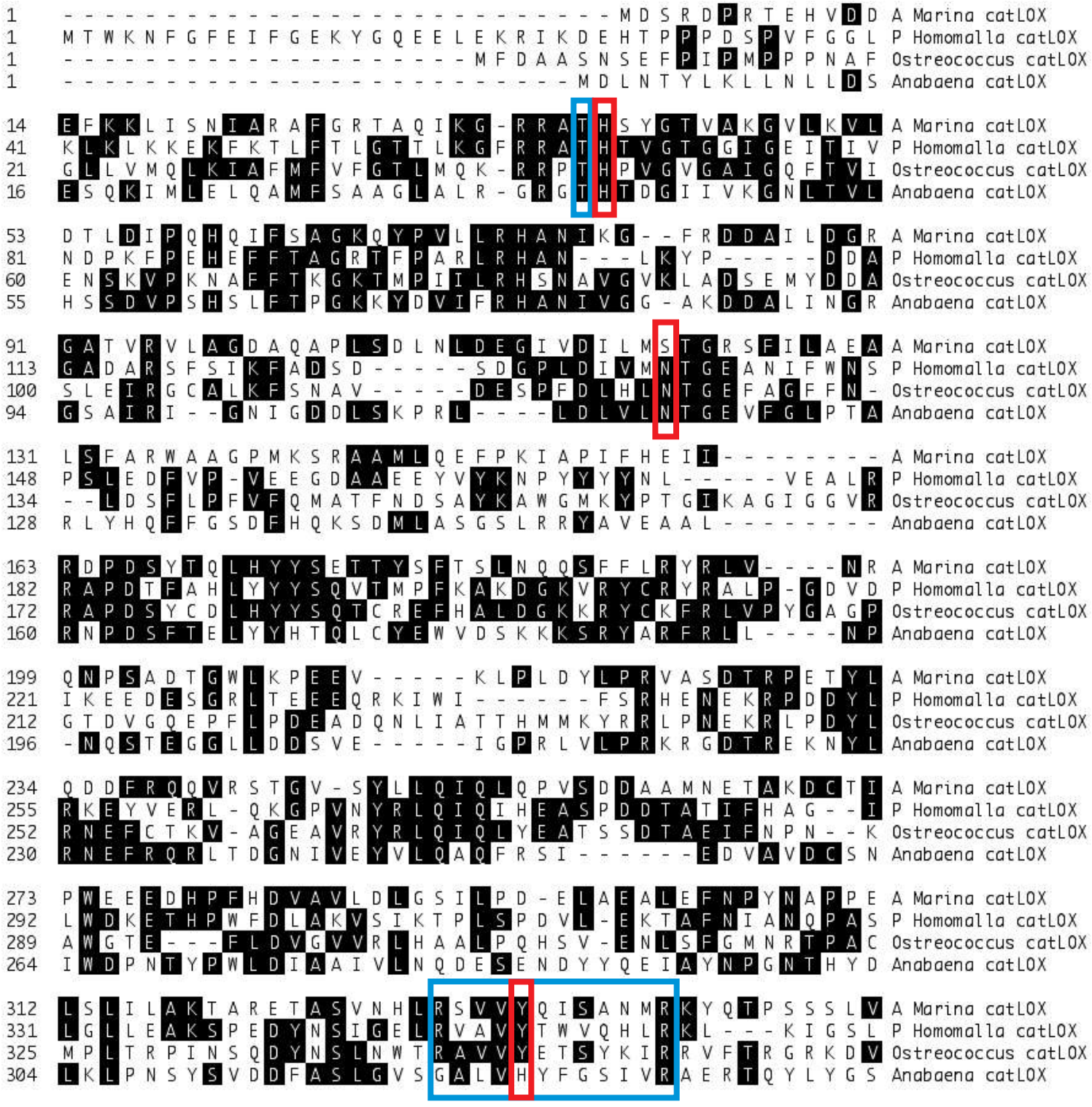
Supplemental Data

Supplemental Data, **Figure S1** (continued on next two pages)
Alignment of the *A. marina* full length catalase-related hemoprotein/lipoxygenase sequence (GenBank accession number ABW27596) with sequences from *Plexaura homomalla* (AF003692) *Ostreococcus lucimarinus* (XM_001418864) and *Anabaena* PCC 7120 (NP_478445.1). The alignment was performed using the Clustal W program in DNAStar with some manual adjustments (mainly to remove spaces corresponding to the different lengths of the N-termini and to make one single long space for the shorter LOX domains of *A. marina* and *Anabaena*).
Page 1: Highlighted in **Red** are the distal heme His and Asn (Ser in *A. marina*) and the Tyr proximal heme ligand. In **Blue** are the Thr next to the distal His and the consensus sequence around the proximal Tyr ligand, RxxxYxxxxxxR)



Alignment **Figure S1**, second page
This shows the first half of the LOX domains
Highlighted in **Red** are the first two ligands to the lipoxygenase non-heme iron

