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Amphiphilic γ-Cyclodextrin–Fullerene Complexes with Photodynamic Activity

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1. Experimental section

1.1. Materials and Methods

Poly(ethylene glycol) monomethyl ether (MW = 2000 and 5000) was purchased from Sigma-Aldrich (USA). 1,8-Diazabicyclo[5.4.0]undeca-7-ene (DBU), sodium hydride, β-nicotinamide adenine dinucleotide disodium salt (reduced form, NADH), sodium chloride (NaCl), tetrahydrofuran (THF), and ethyl acetate (EtOAc) were purchased from nacalai tesque (Japan). 4-Pentynoic acid, DL-α-tocopherol (vitamin E), ethyl bromoacetate, and 2-thiazoline-2-thione were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). *N*,*N'*-Dicyclohexylcarbodiimide (DCC), pentafluorophenol, and 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) were purchased from Watanabe Chemical Industries, Ltd. (Japan). 4-Dimethylaminopyridine (DMAP), copper iodide (CuI), nitro blue tetrazolium chloride (NBT), dichloromethane (CH_2Cl_2), and *N*,*N*-dimethylformamide (DMF) were purchased from Wako Pure Chemicals Industries, Ltd. (Japan). Fullerene was purchased from MTR Ltd. (USA). Deuterium oxide (D_2O) was purchased from Sigma-Aldrich (USA). Dialysis membrane, Spectra/Por 6 (molecular weight cutoff (MWCO): 1000) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

We reported the synthesis of activated amide **5** bearing an indocyanine green dye derivative elsewhere.[1] 9,10-Anthracenedipropinonic acid sodium salt (ADPA sodium salt) was prepared according to the reported procedure.[2] Mono-6-amino-6-deoxy-γ-cyclodextrin **1-NH$_2$**[3] was prepared by the reported procedure. Non-functionalized CFC (γ-cyclodextrin-fullerene 1:2 complex) was synthesized by HSVM.[4]

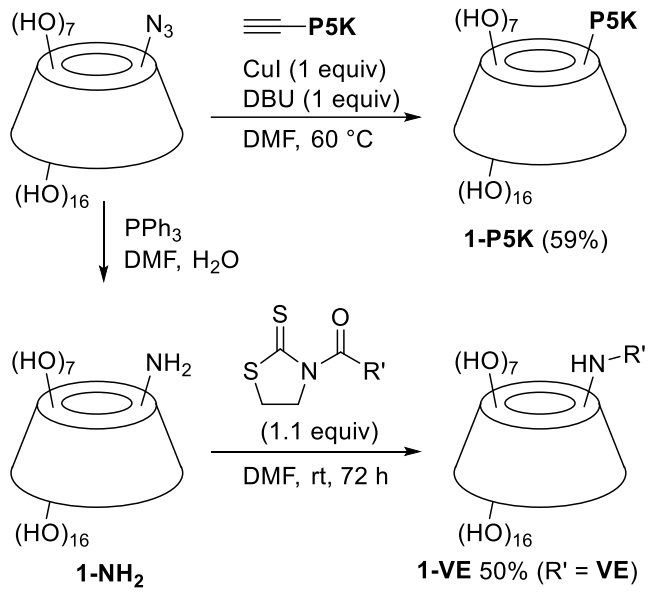
To evaluate the properties and morphology of self-assemblies, amphiphilic CFCs were dissolved in H_2O (5 mg/mL) and stored at room temperature for at least 30 min in dark. The resulting solution was filtered by syringe filter (0.45 μm pore size, PVDF) to prepare a solution of self-assemblies for measurements.

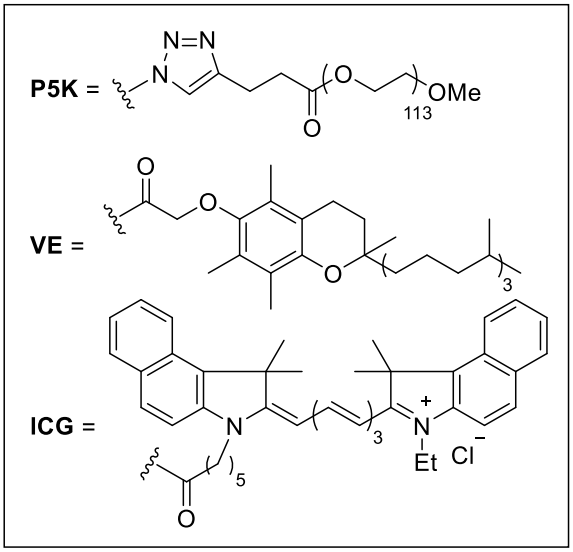
UV-vis absorption spectra were recorded by UV-vis spectrophotometer (V-570, JASCO Corporation, Japan).

Transmission electron microscopy (TEM, JEM-1400, JEOL Ltd., Japan) was used to visualize the morphology of dried self-assemblies. Samples were dropped onto a TEM copper grid covered with a carbon film (200 mesh, Nisshin EM, Japan) and dried for 3 h.

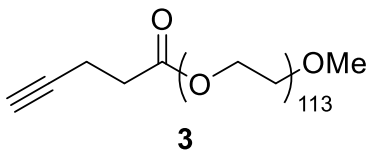
1.2. Synthesis of functionalized γ-CDs 1.

Scheme S1





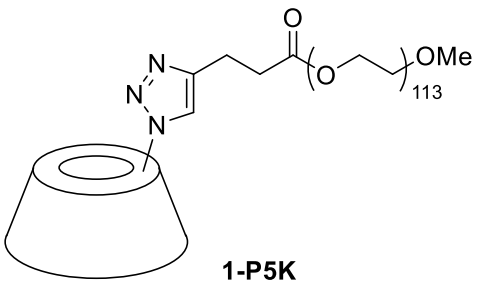
1.2.1. Synthesis of PEG derivative 3.



3

Prior to the condensation reaction poly(ethylene glycol) monomethyl ether (MW = 5000) was dried under reduced pressure at 100 °C for 12 h. To a solution of poly(ethylene glycol) monomethyl ether (2.5 g, 0.50 mmol) in dried THF (20 mL) were added 4-pentynic acid (0.21 g, 2.1 mmol), DCC (0.21 g, 1.0 mmol), and DMAP (19 mg, 0.15 mmol) at 0 °C. After stirring at 55 °C for 3 days, the reaction mixture was filtered through a Celite pad and the filtrate was diluted with Et_2O. The white precipitate was separated by filtration and washed with Et_2O. After drying the white solid under reduced pressure overnight, PEG derivative **3** was obtained as a white solid (1.9 g, 0.38 mmol, 76%). mp 53.5–54.6 °C; IR (KBr) 528, 843, 963, 1061, 1104, 1149, 1242, 1281, 1343, 1360, 1468, 1737, 2165, 2888 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 1.97–2.01 (m, 1H), 2.47–2.55 (m, 2H), 2.55–2.64 (m, 2H), 3.38 (s, 3H), 3.46 (t, J = 4.9 Hz, 2H), 3.40–3.92 (m, 758H), 4.26 (t, J = 4.9 Hz, 2H).

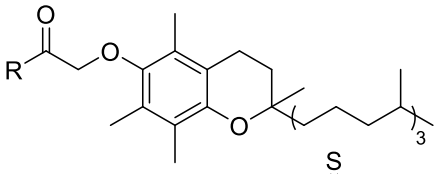
1.2.2. Synthesis of PEG-grafted γ-CDs 1-2K and 1-5K.

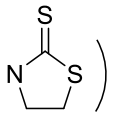


1-P5K

To a solution of mono-6-azido-6-deoxy-γ-cyclodextrin[3] (50 mg, 0.038 mmol) and **3** (0.38 g, 0.076 mmol) in DMF (5.0 mL) were added CuI (7.2 mg, 0.038 mmol) and DBU (5.8 mg, 0.038 mmol). After stirring at 60 °C for 24 h, the organic solvent was removed under reduced pressure. The residue was dissolved in water and dialyzed against H_2O for one day by using Spectra/Por 6 (MWCO = 1000). PEG-grafted γ-CD **1-P5K** (0.23 g, 0.036 mmol, 42%) was obtained as a white solid after lyophilization. mp 53.0–54.2 °C; IR (KBr) 530, 843, 948, 963, 1061, 1107, 1149, 1242, 1281, 1343, 1360, 1413, 1455, 1467, 1634, 1736, 2695, 2741, 2888, 3427 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ 2.62–2.70 (m, 2H), 2.82–2.91 (m, 2H), 3.43–3.60 (m, 6866H), 4.09–4.17 (m, 2H), 4.46–4.71 (m, 16H), 4.82–4.93 (m, 8H), 5.83–5.96 (m, 16H), 7.78 (s, 1H). From ^1H NMR measurement, γ-CD derivative **1-P5K** contains unreacted **3** (~20%). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ 58.1, 60.2, 69.6, 69.8, 70.1, 70.4, 71.3, 72.3. Because of the large signals of PEG, signals of a triazole and γ-CD moiety were not detected in ^{13}C NMR measurement.

1.2.3. Synthesis of amide 4b having a VE moiety.



4a (R = OEt) **4b** (R = 

)

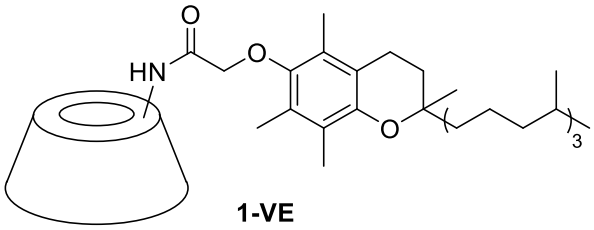
To a solution of DL-α-tocopherol (vitamin E, 1.1 g, 2.5 mmol) in THF (11 mL) was added sodium hydride (0.15 g, 3.8 mmol) at 0 °C. After stirring at room temperature for 1 h, to this mixture was added ethyl bromoacetate (0.31 mL, 2.8 mmol) in THF (3 mL) at room temperature. After stirring overnight, to this mixture were added 2M KOH aqueous solution (7 mL) and THF (4 mL). After stirring at room temperature for 4 h (check the consumption of ethyl ester by TLC), the reaction mixture was poured into water (20 mL) and EtOAc (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL×2). The combined organic layer was washed with brine (10 mL) and dried over Na_2SO_4. The organic solvents were removed under reduced pressure and subjected to column chromatography on SiO_2 with hexane and EtOAc (v:v = 8:1) as eluents to afford vitamin E derivative having a carboxylic acid

moiety (0.93 g, 1.9 mmol, 76%) as a pale brown solid.

For the isolation of **4a**, the reaction mixture was concentrated without the hydrolysis step. The residue was subjected to short column chromatography on SiO_2 with hexane and EtOAc (v:v = 10:1) as eluents to afford **4a** (~90% yield) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 0.84 (d, J = 6.4 Hz, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.86 (d, J = 6.7 Hz, 6H), 1.02–1.61 (m, 24H, including the following two signals, δ 1.23 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H)), 1.71–1.84 (m, 2H), 2.07 (s, 3H), 2.14 (s, 3H), 2.18 (s, 3H), 2.56 (t, J = 6.7 Hz, 2H), 4.30 (q, J = 7.2 Hz, 2H), 4.28 (s, 2H).

To a solution of the product (0.23 g, 0.47 mmol) in CH_2Cl_2 (5 mL) were added 2-thiazoline-2-thione (63 mg, 0.53 mmol) and EDC (0.18 g, 0.95 mmol) at 0 °C. After stirring at room temperature for 24 h, to this mixture was added 0.1N HCl aqueous solution (12 mL). After stirring for 3 h, the organic layer was separated and the aqueous layer was extracted with EtOAc (10 mL×3). The combined organic layer was dried over Na_2SO_4. The organic solvents were removed under reduced pressure and subjected to column chromatography on SiO_2 with hexane and EtOAc (v:v = 4:1) as eluents to afford **4b** (0.24 g, 0.40 mmol, 86%) as a yellow solid. mp 51.0-52.5 °C; IR (KBr) 725, 865, 1025, 1052, 1095, 1175, 1233, 1258, 1282, 1373, 1410, 1462, 1712, 2868, 2926 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 0.83–0.87 (m, 12H), 1.08–1.59 (m, 24H), 1.71–1.85 (m, 2H), 2.07 (s, 3H), 2.13 (s, 3H), 2.17 (s, 3H), 2.56 (t, J = 6.8 Hz, 2H), 3.41 (t, J = 7.3 Hz, 2H), 4.69 (t, J = 7.3 Hz, 2H), 5.18 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 11.7, 11.9, 12.8, 19.6–19.7 (multiple), 20.6, 21.0, 22.7, 23.8, 24.4, 24.9, 28.0, 29.4, 31.2, 31.2, 32.7–32.8 (multiple), 37.3–37.4 (multiple), 39.3, 40.1, 55.6, 74.3, 74.9, 117.6, 122.9, 125.6, 127.5, 147.7, 148.2, 170.5, 200.8. Multiple signals were observed due to diastereoisomers. HRMS (FAB) calcd for $C_{34}H_{55}O_3NS_2Na$ ($[M+Na]^+$): 612.3521, found: 612.3535.

