SUPPLEMENTARY DATA

Steric analysis of epoxyalcohol and trihydroxy derivatives of 9-hydroperoxy-linoleic acid from hematin and enzymatic synthesis

by
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Table S1. ^{1}H NMR spectrum of 9*S*,10*S*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Trihydroxy I, generated from the acid hydrolysis of 9*S,*10*S*-*trans*-epoxy-13*S*-hydroxy-octadec-11*E*-enoic acid)

Table S2. ^{1}H NMR spectrum of 9*S*,12*R*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Trihydroxy IIa, generated from the acid hydrolysis of 9*S*,10*S*-*trans*-epoxy-13*S*-hydroxy-octadec-11*E*-enoic acid)

Table S3. ^{1}H NMR spectrum of 9*S*,10*R*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Trihydroxy IIb, generated from the acid hydrolysis of 9*S*,10*S*-*trans*-epoxy-13*S*-hydroxy-octadec-11*E*-enoic acid)

Table S4. ^{1}H NMR spectrum of 9*S*,12*S*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Trihydroxy III, pinellic acid, a minor acid hydrolysis product of 9*S*,10*S*-*trans*-epoxy-13*S*-hydroxyoctadec-11*E*-enoic acid)

Table S5. ^{1}H NMR spectrum of 9*S*,12*S*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Pinellic acid generated from 9*S*-HPODE in beetroot extract)

Table S6. ^{1}H NMR spectrum of 9*R*-hydroxy-10*E*-12*R,*13*S*-*cis*-epoxyoctadecenoate methyl ester. (Main epoxyalcohol generated by short (5 min) incubation of 9*R*-HPODE in beetroot)

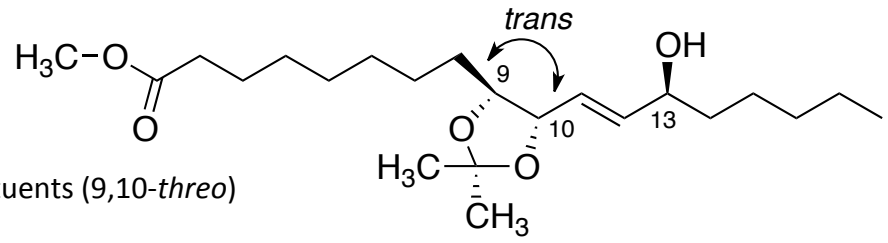
Table S7. ^{1}H NMR spectrum of 9*R*,12*S*,13*S*-trihydroxyoctadecenoate methyl ester, DMP derivative. (Main trihydroxy product of 60 min incubation of 9*R*-HPODE in beetroot)

Table S8. ^{1}H NMR spectrum of 9-hydroxy-11*E*-13-oxo-octadecenoate methyl ester. (Major δ-ketol formed during mild acid hydrolysis of 9*S*,10*S*-*trans*-epoxy-13*S*-hydroxy-octadec-11*E*-enoic acid).

Table S9. ^{1}H-NMR spectrum of 9*S*,10*S*-*trans*-epoxy-11*S*-*erythro*-hydroxy-octadec-12*Z*-enoate methyl ester.

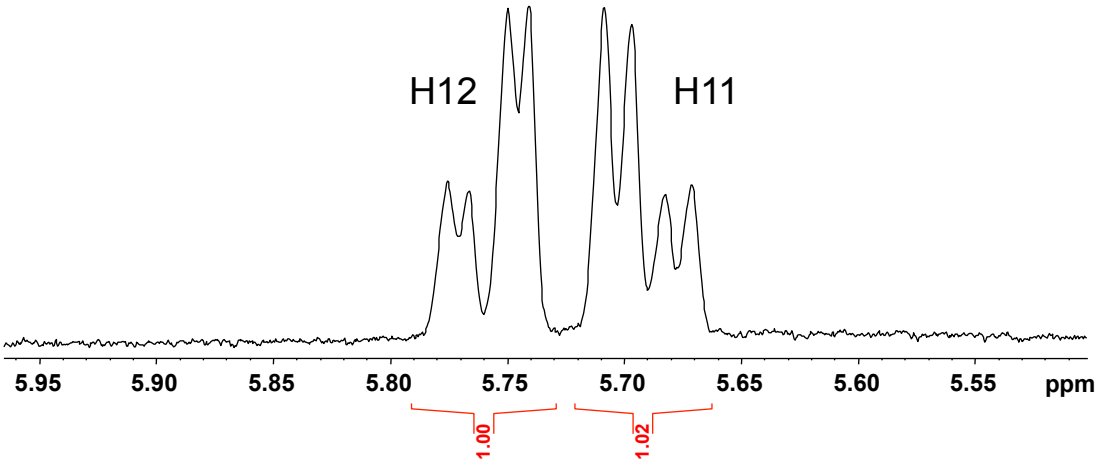
Table S10. ^{1}H-NMR spectrum of 9*S*,10*S*-*trans*-epoxy-11*R*-*threo*-hydroxy-octadec-12*Z*-enoate methyl ester.
and
Assignment of *erythro* versus *threo* 9,10-*trans*-epoxy-11-hydroxy-octadecenoates.

Table S1. ^{1}H NMR of 9(*S*),10(*S*),13(*S*)-trihydroxyoctadecenoate methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. Trihydroxy I is generated from the acid hydrolysis of 9(*S*),10(*S*)-epoxy-13(*S*)-hydroxyoctadecenoic acid.



9*S*,10*S*,13*S*
trans acetonide substituents (9,10-*threo*)
H10 at 4.06 ppm
acetonide methyls at 1.45 & 1.47 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.76	dd	H12 $J_{11,12}$=15.5Hz, $J_{12,13}$=5.5Hz
5.69	dd	H11 $J_{11,12}$= 15.5Hz, $J_{10,11}$ = 6.8Hz
4.06	dd	H10 $J_{10,11}$ = 7.5Hz, $J_{9,10}$ = 7.7Hz
3.91	m	H9
3.72	m	H13
3.35	s	-OCH_3



