Electronic Supplementary Material (ESI) for Green Chemistry.

Enzymatic Synthesis of New Hepoxilins and Trioxilins from Polyunsaturated Fatty Acids†

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Table S1. Chemical names and classification of HXs and TrXs.

Type	Type	Product	Chemical name	Reference
Hepoxilin	A	HXA_3	8-Hydroxy-11,12-epoxyeicosa-5,9,14-trienoic acid	[1]
		HXA_4	8-Hydroxy-11,12-epoxyeicosa-5,10,14,17-tetraenoic acid	[2]
		HXA_5	10-Hydroxy-13,14-epoxydocosa-4,7,11,16,19-pentaenoic acid	[3]
		14,15-HXA_3	11-Hydroxy-14,15-epoxyeicosa-5,8,12-trienoic acid	[4]
	B	HXB_3	10-Hydroxy-11,12-epoxyeicosa-5,8,14-trienoic acid	[5]
		HXB_4	10-Hydroxy-11,12-epoxyeicosa-5,8,14,17-tetraenoic acid	[5]
		HXB_5	12-Hydroxy-13,14-epoxydocosa-4,7,10,16,19-pentaenoic acid	[5]
		14,15-HXB_3 (**4**)	13-Hydroxy-14,15-epoxyeicosa-5,8,11-trienoic acid	[4]
		14,15-HXB_4 (**9**)	13-Hydroxy-14,15-epoxyeicosa-5,8,11,17-tetraenoic acid	This study
		16,17-HXB_5 (**14**)	15-Hydroxy-16,17-epoxydocosa-4,7,10,13,19-pentaenoic acid	This study
		14,15-HXB_2(γ) (**24**)	13-Hydroxy-14,15-epoxyeicosa-8,11-dienoic acid	This study
		16,17-HXB_3 (**19**)	15-Hydroxy-16,17-epoxydocosa-7,10,13-trienoic acid	This study
	D	HXD_3	13-Hydroxy-11,12-epoxyeicosa-5,8,14-trienoic acid	[5]
	E	HXE_3	15-Hydroxy-11,12-epoxyeicosa-5,8,13-trienoic acid	[5]
Trioxilin	A	$TrXA_3$	8,11,12-Trihydroxyeicosa-5,9,14-trienoic acid	[6]
		$TrXA_4$	8,11,12-Trihydroxyeicosa-5,10,14,17-tetraenoic acid	[2]
		$TrXA_5$	10,13,14-Trihydroxydocosa-4,7,11,16,19-pentaenoic acid	[3]
	B	$TrXB_3$	10,11,12-Trihydroxyeicosa-5,8,14-trienoic acid	[5]
		$TrXB_4$	10,11,12-Trihydroxyeicosa-5,8,14,17-tetraenoic acid	[5]
		$TrXB_5$	12,13,14-Trihydroxydocosa-4,7,10,16,19-pentaenoic acid	[5]
		13,14,15-$TrXB_3$ (**5**)	13,14,15-Trihydroxyeicosa-5,8,11-trienoic acid	[7]
		13,14,15-$TrXB_4$ (**10**)	13,14,15-Trihydroxyeicosa-5,8,11,17-tetraenoic acid	This study
		15,16,17-$TrXB_5$ (**15**)	15,16,17-Trihydroxydocosa-4,7,10,13,19-pentaenoic acid	This study
		13,14,15-$TrXB_2$(γ) (**25**)	13,14,15-Trihydroxyeicosa-8,11-dienoic acid	This study
		15,16,17-$TrXB_3$ (**20**)	15,16,17-Trihydroxydocosa-7,10,13-trienoic acid	This study
	C	$TrXC_3$	8,9,12-Trihydroxyeicosa-5,10,14-trienoic acid	[8]
	D	$TrXD_3$	11,12,13-Trihydroxyeicosa-5,8,14-trienoic acid	[5]
	E	$TrXE_3$	11,12,15-Trihydroxyeicosa-5,8,13-trienoic acid	[5]

Table S2. Isolated yields, purities, and regression equations for calibration curves of PUFAs and HFAs, HXs, and TrXs obtained from PUFAs by ARA 15-LOX from *B. thailandensis* without and with EH from *M. xanthus*.

Type	Product	Isolated yield (w/w)	Purity (w/w)	Regression equation[a]	r^2
PUFA	ARA (**1**)	–	≥ 98.5%	$y = 0.00003583x - 0.0562$	0.9936
	EPA (**6**)	–	≥ 99%	$y = 0.00003014x - 0.0941$	0.9994
	DHA (**11**)	–	≥ 98%	$y = 0.00002772x - 0.0171$	0.9948
	ADA (**16**)	–	≥ 98%	$y = 0.0005711x + 0.0119$	0.9978
	DGLA (**21**)	–	≥ 99%	$y = 0.0002046x - 0.0543$	0.9992
HFA	15-HETE (**3**)	–	≥ 95%	$y = 0.00009662x + 0.0056$	0.9922
	15-HEPE (**8**)	–	≥ 98%	$y = 0.0002302x + 0.0735$	0.9765
	17-HDoHE (**13**)	–	≥ 98%	$y = 0.0006845x + 0.0156$	0.9922
	17-HDoTE (**18**)	86%	96%	$y = 0.001988x - 0.0148$	0.9952
	15-HETrE(γ) (**23**)	88%	94%	$y = 0.001069x + 0.0641$	0.9844
HX	14,15-HXB_3 (**4**)	80%	95%	$y = 0.0002206x - 0.0346$	0.9928
	14,15-HXB_4 (**9**)	70%	91%	$y = 0.0002392x - 0.0426$	0.9919
	16,17-HXB_5 (**14**)	82%	93%	$y = 0.0004638x - 0.0195$	0.9950
	16,17-HXB_3 (**19**)	81%	94%	$y = 0.0002720x - 0.0280$	0.9903
	16,17-HXB_2(γ) (**24**)	75%	92%	$y = 0.001083x - 0.000371$	0.9947
TrX	13,14,15-$TrXB_3$ (**5**)	78%	93%	$y = 0.0005837x - 0.00822$	0.9971
	13,14,15-$TrXB_4$ (**10**)	73%	91%	$y = 0.0005802x + 0.00537$	0.9987
	15,16,17-$TrXB_5$ (**15**)	86%	98%	$y = 0.0003952x + 0.04184$	0.9903
	15,16,17-$TrXB_3$ (**20**)	83%	96%	$y = 0.0006904x + 0.0904$	0.9904

[a] x, peak area in HPLC profile; y, concentration of standard in mg mL^{-1}.

Table S3. Specific activity of ARA 15-LOX form *B. thailandensis* (BT) and ARA 11-LOX, ARA 12-LOX, and EH from *M. xanthus* (MX) towards C20 and C22 PUFAs.

Enzyme	Substrate	Product	Specific activity (U mg^{-1})[a]	Reference
BT 15-LOX	ARA (**1**)	15-HpETE (**2**)	23.3 ± 0.3	This study
	EPA (**6**)	15-HpEPE (**7**)	21.4 ± 0.1	This study
	DHA (**11**)	17-HpDoHE (**12**)	15.4 ± 0.5	This study
	DGLA (**21**)	15-HpETrE(γ) (**22**)	4.9 ± 0.2	This study
	ADA (**16**)	17-HpDoTE (**17**)	10.3 ± 0.2	This study
MX EH	14,15-HXB_3 (**4**)	14,15-$TrXB_3$ (**5**)	54.0 ± 0.6	This study
MX 11-LOX	ARA (**1**)	11-HpETE	5.2 ± 0.1	[9]
MX 12-LOX	ARA (**1**)	12-HpETE	15.4 ± 0.1	[10]

15-HpETE, 15-Hydroperoxyeicosatetraenoic acid; 15-HpEPE, 15-Hydroperoxyeicosapentaenoic acid; 17-HpDoHE, 17-Hydroperoxydocosahexaenoic acid; 15-HpETrE(γ), 15-Hydroperoxydocosatrienoic acid(γ); and 17-HpDoTE,17-Hydroperoxydocosatetraenoic acid.

[a]The specific activity calculated by the reaction of purified ARA 15-LOX from *B. thailandensis* or EH from *M. xanthus* with 1 mM substrate for 1 min.

Supporting Figures

Fig. S1 Nomenclature rules of HXs and TrXs. Compounds of HXA and HXB series contain a hydroxyl group at C8 for C20 PUFAs and at C10 for C22 PUFAs; and at C10 for C20 PUFAs and at C12 for C22 PUFAs, respectively. HXD_3 and HXE_3 have a hydroxyl group at C13 and C15, respectively. Compounds of TrXA and TrXB series contain three hydroxyl groups at C8, C11, and C12 for C20 PUFAs and at C10, C13, and C14 for C22 PUFAs; and at C10, C11, and C12 for C20 PUFAs and at C12, C13, and C14 for C22 PUFAs, respectively. $TrXC_3$, $TrXD_3$, $TrXE_3$ have three hydroxyl groups at C8, C9, and C12; C11, C12, and C13; and C11, C12, and C15, respectively. Compounds of HX series contain an epoxide group at C11 and C12 for C20 PUFAs and at C13 and C14 for C22 PUFAs, while compounds of 14,15-HX and 16,17-HX series have an epoxide group at C14 and C15 for C20 PUFAs and at C16 and C17 for C22 PUFAs, respectively. Compounds of TrXA and TrXA series have three hydroxyl groups at C8, C11, and C12 for C20 PUFAs and at C10, C13, and C14 for C22 PUFAs, while compounds of 13,14,15-TrX and 15,16,17-TrX series have three hydroxyl groups at C13, C14, and C15 for C20 PUFAs and at C15, C16, and C17 for C22 PUFAs, respectively.

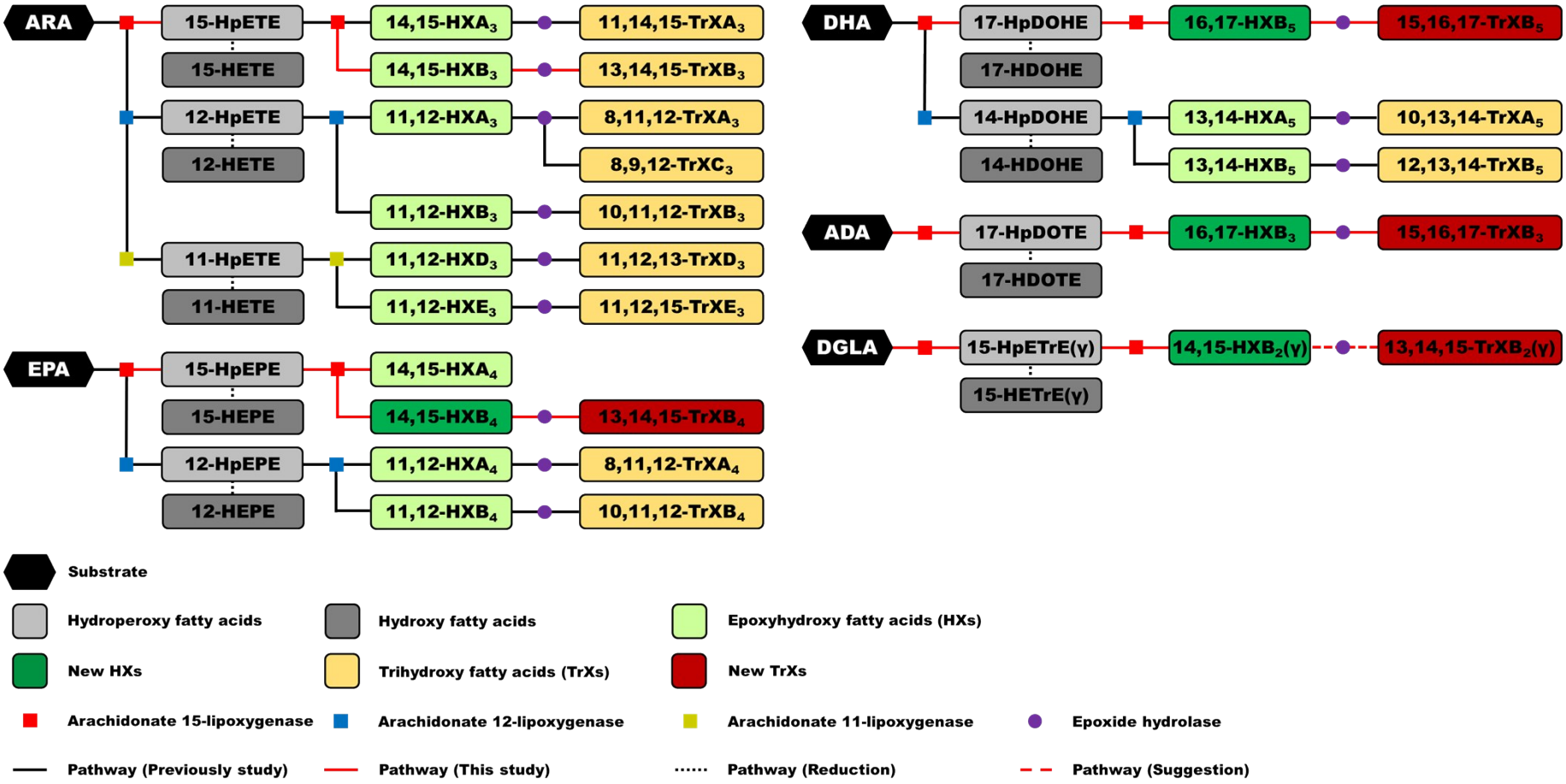


Fig. S2 Biosynthetic pathways of PUFAs into TrXs *via* HXs previously reported and identified in this study.

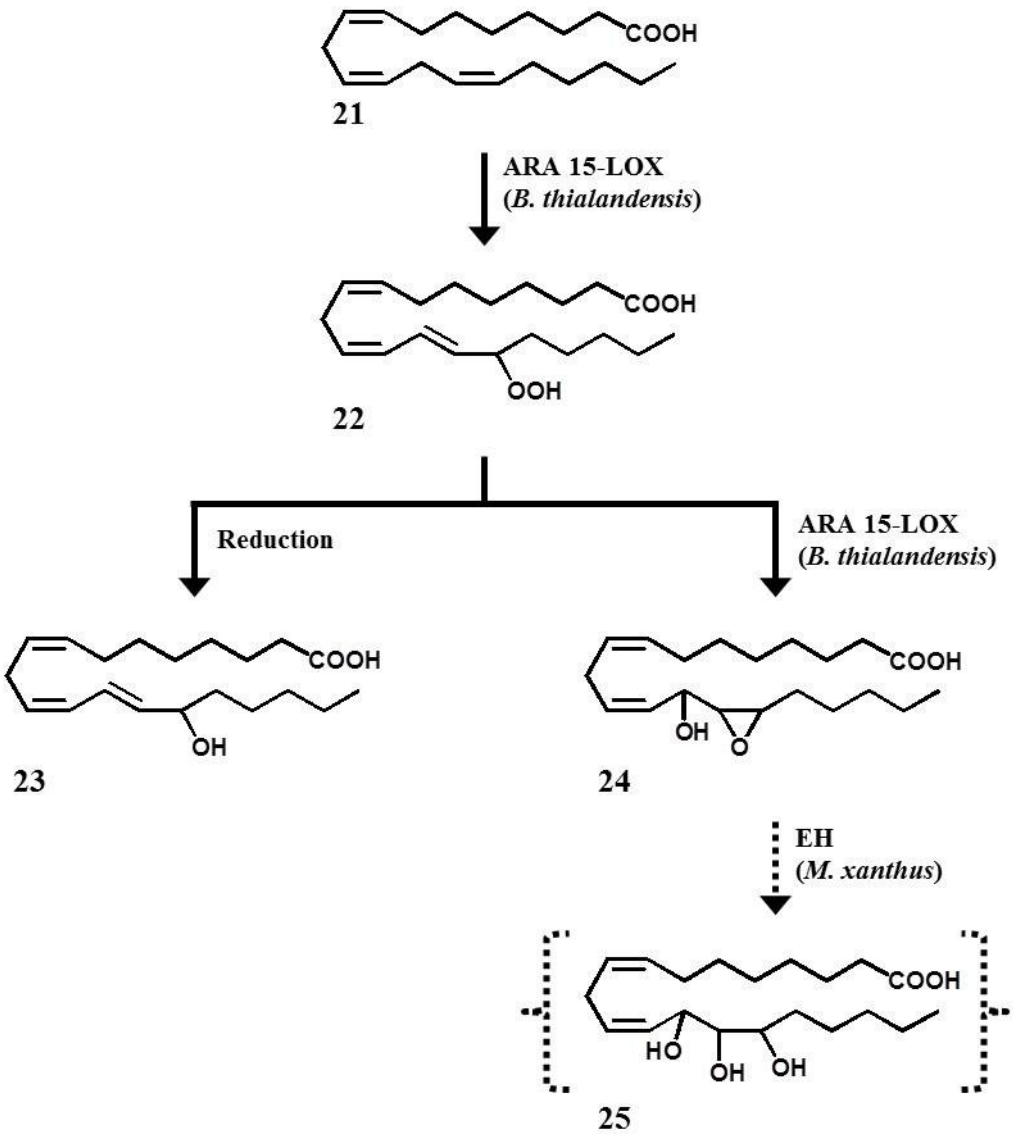


Fig. S3 Biosynthetic pathway of DGLA (**21**) into 13,14,15-TrXB$_2$(γ) (**25**) *via* 14,15-HXB$_2$(γ) (**24**). The compound **25** (dotted bracket) was identified by only LC-MSMS due to the low activity of *E. coli* expressing ARA 15-LOX from *B. thailandensis* and EH from *M. xanthus*.

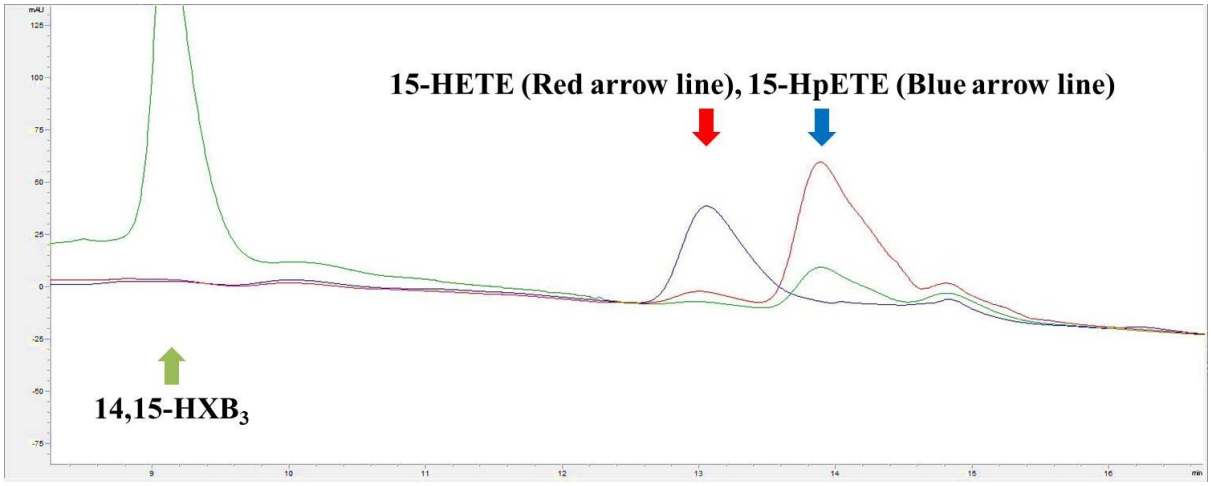


Fig. S4 HPLC chromatograms for the conversion of 15-HpETE to 15-HETE with 50 mM HEPES buffer (red line), *E. coli* cells without plasmid (blue line), and 15-LOX from *B. thailandensis* (green line). The reaction were performed in 50 mM HEPES buffer (pH 7.5) containing 0.5 mM 15-HpETE at 25 °C for 60 min. The concentrations of *E. coli* cells without plasmid and 15-LOX were 6 g L^{-1} and 0.4 g L^{-1}, respectively.

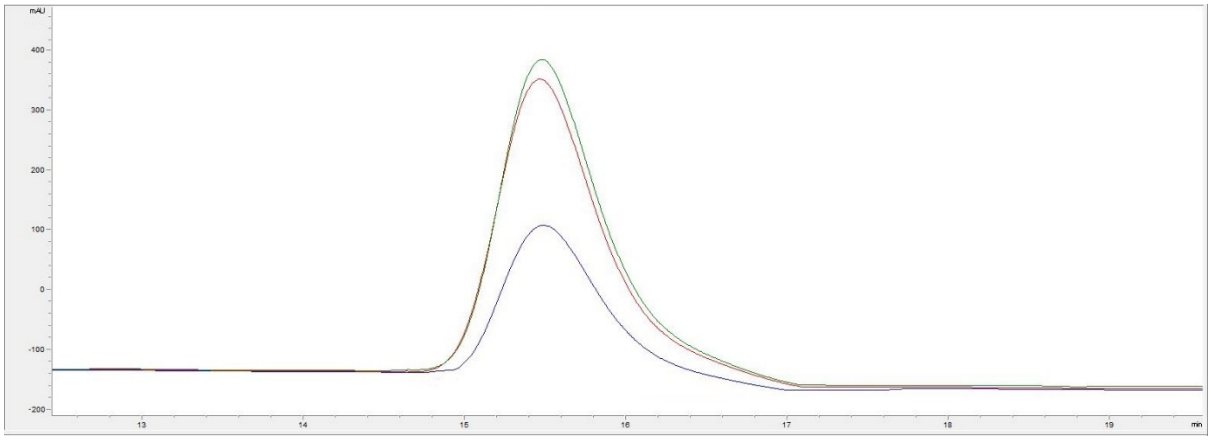


Fig. S5 HPLC chromatograms for the standard and purified compounds of 15-HETE. Green, red, and blue lines represent 15-HETE standard, purified 15-HETE using Prep-LC and SP825 adsorbent resin, and purified 15-HETE using Prep-LC, respectively.

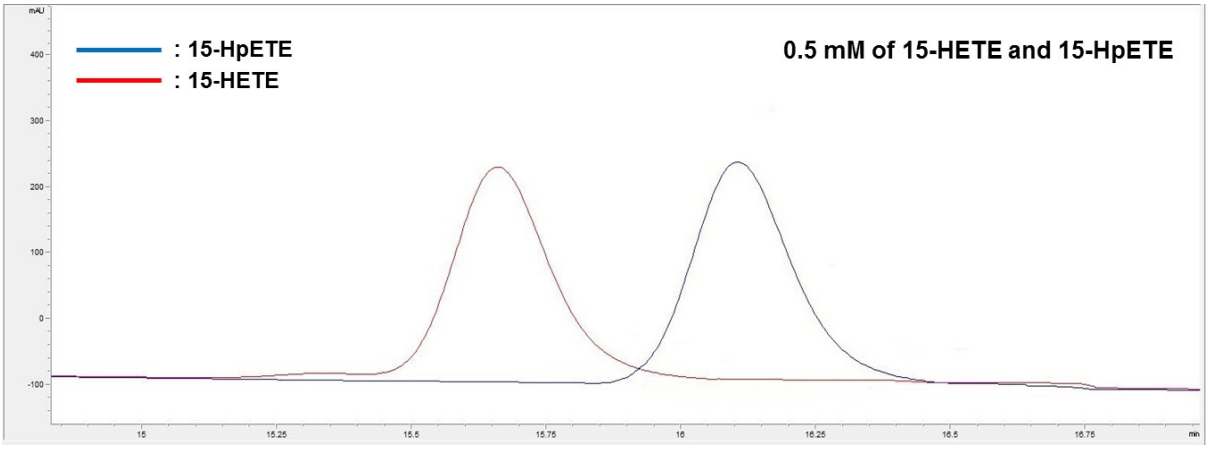


Fig. S6 Peak areas of 15-HpETE and 15-HETE at the same concentrations. The peak area of 15-HpETE is the same as that of 15-HETE.

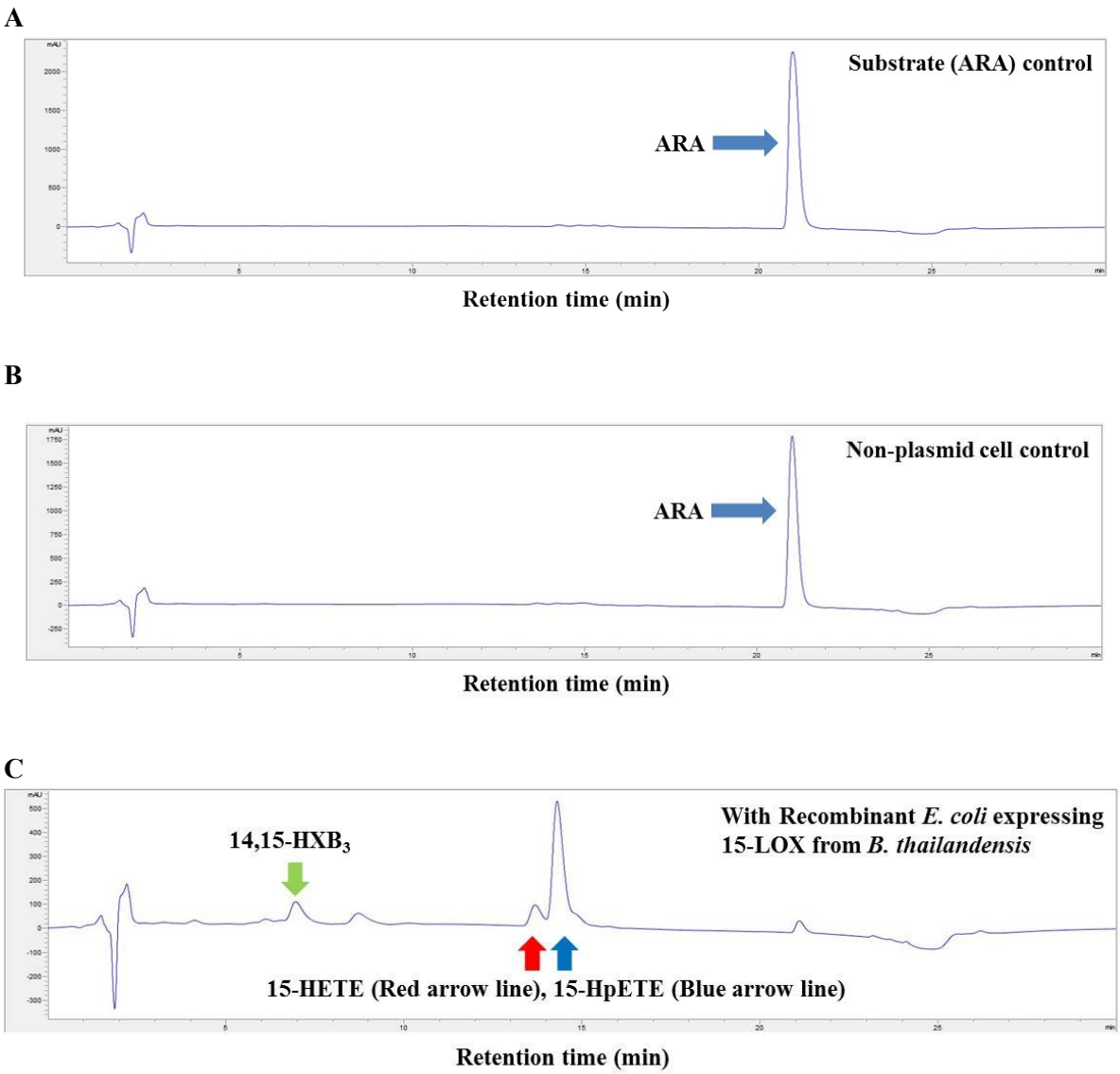


Fig. S7 HPLC profiles of metabolites from ARA by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis*. A) Chromatogram of reaction solution containing 1 mM ARA. **B**) Chromatogram of reaction solution after the reaction with 1 mM ARA and 15 g L^{-1} *E. coli* without plasmid for 60 min. **C**) Chromatogram of reaction solution after the reaction with 1 mM ARA and 15 g L^{-1} *E. coli* expressing ARA 15-LOX from *B. thailandensis* for 60 min.

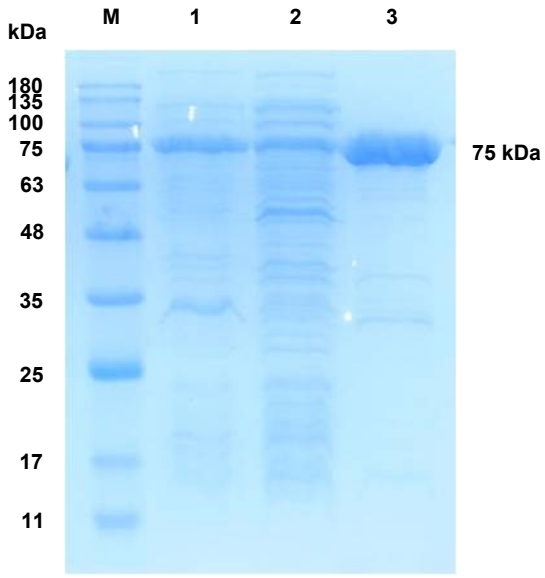


Fig. S8 SDS-PAGE analysis of ARA 15-LOX expressed in recombinant *E. coli* ER2566. Lane M indicates the marker proteins. *M*, molecular mass marker proteins (180,135, 100, 75, 63, 48, 35, 25, 17, and 11 kDa); *lane 1*, crude extract; *lane 2*, pellet, and *lane 3,* purified ARA 15-LOX.

