Supporting Information

Fungal Indole Alkaloid Biosynthesis: Genetic and Biochemical Investigation of Tryptoquialanine Pathway

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Table of Contents

SUPPLEMENTAL METHODS

X-ray Crystallographic data collection

A colorless platelet-like crystal of **1**, approximate dimensions .40mm x .20mm x .10 mm, was used for intensity data. The diffraction data were measured at room temperature on a Bruker SMART Apex2 CCD-based X-ray diffractometer system equipped with a Mo-Kα radiation (λ= 0.71073 Å). The detector was placed at a distance of 6.00 cm from the crystal. A total of 1798 frames were collected with a scan width of 0.5° in ω, with an exposure time of 30 sec./frame. The frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm. [1a] The integration of the data using a orthorhomic unit cell yielded a total of 29867 reflections to a maximum 2Θ angle of 56.64°, of which 3665 were independent (Rint = 5.3%). The final cell constants of a = 14.158 (10) Å, b = 21.005 (14) Å, c = 21.005 (12) Å, V = 5453(6) $Å^3$, are based upon the refinement of the XYZ centroids of 6528 reflections. The structure was solved and refined using the Bruker SHELXTL (Version 6.14) Software Package,[1b] in the space group $C222_1$. The absolute configuration of **1** was fixed based on the possible *R* configuration at C12 position and is consistent with the crystal structure of **4** established by X-ray crystallography.[2] All atoms were refined anisotropically and hydrogen atoms were placed at the calculated positions. The final anisotropic full-matrix least-squares refinement on F^2 converged at R_1 = 4.091%, wR_2= 16.55% and a goodness-of –fit of 1.042.

A colorless needle -like crystal of **18**, approximate dimensions .12mm x .02mm x .02 mm, was used for intensity data. The diffraction data were measured at 100K on a Bruker SMART Apex2 CCD-based X-ray diffractometer system equipped with a Mo-Kα radiation (λ= 0.71073 Å). The detector was placed at a distance of 6.00 cm from the crystal. A total of 1798 frames were collected with a scan width of 0.5° in ω, with an exposure time of 120 sec./frame. The frames were integrated with the Bruker SAINT software package using a narrow-frame integration algorithm.[1a] The integration of the data using a hexagonal unit cell yielded a total of 41893 reflections to a maximum 2Θ angle of 52.72°. The final cell constants of a = 21.974 (10) Å, c = 10.8958 (12) Å, V = 4556(6) $Å^3$, are based upon the refinement of the XYZ centroids of 5907 reflections. The structure

was solved and refined using the Bruker SHELXTL (Version 6.14) Software Package,[1b] in the space group $P6_3$. The absolute configuration of **18** was fixed based on the possible *S* configuration at C27 position and *R* configuration at C12 position and is consistent with incorporation of L-Ala and D-tryptophan moieties. All atoms were refined anisotropically and hydrogen atoms were placed at the calculated positions. The final anisotropic full-matrix least-squares refinement on F^2 converged at R_1 = 7.03%, wR_2= 17.13% and a goodness-of –fit of 1.082.

(1) a) Saint version 7.68 (2009), Bruker AXS Inc., Madison, WI (USA)

b) Shelxtl version 6.14 (2000), Sheldrick, Bruker AXS Inc., Madison, WI (USA)

(2) Springer, J. P. *Tetrahedron Lett.* 1979, 339.

Table S1 Domain architectures of putative NRPSs in *P.aethiopicum* and comparison to the closest homologs in *A.clavatus* and *P. chrysogenum*

	Contig No.	Contig length	Location	NRPS Length (aa)	Domains	Closest homolog in BlastP	Closest *P. chrysogenum* homolog	Closest *A. clavatus* homolog
1	contig00857	73656	6614-13830	2368	A-T-C-A-T-C	Pc21g15480 (87%)	Pc21g15480 (87%)	ACLA 059530 (31%)
2	contig01275	41088	37-12346	4074	C*A-T-C-A-T-E-C-A-T-C	ACLA 017890 (63%)	Pc13g14330 (33%)	ACLA 017890 (63%)
3	contig00865	23983	10454-23163	4209	C-A-T-C-A-T-C-A-T-C	Pc13g14330 (53%)	Pc13g14330 (53%)	ACLA_059530(45%)
4	contig00311	157072	142932-149218	2082	A-T-C-A-T-C	Pc22g20400 (94%)	Pc22g20400 (94%)	AFLA_010620 (68%)
5	contig00107	69917	32541-38452	1921	A-T-C-A-T-C	Pc16g03850 (93%)	Pc16g03850 (93%)	ACLA_061000(42%)
6	contig00904	94631	40241-47585	2409	A-T-C-A-T-C	Pc21g12630 (84%)	Pc21g12630 (84%)	ACLA_076770(33%)
7	contig00185	110569	31302-42930	3864	A-T-C-A-T-C	NFIA_044240 (83%)	Pc21g10790 (31%)	ACLA_095980(34%)
8	contig00284	128491	104811-121323	5397	A-T-C-A-T-C-A-C-A-T-C-A-T-C	AFLA_066720 (54%)	Pc21g10790 (30%)	ACLA_059530(28%)
9	contig00894	67132	8793-27331	6048	A-T-E-C-A-T-C-A-T-E-C-A-T-C-A-T	Pc16g04690 (93%)	Pc16g04690 (93%)	ACLA_025160(57%)
10	contig00025	17604	543-13015	4102	A-T-C-A-T-C-A-C	ABR23346.1 (32%, partial identity)	Pc21g12630 (31%)	ACLA_093780(32%)
11	contig01022	16832	9044 - 12370	1108	A-T-C	ACLA_017900 (67%)	Pc21g10790 (32%)	ACLA_017900(67%)
12	contig01242	215775	55563 - 58001	812	A	Pc22g22580 (85%)	Pc22g22580 (85%)	ACLA_093780(30%)
13	contig01239	68878	23001 - 25741	809	A	Pc21g12840 (91%)	Pc21g12840 (91%)	ACLA_098420(32%)
14	contig00921	69917	32996 - 36780	1215	A	Pc20g12670 (82%)	Pc20g12670 (82%)	ACLA_095980(32%)
15	contig00239	135391	104823 - 111215	2106	A-T-C-A-T-C	Pc21g01710 (94%)	Pc21g01710 (94%)	ACLA_061190(64%)
16	contig00759	116158	26436 - 41994	5096	A-C-A-T-C-A-T-C-A-T-C-A-T-C-A-T-C	Pc13g05250 (89%)	Pc13g05250 (89%)	ACLA_079690(53%)

The NRPS genes in *P. aethiopicum* were identified from the local genome database using A domains of the acetylaszonalenin synthetase of *Neosartorya fischeri NRRL 181*, which activates an anthranilate and a tryptophan.[2] A total of 16 NRPS genes were found. The strategy for the search of *P. aethiopicum* NRPSs genes responsible for the production of **1** was based on assumptions that 1) PaeNRPSs that are highly similar and orthologous (> 80% identity with identical domain architecture) to those in *P. chrysogenum* are unlikely to produce **1** and thus can be excluded; 2) since the PaeNRPSs for **1** in *P. aethiopicum* and **2** in *A. clavatus* are likely to be close homologs of each other, PaeNRPSs genes that are highly similar to those in *A. clavatus* are good candidates. Combining these two search parameters allow us to narrow down the candidate to two (PaeNRPS1275 and PaeNRPS1022). Incidentally, the homologs of the two NRPSs in *A. clavatus* (ACLA_17890 and ACLA_17900) are adjacent to each other in the genome and have a combined total of four modules, correspond to the four amino acid units required to build **1** and **2**. The close similarity of PaeNRPS1275 to the tryptophan- and anthranilate-activating A domains (both 47% identity) of the acetylaszonalenin synthetase of *N. fischeri NRRL 181* supports that PaeNRPS1275 is a highly plausible candidate.

(2) Yin, W. B.; Grundmann, A.; Cheng, J.; Li, S. M. *J. Biol. Chem.* 2009, *284*, 100.

Table S2 Sequences of primers used in this paper

Primer	Sequence
tqaAKO_P1	5’-AATTCCACCAAGCCCTTACA-3’
tqaAKO_P2	5’-ATGACGGTCCAATCTCTCGA-3’
tqaAKO_P3	5’-GCCCGTCACCGAGATTTAGGGTCCTGCCTCCAAGCAGGCG-3’
tqaAKO_P4	5’-CAATATCATCTTCTGTCGACGTCGCCGAGTAAAAGCCCGT-3’
tqaAKO_P5	5’-GATCAAGTTTGTGCTTGCCG-3’
tqaAKO_P6	5’-AATTAGATTTACCGCAGTCA-3’
tqaBKO_P1	5’-AAACCATAAAATCAATTGGA-3’
tqaBKO_P2	5’-ATTTTCAACCCACGAGACAA-3’
tqaBKO_P3	5’-GCCCGTCACCGAGATTTAGGCATTATACAGGTGAACATAT-3’
tqaBKO_P4	5’-CAATATCATCTTCTGTCGACGAAACCTGCGAACTACCGGA-3’
tqaBKO_P5	5’-GTAAGTCTTGGTTGGGGCCT-3’
tqaBKO_P6	5’-GTTCGACCGATTCGAGCAGC-3’
tqaCKO-P1	5’-GAAATCACTCGACAAGGTGGAC -3’
tqaCKO-P2	5’-AGGATGACGCCTCAGGAAATGT-3’
tqaCKO-P3	5’-TGCCCGTCACCGAGATTTAGGCTTCAAGCTCGGGTGTGGAAGG-3’
tqaCKO-P4	5’-TCAATATCATCTTCTGTCGACGAGACCTGCGGATCATTGGGAG-3’
tqaCKO-P5	5’-AGCGTAACGTAAACCTCCCACC-3’
tqaCKO-P6	5’-CTGAAGAAATGAGCCCATAGCA-3’
tqaDKO-P1	5’-TTCCCGGAGACTAGAACTGGCT-3’
tqaDKO-P2	5’-TGCCACGGTAGCAATAGTCAAC-3’
tqaDKO-P3	5’-TGCCCGTCACCGAGATTTAGGGTCCCACAGTATGATCCAAGCAG-3’
tqaDKO-P4	5’-TCAATATCATCTTCTGTCGACCTACAGTCGGATGCTATGGGCT-3’
tqaDKO-P5	5’-GATGCTCAAATGTCGCCAGGTC-3’
tqaDKO-P6	5’-CTGAGGGAGTCAACATGGCAAG-3’
tqaEKO-P1	5’-TCGAACGATGGCTGCAAATCTT-3’
tqaEKO-P2	5’-GCCGATGGAGAGGAGGAAATAC-3’
tqaEKO-P3	5’-TGCCCGTCACCGAGATTTAGGTGGGTTGTTCCACGCAGAAGAC-3’
tqaEKO-P4	5’-TCAATATCATCTTCTGTCGACCAAGGCCGACAATGATGACAGT-3’
tqaEKO-P5	5’-AGGGCTTCCCATTTGATTTGGT-3’
tqaEKO-P6	5’-GTGGTGTCCAATGTGGACCAAA-3’
tqaFKO-P1	5’-ATCGCCTTCCGAAGATAAGAGA-3’
tqaFKO-P2	5’-ATTTCTTCCTGTAACCCTCGTC-3’
tqaFKO-P3	5’-TGCCCGTCACCGAGATTTAGGAGGCGGTATGCGGATAACTGAG-3’
tqaFKO-P4	5’-TCAATATCATCTTCTGTCGACGACTGGCGATTTGACACACTTG-3’
tqaFKO-P5	5’-TGAAGACAGTTCGCTACCGAGC-3’
tqaFKO-P6	5’-CAATTCCCCAGCACTACTGAAG-3’
tqaGKO-P1	5’-ACAATACGGTCTTCCAGGCGCT-3’
tqaGKO-P2	5’-AGCTTGATTATCGCAGCAACCA-3’
tqaGKO-P3	5’-TGCCCGTCACCGAGATTTAGGAGGTGATGTCGGTTCCAAAGGA-3’
tqaGKO-P4	5’-TCAATATCATCTTCTGTCGACCCAATGAGCACGGACACGAAAC-3’
tqaGKO-P5	5’-GTTTGTGACCAAGGAGGAGGTG-3’
tqaGKO-P6	5’-GACTGTGGATGATGGAGAGCGT-3’
tqaHKO-P1	5’-ACCGTAGGCGTTCCACGTCATG-3’
tqaHKO-P2	5’-TGCTGGCTTTCATCAGATTGGT-3’
tqaHKO-P3	5’-TGCCCGTCACCGAGATTTAGGTCCGGTTCCCTAATCCTGACTG-3’
tqaHKO-P4	5’-TCAATATCATCTTCTGTCGACATCTACGTGCGTGGAGTCACAG-3’
tqaHKO-P5	5’-TCTCCAAATCGAGGGCAAGCAT-3’
tqaHKO-P6	5’-GATGGTGGGAGCTGATGGTTGG-3’
tqaIKO-P1	5’-TGCAATGAAGGCATCTGCACTG-3’
tqaIKO-P2	5’-GCCGACATCTTTGACCATCCTG-3’
tqaIKO-P3	5’-TGCCCGTCACCGAGATTTAGGTGGCCAGTATCCAAACATGGGT-3’

tqaIKO-P4	5’-TCAATATCATCTTCTGTCGACAAATGGGCATTGGGAAGAACCT-3’
tqaIKO-P5	5’-TGGCAAGCGACAACAAATCATC-3’
tqaIKO-P6	5’-GCAGTGTACGACCGAGTGGTG-3’
tqaKKO-P1	5’-GCAGCAAATTGCGAAAGCTCTC-3’
tqaKKO-P2	5’-ACCGTCGATGAGGCATCCTTTC-3’
tqaKKO-P3	5’-TGCCCGTCACCGAGATTTAGGGTTTCCTTTCCATCCGGCTCTT-3’
tqaKKO-P4	5’-TCAATATCATCTTCTGTCGACGACATAGCACTTGCAGCCATTG-3’
tqaKKO-P5	5’-TGGCCGACTCGCTTACCGACTA-3’
tqaKKO-P6	5’-AGGTTGAGTTGGGTGGCCGACT-3’
tqaLKO-P1	5’-CAGGATTTCGGAATGAACCATG-3’
tqaLKO-P2	5’-GCTGTCGTGAAGGGAAAGCAGA-3’
tqaLKO-P3	5’-TGCCCGTCACCGAGATTTAGGTCCACGGCCAAGTCTCTGGTCT-3’
tqaLKO-P4	5’-TCAATATCATCTTCTGTCGACGAACCTGTCGGATGGATATGGA-3’
tqaLKO-P5	5’-CAGTCTAGTTGGTGATTTCGTG-3’
tqaLKO-P6	5’-TCGTGGAGCCGGTCATAACTTT-3’
tqaMKO-P1	5’-TCTTGTTCAGCGCATCCACTAC-3’
tqaMKO-P2	5’-GACTGACACAATCATGGCAATG-3’
tqaMKO-P3	5’-TGCCCGTCACCGAGATTTAGGTCAGGCTCTAATTTGGAAGATG-3’
tqaMKO-P4	5’-TCAATATCATCTTCTGTCGACTTGGCTGTATGTAGAGGCGCAG-3’
tqaMKO-P5	5’-CTAGTGCGAATTAGTTCCCCA-3’
tqaMKO-P6	5’-GTCATCAATGAGTGGTTGTCGT-3’
orf4KO-P1	5’-TAGCGATGACAAGCATTGCG
orf4KO-P2	5’-GATCAAGCCAGTCGAGGTTG
orf4KO-P3	5’-TGCCCGTCACCGAGATTTAGGCACGTCTGGGTTGGACTATACTG-3’
orf4KO-P4	5’-TCAATATCATCTTCTGTCGACAGTCGGTGAATGTGCTTCCA-3’
orf4KO-P5	5’-TCCAGCAACACAAGTGAAGCT-3’
orf4KO-P6	5’-GAAATGGCAGAATCCCAGTCTT-3’
orf5KO-P1	5’-CTCTAAAGCGCGGCGAGTCG-3’
orf5KO-P2	5’-GATGGAGACATGGTGGTAATTG-3’
orf5KO-P3	5’-TGCCCGTCACCGAGATTTAGGAGTGAGGGCTCATGTCGTGGAG-3’
orf5KO-P4	5’-TCAATATCATCTTCTGTCGACGTTGCATTGGGTCCTACAGTTC-3’
orf5KO-P5	5’-CAAAGCCTCCGGCTTCCTAGC-3’
orf5KO-P6	5’-TGCTATGTCTCGGGTATTCG-3’
orf6KO-P1	5’-CATGTTTGTTGGAGCAAGCCT-3’
orf6KO-P2	5’-TCGTGGGTACAATCTTCCGA-3’
orf6KO-P3	5’-TGCCCGTCACCGAGATTTAGGGGCAACCTGTCCGGGATTTC-3’
orf6KO-P4	5’-CAATATCATCTTCTGTCGACGTGTTCTCGGAGGACGAACA-3’
orf6KO-P5	5’-CTCTGTTGGCAGGCTACCTC-3’
orf6KO-P6	5’-ACTAGCCGGGCTATACAAGGT-3’
TqaB_SacI_F	5’-AA**GAGCTC**ATGTTTGAGCCAATCGAGACTC-3’
TqaB_SacI_R	5’-TT**AAGCTT**TTACCCGTTGATCTTGGAGAGC-3’
TqaB_CDFduet_SacI_F	5’-AAAAAA**GAGCTC**GATGTTTGAGCCAATCGAGACTC-3’
TqaH_NdeI_F	5’-AAAAAA**CATATG**ACAACCGACCGTCAACCAT-3’
TqaH_EcoRI_R	5’-TTTTTT**GAATTC**TCACCCGCGCTTGTATGTATTC-3’
TqaH_XhoI_R	5’-TTTTTT**CTCGAG**TCACCCGCGCTTGTATGTATTC-3’
TqaD_NheI_F	5’-AA**GCTAGTA**TGACAGTCTCTGAAACAAAGA-3’
TqaD_ XhoI_R	5’-AA**GCTAGTA**TTACGAAATGTATCTTGCAACT-3’
Seq contig 1022B_R	5’-CTAACGTTTGAAGATATAAC-3’
Seq contig 326B_R	5’-GTCTTATGAGTATTCAGCCT-3’

Table S3 NMR Data of **1** and **20** in DMSO-d_6 Measured on 500 MHz Bruker NMR Spectrometer.

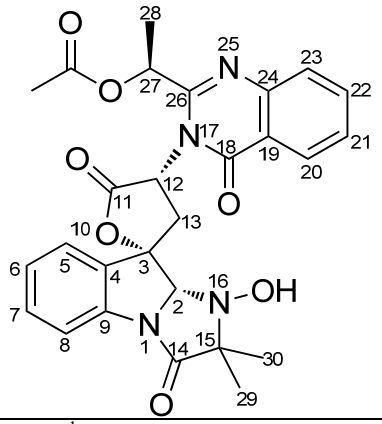
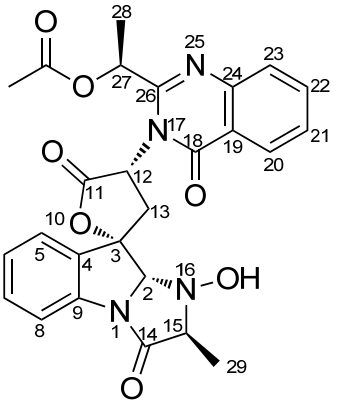
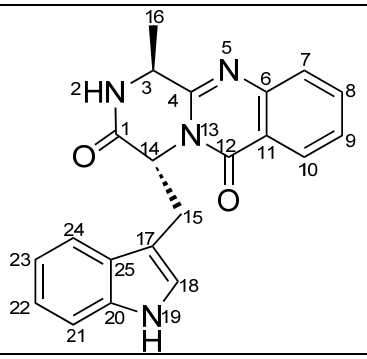
1					**20**			
								
NO.	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC
2	83.9	5.22 (s)	16N-OH, H13	H2	88.4	5.41 (s)	H15, H13, 16N-OH	H2
3	85.5		H2, H13		84.2		H13, H5, H2	
4	134.3		H8, H6, H13		134.3		H8, H6, H13	
5	125.4	7.91(m)	H7	H5	125.4	7.91(m)		H5
6	125.2	7.30 (t, 7.4)	H8	H6	125.4	7.32 (t, 7.5)	H8	H6
7	131.4	7.52 (m)	H5	H7	131.4	7.53 (t, 7.5)	H5, H9	H7
8	114.7	7.52 (m)	H6	H8	115.0	7.50 (t, 7.5)	H6	H8
9	137.1		H5, H7		137.2		H5	
11	169.9		H12, H13		169.8		H12, H13	
12	54.4	5.91 (t, 9.9)	H13	H12	54.5	5.91 (t, 9.9)	H13	H12
13	34.2	3.12 (m)	H12, H2	H13	33.4	3.13 (dd, 13.5, 9.9)	H2	H13
		3.08 (m)		H13		3.04 (dd, 13.5, 9.9)		H13
14	170.8		H29, H30		169.9		H29, H2, H15	H15
15	70.6		H29, H30		67.3	4.07 (q, 7.1)	16N-OH	
16N-OH		7.95 (S)				8.01 (s)		
18	160.4		H20, H12		160.5		H12, H20	
19	119.8		H23, H21		119.8		H23, H21	
20	126.2	8.17 (d, 7.9)	H22	H20	126.2	8.17 (d, 7.9)	H18, H22	H20
21	128.3	7.63 (t, 7.5)	H23	H21	128.1	7.63 (t, 7.6)	H23	H21
22	135.6	7.94 (m)	H20	H22	135.6	7.92 (m)	H20	H22
23	127.4	7.76 (d, 8.2)	H21	H23	127.4	7.76 (d, 8.1)	H21	H23
24	146.0		H20, H22		146.0		H20, H22	
26	154.8		H27, H12, H28		154.9		H27, H28	
27	68.4	6.31 (q, 6.2)	H28	H27	68.4	6.28 (q, 6.3)	H28	H27
CH_3COO^-	170.1		H27, CH_3COO^-		170.1		H27, CH_3COO^-	
CH_3COO^-	20.6	2.07 (s)		CH_3COO^-	20.6	2.08 (s)		CH_3COO^-
28	18.6	1.68 (d, 6.3)	H27	H28	18.6	1.68 (d, 6.3)	H27	H28
29	22.8	1.30 (s)	H30	H29	10.9	1.43 (d, 7.1)	H15	H29
30	16.7	1.34 (s)	H29	H30				

Table S4 NMR Data of **14** in $CDCl_3$ Measured on 500 MHz Bruker NMR Spectrometer.

14



NO.	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC
1	169.3		H2, H14, H15	
2		6.63 (br)		H3
3	49.1	3.13 (q, 6.5, 0.3)	NH2, H16	
4	151.6		H2, H14, H3, H16	
6	147.0		H10, H8	
7	127.3	7.59 (d, 8.1)	H9, H10	H7
8	134.7	7.77 (td, 7.7, 1.7)	H10	H8
9	127.1	7.53 (t, 7.6)	H8, H7	H9
10	126.8	8.36 (dd, 8.0, 1.1)	H8, H9	H10
11	120.2		H7, H9	
12	160.8		H10	
14	57.5	5.68 (m)	NH2, H15	H14
15	27.0	3.70 (dd, 14.8, 5.1) 3.64 (dd, 15.0, 3.6)	H18, H14	H15
16	19.1	1.36 (d, 6.6)	H3	H16
17	109.4		H19, H24, H23, H18, H14, H15	
18	123.5	6.67 (d, 1.7)	H15	H18
19		8.26 (br)		
20	135.9		H19, H24, H22, H18	
21	111.2	7.28 (d, 8.1)	H22	H21
22	122.6	7.12 (t, 7.8)	H24, H21, H23	H22
23	120.0	6.91 (t, 7.6)	H21, H22	H23
24	118.5	7.39 (d, 8.0)	H21, H22	H24
25	127.3		H24, H21, H23, H18, H15	

Table S5 NMR Data of **18** and **19** in DMSO-d_6 Measured on 500 MHz Bruker NMR Spectrometer.

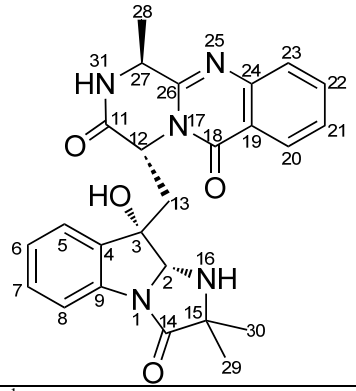
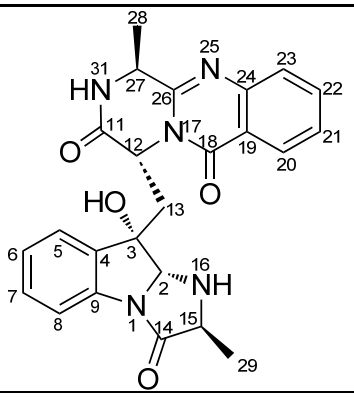
18					**19**			
								
NO.	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC
2	79.0	5.00 (d, 9.0)	H3, 16NH	H2	81.8	5.11 (d, 8.7)		H2
3	74.4				75.0		H2, 3OH	
3OH		5.66(s)				5.60 (s)		
4	138.1		H8		138.1		H8	
5	124.5	7.45 (d, 7.6)	H7	H5	124.6	7.45(d, 7.7)	H7	H5
6	124.8	7.12(m)	H8	H6	124.8	7.13 (dd, 9.9, 6.3)	H8	H6
7	129.7	7.33(m)	H5	H7	129.6	7.33 (m)	H5	H7
8	114.4	7.32(m)	H6	H8	114.6	7.33 (m)	H6	H8
9	137.4		H5, H7		137.5		H5	
11	175.3		H13		174.7		H13	
12	53.0	5.35(dd, 8.0, 3.6)	H13, 31NH	H12	53.0	5.38 (dd, 13.0, 9.9)	31NH	H12
13	37.5	2.81 (dd, 14.4, 7.9)	16NH	H13	37.5	2.83 (dd, 14.8, 3.9)	3OH	H13
		2.69 (dd, 14.4, 7.5)		H13		2.66 (dd, 14.7, 7.4)		
14	168.5		H29, H30		168.5		H13, H29	
15	64.4		H29, H30		60.0	3.65 (m)		H15
16NH		2.84 (d, 3.4)				3.17 (m)		
18	160.3		H20		160.3		H20	
19	120.2		H21, H23		120.1		H21, H23	
20	126.2	8.15 (d, 7.9)	H22	H20	126.3	8.15 (d, 7.9)	H22	H20
21	126.7	7.53 (t, 7.5)	H23	H21	126.8	7.54 (t, 7.6)	H23	H21
22	134.5	7.83 (m)	H20	H22	134.5	7.84 (t, 7.7)	H20	H22
23	126.9	7.66 (d, 8.0)	H21	H23	127.0	7.67 (d, 8.1)	H21	H23
24	147.0		H20, H22		146.9		H20, H22,H27	
26	152.9		H27, 31NH, H28		153.0		H27, 31NH, H28	
27	48.8	4.96 (q, 6.3)	H28, 31NH	H27	48.7	4.96 (q, 6.1)	31NH, H28	H27
28	17.3	1.58 (d, 6.5)	H27	H28	17.1	1.58 (d, 6.6)	H27	H28
29	24.4	1.11(s)	H29	H29	17.2	0.98 (d, 7.1)		H29
30	23.5	0.93(s)	H30	H30				
31NH		8.67(s)				8.67 (s)		

Table S6 NMR Data of **24** and **25** in DMSO-d_6 Measured on 500 MHz Bruker NMR Spectrometer.

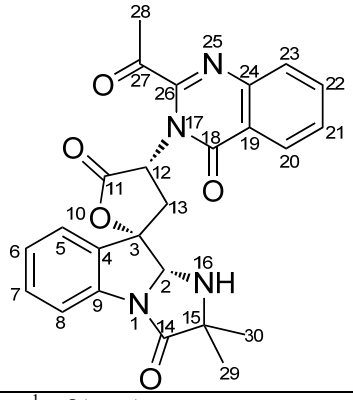
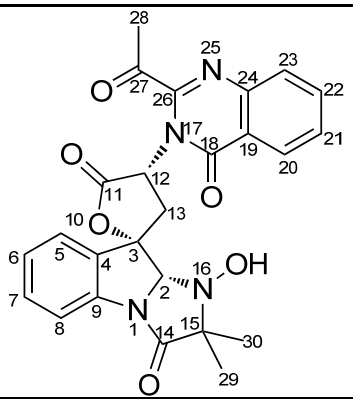
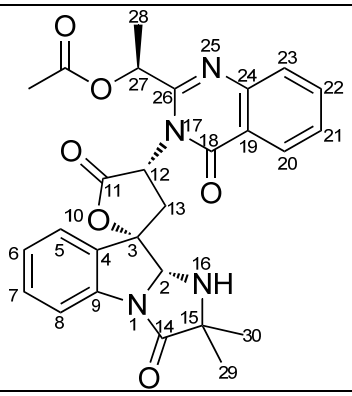
24					**25**			
								
NO.	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC
2	79.8	5.22 (d, 6.8)	16NH, H13	H2	84.3	5.31 (s)	16NOH	H2
3	83.8		H2, H13		83.5		H2,H5,H13	
4	134.6		H6, H8, H13		134.4		H5,H8,H13	
5	124.6	7.59(d, 7.3)	H7	H5	124.6	7.58(d, 7.5)	H7	H5
6	125.5	7.33 (t, 7.4)	H8	H6	125.4	7.31 (dd, 10.0, 3.8)	H8	H6
7	131.4	7.52 (m)	H5	H7	131.5	7.53 (m)	H5	H7
8	115.3	7.49 (m)	H6	H8	114.9	7.50 (m)	H6	H8
9	138.0		H7, H8		137.3		H5, H7,H8	
11	170.0		H13		170.1		H13	
12	55.8	6.08 (t, 9.9)	H2,H13	H12	55.9	6.16(t, 10.4)	H13	H12
13	33.4	3.17(dd, 13.1, 9.8)	H2	H13	35.1	3.21 (m)	H2	
		3.08(dd, 13.1, 9.9)		H13		3.18 (dd, 12.6, 9.2)		H13
14	175.0		H29, H30		171.1		H29, H30	
15	64.9		H29, H30		70.6		H29, H30	
16NH		3.22 (d, 8.4)						
16NOH						8.29 (s)		
18	160.1		H20		160.1		H20	
19	121.4		H21, H23		121.6		H21, H23	
20	126.4	8.22 (d, 8.0)	H22	H20	126.4	8.18 (d, 7.9)	H22	H20
21	129.6	7.74 (t, 7.6)	H23	H21	129.7	7.74 (t, 7.6)	H23	H21
22	135.5	7.98 (t, 7.7)	H20	H22	135.5	7.98 (t, 7.7)	H20	H22
23	128.3	7.89 (d, 8.2)	H21	H23	128.4	7.89 (d, 8.0)	H21	H23
24	145.0		H20, H22		145.0		H20, H22	
26	150.0				150.0		H28	
27	195.2		H28		195.3		H28	
28	27.4	2.77 (s)		H28	27.4	2.77 (s)		H28
29	25.6	1.34 (s)	H30	H29	22.8	1.27 (s)	H30	H29
30	24.9	1.42 (s)	NH, H29	H30	16.3	1.32 (s)	H29	H30

Table S7 NMR Data of **28** in DMSO-d_6 Measured on 500 MHz Bruker NMR Spectrometer.

