Supporting information for

A Side-Chain Engineering Strategy for Constructing Fluorescent Dyes with Direct and Ultrafast Self-Delivery to Living Cells

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Contents

Materials S3
Spectroscopic measurements S3
Calculation methods of molecular frontier orbitals S4
Cytotoxicity measurement S4
Cell culture and staining methods S4
Fluorescence imaging methods S5
Time-dependent dynamic analysis (TDDA) S5
Fluorescent recovery after photobleaching (FRAP) S5
Figure S1 S6
Table S1 S6
Figure S2, S3 S7
Figure S4, S5 S8
Figure S6, S7 S9
Figure S8, S9 S10
Figure S10, S11 S11
Figure S12, S13 S12
Figure S14, S15 S13
Figure S16, S17 S14
Figure S18, S19 S15
Figure S20, S21 S16
Figure S22 S17
Figure S23 S18
Figure S24-26 S19
Table S2 S20
Figure S27 S21
Figure S28, Table S3, Table S4 S22
References S22
Synthesis and characterization of SPs and 9E-BMVCs S23-S46
Materials
Unless otherwise stated, all solvents and reagents were commercially available used without further purification. Thin-layer chromatography (TLC) analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals. MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was from Sigma. MitoTracker® Deep Red FM (MTDR), Mito-Tracker Green (MTG), Mito-Tracker Red (MTR), Rhodamine123 (Rh123) were purchased from Molecular SPs.
The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AVANCE III 300 MHz or 400 MHz Digital NMR Spectrometer, and using DMSO-$d_6$ as solvent and tetramethylsilane (TMS) as internal reference respectively.

Spectroscopic measurements
The UV-visible-near-IR absorption spectra of dilute solutions were recorded on a Hitachi U-2910 spectrophotometer using a quartz cuvette of 1 cm path length. One-photon fluorescence spectra were obtained on a HITACH F-2700 spectrofluorimeter equipped with a 450-W Xe lamp. Two-photon fluorescence spectra were measured on a SpectroPro300i and the pump laser beam came from a mode-locked Ti: sapphire laser system at the pulse duration of 220 fs, a repetition rate of 76 MHz (Coherent Mira900-D).

The fluorescence quantum yields can be calculated by the following equation (1)\(^1\):

$$\Phi_s = \frac{A_r(\lambda_r) n_r^2 F_s}{A_s(\lambda_s) n_s^2 F_r}$$

(1)

Two-photon absorption cross sections are calculated by means of equation (2)\(^2\):

$$\delta_s = \frac{\Phi_r n_r c_r F_s}{\Phi_s n_s c_s F_r}$$

(2)

The subscripts s and r refer to the sample and the reference materials, respectively. $\Phi$ is the quantum yield, $F$ in equation (1) is the one-photon excited fluorescence integrated emission intensity, while in equation (2), it indicates the two-
photon excited fluorescence integral intensity. A stands for the absorbance, and n is the refractive index. \( \delta \) is the two-photon absorption cross-section value, c is the concentration of the solution. In this paper, fluorescein in aqueous NaOH (pH = 13) was selected as the reference. Its \( \Phi \) and \( \delta \) (excitation with 800 nm) are 0.93 and 36GM, respectively.\(^3\)

**Calculation methods of molecular frontier orbitals**

The geometrically optimized structure and the frontier orbitals of the probe molecules were calculated with Gaussian 09 package.\(^4\) Chemical structures were optimized sequentially with the basic set of PM3, B3LYP/3-21g, B3LYP/6-31g, and cam-B3LYP/TZVP. The frontier molecular orbitals were obtained via TD-DFT calculation of the single point of the optimized structure on the basic set of cam-B3LYP/TZVP TD.

