# Supplementary data

A fungal catalase reacts selectively with the 13*S* fatty acid hydroperoxide products of the adjacent lipoxygenase gene and exhibits 13*S*-hydroperoxide-dependent peroxidase activity

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Figure S1: **Chiral column analysis of the 13-HPODE product of Fg-LOX from [14C]Linoleic Acid, analyzed as 13-HODE Me ester**
Chiralpak AD column (25 x 0.46 cm), solvent Hexane/MeOH (100:5 by volume), flow rate 1 ml/min. Unlabelled racemic 13-HODE Me ester was added to the 14C-labelled product and the 14C and UV (235 nm) profiles recorded. The x-axis of the 14C recording is expanded to match the retention time scale of the UV profile.
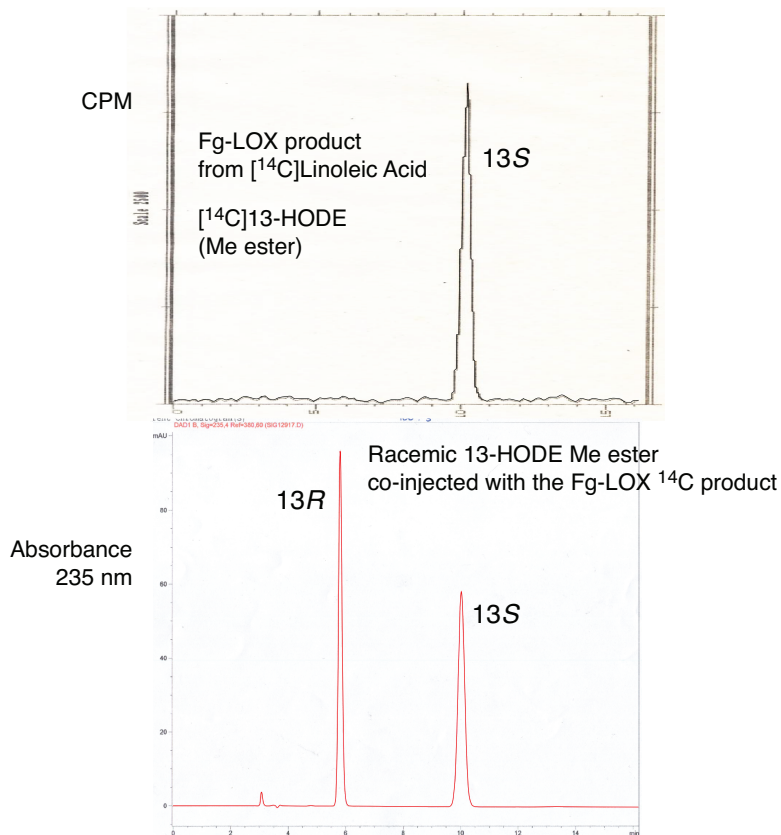
Figure S2: **Chiral column analysis of the 15-HPETE product of Fg-LOX from [14C]Arachidonic Acid, analyzed as 15-HETE Me ester**
Chiralpak AD column (25 x 0.46 cm), solvent Hexane/MeOH (100:2 by volume), flow rate 1 ml/min. Unlabelled racemic 15-HETE Me ester was added to the 14C-labelled product and the 14C and UV (235 nm) profiles recorded. The x-axis of the 14C recording is adjusted to match the retention time scale with the UV profile.
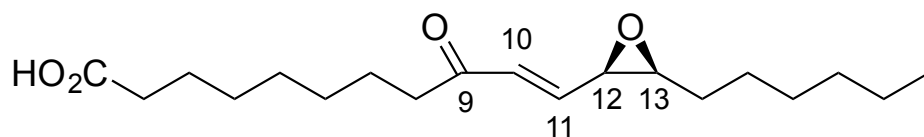

2
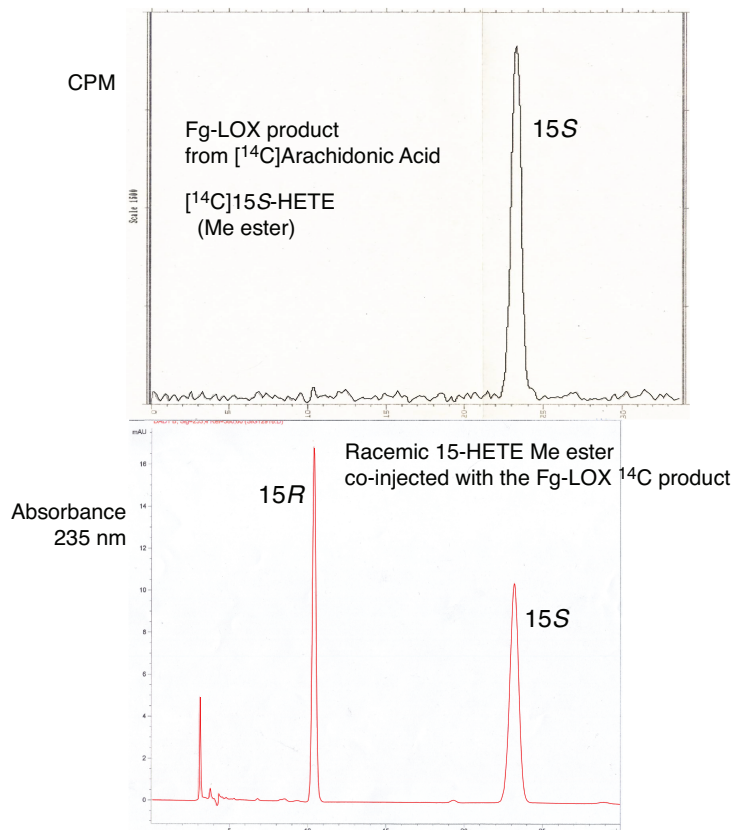
Table S1: **9-oxo-10*E*-12,13-*cis*-epoxide-C18:1, CDCl₃, 400 MHz (Fig. 4, main text)**

Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H11	6.63	$J_{10,11}$ = 15.9 (*trans*) $J_{11,12}$ = 6.6	dd	1
H10	6.38	$J_{10,11}$ = 15.9 (*trans*)	d	1
H12	3.50	$J_{11,12}$ = 6.2 $J_{12,13}$ = 4.9 (*cis epoxy*)	dd	1
H13	3.18	$J_{12,13}$ = 5.0(*cis-epoxy*) $J_{13,14}$ = 5.4	dt	1
H8	2.52	$J_{7,8}$ = 7.4	t	2
H2	2.33	$J_{2,3}$ = 7.4	t	2
H18	0.87	$J_{17,18}$ = 6.9	t	3
Additional signals at ca. 1.5 to 1.65 ppm were not discernable				

Table S2: **The minor *trans*-epoxy isomer - 9-oxo-10*E*-12,13-*trans*-epoxide-C18:1, CDCl₃, 400 MHz (Fig. 4, main text)**
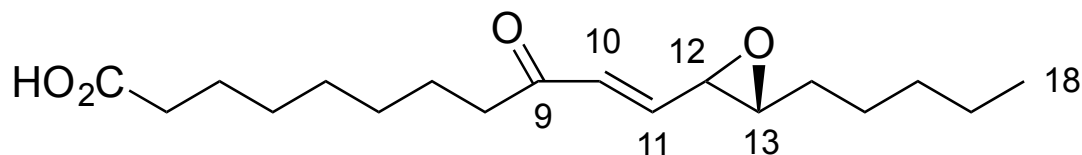
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H11	6.49	$J_{10,11}$ = 16.0 (*trans*) $J_{11,12}$ = 7.1	dd	1
H10	6.35	$J_{10,11}$ = 15.9 (*trans*)	d	1
H12	3.19	$J_{11,12}$ = 6.9 $J_{12,13}$ = 1.5 (*trans epoxy*)	dd	1
H13	2.89	$J_{12,13}$ = 1.7 (*trans epoxy*) $J_{13,14}$ = 5.5	dt	1
H8	2.51	$J_{7,8}$ = 7.5 $J_{6,8}$ = 2.5	dt	2
H2	2.33	$J_{2,3}$ = 7.4	t	2
H18	0.87	$J_{17,18}$ = 7.8	t	3
Additional signals at ca. 1.5 to 1.6 ppm were not discernable				

Table S3: Product 1 (Fig 5, main text) 13-oxo-11*E*-9,10-*trans*-epoxy-C13:1, CDCl$_3$, 400 MHz
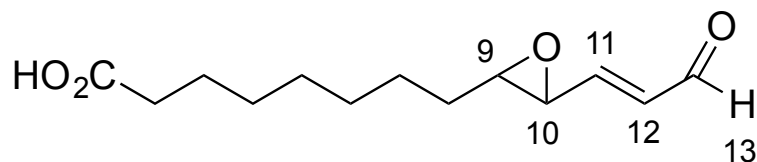
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H13	9.54	$J_{12,13} = 7.7$	d	1
H11	6.52	$J_{11,12} = 15.8$ (*trans*) $J_{10,11} = 6.9$	dd	1
H12	6.37	$J_{11,12} = 15.8$ (*trans*) $J_{12,13} = 7.6$	dd	1
H10	3.30	$J_{10,11} = 7.0$ $J_{9,10} = 1.8$ (*trans epoxy*)	dd	1
H9	2.93	$J_{8,9} = 5.7$ $J_{9,10} = 1.9$ (*trans epoxy*)	dt	1
H2	2.34	$J_{2,3} = 7.5$	t	2
Additional signals at ca. 1.4 to 1.65 ppm were not discernable				

