Supplementary Information

Coordinative Amphiphiles as Tunable siRNA Transporters

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1. Materials and Methods

All reagents were purchased from Sigma–Aldrich, TCI, Across, Alfa Aesar, and Combi-blocks. Column chromatography was performed on silica gel 60 (Merck, 40–63 μm) or basic alumina (Alfa Aesar, Brockmann Grade I, 58 Å) with appropriate air pressure. ^{1}H and ^{13}C NMR spectra were recorded using Bruker 400 MHz and 300 MHz spectrometers (Bruker Corporation, Billerica, MA); the spectra are reported as chemical shifts (δ) in ppm relative to the solvent standard. For ^{1}H NMR spectra, the multiplicities are reported as singlets (s), doublets (d), triplets (t), quartets (q), quintets (quint), doublets of doublets (dd), and triplets of doublets (td) with coupling constants (J) in Hz, or multiplets (m), or broad peaks (br). For 13C NMR spectra, the peaks were assigned for quaternary (s), tertiary (d), secondary (t), and primary (q) carbons. The exact masses of the compounds were measured using a high-resolution mass spectrometer (HRMS, ESI) with a Waters®3100 mass detector (Waters, Milford, MA) at the Korea Institute of Science and Technology (Rep. of Korea) or using a JEOL-JMS700 high-resolution mass spectrometer (JEOL, Japan) (HRMS, FAB) at the Korea Basic Science Institute, Daegu Center (Daegu, Rep. of Korea). Sizes and zeta potentials were measured using a Zatasizer Nano ZS apparatus (Malvern, UK). Cryo-TEM images were recorded using a JEOL-JEM-3011 HR apparatus (JEOL, Japan) operated at 300 kV. TEM images were recorded using a JEM-1400 apparatus (JEOL, Japan) operated at 120 kV.

Abbreviations. Calcd.: Calculated; DCM: Dichloromethane; DIEA: *N,N*-Diisopropylethylamine; DMF: *N,N*-Dimethylformamide; DMSO: *N,N*-Dimethylsulfoxide; DPA: Dipicolylamine; dsRNA: Double-stranded RNA; cryo-TEM: Cryogenic transmission electron microscopy; DW: Deionized water; EDC: *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride; ESI: Electron spray ionization; Et_2O: Diethyl ether; EtOAc: Ethyl acetate; EtOH: Ethanol; FAB: Fast atom bombardment; HATU: 2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Hex: *n*-Hexane; HRMS: High-resolution mass spectrometry; MβCD: methyl-β-cyclodextrin; MeOD: Methanol-d_4; MeOH: Methanol; r.t.: Room temperature; scRNA: scrambled siRNA; TEA: Triethylamine.

2. Synthesis

General Procedure A—Deprotection of Phthalimide. Hydrazine hydrate (3 eq.) was added to a solution of a phthalimide (0.08 M) in DCM and EtOH (1:9, v/v). After heating under reflux for 1 h, the mixture was concentrated under reduced pressure. The residue was dissolved in DCM (0.32 M) and filtered. The filtrate was evaporated and the resulting brown oily compound was used directly in the coupling reaction without further purification.

General Procedure B—Complexation with Zinc Nitrate. Zinc nitrate hexahydrate (1.0 equiv) was added to a solution of a DPA compound in MeOH (5.0 mL). The solvent was evaporated after 30 min. The resulting solid was used without further purification.

Compound 1 was synthesized following the procedure in ref. S1.

Compound 2a. Following general procedure A, a deprotected compound was obtained from **1** (155 mg, 0.39 mmol) and dissolved in DCM (10 mL). Oleic acid (140 µL, 0.43 mmol) and TEA (82 µL, 0.59 mmol) were added. After the mixture had become clear, HATU (226 mg, 0.59 mmol) was added and the mixture was stirred at r.t. for 16 h. The mixture was washed sequentially with 0.5 M $HCl_{(aq.)}$ (2 × 10 mL), saturated $NaHCO_{3(aq.)}$ (2 × 10 mL), and water (10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The crude compound was purified through column chromatography (basic alumina; EtOAc only) to obtain compound **2a** (105 mg, 42%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) 8.51 (d, *J* = 4 Hz, 2H), 7.66 (t, *J* = 7.6 Hz, 2H), 7.60–7.58 (m, 2H), 7.21 (t, *J* = 8 Hz, 1H), 7.13 (t, *J* = 6 Hz, 2H), 7.00–6.96 (m, 2H), 6.75 (d, *J* = 8 Hz, 1H), 5.80 (t, *J* = 5.5 Hz, 1H), 5.35–5.31 (m, 2H), 3.97 (t, *J* = 6 Hz, 2H), 3.81 (s, 4H), 3.66 (s, 2H), 3.32 (q, *J* = 6.4 Hz, 2H), 2.13 (t, *J* = 7.6 Hz, 2H), 2.00–1.99 (m, 4H), 1.81 (t, *J* = 6.8 Hz, 2H), 1.69 (t, *J* = 7.2 Hz, 2H), 1.59–1.64 (m, 2H), 1.28–1.26 (m, 20H), 0.87 (t, *J* = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.1 (s), 159.7 (s), 158.9 (s), 148.9 (d), 140.5 (s), 136.4 (d), 129.9 (d), 129.7 (d), 129.3 (d), 122.7 (d), 121.9 (d), 121.2 (d), 115.0 (d), 112.9 (d), 67.3 (t), 59.9 (t), 58.4 (t), 39.0 (t), 36.8 (t), 31.8 (t), 29.8 (t), 29.7 (t), 29.5 (t), 29.3 (t), 29.2 (t), 29.1 (t), 27.2 (t), 27.1 (t), 26.6 (t), 26.4 (t), 25.8 (t), 22.6 (t), 14.1 (q); HRMS (ESI$^+$) calcd. for $[M + Na]^+$ $C_{41}H_{60}N_4O_2Na^+$: *m*/*z* 663.4608, found: 663.4651.

Compound 2b. Following general procedure A, a deprotected compound was obtained from **1** (155 mg, 0.39 mmol) and dissolved in DCM (10 mL). 1-Pyrene butyric acid (123 mg, 0.43 mmol) and TEA (83 µL, 0.59 mmol) were added. After the mixture had become clear, HATU (224 mg, 0.59 mmol) was added and the mixture then stirred at r.t. for 16 h. The mixture was washed sequentially with 0.5 M $HCl_{(aq.)}$ (2 × 10 mL), saturated $NaHCO_{3(aq.)}$ (2 × 10 mL), and water (10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The crude compound was purified through column chromatography (basic alumina; EtOAc only; R_f = 0.45) to obtain compound **2b** (159 mg, 63%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) 8.46 (d, *J* = 4.2 Hz, 2H), 8.23–8.20 (m, 1H), 8.11–8.08 (m, 2H), 8.07–8.00 (m, 2H), 7.95–7.92 (m, 3H), 7.78–7.76 (m, 1H), 7.57–7.51 (m, 4H), 7.17 (t, *J* = 7.8 Hz, 1H), 7.06–7.02 (m, 2H), 6.96–6.92 (m, 2H), 6.69 (dd, *J* = 8.1, 1.5 Hz, 1H), 5.76 (t, *J* = 6.0 Hz, 1H), 3.88 (t, *J* = 6.0 Hz, 2H), 3.76 (s, 4H), 3.61 (s, 2H), 3.32–3.23 (m, 4H), 2.17–2.12 (m, 4H), 1.74–1.68 (m, 2H), 1.63–1.56 (m, 2H); ^{13}C NMR (75

MHz, $CDCl_3$) 172.6 (s), 159.7 (s), 158.9 (s), 148.9 (d), 140.6 (s), 136.4 (d), 135.9 (s), 131.4 (s), 130.9 (s), 129.9 (s), 129.3 (d), 128.7 (s), 127.4 (d), 127.3 (d), 126.7 (d), 125.8 (d), 125.0 (s), 124. 9(s), 124.9 (d), 124.7 (d), 123.3 (d), 122.7 (d), 121.9 (d), 121.2 (d), 115.1 (d), 113.0 (d), 67.3 (t), 60.0 (t), 58.4 (t), 39.1 (t), 36.0 (t), 32.7 (t), 27.4 (t), 26.7 (t), 26.4 (t); HRMS (ESI^+) calcd. for $[M + Na]^+$ $C_{43}H_{42}N_4O_2Na^+$: *m*/*z* 669.3205, found: 669.3231.

Compound 2c. Following general procedure A, a deprotected compound was obtained from **1** (155 mg, 0.39 mmol) and dissolved in DCM (10 mL). D-Biotin (94 mg, 0.38 mmol) and TEA (70 µL, 0.52 mmol) were added. After the mixture had become clear, HATU (200 mg, 0.52 mmol) was added and the mixture was stirred at r.t. for 16 h. The mixture was washed sequentially with 0.5 M $HCl_{(aq.)}$ (2 × 10 mL), saturated $NaHCO_{3(aq.)}$ (2 × 10 mL), and water (10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The crude compound was purified through column chromatography (basic alumina; DCM/MeOH, 13:1; R_f = 0.2) to obtain compound **2c** (52 mg, 25%) as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$) 8.48 (d, *J* = 4.8 Hz, 2H), 7.64 (td, *J* = 7.6, 1.6 Hz, 2H), 7.56–7.54 (m, 2H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 6.2 Hz, 2H), 6.97–6.92 (m, 2H), 6.72 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.37 (s, 1H), 6.24 (t, *J* = 5.5 Hz, 1H), 5.50 (s, 1H), 4.44–4.39 (m, 1H), 4.26–4.22 (m, 1H), 3.94 (t, *J* = 6.1 Hz, 2H), 3.77 (s, 4H), 3.63 (s, 2H), 3.27 (q, *J* = 6.4 Hz, 2H), 2.85–2.79 (m, 1H), 2.85–2.79 (m, 1H), 2.67–2.62 (m, 1H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.80–1.73 (m, 2H), 1.67–1.61 (m, 6H), 1.42–1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.1 (s), 163.8 (s), 159.6 (s), 158.9 (s), 148.9 (d), 140.5 (s), 136.4 (d), 129.3 (d), 122.8 (d), 122.0 (d), 121.2 (d), 115.1 (d), 113.0 (d), 67.4 (t), 61.7 (d), 60.1 (d), 59.9 (t), 58.4 (t), 55.5 (d), 40.5 (t), 39.1 (t), 36.0 (t), 28.1 (t), 28.0 (t), 26.7 (t), 26.3 (t), 25.6 (t); HRMS (ESI^+) calcd. for $[M + Na]^+$ $C_{33}H_{42}N_6O_3SNa^+$: *m*/*z* 625.2937, found: 625.2937.

