ω-Alkynyl Lipid Surrogates for Polyunsaturated Fatty Acids: Free Radical and Enzymatic Oxidations

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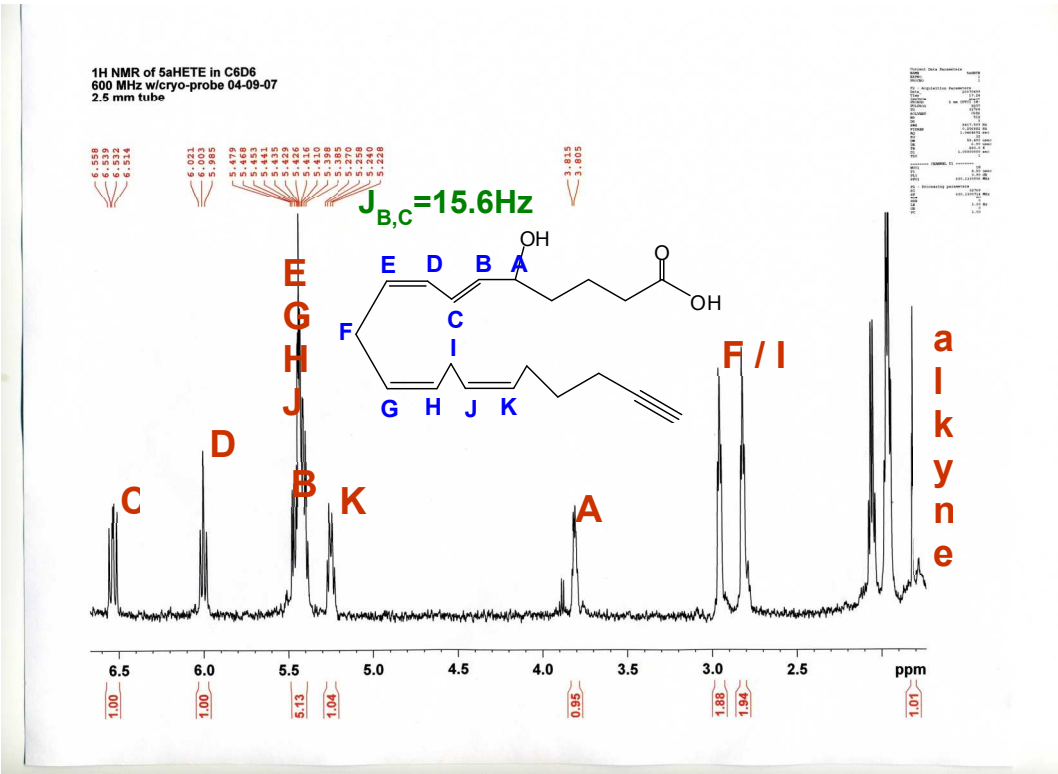
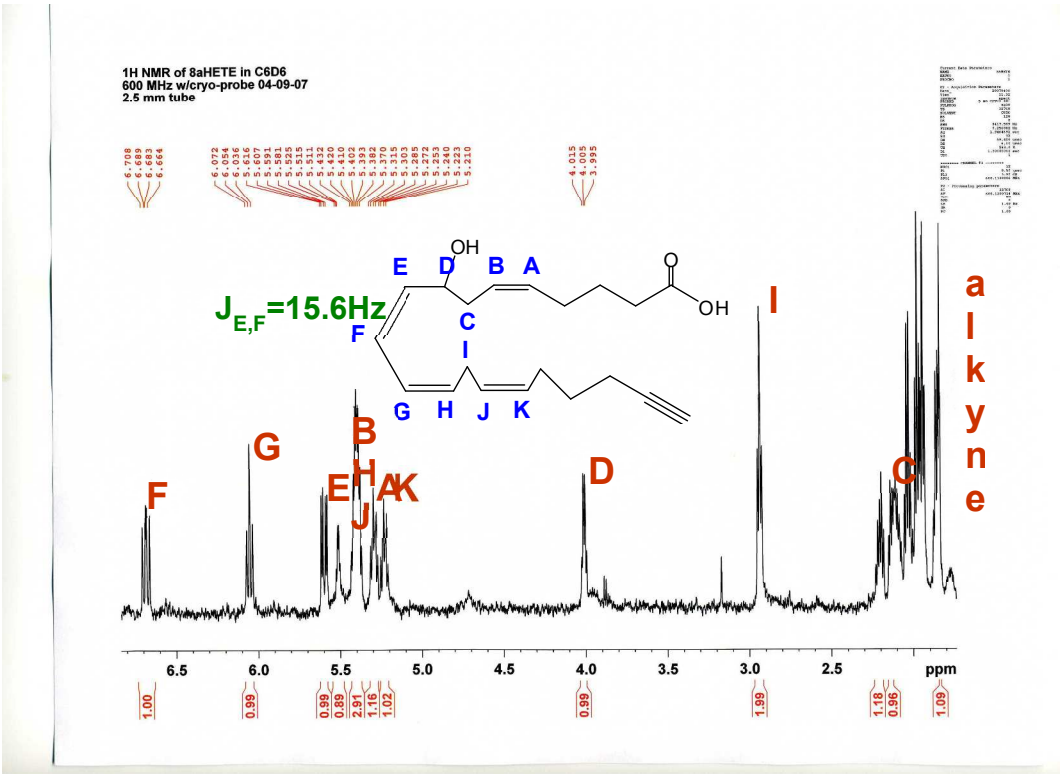
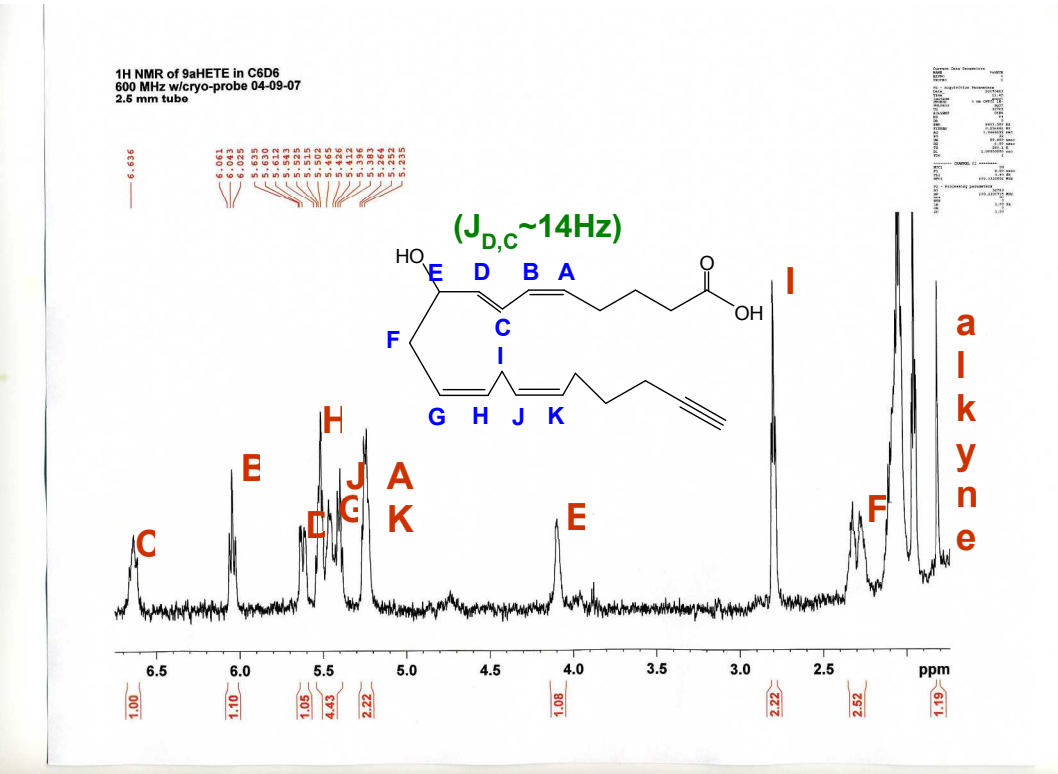