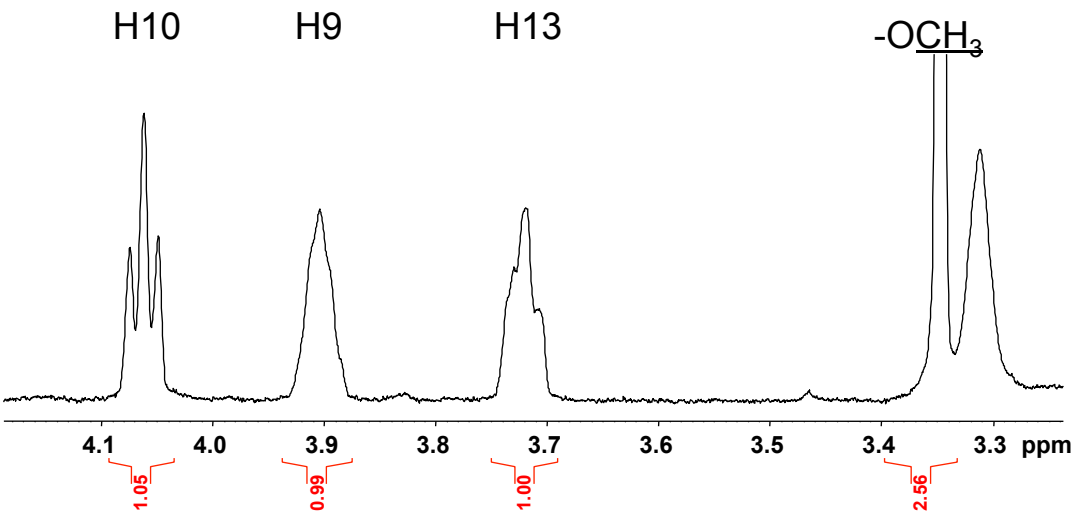
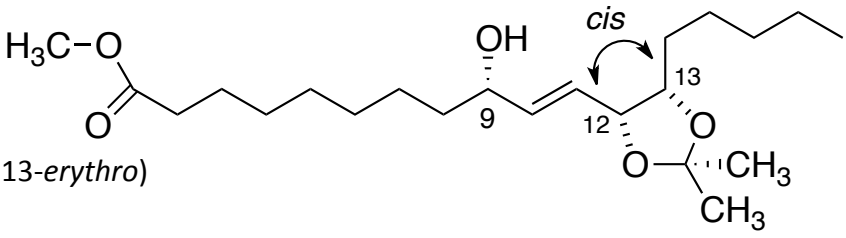
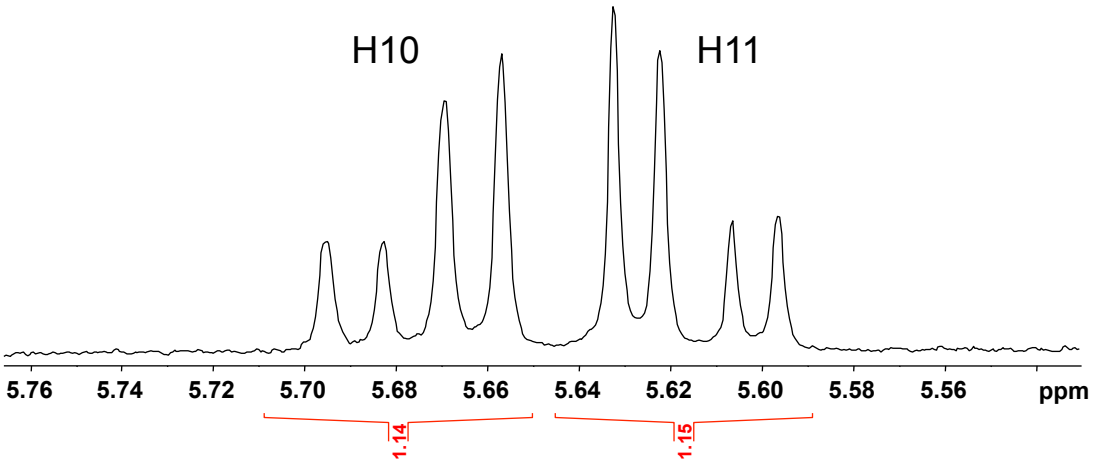


Table S2. ^{1}H NMR spectrum of 9(*S*),12(*R*),13(*S*)-trihydroxyoctadecenoate methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. Trihydroxy IIa is generated from the acid hydrolysis of 9(*S*),10(*S*)-epoxy, 13(*S*)-hydroxyoctadecenoic acid.



9*S*,12*R*,13*S*
cis acetonide substituents (12,13-*erythro*)
H12 at 4.42 ppm
acetonide methyls at 1.38 & 1.56 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.68	dd	H10 $J_{10,11}$=15.5Hz, $J_{9,10}$=7.4Hz
5.62	dd	H11 $J_{10,11}$= 15.5Hz, $J_{11,12}$ = 6.1Hz
4.42	dd	H12 $J_{11,12}$ = 6.8Hz, $J_{12,13}$ = 6.8Hz
4.02	m	H13
3.87	m	H9
3.35	s	-OCH_3



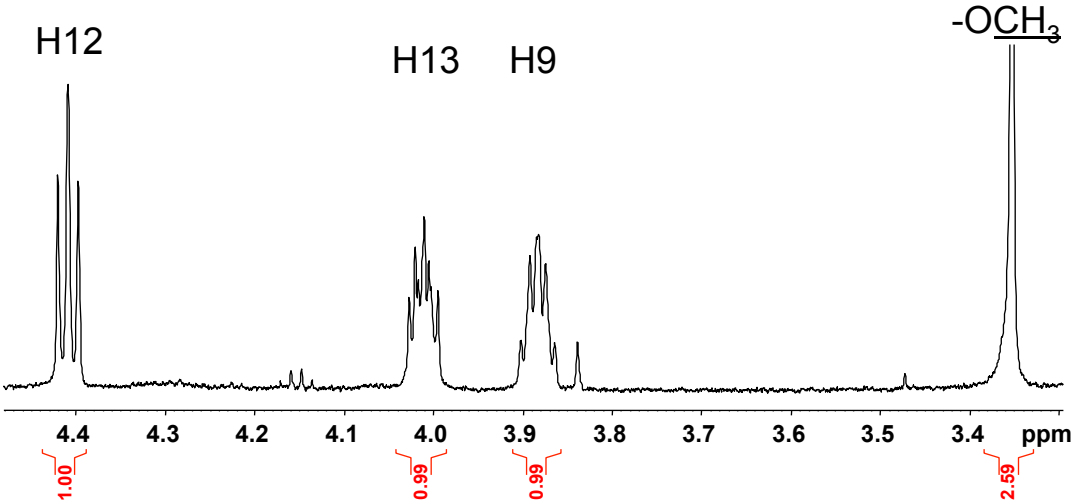
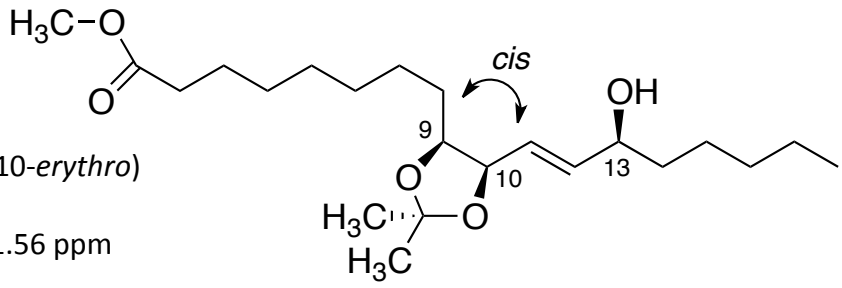
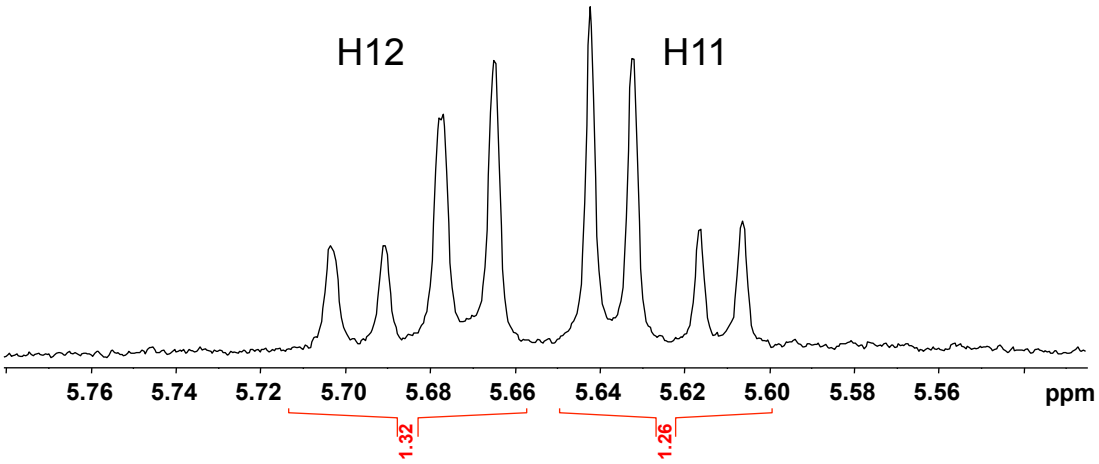


