Electronic Supplementary Information

Study on tunable AIE (AIEE) of boron ketoiminate-based conjugated polymers for live cell imaging

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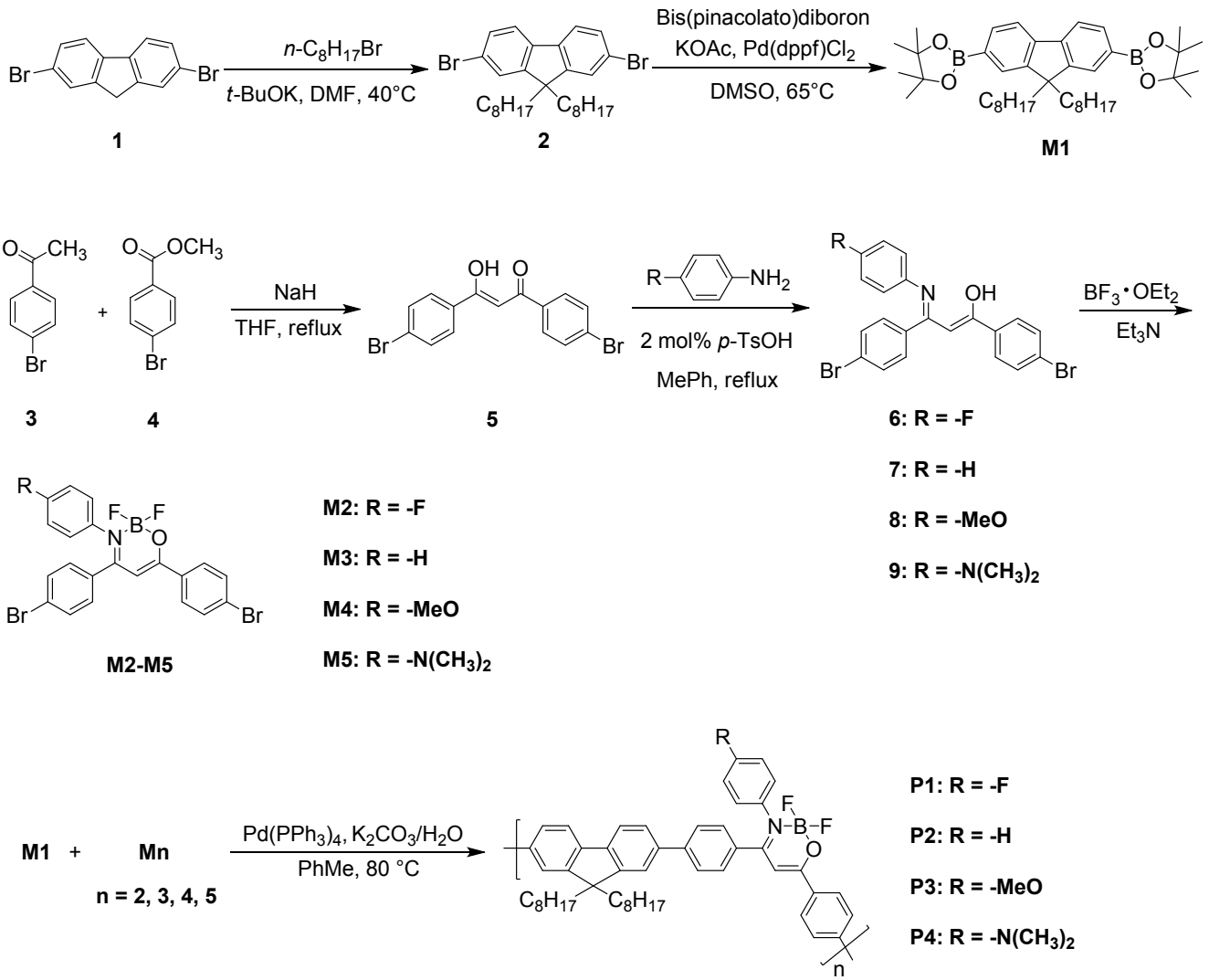
Contents:

ESI 1. Measurements and materials

All solvents and reagents were commercially available and analytical-reagent-grade. THF and Et_3N were further purified by distillation from sodium in the presence of benzophenone. NMR spectra were obtained using a 400-Bruker for ^1H NMR, 376 MHz for ^{19}F NMR and 100 MHz for ^{13}C NMR and reported as parts per million (ppm) from the internal standard TMS. Fluorescence spectra were obtained using a Fluoromax-4 Spectrofluorometer. Time-resolved fluorescence decays were recorded with an Edinburgh Instrument FLS 920 fluorospectrophotometer. UV-vis absorption spectra were obtained using a Perkin-Elmer Lambda 35 spectrophotometer. Mass spectra (MS) were determined on a Micromass GC-TOF (EI). C, H and N of elemental analyses were performed on an Elementar Vario MICRO analyzer. Molecular weight of the polymers were determined by GPC with Waters-244 HPLC pump and THF was used as solvent and relative to polystyrene standards. The particle size distributions of CPNs were measured by dynamic light scattering (DLS) using a particle size analyser (BI-200SM, Brookhaven instruments Corp., Holtsville, NY). The morphology of the CPNs were characterized on field emission scanning electron microscope (FESEM, Hitachi, S-4800) at an accelerating voltage of 5.0 kV. The nanoparticles suspension sample for SEM measurement was dropped onto silicon slice, surface morphology was evaluated after the water evaporation. Confocal laser confocal scanning microscope images of CPNs were taken on a Olympus Fluo-view 1000. 2,7-dibromo-9*H*-fluorene, 1-(4-bromophenyl)ethanone, methyl 4-bromobenzoate, substituted anilines, boron trifluoride etherate, bis(pinacolato)diboron and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from sigma-Aldrich and used without further purification. Milli-Q Water (18.2M) was used to prepare the buffer solutions from the 10×PBS stock buffers.

ESI 2. Synthesis of the monomers and conjugated polymers

The procedures for the monomers **M1**-**M5** and the boron ketoiminate-based conjugated polymers **P1**-**P4** are outlined in Scheme S1. 2,2'-(9,9-dioctyl-9*H*-fluorene-2,7-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (**M1**),[1] (*Z*)-1,3-bis(4-bromophenyl)-3-hydroxyprop-2-en-1-one (**5**),[2] (1*Z*,3*Z*)-1,3-bis(4-bromophenyl)-3-(substituted phenylimino)prop -1-en-1-ol (**6-9**),[3] 4,6-bis(4-bromophenyl)-2,2-difluoro-3-substituted phenyl-2*H*-1,3,2-oxazaborinin-3-ium-2-uide (**M2**-**M5**),[3] were prepared according to the procedure described in the literature. The conjugated polymers **P1**-**P4** were prepared from palladium-catalyzed Suzuki coupling reaction.



Scheme S1. Synthetic procedures for the monomers **M1**-**M5** and the conjugated polymers **P1**-**P4**.

Synthesis of 6 (R = F)

A mixture of compound **5** (3.82 g, 10.0 mmol), 4-fluoroaniline (1.39 g, 12.5 mmol) and *p*-toluenesulfonic acid (0.05 g) in 100 mL of anhydrous toluene was refluxed in a 250 mL flask equipped with Dean-Stark apparatus for 36 h. After cooling to room temperature, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on neutral aluminum oxide column using petroleum ether/ethyl acetate (30:1, *v/v*) as eluent to give the product **6** as a yellow solid (1.33 g, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.78 (s, 1 H, OH), 7.83–7.79 (m, 2H, Ar-H), 7.60–7.56 (m, 2H, Ar-H), 7.49–7.46 (m, 2H, Ar-H), 7.25–7.21 (m, 2H, Ar-H), 6.89–6.84 (m, 2H, Ar-H), 6.79–6.75 (m, 2H, Ar-H), 5.99 [s, 1H, C(O)CHC(N)]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.5, 161.0, 160.9, 158.6, 138.4, 135.1, 134.2, 132.0, 131.7, 130.0, 128.9, 126.3, 125.2, 125.1, 124.3, 115.9, 115.7, 96.4, 77.3, 77.0, 76.7; MS (EI, *m/z*): 475.2 (M^+); anal. calcd for $C_{21}H_{14}Br_2FNO$: C, 53.08; H, 2.97; N, 2.95. Found: C, 52.92; H, 3.07; N, 2.78%.

Synthesis of 7 (R = H)

7 was synthesized from aniline in 32% yield by following the same procedure as the preparation of **6**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.82 (s, 1H, OH), 7.83–7.80 (m, 2H, Ar-H), 7.59–7.56 (m, 2H, Ar-H), 7.48–7.45 (m, 2H, Ar-H), 7.27–7.24 (m, 2H, Ar-H), 7.18–7.14 (m, 2H, Ar-H), 7.05–7.01 (t, J = 7.4 Hz, 1H, Ar-H), 6.79 (d, J = 7.6 Hz, 2H, Ar-H), 5.98 [s, 1H, C(O)CHC(N)]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.3, 160.6, 139.0, 138.5, 134.5, 131.9, 131.6, 130.0, 129.0, 128.9, 126.2, 124.6, 124.2, 123.4, 96.5, 77.3, 77.0, 76.7; MS (EI, *m/z*): 457.1 (M^+); anal. calcd for $C_{21}H_{15}Br_2NO$: C, 55.17; H, 3.31; N, 3.06. Found: C, 55.01; H, 3.40; N, 3.13%.

