

Supplementary Material

Isolation and structural characterization of novel thermal degradation impurity of nafcillin sodium using spectroscopic and computational techniques

Prasad Yaragorla,^a Yesudas Kada,^a Krishnam Raju Ch,^b Arunima Pola,^b Rabin Bera,^{a*} and Sanath Kumar Goud P^b

^aSynthetic Laboratory and ^bAnalytical Research and Development, United States Pharmacopeial Convention – India (P) Ltd., Hyderabad-500101, India

Email: rxb@usp.org

Table of Contents

1. Thermal degradation Impurity (TDI) crude (150 Hr) Chromatogram	S2
2. Mass spectrum of TDI	S3
3. MS ⁿ spectral data of TDI	S4
4. High resolution mass spectrum of NS	S5
5. Elemental composition report of NS	S6
6. High resolution mass spectrum of TDI	S7
7. Elemental composition report of TDI	S8
8. High resolution MSMS spectrum for TDI	S9
9. ¹ H NMR Spectrum of TDI (500 MHz, DMSO-d ₆ , 300° K)	S10
10. ¹³ C NMR Spectrum of TDI (126 MHz, DMSO-d ₆ , 300° K)	S11
11. DEPT90 NMR Spectrum of TDI (126 MHz, DMSO-d ₆ , 300° K)	S12
12. DEPT135 NMR Spectrum of TDI (126 MHz, DMSO-d ₆ , 300° K)	S13
13. COSY NMR Spectrum of TDI (500 MHz, DMSO-d ₆ , 300° K)	S14
14. TOCSY NMR Spectrum of TDI (500 MHz, DMSO-d ₆ , 300° K)	S15
15. HSQC NMR Spectrum of TDI (500 MHz, DMSO-d ₆ , 300° K)	S16
16. HMBC NMR Spectrum of TDI (500 MHz, DMSO-d ₆ , 300° K)	S17
17. IR Spectrum of TDI	S18
18. Optimized geometries of TDI and TDI-1	S19
19. Calculated ¹ H and ¹³ C NMR chemical shifts of TDI and TDI-1	S20

