Supplemental Data

Demonstration of HNE-related aldehyde formation via lipoxygenase-catalyzed synthesis of a *bis*-allylic dihydroperoxide intermediate

Jing Jin, Yuxiang Zheng[1], and Alan R. Brash[*]

Department of Pharmacology, and the Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN 37232

*Address correspondence to Alan R. Brash, Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232-6602; Tel. 615-343-4495; Fax: 615-322-4707 Email: alan.brash@vanderbilt.edu

[1] Present address: Weill Cornell Cancer Center, 1300 York Avenue, New York, NY 10065

Figure S1: Reaction of arachidonic acid (25 µg/ml) with recombinant 8*R*-LOX (10 µg/ml) following over time by repetitive UV scanning (200 – 350 nm).

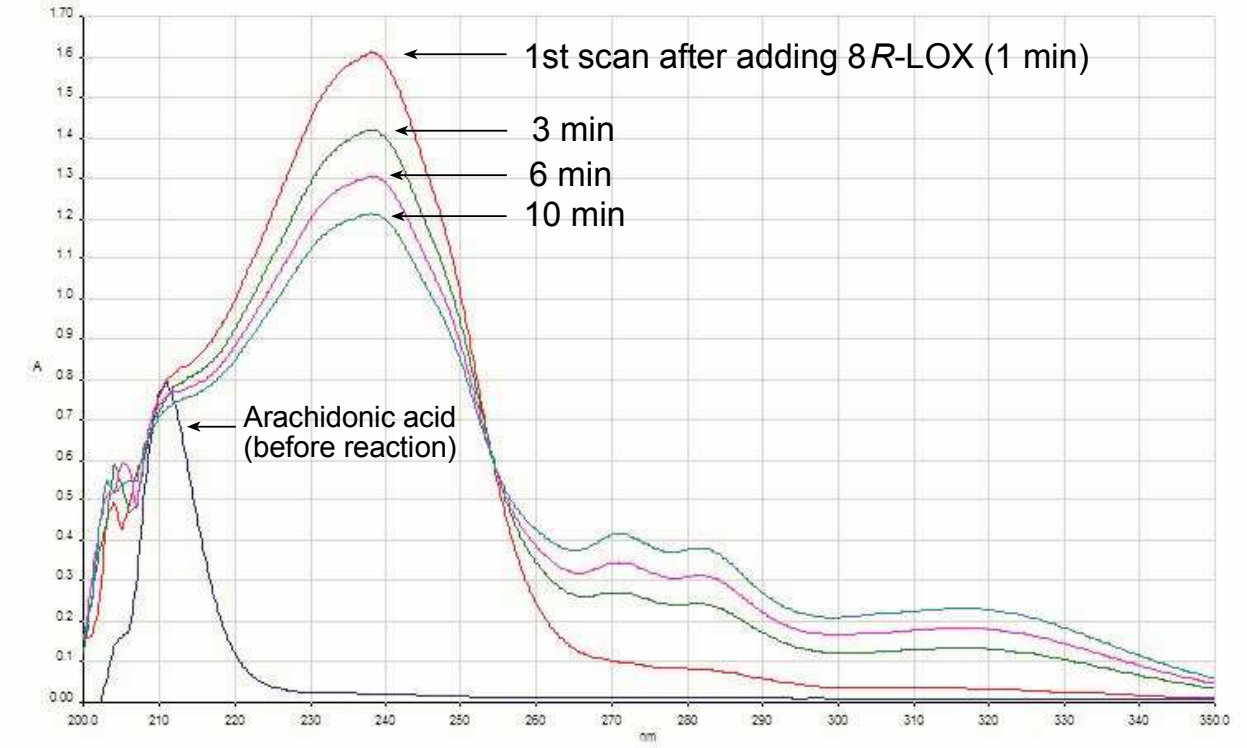
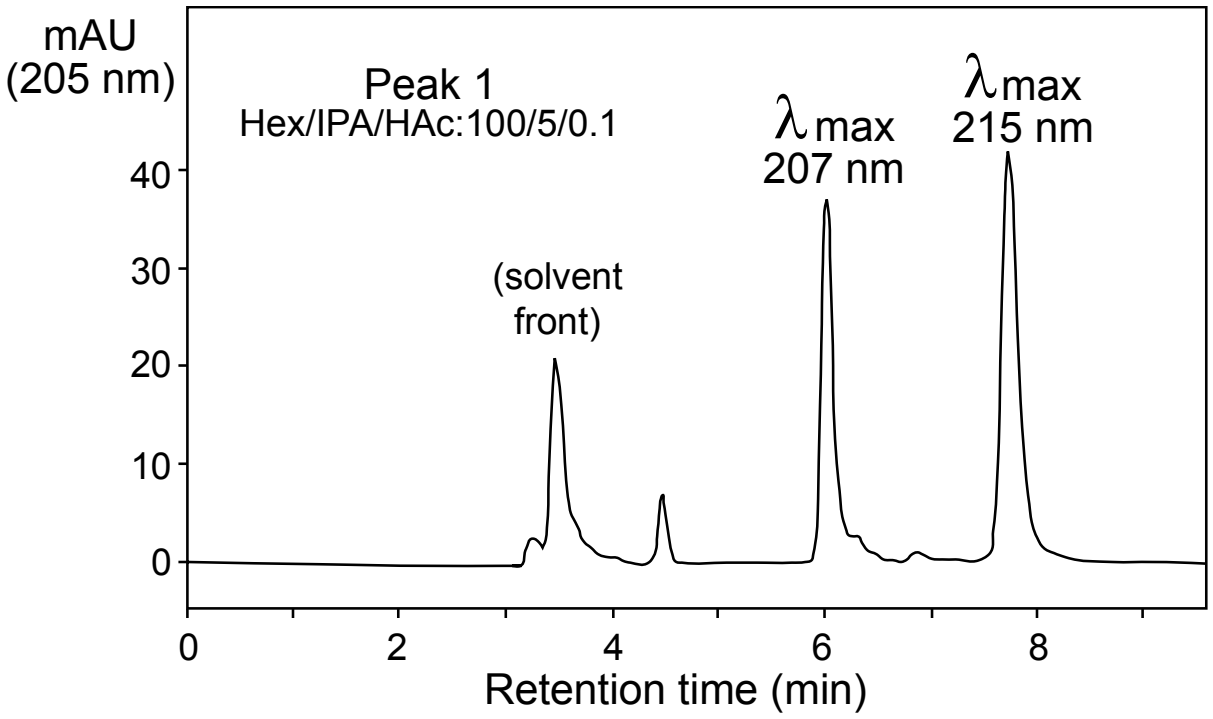