Synthesis of 8 (R = OCH_3)

8 was synthesized from 4-methoxyaniline in 35% yield by following the same procedure as the preparation of **6**: bright yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.82 (s, 1H, OH), 7.81 (d, J = 8.5 Hz, 2H, Ar-H), 7.57 (d, J = 8.5 Hz, 2H, Ar-H), 7.46 (d, J = 8.5 Hz, 2H, Ar-H), 7.23 (d, J = 8.5 Hz, 2H, Ar-H), 6.76–6.74 (m, 2H, Ar-H), 6.71–6.69 (m, 2H, Ar-H), 5.94 [s, 1H, C(O)CHC(N)], 3.74 (s, 1H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.0, 161.2, 156.9, 138.6, 134.6, 131.9, 131.8, 131.6, 130.0, 128.8, 126.0, 125.1, 124.0, 114.2, 95.6, 77.3, 77.0, 76.7, 55.4; MS (EI, *m/z*): 487.2 (M^+); anal. calcd for $C_{22}H_{17}Br_2NO_2$: C, 54.24; H, 3.52; N, 2.88. Found: C, 54.03; H, 3.53; N, 2.97%.

Synthesis of 9 (R = $N(CH_3)_2$)

9 was synthesized from N,N-dimethylbenzene-1,4-diamine in 37% yield by following the same procedure as the preparation of **6**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.88 (s, 1 H, OH), 7.80 (d, J = 8.5 Hz, 2H, Ar-H), 7.56 (d, 2H, J = 8.5 Hz, Ar-H), 7.45 (d, J = 10.6 Hz, 2H, Ar-H), 7.25 (d, J = 8.6 Hz, 2H, Ar-H), 6.70 (d, J = 8.9 Hz, 2H, Ar-H), 6.52 (d, J = 8.7 Hz, 2H, Ar-H), 5.89 [s, 1H, C(O)CHC(N)], 2.89 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 187.5, 161.3, 138.8, 134.9, 131.7, 131.5, 130.0, 128.7, 125.7, 124.9, 123.8, 112.5, 95.0, 77.3, 77.0, 76.7, 40.6; MS (EI, *m/z*): 500.2 (M^+); anal. calcd for $C_{23}H_{20}Br_2N_2O$: C, 55.22; H, 4.03; N, 5.60. Found: C, 55.11; H, 3.93; N, 4.08%.

Synthesis of M2 (R = F)

Compound **6** (0.95 g, 2.0 mmol) was dissolved in 30 mL toluene, followed by the addition of triethylamine (1 mL) and $BF_3{\cdot}Et_2O$ (2 mL). The mixture was stirred under N_2 for 2 h at 80 ºC. After

the toluene was removed, the residues were purified by flash column chromatography on neutral aluminum oxide column using petroleum ether/ethyl acetate (5:1, *v*/*v*) as eluent to afford compound **M2** as a yellow solid (1.04 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90–7.87 (m, 2H, Ar-H), 7.64–7.61 (m, 2H, Ar-H), 7.47–7.44 (m, 2H, Ar-H), 7.12–7.09 (m, 4H, Ar-H), 6.97–6.91 (m, 2H, Ar-H), 6.32 [s, 1H, C(O)CHC(N)]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 169.9, 162.9, 160.4, 136.1, 133.3, 132.2, 132.1, 131.7, 130.0, 129.2, 128.5, 128.4, 128.2, 125.6, 116.1, 115.9, 96.6, 77.3, 77.0, 76.7; ^{19}F NMR (376 MHz, $CDCl_3$): δ -113.25 (s), -133.87 (q, $J_{F\text{-}F}$ = 28.7 Hz, $J_{B\text{-}F}$ = 12.5 Hz); MS (EI, *m/z*): 523.0 (M^+); anal. calcd for $C_{21}H_{13}BBr_2F_3NO$: C, 48.23; H, 2.51; N, 2.68. Found: C, 48.11; H, 2.53; N, 2.81%.

Synthesis of M3 (R = H)

M3 was synthesized from the starting compound **7** in 91% yield by following the same procedure as the preparation of **M2**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91–7.88 (m, 2H, Ar-H), 7.65–7.61 (m, 2H, Ar-H), 7.44–7.41 (m, 2H, Ar-H), 7.28–7.26 (m, 1H, Ar-H), 7.24–7.20 (m, 2H, Ar-H), 7.14–7.10 (m, 4H, Ar-H), 6.32 [s, 1H, C(O)CHC(N)]; ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 169.6, 140.2, 133.6, 132.2, 131.9, 130.1, 129.1, 129.0, 128.0, 127.7, 126.8, 125.4, 96.6, 77.3, 77.0, 76.7; ^{19}F NMR (376 MHz, $CDCl_3$): δ -134.01 (q, $J_{F\text{-}F}$ = 29.0 Hz, $J_{B\text{-}F}$ = 13.0 Hz); MS (EI, *m/z*): 504.96 (M^+); anal. calcd for $C_{21}H_{14}BBr_2F_2NO$: C, 49.95; H, 2.79; N, 2.77. Found: C, 49.83; H, 2.86; N, 2.69%.

Synthesis of M4 (R = OCH_3)

M4 was synthesized from the starting compound **8** in 90% yield by following the same procedure as the preparation of **M2**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90–7.87 (m, 2H, Ar-H), 7.64–7.60 (m, 2H, Ar-H), 7.46–7.42 (m, 2H, Ar-H), 7.14–7.11 (m, 2H, Ar-H), 7.06–7.03 (m, 2H, Ar-H), 6.78–6.74 (m, 2H, Ar-H), 6.30 [s, 1H, C(O)CHC(N)], 3.76 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 169.3, 158.8, 133.7, 133.0, 132.1, 131.9, 130.1, 129.1, 127.9, 127.7, 125.2, 114.2, 96.5, 77.3, 77.0, 76.7, 55.4; ^{19}F NMR (376 MHz, $CDCl_3$): δ -134.40 (q, $J_{F\text{-}F}$ = 28.8 Hz, $J_{B\text{-}F}$ = 12.6 Hz); MS (EI, *m/z*): 535.0 (M^+); anal. calcd for $C_{22}H_{16}BBr_2F_2NO_2$: C, 49.39; H, 3.01; N, 2.62. Found: C, 49.26; H, 3.02; N, 2.49%.

Synthesis of M5 (R = $N(CH_3)_2$)

M5 was synthesized from the starting compound **9** in 93% yield by following the same procedure as the preparation of **M2**: yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89–7.86 (m, 2H, Ar-H), 7.62–7.60 (m, 2H, Ar-H), 7.45–7.43 (m, 2H, Ar-H), 7.16–7.14 (m, 2H, Ar-H), 6.97 (d, J = 8.8 Hz, 2H, Ar-H), 6.55 (d, J = 8.2 Hz, 2H, Ar-H), 6.28 [s, 1H, C(O)CHC(N)], 2.92 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 168.4, 134.2, 132.1, 131.9, 130.2, 129.0, 127.5, 127.2, 125.0, 112.3, 96.7, 77.3, 76.9, 76.7, 40.5; ^{19}F NMR (376 MHz, $CDCl_3$): δ -135.00 (q, $J_{F\text{-}F}$ = 32.3 Hz, $J_{B\text{-}F}$ = 12.3 Hz); MS (EI, *m/z*): 548.0 (M^+); anal. calcd for $C_{23}H_{19}BBr_2F_2N_2O$: C, 50.41; H, 3.49; N, 5.11. Found: C, 50.25; H, 3.45; N, 5.20%.

Synthesis of P1 (R = F)

A mixture of **M1** (321.3 mg, 0.50 mmol), **M2** (252.5 mg, 0.50 mmol), and $[Pd(PPh_3)_4]$ (28.89 mg, 0.025 mmol) was added in a 100 mL Schlenk tube. 20 mL of K_2CO_3 aqueous solution (2 mol/L), and 30 mL of toluene added by syringe after the tube was evacuated and refilled with Ar three times.

The mixed solution was stirred at 80 °C for 24 h under Ar atmosphere. After cooling to room temperature, the resulting mixture was extracted with toluene for three times. Organic layer were combined, washed with water, dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure. The residue was dissolved in a small quantity of THF. The resulting mixture was added into 50 mL of methanol to precipitate the polymer. The polymer **P1** was collected as a dark yellow solid (307.2 mg). Yield: 81%. GPC results: M_w = 43980, M_n = 25390, PDI = 1.73. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19–8.14 (m), 7.85–7.79 (m), 7.69–7.47 (m), 7.40 (d), 7.24–7.14 (m), 7.01–6.97 (t), 6.52 (s), 2.08–2.05 (m), 1.59 (s), 1.26–1.09 (m), 0.88–0.71 (m).

Synthesis of P2 (R = H)

P2 was synthesized from monomers **M1** and **M3** in 85% yield by following the same procedure as the preparation of **P1**: dark yellow solid. GPC results: M_w= 33820, M_n = 14180, PDI = 2.38. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19–8.14 (m), 7.85–7.76 (m), 7.70–7.52 (m), 7.45–7.40 (m), 7.32–7.28 (m), 7.23–7.15 (m), 6.52 (s), 3.76–3.73 (m), 3.66 (s), 3.49 (s), 3.31 (s), 2.35 (s), 2.24–2.20 (t), 2.08–2.01 (m), 1.87–1.84 (m), 1.44 (s), 1.26–1.09 (m), 0.90–0.71 (m).

Synthesis of P3 (R = OCH_3)

P3 was synthesized from monomers **M1** and **M4** in 90% yield by following the same procedure as the preparation of **P1**: dark yellow solid. GPC results: M_w= 33950, M_n = 19480, PDI = 1.74. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18–8.13 (m), 7.85–7.78 (m), 7.69–7.54 (m), 7.47–7.41 (m), 7.18 (d), 6.82–6.77 (m), 6.50 (s), 3.78 (s), 3.49 (s), 2.35 (s), 2.17 (s), 2.07–2.05 (m), 1.87–1.84 (m), 1.42 (s), 1.26–1.09 (m), 0.90–0.71 (m).

