

Supplementary material

for

Unmasking the Aliphatic Repertoire: New Polyunsaturated Metabolites in *Bupleurum falcatum sensu lato* Provide Chemotaxonomic Insights

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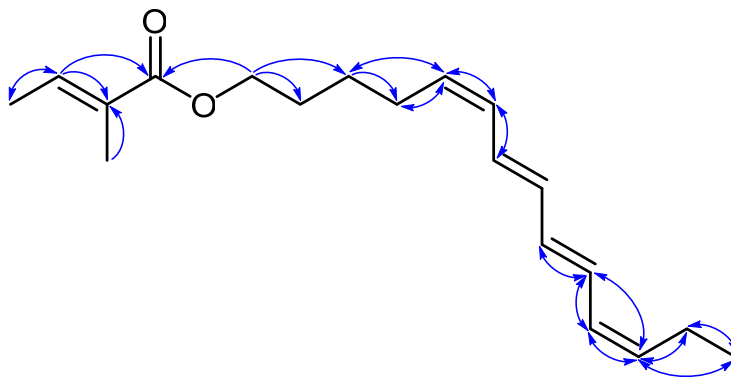


Figure S1. Important HMBC interactions of compound **1**

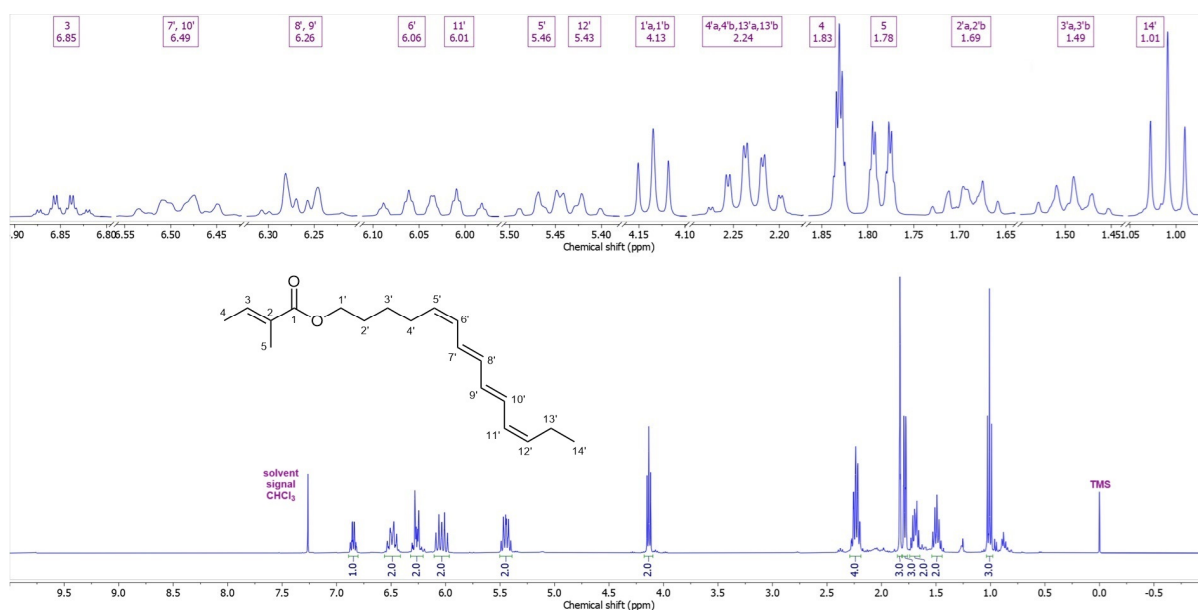


Figure S2. ¹H NMR (400 MHz, CDCl₃) spectrum of compound **1**

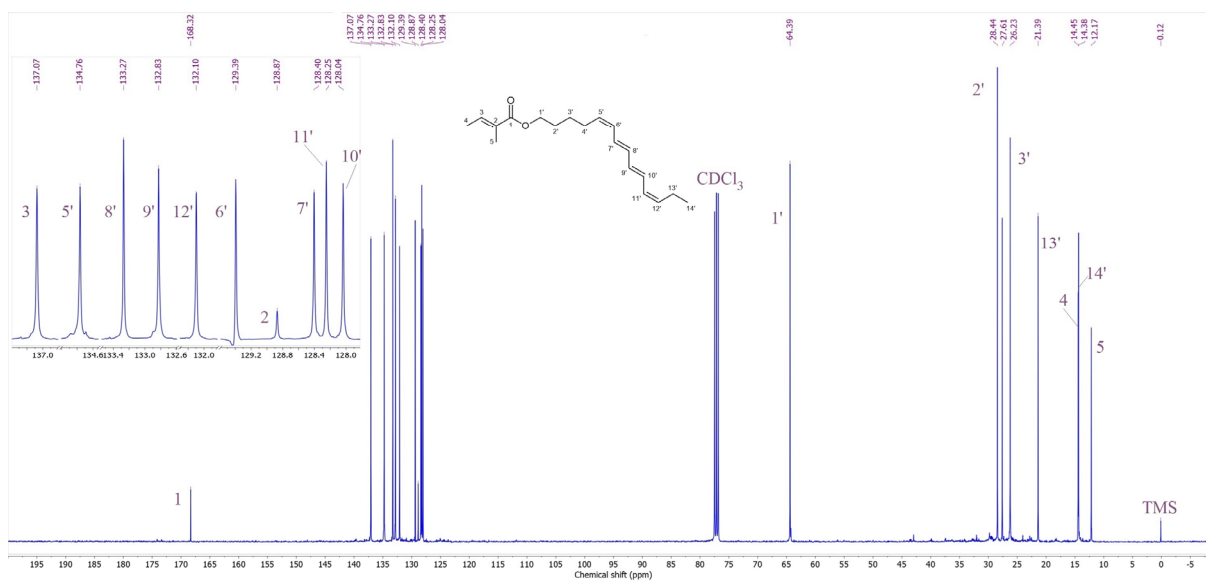


Figure S3. ¹³C NMR (100.6 MHz, CDCl₃) spectrum of compound **1**

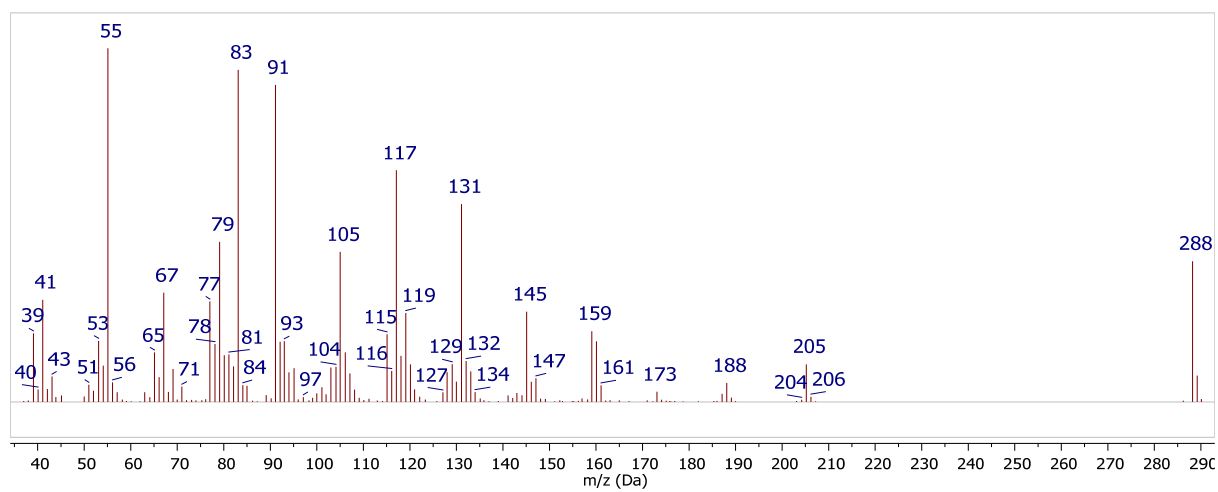


Figure S4. EI (70 eV) mass spectrum of compound **1**

[Mass Spectrum]

Data: MS-BFS-1 Date: 25-Sep-2023 13:18

Instrument : MStation

Sample: -

Note: -

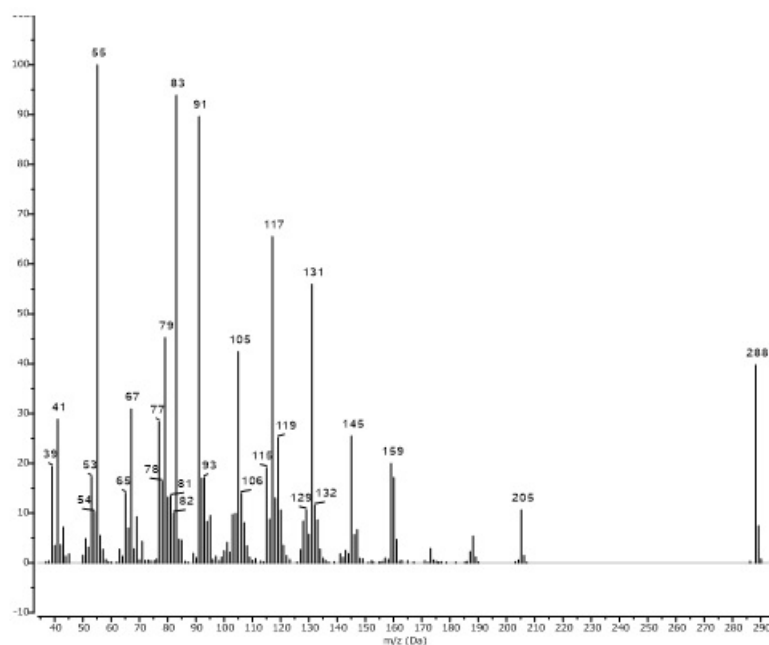
Inlet : Direct Ion Mode : EI+

Spectrum Type : Normal Ion [MF linear]

