Electronic Supplementary Information

A Caveat to Common Hemicyanine Dye Components and Its Resolution

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General information. The chemical reagents were purchased from Sigma Aldrich or Alfa Aesar. Commercially available reagents were used without further purification. Anhydrous solvents for organic synthesis were prepared by passing through a solvent purification tower. All reactions were performed under argon atmosphere unless otherwise stated. Thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 glass plates and visualized with ultraviolet light. ^{1}H and 13C NMR spectra were measured with a Bruker AVANCE III 300MHZ and AVANCE III 500 MHZ FT-NMR spectrometer. Coupling constants (*J* value) are reported in Hertz. The chemical shifts (δ) are shown in ppm. Spectra are referenced to residual $CHCl_3$ (7.26 ppm, ^{1}H; 77.16 ppm, ^{13}C) and DMSO (2.50 ppm, ^{1}H; 39.51 ppm, ^{13}C). High resolution mass spectra was recorded on a JE JMS-700 spectrometer at the Korea Basic Science Center, Kyungpook National University and the values are reported in units of mass to charge (m/z). Na_2S, NaOCl, and $NaHSO_3$ were used as the source of H_2S, HOCl, and HSO_3^-, respectively.

Spectroscopic analysis. UV/Vis absorption spectra were obtained using a HP 8453 UV/Vis spectrophotometer. Fluorescence spectra were recorded on a Photon Technical International Fluorescence System with a 3.0 mL quartz cell with 1.0–cm standard path length. 1.0 mM stock solutions were prepared in DMSO. All photo-physical studies were conducted with solutions of the probe at 10 μM. Quantum yields were calculated using the following equation using Rhodamine 6G (Φ_F = 0.95 in EtOH) as standard, where Φ is the quantum yield, *I* is the measured emission intensity, η is the refractive index of the media and A is the absorbance. Subscript 'r' stands for reference.

$$\Phi = \Phi_r \left(\frac{\eta}{\eta_r}\right)^2 \left(\frac{I}{I_r}\right)\left(\frac{A_r}{A}\right) \quad \text{..... (1)}$$

Synthesis. Compound **5** was prepared following the reported synthetic procedure by us (*Dyes and Pigments* **2019**, *171*, 107718).

NVId. A mixture of 6-(pyrrolidin-1-yl)-2-naphthaldehyde (**2**) (30 mg, 0.133 mmol) and 1,2,3,3-tetramethyl-3*H*-indol-1-ium iodide (60.15 mg, 0.200 mmol) in EtOH (1mL) was refluxed overnight. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography on silica gel to afford **NVId** as a blue solid (80 mg, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz, 298 K), δ 8.547 (s, 1H), 8.24 (d, 1H), 7.95 (m, 2H), 7.47 (m, 5H), 6.89 (q, 1H), 6.59 (s, 1H), 4.24 (s, 3H), 3.39 (s, 4H), 2.04 (s, 4H), and 1.79 (s, 6H); ^{13}C NMR ($CDCl_3$, 500 MHz, 293 K) δ 180.39, 155.54, 149.19, 142.51, 141.71, 139.22, 137.09, 132.63, 129.44, 128.74, 127.16, 127.13, 127.17, 125.71, 122.58, 116.66, 113.91, 108.22, 105.29, 51.74, 48.00, 36.33, 27.48, and 25.54; HRMS: *m/z* calcd. for $C_{27}H_{29}N_2$, 381.53; found, 381.233.

NVBt. A mixture of compound **2** (30 mg, 0.133 mmol) and 2,3-dimethylbenzo[d]thiazol-3-ium iodide (38 mg, 0.133 mmol) in EtOH (2.5 mL) was refluxed overnight. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography on silica gel to afford **NVBt** as a blue solid (82.8 mg, 80%). ^{1}H NMR (DMSO, 500 MHz, 298 K), δ 8.369 (d, 1H), 8.272 (s, 1H), 8.170 (t, 1H), 8.037 (m, 1H), 7.817 (m, 3H), 7.726 (m, 2H), 7.053 (q, 1H), 6.774 (d, 1H), 4.2864 (s, 3H), 3.379 (t, 4H), and 2.008 (t, 4H); ^{13}C NMR (DMSO, 500 MHz, 293 K) δ 171.50, 149.54, 147.77, 141.96, 137.26, 133.95, 130.72, 129.11, 127.92, 127.38, 126.77, 126.42, 124.85, 124.75, 124.00, 116.68, 116.37, 109.98, 104.63, 79.18, 47.43, 35.95, and 24.98; HRMS: *m/z* calcd. for $C_{24}H_{23}N_2S$, 371.16; found, 371.159.

