Electronic Supplementary Information

Rational design of pH-responsive near-infrared spirocyclic cyanines: the effects of substituents and the external environment

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Abbreviations

DMF: *N*,*N*-dimethylformamide
EDC: 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide
HOAt: 1-hydroxy-7-azabenzotriazole
mPEG-NH_2: *O*-(2-aminoethyl)-*O′*-methylpolyethylene glycol
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NHS: *N*-hydroxysuccinimide
PMP: *p*-methoxyphenyl
p-Ts: *p*-toluenesulfonyl
TFA: trifluoroacetic acid
THF: tetrahydrofuran

Materials and methods.

General

^{1}H and ^{13}C NMR spectroscopy was performed using a JEOL JNM-ECA500 or JEOL JNM-ECZ400S spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6. All chemical shifts are relative to the internal standard of tetramethylsilane (δ = 0.0 ppm) or solvent residual peaks ($CDCl_3$; δ = 7.26 ppm, CD_3OD; δ = 3.31 ppm, DMSO-d_6; 2.50 ppm for ^{1}H; $CDCl_3$; δ = 77.06 ppm, CD_3OD; δ = 49.03 ppm, DMSO-d_6; 39.53 ppm for ^{13}C). Coupling constants (*J*) are given in Hz. High-resolution mass spectra (HRMS) were recorded on a LCT Premier XE (Waters, Milford, MA) or MALDI-7090 (SHIMADZU Co. Ltd., Kyoto, Japan) mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel plates (TLC Silica gel 60 F_{254}, Merck KGaA, Darmstadt, Germany). Flash column chromatography on silica gel was performed using Silica gel 60 (0.063–0.200 mm) (Merck KGaA, Darmstadt, Germany), Purifi-Pack EX SI-50 (Shoko Science, Yokohama, Japan), or FlashPure Select Silica (BÜCHI, Flawil, Switzerland). Combined organic extracts were dried over anhydrous Na_2SO_4. All the solvents and reagents were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan), Kanto Chemical (Tokyo, Japan), Tokyo Chemical Industries (Tokyo, Japan), Nakalai Tesque (Kyoto, Japan), or Sigma-Aldrich (St. Louis, MO) and were used as received without further purification. Absorbance measurement was performed using a UV-3600Plus instrument (SHIMADZU Co. Ltd., Kyoto, Japan). Fluorescence measurement was performed using a RF-6000 instrument (SHIMADZU Co. Ltd., Kyoto, Japan).

pH-dependent optical properties of spirocyclic cyanine dyes

The pH-dependent optical properties were evaluated through absorbance and fluorescence measurements in difference pH buffer solutions. Citric acid buffers from pH 4.0 to 6.0 were prepared with 25 mM citric acid and monosodium citrate solutions. Phosphate buffers from pH 6.5 to 8.0 were prepared with 25 mM NaH_2PO_4 and Na_2HPO_4 solutions. Boric acid buffers from pH 10.5 to 11.0 were prepared with 25 mM boric acid and 1 M NaOH solutions. Phosphate buffers from pH 11.5 to 12.0 were prepared with 25 mM Na_2HPO_4 and 1 M NaOH solutions. The stock solutions of the dyes

were prepared with DMSO and were diluted with the corresponding buffer solution (10% DMSO). The pK_{cycl} was determined by a least-squares method. The absorbance (A) and fluorescence intensity (F) were measured and were fitted to the following equation.

$$A(F) = A(F)_{min} + (A(F)_{max} - A(F)_{min}) \frac{1}{1 + 10^{\mathrm{h}(\mathrm{p}K_{\mathrm{cycl}} - \mathrm{pH})}} \quad (1)$$

Where, $A(F)_{min}$, $A(F)_{max}$, and h represent the corresponding minimum and maximum absorbance or fluorescence intensity, and the Hill coefficient, respectively.

Quantum chemical calculation

The quantum chemical calculations of frontier orbital energy levels of **IR-HM** in the open and closed forms were conducted by the Gaussian 16W program.

Preparation of spirocyclic cyanine dye-loaded F127 polymeric micelles

Cyanine dye-loaded F127 polymeric micelles were prepared by a thin-film hydration method.[1] **IR-PAM (*n*-Hex)** and Pluronic F127 (mass ratio 1:1000) were dissolved in CH_2Cl_2, and the solvent was removed by rotary evaporation to form a thin film. The film was solubilized by adding buffer solutions of the appropriate pH or culture medium, followed by sonication to form micelles. Encapsulation efficiency was calculated by comparing the absorbance derived from **IR-PAM (*n*-Hex)** before and after applying an ultrafiltration technique, involving centrifugation for 10 minutes at 4000×g using Amicon Ultra (Millipore Co., USA, MWCO 10kDa), performed three times.

Preparation of spirocyclic cyanine dye-loaded PEG-*b*-PCL polymeric micelles

Cyanine dye-loaded PEG-*b*-PCL polymeric micelles were prepared by a solvent evaporation method.[2] **IR-PAM (*n*-Hex)** and PEG-*b*-PCL (mass ratio 1:1000) were dissolved in THF (0.5 mL), and water (20 mL) was added. THF was removed by rotary evaporation to form micelles. This solution was diluted by buffer solutions of the appropriate pH.

Evaluation of polarity of micelle cores

The solvatofluorchromic dye KSD-3 was synthesized as previously reported.[3] KSD-3 was encapsulated in micelles of F127 and PEG-*b*-PCL by the same procedure as described for the loading of spirocyclic cyanine dyes. Fluorescence spectra were measured to evaluate the polarity of each micelle core.

Fluorescence imaging

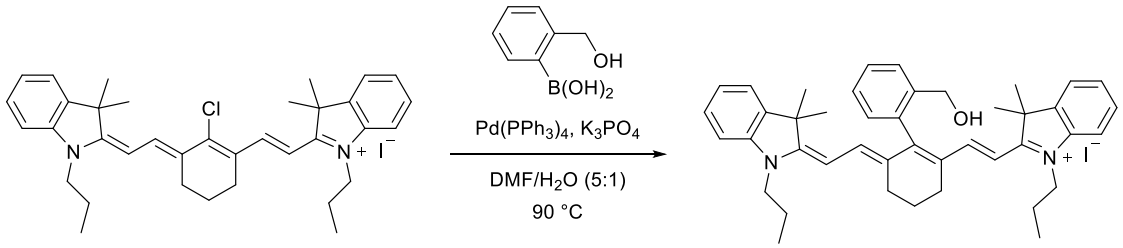
HeLa cells were cultured in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% (v/v; Thermo Fisher Scientific) FBS, 50 U/mL penicillin and 50 μg/mL streptomycin (Nacalai Tesque, Kyoto, Japan) in an incubator maintained at 37 °C and a humidified atmosphere of 5% CO_2. Cells were plated on glass bottom dishes (Iwaki, Tokyo, Japan). HeLa cells were incubated in MEM (Thermo Fisher Scientific) with 10 μM **IR-PAM(*n*-Hex)@F127** for 2 h in the incubator. Then, the cells were washed with MEM to

remove the dye remaining outside the cells. The cells stained with IR-PAM(*n*-Hex)@F127 were incubated with nigericin (5 μg/mL; Wako, Osaka, Japan) in high-concentration K^+ HBSS for 10 min at pH 6.4 or 7.4 (15 mM NaCl; 125 mM KCl; 1.3 mM $CaCl_2$; 0.5 mM $MgCl_2$; 0.4 mM $MgSO_4$; 0.3 mM Na_2HPO_4; 0.4 mM KH_2PO_4; 4.2 mM $NaHCO_3$; 5.6 mM D-glucose; pH was buffered by 10 mM HEPES for pH 6.4 and 7.4), respectively. Fluorescence images were acquired on a fluorescence microscope, BZ-X800 (Keyence, Tokyo, Japan) with a 100x objective. The dye was excited by a metal halide lamp through a 710/75 excitation filter and a 760 nm dichroic mirror, and the fluorescence was observed through an 810/90 nm emission filter. The fluorescence intensity within the cell was determined as an averaged fluorescence intensity in the whole cell body. Fluorescence intensities at pH 6.4 and 7.4 were calculated as averaged fluorescence intensities of all the cells observed at the respective pH.

Synthetic procedures and characterization of new compounds.

Compounds **S1**[4], **S3**[5], **S5**[6], and **S8**[7] were prepared as previously reported.

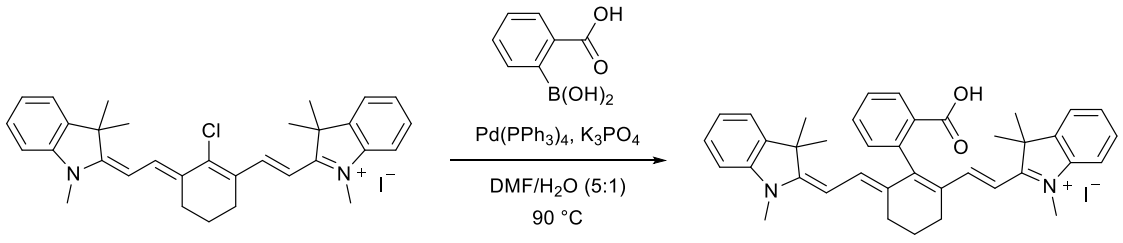
2-[(*E*)-2-(2'-(Hydroxymethyl)-6-{(*E*)-2-[(*E*)-3,3-dimethyl-1-propylindolin-2-ylidene]ethylidene}-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-3,3-dimethyl-1-propyl-3*H*-indol-1-ium iodide (IR-HM).



IR-780 iodide **IR-HM**

The following reaction was carried out under Ar. To a degassed stirred solution of IR-780 iodide (1.02 g, 1.53 mmol), 2-(hydroxymethyl)phenylboronic acid (465 mg, 3.06 mmol), and K_3PO_4 (325 mg, 1.53 mmol) in DMF/H_2O (5:1, 37 mL) was added $Pd(PPh_3)_4$ (175 mg, 0.151 mmol). After being stirred at 90 °C overnight, the mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (three times). The combined extracts were washed with saturated brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 80:20) to provide 370 mg (33%) of **IR-HM** as a green solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, 1H, *J* = 7.7 Hz), 7.55 (dd, 1H, *J* = 7.7, 7.5 Hz), 7.44 (t, 1H, *J* = 7.5 Hz), 7.31 (t, 2H, *J* = 7.6 Hz), 7.19–7.13 (m, 6H), 7.06 (d, 1H, *J* = 7.5 Hz), 7.03 (d, 2H, *J* = 8.0 Hz), 6.03 (d, 2H, *J* = 14.3 Hz), 4.54 (s, 2H), 4.01–3.92 (m, 4H), 2.79–2.73 (m, 2H), 2.67–2.61 (m, 2H), 2.14–2.03 (m, 2H), 1.88–1,81 (m, 4H), 1.17 (s, 6H), 1.13 (s, 6H), 1.03 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 161.3, 147.8, 142.4, 140.9, 140.1, 136.4, 131.4, 128.8, 128.74, 128.69, 128.5, 127.1, 124.8, 122.2, 110.3, 100.0, 61.4, 48.7, 45.9, 28.0, 27.6, 24.7, 21.3, 20.7, 11.7; HRMS (ESI/TOF) calcd for $C_{43}H_{51}N_2O$ (M^+) *m*/*z* 611.3996, found 611.4001.

2-[(*E*)-2-(2'-Carboxy-6-{(*E*)-2-[(*E*)-1,3,3-trimethylindolin-2-ylidene]ethylidene}-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-1,3,3-trimethyl-3*H*-indol-1-ium iodide (S2).

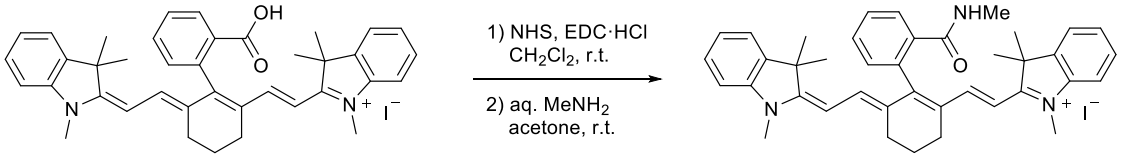


IR-786 iodide (**S1**) **S2**

The following reaction was carried out under Ar. To a degassed stirred solution of **S1** (1.00 g, 1.64 mmol), 2-carboxyphenylboronic acid (543 mg, 3.27 mmol), and K_3PO_4 (347 mg, 1.63 mmol) in DMF/H_2O (5:1, 38 mL) was added $Pd(PPh_3)_4$ (377 mg, 0.326 mmol). After being stirred at 90 °C for 21 h, the mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (three times). The combined extracts were washed with saturated brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 88:12) to provide 208 mg (18%) of **S2** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.19 (dd, 1H, *J* = 7.7, 1.2 Hz), 7.74 (td, 1H, *J* = 7.5, 1.4 Hz), 7.67 (td, 1H, *J* = 7.7, 1.2 Hz), 7.35–7.30 (m, 4H), 7.23–7.14 (m, 5H), 7.20 (d, 2H, *J* = 14.0 Hz), 6.12 (d, 2H, *J* = 14.0 Hz), 3.54 (s, 6H), 2.71 (t, 4H, *J* = 6.2 Hz), 2.12 (m, 1H), 2.02 (m, 1H), 1.19 (s, 6H), 1.13 (s, 6H); ^{13}C NMR (125

MHz, CD_3OD) δ 173.5, 170.0, 165.0, 148.9, 144.4, 142.1, 141.0, 135.0, 132.7, 132.6, 132.1, 131.9, 129.64, 129.62, 125.7, 123.2, 111.3, 100.8, 49.7, 31.3, 28.2, 27.9, 25.8, 22.3; HRMS (ESI/TOF) calcd for $C_{39}H_{41}N_2O_2$ (M^+) *m*/*z* 569.3163, found 569.3168.

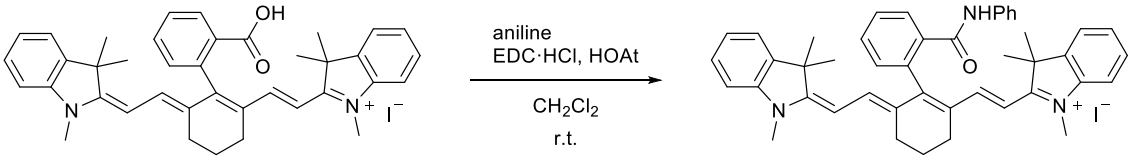
1,3,3-Trimethyl-2-[(*E*)-2-(2'-(methylcarbamoyl)-6-{(*E*)-2-[(*E*)-1,3,3-trimethylindolin-2-ylidene]ethylidene}-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-3*H*-indol-1-ium iodide (IR-MA).



S2 **IR-MA**

The following reaction was carried out under Ar. A solution of **S2** (102 mg, 0.146 mmol), NHS (25.6 mg, 0.222 mmol), and EDC·HCl (54.9 mg, 0.286 mmol) in CH_2Cl_2 (13 mL) was stirred at room temperature for 7 h, and the mixture was concentrated under reduced pressure. The residue was dissolved in acetone (13 mL), and then *ca.* 12 M aqueous $MeNH_2$ (1 mL) was added. After being stirred at room temperature for 16 h, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (three times). The combined extracts were washed with saturated brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 99:1 → 90:10) to provide 37 mg (36%) of **IR-MA** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 7.89 (d, 1H, *J* = 7.2 Hz), 7.73 (t, 1H, *J* = 6.9 Hz), 7.69 (t, 1H, *J* = 7.2 Hz), 7.35 (t, 2H, *J* = 7.5 Hz), 7.32 (d, 2H, *J* = 7.5 Hz), 7.24 (d, 1H, *J* = 7.2 Hz), 7.22–7.16 (m, 6H), 6.15 (d, 2H, *J* = 14.0 Hz), 3.55 (s, 6H), 2.79–2.65 (m, 4H), 2.76 (s, 3H), 2.06 (m, 2H), 1.21 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.7, 169.9, 162.9, 148.8, 144.4, 142.1, 139.4, 137.0, 132.8, 132.4, 131.9, 129.9, 129.7, 129.3, 125.9, 123.2, 111.5, 101.0, 31.4, 28.2, 28.0, 26.9, 25.8, 22.4; HRMS (ESI/TOF) calcd for $C_{40}H_{44}N_3O$ (M^+) *m*/*z* 582.3479, found 582.3476.

1,3,3-Trimethyl-2-[(*E*)-2-(6-{(*E*)-2-[(*E*)-1,3,3-trimethylindolin-2-ylidene]ethylidene}-2'-(phenylcarbamoyl)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-3*H*-indol-1-ium iodide (IR-PAH).

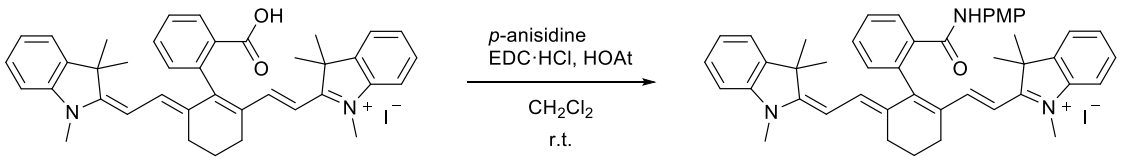


S2 **IR-PAH**

The following reaction was carried out under Ar. A solution of **S2** (24.1 mg, 34.6 μmol), aniline (10 μL, 0.11 mmol), EDC·HCl (10.5 mg, 54.8 μmol), and HOAt (7.8 mg, 57 μmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 17 h, and the mixture was washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, and saturated brine, sequentially. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 97:3 → 92:8) to provide 19.1 mg (70%) of **IR-PAH** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.07 (dd, 1H, *J* = 7.2, 1.4 Hz), 7.81–7.75 (m, 2H), 7.42 (d, 2H, *J* = 8.0 Hz), 7.37–7.27 (m, 8H), 7.23 (t, 2H, *J* = 7.7 Hz), 7.18 (t, 2H, *J* = 7.5 Hz), 7.10

(t, 1H, J = 7.5 Hz), 6.18 (d, 2H, J = 14.0 Hz), 3.56 (s, 6H), 2.76–2.65 (m, 4H), 2.05–1.96 (m, 2H), 1.26 (s, 6H), 1.19 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.9, 167.4, 161.9, 148.5, 144.4, 142.2, 139.3, 137.2, 132.8, 132.5, 132.2, 130.1, 130.0, 129.7, 126.0, 125.9, 123.2, 111.6, 101.3, 49.8, 31.5, 28.3, 27.9, 25.8, 22.3; HRMS (ESI/TOF) calcd for $C_{45}H_{46}N_3O$ (M^+) m/z 644.3635, found 644.3661.

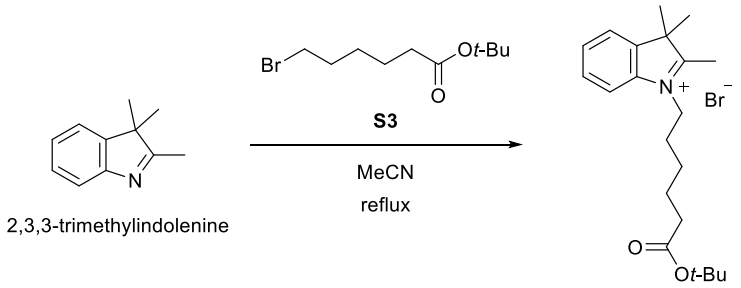
2-[(*E*)-2-(2'-[(4-Methoxyphenyl)carbamoyl]-6-{(*E*)-2-[(*E*)-1,3,3-trimethylindolin-2-ylidene]ethylidene}-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-1,3,3-trimethyl-3*H*-indol-1-ium iodide [IR-PAM (Me)].



