Supplementary Information

Characterization and *in silico* Mutagenic Assessment of a New Betahistine Degradation Impurity

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Table S1. Chromatographic conditions to betahistine degradation products

time / min	Solution A / %	Solution B / %
0.00	98	2
4.50	80	20
5.00	80	20
5.01	98	2
8.00	98	2

Table S2. Standard parameters used for mutagenicity prediction with the ICH M7 tool of the Nexus ecosystem

Parameter	System	
	Derek Nexus	Sarah Nexus
Endpoint	*in vitro* mutagenicity	*in vitro* mutagenicity
Species	bacteria	bacteria
Database	Derek KB 2018 1.1	–
Model	–	2.0
Reasoning type	–	Weighted
Equivocal setting		8%
Sensitivity		8%

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[#]Murilo B. M. de Mello and Antonio A. F. de Oliveira had contributed equality for this work.

Table S3. Impurities detected in each stress condition during forced degradation experiments. Data were acquired by monitoring λ = 260 nm

Analyte	Relative retention time / min	Normal condition / % area		Acid / % area		Alkaline / % area		Oxidative / % area		Metallic ions / % area		Photolytic / % area		Thermic / % area		Humidity / % area	
		RM	Tab	RM	Tab	RM	Tab	RM	Tab	RM	Tab	RM	Tab	RM	Tab	RM	Tab
Unknown impurity	0.38	–	–	–	–	–	–	0.06	0.37	–	–	–	–	–	–	–	–
Unknown impurity	0.49	–	–	–	–	–	–	0.39	14.13	–	–	–	–	–	–	–	–
Unknown impurity	0.55	–	–	–	–	–	0.58	–	–	–	–	–	–	–	–	–	–
Unknown impurity	0.61	–	–	–	–	–	0.36	–	–	–	–	–	–	–	–	–	–
Unknown impurity	0.73	–	–	–	–	0.04	–	–	–	–	–	–	–	–	–	–	–
Impurity A	0.74	–	–	–	–	0.13	0.52	–	–	–	–	–	–	–	0.07	–	–
Unknown impurity	0.82	–	–	–	–	–	1.11	–	–	–	–	–	–	–	–	–	–
Unknown impurity	0.87	–	–	–	–	–	0.80	–	–	–	–	–	–	–	–	–	–
Unknown impurity	0.90	–	–	–	–	–	0.44	–	–	–	–	–	–	–	–	–	–
Unknown impurity	0.91	–	–	–	–	–	0.19	–	–	–	–	–	–	–	0.28	–	–
Unknown impurity	0.95	–	–	–	–	0.17	1.69	–	–	–	–	–	–	–	–	–	–
Betahistine	1.00	100.0	99.88	100.0	99.87	99.53	92.59	99.55	85.49	100.0	99.87	100.0	99.87	99.93	96.60	100.0	99.85
Unknown impurity	1.09	–	–	–	–	–	0.09	–	–	–	–	–	–	–	–	–	–
Unknown impurity	1.14	–	–	–	–	0.13	0.97	–	–	–	–	–	–	–	–	–	–
Unknown impurity	1.29	–	–	–	–	–	0.50	–	–	–	–	–	–	–	–	–	–
Unknown impurity	1.74	–	–	–	–	–	–	–	–	–	–	–	–	–	0.43	–	–
Impurity C	1.80	–	0.12	–	0.13	–	0.21	–	0.13	–	0.13	–	0.13	–	2.62	–	0.15

General

Acetonitrile HPLC grade was purchased from Merck (Darmstadt, Germany). All the other chemicals were of analytical grade. Heptafluorobutyric acid was obtained from Sigma-Aldrich (Saint Louis, MO, USA). Hydrochloride acid, sodium hydroxide, hydrogen peroxide, and copper chloride were purchased from Panreac AppliChem (Barcelona, Spain). 4-Nitroquinoline-*N*-oxide and 2-aminofluorene were acquired from Acros Organic (Geel, Belgium). Sodium azide was obtained from Synth (Diadema, Brazil). Ciclofosfamide was purchased from Baxter (Deerfield, USA). Betahistine dihydrochloride raw material was precedent from LEBSA Laboratories (Barcelona, Spain). Betahistine tablets and placebo were internally manufactured at Aché Laboratórios Farmacêuticos (Guarulhos, Brazil). Additionally, the following equipment was used: water deionizer system OptionQ (Elga Labwater, High Wycombe, UK); ultrasonic (Unique, Indaiatuba, Brazil); vortex mixer model Genie-2 (Scientific Industries, Bohemia, NY, USA); hot air oven (Quimis, Diadema, Brazil); Micropipettes (Gilson, Middleton, WI, USA); photo stability chamber model EC/02/RF (Mecalor, São Paulo, Brazil).

Mass spectrometry analysis of **6**

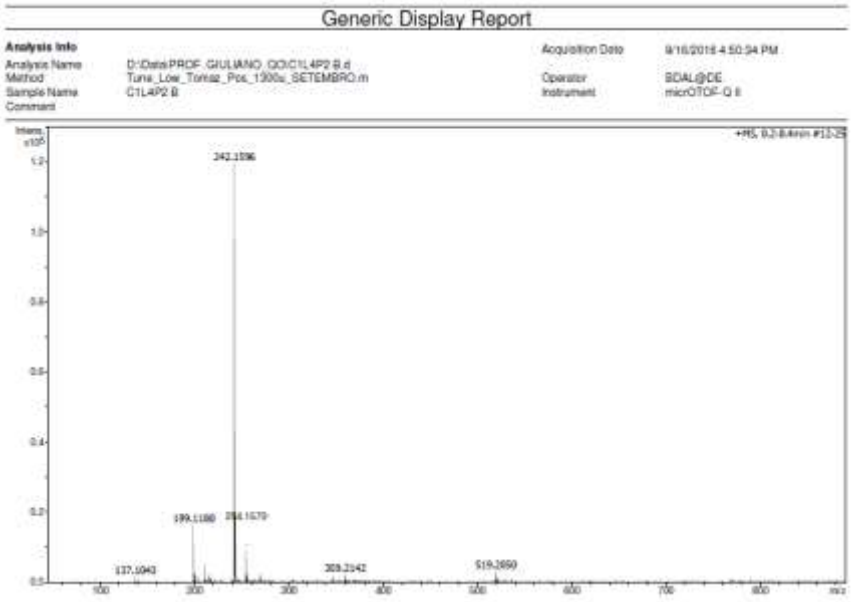

Figure S1. TIC ESI-TOF(+) HRMS of **6**.

