



Phytochemical Constituents of the Aerial Parts of *Artemisia iwayomogi* Kitamura and Their Anti-inflammatory Activities

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Abstract – *Artemisia iwayomogi* Kitamura is a well-known medicinal plant widely utilized in traditional medicine for its various pharmacological properties. In this study, phytochemical investigation of the aerial parts of *A. iwayomogi* led to the isolation and characterization of 13 secondary metabolites, including one new (**1**) and 12 known compounds (**2–13**). The structural elucidation of the new compound was achieved through comprehensive spectroscopic analysis, including NMR and HRMS data, and a modified Mosher's method. This study represents the first report of compounds **2**, **3**, **5**, and **13** from this plant species. Compound **1** was evaluated for its inhibitory activity against NO production in LPS-stimulated macrophages. However, it was found to be inactive against NO production ($IC_{50} > 100 \mu M$). *In silico* target prediction using SwissTargetPrediction identified catechol O-methyltransferase (COMT) as the highest-probability predicted target of compound **1**, providing a hypothesis-generating basis for future biological investigation.

Keywords – *Artemisia iwayomogi*, Anti-inflammatory, NMR, *In silico*

Introduction

The nitric oxide synthase (NOS) family of enzymes comprises the constitutive isoforms endothelial NOS (eNOS) and neuronal NOS (nNOS), along with the inducible form (iNOS). These enzymes are responsible for the synthesis of nitric oxide (NO), an essential intercellular signaling molecule, from L-arginine.^{1,2} Constitutive nitric oxide production under physiological conditions plays a crucial role in supporting innate immune defense against pathogens, modulating neuronal communication, and maintaining vascular homeostasis through the process of vasodilation.² NO exhibits

concentration-dependent biphasic effects. While basal levels of NO are cytoprotective, excessive production, particularly through iNOS induction in response to inflammatory triggers such as lipopolysaccharide (LPS) and pro-inflammatory cytokines, can initiate pathological cascades. Excess NO rapidly reacts with superoxide anions to form peroxynitrite (ONOO⁻), a potent reactive nitrogen species that induces DNA damage, lipid peroxidation, protein nitration, and cellular dysfunction in oxidative conditions.^{2,3} Numerous diseases, including cancer, inflammatory bowel disease, cardiovascular conditions, septic shock, and neurodegenerative disorders, are affected by oxidative and nitrosative stress.² Selective inhibitors of iNOS have emerged as promising therapeutic candidates because of the enzyme's involvement in various pathologies. Natural products serve as an excellent resource for discovering novel NO-modulating agents due to their structural diversity and ability to interact with multiple targets.

Known locally as “Dowijigi” or “Haninjin” in Korea, *Artemisia iwayomogi* Kitamura is a perennial herb that is part of the Asteraceae family. This plant has a long

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history of use in traditional East Asian medicine, where it has been employed to address a range of ailments, particularly those affecting the liver, such as cirrhosis and hepatitis, along with various inflammatory and immunomodulatory disorders.^{4,5} Numerous bioactive secondary metabolites present in the plant have been identified through comprehensive phytochemical analysis. These encompass coumarins such as scopoletin and scopolin, flavonoids including genkwanin and jaceosidin, as well as phenolic acids like chlorogenic acid.^{4,6} Pharmacological investigations have demonstrated significant anti-inflammatory effects by inhibiting pro-inflammatory cytokines (TNF- α , IL-6) through the downregulation of p38 MAPK and NF- κ B signaling pathways, which are crucial transcriptional regulators of iNOS production.⁴ Moreover, compounds like chlorogenic acid and scopoletin demonstrate direct scavenging ability against ONOO⁻, in addition to possessing anti-allergic, bone metabolism-regulating, and antimicrobial characteristics.^{6,7} Recently, we reported the isolation and anti-inflammatory activity of several compounds from *A. iwayomogi*.⁵ Given the plant's rich phytochemical diversity and established anti-inflammatory properties, continued investigation of its secondary metabolites for NO inhibitory potential is warranted. Herein, we describe the isolation and structural elucidation of additional compounds from *A. iwayomogi* and their inhibitory effects on NO production in LPS-induced RAW 264.7 macrophages.

Experimental

General experimental procedures – Optical rotations were measured using a JASCO P-2000 digital polarimeter. NMR spectra were acquired using the Varian Unity Inova 400 MHz spectrometer (Varian, Inc., California, USA). High-resolution electron impact-mass spectrometry (HREIMS) was carried out using a JEOL JMS-AX 300L spectrometer (Jeol, Akishima, Tokyo, Japan). Thin-layer chromatography (TLC) was conducted utilizing RP-18 F254s and Merck precoated silica gel F254 plates. Column chromatography (CC) utilized (RP)-C18 (Merck, 75 mesh) and silica gel 60 (Merck, 230–400 mesh). Preparative HPLC was performed utilizing a YMC Pak ODS-A column (20 $\times$ 250 mm, 5 μ m particle size; YMC Co., Ltd., Japan) and a Waters 2487 controller system equipped with a UV detector (UV/VIS-156).

Plant material – The aerial parts of *A. iwayomogi* were collected at the Medicinal Plants Garden at Daegu Catholic University in October 2020 and authenticated by

Professor Byung Sun Min, one of the authors. A voucher specimen (voucher no. CUD-3030) was deposited at the Laboratory of Pharmacognosy in the College of Pharmacy, Daegu Catholic University, Republic of Korea.

Extraction and isolation – The chopped, air-dried aerial parts of *A. iwayomogi* (7.3 kg) underwent exhaustive extraction with 100% MeOH (3 $\times$ 10 L). The resulting filtrates were pooled and evaporated under reduced pressure using a vacuum rotary evaporator, yielding 750 g of a crude extract. This residue was subsequently suspended in distilled water and sequentially partitioned with *n*-hexane, dichloromethane (MC), ethyl acetate (EA), and *n*-butanol (*n*-BuOH), respectively. The EA extract (150 g) was fractionated via silica gel column chromatography (CC), employing a gradient system of CH₂Cl₂:MeOH (1:0–0:1, *v/v*) to afford 15 subfractions (Fr.1–Fr.15). Fr.3 (1.4 g) was chromatographed on a silica gel column using a *n*-hexane:acetone gradient (3:1–0:1) to provide nine subfractions (Fr.3.1–Fr.3.9). Fr.3.2 (118.8 mg) was purified by preparative HPLC (MeOH:H₂O, 20:80–80:20, 5 mL/min) to yield compounds **2** (1.0 mg), **3** (1.3 mg), **10** (11.5 mg), and **11** (3.3 mg). Fr.3.5 (455.1 mg) was separated by Sephadex LH-20 to obtain **9** (2.1 mg), **12** (7.3 mg), and **13** (5.6 mg). Fr.3.6 (90.0 mg) was purified by preparative HPLC (MeOH:H₂O, 40:60–70:30, 5 mL/min) to yield compounds **1** (5.5 mg), **4** (3.8 mg), and **8** (2.5 mg). Further elution of Fr.3.8 (164.3 mg) through an RP-C18 column (MeOH:H₂O, 1:2–3:1) afforded compounds **5** (3.6 mg), **6** (8.3 mg), and **7** (4.1 mg).

Artemisiwayin (1) – White amorphous powder; [α] +17.5 (*c* 0.015, MeOH); ¹H (400 MHz, methanol-*d*₄) and ¹³C NMR (100 MHz, methanol-*d*₄) data, see Table 1; HREIMS *m/z* 182.0577 [*M*]⁺ (calcd for C₉H₁₀O₄, 182.0579).

***p*-Coumaric acid (2)** – ¹H NMR (400 MHz, methanol-*d*₄) δ _H 7.61 (1H, d, *J* = 15.8 Hz), 7.46 (2H, d, *J* = 8.7 Hz), 6.81 (2H, d, *J* = 8.7 Hz), 6.29 (1H, d, *J* = 15.8 Hz); ¹³C NMR (100 MHz, methanol-*d*₄) δ _C 169.6, 159.7, 145.2, 129.6, 125.8, 115.4, 114.2.

Caffeic acid (3) – ¹H NMR (400 MHz, methanol-*d*₄) δ _H 7.48 (1H, d, *J* = 15.8 Hz), 6.98 (1H, m), 6.88 (1H, m), 6.72 (1H, m), 6.16 (1H, d, *J* = 15.8 Hz); ¹³C NMR (100 MHz, methanol-*d*₄) δ _C 171.1, 149.4, 147.0, 146.7, 127.8, 122.8, 116.5, 115.5, 115.1.

Methyl caffeate (4) – ¹H NMR (400 MHz, methanol-*d*₄) δ _H 7.56 (1H, d, *J* = 15.8 Hz), 7.05 (1H, s), 6.95 (1H, d, *J* = 7.8 Hz), 6.79 (1H, d, *J* = 7.8 Hz), 6.27 (1H, d, *J* = 15.8 Hz), 3.77 (3H, s); ¹³C NMR (100 MHz, methanol-*d*₄) δ _C 169.7, 149.5, 146.9, 146.8, 127.7, 122.9, 116.5, 115.1, 114.8, 52.0.

Hydroquinone (5) – ¹H NMR (400 MHz, methanol-

d_4) δ_H 6.62 (4H, s); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 151.2, 116.8.

4-Hydroxybenzoic acid (6) – ^1H NMR (400 MHz, methanol- d_4) δ_H 7.90 (2H, d, $J = 8.8$ Hz), 6.84 (2H, d, $J = 8.8$ Hz); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 170.0, 163.3, 133.0, 122.7, 116.0.

Protocatechuic acid (7) – ^1H NMR (400 MHz, methanol- d_4) δ_H 7.45 (1H, s), 7.40 (1H, d, $J = 8.2$ Hz), 6.78 (1H, d, $J = 8.2$ Hz); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 173.4, 150.1, 145.6, 127.2, 123.4, 117.8, 115.5.

Eugenyl glucoside (8) – ^1H NMR (400 MHz, methanol- d_4) δ_H 7.08 (1H, d, $J = 8.2$ Hz), 6.82 (1H, d, $J = 1.9$ Hz), 6.72 (1H, dd, $J = 8.2, 1.9$ Hz), 5.95 (1H, ddt, $J = 16.8, 10.0, 6.7$ Hz), 5.02 (1H, m), 4.85 (1H, d, $J = 7.4$ Hz), 3.87 (1H, dd, $J = 12.1, 1.3$ Hz), 3.83 (3H, s), 3.69 (1H, dd, $J = 12.1, 5.1$ Hz), 3.35–3.49 (overlap); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 150.7, 146.3, 139.0, 136.4, 122.1, 118.3, 115.8, 114.1, 103.0, 78.1, 77.8, 74.9, 71.3, 62.5, 56.7, 40.7.

Kaempferol (9) – ^1H NMR (400 MHz, DMSO- d_6) δ_H 8.04 (2H, d, $J = 8.8$ Hz), 6.93 (2H, d, $J = 8.8$ Hz), 6.44 (1H, d, $J = 1.97$ Hz), 6.20 (1H, d, $J = 1.97$ Hz); ^{13}C NMR (100 MHz, DMSO- d_6) δ_C 175.8, 163.8, 160.7, 160.5, 159.1, 156.2, 146.7, 135.6, 129.5, 121.7, 115.4, 103.0, 98.1, 93.5.

Luteolin (10) – ^1H NMR (400 MHz, methanol- d_4) δ_H 7.34 (1H, overlap), 7.32 (1H, overlap), 6.88 (1H, d, $J = 7.2$ Hz), 6.49 (1H, s), 6.40 (1H, s), 6.18 (1H, s); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 183.8, 166.3, 165.9, 163.1, 159.3, 150.9, 146.9, 123.6, 120.3, 116.8, 114.2, 105.3, 103.8, 100.1, 95.0.

Apigenin (11) – ^1H NMR (400 MHz, DMSO- d_6) δ_H 7.91 (2H, d, $J = 8.7$ Hz), 6.92 (2H, d, $J = 8.7$ Hz), 6.76 (1H, s), 6.47 (1H, brs), 6.19 (1H, brs); ^{13}C NMR (100 MHz, DMSO- d_6) δ_C 181.7, 164.1, 163.7, 161.4, 161.2, 157.3, 128.4, 121.2, 115.9, 103.7, 102.8, 98.8, 94.0.

Naringenin (12) – ^1H NMR (400 MHz, methanol- d_4) δ_H 7.33 (2H, d, $J = 8.4$ Hz), 6.84 (2H, d, $J = 8.4$ Hz), 5.91 (2H, overlap), 5.35 (1H, dd, $J = 12.9, 2.8$ Hz), 3.12 (1H, dd, $J = 17.1, 13.0$ Hz), 2.71 (1H, dd, $J = 17.1, 3.0$ Hz); ^{13}C NMR (100 MHz, methanol- d_4) δ_C 197.8, 168.3, 165.5, 164.9, 159.0, 131.1, 129.0, 116.3, 103.4, 97.0, 96.2, 80.5, 44.0.

Eriodictyol (13) – ^1H NMR (400 MHz, acetone- d_6) δ_H 7.04 (1H, s), 6.88 (2H, s), 5.97 (1H, brs), 5.96 (1H, brs), 5.40 (1H, dd, $J = 12.7, 3.0$ Hz), 3.14 (1H, dd, $J = 17.1, 12.7$ Hz), 2.73 (1H, dd, $J = 17.1, 3.0$ Hz); ^{13}C NMR (100 MHz, acetone- d_6) δ_C 197.2, 167.3, 165.3, 165.0, 164.3, 146.3, 146.0, 131.6, 119.3, 116.0, 114.7, 103.2, 96.8, 95.8, 79.9, 43.6.

Determination of absolute configuration – Compound **1** (1.0 mg) was treated separately with (*R*)- and (*S*)-

MTPA-Cl in pyridine. The mixtures were stirred at room temperature until the reactions were complete to yield the (*S*)- and (*R*)-MTPA esters, respectively. After that, ^1H NMR spectra were recorded, and the absolute configuration was assigned based on the sign distribution of $\Delta\delta_{H(S-R)}$ values following the modified Mosher's method.⁸

Determination of NO production and cell viability – RAW 264.7 cells were exposed to either 1 μ g/mL of LPS (Sigma Chemical Co.) or a control for 24 hours, with or without the test substance (1–100 μ M). A total of 100 μ L of Griess reagent (consisting of 0.1% naphthyl ethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% phosphoric acid) was combined with 100 μ L of the cell culture supernatant following incubation. Cell viability was evaluated via an MTT-based colorimetric test.⁹

In silico target prediction – Biological targets for compound **1** were predicted using the SwissTargetPrediction webserver (<http://www.swisstargetprediction.ch/>) against *Homo sapiens*. The structure was submitted as a SMILES string to identify potential interactions with protein classes based on 2D/3D structural similarity to known ligands.¹⁰

Results and Discussion

The methanolic extract from the aerial parts of *A. wayomogi* was sequentially partitioned using *n*-hexane, MC, EA, and *n*-BuOH. Intensive fractionation of the EA-soluble residue (150 g) via silica gel chromatography and preparative HPLC yielded one new (**1**), and 12 known compounds (**2**–**13**) (Fig. 1), which were determined to be *p*-coumaric acid (**2**),¹¹ caffeic acid (**3**),¹² methyl caffeate (**4**),¹² hydroquinone (**5**),¹³ 4-hydroxybenzoic acid (**6**),¹⁴ protocatechuic acid (**7**),¹⁵ eugenyl glucoside (**8**),¹⁶ kaempferol (**9**),¹⁷ luteolin (**10**),¹⁸ apigenin (**11**),¹⁹ naringenin (**12**),²⁰ and eriodictyol (**13**)²¹ by analyzing and comparing their spectroscopic data with those reported in the literature. Notably, this study marks the first isolation of compounds **2**, **3**, **5**, and **13** from this plant species.

Artemisiwayin (**1**) was isolated as a white amorphous powder with a molecular formula of $C_9H_{10}O_4$, which was deduced from the HREIMS that displayed a molecular ion peak at m/z 182.0577 $[M]^+$ (calcd for $C_9H_{10}O_4$, 182.0579). Its ^1H NMR spectrum (Table 1) revealed an ABX coupling system characterized by signals at δ_H 7.43 (2H, overlap, H-2 and H-6) and 6.84 (1H, d, $J = 8.8$ Hz, H-5), along with one methine signal at δ_H 5.11 (1H, q, $J = 6.9$ Hz, H-8) and one methyl signal at δ_H 1.39 (3H, d, $J = 6.9$ Hz, H-9). The ^{13}C NMR spectrum displayed 9 carbon signals, including one carbonyl (δ_C 202.1), six aromatic

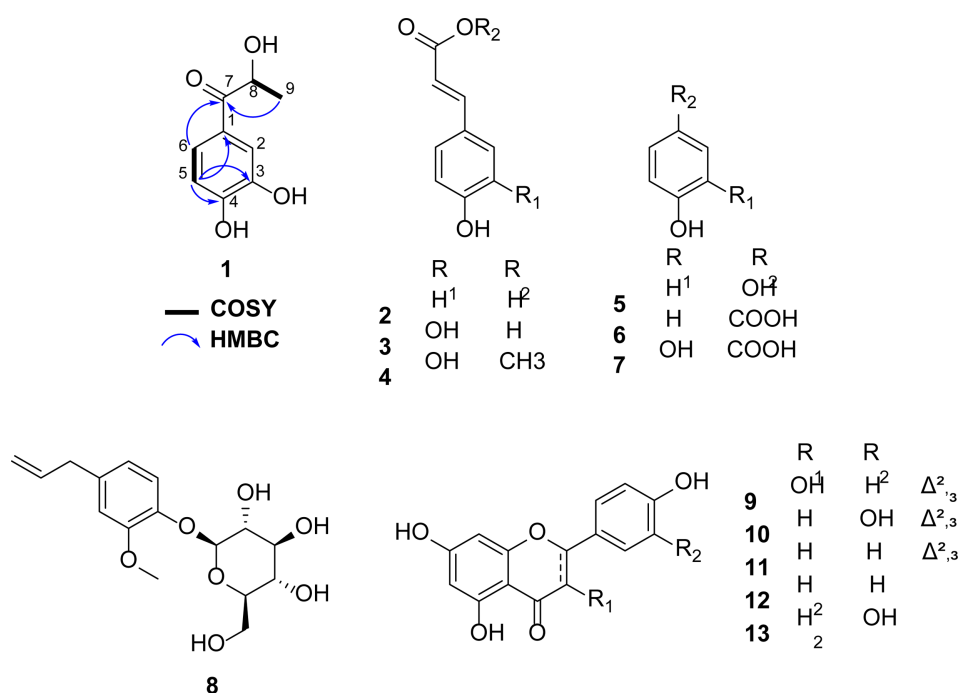


Fig. 1. Chemical structures of compounds **1–13** isolated from *A. iwayomogi* and key COSY and HMBC correlations for the new compound **1**.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data in methanol- d_4 for compound **1**.

