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SPECIAL FEATURE: BRIEF COMMUNICATION

Synthesis of chemically edited derivatives of the endogenous regulator of inflammation 9-PAHSA

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Abstract

Fatty acid esters of hydroxy fatty acids (FAHFAs) are a growing class of natural products found in organisms ranging from plants to humans. The roles these endogenous derivatives of fatty acids play in biology and their novel pathways for controlling inflammation have increased our understanding of basic human physiology. FAHFAs incorporate diverse fatty acids into their structures, however, given their recent discovery non-natural derivatives have not been a focus and as a result structure-activity relationships remain unknown. The importance of the long chain hydrocarbons extending from the ester linkage as they relate to anti-inflammatory activity is unknown. Herein the systematic removal of carbons from either the hydroxy fatty acid or fatty acid regions of the most studied FAHFA, palmitic acid ester of 9-hydroxystearic acid (9-PAHSA), was achieved and these synthetic, abridged analogs were tested for their ability to attenuate IL-6 production. Reduction of the carbon chain lengths of the 9-hydroxystearic acid portion or palmitic acid hydrocarbon chain resulted in lower molecular weight analogs that maintained anti-inflammatory activity or in one case enhanced activity.

Introduction

FAHFAs (Fatty Acid esters of Hydroxy Fatty Acids) are endogenous bioactive lipids with anti-diabetic and anti-inflammatory activities. FAHFAs were discovered and structurally elucidated through lipidomic analyses of adipose tissues (AT) obtained from transgenic mice over-expressing the glucose transporter 4 in adipose tissue (AG4OX). Though obese, AG4OX mice exhibited anti-diabetic characteristics, such as insulin sensitivity and glucose homeostasis when compared with their wild-type counterparts [1]. Further studies of AG4OX mice revealed that a class of lipid-based metabolites was differentially upregulated by more than 16-fold in their AT. Upon structural reconstruction based on the detected masses, fragmentations of these metabolites were derived from a novel class of natural lipids called FAHFAs, which had contributed to the favorable metabolic phenotypes in AG4OX mice. Structurally unique, a FAHFA (Fig. 1) is composed of a fatty acid chain and a hydroxyl fatty acid chain linked by an ester bond. Targeted liquid chromatography-mass spectrometry (LC-MS) found that at least 16 different FAHFA families exist, which consisted of four fatty acids and four hydroxy-fatty acids in different combinations [2]. For each family, multiple regioisomers can be found that differ in the site of the ester functional group at junctions of chains. For instance, palmitic acid esters of hydroxy stearic acids (PAHSAs) was one of the most abundant FAHFA families reported in which eight distinct PAHSA regioisomers (−5, −7, −8, −9, −10, −11, −12, and −13) were identified to be the most upregulated family members in adipose tissue of AG4OX mice [2].

Previous evidence has highlighted the therapeutic potential of FAHFAs in mediating symptoms of insulin resistance and inflammation. Compared with vehicle treated

This study is dedicated to Professor Samuel Danishefsky for his tireless efforts to advance the field of organic chemistry and his tremendous scientific contributions to the exploration of structurally complex, and biologically important, natural products.

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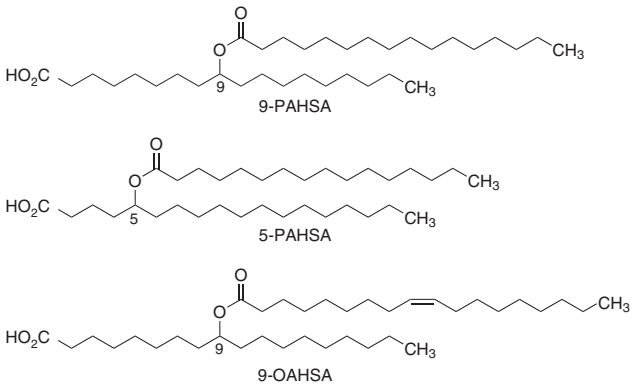


Fig. 1 Representative FAHFA structures; 9-PAHSA, 5-PAHSA, and 9-OAHSA

mice, acute oral administration of 5-PAHSA or 9-PAHSA improved glucose tolerance and enhanced insulin sensitivity in insulin-resistant mice on high-fat diet (HFD). Specifically, consecutive gavage of 9-PAHSA in HFD-fed mice demonstrated significant reduction in levels of proinflammatory cytokines, such as TNF and IL-1β in their AT macrophages. Treatment of LPS-stimulated bone-marrow-derived dendritic cells (BMDCs) with 9-PAHSA also inhibited BMDC maturation and abrogated expressions of cellular inflammation markers such as, CD80, CD86, CD40, and MHCII at a dose-dependent manner. In addition, a mouse colitis model was used to investigate the anti-inflammatory activities of 9-PAHSA and determine whether the effects were broadly applicable or disease specific. Mice with chemically induced colitis that had been treated with 9-PAHSA showed significant improvements in clinical outcomes, suggesting that the inflammation was remediated by the FAHFA treatment [3]. As demonstrated by Kuda et al. treatment of docosahexaenoic acid ester of 13-hydroxy linoleic acid (13-DHAHLA) could also inhibit the secretion of proinflammatory cytokines in LPS-induced mouse macrophage cell line RAW 264.7, and in fact, at a higher potency than 9-PAHSA [4]. Together, FAHFAs are general anti-inflammatory agents with varying degrees of activities that are modulated by their structural compositions. In this study, our goal is to delineate the structure-activity relationships in FAHFAs, specifically PAHSAs, and the anti-inflammatory abilities attributed to their lengths of carbon chains.

The lack of an understanding of the structural components needed for activity led to the syntheses and testing of synthetic 9-PAHSA analogs. We aim to investigate whether modifications in hydrocarbon chains can influence the analogs' abilities to mitigate inflammation following LPS stimulation in vitro. Preliminary data generated indicated that the *R* and *S* 9-PAHSA were equipotent in biological assays (glucose uptake and GPR40 activation) but were hydrolyzed at different rates in liver lysates [5]. The similarity in pharmacodynamic activity could arise through pseudosymmetry of FAHFAs where the fatty acid and the long alkyl chain extending after the ester linkage of the hydroxy-fatty acid would engender similar non-polar interactions. Herein we report the syntheses of 9-PAHSA analogs with varying lengths of the hydrocarbon chains through an efficient synthetic route and the analogs potential in modulating the secretion of a proinflammatory cytokine, IL-6, in LPS-stimulated RAW 264.7 cells.

