Supporting Information

Biosynthesis-driven Structure-Activity Relationship Study of a Polyketide

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1. Crystallography data

SI Table I - Data collection and refinement statistics (molecular replacement).

	PremonensinA•PDE6δ
Data collection	
Space group	*P3221*
Cell dimensions	
a, *b*, *c* (Å)	55.97, 55.97, 115.56
α, β, γ (°)	90.00, 90.00, 120.00
Resolution (Å)	27.99-2.40
R_{sym} or R_{merge}	10.3 (46.6)
$I / \sigma I$	23.89 (6.99)
Completeness (%)	99.9 (100.0)
Redundancy	18.7 (19.27)
Refinement	
Resolution (Å)	2.40
No. reflections	8227
R_{work} / R_{free}	19.1/24.9
No. atoms	
Protein	1182
Ligand/ion	41
Water	63
B-factors	
Protein	34.46
Ligand/ion	73.44
Water	36.75
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (°)	1.549

Numbers in parentheses represent the highest-resolution bin.

2. Feeding experiments

The protocol used was previously reported by Bravo-Rodriguez *et al*. [1]

3. Fermentation of premonensin derivatives

The protocol used was previously reported in Kushnir *et al*. [2] For skipmon isolation (compounds **7** and **8**) the fermentation was carried out for 4 days

4. General procedure for the extraction and purification of premonensin derivatives

The protocol used was previously reported in Bravo-Rodriguez *et al* [1]

	16-Propyl premonensin	Premonensin	
Fermentation volume	1.8 L	1.8 L	
Compound	**1**	**A**	**B**
Isolated amount (mg)	5.0	1.2	2.1

	16-Butyl-Premonensin	Premonensin	
Fermentation volume	1.8 L	1.8 L	
Compound	**2**	**A**	**B**
Isolated amount (mg)	3.8	20.0	5.0

	Skipmon		Shunt product (after module 6)	
Fermentation volume	7.0 L		7.0 L	
Compound	**7**	**8**	**A**	**B**
Isolated amount (mg)	14.5	38.5	9.8	3.2

5. High-Resolution MS-results

High resolution measurement of the compound masses were performed as previously reported by Kushnir *et al*. [2]

16-propylpremonensin (1)

$[M+NH_4]^+$ theor. $C_{36}H_{64}O_6N$: 606.4728, exp.: 606.4724

16-butyl premonensin (2)

$[M+NH_4]^+$ theor. $C_{37}H_{66}O_6N$: 620.4885, exp.: 620,4875

Skipmon B (8)

$[M+H]^+$theor. $C_{29}H_{49}O_6$: 493.35237, exp: 493.35219.

Skipmon A (7)

$[M+H]^+$theor. $C_{30}H_{51}O_6$: 507.36802, exp: 507.36795.

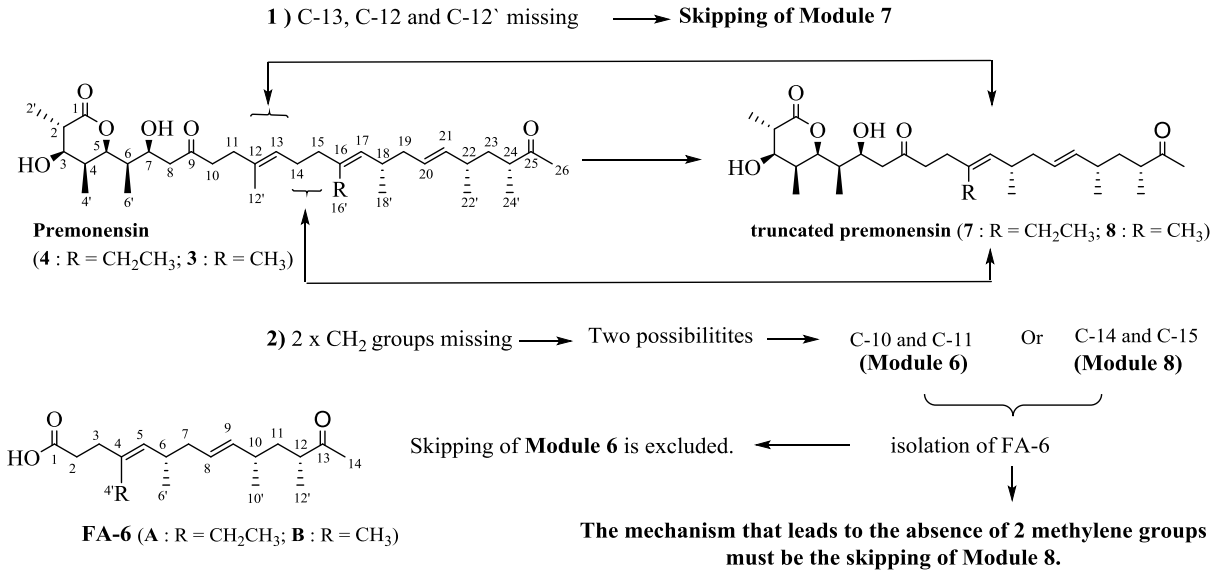
Shunt product 6 A

$[M+H]^+$theor. $C_{19}H_{33}O_3$: 309.24242, exp: 309.24279

Shunt product 6 B

$[M+H]^+$theor. $C_{18}H_{31}O_3$: 295.22677, exp: 295.22705.

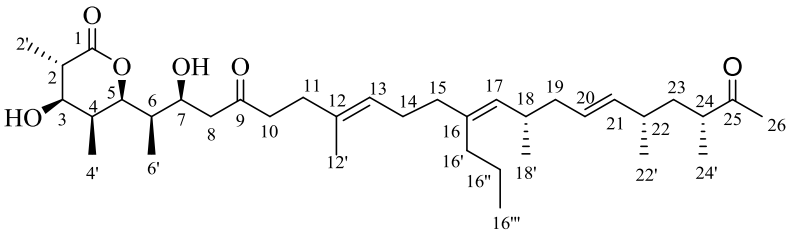
6. Proposed skipping mechanism based on structures of skipmon and the shunt product after module mon6



Scheme 1: The proposed mechanistic logic behind the skipping process of modules 7 and 8, based on NMR data of the isolated compounds.

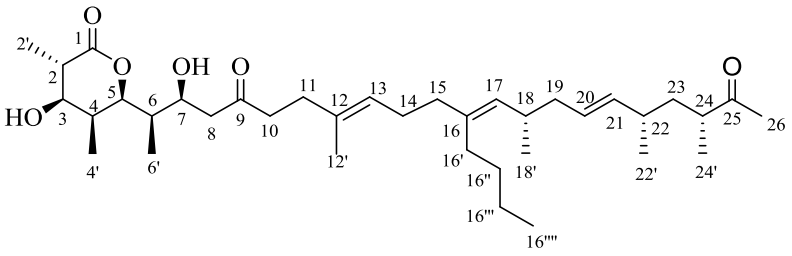
7. NMR analysis of premonensin derivatives

16-propylpremonensin (1)



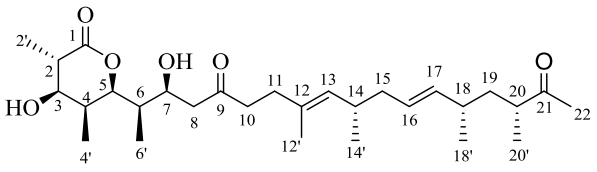
		(1) **($CDCl_3$, 400 MHz)**			
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13-C(ppm)
				C-1	174.3
H-2	2.47	m	---	**C-2**	39.8
H-2'	1.41	d	7.1	**C-2'**	14.6
H-3	3.87	dd	10.4, 4.3	**C-3**	73.9
H-4	2.47	m	---	**C-4**	35.0
H-4'	0.95	d	6.8	**C-4'**	5.00
H-5	4.22	m	---	**C-5**	81.7
H-6	1.74	m	---	**C-6**	38.6
H-6'	1.10	d	7.1	**C-6'**	10.0
H-7	4.22	m	---	**C-7**	66.0
H-8a	2.70	m	---	**C-8**	46.6
H-8b	2.54	m	---	**C-8**	46.6
				C-9	212.8
H-10	2.54	m	---	**C-10**	42.2
H-11	2.25	t	7.6	**C-11**	33.3
				C-12	133.0
H-12'	1.60	s	---	**C-12'**	16.3
H-13	5.11	m	---	**C-13**	125.4
H-14	2.06	m	---	**C-14**	26.8
H-15	1.93	m	---	**C-15**	36.7
				C-16	137.3
H-16'	1.93	m	---	**C-16'**	32.6
H-16''	1.35	m	---	**C-16''**	22.0
H-16'''	0.88	m	---	**C-16'''**	14.5
H-17	4.87	d	9.6	**C-17**	131.3
H-18	2.33	m	---	**C-18**	32.4
H-18'	0.88	m	---	**C-18'**	21.3
H-19	1.92	m	---	**C-19**	40.9
H-20	5.33	m	---	**C-20**	128.6
H-21	5.11	m	---	**C-21**	136.1
H-22	2.05	m	---	**C-22**	35.3
H-22'	0.97	d	6.8	**C-22'**	21.9
H-23a	1.58	m	---	**C-23**	39.8
H-23b	1.20	m	---	**C-23**	39.8
H-24	2.48	m	---	**C-24**	45.3
H-24'	1.02	d	6.8	**C-24'**	15.9
				C-25	214.0
H-26	2.12	s	---	**C-26**	28.3

16-Butyl premonensin (2)



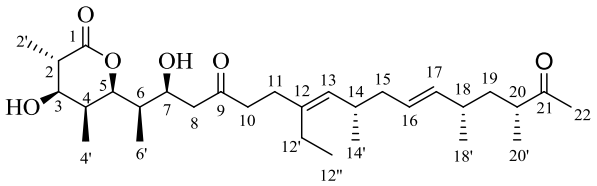
		(2) ($CDCl_3$, 400 MHz)			
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13-C(ppm)
				C-1	174.1
H-2	2.47	m	---	**C-2**	39.8
H-2'	1.41	d	7.1	**C-2'**	14.6
H-3	3.87	dd	10.4, 4.3	**C-3**	73.9
H-4	2.47	m	---	**C-4**	35.0
H-4'	0.95	d	6.8	**C-4'**	5.00
H-5	4.22	m	---	**C-5**	81.7
H-6	1.73	m	---	**C-6**	38.6
H-6'	1.10	d	6.6	**C-6'**	10.0
H-7	4.22	m	---	**C-7**	66.0
H-8a	2.70	m	---	**C-8**	46.8
H-8b	2.54	m	---	**C-8**	46.8
				C-9	212.7
H-10	2.54	m	---	**C-10**	42.1
H-11	2.25	t	7.8	**C-11**	33.2
				C-12	133.0
H-12'	1.60	s	---	**C-12'**	16.3
H-13	5.11	m	---	**C-13**	125.4
H-14	2.04	m	---	**C-14**	26.8
H-15	1.93	m	---	**C-15**	36.7
				C-16	137.5
H-16'	1.93	m	---	**C-16'**	30.1
H-16''	1.30	m	---	**C-16''**	31.0
H-16'''	1.30	m	---	**C-16'''**	23.0
H-16''''	0.90	m	---	**C-16''''**	14.3
H-17	4.86	d	9.6	**C-17**	131.1
H-18	2.37	m	---	**C-18**	32.4
H-18'	0.90	m	---	**C-18'**	21.3
H-19	1.93	m	---	**C-19**	40.8
H-20	5.32	m	---	**C-20**	128.6
H-21	5.11	m	---	**C-21**	136.1
H-22	2.04	m	---	**C-22**	35.3
H-22'	0.97	d	6.6	**C-22'**	21.9
H-23a	1.58	m	---	**C-23**	39.9
H-23b	1.20	m	---	**C-23**	39.9
H-24	2.47	m	---	**C-24**	45.3
H-24'	1.02	d	6.8	**C-24'**	15.9
				C-25	213.8
H-26	2.12	s	---	**C-26**	28.3

Skipmon B (8)



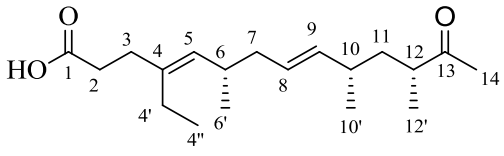
(8) (CDCl$_3$, 500 MHz)					
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13-C(ppm)
				C-1	174.1
H-2	2.47	m	-	**C-2**	40.10
H-2'	1.40	d	7.0	**C-2'**	14.7
H-3	3.84	dd	4.0, 10.0	**C-3**	74.1
H-4	2.44	m	-	**C-4**	35.3
H-4'	0.95	m	-	**C-4'**	5.0
H-5	4.21	m	-	**C-5**	81.6
H-6	1.74	m	-	**C-6**	38.8
H-6'	1.09	d	6.5	**C-6'**	10.0
H-7	4.19	m	-	**C-7**	66.3
H-8a	2.67	dd	10.0, 18.0	**C-8**	46.6
H-8b	2.55	m	-	**C-8**	46.6
				C-9	212.4
H-10	2.56	m	-	**C-10**	42.4
H-11	2.24	t	7.5	**C-11**	33.6
				C-12	131.8
H-12'	1.59	s	-	**C-12'**	16.4
H-13	4.89	d	9.0	**C-13**	131.9
H-14	2.34	m	-	**C-14**	32.9
H-14'	0.88	d	7.0	**C-14'**	20.7
H-15	1.89	m	-	**C-15**	40.6
H-16	5.28	dt	7.0, 14.5	**C-16**	128.0
H-17	5.13	dd	8.5, 15.0	**C-17**	136.7
H-18	2.09	m	-	**C-18**	35.4
H-18'	0.96	m	-	**C-18'**	21.8
H-19a	1.60	m	-	**C-19**	40.09
H-19b	1.21	ddd	5.0; 8.0; 3.5	**C-19**	40.09
H-20	2.50	m	-	**C-20**	45.5
H-20'	1.03	d	7.0	**C-20'**	16.2
				C-21	213.6
H-22	2.10	s	-	**C-22**	28.1

Skipmon A (7)



