

Minutissamides E - L, Antiproliferative Cyclic
Lipodecapeptides from the Cultured Freshwater
Cyanobacterium cf. *Anabaena* sp.

*Hahk-Soo Kang, Megan Sturdy, Aleksej Kronic, Hyunjung Kim, Qi Shen, Steven M. Swanson and Jimmy Orjala**

Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 S.
Wood St., Chicago, Illinois, 60612

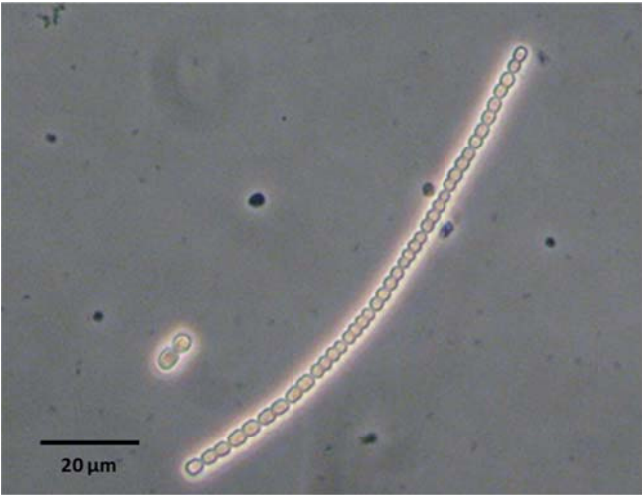
Supplementary Data

Table of Contents

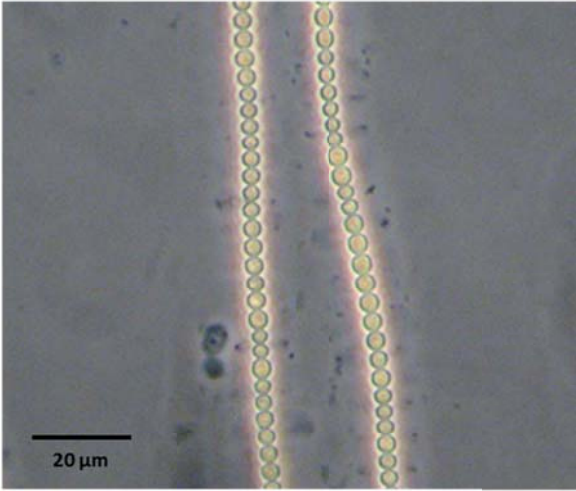
- S1. Morphological comparison between *Anabaena minutissima* UTEX 1613 and cf. *Anabaena* sp. UIC 10035
- S2. Complete phylogenetic tree for taxonomic identification of UIC 10035
- S3. Complete NMR data for minutissamide E (**1**) in DMSO-*d*₆
- S4. Advanced Marfey's analysis of **1** for the determination of amino acid configurations
- S5. Synthesis of OMeThr standards and Marfey's analysis of **1** for the absolute configuration of OMeThr
- S6. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **1**
- S7. DEPTQ spectrum (226 MHz, DMSO-*d*₆) of **1**
- S8. COSY spectrum (600 MHz, DMSO-*d*₆) of **1**
- S9. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **1**
- S10. HSQC spectrum (600 MHz, DMSO-*d*₆) of **1**
- S11. HMBC spectrum (600 MHz, DMSO-*d*₆) of **1**
- S12. T-ROESY spectrum (600 MHz, DMSO-*d*₆) of **1**
- S13. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **2**
- S14. DEPTQ spectrum (226 MHz, DMSO-*d*₆) of **2**
- S15. COSY spectrum (600 MHz, DMSO-*d*₆) of **2**
- S16. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **2**
- S17. HSQC spectrum (600 MHz, DMSO-*d*₆) of **2**
- S18. HMBC spectrum (600 MHz, DMSO-*d*₆) of **2**
- S19. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **3**
- S20. COSY spectrum (600 MHz, DMSO-*d*₆) of **3**
- S21. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **3**
- S22. HSQC spectrum (600 MHz, DMSO-*d*₆) of **3**
- S23. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **4**
- S24. COSY spectrum (600 MHz, DMSO-*d*₆) of **4**
- S25. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **4**
- S26. HSQC spectrum (600 MHz, DMSO-*d*₆) of **4**
- S27. HMBC spectrum (600 MHz, DMSO-*d*₆) of **4**
- S28. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **5**
- S29. DEPTQ spectrum (226 MHz, DMSO-*d*₆) of **5**
- S30. COSY spectrum (600 MHz, DMSO-*d*₆) of **5**
- S31. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **5**
- S32. HSQC spectrum (600 MHz, DMSO-*d*₆) of **5**
- S33. HMBC spectrum (600 MHz, DMSO-*d*₆) of **5**

- S34. Selective HMBC spectrum (900 MHz, DMSO-*d*₆) of **5**
- S35. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of **6**
- S36. COSY spectrum (600 MHz, DMSO-*d*₆) of **6**
- S37. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **6**
- S38. HSQC spectrum (600 MHz, DMSO-*d*₆) of **6**
- S39. HMBC spectrum (600 MHz, DMSO-*d*₆) of **6**
- S40. ¹H NMR spectrum (600 MHz, DMSO-*d*₆) of the mixture of **7** and **8**
- S41. COSY spectrum (600 MHz, DMSO-*d*₆) of the mixture of **7** and **8**
- S42. TOCSY spectrum (600 MHz, DMSO-*d*₆) of the mixture of **7** and **8**
- S43. HSQC spectrum (600 MHz, DMSO-*d*₆) of the mixture of **7** and **8**
- S44. HMBC spectrum (600 MHz, DMSO-*d*₆) of the mixture of **7** and **8**

S1. Morphological comparison between *Anabaena minutissima* UTEX 1613 and cf. *Anabaena* sp. UIC 10035

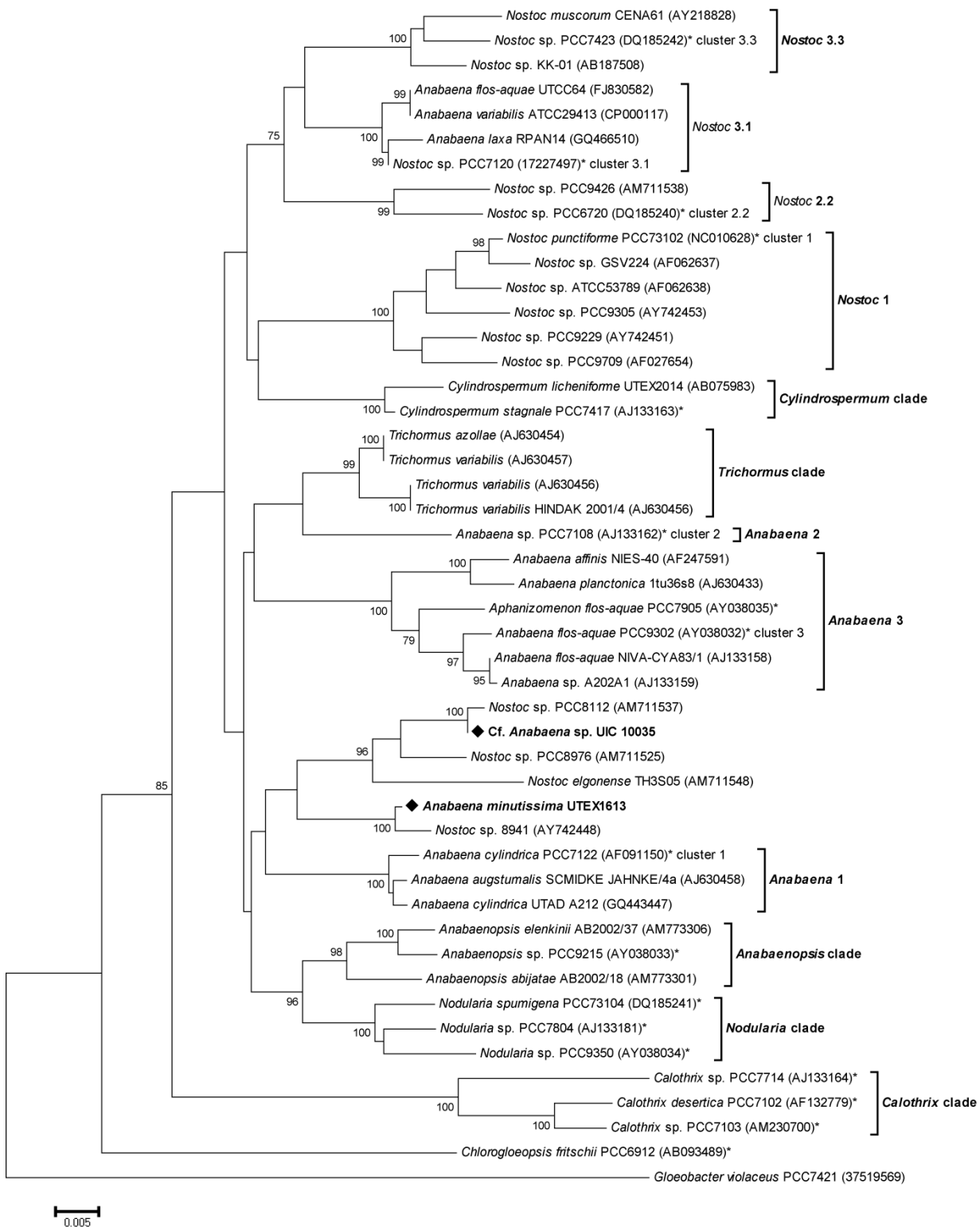


UTEX 1613



UIC 10035

S2. Complete phylogenetic tree for taxonomic identification of UIC 10035



Evolutionary distances were determined using the minimum evolution method with 1,000 replicate bootstrap re-sampling to construct the phylogenetic tree. Strains denoted with an asterisk (*) are “Bergey’s” reference strains. Strains were obtained from NCBI with the accession number given in parentheses. Only bootstrap values greater than or equal to 75% are displayed.

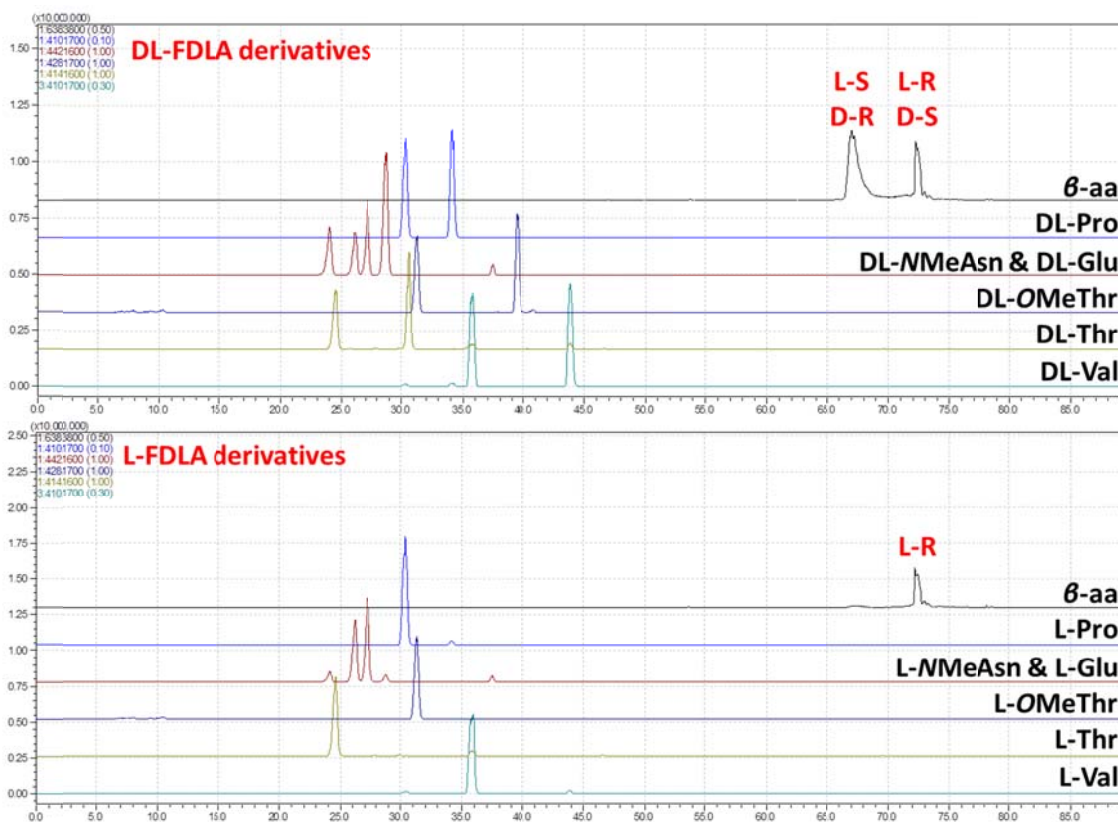
S3. Complete NMR data for minutissamide E (**1**) in DMSO-*d*₆

		Minutissamide E (1)						
		δ_C^a	δ_H^b	mult. (<i>J</i> in Hz)	COSY ^b	HMBC ^b	ROESY ^b	
Ahmoo	1	169.6						
	2	69.6	4.17	d (4.8)	H-3	1	NH	
	3	56.0	3.92	td (10.8, 4.8)	H-2, H-4, NH	1, 2, 4, 4-Me	H-2, H ₂ -5, 4-Me	
	4	32.2	1.68	m	H-3, H-5, 4-Me		H-2	
	5	33.4	1.17	m	H-4		H-3, 4-Me	
			1.62	m				
			1.17	m				
			1.25	m				
			1.25	m				
			1.25	m	overlapped		overlapped	
			1.25	m				
			1.25	m				
			1.25	m				
			1.44	m	H-14, H-12	12, 14, 15		
			2.37	t (7.2)	H-13	12, 13		
			210.6					
			43.8	2.36	t (7.2)	H-17	15, 17, 18	
			16.7	1.47	s (7.2)	H-16, H-18	15, 16, 18	
		13.6	0.83	t (7.2)	H-17	16, 17		
	3-NH		6.77	d (10.8)		3, 1 _{Pro}	H-2, H-4, 4-Me, H-2 _{NMeAsn}	
	4-Me		0.57	d (6.6)		3, 5	H-3, H-5	
Pro	1	171.2						
	2	59.9	4.25	dd (8.4, 2.4)	H-3	1, 3, 4	H-3, H-4, NH _{Ahmoo}	
	3	30.1	1.94	m	H-2, H-4			
			1.99	m				
			1.71	m				
		23.4	1.84	m	H-3, H-5			
			3.11	m				
			4.21	m	H-4		H-2, H-3, H-4, H-2 _{NMeAsn}	
NMeAsn	1	167.4						
	2	49.7	5.52	dd (11.4, 3.6)	H-3	1, 3, 4, N-Me, 1 _{OMeThr}	H-3, NH _{Thr2} , H-5 _{Pro} , H-2 _{Thr2} , NH _{Thr2} , NH _{Ahmoo}	
	3	33.7	1.97	overlapped	H-2	1, 2, 4	H-2	
			3.00	dd (15.6, 12.0)				
			171.5					
	N-Me		30.5	2.93	s	2, 1 _{OMeThr}	H-2 _{OMeThr} , H-3 _{OMeThr}	
				5.99	s			
	NH ₂			7.49	s	3, 4	H-3, H-3 _{Dhb}	
OMeThr	1	169.6						
	2	52.6	4.79	dd (9.0, 1.8)	NH, H-3	3, 4, 1 _{Ala}	H-3, H-4, NH, NMe	
	3	75.1	3.71	qd (6.0, 1.8)	H-2, H-4	OMe	H-2, H-4, NMe, H ₂ -5 _{Pro}	
	4	14.7	0.95	d (6.0)	H-3	2, 3	H-2, H-3, OMe	
	O-Me		55.6	3.13	s		3	H-3
	NH			6.74	d (9.0)	H-2	1 _{Ala}	NH _{Ala}
Ala	1	171.9						
	2	49.1	4.19	p (7.8)	H-3, NH	3	NH	
	3	16.3	1.29	d (7.8)	H-2	1, 2	H-2	
	NH			7.58	d (7.8)	H-2	2, 3, 1 _{Gln}	H-2, H-3, NH _{OMeThr} , H-2 _{Gln}
Gln	1	171.1						
	2	52.9	4.08	m	H-3, NH	3	H-3, H-4, NH	
			1.78	m				
			2.03	m	H-2, H-4		H-2	
			2.15	m	H-3	2, 3, 5	H-2, H-4 _{Thr}	
			173.9					
	NH		7.25	d (9.0)	H-2	2, 3, 1 _{Thr1}	H-2, H-3, H-2 _{Thr1} , H-3 _{Thr2}	
	NH ₂		6.80	s		4, 5	H-4	

7.27 s							
Thr1	1	170.4					
	2	61.3	3.90 m	H-3, NH	3		H-3, H-4
	3	65.2	4.17 overlapped	H-2, H-4			
	4	20.6	1.25 overlapped	H-3	2, 3		H-2
	NH		8.84 brs	H-2			H-2, H-3, H-2 _{Thr2} , H-3 _{Thr2} , NH _{Gln}
Thr2	1	174.2					
	2	56.6	5.02 dd (10.2, 2.4)	H-3, NH	1, 3, 1 _{Dhb}		H-3, H-4, NH, NH _{Thr1}
	3	70.3	4.57 brm	H-2, H-4			H-2, H-4, H-2 _{OMeThr} , NH _{Gln} , NH _{Thr1}
	4	19.0	1.25 overlapped	H-3	2, 3		H-3, H-5 _{Pro}
	NH		8.37 d (10.2)	H-2	1 _{Dhb}		H-2, H-2 _{NMeAsn} , NH _{OMeThr}
Dhb	1	163.9					
	2	132.4					
	3	117.3	5.38 q (7.2)	H-4	1, 2, 4		H-4, NH, NH _{2NMeAsn}
	4	13.2	1.75 d (7.2)	H-3	2, 3		H-3, H-4 _{Thr2}
	NH		9.08 s		1, 2, 3, 1 _{Val}		H-3, H-2 _{Val}
Val	1	168.8					
	2	55.5	4.31 dd (9.0, 6.0)	H-3, NH	3, 4, 4'		H-3, H-4, H-4', NH, NH _{Dhb}
	3	32.7	1.81 m	H-2, H-4, H-4'	1, 2, 4, 4'		
	4	18.4	0.83 d (6.0)	H-3	2, 3, 4'		H-2, H-3
	4'	19.0	0.89 d (6.0)	H-3	2, 3, 4		H-2, H-3
	NH		6.85 d (9.0)				H-2, H-3, H-4, H-2 _{Ahmoo}

^aCarbon chemical shifts were assigned from the DEPT-Q spectrum (226 MHz), ^bMeasured at 600 MHz