28



No	^{13}C δ(ppm)	^{1}H δ(ppm) (m, J_{HH}(Hz))	HMBC	HMQC
2	80.6	5.47 (d, 7.3)	NH, H13	H2
3	84.3		H2, H5, NH, H13	
4	134.5		H6, H13	
5	125.8	7.92(m)	H7	H5
6	125.2	7.32 (t, 7.4)	H8	H6
7	131.3	7.52 (m)	H5	H7
8	114.9	7.49 (m)	H6	H8
9	138.0		H5, H8	
11	170.4		H12, H13	
12	54.1	5.91 (t, 9.6)	H13	H12
13	33.4	3.10 (dd, 13.8, 10.6)	H2, H12, H13	H13
		2.90 (dd, 13.8, 8.9)		H13
14	174.9		NH, H28,H29	
15	64.6		H29, H30	
16NH		3.18 (d, 7.4)		
18	160.4		H20	
19	119.8		H23, H21	
20	126.3	8.20 (d, 7.9)	H22	H20
21	127.9	7.62 (t, 7.6)	H23	H21
22	135.5	7.91 (m)	H20	H22
23	127.4	7.74 (d, 8.1)	H21	H23
24	146.1		H20	
26	155.0		H12, H22, H26, H27	
27	68.3	6.19 (q, 6.3)	H28	H27
CH_3COO^-	170.2		H27, CH_3COO^-	
CH_3COO^-	20.6	2.10 (s)		CH_3COO^-
28	18.7	1.67 (d, 6.3)	H27	H28
29	24.9	1.44 (s)	16NH, H30	H29
30	25.5	1.22 (s)	H29	H30

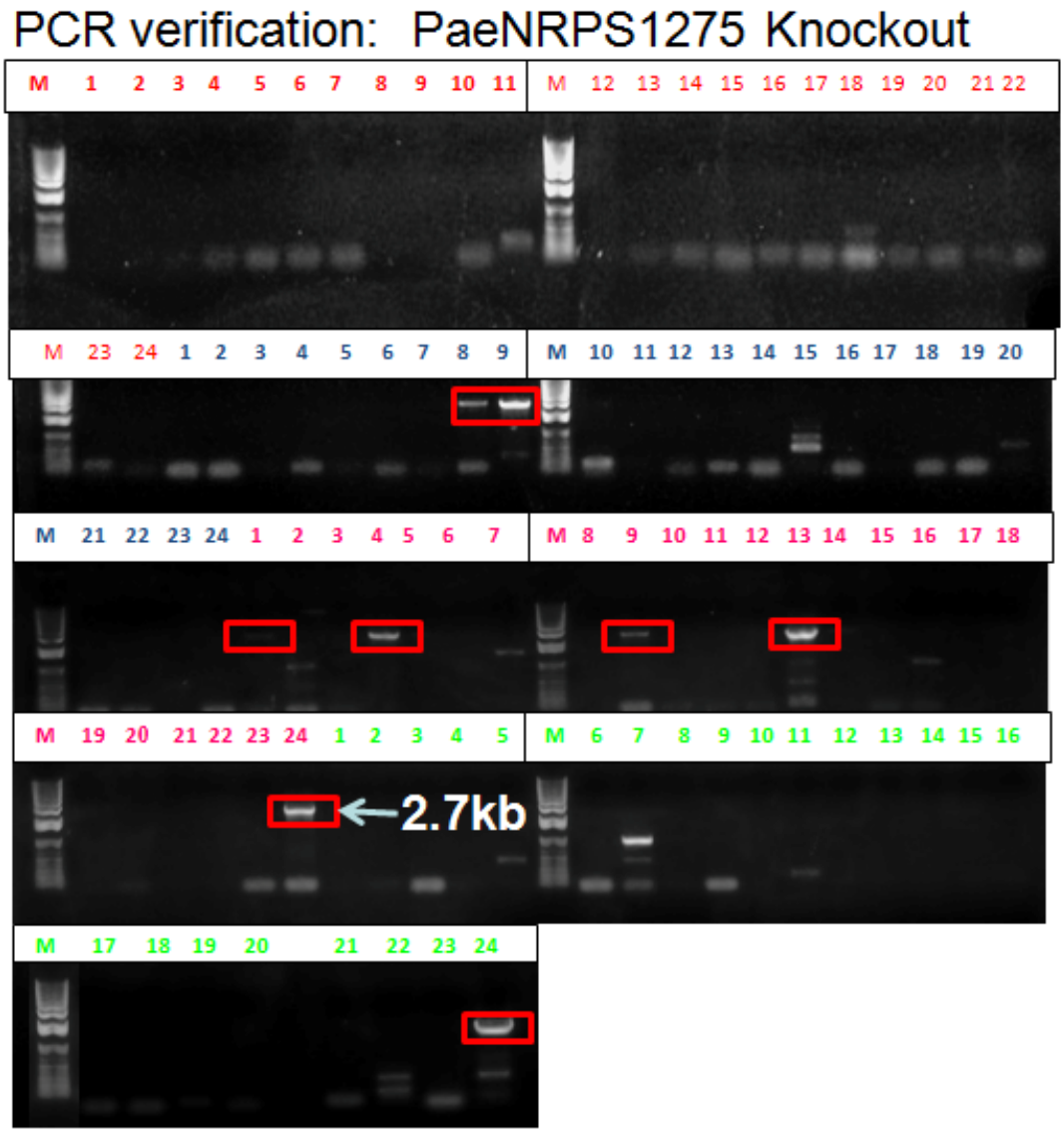


Figure S1 PCR verification of PaeNRPS1275 transformants as an example. 96 transformants were screened by PCR using bar gene primer (BarF) and primer outside of the deletion cassette (PaeNRPS1275KO_P1). Positive transformants showed a band at 2.7kb.

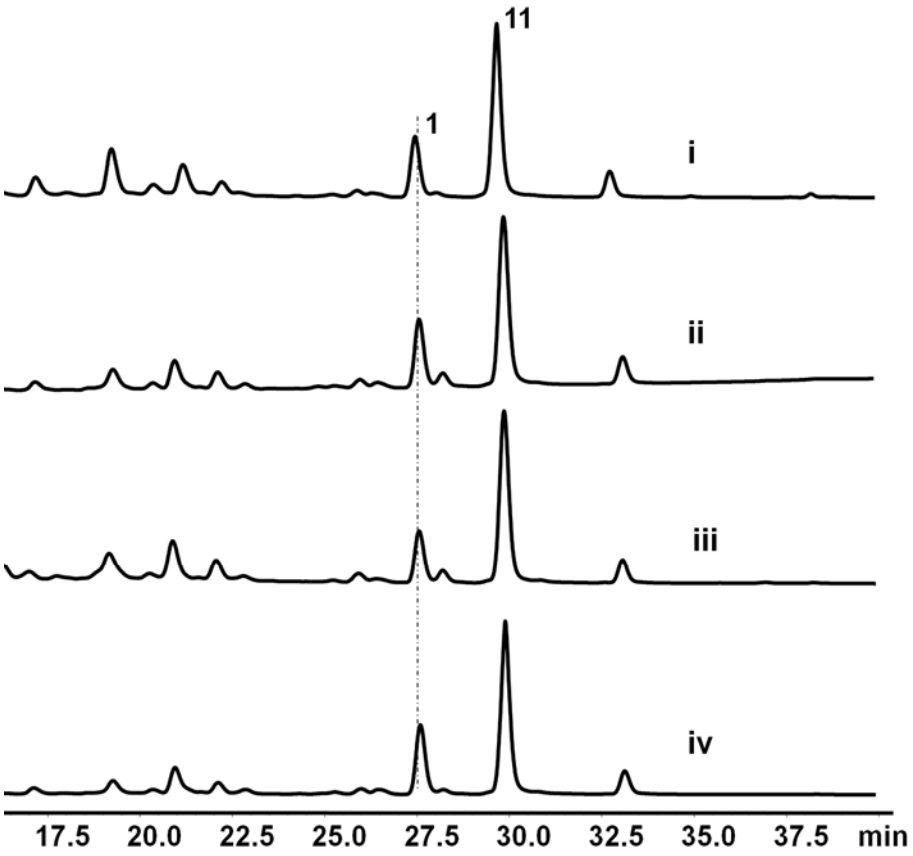


Figure S2 HPLC traces of metabolic extracts from single gene deletion strains of *P. aethiopicum.* Trace i: Δ*gsfA;* trace ii Δ*gsfA/*Δ*orf4*; trace iii: Δ*gsfA/*Δ*orf5;* trace iv: Δ*gsfA/* Δ*orf6*.

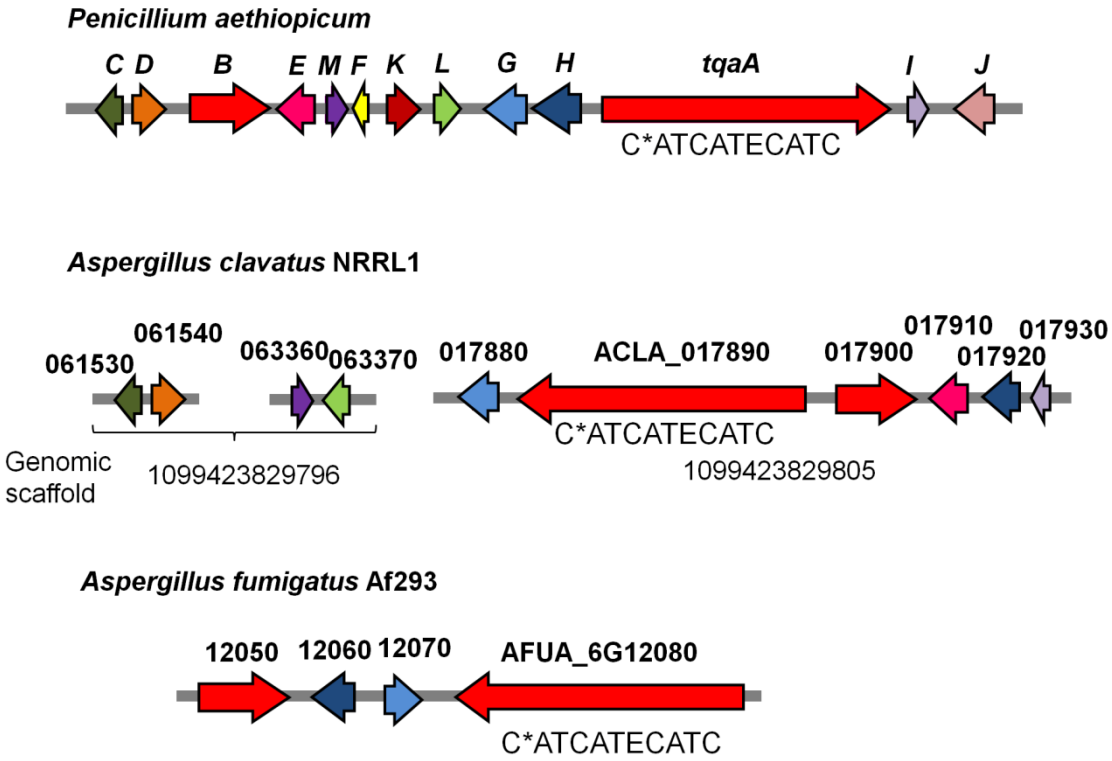


Figure S3 Corresponding *tqa* homologs in *A. clavatus* NRRL1 and *A.* fumigatus Af293 (corresponding homologs are in the same color); domain architecture of TqaA, TqvA (ACLA_017890) and FqaA (AFUA_6G12080). Homologous *tqv* gene cluster are fragmented in three different genomic loci.

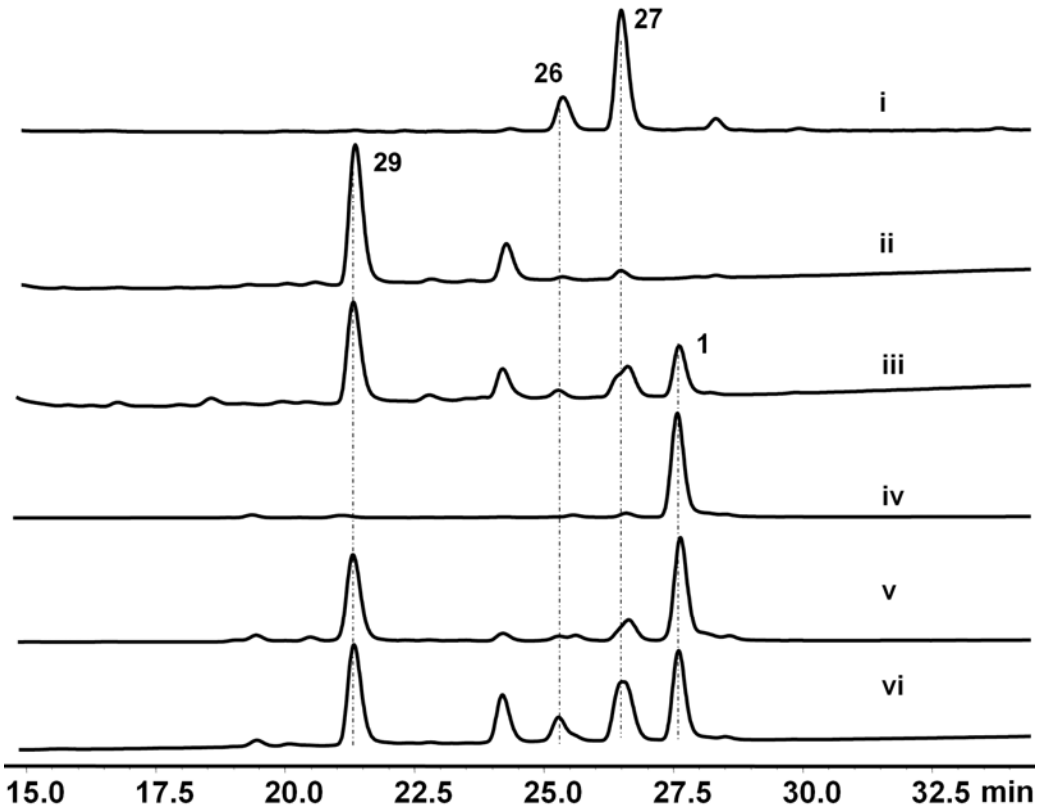


Figure S4 Biochemical characterizations of TqaD. Trace i: Compound **26** and **27** purified from Δ*tqaD* strain as substrate; extracts from overnight in vitro assays in 50 mM HEPES, pH 7.9, trace ii: control with 1 mM **26** and **27** mixture, 4 mM acetyl-CoA; trace iii: assay with 1 mM **26** and **27** mixture, 4 mM acetyl-CoA, 10 μM TqaD; trace iv: control with 1 mM **1**; trace v: assay with 1 mM **1**, 10 μM TqaD; trace vi: assay with 1 mM **1**, 10 μM TqaD. Reaction samples in trace i to v were extracted by EA only; reaction sample in trace vi is extracted by EA+5% TFA.

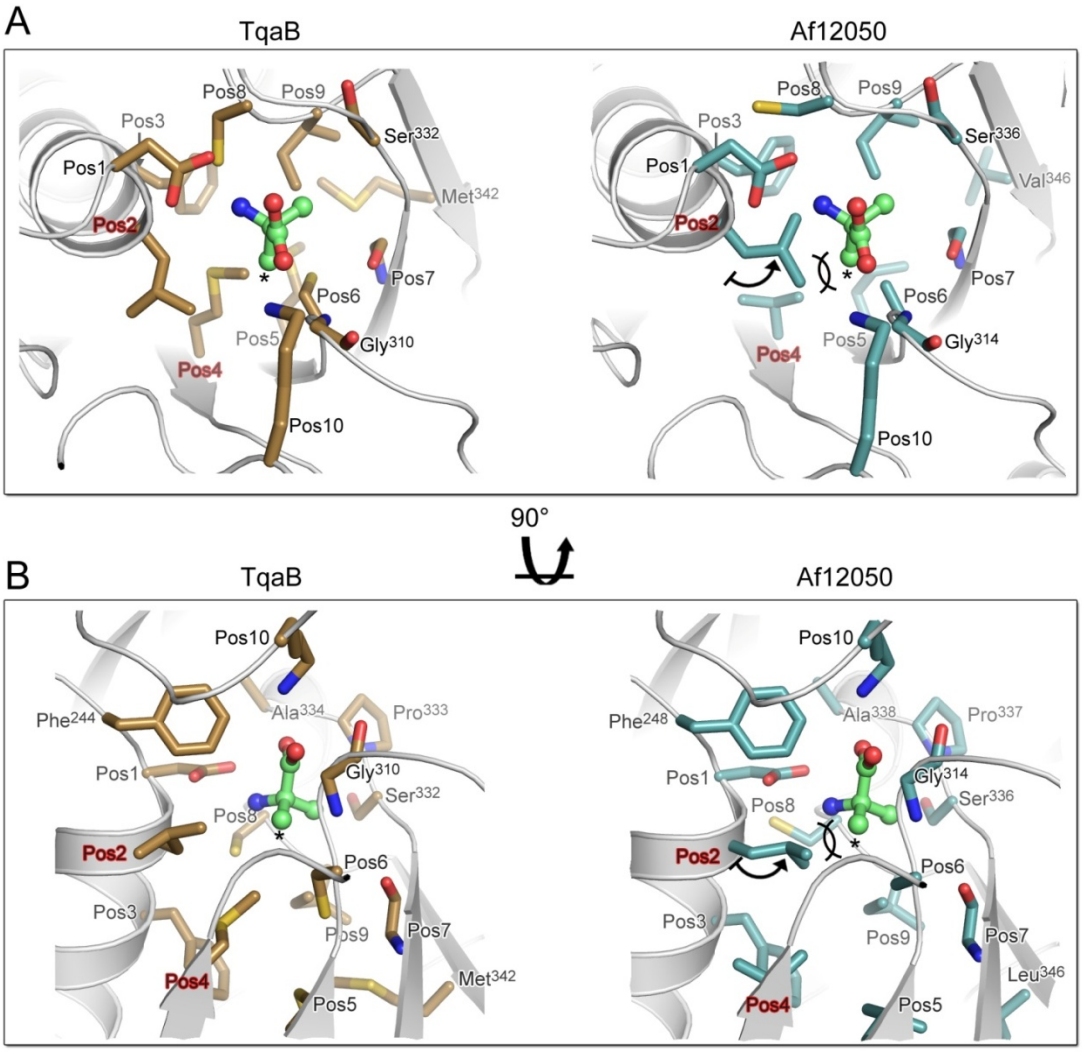


Figure S5 Substrate binding pocket comparison of the A-domains of TqaB and Af12050 generated via homology modeling using HHpred with PDB_{id} 1AMU as template. (**A**) A top-down view of the modeled substrate binding pocket and (**B**) a side-on view generated by rotating 90° about the horizontal plane. Residues are labeled according to their position in the 10AA specificity code or with their respective residue identifier. 2-aminoisobutyric acid (2-AIB, green ball-and-stick) was manually docked into the active site based on the position of the bound Phe of 1AMU. The *pro-R* (D-configuration) methyl of 2-AIB is indicated by an asterisk. The overlapping ")(" symbol indicates steric clash.

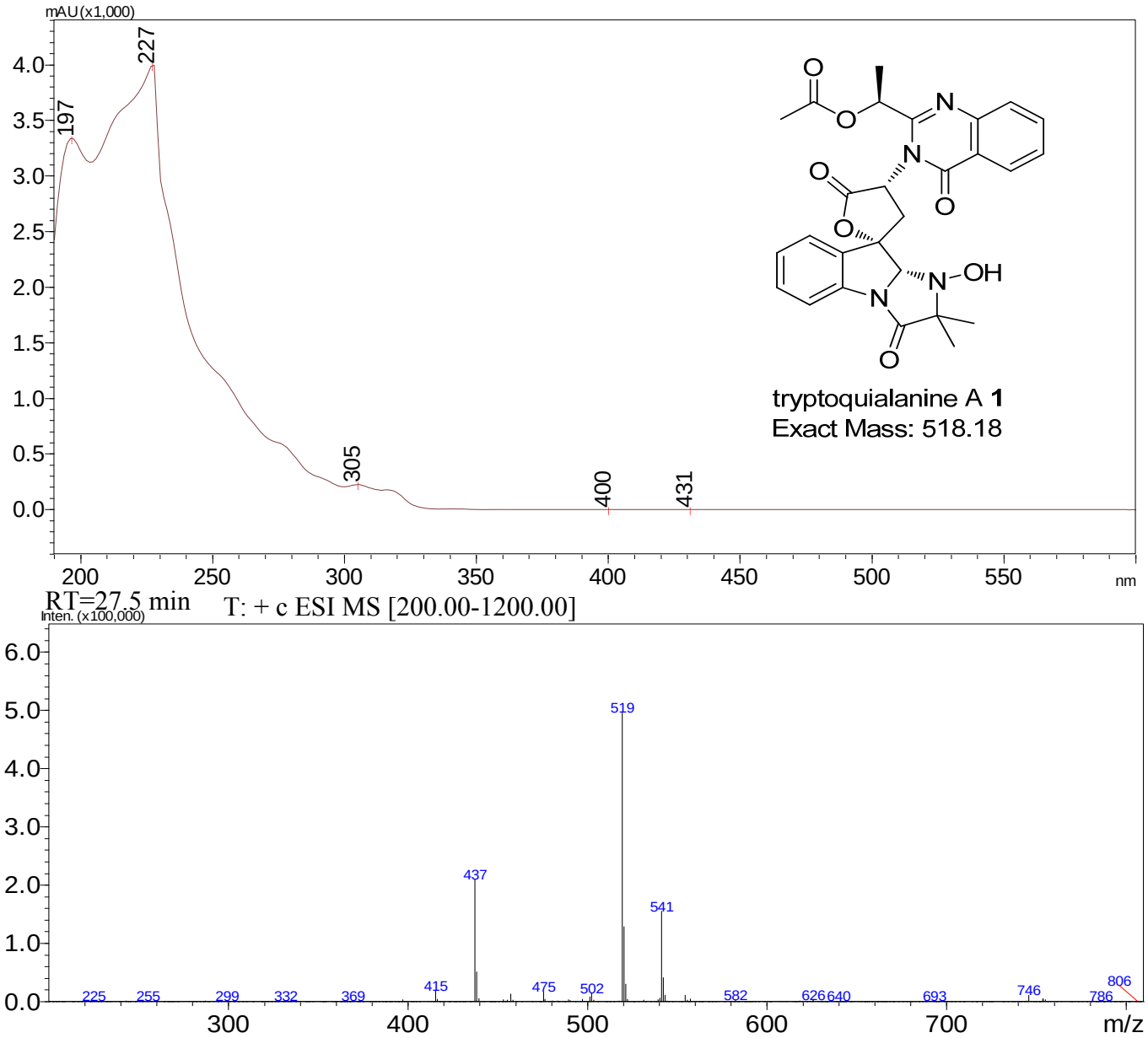


Figure S6.1 UV spectrum and MS Measured during LC-MS for **1**

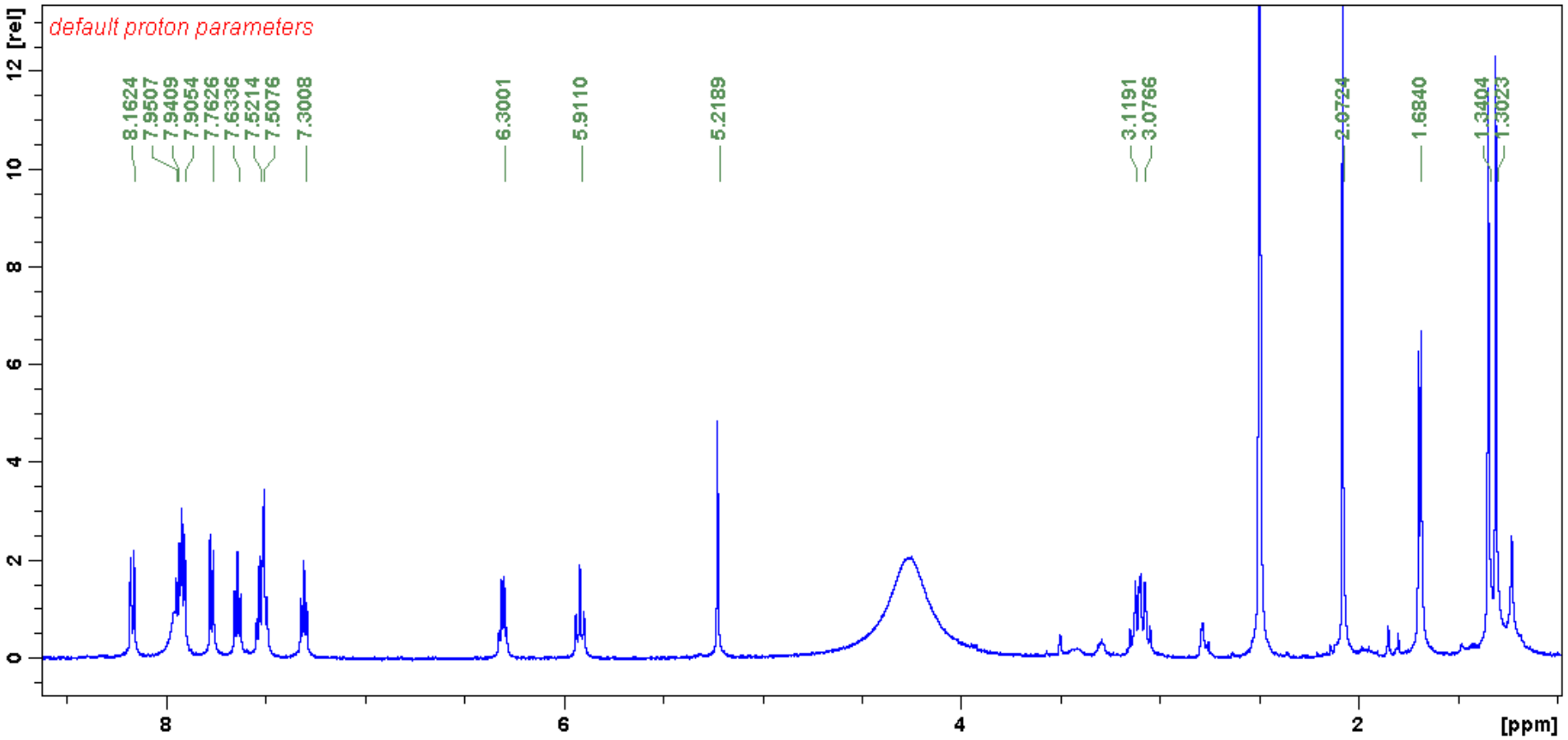


Figure S6.2 ^{1}H NMR spectrum of **1**. Measured in DMSO-d_6 at 500MHz

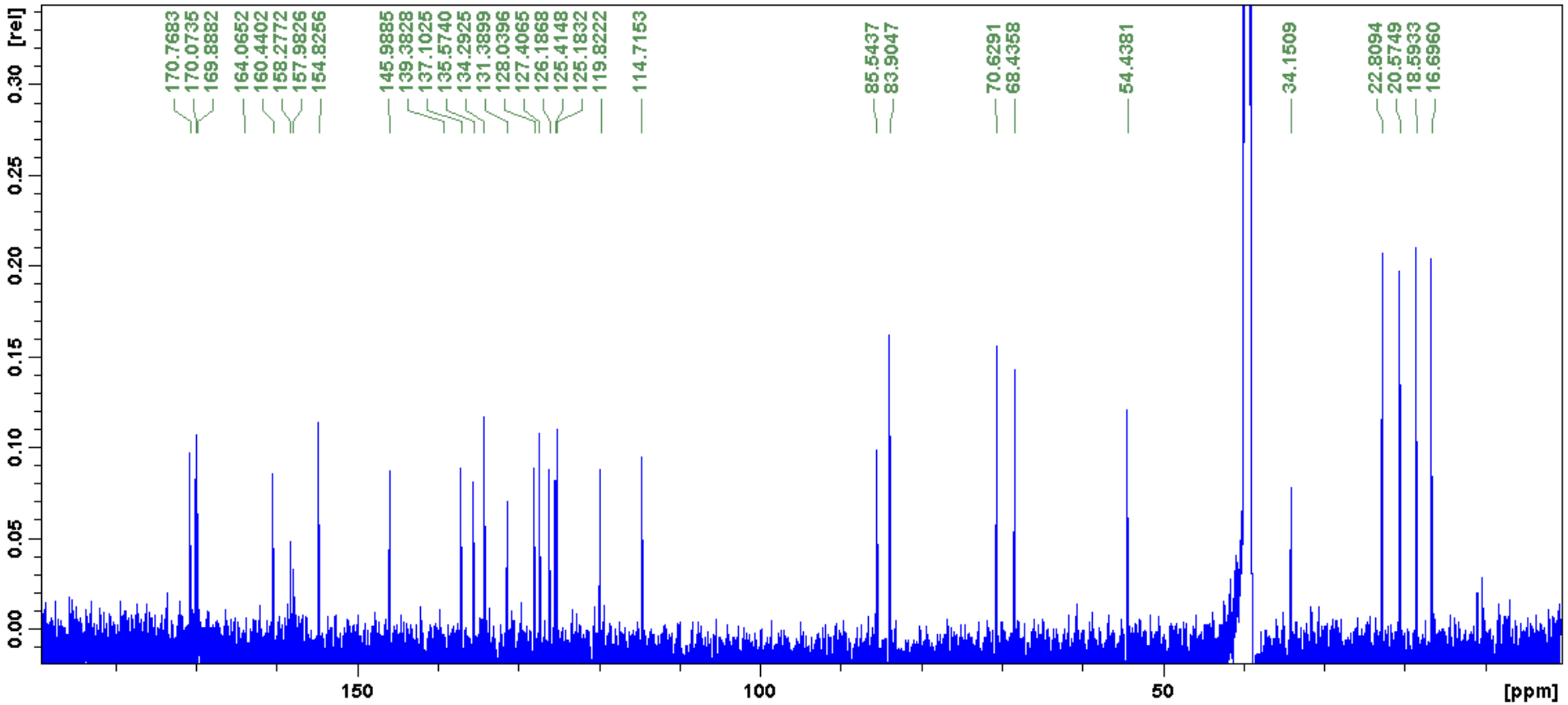


Figure S6.3 ^{13}C NMR spectrum of **1**. Measured in DMSO-d_6 at 125MHz

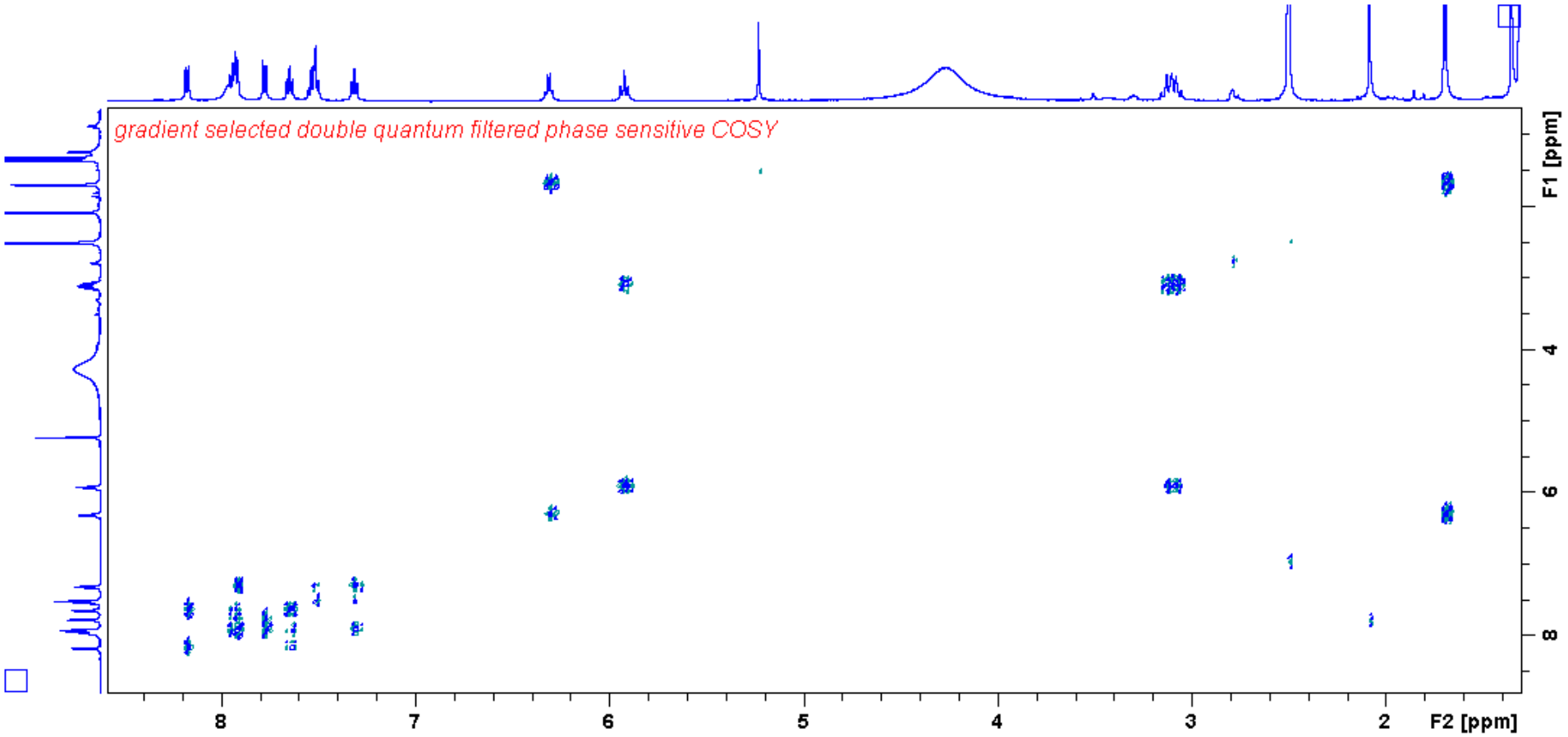


Figure S6.4 ^{1}H-^{1}H COSY spectrum of **1**.

