Supplemental Material for

A Widespread Bacterial Phenazine Forms Conjugates with Biogenic Thiols and Crosslinks Proteins

Daniel Heine, Srividhya Sundaram, Matthias Beudert, Karin Martin and Christian Hertweck

Analytical data of compounds 3 and 4

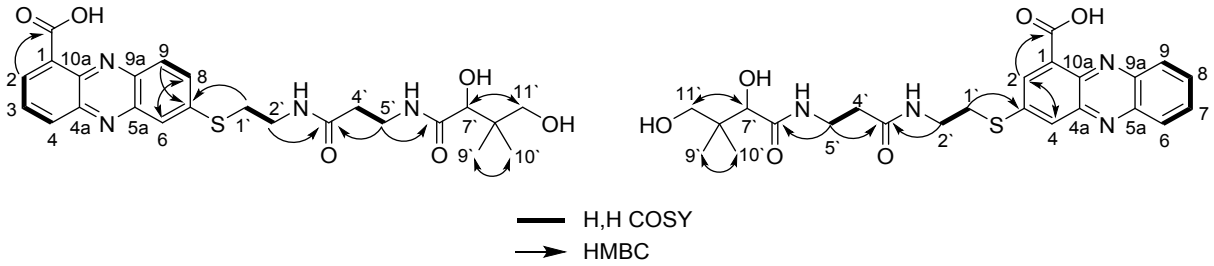


Figure S1: Selected $^{1}H,^{13}C$ HMBC and $^{1}H,^{1}H$ COSY correlations of panphenazines **3** and **4**.

Table S1: NMR spectroscopic data for Compounds **3** and **4** in methanol-*d*4.

	3		**4**	
Position	δ_H[a] (*J* in Hz)	δ_C[b]	δ_H[a] (*J* in Hz)	δ_C[b]
1	-	-[c]	-	-[c]
2	8.62, d (7.0)	135.5	8.32, m	134.7
3	8.03, dd (8.7, 7.0)	131.7	-	143.3
4	8.40, dd (8.7, 1.3)	134.8	8.17, d (2.2)	126.6
4a	-	144.6	-	144.8
5a	-	145.3	-	145.1
6	8.05, d (2.1)	123.2	8.22, dd (7.6, 2.2)	130.2
7	-	145.2	7.96, m	133.0
8	7.87, dd (9.1, 2.1)	134.3	7.96, m	132.8
9	8.18, d (9.1)	129.4	8.28, dd (7.6, 2.2)	129.9
9a	-	141.0	-	142.0
10a	-	140.8	-	140.4
1`	3.38, m	32.0	3.39, m	32.3
2`	3.59,td (6.90, 1.5)	39.3	3.59, t (6.8)	39.2
3`	-	174.1	-	174.1
4`	2.43, t (6.7)	36.5	2.42, t (6.6)	36.5
5`	3.48, m	36.4	3.49, m	36.3
6`	-	176.1	-	176.1
7`	3.87, s	77.3	3.87, s	77.3
8`	-	40.4	-	40.4
9`/10`	0.89, s	21.3/20.9	0.89, s	21.3/20.9
11`	3.39, m	70.4	3.40, m	70.4
COOH	-	169.7	-	170.2

[a] recorded at 600.3 MHz, [b] recorded at 150.9 MHz; [c] signals not detectable.

Binding of phenazine to biogenic thiols

Conjugate formation of PCA with pantetheine following irradiation at 370 nm

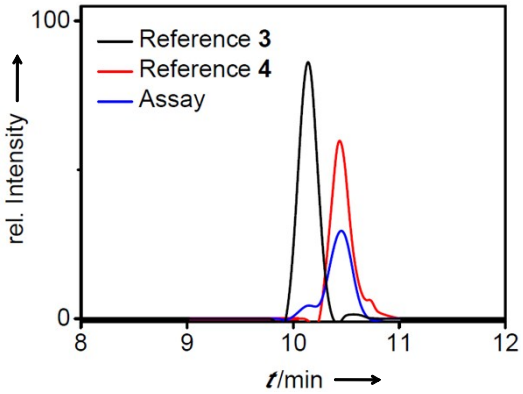


Figure S2. SIM-HRMS (*m*/*z* = 501.1788–501.1828) analysis of the obtained product after incubation of PCA and pantetheine with irradiation at 370 nm; comparison with the isolated reference compounds **3** and **4**. SIM: selected ion monitoring.

Coupling of PCA to cysteine and glutathione

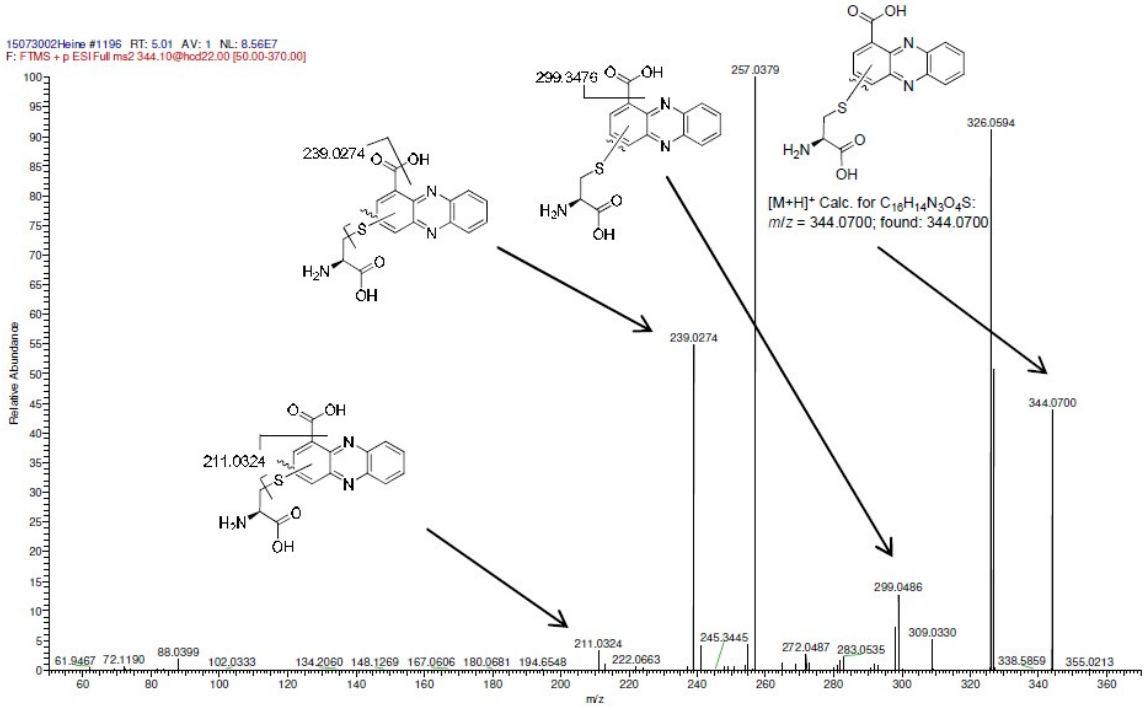


Figure S3. HRMS(/MS) spectrum of PCA-cysteine adduct ([M+H]$^+$) after incubation of PCA with cysteine under sunlight.

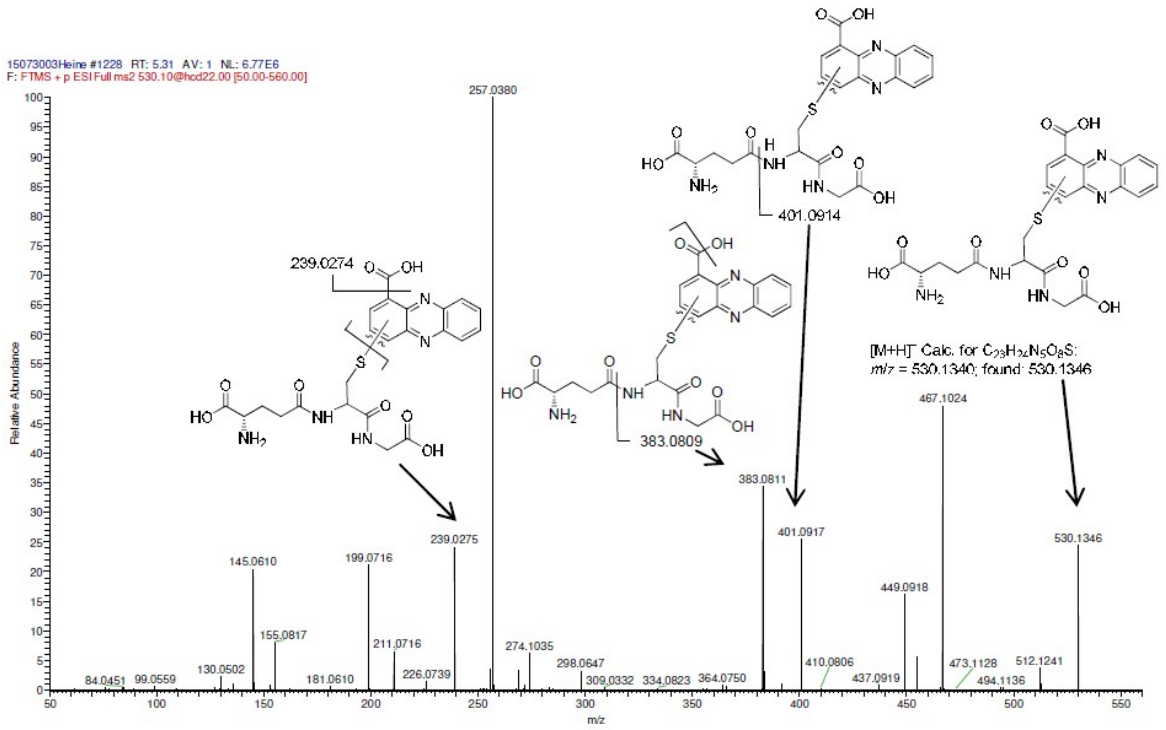


Figure S4. HRMS(/MS) spectrum of PCA-glutathione-adduct ($[M+H]^+$) after incubation of PCA with glutathione under sunlight.

Conjugate formation of PCA with pantetheine in presence of AIBN

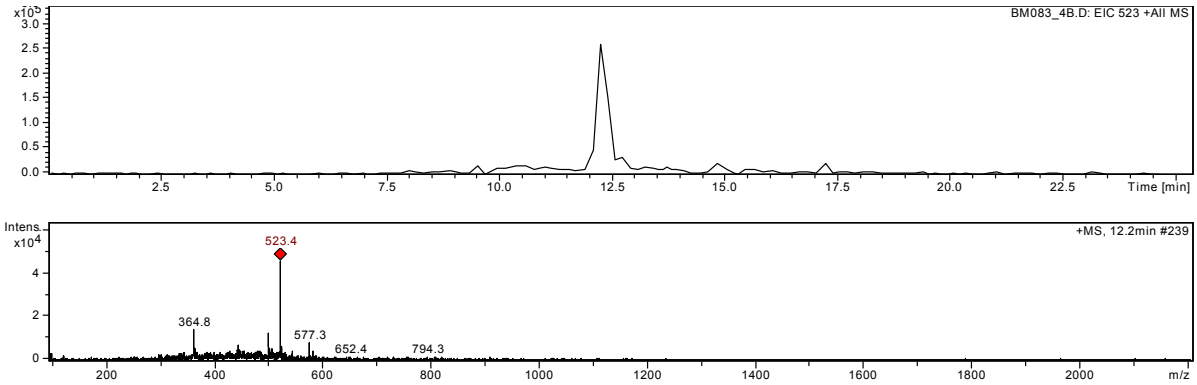


Figure S5. SIM-MS spectrum of the product after incubation of PCA with pantetheine in presence of AIBN in the dark (top). MS spectrum of PCA-pantetheine-adduct ($[M+Na]^+$) (bottom).

Synthesis of phenazine probes 9 (with biotin) and 10 (with rhodamine).

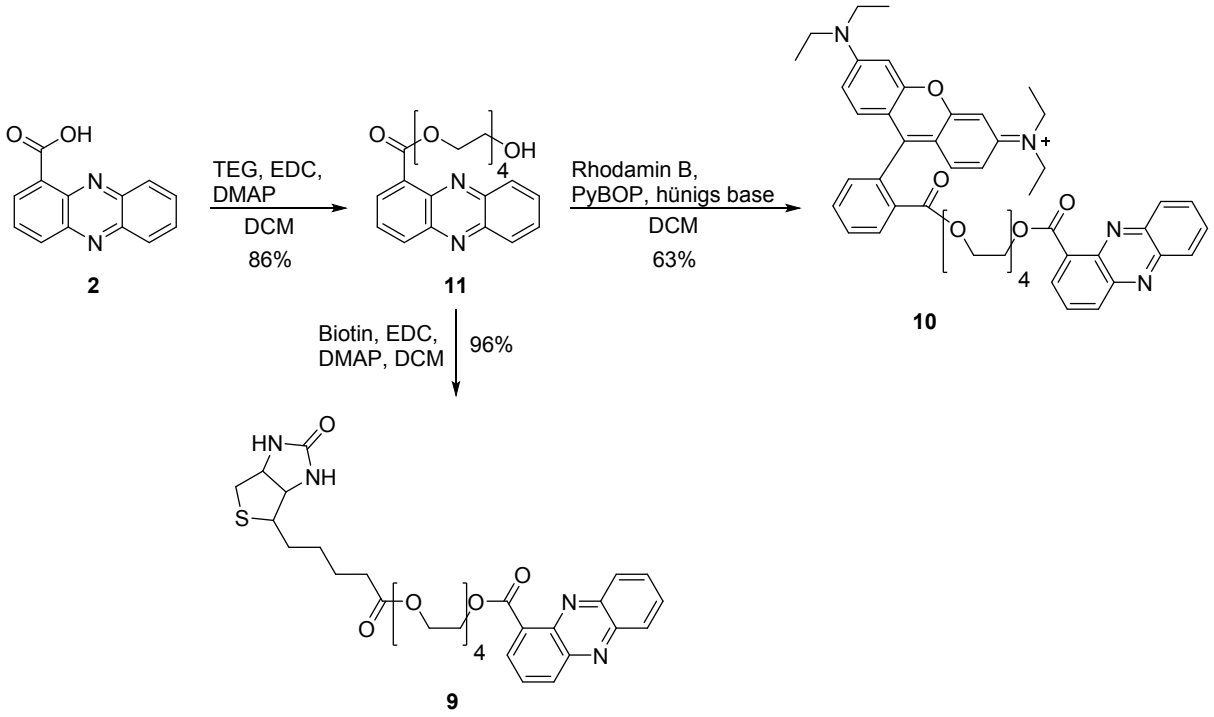


Figure S6: Synthesis of biotin- and rhodamine-B-tagged phenazine probes.