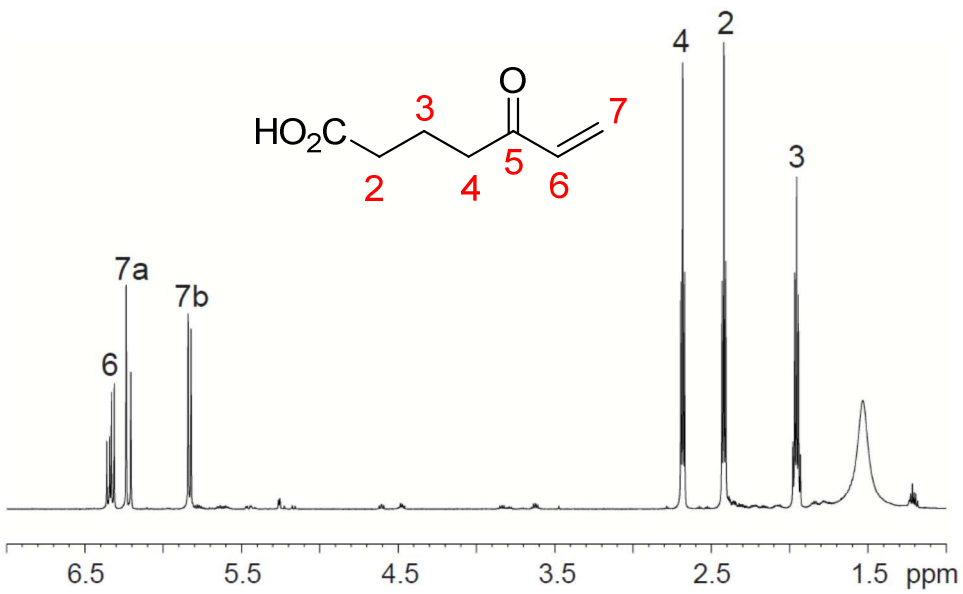
Figure S2: SP-HPLC separation of the two main components of peak 1 in Figure 1 of the main text.



The first of the two peaks was identified as 5-oxo-hept-6-enoic acid (Figure S3); it exhibits a conjugated enone chromophore, λmax 207 nm in the SP-HPLC solvent (and λmax of 212 nm in RP-HPLC solvent, water/CH_3CN/acetic acid, 70:30:0.01).

The later eluting product on the SP-HPLC chromatogram was identified by NMR as 7-oxo-hept-5E-enoic acid (Figure S4). It has a conjugated enone chromophore with λmax of 215 nm in SP-HPLC solvent (and 223 nm in RP-HPLC solvent).

Figure S3: ^{1}H-NMR spectrum of 5-oxo-hept-6-enoic acid (one of the two main components of peak 1 in Figure 1, main text). The spectrum was recorded in d6-benzene at 298K using a Bruker 600 MHz spectrometer.



5-oxo-hept-6-enoic acid. ^{1}H-NMR, 600 MHz, $CDCl_3$, 283K, δ6.34, dd, 1H, H6, $J_{6,7a}$ = 17.6 Hz, $J_{6,7b}$ = 10.6 Hz; 6.22, d, 1H, H7a, $J_{6,7a}$ = 17.6 Hz; 5.83, d, 1H, H7b, $J_{6,7a}$ = 10.6 Hz; 2.69, t, 2H, H4, $J_{3,4}$ = 7.2 Hz; 2.42, t, 2H, H2, $J_{2,3}$ = 7.2 Hz; 1.96, p, 2H, H3, J = 7.2 Hz. Assignments were confirmed by HMBC/HSQC.

The molecular weight was confirmed as 142 by LC-MS (Q-TOF, negative-ESI, $[M-H]^-$ ion, predicted 141.0552, found 141.0557, $C_7H_9O_3$.

The chemical shifts and coupling constants for the enone moiety are very similar to those reported for the synthetic vinyl ketone analogues, 1-octen-3-one and 1,5-octadien-3-one [1].

This class of enone has been shown to exhibit adduction with glycine [2], and in a recent study exhibit reaction specificity with cysteine [3].

A proposed mechanism of formation involves a cleavage route suggested by Blank and colleagues for 1-alkene-3-one formation in the autoxidation of arachidonic acid [4].