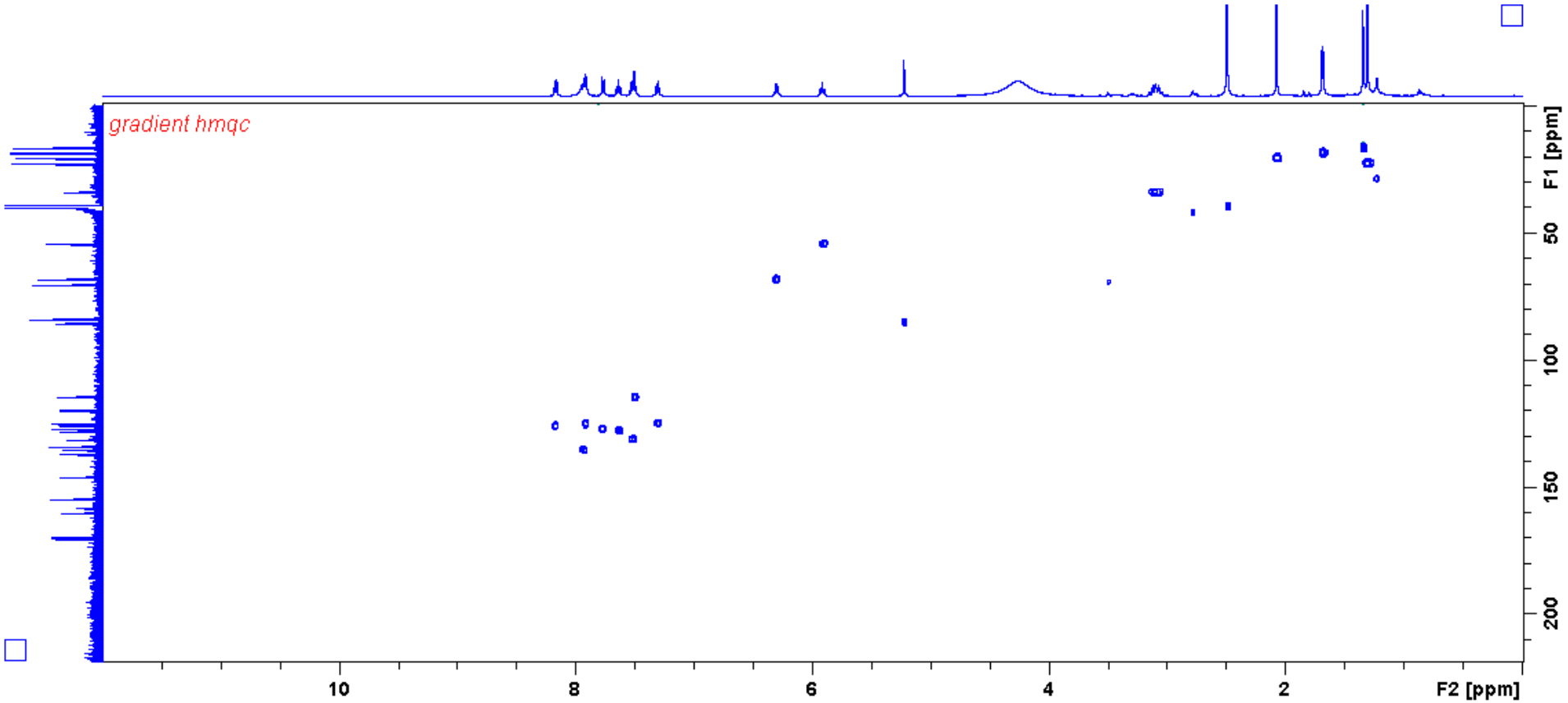


Figure S6.5 HMQC spectrum of **1**.

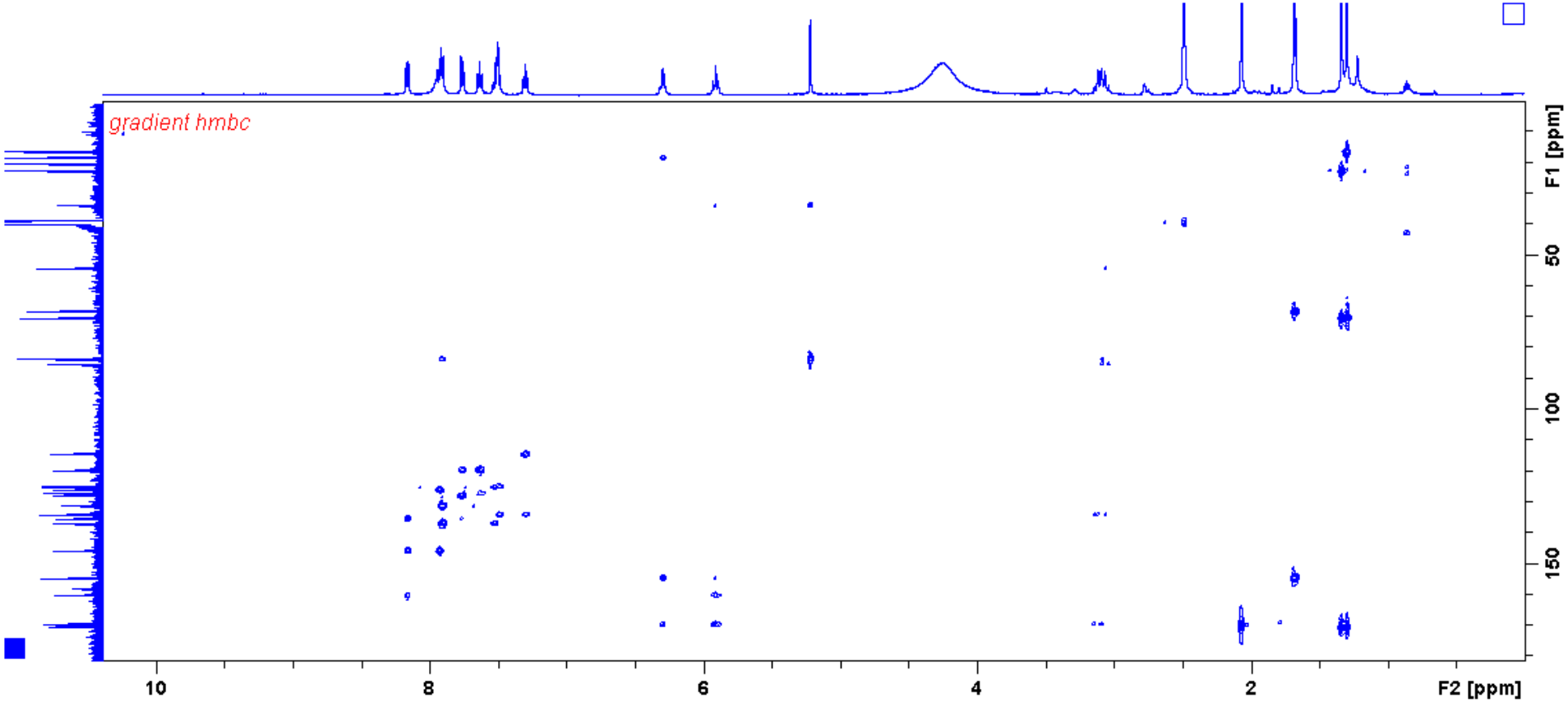
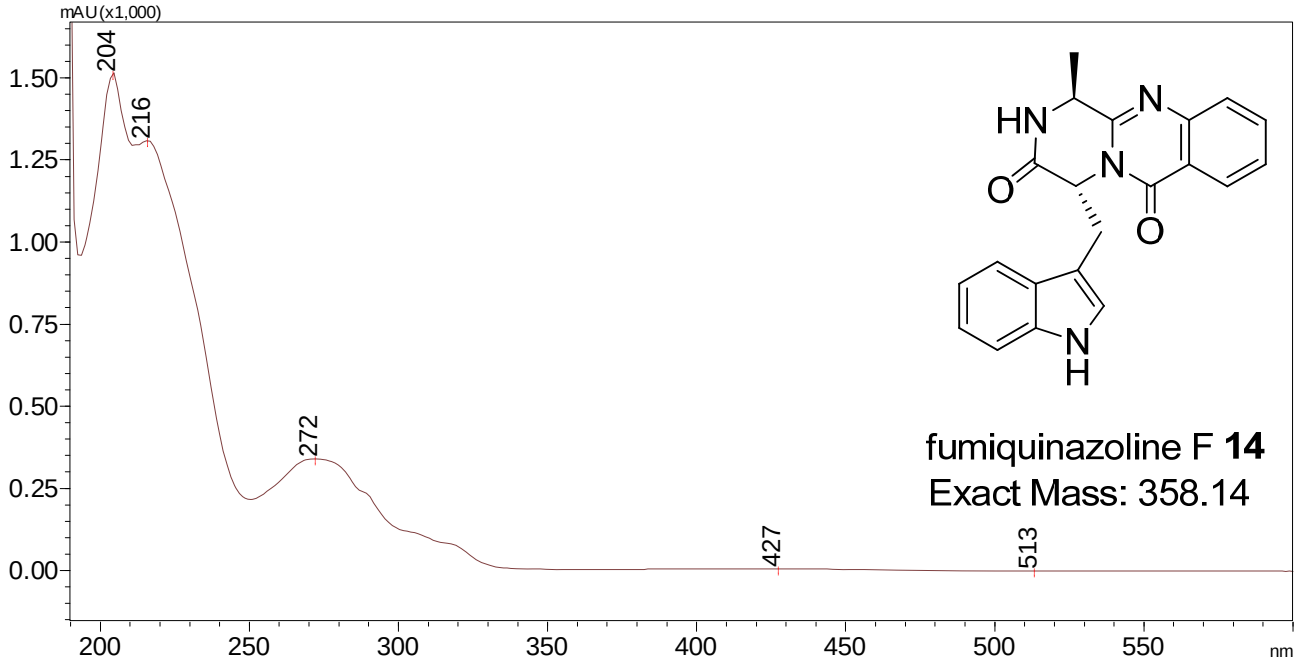


Figure S6.6 HMBC spectrum of **1**



RT=22.8 min T: + c ESI MS [200.00-1200.00]

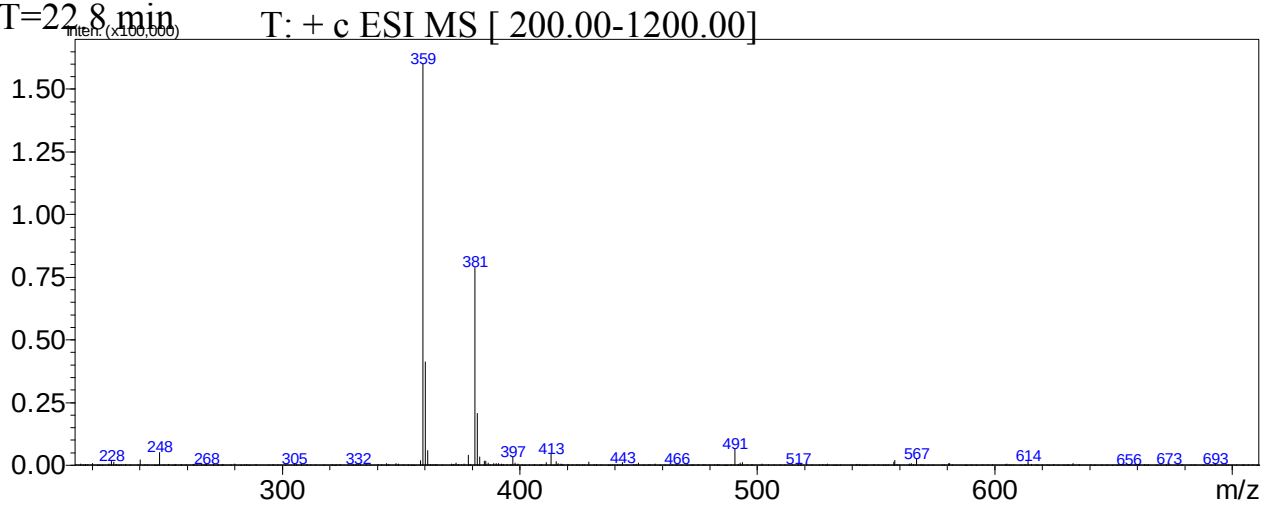


Figure S7.1 UV spectrum and MS Measured during LC-MS for **14**

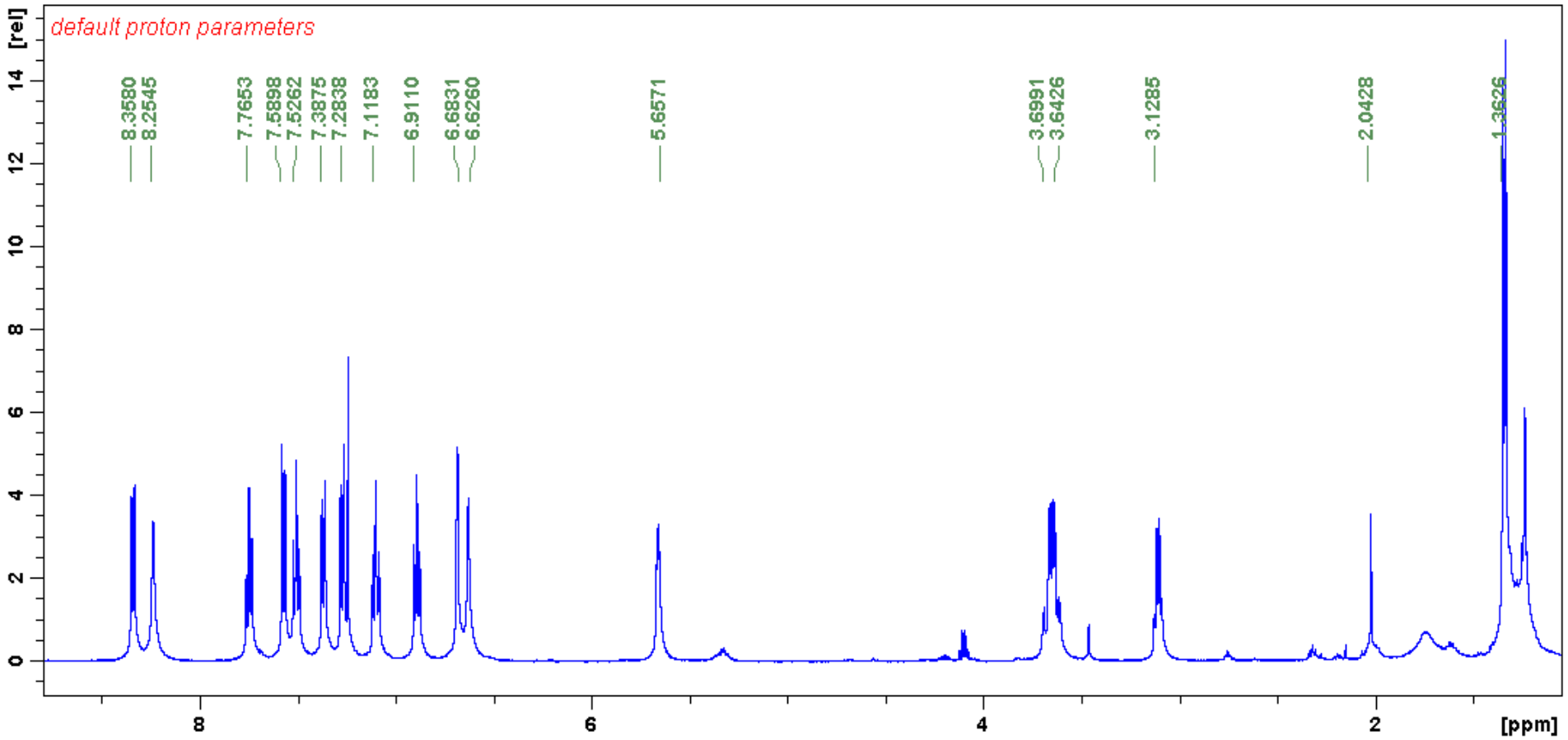


Figure S7.2 ^{1}H NMR spectrum of **14**. Measured in $CDCl_3$ at 500MHz

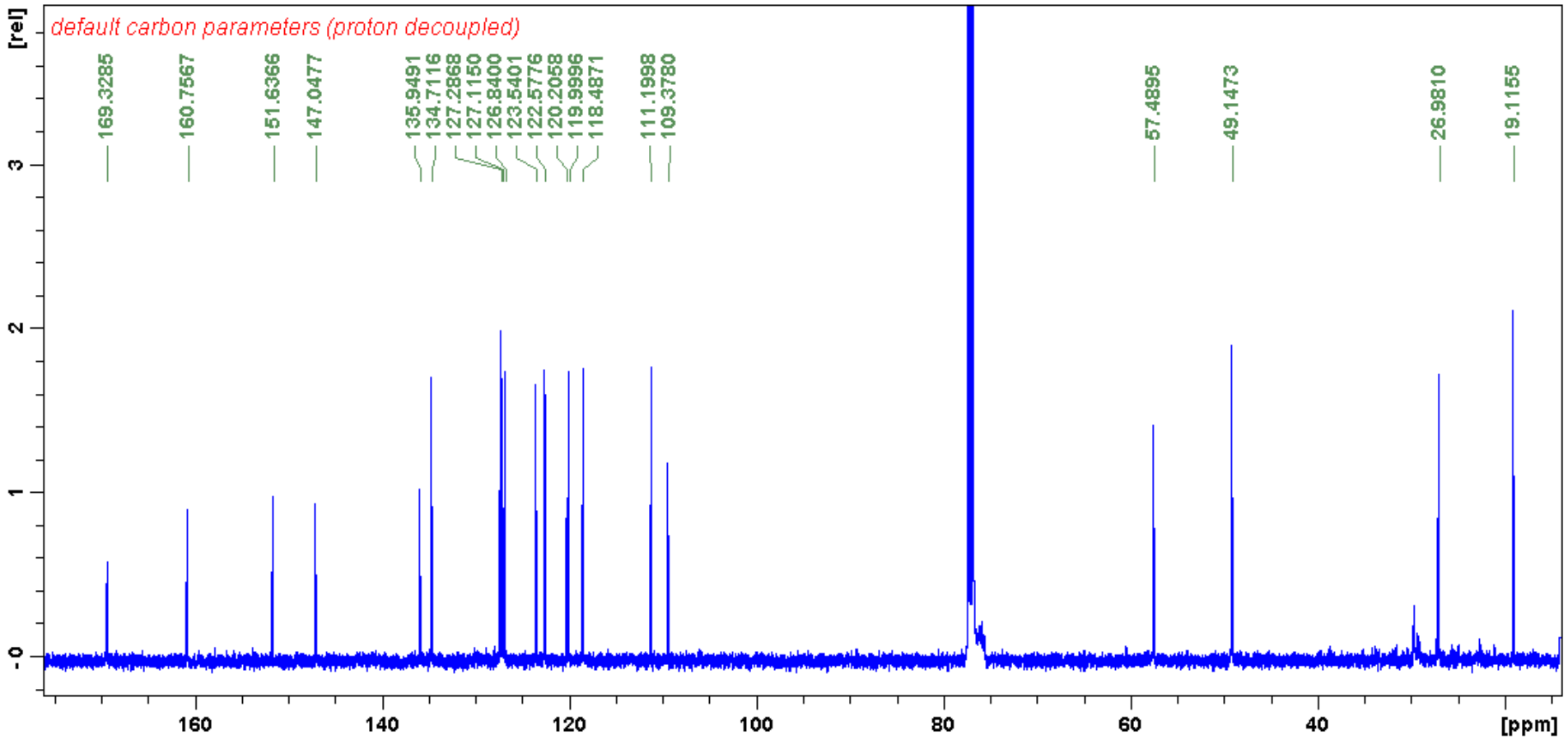


Figure S7.3 ^{13}C NMR spectrum of **14**. Measured in $CDCl_3$ at 125MHz

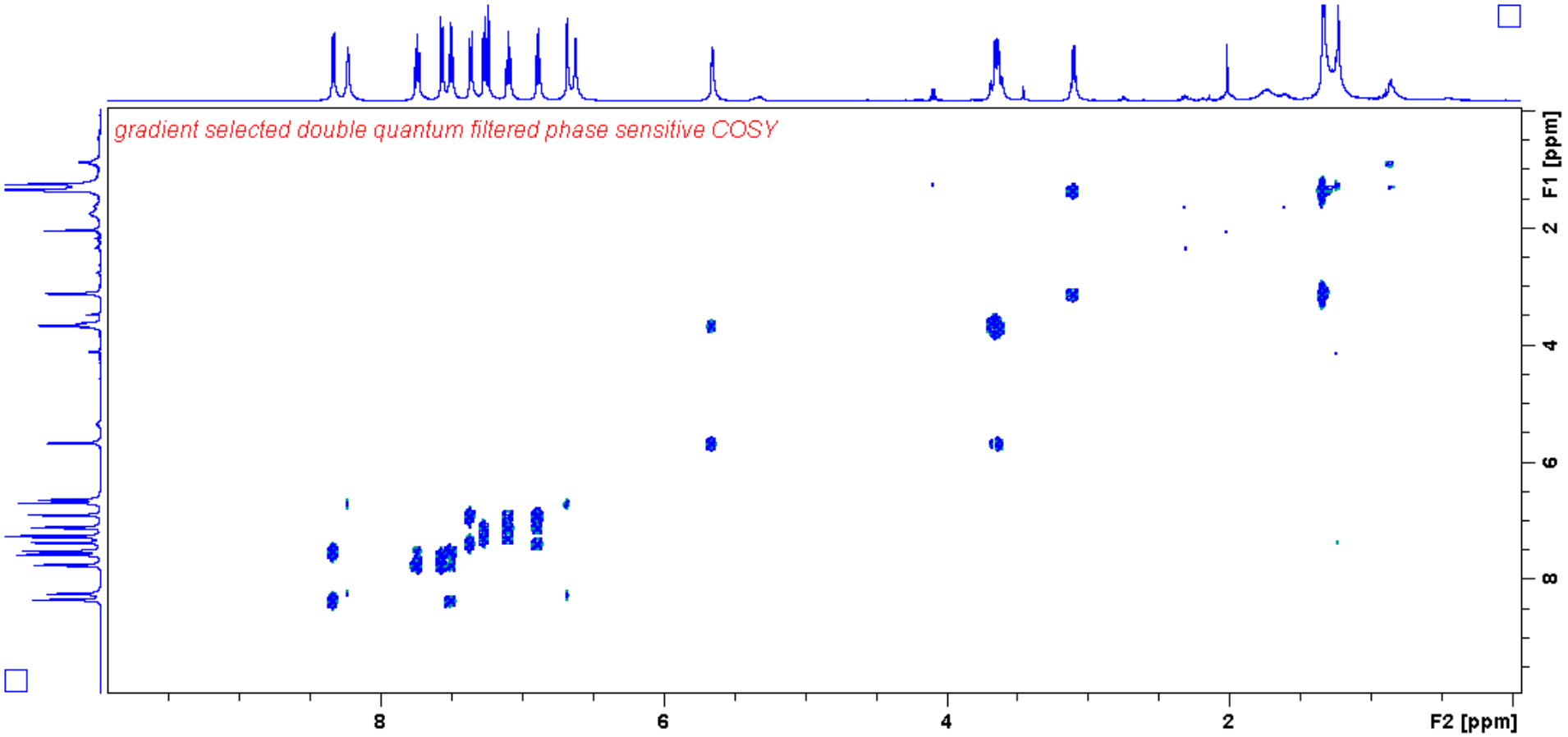


Figure S7.4 ^{1}H-^{1}H COSY NMR spectrum of **14**.

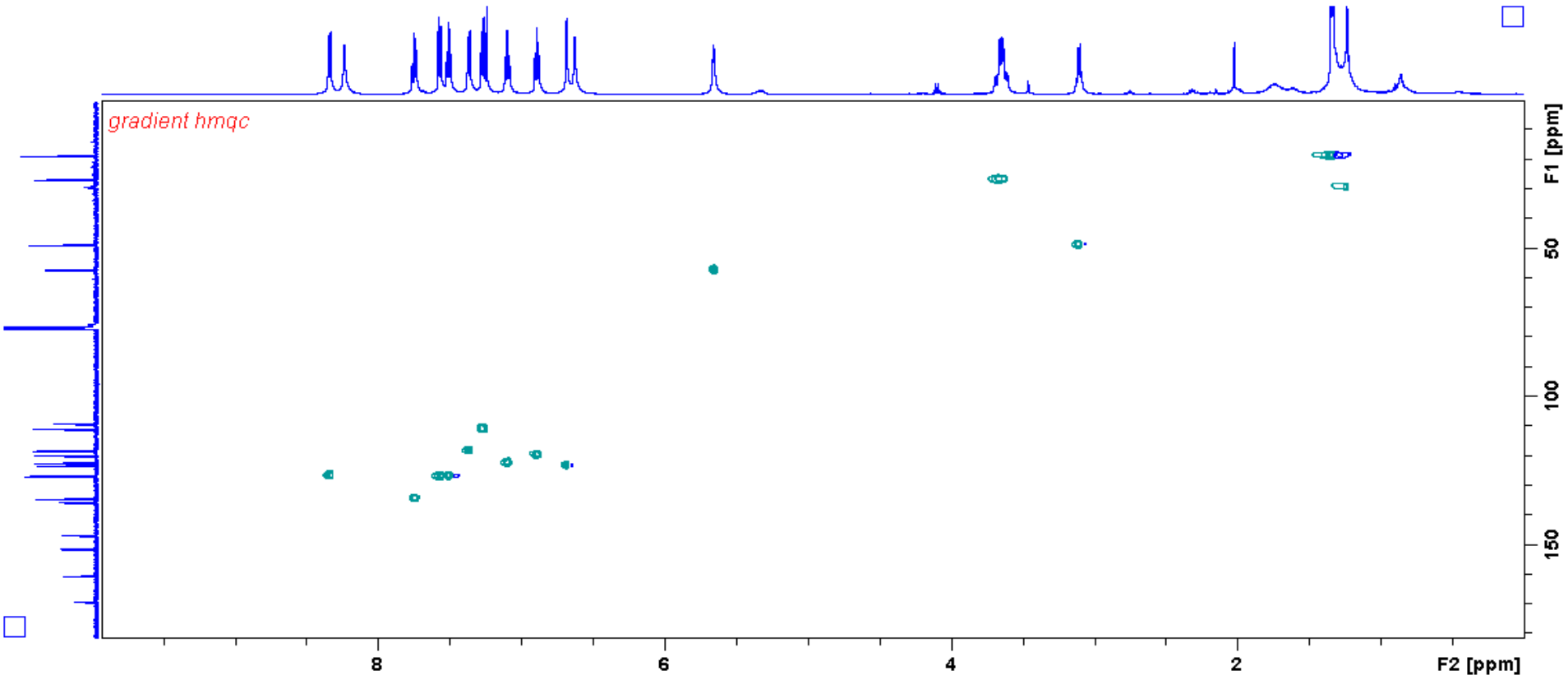


Figure S7.5 ^{1}H-^{13}C HMQC NMR spectrum of **14**.

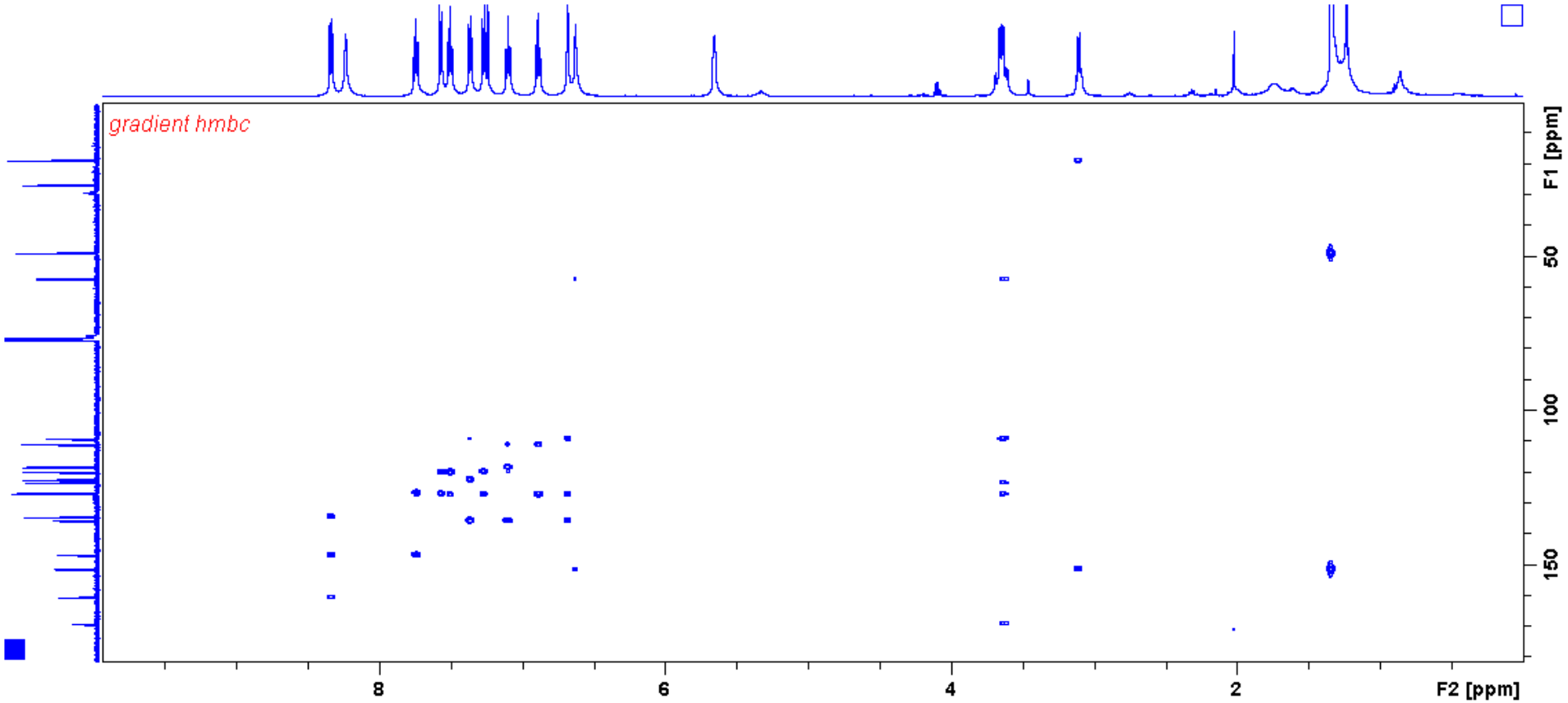
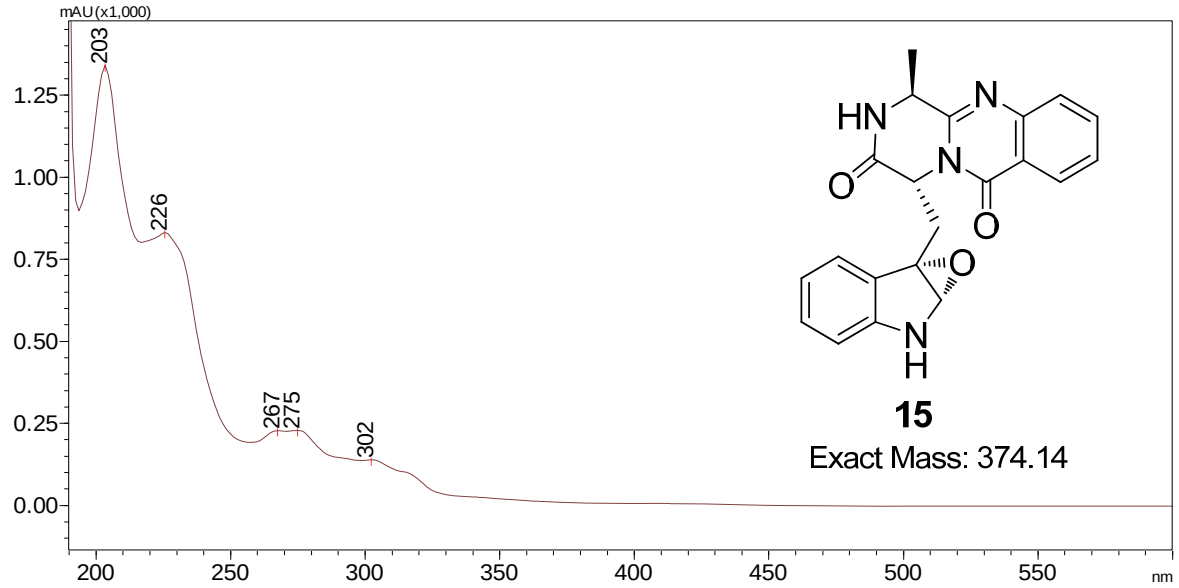


Figure S7.6 ^{1}H-^{13}C HMBC NMR spectrum of **14**.