Phenazine-protein binding assays with KS-B

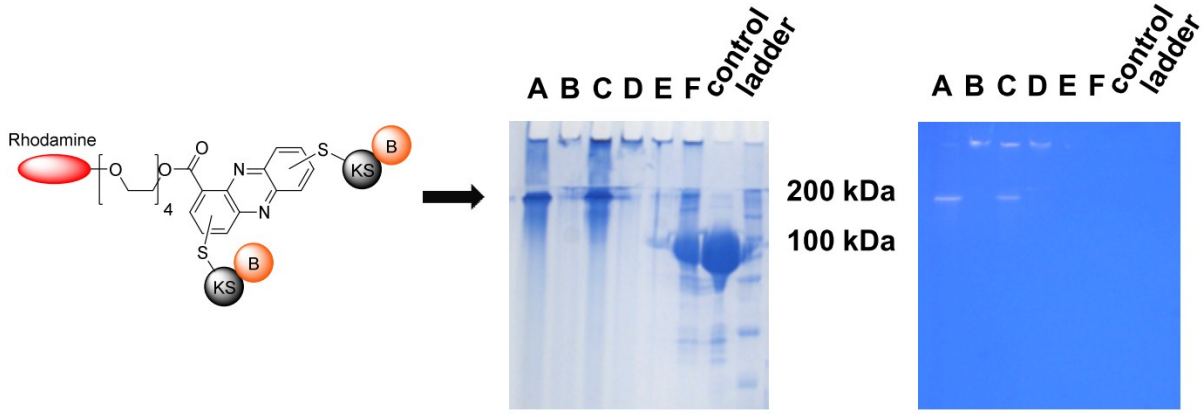


Figure S7. SDS-PAGE (12% gel) after incubation of KS-B with rhodamine-B-labelled phenazine probe **10** under the following conditions: A) λ = 370 nm, 20 h, in H_2O, B) λ = 370 nm, 20 h, DMSO, C) sunlight, 8 h, H_2O, D) D: sunlight, 8 h, DMSO, E) E: AIBN, 20 h, DMSO, F) F: AIBN, 20 h, H_2O.

Tryptic mass fingerprinting of labelled KS-B

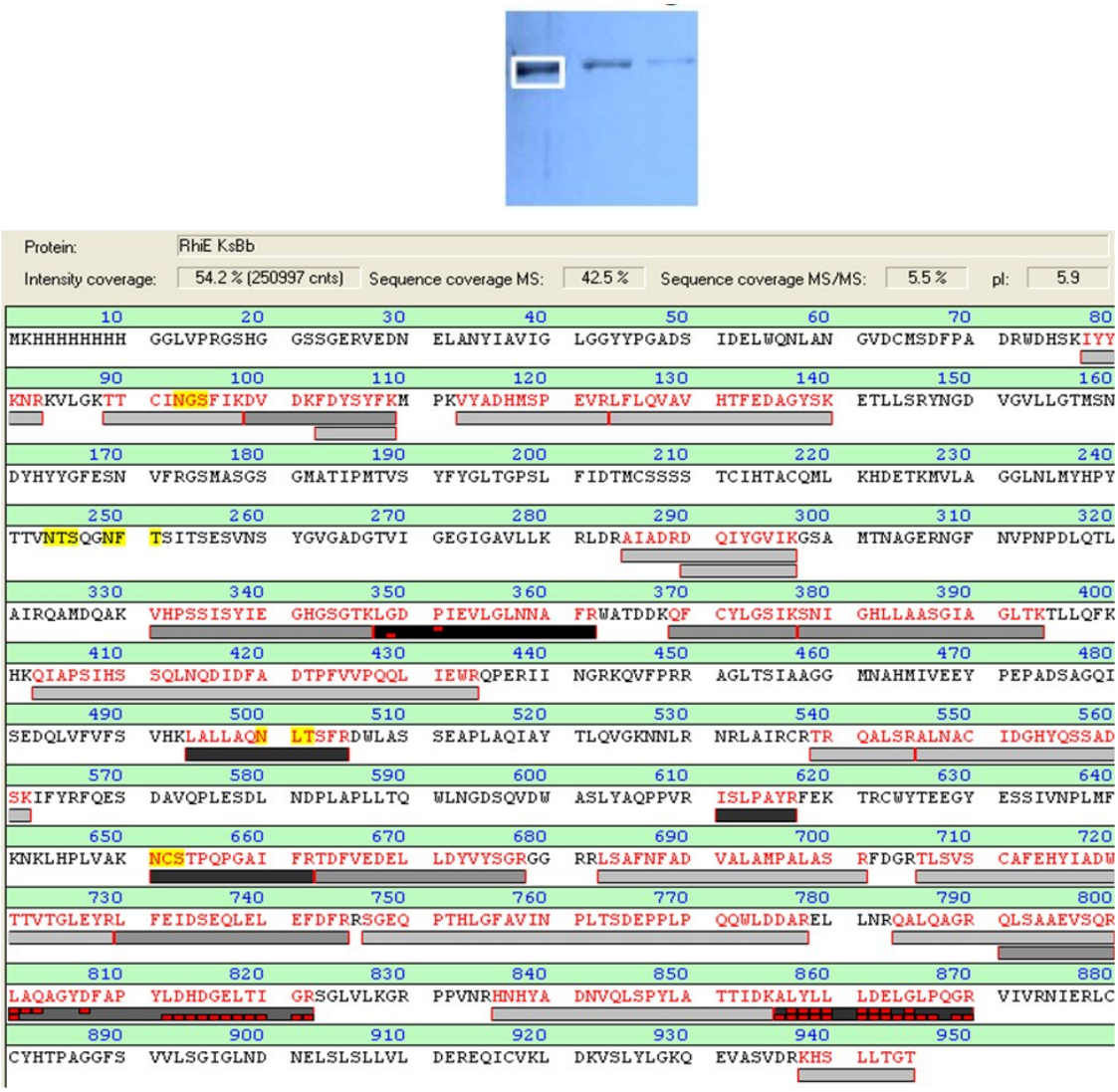


Figure S8.1. Tryptic mass fingerprinting of KS-B after incubation with **9** and AIBN followed by affinity chromatography.

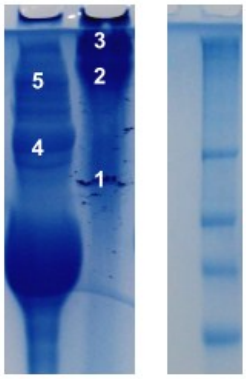


Figure S8.2. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).

Figure S8.3. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).

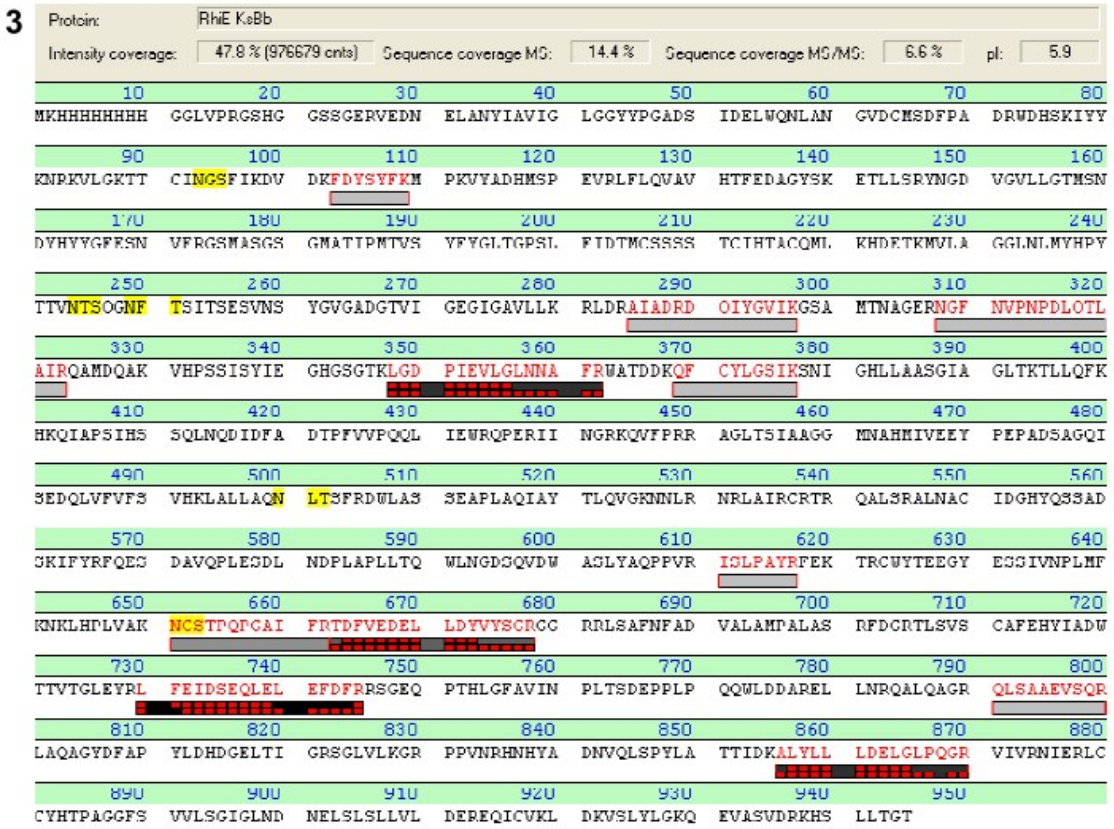


Figure S8.4. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).

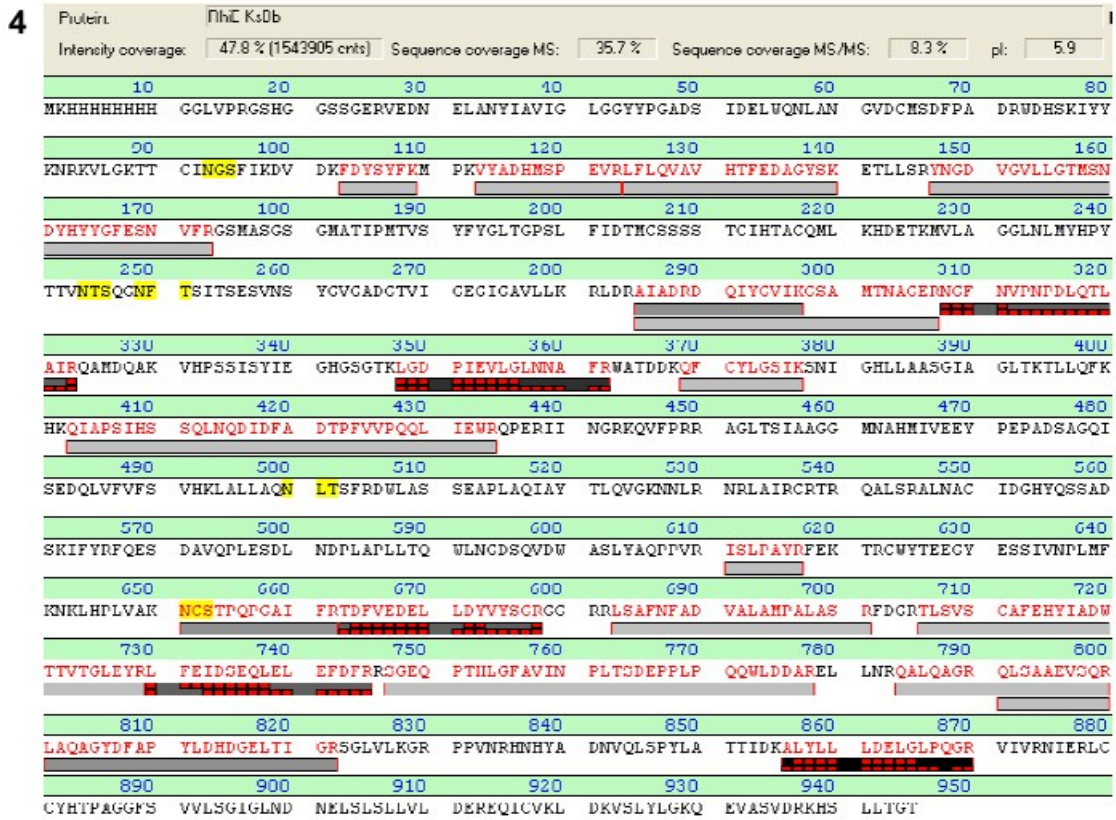


Figure S8.5. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).

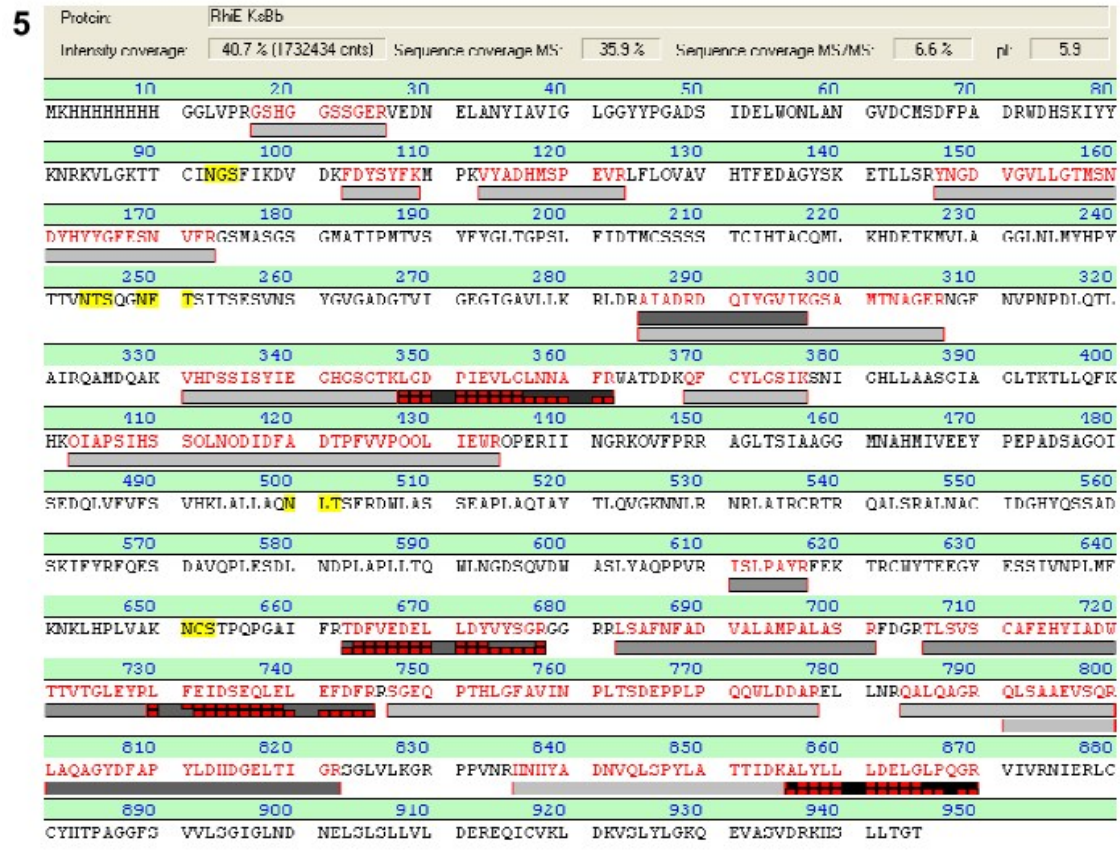


Figure S8.6. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).