NVPy. A mixture of compound **2** (30 mg, 0.133 mmol), piperidine (2.0 µL) and 1,4-dimethylpyridin-1-ium trifluoromethanesulfonate (31 mg, 0.121 mmol) was refluxed in EtOH overnight. The solvent was evaporated under reduced pressure, and the crude product was purified by flash silica gel column chromatography to afford **NVPy** as an orange solid (13.9 mg, 18%). ^{1}H NMR (DMSO, 500 MHz, 298 K), δ 8.778 (d, 2H), 8.160 (d, 2H), 8.084 (d, 1H), 7.977 (s, 1H), 7.769 (m, 2H), 7.769 (m, 2H), 7.696 (d, 1H), 7.421 (d, 1H), 7.010 (q, 1H), 6.797 (d, 1H), 4.217 (s, 3H), 3.389 (t, 4H), and 2.013 (t, 4H), ^{13}C NMR (DMSO, 500 MHz, 293 K) δ 152.93, 146.95, 144.74, 141.67, 136.16, 130.56, 129.90, 127.77, 126.43, 125.18, 123.92, 122.82, 120.32, 116.52, 104.49, 47.44, 46.64, and 25.02; HRMS: *m/z* calcd. for $C_{22}H_{23}N_2$, 315.19; found, 315.186.

Photophysical properties.

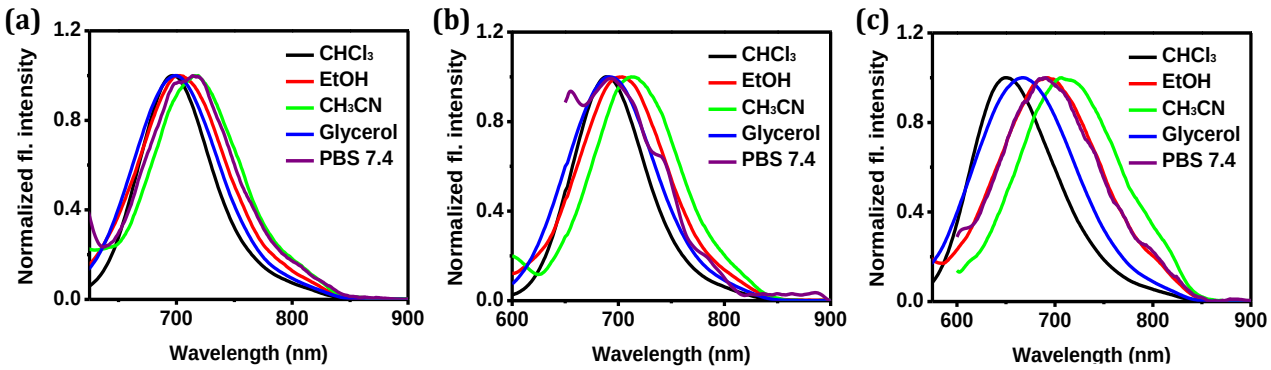


Figure S1. Normalized emission spectra of (a) **NVId**, (b) **NVBt**, and (c) **NVPy** (each at 10 μM) in solvents in the order of increasing dielectric constant: chloroform (4.81), ethanol (24.5), acetonitrile (37.5), glycerol (46.5), and pH 7.4 PBS (80.1) at 25 °C.

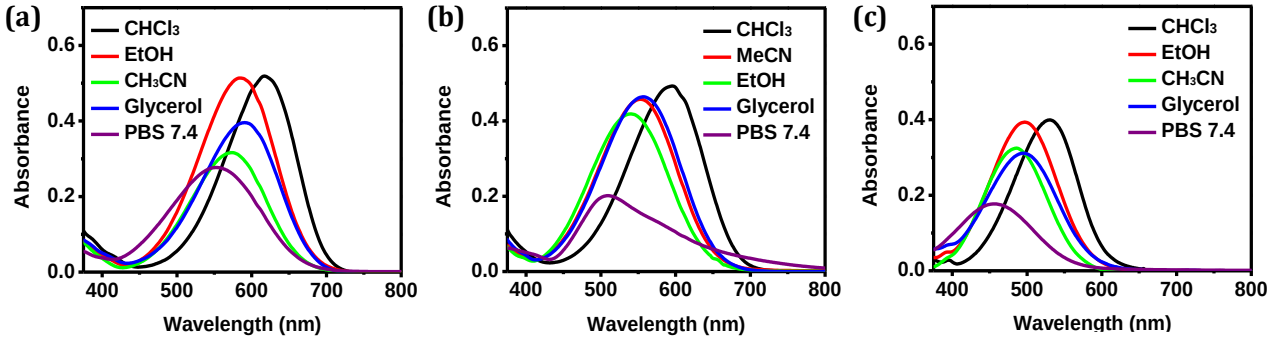


Figure S2. Absorption spectra of (a) **NVId**, (b) **NVBt**, and (c) **NVPy** (each at 10 μM) in solvents in the order of increasing dielectric constant: chloroform (4.81), ethanol (24.5), acetonitrile (37.5), glycerol (46.5), and pH 7.4 PBS 7.4 (80.1) at 25 °C.