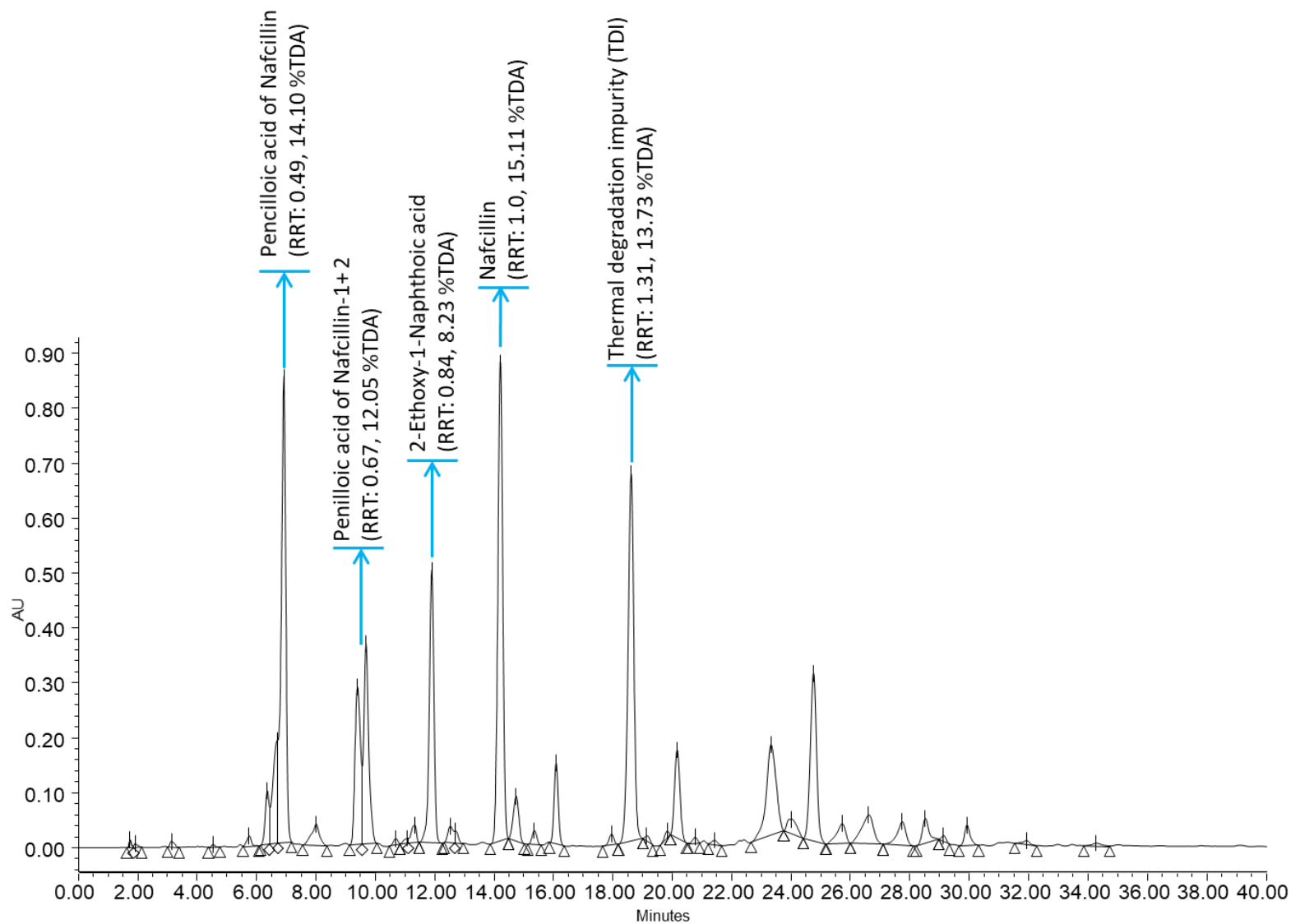


Figure S1. Thermal degradation Impurity (TDI) crude (150 Hr) Chromatogram

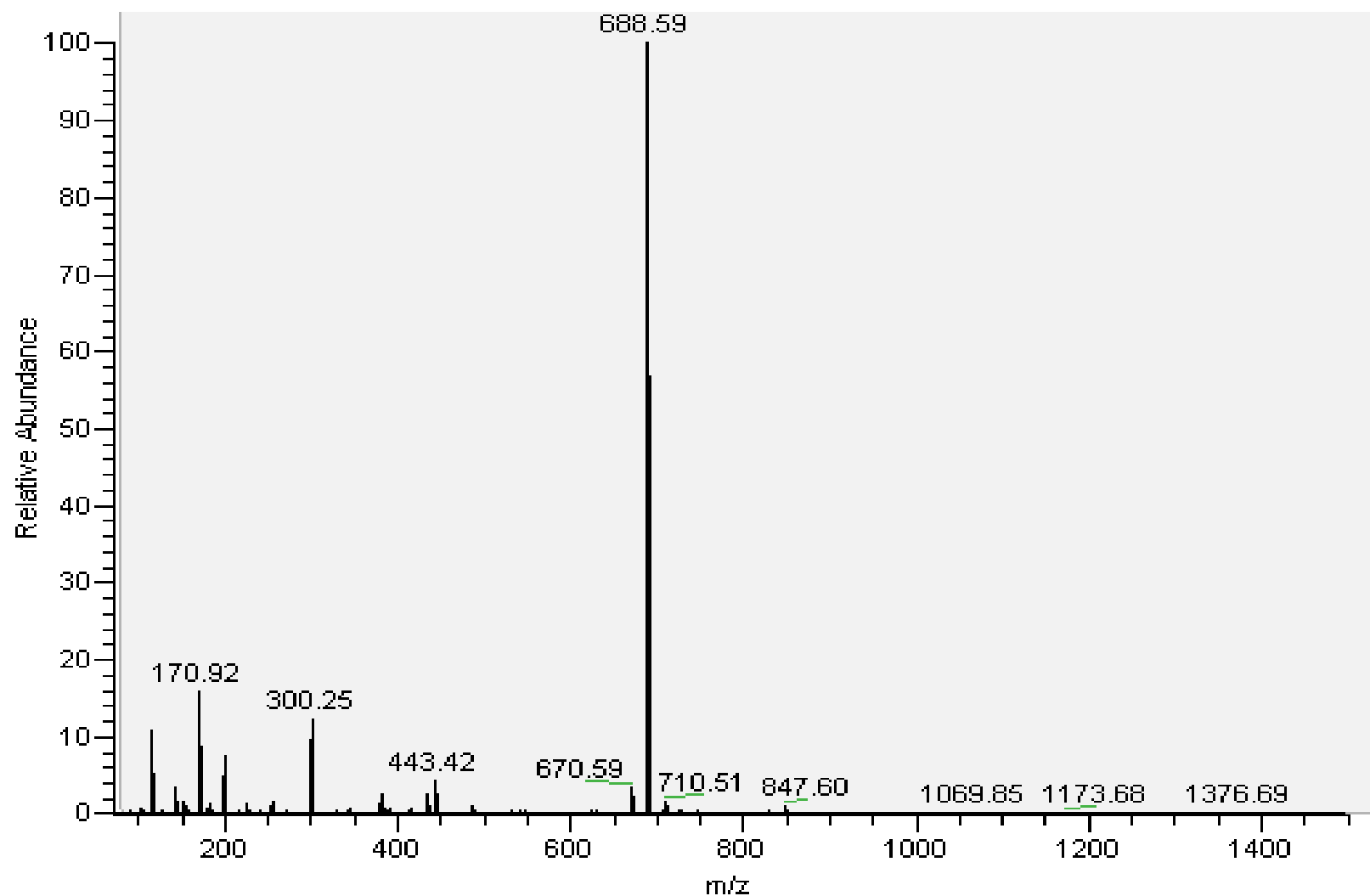


Figure S2. Mass spectrum of TDI

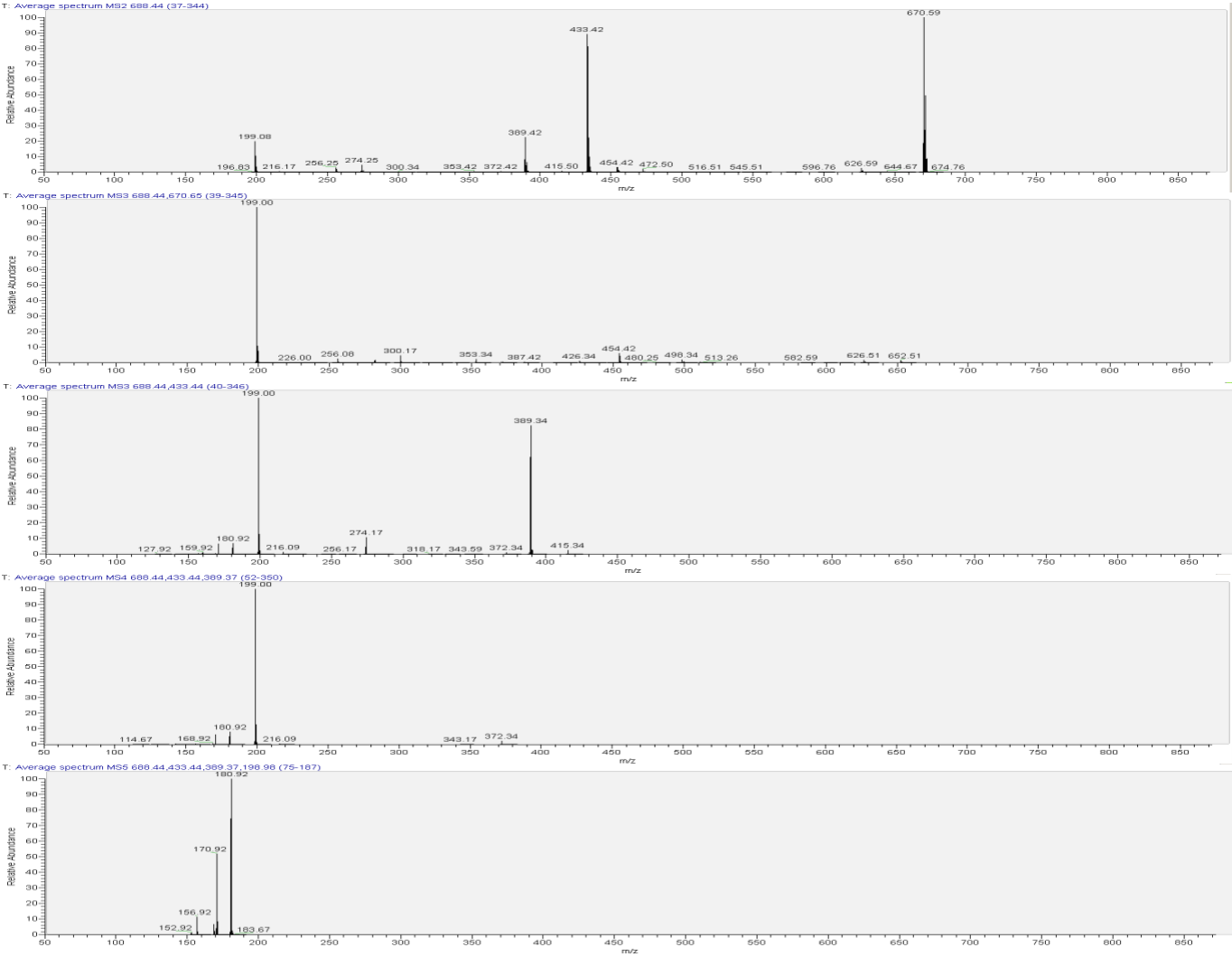


Figure S3. MSⁿ spectral data of TDI

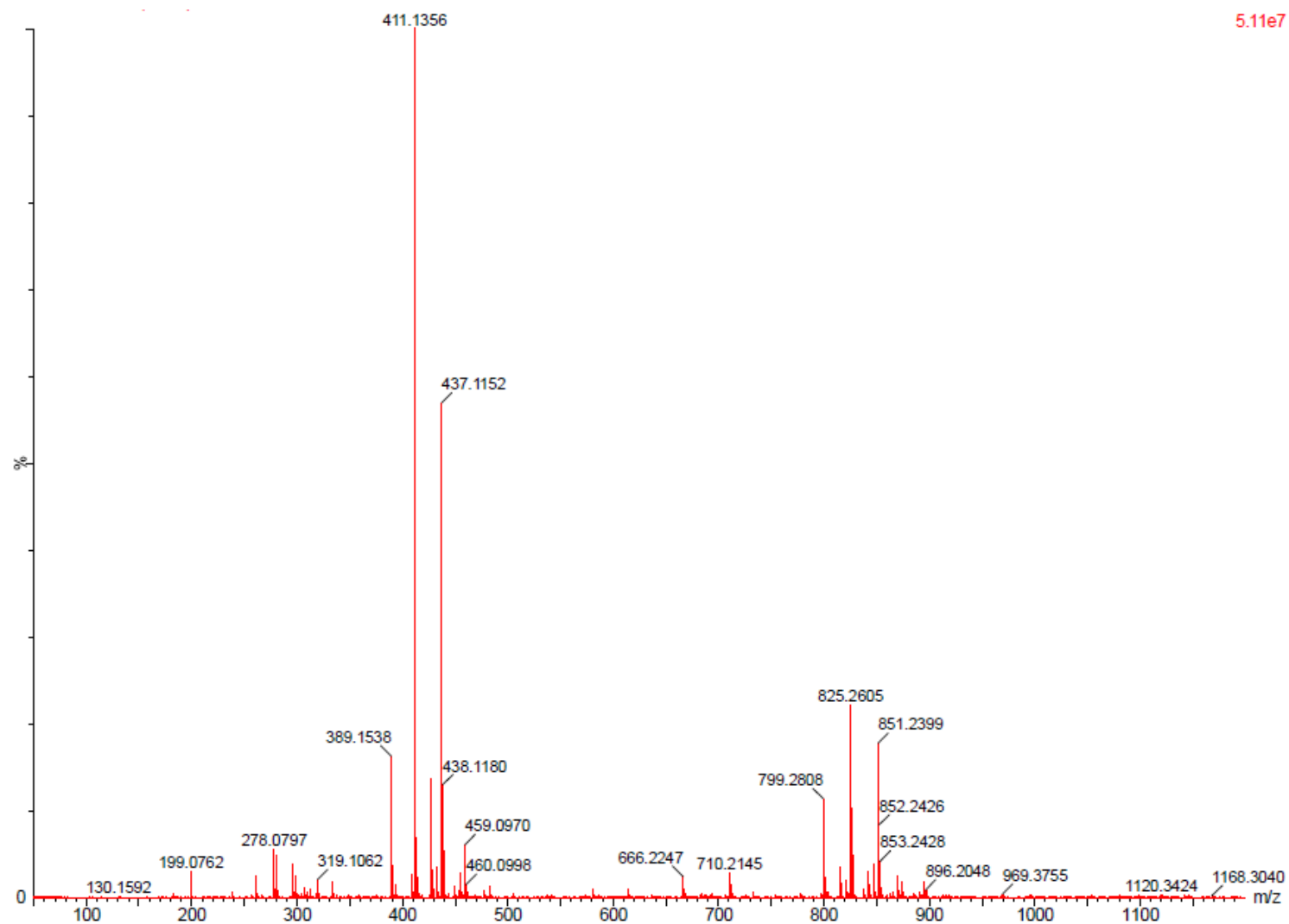


Figure S4. High resolution mass spectrum of NS

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

149 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-25 H: 0-25 N: 0-4 O: 0-6 Na: 0-1 S: 0-1