Phenazine-protein binding assays with carbonic anhydrase III and albumin

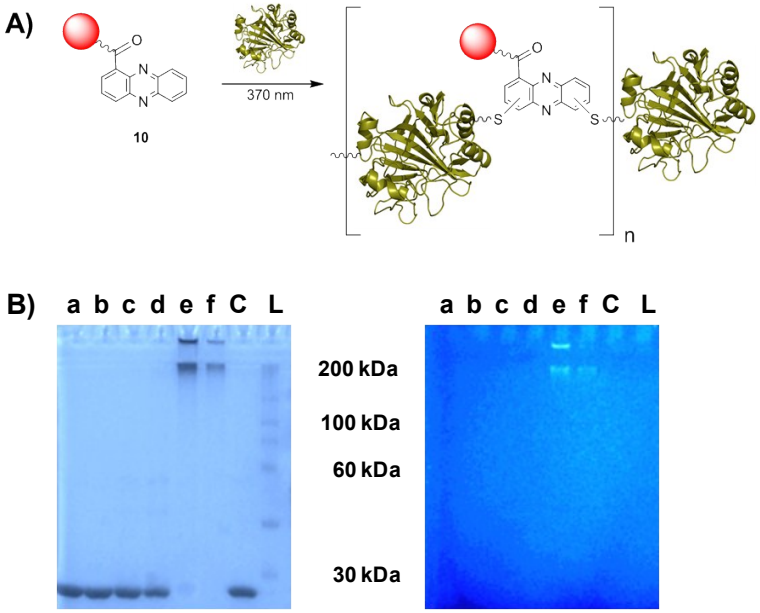


Figure S9. A) Scheme for the functionalization of carbonic anhydrase III by probe **10** after irradiation with UV-light. B) SDS-PAGE (10%) of carbonic anhydrase III after the incubation with probe **10** or rhodamine B in water; a) rhodamine B, 370 nm, 10 h; b) **10**, 370 nm, 15 min; c) **10**, 370 nm, 30 min; d) **10**, 370 nm, 1 h; e) **10**, 370 nm, 5 h; f) **10**, 370 nm, 10 h, C) carbonic anhydrase III control, L: Ladder. The fluorescent bands would correspond to potential conjugates of carbonic anhydrase III and **9** with n>5.

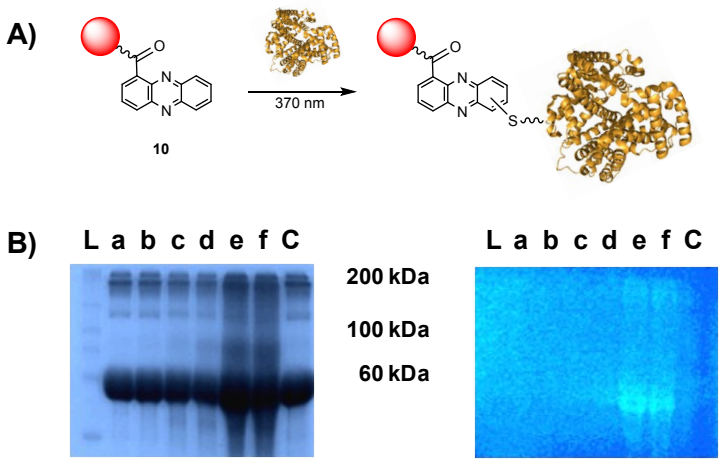
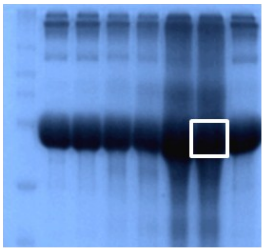


Figure S10. SDS-PAGE (10%) of albumin after the incubation with probe **10** or rhodamine B in water (left picture: under white light; right picture at 370 nm); L, Ladder, a) rhodamine B, 370 nm, 10 h; b) **10**, 370 nm, 15 min; c) **10**, 370 nm, 30 min; d) **10**, 370 nm, 1 h; e) **10**,

370 nm, 5 h; f) **10**, 370 nm, 10 h, C) albumin control. The fluorescent band potentially corresponds to a 70 kDa monoadduct of **10** and albumin.

Tryptic mass fingerprinting of labelled albumin



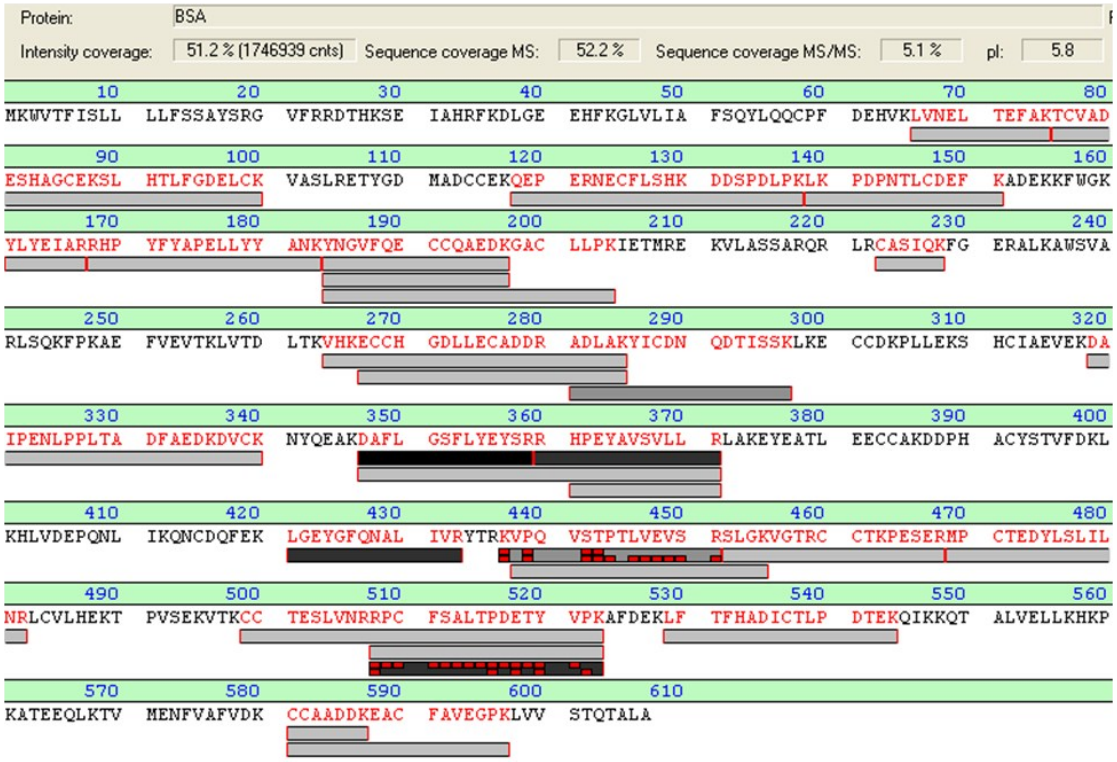


Figure S11. Tryptic mass fingerprinting of albumin after functionalization with **10** under UV light (1).

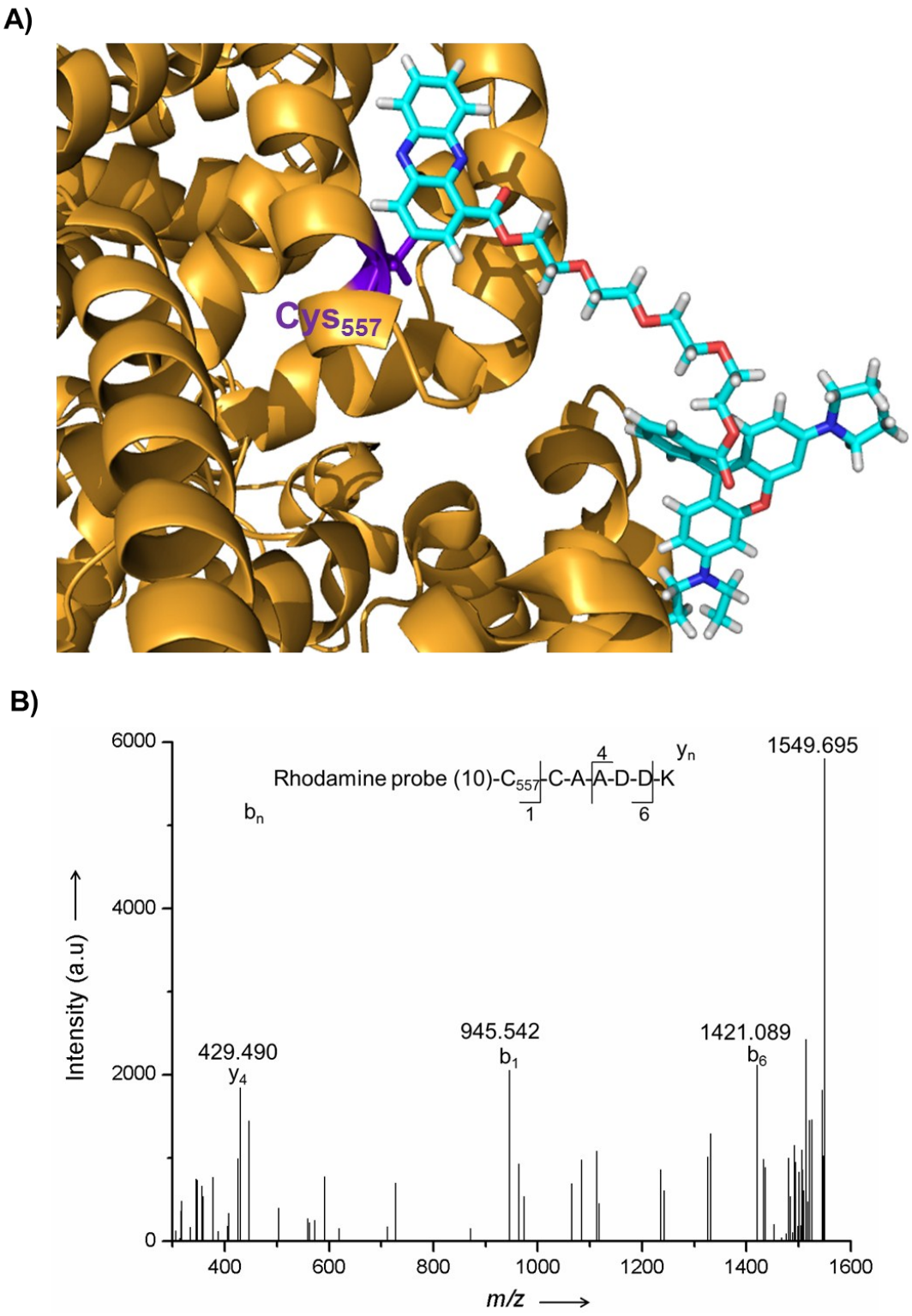


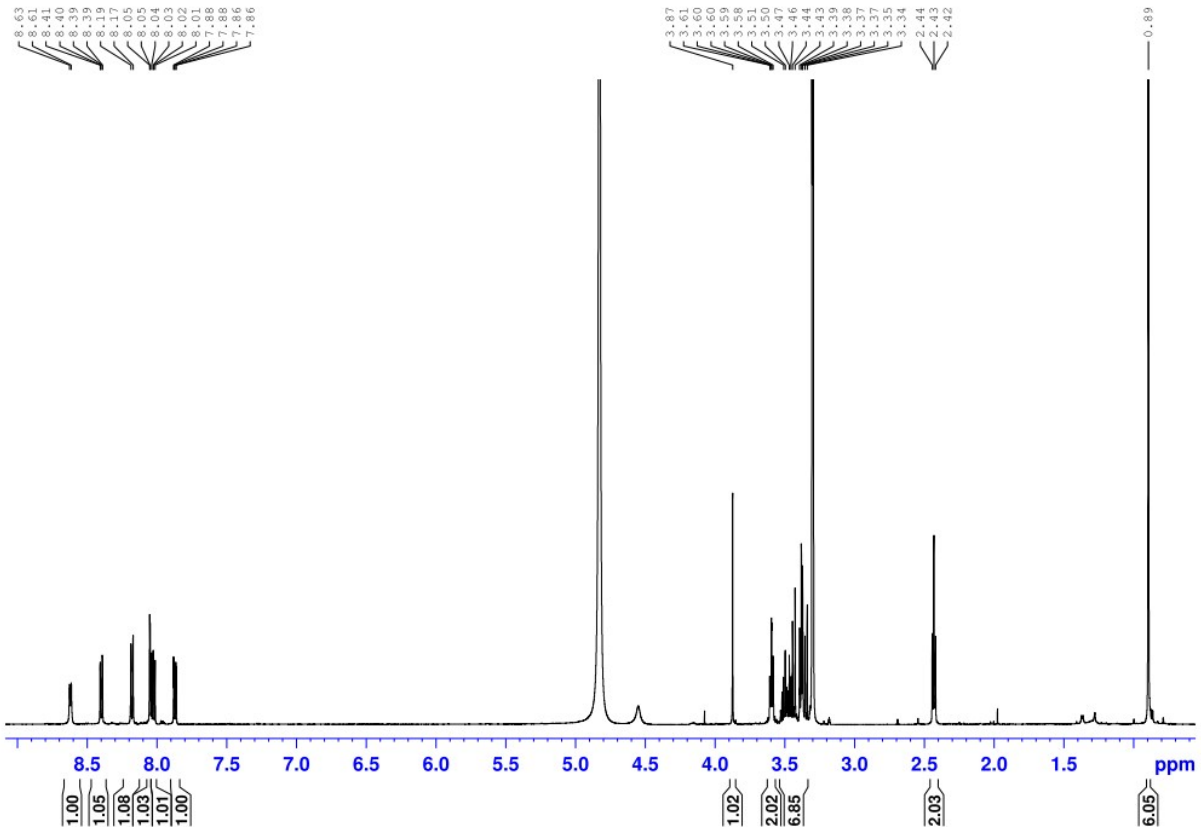
Figure S12. A) Model of the rhodamine-based probe (**10**) crosslinked to the Cys_{557} of albumin under UV light (PDB code 4F5S) B) MALDI-MS/MS spectrum of the rhodamine-based probe (**10**)-linked peptide of albumin.