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352 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A Marina catLOX
368 V P A G Q N A I Y N V E V E T G D R E H A G T D A T I T I R I T G A K G R T D Y P Homomalla catLOX
365 Y A P E A S K K Y T I K V T T H S I A G S G I D G D V V L Q F V G T R G A T P K Ostreococcus catLOX
344 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Anabaena catLOX

352 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A Marina catLOX
408 L K L D K W F H N D F E A G S K E Q Y T V Q G F D V G D I Q L I E L H S D G G G P Homomalla catLOX
405 I V L H D - L G D S F A T G A I S T H F V H A D D I G E P A Y A I I S Q - - - - Ostreococcus catLOX
344 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Anabaena catLOX

352 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A Marina catLOX
448 Y W S G D P D W F V N R V I I I S S T Q D R V - Y S F P C F R W V I K D M V L F P Homomalla catLOX
440 - - T S K S S W N C G R I D V T F T S Q I P T S F T L N V W R Y I G Q G V R F V Ostreococcus catLOX
344 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Anabaena catLOX

352 - - - - - - - - - - - - - - - - - D W G S G H Q P S L P E Q Y P Y G T G K T P S A Marina catLOX
487 P G E A T L P F N E V P - - A I V S E Q R Q K E L E Q R K L T Y Q W D Y V S D D P Homomalla catLOX
478 L P C S I T P T T S L A C R Q L I A D A T E Q Y L S F M K K E F D W S N G S Y - Ostreococcus catLOX
344 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Anabaena catLOX

375 F D N T K P L P A R V K P K P R Y W A N F G L K L I P N Q Q L - - D P D L P E L A Marina catLOX
525 M P G N I K A K T H D D L P R D V Q F T D E K S R S Y Q E S R K A A L V N L G I P Homomalla catLOX
517 L P N H S T Y D D H S K L P I T E Q F S S T K Y K D F F V D T F V G F E N K G V Ostreococcus catLOX
344 - K D D L P G K P V Y F P L P V T E I P S K R F L F L L E K Y N F L T D N S Y P Anabaena catLOX

413 G I - - T G A L D - L M G T S V V S Y M P P N L T R - - - - T R L D K F S D D F A Marina catLOX
565 G S L F T M F E N W D S Y D D Y H I L Y R N W I L G G T P N M A D R W H E D R W P Homomalla catLOX
557 A E M I K D - E E I K S L D D Y Y R L Y A S - - L P P V A I I R D - W R S D E E Ostreococcus catLOX
383 S D G E - - H D K I E A L V S A M P T T A L D L A V G T - - T D P T D I P D S Y Anabaena catLOX

446 F V E R R L N G F N P G K L N R V T G H - A W Q Y Q V C Y D C S K H Q V E P A G A Marina catLOX
605 F G Y Q F L N G A N P V I L T R C D A L P S - N F P V T N E H V N A S L D R G K P Homomalla catLOX
593 F G R Q F M N G T H P N Q V R K V S Q I P A - K F R V T E E H V R G V L P P G K Ostreococcus catLOX
419 F L E R R L N G Y N P G A I R E S S G Q E G W T H E L T H N L A K Y D I K P G L Anabaena catLOX

485 I L P T K I T - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A Marina catLOX
644 N L D E E I K D G H I Y I V D F K V L V G A K S Y G G P V L E D I G Y K V P D H P Homomalla catLOX
632 T L E S E L A A G H I F M V D Y E I L - - - - - - - - - - - - - - - - - - - E D Ostreococcus catLOX
459 H F P D - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Anabaena catLOX

492 - - - - - A R F N F C G Q - - - - - - Y L H P H S - I Q F - T L N G Q T E T Q Q A Marina catLOX
684 L K H D E A D I R Y C A A - P L A L F Y V N K L G H L M P I A I Q I N Q E P G P P Homomalla catLOX
653 I K R G Q G - - - Y C V E N S M A L F F A D K S T A L R P I C I Q - H F Q S G A Ostreococcus catLOX
463 - - - - - - - F V Q C R L - - - - - - F V D K Q N G V K L H S I K I D D H E I T Anabaena catLOX