Minimum:				-1.5				
Maximum:	100.0	10.0		50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula
437.1152	437.1147	0.5	1.1	11.5	773.9	0.221	80.16	C21 H22 N2 O5 Na S
	437.1171	-1.9	-4.3	14.5	775.4	1.634	19.51	C23 H21 N2 O5 S
	437.1113	3.9	8.9	16.5	779.4	5.707	0.33	C24 H18 N2 O5 Na

Figure S5. Elemental composition report of **NS**

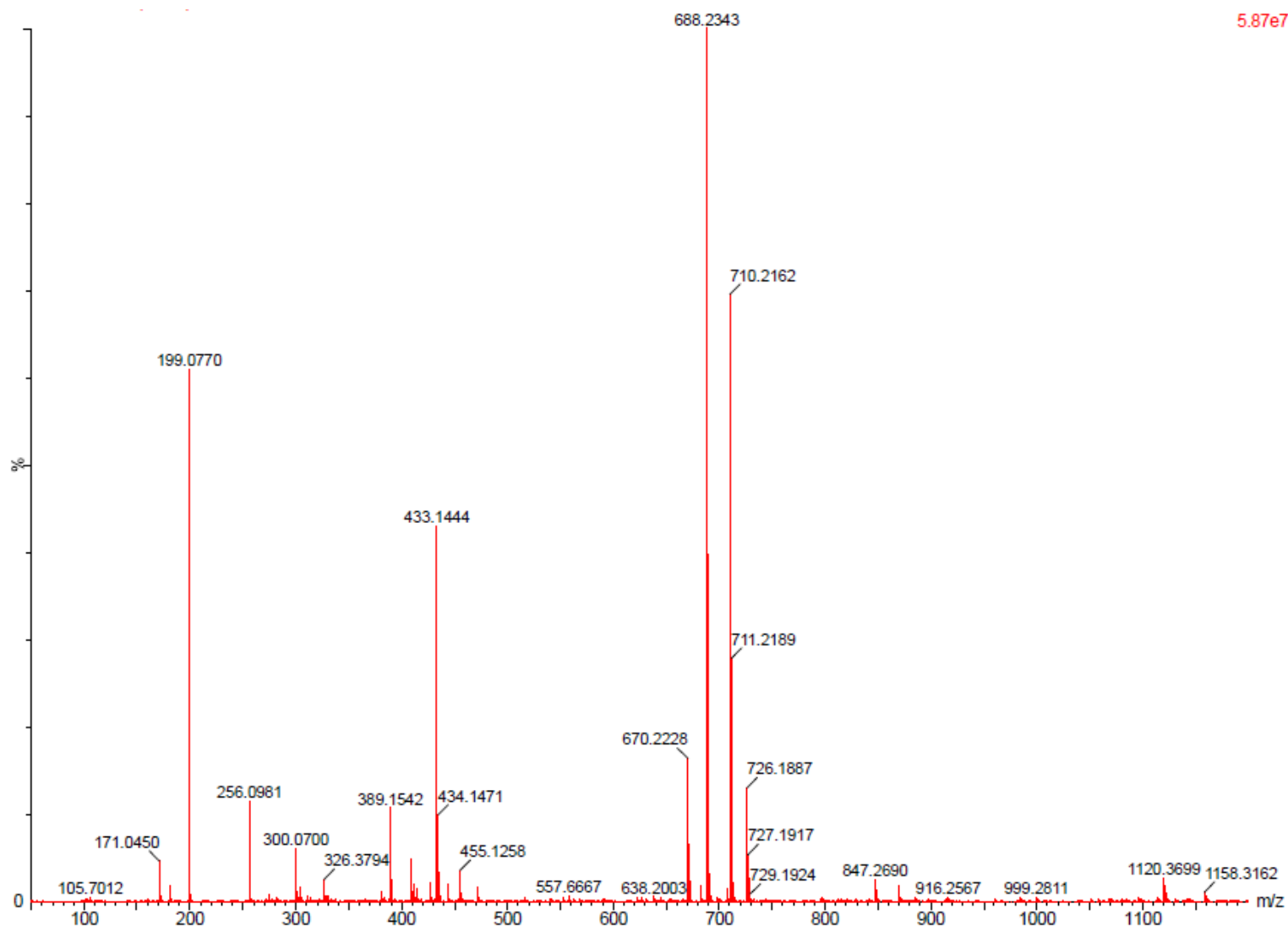


Figure S6. High resolution mass spectrum of TDI

Elemental Composition Report

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

262 formula(e) evaluated with 3 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 0-50 H: 0-50 N: 2-4 O: 0-10 S: 0-1

Minimum:				-1.5					
Maximum:	100.0	10.0		50.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf (%)	Formula	
688.2343	688.2329	1.4	2.0	19.5	806.5	0.000	99.96	C36 H38 N3 O9 S	
	688.2389	-4.6	-6.7	37.5	818.3	11.848	0.00	C50 H30 N3 O	
	688.2295	4.8	7.0	24.5	814.5	7.960	0.03	C39 H34 N3 O9	