S2 **IR-PAM (Me)**

The following reaction was carried out under Ar. A solution of **S2** (20.0 mg, 28.7 μmol), *p*-anisidine (7.1 mg, 58 μmol), EDC·HCl (8.4 mg, 44 μmol), and HOAt (6.0 mg, 44 μmol) in CH_2Cl_2 (2 mL) was stirred at room temperature overnight, and the mixture was washed with 1 M aqueous HCl, saturated aqueous $NaHCO_3$, and saturated brine, sequentially. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 97:3 → 92:8) to provide 17.5 mg (76%) of **IR-PAM (Me)** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.05 (dd, 1H, J = 7.4, 1.4 Hz), 7.80–7.74 (m, 2H), 7.37–7.30 (m, 7H), 7.25 (d, 2H, J = 14.0 Hz), 7.22 (d, 2H, J = 8.0 Hz), 7.18 (td, 2H, J = 7.5, 0.6 Hz), 6.86–6.83 (m, 2H), 6.17 (d, 2H, J = 14.0 Hz), 3.74 (s, 3H), 3.56 (s, 6H), 2.77–2.66 (m, 4H), 2.10–1.97 (m, 2H), 1.26 (s, 6H), 1.19 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.8, 167.4, 162.2, 158.4, 148.6, 144.4, 142.1, 139.3, 137.3, 132.8, 132.5, 132.13, 132.10, 130.1, 129.7, 129.6, 126.0, 123.9, 123.2, 115.1, 111.6, 110.2, 55.9, 49.9, 31.4, 28.3, 27.9, 25.8, 22.3; HRMS (ESI/TOF) calcd for $C_{46}H_{48}N_3O_2$ (M^+) m/z 674.3741, found 674.3737.

1-[5-(*tert*-Butoxycarbonyl)pentyl]-2,3,3-trimethyl-3*H*-indol-1-ium bromide (S4).

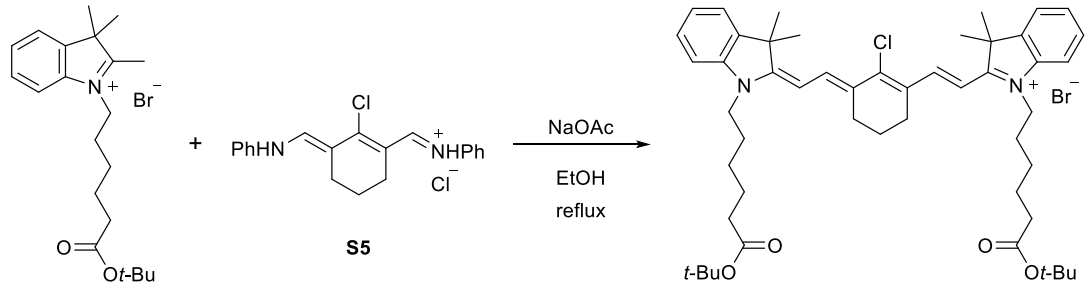


S4

The following reaction was carried out under Ar. A solution of 2,3,3-trimethylindolenine (3.40 g, 21.4 mmol) and **S3** (7.99 g, 31.8 mmol) in MeCN (32 mL) was refluxed for 48 h, and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 90:10) to provide 4.09 g (47%) of **S4** as a highly hygroscopic pink solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (m, 1H), 7.85 (m, 1H), 7.64–7.61 (m, 2H), 4.46 (t, 2H, J = 7.6 Hz), 2.85 (s, 3H), 2.20 (t, 2H, J = 7.3

Hz), 1.84 (m, 2H), 1.57–1.51 (m, 2H), 1.54 (s, 6H), 1.43–1.36 (m, 2H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.6, 172.2, 141.9, 141.1, 129.4, 129.0, 123.5, 115.5, 79.5, 54.2, 47.5, 34.4, 27.8, 27.0, 25.3, 24.2, 22.0, 14.0; HRMS (ESI/TOF) calcd for $C_{21}H_{32}NO_2$ (M^+) *m*/*z* 330.2428, found 330.2428.

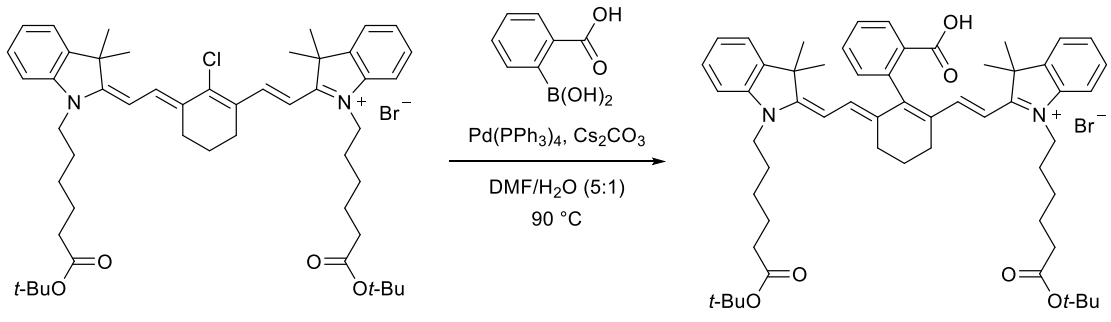
1-[5-(*tert*-Butoxycarbonyl)pentyl]-2-{(*E*)-2-[3-((*E*)-2-{(*E*)-1-[5-(*tert*-butoxycarbonyl)pentyl]-3,3-dimethylindolin-2-ylidene}ethylidene)-2-chlorocyclohex-1-enyl]vinyl}-3,3-dimethyl-3*H*-indol-1-ium bromide (S6).



S4 **S5** **S6**

The following reaction was carried out under Ar. A solution of **S4** (3.35 g, 8.16 mmol), **S5** (1.66 g, 4.63 mmol), NaOAc (669 mg, 8.16 mmol) in EtOH (80 mL) was refluxed for 5 h, and the mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (100 mL × 3) and saturated brine (40 mL), sequentially. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 94:6) to provide 2.14 g (60% from **S4**) of **S6** as green crystals: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (d, 2H, *J* = 14.0 Hz), 7.63 (d, 2H, *J* = 7.5 Hz), 7.46–7.41 (m, 4H), 7.29 (t, 2H, *J* = 7.2 Hz), 6.32 (d, 2H, *J* = 14.0 Hz), 4.23 (t, 4H, *J* = 7.0 Hz), 2.71 (t, 4H, *J* = 5.7 Hz), 2.18 (t, 4H, *J* = 7.5 Hz), 1.86 (quint, 2H, *J* = 5.6 Hz), 1.74 (quint, 4H, *J* = 7.5 Hz), 1.67 (s, 12H), 1.54 (quint, 4H, *J* = 7.5 Hz), 1.40–1.34 (m, 4H), 1.34 (s, 18H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.3, 172.2, 148.0, 143.0, 142.1, 141.1, 128.7, 126.1, 125.2, 122.6, 111.6, 101.7, 79.4, 49.0, 43.7, 34.6, 27.7, 27.5, 26.8, 25.8, 25.6, 24.4, 20.4; HRMS (ESI/TOF) calcd for $C_{50}H_{68}N_2O_4$ (M^+) *m*/*z* 795.4862, found 795.4851.

1-[5-(*tert*-Butoxycarbonyl)pentyl]-2-{(*E*)-2-[6-((*E*)-2-{(*E*)-1-[5-(*tert*-butoxycarbonyl)pentyl]-3,3-dimethylindolin-2-ylidene}ethylidene)-2'-carboxy-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl]vinyl}-3,3-dimethyl-3*H*-indol-1-ium bromide (S7).

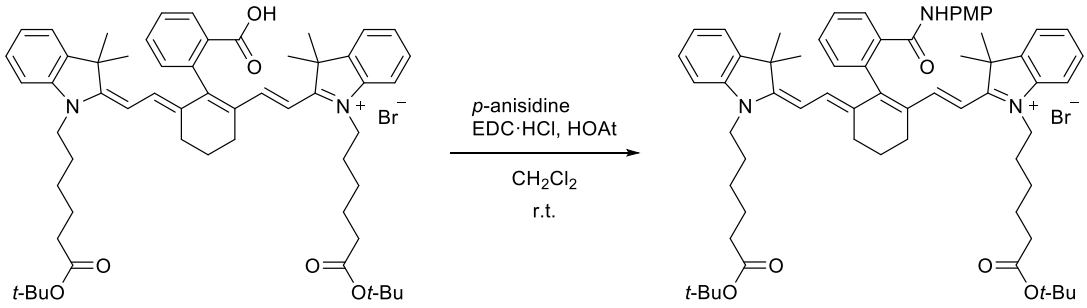


S6 **S7**

The following reaction was carried out under Ar. To a degassed stirred solution of **S6** (1.00 g, 1.14 mmol),

2-carboxyphenylboronic acid (372 mg, 2.24 mmol), and Cs_2CO_3 (366 mg, 1.12 mmol) in DMF/H_2O (5:1, 38 mL) was added $Pd(PPh_3)_4$ (261 mg, 0.226 mmol). After being stirred at 90 °C for 23 h, the mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (three times). The combined extracts were washed with saturated brine, dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 90:10) to provide 131 mg (12%) of **S7** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.18 (dd, 1H, *J* = 7.7, 1.2 Hz), 7.69 (td, 1H, *J* = 7.5, 1.4 Hz), 7.64 (td, 1H, *J* = 7.6, 1.4 Hz), 7.35–7.30 (m, 4H), 7.23 (d, 2H, *J* = 14.0 Hz), 7.19 (dd, 1H, *J* = 7.2, 1.4 Hz), 7.18 (d, 2H, *J* = 8.0 Hz), 7.15 (td, 2H, *J* = 7.5, 0.6 Hz), 6.13 (d, 2H, *J* = 14.0 Hz), 4.05 (t, 4H, *J* = 7.3 Hz), 2.76–2.66 (m, 4H), 2.24 (t, 4H, *J* = 7.2 Hz), 2.14 (m, 1H), 2.02 (m, 1H), 1.78 (quint, 4H, *J* = 7.5 Hz), 1.64 (quint, 4H, *J* = 7.5 Hz), 1.45–1.40 (m, 4H), 1.41 (s, 18H), 1.20 (s, 6H), 1.13 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.7, 172.8, 170.8, 165.9, 149.1, 143.8, 142.2, 140.7, 136.5, 132.7, 132.0, 131.8, 129.6, 129.5, 125.7, 123.3, 111.6, 100.7, 81.5, 49.8, 44.7, 36.1, 28.39, 28.35, 28.04, 28.00, 27.4, 25.9, 22.4; HRMS (ESI/TOF) calcd for $C_{57}H_{73}N_2O_6$ (M^+) *m*/*z* 891.5463, found 891.5499.

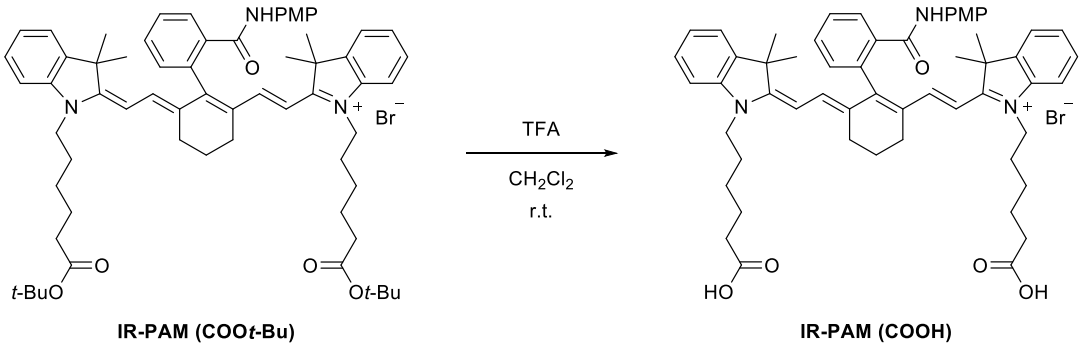
1-[5-(*tert*-Butoxycarbonyl)pentyl]-2-{(*E*)-2-[6-((*E*)-2-{(*E*)-1-[5-(*tert*-butoxycarbonyl)pentyl]-3,3-dimethylindolin-2-ylidene}ethylidene)-2'-[(4-methoxyphenyl)carbamoyl]-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl]vinyl}-3,3-dimethyl-3*H*-indol-1-ium bromide [IR-PAM (COO*t*-Bu)] .



S7 **IR-PAM (COO*t*-Bu)**

The following reaction was carried out under Ar. A solution of **S7** (30.0 mg, 31.2 μmol), *p*-anisidine (5.0 mg, 41 μmol), EDC·HCl (10.0 mg, 52.1 μmol), and HOAt (7.0 mg, 51 μmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 2 h, and then *p*-anisidine (4.4 mg, 36 μmol) was added. After being stirred for 3.5 h, the mixture was washed with saturated aqueous $NaHCO_3$, 1 M aqueous HCl, and saturated brine, sequentially. The organic layer was dried and concentrated under reduced pressure to provide 33.9 mg (quant.) of **IR-PAM (COO*t*-Bu)** as a dark green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (dd, 1H, *J* = 7.6, 1.6 Hz), 7.80–7.74 (m, 2H), 7.37–7.29 (m, 7H), 7.25 (d, 2H, *J* = 14.0 Hz), 7.22 (d, 1H, *J* = 8.0 Hz), 7.19 (t, 2H, *J* = 7.5 Hz), 6.87–6.83 (m, 2H), 6.20 (d, 2H, *J* = 14.0 Hz), 4.08 (t, 4H, *J* = 7.2 Hz), 3.74 (s, 3H), 2.77–2.66 (m, 4H), 2.22 (t, 4H, *J* = 7.2 Hz), 2.03 (quint, 2H, *J* = 6.0 Hz), 1.78 (quint, 4H, *J* = 7.5 Hz), 1.63 (quint, 4H, *J* = 7.5 Hz), 1.39 (s, 18H), 1.26 (s, 6H), 1.19 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.7, 173.2, 167.3, 162.4, 158.4, 148.6, 143.7, 142.2, 139.3, 137.3, 132.8, 132.5, 132.1, 130.1, 129.8, 129.6, 126.0, 123.9, 123.4, 115.1, 111.9, 101.2, 81.5, 55.9, 50.0, 44.8, 36.1, 28.44, 28.38, 28.1, 27.3, 25.9, 22.4; HRMS (ESI/TOF) calcd for $C_{64}H_{80}N_3O_6$ (M^+) *m*/*z* 986.6042, found 986.6016.

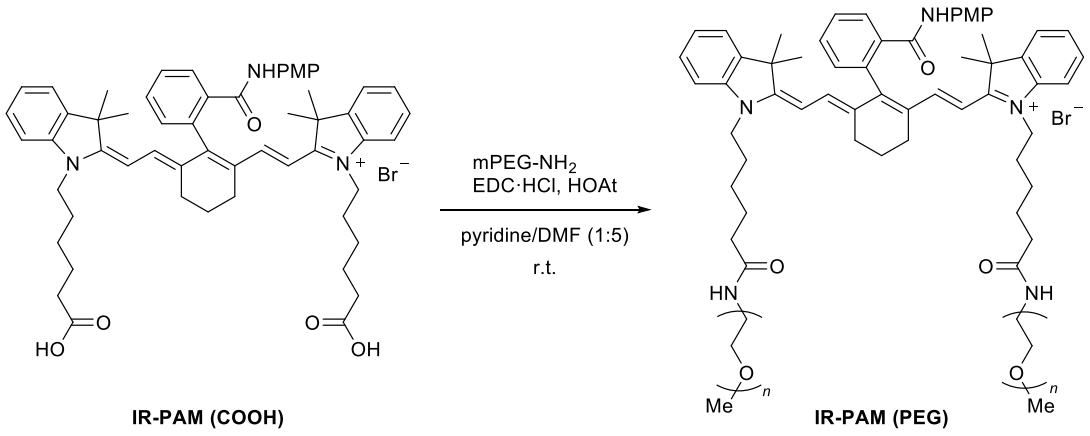
1-(5-Carboxypentyl)-2-[(*E*)-2-(6-{(*E*)-2-[(*E*)-1-(5-carboxypentyl)-3,3-dimethylindolin-2-ylidene]-ethylidene}-2'-[(4-methoxyphenyl)carbamoyl]-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-3,3-dimethyl-3*H*-indol-1-ium bromide [IR-PAM (COOH)].



IR-PAM (COO*t*-Bu) **IR-PAM (COOH)**

The following reaction was carried out under Ar. A solution of **IR-PAM (COO*t*-Bu)** (19.3 mg, 18.1 μmol) in 5% TFA in CH_2Cl_2 (2 mL) was stirred at room temperature for 24 h, and the mixture was directly purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 92:8) to provide 8.9 mg (52%) of **IR-PAM (COOH)** as a green solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.05 (dd, 1H, *J* = 7.6, 1.3 Hz), 7.77 (td, 2H, *J* = 7.5, 1.7 Hz), 7.37–7.32 (m, 5H), 7.29 (td, 2H, *J* = 6.9, 2.0 Hz) , 7.25 (d, 2H, *J* = 14.0 Hz), 7.22 (d, 1H, *J* = 8.0 Hz), 7.18 (t, 2H, *J* = 7.5 Hz), 6.85 (td, 1H, *J* = 6.9, 2.0 Hz), 6.20 (d, 2H, *J* = 14.0 Hz), 4.08 (t, 4H, *J* = 7.6 Hz), 3.74 (s, 3H), 2.74–2.69 (m, 4H), 2.29 (t, 4H, *J* = 7.3 Hz), 2.02 (quint, 2H, *J* = 5.0 Hz), 1.79 (quint, 4H, *J* = 7.0 Hz), 1.67 (quint, 4H, *J* = 7.5 Hz), 1.46 (quint, 4H, *J* = 7.5 Hz), 1.25 (s, 6H), 1.19 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 177.2, 173.2, 167.4, 162.4, 158.5, 148.6, 143.7, 142.2, 139.4, 137.3, 132.9, 132.5, 132.1, 132.0, 130.1, 129.8, 129.6, 126.0, 124.0, 123.4, 115.1, 111.8, 101.2, 55.9, 50.0, 44.8, 34.6, 28.4, 28.1, 28.0, 27.4, 25.8, 25.7, 22.3; HRMS (ESI/TOF) calcd for $C_{56}H_{64}N_3O_6$ (M^+) *m*/*z* 874.4790, found 874.4802.

IR-PAM (PEG).



IR-PAM (COOH) **IR-PAM (PEG)**

The following reaction was carried out under Ar. To a stirred solution of **IR-PAM (COOH)** (5.6 mg, 5.9 μmol), EDC·HCl (4.0 mg, 21 μmol), and HOAt (3.0 mg, 22 μmol) in DMF (1 mL) were added mPEG-NH_2 (average M_n = 500, 14.0 mg, 28.0 μmol) and pyridine (0.2 mL). After being stirred at room temperature for 1 h, EDC·HCl (4.5 mg, 23 μmol) was added. After being stirred at room temperature for 22 h, the mixture

was concentrated under reduced pressure. The residue was diluted with CH_2Cl_2 (8 mL) and washed with 1 M aqueous HCl (10 mL × 2) and saturated brine, sequentially. The organic layer was dried and concentrated under reduced pressure to provide 9.7 mg of the mixture of **IR-PAM (PEG)** and mPEG-NH_2 (1:3) as a green oil: MALDI-TOFMS revealed average M_n of 1825 ± 200 which represents the title compound.

