

## Supplementary Information

# New Deoxyenhygrolides from *Plesiocystis pacifica* Provide Insights into Butenolide Core Biosynthesis

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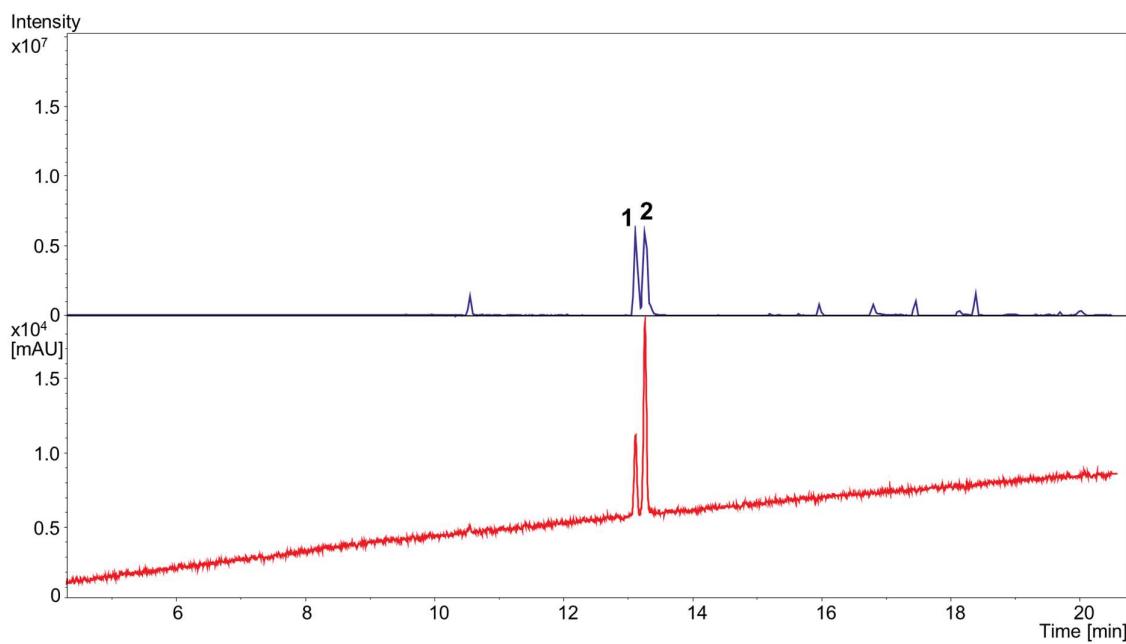
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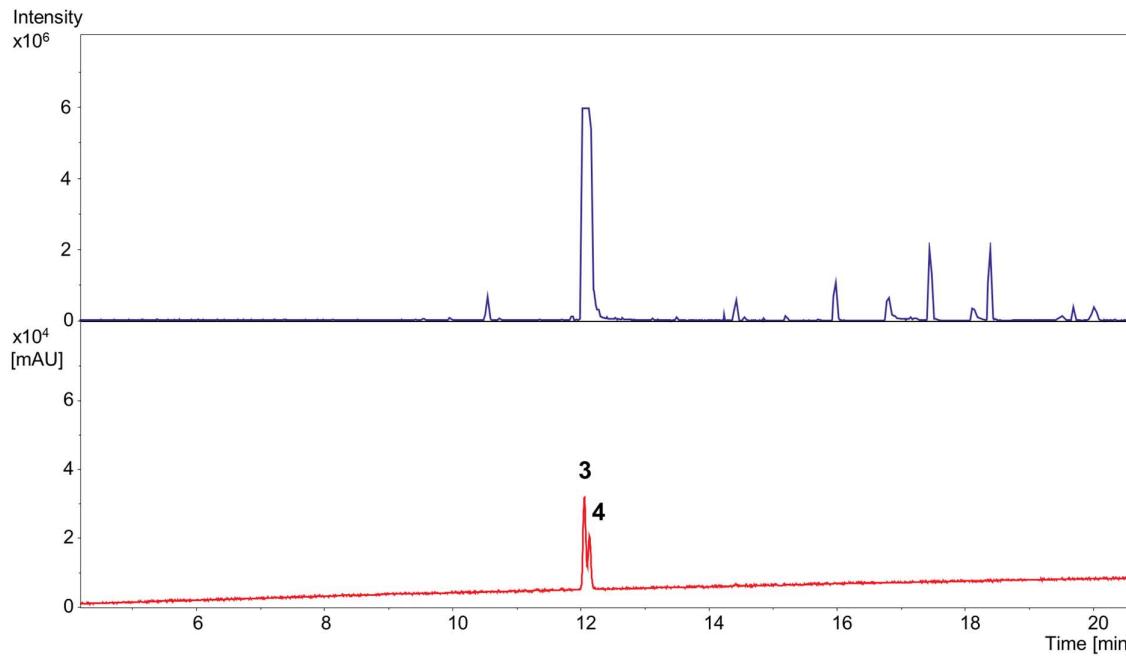
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## 1. MS spectra

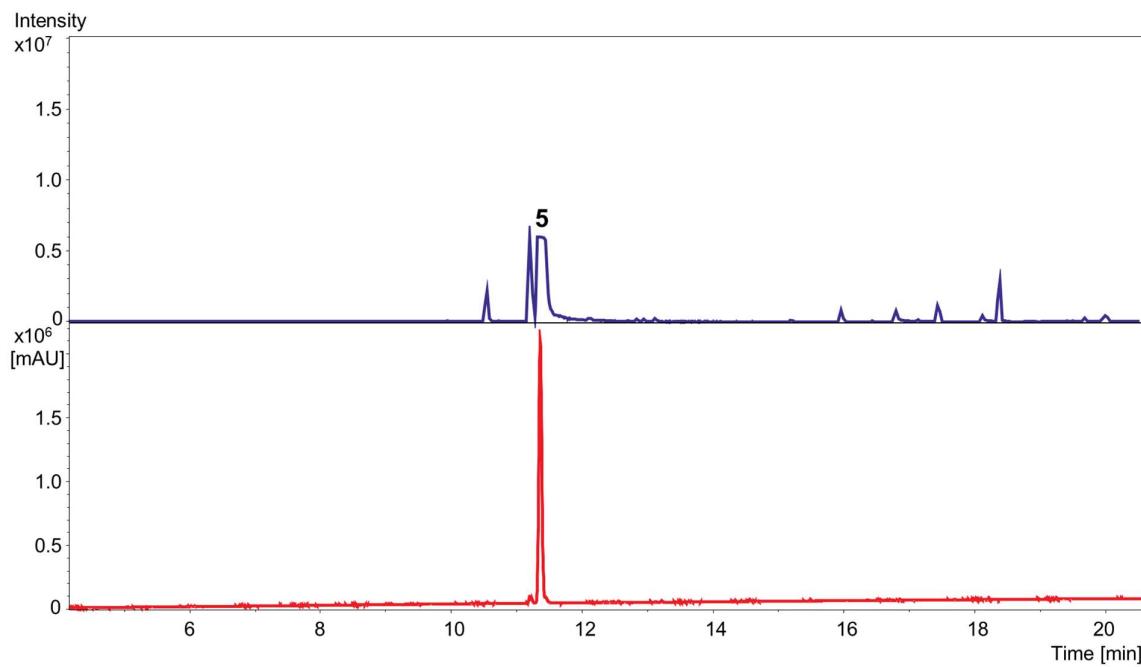
### 1.1 Preparative LC-MS chromatograms



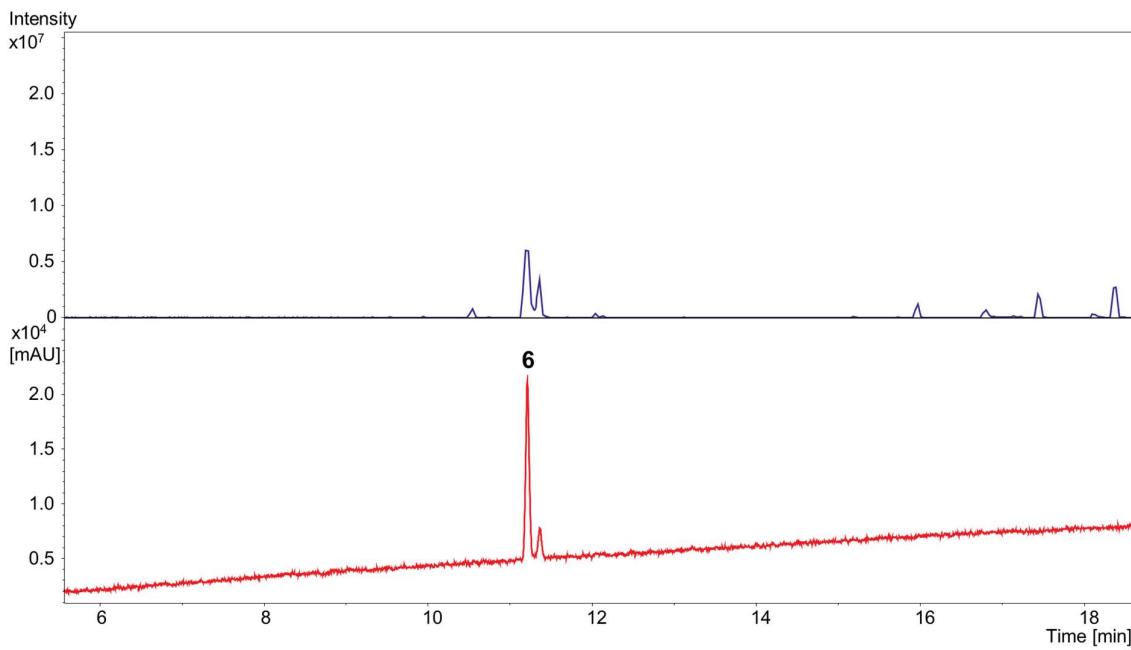
**Figure S1.** HPLC-MS BPC trace of purified **1** and **2** (top, blue) and UV/VIS (bottom, red) trace of **1** and **2**. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.



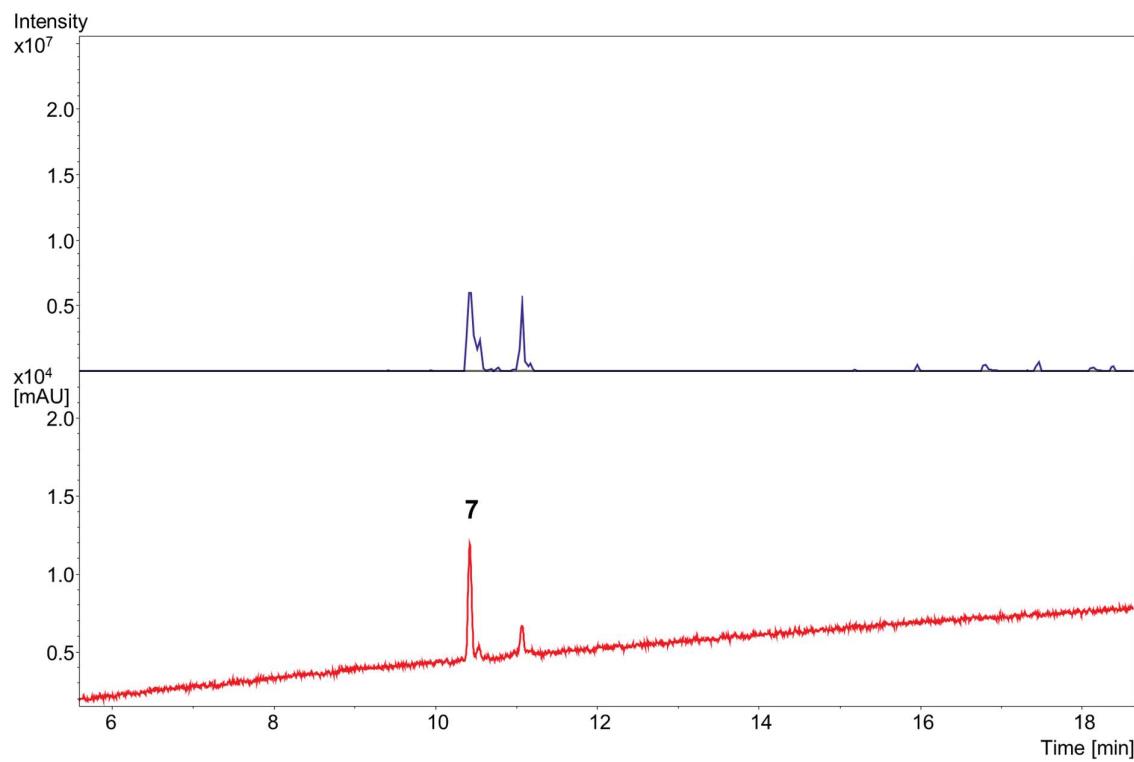
**Figure S2.** HPLC-MS BPC trace of purified **3** and **4** (top, blue) and UV/VIS (bottom, red) trace of **3** and **4**. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.



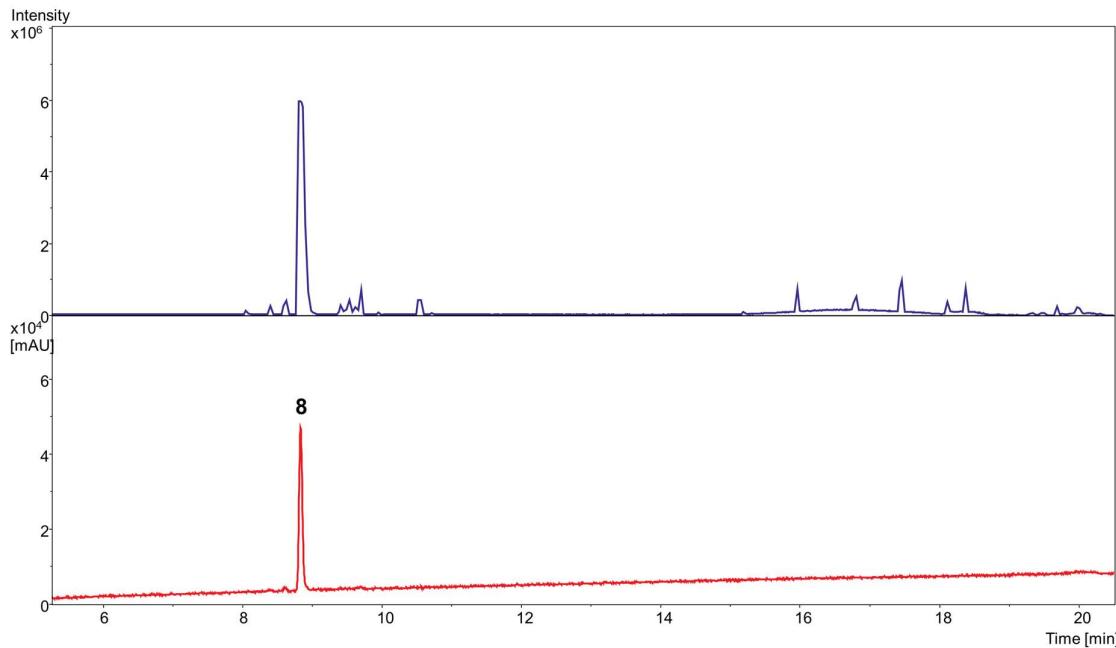
**Figure S3.** HPLC-MS BPC trace of purified 5 (top, blue) and UV/VIS (bottom, red) trace of 5. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.



**Figure S4.** HPLC-MS BPC trace of purified 6 (top, blue) and UV/VIS (bottom, red) trace of 6. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.

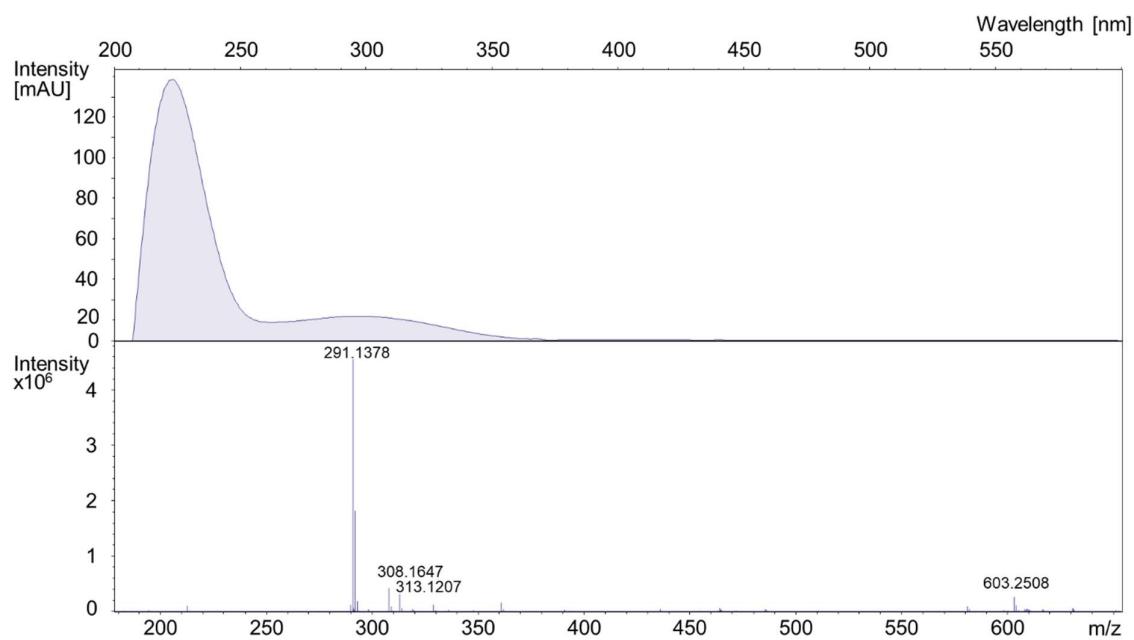


**Figure S5.** HPLC-MS BPC trace of purified **7** (top, blue) and UV/VIS (bottom, red) spectrum of **7**. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.

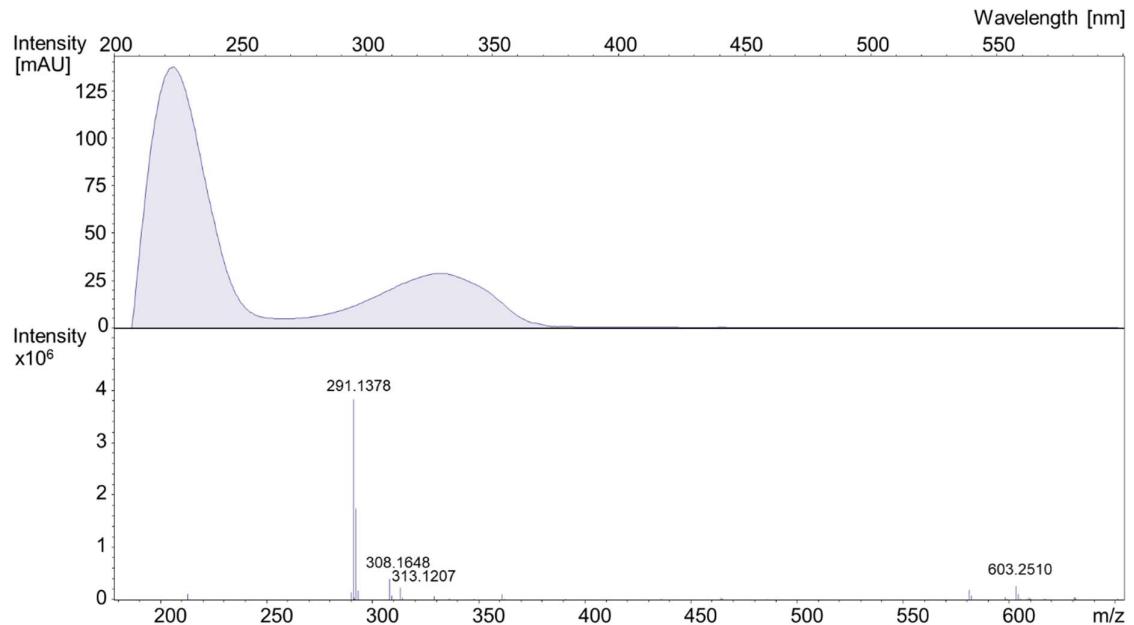


**Figure S6.** HPLC-MS BPC trace of purified **8** (top, blue) and UV/VIS (bottom, red) spectrum of **8**. Mass spectrum was acquired from 150–2000  $m/z$ , in positive mode; UV/VIS detection by a DAD at 200–600 nm.

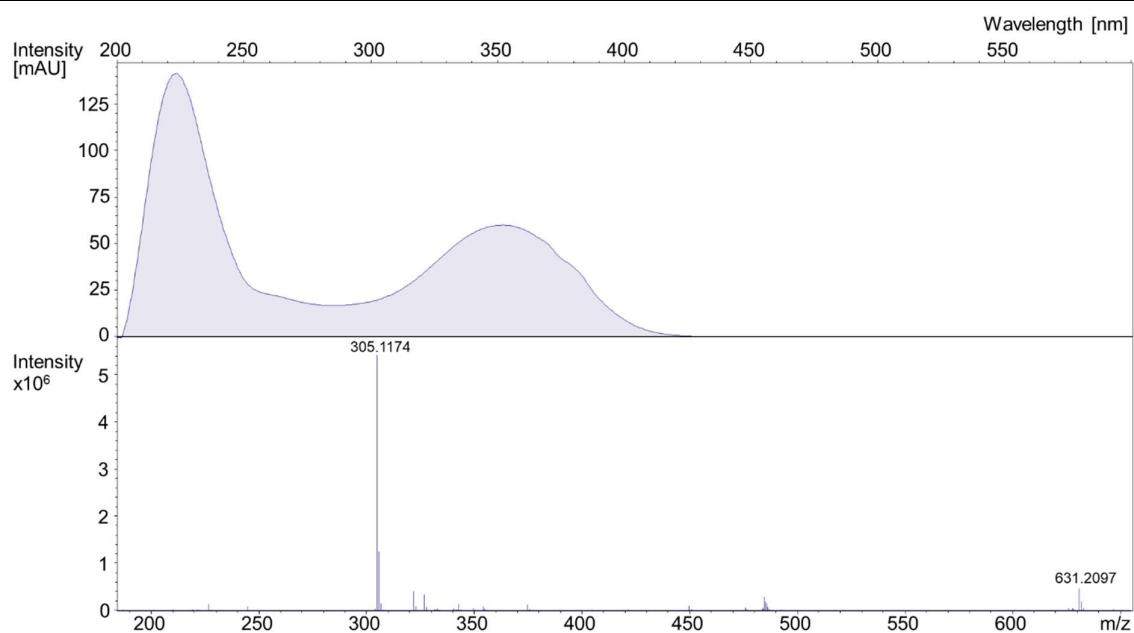
## 1.2 Partial ESI-MS spectra



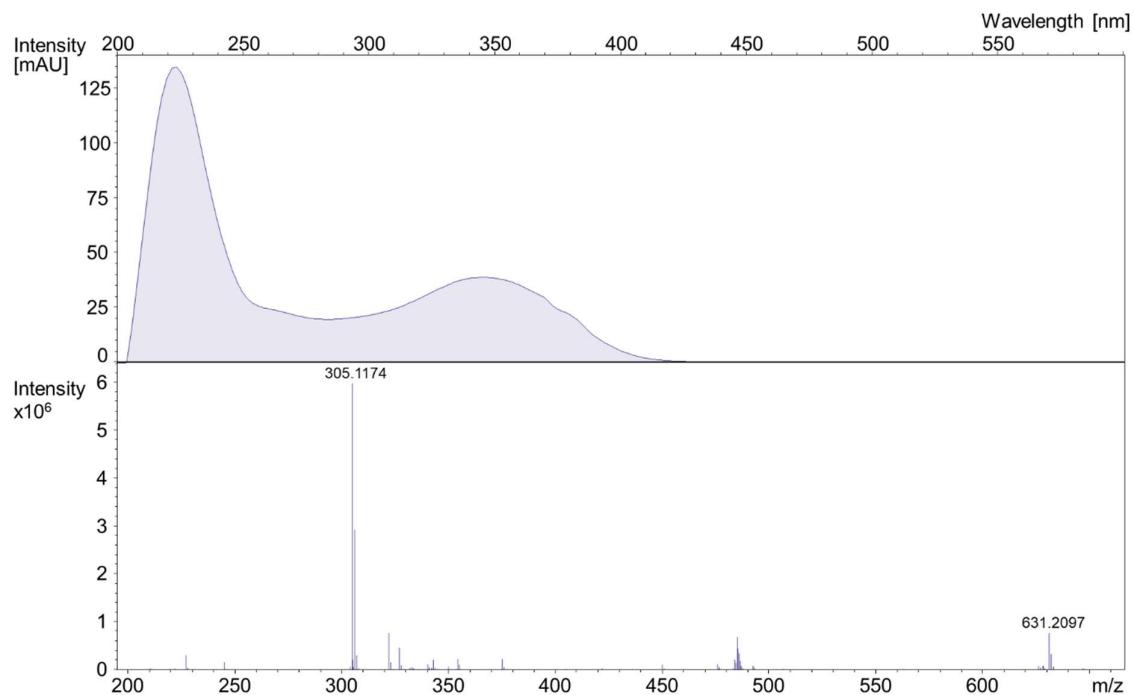
**Figure S7.** UV/VIS and partial ESI+MS spectra of purified **1** (291.1378  $[M+H]^+$ , 308.1647  $[M+NH_4]^+$ , 313.1207  $[M+Na]^+$ , 603.2508  $[2M+Na]^+$ ). RT: 13.05–13.15 min.