Figure S7. Elemental composition report of **TDI**

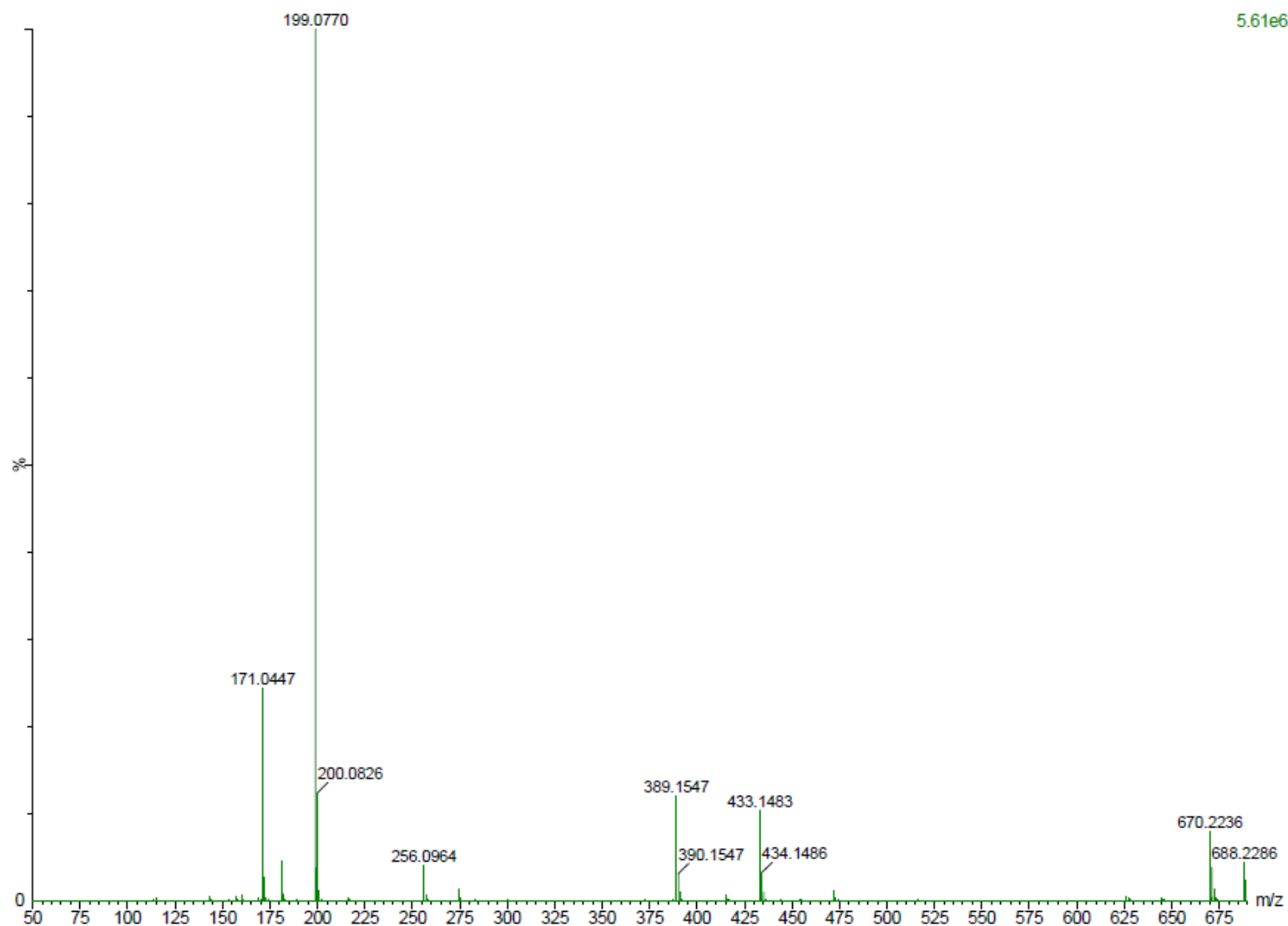


Figure S8. High resolution MSMS spectrum for **TDI**

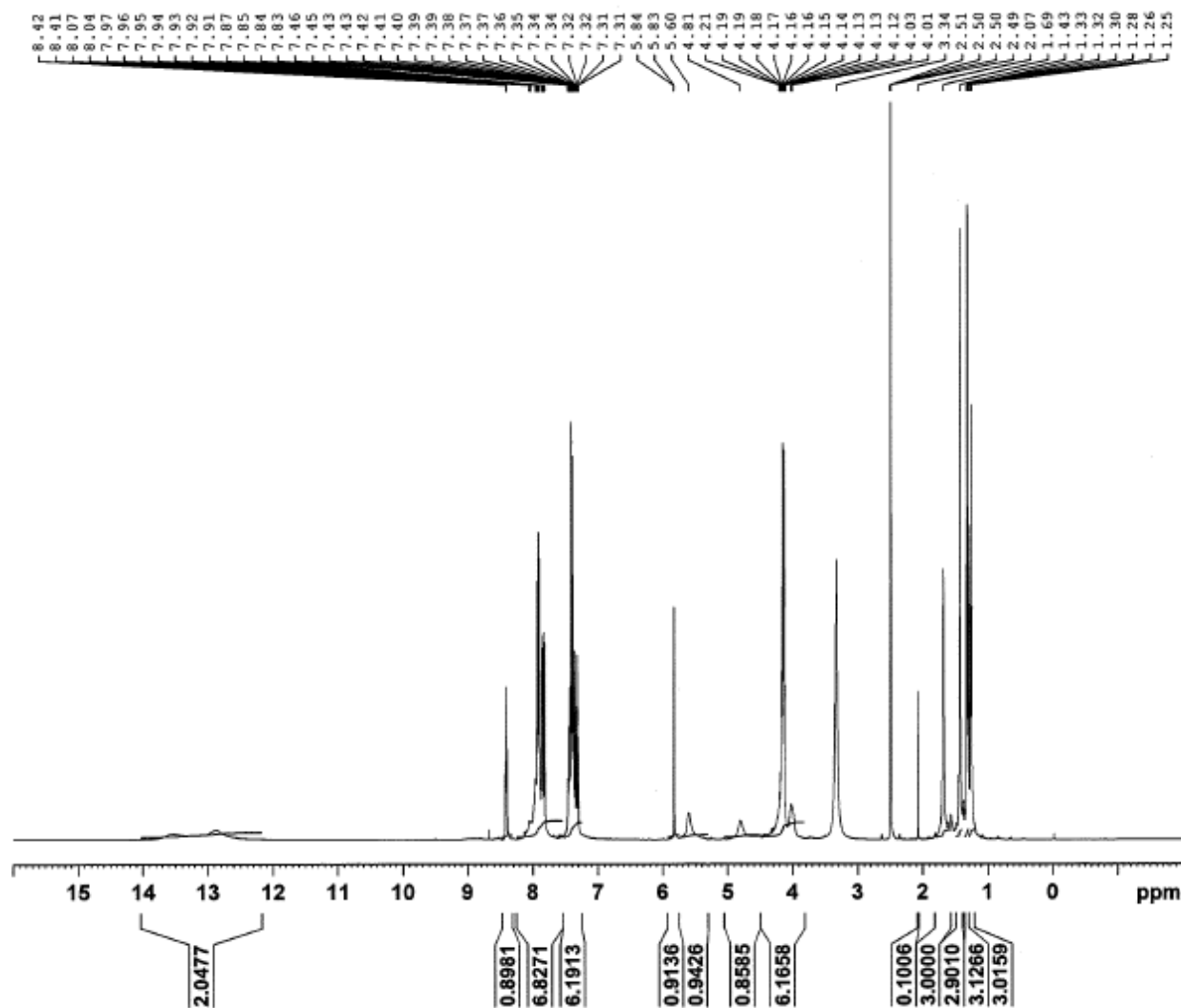


Figure S9. ¹H NMR Spectrum of TDI (500 MHz, DMSO-d₆, 300° K)

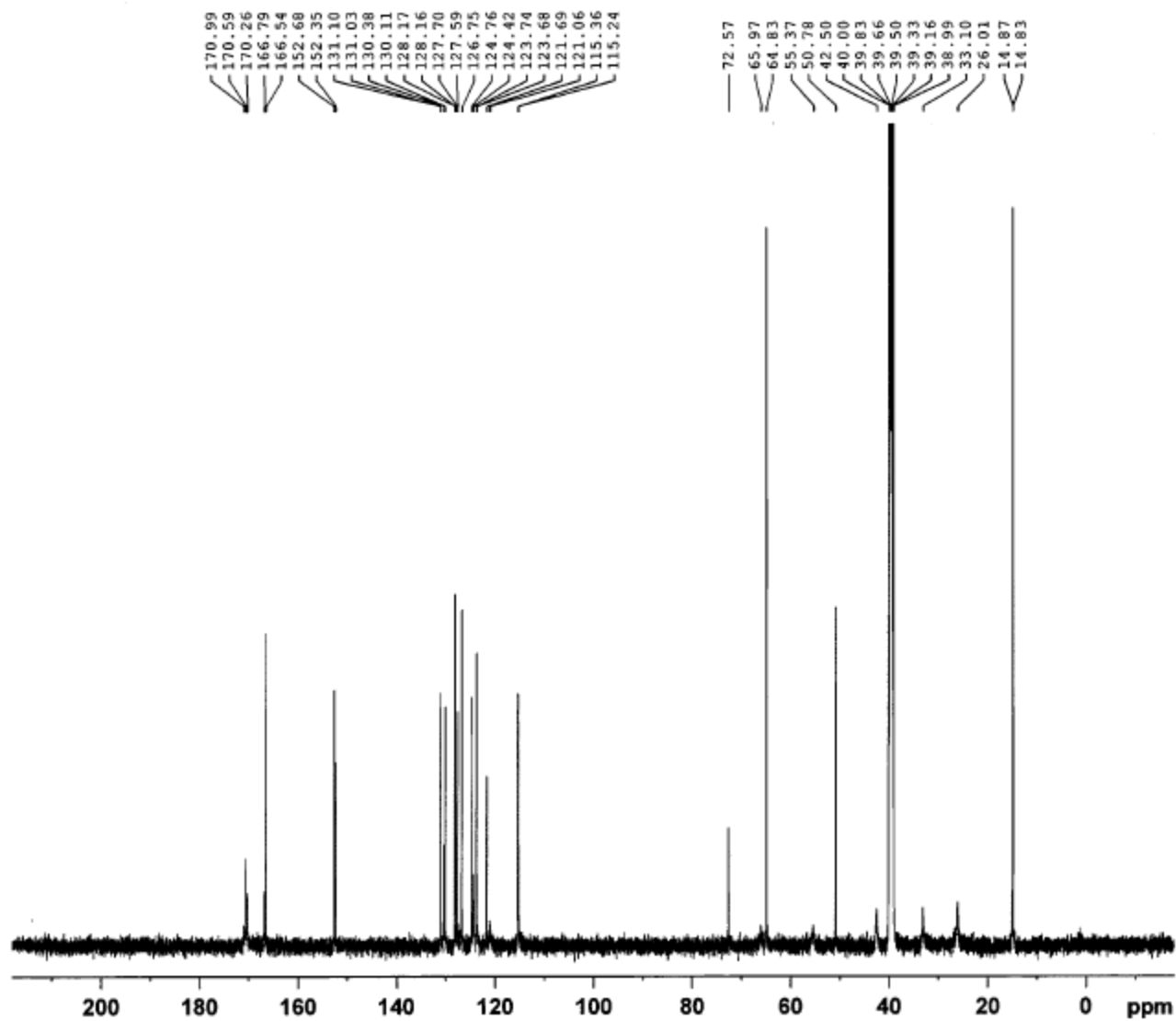


Figure S10. ¹³C NMR Spectrum of **TDI** (126 MHz, DMSO-d₆, 300° K)