519 P G - - - - - - - - - D E N W E W S K R L F R C A E F V F Q E A Q S H L G R T H A Marina catLOX
723 E N P I W T P H E E N E H D W M M A K F W L G V A E S N F H Q L N T H L L R T H P Homomalla catLOX
689 S A P V W T P K D G - E Y E W L L A K A H L M C S D G N V H Q M I S H L L G T H Ostreococcus catLOX
490 P C - - - - - - - - - Q E Q W Q Y A K R T Y L Q A E F L S Q E L K L H L A R C H Anabaena catLOX
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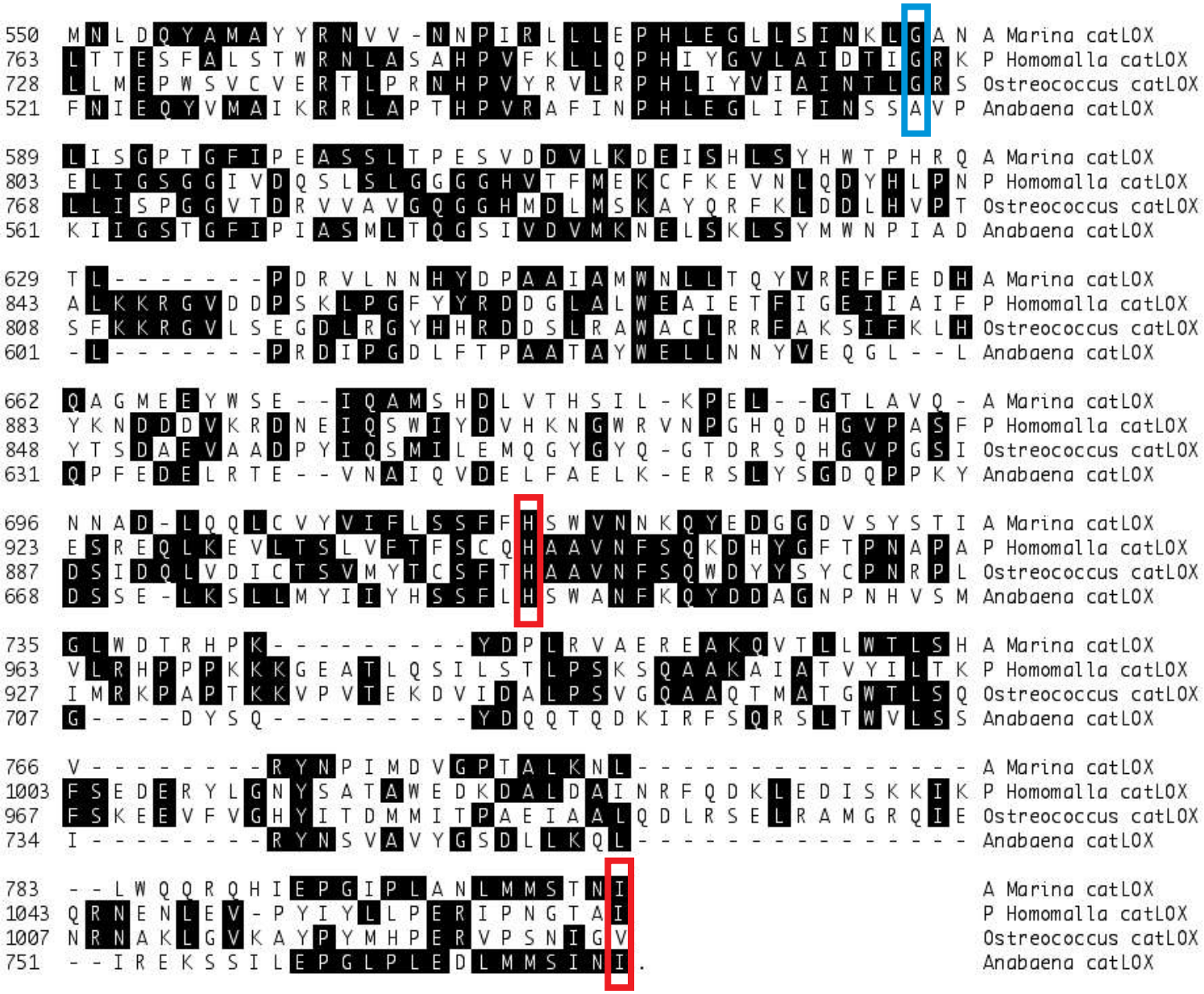
Alignment **Figure S1**, third page
This shows the second half of the LOX domains
Highlighted in **Red** are the third and fourth iron ligands to the lipoxygenase domains and in **Blue** is the Gly-Ala determinant of R-S LOX specificity (Coffa and Brash, PNAS 101:15579, 2004)



Scheme 1. Analysis of the epoxide stereoconfiguration in the linoleic acid-derived epoxyalcohol product of the *A. marina* enzyme by chemical transformations.