RT : 6.55 min Scan# : 40 Temp : -

BP: m/z 55 Int. : 85320

Output m/z range : 30 to 295 Cut Level : 0.00%



Data: MS-BFS-1 Date: 25-Sep-2023 15:48

Instrument : MStation

Sample: -

Note: -

Inlet : Direct Ion Mode : EI+

RT : 6.55 min Scan# : 40

Elements : C 19/0, H 28/0, O 2/0

Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 50mmu if m/z > 50

Unsaturation (U.S.) : -0.5 -20.0

	Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
1	288.2094	39.77	+ 1.7 / + 0.5	6	C19 H28 O2

Figure S5. HRMS spectrum of compound 1

Table S1. ^1H (400 MHz) and ^{13}C (100.6 MHz) NMR spectral data (in CDCl_3) of compounds **1** and **2** (NMR parameters are derived from manual iterative full spin analysis)

Compound 1			Compound 2		
Position	δ_{H} (m, J ¹ (Hz), Integral)	δ_{C} (ppm)	Position	δ_{H} (m, J (Hz), Integral)	δ_{C} (ppm)
1	/	168.32	1	/	168.33
2	/	128.87	2	/	128.85
3	6.8468 (qq, $^3J_{3,4} = 7.1$, $^4J_{3,5} = 1.4$, 1 H)	137.07	3	6.8500 (qq, $^3J_{3,4} = 7.0$, $^4J_{3,5} = 1.5$, 1 H)	137.30
4	1.7846 (d, $^3J_{3,4} = 7.1$, 3 H)	14.45	4	1.7860 (dq, $^3J_{3,4} = 7.0$, $^5J_{4,5} = 1.2$, 3 H)	14.39
5	1.8308 (d, $^4J_{3,5} = 1.4$, 3 H)	12.17	5	1.8312 (dq, $^4J_{3,5} = 1.5$, $^5J_{4,5} = 1.2$, 3 H)	11.78
1'a	4.1343 (dd, $^3J_{1'a,2'b} = 7.7$, $^3J_{1'a,2'a} = 5.5$, 1 H)	64.39	1'a	4.1320 (dd, $^3J_{1'a,2'b} = ^3J_{1'a,2'a} = 7.0$, 1 H)	64.16
1'b	4.1343 (dd, $^3J_{1'b,2'a} = 7.7$, $^3J_{1'b,2'b} = 5.5$, 1 H)		1'b	4.1320 (dd, $^3J_{1'b,2'a} = ^3J_{1'b,2'b} = 7.0$, 1 H)	
2'a	1.6932 (dddd, $^3J_{2'a,3'b} = 9.8$, $^3J_{1'b,2'a} = 7.7$, $^3J_{2'a,3'a} = 5.6$, $^3J_{1'a,2'a} = 5.5$, 1 H)	28.44	2'a	1.6900 (dddd, $^3J_{2'a,3'b} = 9.0$, $^3J_{1'b,2'a} = ^3J_{1'a,2'a} = 7.0$, $^3J_{2'a,3'a} = 5.0$, 1 H)	26.15
2'b	1.6932 (dddd, $^3J_{2'b,3'a} = 9.8$, $^3J_{1'a,2'b} = 7.7$, $^3J_{2'b,3'b} = 5.6$, $^3J_{1'b,2'b} = 5.5$, 1 H)		2'b	1.6900 (dddd, $^3J_{2'b,3'a} = 9.0$, $^3J_{1'a,2'b} = ^3J_{1'b,2'b} = 7.0$, $^3J_{2'b,3'b} = 5.0$, 1 H)	
3'a	1.4922 (dddd, $^3J_{2'b,3'a} = ^3J_{3'a,4'b} = 9.8$, $^3J_{2'a,3'a} = ^3J_{3'a,4'a} = 5.6$, 1 H)	26.23	3'a	1.4820 (dddd, $^3J_{2'b,3'a} = ^3J_{3'a,4'b} = 9.0$, $^3J_{2'a,3'a} = ^3J_{3'a,4'a} = 5.0$, 1 H)	26.95
3'b	1.4922 (dddd, $^3J_{2'a,3'b} = ^3J_{3'b,4'a} = 9.8$, $^3J_{2'b,3'b} = ^3J_{3'b,4'b} = 5.6$, 1 H)		3'b	1.4820 (dddd, $^3J_{2'a,3'b} = ^3J_{3'b,4'a} = 9.0$, $^3J_{2'b,3'b} = ^3J_{3'b,4'b} = 5.0$, 1 H)	
4'a	2.2263 (dddd, $^3J_{3'b,4'a} = 9.8$, $^3J_{4'a,5'} = 7.8$, $^3J_{3'a,4'a} = 5.6$, $^4J_{4'a,6'} = 1.7$, 1 H)	27.61	4'a	2.1210 (dddd, $^3J_{3'b,4'a} = 9.0$, $^3J_{4'a,5'} = 7.3$, $^3J_{3'a,4'a} = 5.0$, $^4J_{4'a,6'} = 1.3$, 1 H)	27.58
4'b	2.2263 (dddd, $^3J_{3'a,4'b} = 9.8$, $^3J_{4'b,5'} = 7.8$, $^3J_{3'b,4'b} = 5.6$, $^4J_{4'b,6'} = 1.7$, 1 H)		4'b	2.1210 (dddd, $^3J_{3'a,4'b} = 9.0$, $^3J_{4'b,5'} = 7.3$, $^3J_{3'b,4'b} = 5.0$, $^4J_{4'b,6'} = 1.3$, 1 H)	
5'	5.4562 (dddd, $^3J_{5',6'} = 11.0$, $^3J_{4'a,5'} = ^3J_{4'b,5'} = 7.8$, $^4J_{5',7'} = -1.7$, 1 H)	134.76	5'	5.3827 (dddd, $^3J_{5',6'} = 10.8$, $^3J_{4'a,5'} = ^3J_{4'b,5'} = 7.3$, $^4J_{5',7'} = -0.8$, 1 H)	132.03
6'	6.0612 (dddd, $^3J_{5',6'} = ^3J_{6',7'} = 11.0$, $^4J_{4'a,6'} = ^4J_{4'b,6'} = 1.7$, $^4J_{6',8'} = -0.7$, 1 H)	129.39	6'	6.0170 (dddd, $^3J_{6',7'} = 11.4$, $^3J_{5',6'} = 10.8$, $^4J_{4'a,6'} = ^4J_{4'b,6'} = 1.3$, $^4J_{6',8'} = -0.7$, 1 H)	129.34
7'	6.5020 (dddd, $^3J_{7',8'} = 15.2$, $^3J_{6',7'} = 11.0$, $^4J_{5',7'} = -1.7$, $^4J_{7',9'} = -0.8$, $^5J_{7',10'} = -0.6$, 1 H)	128.40	7'	6.3642 (dddd, $^3J_{7',8'} = 14.8$, $^3J_{6',7'} = 11.4$, $^4J_{5',7'} = -0.8$, $^4J_{7',9'} = -0.7$, 1 H)	125.86
8'	6.2700 (dddd, $^3J_{7',8'} = 15.2$, $^3J_{8',9'} = 12.0$, $^4J_{8',10'} = -0.8$, $^4J_{6',8'} = -0.7$, 1 H)	133.27	8'	6.1730 (dddd, $^3J_{7',8'} = 14.8$, $^3J_{8',9'} = 10.8$, $^4J_{8',10'} = -0.7$, $^4J_{6',8'} = -0.7$, 1 H)	132.03
9'	6.2599 (dddd, $^3J_{9',10'} = 15.2$, $^3J_{8',9'} = 12.0$, $^4J_{7',9'} = -0.8$, $^4J_{9',11'} = -0.7$, 1 H)	132.83	9'	6.0980 (dddd, $^3J_{9',10'} = 15.0$, $^3J_{8',9'} = 10.8$, $^4J_{7',9'} = -0.7$, $^4J_{9',11'a} = ^4J_{9',11'b} = 1.3$, 1 H)	130.62
10'	6.4780 (dddd, $^3J_{9',10'} = 15.2$, $^3J_{10',11'} = 11.0$, $^4J_{10',12'} = 1.0$, $^4J_{8',10'} = -0.8$, $^5J_{7',10'} = -0.6$, 1 H)	128.04	10'	5.7092 (dddd, $^3J_{9',10'} = 15.0$, $^3J_{10',11'a} = ^3J_{10',11'b} = 6.9$, $^4J_{8',10'} = -0.7$, 1 H)	135.60
11'	6.0093 (dddd, $^3J_{10',11'} = ^3J_{11',12'} = 11.0$, $^4J_{11',13'a} = ^4J_{11',13'b} = 1.6$, $^4J_{9',11'} = -0.7$, 1 H)	128.25	11'a	2.1050 (dddd, $^3J_{10',11'a} = 6.9$, $^3J_{11'a,12'a} = ^3J_{11'a,12'b} = 7.2$, $^4J_{9',11'a} = 1.3$, 1 H)	32.64
			11'b	2.1050 (dddd, $^3J_{10',11'b} = 6.9$, $^3J_{11'b,12'a} = ^3J_{11'b,12'b} = 7.2$, $^4J_{9',11'b} = 1.3$, 1 H)	
12'	5.4346 (dddd, $^3J_{11',12'} = 11.0$, $^3J_{12',13'a} = ^3J_{12',13'b} = 7.8$, $^4J_{10',12'} = 1.0$, 1 H)	132.10	12'a	1.2930 (dddd, $^3J_{12'a,13'b} = 9.0$, $^3J_{11'a,12'a} = ^3J_{11'b,12'a} = 7.2$, $^3J_{12'a,13'a} = 5.0$, 1 H)	31.58
			12'b	1.2930 (dddd, $^3J_{12'b,13'a} = 9.0$, $^3J_{11'a,12'b} = ^3J_{11'b,12'b} = 7.2$, $^3J_{12'b,13'b} = 5.0$, 1 H)	
13'a	2.2367 (ddd, $^3J_{12',13'a} = 7.8$, $^3J_{13'a,14'} = 7.5$, $^4J_{11',13'a} = 1.6$, 1 H)	21.39	13'a	1.3670 (ddd, $^3J_{12'b,13'a} = 9.0$, $^3J_{13'a,14'} = 7.0$, $^3J_{12'a,13'a} = 5.0$, 1 H)	22.55
13'b	2.2367 (ddd, $^3J_{12',13'b} = 7.8$, $^3J_{13'b,14'} = 7.5$, $^4J_{11',13'b} = 1.6$, 1 H)		13'b	1.3670 (ddd, $^3J_{12'a,13'b} = 9.0$, $^3J_{13'b,14'} = 7.0$, $^3J_{12'b,13'b} = 5.0$, 1 H)	
14'	1.0092 (t, $^3J_{13'a,14'} = ^3J_{13'b,14'} = 7.5$, 3 H)	14.38	14'	0.8964 (t, $^3J_{13'a,14'} = ^3J_{13'b,14'} = 7.0$, 3 H)	14.07

