Supporting Information for

Engineering pH-Responsive Switch of Donor-π-Acceptor Chromophore Alignments along Peptide Nanotube Scaffold

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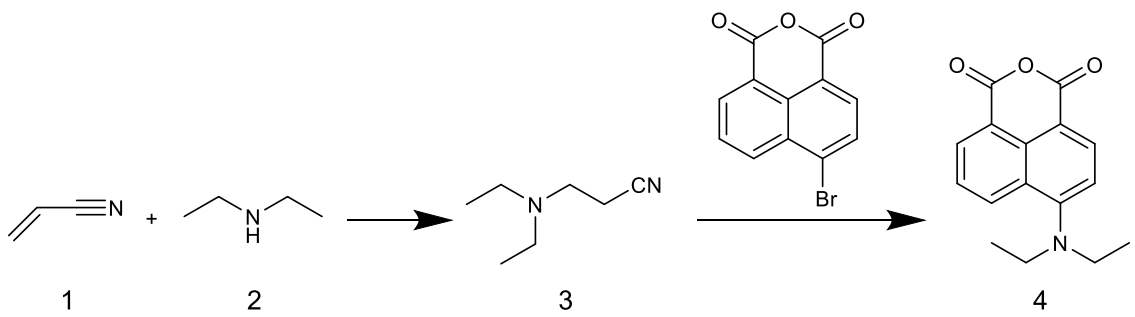
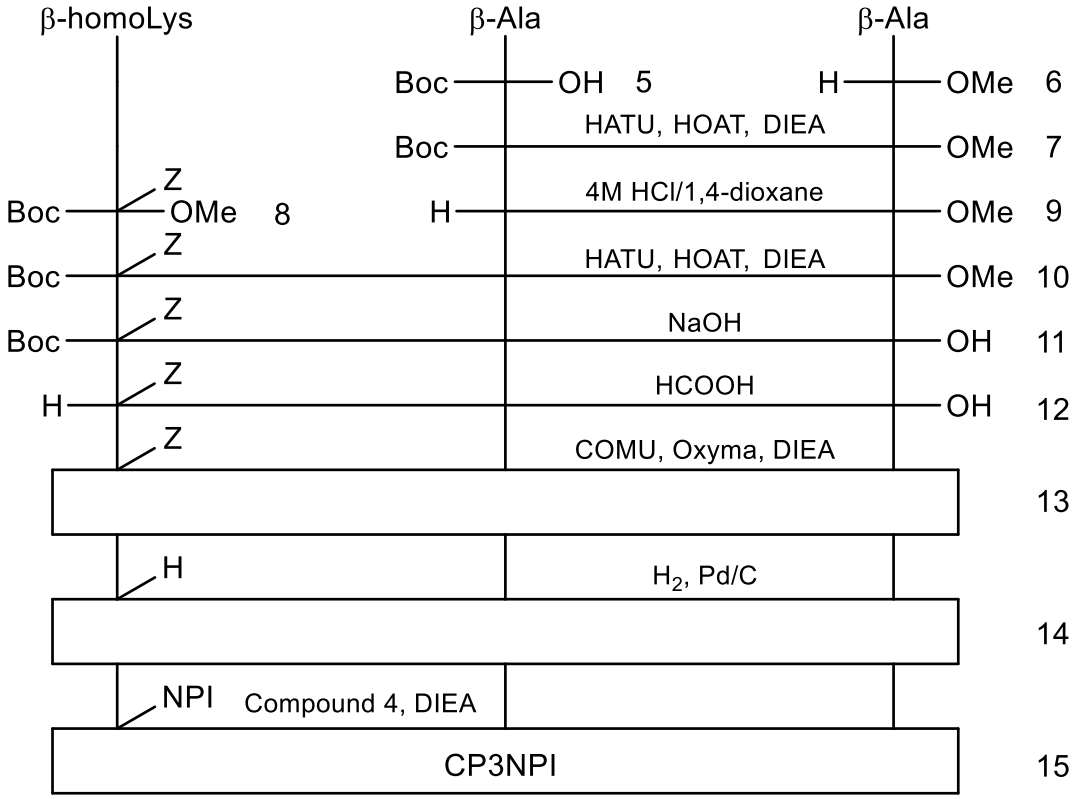
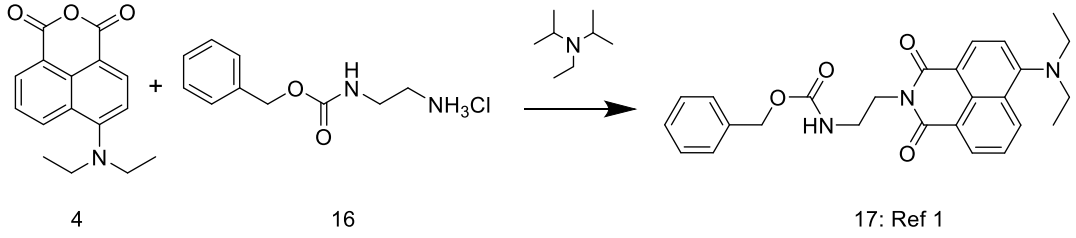
Materials



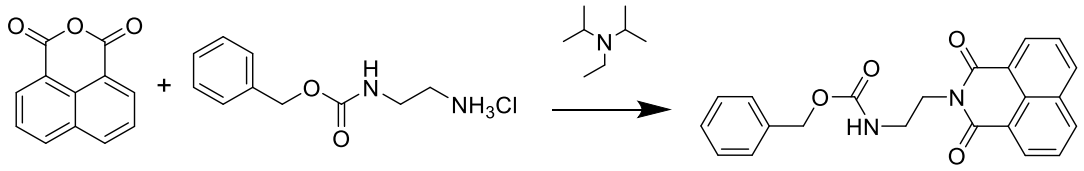
Scheme S1 Synthetic scheme of Compound 4.



Scheme S2 Synthetic schemes for C3NPI.



Scheme S3 Synthetic schemes for Ref 1.



Scheme S4 Synthetic schemes for Compound Ref 2.

All chemicals were purchased from commercial suppliers and used without further purification. CP3NPI, Ref1 and Ref2 were synthesized according to Scheme S1–S4. All the intermediates were identified by ^{1}H NMR spectroscopy (Bruker DPX-400) and the final products were further confirmed by ESI mass spectrometry (Thermo Fisher Scientific Exactive Plus Orbitrap ESI mass). The purity of the intermediates was checked by thin-layer chromatography and that of the final compounds was checked by HPLC (Shimadzu HPLC Prominence 20 system) with ODS column (TSKgel ODS-120T).

***N,N*-diethylaminopropionitrile (3)**

Acrylonitrile (**1**) (25 mL, 379 μmol) and diethylamine (**2**) (26 mL, 250 μmol) were dissolved in water (250 mL) and stirred at room temperature. This solution was extracted by ethyl acetate (200 mL×4) and organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated to afford *N, N*-diethylaminopropionitrile (**3**) as a clear liquid (4.55 g, 36.1 mmol, 29%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.01–1.04 (t, 6H, NCH_2CH_3), 2.40–2.44, 2.77–2.80 (t, 4H, CH_2CH_2CN), 2.51–2.56 (q, 4H, NCH_2CH_3).