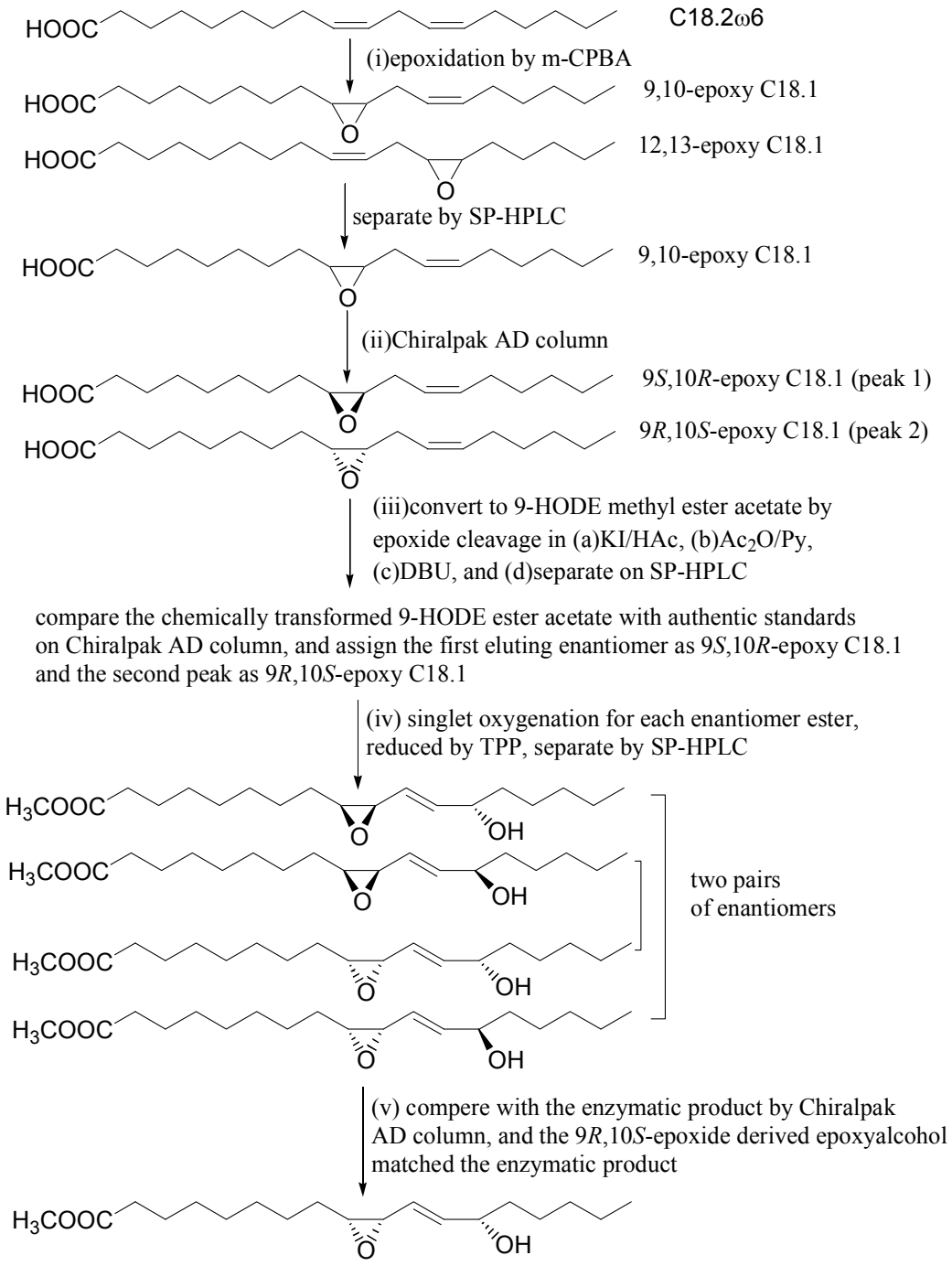
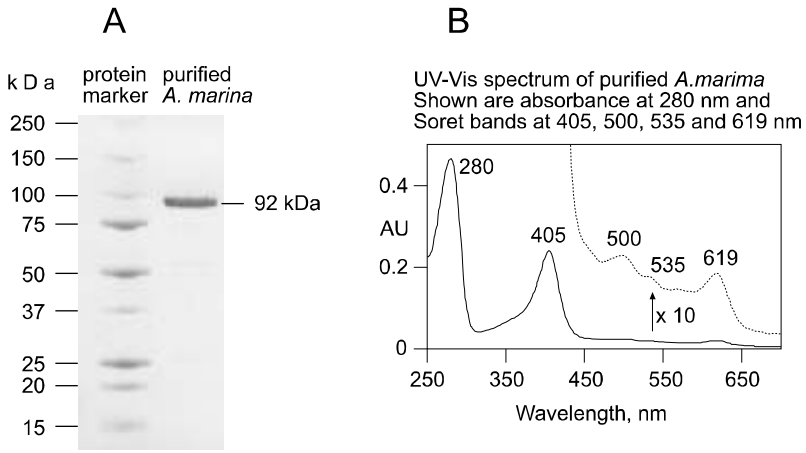
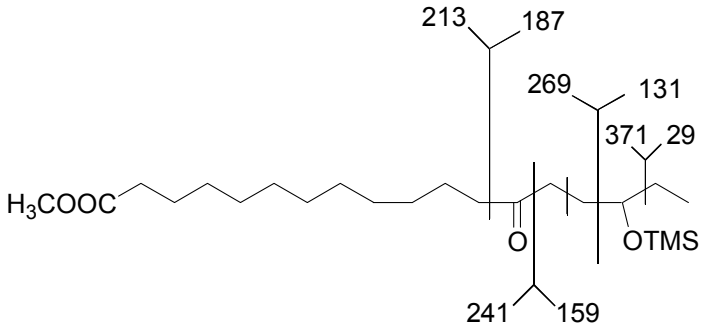