¹ Coupling constant values were initially inferred from ^1H homoselective decoupling NMR experiments and afterward refined through a manual iterative full spin analysis. For details, cf. Experimental part.

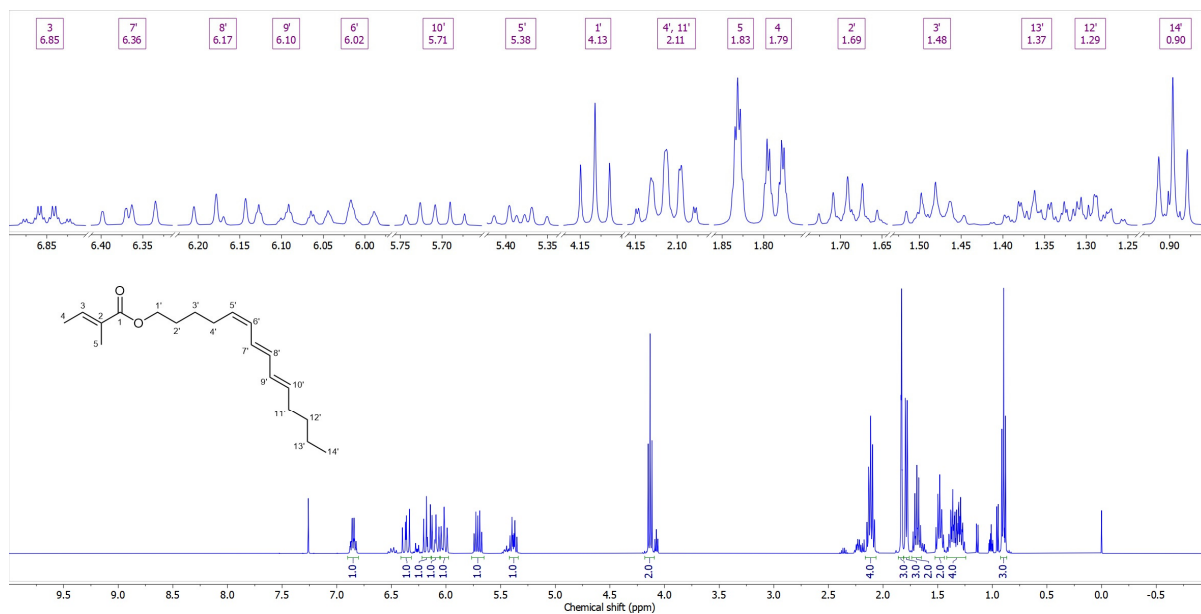


Figure S6. ¹H NMR (400 MHz, CDCl₃) spectrum of compound 2

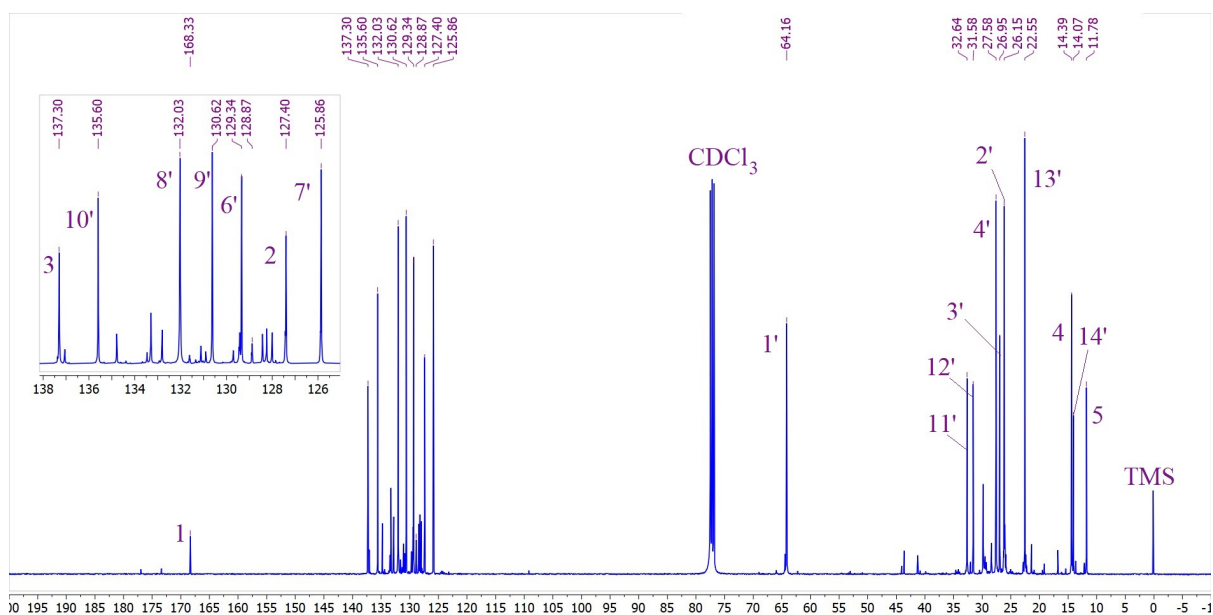


Figure S7. ¹³C NMR (100.6 MHz, CDCl₃) spectrum of compound 2

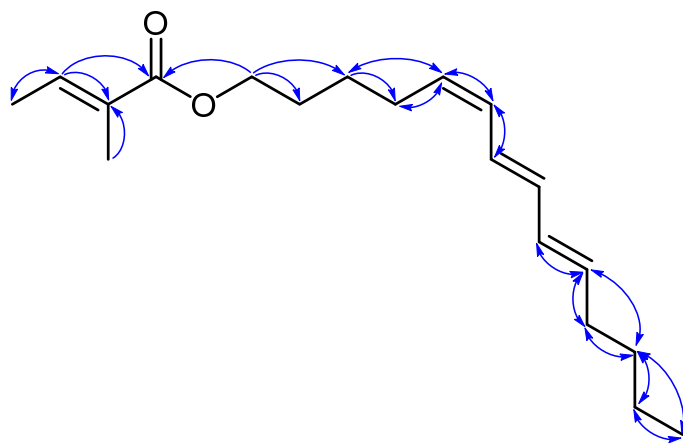


Figure S8. Important HMBC interactions of compound 2

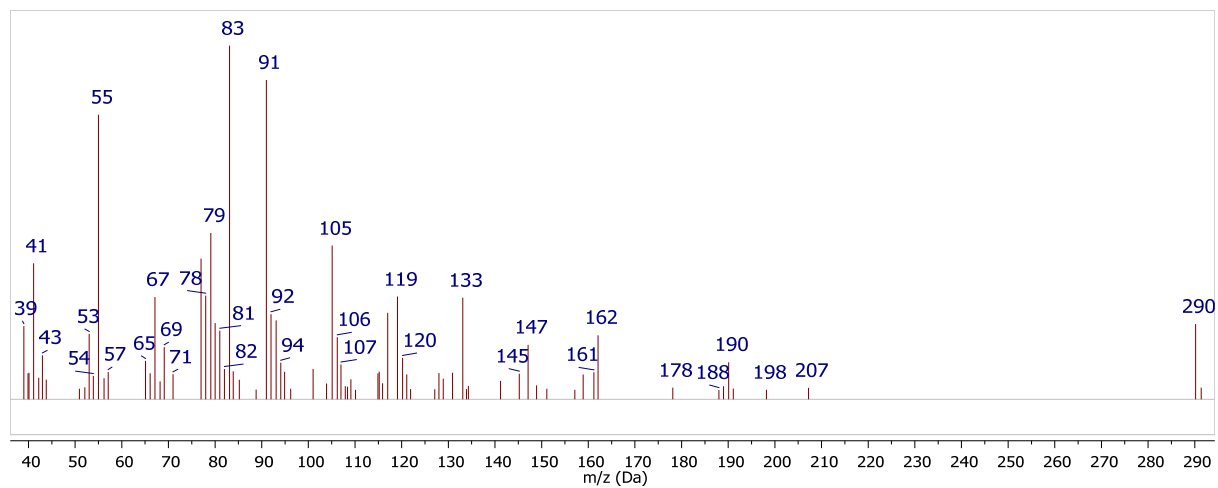


Figure S9. EI (70 eV) mass spectrum of compound 2

[Mass Spectrum]