RT=19.6 min T: + c ESI MS [200.00-1200.00]

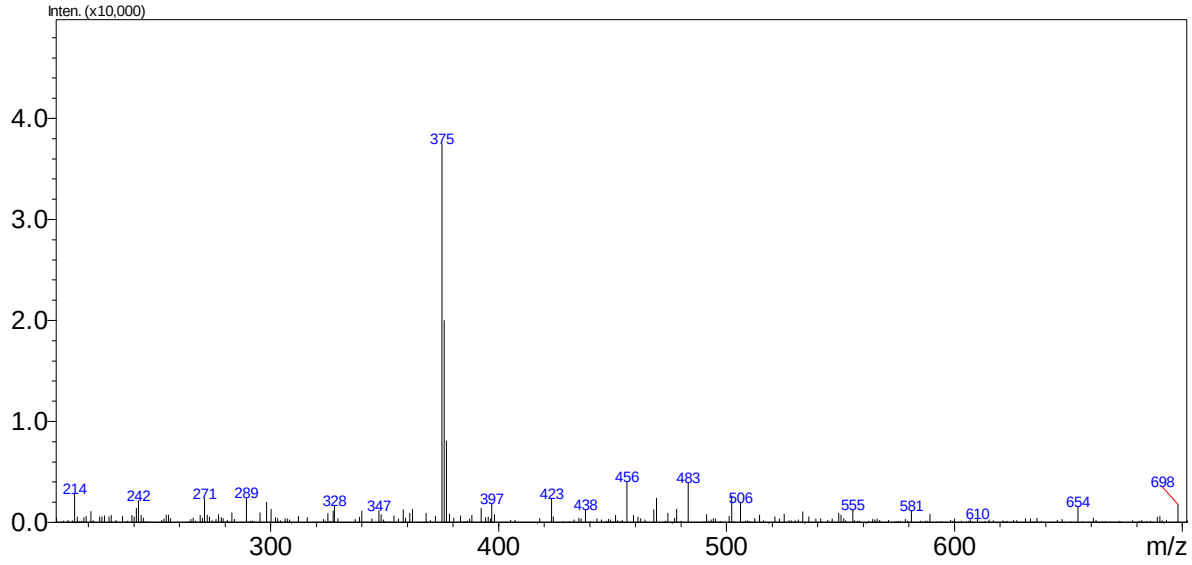


Figure S8 UV spectrum and MS Measured during LC-MS for **15**

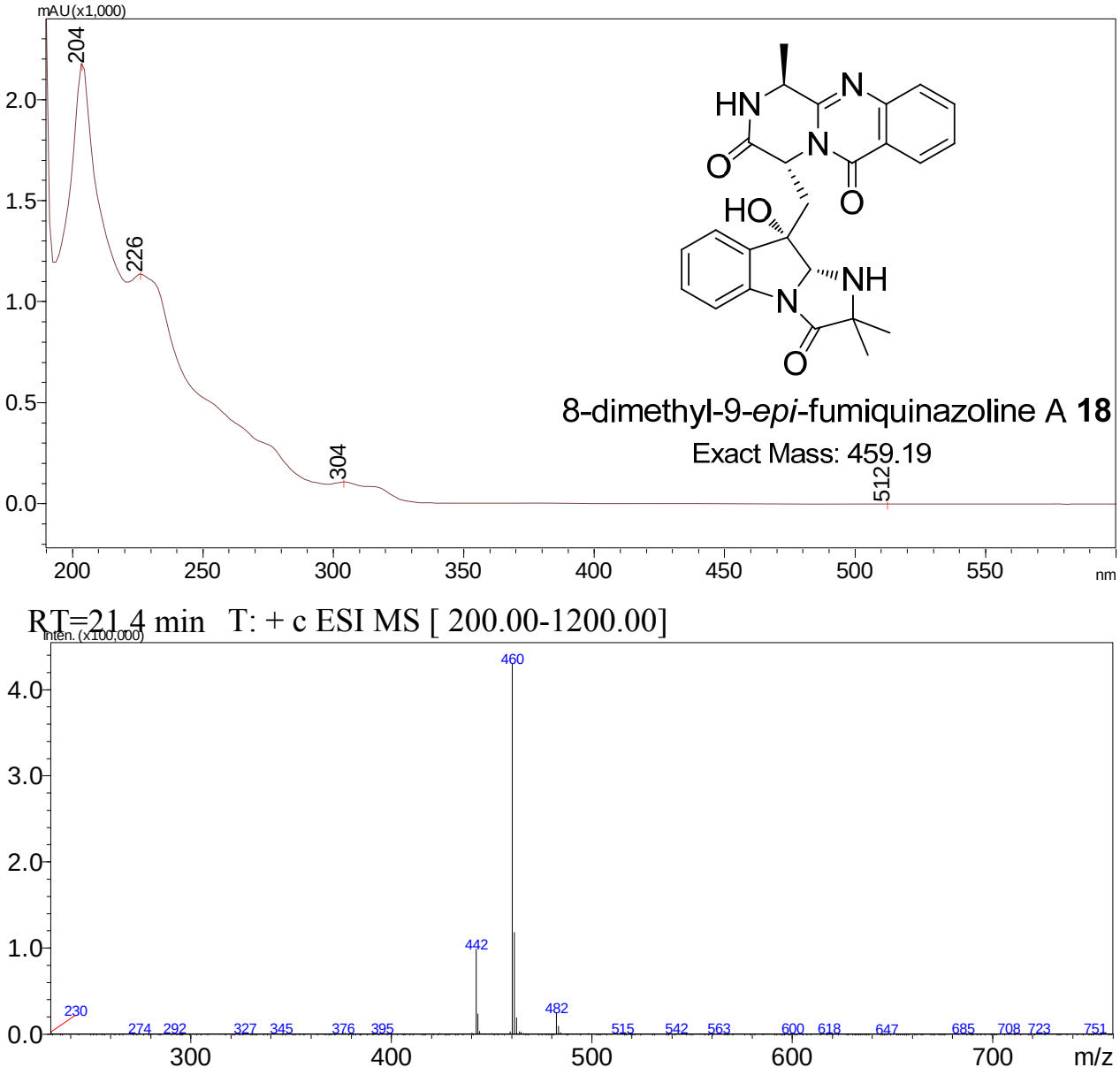


Figure S9.1 UV spectrum and MS Measured during LC-MS for **18**

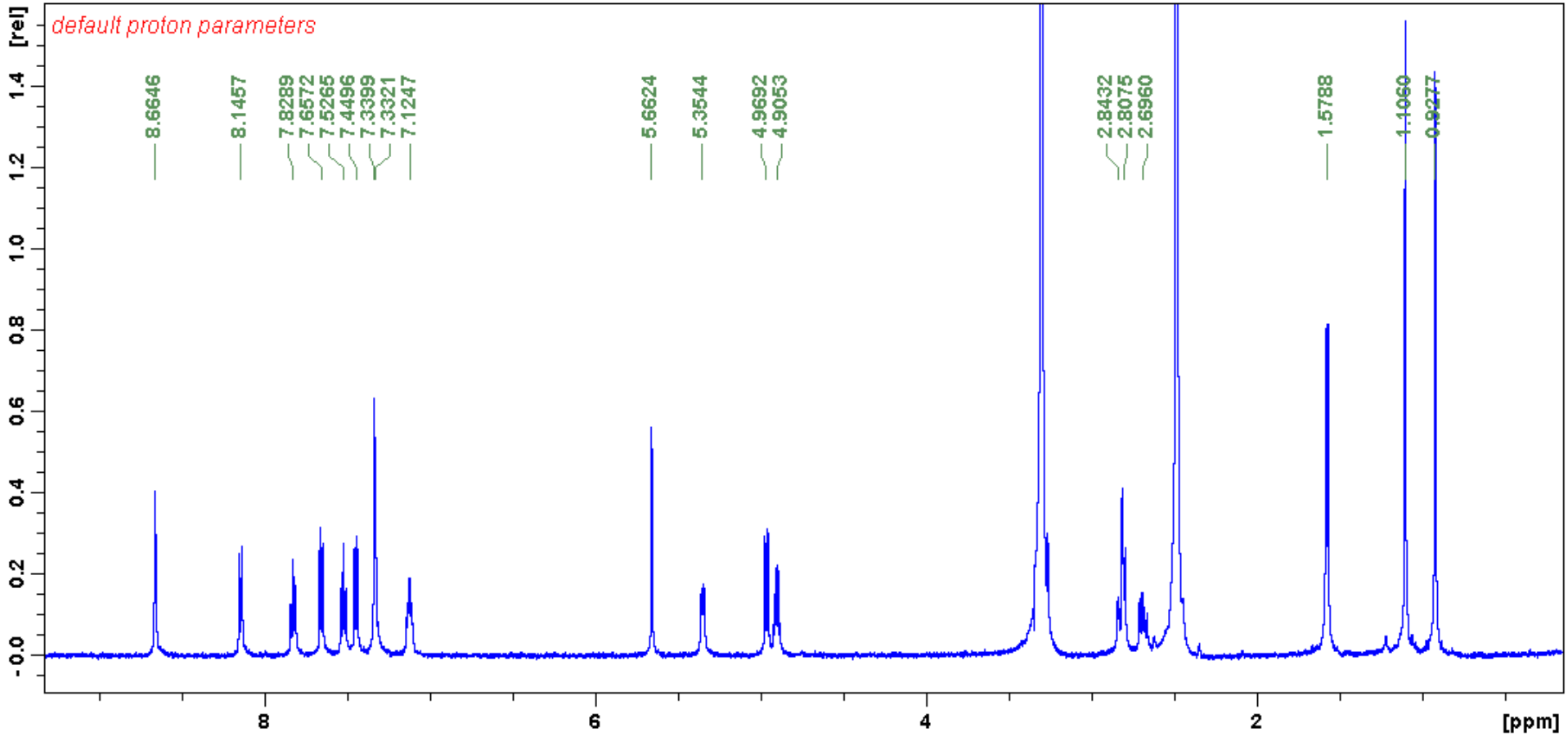


Figure S9.2 ^{1}H NMR spectrum of **18**. Measured in DMSO-d_6 at 500MHz

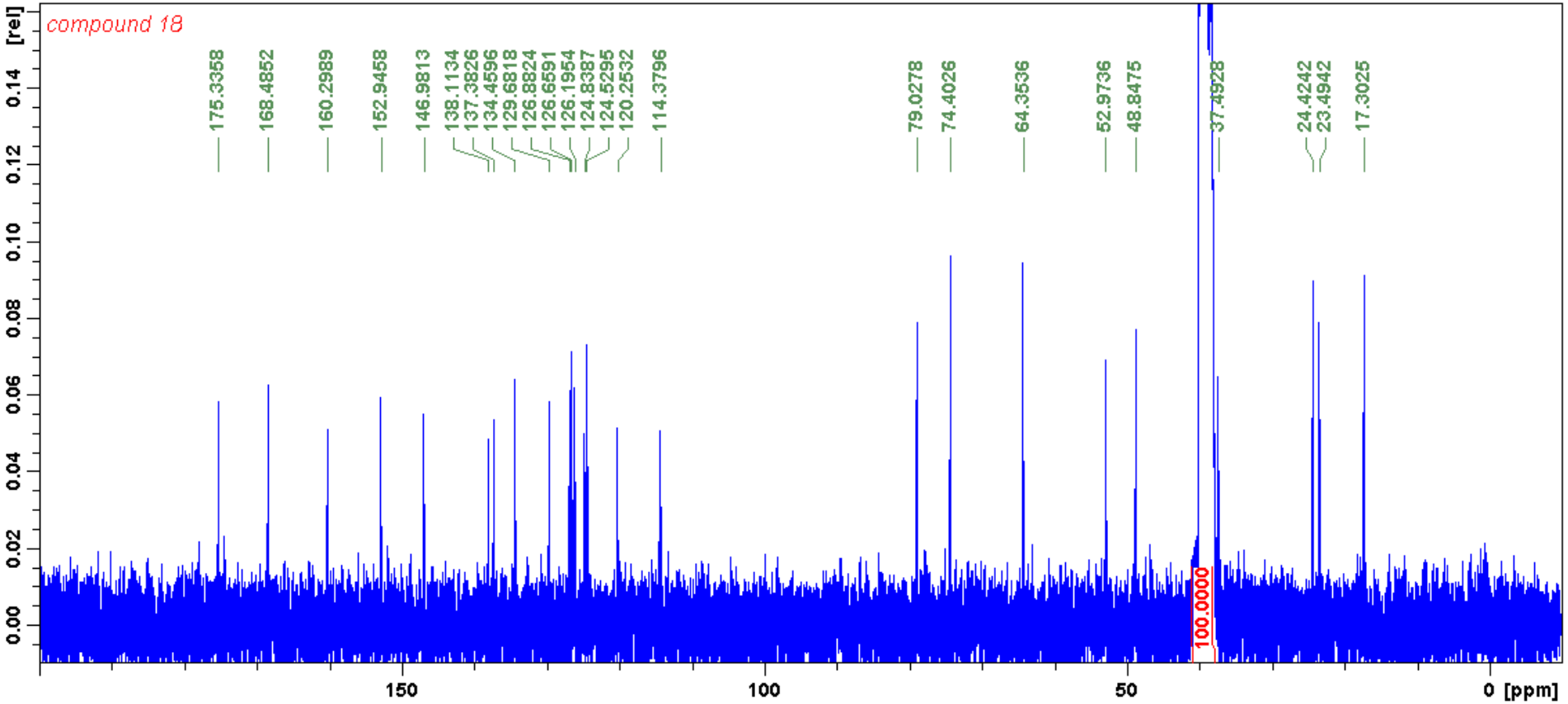


Figure S9.3 ^{13}C NMR spectrum of **18**. Measured in DMSO-d_6 at 125MHz

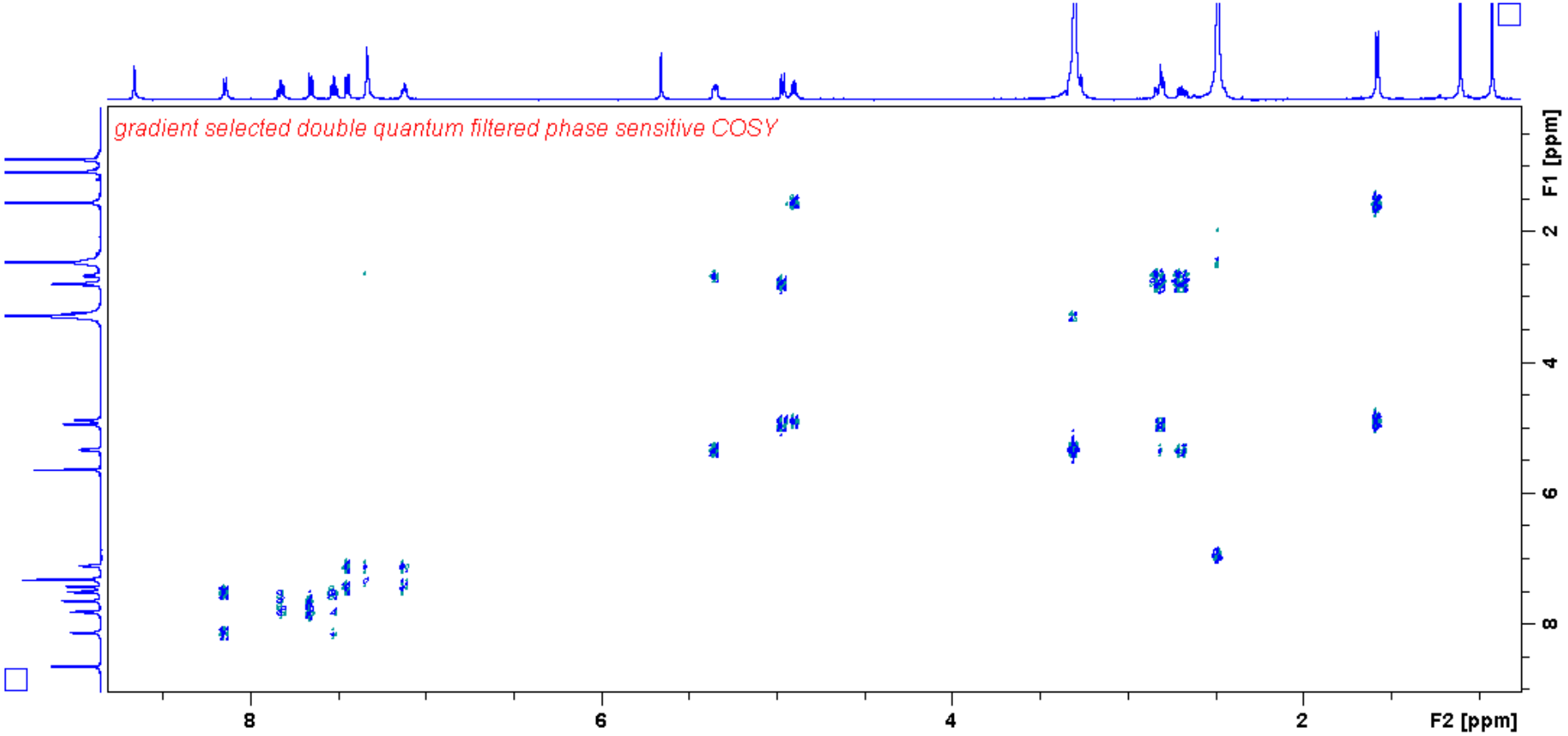


Figure S9.4 ^{1}H-^{1}H COSY NMR spectrum of **18**.

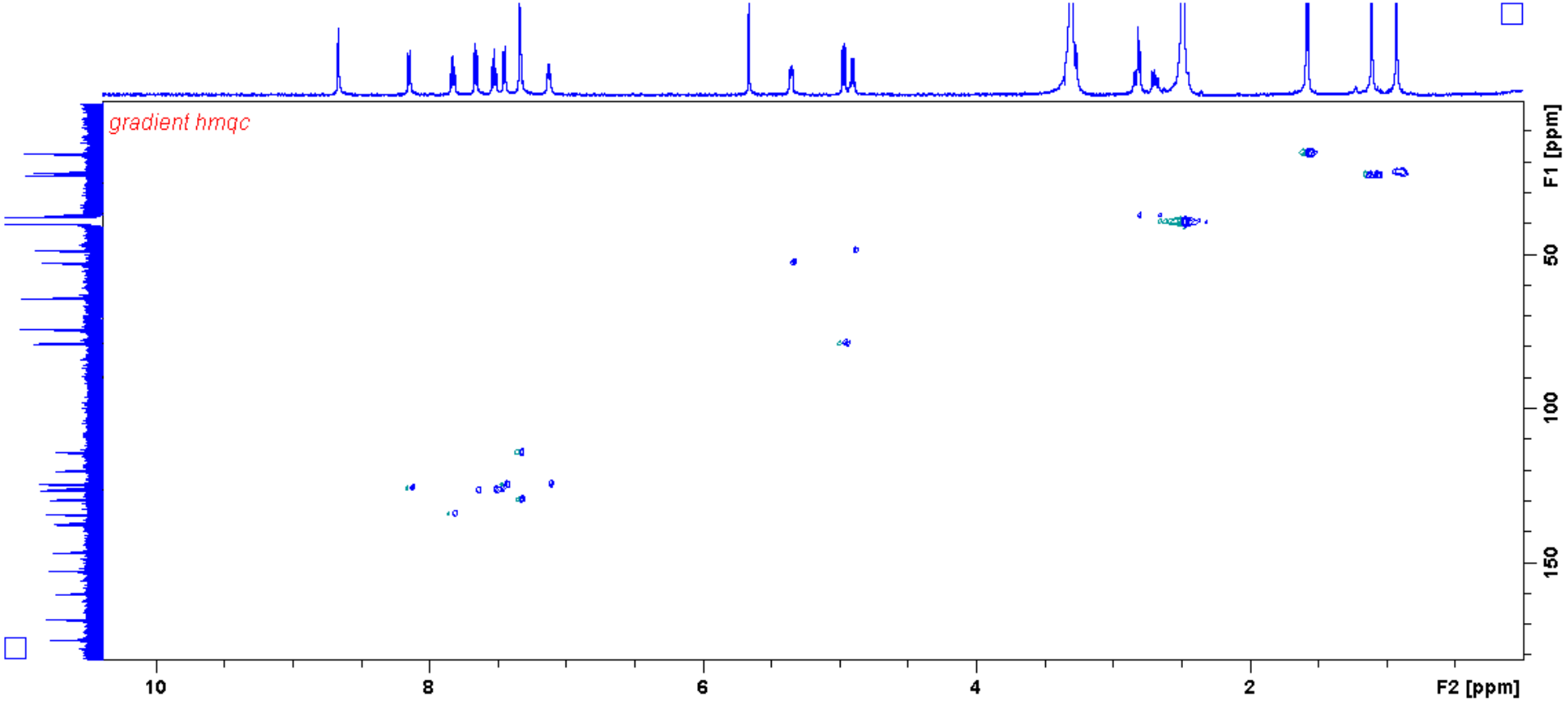


Figure S9.5 ^{1}H-^{13}C HMQC NMR spectrum of **18**.

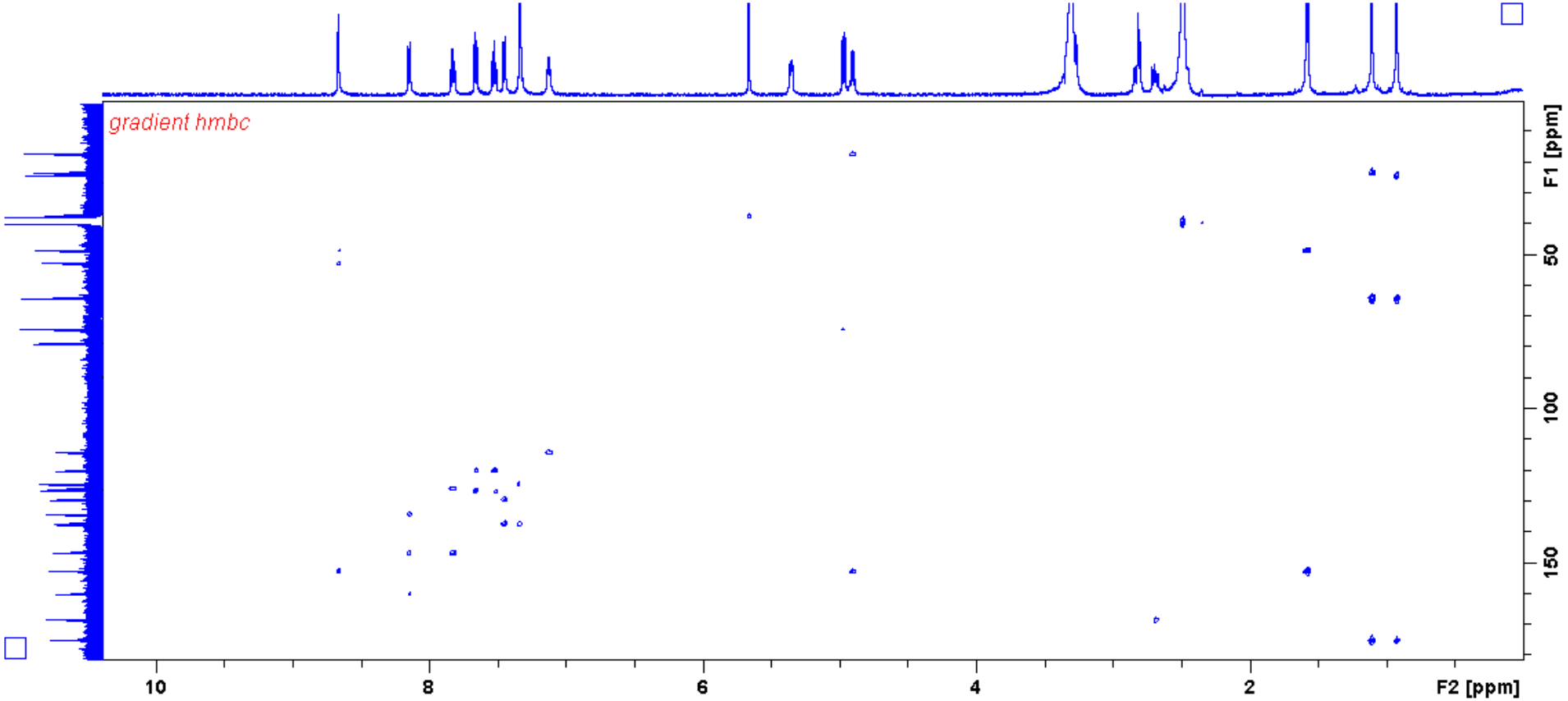
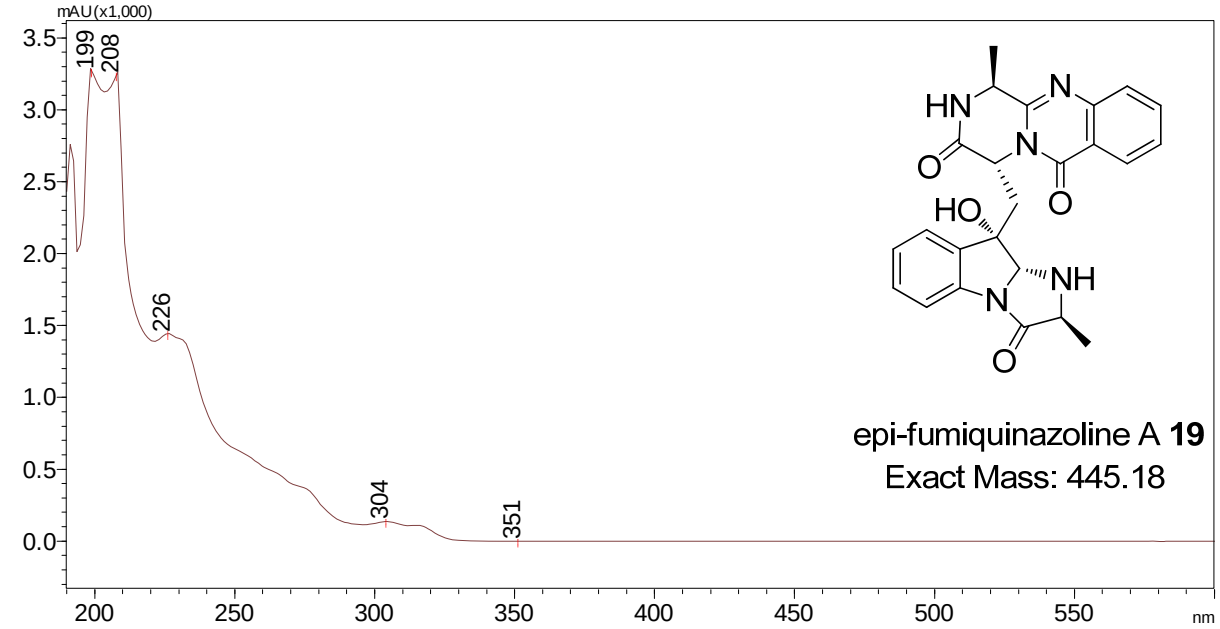


Figure S9.6 ^{1}H-^{13}C HMBC NMR spectrum of **18**.



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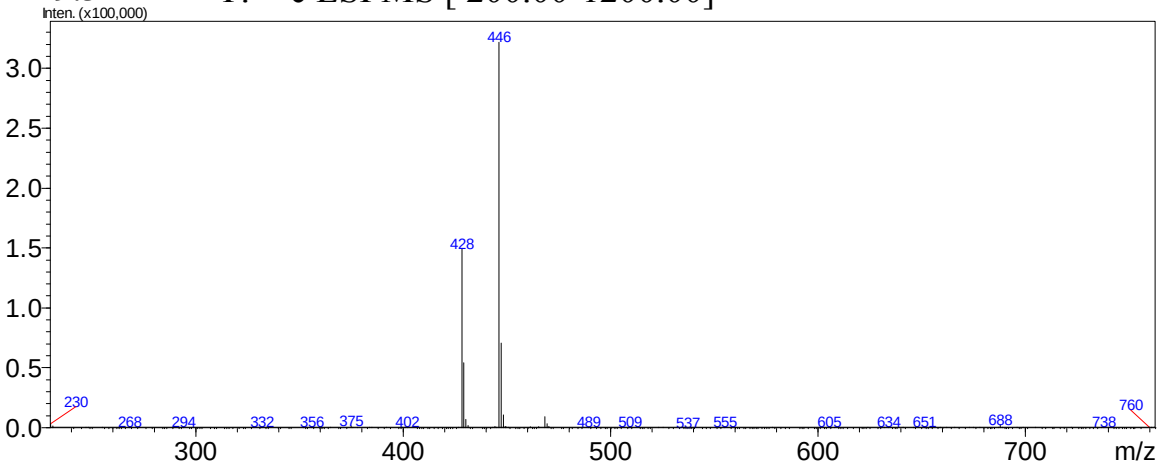