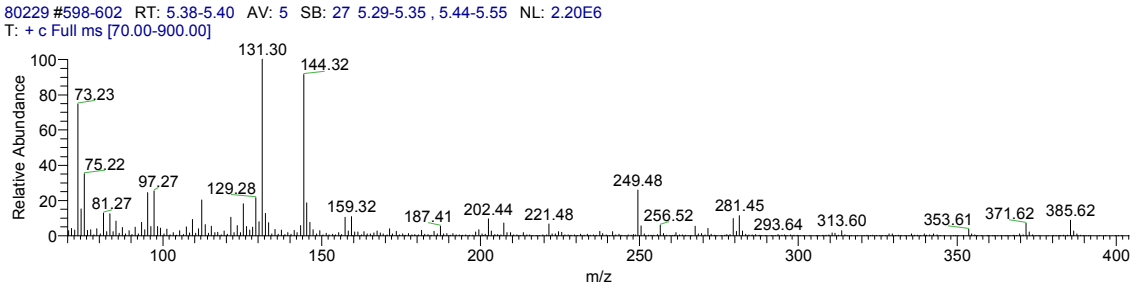
Figure S2. SDS-PAGE and UV-Vis spectrum of *A. marina* fusion protein purified by Ni-NTA affinity column. Panel A: SDS-PAGE indicating an expected 92 kD fusion protein. Panel B: the UV-Vis spectrum showing the main Soret band at 405 nm and the 10 fold expanded y-axis showing more Soret bands at the longer wavelength 500, 535 and 619

nm. The absorbance ratio for $A_{405/280}$ is 0.52, demonstrating a good incorporation of heme in the purified protein.



Identification of peak 1 by GC-MS

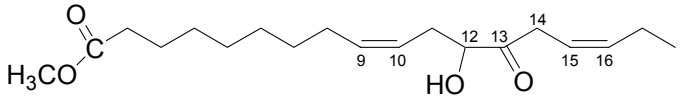




Peak 1 (refers to main text Figure 2A) compound was converted to methyl ester TMS ether derivative and hydrogenated. GC-MS spectrum showed a base ion at m/z of 131 (100%), which is the α-cleavage between C15 and C16. The ion of m/z 144 (93%) was a rearrangement ion produced by cleavage between C14 and C15, followed by H migration from C15 to the carbonyl oxygen (H.W. Gardner et al, Lipids, Vol. 10, P602-608). Other ions are at m/z of 73 (75%), 159 (11%), 187 (5%), 281 (11%, 371-90[TMSOH]), 371 (7%, M-29), 385 (9%, M-15). The fragmentation pattern confirmed a structure of 13-keto-16-hydroxy-octadeca-9,14-dienoic acid for peak 1. Alternatively, peak 1 compound was converted to TMS ester TMS ether methoxime derivatives (with and without

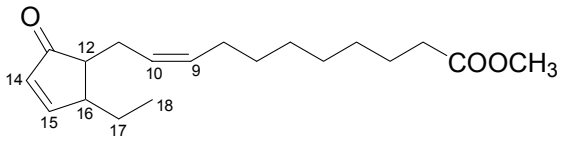
hydrogenation). GC-MS spectrum again confirmed the structure as a γ-ketol of 13-keto-16-hydroxy-octadeca-9,14-dienoic acid.

Table 1. ^{1}H-NMR chemical shift of α-ketol (12-hydroxy-13-keto-octadeca-9,15-dienoic acid). NMR spectra were acquired at room temperature in d-benzene solvent.



Chemical shift	Multiplicity	Assignment, coupling constant
5.57	m	H10
5.45	m	H9, H15, H16
3.99	dt	H12; $J_{12,11}$ = 10.9Hz, $J_{12,OH}$ = 5.2Hz
3.39	d	-OH (on C12); $J_{12,OH}$ = 5.2Hz
3.36	s	-OCH_3
2.97	dd	H11a; $J_{11a,12}$ = 6.8Hz, $J_{11a,10}$ =17.8Hz
2.80	dd	H11b; $J_{11b,12}$ = 7.0Hz, $J_{11b,10}$ =17.8Hz
2.45	m	H14a
2.23	m	H14b
2.11	t	H2, $J_{2,3}$ = 7.4Hz
1.95	m	H8
1.83	quintet	H17; $J_{17,16}$ = 14.9Hz, $J_{17,18}$ = 7.5Hz
1.55	m	H3
1.25	m	H7
1.17	m	H4, H5, H6
0.84	t	H18; $J_{18,17}$ = 7.6Hz