Synthesis of P4 (R = $N(CH_3)_2$)

P4 was synthesized from monomers **M1** and **M5** in 83% yield by following the same procedure as the preparation of **P1**: dark yellow solid. GPC results: M_w= 29310, M_n = 15150, PDI = 1.93. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d), 7.84–7.52 (m), 7.44 (d), 7.15 (d), 6.69 (brs), 6.48 (s), 2.96 (s), 2.05 (s), 1.40 (s), 1.25–1.09 (m), 0.81–0.71 (m).

ESI 3. Preparation of CPNs

The CPNs were prepared *via* reprecipitation method according to the known literature.[4] A stock solution in anhydrous THF (120 μM) of the conjugated polymer was further diluted to 30 μM in THF after stirring overnight at room temperature. The dilute solution was filtered with a 0.22 μm syringe filter. A 2 mL quantity of the above solution was injected rapidly into 8 mL Milli-Q Water under sonication. The mixture was further concentrated by evaporation under reduced pressure, followed by an additional filtration step with a 0.22 μm syringe filter to give the CPNs.

Reference:

1. (a) M. S. Maji, T. Pfeifer, and A. Studer, *Chem. Eur. J.*, 2010, **16**, 5872; (b) N. Berton, F. Lemasson, N. Stürzl, F. Hennrich, M. M. Kappes, and M. Mayor, *Chem. Mater.*, 2011, **23**, 2237.
2. C. Dai, Y. Wang, Y. Quan, Q. Chen, Y. Cheng, C. Zhu, *J. Polym. Sci., Part A: Polym. Chem.*, 2014, **52**, 3080.
3. X. Jiang, X. Liu, Y. Jiang, Y. Quan, Y. Cheng, and C. Zhu, *Macromol. Chem. Phys.*, 2014, **215**,

358.
4. B. Bao, N. Tao, D. Yang, L. Yuwen, L. Weng, Q. Fan, W. Huang, and L. Wang, *Chem. Commun.*, 2013, **49**, 10623.

ESI 4. Cell culture

For cellular imaging study, the human cervical carcinoma HeLa cells in vitro was chosen as an example. The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin), maintaining at 37 ˚C in a humidified atmosphere of 95% air and 5% CO_2. The cells were precultured prior to experiments until 90% confluence was reached.

ESI 5. Photostability of CPNs

The photostability of CPNs was evaluated by monitoring their respective fluorescence intensity changes upon incubation with a phosphate buffer solution (PBS) at 37 °C by using confocal microscopy. The CLSM images of each sample were recorded at 2 min interval under continuous laser scanning at an excitation wavelength of 405 nm with 5 mW laser power. The fluorescence intensity of each image was analyzed by Image Pro Plus software, and was further expressed by I/I_0, where I_0 is the initial fluorescent intensity of fresh CPNs suspension and I is that of CPNs suspension after continuous laser scanning.

ESI 6. Cytotoxicity evaluation

The cytotoxicity of CPNs against HeLa cells was estimated using MTT assays. HeLa cells were seeded in 96-well plates at a density of 4×10^4 cells mL^{-1}. After 24 h incubation, the medium was replaced by CPNs suspension at concentrations of 0, 5, 10, 20 and 40 μM and cultured at 37 °C for additional 48 h. The sample wells were washed twice with 1×PBS buffer and 100 μL of freshly prepared MTT (0.5 mg/mL) solution in culture medium was added into each well. The MTT medium solution was removed after 3 h incubation. DMSO (150 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of individual wells at 570 nm was then monitored by the microplate Reader (BioTek, PowerWave XS2, Vermont, USA). The absorbance of MTT in the sample well was determined by the differentiation between the absorbance of the sample well and that of the corresponding control well. Cell viability was expressed by the ratio of the absorbance of MTT in the sample wells to that of the cells incubated with culture medium only.

ESI 7. Cell staining and imaging

Fresh stock of HeLa cells was seeded into a glass bottom dish with a density of 1×10^5 cells per dish, and incubated for 24 h. Subsequently, the solution was then removed, and the adherent cells were washed with PBS buffer (2 mL×3). After coincubated with CPNs for 2 h, the culture medium was removed and the treated cells were washed three times with PBS (2 mL×3) before observation. Fluorescence imaging was performed with confocal laser scanning microscopy (Olympus, FV-1000; λ_{ex}=405 nm; fluorescent signals were collected at 500–600 nm for **P1**-**P3** NPs and 600–700 nm for **P4** NPs. The images were captured using a photomultiplier.

