

Supporting Information

for

Identification of cyclic depsipeptides and the dedicated synthetase from *Hapsidospora irregularis*

Shuwei Zhang,[†] Yixing Qiu,^{†,‡} Thomas B. Kakule,[§] Zhenyu Lu,[⊥] Fuchao Xu,[†] John G. Lamb,[⊥]
Christopher A. Reilly,[⊥] Yong Zheng,^{||} Shing Wo Simon Sham,[∇] Wei Wang,^{‡*} Lijiang Xuan,^{||}
Eric W. Schmidt[§] and Jixun Zhan^{†,‡*}

[†] Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322, USA

[‡] TCM and Ethnomedicine Innovation & Development Laboratory, School of Pharmacy, Hunan University of Chinese Medicine, Changsha, Hunan 410208, China

[§] Department of Medicinal Chemistry, L. S. Skaggs Pharmacy Institute, University of Utah, Salt Lake City, UT 84112, USA

[⊥] Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, USA

^{||} State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haik Road, Shanghai 202203, China

[∇] Department of Chemistry and Biochemistry, Utah State University, 0300 Old Main Hill, Logan, UT 84322, USA

E-mail: jixun.zhan@usu.edu; wangwei402@hotmail.com

Table S1. Determination of the absolute configuration of amino and hydroxyl acid moieties in **1-7**.

Table S2. Ca²⁺ flux data for **1-7** in primary human lobar bronchial epithelial cells, HEK-293 TRP channel overexpressing cells, and BEAS-2B (immortalized human bronchial epithelial) cells.

Table S3. The extracted signature sequences of the A domains of LACS.

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Figure S29. ¹H NMR spectrum of **7** in CD₃OD.

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Figure S32. HSQC NMR spectrum of **7** in CD₃OD.

Figure S33. HMBC NMR spectrum of **7** in CD₃OD.

Figure S34. Hydrolysis and purification procedure of compounds **2**, **4**, and **6**.

Table S1. Determination of the absolute configuration of amino and hydroxyl acid moieties in **1-7** (recorded on a Rudolph Autopol IV polarimeter using a 10-cm microcell at 19 °C).

Compound	Fragment after hydrolysis	Conc. (g/100 mL)	Solvent	$[\alpha]_D^{19}$
1		1.00	Methanol	-97.8
2		0.30	Methanol	-99.0
	<i>N</i> -Me-L-Phe from 2A	0.095	6 N HCl	+21.1
	3-AIB from 2A	0.10	6 N HCl	-16.0
	L-Leu from 2B	0.15	6 N HCl	+15.7
3		1.17	Methanol	-48.2
4		0.25	Methanol	-68.0
	<i>R</i> -HICA from 4B	0.06	1 N NaOH	+21.7
	L-Val from 4B	0.09	6 N HCl	+27.8
5		0.62	Methanol	-96.1
6		0.50	Methanol	-46.0
	<i>S</i> -HICA from 6A	0.23	1 N NaOH	-23.5
	<i>R</i> -HICA from 6B	0.17	1 N NaOH	+21.8
	L-Leu from 6B	0.19	6 N HCl	+16.9
	L-Tyr from 6A	0.22	6 N HCl	-9.5
7		0.34	Methanol	-52.9
HICA = 2-hydroxy isocaproic acid; 3-AIB = 3-aminoisobutyric acid; Val = valine; Leu = leucine; Tyr = tyrosine; <i>N</i> -Me-L-Phe = <i>N</i> -methyl-L-phenylalanine.				

Table S2. Ca²⁺ flux data for **1-7** in primary human lobar bronchial epithelial cells, HEK-293 TRP channel overexpressing cells, and BEAS-2B (immortalized human bronchial epithelial) cells.

Compound	Cell Identity (Ca ²⁺ Response as % positive control)							
	LOBAR	HEK-293 TRPA1	HEK-293 TRPM8	HEK-293 TRPV3	HEK-293 TRPV4	HEK-293	BEAS-2B TRPV1	BEAS-2B
1	2 ± 1	4 ± 6	19 ± 5	10 ± 3	1 ± 1	10 ± 2	20 ± 20	21 ± 7
2	N.D.	N.D.	1 ± 2	4.40 ± 0.06	1 ± 1	3 ± 3	1 ± 1	10 ± 2
3	0.6 ± 0.4	41 ± 9 (p=0.008)	7 ± 2	6 ± 3	5 ± 2	13 ± 4	1 ± 1	N.D.
4	N.D.	20 ± 10	3 ± 5	2 ± 2	2 ± 1	2 ± 3	N.D.	N.D.
5	4 ± 3	3 ± 7	14 ± 9	8 ± 2	2.6 ± 0.6	N.D.	3 ± 1	8 ± 2
6	37 ± 3 (p=0.001)	88 ± 6 (p=0.0001)	21 ± 6 (p=0.058)	13 ± 3	1.5 ± 0.4	10 ± 4	8 ± 10	16 ± 6
7	N.D.	1 ± 2	10 ± 6	5 ± 2	3.9 ± 0.6	N.D.	1 ± 2	N.D.

Student's t-test, two-tailed p-value in parenthesis (n=3). Lobar cell response values were compared to the buffer only control. HEK-293 TRP-overexpressing cell response values were compared to the HEK-293 control value for the given compound. BEAS-2B TRPV1 cell responses were compared to the normal BEAS-2B response.

Table S3. The extracted signature sequences of the A domains of LACS.

A domain of LACS	Signature sequence	Substrate
A ₁	DIYYVSATAK	β -Alanine
A ₂	GANLIGATVK	<i>S</i> -Leucic acid
A ₃	DAHDIGAPIK	L-Leucine
A ₄	DGLFIGIPVK	<i>S</i> -Leucic acid
A ₅	DPWTYGAVVK	L-Phenylalanine

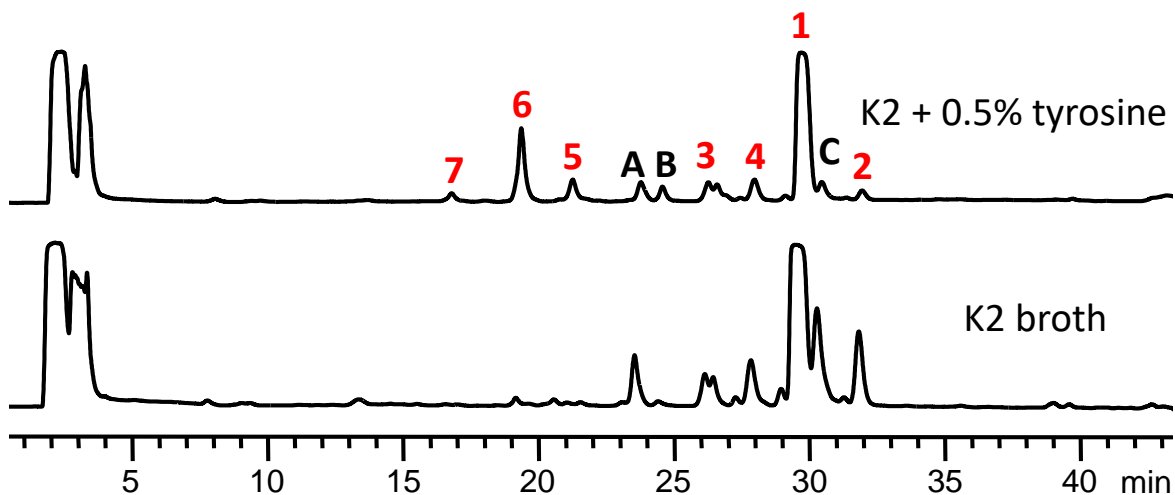


Figure S1. HPLC analysis of the metabolites of *Hapsidospora irregularis* FERM BP-2511 in K2 broth (bottom) and K2 broth supplemented with 0.5% L-tyrosine (top) at 210 nm. 1-7: compounds **1-7**, A: cephalosporin P1, B: isocephalosporin P1, C: mixture of the tetramic acids Sch210971 and Sch210972. HPLC condition: Agilent 1200 HPLC instrument with an Agilent XDB-C18 column (5 μ m, 4.6 mm \times 250 mm), eluted with a gradient of acetonitrile-water (0-5 min: 5%, 5-40 min: 5–100%, 40-45 min: 100%) with 0.1% formic acid over 45 min at a flow rate of 1 mL min⁻¹.

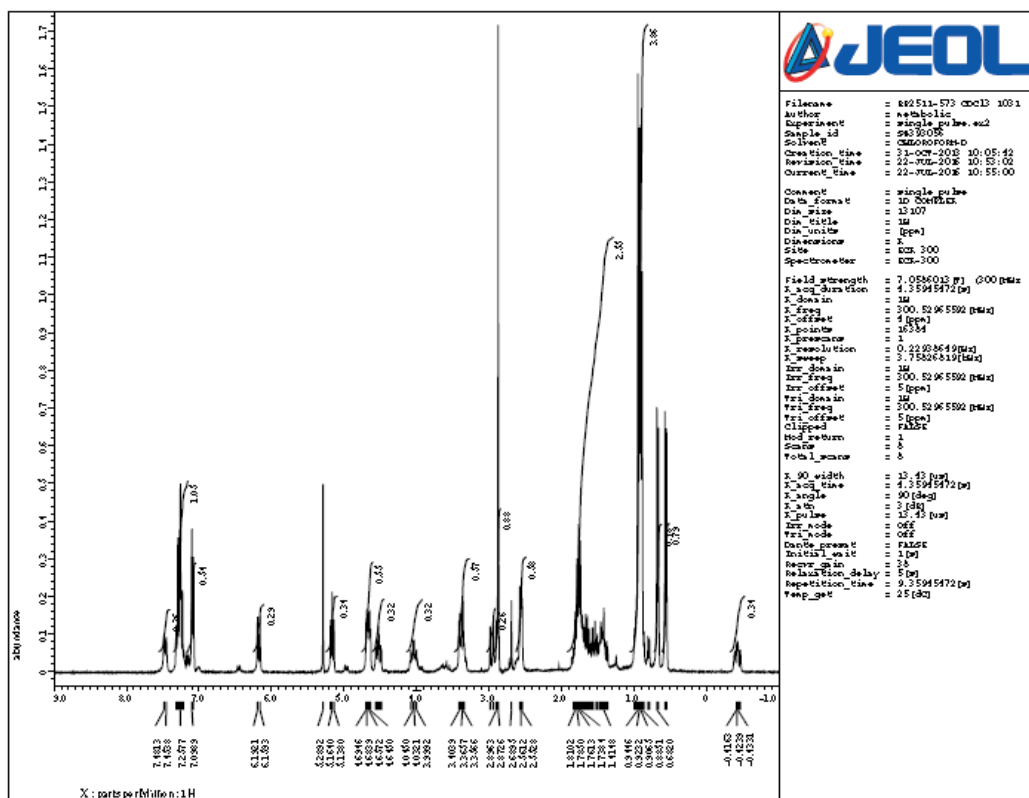


Figure S2. ¹H NMR spectrum of **1** in CDCl₃.