Results and discussion

Chemistry

Synthesis of 9-PAHSA analogs through methylene editing

The synthesis of a set of 9-PAHSA analogs with differing carbon chain length in either the 9-hydroxystearic acid region after the ester linkage or in the palmitic acid fragment required a systematically applicable synthetic strategy. Previous syntheses of 9-PAHSA have been achieved and succeeded in providing the material needed for biological evaluation. In 2014 9-PAHSA was prepared in 11 steps starting from 1,9-nonanediol and the synthesis was pivotal in the early identification and biological characterization of FAHFAs, however, with this step count the initial strategy would not be applicable to the syntheses of a set of analogs (Fig. 2a) [2]. In 2017, an improved synthetic strategy was used to investigate the stereochemistry of endogenous 9-PAHSA (Fig. 2b) [5]. The use of nonracemic epichlorohydrin and two epoxide opening reactions with Grignard reagents proved successful in generating materials which were used to characterize endogenous 9-PAHSA. However, a further reduction of steps is required as removing even one step could have dramatic implications on the number of compounds needed to make a focused set of 9-PAHSA analogs. These syntheses and related syntheses of FAHFAs [4, 6–8] provided insight into route selection. To this goal a revised synthesis of 9-PAHSA and derivatives was achieved over three steps initiated by Grignard addition into methyl 9-oxononanoate (Fig. 2c).

To determine the importance of fatty acid and hydroxy fatty acid hydrocarbon within 9-PAHSA as it relates to anti-inflammatory activity methylenes were iteratively removed from the tail portions of palmitic acid and 9-hydroxy stearic acid (Fig. 3). Within 9-hydroxy stearic acid the carbons spanning the region between the carboxylic acid and ester linkage were left unperturbed. The syntheses of every analog began with the addition of Grignard reagents selectively into the aldehyde of methyl 9-oxononanoate [9, 10], affording different hydroxy-fatty acid methyl esters represented by **13** in good yields (Scheme 1). Esterification

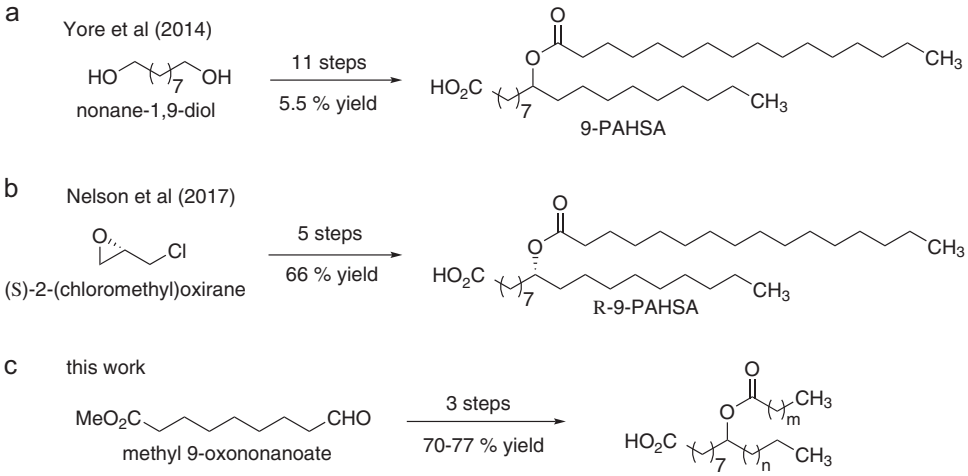


Fig. 2 9-PAHSA syntheses starting in 2014 to access material for biological evaluation

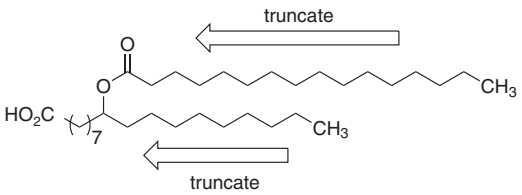


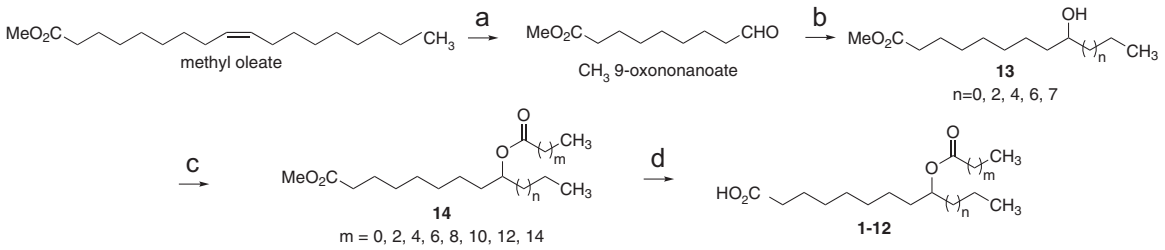
Fig. 3 Methylene deletion from 9-PAHSA to assess the importance of hydrocarbon

of secondary alcohol **13** with the required acid chloride and pyridine provided a FAHFA methyl ester **14** which when subjected to selective hydrolysis of more accessible methyl ester yielded the 9-PAHSA analogs **1–12** in relatively short order. Deletion of hydrocarbon from palmitoyl chloride (C16) down to acetyl chloride (C2) made abridged fatty acid ester derivatives of 9-PAHSA providing **6–12** using the methyl ester of 9-hydroxy stearic acid. More effort was required to access derivatives with excised carbon from 9-hydroxy stearic acid tail. Synthesis of 9-hydroxy stearic acid (C18), 9-hydroxy heptadecanoic acid (C17), 9-hydroxy pentadecanoic acid (C15), 9-hydroxy tridecanoic acid (C13), and 9-hydroxy undecanoic acid (C11) were all achieved to prepare abridged derivatives **1–4** and 9-PAHSA (**5**) (Fig. 4). Similarly, all the steps proceeded in good yield, providing the material for testing the IL-6 lowering capabilities of the analogs. Using this strategy 11 analogs and 9-PAHSA were synthesized through a total of 17 intermediates.