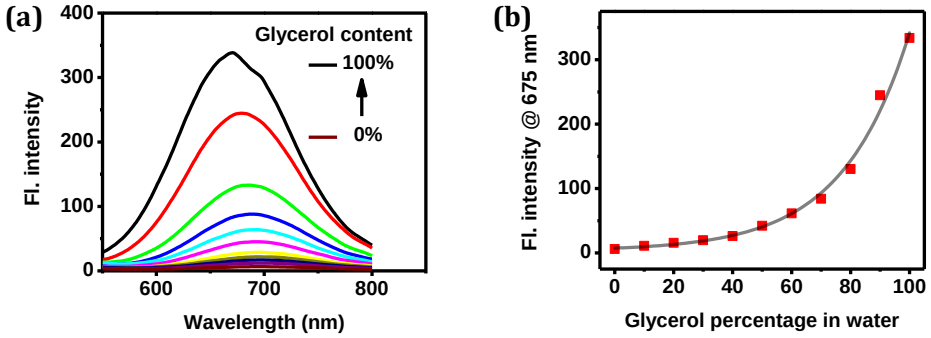


Figure S3. Fluorescence intensity changes of **NVPy** depending the medium viscosity: (a) Fluorescence spectral changes; (b) plot of the fluorescence intensity depending on the glycerol percentage in a glycerol–water binary medium.

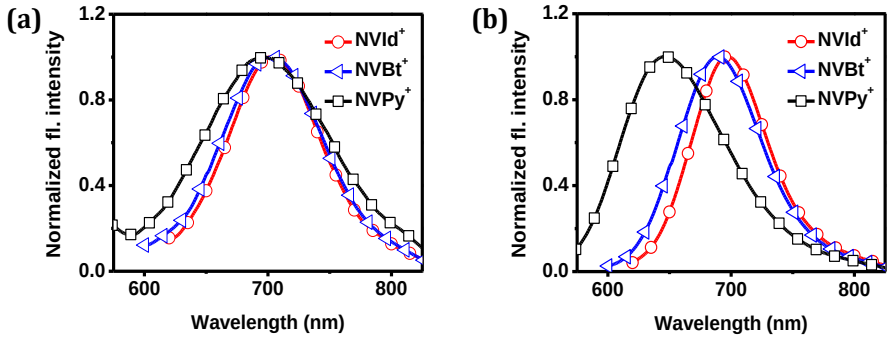


Figure S4. Normalized fluorescence spectra of **NVId**, **NVBt**, and **NVPy** (each at 10 µM) in (a) EtOH and (b) chloroform, obtained under excitation at the maximum absorption wavelength (λ_{max}) of each compound at 25 °C.

Gaussian calculation:

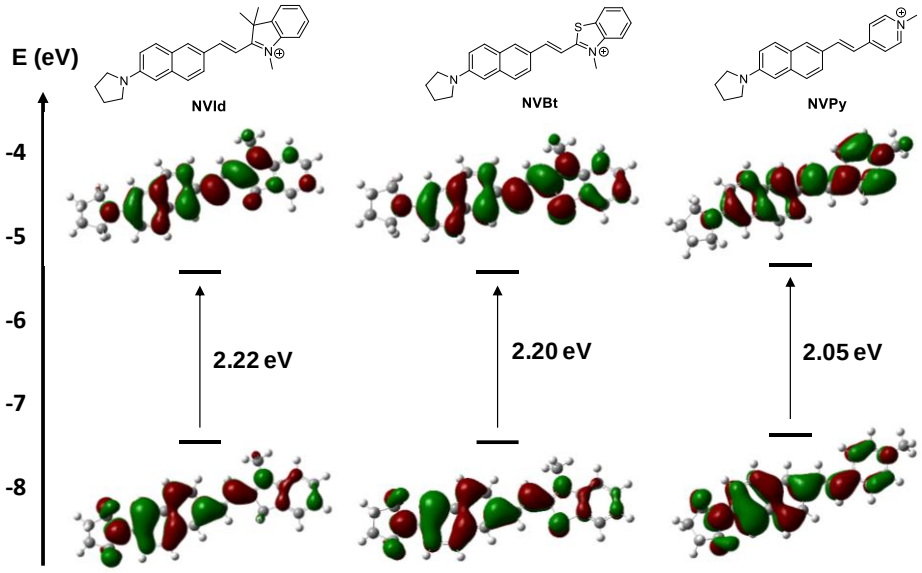


Figure S5. Frontier molecular orbitals of **NVId**, **NVBt** and **NVPy** calculated with Gaussian'09 using hybrid function B3LYP and 6-31 G(d,p) basis set in vacuum.