4-*N,N*-diethylaminonaphthalene-1,8-dicarboxylic anhydride (4)

4-bromo-1,8-naphthalic anhydride (2.50 g, 9.02 μmol) was suspended in isoamyl alcohol (25 mL) and refluxed until dissolved under Ar atmosphere. Then, *N,N*-diethylaminopropionitrile (**3**) was dropwised and stirred under Ar atmosphere for 36 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel, eluent: chloroform/ethyl acetate = 50/1 v/v) to afford 4-*N,N*-diethylaminonaphthalene-1,8-dicarboxylic anhydride (**4**) (802 mg, 2.98 mmol, 33%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.21–1.24 (t, 6H, NCH_2CH_3), 3.46–3.51 (q, 4H, NCH_2CH_3), 7.20–7.22, 7.67–7.71, 8.48–8.50, 8.57–8.59 (d,t,dd, d, 5H, Nap-H).

HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{16}H_{15}NO_3Na$, 292.0944; found, 292.0936.

Boc-(β-Ala)$_2$-OMe (7)

Boc-β-Ala-OH (**5**) (1.061 g, 5.61 mmol), H-β-Ala-OMe (**1-7**) (943 mg, 6.76 mmol), HATU (3.209 g, 8.44 mmol), and HOAt (1.156 g, 8.49 mmol) were dissolved in dry DMF (20 mL) and then, DIEA (6 mL, 34.45 mmol) was added to the mixture at 0 °C and thereafter stirred at room temperature for 18 h under Ar atmosphere. The solvent was removed under reduced pressure. The residue was taken up with chloroform and washed with saturated $NaHCO_3$ aq.

three times, 4 wt% $KHSO_4$ aq. three times and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, eluent: chloroform/methanol = 40/1 v/v) and washed with diisopropyl ether to afford Boc-(β-Ala)$_2$-OMe (**7**) (1.274 g, 4.64 mmol, 83%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.43 (s, 9H, Boc), 2.38, 2.55 (t, 4H, AlaC$^{\alpha}H_2$), 3.41, 3.53 (q, 4H, AlaC$^{\beta}H_2$), 3.71 (s, 3H, OCH_3), 5.15, 6.13 (br, 2H, AlaN*H*).

HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{12}H_{23}N_2O_5$, 275.1601; found, 275.1594.

Boc-β-homoLys(Z)-(β-Ala)$_2$-OMe (10)

Boc-(β-Ala)$_2$-OMe (**7**) (995 mg, 3.63 mmol) was dissolved in dioxane (14 mL). To the solution, 1 M HCl/dioxane (130 mL) was dropwised and stirred at room temperature for 1 h. The solvent was removed under reduced pressure. The residue was washed with diisopropyl ether to afford H-(β-Ala)$_2$-OMe (**9**). Then, obtained deprotected product, Boc-β-homoLys(Z)-OH (**8**) (1.40 g, 3.55 mmol), HATU (2.08 g, 5.46 mmol), and HOAt (0.754 g, 5.54 mmol) were dissolved in dry DMF (25 mL) and then, DIEA (3.82 mL, 21.93 mmol) was added to the mixture at 0 °C and thereafter stirred at room temperature for 20 h under argon atmosphere. The solvent was removed under reduced pressure. The residue was taken up with chloroform and washed with saturated $NaHCO_3$ aq. three times, 4 wt% $KHSO_4$ aq. three times and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, eluent: chloroform/methanol = 40/1 v/v) and washed with diisopropyl ether to afford Boc-β-homoLys(Z)-(β-Ala)$_2$-OMe (**10**) (1.88 g, 0.341 mmol, 93%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.42 (s, 9H, Boc), 1.29–1.58 (m, 6H, HLysC$^{\gamma}H_2$, HLysC$^{\delta}H_2$, HLysC$^{\varepsilon}H_2$), 2.25–2.53 (m, 6H, AlaC$^{\alpha}H_2$, HLysC$^{\alpha}H_2$), 3.16─3.21 (q, 2H, HLysC$^{\zeta}H_2$), 3.46–3.55 (m, 4H, AlaC$^{\beta}H_2$), 3.70 (s, 3H, OCH_3), 3.85 (br, 1H, HLysC$^{\beta}H$), 4.97 (br, 1H, HLysC$^{\zeta}$H$_2$N*H*-Z), 5.09 (s, 2H, $C_6H_5CH_2$), 5.30–5.32 (br, 1H, HLysC$^{\beta}$H$_2$N*H*), 6.60 (br, 2H, AlaN*H*), 7.31–7.36 (m, 5H, aromatic).

HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{43}N_4O_8$, 551.3075; found, 551.3056.

CP3Z (13)

Boc-β-homoLys(Z)-(β-Ala)$_2$-OMe (**1-11**) (404 mg, 0.735 mmol) was dissolved in MeOH (10 mL) and 1,4-dioxane (10 mL). To the solution, 1 M NaOH aq. (5 mL, 5 mmol) was dropwised and stirred at room temperature for 4 h. The solution was acidified to pH 6 with 1 M HCl aq. After evaporation of the solvent, the residue was taken up with chloroform and washed with $KHSO_4$ aq. and brine. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford Boc-β-homoLys(Z)-(β-Ala)$_2$-OH (**11**). Then, this was dissolved in HCOOH (40 mL) and stirred for 1 h. The solvent was removed under reduced pressure. The residue was washed with 1 M HCl in diethyl ether to afford H-β-homoLys(Z)-(β-Ala)$_2$-OH (**12**). This deprotected product was divided into two round bottom flasks, and COMU (1.419 mg, 3.32 mmol) and Oxyma (700 mg, 4.98 mmol) were added to the each flasks, and then, were dissolved in dry DMF (170 mL). To the solution was added DIEA (1.1 mL) in DMF (50 mL) at 0 °C under Ar atmosphere and thereafter stirred at room temperature for 72 h under Ar atmosphere. The solvent was removed under reduced pressure. The precipitate was washed with MeCN and EtOH to afford CP3Z (**13**) (163 mg, 0.390 mmol, 53%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.19–1.24 (m, 2H, HomoLysC$^{\delta}H_2$), 1.37–1.39 (m, 4H, CHCH_2CH$_2$CH_2CH$_2$NH), 2.02–2.46 (m, 6H, HomoLysC$^{\alpha}H_2$, AlaC$^{\alpha}H_2$), 2.93–2.98 (m, 2H, CHCH$_2$CH$_2$CH$_2$CH_2NH), 3.08–3.29 (m, 4H, AlaC$^{\beta}H_2$), 3.88–3.90 (m, 1H, HomoLysC$^{\beta}$H), 5.00 (s, 2H, CH_2Ph), 7.22–7.25 (br, 1H, CHCH$_2$CH$_2$CH$_2$CH$_2$N*H*), 7.30–7.38 (m, 6H, aromatic, HomoLys(Z)N*H*), 7.42–7.44, 7.63–7.66 (t, 2H, AlaNH).
HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{21}H_{30}N_4O_5Na$, 441.2108; found, 441.2095.