Table S4: Product 2 (Fig 5, main text) 9-oxo-10*E*-12,13-*trans*-epoxy-15,16-*trans*-epoxy-C18:1, CDCl₃, 400 MHz
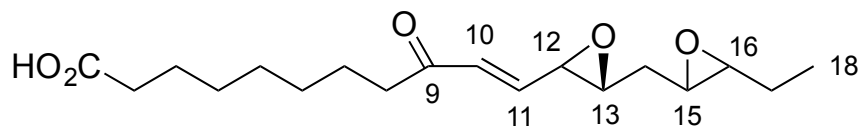
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H11	6.49	$J_{10,11}$ = 16.0 (*trans*) $J_{11,12}$ = 6.7	dd	1
H10	6.39	$J_{10,11}$ = 16.0 (*trans*)	d	1
H12	3.36	$J_{11,12}$ = 6.7 $J_{12,13}$ = 2.0 (*trans epoxy*)	dd	1
H13	3.03	$J_{12,13}$ = 2.0 (*trans epoxy*) $J_{13,14}$ = 5.3	dt	1
H15	2.82	$J_{14a,15}$ = 3.8 $J_{14b,15}$ = 6.2 $J_{15,16}$ = 2.3 (*trans epoxy*)	ddd	1
H16	2.77	$J_{16,17}$ = 5.5 $J_{15,16}$ = 2.2 (*trans epoxy*)	dt	1
H8	2.51	$J_{7,8}$ = 7.4	t	2
H2	2.33	$J_{2,3}$ = 7.4	t	2
H14a	2.10	$J_{14a,14b}$ = 15.0 $J_{14a,13}$ = 4.2 $J_{14a,15}$ = 4.2	ddd	1
H14b	1.81	$J_{14a,14b}$ = 15.2 $J_{14b,13}$ = 5.9 $J_{14b,15}$ = 5.9	ddd	1
H18	0.98	$J_{17,18}$ = 7.5	t	3
Additional signals at ca. 1.55 to 1.62 ppm were not discernable				

Table S5: Product 3 (Fig. 5, main text) 13-oxo-9Z,11E-C13:1, C_6D_6, 600 MHz – (see COSY)
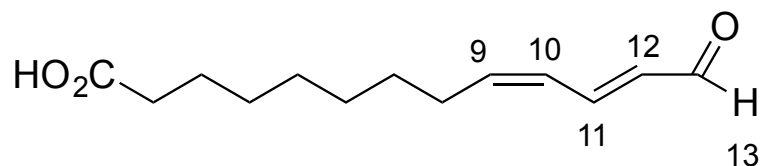
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H13	9.59	$J_{12,13}$ = 7.9	d	1
H11	7.42	$J_{10,11}$ = 11.5 $J_{11,12}$ ~ 15 (*trans*)	dd	1
H10	6.25	$J_{9,10}$ = $J_{10,11}$ = 11.2 (9,10-*cis*)	t	1
H12	6.14	$J_{12,13}$ = 7.9 $J_{11,12}$ = 15.2 (*trans*)	dd	1
H9	5.98	$J_{9,10}$ = 10.6 (*cis*) $J_{8,9}$ = 7.9	dt	1
H2	2.33	$J_{2,3}$ = 7.5	t	2
H8	2.31	$J_{8,9}$ ~ $J_{7,8}$ = 7.9	dt	2
H3	1.64		quintet	2
H7	1.44		quintet	2
H4-H6	1.3 – 1.5		m	(6)

Figure S3: COSY spectrum of Product 3
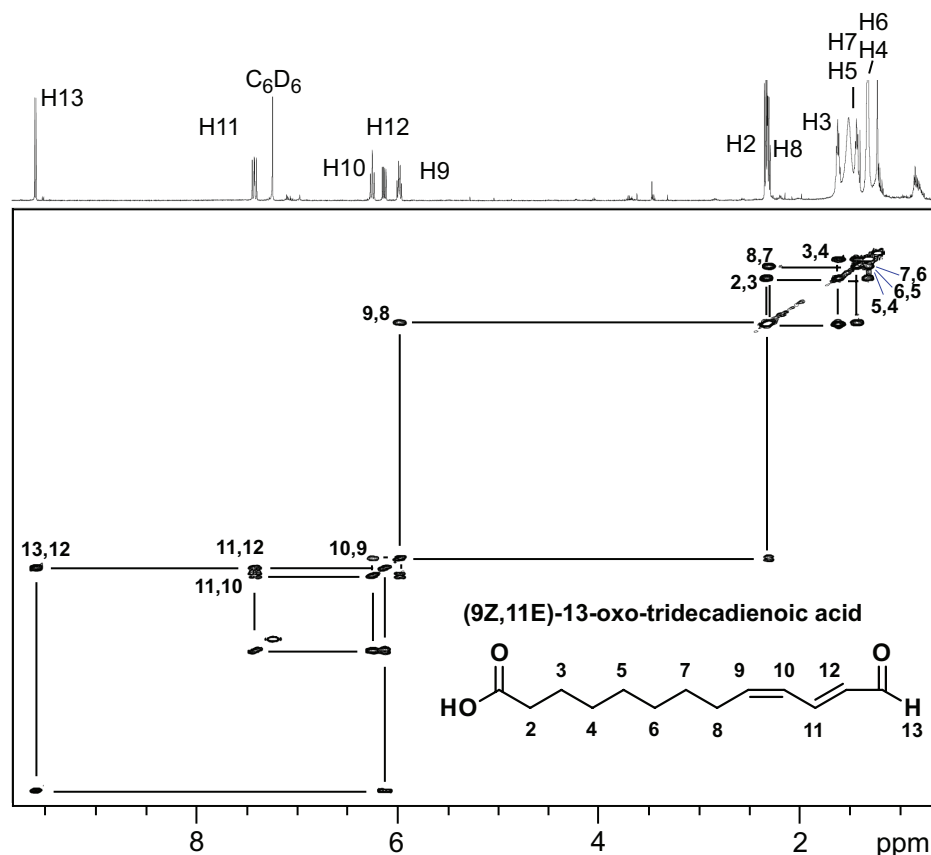
Table S6: Product 4 (Fig 5, main text) 9-oxo-10*E*,15*Z*-12,13-*cis*-epoxy-C18:2, CDCl$_3$, 400 MHz
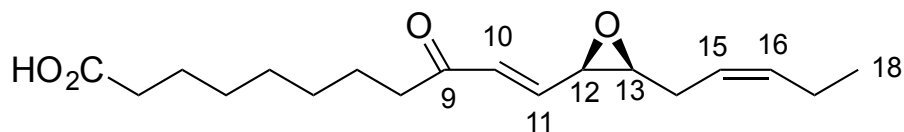
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H11	6.67	$J_{10,11}$ = 15.8 (*trans*) $J_{11,12}$ = 6.3	dd	1
H10	6.40	$J_{10,11}$ = 15.8 (*trans*)	d	1
H16	5.52	$J_{15,16}$ = ~10 (*cis*) $J_{16,17}$ = 7.4	dt (broad)	1
H15	5.31	$J_{15,16}$ = ~10 (*cis*) $J_{15,14}$ = 7.7	dt (broad)	1
H12	3.53	$J_{12,13}$ = 4.9 (*cis epoxy*) $J_{12,11}$ = 5.6	dd	1
H13	3.20	$J_{12,13}$ = 4.8 (*cis epoxy*) $J_{13,14}$ = 6.1	dt	1
H8	2.52	$J_{7,8}$ = 7.4	t	2
H14a	2.39	$J_{14a,14b}$ = 15.0 $J_{14a,13}$ = 6.7 $J_{14a,15}$ = 7.4	ddd	1
H2	2.33	$J_{2,3}$ = 7.4	t	2
H14b	2.17	$J_{14a,14b}$ = 14.8 $J_{14b,13}$ = 6.9 $J_{14b,15}$ = 7.5	ddd	1
H17	2.01	J = 7.4	q	2
H18	0.94	$J_{17,18}$ = 7.5	t	3
Additional signals at ca. 1.5 to 1.65 ppm were not discernable				