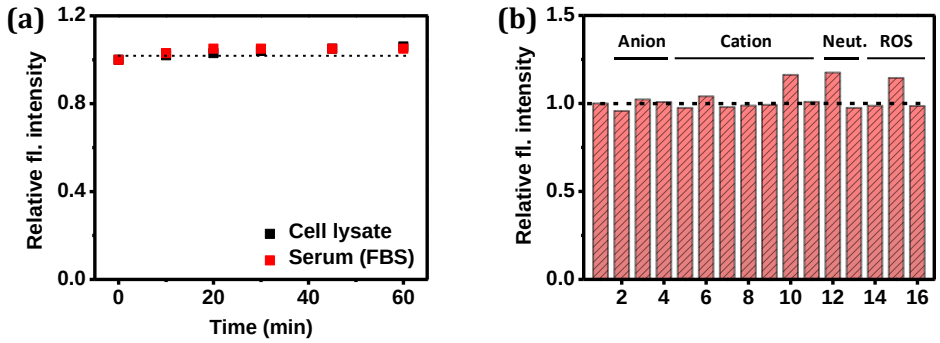


Figure S6. (a) Fluorescence intensity changes of **NVPy** (10 μM) in A549 cell lysate or in serum (FBS), monitored up to 1 h. (b) Fluorescence intensity changes of **NVPy** (10 μM) in the presence of various bio-analytes (200 μM) in PBS 7.4: (1) **NVPy** only, (2) NO_3^-, (3) CN^-, (4) NO^- [source: Angeli's salt ($Na_2N_2O_3$)], (5) Na^+, (6) K^+, (7) Ca^{2+}, (8) Mg^{2+}, (9) Zn^{2+}, (10) Cu^{2+}, (11) Fe^{3+}, (12) NO [source: $Na_2[Fe(CN)_5NO]$ at pH 5], (13) glucose, (14) $^1O_2$ [source: $Na_2MO_4 + H_2O_2$], (15) •OH [Source: $FeSO_4 + H_2O_2$], or (16) O_2^- [Source: solid KO_2]. Data was taken after 30 min of analyte addition under excitation at 460 nm.

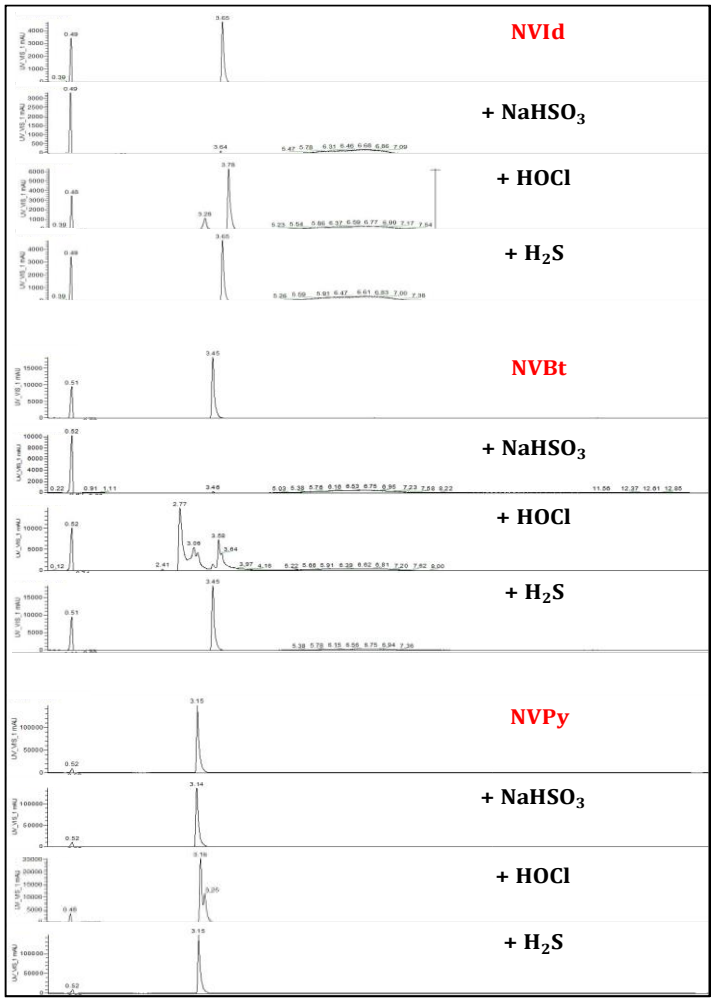


Figure S7. Stability of **NVId**, **NVBt**, and **NVPy** toward $NaHSO_3$, HOCl and H_2S, analyzed by LC-MS.

Solubility assessment of NVPy.

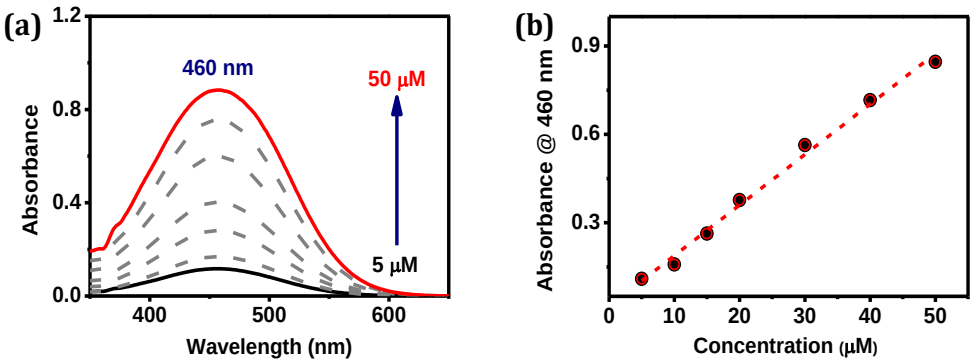


Figure S8. (a) UV/Vis Absorption spectra of **NVPy** at different concentrations (5–50 μM) in PBS (10 mM, pH 7.4) (b) Plot of absorbance vs. concentration.

