Supporting information for

Ansavaricins F – I, New DNA Topoisomerase Inhibitors Produced by *Streptomyces* sp. S012

Zhiqiang Zhang,[a] Xingkang Wu,[a] Rentai Song,[b] Juanli Zhang,[c] Haoxin Wang,[b] Jing Zhu,[b] Chunhua Lu[a] and Yuemao Shen*[ab]

[a]*Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, No. 44 West Wenhua Road, Jinan, Shandong 250012, P. R. China, E-mail: yshen@sdu.edu.cn; Tel:+86-531-88382108*

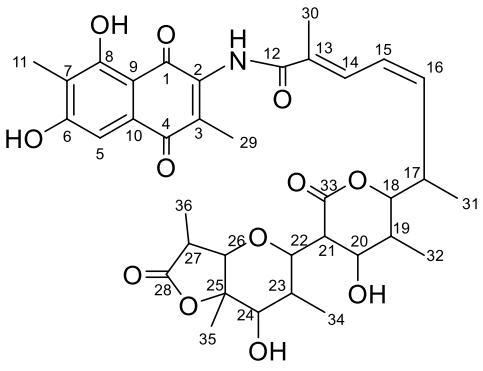
[b]*State Laboratory of Microbial Technology, Shandong University, No. 27 South Shanda Road, Jinan, Shandong 250100, P. R. China*

[c]*Department of pharmacy, Xijing Hospital, The Fourth Military Medical University, Changle West Street 15, Xi'an, Shaanxi 710032, P. R. China*

Table of Contents

Table S1. NMR spectroscopy data (pyridine-d_5) for compound 1

Pos.	δ_H (mult., *J* Hz)	δ_C	^{1}H-^{1}H COSY	HMBC
1		185.3s		
2		140.7s		
3		138.0s		
4		185.1s		
5	7.51 (br s)	108.9d		C-1/C-4, C-7, C-9
6		163.1s		
7		118.2s		
8		164.8s		
9		108.3s		
10		132.6s		
11	2.41 (s)	9.0q		C-6, C-7, C-8
12		168.2s		
13		132.2s		
14	7.95 (d, 11.7)	130.8d	H-15	C-12, C-30
15	6.75 (t, 11.4)	125.4d	H-16	C-17
16	6.24 (t, 10.7)	139.6d	H-15,H-17	C-14
17	3.31 (m)	35.1d	H-31	
18	4.77 (dd, 10.3,1.9)	85.0d	H-19	C-16
19	2.60 (m)	34.5d	H-18, H-20 H-32	
20	4.30 (t, 2.6)	73.8d	H-19, H-21	C-18, C-33
21	3.50 (d, 1.6)	51.3d	H-20	C-20, C-33
22	4.06 (d, 10.3)	78.1d		C-20, C-24, C-33
23	2.82 (m)	38.6d	H-24, H-34	
24	3.99 (d, 11.1)	76.3d	H-23	C-25
25		84.3s		
26	4.52 (d, 11.2)	85.8d	H-27	C-22, C-24, C-25, C-35, C-36
27	3.47 (m)	36.4d	H-26, H-36	
28		176.5s		
29	2.31 (s)	14.3q		C-2, C-3, C-4
30	2.28 (s)	13.8q		C-12, C-13, C-14
31	1.23 (d, 6.9)	19.0q	H-17	C-16, C-17, C-18
32	1.32 (d, 6.8)	14.5q	H-19	C-18, C-19, C-20
33		170.4s		
34	1.36 (d, 6.4)	13.6q	H-23	C-22, C-23, C-24
35	1.77 (s)	18.7q		C-24, C-26
36	1.45 (d, 6.8)	14.0q	H-27	C-26, C-27, C-28
NH	10.05 (s)			C-3



1

Table S2. NMR spectroscopy data (pyridine-d_5) for compound 2

Pos.	δ_H (mult., *J* Hz)	δ_C	^{1}H-^{1}H COSY	HMBC
1		185.3s		
2		138.2s		
3		140.7s		
4		185.1s		
5	7.51 (s)	108.8d		C-1/C-4, C-7, C-9, C-10
6		163.1s		
7		118.3s		
8		164.8s		
9		108.4s		
10		132.2s		
11	2.41 (s)	9.0q		C-6, C-7, C-8
12		168.3s		
13		133.2s		
14	7.80 (s)	130.4d	H-15	C-12, C-30
15	6.60 (t, 11.4)	125.9d	H-16	C-13, C-17
16	5.98 (t, 10.7)	138.4d	H-15, H-17	C-14
17	3.19 (m)	34.4d	H-16, H-31	C-31
18	4.04 (dd, 2.3, 10.9)	86.8d	H-19	C-16, C-17, C-31
19	2.67 (m)	32.9d	H-18, H-20, H-32	C-18, C-20
20	7.05 (d, 2.1)	150.4d	H-19	C-18, C-19, C-22, C-32, C-33
21		130.8s		
22	4.99 (m)	73.1d	H-23	C-20, C-21, C-24, C-33
23	2.26 (m)	40.6d	H-22, H-24, H-34	
24	4.17 (d, 10.9)	76.4d	H-23	C-23, C-25, C-34, C-35
25		84.5s		
26	4.40 (d, 11.1)	86.5d	H-27	C-22, C-24, C-25, C-35, C-36
27	3.62 (br s)	36.5d	H-26, H-36	C-26, C-36
28		176.9s		
29	2.31 (s)	14.5q		C-2, C-3, C-4
30	2.27 (s)	13.826q		C-12, C-13, C-14
31	1.11 (d, 6.9)	18.5q	H-17	C-16, C-17, C-18
32	1.09 (d, 7.2)	16.3q	H-19	C-18, C-19, C-20
33		165.1s		
34	1.30 (d, 6.5)	13.831q	H-23	C-22, C-23, C-24
35	1.84 (s)	18.8q		C-24, C-25, C-26
36	1.37 (d, 6.9)	14.0q		C-26, C-27, C-28
NH	10.06 (s)			

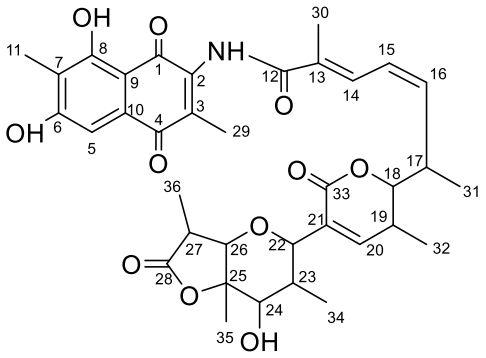


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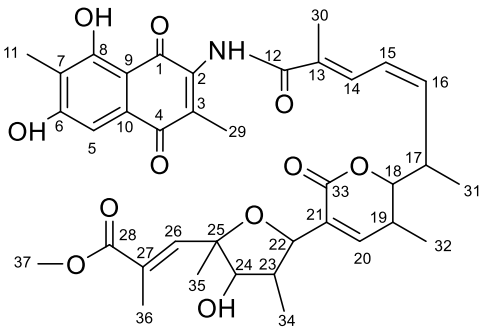
Table S3. NMR spectroscopy data (pyridine-d_5) for compound 3

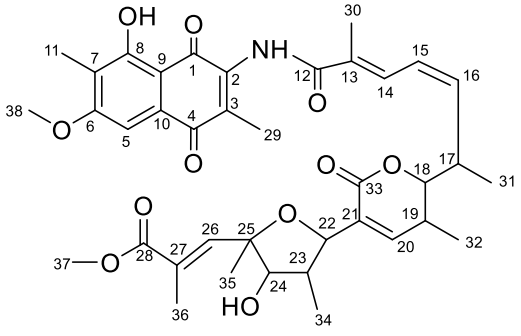
Pos.	δ_H (mult., *J* Hz)	δ_C	^{1}H-^{1}H COSY	HMBC
1		185.5s		
2		141.0s		
3		138.7s		
4		185.4s		
5	7.62 (s)	109.1d		C-1/C-4, C-7, C-9
6		163.3s		
7		118.5s		
8		164.9s		
9		108.6s		
10		132.4s		
11	2.40 (s)	9.1q		C-6, C-7, C-8
12		168.8s		
13		133.3s		
14	7.83 (d, 11.6)	130.7d	H-15	C-12, C-30
15	6.64 (t, 11.4)	126.0d	H-16	C-13, C-17
16	6.00 (t, 10.7)	138.9d	H-15, H-17	C-14
17	3.30 (m)	34.7d	H-16, H-31	
18	4.06 (dd, 10.7, 2.0)	87.2d	H-19	C-16
19	2.53 (m)	32.9d	H-18, H-32	
20	6.73 (d, 1.0)	148.2d		C-32, C-22, C-18, C-33
21		132.2s		
22	4.94 (d, 9.5)	78.5d	H-23	C-20, C-21, C-23, C-33, C-34
23	2.33 (s)	47.1d	H-22, H-24, H-34	C-22, C-25
24	4.03 (d, 9.6)	85.6d	H-23	C-26, C-34, C-35
25		83.0s		
26	7.88 (s)	144.2d		C-25, C-27, C-28, C-36
27		130.8s		
28		170.0s		
29	2.33 (s)	14.5q		C-2, C-3, C-4
30	2.32 (s)	14.0q		C-12, C-13, C-14
31	1.19 (d, 6.7)	18.8q	H-17	C-16, C-17, C-18
32	1.09 (d, 7.1)	16.7q	H-19	C-18, C-19, C-20
33		165.5s		
34	1.38 (d, 6.5)	15.20q	H-23	C-22, C-23, C-24
35	1.65 (s)	25.6q		C-24, C-25, C-26
36	2.33 (s)	15.24q		C-26, C-27, C-28
37	3.74 (s)	52.5q		C-28
NH	10.15 (s)			



3

Table S4. NMR spectroscopy data (pyridine-d_5) for compound 4

Pos.	δ_H (mult., *J* Hz)	δ_C	^{1}H-^{1}H COSY	HMBC
1		185.0s		
2		138.8s		
3		140.5s		
4		184.8s		
5	7.34 (s)	103.4d		C-1/C-4, C-7, C-9
6		164.2s		
7		119.8s		
8		161.5s		
9		107.5s		
10		132.1s		
11	2.21 (s)	8.7q		C-6, C-7, C-8
12		168.4s		
13		133.2s		
14	7.78 (d, 11.7)	130.4d	H-15	C-12, C-30
15	6.59 (t, 11.4)	125.7d	H-16	C-13, C-17
16	5.95 (t, 10.7)	138.5d	H-15, H-17	C-14
17	3.13 (m)	34.4d	H-16, H-18, H-31	
18	3.98 (t, 9.4)	86.8d	H-17, H-19	C-16
19	2.49 (m)	32.7d	H-18, H-20, H-32	
20	6.70 (s)	147.8d		C-18, C-19, C-22, C-32, C-33
21		132.3s		
22	4.94 (d, 9.5)	78.3d	H-23	C-20, C-21, C-34
23	2.31 (m)	47.1d	H-22, H-24, H-34	
24	3.98 (t, 9.4)	85.3d	H-23	C-34, C-35
25		82.7s		
26	7.90 (s)	144.0d		C-25, C-27, C-28, C-36
27		130.7s		
28		169.7s		
29	2.35 (s)	15.1q		C-2, C-3, C-4
30	2.27 (s)	13.8q		C-12, C-13, C-14
31	1.11 (d, 6.8)	18.5q	H-17	C-16, C-17, C-18
32	1.01 (d, 7.1)	16.5q	H-19	C-18, C-19, C-20
33		165.1s		
34	1.36 (d, 6.5)	15.0q	H-23	C-22, C-23, C-24
35	1.61 (s)	25.5q		C-24, C-25, C-26
36	2.33 (s)	14.6q		C-26, C-27, C-28
37	3.70 (s)	52.2q		C-28
38	3.76 (s)	56.5q		C-6
NH	10.16 (s)			