Compound CA1. According to general procedure B, the reaction of **2a** (41 mg, 0.064 mmol) gave **CA1** as a white solid (50 mg, 94%). ^1H NMR (300 MHz, $CDCl_3$) 8.83 (d, *J* = 5.1 Hz, 2H), 8.03 (t, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 6 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.27 (t, *J* = 6.6 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 6.64–6.61 (m, 2H), 6.03 (t, *J* = 6.3 Hz, 1H), 5.37–5.33 (m, 2H), 4.36 (d, *J* = 20.8 Hz, 2H), 4.02–3.96 (m, 4H), 3.66 (d, 2H), 3.37–3.35 (m, 2H), 2.21 (t, *J* = 7.2 Hz, 2H), 2.18–2.02 (m, 4H), 1.84–1.82 (m, 2H), 1.75–1.63 (m, 4H), 1.30–1.28 (m, 21H), 0.89 (t, *J* = 6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 176.7 (s), 159.3 (s), 154.1 (s), 149.1 (d), 140.9 (s), 132.1 (d), 130.0 (d), 129.7 (d), 125.2 (d), 124.2 (d), 123.4 (d), 117.7 (d), 114.6 (d), 67.5 (t), 55.4 (t), 55.0 (t), 39.0 (t), 36.7 (t), 31.8 (t), 29.7 (t), 29.7 (t), 29.5 (t), 29.3 (t), 29.2 (t), 29.1 (t), 27.2 (t), 27.1 (t), 26.4 (t), 26.3 (t), 25.8 (t), 22.6 (q); HRMS(ESI^+) calcd. for $[M + NO_3]^+$ $C_{41}H_{60}N_5O_5Zn^+$: *m*/*z* 766.3880, found: 766.3901.

Compound CA2. According to general procedure B, the reaction of **2b** (11 mg, 0.017 mmol) gave **CA2** (12 mg, 96%). ^1H NMR (300 MHz, $CDCl_3$) 8.82 (d, *J* = 4.8 Hz, 2H), 8.31–8.28 (m, 1H), 8.18–8.07 (m, 4H), 8.04–8.02 (m, 3H), 7.90–7.85 (m, 3H), 7.44 (t, *J* = 6.3 Hz, 2H), 7.34–7.24 (m, 3H), 6.89–6.87 (m, 1H), 6.56–6.54 (m, 2H), 5.83 (t, *J* = 5.4 Hz 1H), 4.26–4.20 (m, 2H), 3.96 (t, *J* = 6 Hz, 2H), 3.91–3.86 (m, 2H), 3.58 (s, 2H), 3.42–3.34 (m, 4H), 2.36–2.32 (m, 2H), 2.27–2.20 (m, 2H), 1.86–1.80 (m, 2H), 1.76–1.69 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 172.9 (s), 159.3 (s), 153.8 (s), 149.1 (d), 140.6 (s), 135.9 (d), 132.0 (s), 131.4

(s), 130.8 (s), 129.9 (s), 129.8 (d), 128.7 (s), 127.4 (d), 127.3 (d), 127.3 (d), 126.7 (d), 125.9 (s), 125.1 (s), 124.9 (d), 124.7 (d), 123.9 (d), 123.4 (d), 123.3 (d), 117.9 (d), 114.4 (d), 67.4 (t), 55.3 (t), 54.8 (t), 53.4 (t), 38.9 (t), 36.1 (t), 32.7 (t), 27.6 (t), 26.4 (t); HRMS (ESI$^+$) calcd. for $[M - H]^+$ $C_{43}H_{41}N_4O_2Zn^+$: *m*/*z* 709.2510, found: 709.2526; calcd. for $[M + NO_3]^+$ $C_{43}H_{42}N_5O_5Zn^{2+}$ *m*/*z*. 772.2472, found: 772.2485.

Compound CA3. According to general procedure B, the reaction of **2c** (40 mg, 0.066 mmol) gave **CA3** (38 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.66 (s, 2H), 8.06 (t, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 6.0 Hz, 2H), 7.54–7.52 (m, 2H), 7.20 (t, *J* = 7.7 Hz, 1H), 6.81 (dd, *J* = 1.8, 8.2 Hz, 1H), 6.76–6.71 (m, 2H), 4.45–4.42 (m, 1H), 4.31–4.25 (m, 3H), 3.93–3.87 (m, 4H), 3.71 (s, 2H), 3.19 (t, *J* = 6.8 Hz, 2H), 3.15–3.10 (m, 1H), 2.84–2.80 (m, 1H), 2.60–2.57 (m, 1H), 2.12 (t, *J* = 7.2 Hz, 2H), 1.74–1.71 (m, 2H), 1.64–1.61 (m, 4H), 1.55–1.53 (m, 2H), 1.34–1.33 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 174.6 (s), 164.8 (s), 159.6 (s), 155.0 (s), 148.2 (d), 141.4 (s), 132.6 (d), 129.6 (d), 125.0 (d), 124.9 (d), 123.4 (d), 117.2 (d), 114.8 (d), 67.2 (t), 62.1 (d), 60.4 (d), 56.4 (t), 55.6 (s), 39.6 (t), 38.5 (t), 35.4 (t), 28.2 (t), 28.0 (t), 26.2 (t), 25.6 (t), 25.5 (t); HRMS (ESI$^+$) calcd. for $[M - H]^+$ $C_{33}H_{41}N_6O_3SZn^+$: *m*/*z* 665.2241, found: 665.2263.

Compound 5 was prepared using a procedure adopted from ref. S2. To a solution of 2-(Chloromethyl)pyridine hydrochloride (673 mg, 4.10 mmol) and benzyl amine (200 mg, 1.86 mmol) in 4 mL of water/acetonitrile (1 : 1, v/v) at 70 °C, 2 mL of aqueous NaOH (327 mg, 8.18 mmol) was added dropwisely for 30 min. The reaction mixture was stirred for additional 2 h and cooled to the ambient temperature. The mixture was extracted with DCM (2 × 10 mL) and the combined organic phase was dried (Na_2SO_4) and concentrated. The resulting brown oil (219 mg, 44%) was a mixture of compound **5** and *N*-benzyl-2-picolylamine (76 : 24, estimated by 1H NMR). The crude mixture was applied directly in the next step without further purification.

ZnBnDPA. According to general procedure B, the reaction of crude compound **5** (110 mg, ca. 0.38 mmol) gave a white precipitate, which was filtered off and washed with Et_2O to yield pure **ZnBnDPA** (106 mg, ca. 62%). ^{1}H NMR (300 MHz, DMSO) 8.67 (d, *J* = 6.4 Hz, 2H), 8.11 (t, *J* = 10.2 Hz, 2H), 7.67–7.62 (m, 4H), 7.50–7.48 (m, 3H), 7.39–7.37 (m, 2H), 4.27 (d, *J* = 16.0 Hz, 2H), 3.75 (s, 2H), 3.71 (d, *J* = 16.0 Hz, 2H); ^{13}C NMR (75 MHz, DMSO) 154.6 (s), 148.3 (d), 141.2 (d), 132.1 (s), 132.0 (d), 129.1 (d), 125.3 (d), 125.2 (d), 57.0 (t), 55.8 (t); HRMS (FAB$^+$) calcd. for $[M + NO_3]^+$ $C_{19}H_{19}N_4O_3Zn^+$: *m*/*z* 415.0746, found: 415.0749.

ZnDPA. According to general procedure B, the reaction of dipicolylamine (100 mg, 0.50 mmol) gave a white precipitate, which was filtered off and washed with Et_2O to yield pure **ZnDPA** (126 mg, 70%). ^{1}H NMR (400 MHz, DMSO) 8.56 (d, *J* = 4.0 Hz, 2H), 7.98 (td, *J* = 7.6, 1.2 Hz, 2H), 7.51–7.49 (m, 4H), 5.15 (s, 1H), 4.45 (dd, *J* = 16.6, 6.6 Hz, 2H), 3.92 (d, *J* = 16.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO) 155.8 (s), 147.3 (d), 140.3 (d), 124.6 (d), 124.1 (d), 52.7 (t); HRMS (FAB$^+$) calcd. for $[M + NO_3]^+$ $C_{12}H_{13}N_4O_3Zn^+$: *m*/*z* 325.0276, found: 325.0279.

Compound 7 was synthesized following the procedure in ref. S3. NMR spectra corresponded with those reported in the literature. ^{1}H NMR (300 MHz, DMSO) 7.20 (s, 1H), 6.70 (s, 1H), 6.07 (s, 1H), 3.87 (s, 2H); ^{13}C NMR (75 MHz, DMSO) 161.4 (s), 151.2 (s), 150.3 (s), 148.7 (s), 143.1 (s), 112.2 (d), 111.2 (d), 102.7 (d), 53.8 (t).