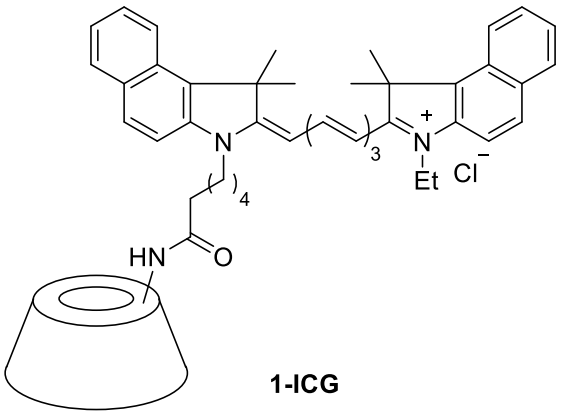
1.2.4. Synthesis of VE-grafted γ-CD 1-VE.



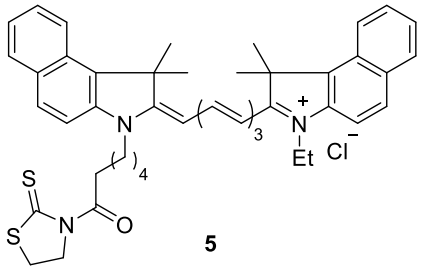
1-VE

To a solution of **1-NH$_2$** (0.22 g, 0.17 mmol) in DMF (4.0 mL) was added **4b** (0.11 g, 0.18 mmol) at room temperature. After stirring for 48 h at room temperature, Et_2O (20mL) was added to the reaction mixture. The precipitate was separated by centrifuge and washed with Et_2O. The crude product was dissolved in MeOH (10 mL) and to this solution was added sodium methoxide (18 mg, 0.34 mmol) at room temperature. After stirring overnight, the organic solvent was removed under reduced pressure and the residue was dispersed in water (10 mL). The aqueous solution was neutralized with 1N HCl aqueous solution. The precipitate was collected by filtration and washed with water. VE-grafted γ-CD **1-VE** (0.15 g, 0.084 mmol, 50%) was obtained as a white solid after drying under reduced pressure. mp 230 °C (dec); IR (KBr) 534, 709, 761, 941, 1031, 1083, 1157, 1253, 1378, 1412, 1458, 1498, 1542, 1561, 1655, 2867, 2927, 3412 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ 0.73–0.90 (m, 12H), 0.95–1.54 (m, 24H), 1.65–1.76 (m, 2H), 1.90–2.12 (m, 9H), 2.40–2.60 (m, 2H, this signal was not clearly observed because the solvent signal was overlapped), 3.00–3.95 (m, signals of γ-CD were overlapped with that of H_2O), 3.98–4.12 (m, 4H), 4.19–4.97 (m, 8H), 5.34–6.37 (m, 8H), 7.74 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C) δ 11.6–11.7 (multiple), 12.4, 12.6, 19.5–19.6 (multiple), 20.0, 20.4, 22.5, 22.6, 23.5, 23.7, 24.2, 27.4, 30.8, 31.9–32.0 (multiple), 36.6–36.8 (multiple), 59.8–60.1 (multiple), 72.0–73.1 (multiple), 74.3, 74.6, 80.4–81.2 (multiple), 101.6-101.8 (multiple), 117.5, 121.9, 125.5, 127.1, 147.0, 147.5, 168.0. Multiple signals were observed due to diastereoisomers. HRMS (FAB) calcd for $C_{79}H_{131}O_{42}NNa$ ($[M+Na]^+$): 1788.8043, found: 1788.8026.

1.2.5. Synthesis of ICG-grafted γ-CD 1-ICG.



1-ICG



5

ICG-grafted γ-CD **1-ICG** was similarly synthesized from **1-NH₂** with ICG derivative **5**.[1] To a solution of **1-NH₂** (0.14 g, 0.11 mmol) in DMF (5.0 mL) was added **7** (90 mg, 0.12 mmol) at room temperature. After stirring overnight, Et_2O (20mL) was added to the reaction mixture. The precipitate was separated by centrifuge and washed with Et_2O (5 mL×3) and water (10 mL×2). ICG-grafted γ-CD **1-ICG** (0.14 g, 0.076 mmol, 70%) was obtained as a dark green solid after drying under reduced pressure. mp >250 °C; IR (KBr) 522, 582, 665, 720, 752, 832, 878, 922, 964, 1007, 1029, 1061, 1096, 1151, 1195, 1306, 1354, 1420, 1475, 1508, 1625, 1654, 2868, 2928, 3368 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ 1.31–1.34 (m, 3H), 1.38–1.55 (m, 2H), 1.71–1.74 (m, 2H), 1.86–1.98 (m, 12H), 2.07–2.09 (m, 2H), 4.14–4.25 (m, 4H), 4.53–4.69 (m, 8H), 4.86–4.92 (m, 8H), 5.67–5.93 (m, 16H), 6.34–6.41 (m, 2H), 6.55–6.59 (m, 2H), 7.47–8.25 (m, 15H); HRMS (FAB) calcd for $C_{91}H_{126}O_{40}N_3$ ($[M]^+$): 1900.7912, found: 1900.7932. In ^{13}C NMR spectrum, only signals (δ 60, 72, 80, and 101 ppm) of γ-CD were observed. Signals of an ICG moiety were not detected probably due to tautomerization.

1.3. Characterization of C_LFC_H complexes 2A.

1.3.1 Mass spectra of C_LFC_H complexes 2A.

Mass spectra of **2A** were measured by MALDI-TOF mass spectrometer (Ultraflex, Bruker Daltonics K.K., Japan) (Figure S1). The mother signals of CFCs were not detected in these cases. Instead, both of γ-CDs bearing lipophilic and hydrophilic substituents were detected.

1.3.2. Evaluation of ^1H NMR spectra of 2A: confirmation of 1:1 ratio of lipophilic and hydrophilic γ-CDs in 2A.

In ^{1}H NMR measurement of CFCs **2A** in D_2O, only broad signals of PEG were detected. Signals of CD and lipophilic substituents were not detected because of the formation of self-assemblies. In ^{1}H NMR measurement in DMSO-d_6, signals of lipophilic moieties as well as those of PEG were detected, however CFCs were totally decomposed. The spectra in DMSO-d_6 were shown in Figure S2 and at the end of the SI.

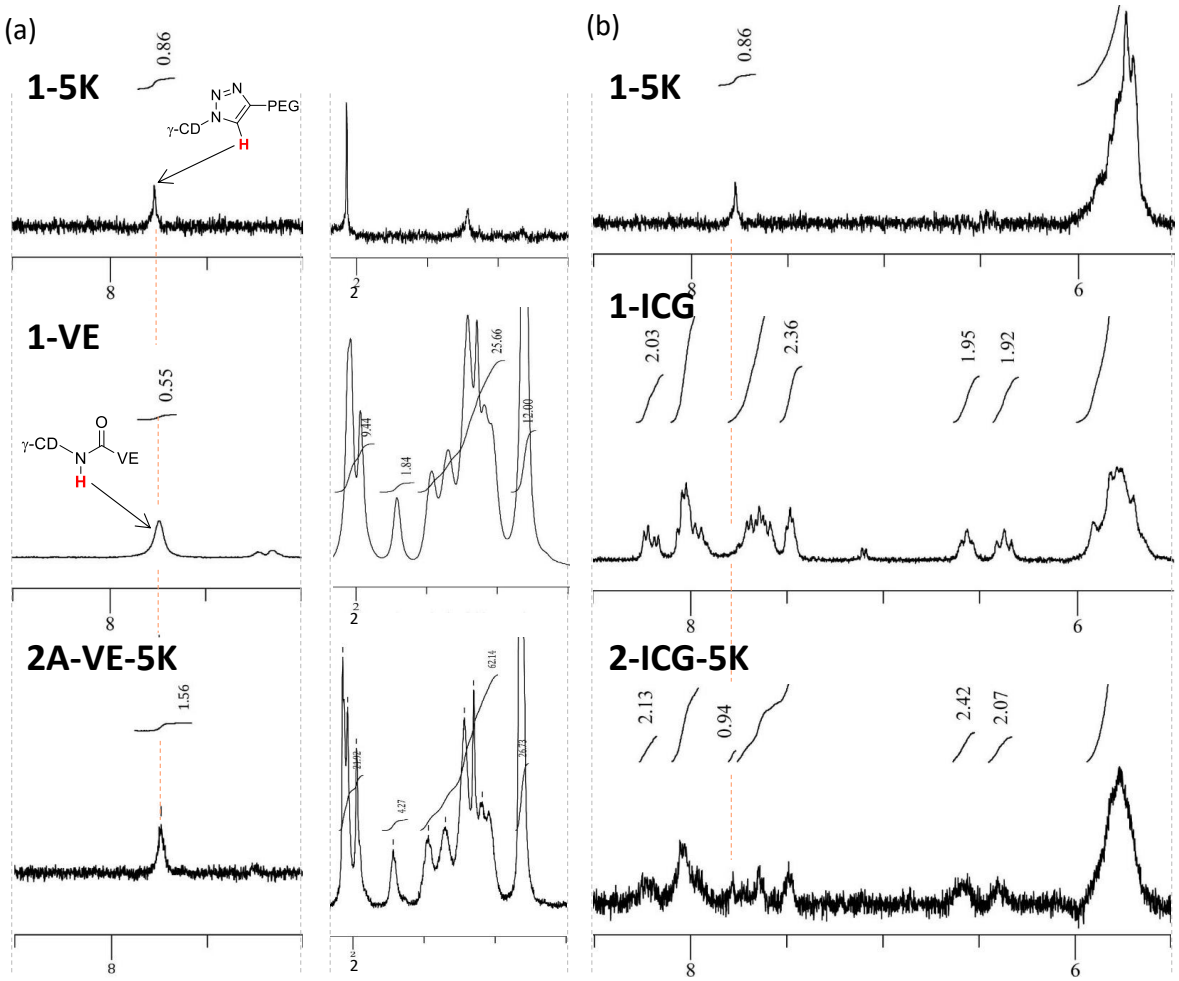


Figure S1. Selected ranges of ^{1}H NMR spectra (DMSO-d_6) of (a) **2A-VE-P5K** and (b) **2A-ICG-P5K** compared with their fragments. Spectra in upfield region shown in (a) were compressed vertically for clarity.

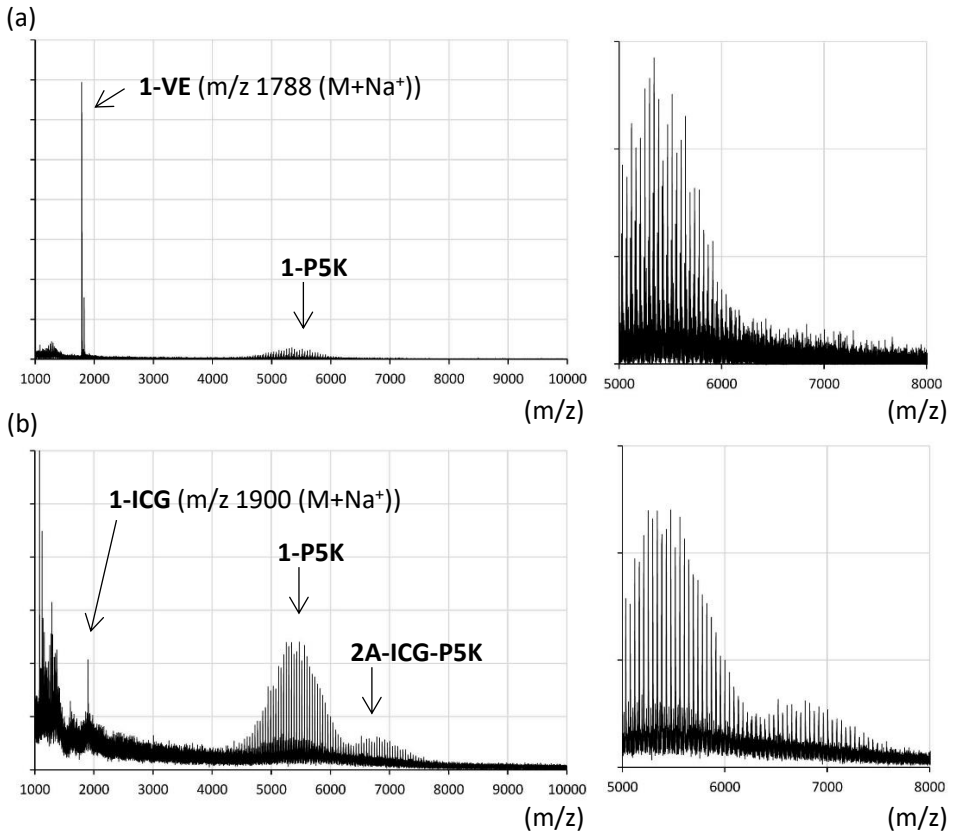


Figure S2. MALDI-TOF mass spectra of (a) **2A-VE-P5K** and (b) **2A-ICG-P5K**. Conditions: refrectron positive mode. Matrix: dithranol with sodium cation.