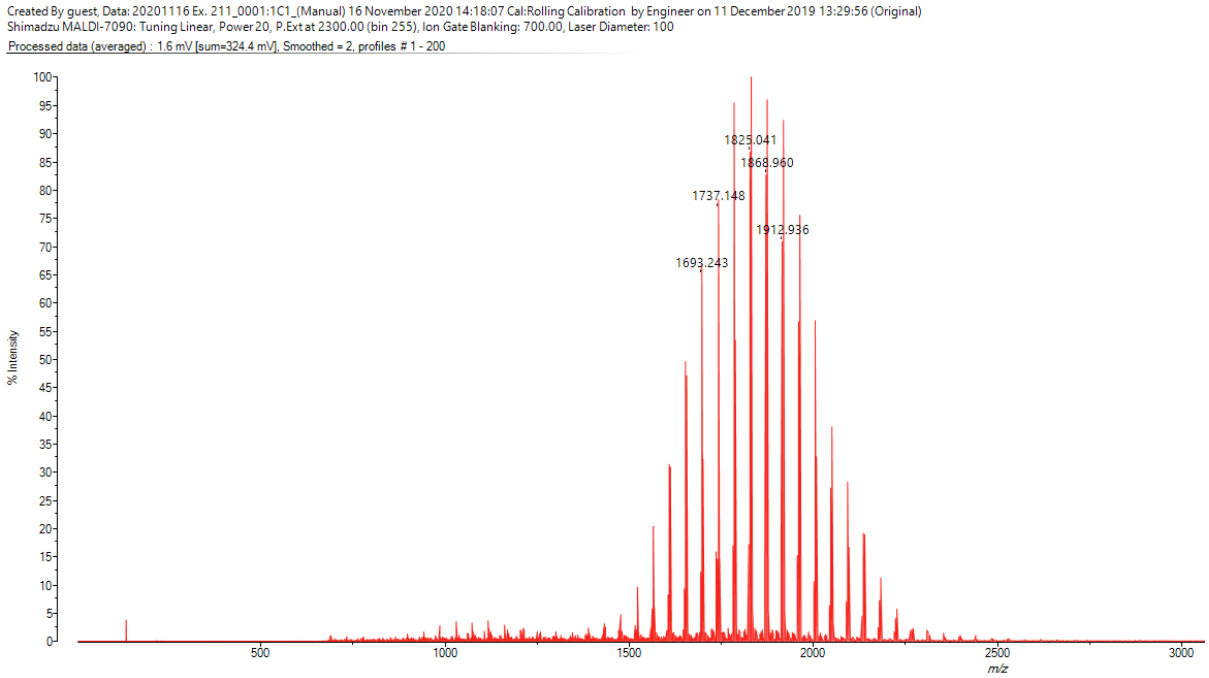
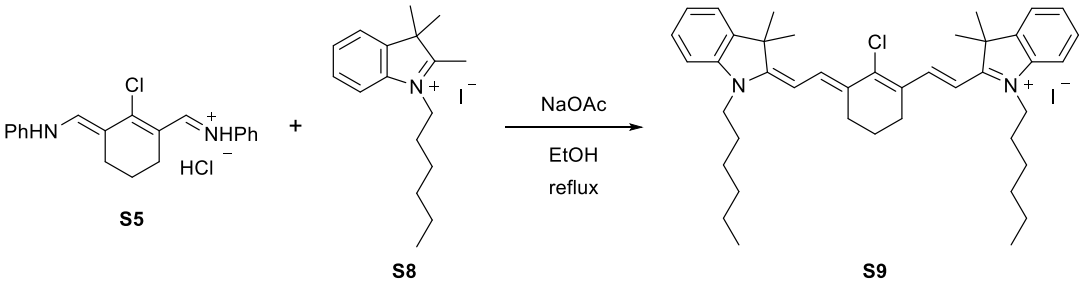


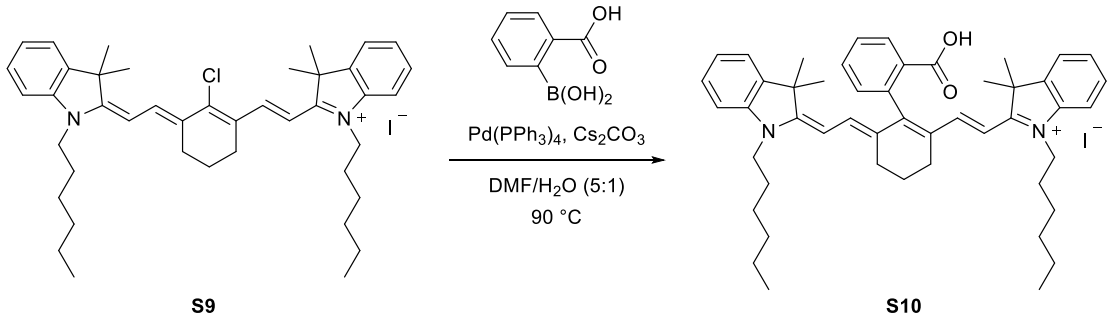
Fig. S1 MALDI-TOFMS spectrum of **IR-PAM (PEG)**.

2-[(*E*)-2-(2-Chloro-3-{(*E*)-2-[(*E*)-1-hexyl-3,3-dimethylindolin-2-ylidene]ethylidene}cyclohex-1-enyl) vinyl]-1-hexyl-3,3-dimethyl-3*H*-indol-1-ium iodide (S9).



The following reaction was carried out under Ar. A solution of **S5** (450 mg, 1.25 mmol), **S8** (1.00 g, 2.69 mmol), and NaOAc (232 mg, 2.83 mmol) in EtOH (20 mL) was stirred at 80 °C for 4 h. The mixture was diluted with H_2O (70 mL) and extracted with CH_2Cl_2 (10 mL × 6). The combined extracts were washed with saturated brine (80 mL), dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 100:0 → 80:20) to provide 625 mg (67%) of **S9** as green solid: The spectroscopic data were consistent with those reported in the literature.[8]

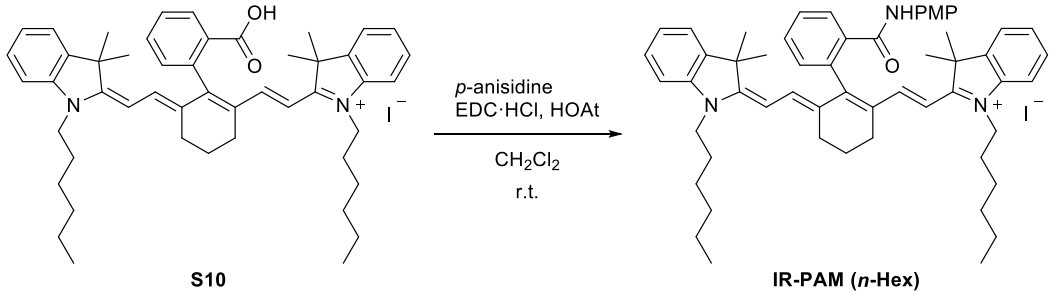
2-[(*E*)-2-(2'-Carboxy-6-{(*E*)-2-[(*E*)-1-hexyl-3,3-dimethylindolin-2-ylidene]ethylidene}-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-1-hexyl-3,3-dimethyl-3*H*-indol-1-ium iodide (S10).



S9 **S10**

The following reaction was carried out under Ar. A solution of **S9** (628 mg, 0.835 mmol), 2-carboxyphenylboronic acid (280 mg, 1.69 mmol), Cs_2CO_3 (271 mg, 0.833 mmol), and $Pd(PPh_3)_4$ (192 mg, 0.166 mmol) in degassed DMF/H_2O (5:1, 30 mL) was stirred at 90 °C for 17 h. The mixture was diluted with H_2O (80 mL) and extracted with CH_2Cl_2 (10 mL × 7). The combined extracts were washed with saturated brine (120 mL), dried, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 80:20) to provide 94.7 mg (14%) of **S10** as green solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (d, 1H, *J* = 7.5 Hz), 7.65–7.54 (m, 2H), 7.30–7.27 (m, 2H), 7.15–7.09 (m, 7H), 6.95 (d, 2H, *J* = 7.7 Hz), 5.91 (d, 2H, *J* = 13.8 Hz), 3.88 (br t, 4H, *J* = 7.2 Hz), 2.74–2.70 (m, 2H), 2.57–2.54 (m, 2H), 2.19 (m, 1H), 1.97 (m, 1H), 1.78–1.72 (m, 4H), 1.43–1.29 (m, 12H), 1.12 (s, 6H), 1.08 (s, 6H), 0.96–0.87 (m, 6H); ^{3}C NMR (100 MHz, $CDCl_3$) δ 170.8, 167.2, 165.9, 148.0, 142.3, 140.8, 139.2, 135.88, 135.86, 132.0, 131.7, 130.4, 129.9, 128.4, 128.1, 124.4, 122.1, 109.8, 99.3, 48.5, 44.1, 31.3, 28.0, 27.6, 27.0, 26.7, 24.7, 22.5, 14.0; HRMS (ESI/TOF) calcd for $C_{49}H_{61}N_2O_2$ (M^+) *m*/*z* 709.4728, found 709.4752.

1-Hexyl-2-[(*E*)-2-(6-{(*E*)-2-[(*E*)-1-hexyl-3,3-dimethylindolin-2-ylidene]ethylidene}-2'-[(4-methoxyphenyl)carbamoyl]-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl]-3,3-dimethyl-3*H*-indol-1-ium iodide [IR-PAM (*n*-Hex)].



S10 **IR-PAM (*n*-Hex)**

The following reaction was carried out under Ar. To a stirred solution of **S10** (20.3 mg, 24.3 μmol), EDC·HCl (9.3 mg, 49 μmol), and HOAt (7.2 mg, 53 μmol) in CH_2Cl_2 (4 mL) was added *p*-anisidine (7.2 mg, 58 μmol). After being stirred at room temperature for 22 h, the mixture was quenched with saturated aqueous $NaHCO_3$ (4 mL) and extracted with CH_2Cl_2 (2 mL × 4). The combined extracts were washed with saturated aqueous $NaHCO_3$ (20 mL), 1 M aqueous HCl (50 mL), and saturated brine (70 mL), sequentially. The organic layer was dried and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 90:10 → 80:20) to provide 15.2 mg (66%) of **IR-PAM (*n*-Hex)** as green solid: ^{1}H

NMR (500 MHz, CD_3OD) δ 8.06 (dd, 1H, *J* = 7.5, 1.7 Hz), 7.78–7.74 (m, 2H), 7.37–7.29 (m, 7H), 7.25 (d, 2H, *J* = 13.9 Hz), 7.22–7.17 (m, 4H), 6.86–6.82 (m, 2H), 6.19 (d, 2H, *J* = 13.9 Hz), 3.74 (s, 3H), 2.76–2.65 (m, 4H), 1.76 (quint, 4H, *J* = 7.2 Hz), 1.43–1.29 (m, 12H), 1.25 (s, 6H), 1.19 (s, 6H), 0.91–0.88 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.2, 167.4, 162.4, 158.4, 153.5, 148.6, 143.7, 142.2, 139.3, 137.3, 132.7, 132.5, 132.2, 132.1, 130.2, 129.7, 129.6, 126.0, 123.9, 126.4, 115.1, 111.8, 101.2, 55.9, 50.0, 44.9, 32.6, 28.4, 28.2, 28.1, 27.6, 25.8, 23.6, 22.3, 14.3; HRMS (ESI/TOF) calcd for $C_{56}H_{68}N_3O_2$ (M^+) *m*/*z* 814.5307, found 814.5324.

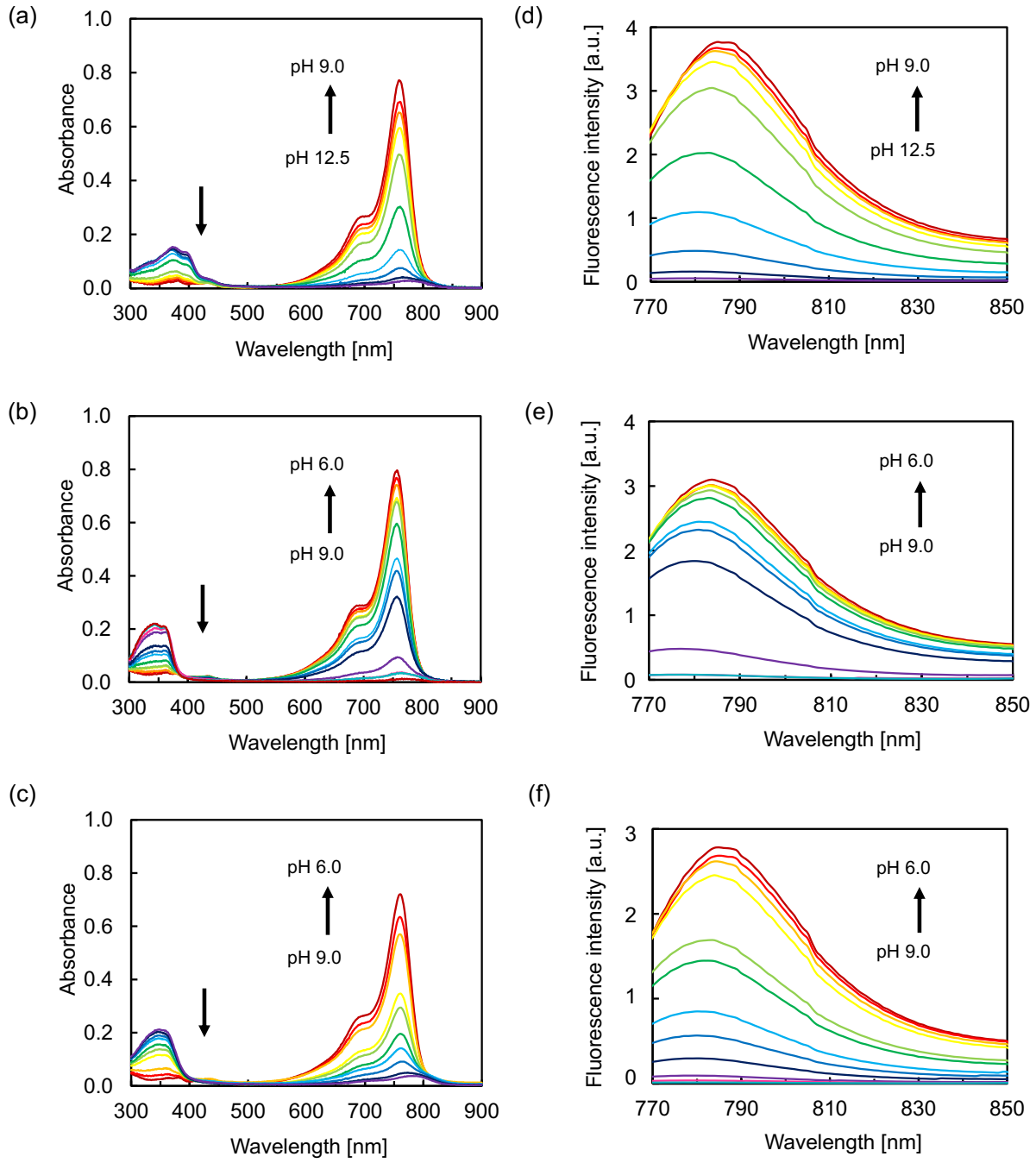
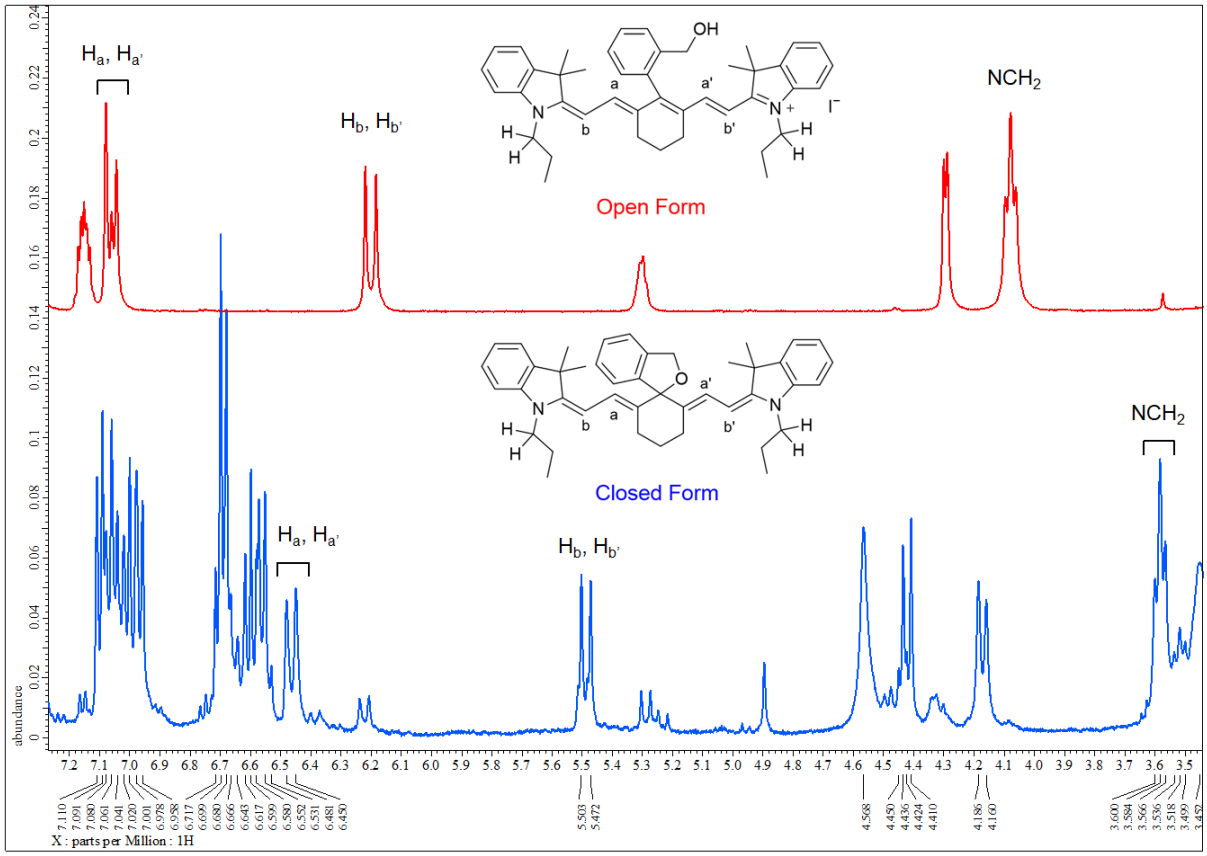


Fig. S2 Absorption spectra of (a) **IR-HM**, (b) **IR-MA**, (c) **IR-PAH**, and fluorescence emission spectra of (d) **IR-HM**, (e) **IR-MA**, (f) **IR-PAH** (4 μM dye in 25 mM buffer solution containing 10% DMSO, λ_{ex} = 740 nm).

(a)



	Chemical shift [ppm]		
	H_a, $H_{a'}$	H_b, $H_{b'}$	NCH_2
Open Form	7.07	6.21	4.09
Closed Form	6.47	5.49	3.58

(b)

Fig. S3 (a) Comparison of ^{1}H NMR spectra (400 MHz, DMSO-d_6) for the open and closed forms of **IR-HM**. The closed form was prepared through the treatment of a solution of **IR-HM** (open form) in CH_2Cl_2 with 1 M aqueous KOH followed by concentration of the organic layer. The chemical shifts of the olefinic methine protons (H_a, $H_{a'}$, H_b, $H_{b'}$) and the nitrogen-adjacent methylene protons (NCH_2) moved upfield with the conversion from the open form to the closed form, reflecting the structural change. (b) The NMR sample solutions of the open (top) and closed (bottom) forms of **IR-HM** in DMSO-d_6. A distinct color difference could be observed between the two solutions.

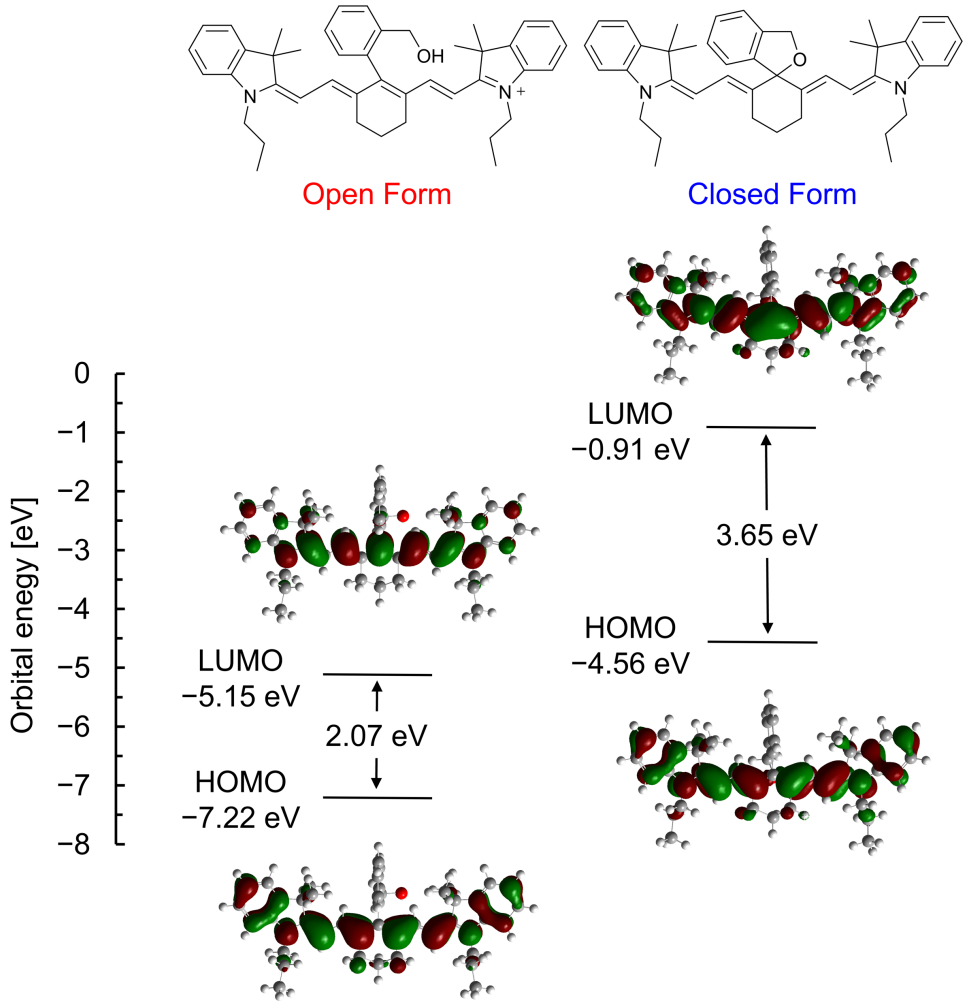


Fig. S4 Quantum chemical calculations of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy levels and their electron density plots of the open and closed forms of **IR-HM** in vacuum using B3LYP/6-31+G(d) theory level.

