

Supplemental Material for

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

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Analytical data of compounds 3 and 4

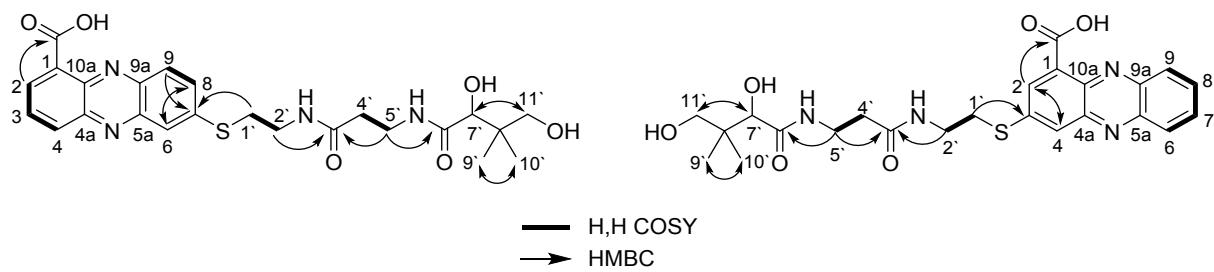


Figure S1: Selected ^1H , ^{13}C HMBC and ^1H , ^1H COSY correlations of panphenazines **3** and **4**.

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

	3		4	
Position	δ_H^a (<i>J</i> in Hz)	δ_C^b	δ_H^a (<i>J</i> 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

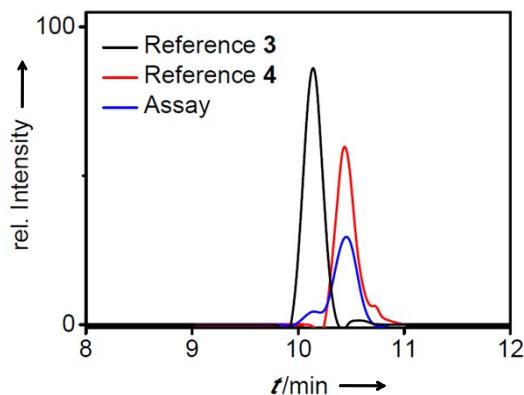


Figure S2. SIM-HRMS ($m/z = 501.1788\text{--}501.1828$) analysis of the obtained product after incubation of PCA and pantetheine with irradiation at 370 nm; comparison with the isolated reference compounds **3** and **4**. SIM: selected ion monitoring.

Coupling of PCA to cysteine and glutathione

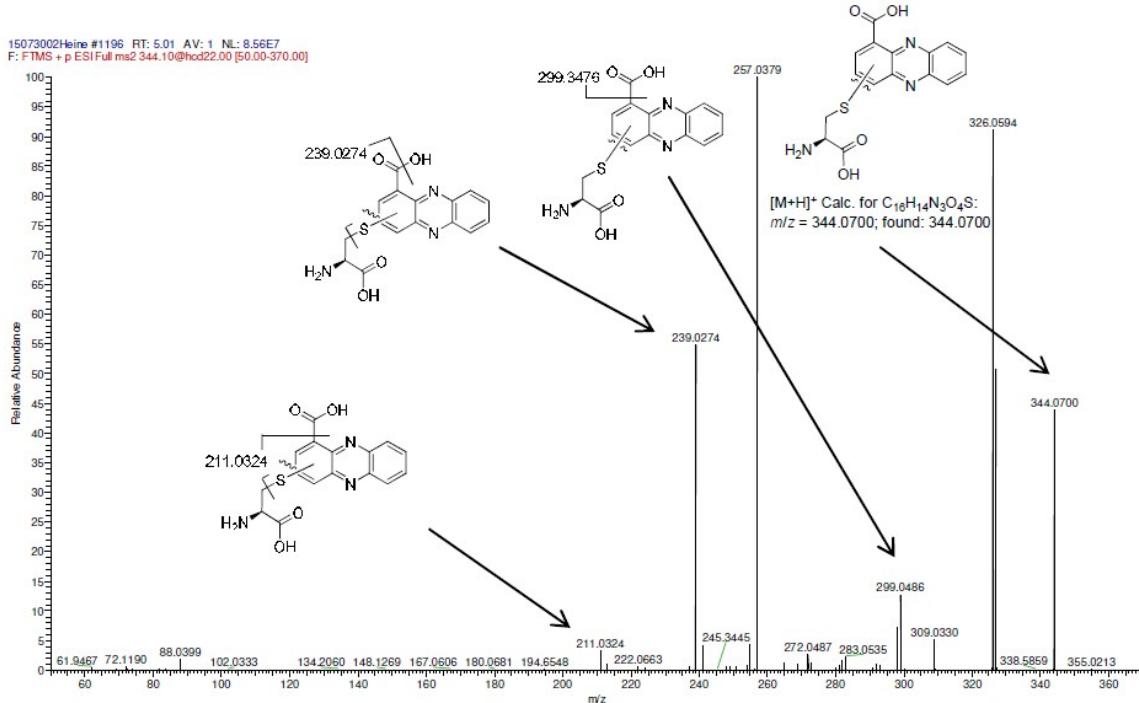


Figure S3. HRMS(/MS) spectrum of PCA-cysteine adduct ($[M+H]^+$) after incubation of PCA with cysteine under sunlight.

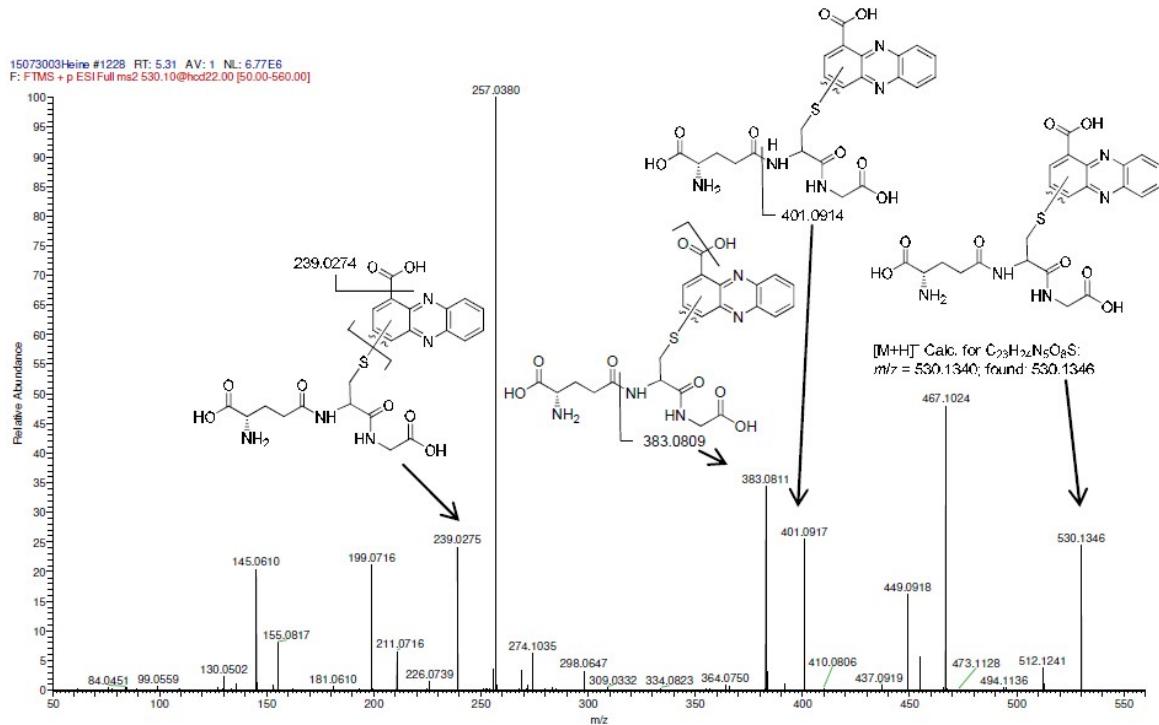


Figure S4. HRMS(/MS) spectrum of PCA-glutathione-adduct ($[M+H]^+$) after incubation of PCA with glutathione under sunlight.

Conjugate formation of PCA with pantetheine in presence of AIBN

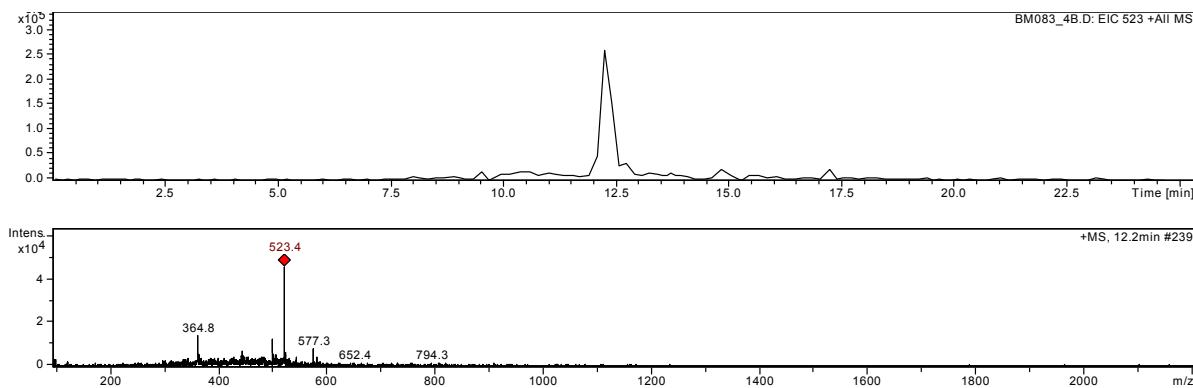


Figure S5. SIM-MS spectrum of the product after incubation of PCA with pantetheine in presence of AIBN in the dark (top). MS spectrum of PCA-pantetheine-adduct ($[M+Na]^+$) (bottom).

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

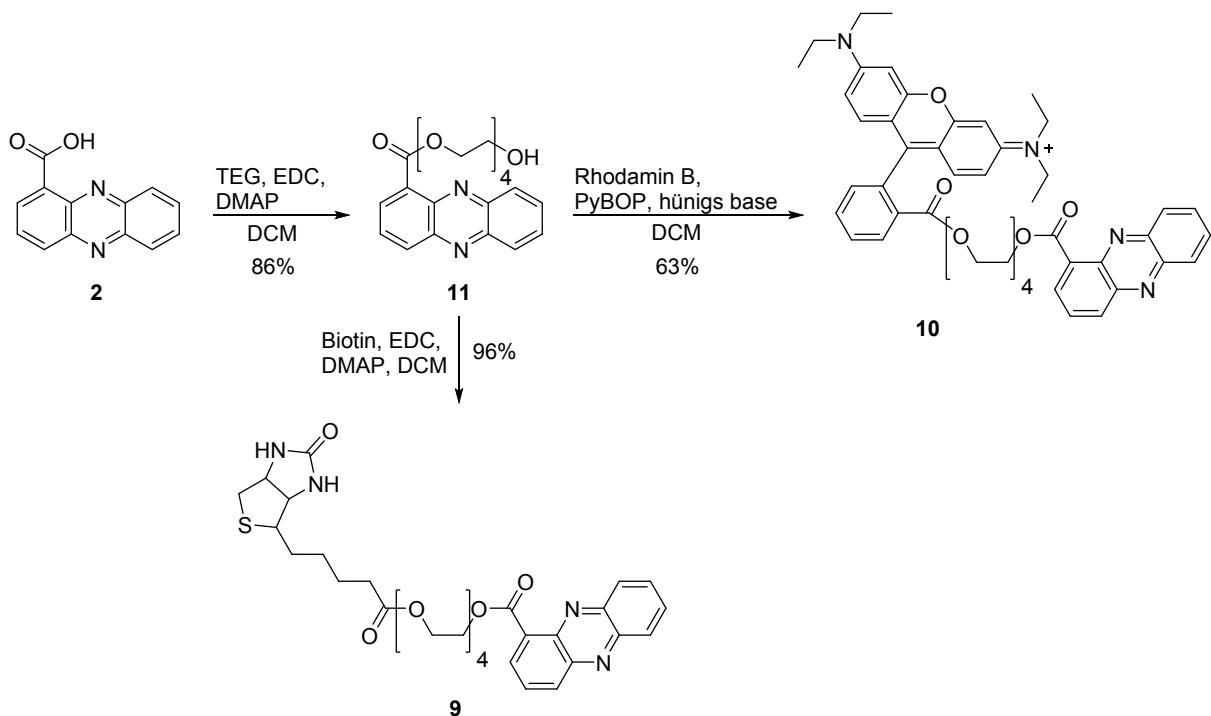


Figure S6: Synthesis of biotin- and rhodamine-B-tagged phenazine probes.

Phenazine-protein binding assays with KS-B

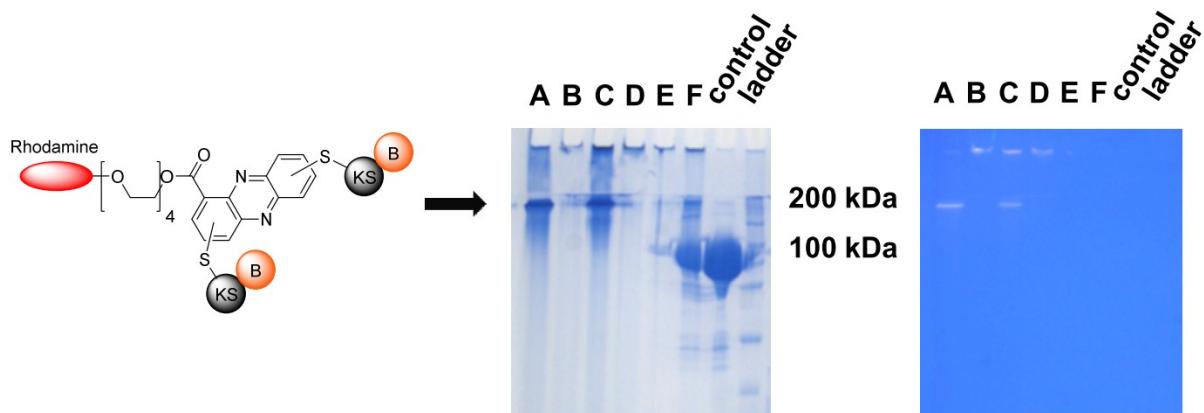


Figure S7. SDS-PAGE (12% gel) after incubation of KS-B with rhodamine-B-labelled phenazine probe **10** under the following conditions: A) $\lambda = 370$ nm, 20 h, in H_2O , B) $\lambda = 370$ nm, 20 h, DMSO, C) sunlight, 8 h, H_2O , D) sunlight, 8 h, DMSO, E) AIBN, 20 h, DMSO, F) AIBN, 20 h, H_2O .

