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Supporting Information

Salinipyrone and Pacificanone Are Biosynthetic By-products of the Rosamicin Polyketide Synthase

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cbic_201500177_sm_miscellaneous_information.pdf

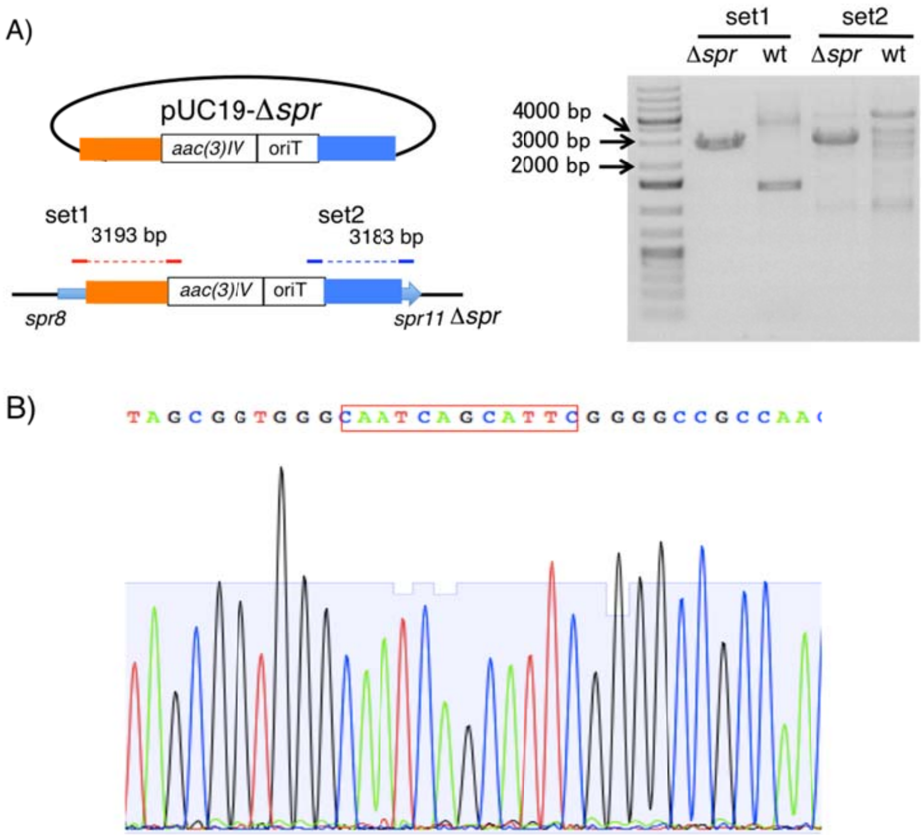

Figure S1 The evaluation of Δ*spr* and *spr10Y1290F* strains. A) PCR amplification by using gDNA from *S. pacifica* CNS-237 and Δ*spr* strain. B) Se- quence result of the 595-bp fragment amplified from *spr10Y1290F* strain. The red frame shows the mutated sequence in this study.

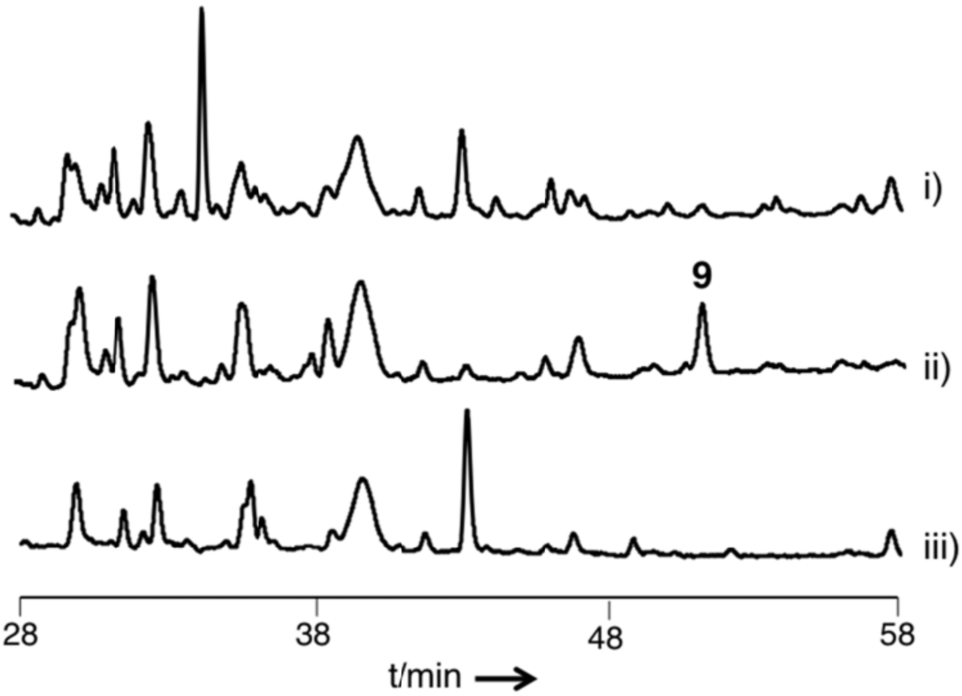

Figure S2 HPLC analyses of the metabolites from i) CNS-237, ii) *spr10*Y**1**290F, and iii) Δ*spr* cultured in A1FeBC medium. The traces represent chromatograms acquired by detection at 280 nm.

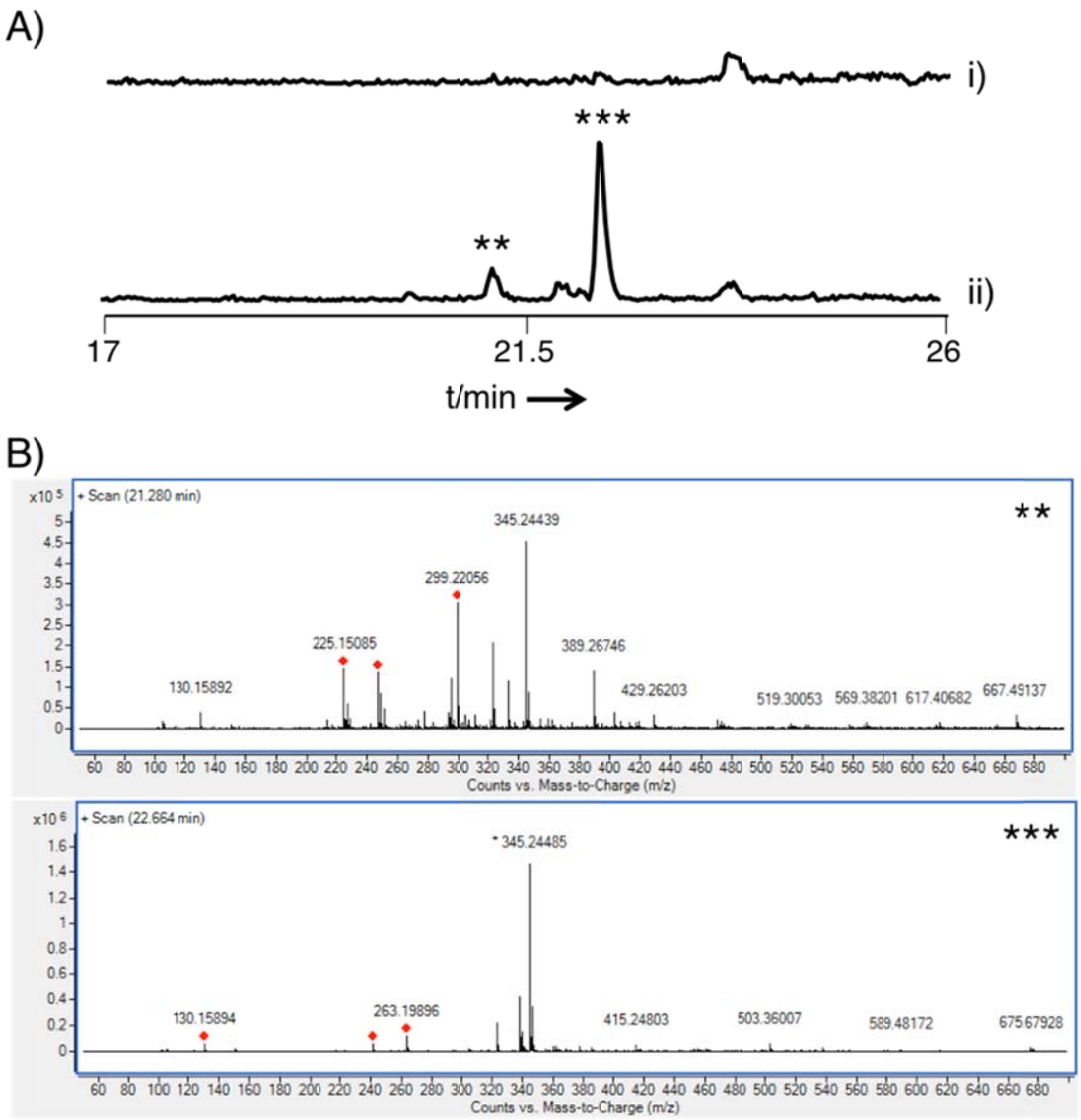

Figure S3 (A) Extracted chromatograms (345.240 m/z) of the metabolites from i) CNS-237 and ii) *spr10*Y1290F cultured in A1+BFe medium (** and *** indicate the compounds whose MSes are identical to **2**). (B) MS spectra of ** and ***.

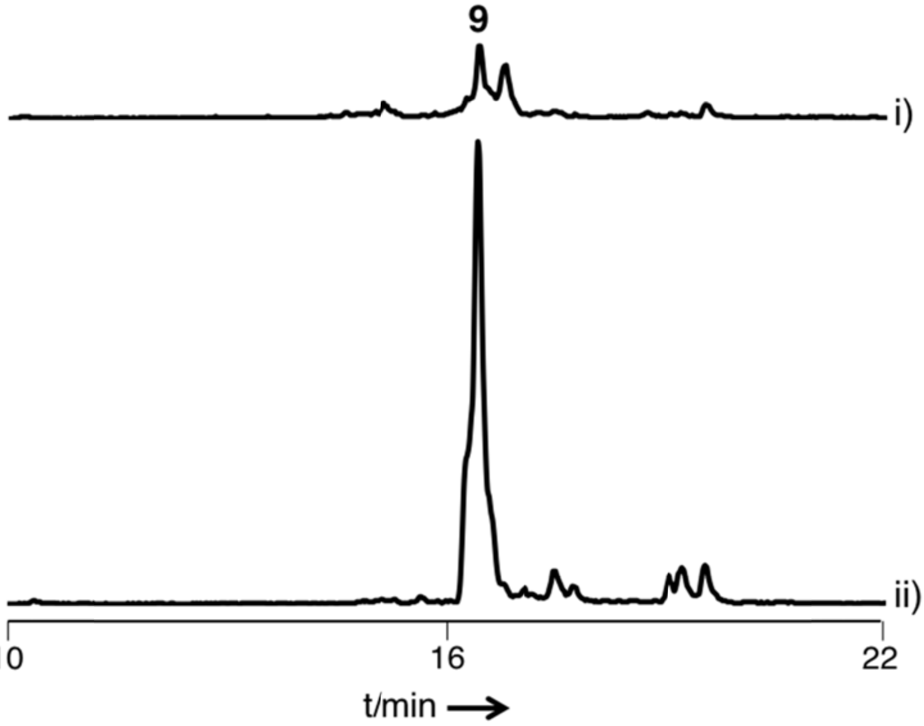

Figure S4 LC-MS analyses of the metabolites from i) CNS-237 and ii) *spr10*Y1290F cultured in A1+BFe medium. The traces represent extracted chromatograms (431.242 m/z).

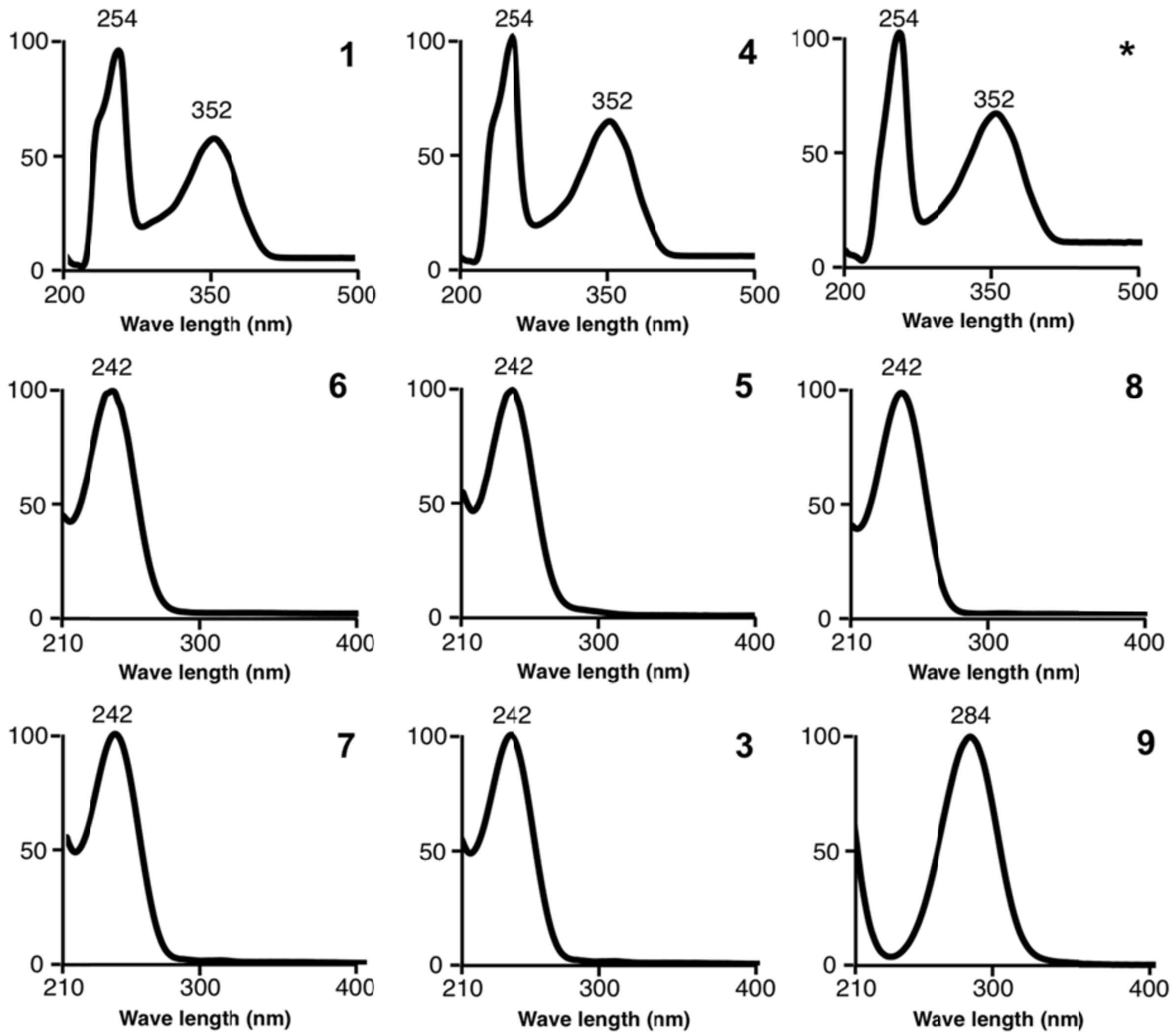

Figure S5 UV spectra of compound **1**, **3**-**9**, and *.