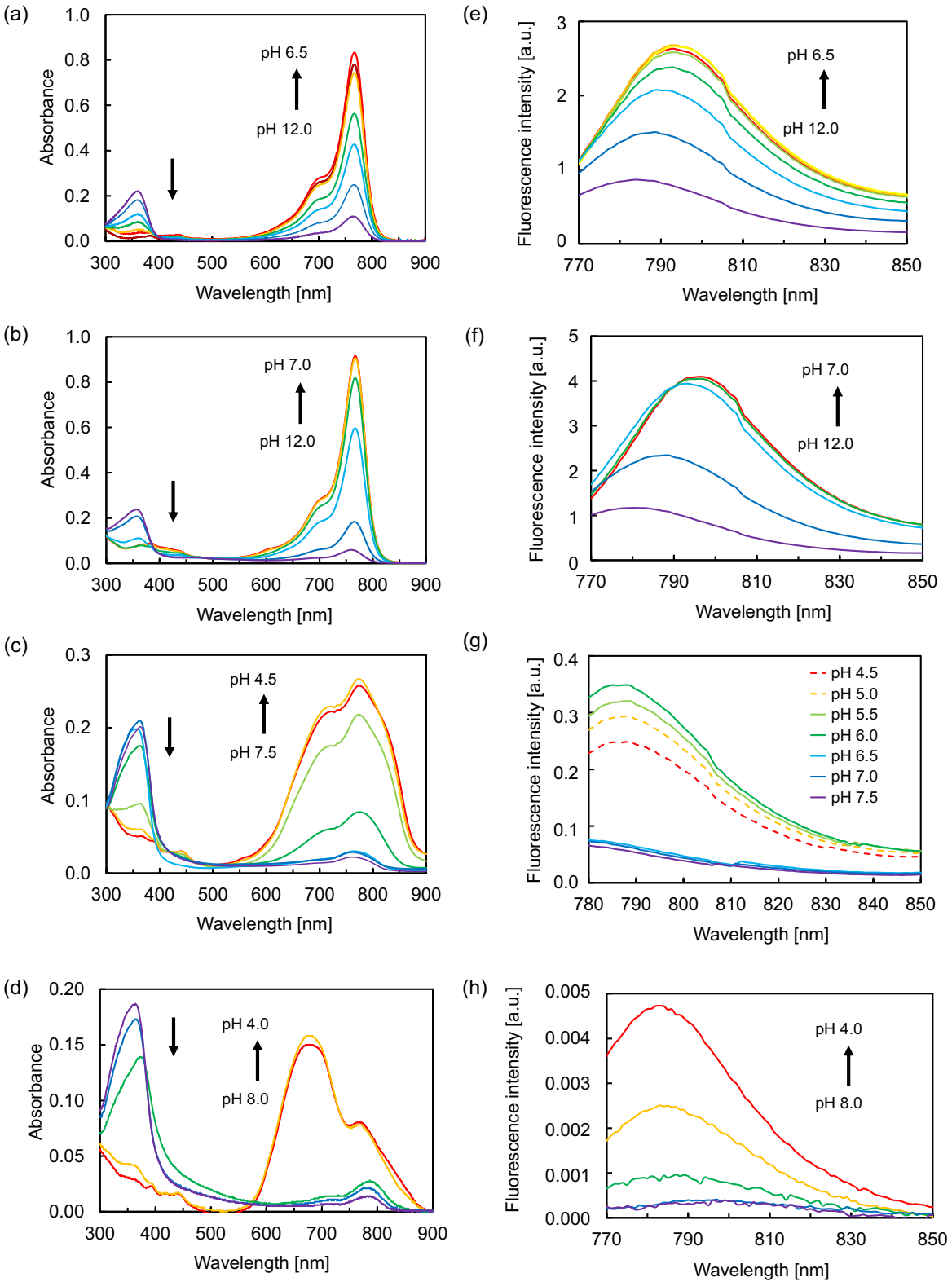


Fig. S5 Absorption spectra of (a) **IR-PAM (COOH)**, (b) **IR-PAM (PEG)**, (c) **IR-PAM (COO*t*-Bu)**, (d) **IR-PAM (*n*-Hex)**, and fluorescence emission spectra of (e) **IR-PAM (COOH)**, (f) **IR-PAM (PEG)**, (g) **IR-PAM (COO*t*-Bu)**, (h) **IR-PAM (*n*-Hex)** (4 μM dye in 25 mM buffer solution containing 10% DMSO, λ_{ex} = 740, 750, 760, 740 nm, respectively).

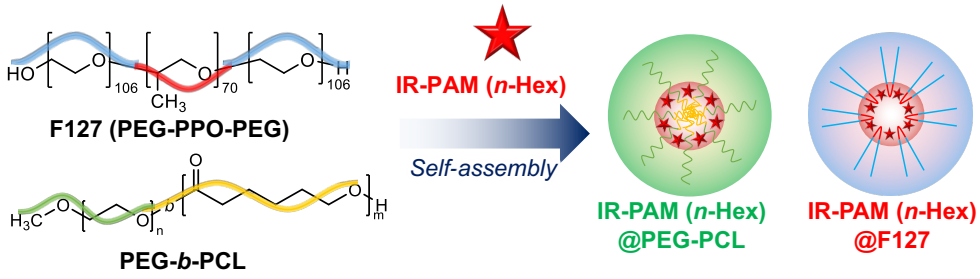


Fig. S6 Scheme of encapsulation in polymeric micelles.

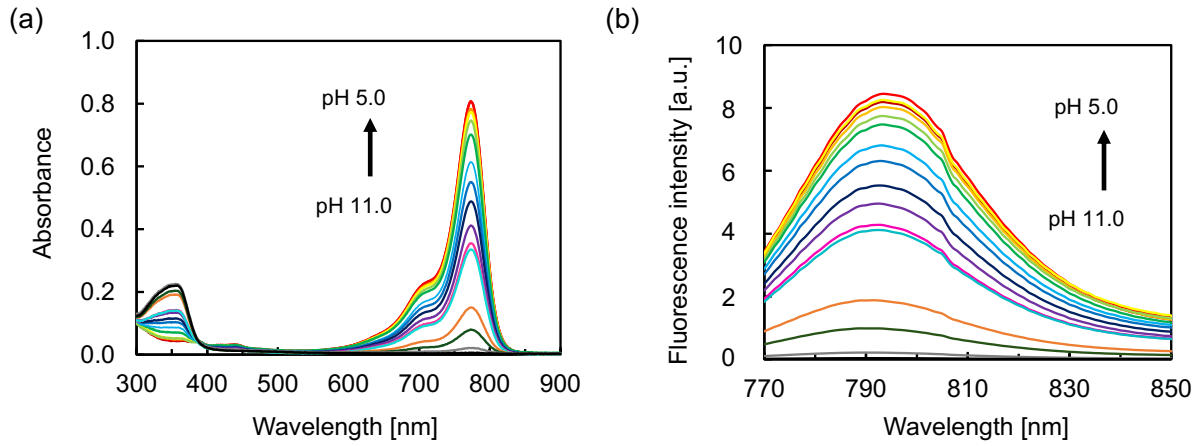


Fig. S7 (a) Absorption and (b) fluorescence emission spectra of **IR-PAM (*n*-Hex)@PEG-*b*-PCL** (2 μM dye, λ_{ex} = 740 nm).

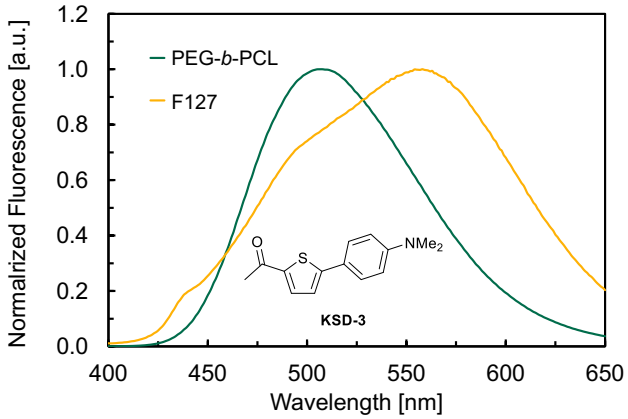


Fig. S8 Evaluation of polarity of micelle cores. To estimate the difference in the hydrophobicity of the cores of PEG-*b*-PCL and Pluronic F127 micelles, fluorescence spectra of the micelles encapsulating a solvatofluorchromic dye KSD-3[3] were measured (λ_{ex} = 380 nm). The inset shows the chemical structure of KSD-3. The emission wavelength of KSD-3 is bathochromically shifted as the polarity of its surrounding environment increases. The fluorescence maximum of KSD-3 in PEG-*b*-PCL micelles is located at shorter wavelength than that in F127 micelles, indicating that the core of PEG-*b*-PCL micelles is more hydrophobic than F127 micelles.

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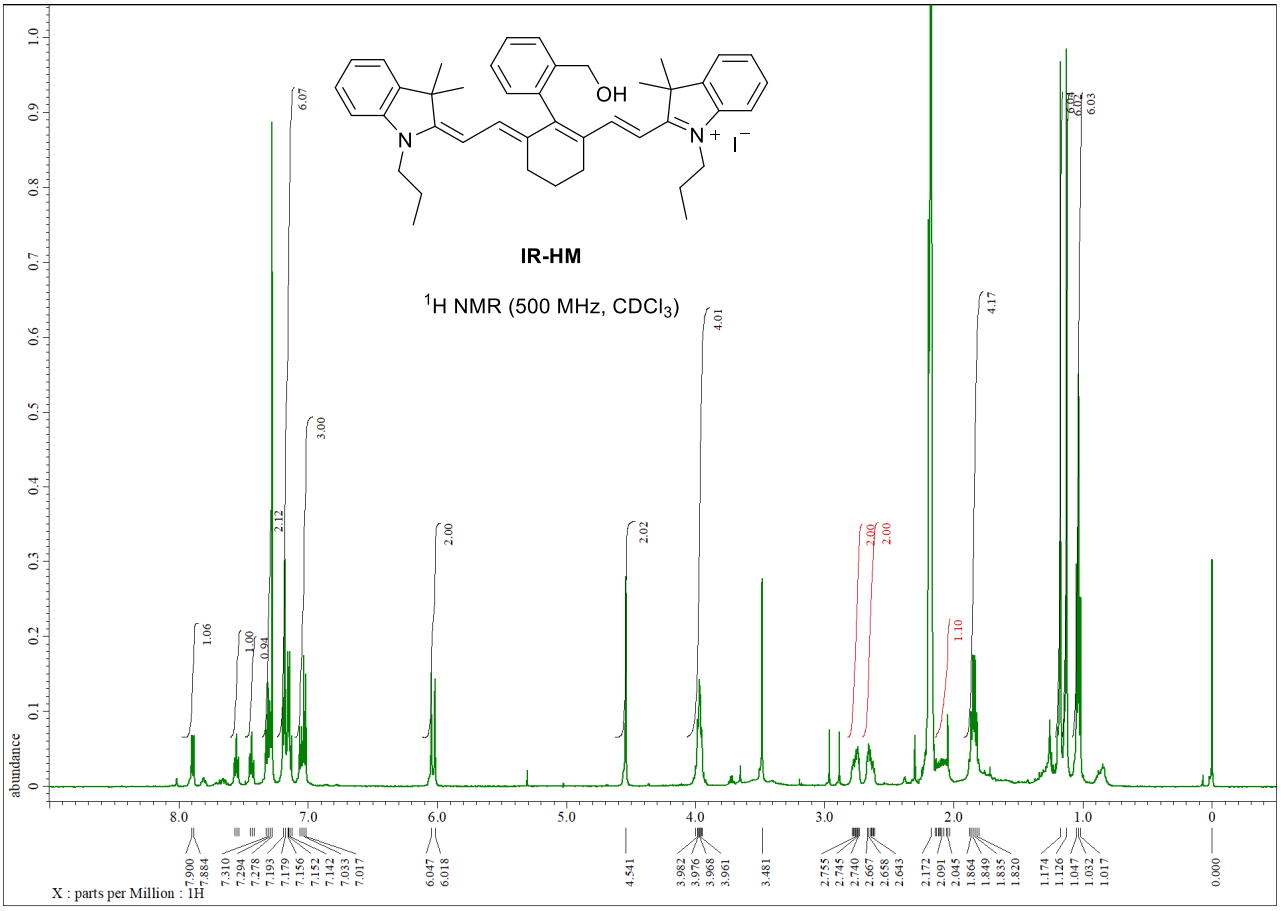
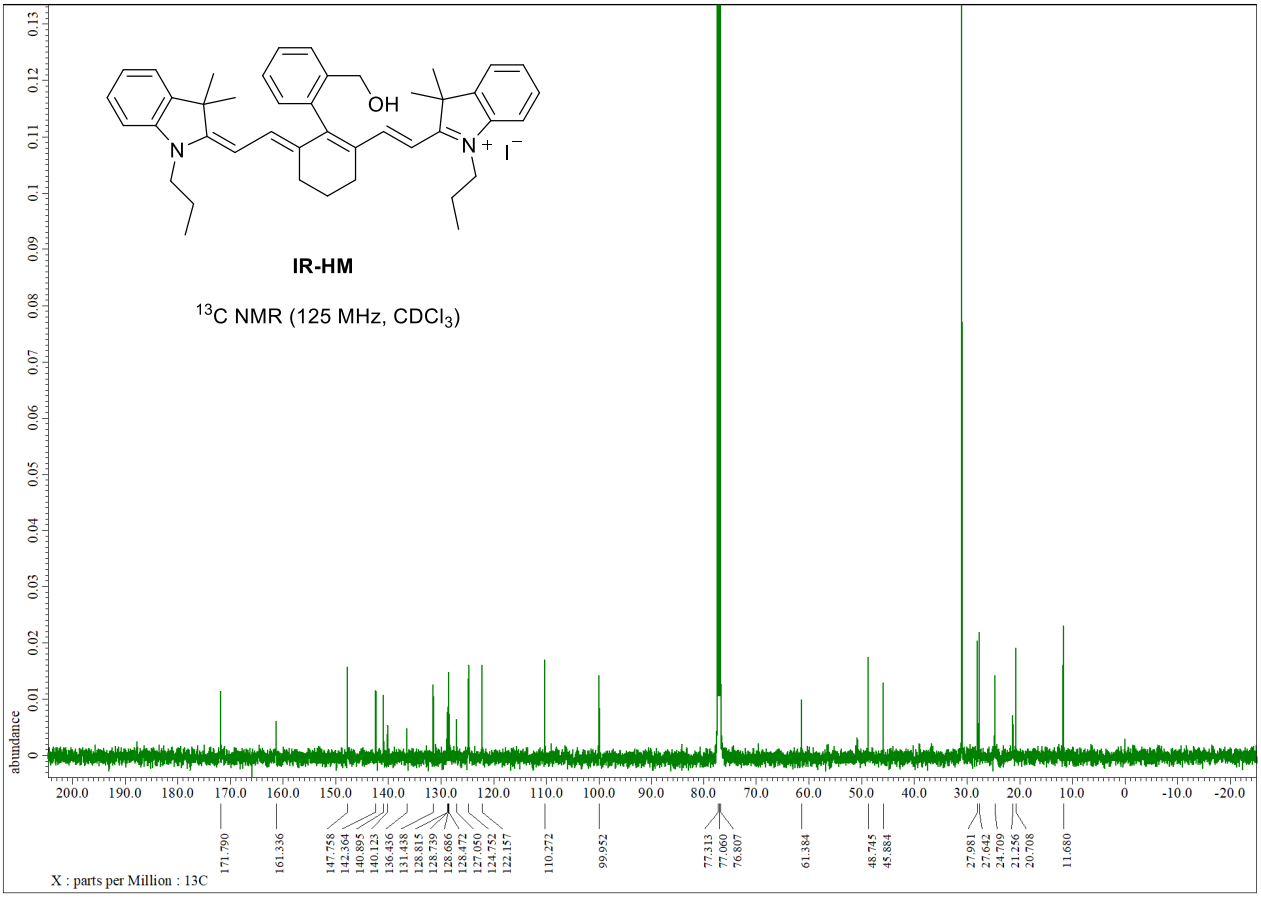
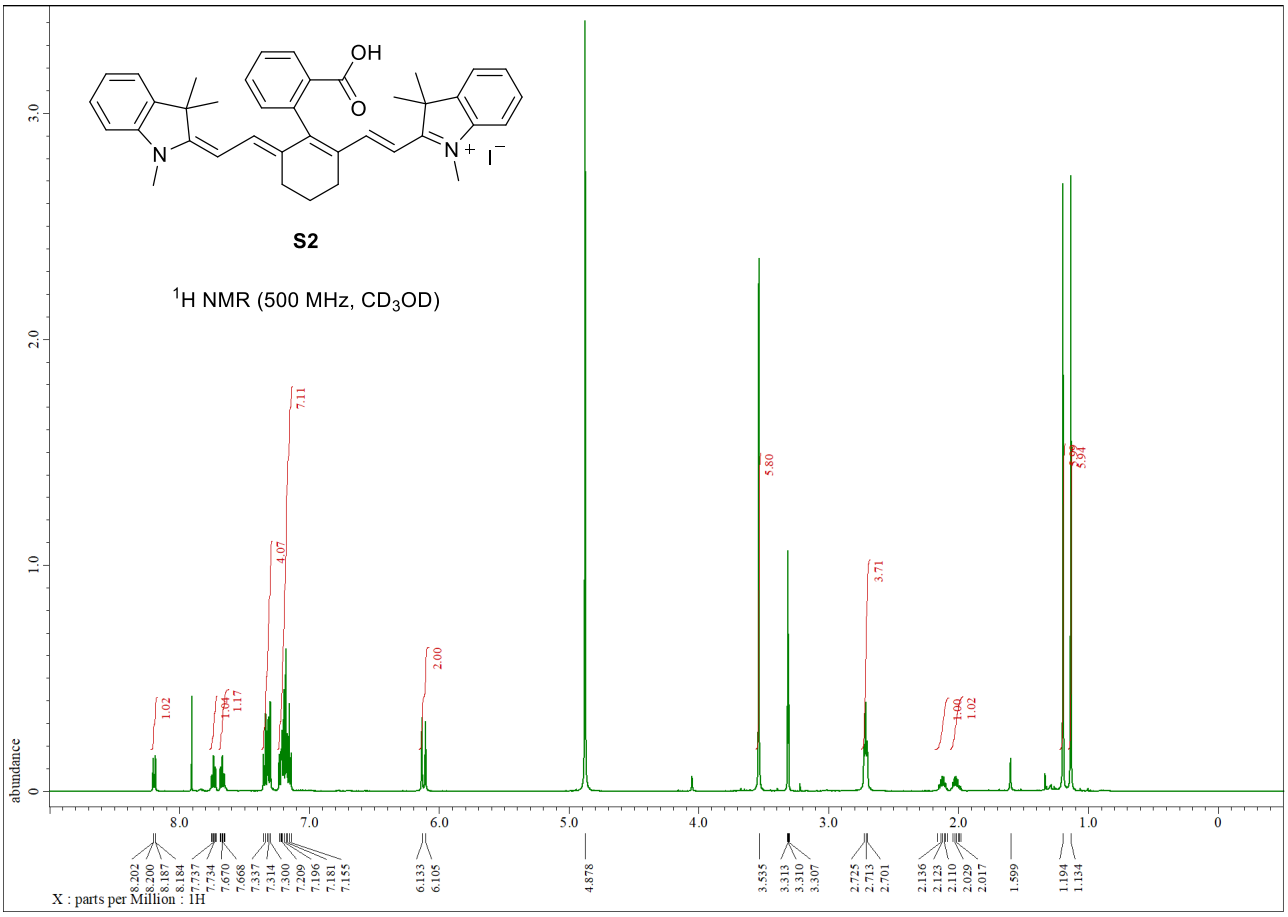
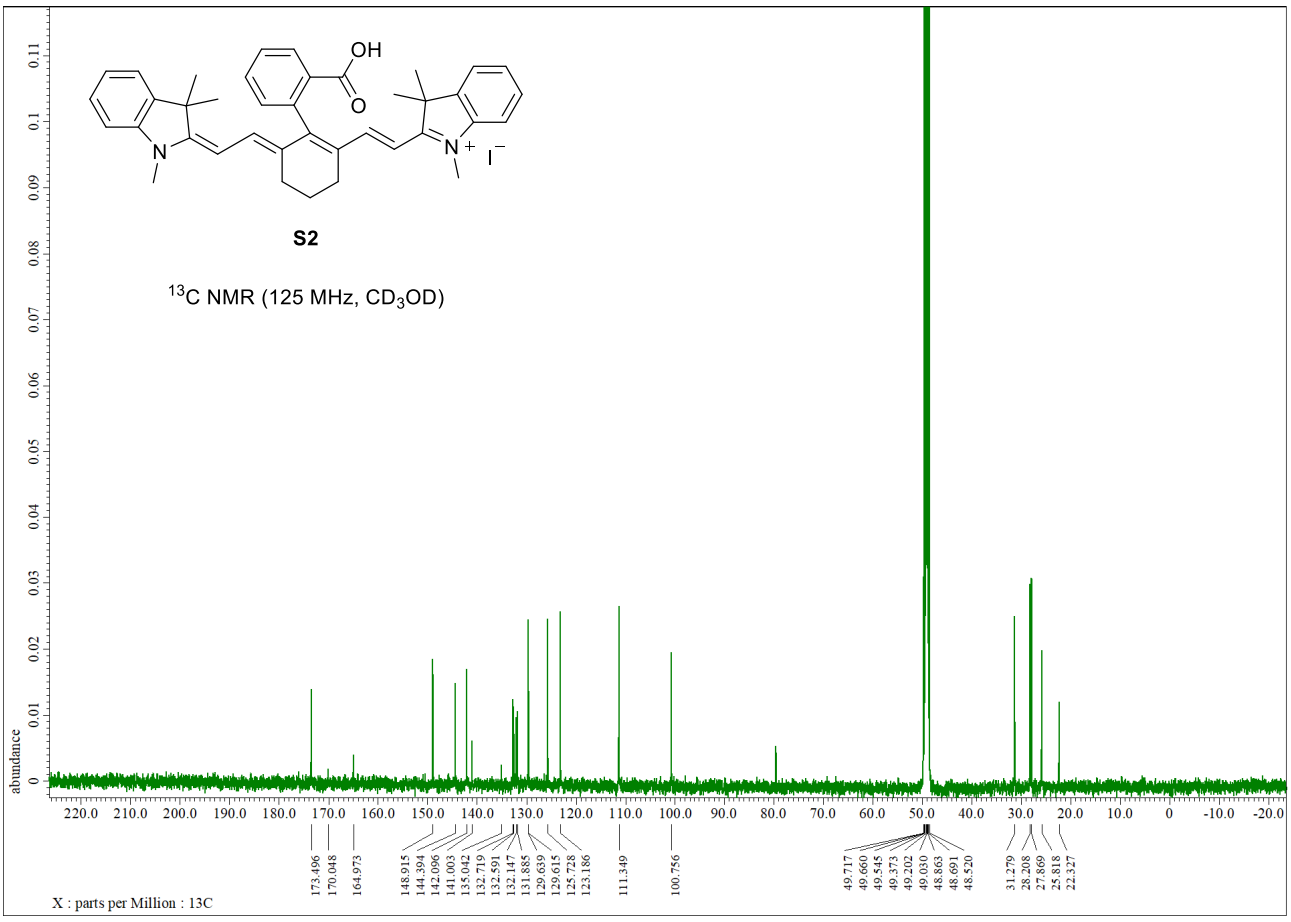
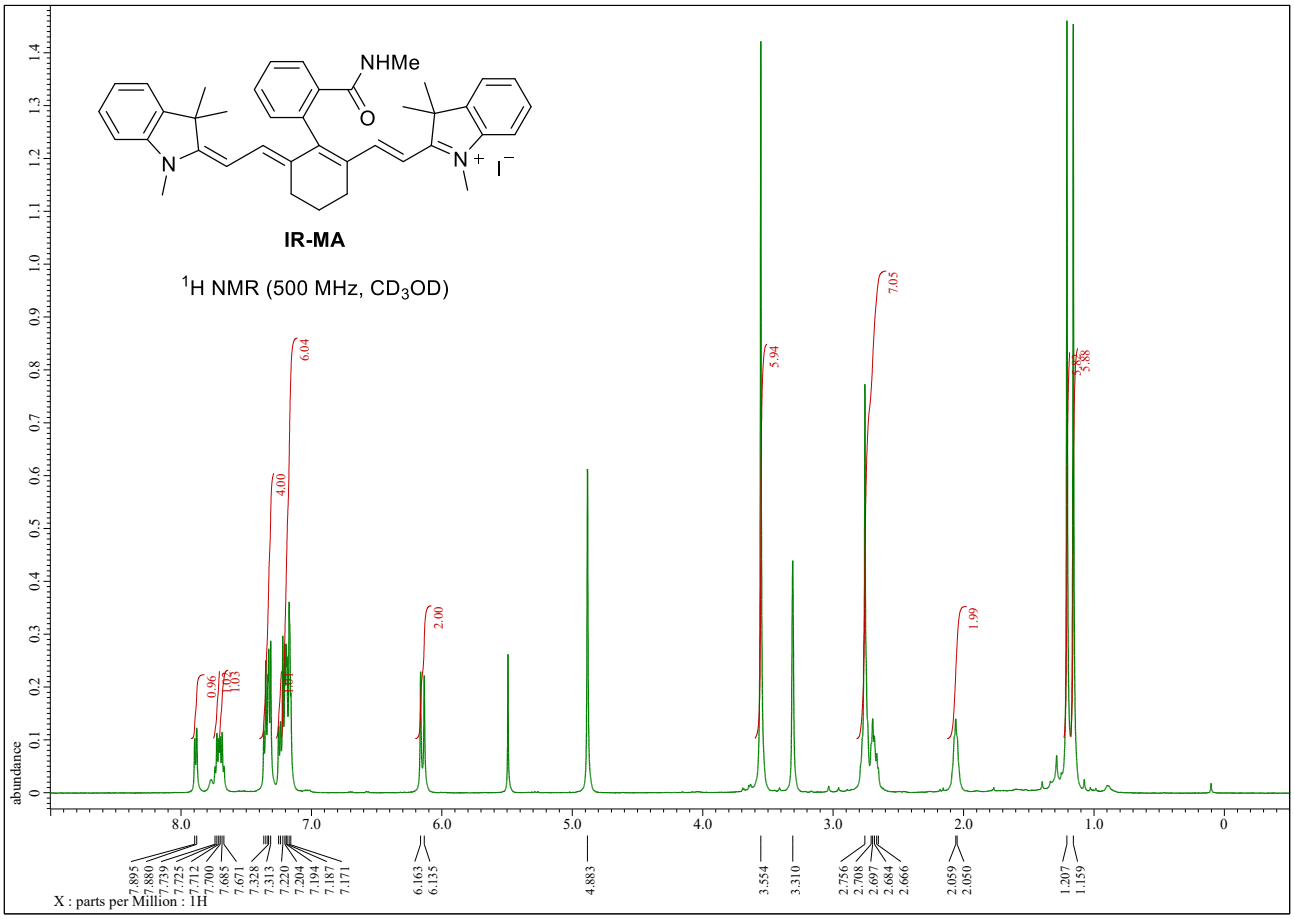
^{1}H and ^{13}C NMR spectra of new compounds