Tryptic mass fingerprinting of labelled KS-B

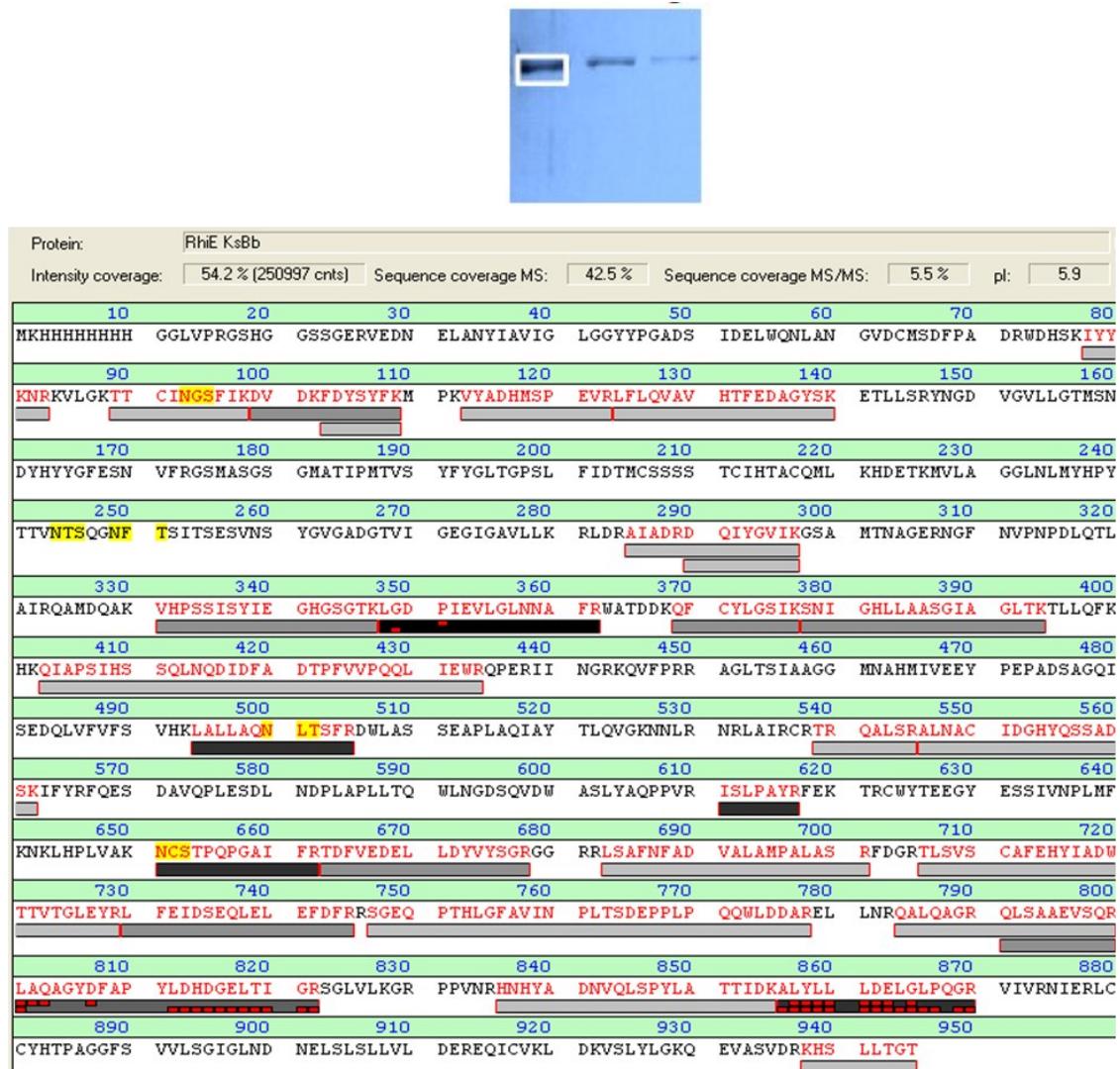
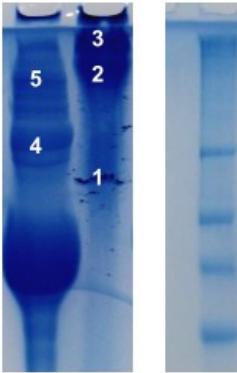


Figure S8.1. Tryptic mass fingerprinting of KS-B after incubation with **9** and AIBN followed by affinity chromatography.



1 Protein: RhoF_KsRh											
Intensity coverage: 49.7 % (2283686 cnts) Sequence coverage MS: 16.3 % Sequence coverage MS+MS: 8.3 % pl: 5.9											
1U	2U	3U	4U	5U	6U	7U	8U				
MKHHHHHHHHHH	GGLVPRGSHG	GSSGERVEDN	ELANYIAVIG	LGGYYPPGADS	IDELCQXLAQ	GVDCMSDFPA	DRWDHSKIIY				
90	100	110	120	130	140	150	160				
KNRKVLGKTT	CINGSFIKDV	DKFDYSYFKM	PKVYADHMSP	EVRLFLQVAV	ETFECLGFSR	ETLLSRYNGD	VGVILLGTMNS				
170	180	190	200	210	220	230	240				
DYHYYGFESN	VFRGSMASGS	GMATIPMTVS	YFYGLTGPSL	FIDTMCSSSE	TCTIEPACQQL	REDETAKVLA	GGNLNMYHPY				
250	260	270	280	290	300	310	320				
TTVNTSQCNF	TSITSESVNS	YVGAGADGTVI	GEGIGAVLLK	RLDRAIAADRC	QIYEWIEGSS	STNAGERNGF	NVPNPDLQTL				
330	340	350	360	370	380	390	400				
AIRQAMDQAK	VHPSSISIYIE	GHGSGTKLGD	PIEVLGLNNAA	FRWATDDKQF	CYLGSIEEQIC	GEELLARSGIA	GLTKTLLQFK				
410	420	430	440	450	460	470	480				
HKQIAPSIHS	SQLNQDIDFA	DTPFVVPQQL	IEWRQPERII	NGRKQVFPPRE	AGLTQDQKGG	ENABEMIVEEY	PEPADSAGQI				
490	500	510	520	530	540	550	560				
SEDQLVVFVFS	VHKLALLAQN	LTSFRDWLAS	SEAPLAQIAY	TLQVGKNNLF	KRLAIECETR	CALSRALNAC	IDGHYQSSAD				
570	580	590	600	610	620	630	640				
SKIFYRFQES	DAVQPLESDL	NDPLAPLLTQ	WLNGDSQVDW	ASLYAQPPVE	I&LFAEEPEER	TCQJYTEEGY	ESSIVNPLMF				
650	660	670	680	690	700	710	720				
KNKLHPLVAK	NCSTPQPGAI	FRTDFVVEDL	LDTVYSGRGG	RRLSAFNFA	VALACEFALS	RFDGRTLSVS	CAFEHYIADW				
730	740	750	760	770	780	790	800				
TTVTGLEYRL	FEIDSEQELEL	EFDFFRSGEQ	PTHLGFAVIN	PLTSDEPPLF	QQGLCCAPEL	INRQALQAGR	QLSAAEVSOR				
810	820	830	840	850	860	870	880				
LAQACGVDFAP	VLHDHGELTT	GRSCLVLKGR	PPVNRHNHYA	DNVQLSPVLA	TTTCFEPFTR	QDQVQDQGR	VTVRNTERIC				
890	900	910	920	930	940	950					
CYHTPAGCFS	VVLSCICLND	NELSLSSLVL	DEREOTCVKL	DKVSLYLCKO	EVAEVDCRRRS	SDCTCT					

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

2

Protein: RhoE_KsBb									
Intensity coverage:		Sequence coverage MS:		Sequence coverage MS/MS:		pl:			
10	20	30	40	50	60	70	80		
MKHHHHHHHHH	GGLVPRGSHG	GSSGERVEDN	ELANYIAVIG	LGGYYPGADS	IDELWQNLAN	GVDCMSDFPA	DRWDHSKIVY		
90	100	110	120	130	140	150	160		
KNRKVVLGKTT	CINGOFIKDV	DKFDYSYFKM	PKVYADHMSP	EVRLFLQVAV	HTFEDAGYSK	ETLLSRYNGD	VGVLGGTMSN		
170	180	190	200	210	220	230	240		
DTHYYGFESN	VFRGSMASGS	GMATIPMTVS	YFYGLTGPSL	FIDTMCS333	TCIHTACQML	KHDETKMVLA	GGLNLMHYHPY		
250	260	270	280	290	300	310	320		
TTVNNTSQGNF	TSITSESVNS	YGVGADGTVI	GEGIGAVLLK	RLDRAIADRD	OIYGVIKGSA	MTNAGERNGF	NVPNPDLQTL		
330	340	350	360	370	380	390	400		
AIRQAMDQAK	VHPSSISYIE	GHGSGTKLGD	PIEVGLNNNA	FRUATDDKQF	CYLGSIKSNI	GHLLAASGIA	GLTKTLLQFK		
410	420	430	440	450	460	470	480		
HKQIAPSIHS	SQLNQDIDFA	DTPFVVVPQLL	IEWRQPERII	NGRKQVFPFR	AGLTS1AAGG	MNAHMIIVEY	PEPADSAGQI		
490	500	510	520	530	540	550	560		
SEDQLVVFVFS	VHKLALLAQN	LTSFRDULAS	SEAPLAQIAY	TLQVGRNNLR	NRLAIRCRTR	QALSRALNAC	IDGHYQSSAD		
570	580	590	600	610	620	630	640		
SKIFYRFQES	DAVQPLESDL	NDPLAPLLTQ	WLNGDSQVDW	ASLYAQPPV	ISLPLAYRFEK	TRCWYTYEEGY	ESSIVNPLMF		
650	660	670	680	690	700	710	720		
KNKLHPLVAK	NC8TPQPGAI	FRTDFVEDEL	LDYVYSGRGG	RRLSAFNAD	VALAMPALAS	RFDGRTLSVS	CAFEHYIADW		
730	740	750	760	770	780	790	800		
TTVTGLEYRL	FEIDSEOLEL	EFDFFRSGEQ	PTHLGFAVIN	PLTSDEPPLP	OOWLDDAREL	LNROALOAGR	OLSAAEVSOR		
810	820	830	840	850	860	870	880		
LAQAGYDFAP	YLDHDGELTI	GRSGLVLUKG	PPVNRHNHYA	DNVQLSPYLA	TTIDKALVLL	LDELGLPQGR	VIVRNIEPLC		
890	900	910	920	930	940	950			
CYHTPAGGFS	VVLSCICLND	NELSLSSLVL	DEREQICVKL	DKVSLYLCRQ	EVASVDRKHS	LLTGT			

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

3

Protein: RhoE_KsBb									
Intensity coverage:		Sequence coverage MS:		Sequence coverage MS/MS:		pl:			
10	20	30	40	50	60	70	80		
MKHHHHHHHHH	GGLVPRGSHG	GSSGERVEDN	ELANYIAVIG	LGGYYPGADS	IDELWQNLAN	GVDCMSDFPA	DRWDHSKIVY		
90	100	110	120	130	140	150	160		
KNRKVVLGKTT	CINGOFIKDV	DKFDYSYFKM	PKVYADHMSP	EVRLFLQVAV	HTFEDAGYSK	ETLLSRYNGD	VGVLGGTMSN		
170	180	190	200	210	220	230	240		
DTHYYGFESN	VFRGSMASGS	GMATIPMTVS	YFYGLTGPSL	FIDTMCS333	TCIHTACQML	KHDETKMVLA	GGLNLMHYHPY		
250	260	270	280	290	300	310	320		
TTVNNTSQGNF	TSITSESVNS	YGVGADGTVI	GEGIGAVLLK	RLDRAIADRD	OIYGVIKGSA	MTNAGERNGF	NVPNPDLQTL		
330	340	350	360	370	380	390	400		
AIRQAMDQAK	VHPSSISYIE	GHGSGTKLGD	PIEVGLNNNA	FRUATDDKQF	CYLGSIKSNI	GHLLAASGIA	GLTKTLLQFK		
410	420	430	440	450	460	470	480		
HKQIAPSIHS	SQLNQDIDFA	DTPFVVVPQLL	IEWRQPERII	NGRKQVFPFR	AGLTS1AAGG	MNAHMIIVEY	PEPADSAGQI		
490	500	510	520	530	540	550	560		
SEDQLVVFVFS	VHKLALLAQN	LTSFRDULAS	SEAPLAQIAY	TLQVGRNNLR	NRLAIRCRTR	QALSRALNAC	IDGHYQSSAD		
570	580	590	600	610	620	630	640		
SKIFYRFQES	DAVQPLESDL	NDPLAPLLTQ	WLNGDSQVDW	ASLYAQPPV	ISLPLAYRFEK	TRCWYTYEEGY	ESSIVNPLMF		
650	660	670	680	690	700	710	720		
KNKLHPLVAK	NC8TPQPGAI	FRTDFVEDEL	LDYVYSGRGG	RRLSAFNAD	VALAMPALAS	RFDGRTLSVS	CAFEHYIADW		
730	740	750	760	770	780	790	800		
TTVTGLEYRL	FEIDSEOLEL	EFDFFRSGEQ	PTHLGFAVIN	PLTSDEPPLP	QQWLDDAREL	LNROALOAGR	QLSAAEVSOR		
810	820	830	840	850	860	870	880		
LAQAGYDFAP	YLDHDGELTI	GRSGLVLUKG	PPVNRHNHYA	DNVQLSPYLA	TTIDKALVLL	LDELGLPQGR	VIVRNIEPLC		
890	900	910	920	930	940	950			
CYHTPAGGFS	VVLSCICLND	NELSLSSLVL	DEREQICVKL	DKVSLYLCRQ	EVASVDRKHS	LLTGT			

