

Stereochemistry of Endogenous Palmitic Acid Ester of 9-Hydroxystearic Acid and Relevance of Absolute Configuration to Regulation

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SUPPORTING INFORMATION

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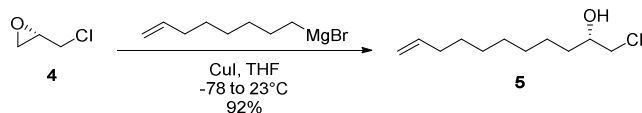
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I. General Information

All reactions were performed in flame- or 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), diethyl ether (Et₂O), and dichloromethane (DCM) were purified with a Pure-Solve MD-5 Solvent Purification System (Innovative Technology). 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. Optical rotations were measured on a JASCO P-2000 polarimeter. Melting points were measured with a MEL-TEMP apparatus without correction. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian 600 MHz (¹H at 600 MHz, ¹³C at 151 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, q = quartet, dd = doublet of doublets, ddd = doublet of doublet of doublets, 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]⁺, [M-H]⁺, or [M-Cl]⁺.

II. General Synthetic Procedures

Grignard addition to epichlorohydrin

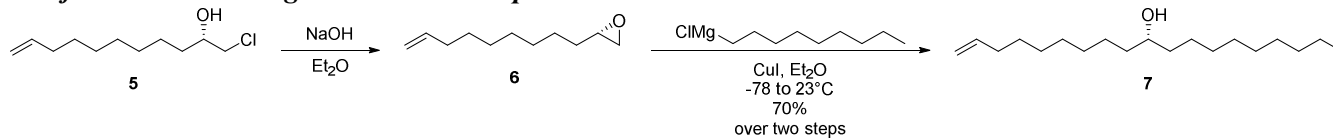


A 250 mL round-bottom flask equipped with stir bar and charged with magnesium turnings (2.96 g, 122 mmol, 3.75 eq) was flame dried under vacuum, cooled to ambient temperature, flushed with nitrogen, and sealed with a rubber septum. Dry THF (66 mL, 2/3 total volume) was added by syringe. Stirring was initiated, affording a heterogeneous mixture of clear solvent and metal turnings. A solution of DIBAL in toluene (0.27 mL, 1.2 M, 0.32 mmol, 0.01 eq) was added via syringe to activate the magnesium turnings. The reaction vessel was submerged in an oil bath, warmed to 35°C, and aged for 5 minutes before adding 8-bromo-1-octene (8.16 mL, 48.6 mmol, 1.5 eq) dropwise over 30 minutes by syringe pump. The reaction turned increasingly brown over the course of addition and the temperature rose to 41°C. After addition was complete, the vessel was warmed to 50°C before being allowed to cool to ambient temperature. The reaction was stirred for an additional 2 hours. A second 250 mL round-bottom flask equipped with stir bar and charged with copper (I) iodide (0.62 g, 3.24 mmol, 0.10 eq) was flame dried under vacuum, cooled to ambient temperature, flushed with nitrogen, sealed with a rubber septum, and maintained under a nitrogen atmosphere. Dry THF (33 mL, 1/3 total volume) and *S*-(-)-epichlorohydrin **4** (2.54 mL, 32.4 mmol, 1 eq) were added by syringe, giving a white, opaque mixture. The reaction vessel was submerged in a dry ice-acetone bath and cooled for 15 minutes. The Grignard reagent prepared above was drawn up in a syringe and added dropwise via syringe pump over 1 hour to the cooled mixture of *S*-(-)-epichlorohydrin **4**. The reaction was allowed to warm to ambient temperature overnight and the color changed to a deep, dark blue. The reaction was carefully quenched with 100 mL saturated aqueous ammonium chloride. The mixture was stirred vigorously until the color changed to a brilliant, royal blue. The mixture was transferred to a separatory funnel and extracted with a mixture of ethyl ether:hexanes (50:50, 50 mL x 3). Combined organics were washed with saturated aqueous ammonium chloride (50 mL x 2), brine (50 mL), dried (sodium sulfate), filtered, and concentrated to give a crude, slightly yellow oil. The residue was purified by silica gel column chromatography, eluting with hexanes:EtOAc (95:5 to 90:10) to afford *S*-**5** (6.11 g, 92%) as a clear oil.

***S*-1-chloroundec-10-en-2-ol 5**

R_f = 0.29 (silica gel, 90:10 hexanes:EtOAc, KMnO_4); $^1\text{H-NMR}$ (600 MHz, CDCl_3): δ 5.81 (m, 1H), 5.02 – 4.96 (m, 1H), 4.95 – 4.90 (m, 1H), 3.83 – 3.77 (m, 1H), 3.63 (dd, J = 11.1, 3.2 Hz, 1H), 3.47 (dd, J = 11.1, 7.2 Hz, 1H), 2.13 (d, J = 4.9 Hz, 1H), 2.04 (q, J = 14.4, 7.0 Hz, 2H), 1.57 – 1.26 (m, 13H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3): δ 139.30, 114.33, 71.61, 50.76, 34.37, 33.91, 29.58, 29.47, 29.15, 29.02, 25.65; **IR** (film, cm^{-1}): 3377; **HRMS** (APCI) calc. for $\text{C}_{11}\text{H}_{21}\text{O}$ $[\text{M}-\text{Cl}]^+$: 169.1587, obs. 169.1584.

Epoxide formation and Grignard addition to epoxide



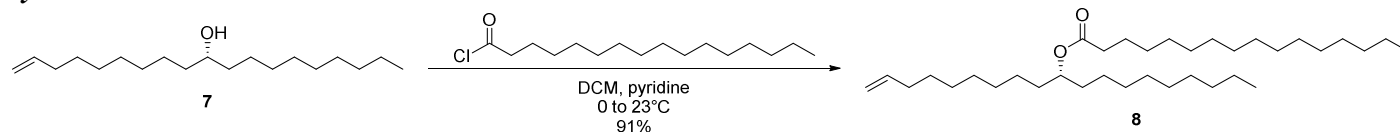
A 100 mL round-bottom flask equipped with an egg-shaped stir bar was charged with chlorohydrin **5** (5.61 g, 27.4 mmol, 1 eq) and dissolved in dry ether (55 mL, 0.5M). Stirring was initiated, giving a clear solution. Freshly crushed sodium hydroxide (6.58 g, 164 mmol, 6 eq) was added in one portion, affording a heterogeneous mixture. The reaction was stirred vigorously (1200 rpm) and monitored by aliquot ¹H-NMR. Conversion was complete within 3 hours. The mixture was vacuum filtered into a dry flask containing 4Å mole sieves, rinsing the filter cake with dry ether (25 mL x 3). The vessel was sealed with a rubber septum and sparged with nitrogen. After aging over sieves for 3 hours, crude epoxide **6** was used without purification.

A 500 mL round-bottom flask charged with magnesium turnings (4.01 g, 165 mmol, 1 eq) and equipped with a stir bar and reflux condenser. The ensemble was flame dried under vacuum, cooled to ambient temperature, flushed with nitrogen, sealed with a rubber septum, and maintained under a nitrogen atmosphere. Dry ether (82.5 mL, 1/2 total volume) was added via syringe. Stirring was initiated, giving a heterogeneous mixture of solvent and turnings. A solution of DIBAL in toluene (1.38 mL, 1.2 M, 1.65 mmol, 0.01 eq) was added by syringe and the reaction was aged for 5 min. A clear solution of 1-chlorooctane (28.0 mL, 165 mmol, 1 eq) in dry ether (82.5 mL, 1/2 total volume) was added dropwise and the reaction was heated to reflux for 3 hours. The reaction yellowed slightly over the course of the reflux and grew increasingly opaque. Most of the magnesium turnings were consumed. The reaction was allowed to cool to ambient temperature. Stirring was arrested and the supernatant solution of 1-octylmagnesium chloride was titrated as 0.52 M using the method of Love and Jones.¹ A 500 mL round-bottom flask equipped with stir bar and charged with copper (I) iodide (0.522 g, 2.75 mmol, 0.1 eq) was flame dried under vacuum, cooled to ambient temperature, flushed with nitrogen, and sealed with a rubber septum. The ethereal solution of epoxide **5** was vacuum filtered into the reaction vessel to remove the molecular sieves. Stirring was initiated, affording an opaque mixture. A dry addition funnel was attached and the reaction was cooled in a dry ice-acetone bath for 30 minutes. The ethereal solution of 1-octylmagnesium chloride (79 mL, 0.52 M, 41.3 mmol, 1.5 eq) prepared above was transferred via cannula to the addition funnel and added dropwise over 1 hour. The reaction mixture was allowed to warm to ambient temperature overnight and the color changed to a deep, dark blue. The reaction was carefully quenched with 200 mL saturated aqueous ammonium chloride. The mixture was stirred vigorously until the color changed to a brilliant, royal blue. The mixture was transferred to a separatory funnel and extracted with a mixture of ethyl ether:hexanes (50:50, 100 mL x 3). Combined organics were washed with saturated aqueous ammonium chloride (50 mL x 2), brine (50 mL), dried (sodium sulfate), filtered, and concentrated to give a crude solid. The residue was purified by silica gel column chromatography, eluting with hexanes:EtOAc (95:5 to 90:10) to yield *R*-**7** (5.45 g, 70%) as a white solid.

R-nonadec-1-en-10-ol **7**

R_f = 0.41 (silica gel, 90:10 hexanes:EtOAc, KMnO₄); **MP** (°C): 51-52; ¹H-NMR (600 MHz, CDCl₃) δ 5.80 (m, 1H), 4.98 (dd, *J* = 17.1, 1.6 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 3.59 – 3.54 (m, 1H), 2.03 (q, *J* = 7.1 Hz, 2H), 1.48 – 1.20 (m, 29H), 0.87 (t, *J* = 7.0 Hz, 3H); ¹³C-NMR (151 MHz, CDCl₃): δ 139.30, 114.25, 72.11, 37.64, 37.61, 33.92, 32.03, 29.86, 29.79, 29.78, 29.72, 29.60, 29.46, 29.22, 29.05, 25.80, 25.78, 22.81, 14.23; **IR** (film, cm⁻¹): 3373; **HRMS** (APCI) calc. for C₁₉H₃₇O [M-H]⁺: 281.2839, obs. 281.2843.

Acylation

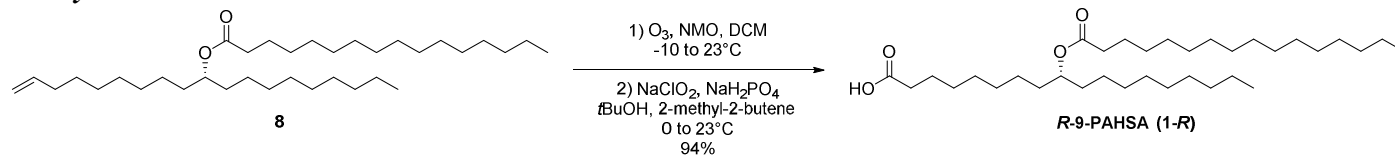


A dry 250 mL round-bottom flask equipped with stir bar, under nitrogen atmosphere, and sealed with a rubber septum was charged with alcohol **7** (4.45 g, 15.8 mmol, 1 eq) and dry dichloromethane (95 mL). Stirring was initiated, giving a clear solution. Dry pyridine (6.37 mL, 79 mmol, 5 eq) was added dropwise via syringe. The reaction vessel was submerged in an ice water bath and aged 15 minutes. Palmitoyl chloride (5.26 mL, 17.3 mmol, 1.1 eq) was added dropwise via syringe. The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was quenched with deionized water (100 mL) and stirred vigorously for 30 minutes. The biphasic mixture was transferred to a separatory funnel. The layers were partitioned and separated. The organic layer was saved while the aqueous layer was extracted with dichloromethane (150 mL x 2). Organics were combined and washed with 0.5 M aqueous hydrochloric acid (100 mL), saturate aqueous sodium bicarbonate (100 mL), and brine (100 mL), dried (sodium sulfate), filtered, and concentrated. The crude product was purified by silica column chromatography, eluting with hexanes:EtOAc (100:0 to 95:5) to give **8** (7.46 g, 91%) as a yellow oil.

