Electronic supplementary materials *Mendeleev Commun.*, 2020, **30**, 150–152

Synthesis and antitumor activity of methanofullerenes equipped with norbornadiene and quadricyclane moieties

**Usein M. Dzhemilev, Arslan R. Akhmetov, Vladimir A. D’yakonov,
Lilya U. Dzhemileva, Milyausha M. Yunusbaeva and Airat R. Tuktarov**

Table of Contents

General Information

All reactions were performed under an argon atmosphere and in anhydrous solvent. The solvents and reagents were dried or refined according to the literature procedures. Fullerene C_{60} (99.5%) was purchased from Sigma-Aldrich. The reaction products were analyzed on a HPLC chromatograph Shimadzu SPD-20A (Japan) equipped with the UV detector at 313 or 340 nm. The mixtures were separated on a preparative column Cosmosil Buckyprep Waters (250×10 mm) at ~20 ^{o}C. Toluene was used as eluent, the flow rate was 3.0 ml min^{-1}. The ^{1}H and ^{13}C NMR spectra were run on a Bruker Avance-500 spectrometer at 500.17 and 125.78 MHz, respectively. A mixture of $CDCl_3$ and CS_2 (1:5) was used as a solvent. The chemical shifts are reported as δ values in ppm relative to internal standard Me_4Si. The coupling constants (*J*) are reported in Hertz. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Germany) operating in linear (TOF) and reflection (TOF/TOF) positive and negative ion modes. Sulfur S_8 and DCTB (*trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) were used as the matrix. For the application on a metal target, toluene solutions of the samples were used. Hamamatsu Lightning cure LC-8 150 W was used for UV irradiation of norbornadienes. Elemental analyses were measured on 1106 Carlo Erba apparatus.

3-Pentylnorbornadiene-2-carboxylic acid triethylene glycol ester 1. A 50 ml glass reactor was charged with 3-pentylnorbornadiene-2-carboxylic acid (3.79 mmol) in dry dichloromethane (30 ml), triethylene glycol (4.17 mmol), DCC (3.79 mmol) and 4-dimethylaminopyridine (0.379 mmol) at room temperature. After 2 h, the reaction mixture was passed through a column with small amount of silica gel and concentrated *in vacuo*. Product **1** was purified by column chromatography, eluent hexane/EtOAc 1:3. Yield 62 %, light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.75 (t, J = 7.2 Hz, 3H, CH_3), 1.08 (m, 6H, $3CH_2$), 1.46 and 1.79 (m, 2H, CH_2), 1.88 (m, 2H, CH_2), 2.2 (m, 2H, CH_2), 3.39 and 3.49 (m, 12H, $6CH_2$), 6.61 and 6.79 (m, 2H, 2CH). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 13.80, 22.36, 24.59, 25.13, 25.55, 26.37, 26.52, 26.55, 29.52, 30.53, 31.01, 31.39, 32.66, 32.71, 49.04, 53.06, 54.10, 59.19, 71.17, 140.81, 142.35, 143.09, 153.96, 159.02, 173.09. Anal.calcd. for $C_{19}H_{33}O_5$: C, 67.43 %; H, 8.94 %; O, 23.64 %; found C, 67.49 %; H 8.89 %.

Ethyl 8-{[(3-pentylbicyclo[2.2.1]hepta-2,5-dien-2-yl)carbonyl]oxy}-3,6-dioxaoctyl malonate 2. A 50 ml glass reactor was charged with norbornadiene ester **2** (3.79 mmol) in dry dichloromethane (30 ml), mono ethyl ester malonic acid (4.17 mmol), DCC (3.79 mmol) and 4-dimethylaminopyridine (0.379 mmol) at room temperature. After 2 h, reaction mixture was passed through a column with small amount of silica gel and concentrated in vacuo. The product **3** was purified by column chromatography, eluent hexane/EtOAc 1:3. Yield 62 %, light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.78 (m, 3H, CH_3), 1.18 (m, 9H, CH_3, $3CH_2$), 1.93 (m, 2H, CH_2), 2.58 (m, 2H, CH_2), 3.28 and 3.41 (m, 2H, 2CH), 3.30 (s, 2H, CH_2), 3.56 and 3.63 (m, 6H, $3CH_2$),4.08 and 4.11 (m, 8H, $4CH_2$), 6.59 and 6.76 (m, 2H, 2CH). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 13.87, 13.91, 22.32, 26.43, 30.25, 31.46, 41.01, 41.29, 50.92, 56.11, 61.36, 62.75, 64.38, 68.77, 69.24, 70.43, 70.47, 70.89, 138.00, 140.63, 143.66, 165.64, 166.33, 166.49, 174.03. Anal.calcd. for $C_{24}H_{36}O_8$: C, 63.70 %; H, 8.02 %; O, 28.28 %; found C, 63.65 %; H 8.10 %.

Ethyl 8-{[(5-pentyltetracyclo[3.2.0.0^{2,7}.0^{4,6}]hept-1-yl)carbonyl]oxy}-3,6-dioxaoctyl malonate 4. Norbornadiene derivative **2** was dissolved in acetonitrile and irradiated with a 150 W UV Lamp for 60 min. The photo isomerization was confirmed by ^{1}H and ^{13}C NMR spectroscopy, the product **4** was not isolated. Yield 100%, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.81 (m, 3H, CH_3), 1.21 (m, 11H, CH_3, $4CH_2$), 1.5 (m, 1H, CH), 1.88 (m, 1H, CH), 1.94 (m, 1H, CH), 2.05 (m, 1H, CH), 2.23 (m, 1H, CH), 2.53 (m, 1H, CH), 3.33 (s, 2H, CH2), 3.43 and 4.22 (m, 14H, 7CH2). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 13.94, 14.03, 17.80, 22.59, 24.56, 27.11, 27.20, 27.46, 30.29, 31.21, 31.89, 37.64, 40.96, 41.33, 53.47, 61.47, 62.76, 64.42, 68.80, 69.22, 70.41, 70.47, 166.42, 166.59, 172.92.

General procedure for the synthesis of norbornadiene and quadricyclane containing methanofullerenes 3 and 5. A 50 ml glass reactor was charged with C_{60} (100 mg, 0.139 mmol) in *o*-xylene (12 ml), malonic ester **2** or **4** (0.2085 mmol), CBr_4 (69 mg, 0.2085 mmol) and DBU (0.031 ml, 0.2085 mmol) at room temperature. The mixture was stirred for 1 h and was quenched with water. The layers were separated and the organic layer was passed through a column with small amount of C18 silica gel. The reaction products and the starting fullerene C_{60} were separated by the semi-preparative HPLC, using toluene as eluent. The samples were washed with hexane and Et_2O before NMR analysis.