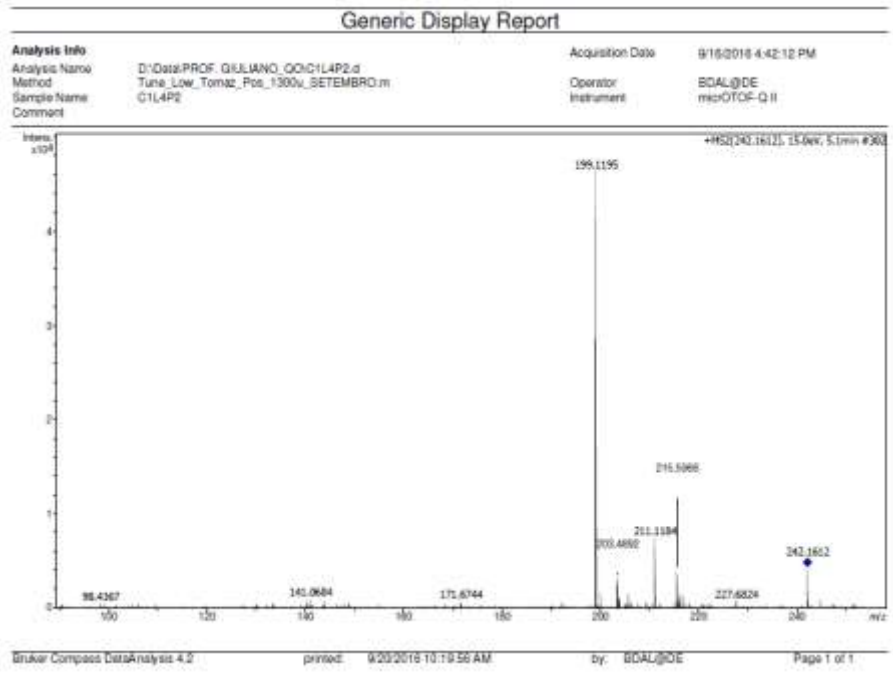

Figure S2. MS/MS ESI-TOF(+) HRMS (15 eV) of **6**.

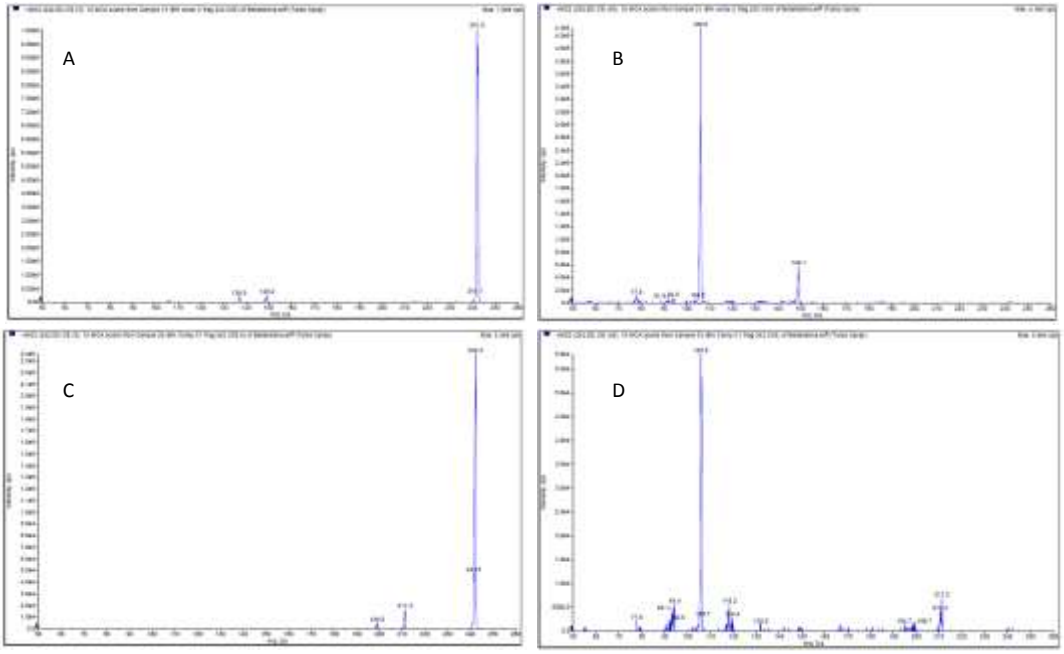

Figure S3. Directed injection on triple-quadruple mass spectra: **5** in A MS/MS (CE5), **6** in B MS/MS (CE5), **5** in C MS/MS (CE45) and **6** in D MS/MS (CE45).

NMR data spectroscopy of **6**

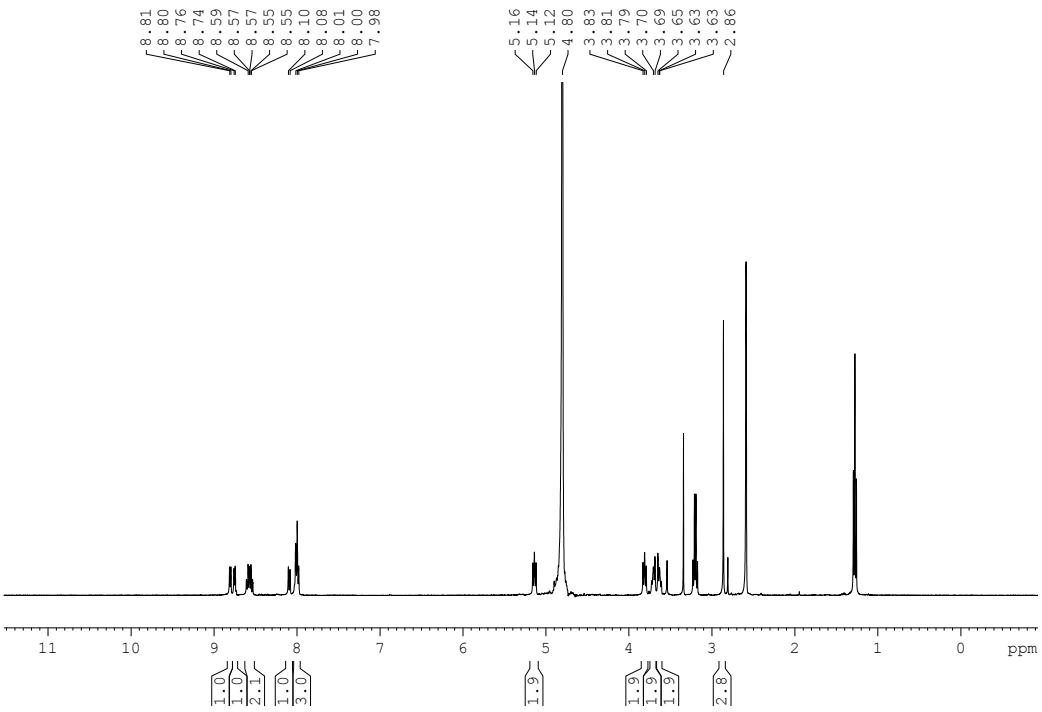

Figure S4. ^{1}H NMR spectrum (400 MHz, D_2O) of **6**.

