Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Structure determination of the bioactive depsipeptide xenobactin from *Xenorhabdus sp.* PB30.3

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Table S1. ROE list with upper and lower distance restraint limits (90%, 110%) including pseudoatom correction from experimentally determined distance for three dimensional modeling of **1**. Average distance, average violation and root mean square violation of single distance restraints over ten conformations from the final MD trajectory (after energy minimization) are shown.

ATOM1	ATOM2	LOW	UPP	AV_DIST	AV_VIOL	RMS_VIOL
4-VAL NH	3-THR γ (pseudo)	3.4	5.2	4.44	0	0
4-VAL NH	4-VAL β	2.9	3.6	3.74	0.14	0.14
4-VAL α	4-VAL NH	2.8	3.4	2.86	0	0
3-THR α	4-VAL NH	2.1	2.5	2.36	0	0
4-VAL NH	5-ILE NH	2.5	3.1	2.73	0	0
3-THR NH	4-VAL NH	3.2	4	4.3	0.29	0.29
3-THR β	4-VAL NH	2.6	4.4	4.61	0.2	0.2
3-THR α	2-TRP NH	3.6	4.5	4.84	0.34	0.34
1-THR α	2-TRP NH	2	2.5	3.1	0.6	0.6
2-TRP α	2-TRP NH	2.6	3.2	3	0	0
1-THR β	2-TRP NH	2.9	3.6	2.35	0.55	0.55
2-TRP NH	6-LEU NH	3.9	4.7	3.47	0.43	0.43
3-THR NH	2-TRP NH	2.4	3	2.35	0.05	0.05
1-THR NH	1-THR γ (pseudo)	2.8	4.4	3.16	0	0
1-THR NH	Acetic Acid α (pseudo)	2.5	4	2.85	0	0
1-THR α	1-THR NH	2.8	3.4	3	0	0
1-THR β	1-THR NH	2.9	3.6	3.72	0.12	0.12
3-THR NH	3-THR γ (pseudo)	3.4	5.2	4.46	0	0
3-THR NH	3-THR β	2.5	3	2.62	0	0
3-THR NH	2-TRP α	2.6	3.2	3.68	0.48	0.48
3-THR NH	5-ILE NH	4.1	5	4.7	0	0
3-THR β	6-LEU NH	4.7	5.7	4.27	0.43	0.43
4-VAL NH	6-LEU NH	3.4	4.1	4.26	0.16	0.16
1-THR α	6-LEU NH	4.8	5.9	5.61	0	0
2-TRP α	6-LEU NH	4.7	5.7	4.45	0.25	0.25
1-THR β	6-LEU NH	4.4	5.4	4.41	0	0
5-ILE NH	6-LEU NH	2.4	2.9	2.62	0	0
4-VAL β	5-ILE NH	3.6	4.4	4.03	0	0
4-VAL α	5-ILE NH	3	3.7	3.63	0	0
3-THR α	5-ILE NH	3.4	4.1	4.36	0.26	0.26
2-TRP α	5-ILE NH	4.2	5.2	3.93	0.27	0.27
1-THR α	1-THR β	2.2	2.7	2.36	0	0
3-THR α	2-TRP α	3.7	4.53	4.68	0.15	0.15
3-THR α	3-THR β	2.6	3.2	3.06	0	0
4-VAL α	4-VAL β	2.7	3.3	2.42	0.28	0.28
1-THR β	1-THR γ (pseudo)	2.4	4	2.5	0	0
1-THR α	1-THR γ (pseudo)	3.3	5.1	3.11	0.19	0.19
3-THR α	3-THR γ (pseudo)	2.9	3.5	3.01	0	0
4-VAL α	3-THR γ (pseudo)	3.8	4.7	4.51	0	0
3-THR β	3-THR γ (pseudo)	2.6	3.2	2.53	0.07	0.07
5-ILE NH	6-ILE β	3.1	3.8	3.74	0	0
4-VAL α	6-Leu γ	4.1	5.1	3.7	0.4	0.4
2-TRP α	6-ILE δ (0.73, pseudo)	3.2	5	3.19	0.01	0.01
3-THR α	6-ILE δ (0.73, pseudo)	4.6	6.7	6.32	0	0
6-LEU NH	6-ILE δ (0.73, pseudo)	3.5	5.2	3.23	0.27	0.27
5-ILE NH	6-ILE δ (0.73, pseudo)	2.6	4.2	3.08	0	0

6-LEU NH	6-Leu γ	2.8	3.4	2.91	0	0
6-ILE β	6-LEU NH	3.6	4.5	4.09	0	0
3-THR NH	6-LEU β 1.79	4.3	5.3	4.15	0.15	0.15
6-LEU NH	6-LEU β 1.79	2.6	3.1	2.64	0	0
2-TRP NH	6-LEU β 1.79	3.5	4.3	4.42	0.12	0.12
6-LEU NH	6-LEU β 1.45	3.5	4.3	3.81	0	0
2-TRP NH	6-LEU β 1.45	4.2	5.2	5.51	0.31	0.31
6-LEU NH	6-LEU δ 0.81	3.9	5.8	4.77	0	0
6-LEU NH	6-LEU δ 0.87	4	6	4.84	0	0
4-VAL α	4-VAL γ (0.88, pseudo)	2.8	4.4	3.17	0	0
4-VAL α	4-VAL γ (0.93, pseudo)	2.9	4.5	3.11	0	0
4-VAL β	4-VAL γ (0.88, pseudo)	2.9	4.5	2.51	0.4	0.4
4-VAL β	4-VAL γ (0.93, pseudo)	2.8	4.4	2.5	0.3	0.3
5-ILE NH	4-VAL γ (0.88, pseudo)	3.4	5.2	3.1	0.3	0.3
4-VAL NH	4-VAL γ (0.88, pseudo)	3.1	4.9	3.88	0	0
4-VAL NH	4-VAL γ (0.93, pseudo)	2.8	4.5	3.03	0	0
5-ILE NH	4-VAL γ (0.93, pseudo)	3.8	5.7	4.95	0	0
1-THR β	3-THR NH	3.9	4.75	4.28	0	0
Average Restraint Violation: 0.118						
Average RMS Restraint Violation: 0.118						

Table S2. Energy [kcal/mol] shown from all conformations obtained by the final MD trajectory after energy minimization. The ten conformations with the lowest energy, which were chosen for the alignment, are colored. The main conformation present in solution is depicted in green the other in orange.

Conformation	Energy	Conformation	Energy
100,000	32.804	1,100,000	25.824
200,000	26.060	1,200,000	28.089
300,000	27.598	1,300,000	27.287
400,000	26.356	1,400,000	27.888
500,000	28.367	1,500,000	28.298
600,000	27.023	1,600,000	28.074
700,000	27.031	1,700,000	27.347
800,000	28.136	1,800,000	31.478
900,000	27.510	1,900,000	26.819
1,000,000	27.807	2,000,000	26.719

Table S3. Chemical shifts [ppm] of all amide protons of **1** are shown at the corresponding temperature.

	300	305	310	315	320	325	330 [K]
1-Thr	8.0597	8.0239	7.9878	7.9510	7.9140	7.8766	7.8389
2-Trp	8.2560	8.2356	8.2149	8.1940	8.1730	8.1516	8.1302
3-Thr	7.7760	7.7516	7.7277	7.7040	7.6808	7.6581	7.6356
4-Val	8.4964	8.4720	8.4476	8.4229	8.3980	8.3731	8.3480
5-Ile	6.7806	6.7823	6.7836	6.7845	6.7852	6.7855	6.7855
6-Leu	7.4283	7.4158	7.4032	7.3908	7.3782	7.3658	7.3535
Indole 1	10.8067	10.7889	10.7708	10.7526	10.7340	10.7167	10.6977

Table S4. Change in the chemical shift [Δδ] between the different temperatures of all amide protons of **1**.

	300	305	310	315	320	325	330 [K]
1-Thr	0	-0.0358	-0.0719	-0.1087	-0.1457	-0.1831	-0.2208
2-Trp	0	-0.0204	-0.0411	-0.0620	-0.0830	-0.1044	-0.1258
3-Thr	0	-0.0244	-0.0483	-0.0720	-0.0952	-0.1179	-0.1404
4-Val	0	-0.0244	-0.0488	-0.0735	-0.0984	-0.1233	-0.1484
5-Ile	0	0.0017	0.0030	0.0039	0.0046	0.0049	0.0049
6-Leu	0	-0.0125	-0.0251	-0.0375	-0.0501	-0.0625	-0.0748
Indole 1	0	-0.0178	-0.0359	-0.0541	-0.0727	-0.0900	-0.1090

Table S5. Temperature coefficients of all amide protons of **1.**

	Δδ/ΔT [ppb/K]
1-Thr	-7.4
2-Trp	-4.2
3-Thr	-4.7
4-Val	-4.9
5-Ile	0.2
6-Leu	-2.5
Indole 1	-3.6

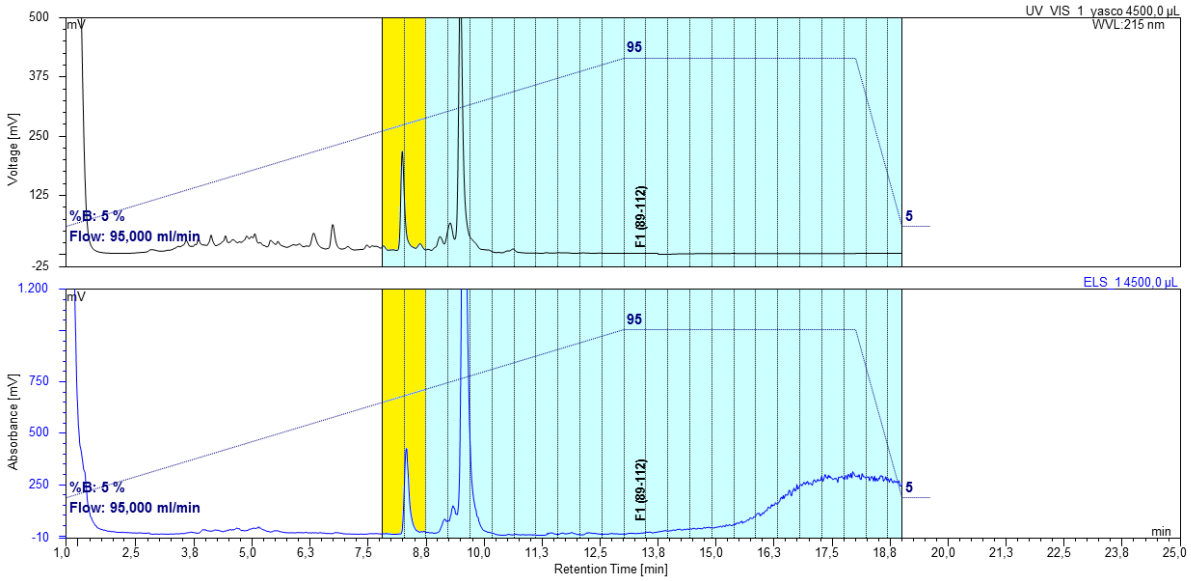


Figure S1. Fractionation of the crude extract of PB30.3; Fraction containing **1** is marked in yellow.