CP3NPI (15)

CP3Z (**13**) (60 mg, 0.143 mmol) was dissolved in HCOOH (20 mL) and 10% Pd/C (20 mg) was added to the solution. Then, the solution was stirred at room temperature for 18 h under H_2 atmosphere. The insoluble part was filtered off and the filtrate was evaporated to obtain deprotected product. To this deprotected product, EtOH (20 mL) was added and the mixture was stirred at 60 °C. DIEA and 4-*N*,*N*-diethylaminonaphthalene-1,8-dicarboxylic anhydride (**4**) was added to the solution and the mixture was refluxed for 18 h under Ar atmosphere. The solvent was removed under reduced pressure and the residue was washed with toluene. Thereafter, the residue was purified by column chromatography (Bio-Gel P-2, eluent HCOOH/water = 1/1 v/v) to obtain CP3NPI (**15**) (25 mg, 0.047 mmol, 33%)
^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.10–1.14 (t, 6H, NCH_2CH$_3$), 1.23–1.31, 1.43–1.46, 1.57–1.60 (m, 6H, HLysC$^{\gamma}H_2$, HLysC$^{\delta}H_2$, HLysC$^{\varepsilon}H_2$), 1.94–2.05, 2.11–2.20, 2.39–2.45 (m, 6H, HLysC$^{\alpha}H_2$, AlaC$^{\alpha}H_2$), 3.01–3.07, 3.16–3.24 (m, 4H, AlaC$^{\beta}H_2$), 3.39–3.43 (m, 4H, NCH$_2$CH_3), 3.90 (m, 1H, HLysC$^{\beta}$H), 3.98–4.02 (t, 2H, HLysC$^{\zeta}H_2$), 7.31–7.36, (m, 2H, HLys(Z)N*H*, aromatic), 7.43, 7.61–7.64 (m, 2H, AlaNH), 7.76–7.80, 8.36–8.38, 8.45–8.46 (m, 4H, aromatic).
HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{29}H_{37}N_5O_5Na$, 558.2687; found, 558.2690.

Compound Ref 1 (17)

Z-ethylenediamine (**16**) (205 mg, 0.889 mmol) was dissolved in EtOH (20 mL) and stirred at 40°C. To the solution, DIEA (260 μL, 0.743 mmol) was dropwised and stirred. Then, 4-*N*,*N*-diethylaminonaphthalene-1,8-dicarboxylic anhydride (**4**) (206 mg, 0.765 mmol) was added to the solution and the mixture was refluxed under Ar overnight. The solution was evaporated under reduced pressure. The precipitate was taken up with $CHCl_3$ and washed with 4 % $KHSO_4$ aq. for 3 times and sat. $NaHCO_3$ aq. for three times and brine. The oraganic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. The precipitate was purified with column chromatography (silica gel, eluent $CHCl_3$/MeOH =50/1) to afford Ref1 (**17**) (279 mg, 626 μmol, yield 85%).
^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.16–1.19 (t, 6H, NCH$_2$CH_3), 3.40–3.45 (q, 4H, NCH_2CH$_3$), 3.59–3.63, 4.38–4.41 (m, 4H, CH_2CH_2), 5.02 (s, 2H, OCH_2), 5.36 (s, 1H, amide), 7.19–8.49 (m, 10H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 12.23 (s), 39.31 (s), 40.73 (s), 47.32 (s), 66.43 (s), 115.04 (s), 116.64 (s), 122.68 (s), 125.11 (s), 127.85 (s), 127.98 (s), 128.33 (s), 130.28 (s), 131.17 (s), 131.33 (s), 132.41 (s), 136.69 (s), 155.42 (s), 156.50 (s), 164.51 (s), 165.02 (s).
HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{26}H_{27}N_3O_4Na$, 468.1894; found, 468.1898.

Compound Ref 2 (19)

Z-ethylenediamine (**16**) (279 mg, 1.12 mmol) was dissolved in EtOH (20 mL) and stirred at 40°C. To the solution, DIEA (352 μL, 2.02 mmol) was dropwised and stirred. Then, 1,8-dicarboxylic anhydride (**18**) (200 mg, 1.01 mmol)

was added to the solution and the mixture was refluxed under Ar overnight. The solution was evaporated under reduced pressure. The precipitate was purified with column chromatography (silica gel, eluent $CHCl_3$/MeOH =20/1) to afford Ref 2 (**19**) (359 mg, 958 μmol, yield 95%).

^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.62–3.65, 4.40–4.43 (m, 4H, CH2CH2), 5.01 (s, 2H, OCH2), 5.27 (s, 1H, urethane), 7.28 (s, 5H, Phe), 7.74–7.79, 8.22–8.25, 8.60–8.62 (t, d, d, 6H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 40.00 (s), 40.77 (s), 66.89 (s), 122.63 (s), 127.31 (s), 128.31 (s), 128.40 (s), 128.46 (s), 128.75 (s), 131.81 (s), 131.86 (s), 134.48 (s), 137.01 (s), 156.93 (s), 164.92 (s).

HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{22}H_{18}N_2O_4Na$, 397.1159; found, 397.1163.