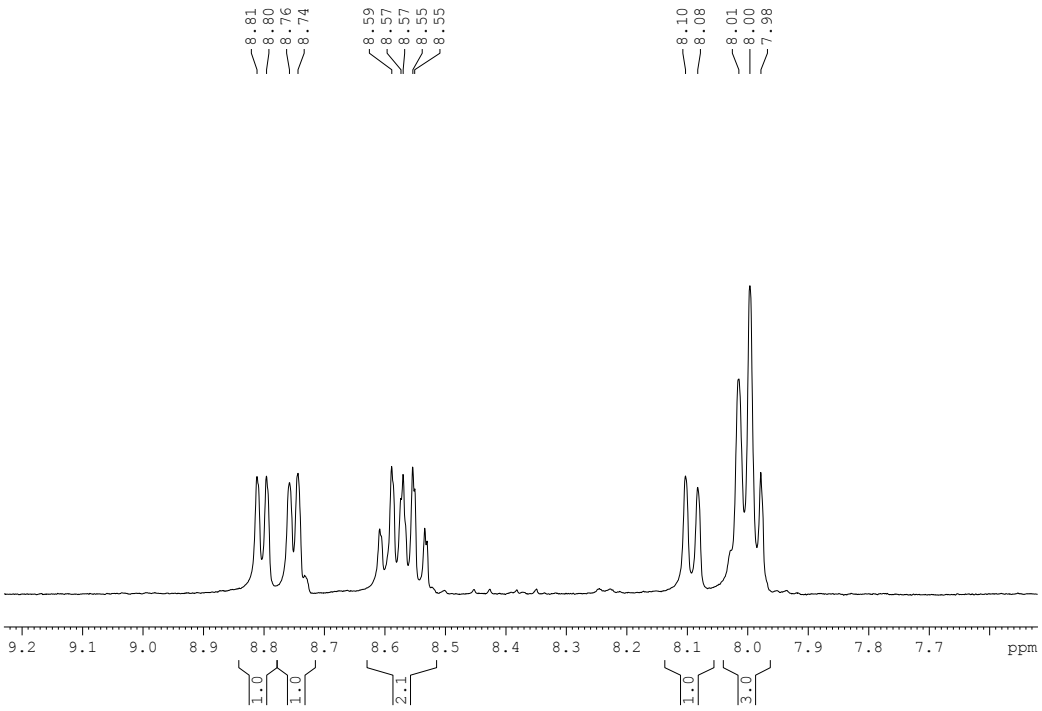

Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O) of **6**. Ampliation in 9.2 to 7.6 ppm.

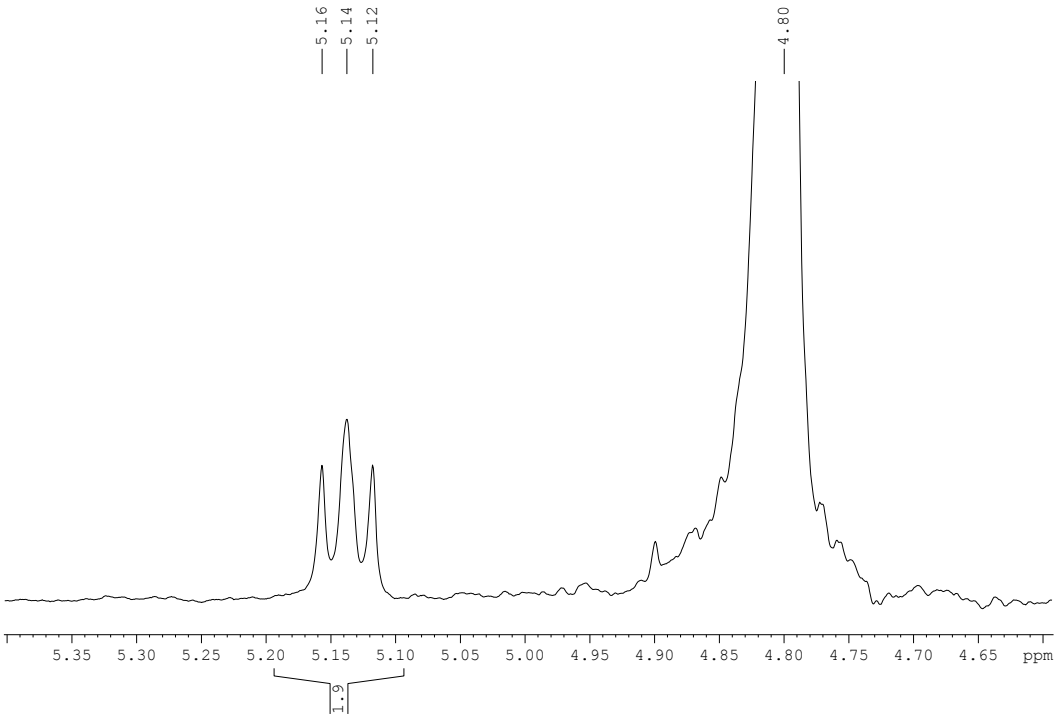

Figure S6. ^{1}H NMR spectrum (400 MHz, D_2O) of **6**. Ampliation in 5.35 to 4.65 ppm.