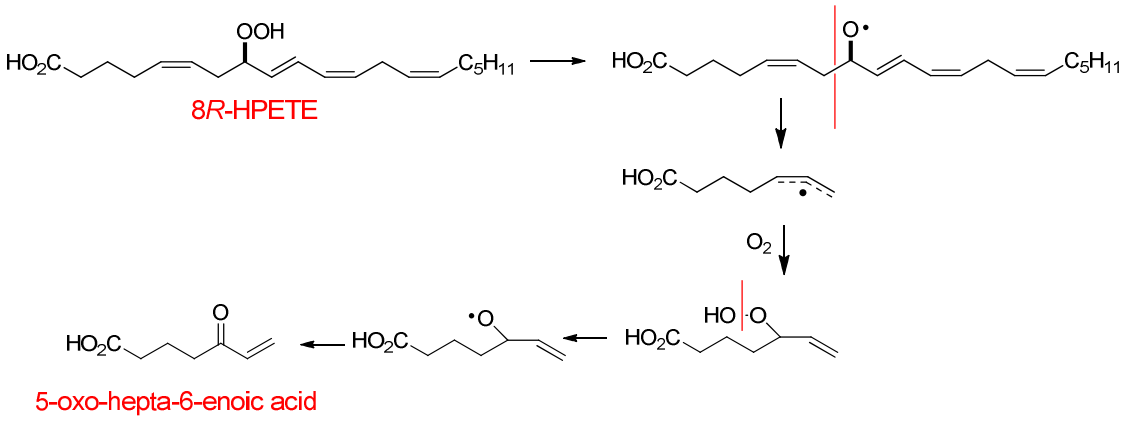
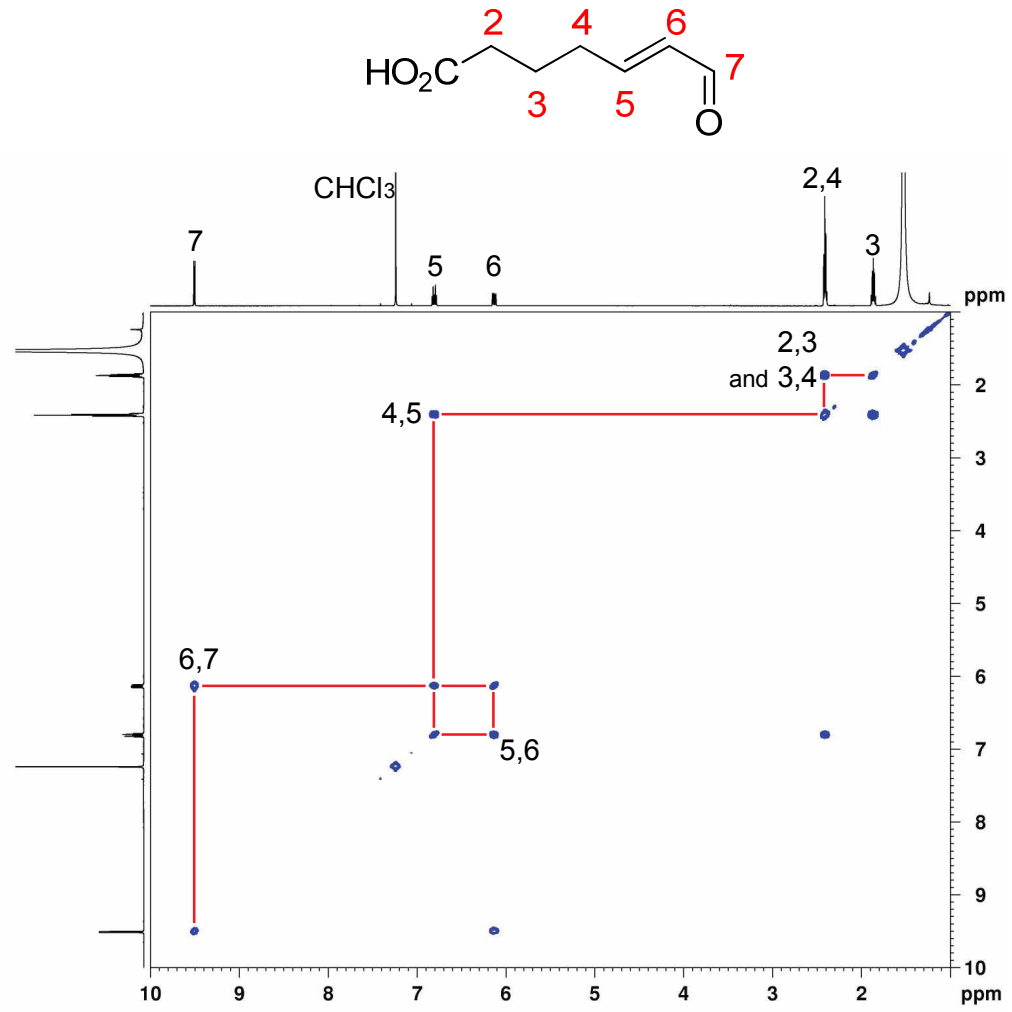
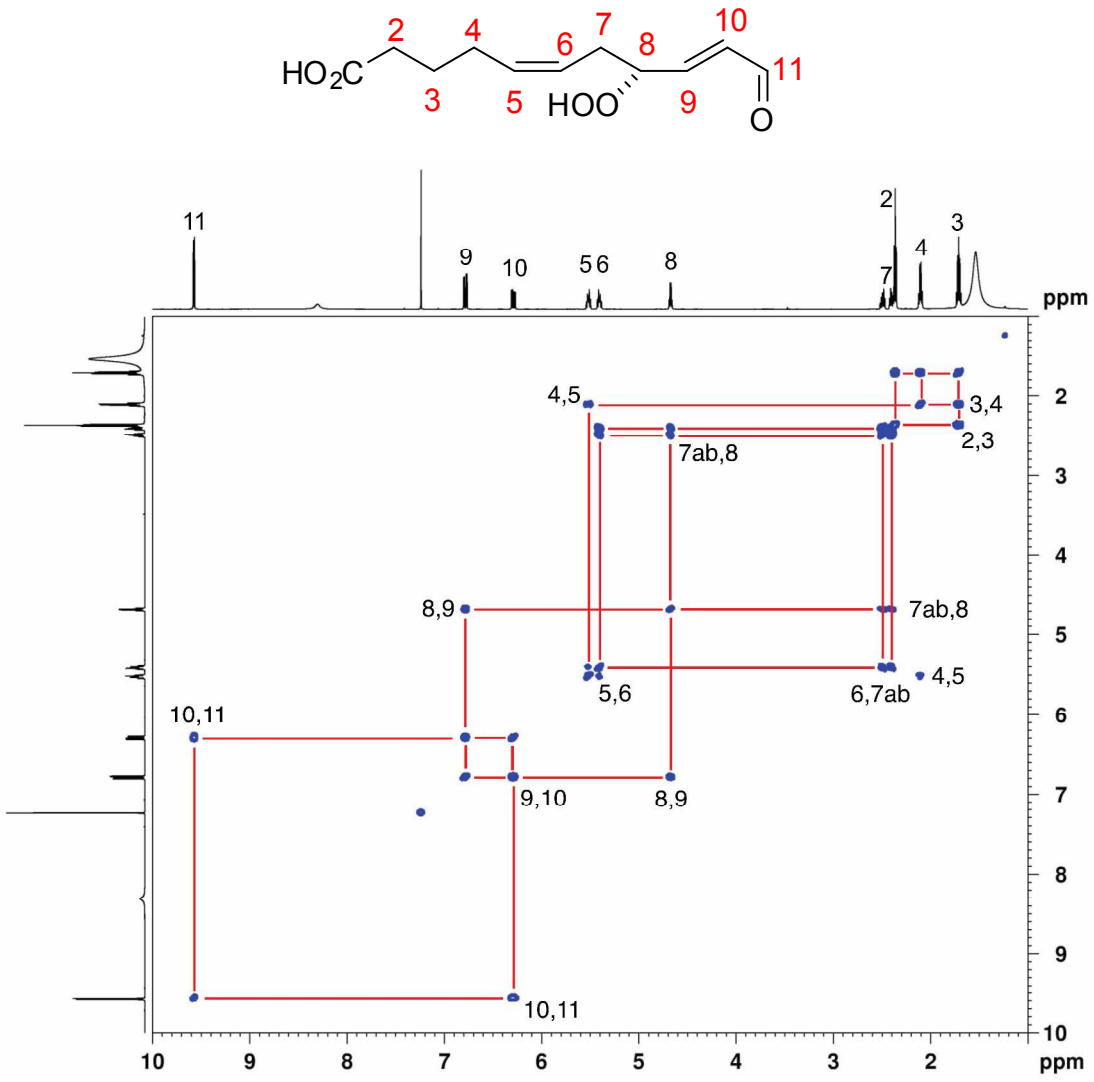


Figure S4: COSY NMR spectrum of 7-oxo-hept-5*E*-enoic acid (the second main component of peak 1 in Figure 1, main text). The spectrum was recorded in d6-benzene at 298K using a Bruker 600 MHz spectrometer.