R-nonadec-1-en-10-yl palmitate **8**

R_f = 0.58 (silica gel, 95:5 hexanes:EtOAc, KMnO₄); ¹H-NMR (600 MHz, CDCl₃): δ 5.80 (m, 1H), 4.98 (dd, J = 17.1, 1.4 Hz, 1H), 4.92 (d, J = 10.2 Hz, 1H), 4.89 – 4.84 (m, 1H), 2.27 (t, J = 7.5 Hz, 2H), 2.03 (q, J = 7.1 Hz, 2H), 1.65 – 1.58 (m, 2H), 1.50 (m, J = 5.0 Hz, 4H), 1.43 – 1.20 (m, 48H), 0.88 (t, J = 7.0 Hz, 6H); ¹³C-NMR (151 MHz, CDCl₃): δ 173.80, 139.27, 114.27, 74.16, 34.89, 34.32, 34.31, 33.93, 32.08, 32.05, 29.85, 29.84, 29.82, 29.81, 29.78, 29.71, 29.69, 29.67, 29.62, 29.53, 29.51, 29.47, 29.35, 29.19, 29.05, 25.47, 25.45, 25.34, 22.84, 22.83, 14.25, 14.24; IR (film, cm⁻¹) 1734; HRMS (APCI) calc. for C₃₅H₆₉O₂ [M+H]⁺: 521.5292, obs. 521.5292.

Ozonolysis and Pinnick Oxidation



4-methylmorpholine *N*-oxide monohydrate (4.05 g, 30.0 mmol, 3 eq) was dehydrated by heating at 90°C under high vacuum overnight. Following the ozonolysis conditions of Drussault,² a 250 mL round-bottom flask equipped with stir bar was charged with olefin **8** (5.21 g, 10.0 mmol, 1 eq), the anhydrous 4-methyl *N*-oxide prepared above, and dry DCM (67 mL). Stirring was initiated, affording a clear solution. The reaction vessel was submerged in an ice-acetone bath and cooled to -10°C for 15 minutes before bubbling in a mixture of ozone/oxygen. Conversion was complete within 7 minutes. The ozone generator was turned off and oxygen was bubbled through the solution for an additional 5 minutes. The reaction vessel was allowed to warm to ambient temperature and aged for 1 hour. The reaction was concentrated and the residue was dissolved in ethyl acetate (150 mL) and washed with water (100 mL) and brine (50 mL x 2), dried (sodium sulfate), filtered, and concentrated. The crude material was used in the next step without further purification.

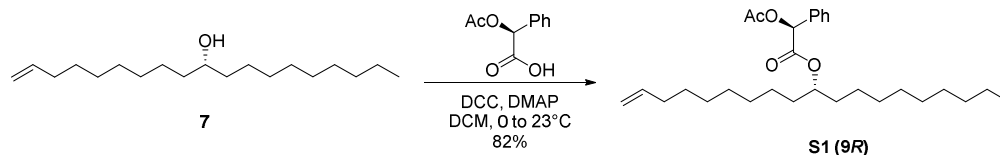
A 250 mL round-bottom flask equipped with stir bar and addition funnel was charged with the crude material above and dissolved in 2-methyl-2-butene (10.6 mL) and *tert*-butanol (50 mL). Stirring was initiated, affording a clear solution. The reaction vessel was cooled in an ice-water bath for 15 minutes before adding an aqueous solution of sodium phosphate monobasic monohydrate (5.52 g, 40.0 mmol, 4 eq) and sodium chlorite (technical grade, 80%, 4.52 g, 40.0 mmol, 4 eq) in deionized water (25 mL) dropwise via addition funnel. The reaction was allowed to warm to ambient temperature and stirred overnight.

Peroxide test strips revealed the presence of peroxides. After adding an aqueous solution of sodium bisulfite (6.24 g, 60.0 mmol, 6 eq) in deionized water (25 mL) dropwise via addition funnel and aging 30 minutes, peroxides were absent. 2-methyl-2-butene and *tert*-butanol were removed by rotavap with water bath at 50°C. The residual aqueous layer was extracted with hexanes:ethyl acetate (50:50, 100 mL x 3). Combined organics were washed with brine (100 mL), dried (sodium sulfate), filtered, and concentrated. The crude product was purified by silica column chromatography, eluting with hexanes:EtOAc (90:10 to 70:30) to yield (**1-R**) (5.17 g, 96%) as an oil.

R-9-PAHSA (1-R)

R_f = 0.29 (silica gel, 80:20 hexanes:EtOAc, KMnO₄); [α]_D²³ 0.1000 (range: -0.1160 to 0.3040) (c 1.00, CHCl₃); **¹H-NMR** (600 MHz, CDCl₃): δ 4.89 – 4.83 (m, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.27 (t, *J* = 7.5 Hz, 2H), 1.65 – 1.58 (m, 4H), 1.50 (m, *J* = 4.8 Hz, 4H), 1.27 (m, *J* = 22.8 Hz, 46H), 0.88 (t, *J* = 6.8 Hz, 6H); **¹³C-NMR** (151 MHz, CDCl₃): δ 179.05, 173.91, 74.17, 34.90, 34.31, 34.28, 33.97, 32.08, 32.05, 29.85, 29.82, 29.81, 29.78, 29.71, 29.69, 29.67, 29.51, 29.46, 29.46, 29.35, 29.27, 29.12, 25.47, 25.40, 25.34, 24.78, 22.84, 22.83, 14.26, 14.25; **IR** (film, cm⁻¹): 1711, 1733, 3427; **HRMS** (ESI) calc. for C₃₄H₆₆O₄Na [M+Na]⁺: 561.4853, obs. 561.4855.

DCC Coupling

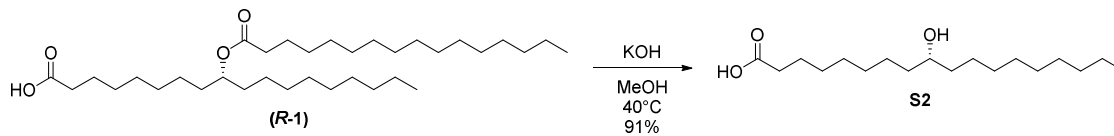


A dry 25 mL round-bottom flask equipped with stir bar, sealed with a rubber septum, and under nitrogen atmosphere was charged with *S*-(+)-*O*-acetylmandelic acid (91 mg, 0.47 mmol, 1.48 eq), DMAP (10.5 mg, 0.086 mmol, 0.27 eq), and dry DCM (2 mL). The reaction vessel was resealed with a rubber septum, sparged with nitrogen, and submerged in an ice-water bath for 15 minutes. A clear solution of alcohol **7** (90 mg, 0.318 mmol, 1 eq) in dry DCM (3 mL) was added via syringe followed by a solution of DCC (97 mg, 0.471 mmol, 1.48 eq) in dry DCM (0.5 mL). The reaction was monitored by TLC (hexanes:EtOAc 90:10). Upon completion, the reaction was filtered through a pad of DCM-wetted silica, washing with DCM (10 mL x 3). The filtrate was concentrated and the residue was purified by silica column chromatography, eluting with hexanes:EtOAc (95:5) to yield **S-1** (120 mg, 82%) as a clear oil.

R*-nonadec-1-en-10-yl (*S*)-2-acetoxy-2-phenylacetate **S1 (9R)*

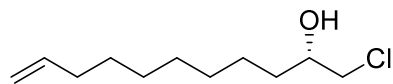
Clear oil. $R_f = 0.45$ (silica gel, 90:10 hexanes:EtOAc, UV, KMnO₄); ¹H-NMR (600 MHz, CDCl₃): δ 7.47 (dd, $J = 7.5, 2.0$ Hz, 2H), 7.39 – 7.33 (m, 3H), 5.88 (s, 1H), 5.81 (m, 1H), 4.99 (ddd, $J = 17.1, 3.5, 1.7$ Hz, 1H), 4.95 – 4.91 (m, 1H), 4.88 (dt, $J = 12.6, 6.2$ Hz, 1H), 2.19 (s, 3H), 2.03 (dd, $J = 14.6, 6.9$ Hz, 2H), 1.59 – 0.86 (m, 30H); ¹³C-NMR (151 MHz, CDCl₃): δ 170.40, 168.87, 139.39, 134.33, 129.23, 128.79, 127.76, 127.75, 114.25, 76.17, 74.91, 34.27, 34.04, 33.93, 32.02, 29.56, 29.54, 29.45, 29.44, 29.42, 29.17, 29.05, 25.28, 24.87, 22.83, 20.89, 14.26; IR (film, cm⁻¹): 1747; HRMS (ESI) calc. for C₂₉H₄₆O₄Na [M+Na]⁺: 481.3288, obs. 481.3292.

Saponification



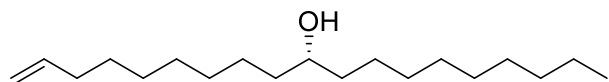
A 25 mL round-bottom flask equipped with stir bar was charged with *R*-9-PAHSA (**R-1**), dissolved in methanol (4 mL) and a few drops of hexanes. A solution of potassium hydroxide in methanol (4 mL, 10% w/v, 400 mg, 7.13 mmol, 15.5 eq) was added dropwise and the reaction was stirred at ambient temperature for 1 hour, then heated to 40°C and stirred overnight. The reaction was cooled to ambient temperature, then ice cubes were added followed by ice-cold 6M HCl until litmus paper turned red. The product was extracted with ethyl acetate (25 mL x 3) and combined organics were washed with brine (25 mL), dried (sodium sulfate), filtered, and concentrated. The residue was purified by flash chromatography, eluting with hexanes:ethyl acetate (90:10 to 50:50) to yield **S2** (126 mg, 91%) as a white solid. Spectra were in agreement with those previously reported.³

III. Characterization Data



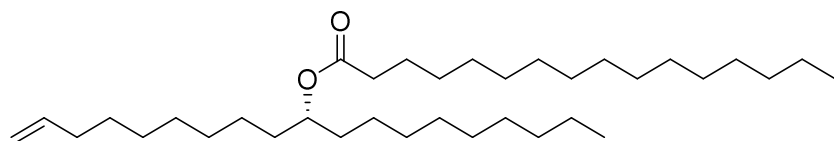
S-1-chloroundec-10-en-2-ol 5

Clear oil. **Yield** = 92%; **R_f** = 0.29 (silica gel, 90:10 hexanes:EtOAc, KMnO₄); **¹H-NMR** (600 MHz, CDCl₃): δ 5.81 (m, 1H), 5.02 – 4.96 (m, 1H), 4.95 – 4.90 (m, 1H), 3.83 – 3.77 (m, 1H), 3.63 (dd, *J* = 11.1, 3.2 Hz, 1H), 3.47 (dd, *J* = 11.1, 7.2 Hz, 1H), 2.13 (d, *J* = 4.9 Hz, 1H), 2.04 (q, *J* = 14.4, 7.0 Hz, 2H), 1.57 – 1.26 (m, 13H); **¹³C-NMR** (151 MHz, CDCl₃): δ 139.30, 114.33, 71.61, 50.76, 34.37, 33.91, 29.58, 29.47, 29.15, 29.02, 25.65; **IR** (film, cm⁻¹): 3377; **HRMS** (APCI) calc. for C₁₁H₂₁O [M-Cl]⁺: 169.1587, obs. 169.1584.



