Supplementary Materials: On the Stability and Degradation Pathways of Venetoclax under Stress Conditions

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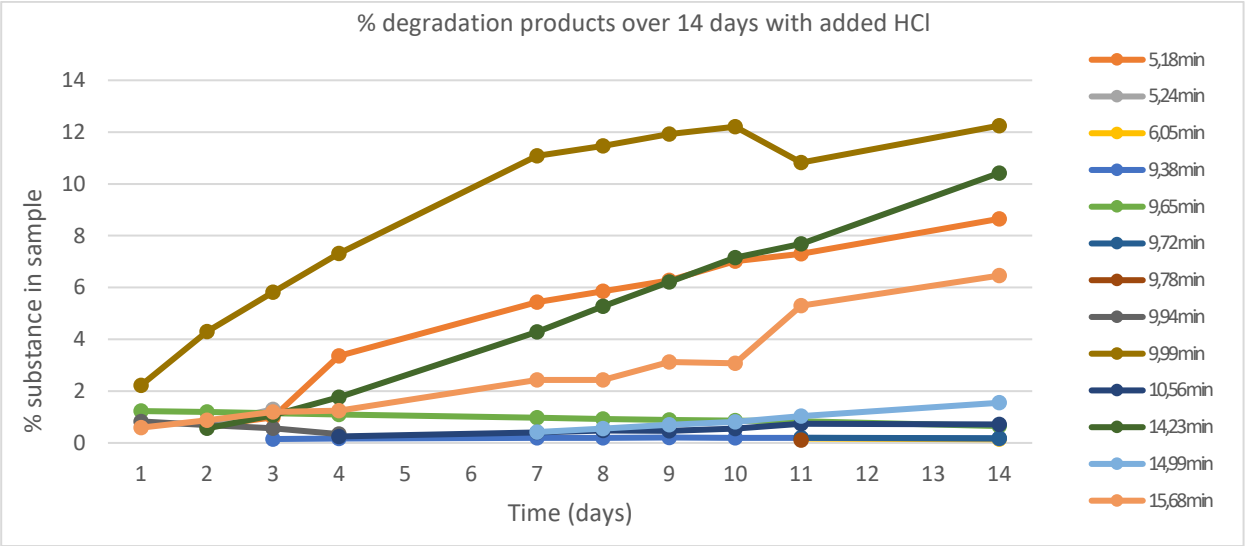
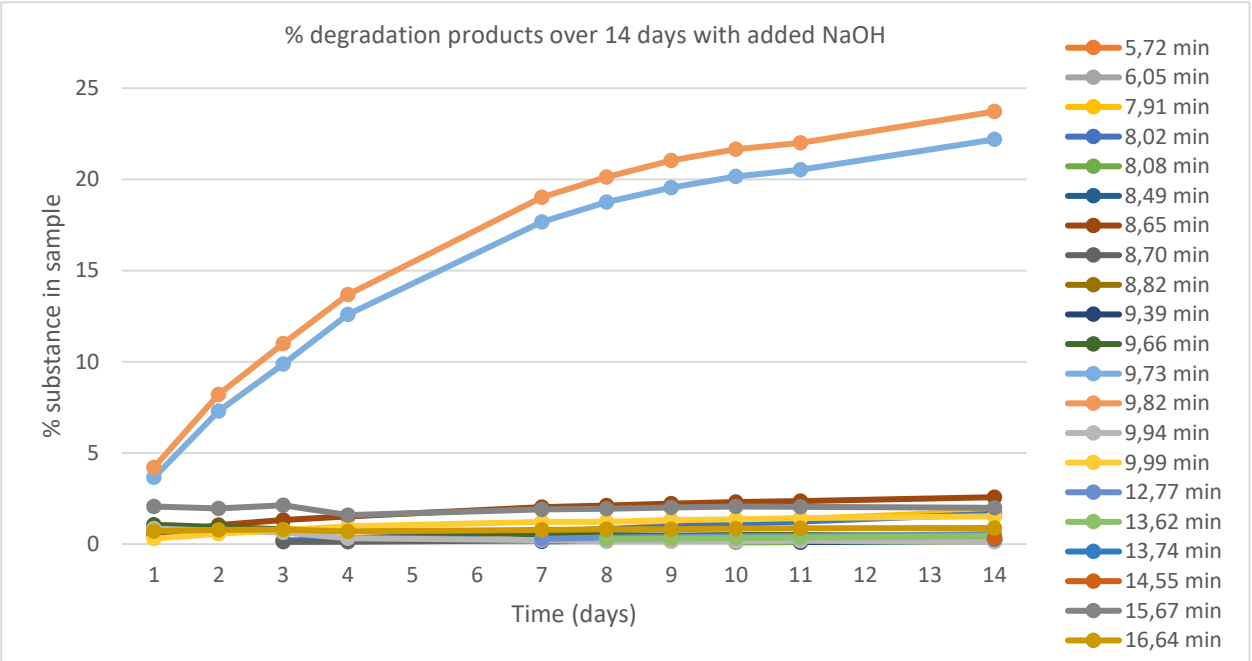
List of supplementary material



(a)



(b)

Figure S1. – Line charts depicting the percentage of degradation products in 14 day degradation testing. a) Line chart representing the percentage of degradation products in the venetoclax stress sample with added HCl (1 M) as a stress medium at 50 °C. Degradation products are named by their approximate retention times.

b) Line chart representing the percentage of degradation products in the venetoclax stress sample with added NaOH (1 M) as a stress medium at 50 °C. Degradation products are named by their approximate retention times.

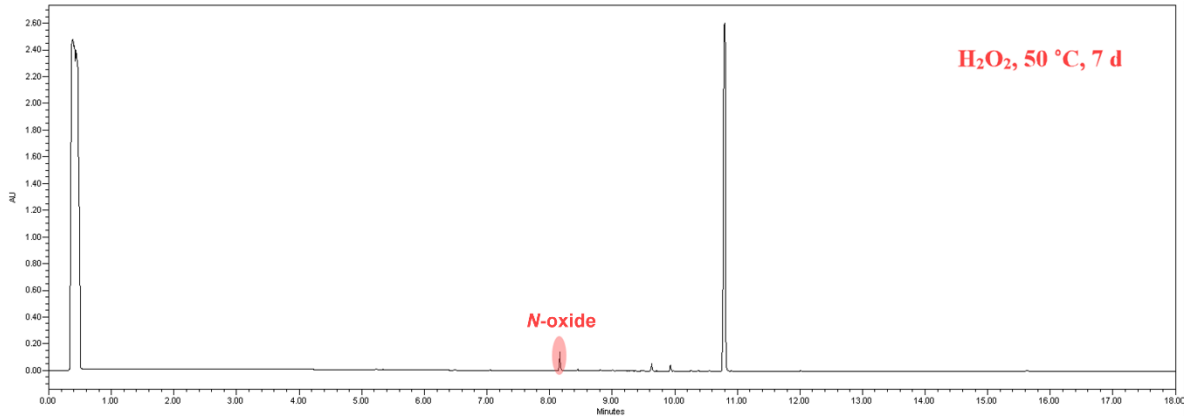


Figure S2. – Chromatogram of the venetoclax stress sample with added 3% H_2O_2 at 50 °C for 7 days. The rise of one degradation product was noticeable, which was later identified as an *N*-oxide venetoclax, where the piperazine moiety is oxidized.

Table S1 – ^{13}C and ^{1}H NMR spectroscopic data for venetoclax and its degradants (125 and 500 MHz, in DMSO-d_6). Chemical shifts (δ) are expressed in ppm with reference to residual solvent signal (2.50 ppm and 39.5 ppm for ^{1}H and ^{13}C, respectively).

Atom No.	Venetoclax		VENE-B1		VENE-B2		VENE-A1		VENE-A2		VENE-A3		VENE-A4	
	^{13}C shift [ppm] & peak multiplicity	**^{1}H shift [ppm], peak** multiplicity & coupling constants [*J*]	**^{13}C shift [ppm] & peak** multiplicity	**^{1}H shift [ppm], peak** multiplicity & coupling constants [*J*]	**^{13}C shift [ppm] & peak** multiplicity	**^{1}H shift [ppm], peak** multiplicity & coupling constants [*J*]**]**	^{13}C shift [ppm] & peak **multiplicity**	^{1}H shift [ppm], peak **multiplicity & coupling constants [*J*]**	^{13}C shift [ppm] & peak **multiplicity**	^{1}H shift [ppm], peak **multiplicity & coupling constants [*J*]**	^{13}C shift [ppm] & peak **multiplicity**	^{1}H shift [ppm], peak **multiplicity & coupling constants [*J*]**	^{13}C shift [ppm] & peak **multiplicity**	^{1}H shift [ppm], peak **multiplicity & coupling constants [*J*]**
2	127.7 s	7.49 – 7.51 m	127.9 s	7.54 m	127.9 s	7.50 – 7.53 m			127.6 s	7.47 m	125.3 s	7.34 (d) 2.5	127.7 s	7.52 m
3	100.0 s	6.38 (dd) 1.8, 3.0	100.1 s	6.43 (dd) 1.9, 3.4	100.0 s	6.42 (dd) 1.9, 3.4			99.9 s	6.38 (dd) 1.9, 3.3	112.6 s		99.9 s	6.42 (dd) 1.9, 3.4
3a	119.8 s		119.9 s		119.9 s				119.8 s		119.5 s		120.0 s	
4	117.9 s	7.54 (d) 2.5	118.5 s	7.62 (d) 2.5	118.3 s	7.60 (d) 2.6			116.3 s	7.38 – 7.41 m	117.3 s	7.66 (d) 2.6	118.8 s	7.63 (dd) 0.5, 2.6
5	146.5 s		146.1 s		146.1 s				148.3 s		145.5 s		146.4 s	
6	135.3 s	8.05 (d) 2.5	135.4 s	8.07 – 8.08 m	135.4 s	8.06 (d) 2.6			134.6 s	7.98 (d) 2.6	135.2 s	7.97 (d) 2.6	135.8 s	8.03 (d) 2.6
7a	145.4 s		145.6 s		145.6 s				144.9 s		145.9 s		145.5 s	
8	157.8 s		158.1 s		158.1 s				158.4 s		158.1 s		159.7 s	
9	102.4 s	6.18 (d) 1.6	102.6 s	6.22 (d) 2.3	102.6 s	6.23 (d) 2.4			105.6 s	6.41 (d) 2.5	102.3 s	6.19 (d) 2.3	104.7 s	6.57 m
10	154.5 s		153.5 s		153.6 s				153.4 s		153.5 s		151.0 s	
11	108.7 s	6.66 (dd) 1.6, 9.2	109.0 s	6.68 (dd) 2.3, 8.9	109.1 s	6.71 (dd) 2.4, 9.0			109.5 s	6.77 (dd) 2.5, 8.9	109.0 s	6.69 (dd) 2.3, 9.0	110.0 s	6.65 (dd) 2.0, 8.1

12	132.1 s	7.49 – 7.51 m	132.1 s	7.47 (d) 8.9	132.3 s	7.50 – 7.53 m			133.5 s	7.78 (d) 8.9	132.2 s	7.51 (d) 9.0	130.3 s	7.18 m
13	112.6 s		113.5 s		113.2 s				113.0 s		113.2 s		108.1 s	6.34 (dd) 2.0, 8.1
14	163.8 s		163.3 s		163.5 s				165.9 s		163.5 s			
16	124.9 s		131.1 s		129.3 s		130.0 s				124.3 s			
17	127.7 s	8.57 (d) 2.2	110.0 s	8.07 – 8.08 m	126.1 s	8.39 (d) 2.4	124.7 s	8.47 (d) 2.3			127.9 s	8.58 (d) 2.3		
18	129.5 s		132.9 s		136.2 s		129.4 s				129.6 s			
19	147.3 s		140.3 s		156.1 s		146.7 s				147.5 s			
20	115.0 s	7.10 (d) 9.3	119.1 s	7.67 m	119.6 s	7.23 (d) 8.9	115.7 s	7.30 (d) 9.2			115.1 s	7.13 (d) 9.4		
21	133.9 s	7.81 (dd) 1.8, 9.3	120.6 s	7.67 m	133.9 s	7.98 (dd) 2.4, 8.9	132.7 s	7.82 (dd) 2.3, 9.2			133.9 s	7.84 (dd) 2.3, 9.4		
23	47.9 s	3.29 m	157.1 s				47.8 s	3.35 m			47.9 s	3.28 m		
24	33.8 s	1.88 m	31.9 s	3.37 m			33.9 s	1.90 m			33.9 s	1.86 m		
25, 28	30.2 s	1.25 m, 1.60 m	29.9 s	1.82 – 1.90 m			66.6 s	1.26 m, 1.61 m			30.1 s	1.23 m, 1.58 m		
26, 27	66.6 s	3.25 m, 3.84 m	66.5 s	3.50 m, 3.96 m			30.1 s	3.26 m, 3.85 m,			66.6 s	3.23 m, 3.82 m,		
2′, 6′	46.5 s	3.06 br.s	43.8 s	3.02 br.s, 3.64 br.s	43.8 s	3.01 br.s, 3.64 br.s				3.06 br.s, 3.74 br.s	43.8 s	3.00 br.s, 3.62 br.s	45.0 s	3.01 br.m, 3.65 br.m
3′, 5′	52.0 s	2.19 br.s	50.5 s	2.74 br.s, 3.26 br.s	50.5 s	2.75 br.s, 3.25 br.s				2.78 br.s, 3.28 br.s	50.5 s	2.74 br.s, 3.20 br.s	50.8 s	2.83 br.m, 3.33 br.m
7′	59.6 s	2.74 s	58.0 s	3.56 s	58.0 s	3.57 s			58.0 s	3.59 s	58.1 s	3.56 s	57.9 s	3.64 s
8′	128.5 s		121.7 s		121.7 s						121.8 br.s		121.7 s	
9′	134.6 s		141.6 s		141.6 s				141.7 s		141.6 br.s		141.7 s	
10′	46.3 s	1.93 s	46.6 s	2.00 s	46.6 s	2.00 s			46.6 s	2.02 s	46.6 s	2.00 s	46.5 s	2.05 s
11′	28.8 s		28.6 s		28.7 s				28.7 s		28.7 s		28.7 s	
12'	34.8 s	1.36 (t) 6.2	34.2 s	1.44 m	34.2 s	1.44 m			34.3 s	1.46 m	34.2 s	1.44 m	34.2 s	1.49 (t) 6.3
13′	25.1 s	2.12 br.m	24.7 s	2.18 br.m	24.8 s	2.17 br.m			24.8 s	2.20 br.m	24.8 s	2.18 br.m	24.8 s	2.23 br.m

1″	141.9 s		140.3 s		140.3 s			140.4 s		140.3 s		140.3 s		
2″, 6″	130.0 s	7.02 m	129.7 s	7.07 m	129.8 s	7.07 m		129.8 s	7.09 m	129.8 s	7.07 m	129.8 s	7.13 m	
3″, 5″	128.1 s	7.32 m	128.7 s	7.38 m	128.7 s	7.39 m		128.7 s	7.38 – 7.41 m	128.7 s	7.37 m	128.7 s	7.43 m	
4″	130.8 s		131.7 s		131.8 s			131.8 s		131.8 s		131.8 s		
3-CH_2										20.8 s	4.03 s			
11′-$(CH_3)_2$	27.9 s	0.90 s	27.8 s	0.93 s	27.8 s	0.94 s		27.9 s	0.95 s	27.8 s	0.93 s	27.8 s	0.97 s	
1-NH		11.70 s		11.77 s		11.74 s			11.64 s		11.64 br.s		11.72 s	
15-NH		11.32 br. s		11.68 s		11.77 br.s					11.43 (d) 2.5			
22-NH		8.61 (t) 5.8					8.75 (t) 6.0				8.62 (t) 6.0			
14 - OH									9.34 br. s					
NH^+(4′)													9.25 br.s	
15-NH_2							7.32 (br.s)							

Venetoclax information

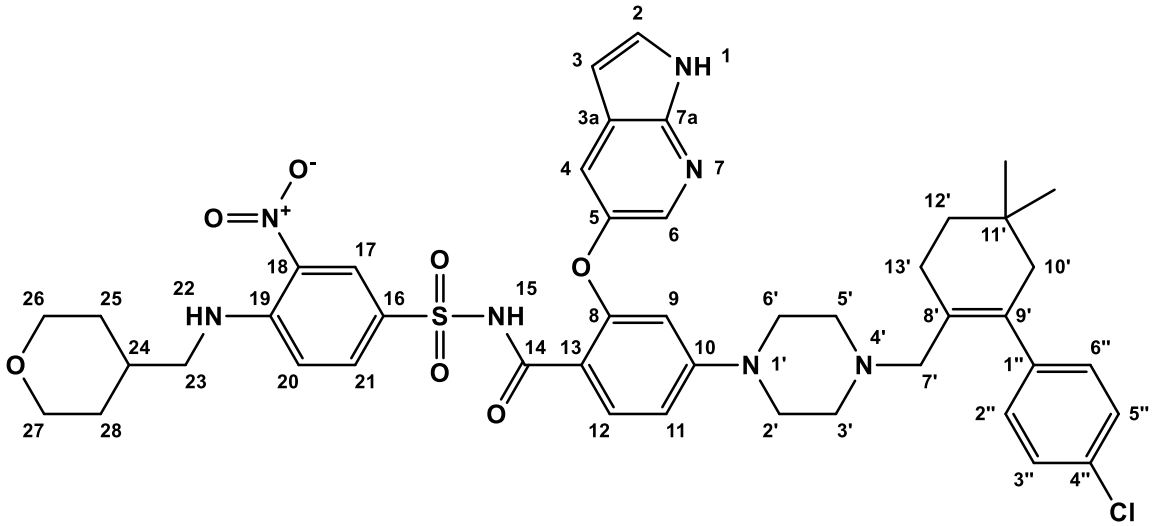


Figure S3. – Venetoclax with NMR assignations.

