Electronic Supplementary Material (ESI) for Chemical Communications

SUPPLEMENTARY INFORMATION

Photodegradation of Lipopolysaccharide and the Inhibition of Macrophage Activation by Anthraquinone-boronic Acid Hybrids

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Supplementary methods

Quantitative three-component ARS assay: A typical procedure for determination of binding constants: To a 1.0×10^{-4} M solution of ARS in 0.1 M sodium phosphate buffer (pH 7.4) (solution A) was added a 2.0×10^{-2} M solution of AQ-boronic acid hybrid in DMF to form a solution of 9.0×10^{-5} M ARS and 2.0×10^{-3} M AQ-boronic acid hybrid in 10% DMF/0.1 M phosphate buffer (pH 7.4) (solution B). The pH was checked and corrected to pH 7.4 if necessary. Solution B was titrated into solution A in order to ensure the mixture had a constant concentration of ARS and a range of concentrations of the AQ-boronic acid hybrid. In general, six different concentrations were used in order to cover as much of the binding curve as possible. Then, 50 μL of the mixture was transferred into a cuvette for absorbance measurement, and the absorbance at 453 nm was recorded. Competitive studies were run in a similar manner. A diol was added to a portion of solution B so that 65–80% of the ARS was in free form (solution C). Solution C was titrated into solution B in order to ensure the mixtures had constant concentrations of ARS and AQ-boronic acid hybrids and a range of diol concentrations.

Photodegradation of glycosides: Each glycoside (1.0 mM) was incubated with **2** or **3** in 10% DMF/0.1 M phosphate buffer (100 μL, pH 7.4) at 25 ºC for 2 h under irradiation with a UV lamp (365 nm, 100 W, Blak-ray (B-100A), UVP. Inc.) placed 10 cm from the mixture, and then the resulting mixture was freeze dried. The progress of the photodegradation was monitored by HPLC/UV (215 nm or 254 nm) analysis after benzyl esterification with BnBr/Cs_2CO_3 at 40 ºC for 14 h in DMF (for **4**), primary alcohol *t*-butyldiphenylsilylation with TBDPSCl/imidazole at 40 ºC for 16 h in DMF (for **5**), or total acetylation with Ac_2O at room temperature for 6 h in Py (for **6-8**) of the resulting products. The percentage degradation was calculated based on the peak area corresponding to the KDOα-OMe benzyl ester,[1] 9-*O*-TBDPS-Neu5Acα-OMe, or each peracetylated glycoside (Mightysil RP-18 GP 5 μm, 4.6 × 150 mm; 40 ºC; detection by UV (215 nm

or 254 nm); 45:55 MeCN/H_2O; flow rate 1.0 mL min^{-1} for **4**; 70:30 MeOH/H_2O; flow rate 1.0 mL min^{-1} for **5**; 35:65 MeOH/H_2O; flow rate 1.0 mL min^{-1} for **6-8**).

Photodegradation of LPS: LPS from *E. coli* was purchased from Enzo Life Science. Photodegradation of LPS (5.0 μg, 2.15 μM) was performed with AQ-boronic acid hybrids (100, 30, 10, 3 equiv. to LPS) in 10% DMF/0.10 M phosphate buffer (pH 7.4) with or without photo-irradiation using a UV lamp (365 nm, 100 W) placed at 10 cm from the vessel at 25 ºC for 2 h. The progress of the photodegradation was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After addition of a 4.8 μL of solution containing SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol) to the samples. Gels (20% SDS/polyacrylamide gel) were run by applying 30 mA for 100 min. The gels were washed three times with distilled water for 5 min, stained with Quick-CBB PLUS (Wako) for 1 h, and destained in distilled water until the bands became to be visible.

Cell culture: Mouse macrophage RAW264.7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 200 μg/mL of kanamycin, 100 units/mL of penicillin G, 600 μg/mL of L-glutamine, and 2.25 g/L of $NaHCO_3$ at 37 ºC under 5% CO_2 plus air.

MTT Assay & Griess Assay: RAW264.7 cells were seeded at 4.0×10^5 per well in 96-well in 10% FBS DMEM (200 μL of complete medium per well) (plate A) and incubated for 24 h at 37 ºC under 5% CO_2 plus air. The cells were treated with a 10% DMF solution (2.0 μL) of LPS (10 μg/mL) and the indicated equiv. of AQ-boronic acid hybrid, which was pre-incubated with or without of 2 h of photo-irradiation using a UV lamp (365 nm, 100 W) placed at 10 cm from the samples at 25 ºC. After 24 h treated at 37 ºC, the supernatant (100 μL) of each well was transferred to a new 96-well (plate B). MTT reagent was added to each well in the plate A and cells were incubated for 2 h additional hours at 37 ºC. The absorbance was measured at 540 nm with a microplate reader

(SAFIRE, TECAN Inc.). In the meantime, Griess reagent was added to each well in the plate B. The concentration of NO was obtained by measuring the absorbance at 570 nm with the microplate reader.

EPR spectrometry: EPR experiments were carried out with a E-500 CW/EPR (Bruker) and recorded under the following conditions: temperature 296 K, microwave frequency 9.39 GHz, microwave power 16 mW, field modulation 0.1 mT at 100 kHz. DMPO was used as a spin-trapping agent. AQ-boronic acid hybrid **3** (200 μM) and DMPO (500 mM) were incubated in 10% DMF/0.1 M phosphate buffer (pH 7.4) containing 1 mM DETAPAC under irradiation with a UV lamp (365 nm, 100 W) placed 40 cm from a flat cell.

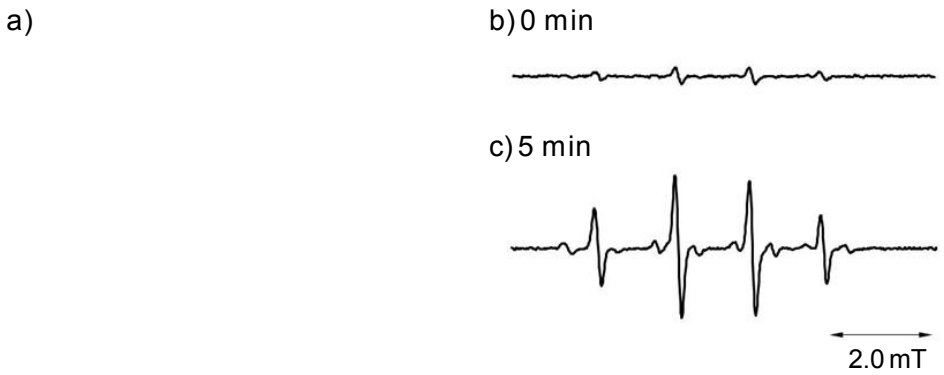

Figure S1 EPR spectra obtained during photo-irradiation of **3** in the presence of DMPO. **3** (1.0 mM) was incubated with DMPO (500 mM) in 10% DMF/0.1 M phosphate buffer (pH 7.4) containing 1.0 mM DETAPAC (diethylenetriamine pentaacetic acid) under irradiation with a UV lamp (365 nm, 100 W) placed 40 cm from a flat cell. a) Formation of DMPO/•OH from DMPO. b) Before irradiation; c) after 5 min irradiation.