1.4. Synthesis of C_LFC_H complexes 2B.

1.4.1. Synthesis of $C_{NH2}FC_{NH2}$.

$C_{NH2}FC_{NH2}$ was synthesized by HSVM. Fullerene (19 mg, 26 μmol) and **1-NH_2** (0.13 g, 0.10 mmol) were weighed into a stainless capsule together with a mixing ball. The materials were thoroughly mixed by HSVM technique (MM400, 1800 rpm) for 20 min. The mixture was dissolved in water (50 mL) and the insoluble was removed by centrifugation. After lyophilization, the residue was washed with water-acetone (40 mL, v:v = 3:1) to afford **$C_{NH2}FC_{NH2}$** (23 mg, 5.8 μmol, 23%, MW(theor) = 3.3K, containing ~10% of free **1-NH_2**) as a brown powder. Purity was determined at 90% by the UV-vis absorption intensity of **$C_{NH2}FC_{NH2}$** at 332 nm, presuming that the molar extinction coefficient of **$C_{NH2}FC_{NH2}$** is same as that of non-

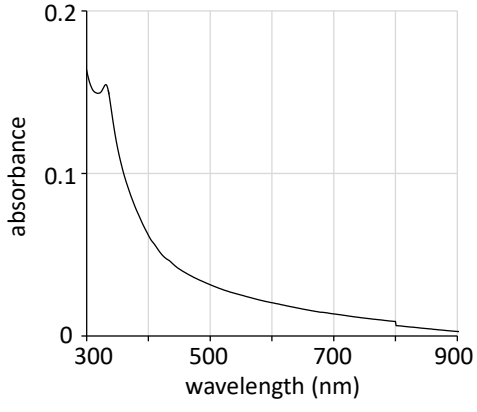


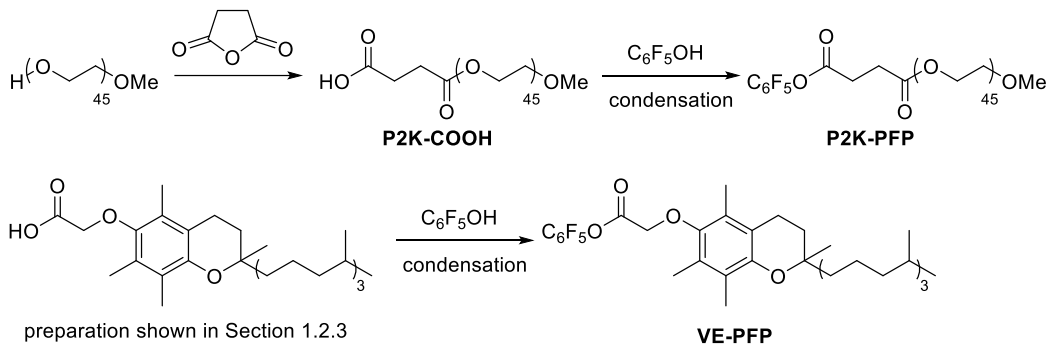
Figure S3. UV-vis absorption spectrum of **$C_{NH2}FC_{NH2}$**.

functionalized CFC ($\varepsilon = 4.27\times10^4$ $M^{-1}cm^{-1}$).[5] IR (ATR) 523, 579, 938, 1023, 1078, 1155, 3334 cm^{-1}. ^1H NMR (500 MHz, D_2O, 25 °C) δ 3.56–3.68 (m, 32H), 3.74–3.80 (m, 16H), 3.87–3.97 (m, 32H), 4.19 (t, J = 9.8 Hz, 16H), 5.05 (d, J = 4.0 Hz, 16H). Because **1-NH$_2$** is much more water-soluble than **C$_{NH2}$FC$_{NH2}$**, the signals of unreacted **1-NH$_2$** were strongly detected by ^1H NMR. UV-vis absorption spectrum was shown in Figure S3.

1.4.2. Synthesis of pentafluorophenyl esters.

Pentafluorophenyl ester having a PEG moiety (MW: 2K) was synthesized according to the reported procedure (Scheme S2).[6] To a solution of **P2K-COOH** (0.50 g, 0.25 mmol) and pentafluorophenol (66 mg, 0.36 mmol) in CH_2Cl_2 (1 mL) were added DCC (80 mg, 0.38 mmol) and DMAP (10 mg, 0.082 mmol) at room temperature. After stirring for 4 h, the organic solvent was removed under reduced pressure and the residue was suspended in water (5 mL). The insoluble was removed by filtration and the solid was washed with water (2 mL×2). After lyophilization, **P2K-PFP** was obtained as a white solid (0.51 g, 0.22 mmol, 89%). IR (ATR) 842, 962, 1061, 1148, 1242, 1280, 1342, 1360, 1467, 1520, 1704, 2884 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ 2.64 (t, J = 6.8 Hz, 2H), 3.03 (t, J = 6.8 Hz, 2H), 3.38 (s, 3H), 3.47–3.79 (m, 231H). ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C) δ –163.4 (s, 1F), –165.2 (t, J = 25.8 Hz, 2F), –171.3 (d, J = 25.8 Hz, 2F).

Scheme S2



Pentafluorophenyl ester of vitamin E (**VE-PFP**) was prepared from the corresponding carboxylic acid. The reaction conditions was similar to those for **P2K-PFP**.

VE-PFP: oil at r.t.; IR (KBr) 736, 759, 909, 992, 1060, 1090, 1259, 1378, 1451, 1519, 1790, 1817, 2868, 2927 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C) δ 0.83–0.88 (m, 12H), 1.08–1.65 (m, 24H), 1.71–1.85 (m, 2H), 2.10 (s, 3H), 2.18 (s, 3H), 2.22 (s, 3H), 2.59 (t, J = 6.6 Hz, 2H), 4.67 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 11.8, 11.9, 12.8, 19.6–19.7 (multiple), 20.6, 21.0, 22.6, 22.7, 23.8, 24.4, 24.8, 28.0, 31.1, 31.2, 32.7–32.8

(multiple), 37.3–37.4 (multiple), 39.4, 40.0, 40.0, 69.0, 75.0, 117.8, 123.3, 125.5, 127.4, 147.8, 148.6, 165.5. Multiple signals were observed due to diastereoisomers. ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C) δ –153.3 (br s, 2F), –158.3 (d, J = 23.8 Hz, 1F), –162.8 (t, J = 23.8 Hz, 2F). HRMS (FAB) calcd for $C_{37}H_{51}O_4F_5Na$ ([M+Na]$^+$): 677.3600, found: 677.3592.

1.4.3. Mass spectra of 2B-P2K.

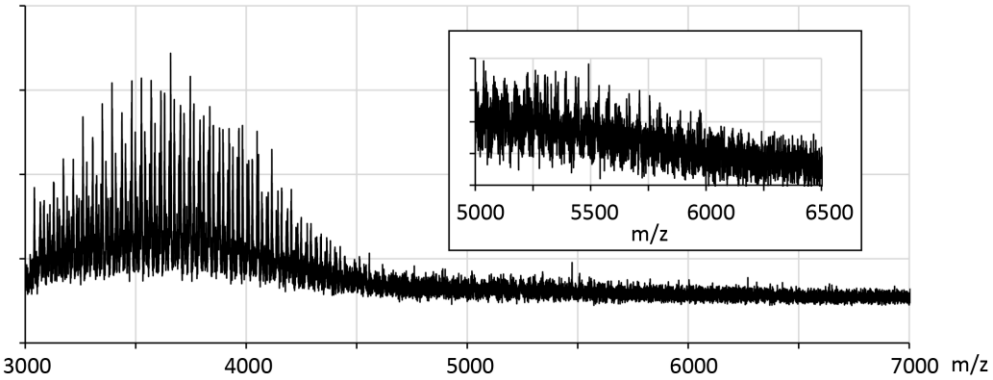


Figure S4. MALDI-TOF mass spectra of **2B-P2K**. Conditions: refrectron positive mode. Matrix: α-cyano-4-hydroxycinnamic acid (CHCA) with sodium cation. The enlarged spectrum was measured by cutting off the stronger signals (m/z < 4500).

1.5. XPS measurement.

X-ray photoelectron spectroscopy (XPS, MgKa, measured by MT-5500 (ULVAC-PHI, Inc.)) spectra of complexes are summarized in Figure S5. The binding energy of the Au 4f7/2 peak was adjusted to 84.0 eV to correct the chemical shift. The signals were observed as a merged signal of C-C and C=C, indicating the existence of C=C in complexes.

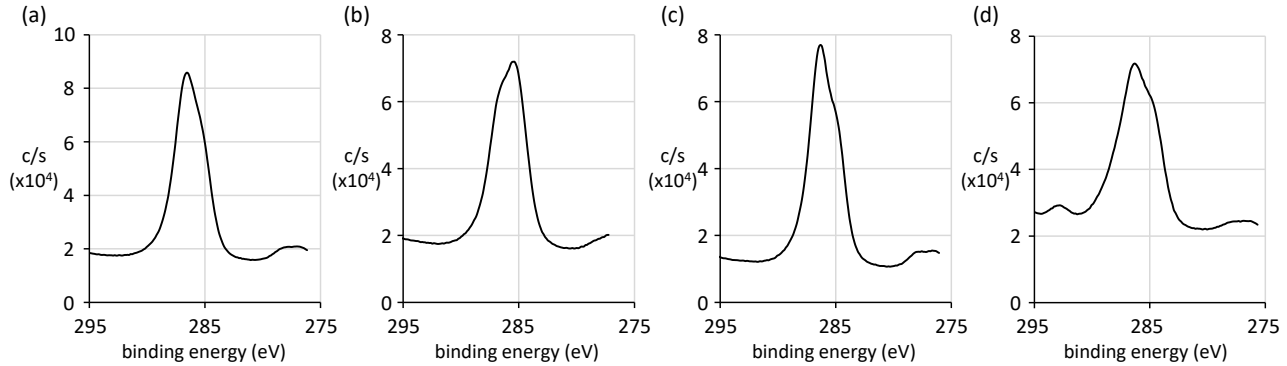


Figure S5. XPS spectra (C1s region) of (a) **2A-ICG-P5K**, (b) **2A-VE-P5K**, (c) **2B-VE-P2K**, and (d) **2B-P2K**.

1.6. Measurement of dynamic light scattering.

Dynamic light scattering was measured on FPAR-1000 (Otsuka Electronics Co., Ltd., Japan) at 20 °C. Before at least 3 h from the measurement, the sample solutions were diluted in H_2O and filtered (syringe filter, 0.45 μm pore size, PVDF). The concentrations of the analyzed sample solutions were 5 mg/mL. The measurements were performed at scattering angles of 90° at 25 °C. The data were summarized in Figure S6.