4

Figure S1. ^{1}H NMR (400 MHz, pyridine-d_5) spectrum for compound 1

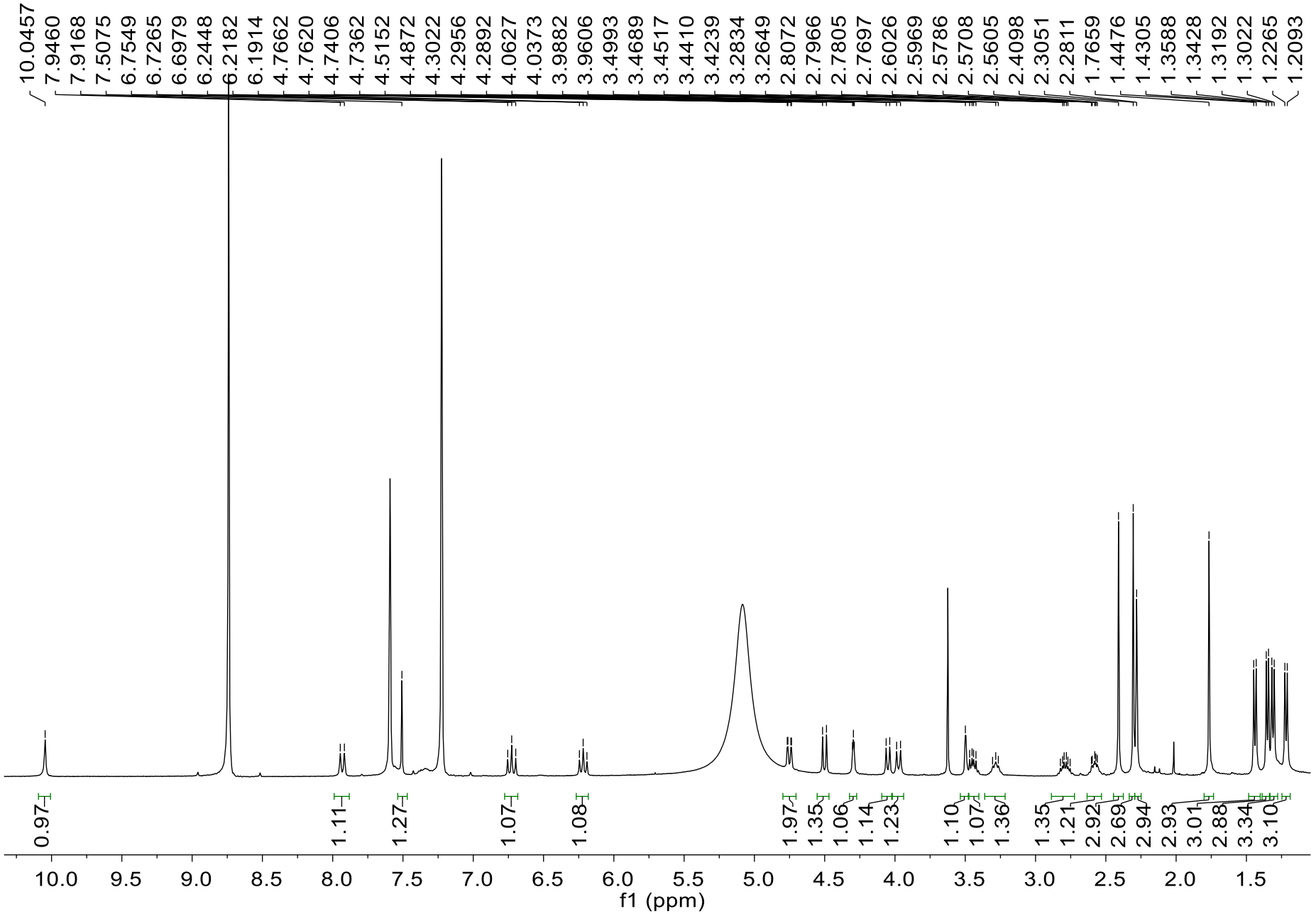


Figure S2. ^{13}C NMR (101 MHz, pyridine-d_5) spectrum for compound 1

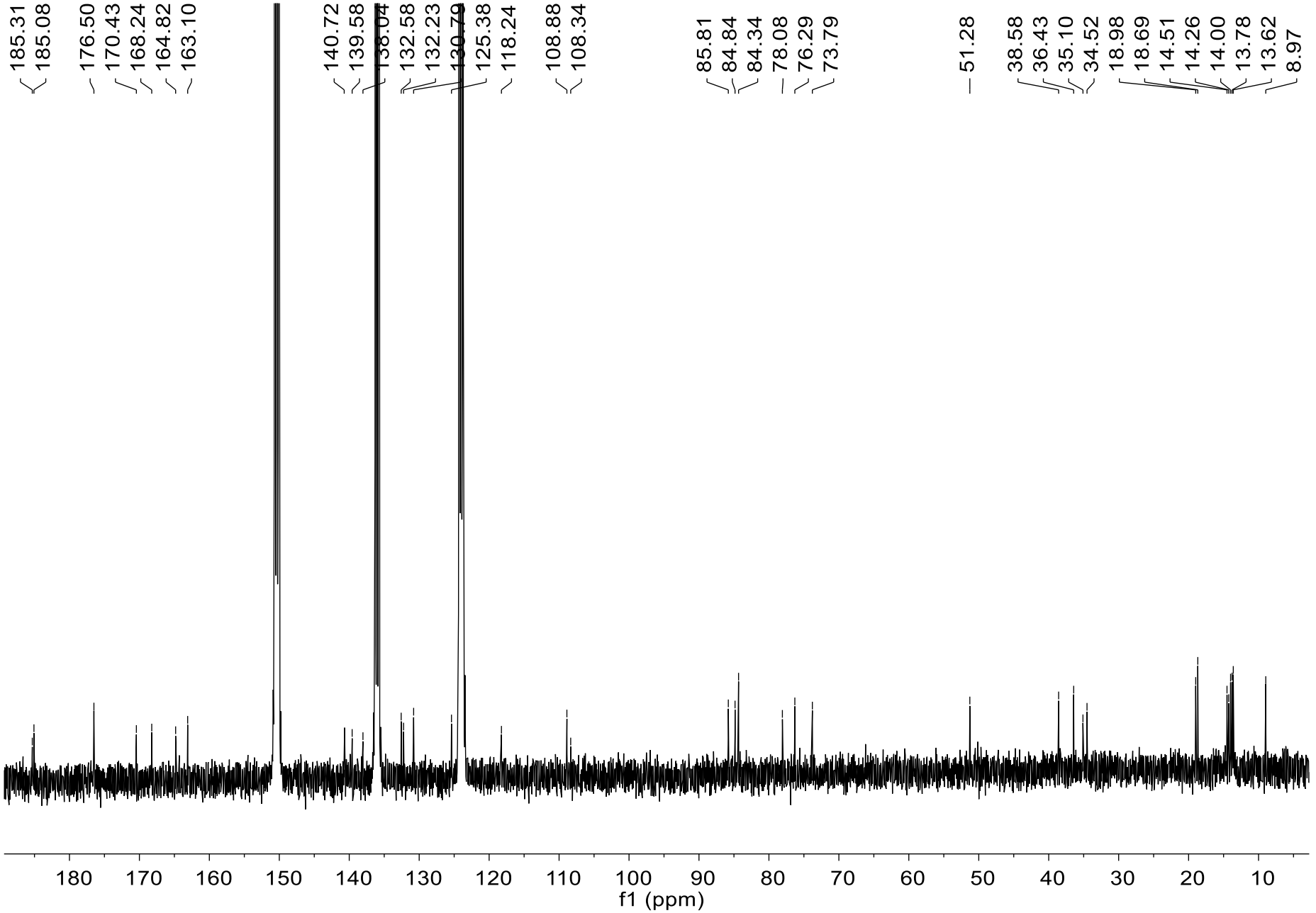


Figure S3. ^{1}H-^{1}H COSY (400 MHz, pyridine-d_5) spectrum for compound 1

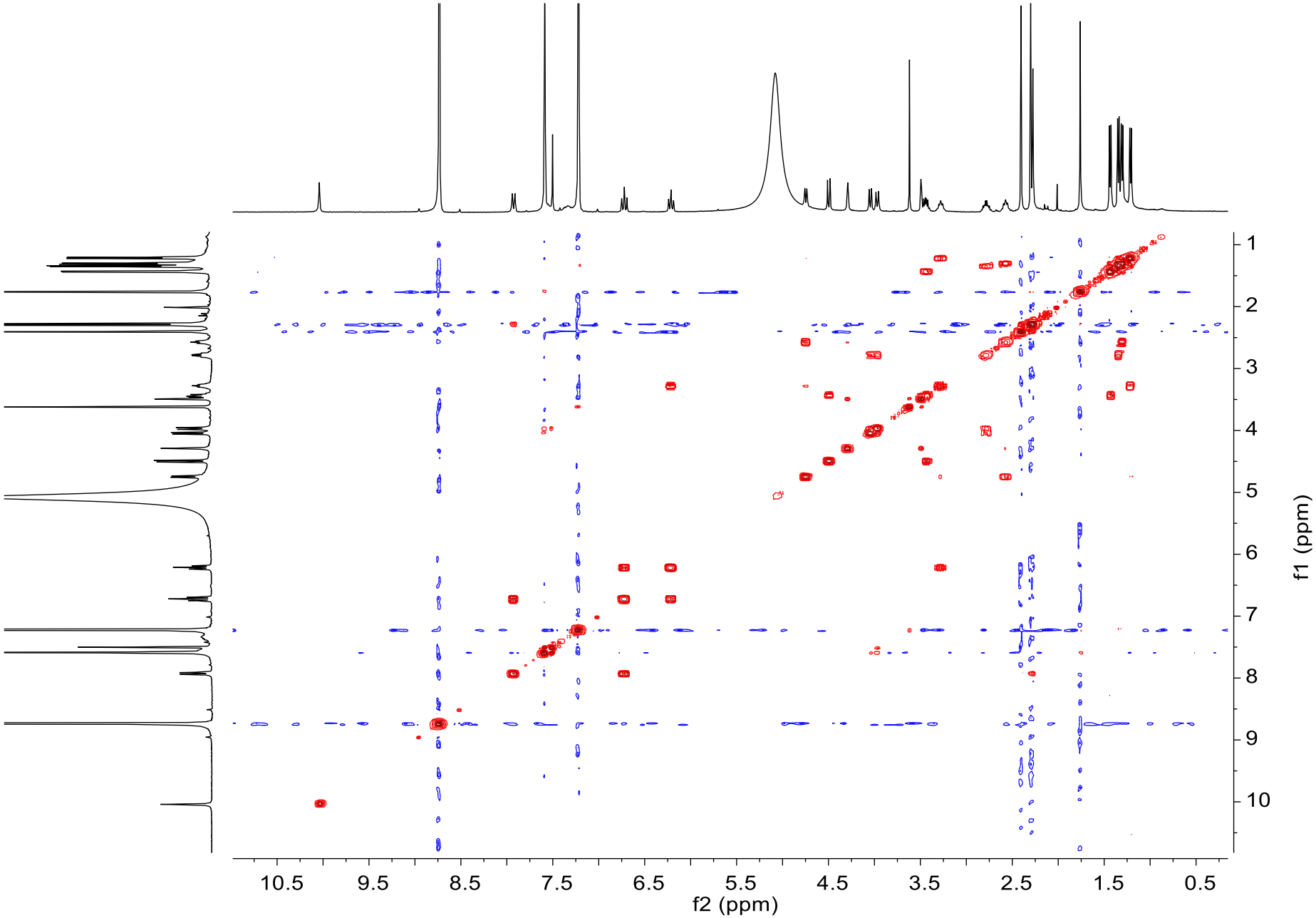


Figure S4. HSQC (400 MHz, pyridine-d_5) spectrum for compound 1

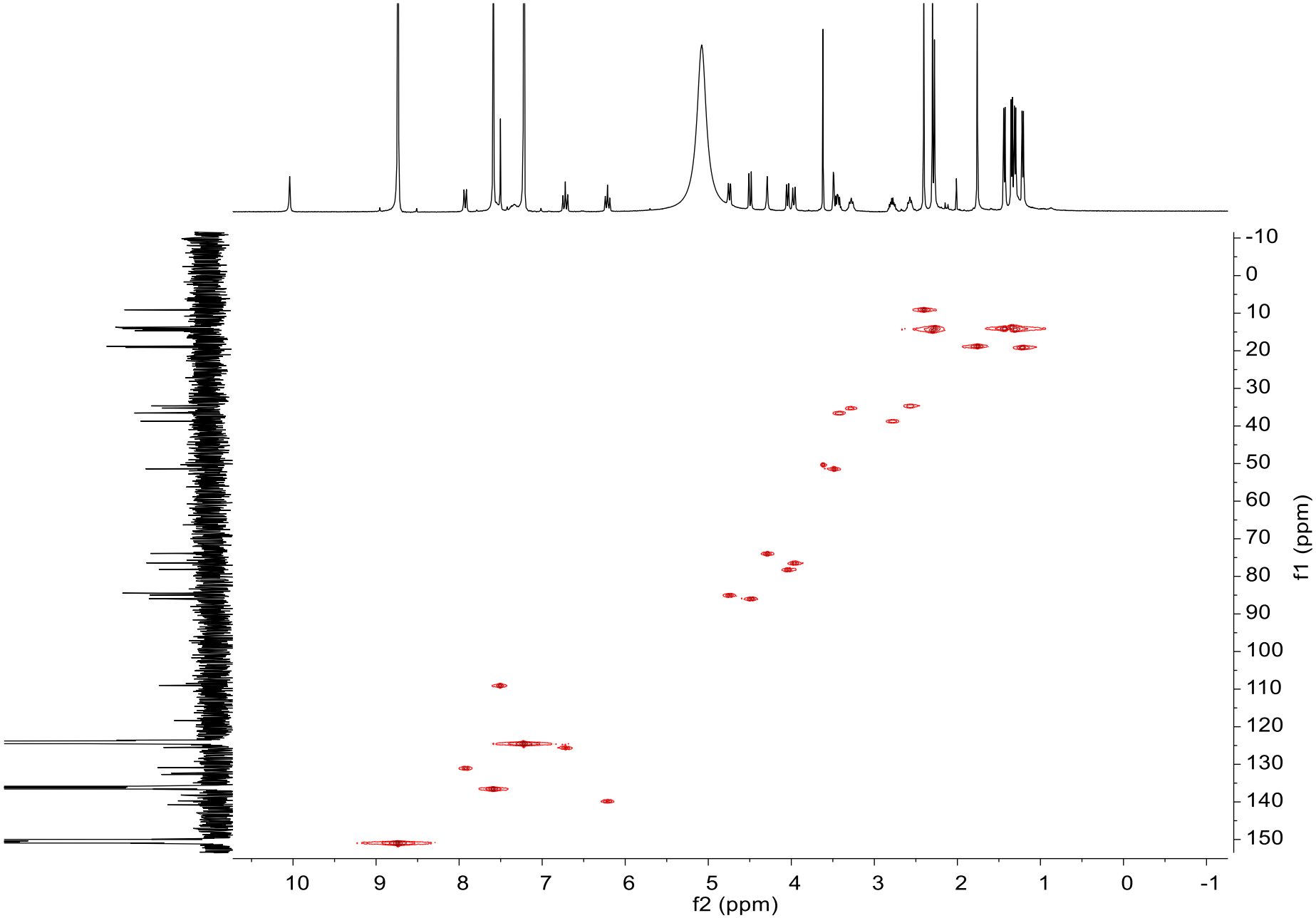


Figure S5. HMBC (400 MHz, pyridine-d_5) spectrum for compound 1

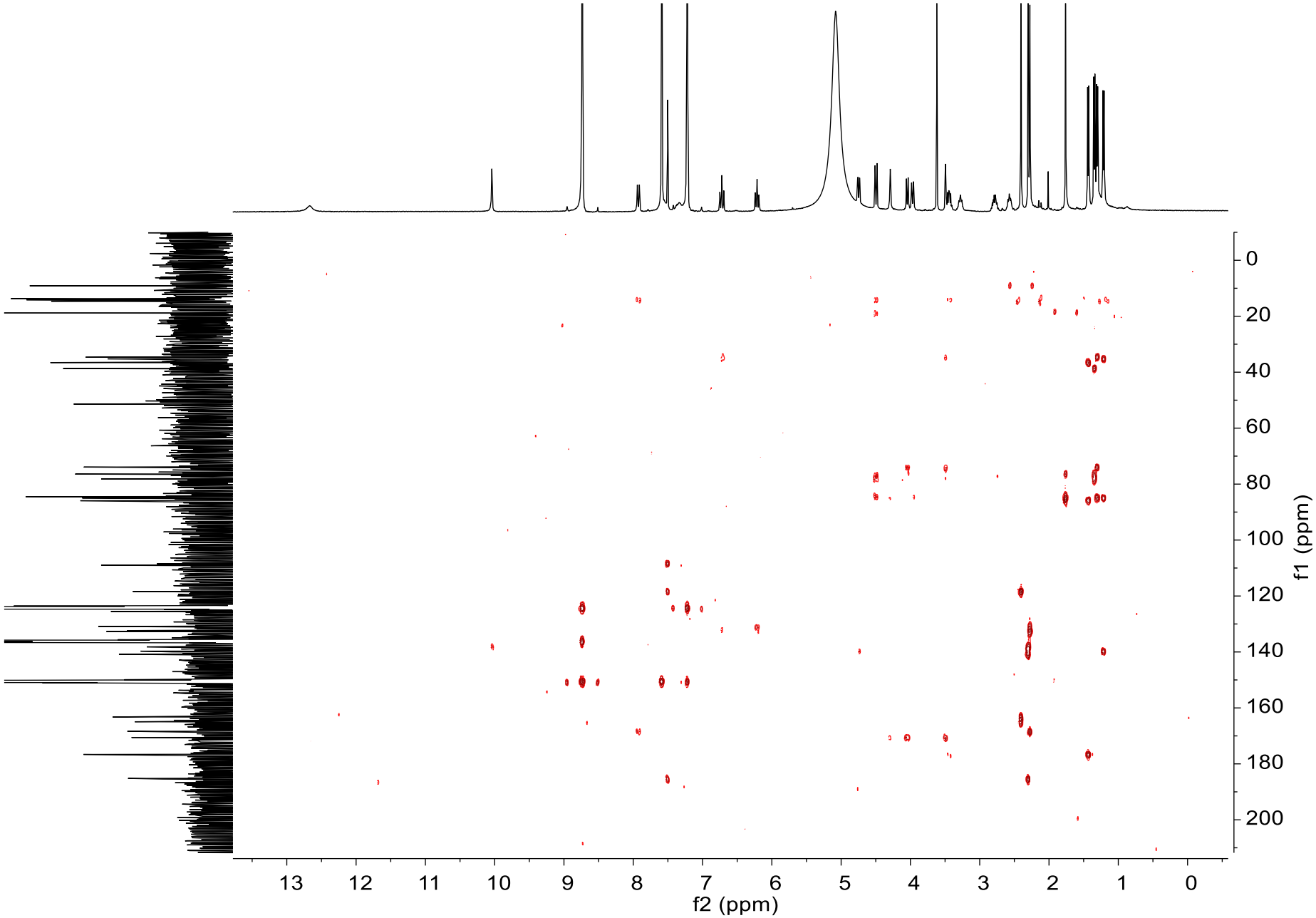


Figure S6. ROESY (400 MHz, pyridine-d_5) spectrum for compound 1

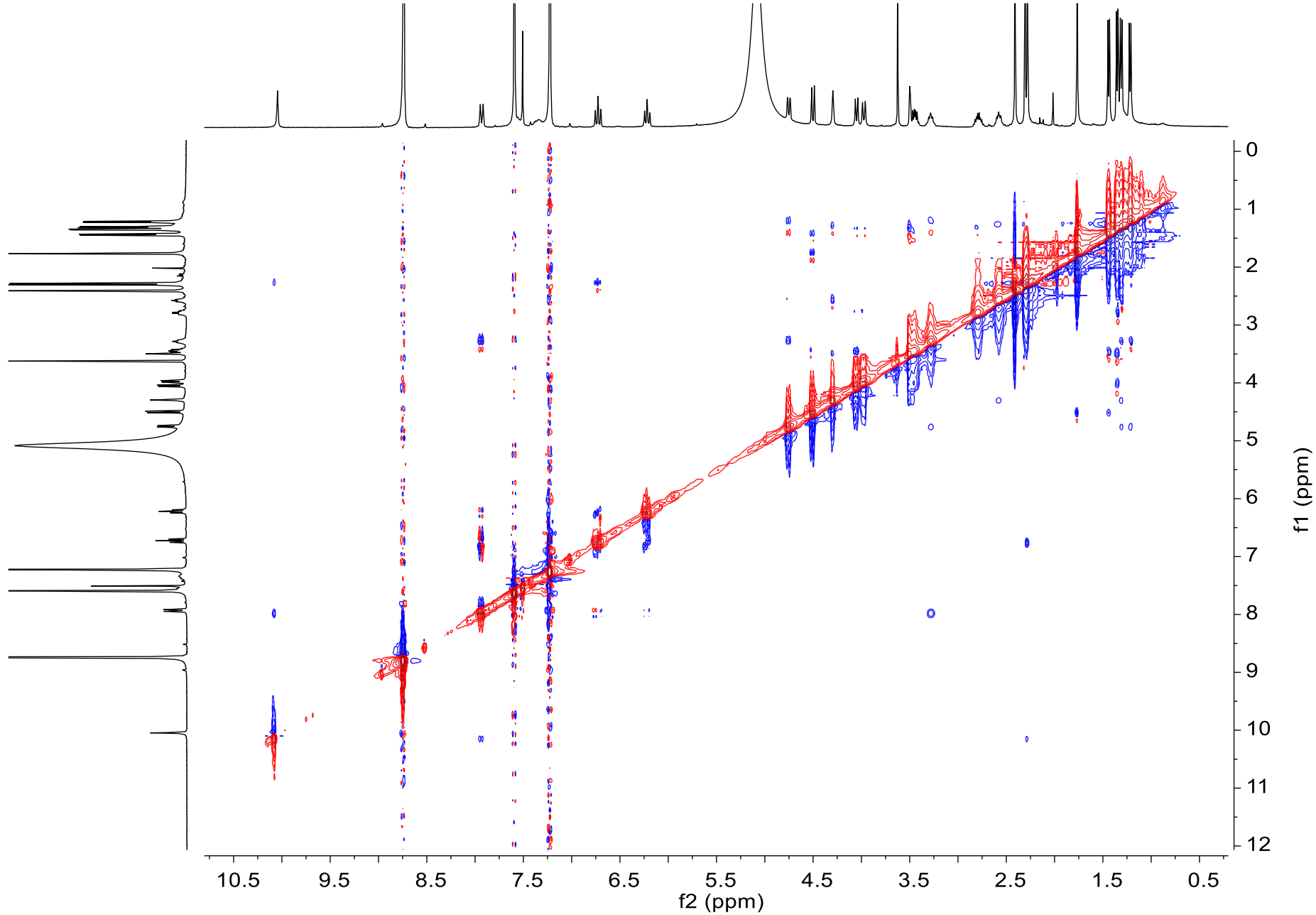


Figure S7. ^{1}H NMR (600 MHz, pyridine-d_5) spectrum for compound 2

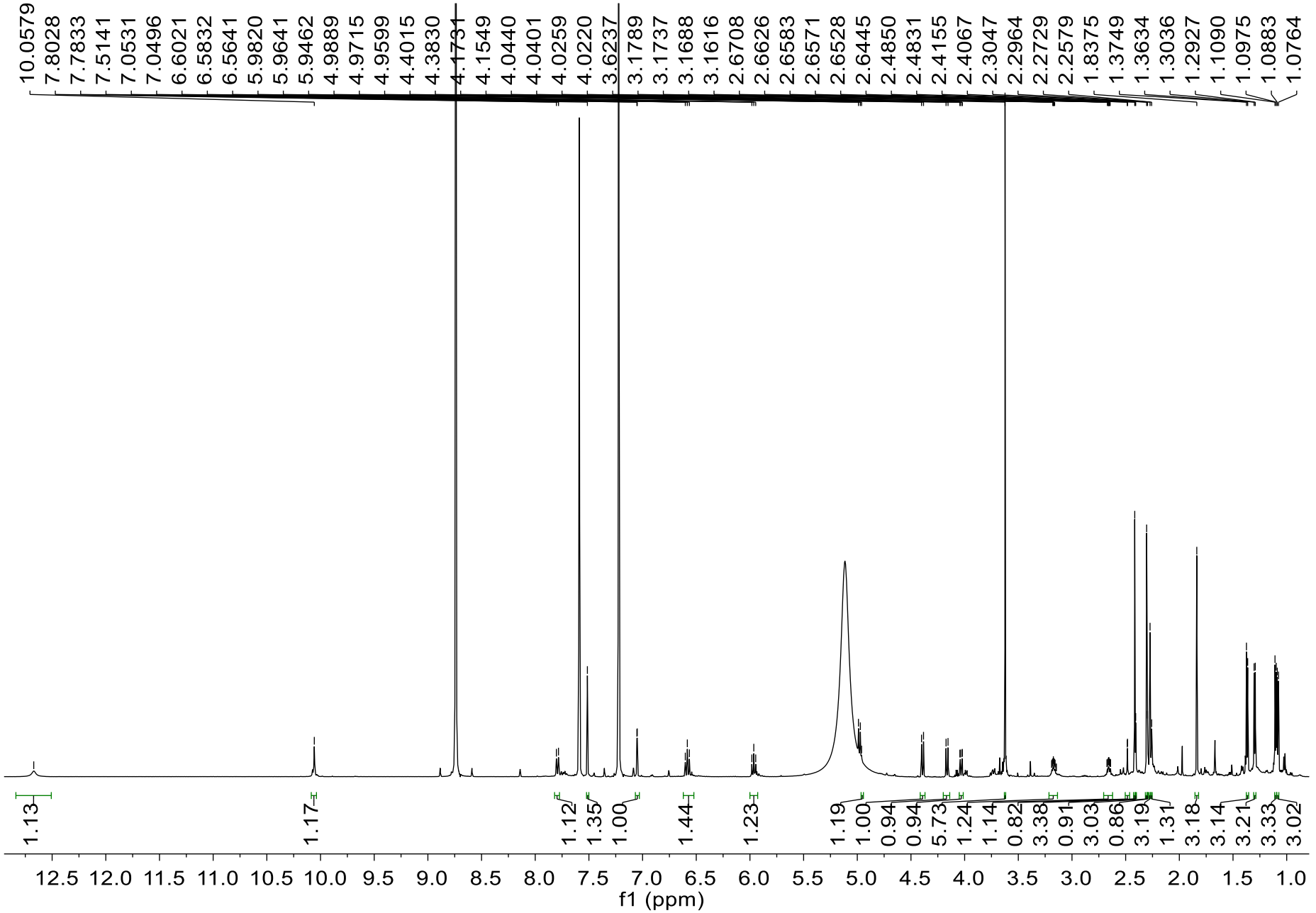


Figure S8. ^{13}C NMR (151 MHz, pyridine-d_5) spectrum for compound 2