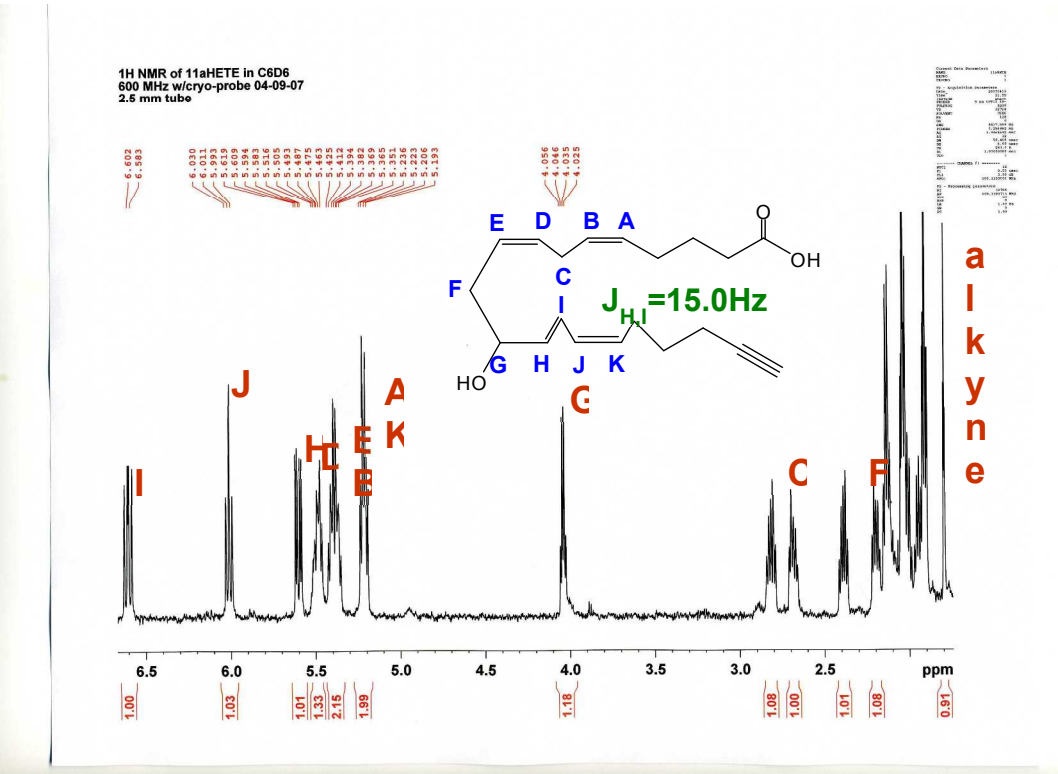
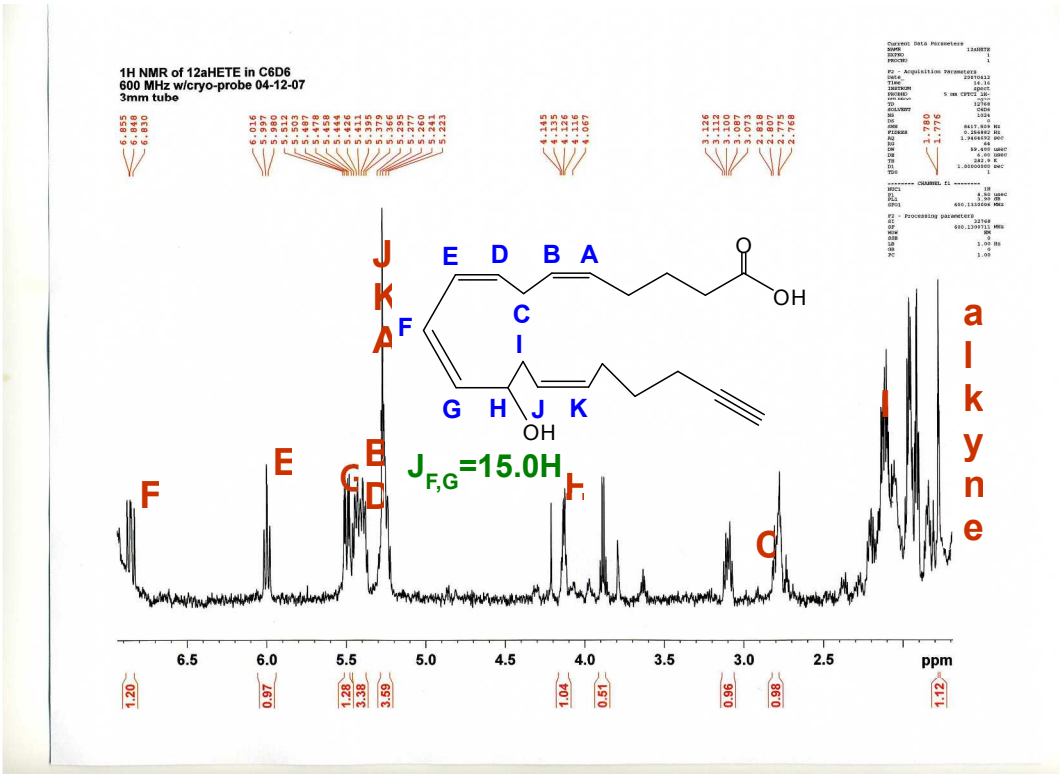
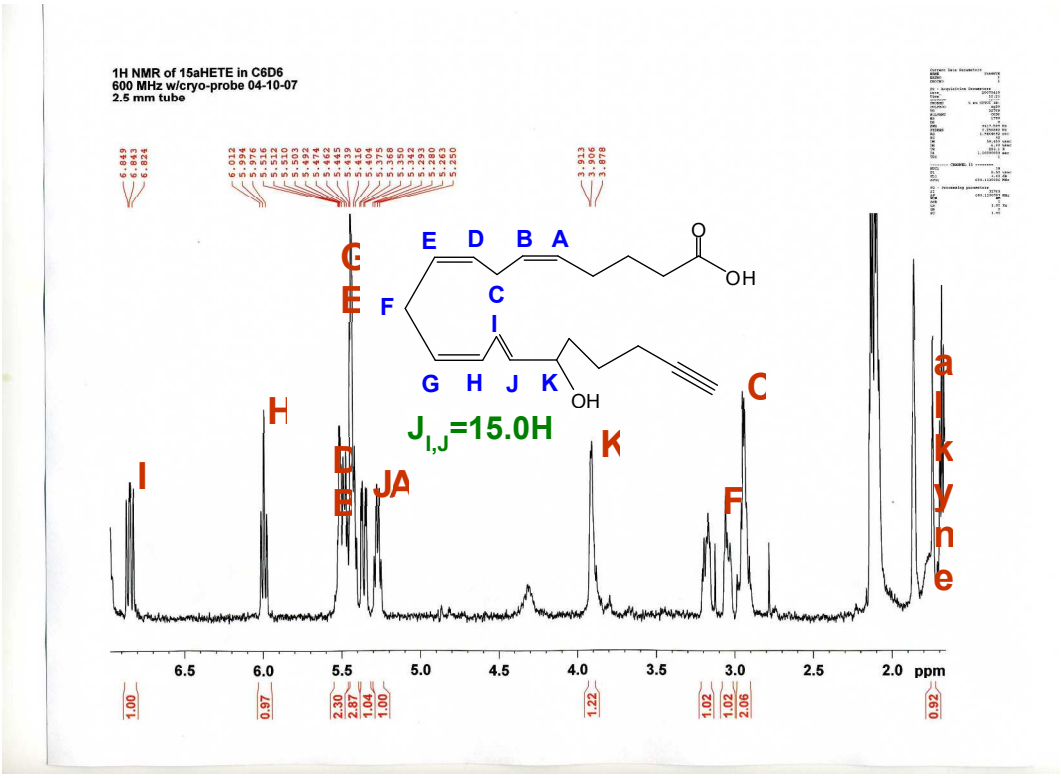