ESI 8. NMR spectra of M2-M5 and P1-P4 in $CDCl_3$

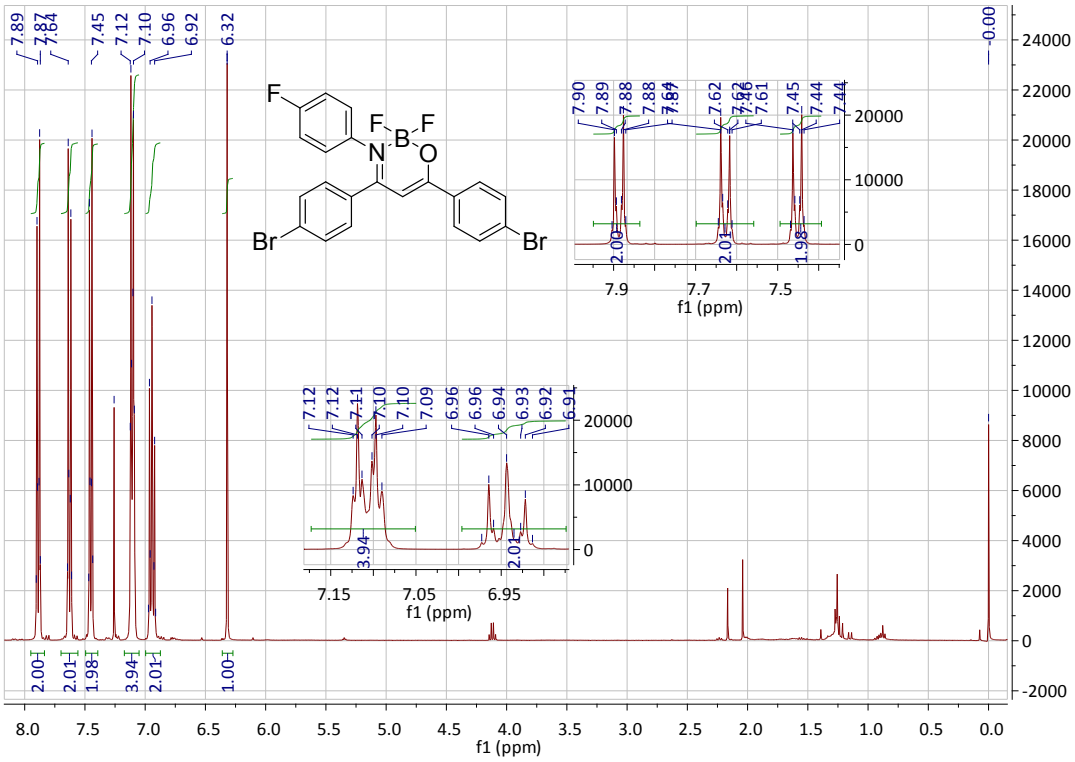


Figure S1. ^{1}H NMR of **M2** in $CDCl_3$

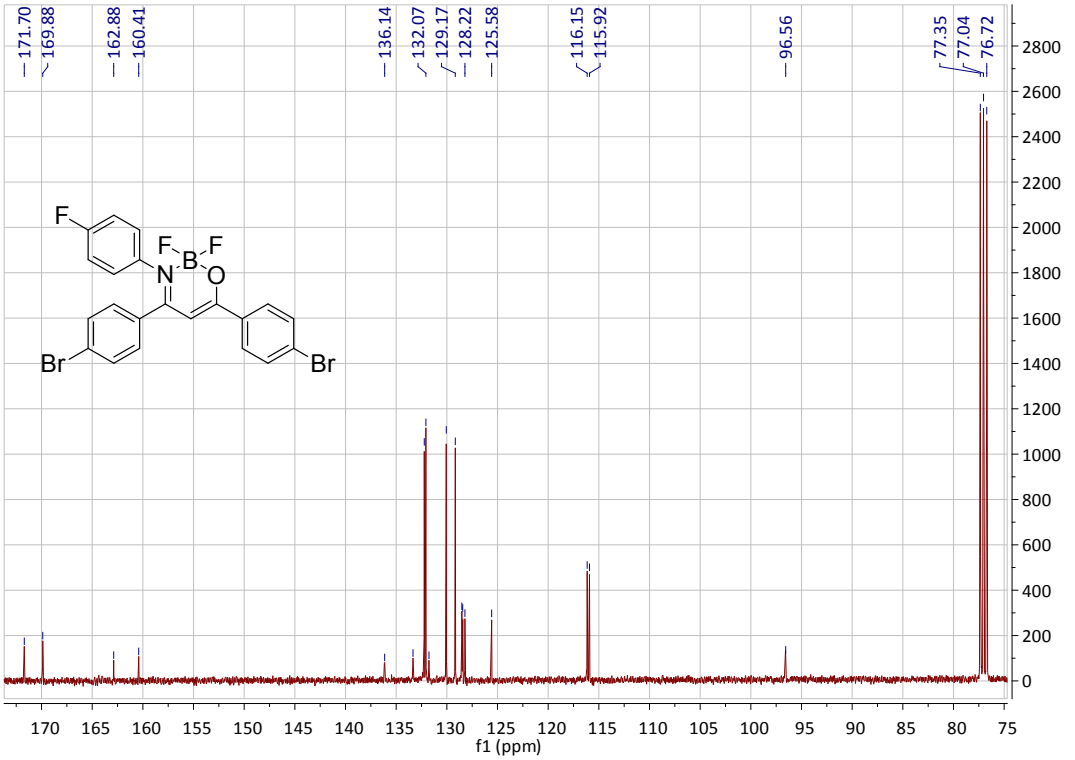


Figure S2. ^{13}C NMR of **M2** in $CDCl_3$

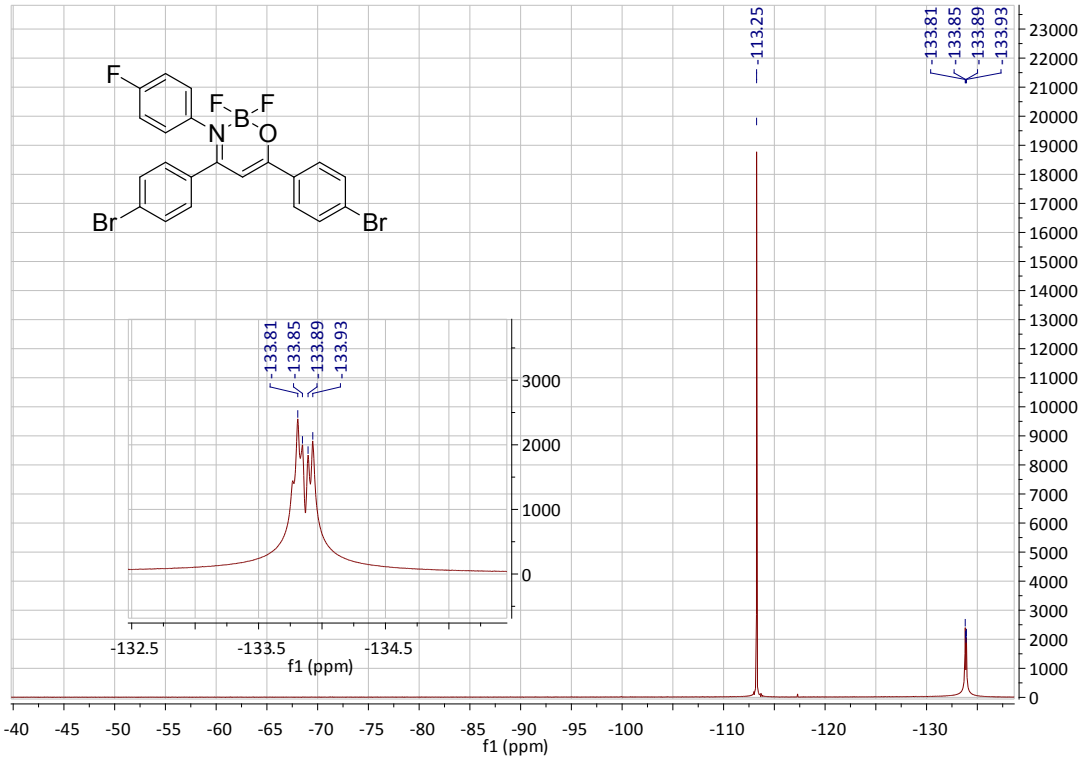


Figure S3. ^{19}F NMR of **M2** in $CDCl_3$

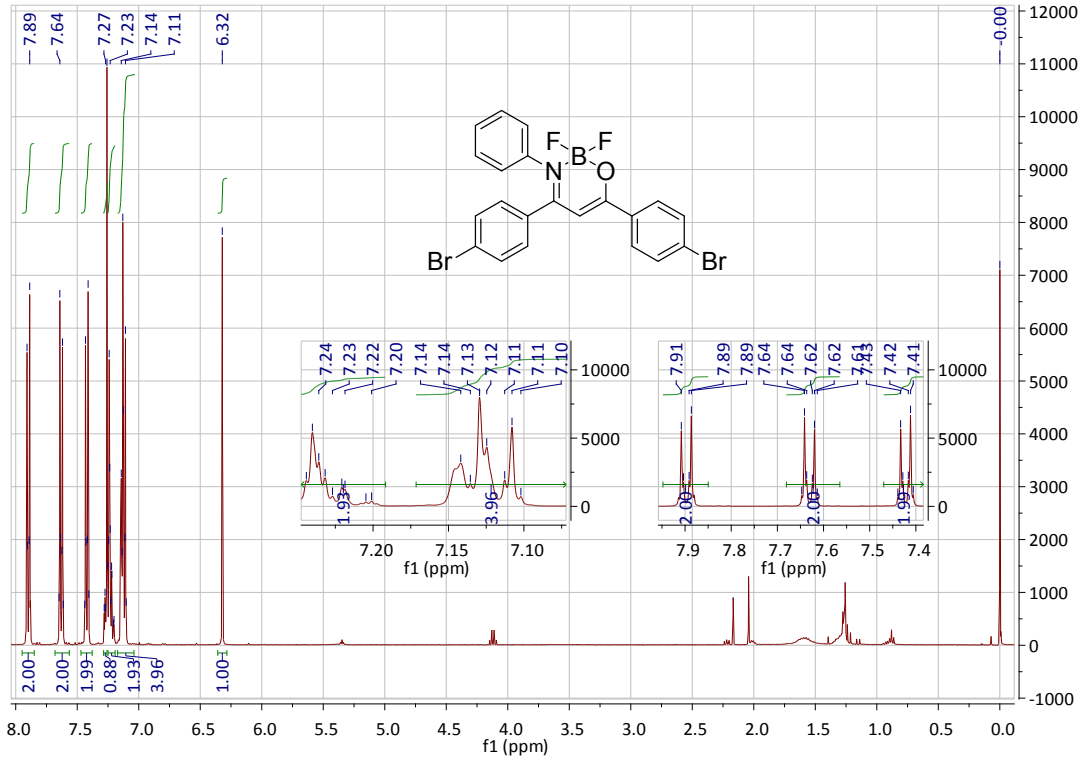


Figure S4. ^{1}H NMR of **M3** in $CDCl_3$