(7) (CDCl$_3$, 500 MHz)					
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13-C (ppm)
				C-1	174.0
H-2	2.47	m	-	**C-2**	40.12
H-2'	1.40	d	7.0	**C-2'**	14.7
H-3	3.84	dd	4.0; 10.0	**C-3**	74.1
H-4	2.44	m	-	**C-4**	35.3
H-4'	0.95	m	-	**C-4'**	5.0
H-5	4.22	m	-	**C-5**	81.6
H-6	1.75	m	-	**C-6**	38.8
H-6'	1.10	d	7.0	**C-6'**	10.0
H-7	4.19	m	-	**C-7**	66.3
H-8a	2.68	dd	10.0; 18.0	**C-8**	46.6
H-8b	2.55	m	-	**C-8**	46.6
				C-9	212.5
H-10	2.56	m	-	**C-10**	42.5
H-11	2.26	t	7.5	**C-11**	30.2
				C-12	137.7
H-12'	2.00	m	-	**C-12'**	23.6
H-12''	0.96	m	-	**C-12''**	13.6
H-13	4.82	d	9.5	**C-13**	131.3
H-14	2.37	m	-	**C-14**	32.6
H-14'	0.88	d	6.5	**C-14'**	21.2
H-15	1.89	dd	7.0; 13.5	**C-15**	40.6
H-16	5.29	m	-	**C-16**	128.1
H-17	5.13	dd	8.5; 15.0	**C-17**	136.7
H-18	2.09	m	-	**C-18**	35.4
H-18'	0.97	m	-	**C-18'**	21.8
H-19a	1.60	ddd	6.0; 8.5; 15.5	**C-19**	40.10
H-19b	1.22	ddd	5.0; 8.0; 13.5	**C-19**	40.10
H-20	2.50	m	-	**C-20**	45.4
H-20'	1.04	d	7.0	**C-20'**	16.3
				C-21	213.5
H-22	2.10	s	-	**C-22**	28.1

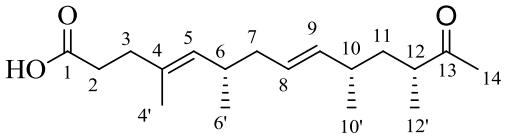
Shunt product 6 A



Shunt product 6 A
($CDCl_3$, 500 MHz)

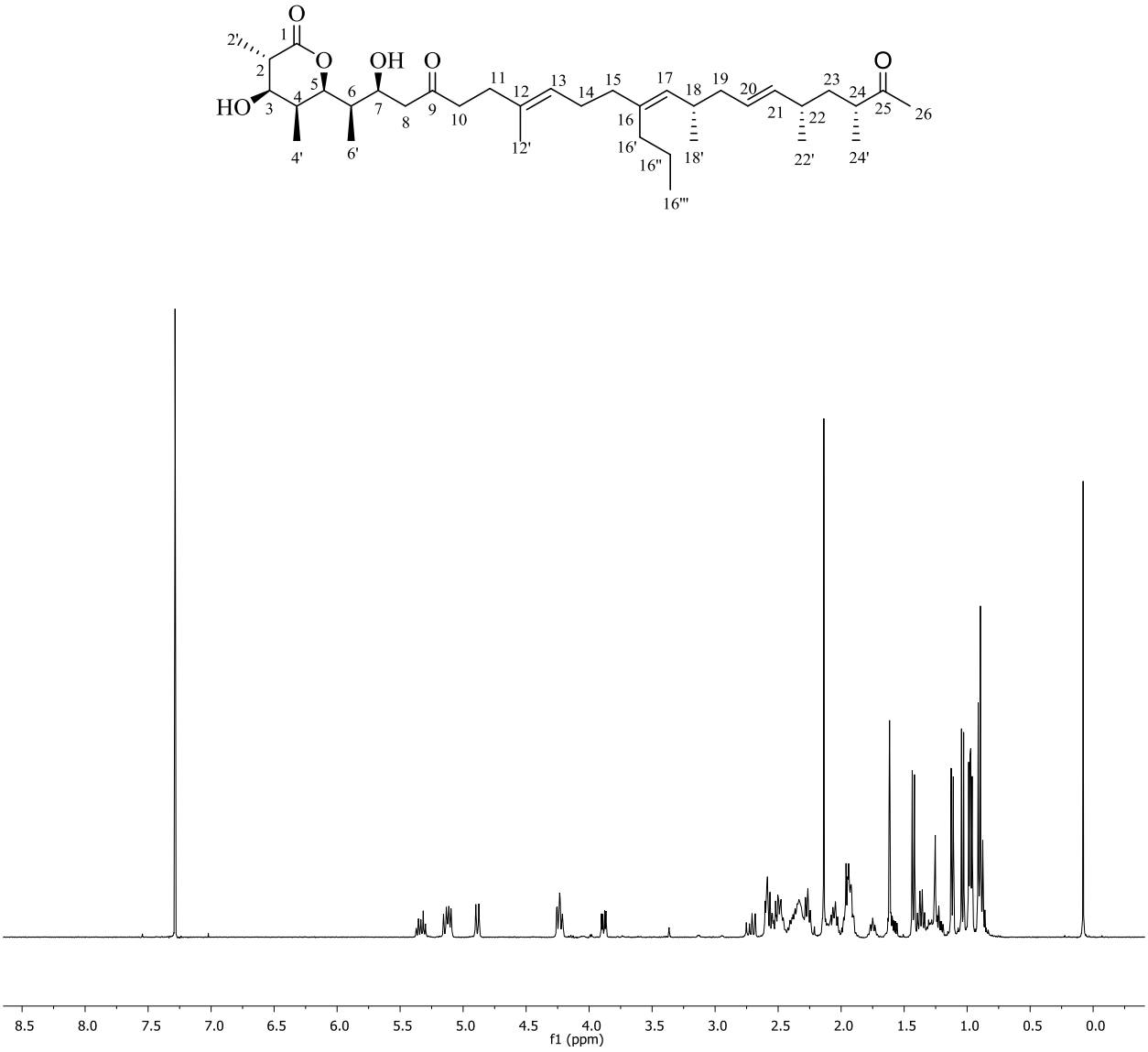
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13C
				C-1	178.3
H-2	2.45	m	-	**C-2**	33.1
H-3	2.31	t	7.5	**C-3**	31.2
			-	**C-4**	137.7
H-4'	2.02	m	-	**C-4'**	23.6
H-4''	0.97	m	-	**C-4''**	13.6
H-5	4.90	d	10.0	**C-5**	131.4
H-6	2.38	m	-	**C-6**	32.7
H-6'	0.90	d	6.5	**C-6'**	21.2
H-7	1.92	m	-	**C-7**	40.8
H-8	5.30	dt	7.0; 14.5	**C-8**	128.2
H-9	5.16	dd	8.5; 15.0	**C-9**	136.5
H-10	2.09	m	-	**C-10**	35.2
H-10'	0.96	d	7.0	**C-10'**	21.8
H-11a	1.61	ddd	6.0; 9.5; 14.0	**C-11**	40.1
H-11b	1.22	ddd	5.5; 8.0; 13.5	**C-11**	40.1
H-12	2.50	m	-	**C-12**	45.4
H-12'	1.04	d	7.0	**C-12'**	16.2
				C-13	213.5
H-14	2.11	s	-	**C-14**	28.1

Shunt product 6 B

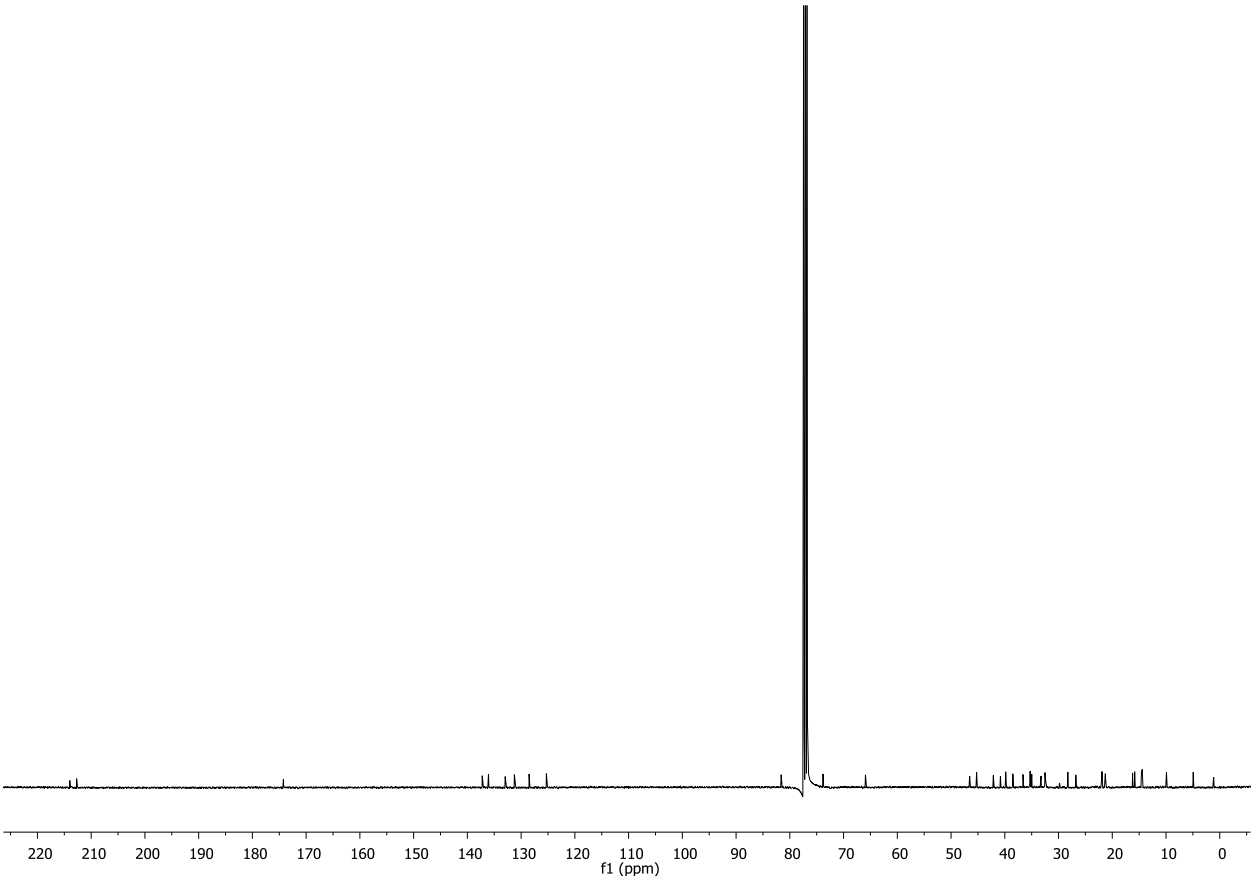


Shunt product 6 B (CDCl$_3$, 500 MHz)					
Protons	δ (ppm)	multiplicity	*J* (Hz)	**Carbons**	13C
			-	**C-1**	177.6
H-2	2.45	m	-	**C-2**	32.91
H-3	2.29	t	7.5	**C-3**	34.5
			-	**C-4**	131.7
H-4'	1.61	s	-	**C-4'**	16.3
H-5	4.96	d	9.0	**C-5**	131.9
H-6	2.37	m	-	**C-6**	32.9
H-6'	0.90	d	7.0	**C-6'**	20.8
H-7	1.92	m	-	**C-7**	40.6
H-8	5.31	dt	7.0; 14.5	**C-8**	128.2
H-9	5.15	dd	8.5; 15.0	**C-9**	136.5
H-10	2.09	m	-	**C-10**	35.2
H-10'	0.96	d	6.5	**C-10'**	21.8
H-11a	1.62	m	-	**C-11**	40.2
H-11b	1.22	ddd	5.0; 8.0; 13.5	**C-11**	40.2
H-12	2.50	m	-	**C-12**	45.4
H-12'	1.04	d	7.0	**C-12'**	16.2
			-	**C-13**	213.6
H-14	2.11	s	-	**C-14**	28.1

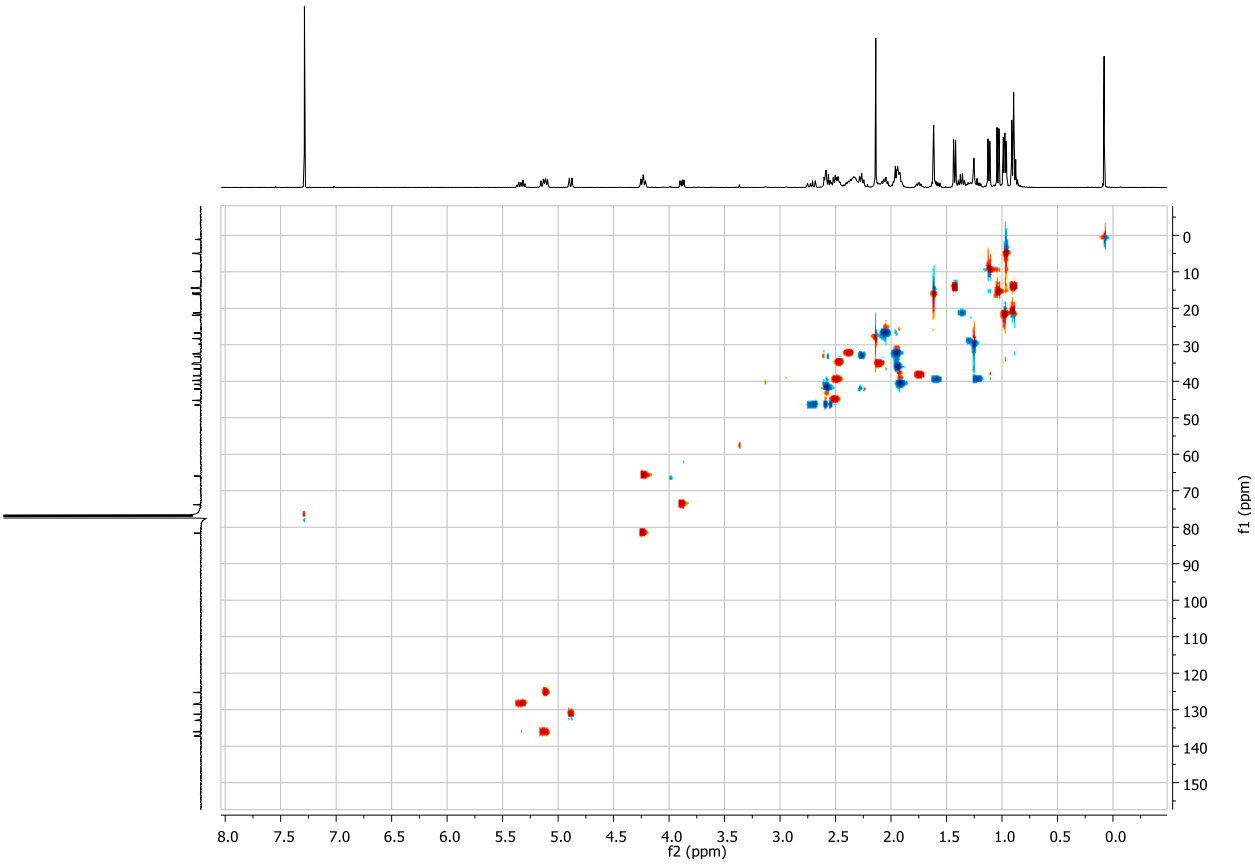
16-propylpremonensin (1)



