Supporting information

Click-Activated Fluorescent Probe for Selective Detection of Hydrazoic Acid and Its Applications to Biological Imagings

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Reagents and Apparatus

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried using standard procedures. Tris-triazoleamine ligand was prepared as described by Chan and Fokin.[1] Electrospray ionization mass spectra (*ESI-MS*) was measured on a Micromass LCTTM system. Fluorescence measurements were performed at room temperature on a Perkin-Elmer LS 50B fluorescence spectrophotometer. ^{1}H-NMR and ^{13}C NMR were measured on a BrukerAV-400 or BrukerAV-300 spectrometer with chemical shifts reported in ppm (in $CDCl_3$ or DMSO-d_6; TMS as internal standard). Data were presented as follows: Chemical shift (in ppm on the scale and TMS as internal standard), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constant (J/Hz), and interpretation. pH measurements were made with a Sartorius basic pH meter PB-10. TLC analysis was performed on silica gel plates. Column chromatography was conducted over silica gel (mesh 200–300), and both were obtained from the Qingdao Ocean Chemicals.

Preparation of the detection system

HAN1

A stock solution of **HAN1** (10 mM) was prepared in DMSO and was stored at -20 °C for spectrum and biological imaging investigation.

Preparation of hydrazoic acid

0.1 M hydrazoic acid solution was prepared by treatment of 0.1 M barium azide solution with 0.1 M sulfuric acid, futher filtered the insoluble barium sulfate.

Preparation of acetic acid/acetate buffer

pH 4.5 (0.2 M Sodium Acetate Solution)**:** Weigh 27.20 g of Sodium Acetate Trihydrate into a one liter volumetric flask. Add 800 mL of deionized water. Mix and dissolve. Bring the pH down to 4.5 with Glacial Acetic Acid. Finally adjust the volume to one liter with deionized water to obtain 1000 mL of solution having a pH of 4.50 ± 0.05.

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Preparation of RNS and ROS

NO_2^-

NO_2^- was prepared from the source of $NaNO_2$ at room temperature in acetic acid/acetate buffer (pH 4.5).

$ONOO^-$

The synthesis of peroxynitrite involved nitrosation of H_2O_2 at pH ≥12.0 by isoamyl nitrite. The

peroxynitrite concentration was determined by using an extinction coefficient of 1670 ± 50 $cm^{-1}(mol/L)^{-1}$ at 302 nm.[2]

OH•

Hydroxyl radicals was generated by the addition of Fe^{2+} (1 mM) and H_2O_2 (1 mM) at room temperature in acetic acid/acetate buffer (pH 4.5), and the mixture was then stirred for 30 min.

HOCl

HOCl was prepared from the source of NaOCl at room temperature in acetic acid/acetate buffer (pH 4.5). The concentration of HOCl was determined by titration with $Na_2S_2O_3$.

O_2•$^-$

Superoxide was prepared from the source of KO_2 at room temperature in acetic acid/acetate buffer (pH 4.5).

NO

Nitric oxide was prepared from a saturated NO aqueous solution (~2 mM) at room temperature in acetic acid/acetate buffer (pH 4.5).

HNO

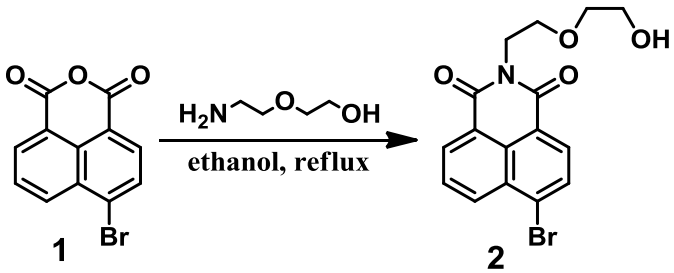
Nitroxyl donor (HNO) was generated from sodium trioxodinitrate ($Na_2N_2O_3$, Angeli's salt). Angeli's salt was prepared as described by King and Nagasawa and was stored at -20 °C until needed.[3]

ROO•

ROO• was generated from 2,2'-azobis(2-amidinopropane)dihydrochloride (CAS: 2997-92-4), which was dissolved in deionized water first and then added into probe testing solutions at room temperature in acetic acid/acetate buffer (pH 4.5) for 30 min.