7-oxo-hept-5*E*-enoic acid. ^{1}H-NMR, 600 MHz, $CDCl_3$, 283K, δ9.50, d, 1H, H7, $J_{6,7}$ = 7.8 Hz; 6.81, dt, 1H, H5, $J_{4,5}$ = 6.7 Hz, $J_{5,6}$ = 15.7 Hz; 6.13, dd, 1H, H6, $J_{5,6}$ = 15.7 Hz, $J_{6,7}$ = 7.8 Hz; 2.38-2.44, m, 4H, H2, H4; 1.86, p, 2H, H3, $J_{2,3}$ = 7.4 Hz, $J_{3,4}$ = 7.4 Hz.

Figure S5: COSY NMR spectrum of 8-hydroperoxy-11-oxo-undeca-5*Z*,9*E*-dienoic acid. The spectrum was recorded in d6-benzene at 298K using a Bruker 600 MHz spectrometer.



8-hydroperoxy-11-oxo-undeca-(5*Z*,9*E*)-dienoic acid. ^{1}H-NMR, 600 MHz, $CDCl_3$, 283K, δ9.57, d, 1H, H11, $J_{10,11}$ = 7.7 Hz; 6.78, dd, 1H, H9, $J_{8,9}$ = 6.0 Hz, $J_{9,10}$ = 16.0 Hz; 6.29, dd, 1H, H10, $J_{9,10}$ = 16.0 Hz, $J_{10,11}$ = 7.7 Hz; 5.52, dt, 1H, H5, $J_{4,5}$ = 7.2 Hz, $J_{5,6}$ = 10.9 Hz; 5.41, dt, 1H, H6, $J_{5,6}$ = 10.9 Hz, $J_{6,7}$ = 7.3 Hz; 4.67, q, 1H, H8, $J_{7,8}$ = 6.0 Hz, $J_{8,9}$ = 6.0 Hz; 2.49, m, 1H, H7a; 2.41, m, 1H, H7b; 2.36, t, 2H, H2, $J_{2,3}$ = 7.2 Hz; 2.10, q, 2H, H4, $J_{3,4}$ = 7.2 Hz, $J_{4,5}$ = 7.2 Hz; 1.71, p, 2H, H3, $J_{2,3}$ = 7.2 Hz, $J_{3,4}$ = 7.2 Hz.

Scheme S1: Mechanism of formation of 8*R*,15-diHETE (*t,t,t*) and 8*R*,15-diHPETE (*t,c,t* & *t,t,t*)

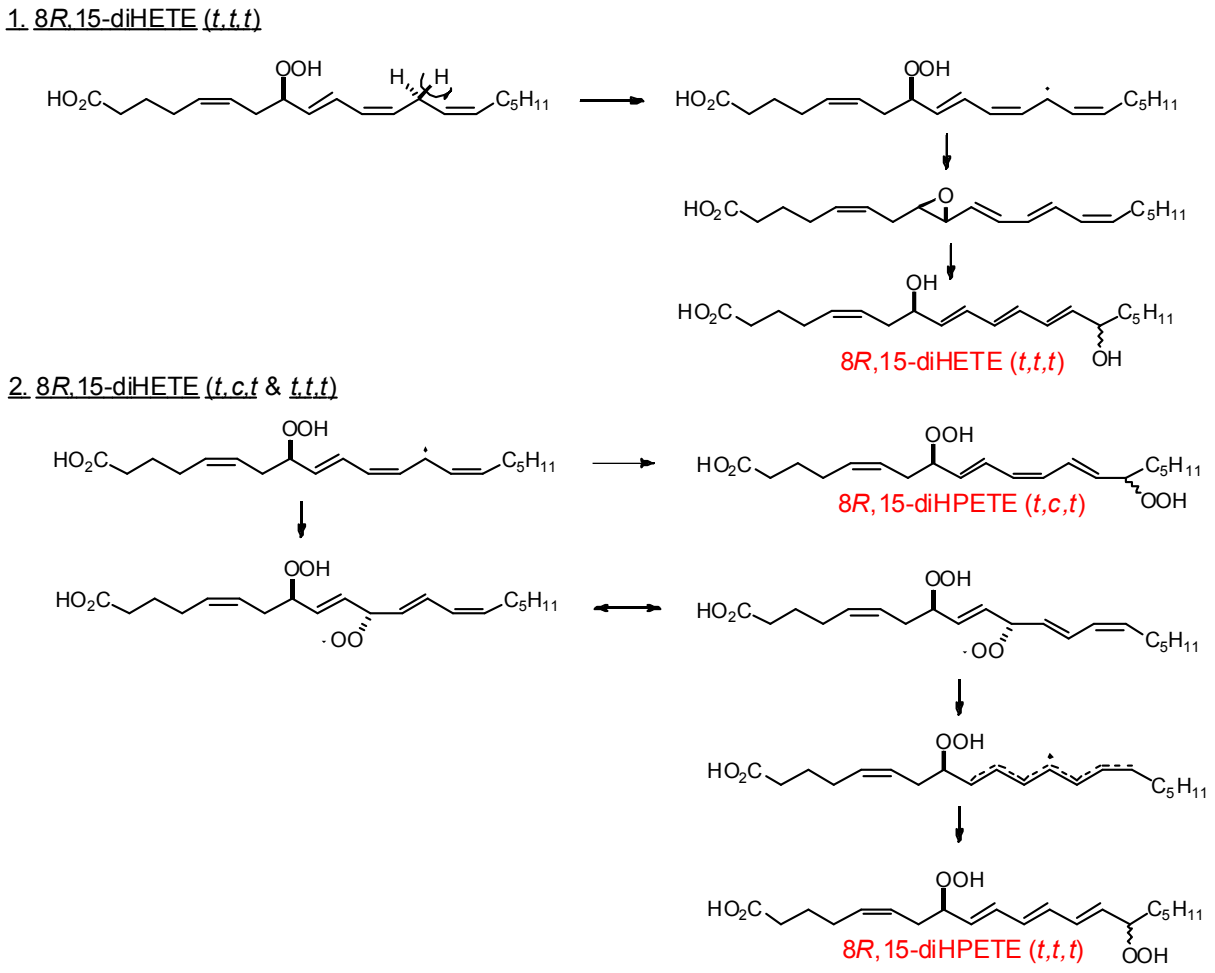


Figure S6: GC-MS analysis of 6-hydroperoxy-(2*E*,4*E*)-undecenal TPP-reduced TMS ether methoxime derivative.