Biology

Interleukin-6 (IL-6) is a proinflammatory cytokine with important roles in both innate and adaptive immune responses and functions as the major mediator during the acute phase of inflammation. Elevated levels of IL-6 have been implicated in chronic inflammatory conditions such as rheumatoid arthritis and cancer [11]. The rationale behind selecting IL-6 as an inflammatory marker in our experiments is that previous knowledge about FAHFAs, specifically 9-PAHSA and DHAHLA, and their abilities to regulate IL-6 secretion in vitro has been established [3, 4]. Based on previous studies, we sought to understand the magnitude of the anti-inflammatory abilities of synthetic 9-PAHSA analogs and whether the activities were correlative to the number of hydrocarbons present in the structure. To quantitate the anti-inflammatory capabilities of these analogs, RAW 264.7 cells were stimulated with DMSO or LPS (100 ng/mL), an endotoxin derived from Gram-negative bacteria. Simultaneously, LPS-stimulated cells were treated with synthetic analogs at either 25 or 50 μM for 20 h before the culture media were collected and subjected to IL-6 quantification using a mouse IL-6 enzyme-linked immunosorbent assay, or ELISA (Fig. 5) (for 50 μM data see supporting information). To monitor potential cytotoxic effects from the treatment, adherent cells were tested for viability after media removal using an MTT assay (see supporting information).

Reduction of the chain length of the 9-hydroxystearic acid region of 9-PAHSA resulted analogs **1**–**4** that approximately maintained the anti-inflammatory activity of 9-PAHSA. Similar activity could be obtained by editing of the palmitic acid chain of 9-PAHSA with the effect maximized by replacement of palmitic acid with hexanoic acid, compound **10**, that shows statistical improvement in activity compared to 9-PAHSA (**5**) (Student's *t*-test, $\#p < 0.05$, compared to 9-PAHSA). This provides insight into the structure-activity relationships of 9-PAHSA and additional assessment of anti-inflammatory activities of the analogs in the future will help statistically resolve the effects of the bio-similar analogs relative to 9-PAHSA (**5**) given the background of the IL-6 measurements.



Scheme 1 Optimized syntheses of truncated 9-PAHSA analogs and 9-PAHSA starting from methyl 9-oxononanate. [a]Conditions: (**a**) O_3, NMO, DCM, –78 °C, 93% yield; (**b**) alkyl-magnesium bromide, THF, –78 °C, 85–90% yield; (**c**) acyl chloride, pyr., CH_2Cl_2, 0 °C to 23 °C, 90–95% yield; (**d**) LiOH·H_2O, THF:H_2O (1:1, v/v), 0 °C to 23 °C, 88–93% yield.

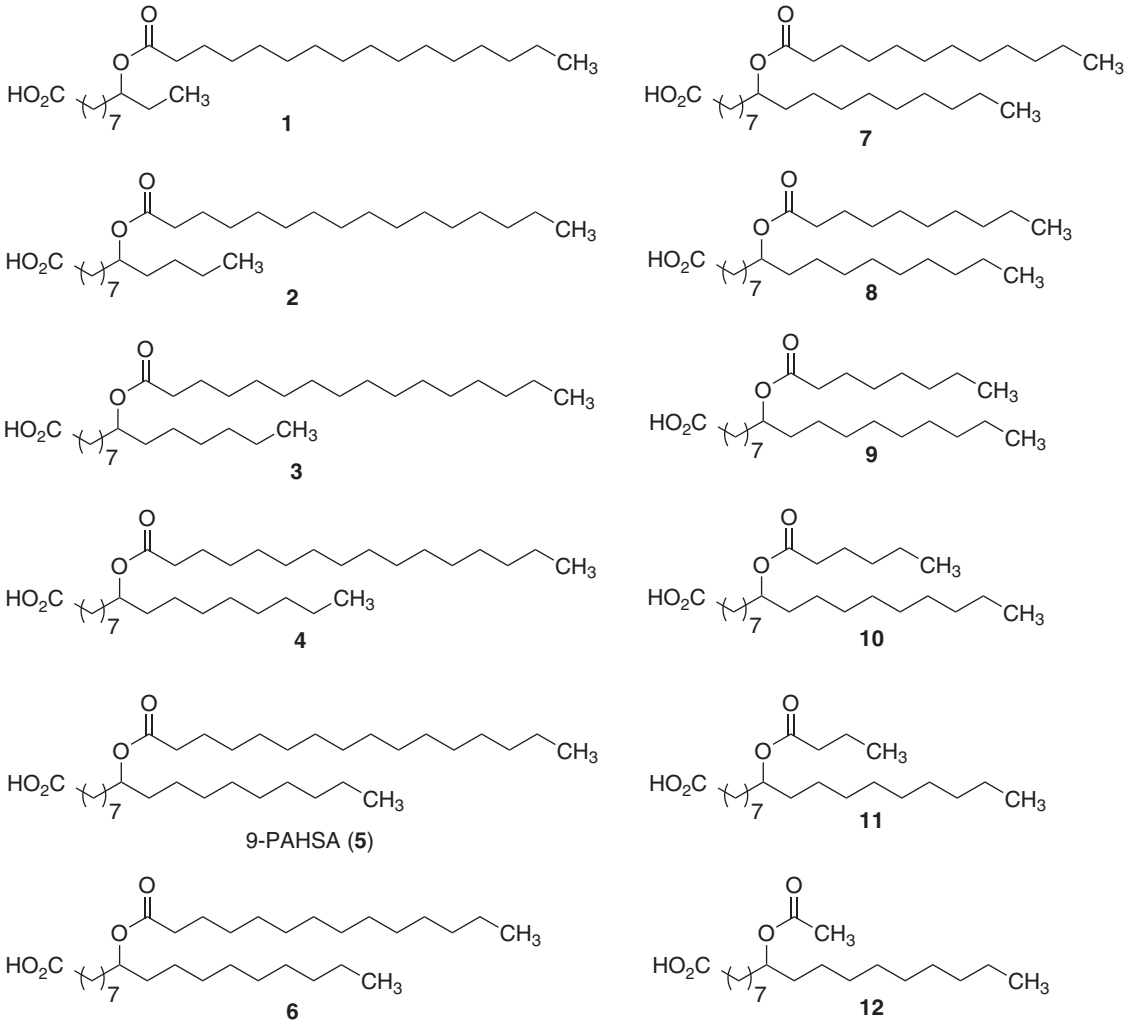


Fig. 4 9-PAHSA and abridged analogs prepared from methyl 9-oxononanate possessing different hydrocarbon in the palmitic acid or 9-hydroxy stearic acid region of 9-PAHSA