Figure S10.1 UV spectrum and MS Measured during LC-MS for **19**

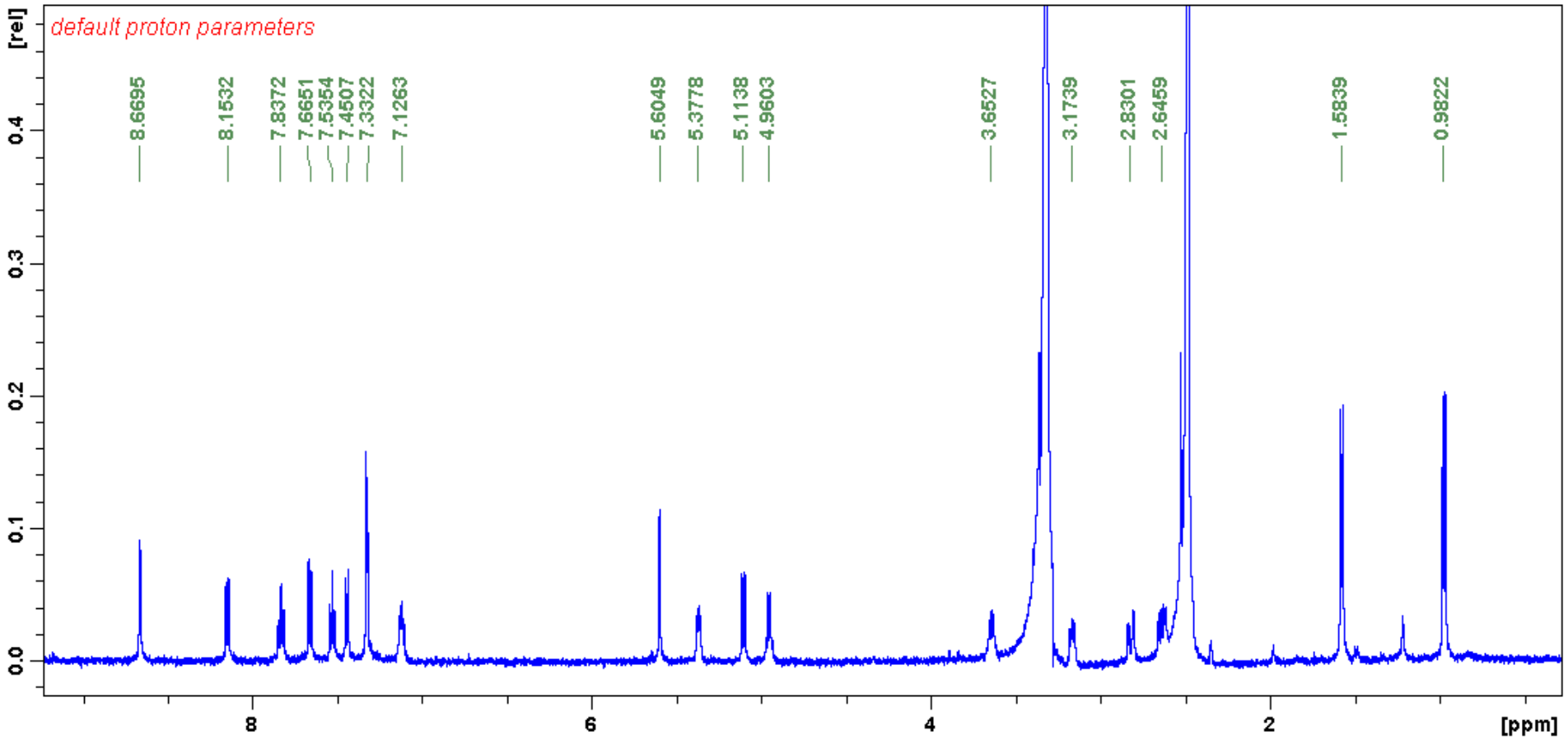


Figure S10.2 ^{1}H NMR spectrum of **19**. Measured in DMSO-d_6 at 500MHz

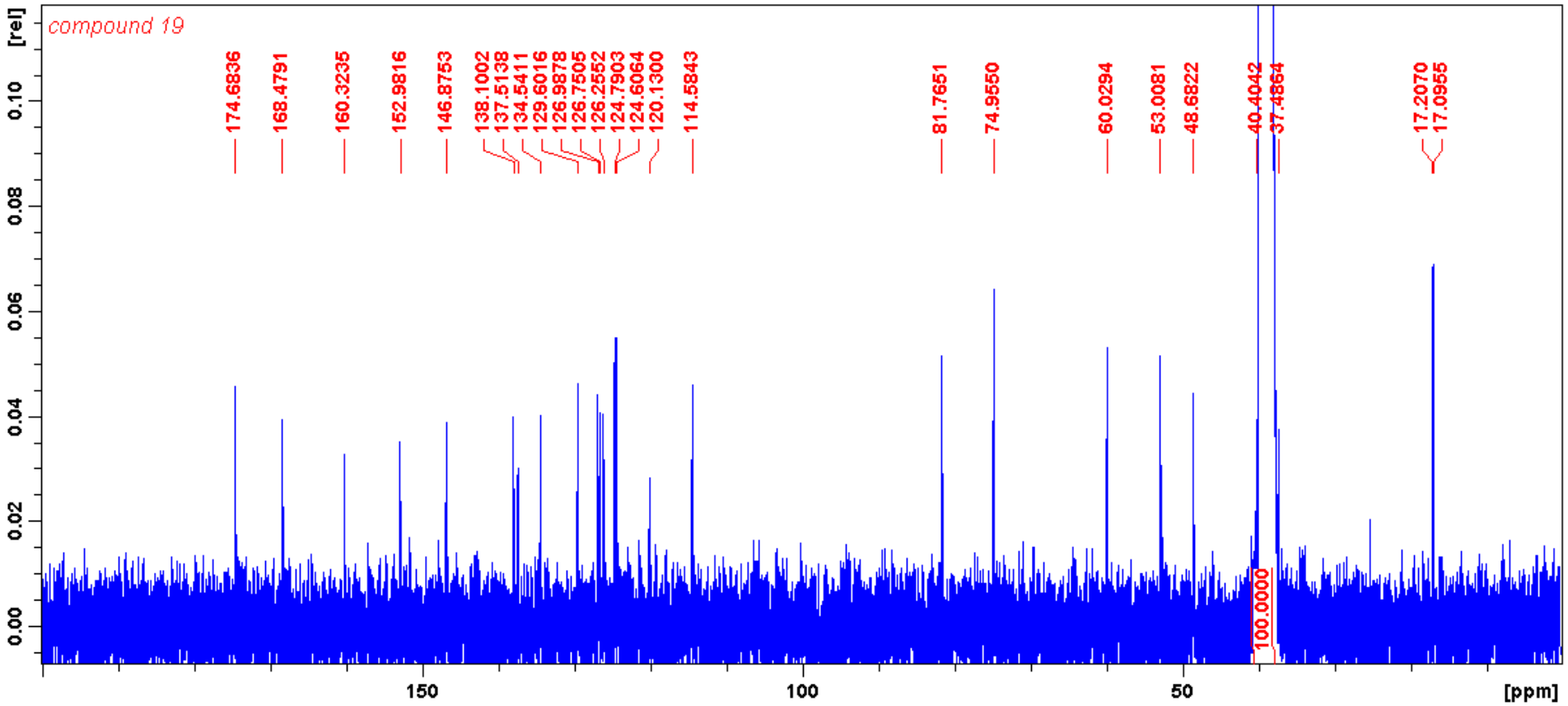


Figure S10.3 ^{13}C NMR spectrum of **19**. Measured in DMSO-d_6 at 125MHz

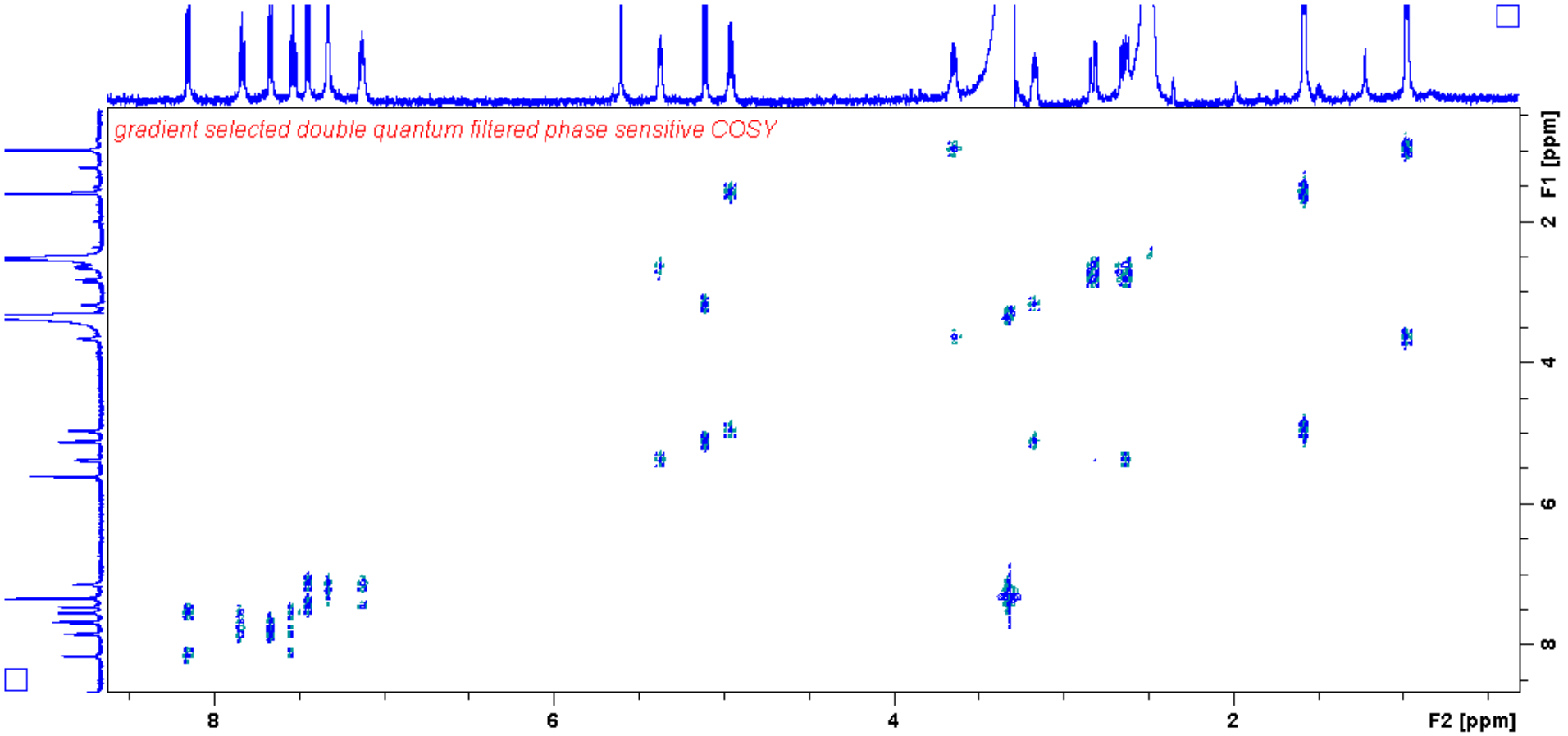


Figure S10.4 ^{1}H-^{1}H COSY NMR spectrum of **19**.

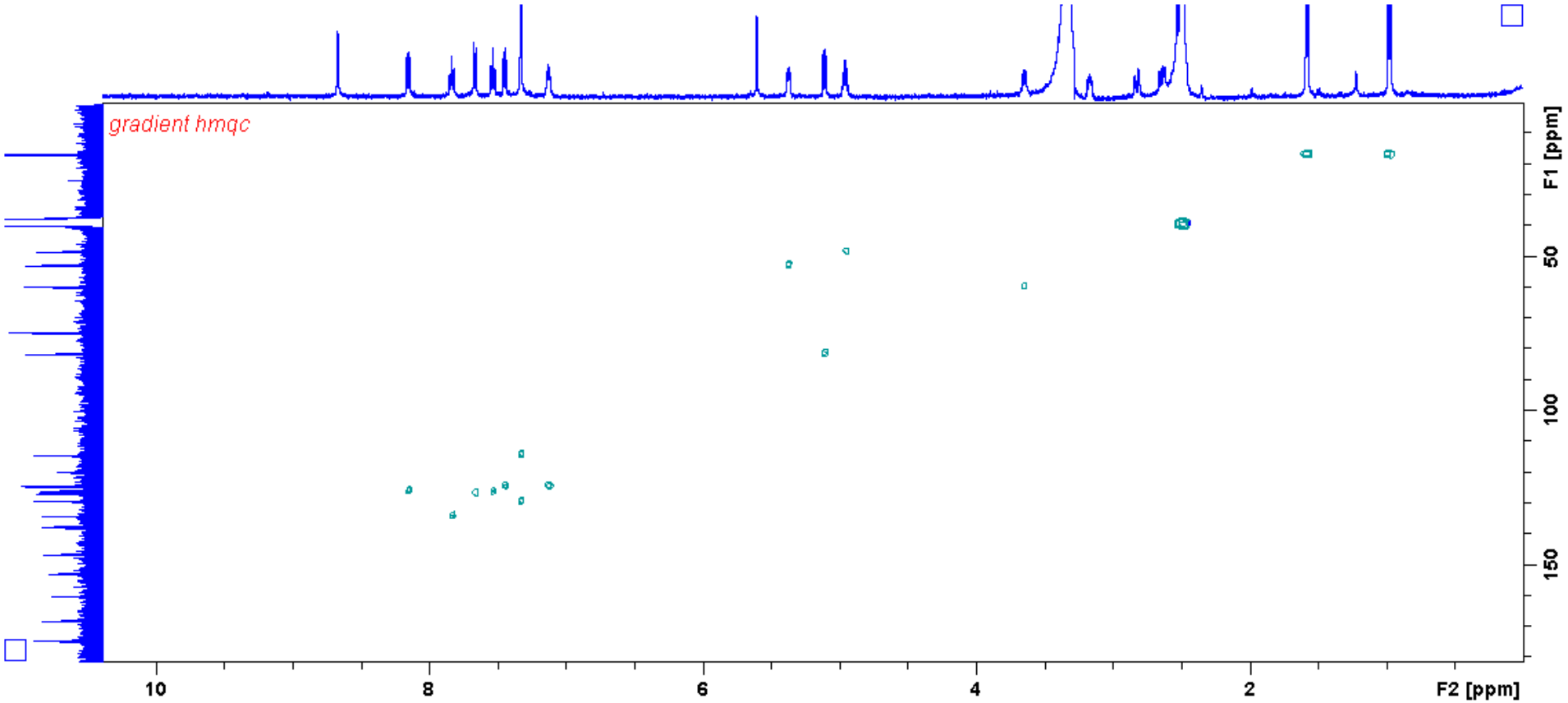


Figure S10.5 ^{1}H-^{13}C HMQC NMR spectrum of **19**.

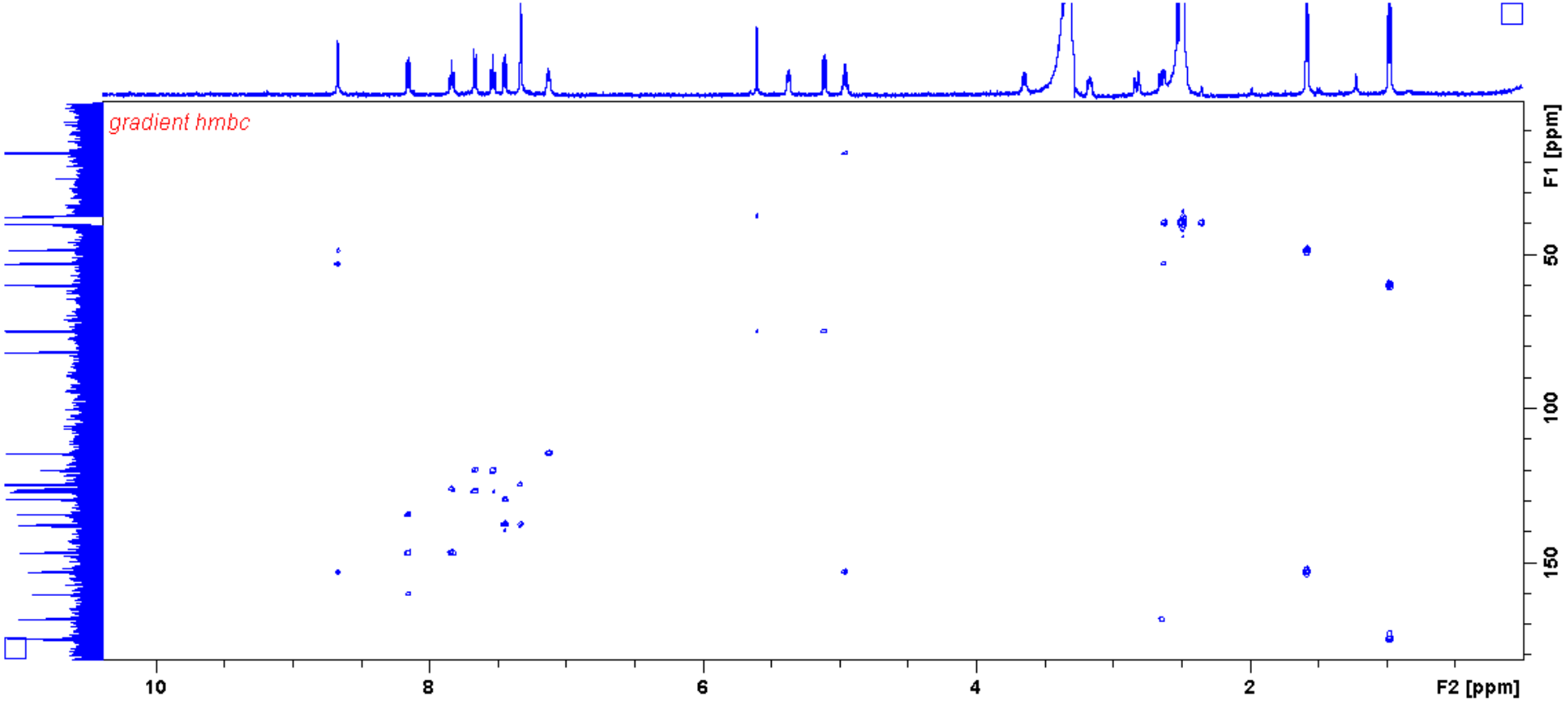
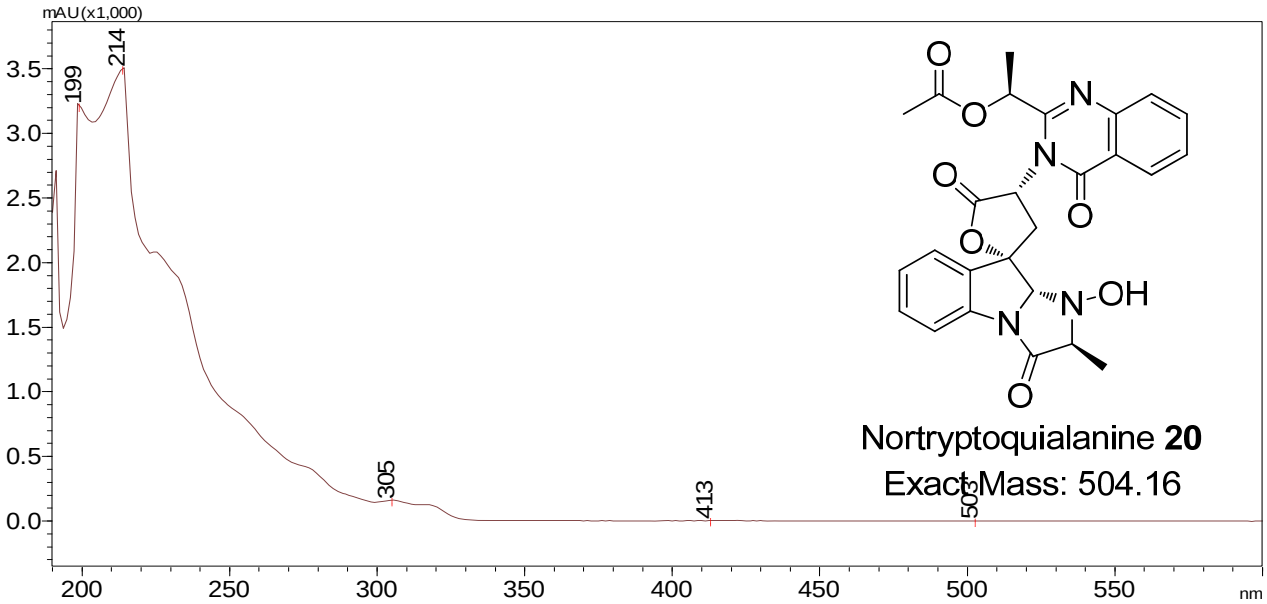


Figure S10.6 ^{1}H-^{13}C HMBC NMR spectrum of **19**.



RT=26.1 min T: + c ESI MS [200.00-1200.00]

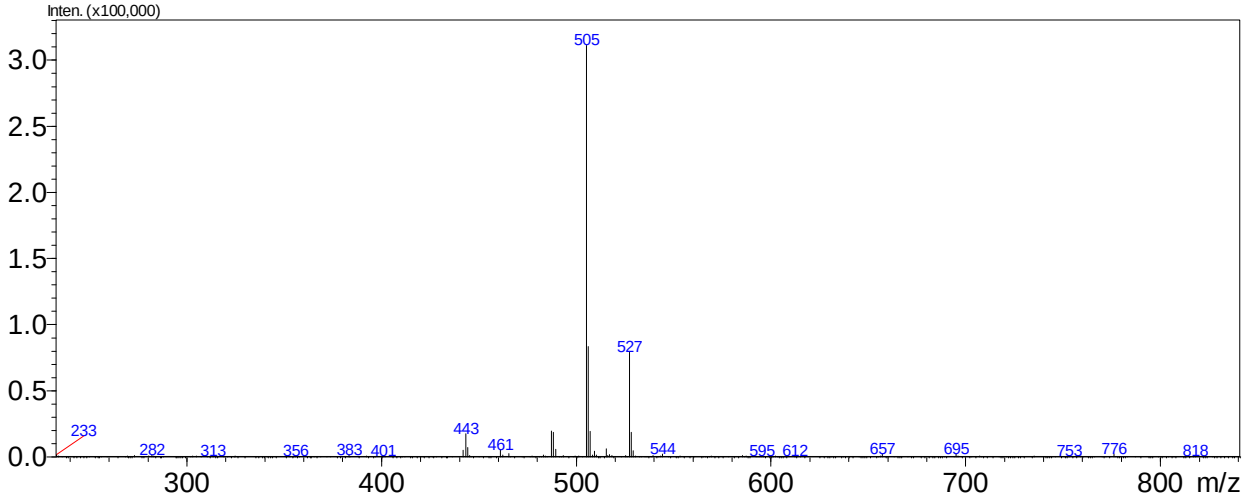


Figure S11.1 UV spectrum and MS Measured during LC-MS for **20**

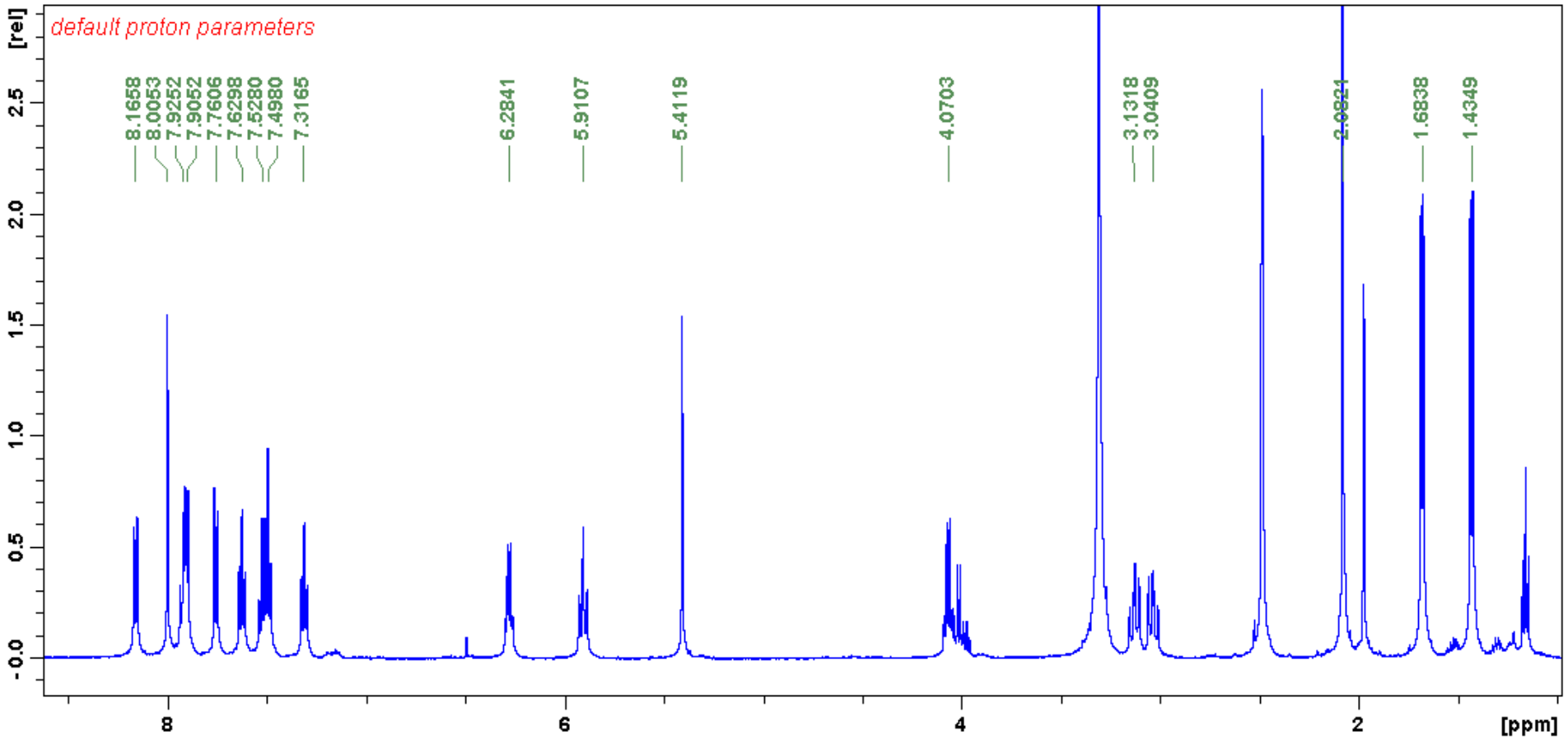


Figure S11.2 ^{1}H NMR spectrum of **20**. Measured in DMSO-d_6 at 500MHz

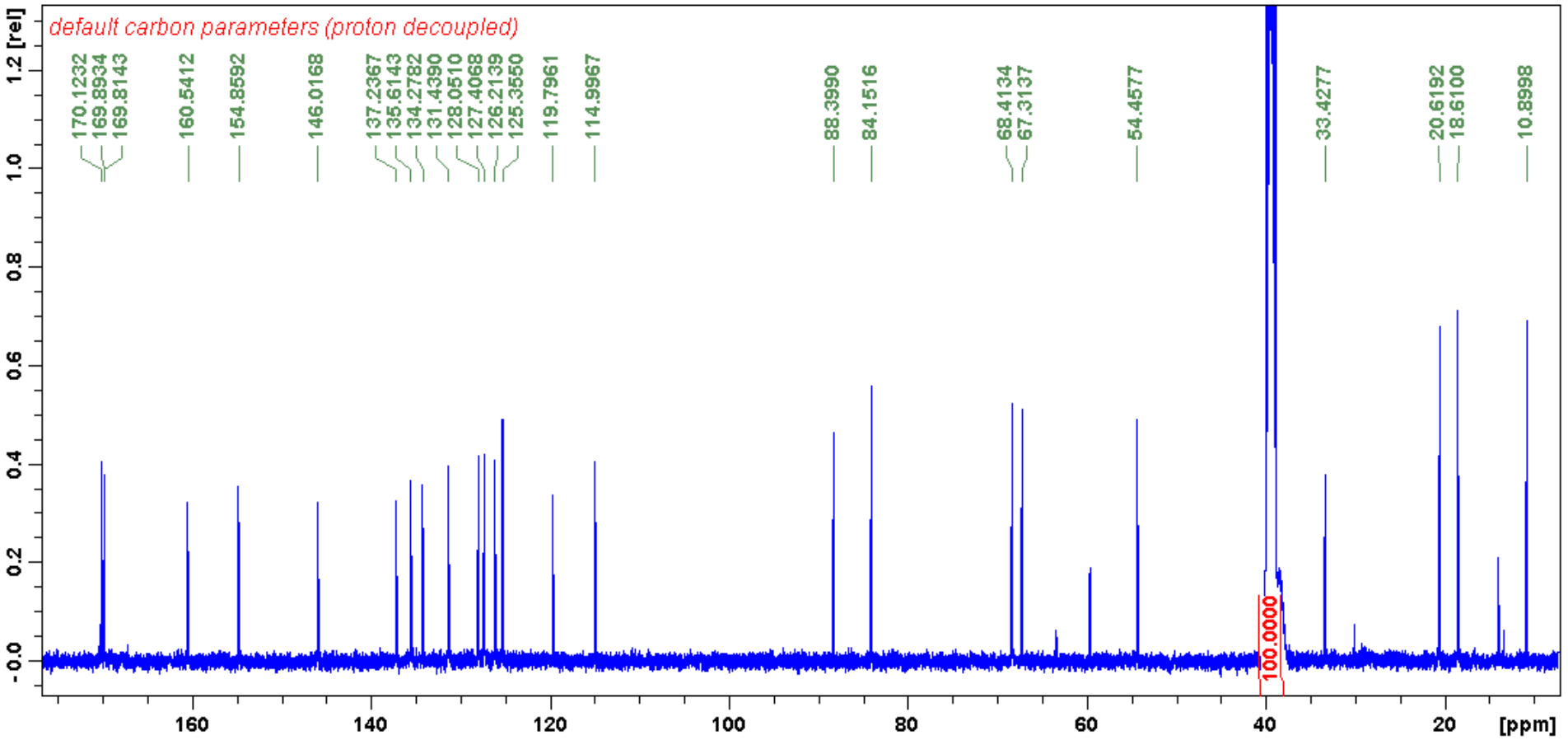


Figure S11.3 ^{13}C NMR spectrum of **20**. Measured in DMSO-d_6 at 125MHz

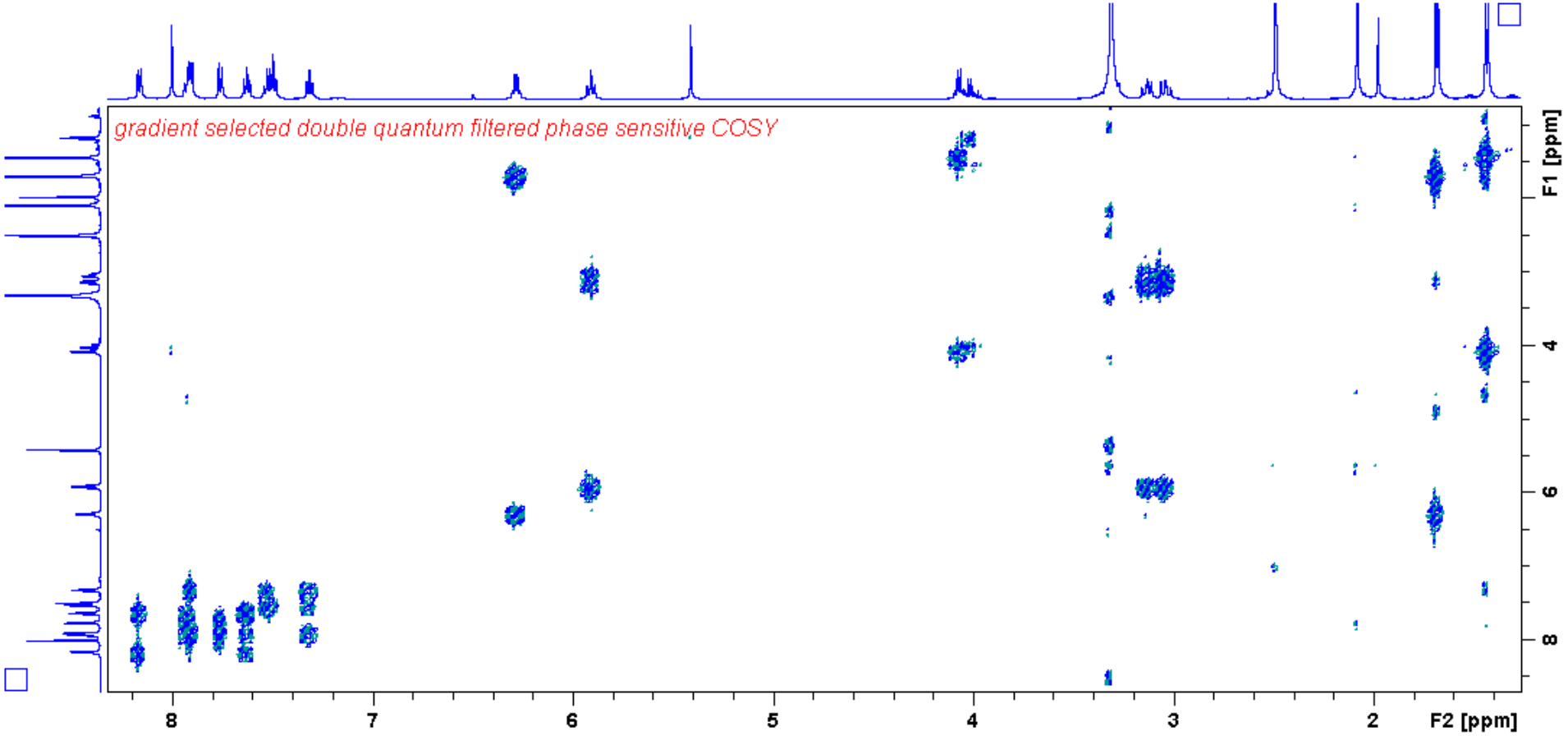


Figure S11.4 ^{1}H-^{1}H COSY NMR spectrum of **20**.

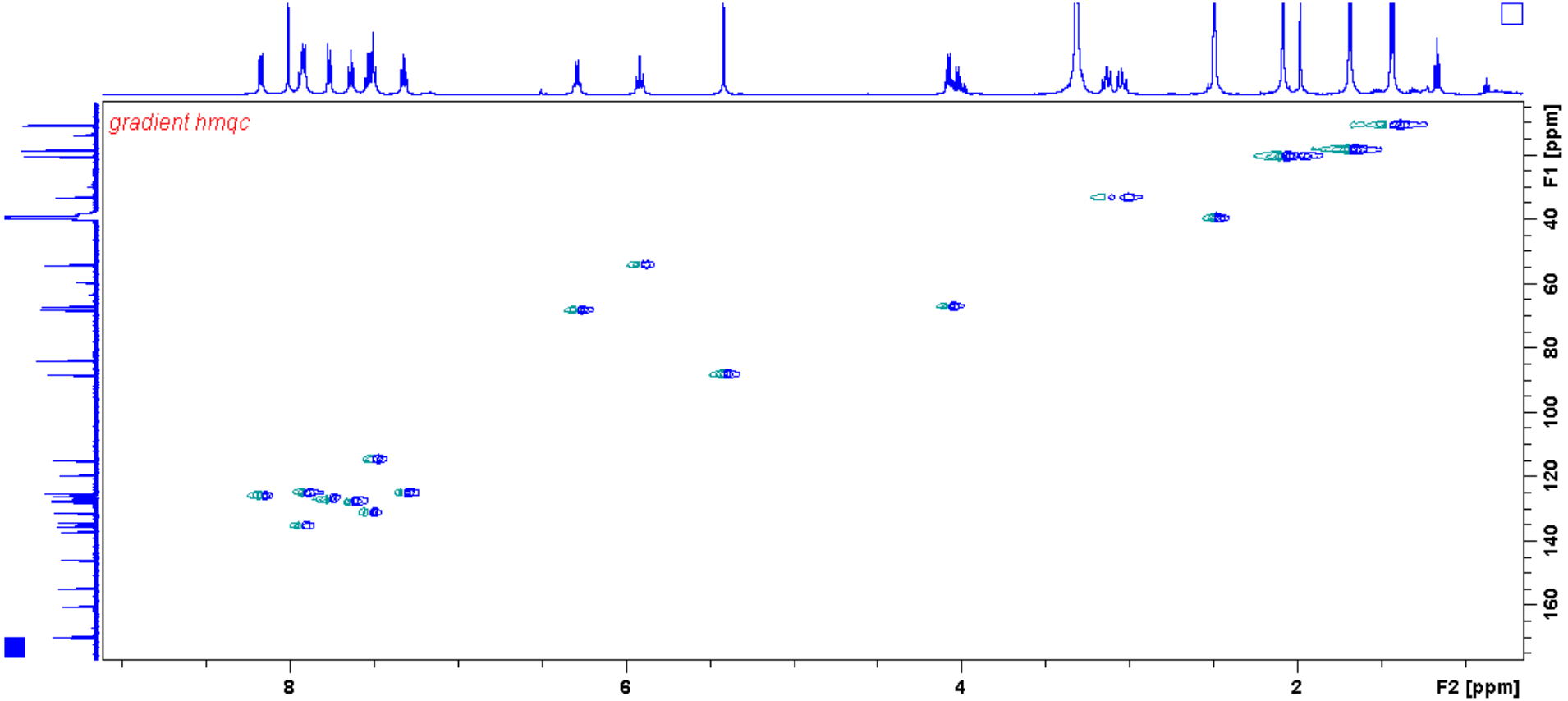


Figure S11.5 ^{1}H-^{13}C HMQC NMR spectrum of **20**.