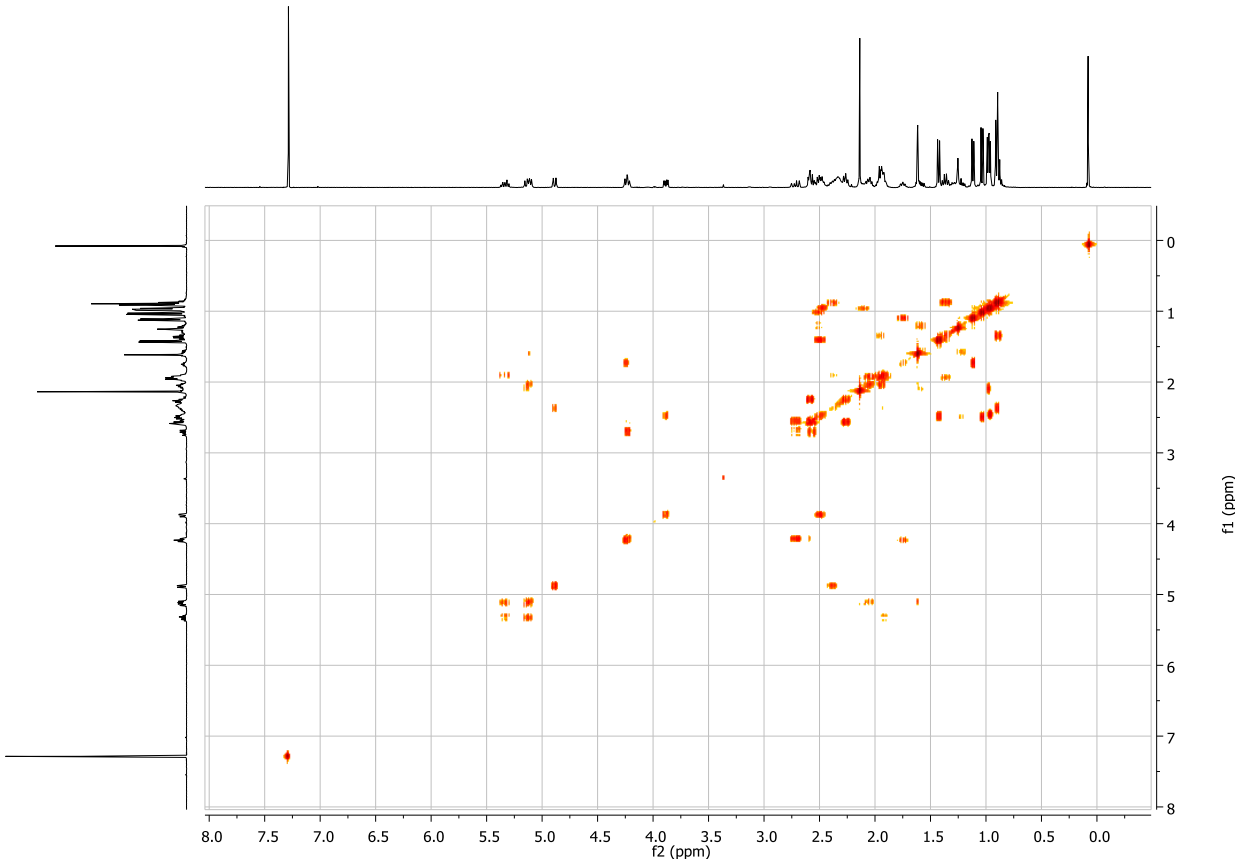
SI Figure 1: ^{1}H-NMR spectra of 16-propyl premonensin (**1**) in $CDCl_3$-d_1



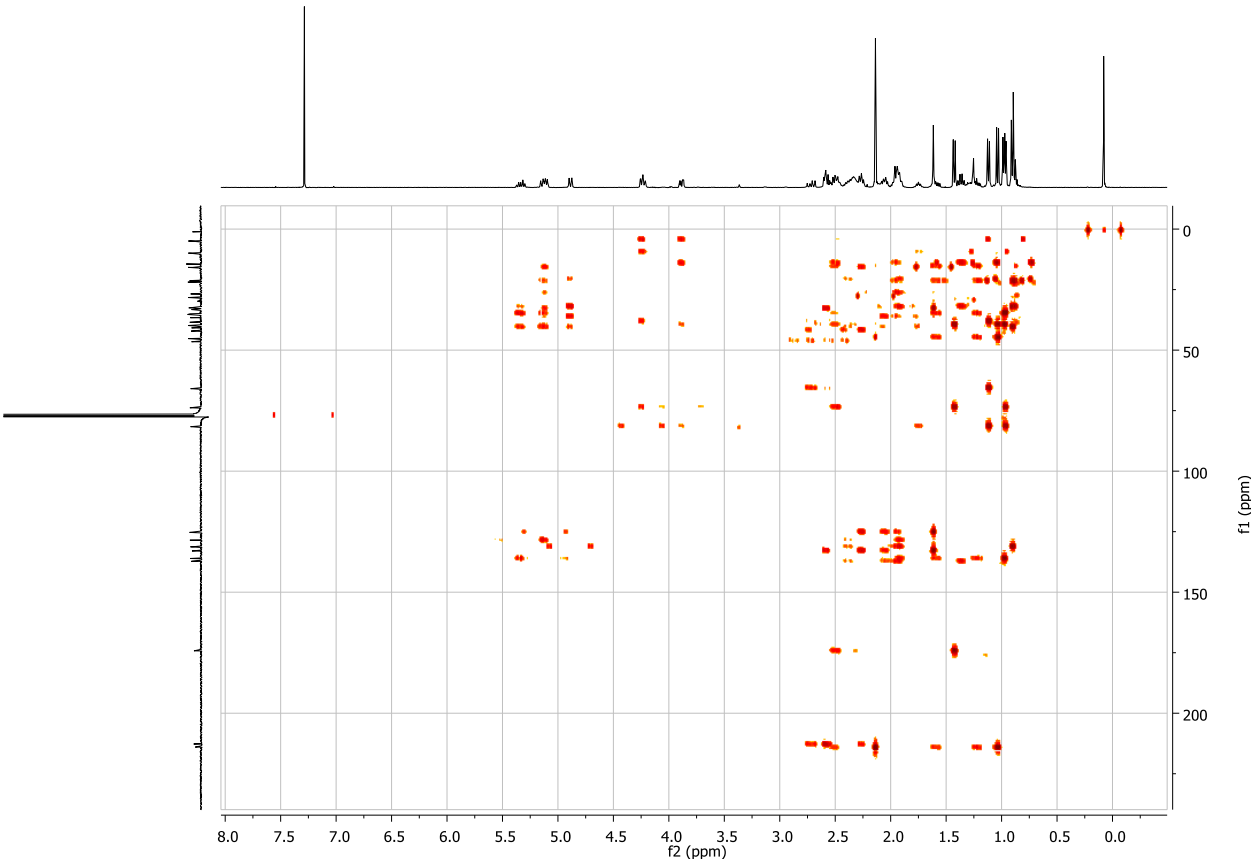
SI Figure 2: ^{13}C-NMR spectra of 16-propyl premonensin (**1**) in $CDCl_3$-d_1