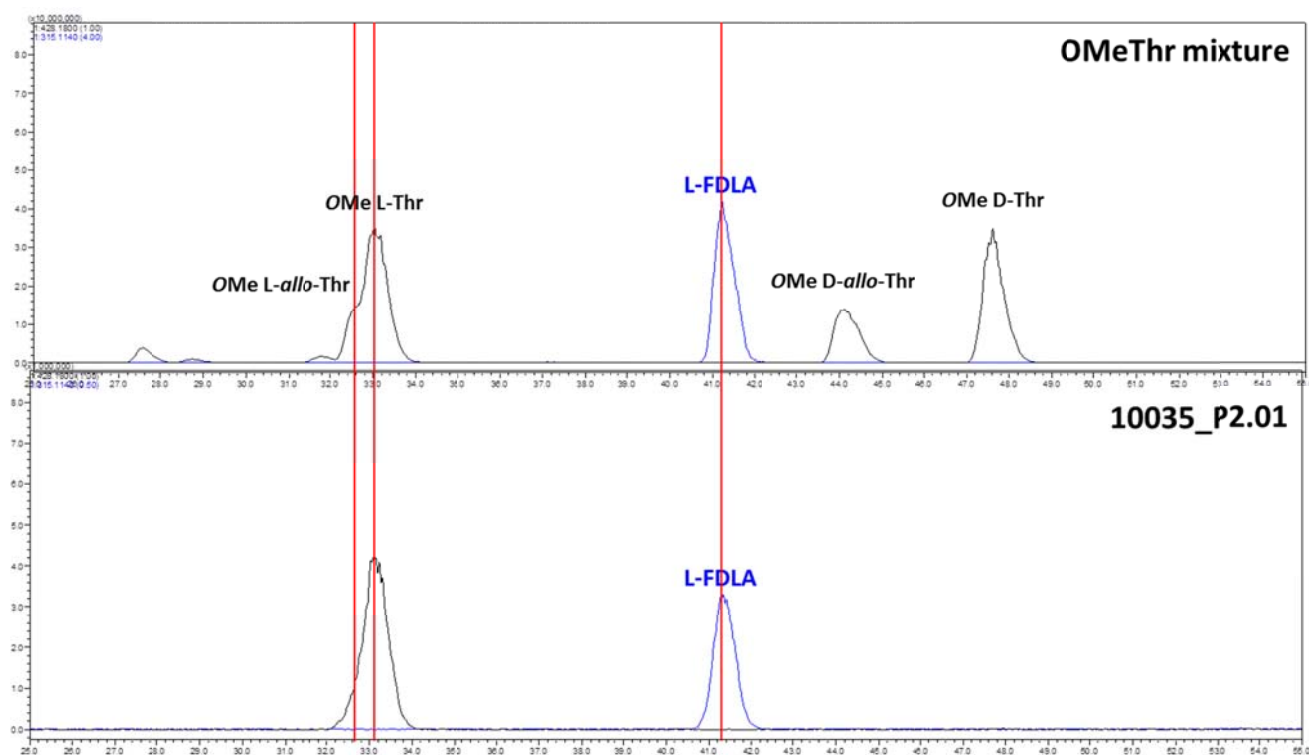
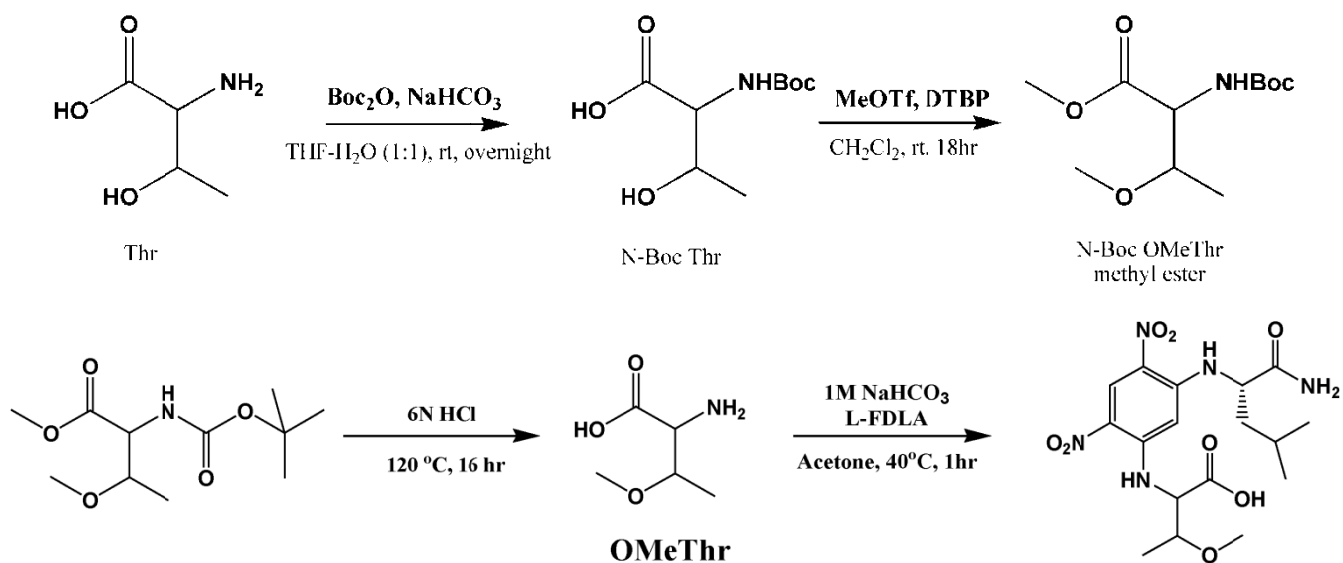
S4. Advanced Marfey's analysis of **1** for the determination of amino acid configurations



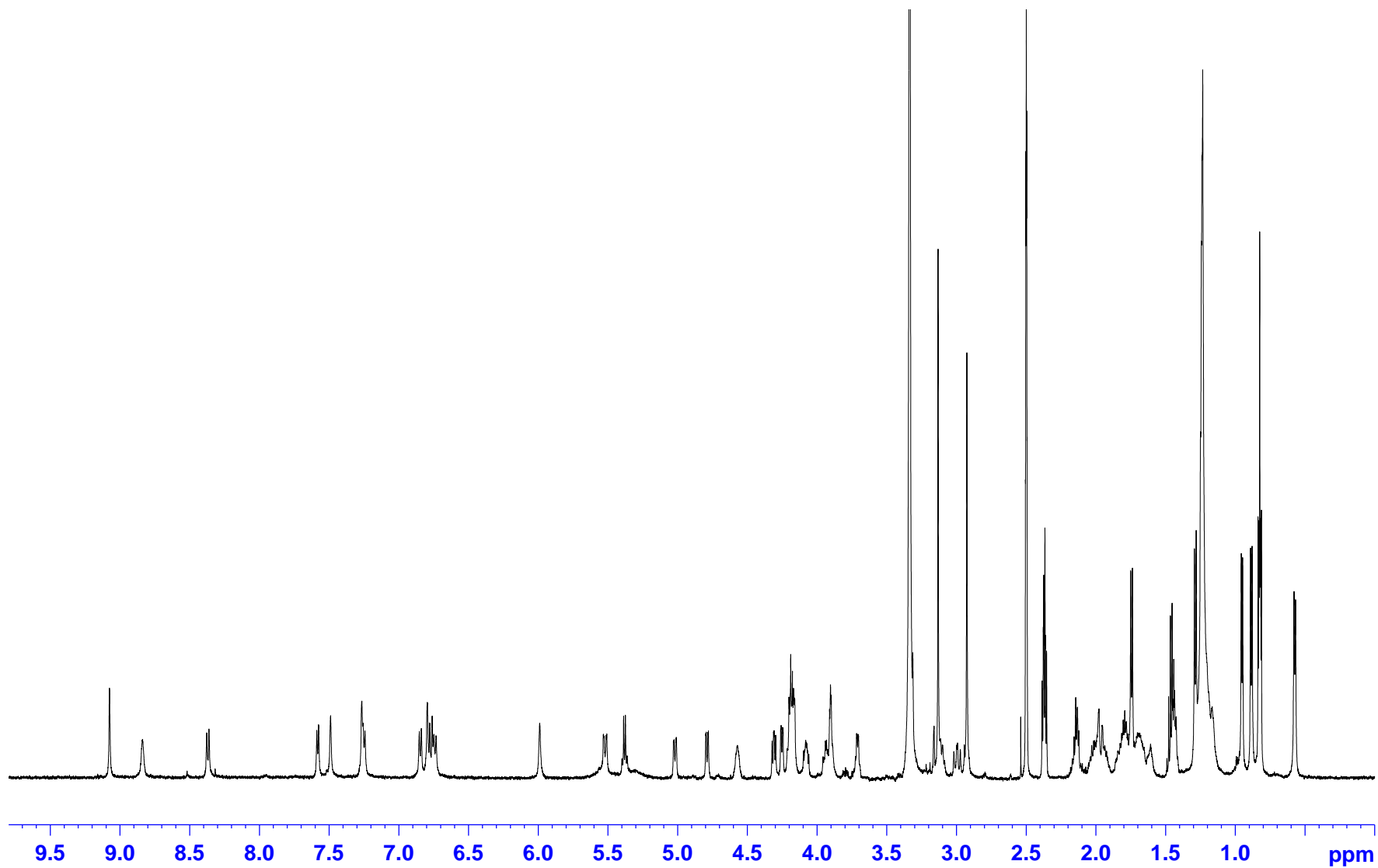
Amino acid	HPLC retention times of DL-FDLA derivative of acid hydrolysate of 1		HPLC retention times of L-FDLA derivative of acid hydrolysate of 1	Assignment
	L	D		
Ahmoo	67.0 min (S)	72.4 min (R)	72.4 min	R
Pro	30.4 min	34.1 min	30.5 min	L
NMeAsn	26.3 min	24.0 min	26.3 min	L
Ala	30.9 min	35.4 min	35.6 min	D
Gln	27.2 min	28.7 min	27.4 min	L
Thr	24.5 min	30.6 min	24.7 min	L
Val	35.8 min	43.8 min	35.9 min	L

- L-FDLA derivative of Thr was further compared with L-FDLA derivatives of four Thr amino acid standards L-Thr, L-*allo*-Thr, D-Thr and D-*allo*-Thr, and corresponded with the retention time of L-Thr.

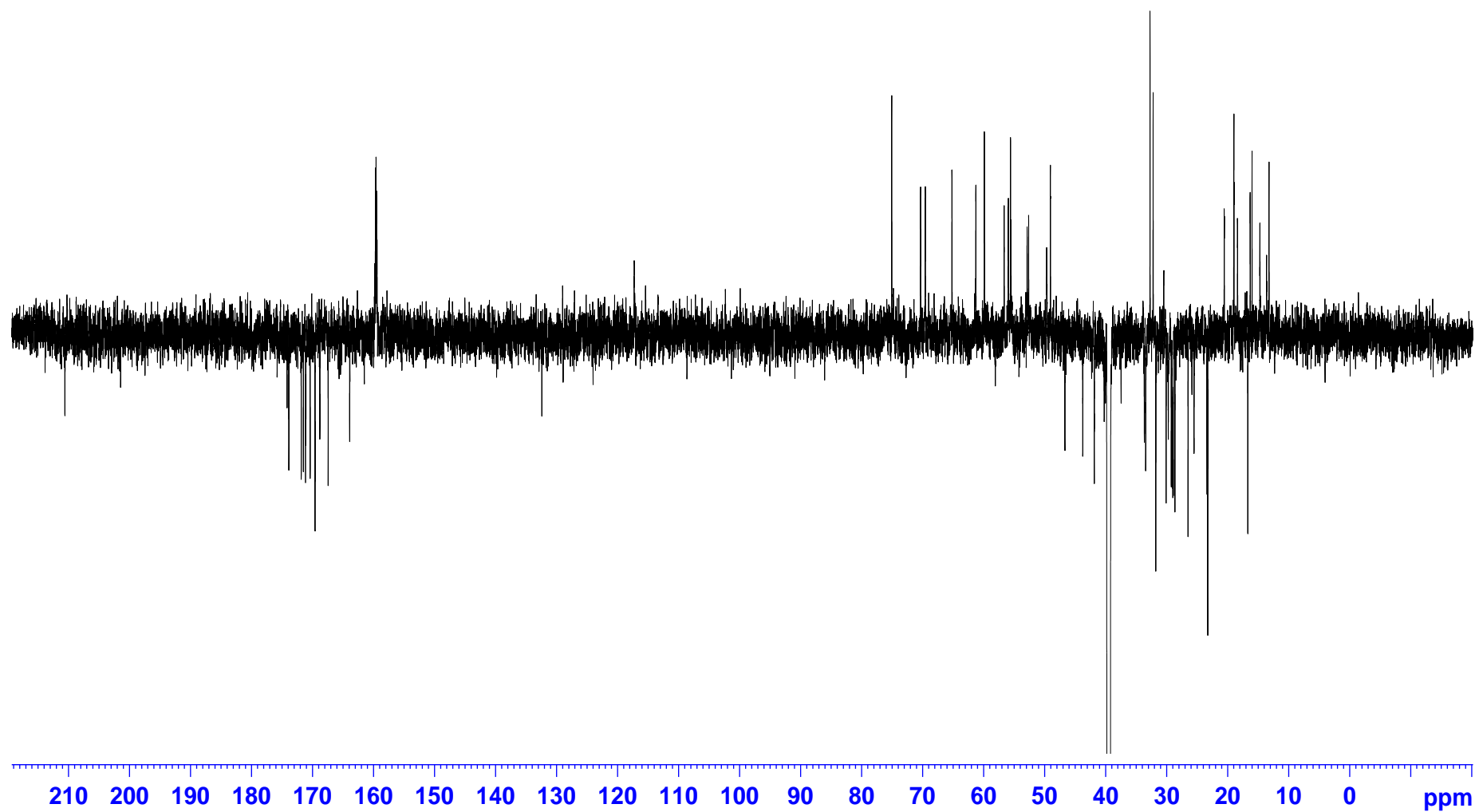
S5. Synthesis of OMeThr standards and Marfey's analysis of **1** for the absolute configuration of OMeThr



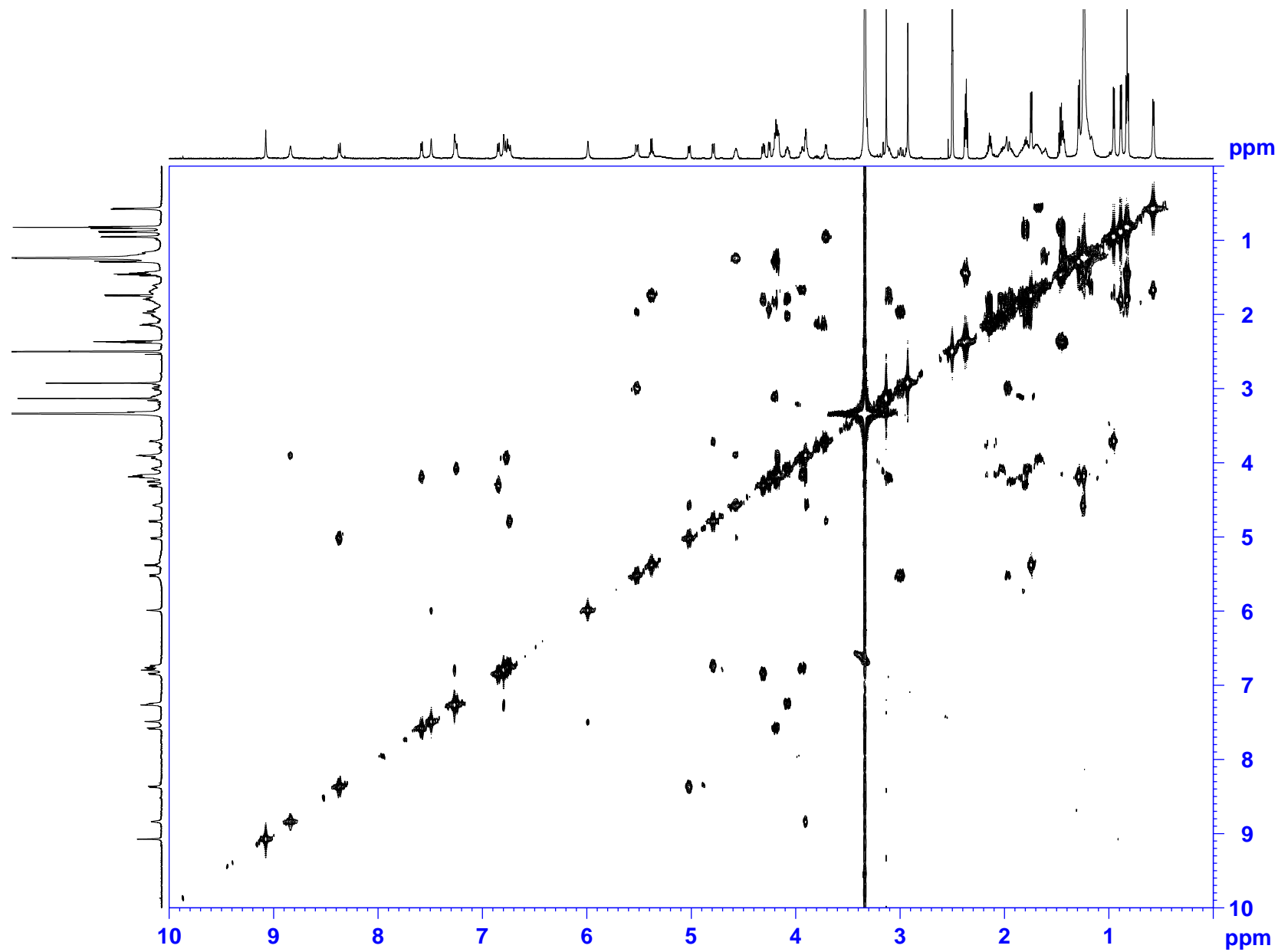
S6. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **1**



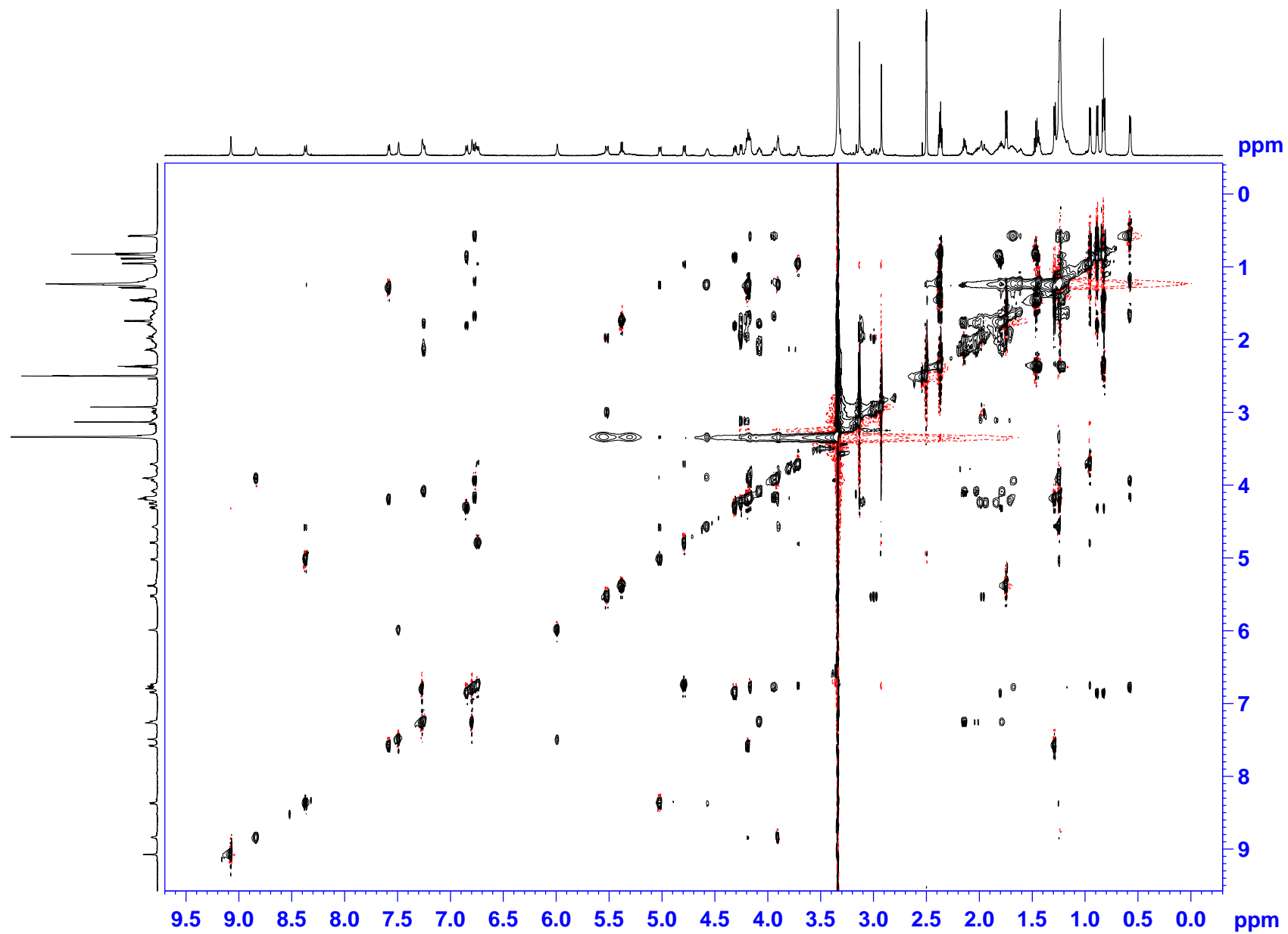
S7. DEPTQ spectrum (226 MHz, DMSO- d_6) of **1**



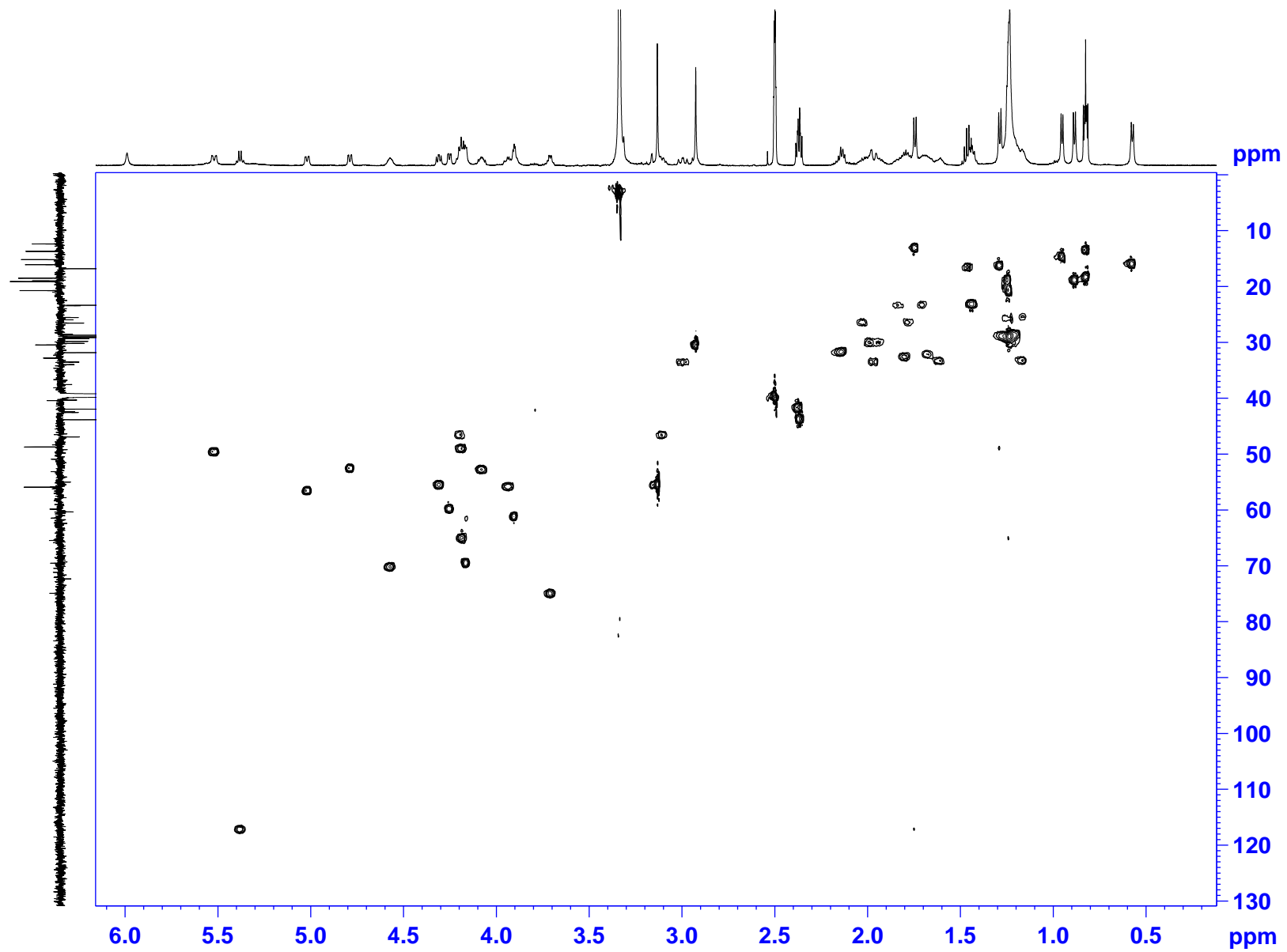
S8. COSY spectrum (600 MHz, DMSO- d_6) of **1**