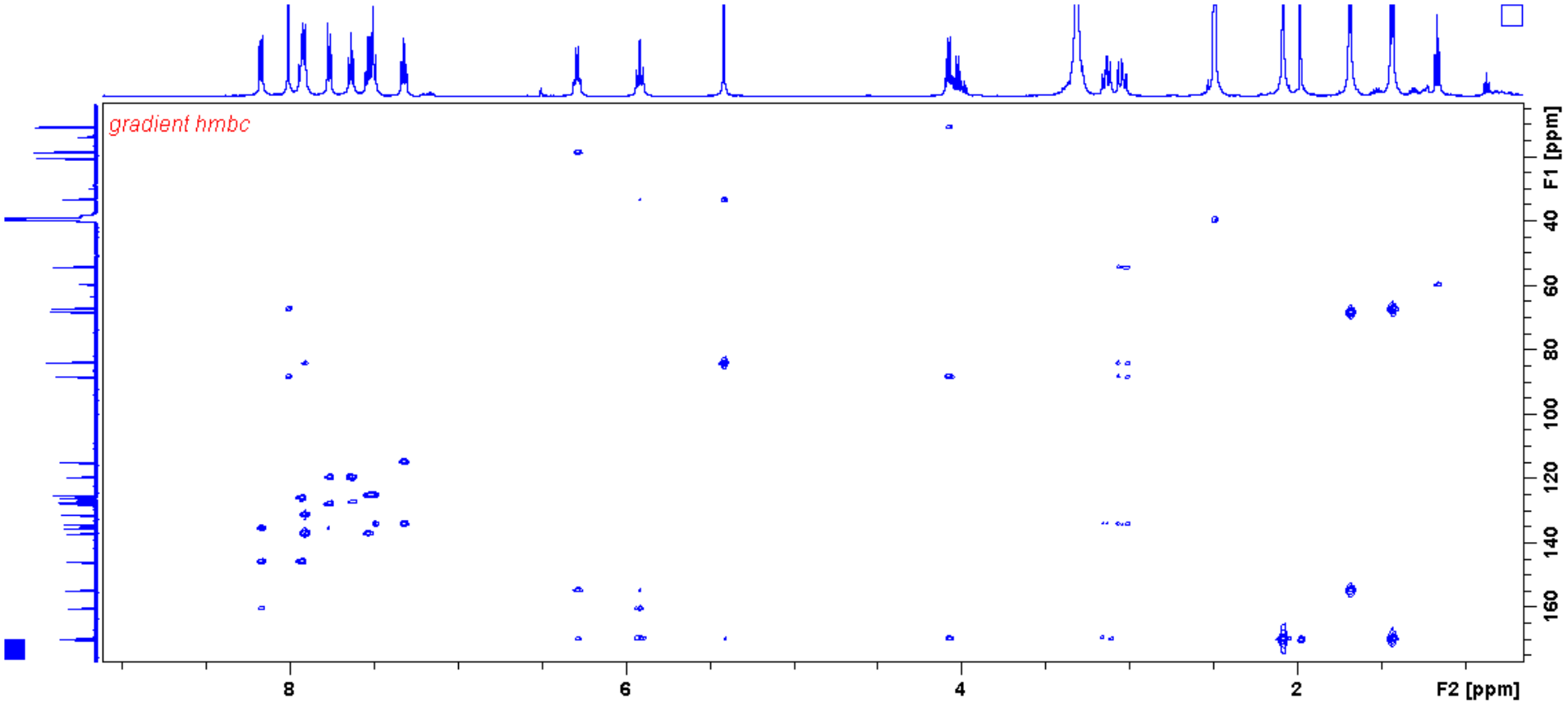


Figure S11.6 ^{1}H-^{13}C HMBC NMR spectrum of **20**.

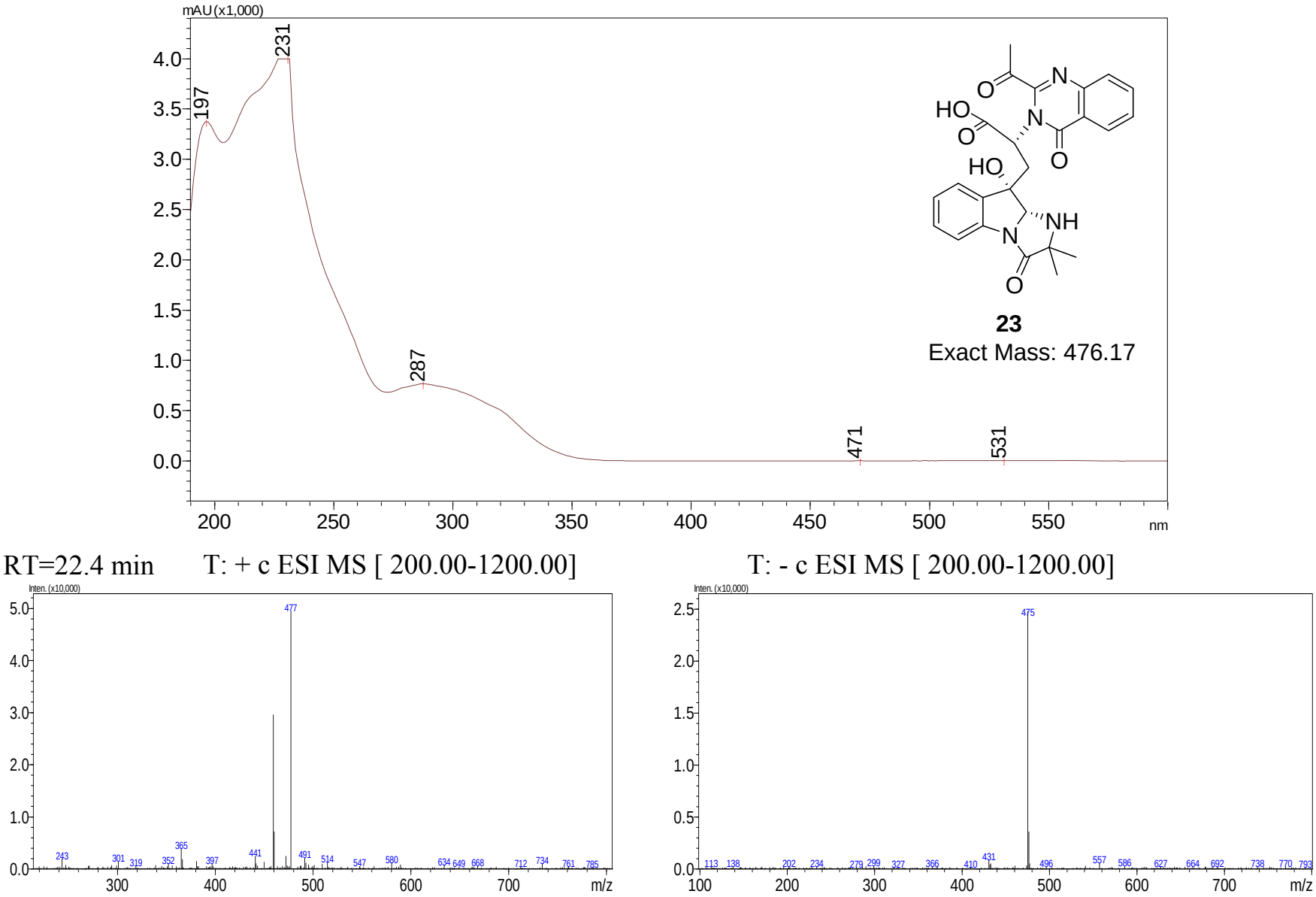
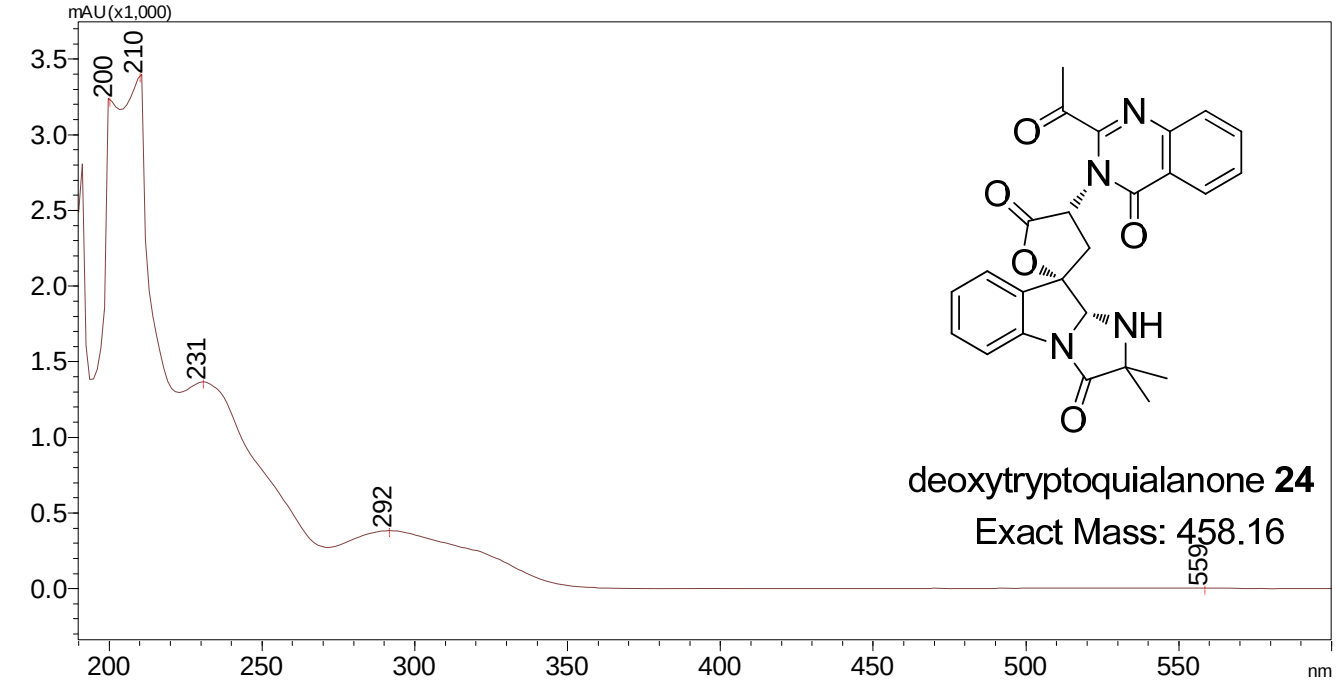


Figure S12 UV spectrum and MS Measured during LC-MS for **23**



RT=25.9 min T: + c ESI MS [200.00-1200.00]

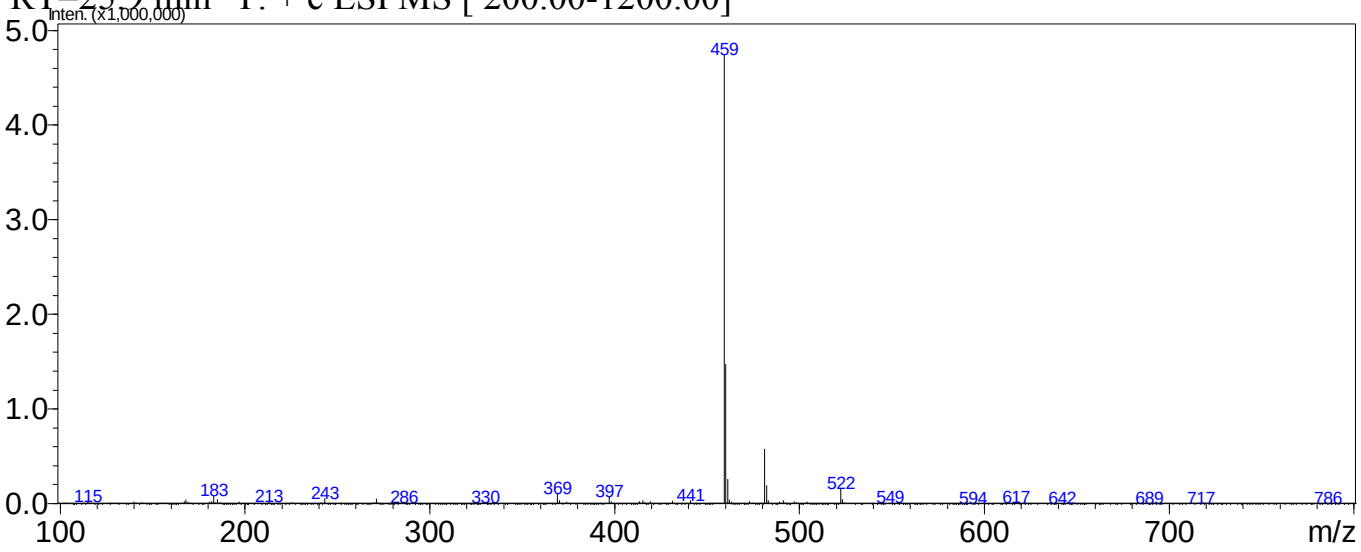


Figure S13.1 UV spectrum and MS Measured during LC-MS for **24**

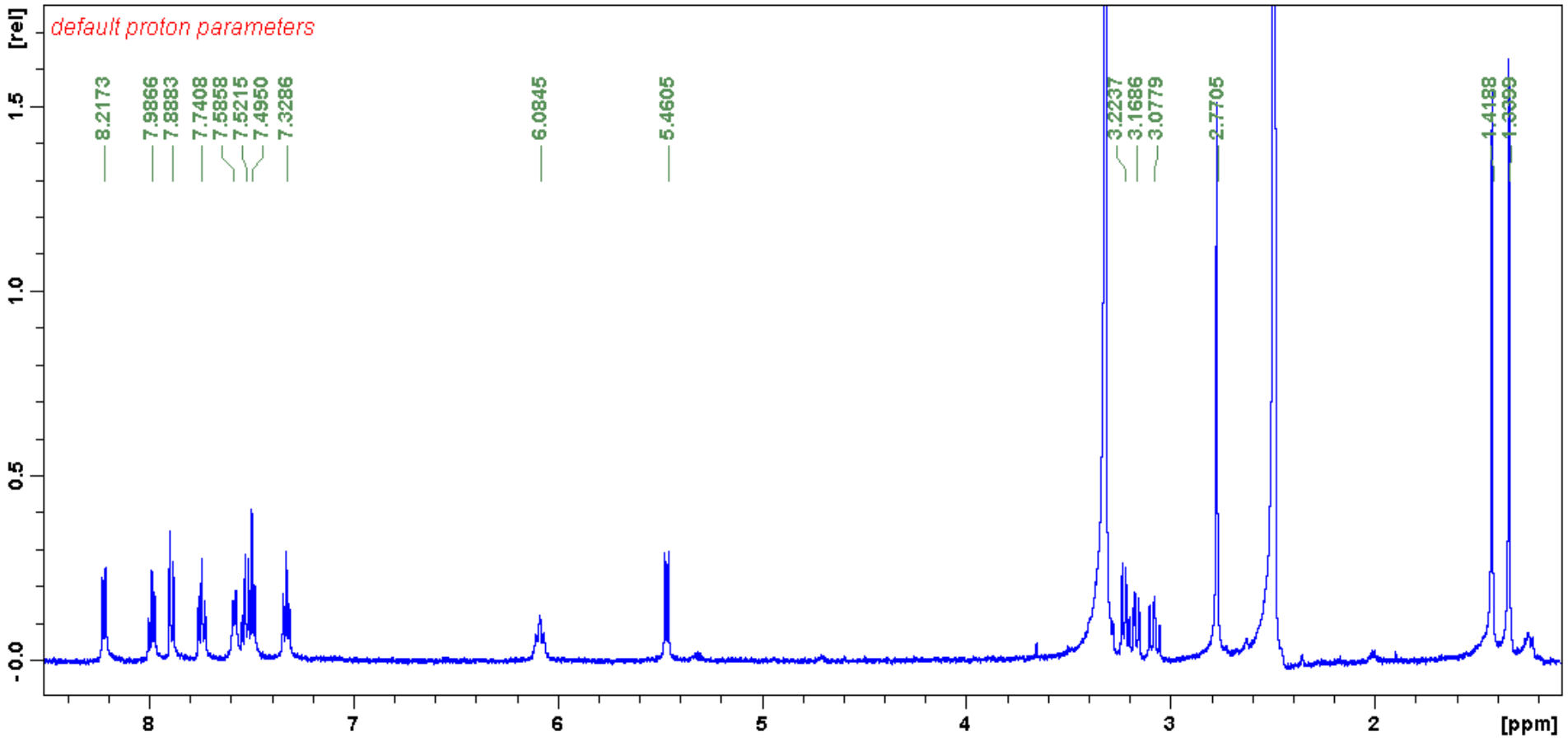


Figure S13.2 ^{1}H NMR spectrum of **24**. Measured in DMSO-d_6 at 500MHz

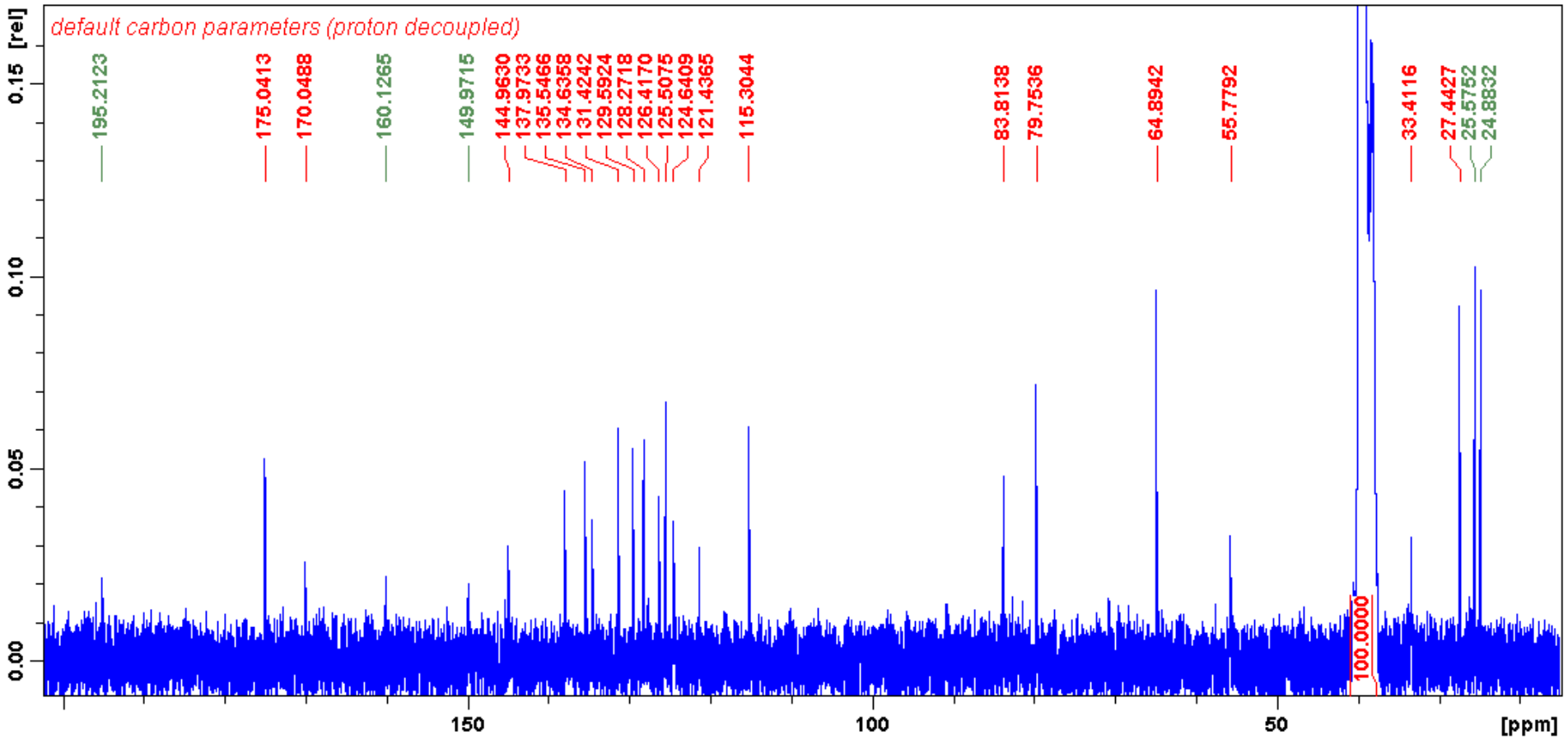


Figure S13.3 ^{13}C NMR spectrum of **24**. Measured in DMSO-d_6 at 125MHz

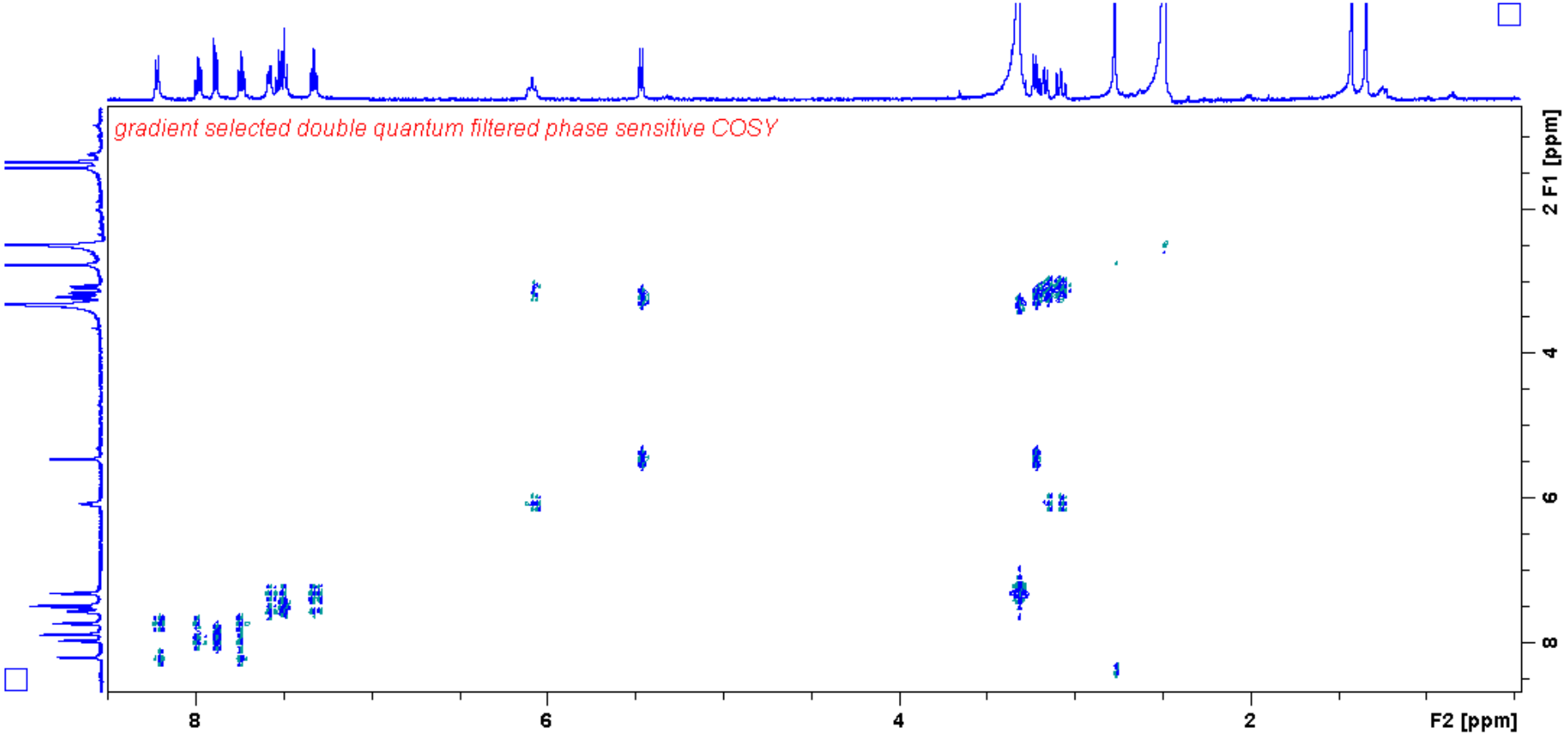


Figure S13.4 ^{1}H-^{1}H COSY NMR spectrum of **24**.

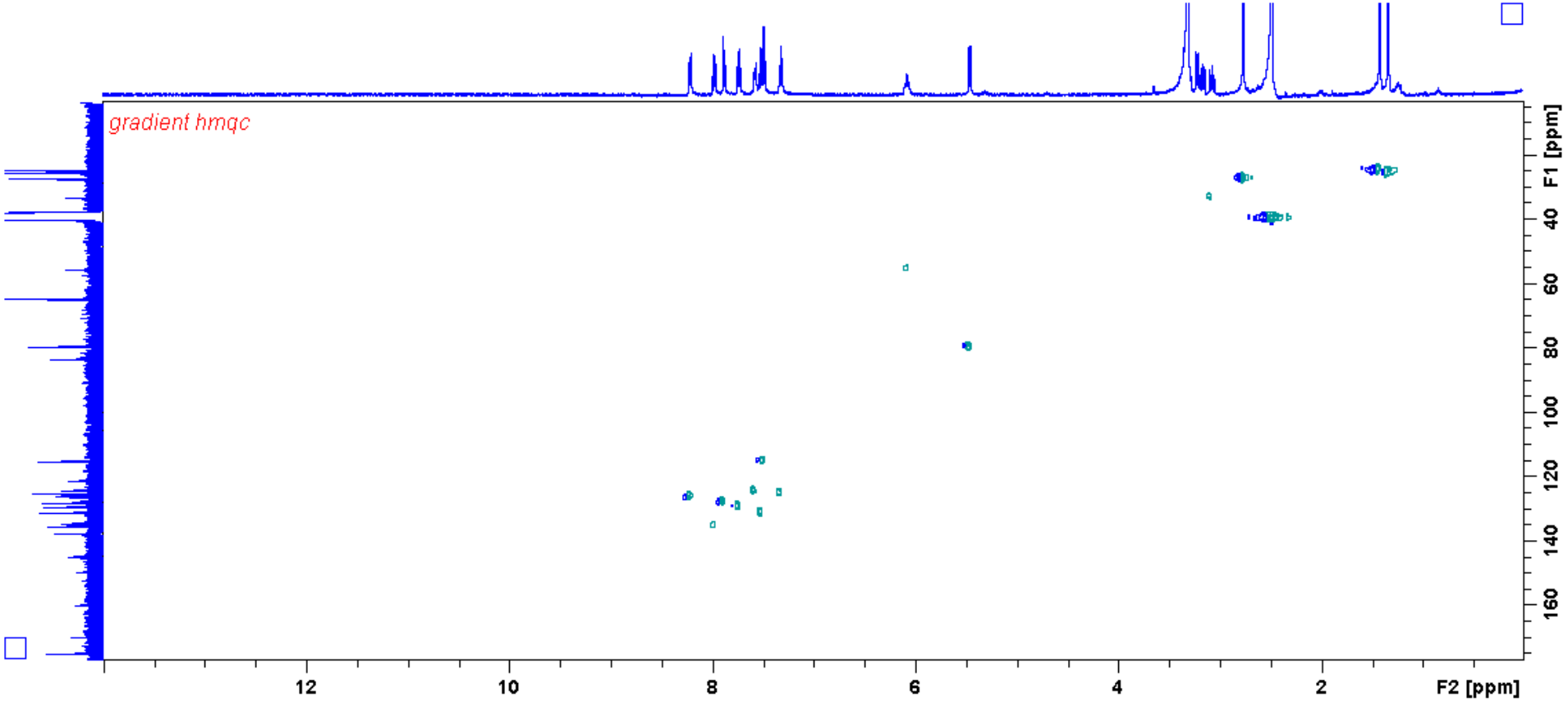


Figure S13.5 ^{1}H-^{13}C HMQC NMR spectrum of **24**.

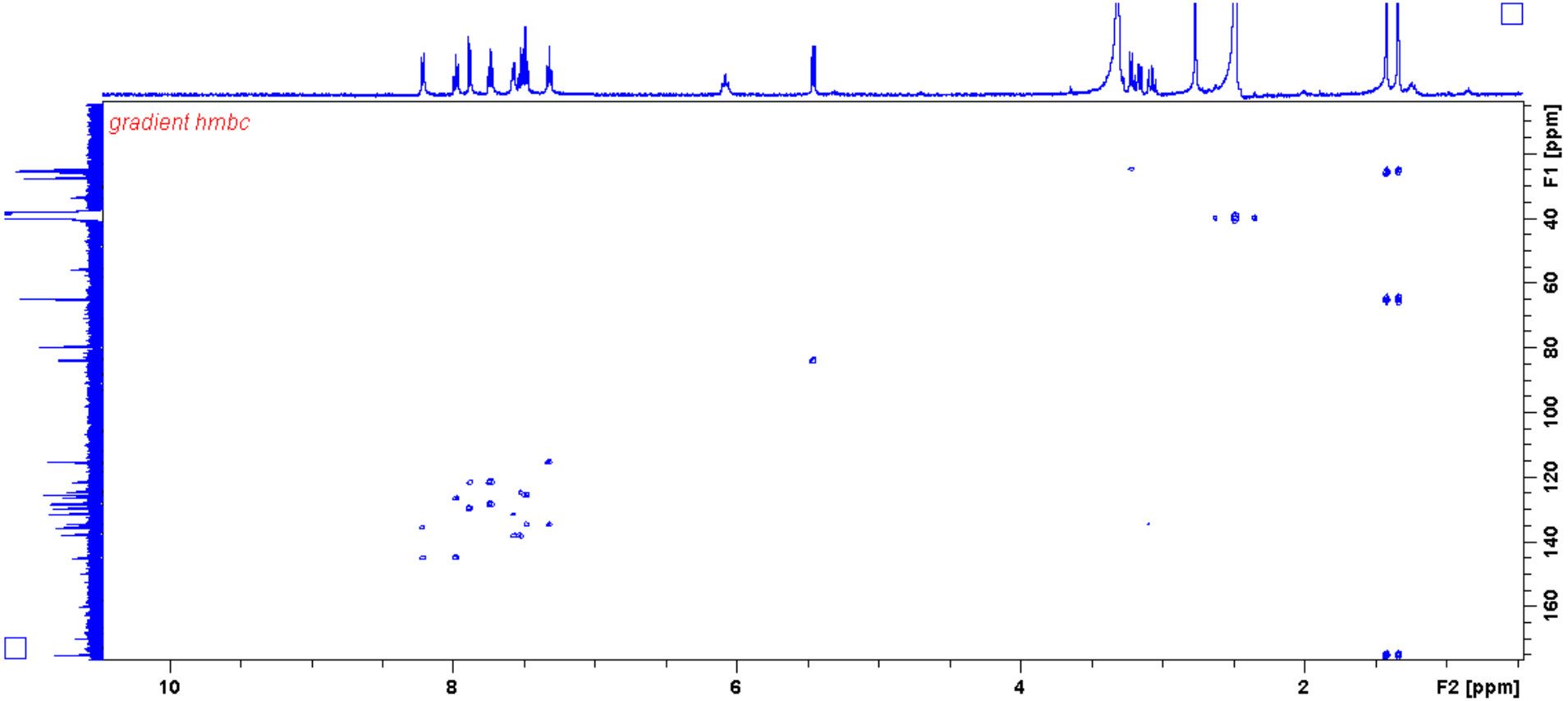


Figure S13.6 ^{1}H-^{13}C HMBC NMR spectrum of **24**.