3'-Ethoxycarbonyl-3'-[12-(5-pentyltetracyclo[3.2.0.0^{2,7}.0^{4,6}]hept-1-yl)-1,12-dioxo-2,5,8,11-tetraoxadodecyl]-3'*H*-cyclopropa[1,9](C$_{60}$-*I*$_h$)[5,6]fullerene 3. Yield 89 mg (52%), brown powder. UV ($CHCl_3$), λ_{max}, nm: 259, 327, 429. ^1H NMR (500 MHz, $CDCl_3$, δ): 0.93 (t, J = 7.2 Hz, 3H, CH_3), 1.33 (m, 6H, $3CH_2$), 1.53 (t, J = 7.2 Hz, 3H, CH_3), 2.01 and 2.04 (m, 2H, 2CH), 2.14 and 2.16 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 2.39 (m, 2H, 2CH), 3.63 (q, J = 7.2 Hz, 2H, CH_2), 3.65 (m, 4H, $2CH_2$), 3.87 (m, 2H, CH_2), 4.16 (m, 2H, CH_2), 4.54 (m, 2H, CH_2), 4.63 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 15.69, 18.49, 23.04, 23.25, 26.99, 27.60, 27.63, 28.05, 29.51, 29.72, 30.39, 31.67, 32.37, 37.71, 52.12, 62.67, 63.35, 66.09, 69.04, 69.59, 70.83, 70.89, 71.58, 139.16, 141.03, 141.90, 142.29, 143.08, 143.16, 143.96, 144.73, 144.92, 145.22, 145.33, 162.82, 163.02, 171.89. HRMS (MALDI TOF), m/z 1170.2258 $[M]^-$, calcd.for $C_{84}H_{34}O_8$ 1170.2254.

3'-Ethoxycarbonyl-3'-[12-(3-pentylbicyclo[2.2.1]hepta-2,5-dien-2-yl)-1,12-dioxo-2,5,8,11-tetraoxadodecyl]-3'*H*-cyclopropa[1,9](C_{60}-I_h)[5,6]fullerene 5. Yield 94 mg (55%), brown powder. UV ($CHCl_3$), λ_{max}, nm: 260, 330, 427. ^1H NMR (500 MHz, $CDCl_3$, δ): 0.91 (t, J = 7.5 Hz, 3H, CH_3), 1.28 (m, 6H, $3CH_2$), 1.52 (t, J = 7.5 Hz, 3H, CH_3), 2.01 and 2.05 (m, 2H, CH2), 2.68 (m, 2H, CH_2), 3.50 and 3.70 (m, 2H, 2CH), 3.68 (m, 6H, 3CH2), 3.7 (m, 2H, CH2), 4.23 (m, 2H, CH_2), 4.53 (m, 2H, CH2), 4.61 (m, 2H, CH2), 6.69 (m, 1H, CH), 6.86 (m, 1H, CH). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 14.49, 23.02, 29.98, 30.60, 31.95, 51.34, 52.11, 56.49, 62.72, 63.34, 66.08, 69.03, 69.85, 70.89, 71.30, 71.57, 138.22, 139.19, 140.69, 140.99, 141.03, 141.88, 141.91, 142.29, 143.07, 143.15, 143.89, 143.95, 144.69, 144.73, 144.91, 145.20, 145.22, 145.31, 145.34, 162.81, 164.98, 173.63. HRMS (MALDI TOF), m/z 1170.2258 $[M]^-$, calcd.for $C_{84}H_{34}O_8$ 1170.2254.

Biological assays

Cell culturing. Jurkat cells and normal fibroblasts were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences, Biolot) and cultured according to standard mammalian tissue culture protocols and sterile technique. All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination. Jurkat cells were maintained in RPMI 1640 (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma) and 100 units/ml penicillin-streptomycin (Sigma). Cells were grown in an atmosphere of 5 % CO_2 at 37 °C. The cells were subcultured at 2-3 days intervals. Cells were then seeded in 24 well plates at $5x10^4$ cells per well and incubated overnight. Jurkat cells were subcultured at 2 day intervals with a seeding density of 1×10^5cells per 24 well plates in RPMI with 10% FBS.

Cytotoxicity assay. Viability (Live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). Cells after treatment with compounds at various concentrations and incubated in an atmosphere of 5 % CO_2 at 37 °C during 24, 48 or 72 h were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400 g for 5 minutes. Cell pellets were resuspended in 200 μl of flow cytometry staining buffer (PBS without $Ca^{2+}$and Mg^{2+}, 2.5 % FBS) and stained with 5 μl of 7-AAD staining solution for 15 minutes at room temperature in the dark. Samples were acquired on NovoCyteTM 2000 FlowCytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in FL4 channel.