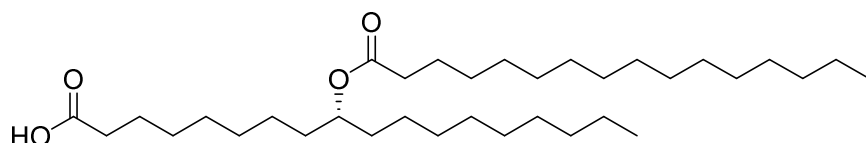
R-nonadec-1-en-10-ol 7

White Solid. **Yield** = 70%; **R_f** = 0.41 (silica gel, 90:10 hexanes:EtOAc, KMnO₄); **MP** (°C): 51-52; **¹H-NMR** (600 MHz, CDCl₃) δ 5.80 (m, 1H), 4.98 (dd, *J* = 17.1, 1.6 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 3.59 – 3.54 (m, 1H), 2.03 (q, *J* = 7.1 Hz, 2H), 1.48 – 1.20 (m, 29H), 0.87 (t, *J* = 7.0 Hz, 3H); **¹³C-NMR** (151 MHz, CDCl₃): δ 139.30, 114.25, 72.11, 37.64, 37.61, 33.92, 32.03, 29.86, 29.79, 29.78, 29.72, 29.60, 29.46, 29.22, 29.05, 25.80, 25.78, 22.81, 14.23; **IR** (film, cm⁻¹): 3373; **HRMS** (APCI) calc. for C₁₉H₃₇O [M-H]⁺: 281.2839, obs. 281.2843.



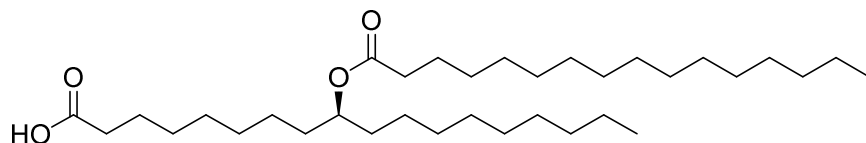
R-nonadec-1-en-10-yl palmitate 8

Clear Oil. **Yield** = 91%; **R_f** = 0.58 (silica gel, 95:5 hexanes:EtOAc, KMnO₄); **¹H-NMR** (600 MHz, CDCl₃): δ 5.80 (m, 1H), 4.98 (dd, *J* = 17.1, 1.4 Hz, 1H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.89 – 4.84 (m, 1H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.03 (q, *J* = 7.1 Hz, 2H), 1.65 – 1.58 (m, 2H), 1.50 (m, *J* = 5.0 Hz, 4H), 1.43 – 1.20 (m, 48H), 0.88 (t, *J* = 7.0 Hz, 6H); **¹³C-NMR** (151 MHz, CDCl₃): δ 173.80, 139.27, 114.27, 74.16, 34.89, 34.32, 34.31, 33.93, 32.08, 32.05, 29.85, 29.84, 29.82, 29.81, 29.78, 29.71, 29.69, 29.67, 29.62, 29.53, 29.51, 29.47, 29.35, 29.19, 29.05, 25.47, 25.45, 25.34, 22.84, 22.83, 14.25, 14.24; **IR** (film, cm⁻¹) 1734; **HRMS** (APCI) calc. for C₃₅H₆₉O₂ [M+H]⁺: 521.5292, obs. 521.5292.



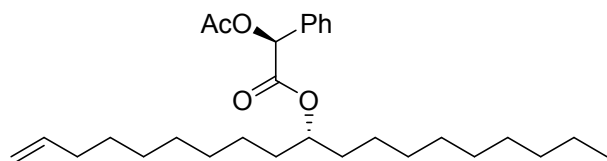
R-9-PAHSA 1-R

Clear oil. **Yield** = 96%; **R_f** = 0.29 (silica gel, 80:20 hexanes:EtOAc, KMnO₄); **[α]_D²³** 0.1000 (range: -0.1160 to 0.3040) (c 1.00, CHCl₃); **¹H-NMR** (600 MHz, CDCl₃): δ 4.89 – 4.83 (m, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.27 (t, *J* = 7.5 Hz, 2H), 1.65 – 1.58 (m, 4H), 1.50 (m, *J* = 4.8 Hz, 4H), 1.27 (m, *J* = 22.8 Hz, 46H), 0.88 (t, *J* = 6.8 Hz, 6H); **¹³C-NMR** (151 MHz, CDCl₃): δ 179.05, 173.91, 74.17, 34.90, 34.31, 34.28, 33.97, 32.08, 32.05, 29.85, 29.82, 29.81, 29.78, 29.71, 29.69, 29.67, 29.51, 29.46, 29.46, 29.35, 29.27, 29.12, 25.47, 25.40, 25.34, 24.78, 22.84, 22.83, 14.26, 14.25; **IR** (film, cm⁻¹): 1711, 1733, 3427; **HRMS** (ESI) calc. for C₃₄H₆₆O₄Na [M+Na]⁺: 561.4853, obs. 561.4855.



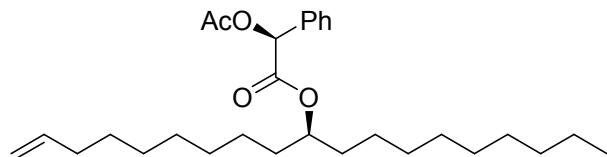
S-9-PAHSA 1-S

Clear oil. As for *S*-9-PAHSA, save **Yield** = 96%; **[α]_D²³** -0.1420 (range: -0.4660 to 0.2040) (c 1.00, CHCl₃).



(R)-nonadec-1-en-10-yl (S)-2-acetoxy-2-phenylacetate S1 (9R)

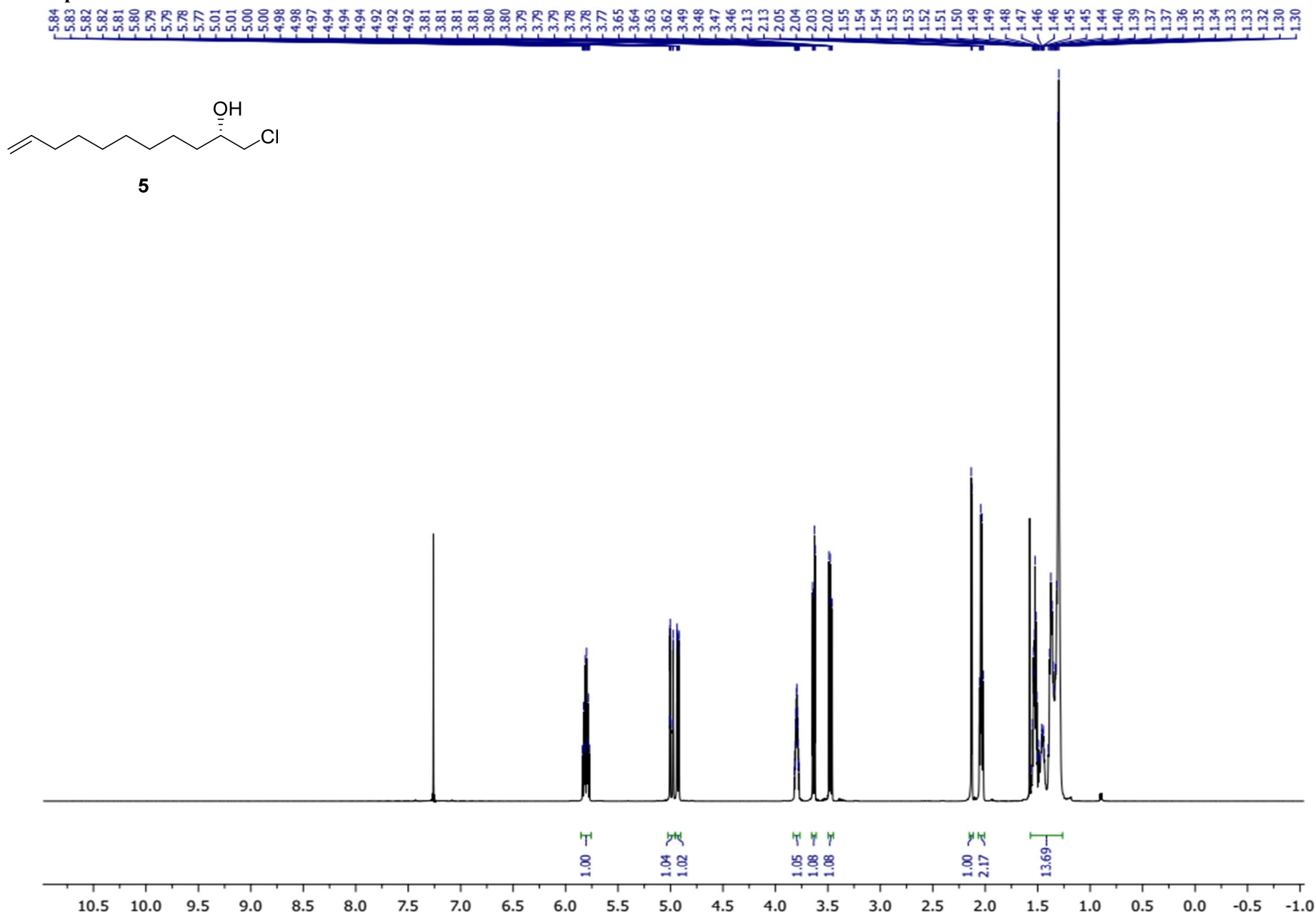
Clear oil. $R_f = 0.45$ (silica gel, 90:10 hexanes:EtOAc, UV, KMnO₄); **¹H-NMR** (600 MHz, CDCl₃): δ 7.47 (dd, $J = 7.5, 2.0$ Hz, 2H), 7.39 – 7.33 (m, 3H), 5.88 (s, 1H), 5.81 (m, 1H), 4.99 (ddd, $J = 17.1, 3.5, 1.7$ Hz, 1H), 4.95 – 4.91 (m, 1H), 4.88 (dt, $J = 12.6, 6.2$ Hz, 1H), 2.19 (s, 3H), 2.03 (dd, $J = 14.6, 6.9$ Hz, 2H), 1.59 – 0.86 (m, 30H); **¹³C-NMR** (151 MHz, CDCl₃): δ 170.40, 168.87, 139.39, 134.33, 129.23, 128.79, 127.76, 127.75, 114.25, 76.17, 74.91, 34.27, 34.04, 33.93, 32.02, 29.56, 29.54, 29.45, 29.44, 29.42, 29.17, 29.05, 25.28, 24.87, 22.83, 20.89, 14.26; **IR** (film, cm⁻¹): 1747; **HRMS** (ESI) calc. for C₂₉H₄₆O₄Na [M+Na]⁺: 481.3288, obs. 481.3292.

