Supplementary Material (ESI) for Organic & Biomolecular Chemistry

A 2,7-carbazole-based dicationic salt for fluorescence detection of nucleic acids and two-photon fluorescence imaging of RNA in nucleoli and cytoplasm

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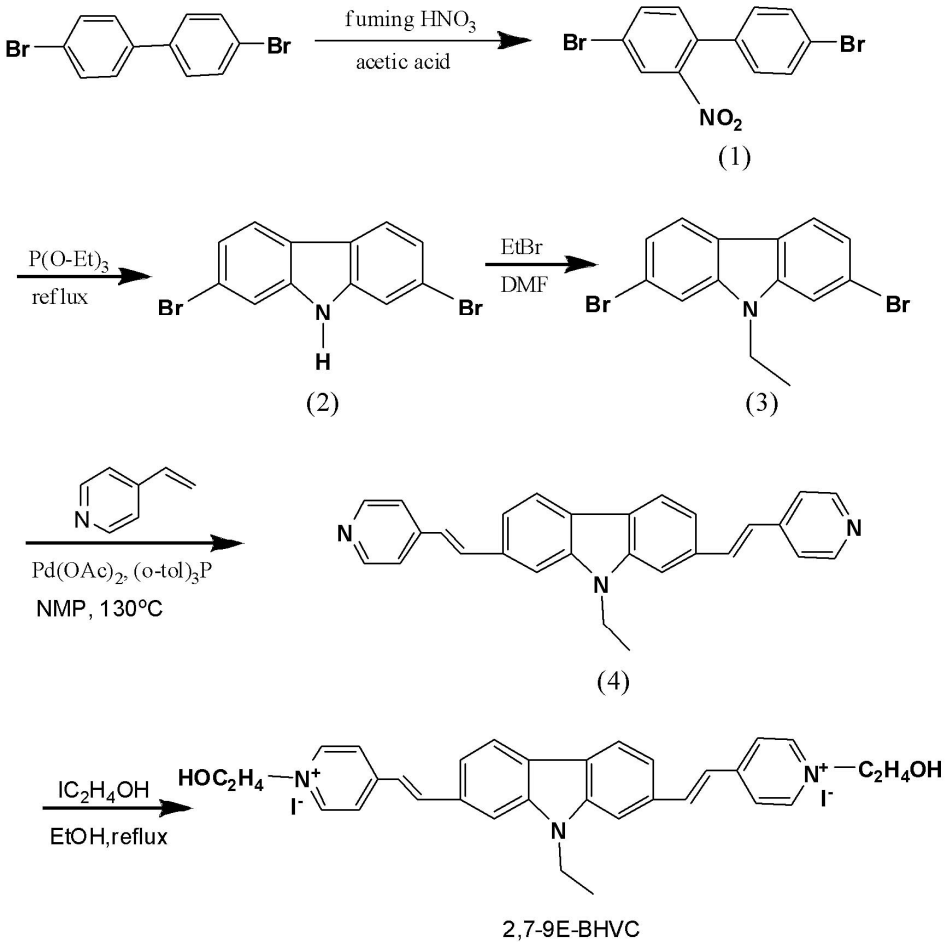
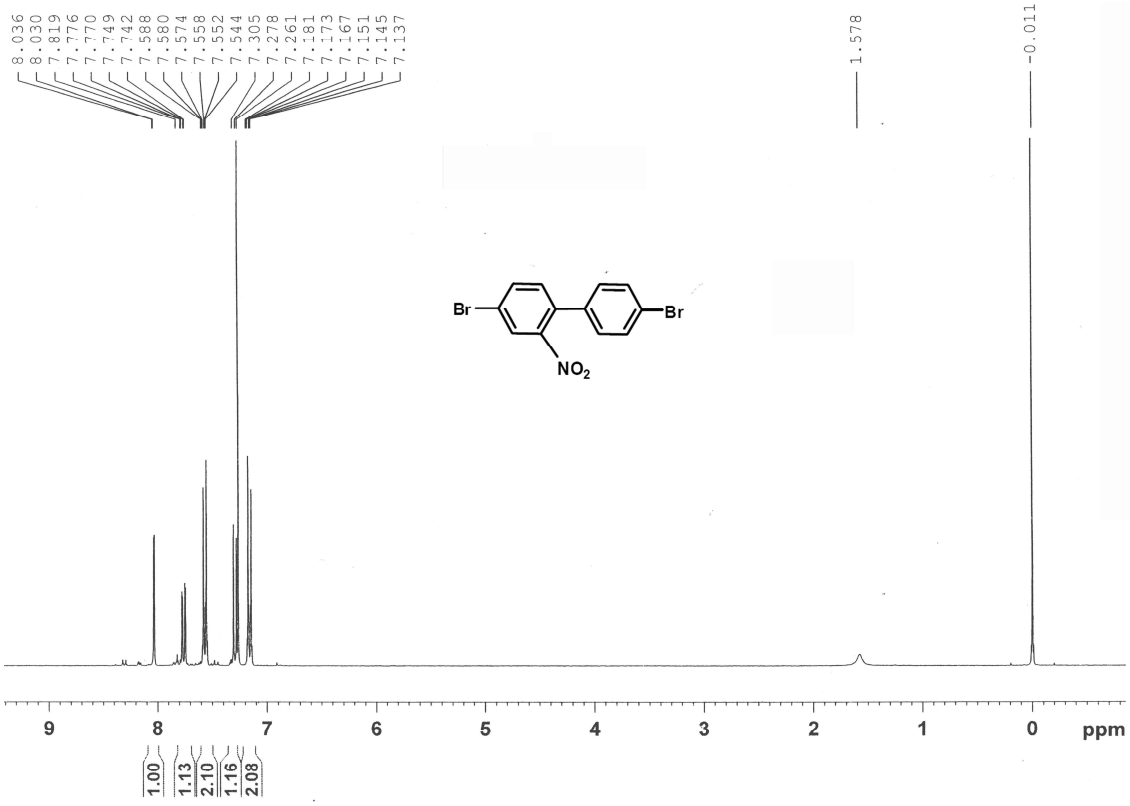