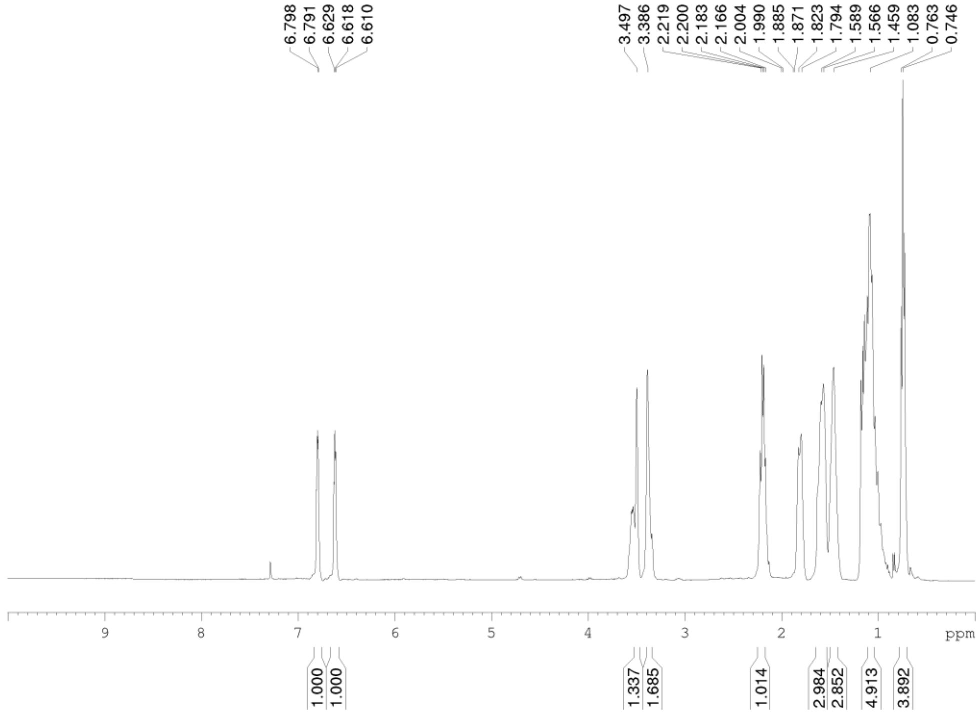

Figure S1. Copies of ^{1}H NMR spectra of compound **1**

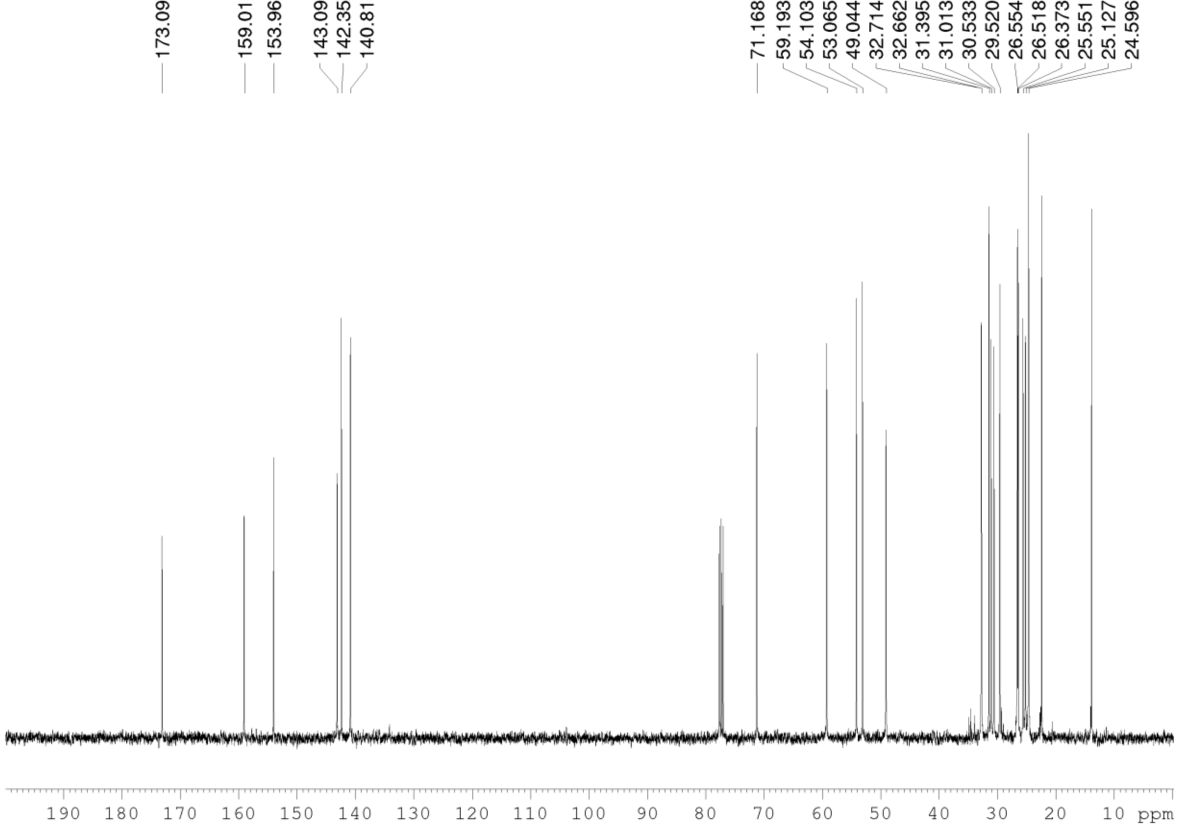

Figure S2. Copies of ^{13}C NMR spectra of compound **1**