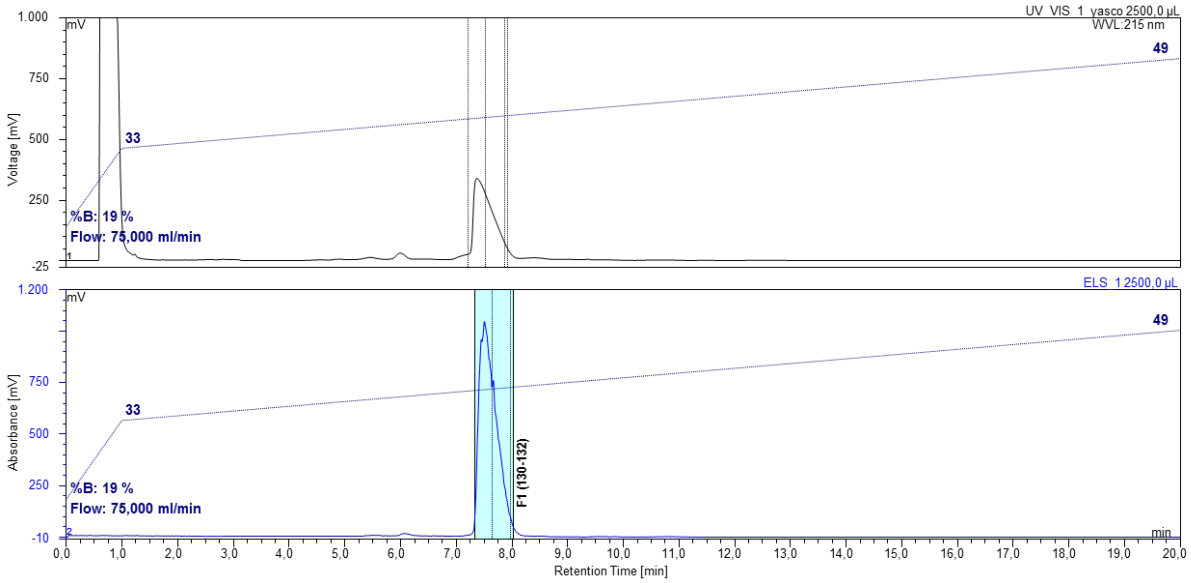


Figure S2. Isolation of **1** from the fraction obtained from the crude exract.

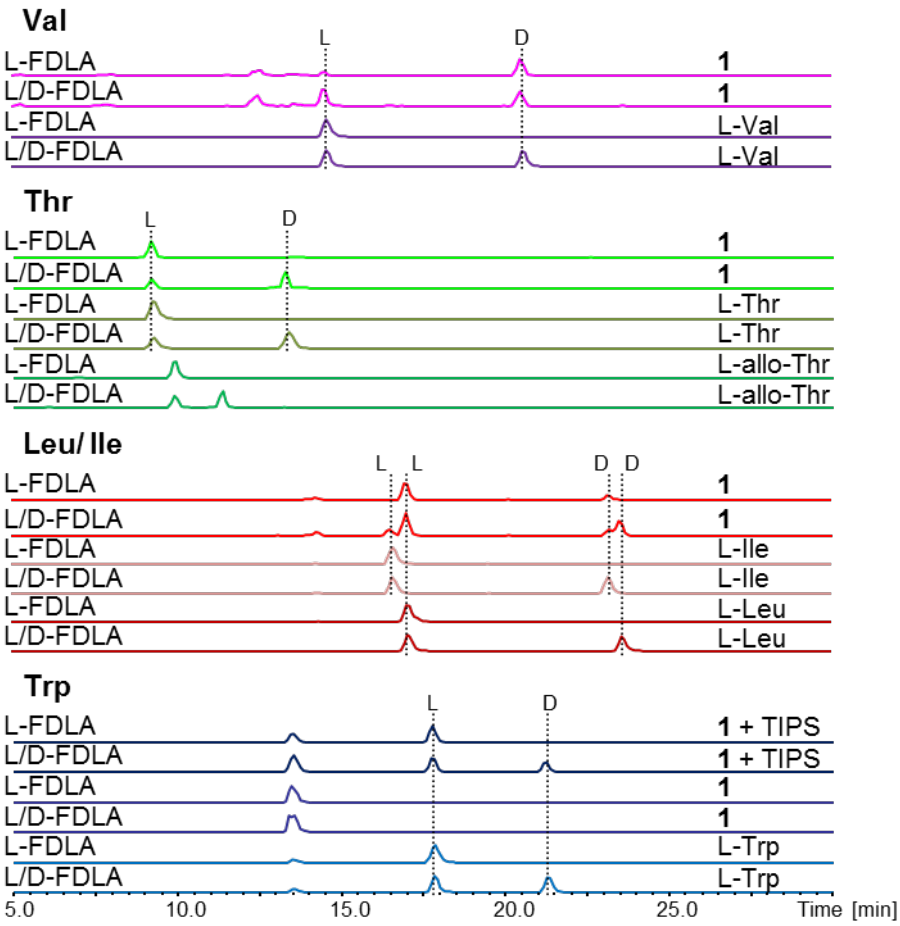


Figure S3. Extracted ion chromatograms for the analysis of the absolute stereochemistry by the modified advanced Marfey´s method. The following m/z ratios were used for single amino acids in the positive ion modus: valine, 412; threonine, 414; leucine/isoleucine, 426; tryptophan, 499. For every amino acid the references are also shown. The analysis of tryptophan was only possible with the addition of TIPS during hydrolysis of the peptide.

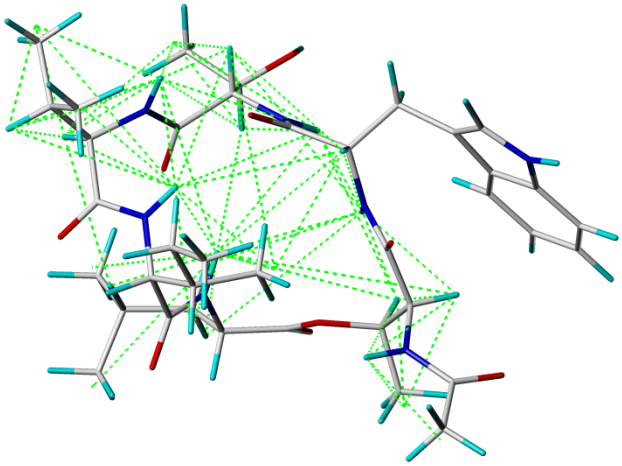


Figure S4. Conformation A of **1** with the lowest energy of all conformations from the final MD run after energy minimization with all protons and used distance restraints shown.

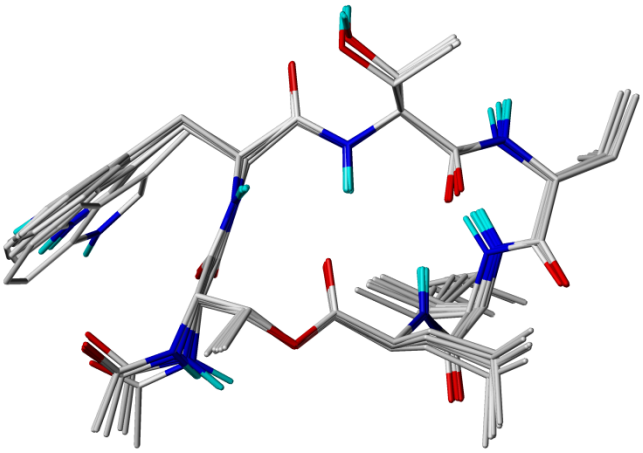


Figure S5. Alignment (six conformations) of conformation A of **1** extracted from the alignment of the final MD run after energy minimization.

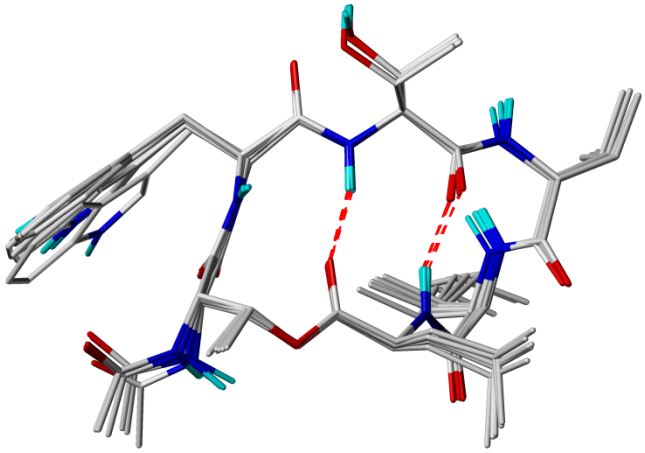


Figure S6. Alignment (six conformations) of conformation A of **1** extracted from the alignment of the final MD run with calculated hydrogen bonds.

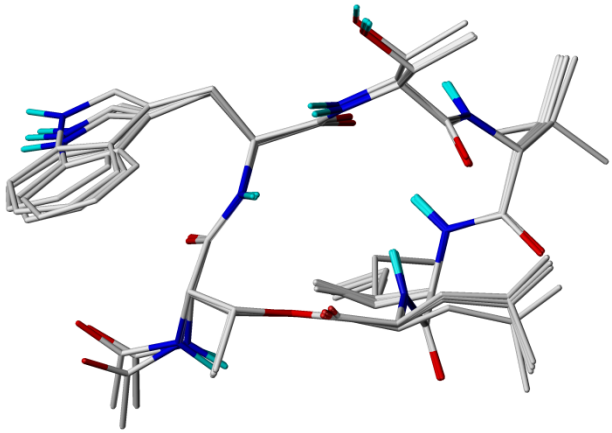


Figure S7. Alignment (four conformations) of conformation B of **1** extracted from the alignment of the final MD run after energy minimization.

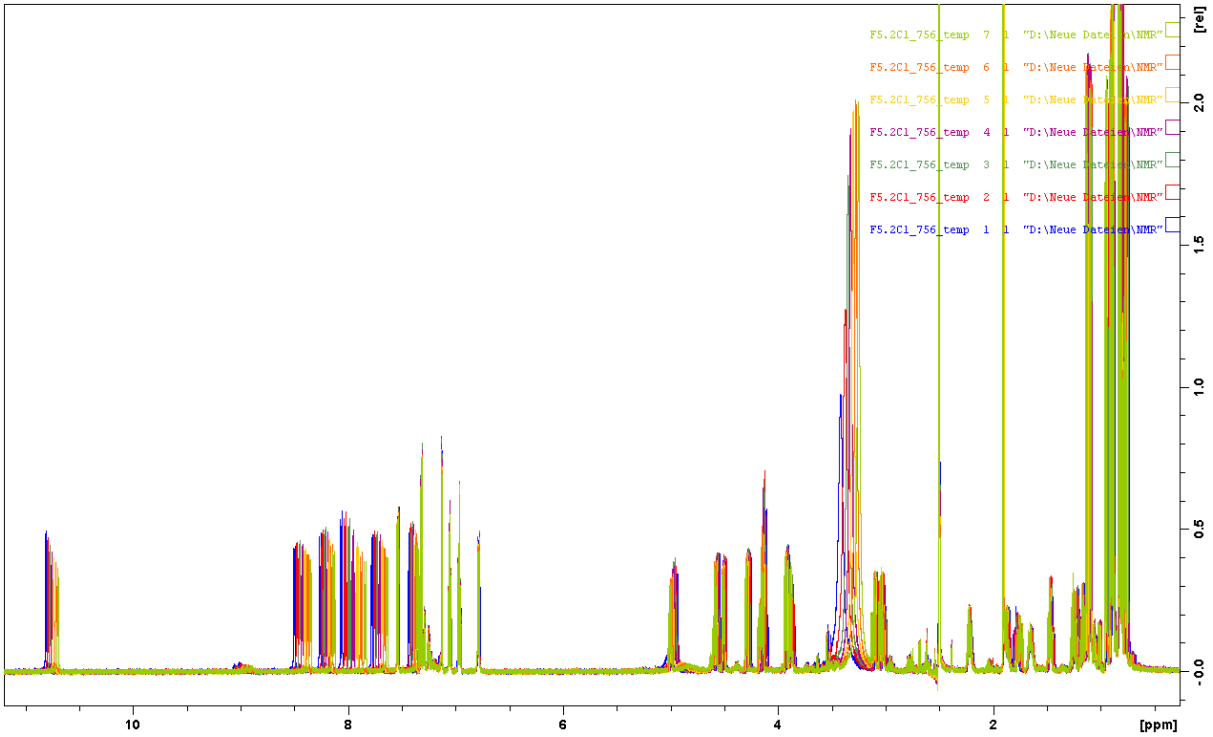


Figure S8. Stacked ^{1}H NMR spectra of **1** at 300 – 330 K.