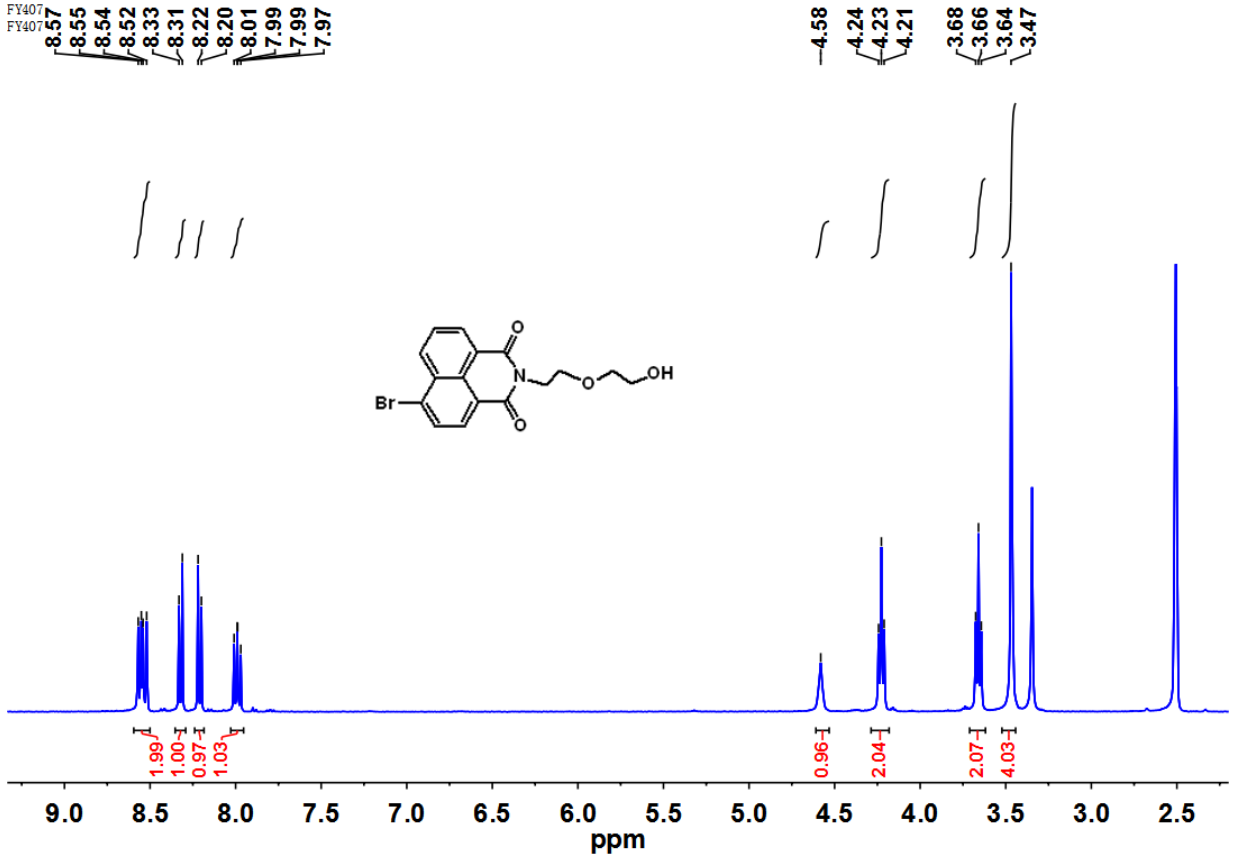
Synthesis and Characterization of Probe

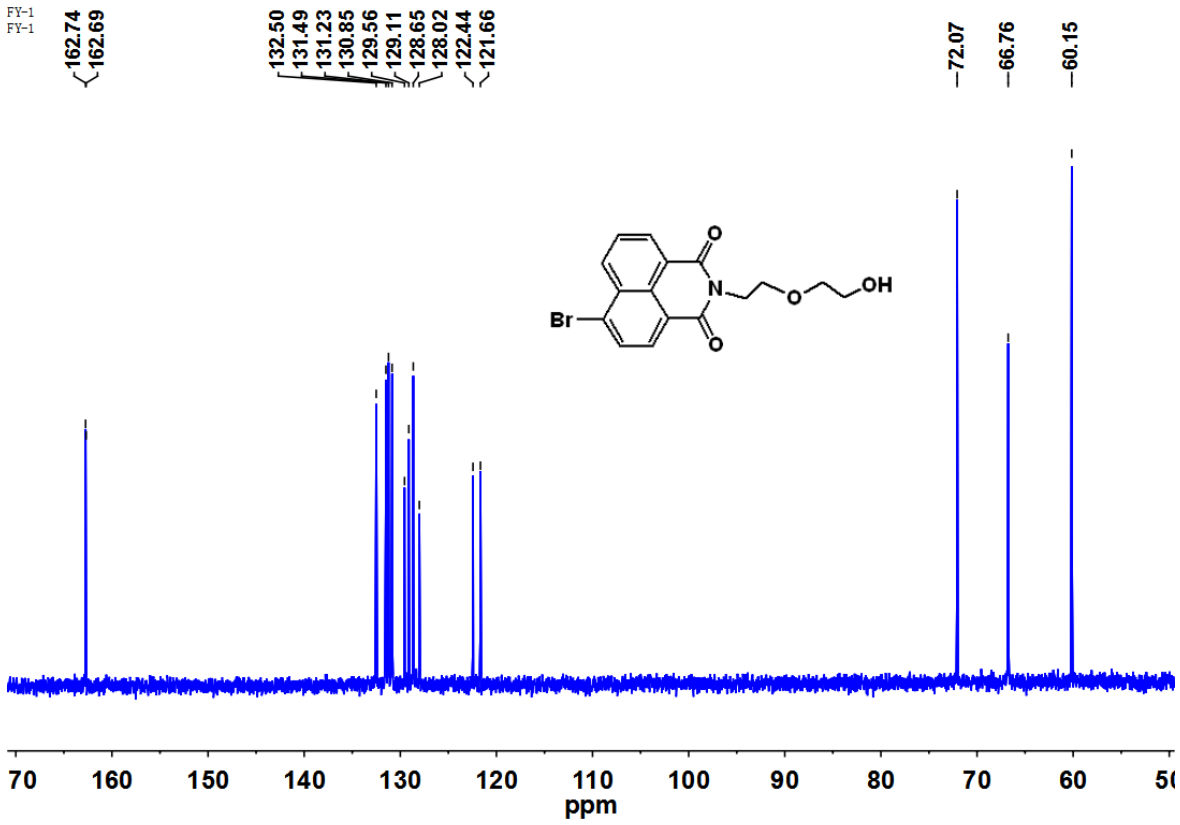
4-Bromo-N-hydroxydiethyl ether-1,8-naphthalimide



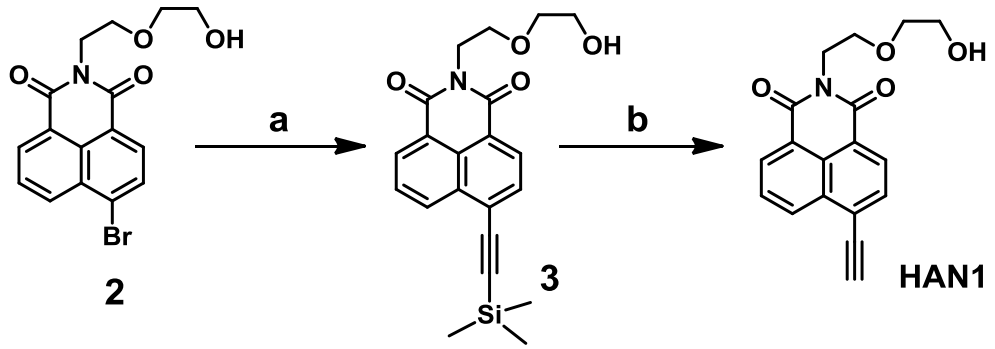
To a solution of 4-bromo-1,8-naphthalic anhydride **1** (2.77 g, 10.0 mmol) in 40 mL ethanol was added 1-amino-2-(2-hydroxyethoxy)ethane (1.57 g, 15.0 mmol) and the mixed solution was refluxed at 80 °C for ~25 h, TLC (PE:EA, 8:2) indicated the formation of the product (R_f = 0.5) with the complete consumption of starting material (R_f = 0.2). After cooling to room temperature, the brown color suspension was put into water. The resulting precipitate was filtered and washed with water, and dried to yield a light yellow product **2** (3.05 g, 84%).^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.55 (dd, *J* = 11.8,

7.9 Hz, 2H, Ar-H), 8.32 (d, *J* = 7.9 Hz, 1H, Ar-H), 8.21 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.99 (dd, *J* = 8.3, 7.4 Hz, 1H, Ar-H), 4.58 (s, 1H,-OH), 4.23 (t, *J* = 6.5 Hz, 2H, -CH_2-), 3.66 (t, *J* = 6.5 Hz, 2H, -CH_2-), 3.47 (s, 4H, -CH_2-).^{13}C NMR (100 MHz, DMSO-d_6): δ = 162.74, 162.69, 132.50, 131.49, 131.23, 130.85, 129.56, 129.11, 128.65, 128.02, 122.44, 121.66, 72.07, 66.76, 60.15. *ESI*-MS: m/z 365.1 $[M+H]^+$, 387.1 $[M+Na]^+$.