3. Phosphate Binding Assay

3.1. Binding Assay with Ligand 7. A solution (1 μL) of a compound in DMSO (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10 mM) or DMSO (as a control; 1 μL) was added to a well of a 96-well opaque plate (30296, SPL Lifesciences, Rep. of Korea). A solution of **7** (1 mM in DW), DW, and HEPES-NaOH buffer (10 mM, pH 7.4) (1:48:50) was freshly prepared; an aliquot (99 μL) of the mixture was added to each well. After mild shaking (400 rpm) at r.t. for 10 min, the fluorescence of **7** (λ_{ex}: 347 nm; λ_{em}: 480 nm) was measured using a Flexstation 3 apparatus (Molecular Devices, Sunnyvale, CA). All signals were averaged from duplicates and normalized to the value obtained when using DMSO only. In the presence of equimolar **CA2**, **CA3**, or **ZnBnDPA**, approximately 75–80% of the fluorescence intensity of **7** was quenched. One more equimolar charge of **CA1** and **ZnDPA** was needed to reach a quenching level of **7** (Figure S1A).

3.1. Binding Assay with Substrates. A solution (1 μL) of a compound in DMSO (1.0 mM for **CA1**, **CA2**, **CA3**, **ZnBnDPA**, and **ZnDPA**; 2.0 mM for **CA1** and **ZnDPA**) or DMSO (as a control; 1 μL) was added to a well of a 96-well opaque plate (30296, SPL Lifesciences, Rep. of Korea). The substrate solutions were prepared by serial dilution; an aliquot (48 μL) of each solution or DW (48 μL) was added to each well. A solution of **7** (1 mM in DW) and HEPES-NaOH buffer (10 mM, pH 7.4) (1:50) was freshly prepared; an aliquot (51 μL) of this mixture was added to each well. 1:1 mixtures for **CA2**, **CA3**, and **ZnBnDPA** and 2:1 mixtures for **CA1** and **ZnDPA** were used to a quenching level similar to that of **7** at the beginning. The concentrations of siRNAs were 24, 12, 6.0, 3.0, 1.5, 0.75, 0.38, and 0.19 μM; the concentration of phosphate groups was obtained by multiplying the concentration of 21-mer siRNAs by 40. The final concentrations of 8-mer dsRNAs were 48, 24, 12, 6.0, 3.0, 1.5, 0.75, and 0.38 μM; in this case, the concentration of phosphate groups was calculated by multiplying the concentration of 8-mer dsRNAs by 14. The concentrations of 21-mer ssRNAs were 48, 24, 12, 6.0, 3.0, 1.5, 0.75, 0.38, 0.19, 0.094, 0.047, 0.023, 0.012, 0.0059, and 0.0029 μM; the concentration of phosphate groups was obtained by multiplying the concentration of 21-mer ssRNAs by 20. The final concentrations of UMP were 4.8, 2.4, 1.2, 0.60, 0.30, 0.15, 0.075, and 0.038 mM. After mild shaking (400 rpm) at r.t. for 10 min, the fluorescence of **7** (λ_{ex}: 347 nm; λ_{em}: 480 nm) was measured using a Flexstation 3 apparatus (Molecular Devices, Sunnyvale, CA). All signals were averaged from duplicates and normalized to the value obtained using DMSO only. The signals were plotted using following simplified equation (Eq. 1):

$F = F_o + F_{max}$ [S] / (K_d + [S]) (Eq. 1)

F = Fluorescence signal
F_o = Fluorescence signal in the absence of substrates
F_{max} = Maximum recovered fluorescence signal in the presence of substrates
[S] = Concentration of substrate (UMP, siRNA, dsRNA)
K_d = Dissociation constant
The dissociation constants, K_d, were obtained using Eq. 1 (Table 1).

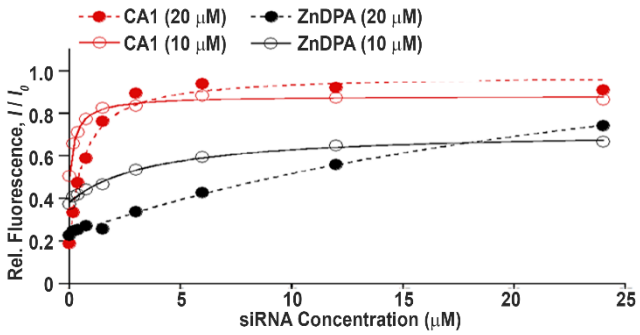

Figure S1. Binding curves for the interactions of siRNAs with **CA1** and **ZnDPA**. Comparison of 1:1 and 2:1 mixtures of Zn/DPA compounds and the ligand **7**. Although the fluorescence recoveries of 1:1 mixtures of **7** with **CA1** and **ZnDPA** were faster than those of their 2:1 mixtures, the saturated values were almost identical. I_o: Fluorescence intensity of **7** alone (10 μM).

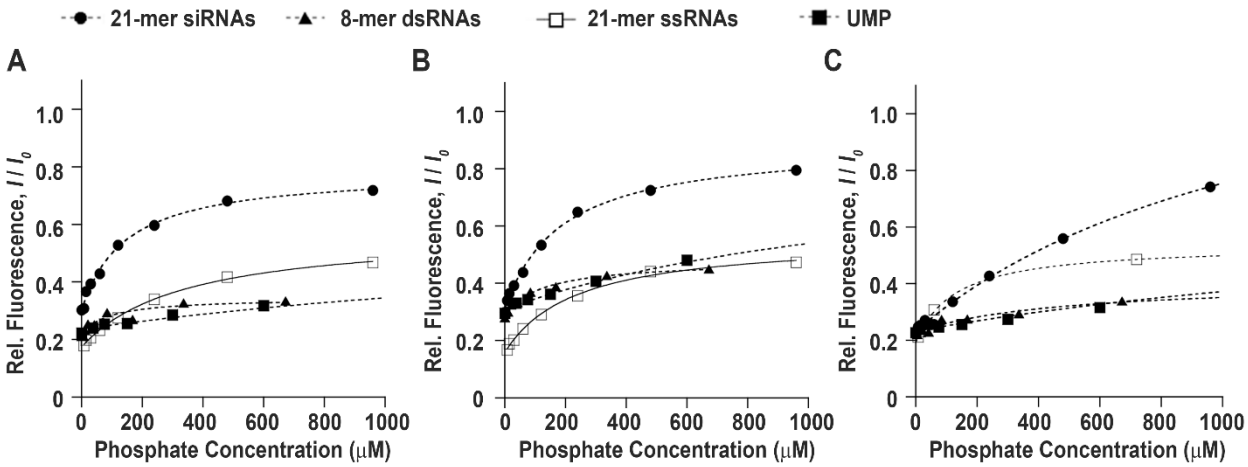

Figure S2. Binding curves for the interactions of the 21-mer siRNA, the 8-mer dsRNA, the 21-mer ssRNA, and UMP with (A) **CA3**, (B) **ZnBnDPA**, and (C) **ZnDPA**. I_o: Fluorescence intensity of **7** alone (10 μM).

4. Gel Electrophoresis

4.1. Gel Retardation Assay. Solutions of the compounds at appropriate concentrations (corresponding to the given molar ratios) in DMSO were freshly prepared. An aliquot (5 μL) of a compound solution and an aliquot (10 μL) of siRNA solution (5 μM in DEPC water, 50 pmol/well) were gently mixed with a pipette. The mixtures were incubated at ambient temperature for 10 min and then the complexes were applied to 1.0% agarose gel. Only with the **CA1** complex, which had the lowest dissociation constant, were some heavy particles detected when using fluorescent siRNAs (Figure S3); their patterns were similar to those formed from conventional cationic lipids.

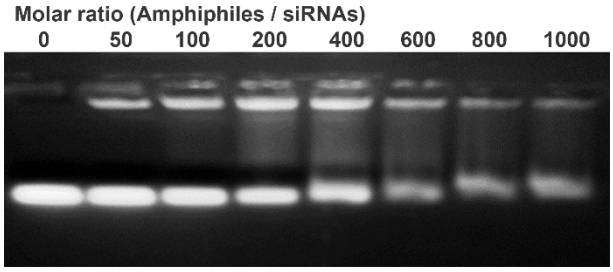

Figure S3. Gel retardation assay of **CA1** and fluorescent siRNAs

4.2. Enzymatic Stability Assay. Solutions of the compounds at appropriate concentrations (corresponding to the given molar ratios) in DMSO were freshly prepared. To an aliquot (10 μL) of a compound solution, siRNA solution (25 μM in DEPC water; 4 μL), DW (6 μL), and Tris-HCl buffer (10 mM, pH 7.4; 16 μL) were added. The mixture of amphiphiles and siRNAs was incubated for 10 min at ambient temperature. RNase A (1U in Tris-HCl buffer containing 100 mM NaCl, pH 7.4) or buffer only (4 μL) was added. After incubating at 37 °C for 30 min and following phenol extraction and precipitation, RNAs were loaded onto 1.0% agarose gel.

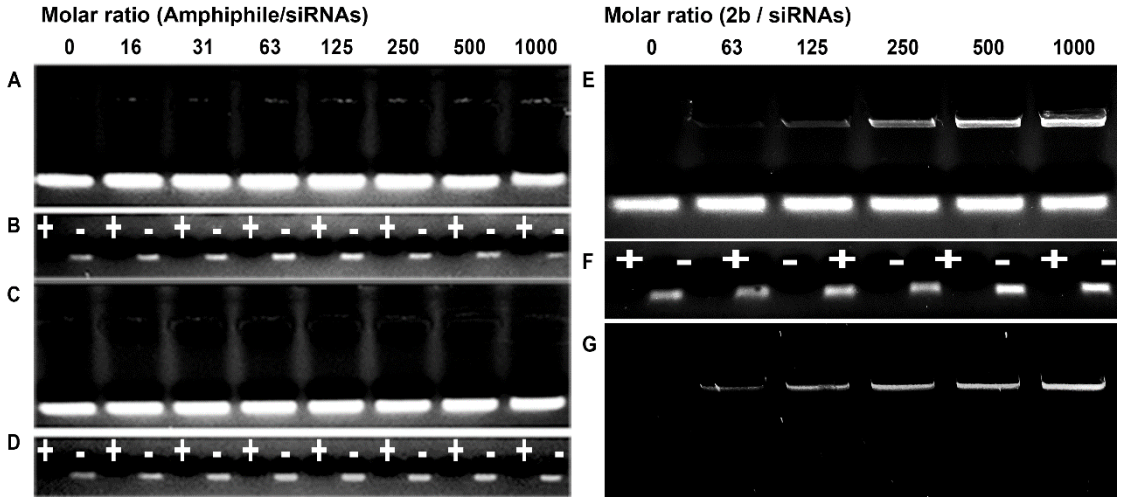

Figure S4. (A, C, E, G) Gel retardation assays and (B, D, F) enzymatic stability tests for complexes of the siRNAs with (A, B) **ZnBnDPA**, (C, D) **ZnDPA**, and (E, F, G) **2b**. Only for (G), used a gel without RNA staining dye. The CA/siRNA complexes were loaded onto 1% agarose gel (A, C, E, G) directly or (B, D, F) after incubation in the presence (+)/absence (–) of RNase A for 30 min at 37 °C. Table S1 lists corresponding charge ratios.