General methods for chemical synthesis

Melting points were determined on a micro hot-stage (Yanako MP-S3). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL ECA-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) and a JEOL Lambda (300 MHz, for ^{1}H) spectrometer in the indicated solvent. ESI-TOF Mass spectra were measured on a Waters LCT premier XE. UV/vis spectra were recorded on a JASCO V-550 spectrometer. The reactions were monitored by thin layer chromatography carried out on Merck TLC 60F-254 (0.25 mm) using UV light and *p*-anisaldehyde or 10% ethanolic phosphomonolybdic acid as developing agent. Column chromatography separations were performed using silica gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.). Reverse phase column chromatography separations were performed using Wakosil 40C18 (Wako). Analytical and preparative reverse-phase high performance liquid chromatography (RP-HPLC) were performed on a JASCO apparatus HPLC analytical Mightysil RP-18 GP 5 µm (4.6×150 mm, Kanto Chemical Co., Inc.) with flow rate of 1.0 mL/min. Detection of products were made by UV detector (JASCO, UV-2075 Plus). All RP-HPLC procedures were carried out with a liner gradient using H_2O and MeCN. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon using oven-dried glassware. In general, organic solvents were purified and dried using an appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 ºC, unless otherwise noted.

Synthesis of the AQ-boronic acid hybrids

Scheme S1 Synthesis of the AQ-boronic acid hybrids.

***N*-[(9,10-Dihydro-9,10-dioxo-2-anthracenyl)carbonyl]glycine *tert*-butyl ester (11):** To a solution of anthraquinone-2-carboxylic acid (**10**) (109 mg, 0.432 mmol) in DMF (10.0 mL) were added *N*-ethylmorpholine (110 μL, 0.864 mmol) and TBTU (185 mg, 0.576 mmol) under Ar atmosphere at room temperature. After the mixture was stirred for 15 min at room temperature, a solution of **9** (48.2 mg, 0.288 mmol) in DMF (5.00 mL) was dropwisely added to the mixture in ice bath. After the mixture was stirred for 12 h at room temperature, the reaction was quenched with saturated NH_4Cl aq. (12.0 mL). The resulting solution was extracted with EtOAc (20 mL×3). The extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (6 g, toluene/EtOAc = 3/1) gave **11** (82.0 mg, 0.224 mmol, 78% yield) as a yellow solid.; R_f = 0.24 (3/1 toluene/EtOAc); mp 193 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$, TMS) δ 8.66 (1H, d, J = 1.7 Hz), 8.39 (1H, d, J = 8.0 Hz), 8.28-8.35 (3H, m), 7.84 (2H, dd, J = 3.5, 5.8 Hz), 6.92 (1H, brs), 4.21 (2H, d, J = 4.9 Hz), 1.53 (9H, s) (**Figure S2**); ^{13}C -NMR (125 MHz, $CDCl_3$) δ 182.0, 181.9, 168.8, 165.6, 138.6, 134.7, 134.1,

133.0, 132.9, 132.7, 127.4, 127.0, 125.2, 82.3, 50.3, 42.5, 27.8 (**Figure S3**); HRMS (ESI-TOF) *m/z* 366.1333 (366.1341 calcd. for $C_{21}H_{20}NO_5$, $[M+H]^+$).

***N*-[(9,10-Dihydro-9,10-dioxo-2-anthracenyl)carbonyl]glycine (12):** To a solution of **11** (82.0 mg, 0.224 mmol) in CH_2Cl_2 (2.00 mL) was added TFA (2.00 mL) under Ar atmosphere in ice bath. After the mixture was stirred for 3 h at room temperature, the mixture was concentrated in *vacuo*. Purification of the residue by recrystallization from MeOH gave **12** (68.6 mg, 0.222 mmol, 99% yield) as a yellow solid.; R_f = 0.32 (3/1/0.1 toluene/EtOAc/AcOH); mp 272 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6, TMS) δ 12.7 (1H, brs), 9.35 (1H, t, J = 5.8 Hz), 8.67 (1H, d, J = 1.5 Hz), 8.20-8.36 (4H, m), 7.95 (2H, dd, J = 3.5, 5.8 Hz), 4.01 (2H, d, J = 6.1 Hz) (**Figure S4**); ^{13}C-NMR (125 MHz, DMSO-d_6); δ 182.6, 171.6, 165.6, 139.2, 135.3, 135.2, 134.1, 133.7, 133.6, 133.3, 127.7, 127.4, 127.3, 126.1, 41.9 (**Figure S5**); HRMS (ESI-TOF) *m/z* 308.0561 (308.0559 calcd. for $C_{17}H_{10}NO_5$ $[M-H]^-$).

AQ-boronic acid hybrid 2: To a solution of *m*-aminophenylboronic acid (23.1 mg, 0.169 mmol) in THF (1.50 mL) was added PS-DEAM[2] (101 mg, 0.169 mmol, 1.68 mmol/g) under Ar atmosphere at room temperature. After the reaction suspension was shaken for 1 h at room temperature, the resulting solution was drained. The resin was then rinsed with THF (1.5 mL×3). The resulting resin was swollen in a solution of *N*-ethylmorpholine (64.3 μL, 0.507 mmol), TBTU (163 mg, 0.507 mmol), and **12** (74.6 mg, 0.254 mmol) in DMF (2.00 mL) under Ar atmosphere at room temperature. After the reaction suspension was shaken for 20 h, the resulting solution was drained. The resin was rinsed with DMF (2.0 mL×3) and CH_2Cl_2 (2.0 mL×5). The resulting resin was treated with CH_2Cl_2/MeOH/TFA (8/1/1, 2.0 mL×3) for 1 min. The solution was drained and concentrated in *vacuo*. Purification of the residue by reverse-phase column chromatography (H_2O/MeCN = 52/48) gave **2** (19.5 mg, 0.046 mmol, 27% yield in 2 steps) as a yellow solid.; mp >300 ºC; ^{1}H-NMR (300 MHz, DMSO-d_6, TMS) δ 10.03 (1H, s), 9.35 (1H, t, J = 5.7 Hz), 8.73 (1H,

d, J = 1.7 Hz), 8.39 (1H, dd, J = 1.7, 8.0 Hz), 8.33 (1H, d, J = 8.0 Hz), 8.22-8.30 (2H, m), 8.02 (2H, s), 7.93-7.99 (2H, m), 7.87 (1H, br-s), 7.72-7.75 (1H, m), 7.49 (1H, d, J = 7.7 Hz), 7.28 (1H, t, J = 7.7 Hz), 4.13 (2H, d, J = 5.8 Hz) (**Figure S6**); ^{13}C-NMR (125 MHz, DMSO-d_6); δ 182.2, 167.3, 165.2, 138.9, 138.0, 134.7×2, 133.2, 133.1, 132.9, 129.1, 127.7, 127.1, 126.9, 126.8, 125.7, 125.3, 121.2, 43.4 (**Figure S7**); HRMS (ESI-TOF) *m/z* 427.1081 (427.1101 calcd. for $C_{23}H_{16}BN_2O_6$, $[M-H]^-$).