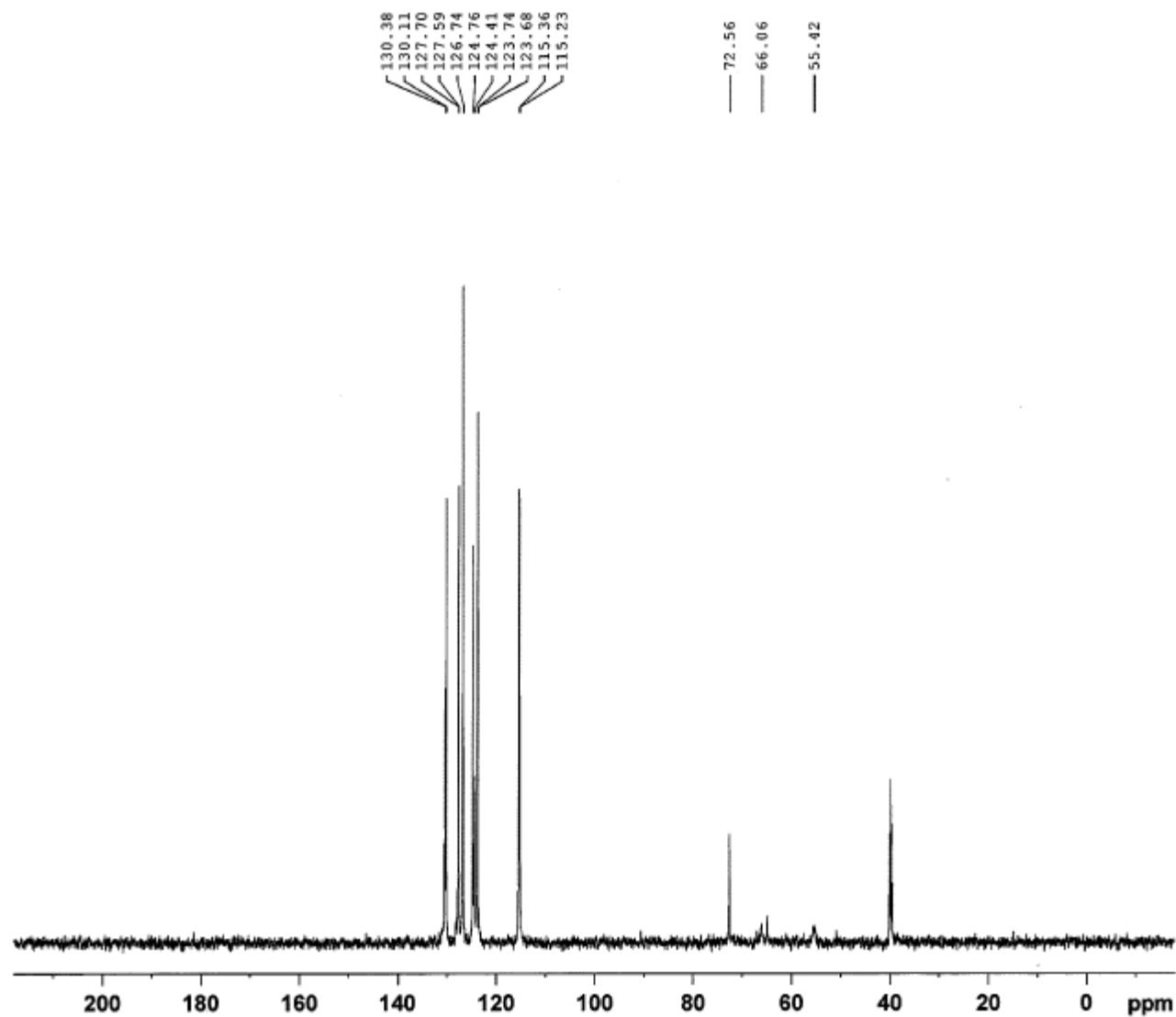


Figure S11. DEPT90 NMR Spectrum of **TDI** (126 MHz, DMSO-d₆, 300° K)

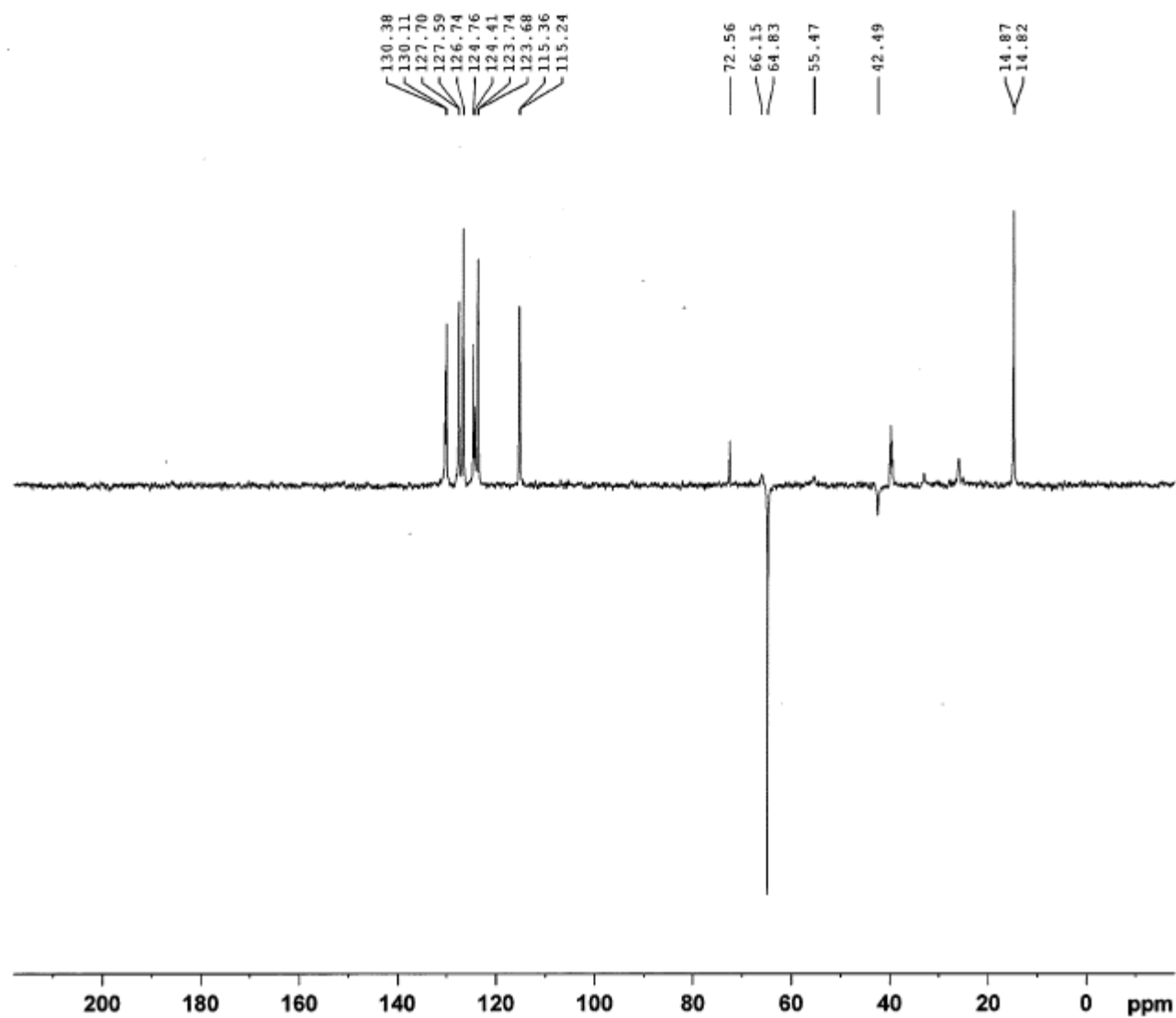


Figure S12. DEPT135 NMR Spectrum of **TDI** (126 MHz, DMSO-d₆, 300° K)