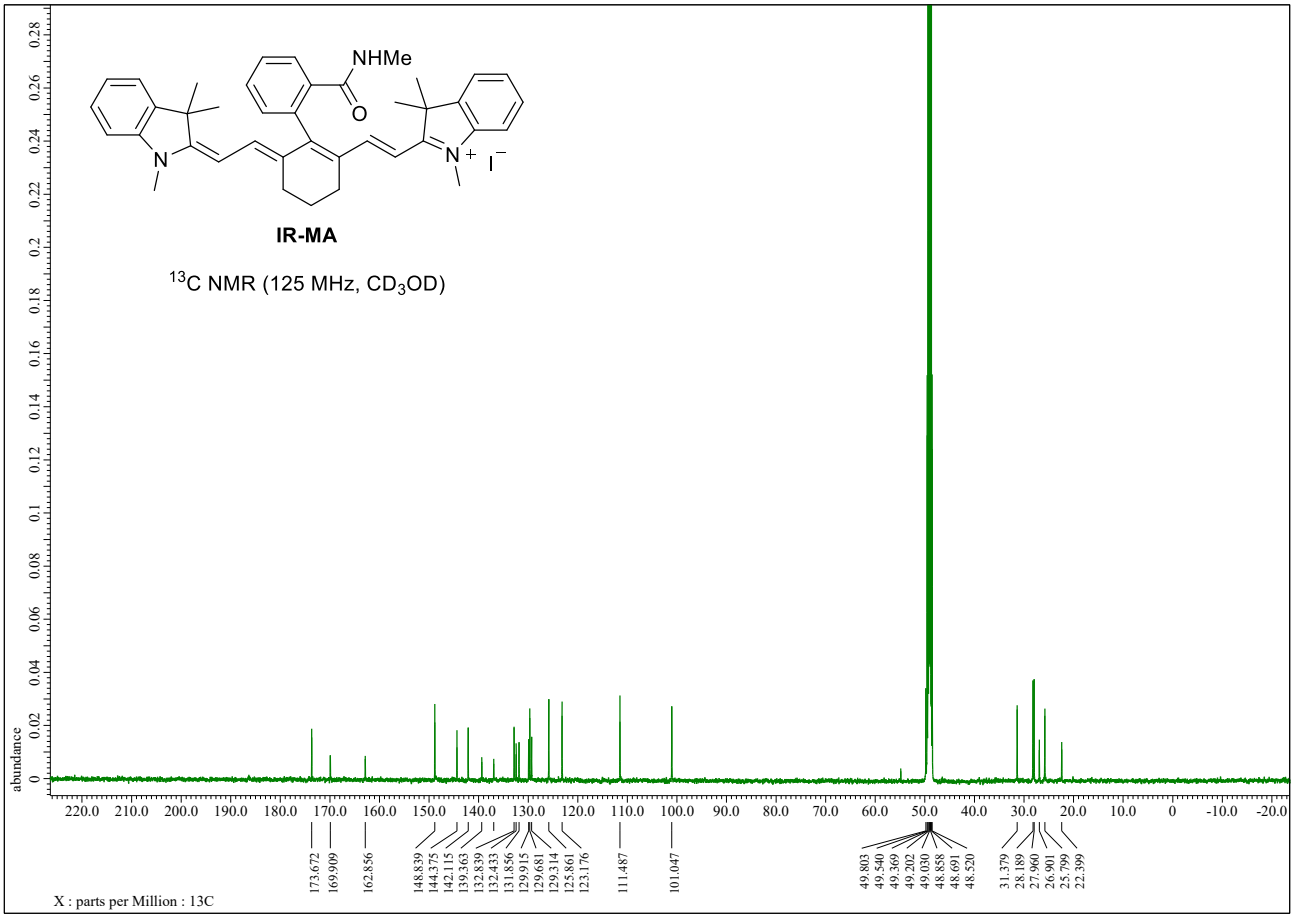


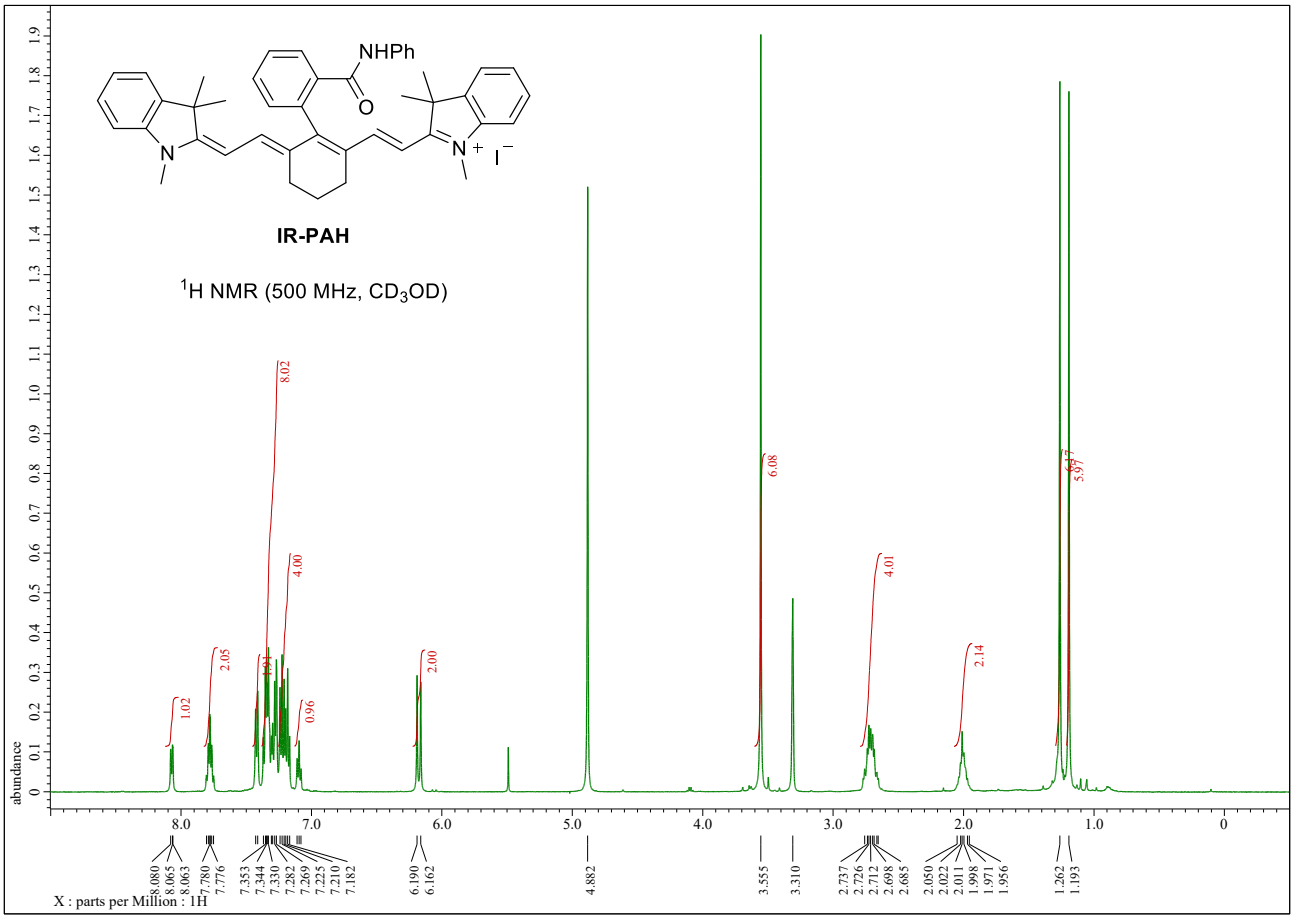


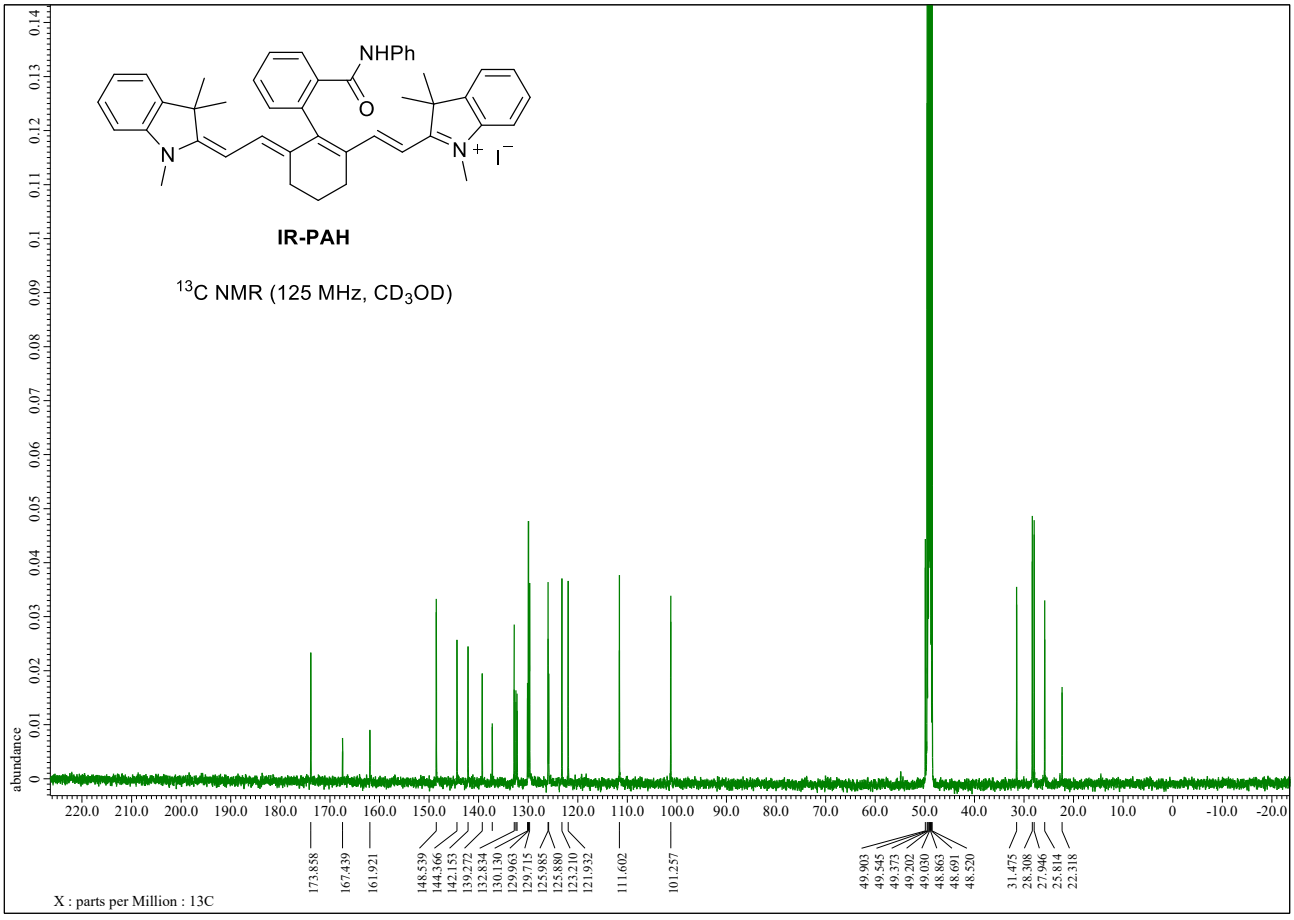

OH
O
N
N+
I−
S2
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

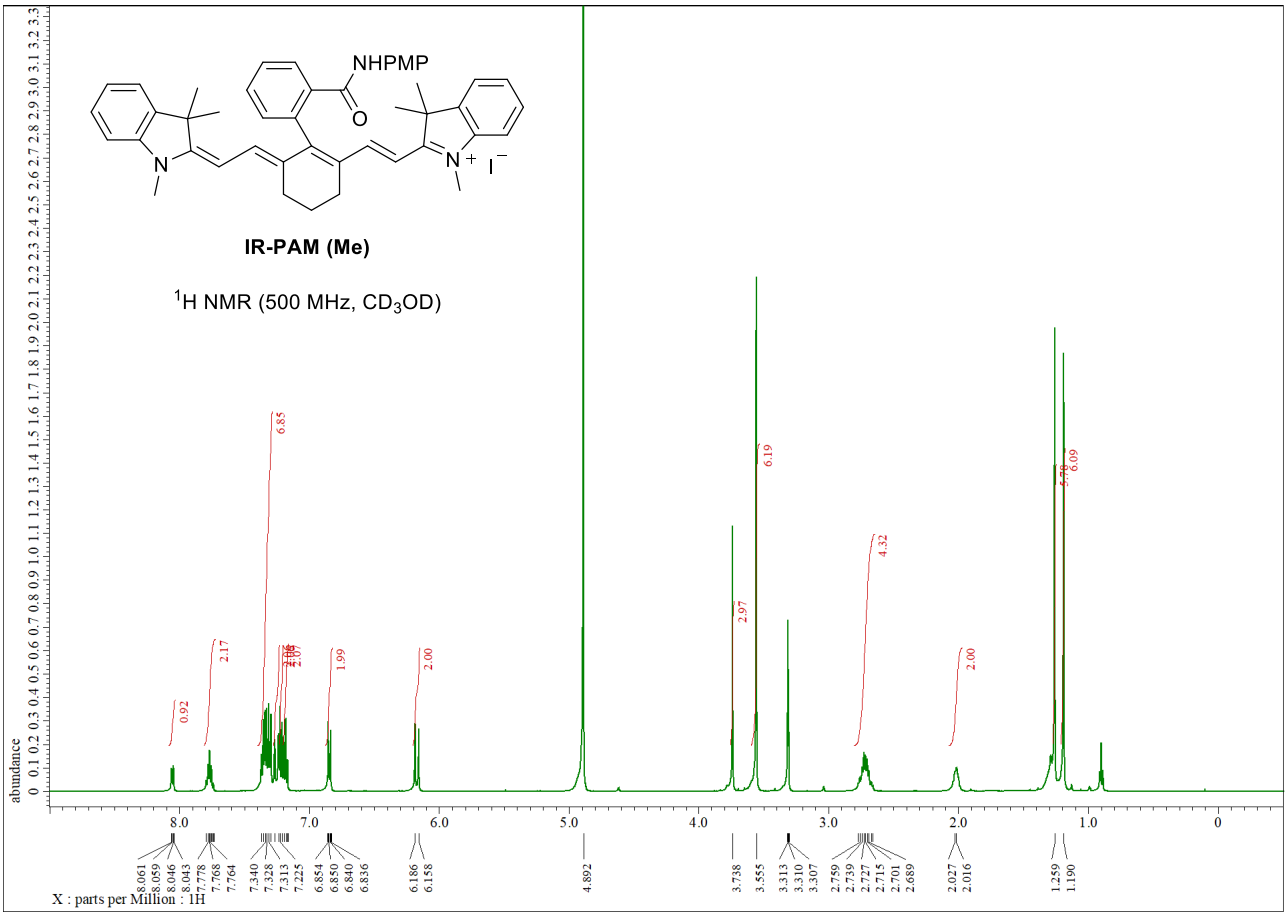

OH
O
N
N+
I−
S2
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C

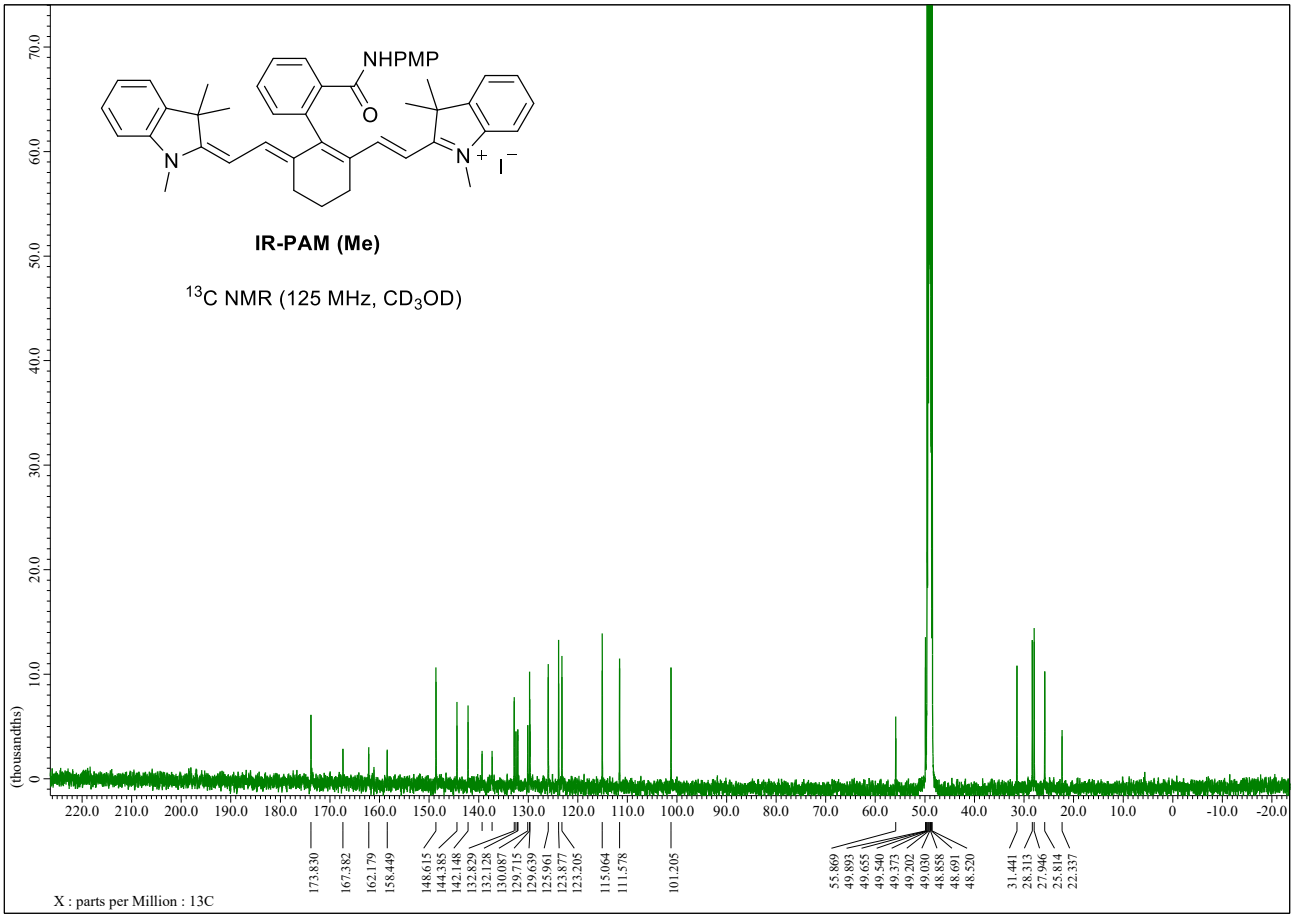

NHMe
O
N
N+
I−
IR-MA
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

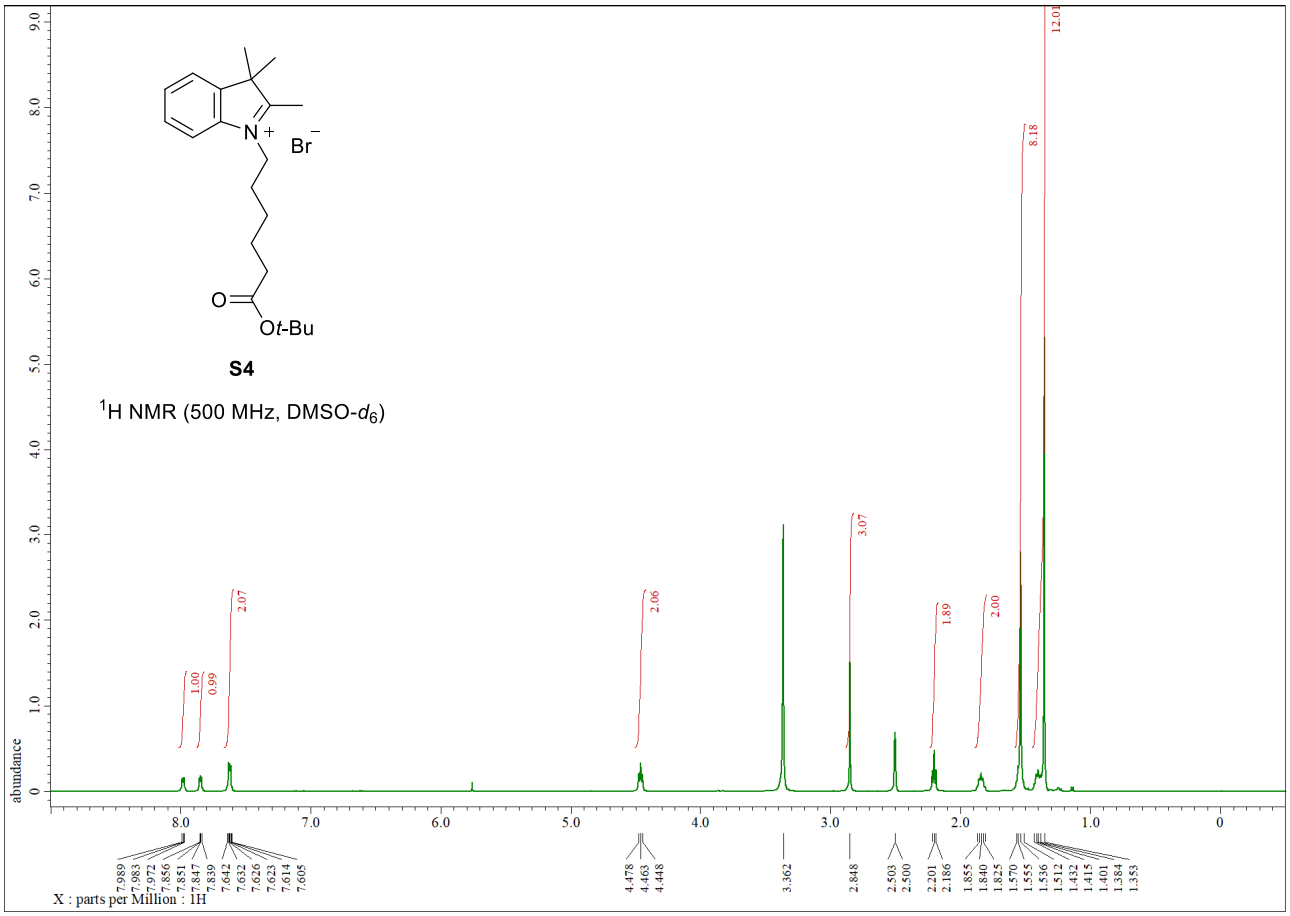