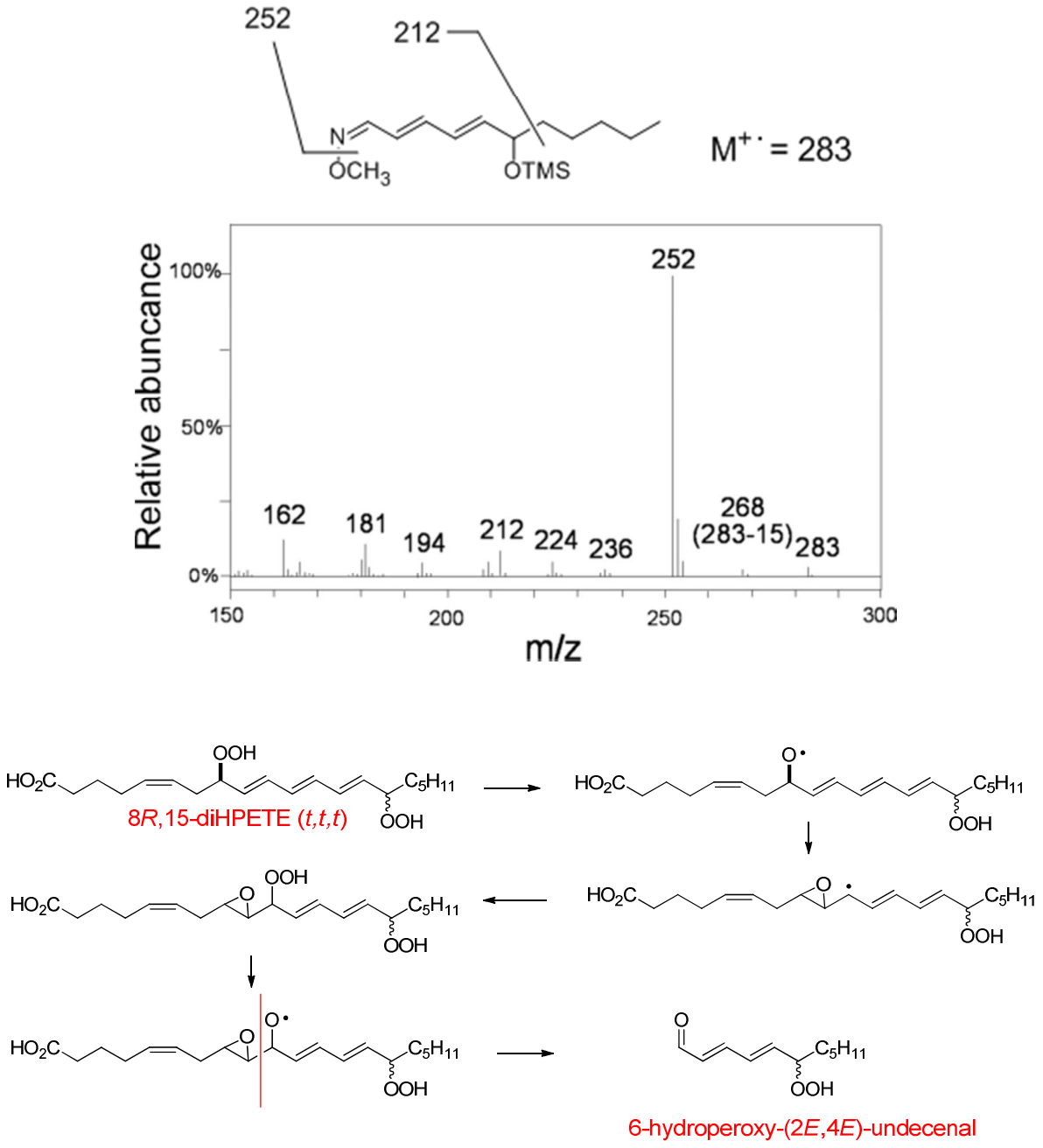
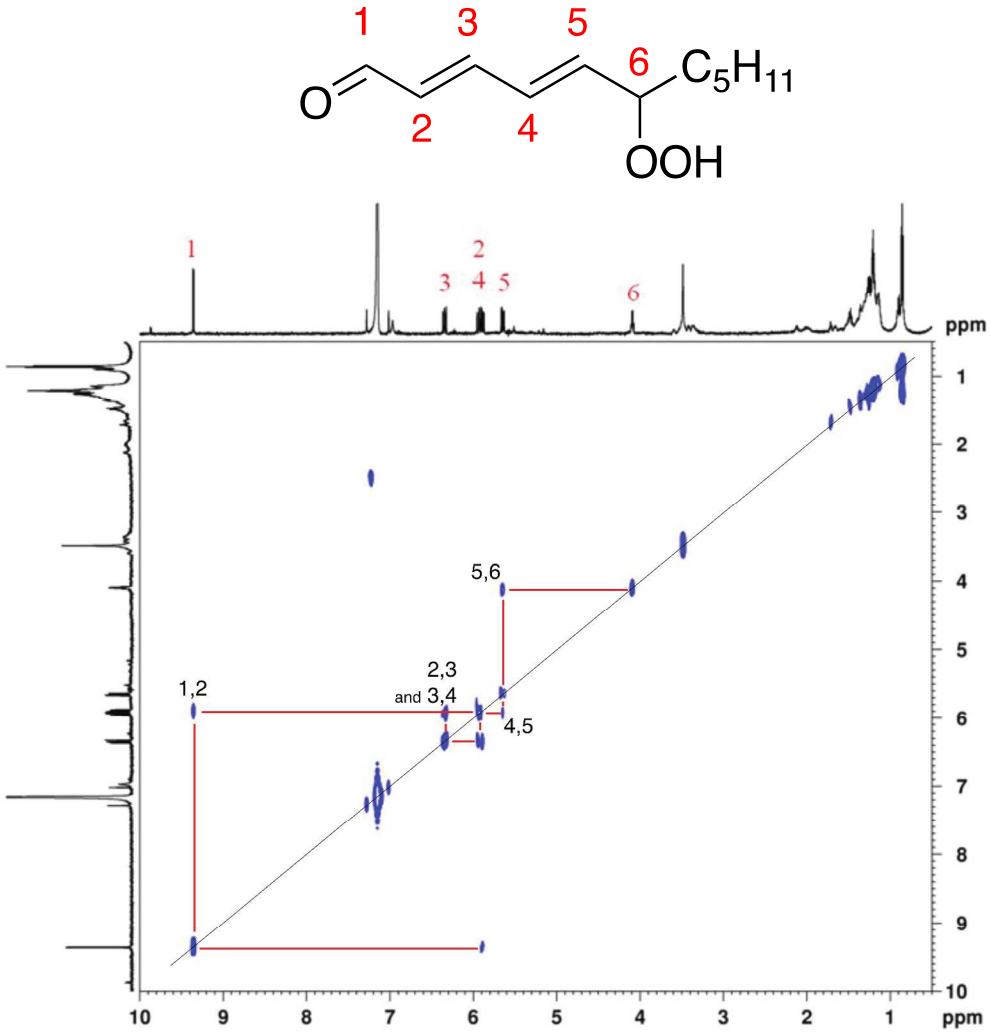
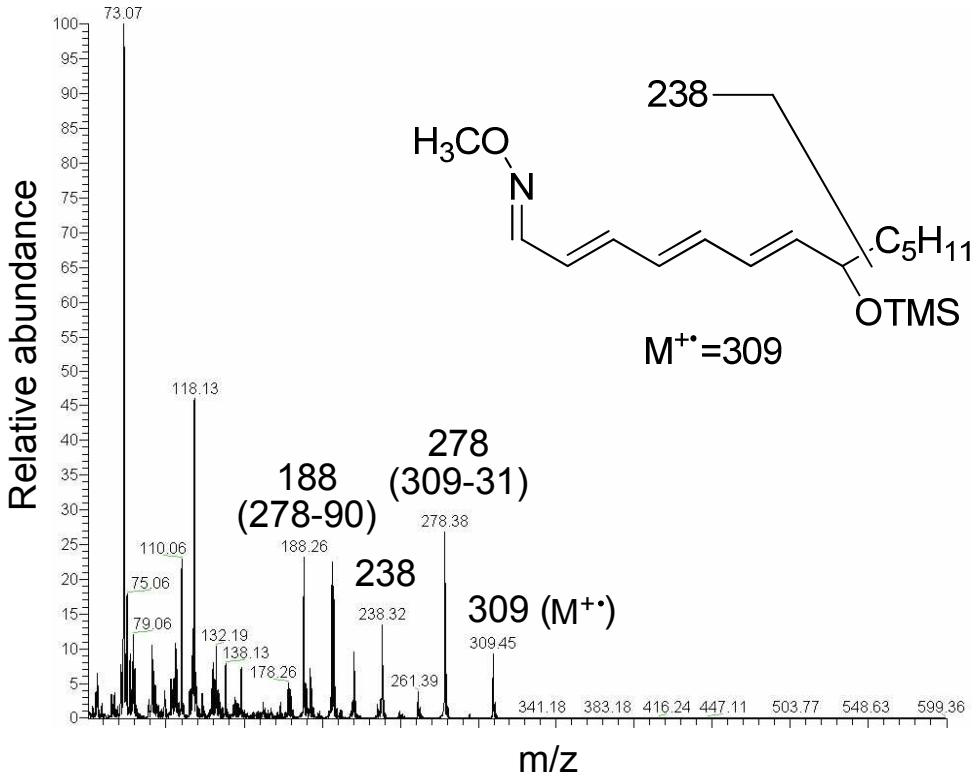