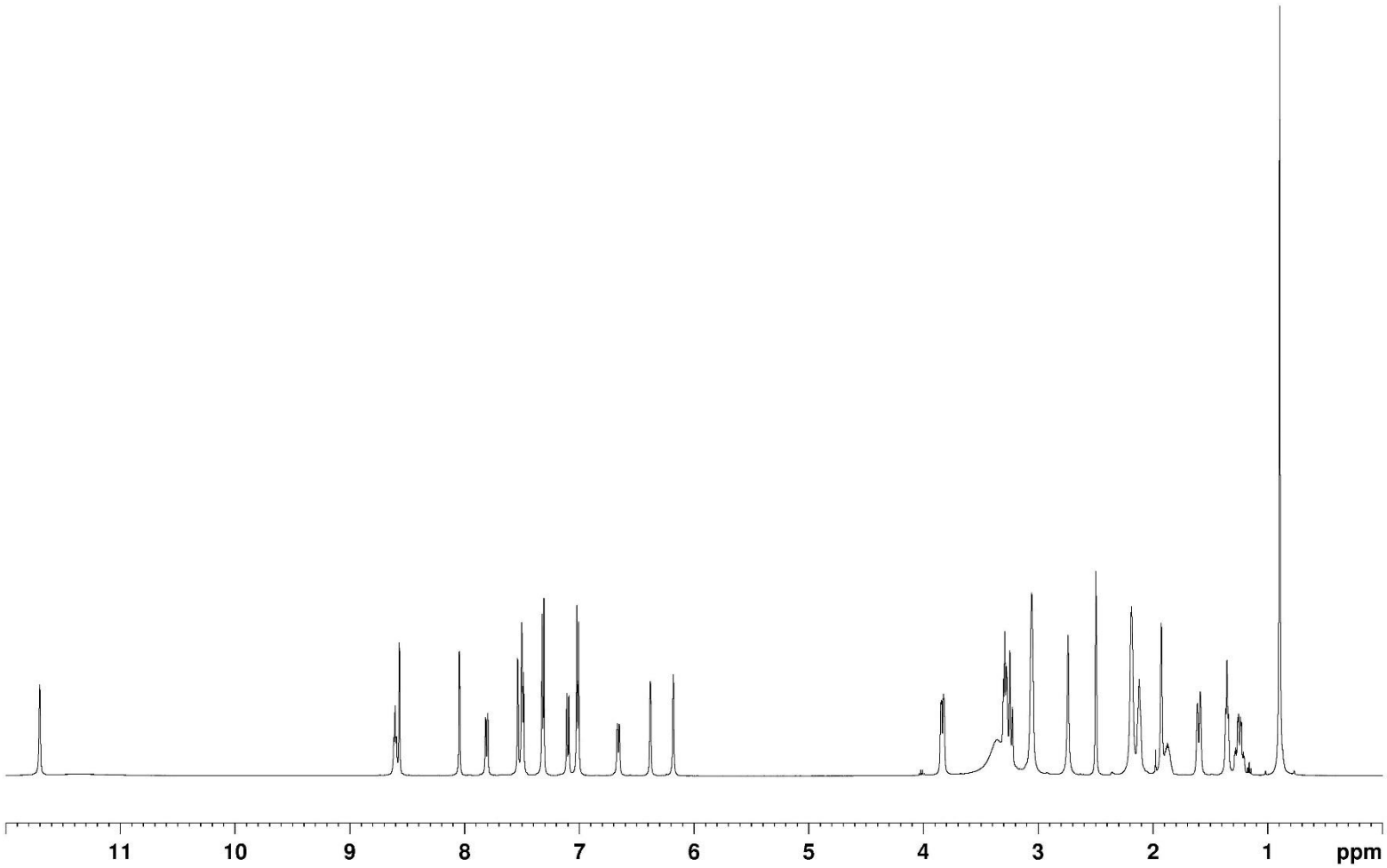


Figure S4 – ^{1}H NMR spectrum of **venetoclax**

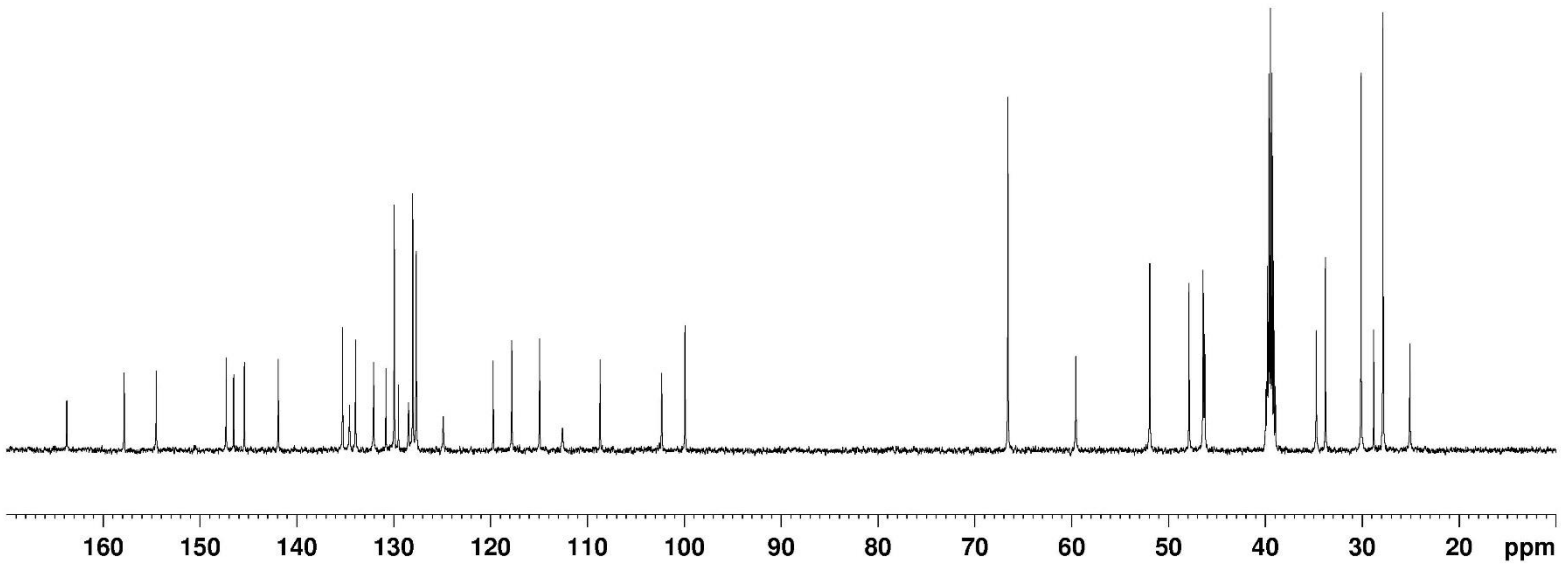


Figure S5 – ^{13}C NMR spectrum of **venetoclax**

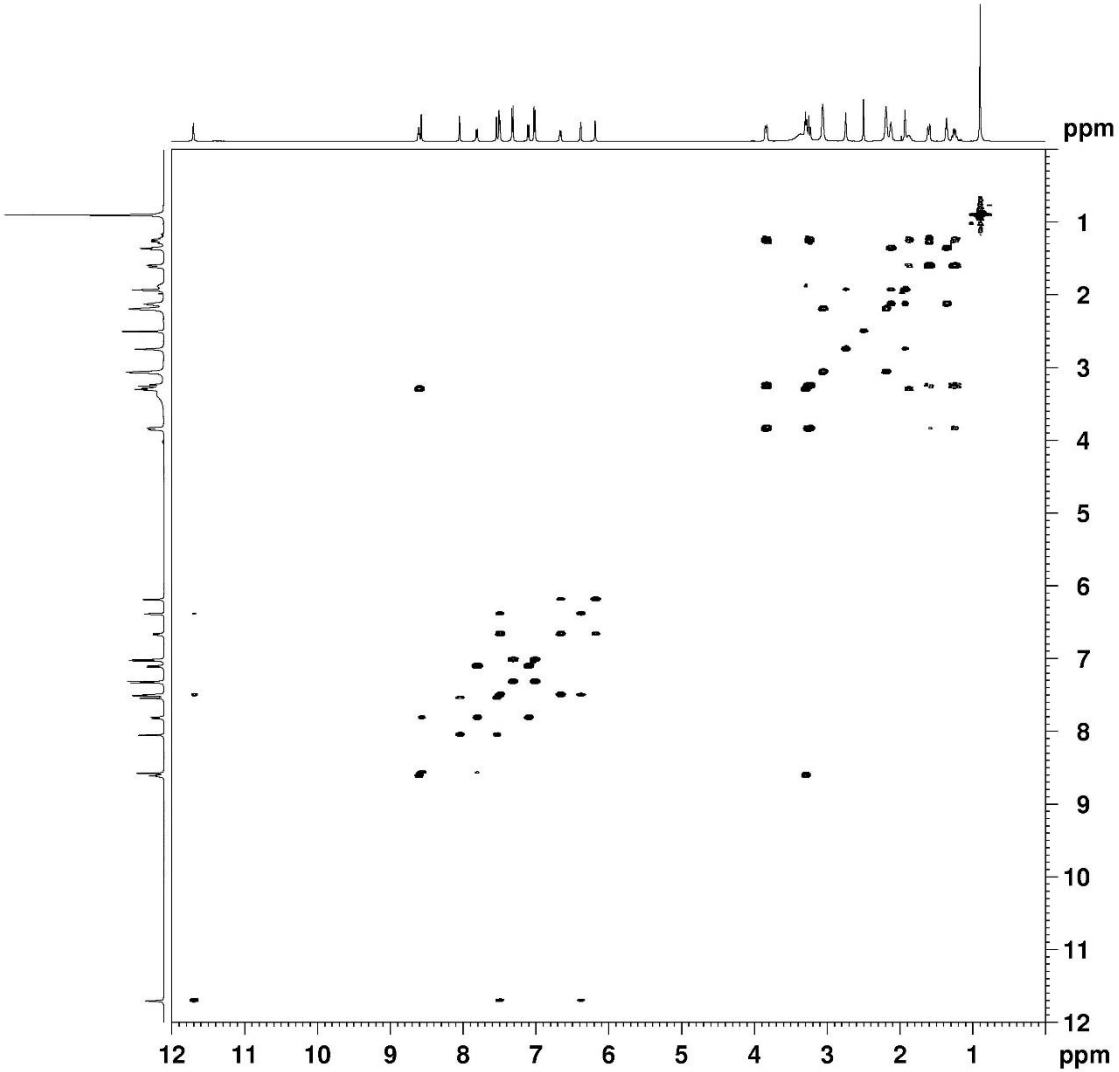


Figure S6 – (^{1}H, ^{1}H)-COSY spectrum of **venetoclax**

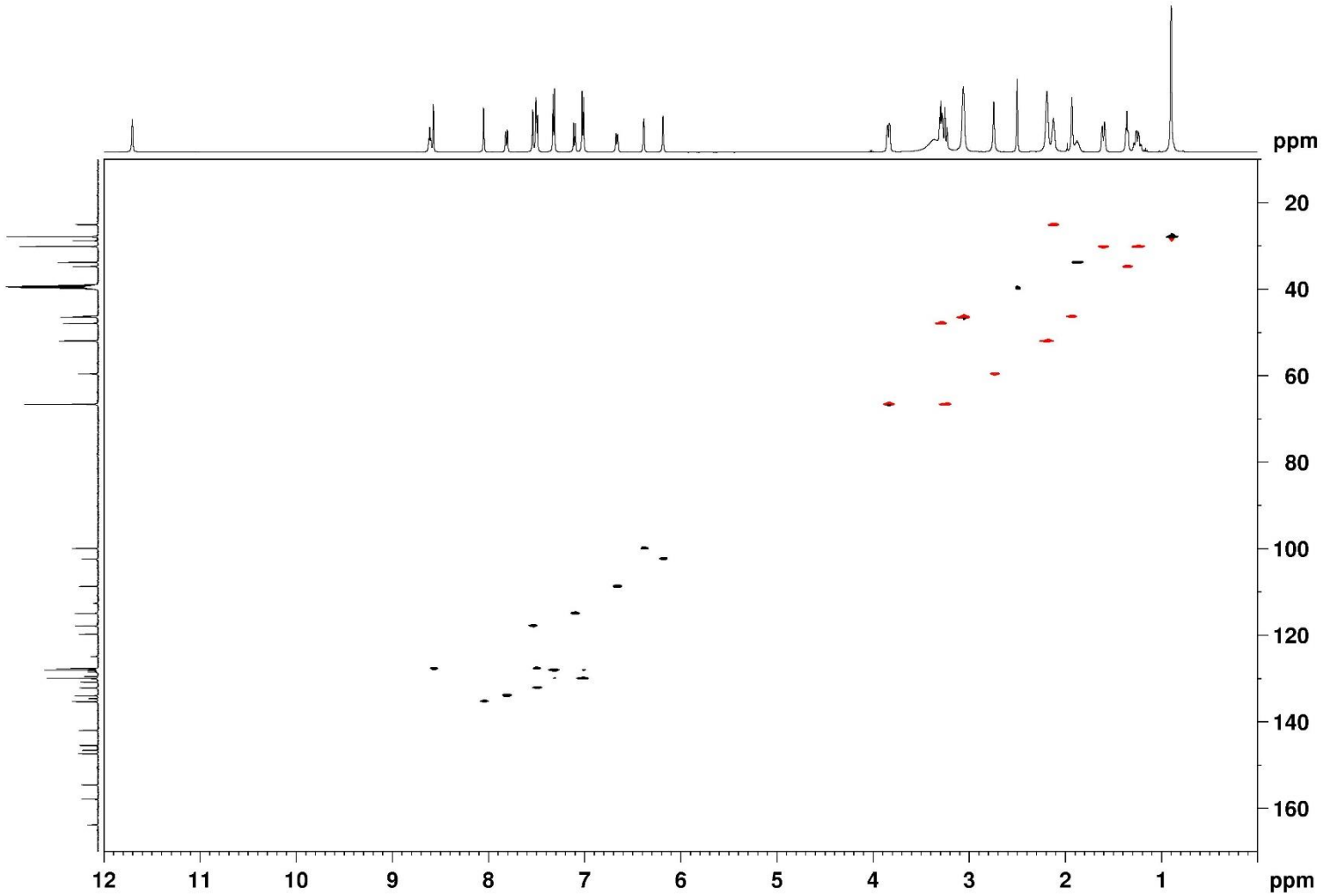


Figure S7 – (^{1}H, ^{13}C)-HSQC spectrum of **venetoclax**

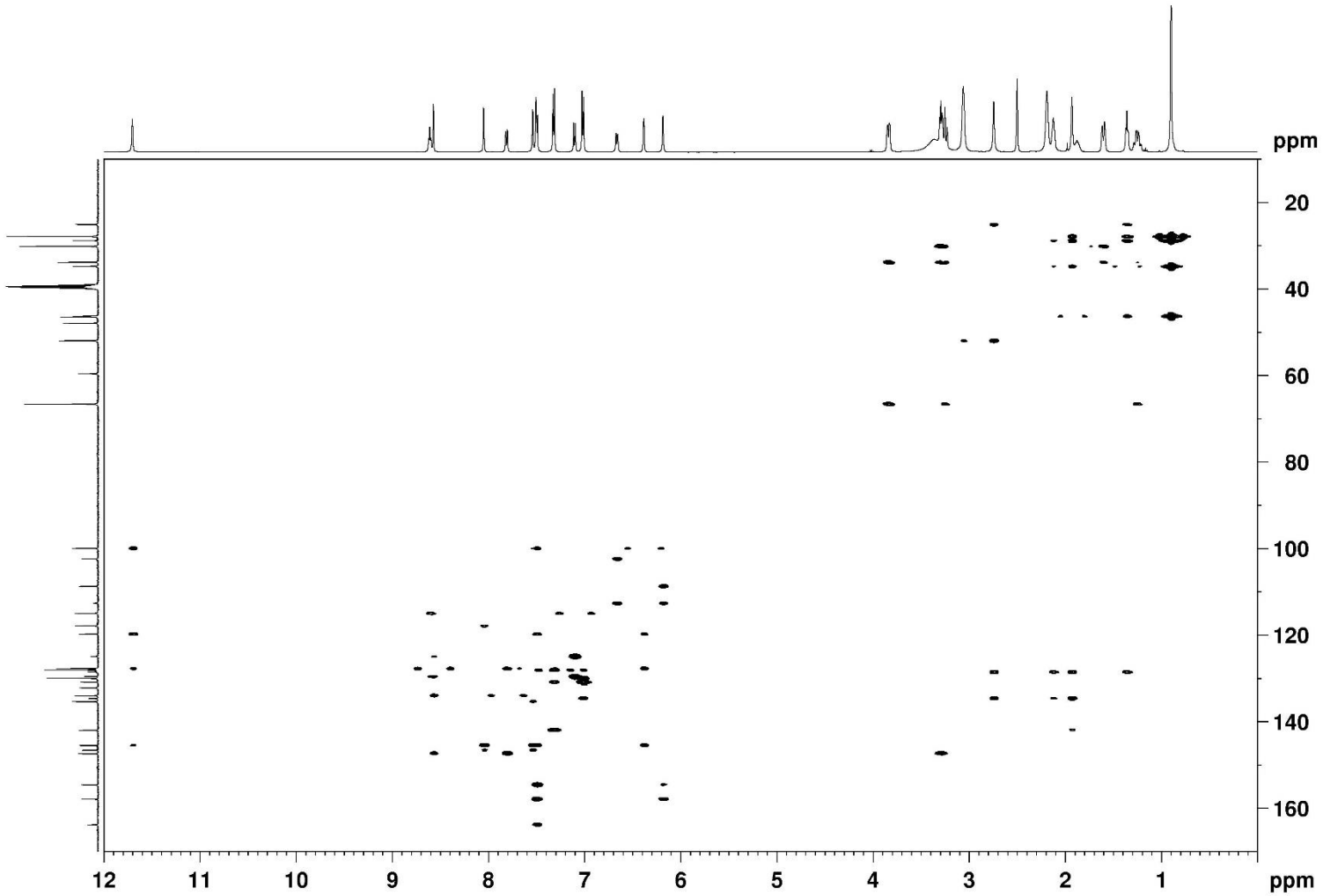


Figure S8 – (^{1}H, ^{13}C)-HMBC spectrum of **venetoclax**

Degradation product A1 information

3-nitro-4-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)benzenesulfonamide: crystalline solid; m.p. 190.3 °C; HRMS $[M+H]^+$: calculated 316.0962, found 316.0958.