SI Figure 3: spectra of 16-propyl premonensin (**1**) in $CDCl_3$-d_1

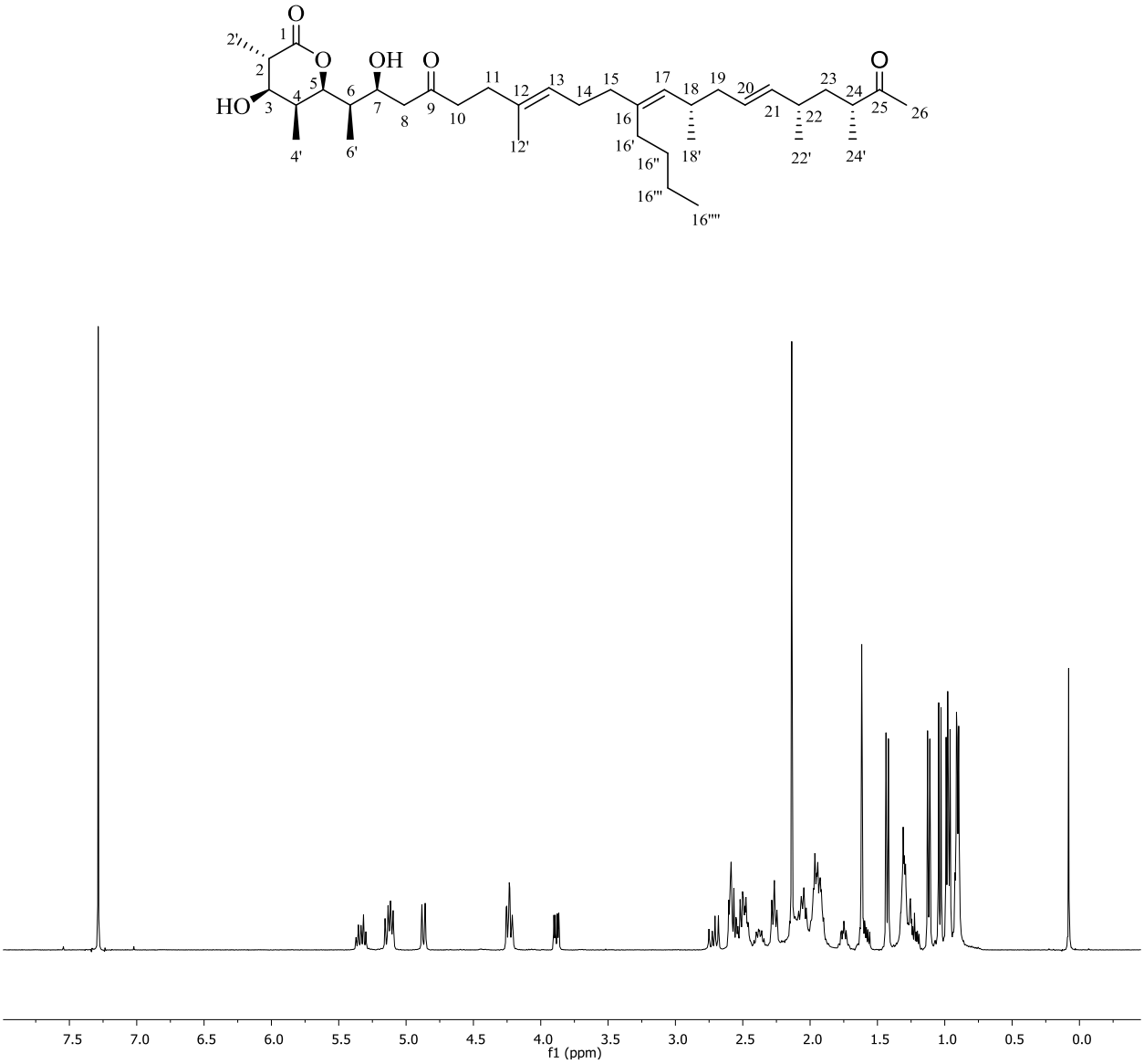


SI Figure 4: gCOSY spectra of 16-propyl premonensin (**1**) in $CDCl_3$-d_1

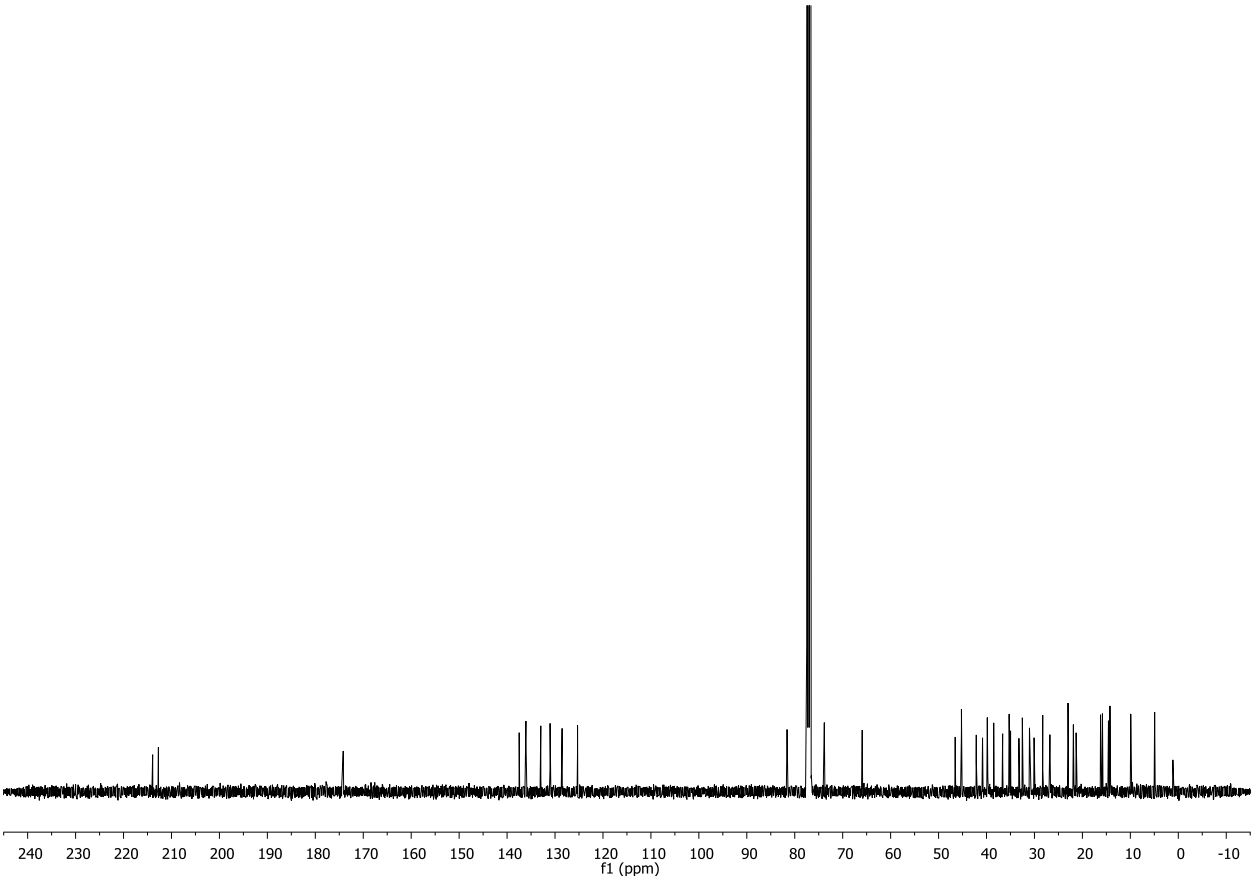


SI Figure 5: gHMBC spectra of 16-propyl premonensin (**1**) in $CDCl_3$-d_1

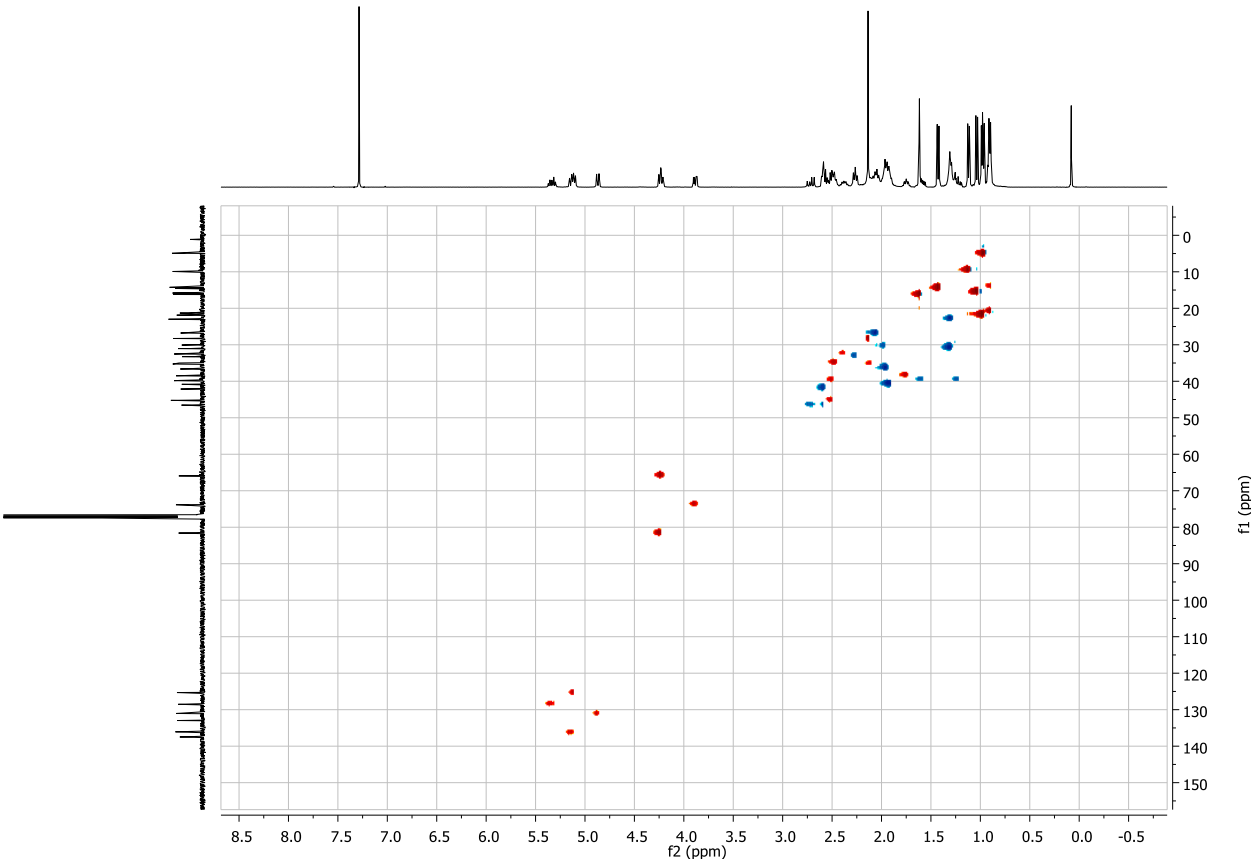
16-Butyl premonensin (2)



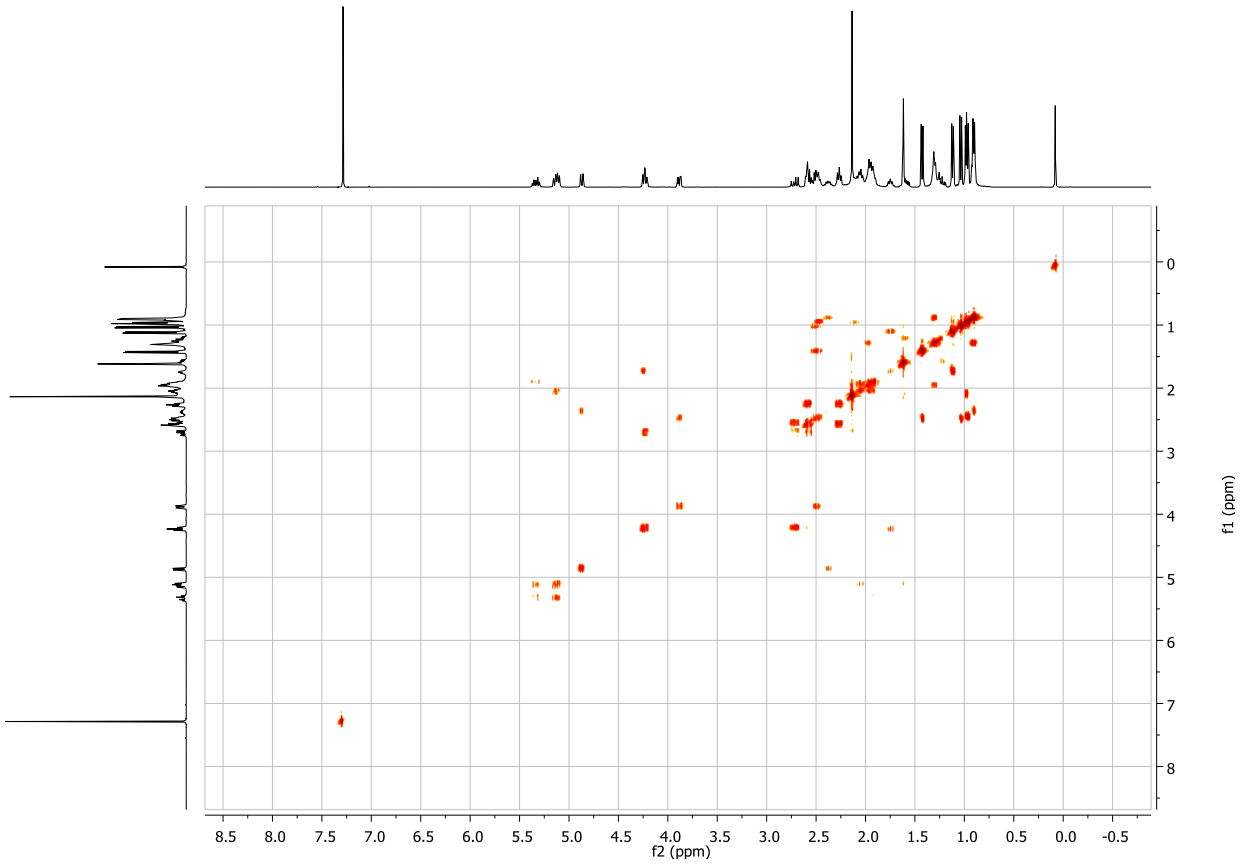
SI Figure 6: ^{1}H-NMR spectra of 16-butyl premonensin (**2**) in $CDCl_3$-d_1



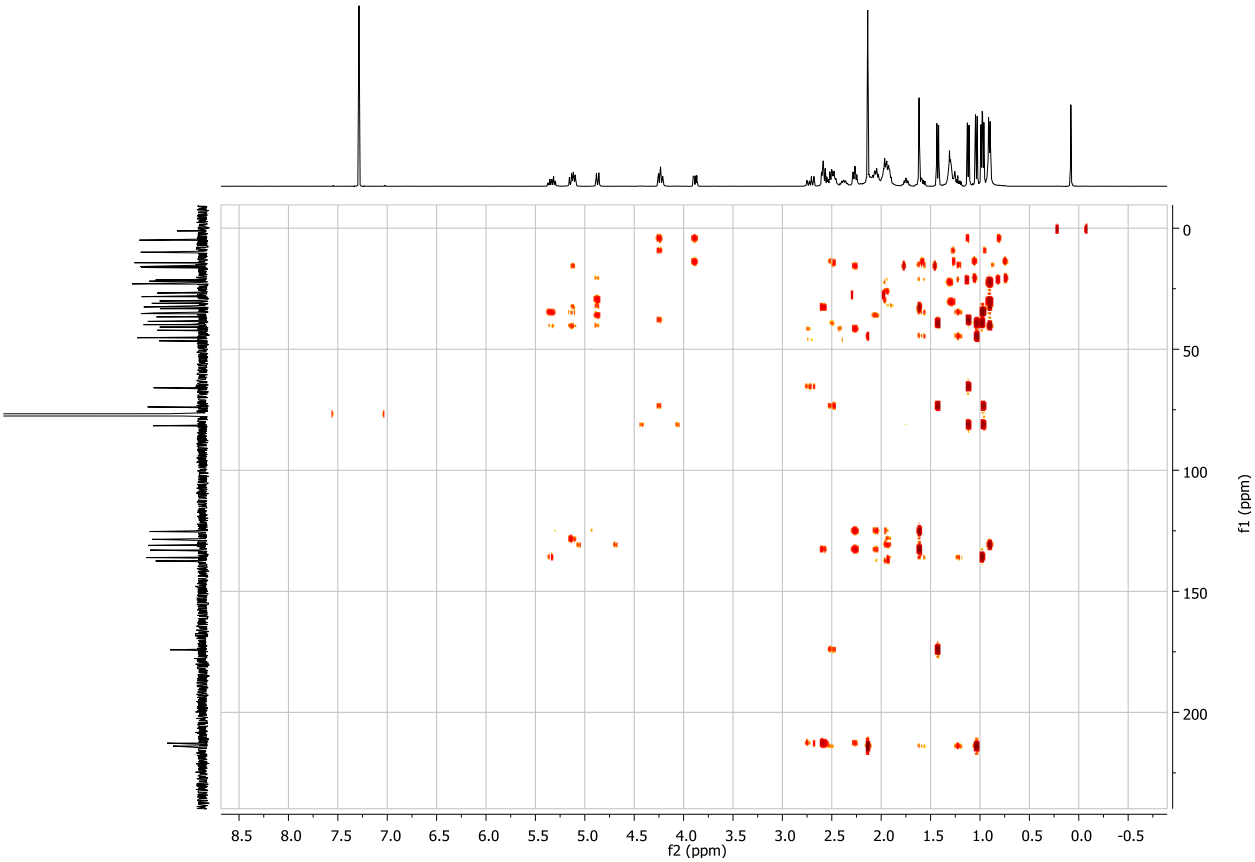
SI Figure 7: ^{13}C-NMR spectra of 16-butyl premonensin (**2**) in $CDCl_3$-d_1