1H NMR of 5aHETE in C6D6
600 MHz w/cryo-probe 04-09-07
2.5 mm tube
$J_{B,C}$=15.6Hz
OH
OH
E D B A
F
C
I
G H J K
E
G
H
J
D
C
B
K
A
F / I
alkyne
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
1.00
1.00
5.13
1.04
0.95
1.88
1.94
1.01

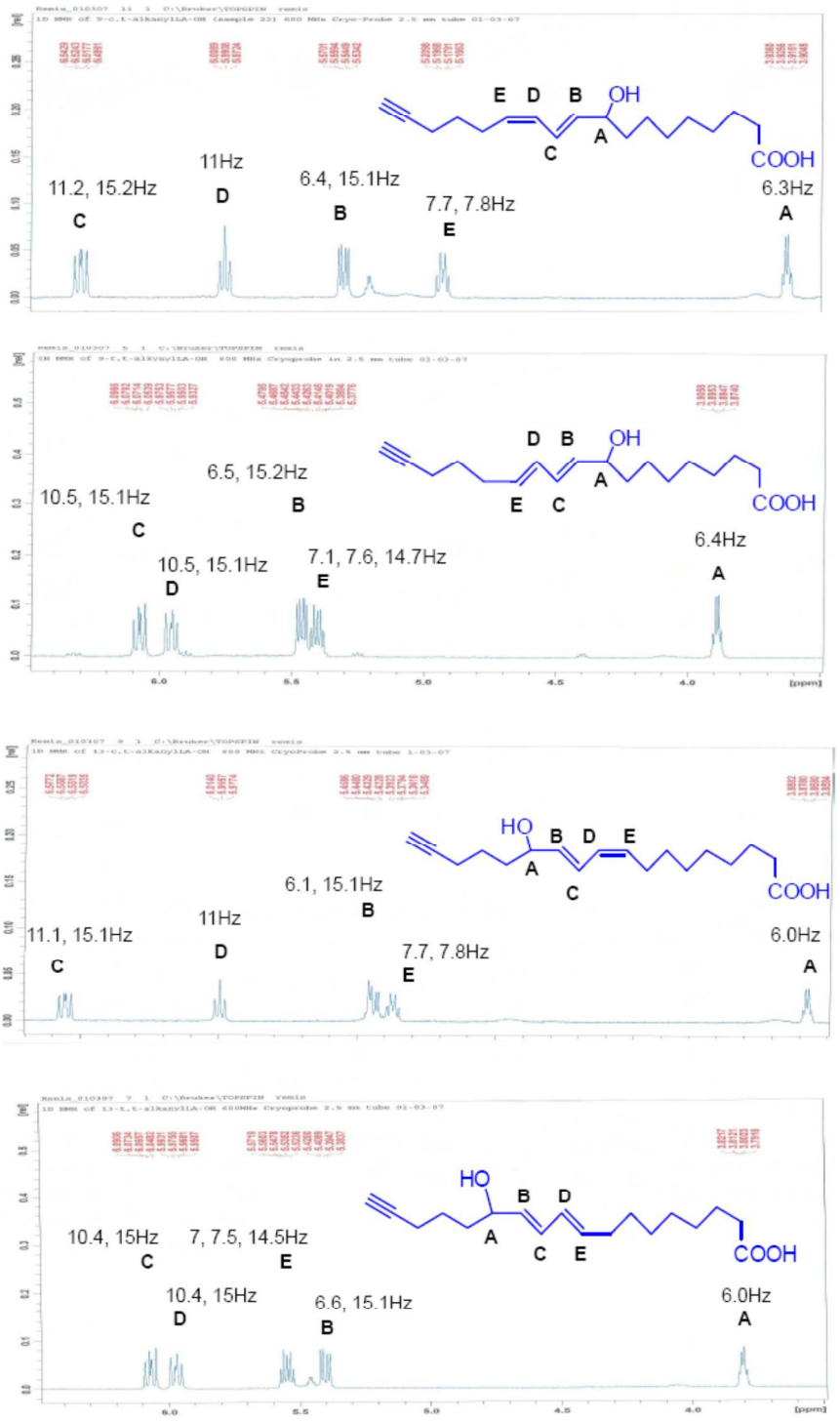

1H NMR of 8aHETE in C6D6
600 MHz w/cryo-probe 04-09-07
2.5 mm tube
$J_{E,F}$=15.6Hz
OH
OH
E D B A
F
C
I
G H J K
F
G
B
E
H
A
K
J
D
I
C
alkyne
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm
1.00
0.99
0.99
0.89
2.91
1.16
1.02
0.99
1.99
1.18
0.96
1.09


1H NMR of 9aHETE in C6D6
600 MHz w/cryo-probe 04-09-07
2.5 mm tube
(J D,C ~14Hz)
HO
E D B A
O
OH
F
C
I
G H J K
C
E
H
J
G
A
K
E
I
F
alkyne
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
1.00 1.10 1.05 4.43 2.22 1.08 2.22 2.52 1.19


1H NMR of 11aHETE in C6D6
600 MHz w/cryo-probe 04-09-07
2.5 mm tube
E D B A
O
OH
F
C
I
J H,I =15.0Hz
G H J K
HO
I
J
H
E
A
K
G
C
F
alkyne
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
1.00 1.03 1.01 1.33 2.15 1.99 1.18 1.08 1.00 1.01 1.08 0.91


1H NMR of 12aHETE in C6D6
600 MHz w/cryo-probe 04-12-07
3mm tube
E D B A
C
I
OH
G H J K
OH
J
K
A
F
E
B
G
D
F
H
C
I
alkyne
$J_{F,G}$=15.0H
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
1.20 0.97 1.28 3.38 3.59 1.04 0.51 0.96 0.98 1.12

