Supporting information for

Heme-catalyzed Reduction of N-Oxide and its application to sensing heme and hemoglobin via a chemiluminescent probe

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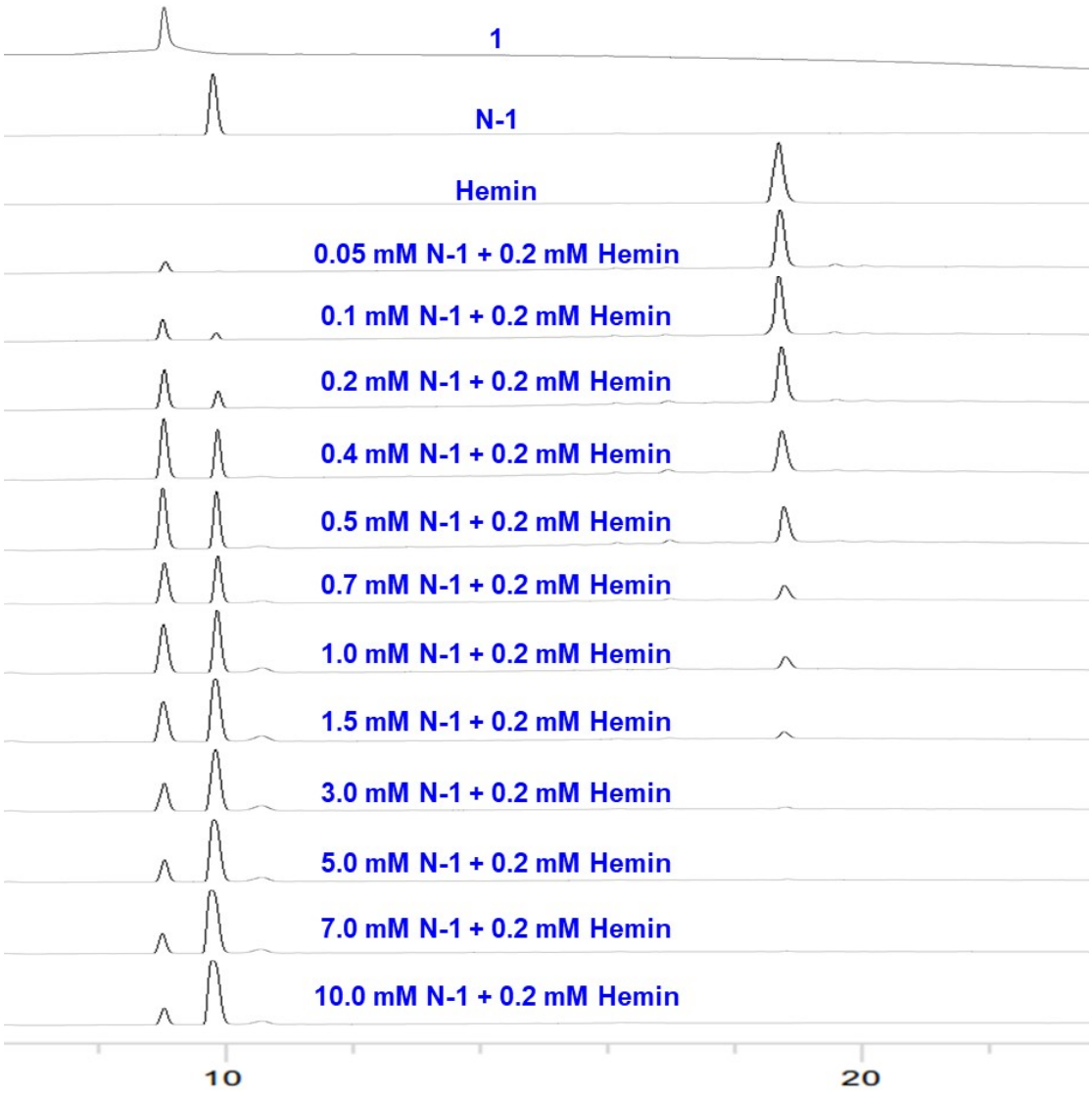

Fig. S1 The HPLC profiles for the kinetic study for hemin (0.2 mM) treated with different concentrations of N-1(0.05-10.0 mM) for 0.5 h at 37 ^{o}C. Detection wavelength of the HPLC: 214 nm.

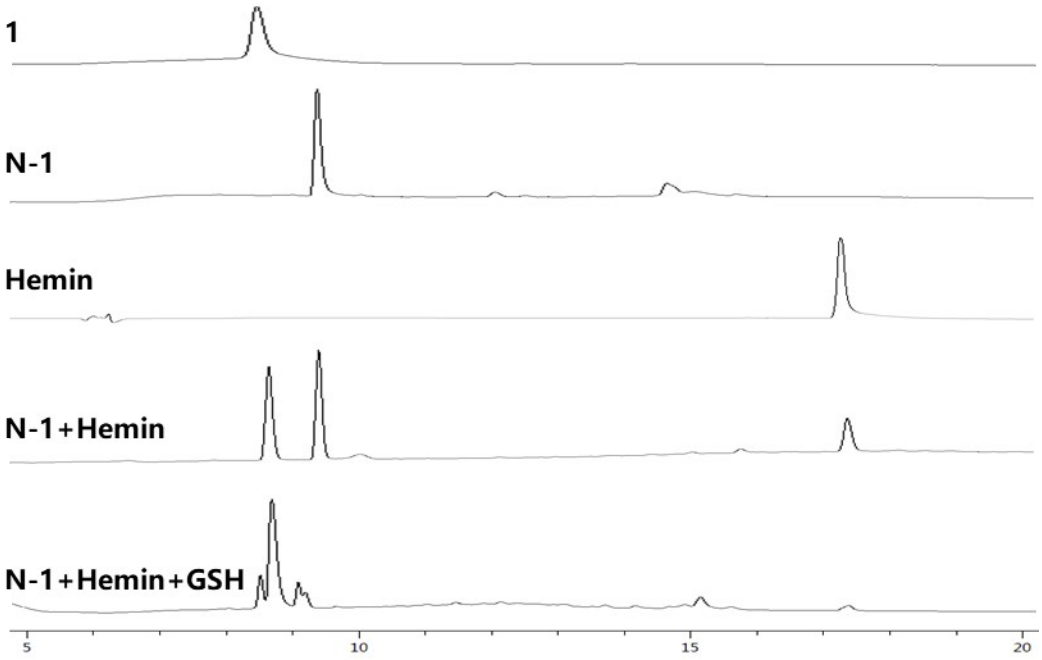

Fig. S2 The HPLC profiles of the reactions between N-1 and hemin in presence or absence of GSH. N-1: 1 mM, Hemin: 0.5 mM, GSH: 5mM, 37 °C/0.5h. Detection wavelength of the HPLC: 214 nm.

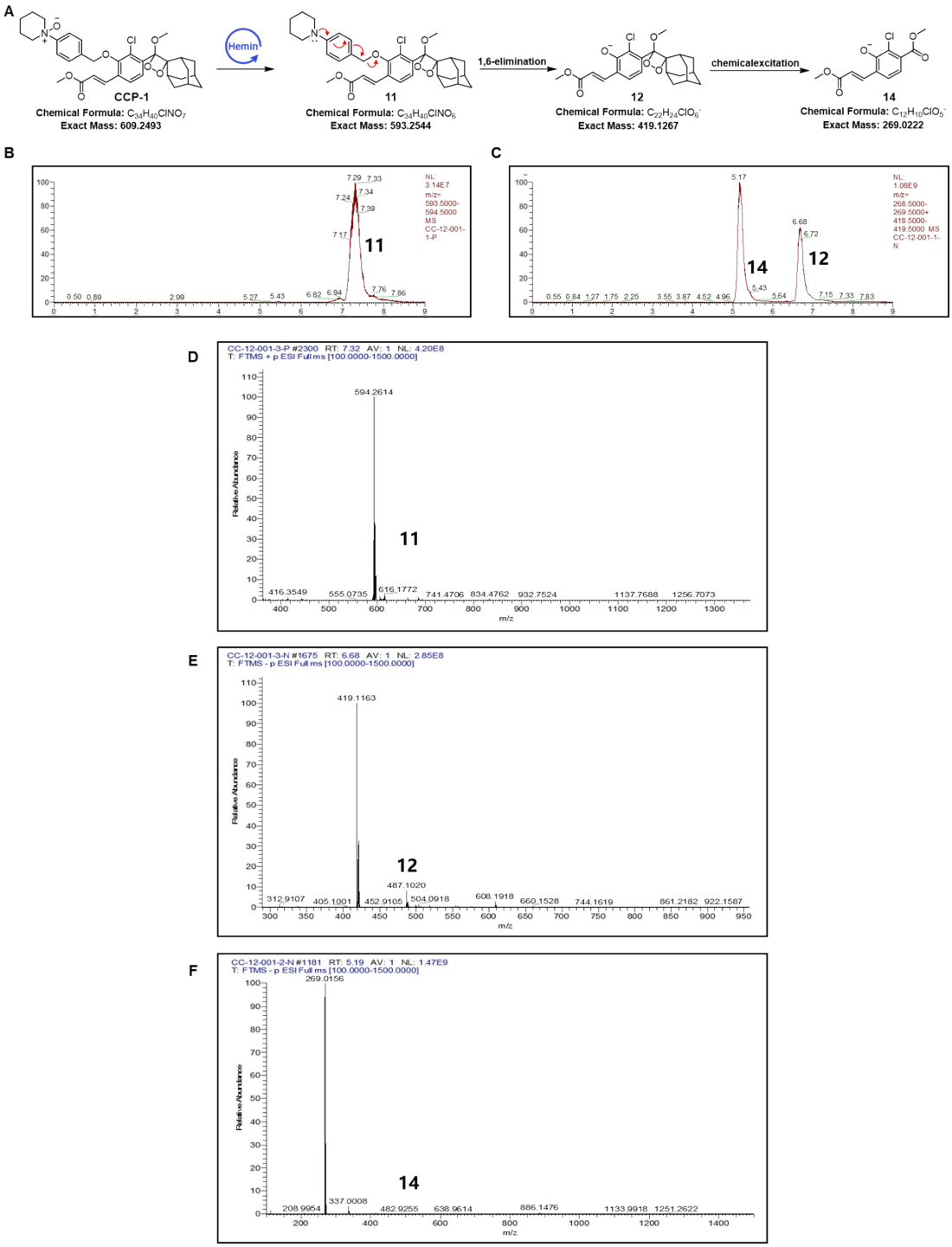

Fig. S3 Investigation of the mechanism of reaction between hemin and CCP-1 by using LC-MS. (A) The proposed mechanism of reaction between hemin and CCP-1. (B) Extracted ion current (EIC) with the m/z value being 593.50-594.50 for the reaction mixture (Positive). (C) Extracted ion current (EIC) with the m/z value being 268.50-269.50 and 418.50-419.50 for the reaction mixture (Negative). (D-F) The HRMS results of probe's reaction intermediate products. CCP-1: 1 mM, Hemin: 1 mM, 37 ºC/0.5 h.