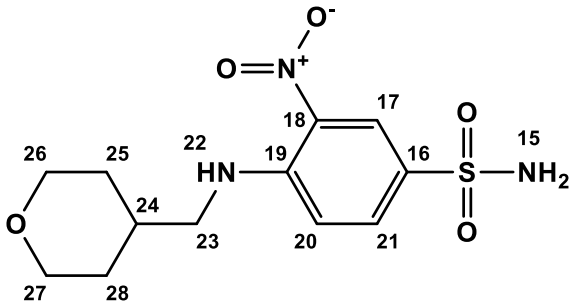


Figure S9 – Degradation product A1 with NMR assignations

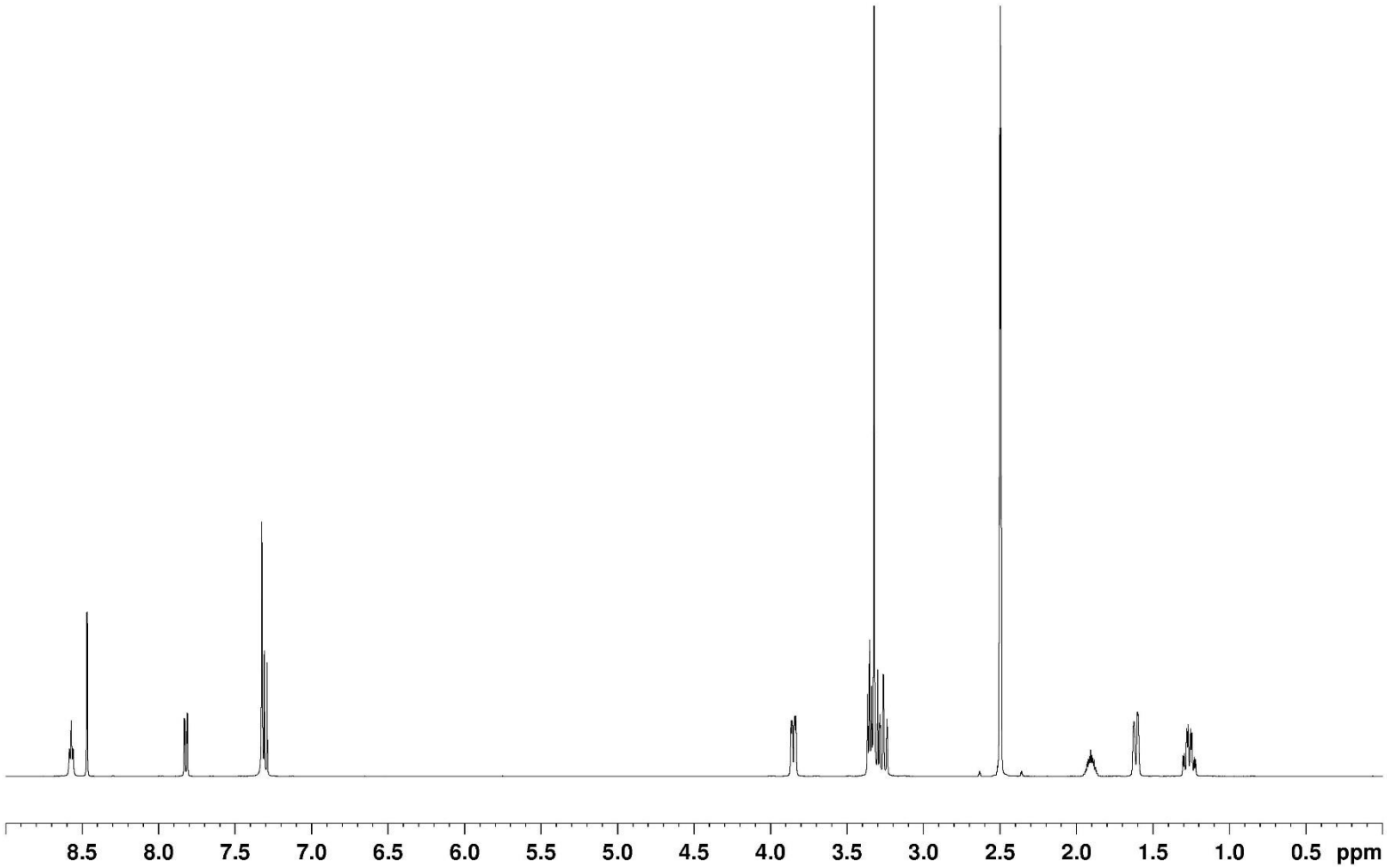


Figure S10 – ^{1}H NMR spectrum of **A1**

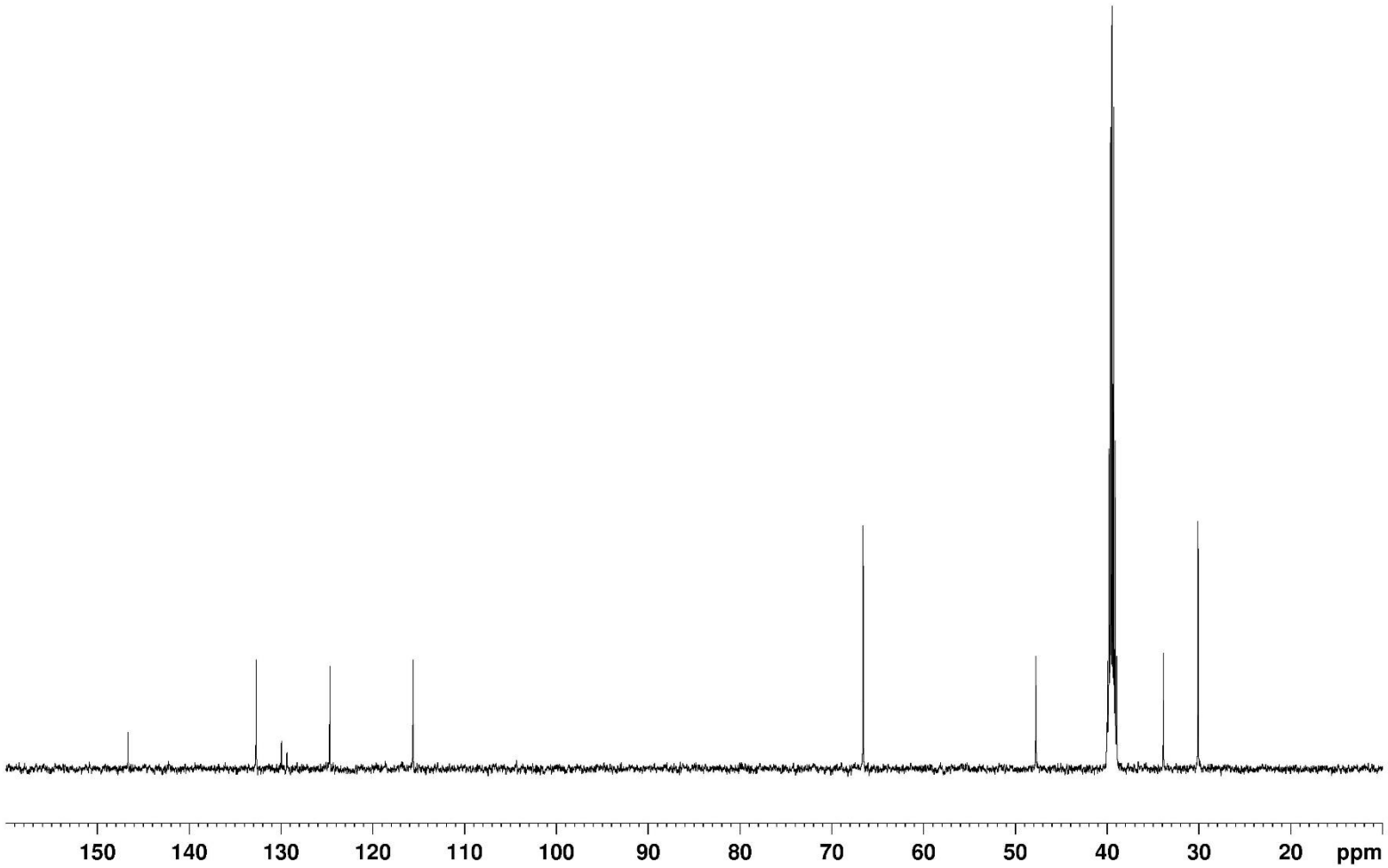


Figure S11 – ^{13}C NMR spectrum of **A1**

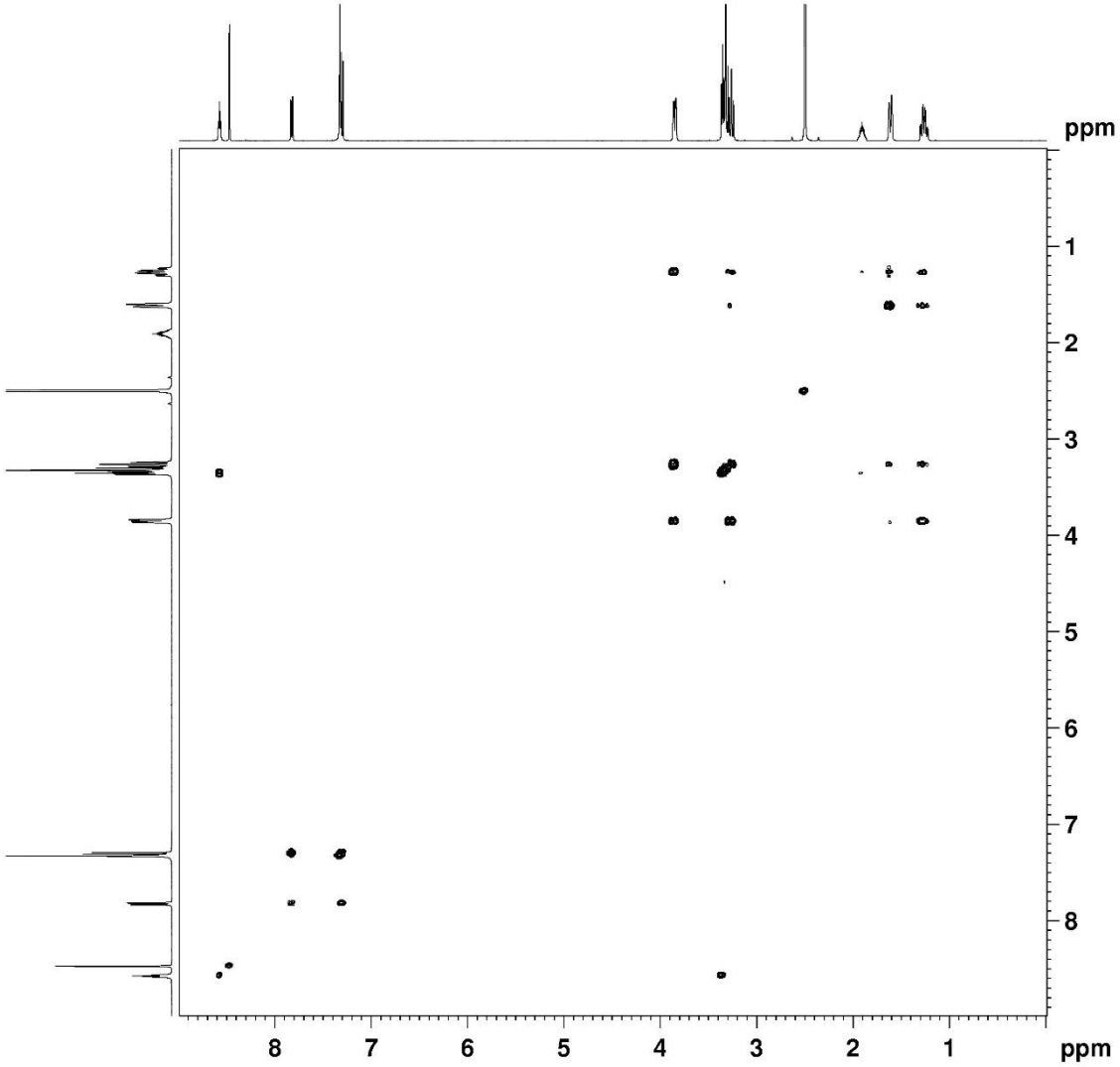


Figure S12 – (^{1}H, ^{1}H)-COSY spectrum of **A1**

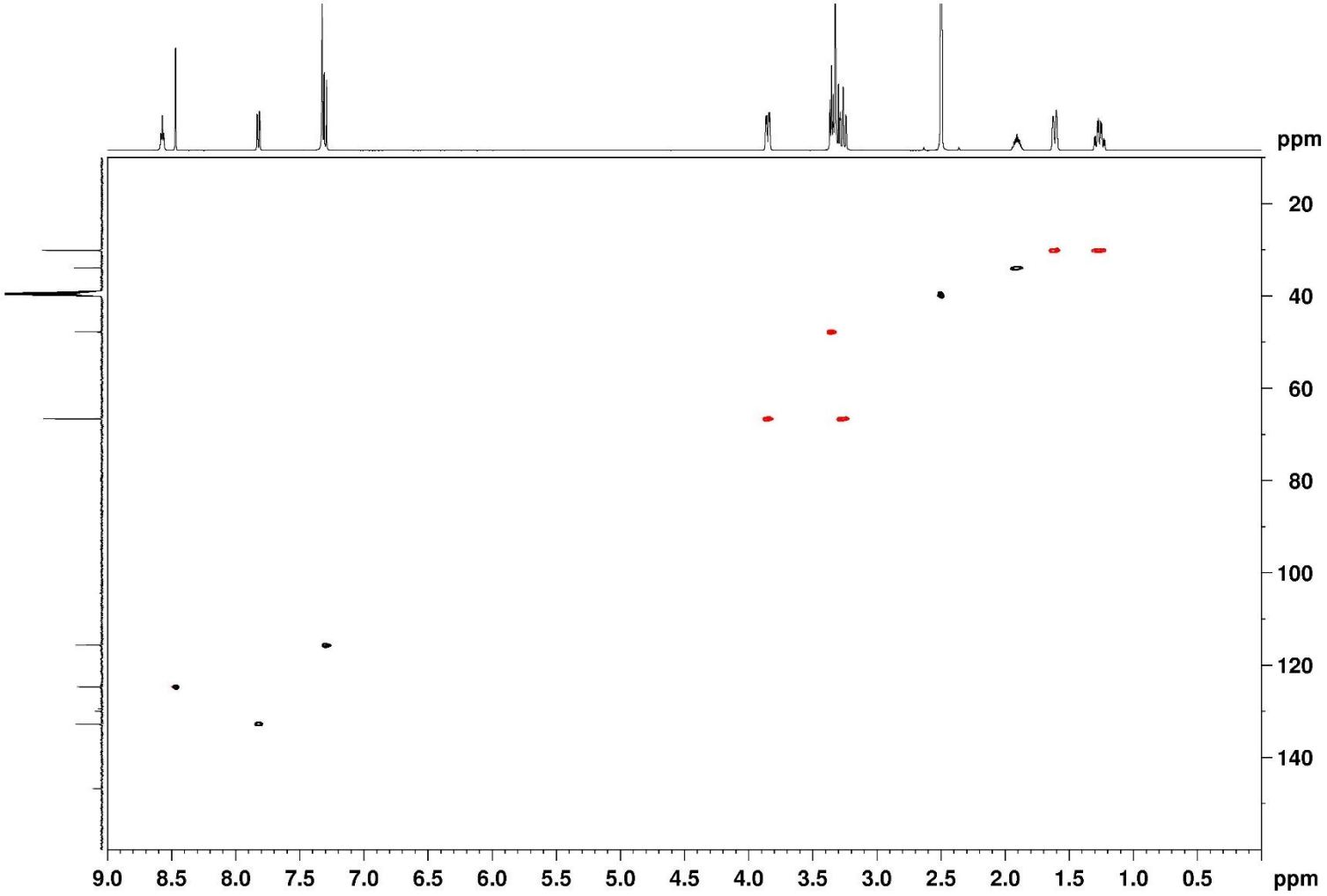


Figure S13 – (^{1}H, ^{13}C)-HSQC spectrum of **A1**

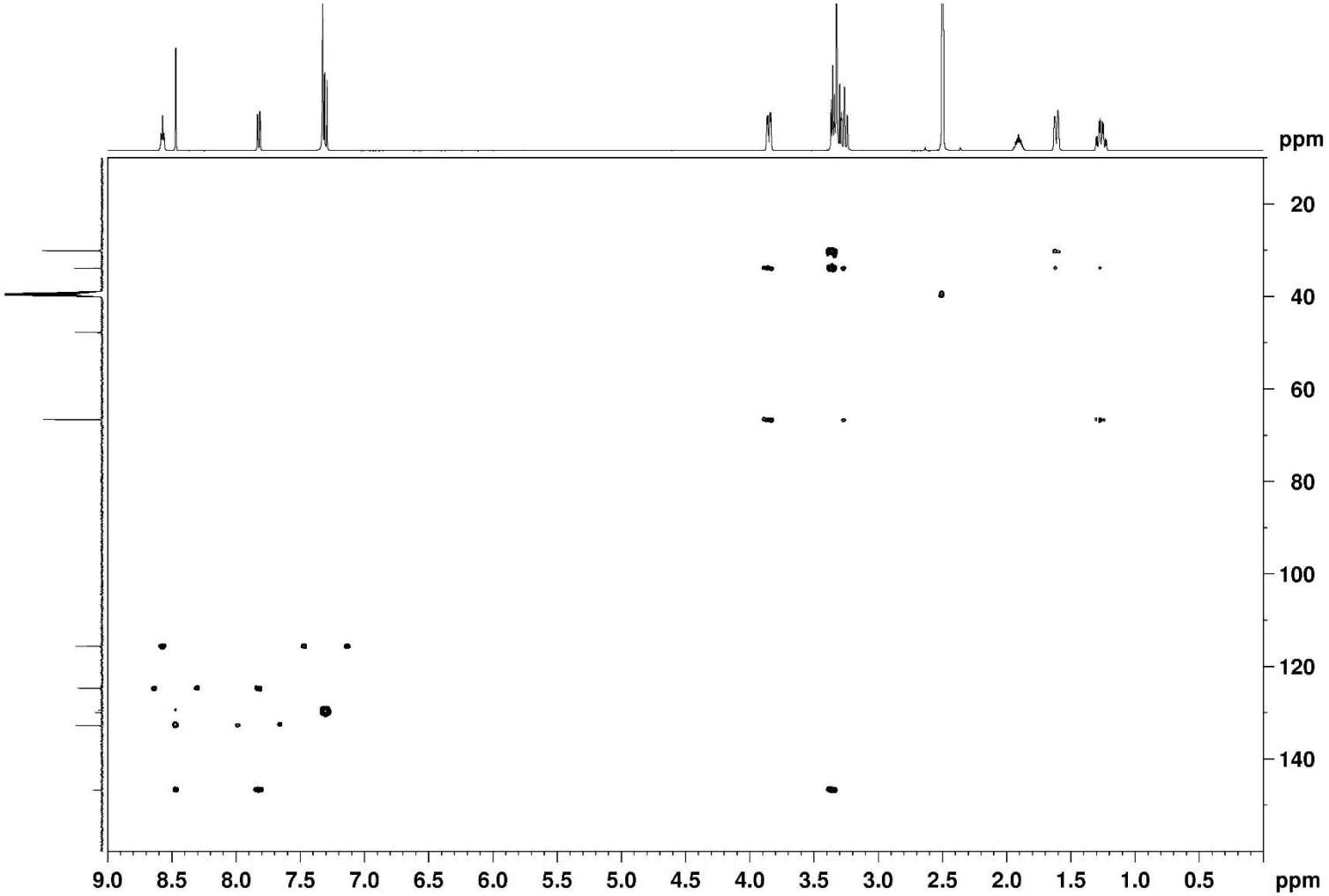


Figure S14 – (^{1}H, ^{13}C)-HMBC spectrum of **A1**

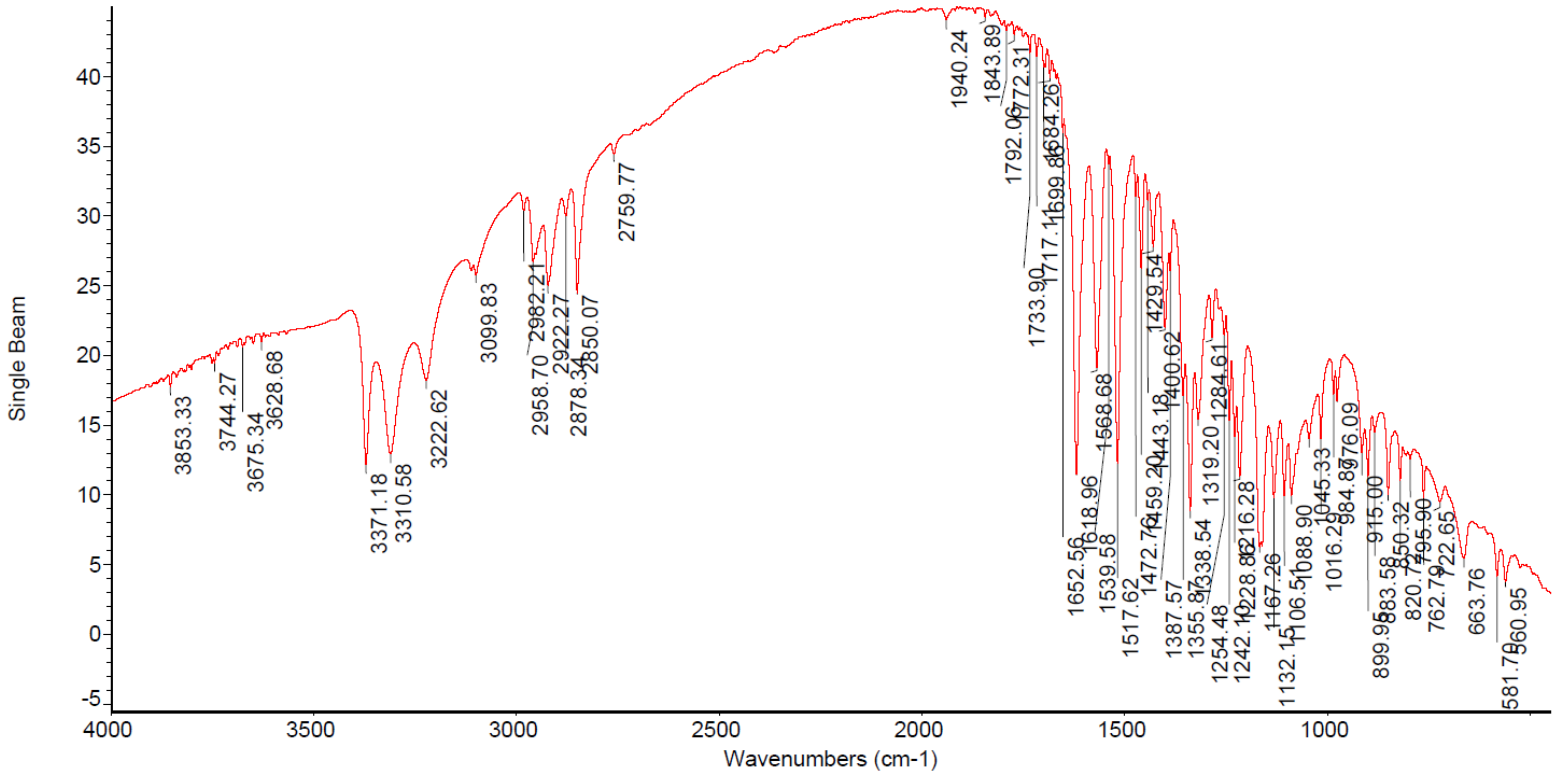


Figure S15 – IR spectrum of **A1**

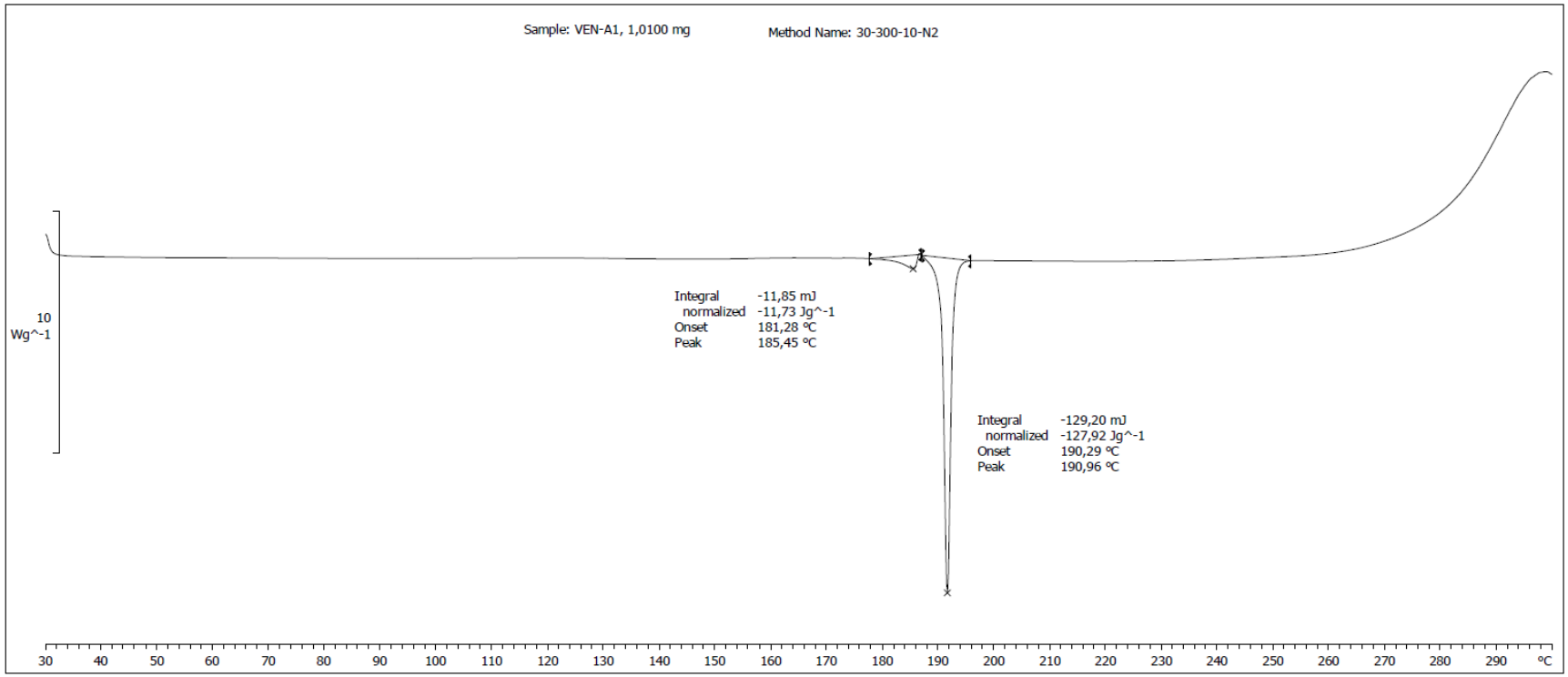


Figure S16 – DSC curve for **A1**

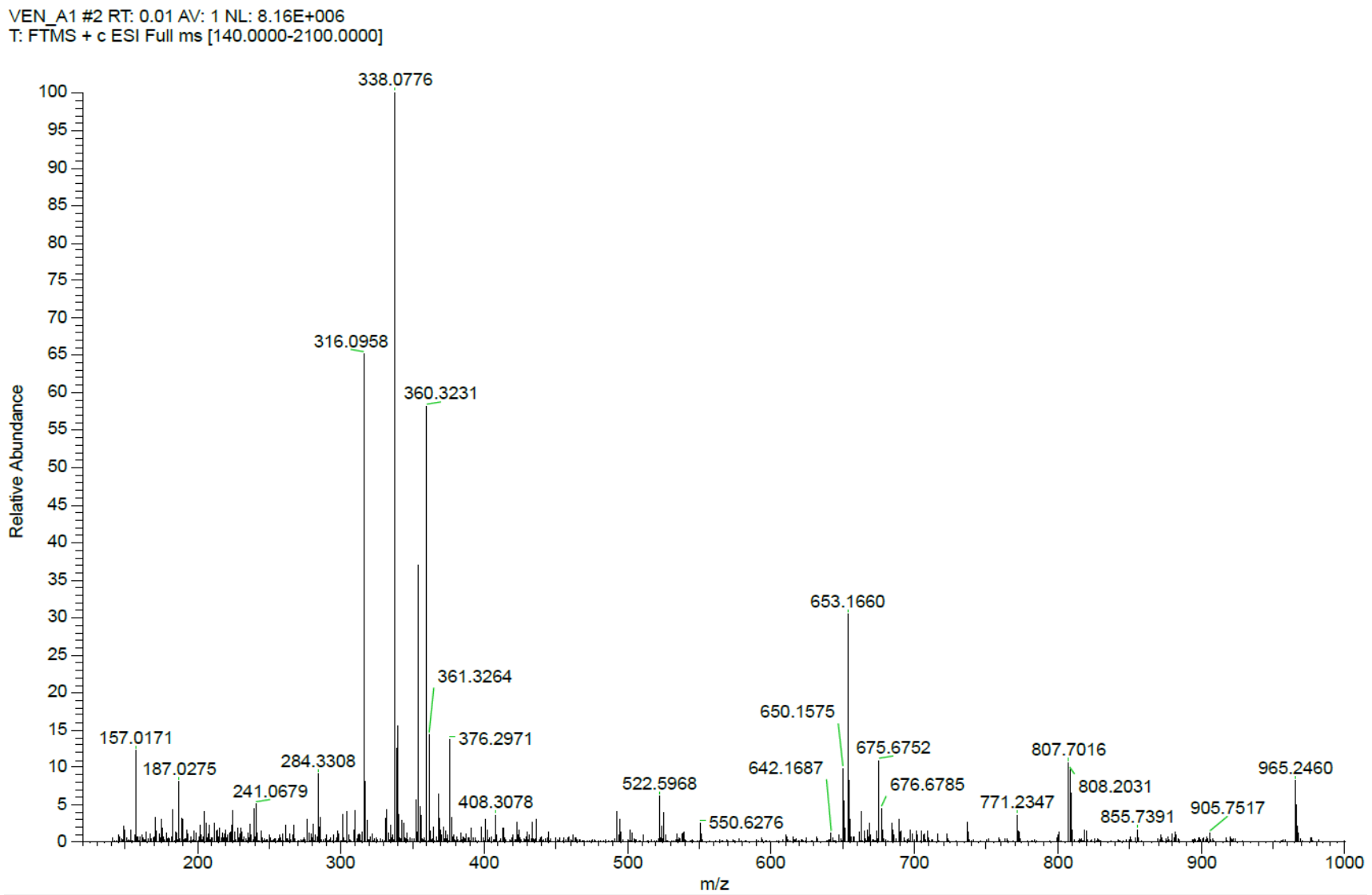


Figure S17 – HRMS spectrum of **A1**

Degradation product A2 information

2-((1*H*-pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoic acid: amorphous solid; HRMS $[M+H]^+$: calculated 571.2470, found 571.2458.

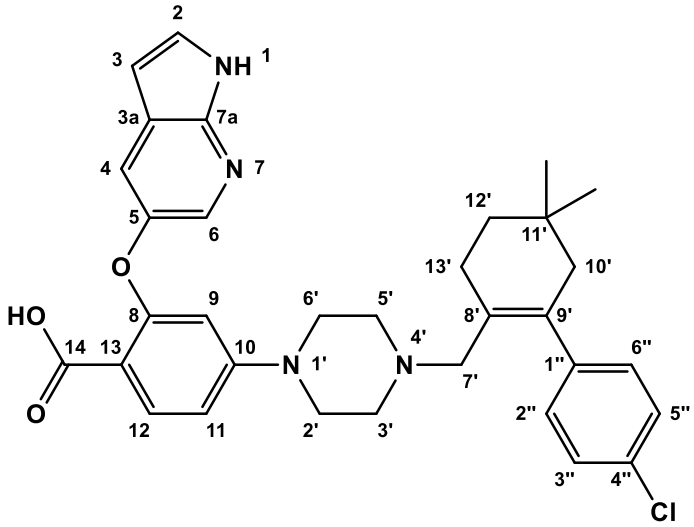


Figure S18 – Degradation product A2 with NMR assignations

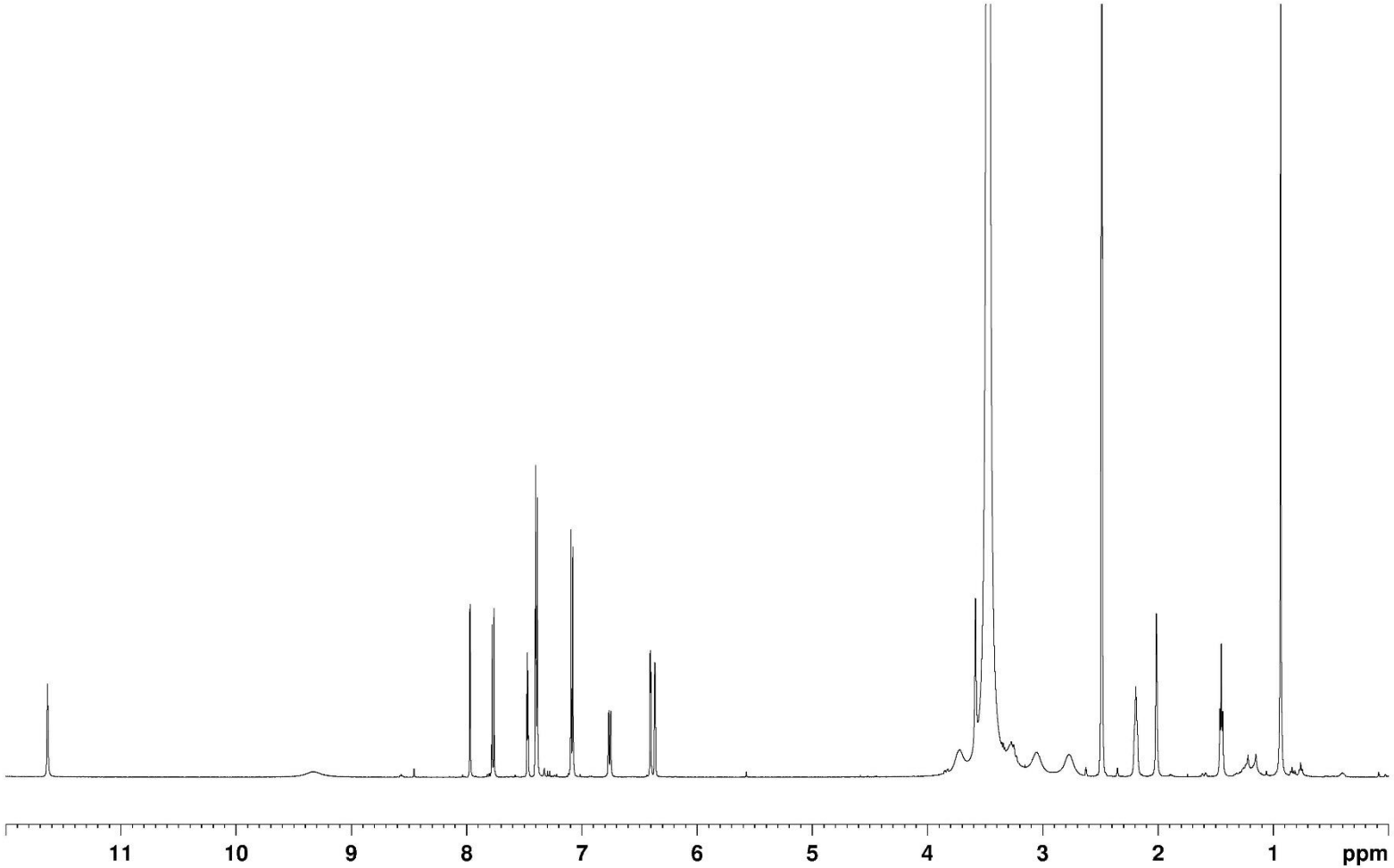


Figure S19 – ^{1}H NMR spectrum of **A2**

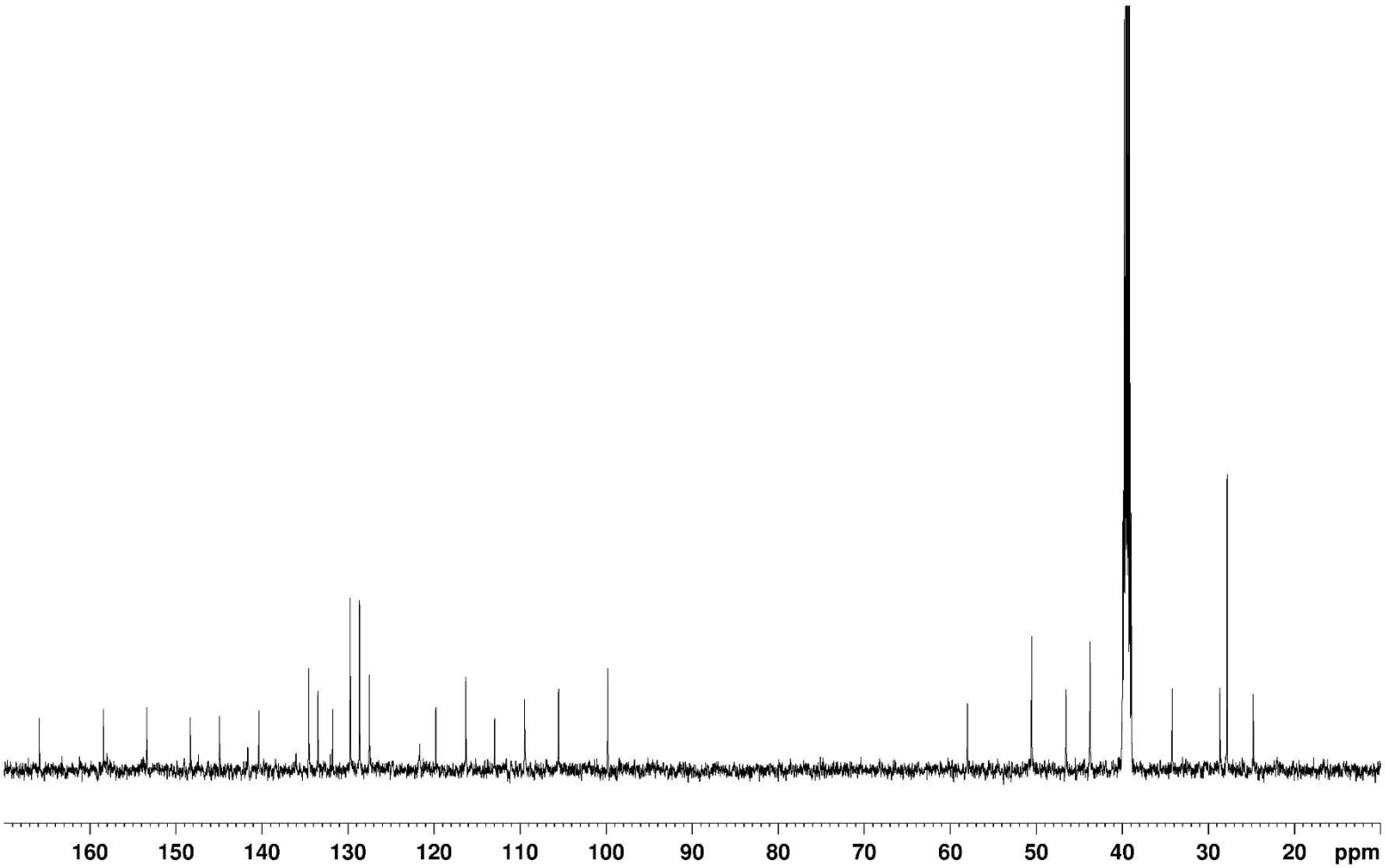


Figure S20 – ^{13}C NMR spectrum of **A2**

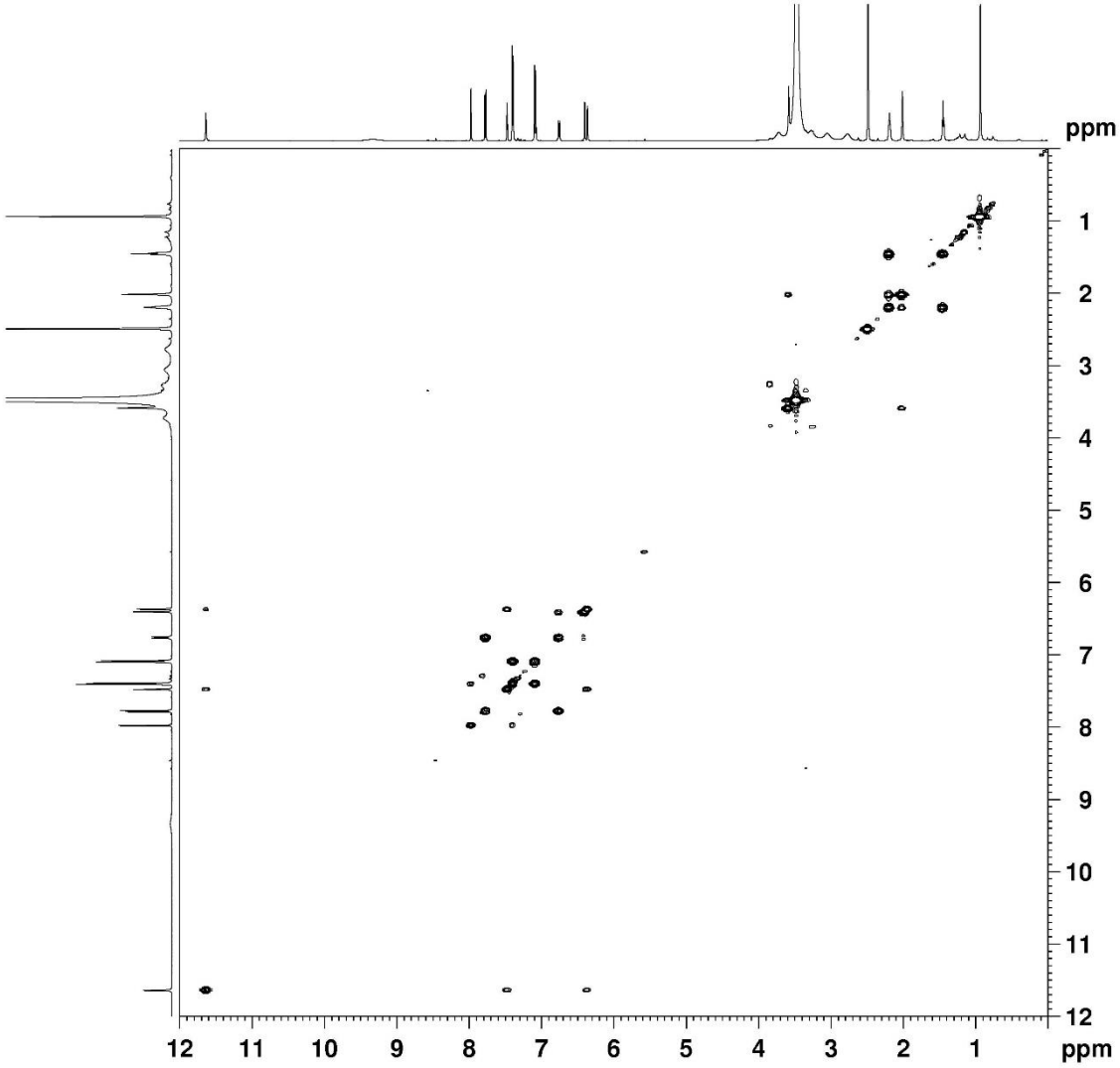


Figure S21 – (^{1}H, ^{1}H)-COSY spectrum of **A2**

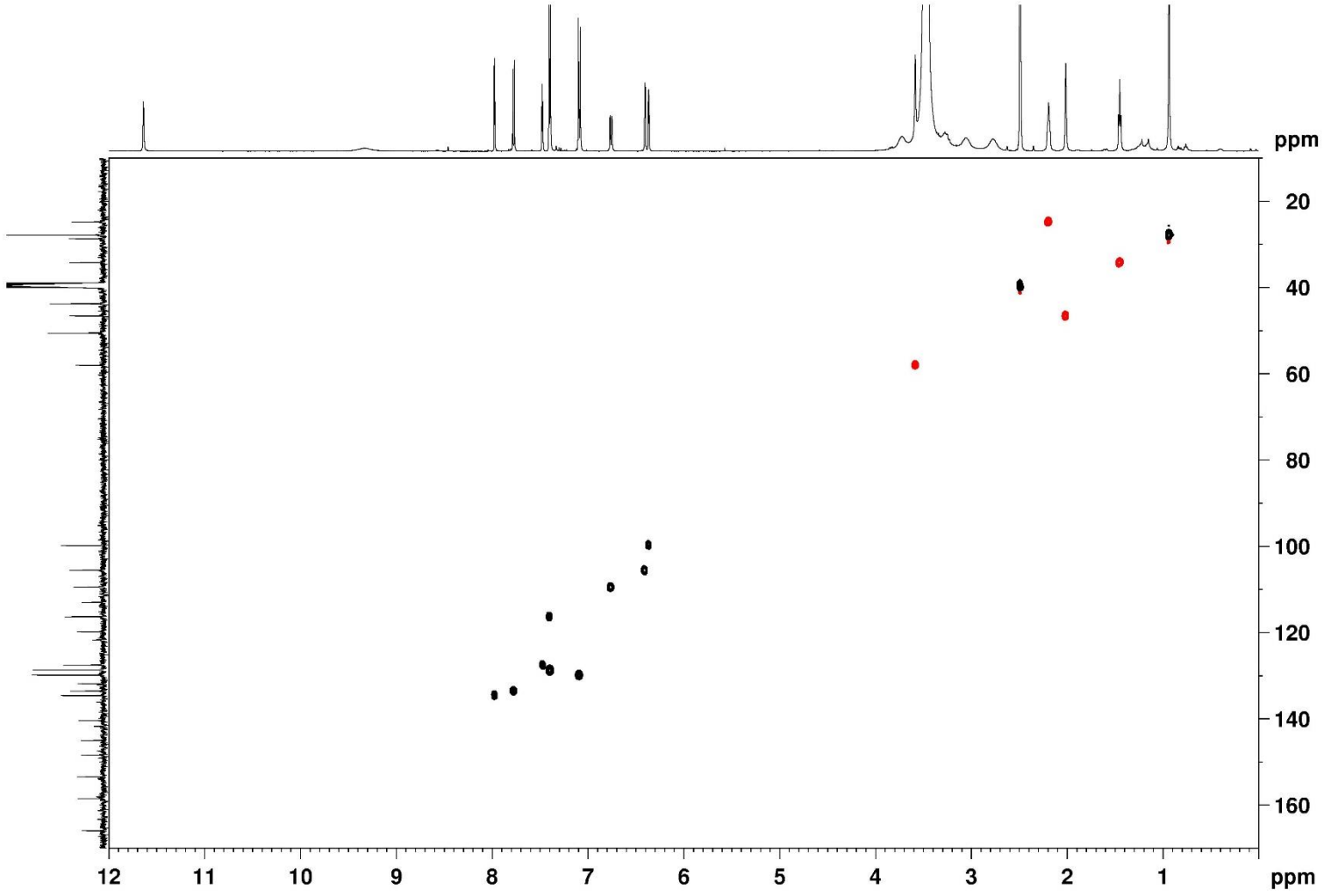


Figure S22 – (^{1}H, ^{13}C)-HSQC spectrum of **A2**

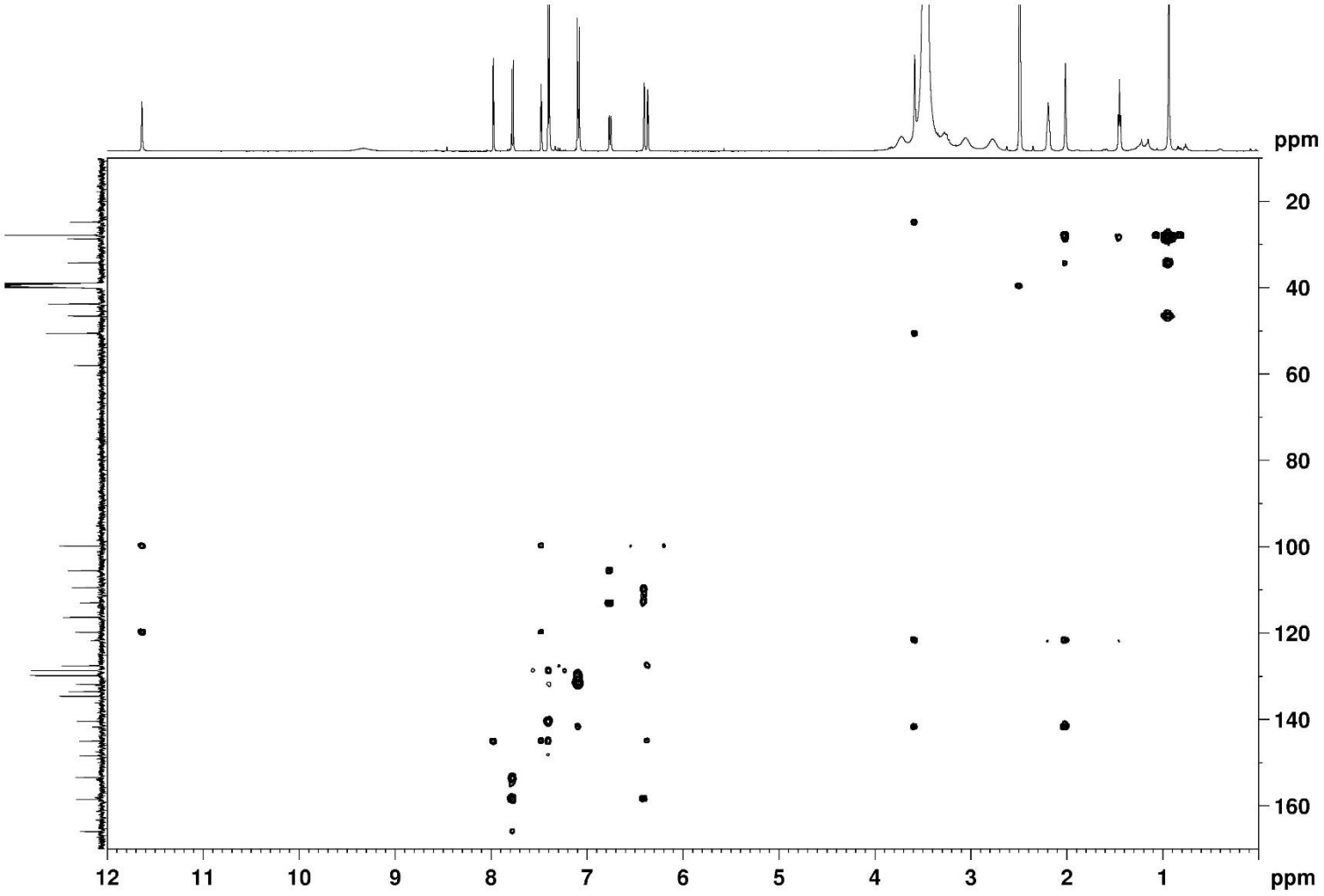


Figure S23 – (^{1}H, ^{13}C)-HMBC spectrum of **A2**

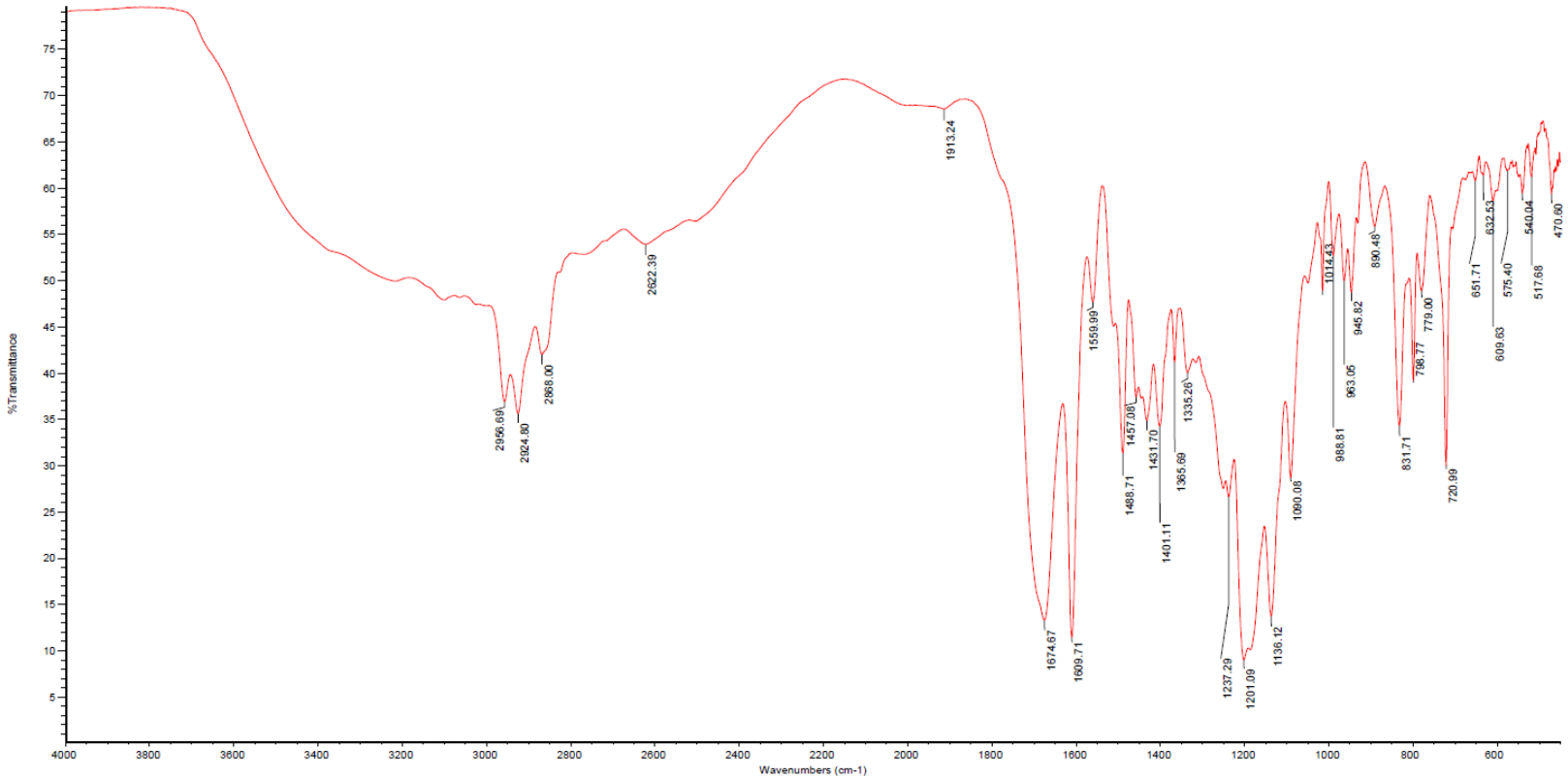


Figure S24 – IR spectrum of **A2**

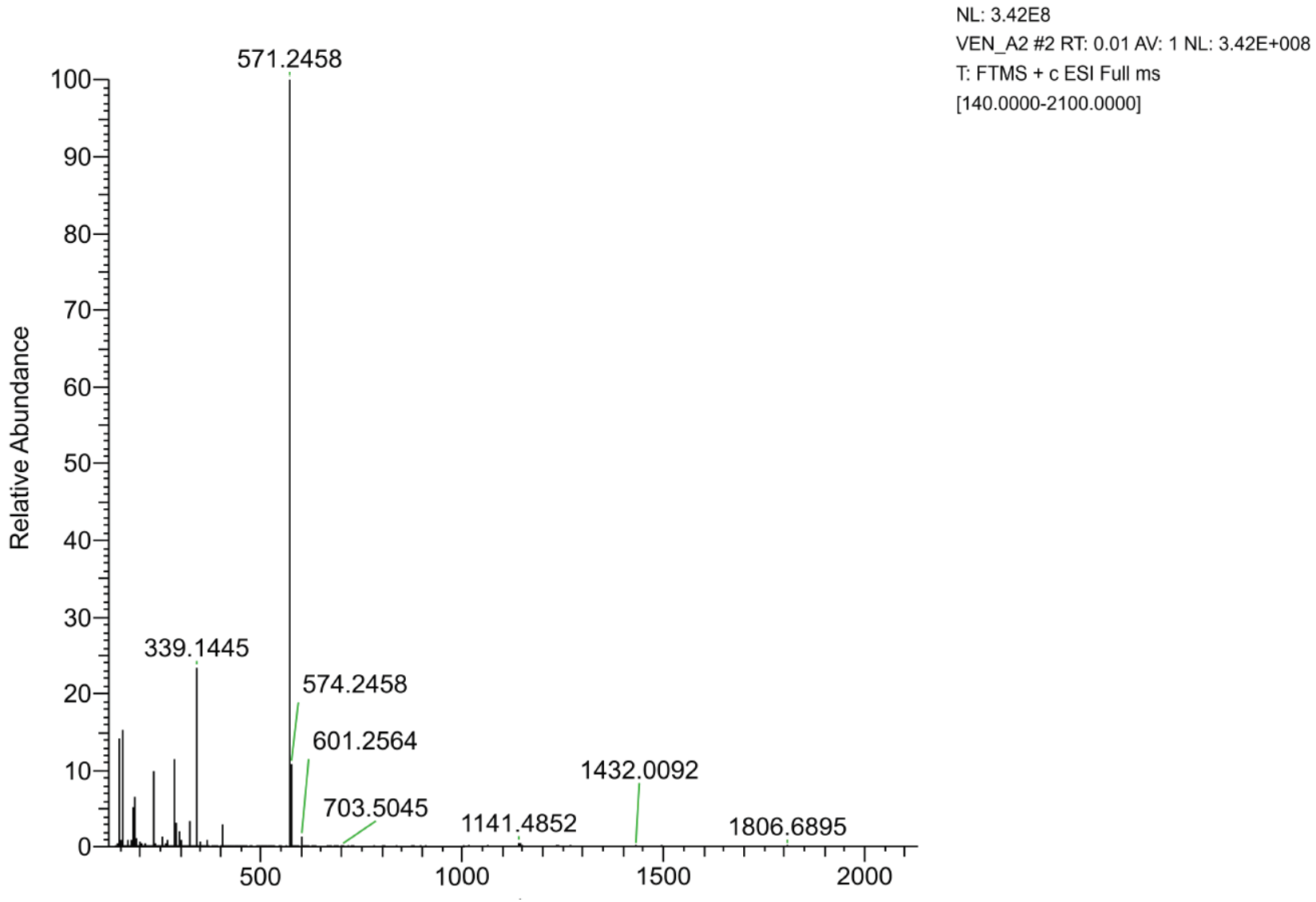


Figure S25 – HRMS spectrum of **A2**

Degradation product A3/B3 information

2,2'-((methylenebis(1*H*-pyrrolo[2,3-b]pyridine-1,5-diyl))bis(oxy))bis(4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((3-nitro-4-(((tetrahydro-2*H*-pyran-4-yl)methyl)amino)phenyl)sulfonyl)benzamide): amorphous solid; HRMS $[M+H]^+$: calculated 1747.6435, found 1747.6434.