<u>Figure S4</u>: Kinetic analysis of ABTS oxidation by Fg-cat using 13*S*-HPOTE as co-substrate

Reactions were conducted in 25 mM phosphate pH 7.5 containing 150 mM NaCl using an Agilent Cary 60 spectrophotometer which allowed rapid acquisition of reaction rates after addition of enzyme (within 1 sec). ABTS concentration was held at 1 mM and concentrations of 13*S*-HPOTE were varied as indicated. The calculated parameters using Prism software were Km = 25.1 μM for 13*S*-HPOTE and for ABTS oxidation kcat = 552 s^{-1}.

Initial rate
AU/min

Vmax = 7.7 AU/min
kcat = 552 s^{-1}

K_M = 25 μM

μM 13*S*-HPOTE
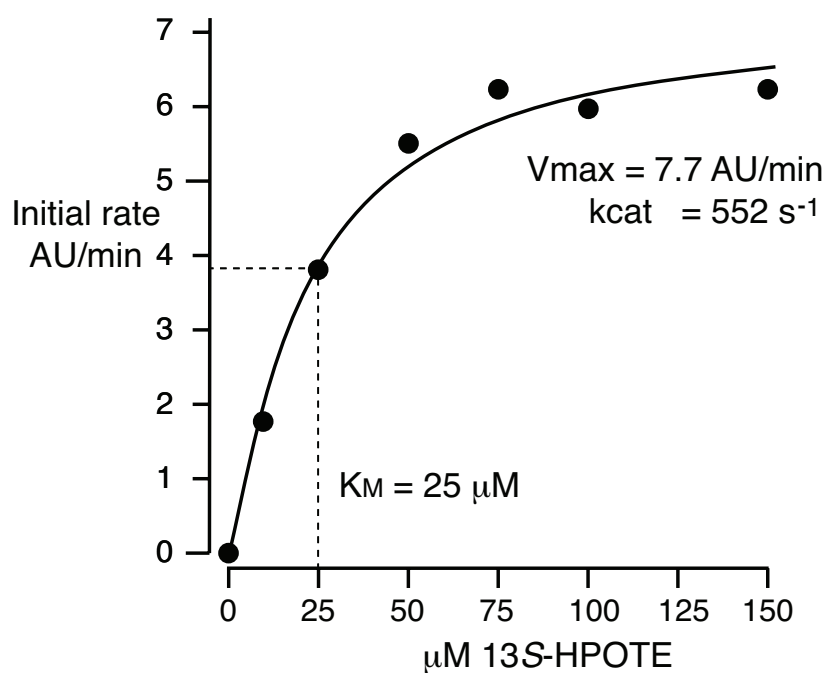
Figure S5: Separation of the peak of diepoxy-hydroperoxides from Figure 8, main text (products 5a, 5b, 5c) by straight-phase HPLC of the methyl ester derivatives