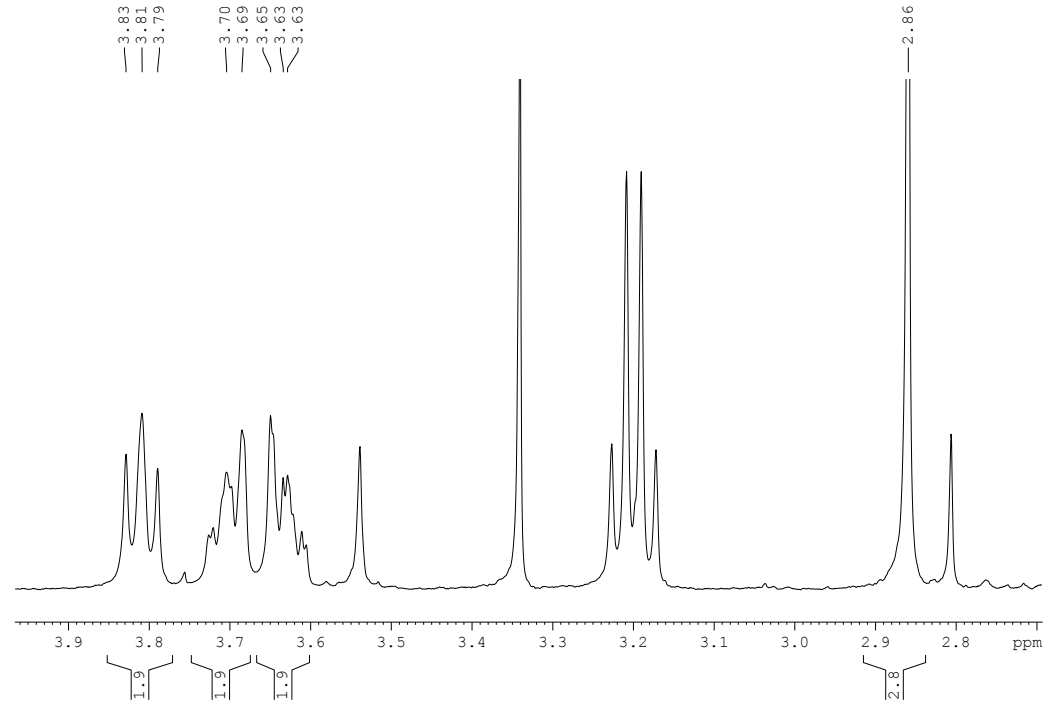

Figure S7. ^{1}H NMR spectrum (400 MHz, D_2O) of **6**. Ampliation in 3.90 to 2.80 ppm.

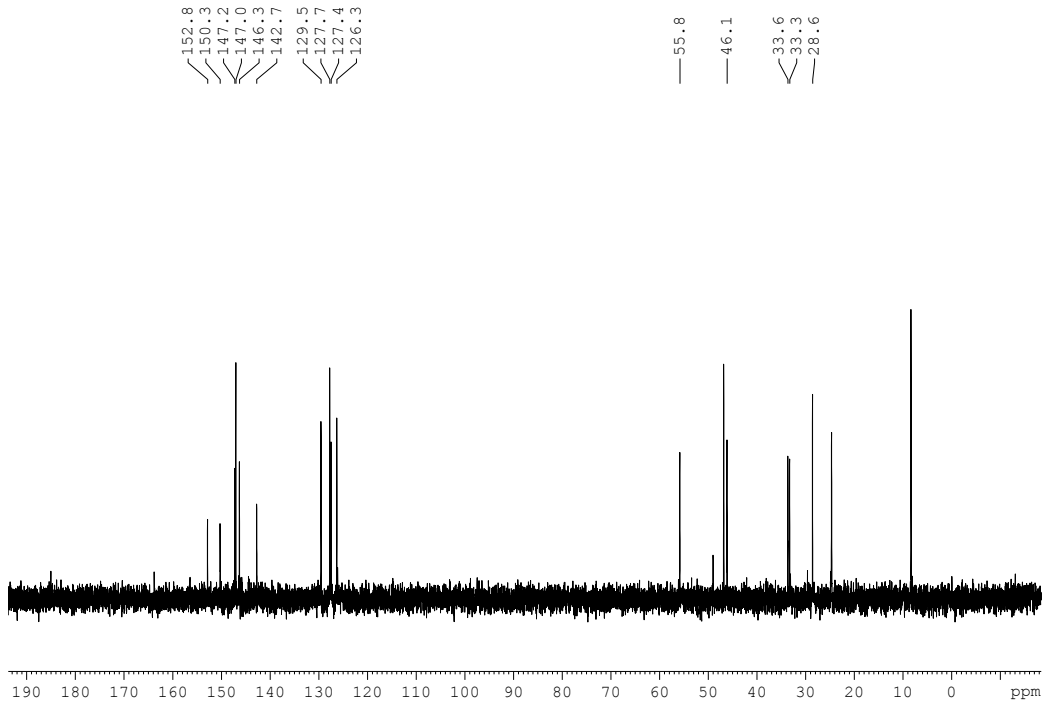

Figure S8. ^{13}C NMR spectrum (100 MHz, D_2O) of **6**.

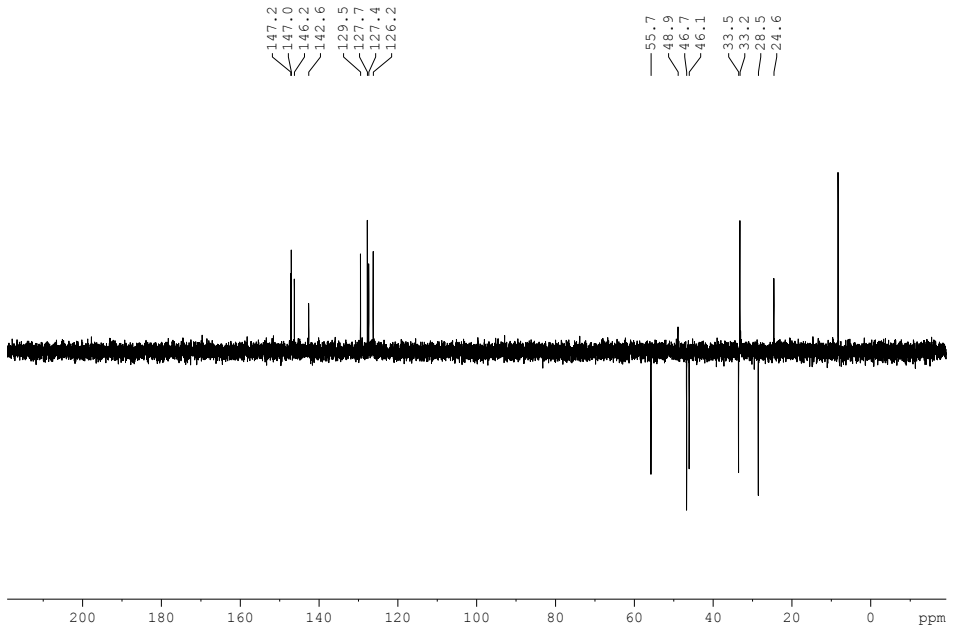

Figure S9. DEPT-135 NMR spectrum (100 MHz, D_2O) of **6**.