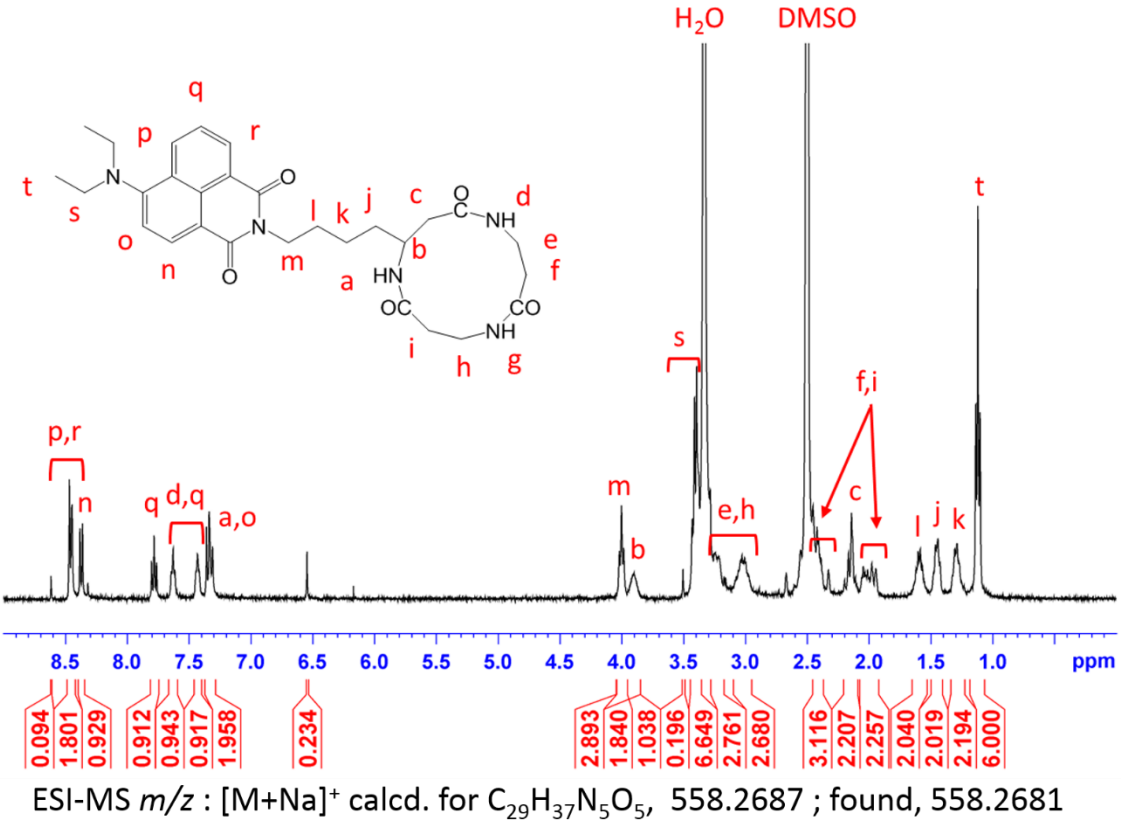


Figure S1 ^{1}H NMR spectrum in DMSO-d_6 and the result of ESI-MS of CP3NPI.

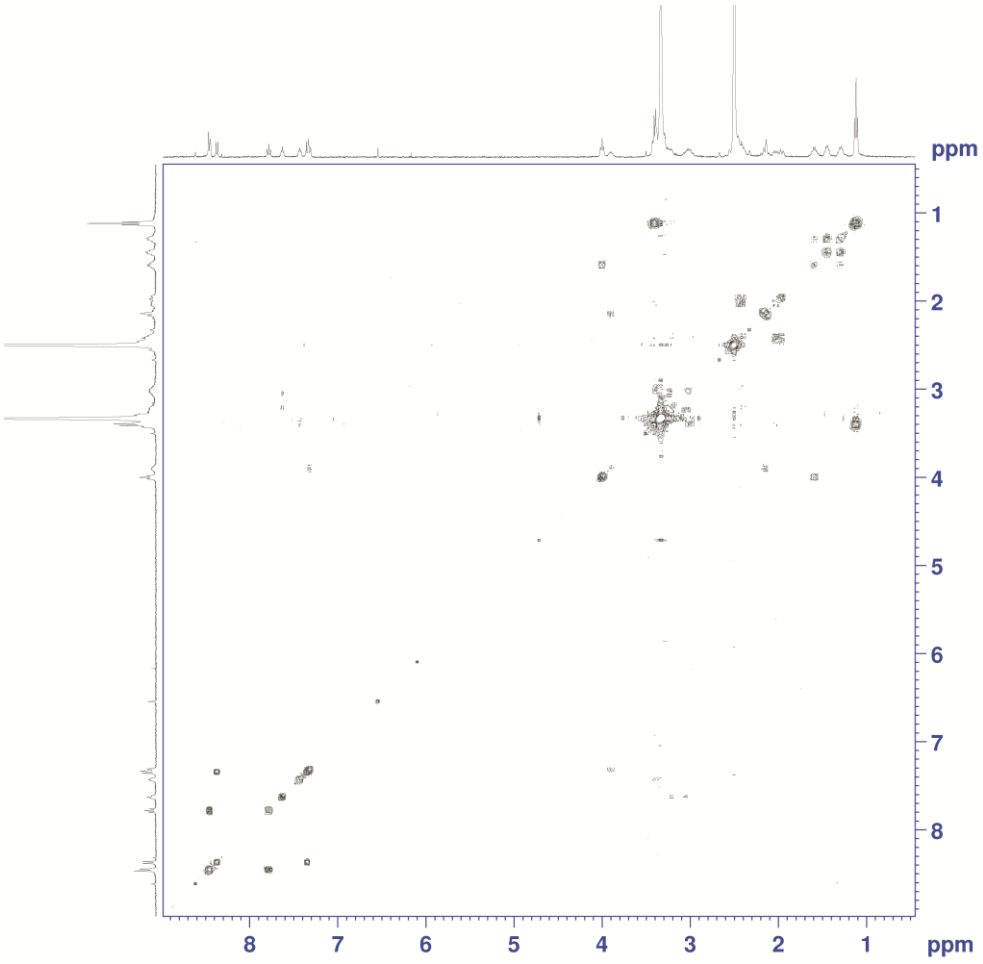


Figure S2 2D-COSY NMR spectrum in DMSO-d_6 of CP3NPI.

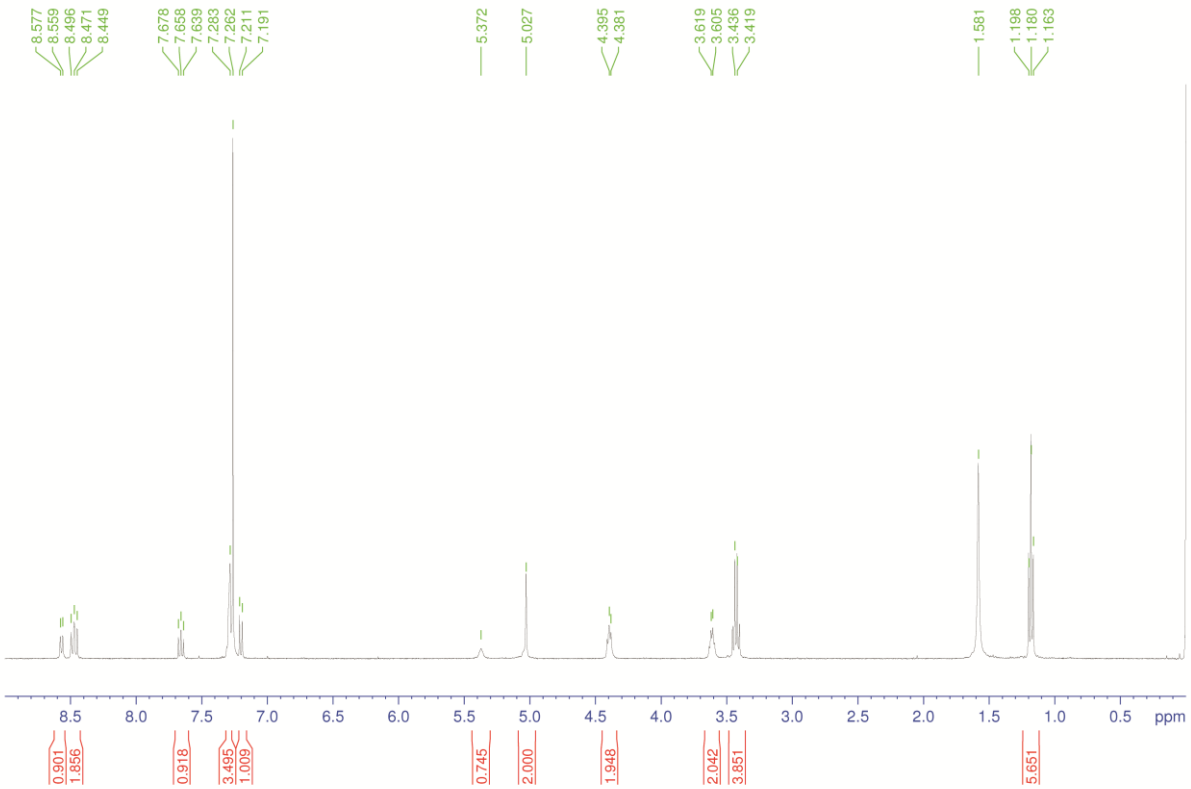


Figure S3 ^{1}H NMR spectrum in $CDCl_3$ of Ref1.