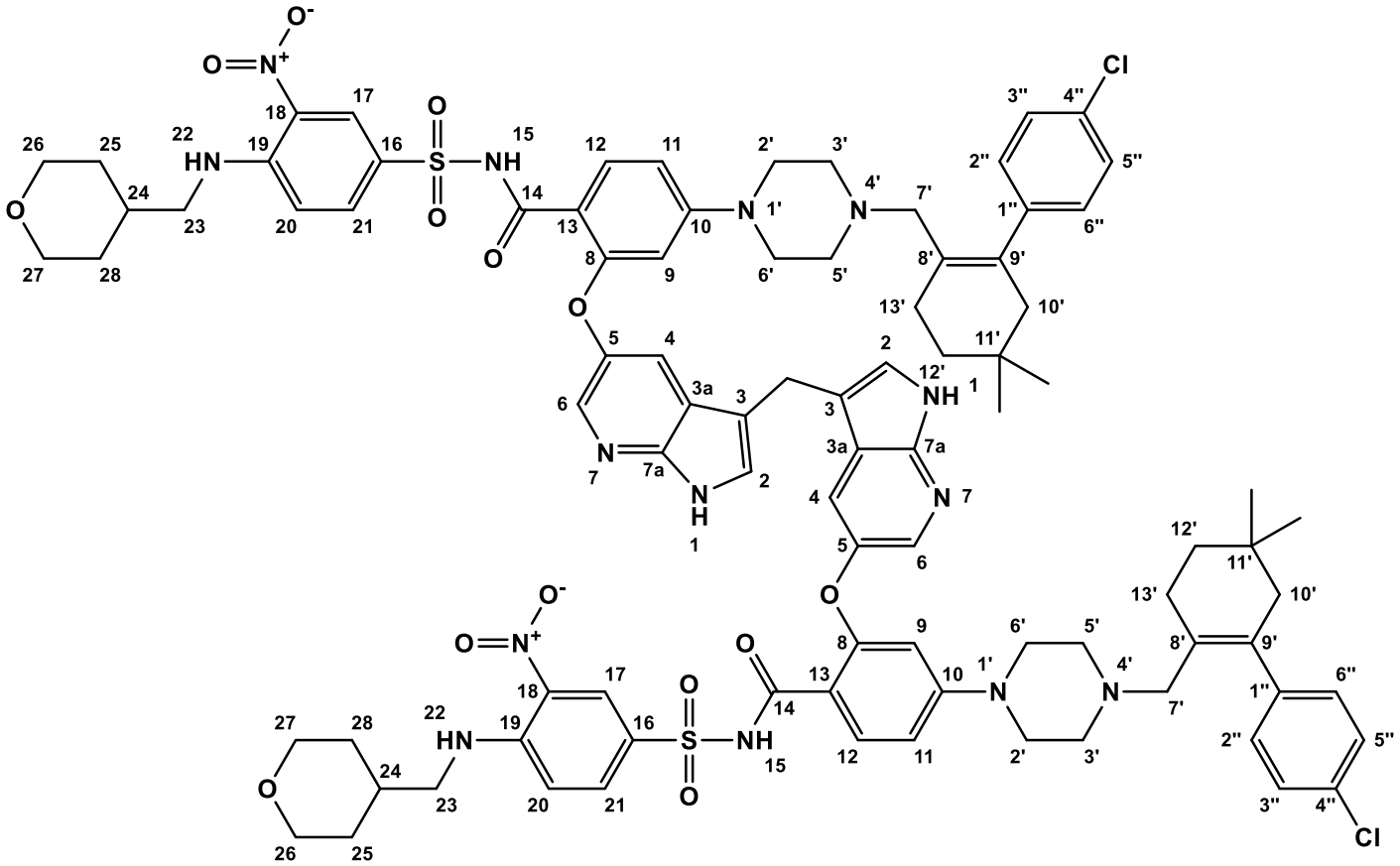


Figure S26 – Degradation product A3 with NMR assignations

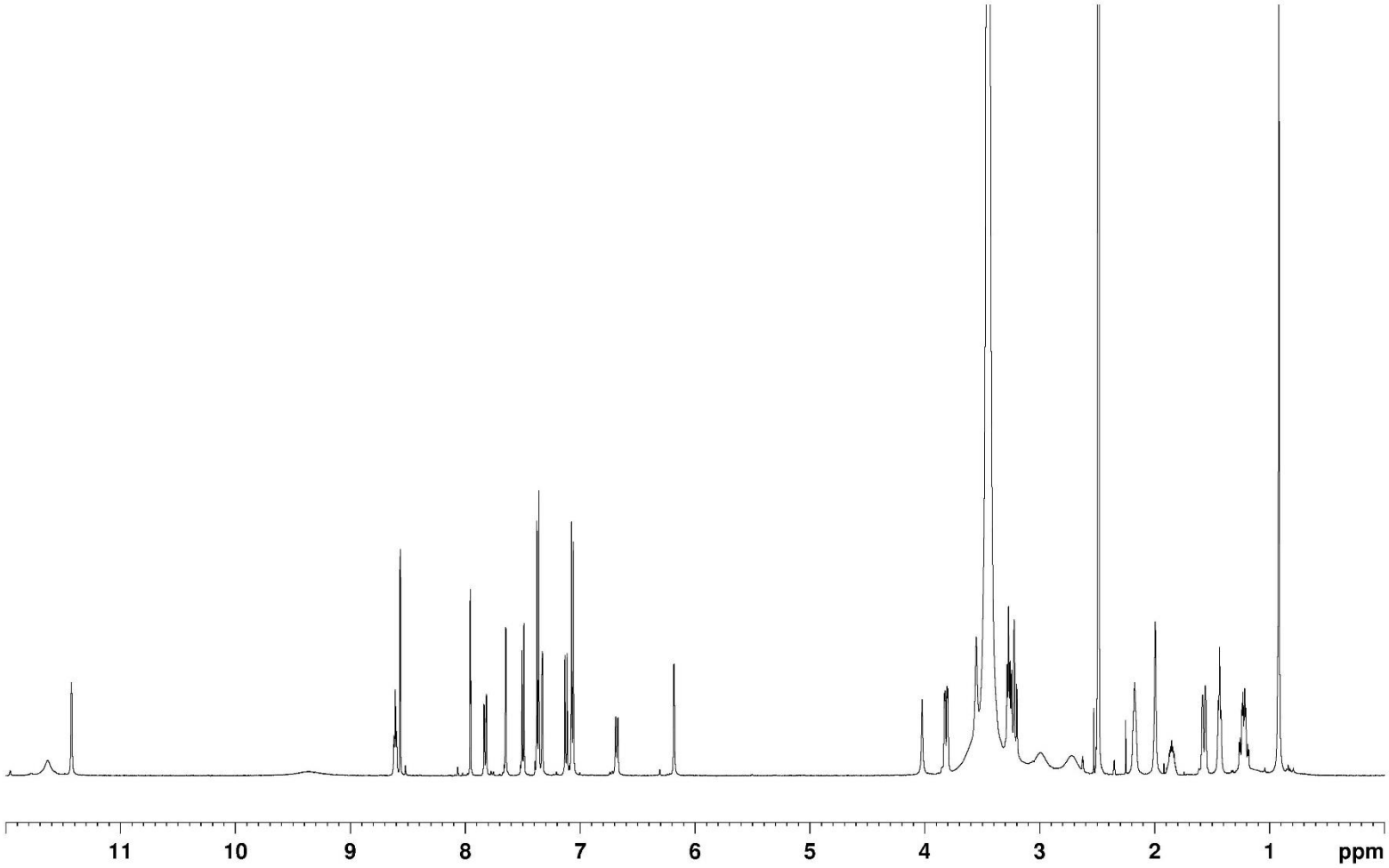


Figure S27 – ^{1}H NMR spectrum of **A3**

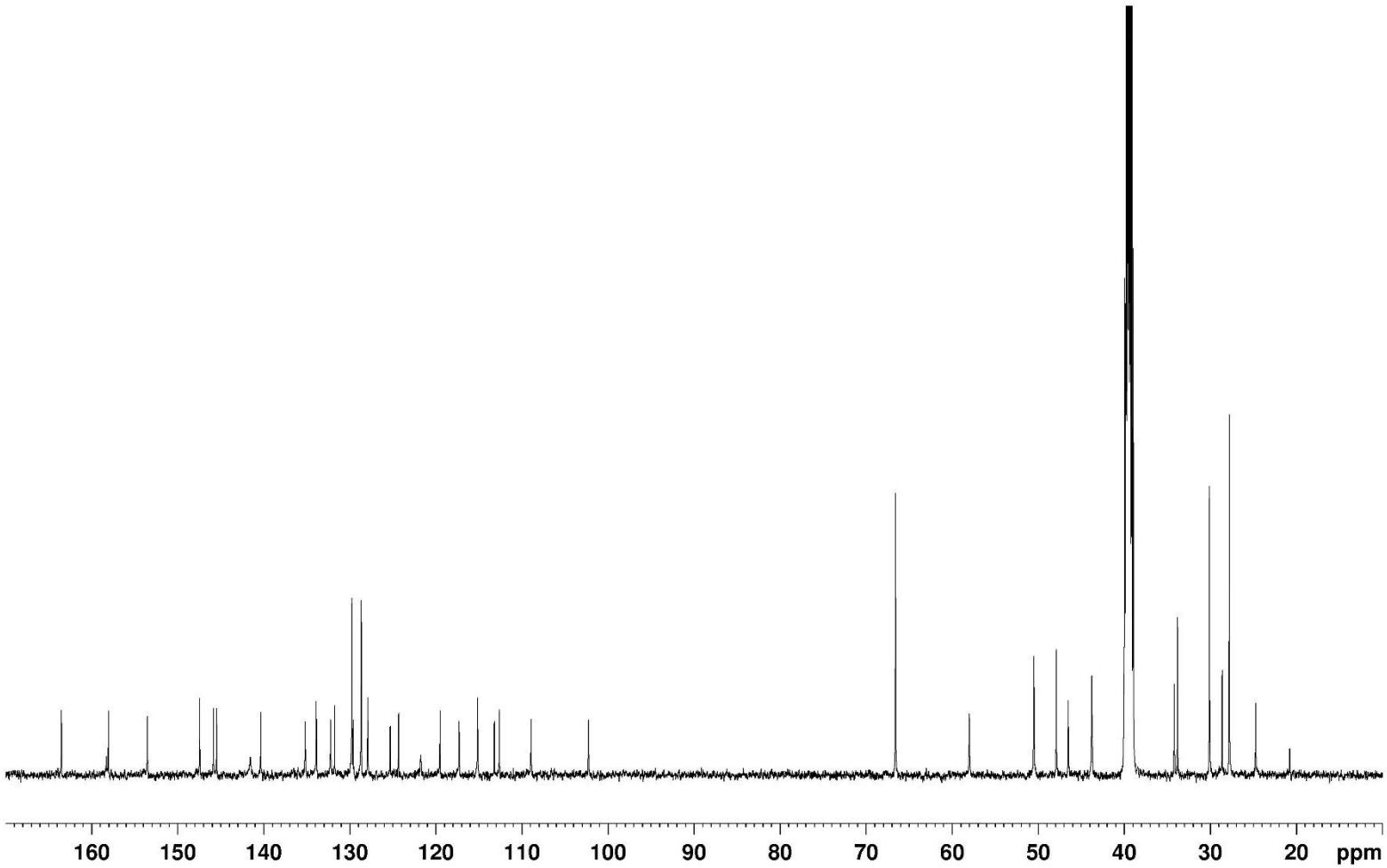


Figure S28 – ^{13}C NMR spectrum of **A3**

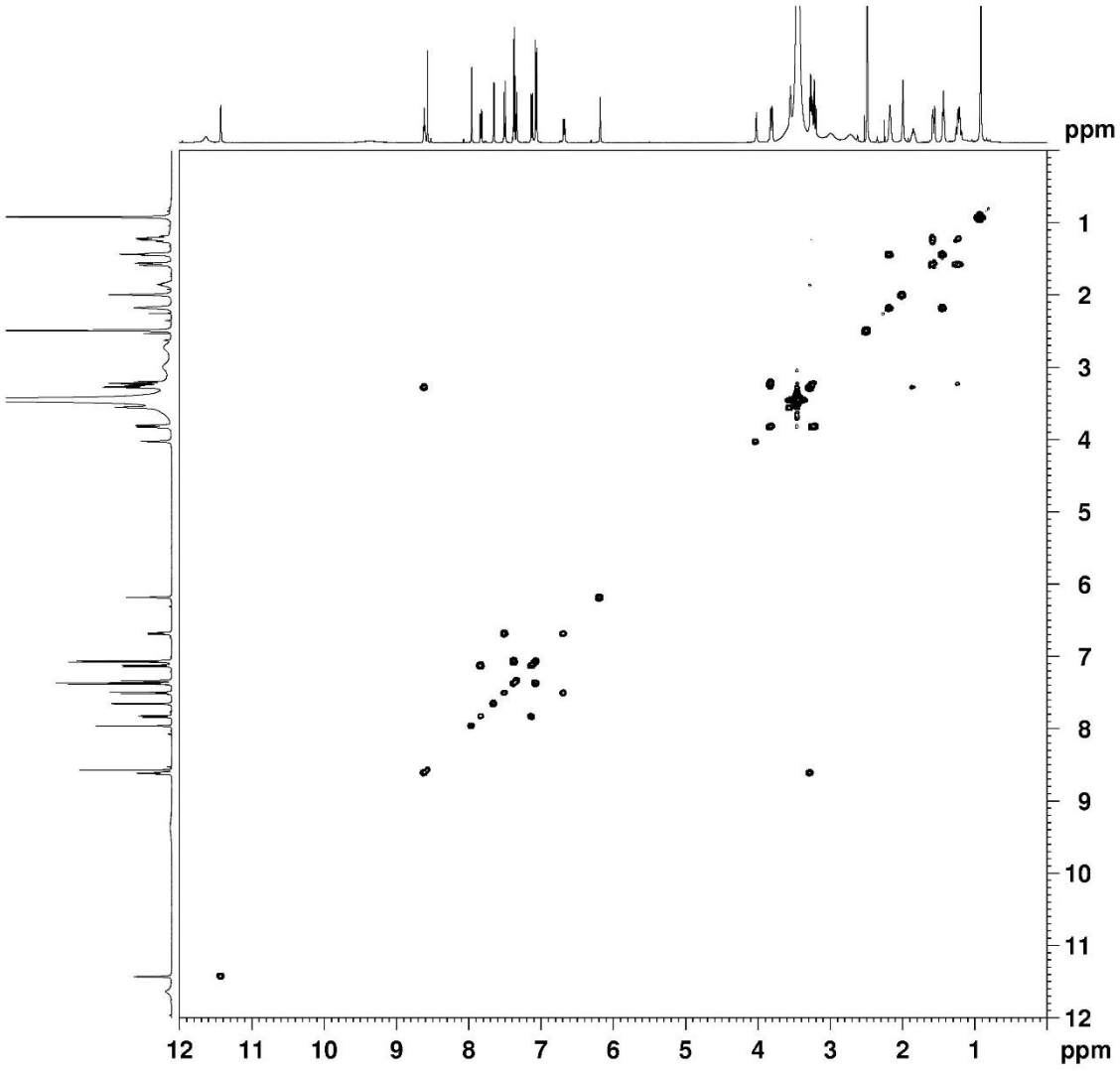


Figure S29 – (^{1}H, ^{1}H)-COSY spectrum of **A3**

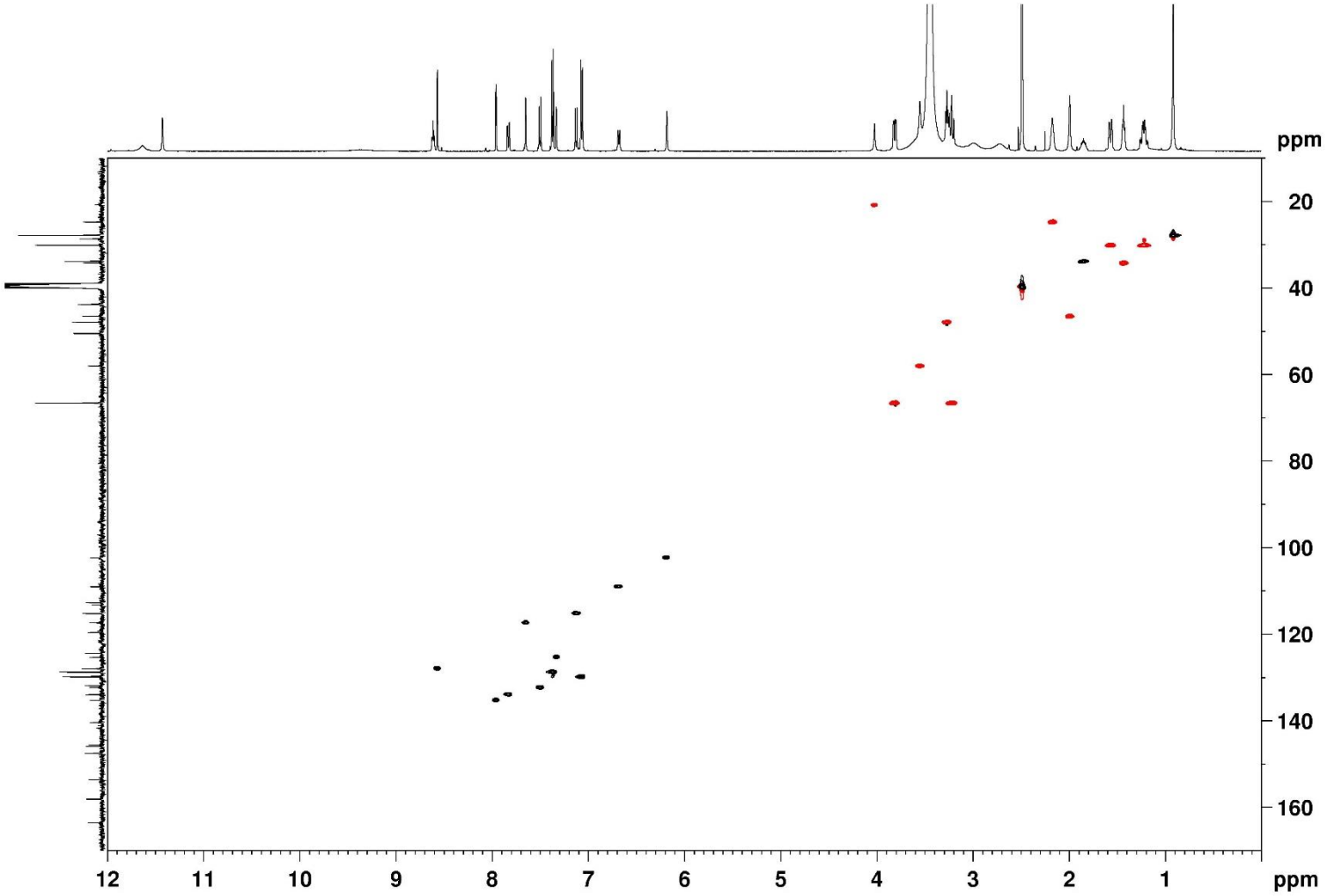


Figure S30 – (^{1}H, ^{13}C)-HSQC spectrum of **A3**

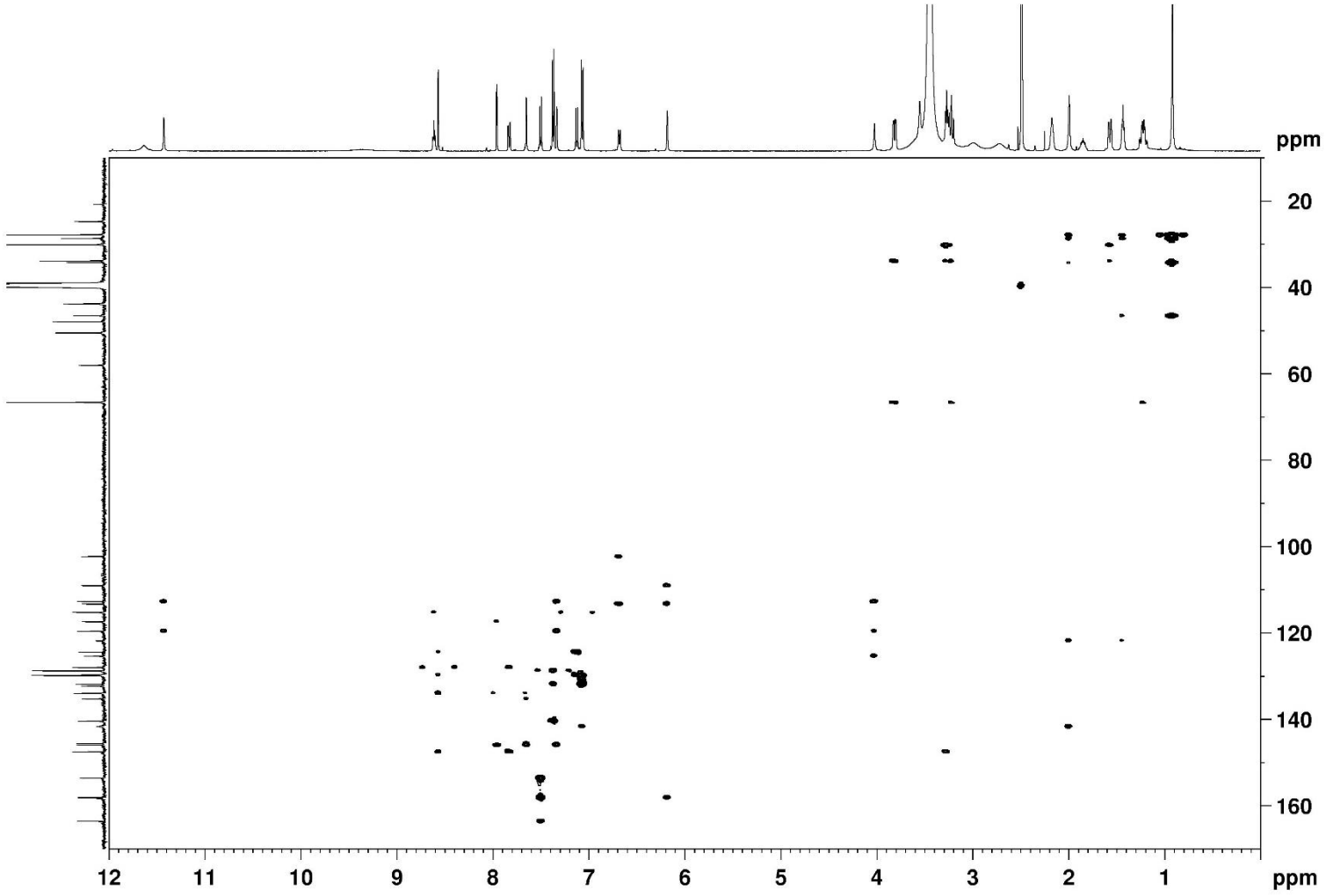


Figure S31 – (^{1}H, ^{13}C)-HMBC spectrum of **A3**

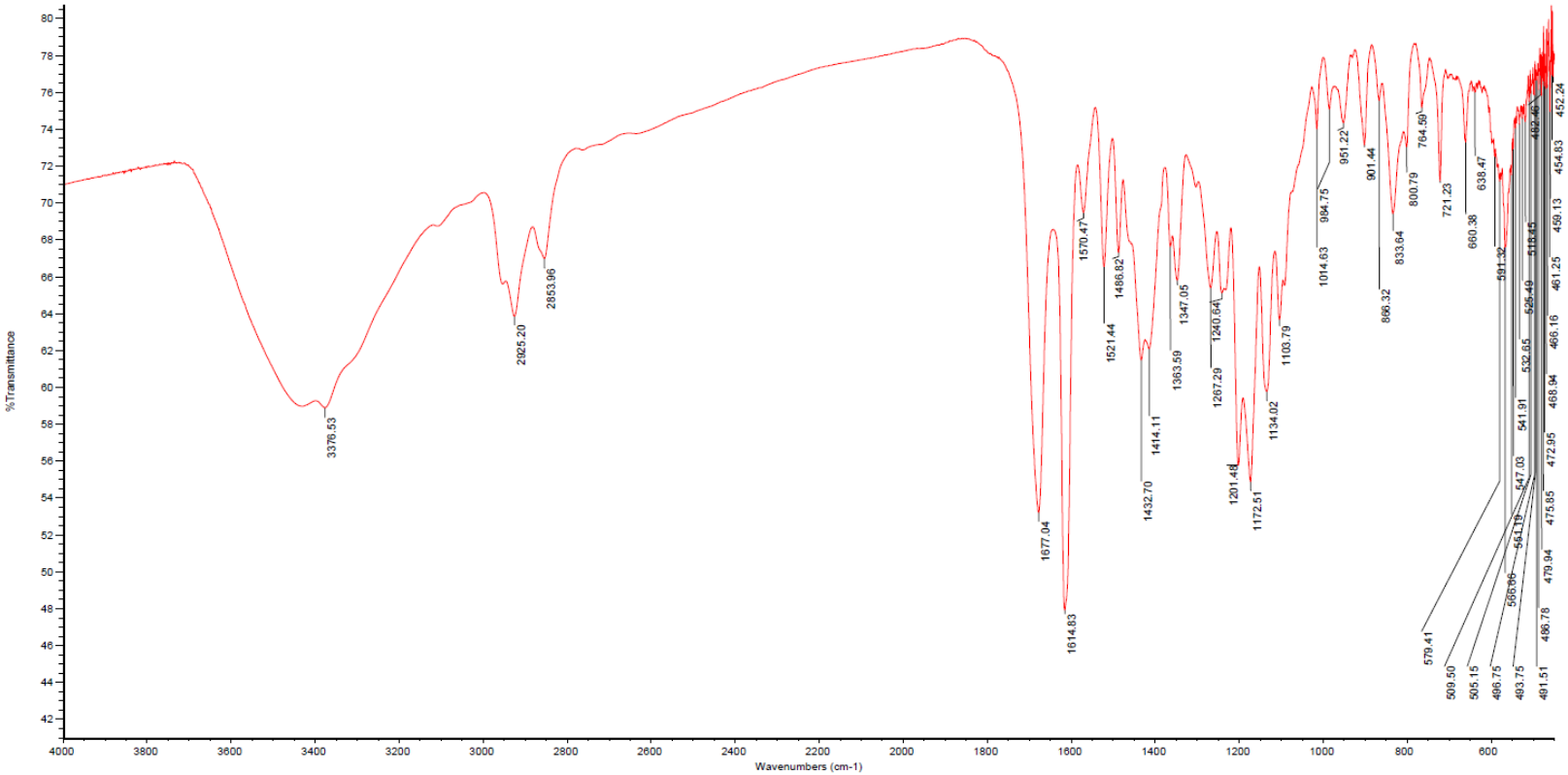


Figure S32 – IR spectrum of **A3**

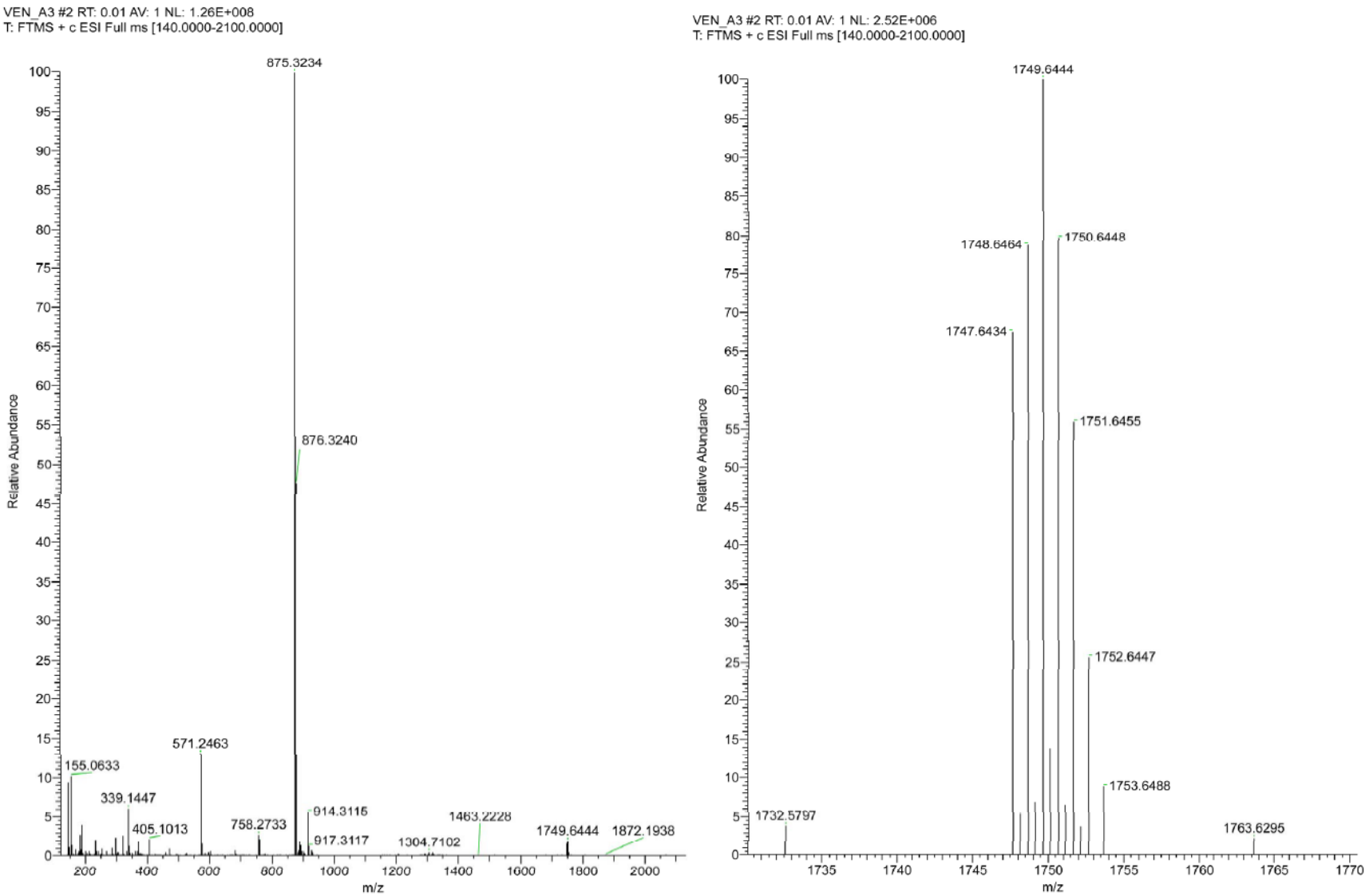


Figure S33 – HRMS spectrum of **A3**. Full spectrum (left) and spectrum of higher m/z (right)