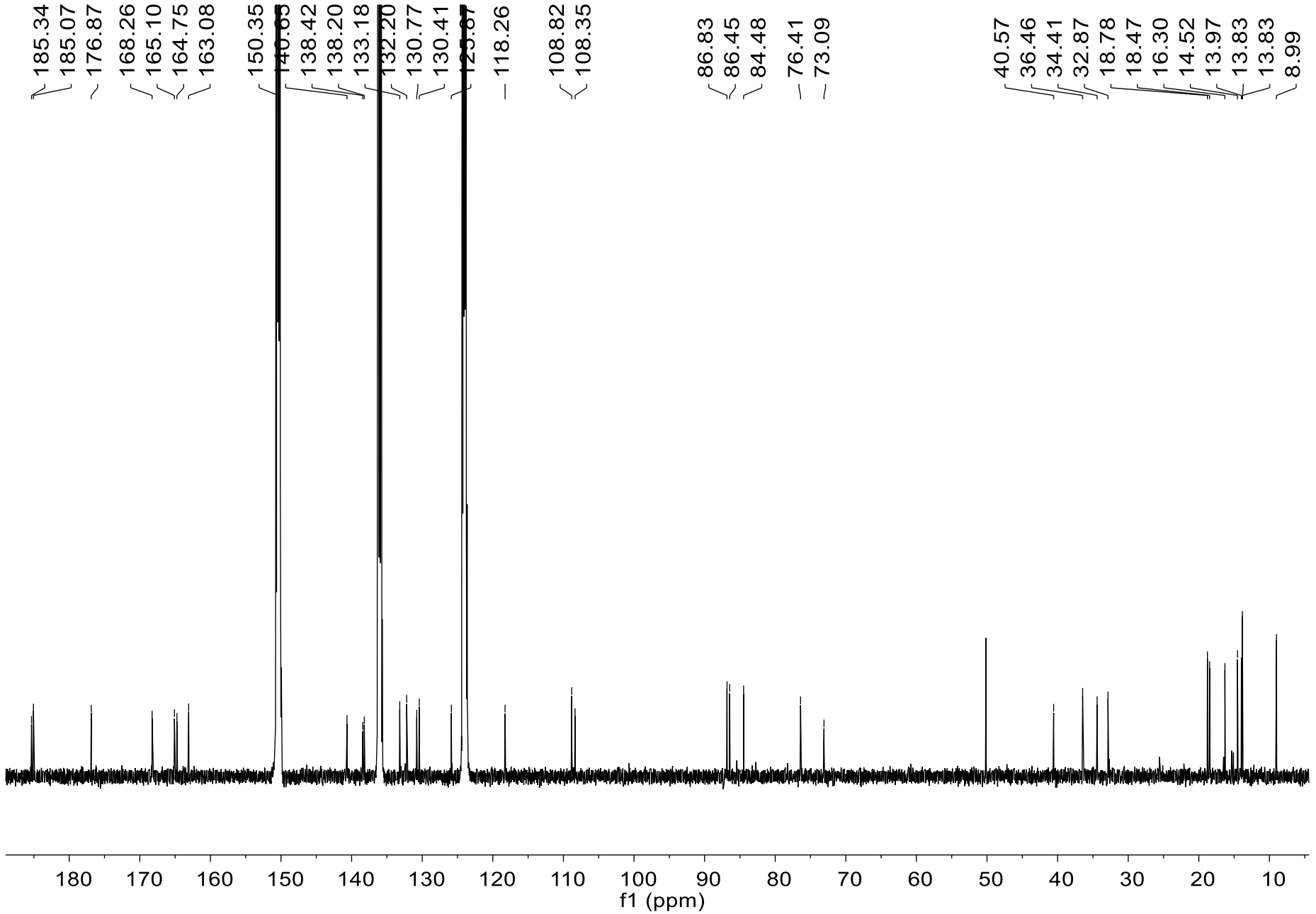


Figure S9. ^{1}H-^{1}H COSY (600 MHz, pyridine-d_5) spectrum for compound 2

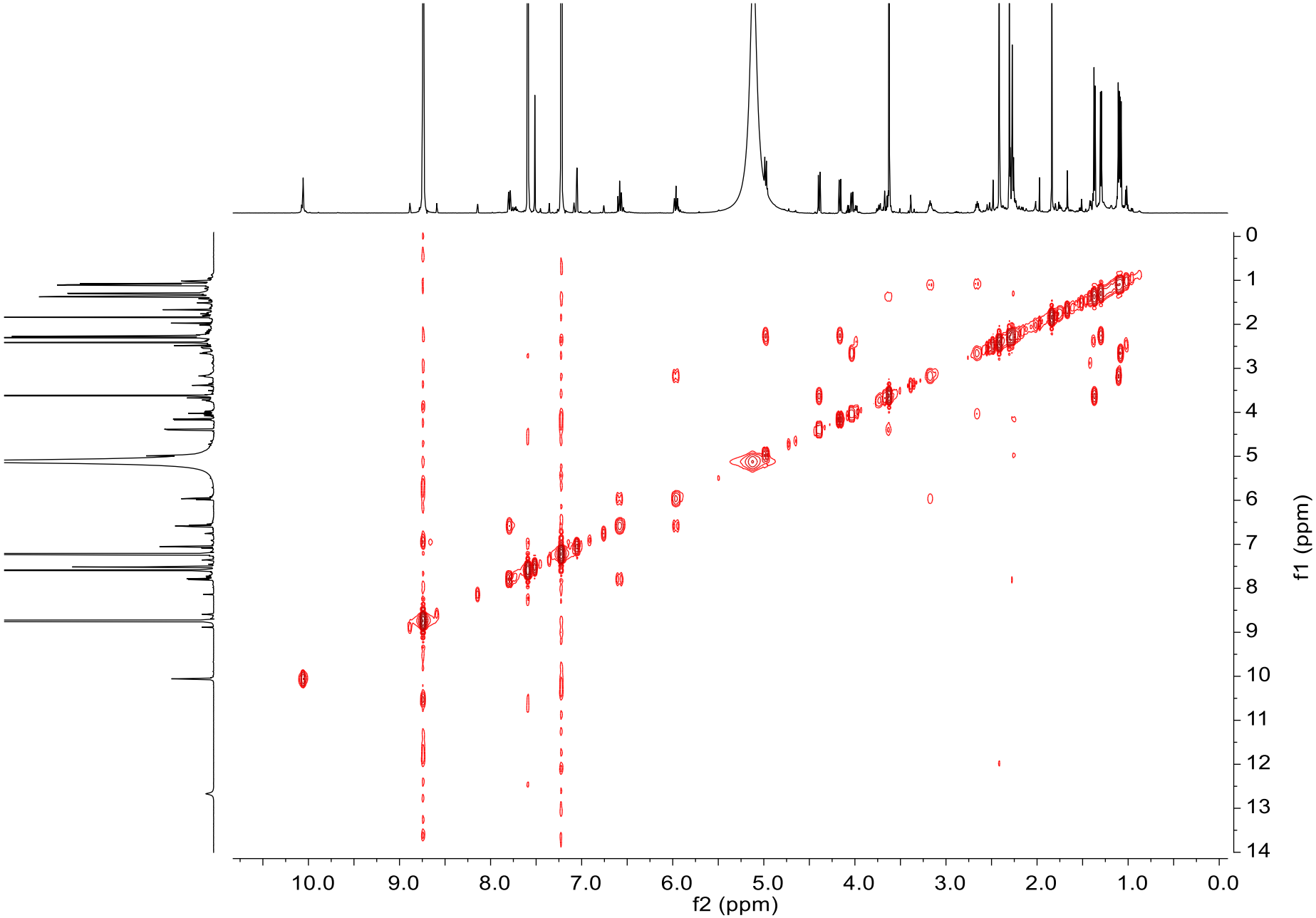


Figure S10. HSQC (600 MHz, pyridine-d_5) spectrum for compound 2

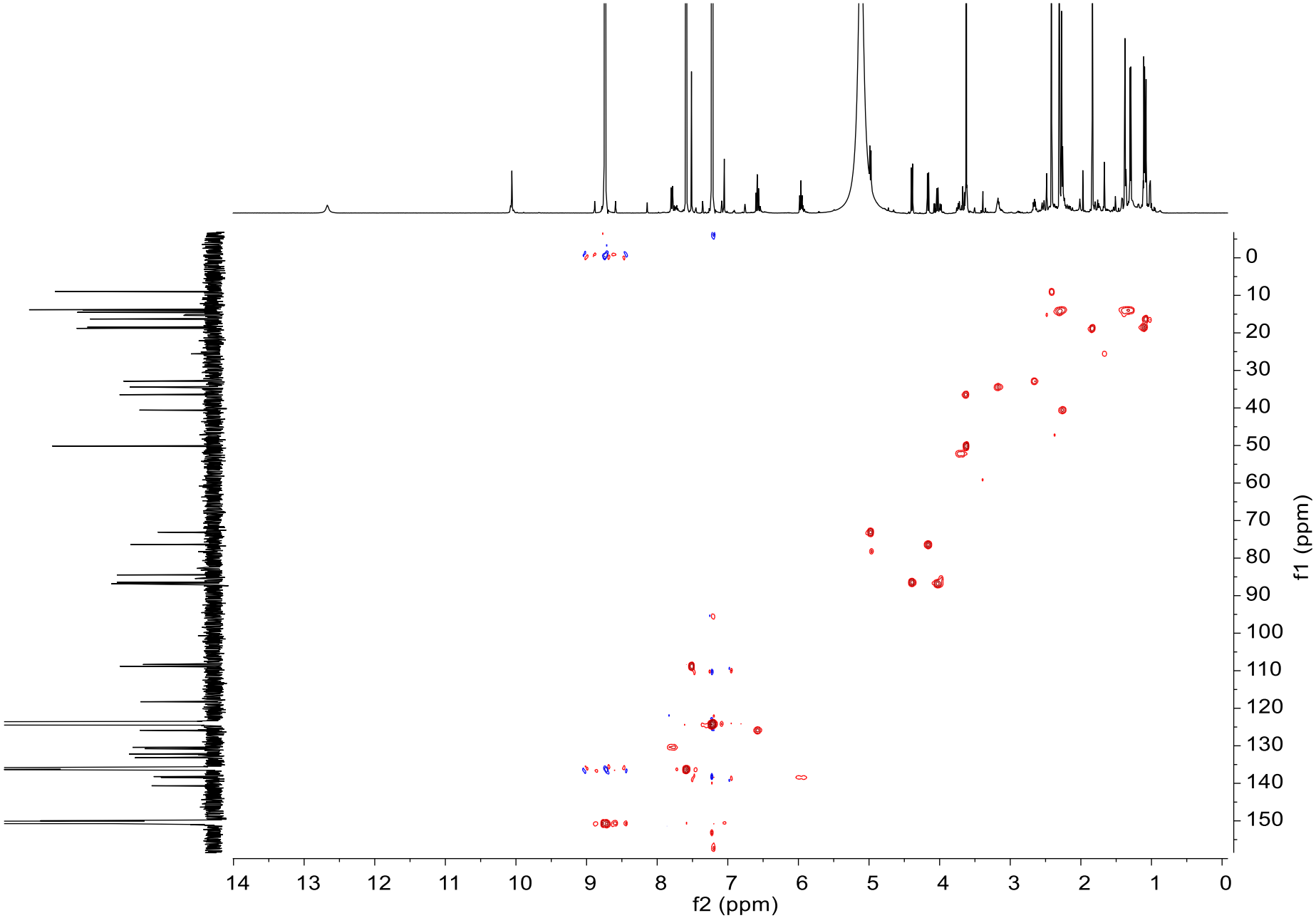


Figure S11. HMBC (600 MHz, pyridine-d_5) spectrum for compound 2

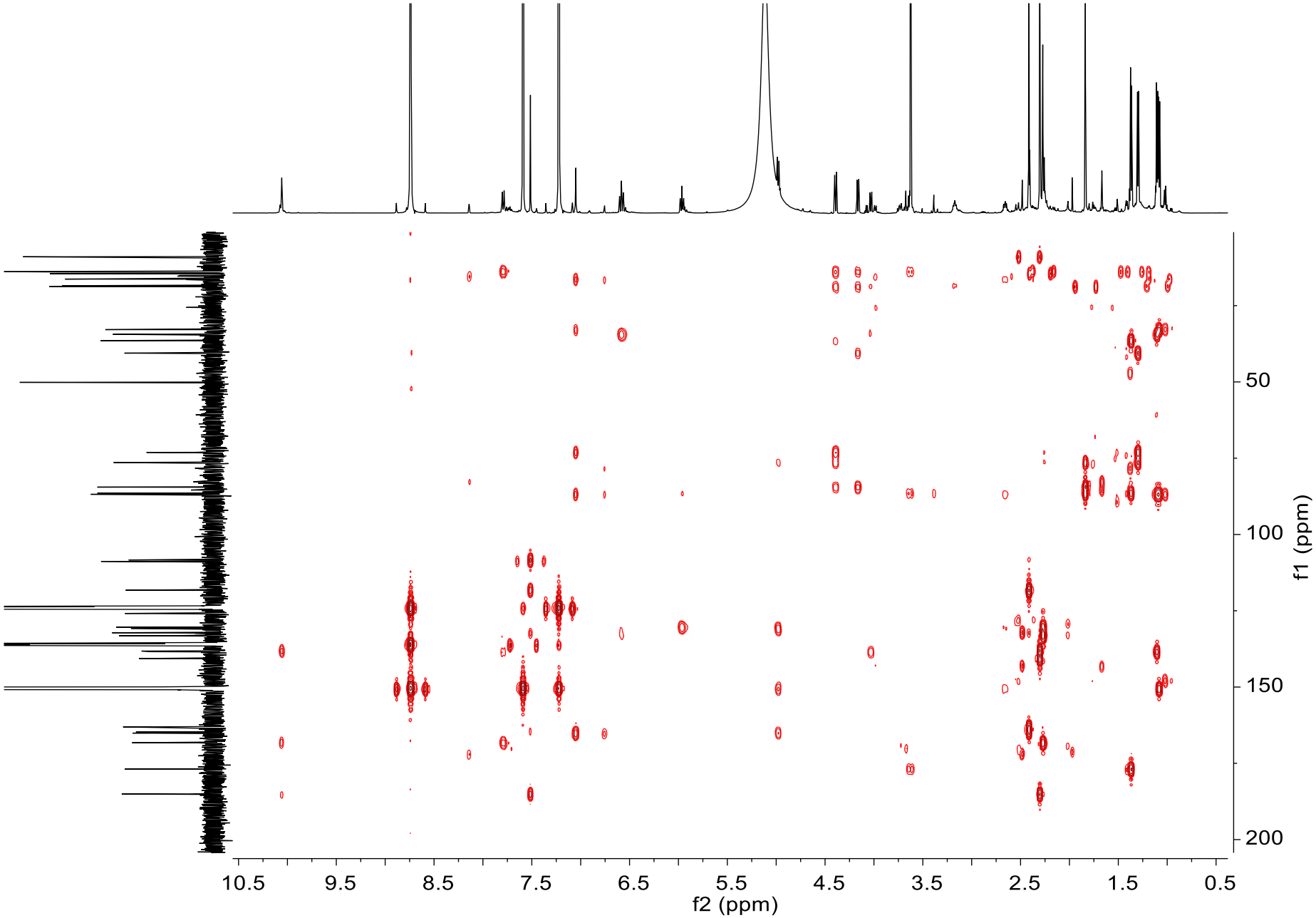


Figure S12. ROESY (600 MHz, pyridine-d_5) spectrum for compound 2

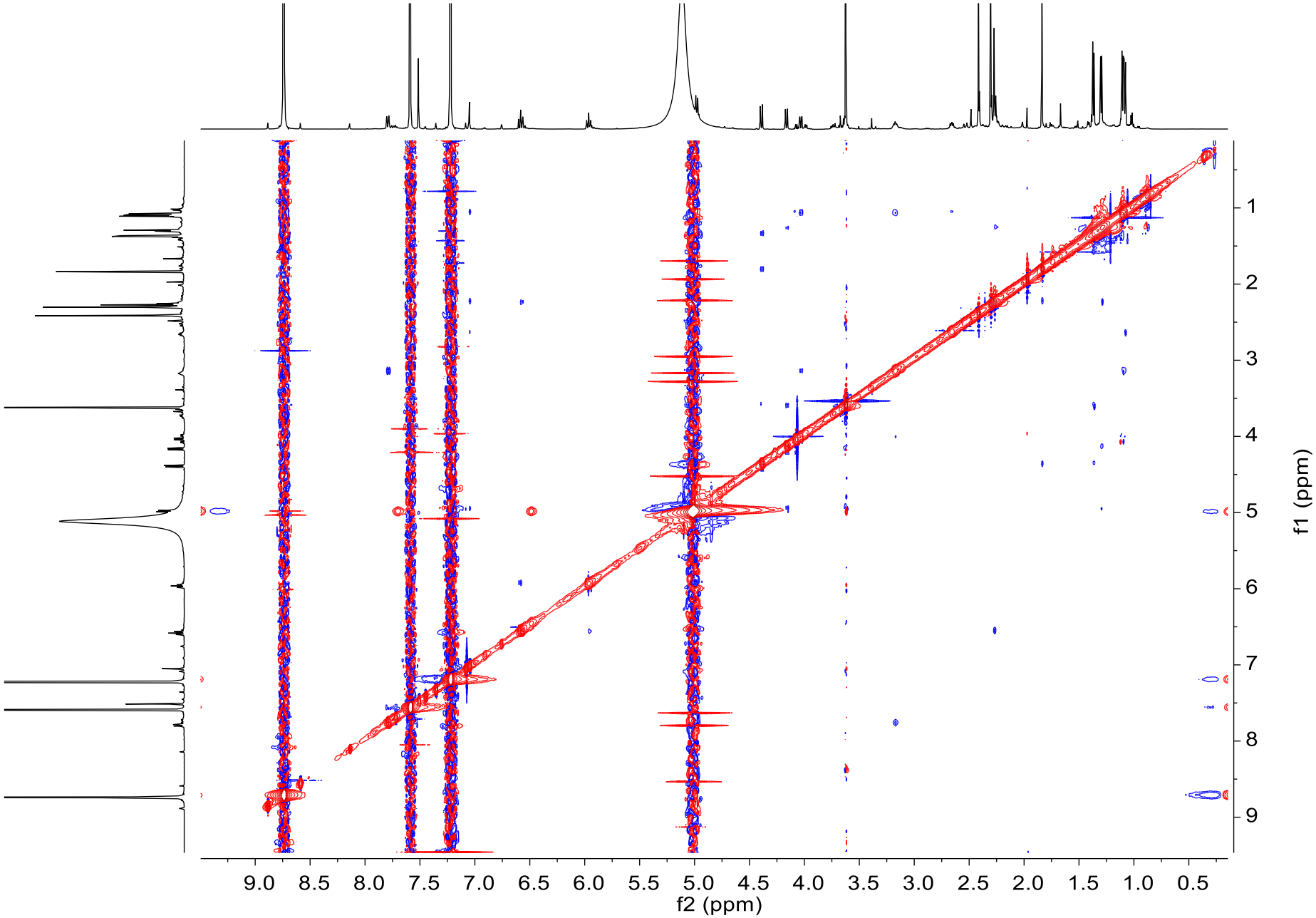


Figure S13. ^{1}H NMR (400 MHz, pyridine-d_5) spectrum for compound 3

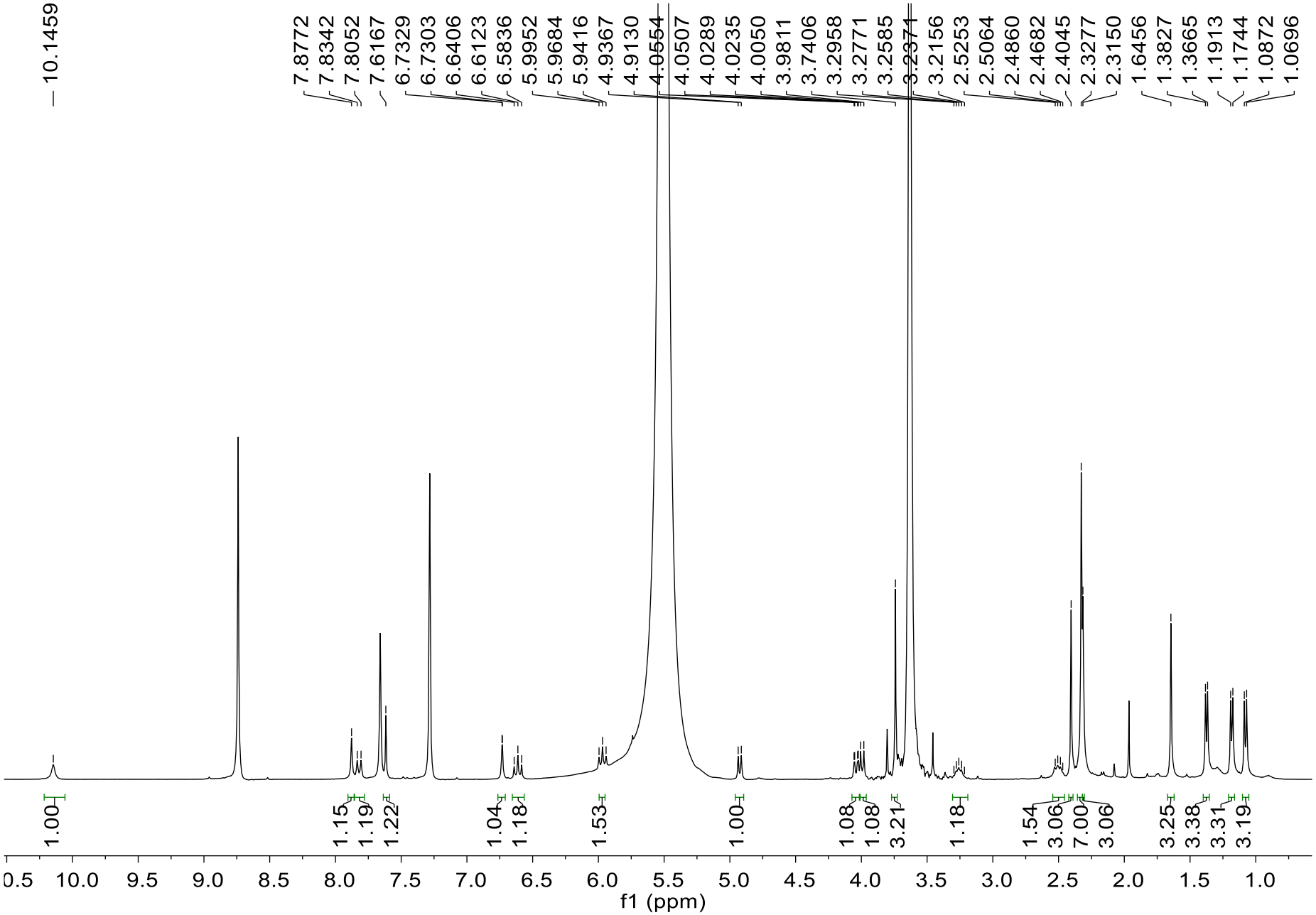


Figure S14. ^{13}C NMR (101 MHz, pyridine-d_5) spectrum for compound 3

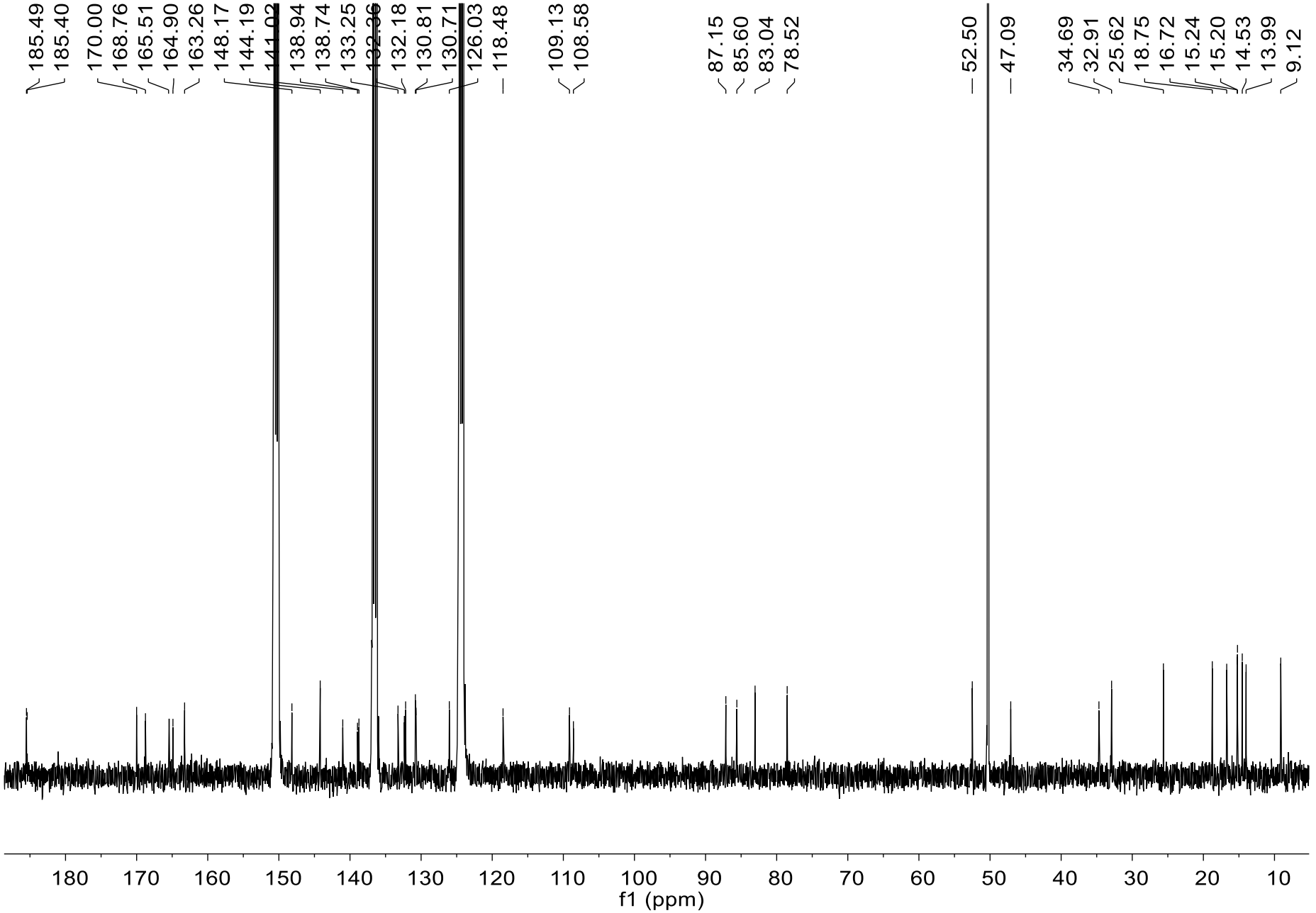


Figure S15. ^{1}H-^{1}H COSY (400 MHz, pyridine-d_5) spectrum for compound 3

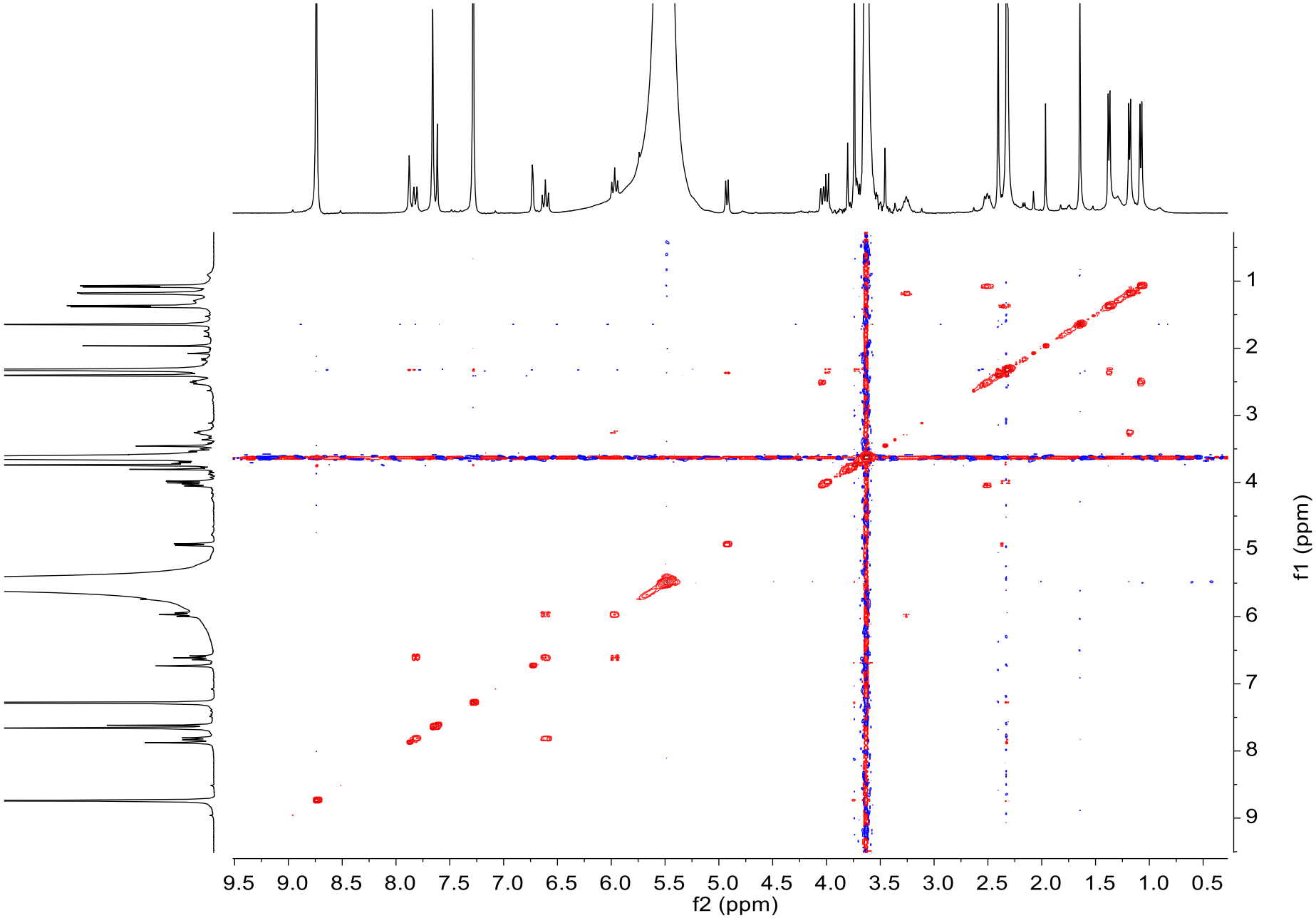


Figure S16. HSQC (400 MHz, pyridine-d_5) spectrum for compound 3