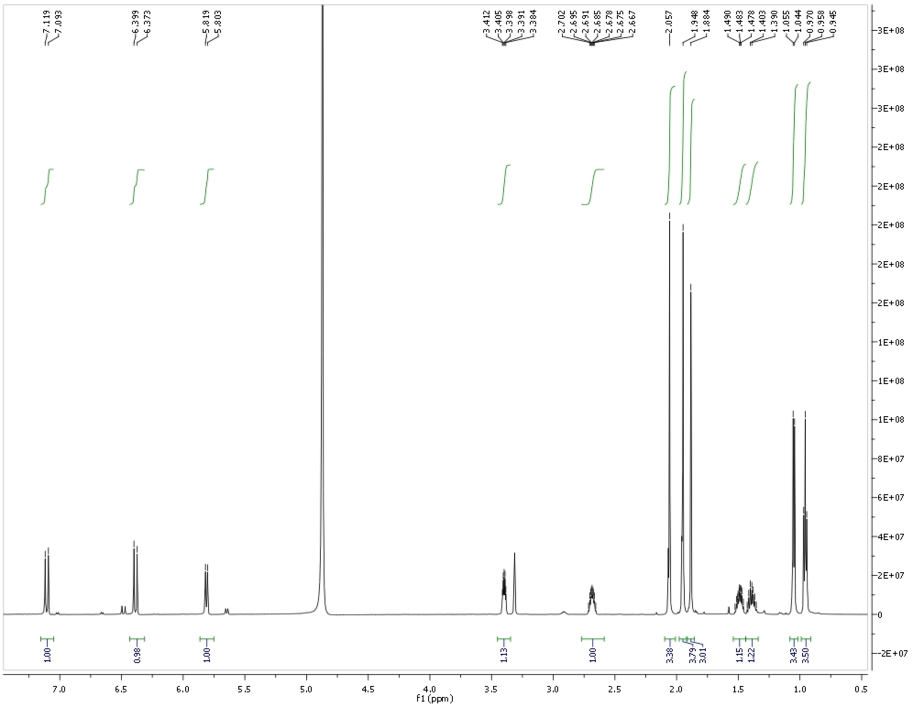

Figure S6 ^{1}H NMR spectrum of **1** in CD_3OD

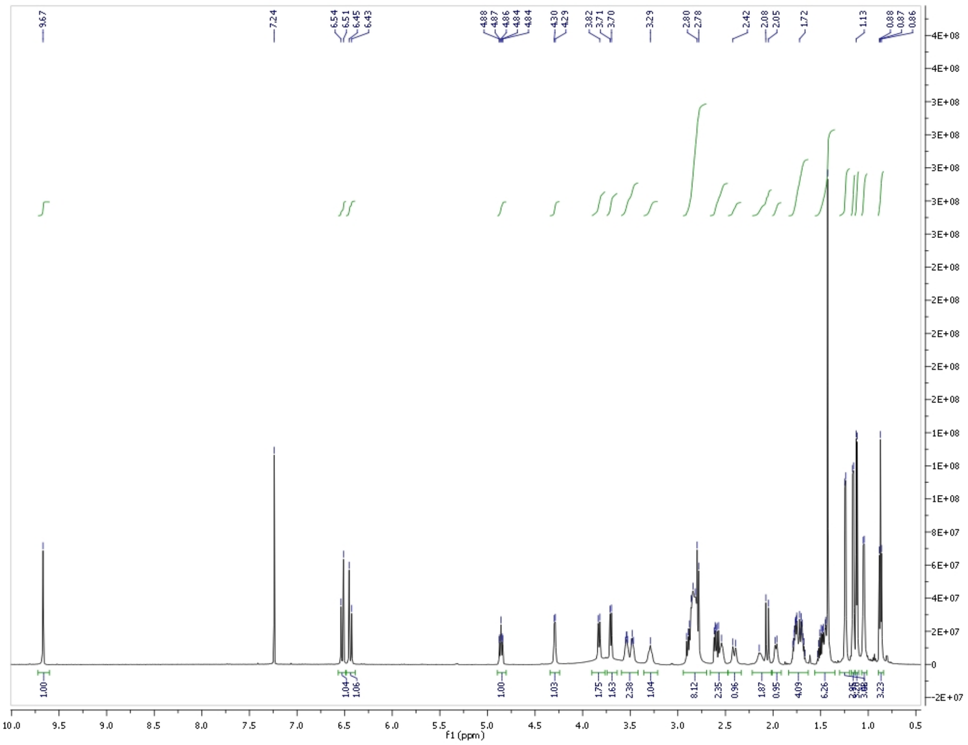

Figure S7 ^{1}H NMR spectrum of **3** in $CDCl_3$

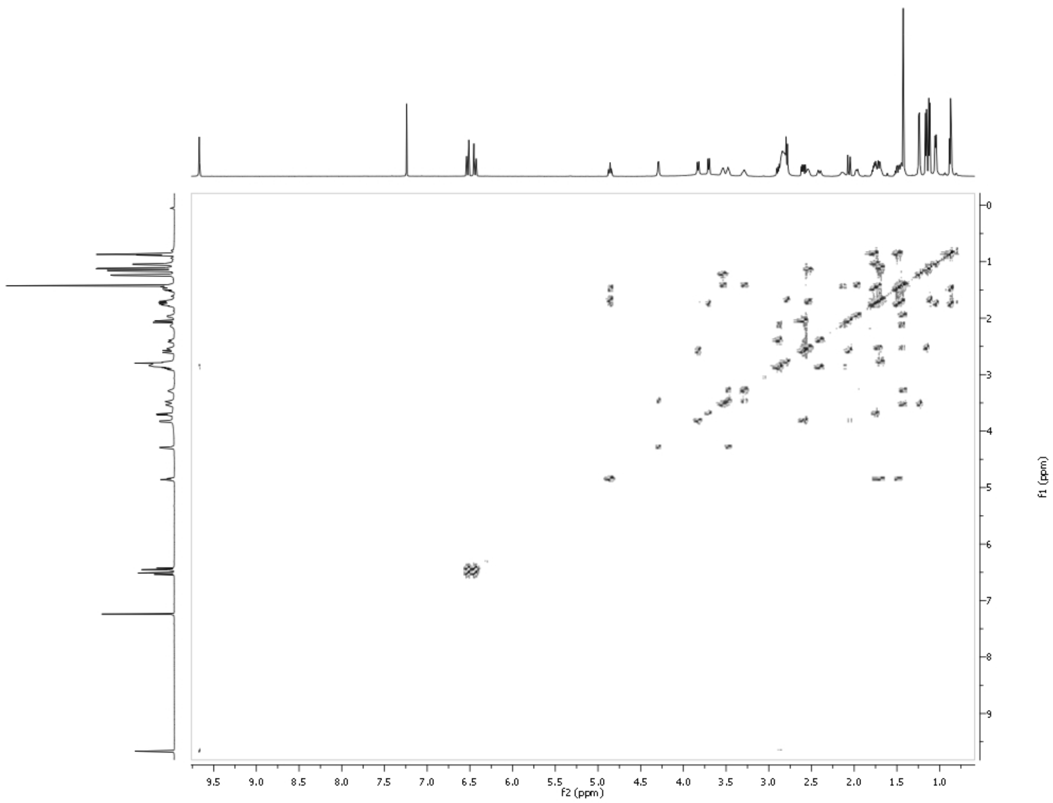

Figure S8 ^{1}H-^{1}H COSY spectrum of **3** in $CDCl_3$

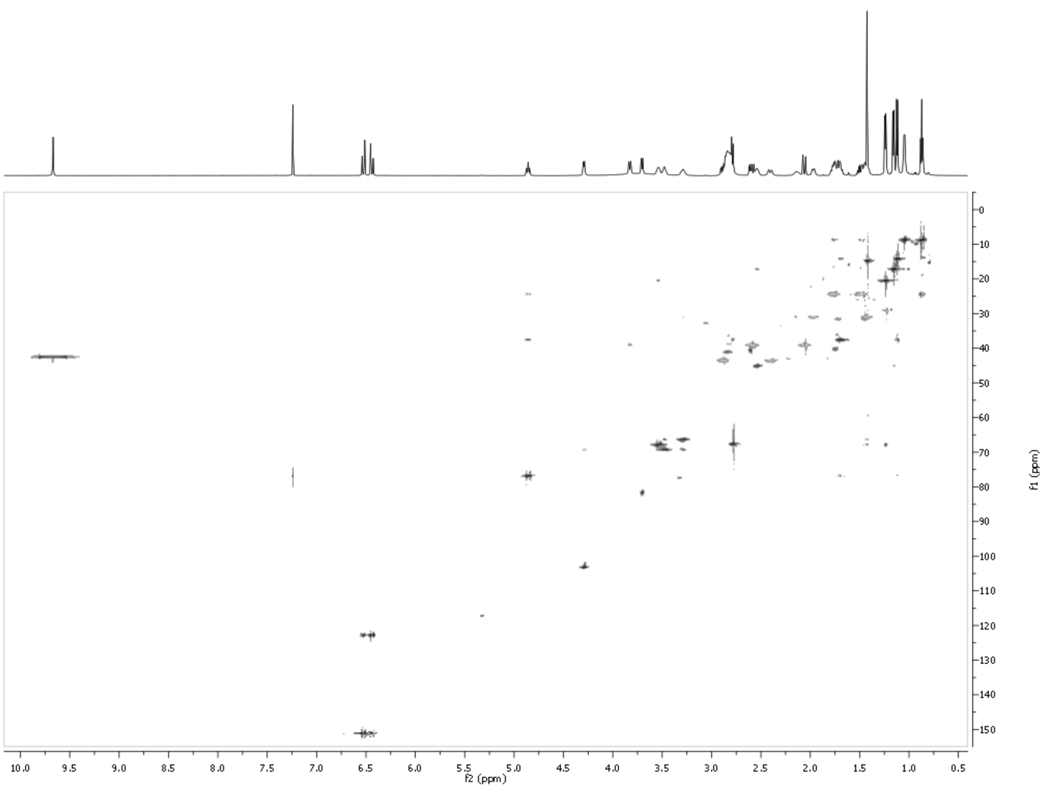

Figure S9 HSQC spectrum of **3** in $CDCl_3$

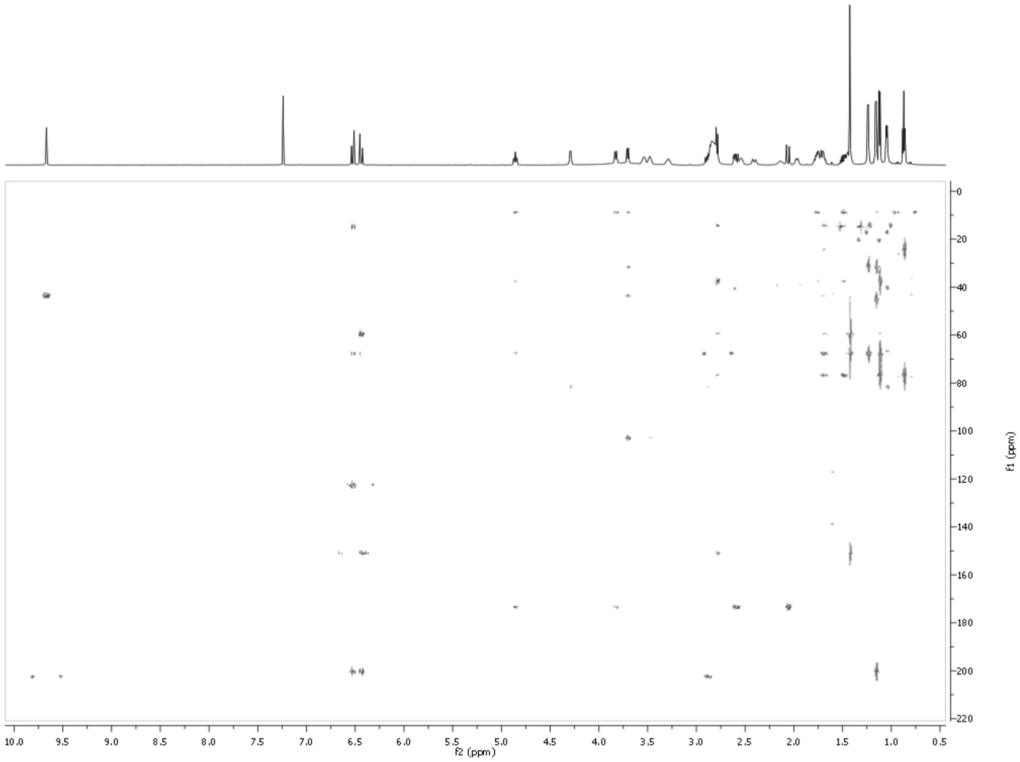

Figure S10 HMBC spectrum of **3** in $CDCl_3$