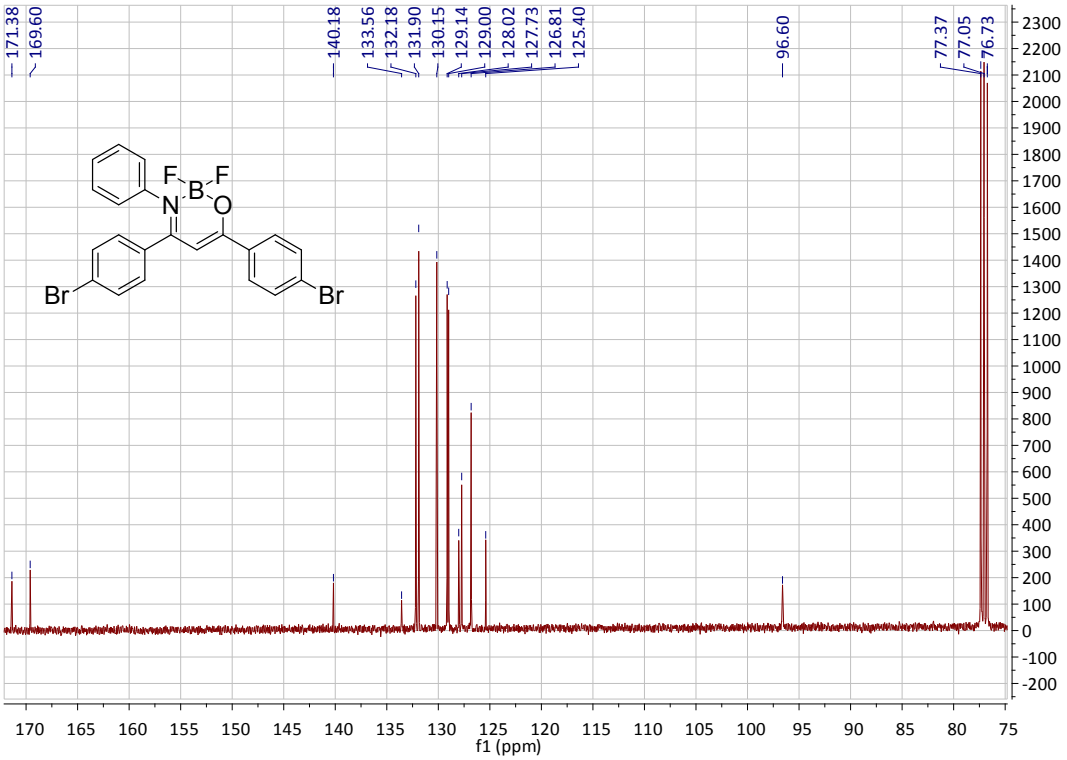


Figure S5. ^{13}C NMR of **M3** in $CDCl_3$

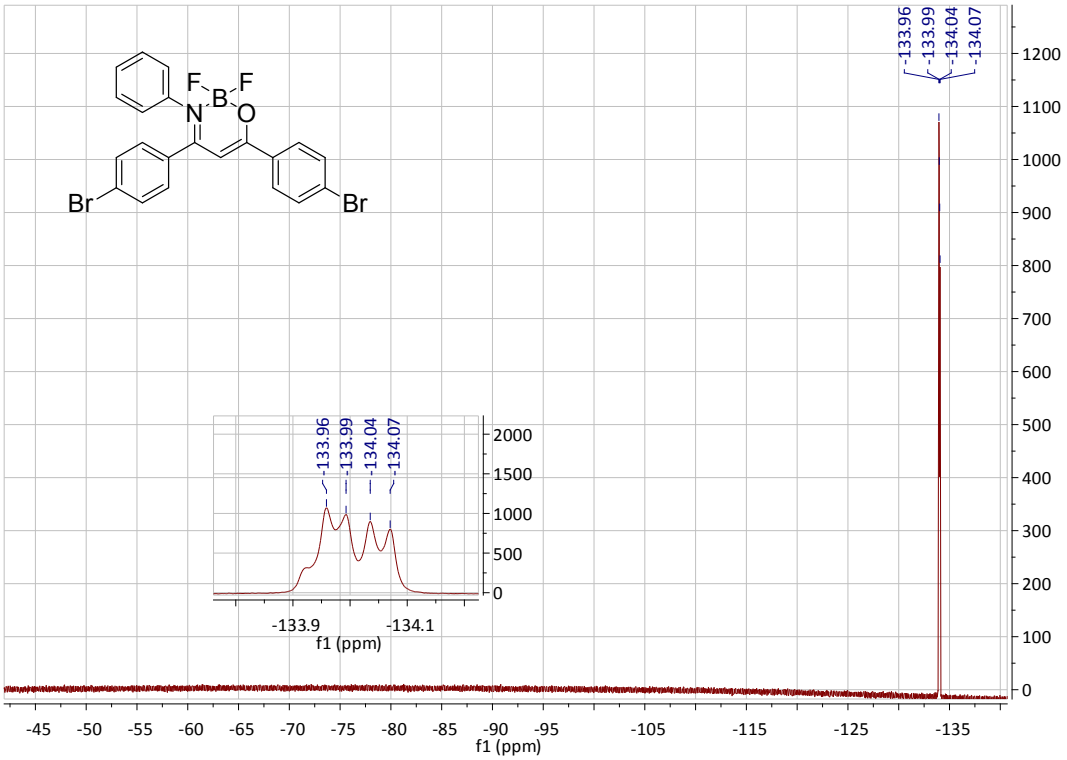


Figure S6. ^{19}F NMR of **M3** in $CDCl_3$

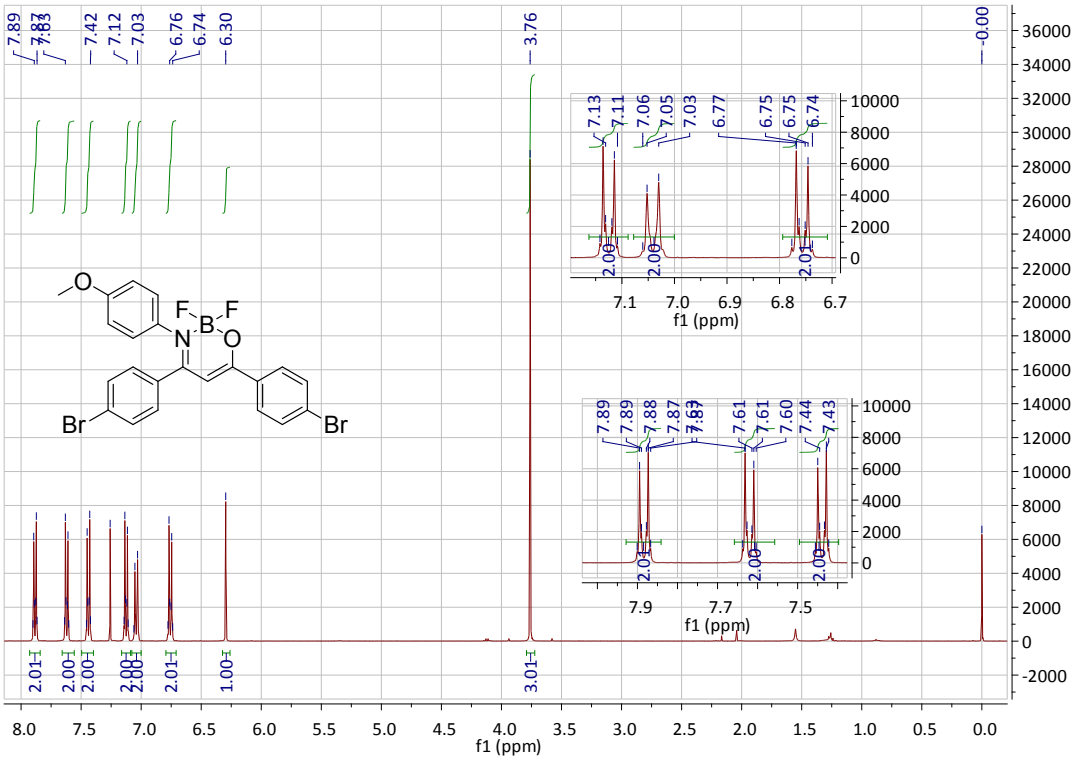


Figure S7. ^{1}H NMR of **M4** in $CDCl_3$

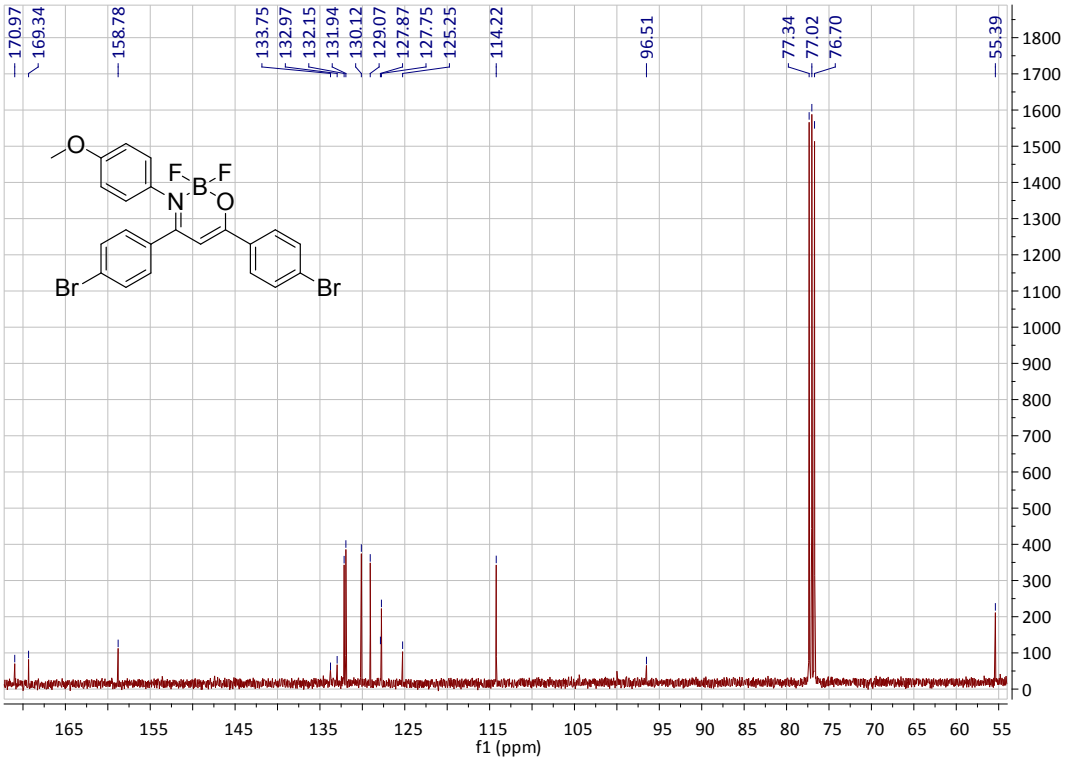


Figure S8. ^{13}C NMR of **M4** in $CDCl_3$

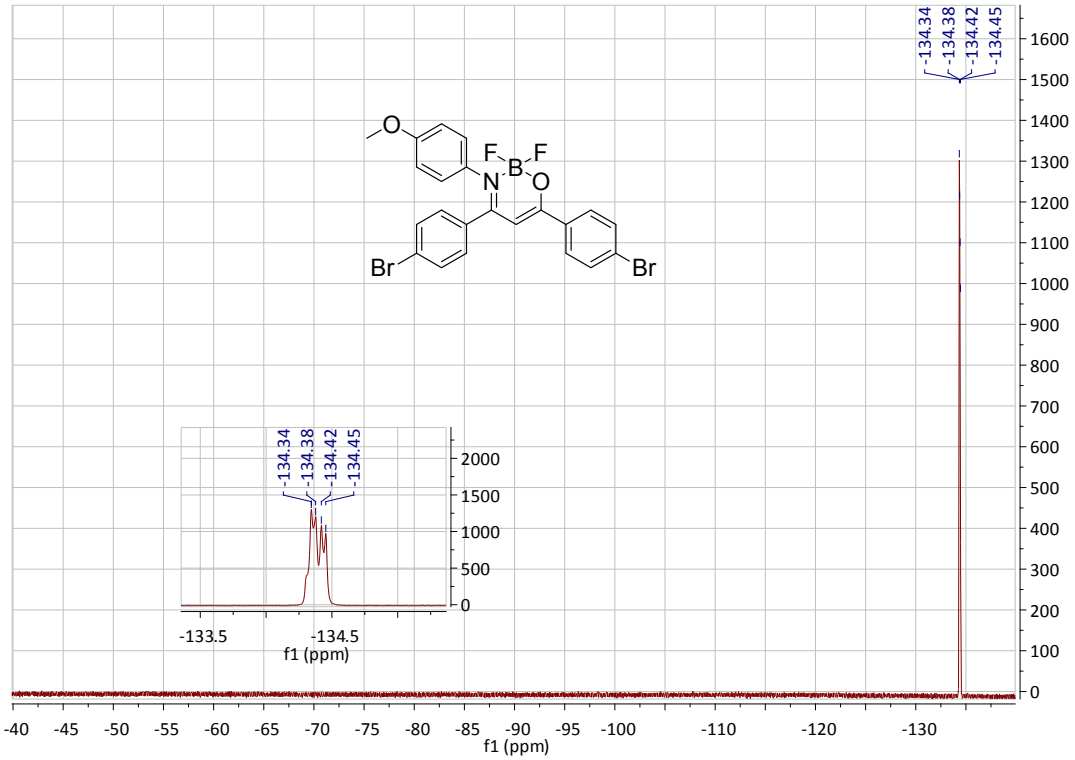