Degradation product A4 information

5-(3-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)phenoxy)-1*H*-pyrrolo[2,3-b]pyridine: HRMS $[M+H]^+$: calculated 527.2572, found 527.2543.

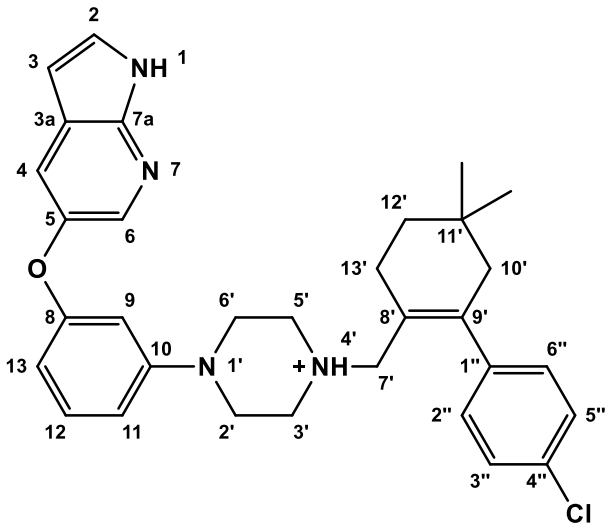


Figure S34 – Degradation product A4 with NMR assignations

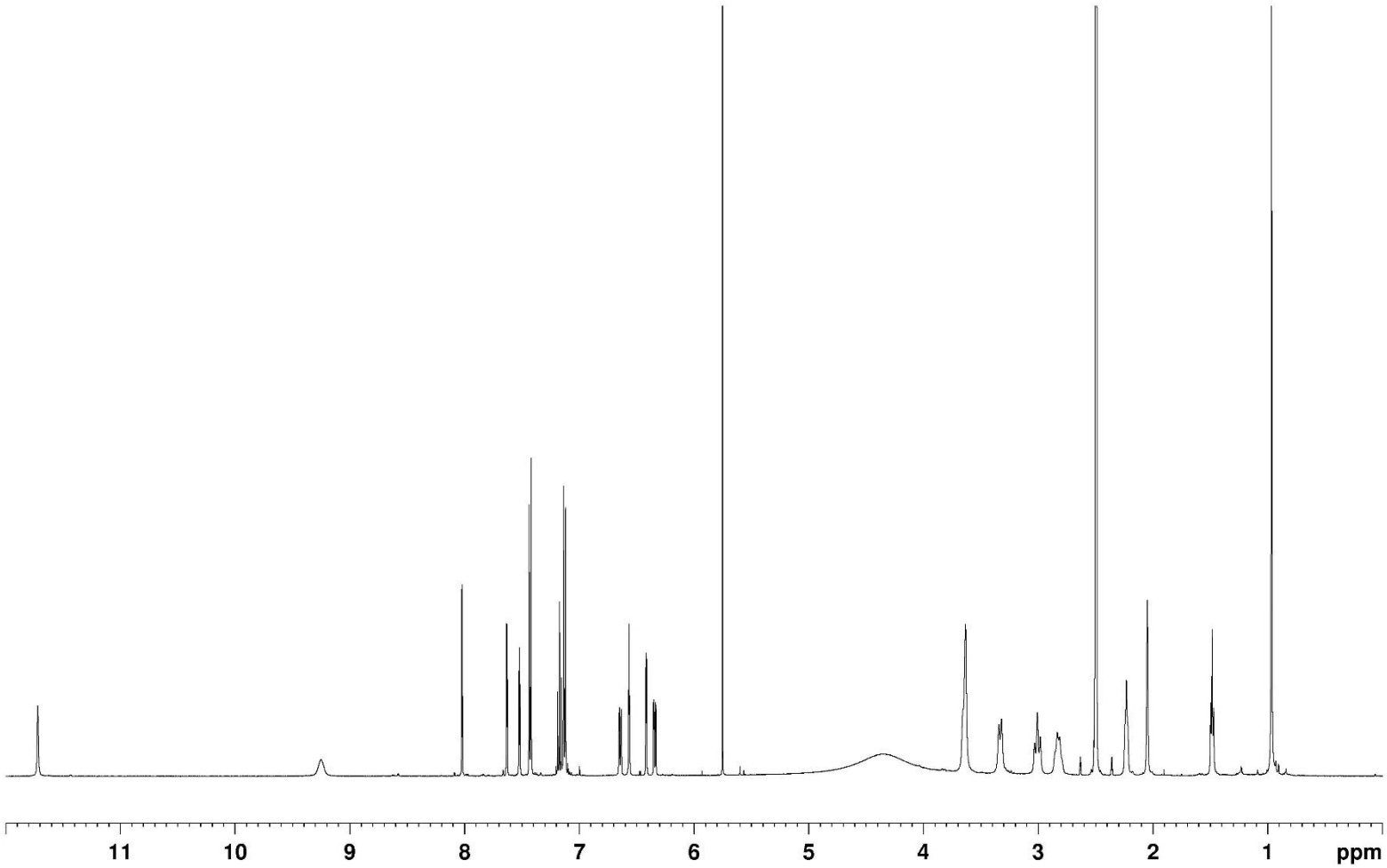


Figure S35 – ^{1}H NMR spectrum of **A4**

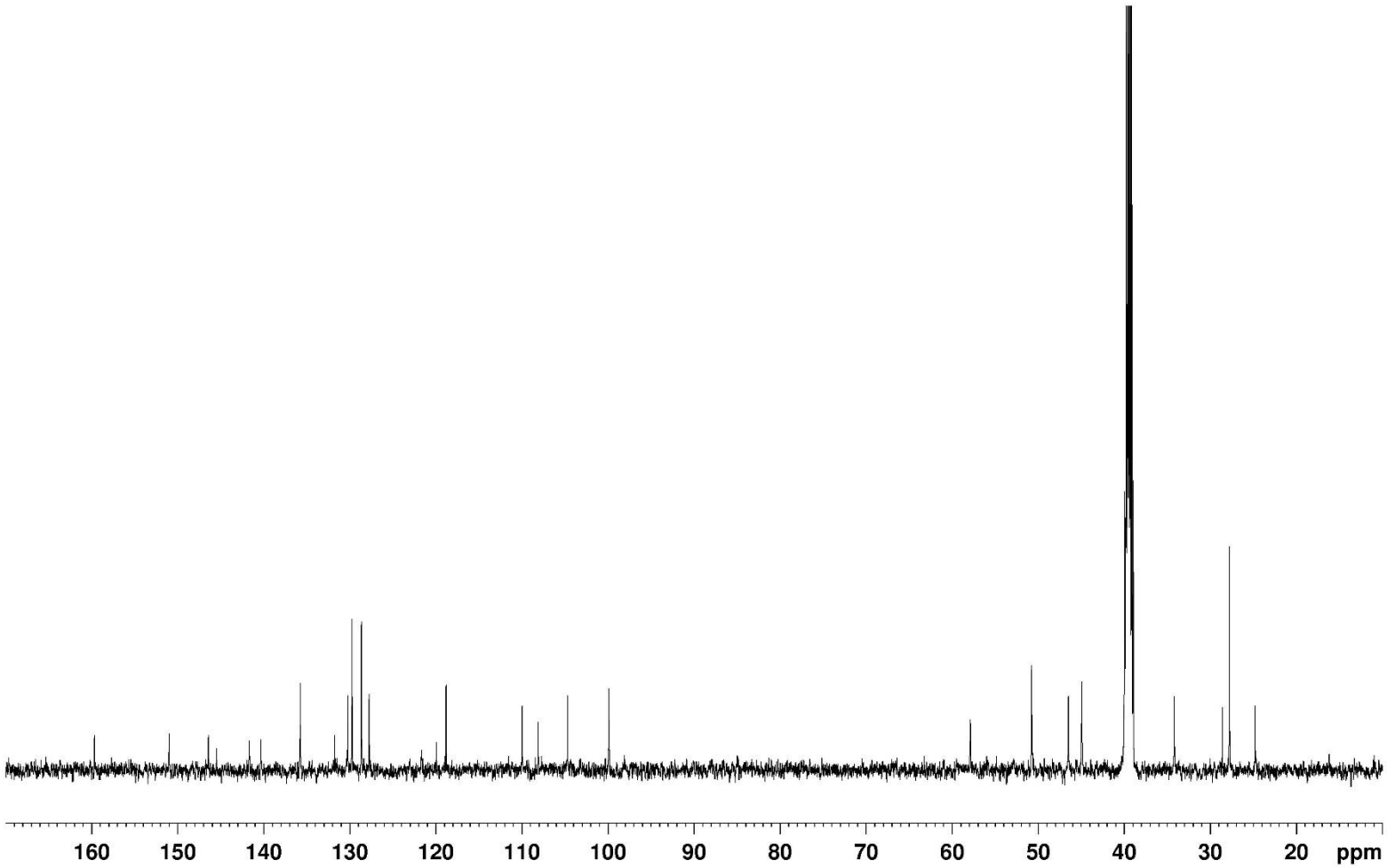


Figure S36 – ^{13}C NMR spectrum of **A4**

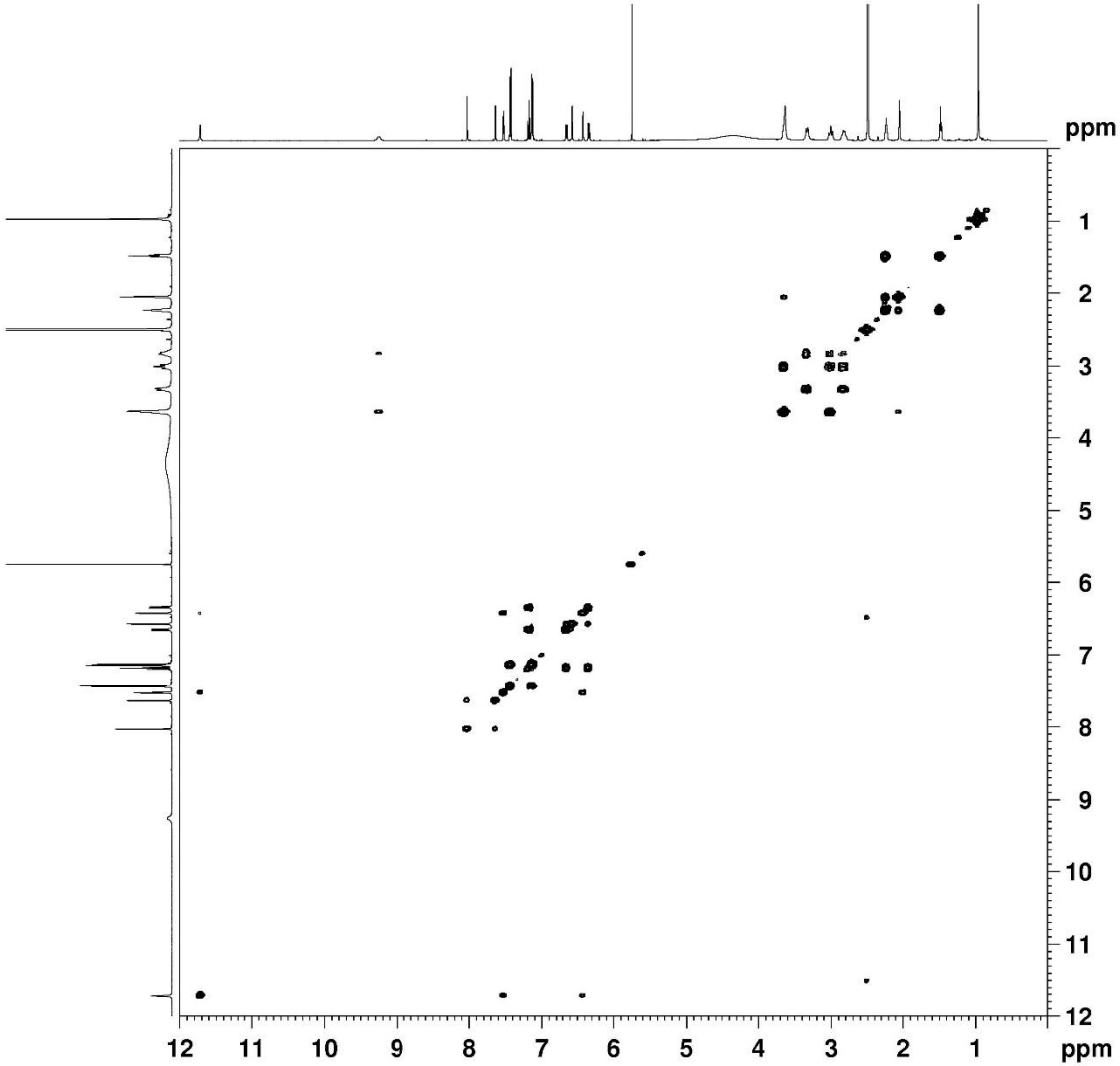


Figure S37 – (^{1}H, ^{1}H)-COSY spectrum of **A4**

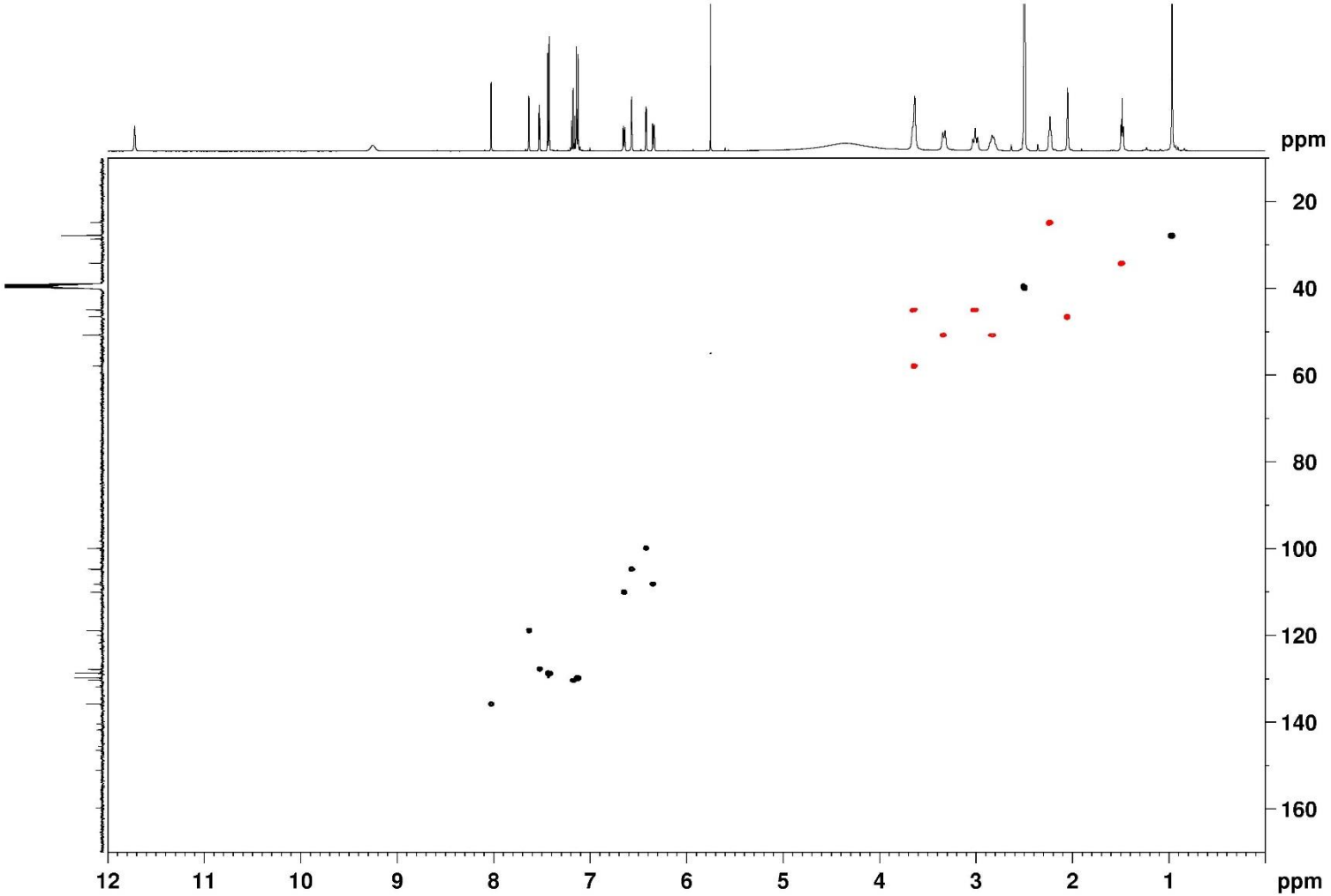


Figure S38 – (^{1}H, ^{13}C)-HSQC spectrum of **A4**

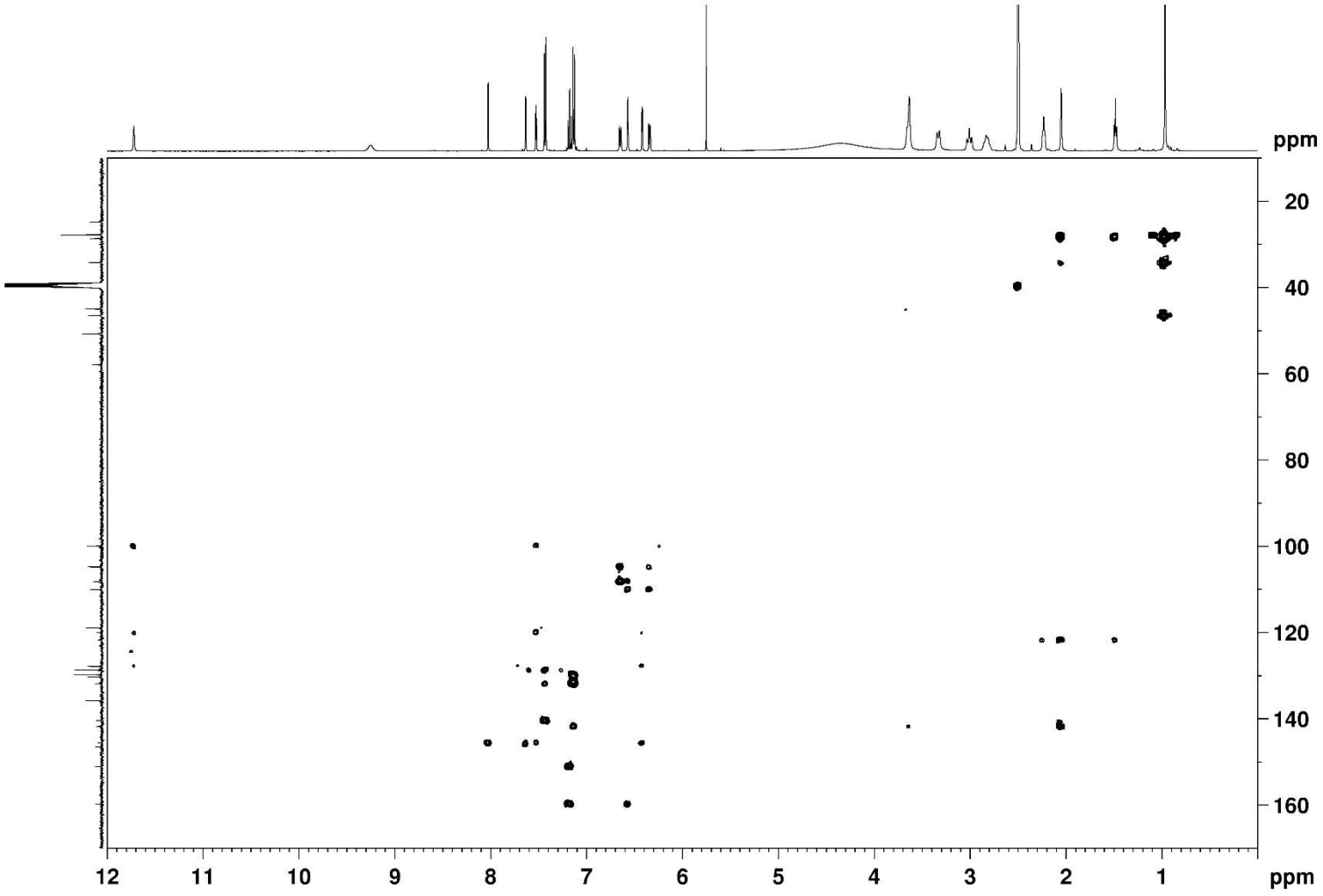


Figure S39 – (^{1}H, ^{13}C)-HMBC spectrum of **A4**

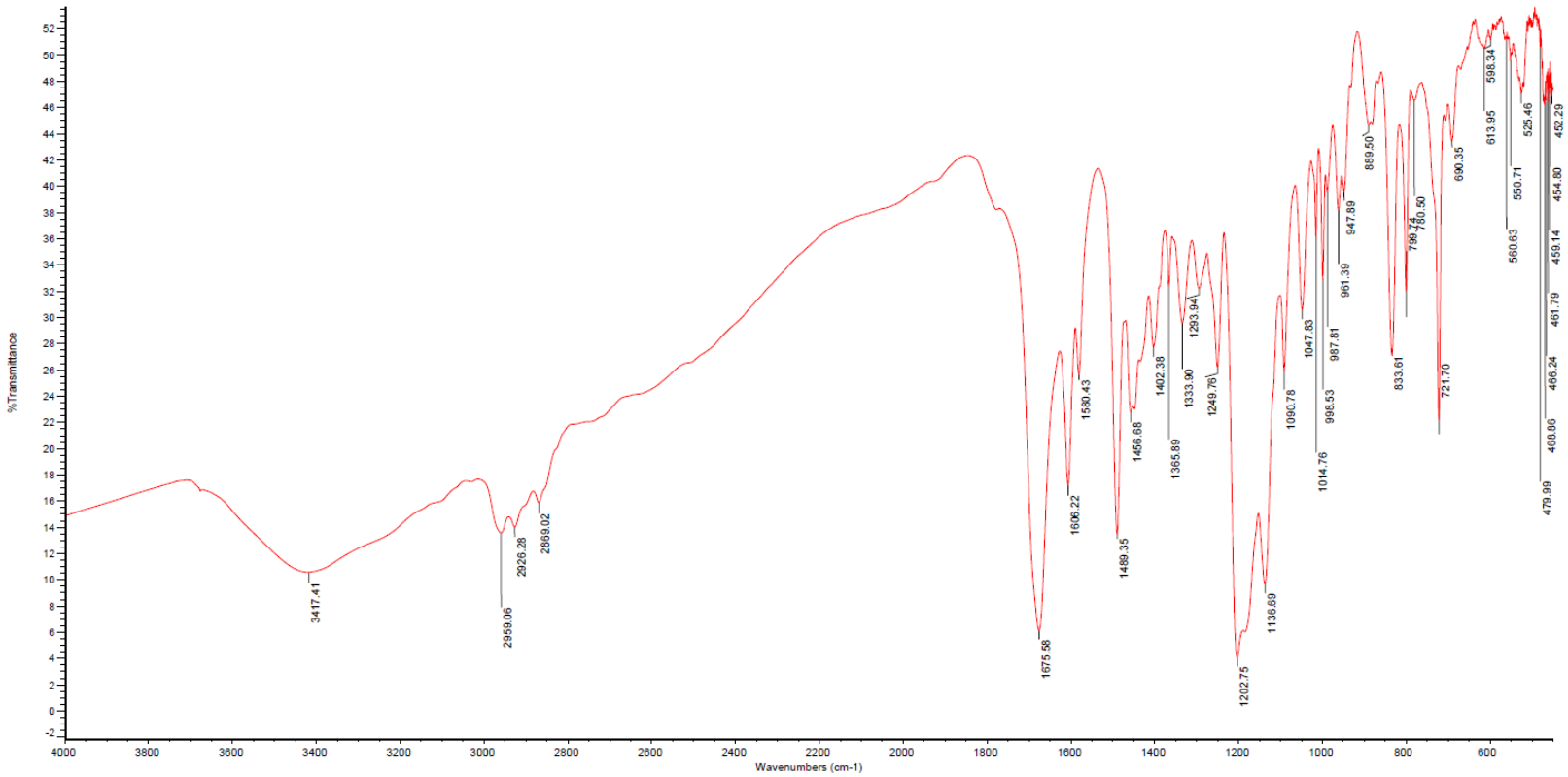


Figure S40 – IR spectrum of **A4**

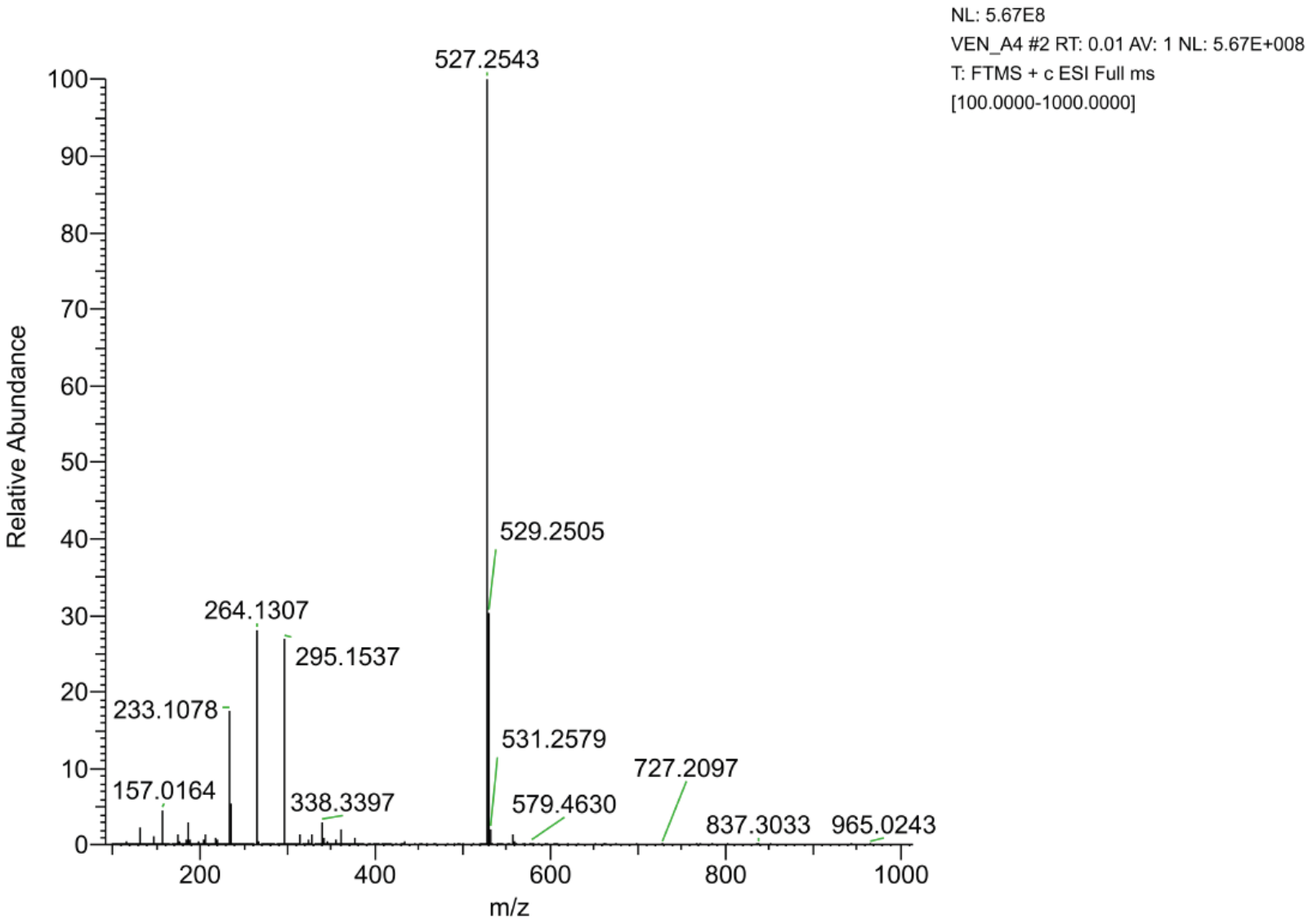


Figure S41 – HRMS spectrum of **A4**

Degradation product B1 information

5-(N-(2-((1*H*-pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-(tetrahydro-2*H*-pyran-4-yl)-1*H*-benzo[d]imidazole 3-oxide

Tautomer:
2-((1*H*-pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((1-hydroxy-2-(tetrahydro-2*H*-pyran-4-yl)-1*H*-benzo[d]imidazol-6-yl)sulfonyl)benzamide: amorphous solid; HRMS $[M+H]^+$: calculated 850.3148, found 850.3132.