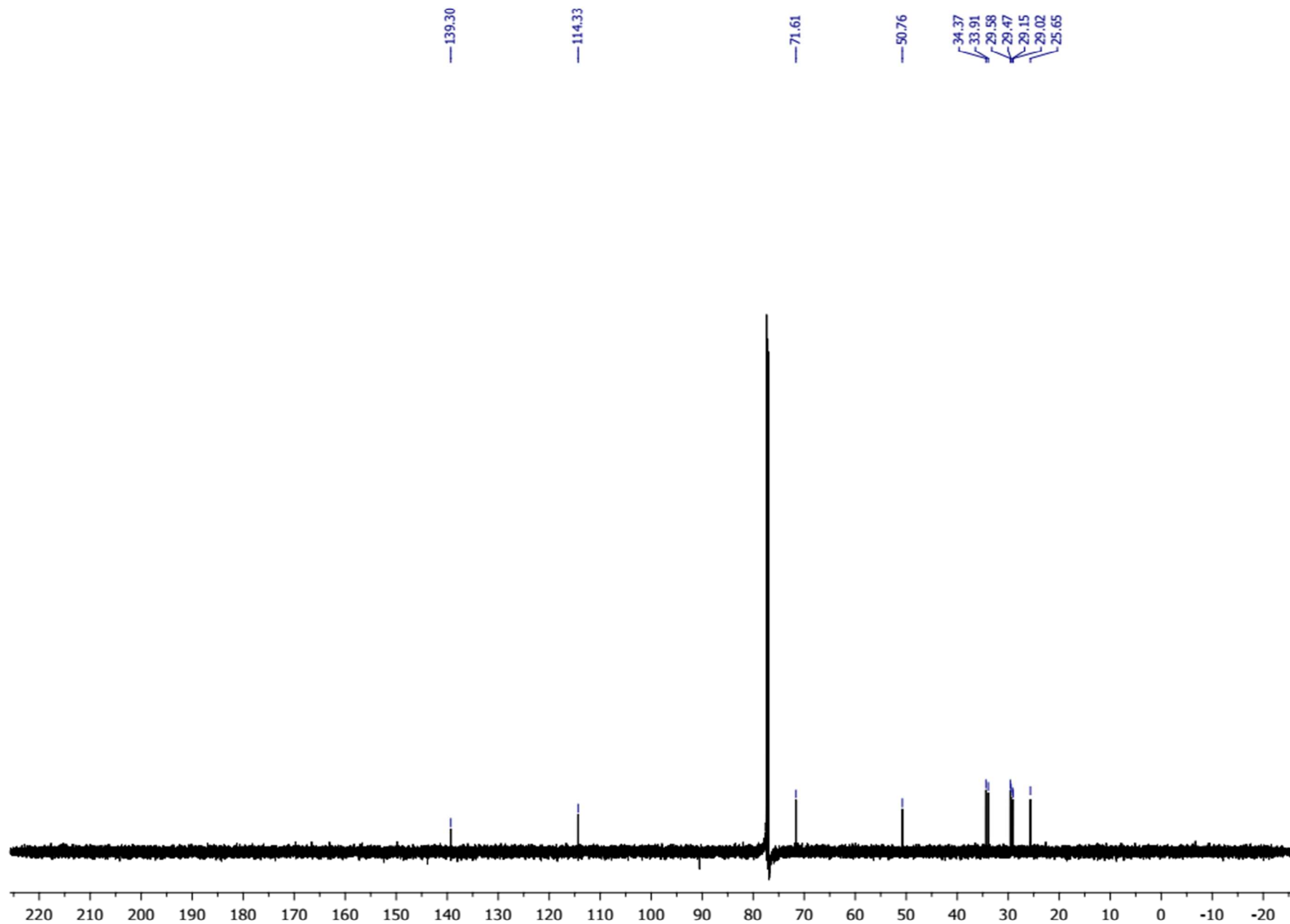


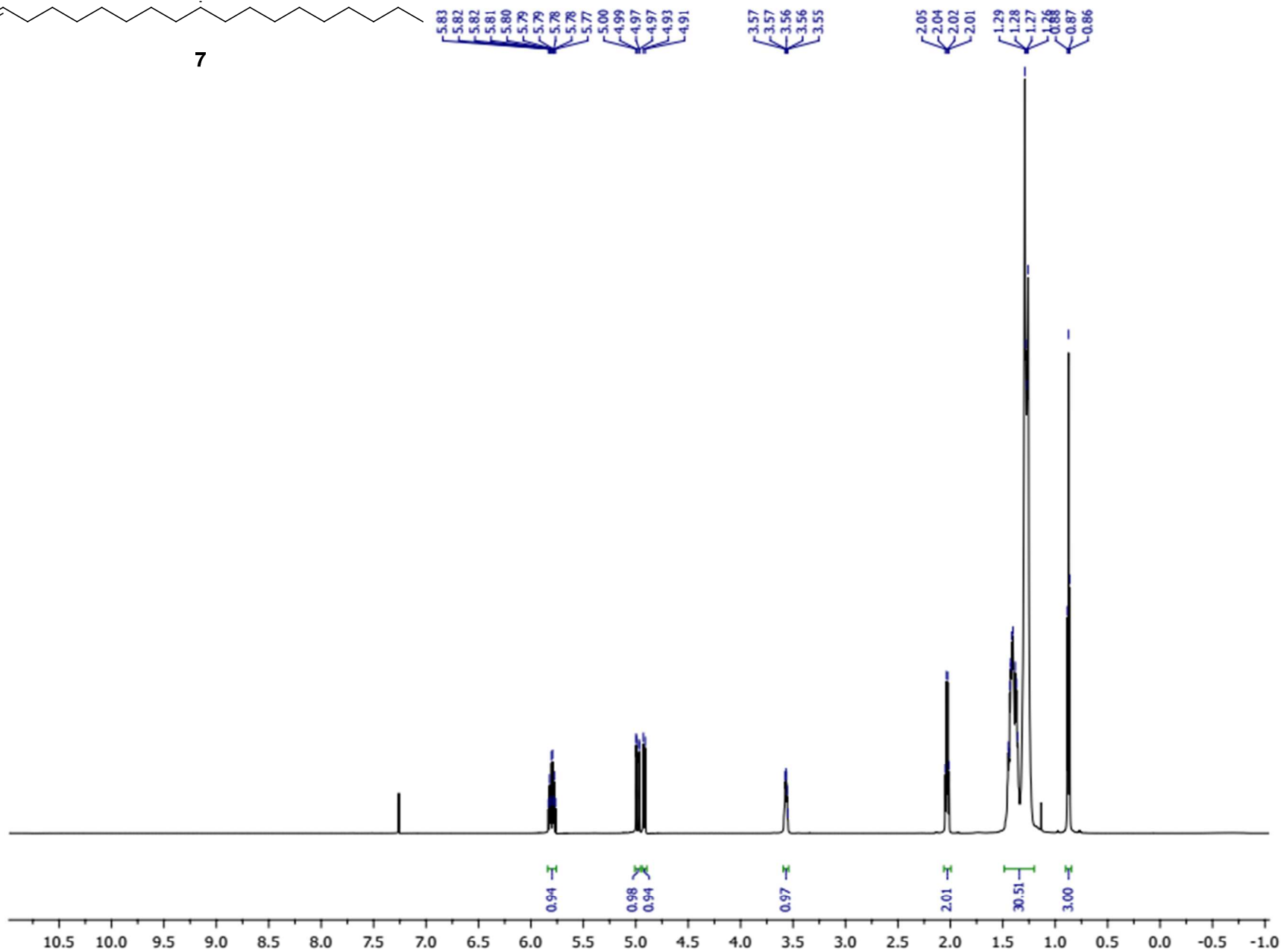
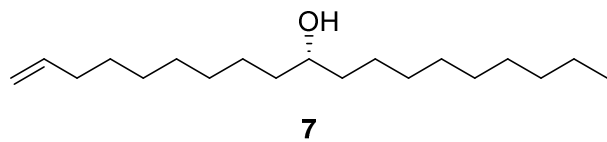
(S)-nonadec-1-en-10-yl (S)-2-acetoxy-2-phenylacetate S1 (9S)

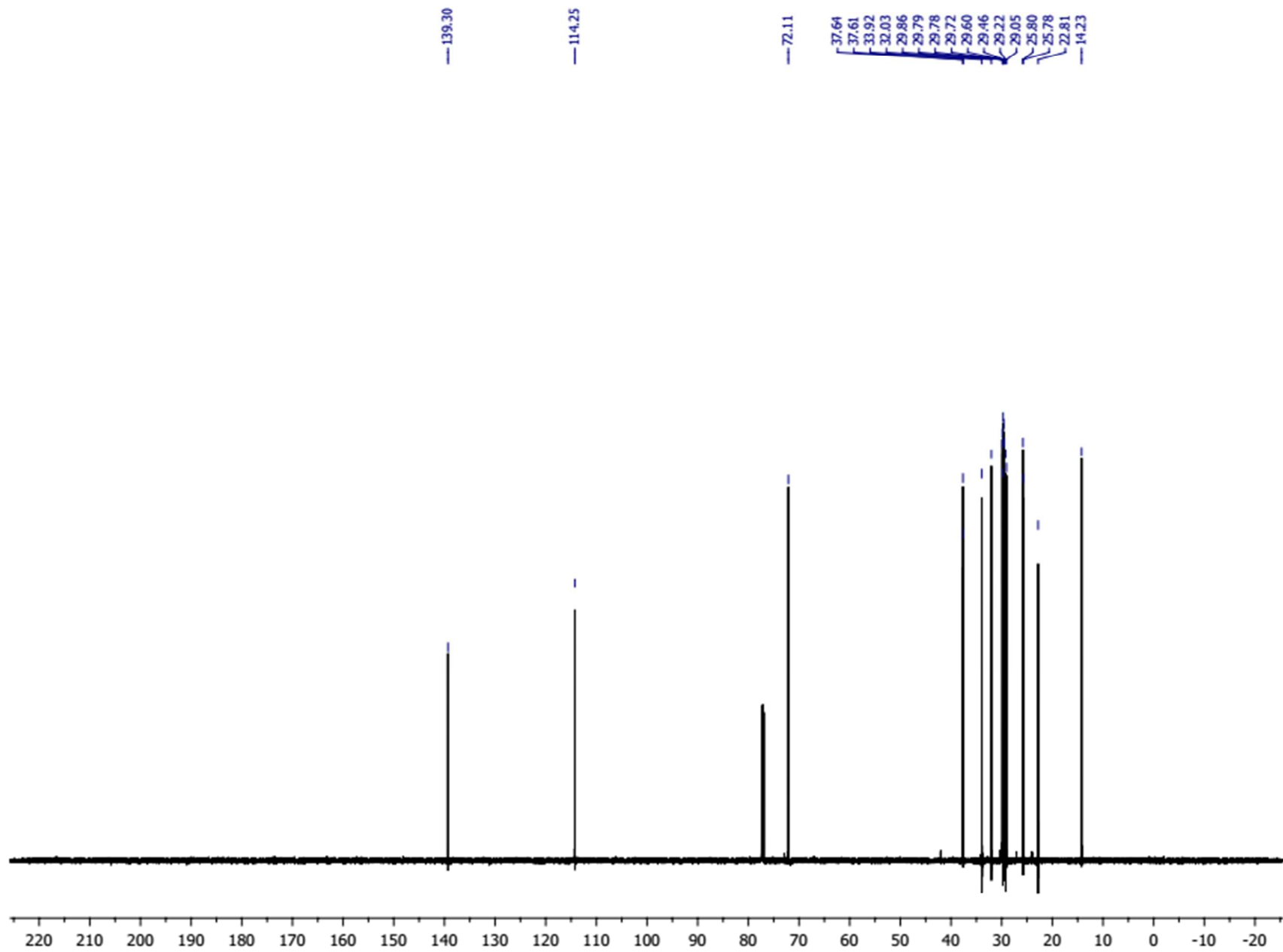
Clear oil. $R_f = 0.45$ (silica gel, 90:10 hexanes:EtOAc, UV, KMnO₄); **¹H-NMR** (600 MHz, CDCl₃): δ 7.49 – 7.45 (m, 2H), 7.39 – 7.34 (m, 3H), 5.88 (s, 1H), 5.84 – 5.76 (m, 1H), 4.99 (ddd, $J = 17.1, 3.5, 1.6$ Hz, 1H), 4.95 – 4.91 (m, 1H), 4.87 (dt, $J = 12.7, 6.1$ Hz, 1H), 2.19 (s, 3H), 2.01 (dd, $J = 14.7, 6.9$ Hz, 2H), 1.58 – 0.86 (m, 31H); **¹³C-NMR** (151 MHz, CDCl₃): δ 170.39, 168.86, 139.33, 134.35, 129.23, 128.78, 127.76, 127.75, 114.27, 76.17, 74.91, 34.28, 34.01, 33.90, 32.04, 29.67, 29.63, 29.61, 29.45, 29.35, 29.34, 29.06, 29.03, 25.31, 24.85, 22.83, 20.89, 14.26; **IR** (film, cm⁻¹): 1747; **HRMS** (ESI) calc. for C₂₉H₄₆O₄Na [M+Na]⁺: 481.3288, obs. 481.3289.