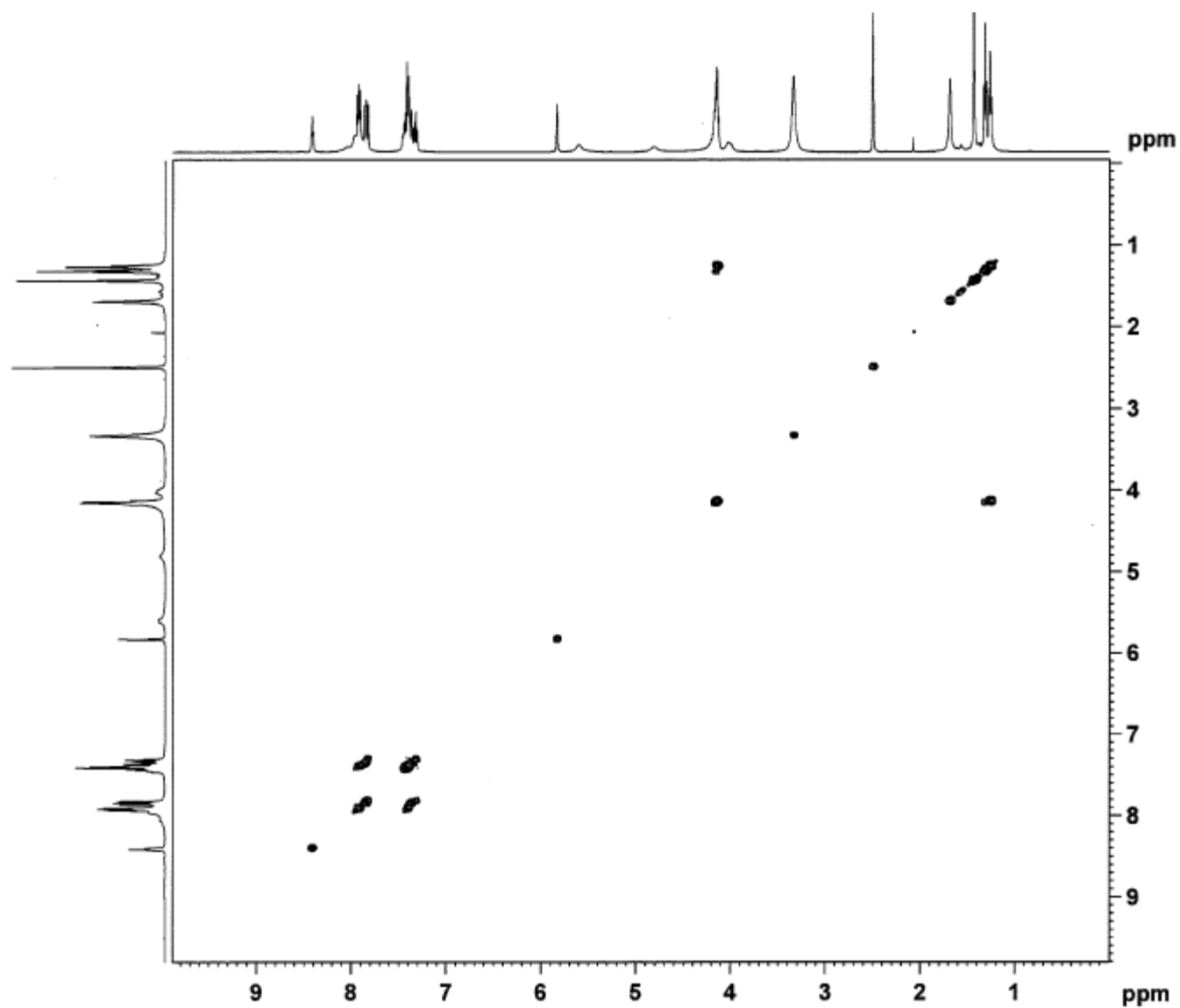


Figure S13. COSY NMR Spectrum of **TDI** (500 MHz, DMSO- d_6 , 300° K)

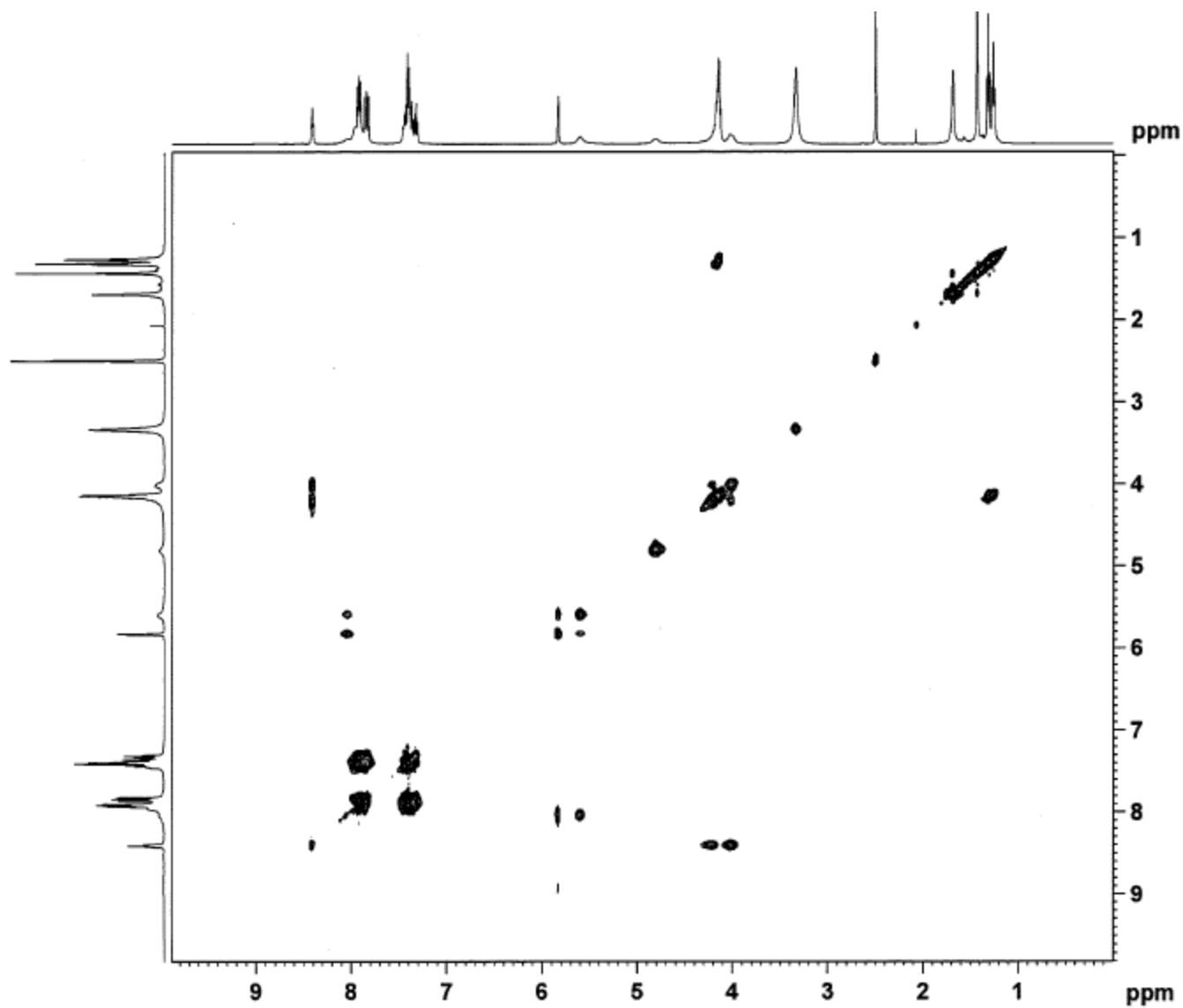


Figure S14. TOCSY NMR Spectrum of **TDI** (500 MHz, DMSO-*d*₆, 300° K)