NHMe
O
N
N+
I−
IR-MA
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C

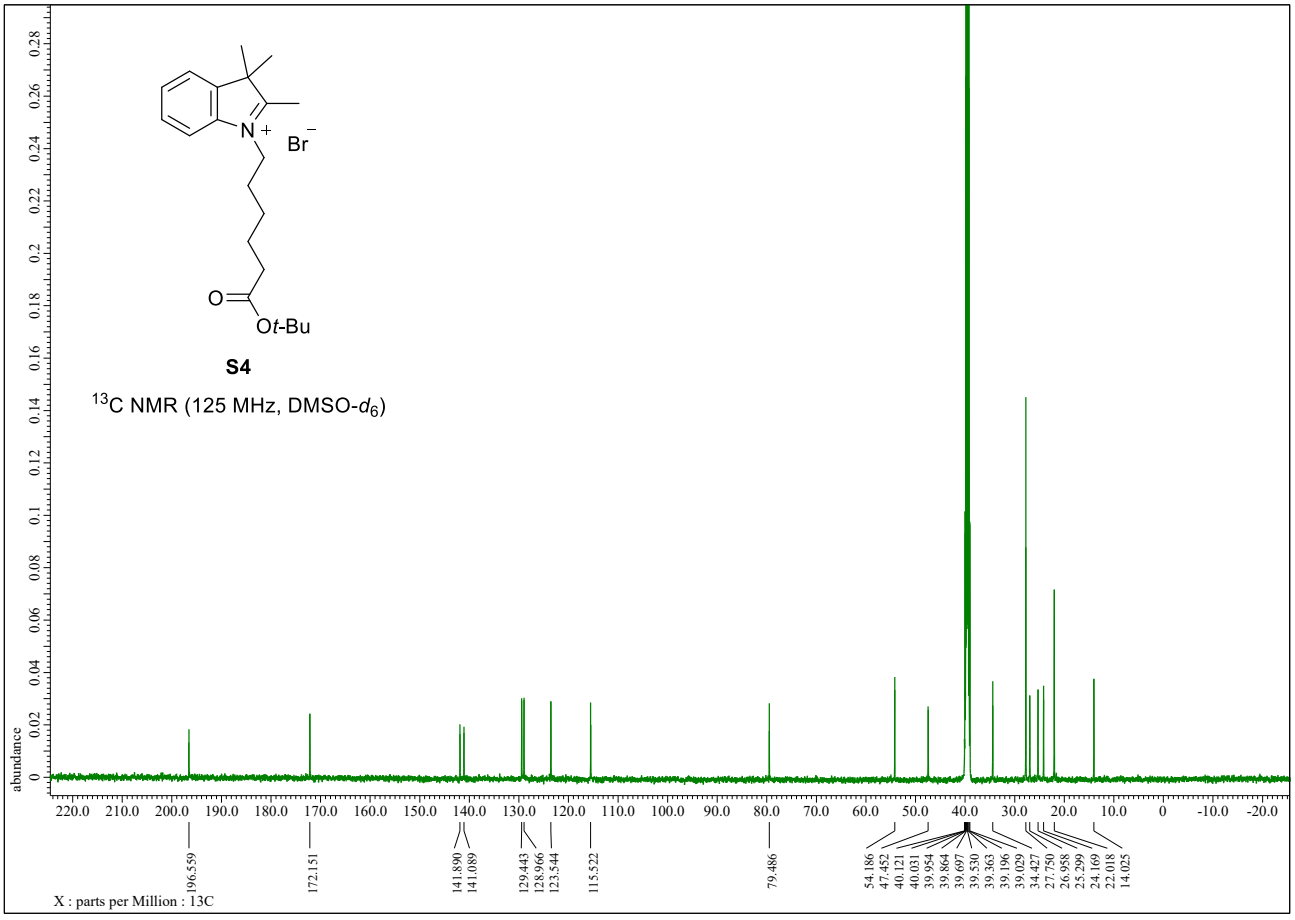

NHPh
O
N
N+
I−
IR-PAH
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

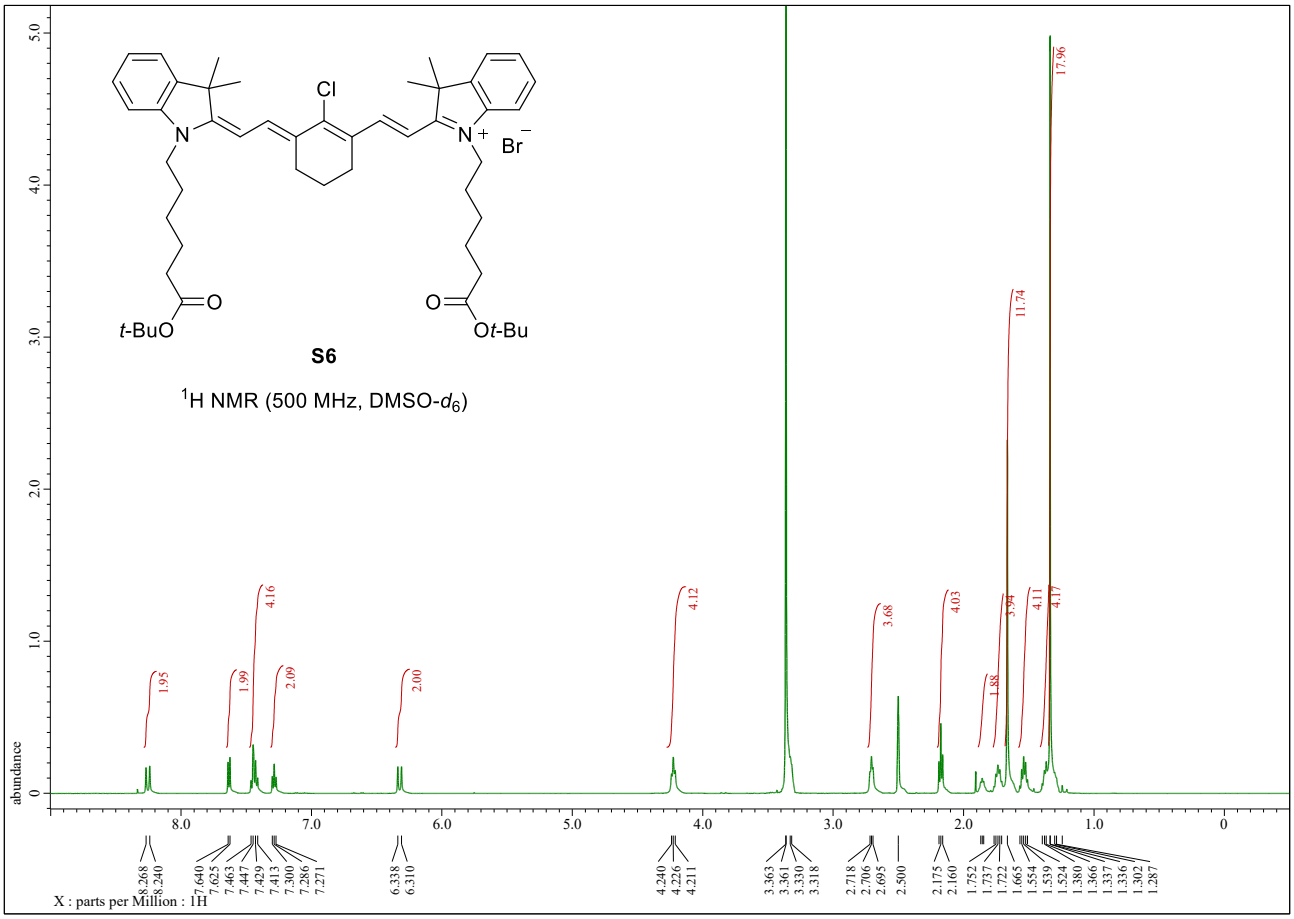

NHPh
O
N
N+
I−
IR-PAH
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C

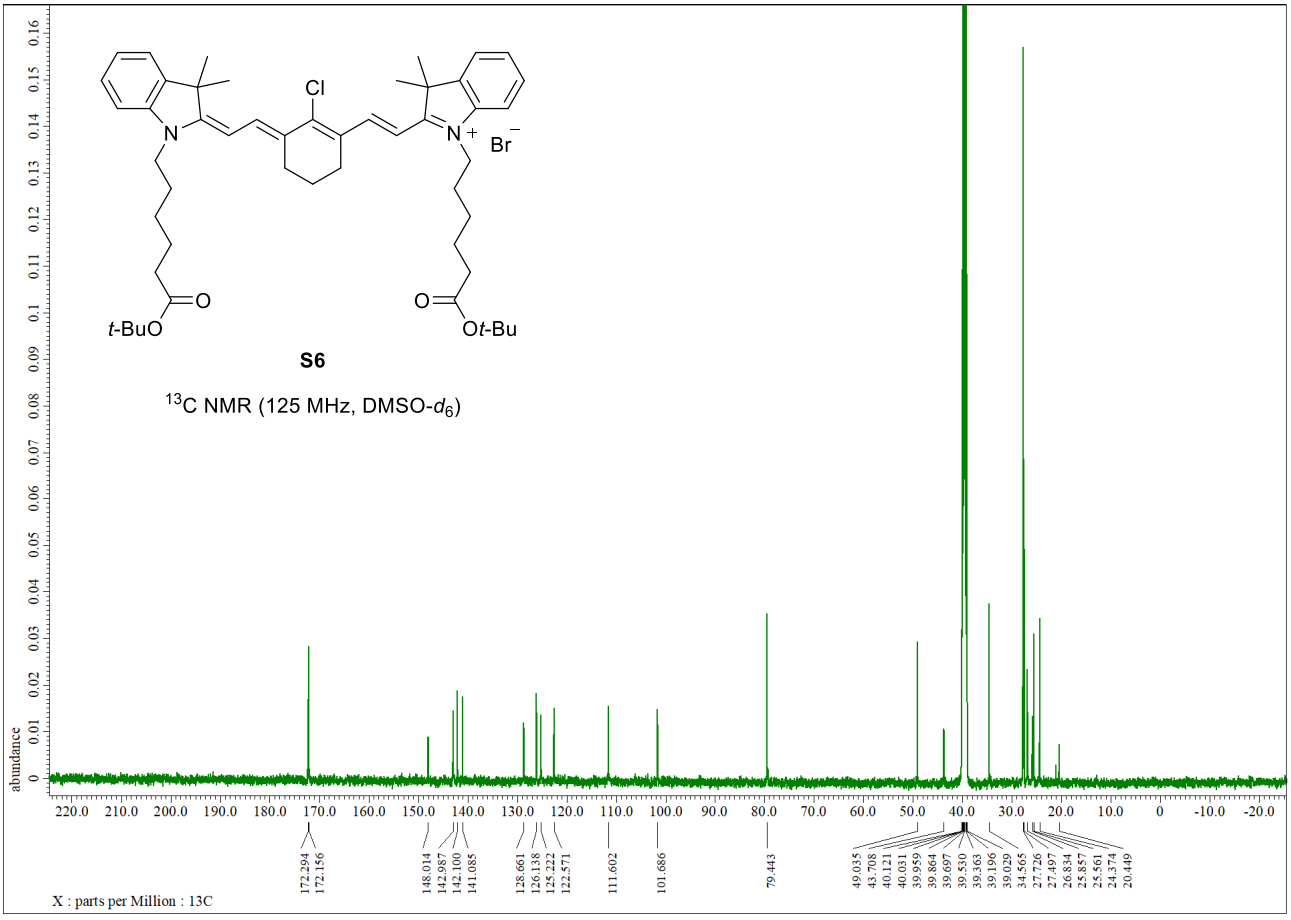

NHPMP
O
N
N+
I−
IR-PAM (Me)
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