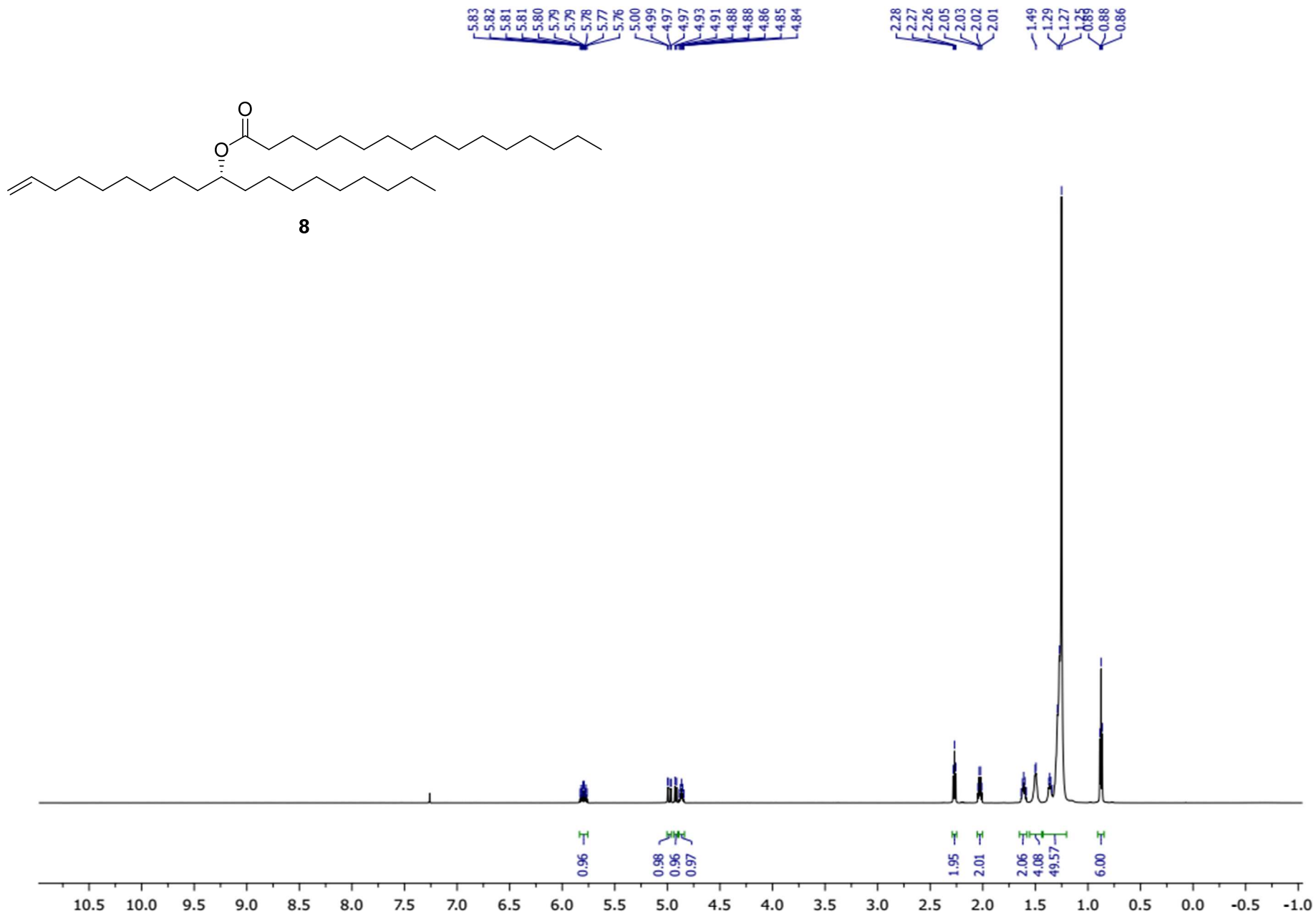
IV. Spectral Data

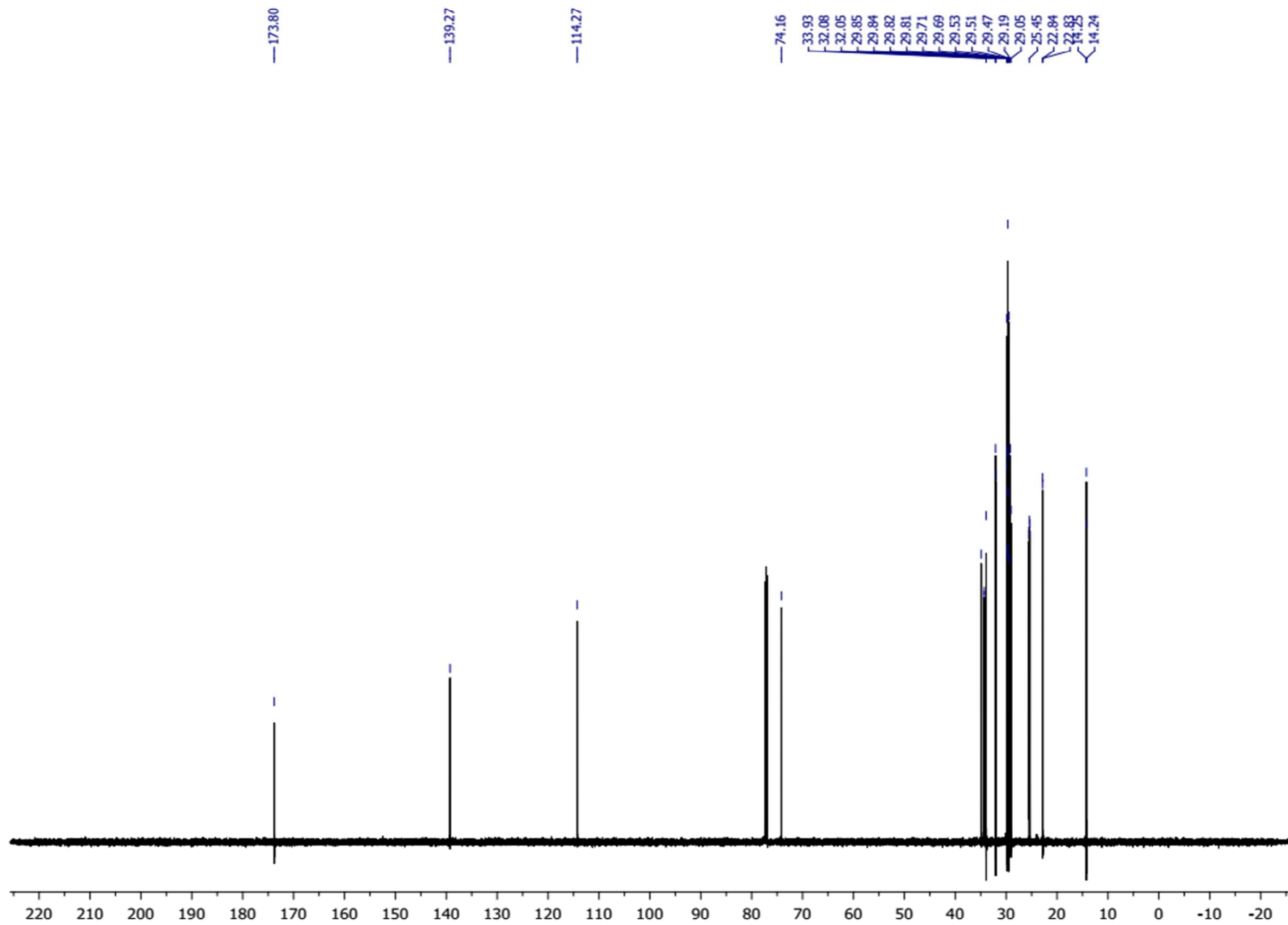


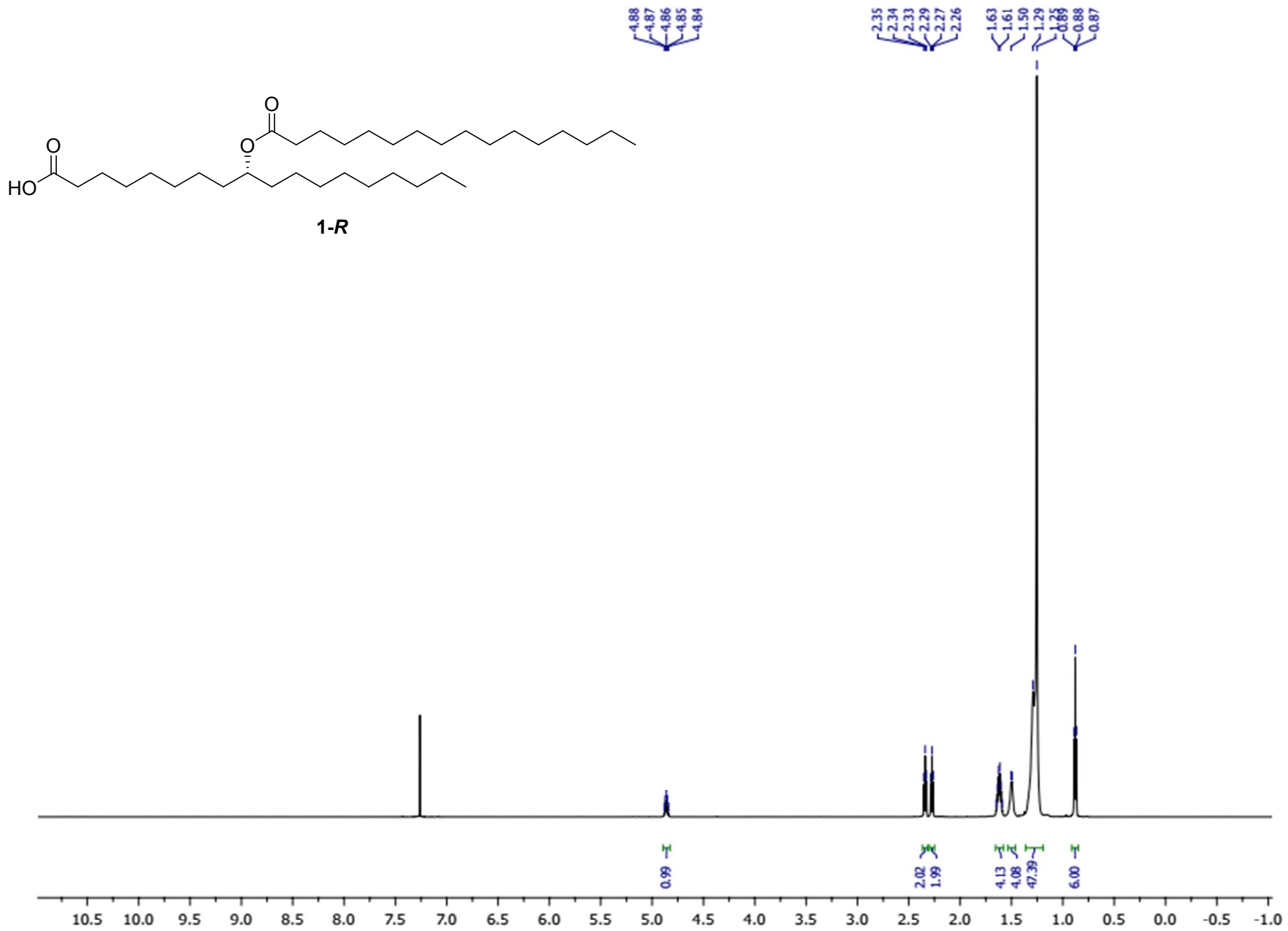


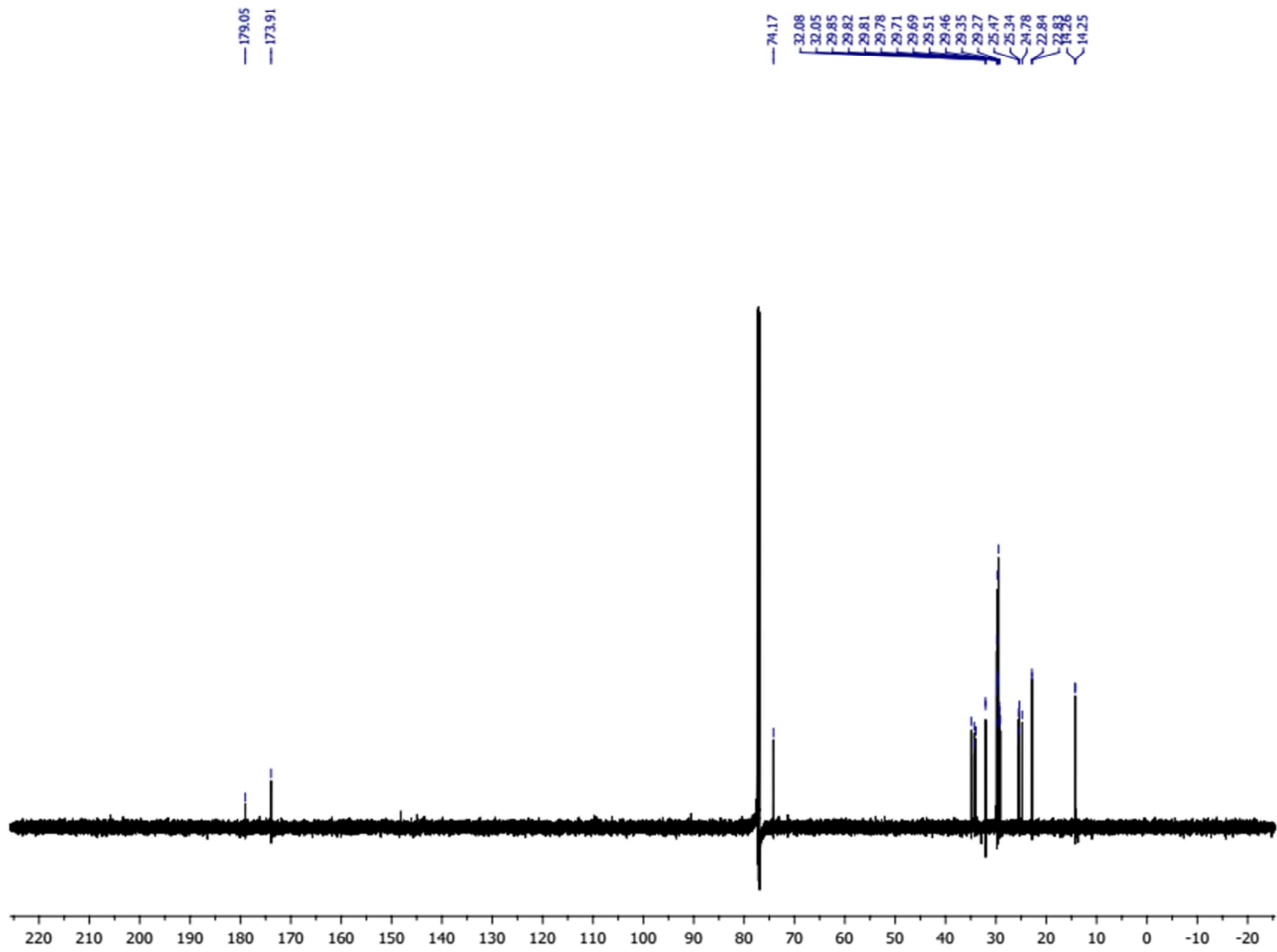


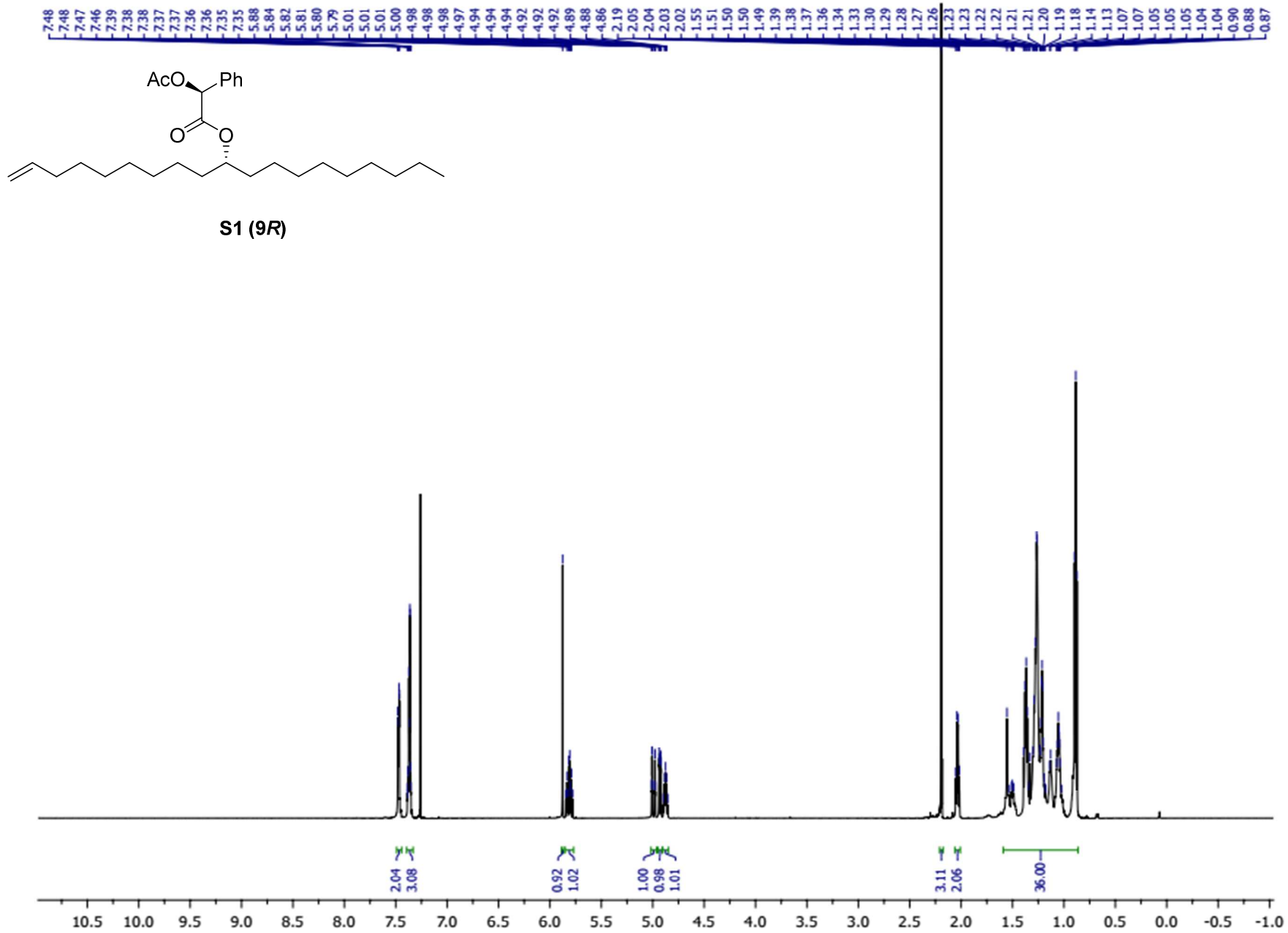


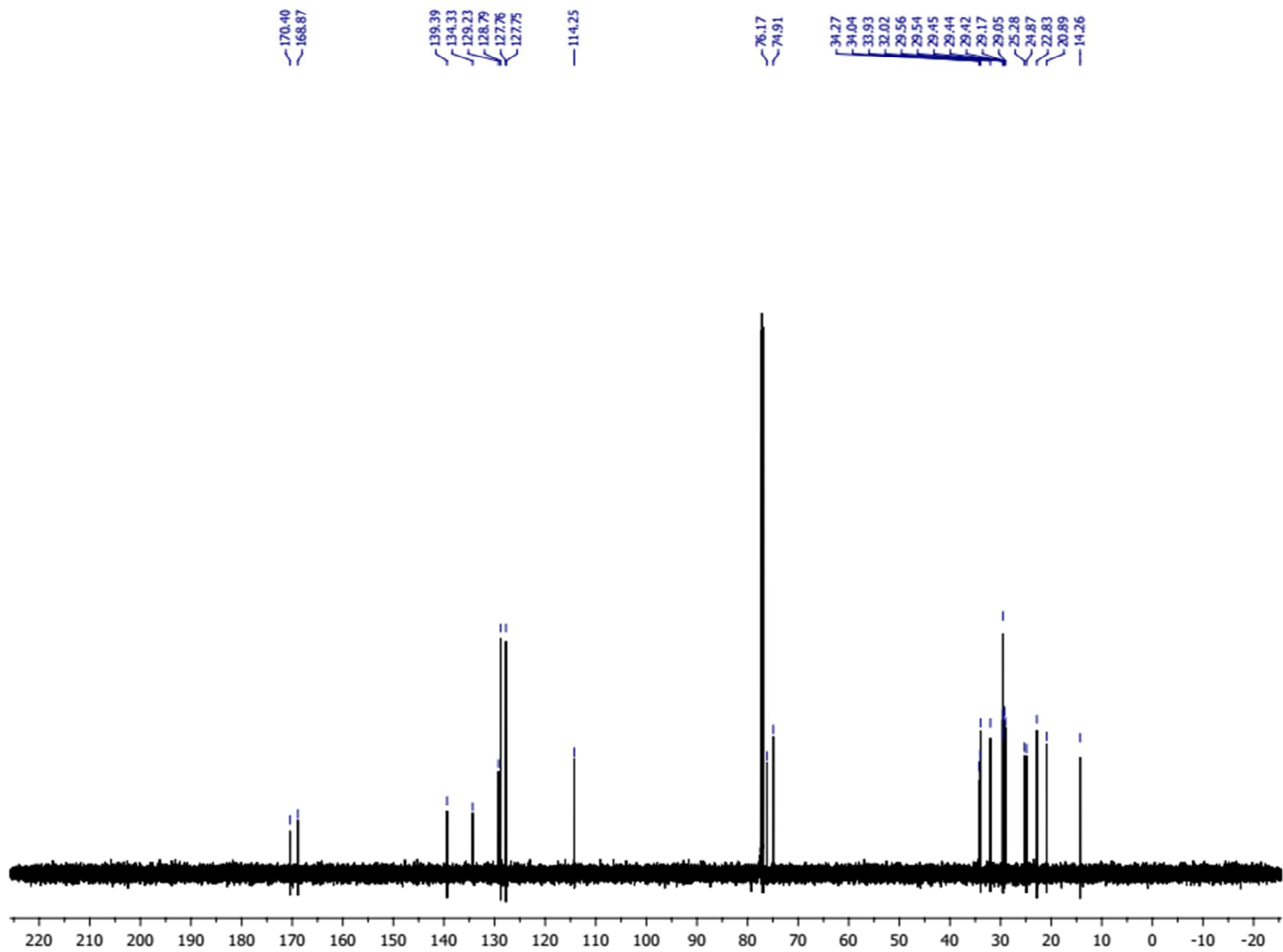


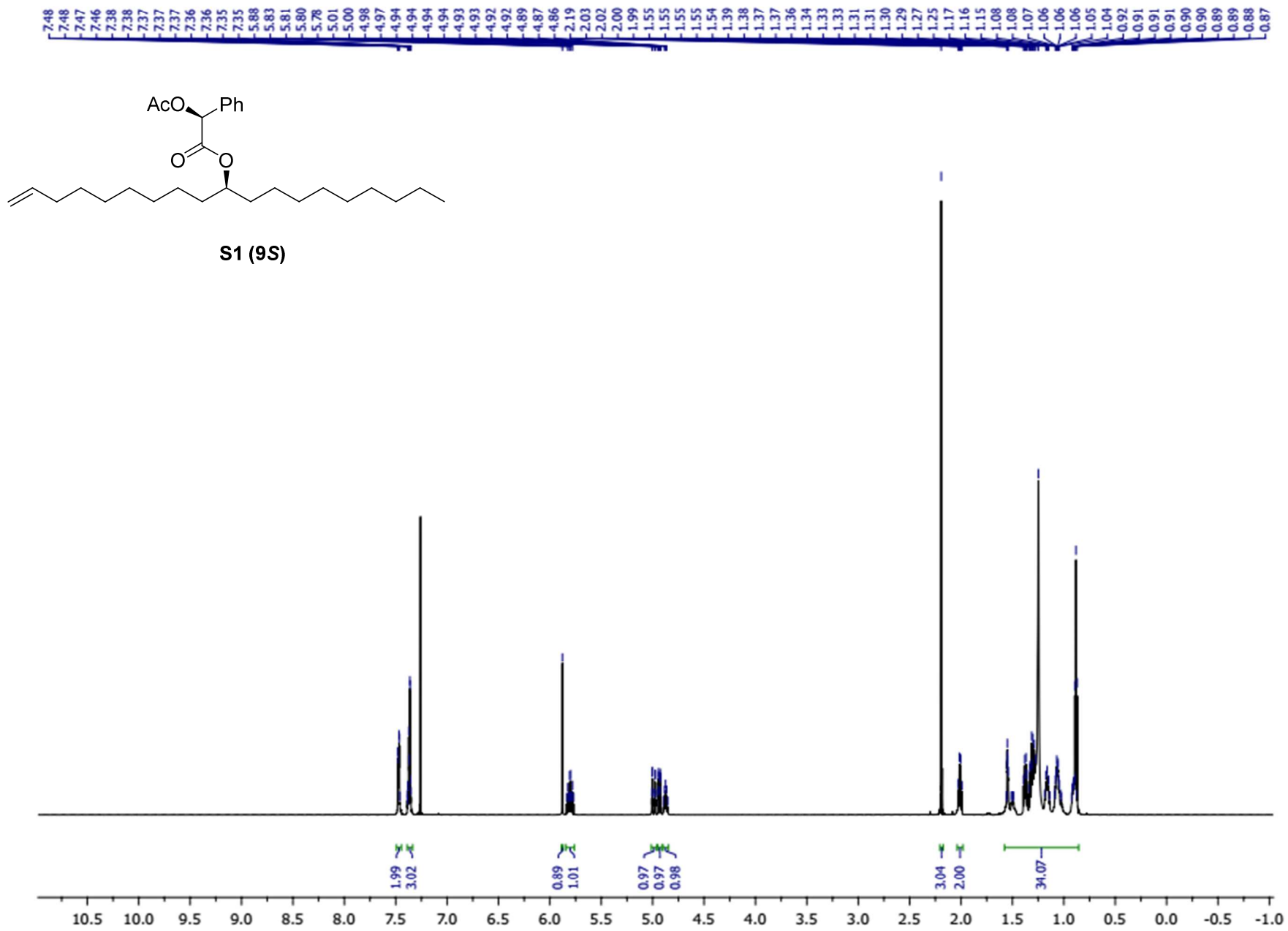