Figure S9. ^{19}F NMR of **M4** in $CDCl_3$

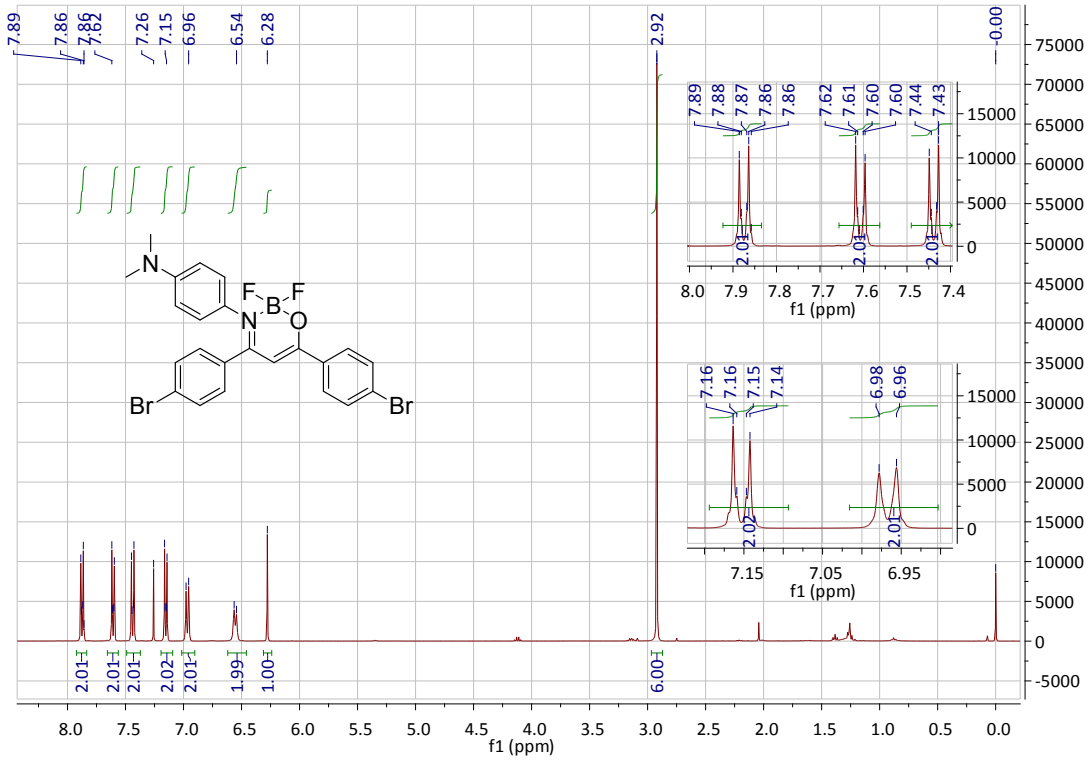


Figure S10. ^{1}H NMR of **M5** in $CDCl_3$

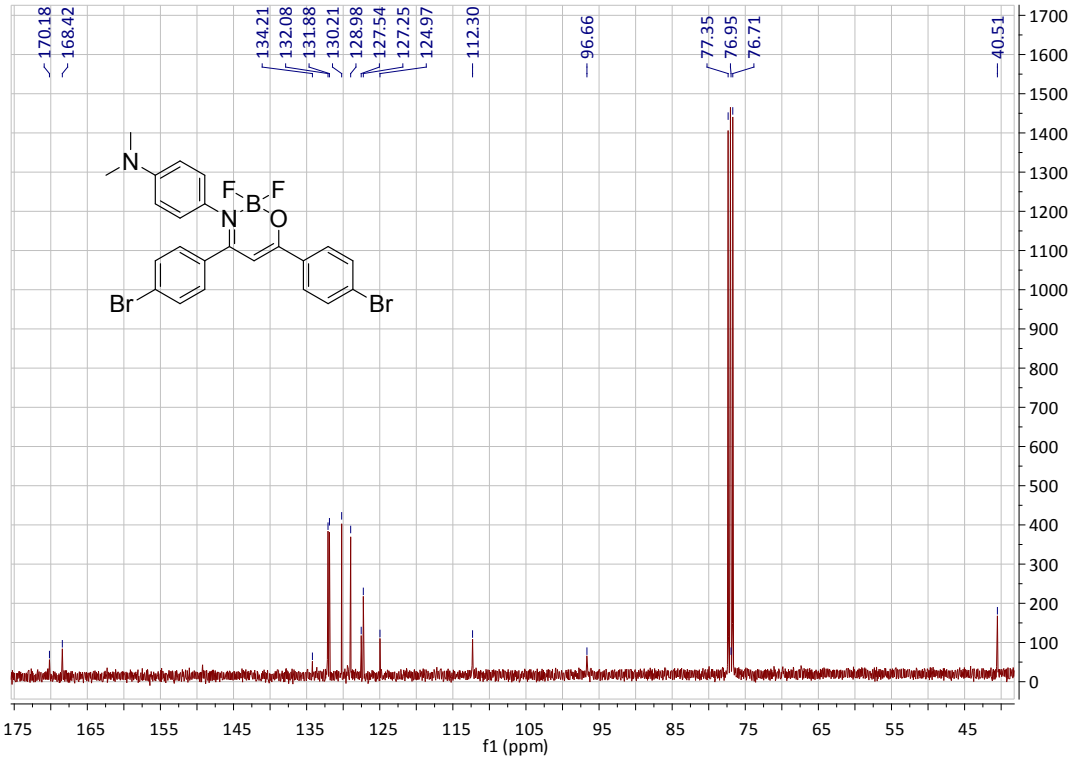


Figure S11. ^{13}C NMR of **M5** in $CDCl_3$

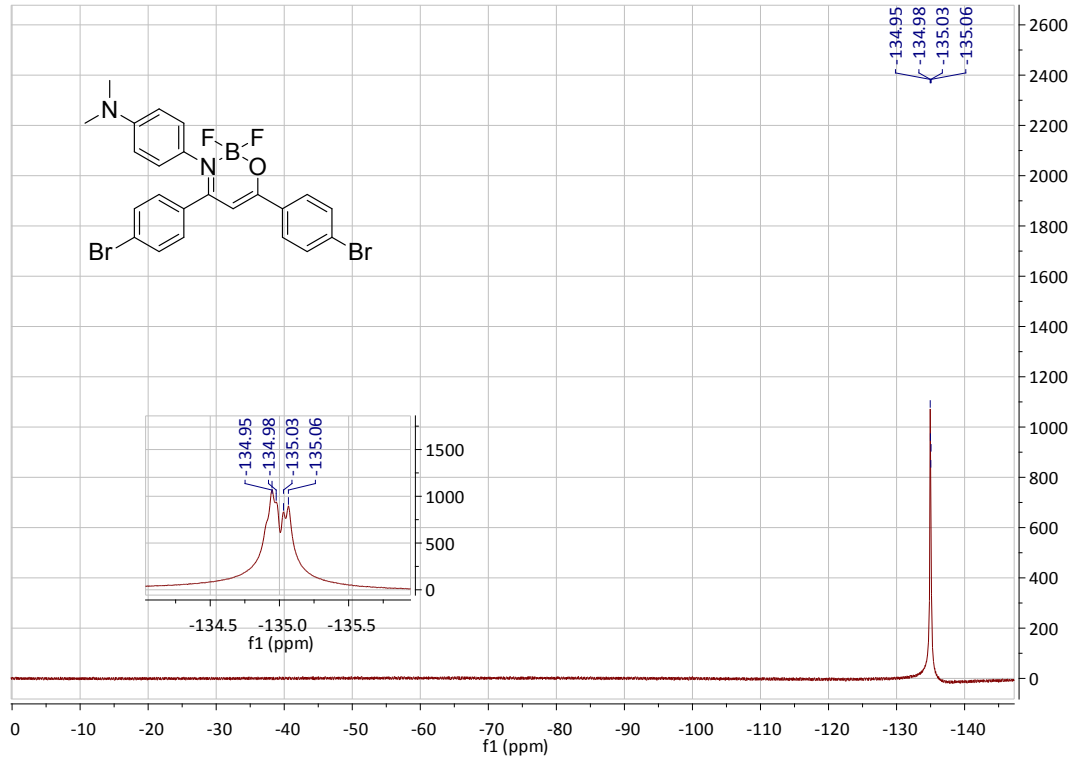


Figure S12. ^{19}F NMR of **M5** in $CDCl_3$

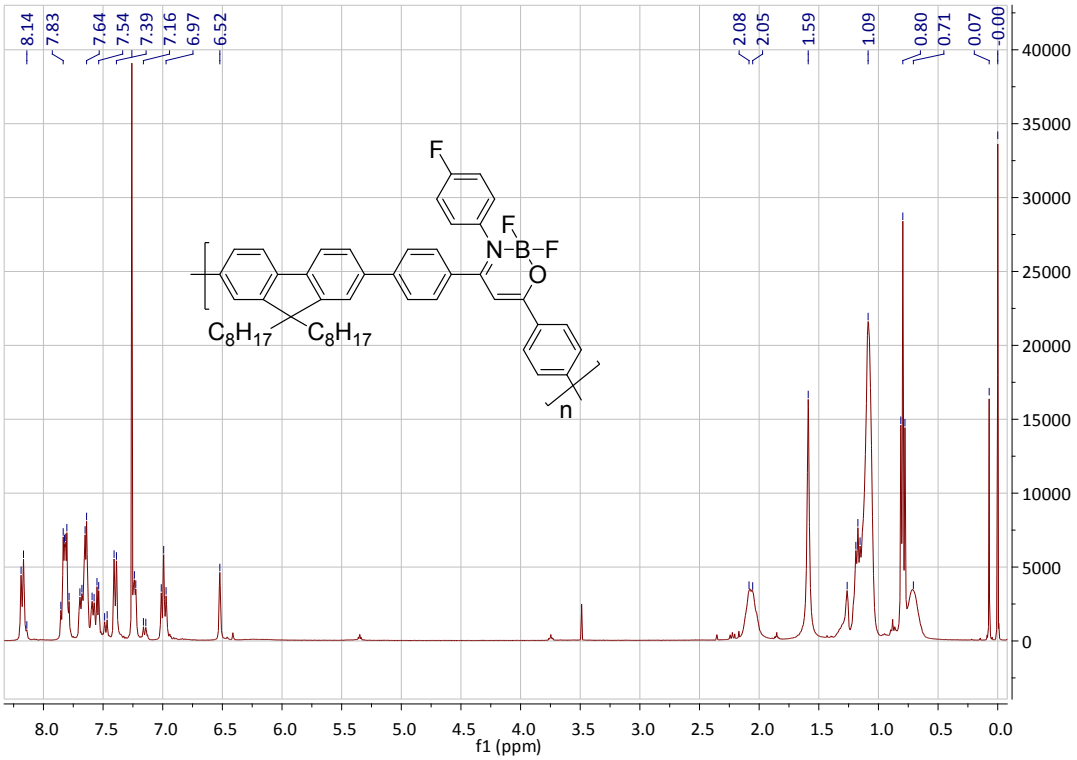


Figure S13. ^{1}H NMR of **P1** in $CDCl_3$