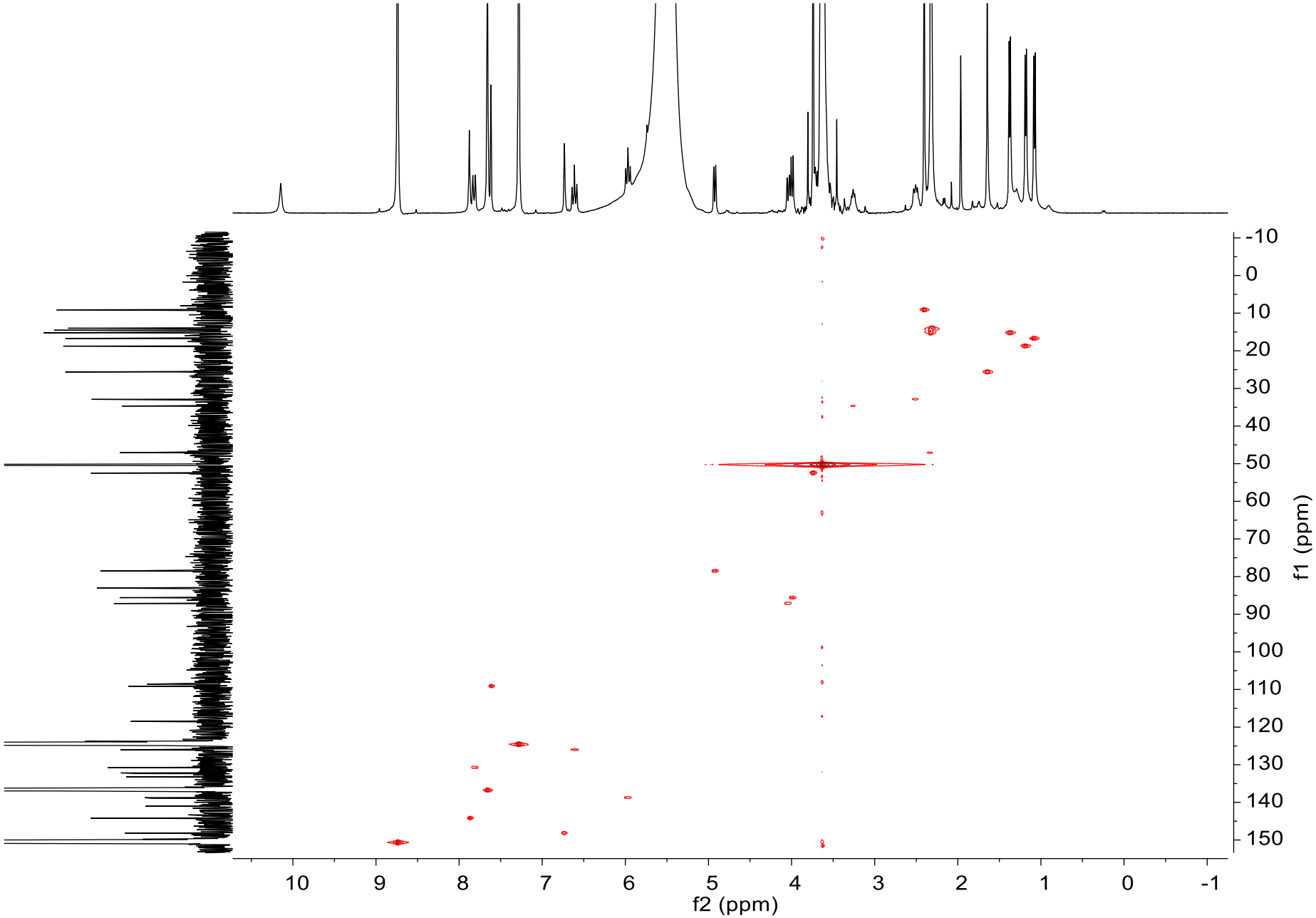


Figure S17. HMBC (400 MHz, pyridine-d_5) spectrum for compound 3

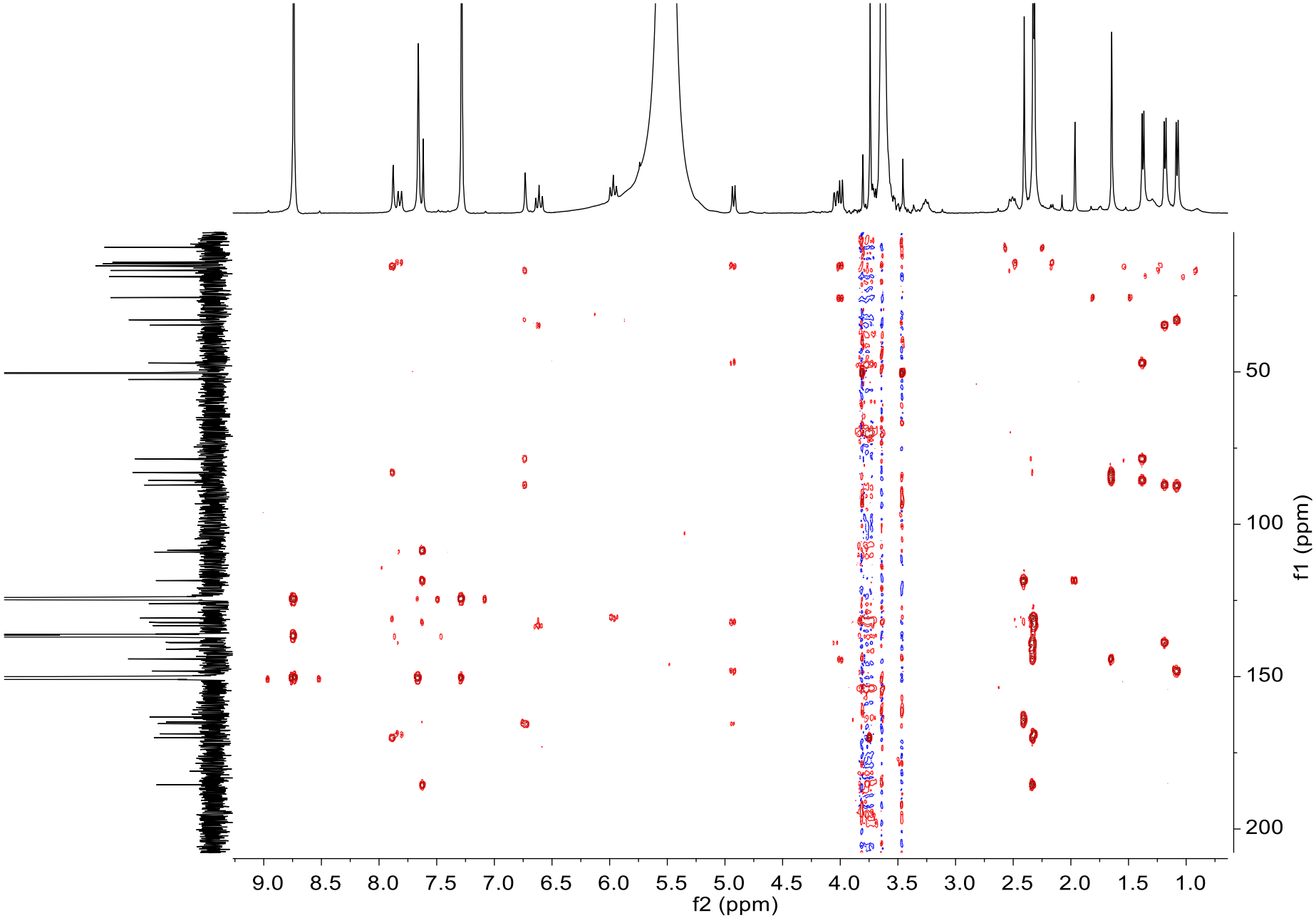


Figure S18. ROESY (400 MHz, pyridine-d_5) spectrum for compound 3

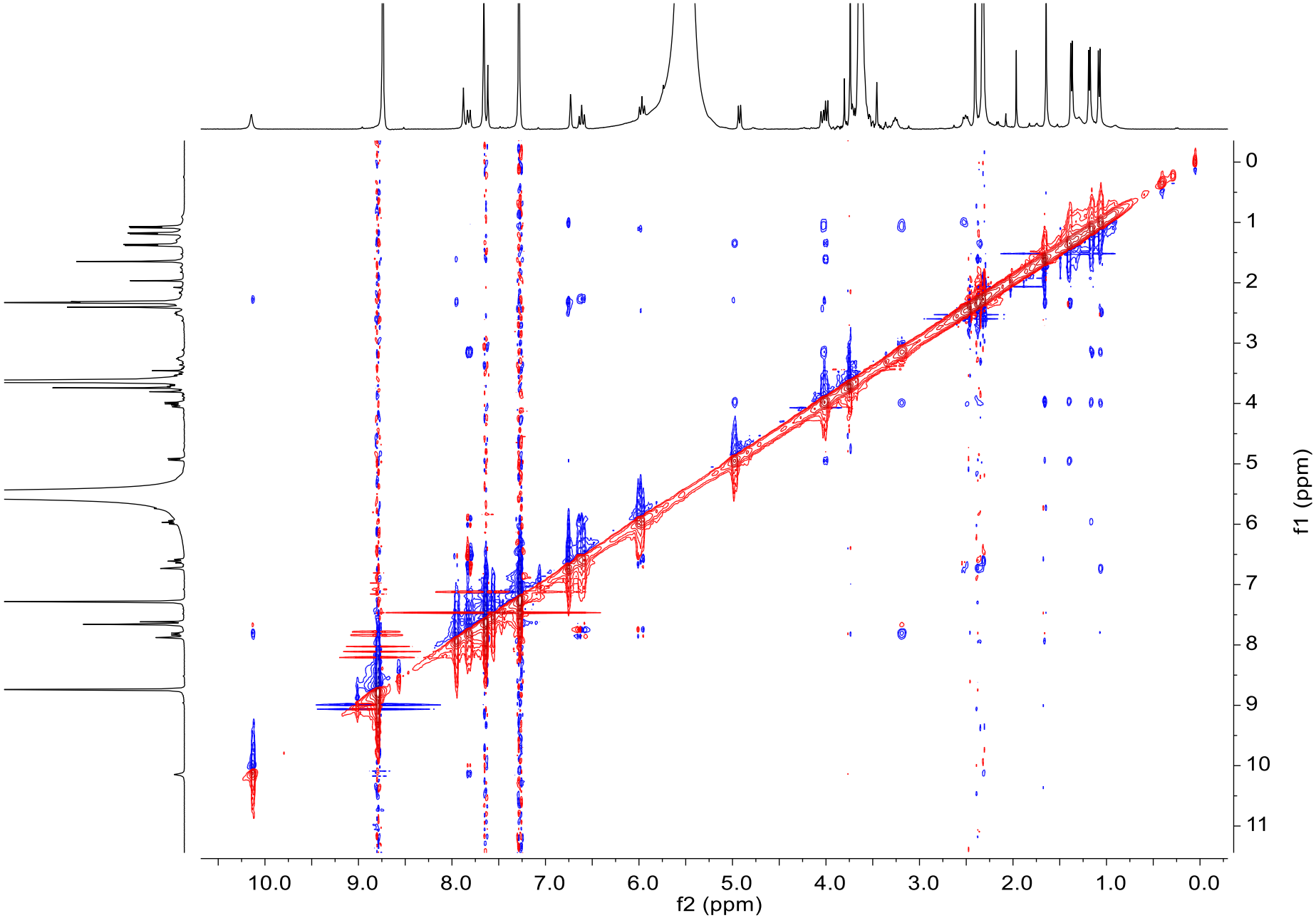


Figure S19. ^{1}H NMR (600 MHz, pyridine-d_5) spectrum for compound 4

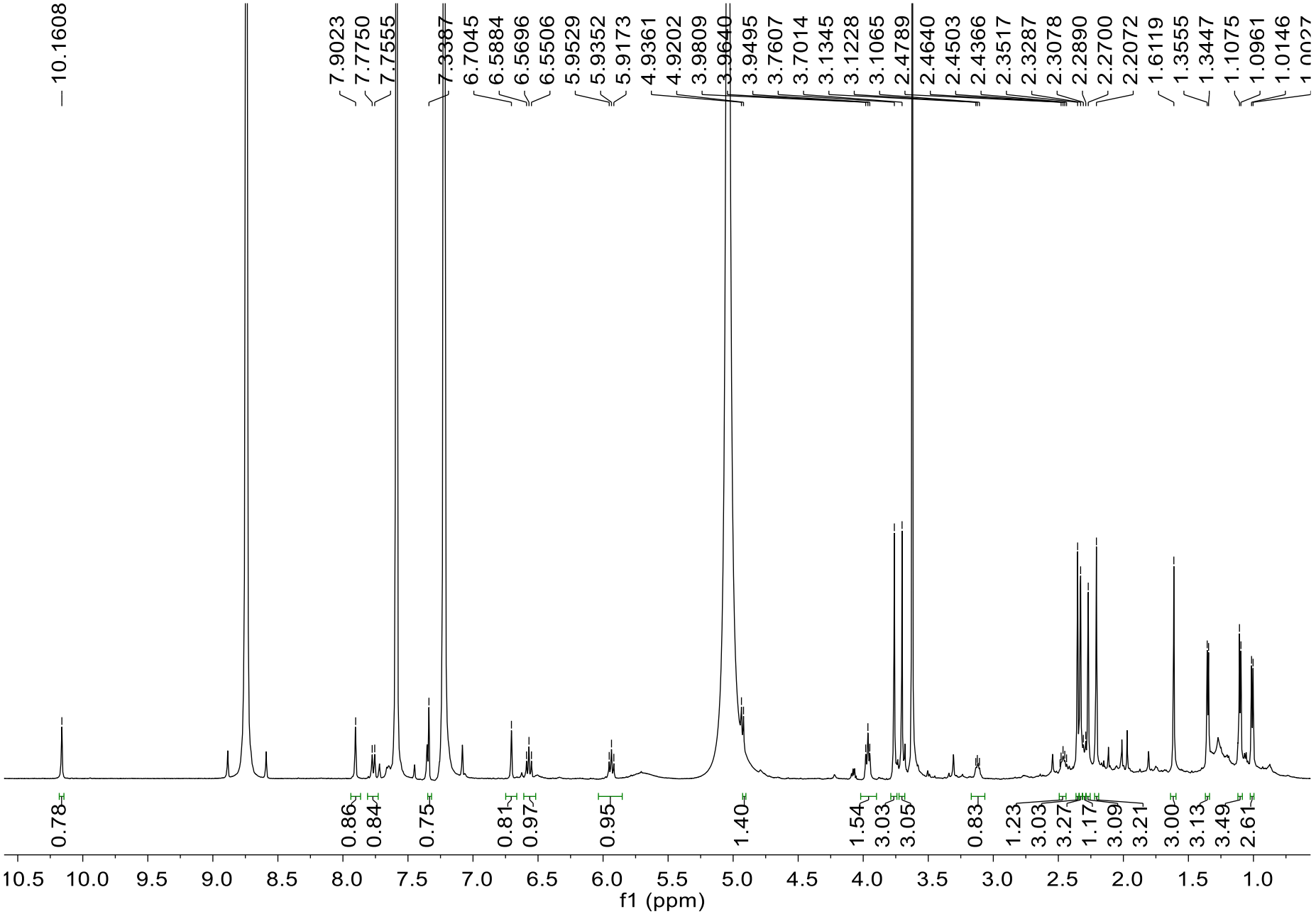


Figure S20. ^{13}C NMR (151 MHz, pyridine-d_5) spectrum for compound 4

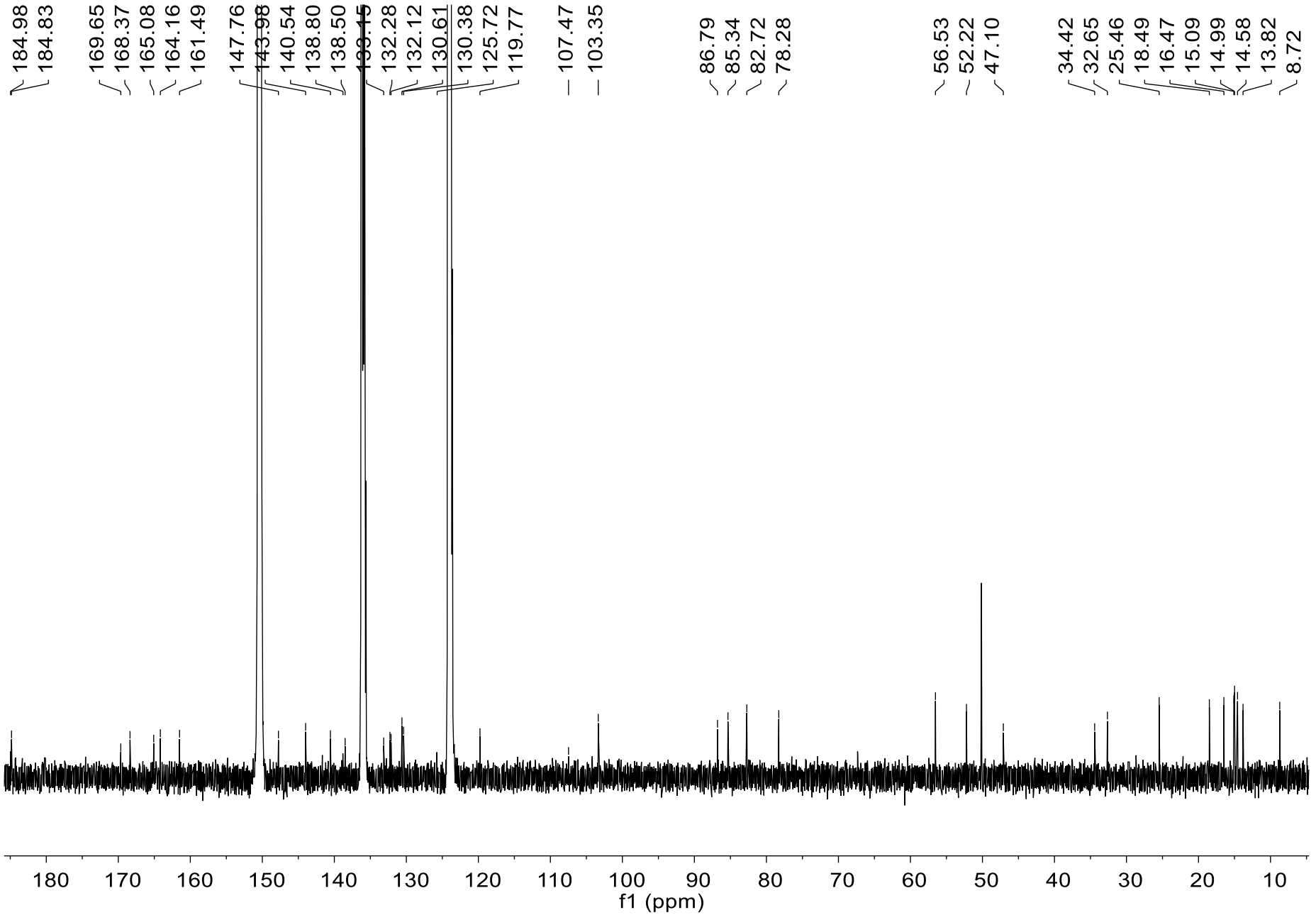


Figure S21. ^{1}H-^{1}H COSY (600 MHz, pyridine-d_5) spectrum for compound 4

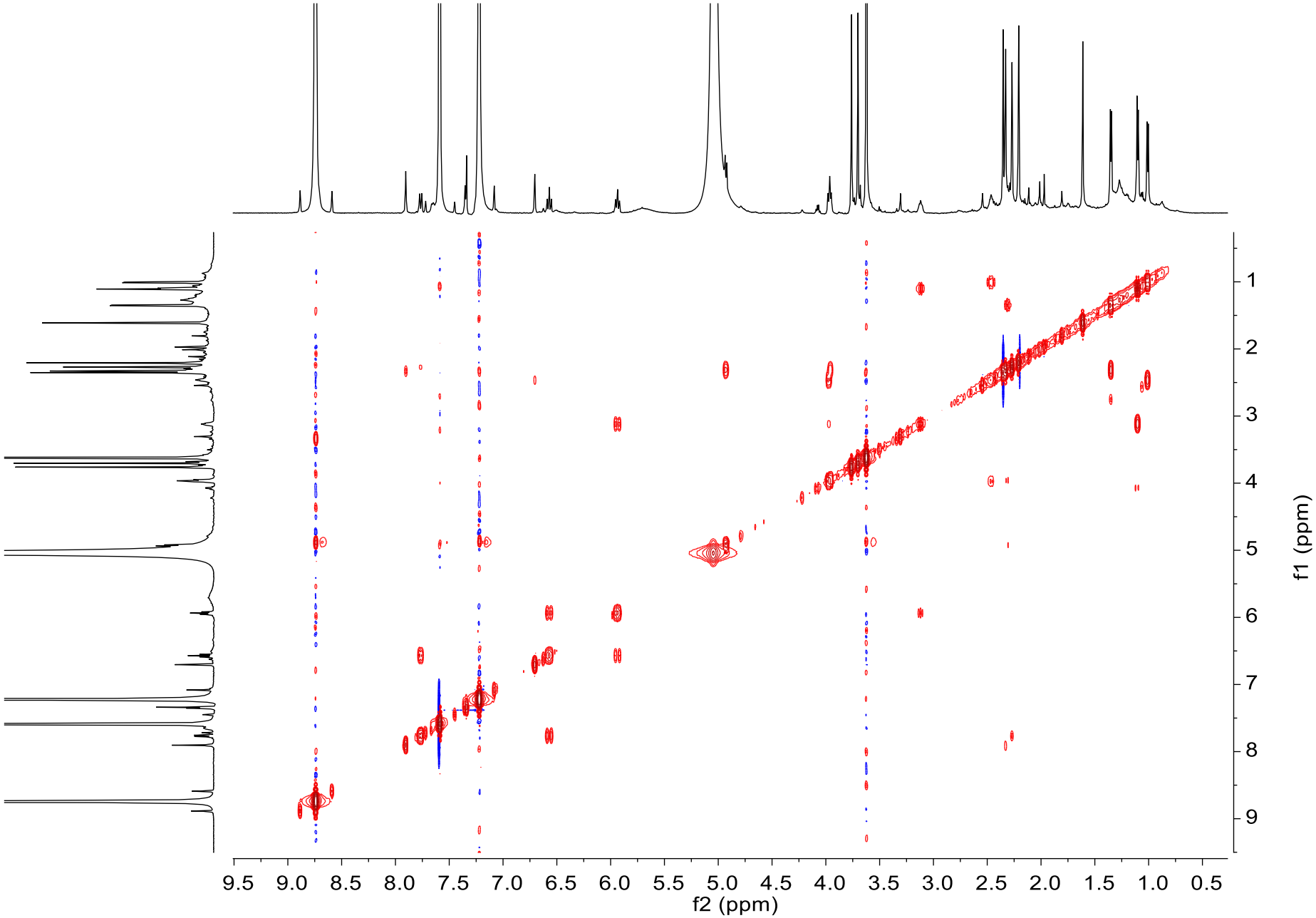


Figure S22. HSQC (600 MHz, pyridine-d_5) spectrum for compound 4

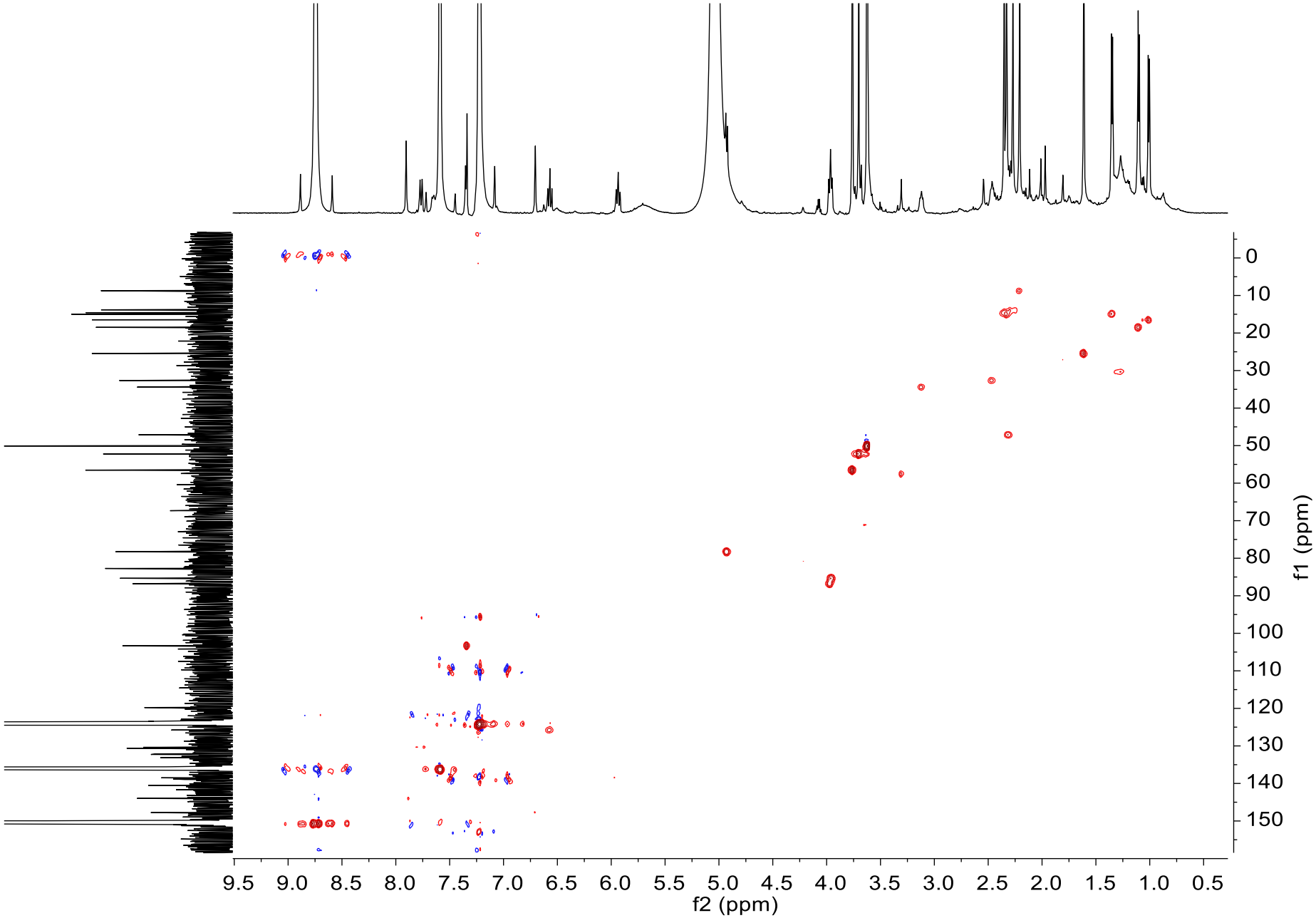


Figure S23. HMBC (600 MHz, pyridine-d_5) spectrum for compound 4

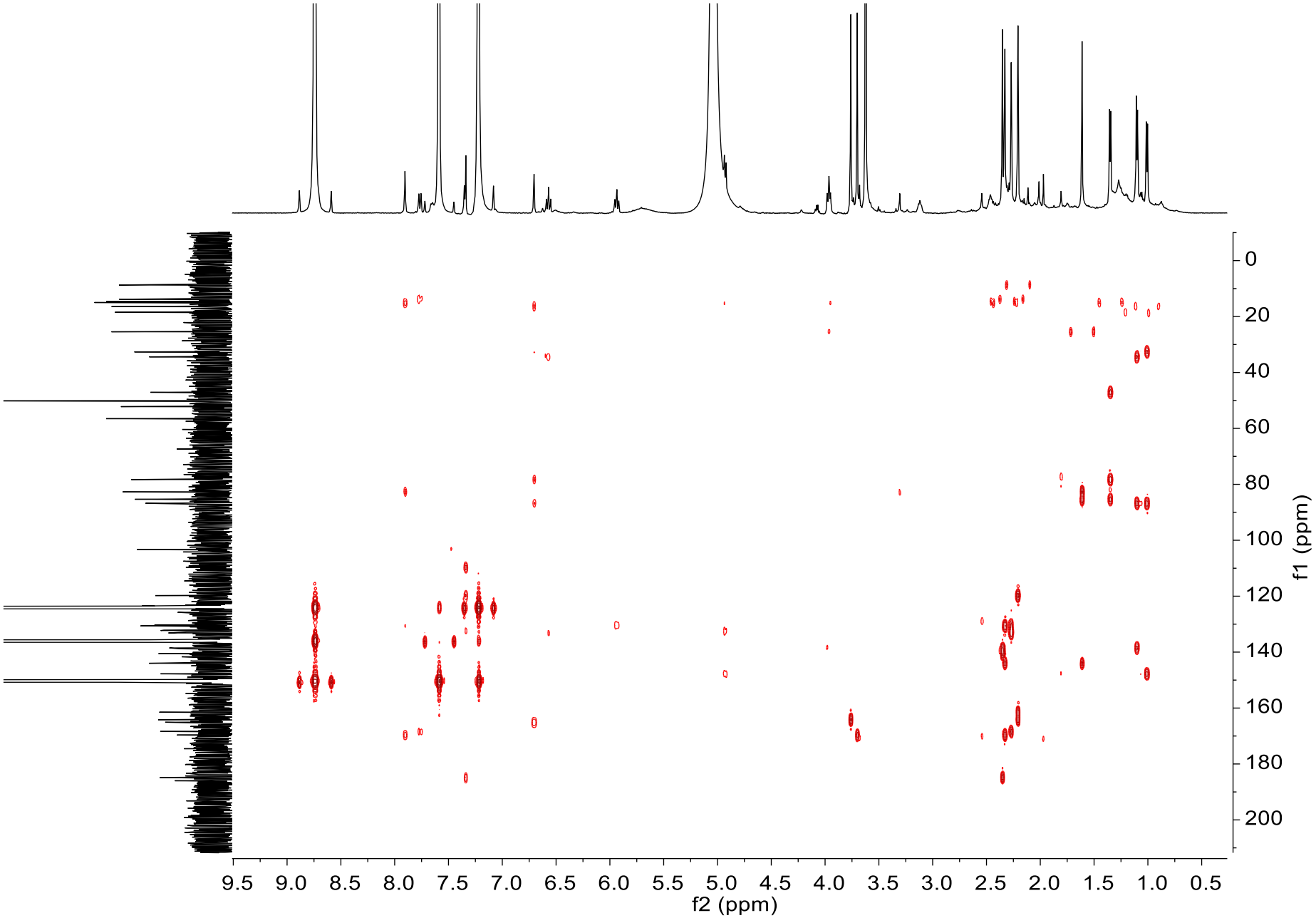