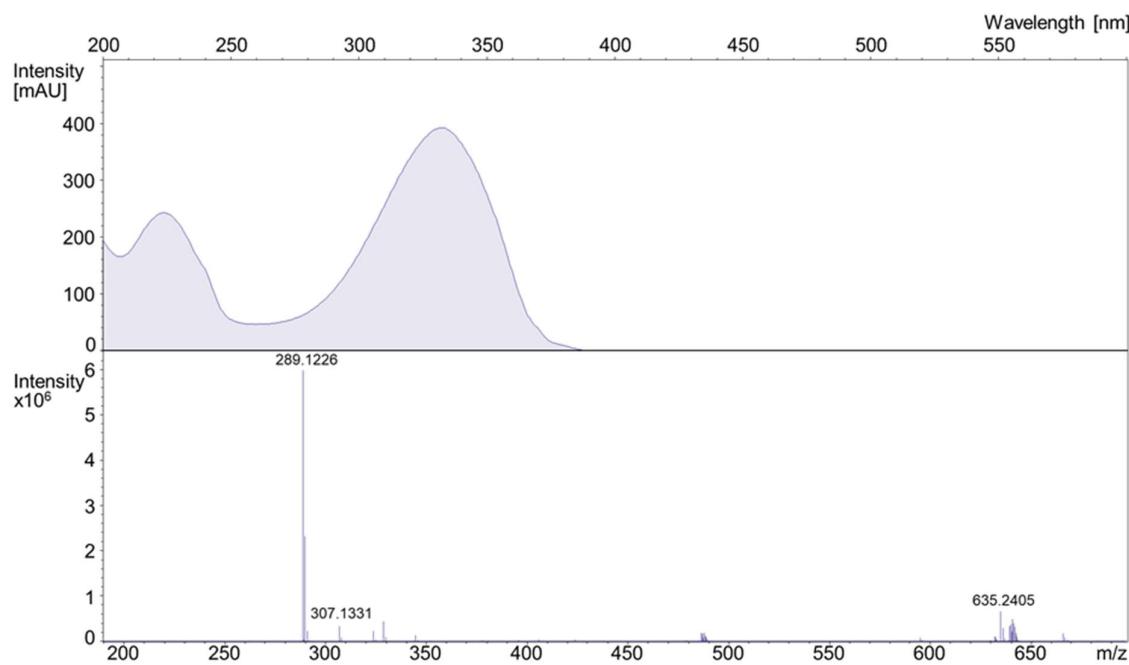
**Figure S8.** UV/VIS and partial ESI+MS spectra of purified **2**. 291.1378  $[M+H]^+$ , 308.1648  $[M+NH_4]^+$ , 313.1207  $[M+Na]^+$ , 603.2510  $[2M+Na]^+$ ). RT: 13.20–13.30 min.



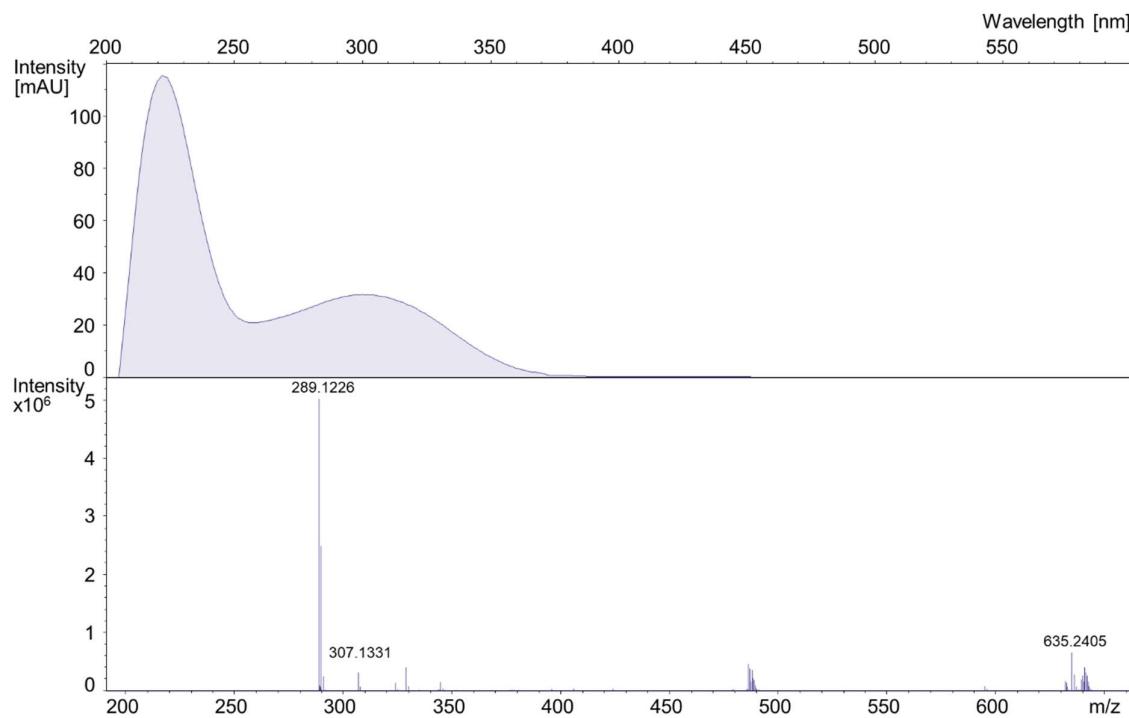
**Figure S9.** UV/VIS and partial ESI+MS spectra of purified **3**.  $305.1174$   $[M+H]^+$ ,  $631.2097$   $[2M+Na]^+$ . RT: 12.00–12.10 min.



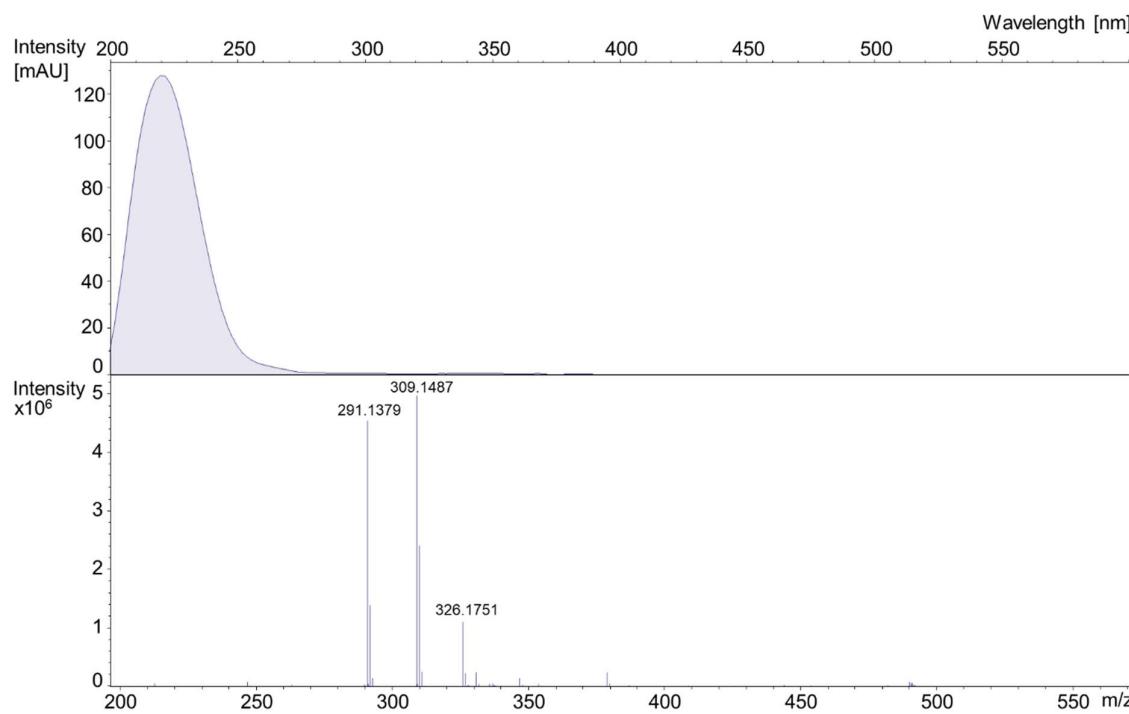
**Figure S10.** UV/VIS and partial ESI+MS spectra of purified **4**.  $305.1174$   $[M+H]^+$ ,  $631.2097$   $[2M+Na]^+$ . RT: 12.10–12.20 min.



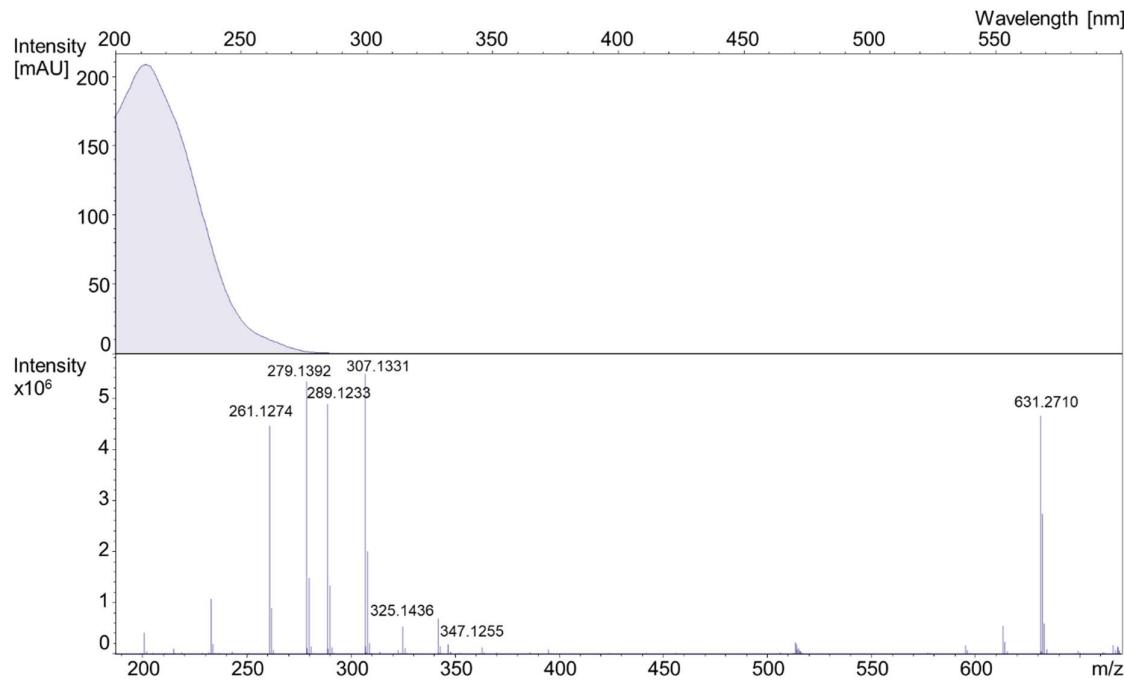
**Figure S11.** UV/VIS and partial ESI+MS spectra of purified **5**. 289.1243 [ $M-H_2O+H$ ] $^+$ , 307.1331 [ $M+H$ ] $^+$ , 635.2405 [ $2M+Na$ ] $^+$ . RT: 11.30–11.45 min.



**Figure S12.** UV/VIS and partial ESI+MS spectra of purified **6**. 289.1226 [ $M-H_2O+H$ ] $^+$ , 307.1331 [ $M+H$ ] $^+$ , 635.2405 [ $2M+Na$ ] $^+$ . RT: 11.15–11.25 min.



**Figure S13.** UV/VIS and partial ESI+MS spectra of purified 7. 291.1379  $[M-H_2O+H]^+$ , 309.1487  $[M+H]^+$ , 326.1751  $[M+NH_4]^+$ . RT: 10.38–10.48 min.

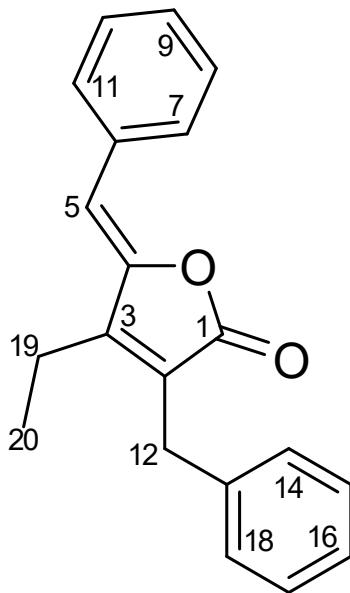


**Figure S14.** UV/VIS and partial ESI+MS spectra of purified 8. 289.12332  $[M-2H_2O+H]^+$ , 261.1274  $[M-2H_2O-C_2H_4+H]^+$ , 307.1331  $[M-H_2O+H]^+$ , 279.1392  $[M-H_2O-C_2H_4+H]^+$ , 325.1436  $[M+H]^+$ , 347.1255  $[M+Na]^+$ , 631.2710  $[2M-H_2O+H]^+$ . RT: 8.80–8.90 min.

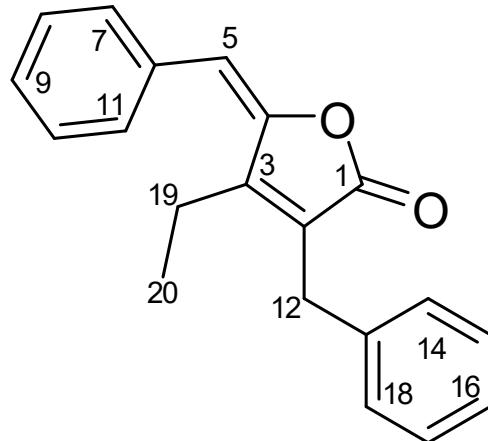
## 2. NMR spectroscopic data for 1–8

**Table S1.** Spectroscopic values of deoxyenhygrolide C and D (**1** and **2**) acquired in  $\text{CDCl}_3$  at 700 MHz.

Position	Deoxyenhygrolide C ( <b>1</b> )			Deoxyenhygrolide D ( <b>2</b> )				
	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY
1	170.7, -	-	-	-	170.1	-	-	-
2	126.1, -	-	-	-	130.8	-	-	-
3	154.9	-	-	-	152.8	-	-	-
4	148.0	-	-	-	149.5	-	-	-
5	109.1	6.02, s	3, 4, (6), 7/11	19	114.7	6.86, s	3, 4, (6), 7/11	-
6	133.3	-	-	-	133.2	-	-	-
7/11	130.6	7.78, m	5, 7/11, 8/9/10	-	129.3	7.30, m	5	-
8/10	128.9	7.38, m	6, 7/11, 8/9/10	-	128.5	7.35, m	-	-
9	128.9	7.31, m	7/11	-	128.9	7.27, m	-	-
12	29.8	3.74, s	1, 2, 3, (4), 13, 14/18-	-	29.7	3.69, s	1, 2, 3, (4), 13, 14/18-	-
13	138.1	-	-	-	137.9	-	-	-
14/18	128.6	7.27, m	12	-	128.7	7.24, m	12	-
15/17	128.9	7.29, m	13	-	128.9	7.28, m	13	-
16	126.8	7.21, m	(13), 14/18	-	128.9	7.27, m	-	-
19	18.4	2.58, q, 7.7	2, 3, 4, 20	5	19.4	2.28, q, 7.6	2, 3, 4, 20	-
20	14.1	1.15, t, 7.7	3, 19	-	12.9	0.64, t, 7.6	3, 19	-



**1**

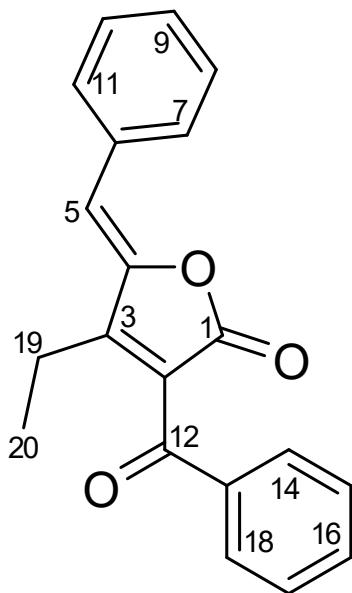
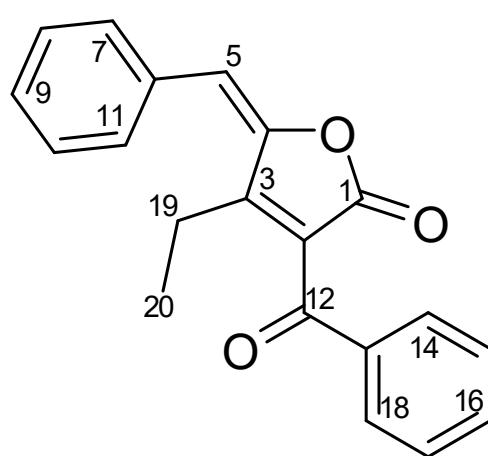


**2**

**Figure S15.** Structure and carbon numbering of deoxyenhygrolide C and D (**1** and **2**).

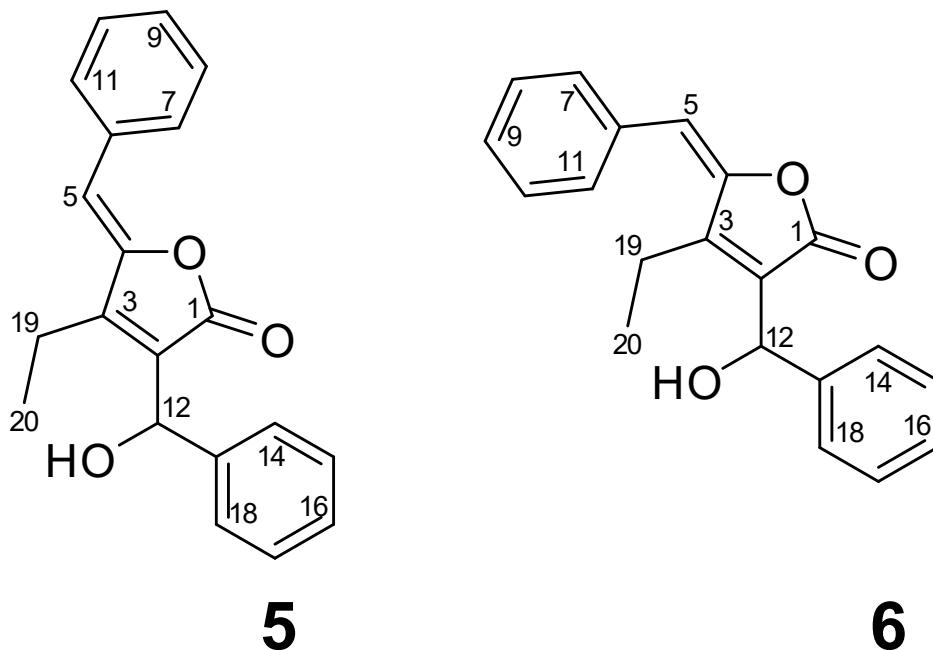
Table S2. Spectroscopic values of deoxyenhygrolide E and F (**3** and **4**) acquired in CDCl<sub>3</sub> at 500 MHz.

Position	Deoxyenhygrolide E ( <b>3</b> )			Deoxyenhygrolide F ( <b>4</b> )				
	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY
1	166.9	-	-	-	166.2	-	-	-
2	124.2	-	-	-	129.4	-	-	-
3	164.7	-	-	-	161.2	-	-	-
4	147.1	-	-	-	148.8	-	-	-
5	113.9	6.36, s	(1), 3, 4, (6), 7/11	19	119.6	7.16, s	3, 4, (6), 7/11	-
6	132.6	-	-	-	132.4	-	-	-
7/11	131.4	7.88, m	5, 7/11	-	129.2	7.37, m	5, 6	19
8/10	129.2	7.45, m	6, 8/10	-	129.2	7.44, m	6, 8/10	-
9	130.1	7.41, m	-	-	128.8	7.41, m	-	-
12	190.0	-	-	-	190.2	-	-	-
13	136.7	-	-	-	136.4	-	-	-
14/18	129.7	7.88, m	12, 14/18, 16	-	129.7	7.87, m	12, 14/18, 16	-
15/17	128.8	7.50, m	13, 14/18, 15/17	-	128.8	7.50, m	13, 14/18, 15/17	-
16	134.2	7.63, m	14/18	-	134.4	7.63, m	14/18	-
19	19.2	2.78, q, 7.5	(1), 2, 3, 4, 20	5	20.3	2.40, q, 7.5	(1), 2, 3, 4, 20	7/11
20	15.0	1.32, t, 7.5	3, 19	-	13.6	0.80, t, 7.5	3, 19	-

**3****4**Figure S16. Structure and carbon numbering of deoxyenhygrolide E and F (**3** and **4**).

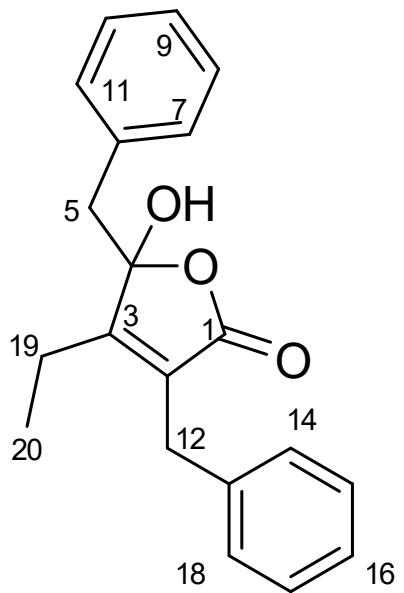
**Table S3.** Spectroscopic values of deoxyenhygrolide G and H (**5** and **6**) acquired in CDCl<sub>3</sub> at 700 MHz.

Position	Deoxyenhygrolide G ( <b>5</b> )			Deoxyenhygrolide H ( <b>6</b> )				
	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	ROESY
1	169.9	-	-	-	169.4	-	-	-
2	126.9	-	-	-	131.2	-	-	-
3	155.1	-	-	-	152.8	-	-	-
4	147.4	-	-	-	149.0	-	-	-
5	110.8	6.11, s	(1), 3, 4, 7/11	7/11, 19	116.8	6.97, s	3, 4, (6), 7/11, (12)	-
6	132.9	-	-	-	132.7	-	-	-
7/11	130.8	7.78, m	5, 9, 7/11	5	129.2	7.31, m	5, 9, 8/10	-
8/10	129.0	7.39, m	6, 7/11, 8/10	-	128.6	7.35–7.39, m	-	-
9	129.3	7.33, m	6, 7/11	-	128.8	7.35–7.39, m	-	-
12	68.9	5.78, br s	1, 2, 3, 13, 14/18	19	68.9	5.69, br s	1, 2, 3, 13, 14/18	19
12-OH	-	3.44, br s	(2)	-	-	3.64, br s	2, 12, (13)	-
13	141.5	-	-	-	141.5	-	-	-
14/18	126.1	7.46, m	12, 14/18, 16	-	126.2	7.42, m	12, 14/18, 16	-
15/17	128.9	7.37, m	13, (14/18), 15/17	-	128.9	7.36, m	13, (14/18), 15/17	-
16	128.2	7.30, m	(13), 14/18	-	128.3	7.30, m	14/18, 15/17	-
19	18.2	2.62, q, 7.7	(1), 2, 3, 4, 20	5, 12	19.2	2.32, q, 7.6	(1), 2, 3, 4, 20	12
20	14.4	1.17, t, 7.7	3, 19	-	13.0	0.67, t, 7.6	3, 19	-

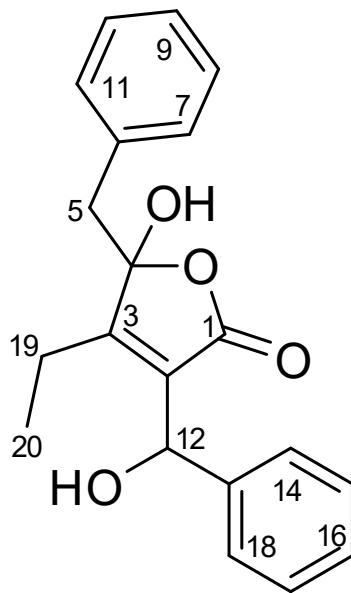

**Figure S17.** Structure and carbon numbering of deoxyenhygrolide G and H (**5** and **6**).

**Table S4.** Spectroscopic values of deoxyenhygrolide I and J (7 and 8) acquired in CD<sub>3</sub>OD at 700/500 MHz, respectively.