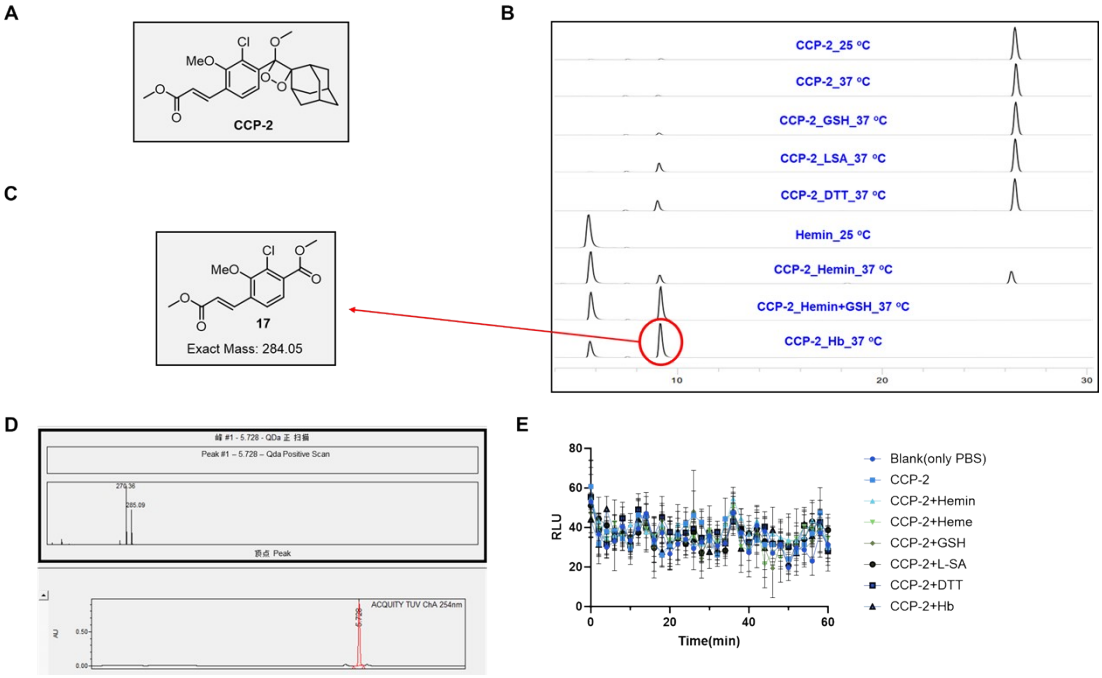

Fig. S4 The study on the decomposition of CCP-2 in the presence of hemin and other related reducing agents. (A) The chemical structure of CCP-2. (B) The HPLC profile of CCP-2 (0.5 mM) was incubated with various reductive reagents for 30 min. GSH was 5 mM while other reducting agents were 0.5 mM. (C) The chemical structure of decomposed product (17) of CCP-2. (D) The LC-MS analysis of decomposed product 17. (E) The luminescence of CCP-2 in the presence of various reductive reagents. CCP-2: 1 μM, GSH: 10 μM, another reagents: 1 μM. Results are shown as the mean ± SD with n = 3.

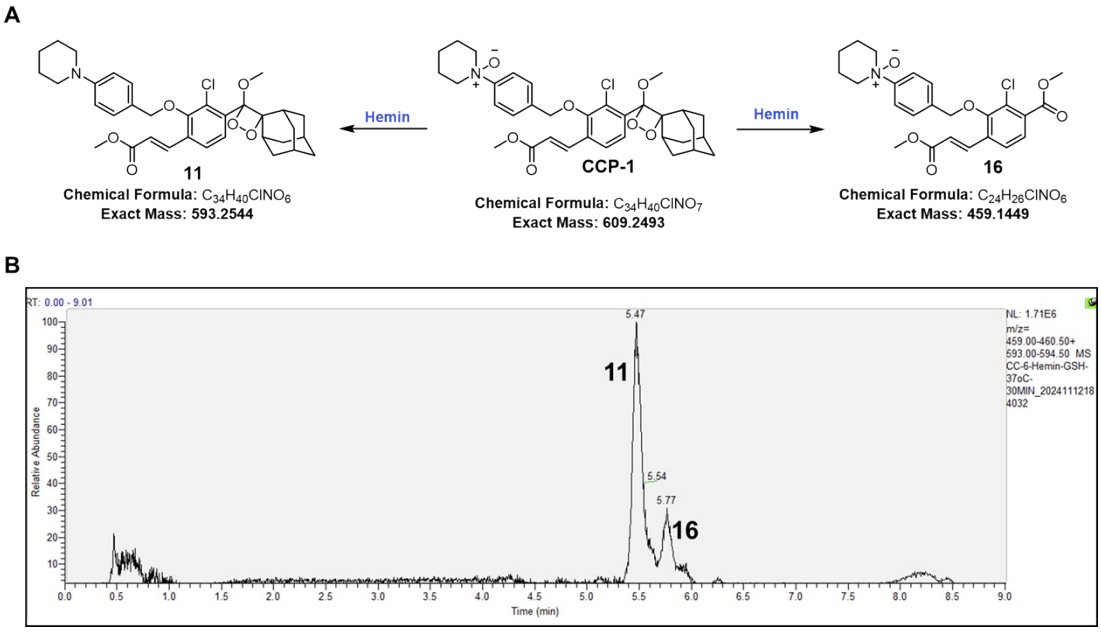

Fig. S5 (A) The intermediates 11 and 16 generated by the reaction between N-Oxide part or and dioxetane part of CCP-1 and hemin. (B) Extracted ion current (EIC, Positive) with the m/z value being 593.00-594.50 (intermediate 15) and 459.00-460.50 (intermediate 16) from the LC-MS analysis of the reaction mixture.

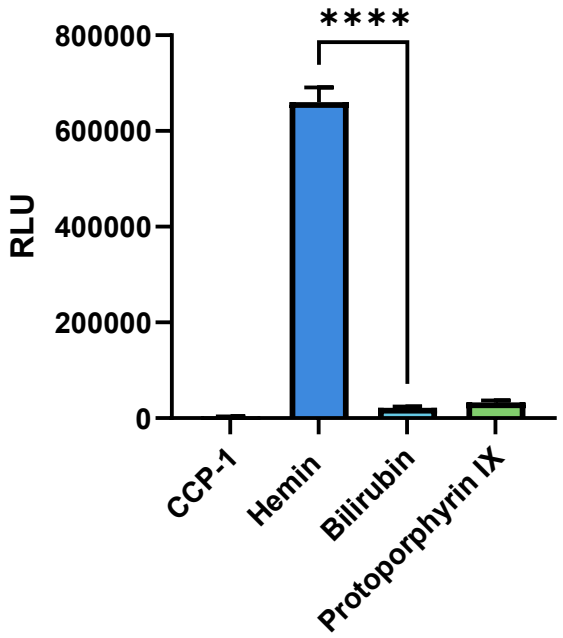

Fig. S6 The chemiluminescence intensity of probe CCP-1 (1 μM) treated with hemin (1 μM), bilirubin (1 μM), or protoporphyrin IX (1 μM) at 37 °C for 16 min. Results are shown as the mean ± SD with n = 3.

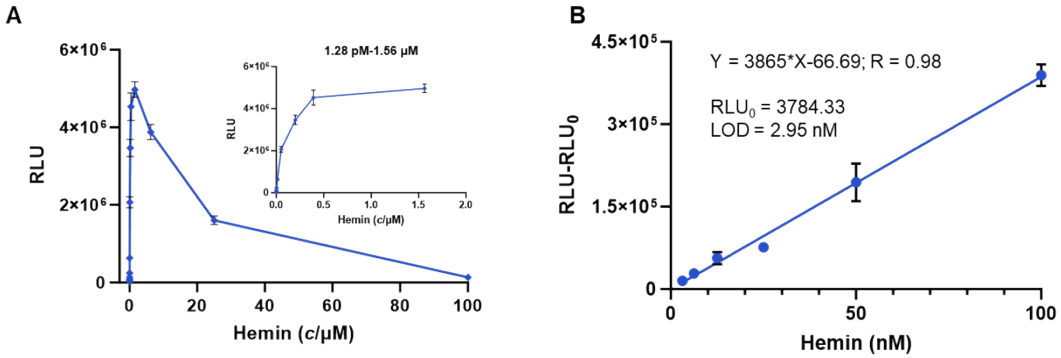

Fig. S7 (A) The chemiluminescence intensity of the probe CCP-1 treated with different concentrations of hemin. (B) The linear range for the detection of hemin by probe CCP-1 and the calculated limit of detection (LOD). The LOD calculated as 3 times the blank value. Results in the curves or bars are shown as the mean ± SD with n = 3.

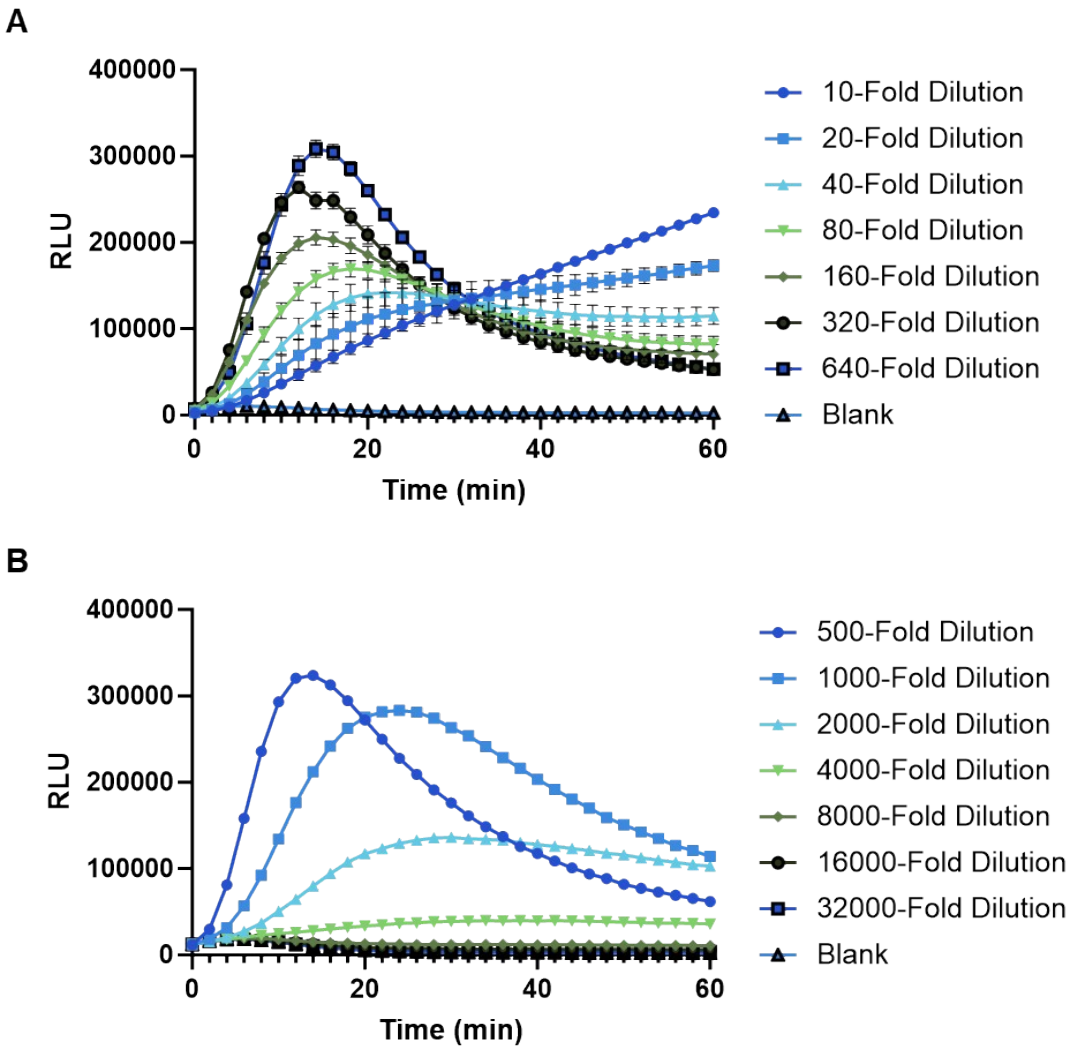

Fig. S8 The chemiluminescence intensity of probe CCP-1 (1 μM) incubated with diluted plasma. (A) The plasma was diluted in the range of 10 to 640 times. (B) The plasma was diluted in the range of 500 to 32000 times.

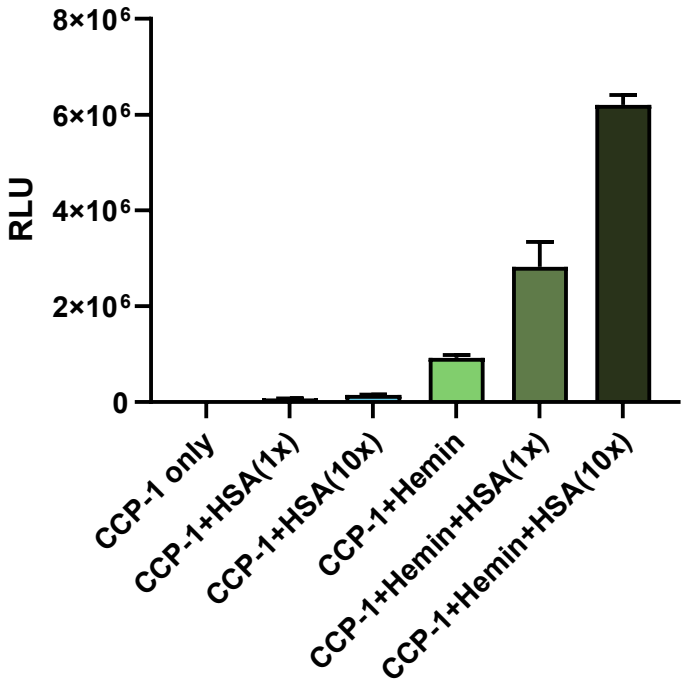

Fig. S9 The luminescence of CCP-1 in the presence of HSA. P-1 μM, HSA-1/10 μM, Hemin-1 μM, 37 °C/0.5 h. Data of 12 min was collected and results are shown as the mean ± SD with n = 3.