Figure S24. ROESY (600 MHz, pyridine-d_5) spectrum for compound 4

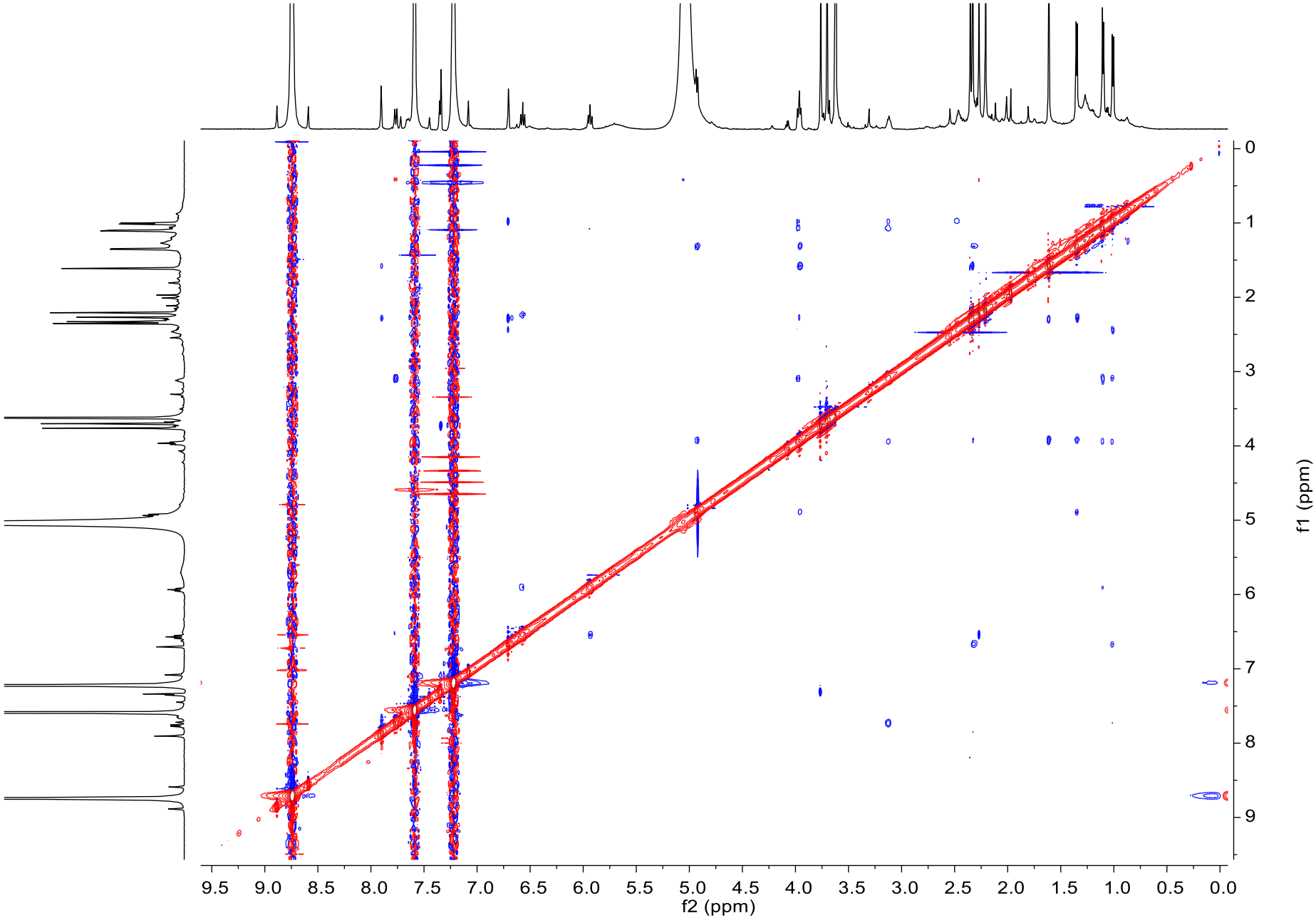


Figure S25. High-resolution ESIMS spectrum for compound 1

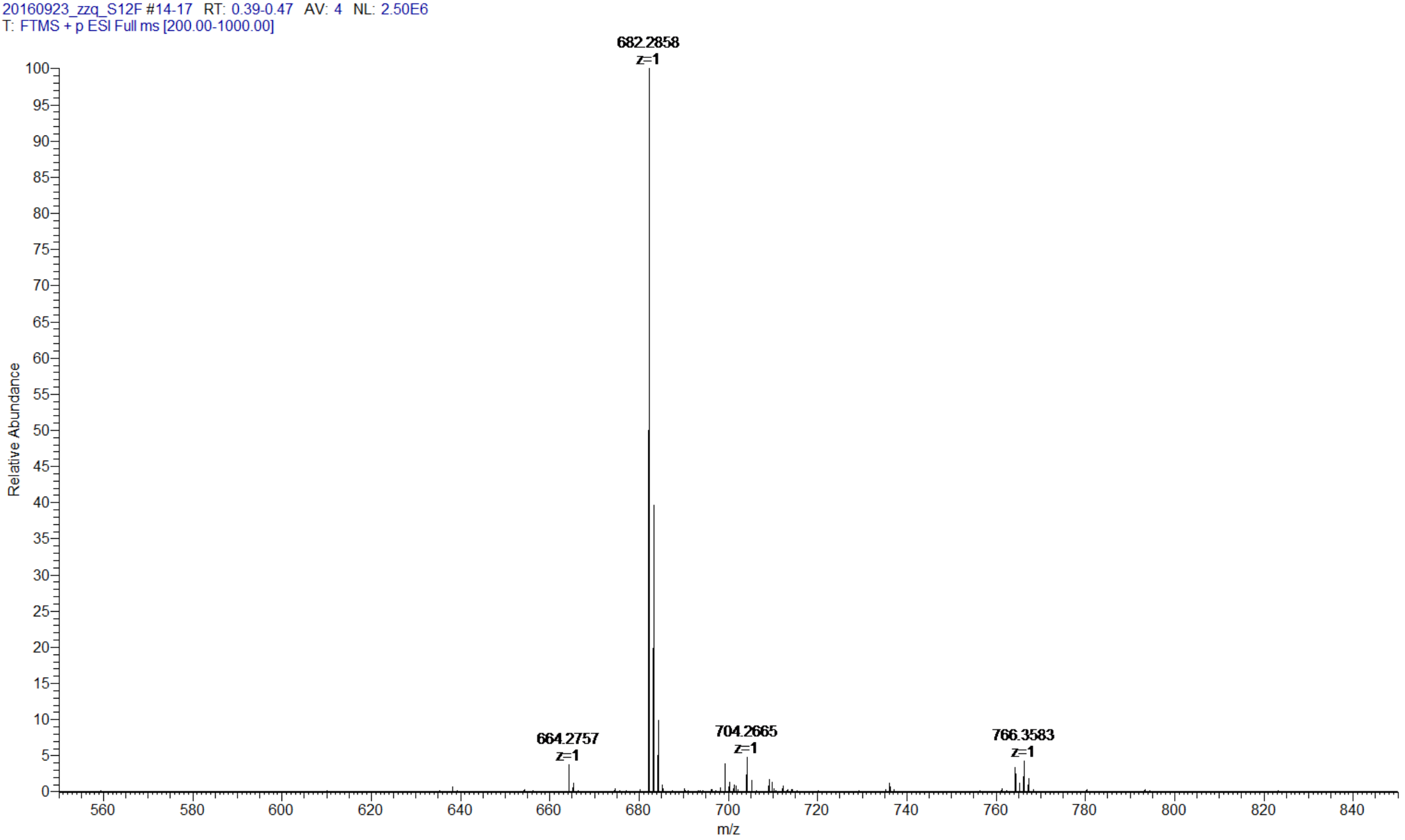


Figure S26. High-resolution ESIMS spectrum for compound 2

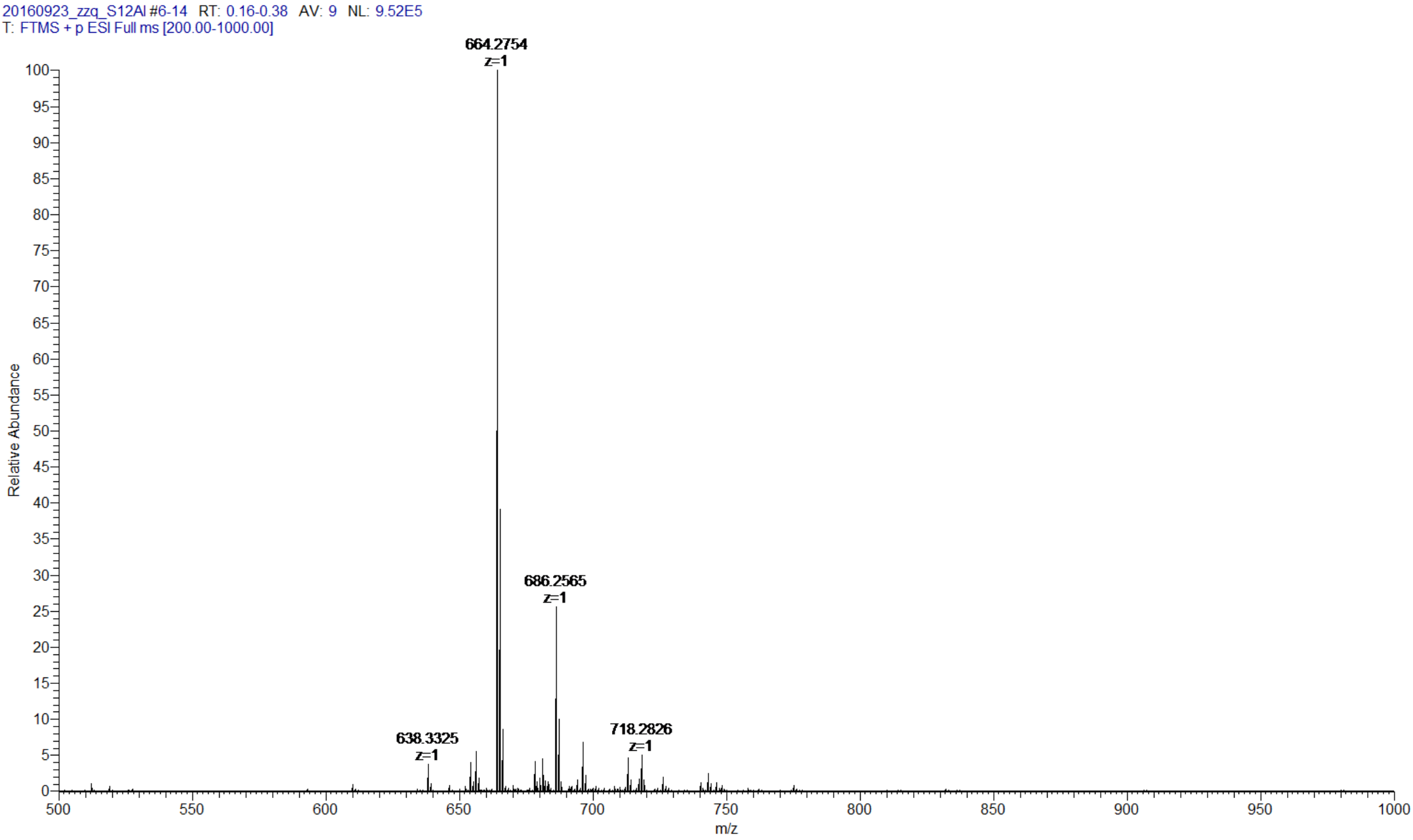


Figure S27. High-resolution ESIMS spectrum for compound 3

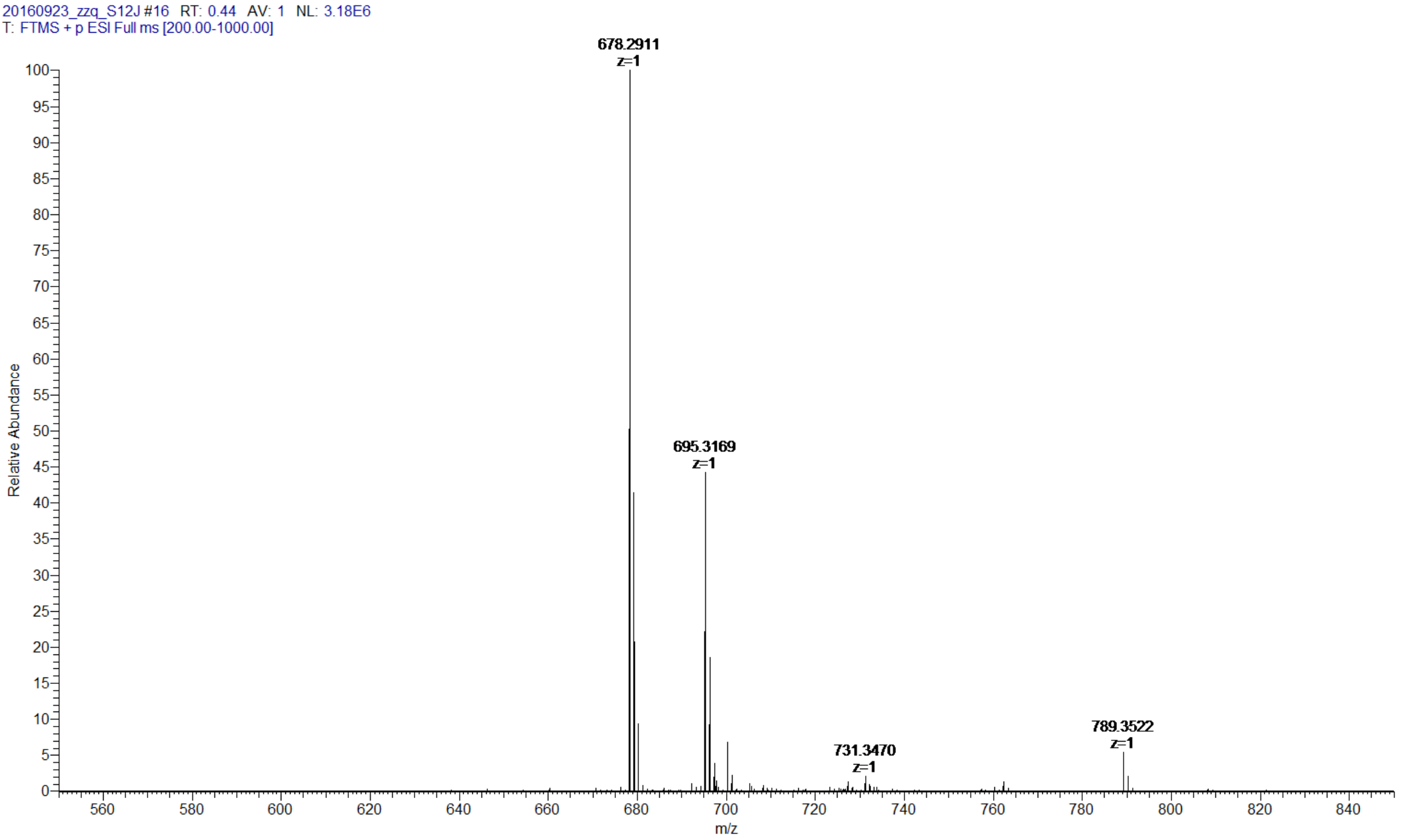


Figure S28. High-resolution ESIMS spectrum for compound 4

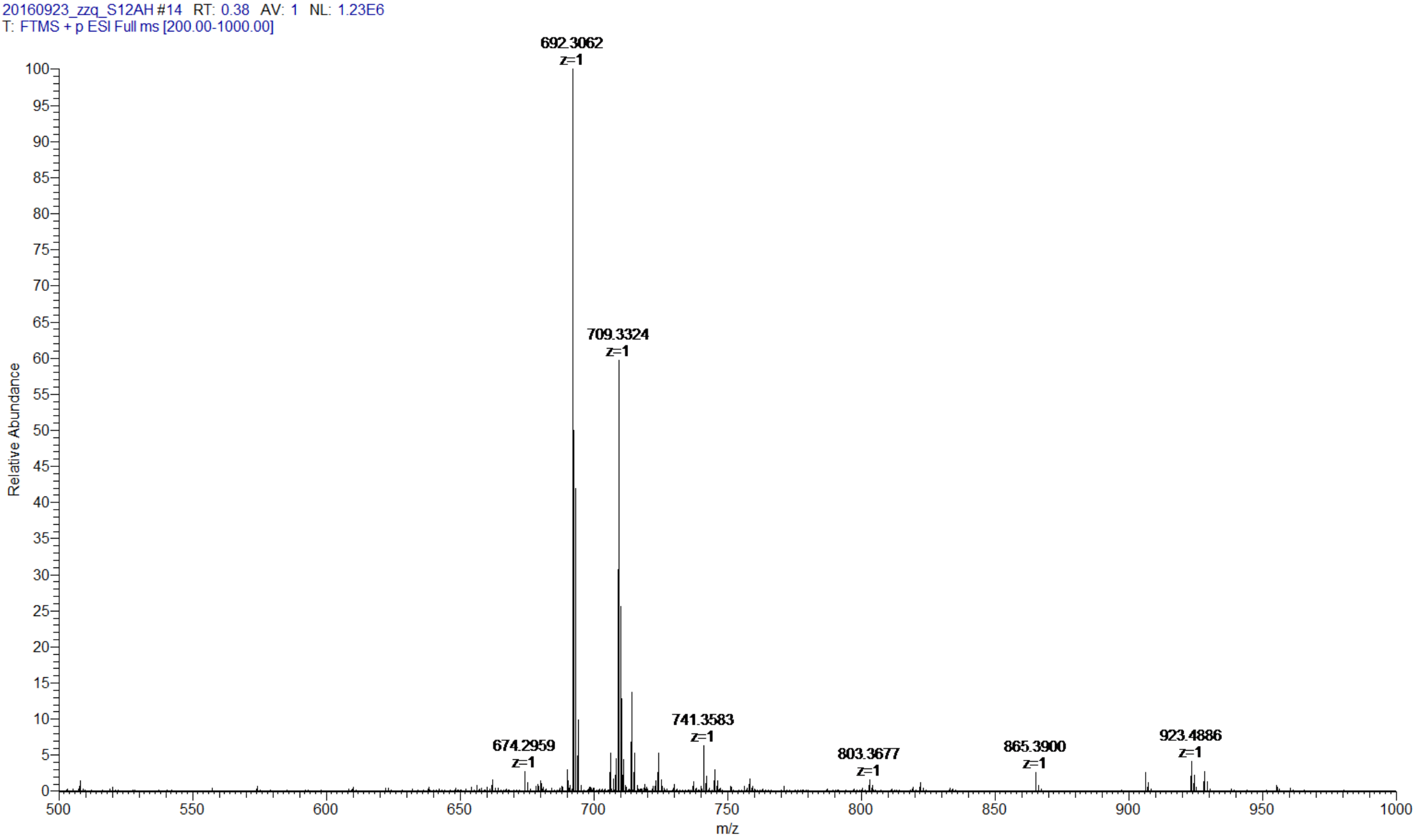


Figure S29. The proposed streptovaricin biosynthetic logic

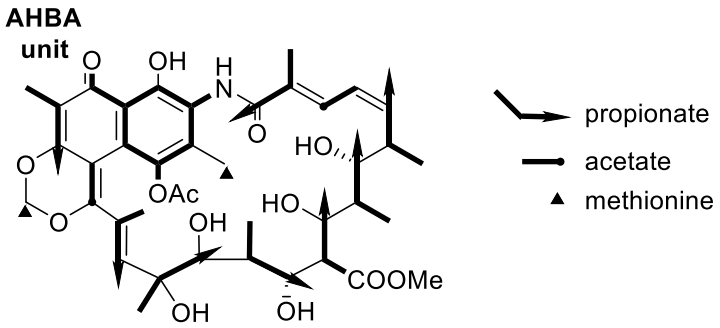


Figure S30. Streak plate of *Streptomyces* sp. S012 culture in ISP3

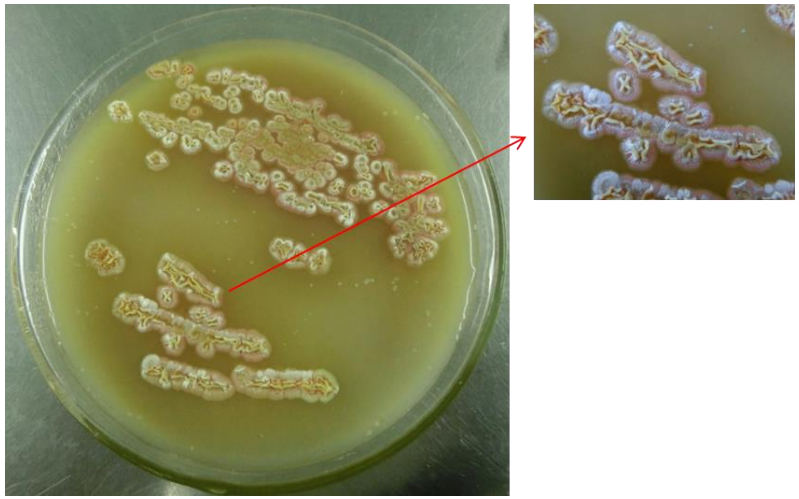


Figure S31. The Topo I-mediated DNA relaxation inhibition rate (%) of compounds 1-9 and CPT.

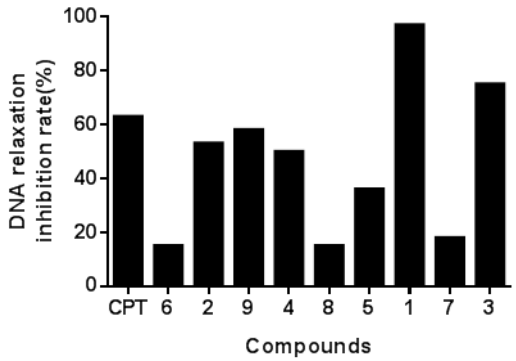