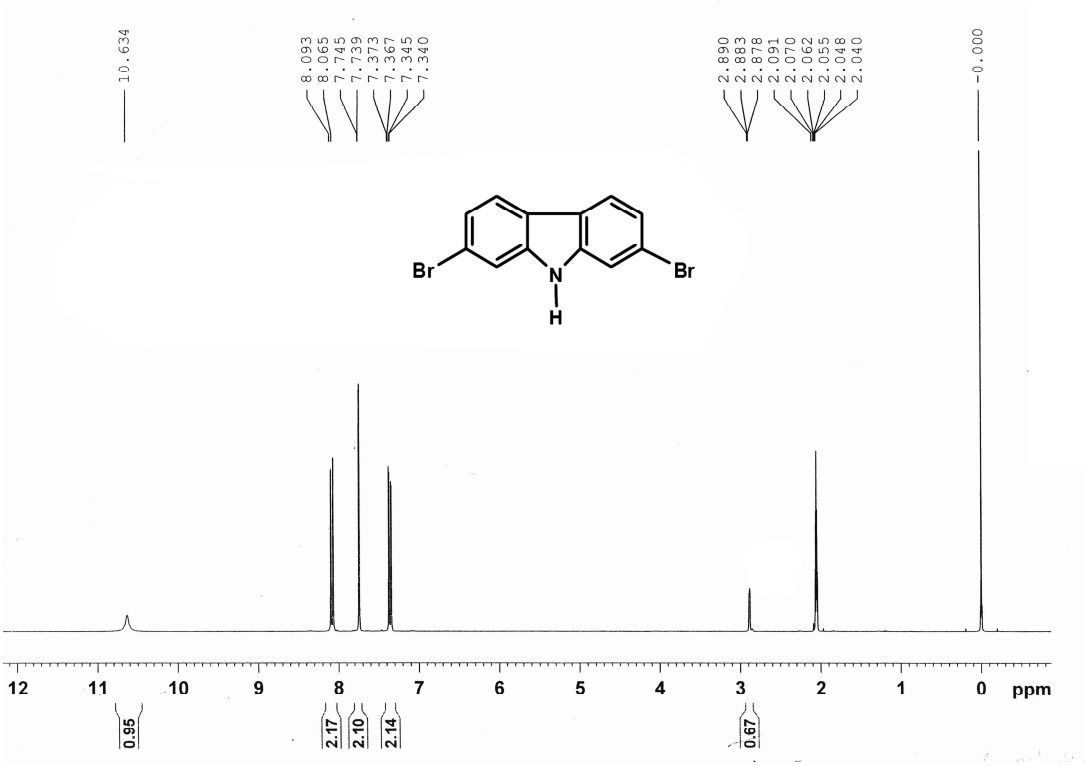
Scheme S1: Synthetic route of 2,7-9E-BHVC