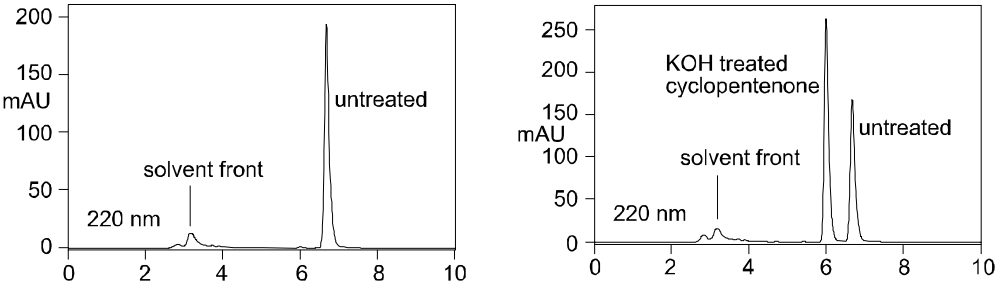
Table 2. ^{1}H-NMR chemical shift of the cyclopentenone. NMR spectra were acquired at room temperature in d-benzene solvent.



Chemical shift	Multiplicity	Assignment, coupling constant
7.01	dd	H15; $J_{15,14}$ = 5.8Hz, $J_{15,16}$ = 2.7Hz
5.99	d	H14; $J_{14,15}$ = 5.9Hz
5.45	quintet	H9, H10; $J_{9,10}$ = 10.6Hz
3.36	s	-OCH_3
2.72	m	H11a
2.33	m	H16

2.19	m	H11b
2.12	t,m	H2, H12
2.02	m	H8
1.55	quintet	H3
1.49	m	H17a
1.29	m	H7
1.18	m	H4, H5, H6
0.83	m	H17b
0.65	t	H18; $J_{18,17}$ = 9.1Hz

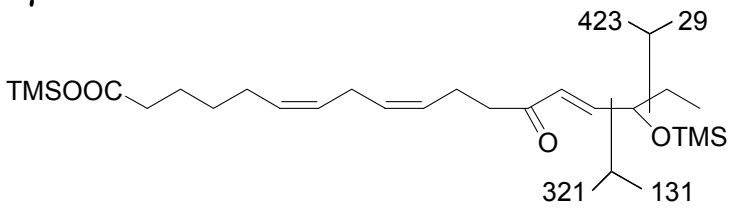
Figure S3. SP-HPLC analysis of the side chain transformation in alkali treated cyclopentenone molecule. The analysis was performed with the solvent hexane/isopropanol (100/1.5, v/v) using the silica column (4.6x250mm) at the flow rate of 1 ml/min.

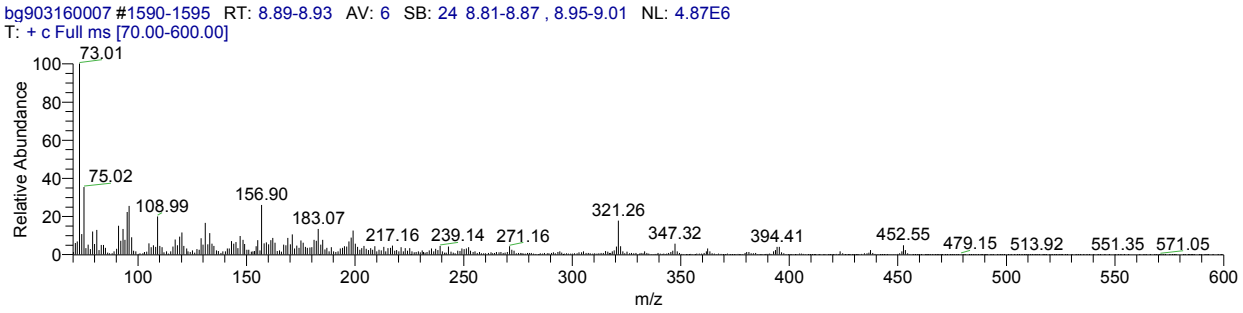


The alkali treatment to the cyclopentenone showed a peak shift to the left on SP-HPLC, confirmed a cis-trans transformation due to the alkali treatment, indicating a cis arrangement of the two side chains in cyclopentenone molecule.

Products analysis of C18.4ω3 incubation with *A. marina* fusion protein by GC-MS

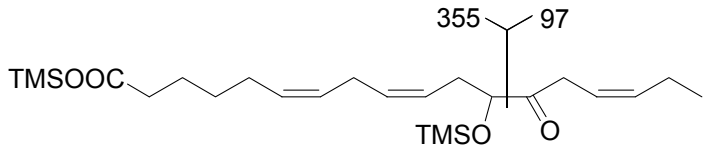
1. γ-ketol identification:

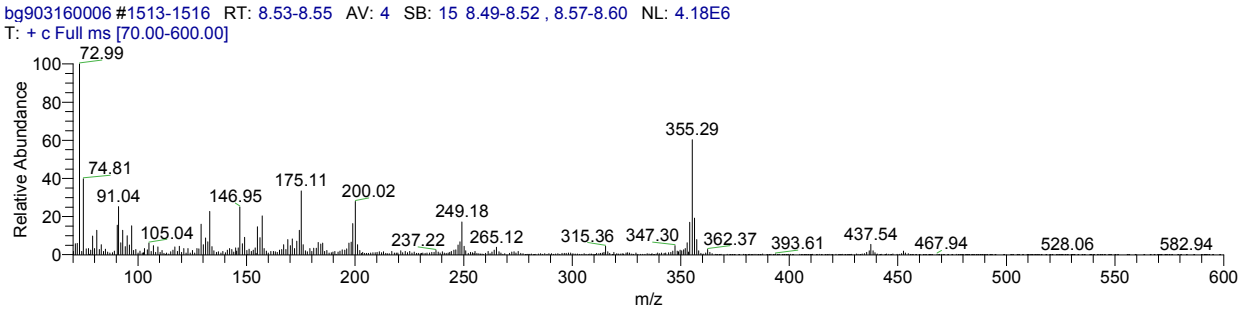




GC-MS spectrum of peak1(refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 73 (100%), 131 (17%), 321 (18%), 423 (0.2%, M-29), 437 (0.3%, M-15), and 452 (0.1%, M). These ions confirmed a molecular structure of 16-hydroxy-13-ketol-octadec-6,9*Z*,14*E* trienoic acid, γ-ketol.

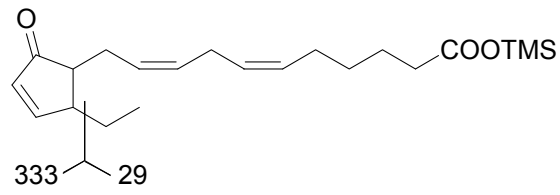
2. α-ketol identification:

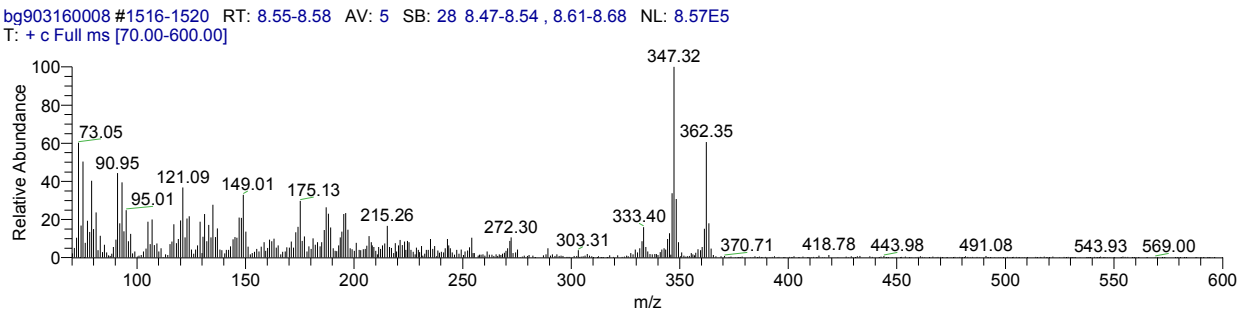




GC-MS spectrum of peak 2 (refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 73 (100%), 355 (60%), 97 (15%), 437 (3%, M-15), and 452 (0.2%, M), indicating a molecular structure of 12-hydroxy-13-ketol-octadec-6,9*Z*,15*Z* trienoic acid, α-ketol.

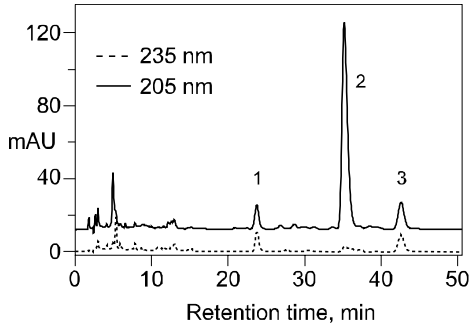
3. Cyclopentenone identification:





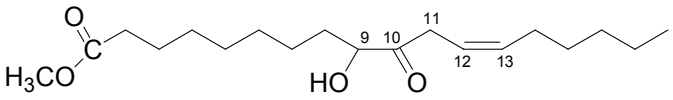
GC-MS spectrum of peak 3 (refers to main text Figure 2B) TMS ester TMS ether derivative showed ions of m/z at 347 (100%, M-15), 362 (61%, M), 73 (60%), 333 (20%, M-29). These ions confirmed a molecular structure of cyclopentenone.

Figure S4. RP-HPLC profile of γ-ketol, α-ketol and cyclopentenone from incubation of C20.4ω6 with *A. marina*. The RP-HPLC was carried out using a Waters Symmetry C18 column (25 x 0.46 cm), a solvent system of methanol/water/acetic acid in the proportions 70/30/0.01 (v/v/v), a flow rate of 1 ml/min and with UV detection at 205 and 235nm.



Peaks 1, 2, 3 of the RP-HPLC profile correspond to γ-ketol, α-ketol and cyclopentenone, the hydrolysis and cyclization products from the allene oxide. They were identified by GC-MS (data not shown) and the typical UV spectra.