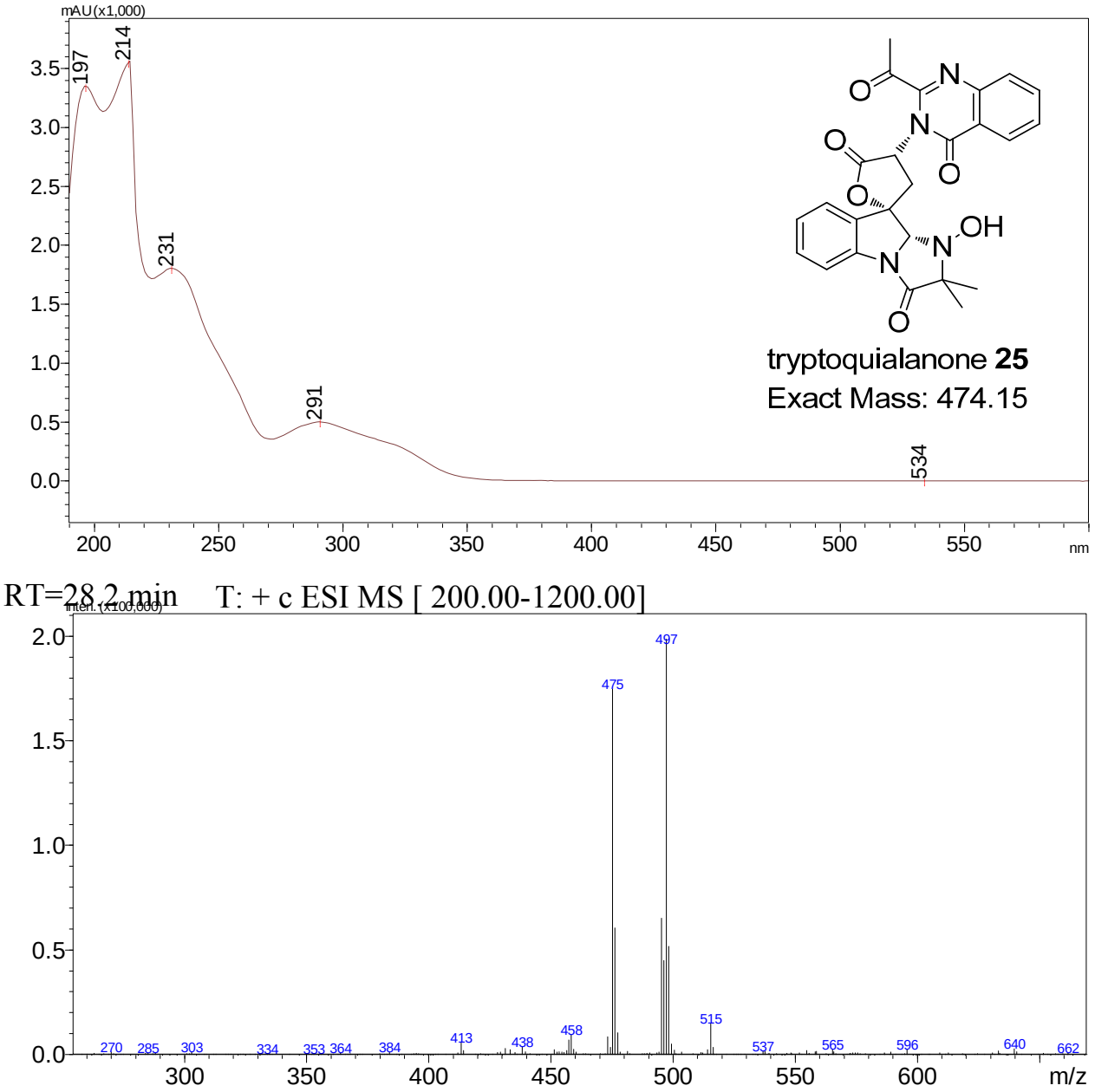


Figure S14.1 UV spectrum and MS Measured during LC-MS for **25**

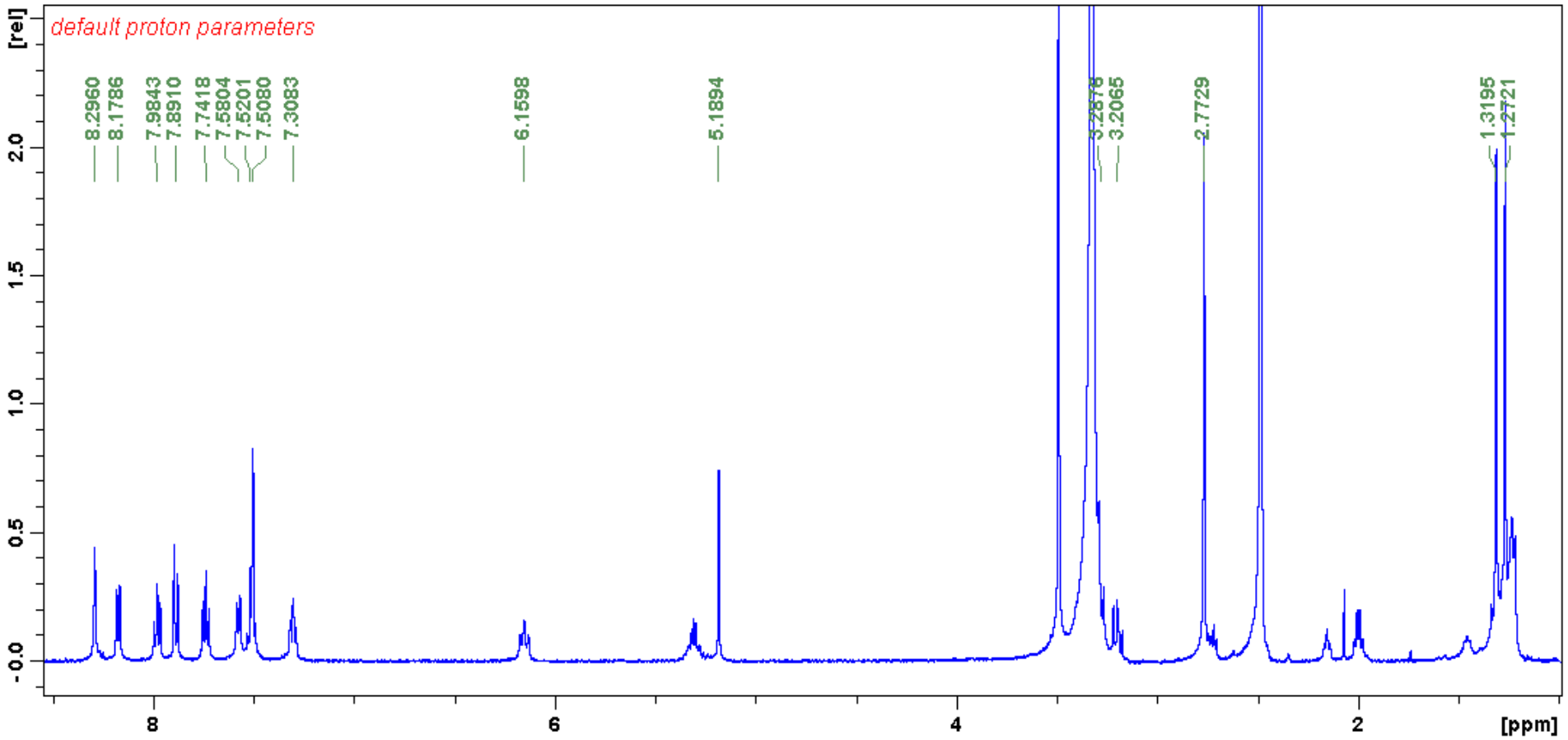


Figure S14.2 ^{1}H NMR spectrum of **25**. Measured in DMSO-d_6 at 500MHz

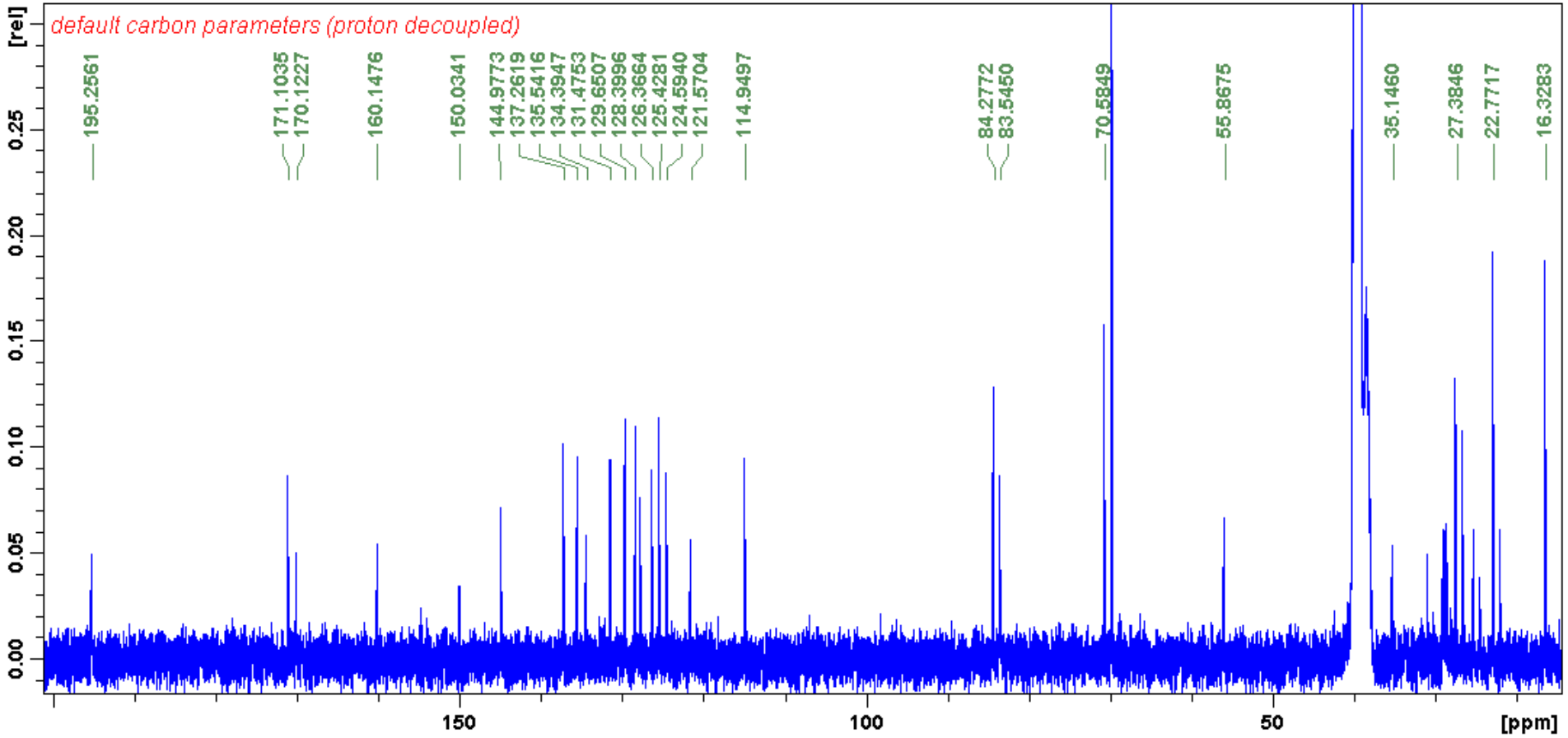


Figure S14.3 ^{13}C NMR spectrum of **25**. Measured in DMSO-d_6 at 125MHz

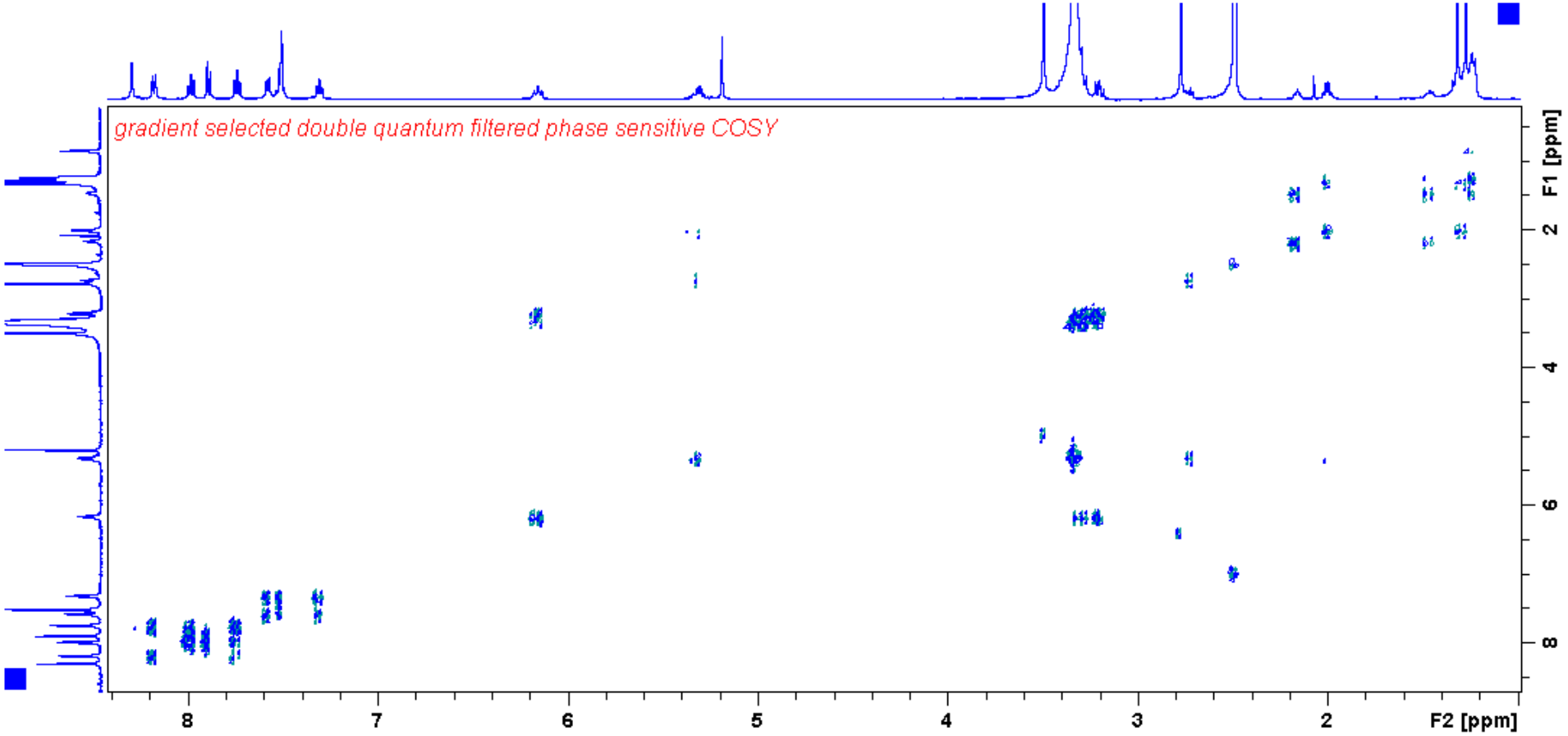


Figure S14.4 ^{1}H-^{1}H COSY NMR spectrum of **25**

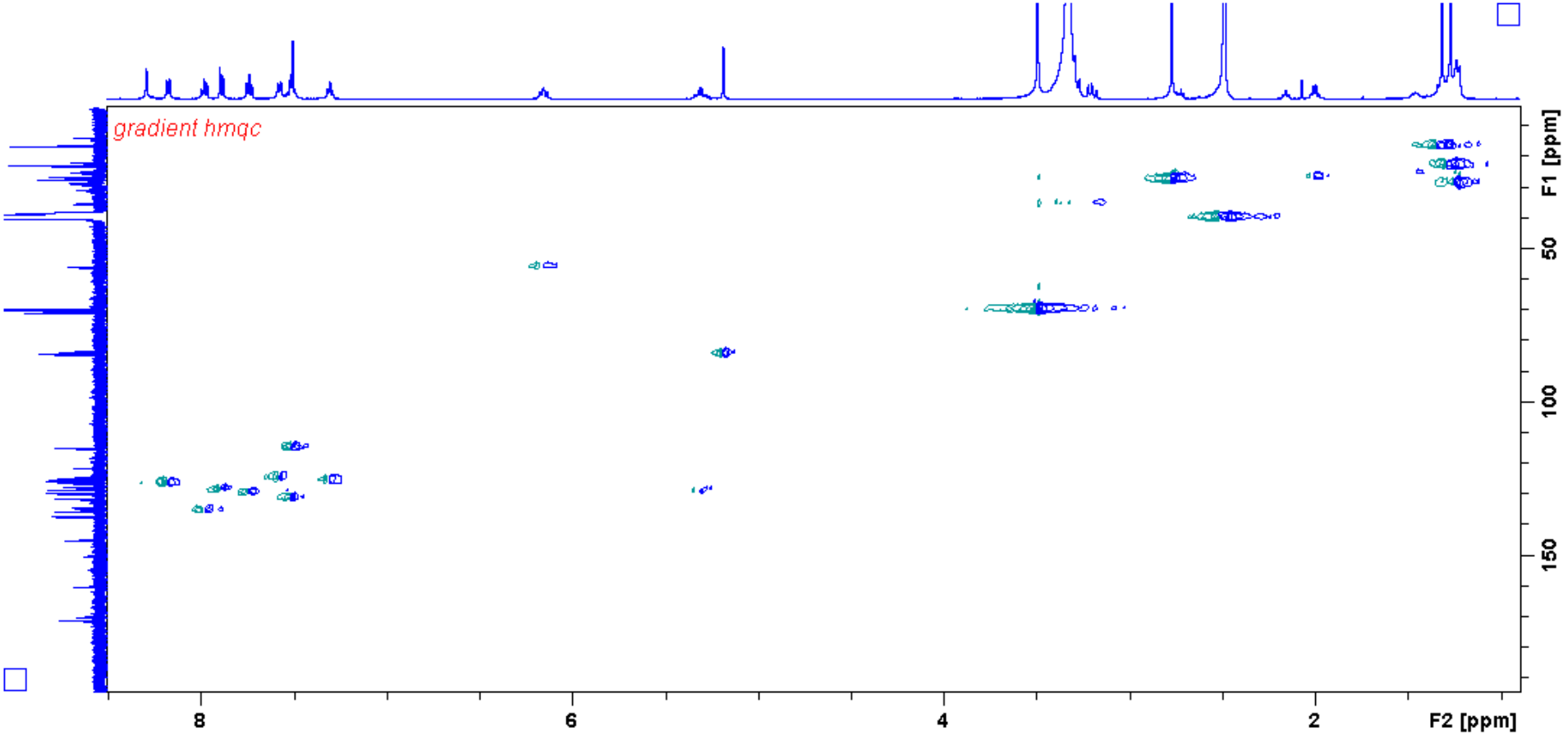


Figure S14.5 ^{1}H-^{13}C HMQC NMR spectrum of **25**.

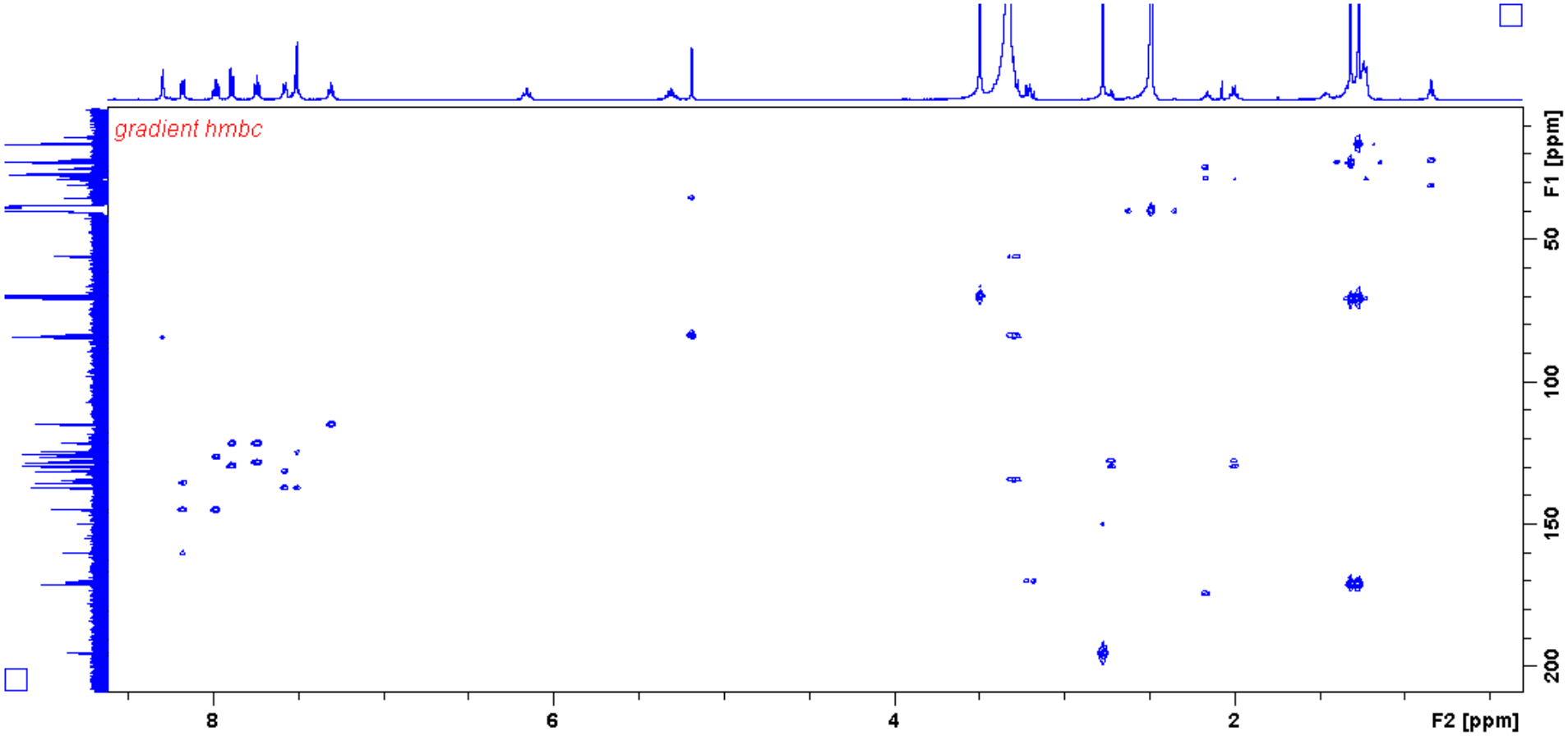


Figure S14.6 ^{1}H-^{13}C HMBC NMR spectrum of **25**.

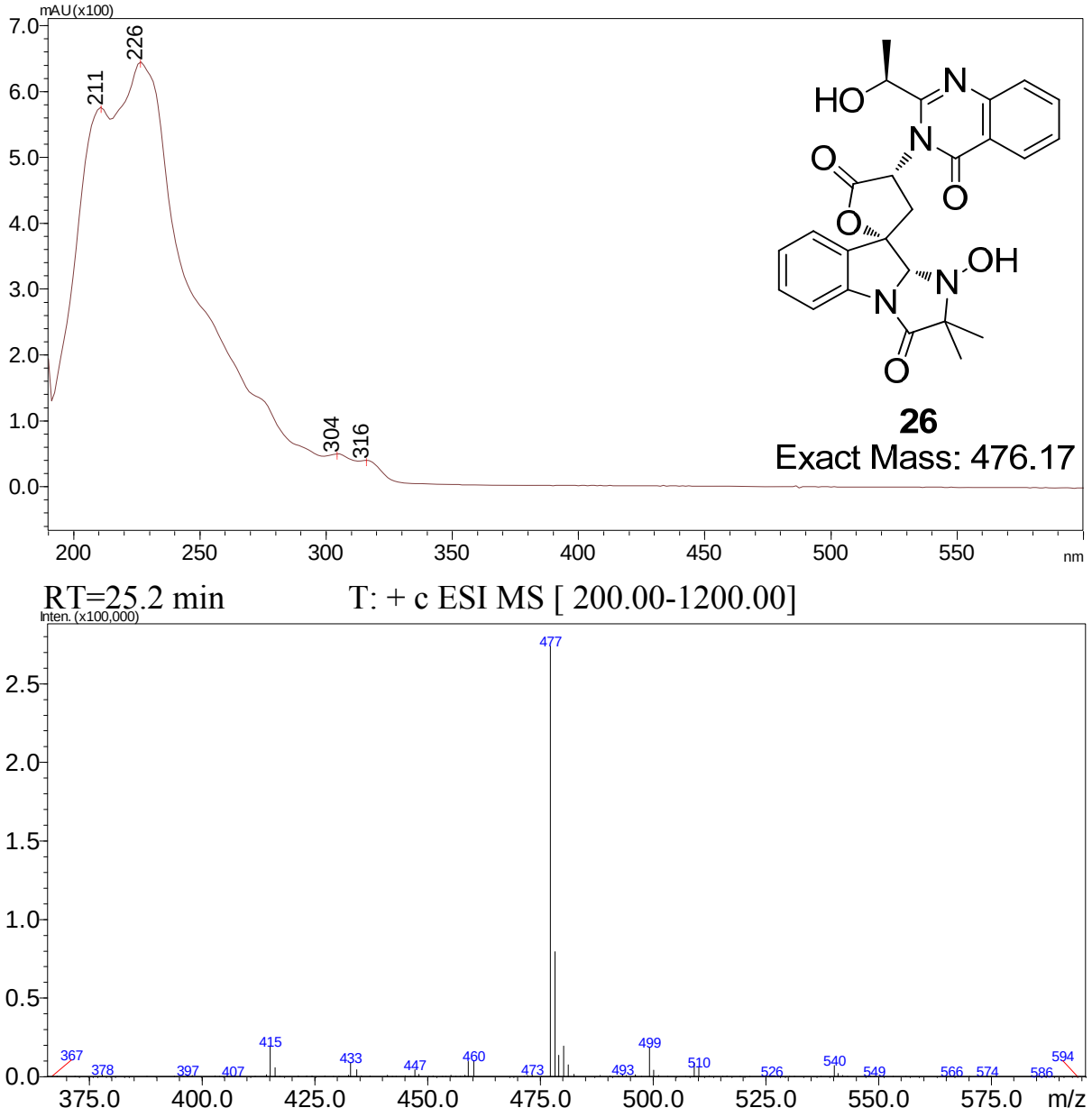


Figure S15 UV spectrum and MS Measured during LC-MS for **26**

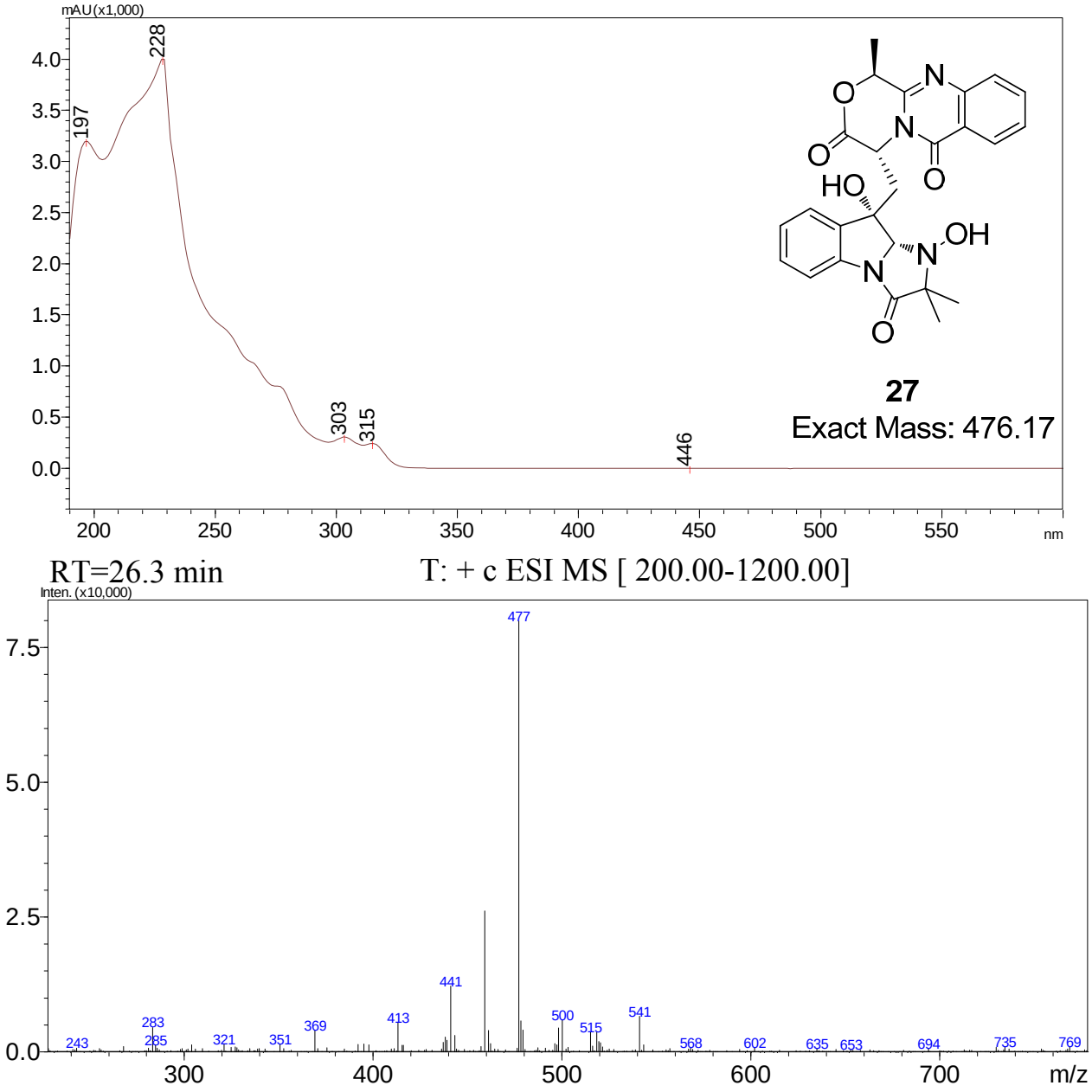
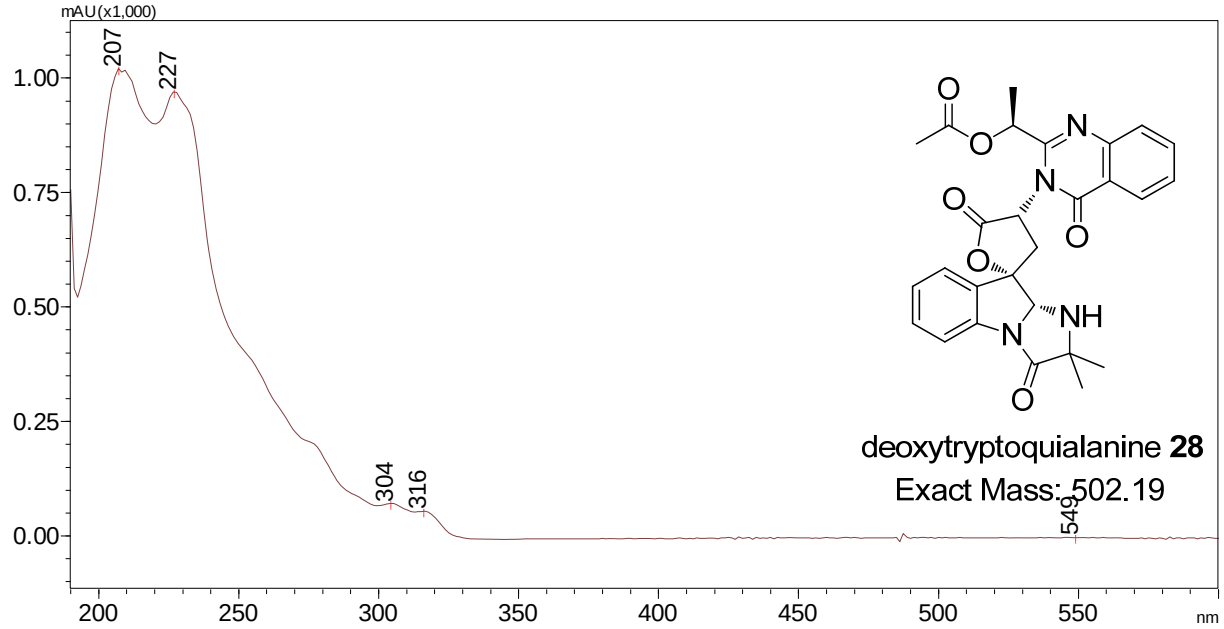