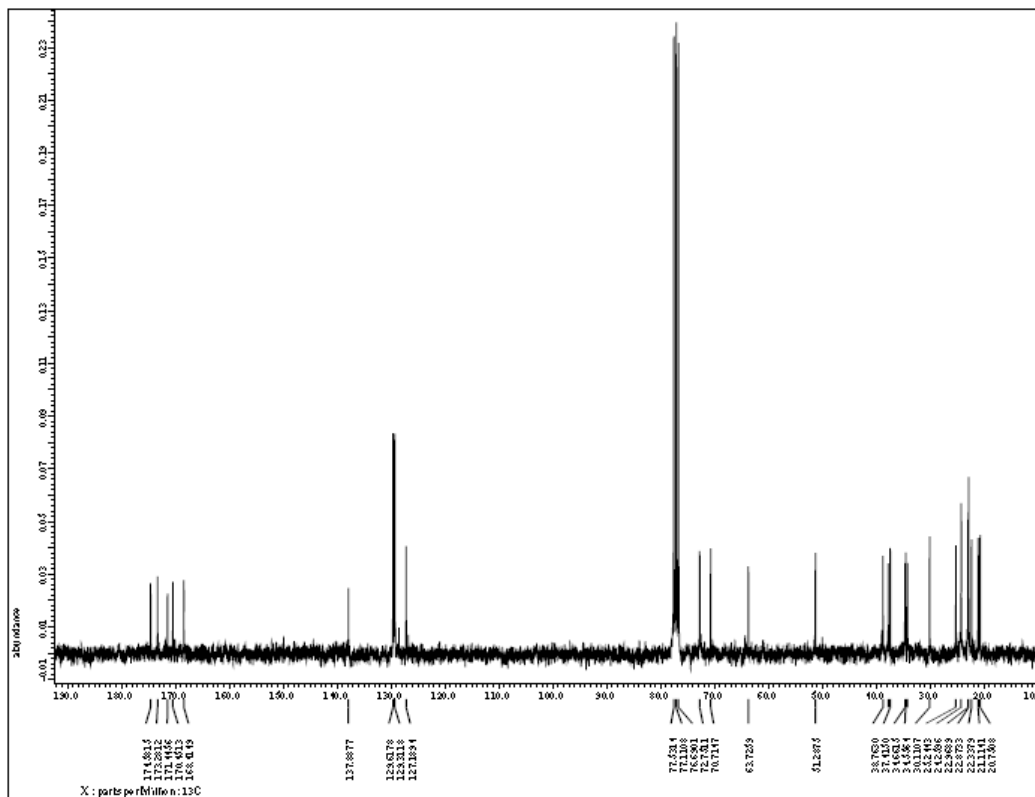


Figure S3. ^{13}C NMR spectrum of compound 1 in CDCl_3 .

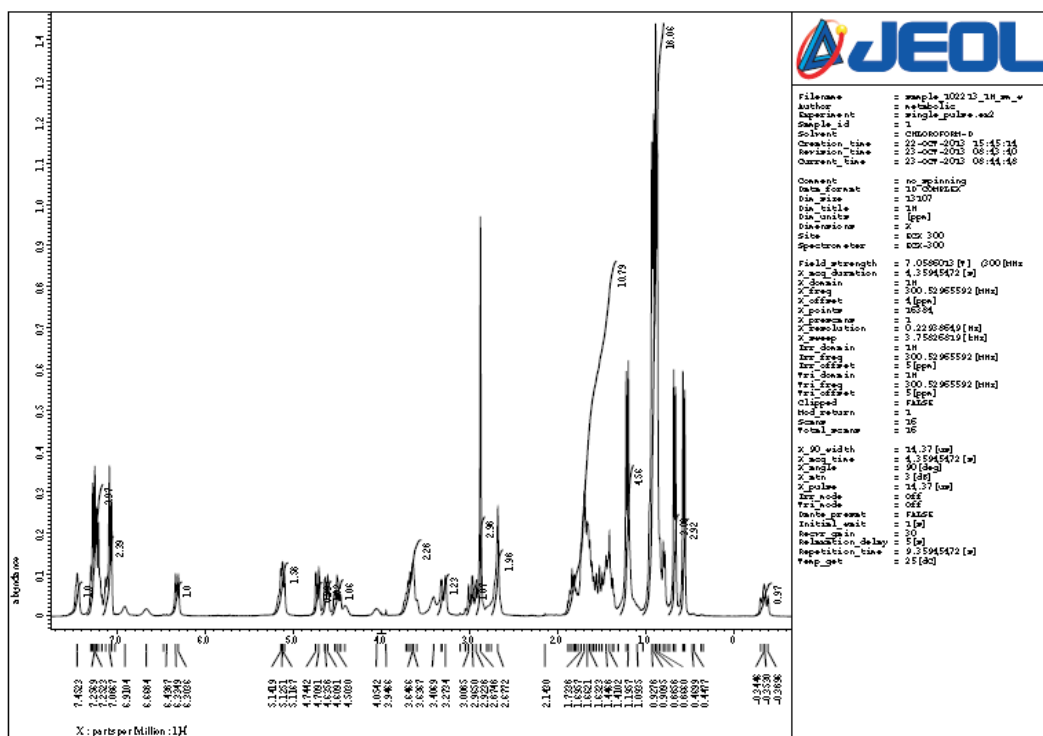


Figure S4. ^1H NMR spectrum of 2 in CDCl_3 .

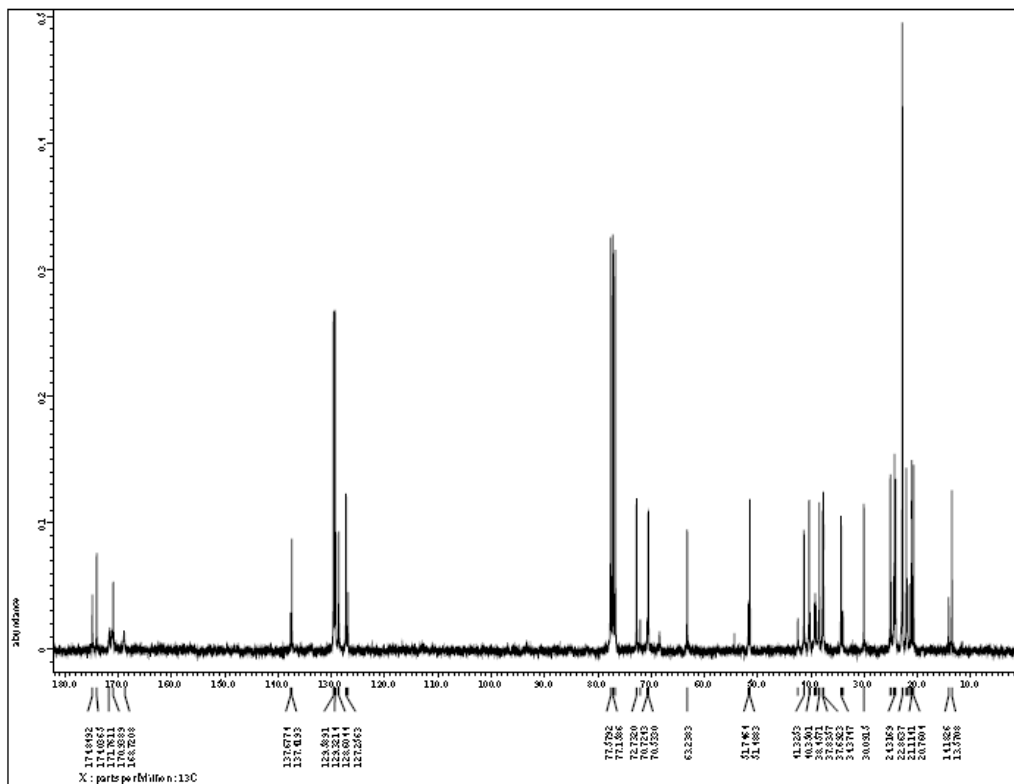


Figure S5. ¹³C NMR spectrum of 2 in CDCl₃.

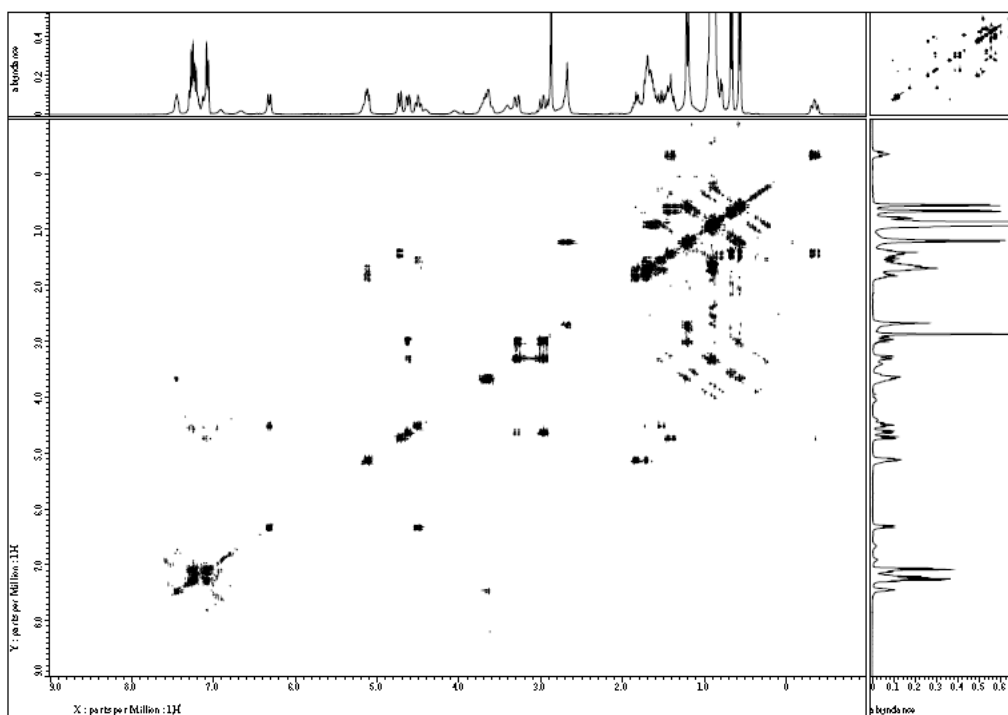


Figure S6. ¹H-¹H COSY NMR spectrum of 2 in CDCl₃.