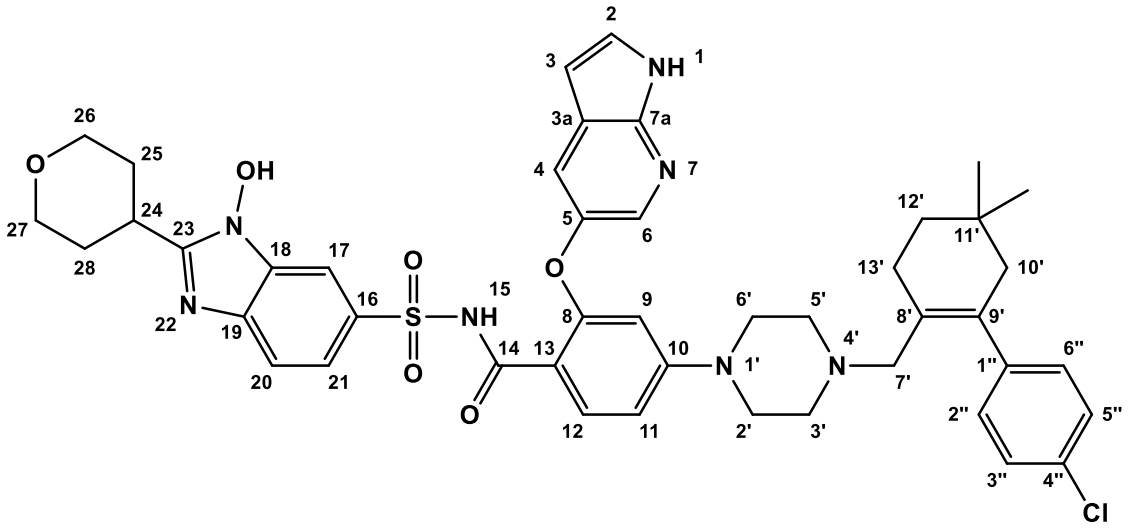


Figure S42 – Degradation product B1 with NMR assignations

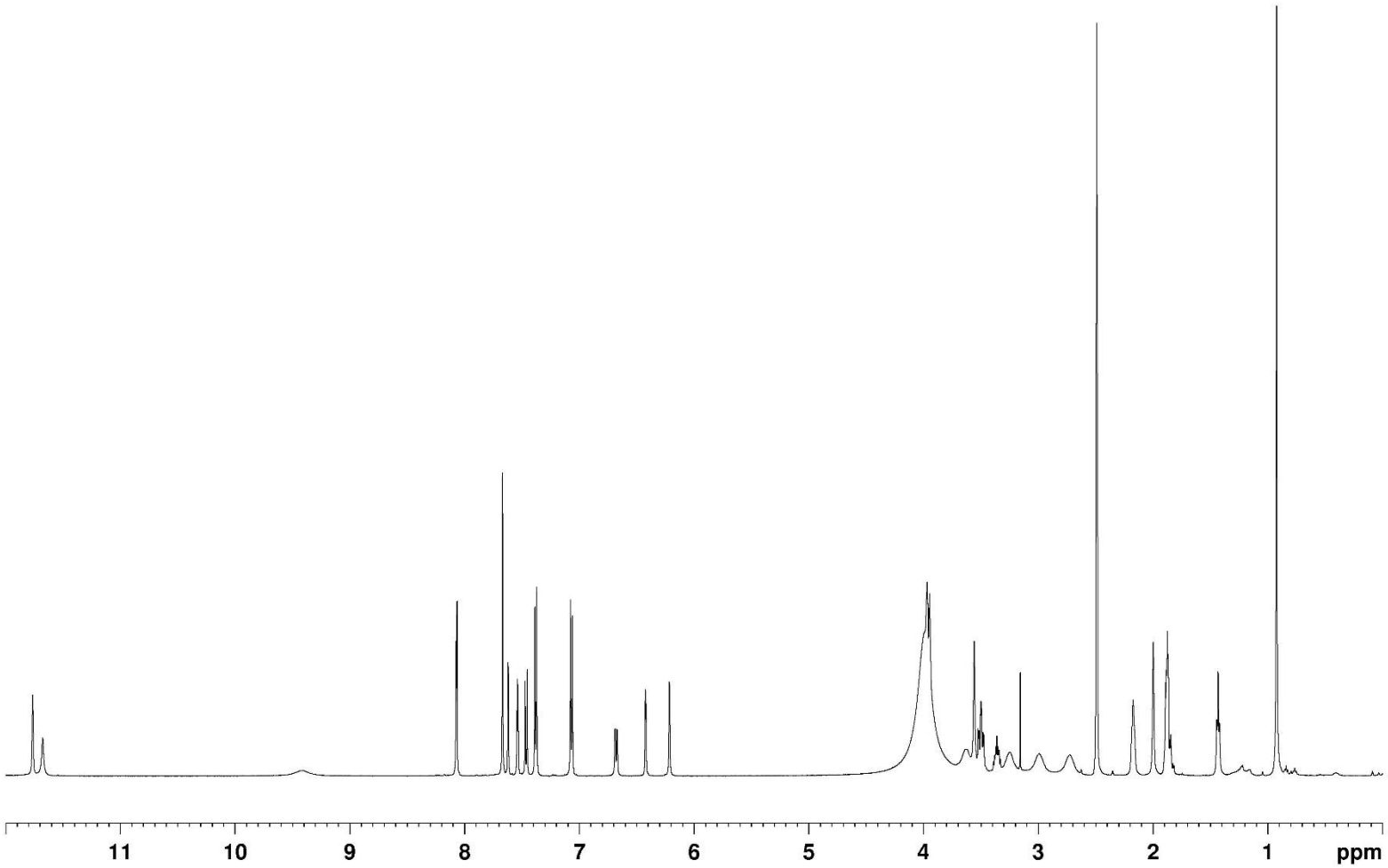


Figure S43 – ^{1}H NMR spectrum of **B1**

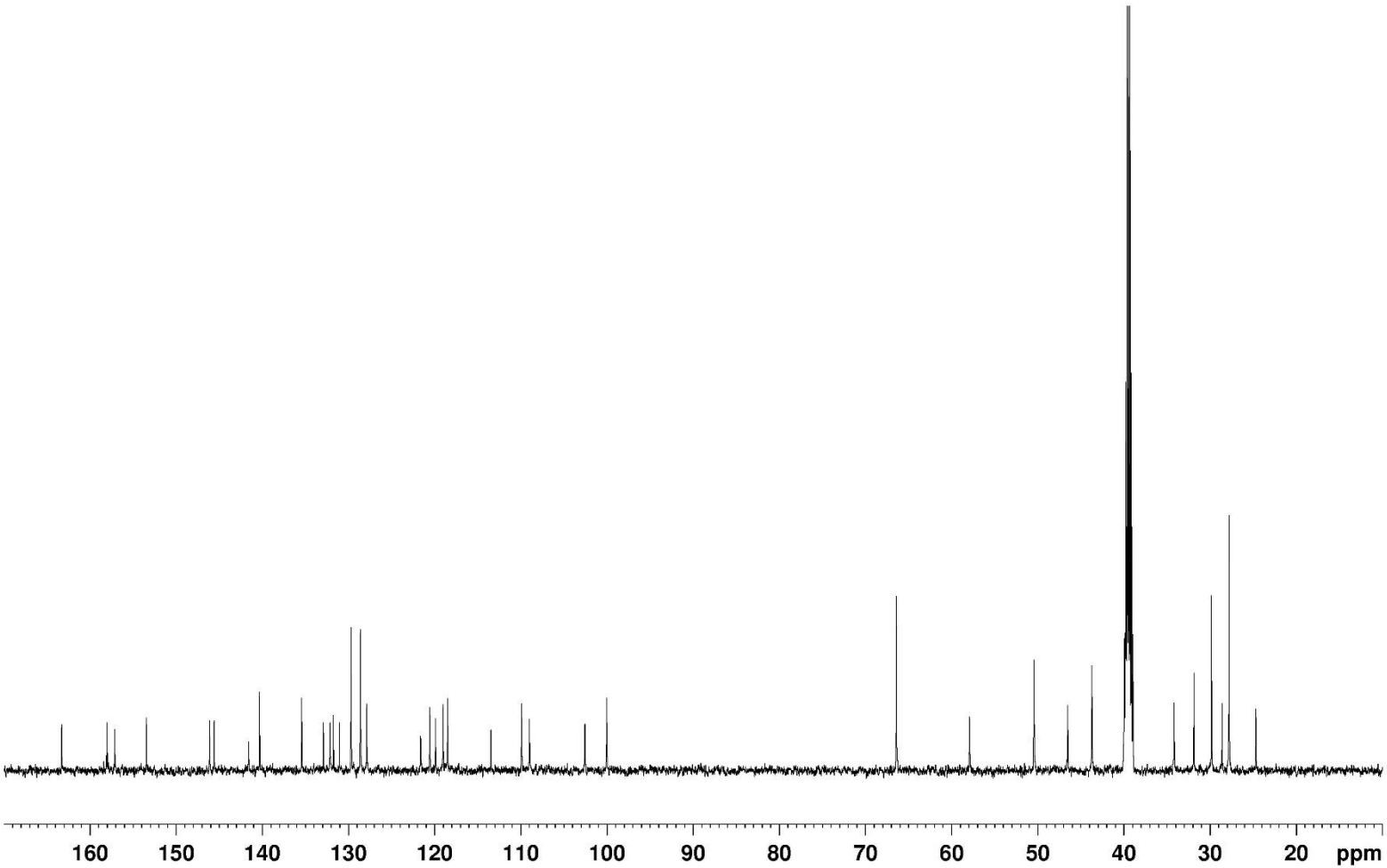


Figure S44 – ^{13}C NMR spectrum of **B1**

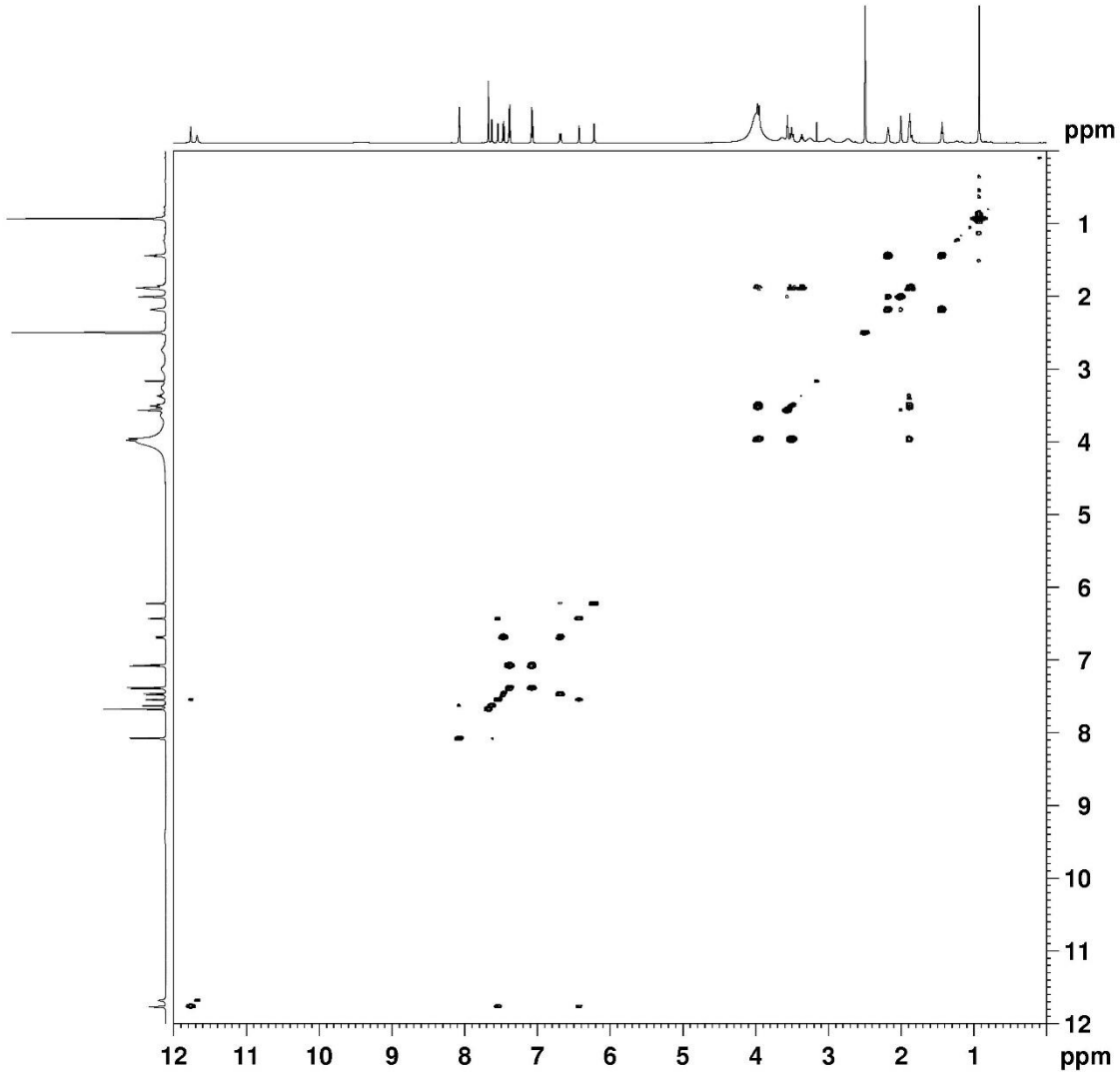


Figure S45 – (^{1}H, ^{1}H)-COSY spectrum of **B1**

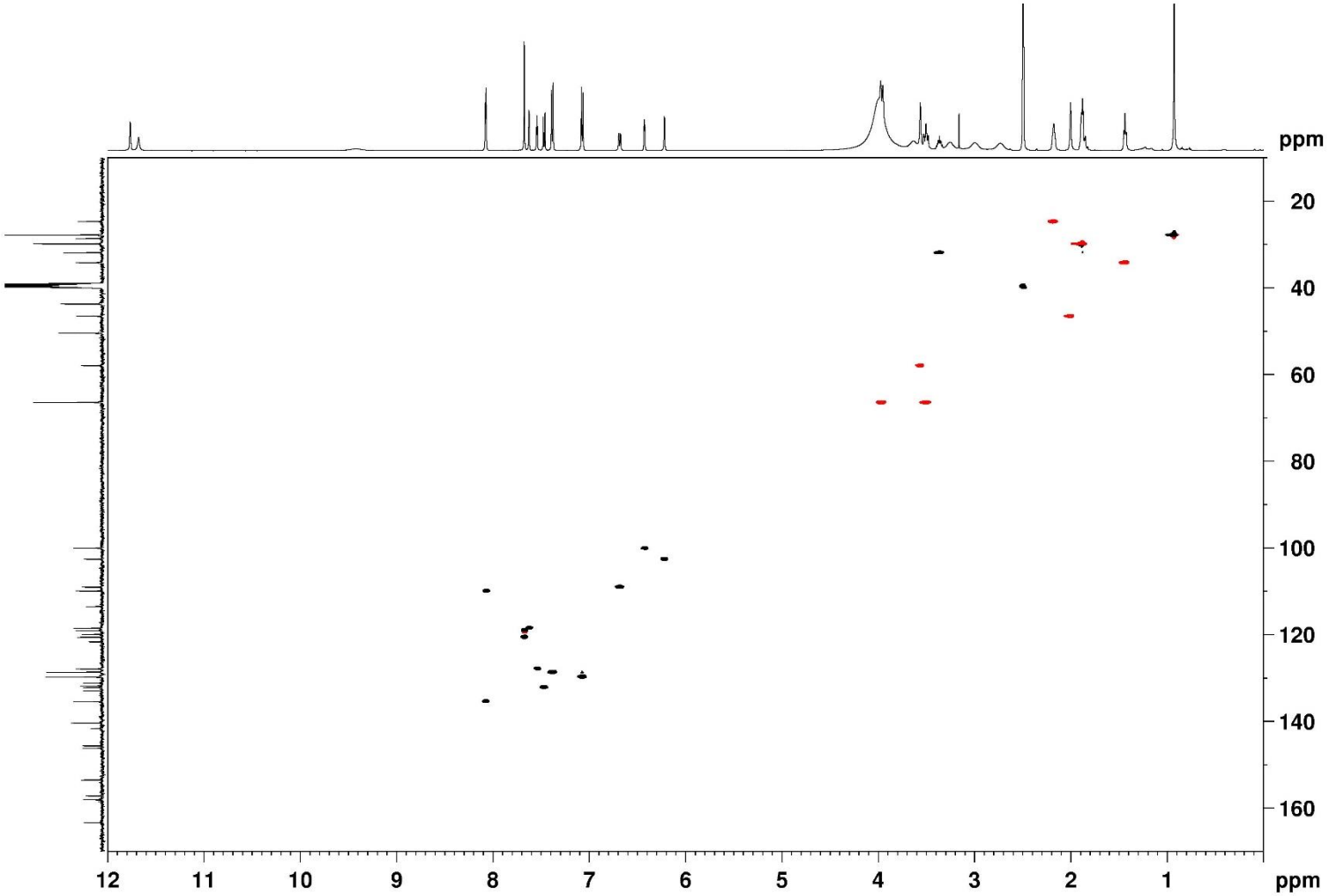


Figure S46 – (^{1}H, ^{13}C)-HSQC spectrum of **B1**

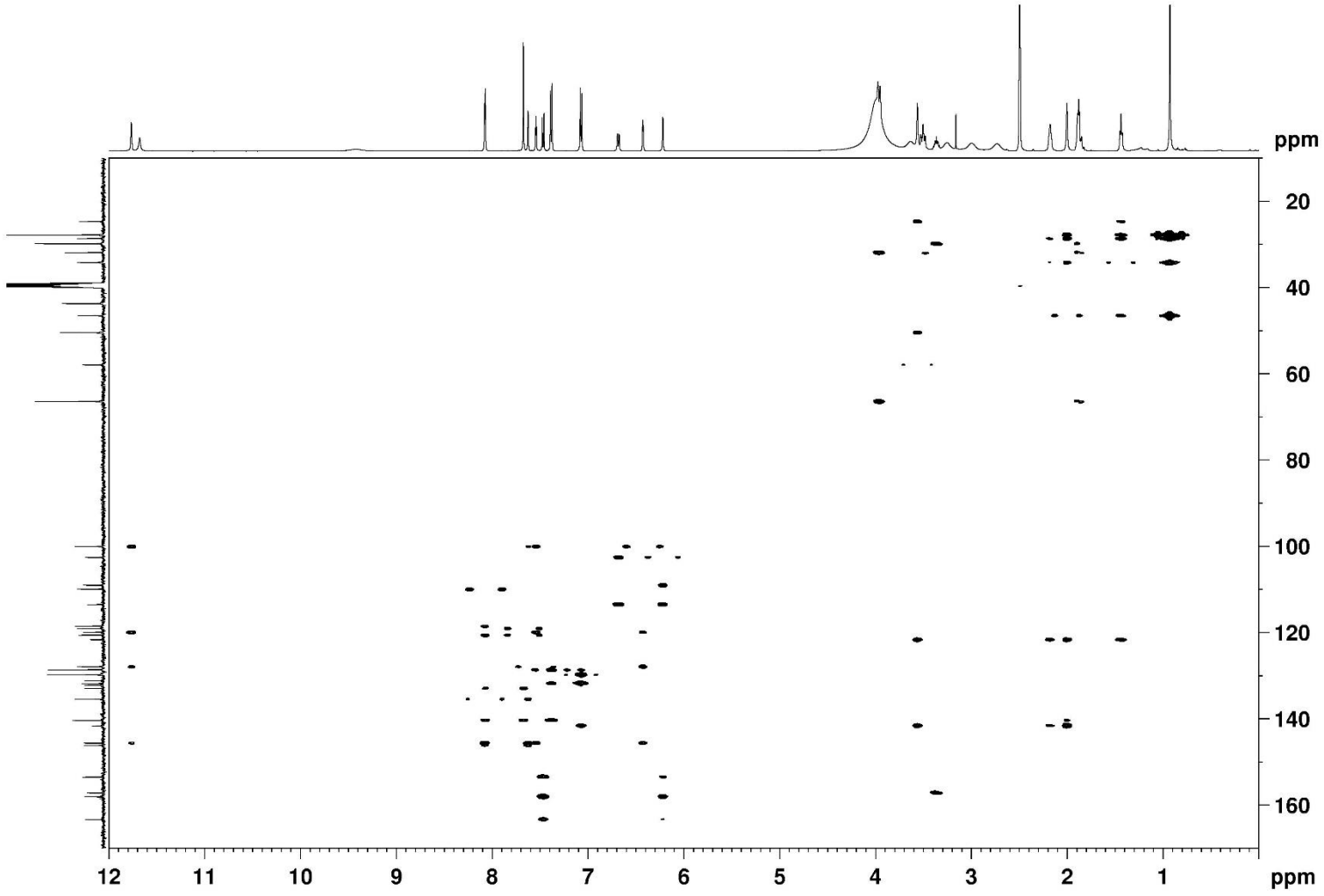


Figure S47 – (^{1}H, ^{13}C)-HMBC spectrum of **B1**

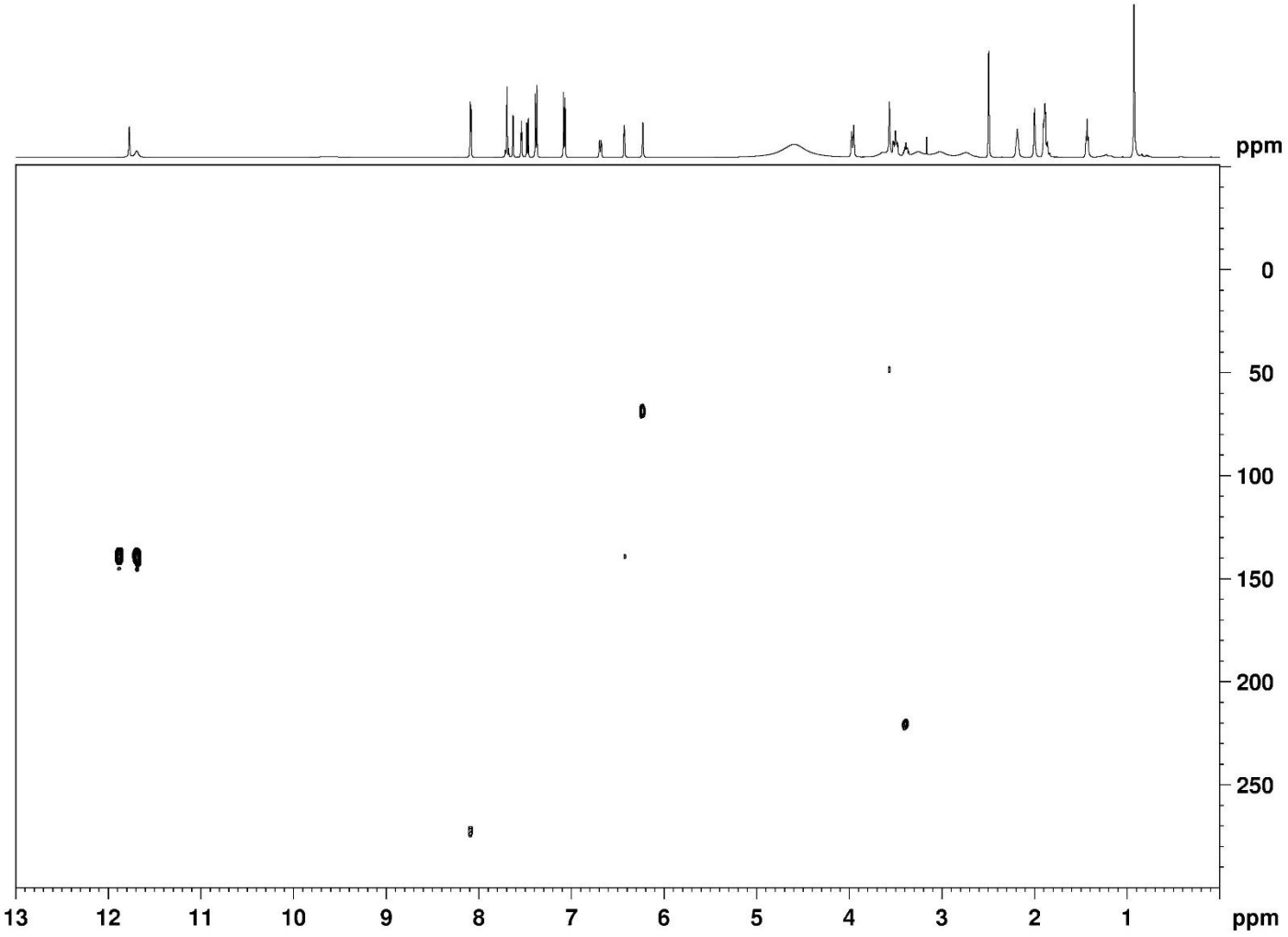


Figure S48 – (^{1}H, ^{15}N)-HMBC spectrum of **B1**

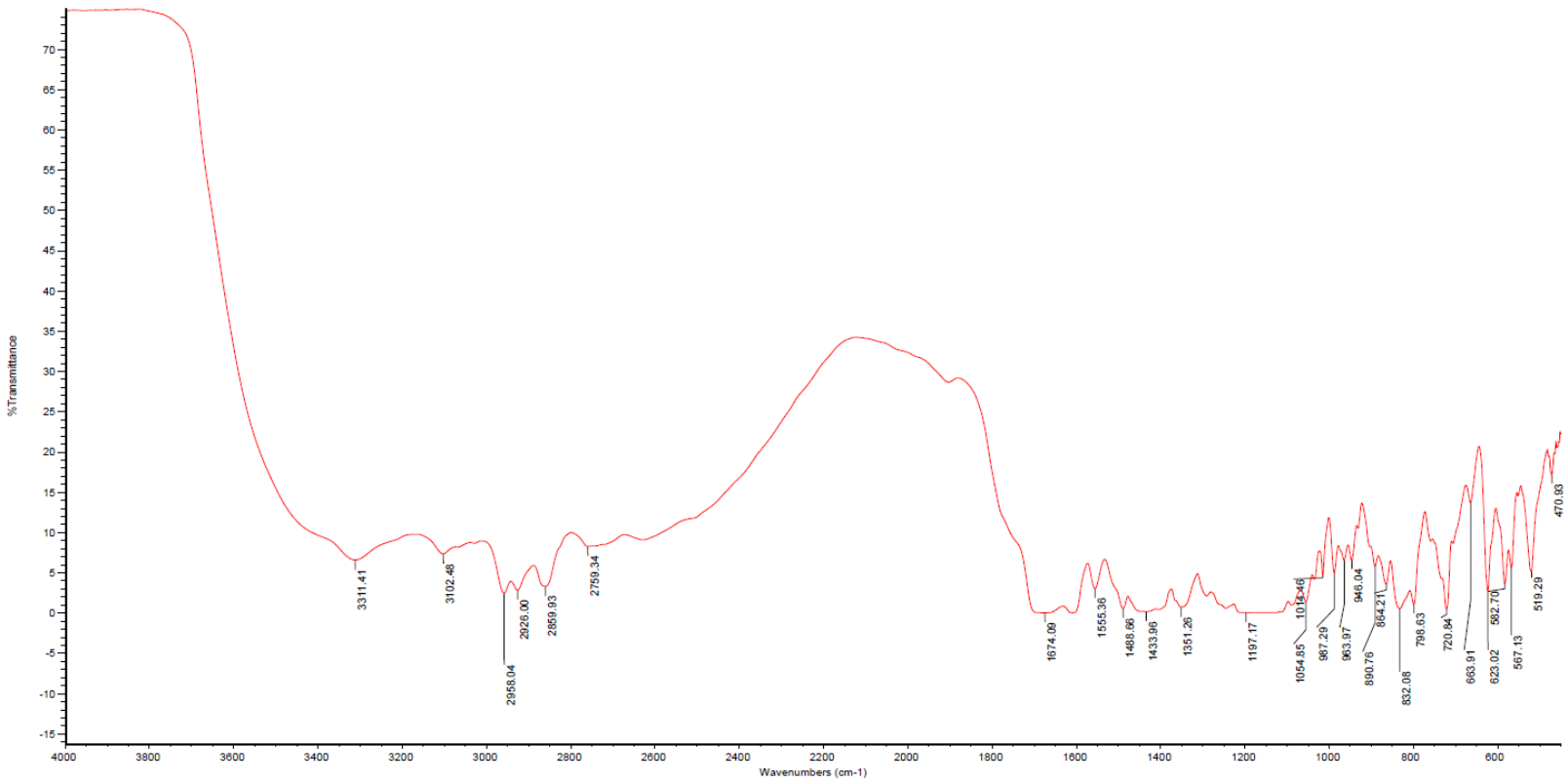


Figure S49 – IR spectrum of **B1**

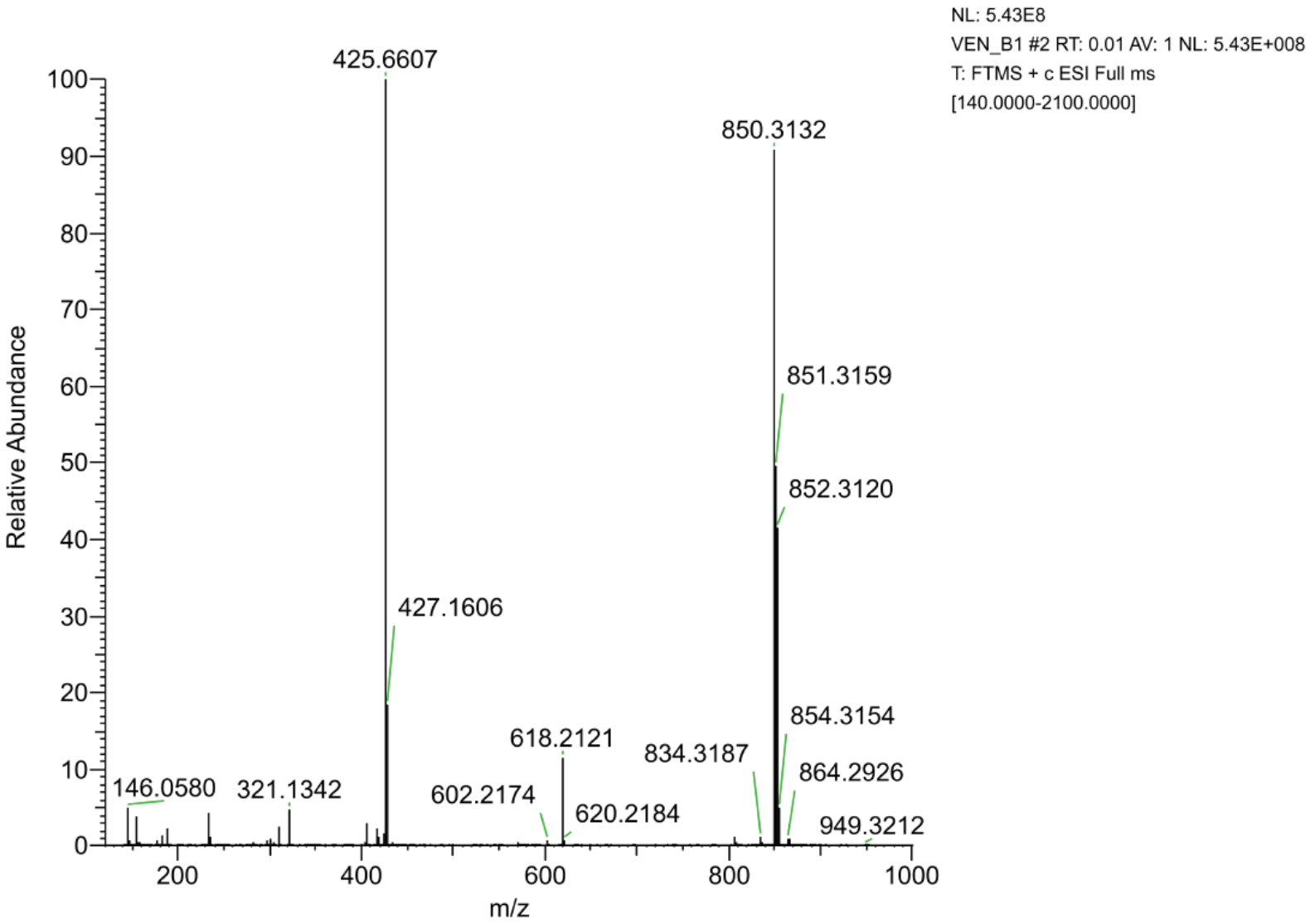


Figure S50 – HRMS spectrum of **B1**

Degradation product B2 information