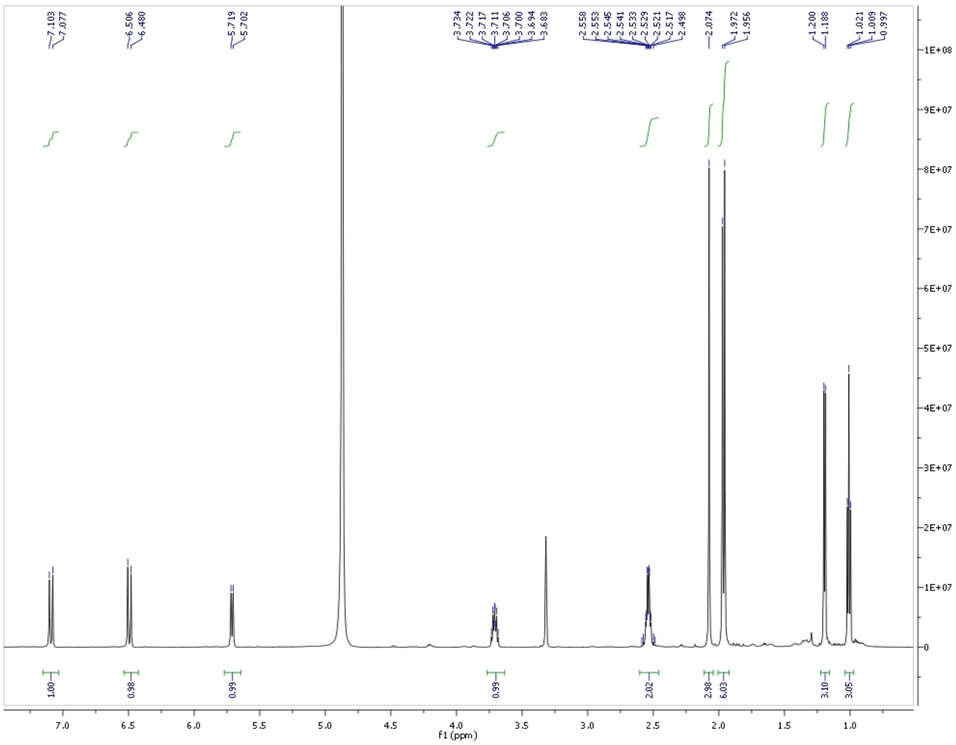

Figure S11 ^1H NMR spectrum of **4** in CD_3OD

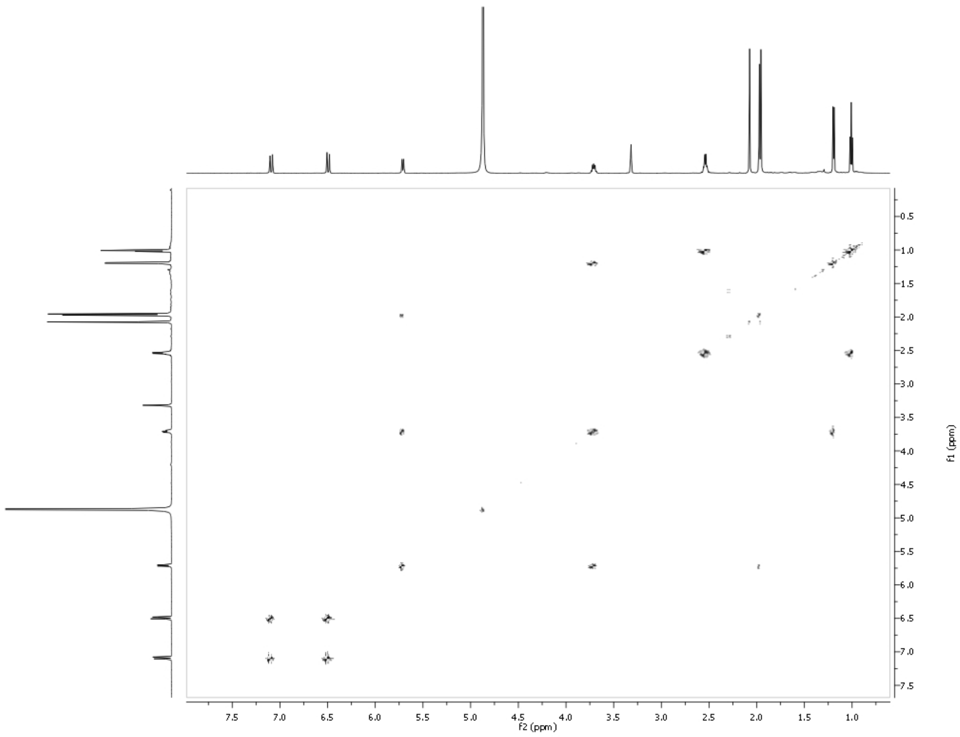

Figure S12 ^{1}H-^{1}H COSY spectrum of **4** in CD_3OD

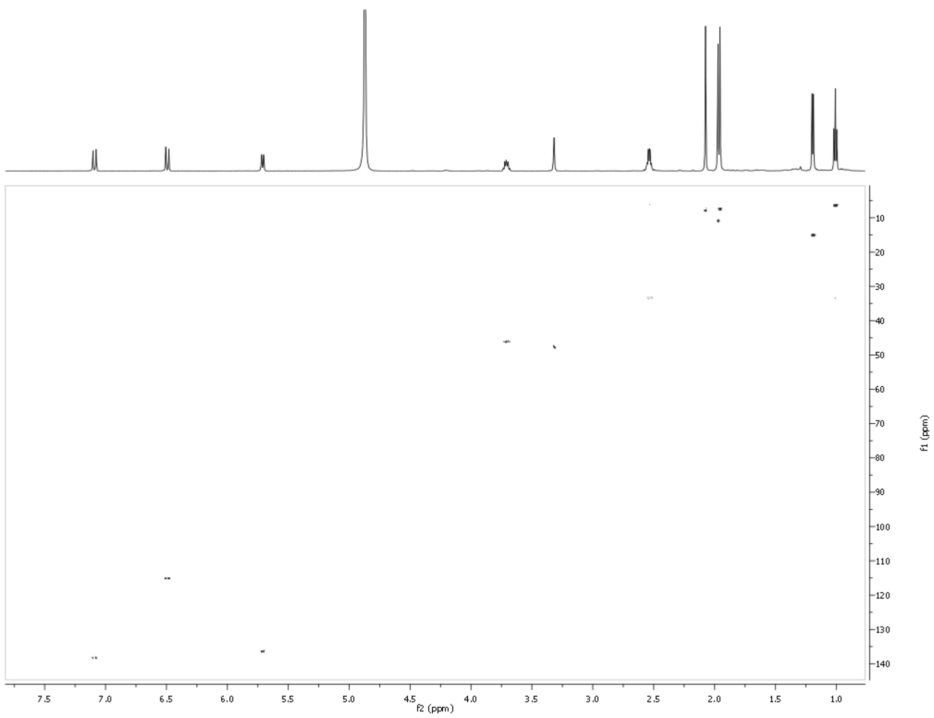

Figure S13 HSQC spectrum of **4** in CD_3OD

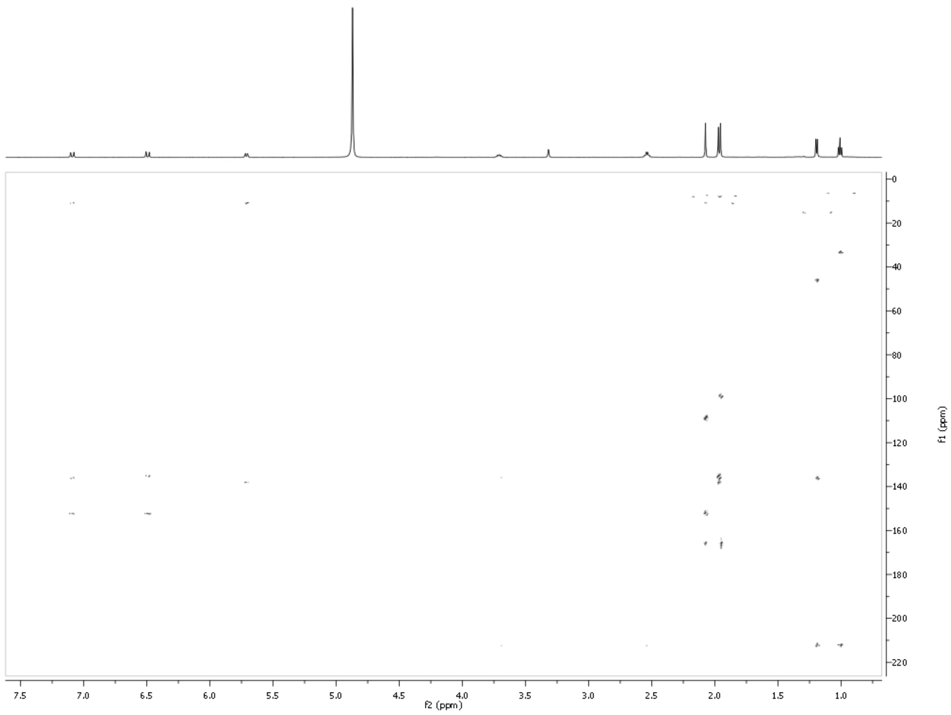

Figure S14 HMBC spectrum of **4** in CD_3OD

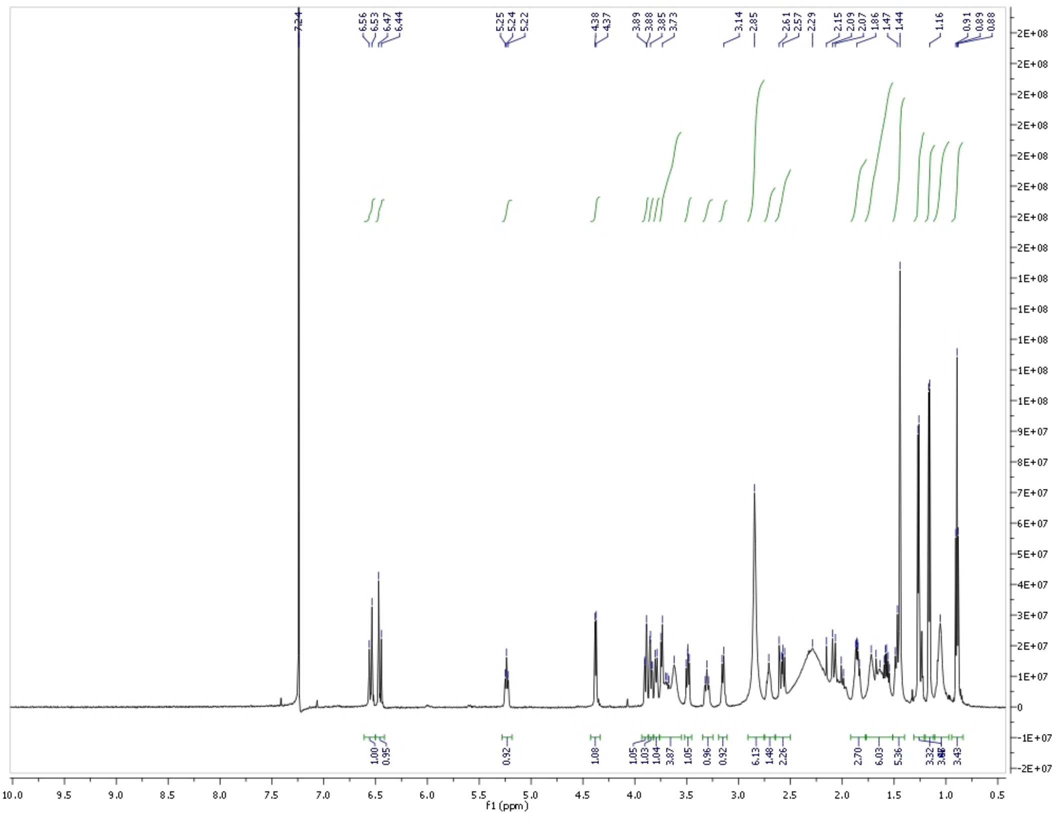

Figure S15 ^1H NMR spectrum of **5** in $CDCl_3$

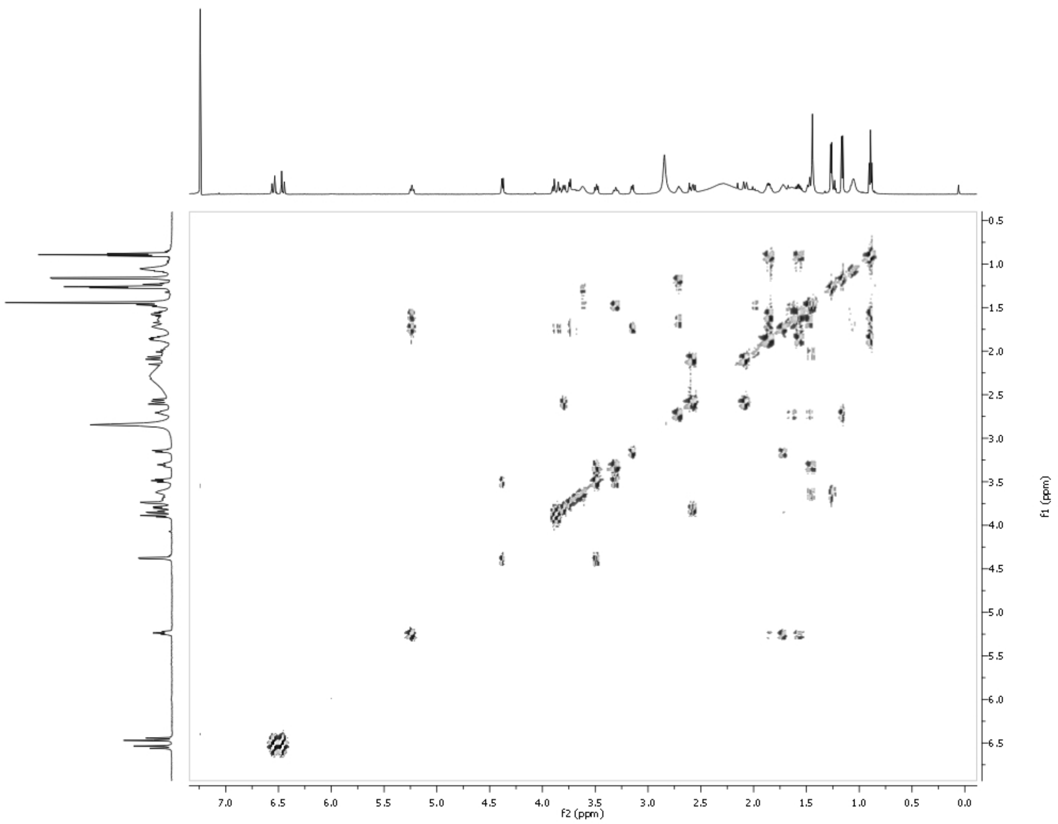