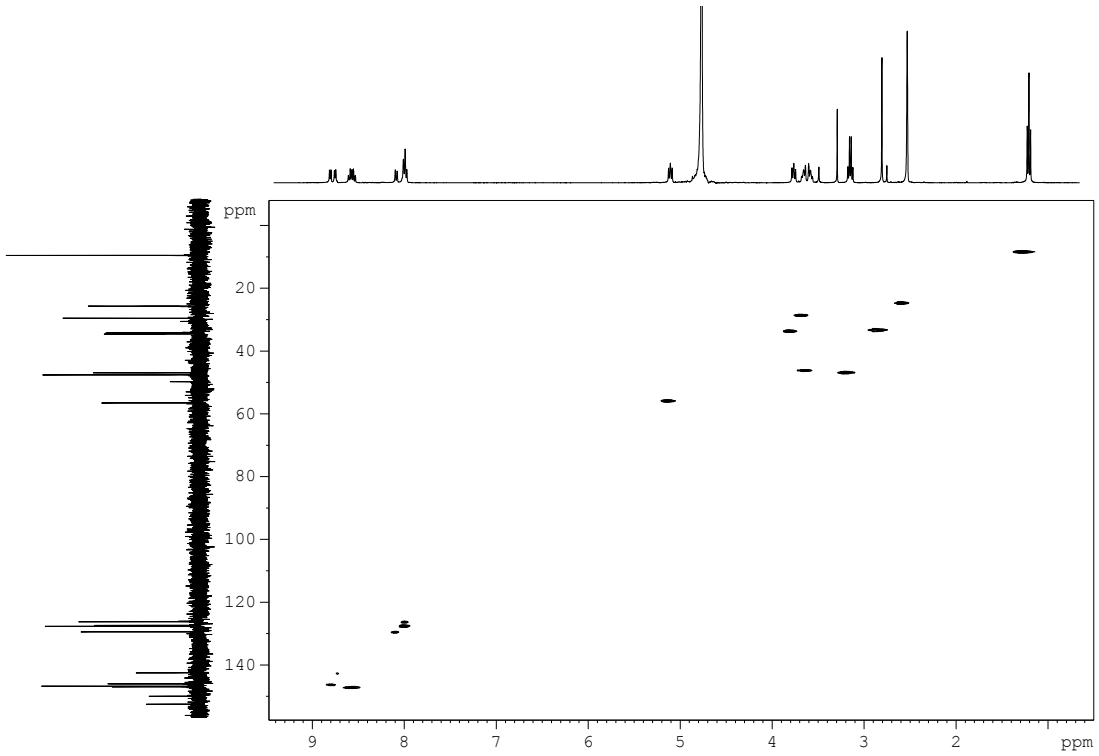

Figure S10. HSQC correlation map of **6**.

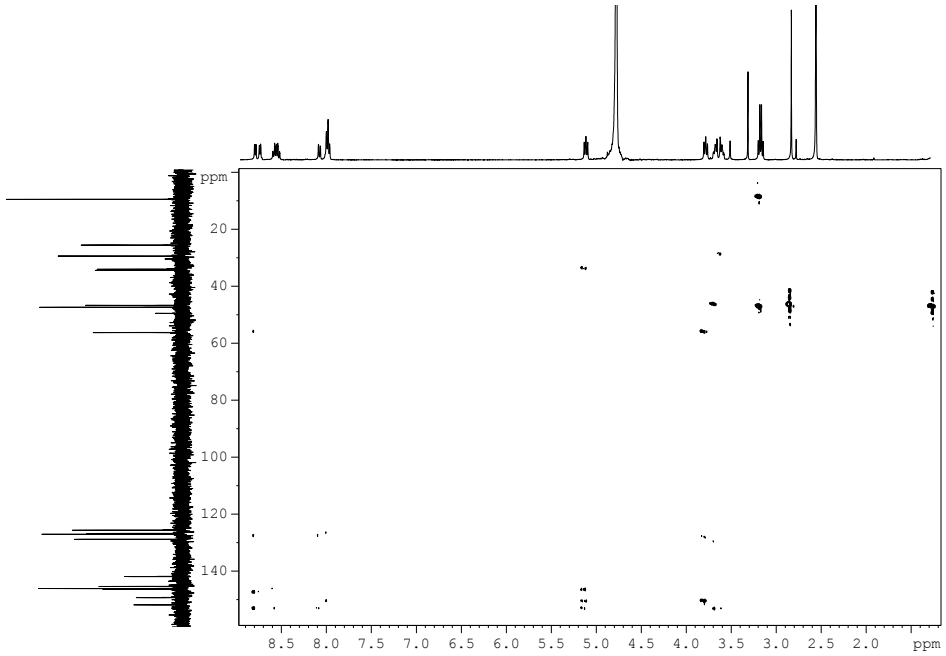

Figure S11. HMBC correlation map of **6**.