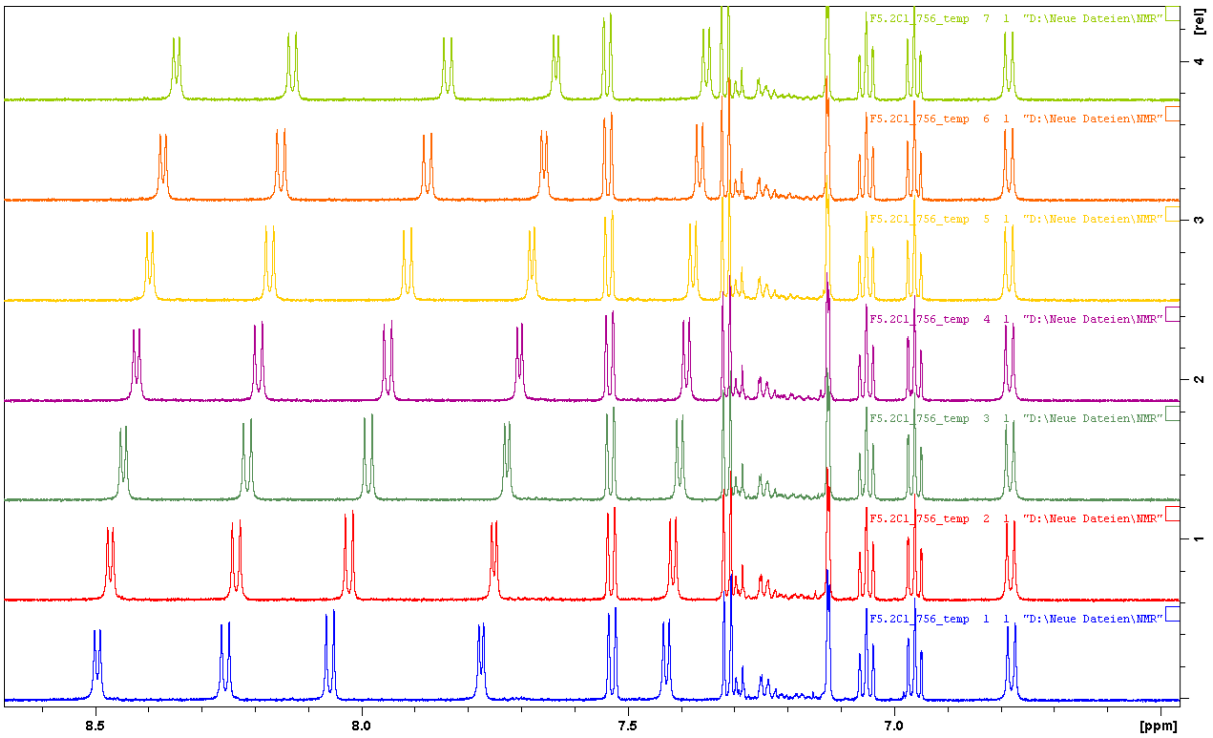


Figure S9. Region of amide protons in ^{1}H NMR spectra of **1** at 300 – 330 K.

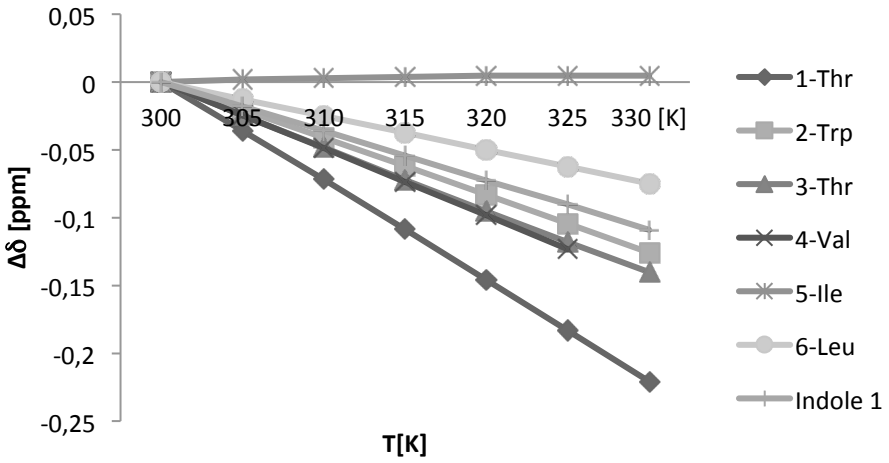
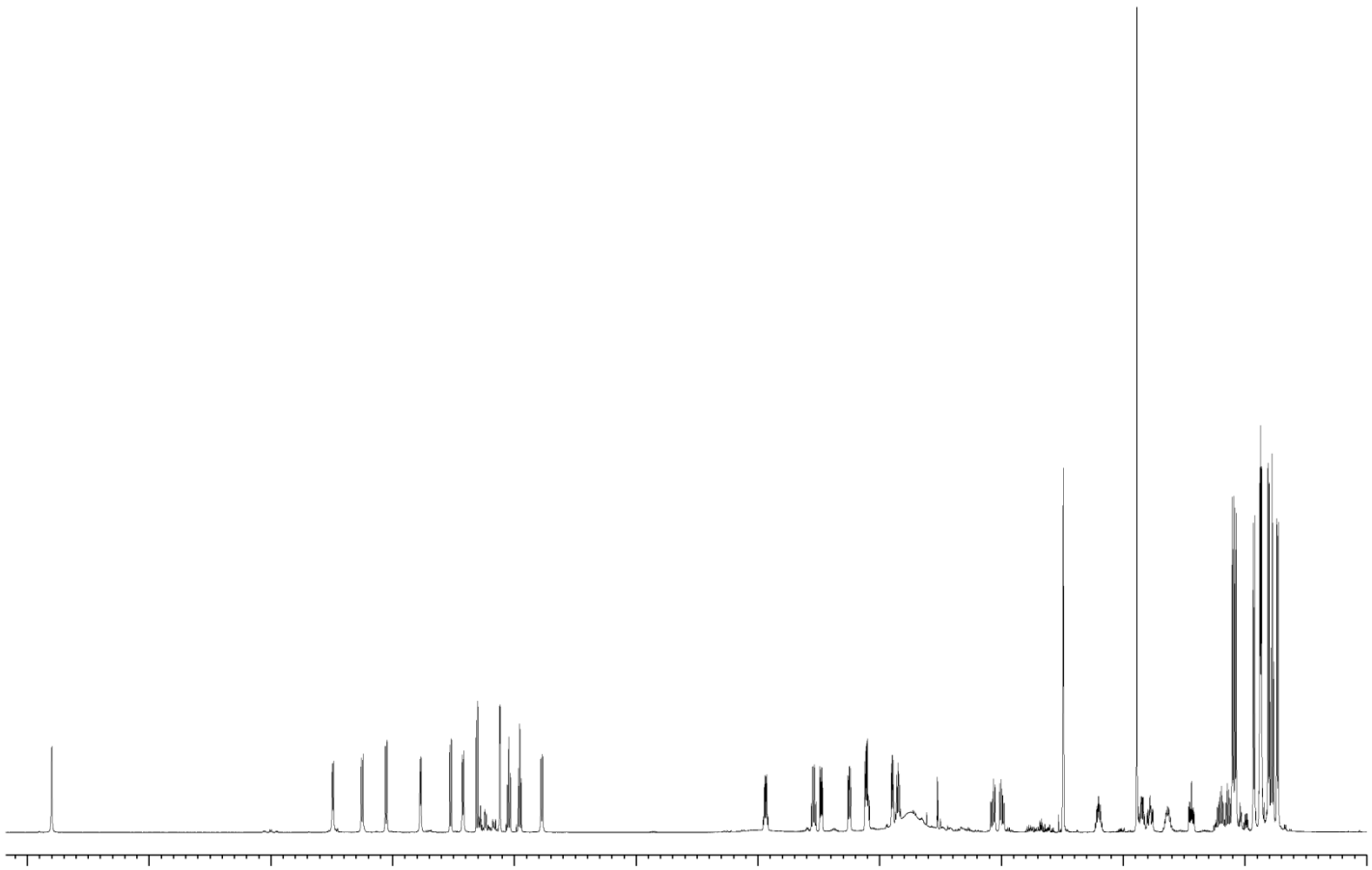
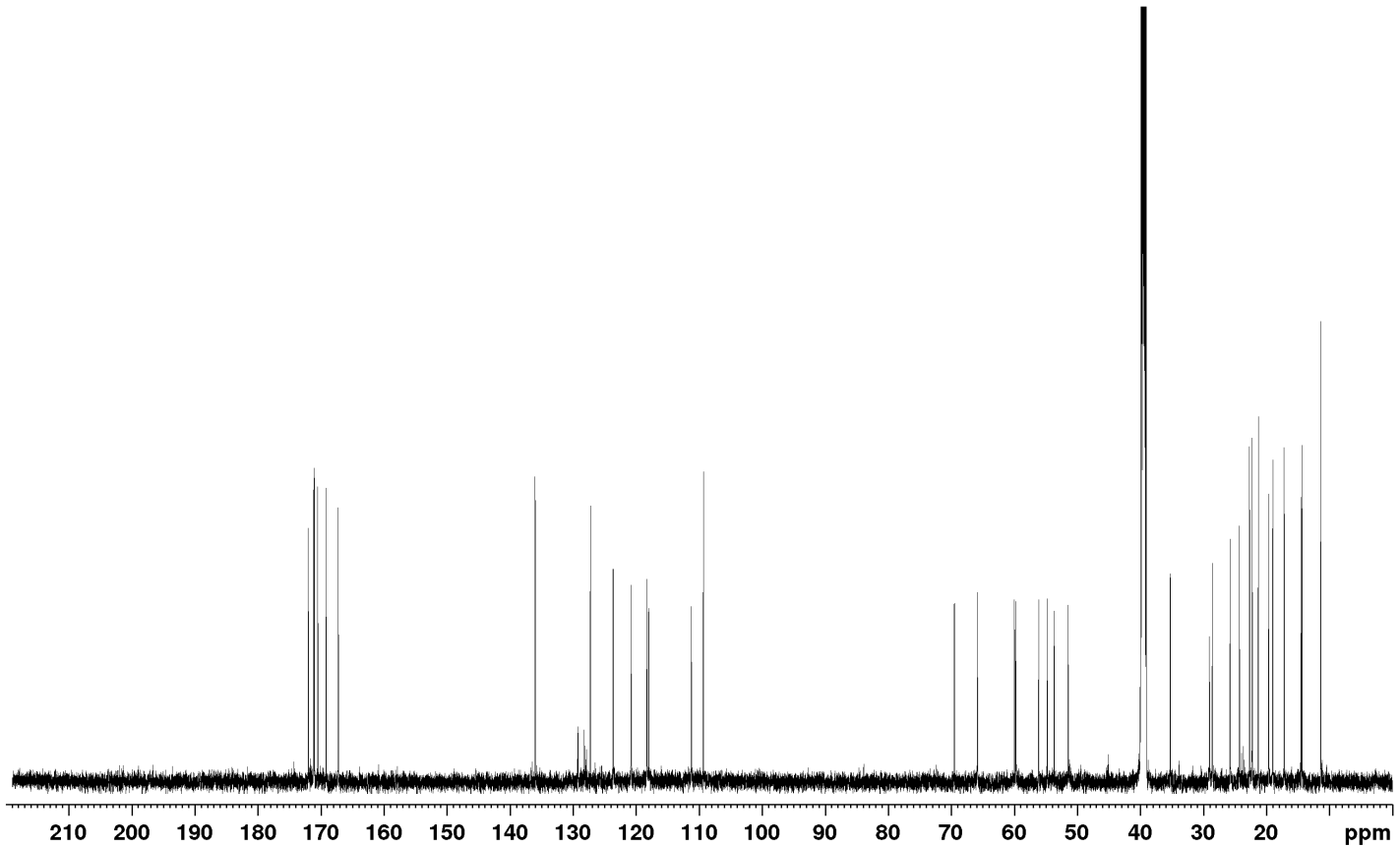
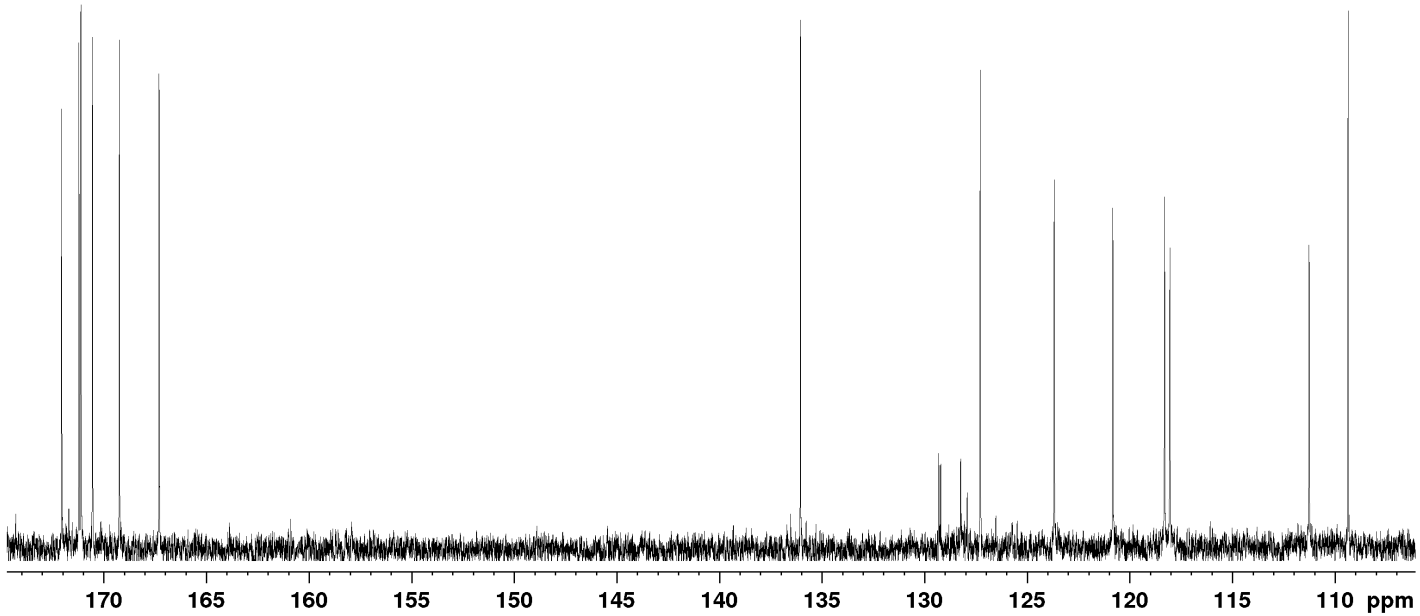