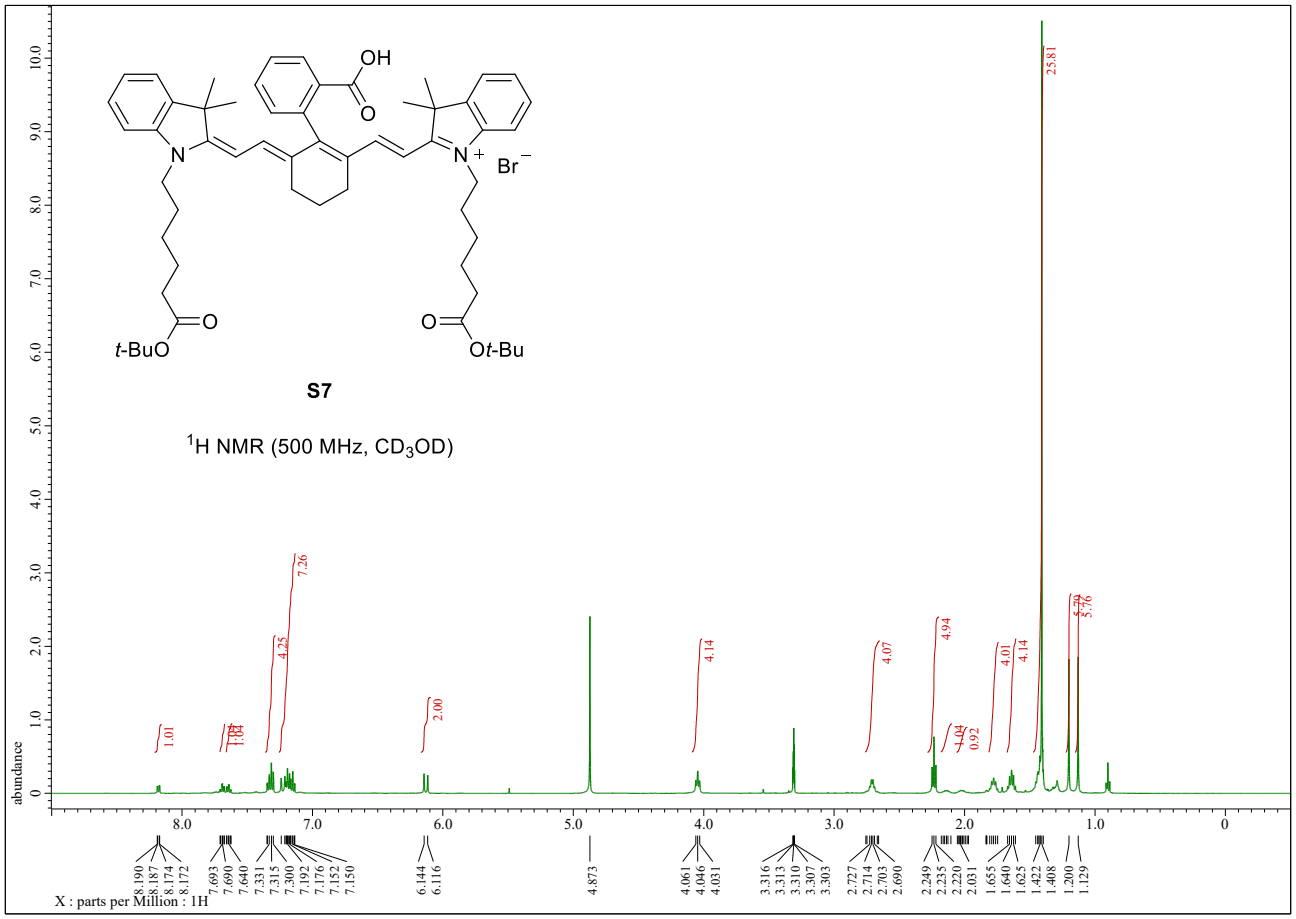

NHPMP
O
N
N+
I−
IR-PAM (Me)
13C NMR (125 MHz, CD3OD)
(thousandths)
X : parts per Million : 13C

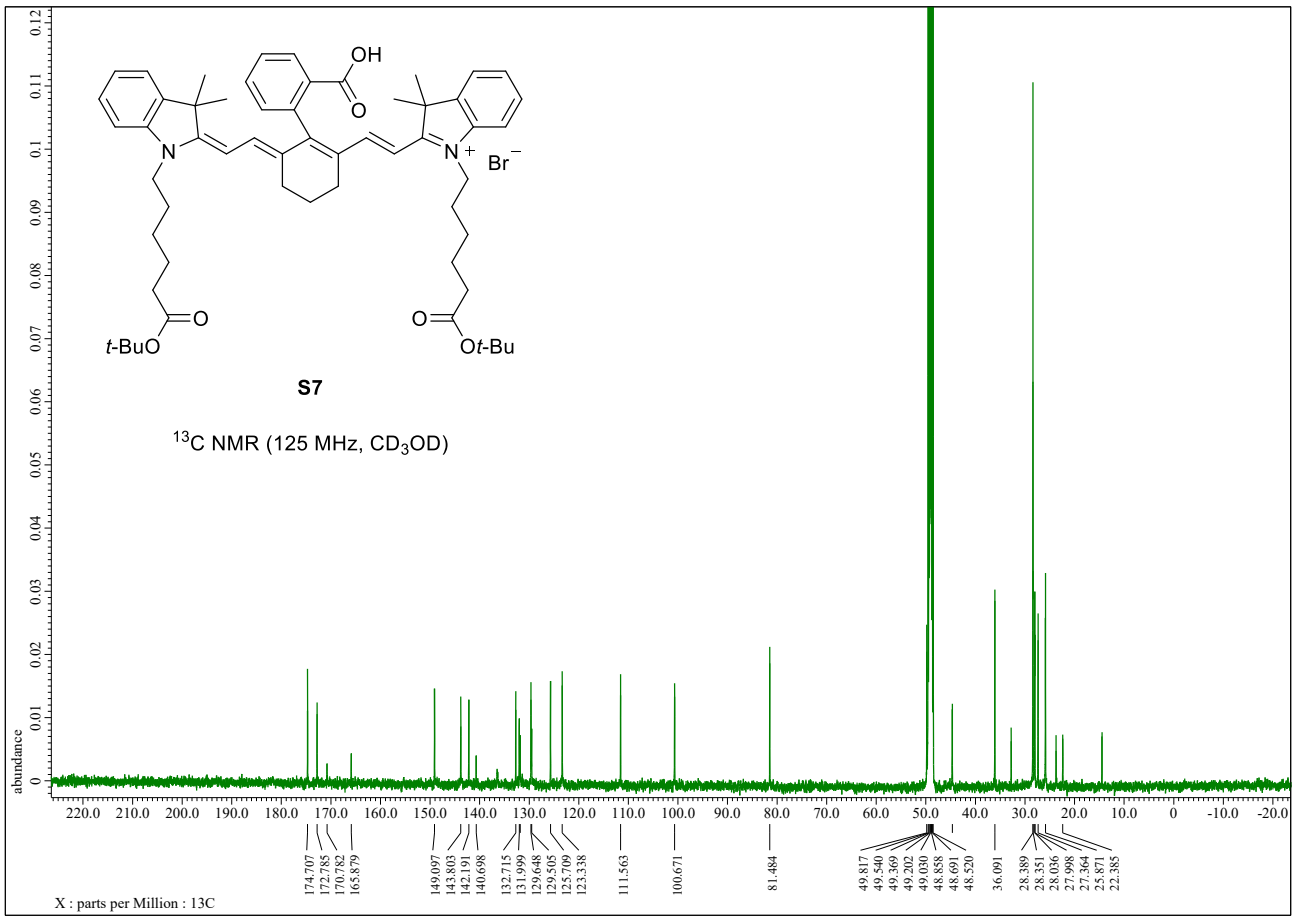

Br
O
Ot-Bu
S4
1H NMR (500 MHz, DMSO-d6)
abundance
X : parts per Million : 1H

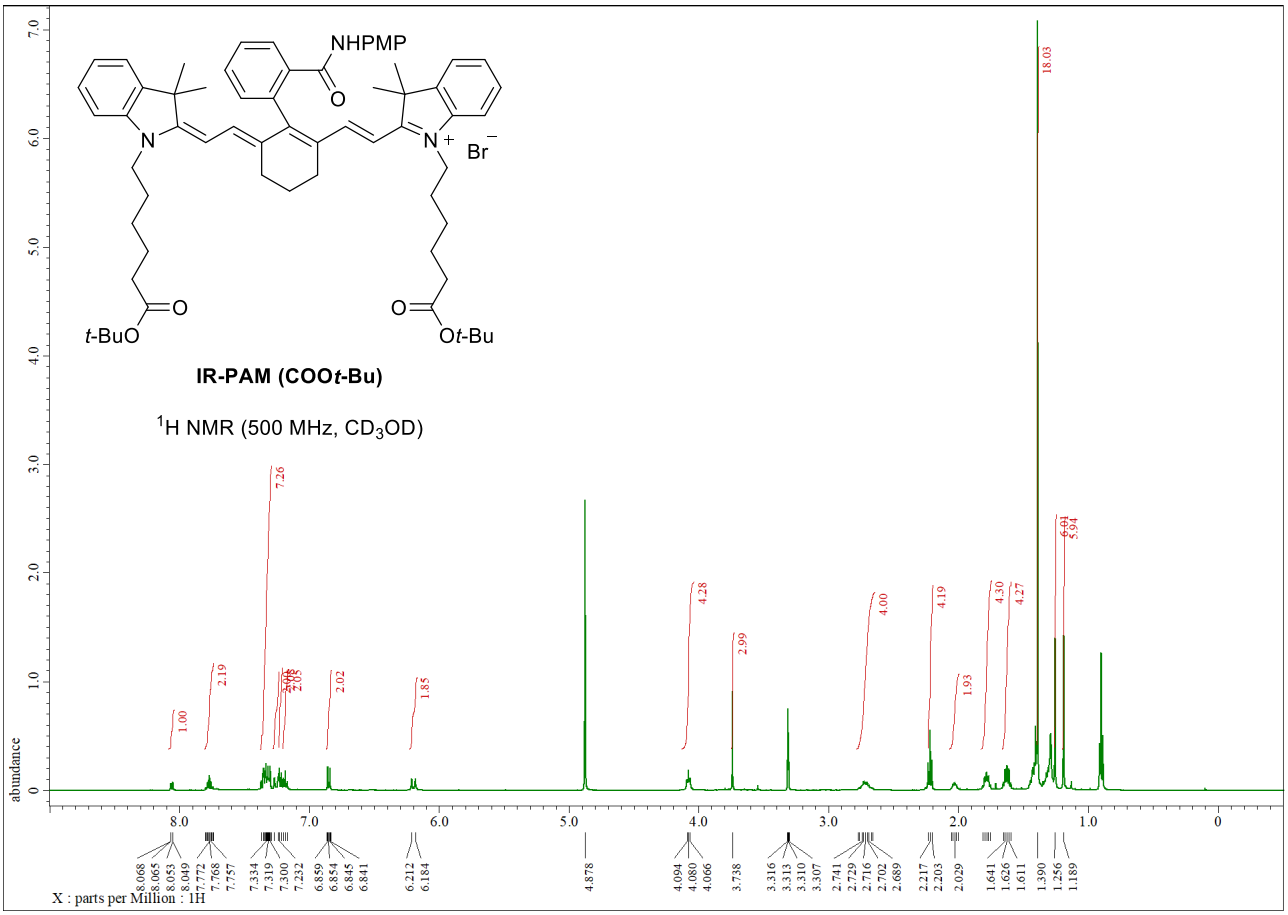

Br
O
Ot-Bu
S4
13C NMR (125 MHz, DMSO-d6)
abundance
X : parts per Million : 13C

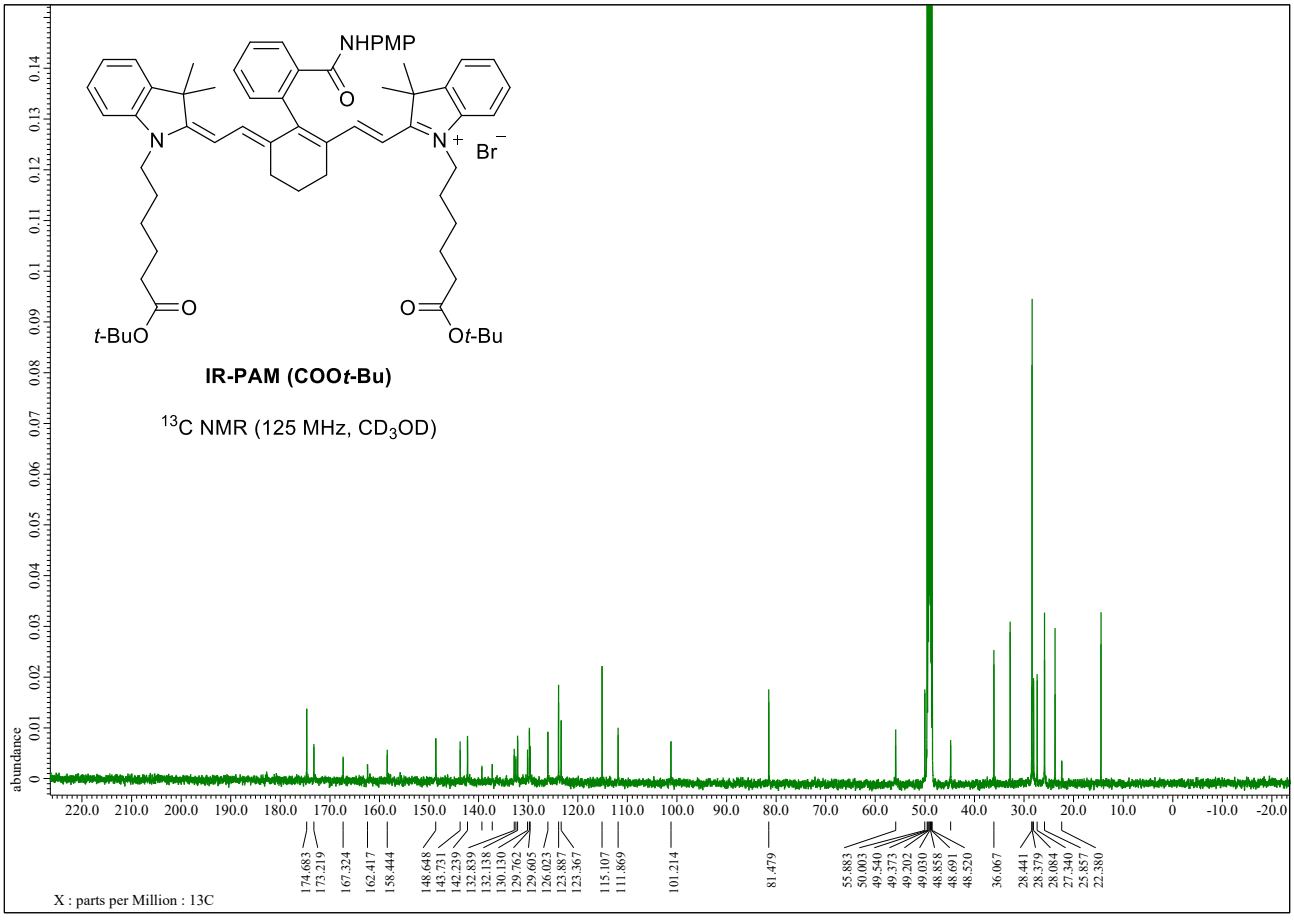

Cl
N
N+
Br−
t-BuO
O
O
Ot-Bu
S6
1H NMR (500 MHz, DMSO-d6)
abundance
X : parts per Million : 1H

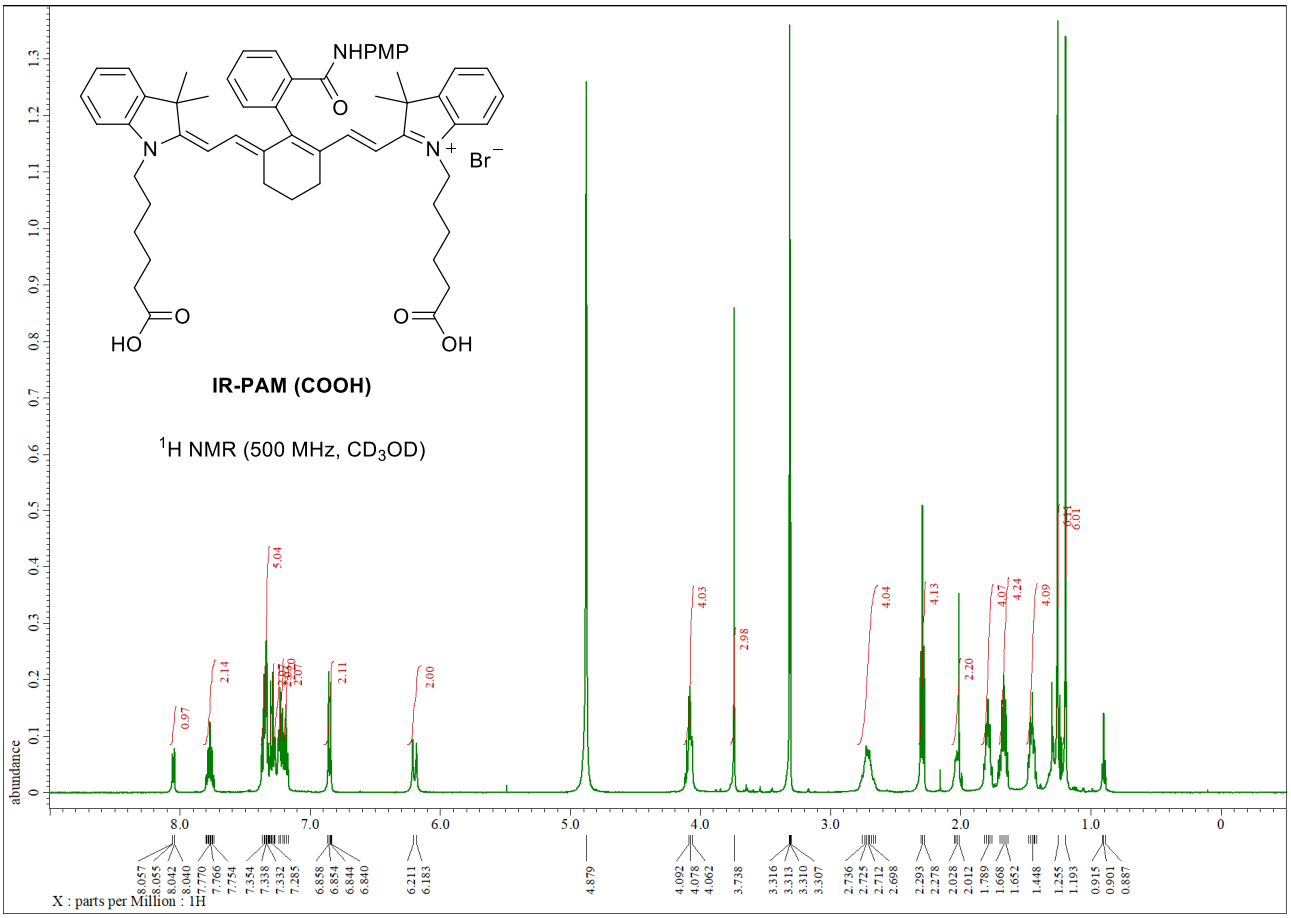

Cl
N
N+
Br−
t-BuO
O
O
Ot-Bu
S6
13C NMR (125 MHz, DMSO-d6)
abundance
X : parts per Million : 13C