4-Ethylnyl-N-hydroxydiethyl ether-1,8-naphthalimide

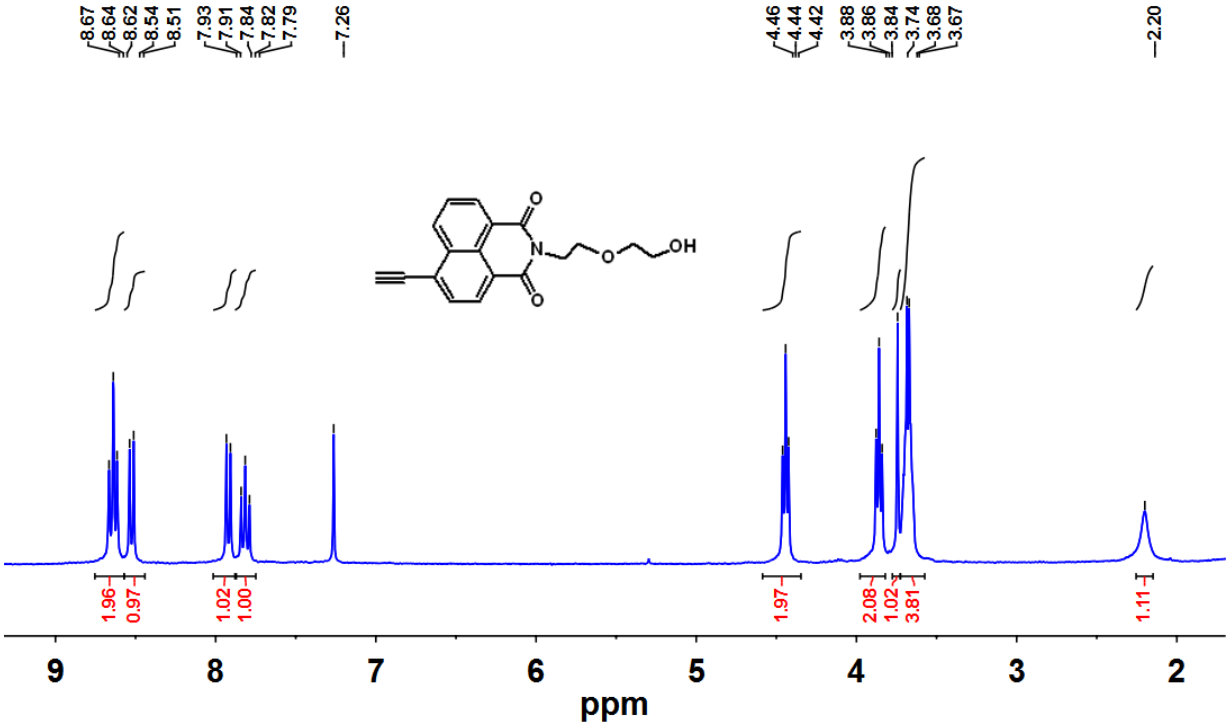


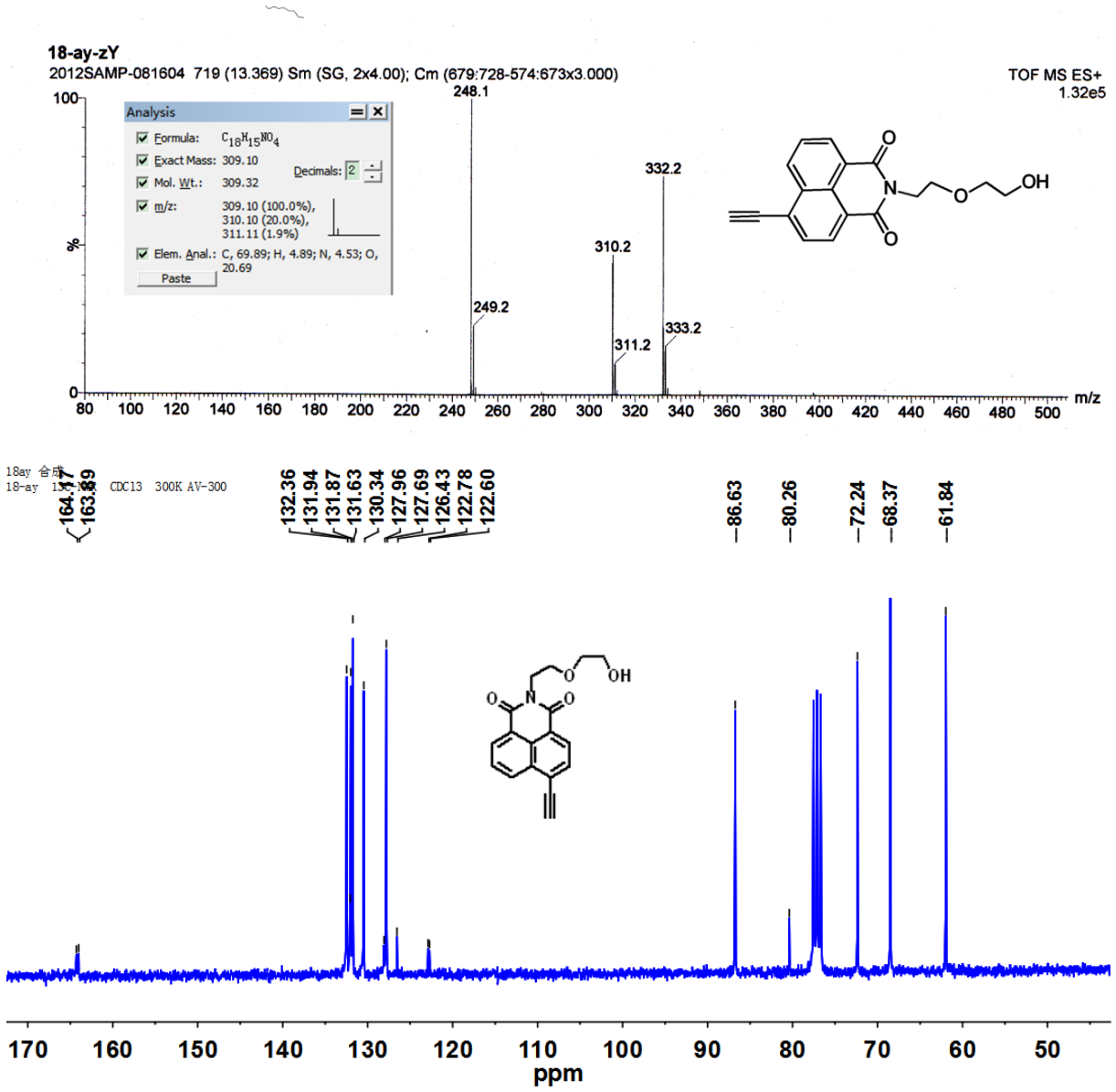
Reagents and conditions:a) $Pd(PPh_3)_2Cl_2/PPh_3/CuI$, tri-methylsilylacetylene, THF/DIPEA, RT, N_2, 69%; b) 1M TBAF in THF, 50 °C, 60 min, 32% (2 steps).