Figure S10. Display of the temperature coefficients of the amide protons of **1** derived from data shown in Table S2 and S3.

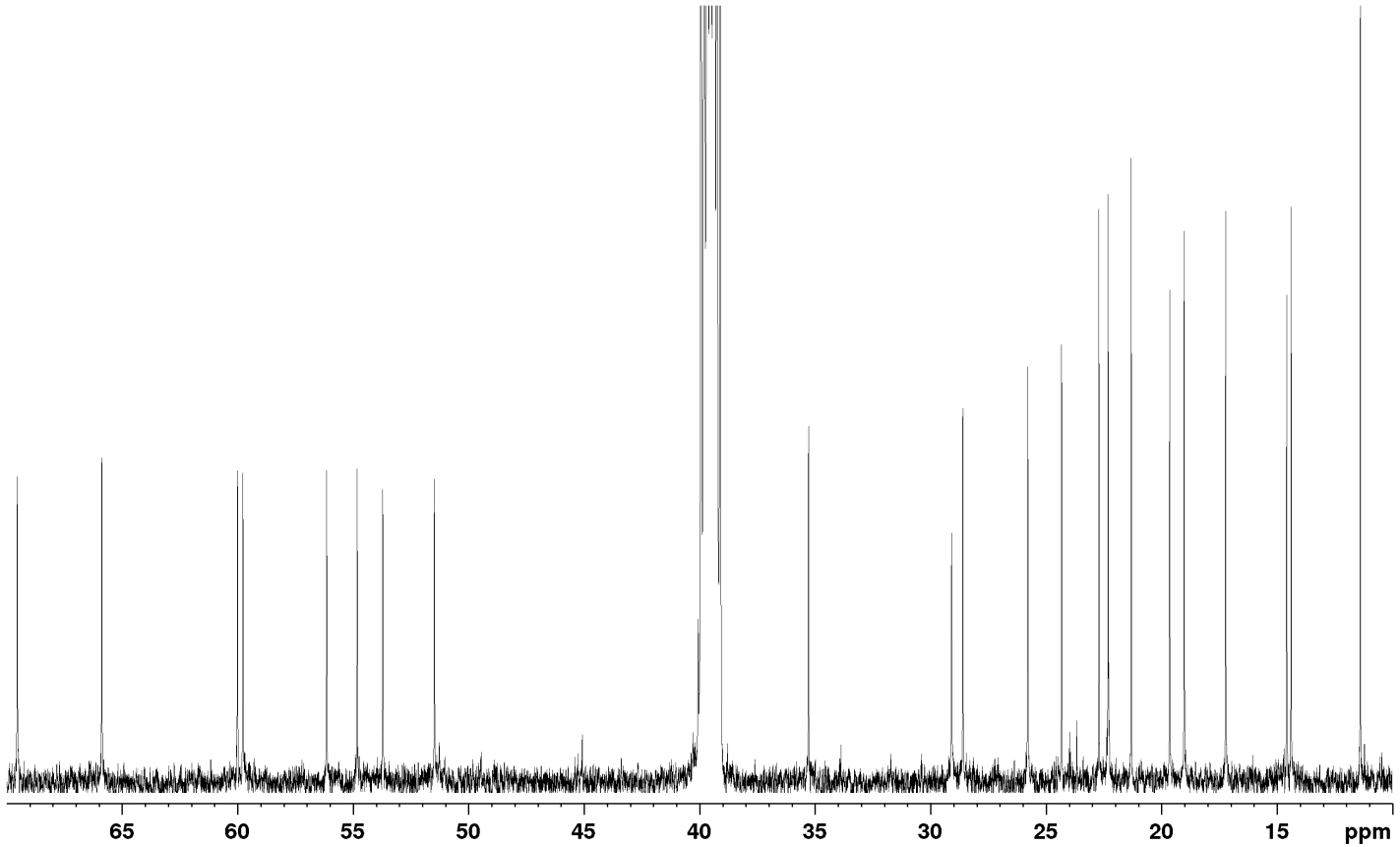




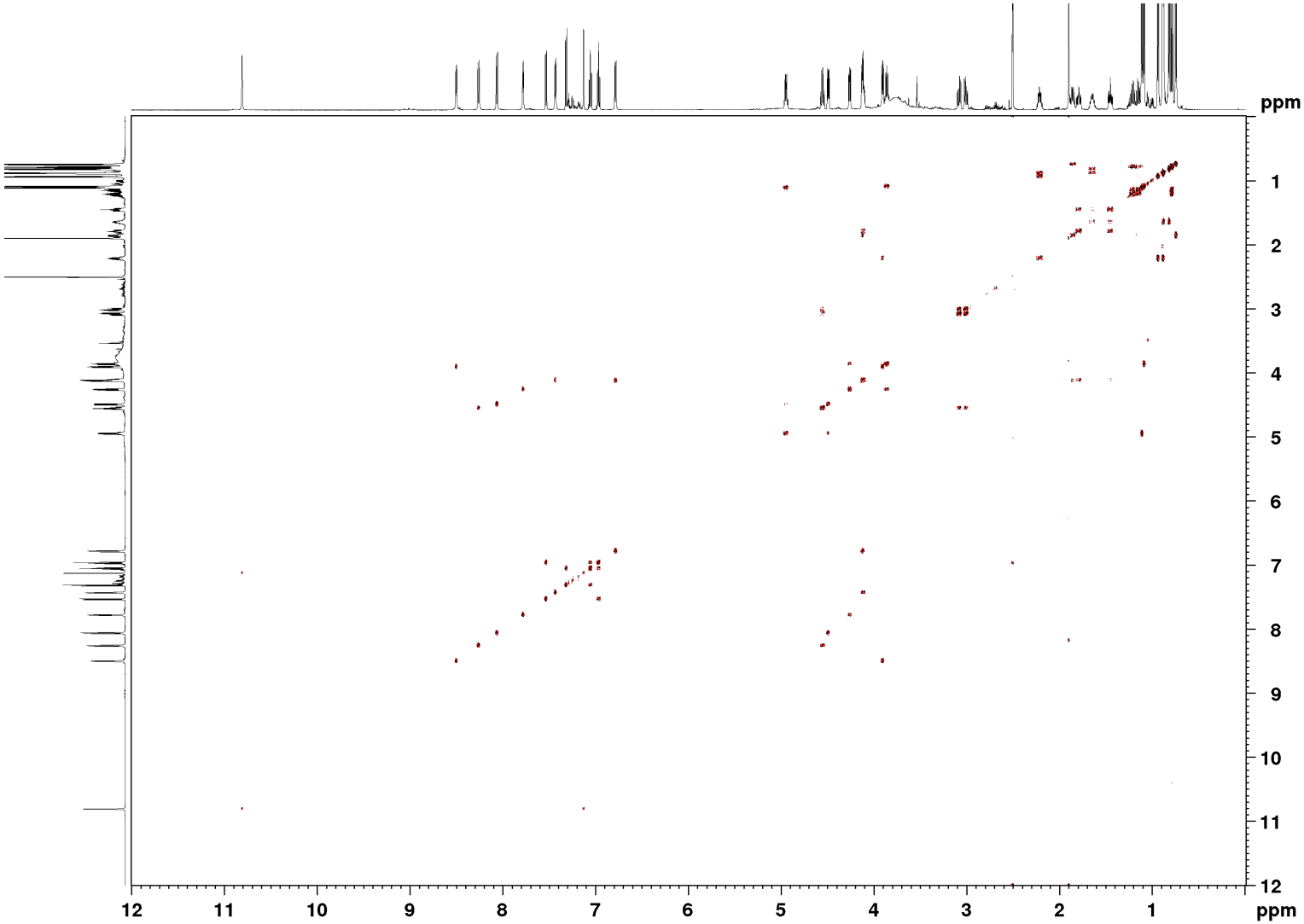
Annex 2. ^{13}C NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz.



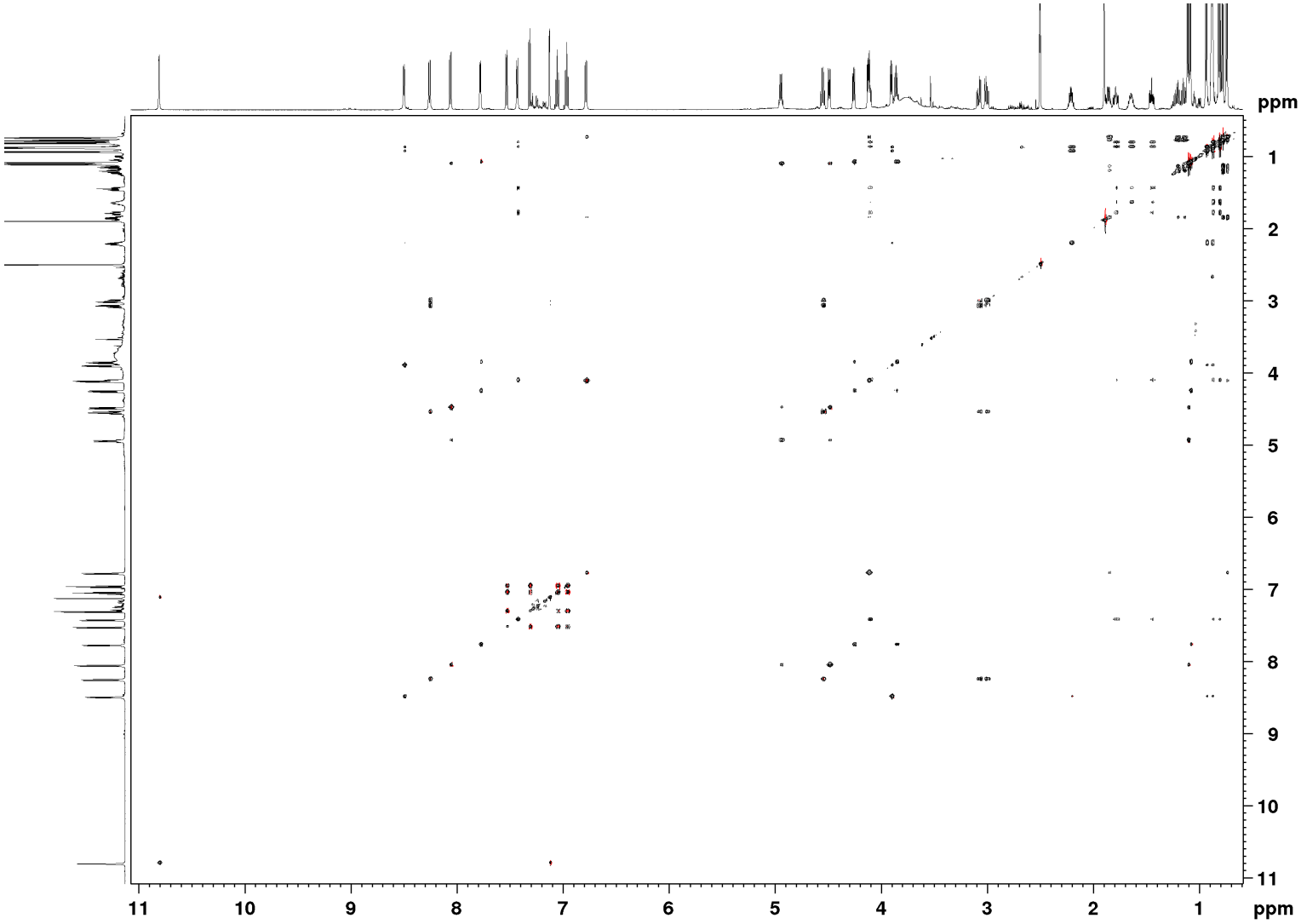
Annex 3. ^{13}C NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aromatic and carbonyl region.