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

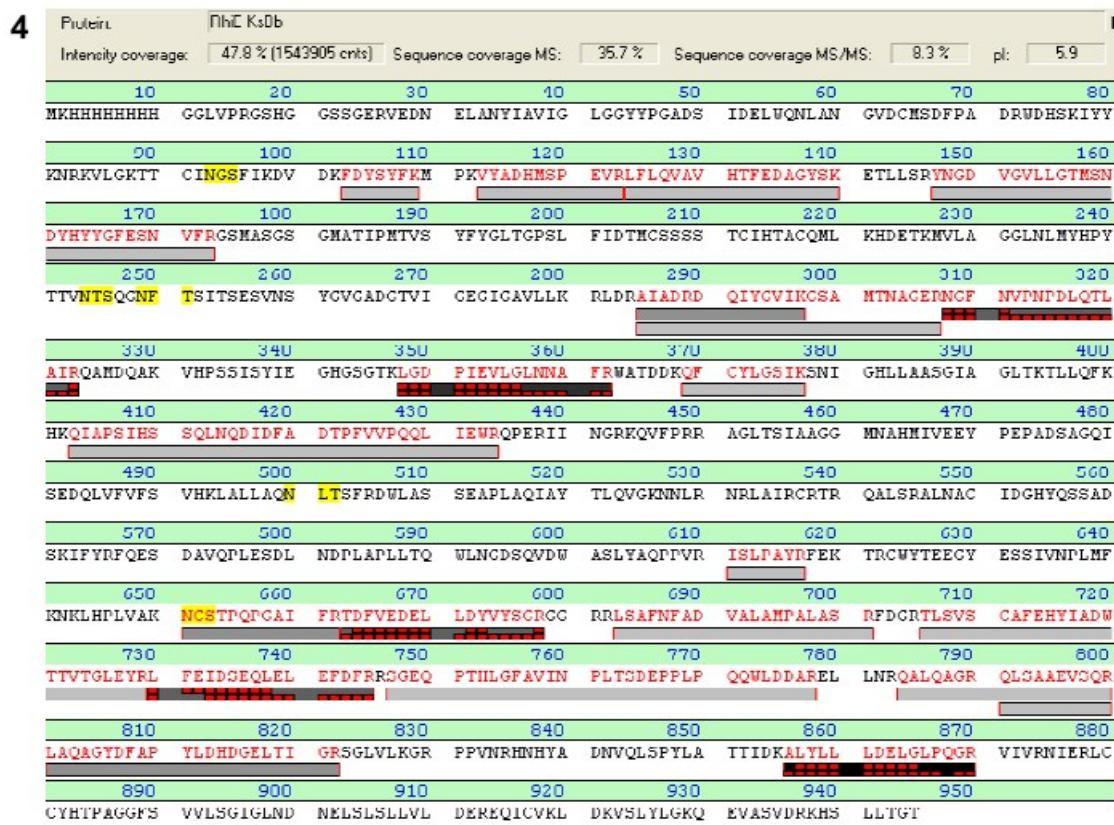


Figure S8.5. Tryptic mass fingerprinting of KS-B after crosslinking with **10** under UV light (1).



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

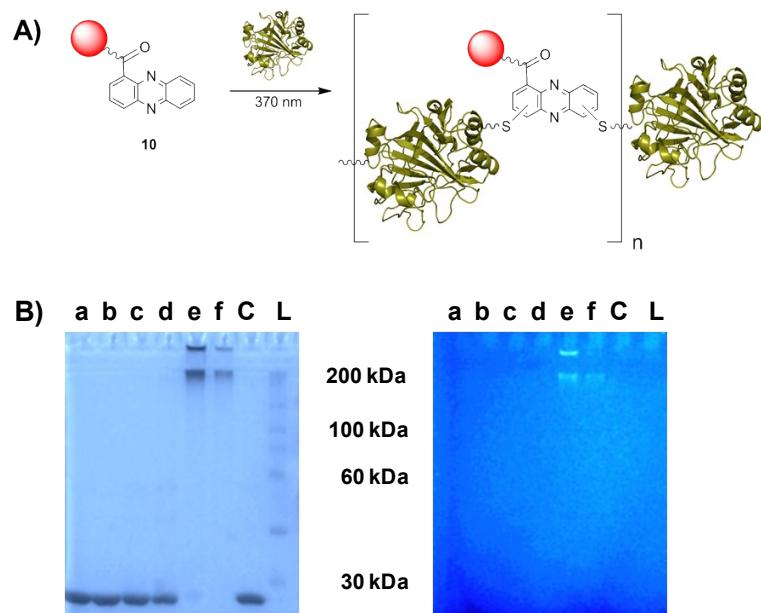


Figure S9. A) Scheme for the functionalization of carbonic anhydrase III by probe **10** after irradiation with UV-light. B) SDS-PAGE (10%) of carbonic anhydrase III after the incubation with probe **10** or rhodamine B in water; a) rhodamine B, 370 nm, 10 h; b) **10**, 370 nm, 15 min; c) **10**, 370 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.

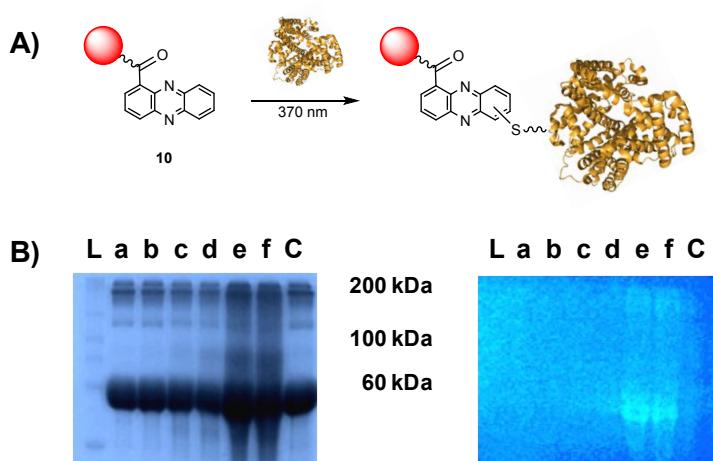


Figure S10. SDS-PAGE (10%) of albumin after the incubation with probe **10** or rhodamine B in water (left picture: under white light; right picture at 370 nm); L, Ladder, a) rhodamine B, 370 nm, 10 h; b) **10**, 370 nm, 15 min; c) **10**, 370 nm, 30 min; d) **10**, 370 nm, 1 h; e) **10**,

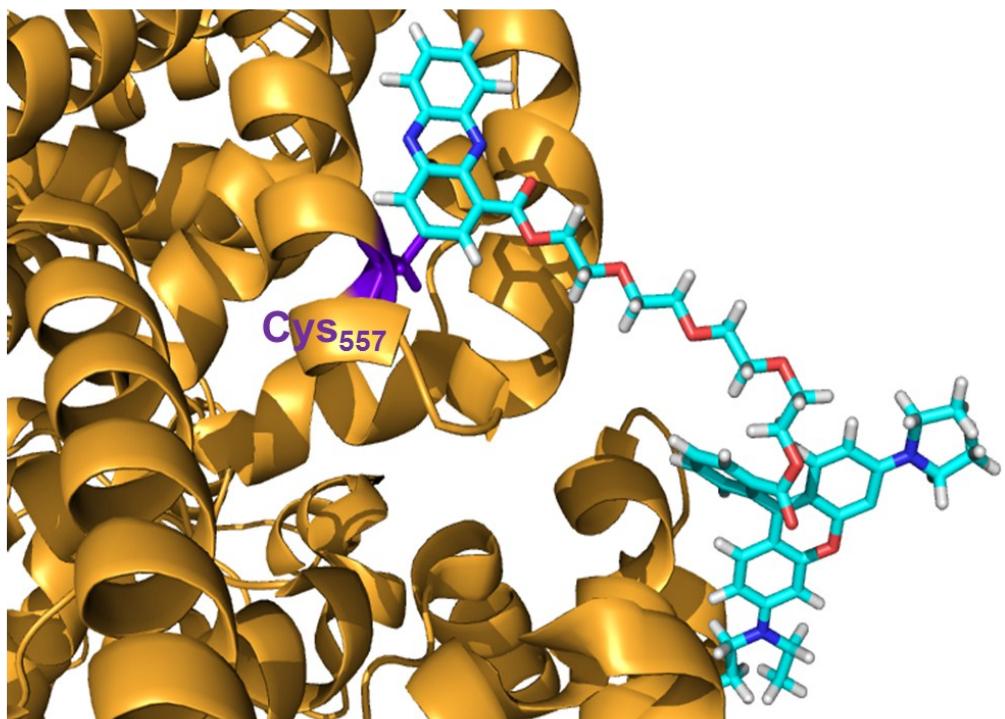
370 nm, 5 h; f) **10**, 370 nm, 10 h, C) albumin control. The fluorescent band potentially corresponds to a 70 kDa monoadduct of **10** and albumin.

Tryptic mass fingerprinting of labelled albumin



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

A)



B)

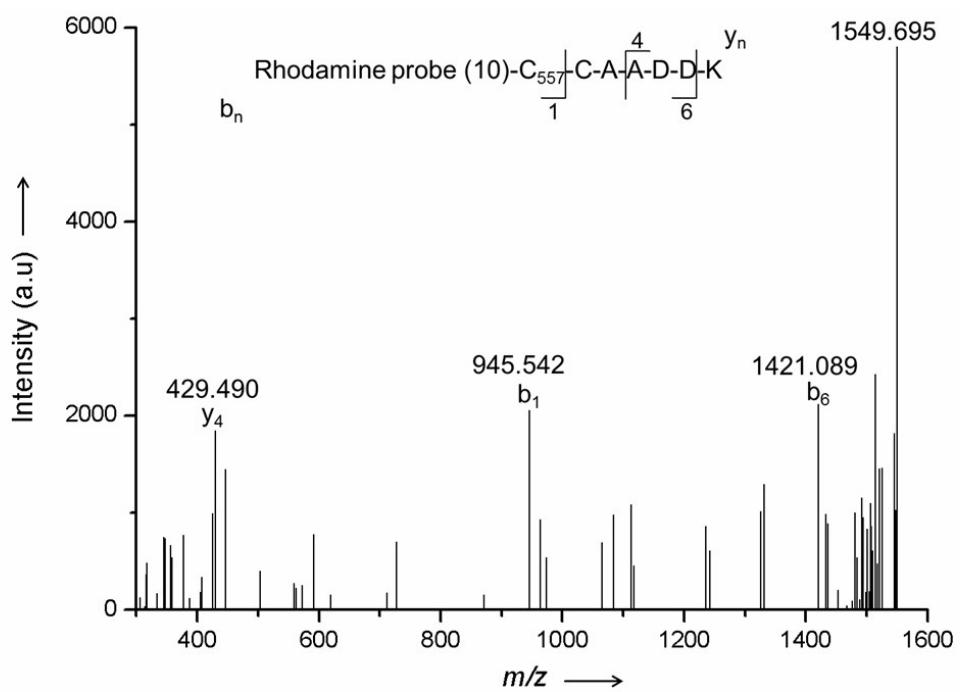


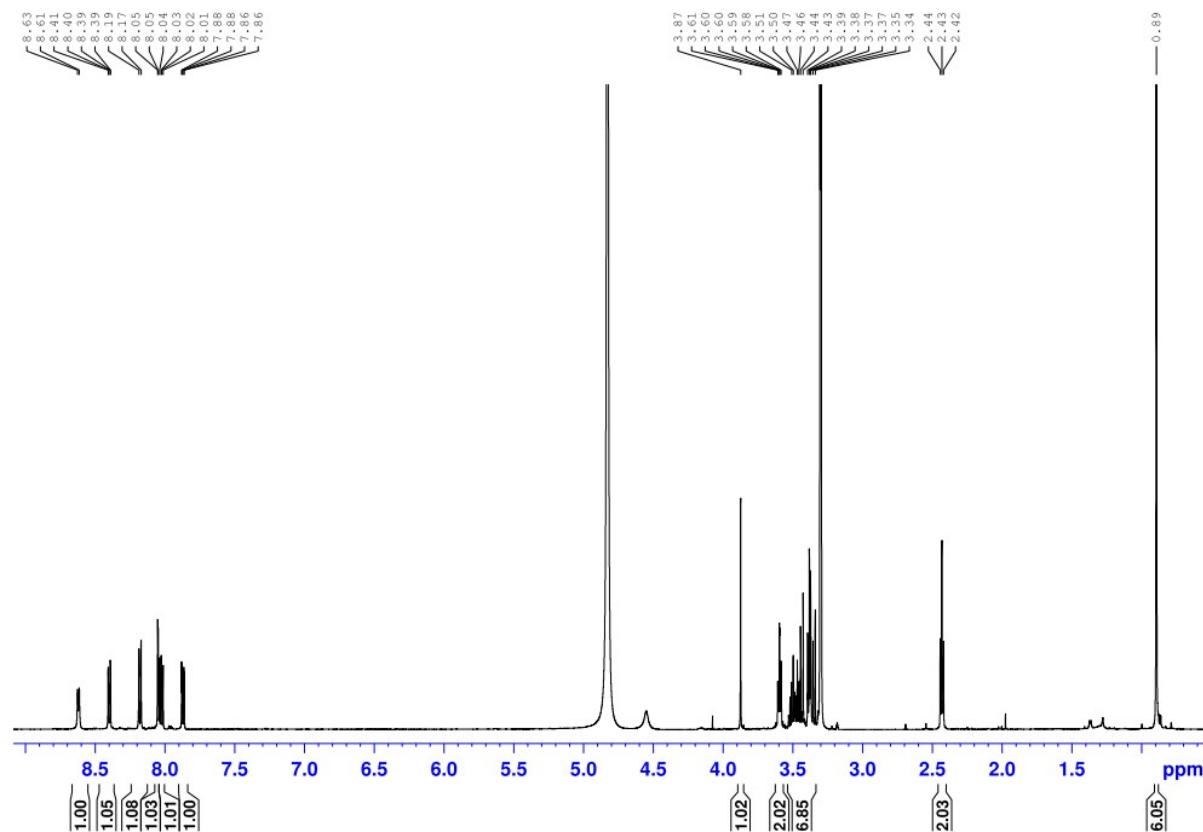
Figure S12. A) Model of the rhodamine-based probe (**10**) crosslinked to the Cys₅₅₇ of albumin under UV light (PDB code 4F5S) B) MALDI-MS/MS spectrum of the rhodamine-based probe (**10**)-linked peptide of albumin.