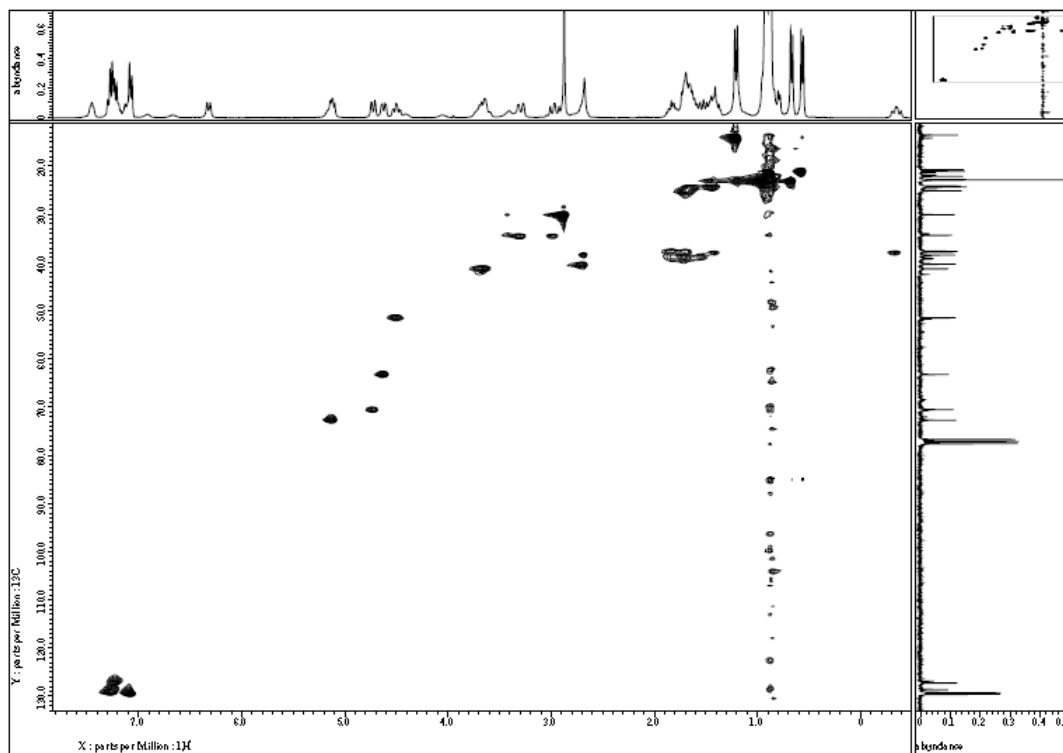


Figure S7. HSQC NMR spectrum of **2** in CDCl₃.

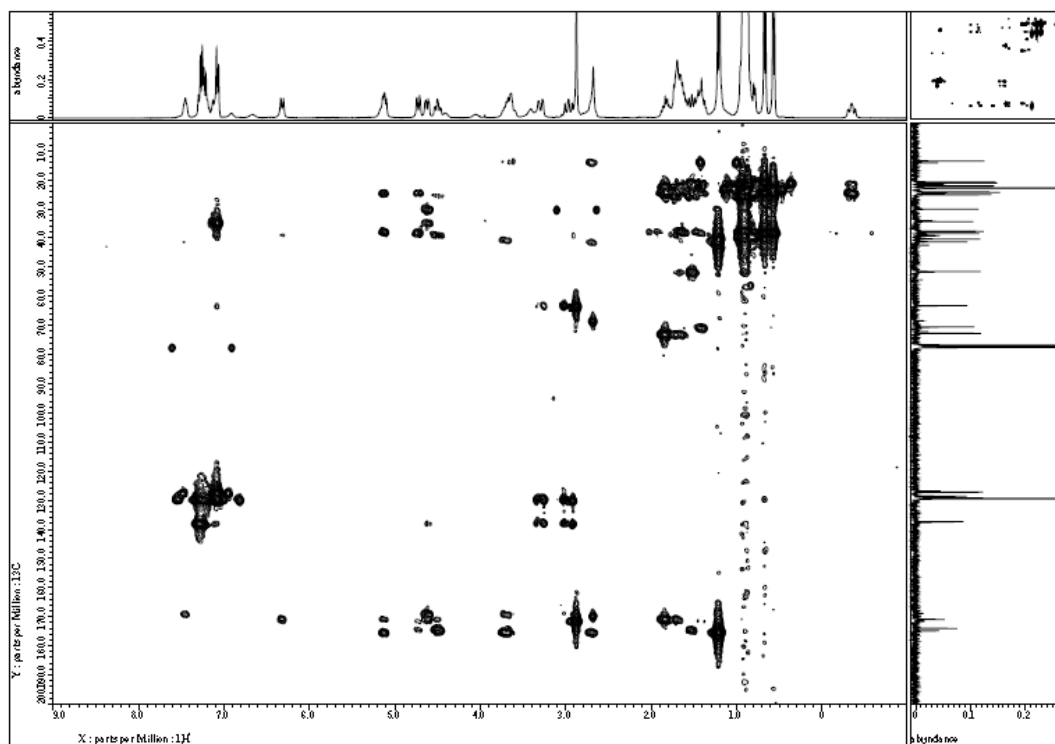


Figure S8. HMBC NMR spectrum of **2** in CDCl₃.

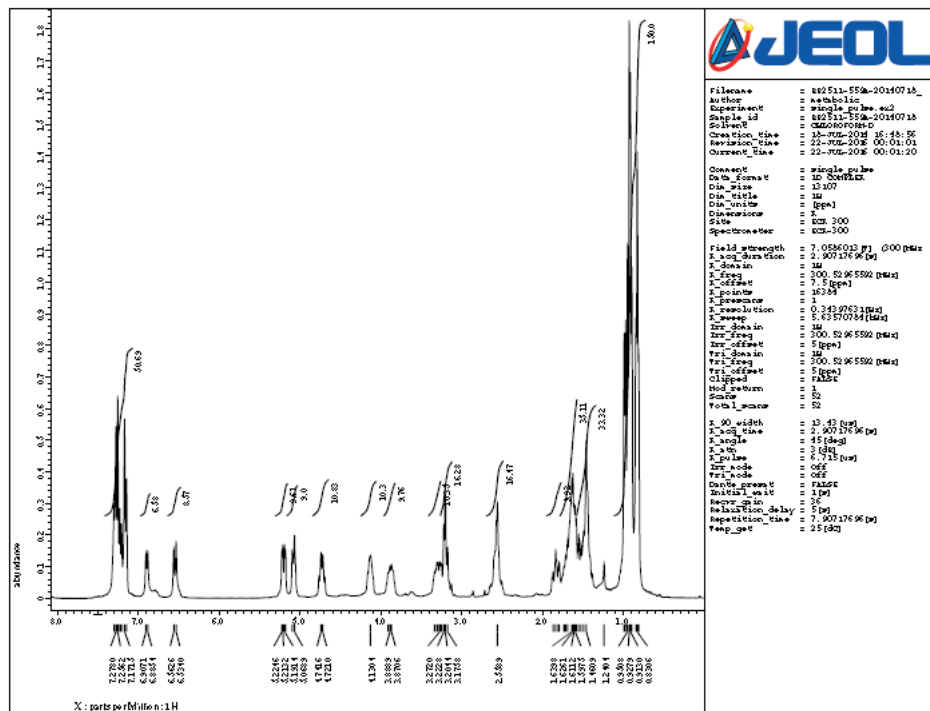


Figure S9. ^1H NMR spectrum of **3** in CDCl_3 .

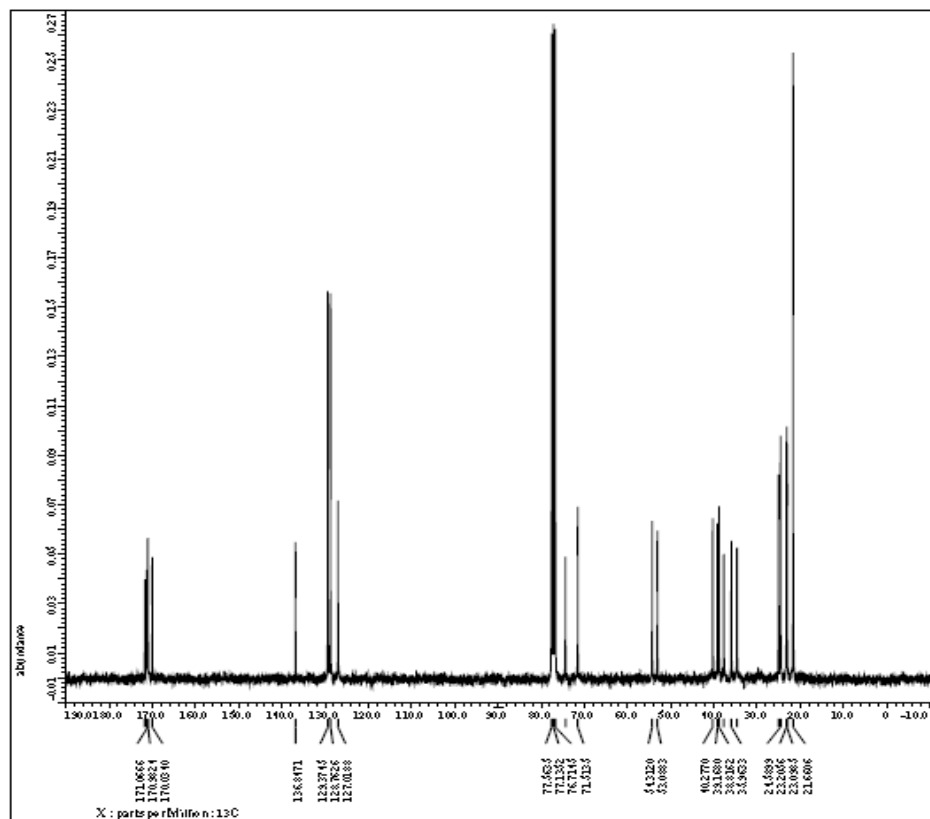


Figure S10. ^{13}C NMR spectrum of **3** in CDCl_3 .

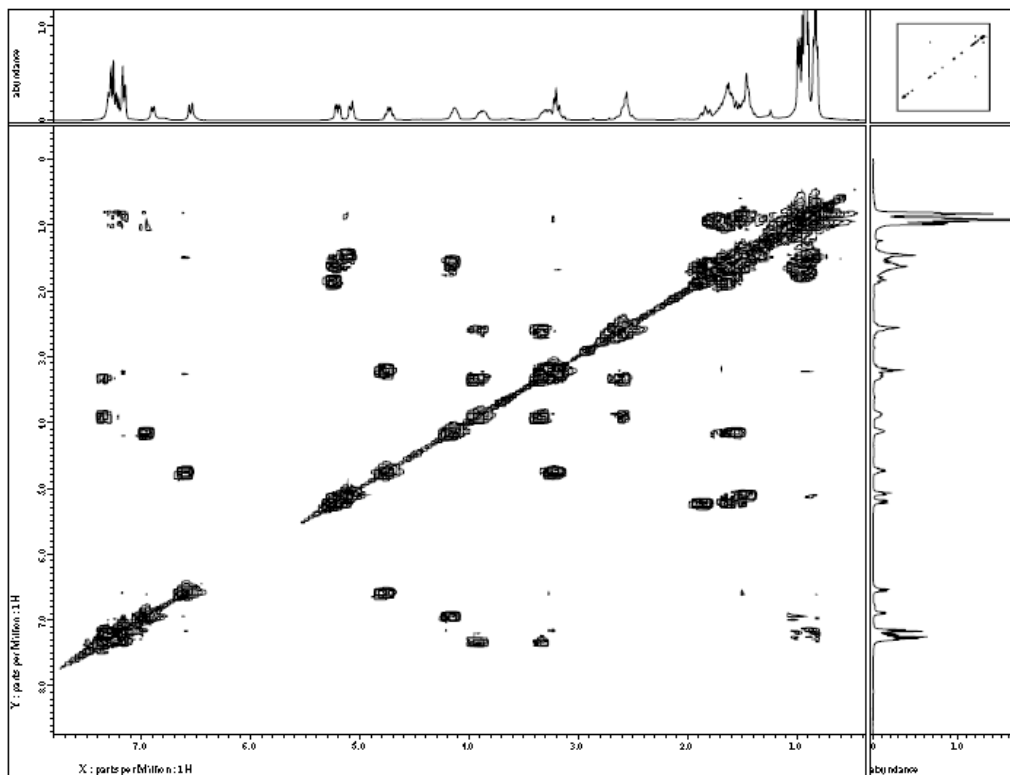


Figure S11. ^1H - ^1H COSY NMR spectrum of **3** in CDCl_3 .