Conclusion

The lack of structure-activity relationships for FAHFAs prompted the syntheses and testing the 9-PAHSA variants with methylene editing to determine the specific contribution of two region of hydrocarbon (fatty acid ester chain and hydroxy stearic acid chain) to bioactivity. The synthetic strategy used provided access to 11 truncate 9-PAHSA analogs and the parent compound in sufficient quantities to test for the compounds' abilities to reduce inflammation. Treatment of RAW 264.7 cells with LPS induced an inflammatory response. Subsequent treatment with

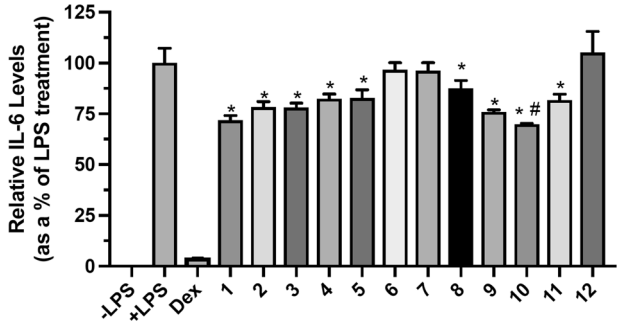


Fig. 5 Testing methylene edited FAHFAs for anti-inflammatory activities. Relative IL-6 levels were quantitated after LPS-stimulated RAW 264.7 cells were co-treated with synthetic 9-PAHSA analogs for 20 h. As a positive control, cells were treated with 25 μM of Dexamethasone, a glucocorticoid used in the clinic as an anti-inflammatory medication. Methylene edited FAHFAs were tested with most lipids showing a reduction in IL-6 levels compared to LPS treated cells (Student's t-test compared to LPS control, *p-value < 0.05) furthermore compound **10** shows statistical improvement in activity compared to 9-PAHSA (**5**) (Student's t-test, #p < 0.05, compared to 9-PAHSA)

9-PAHSA analogs and assessment of the resulting IL-6 levels provided a measurement of the anti-inflammatory activity of each of the compounds. The removal of hydrocarbon was successful in producing analogs that maintained anti-inflammatory activity while also reducing the molecular weight. One compound, **10**, displayed statistically improved activity relative to 9-PAHSA demonstrating the potential importance of this approach. The finding that the reduction of the chain length of 9-hydroxystearic acid of 9-PAHSA and editing of the palmitic acid chain results in active compounds provides avenues for optimization of 9-PAHSA and other FAHFAs. In addition, continued assessment of this series of compounds in related assays evaluating anti-inflammatory activity will provide refinement on the importance of the hydrocarbons of 9-PAHSA as it relates to anti-inflammatory effects.

Experimental

Synthesis of methyl 9-oxononanoate

4-Methylmorpholine *N*-oxide monohydrate (5.9 g, 50.6 mmol, 3.0 equiv) was dehydrated by heating at 90 °C under high vacuum overnight. Following the ozonolysis conditions of Drussault [12], a 250 mL round-bottom flask equipped with stir bar was charged with methyl oleate (5.0 g, 16.9 mmol, 1.0 equiv), the anhydrous 4-methylmorpholine *N*-oxide monohydrate prepared above, and anhydrous DCM (100 mL). Stirring was initiated, affording a clear solution. The reaction mixture was cooled to –78 °C for 15 min before bubbling in a mixture of ozone/oxygen. Conversion was complete within 10 min. The ozone generator was turned off and oxygen was bubbled through the solution for additional 10 min. The reaction mixture was allowed to warm to room temperature and aged for 2 h. The reaction was concentrated and the residue was dissolved in ethyl acetate (200 mL) and washed with water (80 mL) and brine (50 mL), dried over sodium sulfate, filtered, and concentrated to give a yellow oil. The oil was purified by chromatography on silica gel (hexane:EtOAc 2:1) to provide methyl 9-oxononanoate (2.9 g, 93% yield) as a colorless oil. The spectral data were same with the one in published literature [9, 10].

General procedure for preparation of derivatives of the methyl ester of 9-hydroxy stearic acid (13)

To a stirred solution of methyl 9-oxononanoate [9] (500 mg, 2.7 mmol, 1.0 equiv) in dry THF (50 mL) was added the selected Grignard reagent (4.0 mmol, 1.5 equiv) under a nitrogen atmosphere at –78 °C. The reaction mixture was stirred for 1 h at –78 °C and then saturated aqueous NH_4Cl solution (10 mL) was added in a single portion. The resulting mixture was warmed to 23 °C and repeatedly extracted with EtOAc (20 mL × 3). The combined organic extracts were washed with brine (15 mL), dried over sodium sulfate, filtered, and concentrated. The crude compound was purified via careful silica gel column chromatography using hexane: EtOAc to give the esters **13a-e**. All the Grignard reagents we used are shown below and commercially available, ethylmagnesium bromide solution (C_2H_5BrMg, 1.0 M in THF), butylmagnesium chloride solution (C_4H_9ClMg, 2.0 M in THF), hexylmagnesium bromide solution ($C_6H_{13}BrMg$, 0.8 M in THF), octylmagnesium bromide solution ($C_8H_{17}BrMg$, 1.0 M in diethyl ether) and nonylmagnesium bromide solution ($C_9H_{19}BrMg$, 1.0 M in diethyl ether).