General methods

All reactions requiring anhydrous conditions were performed under an argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were A.R. grade. All other commercially available reagents were purchased from suppliers and used without further purification. Thin layer chromatography (TLC): silica gel plates (YingLong GF254), compounds were visualized by irradiation with UV light. Column chromatography was carried out with silica gel (Mietek, particle size 200-300 mesh). ^{1}H-NMR spectra were recorded using Bruker Avance operated at 400MHz, 500MHz. ^{13}C-NMR spectra were recorded using Bruker Avance operated at 101MHz, 126MHz. Chemical shifts were reported in ppm on the δ scale relative to a residual solvent (Chloroform-*d*: δ = 7.26 for ^{1}H-NMR and 77.16 for ^{13}C-NMR; DMSO-d_6: δ = 2.50 for ^{1}H-NMR and 39.52 for ^{13}C-NMR; MeOH-d_4: δ = 3.31 for ^{1}H-NMR and 49.00 for ^{13}C-NMR). HRMS analysis was performed in a Thermo FisherExactive Plus mass spectrometer equipped with a ThermoFisher Accela HPLC system. (ThermoFisher Scientific, Bremen, Germany). HPLC was performed on a Shimadazu LC-6AD instrument with SPD-20A and RID-10A detectors, using an Xtimate C18 column (150 × 10 mm, 5 μm) or Hypersil GOLD C18 column (250 × 4.6 mm, 5 μm). Flow cytometry analysis was performed on a BD FACSVerse Flow Cytometer.

Abbreviations. ACN - Acetonitrile. DCM - Dichloromethane. DEAD - Diethyl azodicarboxylate. DIPEA - N,N-Diisopropylethylamine. DMSO - Dimethyl sulfoxide. DTT - Dithiothreitol. EDCI - n-(3-dimethylaminopropyl)-n'-ethylcarbodiimide hydrochloride. EtOAc - Ethylacetate. HBTU - O-(1H-Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate. HOBt - 1-Hydroxybenzotriazole. LDA - Lithium diisopropylamide. L-SA - Sodium ascorbate. MB - Methyl blue. m-CPBA - 3-Chloroperoxybenzoic acid. MeOH - Methanol. PE - Petroleum ether. NIS - N-Iodosuccinimide. TBAF - Tetrabutylammonium fluoride. TBS-Cl - tert-Butyldimethylsilyl chloride. THF - Tetrahydrofuran.

Kinetics Analyses of the reaction between Hemin and N-1

To determine the kinetic values of the reaction between hemin and **N-1**, reactions were performed with **N-1** from 0.05 mM to 10 mM in PBS (pH 7.4) and in a total volume of 100 μL that contained 0.2 mM Hemin. The mixture was stirred for 30 min at 37℃, and then analyzed by normal-phase HPLC. Data fitting was performed using GraphPad Prism 9, and K_M, k_{cat} and k_{cat}/K_M values represent the mean±s.d. of three independent replicates.

Mechanism investigation of the reaction between Hemin and N-1

The commercial ROSGreen™ H_2O_2 detection kit was used to investigate the reaction mechanism of the reaction between Hemin and **N-1**. **N-1** and Hemin were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS: ACN (1: 1) to a 20 μM working solution. The reaction was performed by incubating 10 μL N-1 with 10 μL Hemin and the total reaction volume was 200 μL (PBS: ACN = 1: 1). The mixture was stirred for 30 min at 37℃, after which 50 μL was taken to mix with 50 μL of the H_2O_2 probe solution (10μM). The mixture was incubated in the dark for 30 min. The fluorescence signal of the reaction mixture was measured at 37℃ every 1 min for 30 min using a Microplate Reader (Tecan Austria GmbH), with excitation and emission wavelength bands centered at 490 nm and 525 nm, respectively. Data at the 30 min were plotted.

The establishment of hemolytic animal model.

C57BL/6J mice were purchased from GemPharmatech (Beijing, China). All animal experiments were performed according to government policies and the Helsinki Declaration. Animal experiments were approved by Institutional Animal Care and Use Committee at the Institute of Basic Medical Sciences, PUMC & CAMS (No. ACUC-A01-2021-032). An anemia model was induced in 8-week-old male mice through intraperitoneal administration of 80mg/kg phenylhydrazine (PHZ, Sigma-Aldrich, p26252). Control mice received saline injections. Peripheral blood was drawn into vacuumed tubes containing EDTA-2K one day post-treatment and centrifuged at 3000 rpm for 10 minutes to isolate plasma.

Measurement of Probe CCP-1 Response to Heme and Fe^{2+}

CCP-1 and hemin were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Similarly, Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) was dissolved in water to prepare a 1 mM stock solution, and then diluted it with PBS to a 20 μM working solution. Additionally, GSH was dissolved in water to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 0.2 mM working solution.

According to Table S1, the chemiluminescence signal intensity under different conditions was measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour, and each experimental group consisted of three independent replicates.

Table S1. Measurement of CL intensity of **CCP-1** incubated with different conditions

Condition	**CCP-1** (μL)	GSH (μL)	hemin (μL)	Fe^{2+} (μL)	PBS (1×) (μL)	total (μL)
1	10				190	200

2	10	10			180	200
3	10	10	10		170	200
4	10	10		10	170	200

Measurement of Probe CCP-1 Response to Heme, Iron Ions, Bilirubin and Protoporphyrin IX of Different Types and Valences

CCP-1, hemin, hematin and protoporphyrin IX were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Similarly, Fe^{2+} and Fe^{3+} were dissolved in water to prepare a 1 mM stock solution, and then diluted it with PBS to a 20 μM working solution. Bilirubin was dissolved in 0.1 M NaOH to prepare a 20 mM stock solution, and then diluted it with PBS to a 20 μM working solution. Additionally, GSH was dissolved in water to prepare a 1 mM stock solution, and subsequently dilute it with PBS to a 0.2 mM working solution.

According to Table S2, the chemiluminescence signal intensity under different conditions was measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour. Data at 16 min was collected and plotted, and each experimental group consisted of three independent replicates.

Table S2. Measurement of CL intensity of **CCP-1** incubated with heme (Cl, OH), hemin, hematin, Fe^{2+}, Fe^{3+}, protoporphyrin IX

Condition	**CCP-1** (μL)	GSH (μL)	hemin (μL)	hematin (μL)	Fe^{2+} (μL)	Fe^{3+} (μL)	protoporphyrin IX (μL)	PBS (1×) (μL)	total (μL)
1	10							190	200
2	10	10						180	200
3	10		10					180	200
4	10	10	10					170	200
5	10			10				180	200
6	10	10		10				170	200
7	10				10			180	200
8	10	10			10			170	200
9	10					10		180	200
10	10	10				10		170	200
11	10						10	180	200
12	10	10					10	170	200

Measurement of Probe CCP-1 Response to Metal Ions of Different Types and Valences

CCP-1 and hemin were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Similarly, Metal ions were dissolved in water to prepare a 1 mM stock solution, and then dilute it with PBS to a 20 μM working solution. Additionally, GSH were dissolved in water to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 0.2 mM working solution.

According to Table S3, the chemiluminescence signal intensity under different conditions was

measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour. Data at 18 min was collected and plotted, and each experimental consisted of three independent replicates.

Table S3. Measurement of CL intensity of **CCP-1** incubated with different metal ions

GSH (μL)	hemin (μL)	Fe^{2+} (μL)	Fe^{3+} (μL)	Cu^{+} (μL)	Cu^{2+} (μL)	K^{+} (μL)	Ca^{2+} (μL)	Ni^{2+} (μL)	Na^{+} (μL)	Mn^{2+} (μL)	Co^{2+} (μL)	Mg^{2+} (μL)	Zn^{2+} μL
10													
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Measurement of Probe CCP-1 Response to L-SA, DTT and Proteins Containing Heme

CCP-1 and hemin were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Similarly, Proteins containing heme, DTT and L-SA were dissolved in water to prepare a 1 mM stock solution, and then diluted it with PBS to a 20 μM working solution. Additionally, GSH was dissolved in water to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 0.2 mM working solution.

According to Table S4, the luminescence signal intensity under different conditions was measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour. Data at 18 min was collected and plotted, and each experimental consisted of three independent replicates.

Table S4. Measurement of CL intensity of **CCP-1** incubated with different heme containing proteins, L-SA and DTT

Condition	**CCP-1** (μL)	GSH (μL)	hemin (μL)	L-SA (μL)	DTT (μL)	Myoglobin (μL)	Catalase (μL)	Cytochrome c (μL)	hemoglobin (μL)	HRP (μL)	PBS (1×) (μL)	total (μL)
1	10										190	200
2	10	10									180	200
3	10		10								180	200
4	10	10	10								170	200
5	10			10							180	200
6	10				10						180	200
7	10					10					180	200
8	10						10				180	200
9	10							10			180	200
10	10								10		180	200
11	10									10	180	200

HRMS-based product Analysis of the Reaction between Probe CCP-1 and Hemin or Its Analogues

CCP-1, hemin and hematin were dissolved in DMSO to prepare a 1 mM working solution. Additionally, GSH was dissolved in water to prepare a 10 mM working solution. The reaction products from probe CCP-1 under different conditions was detected using a HRMS.

Table S5. Measurement of CL intensity of **CCP-1** incubated with different conditions

Condition	**CCP-1** (μL)	GSH (μL)	Hemin (μL)	Hematin (μL)	PBS: ACN (1: 1) (μL)	total (μL)
1	10		10		180	200
2	10	10	10		170	200

3	10		10	180	200
4	10	10	10	170	200

Measurement of Probe CCP-1 Response to Hemin at Different Concentrations

CCP-1 and hemin were dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Added 10 μL of each of the two solutions mentioned above to 180 μL of PBS.

The chemiluminescence signal intensity under different conditions was measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour. Data of 18 min was collected, and each experimental consisted of three independent replicates.

Measurement of Total Heme Concentration in Plasma Samples with Commercial Heme Assay Kit

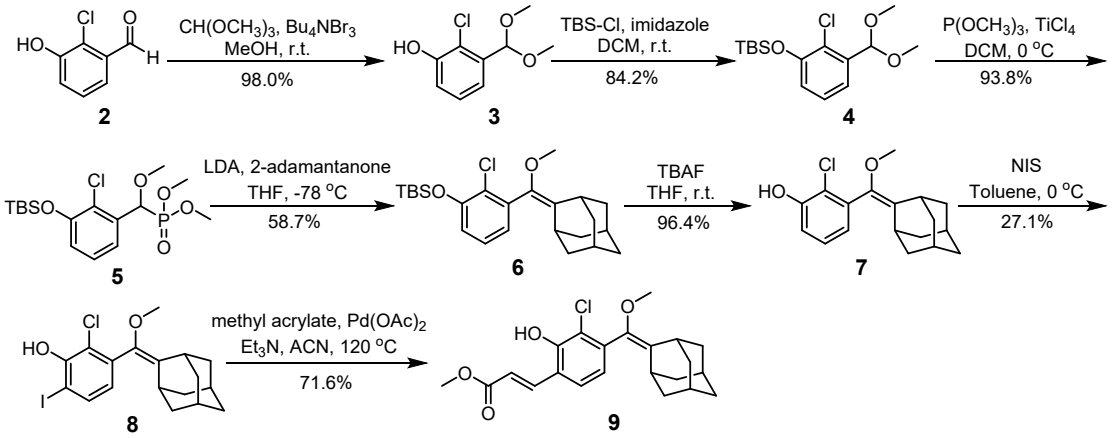
The commercial Sigma-Aldrich® Heme Assay Kit was used. Plasma samples was diluted 50-fold in ultrapure water. Then, 50 μL samples, ultrapure water and heme calibrator and 200 μL heme reagent were added into 96-plate wells respectively, followed by incubation for 5 min at room temperature. The absorbance at 400 nm was measured using a Microplate Reader (Tecan Austria GmbH) at room temperature. The diluted heme calibrator is equivalent to 62.5 μM hemin. Each experiment consisted of three independent replicates.