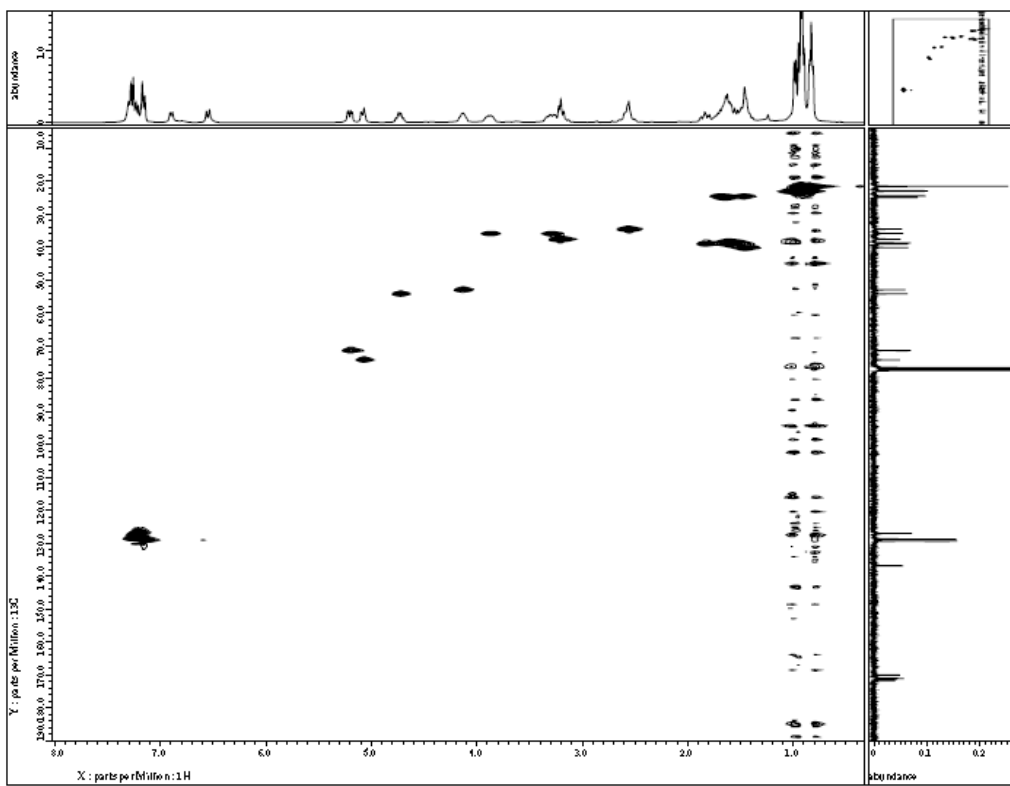


Figure S12. HSQC NMR spectrum of **3** in CDCl_3 .

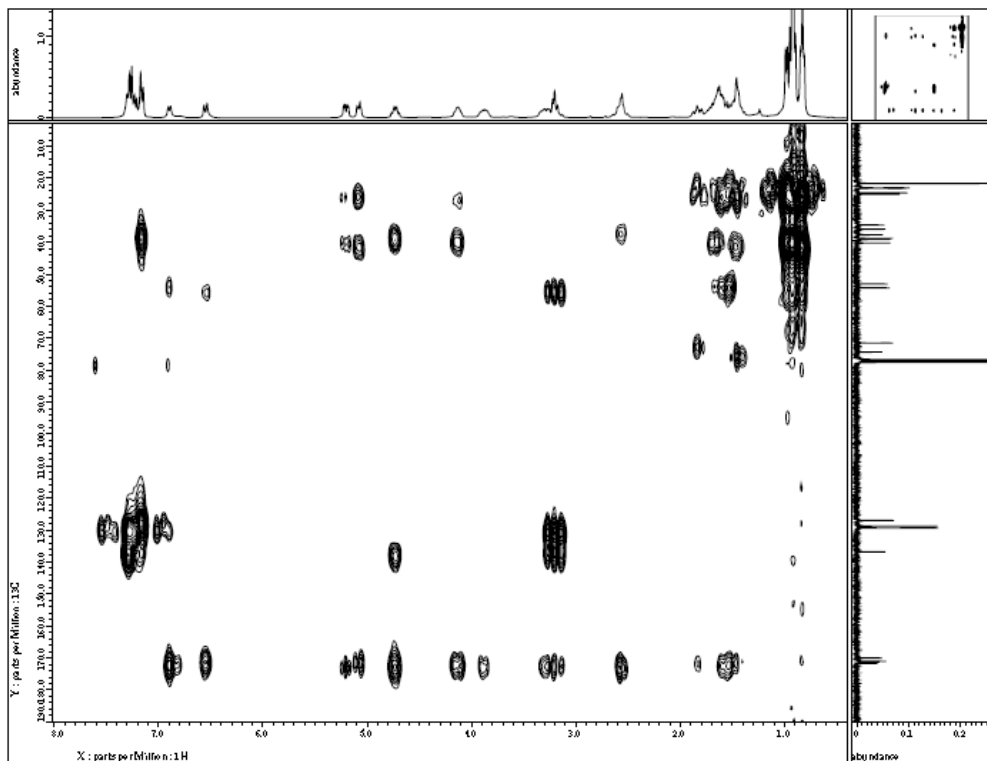


Figure S13. HMBC NMR spectrum of **3** in CDCl_3 .

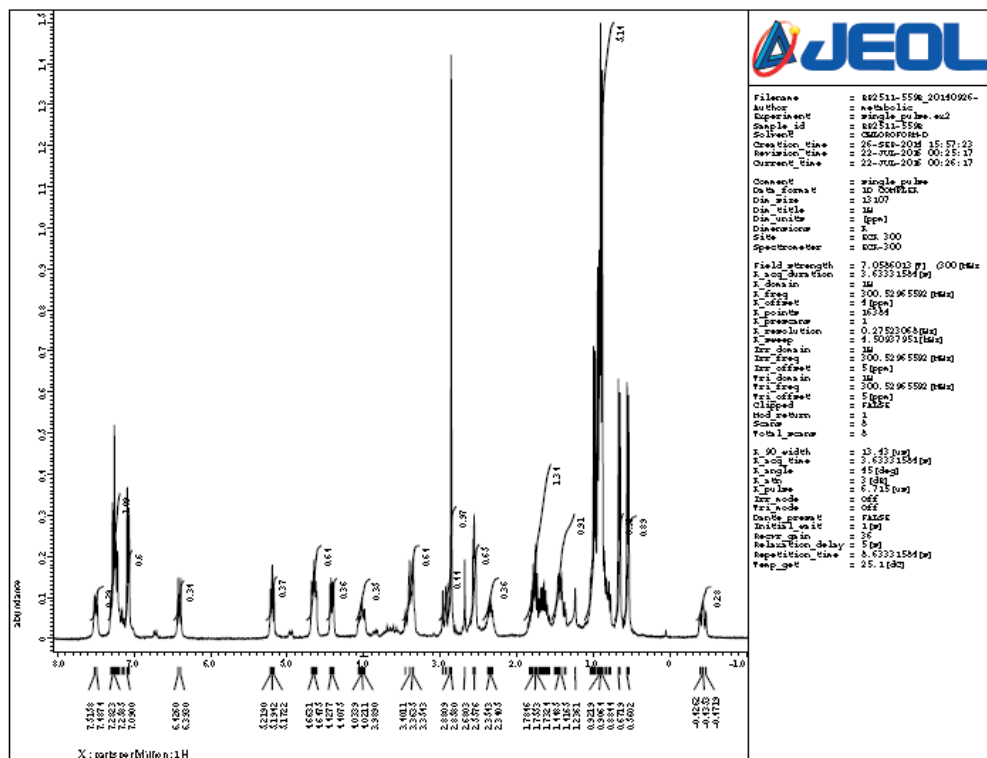


Figure S14. ^1H NMR spectrum of **4** in CDCl_3 .

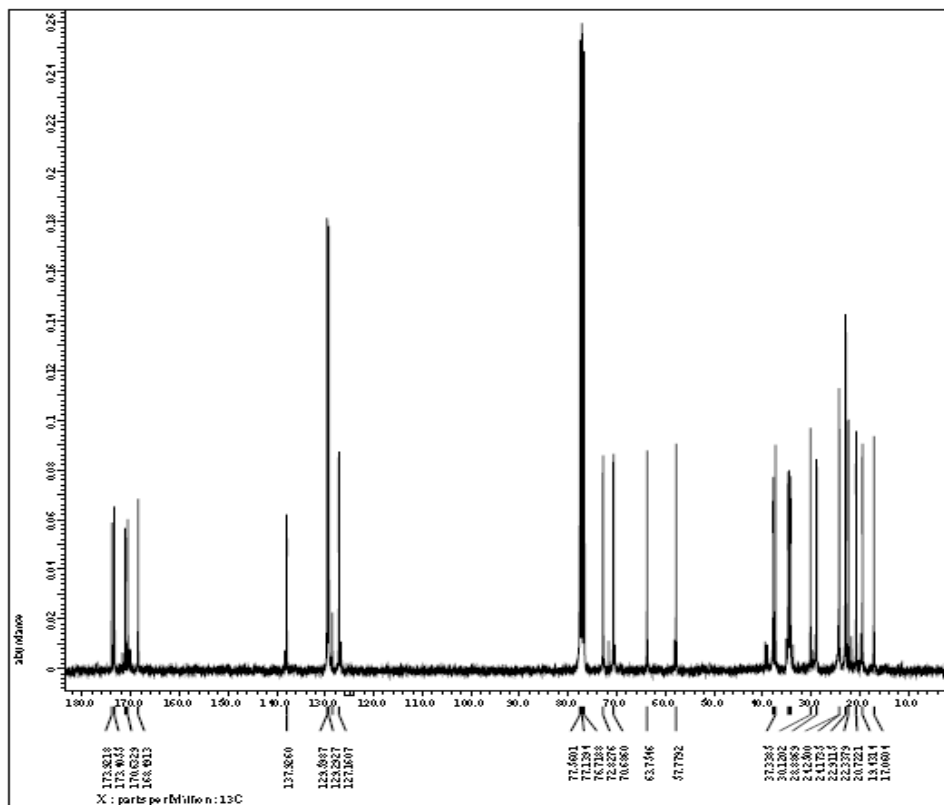


Figure S15. ^{13}C NMR spectrum of **4** in CDCl_3 .