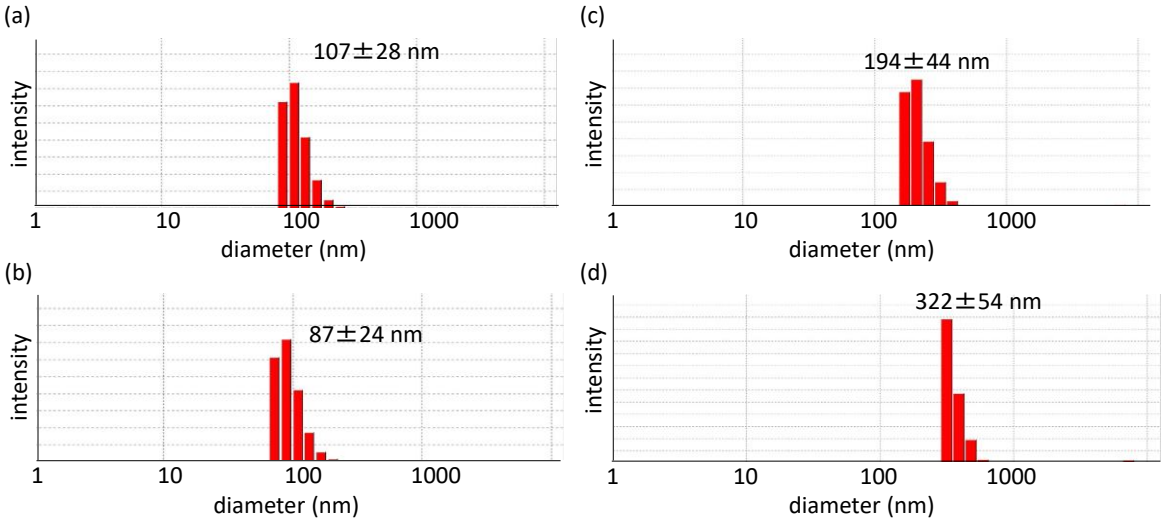


Figure S6. Size distribution of self-assemblies of (a) **2A-VE-P5K**, (b) **2A-ICG-P5K**, (c) **2B-VE-P2K**, and (d) **2B-P2K**. Intensity: number of nanoparticles.

1.7. Detection of singlet oxygen.

Solutions of C_LFC_H complex (15 μM) and ADPA (sodium salt, 25 μM) in D_2O (2 mL, 0.90 wt% of NaCl) as well as ADPA (sodium salt, 25 μM) in D_2O (2 mL, 0.90 wt% of NaCl) were prepared. The oxygen bubbling in each solution was carried out for 30 min. All solutions were transferred into quartz cells and purged with oxygen. The cells were irradiated by 150 W or 300 W Xenon light source (MAX-150 or MAX-303, Asahi Spectra Co., Ltd., Japan, illuminance: 17 $mW \cdot cm^{-2}$ at the sample level, wavelength: 400–800 nm) with a visual light module and an optical filter. The time-dependent generation of singlet oxygen was determined by measuring the UV-vis absorption intensities (Abs) of ADPA at 400 nm. Abs and Abs_0 (Abs of ADPA before irradiation) are mean values of absorbance measured in two independent experiments. We confirmed that the control experiment, the photoirradiation of ADPA in D_2O under the identical conditions, did not show the significant decrement of absorbance at 400 nm (data not shown).

1.8. Detection of superoxide anion.

Superoxide anion is reacted with nitro blue tetrazolium (NBT) to afford formazan efficiently.[7] Based on this reaction, the titration of superoxide anion was carried out. Solutions of CFCs **2A** (15 μM) and nitro blue tetrazolium (NBT, 200 μM) in D_2O (2 mL, 0.90 wt% of NaCl) as well as non-functionalized CFC (control, 50 μM), NADH (500 μM) and NBT (200 μM) in D_2O (2 mL, 0.90 wt% of NaCl) were prepared. The oxygen bubbling in each solution was carried out for 30 min. All solutions were transferred into quartz cells and purged with oxygen. The cells were irradiated by 150 W Xenon light source (MAX-150, Asahi Spectra Co., Ltd., Japan, illuminance: 5.1 $mW \cdot cm^{-2}$ at the sample level, wavelength: 400–800 nm) with a visual light module and an optical filter. The time-dependent generation of superoxide anion was determined by measuring the UV-vis absorption intensities of formazan at 560 nm (Figure S7). No superoxide anion generation were observed in the cases of CFCs **2A**.

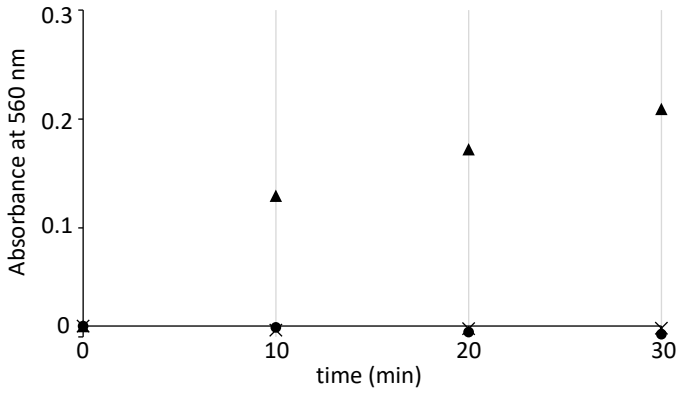


Figure S7. Time-dependent changes of the absorbance at 560 nm (formazan) with CFCs **2A-VE-P5K** (cross), **2A-ICG-P5K** (circle), and non-functionalized CFC with NADH (control, triangle) under visible light irradiation (5.1 $mW \cdot cm^{-2}$).

1.9. Delivery of 2A-ICG-P5K at a cellular level.

The uptake of self-assemblies of **2A-ICG-P2K** by human cervix epitheloid carcinoma (HeLa, American type Culture Collection) cells was examined by utilizing near-infrared fluorescence imaging. HeLa cells (1.0×10^3 cells/well in 96-well plate) were cultured with/without **2A-ICG-P5K** (0.11, 0.33, 1.0, 3.0, and 9.0 μM) in Dulbecco's modified Eagle's medium for 24 h (37 °C, 5% CO_2, dark). The culture medium was, then, removed and cells were washed twice with phosphate buffered saline (PBS, pH = 7.4). The fluorescence intensities of **2A-ICG-P5K** internalized into cells were measured by IVIS-SPECTRUM in vivo imaging device (PerkinElmer Inc., USA, irradiated wavelength: λ_{ex} = 745 nm, detected wavelength: λ_{em} = 840 nm). Images were analyzed using Living Image 2.50-Igor Pro 4.09 software (PerkinElmer Inc.), according to the instruction from the manufacture. Images were shown in Figure S8.

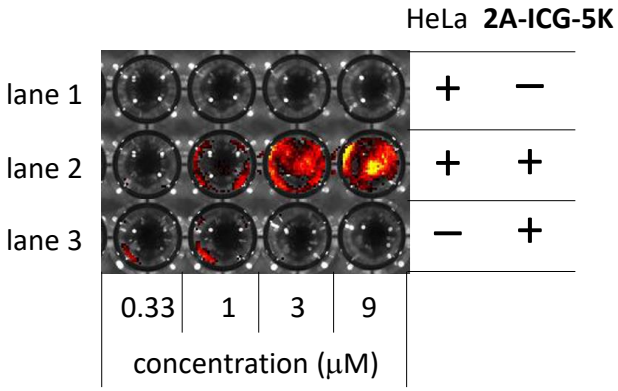


Figure S8. Fluorescence images of HeLa cells treated with aqueous solutions of **2A-ICG-P5K** with various concentrations in culture medium. HeLa cells were seeded in lanes 1 and 2. Aqueous solutions of **2A-ICG-P5K** were treated in lanes 2 and 3.

1.10. Cytotoxicity of C_LFC_H complexes under visible light irradiation.

HeLa cells (1.0×10^3 cells/well in 96-well plate) were cultured with C_LFC_H complex (8.0 μM) in Dulbecco's modified Eagle's medium for 24 h (37 °C, 5% CO_2, dark). The cells were, then, irradiated by 150 W or 300 W Xenon light source (MAX-150 or MAX-303, Asahi Spectra Co., Ltd., Japan, illuminance: 5.1 $mW\cdot cm^{-2}$ at the cell level, wavelength: 400–800 nm) with a visual light module and an optical filter. After irradiation for 1 h, the cells were cultured for 24 h (37 °C, 5% CO_2, dark). The numbers of viable cells were quantified by cell count reagent SF (nacalai tesque, Japan) and were evaluated by MTT assay, according to the manufacturer's instructions. For the control experiment, HeLa cells were cultured in another 96-well plate in similar manner without irradiation, and the cell viability were quantified.

2. References

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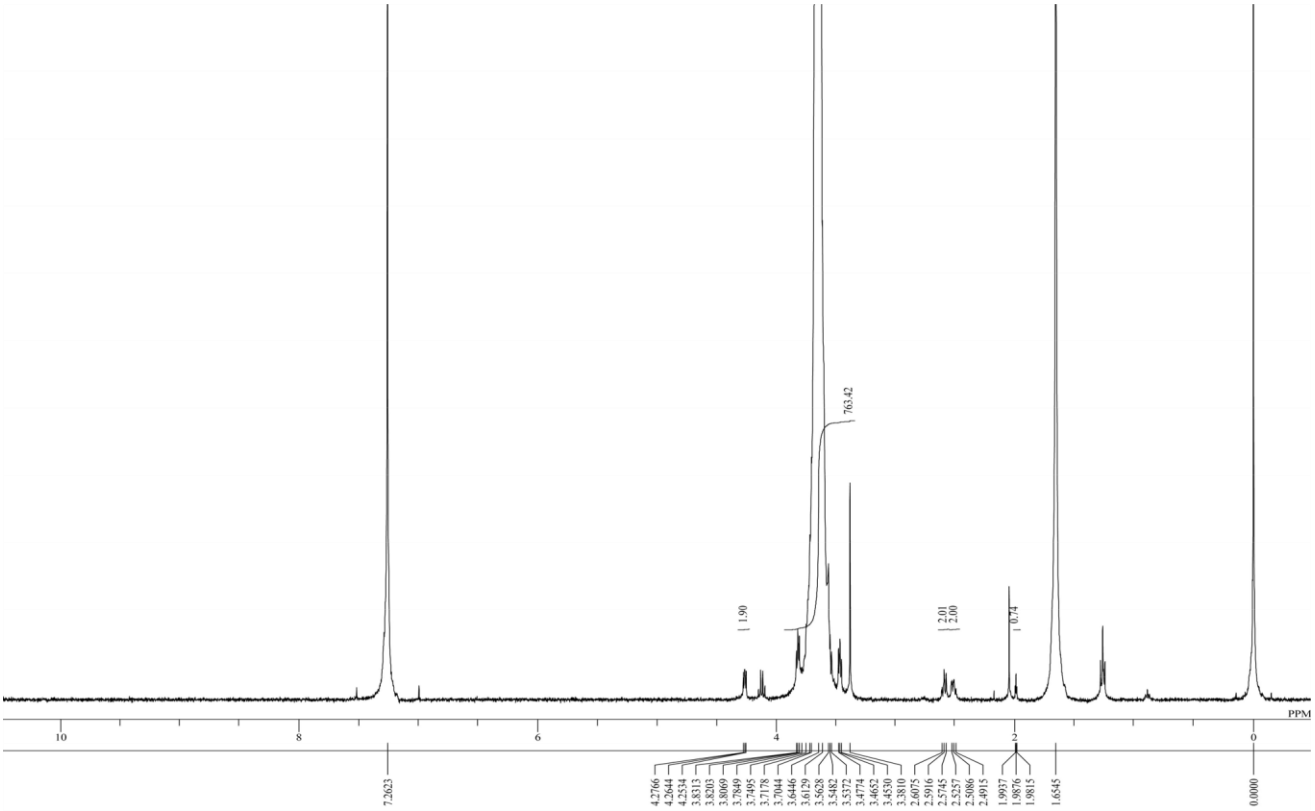
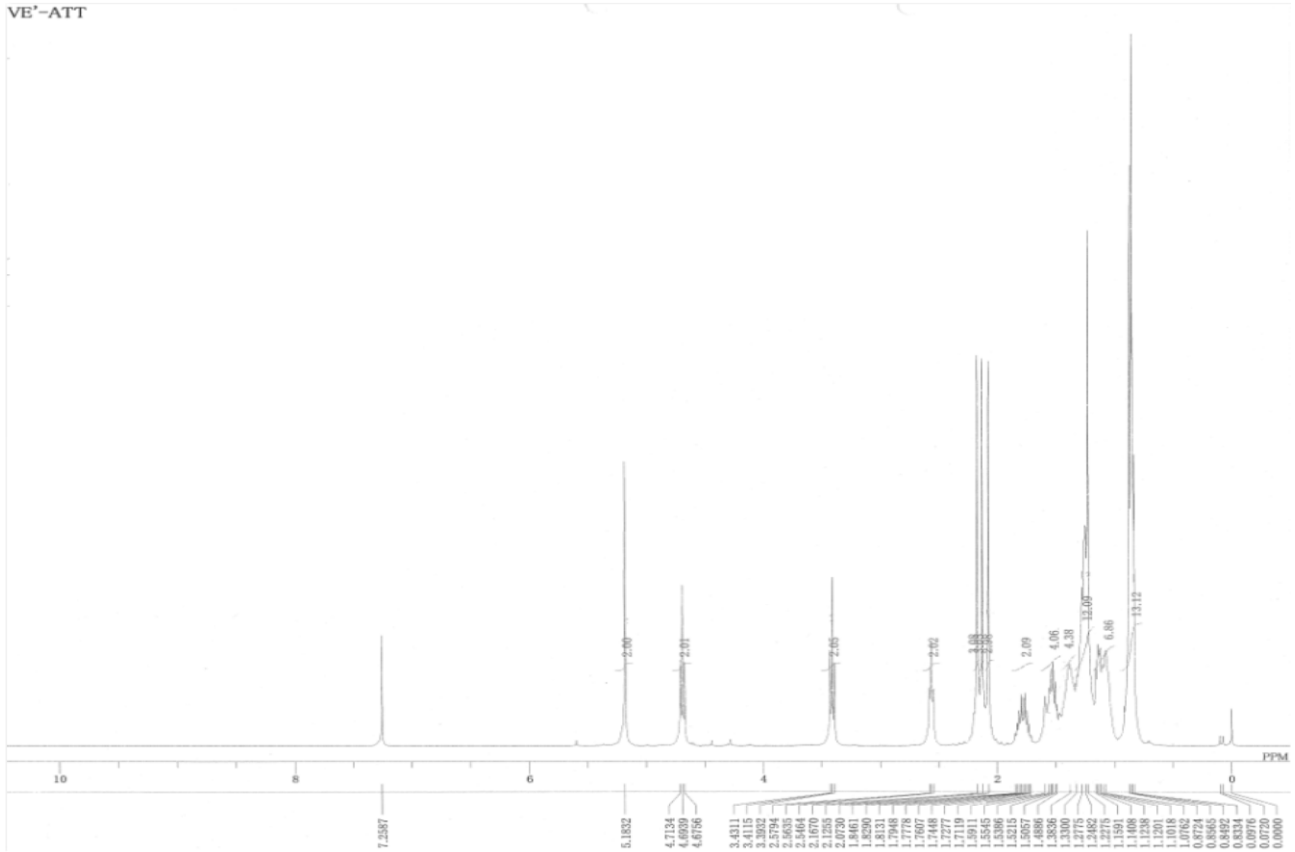
3. NMR spectra