Activatable hemicyanine probes selected:

Table S1. Selected examples of activatable probes based on hemicyanine scaffolds.

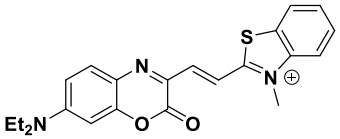
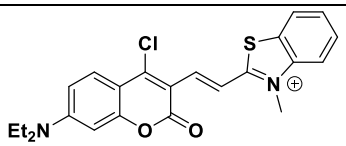
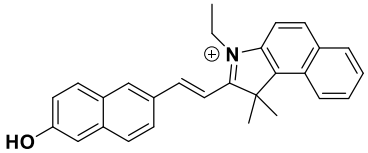
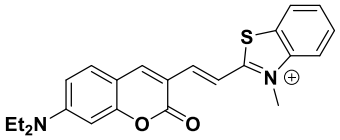
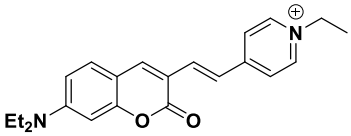
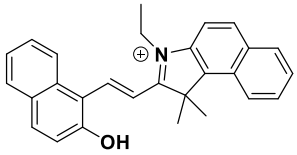
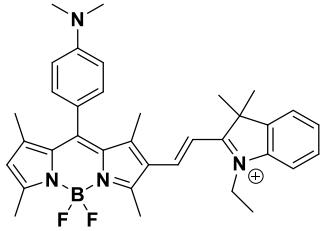
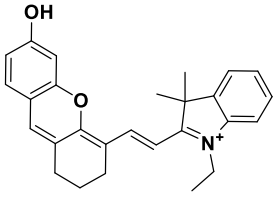
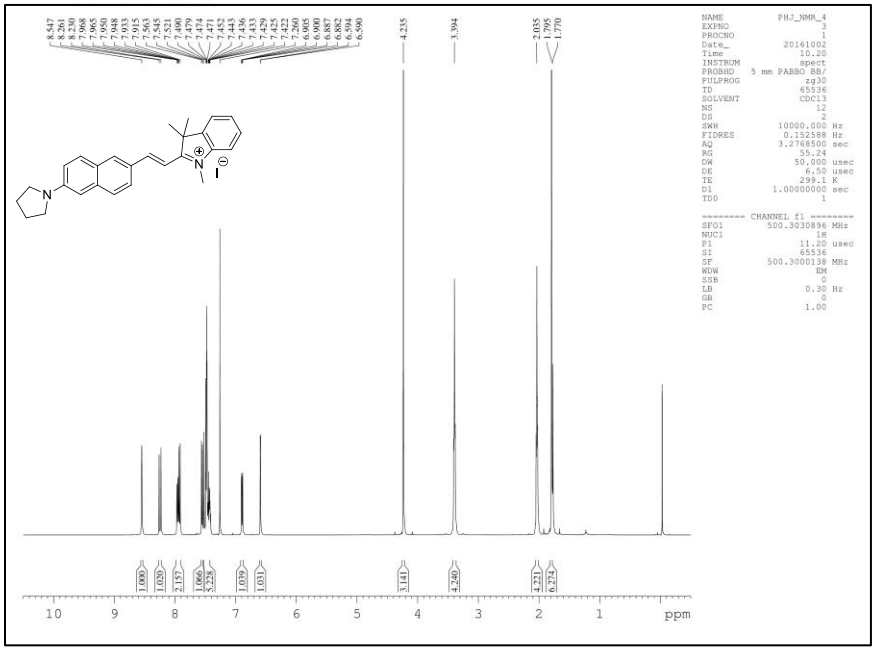
Analyte	Probe structure	Reference
Cys		*Sensors and Actuators B* **2013**, *178*, 525
GSH		*J. Am. Chem. Soc.* **2014**, *136*, 574
HSO_3^-		*Org. Biomol. Chem.* **2017**, *15*, 2734
		*Chem. Sci.,* **2013**, *4*, 2892
HOCl		*RSC Adv.* **2014**, *4*, 59535
		*J. Chem. Sci.* **2019**, 131:36
H_2S		*Chem. Commun.,* **2016**, *52*, 6415
$ONOO^-$		*Chem. Commun.,* **2018**, *54*, 11590