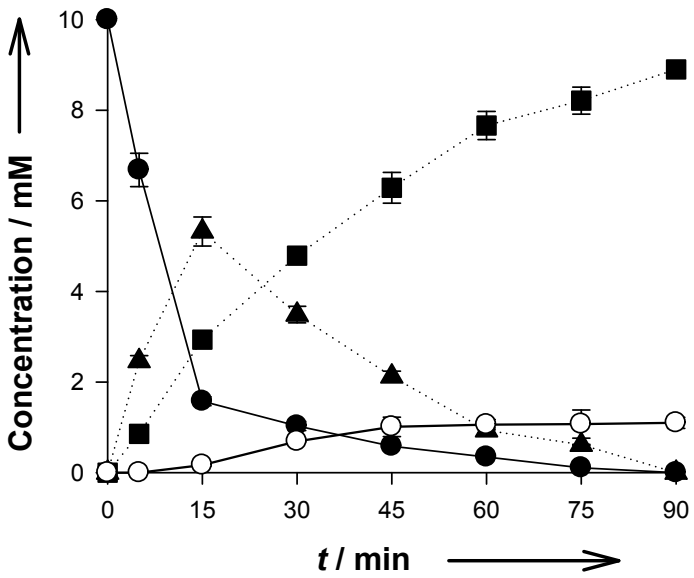


Fig. S9 Biotransformation of DGLA (**21**) into 14,15-$HXB_2(\gamma)$ (**24**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis*. The symbols indicate the concentrations of DGLA (●; **21**), 15-hydroxyperoxyeicosa-8,11,13-trienoic acid (▲; **22**), 15-hydroxyeicosa-8,11,13-trienoic acid (■; **23**), and 14,15-$HXB_2(\gamma)$ (○; **24**). Data represent the means of three experiments and error bars represent the standard deviation.

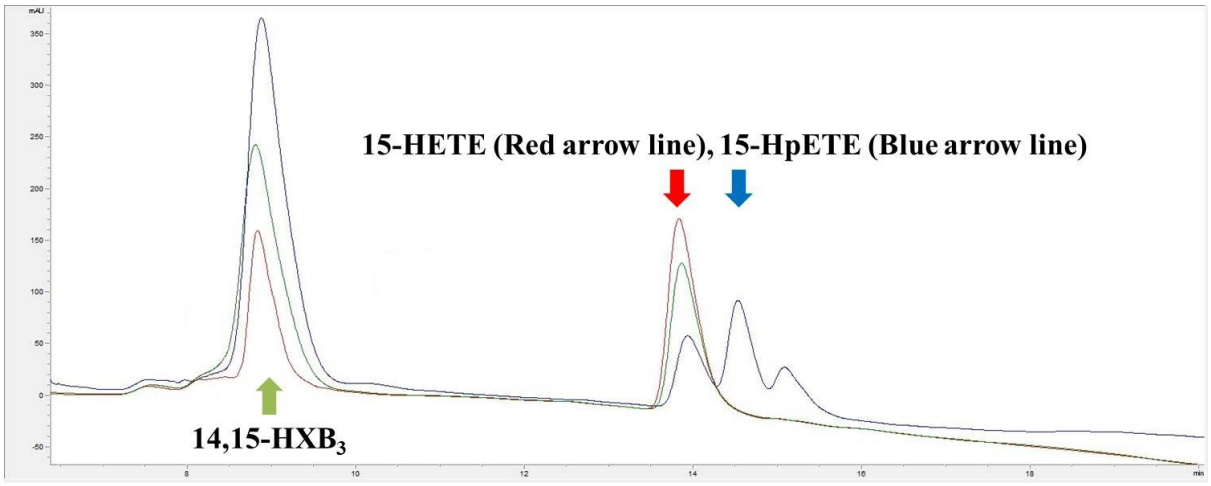
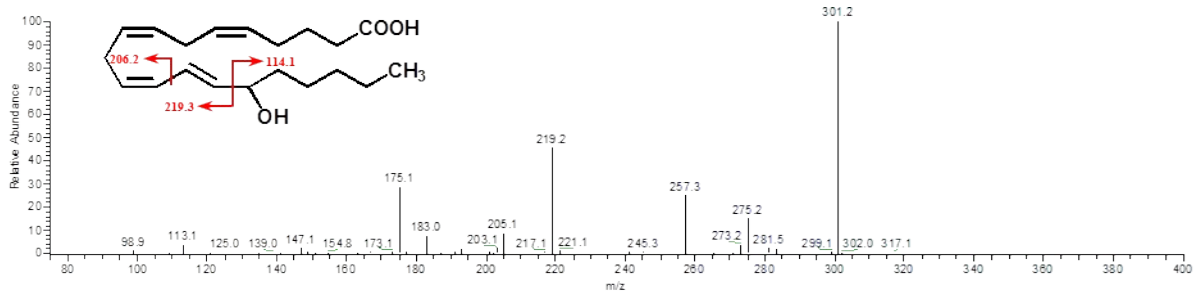


Fig. S10 Increase of the ratio of HFAs to HXs by adding cysteine as a reducing agent. Blue line represents the chromatogram of reaction without cysteine, green line represents the chromatogram of reaction by adding 50 mM cysteine, and red line represent the chromatogram of reaction by adding 100 mM cysteine. All the reaction performed in 50 mM HEPES buffer (pH 7.5) containing 1 mM ARA and 15 g L^{-1} *E. coli* cells at 25 °C for 60 min.

A



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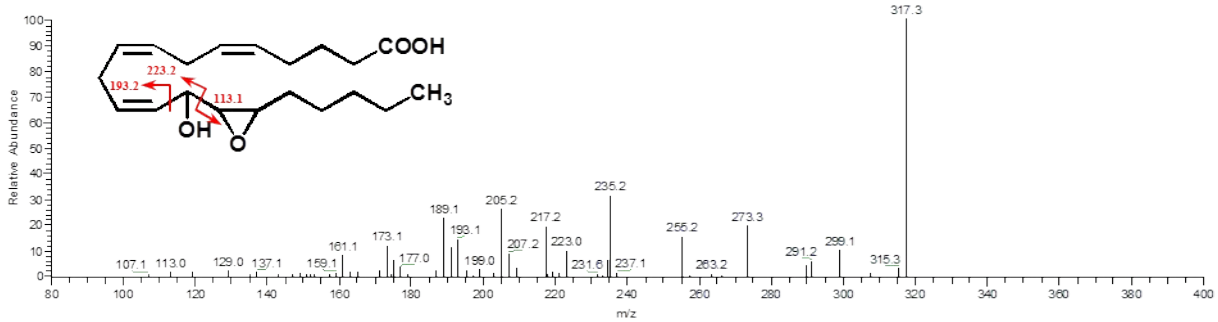

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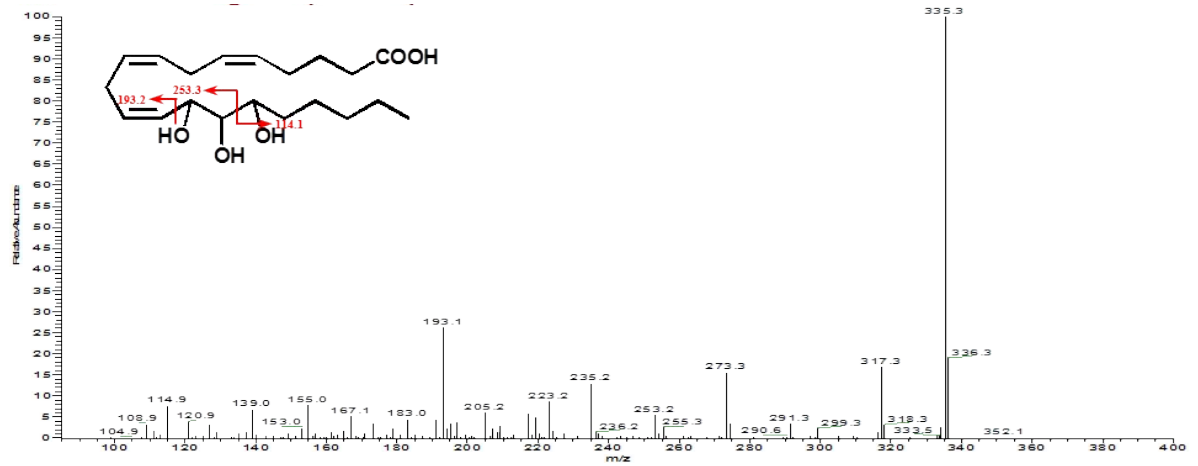
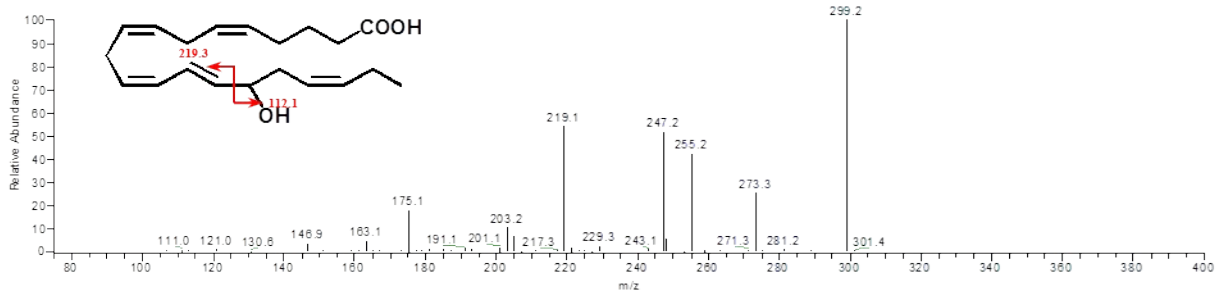


Fig. S11 LC-MS/MS analysis of the products obtained after biotransformation of ARA (**1**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HETE (**3**). B) 14,15-HXB_3 (**4**). C) 13,14,15-$TrXB_3$ (**5**).

A



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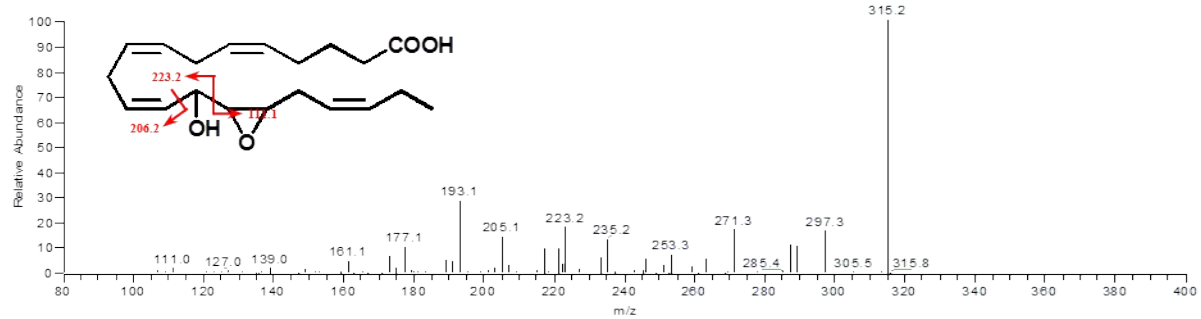

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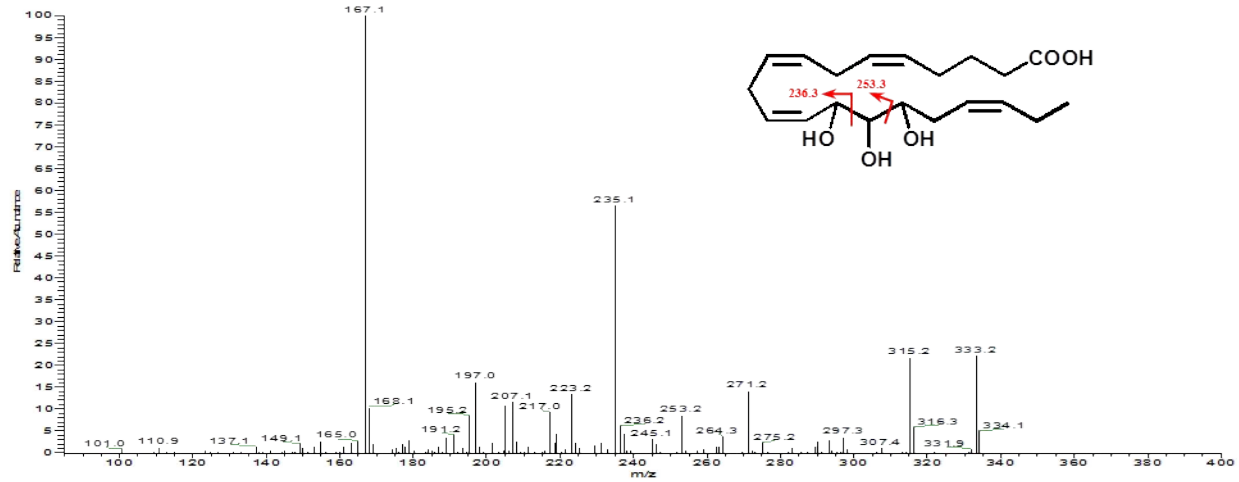
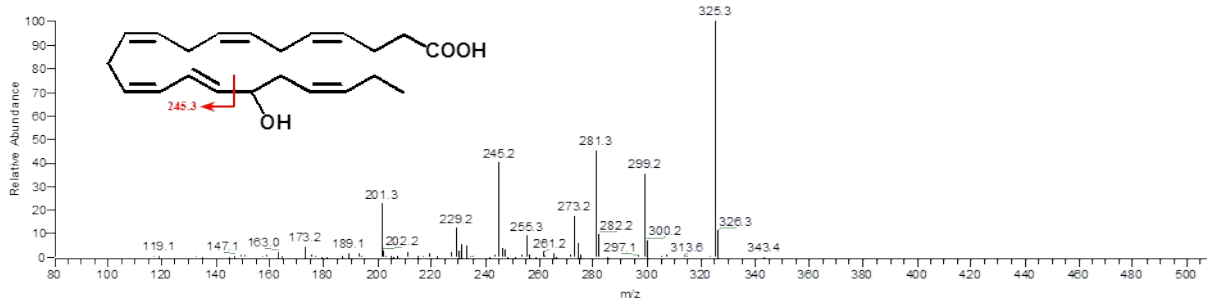


Fig. S12 LC-MS/MS analysis of the products obtained after biotransformation of EPA (**6**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HEPE (**8**). B) 14,15-HXB_4 (**9**). C) 13,14,15-$TrXB_4$ (**10**).

A



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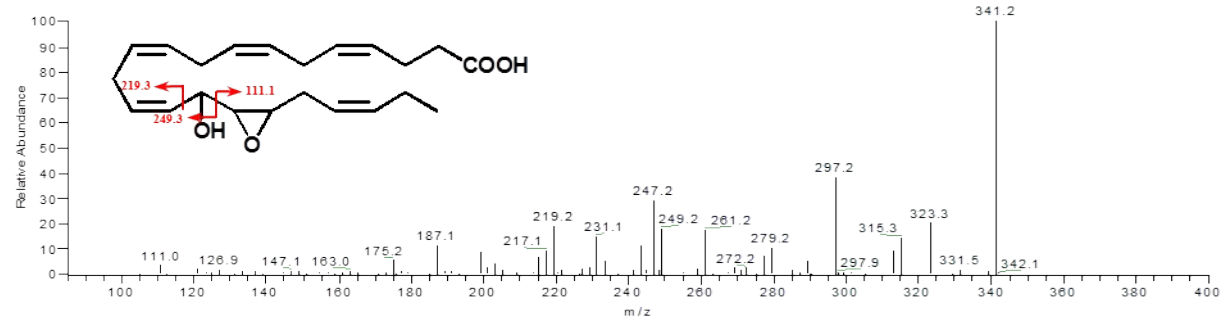

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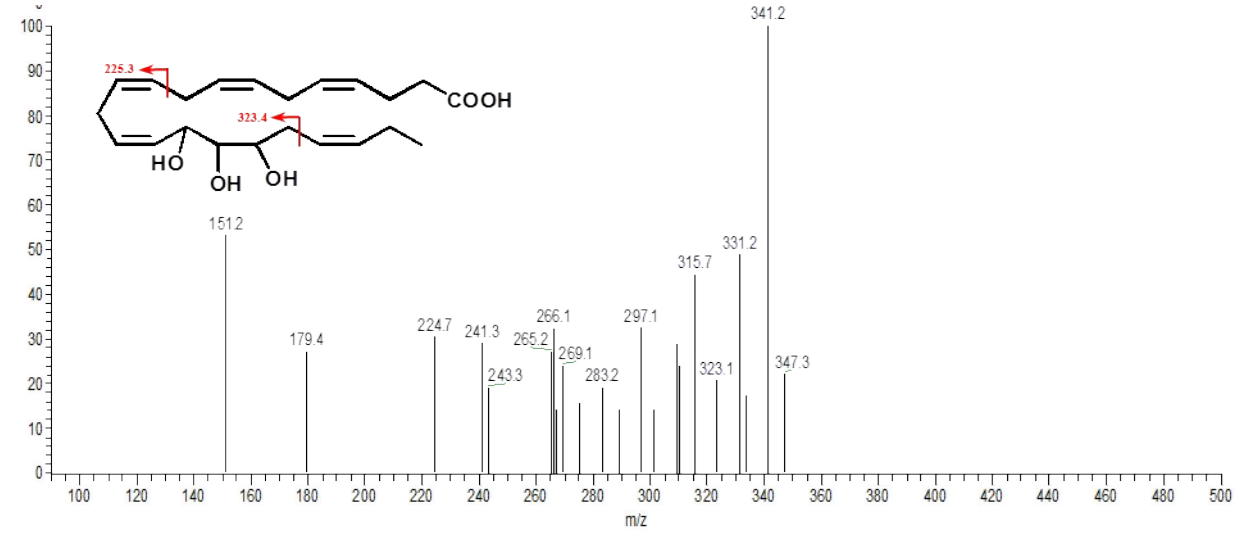
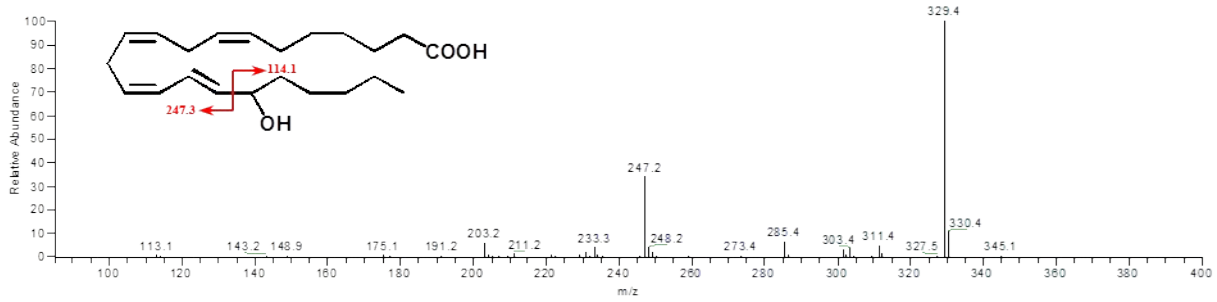


Fig. S13 LC-MS/MS analysis of the products obtained after biotransformation of DHA (**11**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 17-HDOHE (**13**). B) 16,17-HXB$_5$ (**14**). C) 15,16,17-TrXB$_5$ (**15**).

A



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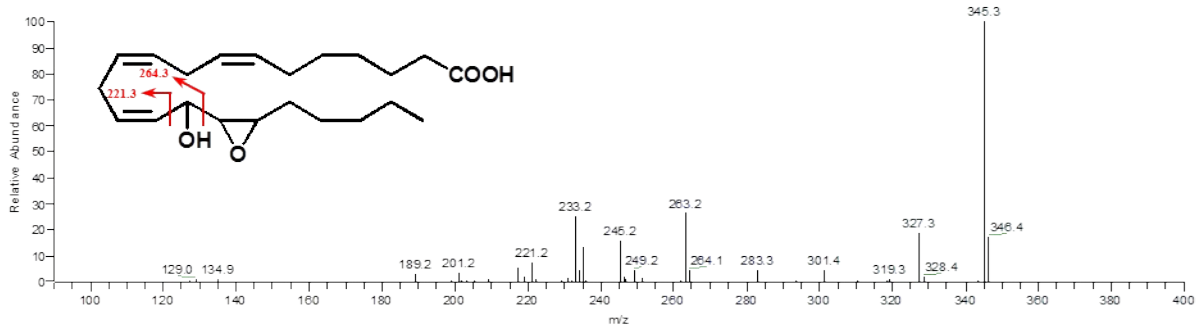


C

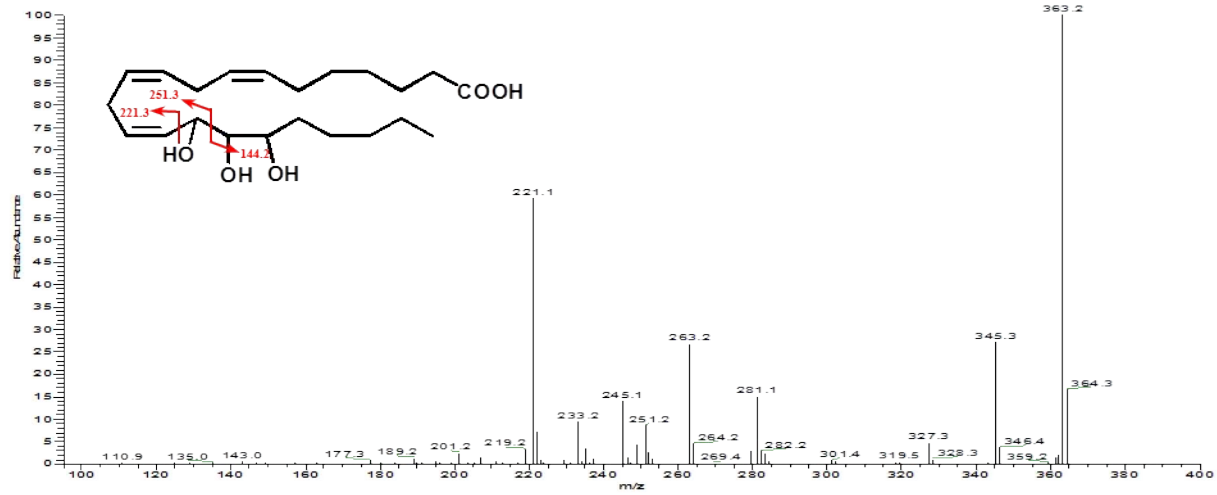
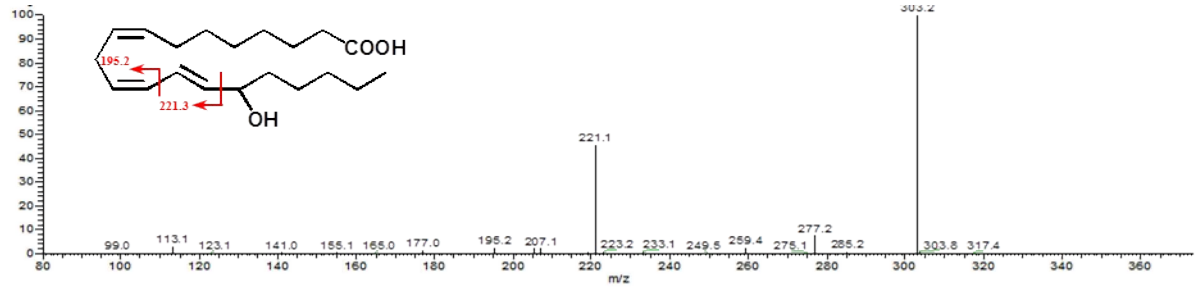


Fig. S14 LC-MS/MS analysis of the products obtained after biotransformation of ADA (**16**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 17-HDOTE (**18**). B) 16,17-HXB$_3$ (**19**). C) 15,16,17-TrXB$_3$ (**20**).

A



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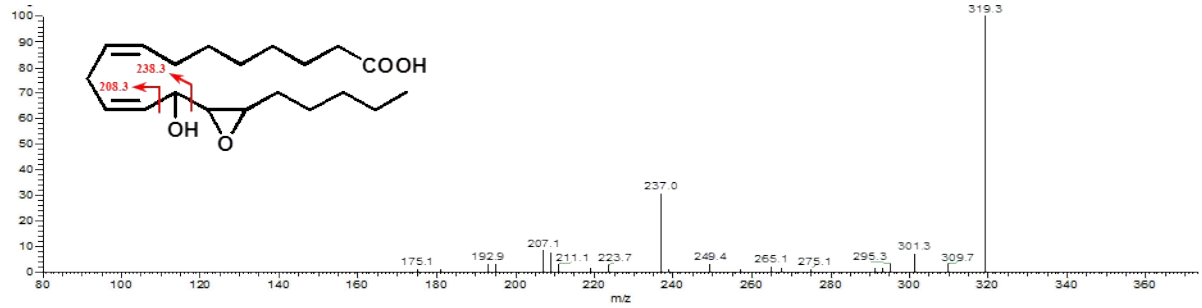

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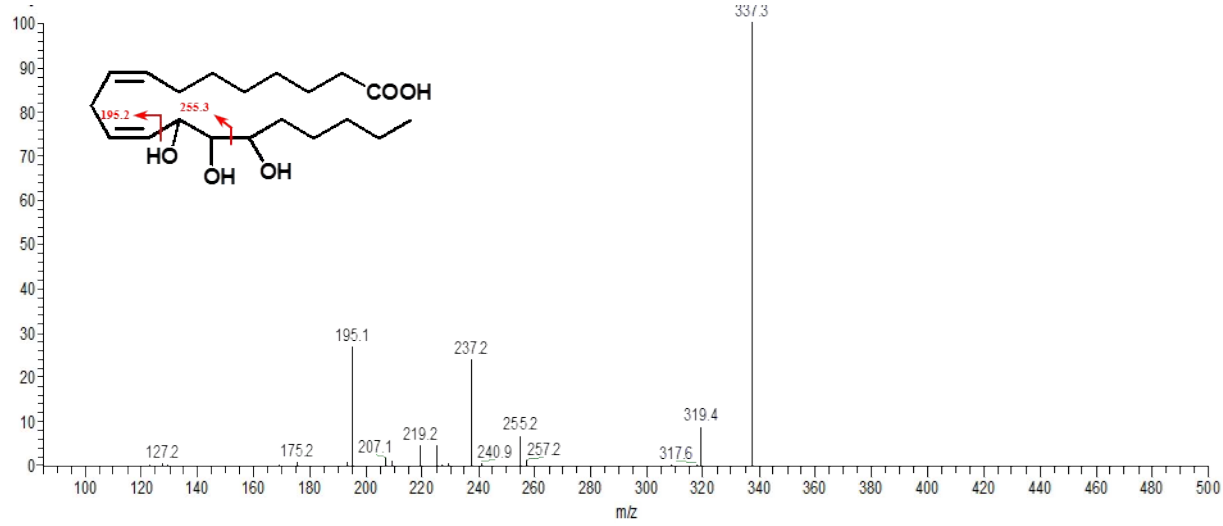
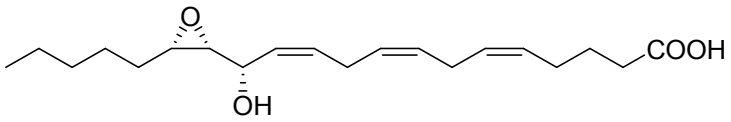
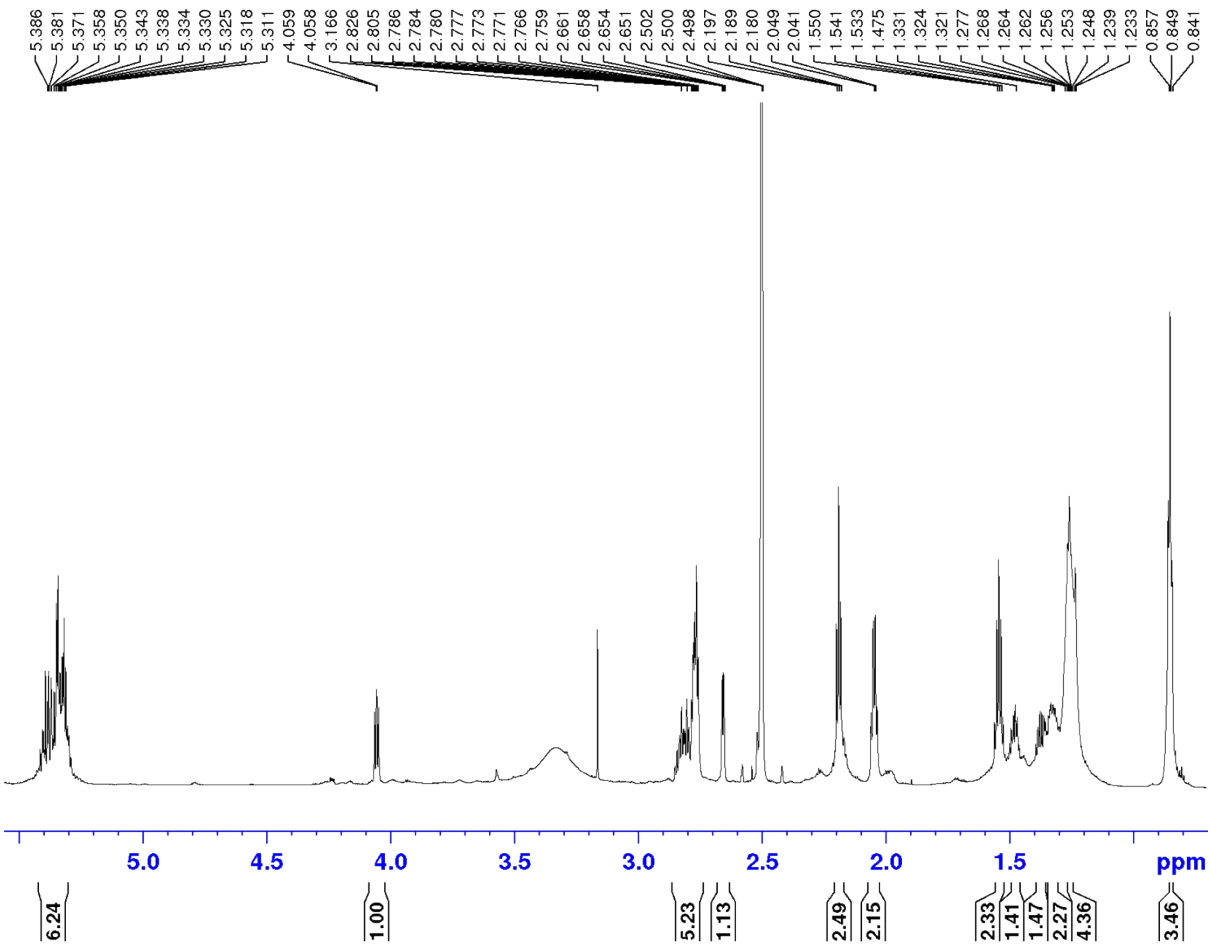


Fig. S15 LC-MS/MS analysis of the products obtained after biotransformation of DGLA (**21**) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HETrE(γ) (**23**). B) 14,15-HXB$_2$(γ) (**24**). C) 13,14,15-TrXB$_2$ (γ) (**25**).