Table S3. ^{1}H NMR spectrum of 9(*S*),10(*R*),13(*S*)-trihydroxyoctadecenoate methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. Trihydroxy IIb is generated from the acid hydrolysis of 9(*S*),10(*S*)-epoxy, 13(*S*)-hydroxyoctadecenoic acid.



9*S*,10*R*,13*S*
cis acetonide substituents (9,10-*erythro*)
H10 at 4.42 ppm
acetonide methyls at 1.38 & 1.56 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.68	dd	H12 $J_{11,12}$=15.8Hz, $J_{12,13}$=7.6Hz
5.62	dd	H11 $J_{11,12}$= 15.4Hz, $J_{10,11}$ = 5.8Hz
4.42	dd	H10 $J_{10,11}$ = 6.7Hz, $J_{9,10}$ = 7.0Hz
4.01	m	H9
3.90	m	H13
3.35	s	-OCH_3



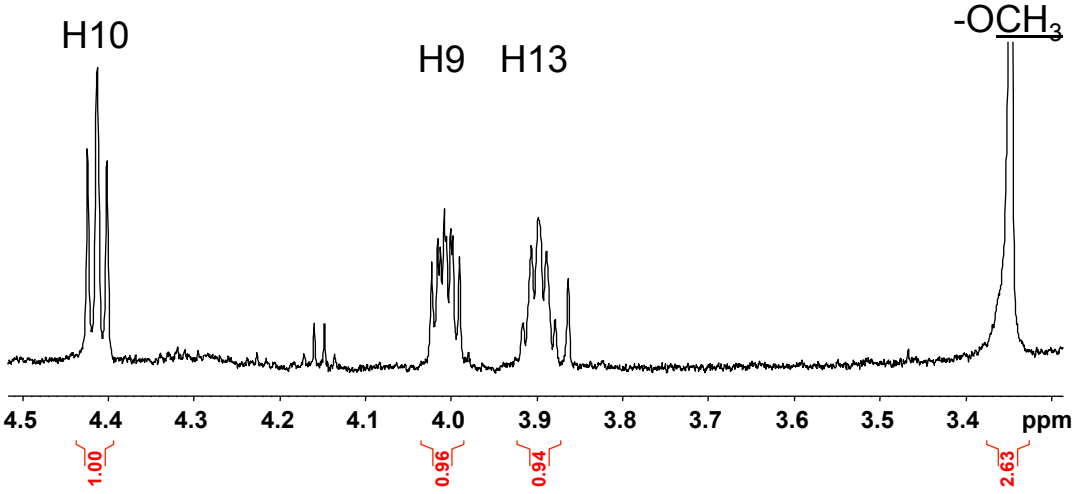
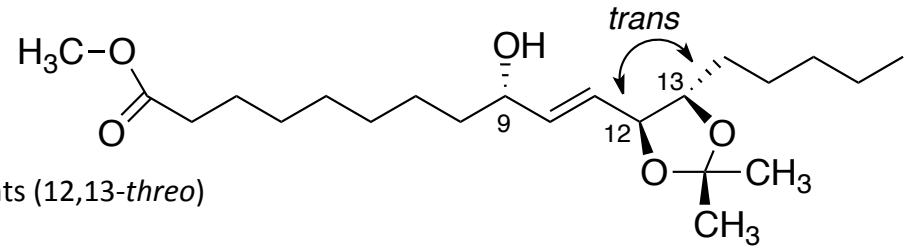
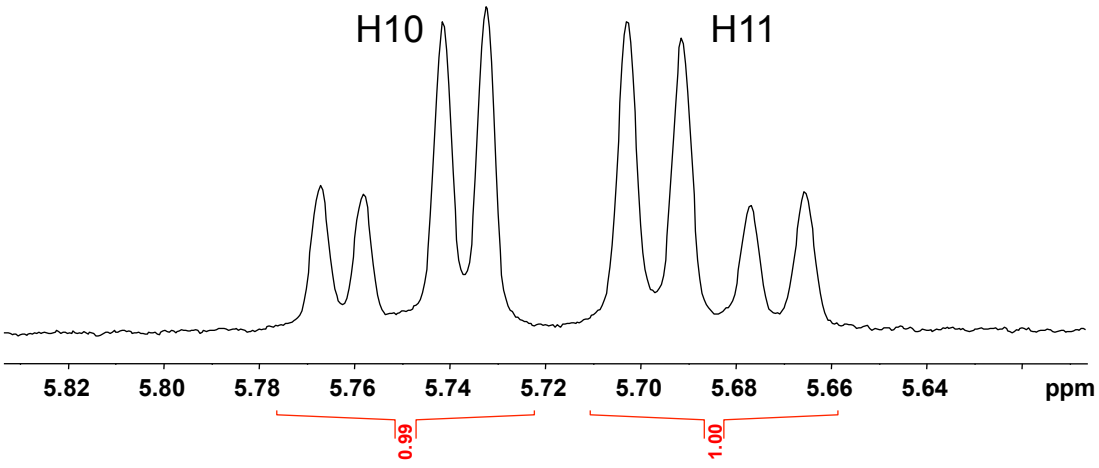


Table S4. ^{1}H NMR spectrum of 9(*S*),12(*S*),13(*S*)-trihydroxyoctadecenoate (pinellic acid) methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. Trihydroxy III is generated from the acid hydrolysis of 9(*S*),10(*S*)-epoxy-13(*S*)-hydroxyoctadecenoic acid.