Figure S16 ^{1}H-^{1}H COSY spectrum of **5** in $CDCl_3$

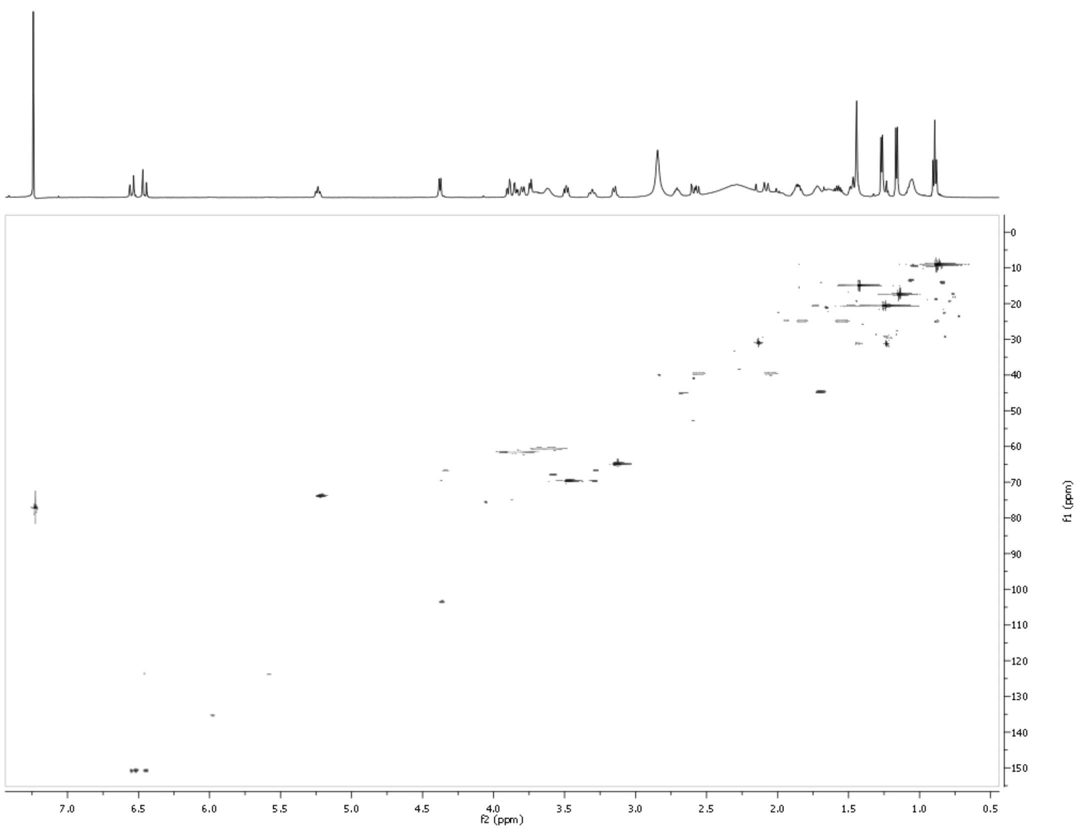

Figure S17 HSQC spectrum of **5** in $CDCl_3$

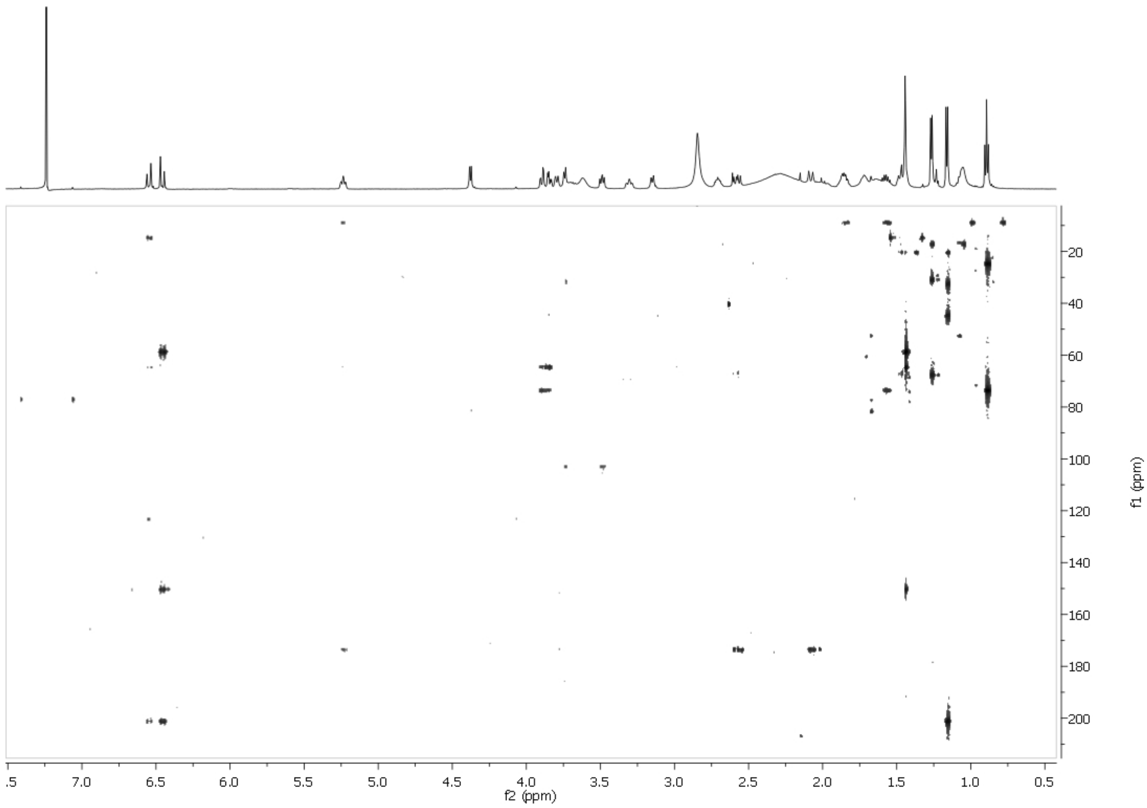

Figure S18 HMBC spectrum of **5** in $CDCl_3$

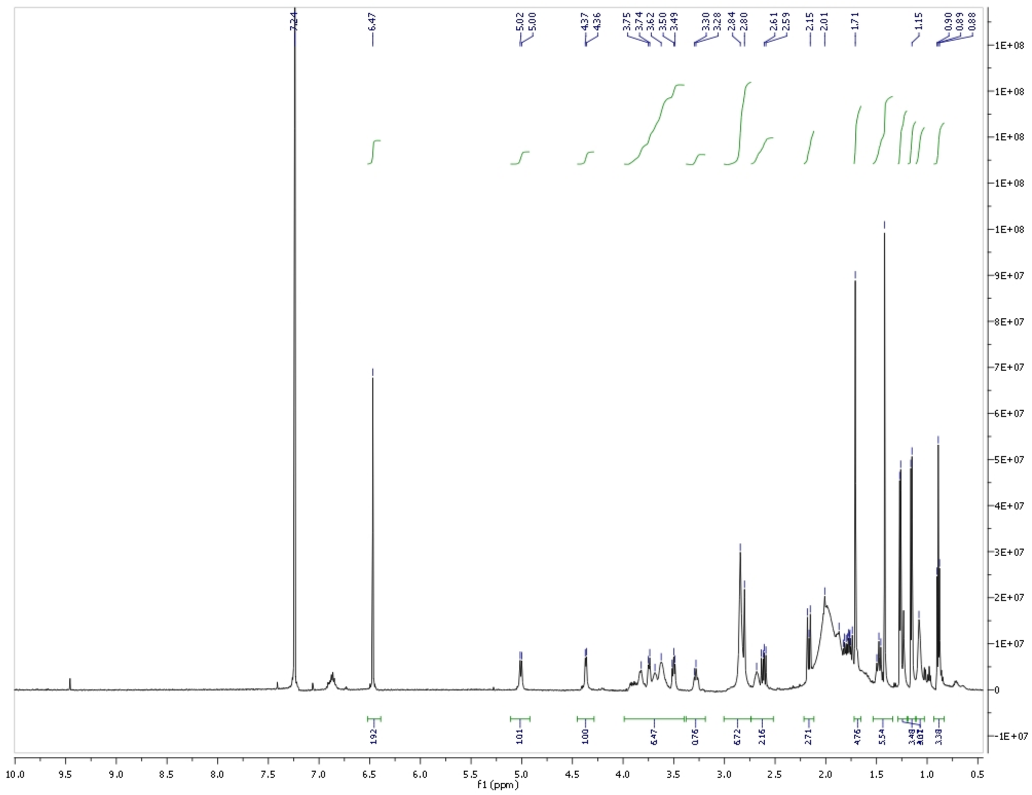

Figure S19 ^{1}H NMR spectrum of **6** in $CDCl_3$

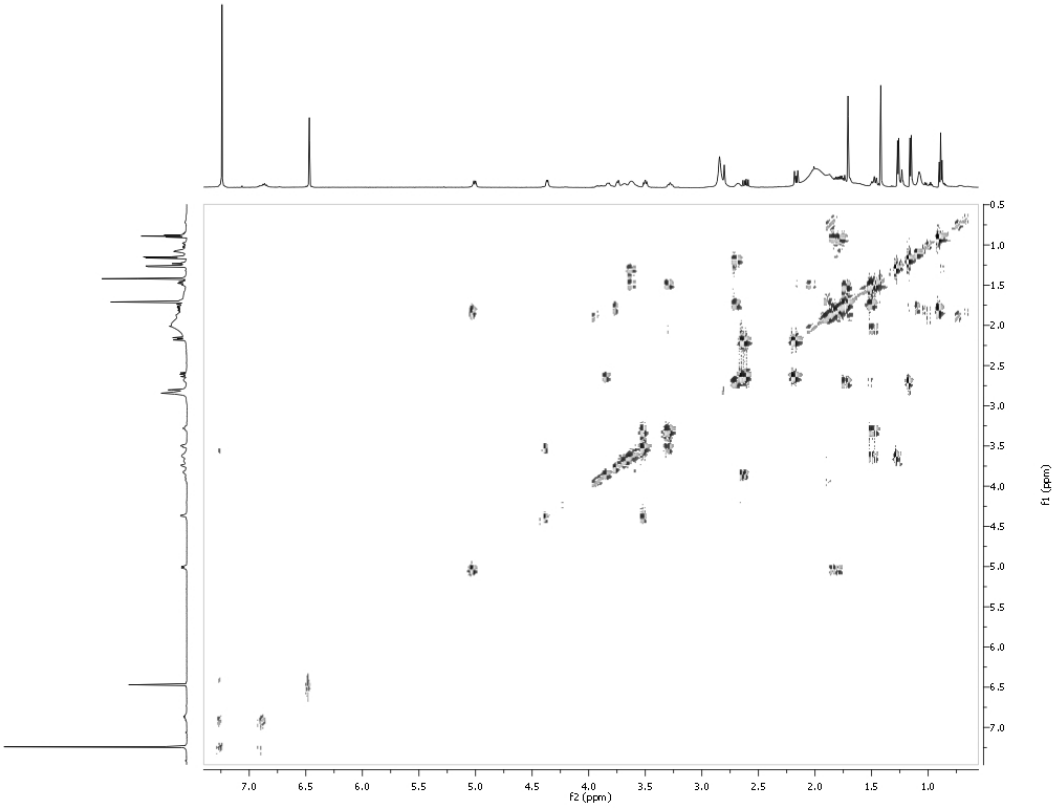

Figure S20 ^{1}H-^{1}H COSY spectrum of **6** in $CDCl_3$

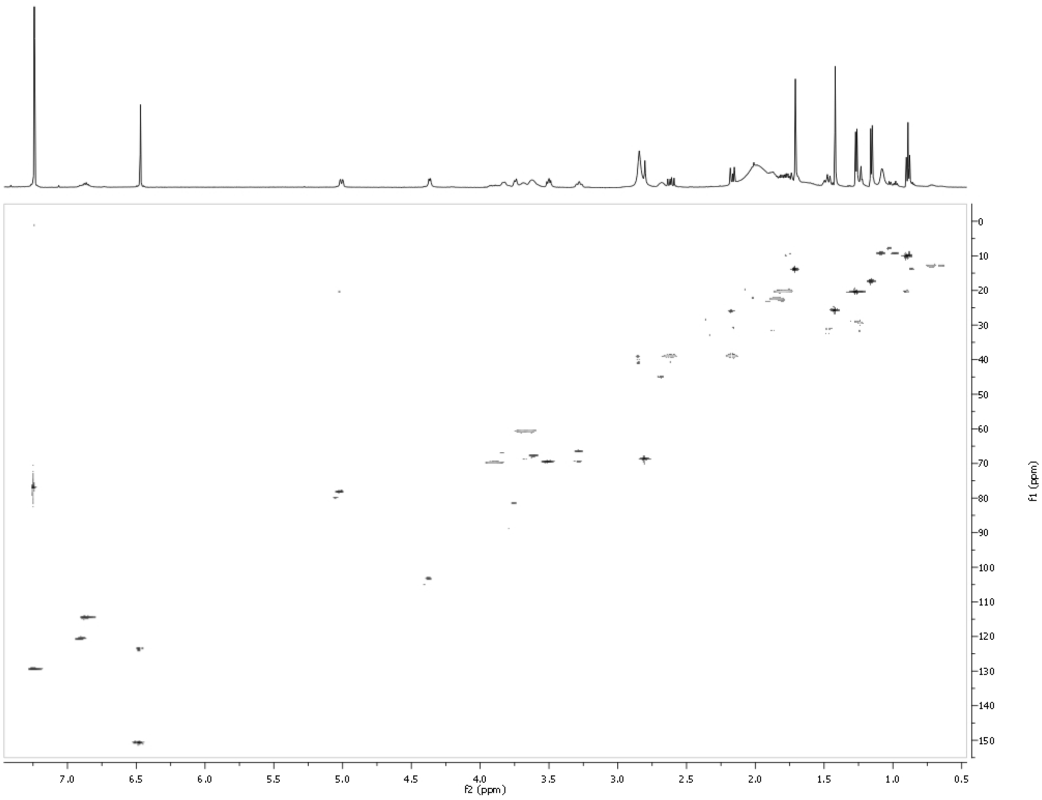