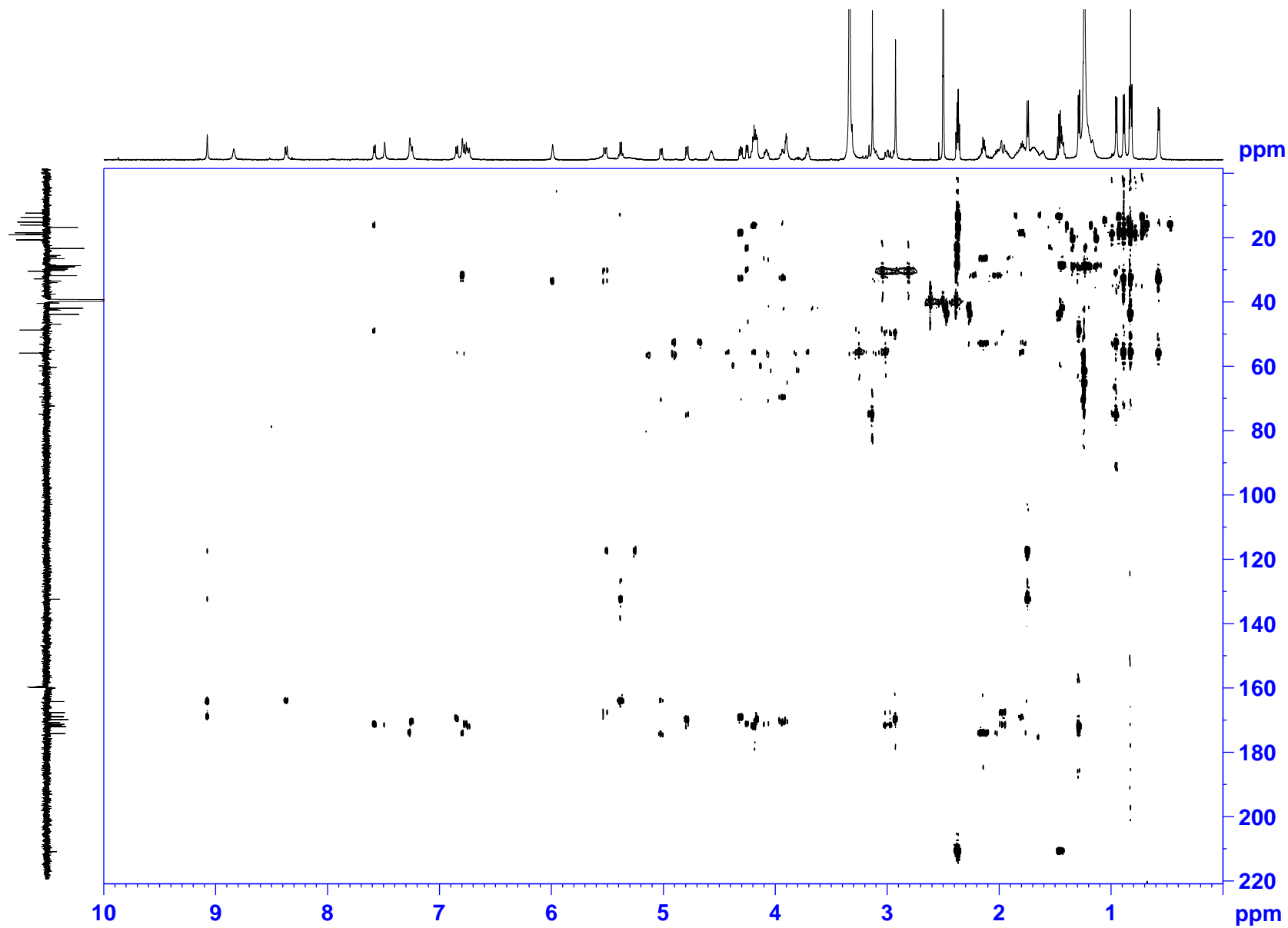
S9. TOCSY spectrum (600 MHz, DMSO-*d*₆) of **1**



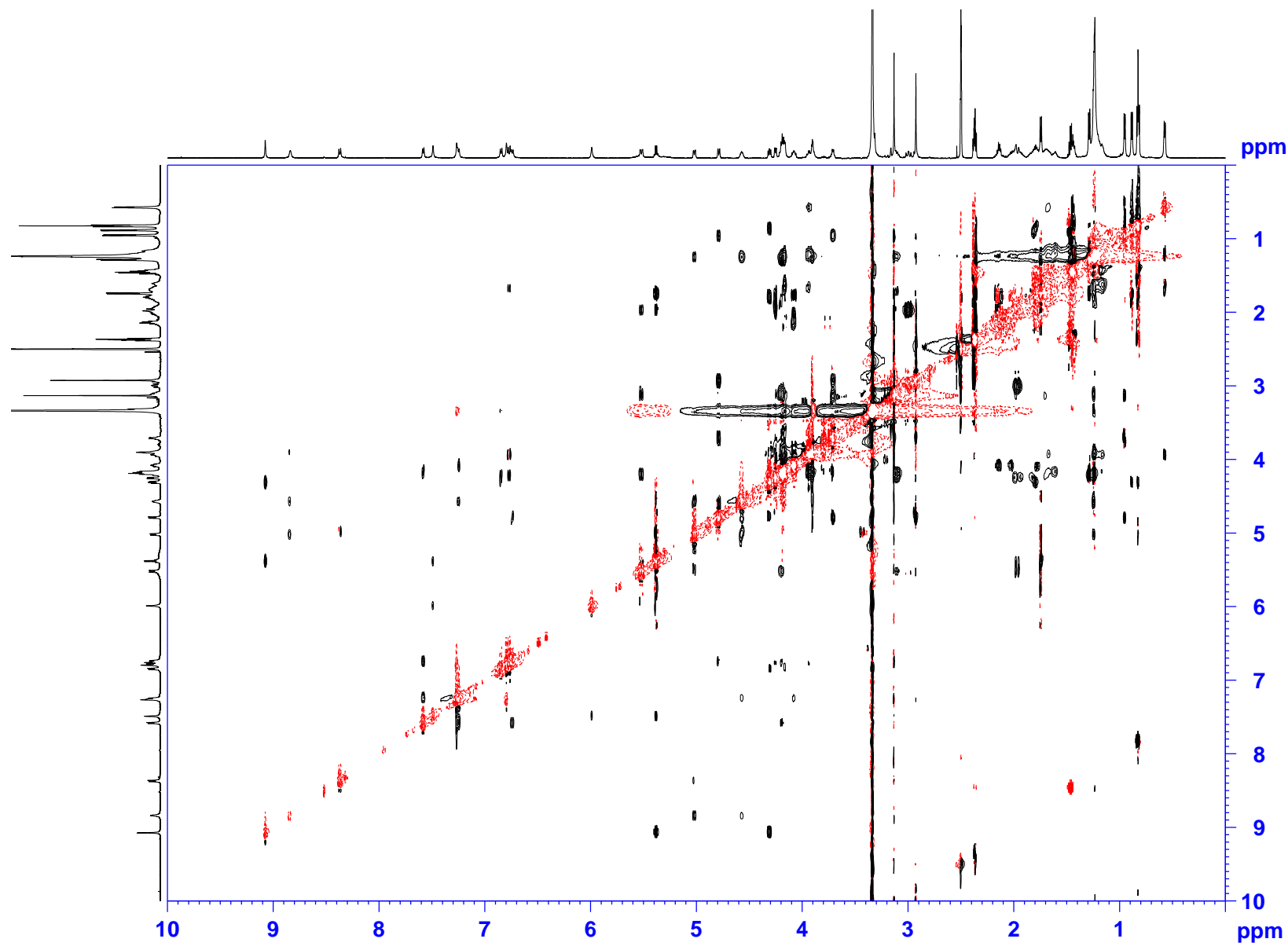
S10. HSQC spectrum (600 MHz, DMSO- d_6) of **1**



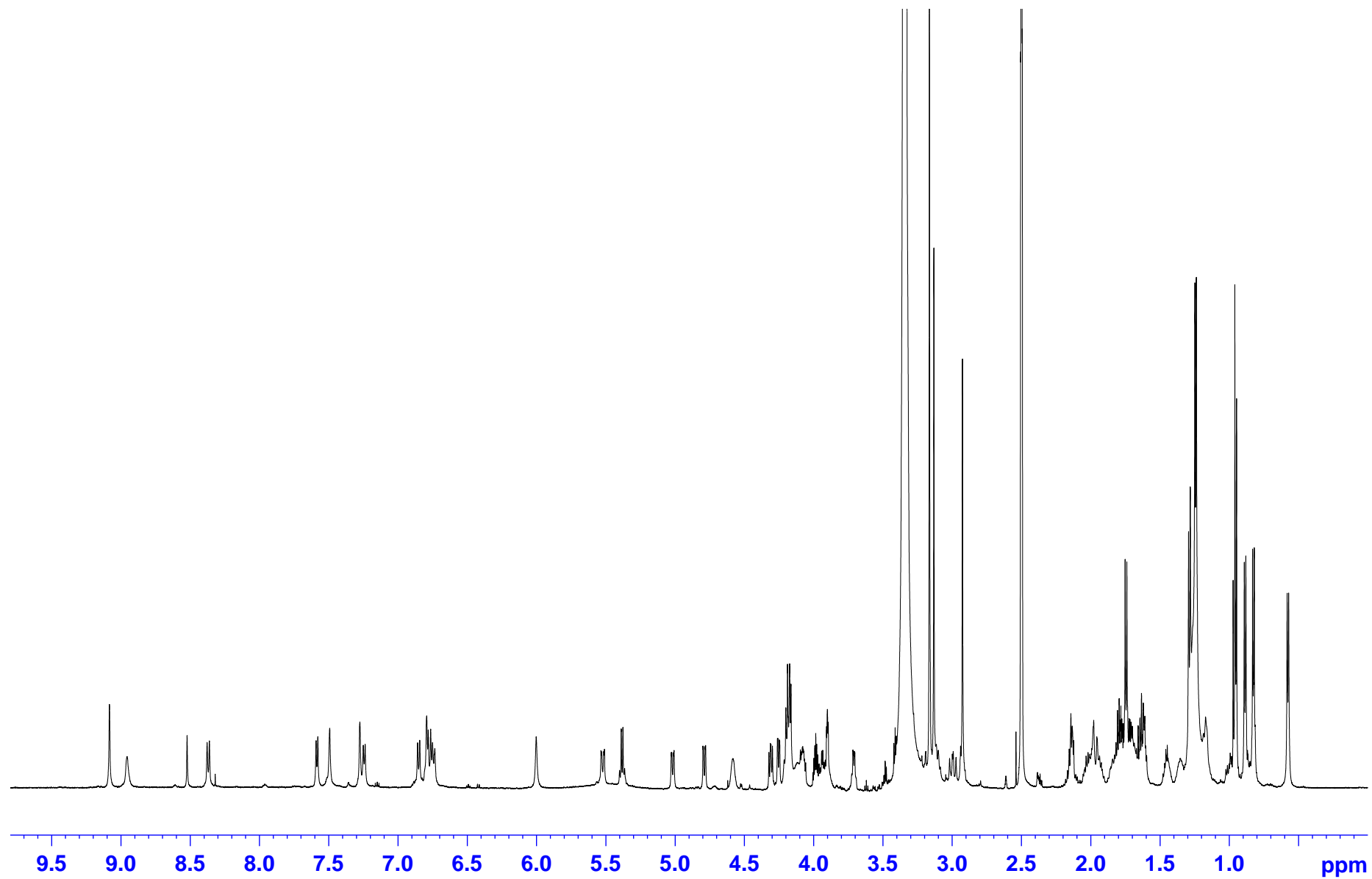
S11. HMBC spectrum (600 MHz, DMSO-*d*₆) of **1**



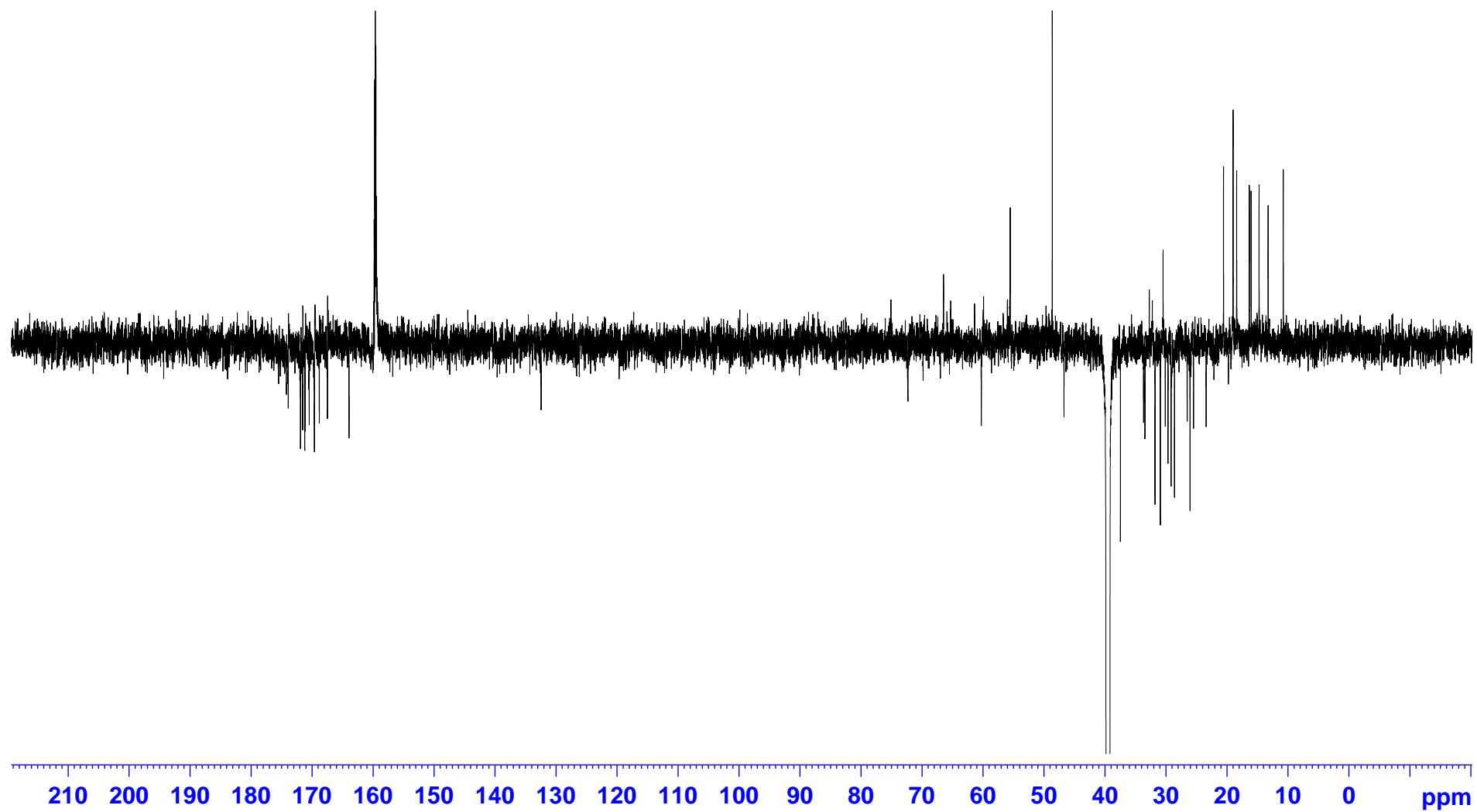
S12. T-ROESY spectrum (600 MHz, DMSO- d_6) of **1**



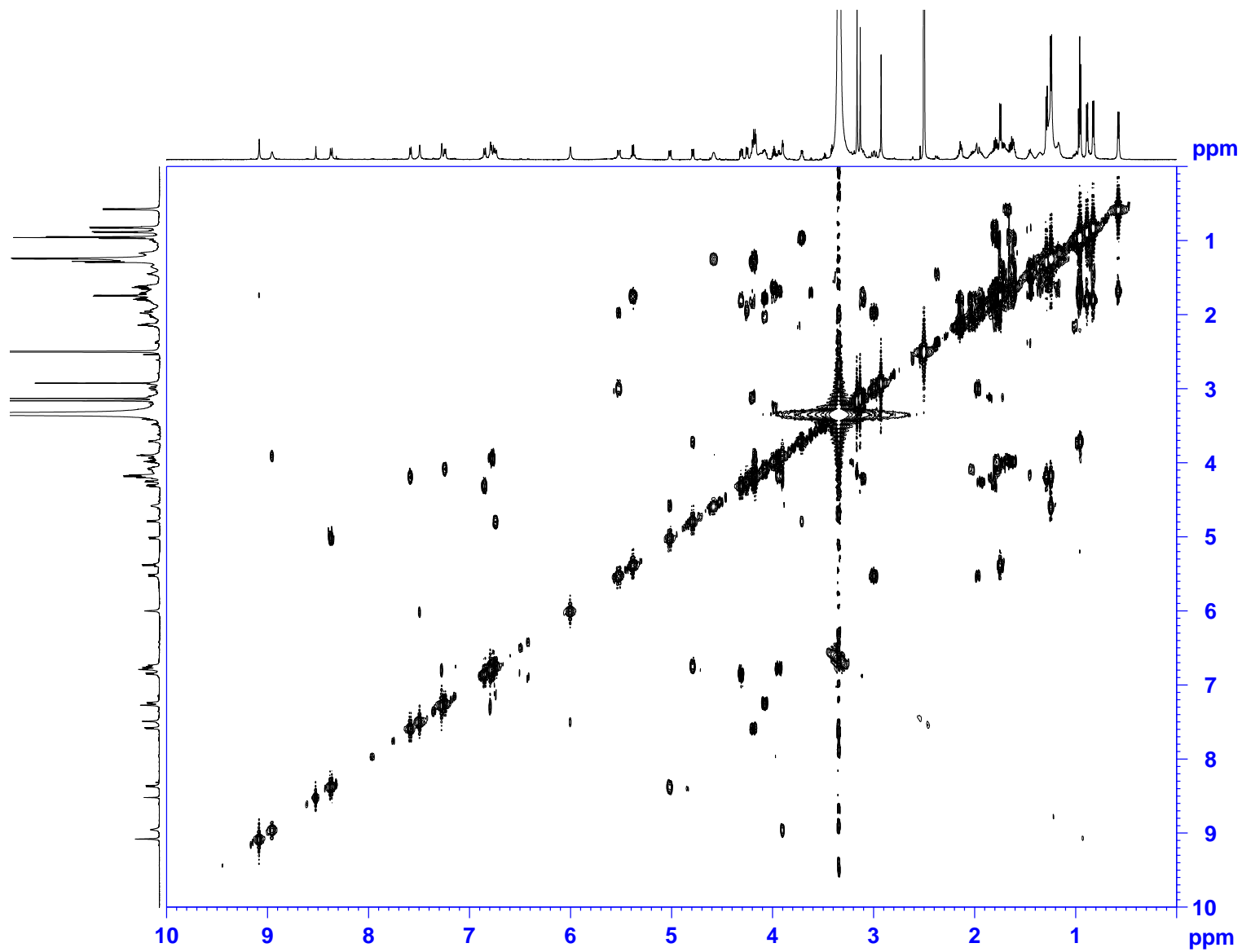
S13. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **2**