Position	Deoxyenhygrolide I (7)			Deoxyenhygrolide J (8)		
	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC	$\delta_c$	$\delta_h$ [m, J (Hz)]	HMBC
1	173.5	-	-	172.8	-	-
2	129.3	-	-	132.3	-	-
3	165.2	-	-	166.4	-	-
4	109.1	-	-	109.3	-	-
5	43.2	3.21, br s 3.35, br s	-	43.6	3.21, br s 3.33, br s	-
6	135.4	-	-	135.5	-	-
7/11	131.1	7.17, m	5, 9, 7/11	128.2	7.23, m	(5), 9, 8/10
8/10	129.0	7.20, m	6, 8/10	129.4	7.20, m	6, 8/10
9	127.9	7.23, m	7/11, (8/10)	131.4	7.15, m	-
12	29.3	3.42, br s	1, 2, 3, 13, 14/18	68.2	5.43, br s	1, 2, 3, 13, 14/18
13	138.5	-	-	142.7	-	-
14/18	128.8	6.65, m	12, (13), 14/18, 16	126.9	6.81, br s	-
15/17	129.1	7.09, m	13, 15/17	129.2	7.15, m	(12), 13, 15/17
16	126.8	7.09, m	-	128.1	7.15, m	14/18
19	20.6	2.42, q, 7.7	2, 3, 4, 20	20.8	2.48, q, 7.6	2, 3, 4, 20
20	12.1	1.17, t, 7.7	3, 19	13.4	1.22, t, 7.6	3, 19



**7**

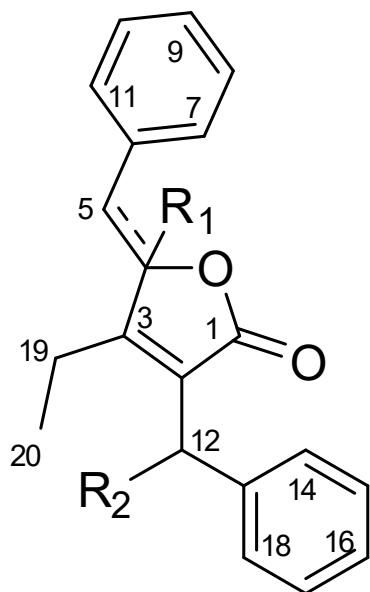


**8**

**Figure S18.** Structure and carbon numbering of deoxyenhygrolide I and J (7 and 8).

**Table S5.** Comparison of  $^{13}\text{C}$  chemical shifts for **1–8**.

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>Pos.</b>	$\text{CDCl}_3$	$\text{CDCl}_3$	$\text{CDCl}_3$	$\text{CDCl}_3$	$\text{CDCl}_3$	$\text{CDCl}_3$	$\text{CD}_3\text{OD}$	$\text{CD}_3\text{OD}$
1	170.7	170.1	166.9	166.2	169.9	169.4	173.5	172.8
2	126.1	130.8	124.2	129.4	126.9	131.2	129.3	132.3
3	154.9	152.8	164.7	161.2	155.1	152.8	165.2	166.4
4	148.0	149.5	147.1	148.8	147.4	149.0	109.1	109.3
5	109.1	114.7	113.9	119.6	110.8	116.8	43.2	43.6
6	133.3	133.2	132.6	132.4	132.9	132.7	135.4	135.5
7/11	130.6	129.3	131.4	129.2	130.8	129.2	131.1	128.2
8/10	128.9	128.5	129.2	129.2	129.0	128.6	129.0	129.4
9	128.9	128.9	130.1	128.8	129.3	128.8	127.9	131.4
12	29.8	29.7	190.0	190.2	68.9	68.9	29.3	68.2
13	138.1	137.9	136.7	136.4	141.5	141.5	138.5	142.7
14/18	128.6	128.7	129.7	129.7	126.1	126.2	128.8	126.9
15/17	128.9	128.9	128.8	128.8	128.9	128.9	129.1	129.2
16	126.8	128.9	134.2	134.4	128.2	128.3	126.8	128.1
19	18.4	19.4	19.2	20.3	18.2	19.2	20.6	20.8
20	14.1	12.9	15.0	13.6	14.4	13.0	12.1	13.4


**1–8**
**Figure S19.** General structure and carbon numbering of deoxyenhygrolide A–H (**1–8**). (1) R1:  $-\text{H}$ , R2:  $-\text{H}$ , (Z); (2) R1:  $-\text{H}$ , R2:  $-\text{H}$ , (E); (3) R1:  $-\text{H}$ , R2:  $=\text{O}$ , (Z); (4) R1:  $-\text{H}$ , R2:  $=\text{O}$ , (E); (5) R1:  $-\text{H}$ , R2:  $-\text{OH}$ , (E); (6) R1:  $-\text{H}$ , R2:  $-\text{OH}$ , (Z); (7) R1:  $-\text{OH}$ , R2:  $-\text{H}$ ; (8) R1:  $-\text{OH}$ , R2:  $-\text{OH}$ .

### 3. Genetic and biosynthetic investigations

#### 3.1 Identification of *cybE* and *cybF* homologs in different myxobacteria

The myxobacterial gene homologs of *cybE* and *cybF* were identified in *Enhygromyxa salina* SWB005 (GenBank accession number: PVNK00000000.1) on contig000249 (GenBank accession number: PVNK01000249.1), in *Enhygromyxa salina* SWB007 (GenBank accession number: PVNL00000000.1) on contig000074 (GenBank accession number: PVNL01000074.1) and in *Plesiocystis pacifica* DSM 14875<sup>T</sup> (GenBank accession number: ABCS00000000.1) on contig1000004 (GenBank accession number: ABCS01000004.1). Interestingly, between the co-localized gene homologs *cybE* and *cybF* in *E. salina* SWB007 (not in *E. salina* SWB005), an open reading frame (ORF) is located, which is putatively encoding a homolog of a flavin-dependent tryptophan halogenase (PrnA) which was described in the biosynthesis of pyrrolnitrin [1]. The size in base pairs (bp) and locus tag of the respective *cybE* or *cybF* are given in **Table S6**.

**Table S6.** *cybE* and *cybF* homologs identified in different myxobacterial genome sequences.

Organism	<i>cybE</i>		<i>cybF</i>	
	Locus Tag	Size [bp]	Locus Tag	Size [bp]
<i>Tolypothrix</i> sp. PCC 9009	Tol9009DRAFT_00039900	1872	Tol9009DRAFT_000398901131	
<i>Plesiocystis pacifica</i> DSM 14875 <sup>T</sup>	PPSIR1_40640	1536	PPSIR1_40635	1107
<i>Enhygromyxa salina</i> SWB005	ENSA5_56740	1674	ENSA5_56750	1113
<i>Enhygromyxa salina</i> SWB007	151239–152885 <sup>(1)</sup>	1647	ENSA7_38410	1125

(1) No gene annotation available; interval of open reading frame is provided

Since the gene annotation in the publicly available myxobacterial genome sequence display shorter *cybE* homologs (1536/1674 bp vs. 1872 bp) or no gene annotation at all (SWB007), we further investigated the genome sequence of *P. pacifica* DSM 14875<sup>T</sup> for the presence of a longer open reading frame and annotated the *cybE* homolog accordingly. The slightly different annotation is presented in **Table S7** alongside the pairwise sequence identity and positive percentage using BLSM62 substitution-scoring matrix [2] obtained by a pairwise alignment with *CybE* from *Tolypothrix* sp. PCC 9009.

**Table S7.** Changed locus tag of *cybE* homolog.

Organism	Locus Tag	Size [bp]	Identity	Similarity
<i>Tolypothrix</i> sp. PCC 9009	Tol9009DRAFT_000399001872		---	---
<i>P. pacifica</i> DSM 14875 <sup>T</sup>	134868–136562	1695	44.0%	61.6%
<i>E. salina</i> SWB005	ENSA5_56740	1674	43.3%	60.8%
<i>E. salina</i> SWB007	151239–152885 <sup>(1)</sup>	1647	44.2%	61.8%

(1) (No) gene annotation available; interval of open reading frame is provided

---

Amino acid sequence alignment of the CybE/F homolog from *P. pacifica* DSM 14875<sup>T</sup> (see 3.2) show comparatively low but significant similarity to the *in vitro* investigated thiamine pyrophosphate (TPP)-binding protein CybE from *Tolypothrix* sp. PCC 9009 [3]. In addition to support the assigned function of the identified CybE homolog from *P. pacifica* DSM 14875<sup>T</sup>, both CybE sequences were evaluated by *in silico* protein homology analogy recognition engine 2 (Phyre2), and the results indeed underline the structural similarity of both CybE enzymes to a structurally characterized flavin-dependent pyruvate oxidase from *Escherichia coli* (*EcPOX*, PDB: 3EY9) [4]. In contrast, Phyre2 analysis of both CybF enyzmes led to slightly different homology models; the CybE homolog from *P. pacifica* DSM 14875<sup>T</sup> displays *in silico* structural similarity to a 3-Ketoacyl-ACP synthase (KAS III) from *Propionibacterium acnes* (PDB: 6A9N, [5]), whereas CybE from *Tolypothrix* sp. PCC 9009 seems structurally closer related to KAS III from *Vibrio cholerae* (PDB: 4X0O, [6]).

### 3.2 Sequence alignments and Phyre2 structures.

CybE

	1	10	20	30	40	50	60	70	80	90
Consensus	MTTGAQMLXAA	XEMGVRAFGVXGGAXXFX	GALHRAVGVX	XHARHESGAXFMAXFAKXASD	XPXVYTT	TGPATNA	TLVSA	AWDGAKXX	XV	XV
Cybe_Pacifica	MFI	LEGRS	SMGLQH-AFC	IGMGA	VVFH	GALERSP	VCF	NCRHS	GAFA	ELA
Cybe_SWB005	MTTGAO	LAALAA	I	EMGVRA	FGV	GALGV	EV	SAMHR	QI	HARHES
Cybe_SWB007	M	LAAAL	VEL	GVR	FGV	FLV	GGA	IA	RC	GHRA
Consensus	100	110	120	130	140	150	160	170	180	190
	GAT	XXX	PER	GRIXA	QFET	XXX	A	D	L	XX
Cybe_Pacifica	GYTP	PAVHR	GRFA	NE	NSNGH	A	ASEL	YI	LSRGM	PD
Cybe_SWB005	GAT	NVP	TER	GRMA	POFTA	-	-	-	DLSPW	LSN
Cybe_SWB007	GATE	LEP	GRKLR	QI	QFET	GR	PRIS	D	LGSLW	DT
Consensus	200	210	220	230	240	250	260	270	280	290
	VXEX	XXX	RXL	XXXX	BAL	WVXG	GAR	XG	XXX	XX
Cybe_Pacifica	I	SEV	HTR	ADP	BAL	WVXG	WAH	K	SRG	APM
Cybe_SWB005	V	AEFH	RH	SS	BV	WV	YCG	AEH	GR	AVH
Cybe_SWB007	VNOY	MLE	LDGS	BAL	WV	YCG	KAQ	GL	LEA	RS
Consensus	300	310	320	330	340	350	360	370	380	390
	XLP	XXX	FHLV	D	DPX	FGV	AYPE	ETL	G	VL
Cybe_Pacifica	R	LI	P	PGGL	H	DPV	APV	GA	A	PE
Cybe_SWB005	A	TS	S	SS	V	DPV	SVF	GA	PE	ETL
Cybe_SWB007	TF	TRA	FR	TF	DPV	DA	T	FGV	PE	ETL
Consensus	400	410	420	430	440	450	460	470	480	
	IQR	V	VFG	SDX	V	XL	ADVG	NST	W	
Cybe_Pacifica	IQR	VE	VFG	SDX	V	XL	ADVG	NST	W	
Cybe_SWB005	IQR	V	VFG	SDX	V	XL	ADVG	NST	W	
Cybe_SWB007	IQR	V	VFG	SDX	V	XL	ADVG	NST	W	
Consensus	490	500	510	520	530	540	550	560	570	574
	YGM	TR	HGM	LAX	GMX	PPXX	TX	IP	TD	PE
Cybe_Pacifica	FGM	D	QGM	L	ALC	KPS	VSH	P	VDE	AL
Cybe_SWB005	YGM	R	HGM	A	GM	PE	E	T	PT	DYAR
Cybe_SWB007	YGMV	R	HGM	A	GM	DPR	GT	A	T	EN

**Figure S20.** Amino acid alignment of myxobacterial CybE homologs from *P. pacifica* DSM 14875<sup>T</sup> (CybE\_Pacifica), *E. salina* SWB005 (CybE\_SWB005) and *E. salina* SWB007 (CybE\_SWB007). Pairwise identity: 45.0%, pairwise positive (BLSM62): 61.2%.

Consensus	MTTGAQXLAXTLEXVGVRRAFGVXGGAJXTXFXXAHRAGXXAHRFXXXAXEMAXFAXXAXXXPVXXTXXGPGATNAITGLVSXAXDGAKVY
1. CybE_SWB005	MTTGAAQXLAAVLELLEMVGVRRAFGVFGSALGVBYVSAHFRAGIQIVHARHESGAAPMASEAYFASGSIGVSAVFTISGPAGATNAITGLVSXAXDGAKVY
2. CybE_SWB007	MATTAALVFLVTVGVRRAFGVFGSALGVBYVSAHFRAGIQIVHARHESGAAPMASEAYFASGSIGVSAVFTISGPAGATNAITGLVSXAXDGAKVY
Consensus	XVXGATAXBXERGRBAXQFDTTXXXLIDIXXWVXBXSXKXYLXXTLEXXXQXBXPLRXLQXAXLXKXBXGFLAXLXPLRXPXXXXXXXXX XXXX XXXPXX
1. CybE_SWB005	AVAGENATINVERGSRMAGAOFETA - ALDPSLWNSNINYLWVQFSPSOLPISPLRQLAAGLARNGEFAANLMEFSSQVAPAPLELGGPLLEAPQKVV
2. CybE_SWB007	VVTGATTELEPRGRBLAOFETGPRSLDGSWVNDTSWLYLHEVYIISDQAQHFLRORLPAHUGKGCGGETSFLVQGTTDMALARATVTPRSV
Consensus	XXXBXVXLVXVXXVXXXLXXXFXVIIWVGXGARXGXGXXUNEXKAERSGAVNMXSKPRAKGFVPESHPDQVLYGTVGMFGCOPCPVXXXXXXXXXXPXXVL1VXGKX
1. CybE_SWB005	GAEEFLVAVHRRRLNRSGSSPFVIIWVGXGARERHRAIPEVFAERSGARVMSSKPRAKGFVPESHPDQVLYGTVGMFGCOPCPVXXXXXXXXXXPXXVL1VXGKX
2. CybE_SWB007	SVCBALVNGVYVLELDQGSKELVWVGFGARKIAGAQLLELAERSGAVPMASKPRAKGFVPESHPDQVLYGTVGMFGCOPCPVWHDYFLFRANRPDVYLVLGSR
Consensus	GZXXTSCFDXXXXBXXXXBXXHVDDIXXXEXGVAYPEXEITLGVAXXXLXILALIXDXLXXXBPVSXAPAXXXXXXXXTXXXEXXXXXGPVXPXKLMALCQ
1. CybE_SWB005	GETTSCDFEBALTSSSEHVHDQPSVYGVAYPEXEITLGVADYVEFLAISLNRWRSSPSVSAAPYNTDWNRSPLKTEAEPAGGPVPRMLAMALCQ
2. CybE_SWB007	QGQSSTCSDPTFBARRAEHVHDQDATETGVAYPEXEITLGVADYVEFLAISLNLALINDL - RPSVEAIIKIVAAAGTEAVEDEDPVPRMLAMALCQ
Consensus	RVVKWXXS0X0XXLADVGNFAXXXZLRFXXGXRGRYRXPXGSXMSMTXAXXCVGVAALASAXXXXWVJYVGDGAMMGNEVSTAVOYVXXVAKWNVLDN
1. CybE_SWB005	RVWVWDGSLVLLADVGNFATWSETLRFQDFGRYRPAEGVMSMIAVWVQGVAALASAKPVALVYVGDGAMMGNEVSTAVOYVEMVAKWNVLDN
2. CybE_SWB007	RVJTMERSDATVLEADGVNFSAWNAQLRFERAAGRYRPTPSGSMINATTGVGVAALASDAAVAAIVYGDGAMMGNEVSTAVOYDPMVAKWNVLDN
Consensus	XYGMIXRHGMALAGMXPXXTIEPTTBAXXAXALGAXGLVXWSAMELIXXALIXXXLATGPVGVVVDVRLPDAVAPFGRSKTMELKREGDAR
1. CybE_SWB005	GYGMUJRHGMALAGMXPETEIEPTTDVQARALAGATGLSGAASAEPELARALATGPVGVVVDVRLPDAVAPFGRSKTMELKREGDAR
2. CybE_SWB007	SYGMVRHGMALAGMDPRTGPPATPTNEAMLAALGAKGLFVNNSASELDALIVENLATGPVGVVVDVRLPDAVAPFGRSKTMELKREGDAR

**Figure S21.** Amino acid alignment of myxobacterial CybE homologs from *E. salina* SWB005 (CybE\_SWB005) and *E. salina* SWB007 (CybE\_SWB007). Pairwise identity: 57.3%, pairwise positive (BLSM62): 72.3%.

**Figure S22.** Amino acid alignment of myxobacterial CybE homolog from *P. pacifica* DSM 14875 (CybE\_Pacifica) and CybE from *Tolypothrix sp.* PCC 9009 (CybE\_Tol9009). Pairwise identity: 44.0%, pairwise positive (BLSM62): 61.6%.

CybF

	1	10	20	30	40	50	60	70
Consensus	MHSTMXXXXPVX	IRSLAVAAPK	TIRTNDELXRHRPEI	-VXRXXESTIGIXSNTXSXPXMXII	DASIVPYLKDPPFRG			
CybF_Pacifica	MHSTIKHNRRGA	LRAVAVAFPETVRNQWWEDH	HHPAL-VANARFATI	AKLWNDFADPSTL	GAYDRAFPYLRDPWRC			
CybF_SWB005	MAADLHPVVI	IRSLSLVAAPSR	IRTNDELRRGRPEL	VSVRPGESTQQLISNTESQP	EMATIDASIVPYLNDPFRG			
CybF_SWB007	MSTV	IPVCIRSLAISAPATIR	RTNDELRRGRPEL	VQRSEQSTLGRIMSNTDSPDMSI	DASIVPYLNDPFRG			
	80	90	100	110	120	130	140	150
Consensus	VTERRVILGPXEX	SIDLVE	RAGAAFXEXAGLXPDX	DIDLLISVGFLPD	PDXVGICNAVXVAKXLGLRGGGXNL	ETACAGPL		
CybF_Pacifica	TVERRVMGP	EDITARSLISARAIR	REAMEAGGYGPDD	IDLILLVNALRPDSHV	VCDAAFLVGELGLRAPAIDF	ETACSSAL		
CybF_SWB005	VTERRVILEP	GEGSIDLVE	RAGAAFAIRAGLEP	GDDIDLILLISVGFLPD	NVGTIGVAKHQVAKLQLQGGGFNL	ESACAGPL		
CybF_SWB007	VTERRVILGPDESS	IIDLVE	QAGAAFAELEGALAPES	SVDLLISVGFLPHV	GICNAVYVAKRLGLRGGGWNL	ETACAGPL		
	160	170	180	190	200	210	220	230
Consensus	TALQTASALVRAGE	YERIL	LVTXSCXYSRYI	AREDDTL	SWFLGDGCGGAFIVSRVXXGX	--GXLSGYTMTHTDTCGSWY		
CybF_Pacifica	VGVHLASDL	IIAAGRAYERIL	LVTCTGYTRVDQADSD	SWFLGDGAGAMIV	EAPEAHGLLAHTIPTVETCGABA			
CybF_SWB005	TALQTASALVRAGE	YERVLIT	LGSCYSRYI	AREDDTL	SWFLGDAGGAFIVSRGTVGA	--QWLSGYTMTHTDTCGSWY		
CybF_SWB007	TALQTAFALVRAGE	YETIL	LVTVSCAYSRV	AREDDTL	SWFLGDGCGGAFIVSRVASGT	--GYLSGYTMTHTDTCGSWY		
	240	250	260	270	280	290	300	
Consensus	YXLELDGGAPA	-RVMRAKPxTGR	I	LRXTVEKH	RRCCDGAXAXAGKS	LDVDFFVFHTPTAWFAEFCVDALG	IDPXR	
CybF_Pacifica	YHLELVD	-GAPALRIVGNKKL	LAGRS	IRDHSERYL	TCVGDALEAGASL	SEVFDCVNCNTPTAWFGPFCADVL	GF-EP	
CybF_SWB005	YQLELDDDDAPA	-RVMRAHPQTGR	I	LRKTVOVKH	I	RCCRGAVALAGITL	DDFDVFVFHTPTAWFAEFCVDALG	DPA
CybF_SWB007	YITLELDDDGPA	-RVMRAK	PETGQI	LRRTVEKH	I	RRCCDGAAAKAGISL	DDVDFFVFHTPTAWFAEFAVSALG	IDPE
	310	320	330	340	350	360	370	380 383
Consensus	RTXXXYQX	YANVGPALTPXNLHYA	AXTKRIPXPGDVL	XYPGPGSVKA	AAVIALRWRGEVAL	GXPAGMDXXXF	FSSSR	
CybF_Pacifica	RFVDNPYPR	FANC	GPALMPNNLHTALCEGRVRPGD	VLVLYGYS	I	GSVSTACAVLLRVGEVA	VGQPPAGMDYCS	
CybF_SWB005	KIAATVYQYANVGPAL	PT	NLHYAATKRI	PGDVLVYGPGS	SAAAALI	LRWGEVGLG	PPAGMDYCS	
CybF_SWB007	RTTSVSYQYANVGPAL	PT	NLHYAAHTKRITAGD	TVLVYGPGSV	SSAAAAMIR	MRWGDVAL	PPAGMDAYF	FSSSR

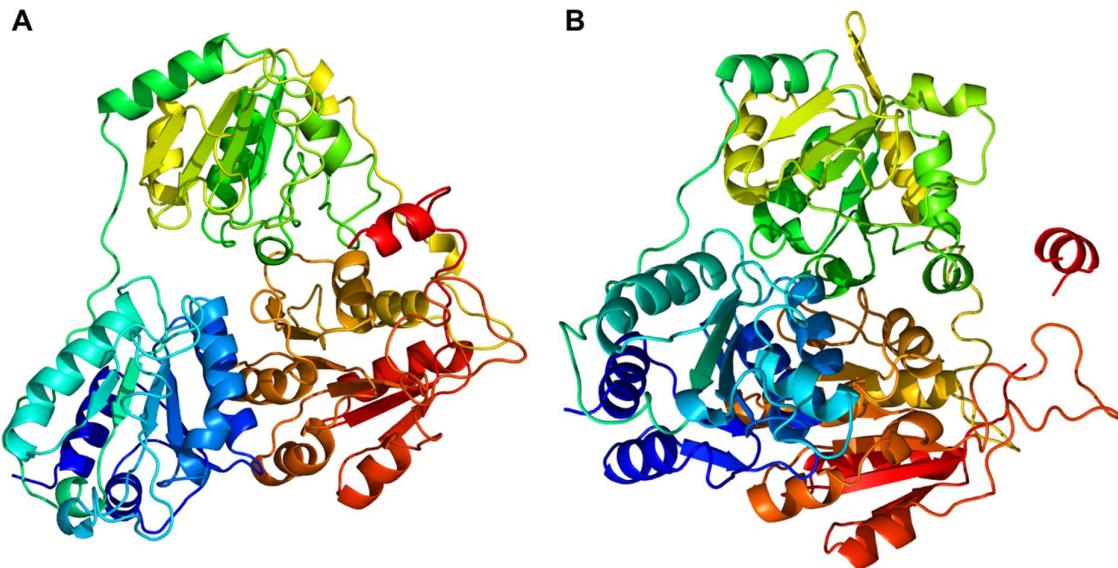
**Figure S23.** Amino acid alignment of myxobacterial CybF homologs from *P. pacifica* DSM 14875<sup>T</sup> (CybF\_Pacifica), *E. salina* SWB005 (CybF\_SWB005) and *E. salina* SWB007 (CybF\_SWB007). Pairwise identity: 50.8%, pairwise positive (BLSM62): 66.5%.