Antimicrobial properties of isolated phenazines **3** and **4**

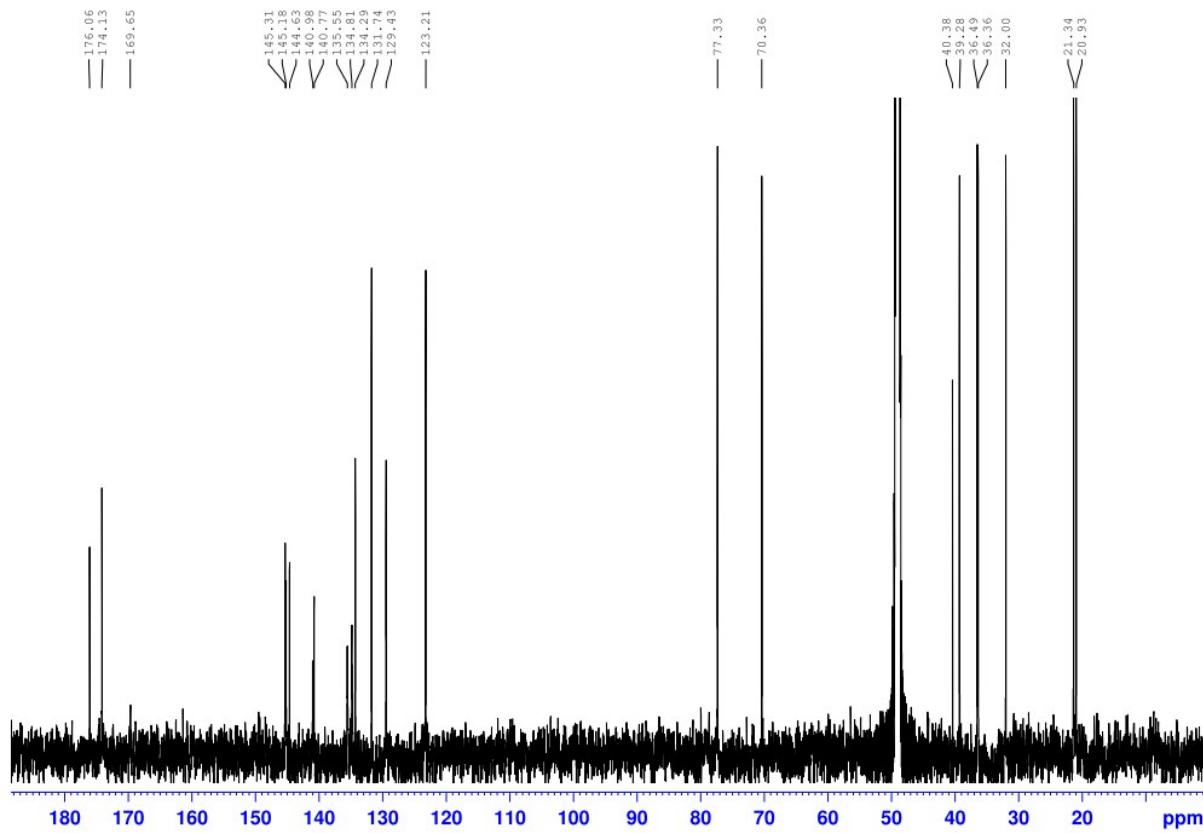
Cpd.	Inhibition zone (mm)									
	<i>B. sub</i>	<i>S. aur</i>	<i>E. col</i>	<i>P. aer</i>	MRSA	VRE	<i>M. vac</i>	<i>S. sal</i>	<i>C. alb</i>	<i>P. not</i>
3	0	0	17	0	0	15	13	20	0	0
4	10	0	0	15	0	0	13	0	0	0
PCA (2)	31	25	32	0	20	0	27	20	35	16

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**).

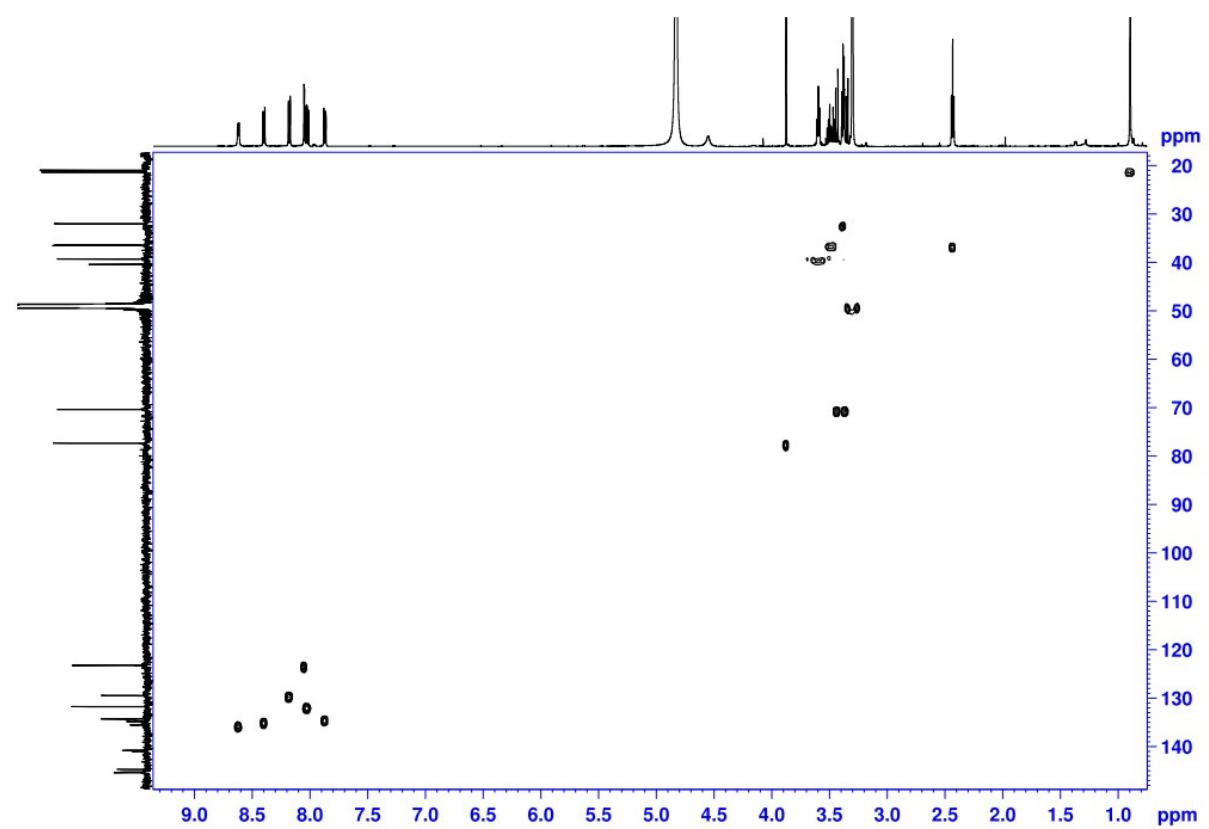
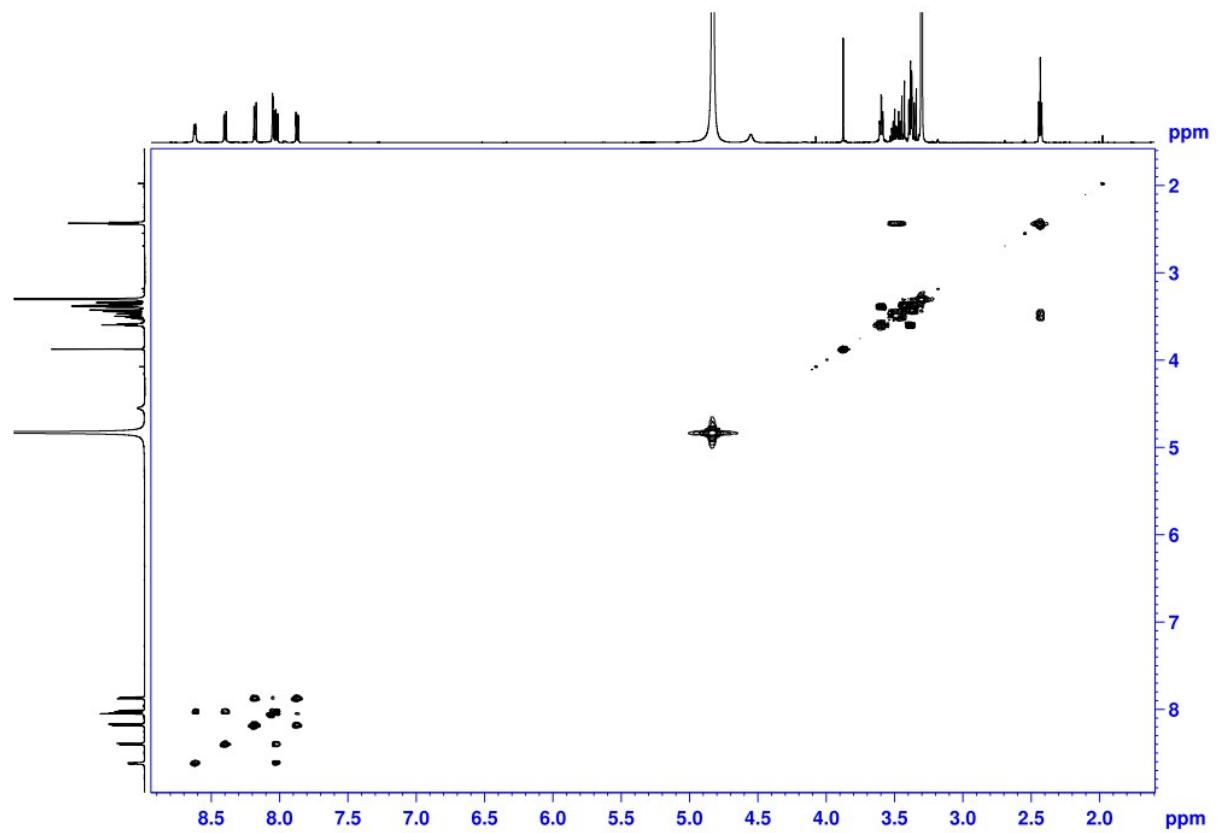
NMR Spectra



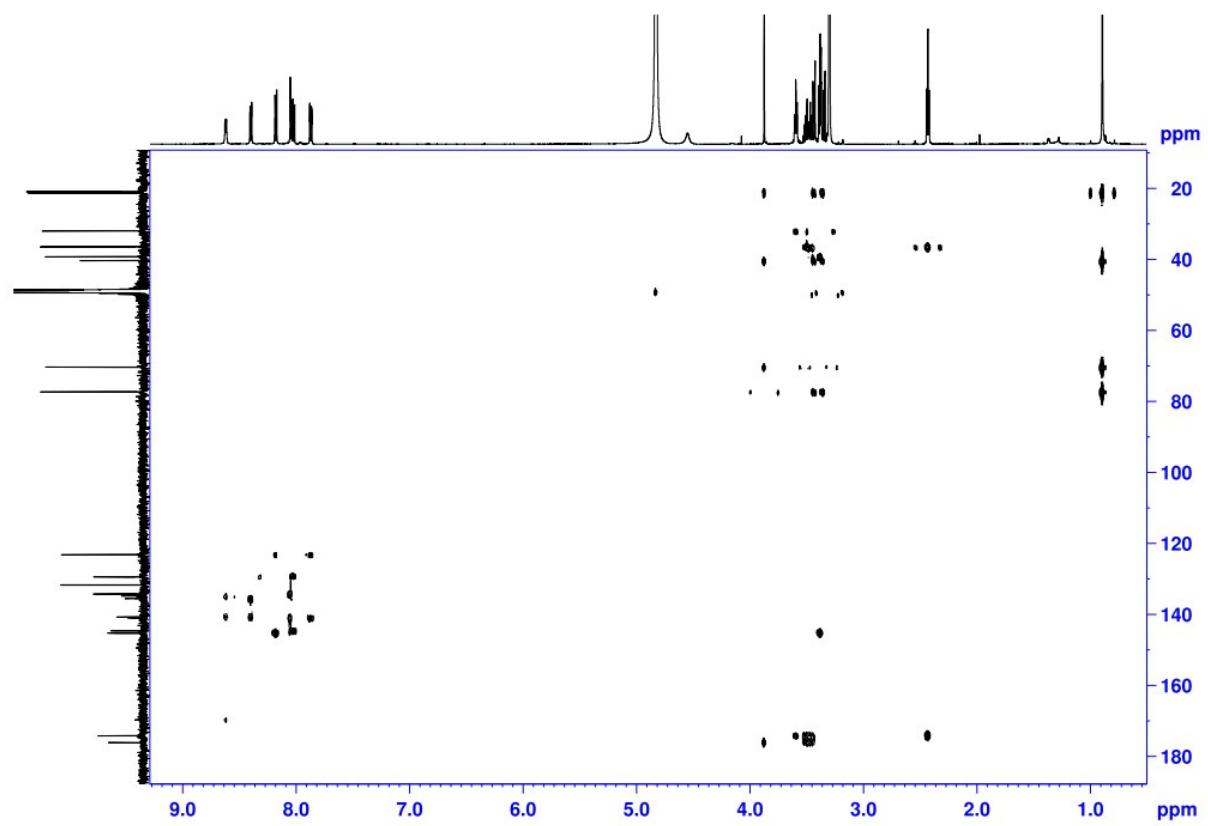
¹H NMR spectrum (600 MHz, MeOD) of **3**.