Figure S7: COSY NMR spectrum of 6-hydroperoxy-(2*E*,4*E*)-undecenal. The spectrum was recorded in d6-benzene at 298K using a Bruker 600 MHz spectrometer.



6-hydroperoxy-(2*E*,4*E*)-undecenal. ^{1}H-NMR, 600 MHz, C_6D_6, 283K, δ9.35, d, 1H, H1, $J_{1,2}$ = 7.7 Hz; 6.34, dd, 1H, H3, $J_{2,3}$ = 15.4 Hz, $J_{3,4}$ = 11.0 Hz; 5.86-5.98, m, 2H, H2,H4; 5.65, dd, 1H, H5, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = 7.3 Hz; 4.09, dt, 1H, H6, $J_{5,6}$ = 7.3 Hz; $J_{6,7}$ = 6.7 Hz.

Figure S8: GC-MS analysis of 8-hydroperoxy-(2,4,6)-tridecenal TPP-reduced TMS ether methoxime derivative



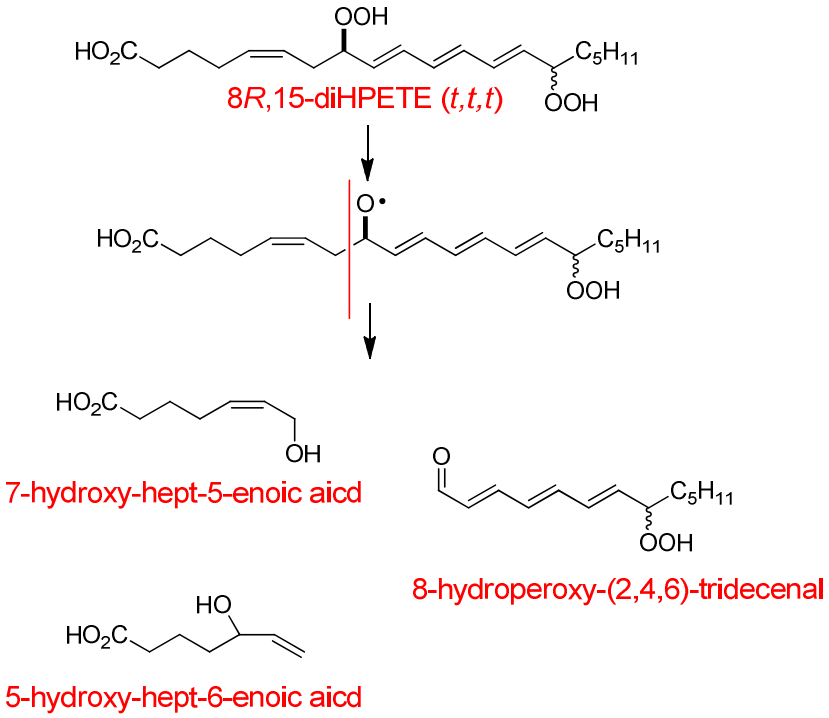
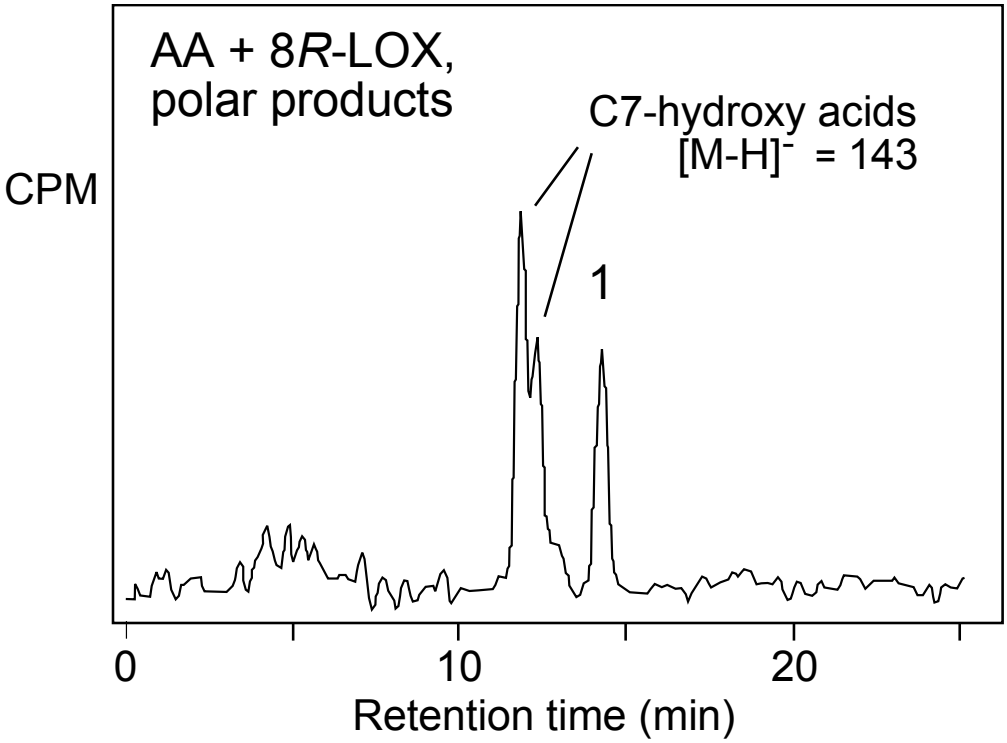