Consensus	1	10	20	30	40	50	60	70
1. Cybf_SWB005	MXXIXXPVXIRSLIXXXAPVX	I	R	T	N	D	L	R
2. Cybf_SWB007	MADLHPVVLIRSLISVAAPS	R	T	N	D	R	G	P
Consensus	MSTV	I	PV	C	T	RSLAISAPAT	T	R
1. Cybf_SWB005	RRVLPXPEXNSIDLEVXAGA	E	X	A	J	X	A	G
2. Cybf_SWB007	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
Consensus	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
1. Cybf_SWB005	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
2. Cybf_SWB007	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
Consensus	80	90	100	110	120	130	140	150
1. Cybf_SWB005	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
2. Cybf_SWB007	RRVLPXPEGSNSIDLEVRA	G	E	A	F	A	R	G
Consensus	160	170	180	190	200	210	220	
1. Cybf_SWB005	LQTAKALVRAGEYEEXLX	T	X	S	C	X	G	L
2. Cybf_SWB007	LQTAKALVRAGEYEERVL	T	I	S	C	S	G	E
Consensus	LQTAKALVRAGEYEERVL	T	I	S	C	S	G	E
1. Cybf_SWB005	LQTAKALVRAGEYEERVL	T	I	S	C	S	G	E
2. Cybf_SWB007	LQTAKALVRAGEYEERVL	T	I	S	C	S	G	E
Consensus	230	240	250	260	270	280	290	300
1. Cybf_SWB005	LDDDXXPARVMRAKPTGK	I	L	R	V	K	H	X
2. Cybf_SWB007	LDDDDAPARVMRAKPTGK	I	L	R	V	K	H	X
Consensus	LDDDDAPARVMRAKPTGK	I	L	R	V	K	H	X
1. Cybf_SWB005	LDDDDAPARVMRAKPTGK	I	L	R	V	K	H	X
2. Cybf_SWB007	LDDDDAPARVMRAKPTGK	I	L	R	V	K	H	X
Consensus	310	320	330	340	350	360	370	375
1. Cybf_SWB005	VYQXYANVGPA	L	T	P	X	N	L	X
2. Cybf_SWB007	VYQXYANVGPA	L	T	P	X	N	L	X
Consensus	VYQXYANVGPA	L	T	P	X	N	L	X
1. Cybf_SWB005	VYQXYANVGPA	L	T	P	X	N	L	X
2. Cybf_SWB007	VYQXYANVGPA	L	T	P	X	N	L	X

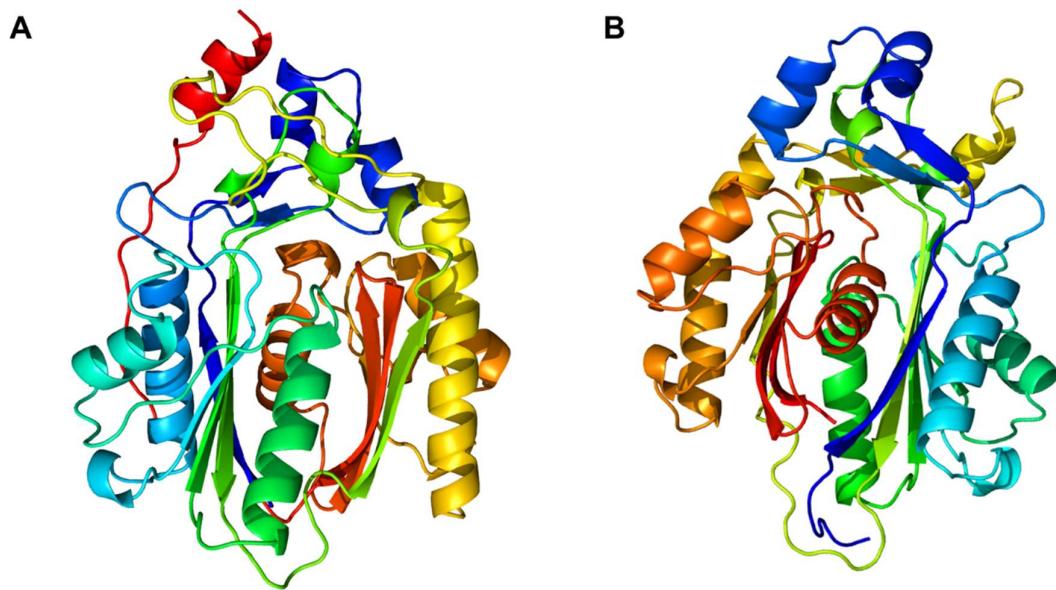
**Figure S24.** Amino acid alignment of myxobacterial CybF homologs from *E. salina* SWB005 (CybF\_SWB005) and *E. salina* SWB007 (CybF\_SWB007). Pairwise identity: 74.1%, pairwise positive (BLSM62): 86.5%.

Consensus	1	10	20	30	40	50	60	70
1. CyBF_Pacifica	MH S T K H X X X X X   X X X A V F P X X X R X N X X X X X X P X L X A X X Z X   T L X K X X X X A D X X X X X X X A P Y L X D P X R G X V							
2. CyBF_Tol9009	MH S T K H N R R G A L R A V A V A F E P T W B R N Q W E D H P R A L V A N A R E A T A L A K L W D N F A D P S T L G A Y D R A F A P Y L R D P W R G T V M F Q P V G I H S L A V S E P S V I R T N D Y Y R E N Y P E L I A Q V E Q K T L S K V F S P - A D S T P S N E F E V E M A P Y L Q D P F R G S V							
Consensus	80	90	100	110	120	130	140	150
1. CyBF_Pacifica	E R R V X G P X X T X X X L S X R A X X X A X E A X X X X X B   I D L X L V X X U X P X X X V X G B A F J X G E L G X X X X A X B X X X C S S A X X X X X							
2. CyBF_Tol9009	E R R V M G P E D I T A R S L S C R A I R E A M E A G G Y G P D D   D I L V I N A U R P D S H V V G D G A F L V G E L G R A P A   D F E T A C S S A L V G W E R R V L G P G I F T S L I S C R A K N A L E A N L S V K N D I M L V I A T I F P Q I O V P G N A A F I A G E L G F Q G A W N L D S T C S S A A I							
Consensus	160	170	180	190	200	210	220	230
1. CyBF_Pacifica	X X A S X L X I X A G K Y X X L V V X C X X X X X D Z X D X V S X M F L G D G A G A X X V X X X X X X X X Z G X L X X X X X X T X X T C G X F X X X X L X							
2. CyBF_Tol9009	H L A S D L I I A A G R Y E R I I L V T C C T Y I T R D V D Q A D S F S W F L G D G A G A M I V E A V E A S P A H E G L L A A H T I I P T V E I T C G A F A Y H L E Q S A S A L V M A R G E Y R I V N V L I I C T N Y S P F A D E N D T I S F L G D G A G A F V V S S L K -- T N Q G V L G T K I V N T A S T C G T F F N Q L T							
Consensus	240	250	260	270	280	290	300	310
1. CyBF_Pacifica	X X X X G P X X X T I X X X R X X X X X X X X X X X X L R X C X X G A L X X A G X X X E B F X X X T P T A W Y X X X C X X L G X X X X R X X D X							
2. CyBF_Tol9009	L - V D G A P A L R I V G N K L A L G R S R D H S E R Y L R T C V D G A L S E E A G A S S E U D F E L V C N T P T A W Y G P F C A D V L G F E - G R F W D N T D A Q G N P R M Y I R A A K G - T N K V L S E T A A P L L R Q C C M G A L E V A G V T U D E I N F A F N P T A W Y A S I C I Q A G L I D P E R T I D L							
Consensus	320	330	340	350	360	370	380	397
1. CyBF_Pacifica	X X R X A N X G P X L X X X N L X X X X X G X X R X X D L V L X X I G S V S X A X A X X R X G X V A G X X P A P P V S F D L Q A D K V F S L R M P R F A N C G P A L W P N N H L T A C E F G R V P D G L V G S I G S V S T A C A V L R I V G E V A G A Q P							
2. CyBF_Tol9009	H K R Y A N I G P M P L T P A N L Y H G A S S G I R E N D L V L V N Y I G S V S N A G A T V M R W G D V A L G P A P A P P V S F D L Q A D K V F S L R							

**Figure S25.** Amino acid alignment of myxobacterial CybF homolog from *P. pacifica* DSM 14875<sup>T</sup> (CybF\_Pacifica) and CybF from *Tolypothrix* sp. PCC 9009 (CybE\_Tol9009). Pairwise identity: 39.3%, pairwise positive (BLSM62): 59.6%.



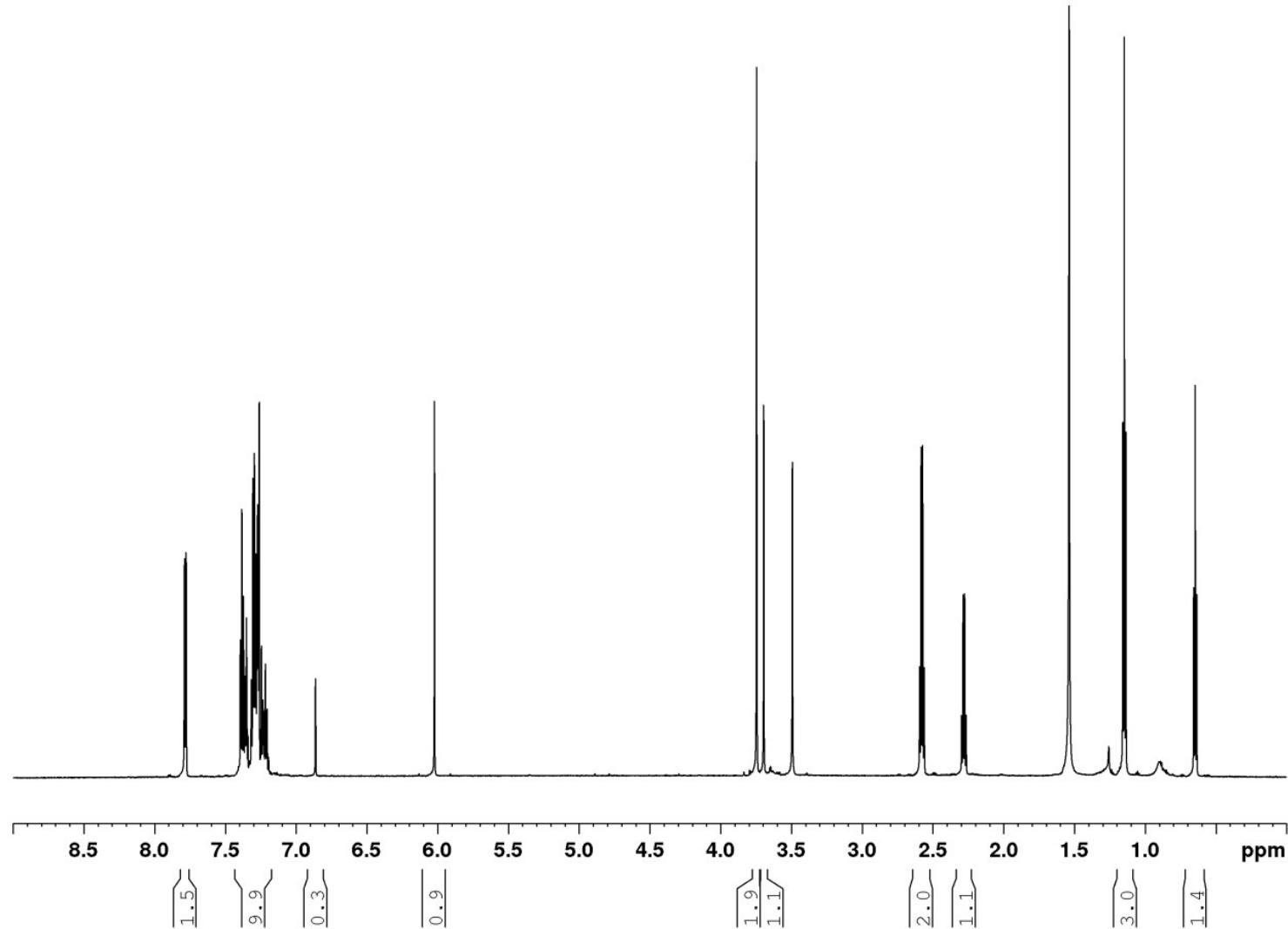
**Figure S26.** Phyre2 structure homology model of the thiamine pyrophosphate-binding protein (TPP) encoded by *cybE* from *Tolypothrix* sp. PCC 9009 (A) and the identified *cybE* homolog from *P. pacifica* DSM 14875<sup>T</sup> (models are based on the template c3ey9B; figure colored by rainbow N → C terminus).



**Figure S27.** Phyre2 structure homology model of the furanolide encoded by *cybF* from *Tolypothrix* sp. PCC 9009 (A) and the identified *cybF* homolog from *Plesiocystis pacifica* DSM 14875<sup>T</sup>. A) Model based on template c4x0oG. B) Model based on template c6a9nA; figure colored by rainbow N → C terminus).

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**4.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for 1–8****4.1 NMR spectra of 1 and 2**



**Figure S28.** <sup>1</sup>H NMR spectrum of **1** and **2** (700 MHz, CDCl<sub>3</sub>).

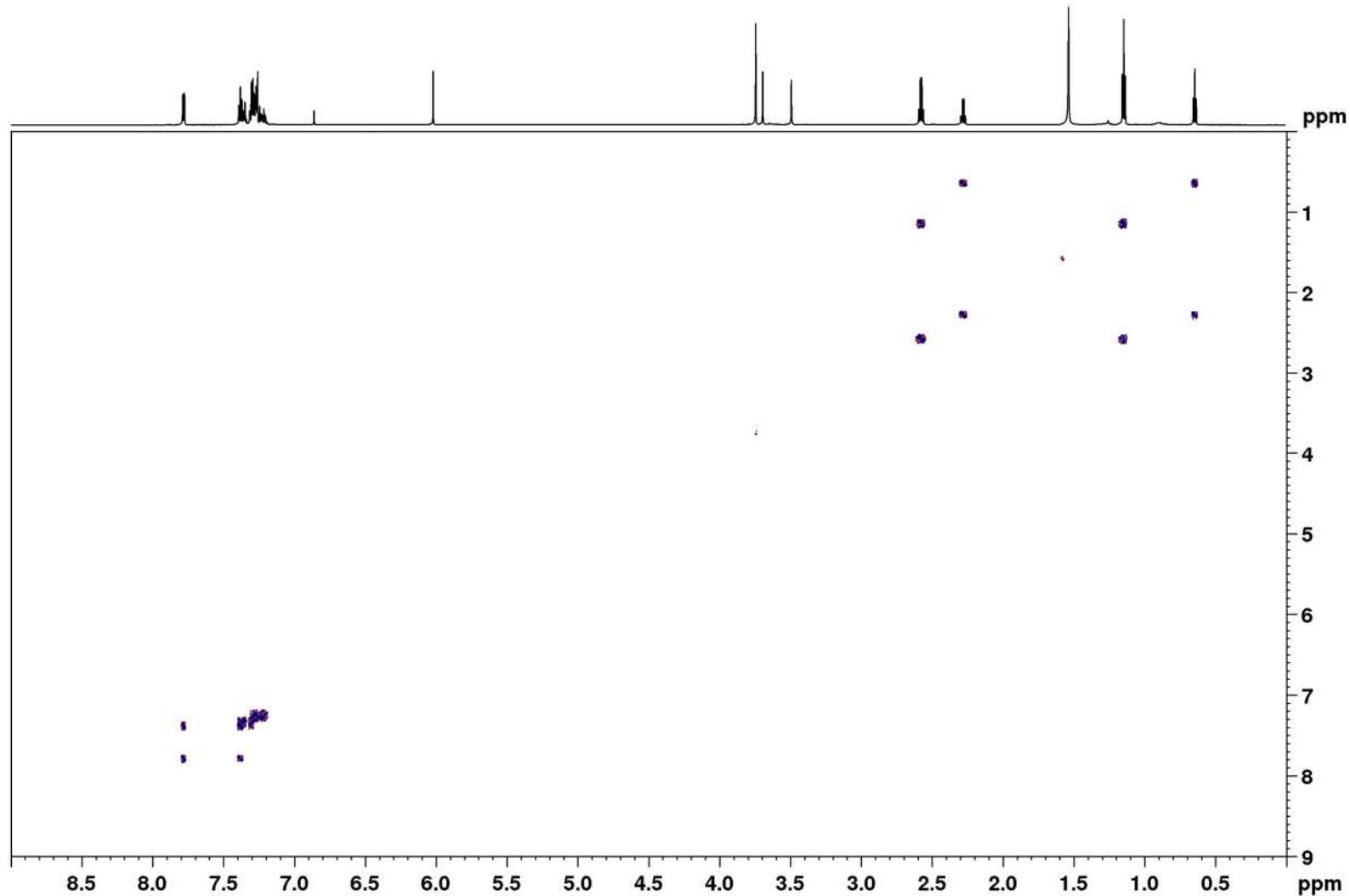


Figure S29. DQF-COSY spectrum of **1** and **2** (700 MHz,  $\text{CDCl}_3$ ).

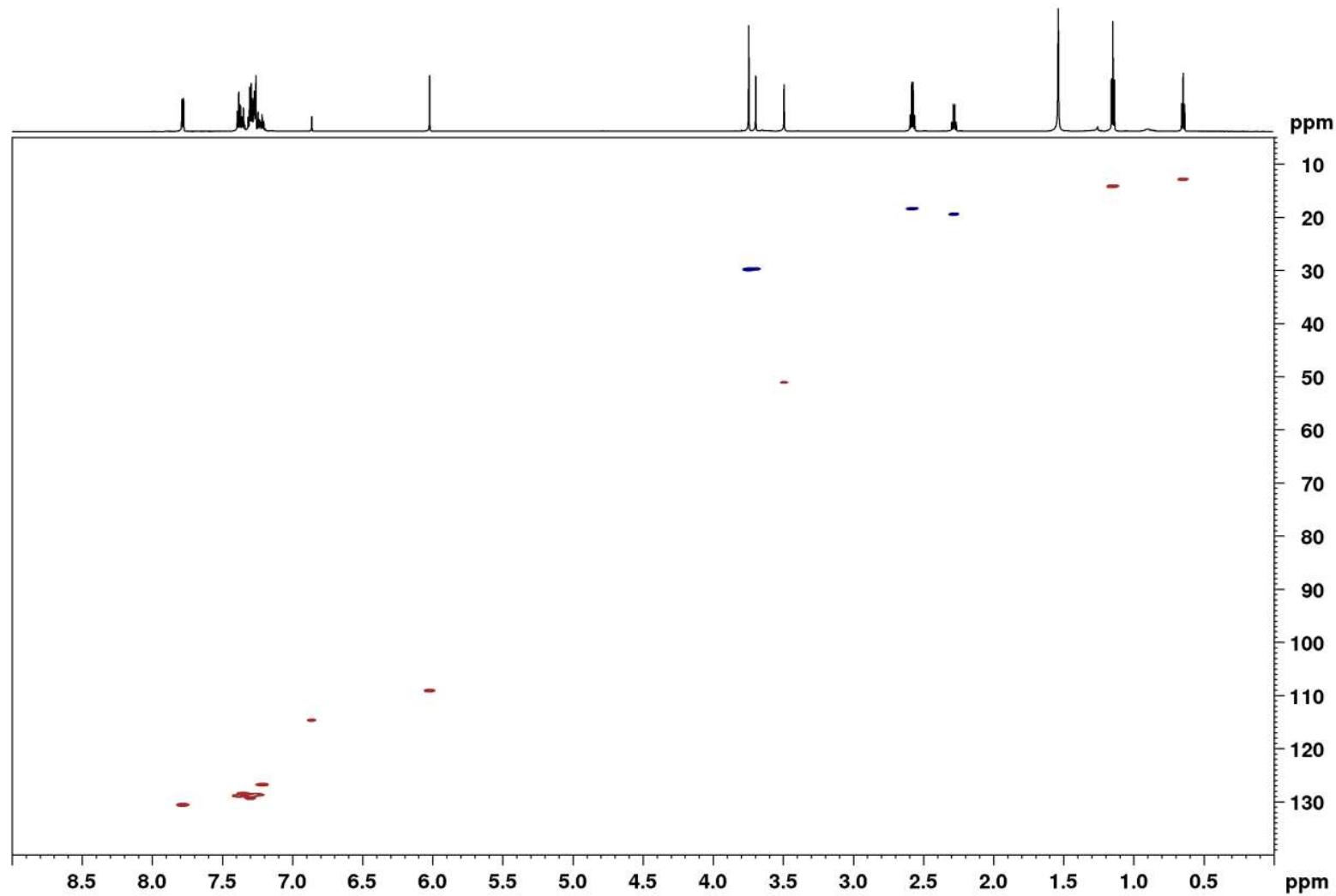


Figure S30. HSQC spectrum of **1** and **2** (700 MHz,  $\text{CDCl}_3$ ).

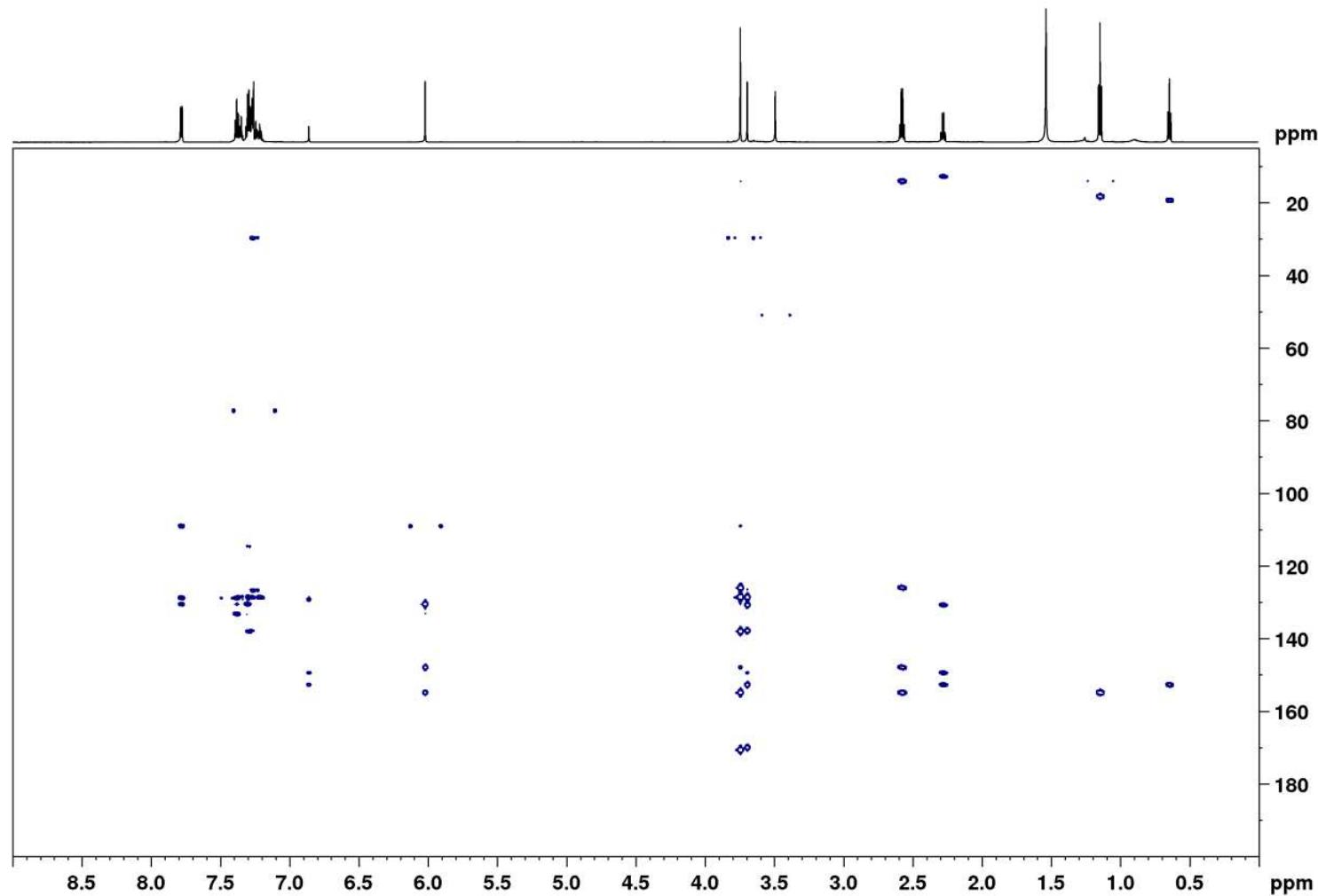


Figure S31. HMBC spectrum of **1** and **2** (700 MHz,  $\text{CDCl}_3$ ).

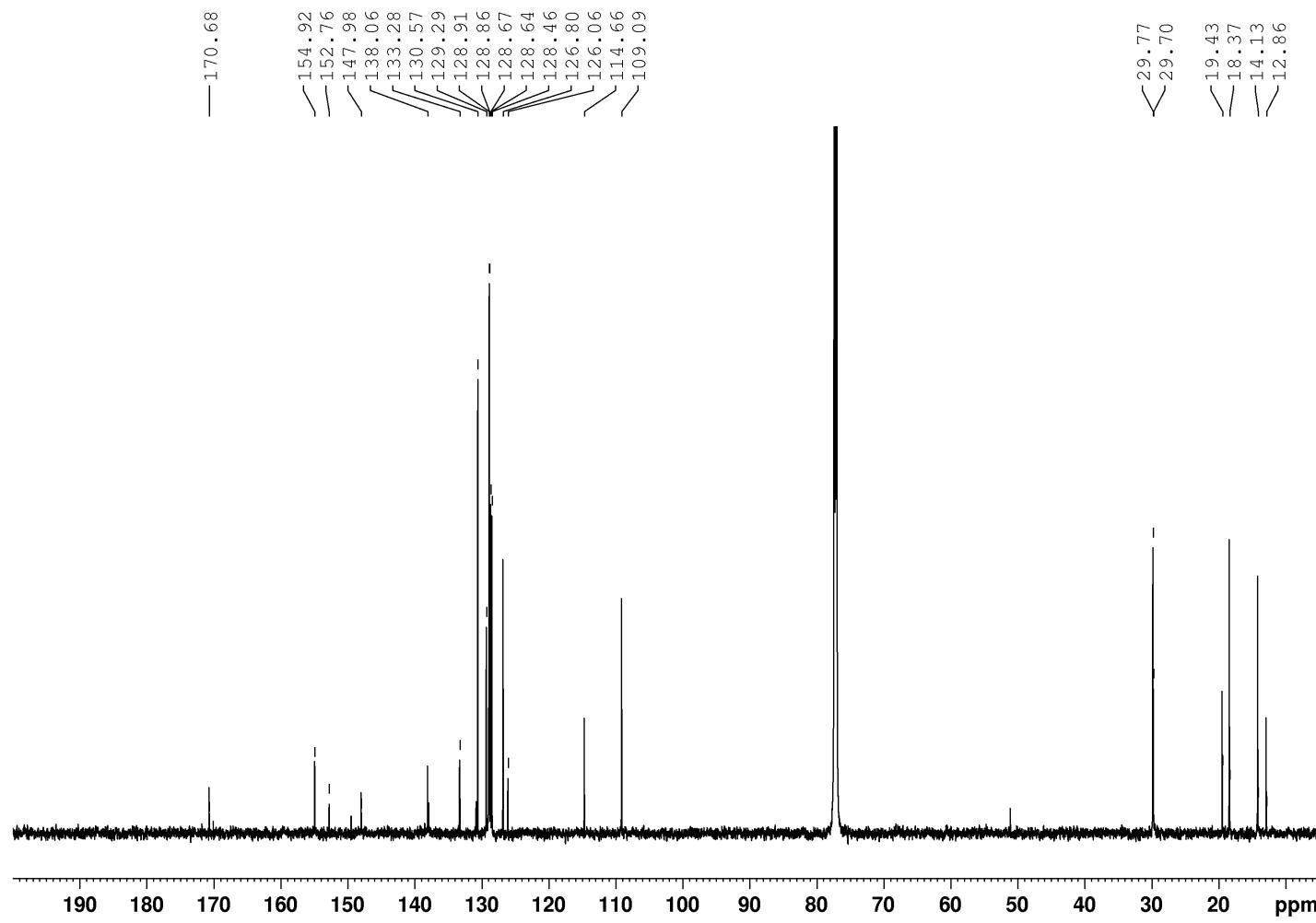


Figure S32. <sup>13</sup>C NMR spectrum of **1** and **2** (700 MHz, CDCl<sub>3</sub>).