SI Figure 8: gHSQC spectra of 16-butyl premonensin (**2**) in $CDCl_3$-d_1

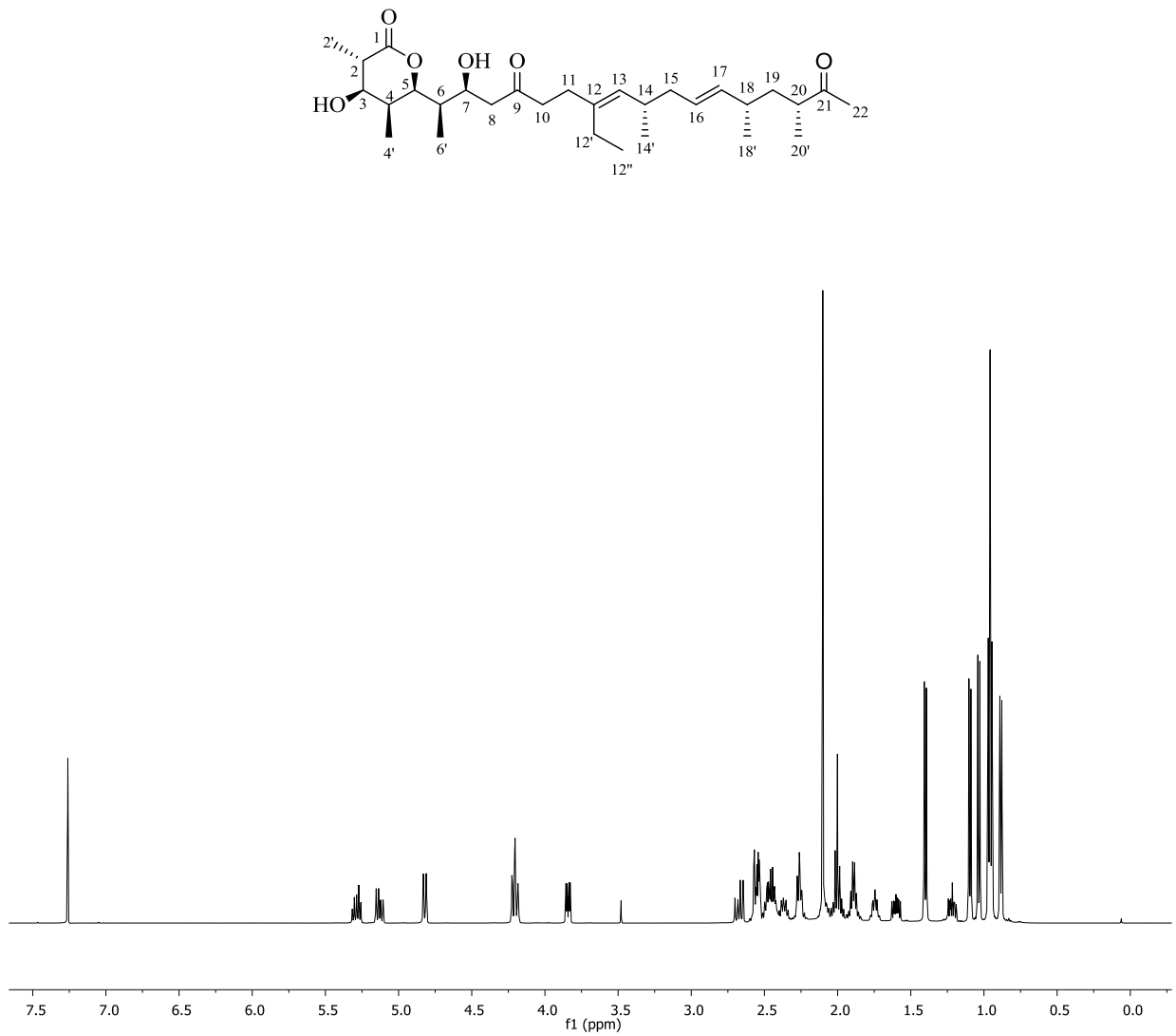


SI Figure 9: gCOSY spectra of 16-butyl premonensin (**2**) in $CDCl_3$-d_1

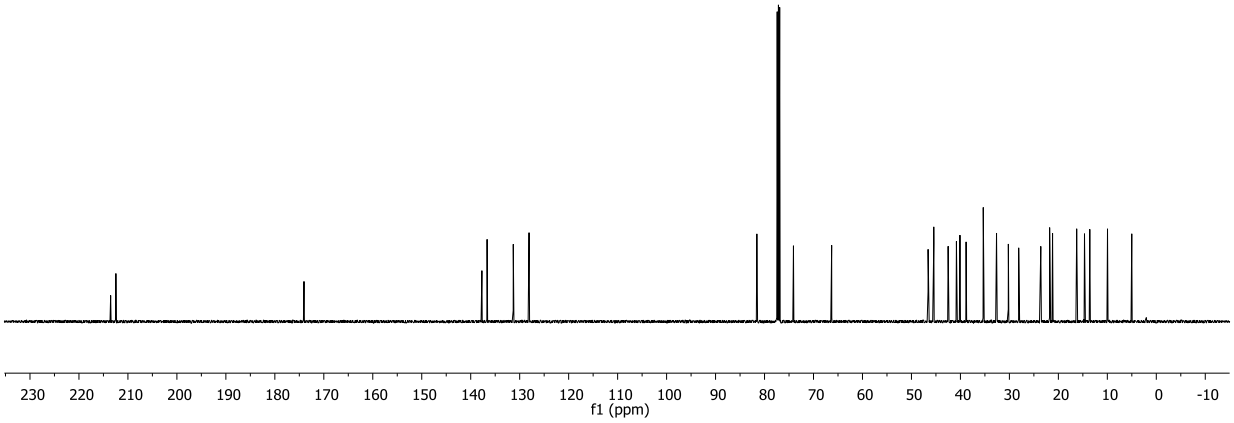


SI Figure 10: gHMBC spectra of 16-butyl premonensin (**2**) in $CDCl_3$-d_1

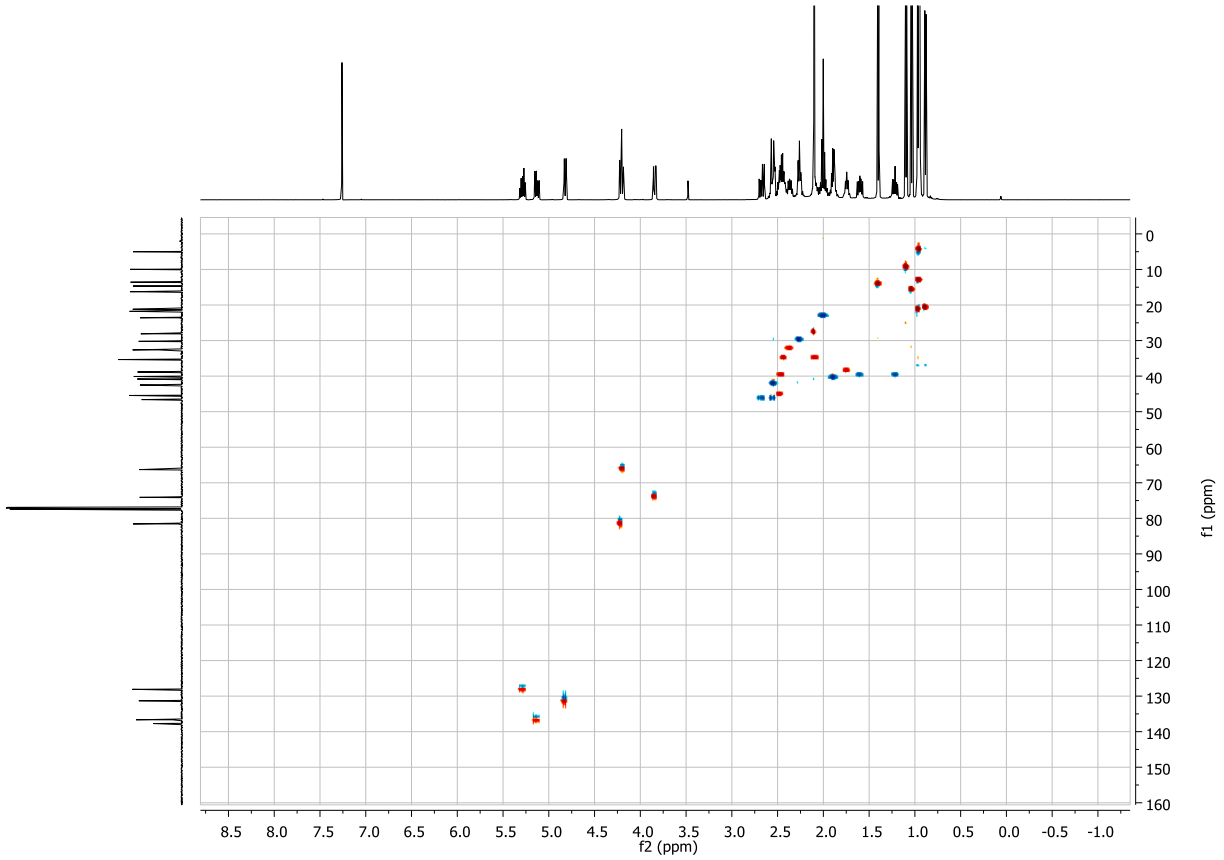
Skipmon A (7)



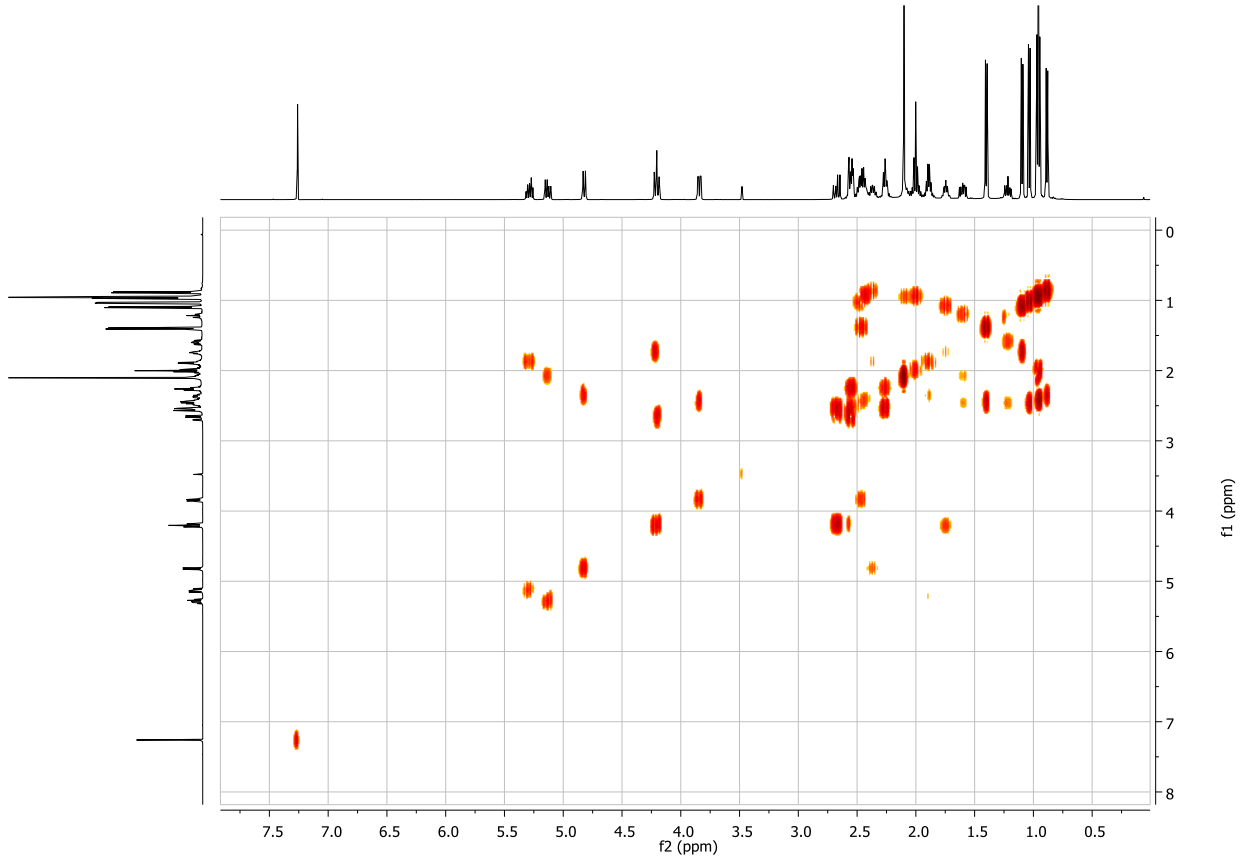
SI Figure 11: ^{1}H-NMR spectra of skipmon A (**7**) in $CDCl_3$-d_1



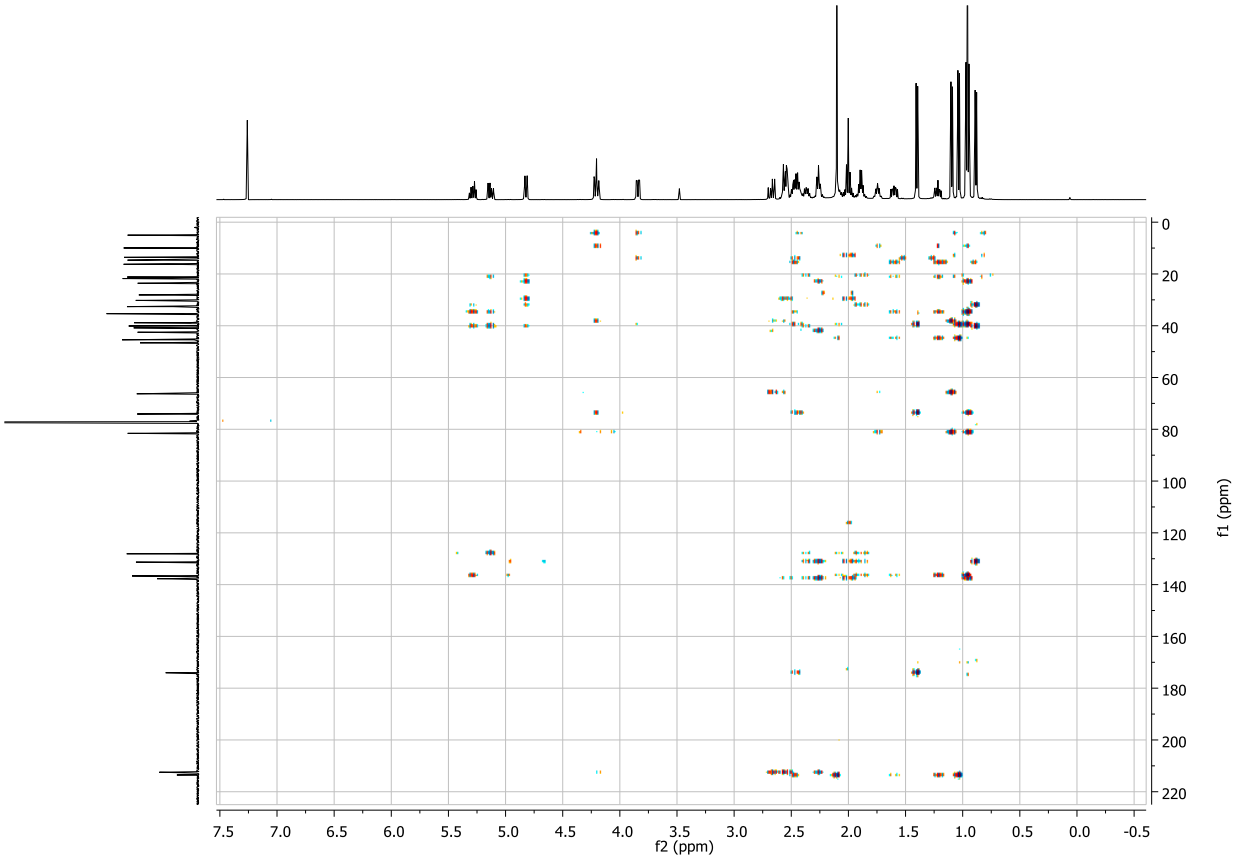
SI Figure 12: ^{13}C-NMR spectra of skipmon A (**7**) in $CDCl_3$-d_1