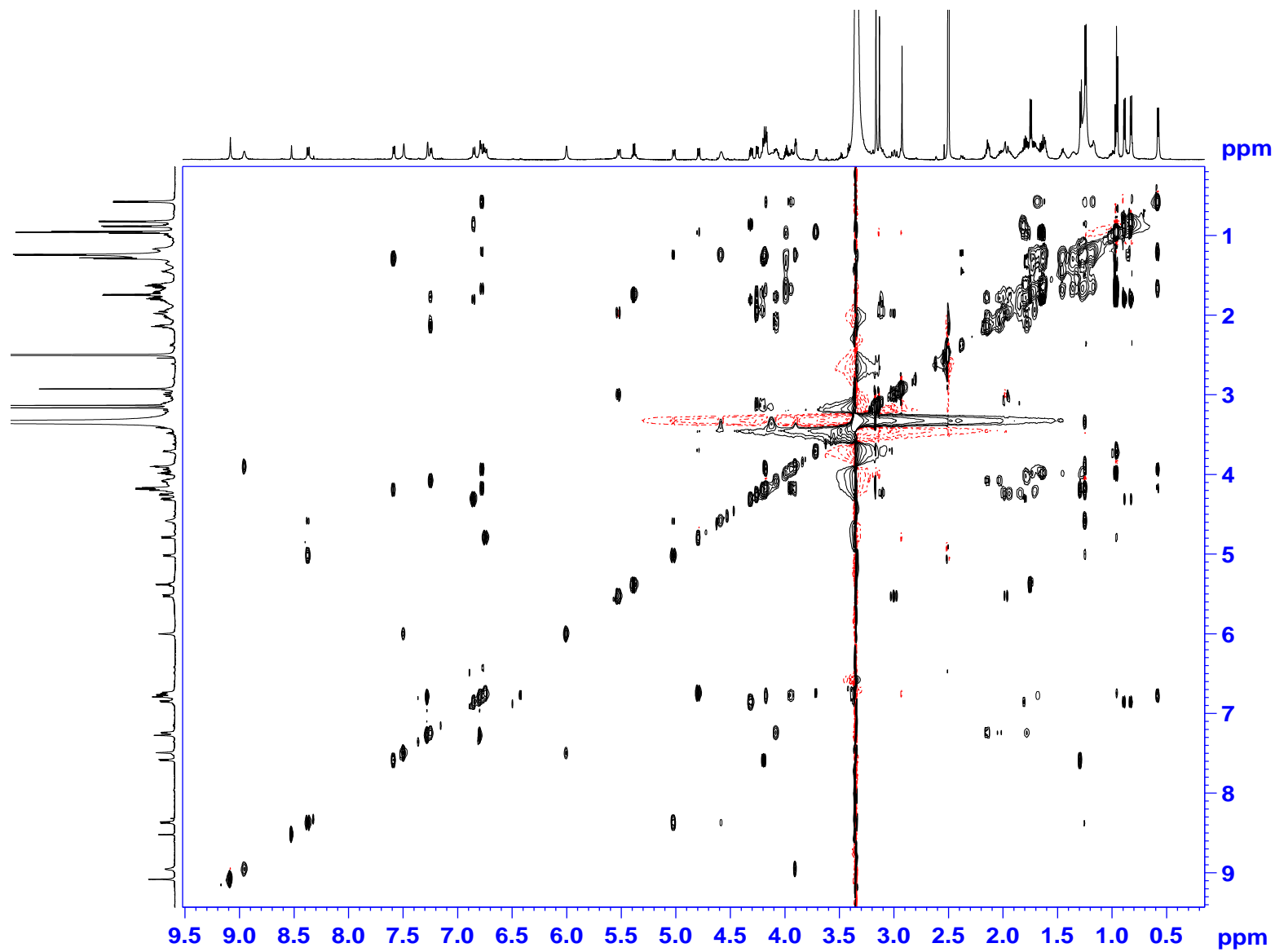
S14. DEPTQ spectrum (226 MHz, DMSO- d_6) of **2**



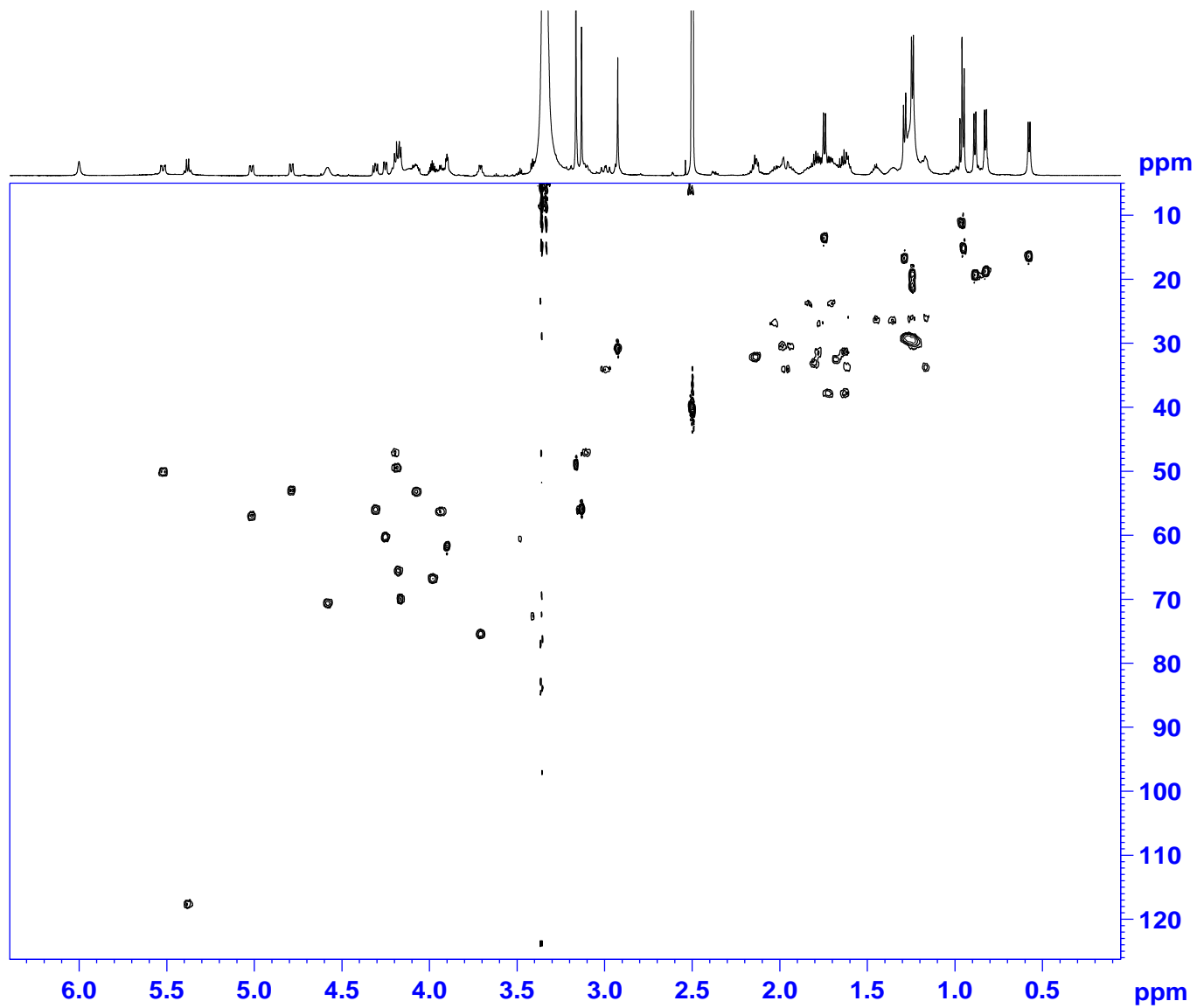
S15. COSY spectrum (600 MHz, DMSO- d_6) of 2



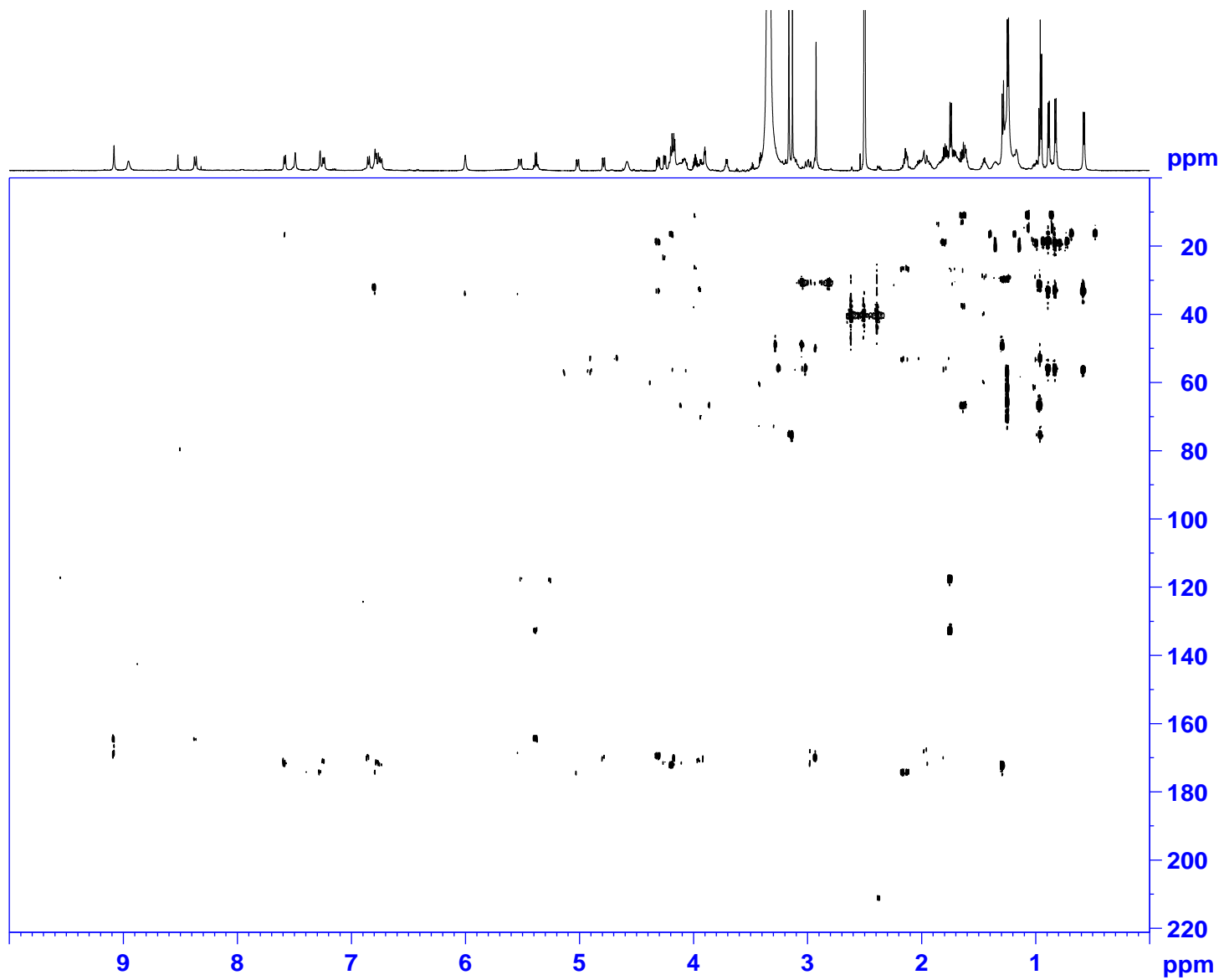
S16. TOCSY spectrum (600 MHz, DMSO- d_6) of **2**



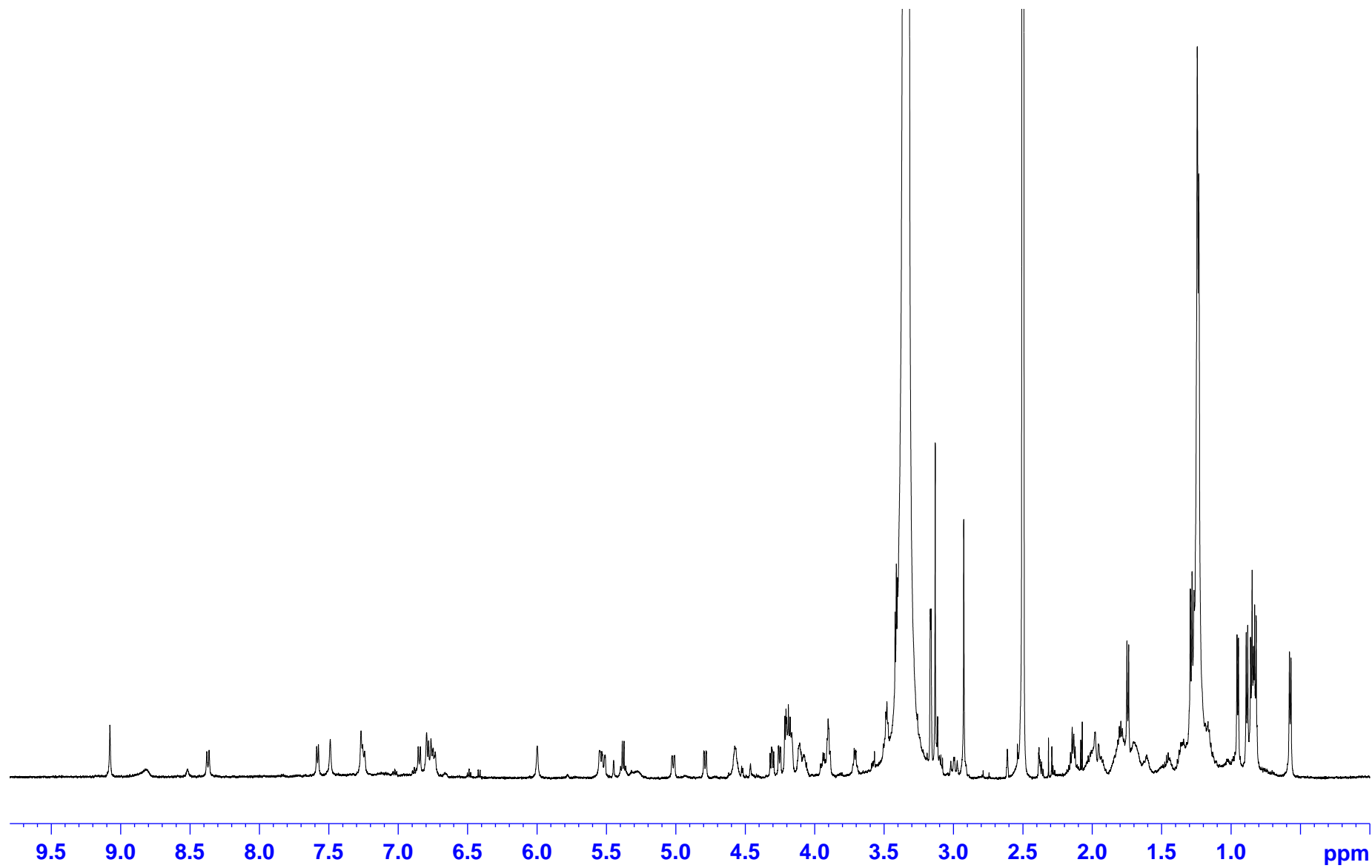
S17. HSQC spectrum (600 MHz, DMSO- d_6) of **2**



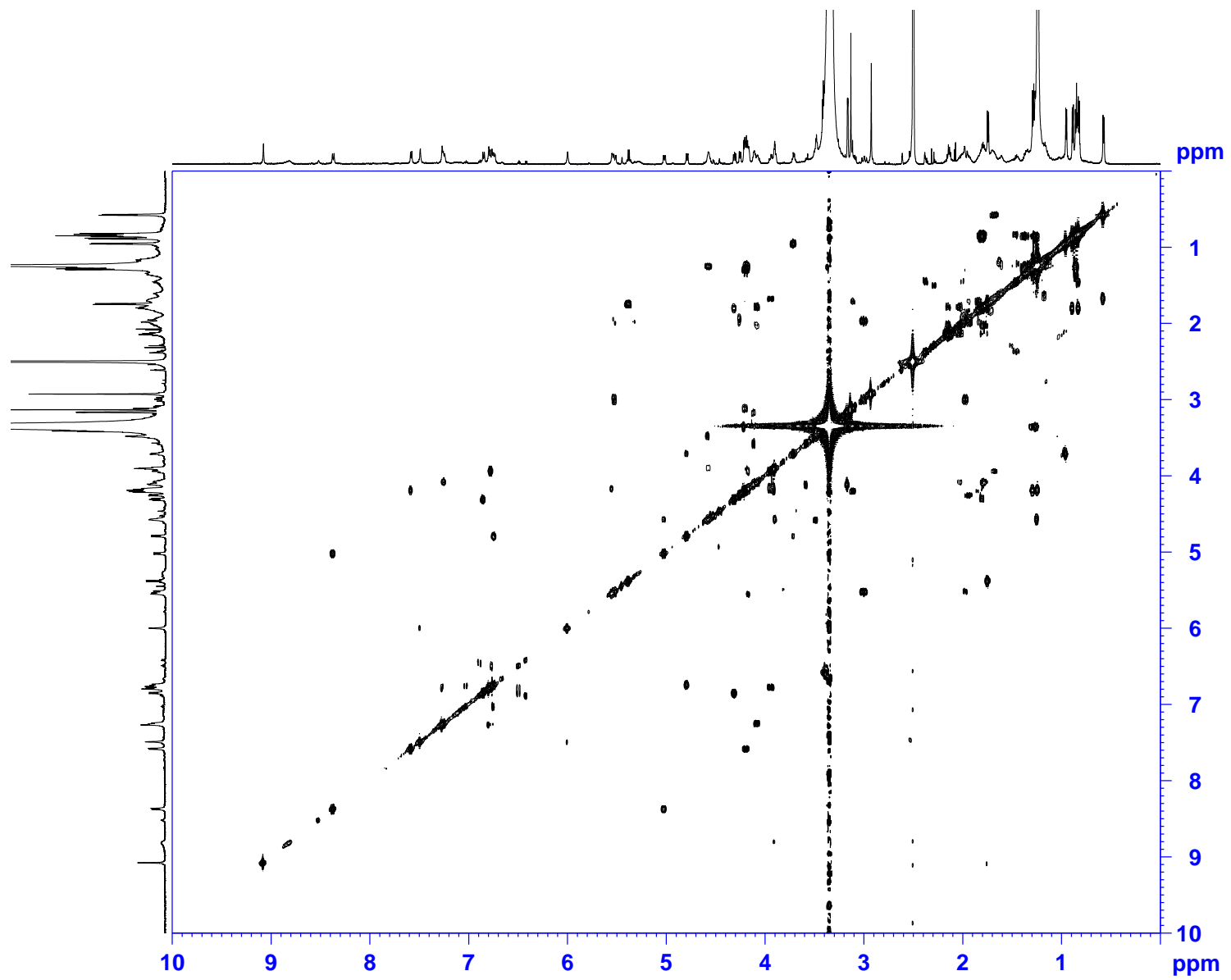
S18. HMBC spectrum (600 MHz, DMSO-*d*₆) of **2**



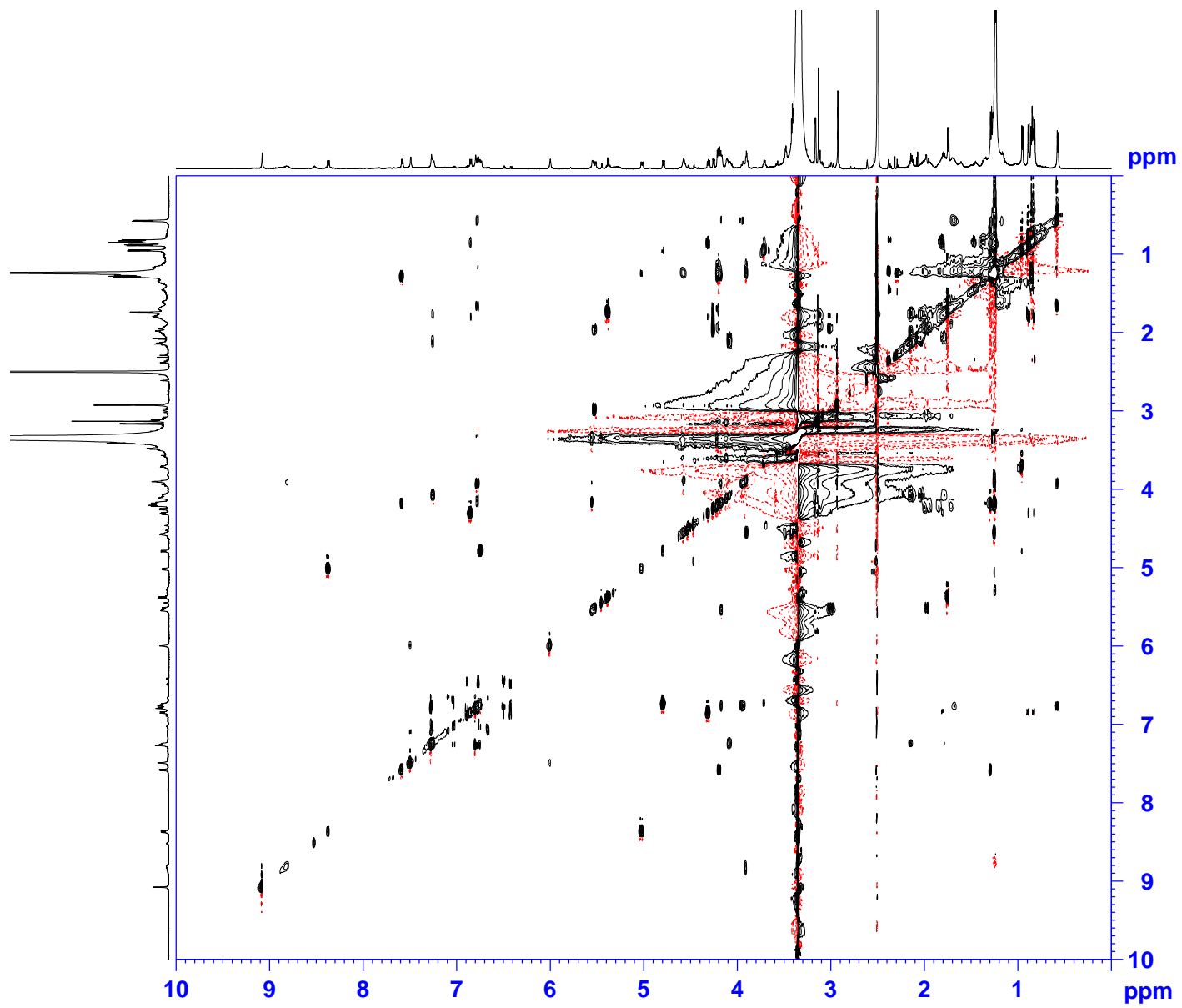
S19. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **3**