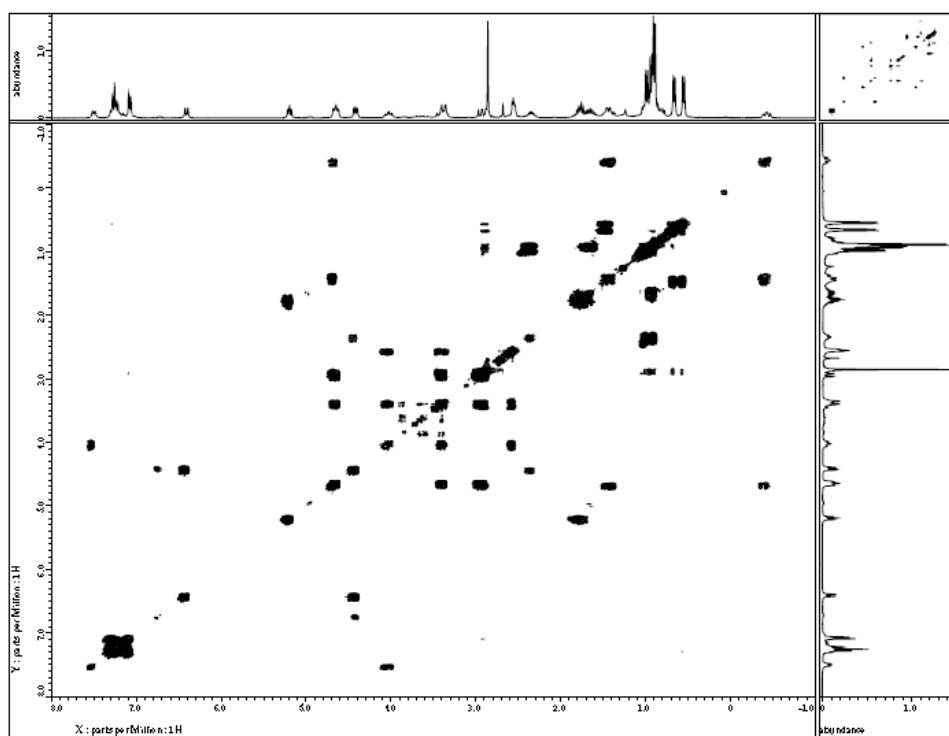


Figure S16. ^1H - ^1H COSY NMR spectrum of **4** in CDCl_3 .

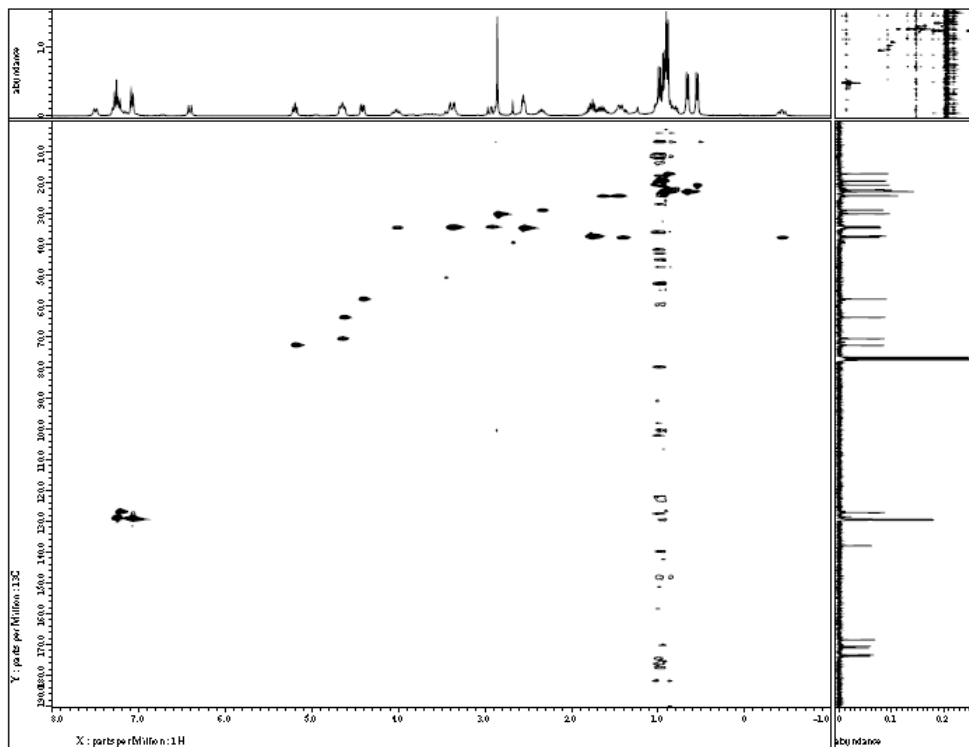


Figure S17. HSQC NMR spectrum of **4** in CDCl_3 .

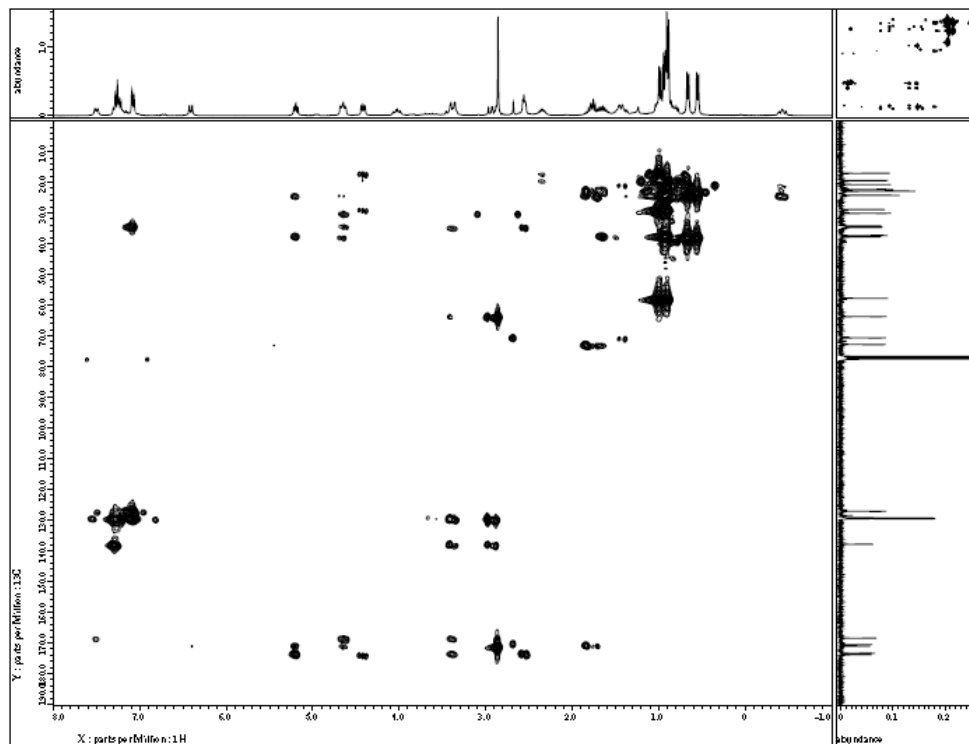


Figure S18. HMBC NMR spectrum of **4** in CDCl_3 .

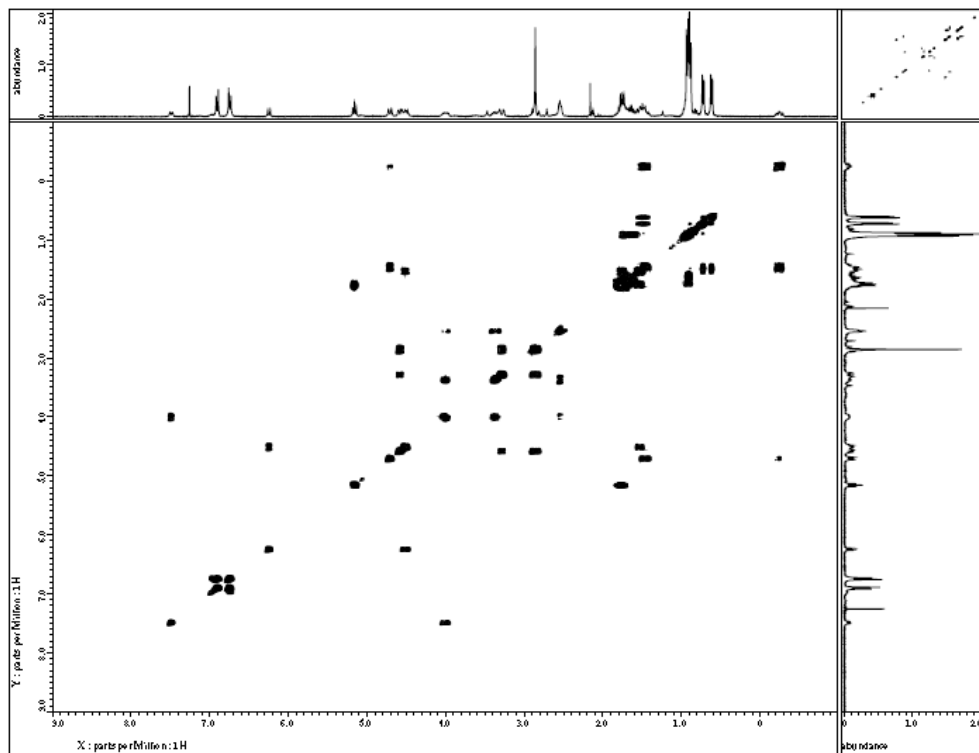


Figure S21. ^1H - ^1H COSY NMR spectrum of **5** in CDCl_3 .