4-(9,10-Dihydro-9,10-dioxo-anthracene-2-carboxamide)butanoate *tert*-butyl ester (15): To a solution of anthraquinone-2-carboxylic acid (**10**) (256 mg, 1.01 mmol) in CH_2Cl_2 (10.0 mL) were added DIEA (236 μL, 1.35 mmol) and PyBop (703 mg, 1.35 mmol) under Ar atmosphere at room temperature. After the mixture was stirred for 15 min at room temperature, the solution of **14** (132 mg, 0.677 mmol) in CH_2Cl_2 (5.00 mL) was dropwisely added to the mixture under Ar atmosphere in ice bath. After the mixture was stirred for 3 h at room temperature, the reaction was quenched with 5% citric acid aq. (10 mL). The resulting mixture was extracted with $CHCl_3$ (30 mL×3). The extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the residue by silica-gel column chromatography (20 g, hexane/EtOAc/$CHCl_3$ = 12/8/5) gave **15** (359 mg, 0.913 mmol, 90% yield) as a yellow solid.; R_f = 0.34 (3/1/0.1 hexane/EtOAc/AcOH); mp 129 ºC; ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ 8.52 (1H, d, J = 1.4 Hz), 8.15-8.24 (4H, m), 7.72-7.76 (2H, m), 7.55 (1H, brs), 3.58 (2H, m), 2.44 (2H, t, J = 7.2 Hz), 2.01 (2H, quint, J = 7.2 Hz), 1.46 (9H, s) (**Figure S8**); ^{13}C-NMR (125 MHz, $CDCl_3$); δ 182.3, 182.2, 173.2, 165.8, 139.7, 134.8, 134.3, 133.3, 133.2, 132.9, 127.6, 127.2, 125.3, 80.9, 40.2, 33.4, 28.1, 24.4 (**Figure S9**); HRMS (ESI-TOF) *m/z* 394.1653 (394.1654 calcd. for $C_{23}H_{24}NO_5$, $[M+H]^+$).

4-(9,10-Dihydro-9,10-dioxo-anthracene-2-carboxamide)butanoic acid (16): To a solution of **15** (95.3 mg, 0.242 mmol) in CH_2Cl_2 (2.50 mL) was added TFA (2.50 mL) under Ar atmosphere in ice

bath. After the mixture was stirred for 3 h at room temperature, the mixture was concentrated in *vacuo*. Purification of the residue by recrystallization from MeOH gave **16** (77.6 mg, 0.223 mmol, 95% yield) as a yellow solid.; R_f = 0.38 (3/1/0.1 toluene/EtOAc/AcOH); mp 216 ^{o}C; ^{1}H-NMR (300 MHz, DMSO-d_6, TMS) δ 8.84 (1H, brs), 8.65 (1H, s), 8.24-8.34 (4H, m), 8.20-8.34 (4H, m), 7.94-7.97 (2H, m), 3.35 (2H, m), 2.32 (2H, t, J = 7.2 Hz), 1.82 (2H, quint, J = 7.2 Hz) (**Figure S10**); ^{13}C-NMR (125 MHz, DMSO-d_6); δ 182.7, 174.8, 165.2, 139.9, 135.3, 135.2, 135.0, 133.6, 133.3, 127.6, 127.4, 126.0, 31.7, 24.9 (**Figure S11**); HRMS (ESI-TOF) *m/z* 366.0864 (366.0872 calcd for $C_{19}H_{14}NO_5$, $[M-H]^-$).

AQ-boronic acid hybrid 3: To a solution of *m*-aminophenylboronic acid (23.1 mg, 0.234 mmol) in THF (1.50 mL) was added PS-DEAM (140 mg, 0.234 mmol) under Ar atmosphere at room temperature. After the vessel was shaken for 1 h at room temperature, the resin was rinsed with THF (1.5 mL×3). The resulting resin was swollen in a solution of DIEA (122 μL, 0.702 mmol), PyBop (244 mg, 0.468 mmol), and **16** (118 mg, 0.351 mmol) in DMF (2.00 mL) under Ar atmosphere at room temperature. After the vessel was shaken for 20 h, the resulting solution was drained. The resin was rinsed with DMF (2.0 mL×3) and CH_2Cl_2 (2.0 mL×5). The resulting resin was treated with CH_2Cl_2/MeOH/TFA (8/1/1, 2.0 mL×3) for 1 min. The solution was drained and concentrated in *vacuo*. Purification of the residue by reverse-phase column chromatography (H_2O/MeCN = 54/46) gave **3** (28.8 mg, 0.063 mmol, 27% yield in 2 steps) as a yellow solid.; ^{1}H-NMR (500 MHz, DMSO-d_6, TMS) δ 9.70 (1H, s), 8.86 (1H, s), 8.64-8.71 (1H, m), 8.16-8.60 (4H, m), 7.95 (2H, dd, J = 3.5, 5.8 Hz), 7.80 (2H, d, J = 33 Hz), 7.70 (1H, t, J = 7.6 Hz), 7.39-7.45 (1H, m), 7.23 (1H, t, J = 7.6 Hz), 3.41 (2H, m), 2.41 (2H, t, J = 7.5 Hz), 1.93 (2H, quint, J = 7.5 Hz) (**Figure S12**); ^{13}C-NMR (125 MHz, DMSO-d_6); δ 182.2, 170.7, 164.6, 139.4, 138.4, 134.7, 134.5, 133.1×2, 132.8, 128.8, 127.5, 127.0, 126.9, 126.8, 125.5, 125.1, 121.1, 48.7, 33.8, 25.0 (**Figure S13**); HRMS (ESI-TOF) *m/z* 455.1435 (455.1414 calcd. for $C_{25}H_{20}BN_2O_6$, [M−H]

⁻).