Methyl 9-hydroxyundecanoate (**13a**): R_f = 0.2 (silica gel, 60:10 hexanes:EtOAc, $KMnO_4$); 522 mg, 90% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (s, 3 H), 3.50 (m, 1H), 2.29 (t, J = 7.6 Hz, 2 H), 1.65–1.55 (m, 2 H), 1.54–1.35 (m, 4 H), 1.30 (m, 8 H), 0.92 (t, J = 7.5 Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 73.4, 51.6, 37.0, 34.2, 30.3, 29.6, 29.3, 29.2, 25.7, 25.0, 10.0; IR (film, cm^{-1}): 2928, 2855, 1738, 1460, 1436, 1245, 1197, 1171; HRMS (ESI) calc. for $C_{12}H_{24}O_3Na$ $[M + Na]^+$: 239.1618, obs. 239.1619.

Methyl 9-hydroxytridecanoate (**13b**): R_f = 0.2 (silica gel, 60:10 hexanes:EtOAc, $KMnO_4$); 557 mg, 85% yield, light yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.64 (s, 3H), 3.55 (m, 1H), 2.27 (t, J = 7.5 Hz, 2 H), 1.59 (m, 2H), 1.47–1.34 (m, 4 H), 1.34–1.21 (m, 12 H), 0.88 (t, J = 6.9 Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 72.0, 51.6, 37.5, 37.3, 34.2, 29.6, 29.3, 29.2, 28.0, 25.7, 25.0, 22.9, 14.2; IR (film, cm^{-1}): 2928, 2856, 1740, 1436, 1249, 1197, 1171; HRMS (ESI) calc. for $C_{14}H_{28}O_3Na$ $[M + Na]^+$: 267.1931, obs. 267.1933.

Methyl 9-hydroxypentadecanoate (**13c**): $R_f = 0.3$ (silica gel, 60:10 hexanes:EtOAc, $KMnO_4$); 643 mg, 88% yield, light yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.66 (s, 3H), 3.58 (m, 1H), 2.30 (td, $J = 7.6$, 2.1 Hz, 2 H), 1.61 (m, 2H), 1.48–1.35 (m, 4 H), 1.29 (t, $J = 9.0$ Hz, 16 H), 0.88 (td, $J =$ 6.9, 2.6 Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 72.1, 51.6, 37.6, 37.5, 34.2, 32.0, 29.6, 29.5, 29.4, 29.2, 25.8, 25.7, 25.1, 22.8, 14.3. IR (film, cm^{-1}): 2926, 2854, 1740, 1458, 1436, 1197, 1171; HRMS (ESI) calc. for $C_{16}H_{32}O_3Na$ $[M + Na]^+$: 295.2244, obs. 295.2243.

Methyl 9-hydroxyheptadecanoate (**13d**): $R_f = 0.2$ (silica gel, 70:10 hexanes:EtOAc, $KMnO_4$); 693 mg, 86% yield; white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.64 (s, 3 H), 3.55 (m, 1H), 2.28 (t, $J = 7.6$ Hz, 2 H), 1.60 (m, 2H), 1.44–1.33 (m, 4 H), 1.26 (m, 20H), 0.86 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 72.0, 51.6, 37.6, 37.5, 34.2, 32.0, 29.8, 29.7, 29.6, 29.4, 29.3, 29.2, 25.8, 25.7, 25.0, 22.8, 14.2; IR (film, cm^{-1}): 2926, 2853, 1740,1436, 1197, 1171; HRMS (ESI) calc. for $C_{18}H_{36}O_3Na$ $[M + Na]^+$: 323.2557, obs. 323.2558.

Methyl 9-hydroxyoctadecanoate (**13e**): $R_f = 0.2$ (silica gel, 70:10 hexanes:EtOAc, $KMnO_4$); 751 mg, 89% yield; white solid; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (s, 3 H), 3.56 (m, 1H), 2.29 (t, $J = 7.6$ Hz, 2 H), 1.60 (m, 2H), 1.40 (m, 4H), 1.28 (m, 22H). 0.86 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 72.1, 51.6, 37.6, 37.5, 34.2, 32.1, 29.8, 29.7, 29.6, 29.5, 29.3, 29.2, 25.8, 25.7, 25.0, 22.8, 14.3; IR (film, cm^{-1}): 2917, 2848, 1740, 1436, 1173; HRMS (ESI) calc. for $C_{19}H_{38}O_3Na$ $[M + Na]^+$: 337.2713, obs. 337.2713.

General procedure for the preparation the methyl ester of 9-PAHSA and analogous derivatives 14

To a stirred solution of 9-hydroxyl methyl ester **13a-e** (0.36 mmol, 1.0 equiv) in dry dichloromethane (15 mL) was added neat, dry pyridine (1.8 mmol, 5.0 equiv) at 0 °C and the solution was stirred for 15 min. Neat acyl chloride (0.43 mmol, 1.2 equiv) was added to the reaction mixture. The reaction was allowed to warm to 23 °C and stirred 12 h. The unreacted acyl chloride was quenched with saturated aqueous ammonium chloride (10 mL). The mixture was extracted with dichloromethane (10 mL × 3). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The crude product was purified via silica gel column chromatography using hexane:EtOAc to give the methyl esters of 9-PAHSA derivatives **14a–l**.

11-Methoxy-11-oxoundecan-3-yl palmitate (**14a**): $R_f =$ 0.7 (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 155 mg, 95% yield, colorless oil;^{1}H NMR (600 MHz, $CDCl_3$) δ 4.78 (m, 1H), 3.63 (s, 3 H), 2.26 (m, 4H), 1.58 (m, 4H), 1.54–1.44 (m, 4 H), 1.29 (m, 32 H), 0.85 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3, 173.8, 75.2, 51.5, 34.8, 34.1, 33.7, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.1, 25.4, 25.3, 25.0, 22.8, 14.2, 9.7; IR (film, cm^{-1}): 2923, 2853, 1733, 1463, 1246, 1172; HRMS (ESI) calc. for $C_{28}H_{55}O_4$ $[M + H]^+$: 455.4095, obs. 455.4095.