Figure S21 HSQC spectrum of **6** in $CDCl_3$

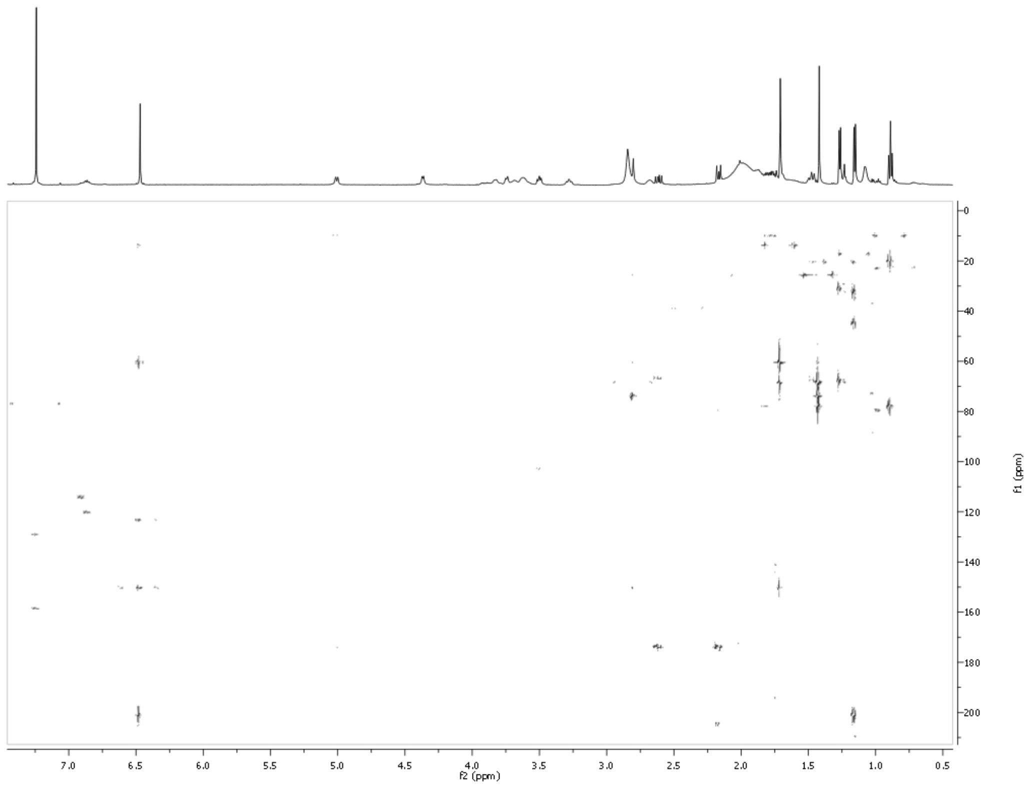

Figure S22 HMBC spectrum of **6** in $CDCl_3$

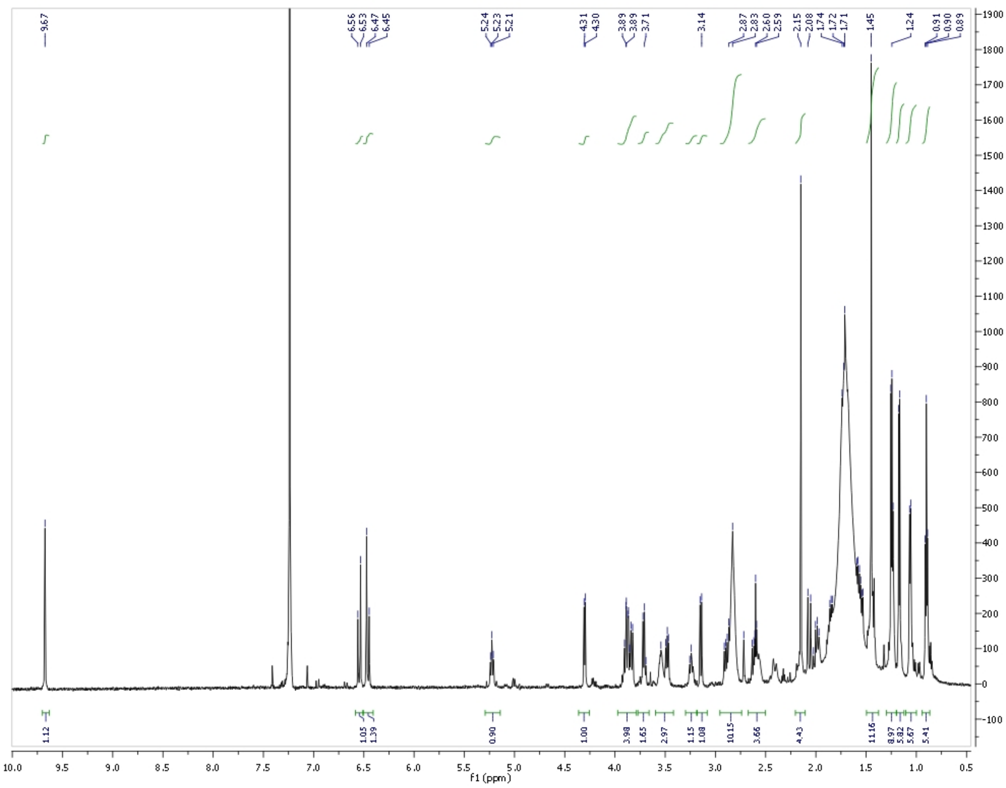

Figure S23 ^{1}H NMR spectrum of **7** in $CDCl_3$

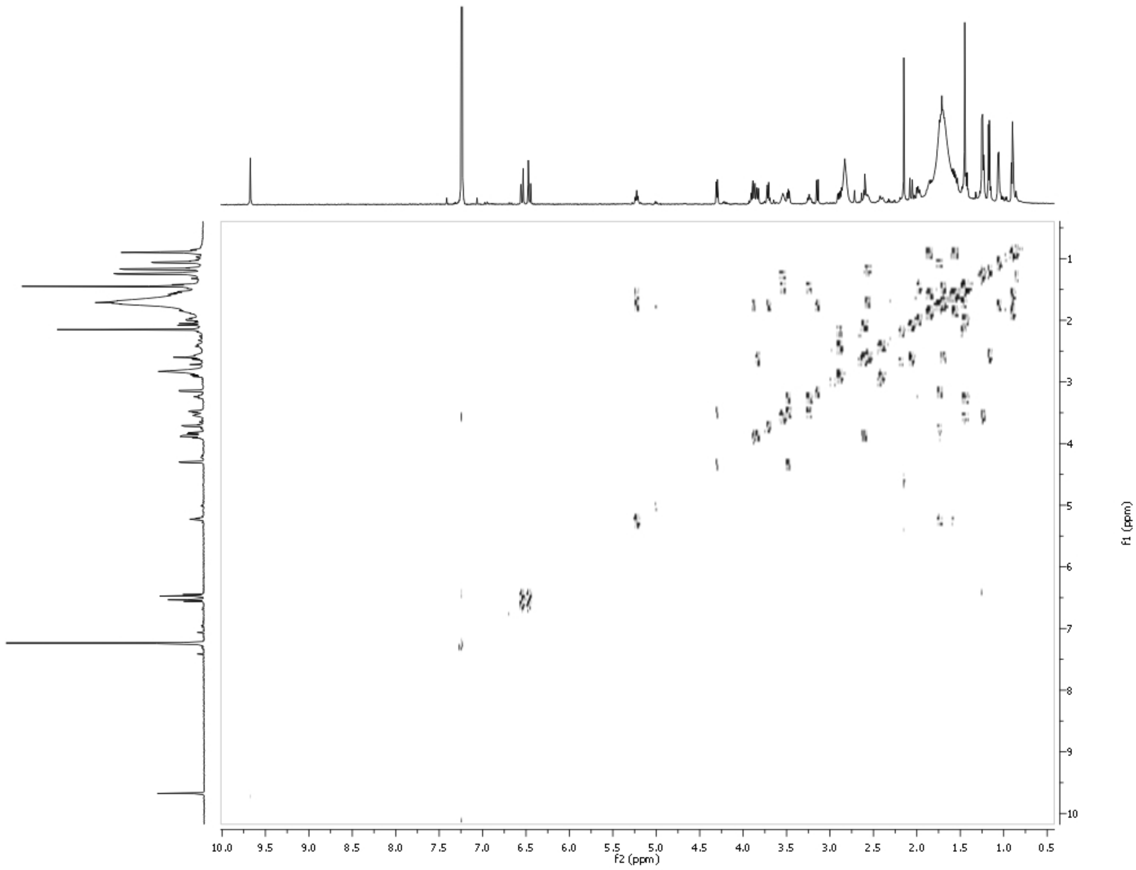

Figure S24 ${}^{1}H$-${}^{1}H$ COSY spectrum of **7** in $CDCl_3$

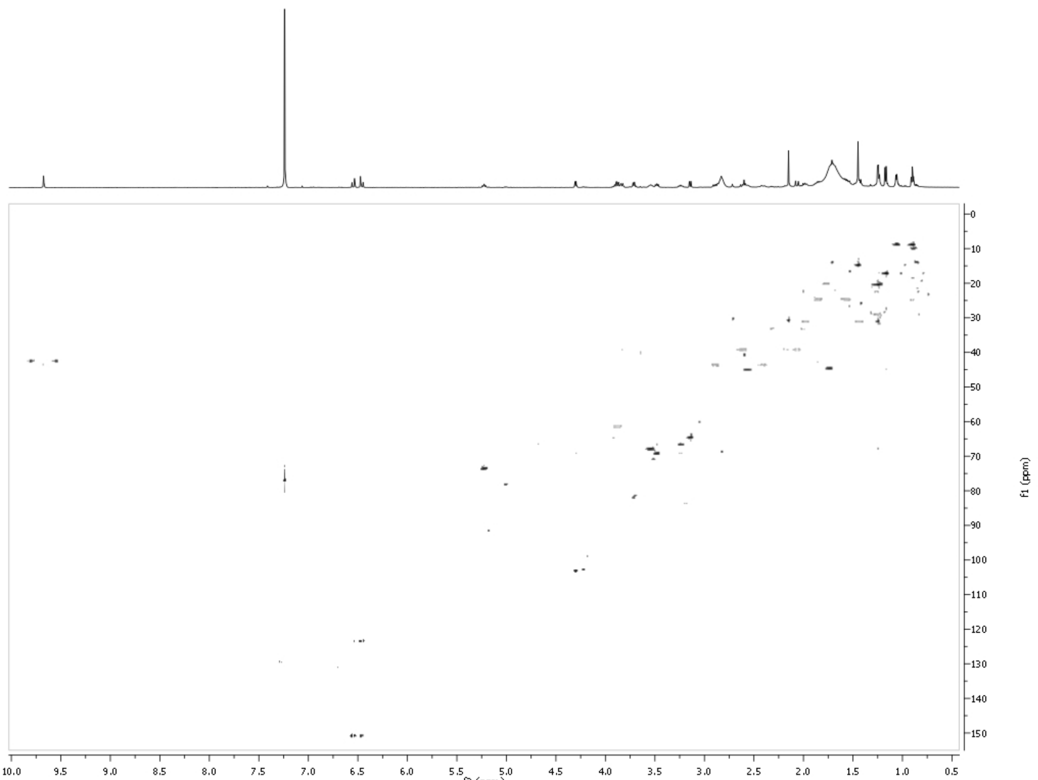

Figure S25 HSQC spectrum of **7** in $CDCl_3$

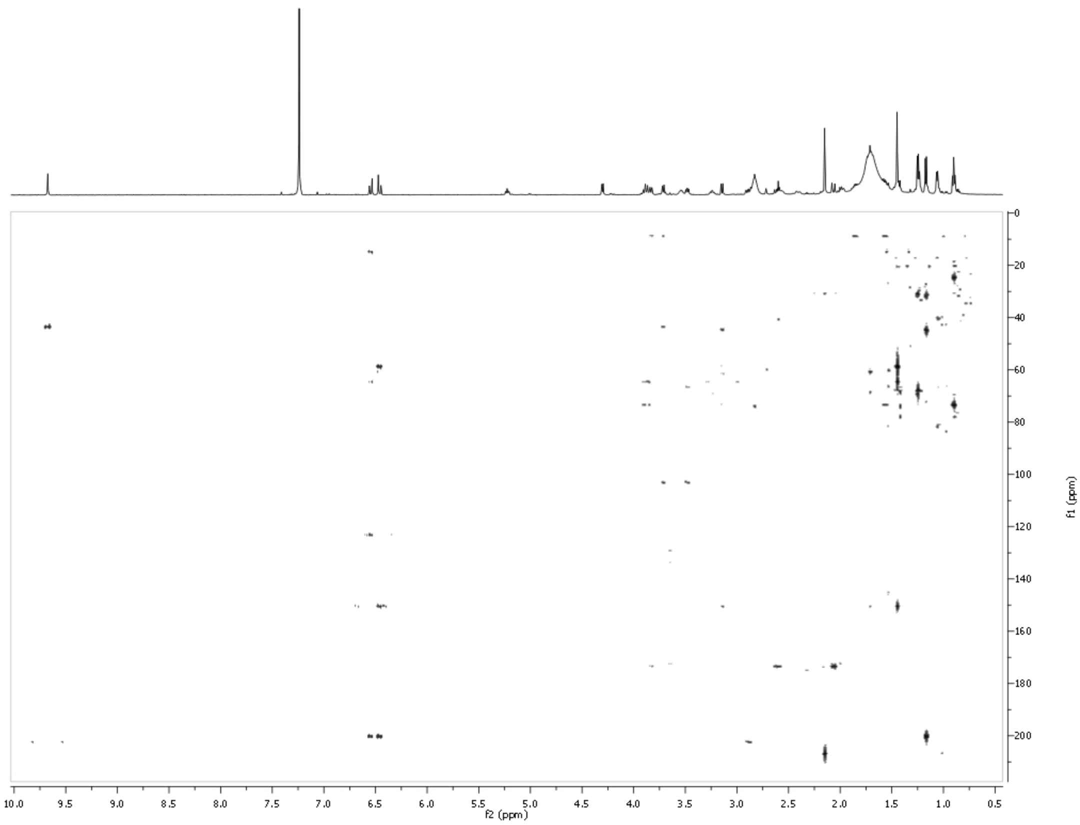