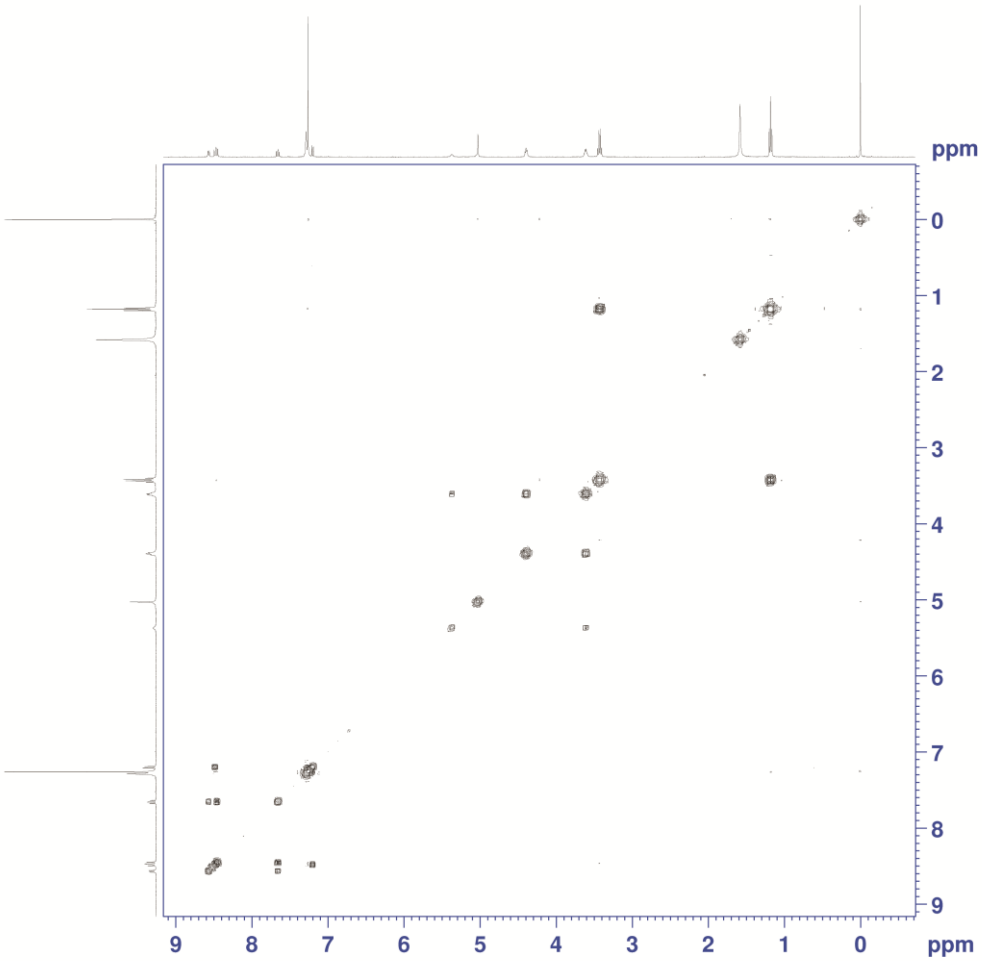


Figure S4 2D-COSY NMR spectrum in $CDCl_3$ of Ref1.

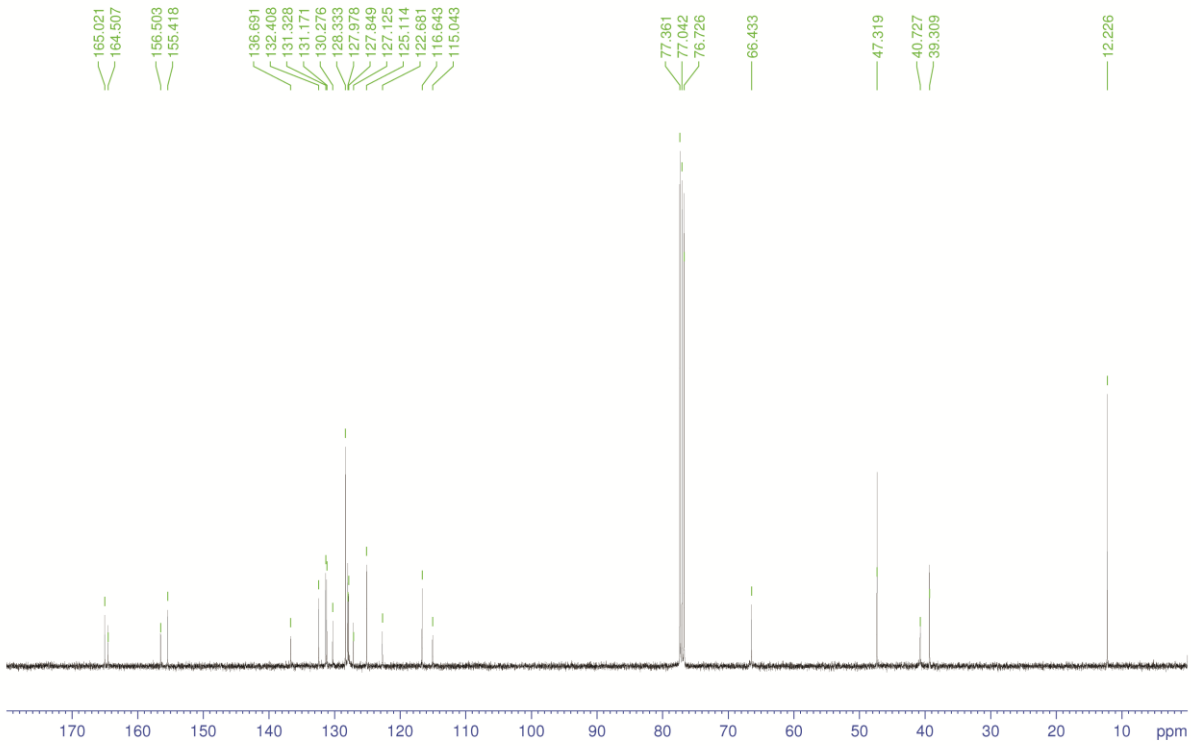


Figure S5 ^{13}C NMR spectrum in $CDCl_3$ of Ref1.