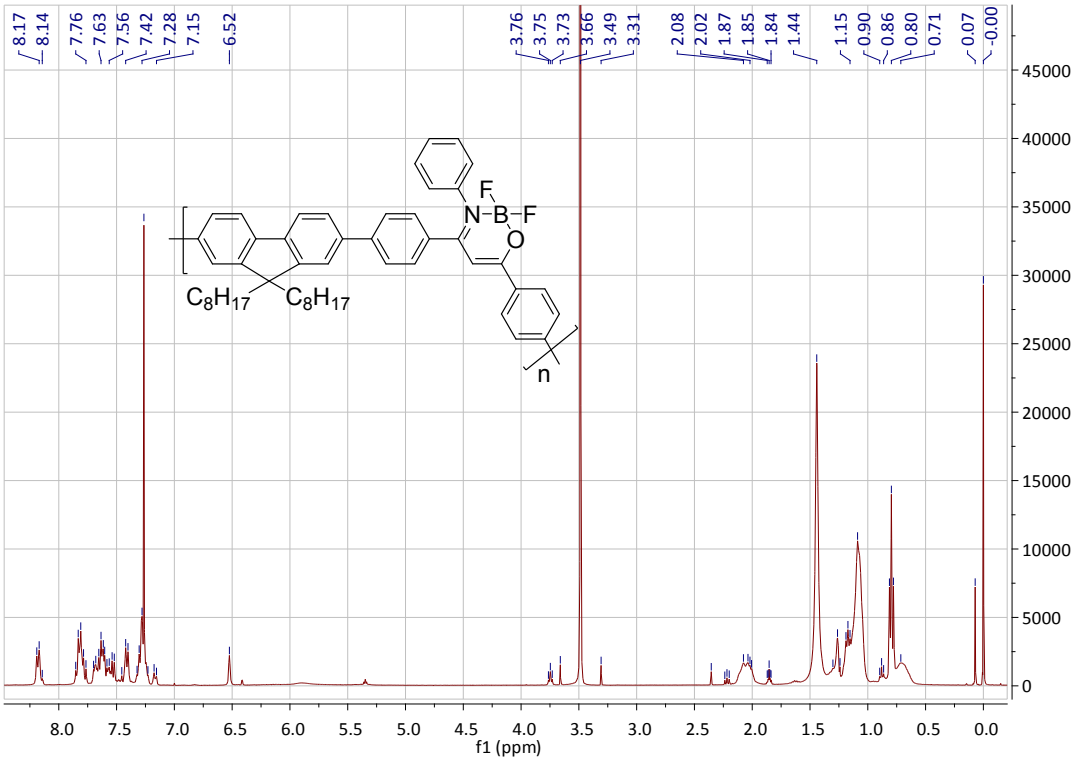


Figure S14. ^{1}H NMR of **P2** in $CDCl_3$

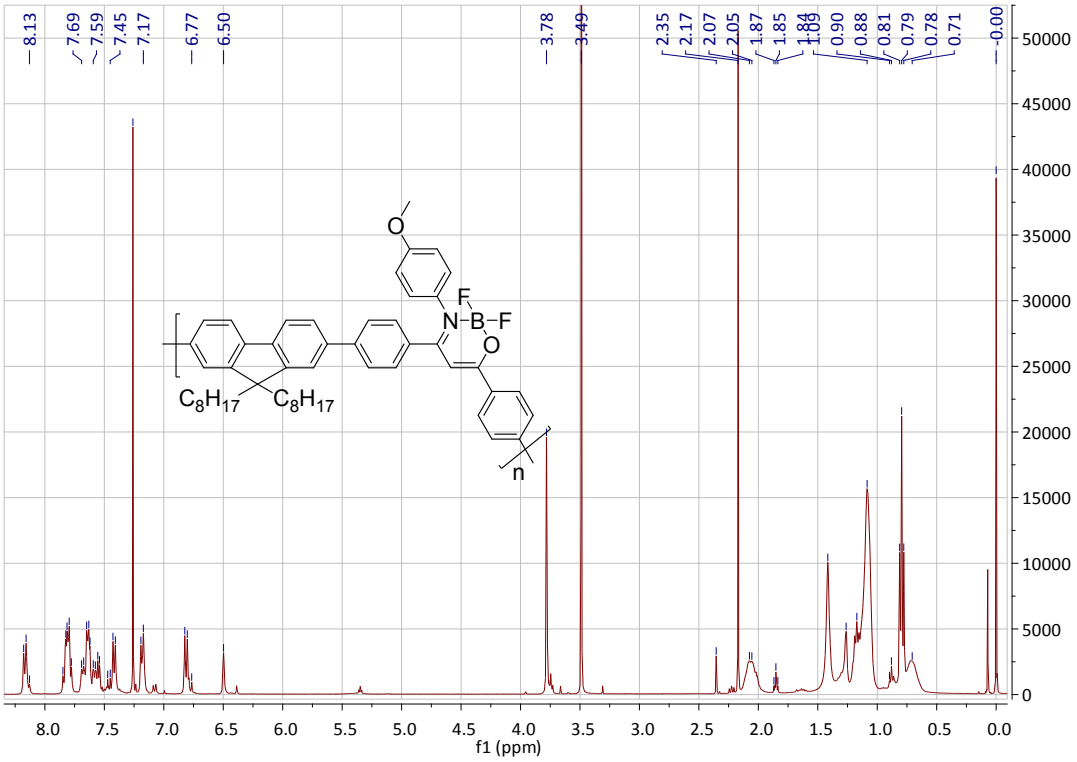


Figure S15. ^{1}H NMR of **P3** in $CDCl_3$

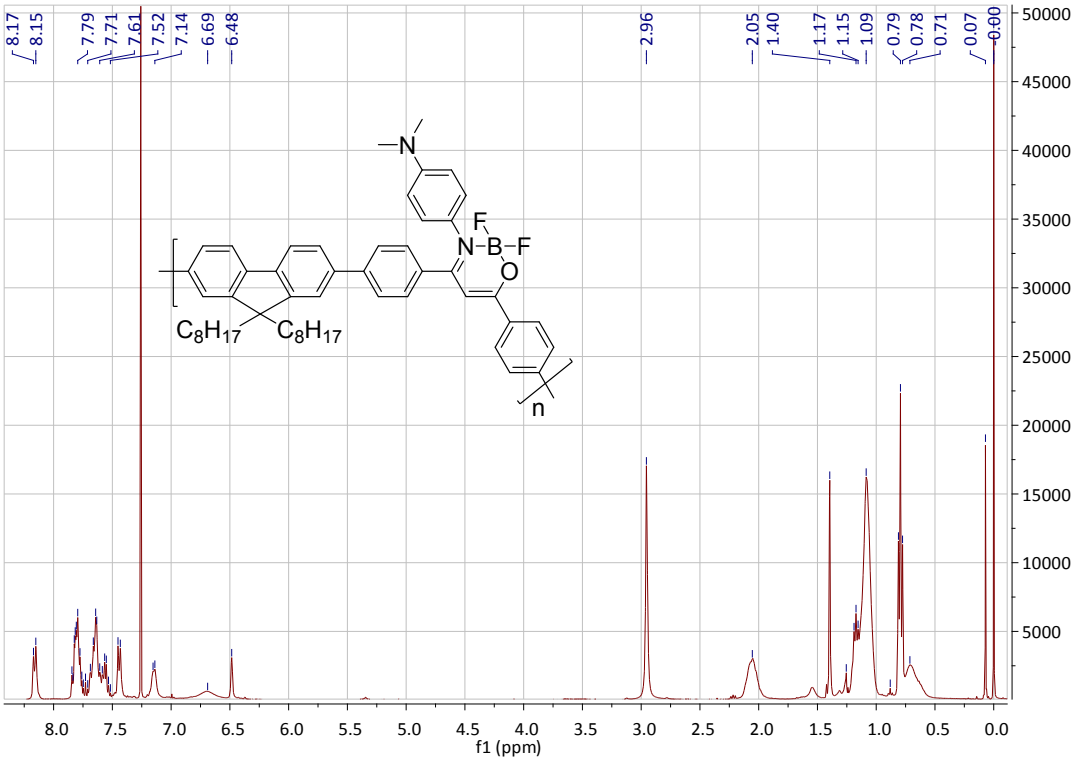
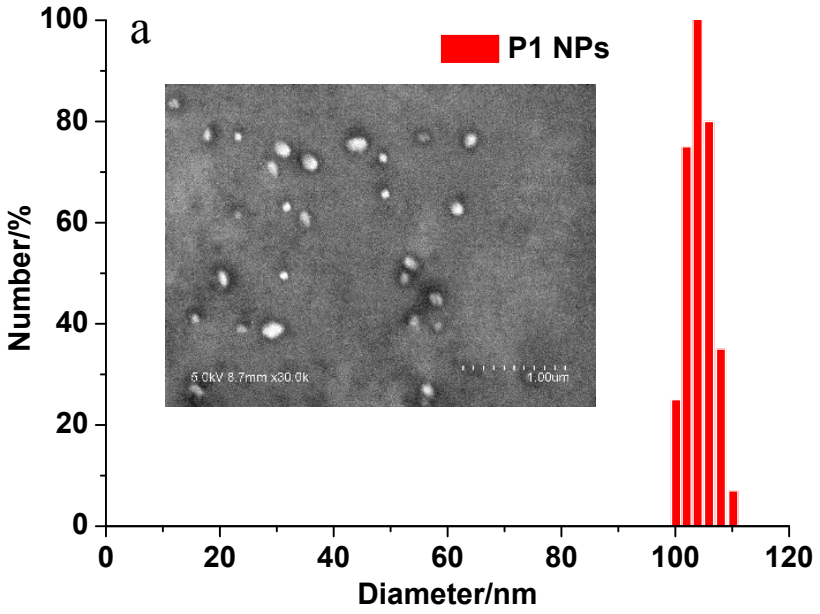
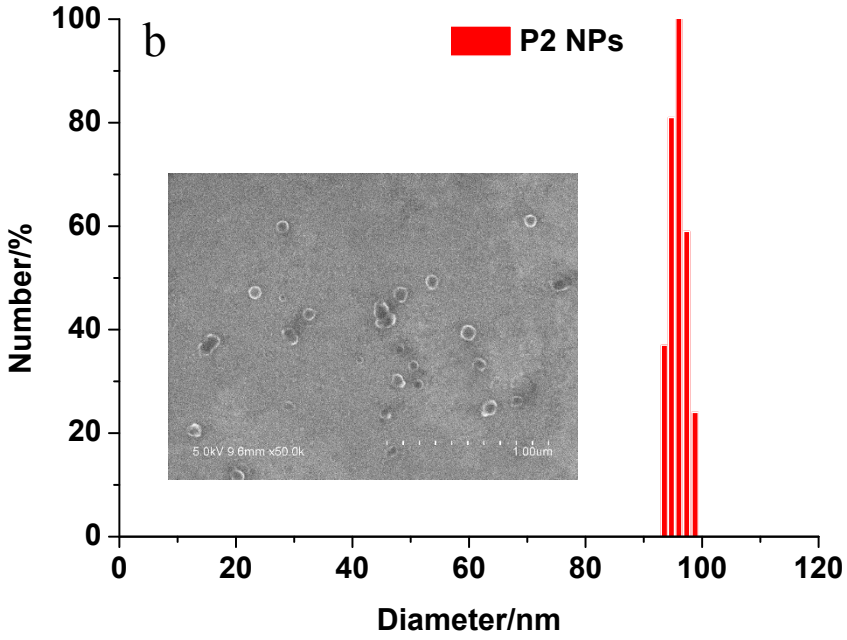
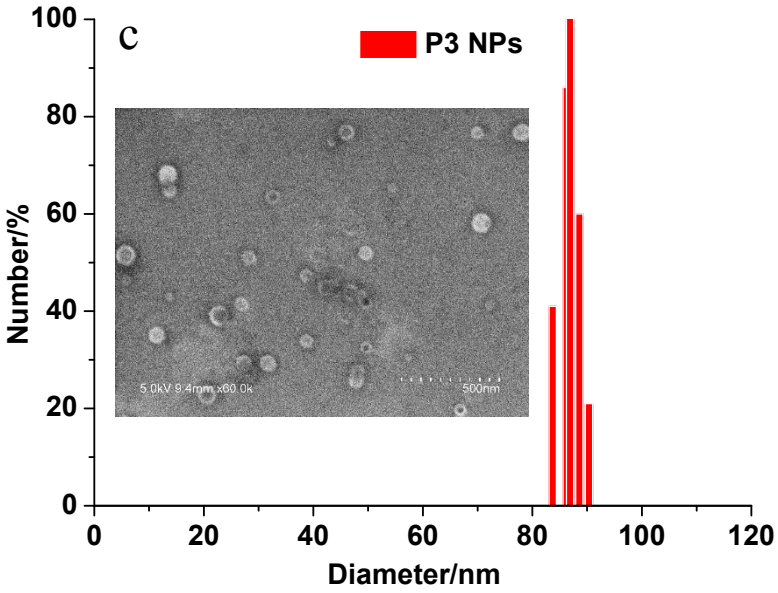


Figure S16. ^{1}H NMR of **P4** in $CDCl_3$