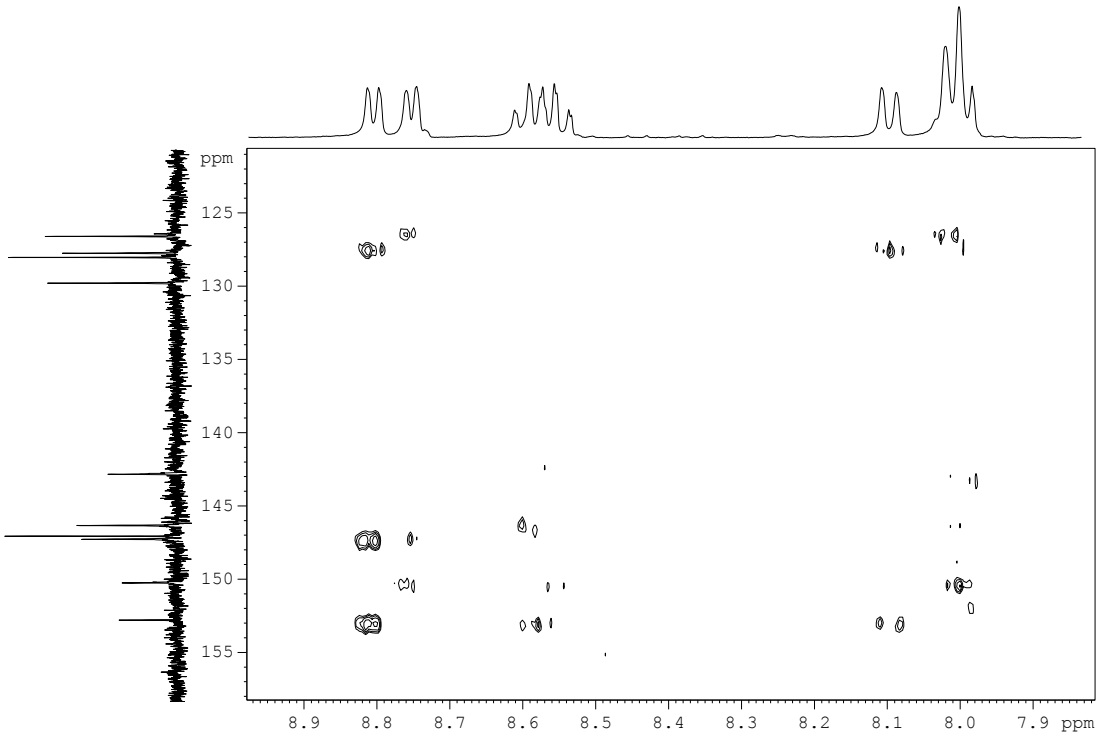

Figure S12. HMBC correlation map of **6**. Ampliation in 8.90 to 7.9 ppm for ^{1}H NMR.

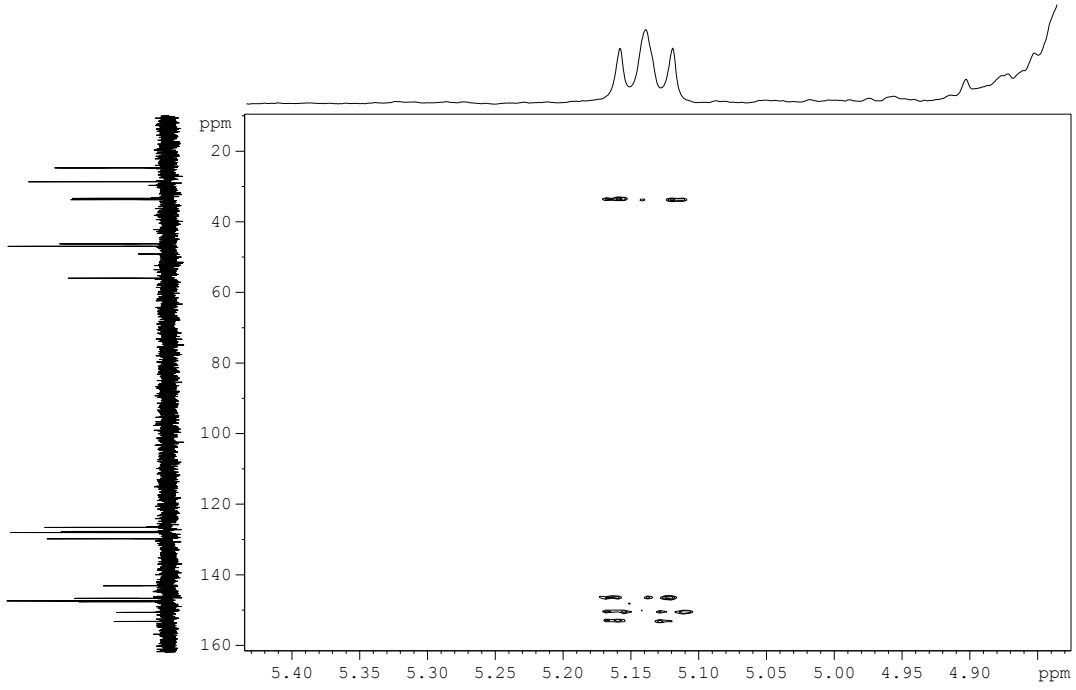

Figure S13. HMBC correlation map of **6**. Ampliation in 5.40 to 4.90 for ^{1}H NMR.

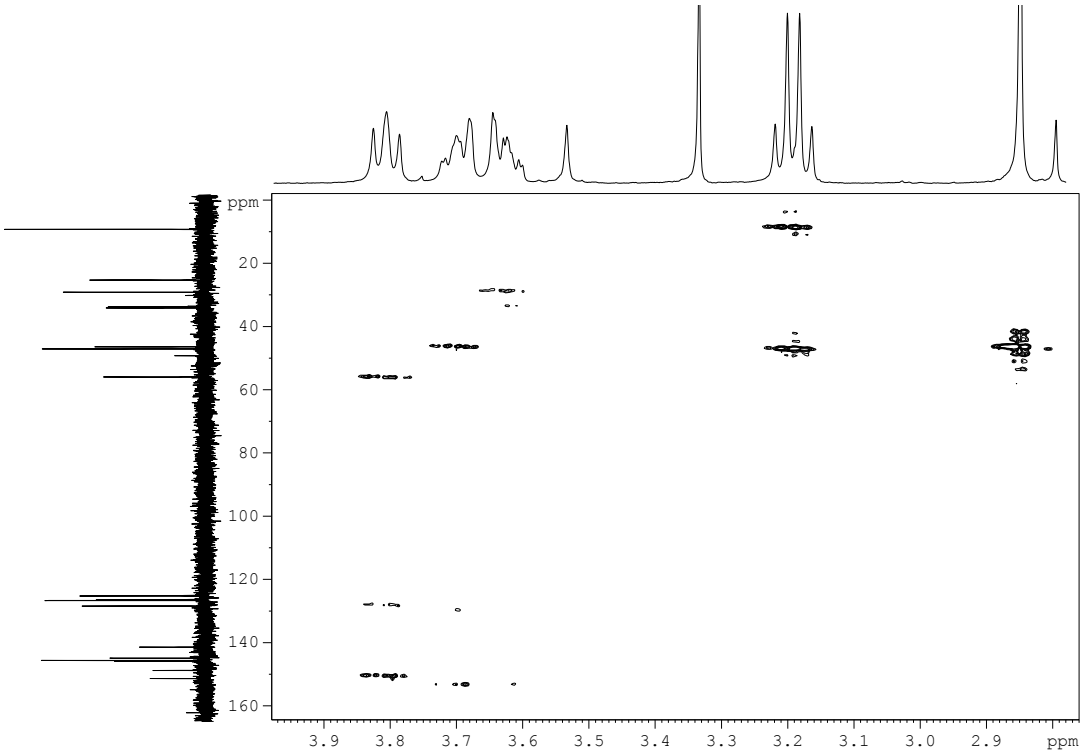

Figure S14. HMBC correlation map of **6**. Ampliation in 3.90 to 2.90 ppm for ^{1}H NMR.