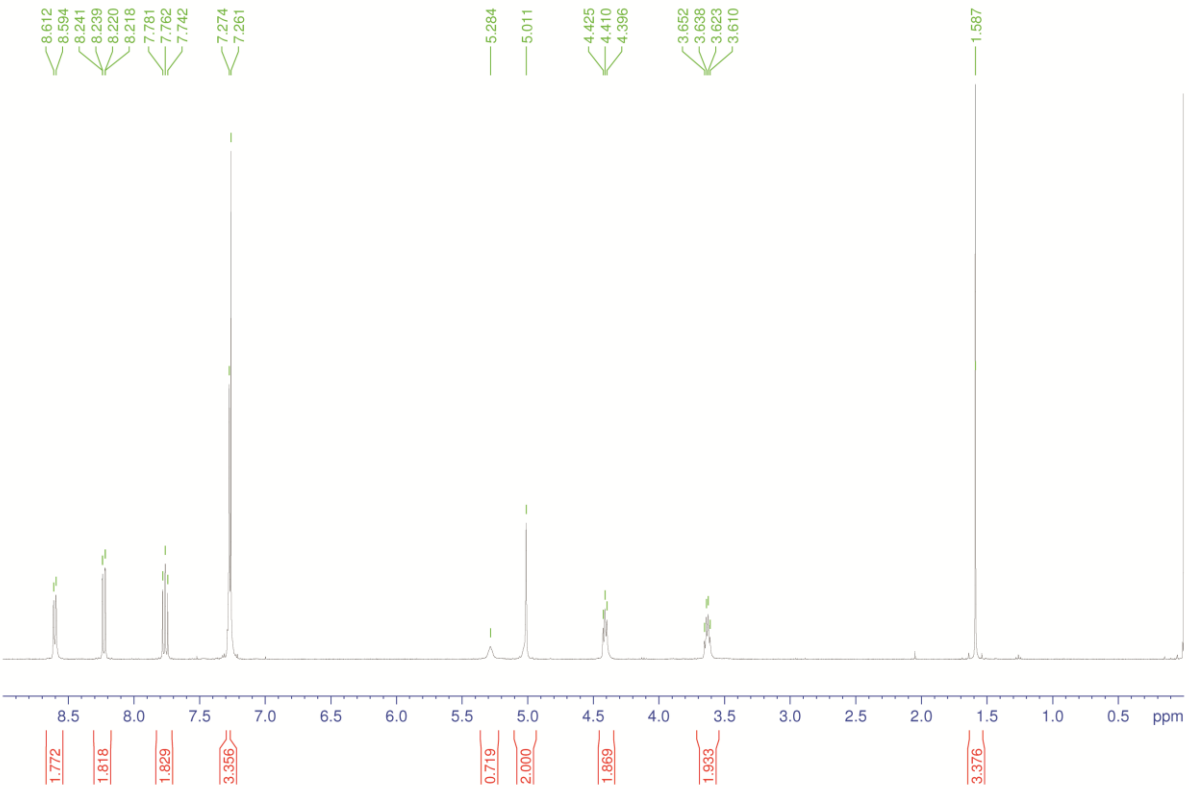


Figure S6 ^{1}H NMR spectrum in $CDCl_3$ of Ref2.

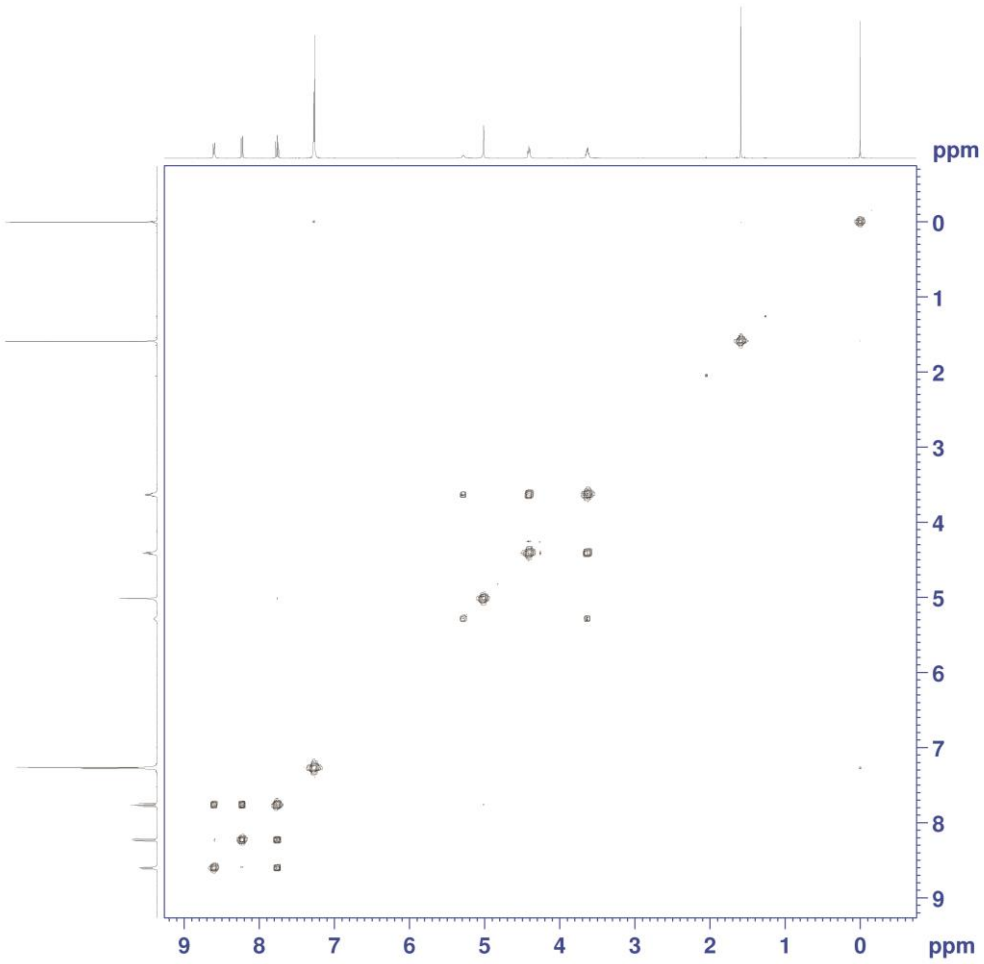


Figure S7 2D-COSY NMR spectrum in $CDCl_3$ of Ref1.