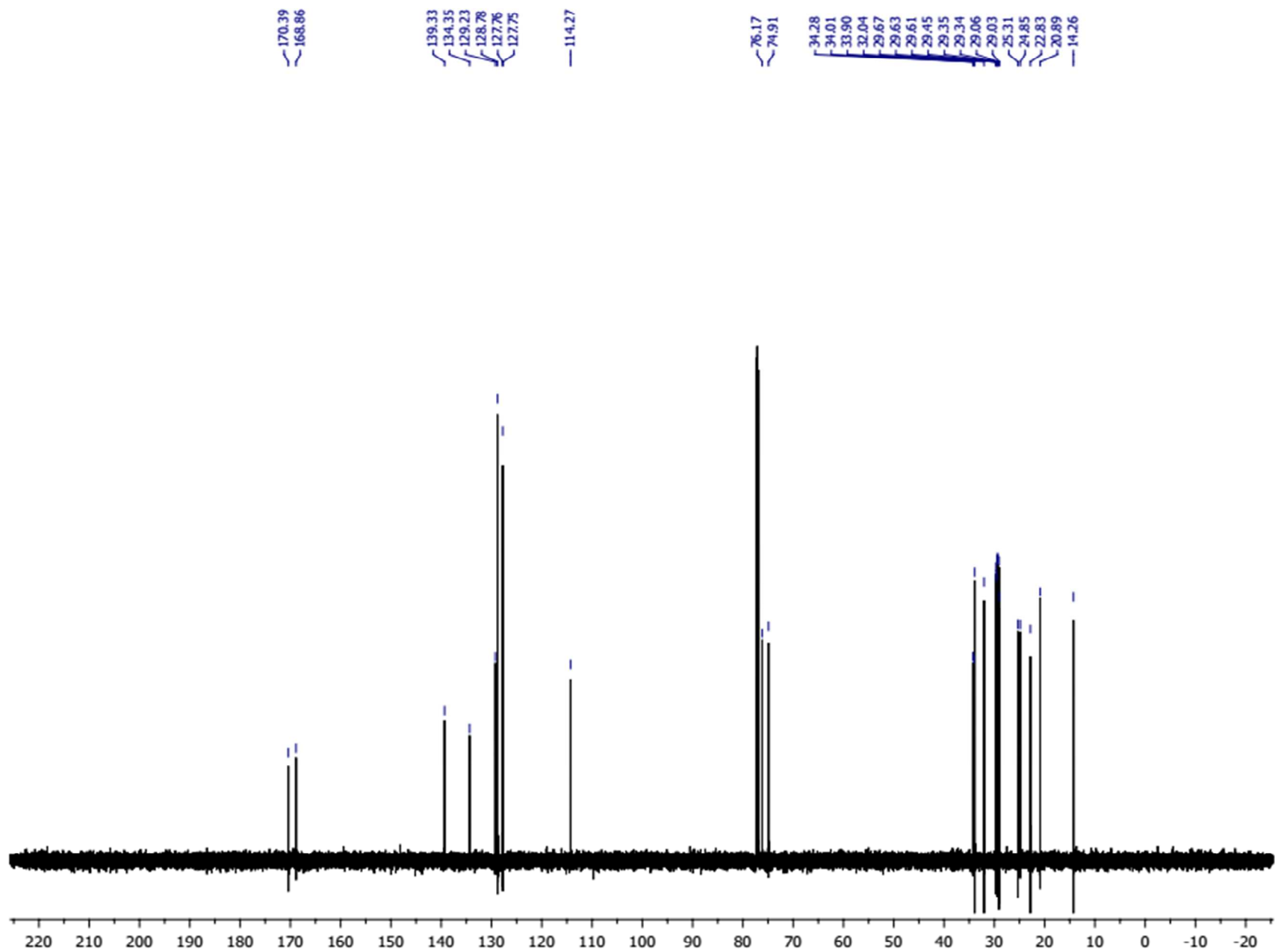


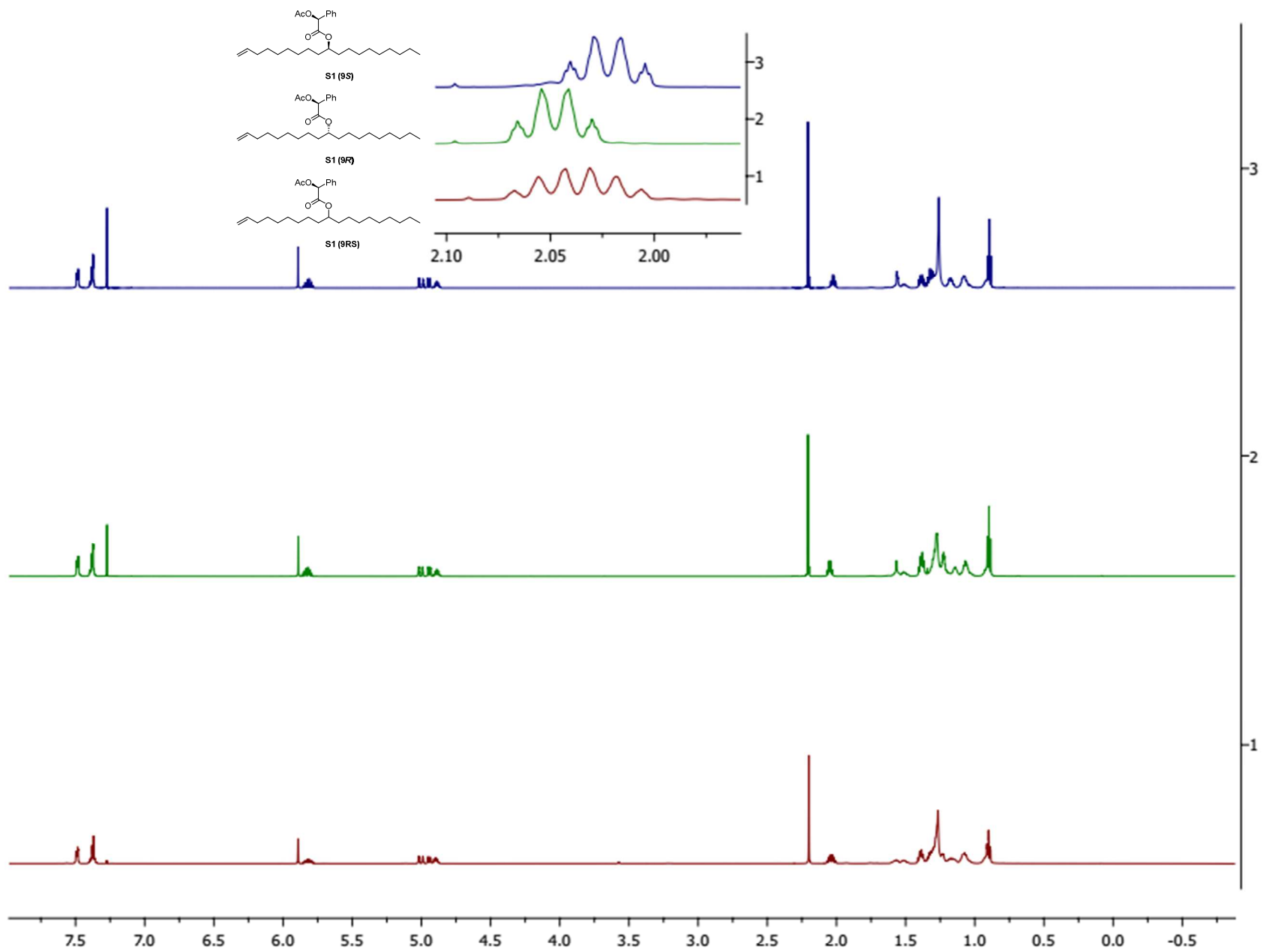












V. Analytical and Biological Assays

Materials. All chemical reagents were purchased from Sigma-Aldrich unless otherwise stated. 5-, 9-, 10- 12-PAHSA LC-MS standards were purchased from Cayman Chemical. [¹³C16]-Palmitic acid was purchased from Cambridge Isotopes and used to synthesize the 13C-9-PAHSA used as an internal standard. Organic solvents for chemical synthesis were purchased from EMD Millipore (Billerica, MA). Solvents for HPLC were purchased from EMD Millipore and solvents for LC-MS were from Honeywell Burdick & Jackson.