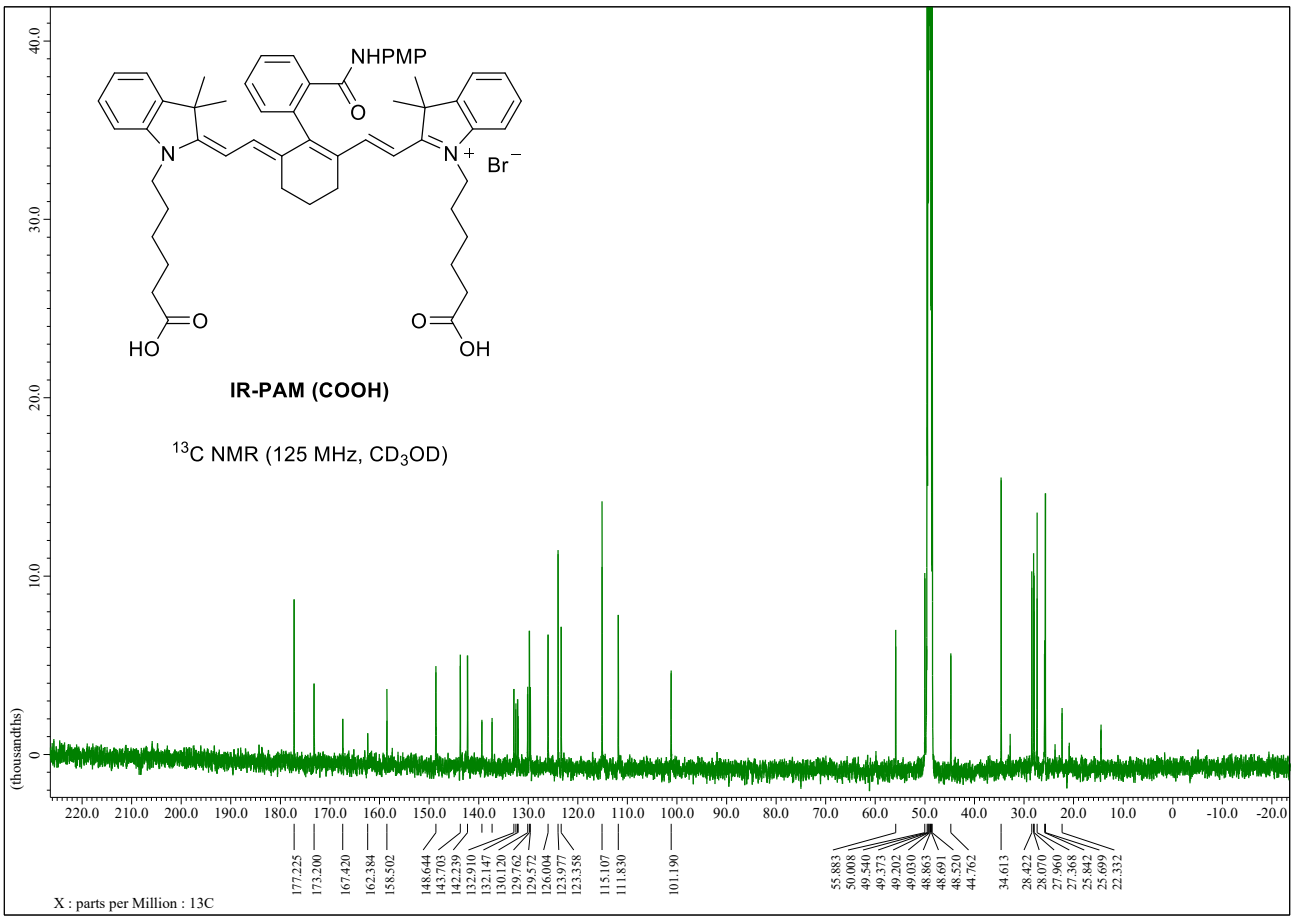

OH
O
N
N+
Br−
t-BuO
O
O
Ot-Bu
S7
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

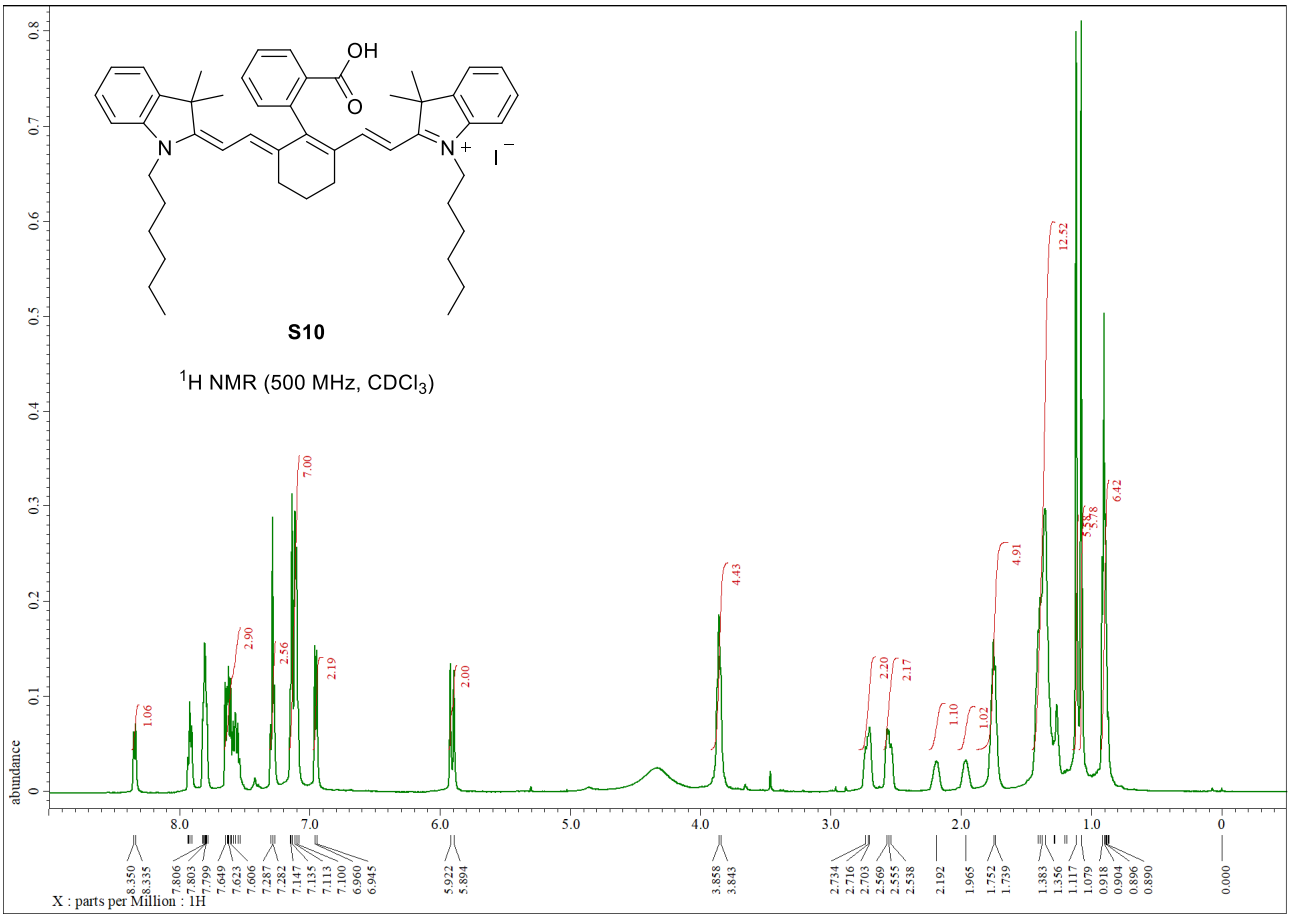

OH
O
N
N+
Br−
t-BuO
O
O
Ot-Bu
S7
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C

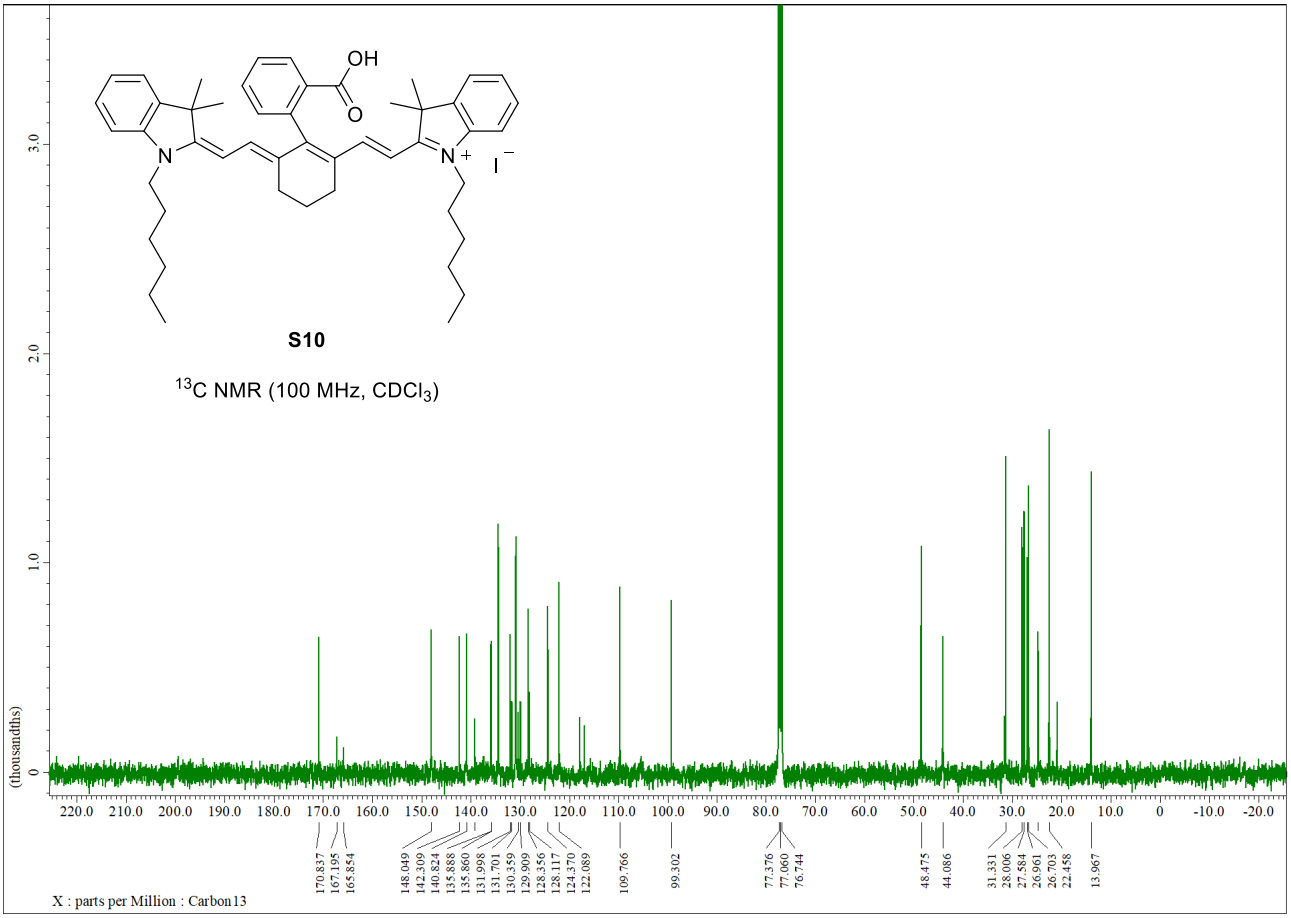

NHPMP
O
N
N+
Br−
t-BuO
O
O
Ot-Bu
IR-PAM (COOt-Bu)
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H

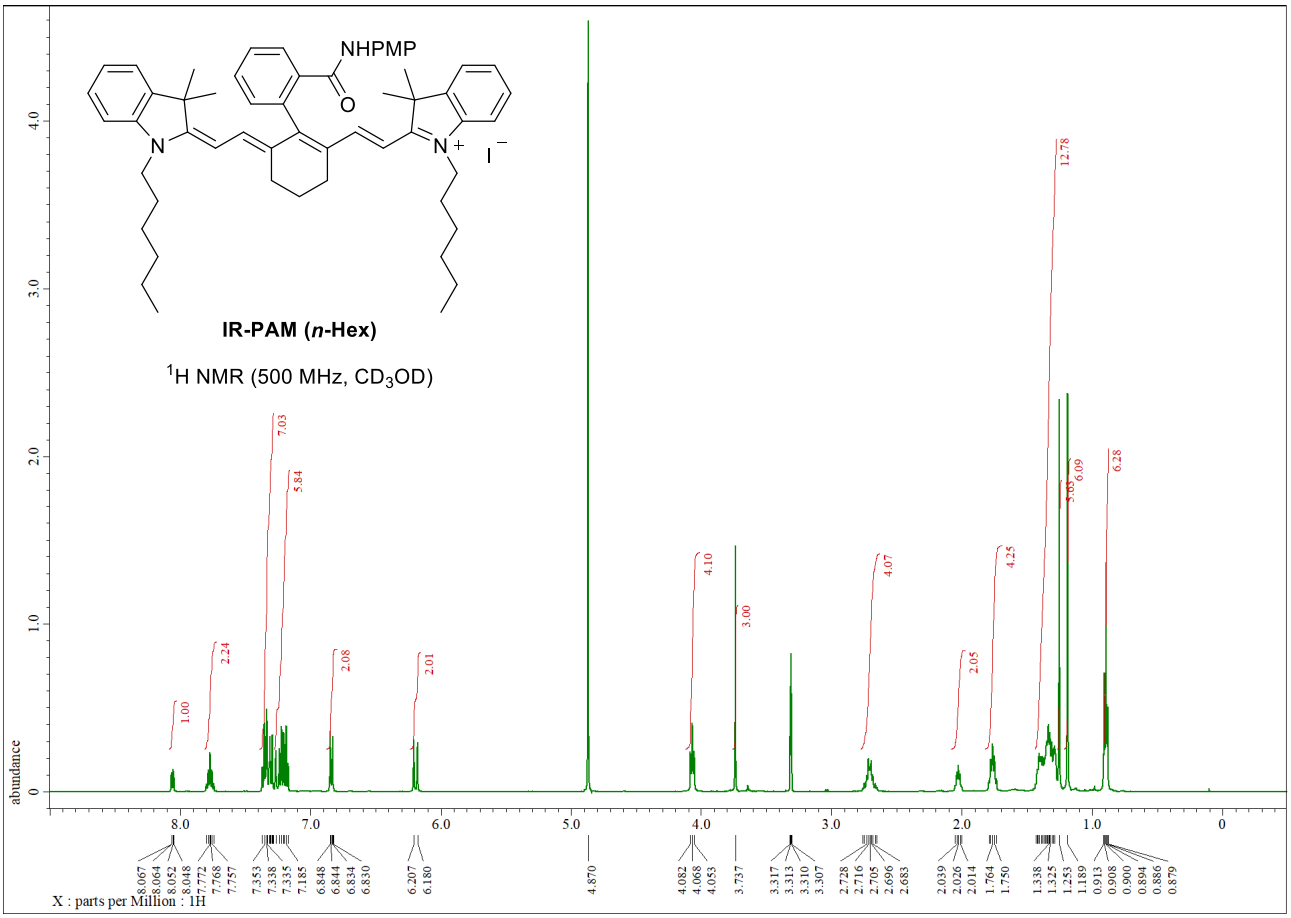

NHPMP
O
N
N+
Br−
t-BuO
O
O
Ot-Bu
IR-PAM (COOt-Bu)
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C

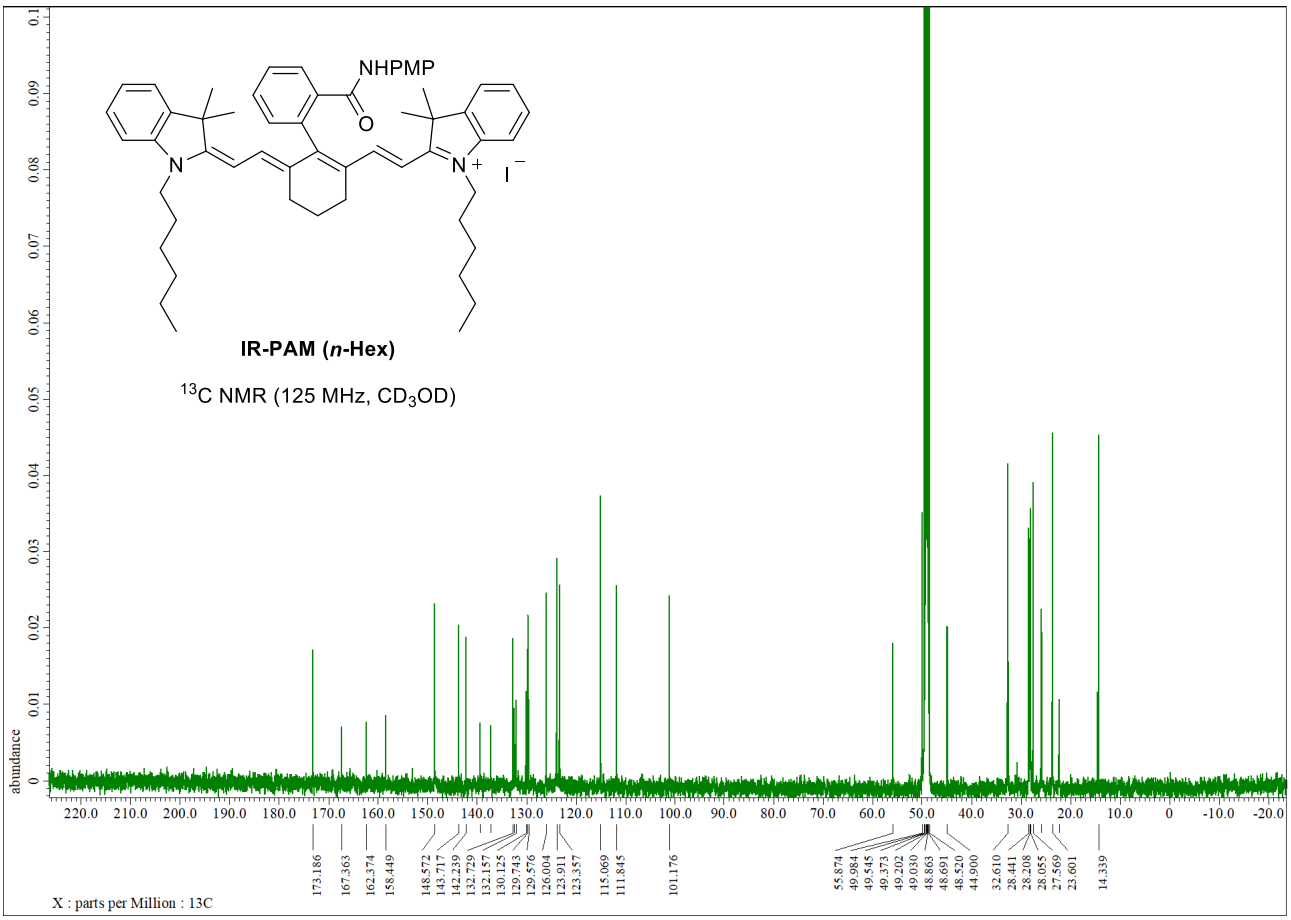

NHPMP
O
N
N+
Br−
HO
O
O
OH
IR-PAM (COOH)
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H


NHPMP
O
N
N+
Br−
HO
O
O
OH
IR-PAM (COOH)
13C NMR (125 MHz, CD3OD)
(thousandths)
X : parts per Million : 13C


OH
O
N
N+
I−
S10
1H NMR (500 MHz, CDCl3)
abundance
X : parts per Million : 1H


OH
O
N
N+
I−
S10
13C NMR (100 MHz, CDCl3)
(thousandths)
X : parts per Million : Carbon13


NHPMP
O
N
N+
I−
IR-PAM (n-Hex)
1H NMR (500 MHz, CD3OD)
abundance
X : parts per Million : 1H


NHPMP
O
N
N+
I−
IR-PAM (n-Hex)
13C NMR (125 MHz, CD3OD)
abundance
X : parts per Million : 13C