Position	δ_{H} mult., (J in Hz)	δ_{C} , type
1		127.6, C
2	7.43, overlap	116.4, CH
3		146.6, C
4		152.5, C
5	6.84, d (8.8)	116.0, CH
6	7.43, overlap	123.5, CH
7		202.1, CO
8	5.11, q (6.9)	69.9, CH
9	1.39, d (6.9)	22.2, CH ₃

carbons (δ_{C} 152.5, 146.6, 127.6, 123.5, 116.4, 116.0), one oxygenated methine (δ_{C} 69.9), and one methyl (δ_{C} 22.2). Comparison with 1-(3,4-dihydroxyphenyl)-1-hydroxy-2-propanone revealed close structural similarities, with the key difference being the positions of the carbonyl and hydroxy groups.²² In compound **1**, these groups were located at C-7 and C-8, respectively, whereas in the reference compound, they were at C-8 and C-7. The structural assignment was confirmed by HMBC correlations from H-6 to C-7 and H-9 to both C-8 and C-7, as well as a COSY cross-peak between H-9 and H-8 (Fig. 1). Based on these findings, a planar structure was proposed for

compound **1**. The absolute configuration at C-8 of **1** was determined through application of the modified Mosher's method.⁸ Treatment of compound **1** with (*R*)- and (*S*)-MTPA-Cl independently yielded the corresponding (*S*)- and (*R*)-MTPA esters, respectively. Analysis of the $\Delta\delta_{\text{H}(\text{S-R})}$ values revealed a positive value for H-5 and a negative value for H₃-9, thereby assigning the *S* configuration to C-8 (Fig. 2). Therefore, the structure of **1** was deduced and given the trivial name artemisiwayin.

Compound **2** was identified as *p*-coumaric acid. The ^1H NMR spectrum exhibited characteristic signals for a *para*-disubstituted benzene ring at δ_{H} 7.46 (2H, d, J = 8.7 Hz, H-2/6) and 6.81 (2H, d, J = 8.7 Hz, H-3/5). The presence of a *trans*-configured olefinic side chain was confirmed by two coupled doublets at δ_{H} 7.61 (1H, d, J = 15.8 Hz,

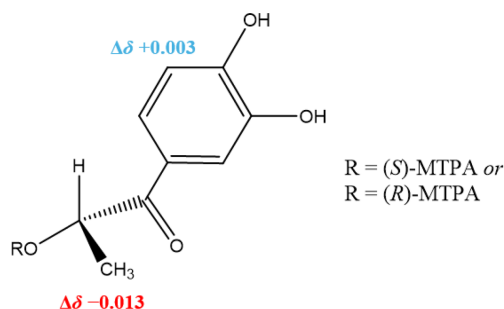


Fig. 2. $\Delta\delta_{\text{H}(\text{S-R})}$ values obtained from the ^1H NMR spectra of the MTPA esters of compound **1**.

H-7) and 6.29 (1H, d, $J = 15.8$ Hz, H-8). The ^{13}C NMR spectrum displayed seven signals, including a carbonyl carbon at δ_{C} 169.6 (C-9), an oxygenated aromatic carbon at δ_{C} 159.7 (C-4), and two olefinic carbons at δ_{C} 145.2 (C-7) and 114.2 (C-8). These spectroscopic data were in full agreement with literature values for *p*-coumaric acid.¹¹

The NMR spectra of **3** were closely related to those of **2**, except for the aromatic region, which exhibited a 1,2,4-trisubstituted benzene system (δ_{H} 6.98, 6.88, and 6.72) instead of the *para*-disubstituted pattern found in **2**. The presence of two oxygenated aromatic carbons at δ_{C} 147.0 (C-3) and 146.7 (C-4) further confirmed the catechol moiety. These spectroscopic data were consistent with literature values for caffeic acid.¹²

Compound **5** was identified as hydroquinone. The NMR spectra revealed a high degree of structural symmetry. The ^1H NMR spectrum exhibited a single resonance at δ_{H} 4.85 (4H, s), corresponding to the four equivalent aromatic protons. Consistently, the ^{13}C NMR spectrum displayed only two signals: one oxygenated aromatic carbon at δ_{C} 151.2 (C-1/4) and one aromatic carbon at δ_{C} 116.8 (C-2/3/5/6). These spectroscopic characteristics were in complete agreement with the structure of hydroquinone.¹³

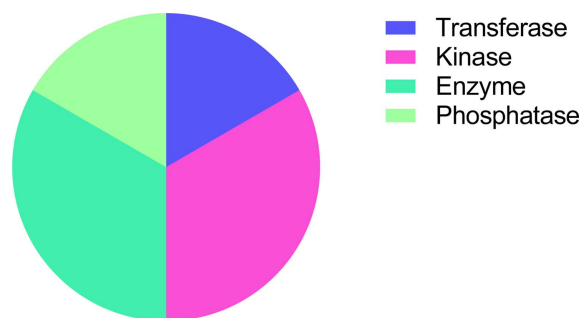


Fig. 3. *In silico* target prediction of compound **1** using SwissTargetPrediction.

The NMR data for **13** were diagnostic of a flavanone skeleton. The ^1H NMR spectrum displayed a typical ABX system for the C-ring at δ_{H} 7.04 (1H, s) and 6.88 (2H, s). The A-ring substitution pattern was evidenced by two meta-coupled aromatic signals at δ_{H} 5.97 and 5.96 (each 1H, brs, H-6 and H-8). In the ^{13}C NMR spectrum, the presence of a flavanone skeleton was further confirmed by the carbonyl resonance at δ_{C} 197.2 (C-4), along with aliphatic signals at δ_{C} 79.9 (C-2) and 43.6 (C-3). Comparison of these data with literature values confirmed the identity of **13** as eriodictyol.²¹

To assess the anti-inflammatory potential of the isolated

Table 2. List of the top 15 predicted protein targets ranked by probability.*

No.	Target	Common name	Target class	Probability*
1	Catechol <i>O</i> -methyltransferase	COMT	Transferase	0.089370474
2	Insulin-like growth factor I receptor	IGF1R	Kinase	0.071787163
3	Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	Enzyme	0.071787163
4	Adenosylhomocysteinase	AHCY	Enzyme	0.071787163
5	Protein-tyrosine phosphatase 2C	PTPN11	Phosphatase	0.071787163
6	Protein kinase C epsilon	PRKCE	Kinase	0.071787163
7	Adenosine A1 receptor (by homology)	ADORA1	Family A G protein-coupled receptor	0
8	Adenosine kinase	ADK	Enzyme	0
9	Purine nucleoside phosphorylase	PNP	Enzyme	0
10	Adenosine A2a receptor	ADORA2A	Family A G protein-coupled receptor	0
11	Adenosine A2b receptor	ADORA2B	Family A G protein-coupled receptor	0
12	Gamma-amino-N-butyrate transaminase (by homology)	ABAT	Transferase	0
13	Glutathione reductase	GSR	Oxidoreductase	0
14	Amine oxidase, copper containing	AOC3	Enzyme	0
15	Beta-glucocerebrosidase	GBA	Enzyme	0

*Entries 7–15 (probability = 0) are listed for completeness only and do not represent supported interactions.

constituents, their ability to inhibit NO production was considered. Given that compounds **2–13** are well-known metabolites with established inhibitory profiles in the literature, further testing was focused exclusively on the new compound **1**. However, compound **1** was found to be inactive under the tested conditions, with an IC_{50} value exceeding 100 μ M. Cell viability was assessed in parallel with the NO inhibition assay across the same concentration range, and compound **1** showed no cytotoxic effect ($IC_{50} > 100 \mu$ M).

To further elucidate the pharmacological potential of the new compound **1**, an *in silico* target prediction analysis was performed using the SwissTargetPrediction platform. SwissTargetPrediction returned 15 putative targets, of which six had a non-zero probability score, spanning four target classes (kinase, enzyme, transferase, phosphatase) (Fig. 3, Table 2). Catechol *O*-methyltransferase (COMT) received the highest probability score; a further five targets, including insulin-like growth factor I receptor (IGF1R), shared an identical, lower score. The remaining nine targets (Table 2, entries 7–15) were assigned a probability of zero and are reported for completeness only, without implying a supported interaction. No NOS-family target was identified among the predictions, which may be in line with the lack of NO-inhibitory activity observed for compound **1**. These results are hypothesis-generating; experimental validation, particularly against COMT, would be needed before any predicted interaction can be considered biologically relevant.

In conclusion, 13 compounds were successfully isolated from the aerial parts of *A. iwayomogi* through various chromatographic techniques. The structural elucidation was confirmed by detailed spectroscopic analysis, identifying one new metabolite along with 12 known compounds. Notably, four of these (**2**, **3**, **5**, and **13**) were reported for the first time from this plant source. Although compound **1** was found to be inactive against NO production ($IC_{50} > 100 \mu$ M), the comprehensive characterization of these isolates enhances our knowledge of the plant's chemical constituents. This study provides a scientific basis for the further exploration of *A. iwayomogi* as a source of diverse natural products, and future research should focus on evaluating the potential of compound **1** in other biological models or alternative therapeutic targets.

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Conflicts of Interest

The authors declare no competing financial interest.

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SUPPLEMENTARY DATA

Phytochemical Constituents of the Aerial Parts of *Artemisia iwayomogi* Kitamura and Their Anti-inflammatory Activities

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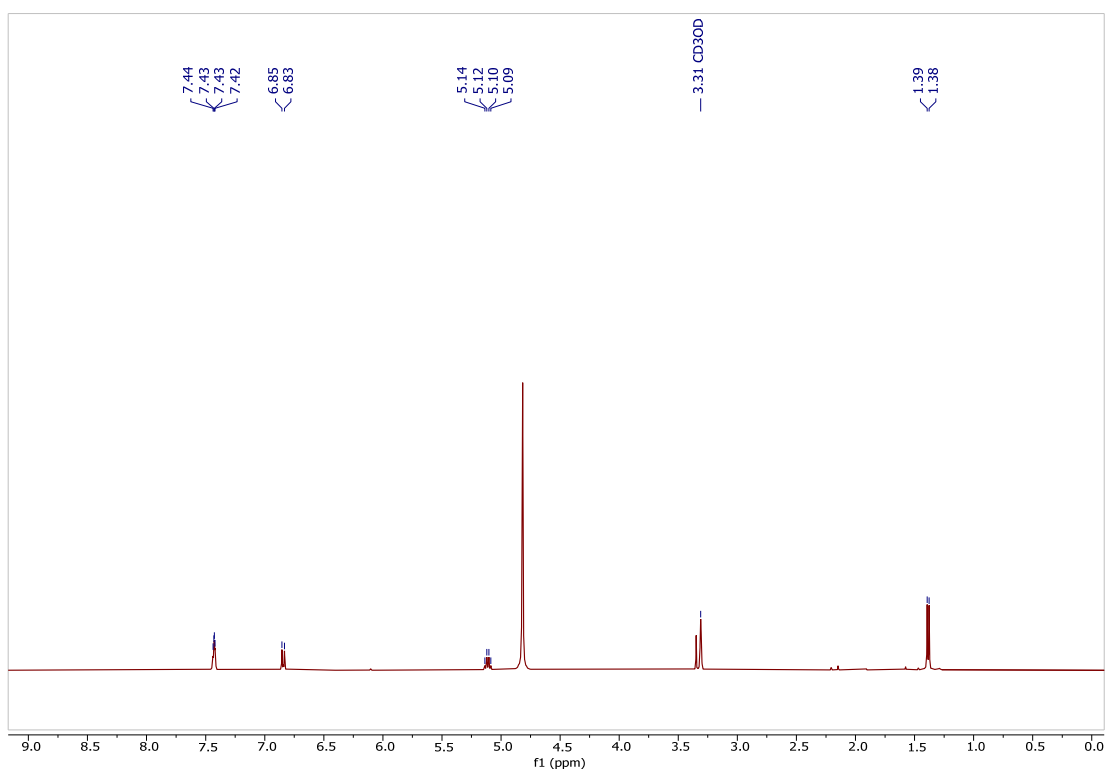