Measurement of Total Heme Concentration in Plasma Samples with CCP-1

CCP-1 was dissolved in DMSO to prepare a 1 mM stock solution, and subsequently diluted it with PBS to a 20 μM working solution. Similarly, hemin was dissolved in DMSO to prepare a 1 mM stock solution, and then diluted it with PBS to a 4 μM initial concentration working solution, then continued to dilute it with PBS until the working concentration is 62.5 nM (repeated 2-fold dilution). Hemolytic plasma samples were first diluted 1500-fold in PBS. Then 10 μL CCP-1 solution and 10 μL diluted plasma solution or hemin working solution were added to 180 μL of PBS. The hemin concentrations used for standard curves are as follows: 3.13, 6.25, 12.5, 25, 50, 100, 200 nM.

The chemiluminescence signal intensity was measured using a Microplate Reader (Tecan Austria GmbH) at 37 °C. The detection interval was set to 1 minute, the total detection duration was 1 hour. Data of 14 min was collected, and each experimental consisted of three independent replicates.

Synthetic schemes and experiment procedures



The synthesis of compound 3

Triethyl orthoformate (10.2 g, 96 mmol) and tetramethylammonium tribromide (1.53 g, 3.2 mmol) were added to a mixture of **2** (10.0 g, 64 mmol) in MeOH (100 mL). The mixture was stirred for 24 h. Subsequently, the mixture was diluted with EtOAc (500 mL) and washed with aq. $NaHCO_3$ (0.01M, 500 mL). The organic phase was dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=100:1~20:1) afforded **3** as a white solid (12.75 g, 98.05% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21-7.18 (m, 2H), 7.01 (q, 1H), 5.58 (s, 1H), 3.37 (s, 6H). HRESI-MS m/z: 201.0283 $[M-H]^-$ (calcd 201.0324 for $C_9H_{10}ClO_3^-$).

The synthesis of compound 4

3 (18 g, 89.11 mmol), tert-butyldimethylsilyl chloride (16.1 g, 106.62 mmol) and andimidazole (12.1 g, 177.94 mmol) were dissolved in DCM (120 mL). The mixture was stirred for 1 h, then filtered to obtain filtrate. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=100:1~50:1) afforded **4** as colorless oil (23.66 g, 84.20% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (dd, 1H), 7.14 (t, 1H), 6.88 (dd, 1H), 5.63 (s, 1H), 3.38 (s, 6H), 1.04 (s, 9H), 0.23 (s, 6H). HRESI-MS m/z: 285.1072 $[M-CH_3O]^+$ (calcd 285.1072 for $C_{14}H_{22}ClO_2Si^+$).

The synthesis of compound 5

4 (23.66 g, 74.88 mmol) and trimethyl phosphite (12.07g, 97.35 mmol) were dissolved in DCM (150 mL). The mixture was cooled to 0℃, then titanium tetrachloride (17.07 g, 89.86 mmol) was added dropwise, and it was stirred for 2 h at 0℃. Subsequently, the saturated aq. $NaHCO_3$ (300 mL) was added and stirred for 10 min at 0℃. The mixture was diluted with DCM (300 mL), then the organic phase was dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=100:1~1:1) afforded **5** as colorless oil (27.67 g, 93.80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (dt, 1H), 7.19 (t, 1H), 6.88 (dt, 1H), 5.19 (d, 1H), 3.79 (d, 3H), 3.64 (d, 3H), 3.35 (s, 3H), 1.03 (s, 9H), 0.22 (s, 6H). HRESI-MS m/z: 395.1206 $[M+H]^+$ (calcd 395.1205 for $C_{16}H_{29}ClO_5PSi^+$).

The synthesis of compound 6

5 (27.67 g, 70.07 mmol) was dissolved in anhydrous THF (300 mL). LDA (70 mL, 2M in THF) was added dropwise at -78℃ under argon, then stirred for 20 min. 2-adamantanone was dissolved in anhydrous THF (100 mL), then added dropwise in the mixture. After be stirred at -78℃ for 15 min, the mixture was heated up to 25℃ to stirred for 5h. Subsequently, the mixture was diluted with EtOAc (800 mL) and washed with brine (800 mL). the organic phase was dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=100:1~10:1) afforded **6** as a white solid (17.25 g, 58.70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12-7.08 (m, 1H), 6.87 (dq, 2H), 3.31 (s, 3H), 3.27 (s, 1H), 2.06 (s, 1H), 1.96-1.65 (m, 12H), 1.04 (s, 9H), 0.23 (s, 6H). HRESI-MS m/z: 419.1805 $[M+H]^+$ (calcd 419.2168 for $C_{24}H_{36}ClO_2Si^+$).

The synthesis of compound 7

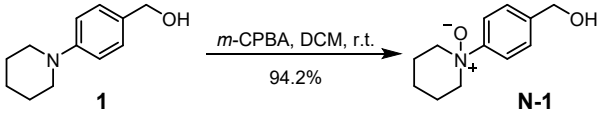
6 (17.25 g, 41.16 mmol) and tetrabutylammonium fluoride were dissolved in THF (150 mL), then stirred for 3 h. Subsequently, the mixture was diluted with EtOAc (750 mL) and washed with 1M HCl (500 mL). the organic phase was dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=50:1~30:1) afforded **7** as a white solid (12.06 g, 96.40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, 1H), 7.00 (dd, 1H), 6.83 (dd, 1H), 5.70 (s, 1H), 3.31 (s, 3H), 3.28 (s, 1H), 2.11 (t, 1H), 1.95-1.73 (m, 12H). HRESI-MS m/z: 303.1049 $[M-H]^-$ (calcd 303.1157 for $C_{18}H_{20}ClO_2^-$).

The synthesis of compound 8

7 (17.25 g, 41.16 mmol) was dissolved in anhydrous toluene (550 mL) at 0℃, and then N-Iodosuccinimide (12.75 g, 56.60 mmol) was added in a batches. The mixture was stirred at 0℃ for 1 h. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=60: 1) afforded **8** as a white solid (4.40 g, 27.10% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 1H), 6.62 (d, 1H), 6.13 (s, 1H), 3.31 (s, 3H), 3.26 (s, 1H), 2.09 (s, 1H), 1.95-1.68 (m, 12H). HRESI-MS m/z: 428.9958 $[M-H]^-$ (calcd 429.0124 for $C_{18}H_{19}ClIO_2^-$).

The synthesis of compound 9

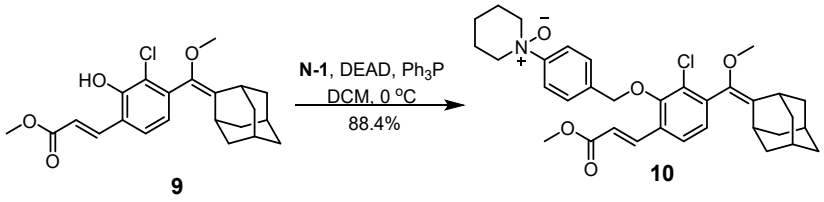
8 (17.25 g, 41.16 mmol), methyl acrylate (600 mg, 6.98 mmol), triethylamine (353 mg, 3.50 mmol), palladium acetate (26.40 mg, 0.12 mmol) and tri(o-tolyl)phosphine (7.08 mg, 0.02 mmol) were dissolved in anhydrous ACN (50 mL) under argon. The mixture was stirred at 120℃ for 5 h. Subsequently, the mixture was diluted with EtOAc (200 mL) and washed with saturated aq. NH_4Cl (200 mL x 2). the organic phase was dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=50:1~7:1) afforded **9** as a pale yellow solid (647 mg, 71.60% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, 1H), 7.38 (d, 1H), 6.86 (d, 1H), 6.61 (d, 1H), 6.21 (s, 1H), 3.82 (s, 3H), 3.31 (s, 3H), 3.27 (s, 1H), 2.12 (s, 1H), 1.96-1.72 (m, 12H). HRESI-MS m/z: 389.1515 $[M+H]^+$ (calcd 389.1514 for $C_{22}H_{26}ClO_4^+$).



1 N-1

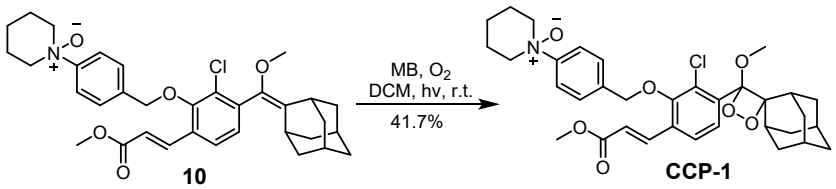
The synthesis of compound N-1

1 (191 mg, 1.00 mmol) and m-CPBA (259 mg, 1.50 mmol) were dissolved in DCM (10 mL), and then stirred for 3 h. Upon solvent evaporation, purification by column chromatography (DCM: MeOH=100:1~10:1) afforded **N-1** as a white solid (195 mg, 94.20% yield). ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.97 (d, 2H), 7.52 (d, 2H), 4.66 (s, 2H), 3.91 (t, 2H), 3.26 (d, 2H), 2.50-2.41 (m, 2H), 1.87-1.55 (m, 4H). ^{13}C NMR (100 MHz, MeOH-d_4) δ 154.43, 144.64, 128.60, 121.38, 69.31, 64.09, 22.37, 22.24. HRESI-MS m/z: 208.1328 $[M+H]^+$ (calcd 208.1332 for $C_{12}H_{18}NO_2^+$).



The synthesis of compound 10

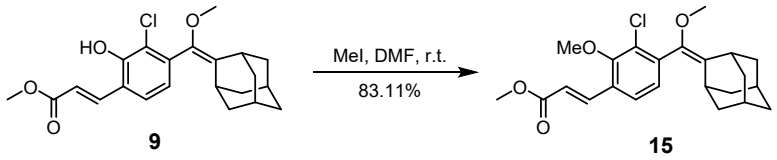
9 (281 mg, 0.72 mmol), **N-1** (150 mg, 0.72 mmol) and triphenylphosphine were dissolved in anhydrous DCM (30 mL). The mixture was cooled to 0℃ under argon, and then diethyl azodicarboxylate was added to it. After the mixture was stirred for 5 h, the solvent was evaporated, and then purification by column chromatography (DCM: MeOH=10:1~10:1) afforded **10** as a pale yellow solid (370 mg, 88.40% yield). ^{1}H NMR (500 MHz, MeOH-d_4) δ 8.06-8.03 (m, 2H), 7.86 (d, 1H), 7.65 (dd, 3H), 7.14 (d, 1H), 6.55 (d, 1H), 5.08 (s, 2H), 3.93 (td, 2H), 3.77 (s, 3H), 3.31 (s, 3H), 3.26 (d, 2H), 2.52-2.42 (m, 2H), 2.07 (s, 1H), 2.01-1.70 (m, 16H), 1.66-1.58 (m, 1H). ^{13}C NMR (125 MHz, MeOH-d_4) δ 168.59, 155.77, 154.86, 140.97, 139.67, 139.43, 139.02, 133.19, 131.08, 130.67, 130.64, 129.36, 126.52, 121.74, 121.06, 75.97, 69.39, 57.46, 52.30, 40.13, 40.00, 39.71, 39.57, 38.11, 34.43, 31.07, 29.81, 29.68, 22.37, 22.26. HRESI-MS m/z: 578.2662 $[M+H]^+$ (calcd 578.2668 for $C_{34}H_{41}ClNO_5^+$).