Antimicrobial properties of isolated phenazines 3 and 4

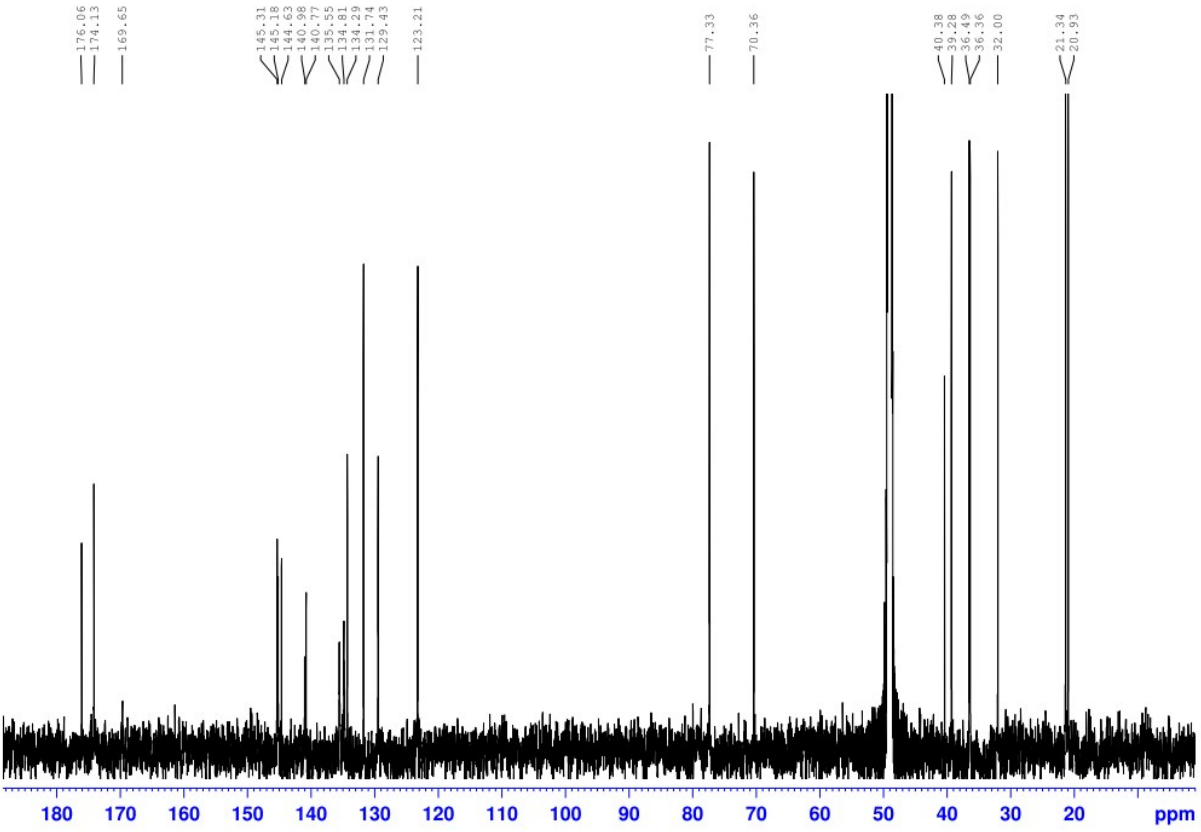
	Inhibition zone (mm)									
Cpd.	***B. sub***	***S. aur***	***E. col***	***P. aer***	**MRSA**	**VRE**	***M. vac***	***S. sal***	***C. alb***	***P. not***
3	0	0	17	0	0	15	13	20	0	0
4	10	0	0	15	0	0	13	0	0	0
PCA (2)	31	25	32	0	20	0	27	20	35	16

Table S2. Antimicrobial properties of the phenazine-pantetheine conjugates **3** and **4** in comparison with PCA (**2**). *B. sub*: *Bacillus subtilis* ATCC 6633; *S. aur*: *Staphylococcus aureus* SG 511; *E. col*: *Escherichia coli* SG 458; *P. aer*: *Pseudomonas aeruginosa* SG 137; MRSA: Methicillin- and quinolone-resistant *Staphylococcus aureus* 134/93; VRE: Vancomycin-resistant *Enterococcus faecalis* 1528*; M. vac*: *Mycobacterium vaccae* IMET 10670; *S. sal*: *Sporobolomyces salmonicolor* SBUG 549; *C. alb*: *Candida albicans* BSMY 212; *P. not*: *Penicillium notatum* JP36; cpd, compound; PCA: phenazine-1-carboxylic acid (**2**).

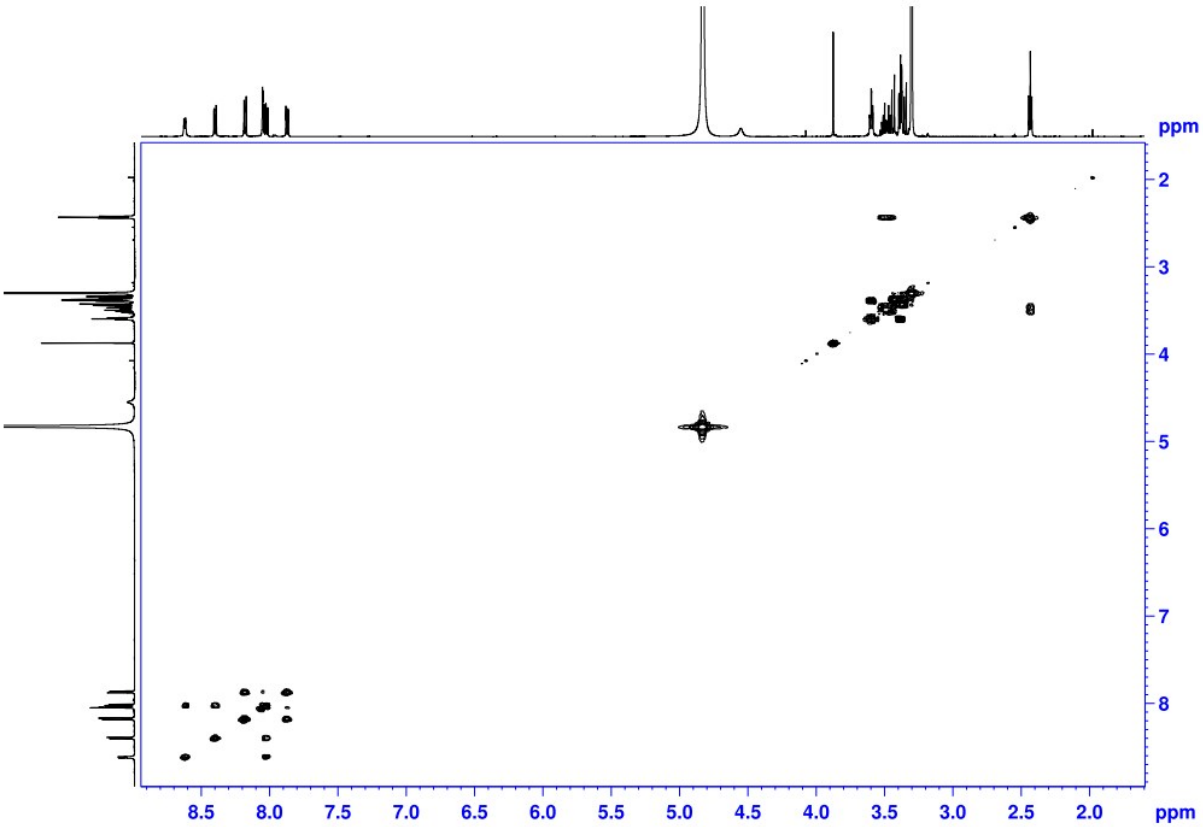
NMR Spectra

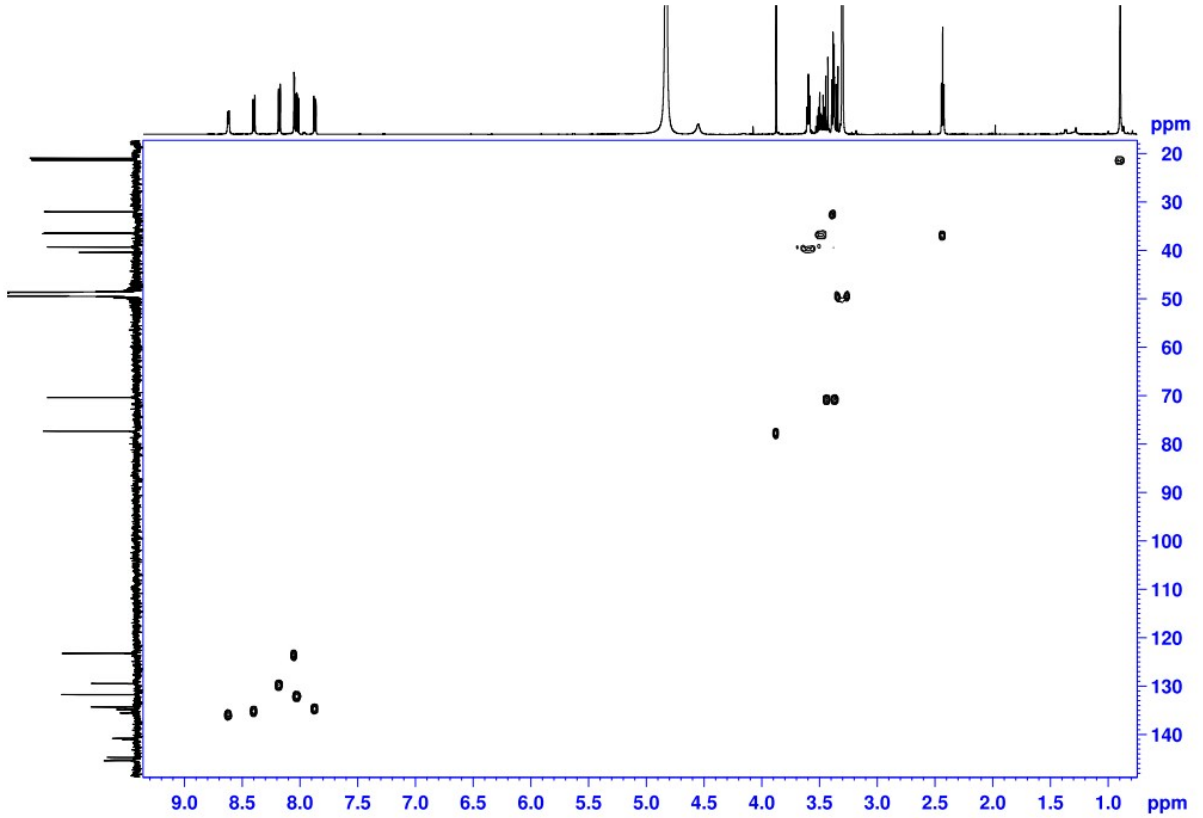


^{1}H NMR spectrum (600 MHz, MeOD) of **3**.

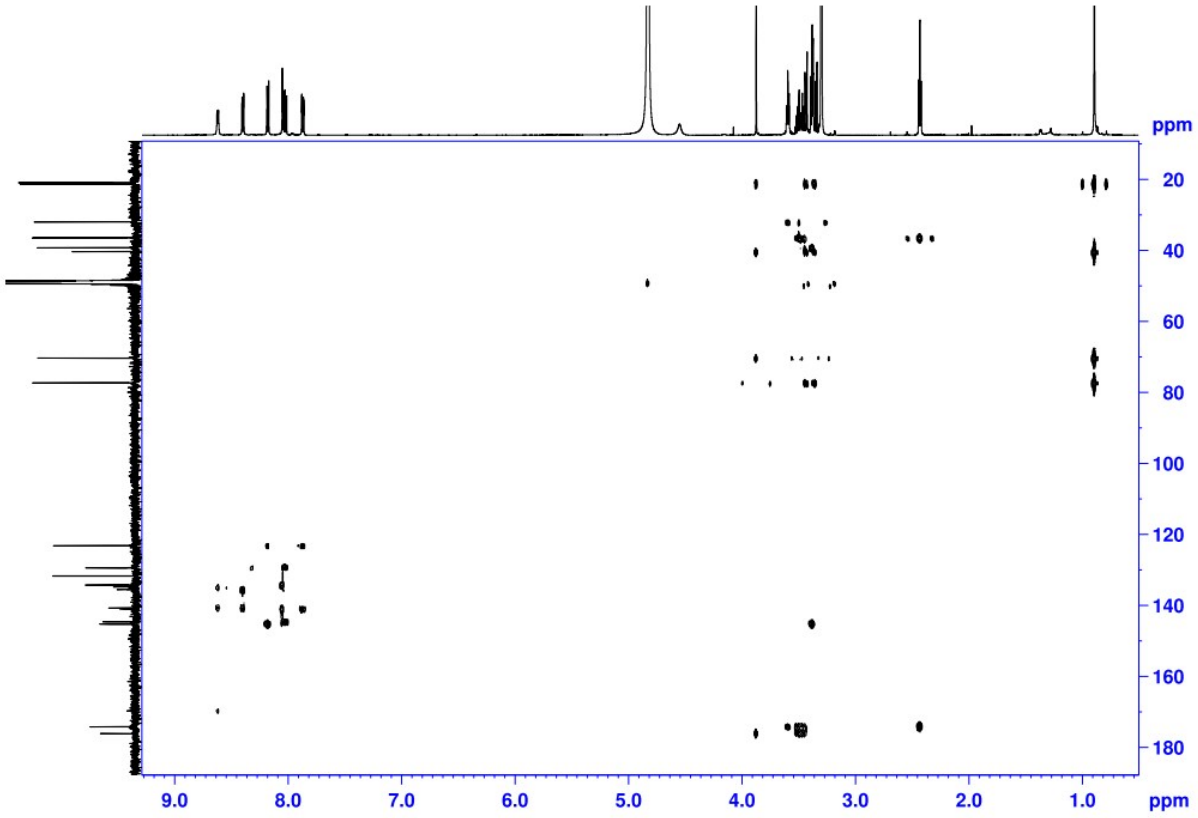


^{13}C NMR spectrum (150 MHz, MeOD) of **3**.