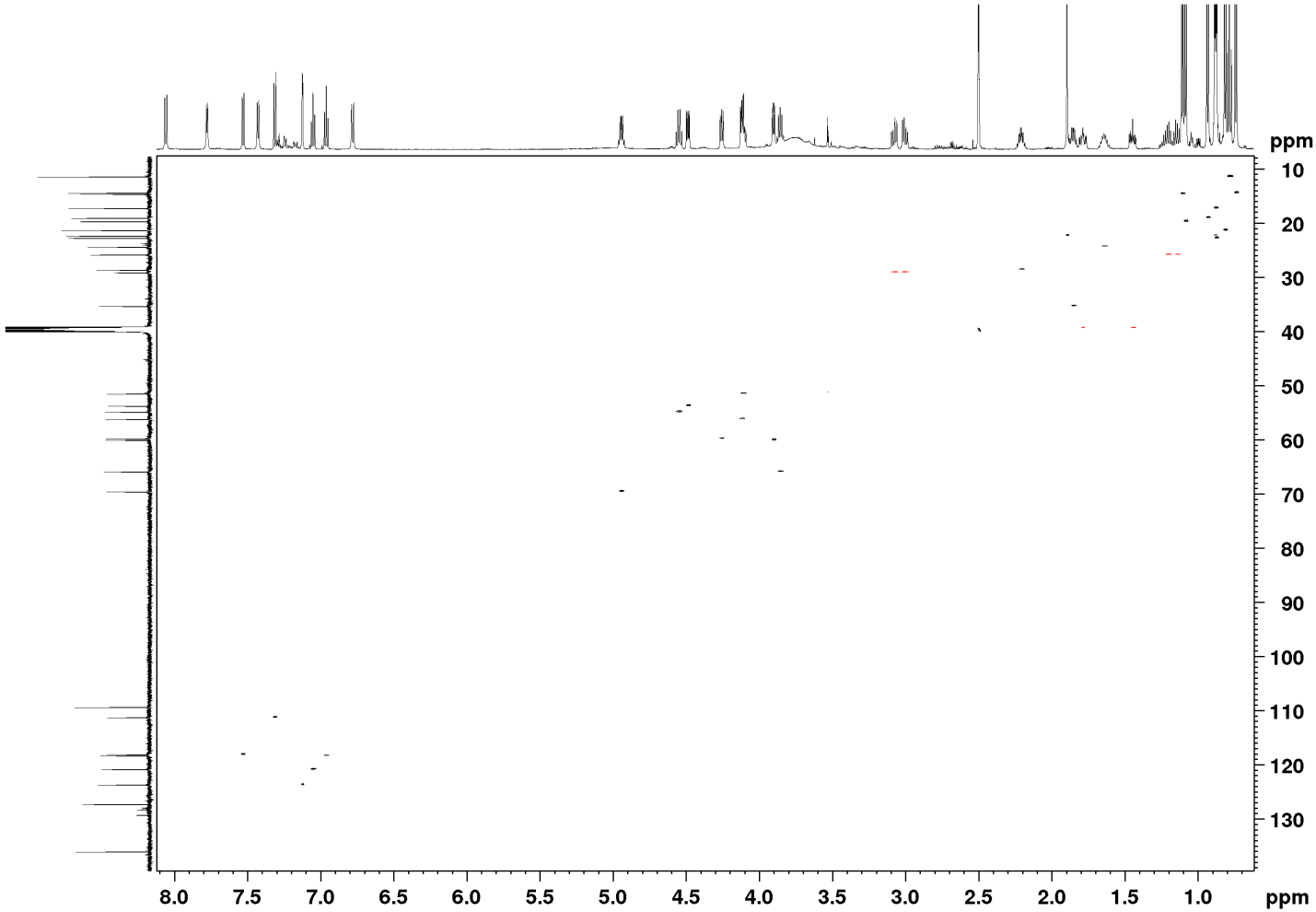
Annex 4. ^{13}C NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aliphatic region.



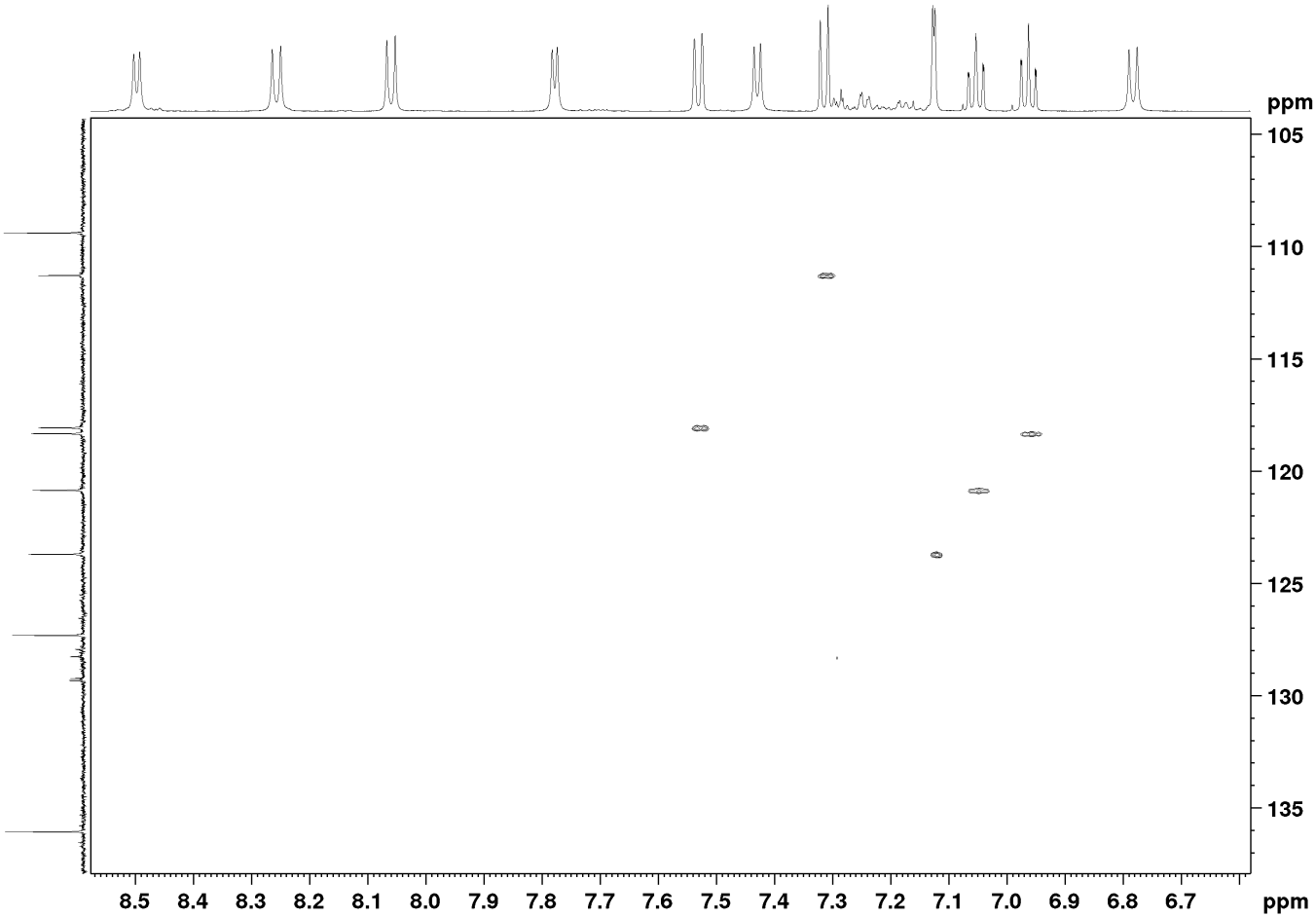
Annex 5. ^{1}H-^{1}H COSY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aliphatic region.



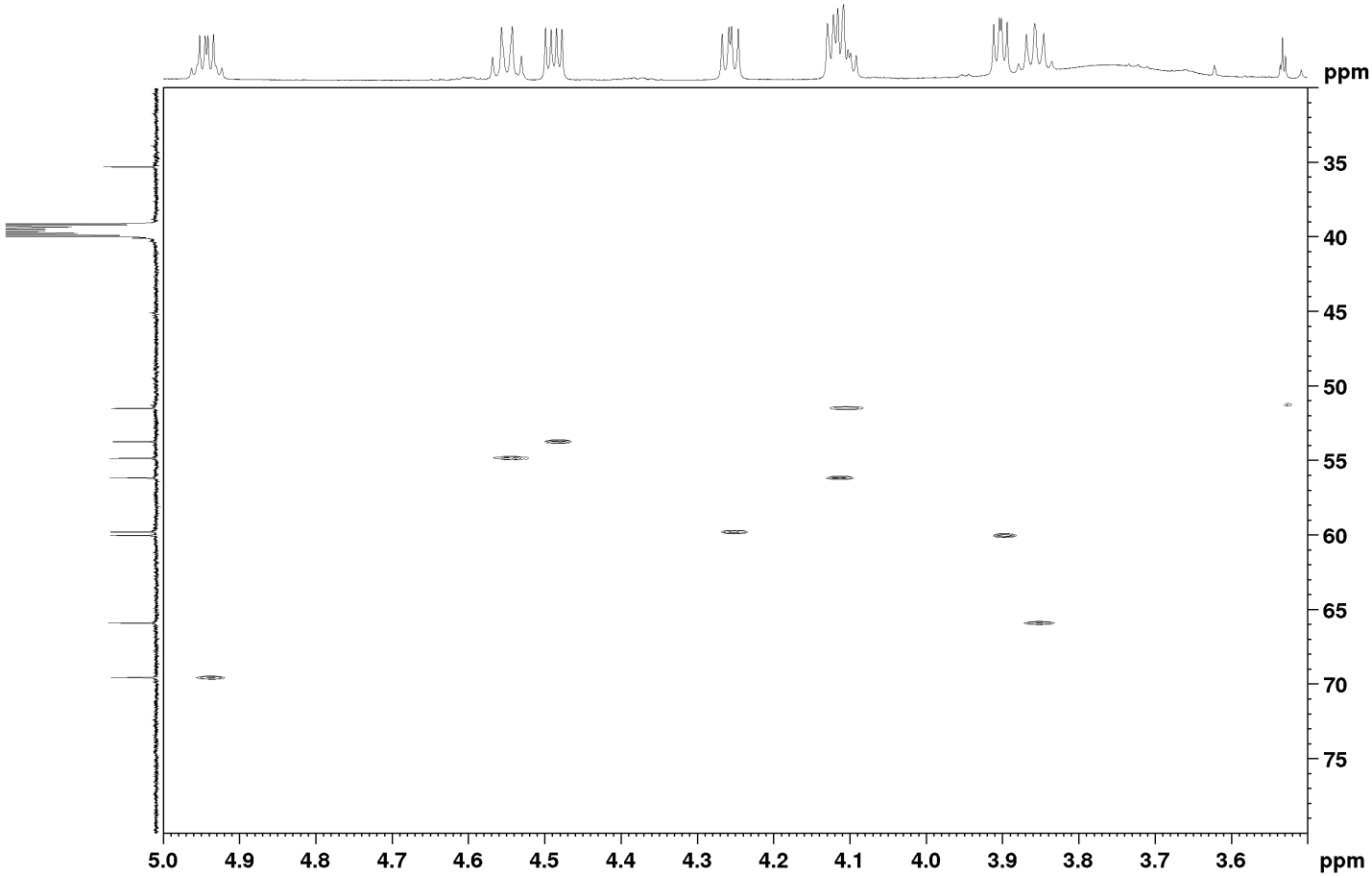
Annex 6. ^{1}H-^{1}H TOCSY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aliphatic region.