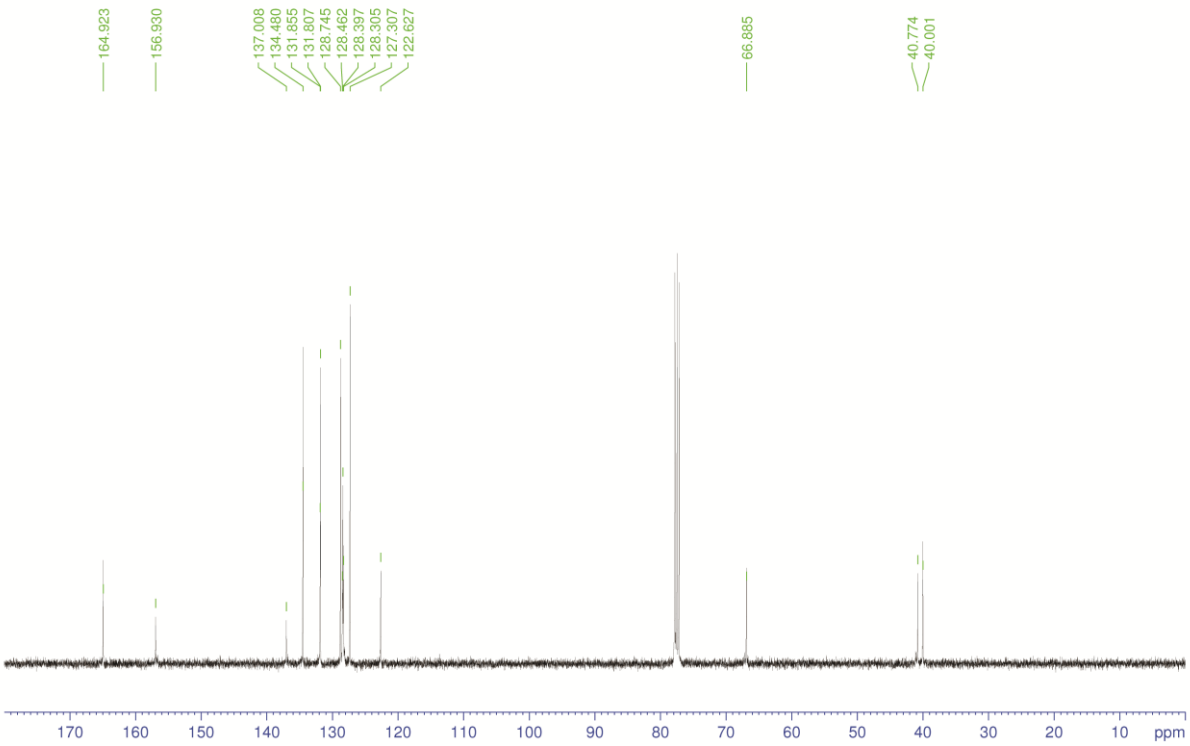


Figure S8 ^{13}C NMR spectrum in $CDCl_3$ of Ref21.

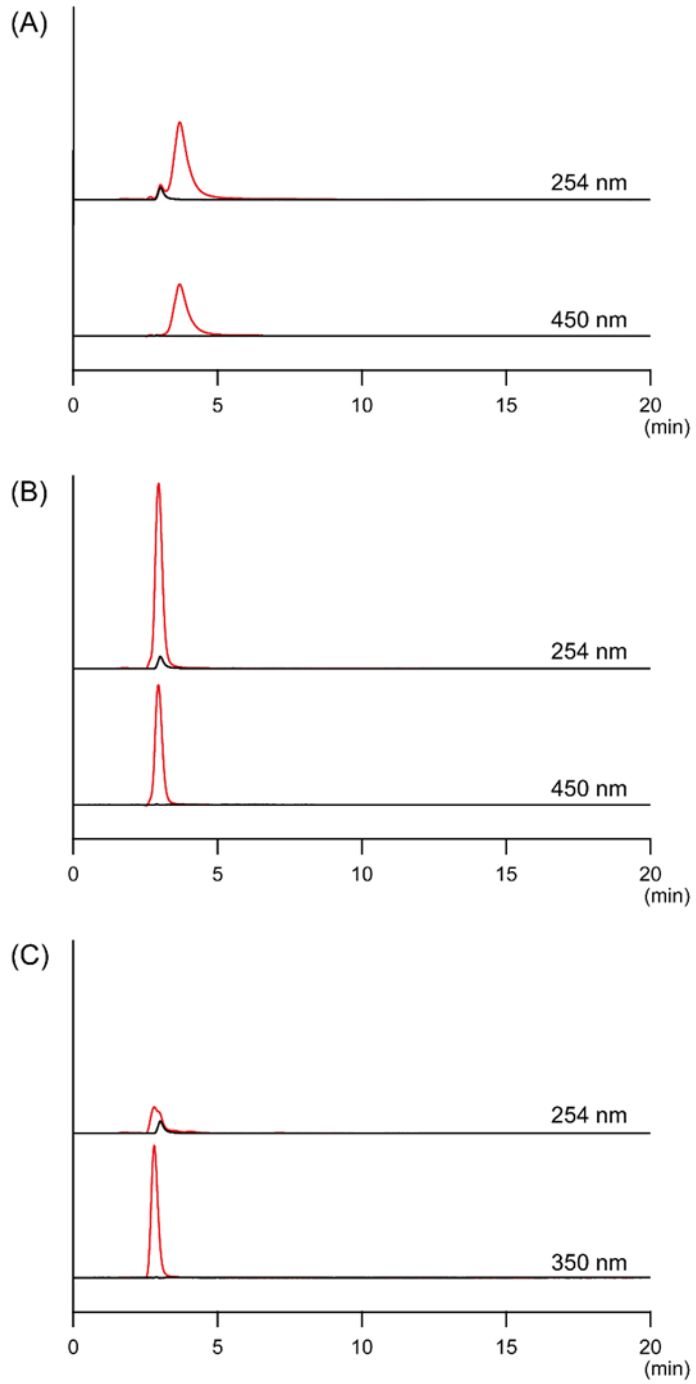


Figure S9 HPLC chromatograms of compounds (red line; CP3NPI (A), Ref1 (B) and Ref2 (C)) and methanol only (black line).

Table S1. HPLC conditions.

Column	TSKGel ODS-120T 2.0 x 150 mm
Mobile phase	Methanol
Column temperature	40 °C,
Injection volume	10 μL
Flow rate	0.20 mL/min
Detection	UV-Vis 254, 350 and 450 nm
Run time	20 minutes

Spectroscopy

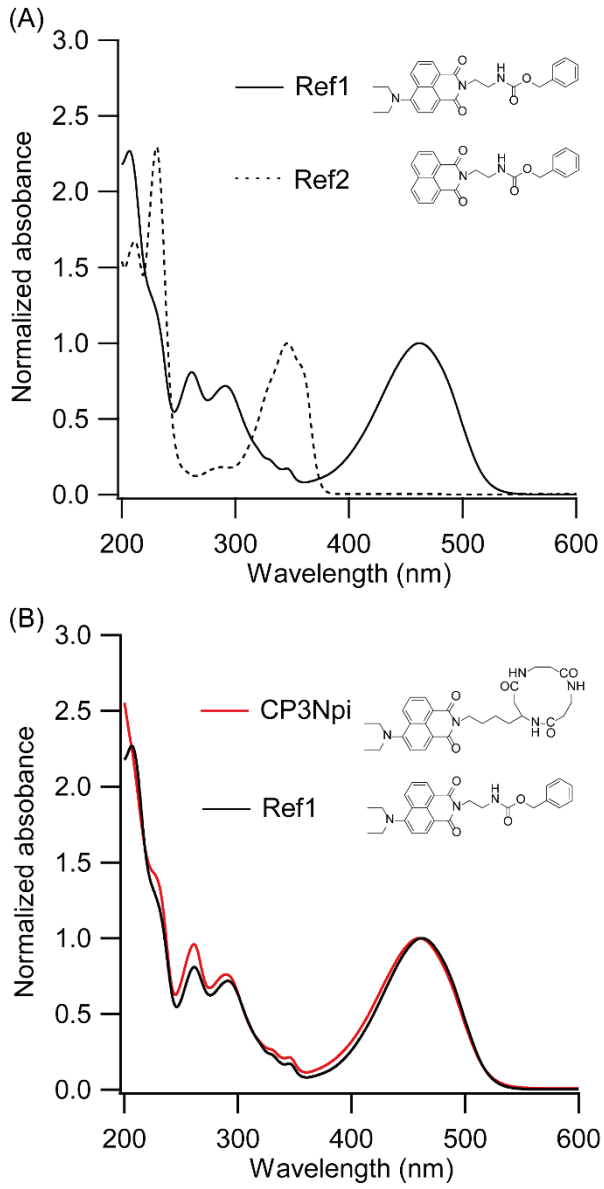


Figure S10 Normalized UV-vis absorption spectra of compound Ref1 and Ref2 (A), CP3Npi and compound Ref1 (B) in HFIP.