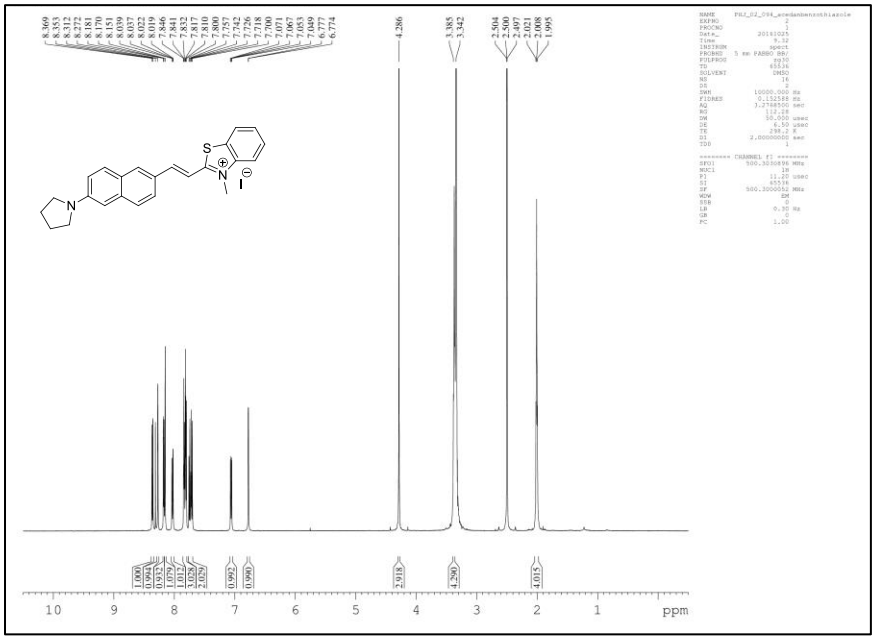
Table S2. Photophysical properties of **NVId**, **NVBt**, and **NVPy**.

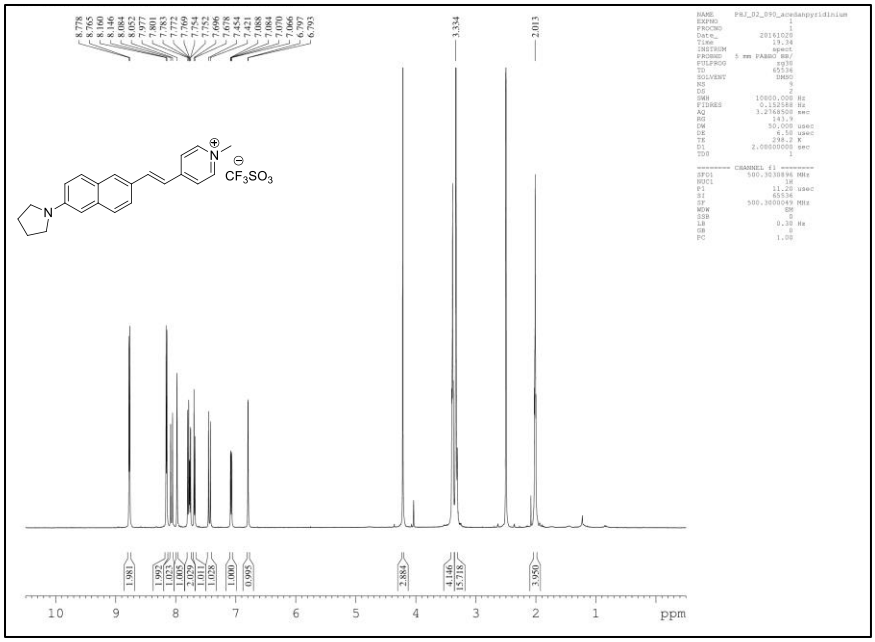
Dye[a]	Solvent	λ_{abs} (nm)	ε[b]	λ_{em}[c] (nm)	Φ_F[d]	Stokes shift
NVId	$CHCl_3$	617	51880	697	0.024	80
	EtOH	583	51371	703	0.011	120
	CH_3CN	573	31622	717	n.d.	144
	Glycerol	591	39551	698	0.05	107
	PBS	554	27673	715	n.d.	161
NVBt	$CHCl_3$	596	49272	690	0.03	94
	EtOH	552	45744	702	0.016	150
	CH_3CN	541	41855	715	n.d.	174
	Glycerol	558	46389	692	0.05	134
	PBS	510	20144	695	n.d.	185
NVPy	$CHCl_3$	530	39,900	652	0.04	122
	EtOH	497	39,300	692	0.014	195
	CH_3CN	485	33,600	715	n.d.	230
	Glycerol	495	31,100	668	0.04	171
	PBS	455	17,700	692	n.d.	237

[a] All the measurements were conducted at 25 °C at 10 µM dye concentration. [b] in unit, L M^{-1} cm^{-1}. [c] Measured under excitation at the maximum absorption wavelength in the given solvent. [d] Quantum yields determined using rhodamine 6G as a reference (Φ_F = 0.95 in EtOH). The n.d. stands for "not determined".

^{1}H NMR data.