A


O
COOH
OH

B


5.386
5.381
5.371
5.358
5.350
5.343
5.338
5.334
5.330
5.325
5.318
5.311
4.059
4.058
3.166
2.826
2.805
2.786
2.784
2.780
2.777
2.773
2.771
2.766
2.759
2.661
2.658
2.654
2.651
2.502
2.500
2.498
2.197
2.189
2.180
2.049
2.041
1.550
1.541
1.533
1.475
1.331
1.324
1.321
1.277
1.268
1.264
1.262
1.256
1.253
1.248
1.239
1.233
0.857
0.849
0.841
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
6.24
1.00
5.23
1.13
2.49
2.15
2.33
1.41
1.47
2.27
4.36
3.46

C

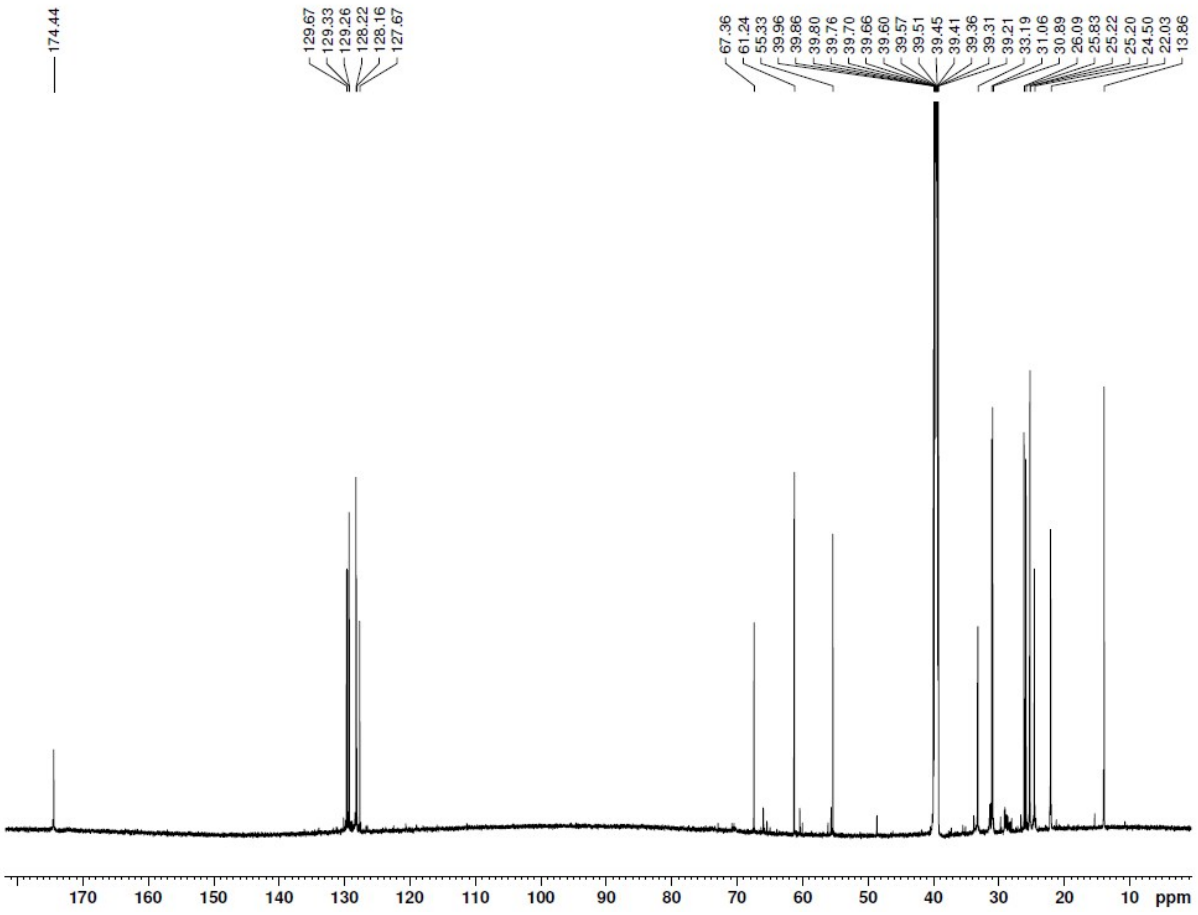
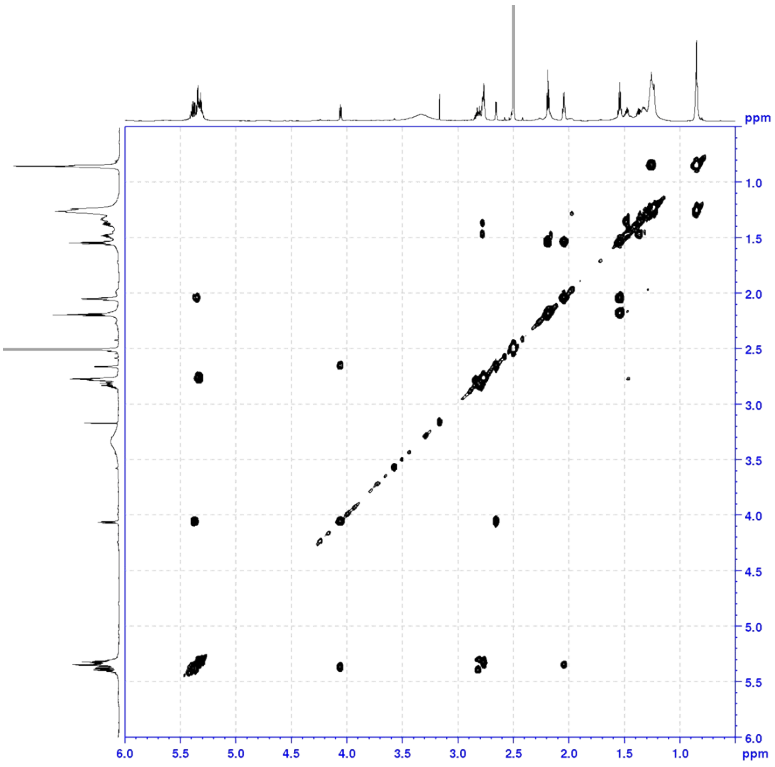
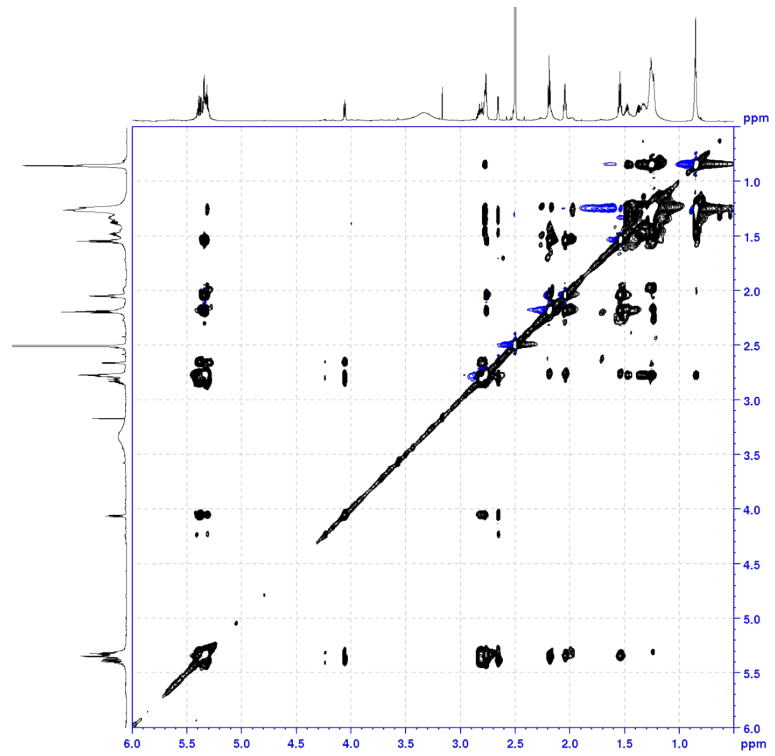


Fig. S16 NMR data of 14,15-HXB_3 (**4**). A) Chemical structure of compound **4**. B) ^{1}H NMR peak of compound **4**. C) ^{13}C NMR peak of compound **4**.

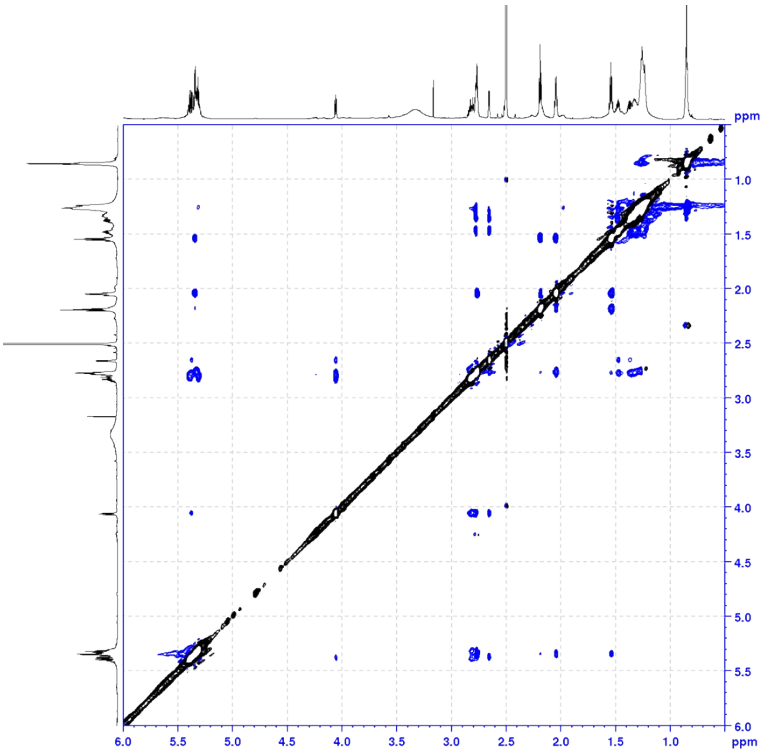
A

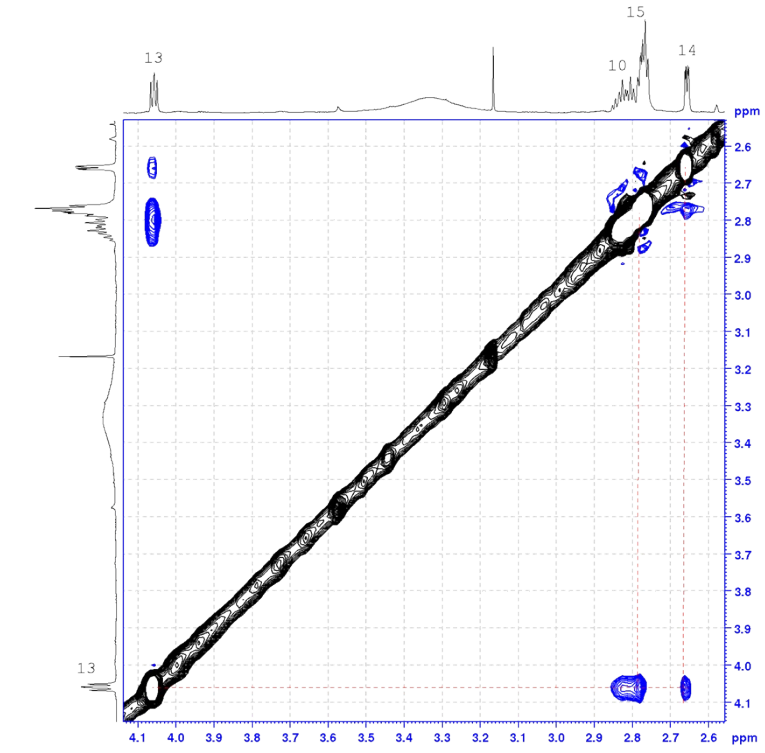

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

B

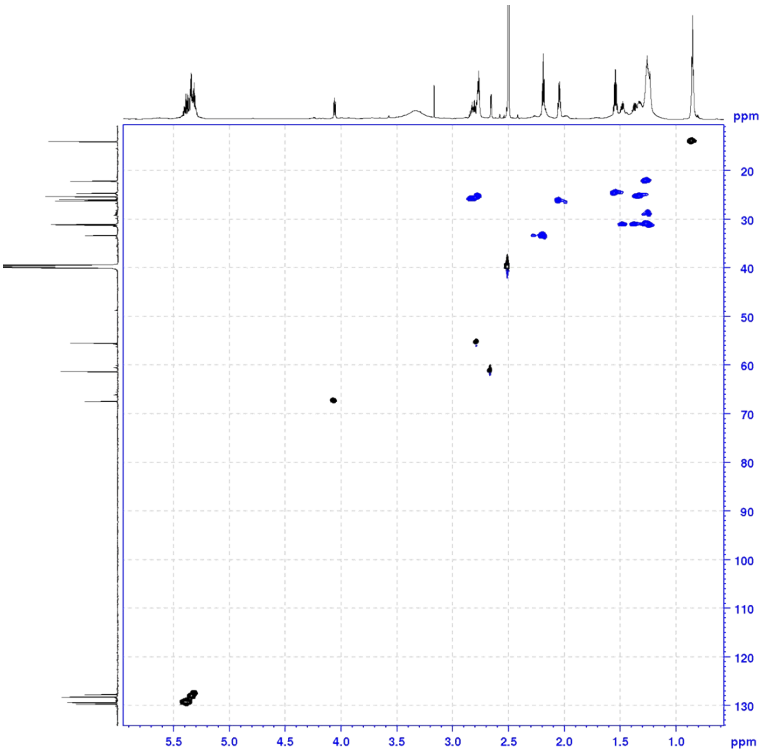

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm


15
13
10
14
ppm
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
13
4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 ppm

D


ppm
20
30
40
50
60
70
80
90
100
110
120
130
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

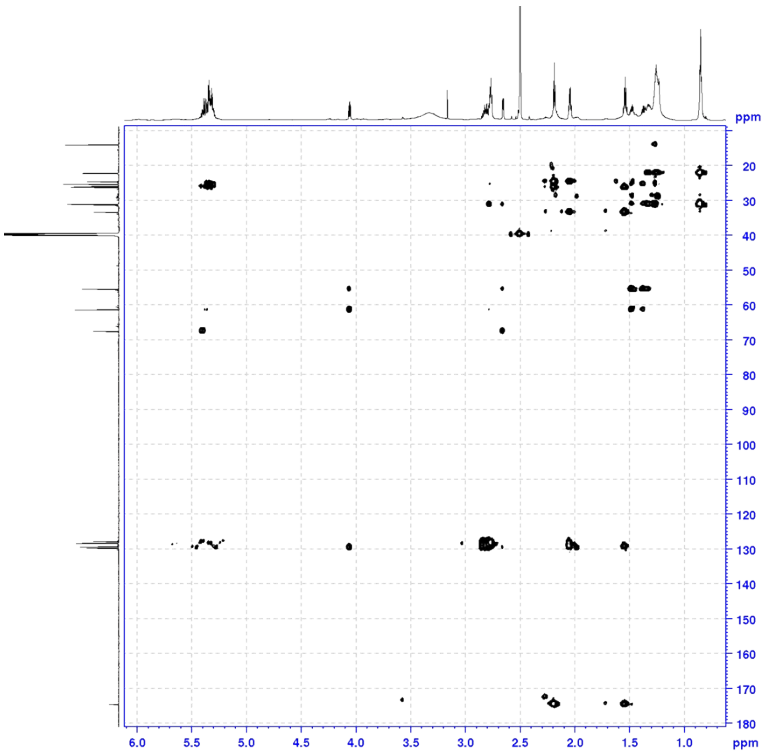
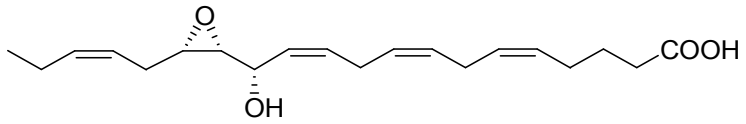
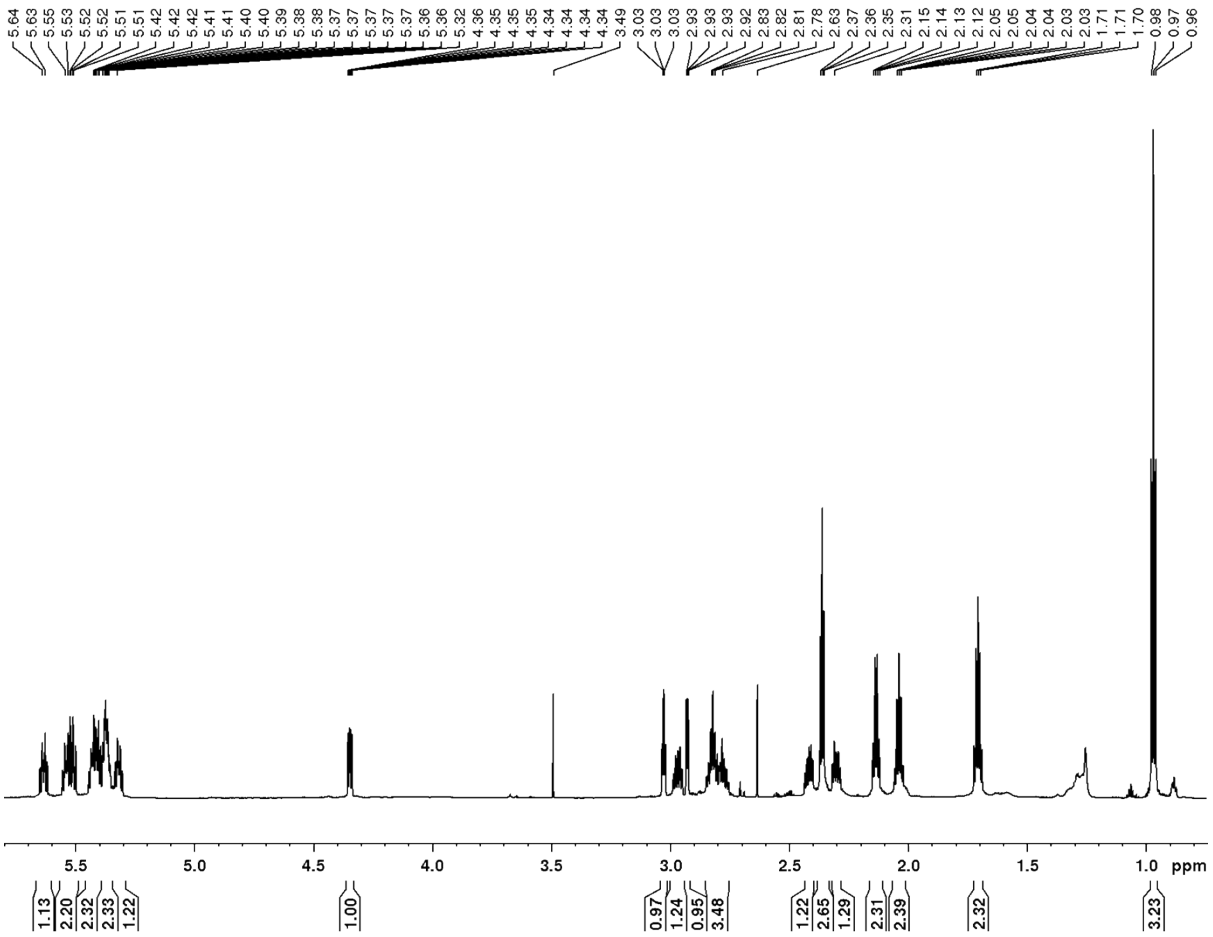

ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S17 2D NMR data of 14,15-HXB_3 (**4**). **A**) COSY spectrum of compound **4**. B) TOCSY spectrum of compound **4**. C) ROESY spectrum of compound **4**. D) HSQC spectrum of compound **4**. E) HMBC spectrum of compound **4**.

A


O
COOH
OH

B


5.64 5.63 5.55 5.53 5.52 5.52 5.51 5.51 5.42 5.42 5.42 5.41 5.41 5.40 5.40 5.39 5.38 5.38 5.37 5.37 5.37 5.37 5.36 5.36 5.32 4.36 4.35 4.35 4.34 4.34 4.34 4.34 3.49 3.03 3.03 3.03 2.93 2.93 2.92 2.83 2.82 2.81 2.78 2.63 2.37 2.36 2.35 2.31 2.15 2.14 2.13 2.12 2.05 2.05 2.04 2.04 2.03 2.03 1.71 1.71 1.70 0.98 0.97 0.96
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.13 2.20 2.32 2.33 1.22 1.00 0.97 1.24 0.95 3.48 1.22 2.65 1.29 2.31 2.39 2.32 3.23

C

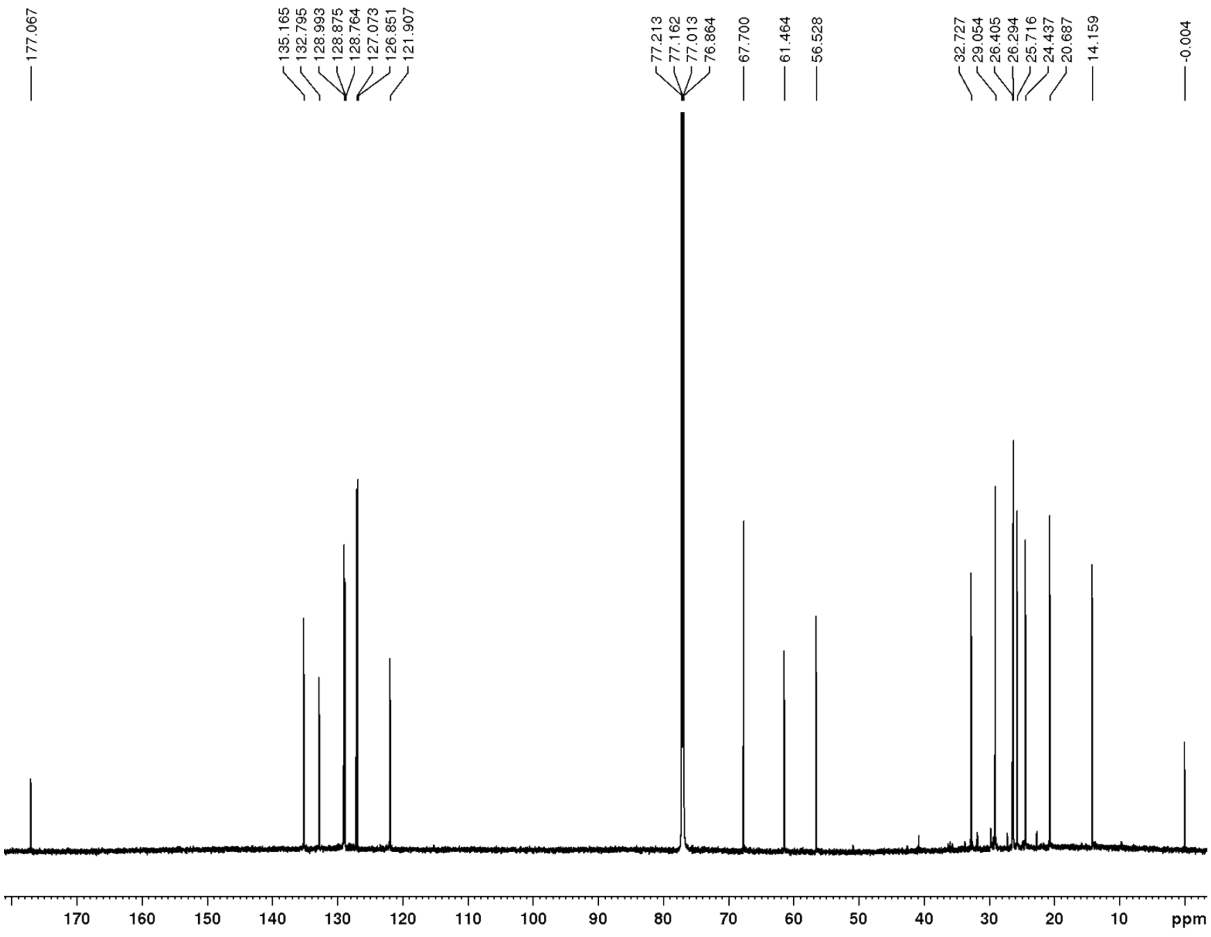
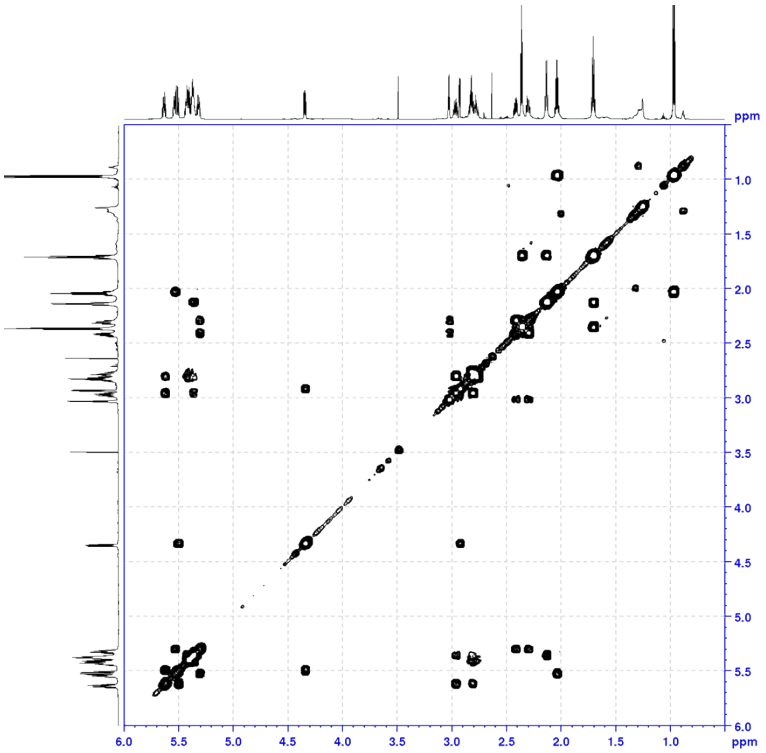
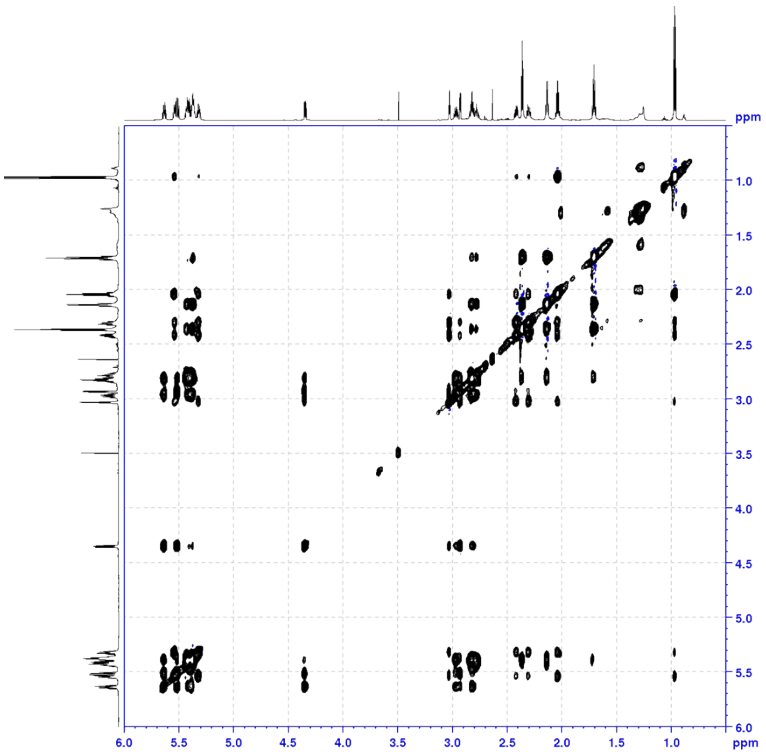


Fig. S18 NMR data of 14,15-HXB_4 (**9**). A) Chemical structure of compound **9**. B) ^{1}H NMR peak of compound **9**. C) ^{13}C NMR peak of compound **9**.