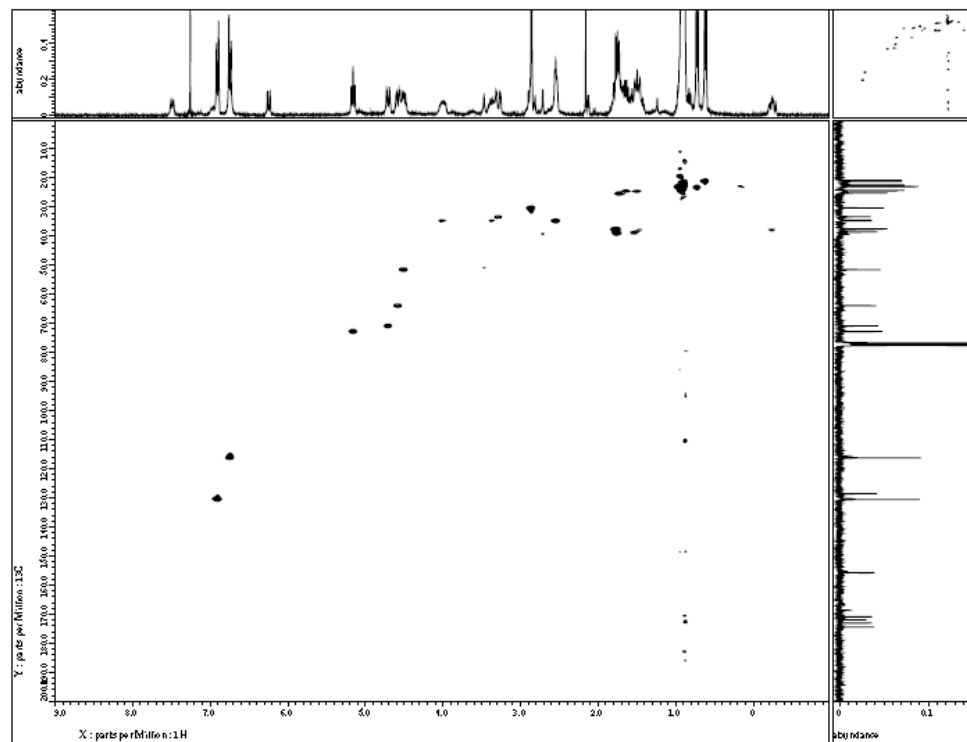


Figure S22. HSQC NMR spectrum of **5** in CDCl_3 .

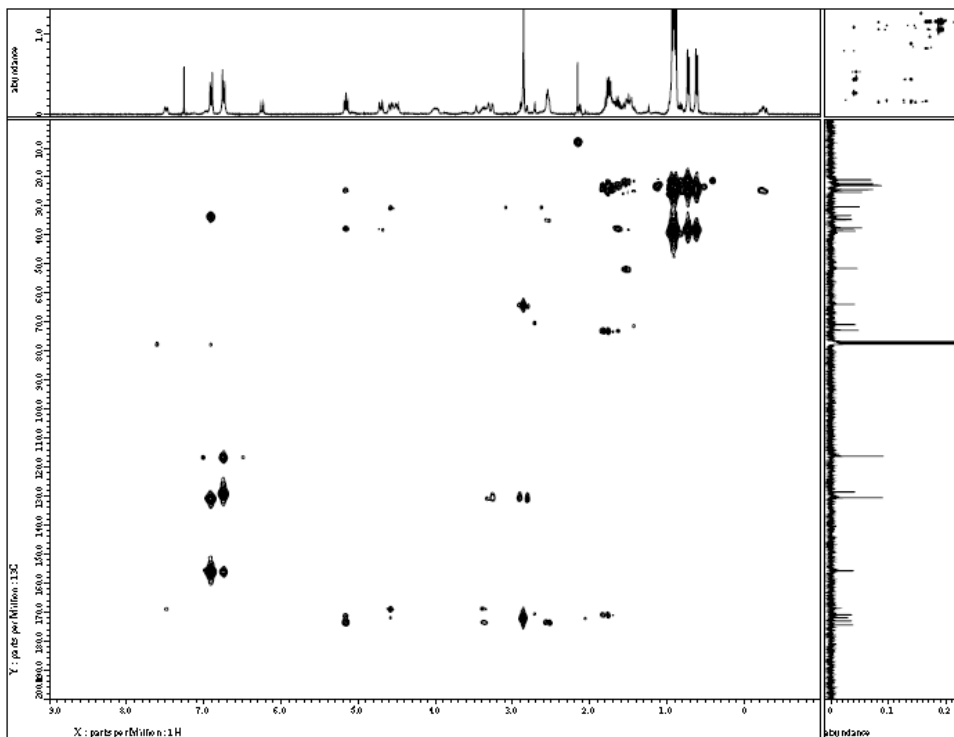


Figure S23. HMBC NMR spectrum of **5** in CDCl_3 .

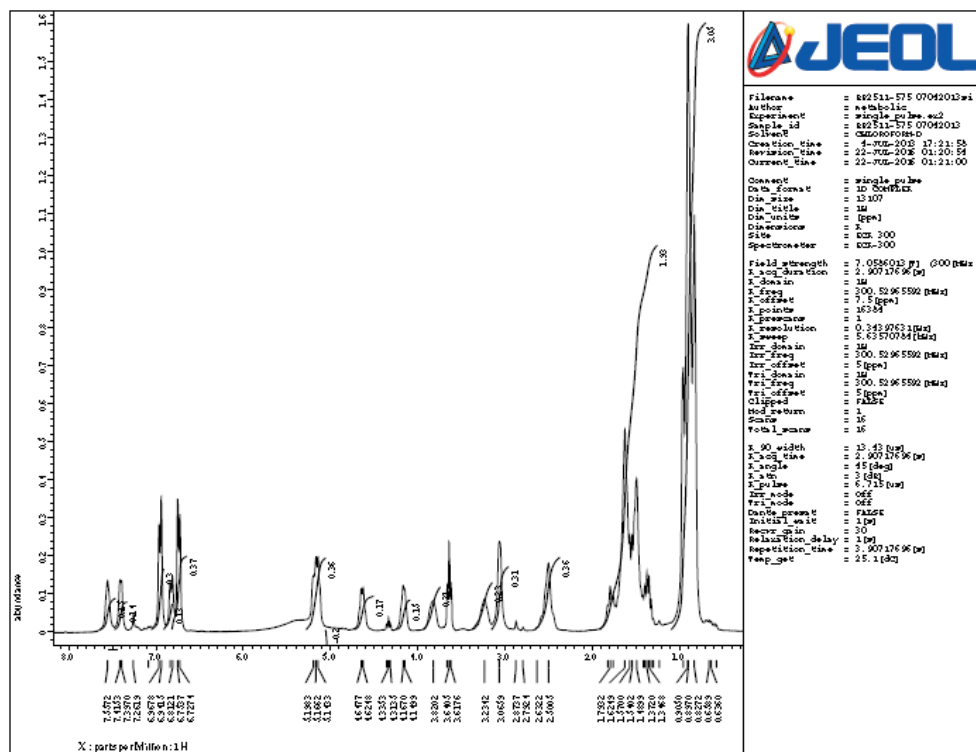


Figure S24. ^1H NMR spectrum of **6** in CDCl_3 .

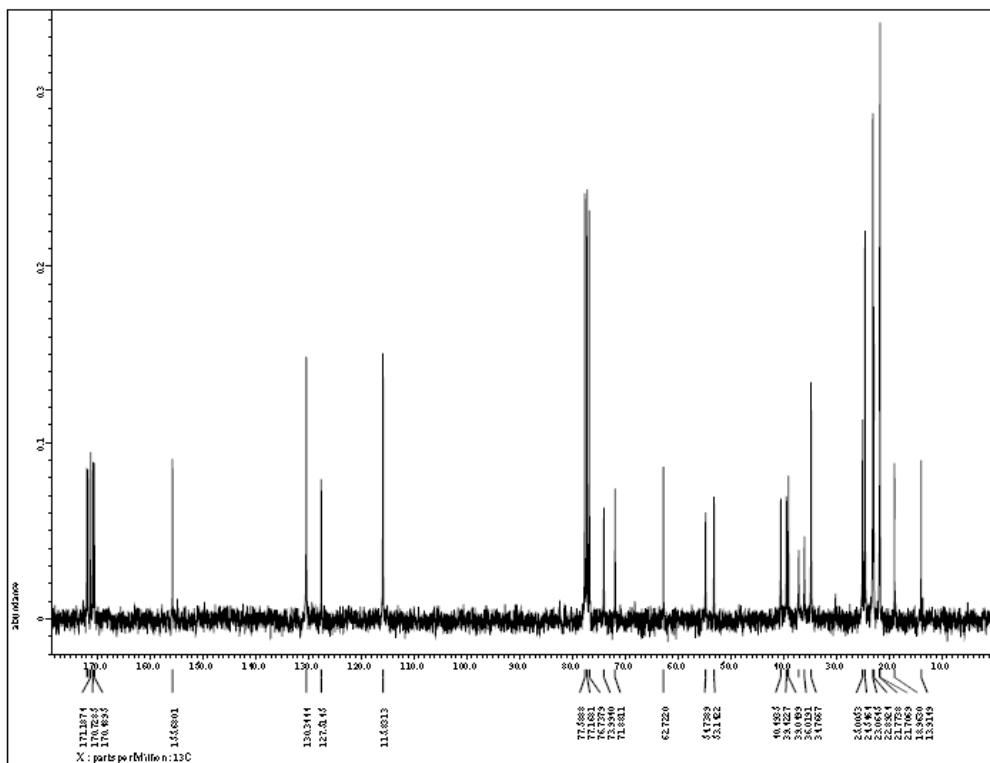


Figure S25. ^{13}C NMR spectrum of **6** in CDCl_3 .

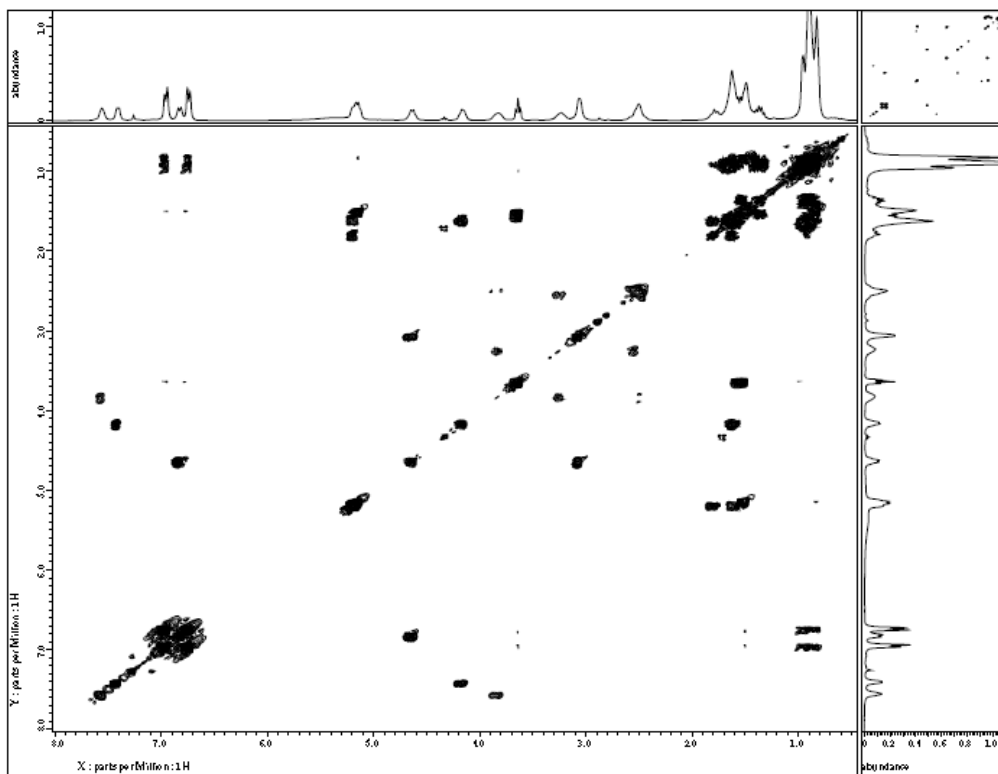


Figure S26. ^1H - ^1H COSY NMR spectrum of **6** in CDCl_3 .