Data: MS-BFS-2 Date: 1-Oct-2023 10:39

Instrument : MStation

Sample: -

Note: -

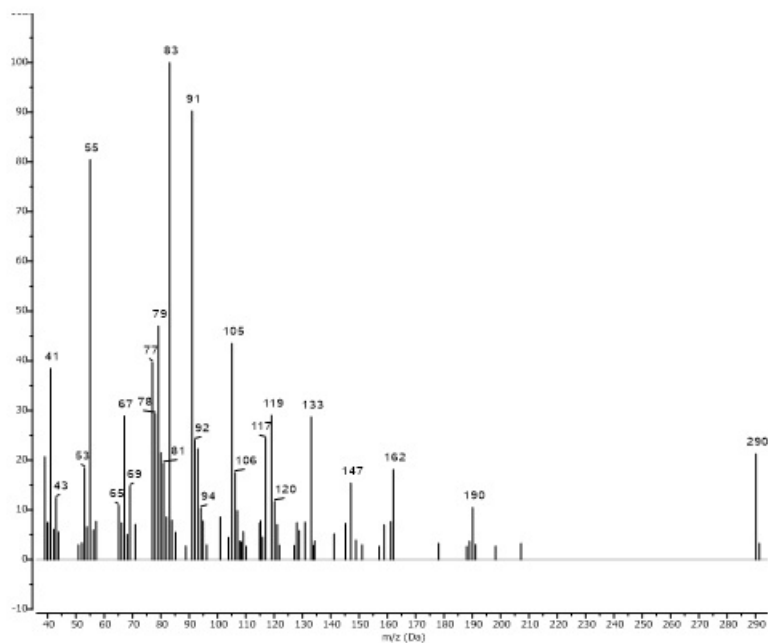
Inlet : Direct Ion Mode : EI+

Spectrum Type : Normal Ion [MF linear]

RT : 6.28 min Scan# : 38 Temp : -

BP: m/z 83 Int. : 5732

Output m/z range : 35 to 295 Cut Level : 0.00%



Data: MS-BFS-2 Date: 1-Oct-2023 11:58

Instrument : MStation

Sample: -

Note: -

Inlet : Direct Ion Mode : EI+

RT : 6.28 min Scan# : 38

Elements : C 19/0, H 30/0, O 2/0

Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 50mmu if m/z > 50

Unsaturation (U.S.) : -0.5 -20.0

	Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
1	290.2251	21.28	+ 1.7 / + 0.5	5	C19 H30 O2

Figure S10. HRMS spectrum of compound 2

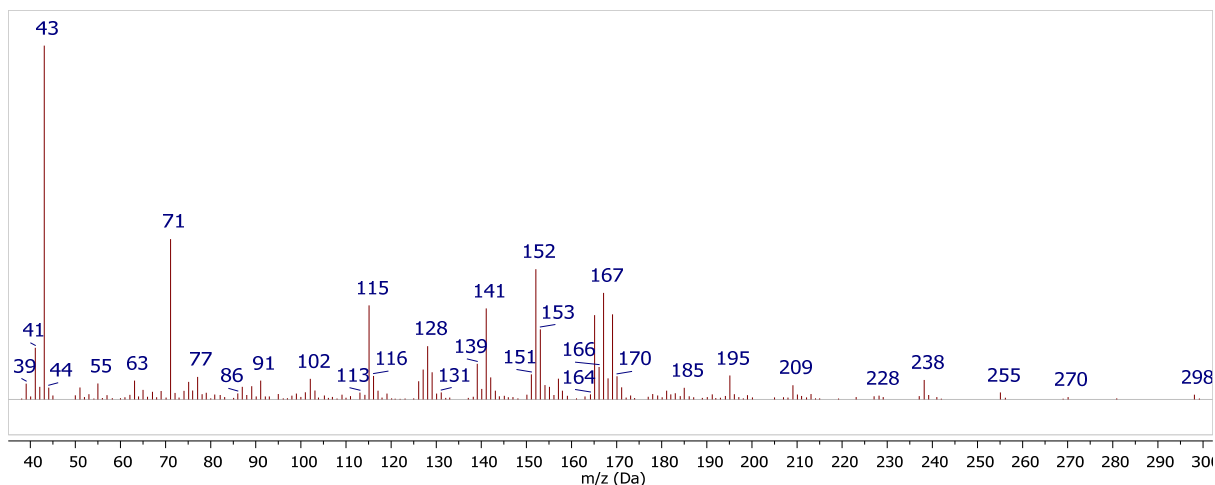


Figure S11. EI (70 eV) mass spectrum of compound **6**

[Mass Spectrum]

Data: MS-BFS-6 Date: 28-Oct-2023 15:28

Instrument : MStation

Sample: -

Note: -

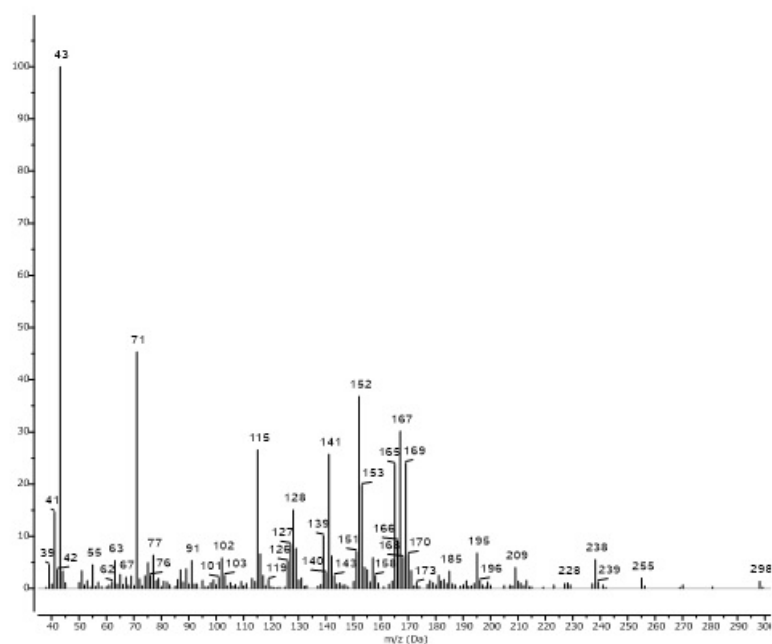
Inlet : Direct Ion Mode : EI+

Spectrum Type : Normal Ion [MF linear]

RT : 7.76 min Scan# : 49 Temp : -

BP: m/z 43 Int. : 75544

Output m/z range : 35 to 305 Cut Level : 0.00%



Data: MS-BFS-6 Date: 28-Oct-2023 17:53

Instrument : MStation

Sample: -

Note: -

Inlet : Direct Ion Mode : EI+

RT : 7.76 min Scan# : 49

Elements : C 19/0, H 22/0, O 3/0

Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 50mmu if m/z > 50

Unsaturation (U.S.) : -0.5 -20.0

	Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
1	298.1574	2.1	+ 1.7 / + 0.5	9	C19 H22 O3