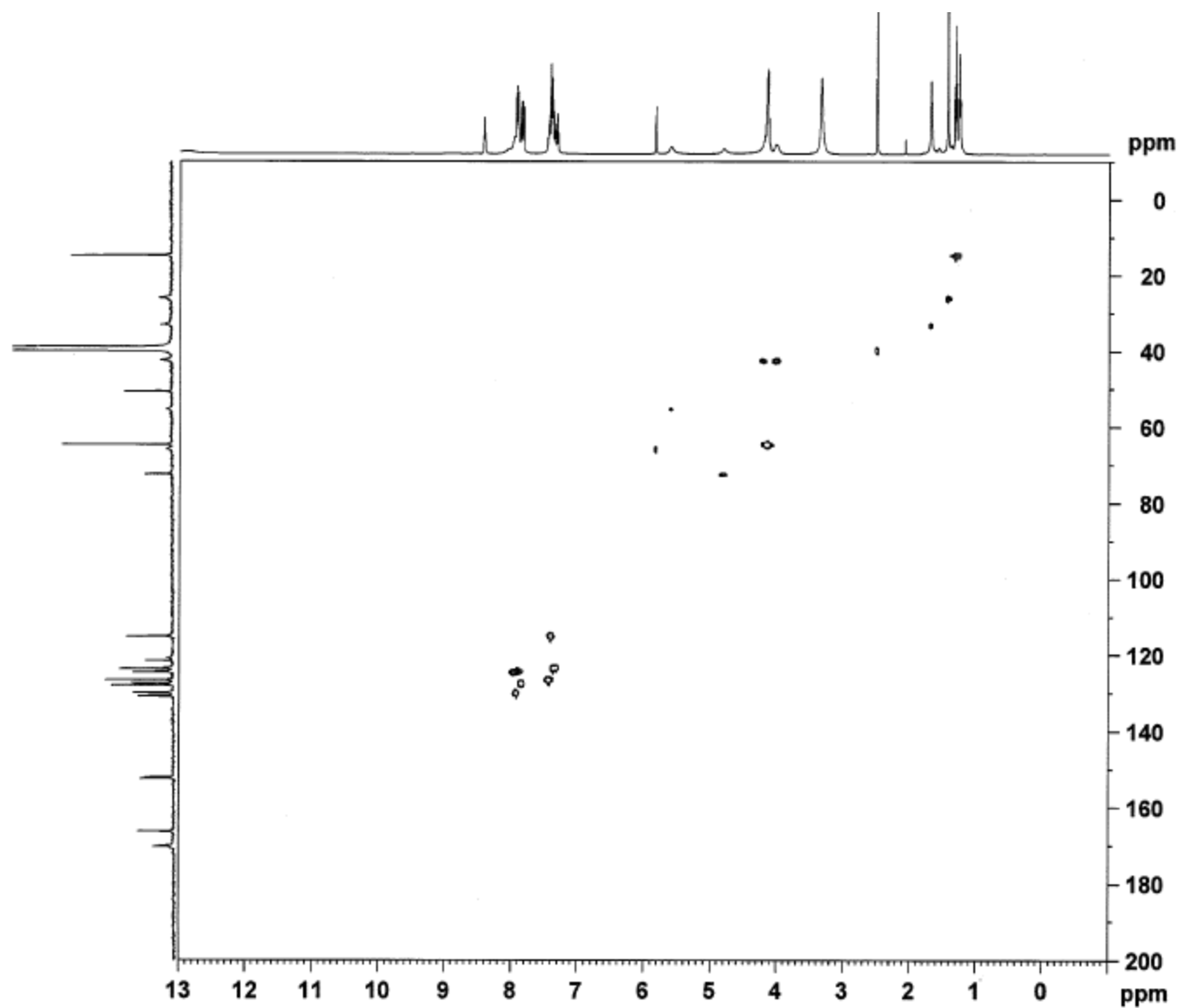


Figure S15. HSQC NMR Spectrum of **TDI** (500 MHz, DMSO- d_6 , 300° K)

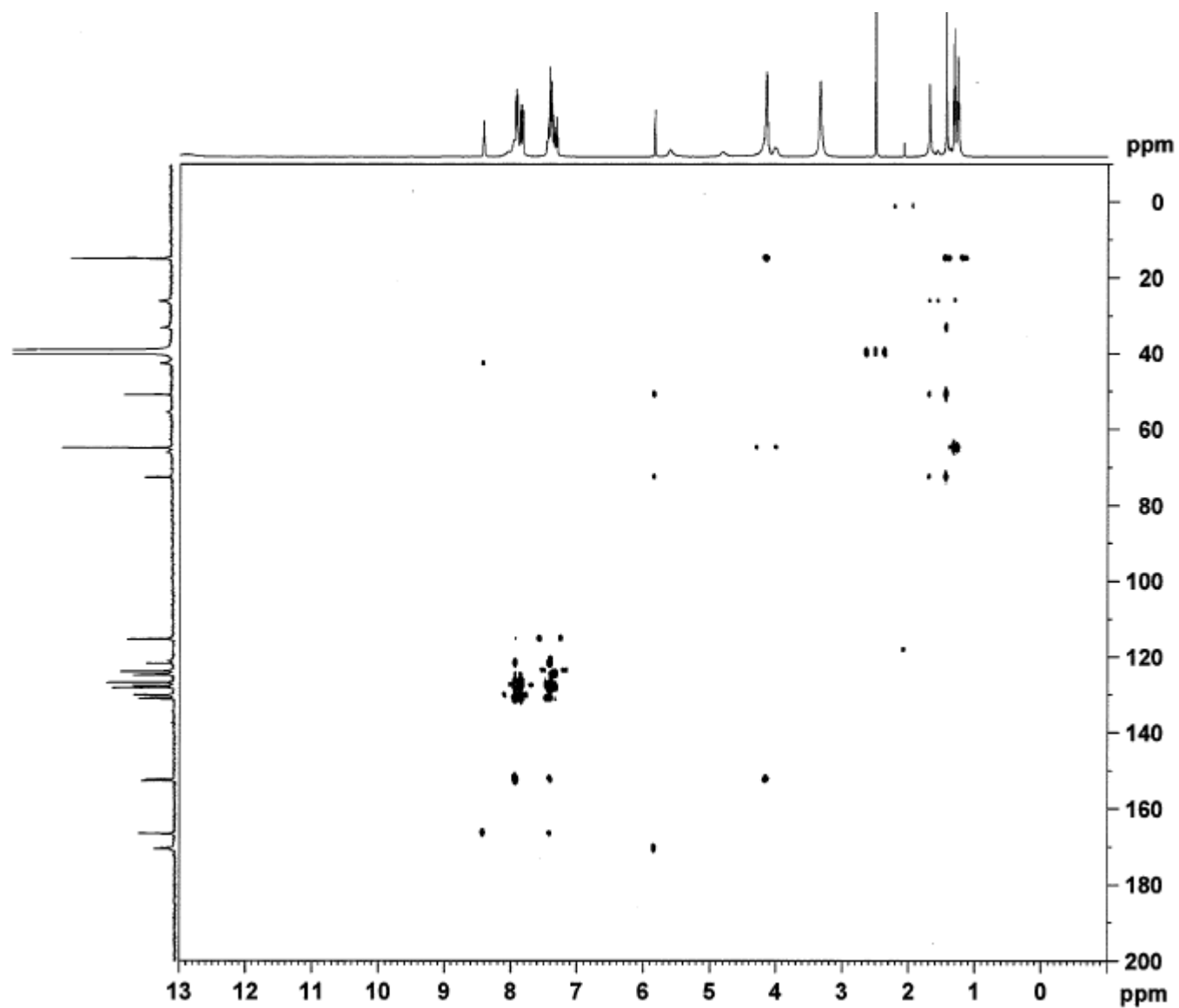


Figure S16. HMBC NMR Spectrum of TDI (500 MHz, DMSO- d_6 , 300° K)