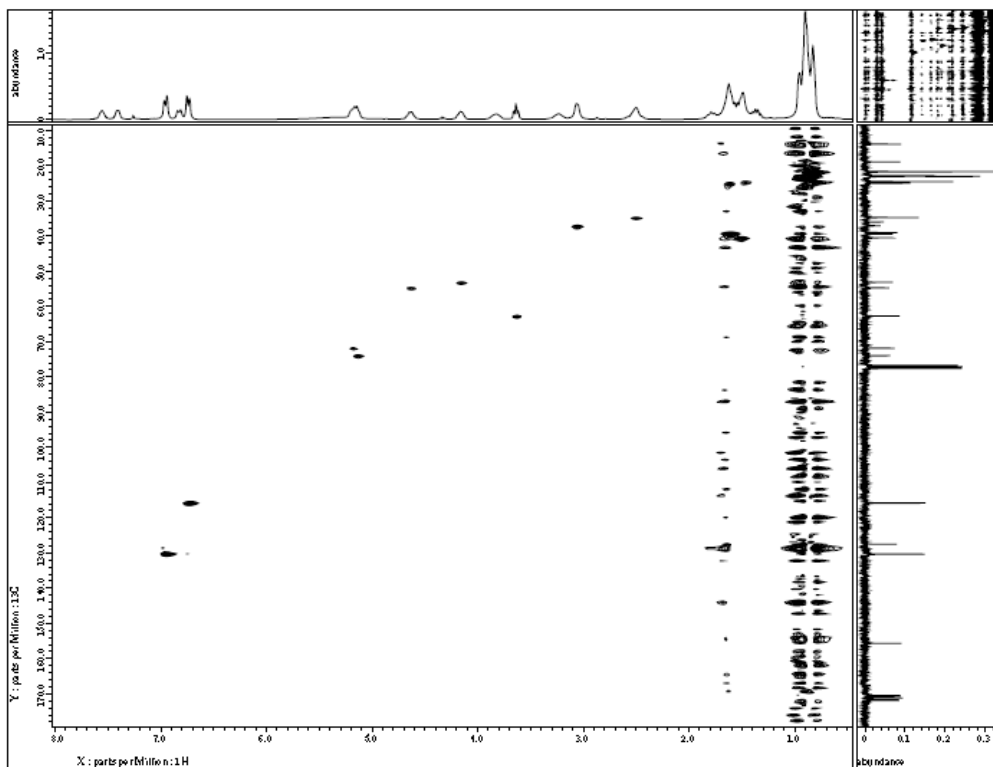


Figure S27. HSQC NMR spectrum of **6** in CDCl_3 .

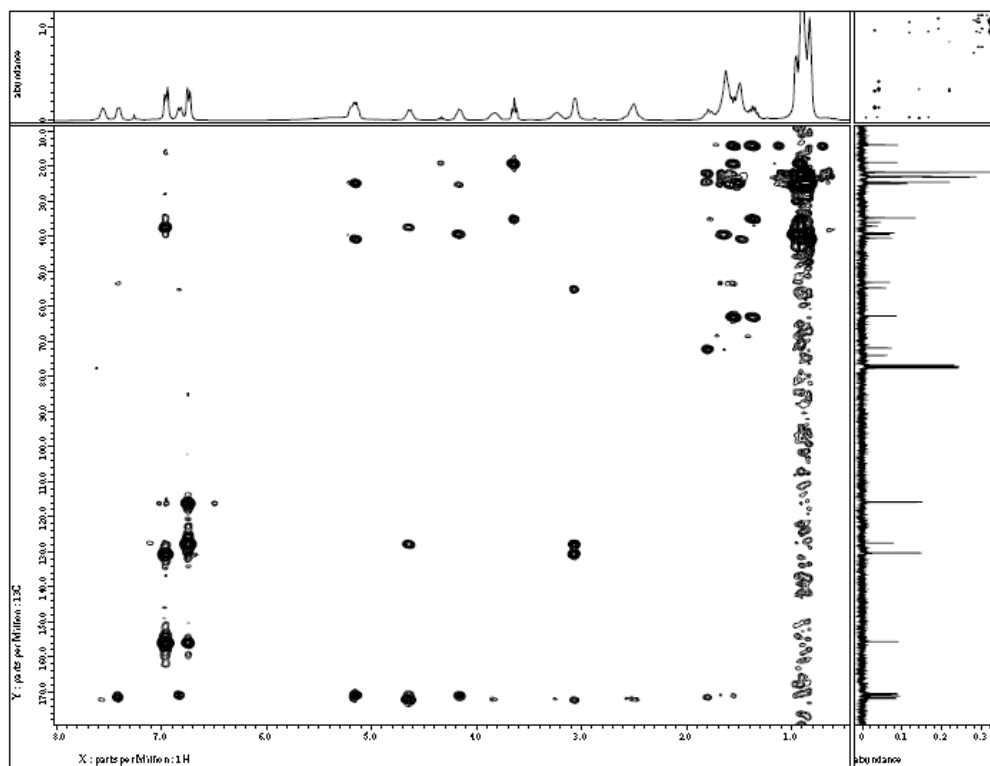


Figure S28. HMBC NMR spectrum of **6** in CDCl_3 .

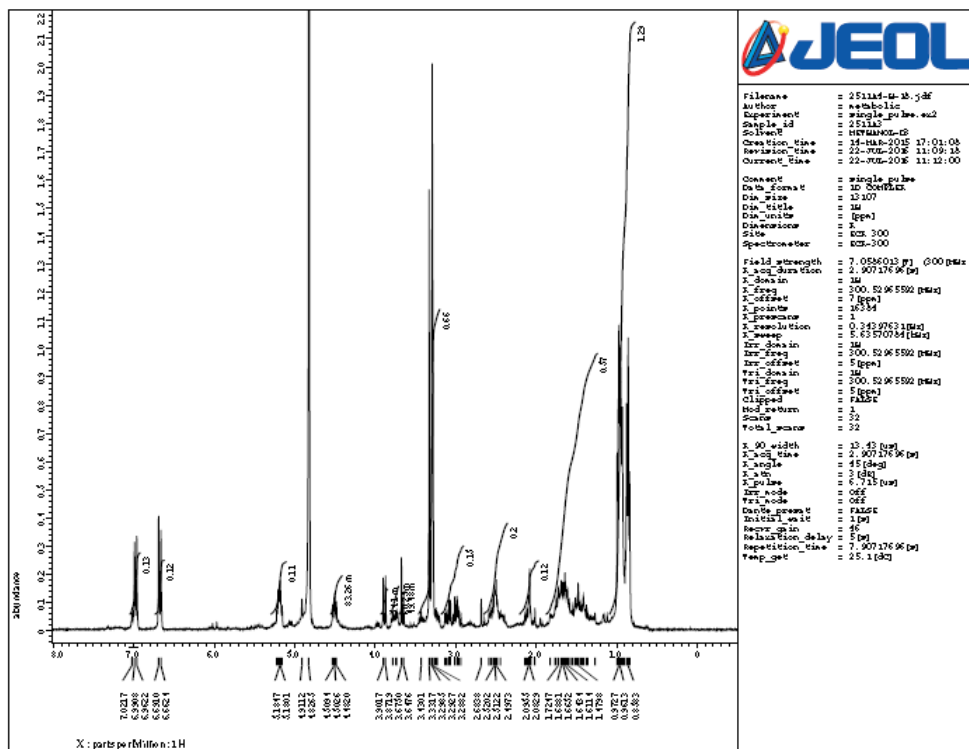


Figure S29. ¹H NMR spectrum of **7** in CD₃OD.

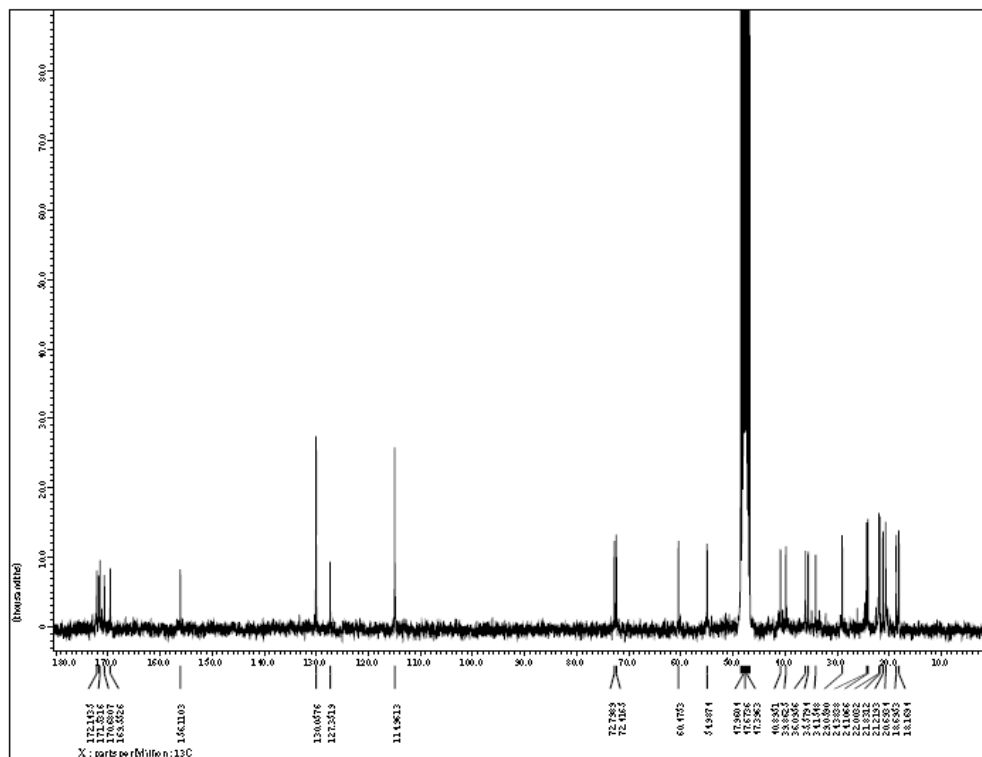


Figure S30. ¹³C NMR spectrum of **7** in CD₃OD.