ESI 9. Particle size and morphology characterization of P1-P4 NPs







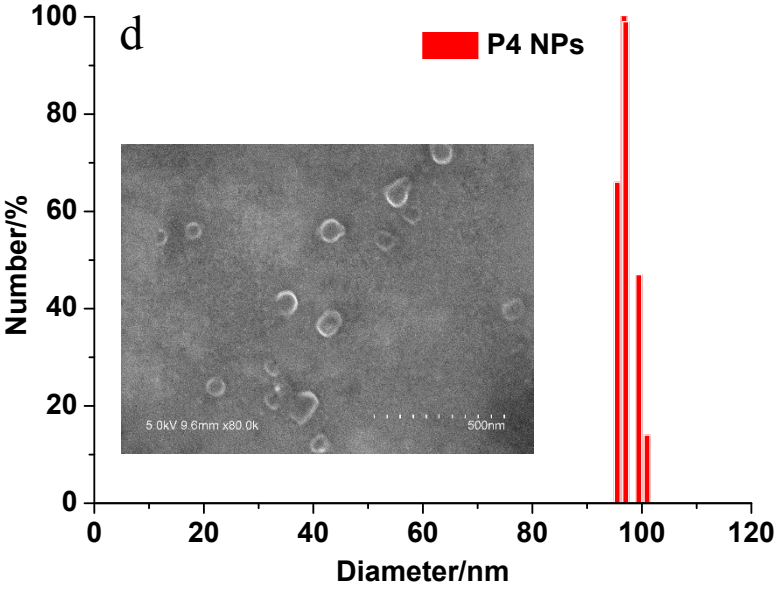


Figure S17. SEM images and histograms of the size distribution (measured by DLS) of CPNs (a-d).

ESI 10. The mean square deviation χ^2 (CHISQ) of P1-P4 NPs

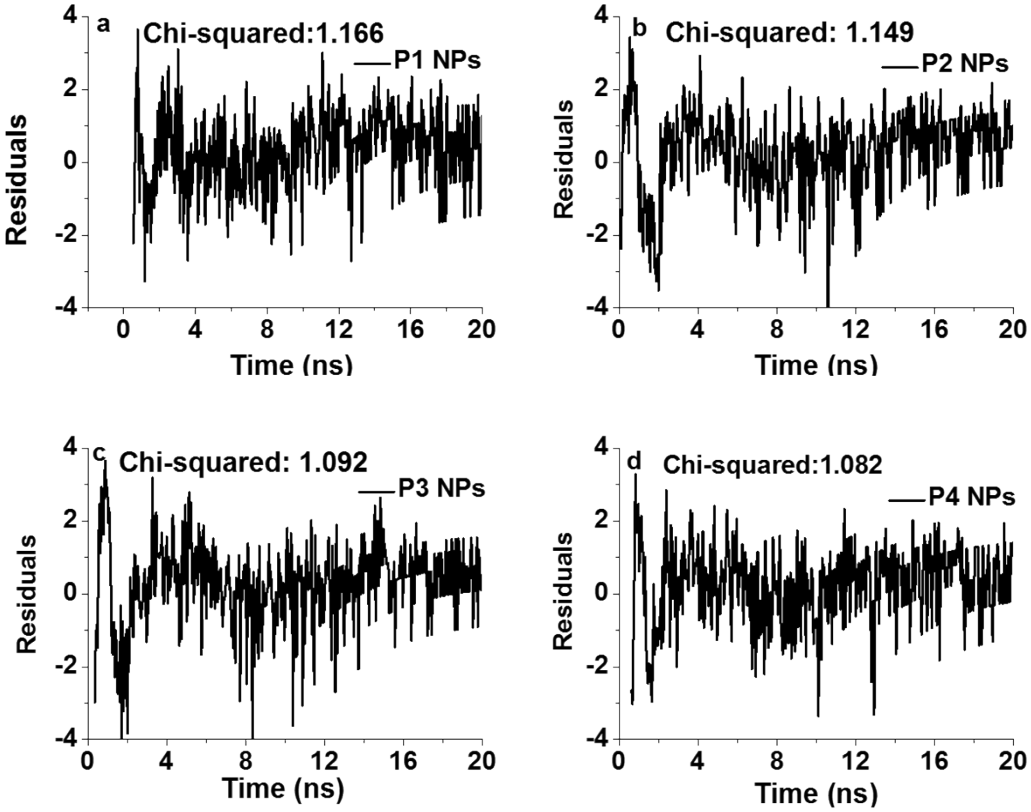


Figure S18. The mean square deviation χ^2 (CHISQ) of **P1-P4** NPs.