9*S*,12*S*,13*S*
trans acetonide substituents (12,13-*threo*)
H12 at 4.05 ppm
acetonide methyls at 1.45 & 1.47 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.75	dd	H10 $J_{10,11}$=15.5Hz, $J_{9,10}$=5.5Hz
5.68	dd	H11 $J_{10,11}$= 15.5Hz, $J_{11,12}$ = 6.8Hz
4.06	dd	H12 $J_{11,12}$ = 7.6Hz, $J_{12,13}$ = 7.6Hz
3.88	m	H13
3.73	m	H9
3.35	s	-OCH_3



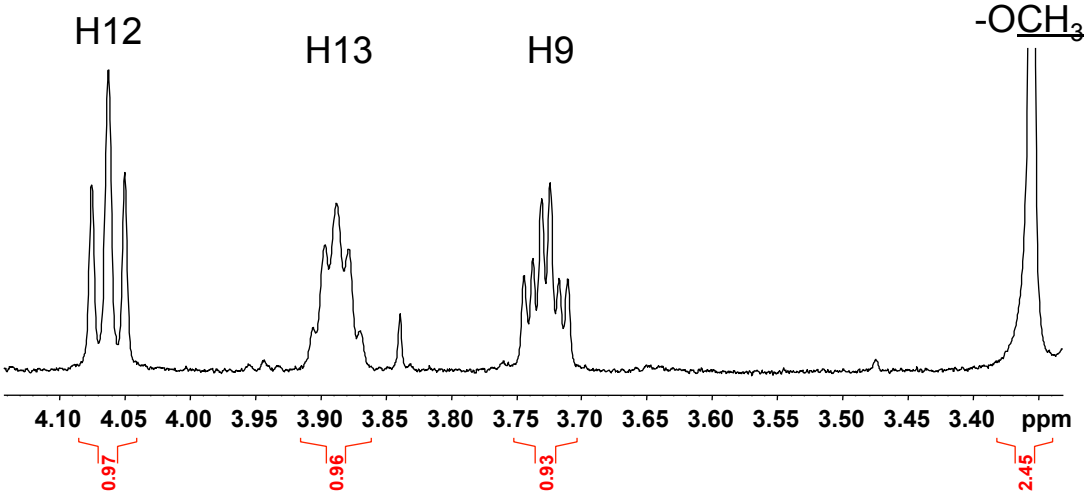
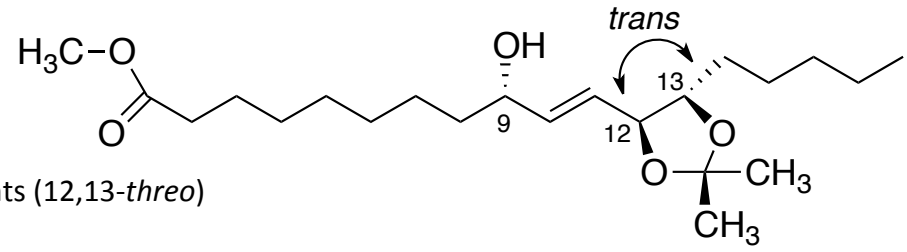


Table S5. ^{1}H NMR spectrum of 9(*S*),12(*S*),13(*S*)-trihydroxyoctadecenoate methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. Pinellic acid is generated from the incubation of 9(*S*)-HPODE with beetroot extract.



9*S*,12*S*,13*S*
trans acetonide substituents (12,13-*threo*)
H12 at 4.06 ppm
acetonide methyls at 1.45 & 1.47 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.75	ddd	H10 $J_{10,11}$=15.5Hz, $J_{9,10}$=5.5Hz, $J_{10,12}$ = 0.3Hz
5.68	ddd	H11 $J_{10,11}$= 15.5Hz, $J_{11,12}$ = 6.9Hz, $J_{9,11}$ = 0.9Hz
4.06	dd	H12 $J_{11,12}$ = 7.3Hz, $J_{12,13}$ = 7.1Hz
3.89	m	H13
3.72	m(dt)	H9
3.36	s	-OCH_3

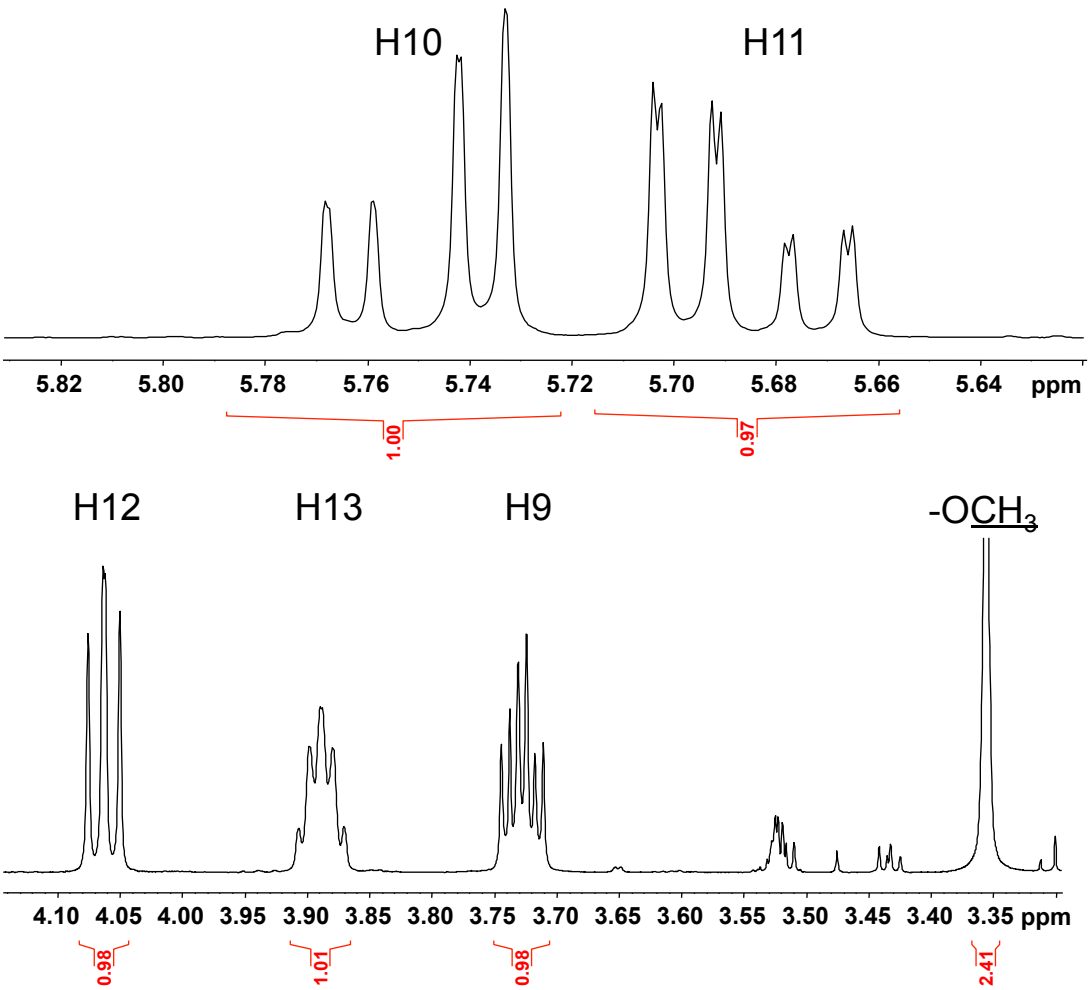
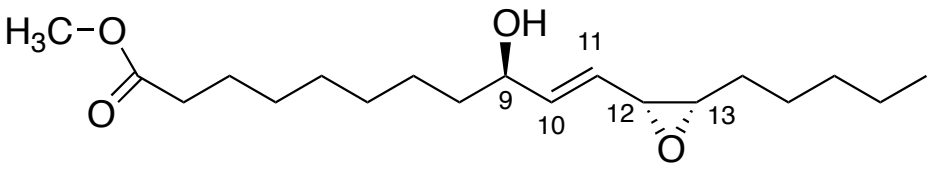
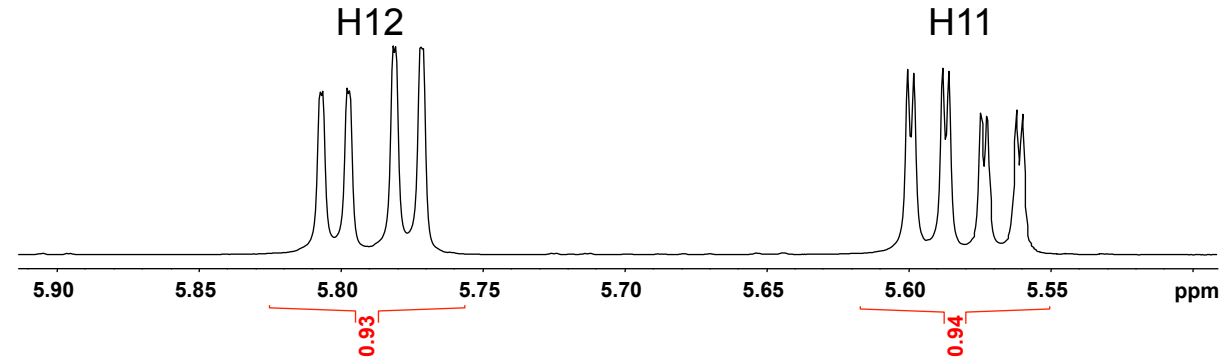
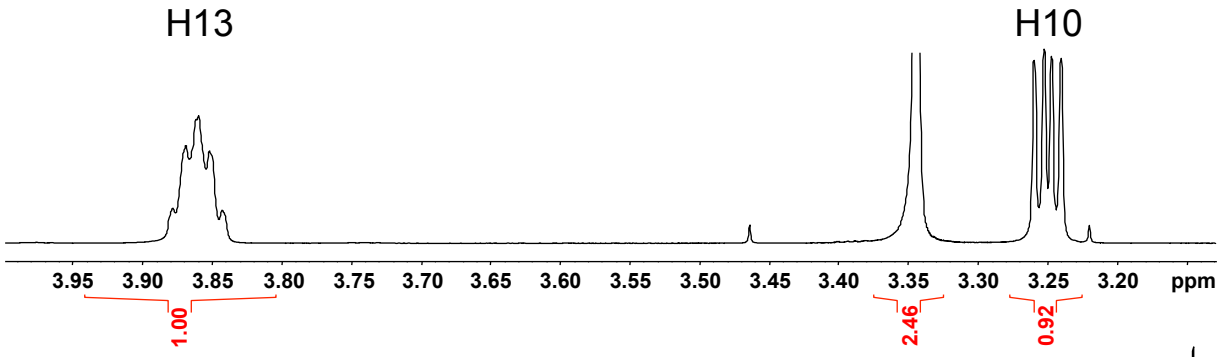