2-((1*H*-pyrrolo[2,3-b]pyridin-5-yl)oxy)-4-(4-((4'-chloro-5,5-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-hydroxy-3-nitrophenyl)sulfonyl)benzamide: HRMS $[M+H]^+$: calculated 771.2362, found 771.2351.

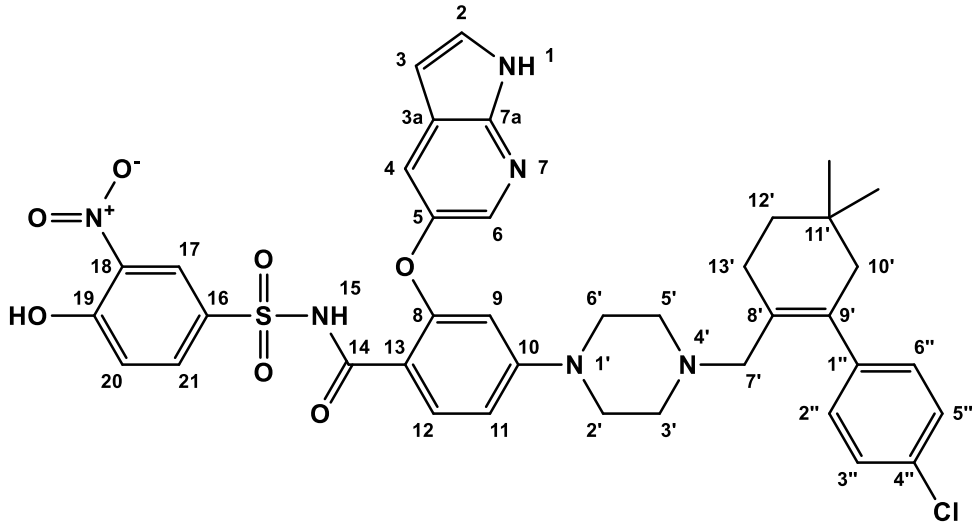


Figure S51 – Degradation product B2 with NMR assignations

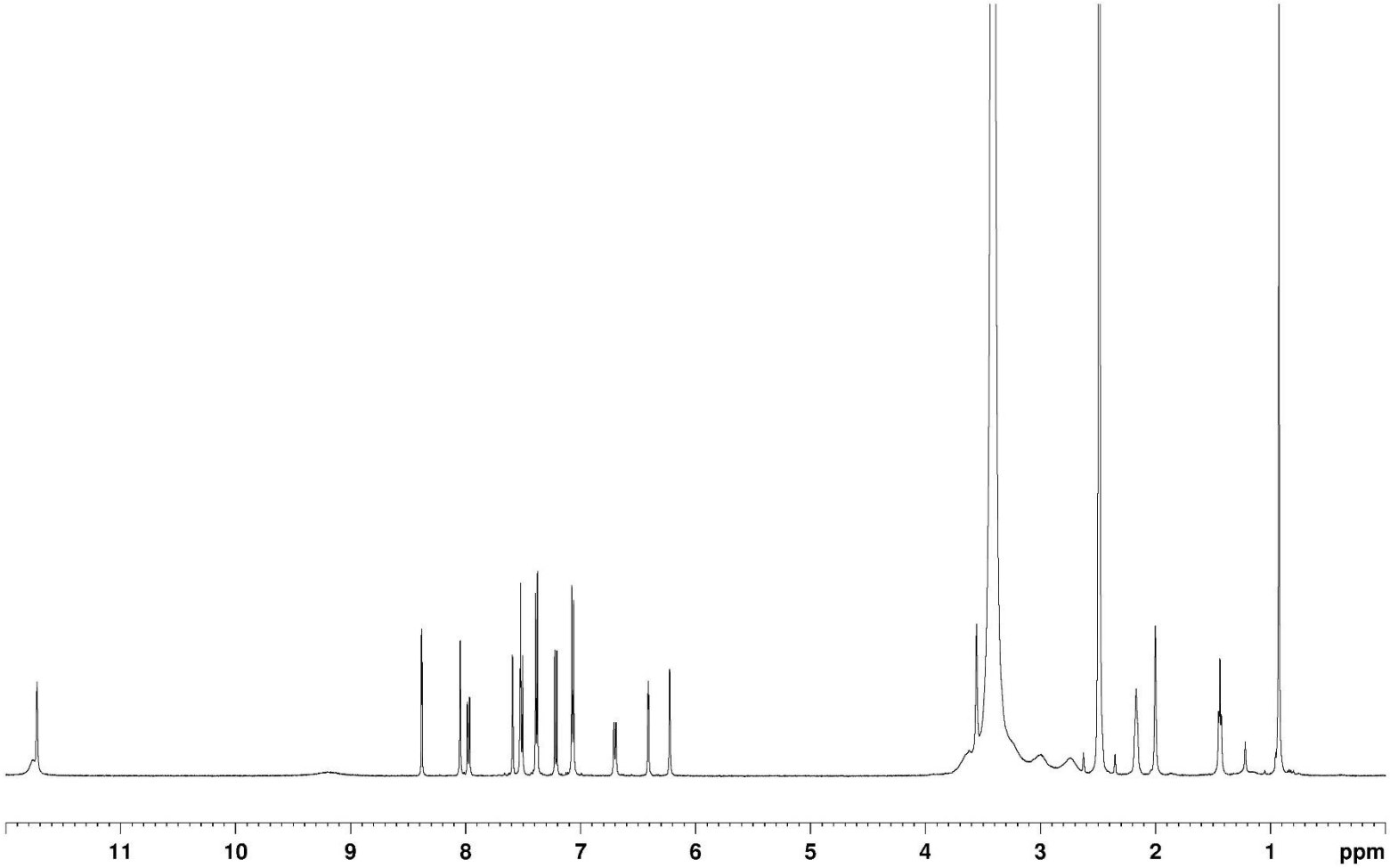


Figure S52 – ^{1}H NMR spectrum of **B2**

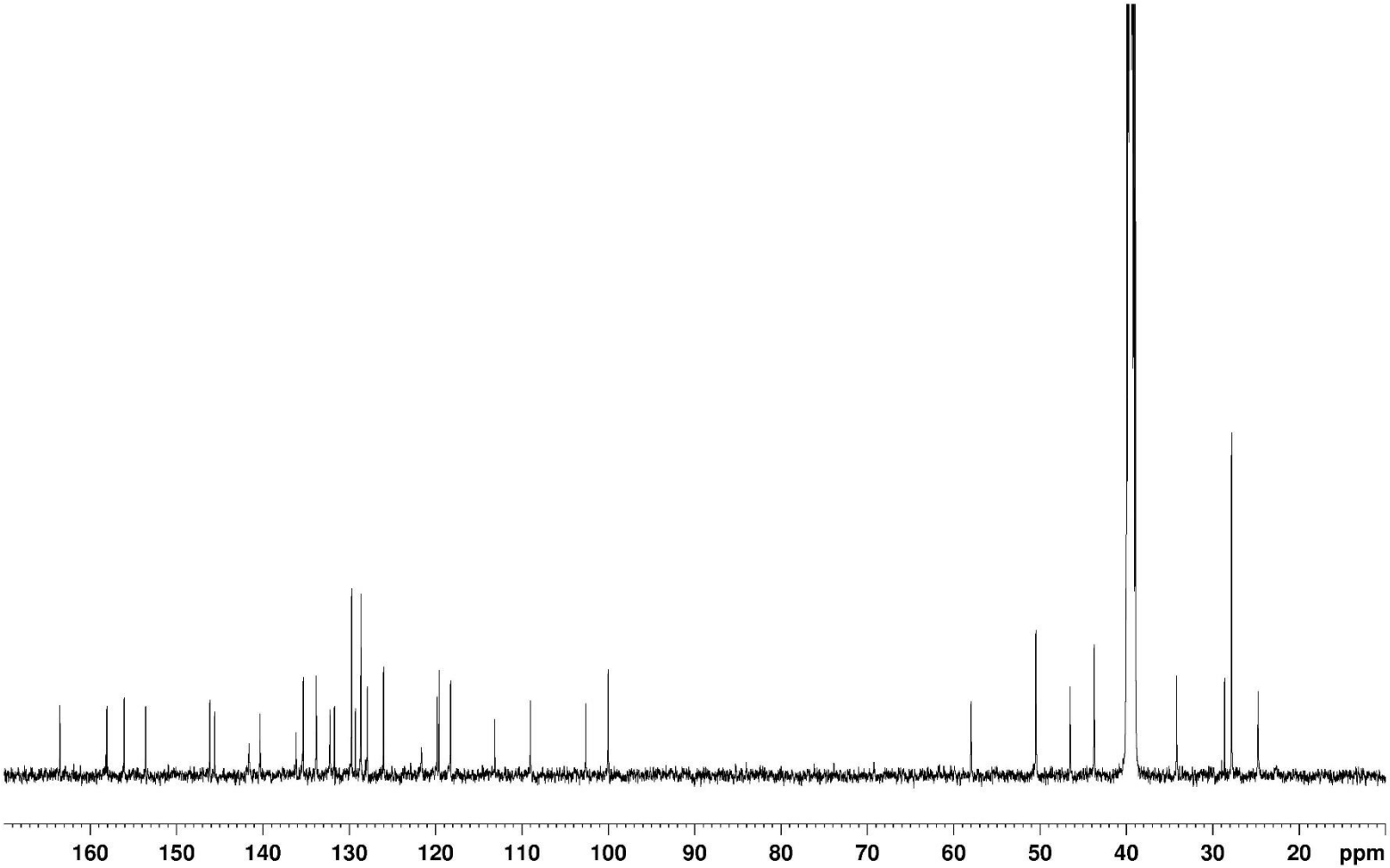


Figure S53 – ^{13}C NMR spectrum of **B2**

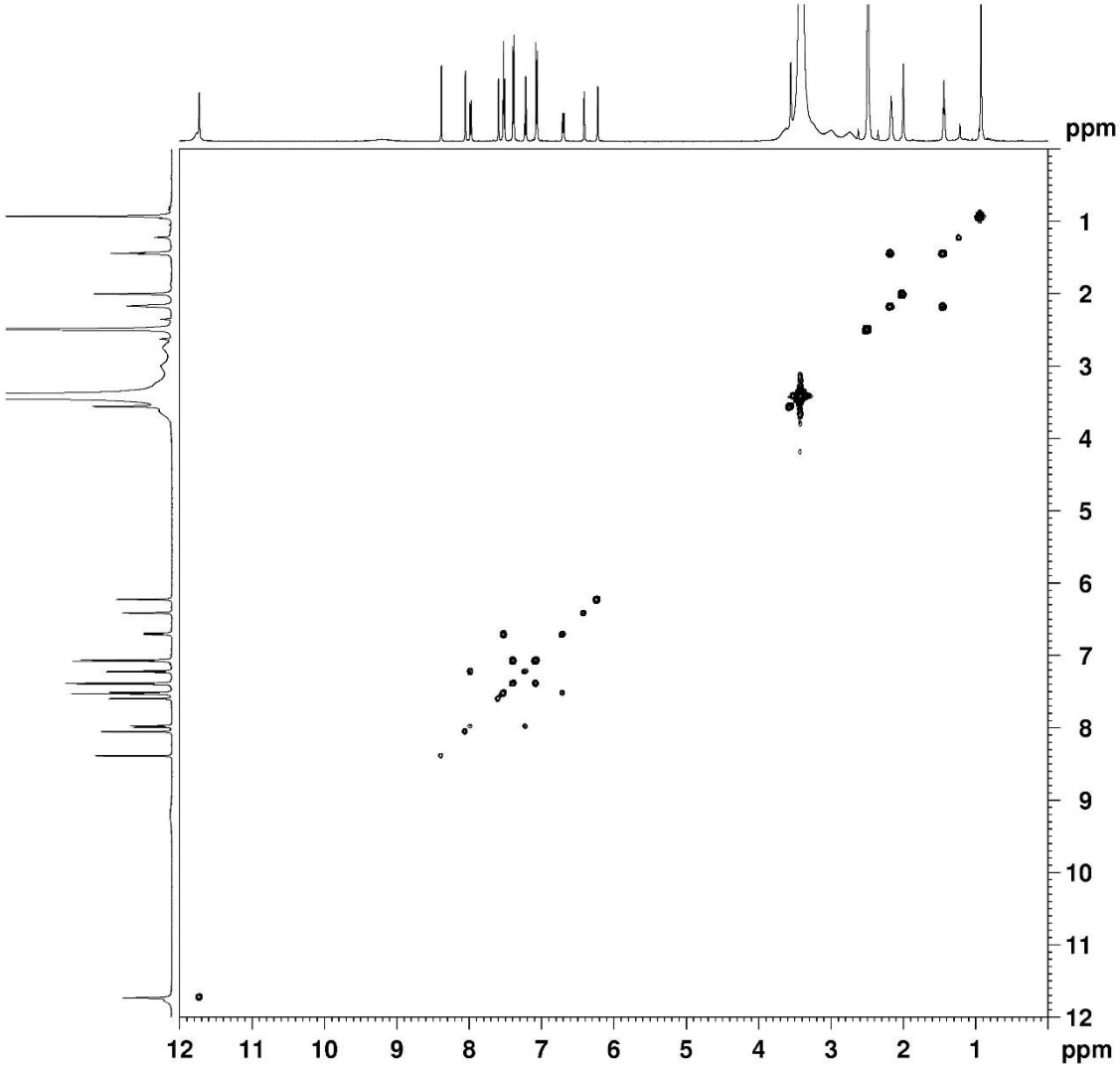


Figure S54 – (^{1}H, ^{1}H)-COSY spectrum of **B2**

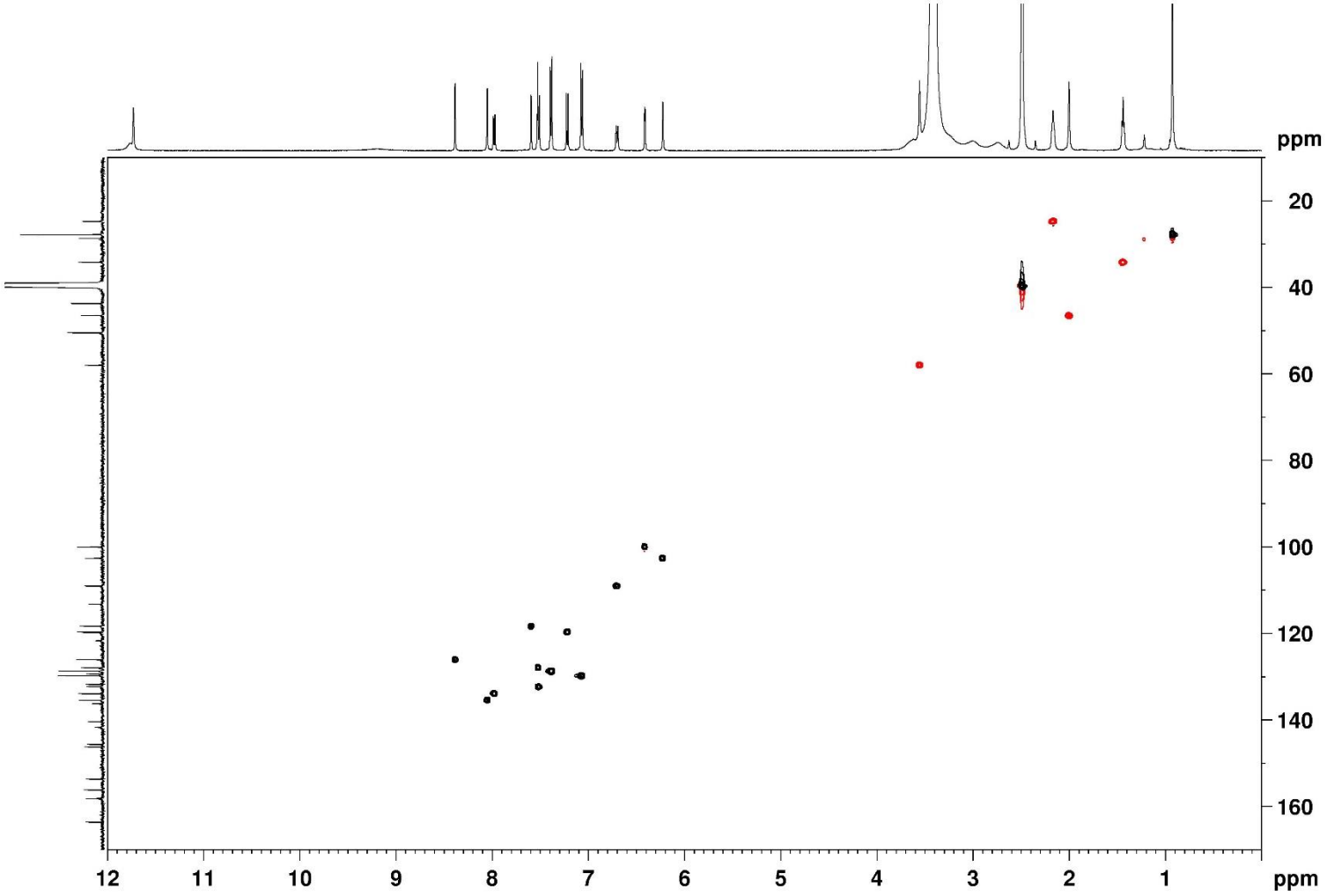


Figure S55 – (^{1}H, ^{13}C)-HSQC spectrum of **B2**

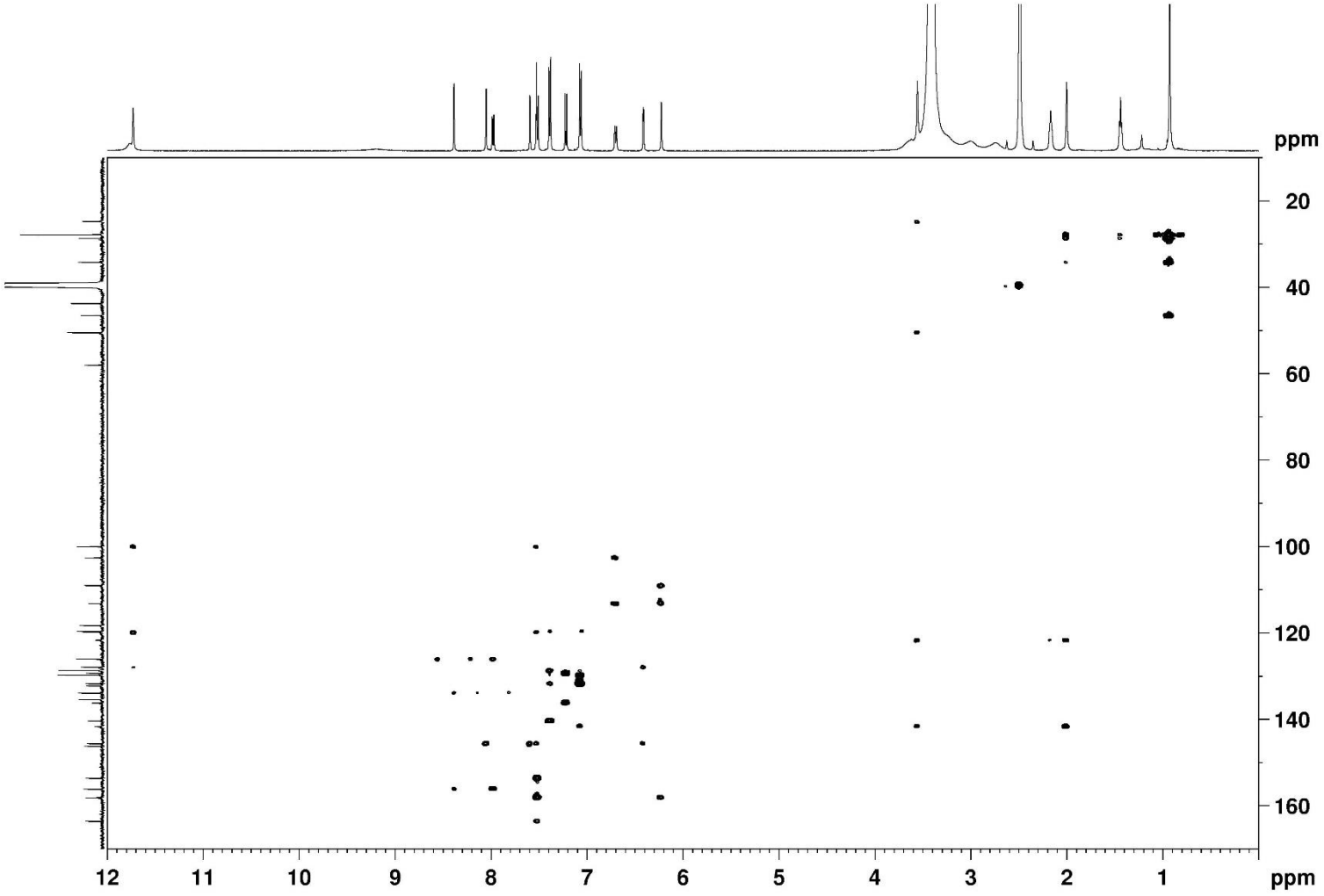


Figure S56 – (^{1}H, ^{13}C)-HMBC spectrum of **B2**

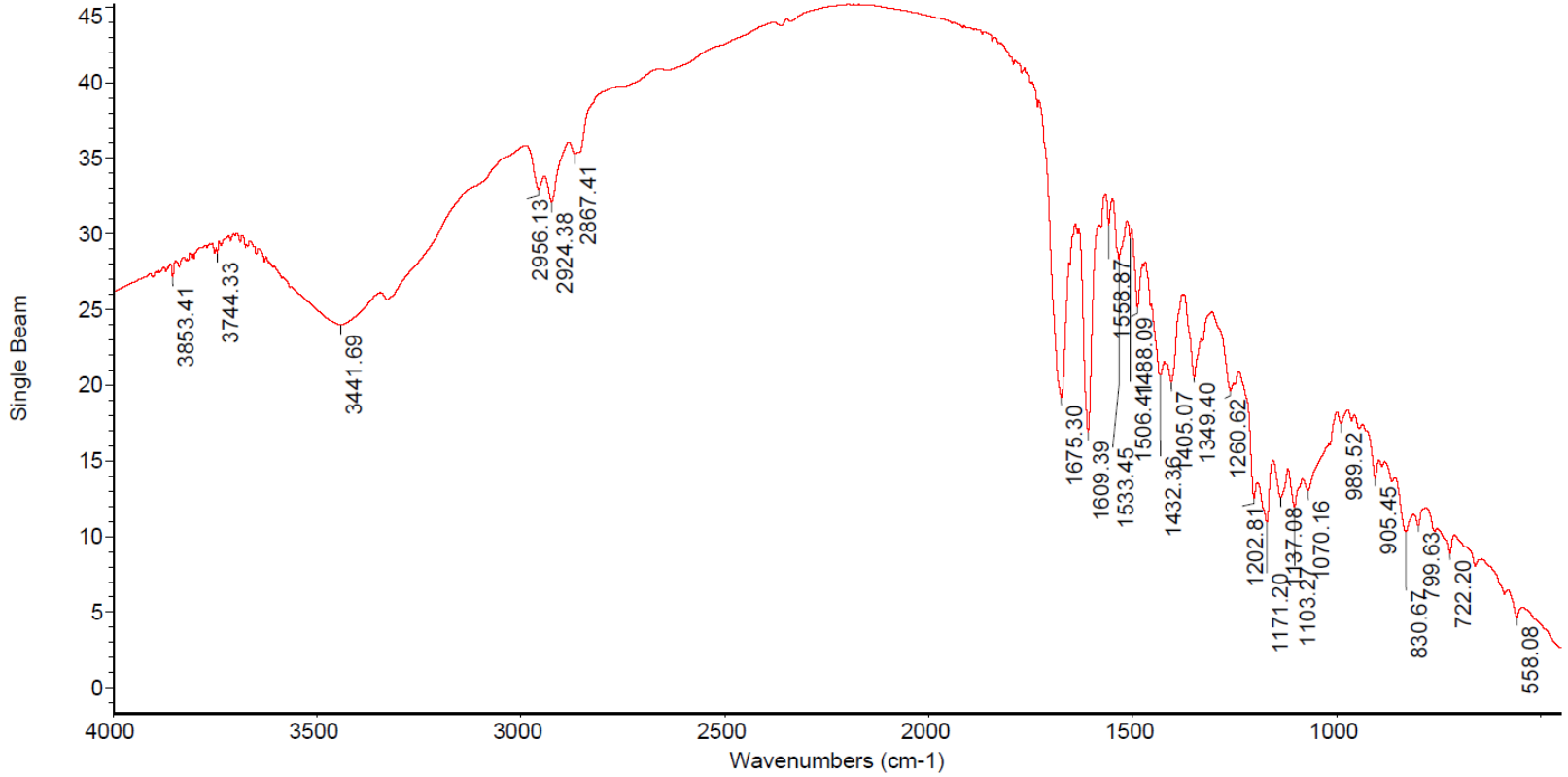


Figure S57 – IR spectrum of **B2**

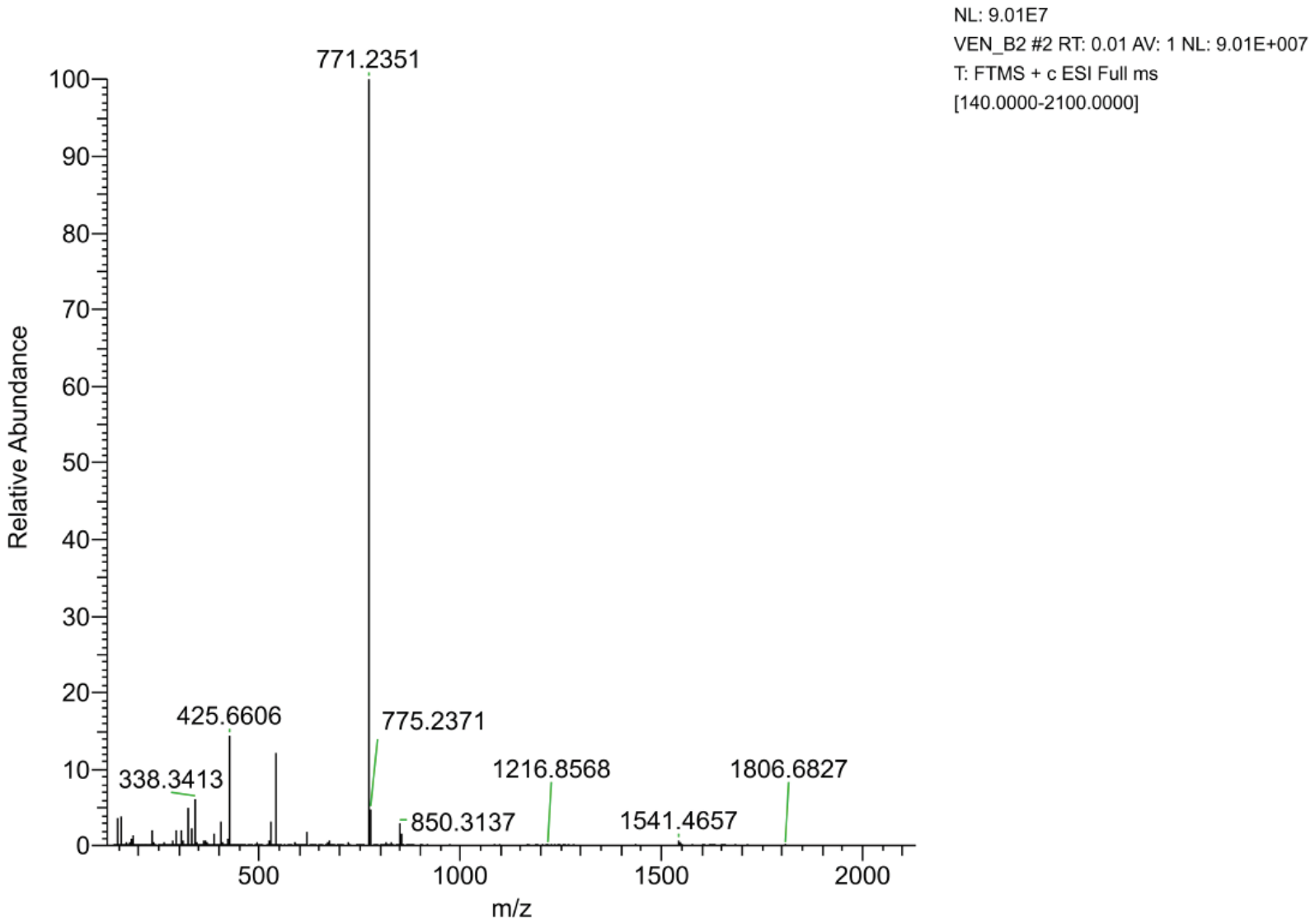


Figure S58 – HRMS spectrum of **B2**

Degradation product B3 information (see Figure S25 – Degradation product A3)