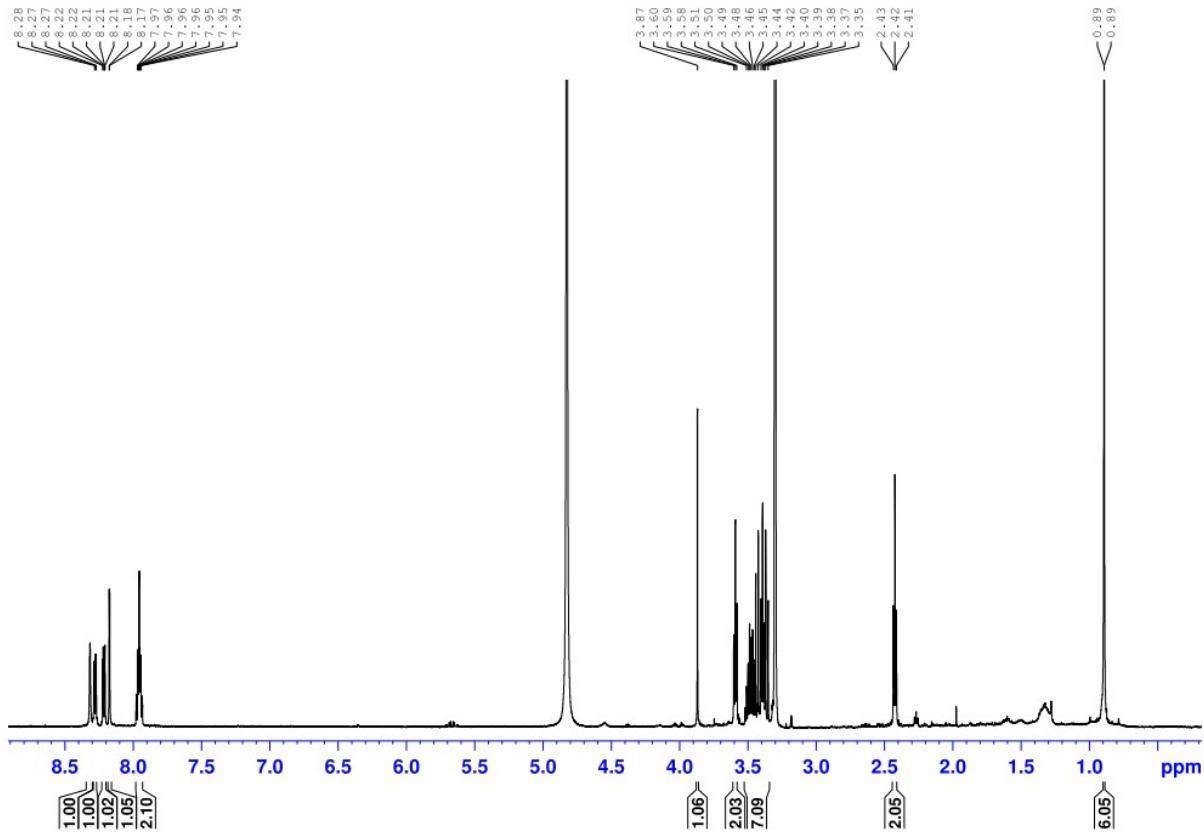
¹³C NMR spectrum (150 MHz, MeOD) of **3**.



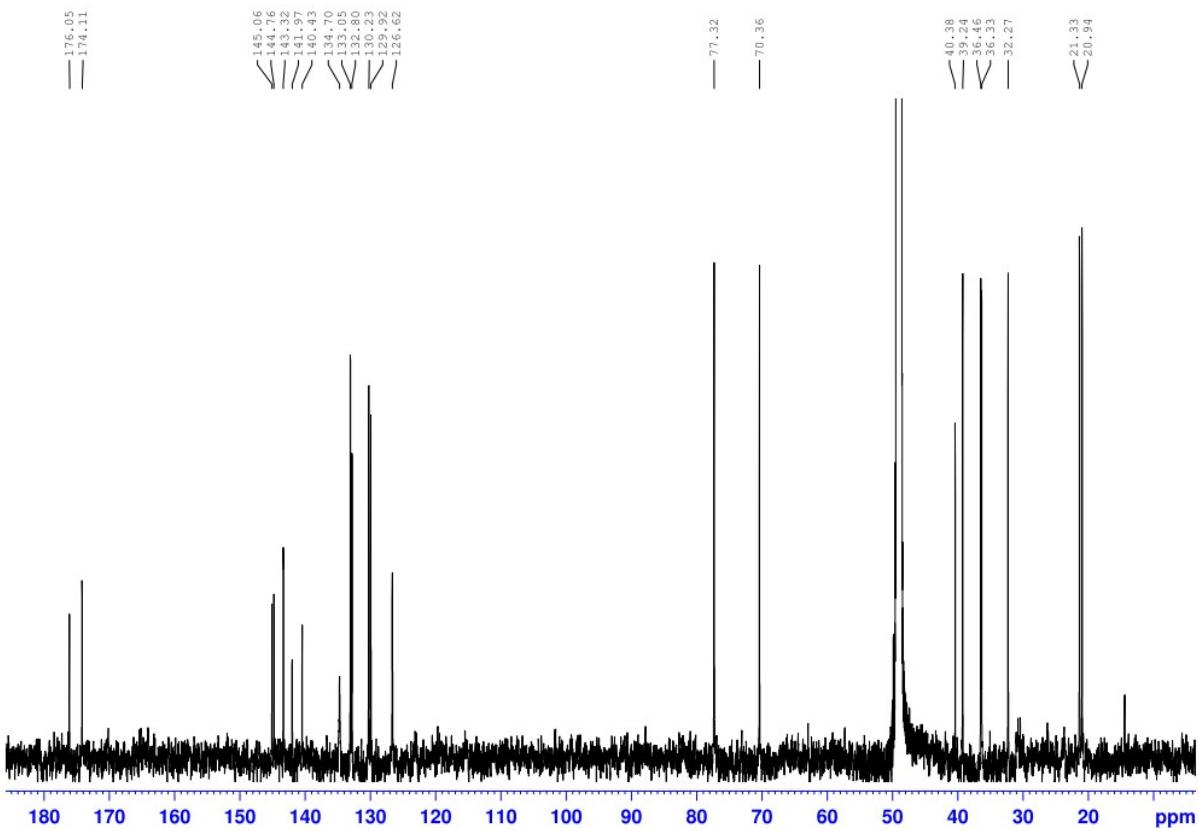
COSY and HSQC spectra of compound 3.



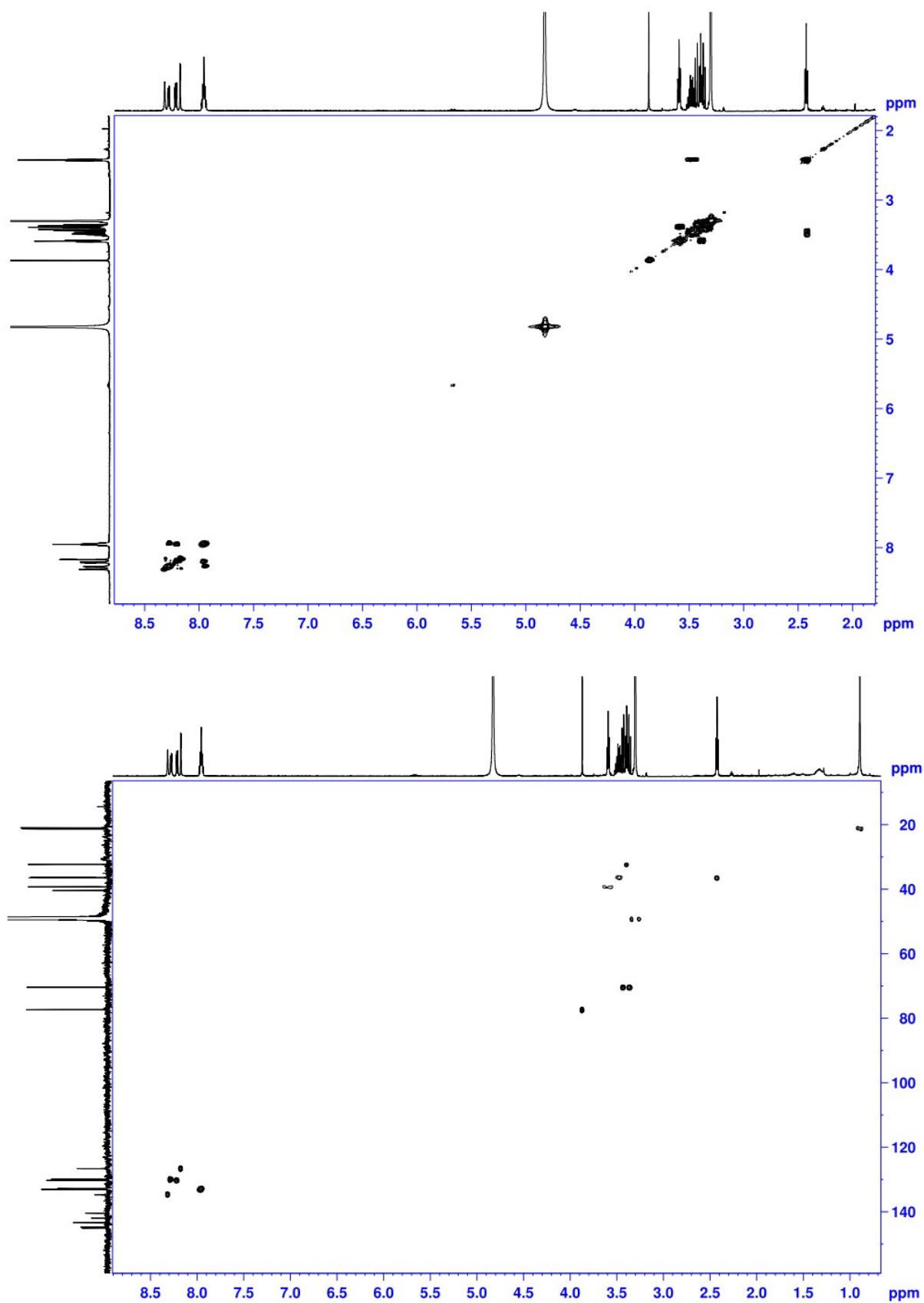
HMBC spectrum of compound **3**.



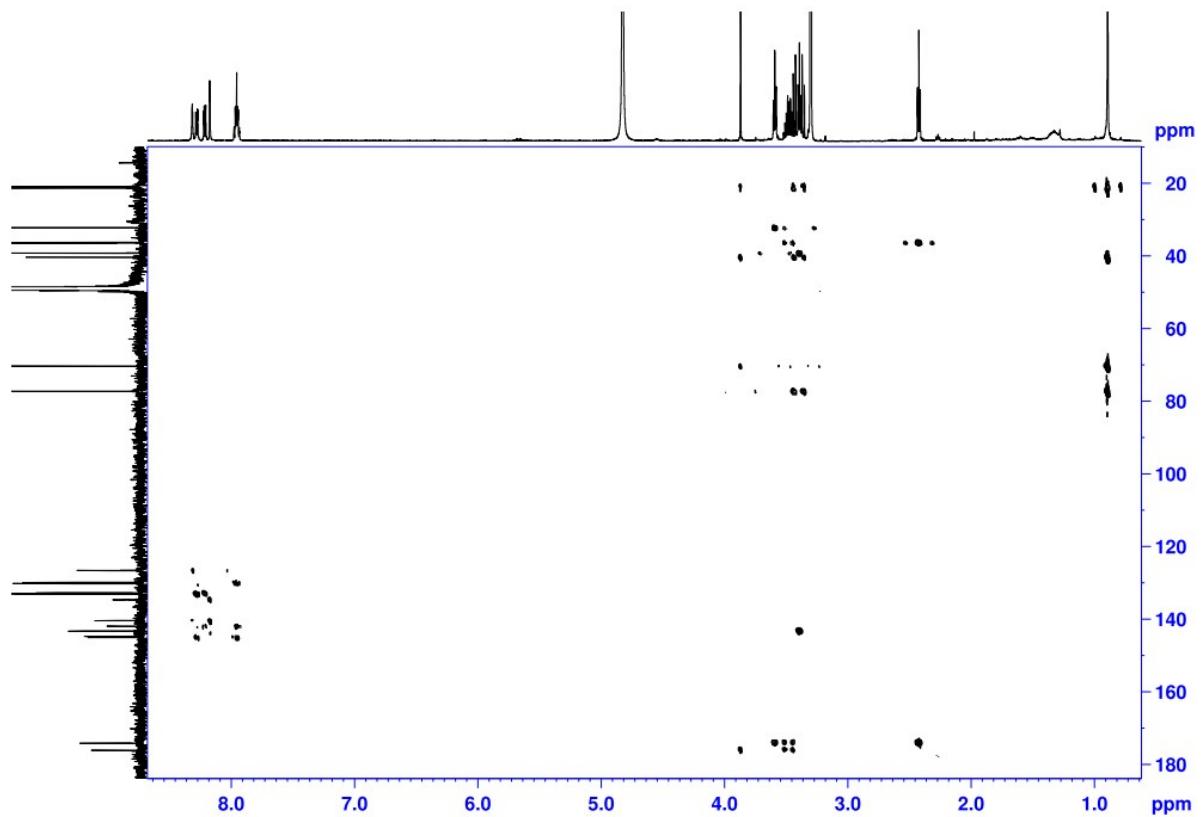
^1H NMR spectrum (600 MHz, MeOD) of **4**.