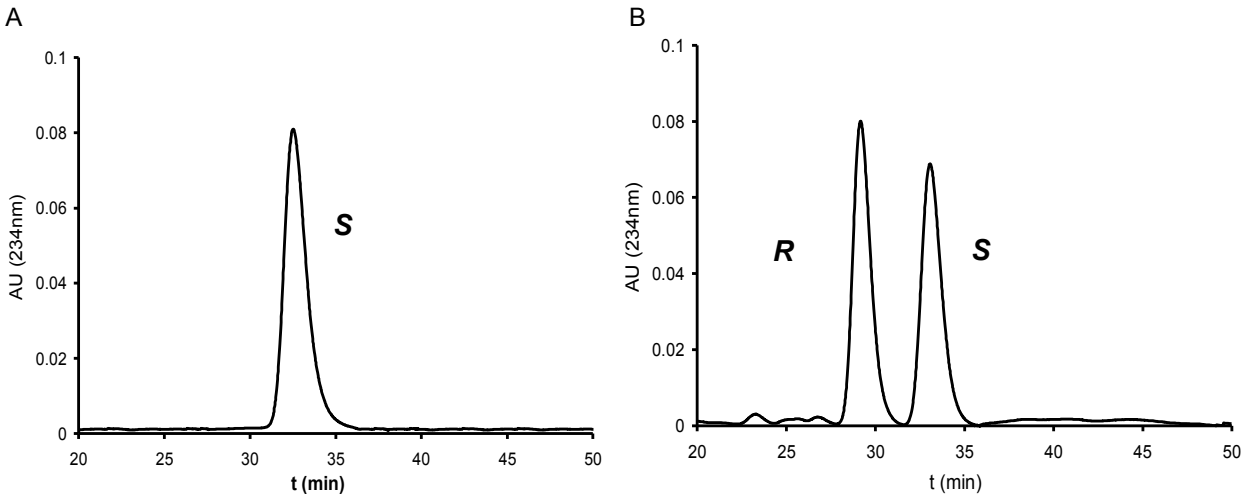

1H NMR of 15aHETE in C6D6
600 MHz w/cryo-probe 04-10-07
2.5 mm tube
E D B A
C
I
OH
G H J K
OH
G
E
F
H
I
D
B
JA
K
C
F
alkyne
$J_{I,J}$=15.0H
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
1.00 0.97 2.30 2.87 1.04 1.00 1.22 1.02 1.02 2.06 0.92



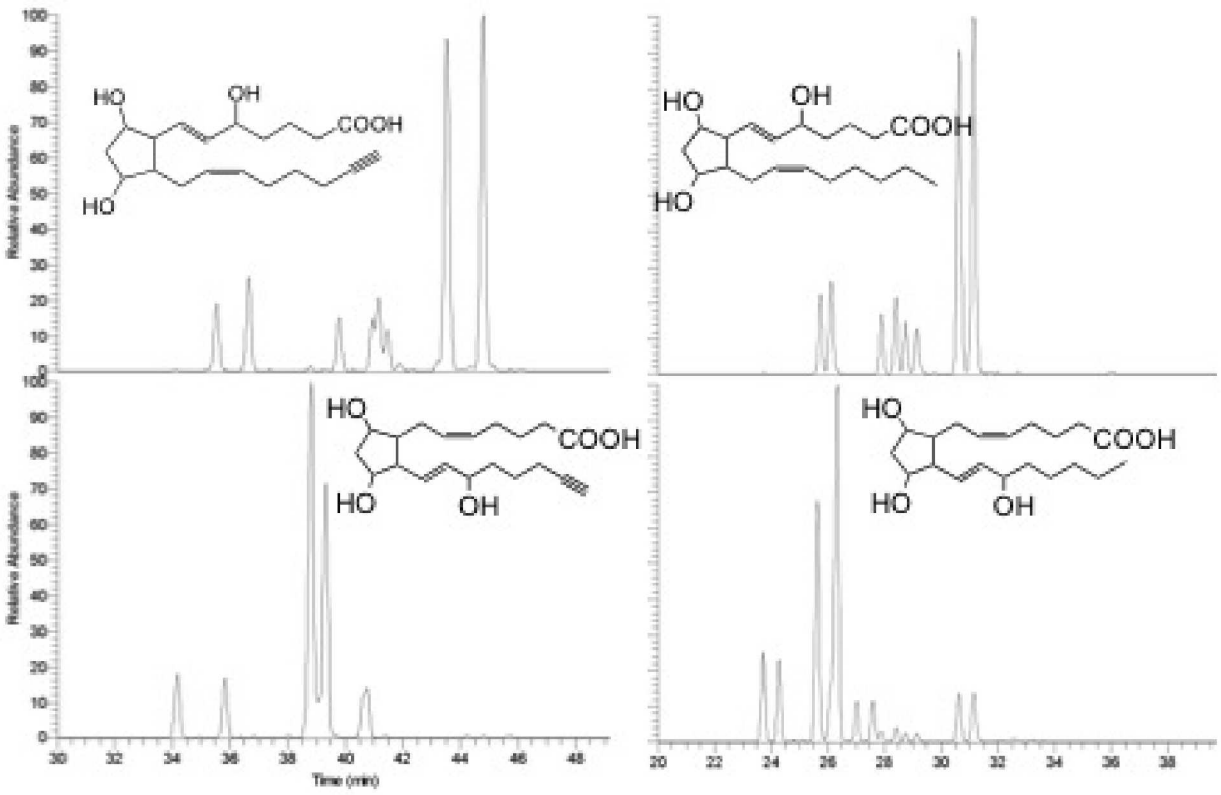
Supplemental Figure 1. ^{1}H NMR spectra of alkynyl HETE and HODE products, and coupling constants assigning the double bonds as either cis or trans.

	Parent ion (m/z)	Products of in source dehydration/decarboxylation (m/z)	Fragment ions (m/z)
5-*a*HETE	314.8	253.2, 297.2	115.2, 199.2
8-*a*HETE	315.3	253.4, 297.1	155.0, 159.1
9-*a*HETE	315.0	253.4, 296.9	123.1, 147.0, 166.9, 175.0
11-*a*HETE	315.1	297.3	166.9
12-*a*HETE	315.1	253.1, 297.2	179.2, 208.2
15-*a*HETE	315.2	253.2, 297.3	175.0, 218.9