Figure S12. HRMS spectrum of compound **6**

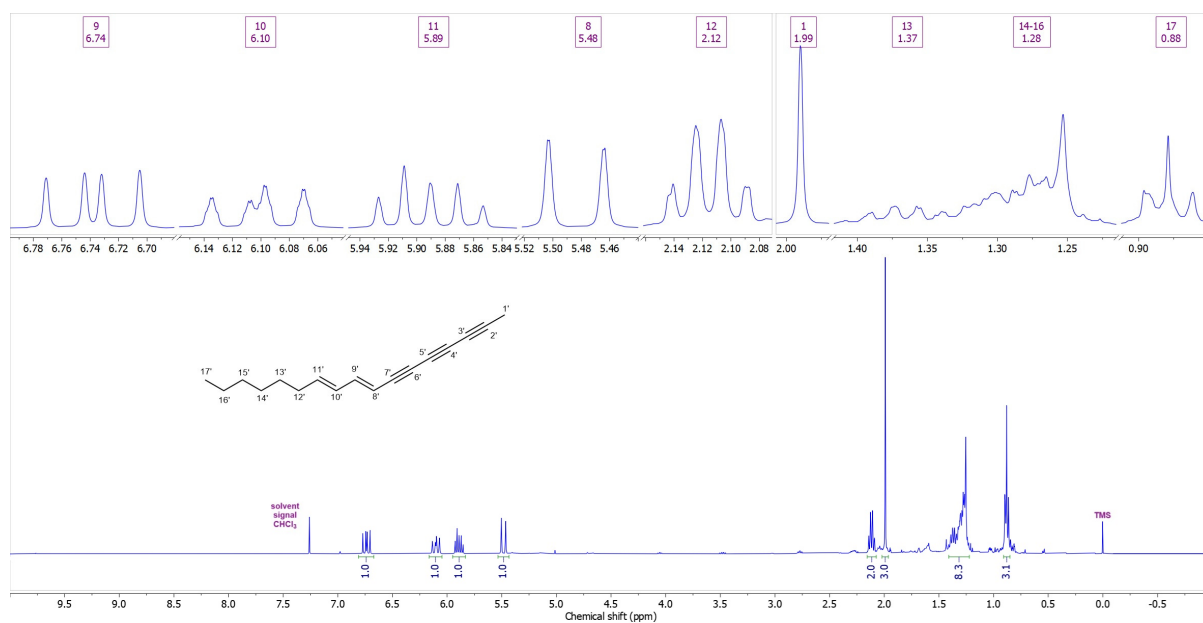


Figure S13. ^1H NMR (400 MHz, CDCl_3) spectrum of compound **8**

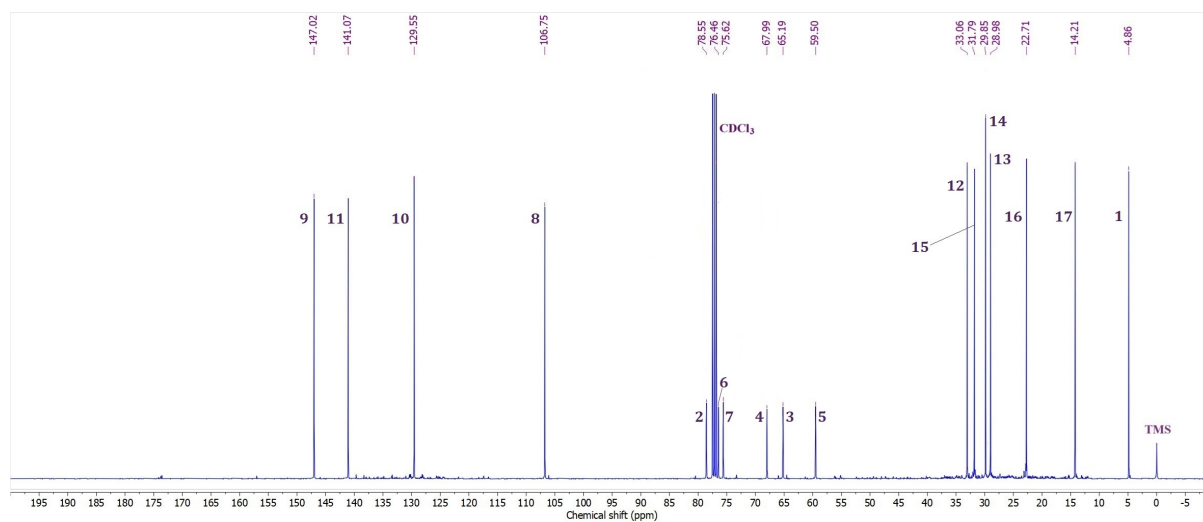


Figure S14. ^{13}C NMR (100.6 MHz, CDCl_3) spectrum of compound **8**

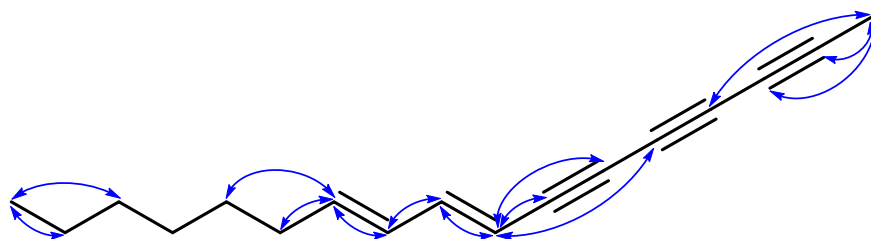


Figure S15. Important HMBC interactions of compound **8**

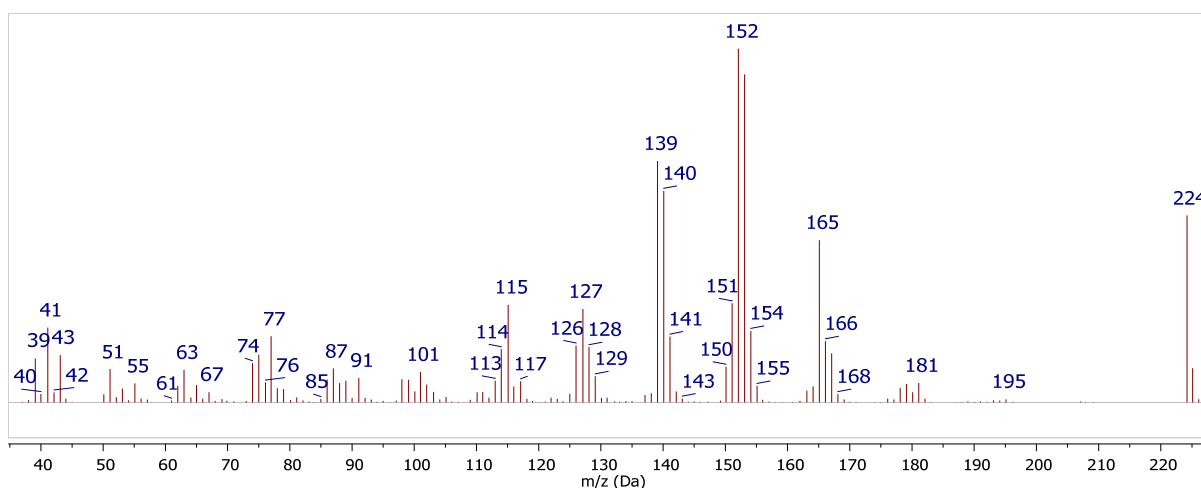


Figure S16. EI (70 eV) mass spectrum of compound 8

[Mass Spectrum]

Data: MS-BFG-8 Date: 15-Aug-2023 18:37

Instrument : MStation

Sample: -

Note: -

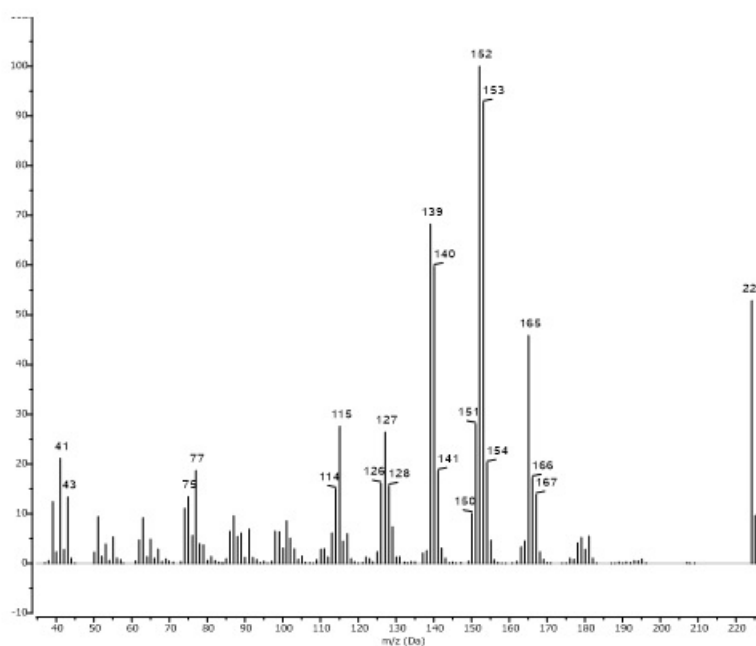
Inlet : Direct Ion Mode : EI+

Spectrum Type : Normal Ion [MF linear]

RT : 6.01 min Scan# : 36 Temp : -

BP: m/z 152 Int. : 162752

Output m/z range : 35 to 230 Cut Level : 0.00%



Data: MS-BFG-8 Date: 15-Aug-2023 21:09

Instrument : MStation

Sample: -

Note: -

Inlet : Direct Ion Mode : EI+

RT : 6.01 min Scan# : 36

Elements : C 17/0, H 20/0

Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 50mmu if m/z > 50

Unsaturation (U.S.) : -0.5 -20.0

	Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
1	224.1570	52.90	+ 2.2 / + 0.5	8	C17 H20

Figure S17. HRMS spectrum of compound 8

Table S2. ^1H (400 MHz) and ^{13}C (100.6 MHz) NMR spectral data (in CDCl_3) of compound **8** (NMR parameters are derived from manual iterative full spin analysis)