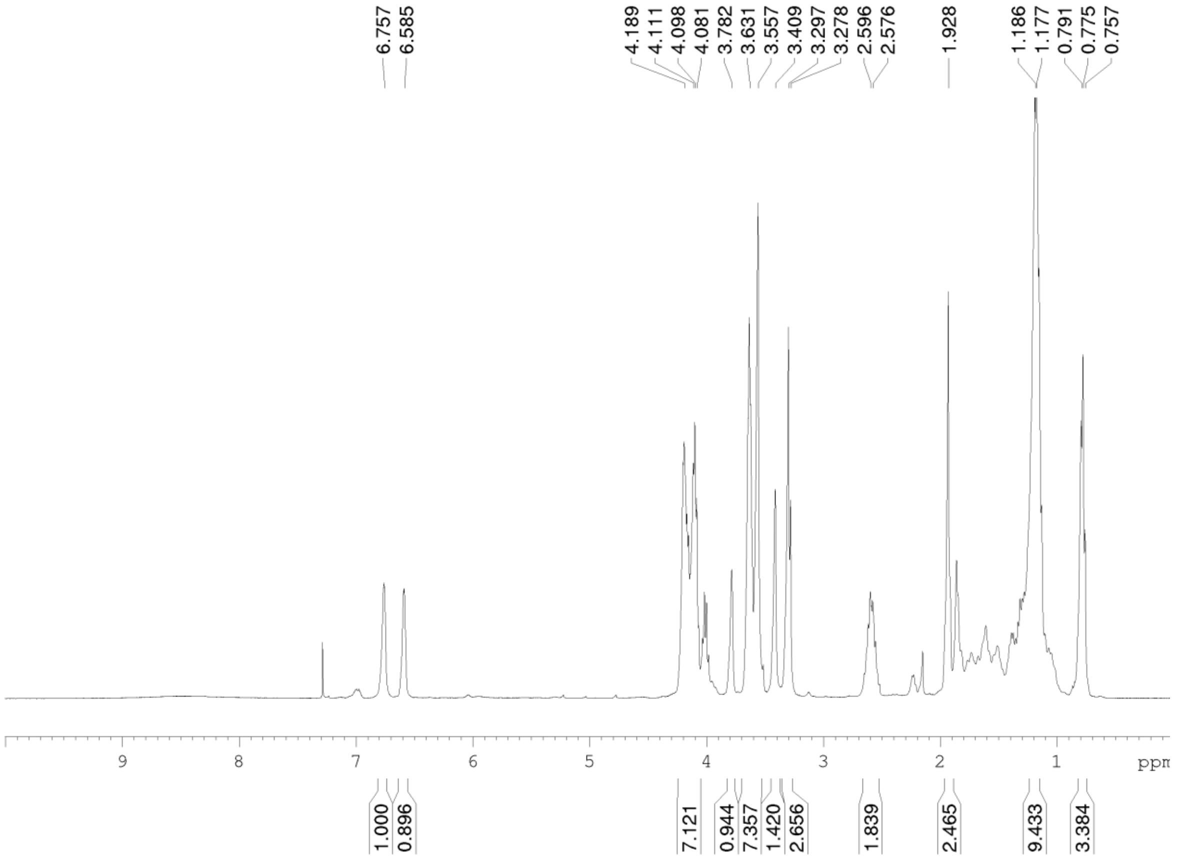

Figure S3. Copies of ^{1}H NMR spectra of compound **2**

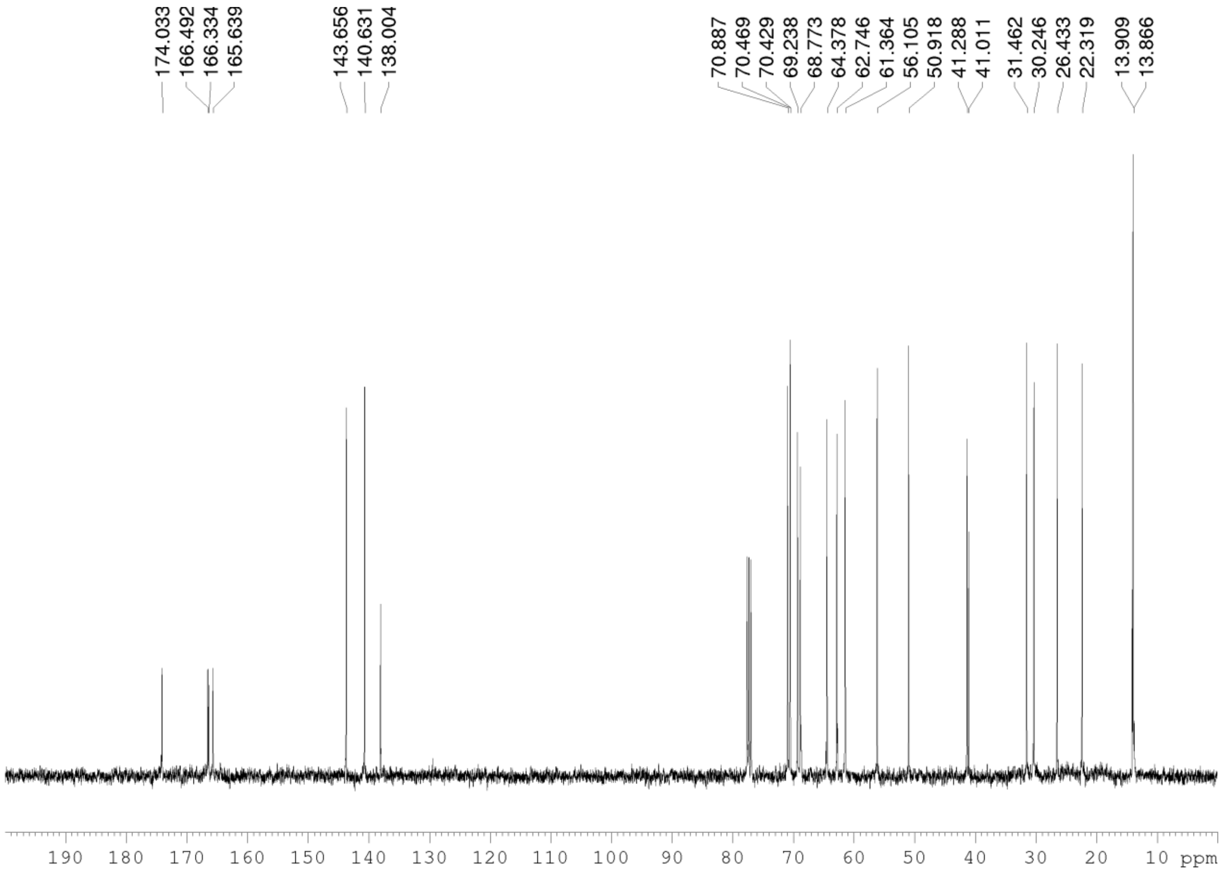

Figure S4. Copies of ^{13}C NMR spectra of compound **2**