4,4'-Dibromo-2-nitrobiphenyl **(1)**: 4,4'-Dibromobiphenyl 10 g (32 mmol) was dissolved in glacial acetic acid (120 mL), and the mixture was stirred and heated to 100 °C. Then, fuming concentrated nitric acid (95%, 40 mL) was added and the resulting mixture was allowed to react for another 30 min. After the reaction solution was cooled to room temperature, the crude product was filtered. After recrystallization from ethanol, the title compound was obtained in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$), δ(ppm): 8.03 (d, J = 1.8 Hz, 1H), 7.76 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H), 7.54–7.59 (m, 2H), 7.31 (s, 1H), 7.14–7.18 (m, 2H).



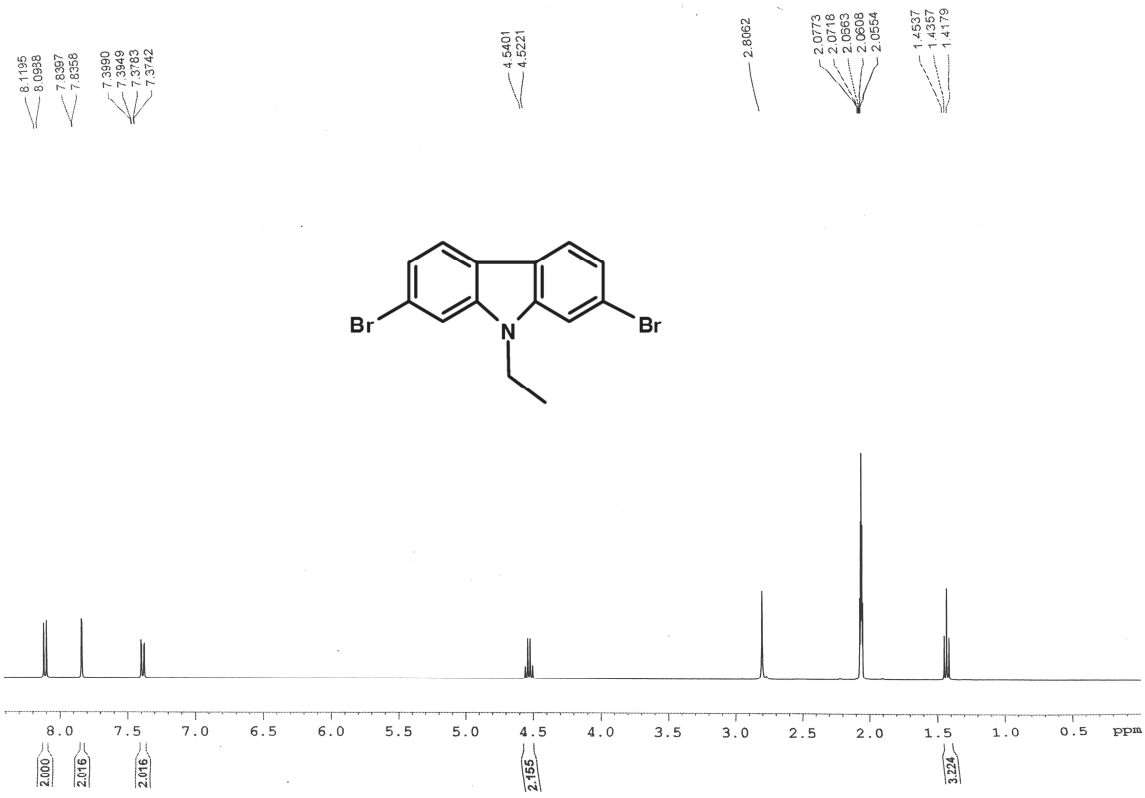
^{1}H NMR spectrum of **1** in $CDCl_3$

***2,7-Dibromocarbazole* (2)**: 7.8 g (22 mmol) of **1** was dissolved in phosphorous acid triethyl ester (30 mL) and the mixture was heated to 150 ^{o}C under the protection of argon. The system was allowed to react for 24 h and a brown solution was obtained. The volatile solvents were then removed by vacuum distillation. The solution left was purified by column chromatography with ethyl acetate/petroleum ether (10:1, v/v) as the eluent. Finally, a white solid was obtained for **2**, in 48% yield. ^{1}H NMR (300 MHz, d_6-Acetone), δ (ppm): 10.64 (s, 1H), 8.09 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 1.8 Hz, 2H), 7.37 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 2H).