Thomson 5μ silica column (25 x 0.46 cm), Hex/IPA 100:2.5, 1 mL/min
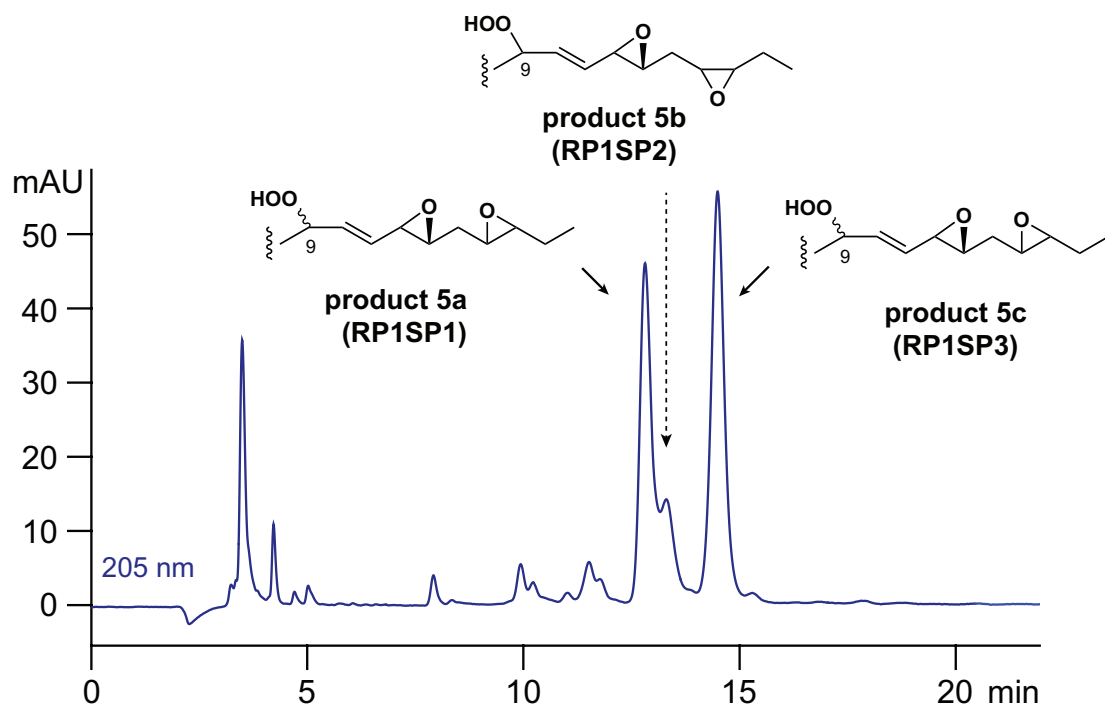
Figure S6: Separation of the peak of mono-epoxy-hydroperoxides from Figure 8 , main text (products 6a, 6b) by straight-phase HPLC of the methyl ester derivatives

Thomson 5μ silica column (25 x 0.46 cm), Hex/IPA 100:1, 1 mL/min

(The unequal abundance of products of 6a and 6b suggests some interaction with Fg-cat during their formation).

Retention time (min)
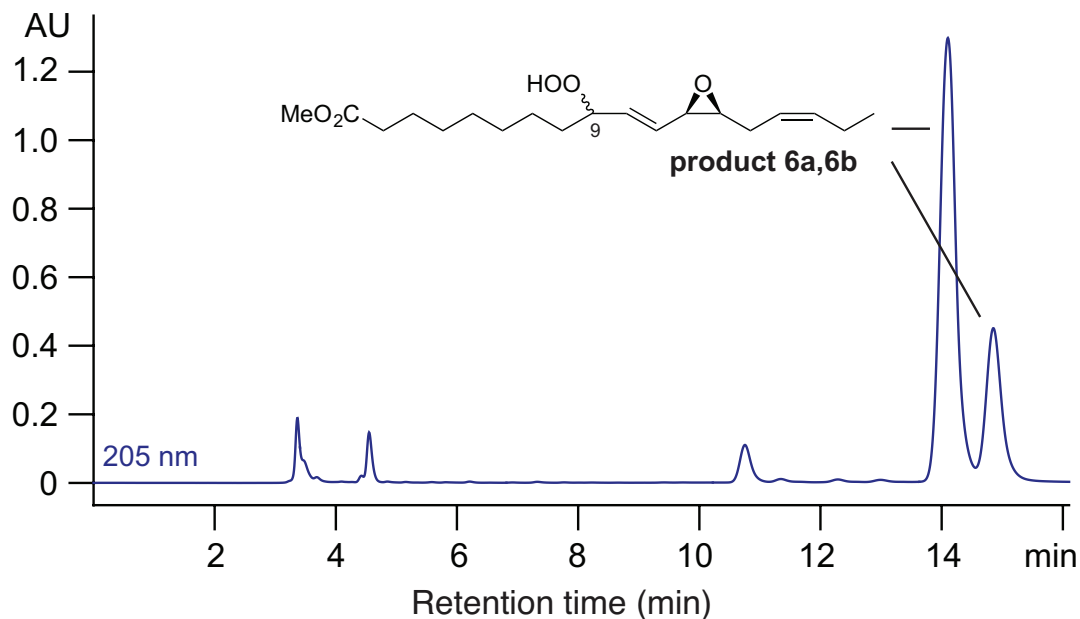
9-OOH-12,13-15,16-diepoxide (C18:1) in C6D6   RP1SP1, product 5a