SI Figure 13: gHSQC spectra of skipmon A (**7**) in $CDCl_3$-d_1

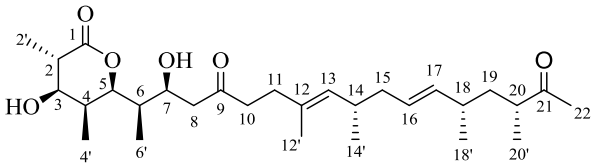


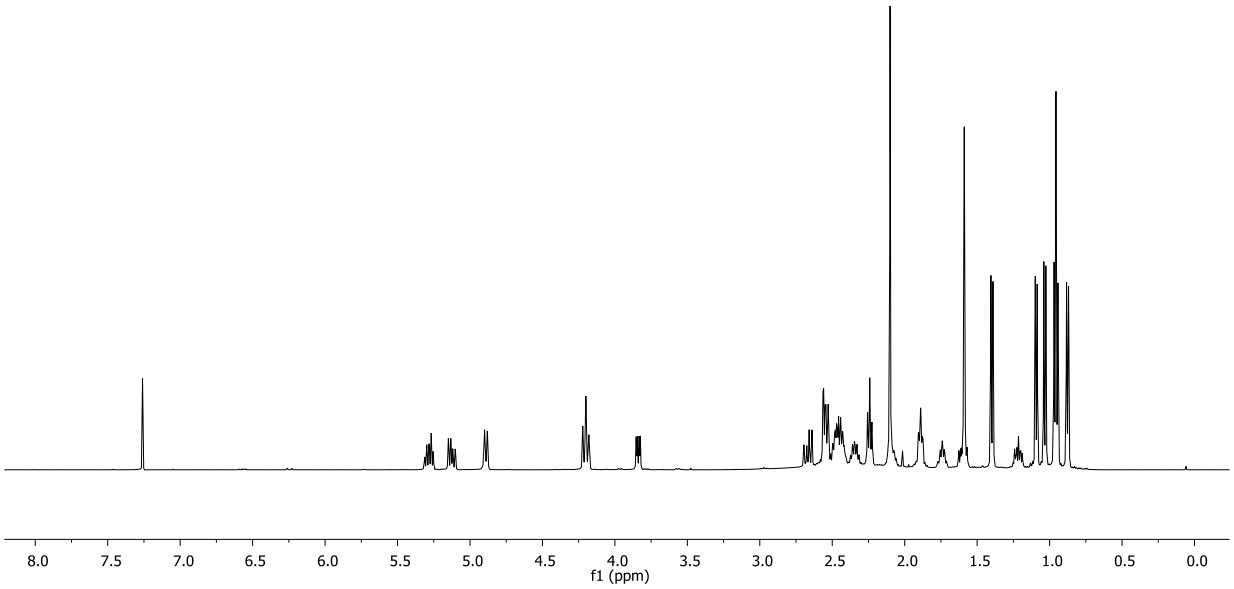
SI Figure 14: gCOSY spectra of skipmon A (**7**) in $CDCl_3$-d_1



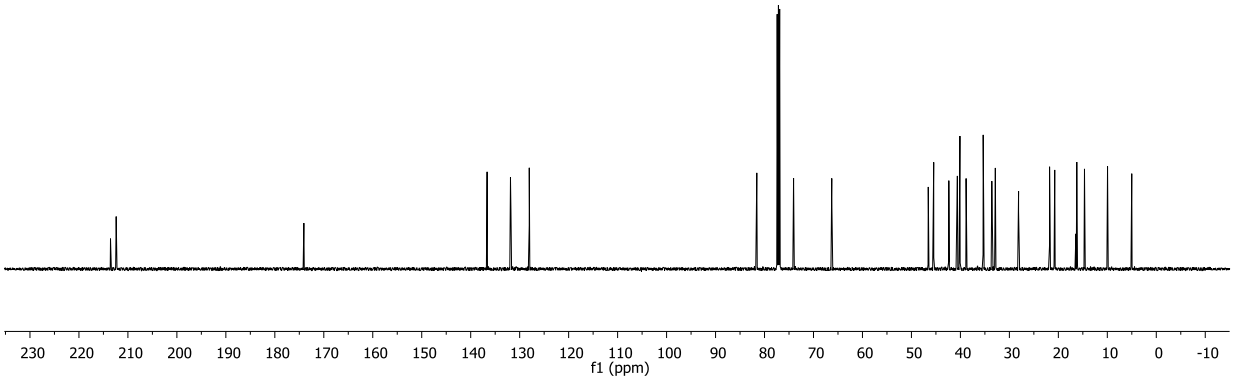
SI Figure 15: gHMBC spectra of skipmon A (**7**) in $CDCl_3$-d_1

Skipmon B (8)

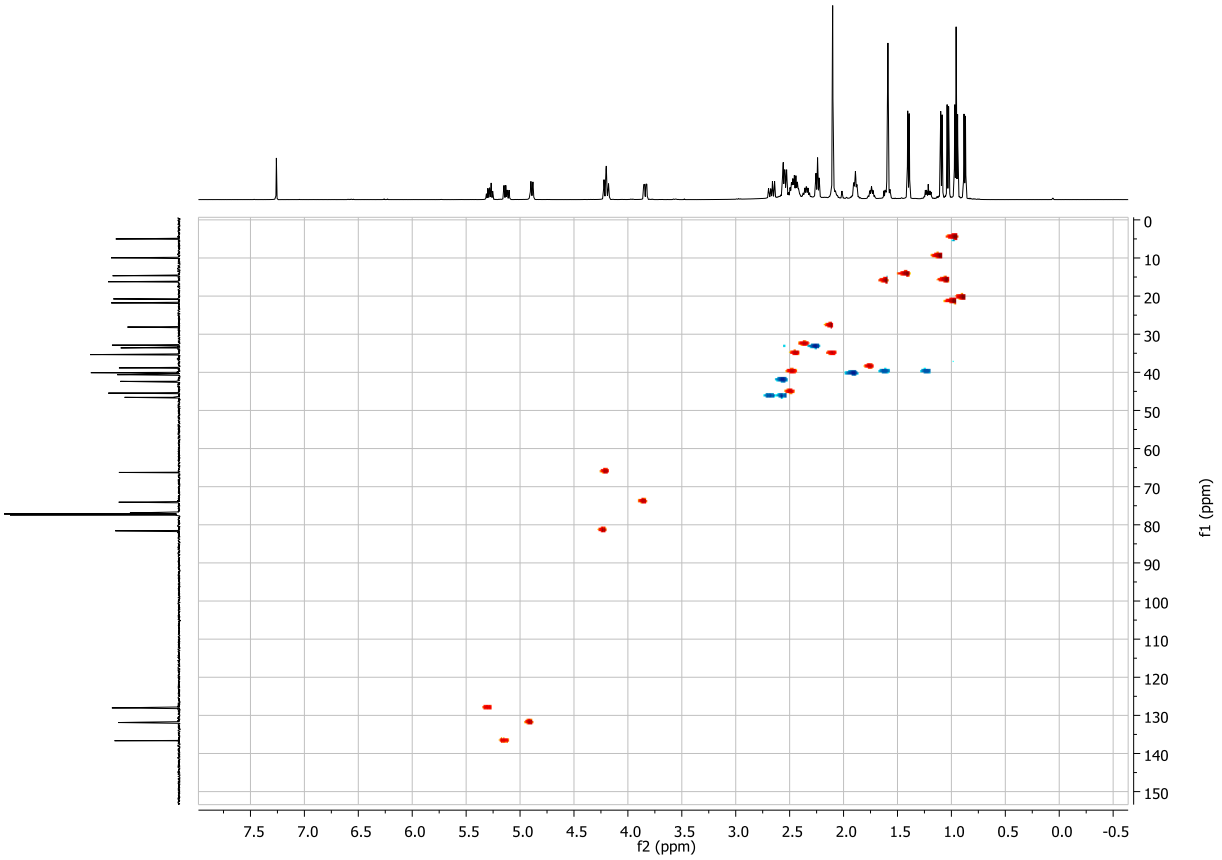




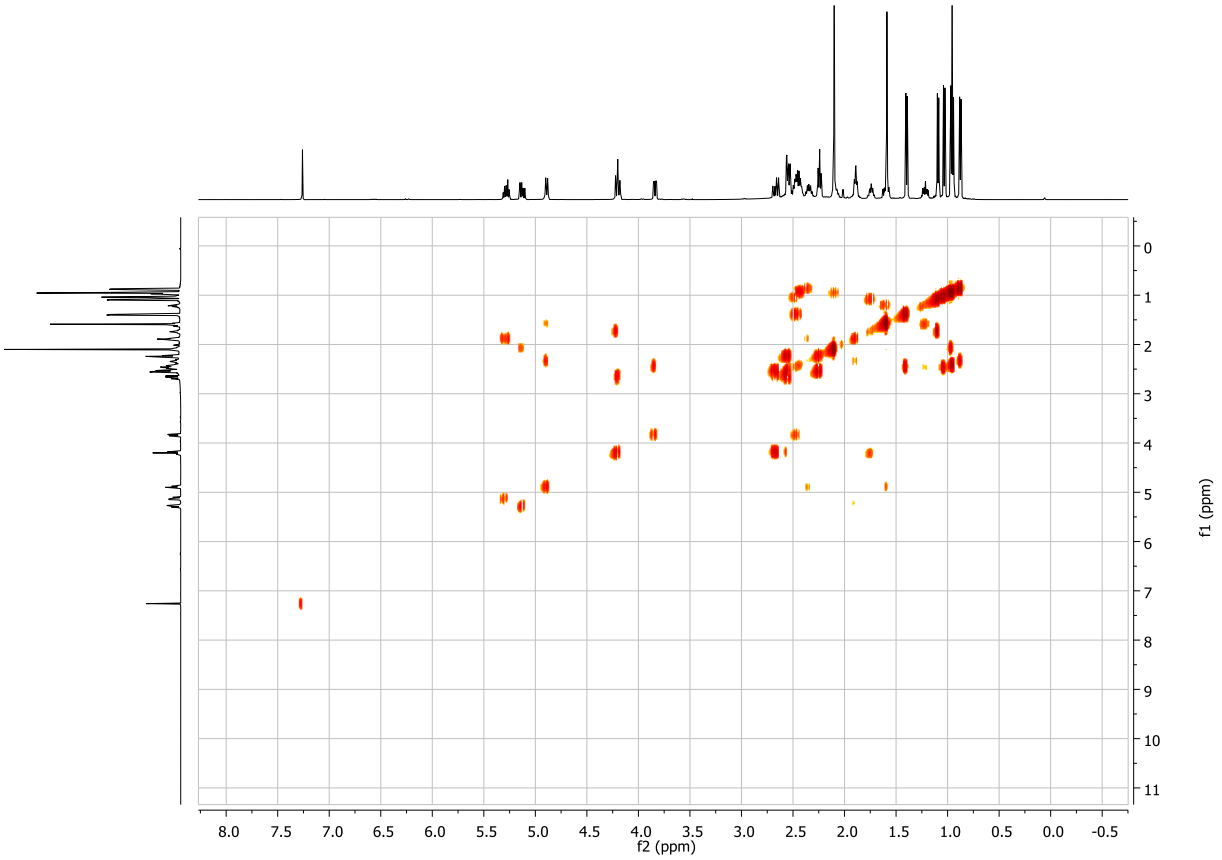
SI Figure 16: ^{1}H-NMR spectra of skipmon B (**8**) in $CDCl_3$-d_1



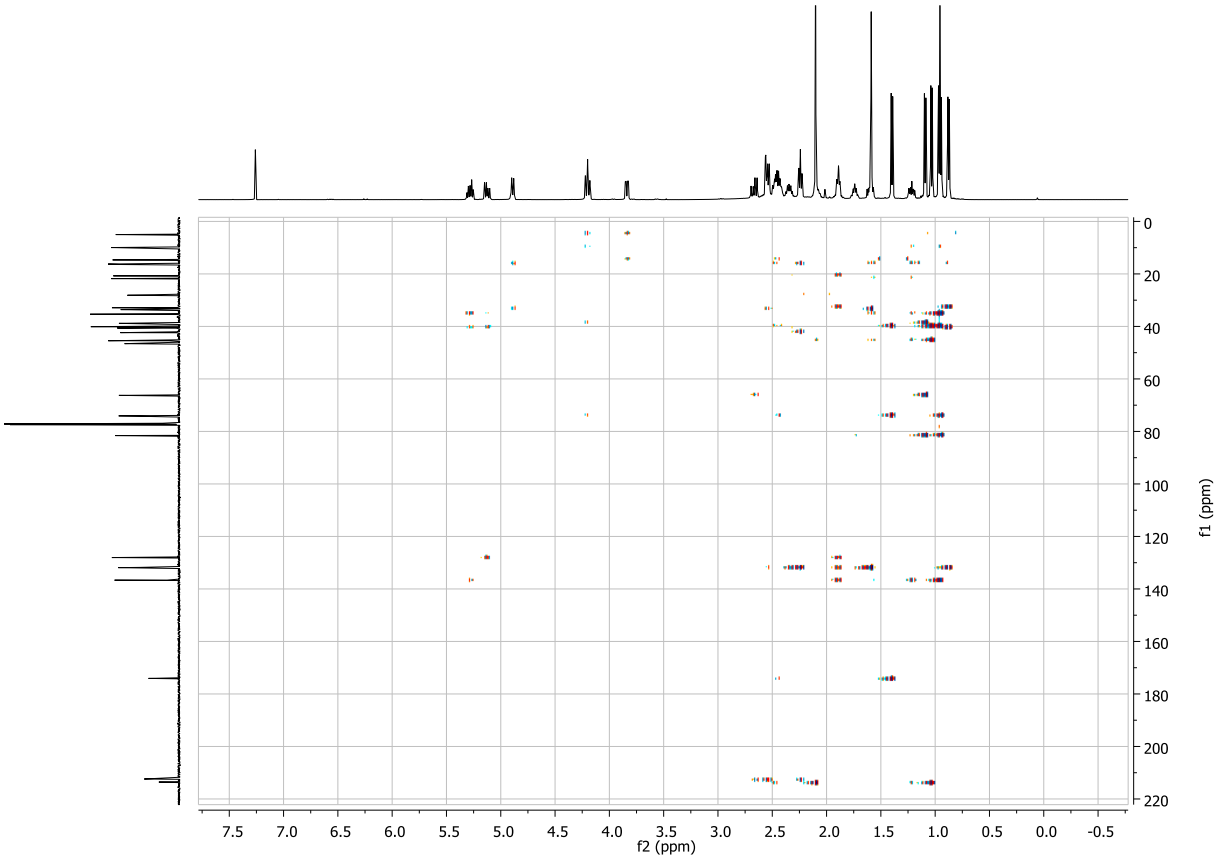
SI Figure 17: ^{13}C-NMR spectra of skipmon B (**8**) in $CDCl_3$-d_1