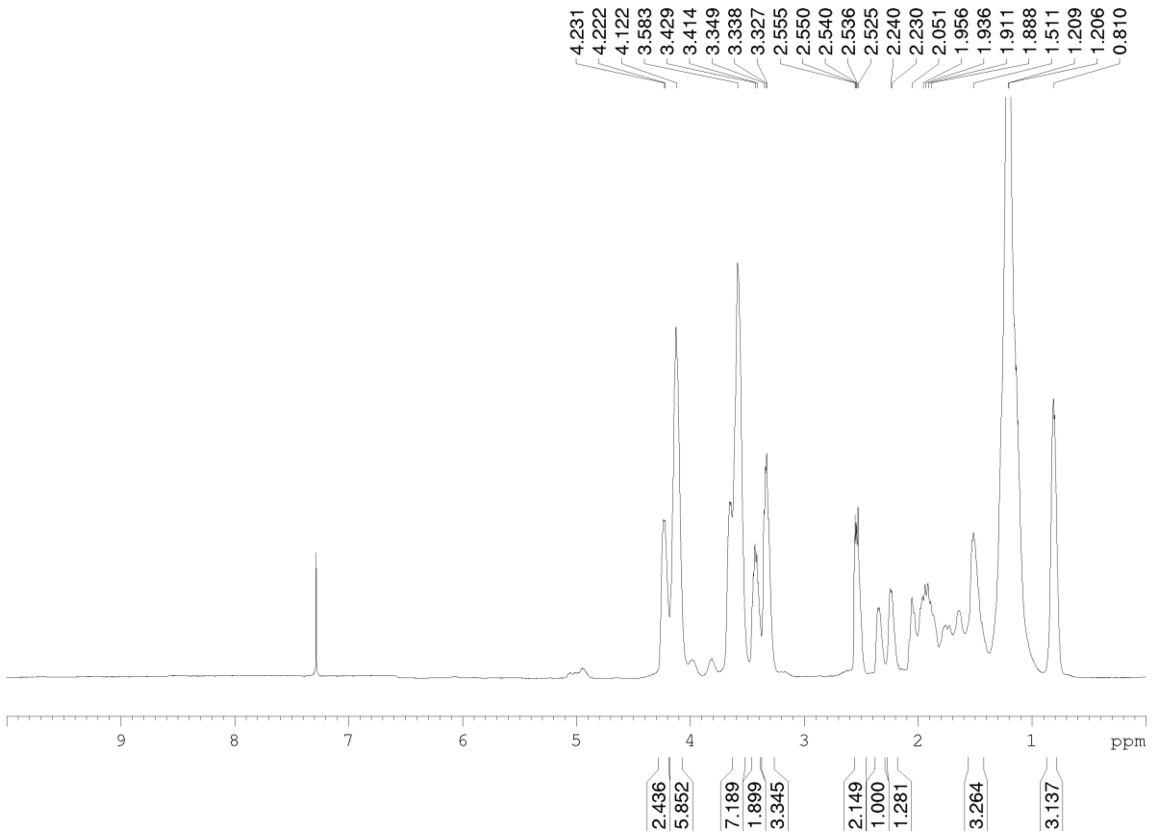

Figure S5. Copies of ^{1}H NMR spectra of compound **4**

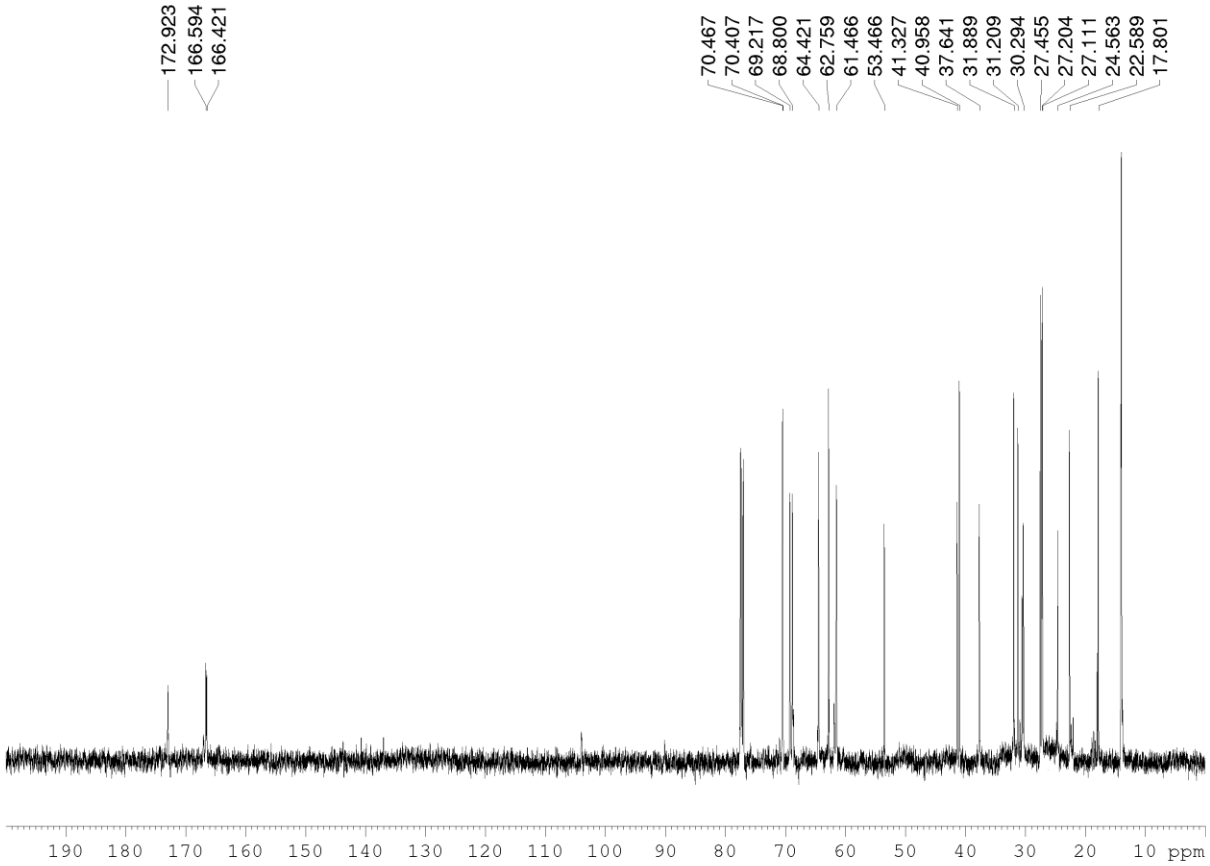

Figure S6. Copies of ^{13}C NMR spectra of compound **4**