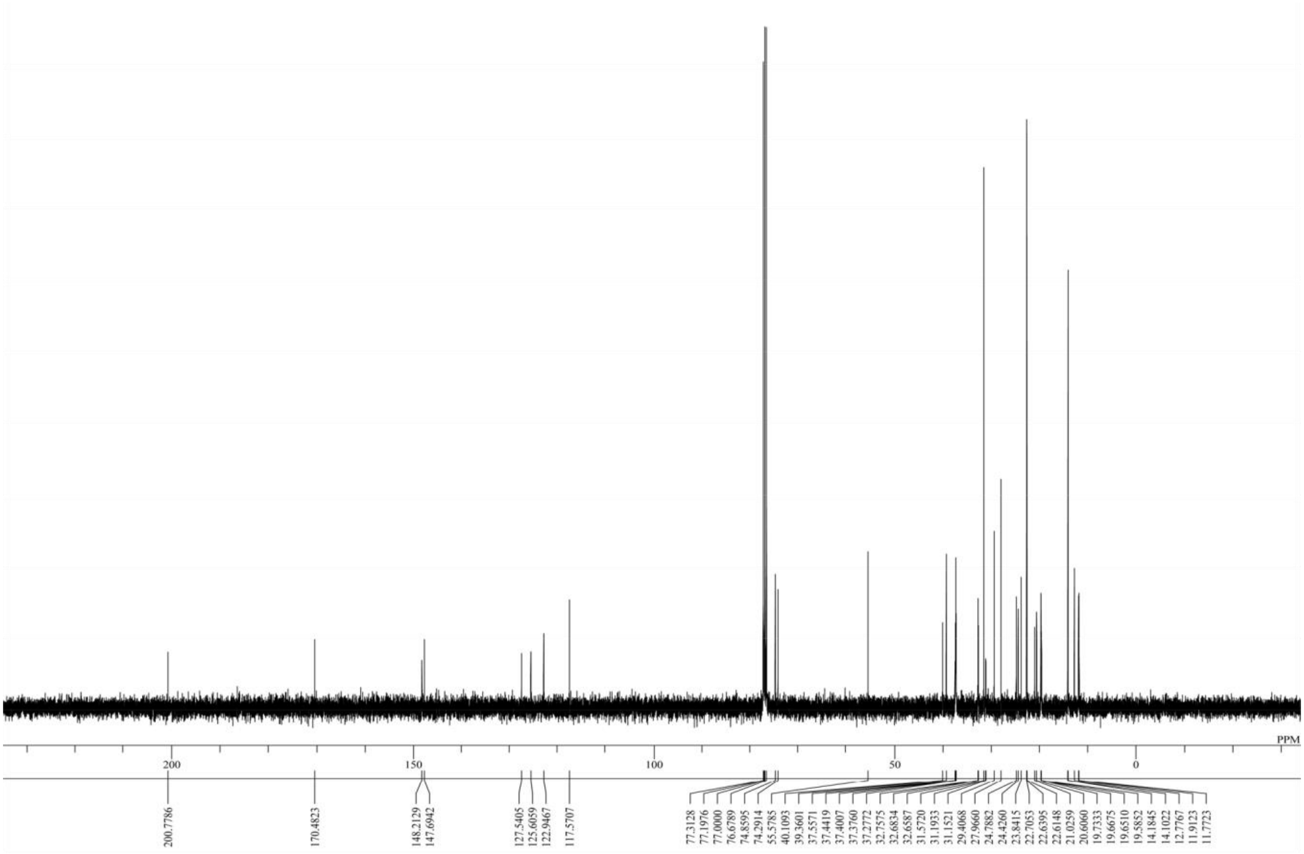
^{1}H NMR (compound **3**)



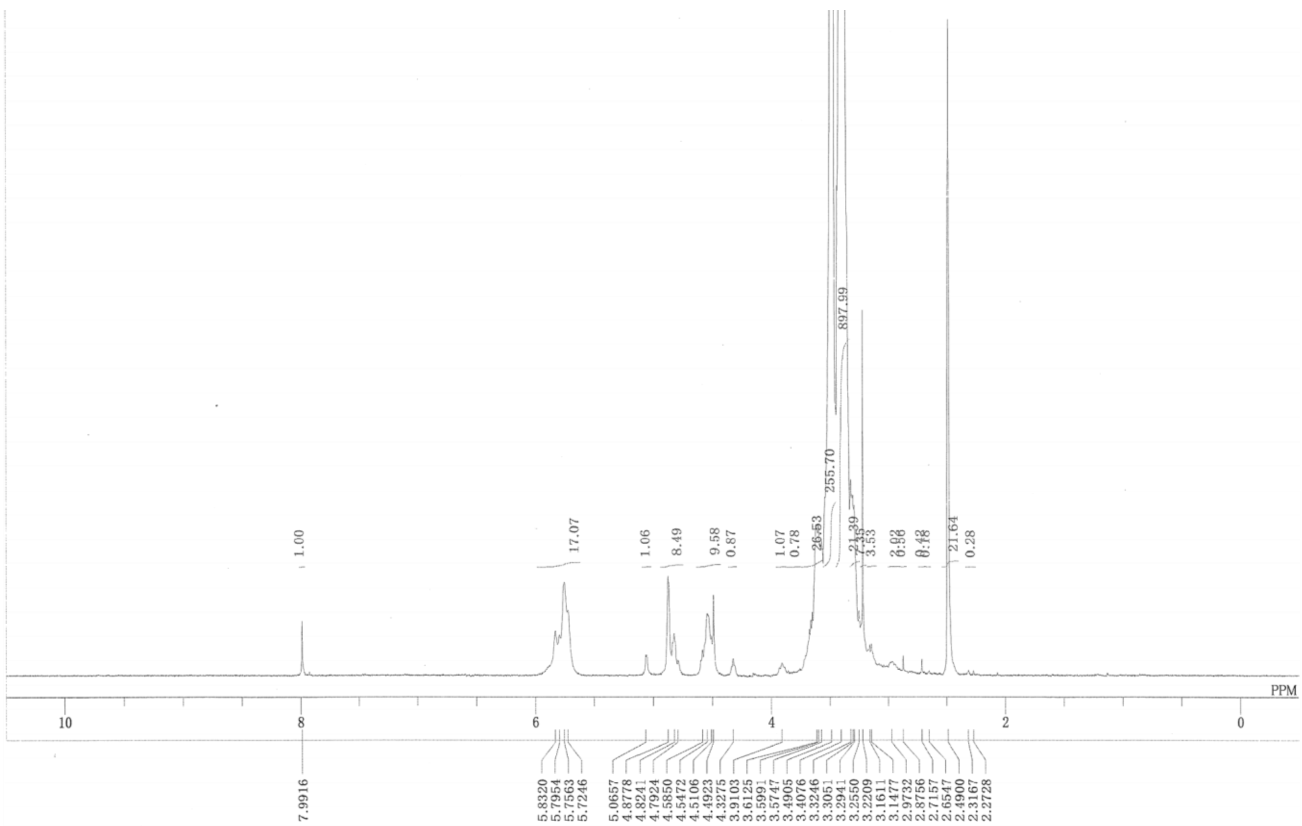
^{1}H NMR (compound **4b**)



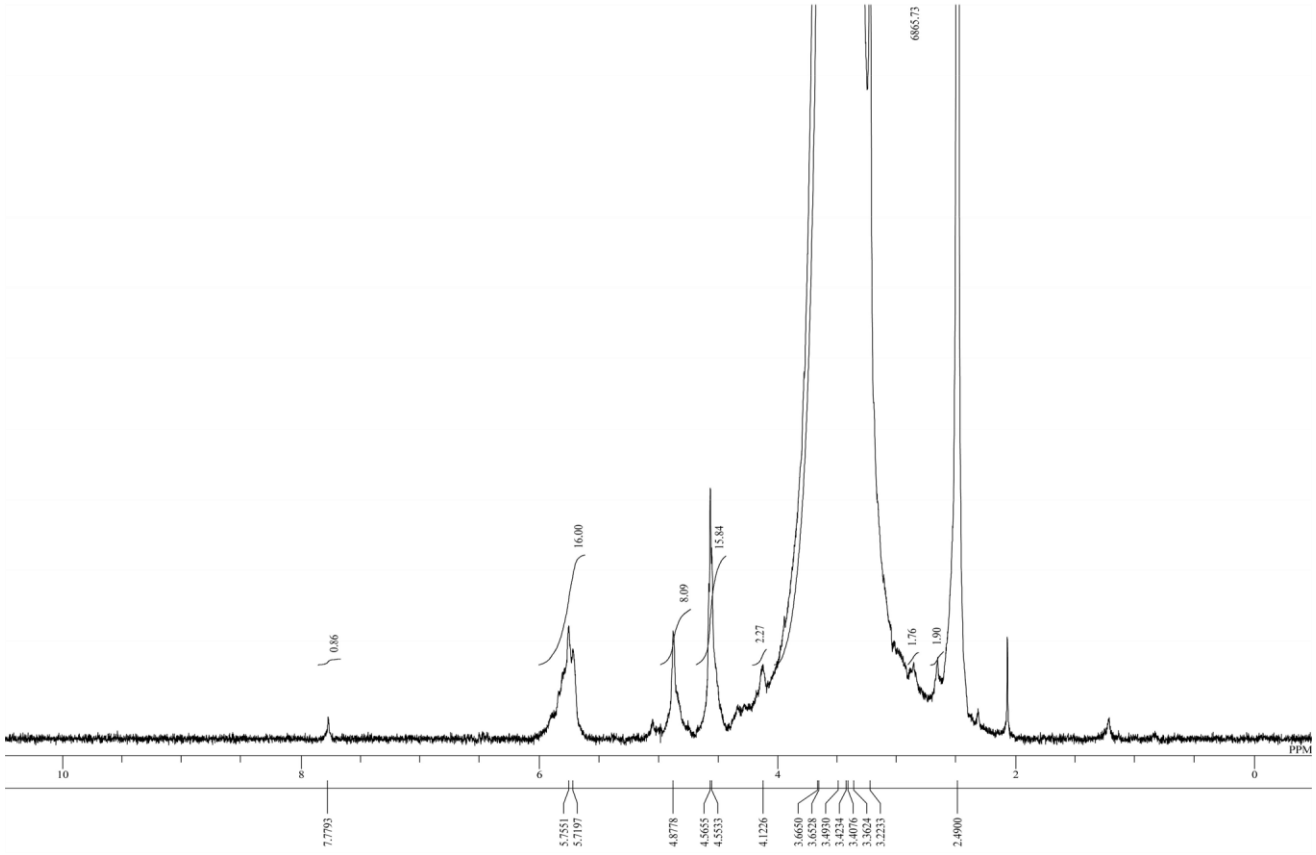
^{13}C NMR (compound **4b**)