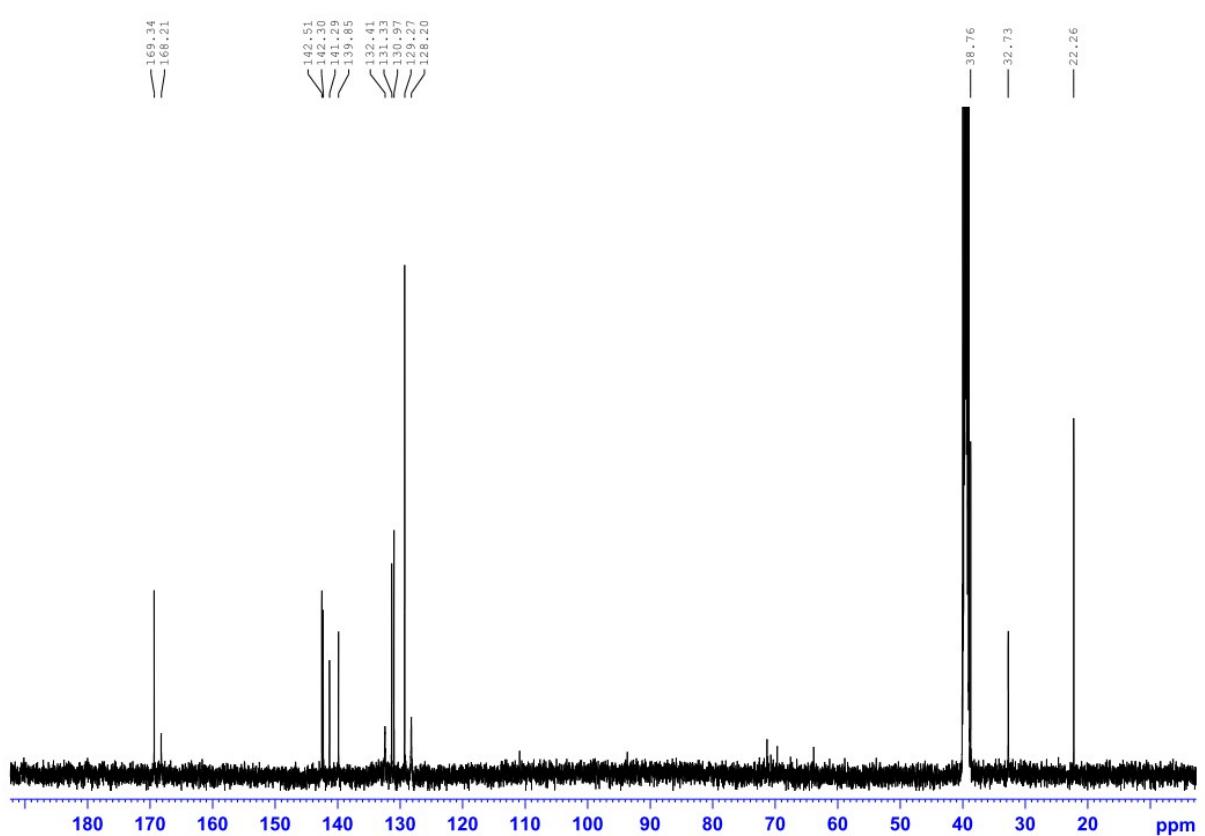
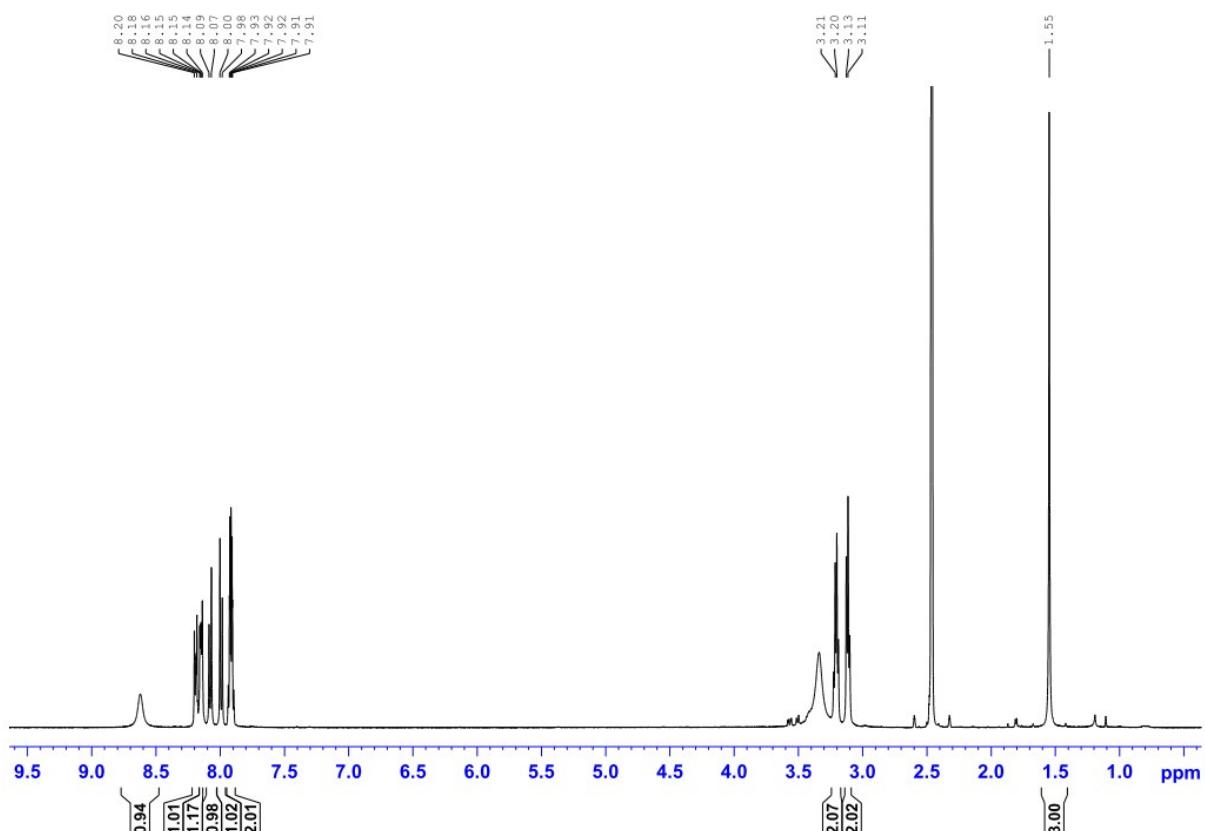
^{13}C NMR spectrum (150 MHz, MeOD) of **4**.



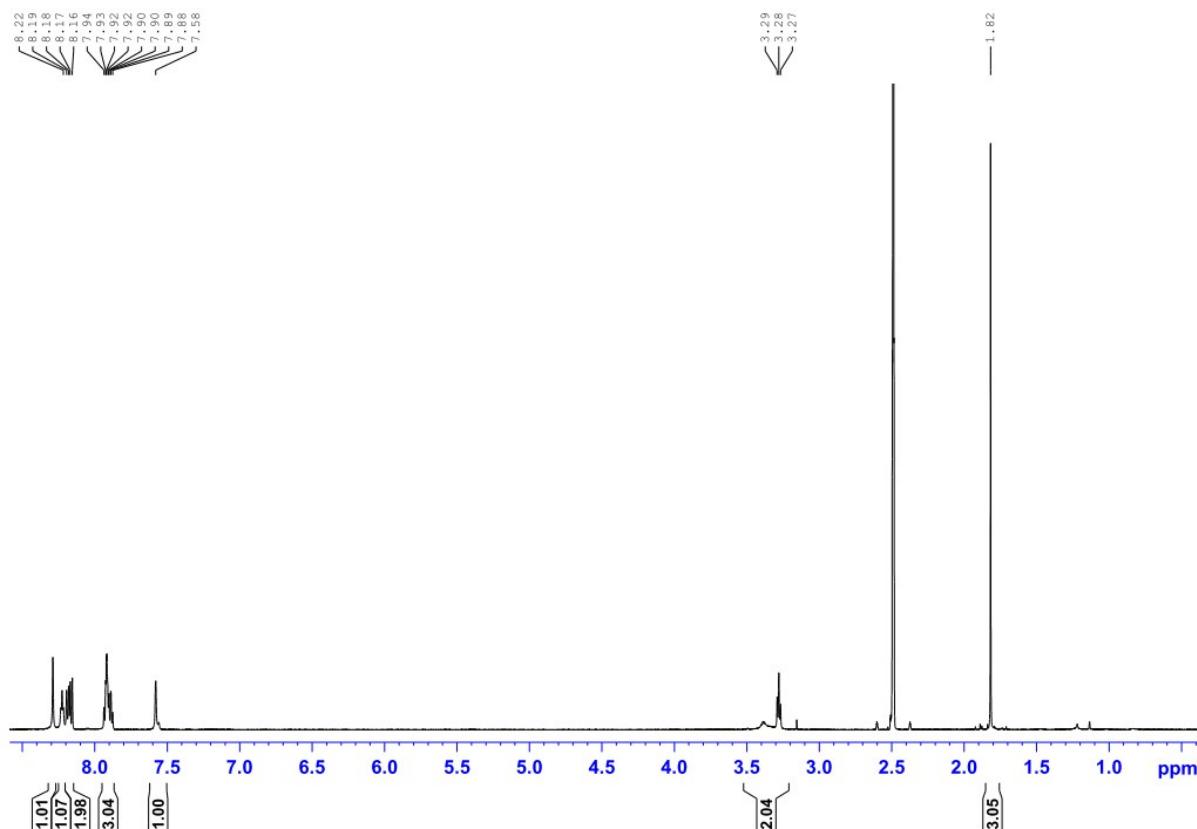
COSY and HSQC spectra of compound 4.



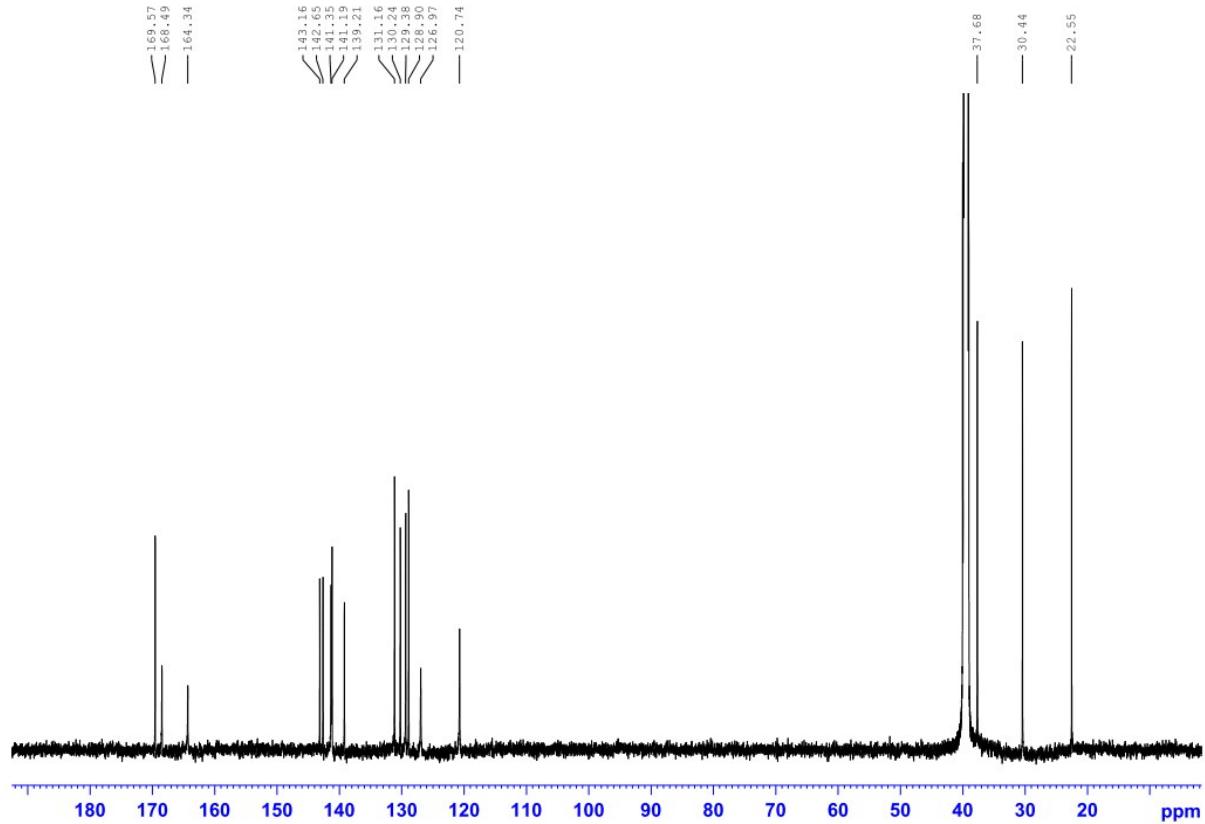
HMBC spectrum of compound **4**.



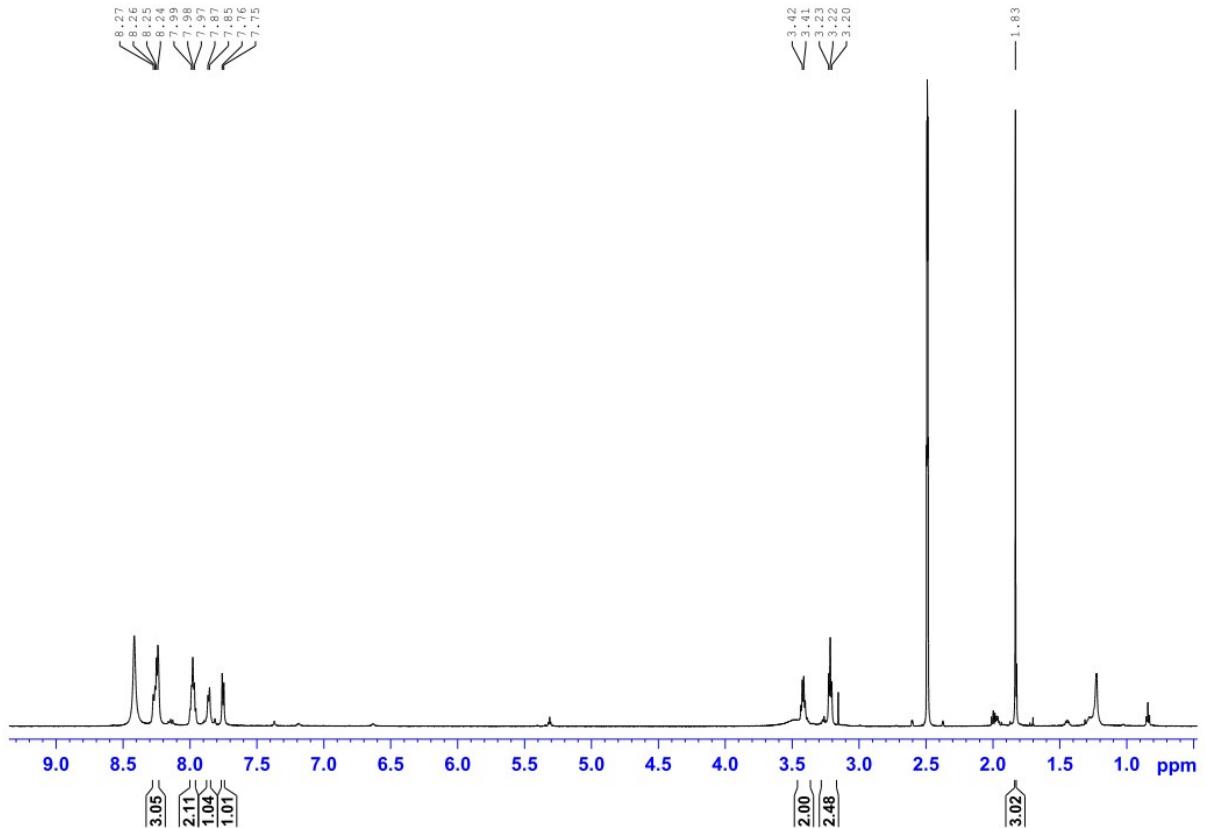
^{13}C NMR spectrum (125 MHz, DMSO- D_6) of **5**.