Figure S32. The comparison of Topo I-mediated DNA relaxation inhibition rate (%) of compounds 1, 3 and CPT at different concentrations.

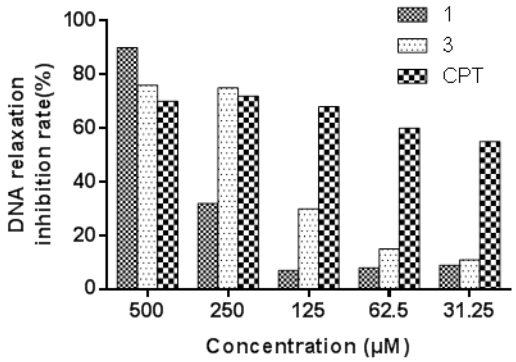


Figure S33. The Topo II*α*-mediated DNA relaxation inhibition rate (%) of compounds 1-9, CS1 and VP16.

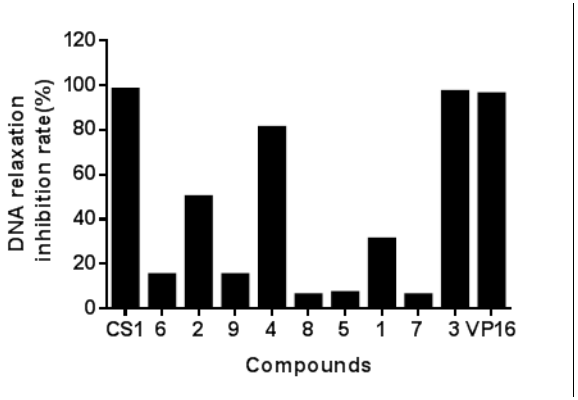


Figure S34. The Topo II*α*-mediated kDNA decatenation inhibition rate (%) of compounds 1-9, CS1 and VP16.

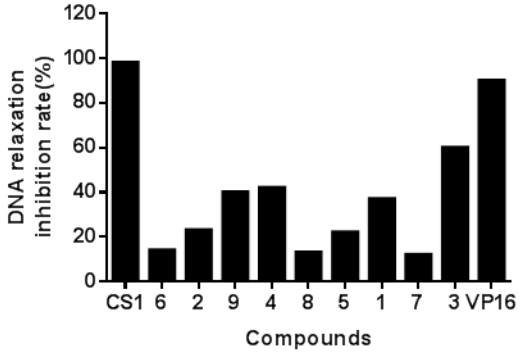


Figure S35. The comparison of Topo IIα-mediated kDNA decatenation inhibition rate (%) of compound 3, VP16 and CS1 at different concentrations.

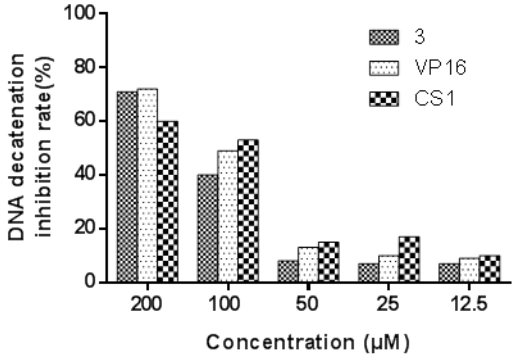


Figure S36. The cell growth inhibition against six human tumor cell lines of compound 3.

	The cell growth inhibition against six human tumor cell lines of compound **3** (mean ± SD)					