13-Methoxy-13-oxotridecan-5-yl palmitate (**14b**): $R_f =$ 0.7 (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 160 mg, 92% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.87 (m, 1H), 3.66 (s, 3 H), 2.29 (m, 4H), 1.67–1.58 (m, 4 H), 1.55–1.47 (m, 4 H), 1.29 (s, 36 H), 0.88 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.3, 173.7, 74.0, 51.5, 34.8, 34.2, 34.1, 34.0, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.6, 25.3, 25.0, 22.8, 22.7, 14.2, 14.1; IR (film, cm^{-1}): 2923, 2853, 1733, 1465, 1172; HRMS (ESI) calc. for $C_{30}H_{59}O_4$ $[M + H]^+$: 483.4408, obs. 483.4405.

15-Methoxy-15-oxopentadecan-7-yl palmitate (**14c**): $R_f = 0.6$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 174 mg, 95% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.89–4.83 (m, 1 H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.60 (m, 4H), 1.49 (m, 4H), 1.28 (m, 40H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.9, 74.1, 51.6, 34.9, 34.3, 34.2, 32.1, 32.0, 29.9, 29.8, 29.7, 29.5, 29.3, 29.2, 25.5, 25.4, 25.3, 25.0, 22.8, 14.3; IR (film, cm^{-1}): 2922, 2853, 1733, 1465, 1170; HRMS (ESI) calc. for $C_{32}H_{62}O_4Na$ $[M + Na]^+$: 533.4540, obs. 533.4536.

Methyl 9-(palmitoyloxy)heptadecanoate (**14d**): $R_f = 0.6$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 180 mg, 93% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.84 (m, 1H), 3.64 (s, 3 H), 2.27 (m, 4H), 1.59 (m, 4H), 1.53–1.43 (m, 4 H), 1.28 (s, 44 H), 0.86 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.8, 74.1, 51.6, 34.8, 34.3, 32.1, 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 25.4, 25.3, 25.0, 22.8, 14.3, 14.2; IR (film, cm^{-1}): 2922, 2853, 1733, 1465, 1170; HRMS (ESI) calc. for $C_{34}H_{66}O_4Na$ $[M + Na]^+$: 561.4853, obs. 561.4849.

Methyl 9-(palmitoyloxy)octadecenoate (**14e**): $R_f = 0.6$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 188 mg, 95% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.85 (m, 1H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.60 (m, 4H), 1.49 (m, 4H), 1.30–1.18 (m, 46 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.9, 74.1, 51.6, 34.9, 34.3, 34.2, 32.1, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.2, 25.4, 25.3, 25.0, 22.8, 22.7, 14.3; IR (film, cm^{-1}): 2922, 2853, 1733, 1465, 1171; HRMS (ESI) calc. for $C_{35}H_{68}O_4Na$ $[M + Na]^+$: 575.5010, obs. 575.5006.

Methyl 9-(tetradecanoyloxy)octadecenoate (**14 f**): $R_{f=}$ 0.7 (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 172 mg, 91% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.89–4.82 (m, 1 H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.66–1.57 (m, 4 H), 1.49 (m, 4H), 1.30–1.20 (m, 42 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.9, 74.2, 51.7, 34.9, 34.3, 34.2, 32.1, 32.0, 29.8, 29.7, 29.5, 29.3, 29.2, 25.4, 25.3, 25.1, 22.8, 14.3; IR (film, cm^{-1}): 2922, 2853, 1733, 1464, 1170; HRMS (ESI) calc. for $C_{33}H_{64}O_4Na$ $[M + Na]^+$: 547.4697, obs. 547.4692.

Methyl 9-(dodecanoyloxy)octadecenoate (**14 g**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 168 mg, 94% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.85 (m, 1H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.60 (m, 4H), 1.49 (m, 4H), 1.25 (m, 38H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.9, 74.1, 51.6, 34.9, 34.3, 34.2, 32.1, 32.0, 29.8, 29.7, 29.5, 29.3, 29.2, 25.5, 25.4, 25.3, 25.1, 22.8, 14.3; IR (film, cm^{-1}): 2922, 2853, 1733, 1465, 1169; HRMS (ESI) calc. for $C_{31}H_{60}O_4Na$ $[M + Na]^+$: 519.4384, obs. 519.4379.

Methyl 9-(decanoyloxy)octadecenoate (**14 h**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 156 mg, 93% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.85 (m, 1H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.61 (m, 4H), 1.49 (m, 4H), 1.29 (s, 34 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 173.9, 74.1, 51.6, 34.9, 34.3, 34.2, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 25.5, 25.4, 25.3, 25.1, 22.8, 14.2. IR (film, cm^{-1}): 2924, 2855, 1734, 1465, 1170; HRMS (ESI) calc. for $C_{29}H_{57}O_4$ $[M + H]^+$: 469.4251, obs. 469.4244.

Methyl 9-(octanoyloxy)octadecenoate (**14i**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 150 mg, 95% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 3.66 (s, 3 H), 2.28 (m, 4H), 1.60 (m, 4H), 1.48 (m, 4H), 1.32–1.21 (m, 30 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 173.9, 74.2, 51.7, 34.9, 34.3, 34.2, 32.0, 31.9, 29.7, 29.5, 29.3, 29.2, 29.1, 25.5, 25.4, 25.3, 25.1, 22.8, 14.3, 14.2; IR (film, cm^{-1}): 2924, 2854, 1732, 1463, 1168; HRMS (ESI) calc. for $C_{27}H_{53}O_4$ $[M + H]^+$: 441.3938, obs. 441.3936.

Methyl 9-(hexanoyloxy)octadecenoate (**14j**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 133 mg, 90% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.91–4.80 (m, 1 H), 3.65 (s, 3 H), 2.28 (m, 4H), 1.60 (m, 4H), 1.53–1.45 (m, 4 H), 1.31–1.22 (m, 26 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.4, 173.9, 74.1, 51.6, 34.8, 34.3, 34.2, 32.0, 31.5, 29.7, 29.5, 29.3, 29.2, 25.5, 25.4, 25.0, 22.8, 22.5, 14.3, 14.1; IR (film, cm^{-1}): 2925, 2855, 1732, 1463, 1171; HRMS (ESI) calc. for $C_{25}H_{49}O_4$ $[M + H]^+$: 413.3625, obs. 413.3625.