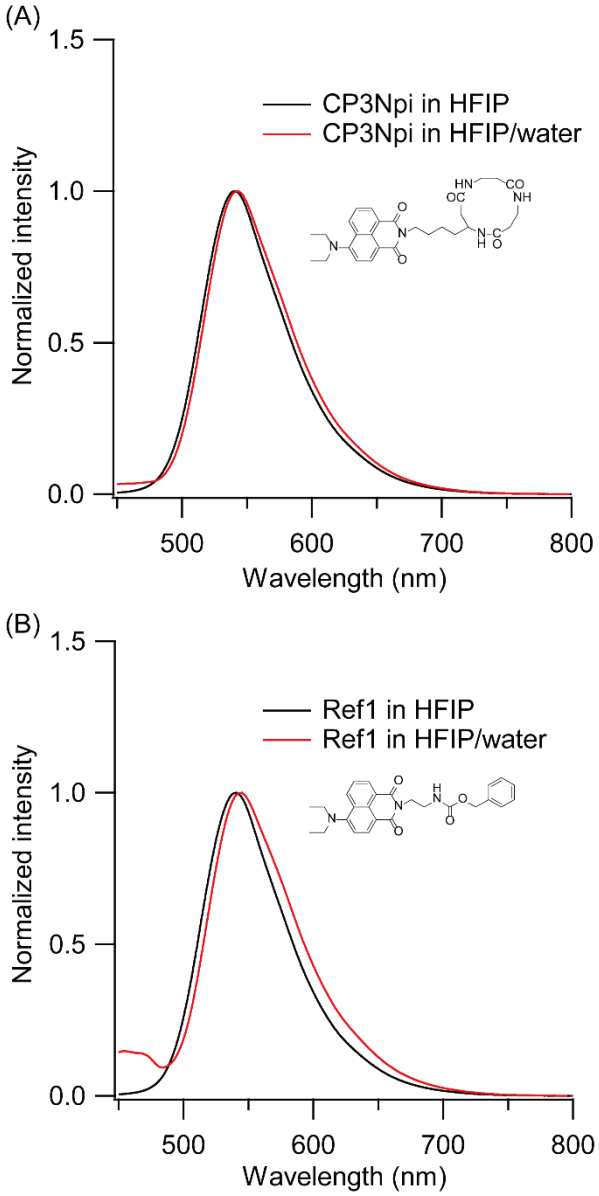


Figure S11 Normalized emission spectra of CP3Npi (A), and compound Ref1 (B) in HFIP and HFIP/water (1/39 v/v).

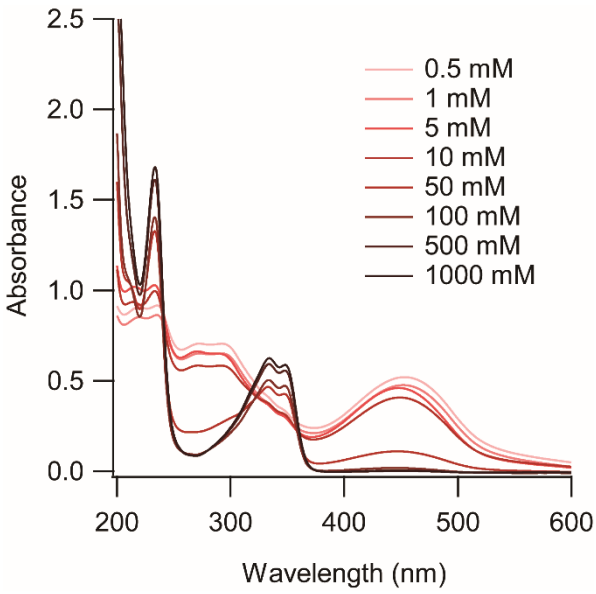


Figure S12 Normalized UV spectra of compound Ref1 in HFIP/HCl aq. with various concentration of HCl (0.5, 1, 5, 10, 50, 100, 500, 1000 mM).

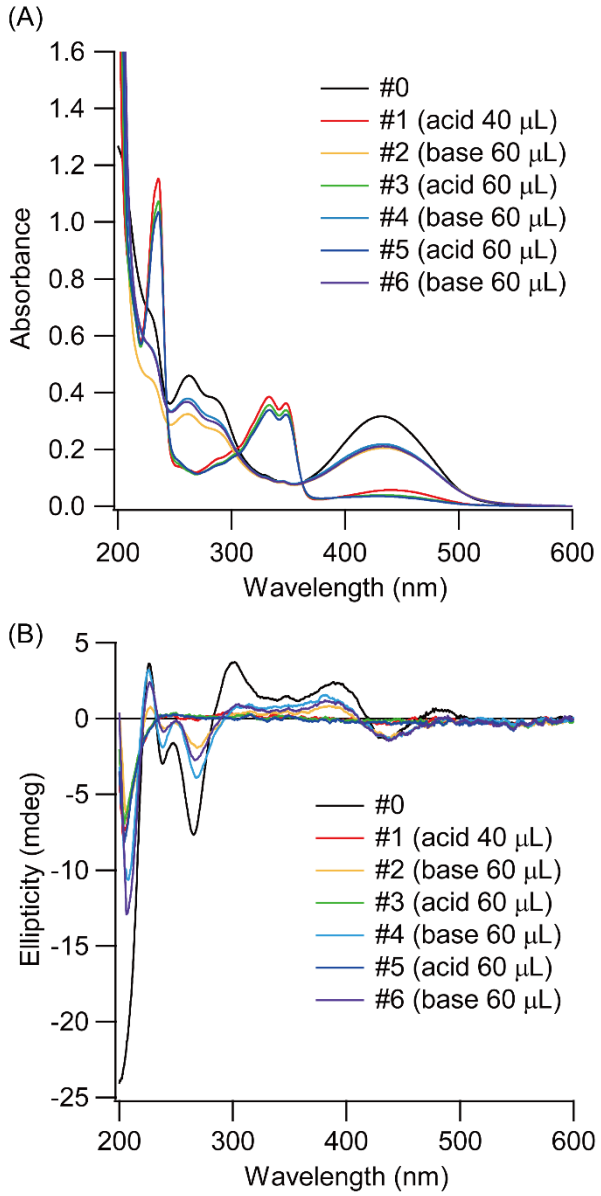


Figure S13 UV and CD spectra of CP3Npi in response of pH changes.