^{1}H- and ^{13}C-NMR spectrum charts of new compounds

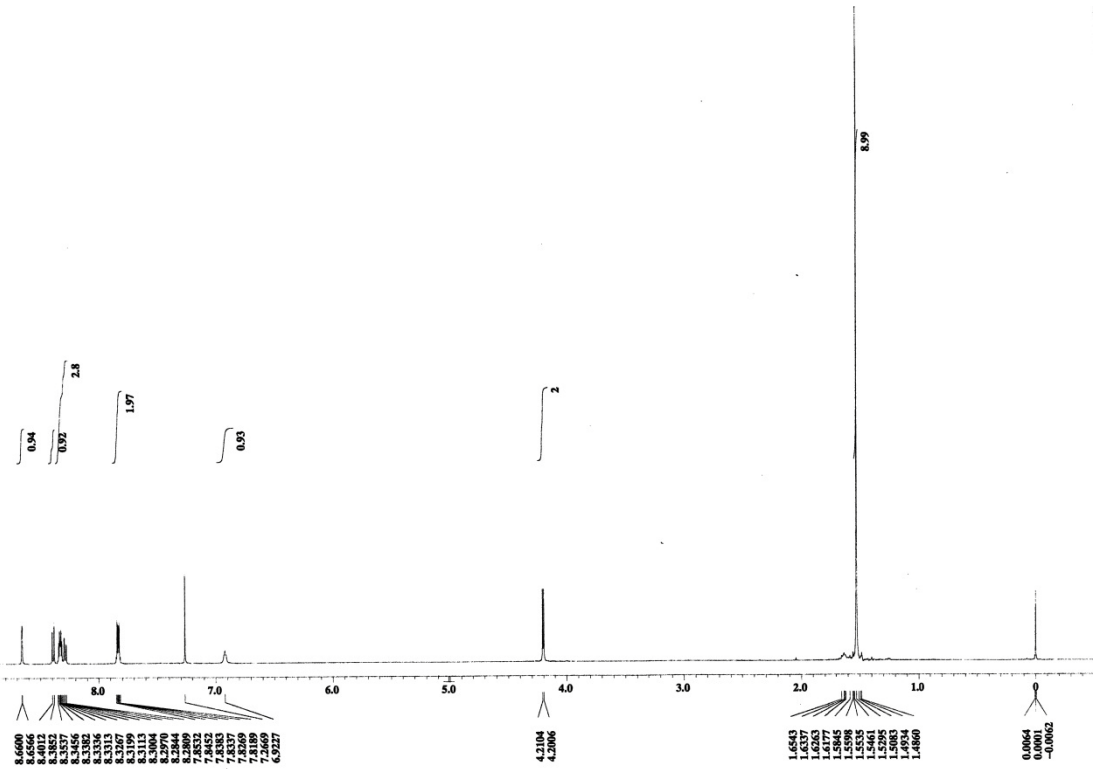

Figure S2 ^{1}H-NMR spectrum of **11**

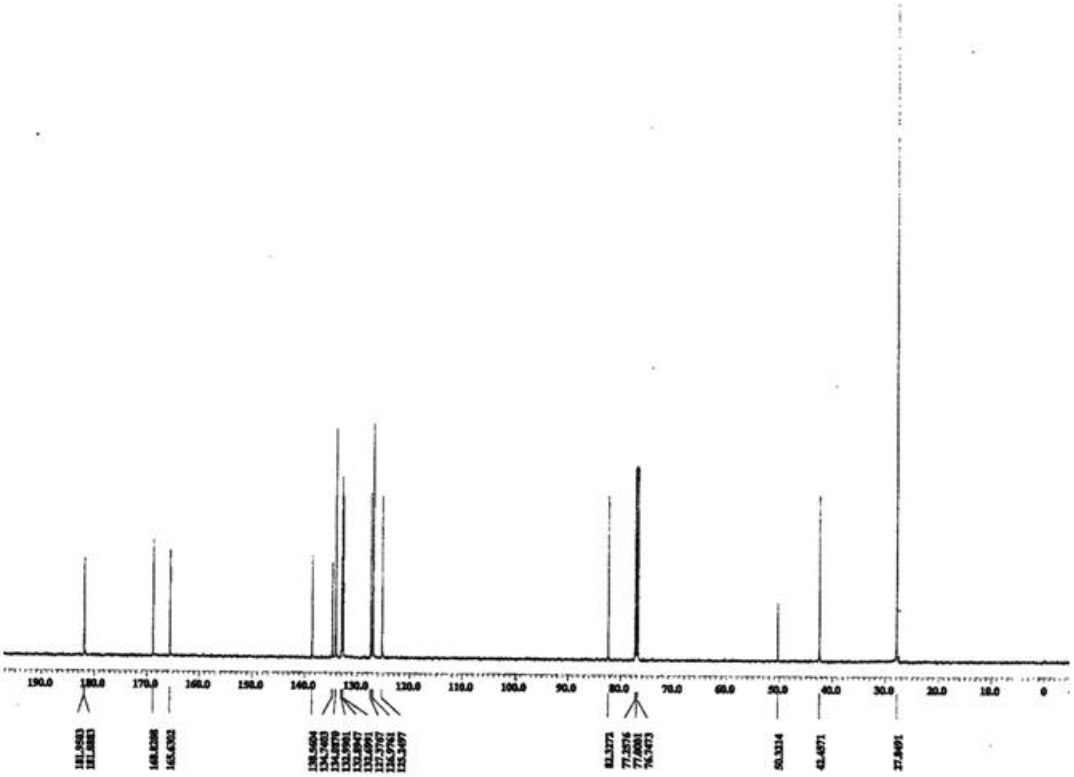

Figure S3 ^{13}C-NMR spectrum of **11**

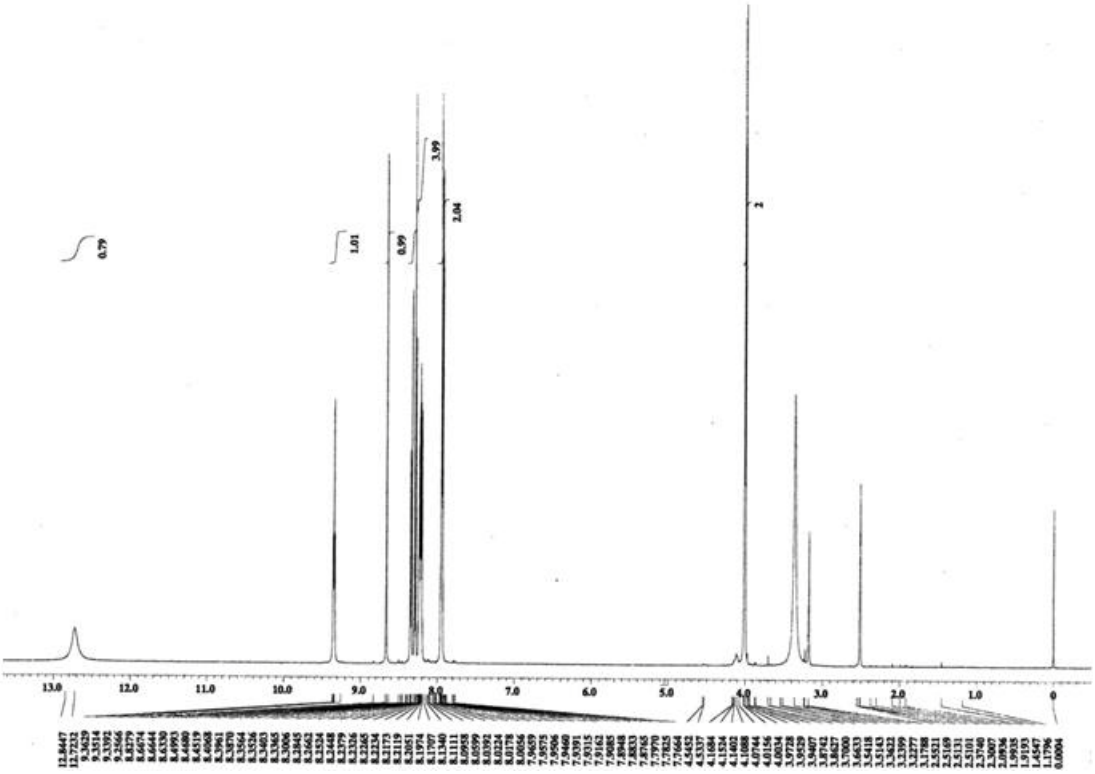