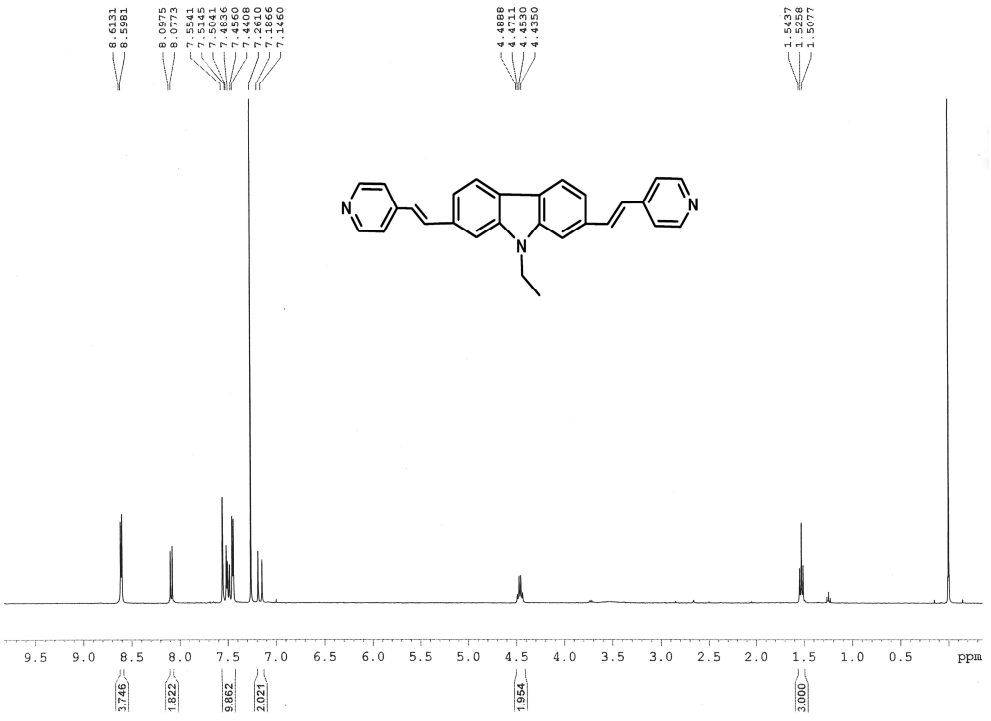
^{1}H NMR spectrum of **2** in d_6-acetone

2,7-Dibromo-9-ethylcarbazole **(3)**: 20 g KOH was added in DMF (70 mL) and the resulting solution was stirred for 30 min. 3.3 g (10 mmol) of **2** was then added and the mixture was stirred for another 40 min. Finally, bromoethane (1.6 g, 15 mmol) was added dropwise and the mixture reacted for 18 h at room temperature. White solid was found when the mixture was poured into water (800 mL). The crude residue was filtered and washed with ethanol for 3 times. A white solid was obtained for **3** after recrystallization from ethanol with a yield of 93%. ^{1}H NMR (400 MHz, d_6-Acetone), δ (ppm): 8.1 (d, J = 8.3 Hz, 2H), 7.84 (d, J = 1.6 Hz, 2H), 7.39 (dd, J_1 = 8.3 Hz, J_2 = 1.6 Hz, 2H), 4.53 (q, J = 7.2 Hz, 2H), 1.44 (t, J = 7.2 Hz, 3H).