The synthesis of compound CCP-1

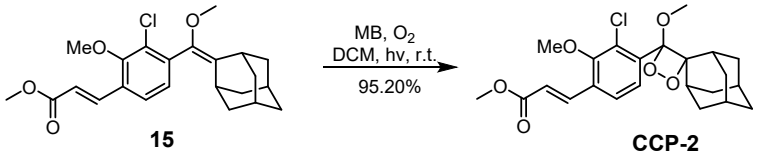
10 (350 mg, 0.61 mmol) and MB (19.4 mg, 0.06 mmol) were dissolved in anhydrous DCM (50 mL). Oxygen was bubbled through the solution while irradiating with yellow light for 50 min. The reaction was monitored by HPLC. After completion, the solvent was evaporated, and then purification by column chromatography (DCM: MeOH=10:1~10:1) afforded **CCP-1** as a pale yellow solid (154 mg, 41.70% yield). ^{1}H NMR (500 MHz, MeOH-d_4) δ 8.04 (d, 2H), 7.92-7.78 (m, 3H), 7.64 (d, 2H), 6.60 (d, 1H), 5.04 (s, 2H), 3.93 (t, 2H), 3.78 (s, 3H), 3.27 (s, 1H), 3.20 (s, 3H), 2.96 (s, 1H), 2.44 (dq, 3H), 1.98-1.60 (m, 14H), 1.51 (d, 1H), 1.40 (s, 2H). ^{13}C NMR (125 MHz, MeOH-d_4) δ 168.35, 155.60, 155.46, 139.16, 138.90, 136.69, 132.94, 130.74, 130.31, 128.82, 126.72, 122.26, 121.79, 112.80, 97.18, 76.10, 69.37, 52.40, 50.02, 37.59, 35.07, 34.92, 33.65, 33.14,

32.92, 32.66, 27.65, 27.31, 22.36, 22.22. HRESI-MS m/z: 610.2615 $[M+H]^+$ (calcd 610.2566 for $C_{34}H_{41}ClNO_7^+$).



The synthesis of compound 15

9 (50 mg, 0.13 mmol), potassium carbonate (53 mg, 0.39 mmol) and iodomethane (16 μL, 0.26 mmol) were dissolved in anhydrous DMF (2 mL), and then stirred for 2 h. Subsequently, the mixture was diluted with water (20 mL) and washed with DCM (10 mL x 3). the organic phase was washed with brine (20 mL x 3) and dried over Na_2SO_4 and filtered. Upon solvent evaporation, purification by column chromatography (PE: EtOAc=10:1~6:1) afforded **15** as a colorless transparent oil (43 mg, 83.11% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.94 (d, 1H), 7.44 (d, 1H), 7.06 (d, 1H), 6.52 (d, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.32 (s, 3H), 3.27 (s, 1H), 2.08 (s, 1H), 1.83 (m, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 167.27, 155.44, 139.47, 138.72, 138.22, 132.55, 129.59, 129.10, 127.65, 125.29, 120.10, 61.72, 57.31, 51.84, 39.20, 39.06, 38.62, 37.06, 32.92, 29.70, 28.36, 28.19. HRESI-MS m/z: 403.1674 $[M+H]^+$ (calcd 403.1671 for $C_{23}H_{28}ClO_4^+$).

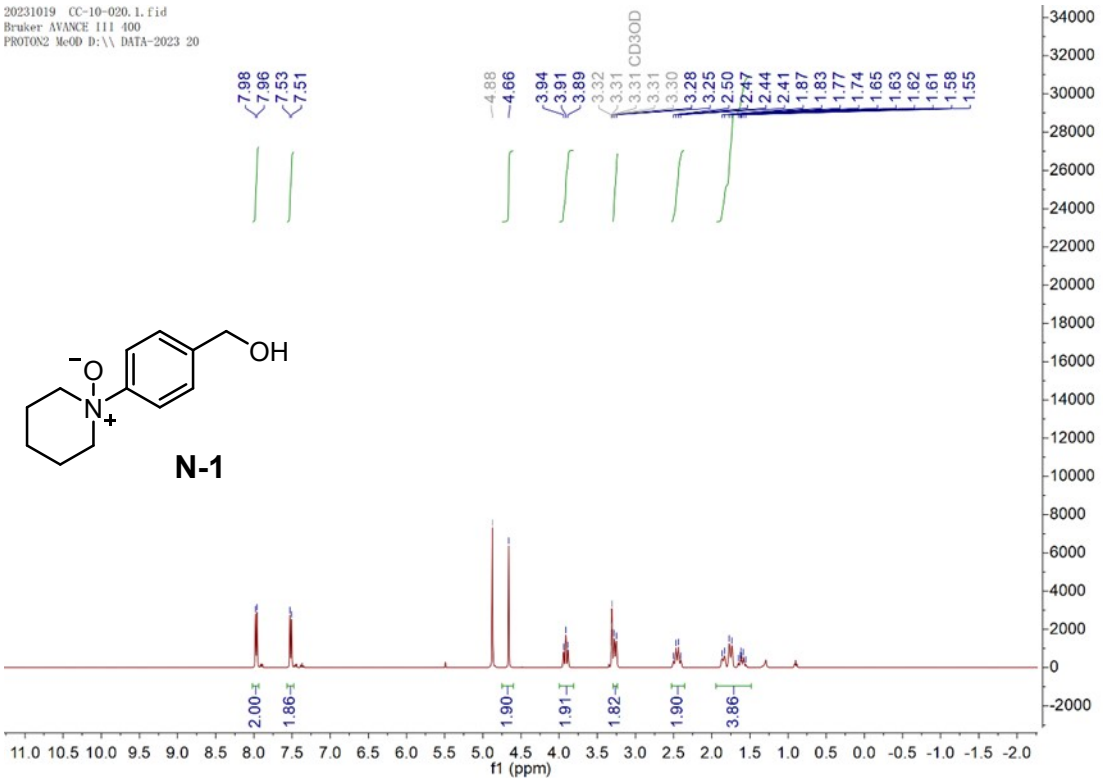


The synthesis of compound CCP-2

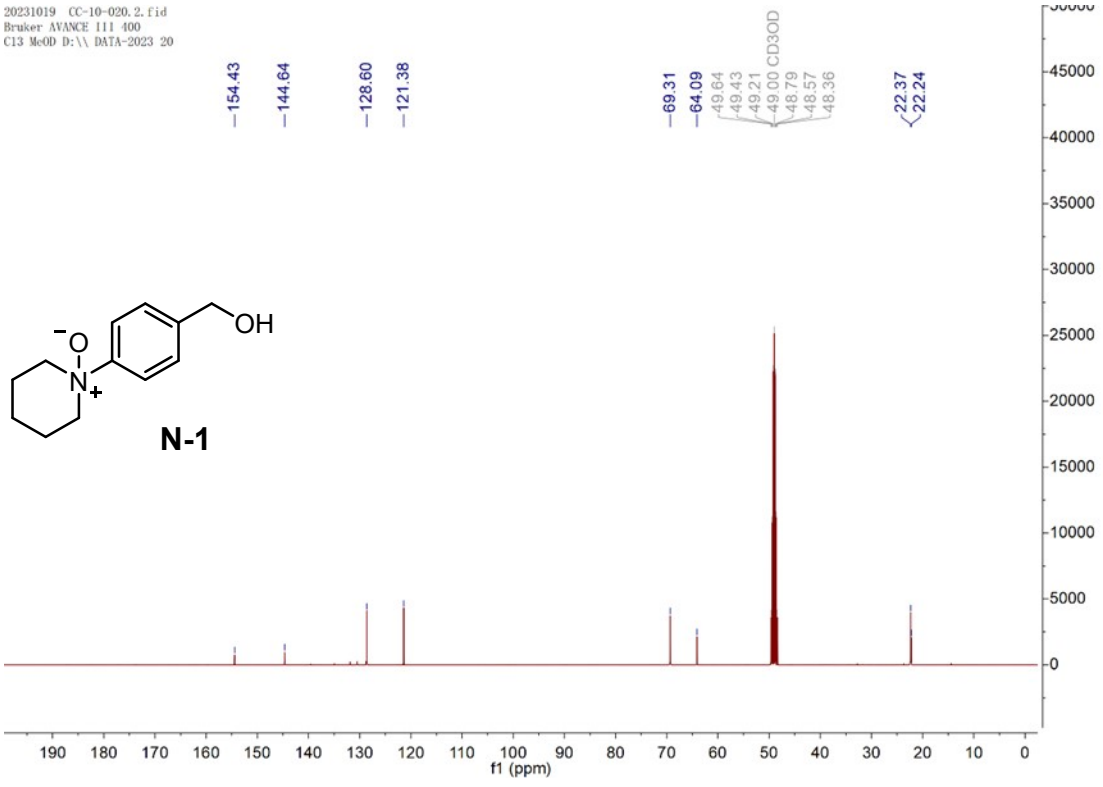
15 (43 mg, 0.11 mmol) and MB (3.6 mg, 0.01 mmol) were dissolved in anhydrous DCM (20 mL). Oxygen was bubbled through the solution while irradiating with yellow light for 30 min. The reaction was monitored by HPLC. After completion, the solvent was evaporated, and then purification by column chromatography (PE: EtOAc=10:1~6:1) afforded **CCP-2** as a white solid (44 mg, 95.20% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.91 (m, 2H), 7.58 (d, 1H), 6.58 (d, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.22 (s, 3H), 3.02 (s, 1H), 2.32 (d, 1H), 1.99 (s, 1H), 1.74 (m, 8H), 1.46 (dd, 1H), 1.32 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 167.00, 155.95, 138.15, 135.24, 130.98, 128.79, 127.58, 125.50, 121.42, 111.74, 96.37, 61.73, 51.93, 49.73, 36.59, 33.89, 33.59, 32.58, 32.22, 31.59, 31.52, 26.16, 25.82. HRESI-MS m/z: 435.1569 $[M+H]^+$ (calcd 435.1560 for $C_{23}H_{28}ClO_6^+$).