Position	δ_{H} (m, J^1 (Hz), Integral)	δ_{C} (ppm)
1	1.9898 (s, 3 H)	4.86
2	/	78.55
3	/	65.19
4	/	67.99
5	/	59.50
6	/	76.46
7	/	75.62
8	5.4839 (ddd, $^3J_{8,9} = 15.6$, $^4J_{8,10} = 0.7$, $^5J_{8,11} = 0.6$, 1 H)	106.75
9	6.7374 (ddd, $^3J_{8,9} = 15.6$, $^3J_{9,10} = 10.9$, $^4J_{9,11} = 0.6$, 1 H)	147.02
10	6.1009 (ddtd, $^3J_{10,11} = 15.1$, $^3J_{9,10} = 10.9$, $^4J_{10,12} = 1.3$, $^4J_{8',10'} = 0.7$, 1 H)	129.55
11	5.8917 (dtdd, $^3J_{10,11} = 15.1$, $^3J_{11,12} = 7.2$, $^4J_{9,11} = ^5J_{8',11'} = 0.6$, 1 H)	141.07
12	2.1153 (m, $^3J_{11,12} = 7.2$, $^4J_{10,12} = 1.3$, 2 H)	33.06
13 ²	1.3723 (overlapped multiplets, 2 H)	28.98
14 ²	1.3070 (overlapped multiplets, 2 H)	29.85
15 ²	1.2550 (overlapped multiplets, 2 H)	31.79
16 ²	1.2880 (m, $^3J_{16,17} = 7.2$, 2 H)	22.71
17	0.8790 (t, $^3J_{16,17} = 7.2$, 3 H)	14.21

¹ Coupling constant values were initially inferred from ^1H homoselective decoupling NMR experiments and afterward refined through a manual iterative full spin analysis. For details, cf. Experimental part. ² Severely overlapping multiplets; the signals were not simulated.