Figure S26 HMBC spectrum of **7** in $CDCl_3$

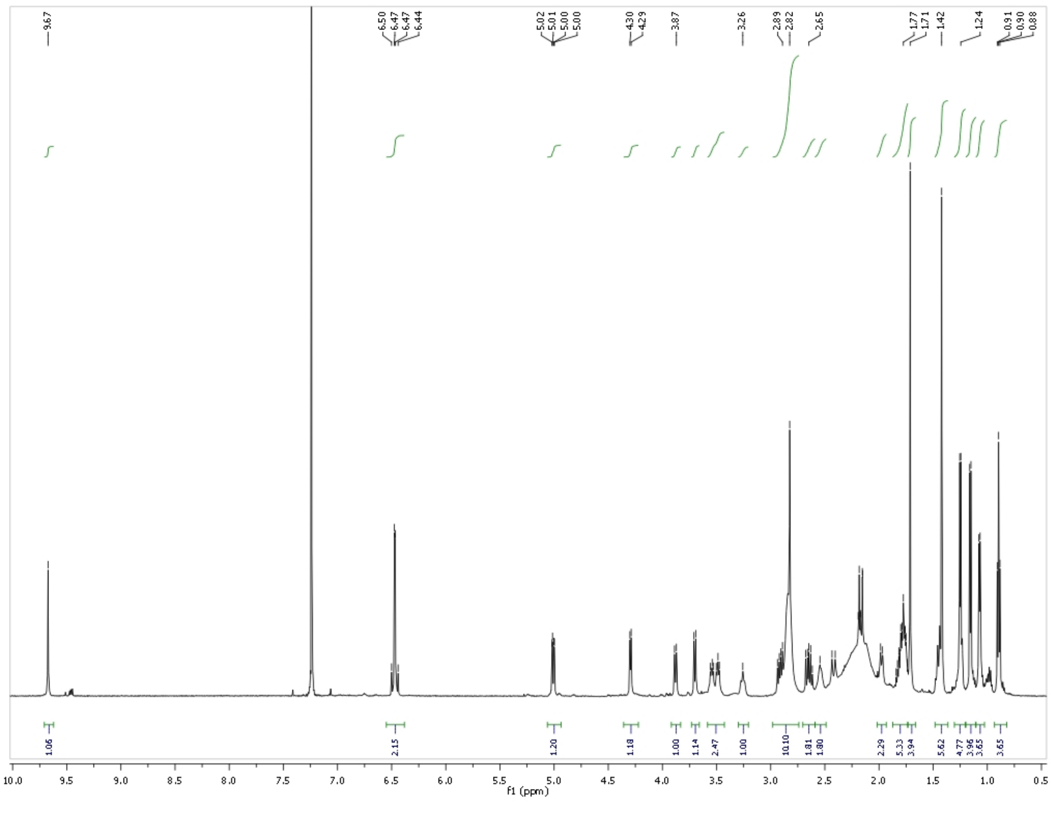

Figure S27 ^1H NMR spectrum of **8** in $CDCl_3$

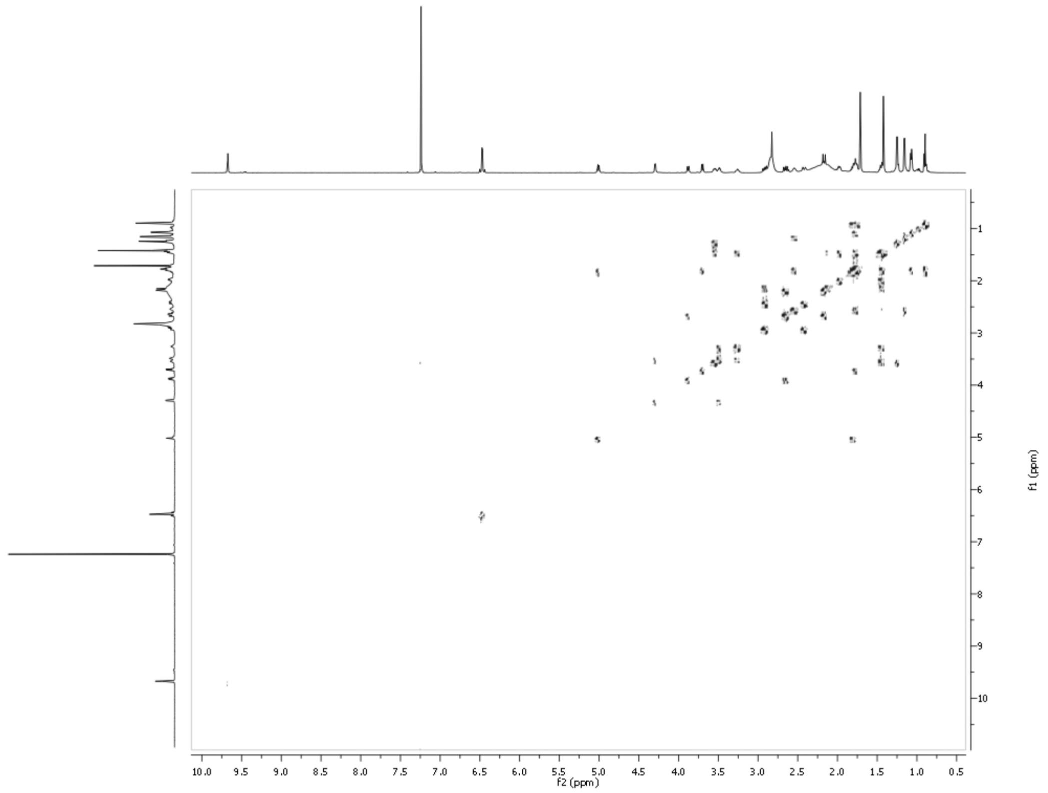

Figure S28 ^{1}H-^{1}H COSY spectrum of **8** in $CDCl_3$

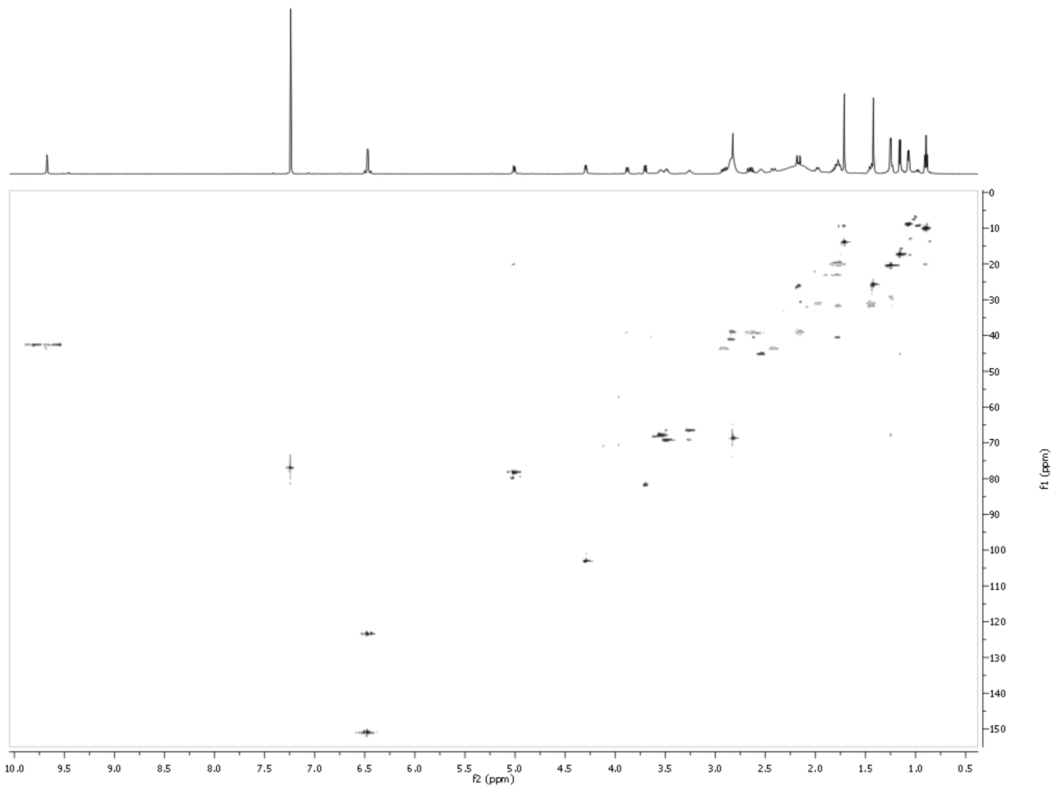

Figure S29 HSQC spectrum of **8** in $CDCl_3$

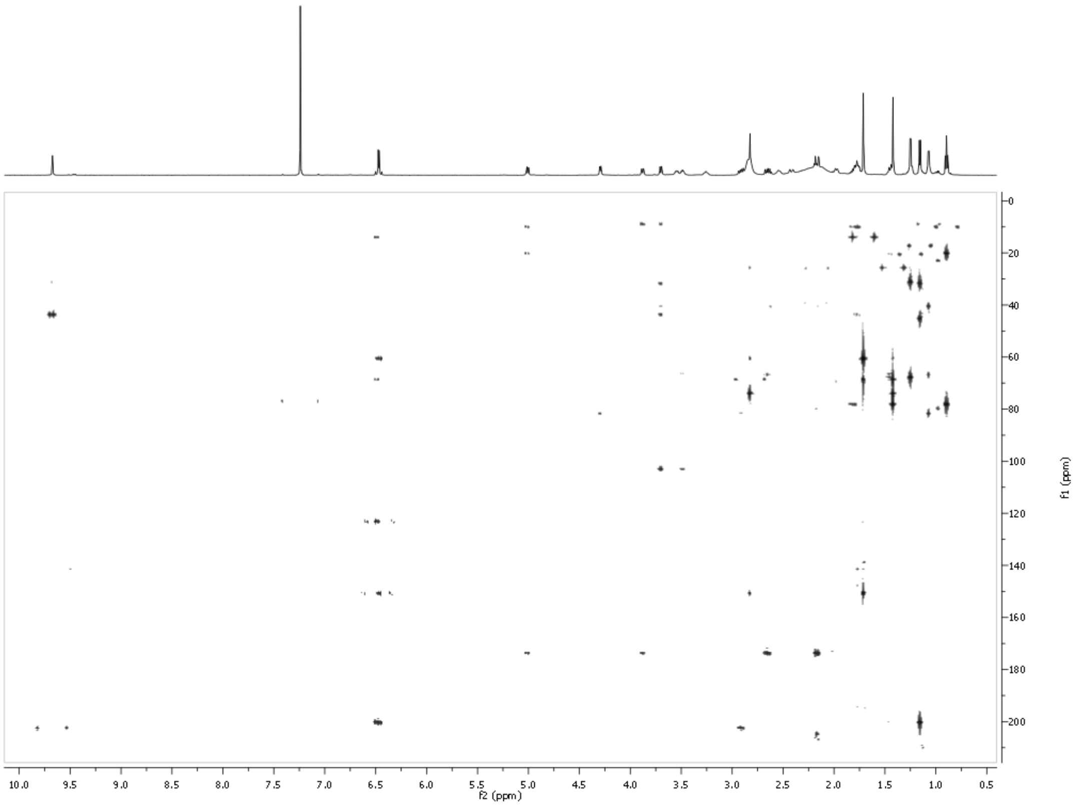

Figure S30 HMBC spectrum of **8** in $CDCl_3$

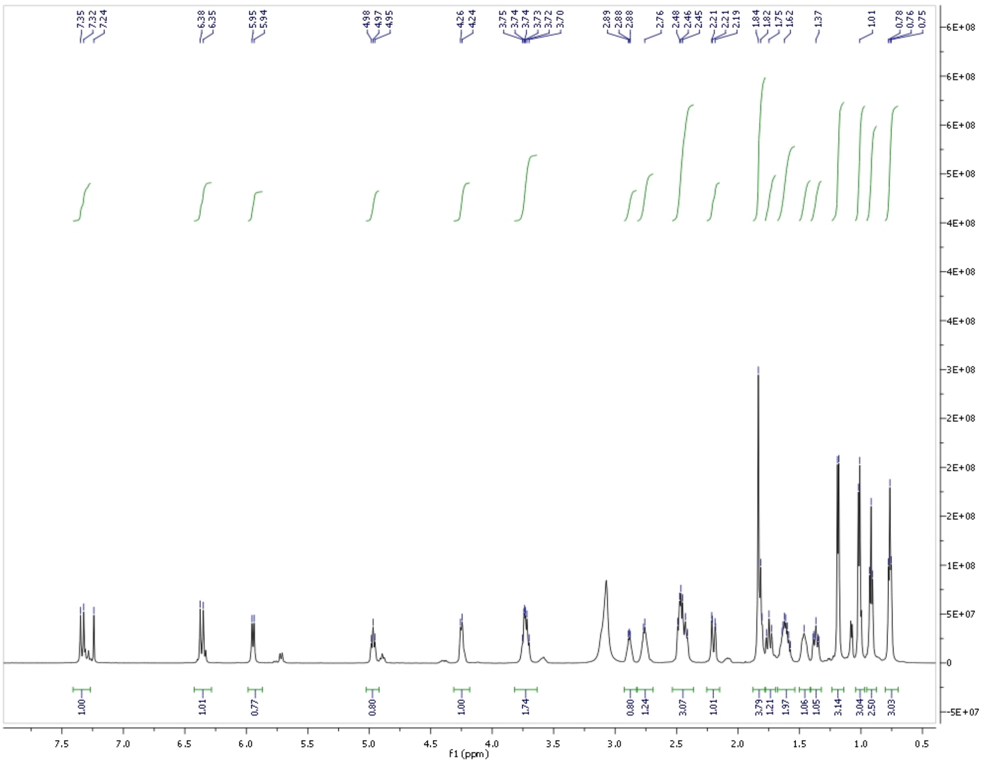

Figure S31 ^{1}H NMR spectrum of **9** in $CDCl_3$