Figure S1. ^1H NMR spectrum of **1** in methanol- d_4

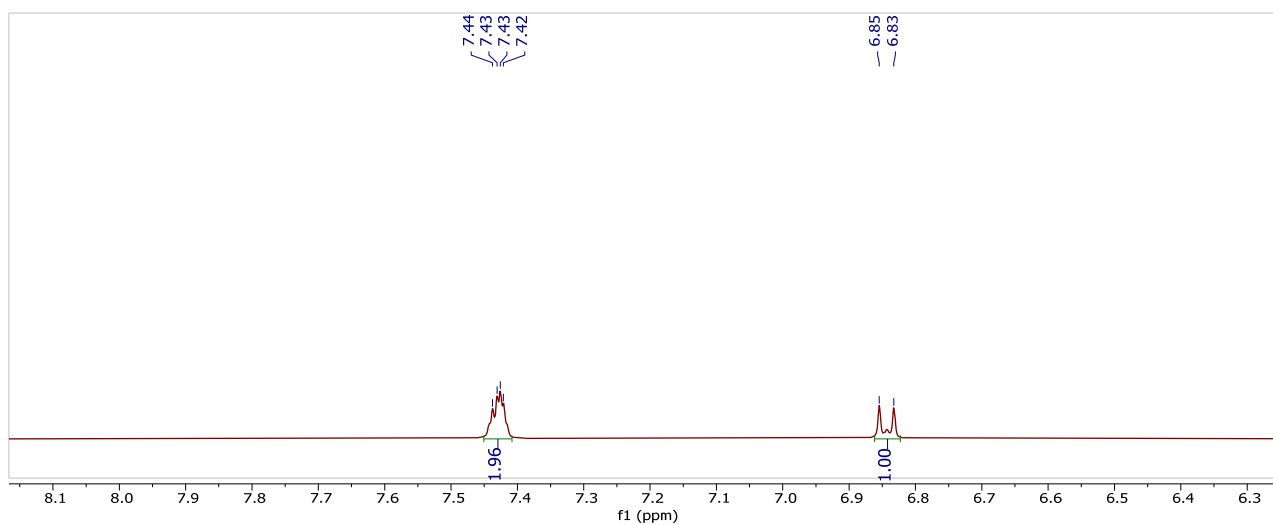


Figure S2. Expanded ^1H NMR spectrum of **1** in methanol- d_4

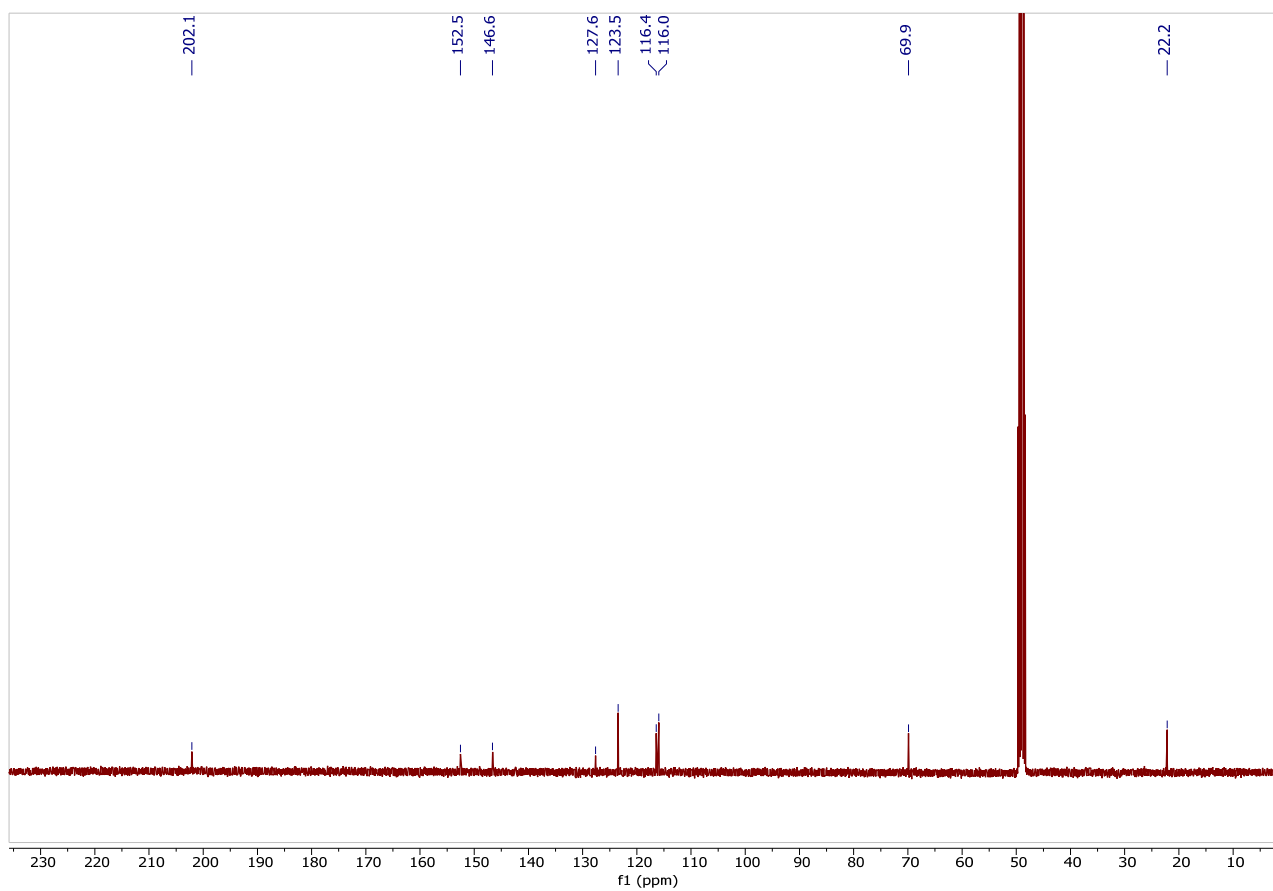


Figure S3. ^{13}C NMR spectrum of **1** in methanol- d_4

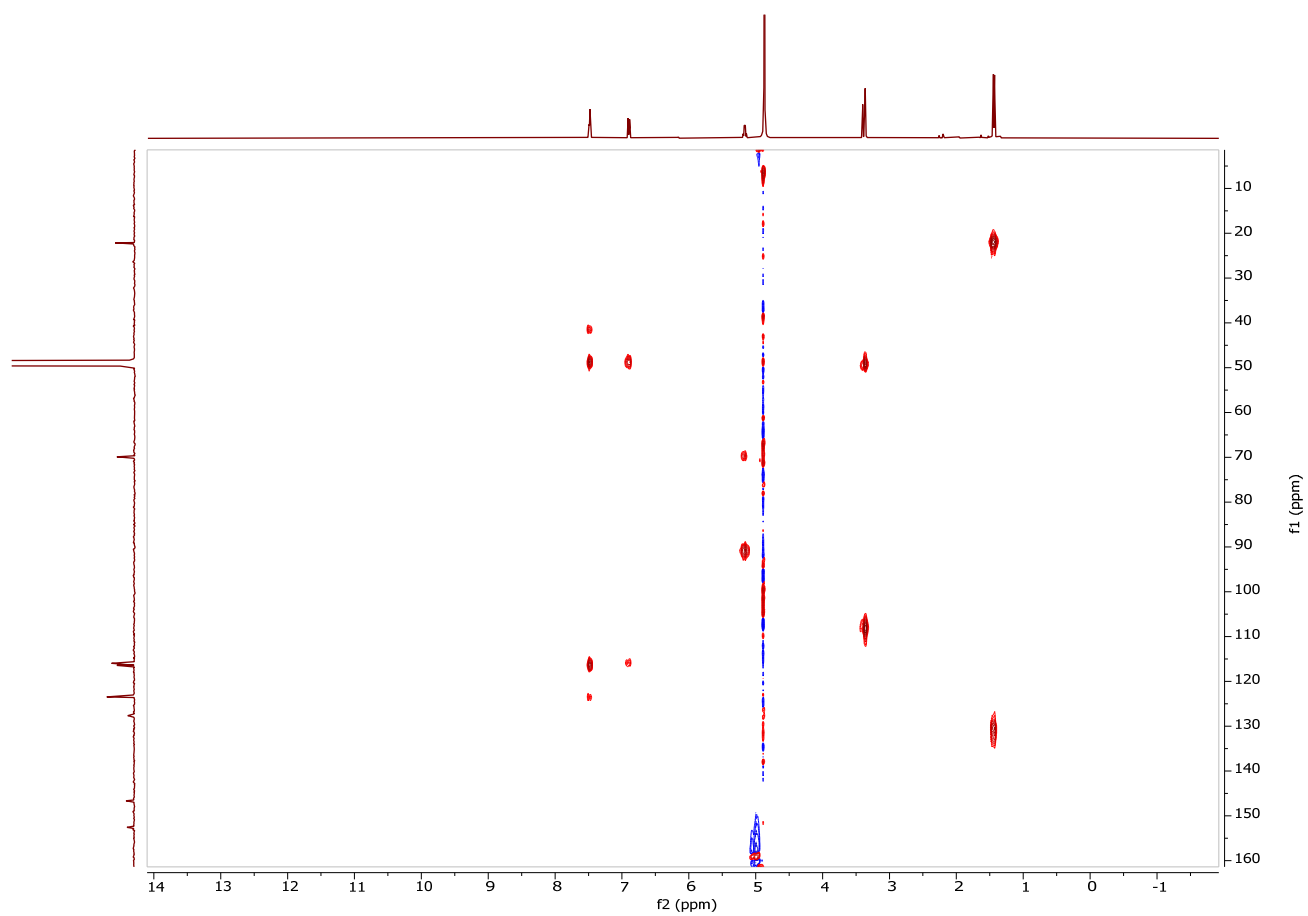


Figure S4. HMQC spectrum of **1** in methanol-*d*₄

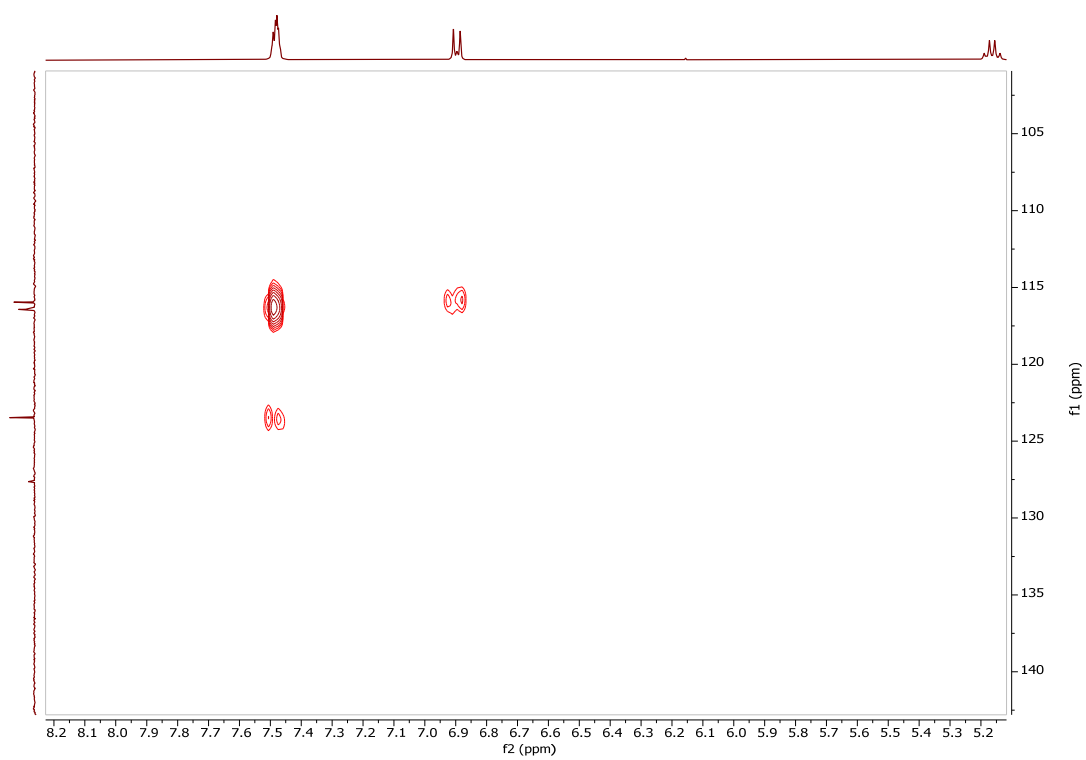


Figure S5. Expanded HMQC spectrum of **1** in methanol-*d*₄

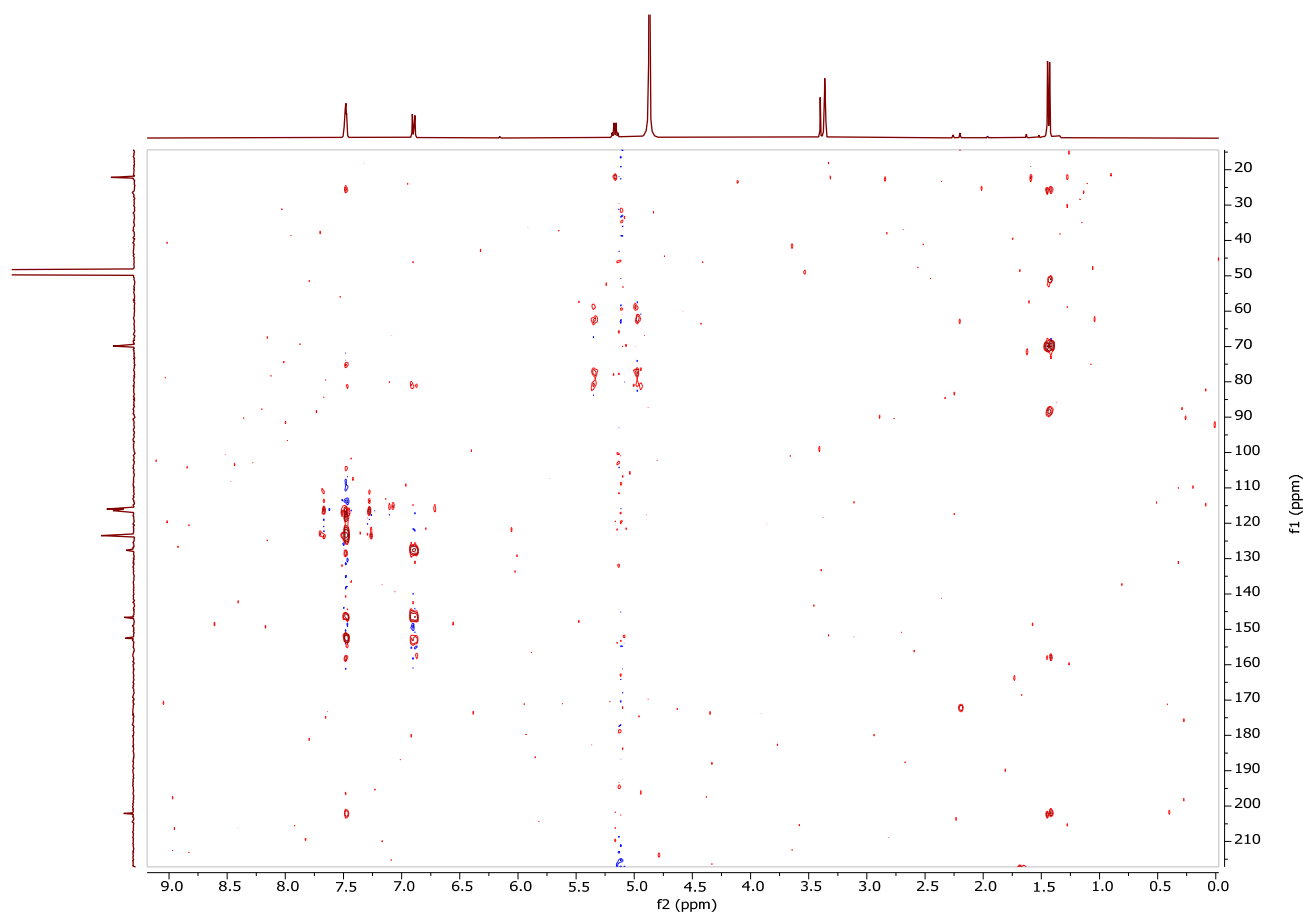


Figure S6. HMBC spectrum of **1** in methanol-*d*₄

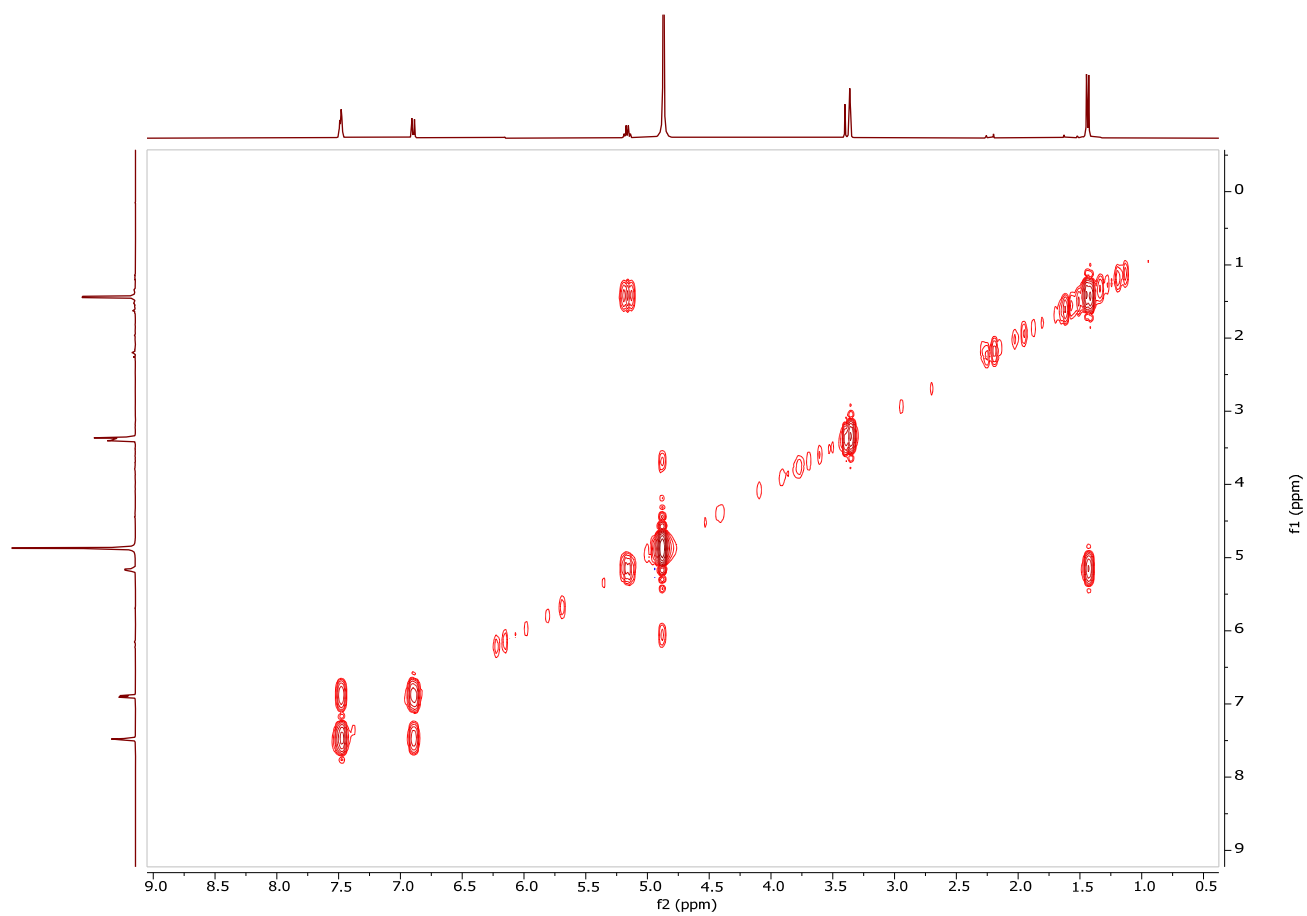


Figure S7. ^1H - ^1H COSY spectrum of **1** in $\text{methanol-}d_4$

[Mass Spectrum]
 Data : AIE6.3.8.2.2.1 Date : 03-Jun-2021 10:40
 Sample : -
 Note : -
 Inlet : Direct Ion Mode : EI+
 Spectrum Type : Normal Ion [EF-Linear]
 RT : 0.70 min Scan# : 22
 BP : m/z 182.0577 Int. : 38.82
 Output m/z range : 173.1869 to 191.2582 Cut Level : 0.00 %
 426854

[Elemental Composition]
 Data : AIE6.3.8.2.2.1 Date : 03-Jun-2021 10:40
 Sample : -
 Note : -
 Inlet : Direct Ion Mode : EI+
 RT : 0.70 min
 Elements : C 9/0, H 10/0, O 4/0
 Mass Tolerance : 1000ppm, 3mmu if m/z > 3
 Unsaturation (U.S.) : -0.5 - 100.0

Page: 1

Observed m/z	Int	Err(ppm / mmu)	U.S. Composition
153.0314	26.8		
164.0469	49.8	-2.7 / -0.4	6.0 C 9 H 8 O 3
165.0508	15.1		
166.0632	91.2	+1.3 / +0.2	5.0 C 9 H 10 O 3
167.0342	10.5	-1.6 / -0.3	5.5 C 8 H 7 O 4
167.0689	34.7		
182.0577	100.0	-1.2 / -0.2	5.0 C 9 H 10 O 4
183.0639	30.2		
193.0369	13.8		