Methyl 9-(butyryloxy)octadecenoate (**14k**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 127 mg, 92% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 3.66 (s, 3 H), 2.27 (m, 4H), 1.63 (m, 4H), 1.49 (m, 4H), 1.28 (m, 22H), 0.94 (t, $J = 7.4$ Hz, 3 H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 173.7, 74.2, 51.6, 36.8, 34.3, 34.2, 32.0, 29.7, 29.5, 29.3, 29.2, 25.5, 25.4, 25.0, 22.8, 14.3, 13.9; IR (film, cm^{-1}): 2926, 2855, 1733, 1459, 1182; HRMS (ESI) calc. for $C_{23}H_{45}O_4$ $[M + H]^+$: 385.3312, obs. 385.3308.

Methyl 9-acetoxyoctadecanoate (**14 l**): $R_f = 0.7$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 124 mg, 95% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.89–4.79 (m, 1 H), 3.66 (s, 3 H), 2.29 (m, 2H), 2.03 (s, 3 H), 1.62 (m, 2H), 1.49 (m, 4H), 1.27 (m, 22H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.5, 171.1, 74.6, 51.6, 34.3, 34.2, 32.0, 29.7, 29.5, 29.3, 29.2, 25.5, 25.4, 25.0, 22.8, 21.5, 14.3; IR (film, cm^{-1}): 2925, 2855, 1737, 1436, 1240; HRMS (ESI) calc. for $C_{21}H_{41}O_4$ $[M + H]^+$: 357.2999, obs. 357.2996.

General procedure for preparation 9-PAHSA and truncated 9-PAHSA analogs

The methyl esters **14a–l** (0.22 mmol) were dissolved in THF: H_2O (5 mL: 5 mL) and cooled to 0 °C for 15 min before adding solid LiOH (1.1 mmol, 5 equiv) in one portion. The reaction was allowed to warm to 23 °C and stirred 12 h. Excess hydroxide was quenched with 1 N HCl. The product was extracted with ethyl acetate (5 mL × 3) and combined organic extracts were washed with brine (5 mL), dried over sodium sulfate, filtered, and concentrated. The crude products were purified by silica gel column chromatography, eluting with hexanes:EtOAc to yield compounds **1–12**.

9-(Palmitoyloxy)undecanoic acid (**1**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 87 mg, 90% yield, white solid; ^1H NMR (600 MHz, $CDCl_3$) δ 4.84–4.77 (m, 1 H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.28 (t, $J = 7.5$ Hz, 2 H), 1.62 (m, 4H), 1.58–1.47 (m, 4 H), 1.31–1.23 (m, 32 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.3, 174.0, 75.3, 34.8, 34.2, 33.7, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 27.1, 25.4, 25.3, 24.7, 22.8, 14.3, 9.7; IR (film, cm^{-1}): 2926, 2854, 1930, 1711, 1465; HRMS (ESI) calc. for $C_{27}H_{51}O_4$ $[M–H]^-$: 439.3793, obs. 439.3790.

9-(Palmitoyloxy)tridecanoic acid (**2**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 91 mg, 89% yield, white solid; ^1H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.33 (t, $J = 7.5$ Hz, 2 H), 2.27 (t, $J = 7.4$ Hz, 2 H), 1.60 (m, 4H), 1.52–1.47 (m, 4 H), 1.27 (m, 36H), 0.89–0.85 (m, 6 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.1, 174.0, 74.1, 34.9, 34.3, 34.2, 34.0, 32.1, 29.8, 29.7, 29.5, 29.3, 29.1, 27.6, 25.4, 25.3, 24.8, 22.9, 22.8, 14.3, 14.2; IR (film, cm^{-1}): 2925, 2855, 1931, 1709, 1465; HRMS (ESI) calc. for $C_{29}H_{55}O_4$ $[M–H]^-$: 467.4106, obs. 467.4102.

9-(Palmitoyloxy)pentadecanoic acid (**3**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 100 mg, 92% yield, colorless oil; ^1H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.27 (t, $J = 7.5$ Hz, 2 H), 1.61 (m, 4H), 1.53–1.47 (m, 4 H), 1.31–1.22 (m, 40 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.6, 173.9, 74.2, 34.9, 34.3, 33.9, 32.1, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3; IR (film, cm^{-1}): 2925, 2854, 1930, 1710, 1465; HRMS (ESI) calc. for $C_{31}H_{59}O_4$ $[M–H]^-$: 495.4419, obs. 495.4414.

9-(Palmitoyloxy)heptadecanoic acid (**4**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 106 mg, 92% yield,

colorless oil; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 4.86 (m, 1H), 2.34 (d, $J = 7.8$ Hz, 2 H), 2.27 (t, $J = 7.5$ Hz, 2 H), 1.62 (m, 4H), 1.50 (m, 4H), 1.29 (m, 44H), 0.88 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.5, 174.0, 74.2, 34.9, 34.3, 32.1, 32.0, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3. IR (film, cm^{-1}): 2922, 2853, 1732, 1709, 1465, 1377, 1174; HRMS (ESI) calc. for $C_{33}H_{63}O_4$ [M–H]$^-$: 523.4732, obs. 523.4727.

9-(Palmitoyloxy)octadecanoic acid (**5**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 108 mg, 92% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.92–4.80 (m, 1 H), 2.31 (dt, $J = 38.9$, 7.7 Hz, 4 H), 1.61 (m, 4H), 1.50 (m, 4H), 1.27 (m, 46H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.7, 174.0, 74.2, 35.0, 34.4, 34.2, 32.0, 30.0, 29.9, 29.8, 29.6, 29.5, 29.4, 29.2, 25.5, 25.4, 24.9, 22.9, 22.8, 14.4, 14.3; IR (film, cm^{-1}): 2917, 2848, 1733, 1714, 1470, 1156; HRMS (ESI) calc. for $C_{34}H_{65}O_4$ [M–H]$^-$: 537.4888, obs. 537.4887.