SI Figure 18: gHSQC spectra of skipmon B (**8**) in $CDCl_3$-d_1

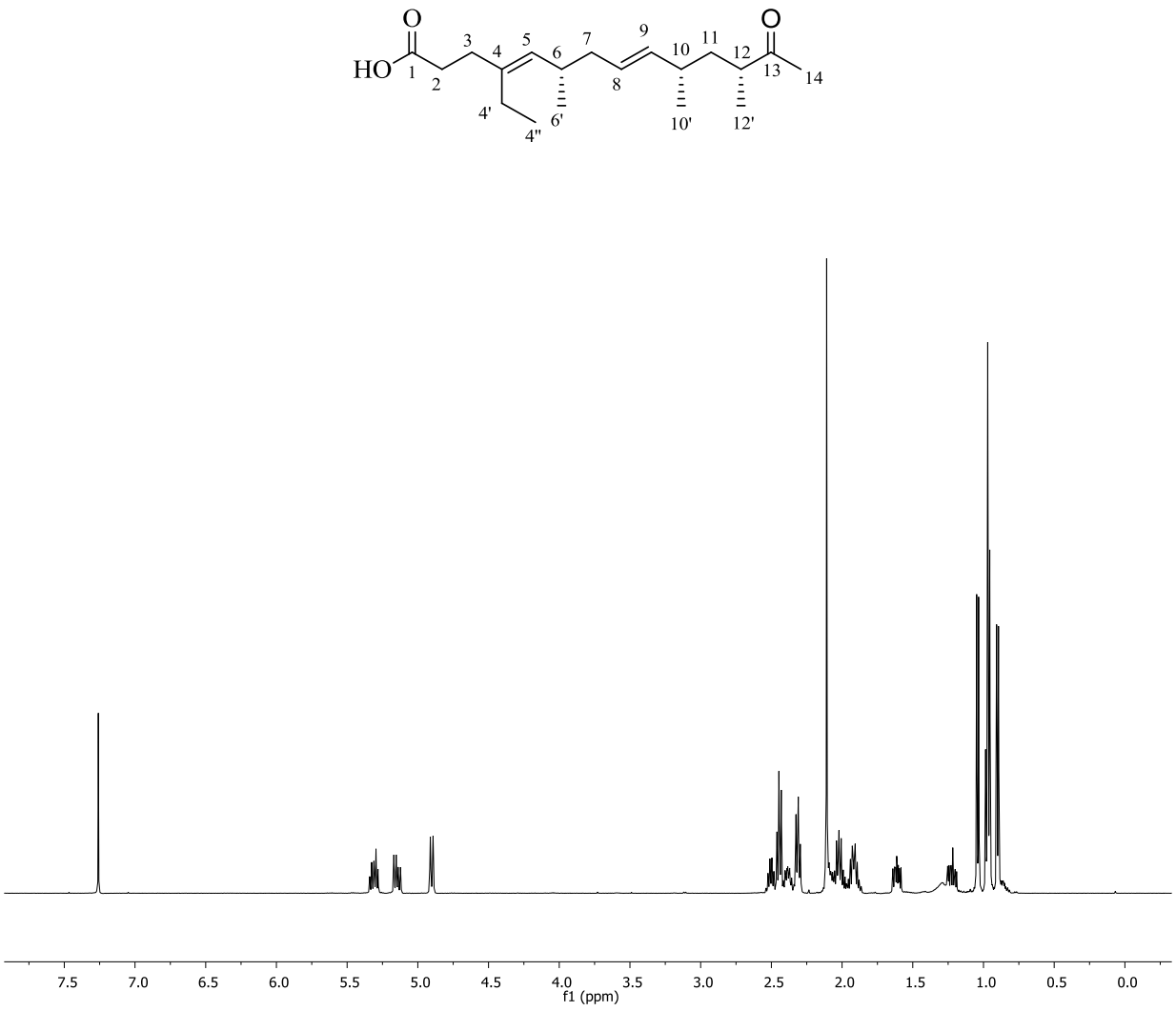


SI Figure 19: gCOSY spectra of skipmon B (**8**) in $CDCl_3$-d_1

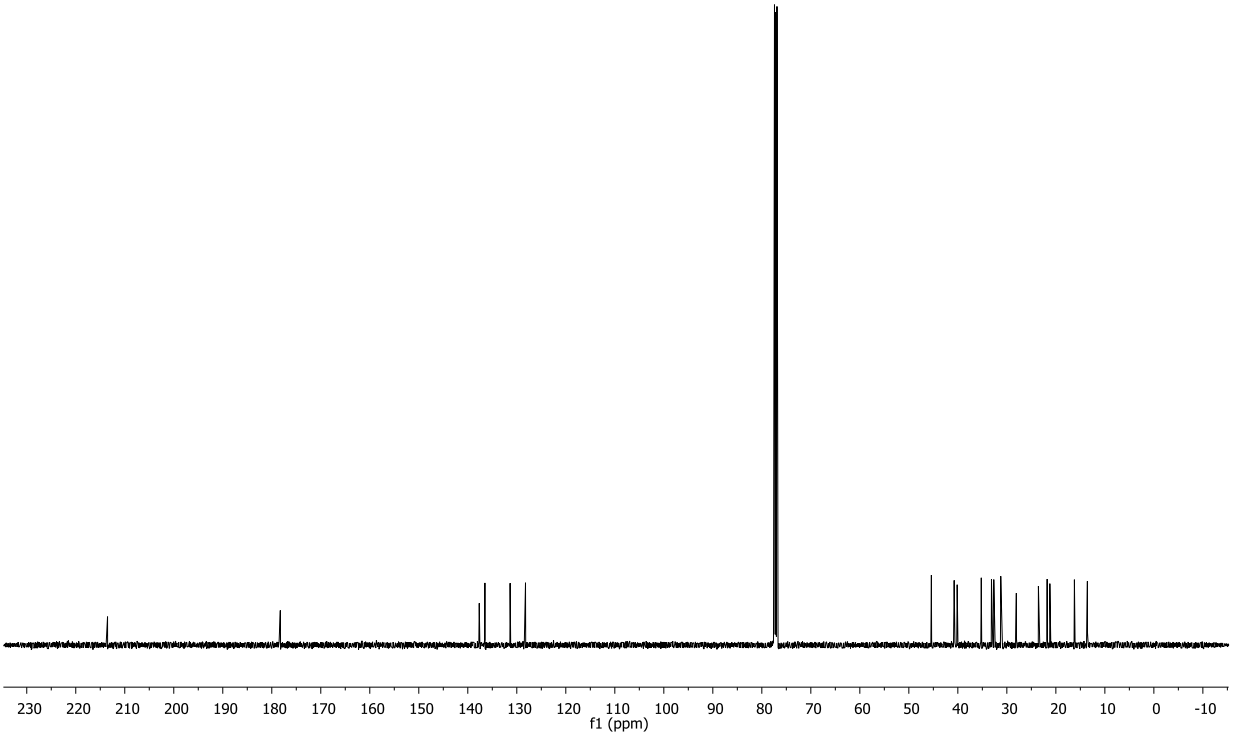


SI Figure 20: gHMBC spectra of skipmon B (**8**) in $CDCl_3$-d_1

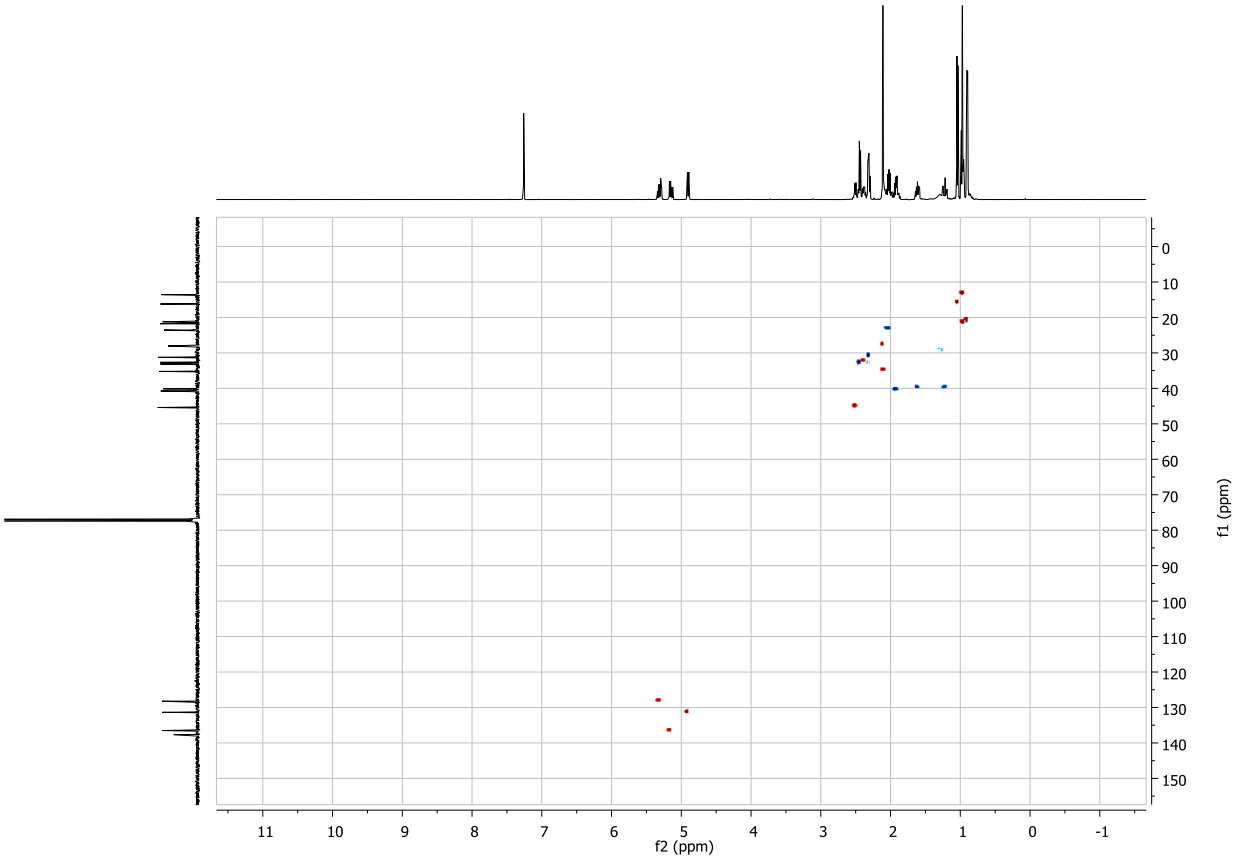
Shunt product 6 A



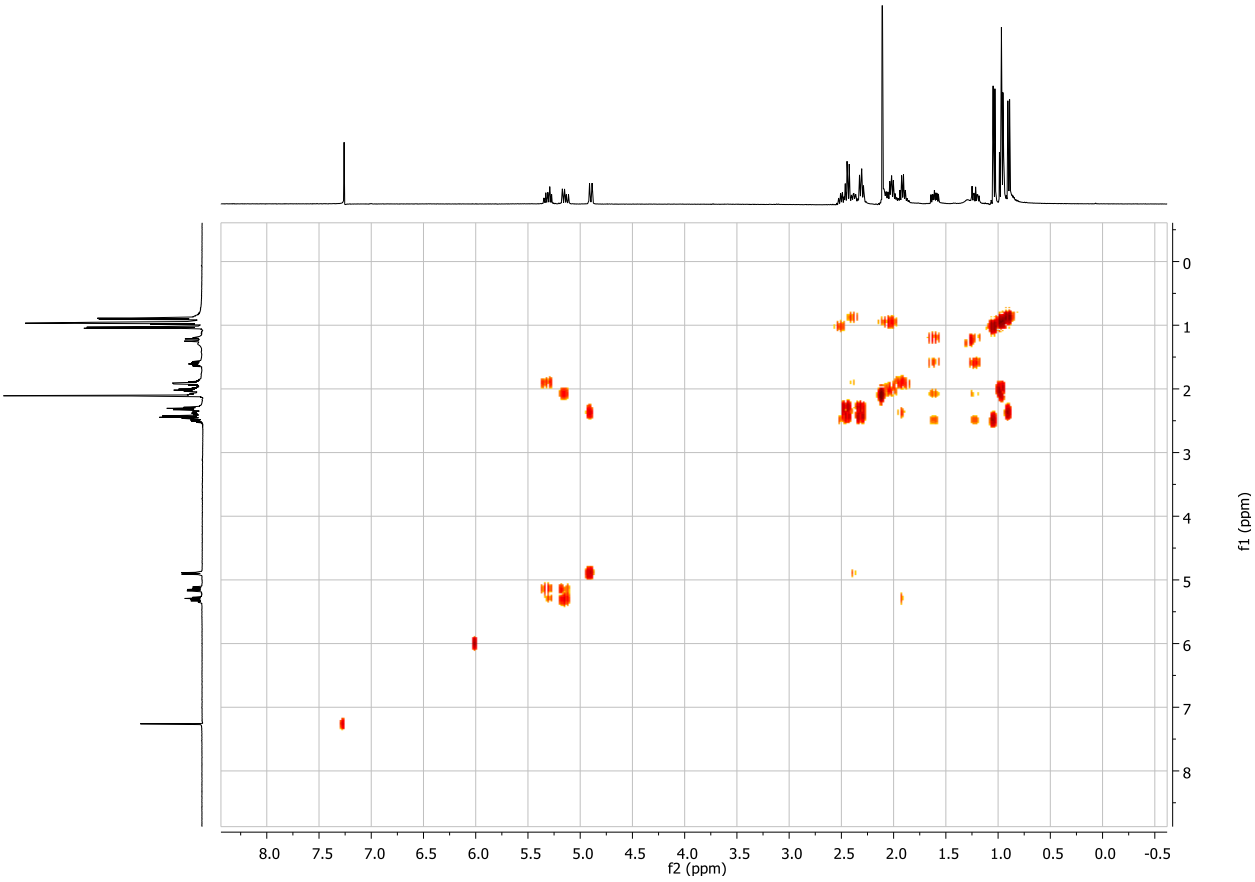
SI Figure 21: ^{1}H-NMR spectra of shunt product 6 A in $CDCl_3$-d_1