Table S6. ^{1}H NMR spectrum of 9(*R*)-hydroxy-12(*R*),13(*S*)-epoxy-octadecenoate methyl ester. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. The epoxyhydroxy is generated from the short duration incubation of 9(*R*)-HPODE with beetroot extract.



Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.79	dd	H12 $J_{11,12}$=15.5Hz, $J_{12,13}$=5.8Hz
5.58	ddd	H11 $J_{11,12}$= 15.5Hz, $J_{10,11}$ = 7.4Hz, $J_{11,13}$ = 1.3Hz
3.86	m	H13
3.35	s	-OCH_3
3.25	dd	H10 $J_{10,11}$ = 7.2Hz, $J_{9,10}$ = 4.4Hz
2.86	m	H9
2.09	t	H2





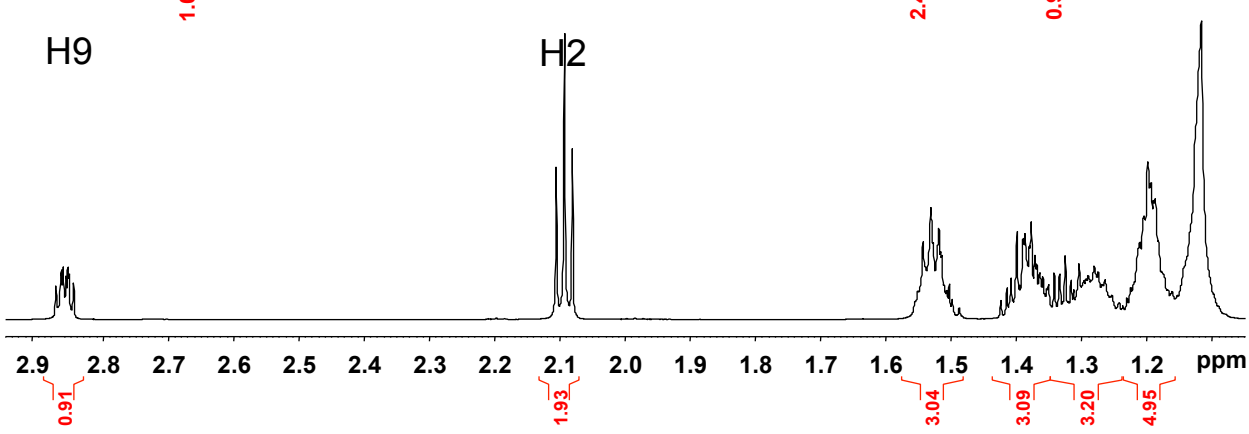
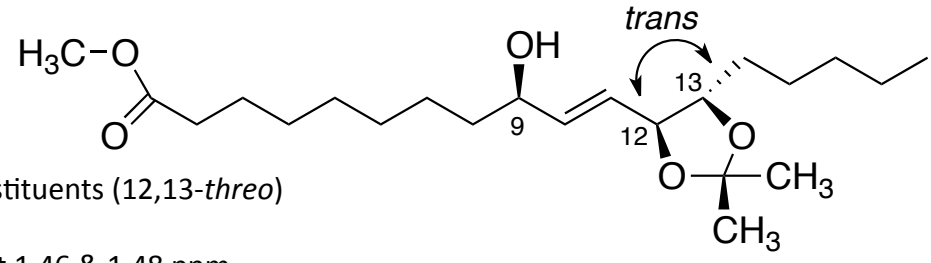
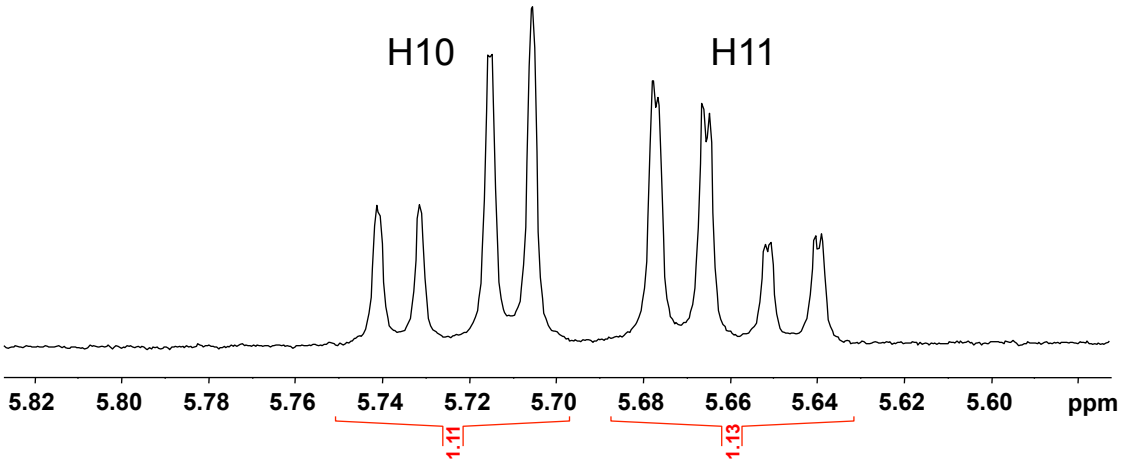