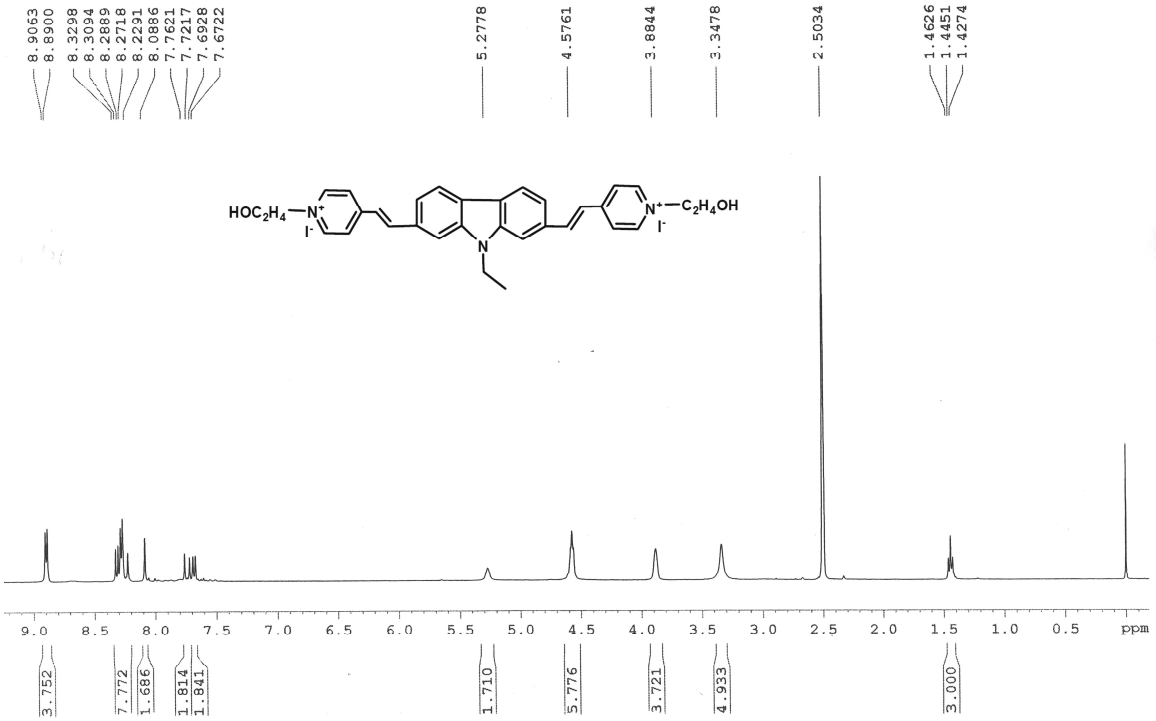
^{1}H NMR spectrum of **3** in d_6-acetone

9-Ethyl-2,7-bis(2-vinyl(pyridin-4-yl))carbazole **(4)**: 2.8 g (8.0 mmol) of **3** was added into a flask containing a mixture of palladium(II) acetate (0.18 g, 0.8 mmol), tri-*o*-tolylphosphine (0.72 g, 2.4 mmol) and K_2CO_3 (8.8 g, 64.0 mmol), and to this mixture *N*-methyl-2-pyrrolidone (NMP, 40 mL) and 4-vinylpyridine (3.4 g, 32.0 mmol) was then added. The system was heated to 130 °C for 2 days under the protection of argon. A dark-red suspension was obtained. When the resulting mixture was cooled to room temperature, it was poured into H_2O (500 mL) and extracted with CH_2Cl_2. Then the organic phases were separated, the excess organic solvent was removed by vacuum distillation and a dark-red solution was obtained. The title product was obtained as a yellow solid after the residue was recrystallized from ethanol (yield: 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.61 (d, J = 6.0 Hz, 4H), 8.09 (d, J = 8.1 Hz, 2H), 7.44–7.55 (m, 10H), 7.17 (d, J = 16.2 Hz, 2H), 4.46 (q, J = 7.2 Hz, 2H), 1.53 (t, J = 7.2 Hz, 3H).