Figure S4 ^{1}H-NMR spectrum of **12**

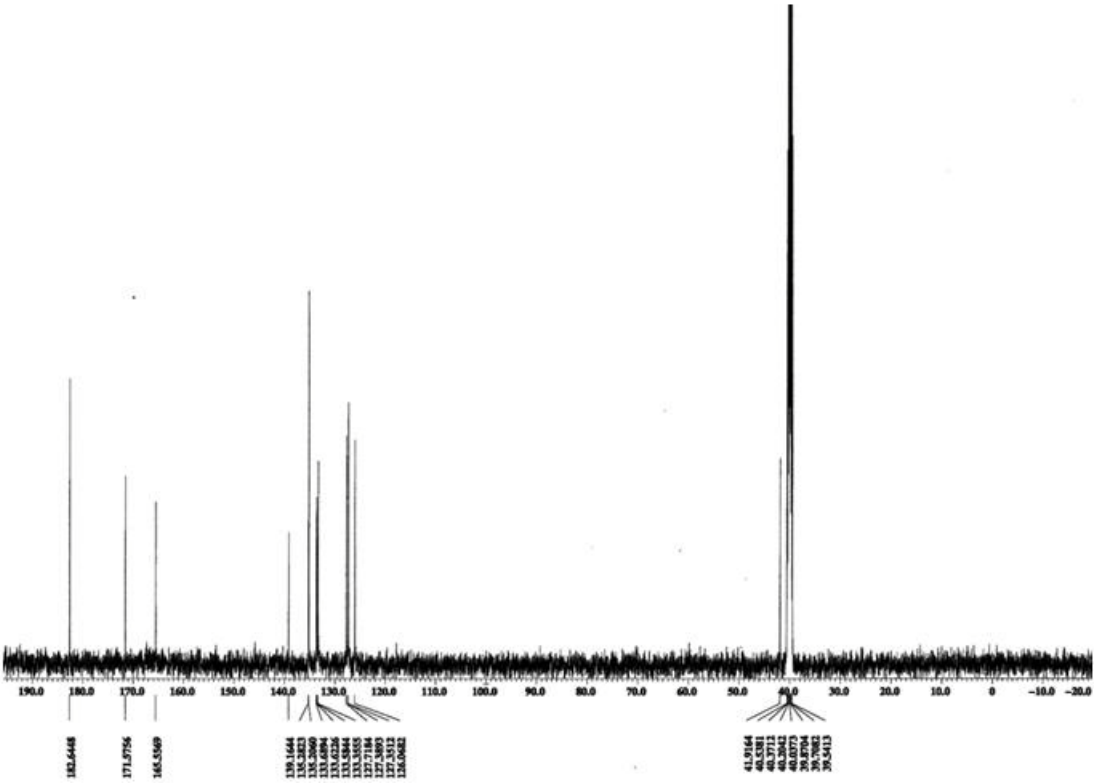

Figure S5 ^{13}C-NMR spectrum of **12**

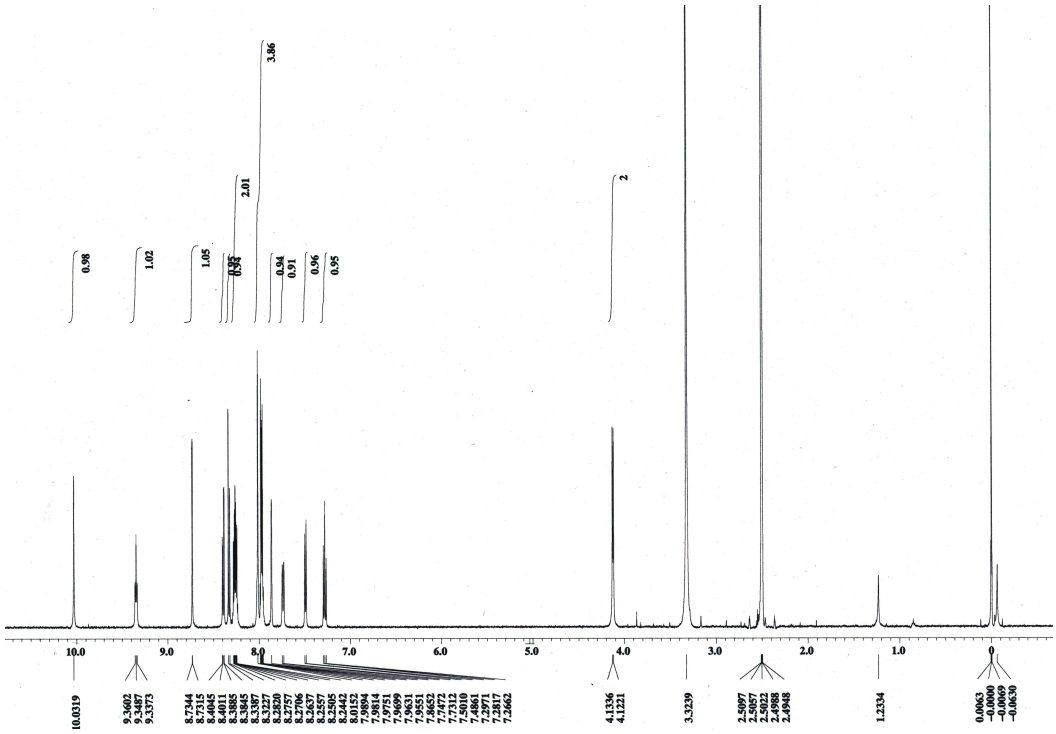

Figure S6 ^{1}H-NMR spectrum of **2**

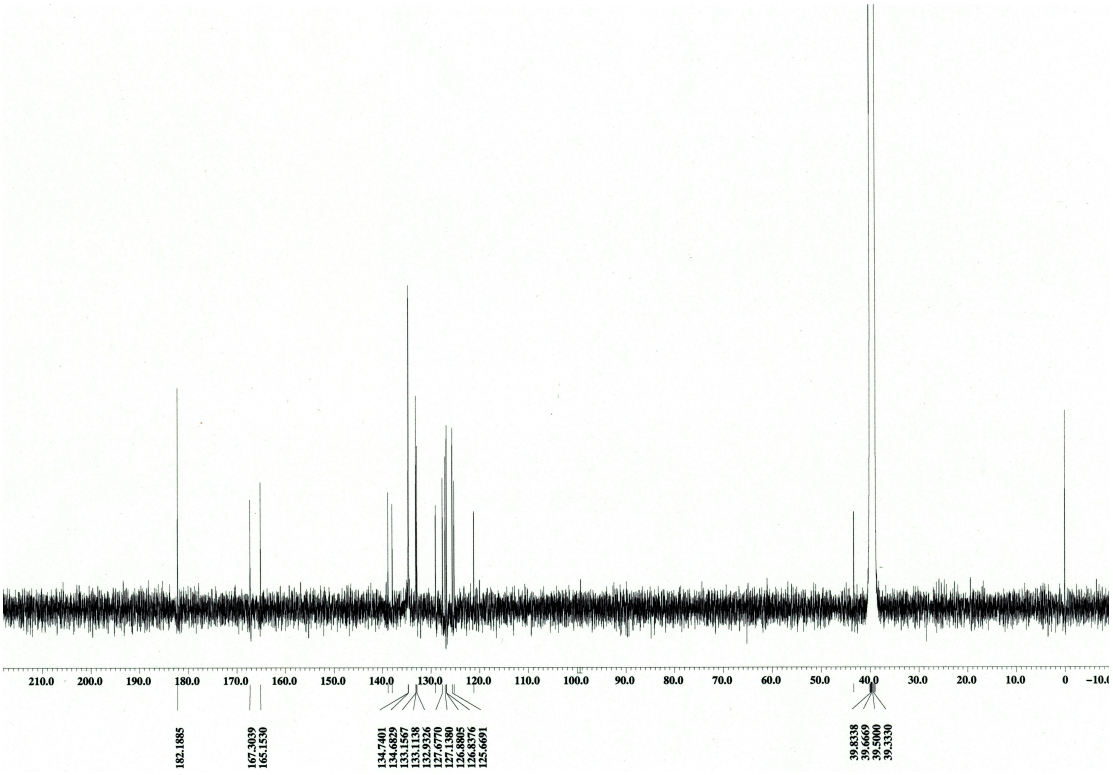

