, the known alkyne \(19\) (270 mg, 2 mmol, 1.5 eq) was dissolved in anhydrous N, N-dimethyformide (15 mL). Stirring was initiated, giving a clear
solution. The reaction was cooled to 0 °C and stirred for 15 min. To the reaction was added NaI (305 mg, 2 mmol, 1.5 eq), CuI (387 mg, 2.0 mmol, 1.5 eq) and Cs₂CO₃ (663 mg, 2.0 mmol, 1.5 eq). The reaction mixture was stirred at 0 °C for 20 min, and the color changed to green. A solution of (S)-8-bromooct-6-yn-4-ol (18-S) (277 mg, 1.3 mmol, 1.0 eq) in anhydrous N, N-dimethyiformaide (5 mL) was added into the reaction mixture at 0 °C and stirred for 10 min. The reaction was warmed to 23 °C and stirred overnight. The reaction was diluted with saturated aqueous ammonium chloride (10 mL). The mixture was stirred vigorously until the color changed to blue. The resulting mixture was extracted with ethyl acetate (15 mL × 3). Combined ethyl acetate solution was washed with brine (10 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (8:1 to 4:1) to yield 20-S (326 mg, 82%) as an oil, which was dissolved in ethyl acetate for storage and used for next step as soon as possible.

A flask was charged with nickel acetate tetrahydrate (265 mg, 1.1 mmol, 1.0 eq) and 95% ethanol (20 mL). The reaction mixture was purged with H₂ gas, and then a suspension of NaBH₄ (42 mg, 1.7 mmol, 1.0 eq) in ethanol (5 mL) was added at 23 °C. The reaction immediately turned a black color. The reaction was stirred at 23 °C for 20 min. Neat Ethylenediamine (0.22 mL, 3.3 mmol, 3.0 eq) was added, followed by adding a solution of the skipped diyne 20-S (326 mg, 1.1 mmol, 1.0 eq) in ethanol (5 mL). The reaction was stirred vigorously under H₂ atmosphere at 23 °C. After 30 min, the reaction was filtered through celite and washed with ethyl acetate. The filtrate was concentrated in vacuo. The crude product was diluted in hexanes:EtOAc (4:1, 50 mL) and the organic solution was washed with brine (10 mL × 2), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (12:1 to 8:1) to yield 8-S (293 mg, 86%) as an oil.

**methyl (S,9Z,12Z)-15-hydroxyoctadeca-9,12-dienoate (8-S)**

Rᵣ = 0.50 (silica gel, 4:1 hexanes:EtOAc, K MünO₄); ¹H NMR (600 MHz, Chloroform-d) δ 5.58 – 5.50 (m, 1H), 5.47 – 5.29 (m, 3H), 3.66 (s, 4H), 2.80 (t, J = 7.5 Hz, 2H), 2.30 (t, J = 7.6 Hz, 2H), 2.24 (m, 2H), 2.04 (m, 2H), 1.66 – 1.56 (m, 2H), 1.46 (m, 2H), 1.39 – 1.32 (m, 2H), 1.29 (m, 8H), 0.93 (t, J = 6.8 Hz, 3H).¹³C NMR (150 MHz, CDCl₃) δ 174.5, 131.6, 130.6, 127.6, 125.7, 71.3, 51.6, 39.2, 35.5, 34.2, 29.7, 29.3, 29.2, 27.4, 25.9, 25.1, 19.1, 14.3. IR (film, cm⁻¹): 2929, 2855, 1735, 1438, 1170; HRMS (ESI) calc. for C₁₉H₃₄O₃Na [M+Na]⁺: 333.2400, obs. 333.2397.
Under an atmosphere of nitrogen, neat linoleic acid (0.2 mL, 0.65 mmol, 2.0 eq) was dissolved into anhydrous CH$_2$Cl$_2$ (10 mL). Stirring was initiated, giving a clear solution. The reaction was cooled to 0 °C for 15 min before adding solid EDCI (124 mg, 0.65 mmol, 2.0 eq) and DMAP (79 mg, 0.65 mmol, 2.0 eq). The reaction mixture was stirred at 0 °C for 20 min. A solution of 8-$S$ (100 mg, 0.32 mmol, 1.0 eq) in anhydrous CH$_2$Cl$_2$ (5 mL) was added. The reaction was warmed to reflux and stirred overnight. The solvent was then removed under reduced pressure and the crude material was purified via flash column chromatography using hexanes:EtOAc (10:1 to 6:1) to get the intermediate (165 mg).