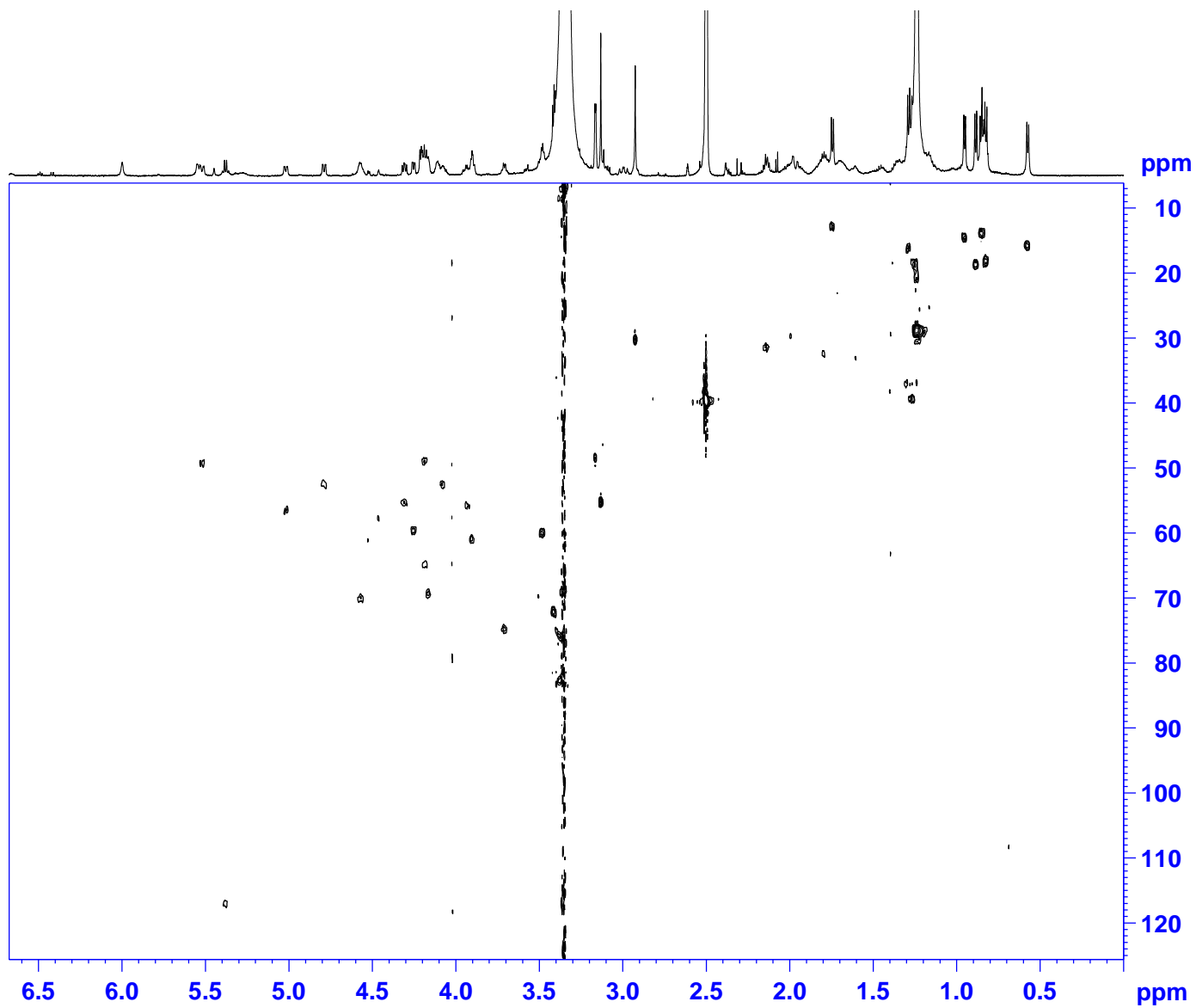
S20. COSY spectrum (600 MHz, DMSO- d_6) of **3**



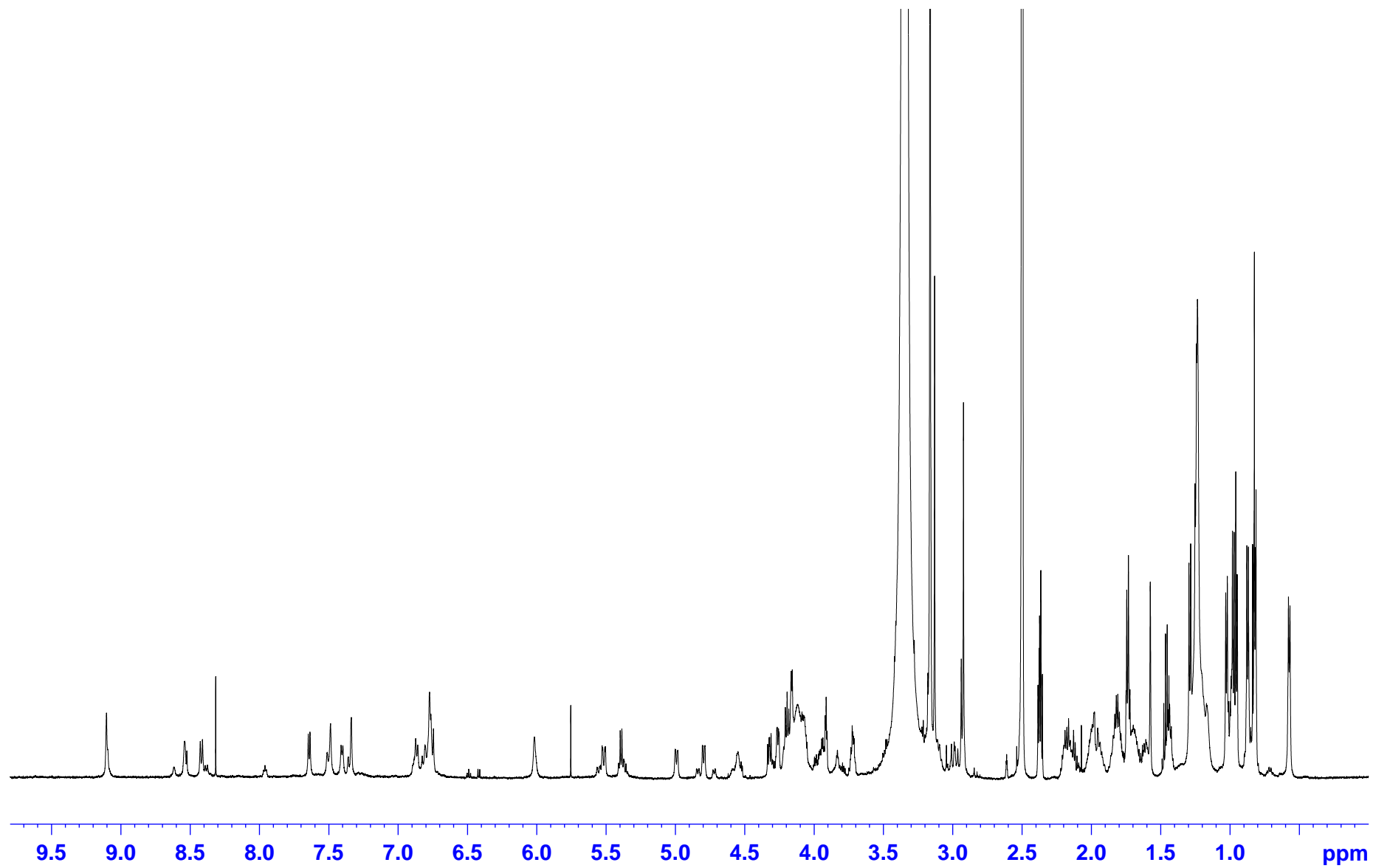
S21. TOCSY spectrum (600 MHz, DMSO- d_6) of **3**



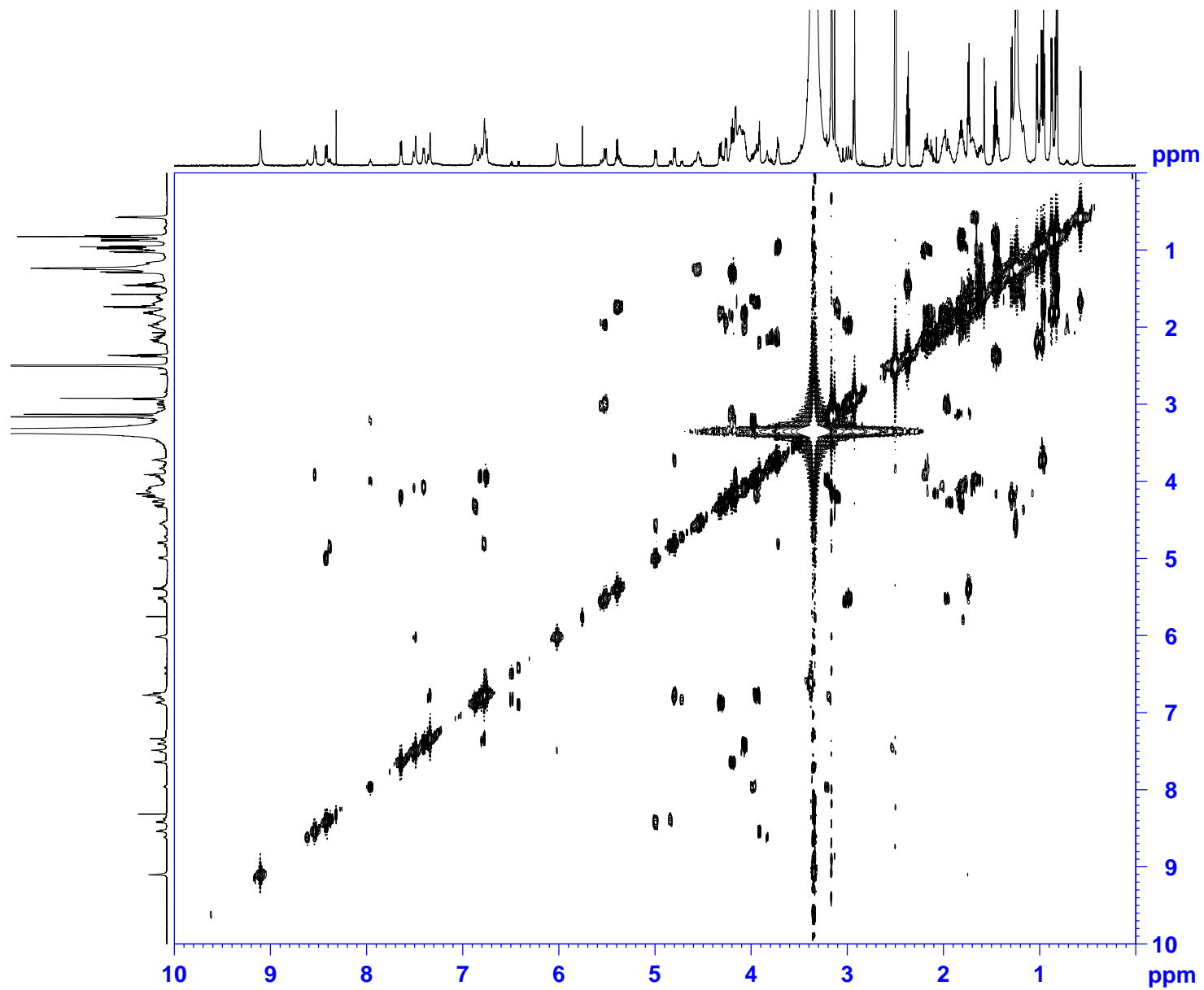
S22. HSQC spectrum (600 MHz, DMSO- d_6) of **3**



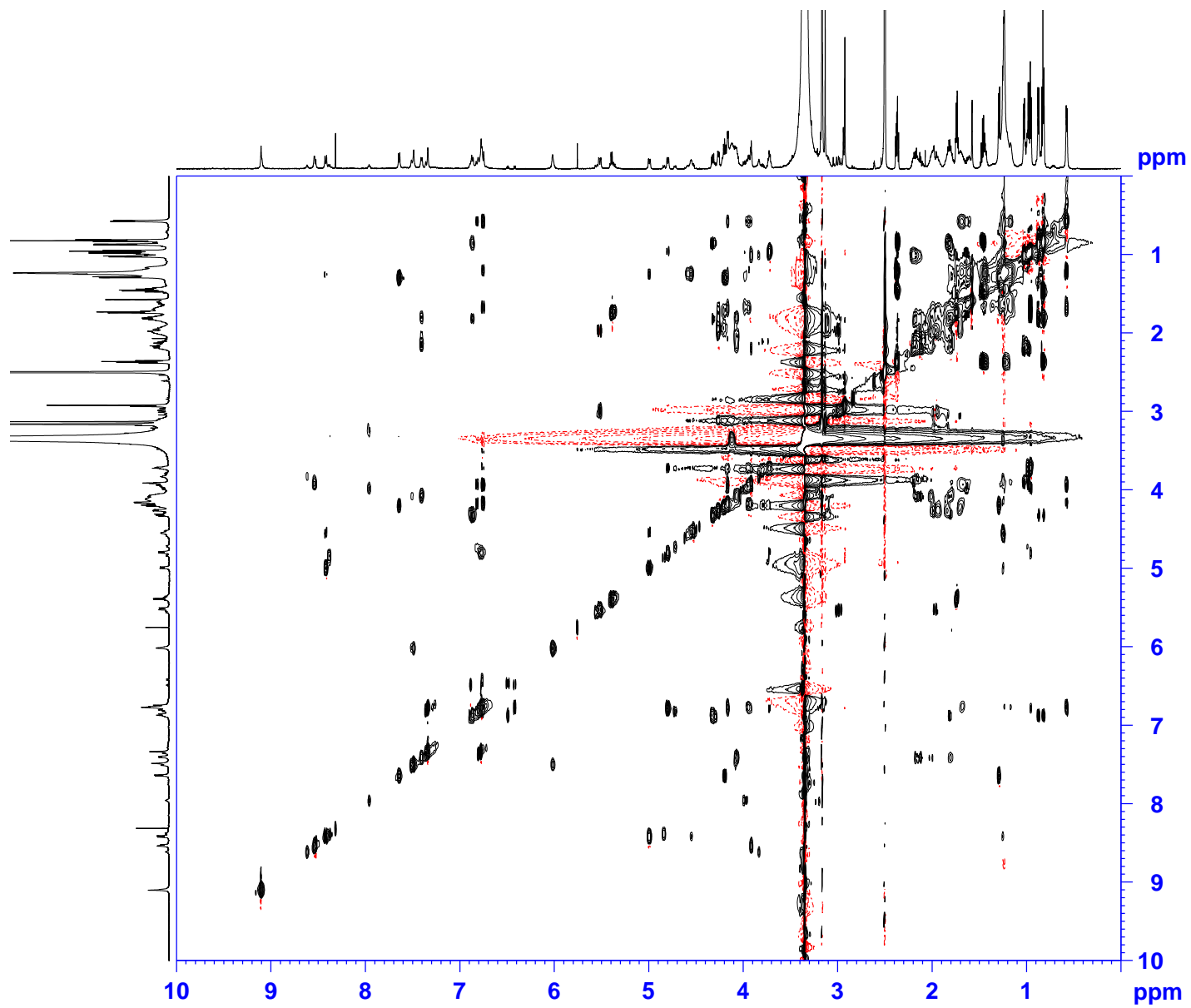
S23. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **4**



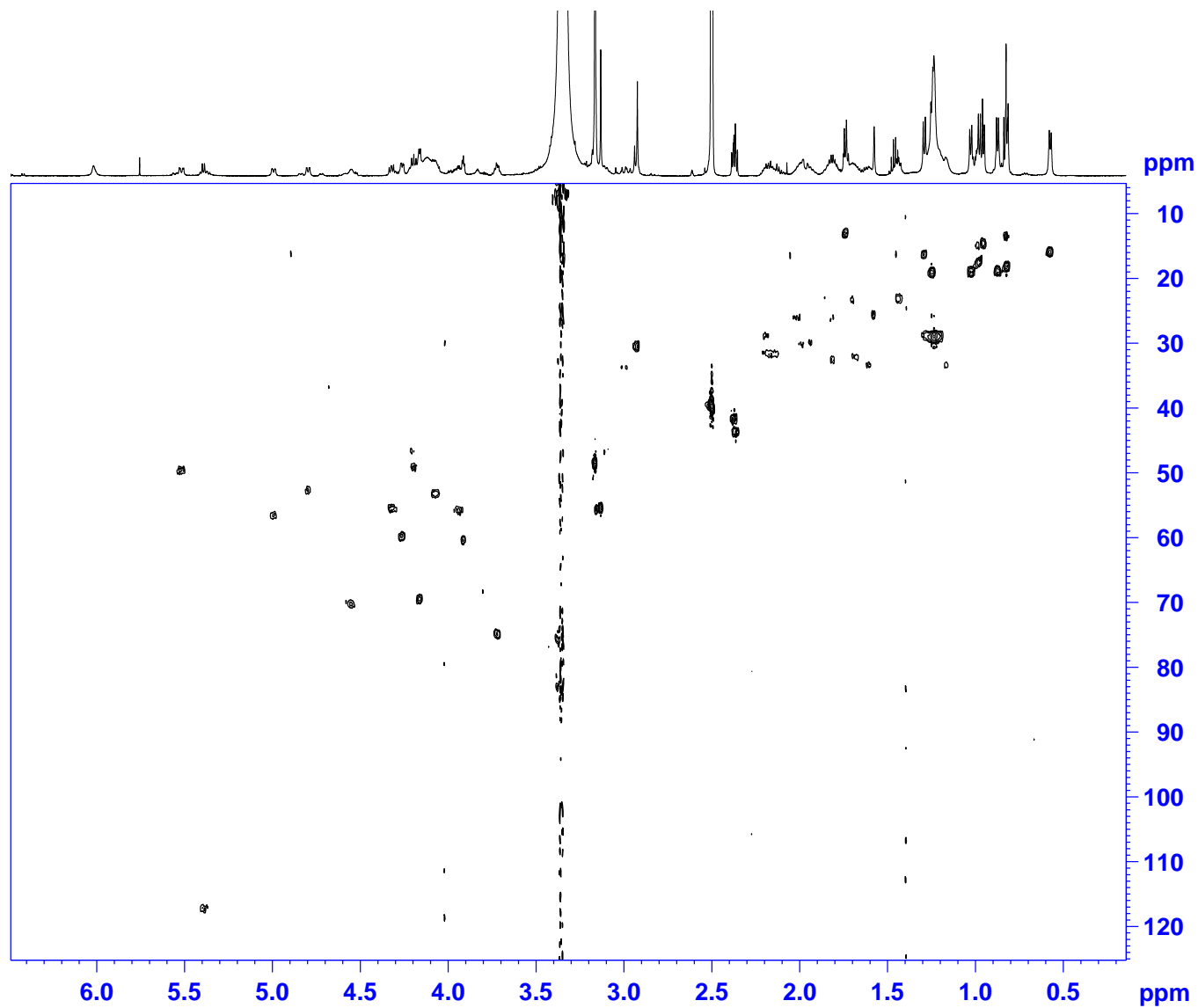
S24. COSY spectrum (600 MHz, DMSO- d_6) of **4**