Table S7. ^{1}H NMR spectrum of 9(*R*),12(*S*),13(*S*)-trihydroxyoctadecenoate methyl ester, DMP derivative. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. The trihydroxy is generated from the incubation of 9(*R*)-HPODE with beetroot extract.



9*R*,12*S*,13*S*
trans acetonide substituents (12,13-*threo*)
H12 at 4.06 ppm
acetonide methyls at 1.46 & 1.48 ppm

Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.73	dd	H10 $J_{10,11}$=15.6Hz, $J_{9,10}$=5.8Hz
5.66	dd	H11 $J_{10,11}$= 16.3Hz, $J_{11,12}$ = 6.9Hz
4.06	dd	H12 $J_{11,12}$ = 7.3Hz, $J_{12,13}$ = 7.3Hz
3.87	m	H13
3.72	m(dt)	H9
3.36	s	-OCH_3



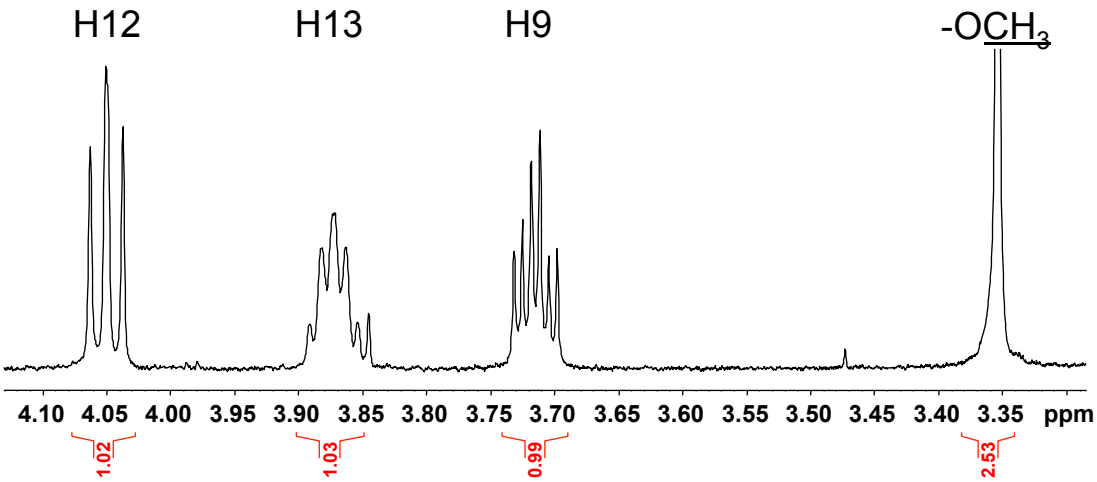
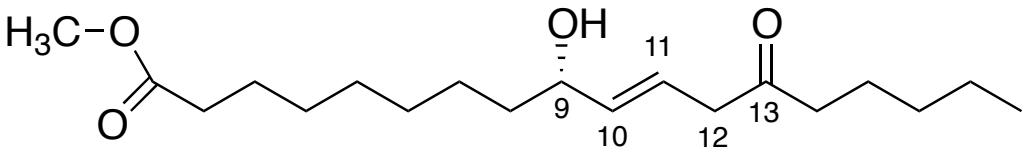
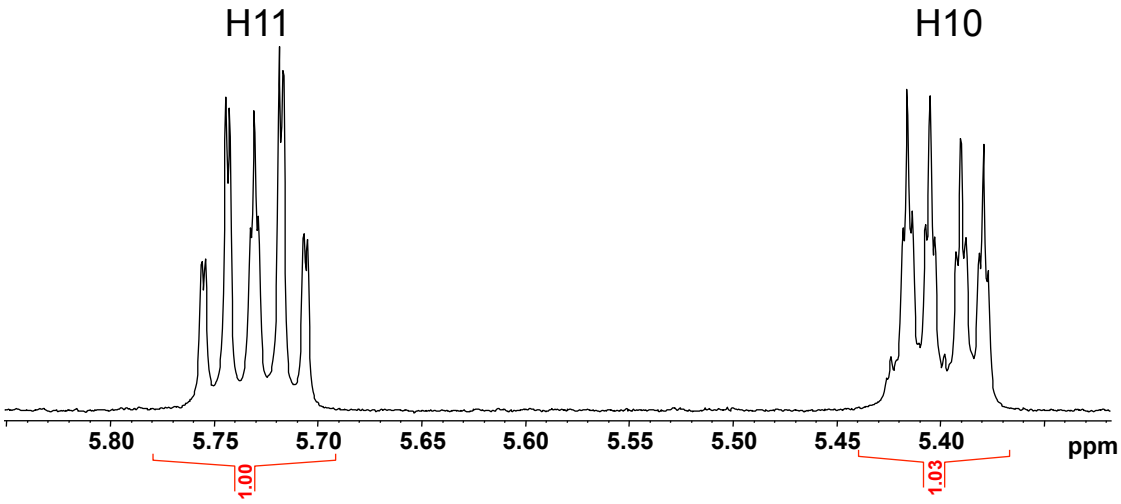


Table S8. ^{1}H NMR spectrum of 9(*S*)-hydroxy,11-*trans*-13-ketooctadecenoic methyl ester. The spectrum (600MHz) was acquired at room temperature in d_6-benzene solvent. The δ-ketol by-product is generated during the acid hydrolysis of 9(*S*),10(*S*)-t*rans*-epoxy-13(*S*)-hydroxyoctadecenoic acid.



Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.74	dt	H11 $J_{10,11}$ = 15.5Hz, $J_{11,12}$ = 7.0Hz, $J_{9,11}$ = 1.1Hz
5.40	dd	H10 $J_{10,11}$= 15.5Hz, $J_{9,10}$ = 6.6Hz, $J_{10,12}$ = 1.7Hz
3.87	m	H9
2.73	d	H12 $J_{11,12}$ = 7.0Hz



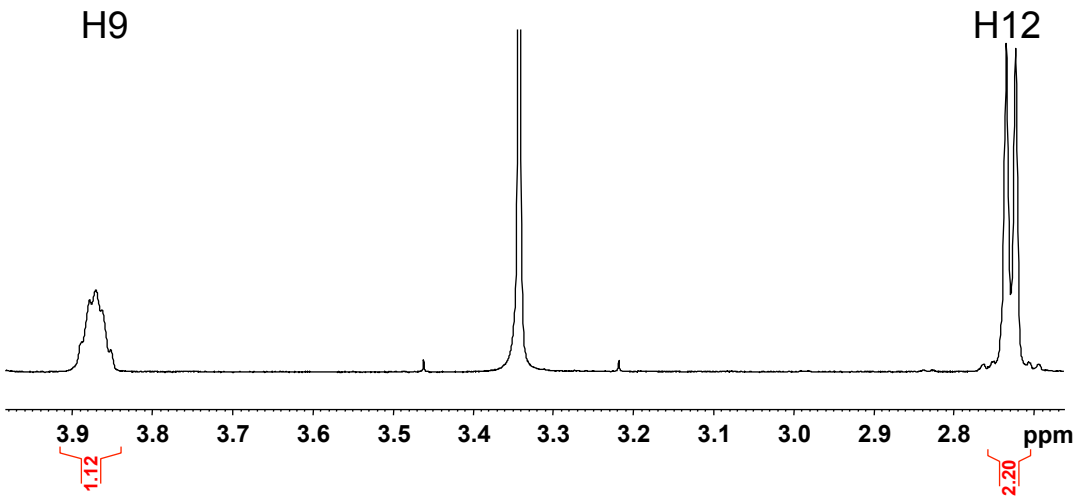
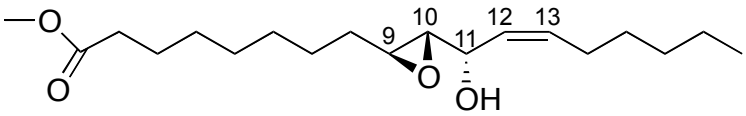


Table S9 . ^{1}H-NMR spectrum of 9*S*,10*S*-*trans*-epoxy-11*S*-*erythro*-hydroxy-octadec-12*Z*-enoate methyl ester. The spectrum (600MHz) was acquired at room temperature in d6- benzene solvent.



Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.45-5.52	dd/dt (m)	H12,13 $J_{12,13}$=11.1Hz
4.54	(dt) ddd	H11 $J_{\text{-OH},11} \approx J_{10,11}$ = 2.5-3Hz, $J_{11,12}$ = 6.7Hz
3.35	s	-OCH_3
2.94	dt	H9 $J_{8,9}$ = 5.6Hz, $J_{9,10}$ = 2.2Hz
2.69	dd	H10 $J_{9,10}$ = 2.2Hz, $J_{10,11}$ =3.12Hz
2.09	t	H2 $J_{2,3}$ = 7.5Hz
1.94	m	H14
1.63	d	11-OH J = 2.5Hz
1.52	p	H3