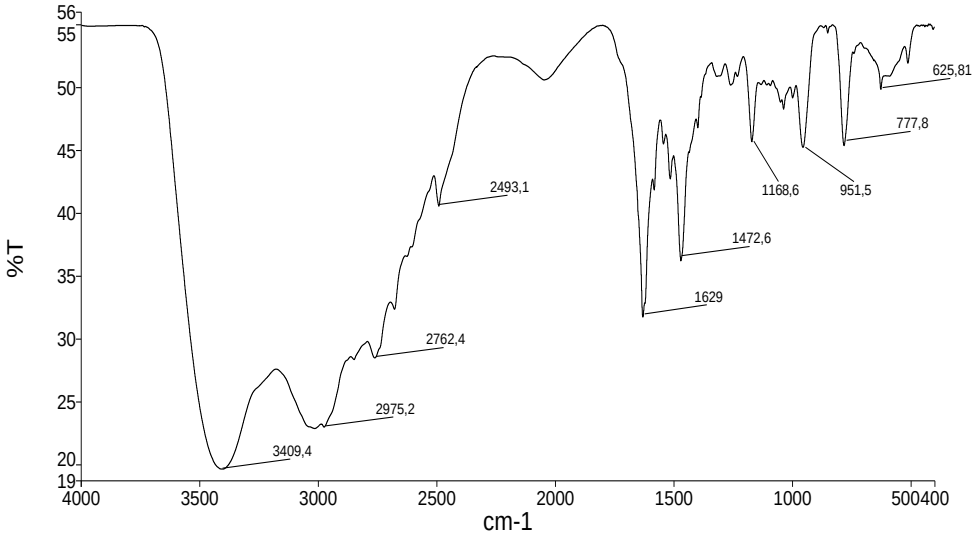

Figure S15. Infrared spectrum (KBr) of **6**.

NMR spectra of **5**

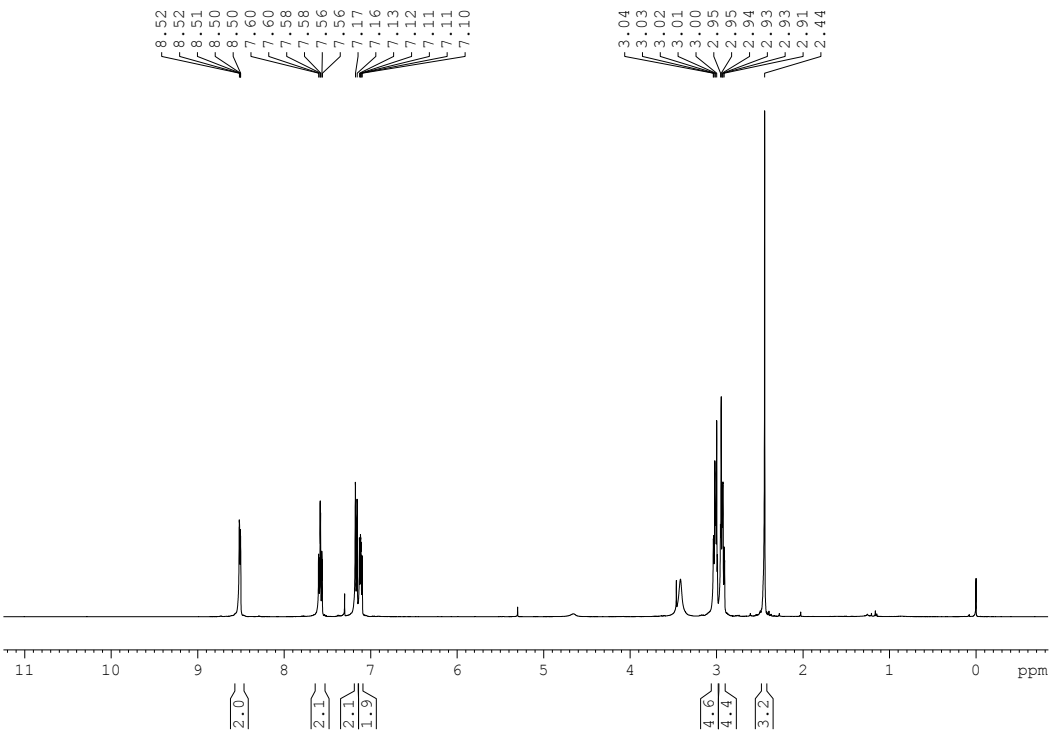

Figure S16. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **5**.