**Cytotoxicity measurement**

The effects of **SPs** on cell viability were carried out using the methylthiazolyldiphenyl-tetrazolium bromide (MTT), purchased from Dojindo. SiHa cells growing in log phase were seeded into 96-well plates (ca. 1 × 10\(^4\) cells/well) and allowed to adhere for 24 h. **SPs** (100 \( \mu \)L/well) at different concentrations (200 nM and 1 nM) was added into the wells of the treatment group, and 100 \( \mu \)L/well DMSO diluted in DMEM at corresponding concentrations to the negative control group, respectively. The cells were incubated for 2, 10, and 24 h at 37 \( ^\circ \)C under 5% \( \text{CO}_2 \), then 10 \( \mu \)L of MTT was added into each well. After incubation for 4 h, the culture medium in each well was removed and DMSO (100 \( \mu \)L) was added to dissolve the purple crystals. After 20 min, the absorbance was measured at 492 nm with a microplate reader. Finally, the cell survival rate can be calculated using the following equation:

\[
\text{Survival rate} = \frac{(A_{\text{Sample}} - A_{\text{DMSO}})}{(A_{\text{Sample}} - A_{\text{Blank}})}
\]

**Cell culture and staining methods**
SiHa and HeLa cells were cultured in H-DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. Mesenchymal stem cells (MSC) were grown in alpha-MEM supplemented with 10% FBS and 1% penicillin and streptomycin. All above cells were cultured in a 5% CO$_2$ incubator at 37 °C. Before cell staining, all cells were placed on glass coverslips and allowed to adhere for 24 h.

For living cells staining experiment, adherent cells were stained with SPs or MTG or MTR (detailed staining concentration and time were illustrated in corresponding figure annotations), and then washed with unbounded SPs and imaged with fluorescence microscopy.

For co-staining experiments, cells were treated with MDTR (200 nM, 20 min) followed by rinsed with PBS twice and then stained with SPs (200 nM, 10 min), and then washed with unbounded SPs and imaged with fluorescence microscopy.

**Fluorescence imaging methods**

Confocal fluorescence imaging was obtained with Olympus FV 1200 laser confocal microscope. In two-photon experiments, excitation wavelength was 900 nm from a Ti:sapphire femtosecond laser source (Coherent Chamelon Ultra), and the incident power on samples was modified by means of an attenuator and examined with Power Monitor (Coherent). A multiphoton emission filter (FF01-750; Semrock) was used to block the IR laser.

**Time-dependent dynamic analysis (TDDA)**

Adherent cells were treated with different concentrations SPs or MTG or MTR, and then immediately observed by confocal microscopy without a washing step.

**Fluorescent recovery after photobleaching (FRAP)**

Adherent cells were pre-stained with 200 nM SPs for 10 min and washed with PBS twice, and then imaged by confocal microscopy. Next, keeping the imaging position unchanged, 200 nM SPs pre-dissolved in medium were added into the cells, and they were immediately observed under confocal microscopy without washing.
Figure S1. The frontier molecular orbital (a) and absorption spectra (b) of SP-1. Testing concentration: 10 μM.

Table S1 Optical properties of SP-1 compared with references (Rhodamin B and Fluorescein)

| Dyes     | Solvents | $\lambda_1^{\text{max}}$ | $\lambda_2^{\text{max}}$ | $\Phi$ (%) | $\delta$ | $\delta \times \Phi$ |
|----------|----------|--------------------------|--------------------------|------------|----------|---------------------|
| SP-1     | EtOH     | 590                      | 607                      | 0.40       | 315      | 1.26                |
|          | Ace      | 599                      | 623                      | 0.11       | 610      | 0.67                |
|          | DMF      | 599                      | 622                      | 0.20       | 291      | 0.58                |
|          | DMSO     | 608                      | 625                      | 0.38       | 144      | 0.54                |
|          | THF      | 580                      | 612                      | 1.04       | 51       | 0.53                |
|          | H$_2$O   | 586                      | 616                      | 0.04       | 1066     | 0.45                |
|          | MeOH     | 588                      | 617                      | 0.13       | 271      | 0.36                |
|          | Gly      | 589                      | 614                      | 7.79       | 114      | 8.86                |
| Rhodamin B | MeOH     | 567                      | 570                      | 59         | 13       | 7.67                |
| Fluorescein | NaOH     | 512                      | 513                      | 89         | 15       | 13.35               |

$\lambda_1^{\text{max}}$: the maximum OPEF wavelength, $\lambda_2^{\text{max}}$: the maximum TPEF wavelength (unit: nm); $\Phi$: fluorescence quantum yield; $\delta$: the two-photo absorption cross-section; $\delta \times \Phi$: the two-photon active absorption cross-section. The two-photon excitation wavelength: 900 nm.
Figure S2. $\delta \times \Phi$ of SP-1 in various solvents under different two-photon excitation wavelengths from 800 nm to 900 nm.