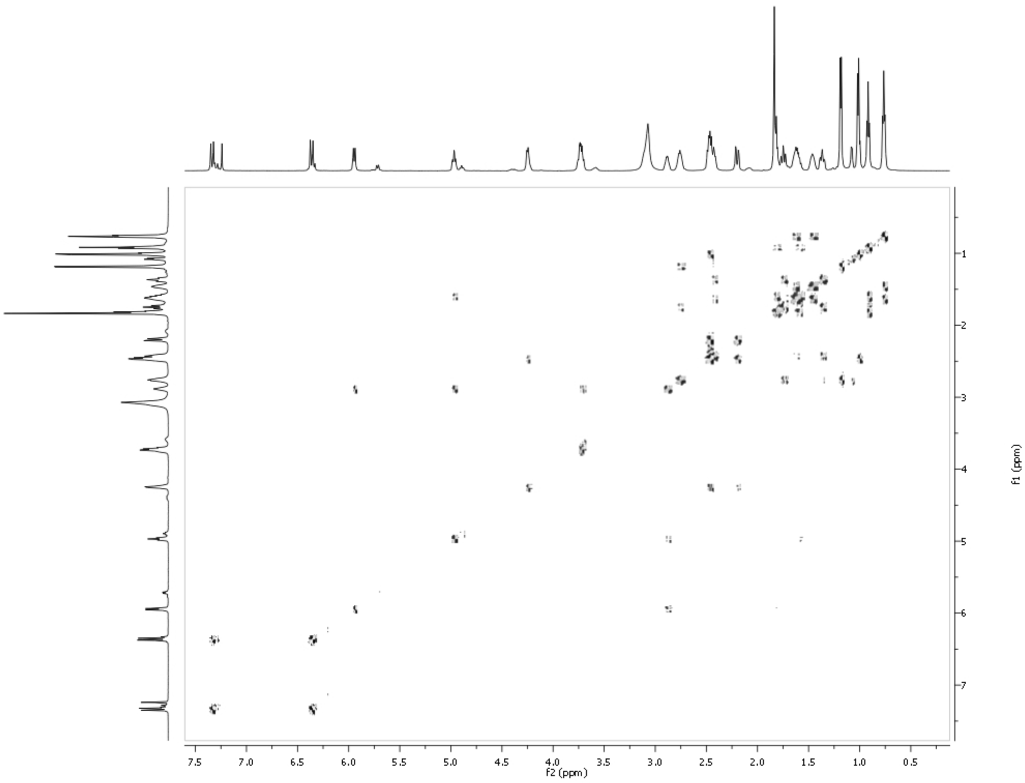

Figure S32 ^{1}H-^{1}H COSY spectrum of **9** in $CDCl_3$

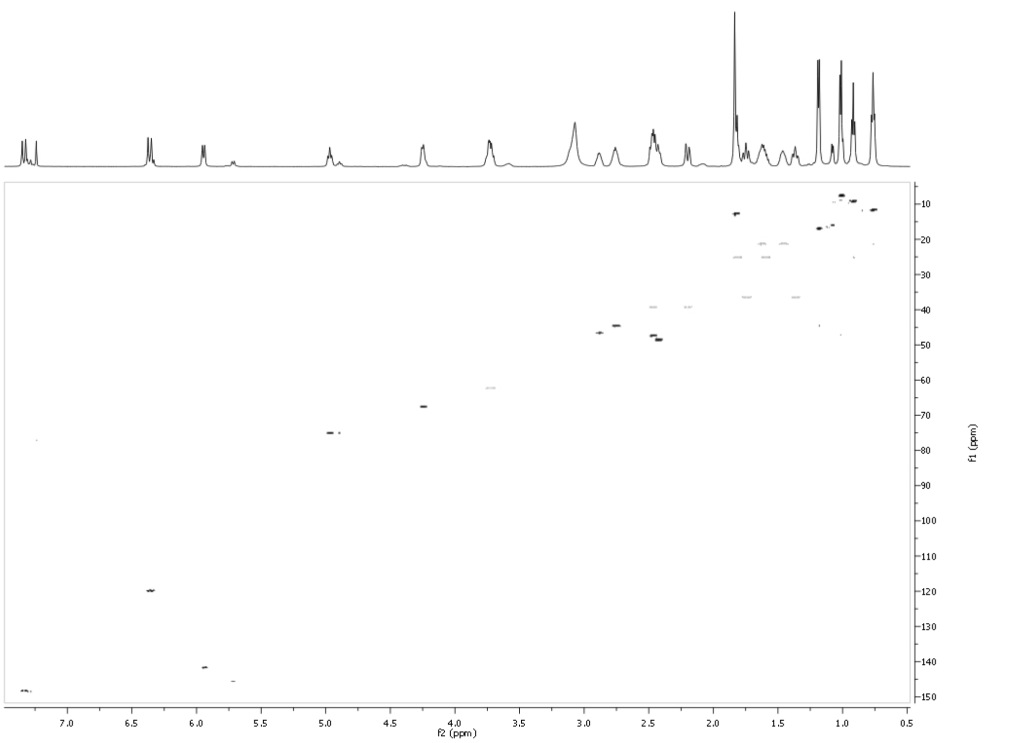

Figure S33 HSQC spectrum of **9** in $CDCl_3$

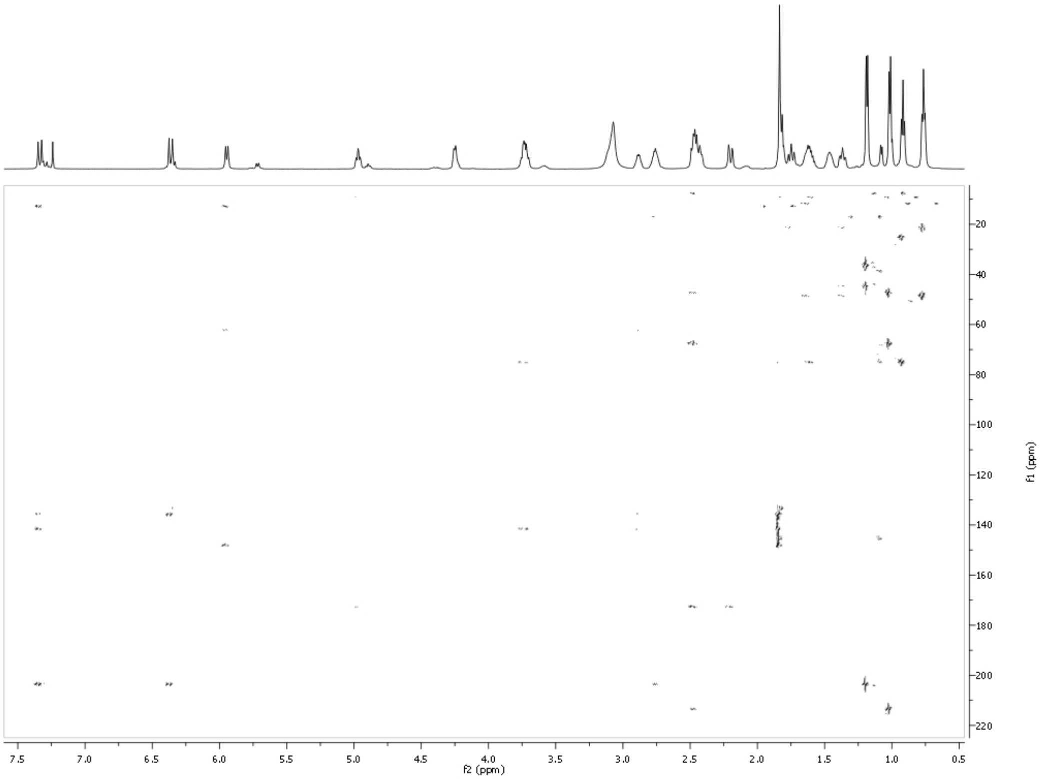

Figure S34 HMBC spectrum of **9** in $CDCl_3$

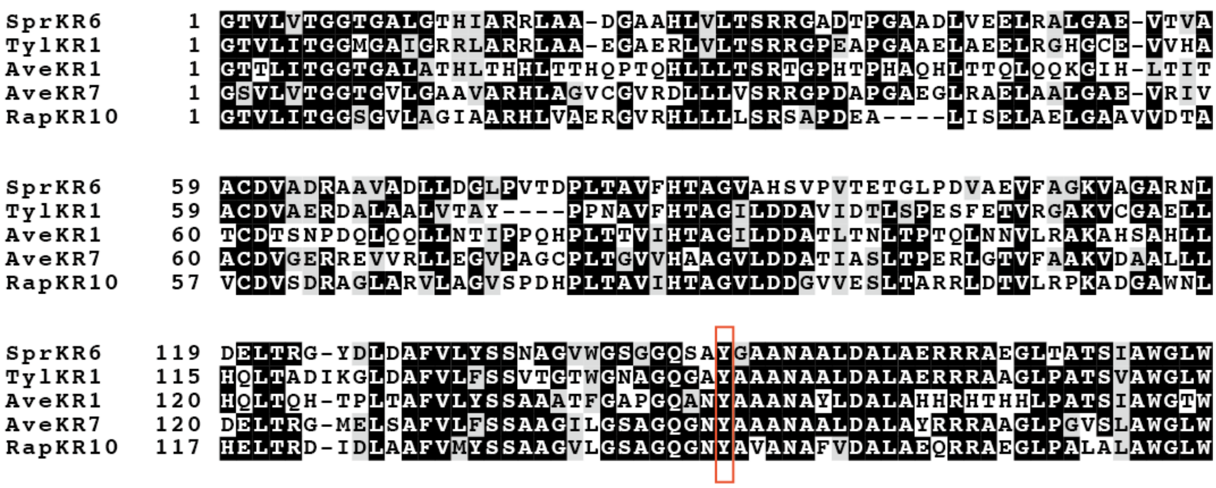

Figure S35 Alignment of SprKR6 (KR in the module 7) with known ketoreductase domains. The red frame shows the catalytic tyrosine residue.