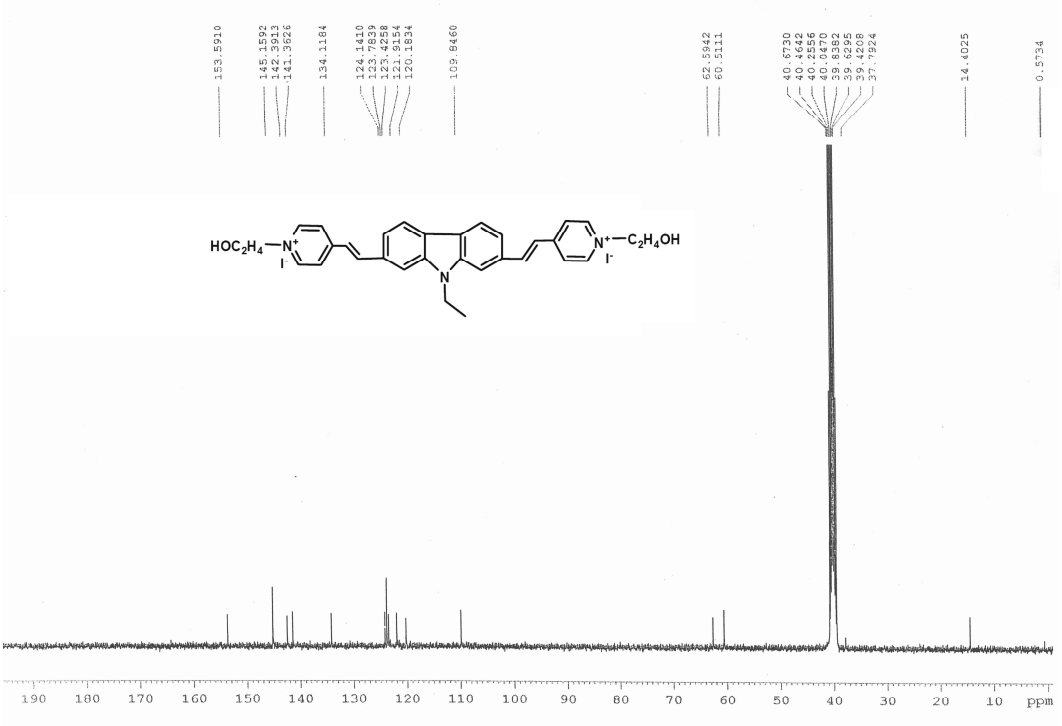


^{1}H NMR spectrum of **4** in $CDCl_3$

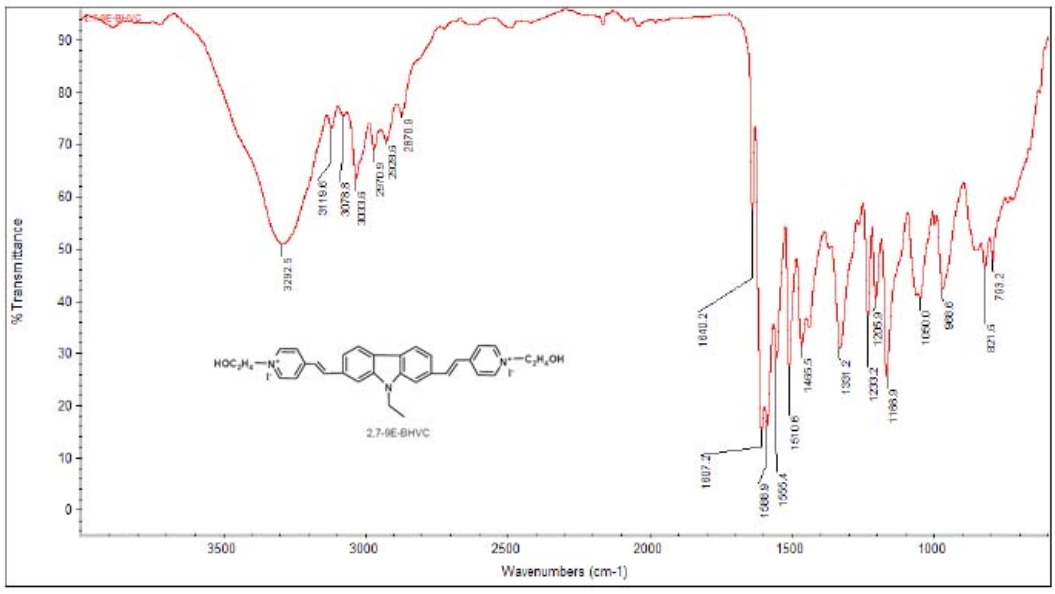
2,7-bis(1-hydroxyethyl-4-vinylpyridium iodine)-N-ethylcarbazole (2,7-9E-BHVC): 0.8 g (2 mmol) of **4** and excess 2-iodoethanol were dissolved in ethanol and stirred for 2 h at room temperature. Then the mixture was refluxed for another 12 h and a red residue was obtained. The residue was filtered and then washed with methanol for 3 times. The title product was obtained as a red solid after the residue was recrystallized from ethanol (Yield: 75%). IR (cm^{-1}): 969 ($\upsilon_{trans\ =C-H}$). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.90 (d, J = 6.5 Hz, 4H), 8.23–8.33 (m, 8H), 8.09 (s, 2H), 7.74 (d, J = 16.2 Hz, 2H), 7.68 (d, J = 8.2 Hz, 2H), 5.28 (s, 2H), 4.58 (s, 6 H), 3.88 (s, 4H), 1.45 (t, J = 7.04 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 153.6, 145.2, 142.4, 141.4, 134.1, 124.1, 123.8, 123.4, 121.9, 120.2, 109.8, 62.6, 60.5, 37.8, 14.4. HRMS (m/z): $[M\text{-}I]^+$ calcd for $C_{32}H_{33}IN_3O_2$, 618.1612; found, 618.1587. Elemental analysis calcd (%) for $C_{32}H_{33}I_2N_3O_2$ (745.43): C 51.56, H 4.46, N 5.64; found: C 51.27, H 4.36, N 5.28.