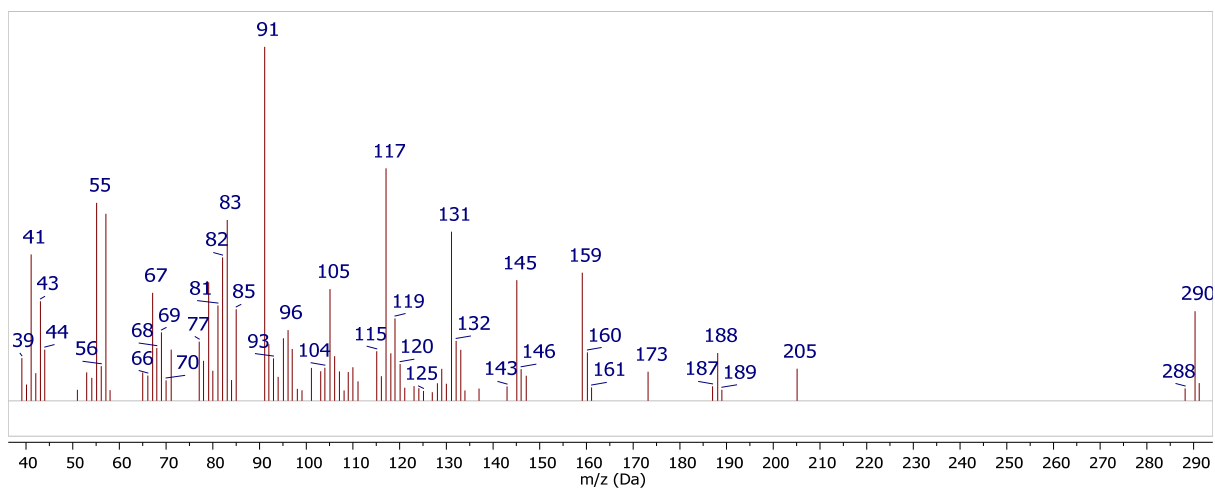


Figure S18. EI (70 eV) mass spectrum of praealtaester B

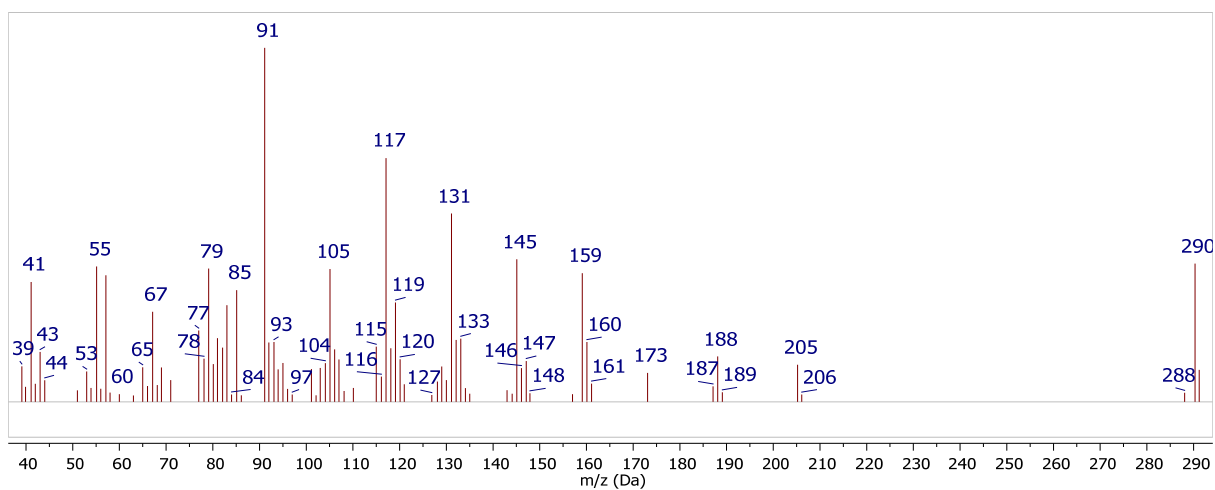


Figure S19. EI (70 eV) mass spectrum of praealtaester B stereoisomer 1

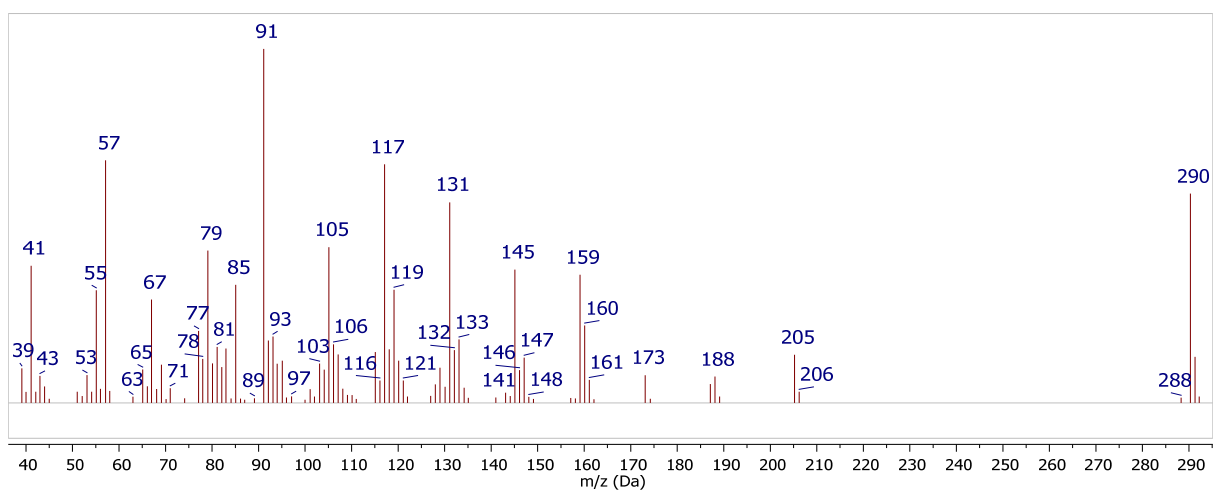


Figure S20. EI (70 eV) mass spectrum of praealtaester B stereoisomer 2

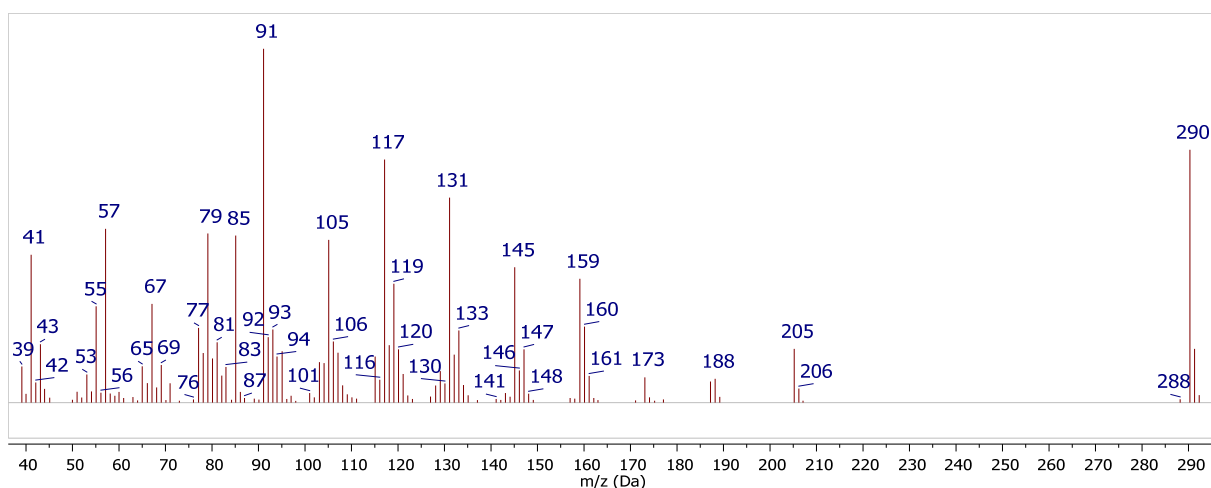


Figure S21. EI (70 eV) mass spectrum of praealtaester B stereoisomer 3

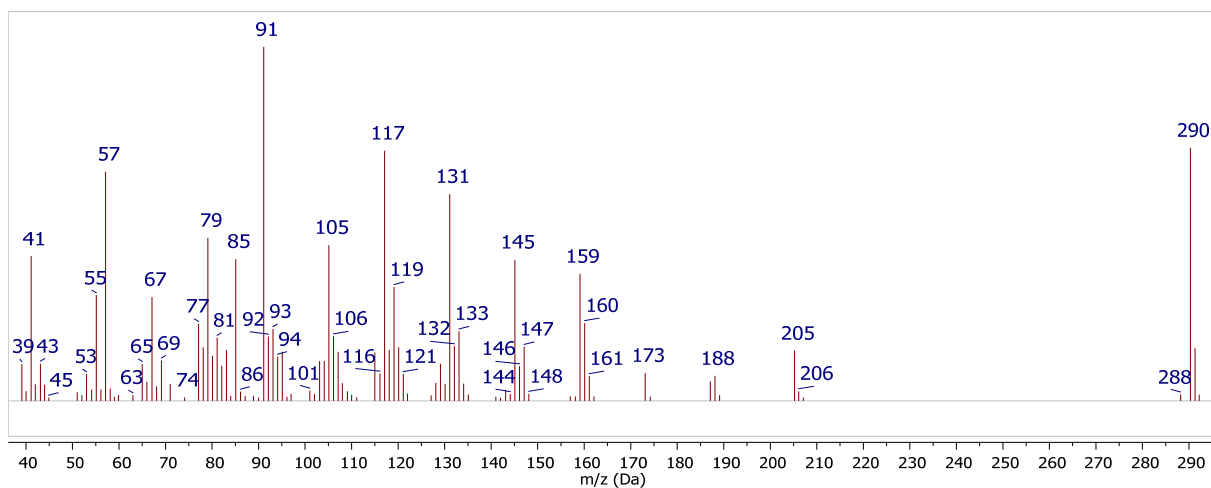


Figure S22. EI (70 eV) mass spectrum of praealtaester B stereoisomer 4

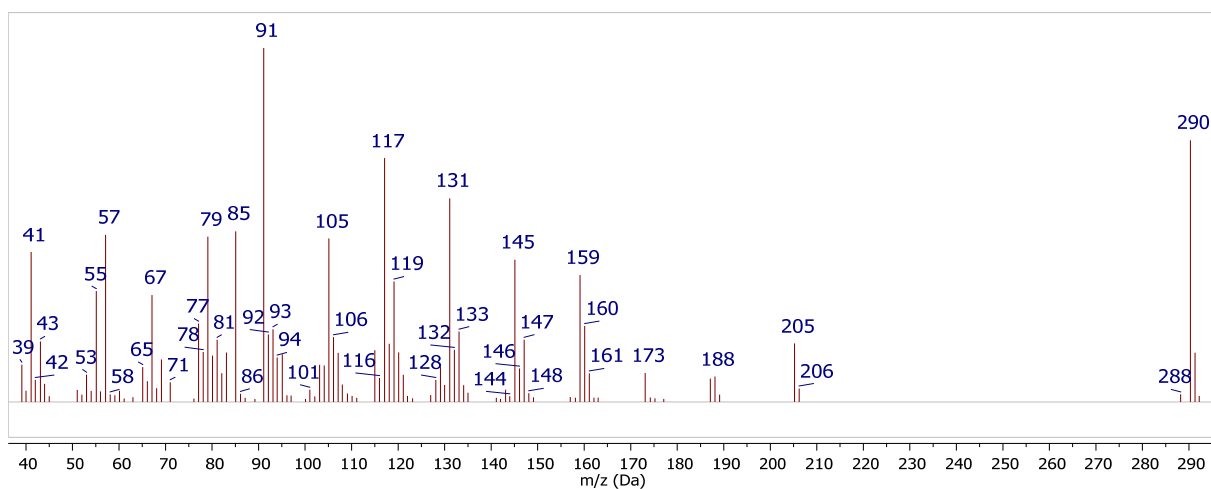


Figure S23. EI (70 eV) mass spectrum of praealtaester B stereoisomer 5

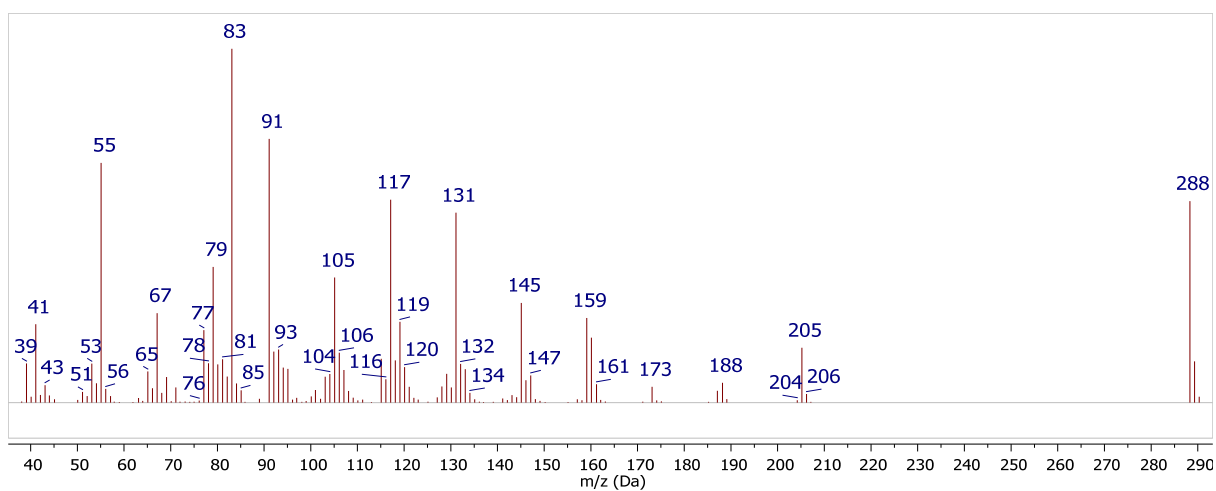


Figure S24. EI (70 eV) mass spectrum of compound 1 stereoisomer 1

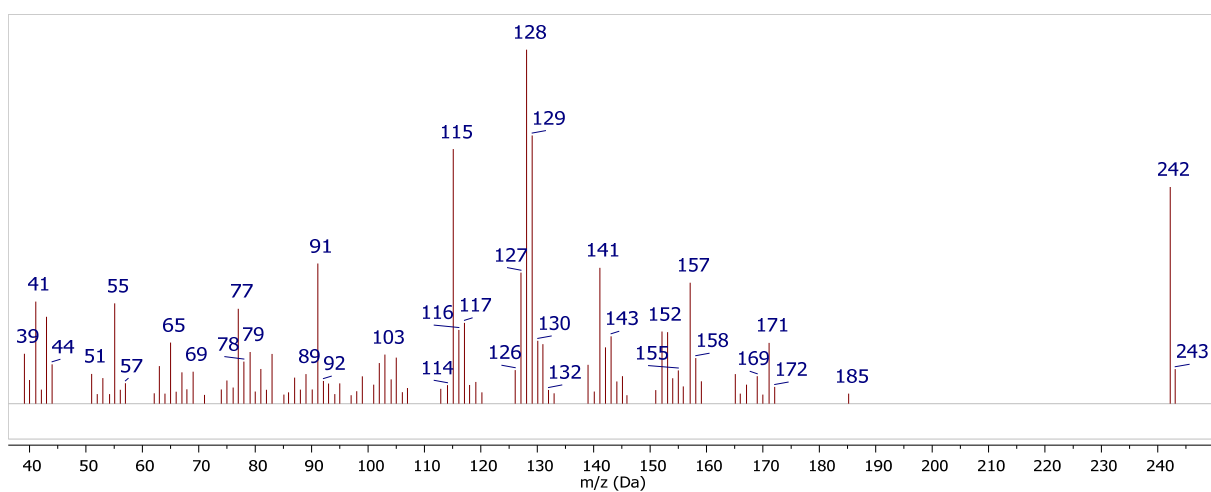


Figure S25. EI (70 eV) mass spectrum of compound 15 stereoisomer 1

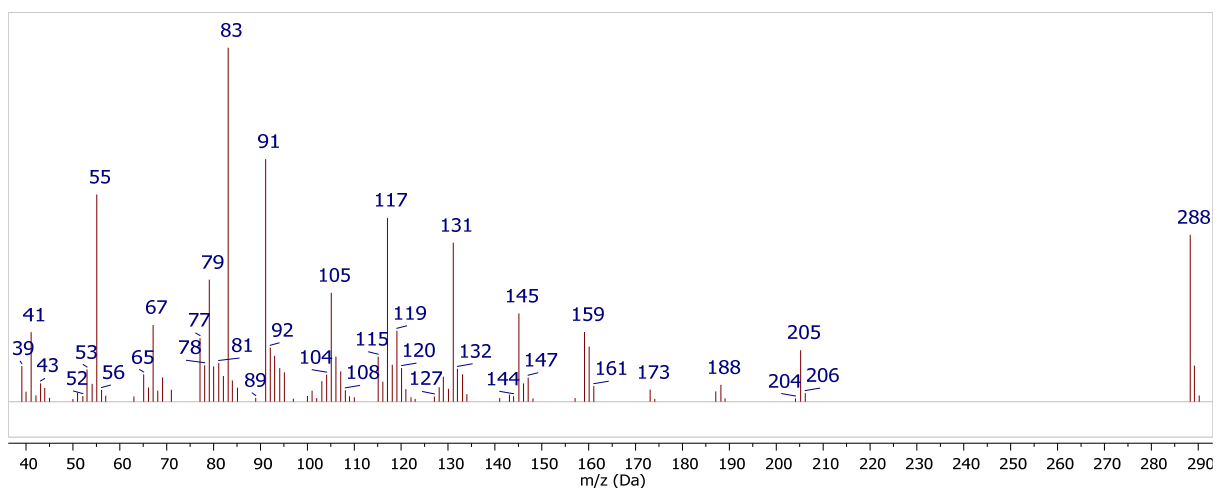


Figure S26. EI (70 eV) mass spectrum of compound 1 stereoisomer 2

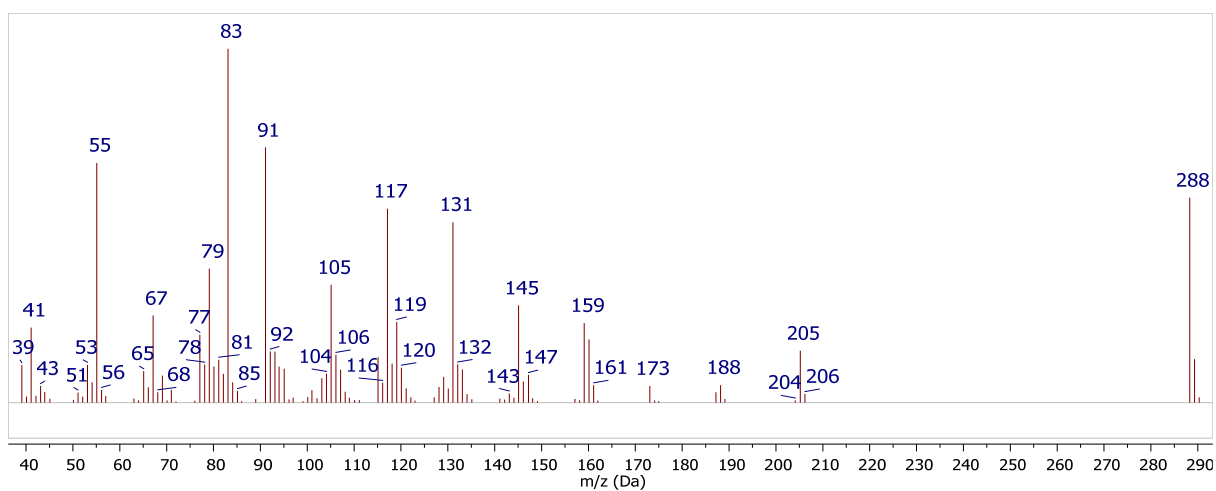


Figure S27. EI (70 eV) mass spectrum of compound 1 stereoisomer 3

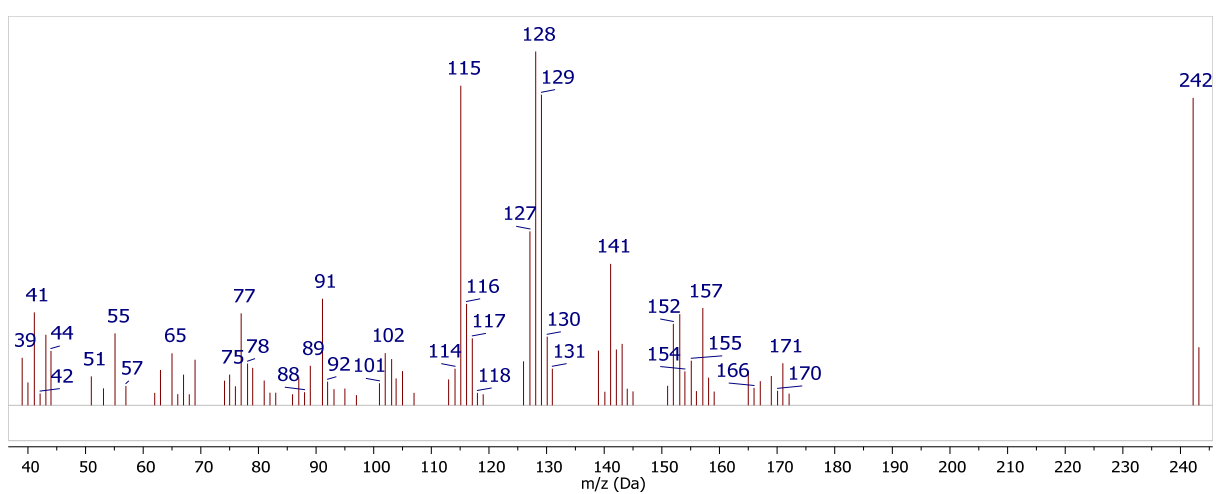


Figure S28. EI (70 eV) mass spectrum of compound 15 stereoisomer 2

Table S3. ^1H (400 MHz) and ^{13}C (100.6 MHz) NMR spectral data (in CDCl_3) of compound 5 (NMR parameters are derived from manual iterative full spin analysis)

Position	δ_{H} (m, J^1 (Hz), Integral)	δ_{C} (ppm)
1	/	168.38
2	/	128.90
3	6.8454 (qq, $^3J_{3,4} = 7.1$, $^4J_{3,5} = 1.5$, 1 H)	136.91
4	1.7862 (dq, $^3J_{3,4} = 7.1$, $^5J_{4,5} = 1.2$, 3 H)	14.43
5	1.8314 (dq, $^4J_{3,5} = 1.5$, $^5J_{4,5} = 1.2$, 3 H)	12.15