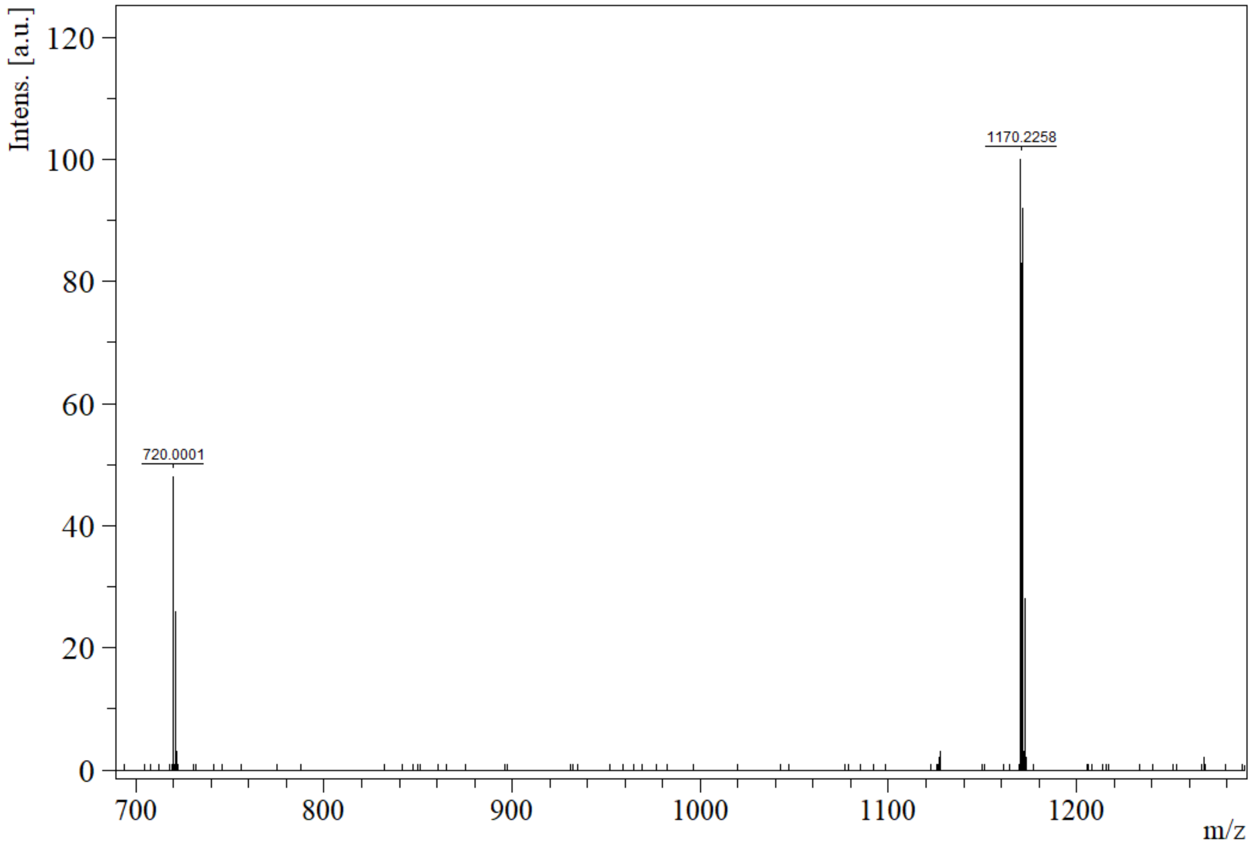

Figure S7. Copies of MALDI TOF spectra of compound **3**

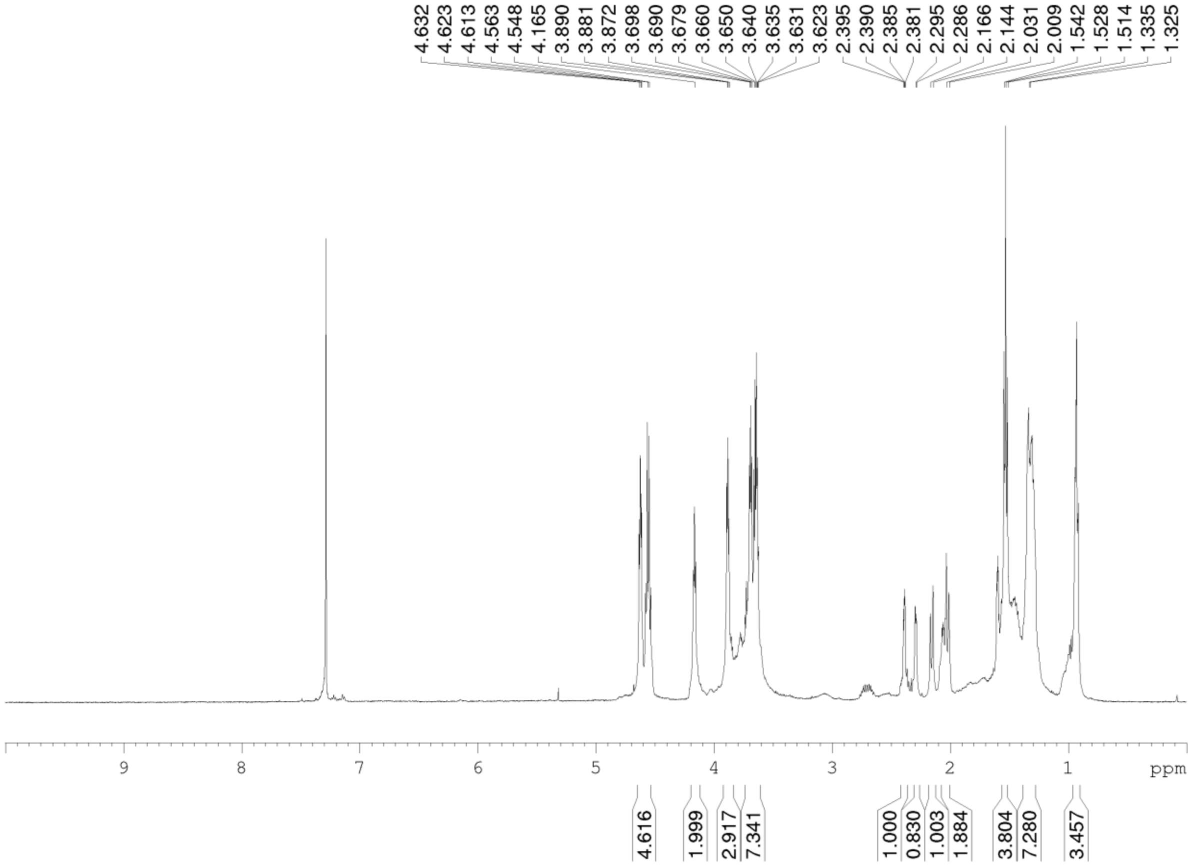

Figure S8. Copies of ^{1}H NMR spectra of compound **3**