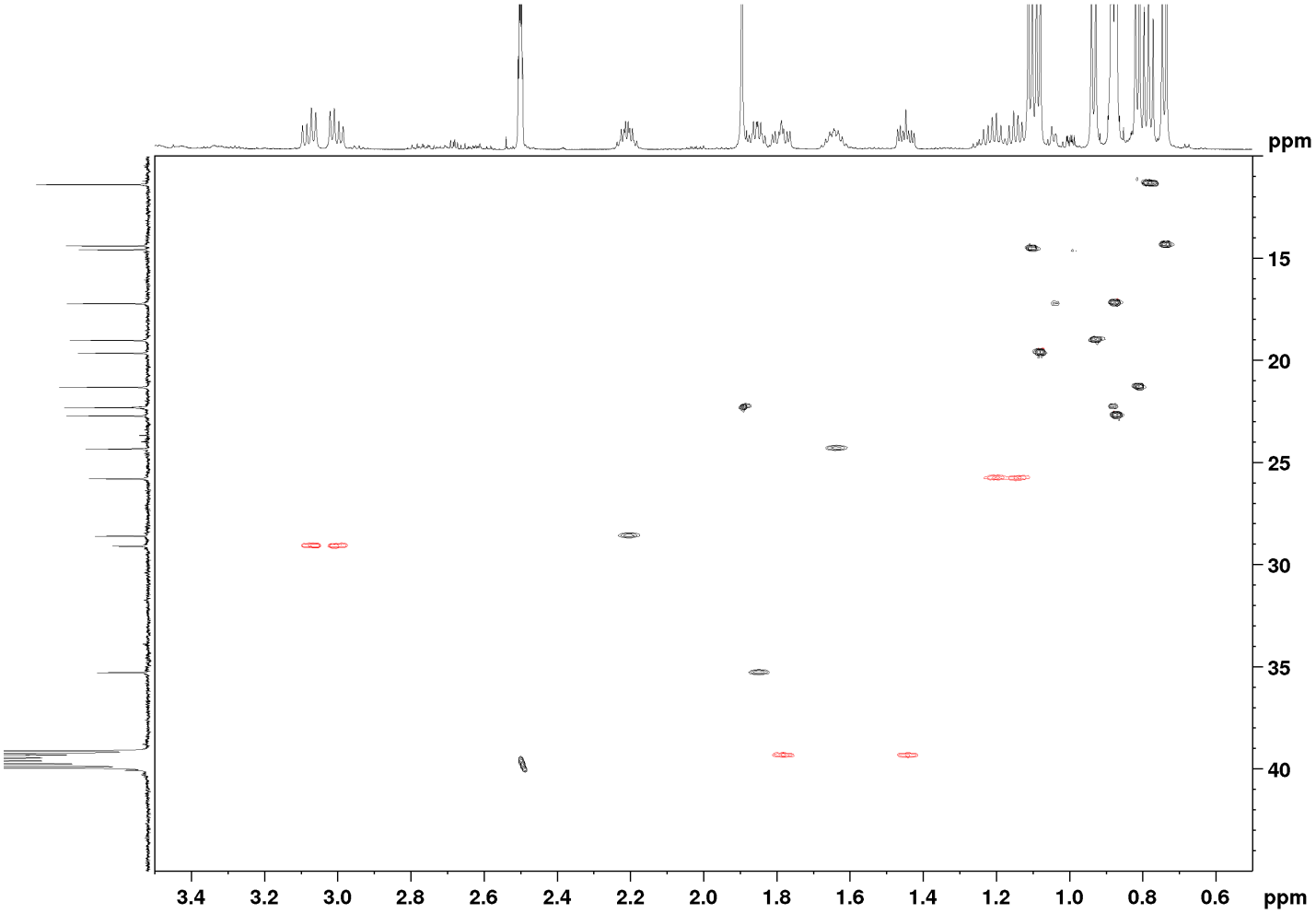
Annex 7. ^{1}H-^{13}C HSQC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz.



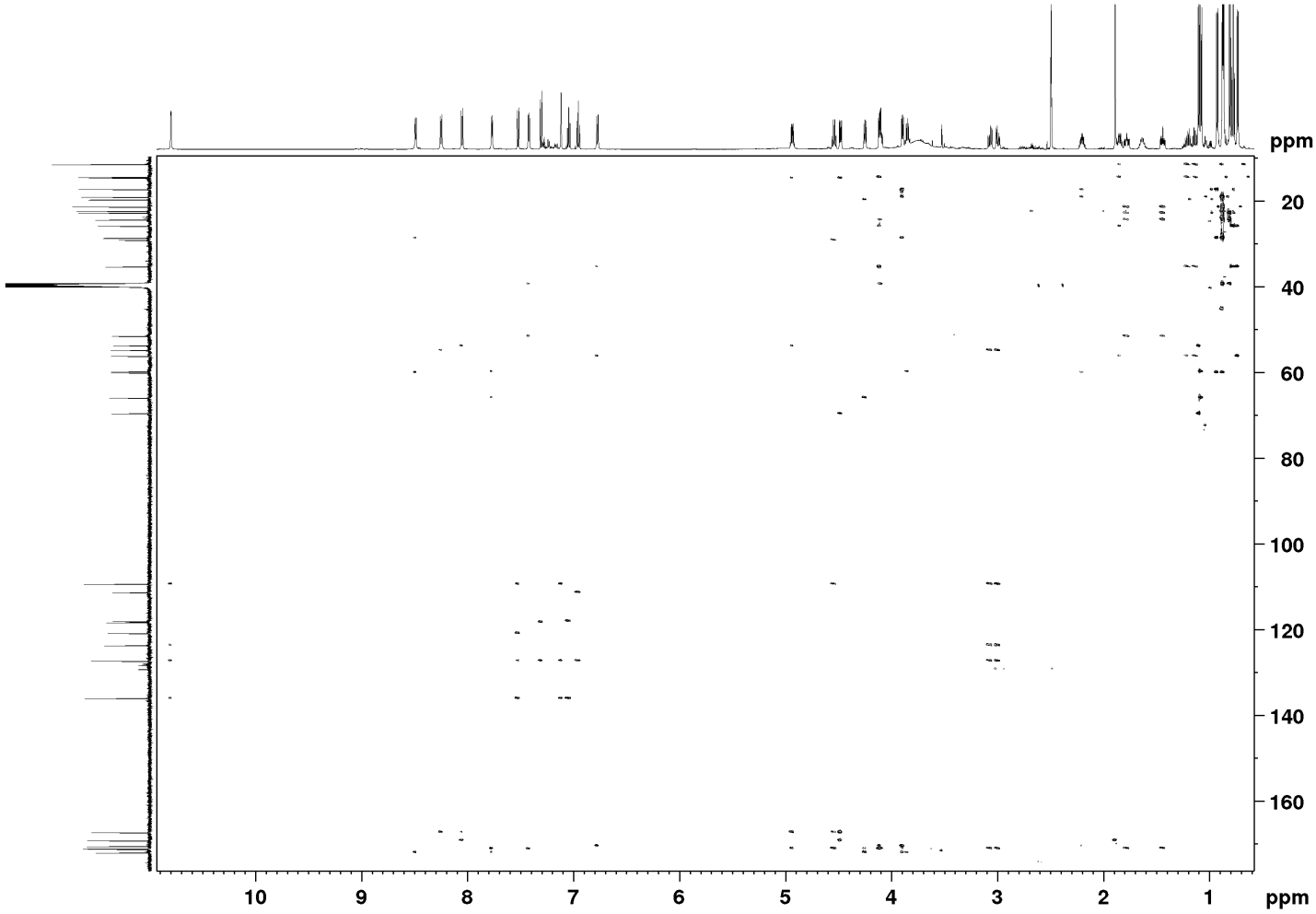
Annex 8. ^{1}H-^{13}C HSQC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aromatic region.



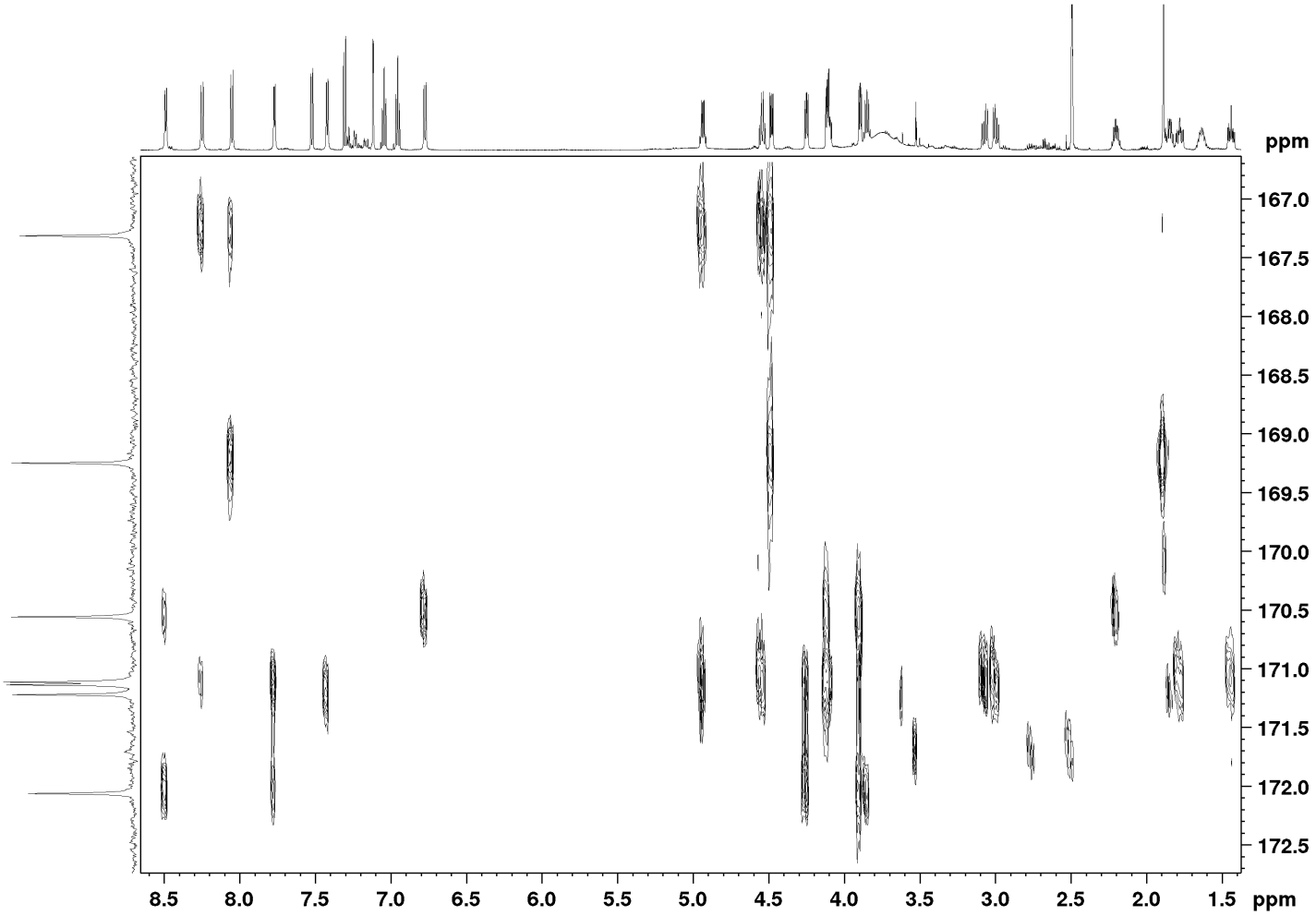
Annex 9. ^{1}H-^{13}C HSQC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the Hα region.