Step 1: 4-Bromo-N-hydroxydiethyl ether-1,8-naphthalimide **2** (1.82 g, 5.0 mmol), $Pd(PPh_3)_2Cl_2$ (351 mg, 0.5 mmol), PPh_3(262 mg, 1.0 mmol) and copper iodide (190 mg, 1.0 mmol) were dissolved in THF (dry, 25 mL) and DIPEA (1.0 mL, 5.74 mmol) under nitrogen atmosphere. Trimethylsilylacetylene (1.06 mL, 0.75 mmol) was added and the mixture was stirred at room temperature for 12 hours. TLC (EA:DCM, 1:9) indicated the formation of product (R_f = 0.35) with the complete consumption of starting material. The reaction mixture was then poured into water and extracted with chloroform. The organic layer was washed with sat. aqueous NH_4Cl and dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (EA:DCM, 1:9) to afford a light

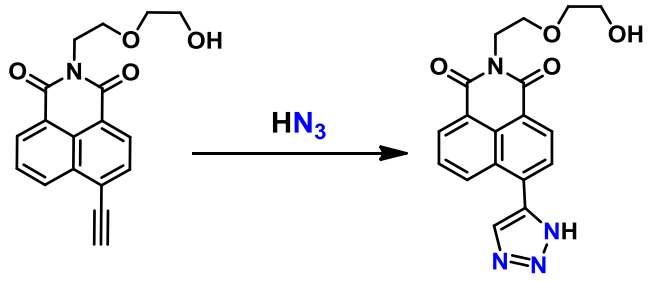
yellow intermediate **3** (1.31 g, yield: 69%). M.p. 116.4~118.5 °C. *ESI*-MS: m/z 382.1 $[M+H]^+$.

Step 2: To a solution of the intermediate **3** (381 mg, 1.0 mmol) in THF (dry, 10 mL) was added tetra-n-butylammonium fluoride (TBAF, 1 M in THF, 5.0 mmol) and the mixture was stirred at 50 °C for 60 min under nitrogen atmosphere. TLC (EA:DCM, 1:4) indicated the formation of product (R_f = 0.25) with the complete consumption of starting material. The reaction mixture was diluted with water, and the precipitates were filtered. The solid was purified by column chromatography (EA:DCM, 1:3) resulting in a light yellow solid (98.9 mg, 32%). M.p. 137.0~137.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.64 (t, *J* = 7.5 Hz, 2H, Ar-H), 8.52 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.92 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.82 (t, *J* = 7.9 Hz, 1H, Ar-H), 4.44 (t, *J* = 5.6 Hz, 2H, -CH_2-), 3.86 (t, *J* = 5.6 Hz, 2H, -CH_2-), 3.74 (s, 1H, C≡H), 3.68 (d, *J* = 4.0 Hz, 4H, -CH_2-), 2.20 (s, 1H, -OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.17, 163.89, 132.36, 131.94, 131.87, 131.63, 130.34, 127.96, 127.69, 126.43, 122.78, 122.60, 86.63, 80.26, 72.24, 68.37, 61.84. *ESI*-MS: m/z 310.2 $[M+H]^+$, 332.2 $[M+Na]^+$.