9-(Tetradecanoyloxy)octadecanoic acid (**6**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 100 mg, 89% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.89–4.83 (m, 1 H), 2.34 (td, $J = 7.5$, 3.1 Hz, 2 H), 2.27 (td, $J = 7.6$, 3.1 Hz, 2 H), 1.61 (m, 4H), 1.49 (m, 4H), 1.28 (m, 42H), 0.87 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.9, 174.0, 74.2, 34.9, 34.3, 34.1, 32.1, 29.8, 29.7, 29.5, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3; IR (film, cm^{-1}): 2923, 2854, 1733, 1710, 1465, 1177; HRMS (ESI) calc. for $C_{32}H_{61}O_4$ [M–H]$^-$: 509.4575, obs. 509.4572.

9-(Dodecanoyloxy)octadecanoic acid (**7**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 95 mg, 90% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.33 (td, $J = 7.6$, 1.5 Hz, 2 H), 2.27 (td, $J = 7.5$, 1.5 Hz, 2 H), 1.61 (m, 4H), 1.50 (m, 4H), 1.26 (m, 38 H), 0.87 (m, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.2, 174.0, 74.2, 34.9, 34.3, 34.2, 32.1, 32.0, 29.8, 29.7, 29.5, 29.4, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3; IR (film, cm^{-1}): 2923, 2854, 1433, 1709, 1465, 1174; HRMS (ESI) calc. for $C_{30}H_{57}O_4$ [M–H]$^-$: 481.4266, obs. 481.4260.

9-(Decanoyloxy)octadecanoic acid (**8**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 88 mg, 88% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.27 (t, $J = 7.5$ Hz, 2 H), 1.62 (m, 4H), 1.49 (m, 4H), 1.29 (m, 34H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.7, 173.9, 74.2, 34.9, 34.3, 33.9, 32.1, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3; IR (film, cm^{-1}): 2925, 2855, 1733, 1710, 1465, 1106; HRMS (ESI) calc. for $C_{28}H_{53}O_4$ [M–H]$^-$: 453.3949, obs. 453.3948.

9-(Octanoyloxy)octadecanoic acid (**9**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 85 mg, 91% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.28 (t, $J = 7.5$ Hz, 2 H), 1.61 (m, 4H), 1.54–1.46 (m, 4 H), 1.32–1.21 (m, 30 H), 0.87 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.5, 174.0, 74.2, 34.9, 34.3, 34.0, 32.0, 31.9, 29.7, 29.5, 29.3, 29.1, 25.5, 25.4, 25.3, 24.8, 22.8, 14.3, 14.2; IR (film, cm^{-1}): 2924, 2855, 1732, 1709, 1464, 1169; HRMS (ESI) calc. for $C_{26}H_{49}O_4$ [M–H]$^-$: 425.3636, obs. 425.3632.

9-(Hexanoyloxy)octadecanoic acid (**10**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 81 mg, 93% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.86 (m, 1H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.28 (t, $J = 7.5$ Hz, 2 H), 1.62 (m, 4H), 1.54–1.46 (m, 4 H), 1.32–1.22 (m, 26 H), 0.88 (m, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.2, 174.0, 74.2, 34.8, 34.3, 34.2, 32.0, 31.5, 29.7, 29.4, 29.3, 29.1, 25.4, 25.0, 24.7, 22.8, 22.5, 14.2, 14.1; IR (film, cm^{-1}): 2925, 2855, 1732, 1709, 1464, 1174; HRMS (ESI) calc. for $C_{24}H_{45}O_4$ [M–H]$^-$: 397.3323, obs. 397.3321.

9-(Butyryloxy)octadecanoic acid (**11**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 73 mg, 90% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.89–4.84 (m, 1 H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.26 (t, $J = 7.4$ Hz, 2 H), 1.63 (m, 4H), 1.54–1.46 (m, 4 H), 1.27 (m, 22H), 0.94 (t, $J = 7.4$ Hz, 3 H), 0.87 (t, $J = 7.0$ Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.8, 173.8, 74.2, 36.8, 34.3, 34.1, 32.0, 29.7, 29.5, 29.4, 29.3, 29.1, 25.5, 25.4, 24.8, 22.8, 18.8, 14.3, 13.9; IR (film, cm^{-1}): 2926, 2855, 1733, 1709, 1465, 1184; HRMS (ESI) calc. for $C_{22}H_{41}O_4$ [M–H]$^-$: 369.3010, obs. 369.3008.

9-Acetoxyoctadecanoic acid (**12**): $R_f = 0.2$ (silica gel, 80:20 hexanes:EtOAc, $KMnO_4$); 66 mg, 88% yield, colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.88–4.82 (m, 1 H), 2.34 (t, $J = 7.5$ Hz, 2 H), 2.04 (s, 3 H), 1.62 (m, 2H), 1.50 (m, 4H), 1.27 (m, 22H), 0.87 (t, $J = 7.0$ Hz, 3 H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 180.0, 171.2, 74.6, 34.3, 34.2, 34.1, 32.0, 29.7, 29.4, 29.3, 29.1, 25.5, 25.4, 24.8, 22.8, 21.5, 14.3; IR (film, cm^{-1}): 2926, 2855, 1737, 1710, 1464, 1242; **HRMS** (ESI) calc. for $C_{20}H_{37}O_4$ [M–H]$^-$: 341.2697, obs. 341.2698.

Biology

Culturing

RAW 264.7 cells were cultured in RPMI 1640 (Gibco), supplemented with L-glutamine (2 mM), 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. Adherent cells were then treated with DMSO or with LPS (100 ng/mL) and individual compounds at 25 μM or 50 μM. Supernantants were collected 20 h post-treatment and subjected to IL-6

quantification using mouse IL-6 ELISA MAX™ Deluxe Kits (BioLegend). Adherent cells were subjected to MTT assay to measure cell viabilities.

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 h. 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.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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