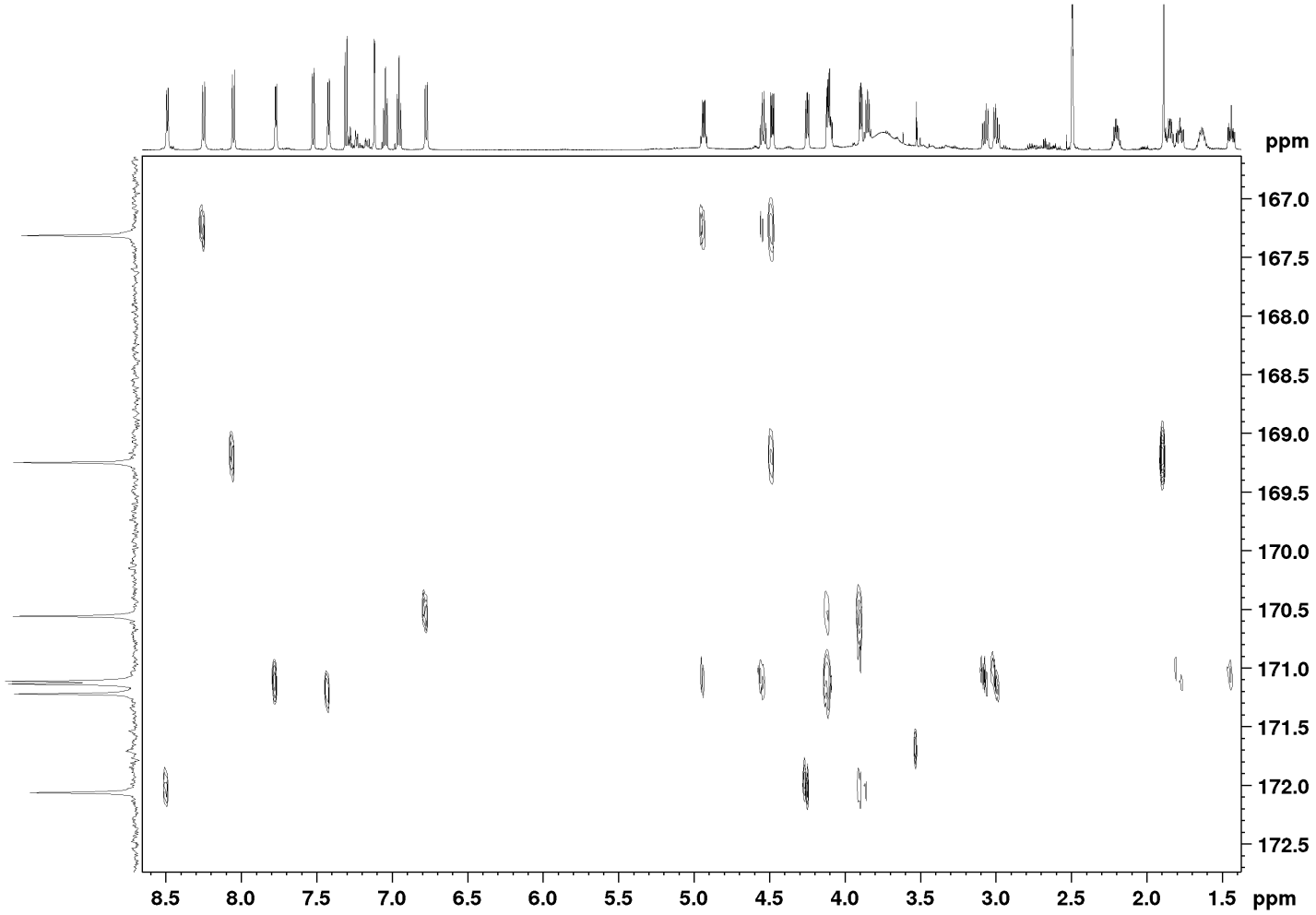
Annex 10. ^{1}H-^{13}C HSQC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the aliphatic region.



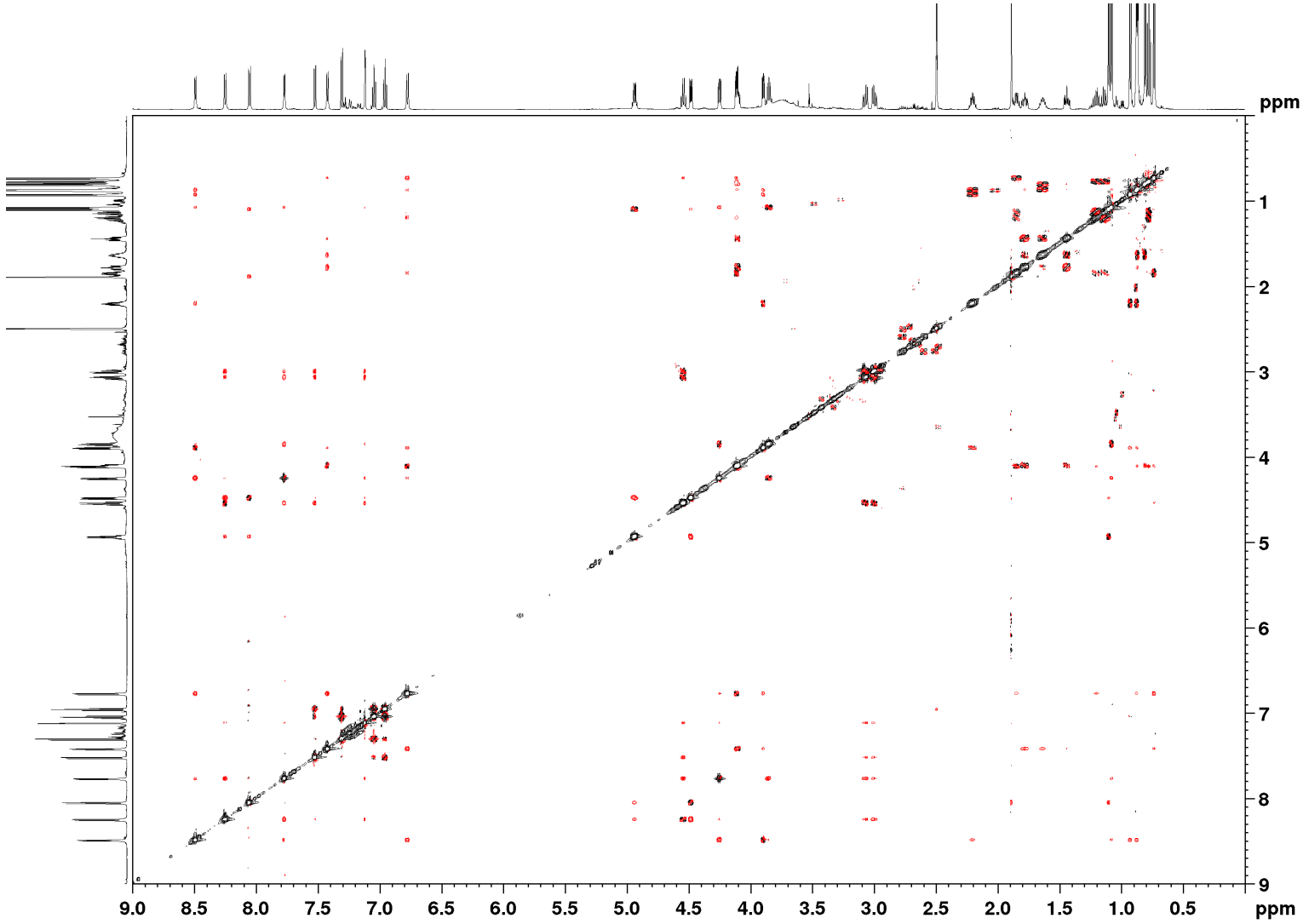
Annex 11. ^{1}H-^{13}C HMBC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz.



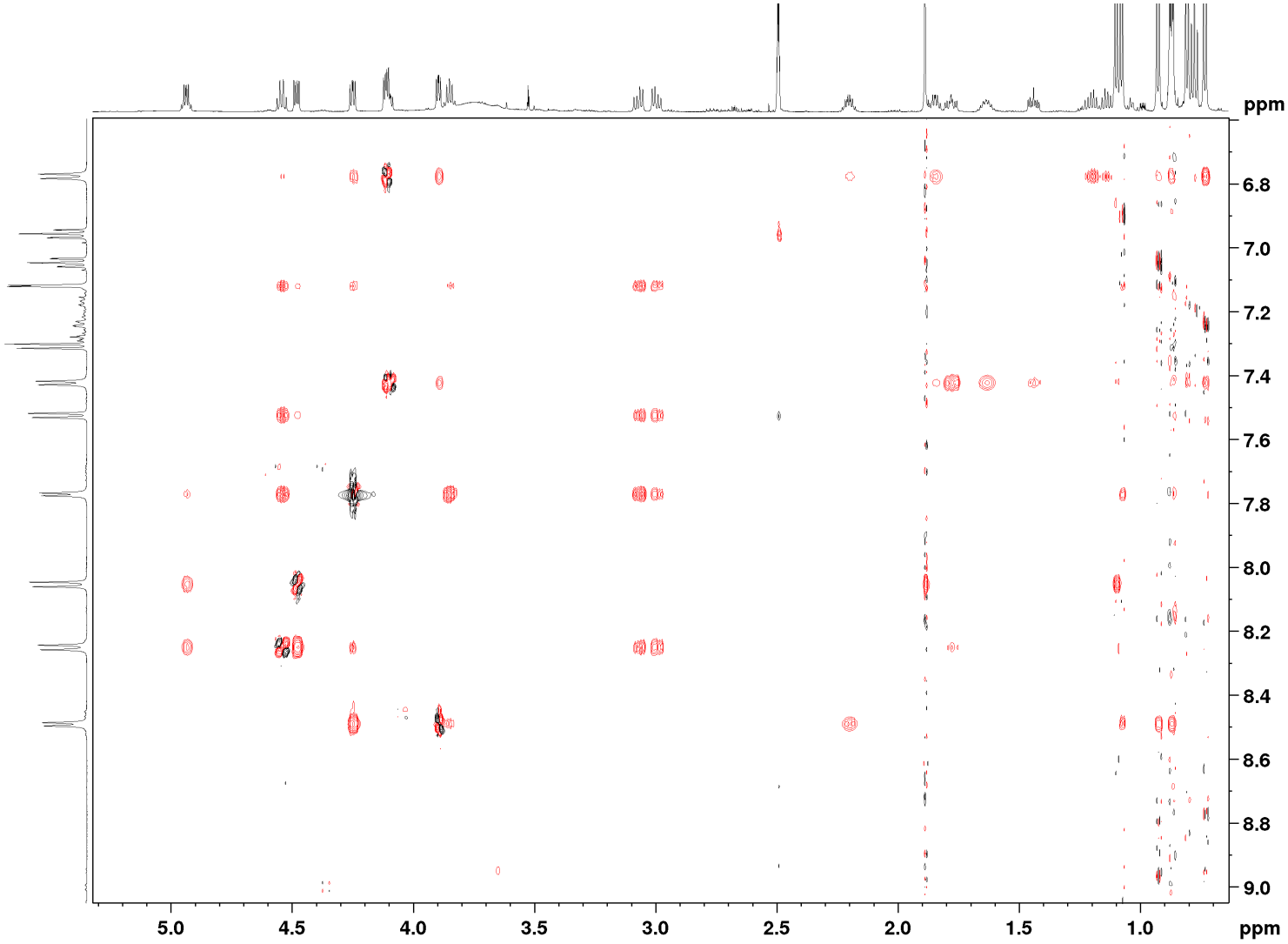
Annex 12. ^{1}H-^{13}C HMBC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the carbonyl region.