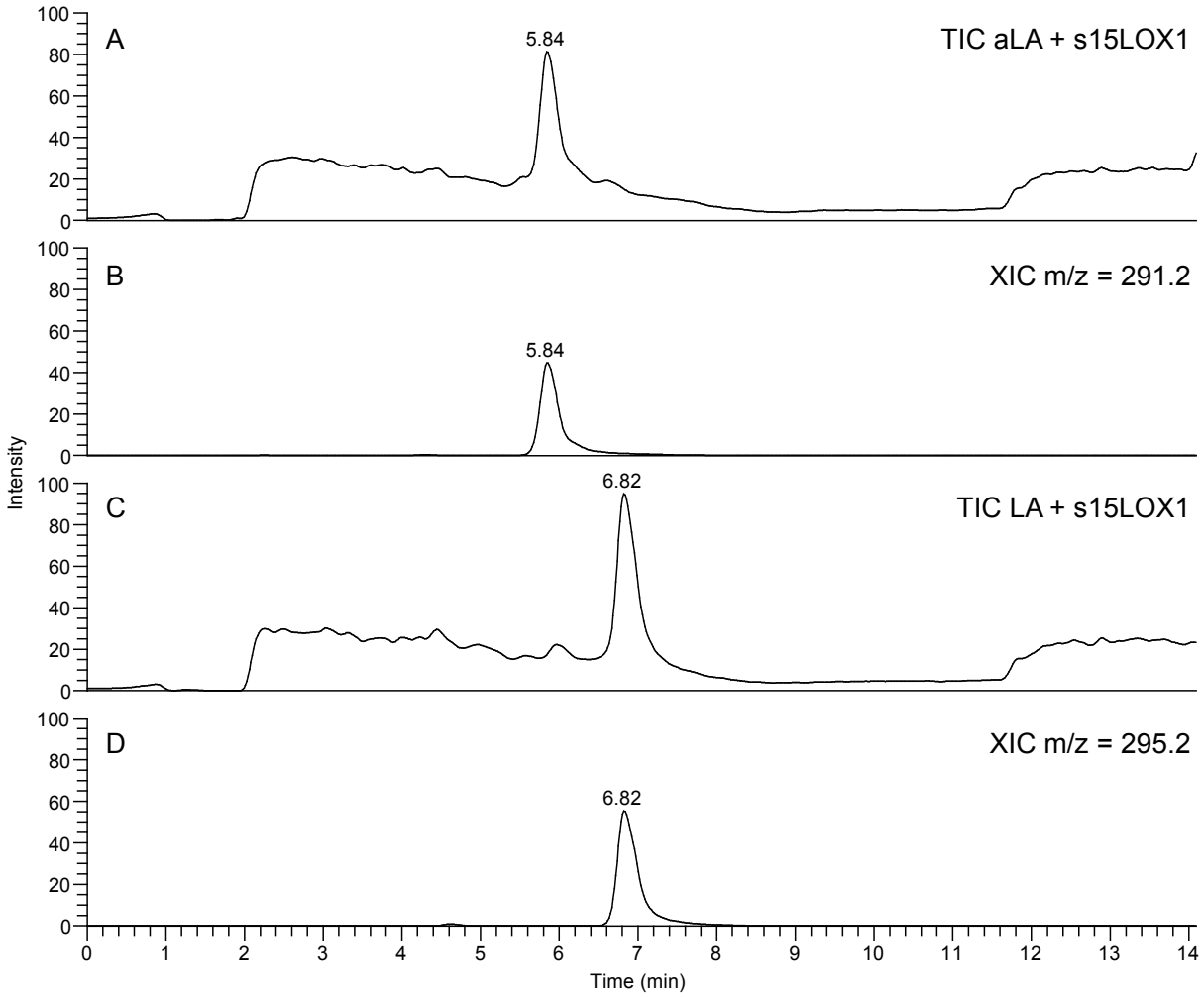
Supplemental Table 1. MS^2 fragmentation patterns for *a*HETE (MS signal optimized with 15-HETE). Red and blue numbers represent non-regiospecific fragments.