SI Figure 22: ^{13}C-NMR spectra of shunt product 6 A in $CDCl_3$-d_1

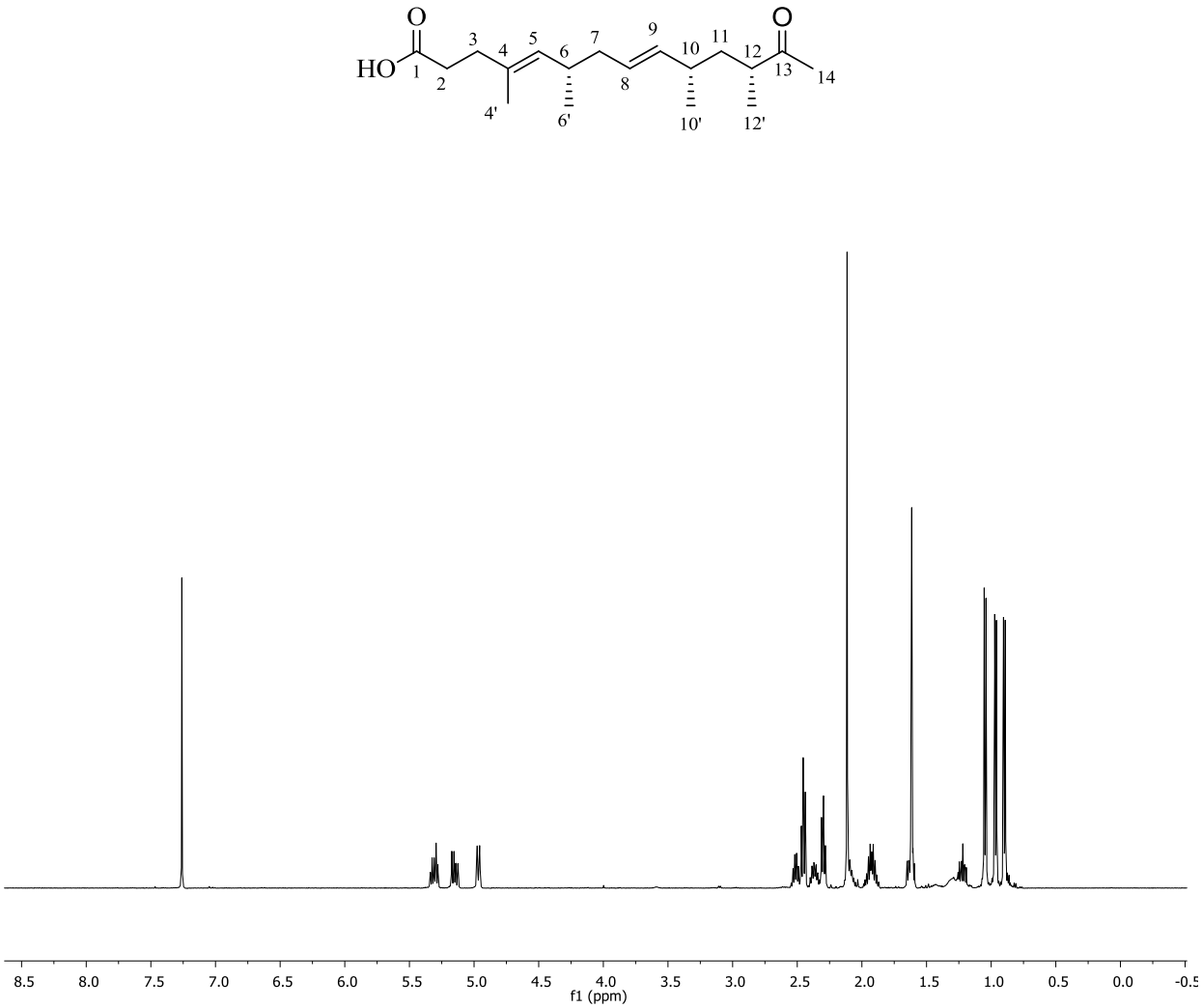


SI Figure 23: gHSQCspectra of shunt product 6 A in $CDCl_3$-d_1

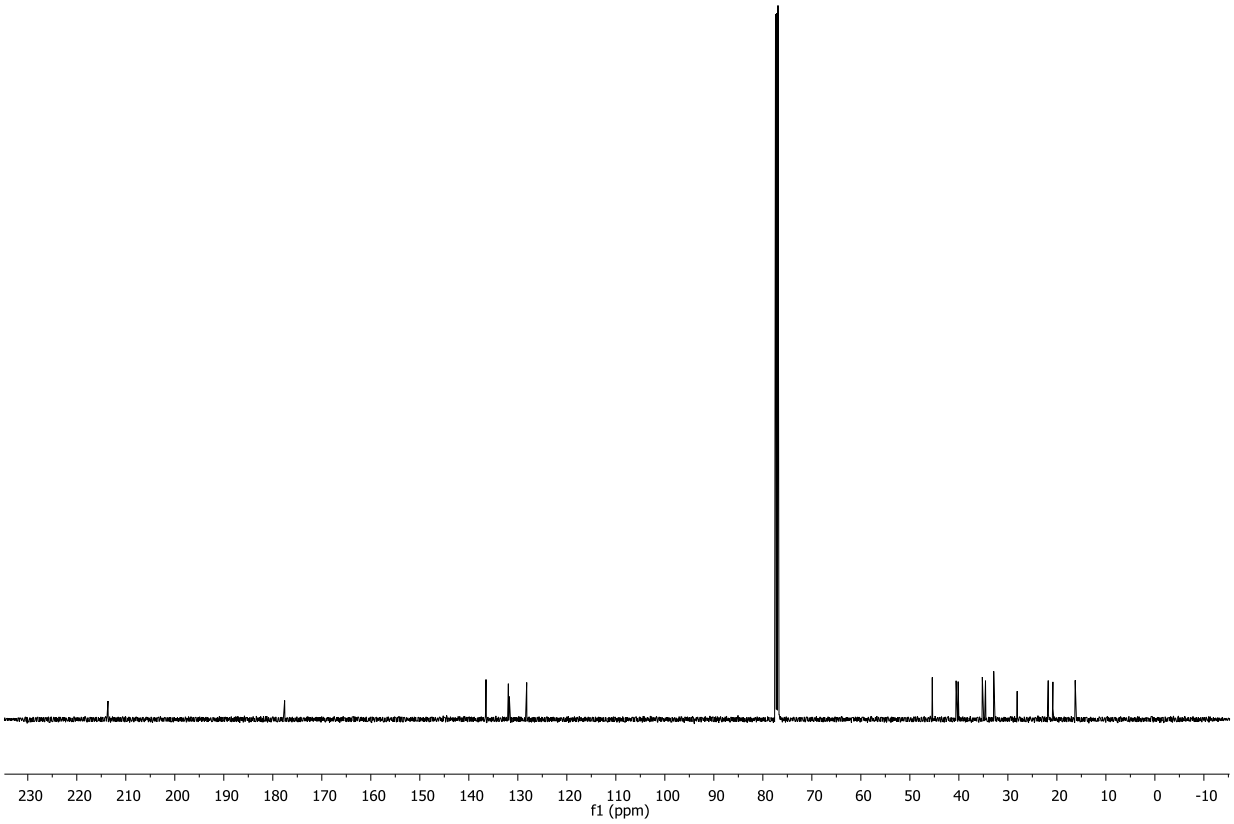


SI Figure 24: gCOSY spectra of shunt product 6 A in $CDCl_3$-d_1

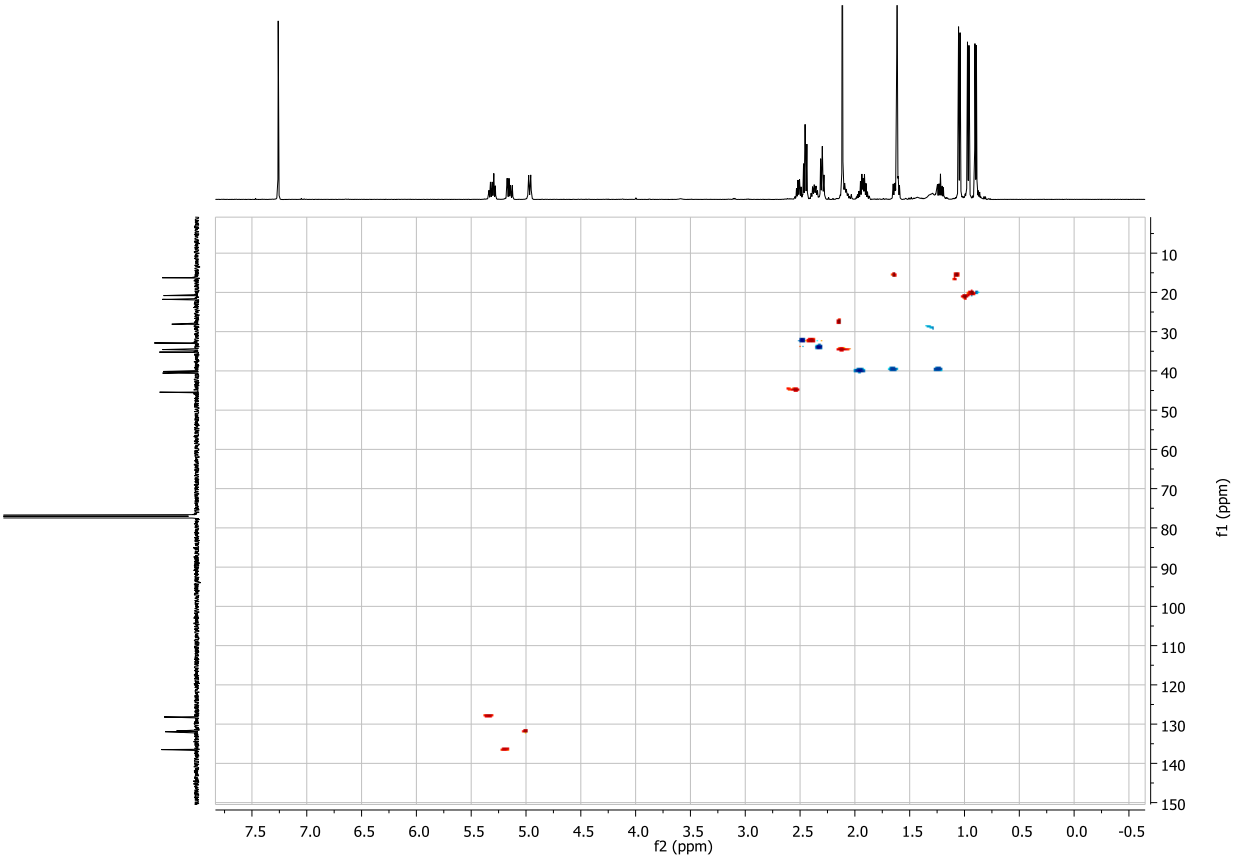
Shunt product 6 B



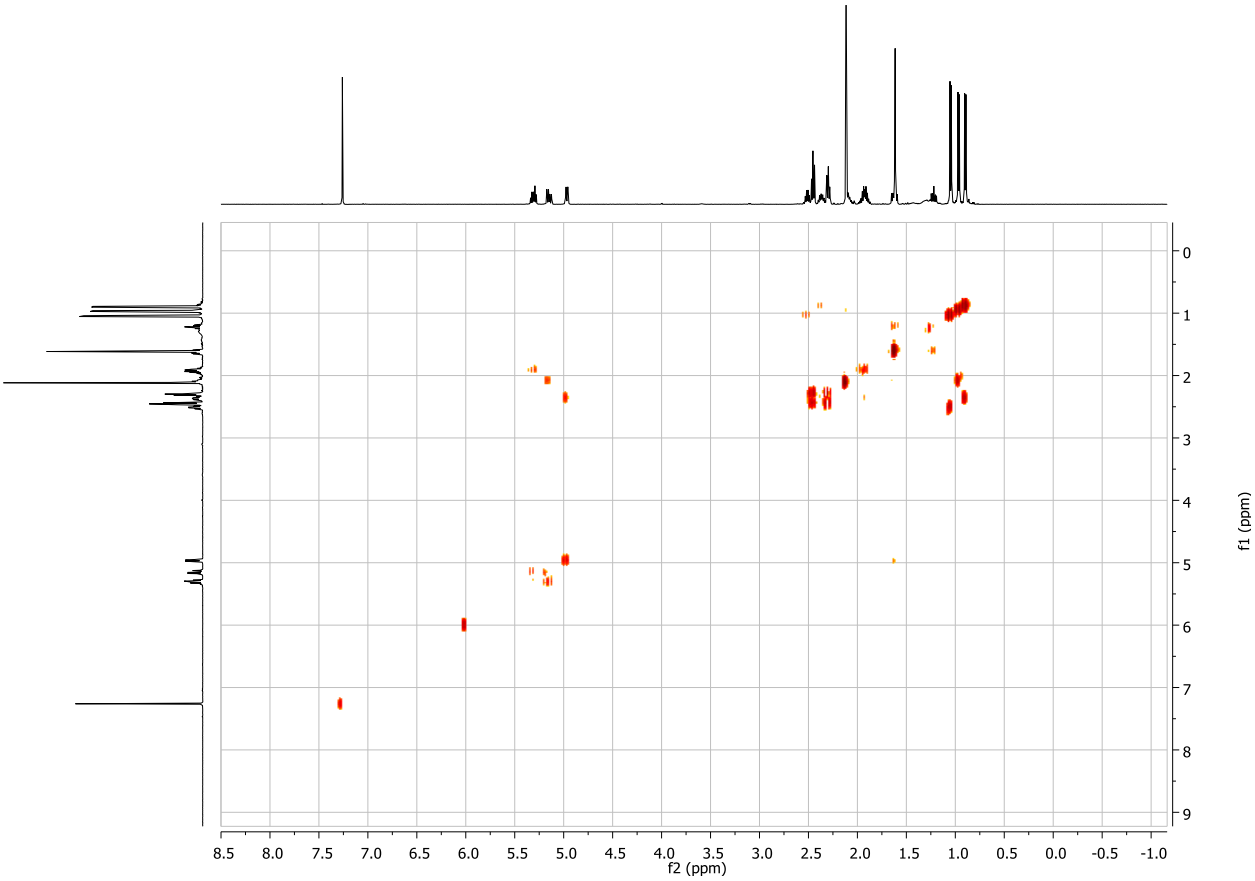
SI Figure 25: ^{1}H-NMR spectra of shunt product 6 B in $CDCl_3$-d_1



SI Figure 26: ^{13}C-NMR spectra of shunt product 6 B in $CDCl_3$-d_1



SI Figure 27: gHSQCspectra of shunt product 6 B in $CDCl_3$-d_1



SI Figure 28: gCOSY spectra of shunt product 6 B in $CDCl_3$-d_1

8. Proliferation assays

Proliferation assays were conducted in the Lead Discovery Centre (LDC) in Dortmund.

SI Table 2: RPMI-8226 IC_{50} values

Compound	IC_{50} (µM)
Skipmon A (**7**)	18.6
Skipmon B (**8**)	25.2
Premonensin A (**4**)	8.2
Premonensin B (**3**)	11.2

9. References

[1] K. Bravo-Rodriguez, A. F. Ismail-Ali, S. Klopries, S. Kushnir, S. Ismail, E. K. Fansa, A. Wittinghofer, F. Schulz, E. Sanchez-Garcia, *ChemBioChem* **2014**, *15*, 1991-1997.

[2] S. Kushnir, U. Sundermann, S. Yahiaoui, A. Brockmeyer, P. Janning, F. Schulz, *Angewandte Chemie International Edition* **2012**, *51*, 10664-10669.