^{1}H, ^{13}C-NMR spectra of synthesized compounds

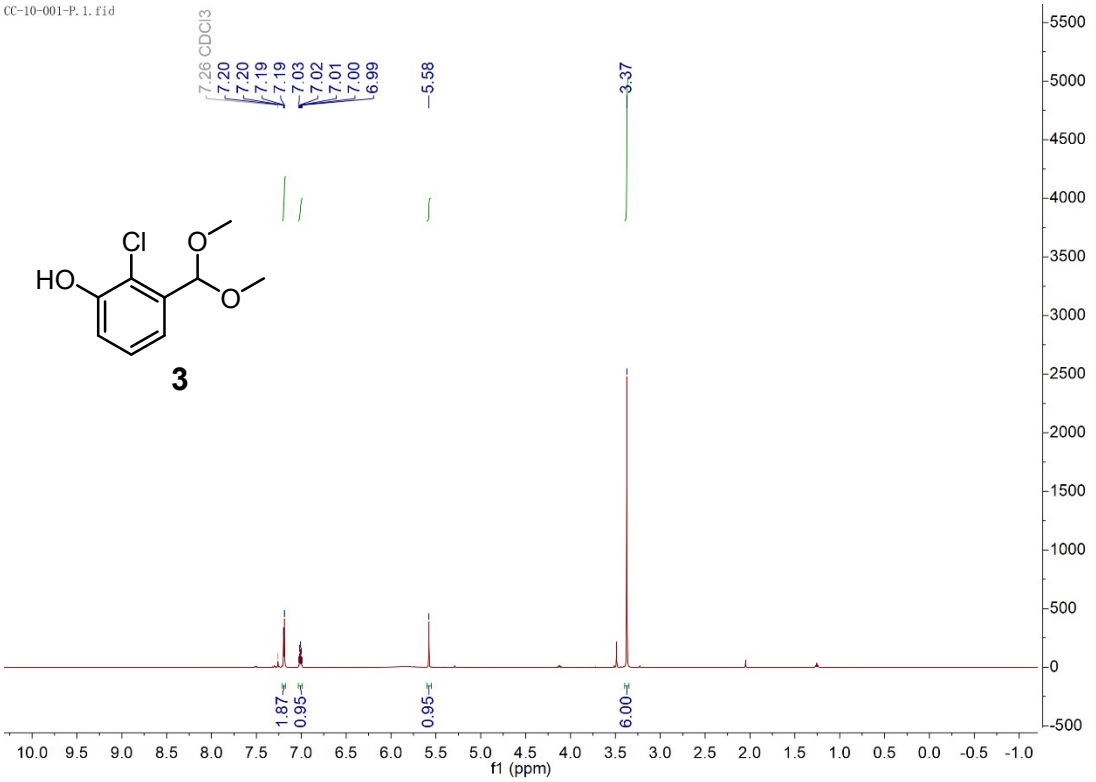
^{1}H-NMR of N-1



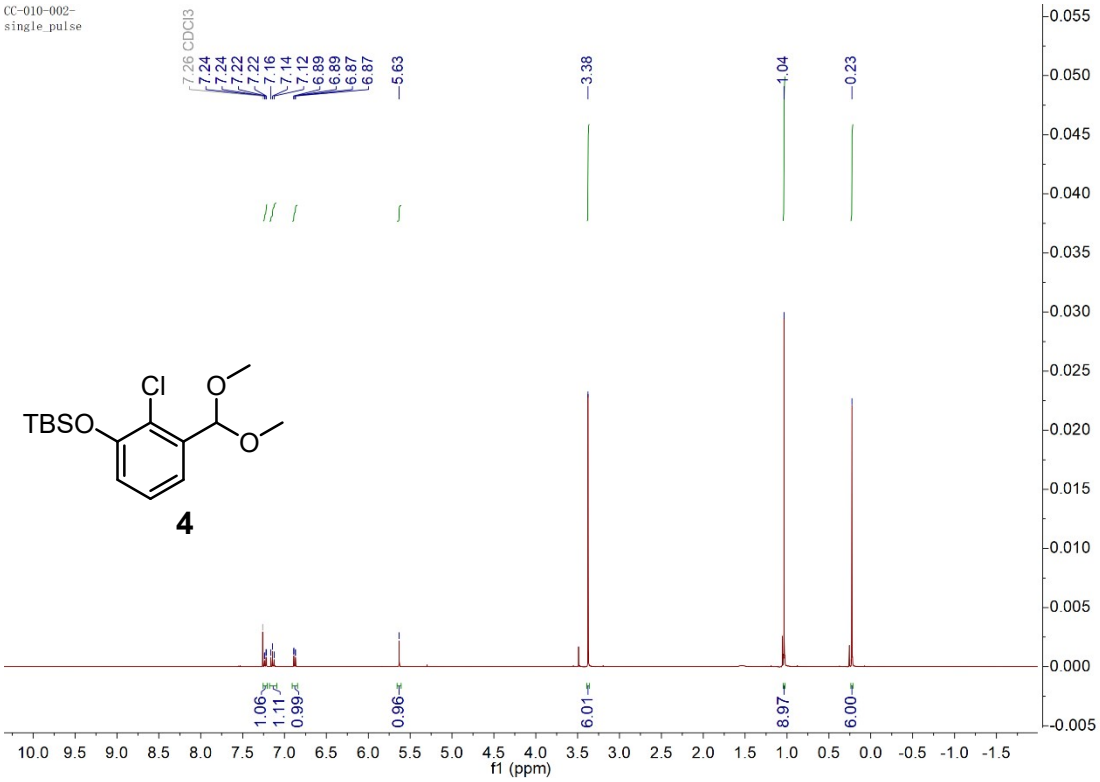
^{13}C-NMR of N-1



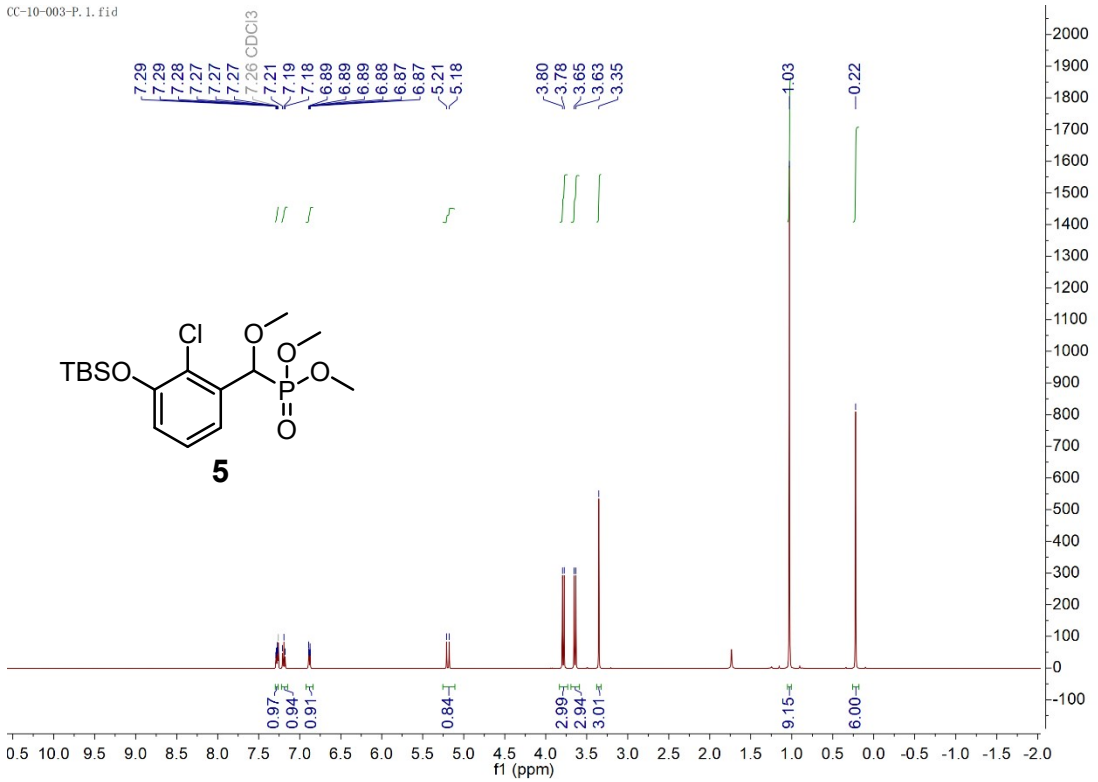
^{1}H-NMR of 3



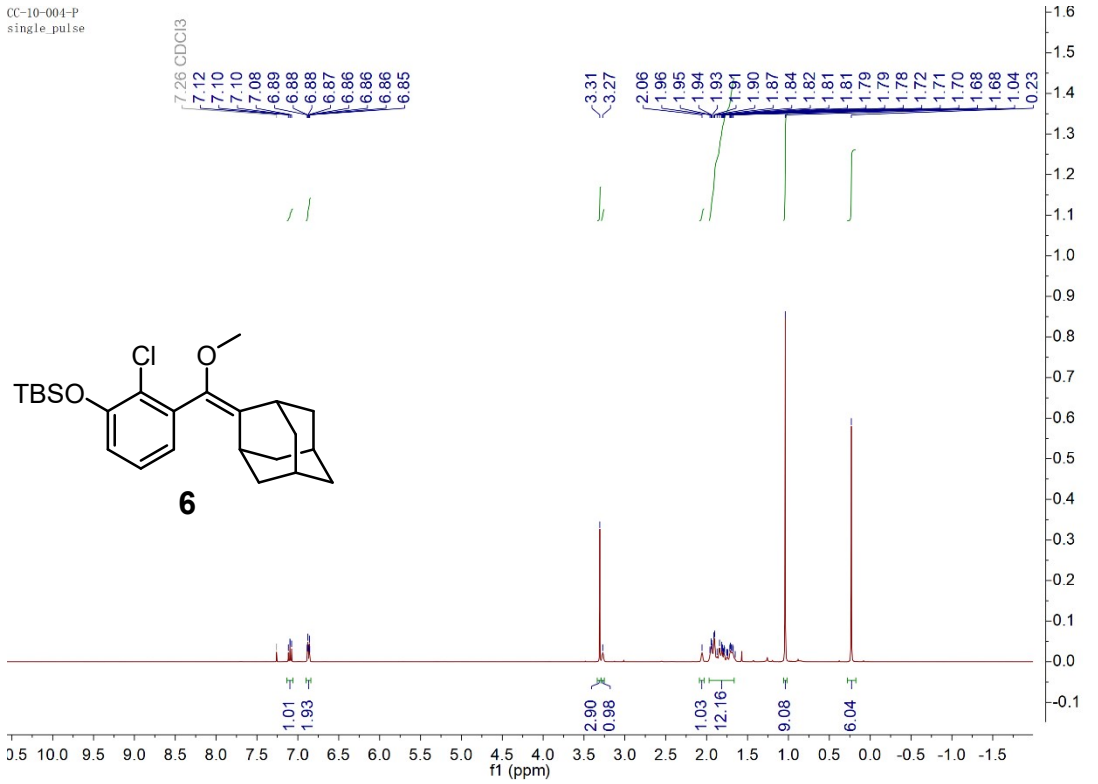
^{1}H-NMR of 4



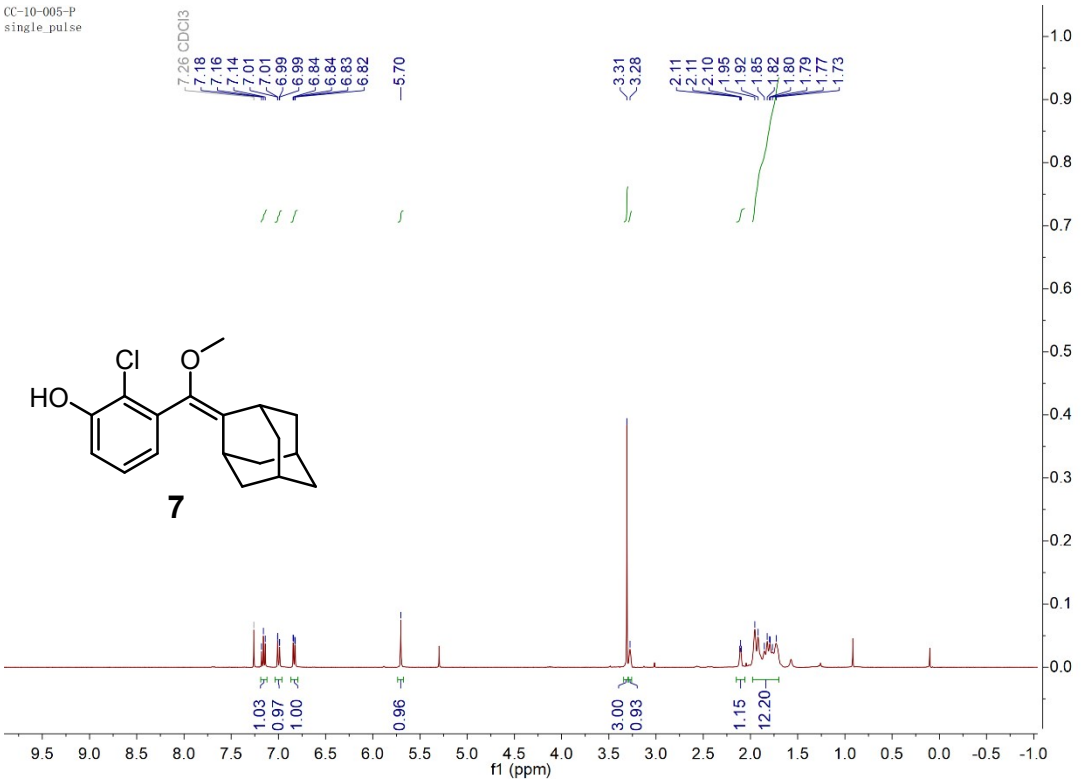