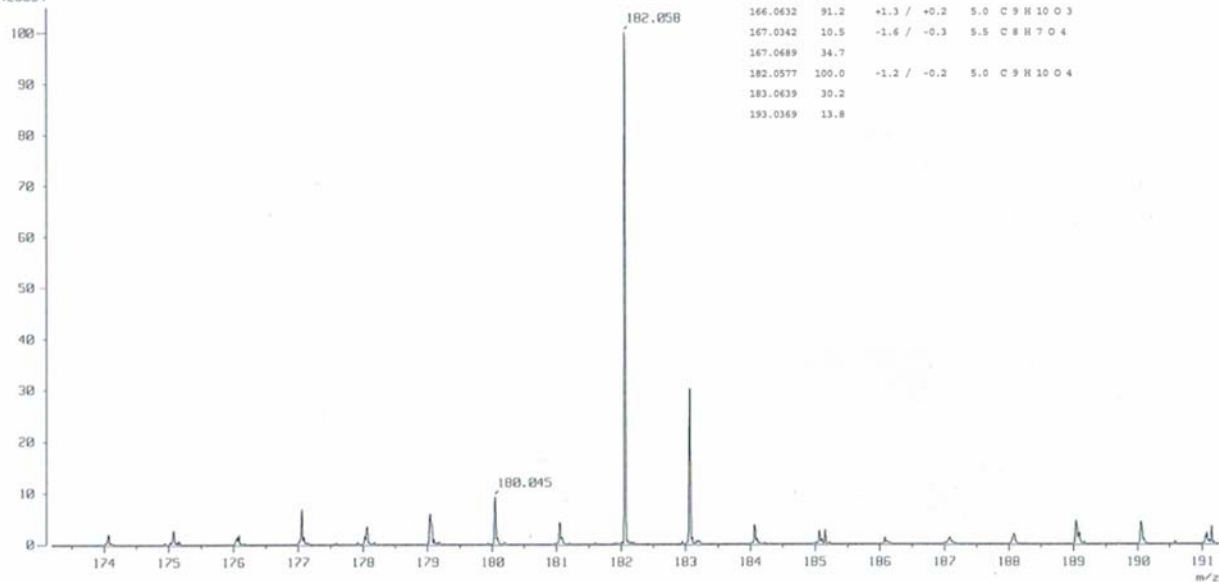


Figure S8. HREIMS spectrum of **1**

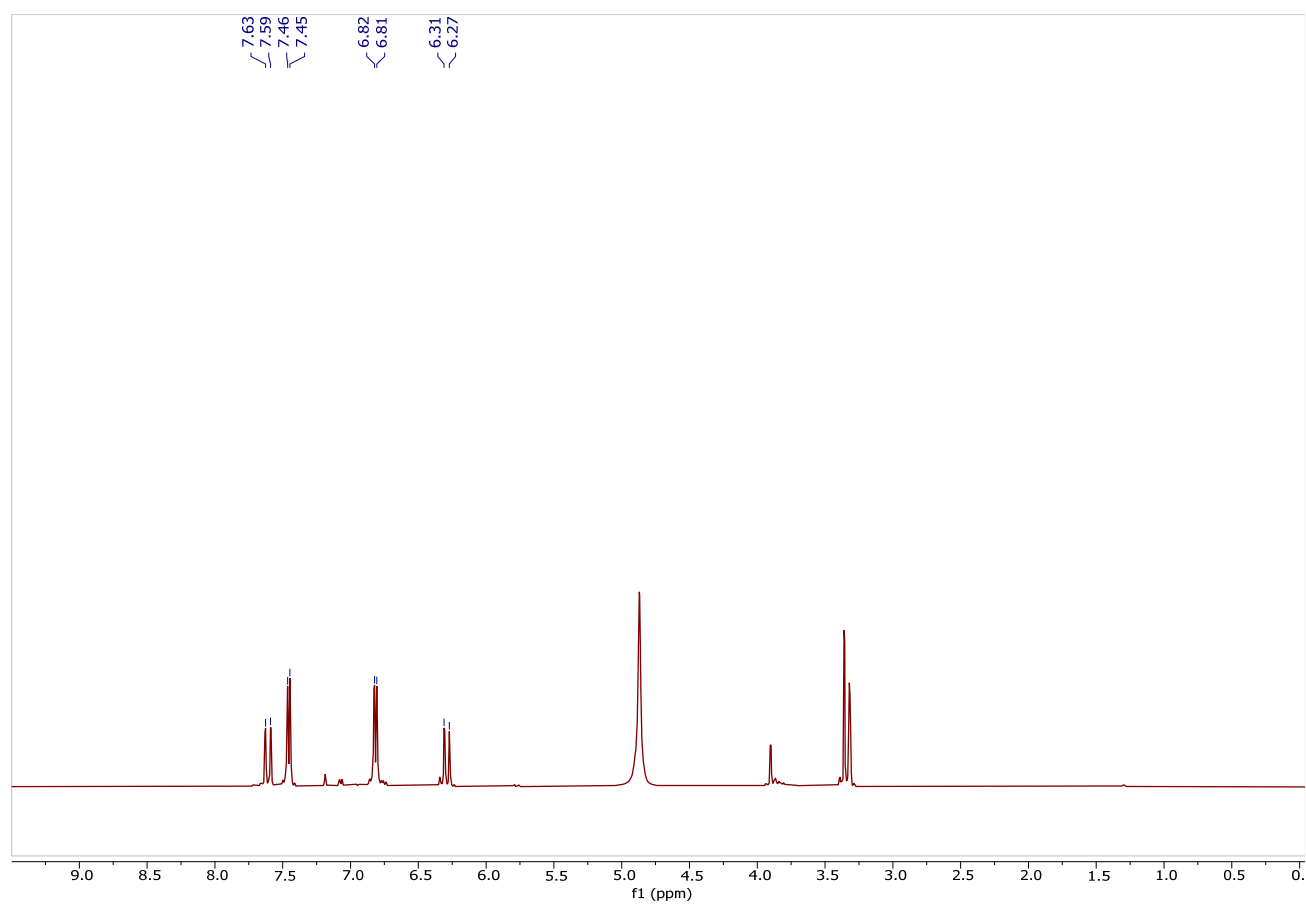


Figure S9. ¹H NMR spectrum of **2** in methanol-*d*₄

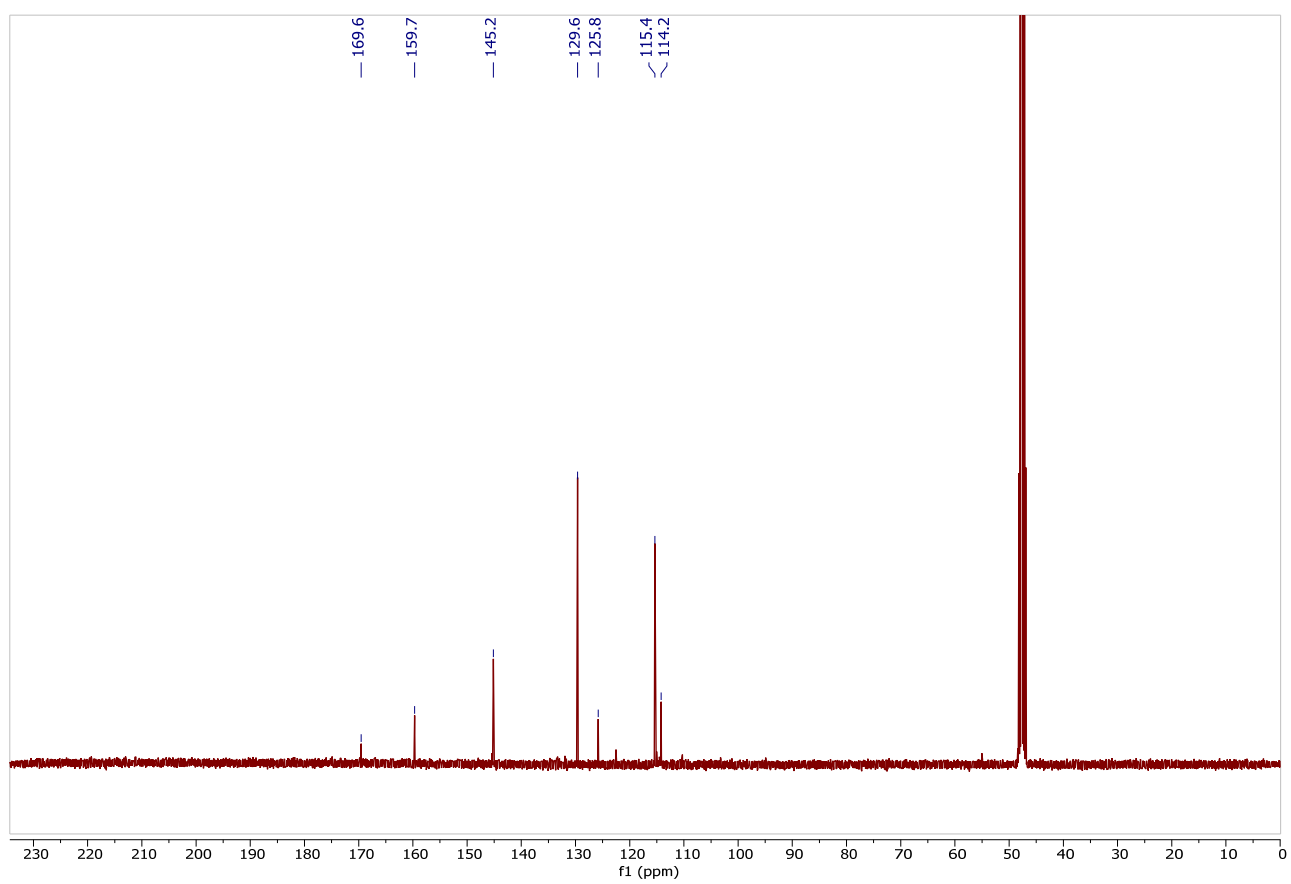


Figure S10. ^{13}C NMR spectrum of **2** in methanol- d_4

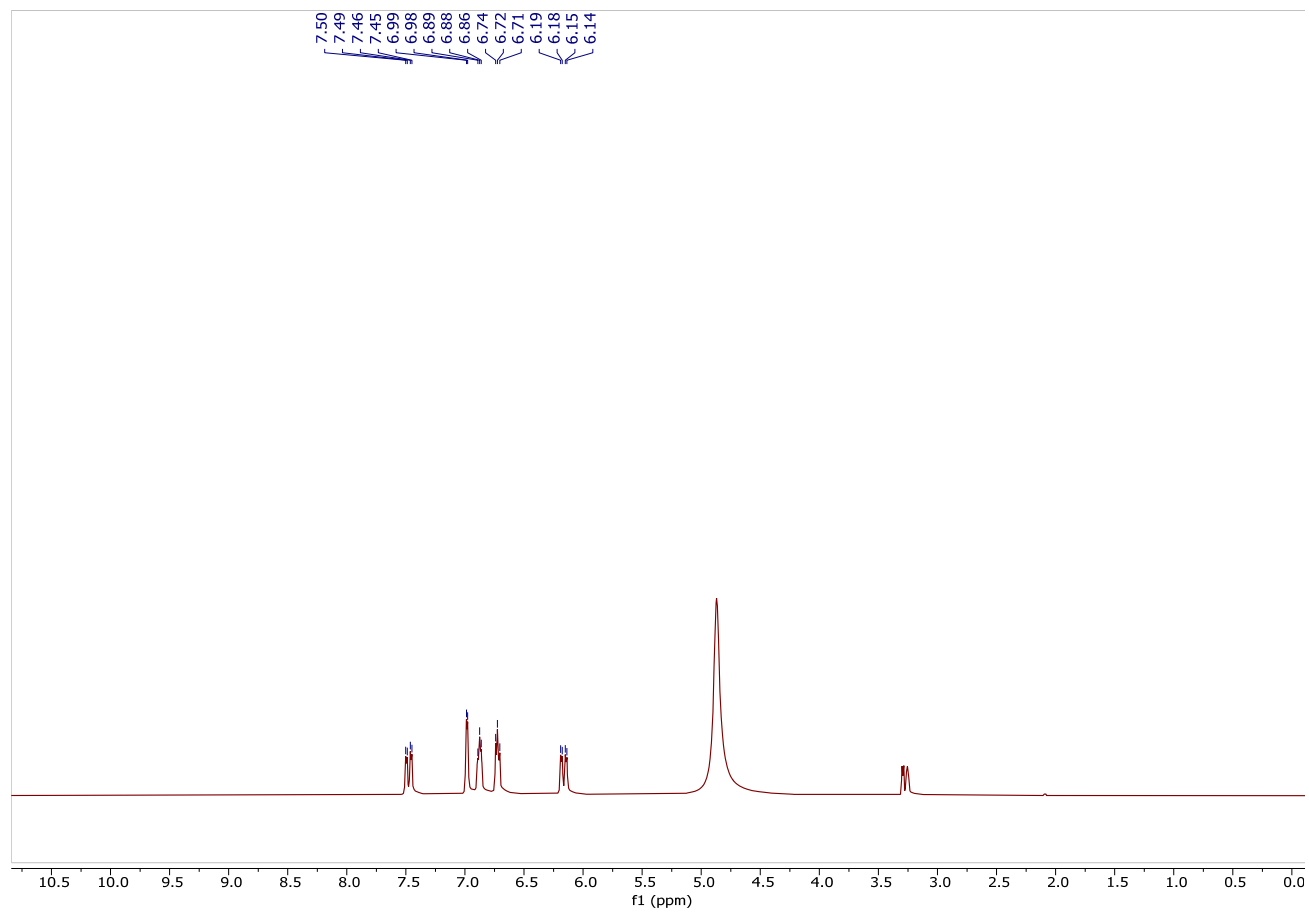


Figure S11. ¹H NMR spectrum of **3** in methanol-*d*₄

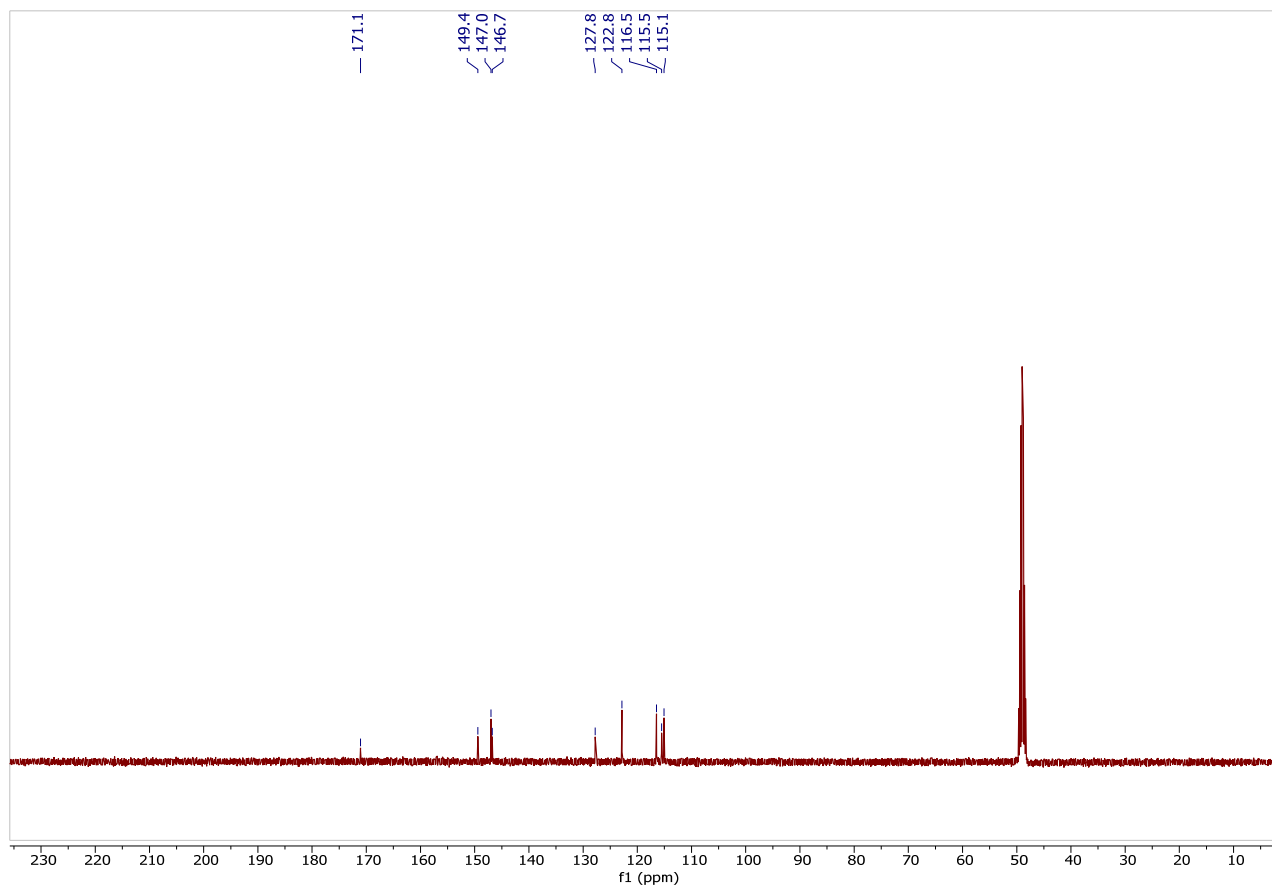


Figure S12. ^{13}C NMR spectrum of **3** in methanol- d_4

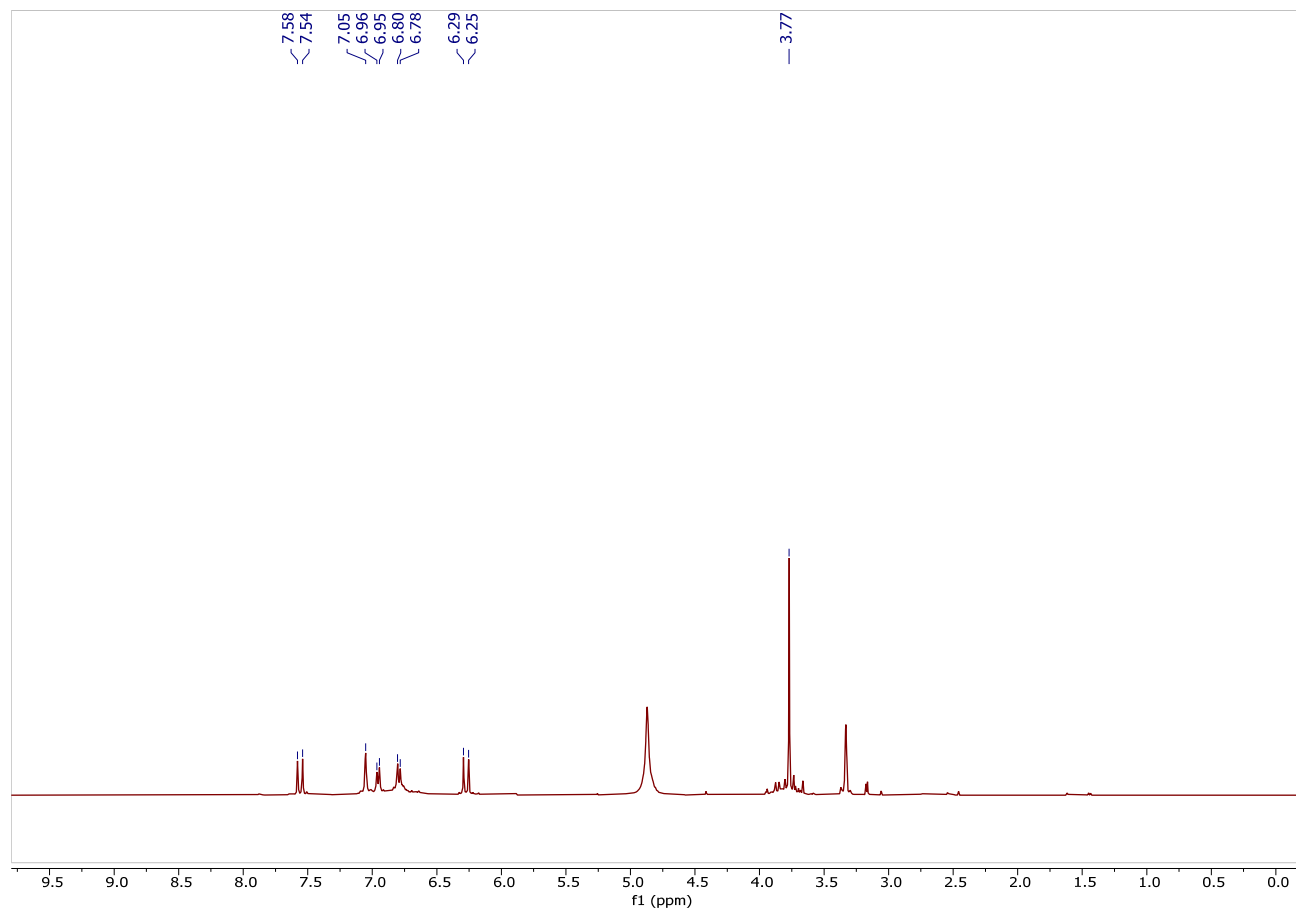