Reaction probe HAN1 with HN_3 in *aqueous* media

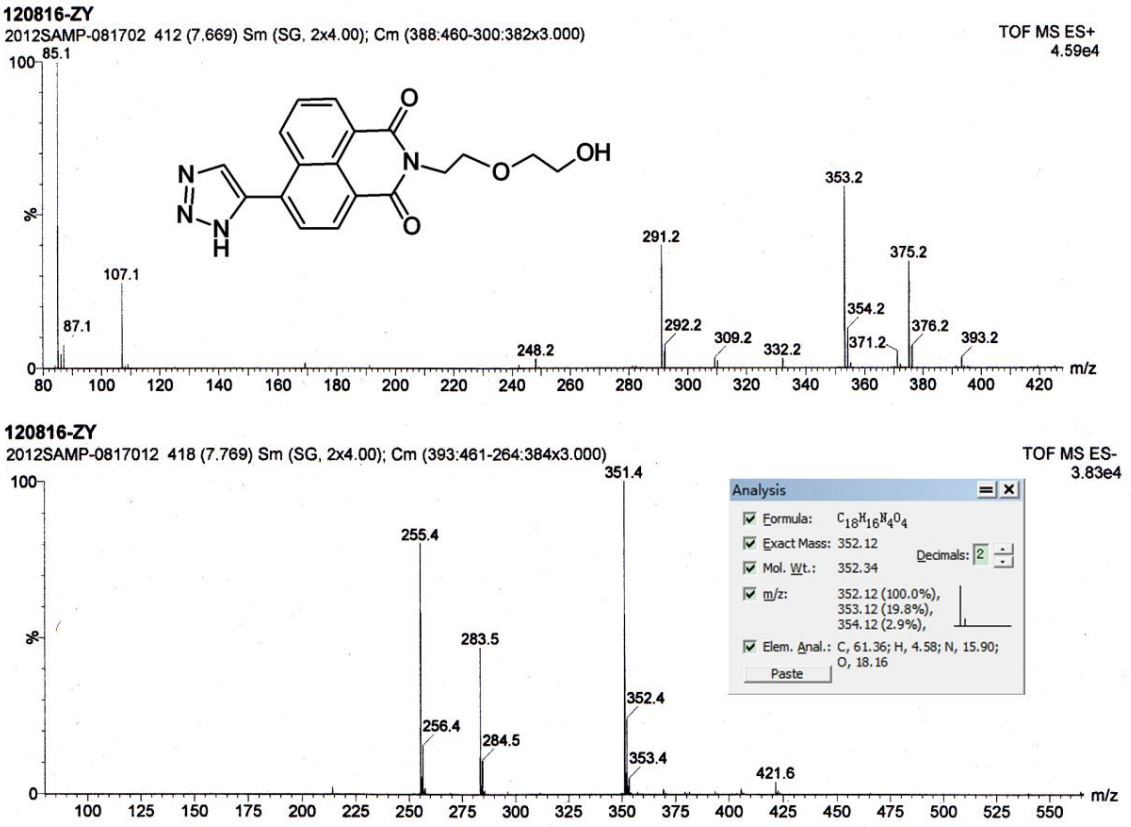


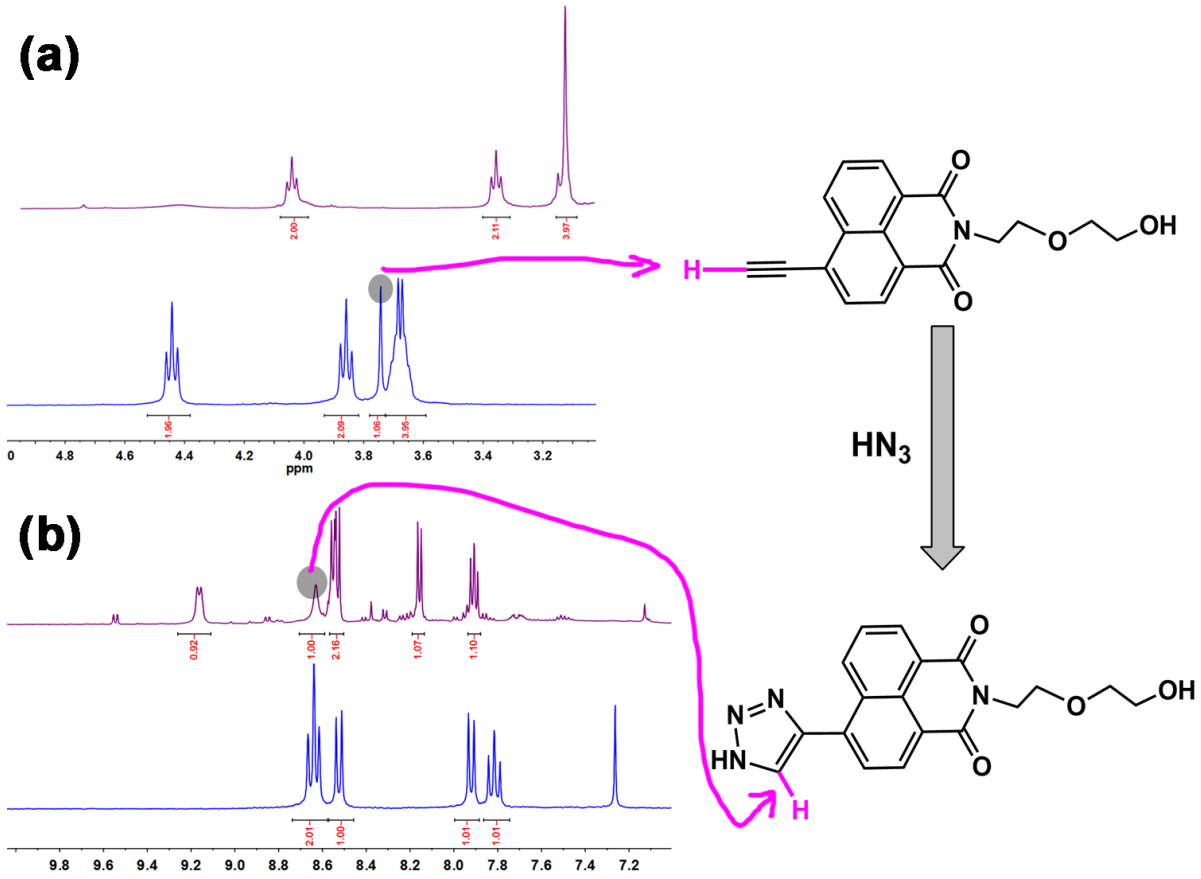
HAN1 (31.0 mg, ~0.1 mmol) was dissolved in 10% CH_3CN/H_2O (5 mL, Containing 10.0 mM Tris-triazoleamine and 5.0 mM CuBr catalyst) and followed by the addition of 0.5 mol/L HN_3(5 mL, Prepared by treatment of 0.5 mol/L barium azide solution with 0.5 mol/L dilute sulfuric acid). The reaction mixture was stirred at room temperature for 4 h and conversion was checked by analytical

HPLC/MS (Trizole yield: >80%). (4.6 mm x 150 mm 5 μm C18 column; 5 μL injection; 10% CH_3CN/H_2O, linear gradient, with constant 0.1 % v/v TFA additive; 20 min run; 1 mL/min flow; ESI; UV detection at 254 nm); *ESI*-MS: (positive ion mode) m/z 353.2 $[M+H]^+$, 375.2 $[M+Na]^+$; (negative ion mode) m/z 351.4 $[M-H]^-$.

Then, the reaction mixture was removed of solvent and the residue was checked by NMR without purify. ^1H NMR (400 MHz, DMSO-d_6): δ = 9.08 (d, *J* = 8.2 Hz, 1H, Ar-H), 8.57 (s, 1H, trizole), 8.48 (dd, *J* = 10.4, 7.4 Hz, 2H, Ar-H), 8.11 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.89-7.84 (m, 1H, Ar-H), 4.23 (t, *J* = 6.5 Hz, 2H, -CH_2-), 3.67 (t, *J* = 6.5 Hz, 2H, -CH_2-), 3.48 (s, 4H,-CH_2-).

***ESI*-MS and ^1H NMR clear indicated that the reaction of HAN1 and HN_3 proceed through a cycloaddition route.**





Kinetic Studies

The kinetic studies of probe **HAN1** (50 μM) with HN_3 (200 μM) was determined at room temperature in acetic acid/acetate buffer (pH 4.5). The *pseudo*-first-order rate constant value was fitted from the emission intensity data at 463 nm following the modified *pseudo*-first-order equation: [4]

$$\mathbf{Ln\ [(\Delta I_{max} - \Delta I_t)/\Delta I_{max}] = - k't}$$

Here $\Delta I_t = I_t - I_{min}$ and $\Delta I\ max = I_{max} - I_{min}$, where I_{min}, I_t, and I_{max} are the fluorescence intensities of **HAN1** considered in the absence of F^-, at an intermediate time t, and at the time a reaction complete. *k'* is the *pseudo*-first-order rate constant. The *pseudo*-first-order plot for the reaction of **HAN1** with HN_3 is shown in Figure S1. From the plot of $Ln[(\Delta I_{max} - \Delta I_t)/\Delta I_{max}]$ against t, the value of *k'* was determined by fluorescence time course method for **HAN1** with HN_3 : $k' = 1.092\times10^{-4}\ s^{-1}$.