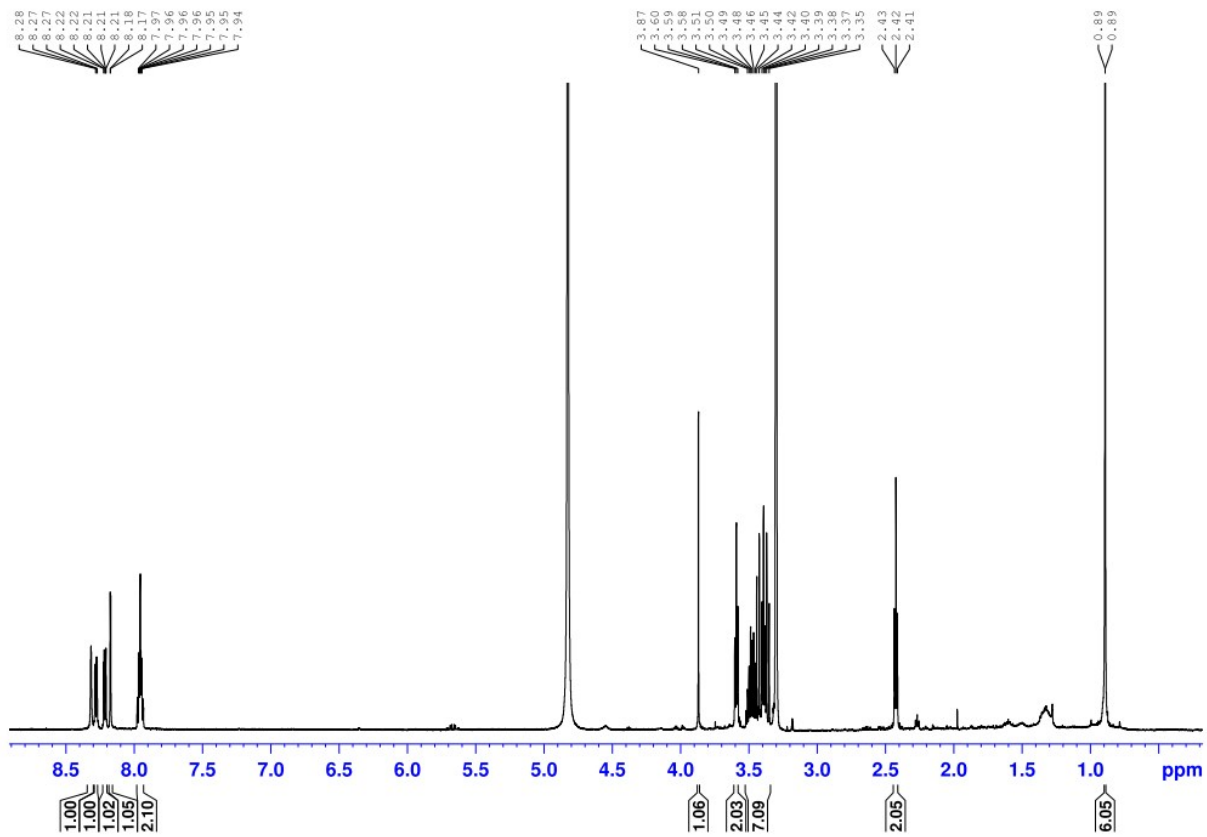




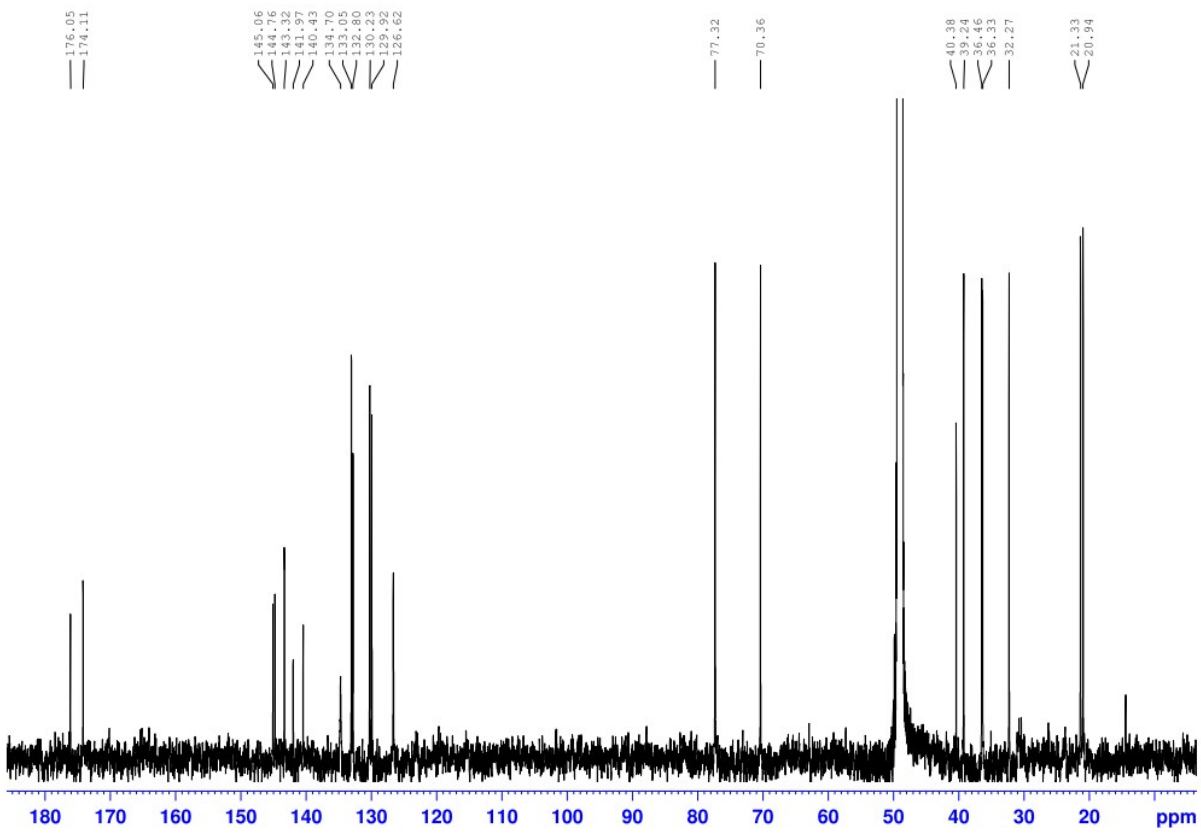
COSY and HSQC spectra of compound **3**.



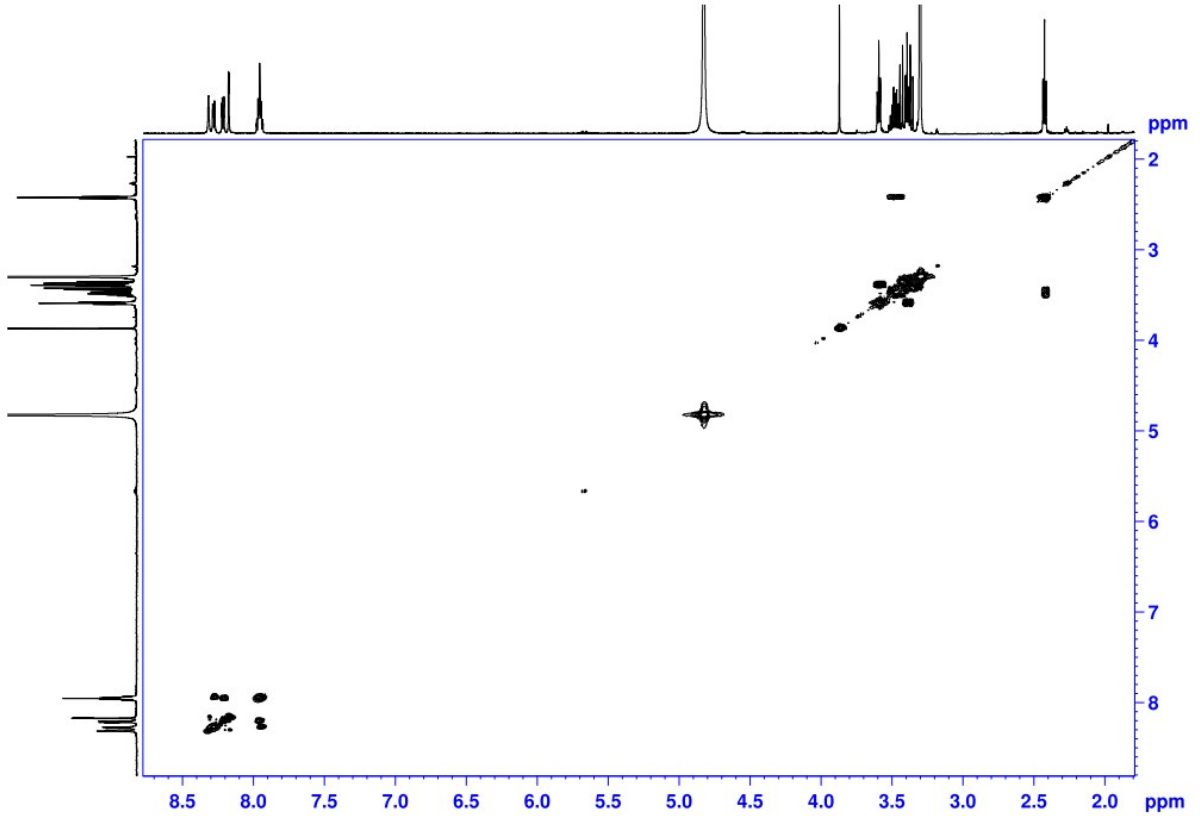
HMBC spectrum of compound **3**.

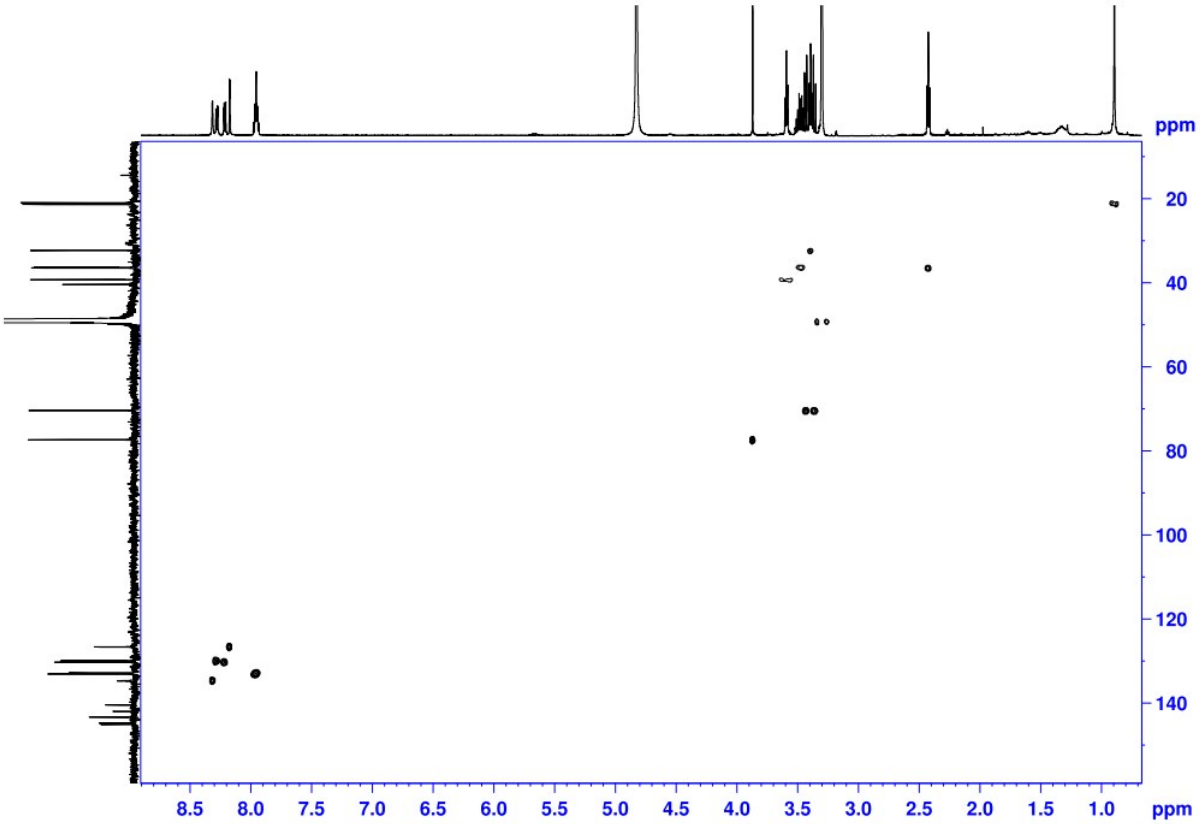


^{1}H NMR spectrum (600 MHz, MeOD) of **4**.

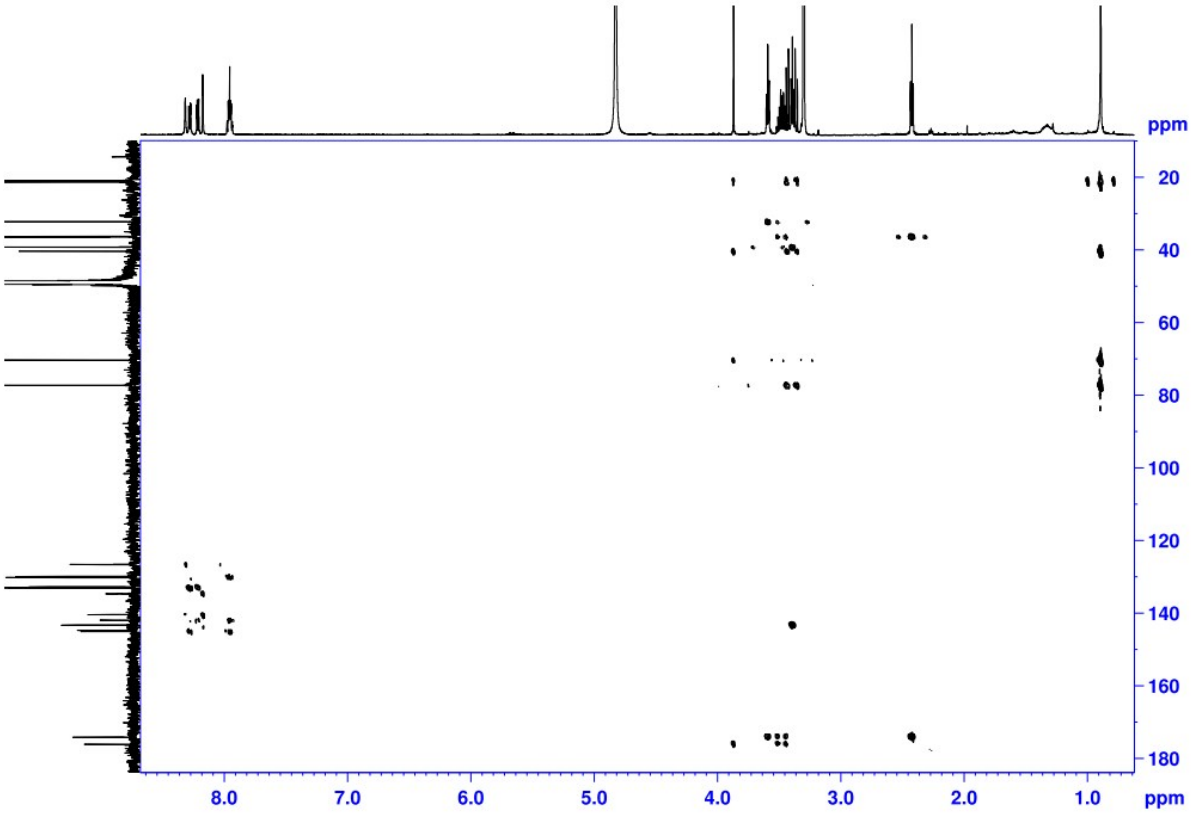


^{13}C NMR spectrum (150 MHz, MeOD) of **4**.