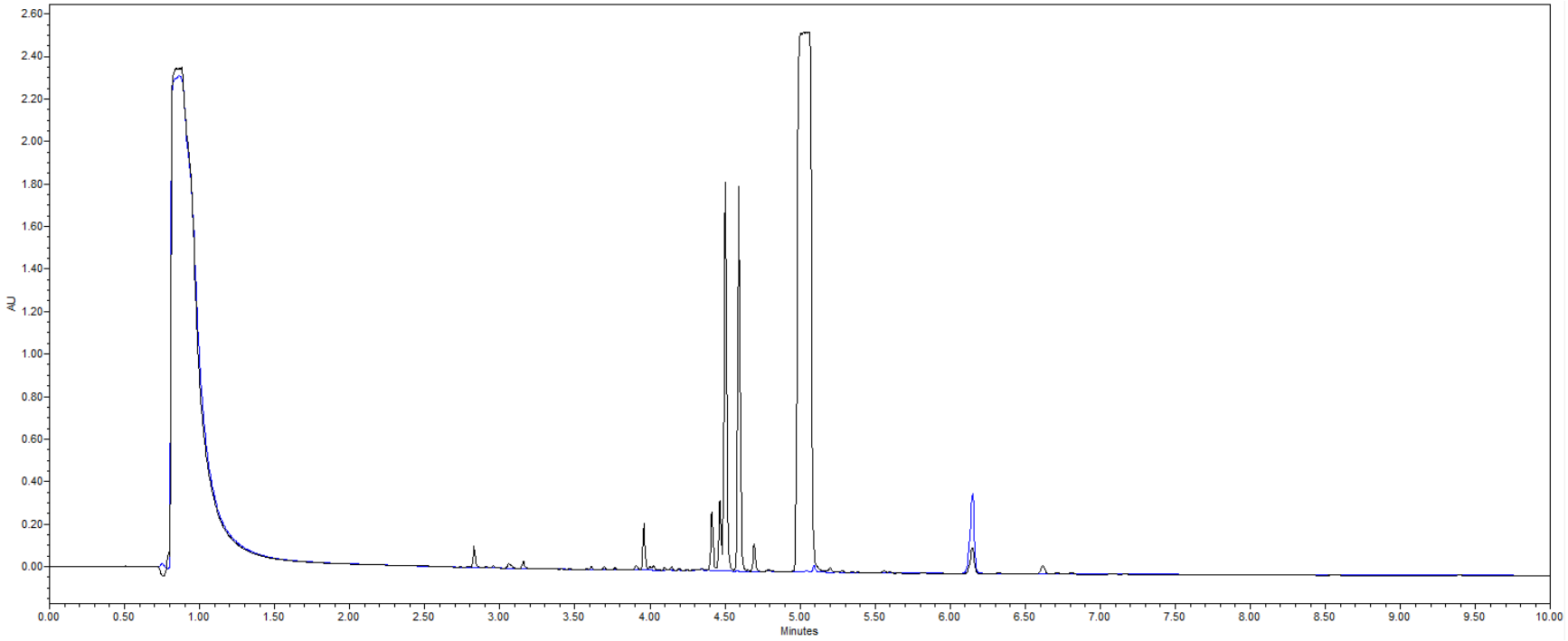


Figure S59 – Overlay chromatogram of a stress sample of venetoclax with added 1M NaOH after 1 day at 50 °C (black) and degradation product A3 (blue).

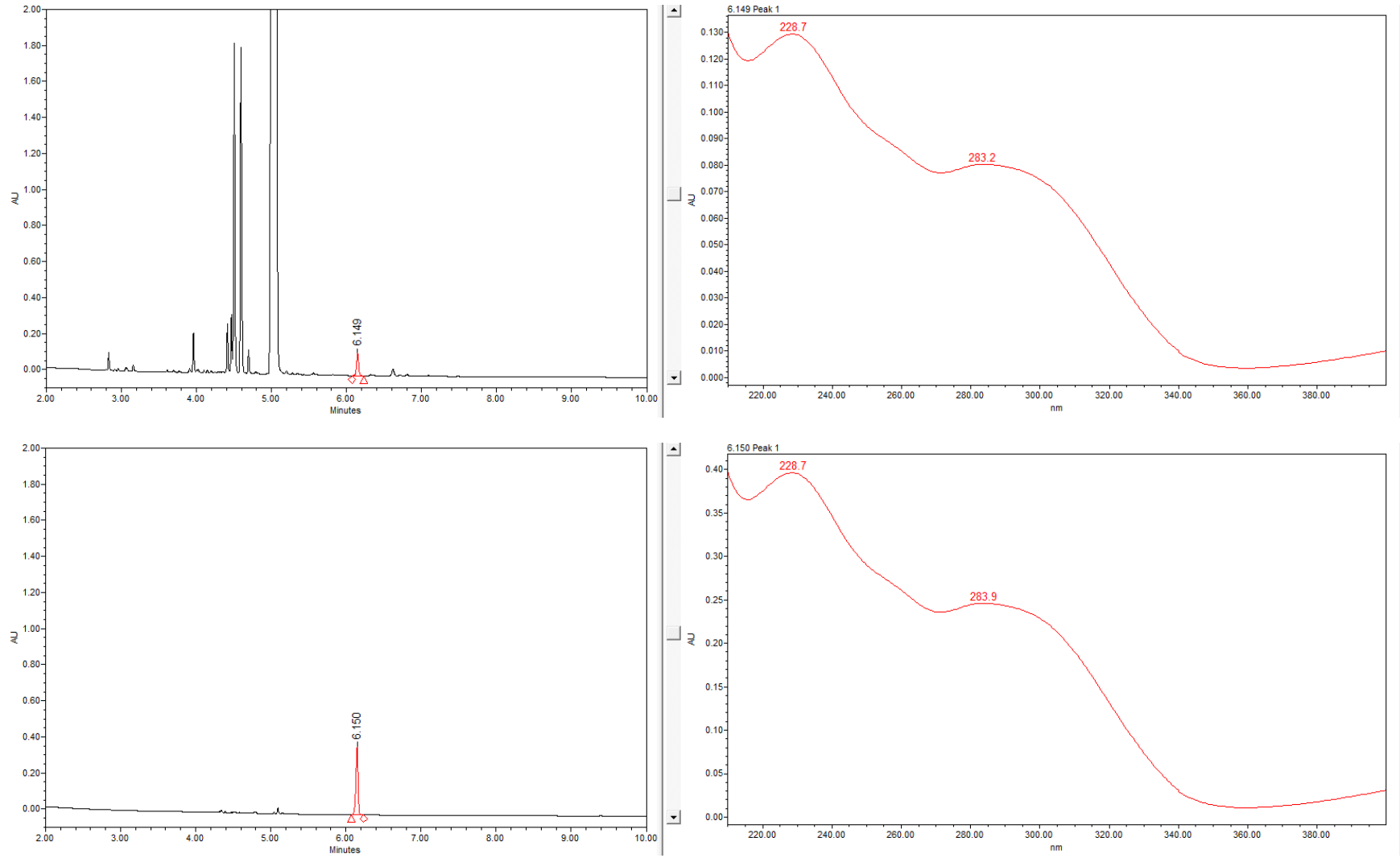


Figure S60 – Chromatograms (left) and UV spectra (right) of **degradation product B3** in stress sample of venetoclax with added 1M NaOH after 1 day at 50 °C (top) and **degradation product A3** (bottom)

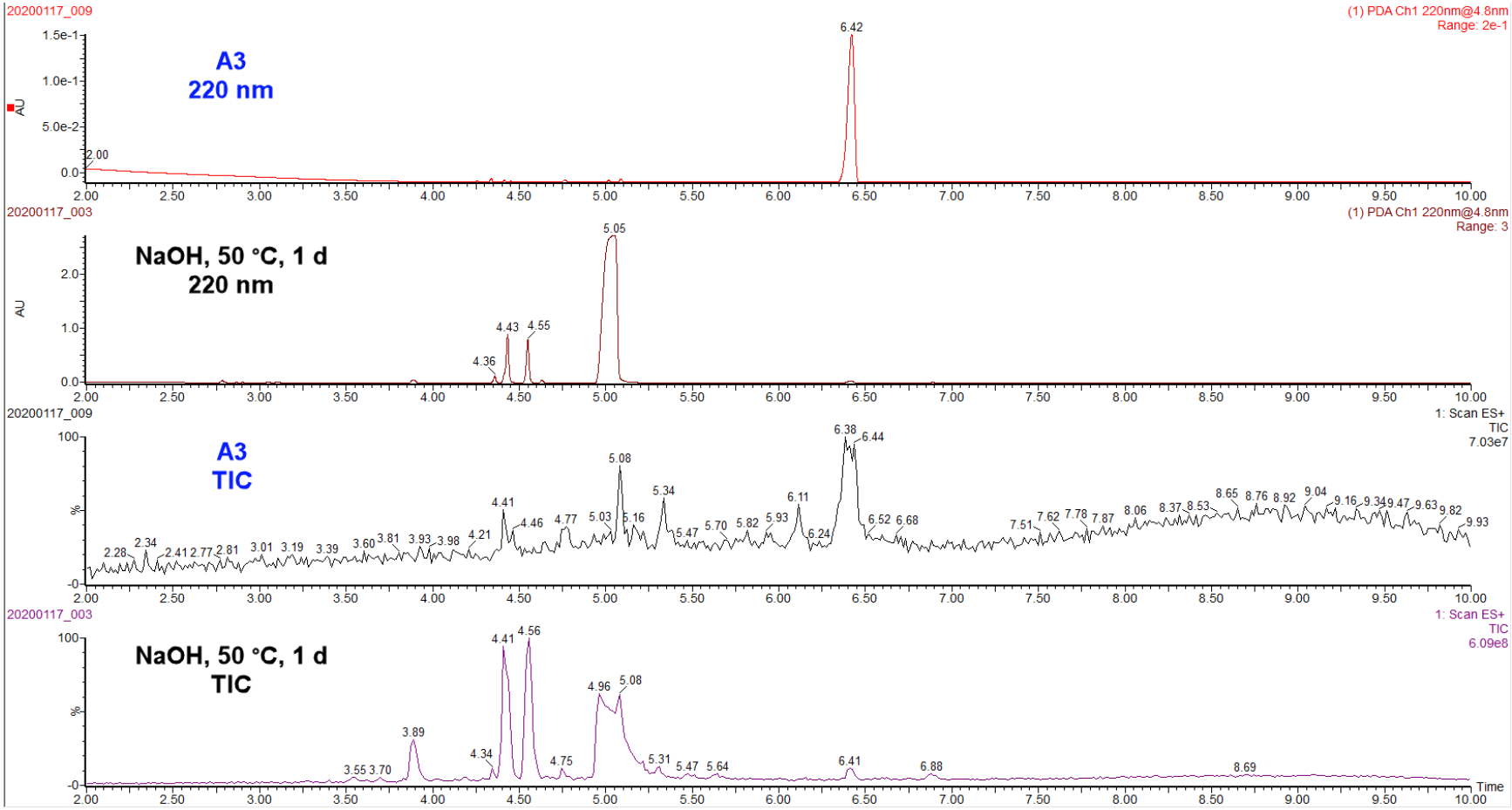


Figure S61 – Chromatograms of a stress sample of venetoclax with added 1M NaOH after 1 day on 50 °C and degradation product A3 obtained with UV and MS detector

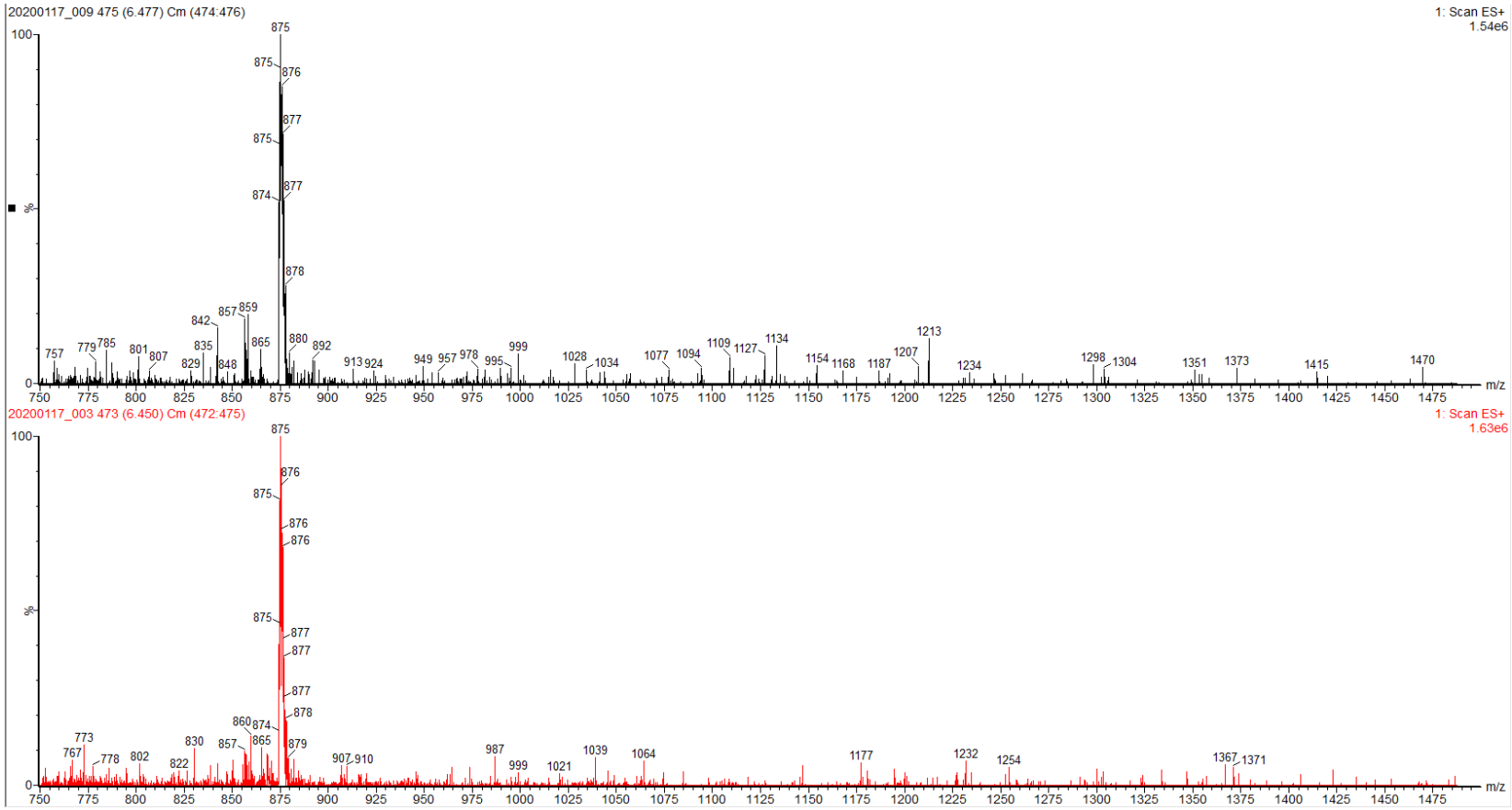


Figure S62 – MS spectra of **degradation product A3** (top) and **degradation product B3** (bottom).

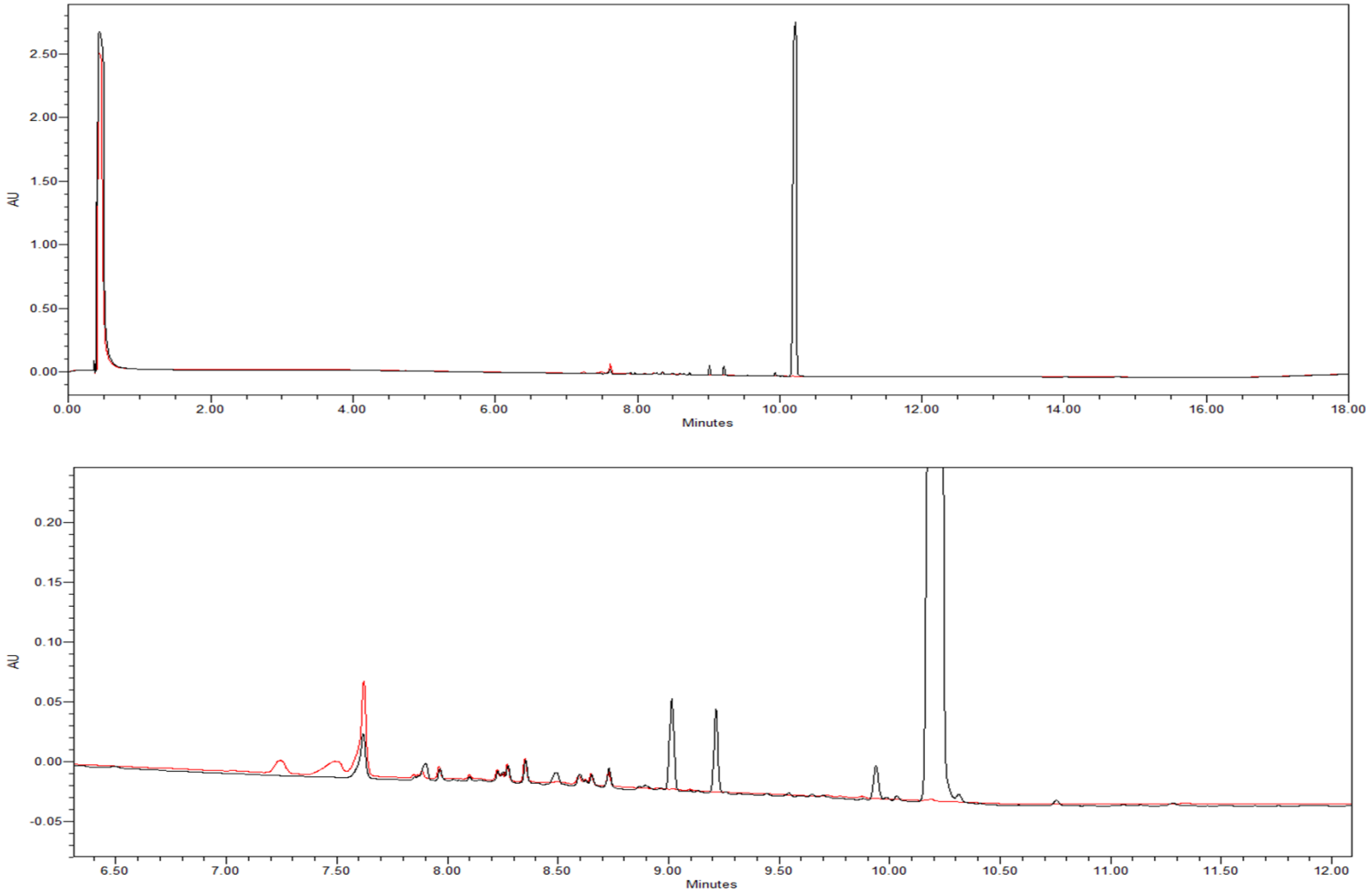


Figure S63 – Overlay chromatogram of a stress sample of venetoclax with added 3% H_2O2 after 7 days at 50 °C (black) and commercially obtained *N*-oxide venetoclax impurity (red) (top) and a close up of the same chromatogram from 6.3 to 12.1 min (bottom). The impurities eluting at approximately 9 min and 9.2 min are process related impurities present in the venetoclax substance.

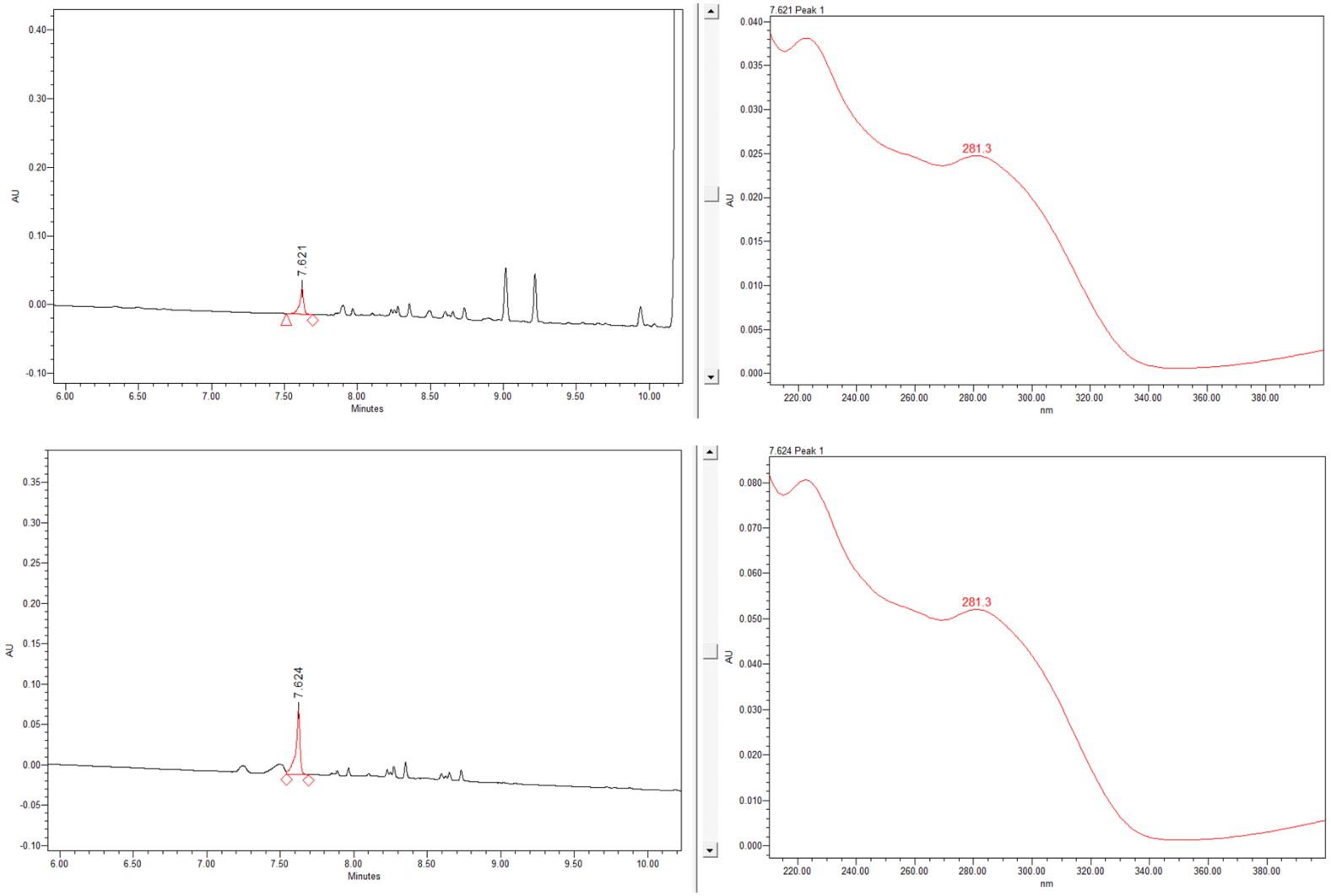


Figure S64 – Chromatograms (left) and UV spectra (right) of **degradation product *N*-oxide** in stress sample of venetoclax with added 3% H_2O_2 after 5 days at room temperature (top) and commercially obtained ***N*-oxide venetoclax** (bottom).

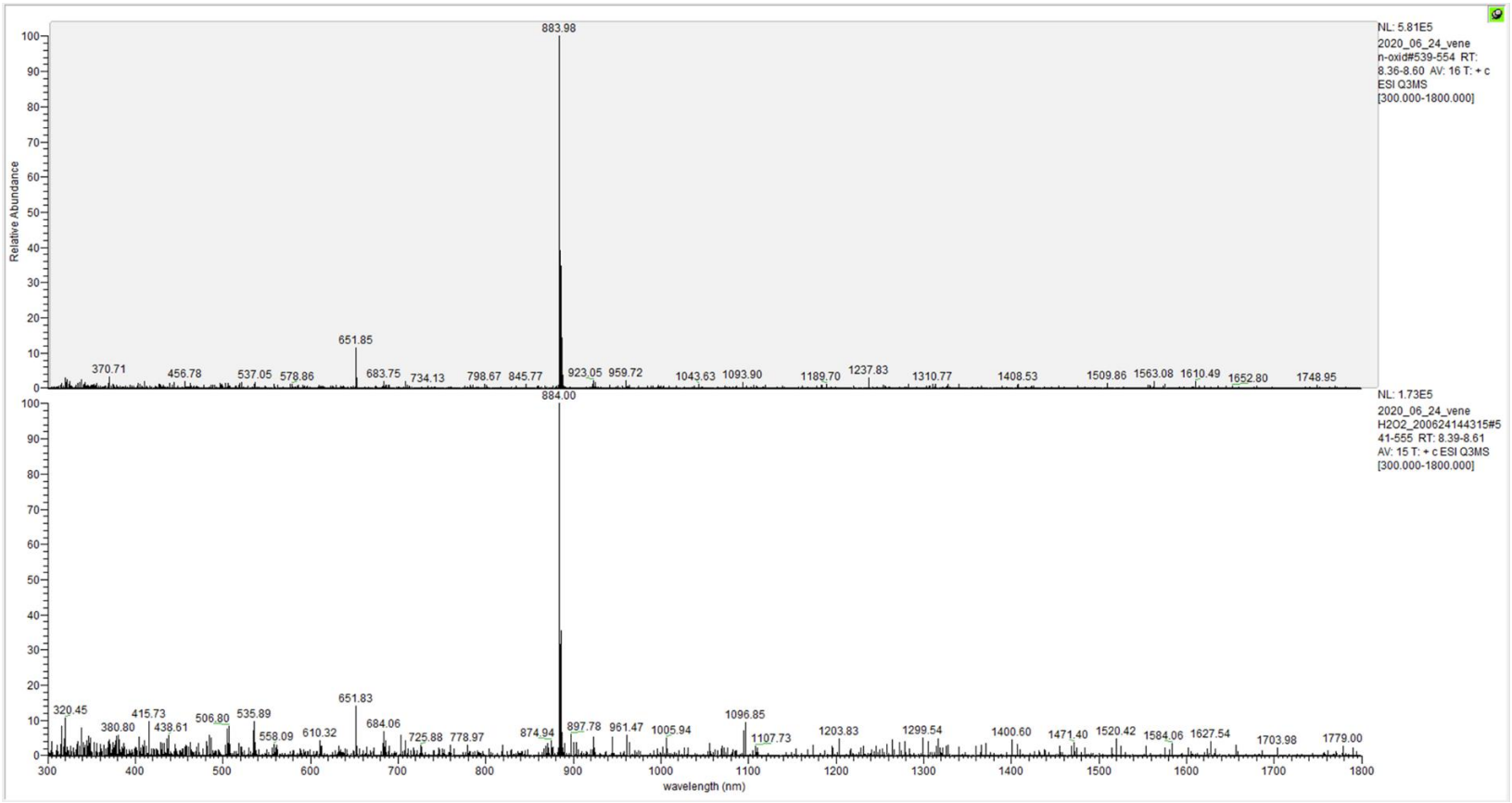


Figure S65 – MS spectra of a commercially obtained ***N*-oxide venetoclax** (top) and **degradation product *N*-oxide** in stress sample of venetoclax with added H_2O_2 (bottom). MS spectra was obtained with a triple quad mass spectrometer.