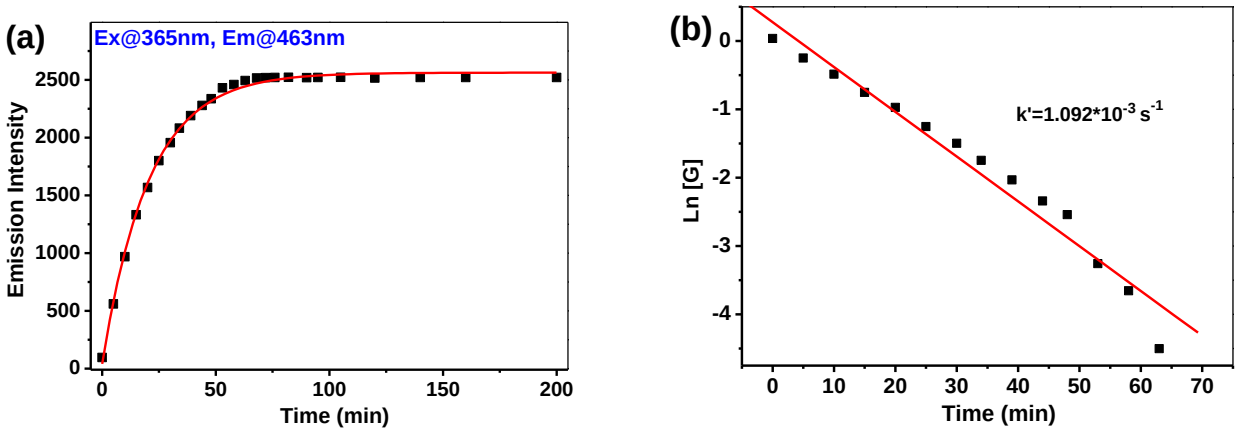


Figure S1: (a) Time course of reaction of **HAN1** (50 μM) with HN_3 (200 μM) at room temperature in acetic acid/acetate buffer (pH 4.5) for 0-200 min. (b) *Pseudo*-first-order kinetic plot of the reaction of **HAN1** (50 μM) with HN_3 (200 μM). [G] = $(\Delta I_{max} - \Delta I_t / \Delta I_{max})$, Slope = 1.092×10^{-3} s^{-1}, R= 0.98091.

Detection limit

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of probe **HAN1** in the absence of HN_3 was measured. The value of **[DL]** was estimated on the basis of the signal-to-noise ratio: For **HAN1** with HN_3: **[DL]**= ~42.1 μM,.

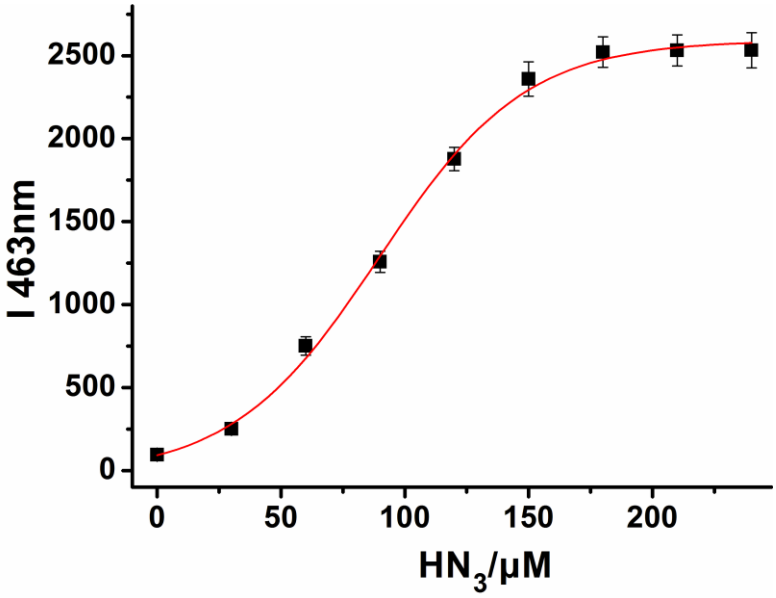


Figure S2: (a) Fluorescence titration of **HAN1** (50 μM) upon addition of HN_3 (0 ~ 250 μM) at room temperature in acetic acid/acetate buffer (pH 4.5) with excitation at 365 nm.

Photophysical properties

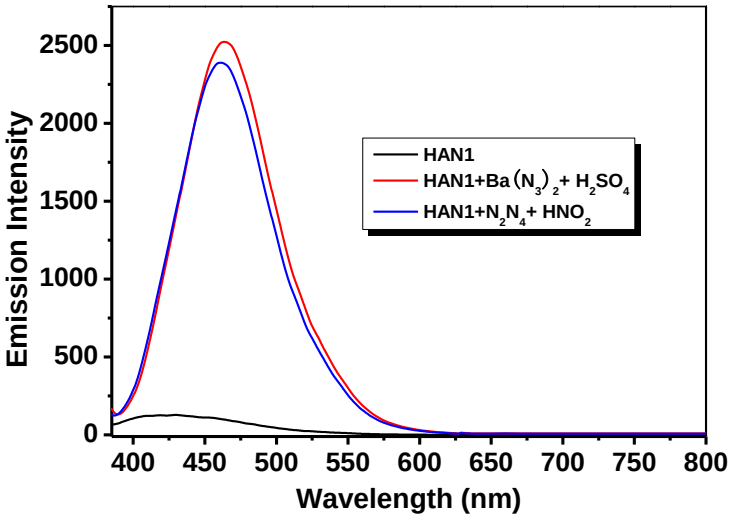


Figure S3: Fluorescence spectra of **HAN1** (50 μM) in acetic acid/acetate buffer (pH 4.5) recorded 60 min after the addition of HN_3 (200 μM). Red line: HN_3 prepared by the reaction of barium azide solution with sulfuric acid; Blue line: HN_3 prepared by the reaction of aqueous hydrazine with nitrous acid.[5]

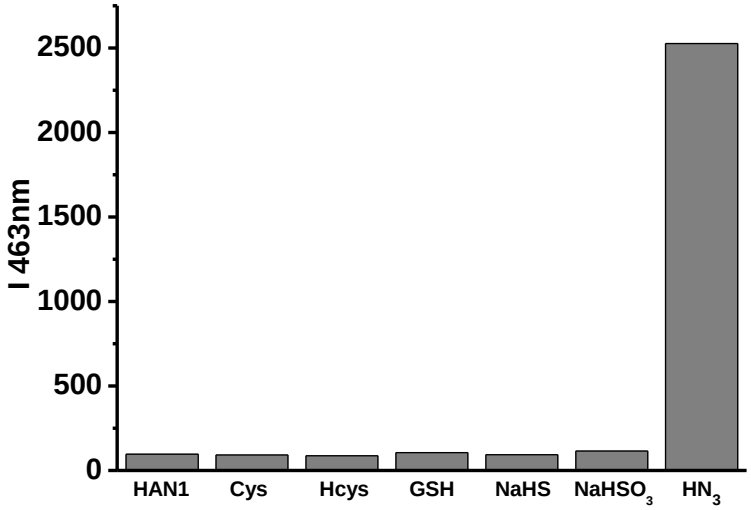


Figure S4: Fluorescence responses of **HAN1** (50 μM) with RSS species: Cys (200 μM), Hcys (200 μM), GSH (200 μM), NaSH (200 μM), and $NaHSO_3$ (200 μM).