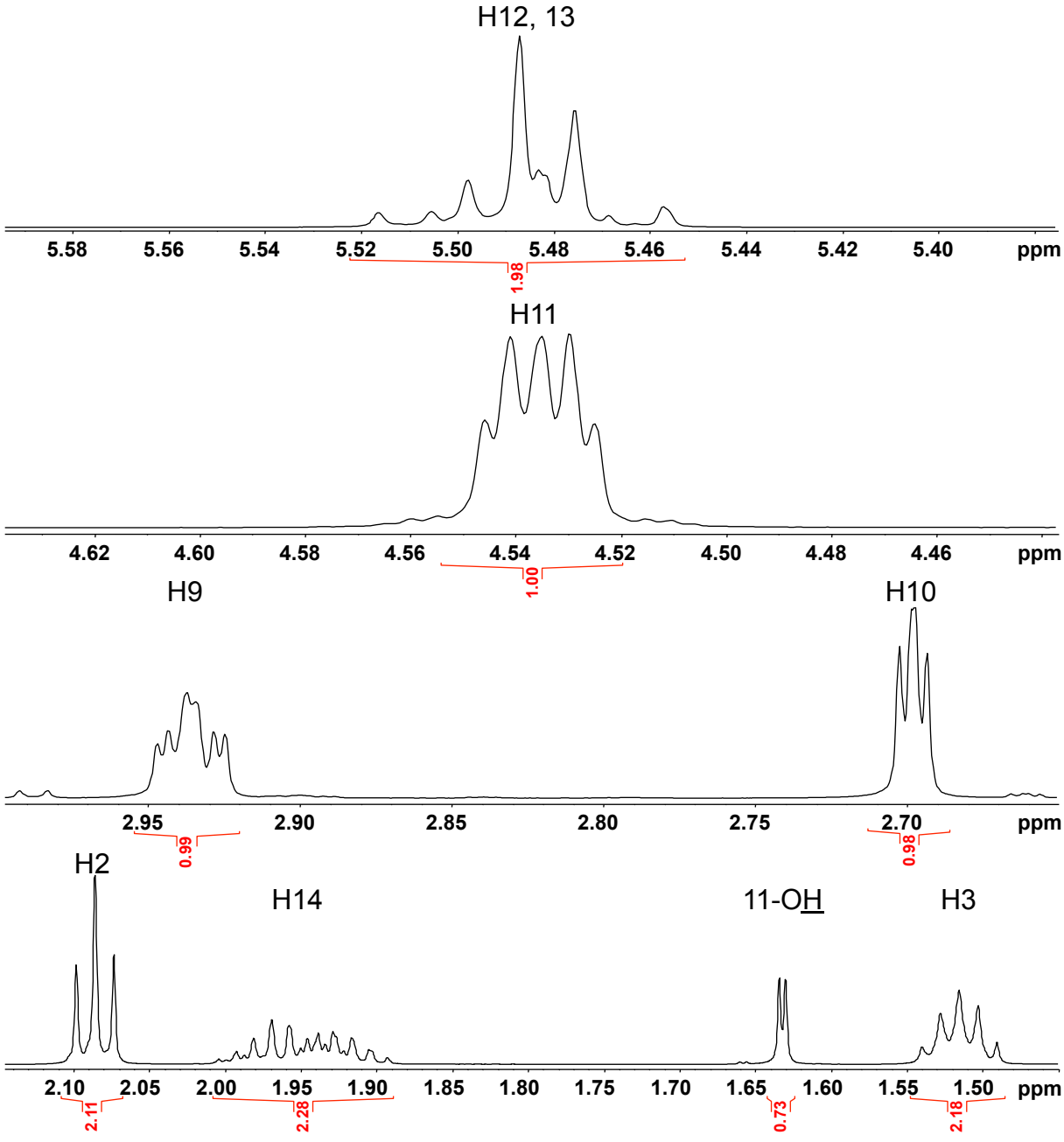
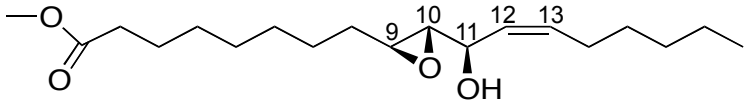
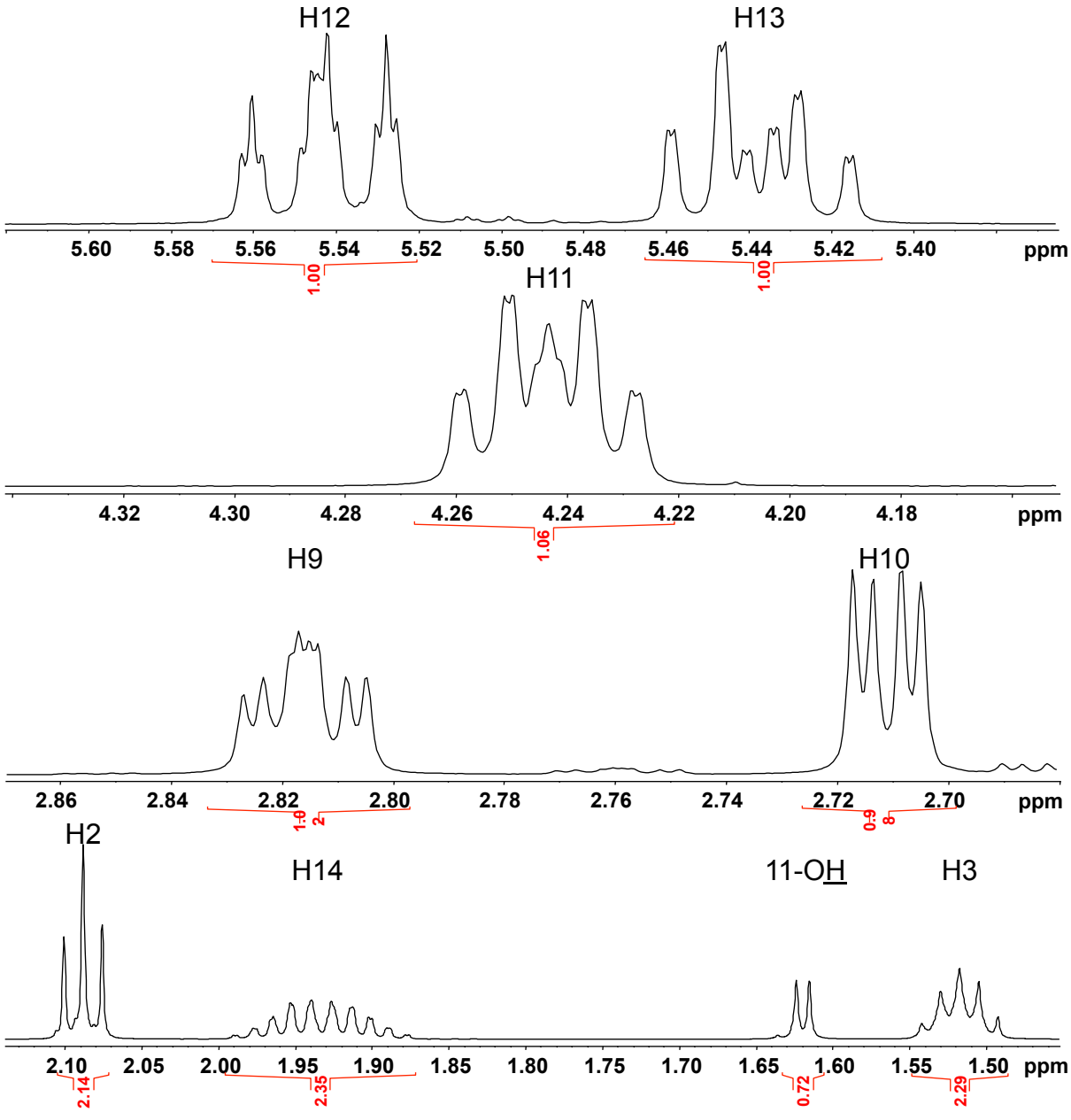


Table S10 . ^{1}H-NMR spectrum of 9*S*,10*S*-*trans*-epoxy-11*R*-*threo*-hydroxy-octadec-12*Z*-enoate methyl ester. The spectrum (600MHz) was acquired at room temperature in d6- benzene solvent.



Chemical shift (ppm)	Multiplicity	Assignment, coupling constant
5.54	dd (+ 1.5 Hz allylic coupling)	H12 $J_{11,12}$=8.6Hz, $J_{12,13}$=11.0Hz
5.44	dt	H13 $J_{12,13}$= 11.0Hz, $J_{13,14}$ = 7.5Hz
4.25	(dt) ddd	H11 $J_{\text{-OH},11} \approx J_{10,11}$ = 5.2Hz, $J_{11,12}$ = 8.6Hz
3.35	s	-OCH_3
2.82	dt	H9 $J_{8,9}$ = 5.6Hz, $J_{9,10}$ = 2.2Hz
2.71	dd	H10 $J_{9,10}$ = 2.2Hz, $J_{10,11}$ = 5.2Hz
2.09	t	H2 $J_{2,3}$ = 7.5Hz
1.94	m	H14
1.62	d	11-OH J = 5.2Hz
1.52	p	H3



Assignment of *erythro* versus *threo* 9,10-*trans*-epoxy-11-hydroxy-octadecenoates.

COSY analysis confirmed the overall structures for the two diastereomers in Supplementary Tables S9 and S10 as 9,10-epoxy-11-hydroxy-octadec-12*Z*-enoates. In both diastereomers, the coupling constant for the epoxide protons, J = 2.2 Hz, established the *trans* epoxide configuration. The *erythro* versus *threo* assignment is confirmed on the basis of three criteria (Bernart and Gerwick, 1994):

1) According to precedents for *erythro* and *threo* epoxyalcohols of this type, the mobility of the two diastereomers on silica (SP-HPLC or TLC) is in the order of the more mobile *erythro* followed by *threo* (Corey and Mehrotra, 1983; Corey et al., 1983; Dix and Marnett, 1985; Gardner and Crawford, 1981; Vasiljeva et al., 1993). Our *erythro* product eluted at 9.3 min on SP-HPLC (Fig. 1A, main text), while the *threo* diastereomer eluted later at 11 min (co-chromatographing with the first of the allylic epoxyalcohols (i.e. 9*S*,10*S*-*cis*-epoxy-10*E*-13-hydroxy-octadecenoate).

2) The α-hydroxy proton in the *erythro* isomer is the further downfield. We observed values of 4.535 ppm (*erythro*) and 4.35 ppm (*threo*).

3) The ^{1}H-NMR coupling constant for the 10-epoxide and 11-hydroxyl protons should be ~3.2 Hz for the *erythro* isomer (observed here as $J_{10,11}$ = 3.1 Hz, Fig. S9) and at least 5 Hz for *threo* ($J_{10,11}$ = 5.2 Hz, Table S10) (Mercier and Agoh, 1974).

REFERENCES

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