Figure S9: RP-HPLC of the reaction of [1-^{14}C]arachidonic acid with recombinant 8*R*-LOX after TPP treatment. Column: Waters Symmetry C18, 25 x 0.46 cm; solvent, CH_3CN/H_2O/HAc (10/90/0.01, by volume); flow rate, 1 ml/min; radioactive monitoring (Radiomatic Flo-One).



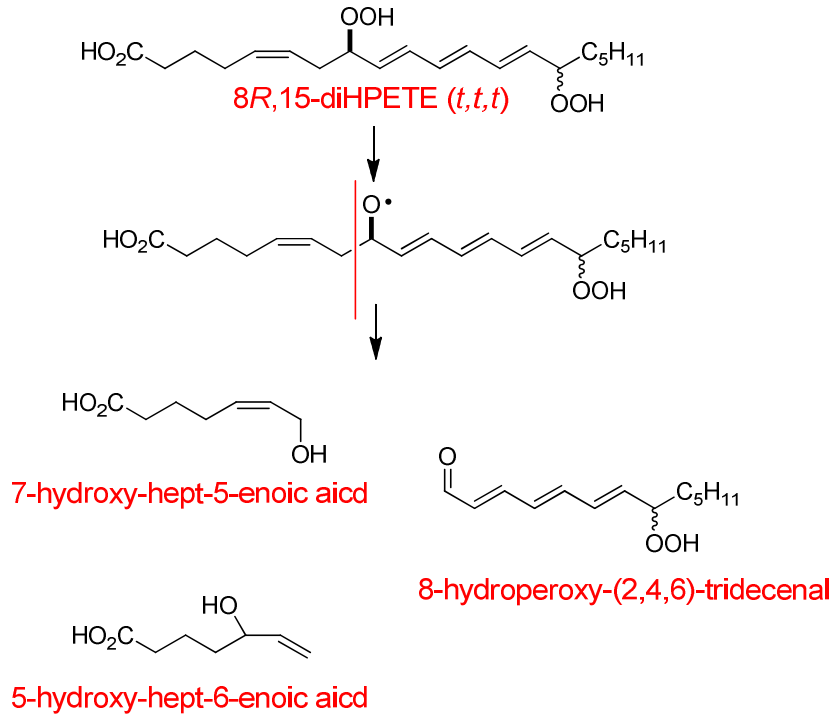


Figure S10: RP-HPLC analyses of room temperature reactions of 15*S*-HPETE with mouse platelet-type 12*S*-LOX (top) and human 15-LOX-1 (below). Column: Waters Symmetry C18, 25x 0.46 cm; solvent, CH_3CN/H_2O/HAc (45/55/0.01, by volume); flow rate, 1 ml/min; on-line diode array detection. (The retention time difference between the two chromatograms is due to the use of different columns).