Cell Culture and Fluorescence Imaging

The **HAN1** working solution for cell staining was prepared from a 10 mM stock DMSO solution by diluting with PBS (pH~5.2) to a final concentration of 20 μM. Hela cells (cervical cancer cells) were dropped on the poly-D-lysine-coated 35 mm glass bottom dishes (Mat Tek Corp) at a density of 2×10^3 cells per well in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% fetal

bovine serum (FBS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air for 24 h prior to staining. All cellular fluorescent images were collected on an FV1000-IX81 confocal microscope. Images were collected using IPP software (Olympus) by confocal microscope.

To ascertain the cytotoxic effect of **HAN1** treatment over a 12 h, the MTT assay was performed. HeLa cells (5×10^4) in the log phase were seeded in 96-well plates. After 24 h incubation, the cells were treated with different concentrations of 20 μM **HAN1**/5 μM Tris-triazoleamine catalyst/5 μM CuBr in PBS pH~7.4 for 12 h. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solutions were added after treatments and incubated for additional 4 h. DMSO was added to solubilize the formazan crystal, and he absorbance of each well was measured by a microplate reader (SPECTRA SLT; Labinstruments, Salzburg, Austria). The cell viability fraction (%) was calculated as follows: cell viability fraction (%) = $OD_{492nmin\ test\ cells}/OD492_{nmin\ control\ cells}$ ×100%.

Table S1. Cell viability was quantified by the MTT assay

HAN1/catalyst/ CuBr	1	2	3
Cell viability	86.4%	83.1%	84.6%

Zebrafish were kept at 28.5°C and maintained at optimal breeding conditions. For mating, male and female zebrafish were maintained in one tank at 28.5°C on a 12 h light/12 h dark cycle and then the spawning of eggs were triggered by giving light stimulation in the morning. Almost all the eggs were fertilized immediately. The 19 dpf old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10-5% methylene blue; pH 7.5).[6] Experiments to detect HN_3 were performed in PBS (pH~5.2) media with 20 μM **HAN1** for 30 min. All zebrafish fluorescent images were collected on a fluorescent dissecting microscope (Leica) equipped with a DP70 digital imaging system (Olympus, Tokyo, Japan) with a GFP filter set.

Theoretical and Computational Methods

The Gaussian 03 package refer to Gaussian 03, Revision D.01: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.;

Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, RevisionD.01, Gaussian, Inc., Wallingford, CT, 2004.

The Density functional theory (DFT) and time dependent Density functional theory (TD-DFT) methods have been carried out to study the ground state structure and electron transition for compounds **HAN1** and **HAN1-Trizole**. The latter method has been confirmed to be an effective candidate to carry out the electron transition. Herein, the Becke's three-parameter hybrid exchange functional with lee-Yang-Parr gradient-corrected correlation (B3LYP functional)[7,8] has been used with the 6-31G(d) basis sets to be an appropriate basis set. The geometries for **HAN1** and **HAN1-Trizole** were fully optimized without symmetry constraints, and all the local minima were confirmed by the absence of an imaginary mode in vibration analysis. The electronic distributions and localizations were calculated using the electron density difference maps (EDDMs) with Gauss-Sum2.2.5 software package.[9] An EDDM is a representation of the changes in electron density that occur for a given electronic transition.

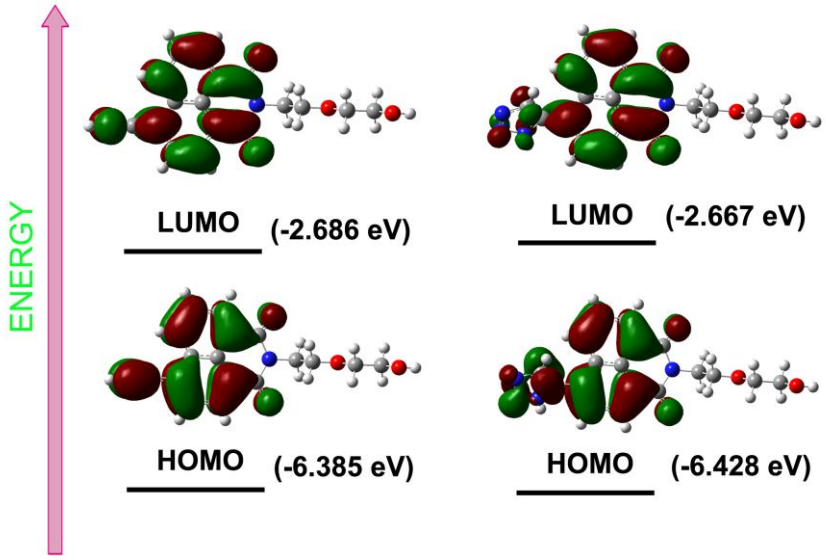


Figure S5: Calculated HOMOs and LUMOs of **HAN1** (left) and **HAN1-Trizole** (right).

The ICT mechanism was confirmed *via* time-dependent density functional theory (TD-DFT) method with 6-31G(d) basis sets. Molecular excitation energies, oscillator strengths (*f*) and electron transitions were listed in **Table S2** using conductor-like polarizable continuum model (C-PCM) for water. Figure S4 displayed that both of the molecules showed the main transition assigned to $S_0 \rightarrow S_1$ from HOMO to LUMO with the largest *f* ~4.0.

Electrons in HOMO for the two molecules were delocalized over the conjugated platform of naphthalimide. For **HAN1,** the electrons in LUMO exhibit large overlap with those in HOMO to result in localized state (LE), and consequently triggering strong fluorescence emission. With **HAN1-Trizole**, the compound exhibited the LE state over the conjugated platform of naphthalimide including the electron transition between π-orbital of naphthalimide moiety and the expanded π-conjugated system of

triazole group. These calculations were consisted with the experimental results and rationalize the ICT process.