9-OOH-12,13-15,16-diepoxide (C18:1)   RP1SP3, product 5c

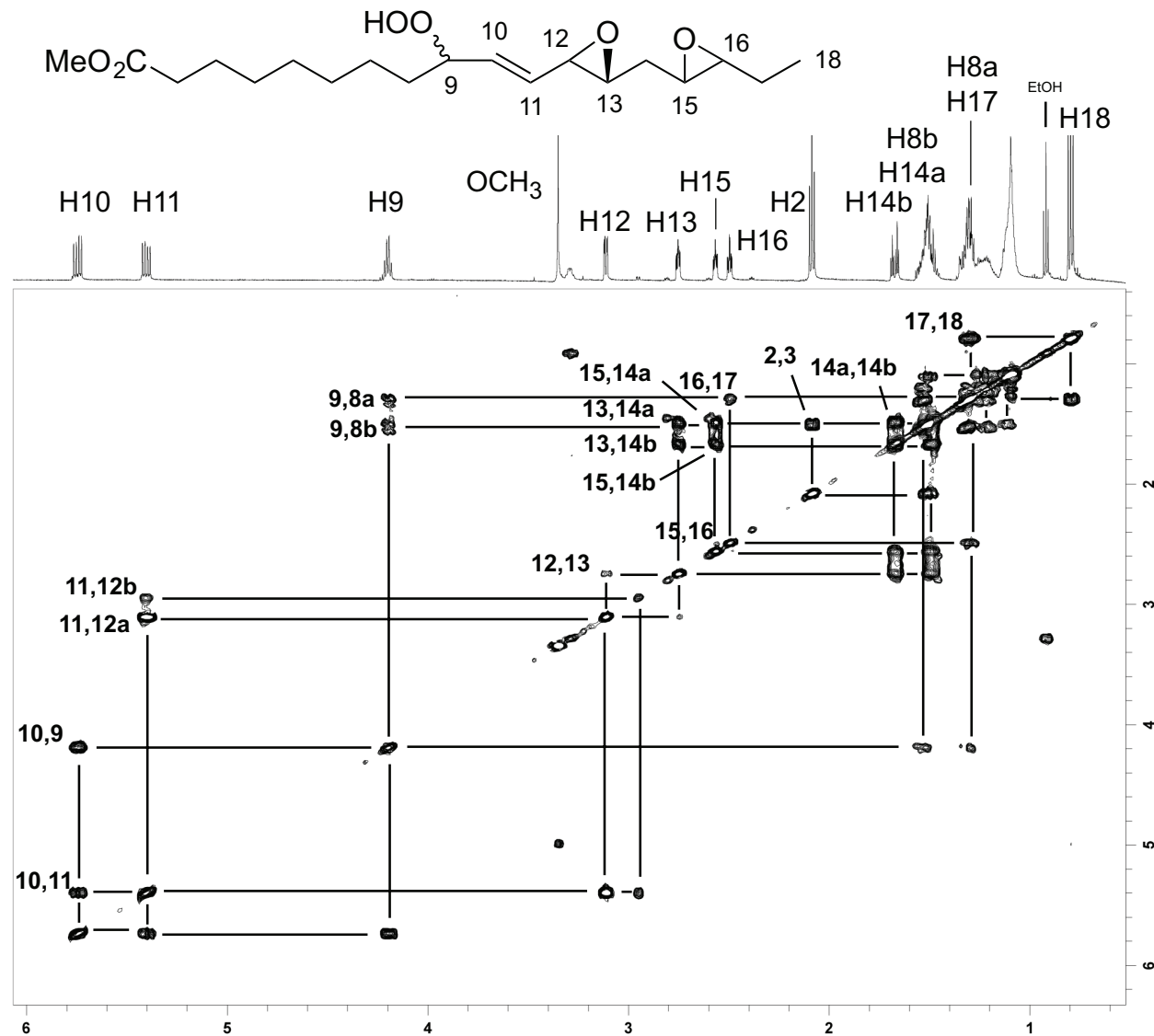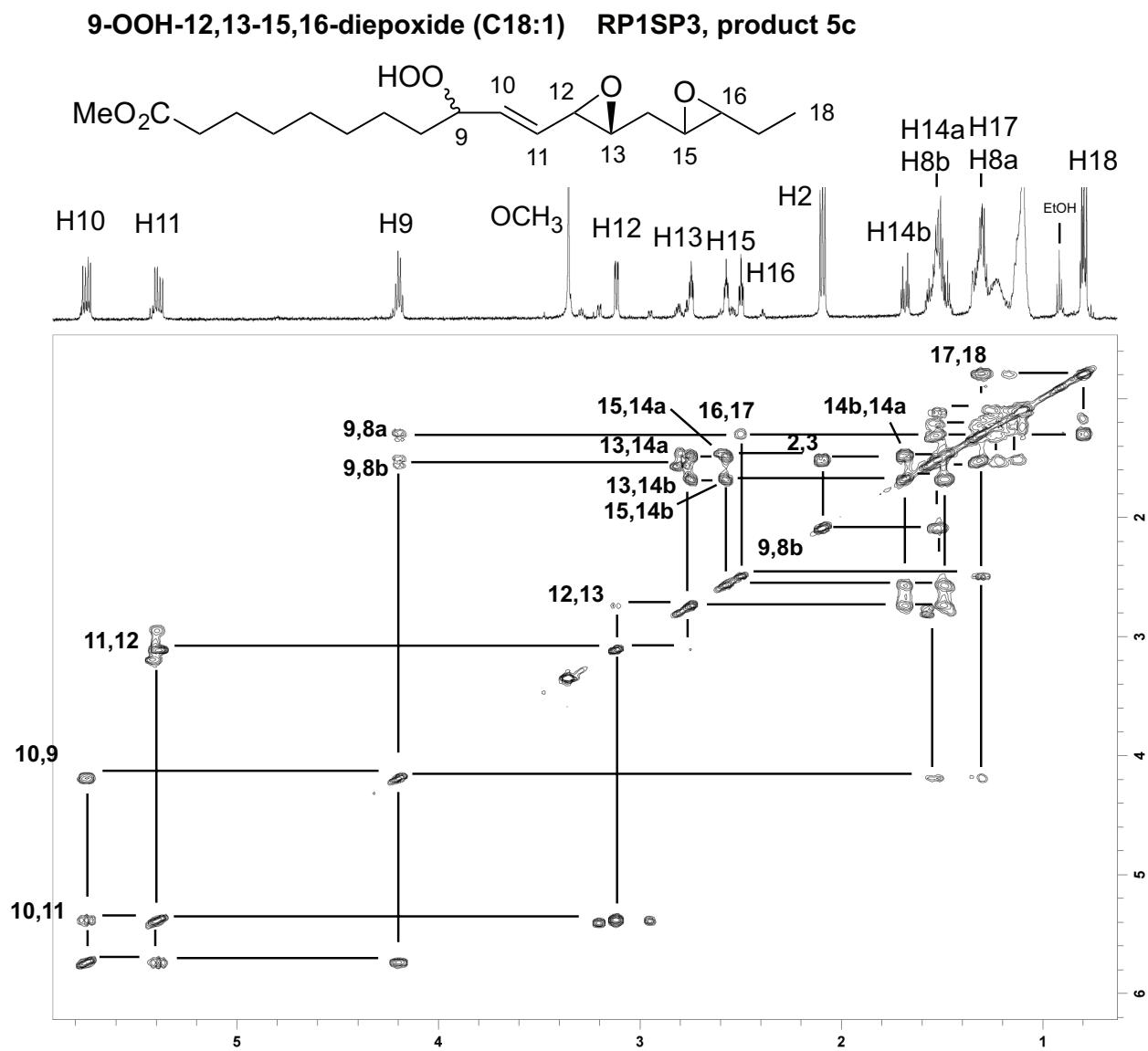**Table S7:** **9-hydroperoxy-10***E***-12,13-epoxy-15,16-diepoxy-C18:1 in C$_6$D$_6$, 600 MHz (RP1SP1, product 5a, Me ester, COSY as Fig. S6)***
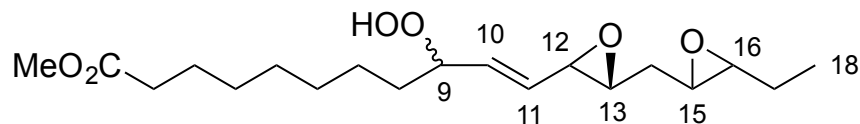
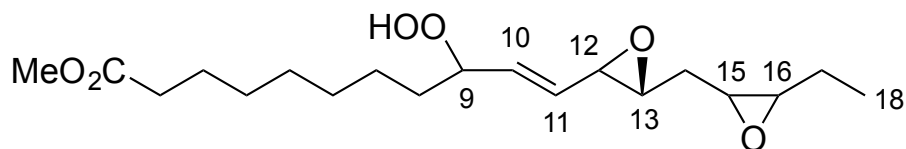| Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H10	5.85	$J_{10,11}$ = 7.6 $J_{10,9}$ = 15.7 (*trans*)	dd	1
H11	5.40	$J_{11,10}$ = 7.6 $J_{11,12}$ = 15.7 (*trans*)	dd	1
H9	4.20	$J_{8,9}$ = $J_{9,10}$ = 6.8	q (dt)	1
-OMe	3.35		s	3
H12	3.11	$J_{12,13}$ = 2.2 (*trans-epoxy*) $J_{12,14}$ = 7.6	dd	1
H13	2.75	$J_{13,12}$ = 2.1 (*trans-epoxy*) $J_{13,14}$ = 4.3, 5.5	ddd	1
H15	2.57	$J_{15,16}$ = 2.2 (*trans-epoxy*) $J_{14,15}$ = 4.4, 5.8	ddd	1
H16	2,49	$J_{15,16}$ = 2.1 (*trans-epoxy*) $J_{16,17}$ = 5.5	dt (ddd)	1
H2	2.08	$J_{2,3}$ = 7.5	t	2
H14b	1.67	$J_{14,15}$ = $J_{14,15}$ 4.3 $J_{14a,14b}$ = 14.8	dt	
H14a, H3, H8b	1.45 – 1.57		m	(5)
H17, H8a	1.3		m	(5)
H4 – H7	1.05 – 1.26		m	(8)
H18	0.79	7.5	t	3