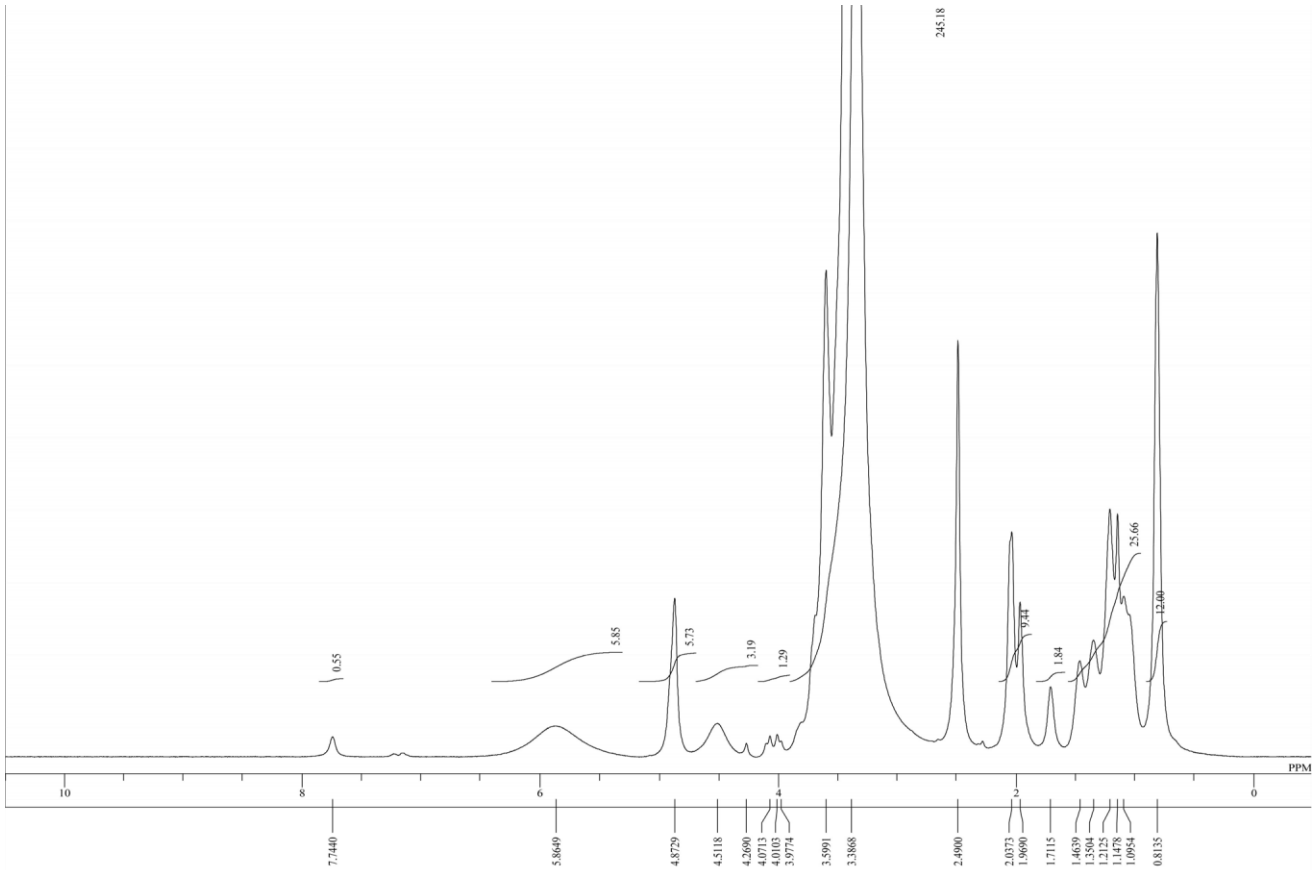
^{1}H NMR (compound **1-P2K**)



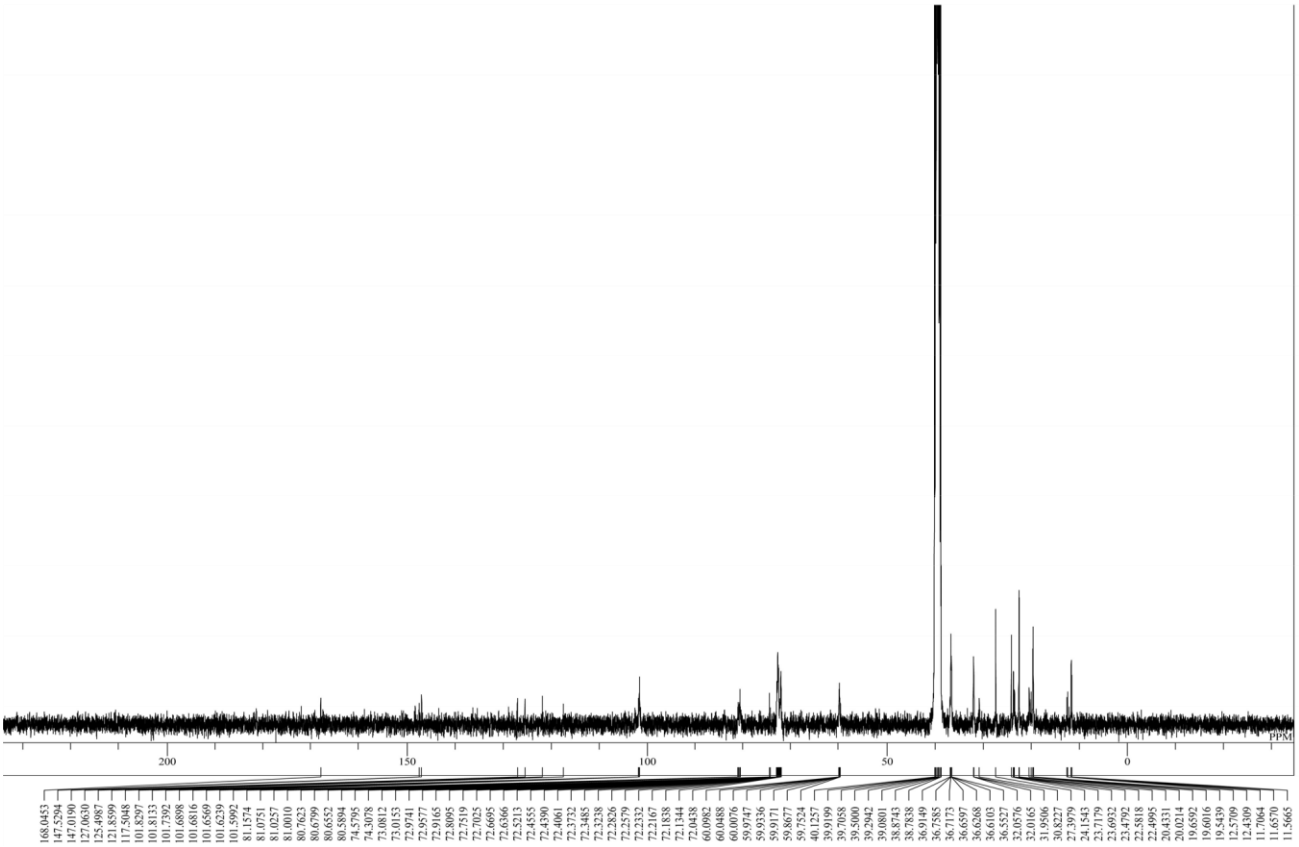
^{1}H NMR (compound **1-P5K**)



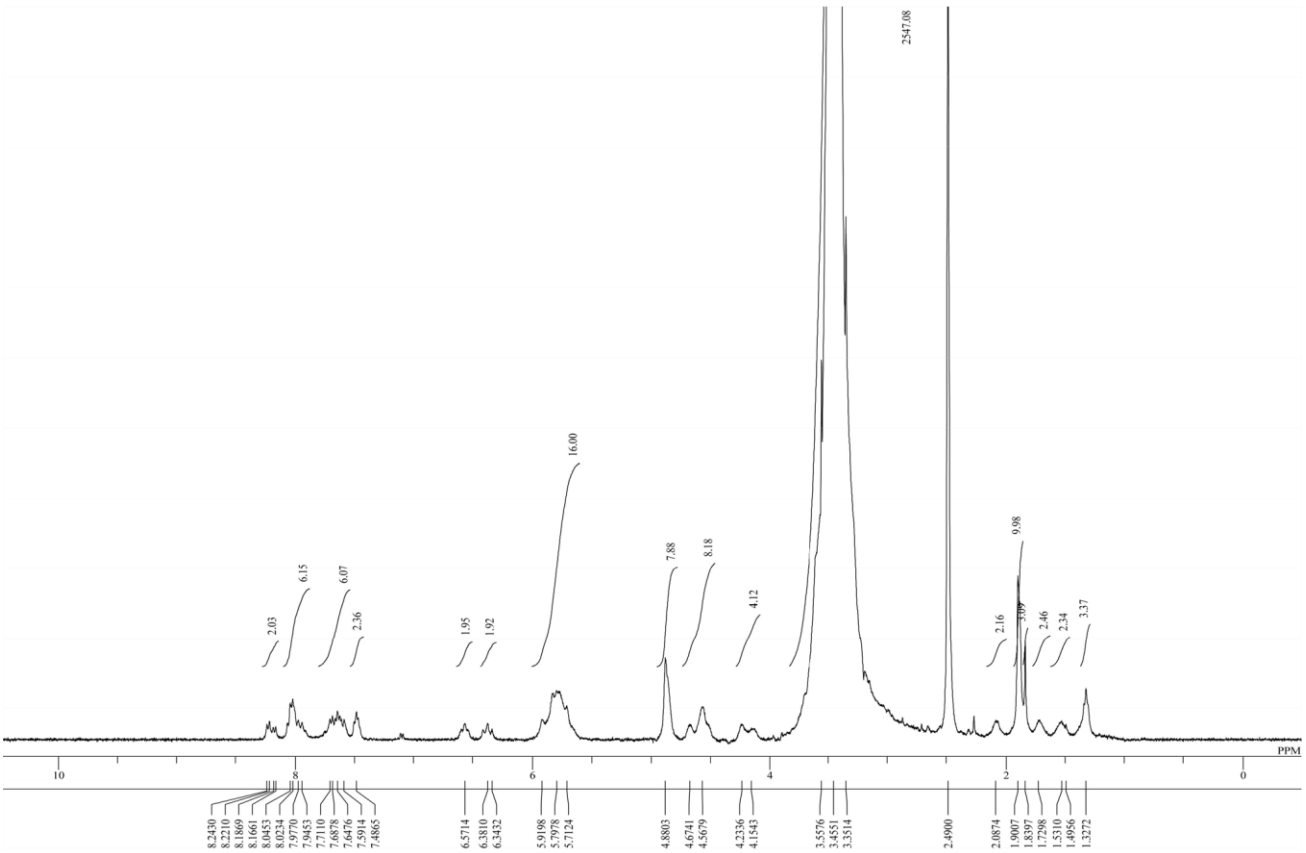
^{1}H NMR (compound **1-VE**)