^{1}H-NMR of 5



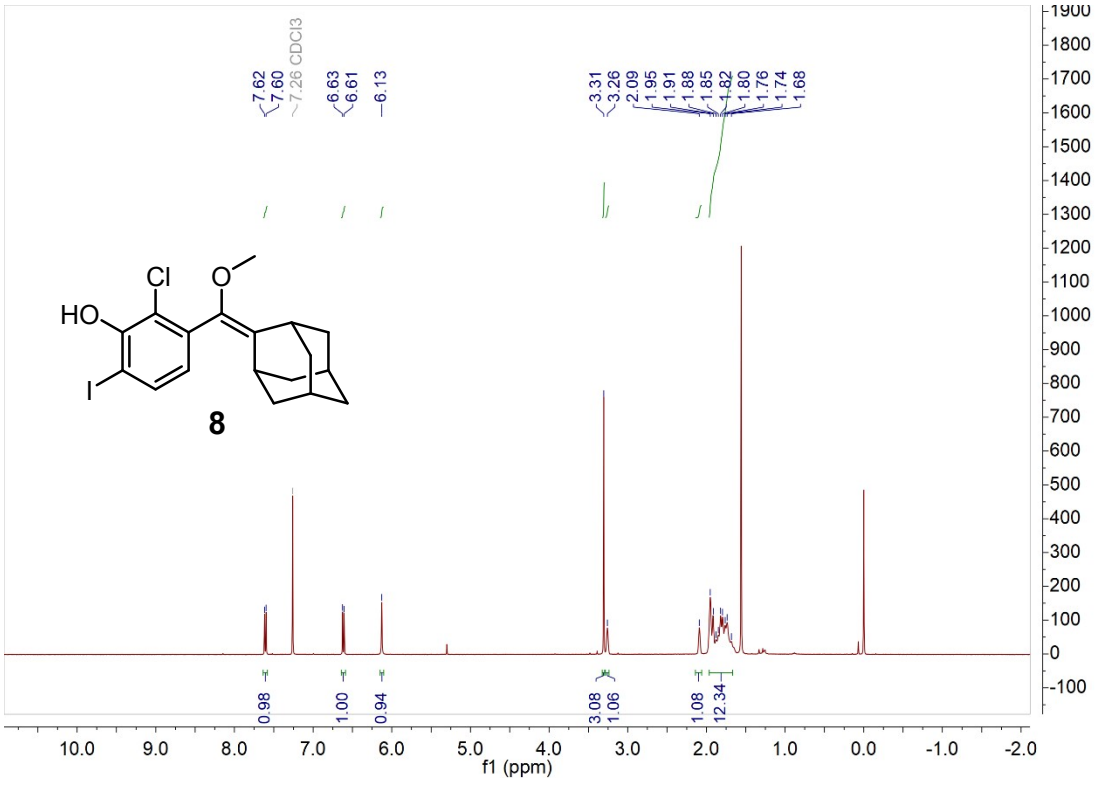
^{1}H-NMR of 6



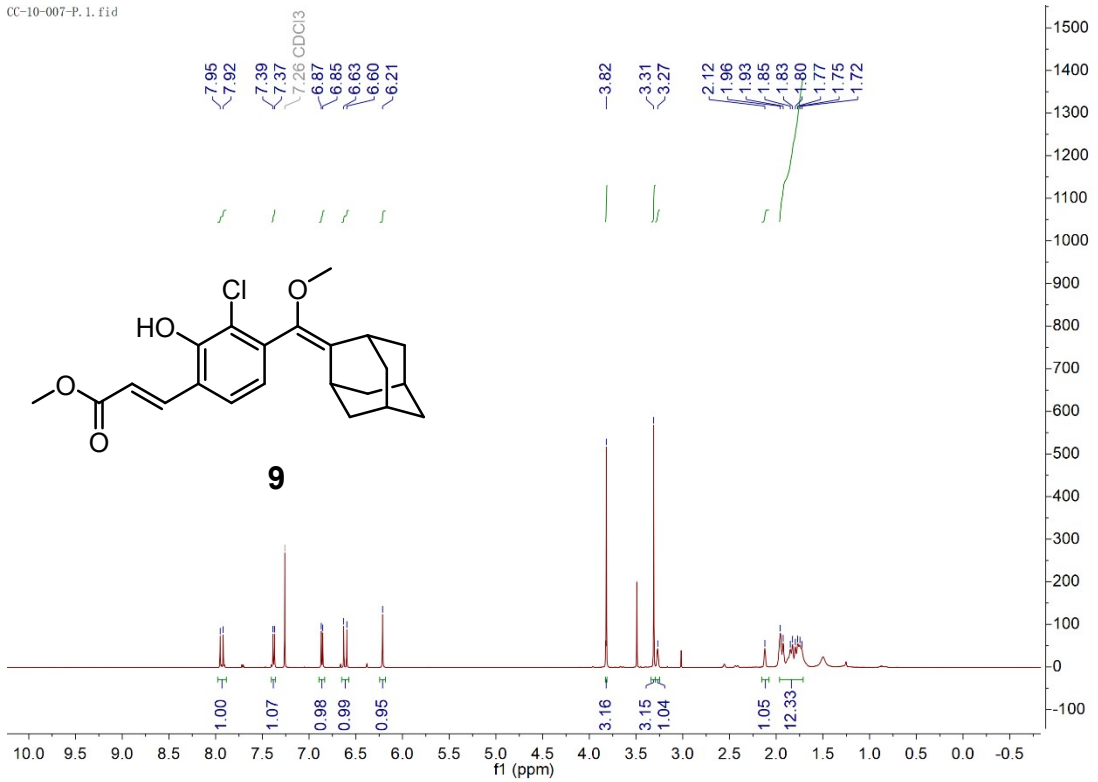
^{1}H-NMR of 7



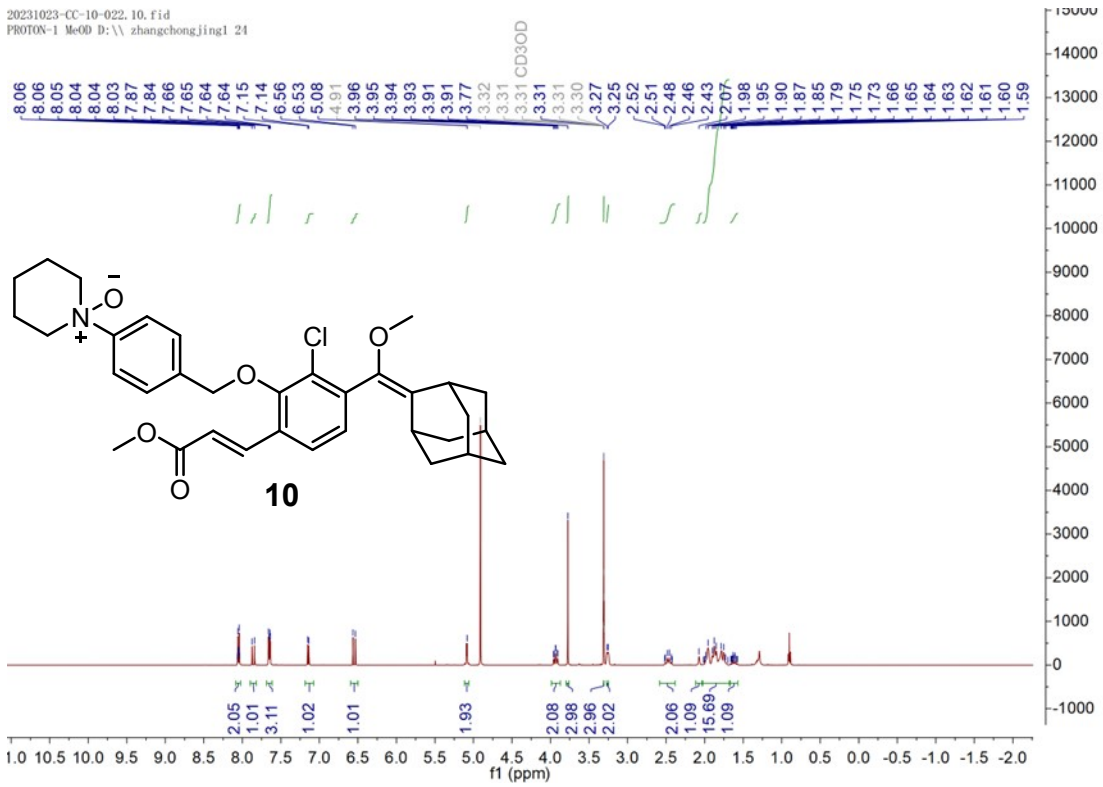
^{1}H-NMR of 8



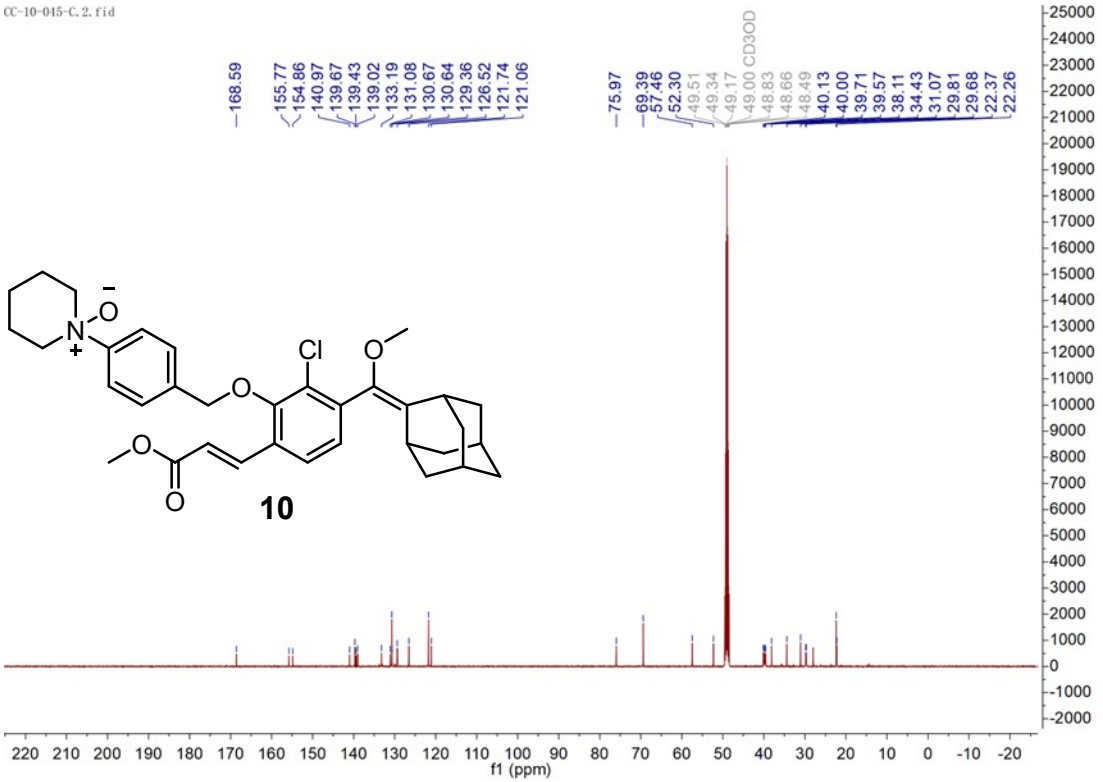
^{1}H-NMR of 9



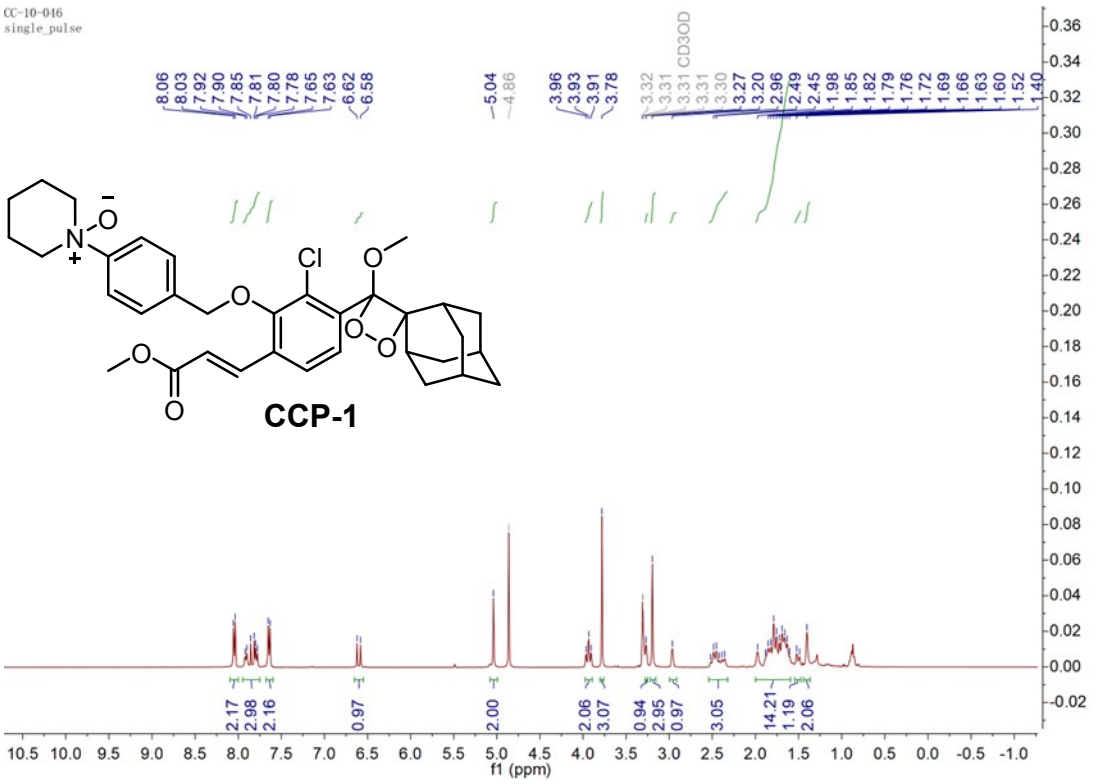