Figure S7 ^{13}C-NMR spectrum of **2**

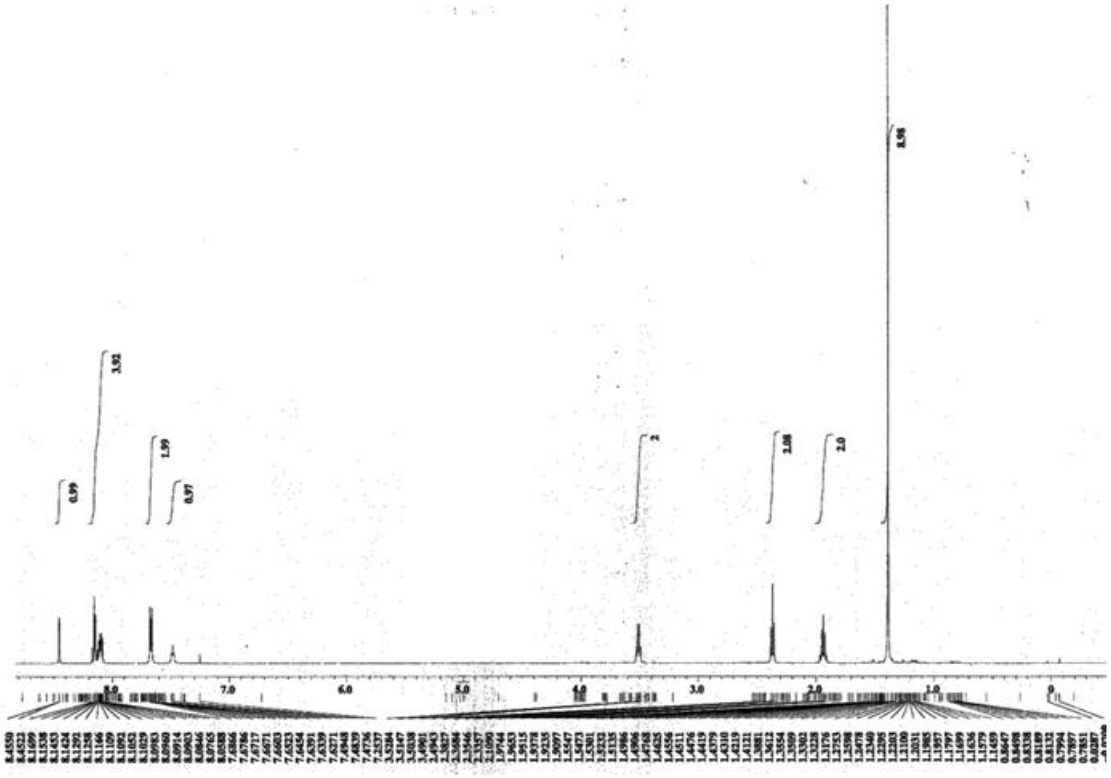

Figure S8 ^{1}H-NMR spectrum of **15**

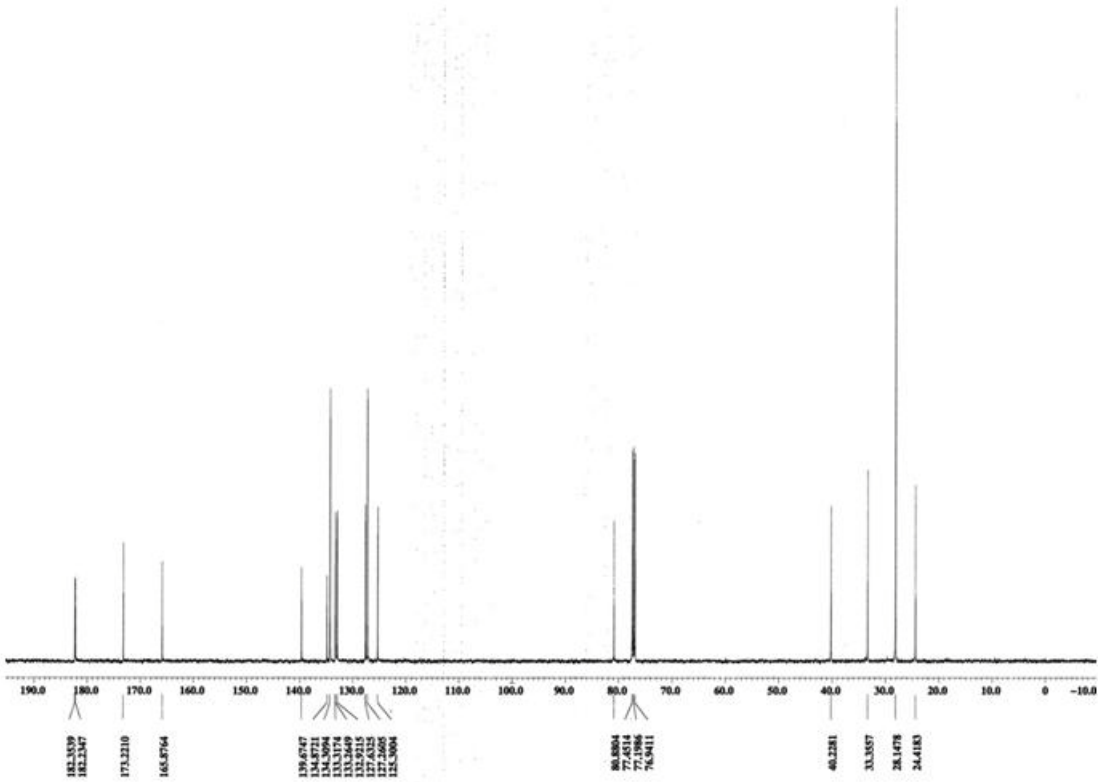

Figure S9 ^{13}C-NMR spectrum of **15**

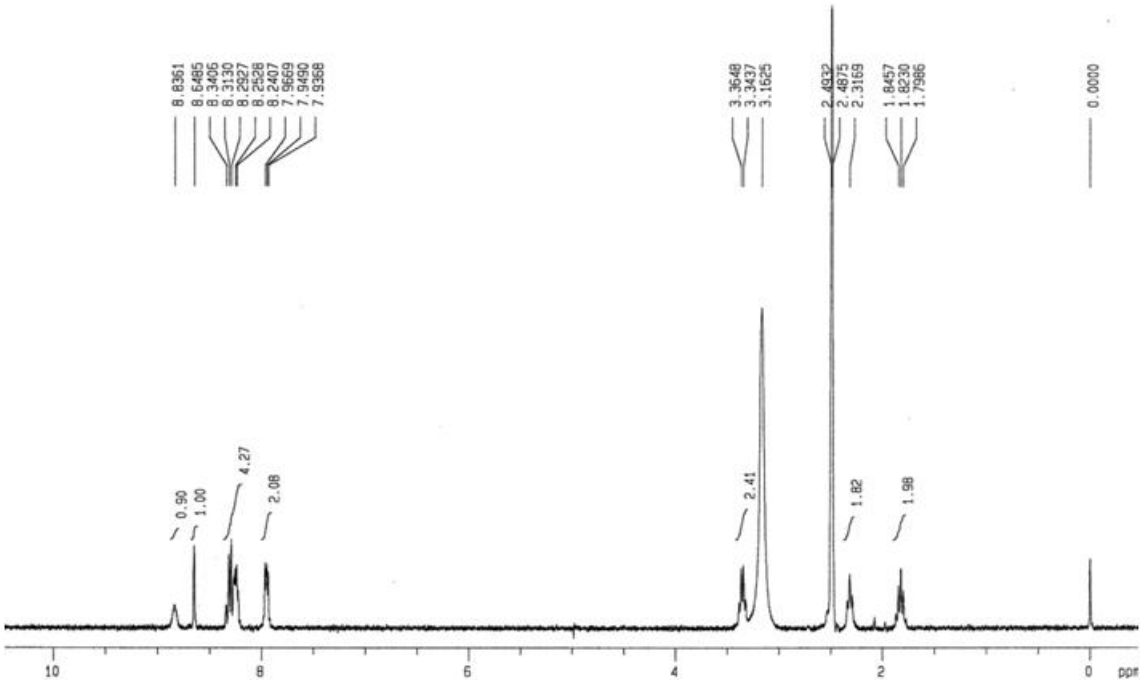