* (RP1SP3, product 5c, Me ester) – see COSY, Fig. S7
9-hydroperoxy-10*E-trans*-12,13-epoxy-*trans*-15,16-epoxy-C18:1 (RP1SP3)
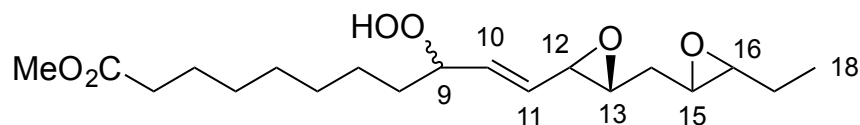
RP1SP3 is an isomer of RP1SP1 with almost identical NMR values and coupling constants – probably a C-9 hydroperoxide epimer
(data not tabulated – see COSY Fig. S7).

Table S8: 9-hydroperoxy-*trans*-12,13-epoxy-*cis*- 15,16-epoxy-C18:1 in C_6D_6, 600 MHz (RP1SP2) (the minor isomer, **RP1SP2, product 5b, Me ester**)

Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H10	5.76	$J_{9,10} = 7.5$ $J_{10,11} = 15.7$ (*trans*)	dd	1
H11	5,42	$J_{11,12} = 7.5$ $J_{10,11} = 15.7$ (*trans*)	dd	1
H9	4.21	$J = 6.8$	q	1
-OMe	3.35		s	3
H12	3.20	$J_{12,13} = 1.9$ (*trans-epoxy*) $J_{11,12} = 7.6$	dd	1
H15	2.81	$J_{15,16} = 5.0$ (*cis-epoxy*) $J_{14,15} = 7.2$	ddd	1
H13	2.77	$J_{12,13} = 2.0$ (*trans-epoxy*) $J_{13,14} = 4.5$	dt	1
H16 (and ?)	2.56	(overapping signals - not discernable)	m	2
H2(and H14a?)	2.09		t and m	
H3 (and H8?)	1.45 – 1.6		m	(4+)
H4 –H7, H17	1.05 – 1.35		m	(10)
H18	0.78	7.5	t	3

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Table S9: 9-hydroperoxy-12,13-epoxy-C18:2 in C_6D_6, 600 MHz (RP4SP1, product 6a, Me ester)
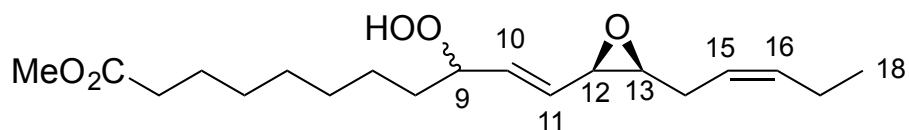
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H10	5.77	$J_{9,10}$ = 7.6 $J_{10,11}$ = 15.7 (*trans*)	dd	1
H11	5.6	$J_{11,12}$ = 6.8 $J_{10,11}$ = 15.7 (*trans*)	dd	1
H16	5.46	(typical *cis*)	m	1
H15	5.38	(typical *cis*)	m	1
H9	4.22	$J_{8,9}$ = $J_{9,10}$ = ~7	q	1
-OMe	3.35		s	3
H12	3.20	$J_{12,13}$ = 4.3 (*cis epoxy*) $J_{11,12}$ = 6.5	dd	1
H13	2.88	$J_{12,13}$ = 4.2 (*cis epoxy*) $J_{13,14}$ = 6.3	dt	1
H14b	2.36		m	1
H14a	2.12		m	1
H2	2.09	$J_{2,3}$ = 7.4	t	2
H17	1.92	$J_{16,17}$ = $J_{17,18}$ = 7.4	quintet (dq)	2
H8b	1.55		m	1
H3	1.52		quintet	2
H4 – H7	1.07 – 1.27		4	8
H18	0,87	7.6	t	3

Table S10: **9-hydroperoxy-12,13-*cis*-epoxy-15Z-C18:2 in C_6D_6, 600 MHz (RP4SP2, product 6b, Me ester)**
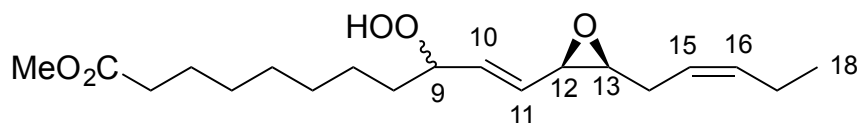
Hydrogen	Chemical shift (δ)	Coupling constant (Hz)	Multiplicity	Number of protons
H10	5.76	$J_{9,10} \sim 7.3$ $J_{10,11} \sim 15.8$ (*trans*)	dd	1
H11	5.6	$J_{11,12} = 6.8$ $J_{10,11} = 15.6$ (*trans*)	dd	1
H16	5.46	$J_{15,16} \sim 11$	m	1
H15	5.39	$J_{15,16} \sim 11$	m	1
H9	4.22	$J_{8,9} = J_{9,10}$ ~ 7	q (dt)	1
-OMe	3.35		s	3
H12	3.20	$J_{11,12} = 6.6$ $J_{12,13} = 4.3$ (*cis epoxy*)	dd	1
H13	2.88	$J_{12,13} = 4.1$ (*cis epoxy*) $J_{12,14} = 6.3$	ddd	1
H14b	2.34		m	1
H14a	2,12		m	1
H2	2.09	$J_{2,3} = 7.5$		
H17	1.92	$J_{16,17} \sim J_{17,18}$ ~ 7.5	p (dq)	2
H3, H8b	1.52		m	1
H3	1.55		m	2
H8a	1.34		m	1 (2 ?)
H4 - H7	1.07 – 1.3		m	8
H18	0.87	$J_{17,18} = 7.5$	t	3

Figure S9: COSY spectrum of Product 6a (Me ester)

9-OOH-12,13-epoxide (C18:2)   RP4SP1, product 6a (Me ester)