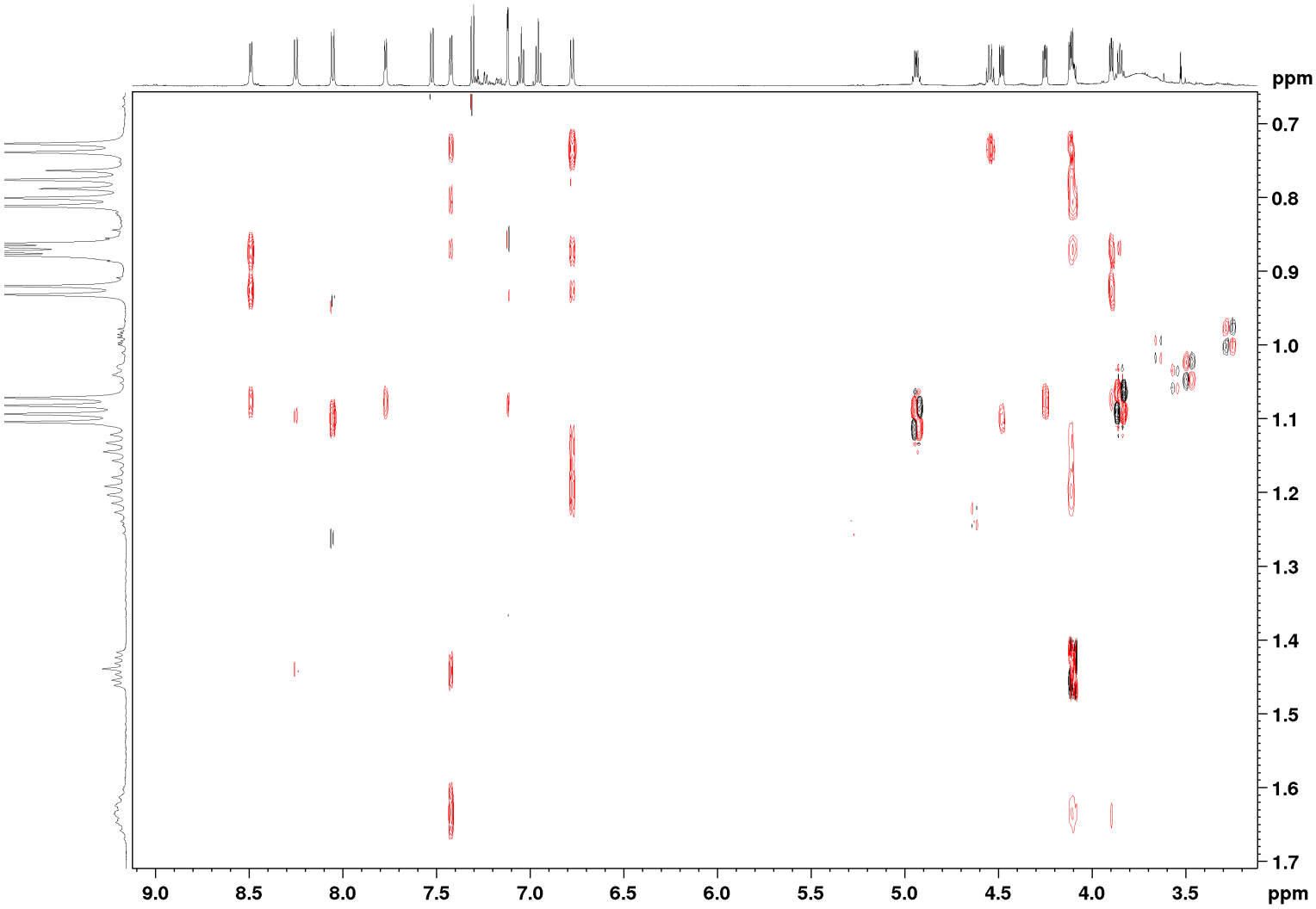
Annex 13. ^{1}H-^{13}C HMBC NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom in the carbonyl region.



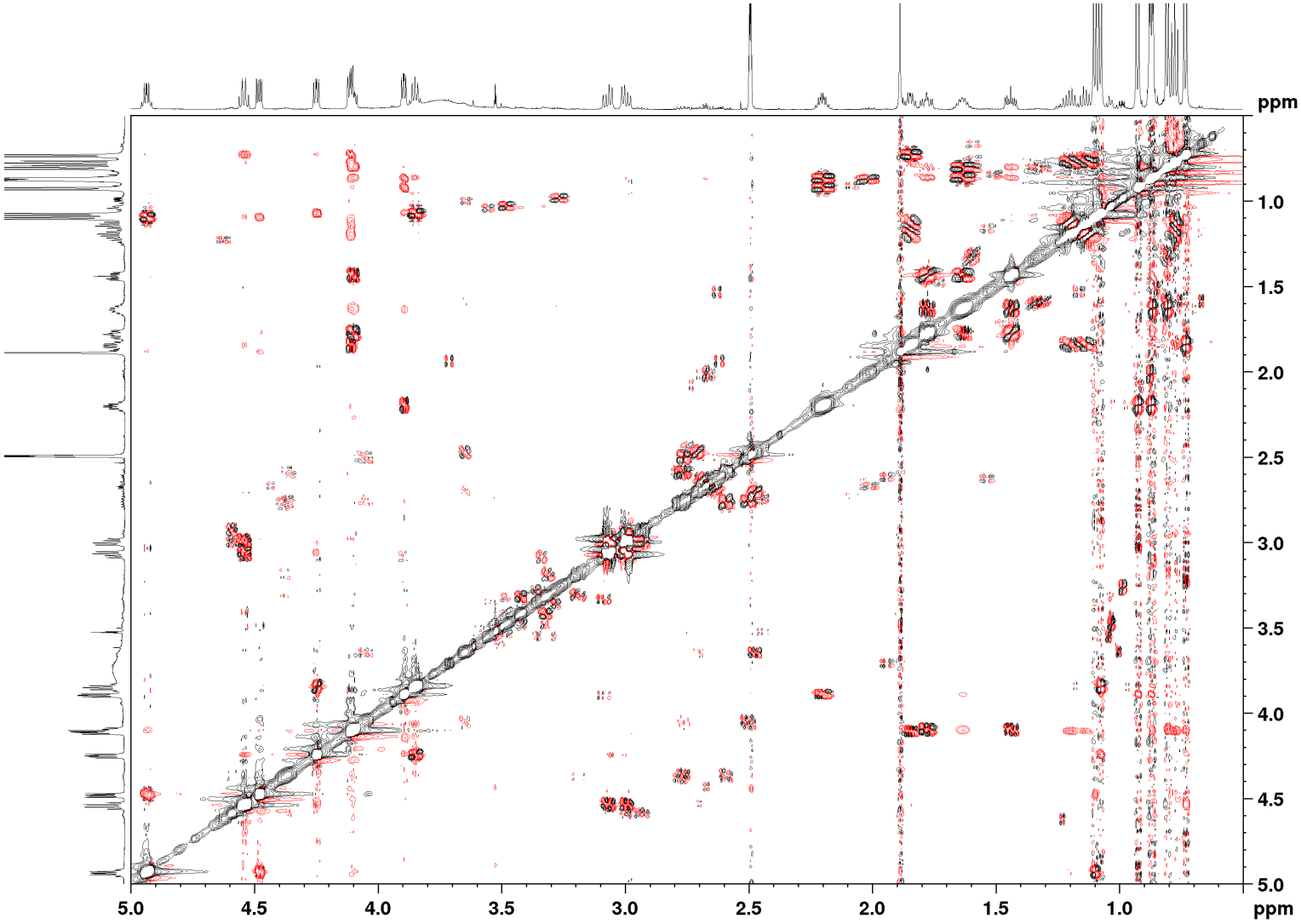
Annex 14. ^{1}H-^{1}H ROESY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz.



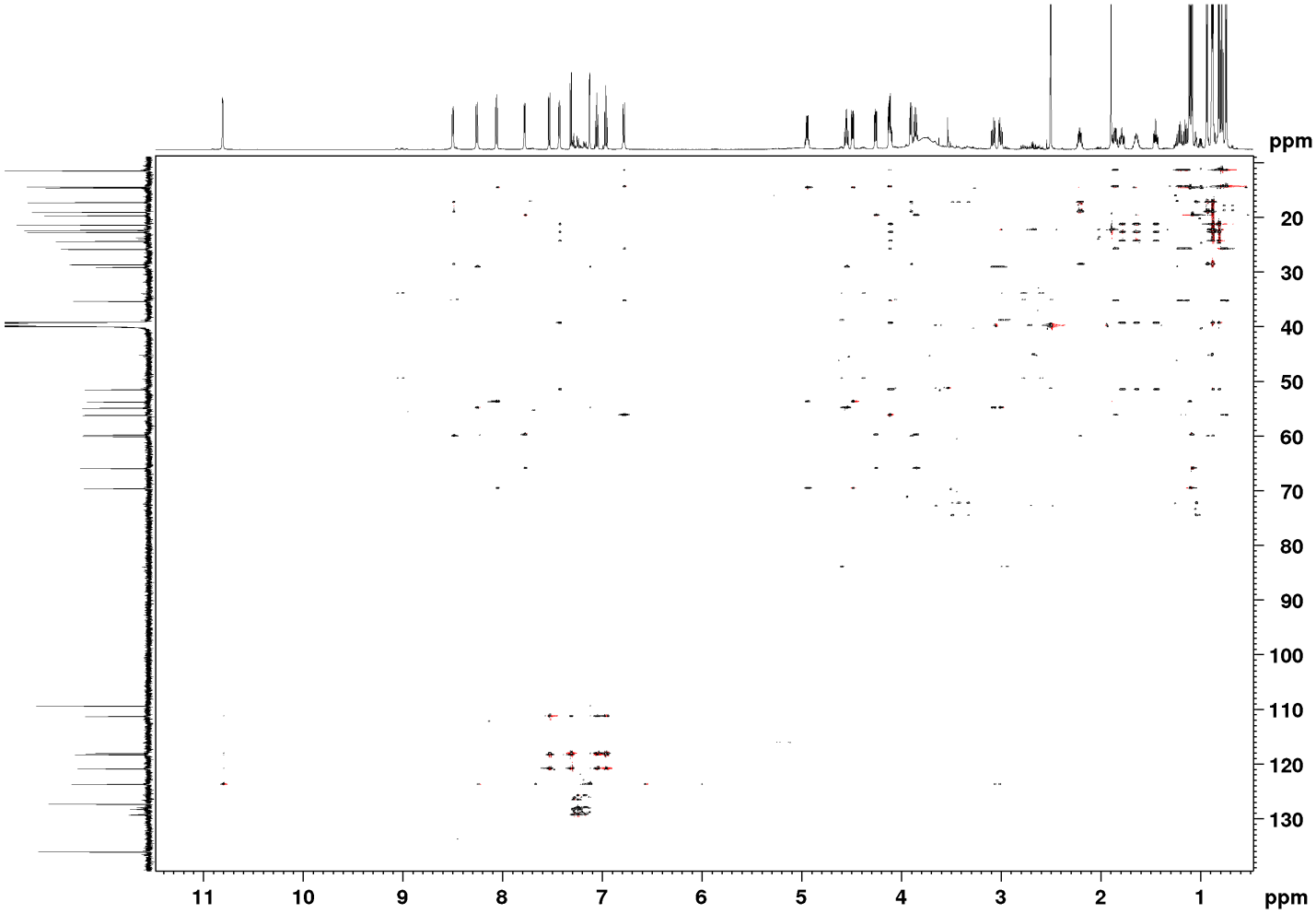
Annex 15. ^{1}H-^{1}H ROESY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom.



Annex 16. ^{1}H-^{1}H ROESY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom.



Annex 17. ^{1}H-^{1}H ROESY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz, zoom.



Annex 18. ^{1}H-^{13}C HSQC-TOCSY NMR spectra of **1**, d_6-DMSO, 300 K, 600MHz.