Table S1. Charge ratios of mixtures of amphiphiles and siRNAs (positive charges of amphiphiles/negative charges of siRNAs)

Molar ratio	Charge ratio	Molar ratio	Charge ratio
16	0.78	50	2.5
31	1.6	100	5.00
63	3.1	200	10.0
125	6.25	400	20.0
250	12.5	600	30.0
500	25.0	800	40.0
1000	50.0		

5. TEM Images

5.1. TEM Characterization

Transmission Electron Microscopy (TEM). A drop of each sample in solution was placed on a carbon-coated copper grid and allowed to evaporate under ambient conditions. When the sample was stained, a drop of uranyl acetate solution (2 wt%) was placed on the surface of the sample-loaded grid. The sample was deposited for approximately 2 min; the excess solution was wicked off using filter paper. The specimen was observed using a JEM-1400 microscope operated at 120 kV. The data were analyzed using Gatan Digital Micrograph software.

Cryogenic TEM (Cryo-TEM). Cryo-TEM experiments were performed using a thin film of a solution of sample (4 μL) transferred to a lacey supported grid through the plunge/dipping method. To prevent evaporation of water from the sample solutions, the thin aqueous films were prepared at ambient temperature and a humidity of 97–99% within a custom-built environmental chamber. The excess liquid was blotted away by applying filter paper for 2–3 s; the thin aqueous films were rapidly vitrified through plunging into liquid ethane (cooled by liquid N_2) at its freezing point. The specimen was observed using a JEOL-JEM-3011 HR apparatus operated at 300 kV. The data were analyzed using Gatan Digital Micrograph software.

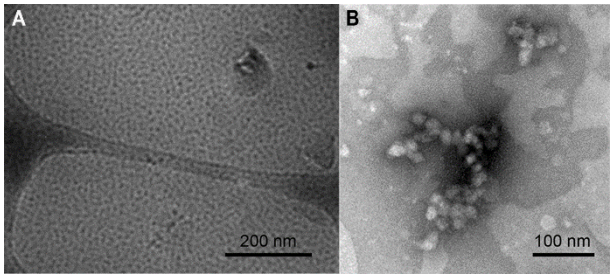

Figure S5. (A) Cryo-TEM image and (B) TEM image of complexes formed from siRNAs and **ZnDPA** (0.5 mM); molar ratio: 400.

6. Size and Zeta Potential Measurements

A compound solution (10 mM in DMSO; 5 μL) was added to a siRNA solution [in filtered DW (0.45-μm membrane filter); 995 μL] at an appropriate concentration corresponding to the given molar ratio, then gently mixed using a micropipette. The mixtures were applied directly to a Zatasizer ZS apparatus (Malvern, UK) to measure their hydrodynamic sizes and zeta potentials.

7. RNA Cleavage Tests

To a mixture of siRNA solution (25 μM in DEPC water; 20 μL), DW (10 μL), and HEPES-NaOH buffer (10 mM, pH 7.4; 50 μL), an aliquot (10 μL) of a compound solution (10 mM in DMSO) or DMSO was added and mixed carefully. The mixture was incubated at 37 °C. After 8, 16, 24, and 48 h, an aliquot (20 μL) of the mixture was applied for phenol extraction and precipitation, RNAs were loaded onto 1% agarose gel.

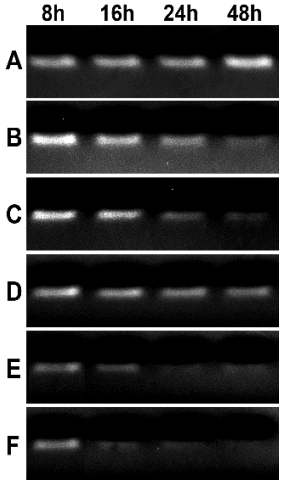

Figure S6. RNA cleavage tests by gel electrophoresis in 1% agarose gel. For the complexes of the siRNAs with (A) DMSO, (B) **CA1**, (C) **CA2**, (D) **CA3**, (E) **ZnBnDPA**, and (F) **ZnDPA** at the molar ratio of 400, after incubation of compound/siRNAs complexes for 8 h, 16h, 24 h, and 48 h at 37 °C in the HEPES-NaOH buffer (10 mM, pH 7.4).

8. *In Vitro* Experiments

8.1. Cell Viability. The in vitro cell viabilities of all of the CAs were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in HeLa, HepG2 and HCT116 cells. Cells were seeded on a 24-well culture plate at an initial density of 2×10^4 cells/well in growth medium (0.5 mL) and allowed to grow to 80% confluency prior to sample treatment. After complexation of 20 nM anti-luciferase siRNA (siLuc) with all of the compounds (**CA1**, **CA2**, **CA3**, **ZnBnDPA**, **ZnDPA**) at various molar ratios (50, 100, 200, 400, 600, 800, 1000), the complexes were placed into individual wells and incubated in serum-free media (0.5 mL) for 4 h. The commercial transfection agent Lipofectamine™ (Invitrogen) was used as a control reagent. The medium was

changed to serum-containing medium after 4 h and then the mixtures were incubated for a further 20 h. After incubation, MTT solution (5 mg/mL, 0.5 mL) was added and the mixtures incubated for 4 h. The medium was then removed and DMSO (500 µL) was added to form the formazan salt solution. After the transfer of aliquots (150 µL) from each well, the absorbance at 570 nm was measured using a microplate spectrofluorometer (Victor3 V Multilabel Counter, PerkinElmer, Wellesley, MA). All MTT experiments were performed in triplicate and repeated at least three times.

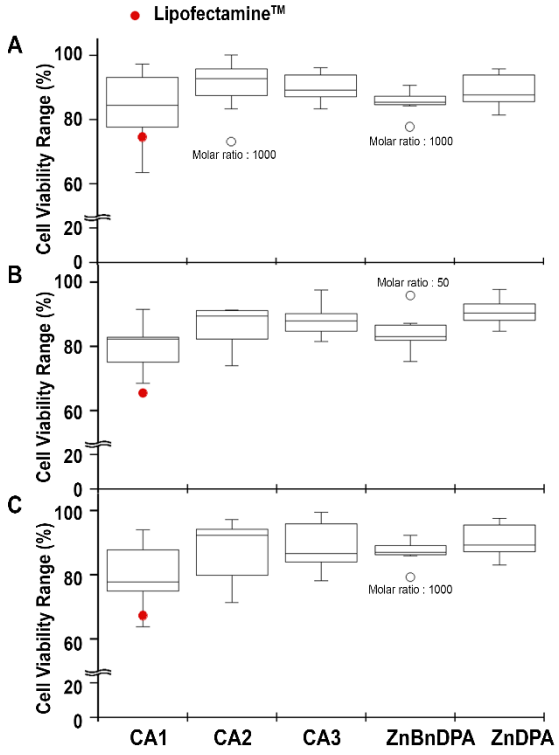

Figure S7. Cell viabilities, determined through MTT assays, of CAs in (A) HeLa, (B) HepG2, and (C) HCT116 cells. The complexes were examined at molar ratios of 50, 100, 200, 400, 600, 800, and 1000; the range of cell viability is illustrated. Generally, the viability decreased slightly upon increasing the molar ratio. In most cases, the compounds were not as cytotoxic as Lipofectamine™.

8.2. Cellular Uptake Efficiency. The transfection efficiencies of the CAs were screened in HepG2 and HCT116 cells using an *in vitro* luciferase reporter gene assay. Cells were seeded on 24-well culture plates at an initial density of 2×10^4 cells/well. To develop luciferase-expression cells, cells were transfected with ssPEI/CMV-LUC (1 µg) prior to transfection. After 6 h, each compound was mixed with 20 nM siLuc at various molar ratios (50, 100, 200, 400, 600, 800, 1000) and then the complexes were added to the luciferase-expression cells. After 4 h, the medium was replaced with 10% serum-containing medium (0.5 mL), followed by incubation for 24 h. Lipofectamine™ was also used as a control. The cells were then washed with Dulbecco's phosphate-buffered saline (DPBS) and lysed using lysis buffer (200 mL). The expression of luciferase reporter gene was measured using a microplate spectrofluorometer (VICTOR 3 V Multilabel Counter, PerkinElmer, Wellesley, MA).

The targeting efficiency of biotin-conjugated **CA3** to biotin receptor-rich HepG2 cells was evaluated through a competitive inhibition assay using free biotin and subsequent comparison with those of biotin receptor–poor HCT116 cells. HepG2 and HCT116 cells were pretreated with 0.5 and 5 mM free biotin for 30 min before adding the **CA3**/siLuc complex at a fixed molar ratio of 400. After incubation for 4 h, the medium was replaced with serum-containing medium and then the mixture was further incubated for 24 h. All experiments were performed in triplicate and repeated at least three times.