Figure S13. ¹H NMR spectrum of **4** in methanol-*d*₄

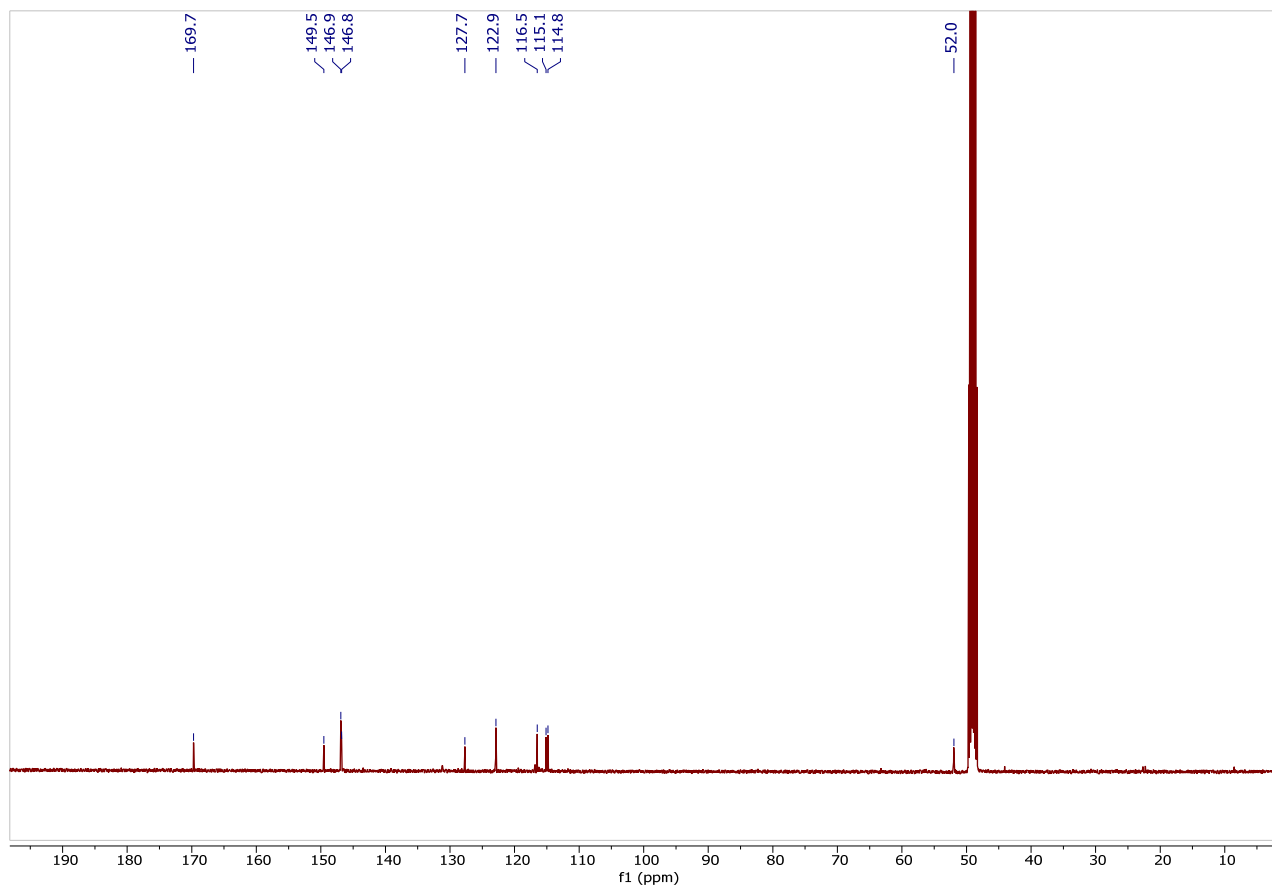


Figure S14. ¹³C NMR spectrum of **4** in methanol-*d*₄

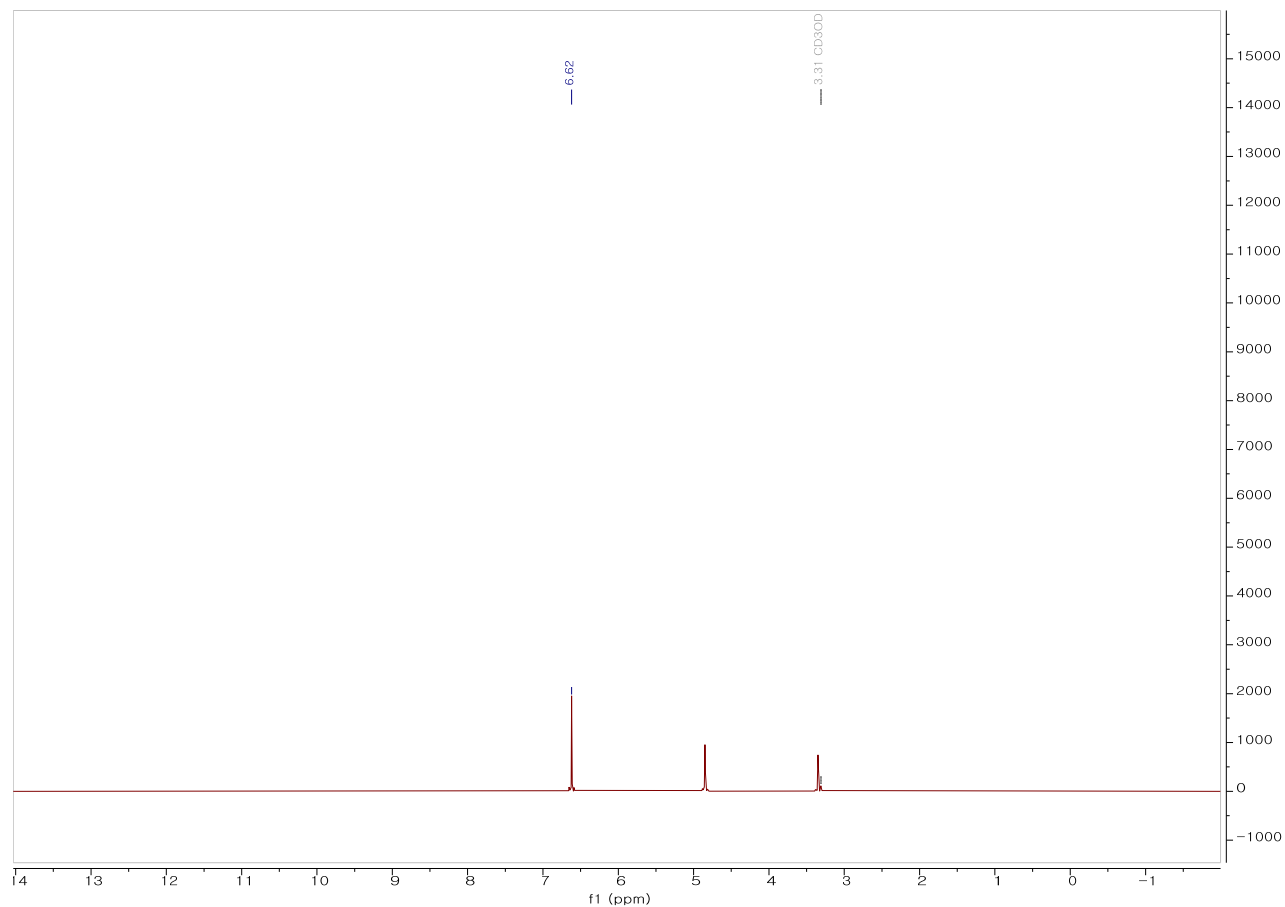


Figure S15. ^1H NMR spectrum of **5** in $\text{methanol-}d_4$

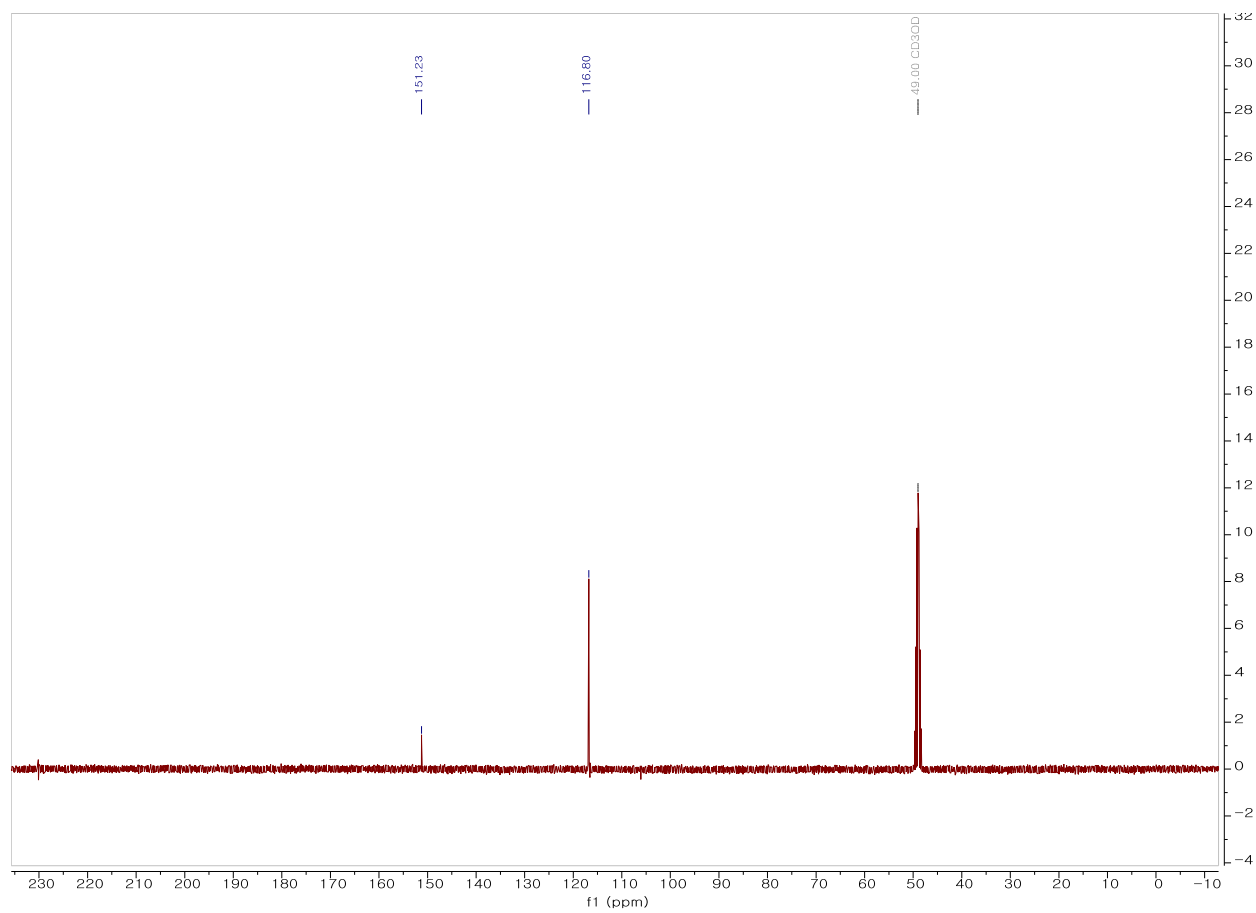


Figure S16. ^{13}C NMR spectrum of **5** in methanol- d_4

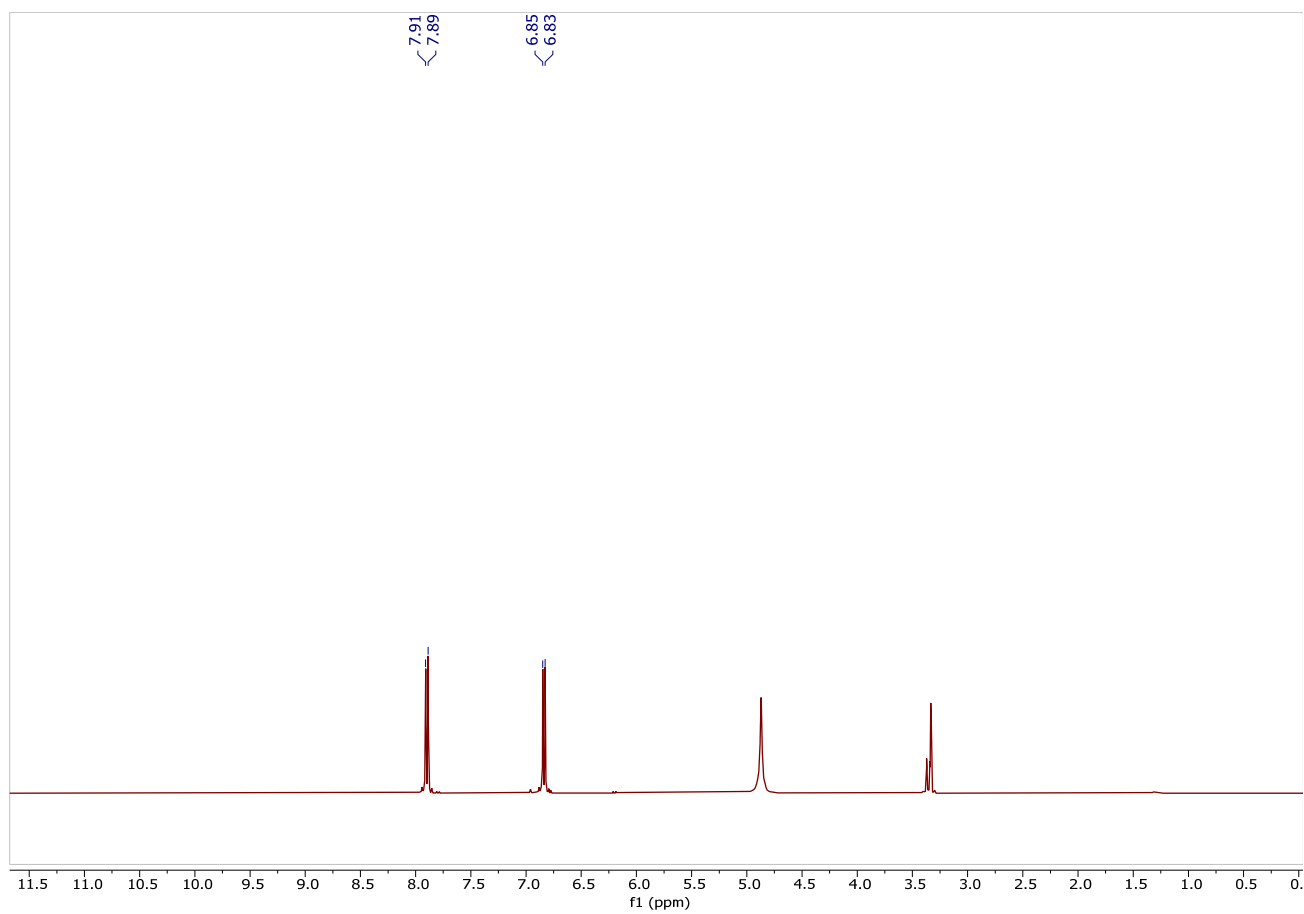


Figure S17. ¹H NMR spectrum of **6** in methanol-*d*₄

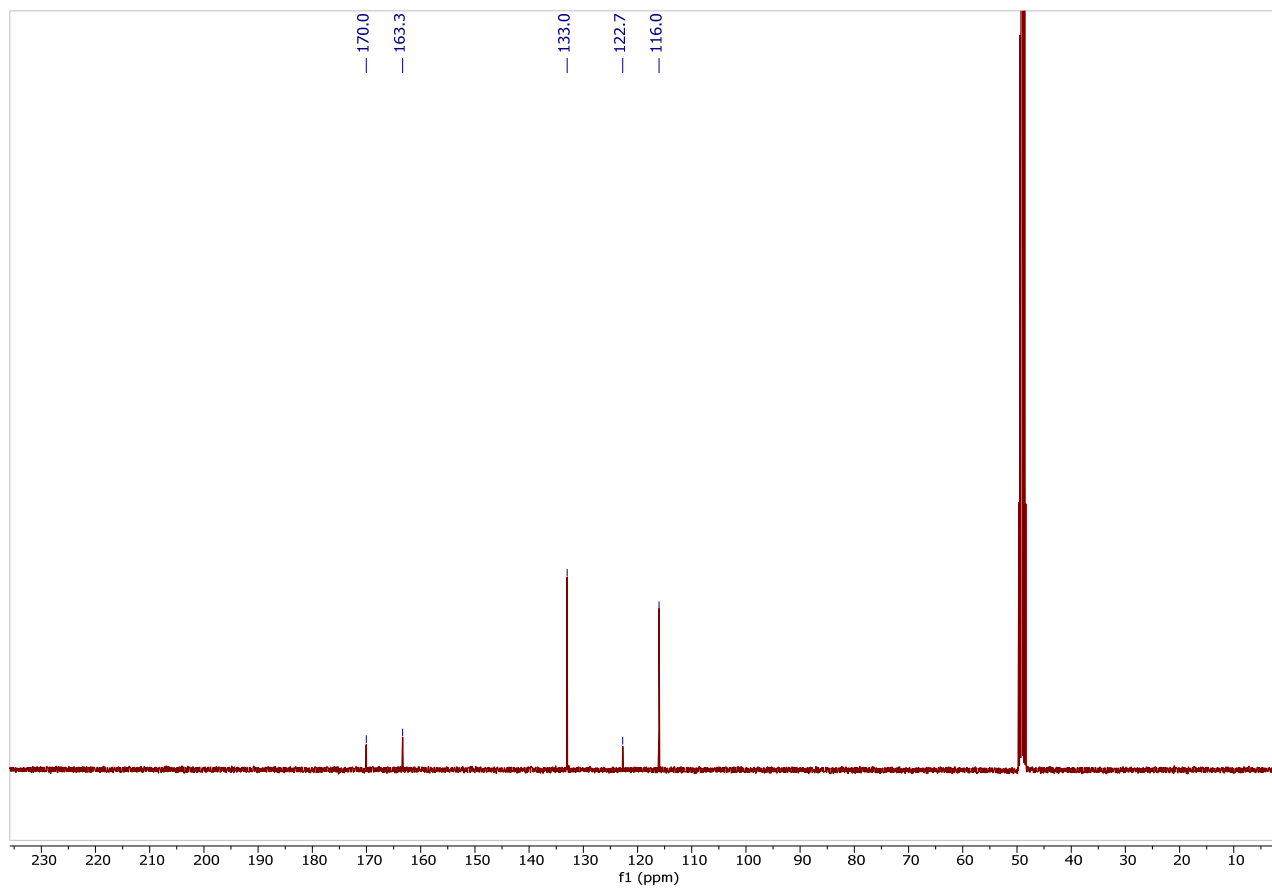


Figure S18. ¹³C NMR spectrum of **6** in methanol-*d*₄