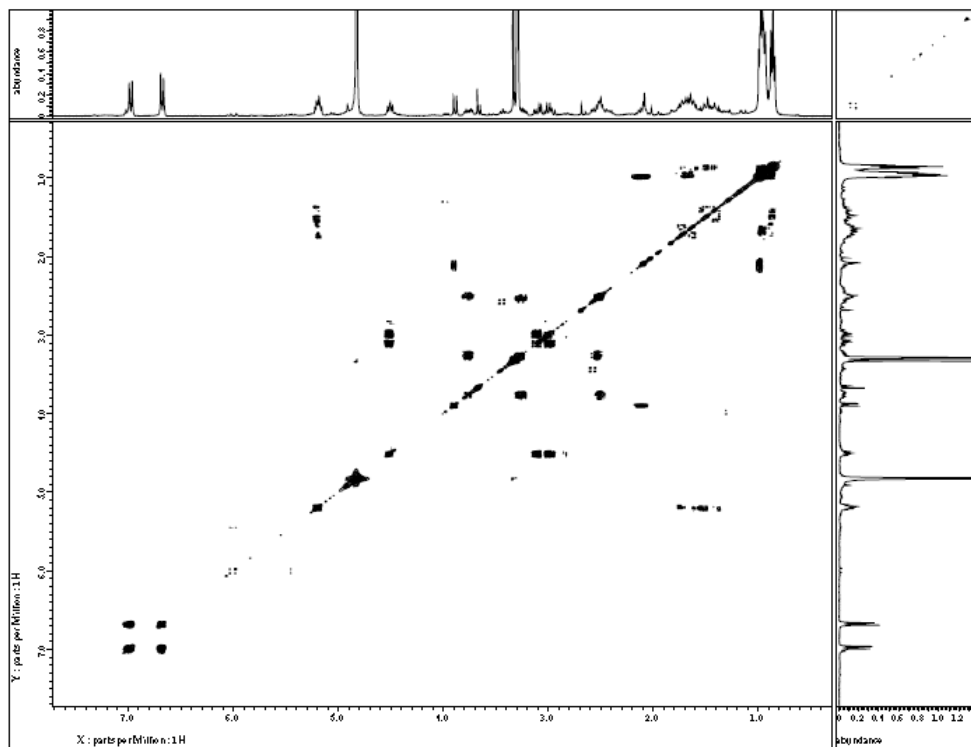


Figure S31. ^1H - ^1H COSY NMR spectrum of **7** in CD_3OD .

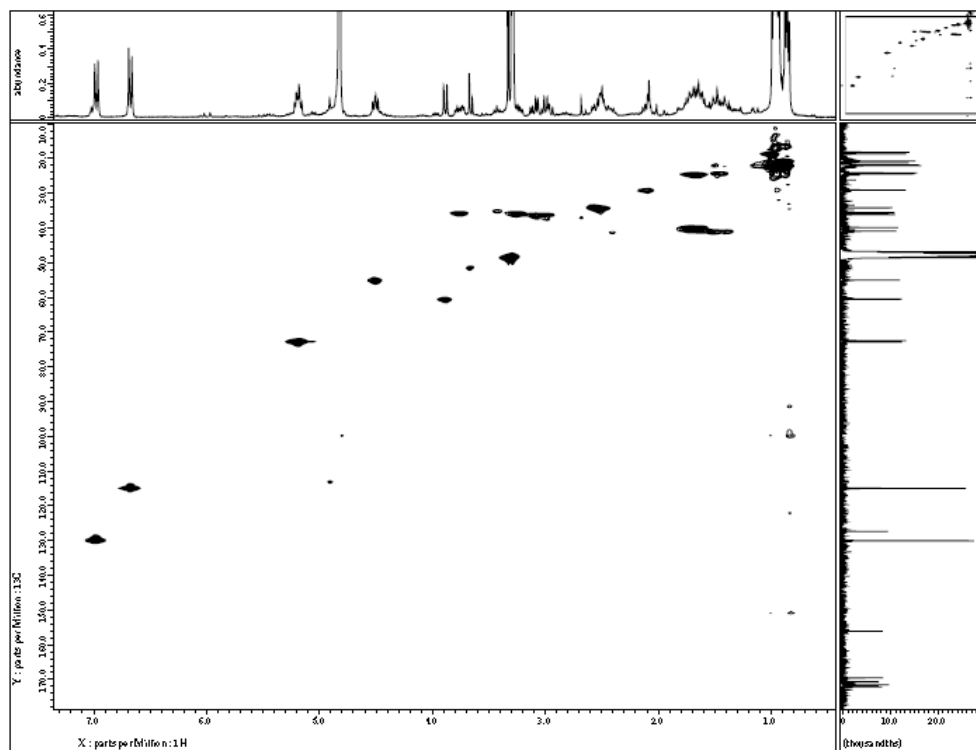


Figure S32. HSQC NMR spectrum of **7** in CD_3OD .

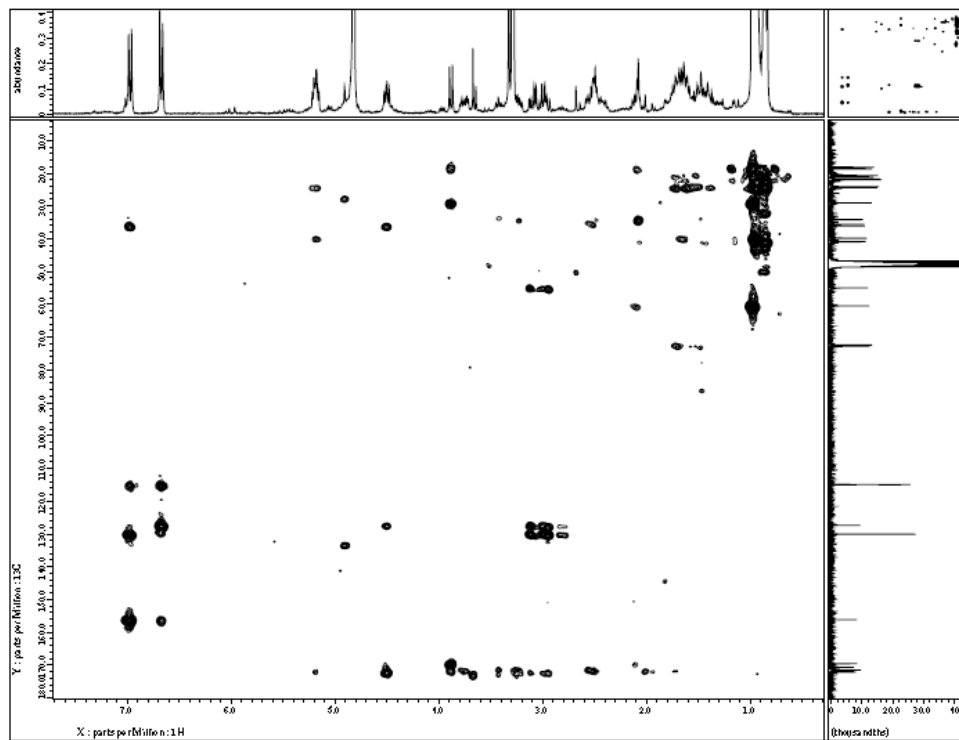


Figure S33. HMBC NMR spectrum of 7 in CD₃OD.

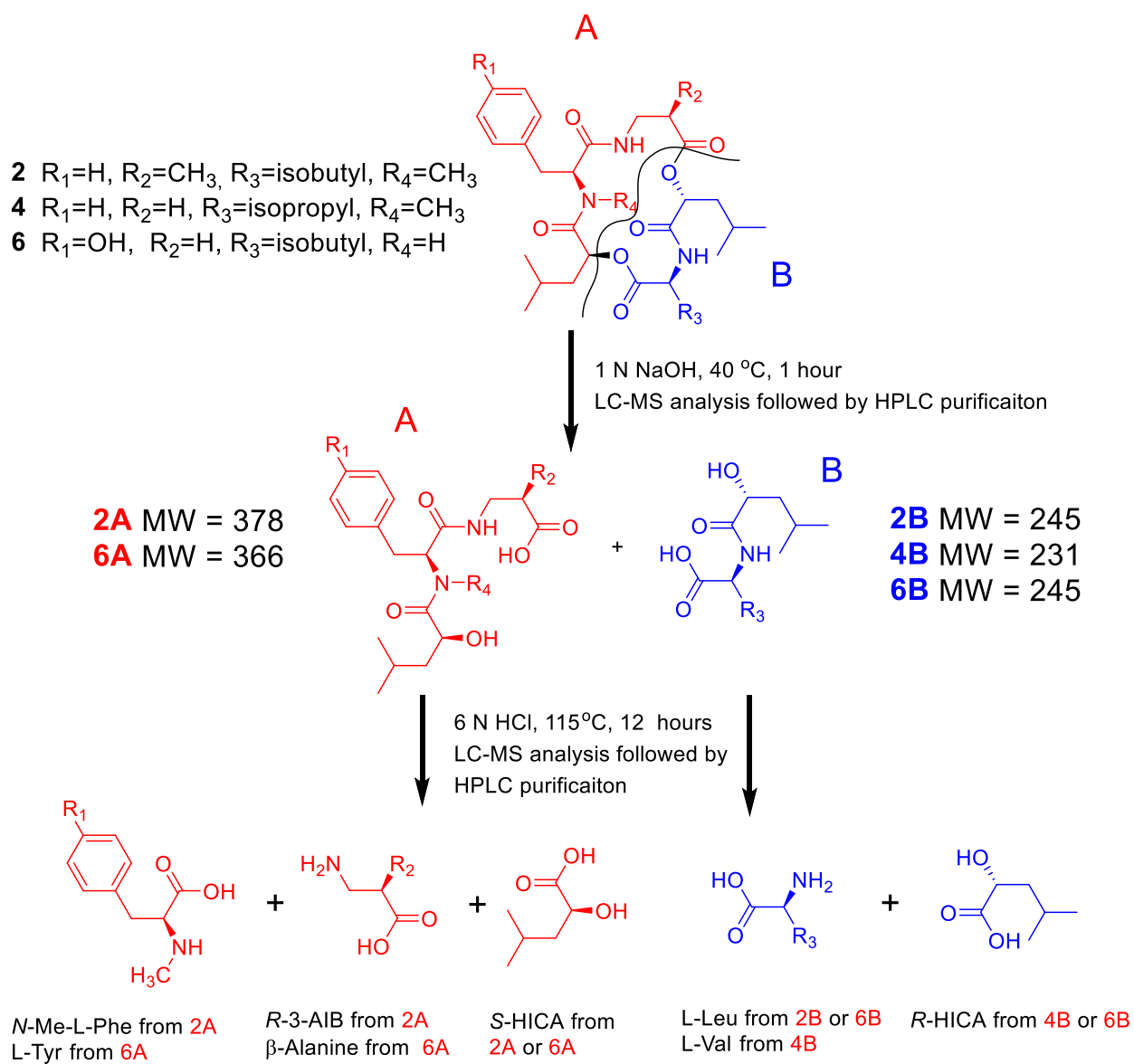


Figure S34. Hydrolysis and purification procedure of compounds **2**, **4**, and **6**.