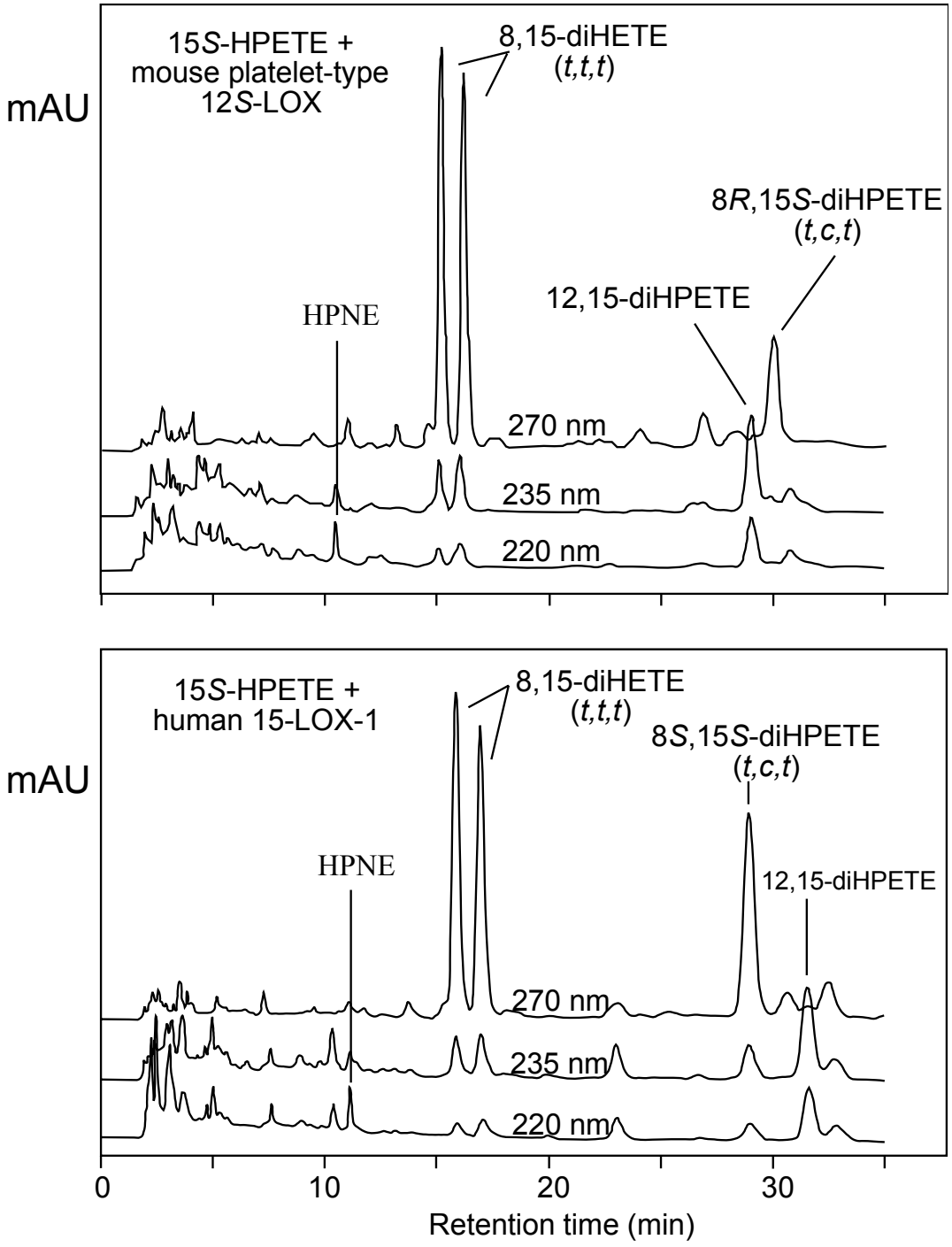
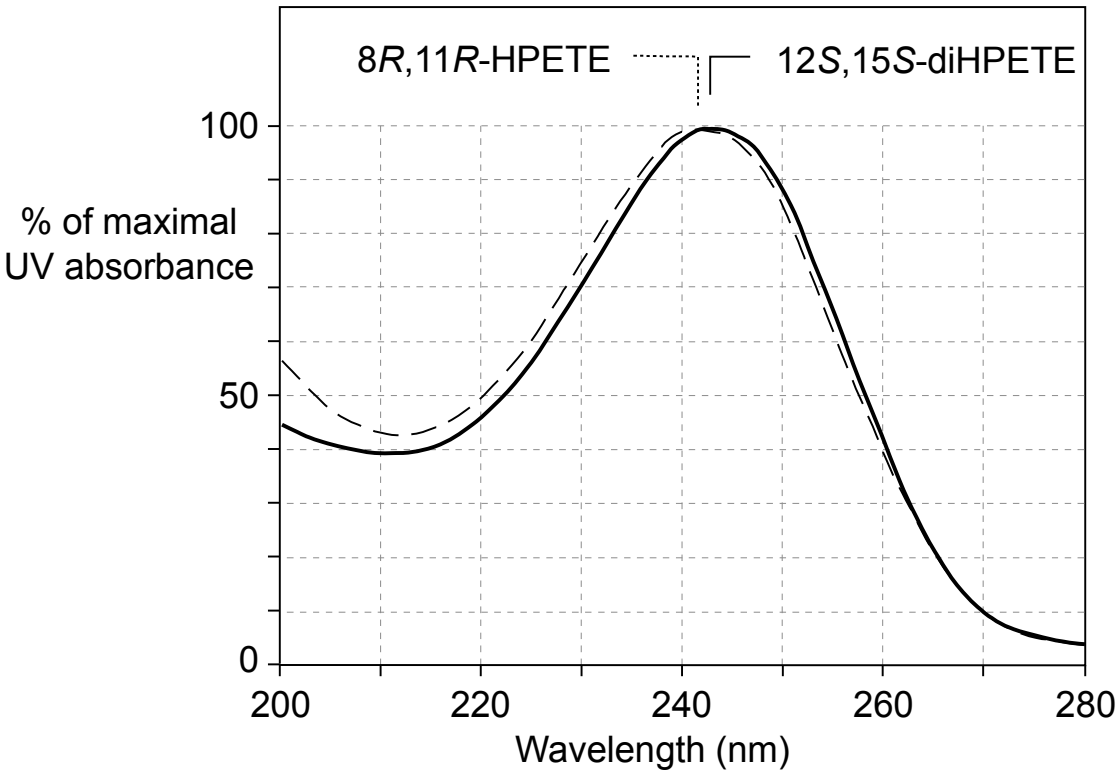
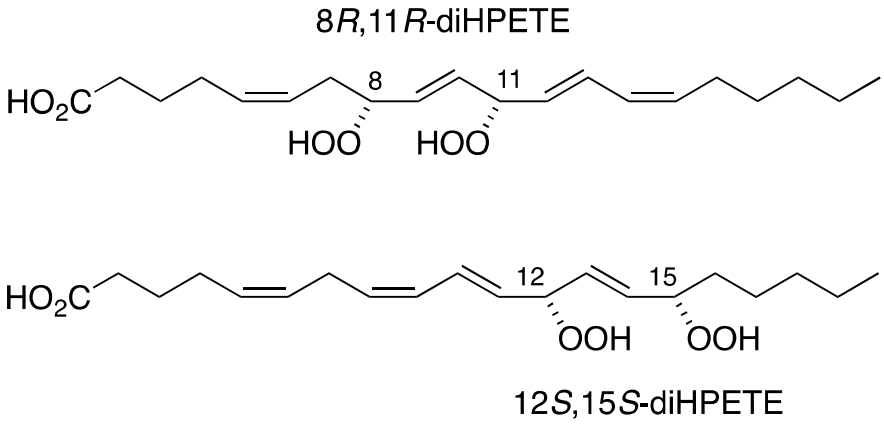


Figure S11: Overlay of the UV spectra of 8*R*,11*R*-HPETE and 12*S*,15*S*-diHPETE





Supplementary References

(1) Lin, J., Welti, D. H., Vera, F. A., Fay, L. B., and Blank, I. (1999) Synthesis of deuterated volatile lipid degradation products to be used as internal standards in isotope dilution assays. 2. Vinyl ketones. *J. Agric. Food Chem.*, *47*, 2822-2829.

(2) Cheng, Y., Huynh-Ba, T., Blank, I., and Robert, F. (2008) Temporal changes in aroma release of Longjing tea infusion: interaction of volatile and nonvolatile tea components and formation of 2-butyl-2-octenal upon aging. *J. Agric. Food Chem.*, *56*, 2160-2169.

(3) Weerapana, E., Simon, G. M., and Cravatt, B. F. (2008) Disparate proteome reactivity profiles of carbon electrophiles. *Nature Chem. Biol.*, *4*, 405-407.

(4) Lin, J., Fay, L. B., Welti, D. H., and Blank, I. (2001) Quantification of key odorants formed by autoxidation of arachidonic acid using isotope dilution assay. *Lipids*, *36*, 749-756.