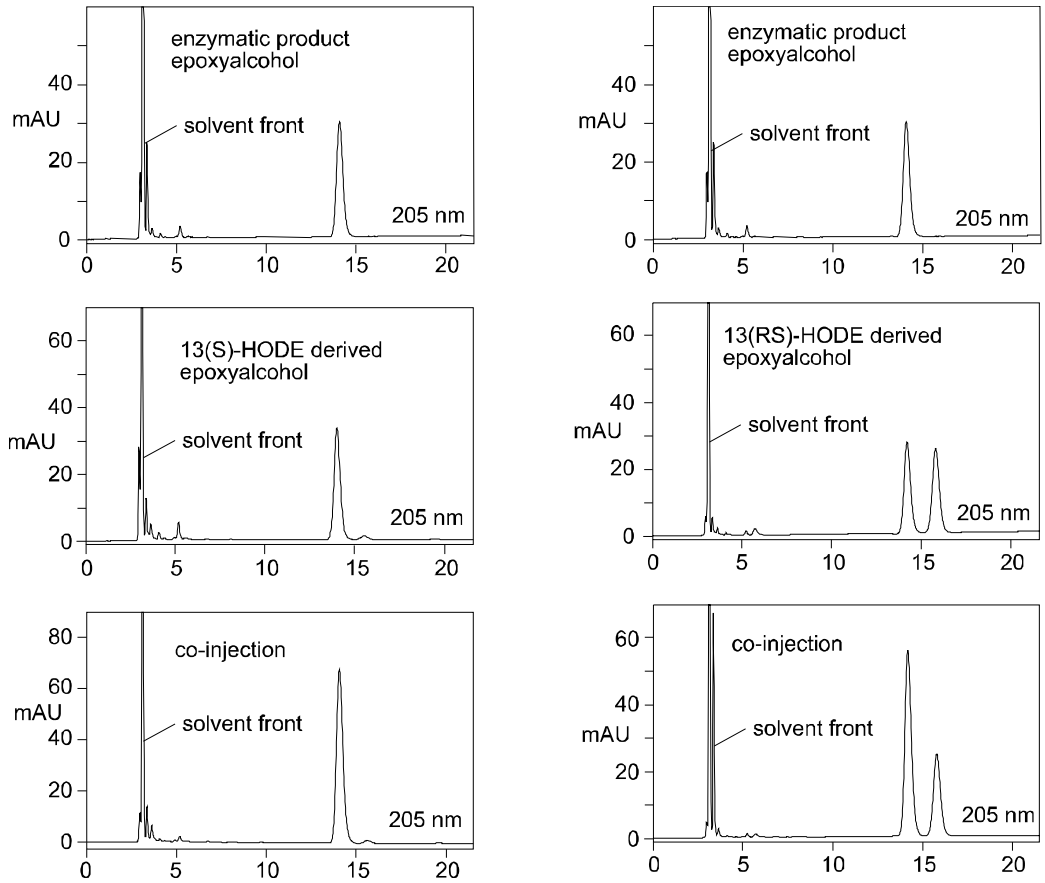
Table 3. ^{1}H-NMR chemical shift of α-ketol (9-hydroxy-10-keto-octadec-12*Z*-enoic acid). NMR spectra were acquired at room temperature in d-benzene solvent.



Chemical shift	Multiplicity	Assignment, coupling constant
5.59	m	H12
5.48	m	H13
3.95	m	H9
3.38	s	-OH
3.35	s	$-OCH_3$
2.98	dd	H11a; $J_{11a,12} = 14.3$Hz , $J_{11a,13} = 6.0$Hz

2.81	dd	H11b; $J_{11b,12}$ = 14.3Hz, $J_{11b,13}$ = 7.1Hz
2.09	t	H2; $J_{2,3}$ = 7.1Hz
1.87	dt	H14; $J_{14,13}$ = 12.4Hz, $J_{14,15}$ = 6.2Hz
1.58	m	H8b
1.53	m	H3
1.32-1.18	m	H4, H5, H6, H7, H8a, H15, H16, H17
0.87	t	H18; $J_{18,17}$ = 7.1Hz

Figure S5. 13-hydroxy stereoconfiguration analysis by Chiralpak AD column.
The analysis was performed with the solvent hexane/ethanol (100/5, v/v) using the Chiralpak AD column (4.6x250mm) at the flow rate of 1 ml/min.



The three panels on the left side are from enzymatic product alone, 13*S*-HODE derived epoxyalcohol and coinjection of enzymatic product with the 13*S*-HODE derived epoxyalcohol, respectively. The13*S*-HODE derived epoxyalcohol cochromatographed with the enzymatic product at the retention time 14.2 min, indicating a 13*S*-hydroxyl stereoconfiguration in the enzymatic epoxyalcohol. The three panels on the right side are from enzymatic product alone, 13*RS*-HODE derived epoxyalcohol and coinjection of enzymatic product with the 13*RS*-HODE derived epoxyalcohol, respectively. The results further confirmed a 13*S*-hydroxyl configuration in the enzymatic product.