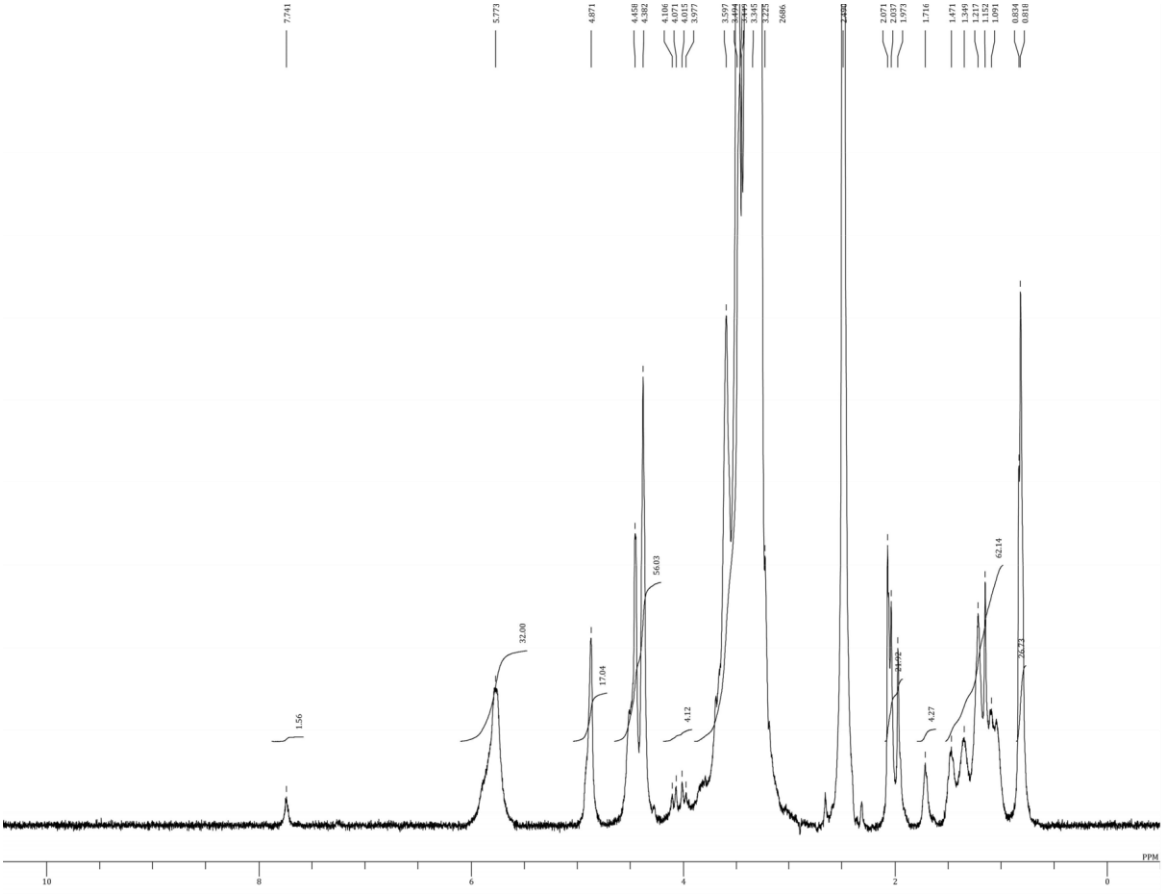
^{13}C NMR (compound **1-VE**)



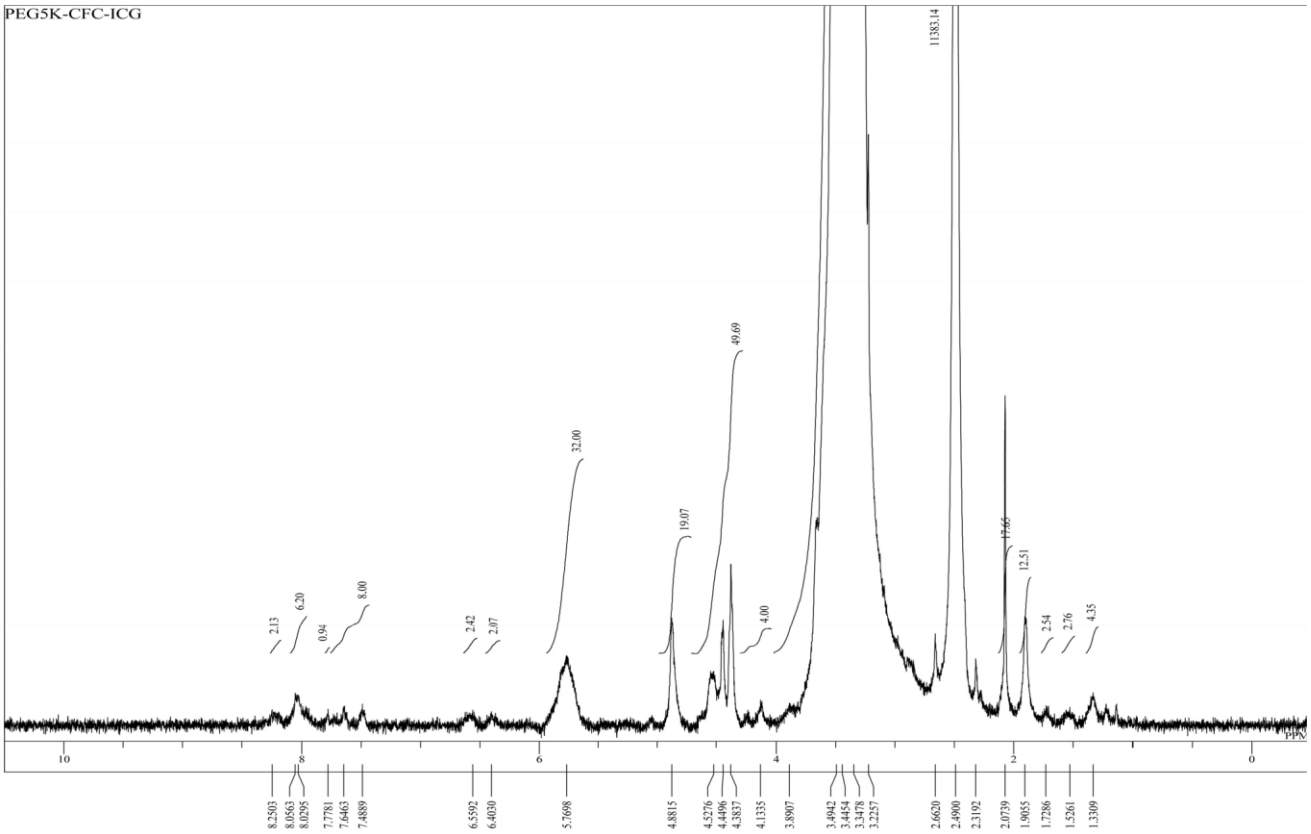
^{1}H NMR (compound **1-ICG**)



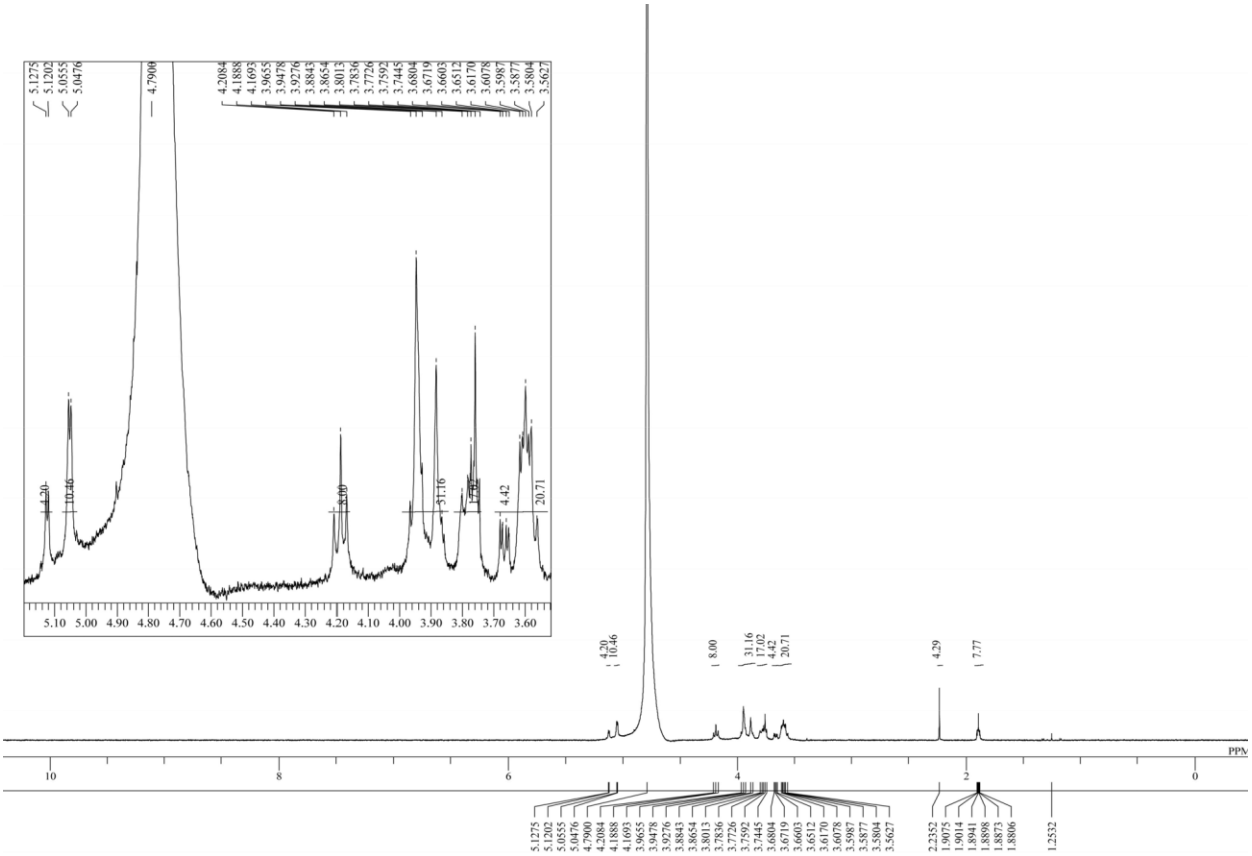
^{1}H NMR (compound **2A-VE-P5K**)



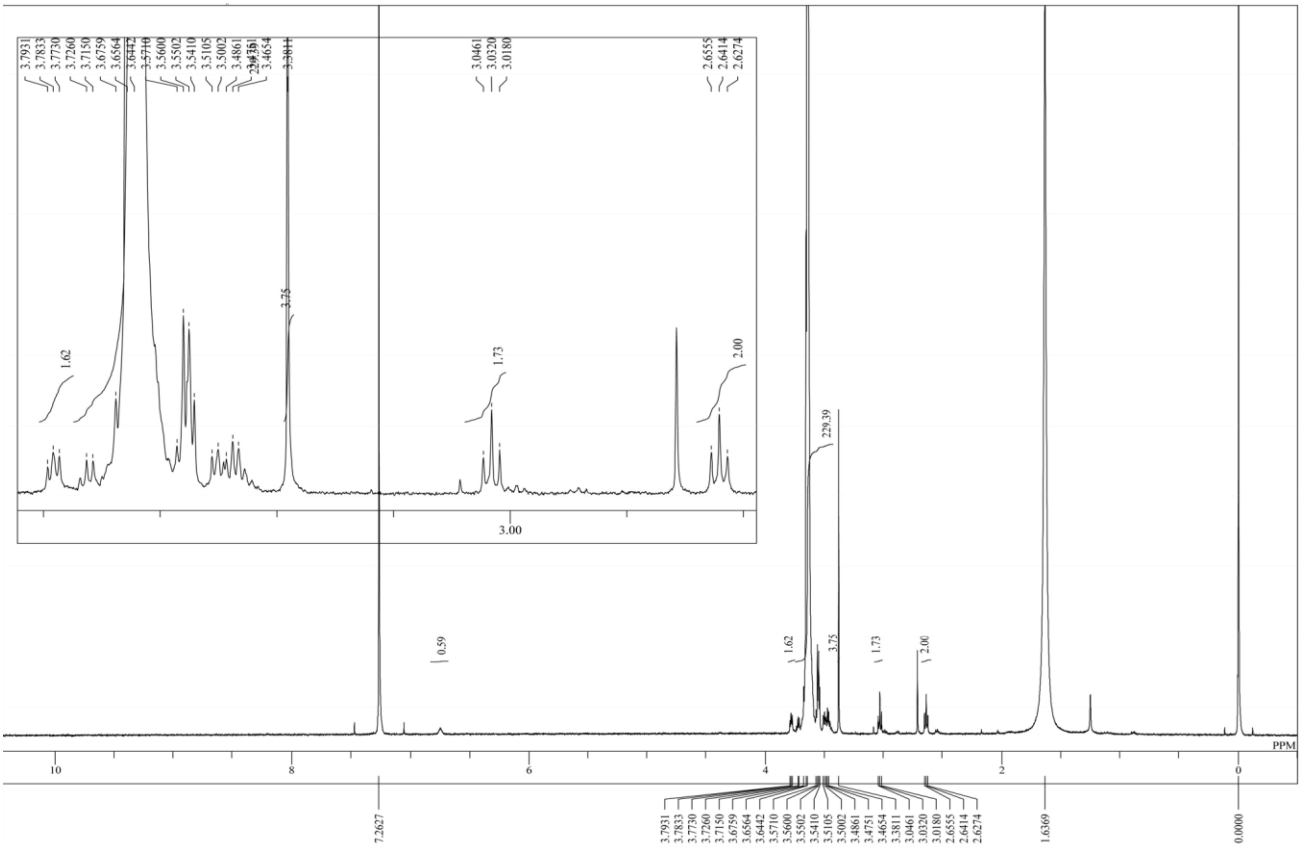
^{1}H NMR (compound **2A-ICG-P5K**)