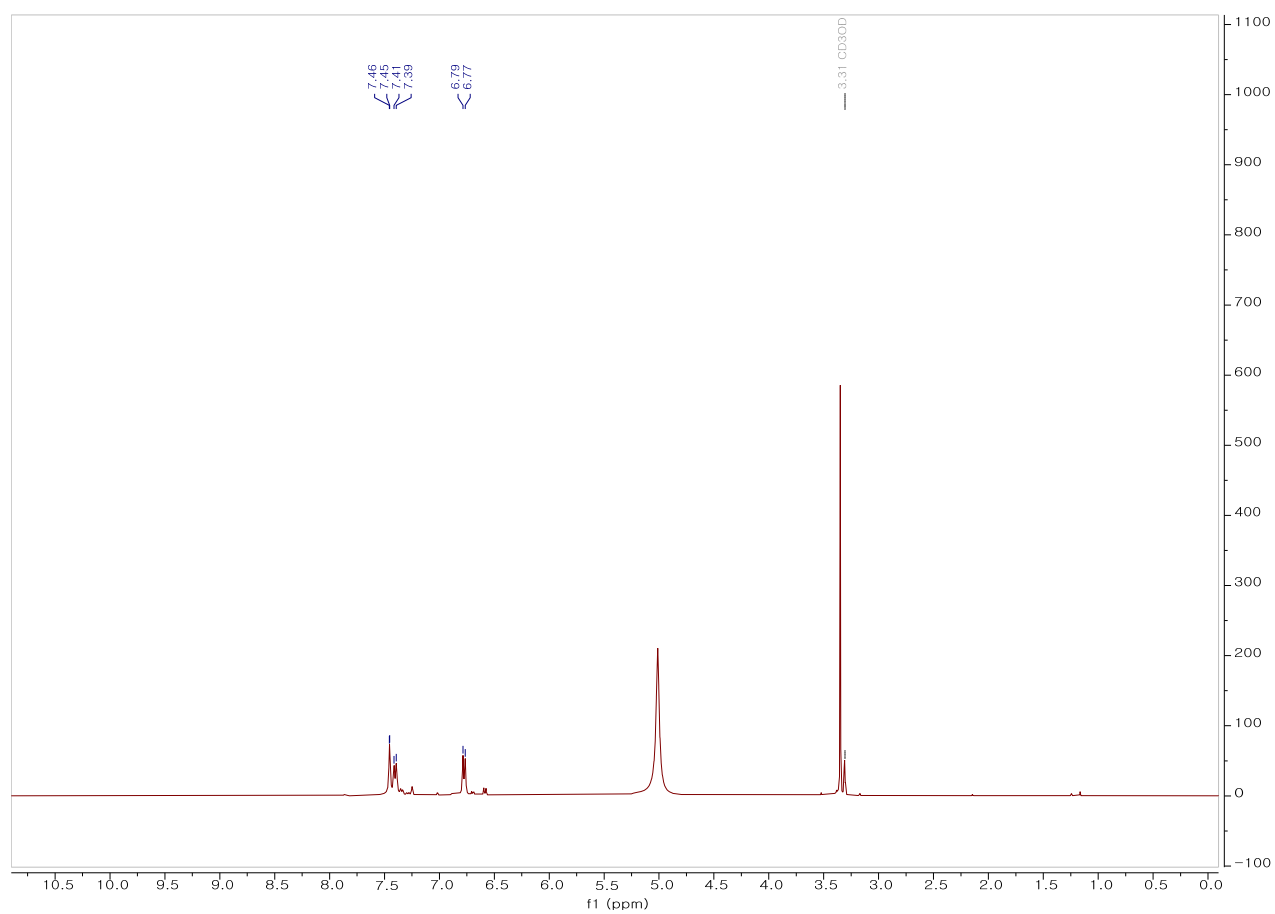


Figure S19. ^1H NMR spectrum of **7** in $\text{methanol-}d_4$

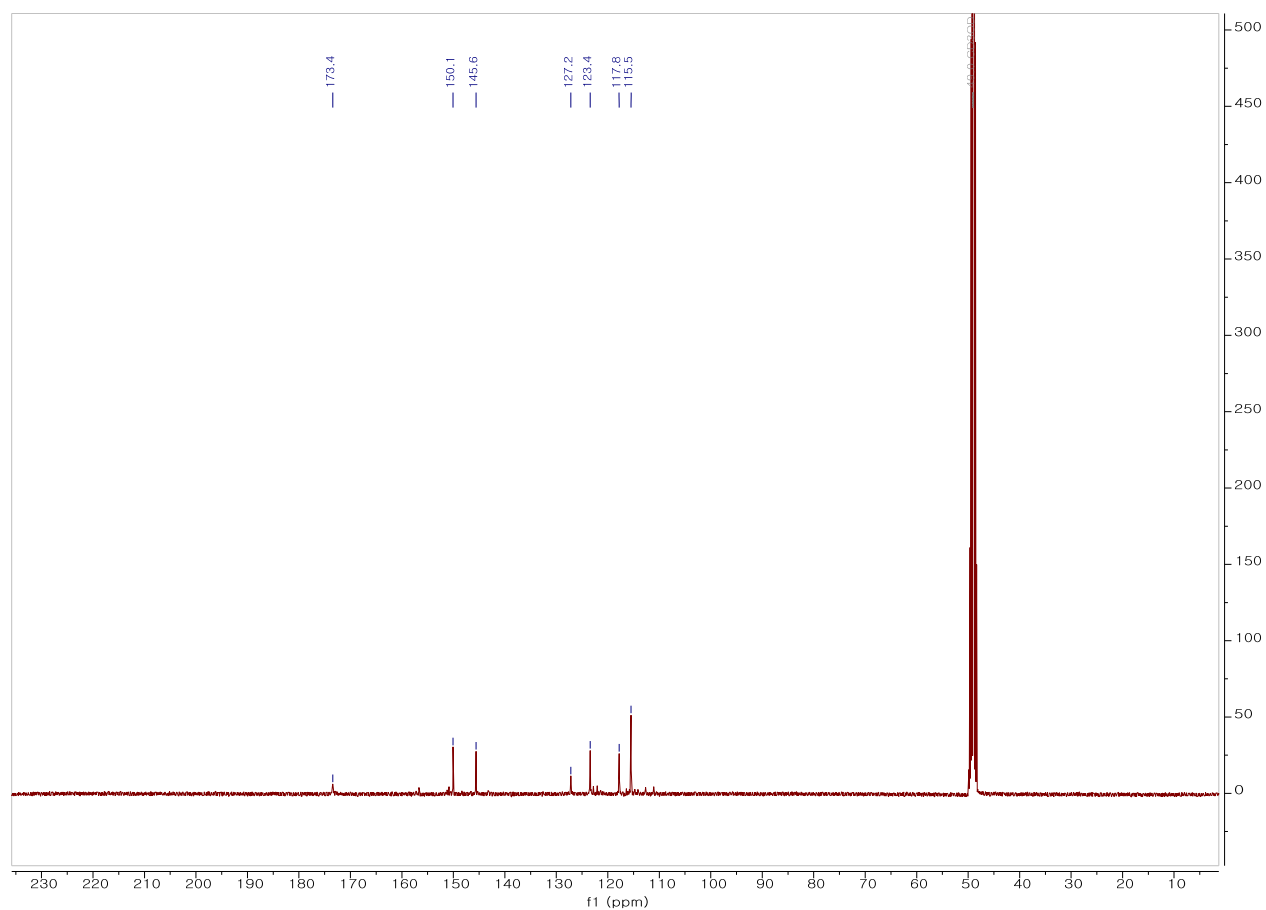


Figure S20. ¹³C NMR spectrum of 7 in methanol-*d*₄

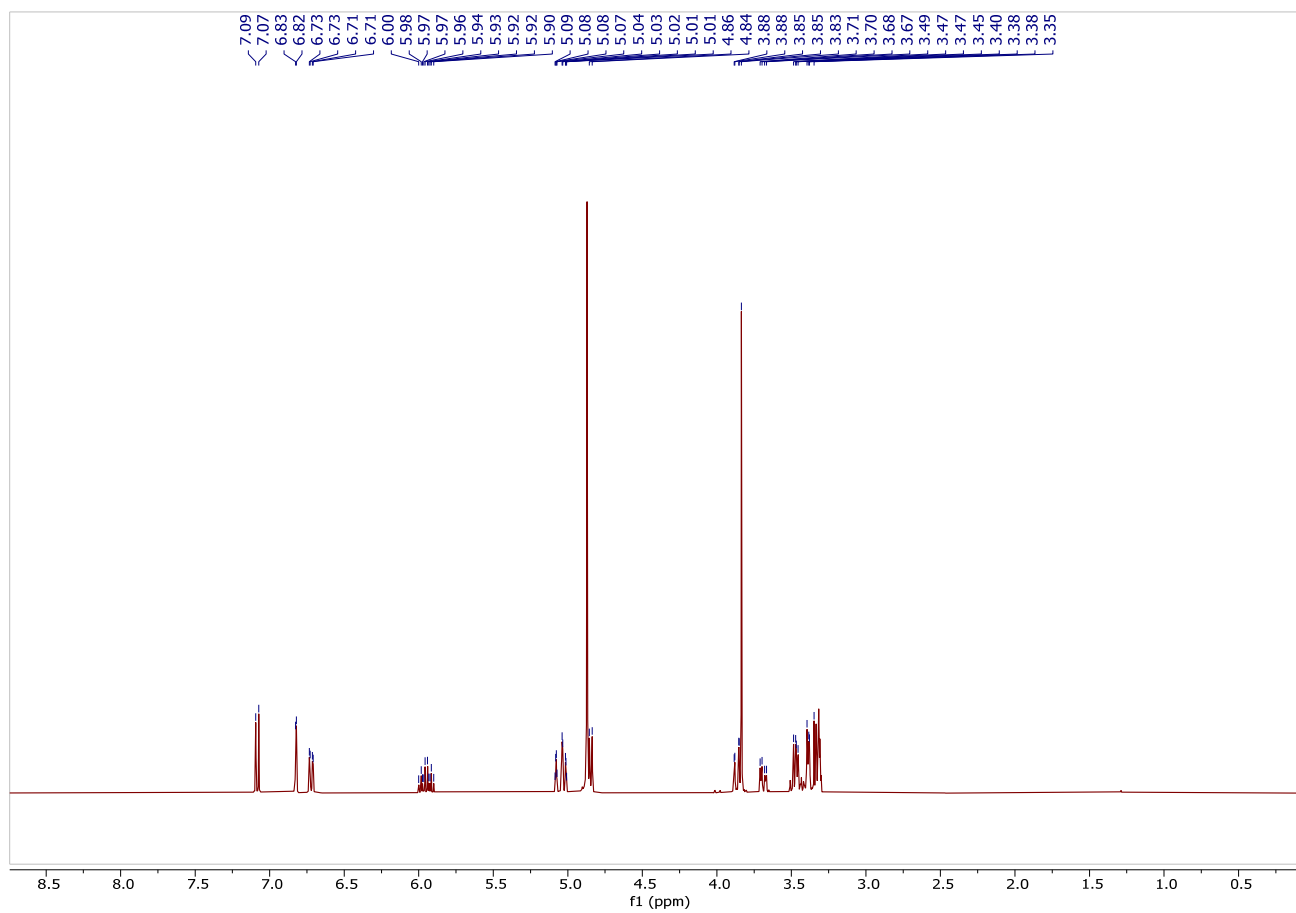


Figure S21. ^1H NMR spectrum of **8** in methanol- d_4

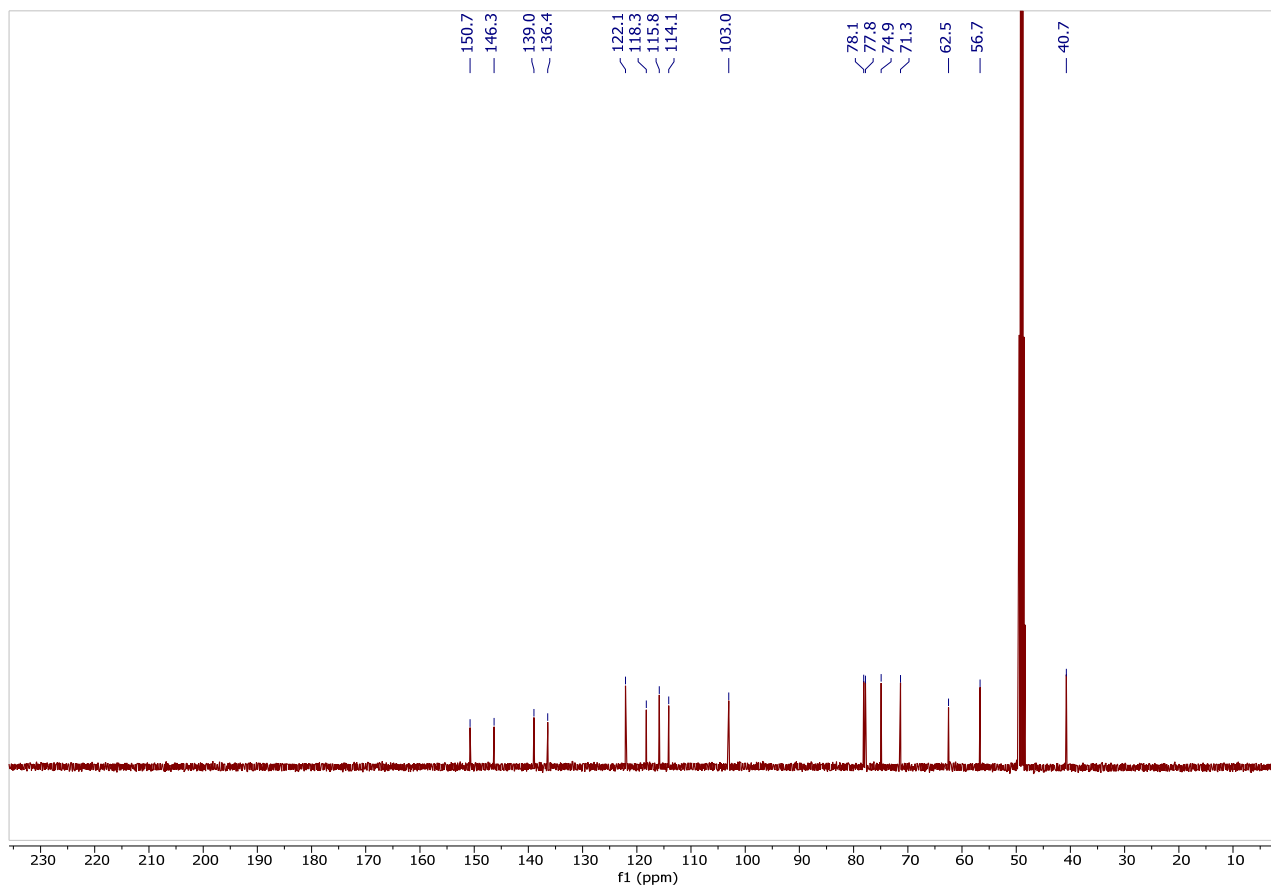


Figure S22. ¹³C NMR spectrum of **8** in methanol-*d*₄

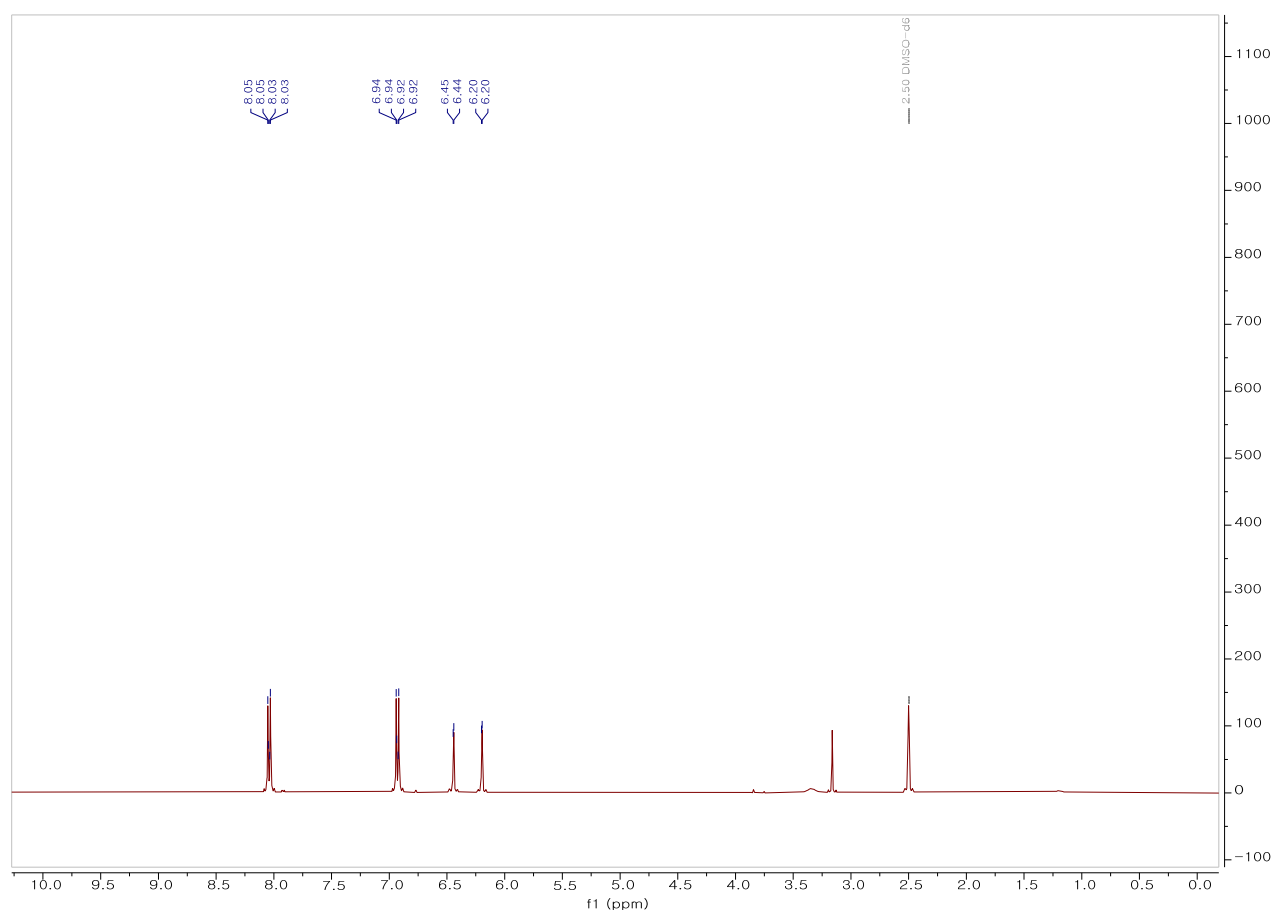


Figure S23. ¹H NMR spectrum of **9** in DMSO-*d*₆

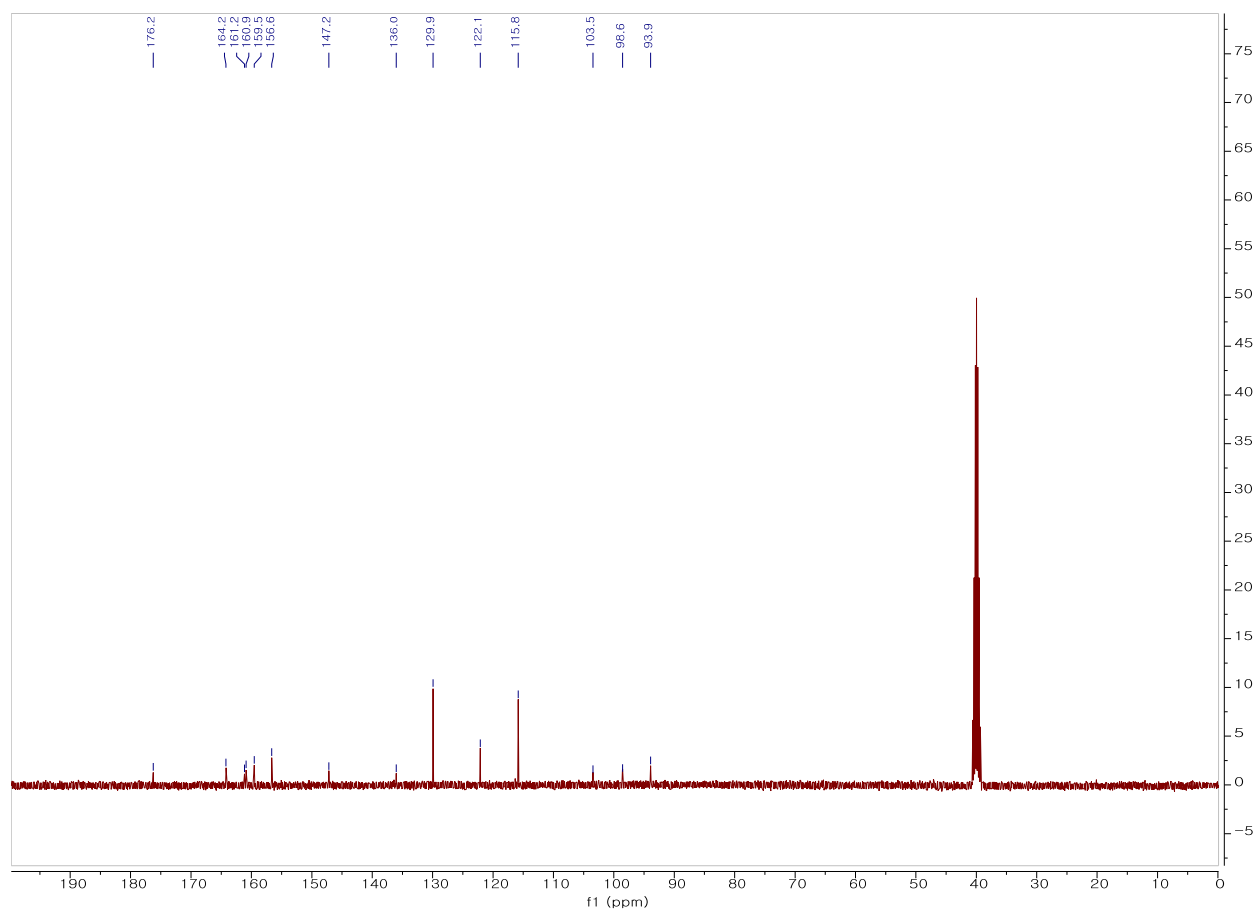