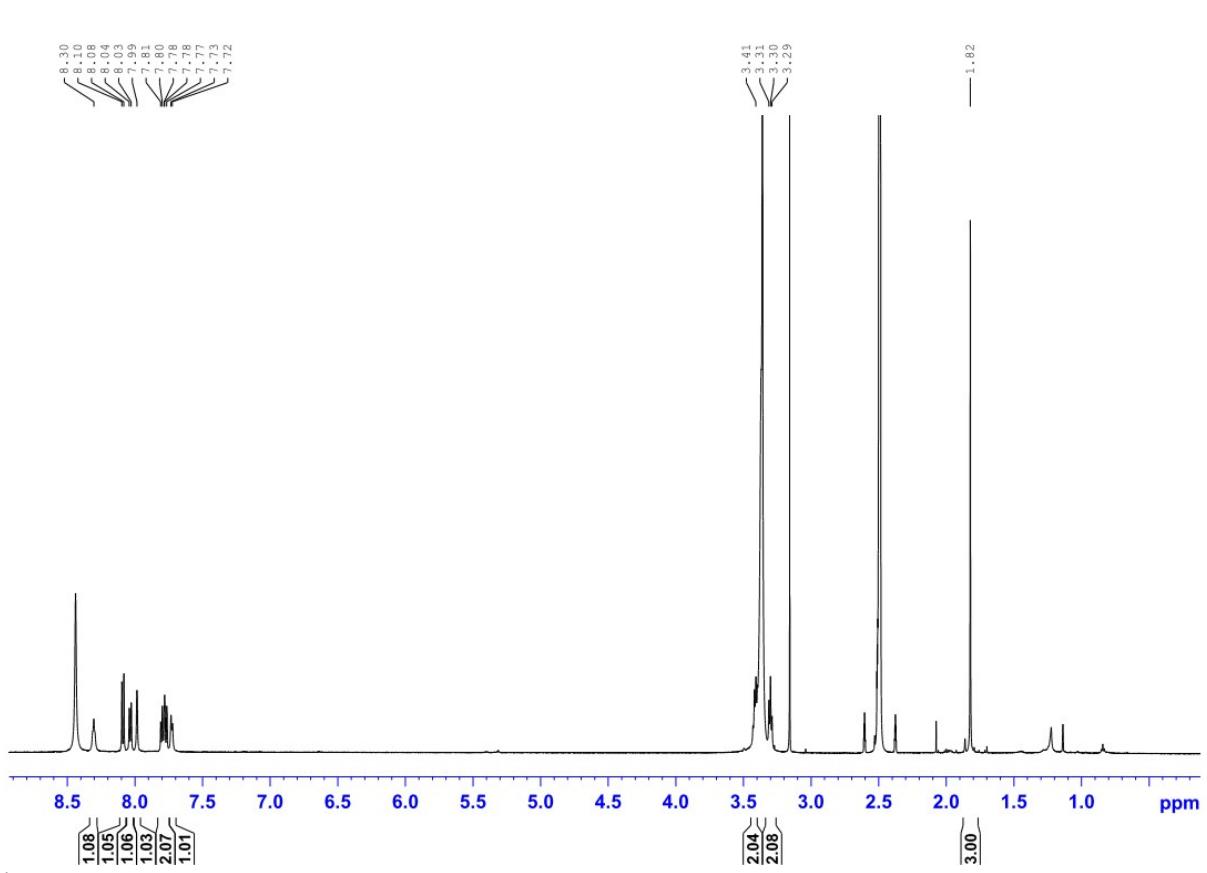
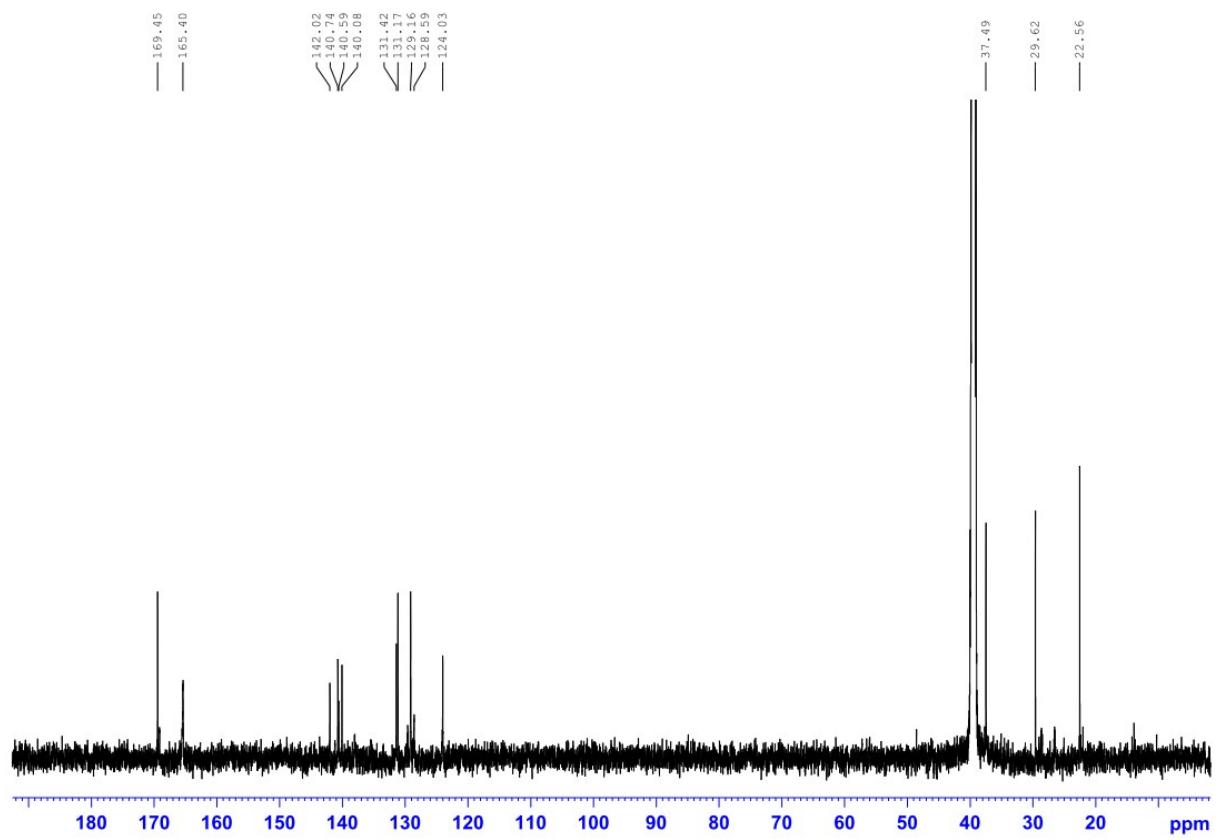
^1H NMR spectrum (600 MHz, DMSO- D_6) of **6**.

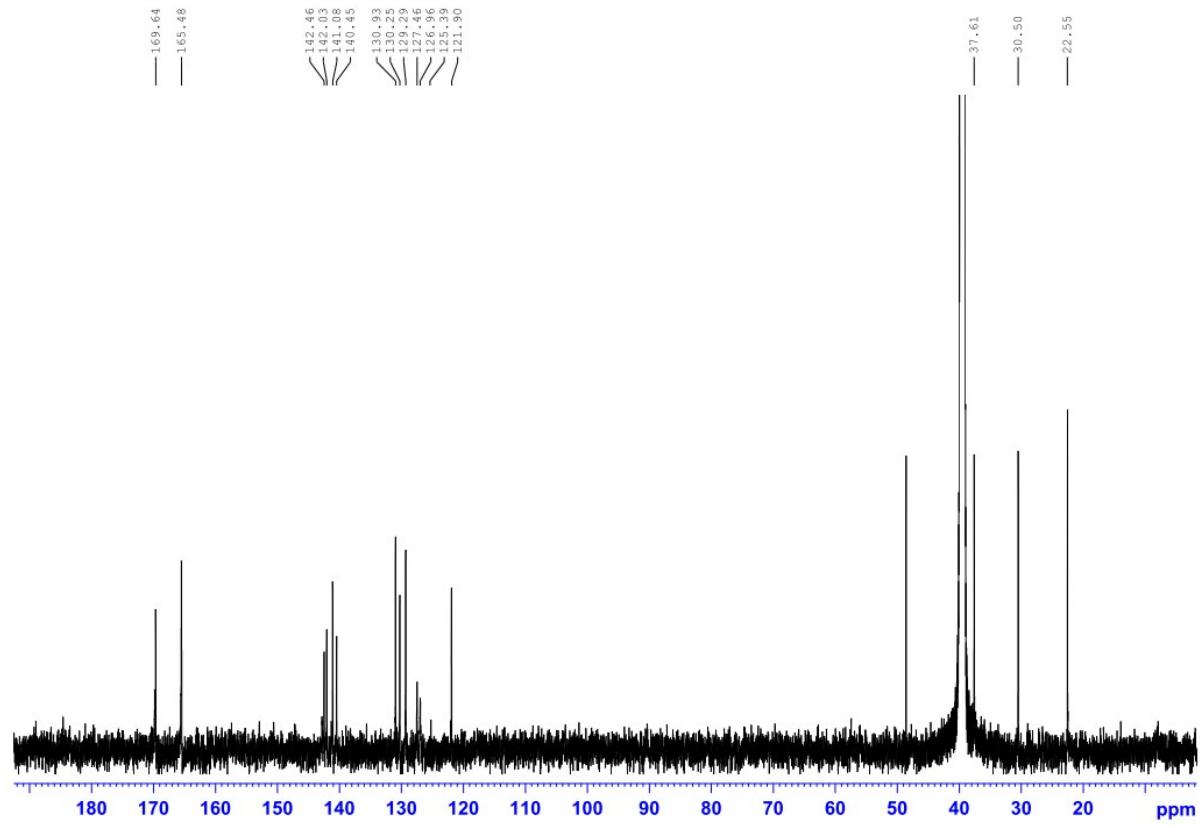


^{13}C NMR spectrum (150 MHz, DMSO- D_6) of **6**.

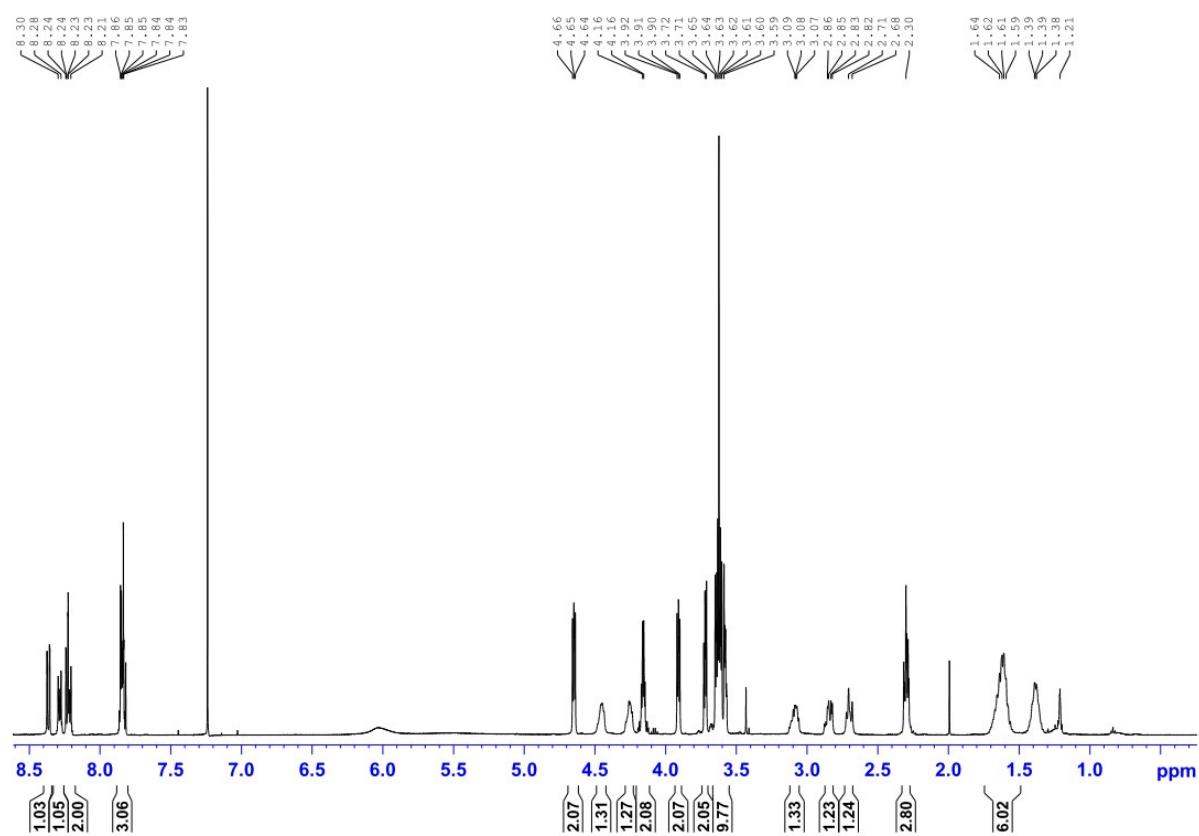


^1H NMR spectrum (500 MHz, DMSO- D_6) of **7**.