8.3. Mechanistic Study. The route of CA uptake was analyzed by inhibiting various endocytosis pathways and then observing their subsequent effects on transfection. Luciferase-expression HepG2 cells were pretreated with each inhibitor [genistein (300 μM), MβCD (5 mg/mL), wortmanin (200 nM), chlorpromazine (2.5 μg/mL)] and then incubated for 1 h at 37 °C before the addition of each sample. Each compound was mixed with 20 nM siLuc at a fixed molar ratio of 400. To investigate the energy-dependency of endocytosis, one plate was placed in a refrigerator at 4 °C while the other was placed in an incubator at 37 °C during the incubation of each CA compound. The incubation, for determining luciferase activity, was performed for 4 h. The medium was replaced with serum-containing medium and then the plates from both groups were returned back to the incubator at 37 °C. The cells were then washed with DPBS and lysed using lysis buffer (200 mL). The expression of the luciferase reporter gene was measured using a microplate spectrofluorometer (VICTOR 3 V Multilabel Counter, PerkinElmer, Wellesley, MA).

8.4. Confocal Microscopic Images.

HepG2 cells were seeded at an initial density of 2×10^4 cells/well in a 12-well culture plate over glass coverslips. After 24 h incubation in a 5% CO_2 humidified incubator, cells were treated with CA/FITC-labelled siRNAs (FITC-siLuc) complexes at a fixed molar ratio of 400 for 2hrs in culture media and then incubated in 5uM Lysotracker Red DND-99 (Thermo Fisher Scientific) for 5 min. Cellular uptake was quenched by adding cold DPBS, followed by washing two times with DPBS. After washing, cells on coverslip were mounted in Vectashield anti-fade mounting medium with DAPI (Vector Laboratories), and the image was obtained with an Olympus FV-1000 instrument and analyzed with Olympus Fluoview version 1.5 viewer software.

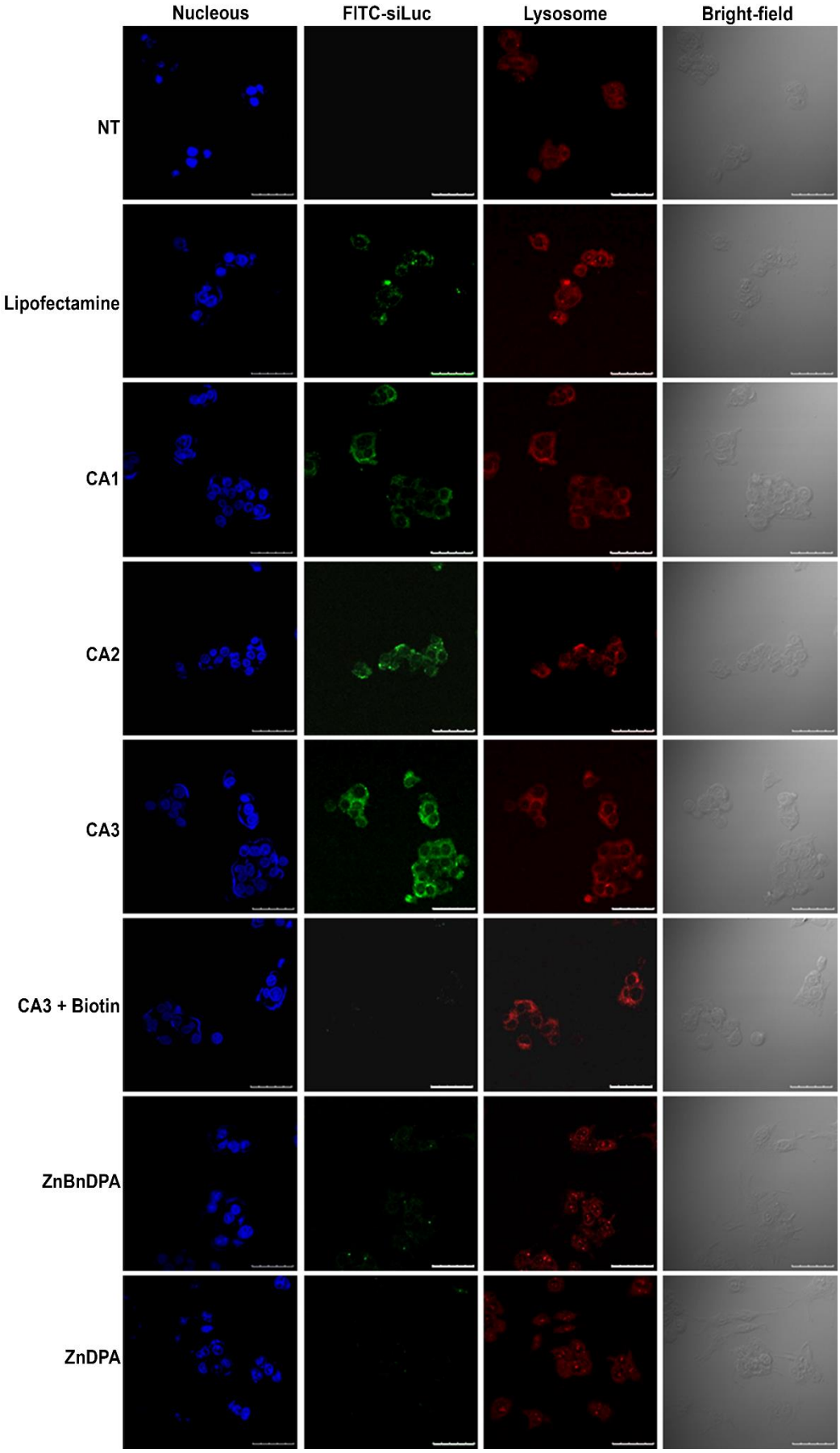

Figure S8. Confocal microscopic images. Uptake images of HeG2 cells treated with of CA/FITC-siLuc complexes. Nucleus were stained with DAPI (blue) and endo/lysosomes were stained with Lysotracker (red). Scale bars = 50 μm. NT, Non-Treated.