Figure S10 ^{1}H-NMR spectrum of **16**

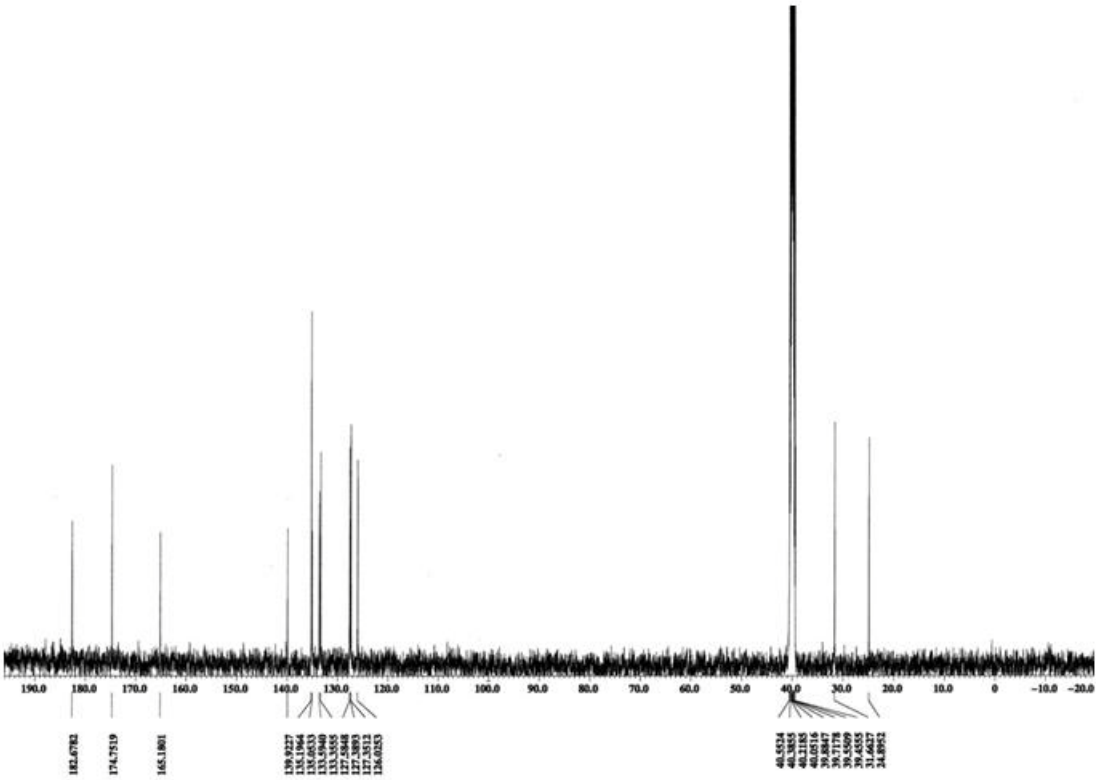

Figure S11 ^{13}C-NMR spectrum of **16**

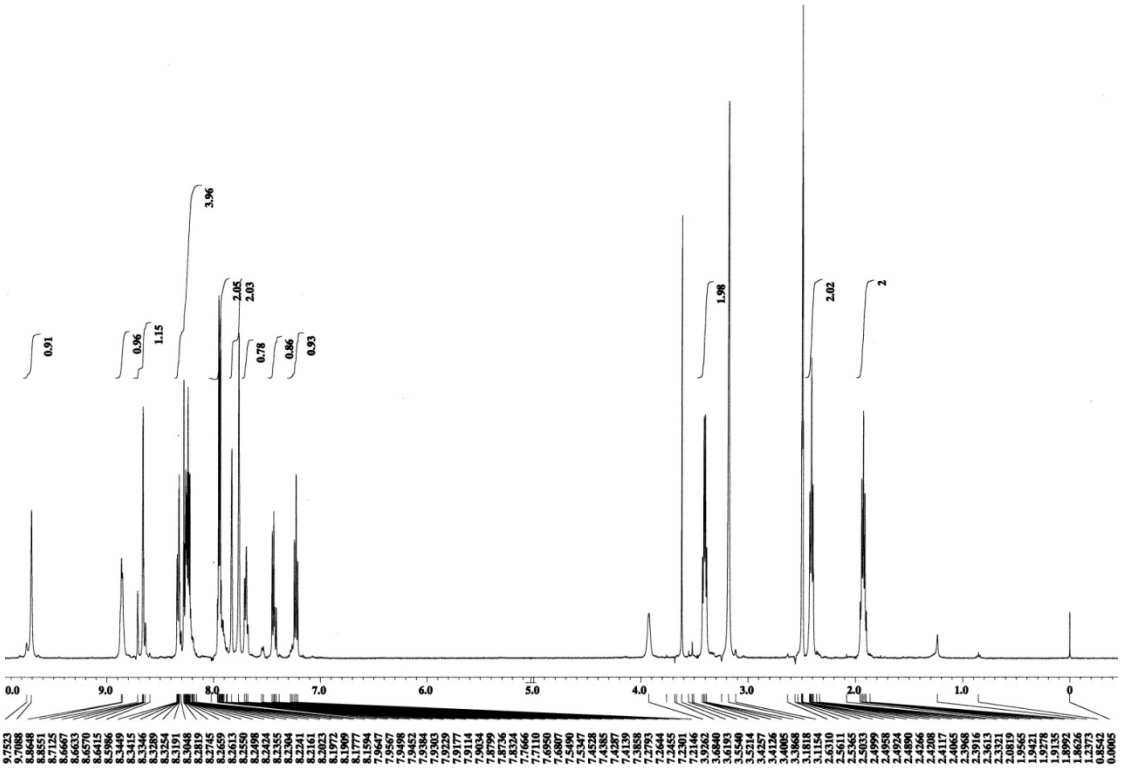

Figure S12 ^{1}H-NMR spectrum of **3**

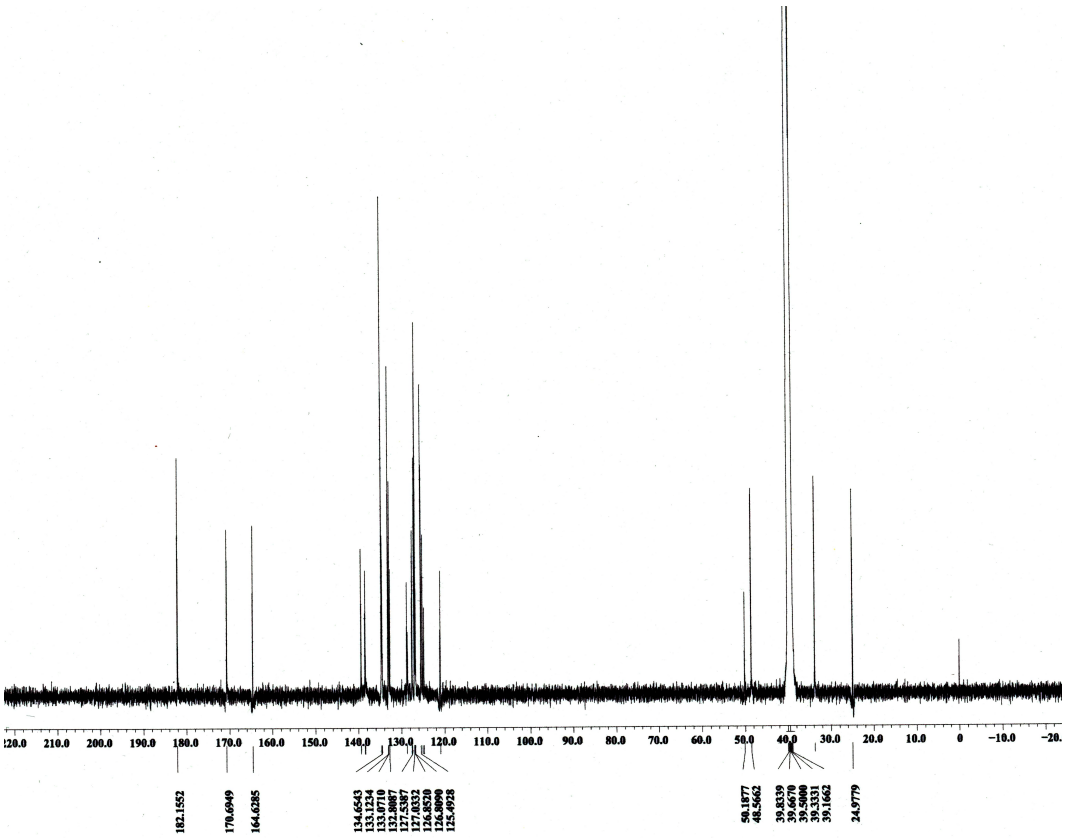

Figure S13 ^{13}C-NMR spectrum of **3**

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[2] D. G. Hall, J. Tailor and M. Gravel, *Angew. Chem. Int. Ed.*, 1999, **38**, 3064; M. Gravel, K. A. Thompson, M. Zak, C. Bérubé and D. G. Hall, *J. Org. Chem.*, 2002, **67**, 3.