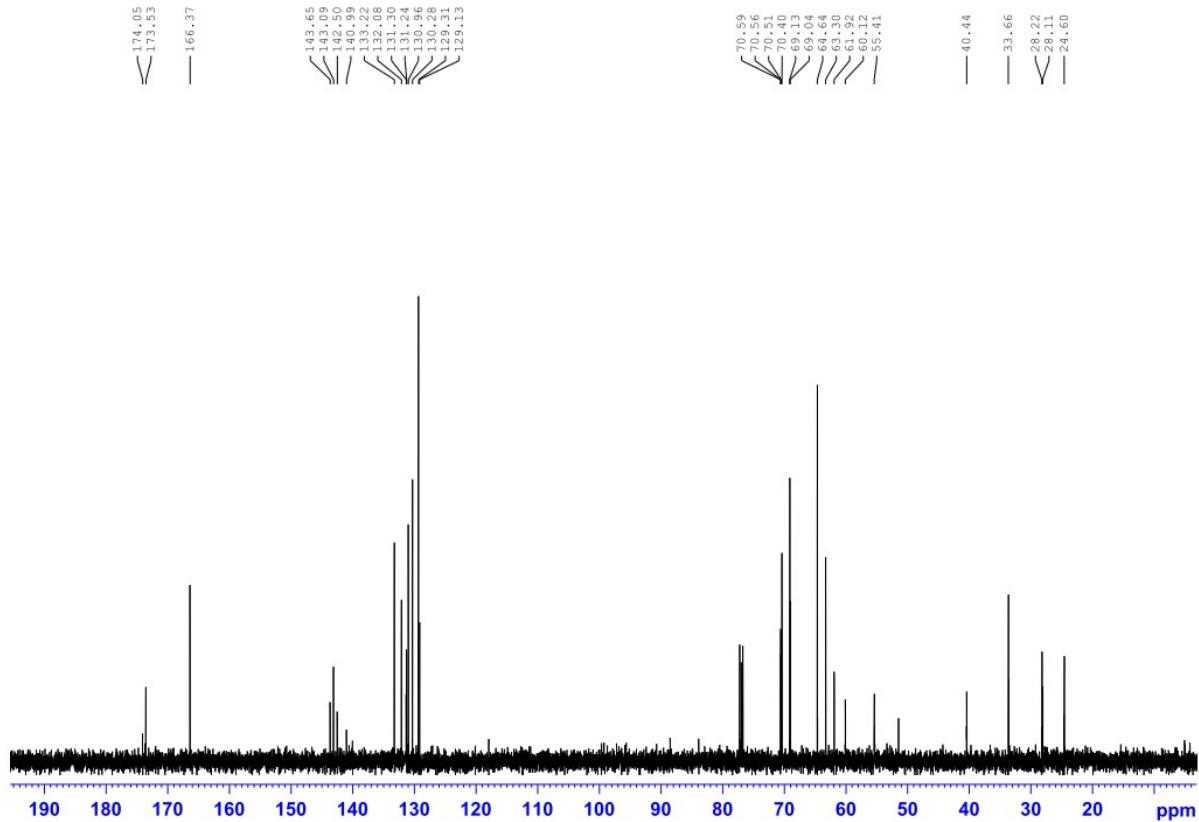




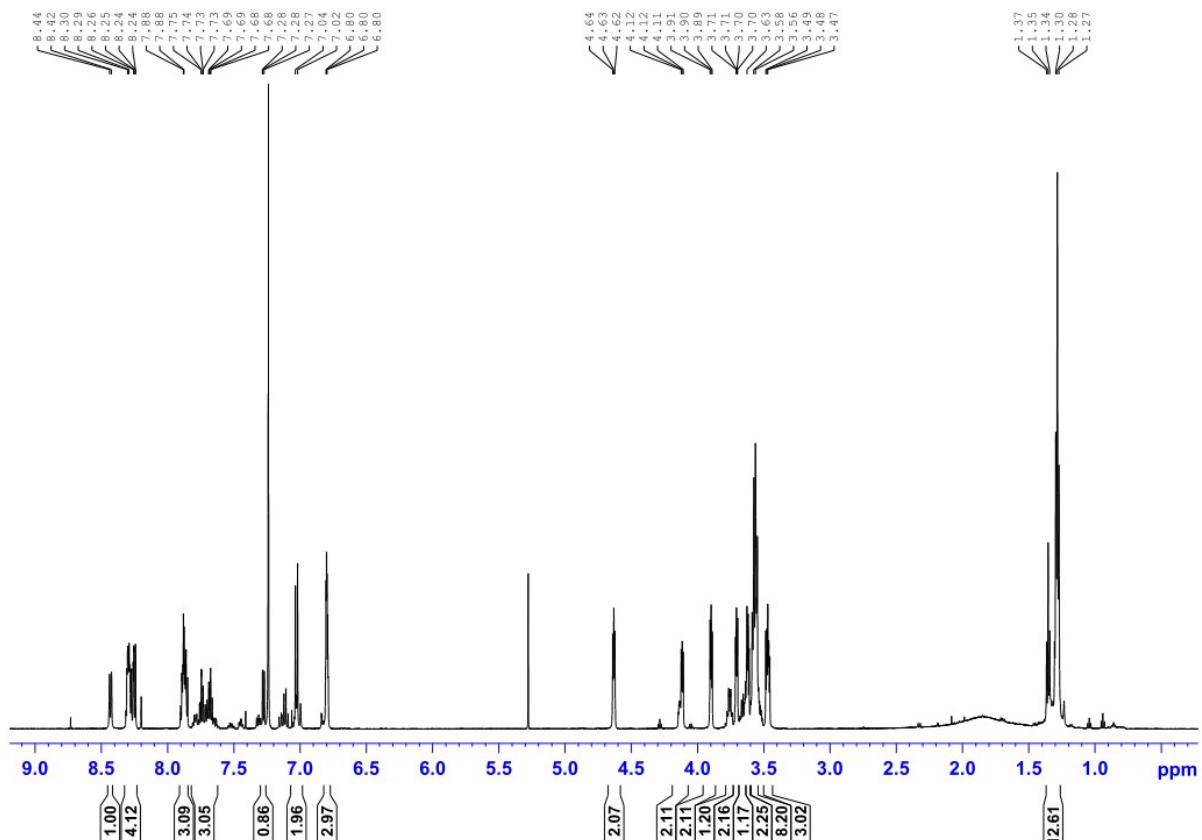
^{13}C NMR spectrum (150 MHz, DMSO- D_6) of **8**.



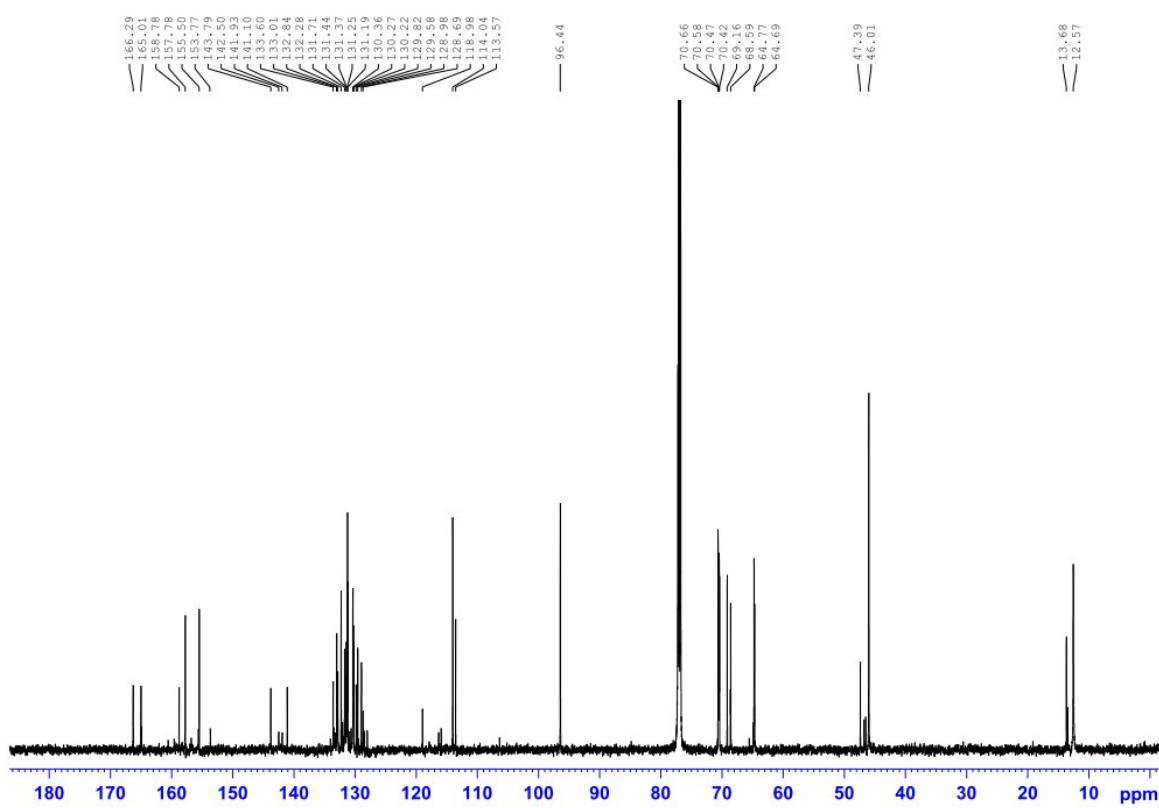
^1H NMR (500 MHz, CDCl_3) spectrum of **9**.



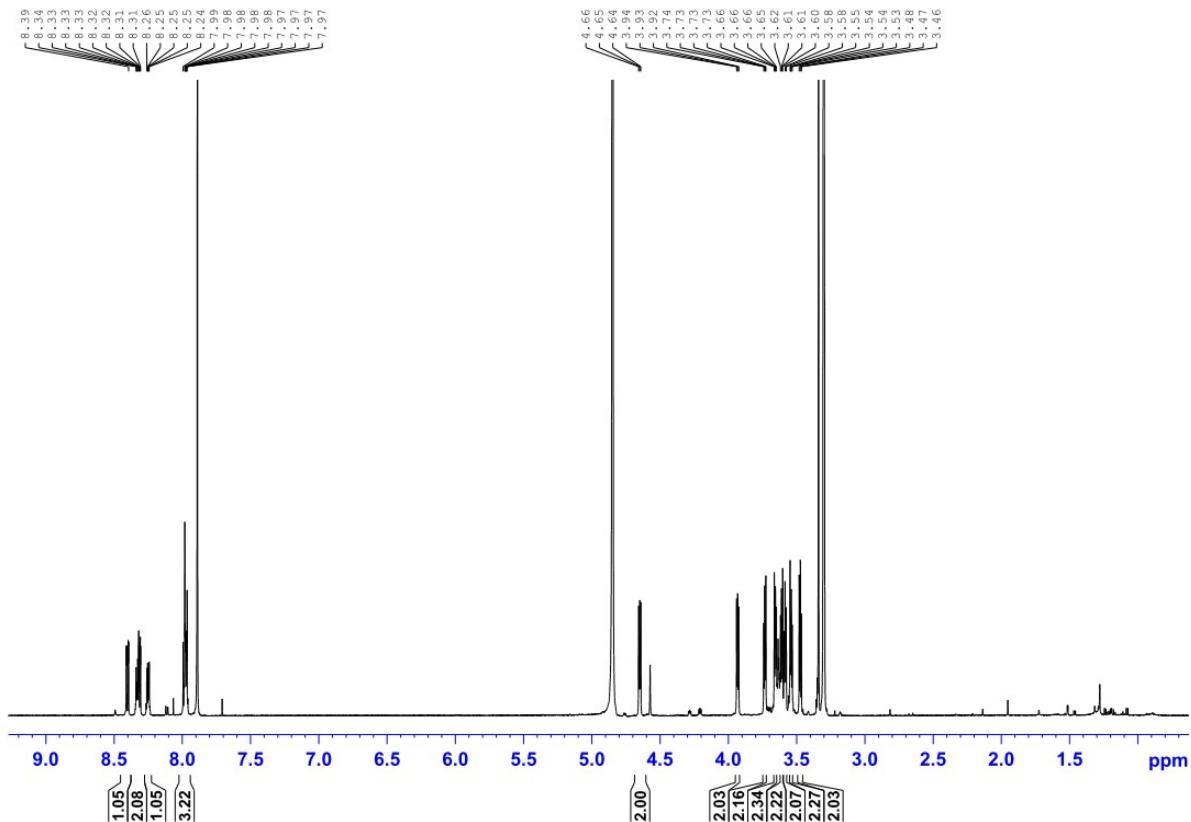
¹³C NMR (150 MHz, CDCl₃) spectrum of **9**.



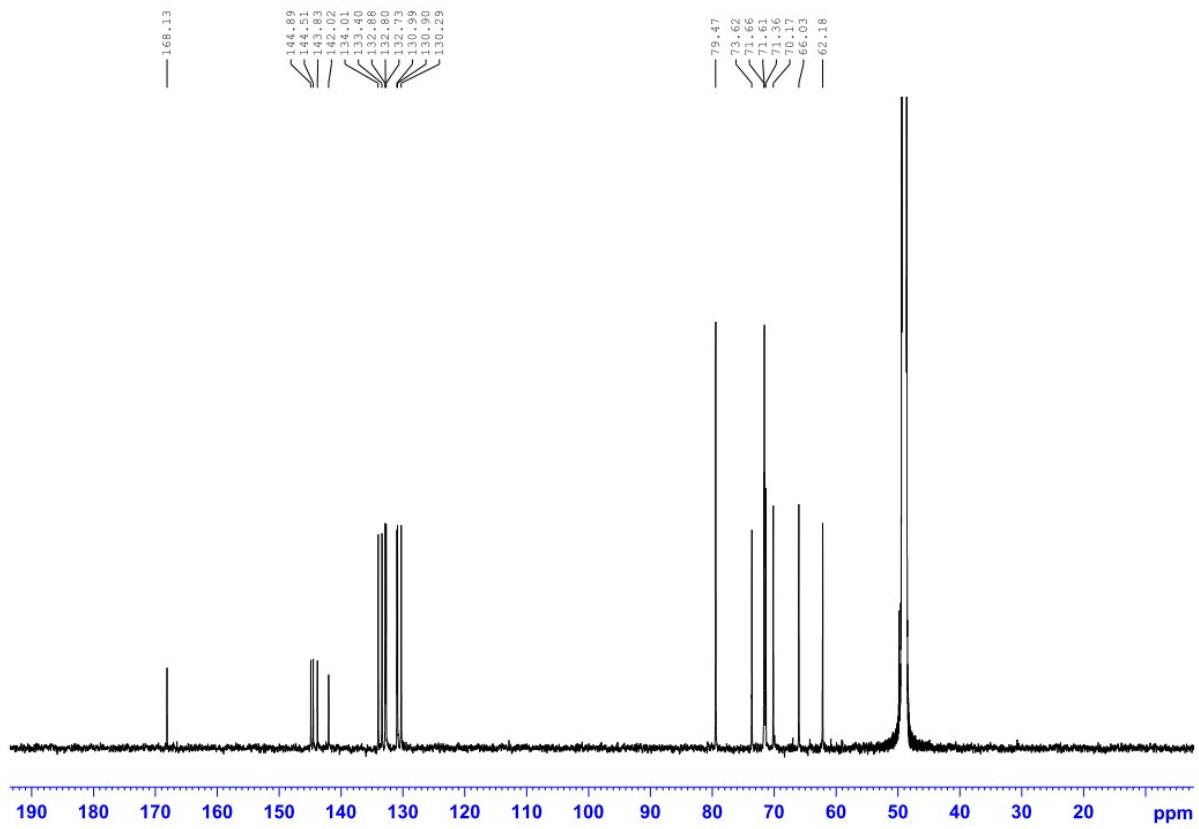
¹H NMR spectrum (600 MHz, CDCl₃) of **10**.



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



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



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