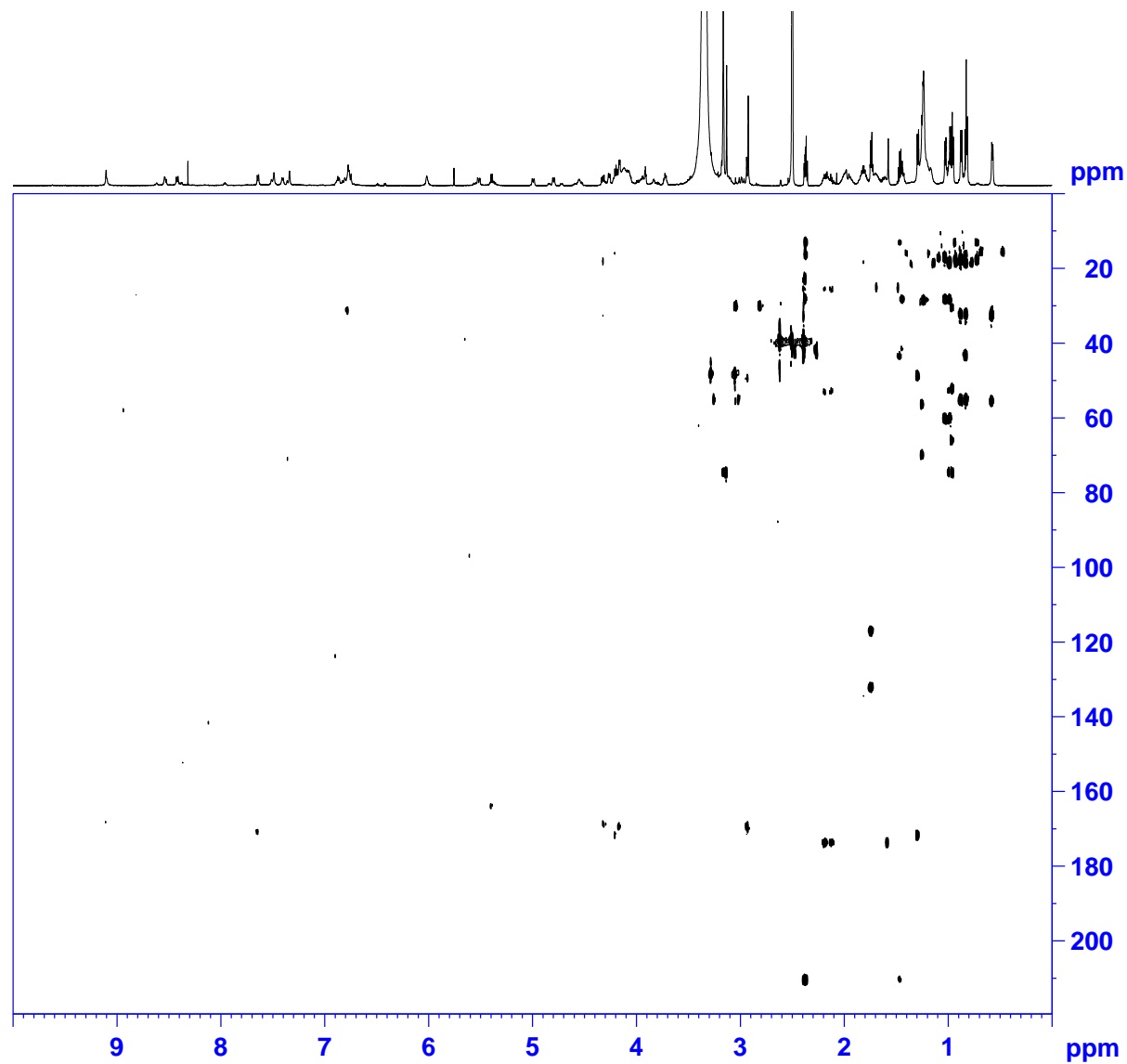
S25. TOCSY spectrum (600 MHz, DMSO- d_6) of **4**



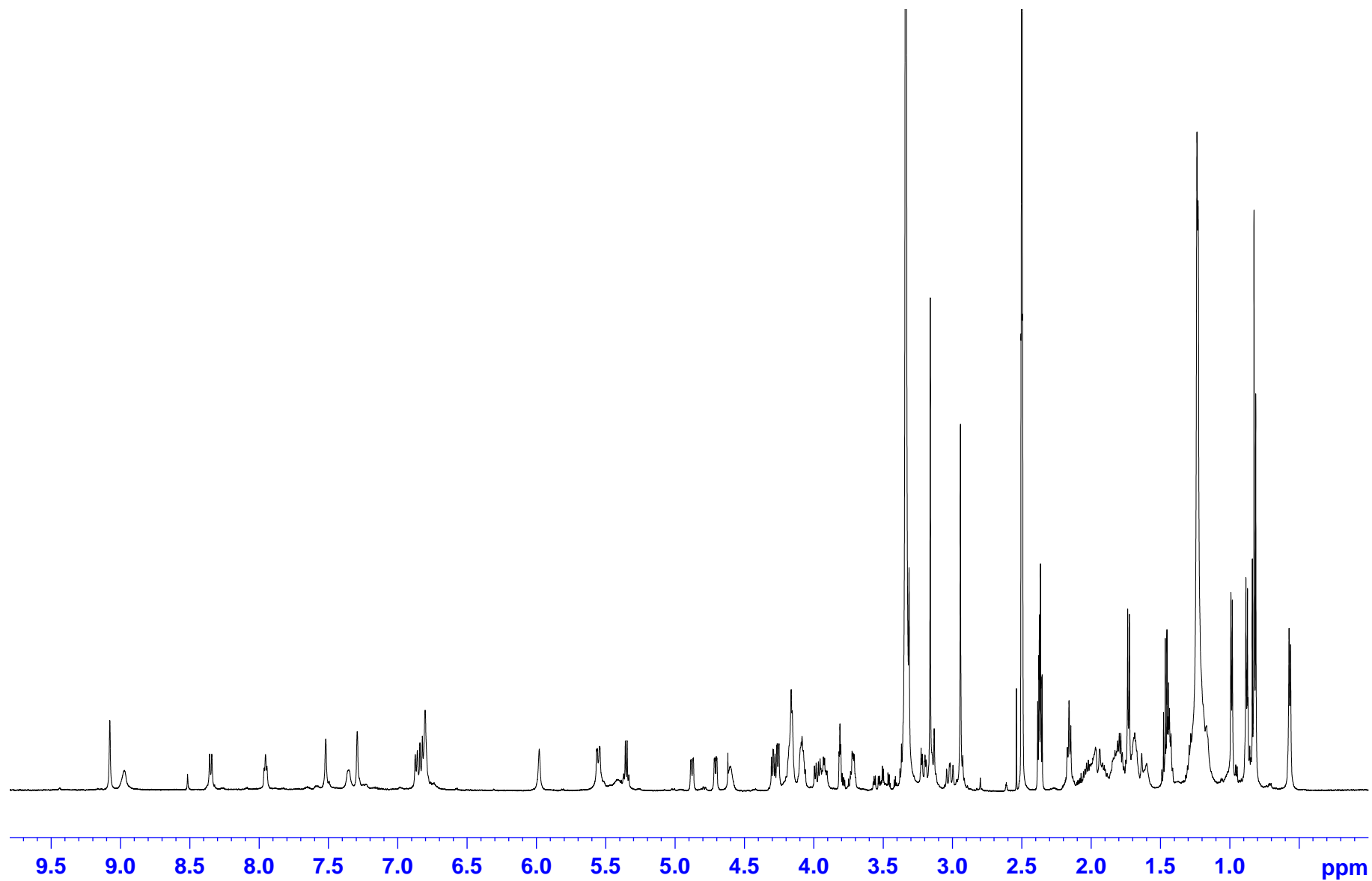
S26. HSQC spectrum (600 MHz, DMSO- d_6) of **4**



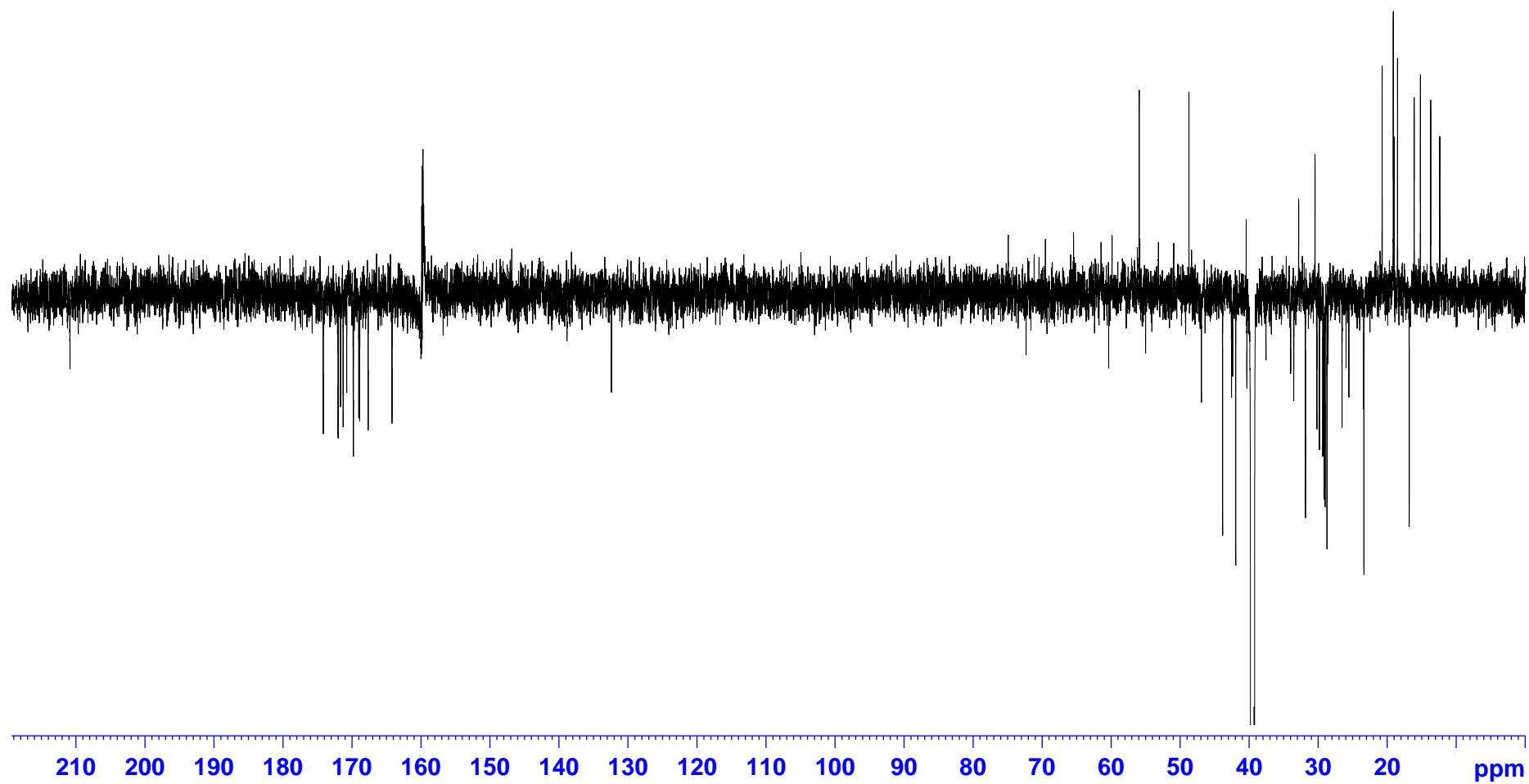
S27. HMBC spectrum (600 MHz, DMSO-*d*₆) of **4**



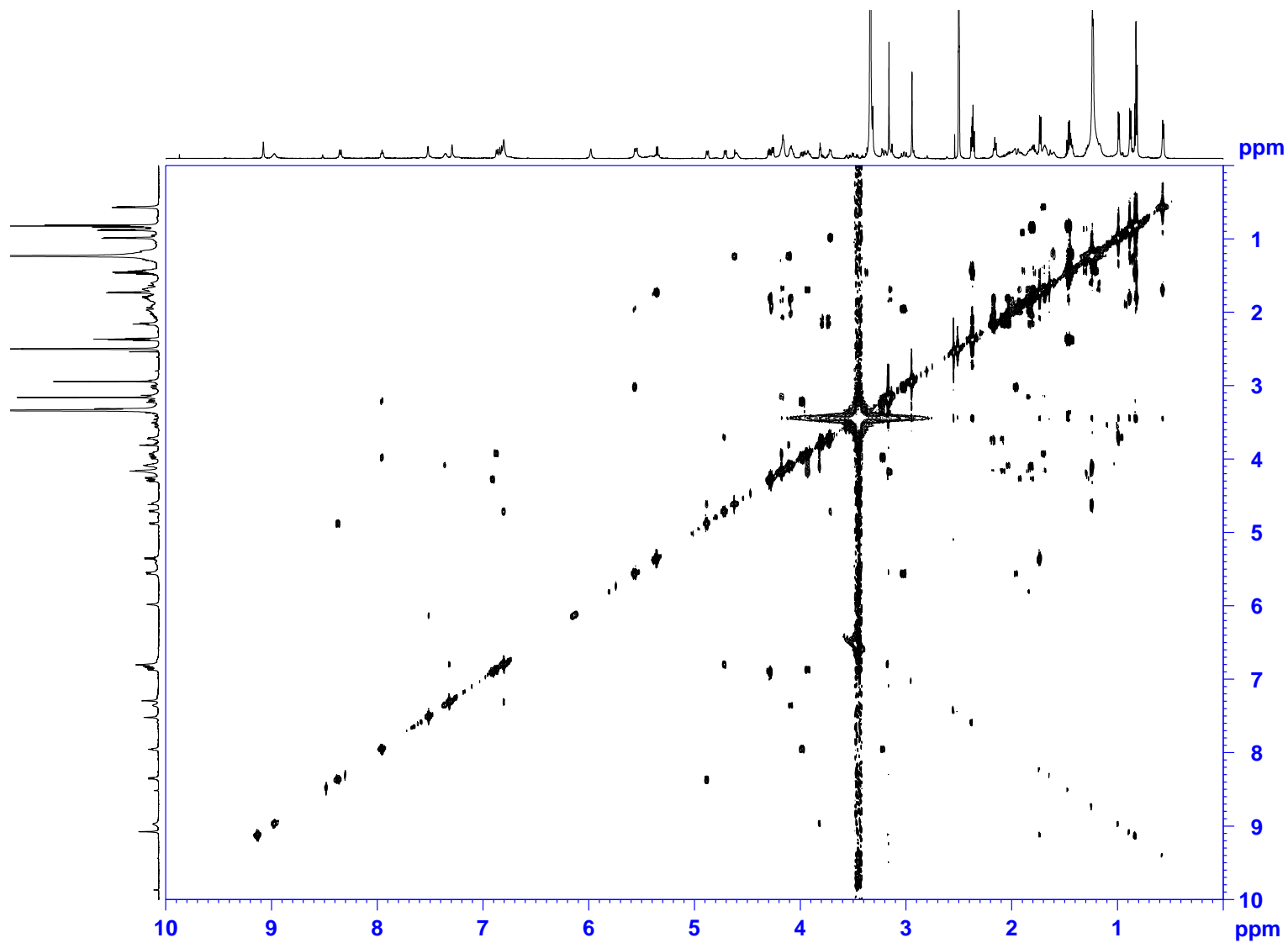
S28. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **5**



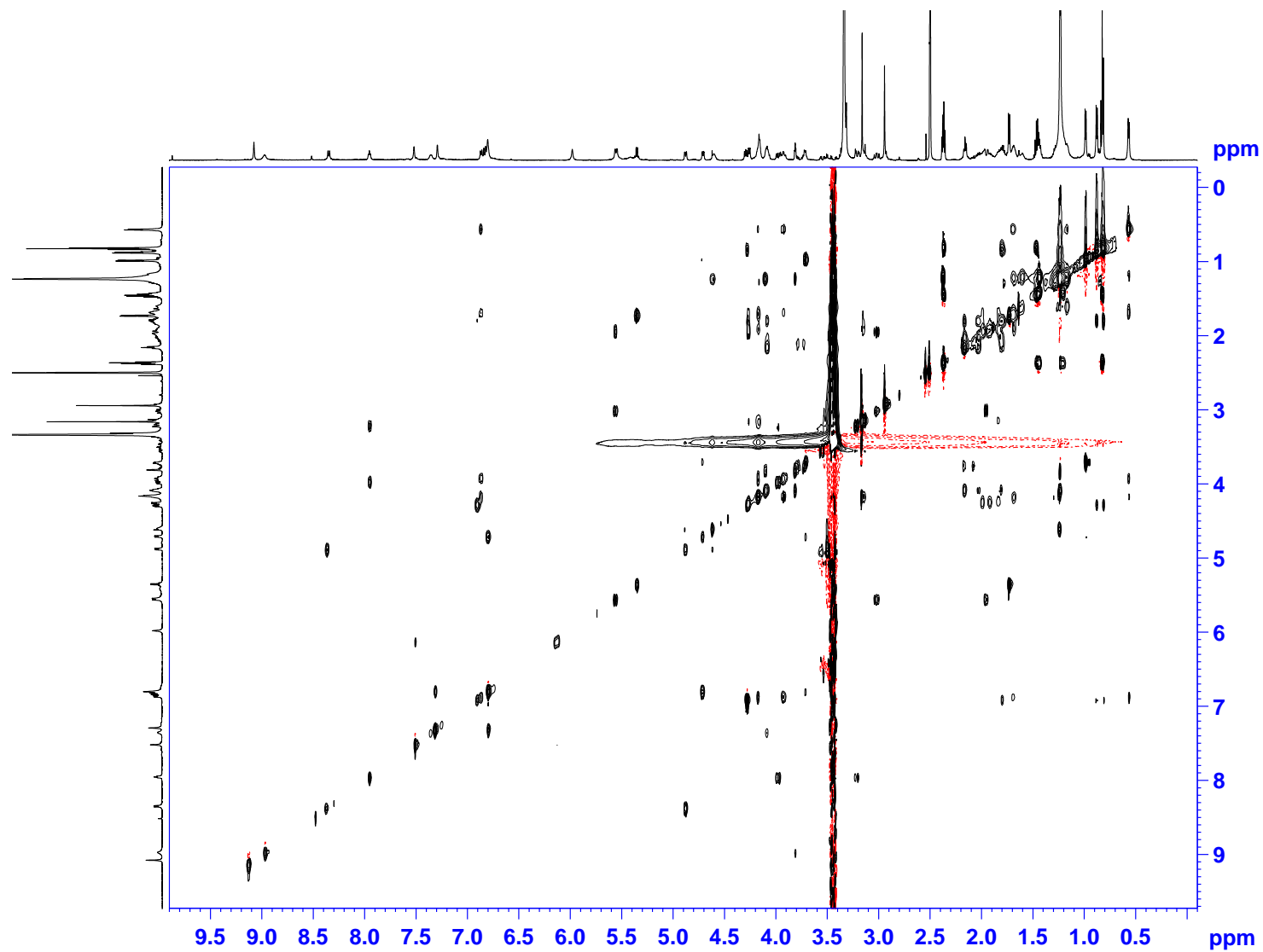
S29. DEPTQ spectrum (226 MHz, DMSO- d_6) of **5**



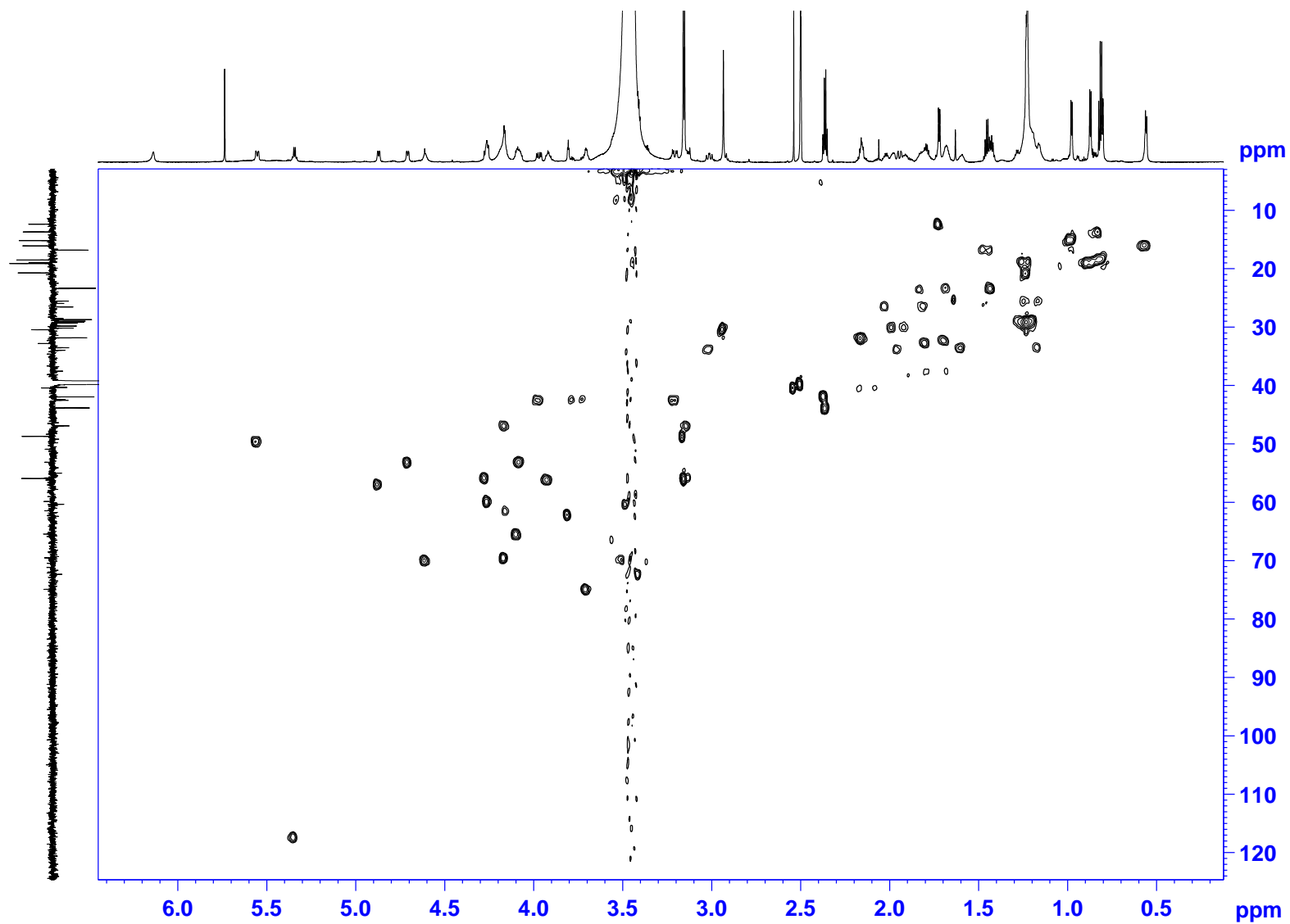
S30. COSY spectrum (600 MHz, DMSO- d_6) of **5**