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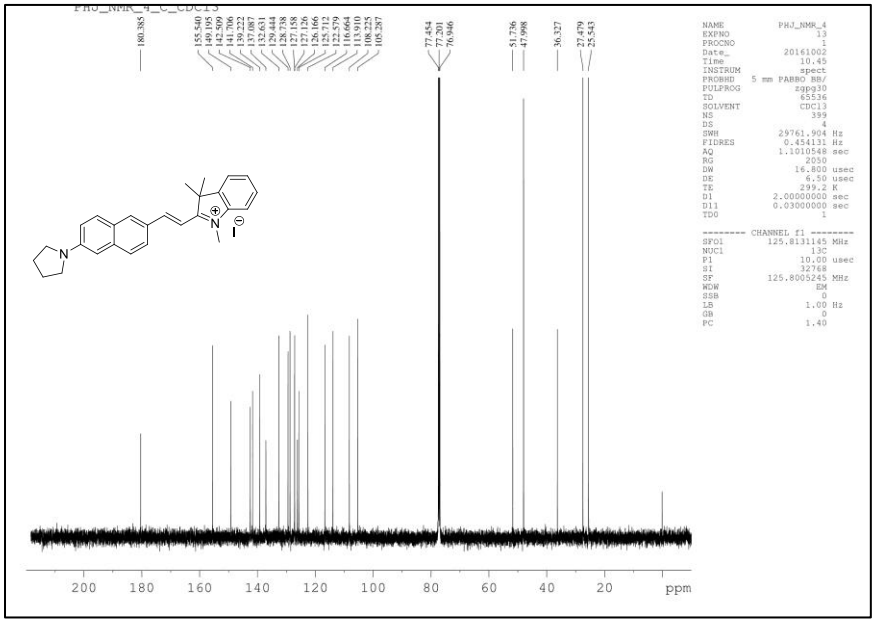
ppm