4.2 NMR spectra of **3** and **4**

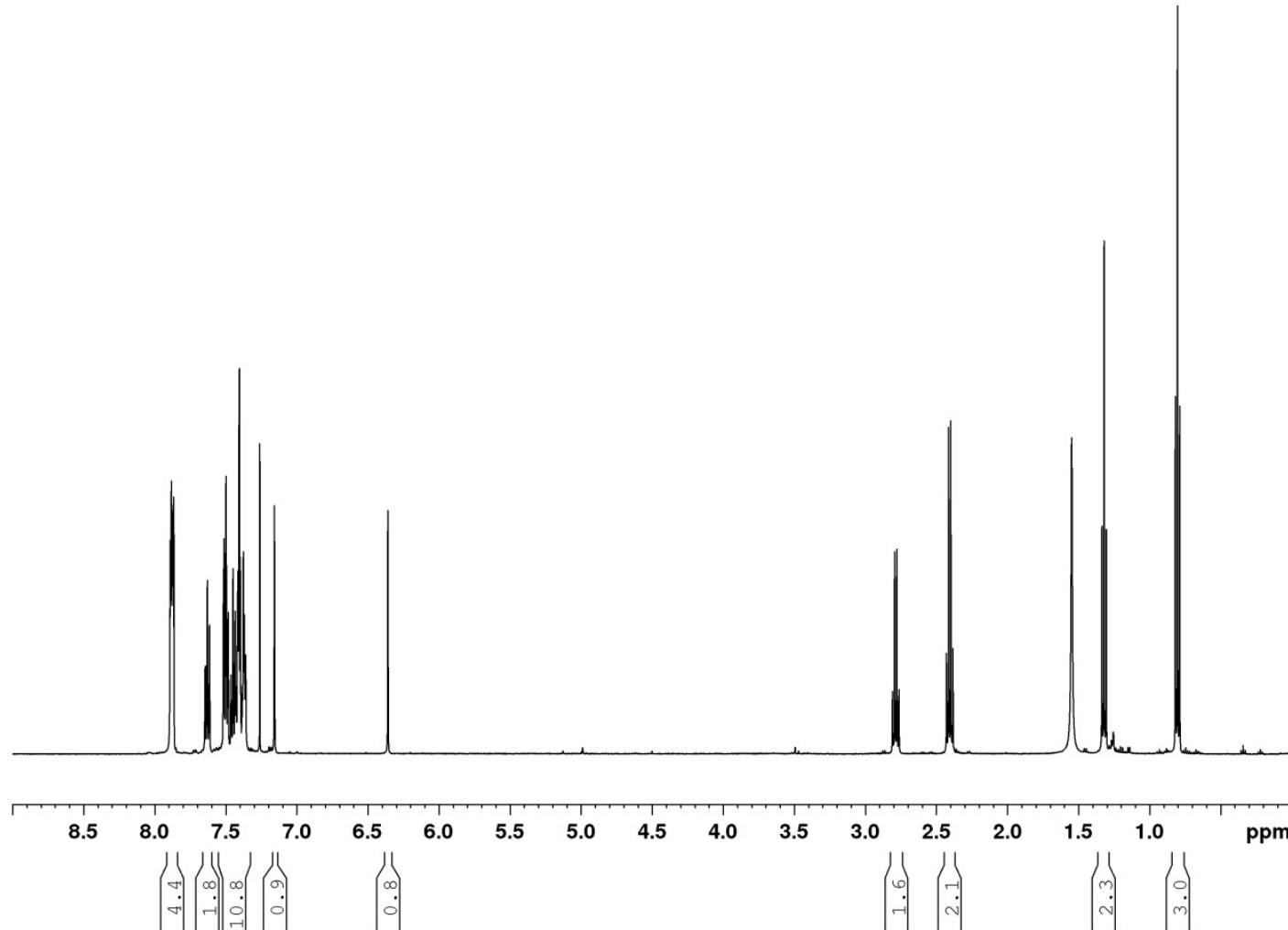


Figure S33. <sup>1</sup>H NMR spectrum of 3 and 4 (500 MHz, CDCl<sub>3</sub>).

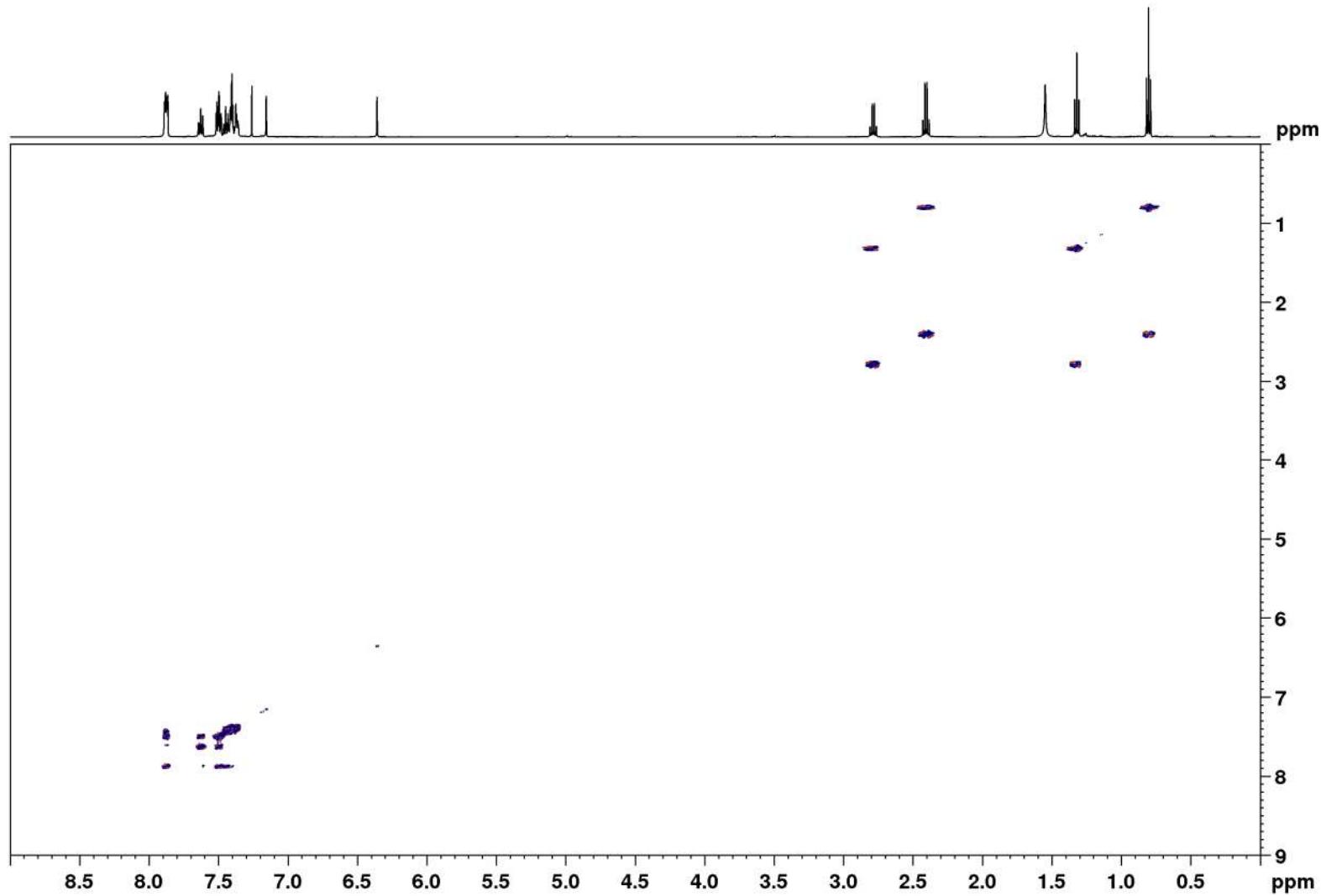


Figure S34. DQF-COSY spectrum of **3** and **4** (500 MHz,  $\text{CDCl}_3$ ).

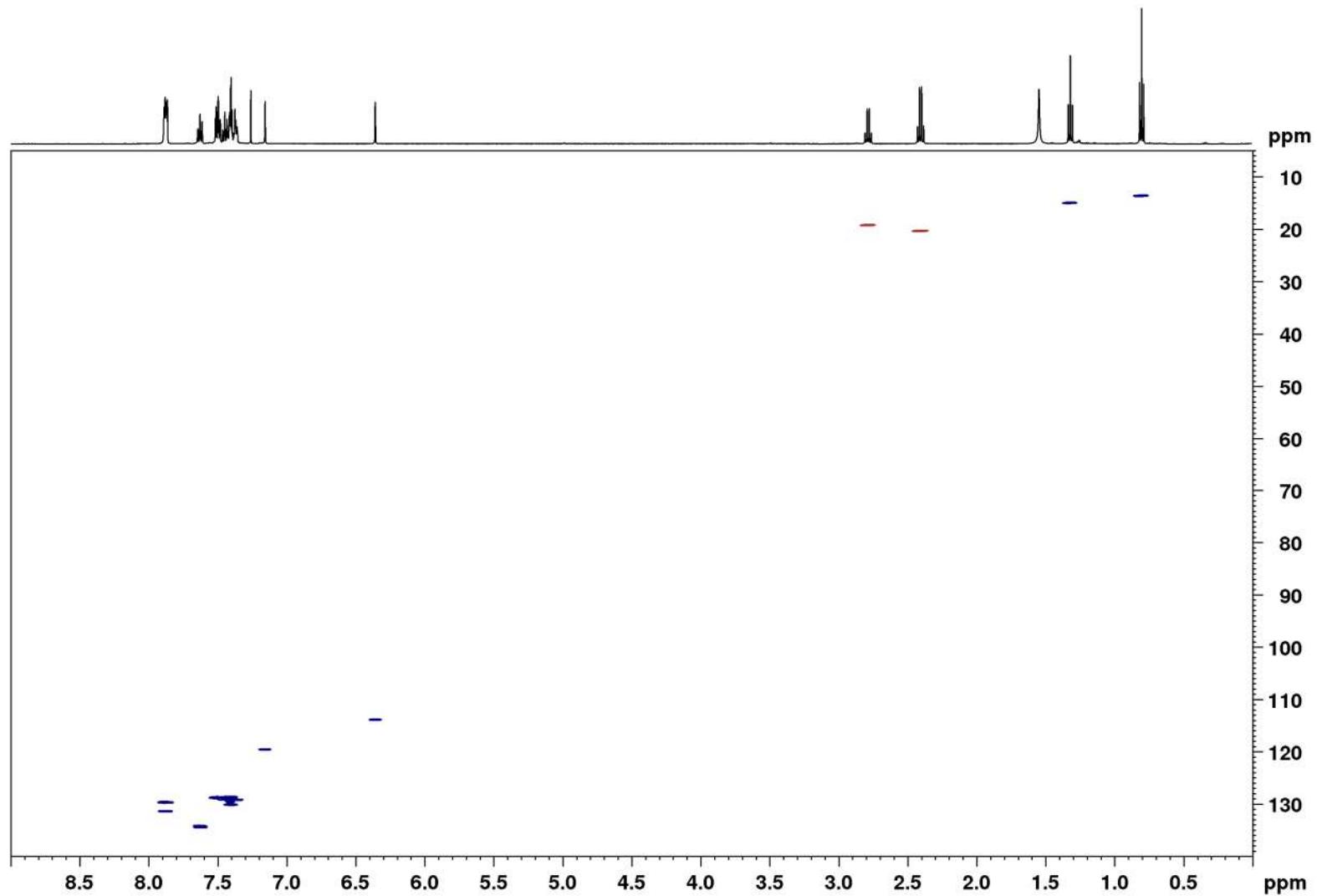


Figure S35. HSQC spectrum of 3 and 4 (500 MHz, CDCl<sub>3</sub>).

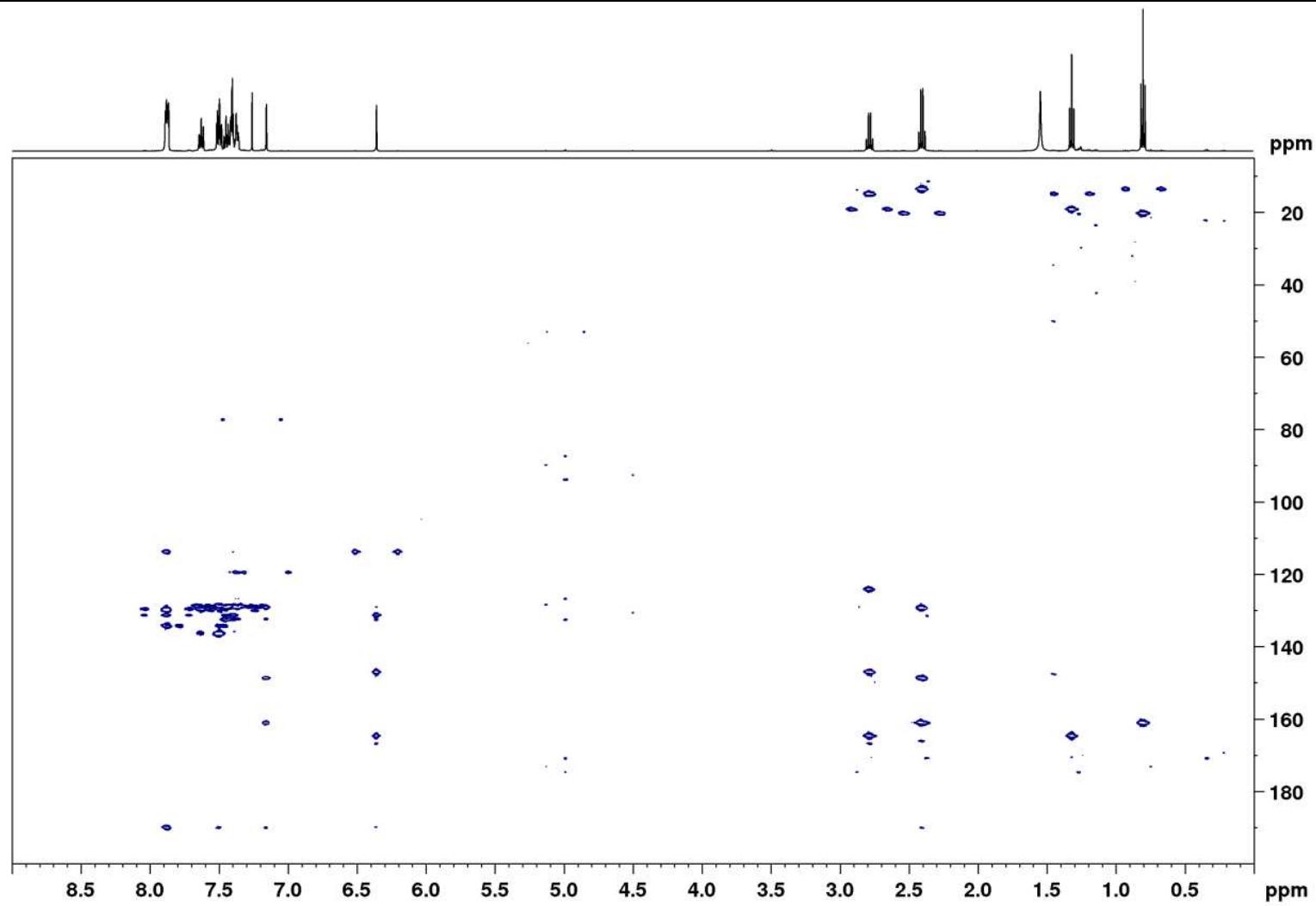
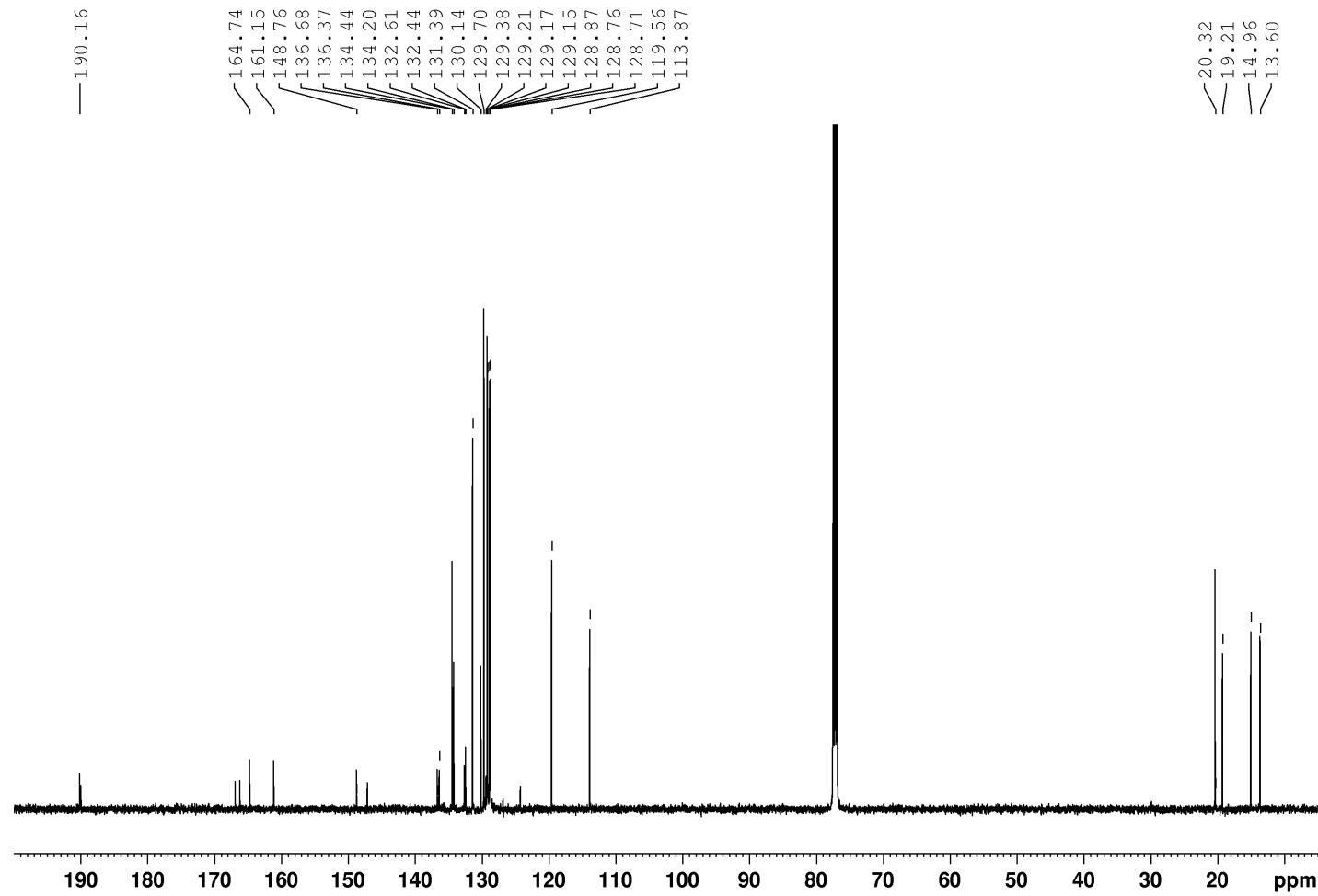


Figure S36. HMBC spectrum of **3** and **4** (500 MHz,  $\text{CDCl}_3$ ).



— 190.16



**Figure S37.** <sup>13</sup>C NMR spectrum of **3** and **4** (125 MHz, CDCl<sub>3</sub>).

## 4.3 NMR spectra of 5

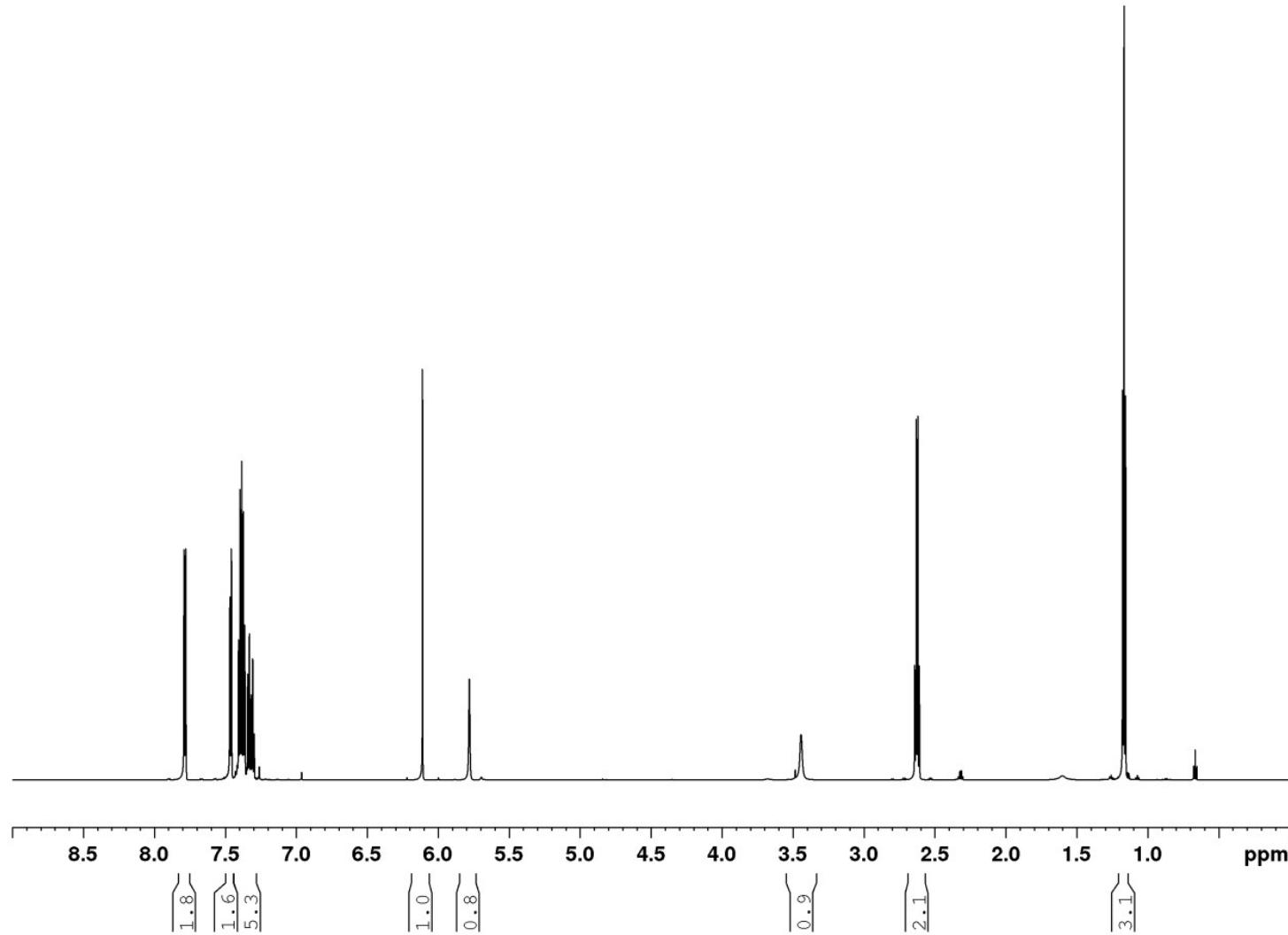


Figure S38.  $^1\text{H}$  NMR spectrum of 5 (700 MHz,  $\text{CDCl}_3$ ).