The intermediate (165 mg) was dissolved in THF:H$_2$O (8 mL : 8 mL) and cooled to 0 °C and stirred for 15 min before adding LiOH·H$_2$O (73 mg, 1.7 mmol, 6.0 eq) in one portion. The reaction was warmed to 23 °C and stirred overnight. The reaction was diluted with 1N aqueous hydrochloric acid (2 mL) and extracted with ethyl acetate (10 mL × 3). The combined ethyl acetate solution was washed with brine (5 mL), dried over sodium sulfate, filtered, and then concentrated in vacuo. The crude material was purified by silica gel column chromatography using hexanes:EtOAc:acetic acid (75:23:2) to yield S-15-LAH LA (5-$S$, 128 mg, 72% over two steps) as an oil.

(9$Z$,12$Z$)-15-(((9$Z$,12$Z$)-octadeca-9,12-dienoyl)oxy)octadeca-9,12-dienoic acid (S-15-LAH LA, 5-$S$)

$R_f$ = 0.20 (silica gel, 3:1 hexanes:EtOAc, KMnO$_4$); $^1$H NMR (600 MHz, Chloroform-$d$) $\delta$ 5.44 (m, 1H), 5.34 (m, 7H), 4.91 (m, 1H), 2.77 (t, $J$ = 7.0 Hz, 4H), 2.34 (m, 3H), 2.31 (m, 1H), 2.27 (m, 2H), 2.04 (m, 6H), 1.62 (m, 4H), 1.57 – 1.47 (m, 2H), 1.31 (m, 24H), 0.89 (m, 6H). $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 179.6, 173.8, 130.8, 130.5, 130.4, 130.2, 128.2, 128.0, 127.6, 124.7, 73.5, 36.0, 34.8, 34.1, 32.2, 31.7, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 27.3, 25.9, 25.8, 25.2, 24.8, 22.7,

**Preparation of R-15-LAHLA (5-R)**

![Structural diagram of R-15-LAHLA (5-R)]

R-15-LAHLA (5-R) was obtained through the same ten step sequence with substituting (S)-(+)-epichlorohydrin at the start of the synthesis (in place of (R)-(−)-epichlorohydrin). NMR and mass spectrometry were in complete agreement with S-15-LAHLA (5-S) values. \([\alpha]_D^{23} = +7.3\) (c 1.2, CHCl₃).

**Determination of % ee by the Mosher ester method.**

![Mosher ester reaction scheme]

**General procedure**

Under an atmosphere of nitrogen, to a stirred solution of alcohol 16-S (50 mg, 0.22 mmol, 1.0 eq) in dry CH₂Cl₂ (10 mL) was added neat Et₃N (37 µL, 0.27 mmol, 1.2 eq), solid DMAP (3 mg, 0.02 mmol, 0.1 eq) and neat S-(+)-MTPA-Cl (50 µL, 0.27 mmol, 1.2 eq) at 0 °C. The reaction mixture was warmed to 23 °C and stirred overnight. The reaction was diluted with saturate aqueous sodium bicarbonate (2 mL) and extracted with CH₂Cl₂ (5 mL × 3). The combined CH₂Cl₂ solution
was washed with brine (8 mL), dried over Na₂SO₄, filtered, and then concentrated in vacuo. The crude material was purified via silica gel column chromatography using hexanes:EtOAc (10:1 to 5:1) to yield R-MTPA-ester of 16-S. R-MTPA-ester of 16-R was obtained under the same procedure starting from 16-R.

R<sup>f</sup> = 0.5 (silica gel, 4:1 hexanes:EtOAc, KMnO₄); ¹H NMR (600 MHz, Chloroform-<i>d</i>) δ 7.54 (m, 2H), 7.42 – 7.37 (m, 3H), 5.20 – 5.12 (m, 1H), 4.74 (m, 1H), 4.23 – 4.09 (m, 2H), 3.81 (m, 1H), 3.54 (s, 3H), 3.50 (m, 1H), 2.55 (m, 2H), 1.85 – 1.67 (m, 4H), 1.62 – 1.55 (m, 2H), 1.55 – 1.30 (m, 4H), 0.93 (t, <i>J</i> = 7.4 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 166.3, 132.2, 129.7, 128.5, 127.6, 123.4 (q, <i>J</i> = 288.4 Hz), 96.8, 81.1, 78.5, 74.8, 62.2, 55.6, 54.4, 35.2, 30.4, 25.4, 24.1, 19.2, 18.5, 13.9. IR (film, cm⁻¹): 2958, 2856, 1738. HRMS (ESI) calc. for C<sub>23</sub>H<sub>29</sub>F<sub>3</sub>O<sub>5</sub>NH<sub>4</sub> [M+NH₄]⁺: 460.2305, obs. 460.2298. The % ee was determined from the analysis by ¹H NMR. The methoxy group of R-MTPA-ester of 16-S with signals at δ 3.60 and 3.54 in a 1:99 ratio and 98% ee for 16-S.