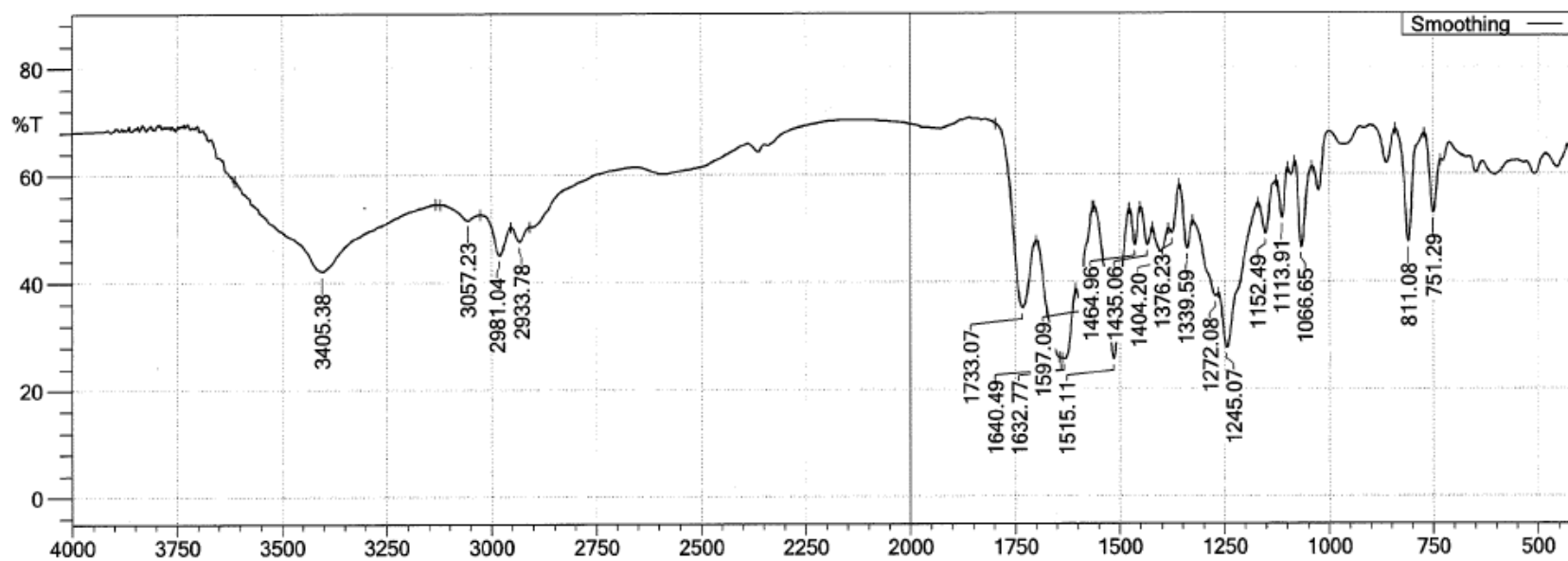


Figure S17. IR Spectrum of TDI

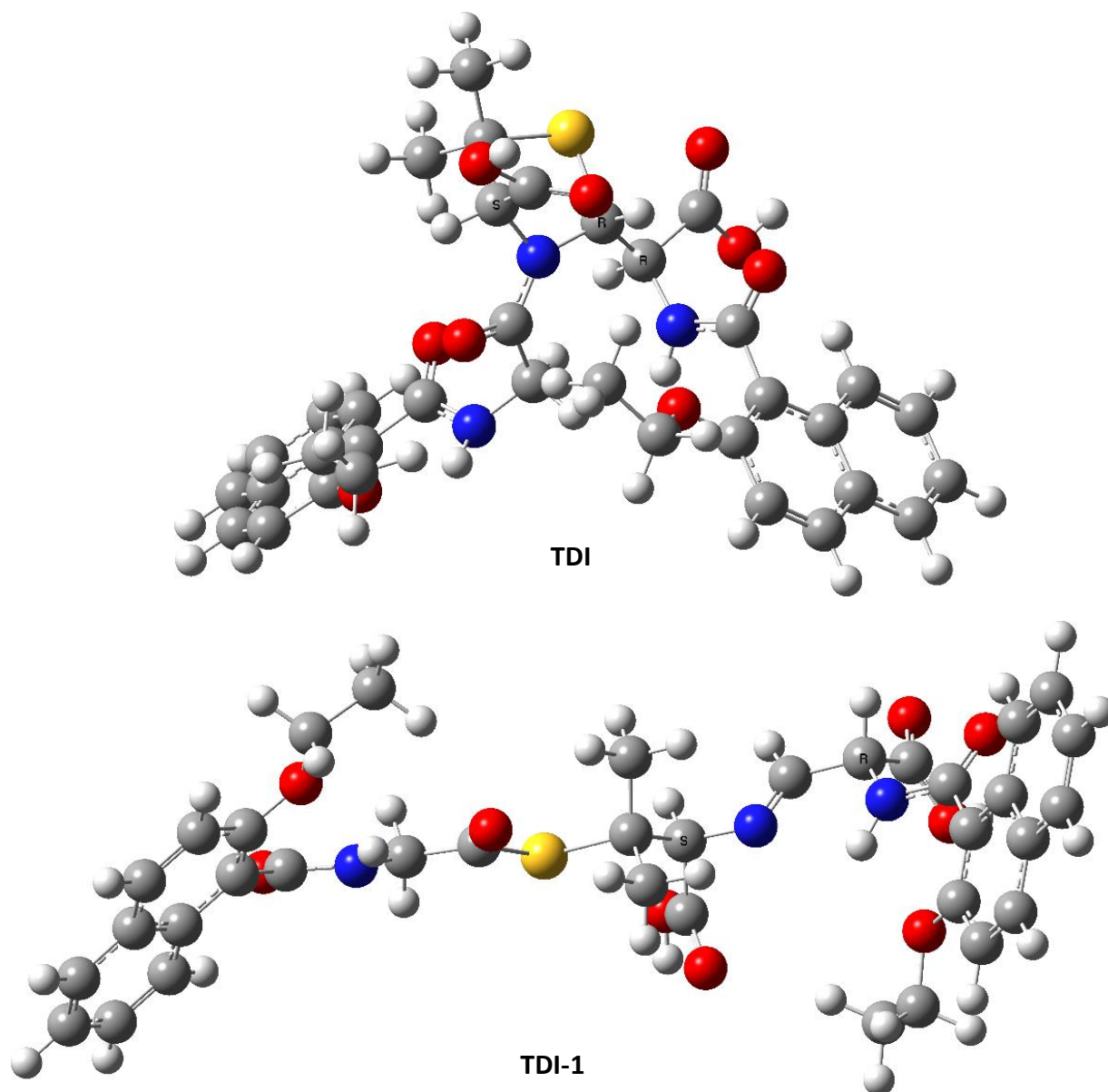


Figure S18. Optimized geometries of **TDI** and **TDI-1** obtained at the B3LYP/6-31+G(d,p) level of the theory in gas phase.

Table S1. Calculated ^1H and ^{13}C NMR chemical shifts (δ) and the difference values[†] ($\Delta\delta$) of **TDI** and **TDI-1** obtained at B3LYP/6-31+G(d,p) level of the theory in the DMSO solvent

Proton #	δ_{exp}	$\delta_{\text{cal}}^{\text{TDI}}$	$\delta_{\text{cal}}^{\text{TDI-1}}$	$\Delta\delta^{\text{TDI}}$	$\Delta\delta^{\text{TDI-1}}$	Carbon #	δ_{exp}	$\delta_{\text{cal}}^{\text{TDI}}$	$\delta_{\text{cal}}^{\text{TDI-1}}$	$\Delta\delta^{\text{TDI}}$	$\Delta\delta^{\text{TDI-1}}$
H1	1.32	1.67	1.43	-0.35	-0.11	C1	14.84	18.22	17.85	-3.38	-3.01
H2	4.16	4.29	4.28	-0.13	-0.12	C2	64.85	71.69	67.25	-6.84	-2.40
H5	7.42	7.71	7.57	-0.29	-0.15	C4	152.52	154.74	154.32	-2.22	-1.80
H6	7.94	8.28	8.12	-0.34	-0.18	C5	115.31	115.43	110.71	-0.13	4.59
H8	7.95	8.19	8.16	-0.24	-0.21	C6	130.26	130.87	132.96	-0.62	-2.70
H9	7.45	7.85	7.65	-0.40	-0.20	C7	129.62	129.76	130.06	-0.13	-0.44
H10	7.34	7.92	7.90	-0.58	-0.56	C8	126.12	125.09	124.58	1.03	1.54
H11	7.85	9.33	8.93	-1.48	-1.08	C9	125.23	123.53	123.87	1.70	1.36
H16a	4.23	5.07	4.74	-0.84	-0.51	C10	125.23	123.53	123.87	1.70	1.36
H16b	4.02	3.45	4.40	0.57	-0.38	C11	126.12	125.09	124.58	1.03	1.54
H19	4.81	5.50	4.26	-0.69	0.55	C12	129.62	129.76	130.06	-0.13	-0.44
H23	1.70	1.48	2.14	0.22	-0.44	C13	121.35	119.42	115.22	1.93	6.13
H23'	1.44	1.71	1.53	-0.27	-0.09	C14	166.80	165.51	159.77	1.29	7.03
H25	5.85	7.13	9.02	-1.28	-3.17	C16	42.70	45.47	53.51	-2.77	-10.81
H26	5.61	4.74	4.60	0.87	1.01	C17	170.26	169.48	199.09	0.78	-28.83
H33	7.85	8.61	9.13	-0.76	-1.28	C19	72.60	72.62	82.42	-0.02	-9.82
H34	7.34	7.95	7.80	-0.61	-0.46	C20	170.90	172.50	168.95	-1.60	1.95
H35	7.45	7.76	7.79	-0.31	-0.34	C22	50.78	65.86	69.47	-15.08	-18.69
H36	7.95	8.11	8.15	-0.16	-0.19	C23	29.51	30.57	24.02	-1.06	5.49
H38	7.92	8.21	8.34	-0.29	-0.42	C23'	29.51	30.57	24.02	-1.06	5.49
H39	7.40	7.42	7.57	-0.02	-0.17	C25	66.02	70.96	165.18	-4.94	-99.16
H42	4.14	4.27	4.56	-0.13	-0.42	C26	55.45	64.94	64.10	-9.49	-8.65
H43	1.27	1.61	1.66	-0.34	-0.39	C27	170.58	170.08	170.18	0.50	0.40
						C30	166.54	164.95	169.66	1.59	-3.12
						C31	121.35	119.42	115.22	1.93	6.13
						C32	129.62	129.76	130.06	-0.13	-0.44
						C33	126.12	125.09	124.58	1.03	1.54
						C34	125.23	123.53	123.87	1.70	1.36
						C35	125.23	123.53	123.87	1.70	1.36
						C36	126.12	125.09	124.58	1.03	1.54
						C37	129.62	129.76	130.06	-0.13	-0.44
						C38	130.26	130.87	132.96	-0.62	-2.70
						C39	115.31	115.43	110.71	-0.13	4.59
						C40	152.52	154.74	154.32	-2.22	-1.80
						C42	64.85	71.69	67.25	-6.84	-2.40
						C43	14.84	18.22	17.85	-3.38	-3.01

[†] $\Delta\delta^{\text{TDI}} = \delta_{\text{cal}}^{\text{TDI}} - \delta_{\text{exp}}$ and $\Delta\delta^{\text{TDI-1}} = \delta_{\text{cal}}^{\text{TDI-1}} - \delta_{\text{exp}}$