^{1}H NMR spectrum of 2, 7-9E-BHVC in DMSO-d_6



^{13}C NMR spectrum of 2,7-9E-BHVC in DMSO-d_6



IR spectrum of 2,7-9E-BHVC

2. Measurement equipments and materials

The UV-visible-near-IR absorption spectra of dilute solutions were recorded on a Cary 50 spectrophotometer using a quartz cuvette having 1 cm path length. One-photon fluorescence spectra were obtained on a HITACHI F-4500 spectrofluorimeter equipped with a 450-W Xe lamp. Two-photon fluorescence spectra were noted on a SpectroPro300i and the pump laser beam comes from a mode-locked Ti:sapphire laser system at the pulse duration of 200 fs, with a repetition rate of 76 MHz (Coherent Mira900-D). The double-stranded DNA-specific dye DAPI was purchased from Molecular Probes. Calf thymus DNA and torula yeast RNA, which were used as the model of DNA and RNA, and ribonuclease A (RNase) were obtained from Sigma.

3. Measurement of TPA cross sections

TPA cross sections have been measured using the two-photon induced fluorescence method[1,2]. Fluorescein (pH = 11, cyan diamond) in aqueous NaOH was used as the standard, whose two-photon properties have been well characterized in the literature[3], and thus cross sections can be calculated by means of equation (1)

$$\delta_s = \delta_r \frac{\Phi_r}{\Phi_s} \frac{c_r}{c_s} \frac{n_r}{n_s} \frac{F_s}{F_r} \qquad (1)$$

where the subscripts *s* and *r* refer to the sample and the reference material, respectively. δ is the TPA cross sectional value, *c* is the concentration of the solution, *n* is the refractive index of the solution, *F* is the two-photon excited fluorescence integral intensity and Φ is the fluorescence quantum yield.

4. Cell culture and staining

HeLa and SiHa cancer cells and MS1 normal cells were cultured in Dulbecco's modified Eagle's medium supplemented with penicillin/streptomycin and 10% bovine calf serum in a 5% CO_2

incubator at 37°C. 2,7-9E-BHVC was dissolved in DMSO at a concentration of 1 mM and DAPI (Invitrogen) was prepared as 1 mM aqueous solution. Before staining experiments, cultured cells grown on glass coverslips were pretreated as the following procedure: cells were first fixed by 4% paraformaldehyde for 30 min and then permeabilized by 1% Trition X-100 for 2 min at ambient temperature.

For wide-field fluorescence microscopy and TPM imaging of cells stained with 2,7-9E-BHVC: pretreated HeLa, SiHa and MS1 cells were stained with 5 μM 2,7-9E-BHVC for 30 min at ambient temperature and then imaged with wide-field fluorescence microscopy or TPM.