Concentration (μM)	HeLa	MDA-MB-453	MDA-MB-231	THP-1	HepG2	HL7702
200	84.03 ± 1.00	89.98 ± 2.34	36.93 ± 3.25	51.13 ± 2.13	68.38 ± 2.78	96.56 ± 0.25
100	65.61 ± 2.92	83.13 ± 1.52	16.62 ± 3.72	48.76 ± 4.08	57.50 ± 2.92	96.40 ± 0.32
50	52.13 ± 1.20	65.91 ± 4.44	9.55 ± 2.11	36.32 ± 3.57	31.60 ± 1.26	91.98 ± 0.64
25	26.06 ± 2.64	26.30 ± 4.30	6.88 ± 4.52	18.88 ± 2.99	8.01 ± 2.38	33.74 ± 2.80
12.5	14.71 ± 4.75	14.58 ± 4.73	4.67 ± 2.69	14.05 ± 1.03	0.050 ± 3.10	1.66 ± 3.74
6.25	11.70 ± 4.29	3.89 ± 4.74	6.44 ± 2.48	10.66 ± 4.39	-4.68 ± 4.26	-7.89 ± 4.15

Figure S37. The cell growth inhibition against six human tumor cell lines of compound VP-16.

	The cell growth inhibition against six human tumor cell lines of compound **VP-16** (mean ± SD)					
Concentration (μM)	HeLa	MDA-MB-453	MDA-MB-231	THP-1	HepG2	HL7702
3.125	52.80 ± 2.53					
6.250		52.40 ± 4.29				
3.125			47.50 ± 3.38			