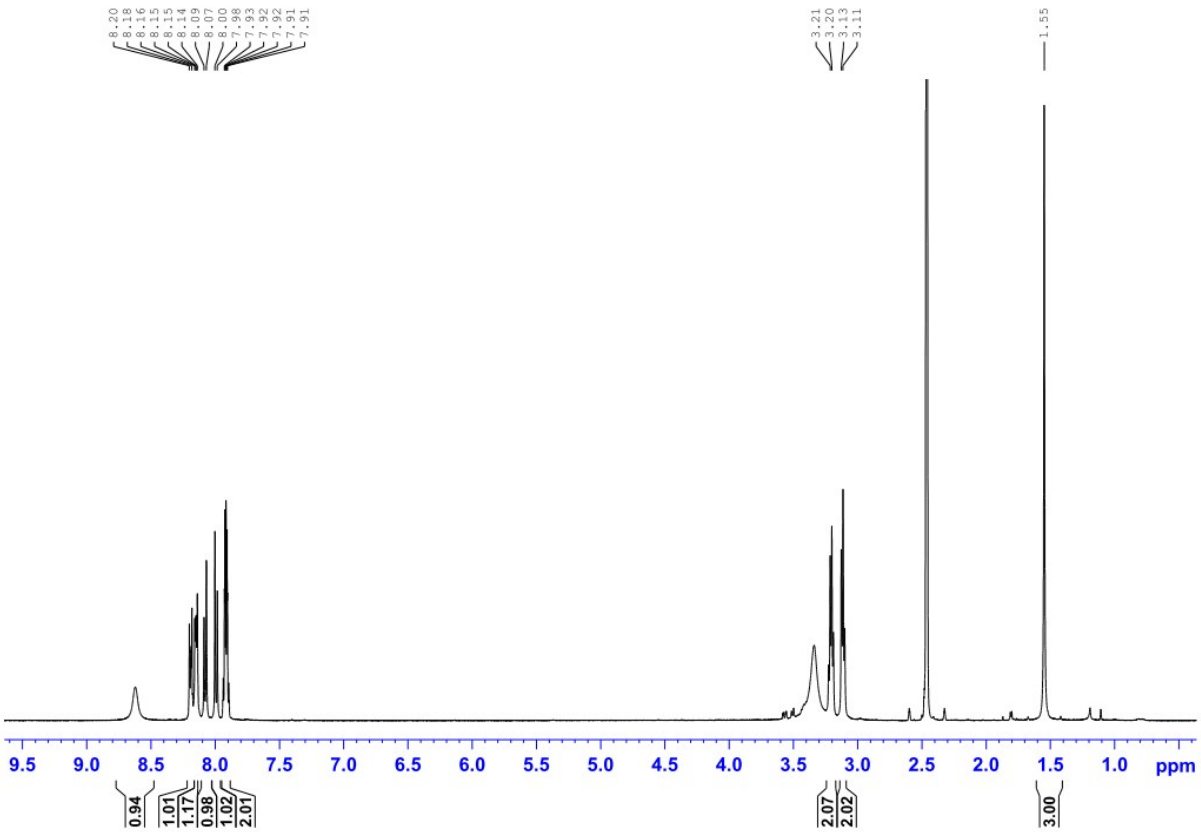




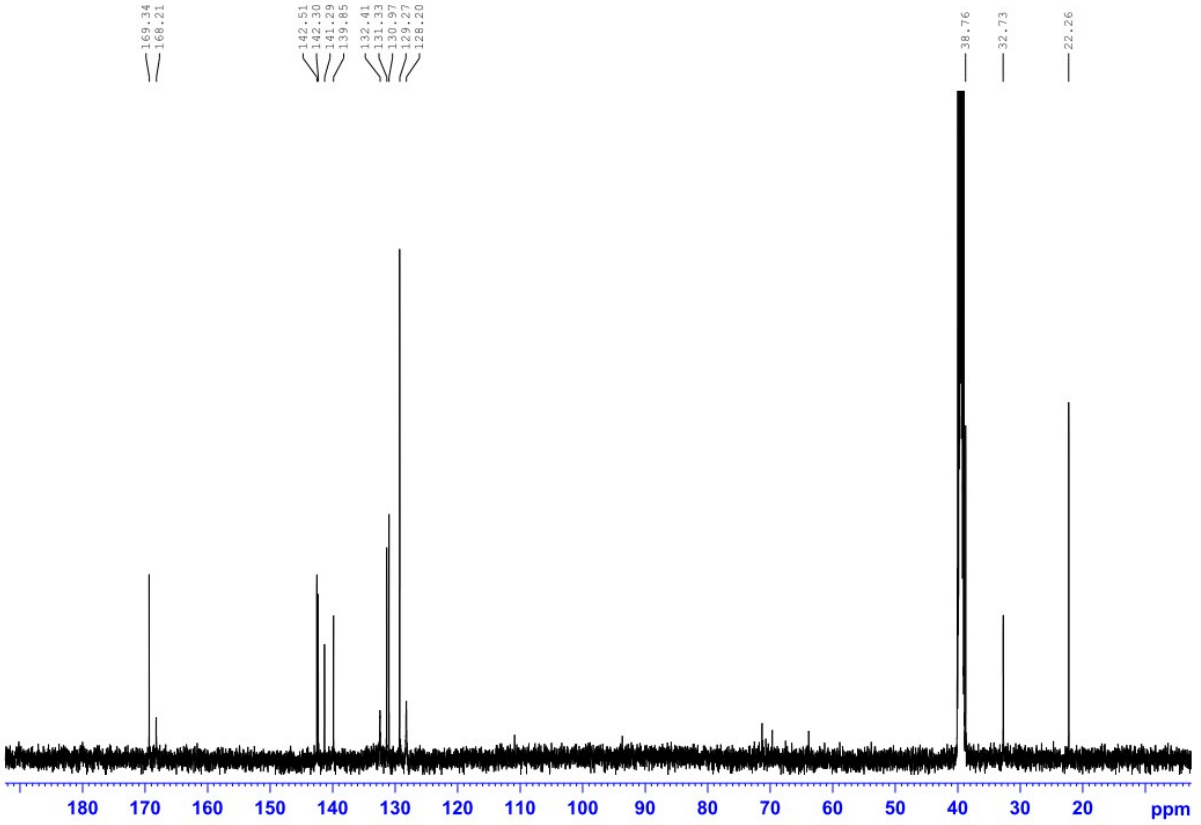
COSY and HSQC spectra of compound **4**.



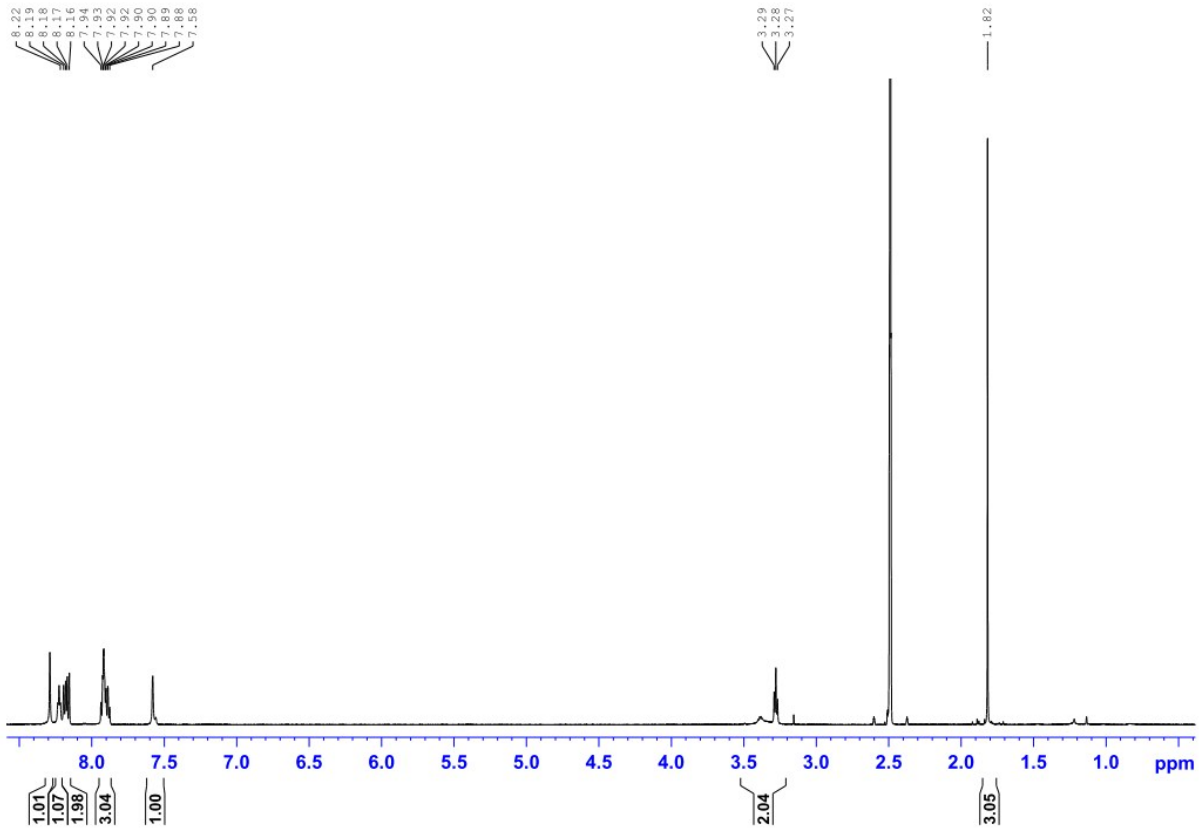
HMBC spectrum of compound **4**.



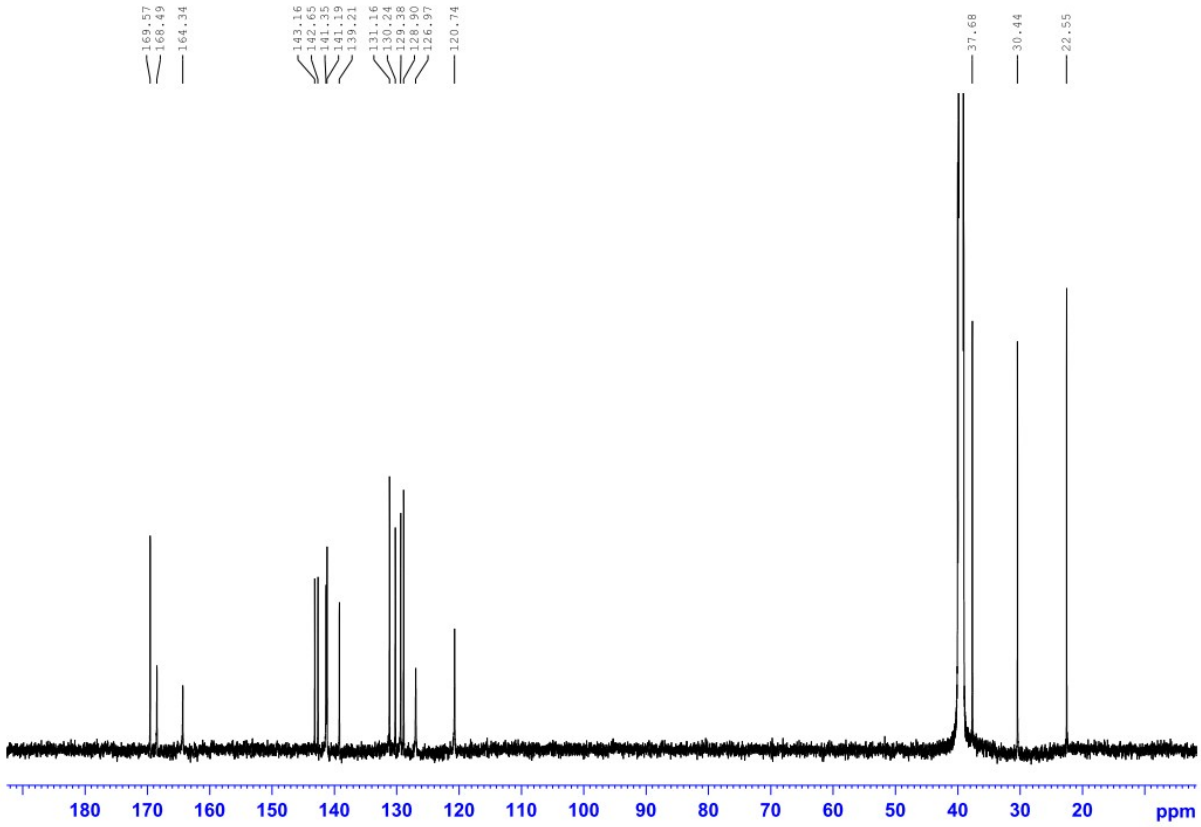
^{1}H NMR spectrum (500 MHz, DMSO-D_6) of **5**.



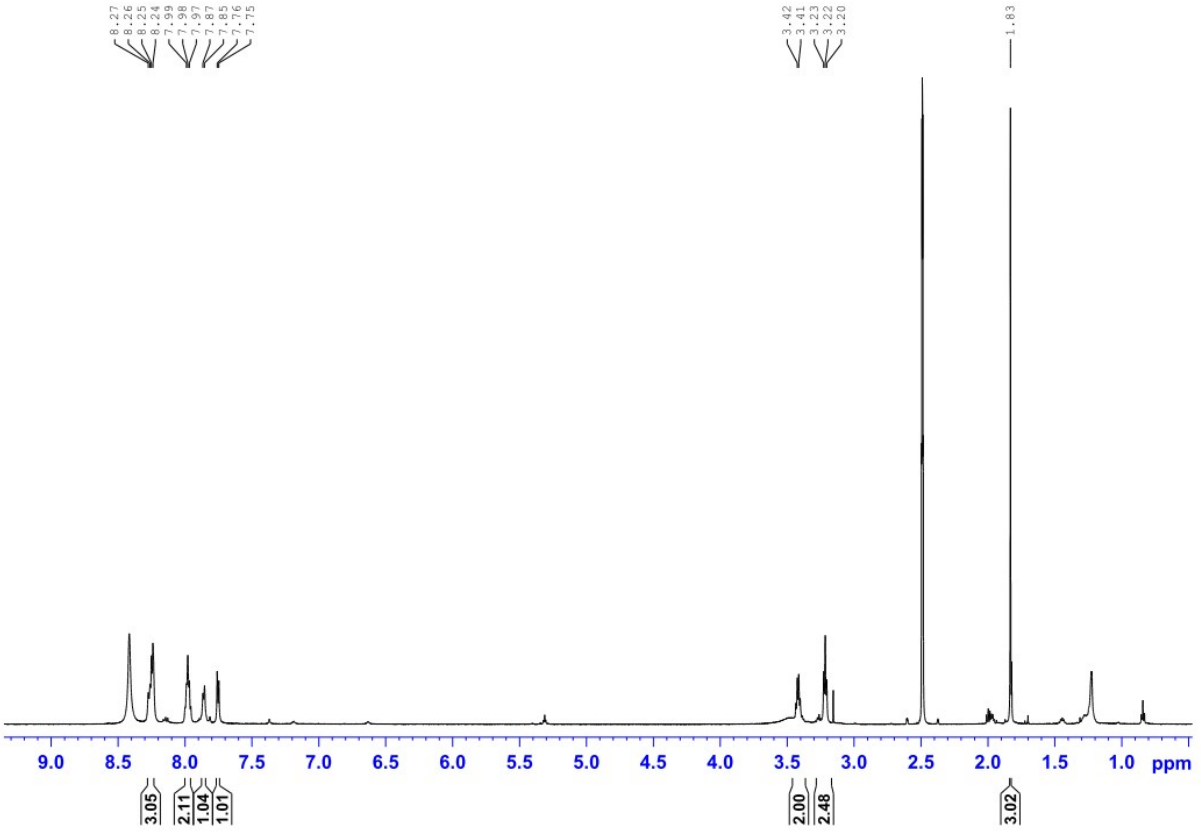
^{13}C NMR spectrum (125 MHz, DMSO-D_6) of **5**.