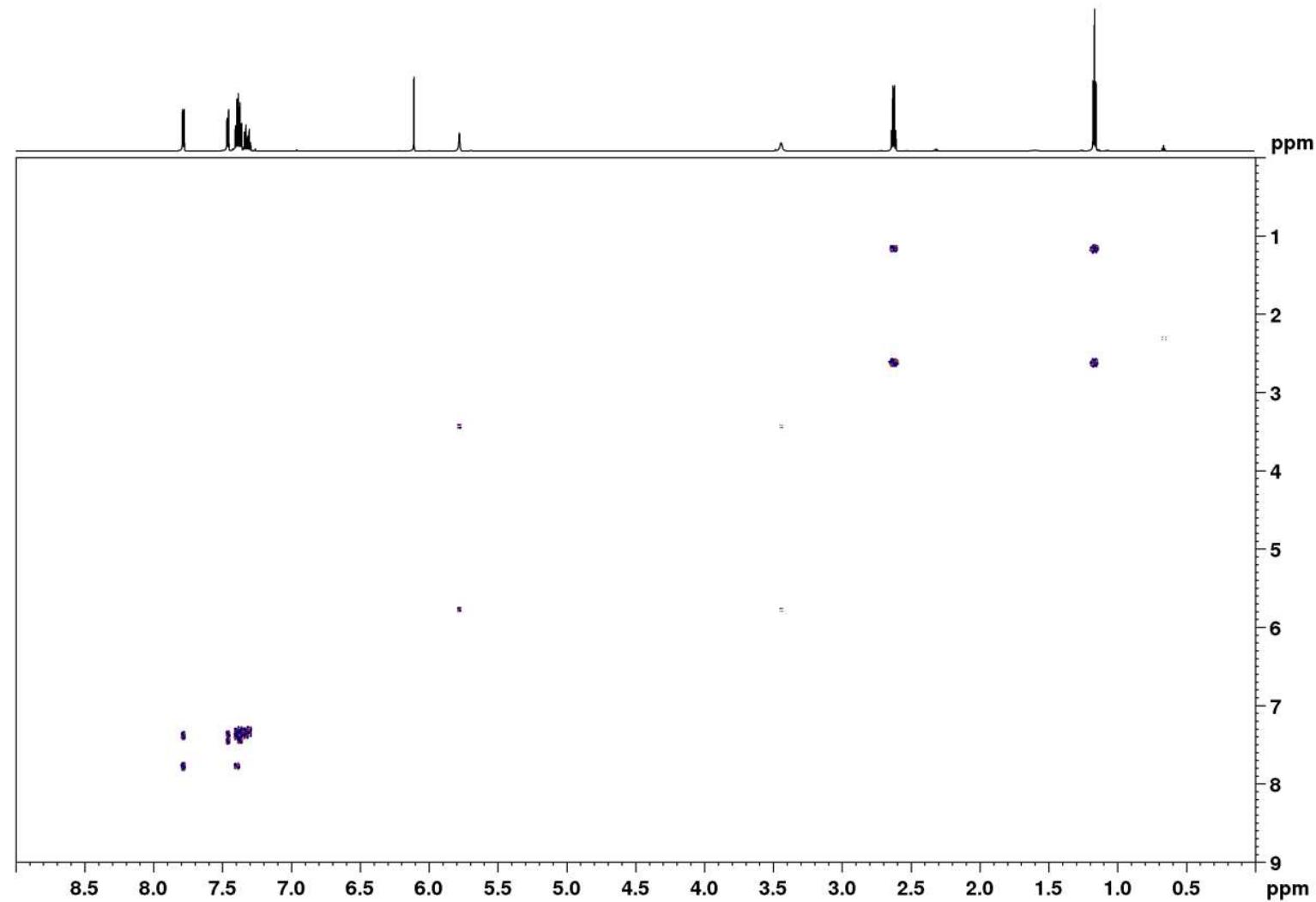


Figure S39. DQF-COSY spectrum of **5** (700 MHz,  $\text{CDCl}_3$ ).

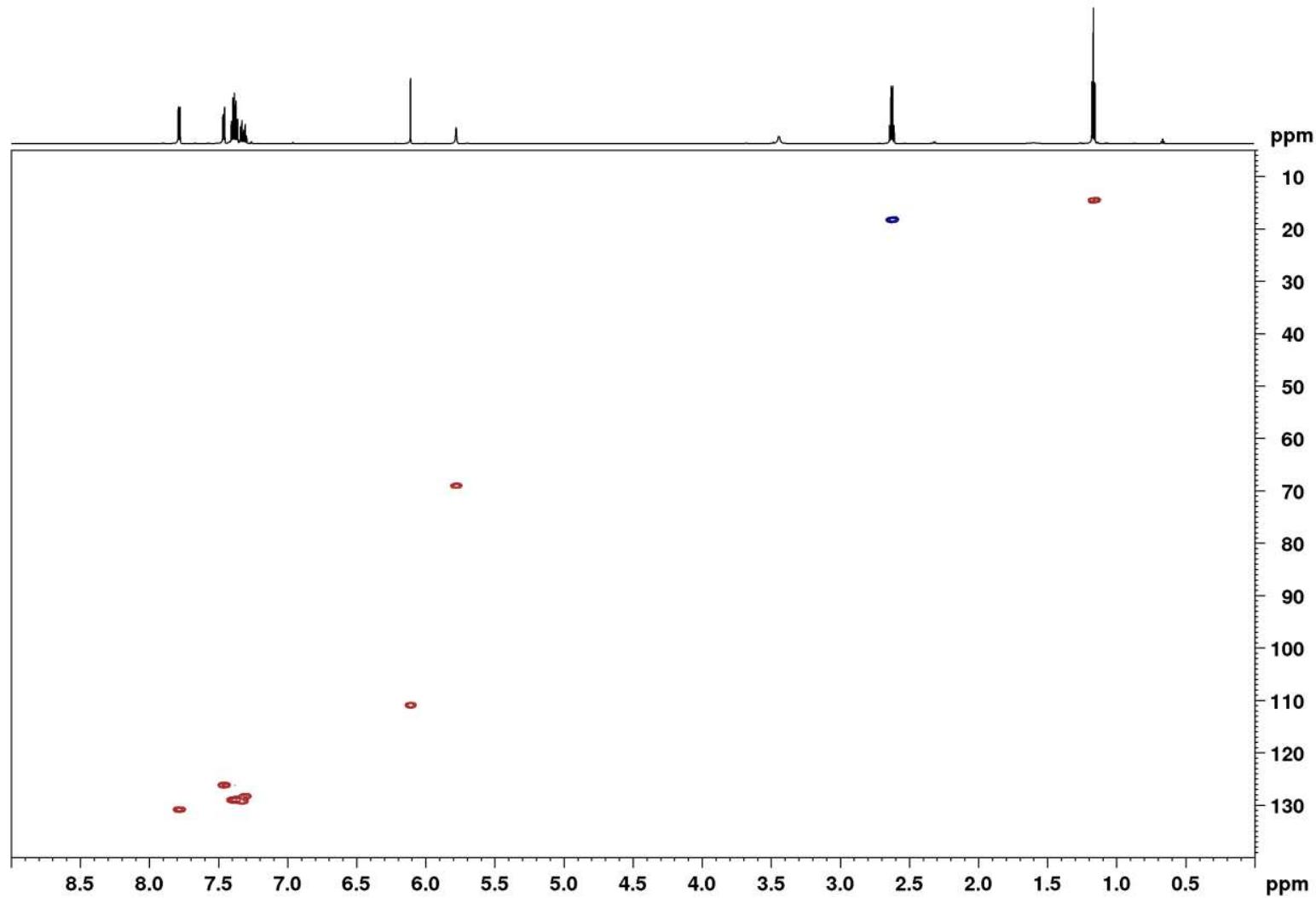


Figure S40. HSQC spectrum of 5 (700 MHz,  $\text{CDCl}_3$ ).

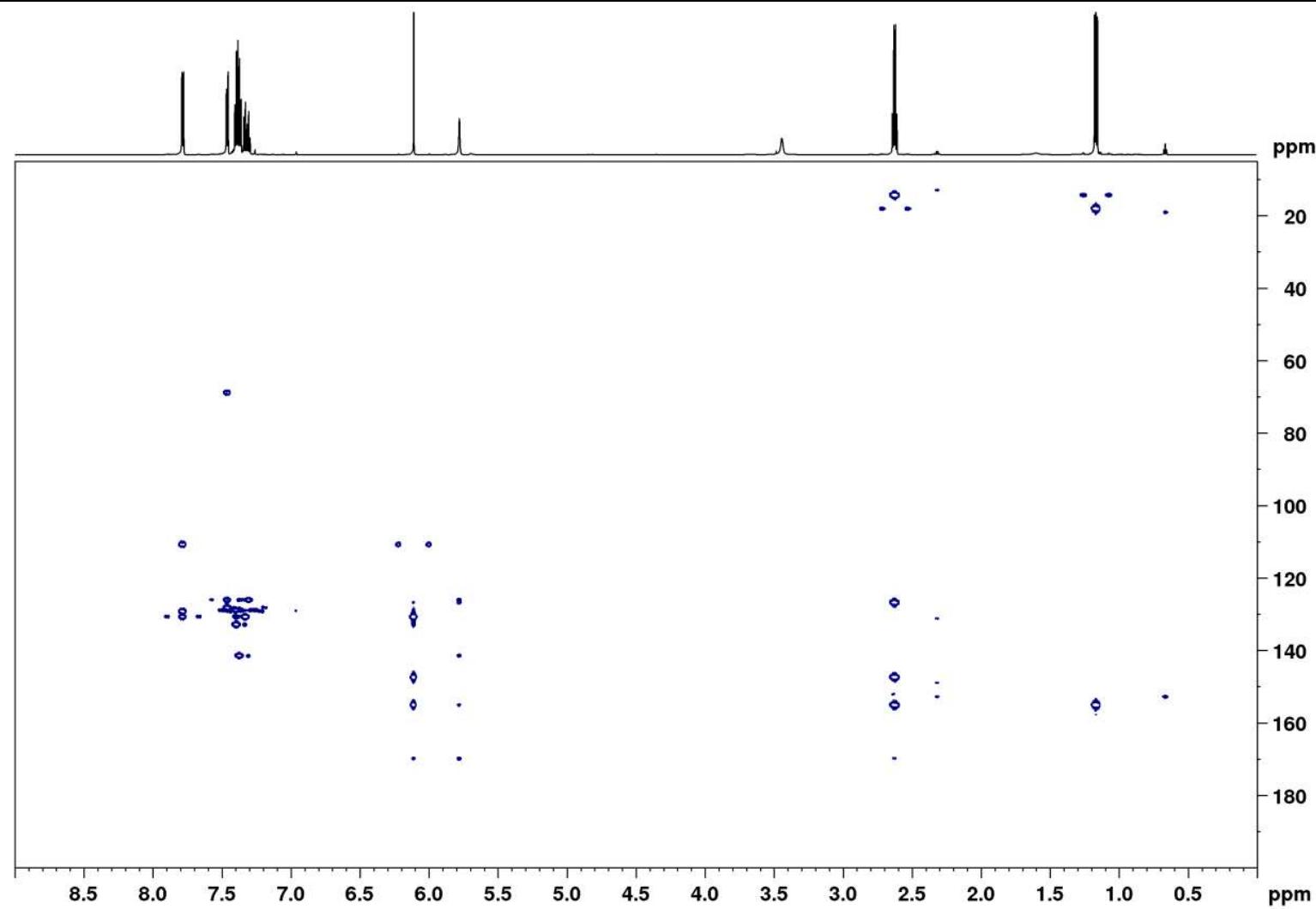
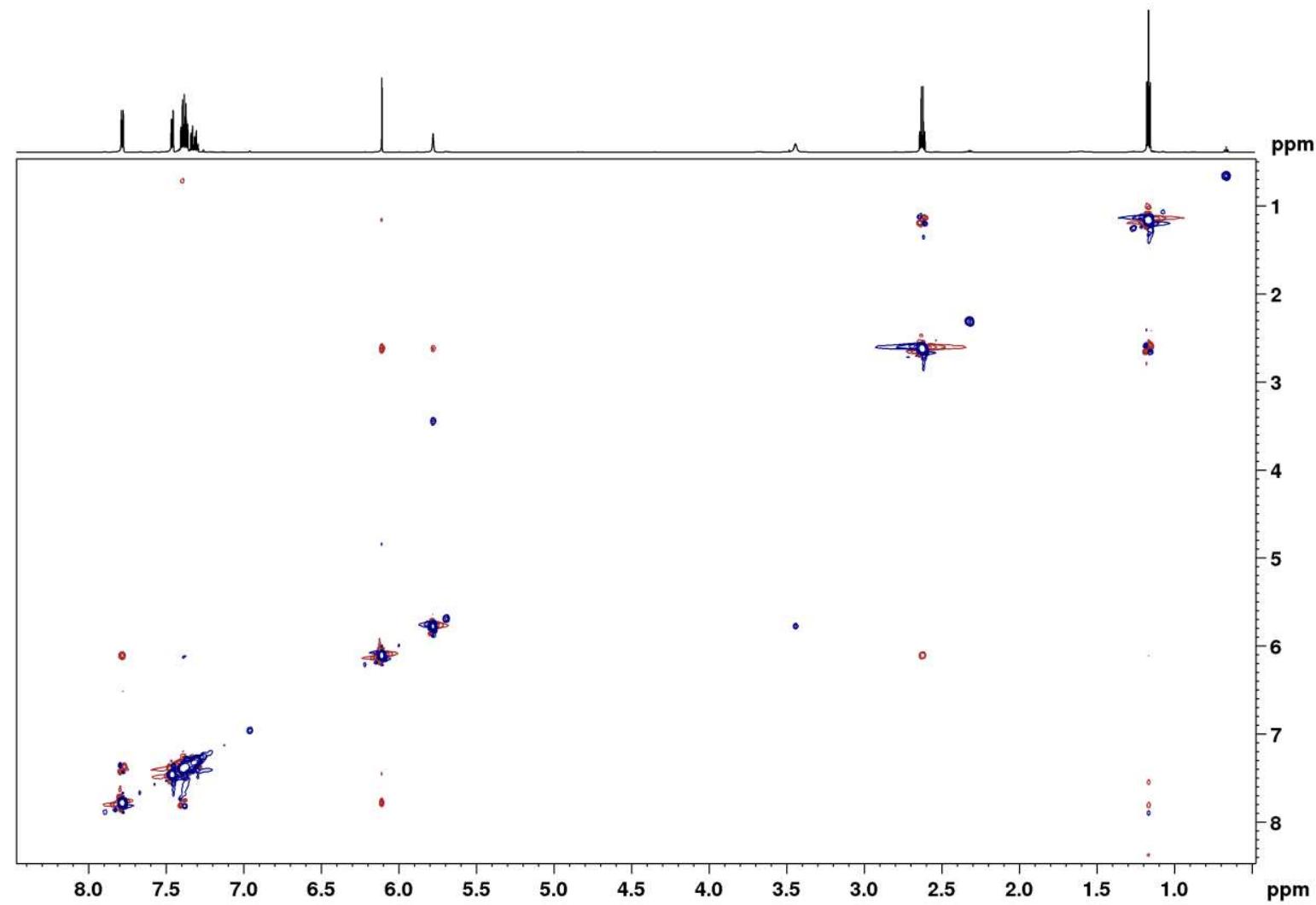
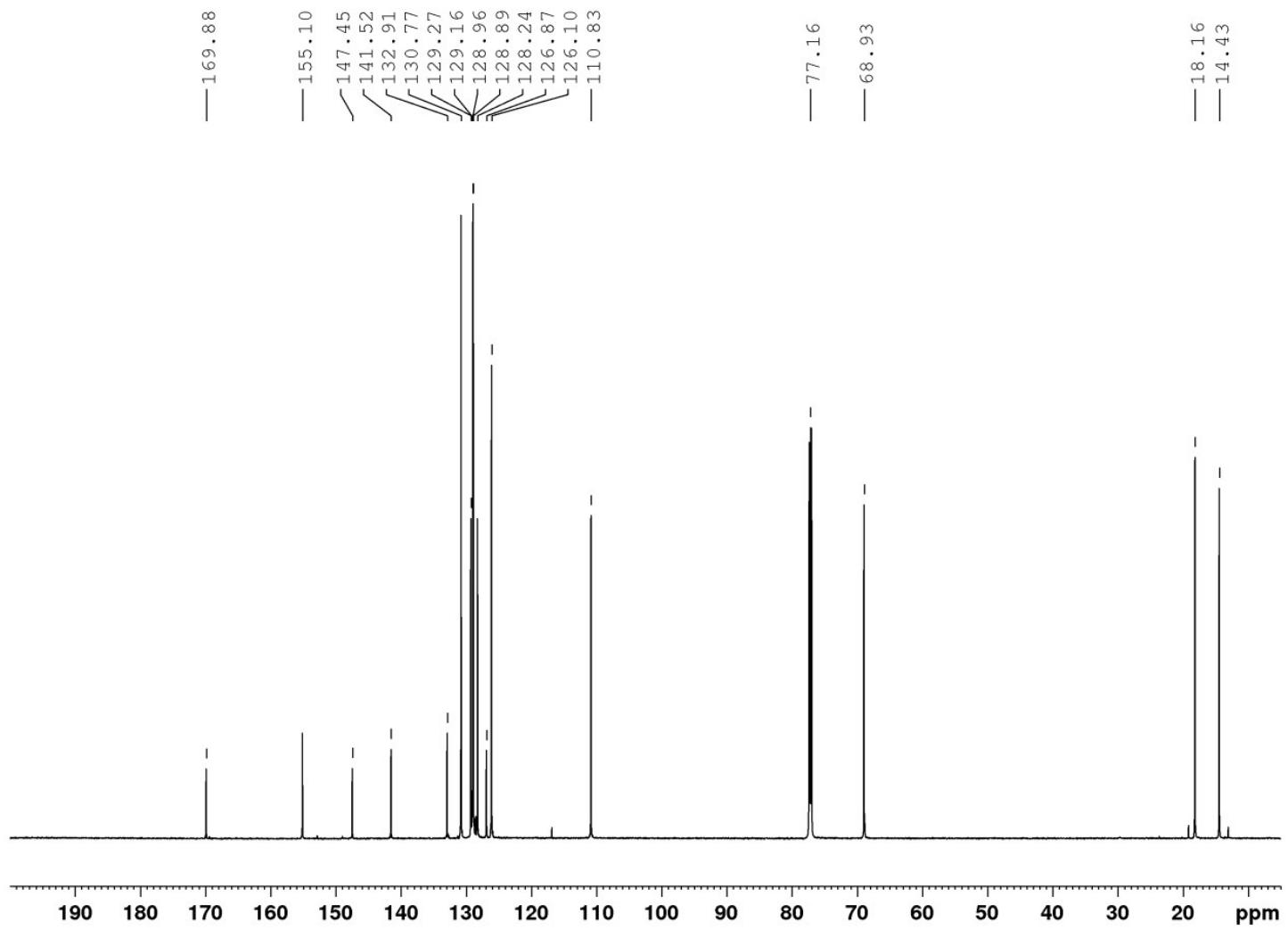


Figure S41. HMBC spectrum of 5 (700 MHz,  $\text{CDCl}_3$ ).

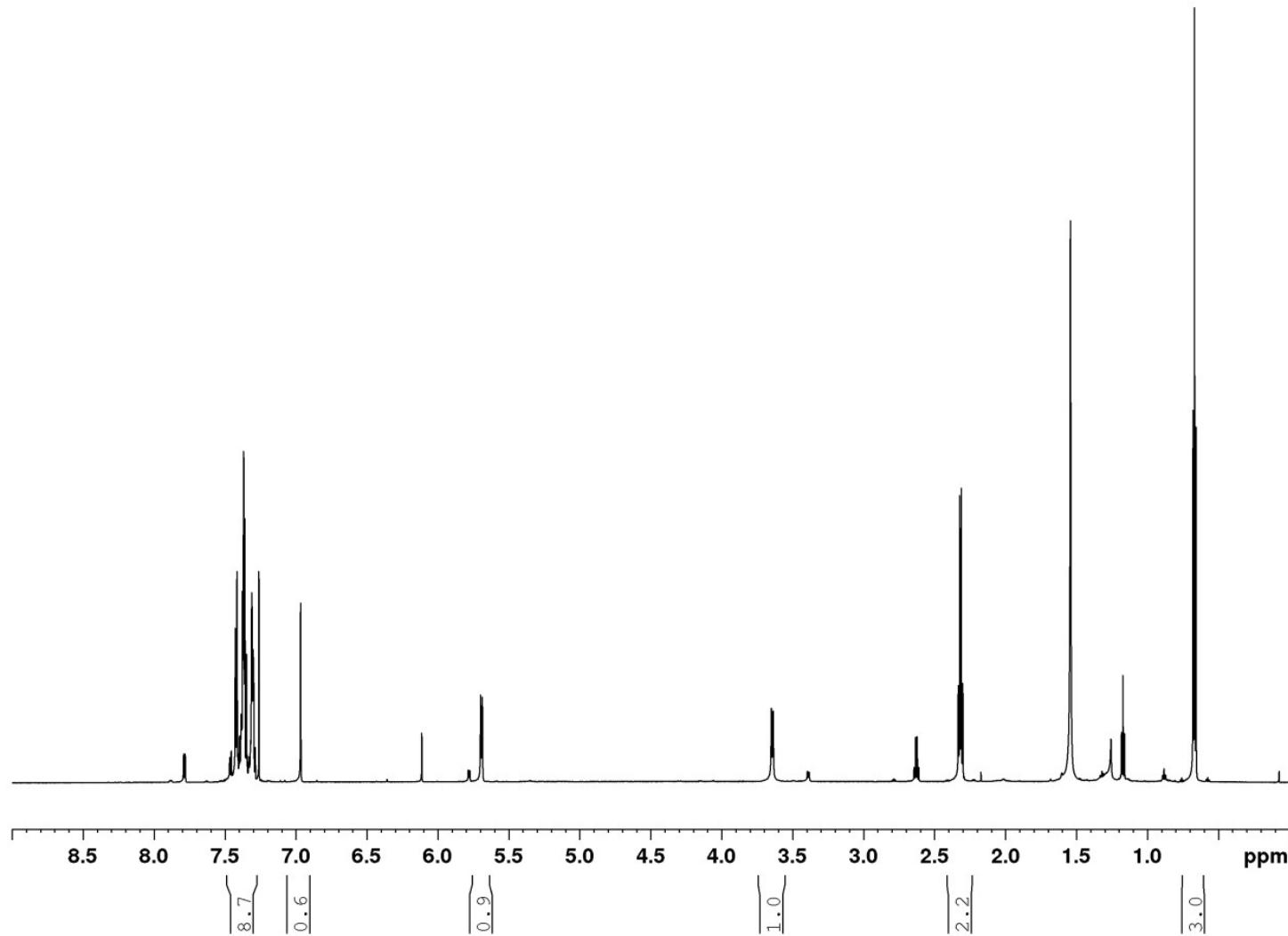


**Figure S42.** ROESY spectrum of **5** (700 MHz,  $\text{CDCl}_3$ , 400 ms).



**Figure S43.**  $^{13}\text{C}$  NMR spectrum of 5 (175 MHz,  $\text{CDCl}_3$ ).

4.4 NMR spectra of **6**



**Figure S44.**  $^1\text{H}$  NMR spectrum of **6** (700 MHz,  $\text{CDCl}_3$ ).

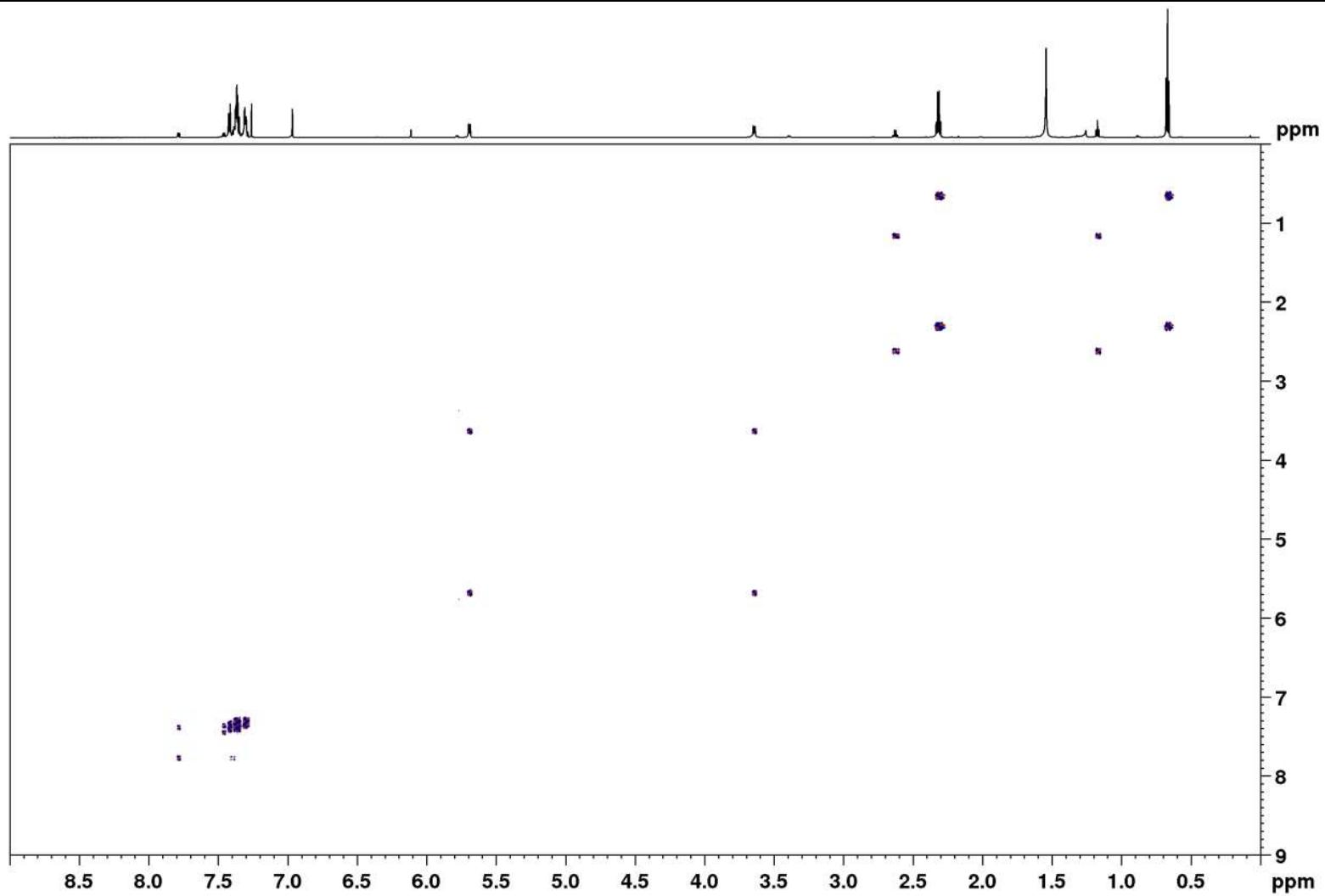


Figure S45. DQF-COSY spectrum of **6** (700 MHz,  $\text{CDCl}_3$ ).