^{13}C NMR data.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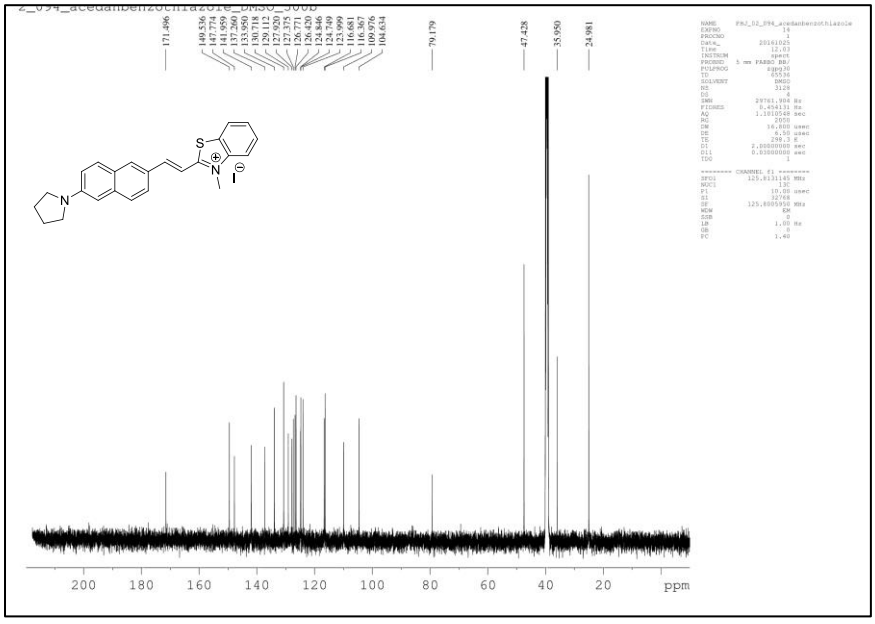
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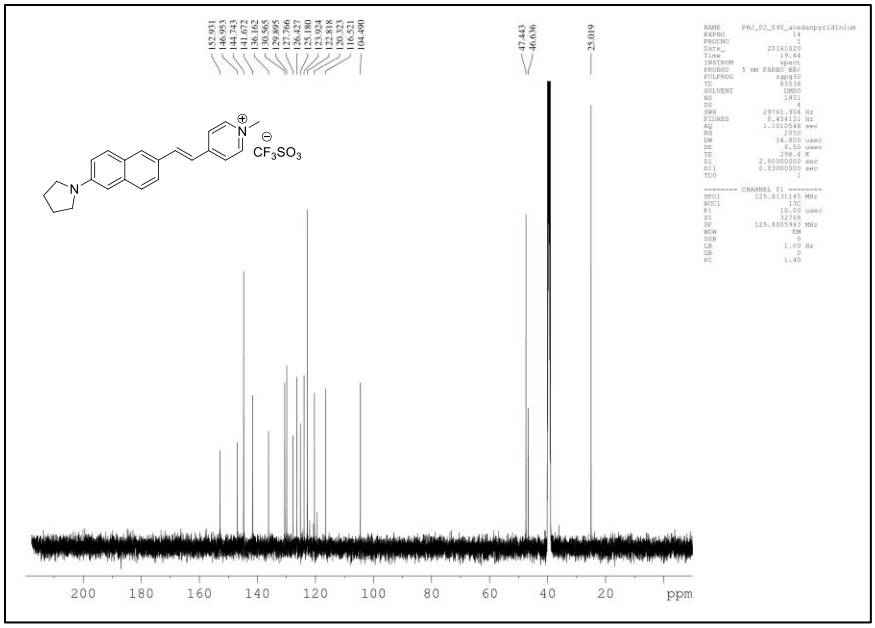
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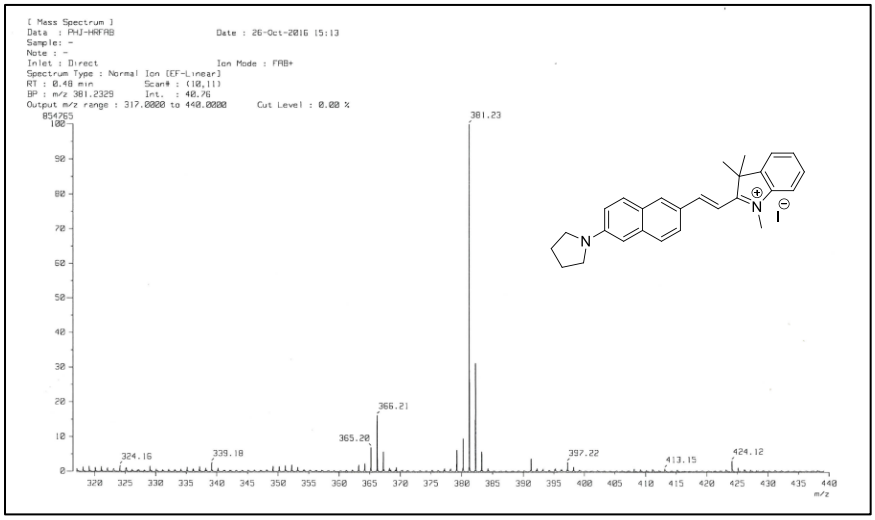
HRMS data: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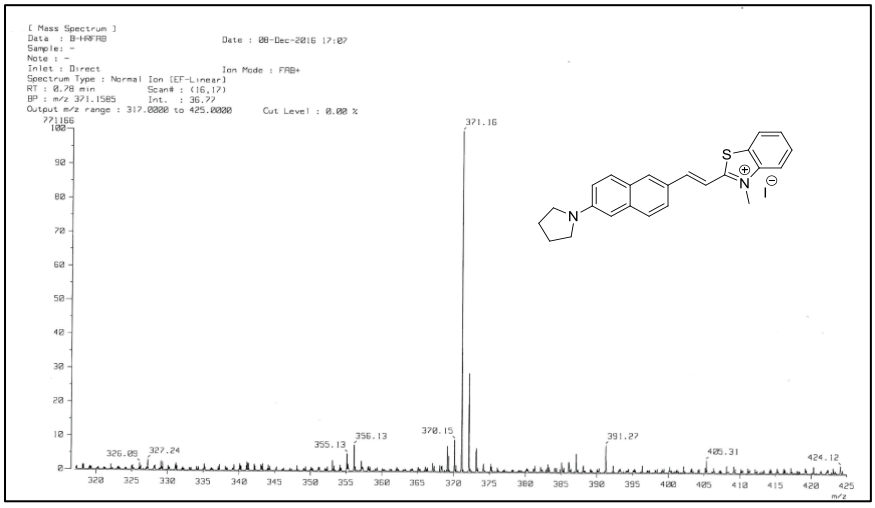
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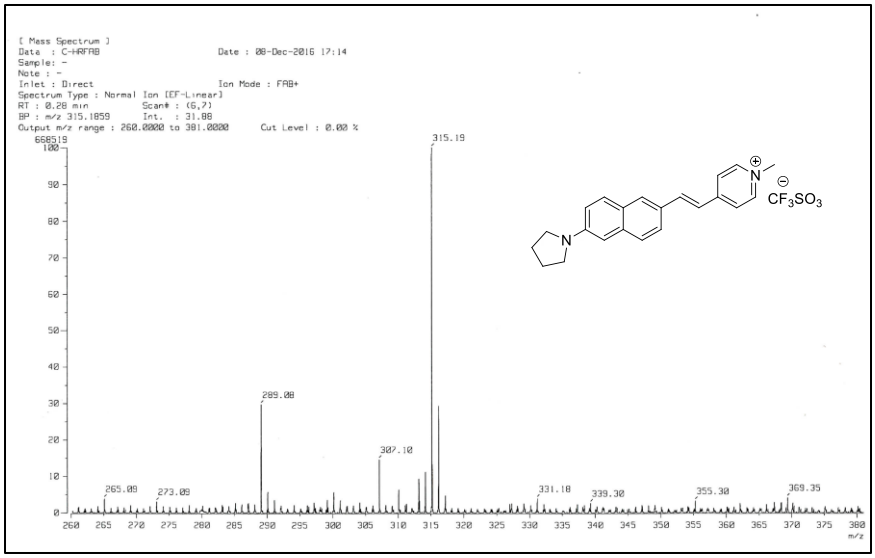
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m/z
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