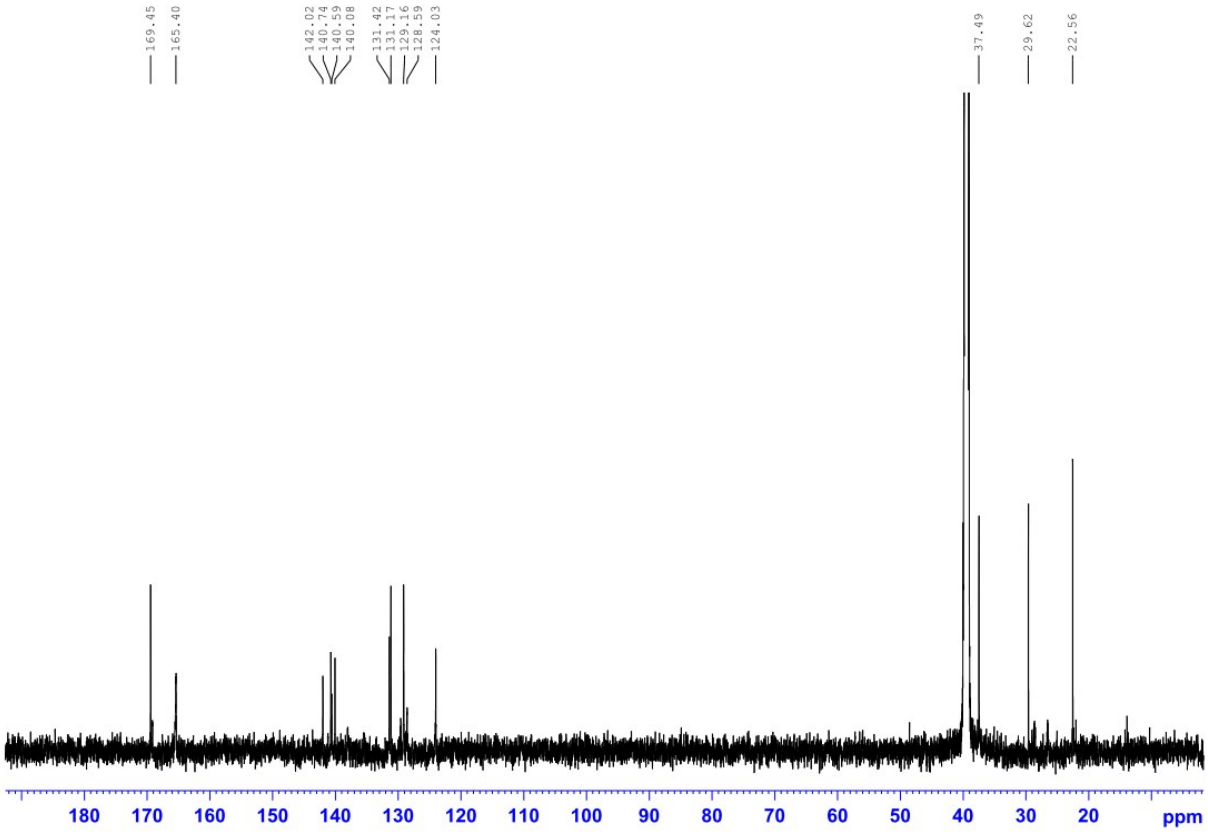
^{1}H NMR spectrum (600 MHz, DMSO-D_6) of **6**.



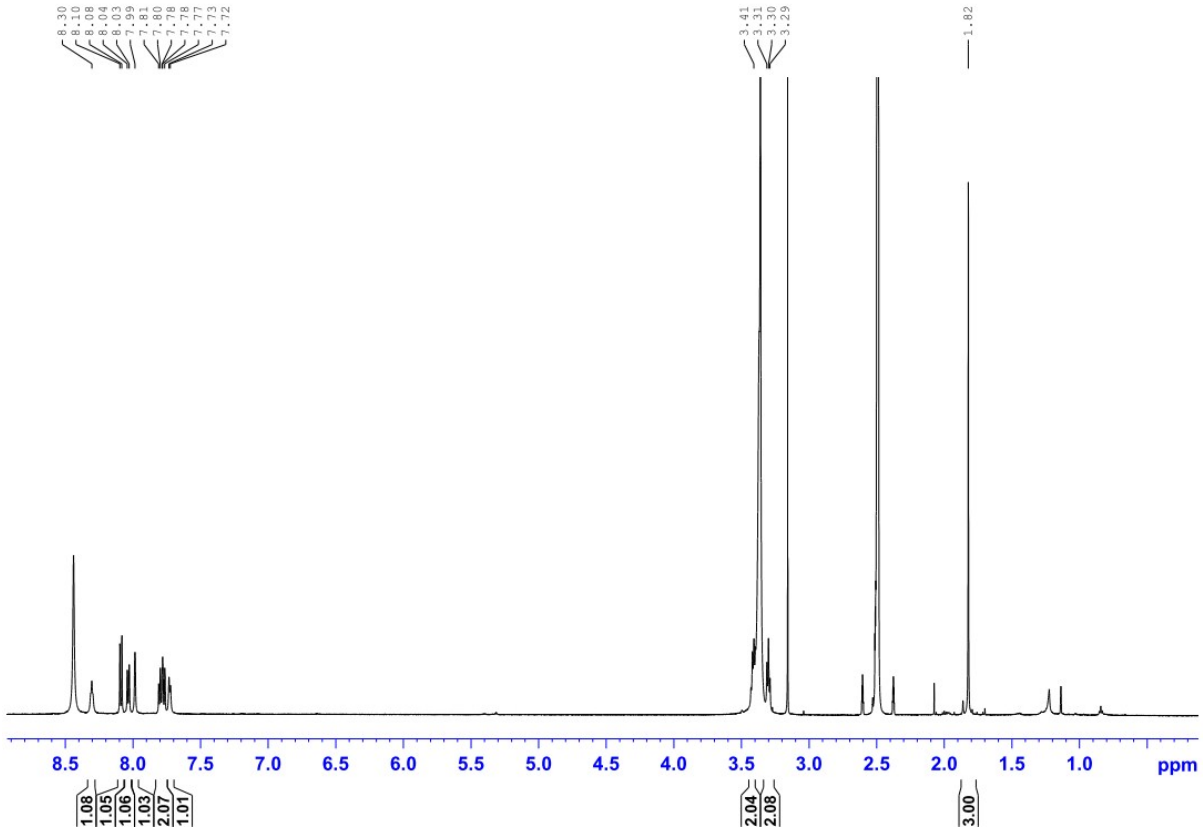
^{13}C NMR spectrum (150 MHz, DMSO-D_6) of **6**.



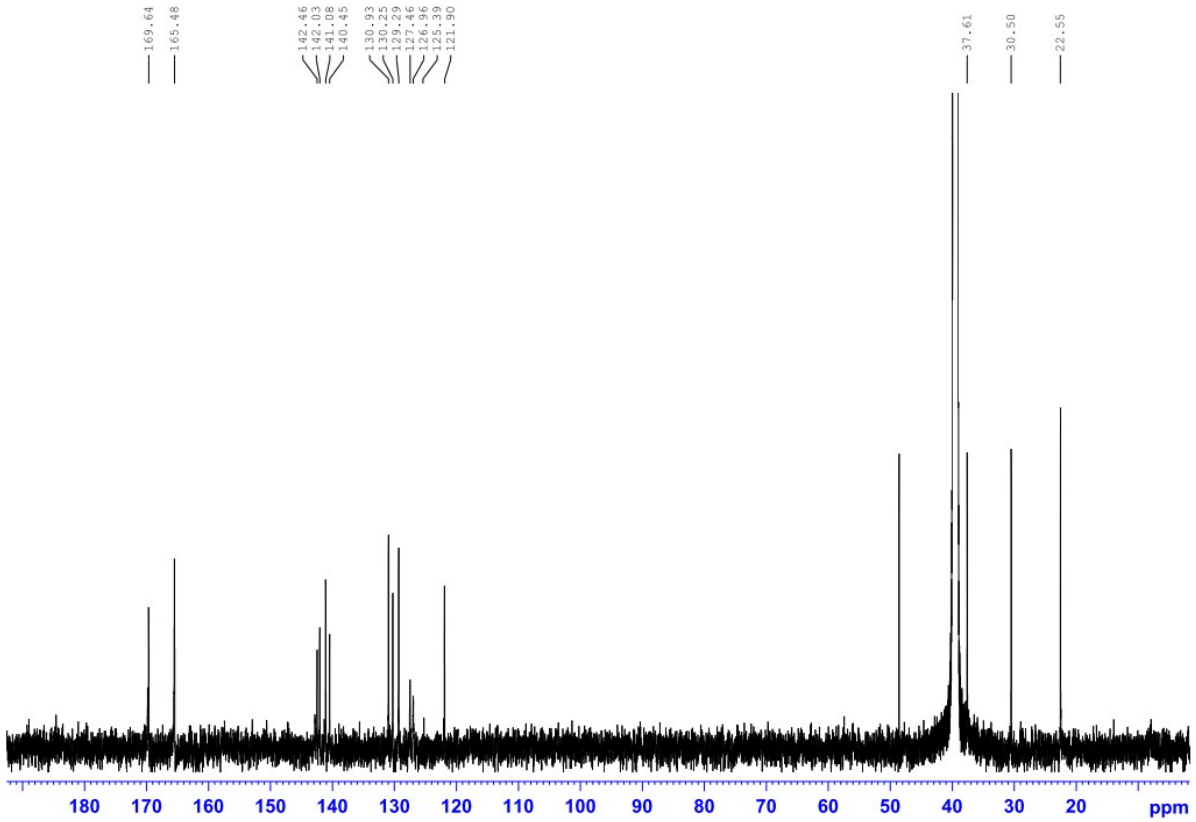
^{1}H NMR spectrum (500 MHz, DMSO-D_6) of **7**.



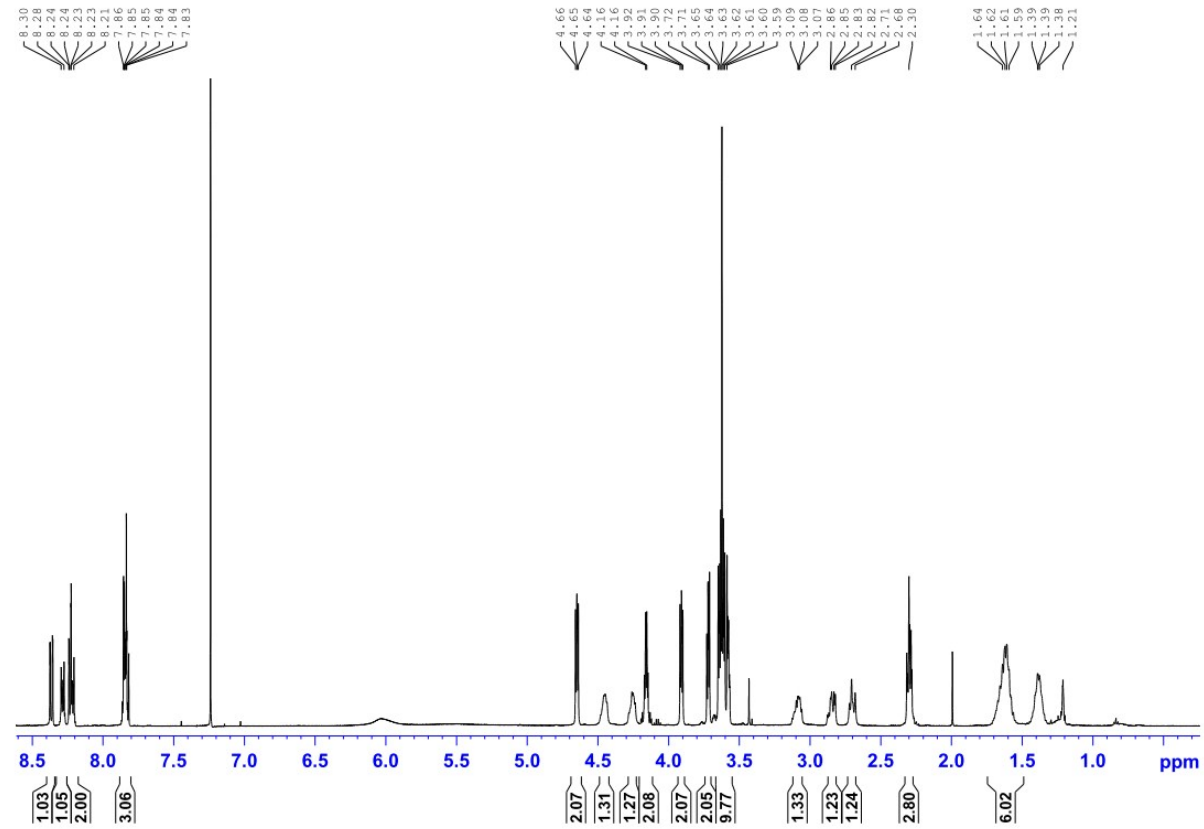
^{13}C NMR spectrum (125 MHz, DMSO-D_6) of **7**.