Table S2. Calculated electronic transitions energies for **HAN1** and **HAN1-Trizole** obtained from TD-DFT/B3LYP/TZVP calculations

HAN1

Transitions	λ_{cal} (nm)	*f*	CI expansion coefficients
S0-S1	367.5	0.4098	0.64245(HOMO-LUMO)
S0-S2	323.7	0.0001	0.64652(HOMO-4-LUMO)
S0-S3	317.5	0.0375	0.63658(HOMO-2-LUMO)
S0-S4	308.0	0.0002	0.66382(HOMO-1-LUMO)
S0-S5	294.7	0.0372	0.55565(HOMO-5-LUMO)

HAN1-Trizole

Transitions	λ_{cal} (nm)	*f*	CI expansion coefficients
S0-S1	366.0	0.4344	0.64932(HOMO-LUMO)
S0-S2	321.4	0.0006	0.64775(HOMO-5-LUMO)
S0-S3	314.9	0.0327	0.639(HOMO-2-LUMO)
S0-S4	306.6	0.0007	0.66129(HOMO-1-LUMO)
S0-S5	297.4	0.0217	0.66137(HOMO-4-LUMO)

In order to provide further demonstration for the molecular electron transition in the two compounds, the electron density difference maps (EDDMS) of the main states have been calculated using Gauss-Sum2.2.5 sofeware package. The EDDMS obtained the same results as TD-DFT to reveal the ICT mechanism.

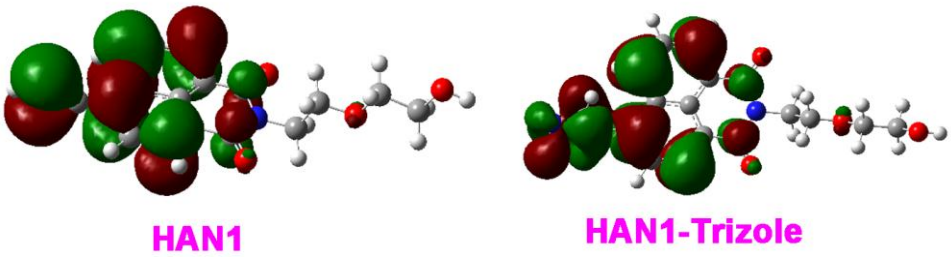


Figure S6: The EDDMS for the first excited state of **HAN1** and **HAN1-Trizole**. The green mark showed the increasing electron density and the red region showed the the decreasing electron density.

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XYZ Coordinates (angstrom) and SCF Energies (a.u.)

Note: upper case letters before the atomic coordinates indicate the atomic symbol of the atoms involved in the calculations.

HAN1 SCF -1050.57007281

C	-3.36674500	-0.62934900	0.06277400
C	-2.56840300	0.56318500	0.00301000
C	-1.16533300	0.43126500	-0.24658600
C	-0.59225000	-0.84839300	-0.45484100
C	-1.38925400	-1.97702500	-0.41686400
C	-2.76470500	-1.86431100	-0.15533400
C	-0.34495100	1.58780500	-0.29471300
C	0.86204500	-0.99916300	-0.71426400
C	1.11501900	1.47105000	-0.54277200
H	-3.36232400	-2.76852100	-0.08197500
C	-3.09835200	1.87251600	0.15251400
C	-2.28003600	2.98296200	0.09831700
C	-0.89525000	2.84380100	-0.11530100
H	-4.16554000	1.99861600	0.29566900
H	-2.70829800	3.97411800	0.21364700
N	1.62127400	0.17957300	-0.76549000
H	-0.92901600	-2.94656100	-0.57490000
H	-0.24159500	3.70864100	-0.15646600
O	1.38700800	-2.09137200	-0.88199800
O	1.85645800	2.44333900	-0.56160300
C	3.05884900	0.05155800	-1.05272100
C	3.88116900	-0.13066100	0.22226300
H	3.37666000	0.95806500	-1.56704800
H	3.19253200	-0.81363200	-1.70165200
H	3.73835000	0.73367100	0.88990800
H	3.55504700	-1.03773000	0.75521700
O	5.23232100	-0.24023100	-0.17751600
C	6.12439200	-0.41900000	0.90675400
C	7.53059700	-0.51772100	0.33555300
H	5.88706100	-1.33673400	1.46818800

H	6.06874000	0.42741700	1.60978900
H	7.57386900	-1.36203500	-0.36925500
H	7.75537800	0.40150000	-0.22657800
O	8.41207100	-0.69762000	1.43702100
H	9.31345200	-0.76044300	1.08900300
C	-4.80358300	-0.58281700	0.35859000
N	-6.87707000	-0.32478300	1.17458600
N	-6.96910400	-1.17837200	0.19143200
C	-5.57992500	0.06645300	1.30636400
H	-5.27270000	0.75036500	2.08366400
N	-5.72372600	-1.34918500	-0.29600900
H	-5.58379800	-1.94321900	-1.10252800

HAN1-Trizole SCF -1215.46393883

C	-3.36674500	-0.62934900	0.06277400
C	-2.56840300	0.56318500	0.00301000
C	-1.16533300	0.43126500	-0.24658600
C	-0.59225000	-0.84839300	-0.45484100
C	-1.38925400	-1.97702500	-0.41686400
C	-2.76470500	-1.86431100	-0.15533400
C	-0.34495100	1.58780500	-0.29471300
C	0.86204500	-0.99916300	-0.71426400
C	1.11501900	1.47105000	-0.54277200
H	-3.36232400	-2.76852100	-0.08197500
C	-3.09835200	1.87251600	0.15251400
C	-2.28003600	2.98296200	0.09831700
C	-0.89525000	2.84380100	-0.11530100
H	-4.16554000	1.99861600	0.29566900
H	-2.70829800	3.97411800	0.21364700
N	1.62127400	0.17957300	-0.76549000
H	-0.92901600	-2.94656100	-0.57490000
H	-0.24159500	3.70864100	-0.15646600
O	1.38700800	-2.09137200	-0.88199800
O	1.85645800	2.44333900	-0.56160300
C	3.05884900	0.05155800	-1.05272100
C	3.88116900	-0.13066100	0.22226300
H	3.37666000	0.95806500	-1.56704800
H	3.19253200	-0.81363200	-1.70165200
H	3.73835000	0.73367100	0.88990800
H	3.55504700	-1.03773000	0.75521700
O	5.23232100	-0.24023100	-0.17751600
C	6.12439200	-0.41900000	0.90675400
C	7.53059700	-0.51772100	0.33555300
H	5.88706100	-1.33673400	1.46818800

H	6.06874000	0.42741700	1.60978900
H	7.57386900	-1.36203500	-0.36925500
H	7.75537800	0.40150000	-0.22657800
O	8.41207100	-0.69762000	1.43702100
H	9.31345200	-0.76044300	1.08900300
C	-4.80358300	-0.58281700	0.35859000
N	-6.87707000	-0.32478300	1.17458600
N	-6.96910400	-1.17837200	0.19143200
C	-5.57992500	0.06645300	1.30636400
H	-5.27270000	0.75036500	2.08366400
N	-5.72372600	-1.34918500	-0.29600900
H	-5.58379800	-1.94321900	-1.10252800