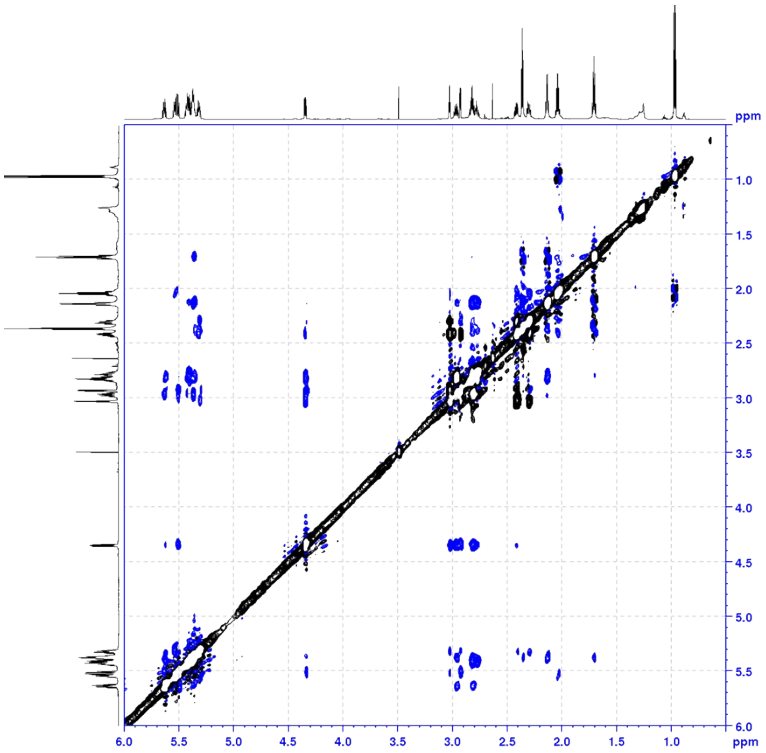
A

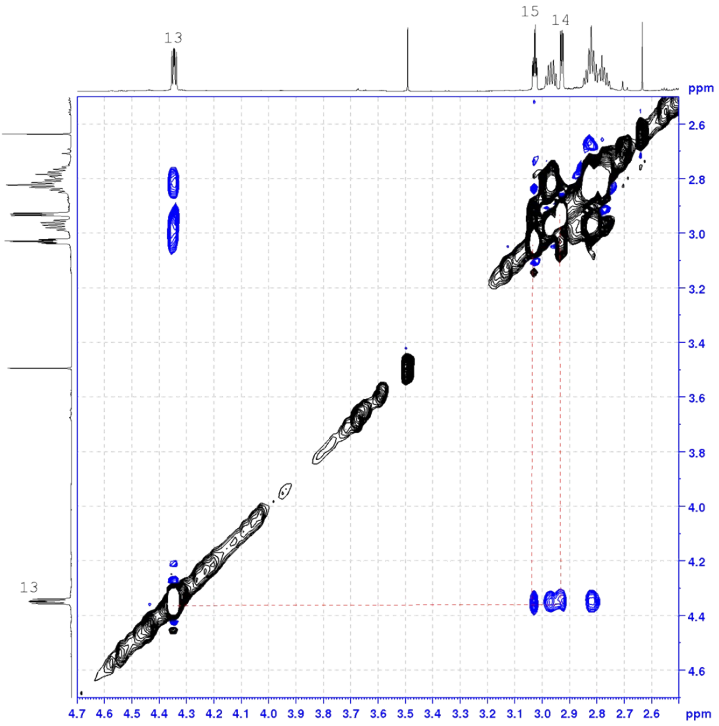

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

B

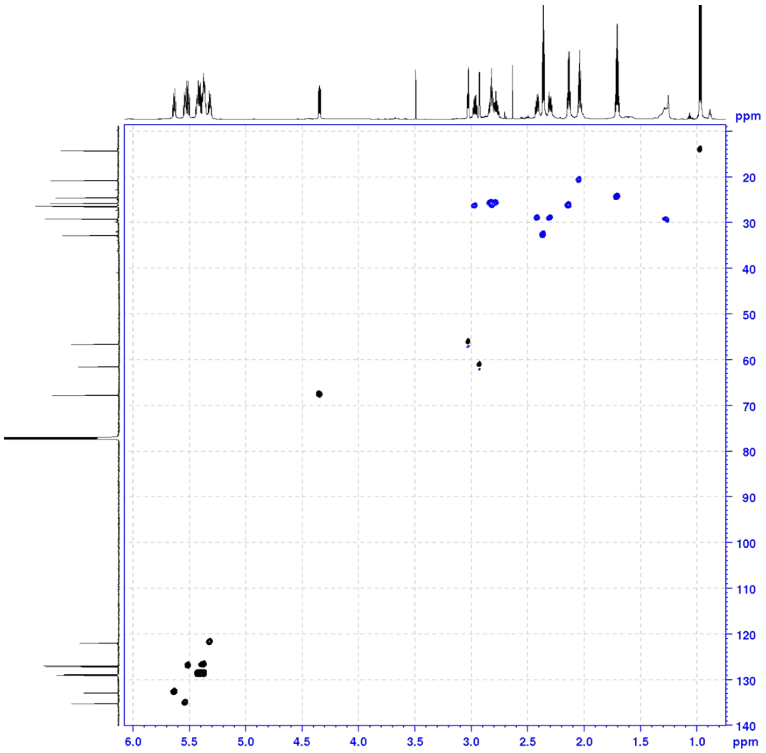

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm


13
15
14
ppm
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
13
4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6
ppm

D


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

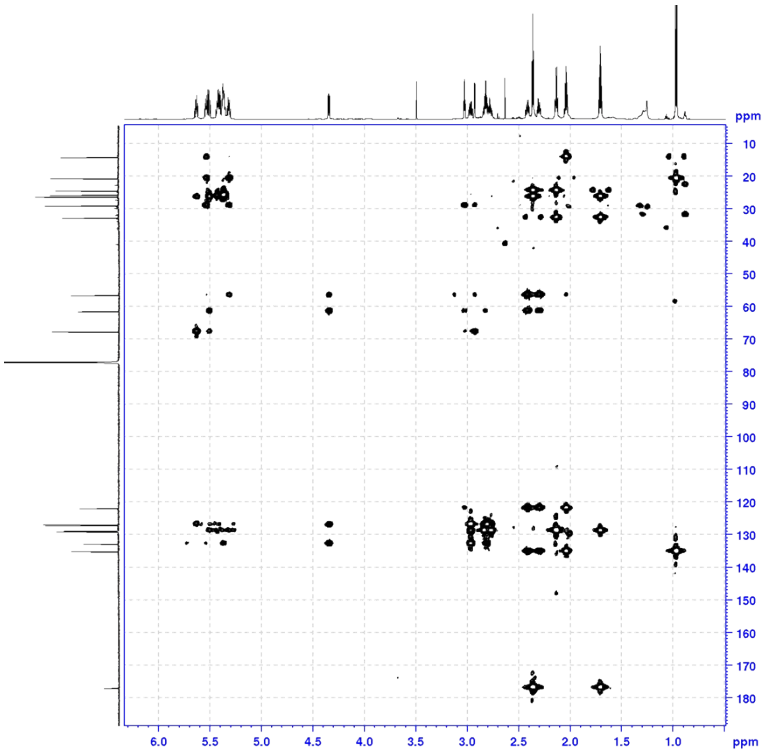
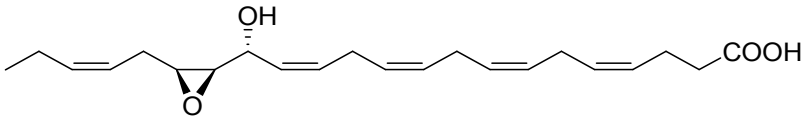
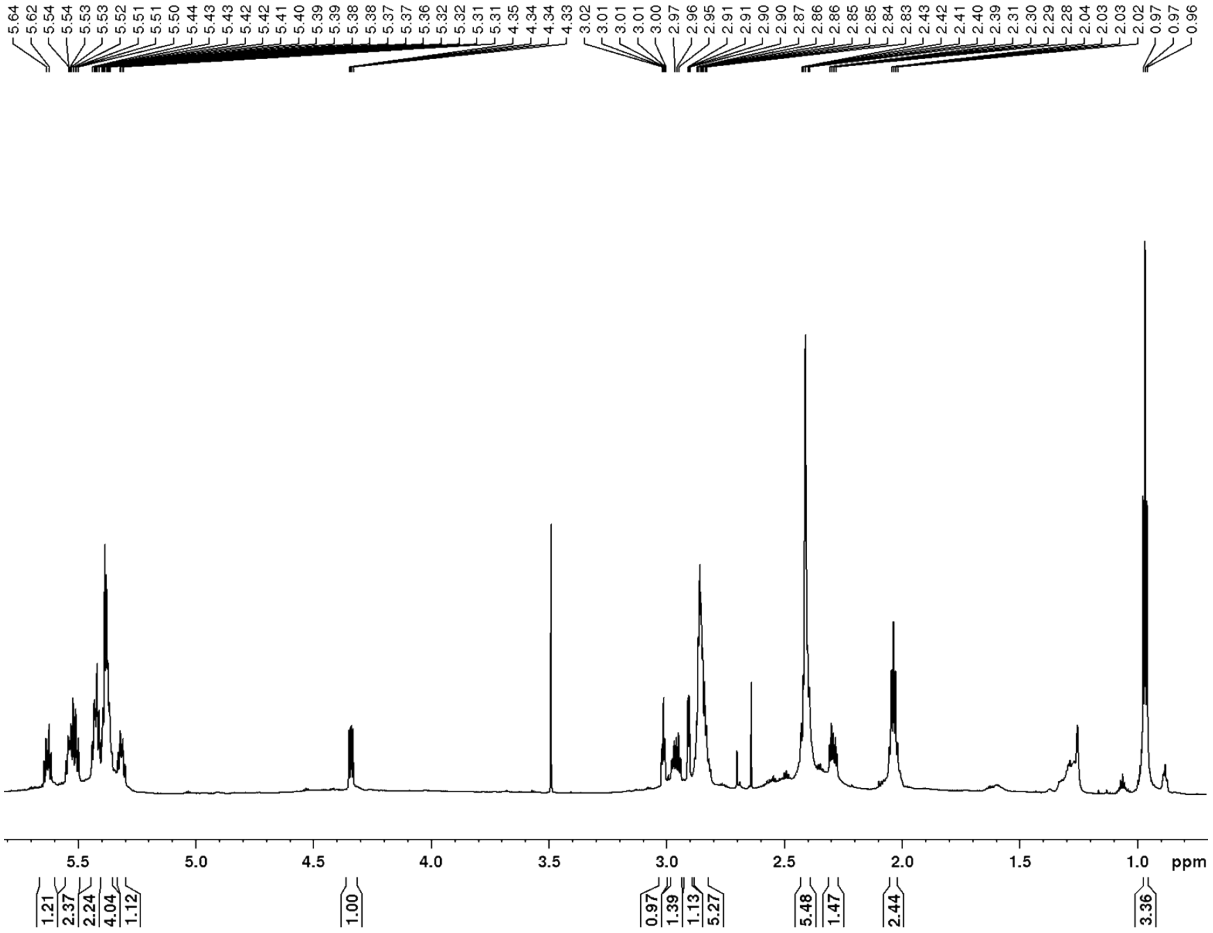

ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S19 2D NMR data of 14,15-HXB_4 (**9**). A) COSY spectrum of compound **9**. B) TOCSY spectrum of compound **9**. C) ROESY spectrum of compound **4**. D) HSQC spectrum of compound **9**. E) HMBC spectrum of compound **9**.

A


OH
COOH
O

B


5.64 5.62 5.54 5.54 5.53 5.53 5.52 5.51 5.51 5.50 5.44 5.43 5.43 5.42 5.42 5.41 5.40 5.39 5.39 5.38 5.38 5.37 5.37 5.36 5.32 5.32 5.31 5.31 4.35 4.34 4.34 4.33
3.02 3.01 3.01 3.01 3.00 2.97 2.96 2.95 2.91 2.91 2.90 2.90 2.87 2.86 2.86 2.85 2.85 2.84 2.83 2.43 2.42 2.41 2.40 2.39 2.31 2.30 2.29 2.28 2.04 2.03 2.03 2.02 0.97 0.97 0.96
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.21 2.37 2.24 4.04 1.12 1.00 0.97 1.39 1.13 5.27 5.48 1.47 2.44 3.36

C

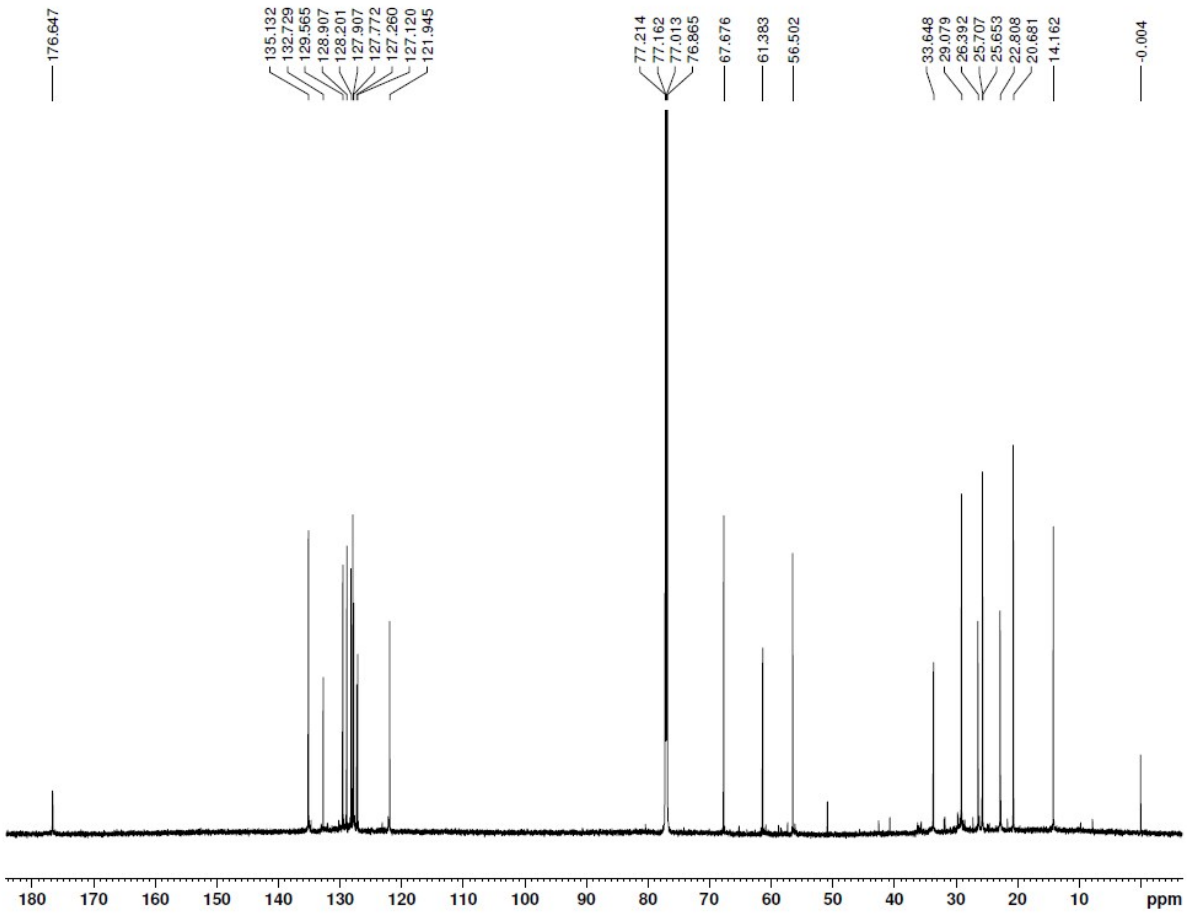
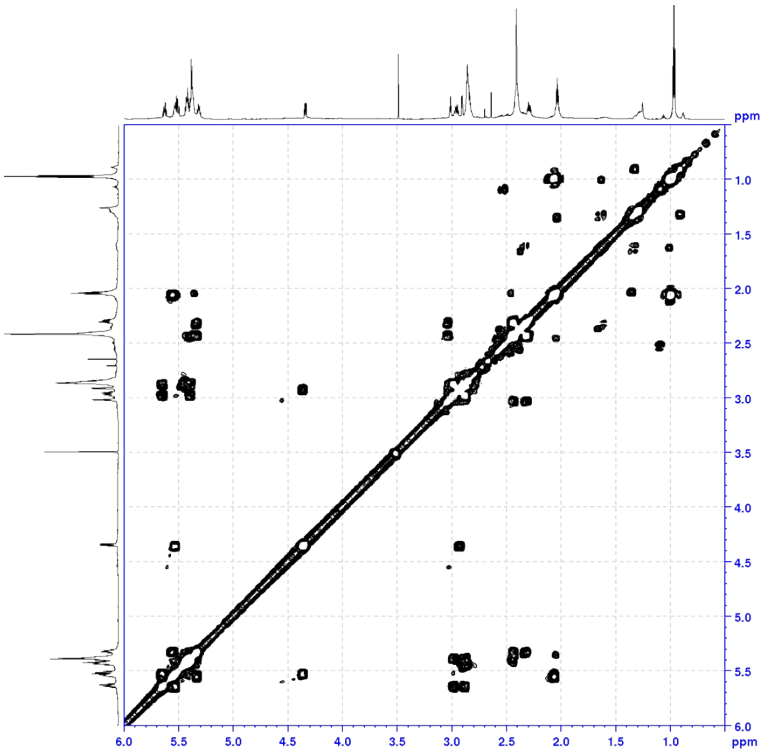
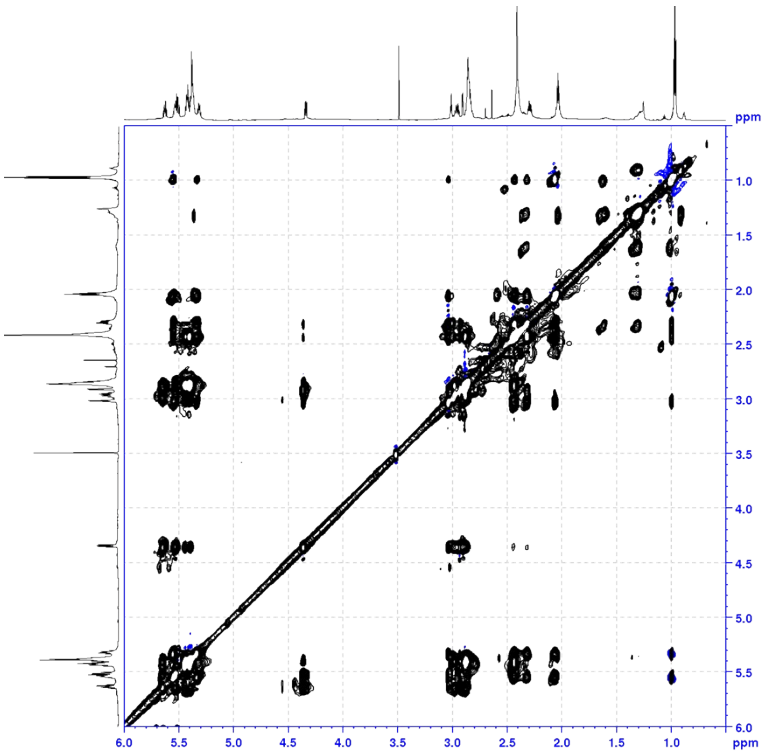


Fig. S20 NMR data of 16,17-HXB$_5$ (**14**). A) Chemical structure of compound **14**. B) ^{1}H NMR peak of compound **14**. C) ^{13}C NMR peak of compound **14**.

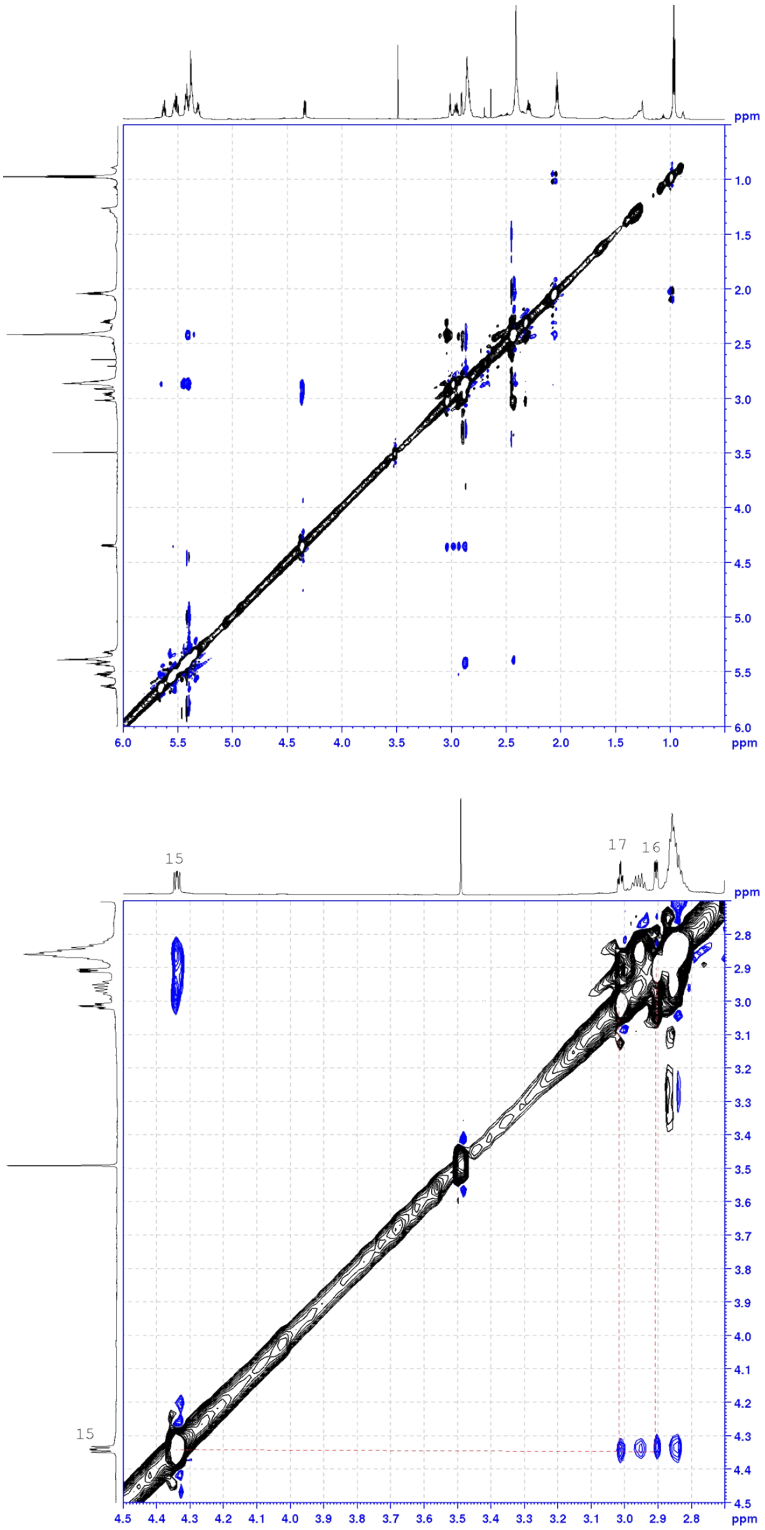
A


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

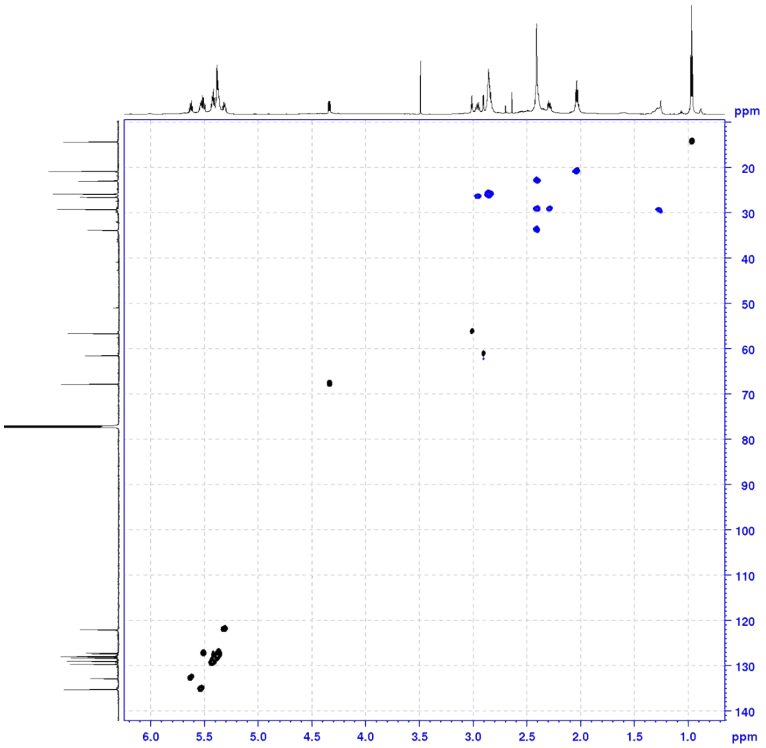
B


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
15
17
16
ppm
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
15
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 ppm

D


ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

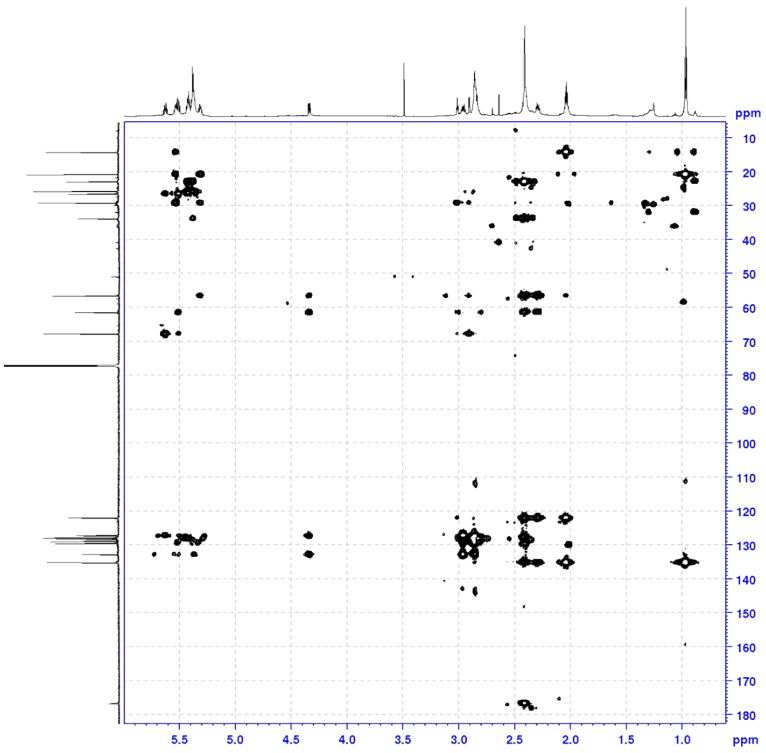
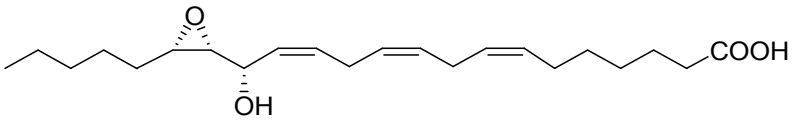
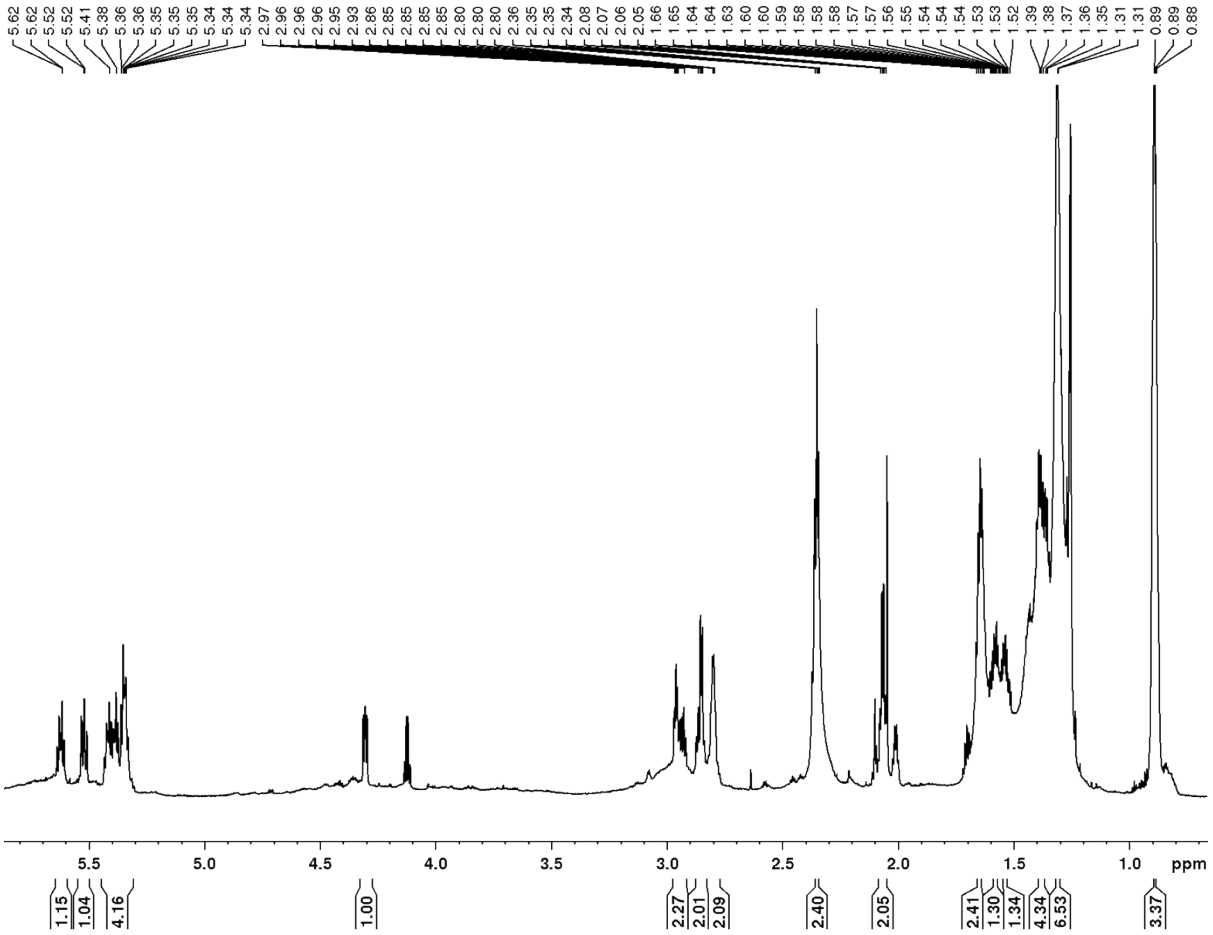

ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S21 2D NMR data of 16,17-HXB_5 (**14**). A) COSY spectrum of compound 14. B) TOCSY spectrum of compound **14**. C) ROESY spectrum of compound **14**. D) HSQC spectrum of compound **14**. E) HMBC spectrum of compound **14**.