Table S1 Gene organization of *spr* cluster

Gene	Size (a.a.)	Predicted function	Closest homologue (% Protein identity)	Accession Number
Spr1	210	α/β hydrolase	-	H303DRAFT_03279
Spr2	211	Thioesterase		H303DRAFT_03280
Spr3	550	ABC transporter		H303DRAFT_03281
Spr4	372	Aminotransferase DesV		H303DRAFT_03282
Spr5	405	P-450 RosC		H303DRAFT_03283
Spr6	403	P-450 RosD		H303DRAFT_03284
Spr7	4428	PKS (KSq-AT-ACP-KS-AT-KR-ACP-KS-AT-DH-KR-ACP)	*Micromonospora carbonacea* (74)	AX697987
Spr8	1889	PKS (KS-AT-DH-KR-ACP)	*Micromonospora carbonacea* (74)	AX697989
Spr9	3719	PKS (KS-AT-KR-ACP-KS-AT-DH-ER-KR-ACP)	*Micromonospora carbonacea* (77)	AX697991
Spr10	1574	PKS (KS-AT-KR-ACP)	*Micromonospora carbonacea* (80)	AX697993
Spr11	1796	PKS (KS-AT-KR-ACP-TE)	*Micromonospora carbonacea* (77)	AX697995
Spr12	423	P-450 DesVIII		H303DRAFT_04085
Spr13	440	Glycosyltransferase DesVII		H303DRAFT_04084
Spr14	238	Methyltransferase DesVI		H303DRAFT_04083
Spr15	67	Hypothetical protein		H303DRAFT_04082
Spr16	671	Transcriptional regulator		H303DRAFT_04081
Spr17	404	Transposase		H303DRAFT_04080
Spr18	482	GTP binding		H303DRAFT_04079
Spr19	381	Aminotransferase DesII		H303DRAFT_04078

Spr20	480	Fe-S oxidoreductase DesI		H303DRAFT_04077
Spr21	277	Dimethyladenosine transferase		H303DRAFT_04076
Spr22	329	Dehydratase Des IV		H303DRAFT_04075
Spr23	294	Pyrophosphorylase DesIII		H303DRAFT_04074

Table S2 ^{1}H-NMR data for **1** in CD_3OD (δ in ppm, *J* in Hz)[a]

No.	δ_H
6	6.37 (d, 1H, *J* = 15.6)
7	7.09 (d, 1H, *J* = 15.6)
9	5.81 (d, 1H, *J* = 9.6)
10	2.69 (m, 1H)
11	3.40 (ddd, 1H, *J* = 4.2, 4.2, 8.4)
12	1.39 (m, 1H), 1.49 (m, 1H)
13	0.96 (t, 3H, *J* = 7.2)
14	1.95 (s, 3H)
15	2.06 (s, 3H)
16	1.88 (s, 3H)
17	1.05 (d, 3H, *J* = 8.4)

[a] **1** was identified as salinipyrone A by comparing its ^{1}H chemical shift values and optical rotation values with the data reported in Oh, D.; Gontang, E. A.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Nat. Prod*. **2008**, *71*, 570.

Table S3 ^{1}H- and ^{13}C-NMR data for **3** in $CDCl_3$ (δ in ppm, J in Hz)[a]

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	173.5	16	1.05 (d, 3H, J = 6.0)	8.9
2	2.08 (d, 1H, J = 16.8), 2.60 (m, 1H)	39.3	17	2.41 (1H, m), 2.82 (1H, m)	43.7
3	3.84 (br d, J = 10.8, 1H)	66.8	18	9.67 (1H, s)	202.6
4	1.75 (m, 1H)	40.3	19	1.16 (d, 3H, J = 6.6)	17.3
5	3.70 (br d, J = 9.0, 1H)	81.6	20	1.43 (s, 3H)	14.8
6	1.97 (br d, J = 11.4, 1H)	31.0	21	1.12 (d, 3H, J = 6.6)	14.5
7	1.45 (m, 2H)	31.1	22	1.50 (m, 1H), 1.75 (m, 1H)	24.4
8	2.54 (m, 1H)	45.2	23	0.87 (t, 3H, J = 7.2)	8.9
9	-	200.7	1'	4.29 (d, 1H, J = 6.6)	103.1
10	6.44 (d, 1H, J = 15.6)	122.9	2'	3.48 (m, 1H)	69.1
11	6.53 (d, 1H, J = 15.6)	151.1	3'	3.29 (m, 1H)	66.2
12	-	59.7	4'	1.42 (m, 2H)	30.8
13	2.79 (m, 1H)	67.5	5'	3.53 (m, 1H)	67.9
14	1.67 (m, 1H)	37.5	6'	1.24 (d, 3H, J = 6.6)	20.5
15	4.86 (m, 1H)	76.9	7', 8'	2.83 (s, 6H)	41.0

[a] **3** was identified as rosamicin A by comparing its ^{1}H and ^{13}C chemical shift values and optical rotation values with the data reported in Nakajima, S., Kojiri, K., Morishima, H., and Okanishi, M. *J. Antibiot*. **1990**, *43*, 1006; and US patent 4,161,523, 1979.

Table S4 ^{1}H- and ^{13}C-NMR data for **4** in CD_3OD (δ in ppm, *J* in Hz)

No.	δ_H	δ_C
1	-	166.2
2	-	98.8
3	-	166.0
4	-	108.7
5	-	152.3
6	6.49 (d, 1H, *J* = 15.6)	115.2
7	7.09 (d, 1H, *J* = 15.6)	138.3
8	-	135.3
9	5.71 (d, 1H, *J* = 10.2)	136.4
10	3.71 (m, 1H)	46.2
11	-	212.2
12	2.54 (m, 2H)	33.6
13	1.01 (t, 3H, *J* = 7.2)	6.5
14	1.96 (s, 3H)	7.6
15	2.07 (s, 3H)	8.0
16	1.97 (s, 3H)	11.1
17	1.18 (d, 3H, *J* = 7.2)	15.2

Table S5 ^{1}H- and ^{13}C-NMR data for **5** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	174	16	1.05 (br s, 3H)	9.3
2	2.60 (m, 2H)	39.5	17	1.45 (m, 2H)	31.1
3	3.79 (m, 1H)	67.0	18	3.61 (m, 1H), 3.70 (m, 1H)	60.7
4	1.72 (m, 1H)	44.7	19	1.16 (d, 3H, J = 6.6)	17.4
5	3.74 (m, 1H)	81.4	20	1.44 (s, 3H)	15.0
6	1.44 (m, 1H)	31.1	21	3.89 (dd, 1H, J = 3.0, 10.8), 3.84 (m, 1H)	61.5
7	1.63 (m, 2H)	28.9	22	1.60 (m, 1H), 1.88 (m, 1H)	24.9
8	2.71 (m, 1H)	45.0	23	0.89 (t, 3H, J = 7.2)	9.2
9	-	201.3	1'	4.38 (d, 1H, J = 7.2)	103.3
10	6.46 (d, 1H, J = 15.6)	123.6	2'	3.49 (m, 1H)	69.5
11	6.55 (d, 1H, J = 15.6)	150.6	3'	3.32 (m, 1H)	66.5
12	-	59.2	4'	1.45 (m, 1H), 1.99 (m, 1H)	31.2
13	3.15 (d, 1H, J = 9.6)	64.8	5'	3.62 (m, 1H)	68.0
14	1.75 (m, 1H)	20.6	6'	1.27 (d, 3H, J = 6.0)	20.6
15	5.23 (m, 1H)	73.8	7', 8'	2.84 (s, 6H)	39.8

Table S6 ^{1}H- and ^{13}C-NMR data for **6** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	174.2	16	1.08 (br s, 3H)	9.4
2	2.61 (m, 2H)	39.2	17	1.48 (m, 2H)	31.3
3	3.83 (m, 1H)	67.1	18	3.62 (m, 1H), 3.69 (m, 1H)	60.8
4	1.75 (m, 1H)	40.5	19	1.16 (d, 3H, J = 6.6)	17.5
5	3.74 (m, 1H)	81.6	20	1.71 (s, 3H)	14.2
6	1.47 (m, 1H)	32.8	21	1.42 (s, 3H)	25.9
7	1.71 (m, 2H)	32.8	22	1.82 (m, 2H)	20.5
8	2.68 (m, 1H)	45.2	23	0.89 (t, 3H, J = 7.2)	10.3
9	-	201.1	1'	4.37 (d, 1H, J = 6.6)	103.3
10	6.47 (brs, 1H)	123.7	2'	3.50 (dd, 1H, J = 7.8, 9.6)	69.6
11	6.47 (brs, 1H)	150.6	3'	3.28 (m, 1H)	66.7
12	-	60.9	4'	1.46 (m, 1H), 2.03 (m, 1H)	31.4
13	2.80 (s, 1H)	68.8	5'	3.60 (m, 1H)	68.0
14	-	74.3	6'	1.27 (d, 3H, J = 6.0)	20.7
15	5.01 (m, 1H)	78.3	7', 8'	2.85 (s, 6H)	39.8

Table S7 ^{1}H- and ^{13}C-NMR data for **7** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	173.2	16	1.06 (d, 3H, J = 7.2)	9.1
2	2.64 (m, 2H)	39.5	17	2.32 (m, 1H), 2.91 (m, 1H)	43.9
3	3.84 (m, 1H)	61.5	18	9.67 (s, 1H)	202.7
4	1.74 (m, 1H)	44.8	19	1.17 (d, 3H, J = 7.2)	17.4
5	3.71 (m, 1H)	81.9	20	1.45 (s, 3H)	15.0
6	1.46 (m, 1H)	31.3	21	3.89 (m, 1H), 3.90 (dd, 1H, J = 3.0, 10.8)	61.6
7	1.69 (m, 2H)	31.8	22	1.56 (m, 1H), 1.84 (m, 1H)	24.9
8	2.56 (m, 1H)	45.2	23	0.90 (t, 3H, J = 7.2)	9.1
9	-	200.7	1'	4.30 (d, 1H, J = 7.2)	103.6
10	6.46 (d, 1H, J = 15.6)	123.5	2'	3.48 (m, 1H)	69.3
11	6.55 (d, 1H, J = 15.6)	151.8	3'	3.24 (m, 1H)	66.8
12	-	59.0	4'	1.43 (m, 1H), 1.99 (m, 1H)	31.3
13	3.14 (m, 1H)	64.8	5'	3.54 (m, 1H)	68.0
14	1.75 (m, 1H)	20.3	6'	1.25 (d, 3H, J = 5.4)	20.7
15	5.24 (m, 1H)	73.7	7', 8'	2.83 (s, 6H)	39.0

Table S8 ^{1}H- and ^{13}C-NMR data for **8** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	173.9	16	1.07 (d, 3H, J = 6.6)	9.2
2	2.64 (d, 2H, J = 10.2)	39.4	17	2.45 (m, 1H), 2.91 (m, 1H)	43.8
3	3.88 (m, 1H)	67.0	18	9.67 (s, 1H)	202.7
4	1.77 (m, 1H)	40.6	19	1.16 (d, 3H, J = 7.2)	17.5
5	3.70 (m, 1H)	81.8	20	1.71 (s, 3H)	14.1
6	1.45 (m, 1H)	31.4	21	1.42 (s, 3H)	25.9
7	1.78 (m, 2H)	31.9	22	1.82 (m, 2H)	20.7
8	2.63 (m, 1H)	45.4	23	0.90 (t, 3H, J = 7.2)	10.2
9	-	200.5	1'	4.29 (d, 1H, J = 7.2)	103.3
10	6.49 (d, 1H, J = 15.6)	123.4	2'	3.49 (m, 1H)	69.3
11	6.51 (d, 1H, J = 15.6)	151.0	3'	3.26 (m, 1H)	66.6
12	-	61.0	4'	1.46 (m, 1H), 2.01 (m, 1H)	31.2
13	2.91 (s, 1H)	68.7	5'	3.54 (m, 1H)	67.9
14	-	74.3	6'	1.25 (d, 3H, J = 6.0)	20.7
15	5.01 (dd, 1H, J = 2.4, 10.8)	78.3	7', 8'	2.83 (s, 6H)	39.3

Table S9 ^{1}H- and ^{13}C-NMR data for **9** in $CDCl_3$ (δ in ppm, J in Hz)

No.	δ_H	δ_C	No.	δ_H	δ_C
1	-	172.8	16	1.02 (d, 3H, J = 6.6)	7.8
2	2.20 (dd, 1H, J = 3.0, 15.9), 2.47 (m, 1H)	39.4	17	1.46 (m, 1H), 1.62 (m, 1H)	21.5
3	4.25 (m, 1H)	67.7	18	0.77 (t, 3H, J = 6.6)	11.8
4	2.45 (m, 1H)	47.5	19	1.19 (d, 3H, J = 6.6)	17.1
5	-	213.5	20	1.84 (s, 3H)	12.9
6	2.42 (m, 1H)	48.7	21	3.71 (m, 1H), 3.74 (m, 1H)	62.4
7	1.37 (m, 1H), 1.75 (m, 1H)	36.6	22	1.61 (m, 1H), 1.82 (m, 1H)	25.2
8	2.76 (m, 1H)	44.8	23	0.92 (t, 3H, J = 7.2)	9.3
9	-	203.5			
10	6.36 (d, 1H, J = 15.6)	119.8			
11	7.34 (d, 1H, J = 15.6)	148.3			
12	-	135.8			
13	5.95 (d, 1H, J = 10.2)	141.7			
14	2.89 (m, 1H)	46.7			
15	4.97 (m, 1H)	75.1			

Table S10 Minimum inhibitory concentration (MIC) values (μg/mL) of **5-8** and erythromycin against a panel of medically-important human bacterial pathogens

[a] Testing performed in RPMI with 5% LB, [b]Testing performed in CA-MHB media with 5% lysed horse blood

	5	**6**	**7**	**8**	**erythromycin**
Acinetobacter baumannii **ATCC17978[a]**	50	100	50	50	1.56
Uropathogenic *Escherichia coli* CFT073[a]	50	50	100	50	6.25
Pseudomonas aeruginosa **PA01[a]**	50	100	100	25	6.25
Streptococcus pyogenes **5448[b]**	50	>100	100	6.125	0.39
Streptococcus pyogenes **NZ131[b]**	50	>100	50	6.125	0.19
Staphylococcus aureus **USA300[b]**	12.5	25	6.25	1.56	1.56