Figure S16 UV spectrum and MS Measured during LC-MS for **27**



RT=24.7 min T: + c ESI MS [200.00-1200.00]

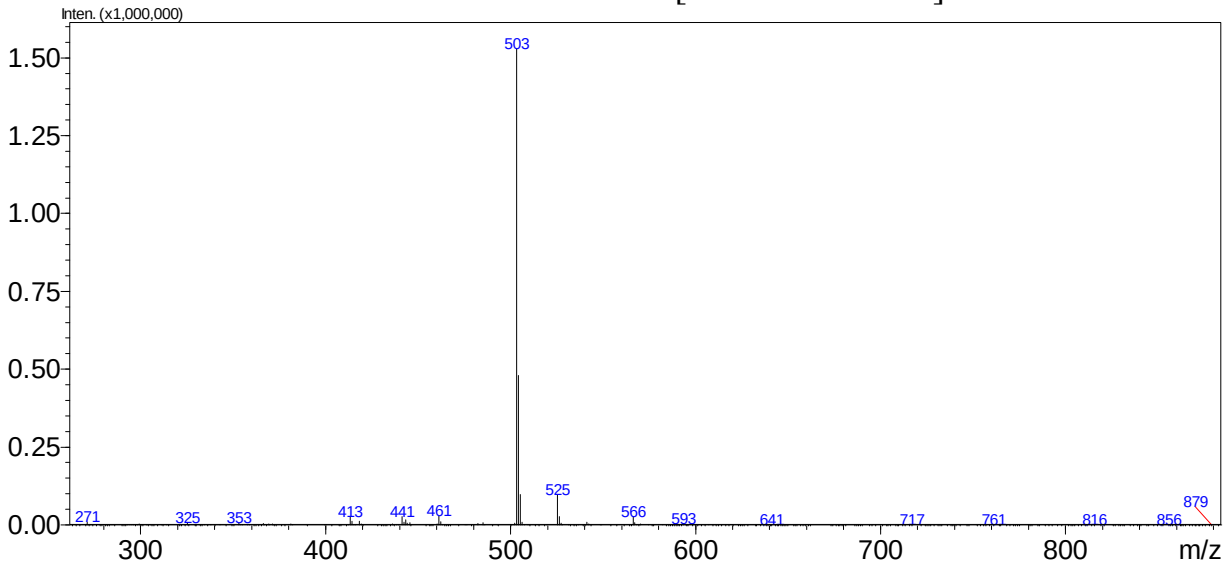


Figure S17.1 UV spectrum and MS Measured during LC-MS for **28**

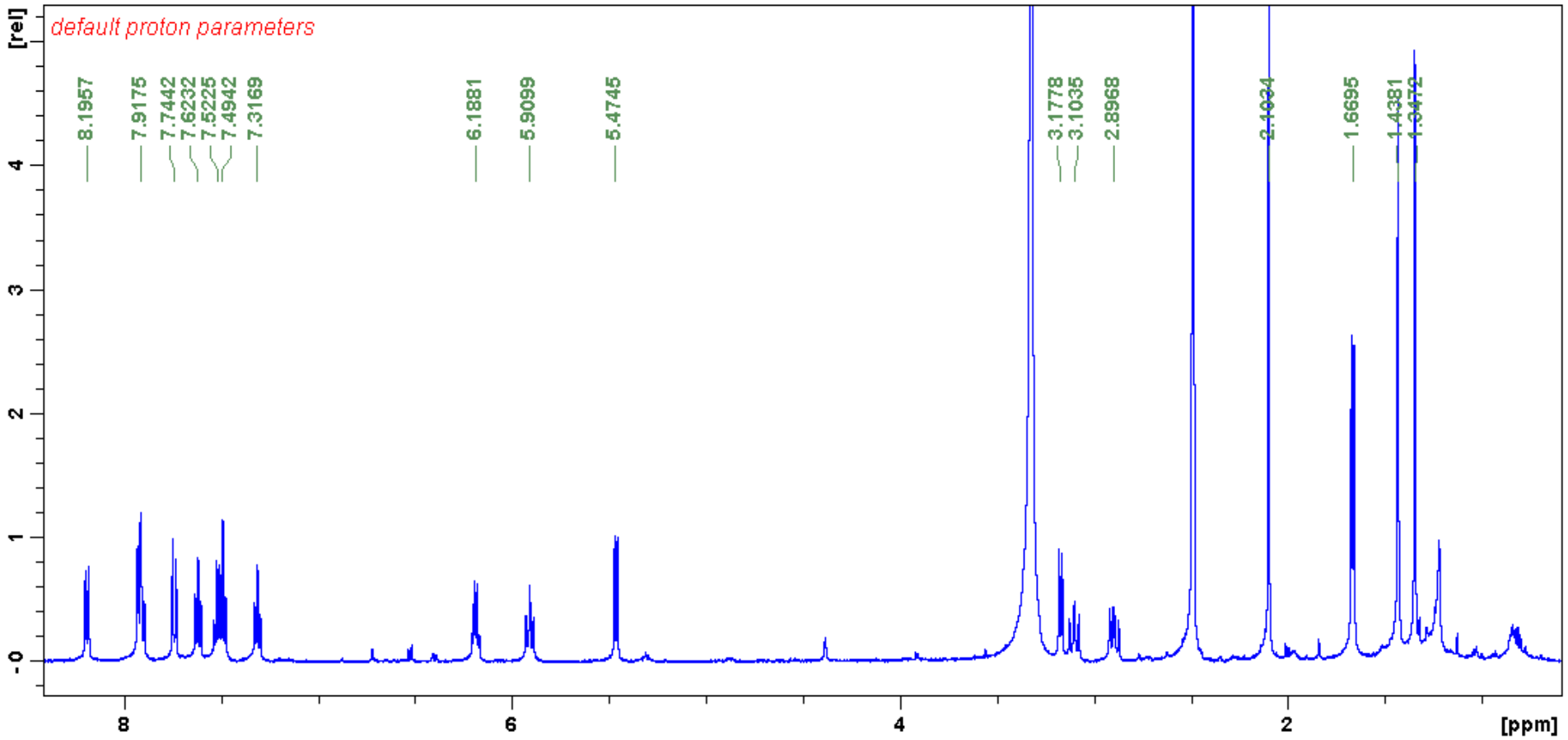


Figure S17.2 ^{1}H NMR spectrum of **28**. Measured in DMSO-d_6 at 500MHz

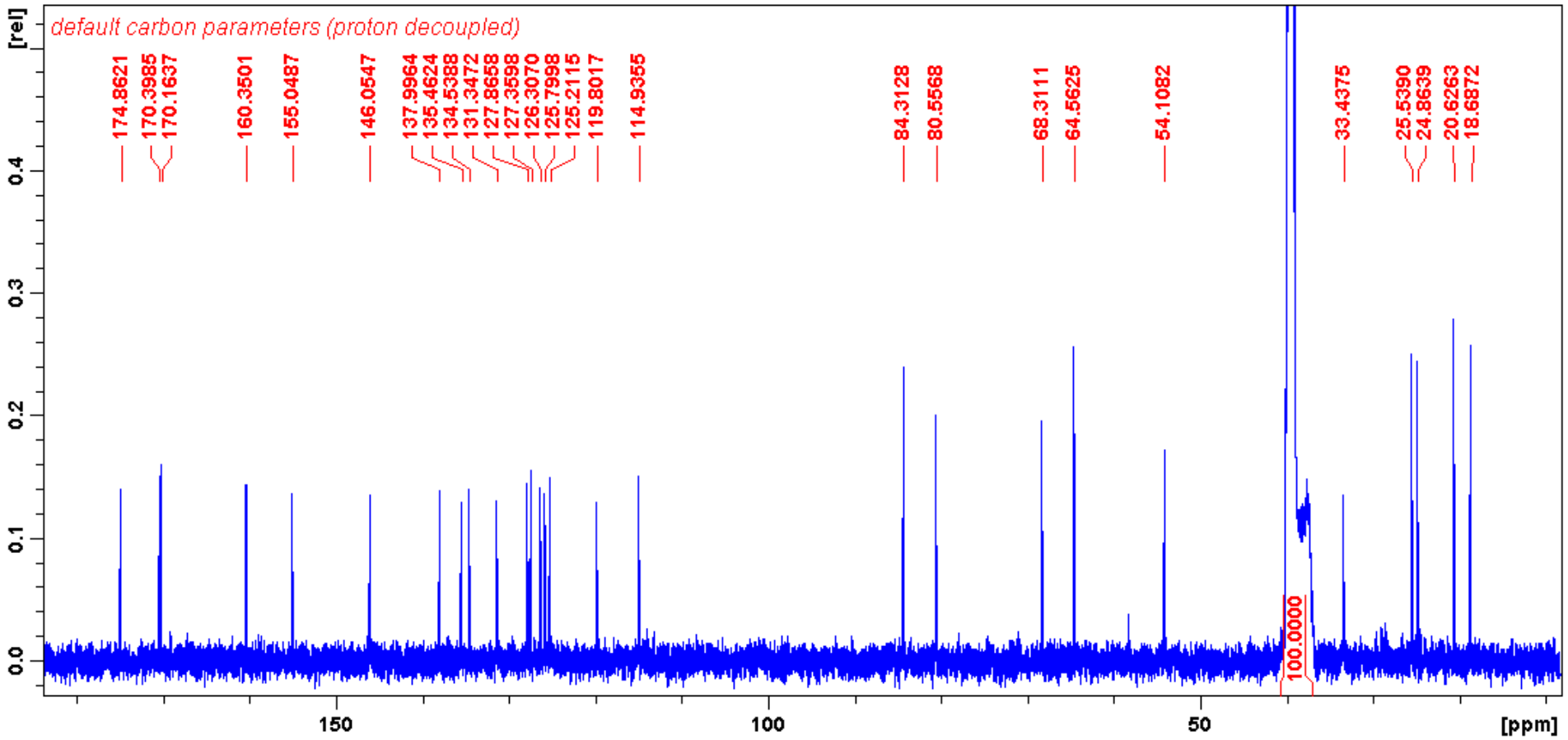


Figure S17.3 ^{13}C NMR spectrum of **28**. Measured in DMSO-d_6 at 125MHz

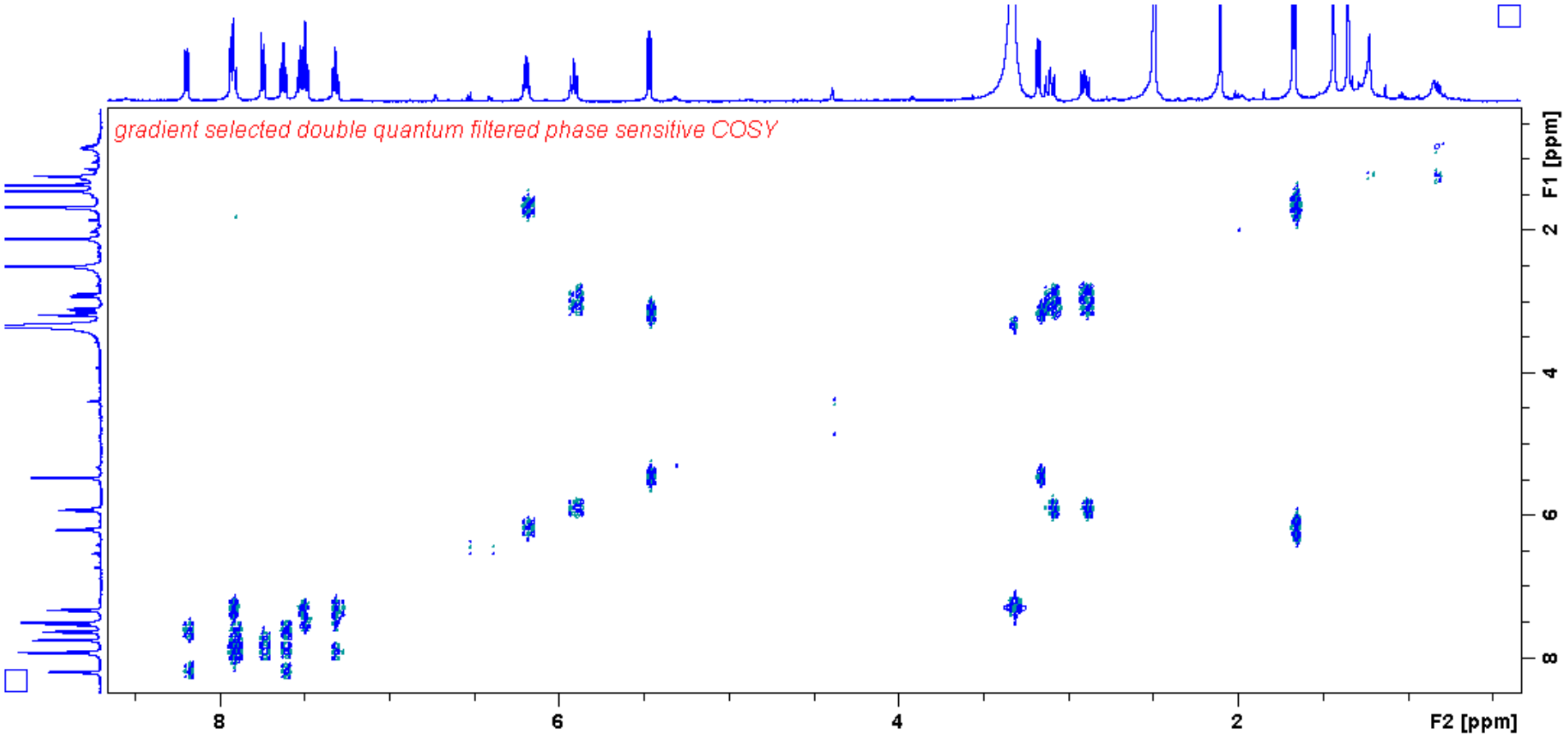


Figure S17.4 ^{1}H-^{1}H COSY NMR spectrum of **28**

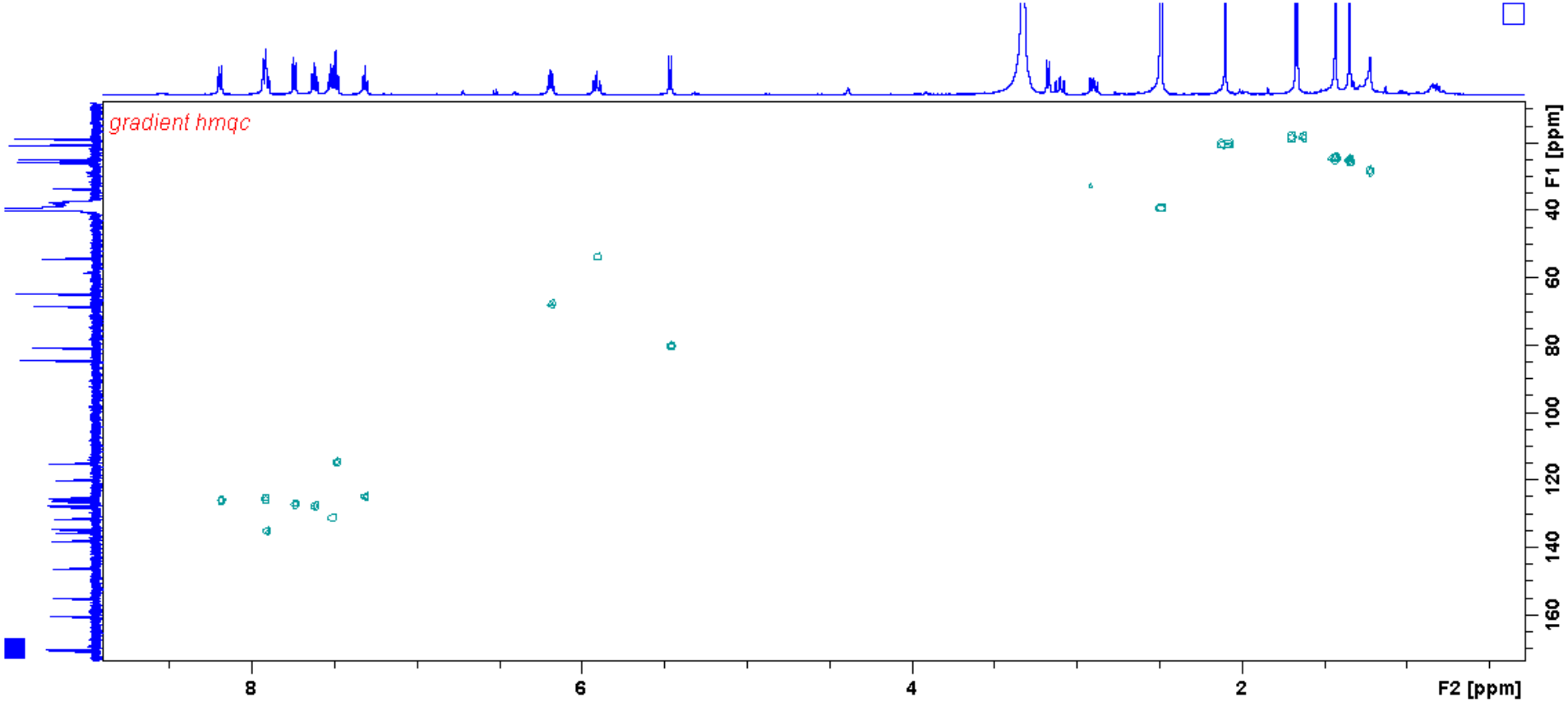


Figure S17.5 ^{1}H-^{13}C MQC NMR spectrum of **28**.

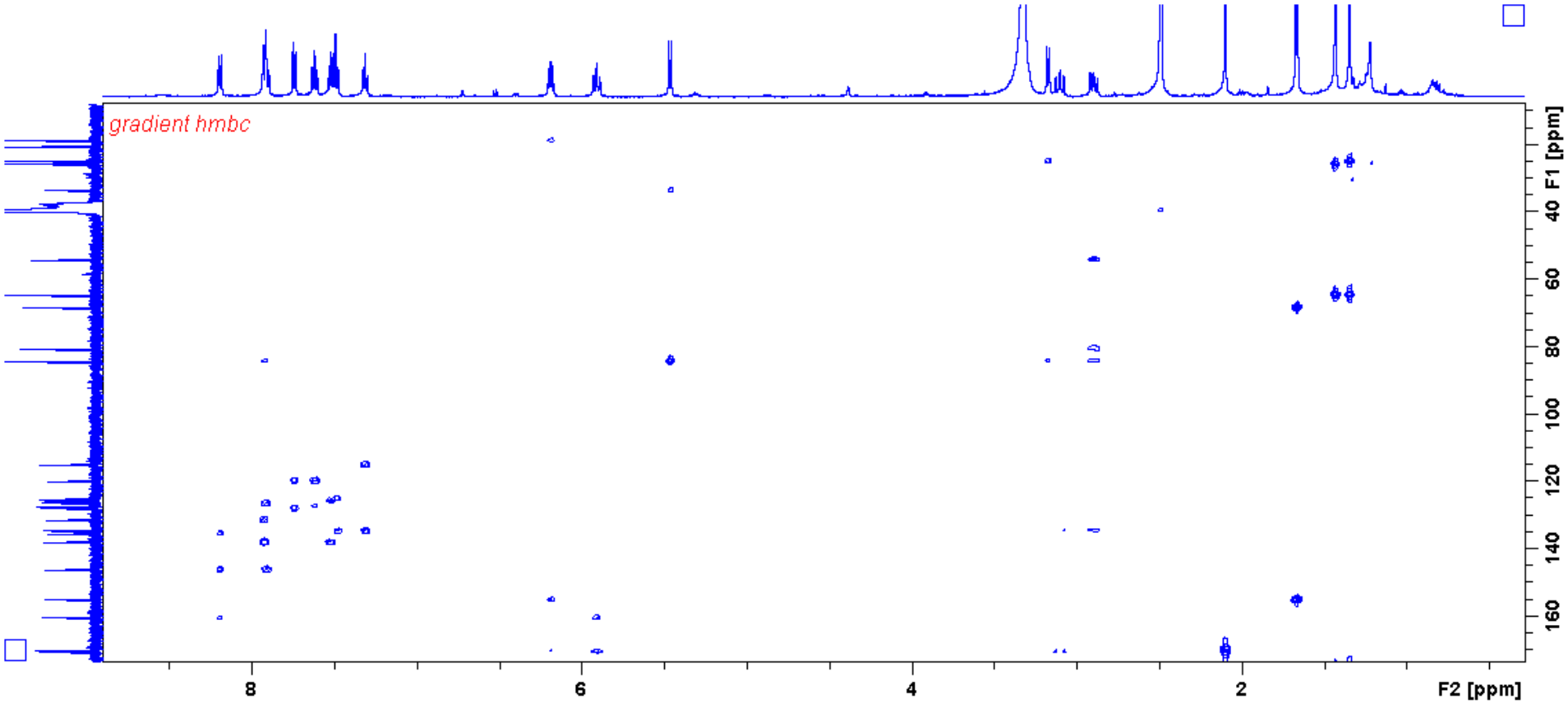
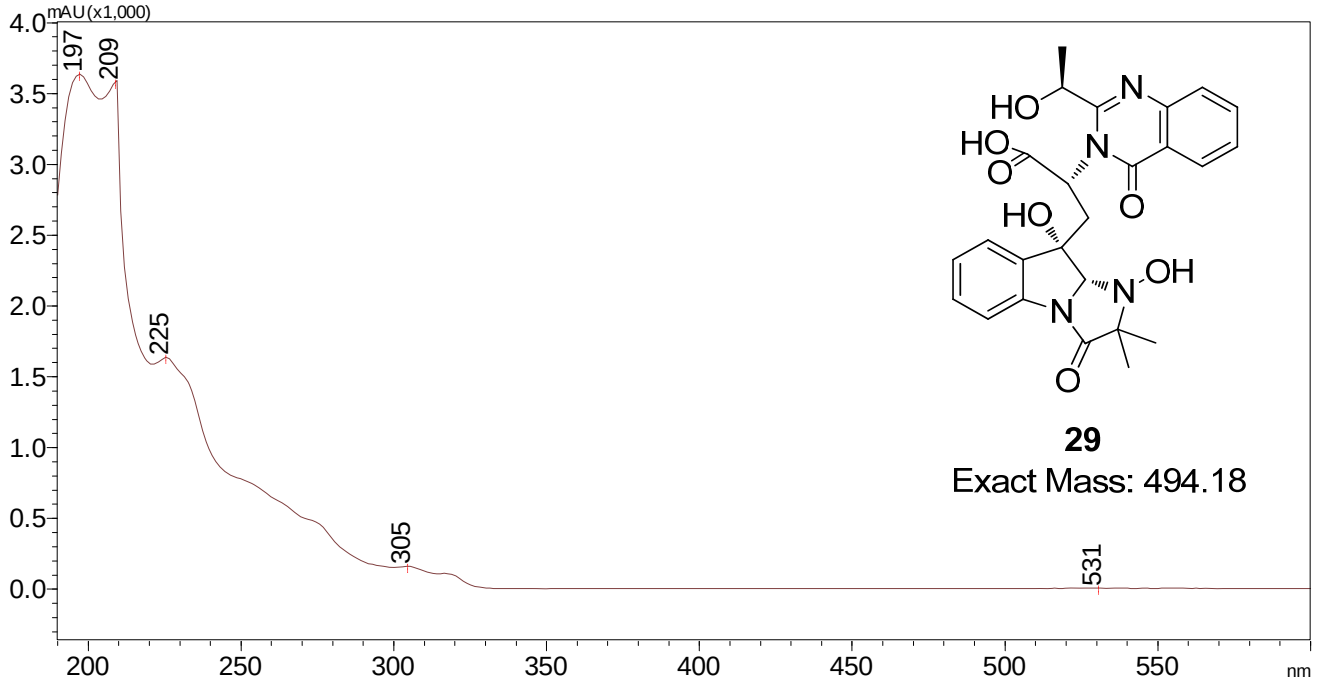
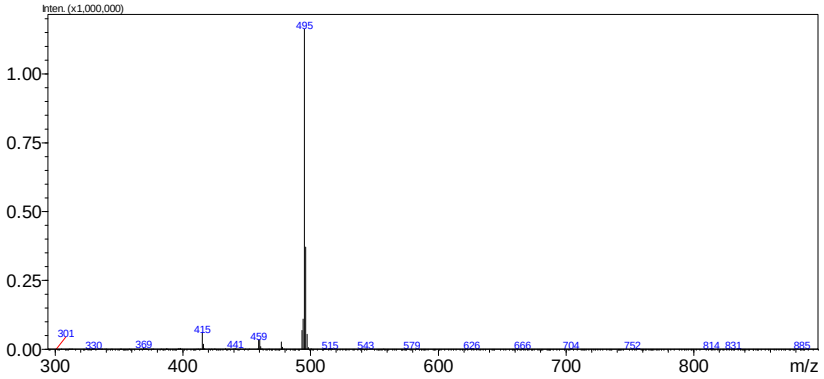


Figure S17.6 ^{1}H-^{13}C HMBC NMR spectrum of **28**.



RT=21.5 min T: + c ESI MS [200.00-1200.00] T: - c ESI MS [200.00-1200.00]



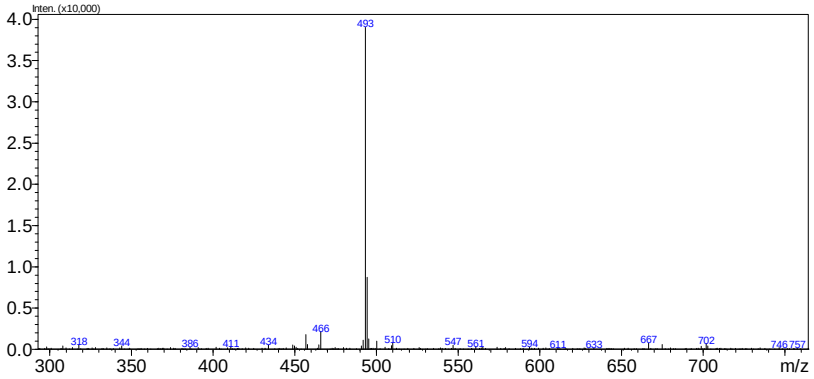
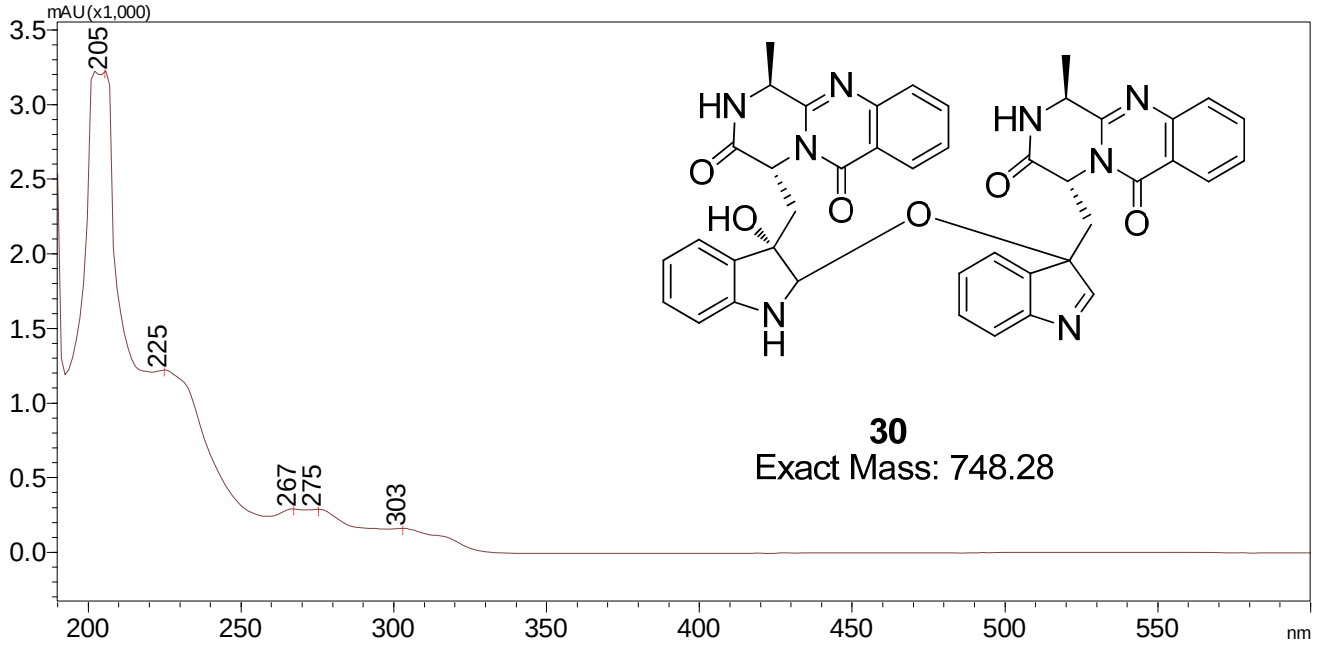


Figure S18 UV spectrum and MS Measured during LC-MS for **29**



RT=25.8 min T: + c ESI MS [200.00-1200.00]

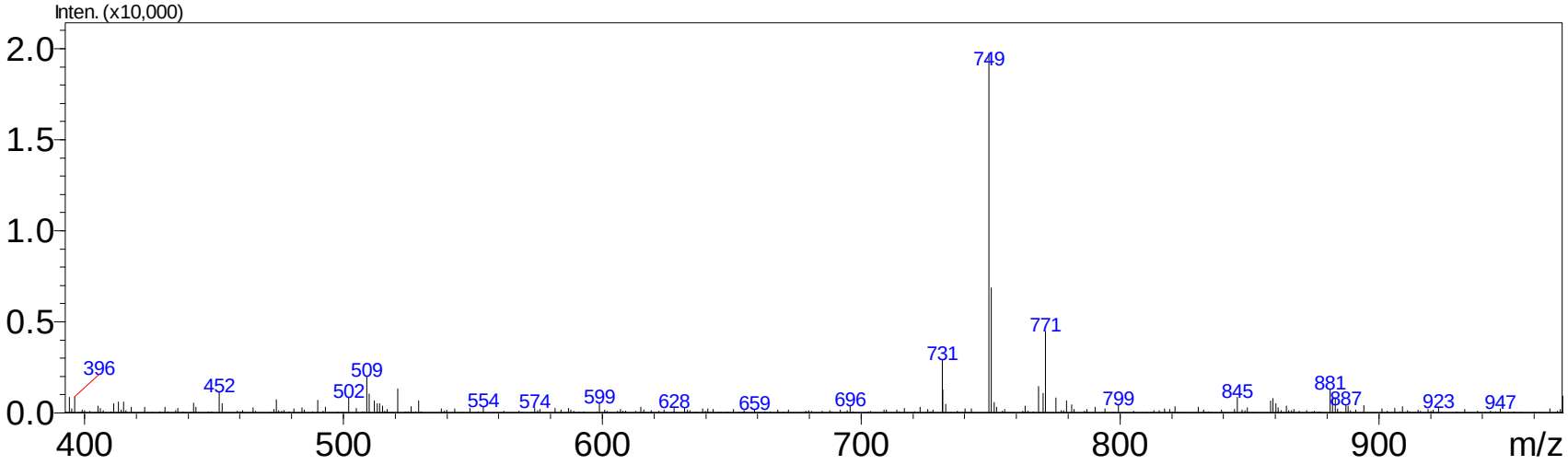


Figure S19 UV spectrum and MS Measured during LC-MS for **30**

Supplementary reference

(41) Fedorova, N. D.; Khaldi, N.; Joardar, V. S.; Maiti, R.; Amedeo, P.; Anderson, M. J.; Crabtree, J.; Silva, J. C.; Badger, J. H.; Albarraq, A.; Angiuoli, S.; Bussey, H.; Bowyer, P.; Cotty, P. J.; Dyer, P. S.; Egan, A.; Galens, K.; Fraser-Liggett, C. M.; Haas, B. J.; Inman, J. M.; Kent, R.; Lemieux, S.; Malavazi, I.; Orvis, J.; Roemer, T.; Ronning, C. M.; Sundaram, J. P.; Sutton, G.; Turner, G.; Venter, J. C.; White, O. R.; Whitty, B. R.; Youngman, P.; Wolfe, K. H.; Goldman, G. H.; Wortman, J. R.; Jiang, B.; Denning, D. W.; Nierman, W. C. *PLos Genet.* **2008**, *4*.