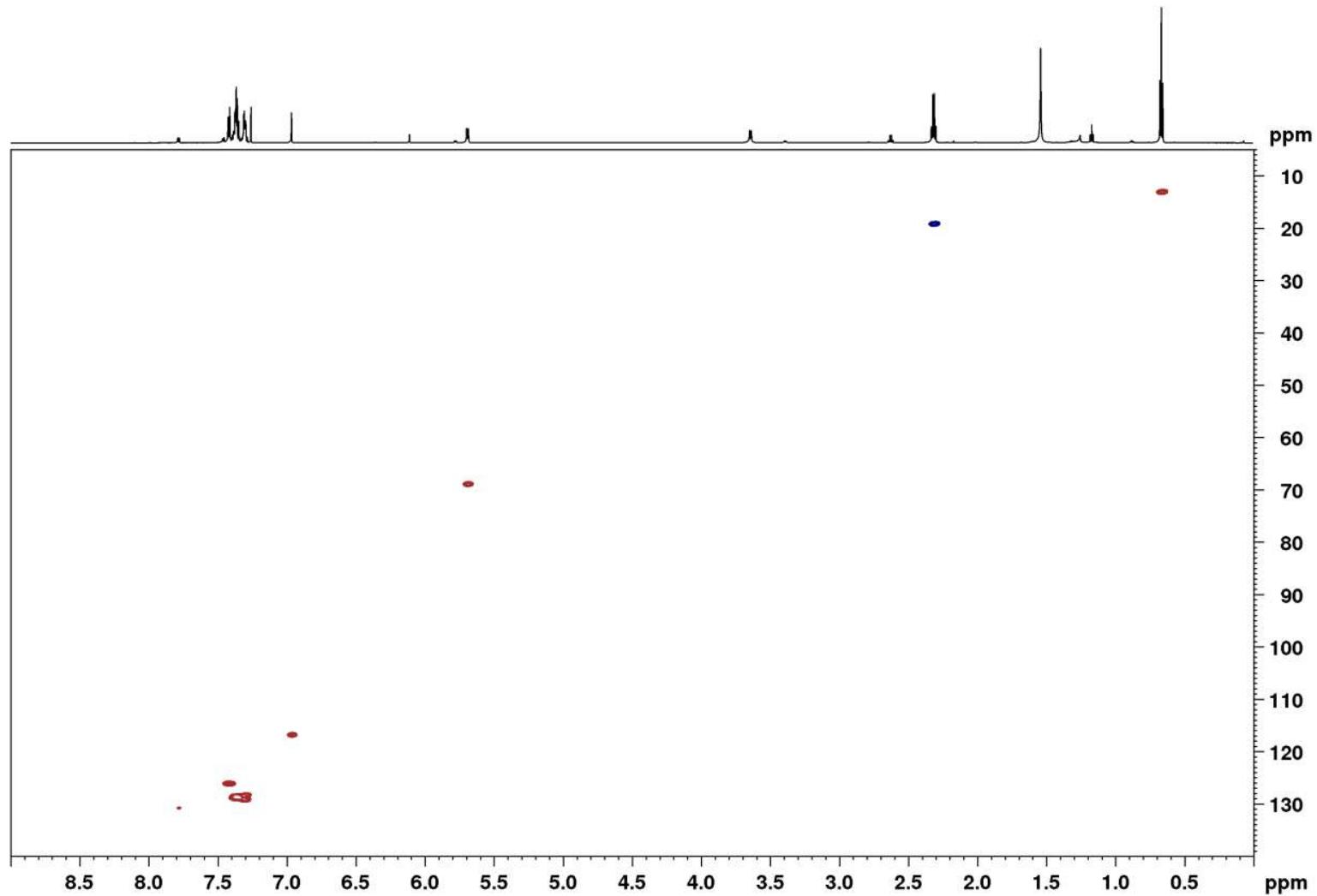


Figure S46. HSQC spectrum of **6** (700 MHz,  $\text{CDCl}_3$ ).

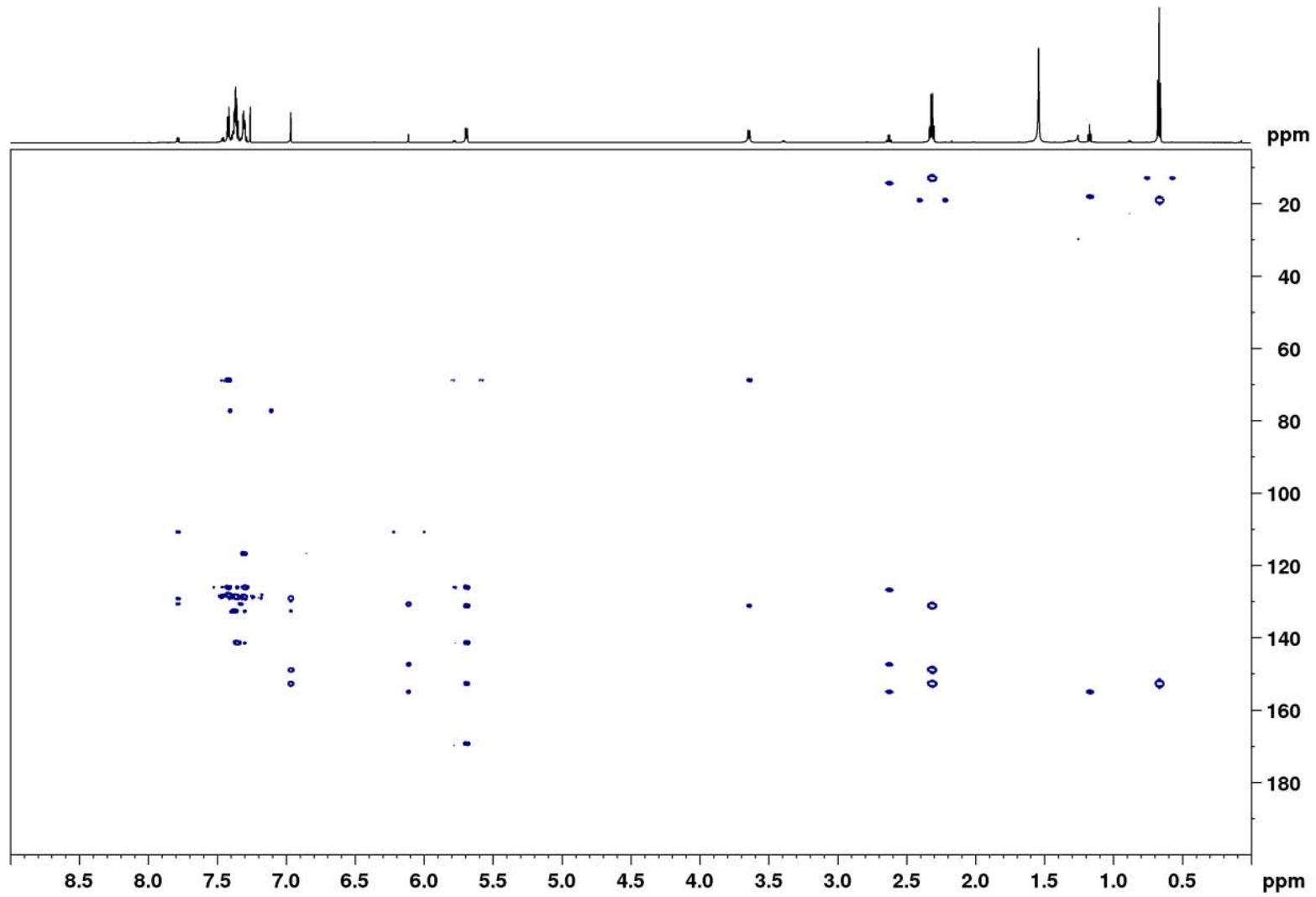


Figure S47. HMBC spectrum of **6** (700 MHz,  $\text{CDCl}_3$ ).

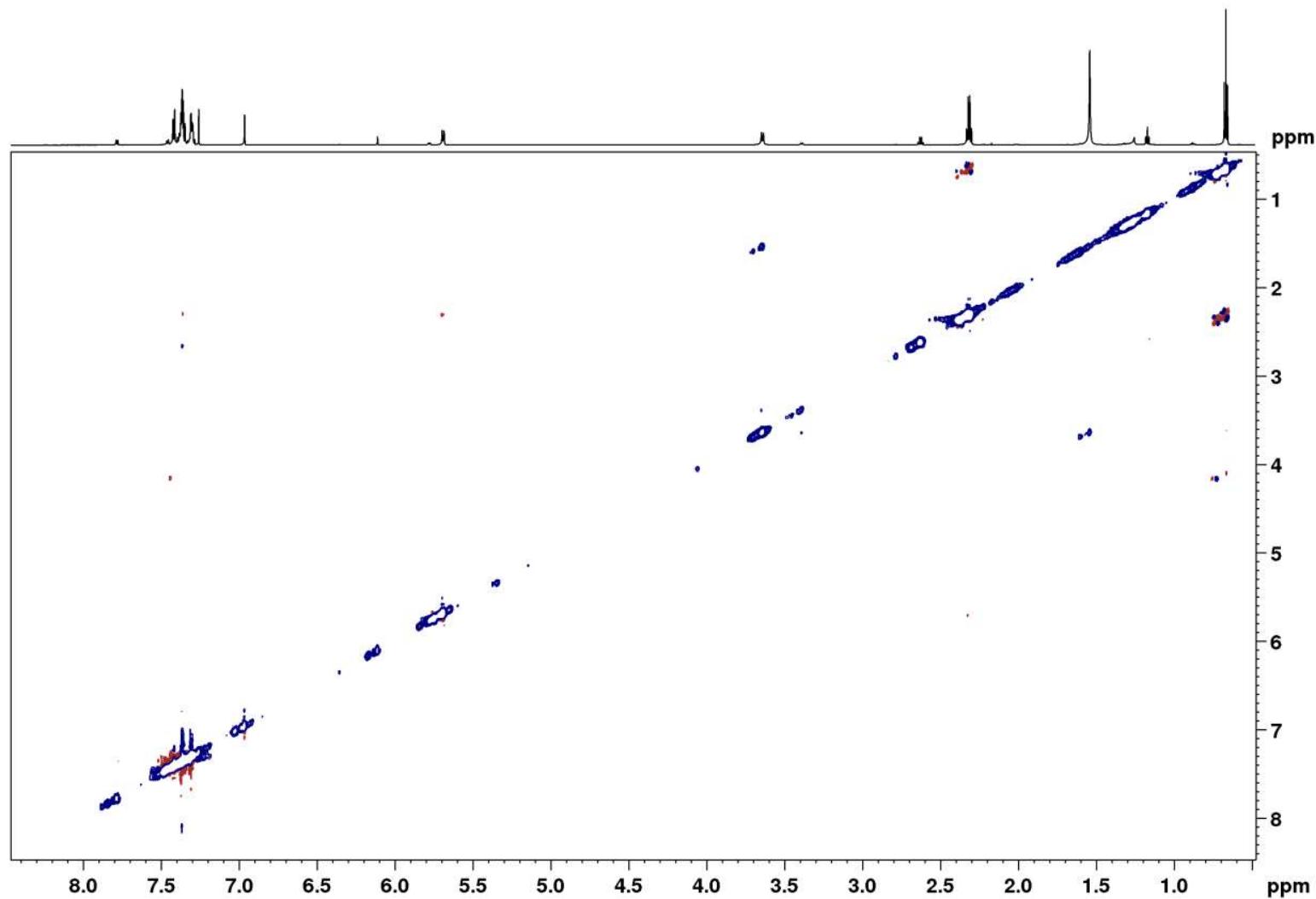
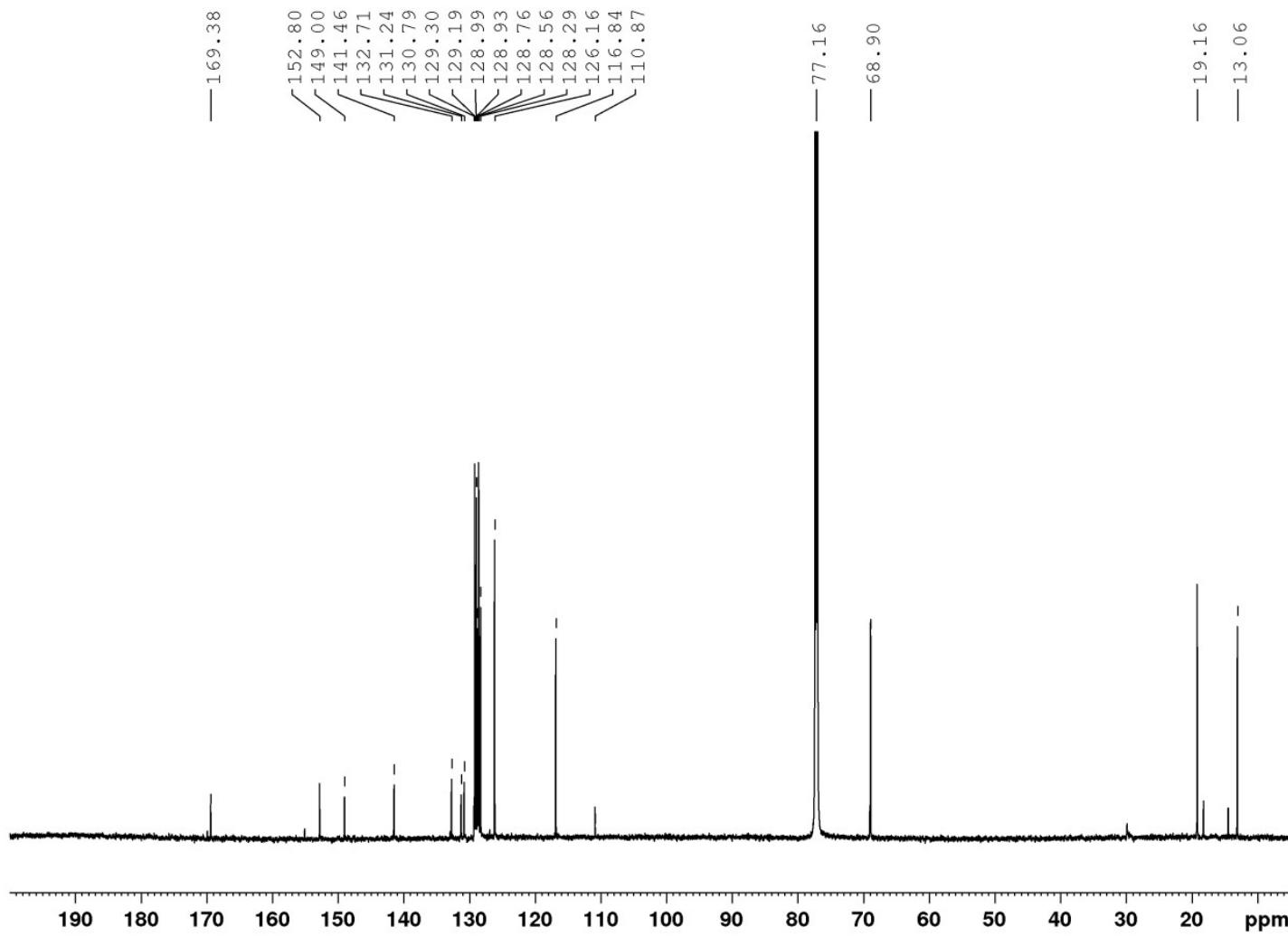


Figure S48. ROESY spectrum of 6 (700 MHz,  $\text{CDCl}_3$ , 300 ms).



**Figure S49.**  $^{13}\text{C}$  NMR spectrum of 6 (175 MHz, CDCl<sub>3</sub>).



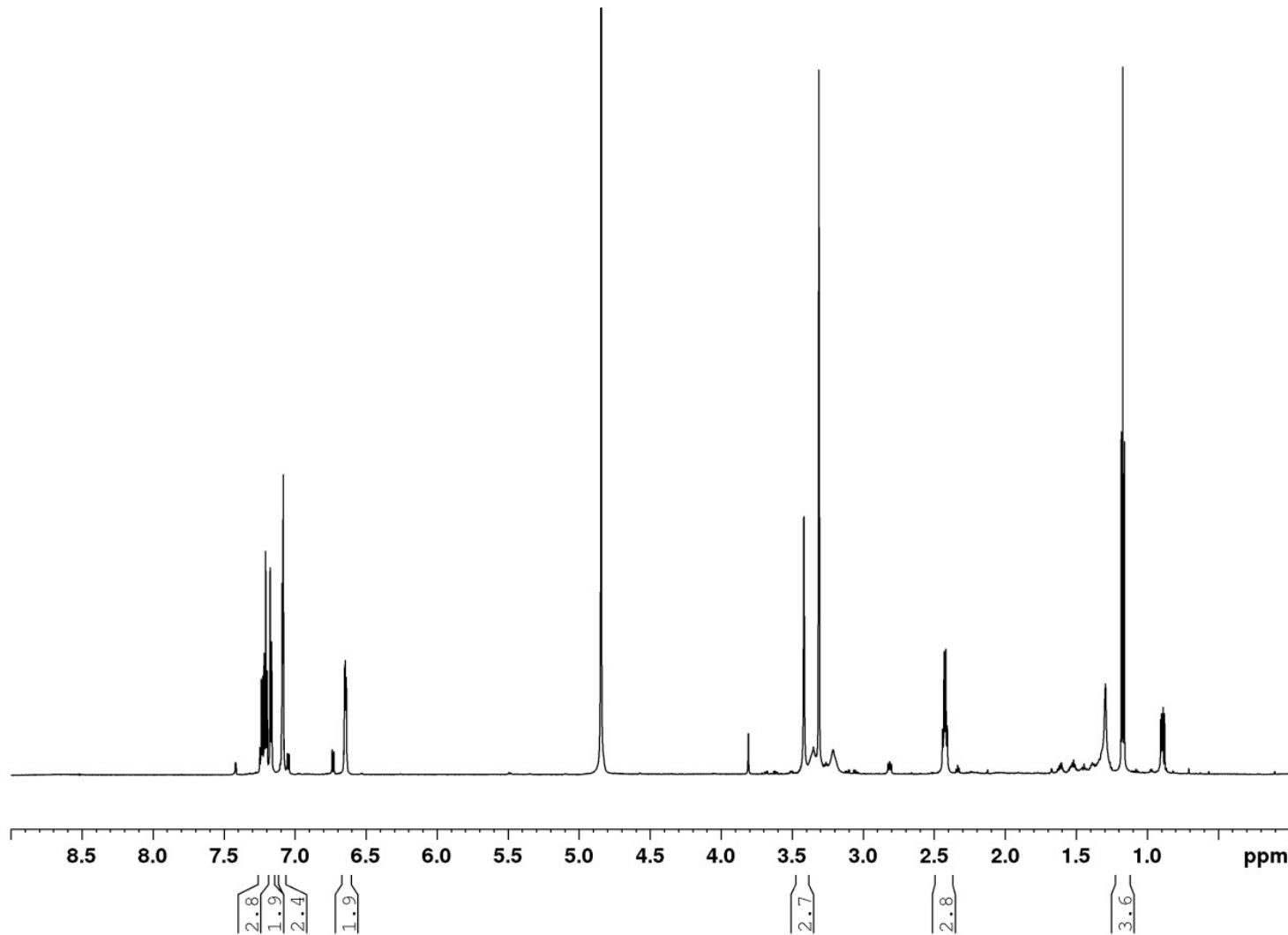


Figure S50.  $^1\text{H}$  NMR spectrum of 7 (700 MHz,  $\text{CD}_3\text{OD}$ ).

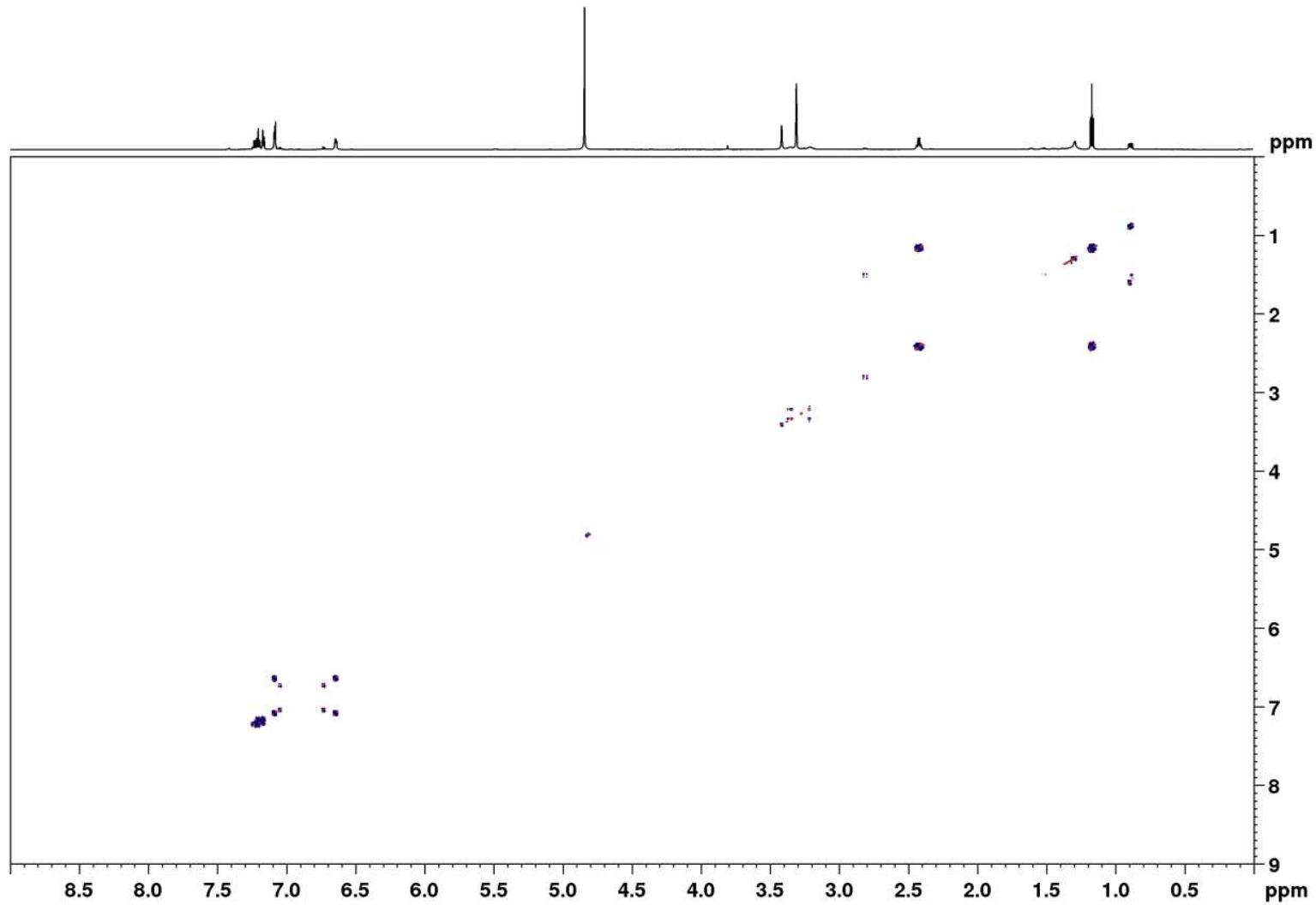


Figure S51. DQF-COSY spectrum of 7 (700 MHz,  $\text{CD}_3\text{OD}$ ).