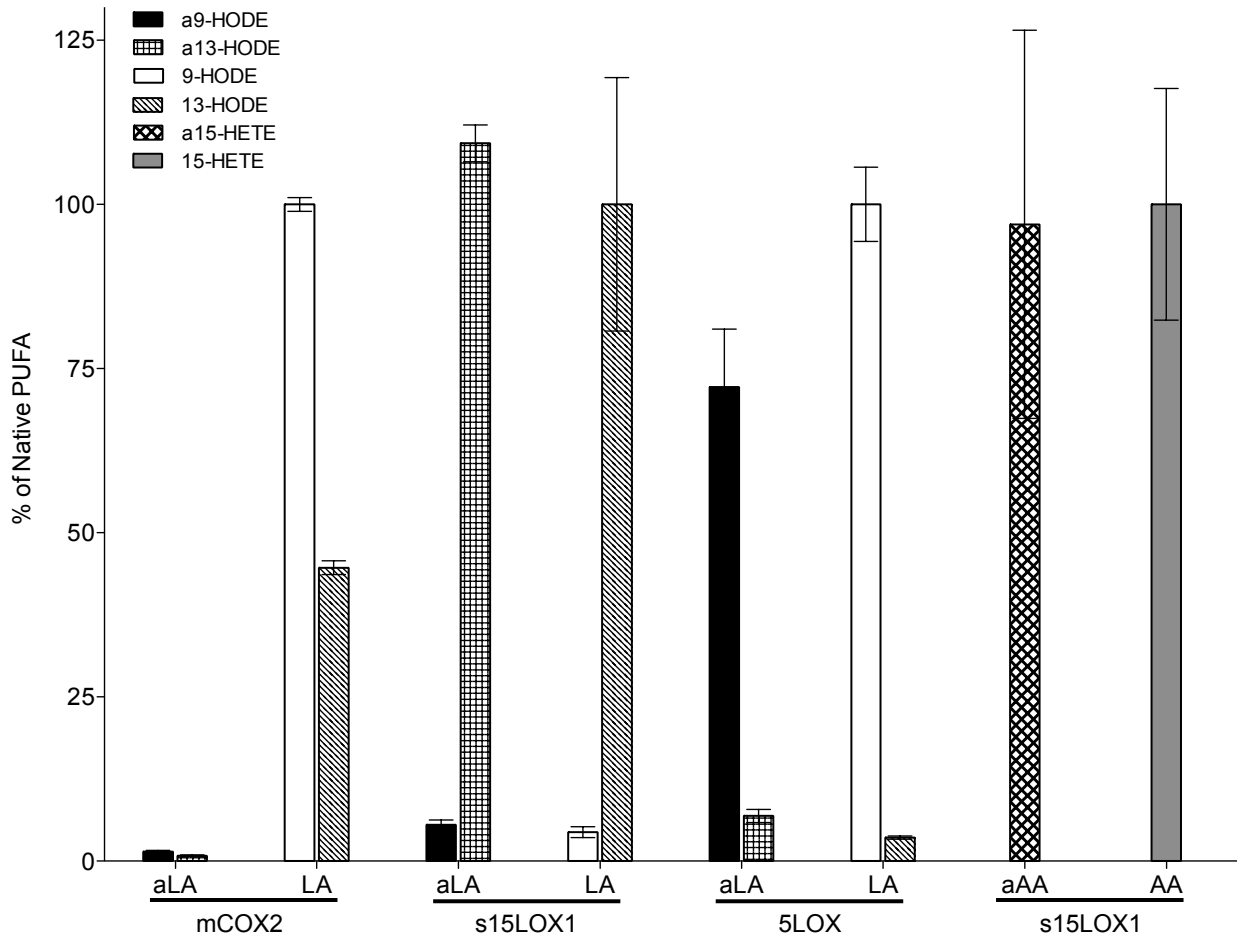
Supplemental Figure 2. Chiral analysis of alkynyl 13-c,t HODE methyl ester produced enzymatically by the reaction with s15LOX1 giving an optically pure product (A), and nonenzymatically giving mixture of optical enantiomers (B).



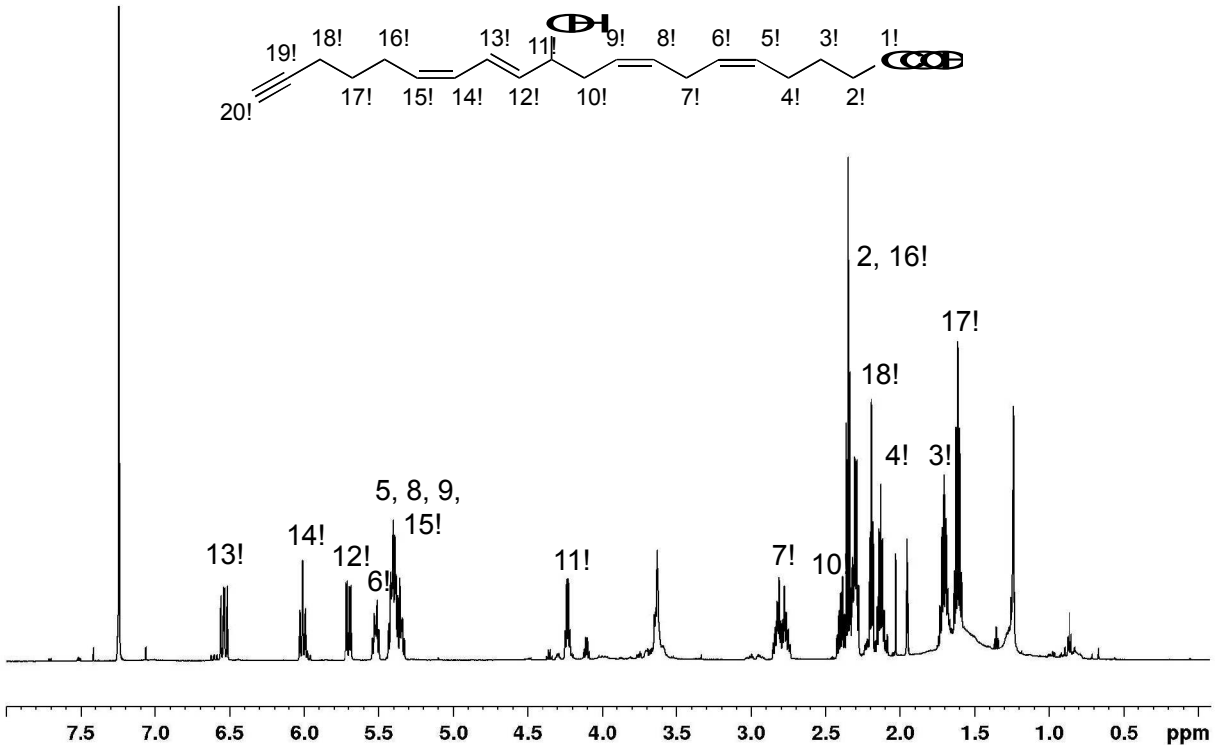
Supplemental Figure 3. RP-LC-MS (SRM) profiles of aF$_{2\alpha}$IsoPs generated upon MeOAMVN initiated oxidations of aAA compared to F$_2$-IsoPs formed from AA. Chromatography was with a MeCN/MeOH 2mM aq. NH_4OAc gradient as described in the Experimental Section.



Supplemental Figure 4. A comparison of the metabolite profiles of *a*LA (A) and LA (C) with s15LOX1. The metabolism of aLA by s15LOX1 shows a single product showing the addition of oxygen. The metabolism of LA by s15LOX1 also shows a single product showing the addition of oxygen.