R<sup>f</sup> = 0.5 (silica gel, 4:1 hexanes:EtOAc, KMnO₄); ¹H NMR (600 MHz, Chloroform-<i>d</i>) δ 7.58 (m, 2H), 7.42 – 7.37 (m, 3H), 5.18 (m, 1H), 4.79 – 4.75 (m, 1H), 4.29 – 4.16 (m, 2H), 3.82 (m, 1H), 3.61 (s, 3H), 3.53 – 3.48 (m, 1H), 2.70 – 2.54 (m, 2H), 1.86 – 1.77 (m, 1H), 1.70 (m, 2H), 1.64 – 1.48 (m, 5H), 1.31 – 1.14 (m, 2H), 0.85 (t, <i>J</i> = 7.4 Hz, 3H). ¹³C NMR (150 MHz, CDCl₃) δ 166.3, 132.5, 129.7, 128.5, 127.4, 123.4 (q, <i>J</i> = 288.4 Hz), 96.9, 81.4, 78.9, 74.8, 62.2, 55.8, 54.5, 35.1, 30.4, 25.5, 24.3, 19.3, 18.2, 13.8. IR (film, cm⁻¹): 2958, 2856, 1738. HRMS (ESI) calc. for C<sub>23</sub>H<sub>29</sub>F<sub>3</sub>O<sub>5</sub>NH<sub>4</sub> [M+NH₄]⁺: 460.2305, obs. 460.2300. The % ee was determined from the analysis
by $^1$H NMR. The methoxy group of $R$-MTPA-ester of 16-$R$ with signals at $\delta$ 3.61 and 3.54 in a 99:1 ratio and 98% ee for 16-$R$.

Analytical and Bioactivity Assays

Figure S1. LC-MS chromatograms of S-15-LAHLA (top panel) and R-15-LAHLA (bottom panel).

Culturing. RAW 264.7 cells were cultured in RPMI 1640 (Gibco), supplemented with L-glutamine (2mM), 10% FBS at 37 °C and 5% CO$_2$. All experiments were performed on or prior to passage 15.

Quantification of IL-6 upon LPS Stimulation Assay. RAW 264.7 cells were seeded onto 48-well plates (2.5 × 10$^4$ cells per well) a day prior to treatment. Once adhered to the wells, cell were treated with DMSO or LPS (100 ng/mL) and compounds of interest individually at 25 µM. Supernatants were collected 20 hours post-treatment and subjected to IL-6 quantification using mouse IL-6 ELISA MAX$^{\text{TM}}$ Deluxe Kits (BioLegend). Adherent cells were subjected to MTT cell viability assay to quantify the percentage of viable cells.

Post-Treatment Analysis of Cell Proliferation using MTT Assay. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved in sterile PBS (5 mg/mL) and filtered through a 0.22 µm Sterile Millex Filter to prepare a 500 µg/mL solution in RPMI. Prepared solution was added to adherent cells and incubated at 37 °C for 4 hours. Sterile DMSO was then
supplied upon removal of MTT solution. Relative cell viabilities were quantified using a plate reader at an absorbance of 570 nm.

Figure S2. Measuring cell viability of RAW 264.7 cells. Upon removal of media post-treatment, adherent cells were subjected to MTT cell viability assay. Data are means ± S.E.M with n = 3 per treatment. *p, < 0.05; **p, < 0.01 versus DMSO by one-way ANOVA.
Figure S3. Inhibition of Interleukin-6 (IL-6) by 9-PAHSA. Quantitation of IL-6 following a 20-hour treatment of 9-PAHSA at 25 μM. Data are means ± S.E.M with n = 3 per treatment group. ****p, < 0.0001 versus DMSO by one-way ANOVA.

Change “****p” to “**p”.

Figure S4. Inhibition of Interleukin-6 (IL-6) by 9-PAHSA. Quantitation of IL-6 following 20-hour treatments of 9-PAHSA. Higher treatment dosages of 9-PAHSA contributed to prominent cell death (data not shown) and failed to abolish IL-6 signaling effectively. Data are means ± S.E.M with n = 3 per treatment dosage group. All p values are ≤ 0.0001 unless indicated otherwise. *p, < 0.05 versus DMSO by one-way ANOVA.

Spectral Data
13-LAHGA (3)
15-LAHLA (5)
S-15-LAHLA (5-5)
R-MTPA-ester of 16-R

\[
\text{THPO} \quad \begin{array}{c}
\text{O} \\
\text{OMe} \\
\text{Me}
\end{array}
\]
R-MTPA-ester of 16-R
References