^{1}H-NMR of 10



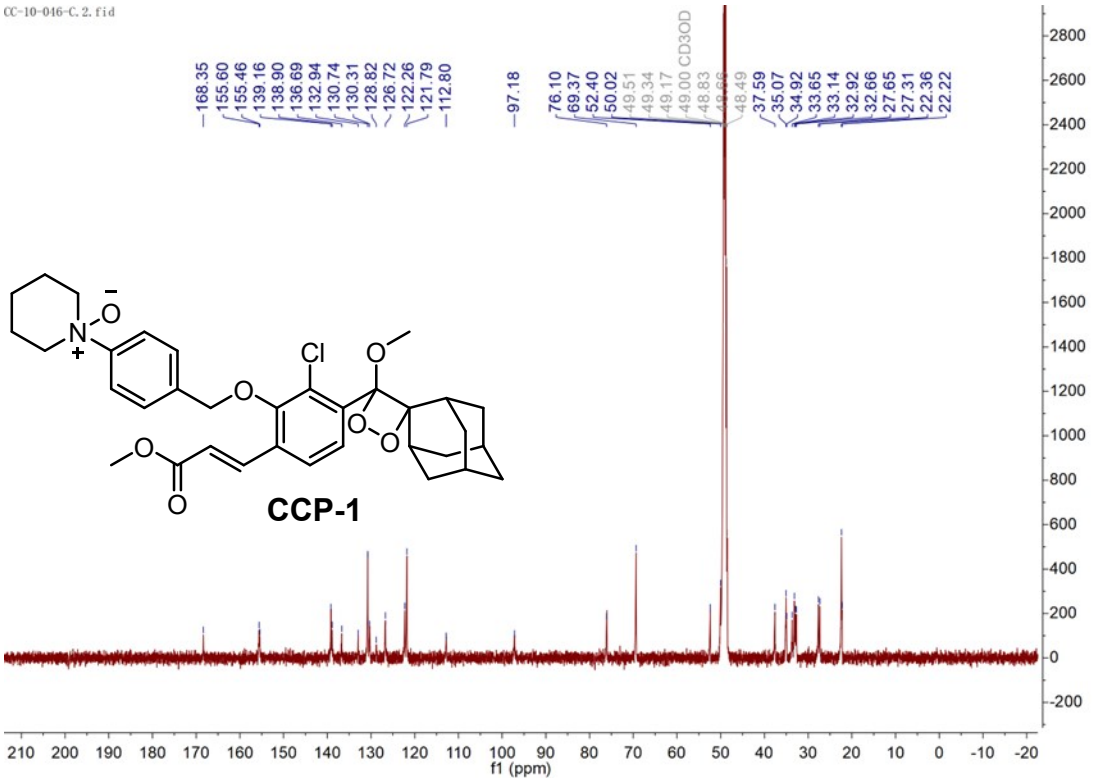
^{13}C-NMR of 10



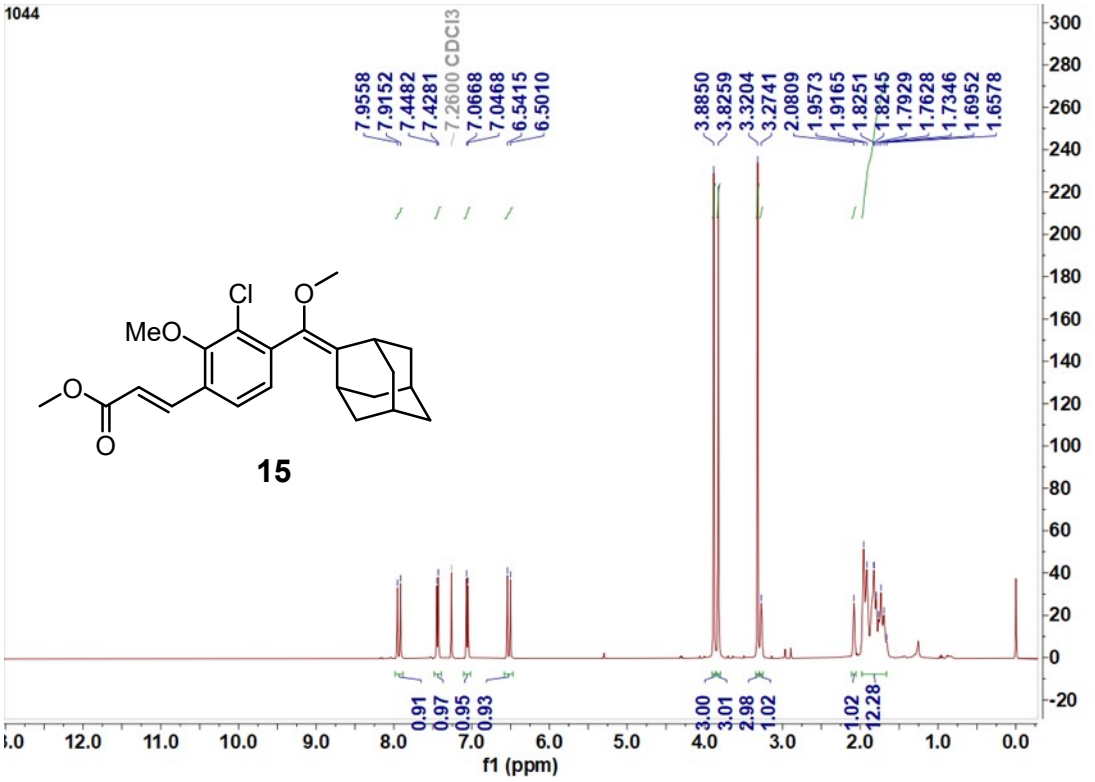
^{1}H-NMR of CCP-1



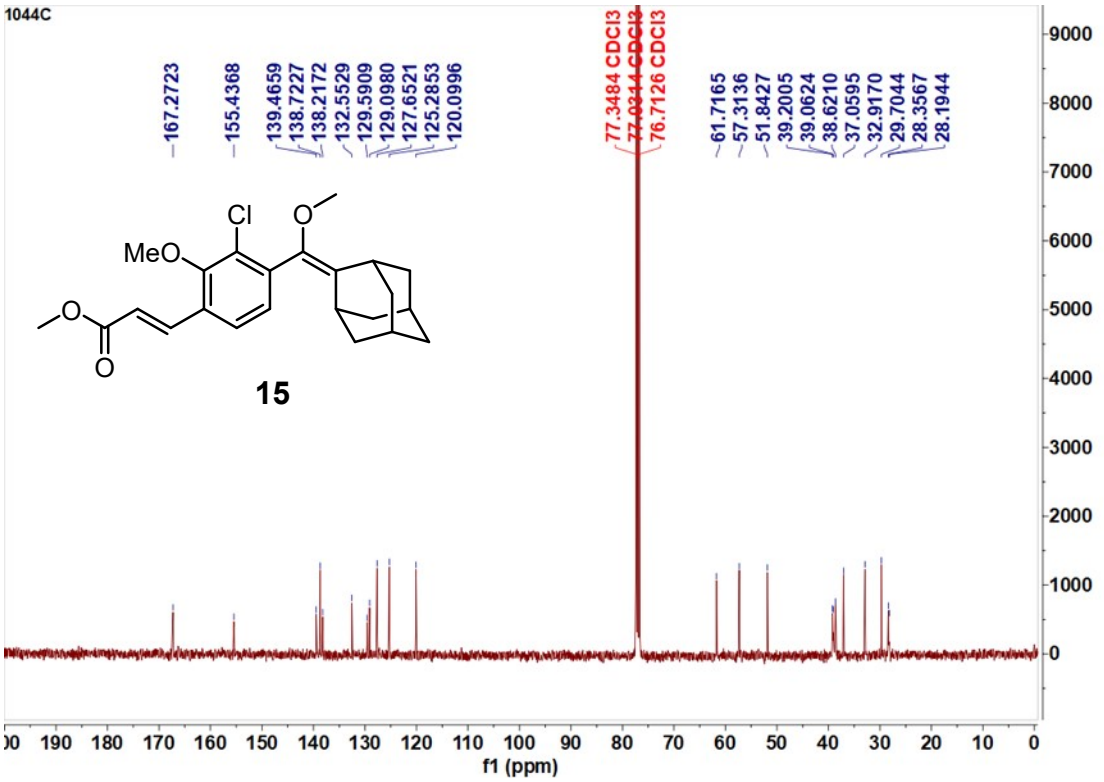
^{13}C-NMR of CCP-1



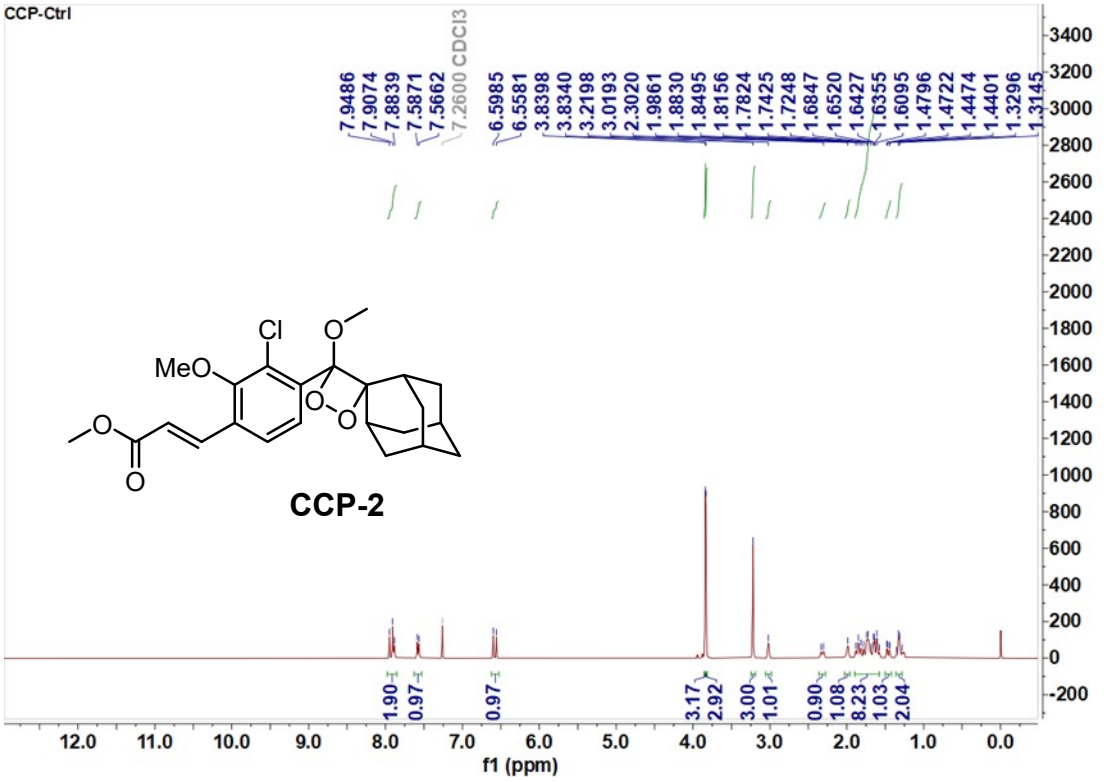
^{1}H-NMR of 15



^{13}C-NMR of 15



^{1}H-NMR of CCP-2



^{13}C-NMR of CCP-2