A


O
COOH
OH

B


5.62 5.62 5.52 5.52 5.41 5.38 5.36 5.36 5.35 5.35 5.35 5.34 5.34 5.34
2.97 2.96 2.96 2.96 2.95 2.93 2.86 2.85 2.85 2.85 2.80 2.80 2.80 2.36 2.35 2.35 2.34 2.08 2.07 2.06 2.05 1.66 1.65 1.64 1.64 1.63 1.60 1.60 1.59 1.58 1.58 1.58 1.57 1.57 1.56 1.55 1.54 1.54 1.54 1.53 1.53 1.52
1.39 1.38 1.37 1.36 1.35 1.31 1.31
0.89 0.89 0.88
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.15 1.04 4.16 1.00 2.27 2.01 2.09 2.40 2.05 2.41 1.30 1.34 4.34 6.53 3.37

C

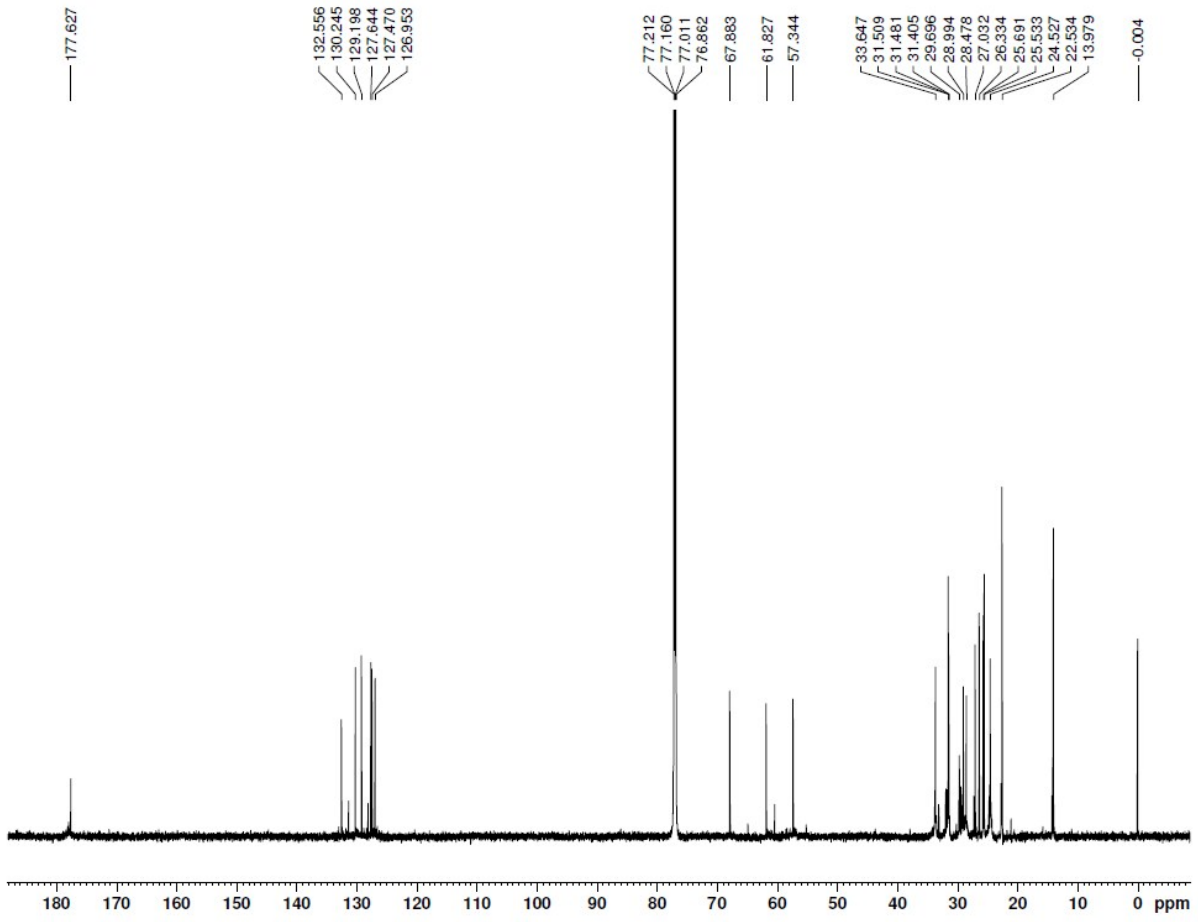
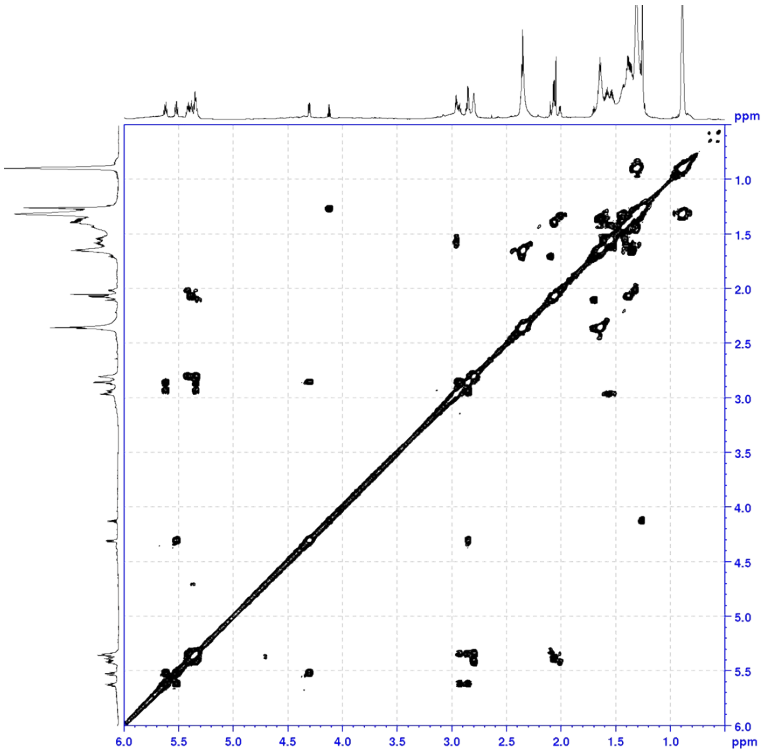
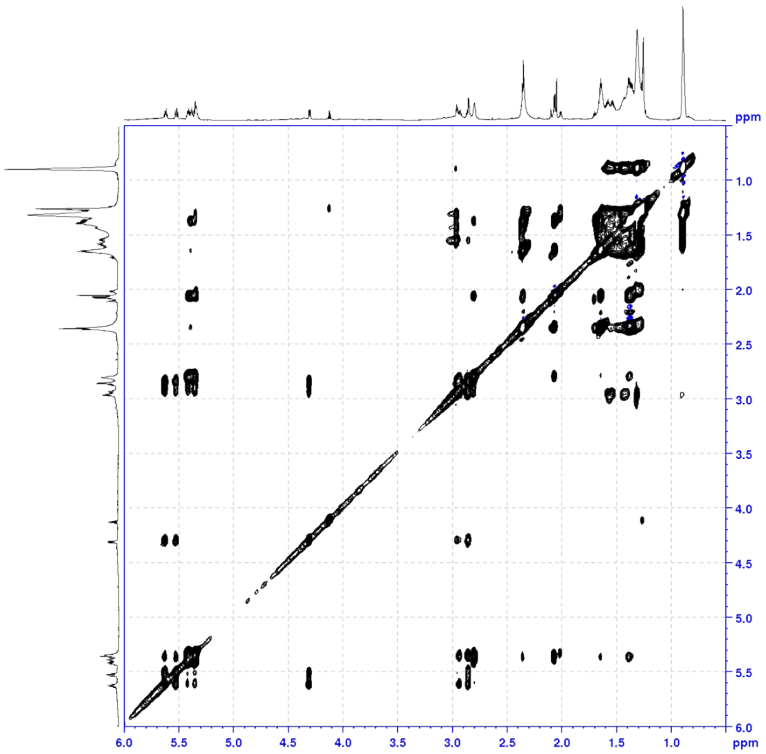


Fig. S22 NMR data of 16,17-HXB_3 (**19**). A) Chemical structure of compound **19**. B) ^{1}H NMR peak of compound **19**. C) ^{13}C NMR peak of compound **19**.

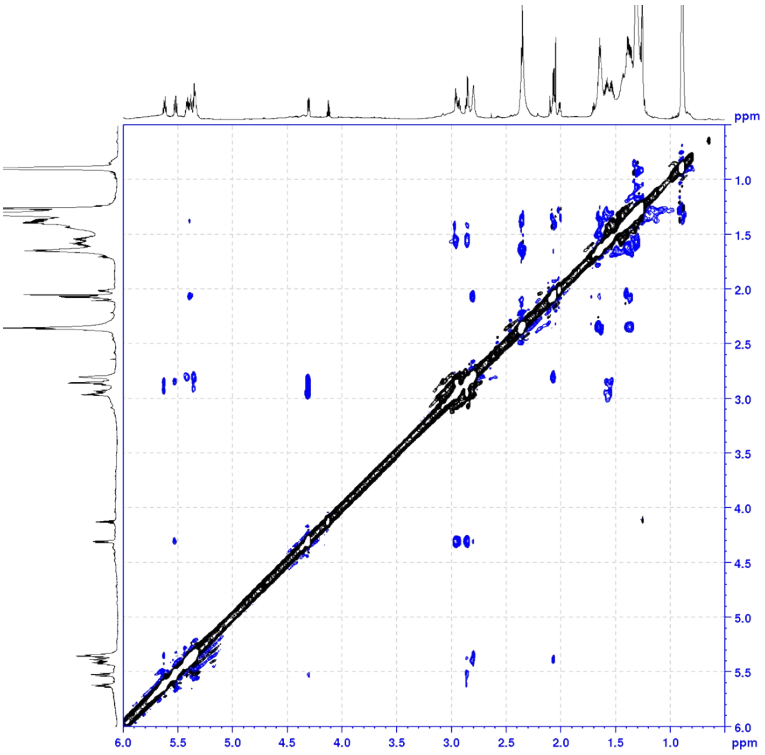
A

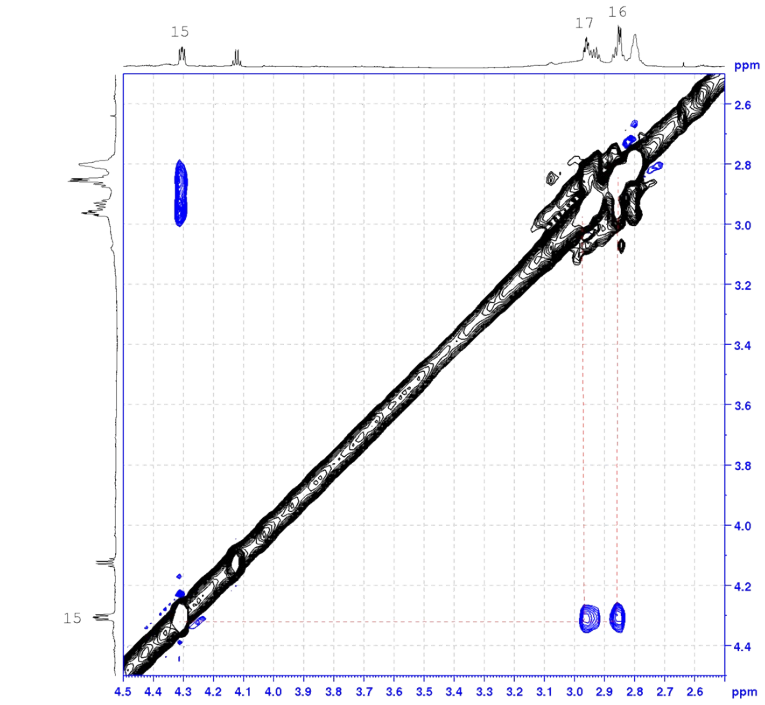

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

B

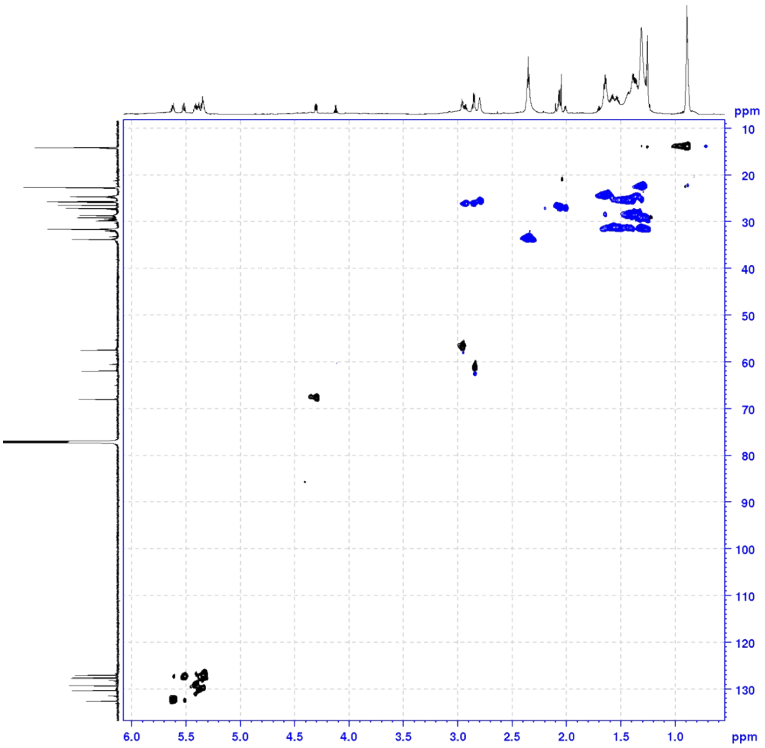

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm


15
17
16
ppm
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
15
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6
ppm

D


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

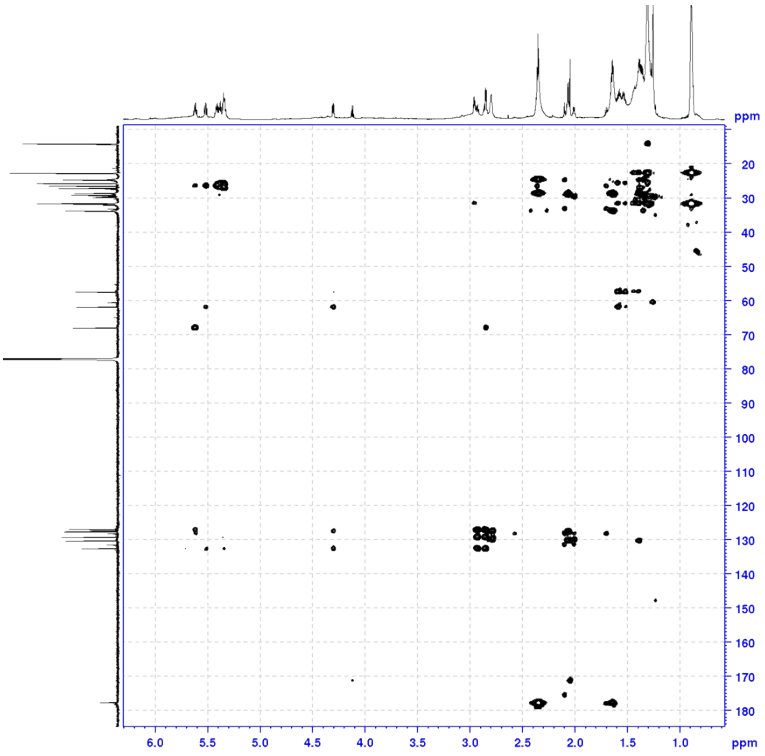
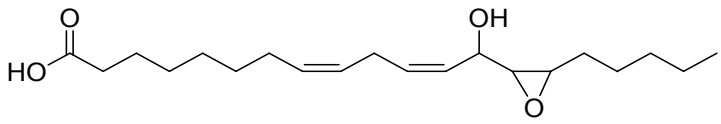
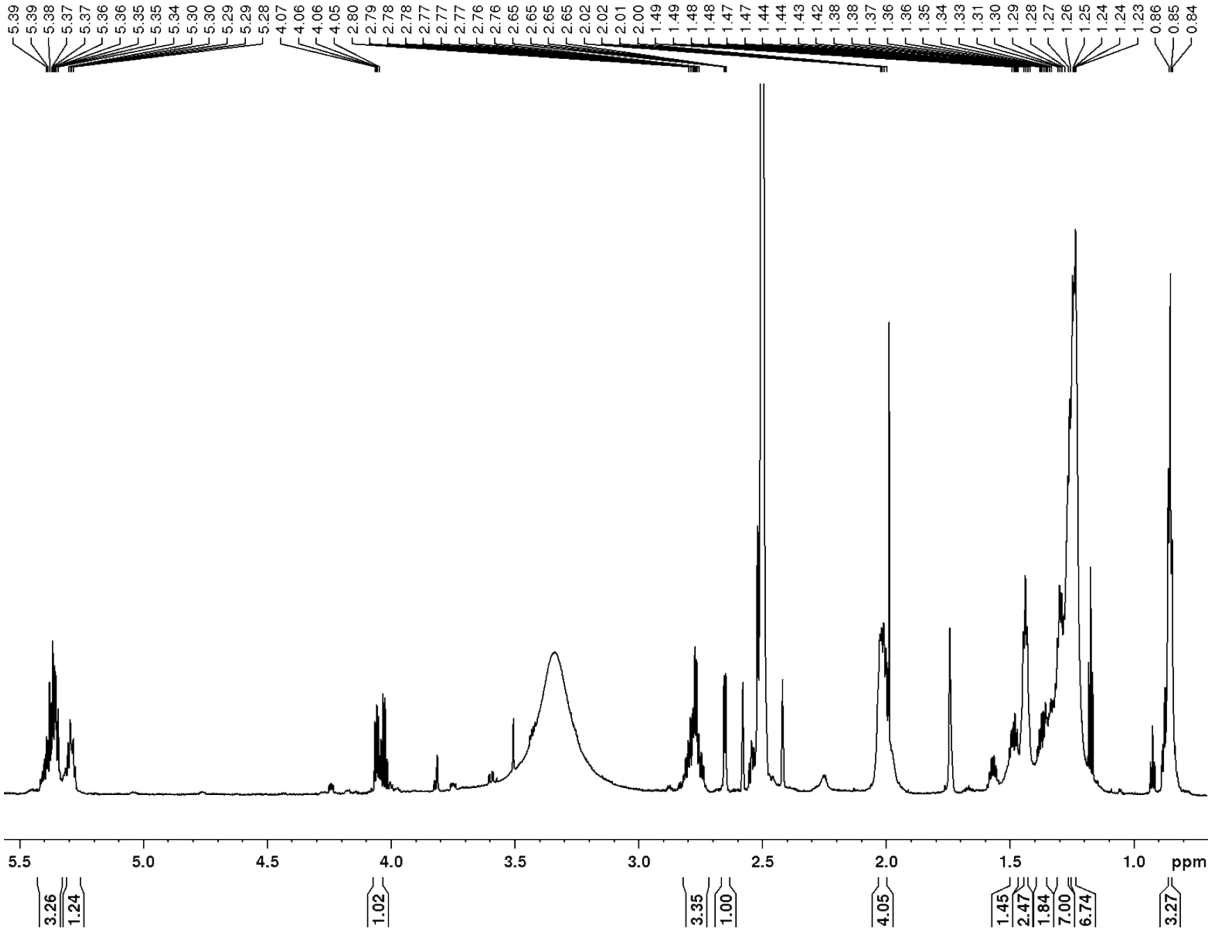

ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S23 2D NMR data of 16,17-HXB_3 (**19**). A) COSY spectrum of compound **19**. B) TOCSY spectrum of compound **19**. C) ROESY spectrum of compound **19**. D) HSQC spectrum of compound **14**. E) HMBC spectrum of compound **19**.

A


O
HO
OH
O

B


5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
3.26
1.24
1.02
3.35
1.00
4.05
1.45
2.47
1.84
7.00
6.74
3.27

C

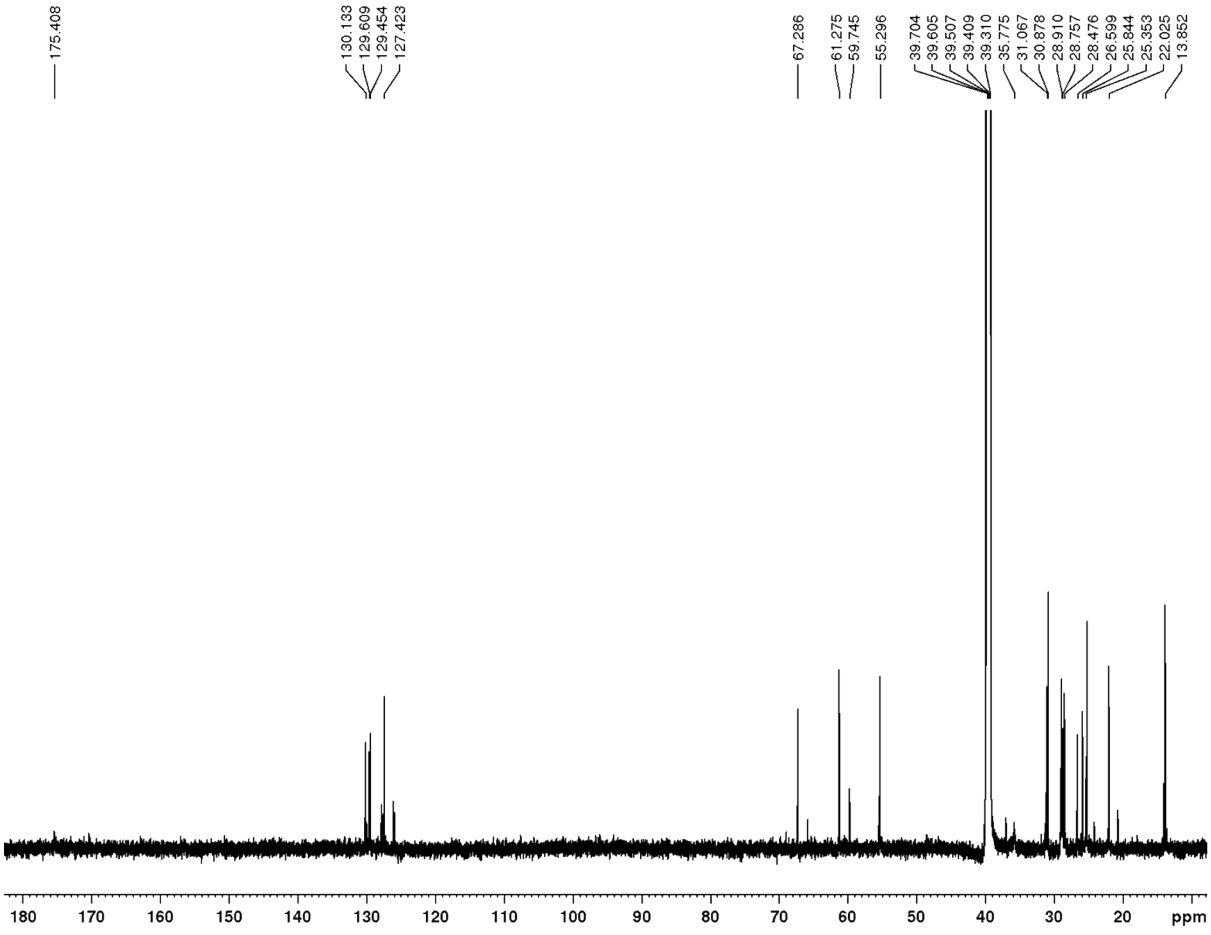
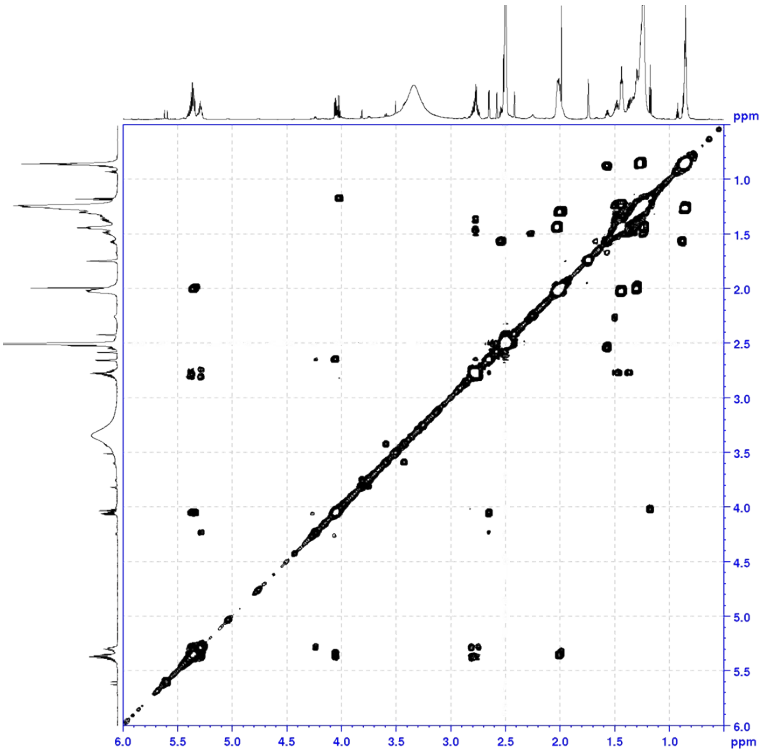
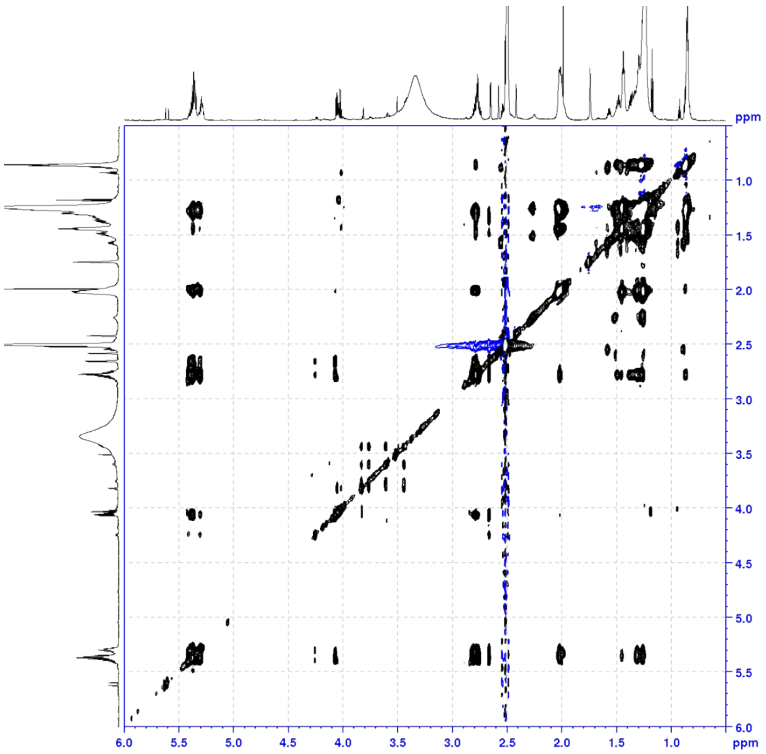


Fig. S24 NMR data of 14,15-HXB_2(γ) (**24**). A) Chemical structure of compound **24**. B) ^{1}H NMR peak of compound **24**. C) ^{13}C NMR peak of compound **24**.