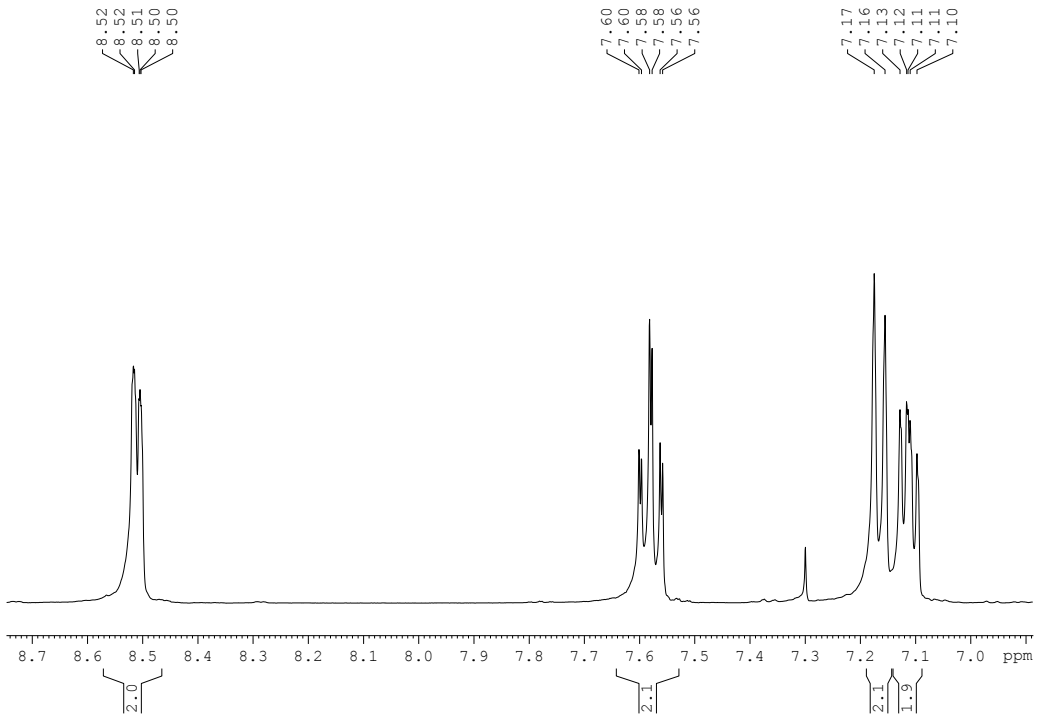

Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **5**. Ampliation in 8.70 to 7.00 ppm.

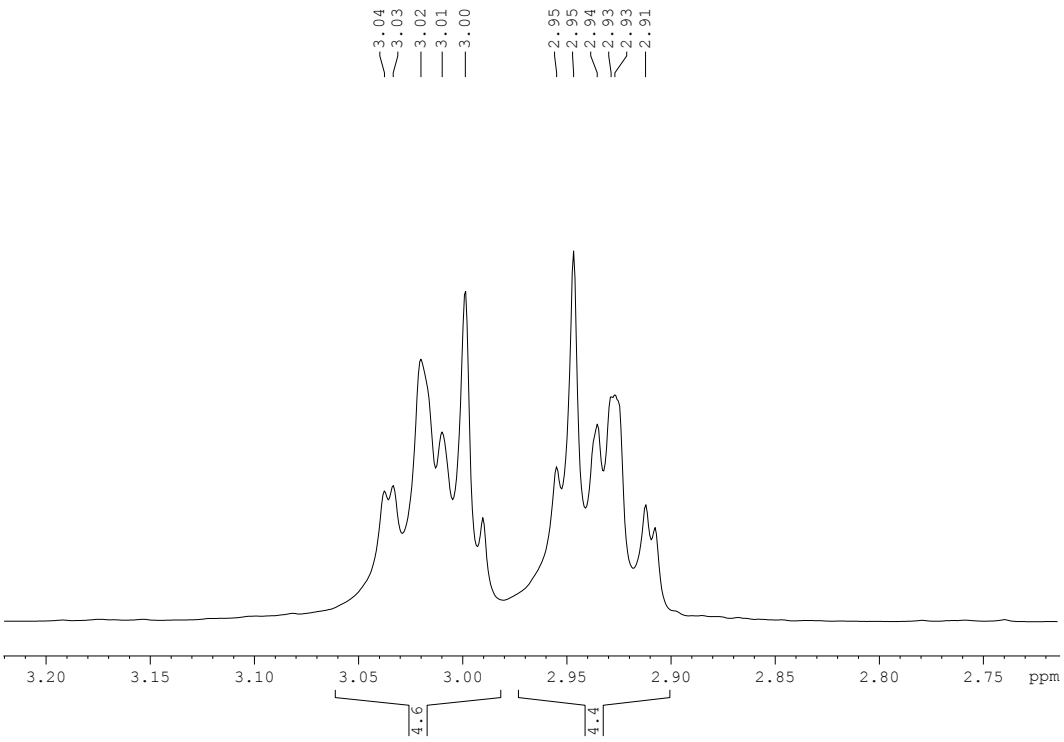

Figure S18. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **5**. Ampliation in 3.20 to 2.75 ppm.

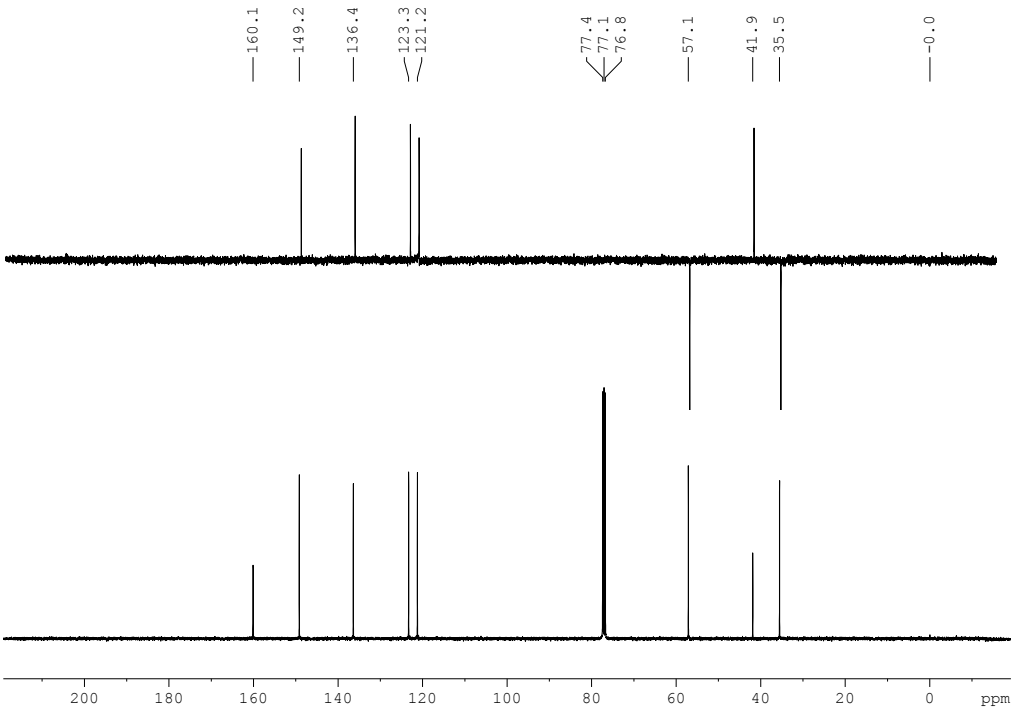

Figure S19. DEPT-135 NMR spectrum (A) and ^{13}C NMR spectrum (400 MHz, $CDCl_3$) (B) of **6**.