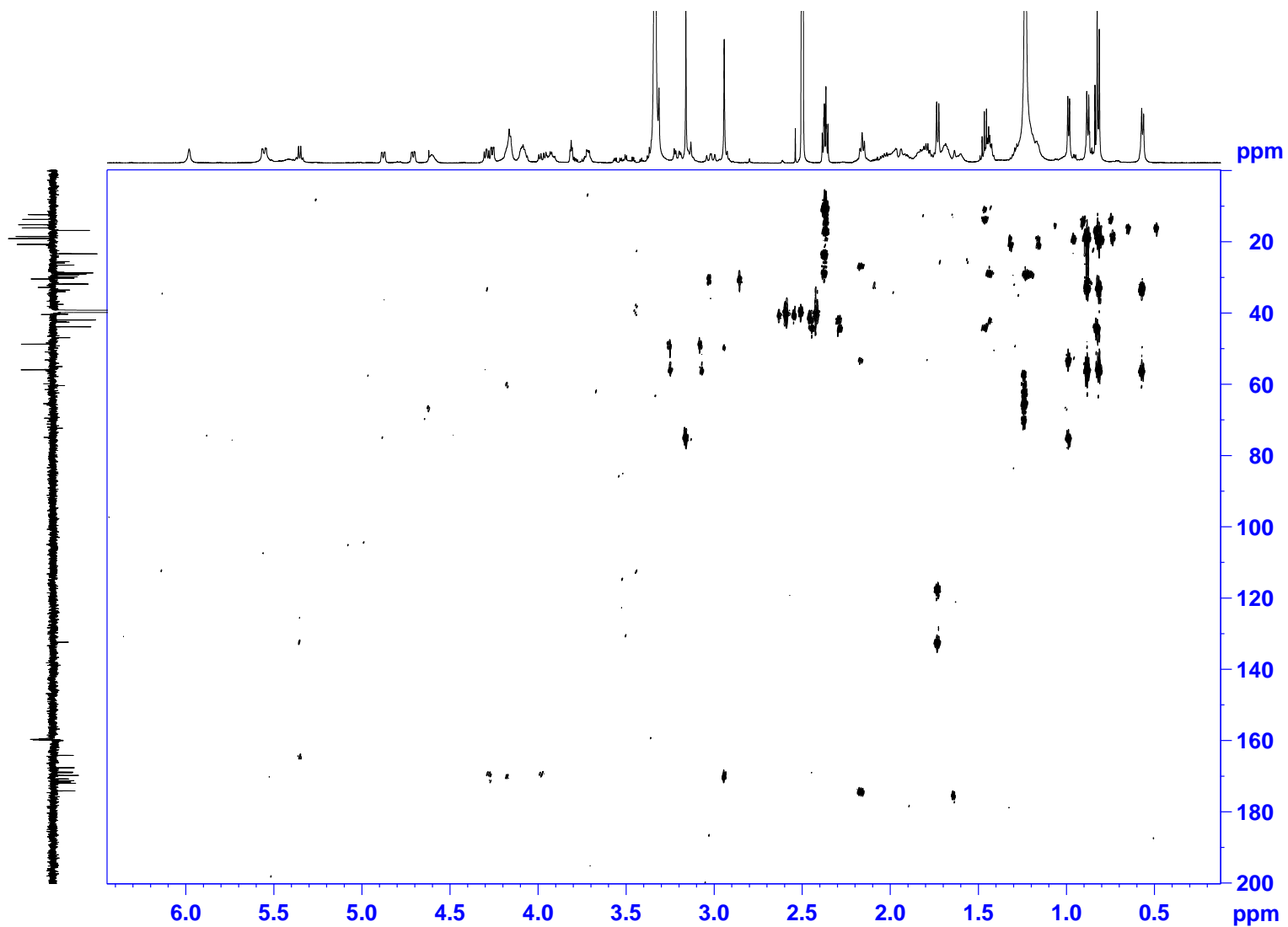
S31. TOCSY spectrum (600 MHz, DMSO- d_6) of **5**



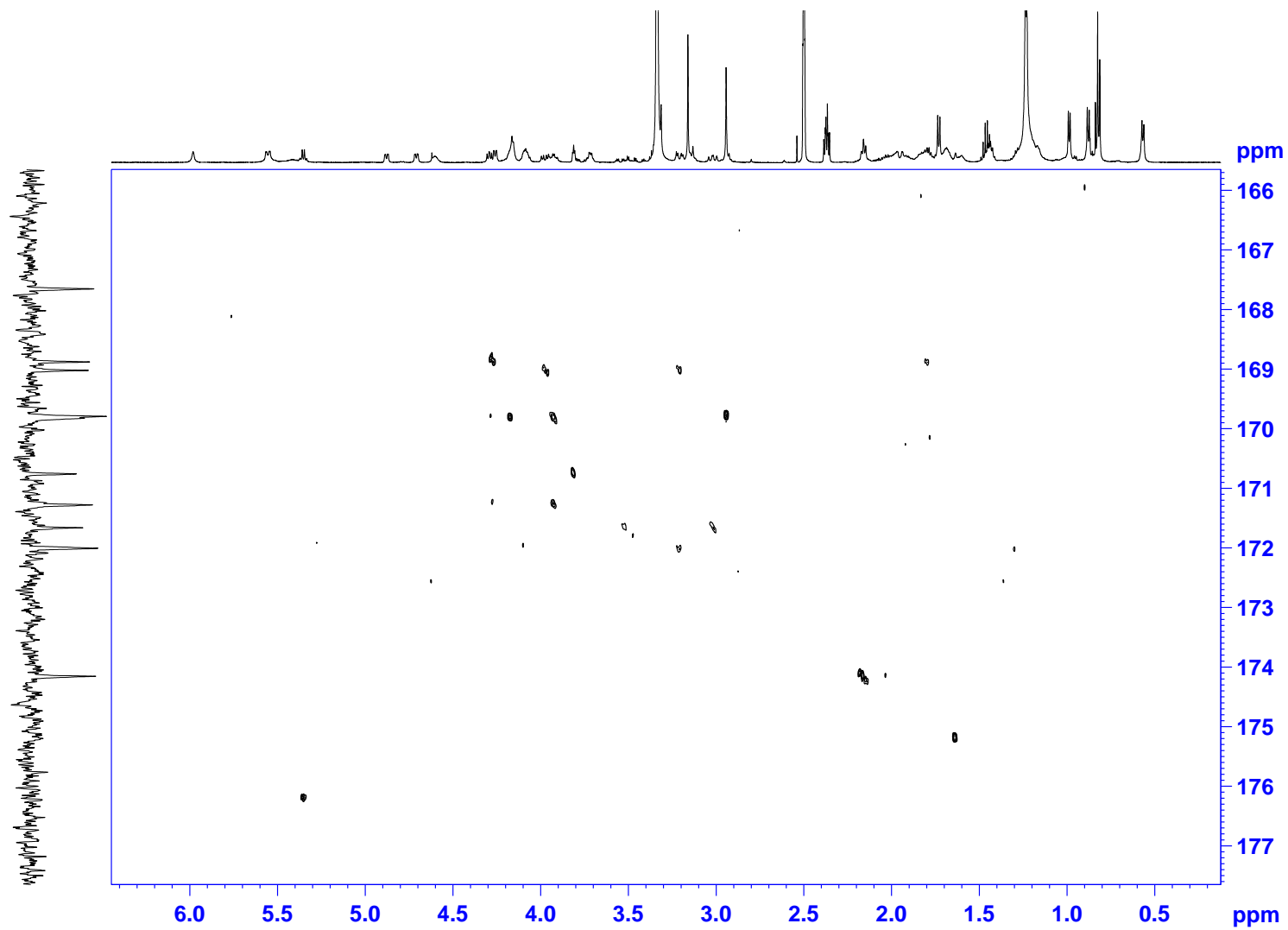
S32. HSQC spectrum (600 MHz, DMSO- d_6) of **5**



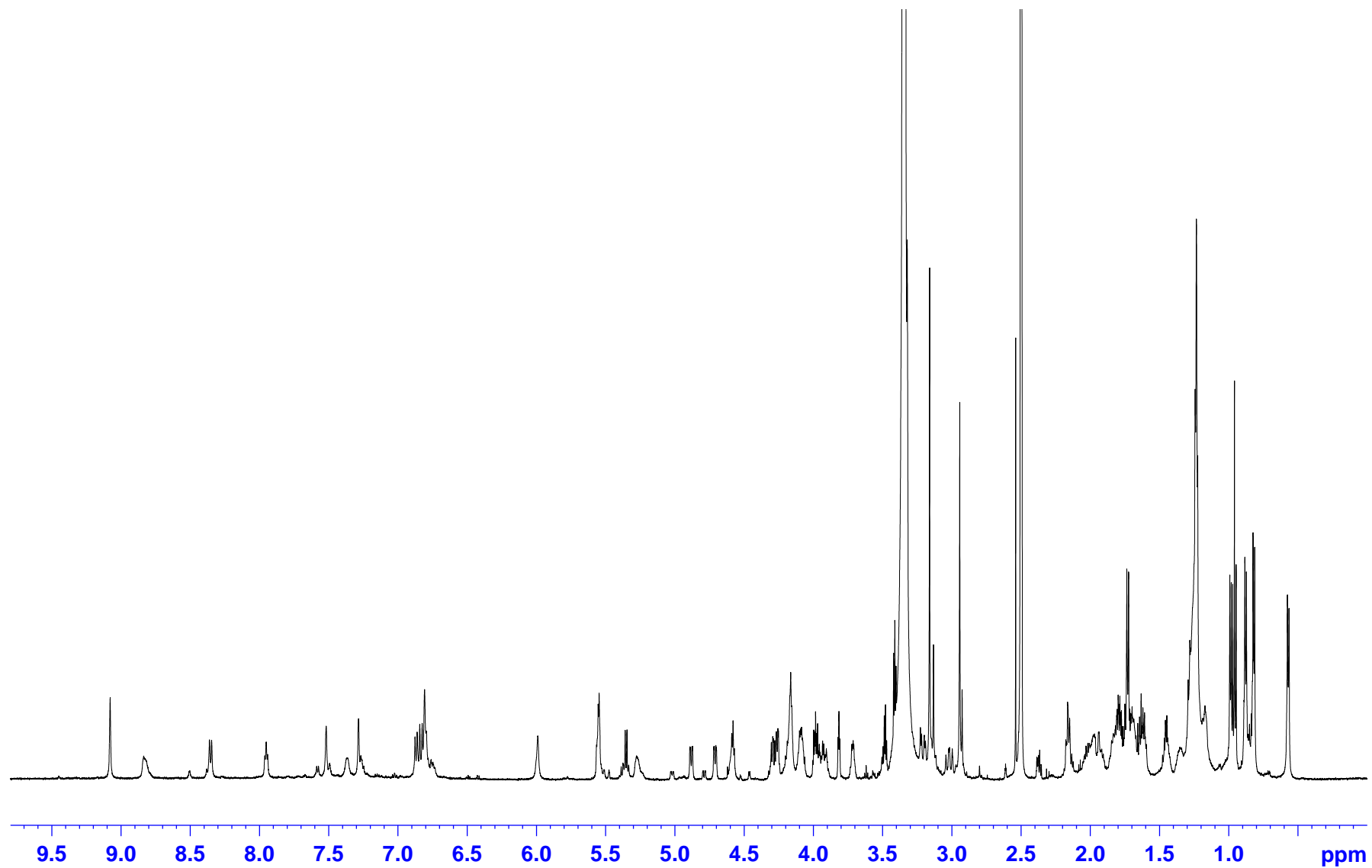
S33. HMBC spectrum (600 MHz, DMSO- d_6) of **5**



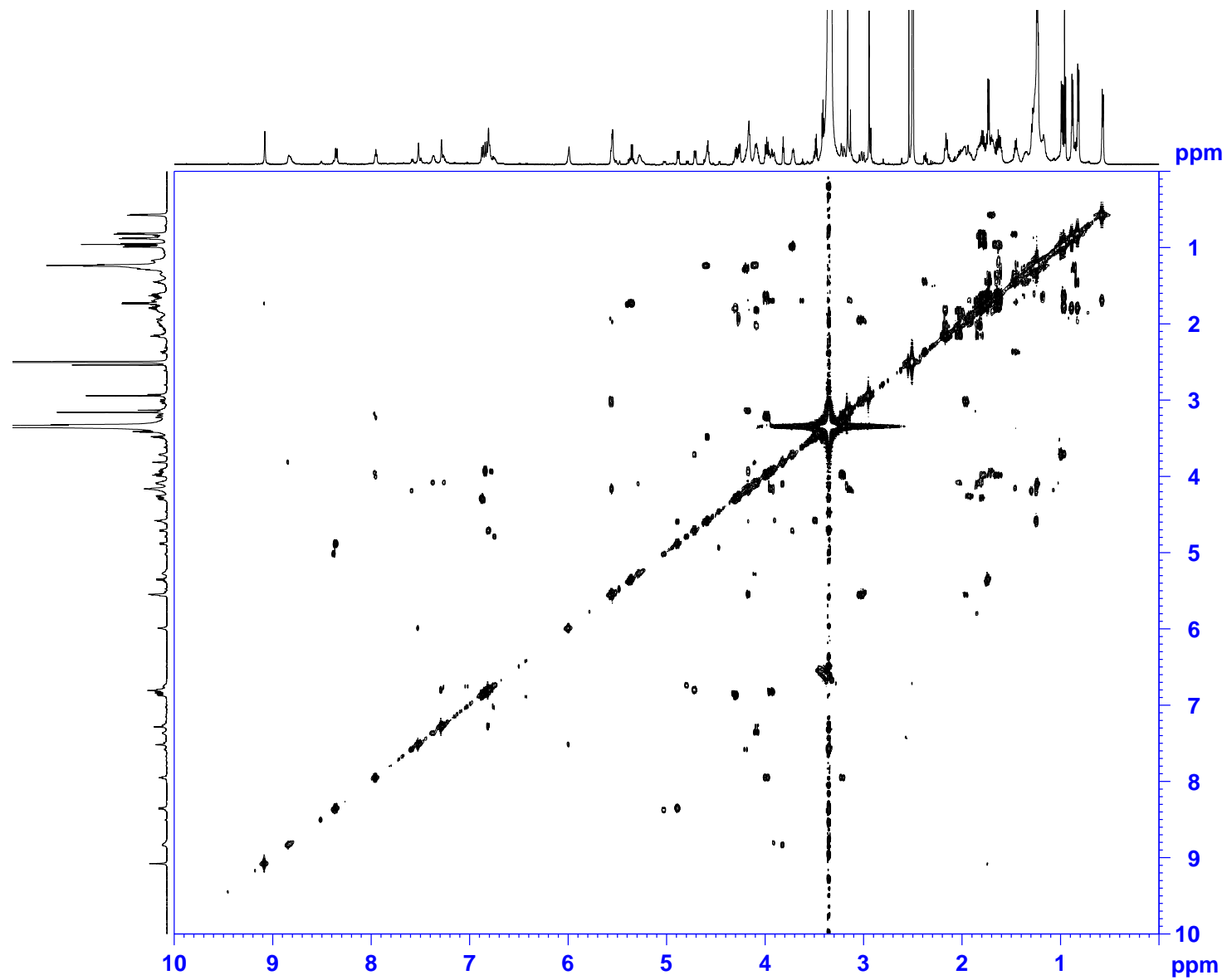
S34. Selective HMBC spectrum (900 MHz, DMSO-*d*₆) of **5**



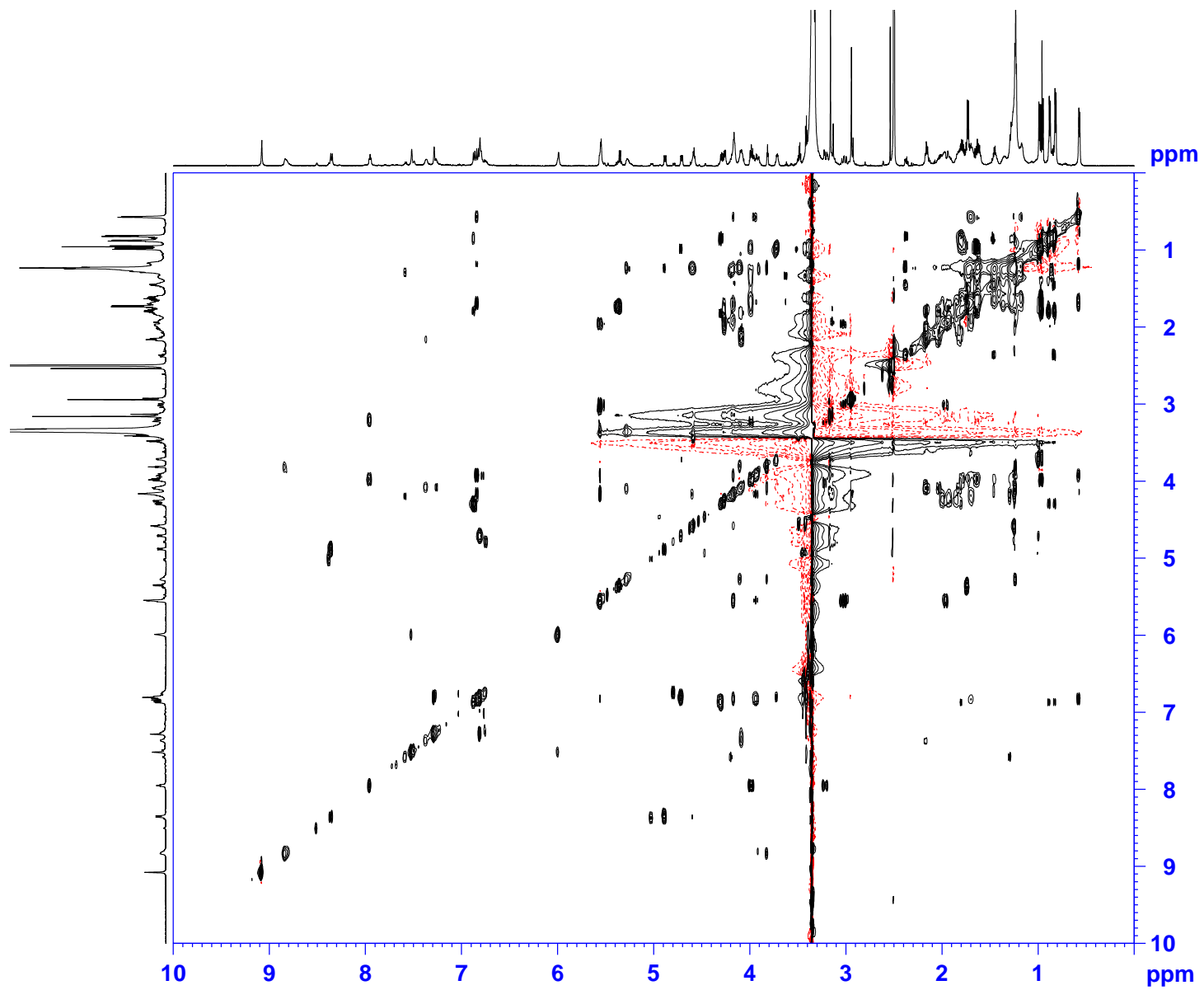
S35. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of **6**



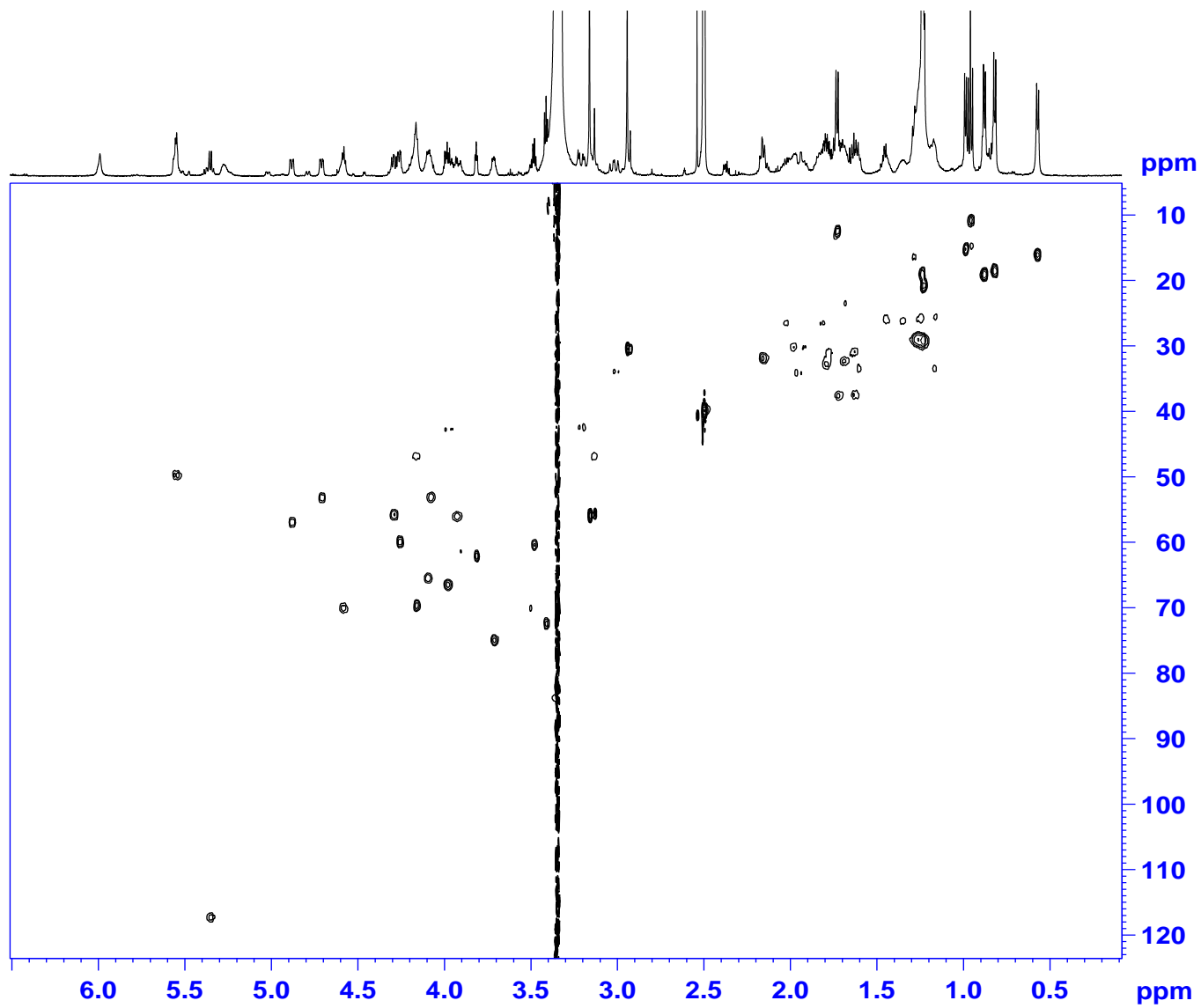
S36. COSY spectrum (600 MHz, DMSO- d_6) of **6**