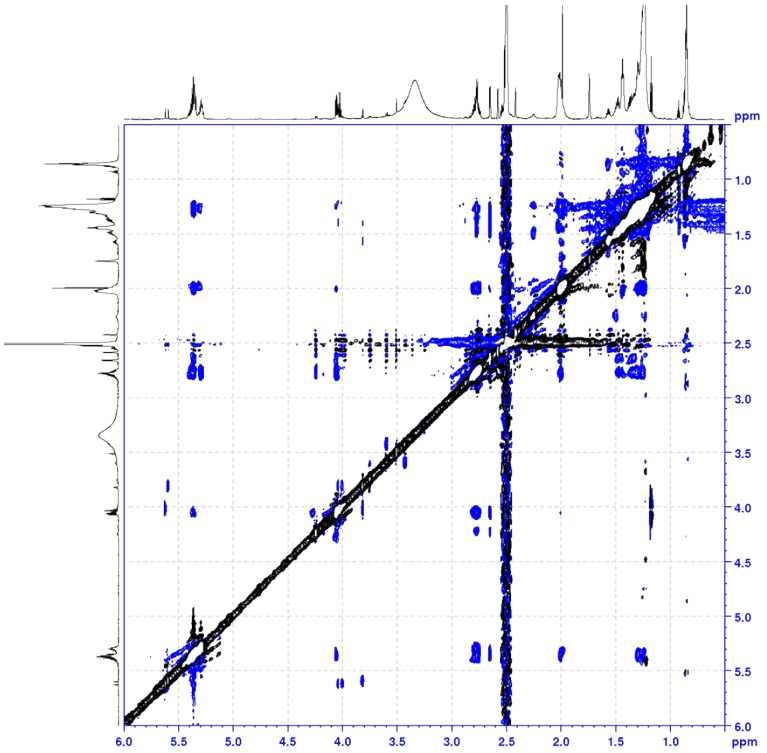
A

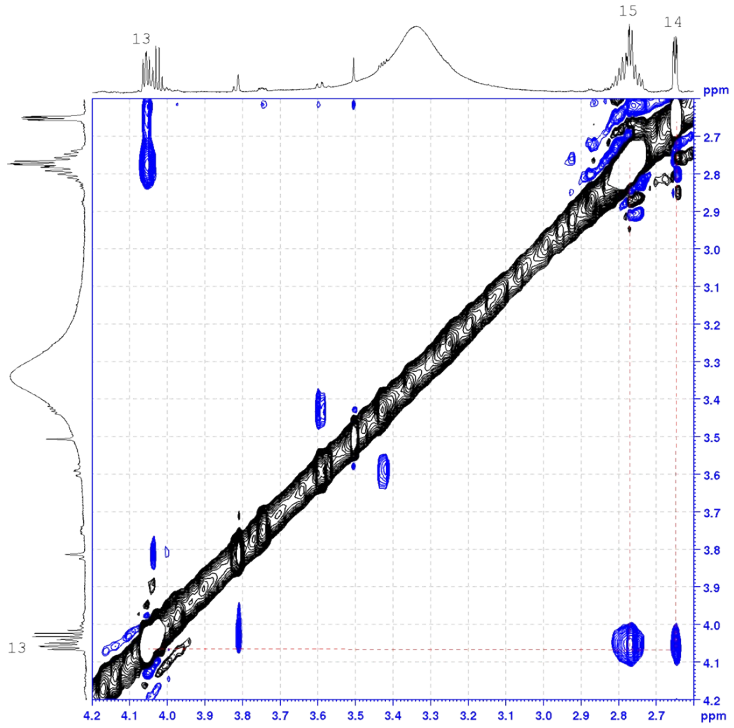

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

B

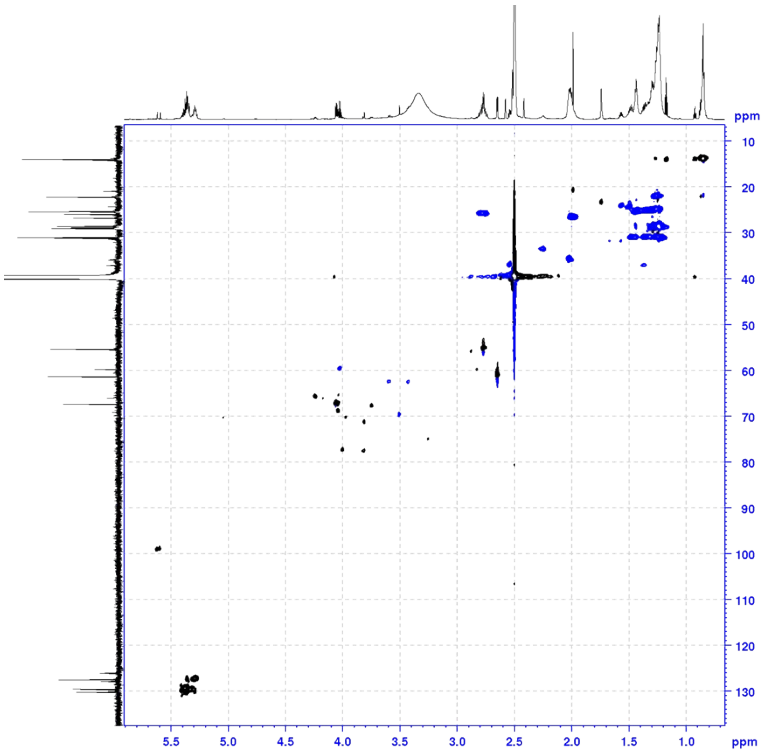

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C

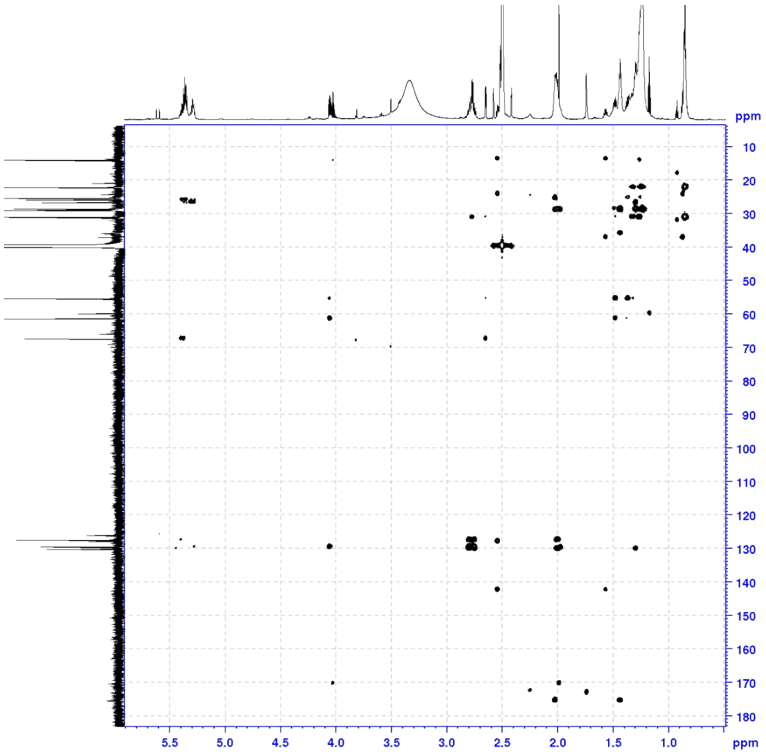

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm


13
15
14
ppm
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
13
4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7
ppm

D


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E


ppm
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20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S25 2D NMR data of 14,15-$HXB_2(\gamma)$ (**24**). A) COSY spectrum of compound **24**. B) TOCSY spectrum of compound **24**. C) ROESY spectrum of compound **24**. D) HSQC spectrum of compound **24**. E) HMBC spectrum of compound **24**.

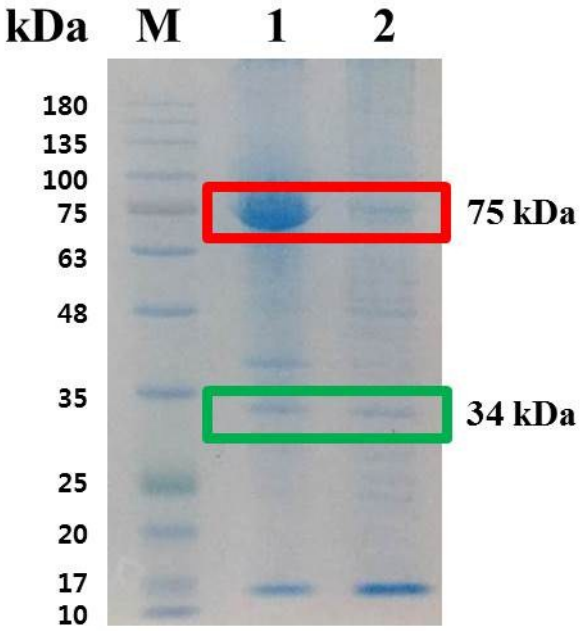
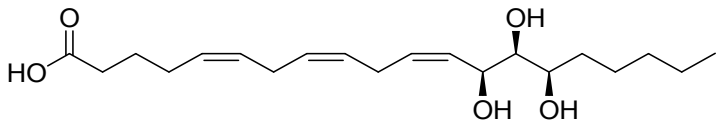
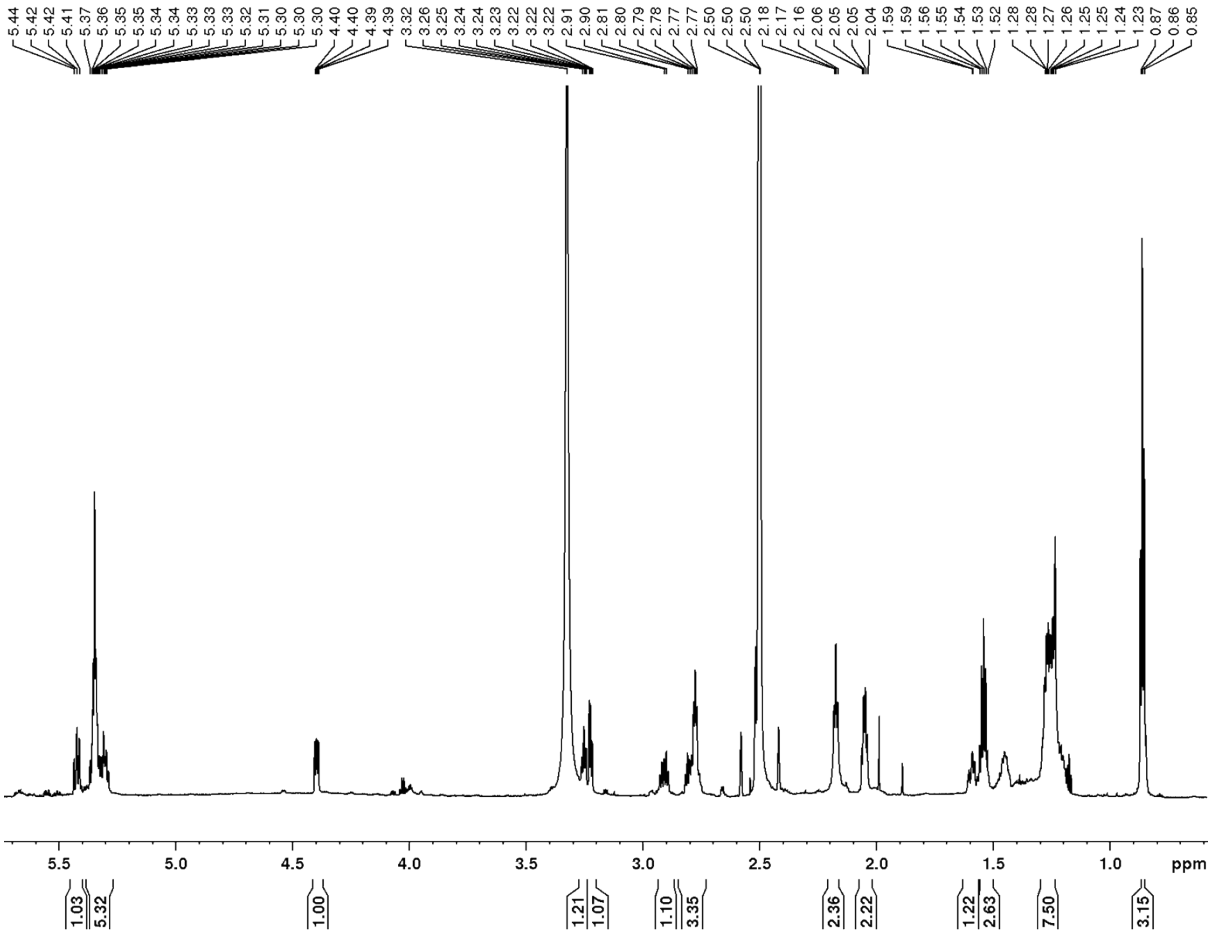


Fig. S26 SDS-PAGE analysis of ARA 15-LOX and EH expressed in recombinant *E. coli* ER2566. Lane M indicates the marker proteins. The crude extract (lane 1) and pellet (lane 2) of the cell lysate of *E. coli* expressing *B. thailandensis* ARA 15-LOX and *M. xanthus* EH. Red box and green box indicate the proteins, *B. thailandensis* ARA 15-LOX, and *M. xanthus* EH, respectively.

A


O
HO
OH
OH
OH

B


5.44 5.42 5.42 5.41 5.37 5.36 5.35 5.35 5.34 5.34 5.33 5.33 5.33 5.32 5.31 5.30 5.30 5.30 4.40 4.40 4.39 4.39 3.32 3.26 3.25 3.24 3.24 3.23 3.22 3.22 3.22 2.91 2.90 2.81 2.80 2.79 2.78 2.77 2.77 2.50 2.50 2.50 2.18 2.17 2.16 2.06 2.05 2.05 2.04 1.59 1.59 1.56 1.55 1.54 1.53 1.52 1.28 1.28 1.27 1.26 1.25 1.25 1.24 1.23 0.87 0.86 0.85
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.03 5.32 1.00 1.21 1.07 1.10 3.35 2.36 2.22 1.22 2.63 7.50 3.15

C

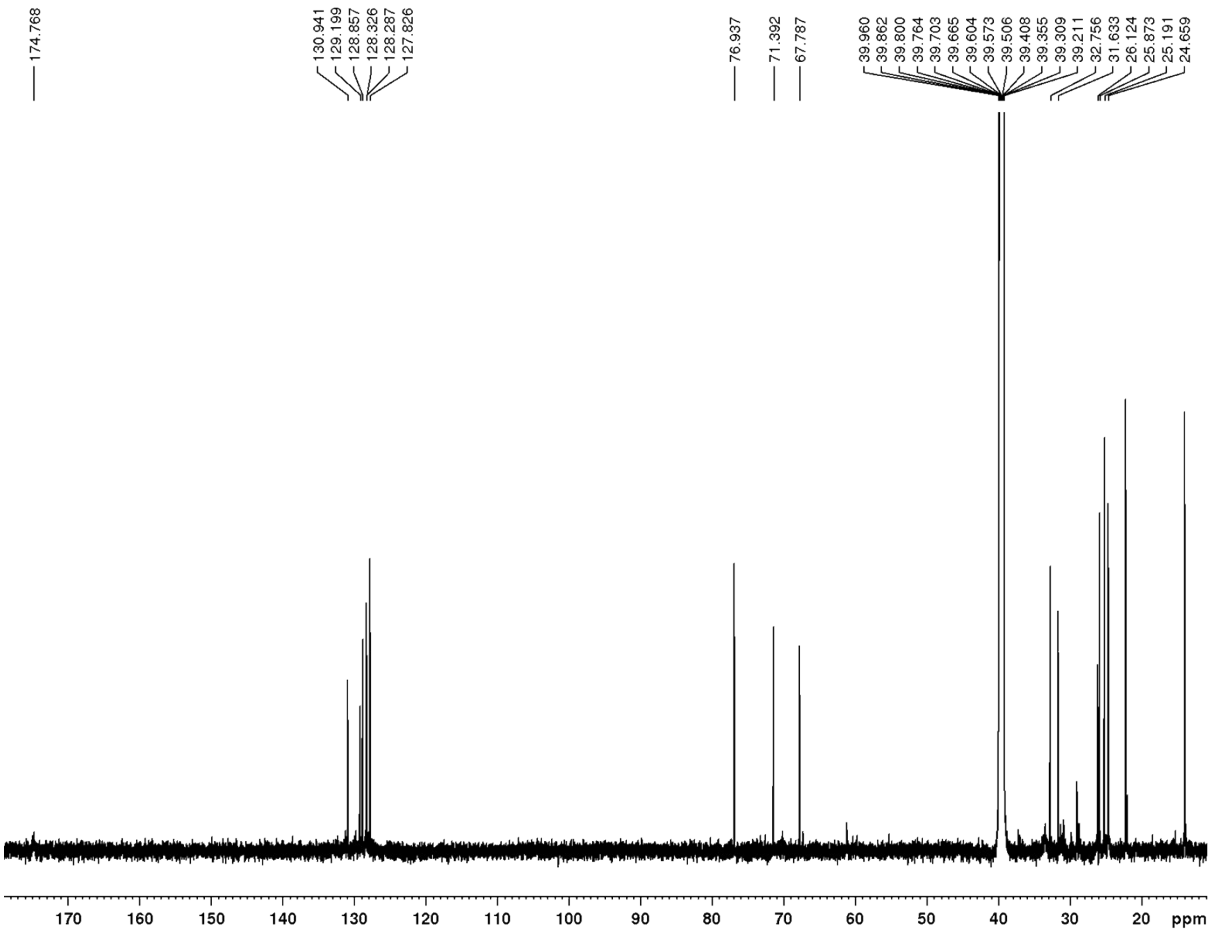
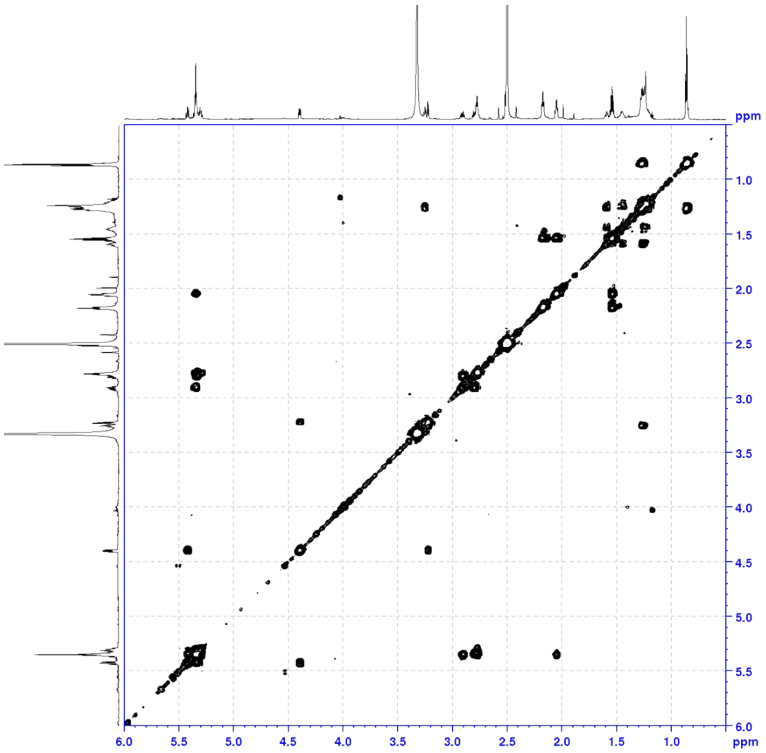
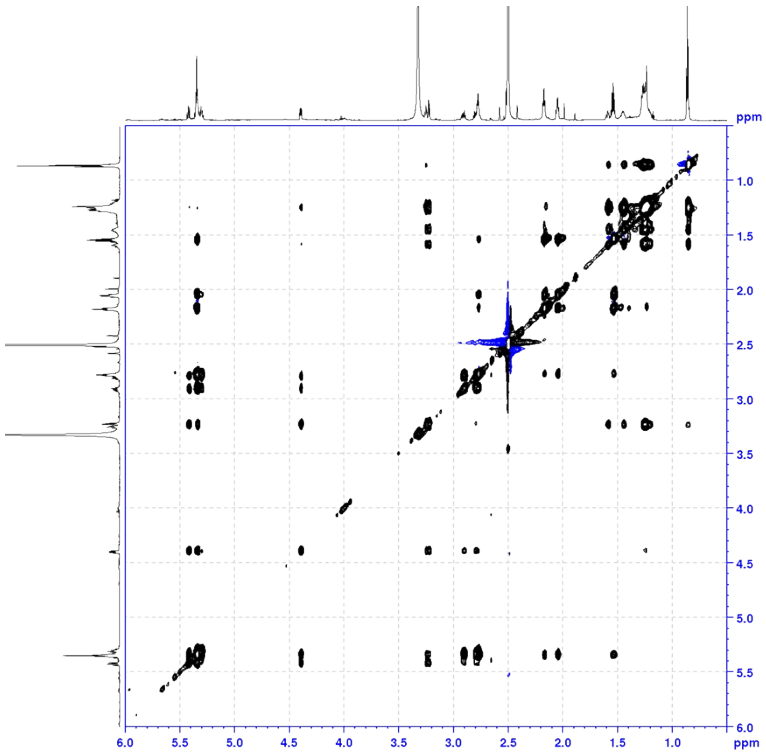


Fig. S27 NMR data of 13,14,15-TrXB_3 (**5**). A) Chemical structure of compound **5**. B) ^{1}H NMR peak of compound **5**. C) ^{13}C NMR peak of compound **5**.

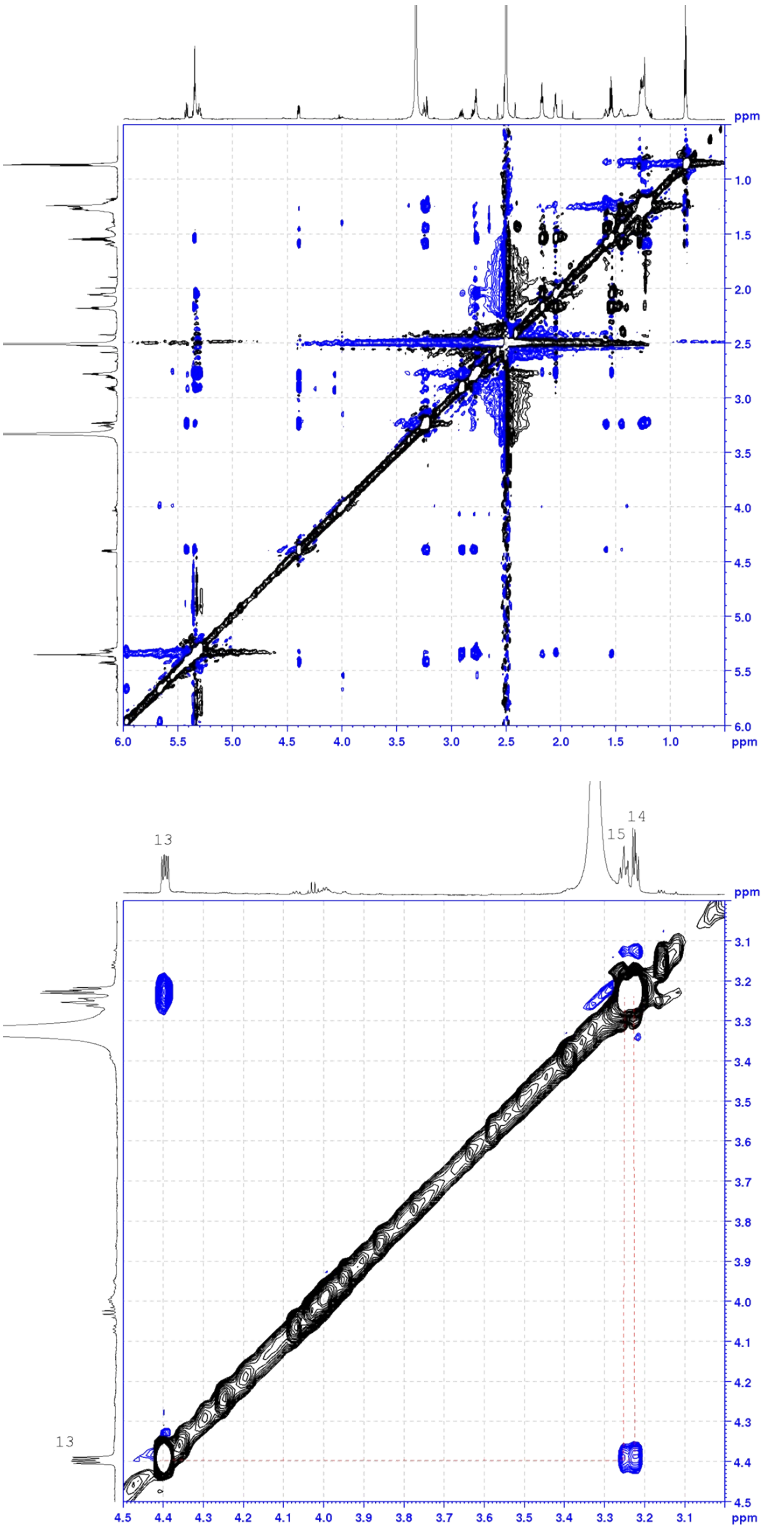
A


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

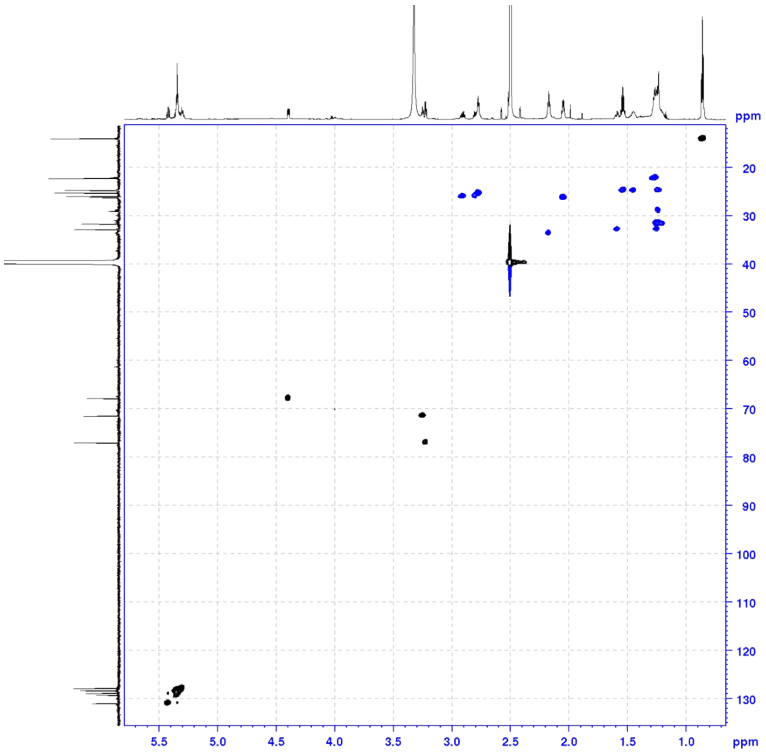
B


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
13
15
14
ppm
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
13
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 ppm

D


ppm
20
30
40
50
60
70
80
90
100
110
120
130
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

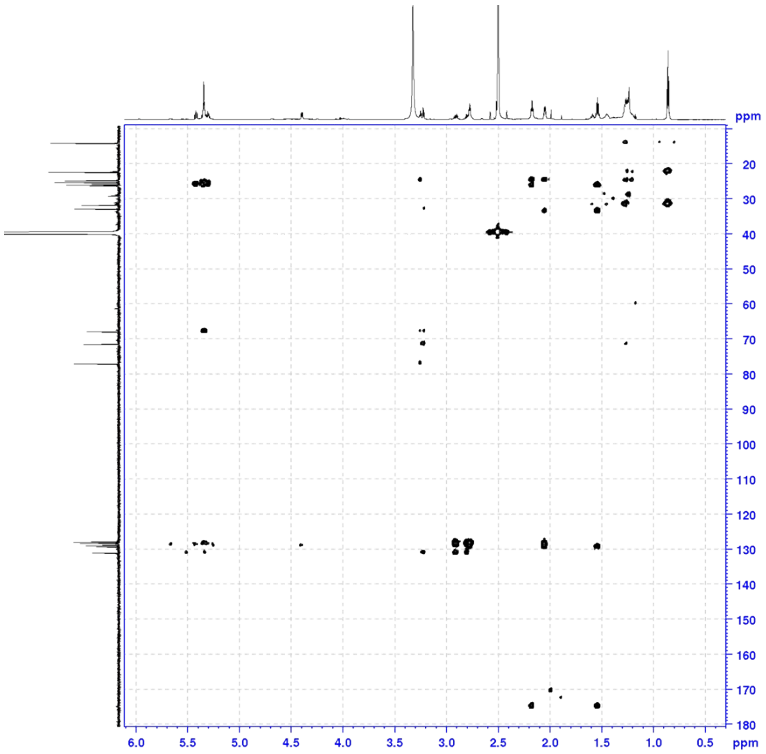
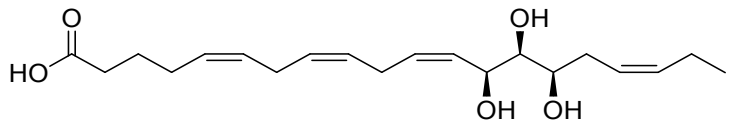
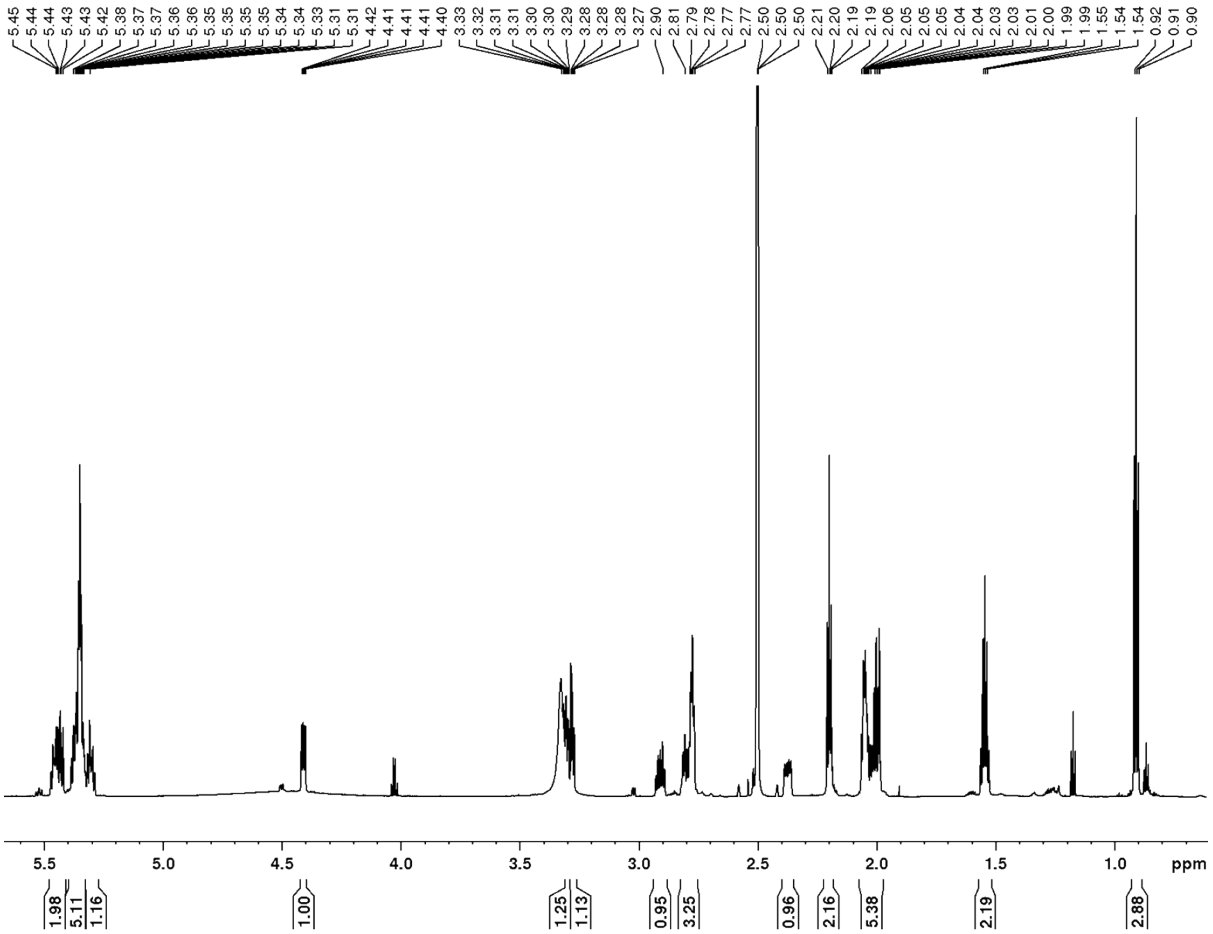

ppm
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

Fig. S28 2D NMR data of 13,14,15-TrXB_3 (**5**). A) COSY spectrum of compound **5**. B) TOCSY spectrum of compound **5**. C) ROESY spectrum of compound **5**. D) HSQC spectrum of compound **5**. E) HMBC spectrum of compound **5**.