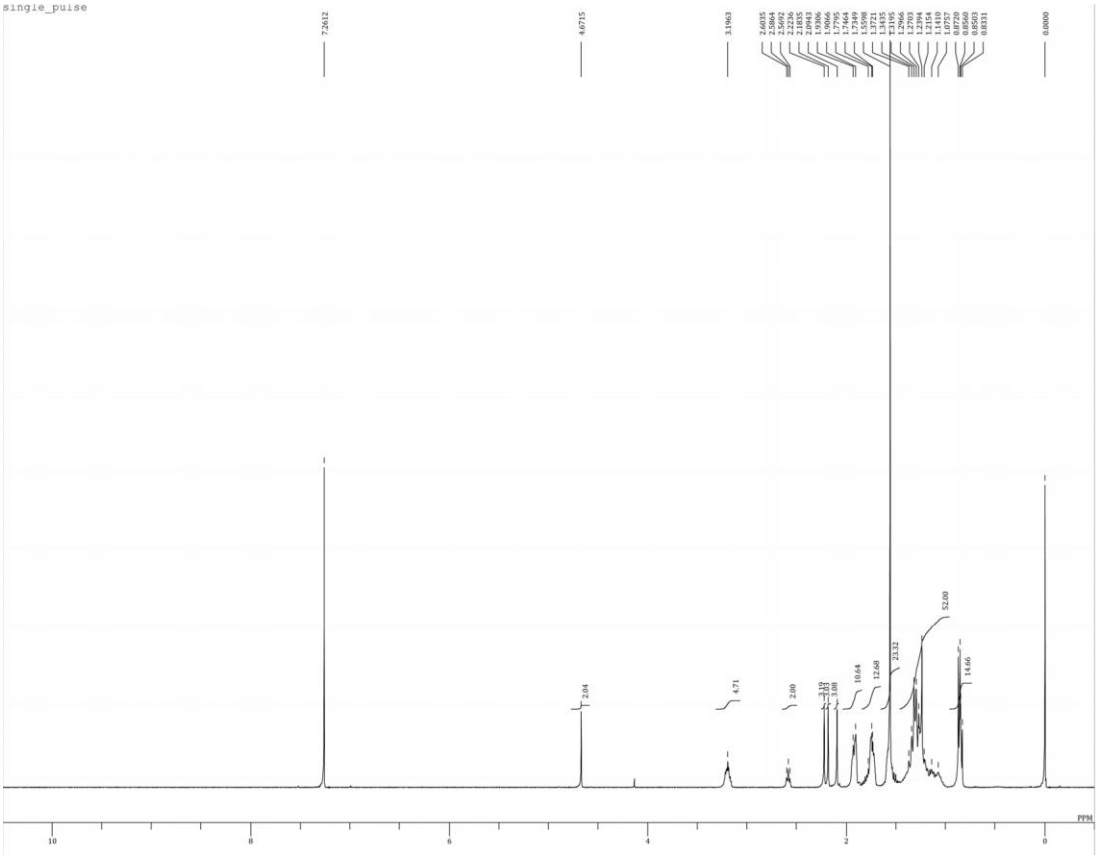
^{1}H NMR (compound $\mathbf{C_{NH2}FC_{NH2}}$)



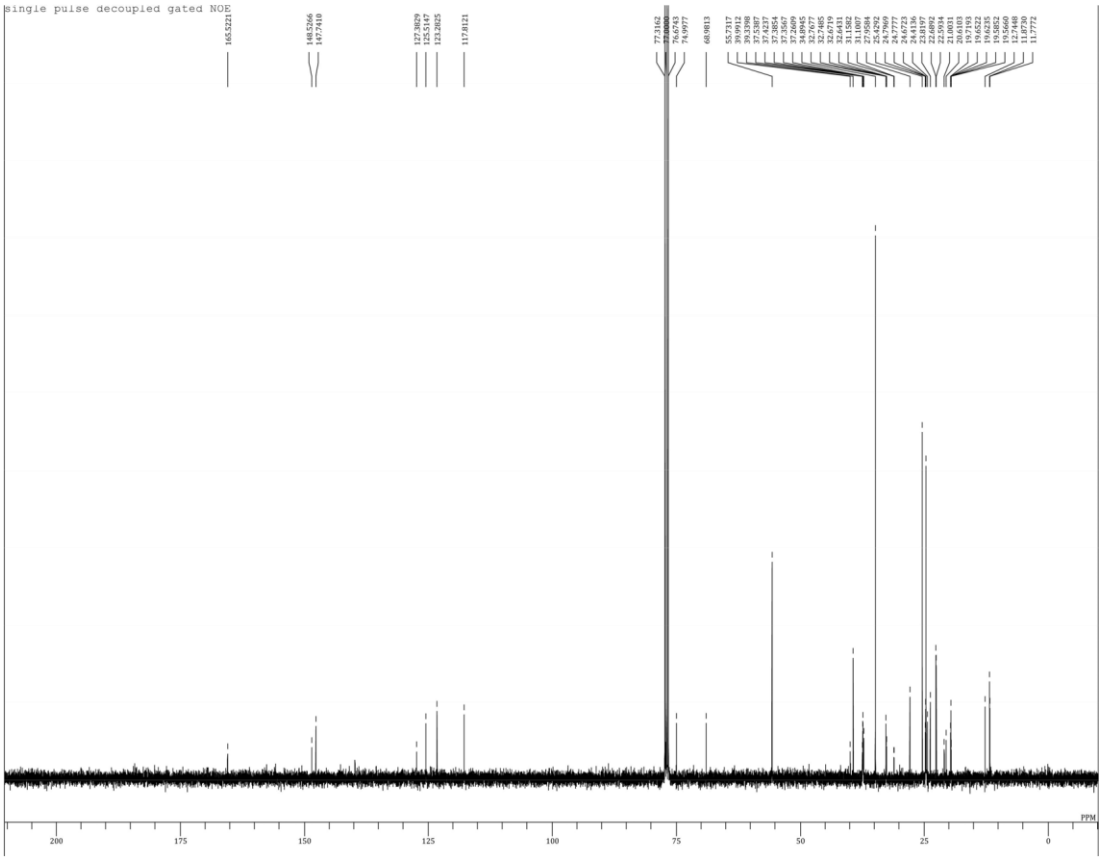
^{1}H NMR (compound **P2K-PFP**)



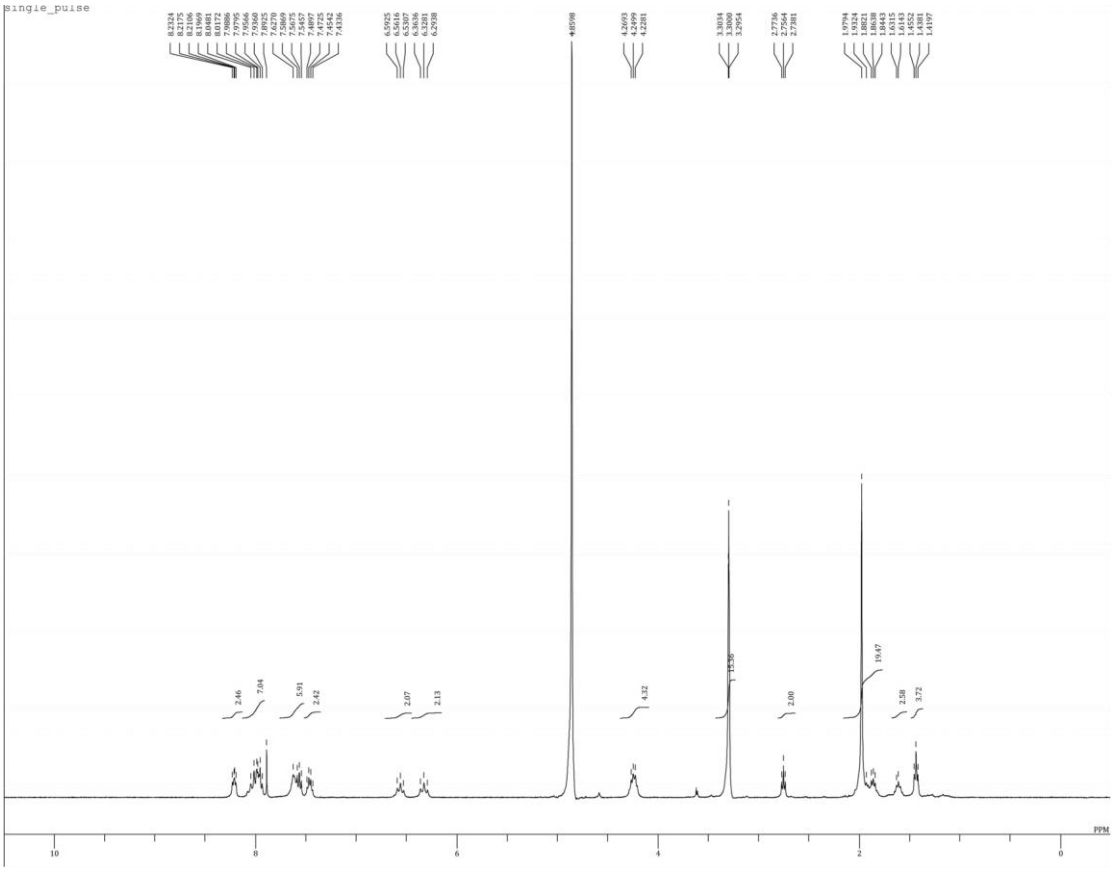
^{1}H NMR (compound **VE-PFP**)



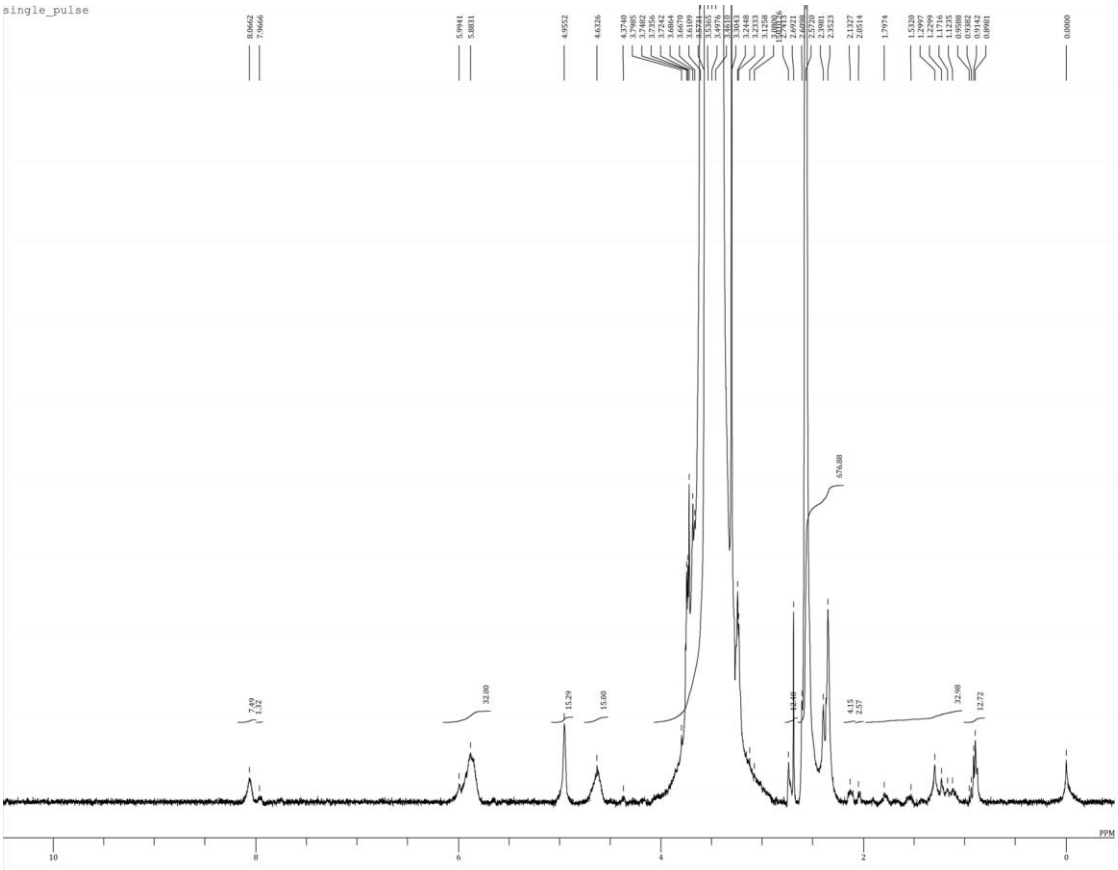
^{13}C NMR (compound **VE-PFP**)



^{1}H NMR (compound **ICG-PFP**)



^{1}H NMR (compound **2B-VE-P2K**)



^{1}H NMR (compound **2B-P2K**)