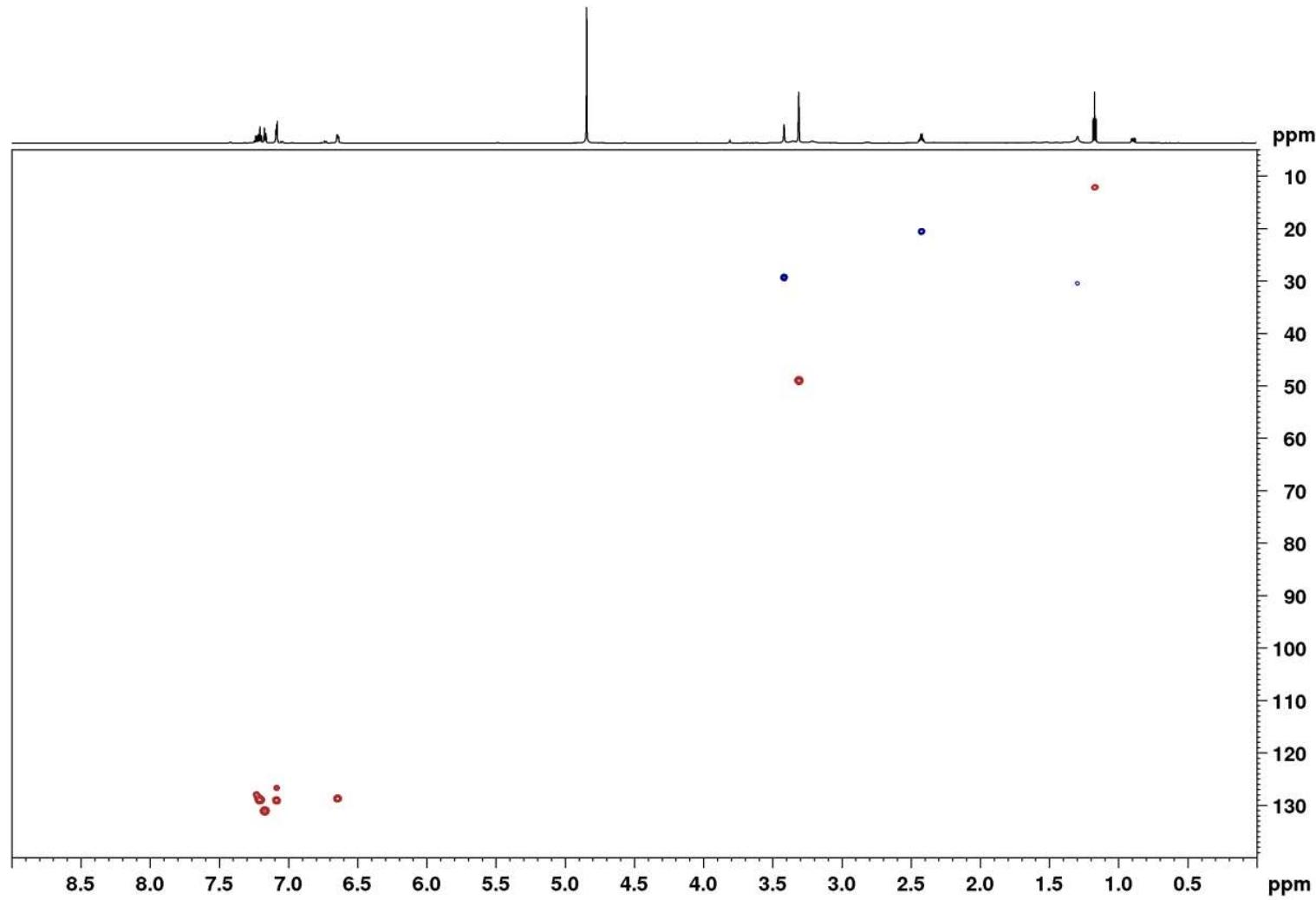


Figure S52. HSQC spectrum of 7 (700 MHz, CD<sub>3</sub>OD).

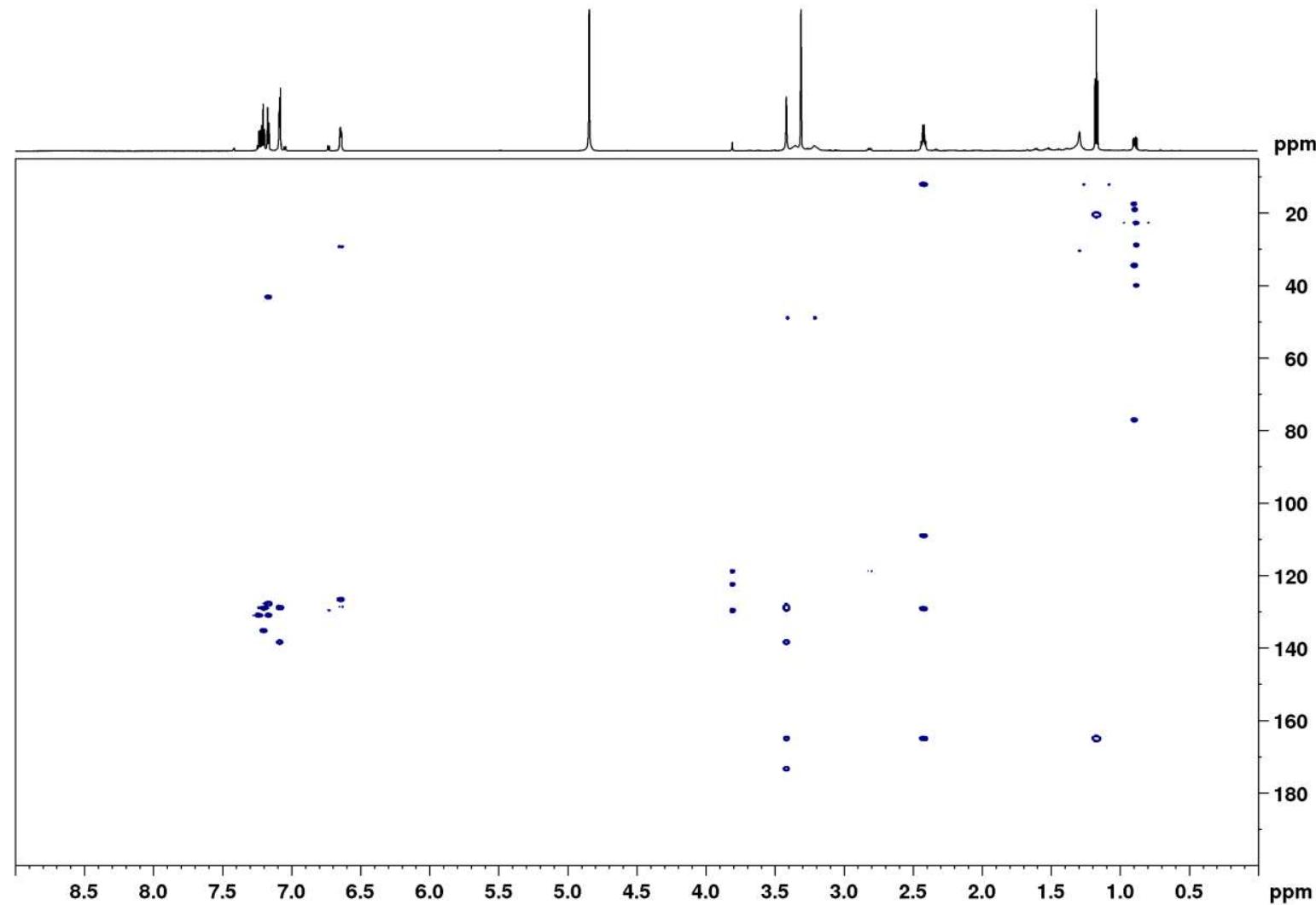


Figure S53. HMBC spectrum of 7 (700 MHz,  $\text{CD}_3\text{OD}$ ).



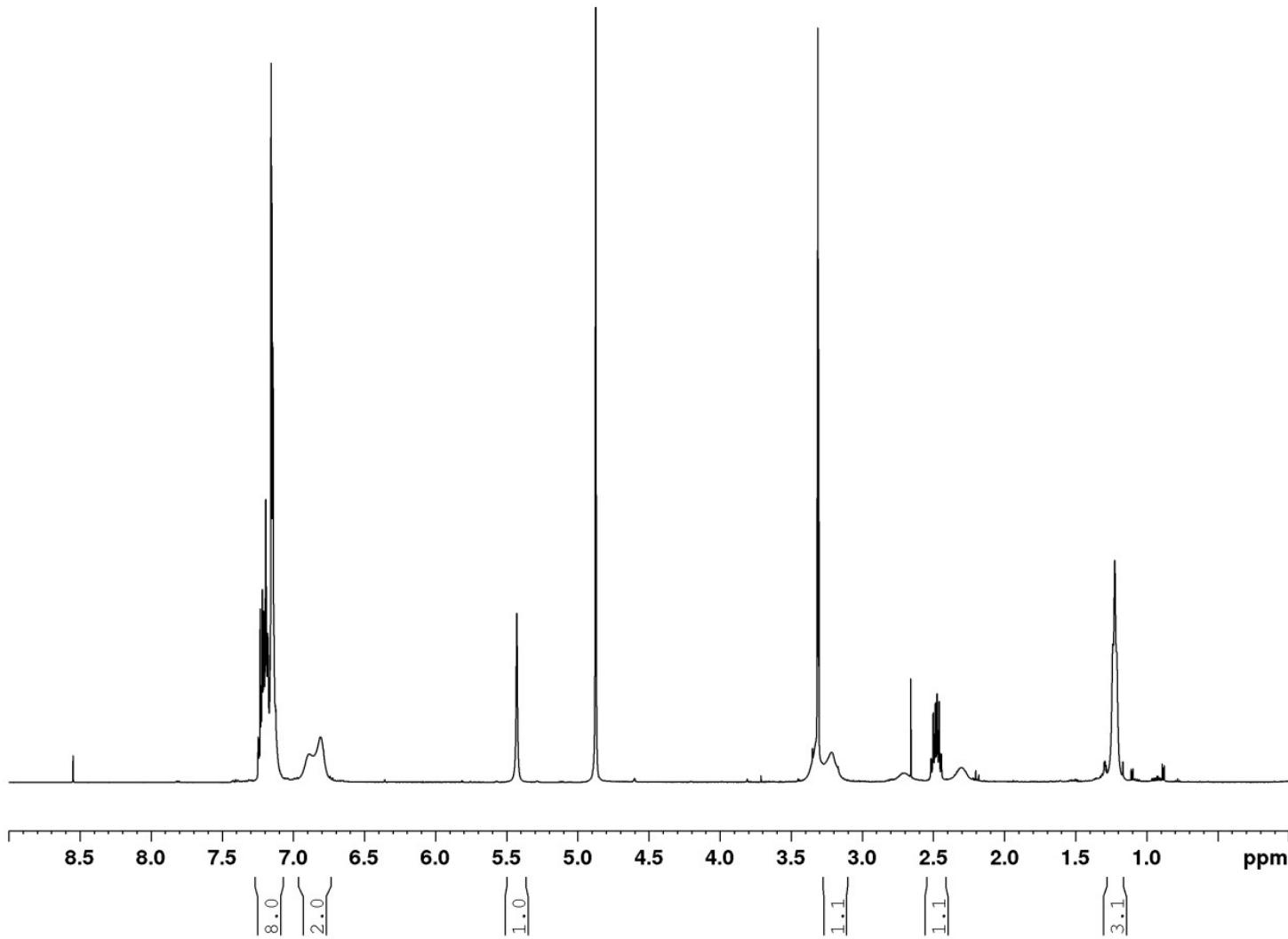


Figure S54.  $^1\text{H}$  NMR spectrum of 8 (500 MHz,  $\text{CD}_3\text{OD}$ ).

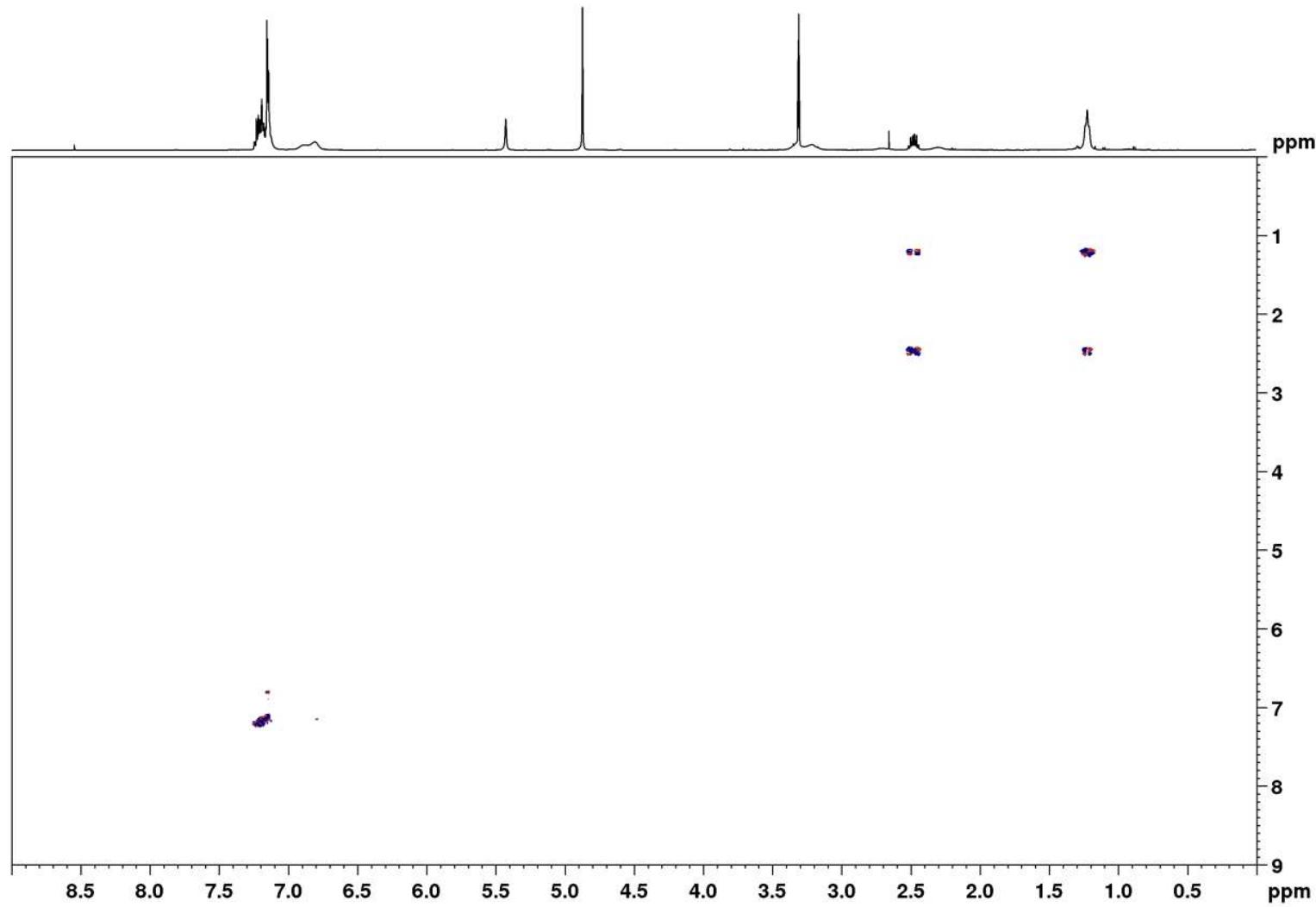


Figure S55. DQF-COSY spectrum of **8** (500 MHz,  $\text{CD}_3\text{OD}$ ).

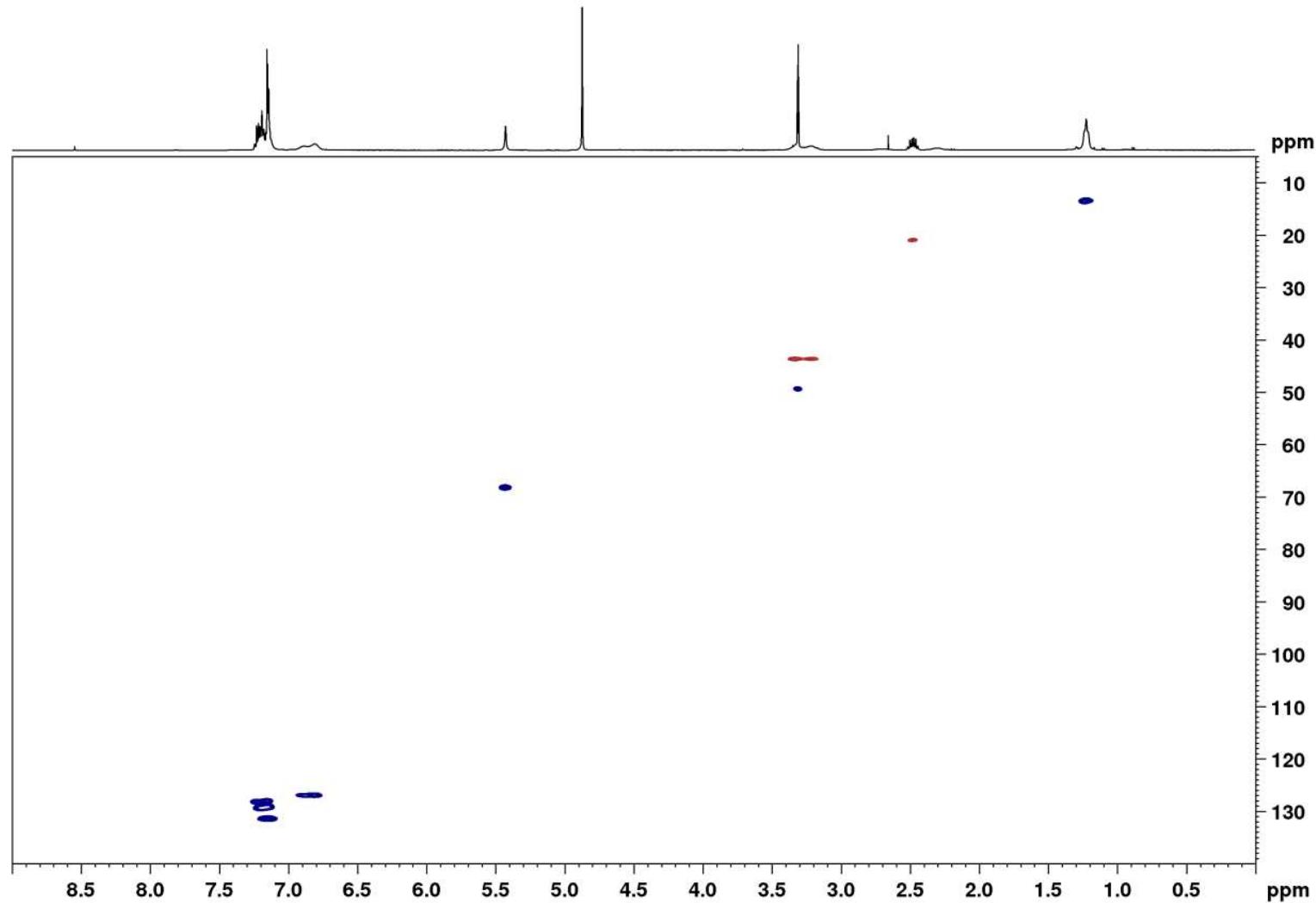


Figure S56. HSQC spectrum of 8 (500 MHz,  $\text{CD}_3\text{OD}$ ).

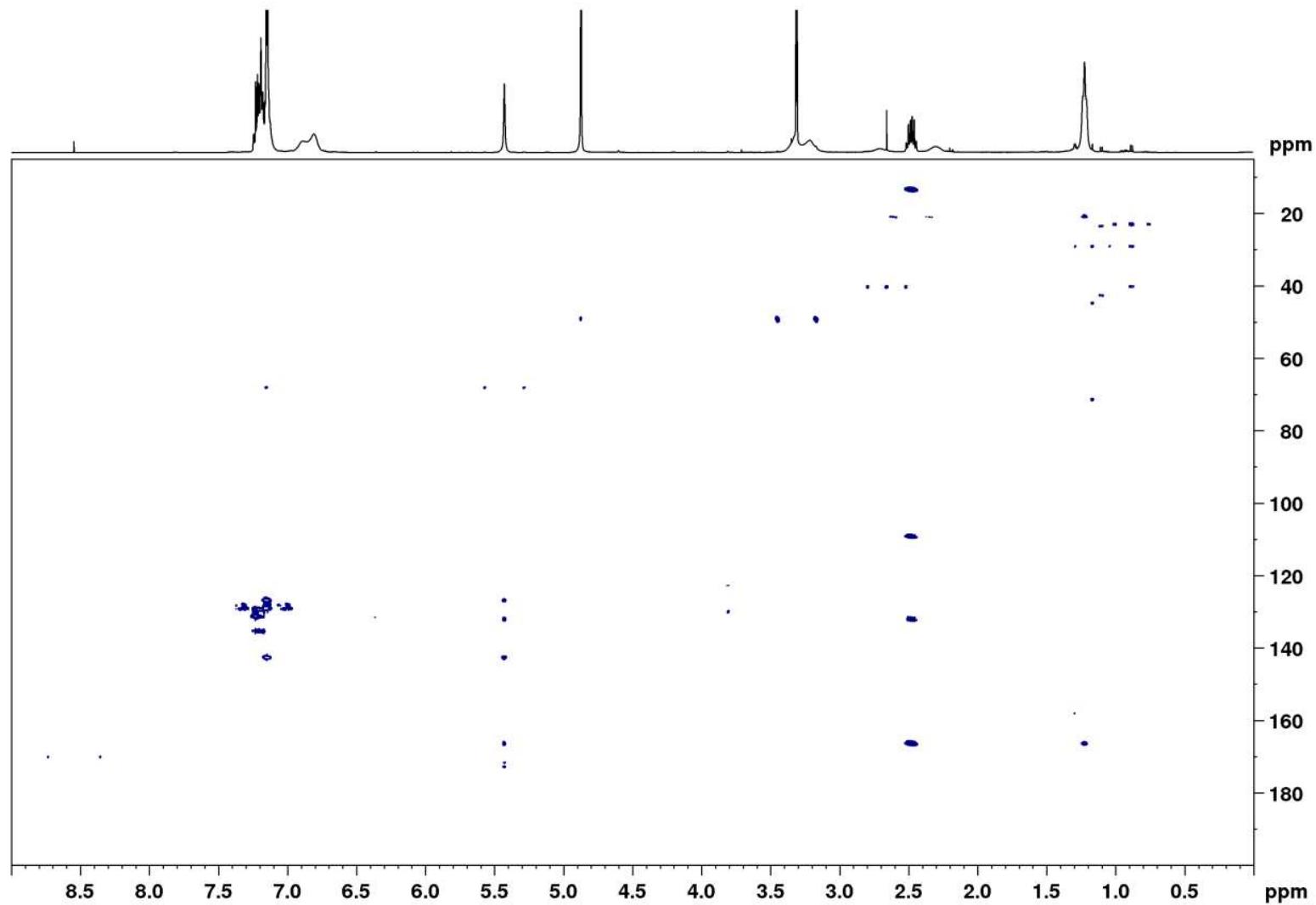
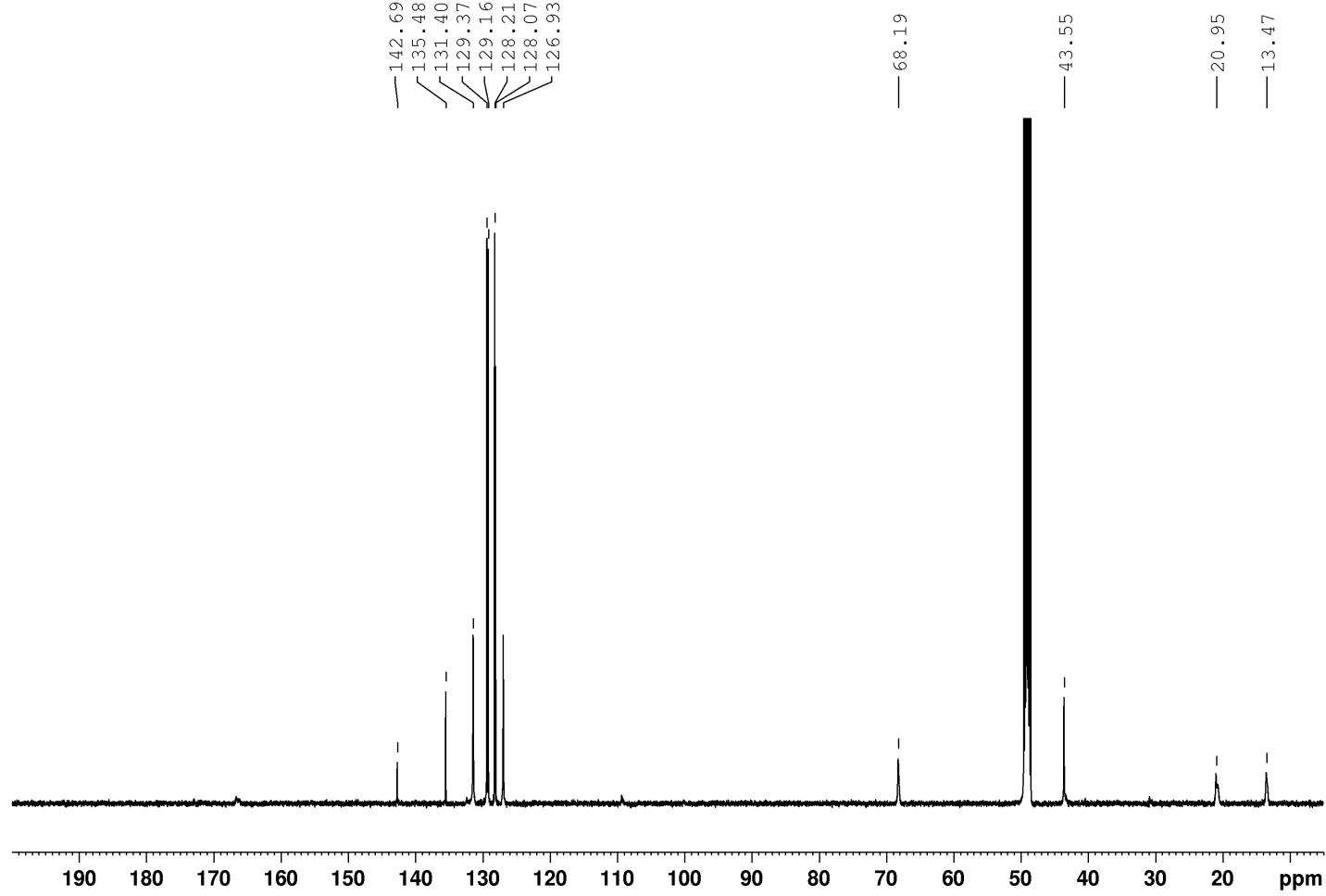


Figure S57. HMBC spectrum of 8 (500 MHz,  $\text{CD}_3\text{OD}$ ).



**Figure S58.**  $^{13}\text{C}$  NMR spectrum of 8 (125 MHz,  $\text{CD}_3\text{OD}$ ).

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## 5. References

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