Biological Sample Preparation. Lipid extraction was performed based on known protocol.^{4, 5} Murine tissues (60–150 mg) were Dounce homogenized on ice for 40 strokes in a mixture of 1.5 ml: 1.5 ml: 3 ml citric acid buffer (100 mM trisodium citrate, 1 M NaCl, pH 3.6): methanol: chloroform. 13C-9-PAHSA standard (5 pmol) was added to chloroform prior to extraction. The resulting mixture was centrifuged at 2200 g, 6 min, 4 °C to separate organic and aqueous phases, and the organic phase containing extracted lipids was removed with a Pasteur pipette, dried under a gentle stream of Nitrogen and stored at -80 °C prior to solid phase extraction (SPE). SPE was performed at room temperature via gravity flow. SPE cartridge (500 mg silica, 6 ml, Thermo Scientific, 60108-411) was conditioned with 15 ml hexane. Extracted lipids (reconstituted in 200 µl chloroform) were loaded onto column. Vial containing lipids was washed with an additional 100 ml chloroform and the wash also loaded onto the column. Neutral lipids were eluted with 16 ml 5% ethyl acetate in hexane, followed by elution of FAHFAs with 16 ml ethyl acetate. FAHFA fraction was dried under nitrogen and stored at -80 °C prior to LC-MS.

9-PAHSA Enantiomer Hydrolysis Assay. *Rac*-, *S*-, and *R*-9-PAHSA hydrolysis was measured and analyzed as described.⁶ Briefly, tissue/cell membrane lysates (20 µg) were incubated with *rac*-, *R*-, or *S*-9-PAHSA (100 µM) in PBS (200 µL) at 37 °C while being shaken. Lipids were then extracted using a 2:1 CHCl₃/MeOH mixture (400 µL) spiked with 9-hydroxyheptadecanoic acid (50 pmol) as an internal standard. This mixture was vortexed, and spun for 5 min at 2200 g. The bottom organic layer was isolated and dried. The extract was then dissolved in 100 µL MeOH and 10 µL was subjected to LC-MS using a Thermo TSQ Quantiva MS fitted with an Acquity UPLC BEH C18 column (Waters).

Targeted LC-MS Analysis of PAHSAs. PAHSAs were measured on a TSQ Quantiva LC-MS via Multiple Reaction Monitoring (MRM) in negative ionization mode as described.⁷ The following MS source parameters were used: spray voltage, 3.5 kV; ion transfer tube temperature, 325 °C; vaporizer temperature, 275 °C; sheath gas, 2.7 L/min; aux gas, 5.0 L/min; and sweep gas, 1.5 L/min. A Luna C18(2) reversed-phase column (3 µm, 250 x 2.0 mm, Phenomenex) was used. PAHSAs were resolved with isocratic flow at 0.2 mL/min using 93:7 MeOH: H₂O with 5 mM ammonium acetate and 0.01% ammonium hydroxide over 100 minutes. MRM [collision energy (CE) of 28 V, RF lens set at 106 V] was used to detect PAHSA (m/z 537.6 → 255.2).

Targeted LC-MS Analysis of PAHSA enantiomers. PAHSA enantiomers were analyzed on a TSQ Quantiva LC-MS in negative mode with the same parameters mentioned above. Resolution of 9-PAHSA enantiomers was achieved using a Lux Cellulose-3 chiral column (3 µm, 250 x 4.6 mm, Phenomenex) with an isocratic flow rate of 0.4 mL/min of a 96:4 MeOH:H₂O + 0.1% formic acid solution.

FAHFA biosynthesis assay. Prior to seeding HEK293T cells, 6-well plates were pretreated with 50 mg/mL poly-lysine for 2 hours. Cells were grown in DMEM supplemented with 10% FCS. After cells reached 90-95% confluence, the media (2 mL/well) was replaced with 100 mM *R*- or *S*-12-HSA. After incubation at 37 °C, 5% CO₂ for 2 hours, cells were washed with sterile PBS (3x) and harvested by scraping. Cells were re-suspended in 1 mL sterile PBS and mixed with 3 mL of 2:1 CHCl₃:MeOH with internal standard (5 pmol 13C-9-PAHSA). The sample was vortexed and centrifuged at 2200 g. The bottom organic layer was isolated and dried. The extract was then dissolved in 100 µL MeOH and 10 µL was subjected to LC-MS.

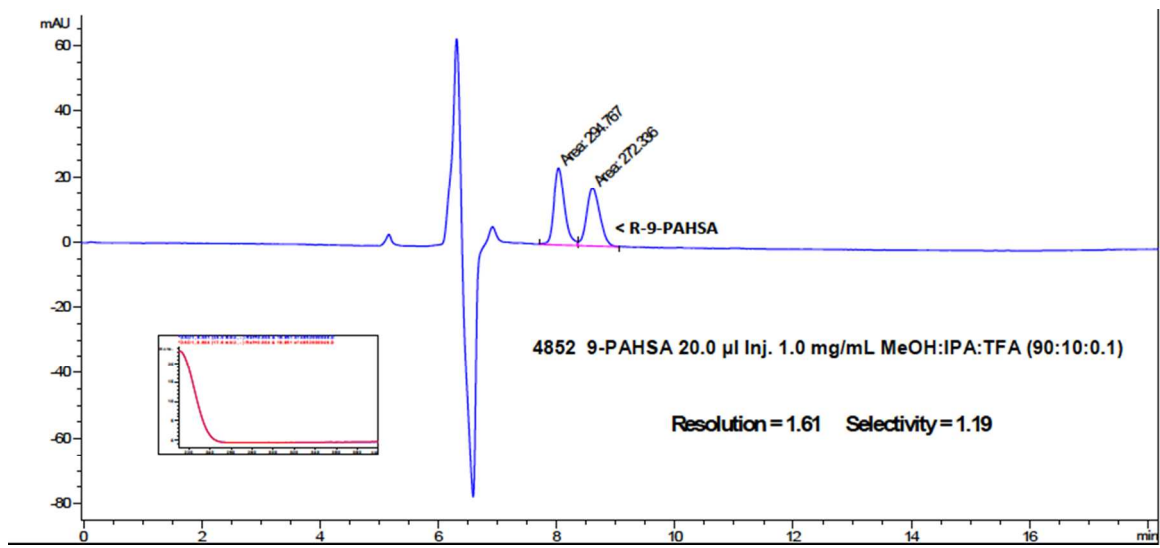


Figure S1. UV trace (abs=210 nm) from Phenomenex's chiral screening service showing resolution of rac-9-PAHSA.

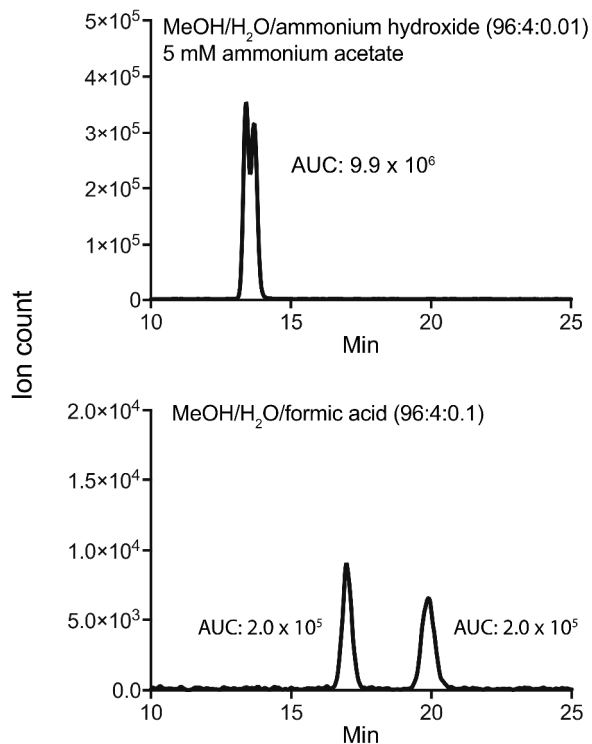


Figure S2. LC-MS traces of *rac*-9-PAHSA (2 pmol) with different modifiers in the mobile phase ran on a Lux 3 μ m Cellulose-3 column. AUC, area under the curve. With the presence of the basic modifier, ammonium hydroxide, the *R*- and *S*-9-PAHSA stereoisomers were not well resolved (top trace). With the acidic modifier, formic acid, the stereoisomers were resolved, but with lower ionization efficiency (bottom trace).

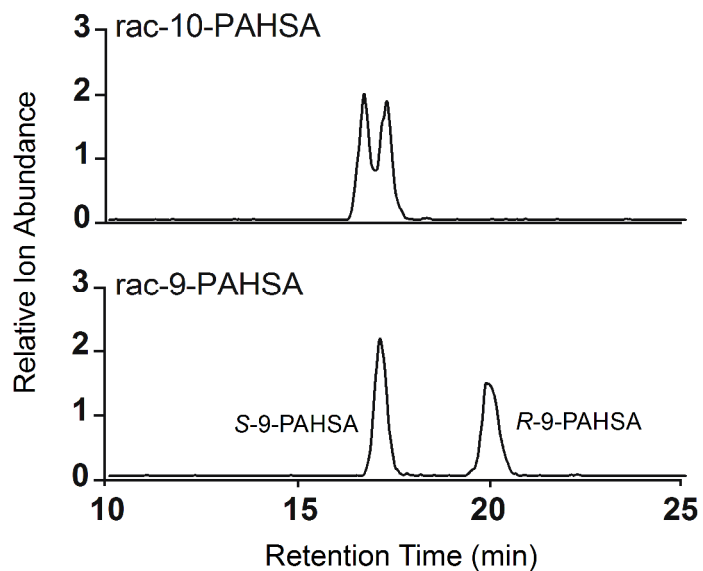


Figure S3. LC-MS traces showing the retention times of rac-10-PAHSA and rac-9-PAHSA. PAHSAs were run on a 3 μ m Lux Cellulose-3 column with an isocratic flow rate of 0.4 mL/min of a 96:4 MeOH:H₂O + 0.1% formic acid solution.

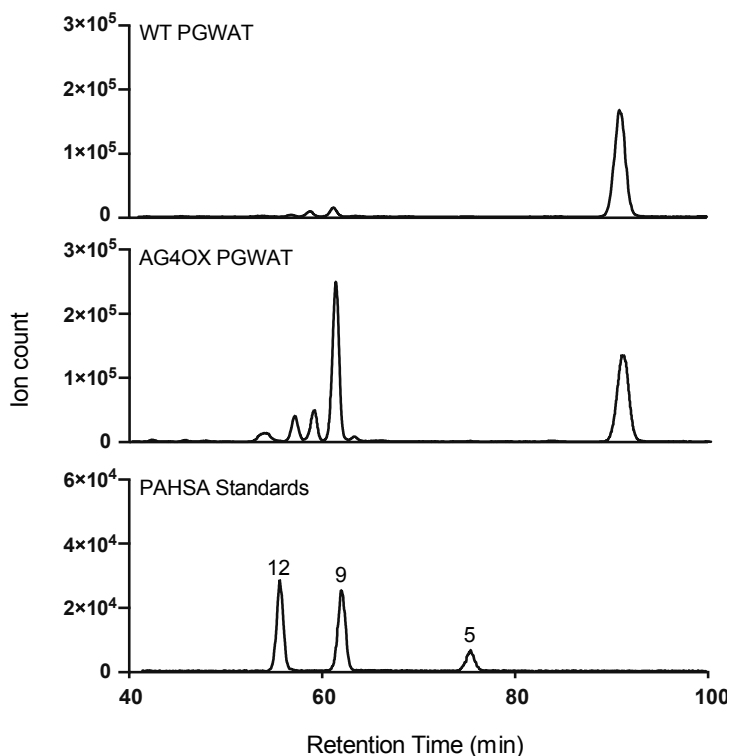


Figure S4. Analysis of the WT and AG4OX WAT samples using our standard targeted LC-MS conditions (isocratic flow at 0.2 mL/min using 93:7 MeOH: H₂O with 5 mM ammonium acetate and 0.01% ammonium hydroxide on a Luna C18(2) reversed-phase column). The increase in 9-PAHSA between AG4OX and WT PGWAT is evident and the fold change is similar to the increase of *R*-9-PAHSA levels (Figure 3).

VI. References

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