0.800				53.26 ± 4.58		
20.000					49.05 ± 1.45	
1.250						64.42 ± 1.49

Figure S38. The gene sequence of AHBA synthase in *Streptomyces* sp. S012.

GTGACCCCCAGGACCCGGGTGATCATGCCCGTGCACATGGCGGGCTACATGGCGGACATGGACGCCCTCGCCAAGCTCTCCGCCGACACGGGCGTGCCGCTGCTCCAGG

ACGCCGCGCACGCGCACGGCGCCCGCTGGCGCGGCAAGCGCGTCGGCGAACTCGGCTCCGTCGCCGCGTTCAGCTTCCAGAACGGCAAGCTGATGACCGCCGGCGAG

GGCGGCGCCGTCCTGTTCCCCGACACCGAGCTGTACGAGACCGCGTTCCTGCGGCACAGCTGCGGGCGGCCCCGCACCGACCGCAGGTACCTGCACGAGATCGCCGGA

TCCAATCTGCGGCTCAACGAGTTCTCCGCGGCGGTGCTCCGGGCCCAGCTCGGCCGCCTCGACCAGCAGGTGACCACCCGTCAGAAGCGCTGGGCGCTGCTGTCCGGG

CTCCTCGGCGCGATCGACGGAGTGACCCCGCAGCGCGGCGACGAGCGCACCGACTTCCCCTCCCACTACATGGCGATGTTCCGGGTCCCCGGCATCGGCGAGGAGGGG

CGCAACGCCCTCGTCGACCGGCTCGTCGAGGCGGGCGTCCCGGCGTTCGCGGGCTTCCGCGCGATCTACCGCACCGCCGCCTTCTGGGAGTTCGGCGCGCCCGACGAG

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ACCGCCGCGATCGT