Figure S24. ^{13}C NMR spectrum of **9** in $\text{DMSO-}d_6$

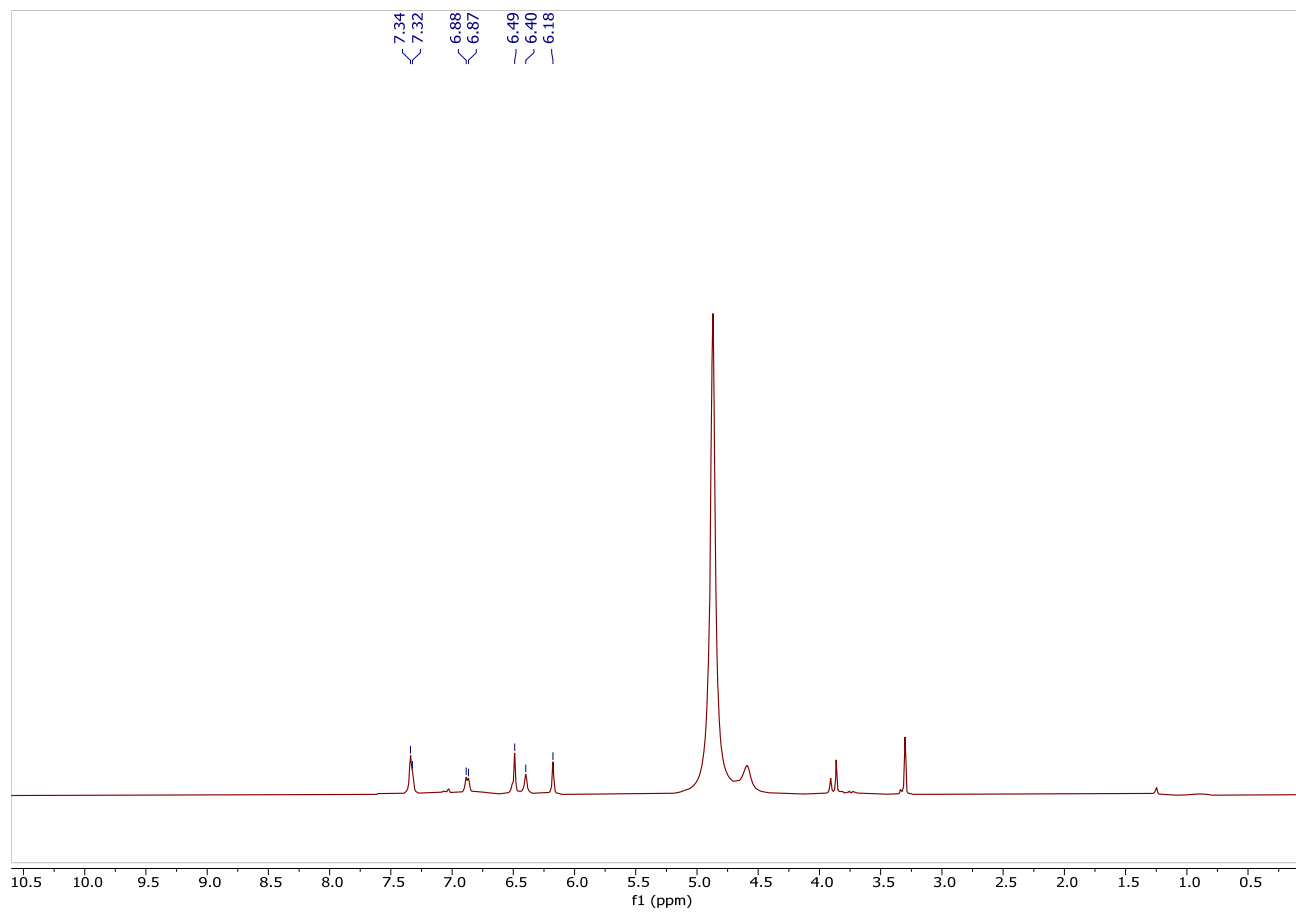


Figure S25. ¹H NMR spectrum of **10** in methanol-*d*₄

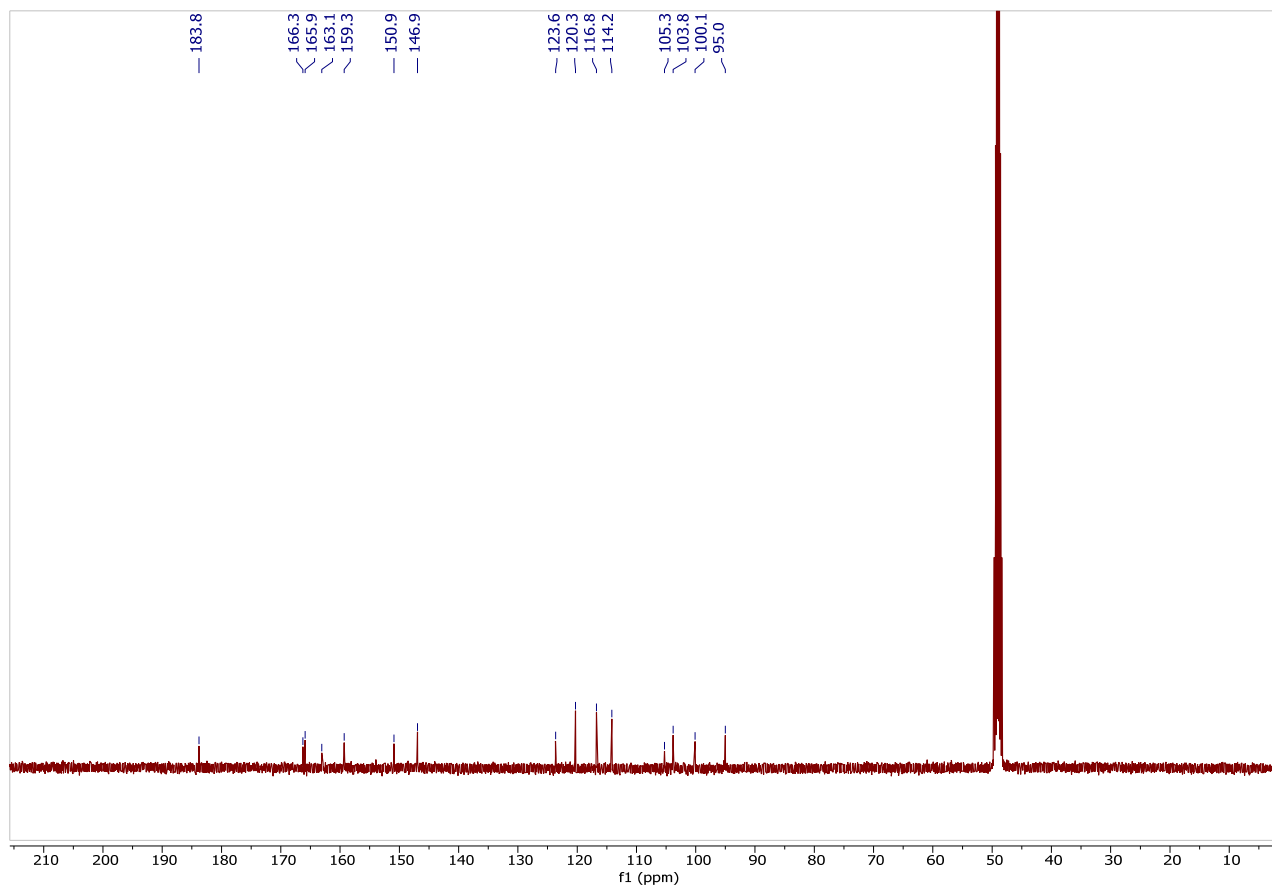


Figure S26. ¹³C NMR spectrum of **10** in methanol-*d*₄

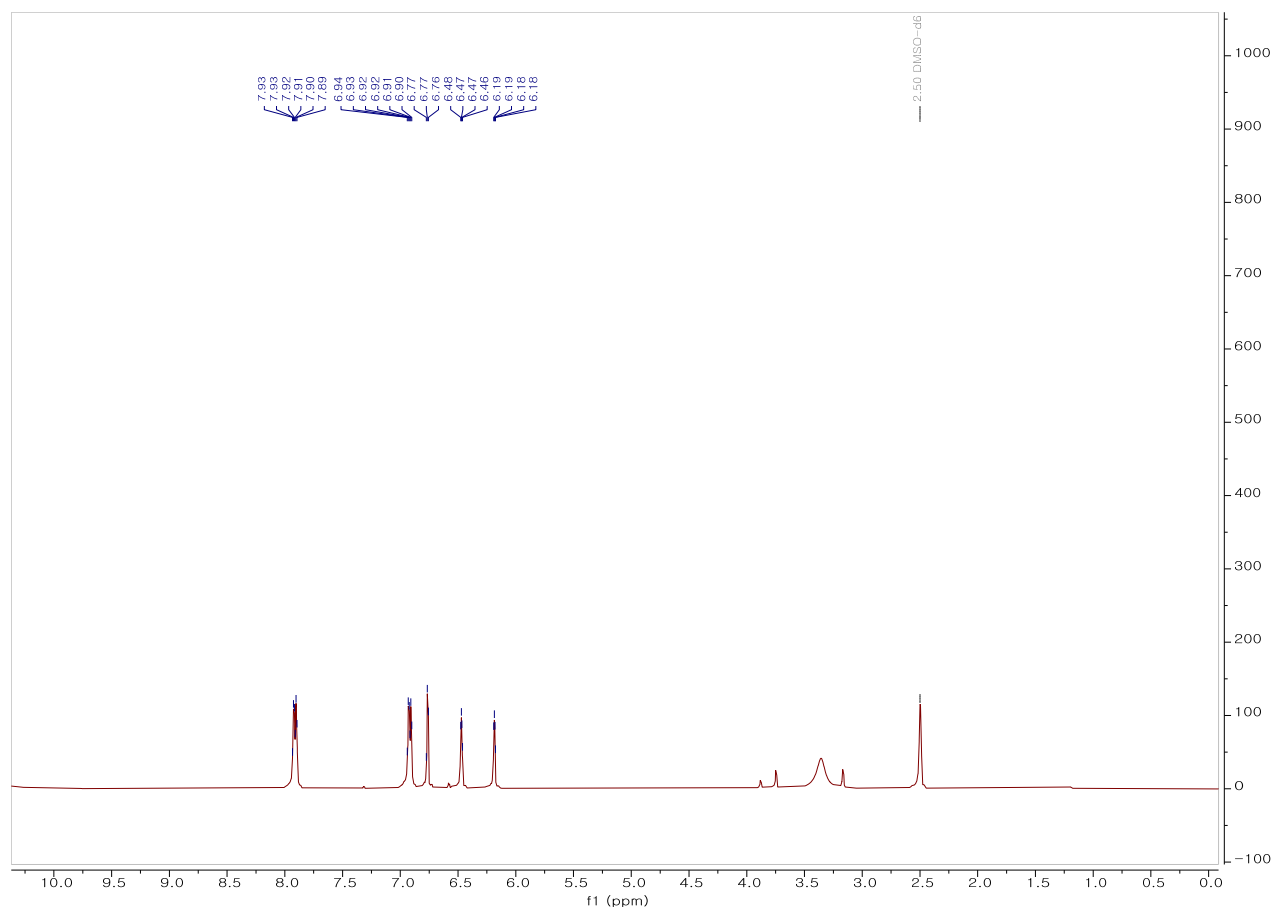


Figure S27. ¹H NMR spectrum of **11** in DMSO-*d*₆

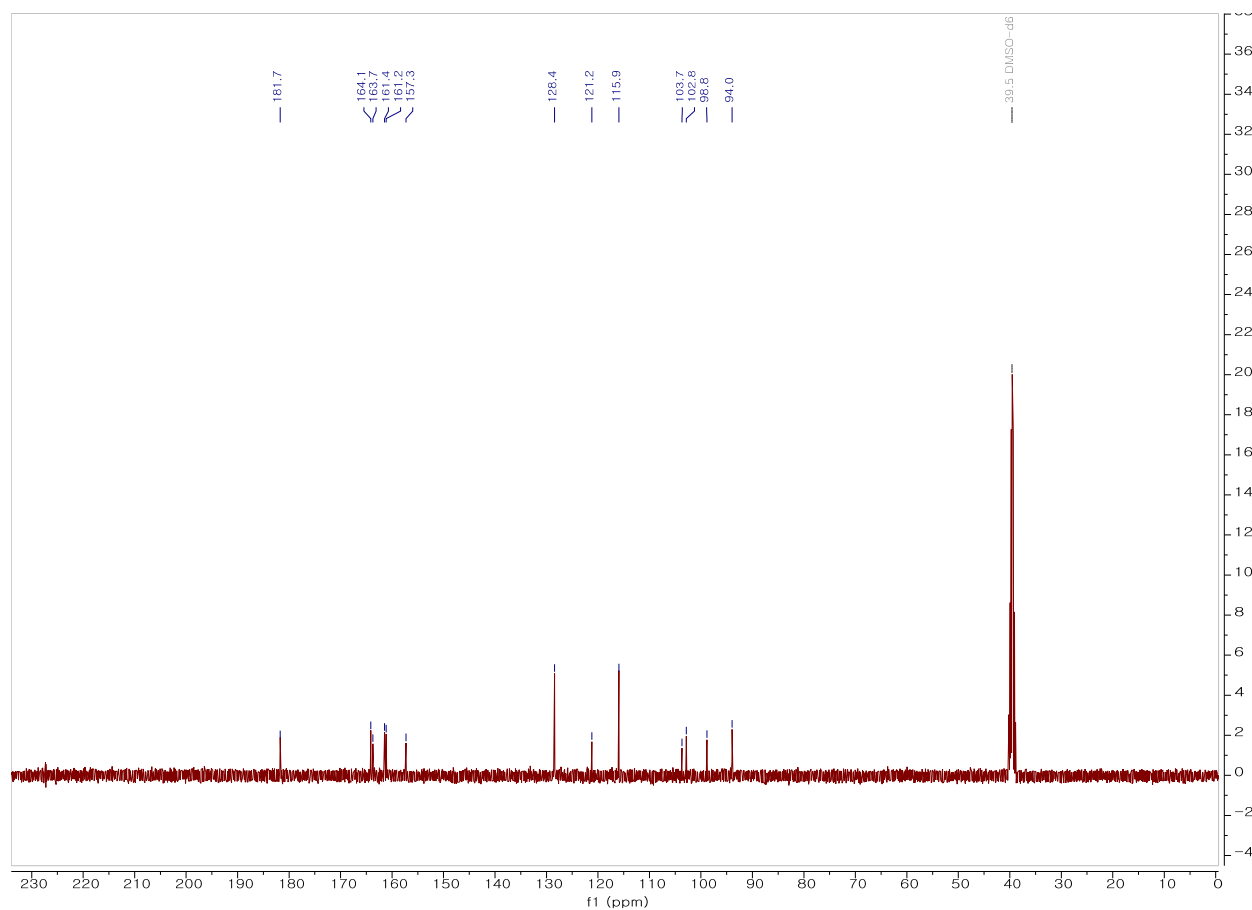


Figure S28. ¹³C NMR spectrum of **11** in DMSO-*d*₆

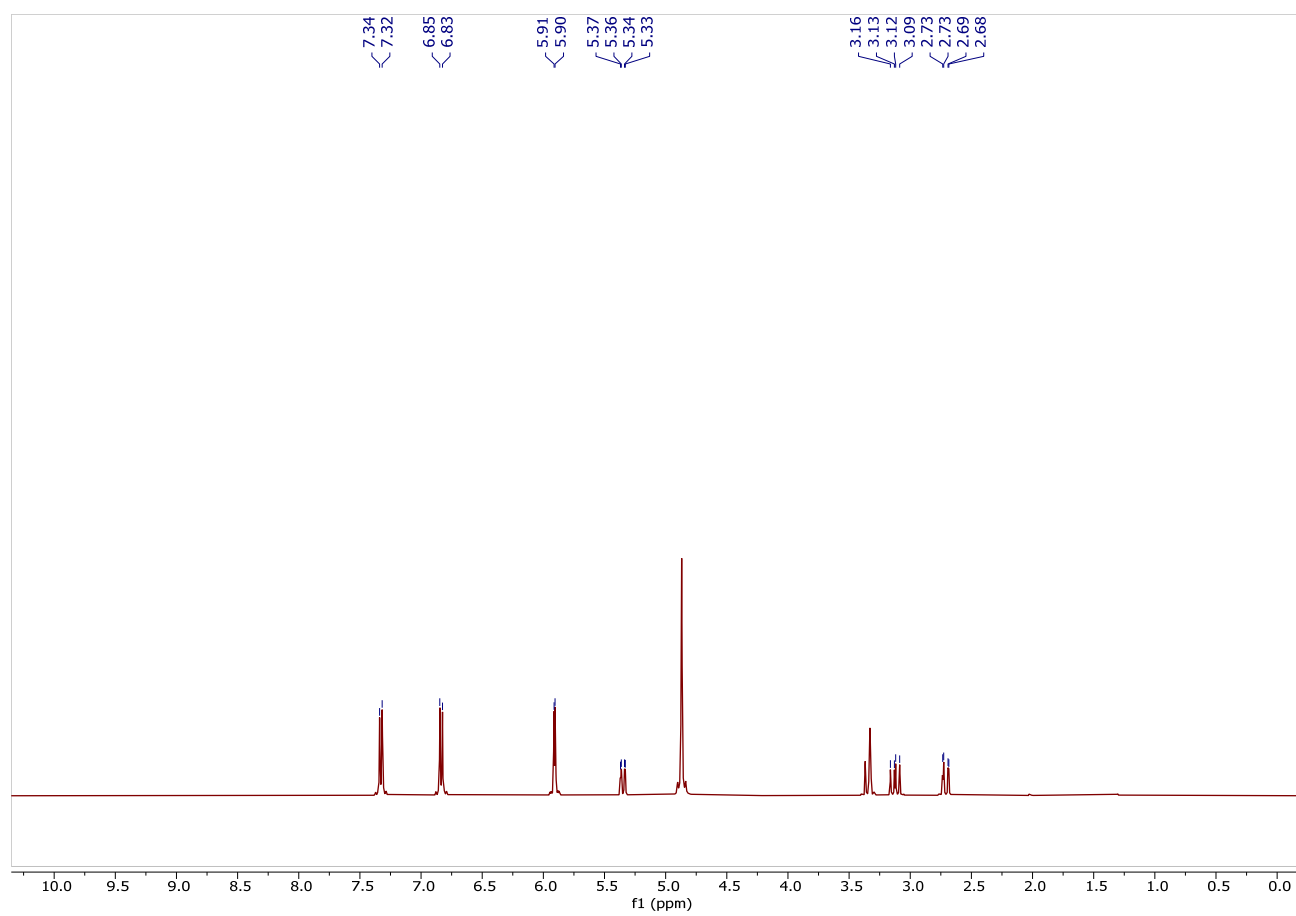


Figure S29. ¹H NMR spectrum of **12** in methanol-*d*₄