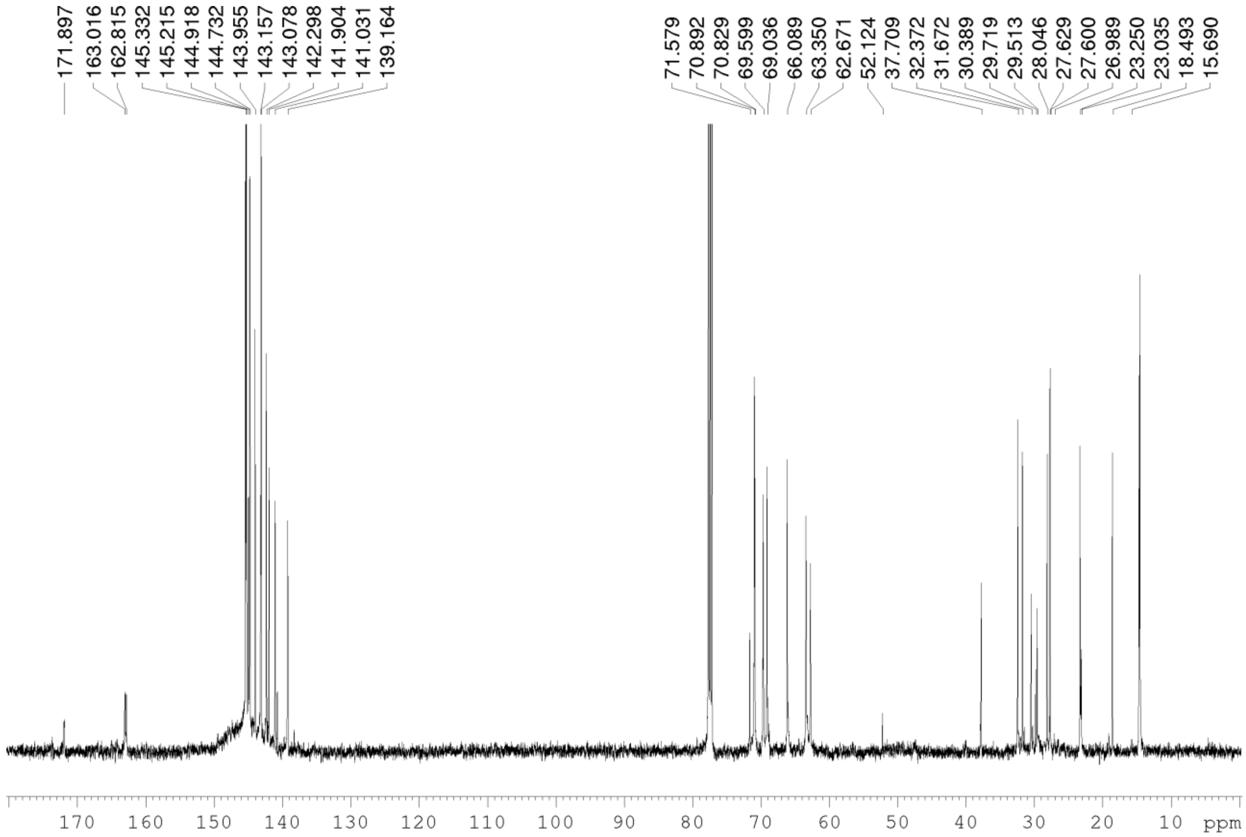

Figure S9. Copies of ^{13}C NMR spectra of compound **3**

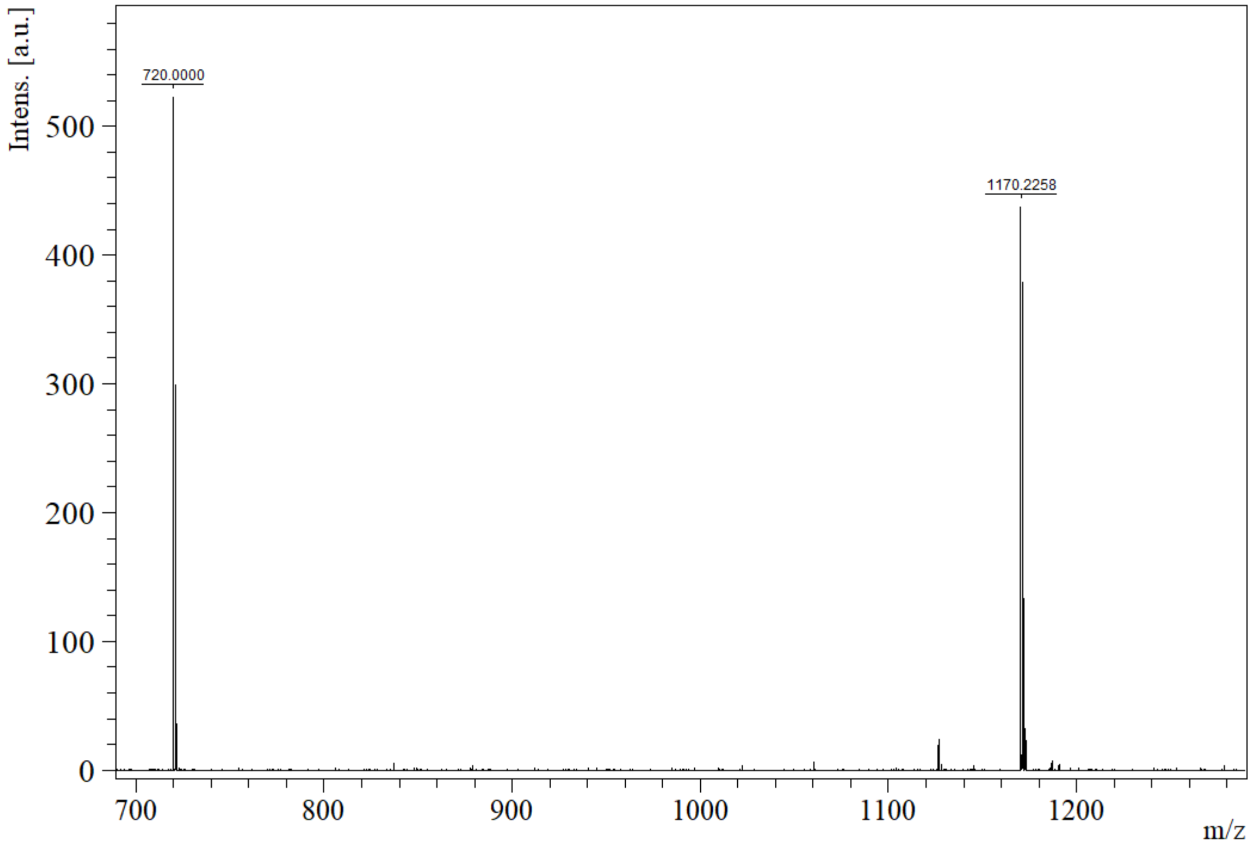

Figure S10. Copies of MALDI TOF spectra of compound **5**