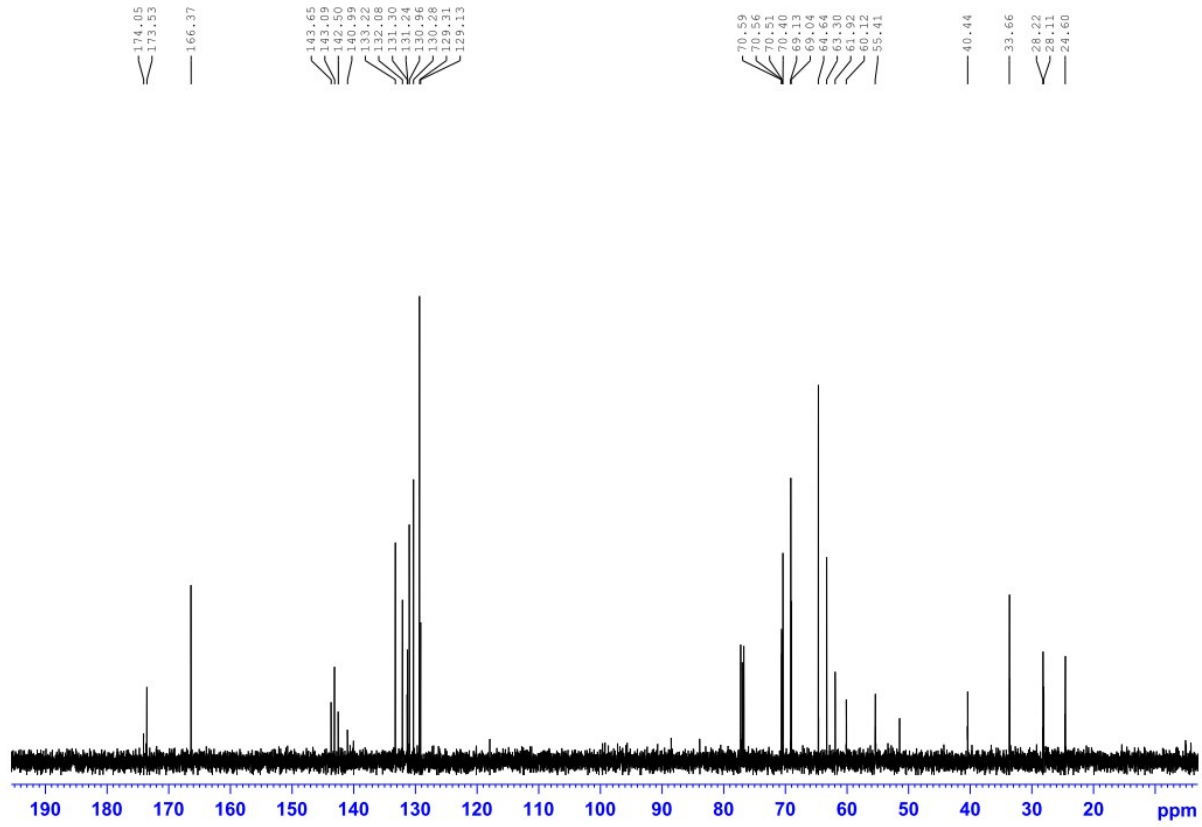
^{1}H NMR spectrum (600 MHz, DMSO-D_6) of **8**.



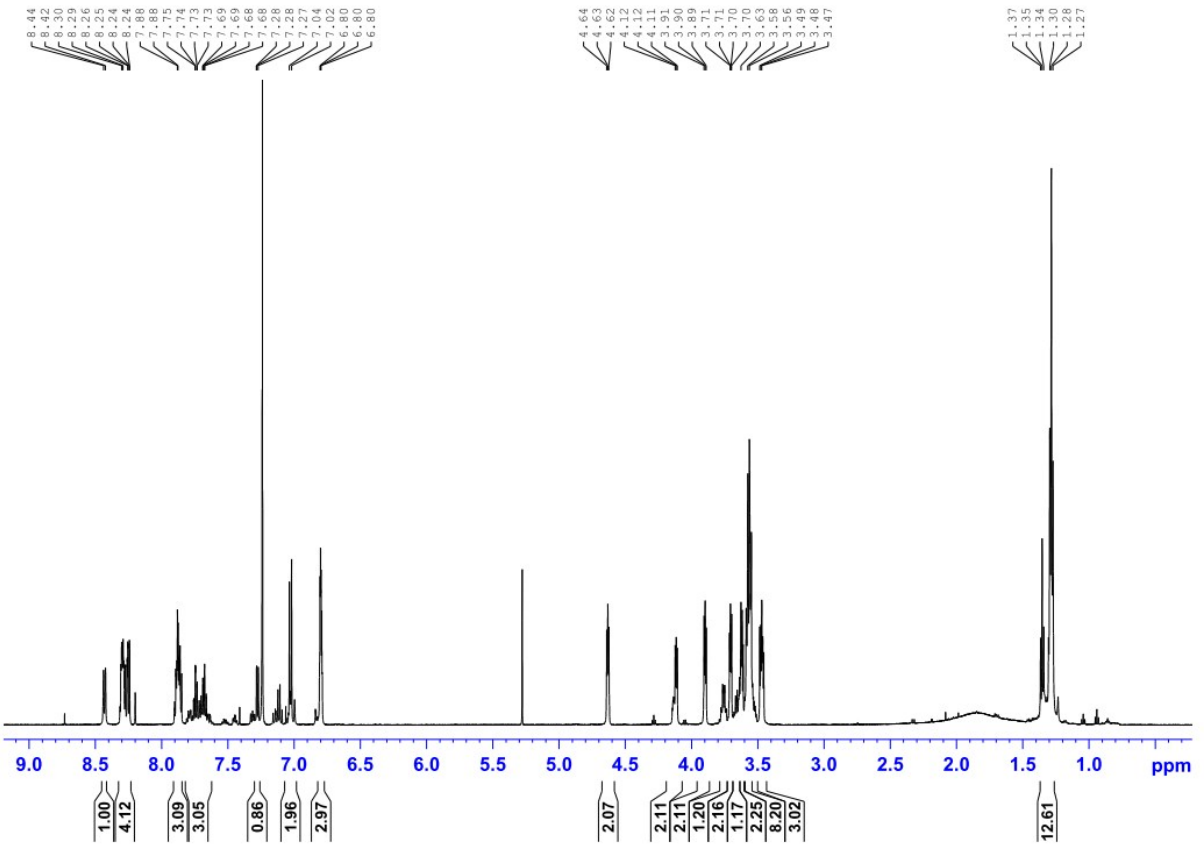
^{13}C NMR spectrum (150 MHz, DMSO-D_6) of **8**.



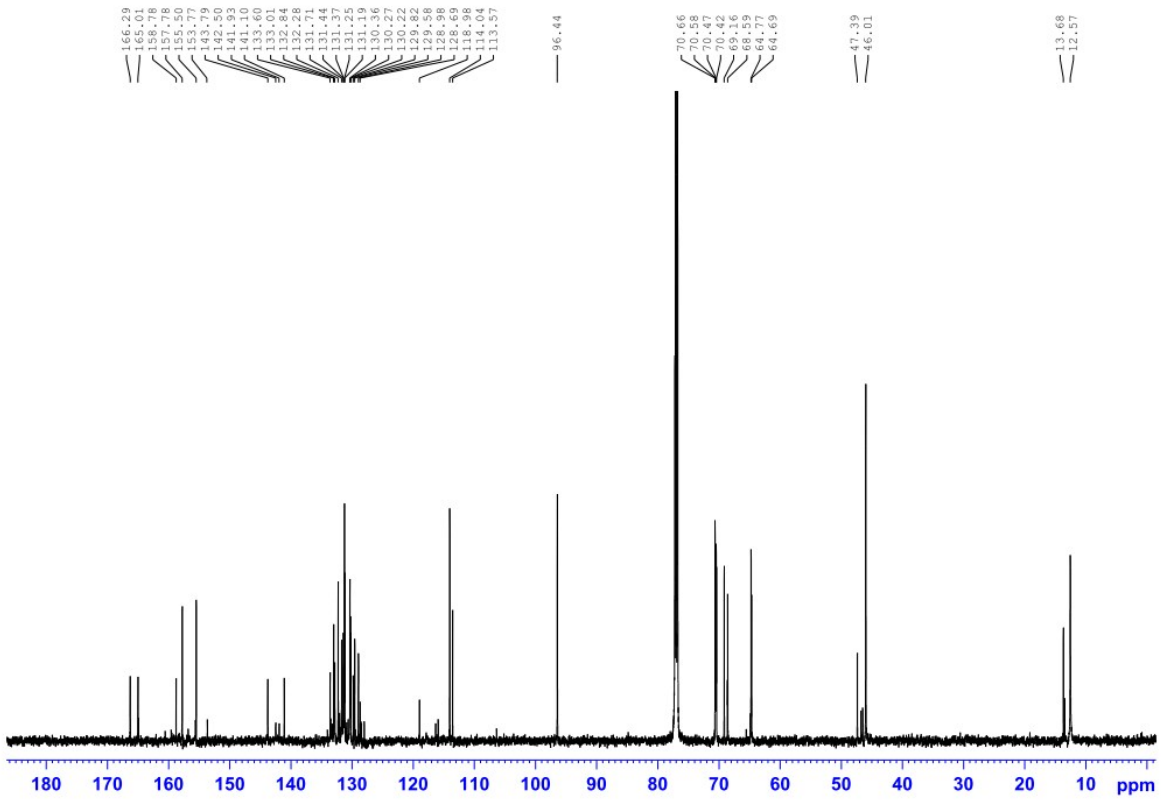
^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **9**.



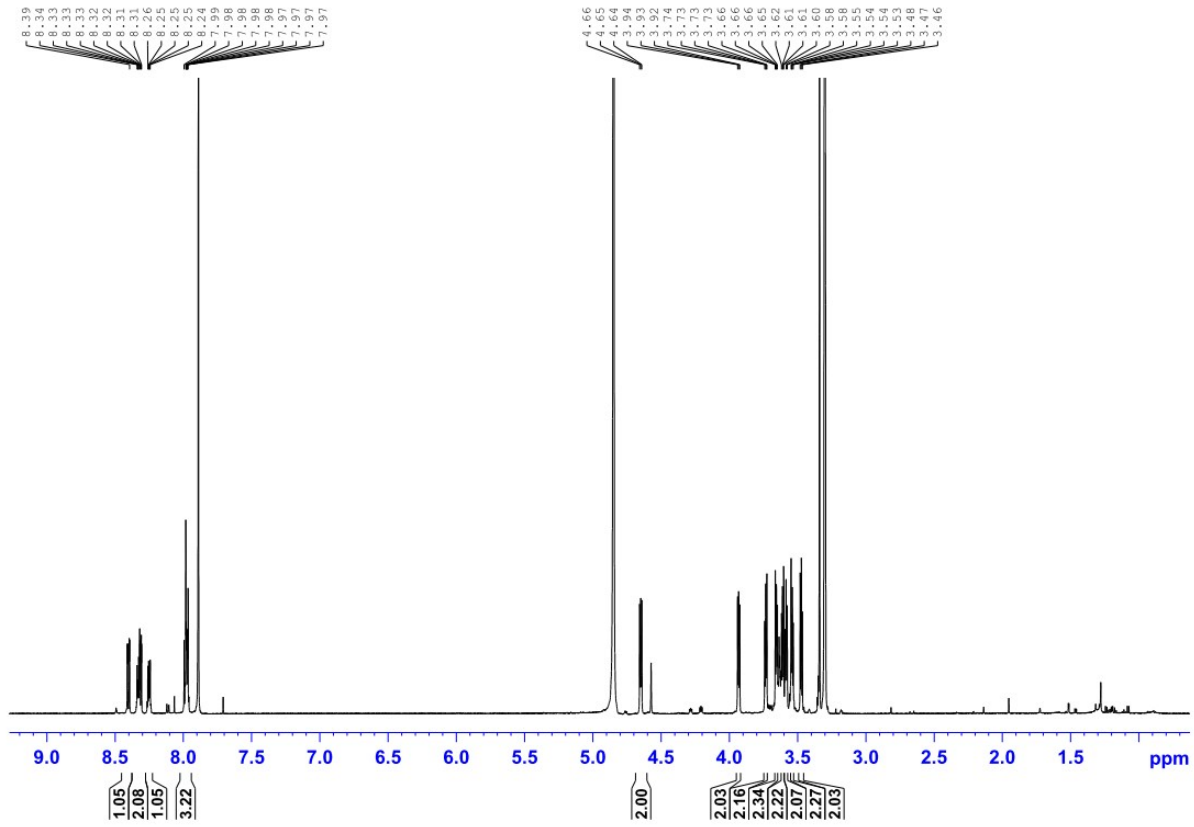
^{13}C NMR (150 MHz, $CDCl_3$) spectrum of **9**.



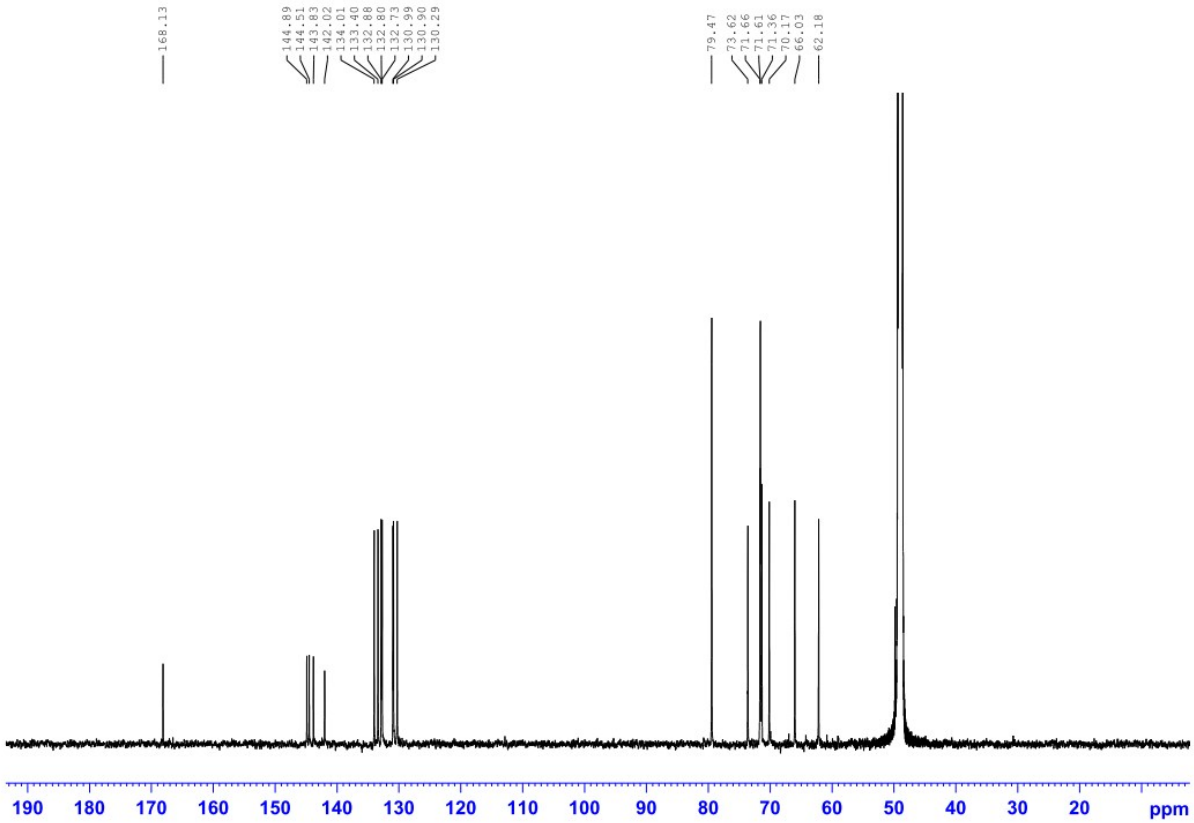
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **10**.



^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **10**.



^{1}H NMR spectrum (600 MHz, MeOD) of **11**.



^{13}C NMR spectrum (150 MHz, MeOD) of **11**.