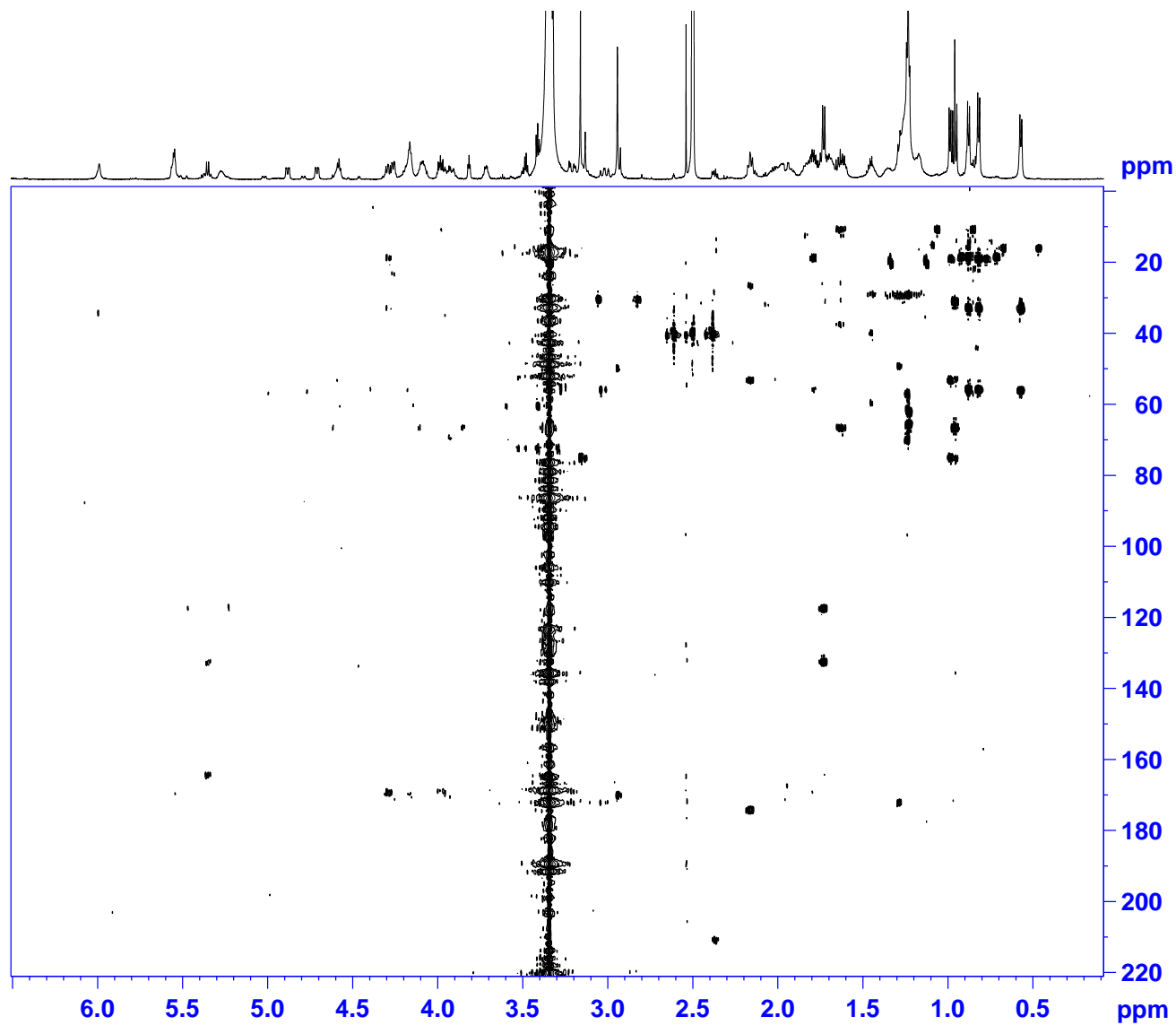
S37. TOCSY spectrum (600 MHz, DMSO- d_6) of **6**



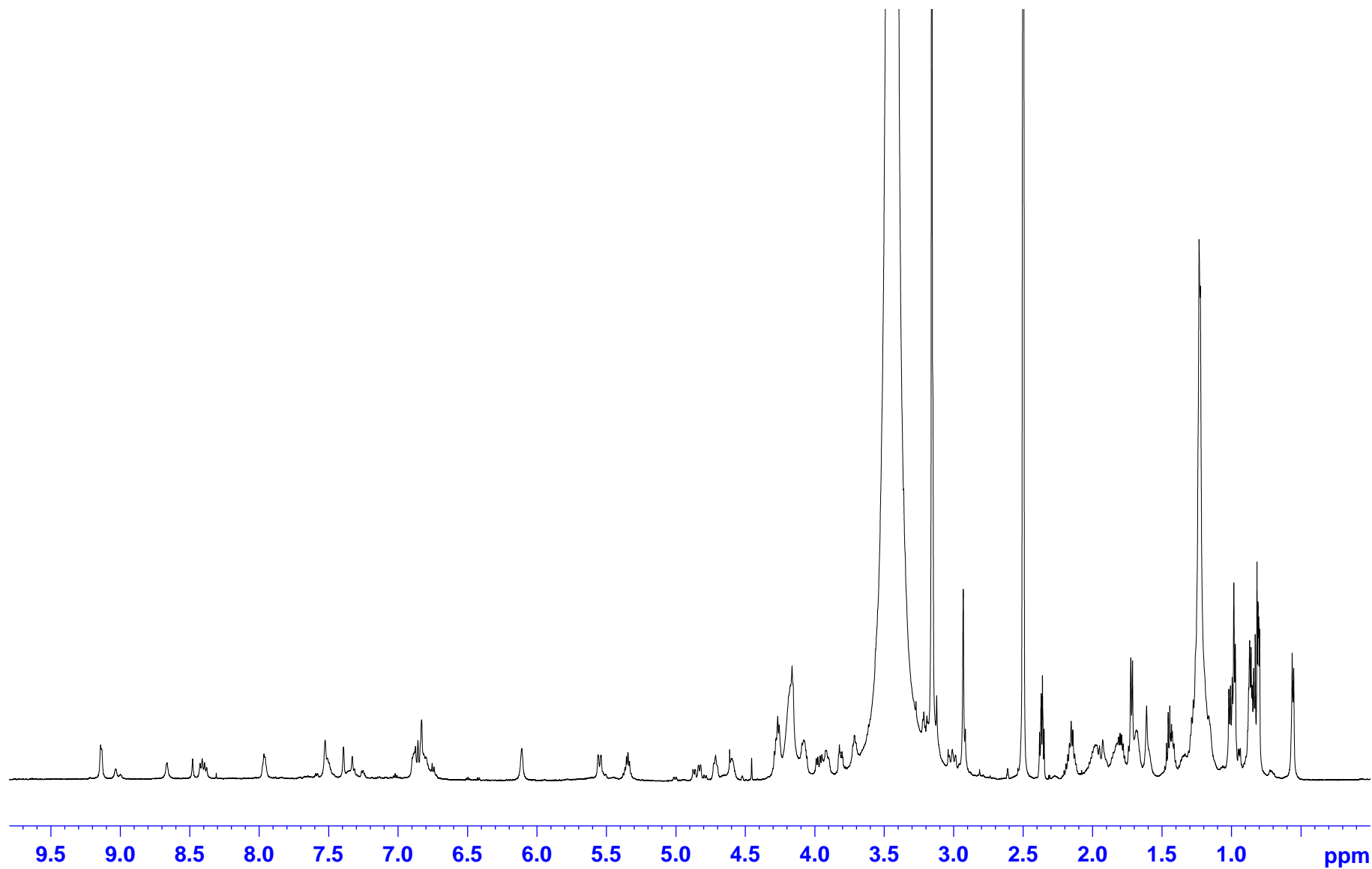
S38. HSQC spectrum (600 MHz, DMSO- d_6) of **6**



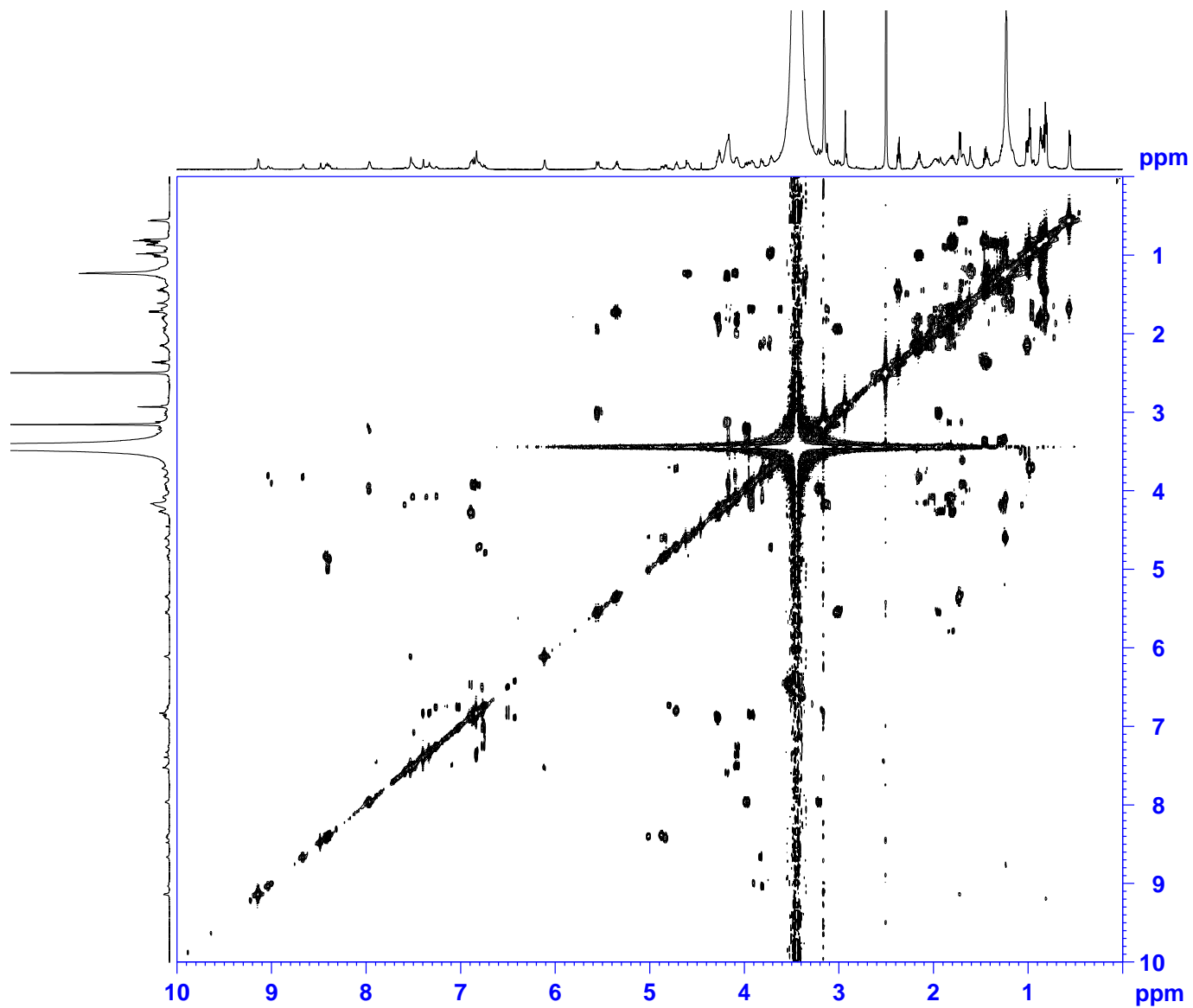
S39. HMBC spectrum (600 MHz, DMSO-*d*₆) of **6**



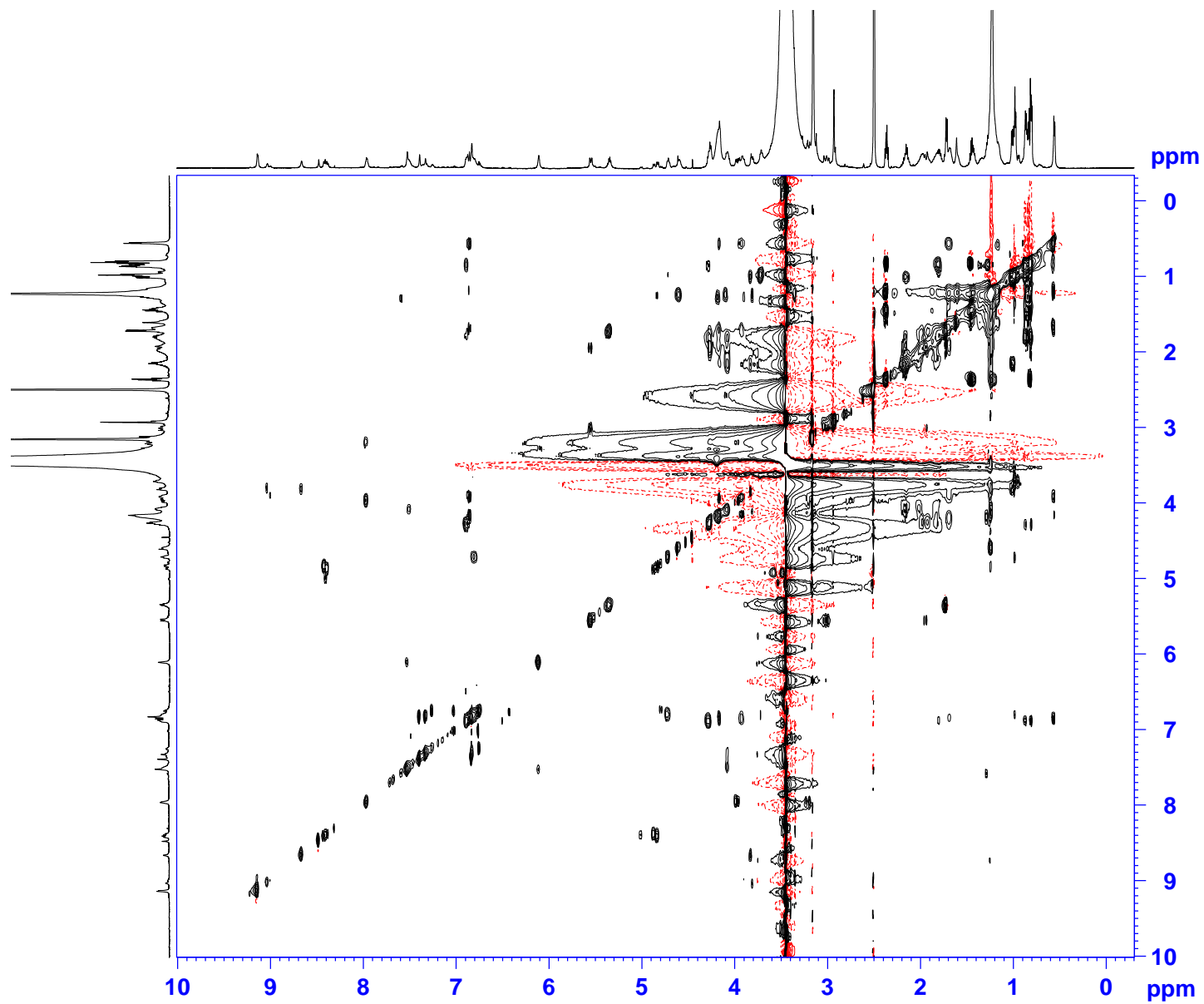
S40. ^1H NMR spectrum (600 MHz, $\text{DMSO-}d_6$) of inseparable mixture of **7** and **8**



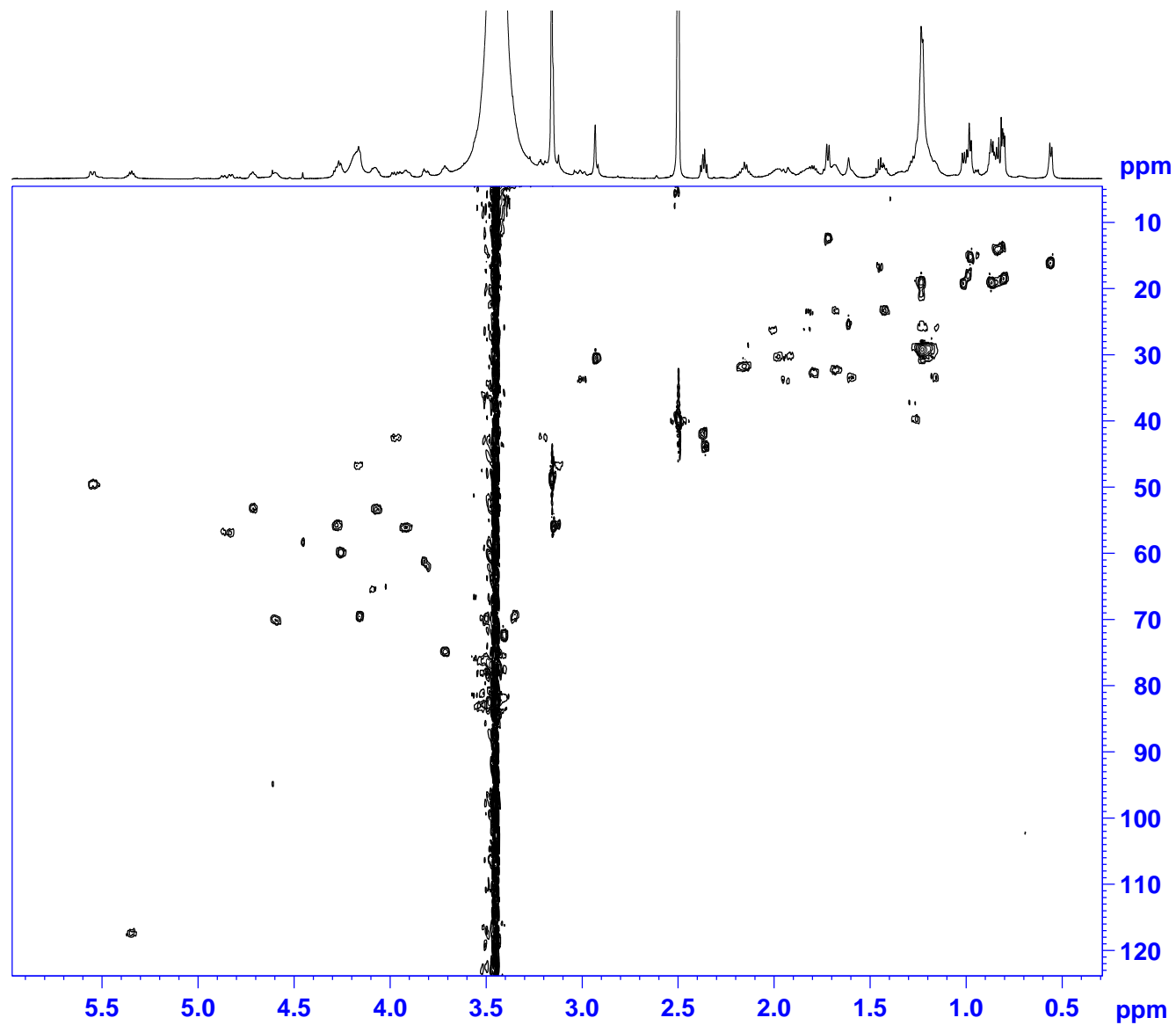
S41. COSY spectrum (600 MHz, DMSO- d_6) of inseparable mixture of **7** and **8**



S42. TOCSY spectrum (600 MHz, DMSO- d_6) of inseparable mixture of **7** and **8**



S43. HSQC spectrum (600 MHz, DMSO- d_6) of inseparable mixture of **7** and **8**



S44. HMBC spectrum (600 MHz, DMSO-*d*₆) of inseparable mixture of **7** and **8**