1'a	4.1138 (dd, $^3J_{1'a,2'b} = ^3J_{1'a,2'a} = 7.0$, 1 H)	64.67
1'b	4.1138 (dd, $^3J_{1'b,2'a} = ^3J_{1'b,2'b} = 7.0$, 1 H)	
2'a	1.6729 (dddd, $^3J_{2'a,3'b} = 9.0$, $^3J_{1'b,2'a} = ^3J_{1'a,2'a} = 7.0$, $^3J_{2'a,3'a} = 5.0$, 1 H)	28.81
2'b	1.6729 (dddd, $^3J_{2'b,3'a} = 9.0$, $^3J_{1'a,2'b} = ^3J_{1'b,2'b} = 7.0$, $^3J_{2'b,3'b} = 5.0$, 1 H)	
3'	1.36 (overlapped multiplets, 1 H)	26.13
4'-7'	1.25-1.40 (overlapped multiplets, 8 H)	28.32- 29.50
8'	2.17 (overlapped multiplets, 2 H)	27.94
9'	5.3927 (dtd, $^3J_{9',10'} = 10.6$, $^3J_{8',9'} = 7.7$, $^4J_{9',11'} = -0.8$, 1 H)	131.99
10'	5.9904 (ddtd, $^3J_{10',11'} = 11.4$, $^3J_{9',10'} = 10.6$, $^4J_{8',10'} = -1.4$, $^4J_{10',12'} = -0.7$, 1 H)	128.80
11'	6.3785 (dddd, $^3J_{11',12'} = 14.8$, $^3J_{10',11'} = 11.4$, $^4J_{9',11'} = -0.8$, $^4J_{11',13'} = -0.7$, 1 H)	126.07
12'	6.1630 (dddd, $^3J_{11',12'} = 14.8$, $^3J_{12',13'} = 10.8$, $^4J_{12',14'} = ^4J_{10',12'} = -0.7$, 1 H)	132.91
13'	6.1050 (ddtd, $^3J_{13',14'} = 15.0$, $^3J_{12',13'} = 10.8$, $^4J_{13',15'} = 1.3$, $^4J_{11',13'} = -0.7$, 1 H)	130.66
14'	5.6992 (dtd, $^3J_{13',14'} = 15.0$, $^3J_{14',15'} = 6.9$, $^4J_{12',14'} = -0.7$, 1 H)	135.29
15'	2.1050 (overlapped multiplets, 2 H)	32.62
16'	1.36 (overlapped multiplets, 2H)	31.58
17'	1.33 (overlapped multiplets, 2H)	22.35
18'	0.8942 (t, $^3J_{17',18'} = 3$ H)	14.06

¹Coupling constant values were initially inferred from ^1H homoselective decoupling NMR experiments and afterward refined through a manual iterative full spin analysis. For details, cf. Experimental part. ² Severely overlapping multiplets; the signals were not simulated.