A


O
HO
OH
OH
OH

B


5.45 5.44 5.44 5.43 5.43 5.42 5.38 5.37 5.37 5.36 5.36 5.35 5.35 5.35 5.35 5.34 5.34 5.33 5.31 5.31 4.42 4.41 4.41 4.41 4.40
3.33 3.32 3.31 3.31 3.30 3.30 3.29 3.28 3.28 3.28 3.27
2.90 2.81 2.79 2.78 2.77 2.77
2.50 2.50 2.50 2.21 2.20 2.19 2.19 2.06 2.05 2.05 2.05 2.04 2.04 2.03 2.03 2.01 2.00 1.99 1.99 1.55 1.54 1.54 0.92 0.91 0.90
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.98 5.11 1.16 1.00 1.25 1.13 0.95 3.25 0.96 2.16 5.38 2.19 2.88

C

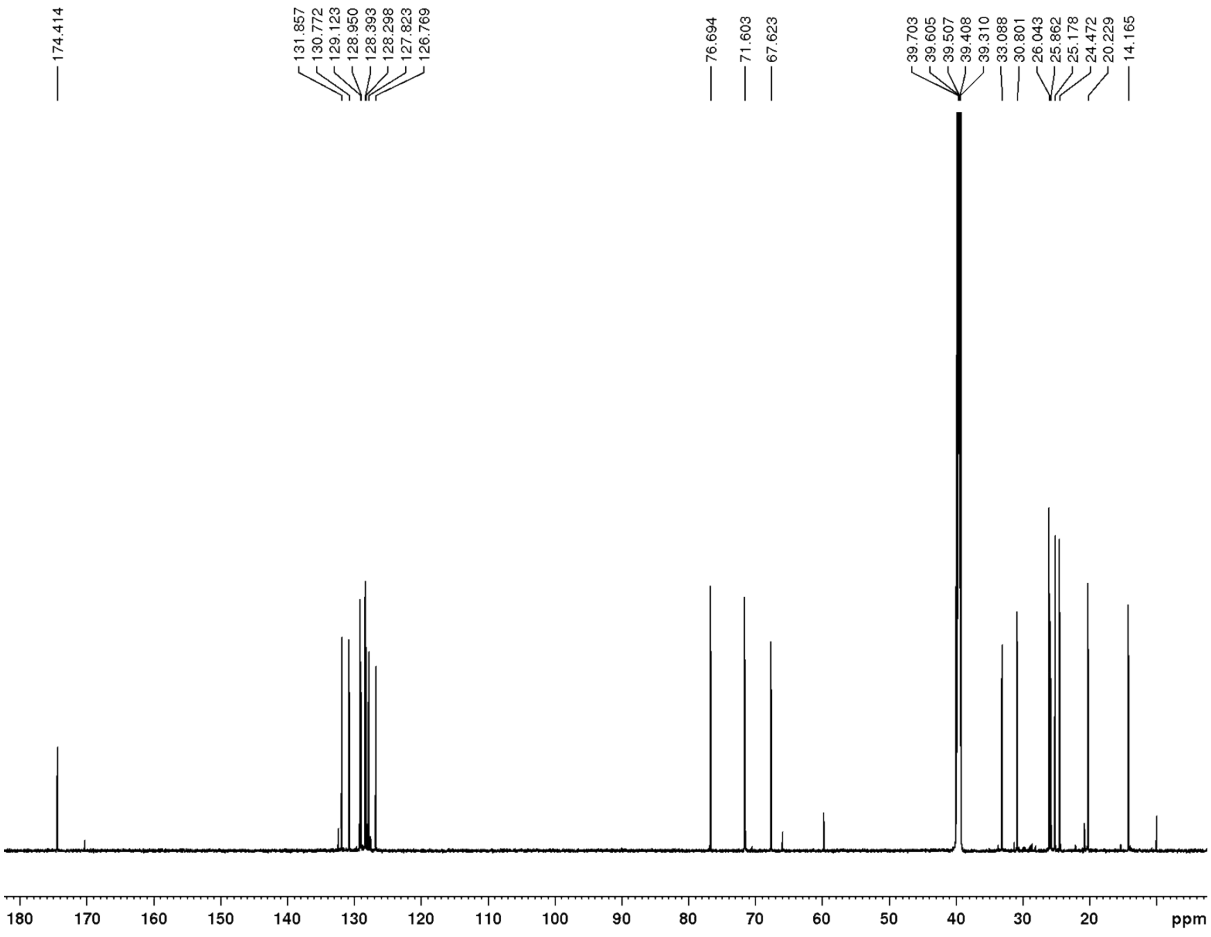
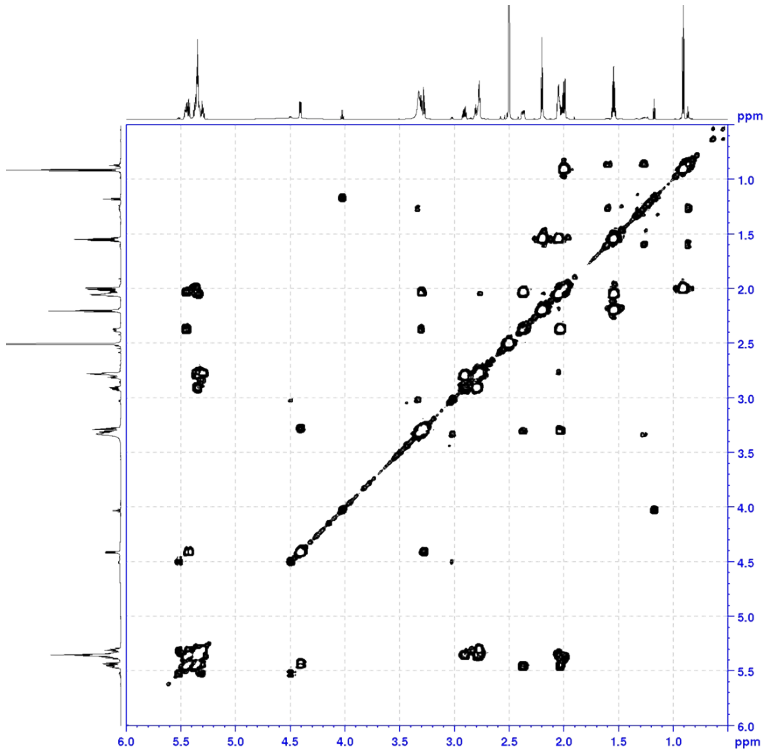
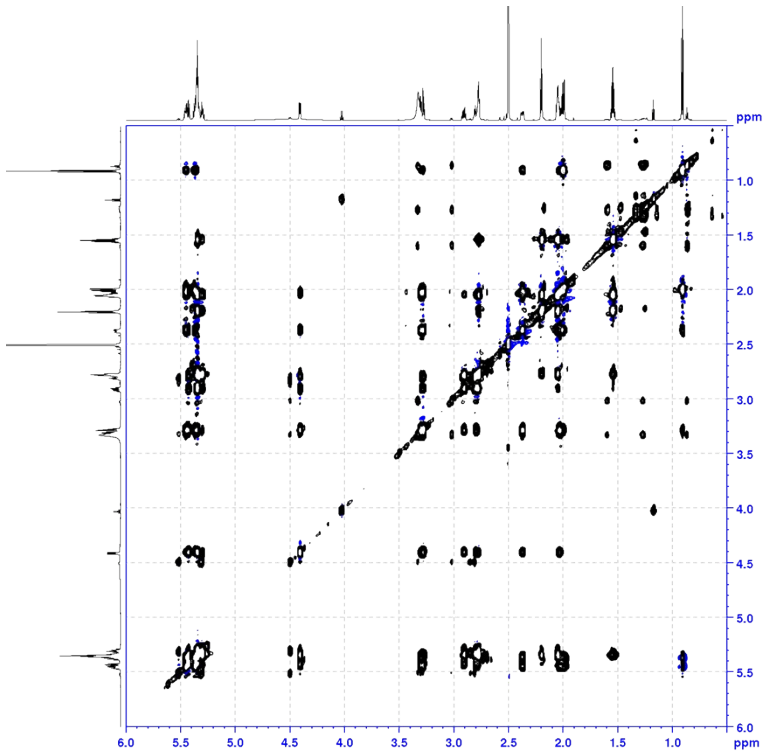


Fig. S29 NMR data of 13,14,15-TrXB_4 (**10**). A) Chemical structure of compound **10**. B) ^{1}H NMR peak of compound **10**. C) ^{13}C NMR peak of compound **10**.

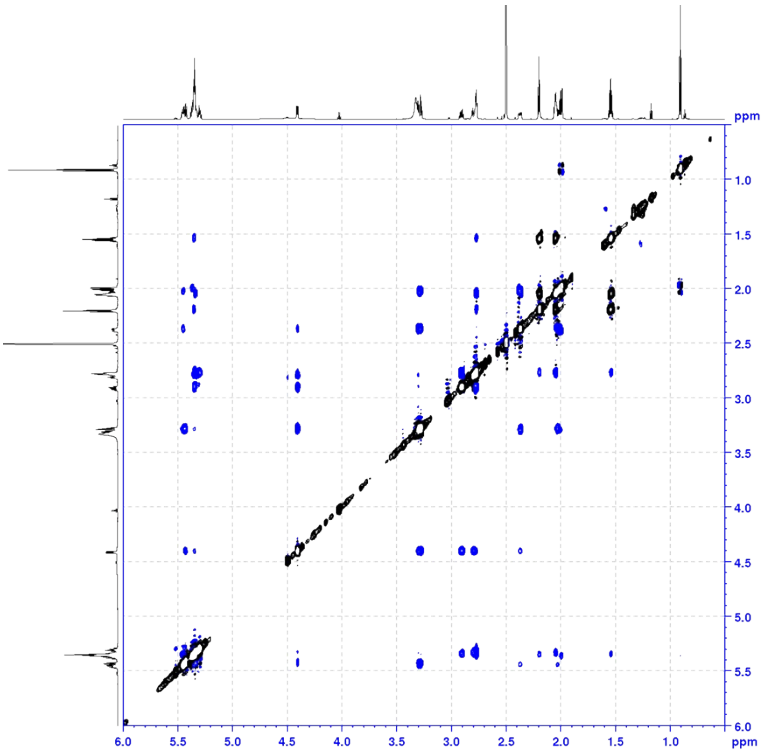
A

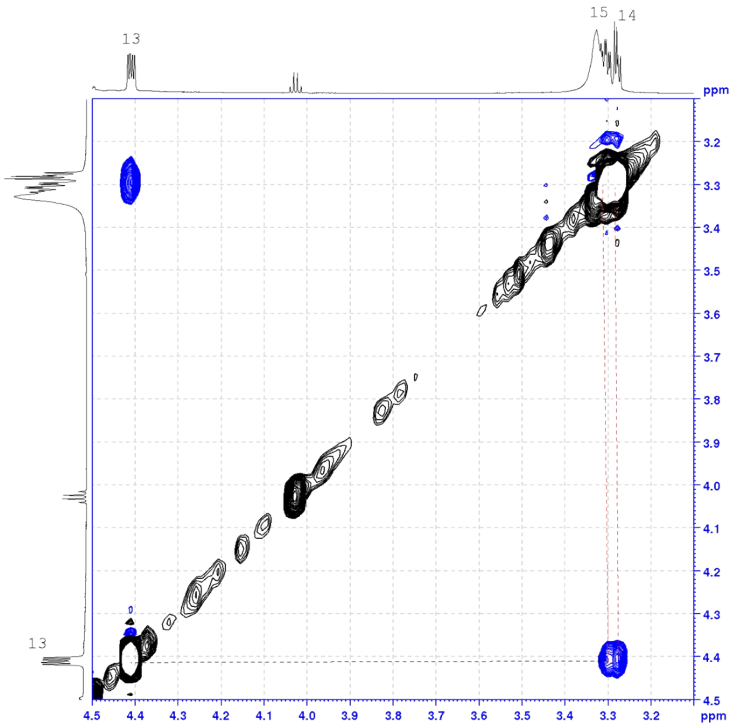

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

B

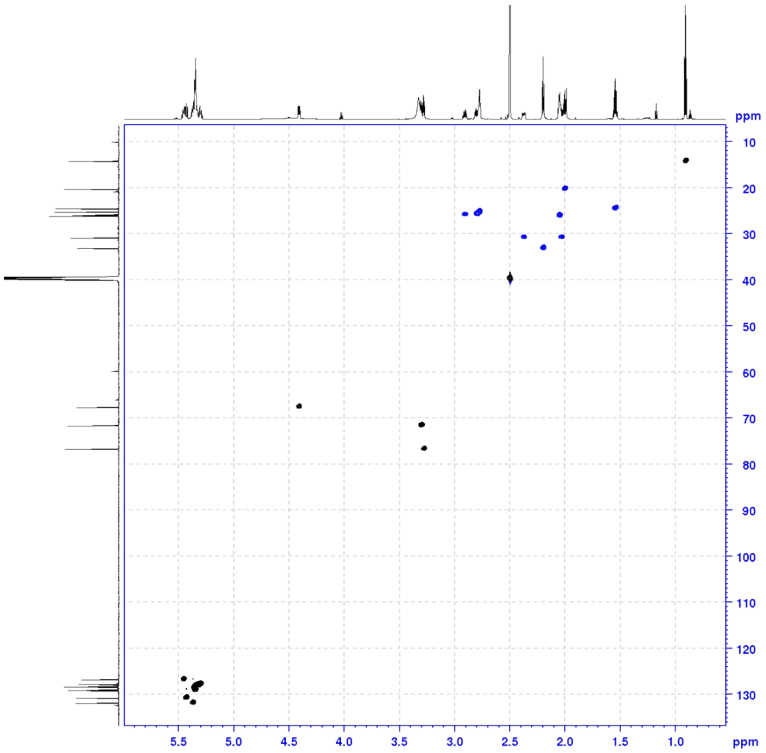

ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


13
15
14
ppm
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
13
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
ppm

D


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

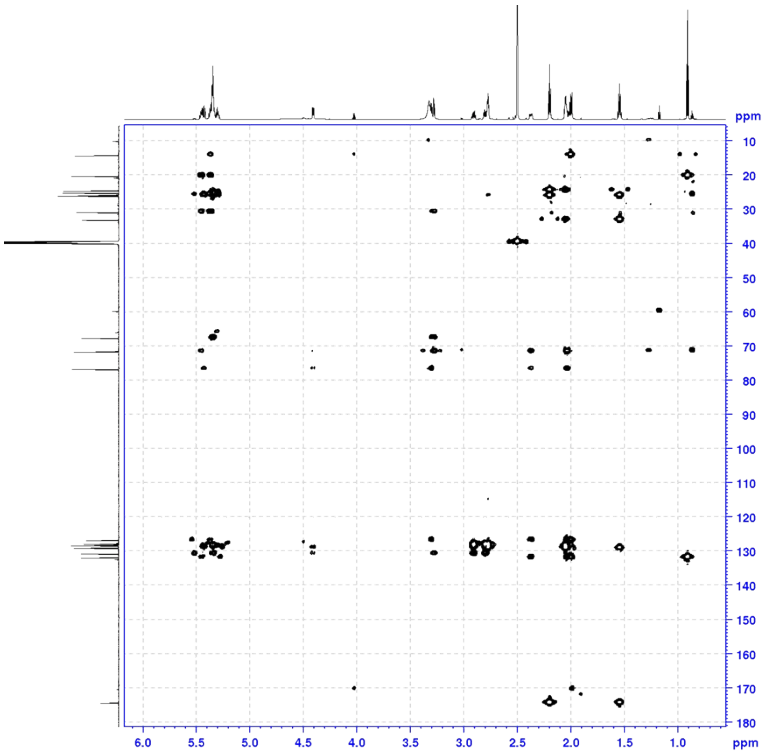
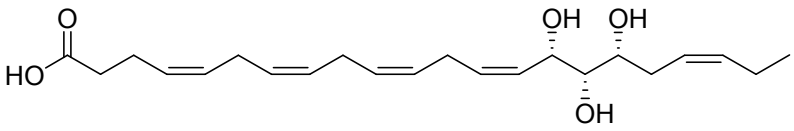
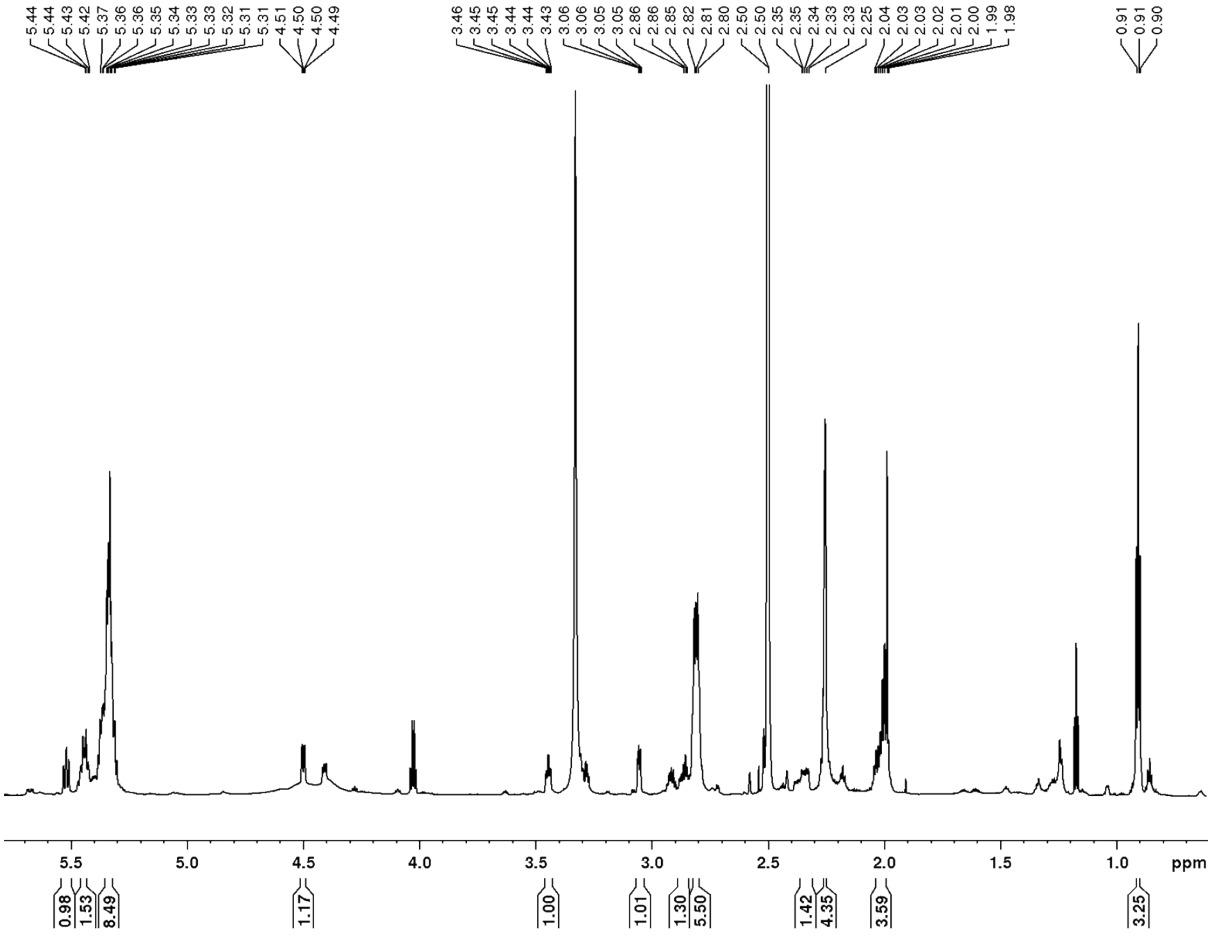

ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S30 2D NMR data of 13,14,15-TrXB_4 (**10**). A) COSY spectrum of compound **10**. B) TOCSY spectrum of compound **10**. C) ROESY spectrum of compound **10**. D) HSQC spectrum of compound **10**. E) HMBC spectrum of compound **10**.

A


O
HO
OH
OH
OH

B


5.44
5.44
5.43
5.42
5.37
5.36
5.36
5.35
5.34
5.33
5.33
5.32
5.31
5.31
4.51
4.50
4.50
4.49
3.46
3.45
3.45
3.44
3.44
3.43
3.06
3.06
3.05
3.05
2.86
2.86
2.85
2.82
2.81
2.80
2.50
2.50
2.35
2.35
2.34
2.33
2.33
2.25
2.04
2.03
2.03
2.02
2.01
2.00
1.99
1.98
0.91
0.91
0.90
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.98
1.53
8.49
1.17
1.00
1.01
1.30
5.50
1.42
4.35
3.59
3.25

C

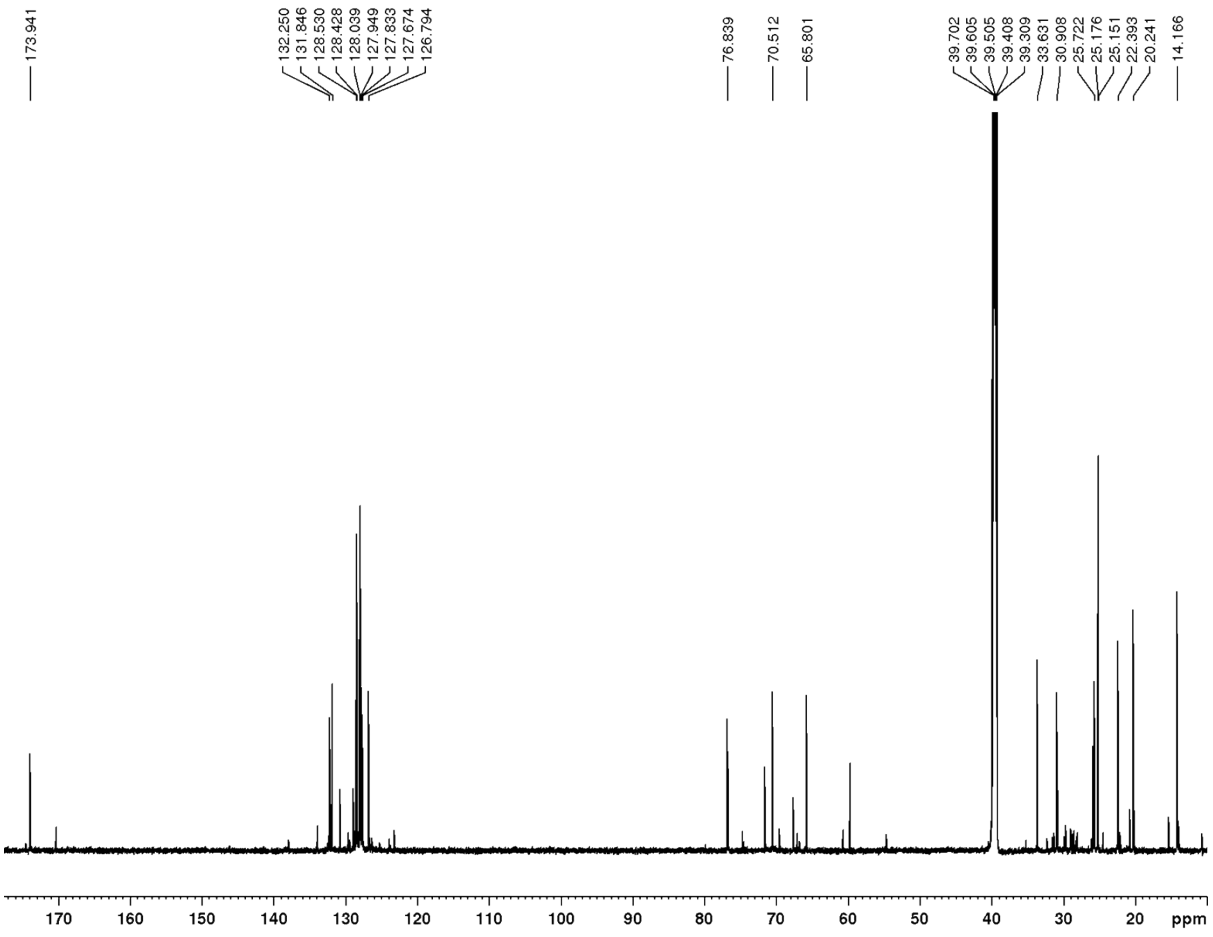
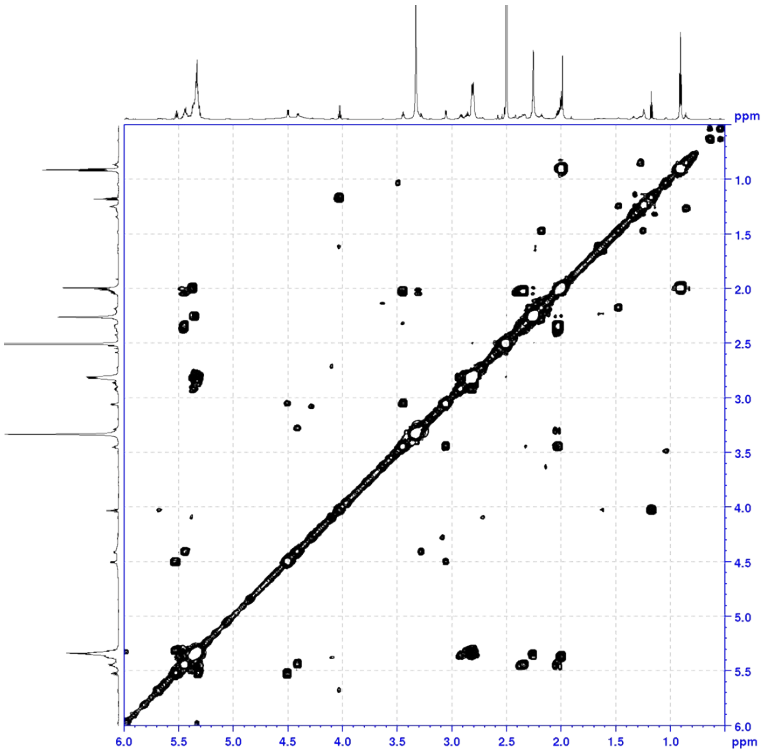


Fig. S31 NMR data of 15,16,17-TrXB_5 (**15**). A) Chemical structure of compound **15**. B) ^{1}H NMR peak of compound **15**. C) ^{13}C NMR peak of compound **15**.