9. NMR Spectra

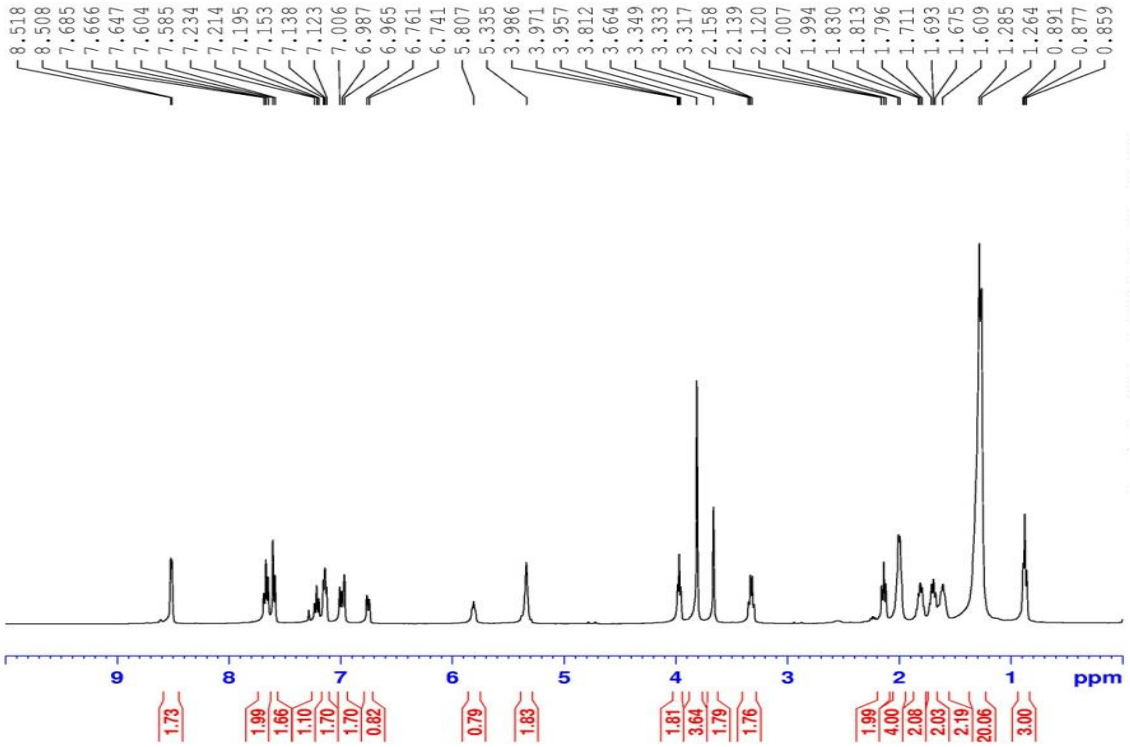

Figure S9. ^{1}H NMR spectrum of compound **2a**

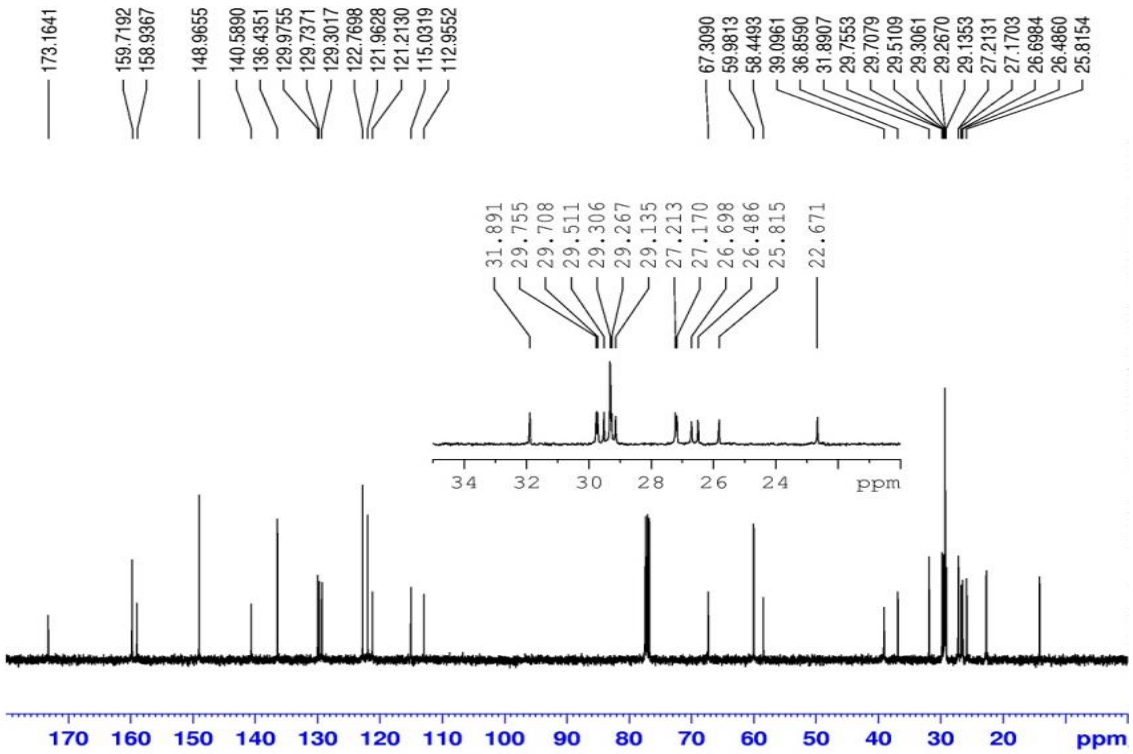

Figure S10. ^{13}C NMR spectrum of compound **2a**

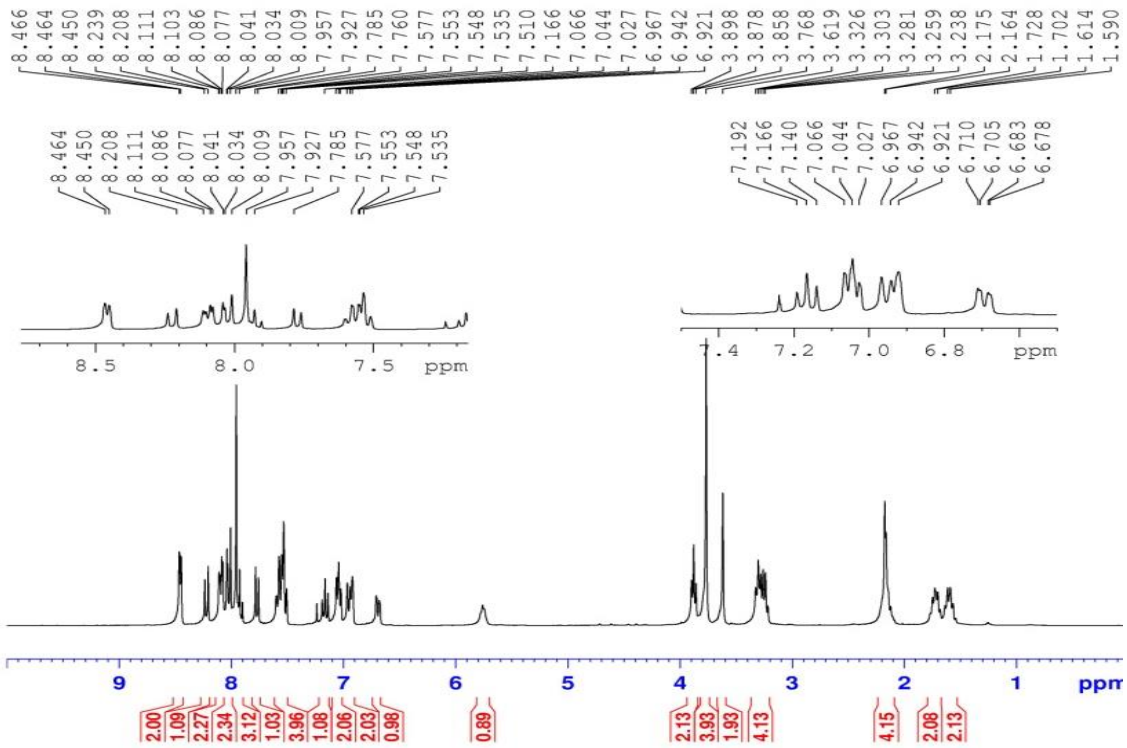

Figure S11. [1]H NMR spectrum of Compound **2b**

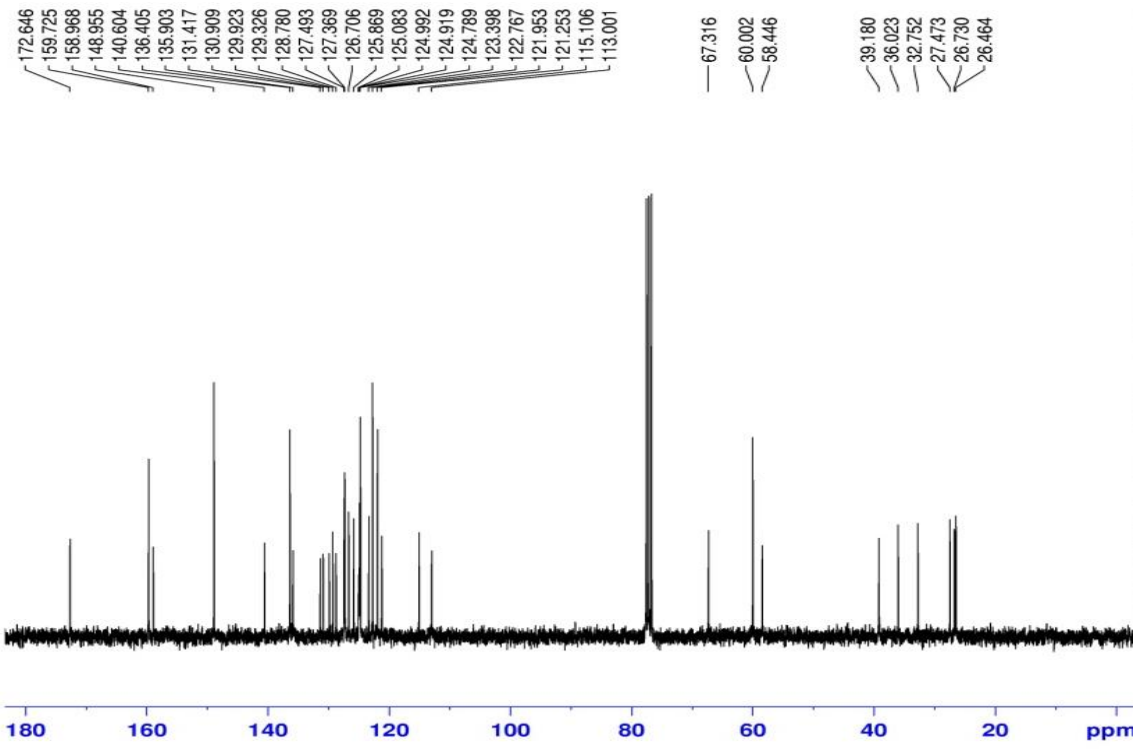

Figure S12. [13]C NMR spectrum of compound **2b**

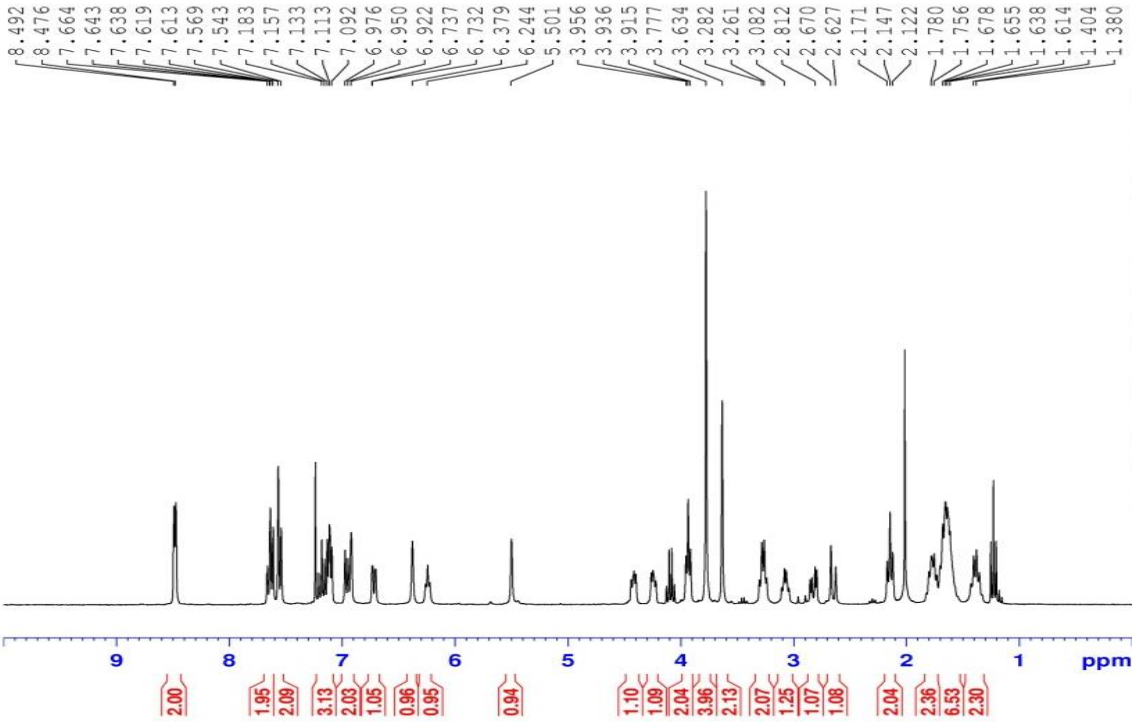

Figure S13. [1]H spectrum of compound **2c**