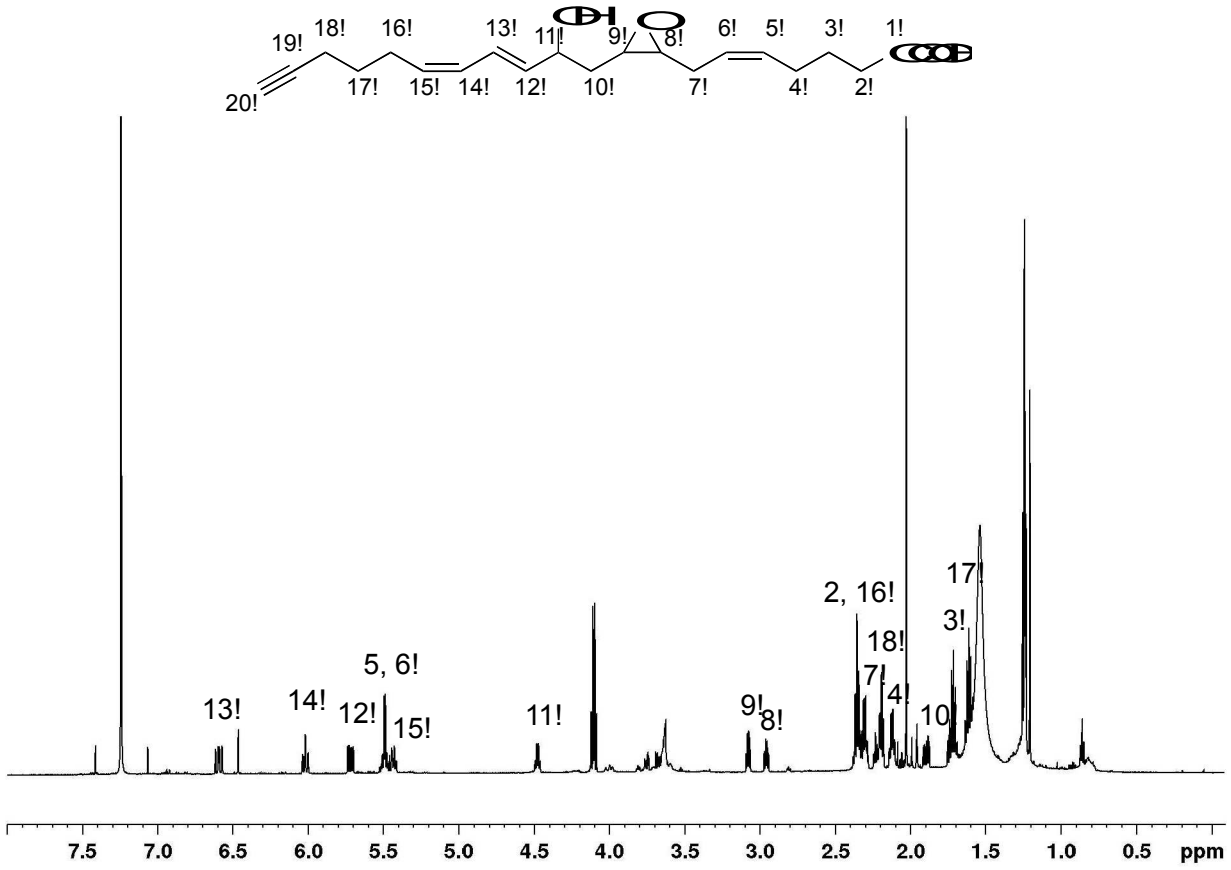
Supplemental Figure 5. Quantification of single oxygenation metabolites for the reaction between the PUFA pairs *a*LA/LA and *a*AA/AA with murine COX2, soybean 15LOX1, and potato 5LOX. Fatty acid pairs are normalized to the most abundant native (LA or AA) metabolite for each enzyme.



Supplemental Figure 6. ^{1}H NMR spectrum of *a*11-HETE in $CDCl_3$

Chemical shift	Multiplicity	Proton(s)	Coupling Constant
ppm		*carbon no.*	*Hz*
6.54	*dd*	H13	$J_{12,13}$ = 15.1
6.01	*t*	H14	$J_{14,15}$ = 11.0
5.70	*dd*	H12	$J_{12,13}$ = 15.2
5.51	*m*	H6	
5.39	*m*	H5, 8, 9, 15	
4.23	*q*	H11	
2.78	*m*	H7	
2.34	*m*	H2a, 10, 16	
2.19	*m*	H18	
2.12	*quint*	H4	
1.70	*m*	H3	
1.61	*quint*	H17	

Supplemental Table 2. ^{1}H-NMR chemical shifts and coupling constants of the collected LCMS peak with m/z = 315.2, which was identified to be *a*11-HETE.



Supplemental Figure 7. ^{1}H NMR spectrum of *a*11-8,9-HEET in $CDCl_3$

Chemical shift	Multiplicity	Proton(s)	Coupling Constant
ppm		*carbon no.*	*Hz*
6.59	*dd*	H13	$J_{12,13}$ = 15.1
6.02	*t*	H14	$J_{14,15}$ = 11.0
5.72	*dd*	H12	$J_{12,13}$ = 15.2
5.51	*m*	H5,6	
5.45	*dt*	H15	$J_{14,15}$ = 10.6
4.48	*q*	H11	
3.08	*quint*	H9	$J_{8,9}$ = 4.2
2.95	*dt*	H8	$J_{8,9}$ = 4.3
2.24	*m*	H2,4,7,16,18	
1.90	*m*	H10a	
1.72	*m*	H10b	
1.61	*quint*	H17	

Supplemental Table 3. ^{1}H-NMR chemical shifts and coupling constants of the collected LCMS peak with m/z = 331.2, which was identified to be *a*11-8,9-HEET.