A


ppm
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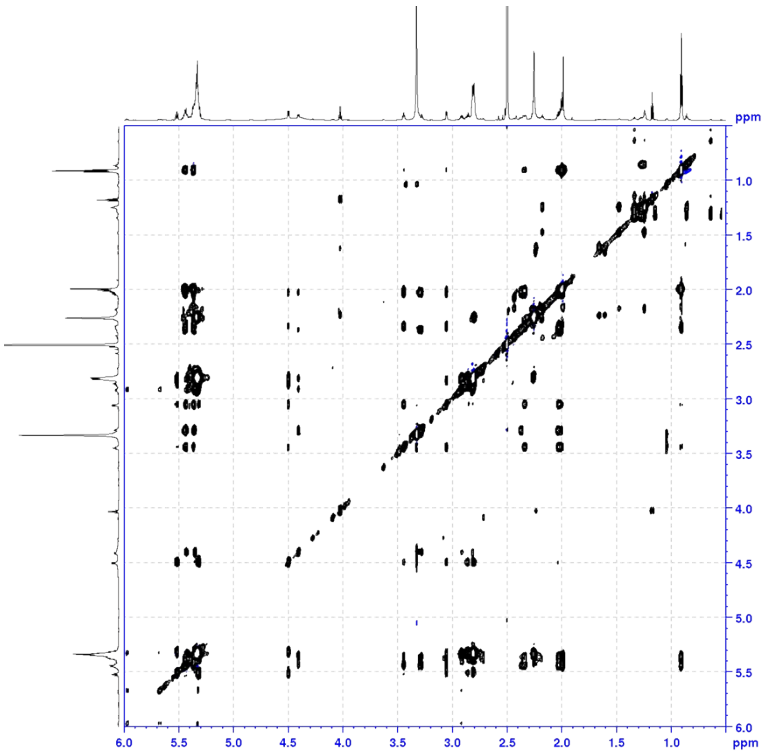
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

B


ppm
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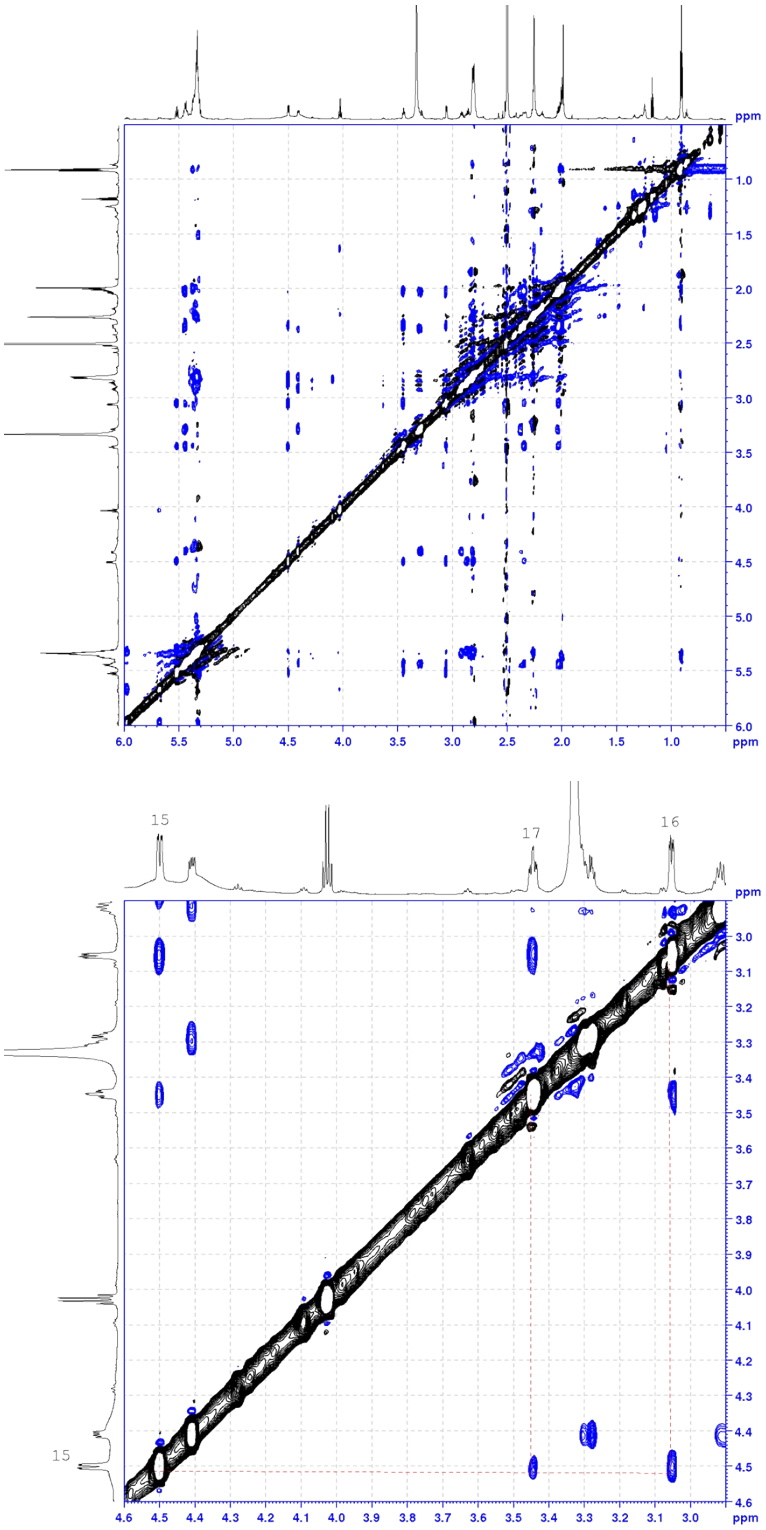
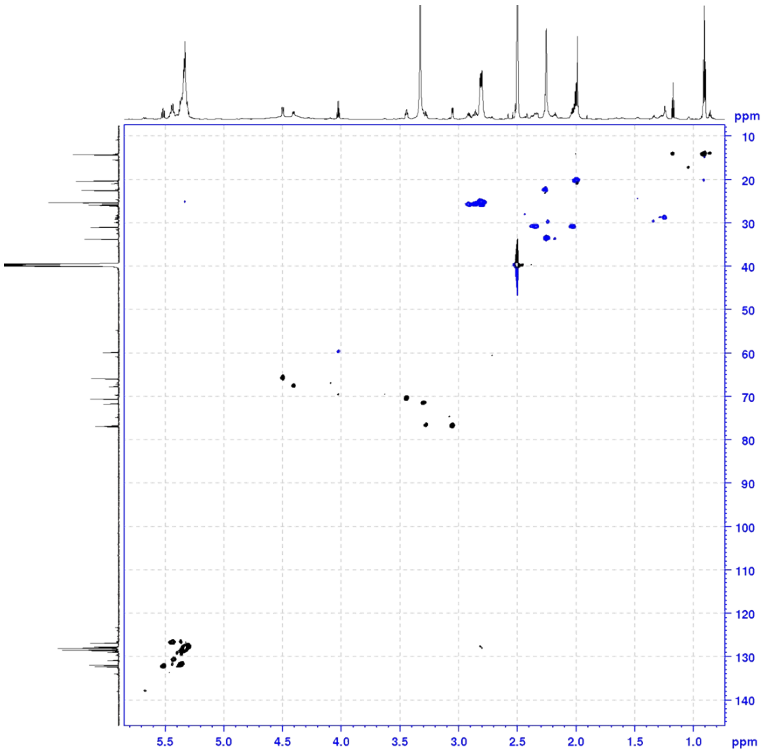
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

C


ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
15
17
16
ppm
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
15
4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 ppm

D


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E

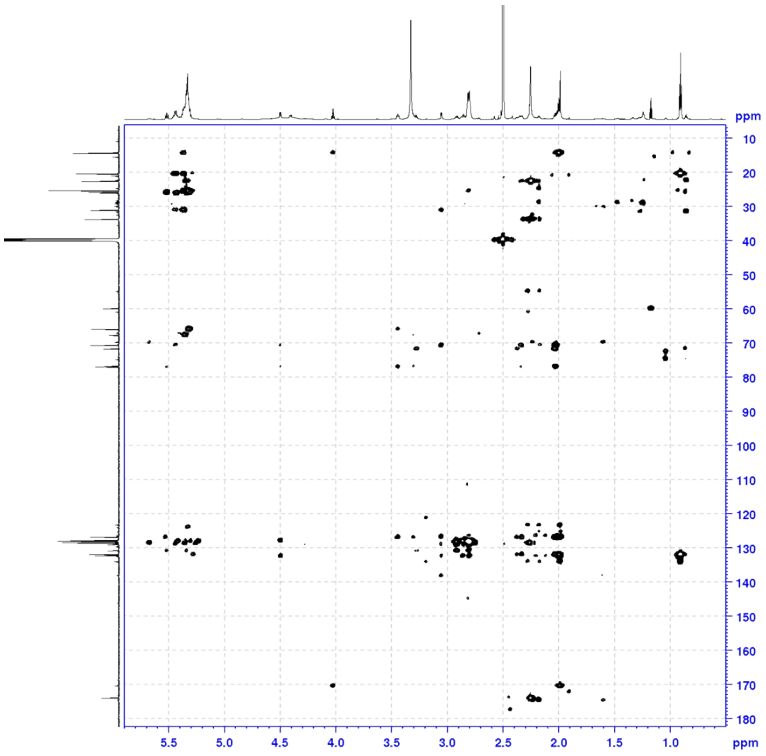
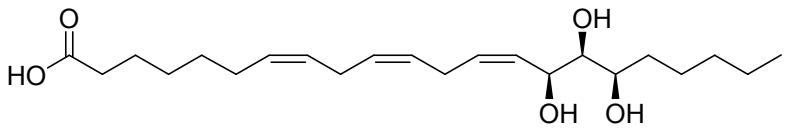
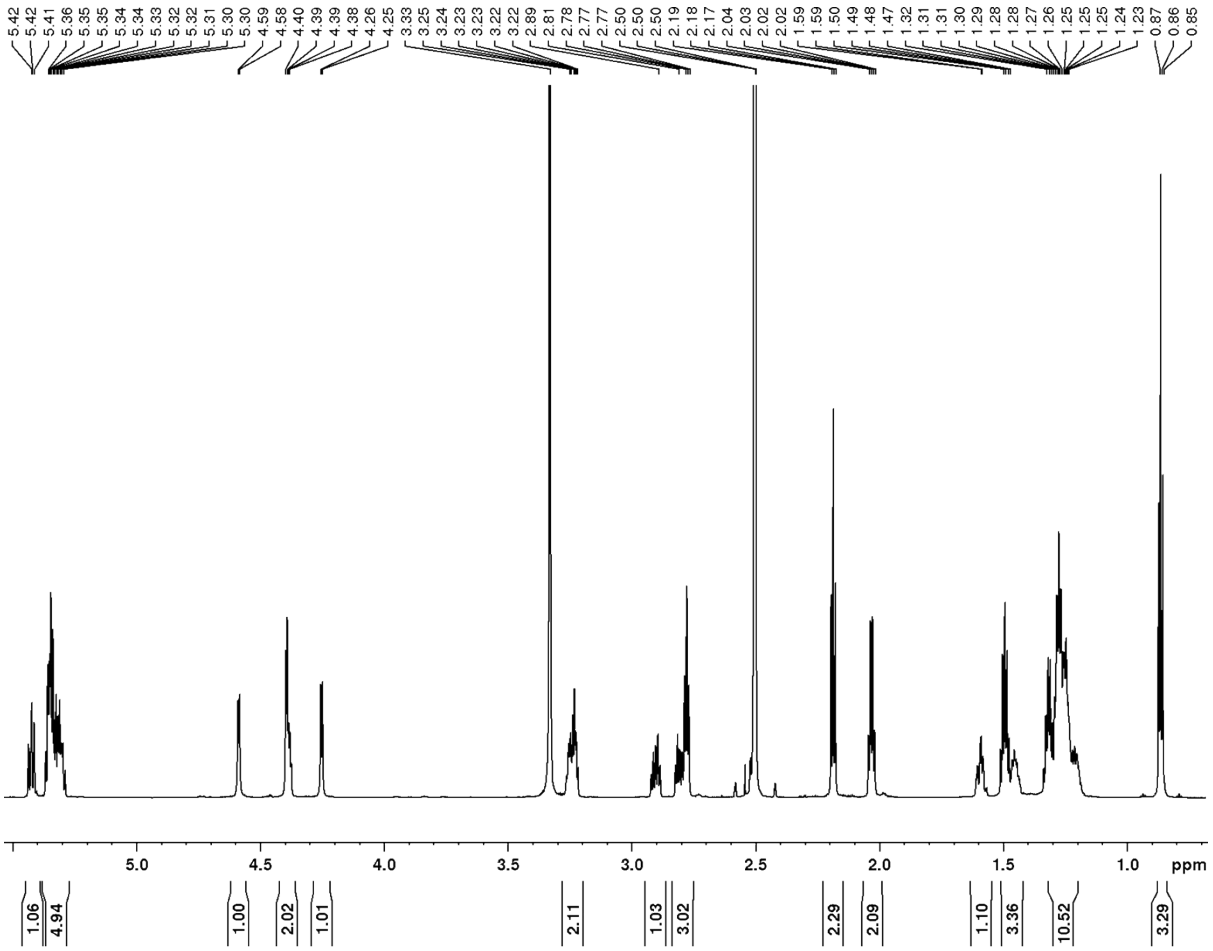

ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

Fig. S32 2D NMR data of 15,16,17-TrXB_5 (**15**). A) COSY spectrum of compound **15**. B) TOCSY spectrum of compound **15**. C) ROESY spectrum of compound **15**. D) HSQC spectrum of compound **15**. E) HMBC spectrum of compound **15**.

A


O
HO
OH
OH
OH

B


5.42 5.42 5.41 5.36 5.35 5.35 5.34 5.34 5.33 5.32 5.32 5.31 5.30 5.30 4.59 4.58 4.40 4.39 4.39 4.38 4.26 4.25 3.33 3.25 3.24 3.23 3.23 3.22 3.22 2.89 2.81 2.78 2.77 2.77 2.50 2.50 2.50 2.19 2.18 2.17 2.04 2.03 2.02 2.02 1.59 1.59 1.50 1.49 1.48 1.47 1.32 1.31 1.31 1.30 1.29 1.28 1.28 1.27 1.26 1.25 1.25 1.25 1.24 1.23 0.87 0.86 0.85
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.06 4.94 1.00 2.02 1.01 2.11 1.03 3.02 2.29 2.09 1.10 3.36 10.52 3.29

C

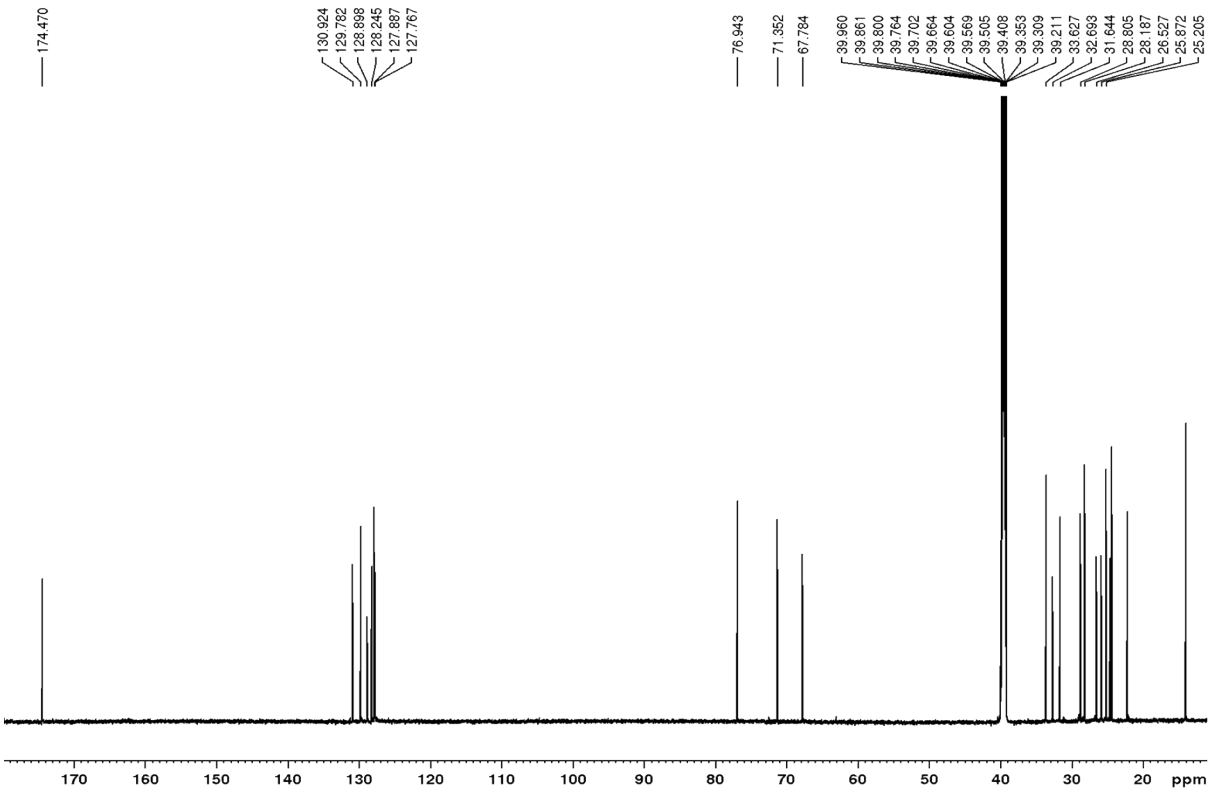
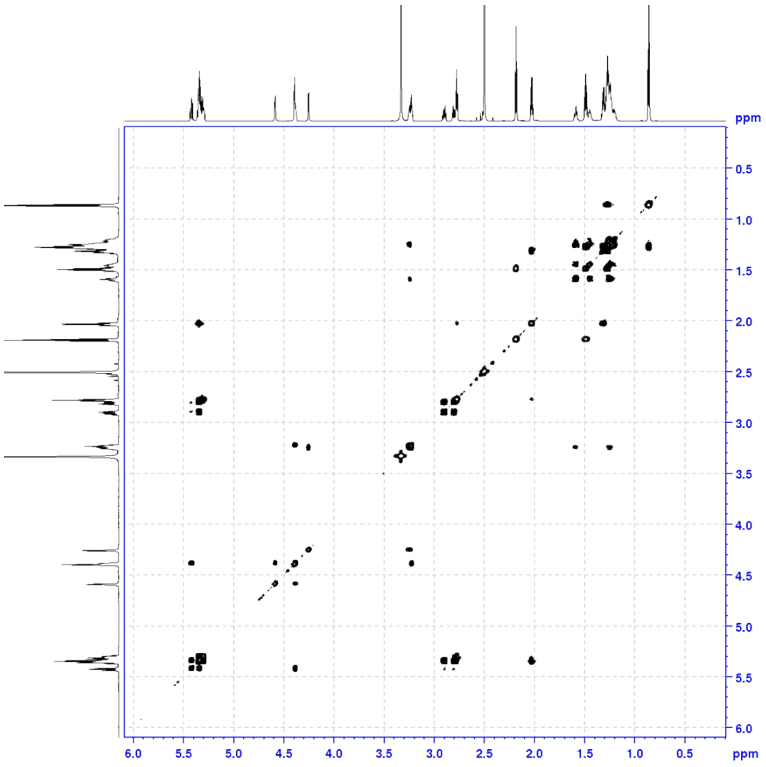
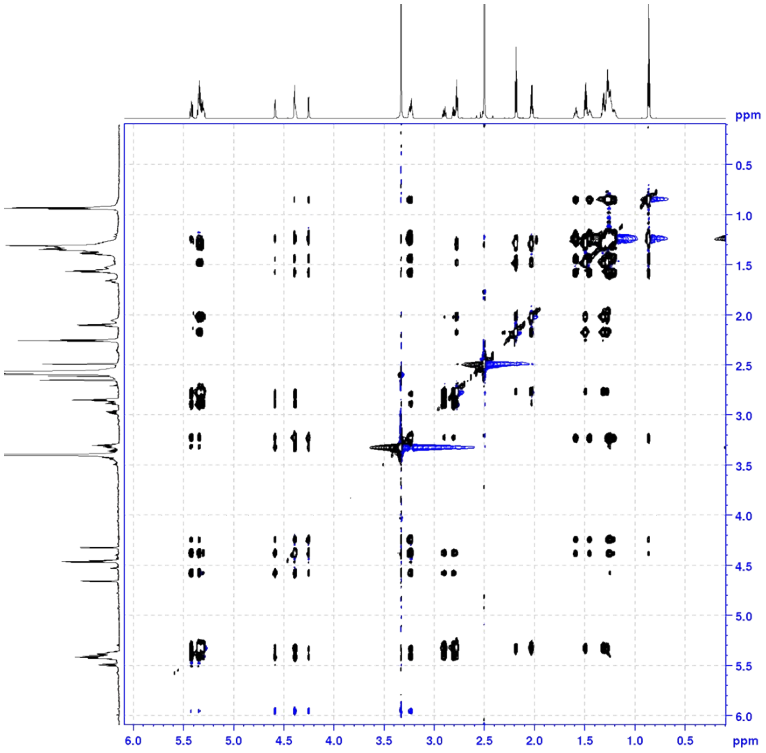


Fig. S33 NMR data of 15,16,17-TrXB_3 (**20**). A) Chemical structure of compound **20**. B) ^{1}H NMR peak of compound **20**. C) ^{13}C NMR peak of compound **20**.

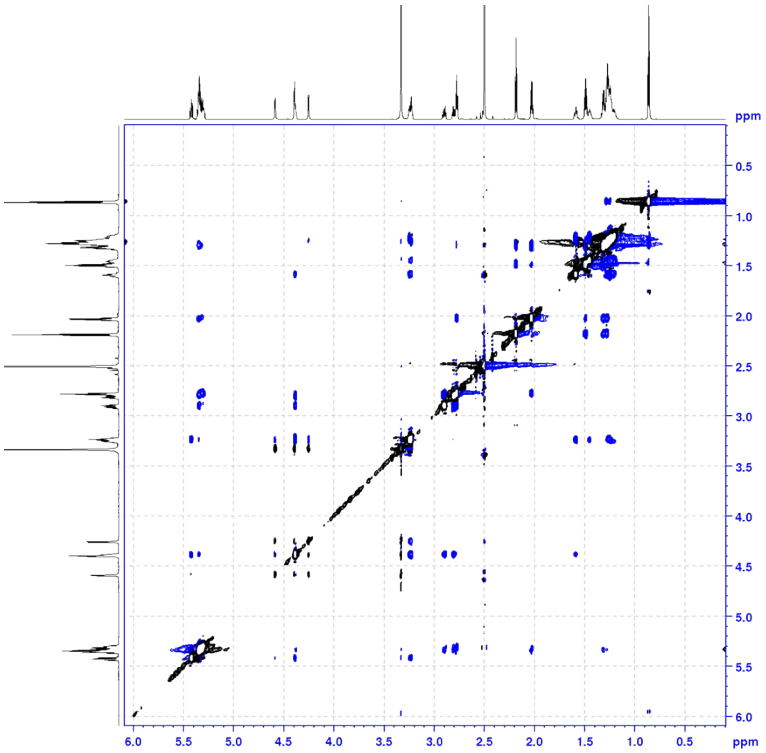
A


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

B


ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

C


ppm
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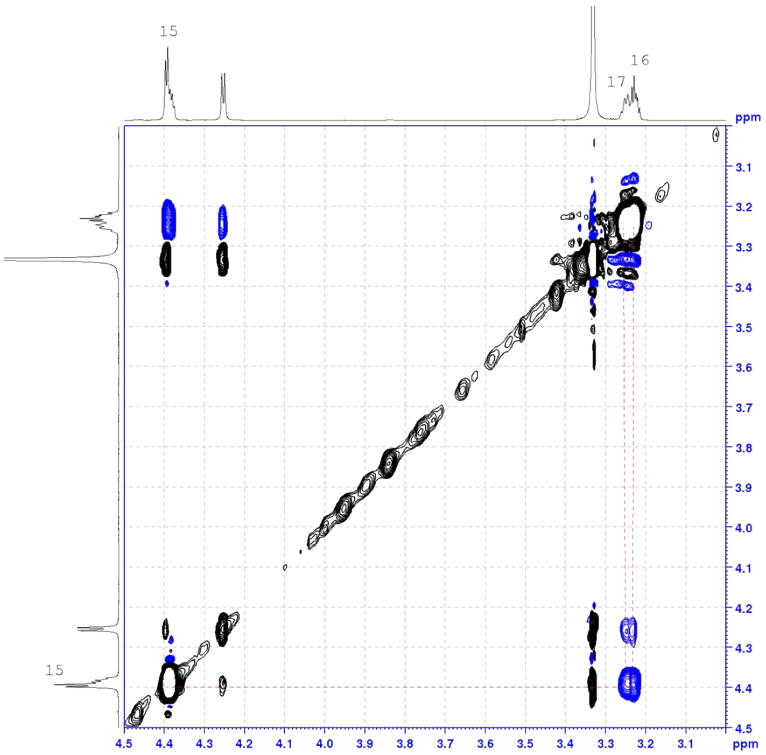
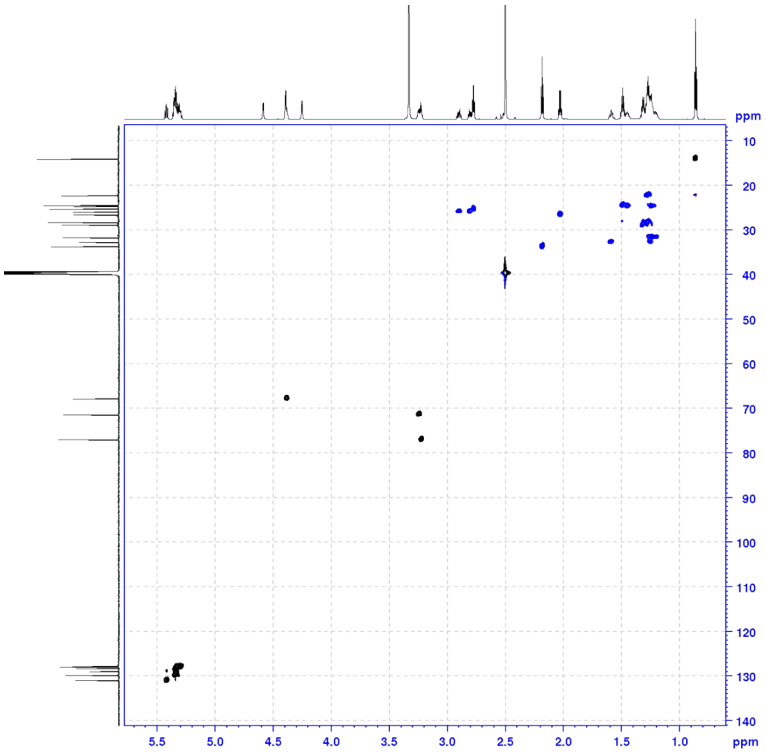
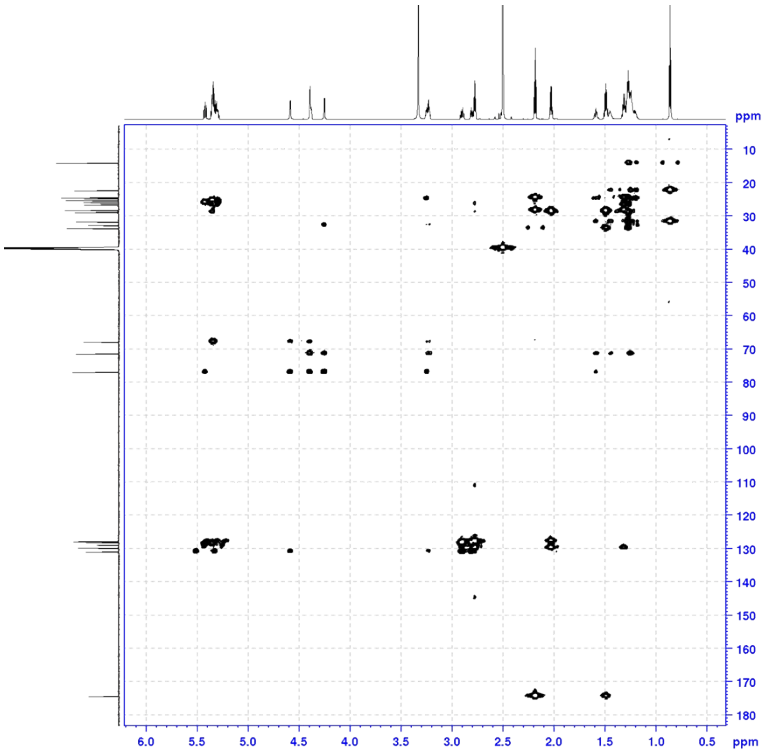
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm


15
16
17
ppm
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
15
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1
ppm

D


ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

E


ppm
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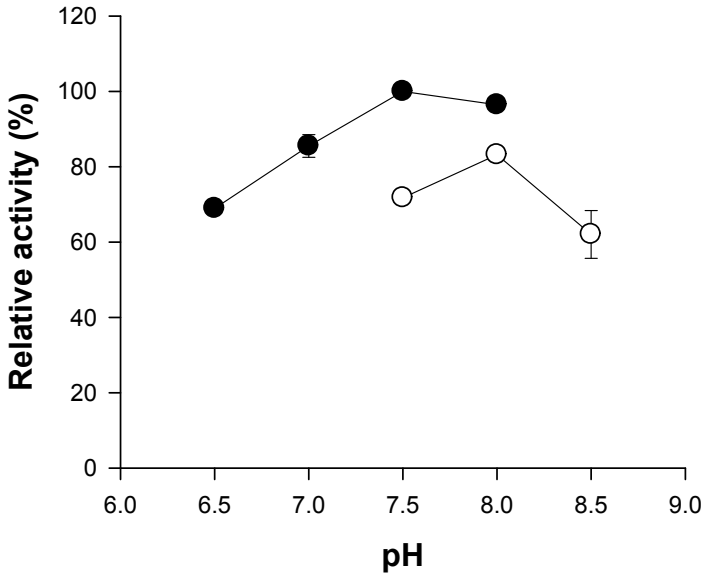
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

Fig. S34 2D NMR data of 15,16,17-TrXB_3 (**20**). A) COSY spectrum of compound **20**. B) TOCSY spectrum of compound **20**. C) ROESY spectrum of compound **20**. D) HSQC spectrum of compound **20**. E) HMBC spectrum of compound **20**.

A



B

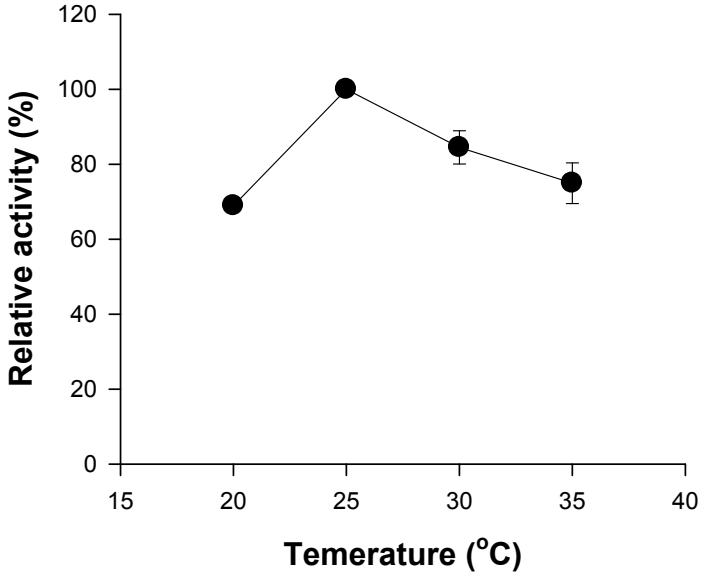


Fig. S35 Effects of pH and temperature on the activity of ARA 15-LOX from *B. thailandensis.* A) Effect of pH. The buffers used were 50 mM HEPES buffer (●, pH 6.5–7.5) and 50 mM Tris-HCl buffer (○, pH 7.5–9) and the reactions were performed at 25 °C with 1 mM ARA and 6 g L^{-1} cells for 5 min. B) Effect of temperature. The reactions were performed in 50 mM HEPES buffer (pH 7.5) containing 1 mM ARA and 6 g L^{-1} cells for 5 min. Data represent the means of three experiments and error bars represent the standard deviation.

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