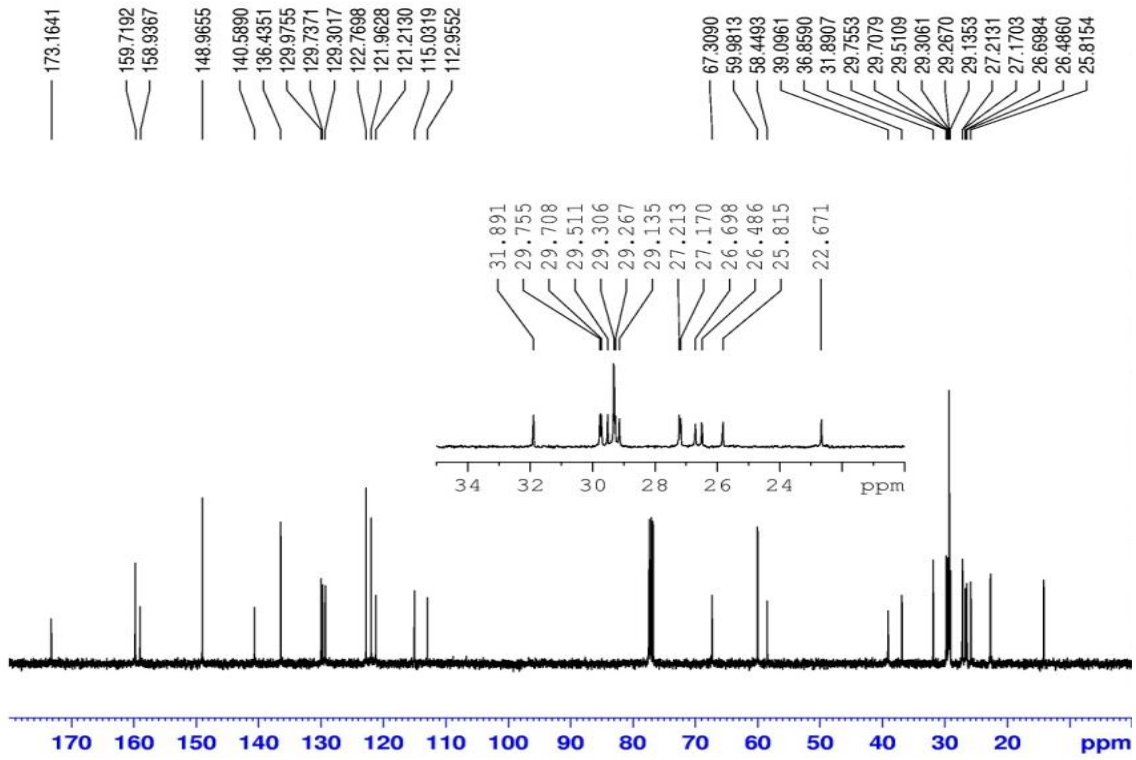

Figure S14. [13]C NMR spectrum of compound **2c**

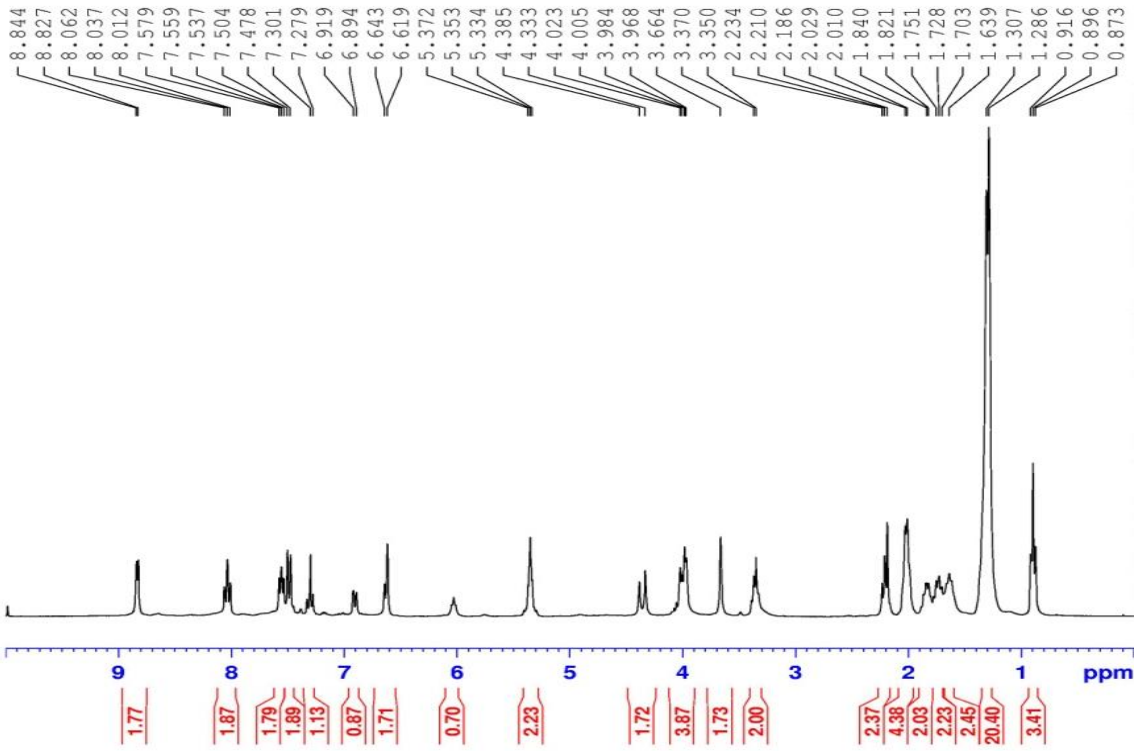

Figure S15. ^{1}H NMR spectrum of compound **CA1**

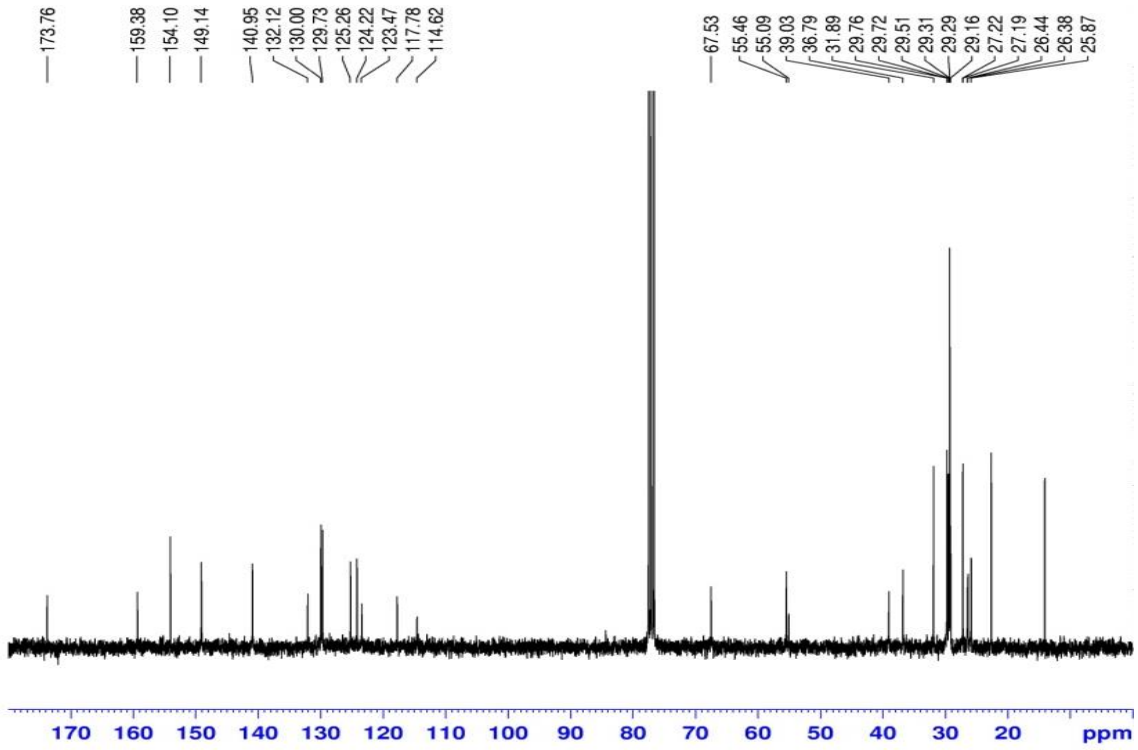

Figure S16. ^{13}C NMR spectrum of compound **CA1**

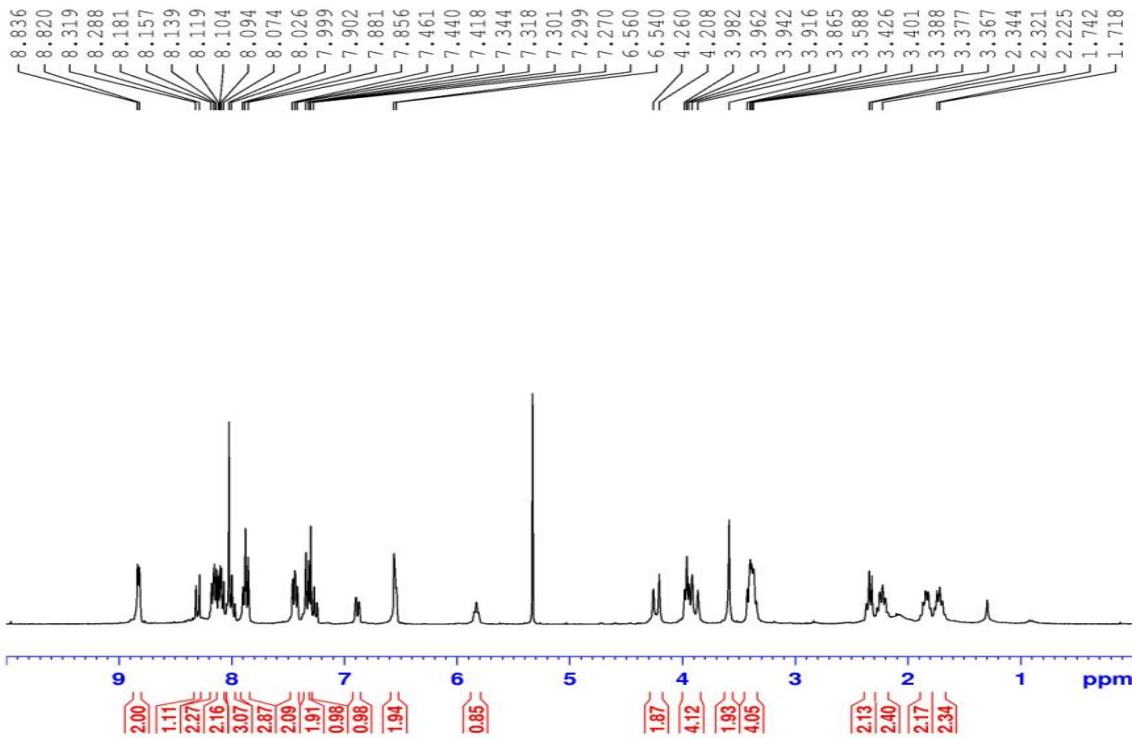

Figure S17. [1]H NMR spectrum of compound **CA2**

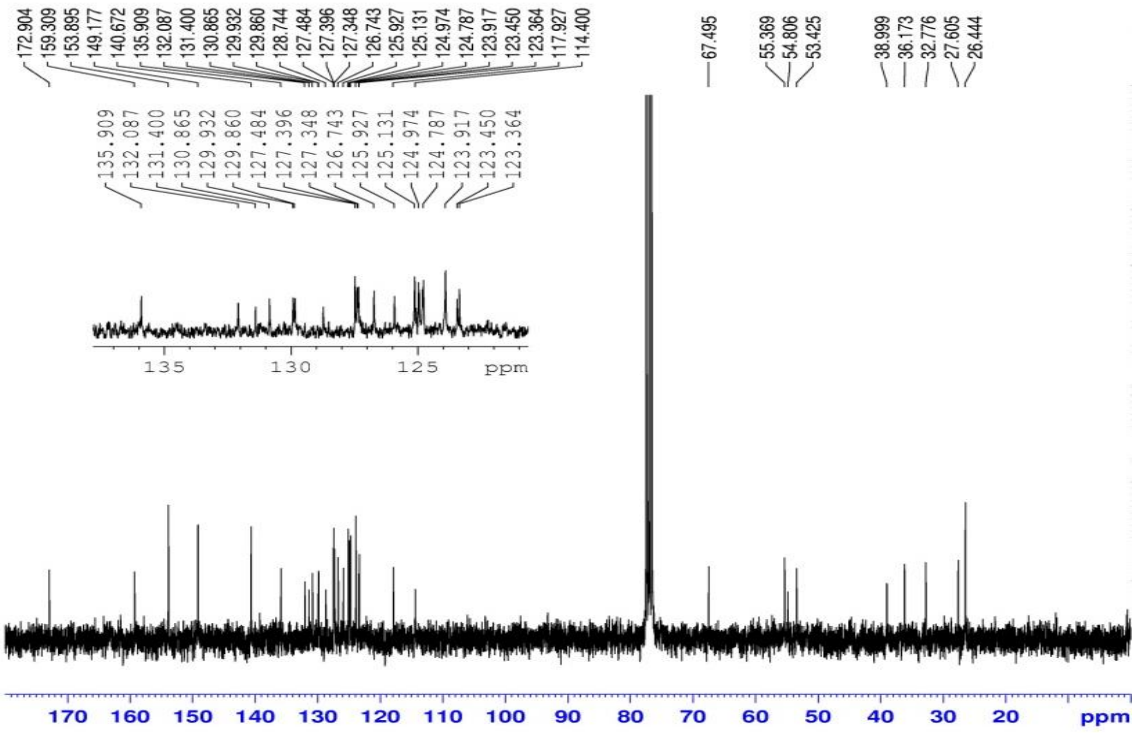

Figure S18. [13]C NMR spectrum of compound **CA2**

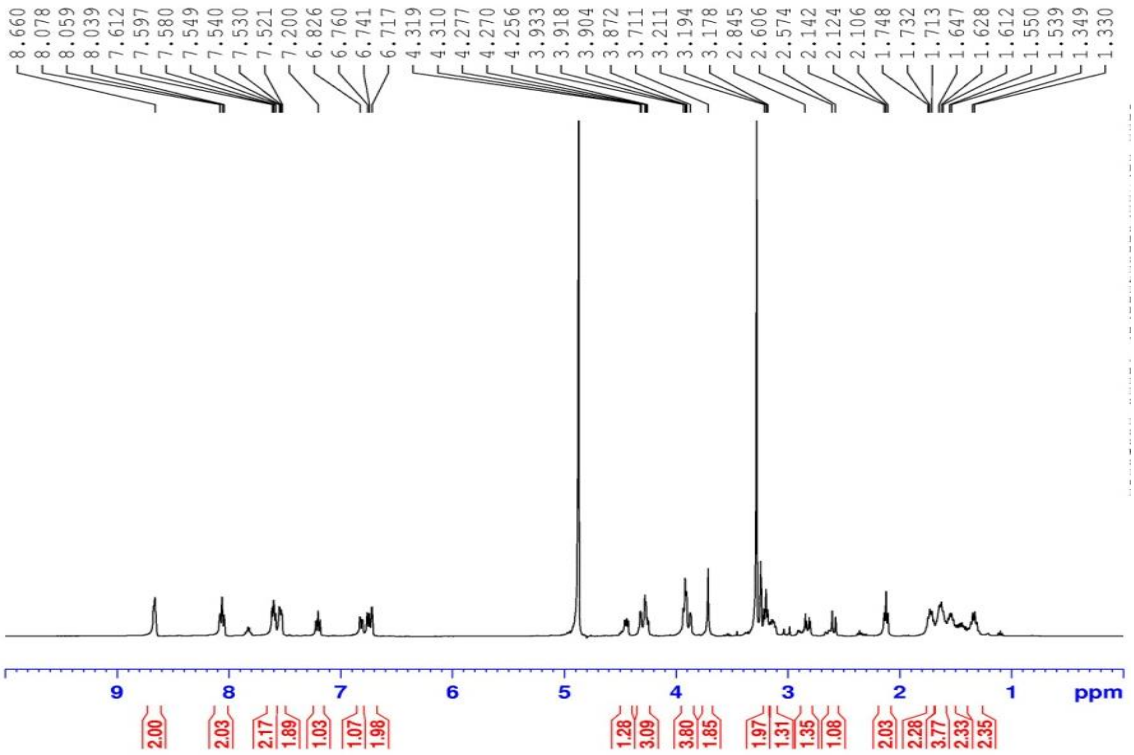