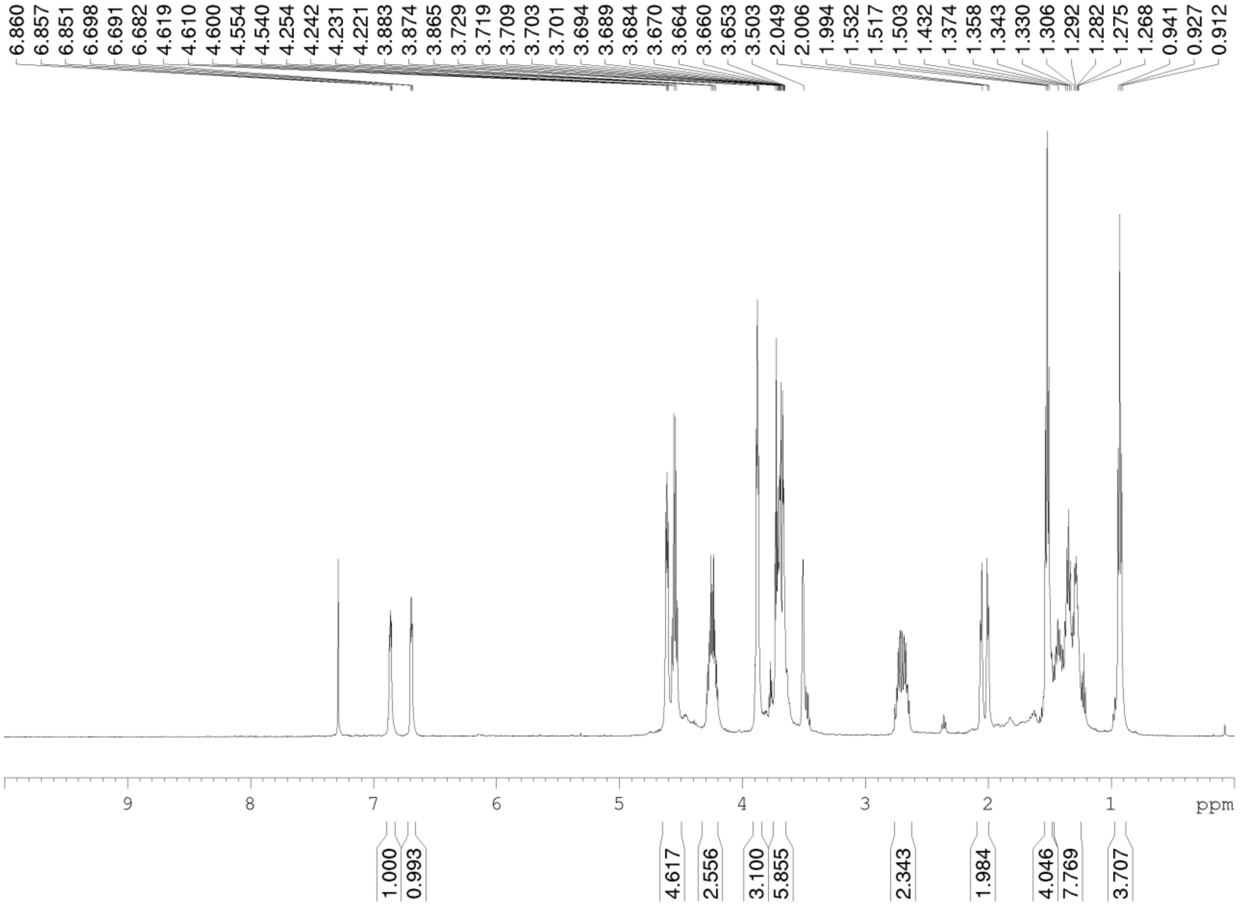

Figure S11. Copies of ^{1}H NMR spectra of compound **5**

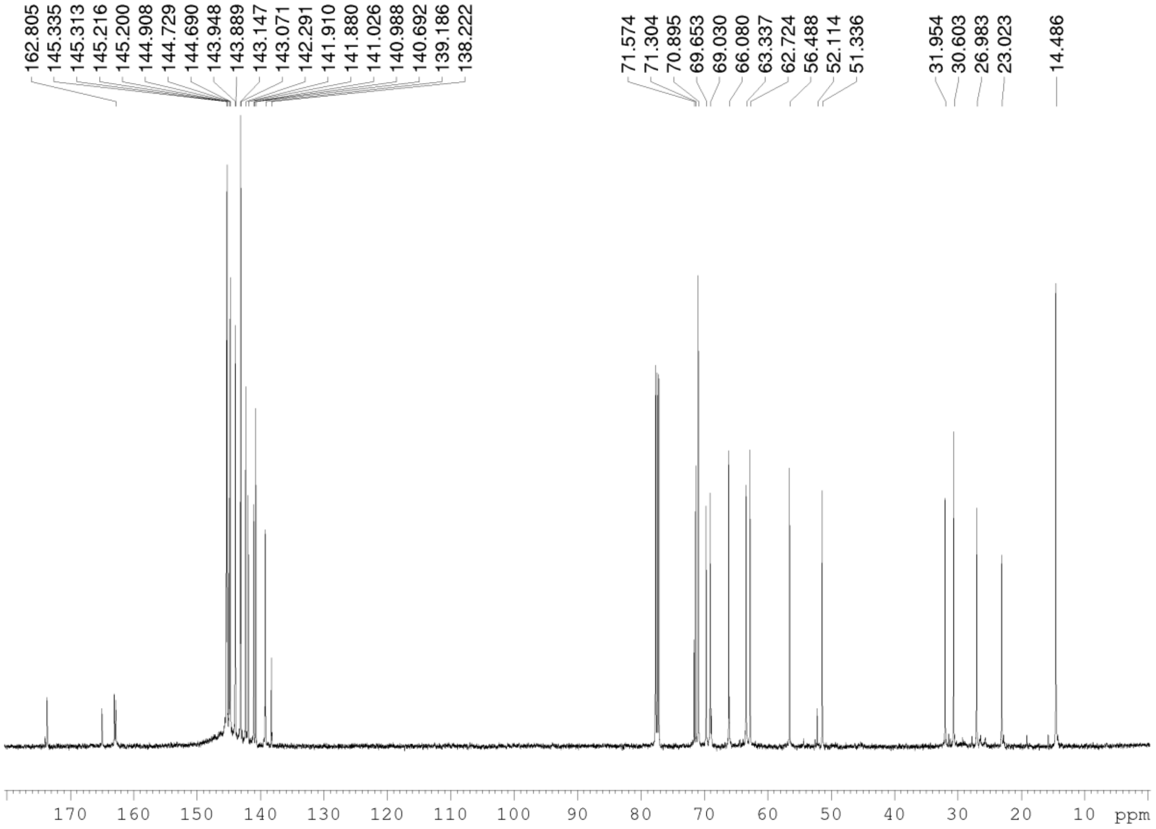

Figure S12. Copies of ^{13}C NMR spectra of compound **5**