For RNase digest test, two sets of pretreated HeLa cells were stained with 5 μM 2,7-9E-BHVC for 30 min. After rinsing with PBS twice, a total 1 ml PBS (as control experiment) was added into a set of cells and 25 mg/ml DNase-Free RNase (GE) was added into the other set of cells, and then two sets of cells were incubated at 37ºC in 5% CO_2 for 2 hr. After rinsing with PBS twice, both two sets of cells were imaged with wide-field fluorescence microscopy. In addition, the RNase digest test of cells stained with 1 μM DAPI was also carried out for comparison experiment.

For cells counterstain experiment with 2,7-9E-BHVC and DAPI: pretreated Hela cells were stained with 5 μM 2,7-9E-BHVC for 30 min. After rinsing with PBS twice, the same sample was stained with 1 μM DAPI for 30 min and then imaged with TPM.

5. Wide-field fluorescence microscopy imaging and two-photon fluorescence microscopy imaging experiment

wide-field fluorescence microscopy images were acquired with an Olympus IX71 inverted microscope coupling with a CCD and display controller software. The fluorescence of

2,7-9E-BHVC and DAPI were excited and collected through U-MNIBA3 and U-MWU2, respectively. All of TPM microscopic photos were obtained with Olympus FV 300 Laser Confocal System with a 60× water objective (N.A. =1.25) and photomultiplier tubes. A Ti:sapphire laser (Coherent) was used to excite specimen at 800 nm. The total power provided by laser source can maintain stable and the incident power was examined with Power Monitor (Coherent) directly. Fluorescence of 2,7-9E-BHVC was collected with a beam splitter DM570 and BA565IF long pass emission filter combination. Fluorescence of DAPI was collected with a beam splitter DM570 and BA430-470 band pass emission filter combination. The DIC image was taken with a 488 nm Arion laser.

6. Fig. S1

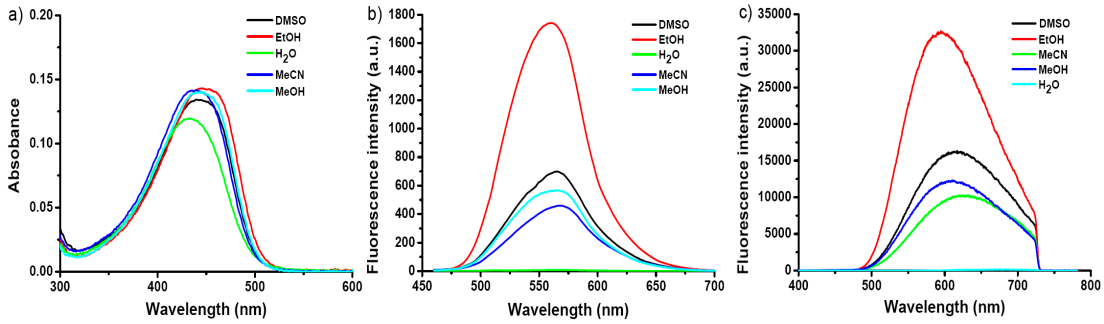


Fig. S1 (a) UV-vis absorption, (b) single-photon fluorescence and (c) two-photon fluorescence spectra of 2,7-9E-BHVC in various solvents. Concentration: 2×10^{-6} for (a) and (b), 1×10^{-5} for (c).

7. Table S1

Table S1 The photophysical properties of 2,7-9E-BHVC

	λ^1_{max}/nm	λ^2_{max}/nm	λ^3_{max}/nm	ε/M^{-1}·cm^{-1}	Φ/%	δ/GM	$\delta\times\phi$/GM
Buffer	434	567	655	6.10×10^4	0.04	4172	1.67
DNA	450	565	608	5.05×10^4	1.00	3156	31.56
RNA	452	567	605	4.95×10^4	1.00	2742	27.42

λ^1, λ^2 and λ^3 are linear absorption, single-fluorescent and two-photon fluorescent maximum peak respectively; ε is molar absorptivity; Φ is single-photon fluorescence quantum yield determined using fluorescein (Φ = 0.95) as the standard. δ is two-photon absorption cross sections determined using fluorescein (δ = 36 GM) as the standard at 800 nm. $\delta\times\phi$ is two-photon action absorption cross sections. 1GM = 10^{-50} cm^4·s·photon^{-1}; Concentration of samples for ϕ: 2×10^{-6} M; error limit: 10%. Concentration of samples for δ: 1×10^{-5} M; error limit: 30%. [NA]/[dyes] ratio: 100:1.

References

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