Figure S19. ^{1}H NMR spectrum of compound **CA3**

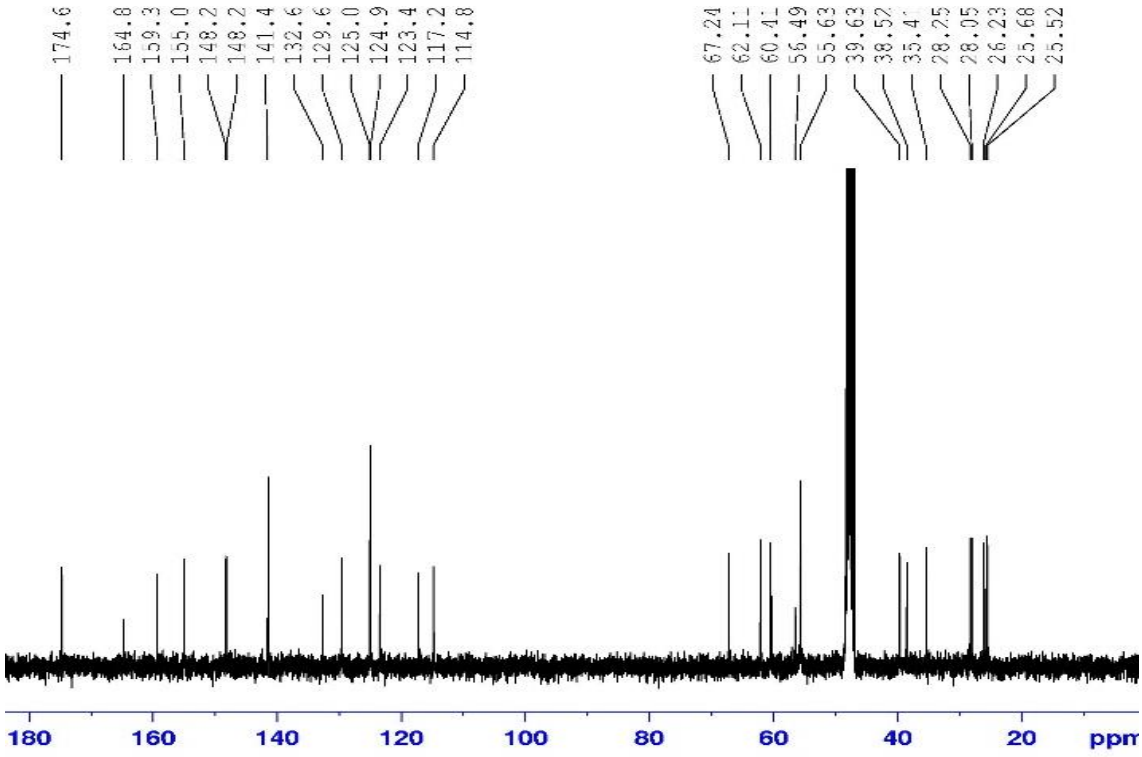

Figure S20. ^{13}C NMR spectrum of compound **CA3**

10. References

S1. Jiang, H.; Smith, B. D. *Chem. Commun.* **2006**, 1407-1409.

S2. Wang, W.; Lee, Y. A.; Kim, G.; Kim, S. K.; Lee, G. Y.; Kim, J.; Kim, Y.; Park, G. J.; Kim, C. *J. Inorg. Biochem*. **2015**, *153*, 143-149.

S3. Hanshaw, R. G.; Hilkert, S. M.; Jiang, H.; Smith, B. D. *Tetrahedron Lett.* **2004**, *45*, 8721–8724.