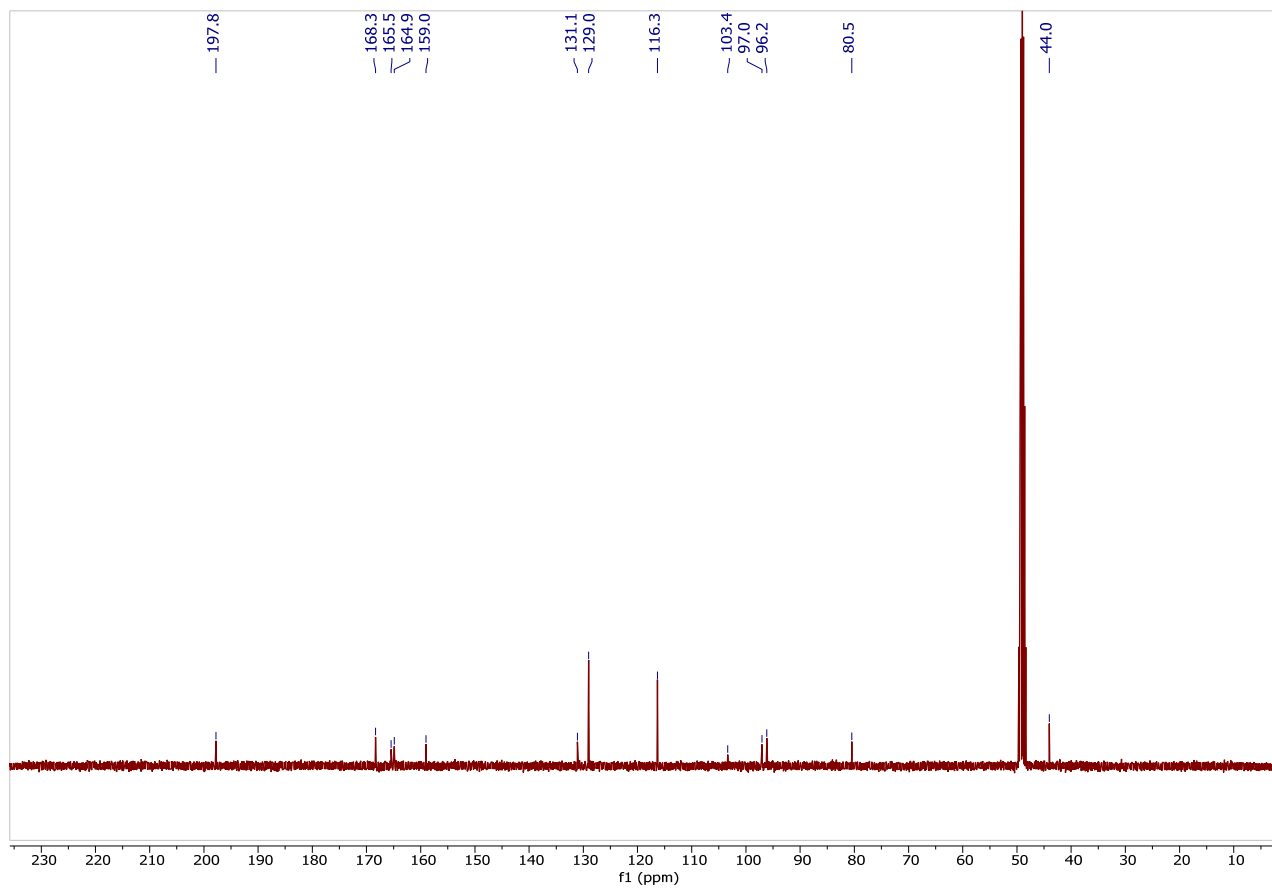


Figure S30. ¹³C NMR spectrum of **12** in methanol-*d*₄

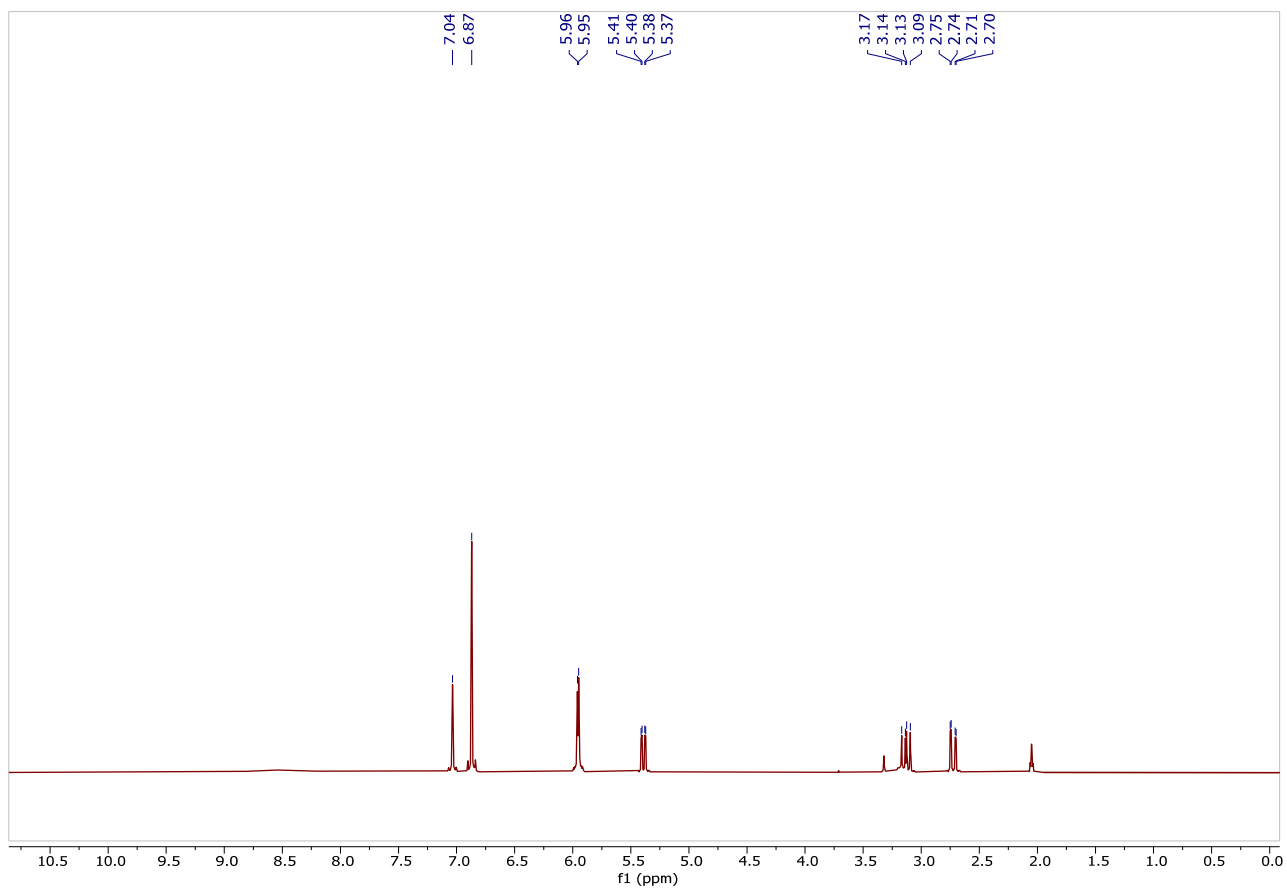


Figure S31. ¹H NMR spectrum of **13** in acetone-*d*₆

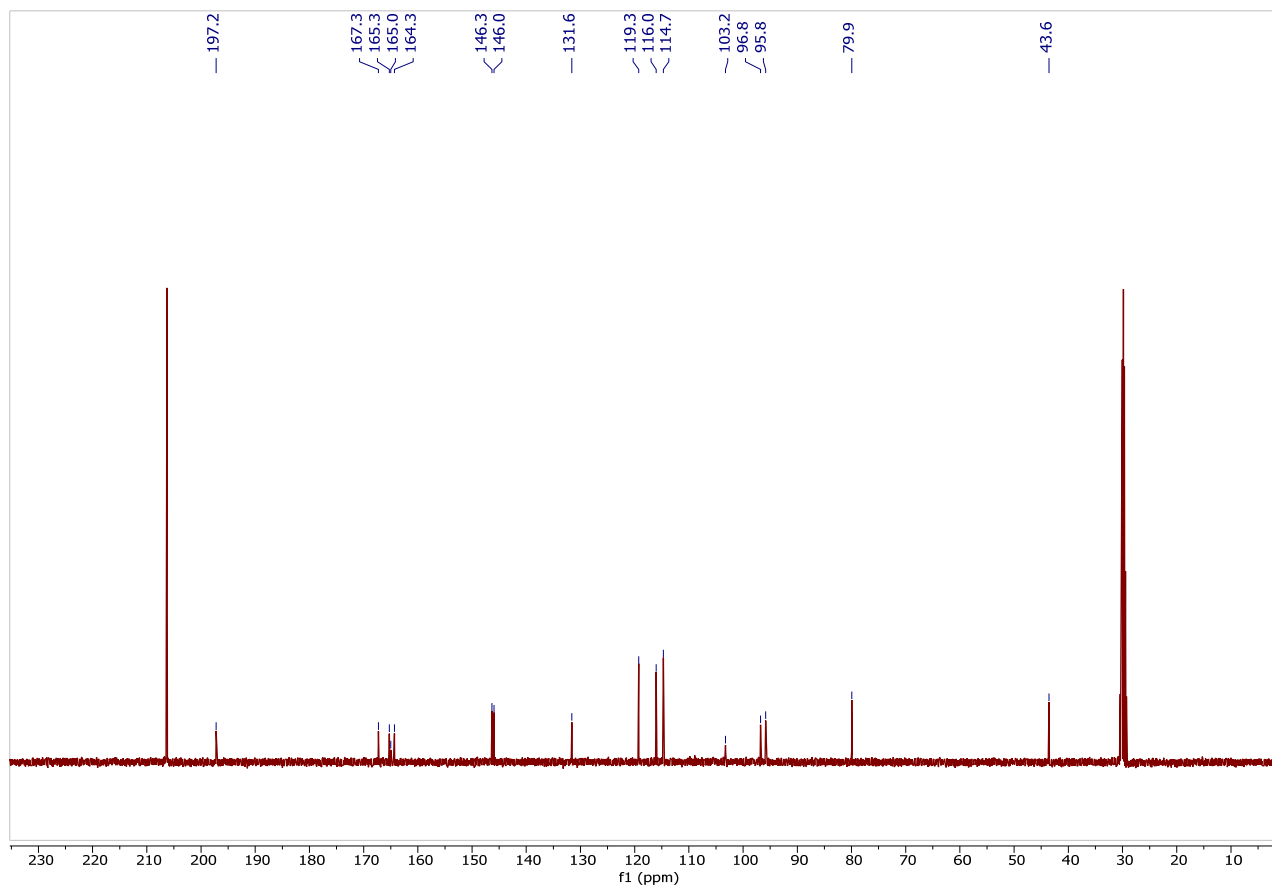


Figure S32. ^{13}C NMR spectrum of **13** in acetone- d_6

Table S1. ^1H NMR chemical shift differences between (*S*)- and (*R*)-MTPA esters of **1** (pyridine-*d*₅).

Position	(<i>S</i>)-MTPA	(<i>R</i>)-MTPA	$\Delta\delta_{\text{H}(\textit{S-R})}$
5	8.3022	8.2995	0.003
9	1.5971	1.6099	−0.013