Figure S3. Naked-eye images of SP-1 under UV light with 365 nm excitation (a), OPEF (b), and TPEF (c) spectra in Gly-MeOH solvents with different viscosity (unit: cp). $\lambda_{\text{ex}}$ (OPEF) = 473 nm; $\lambda_{\text{ex}}$ (TPEF) = 900 nm. Concentration: 10 μM.
Figure S4. $^1$H NMR spectra in the low field of chemical shift of total SPs with DMSO-$d_6$ as the solvent.

Figure S5. The ORTEP drawing (a), unit cell (b), side view (c), top view (d), and intramolecular short-contact interactions (e) of SP-1. The distance between the adjacent parallel benzene rings: 3.175 Å. Dihedral angle between two aromatic rings marked by red: 11°.
Figure S6. The ORTEP drawing (a), unit cell (b), side view (c), top view (d), and intramolecular short-contact interactions (e) of SP-2. The distance between the adjacent parallel benzene rings: 3.374 Å. Dihedral angle between two aromatic rings marked by red: 16.38°.

Figure S7. The ORTEP drawing (a), unit cell (b), side view (c), top view (d), and intramolecular short-contact interactions (e) of SP-6. The distance of between the adjacent antiparallel benzene rings: 4.092 Å, the distance between the adjacent parallel benzene rings: 13.018 Å. Dihedral angle between two aromatic rings marked by red: 5.77°.
**Figure S8.** The anti-photobleaching results of **SPs** in two different solvents (a and b: MeOH, c and d: DMF) under continuous excitation with 473 nm laser. a and c: The normalized fluorescence intensity at different time; b and d: the fluorescence intensity ratios of **SPs** at 900 s relative to its original intensity.

**Figure S9.** MTT assay of SiHa cells after incubation with 200 nM **SPs** for 24 h.
Figure S10. (a) LSCM images of SiHa cells stained with SP-1, SP-6, SP-12, and SP-14 (200 nM, 10 min) at different incubation temperatures (37°C and 4°C), and the relative fluorescence intensity (b).

Figure S11. The absorption (a) and emission (b, c) spectra of SP-1 in mixture solvent of 70% Gly-30% MeOH as well as MTDR in DMSO. Concentration: 10 μM. $\lambda_{ex}$ (b) = 473 nm; $\lambda_{ex}$ (c) = 633 nm.
Figure S12. TDDA for diffusion dynamics of SPs in MSC. (a) LSCM images of cells stained with SPs (200 nM) at different time points (3, 25, and 40 min) and the corresponding DIC images. (b) Time-dependent fluorescence intensity of SPs recorded at different time points. (c) The fluorescence intensity plots of SPs over time. $\lambda_{ex} = 473$ nm, $\lambda_{em} = 550-650$ nm. Bar = 20 $\mu$m.

Figure S13. The calculation of delivery rate constants of SPs in MSC. (a) Top: the established physical model for the transport of SPs through the membrane; Bottom: the corresponding activation energy profile. (b) The experimental intracellular normalized fluorescence intensity curves of SPs (blue) at different normalized time as well as the corresponding fitting curves (red). (c) The calculated delivery rate constants of SPs. (d) The normalized delivery rate constants and FL intensity at 25 min of TDDA experiment (Figure S12b) of SPs.
Figure S14. LSCM imaging parameters (a) and images (b) of SiHa cells stained with SP-6 of different concentrations ranging from 200 nM to 1 nM. $\lambda_{ex} = 473$ nm; $\lambda_{em} = 550-650$ nm. Bar = 20 $\mu$m.

| Dye | Loading conditions | Percentage laser transmission | PMT gain | Offset |
|-----|--------------------|-----------------------------|----------|--------|
| SP-6 | 200 nM / 30 s     | 200 nM / 3 min              | 15%      | 600    | 10%    |
|     | 50 nM / 5 min     |                            |          |        |        |
|     | 10 nM / 10 min    |                            |          |        |        |
|     | 5 nM / 15 min     |                            |          |        |        |
|     | 1 nM / 20 min     |                            |          |        |        |

Figure S15. LSCM imaging parameters (a) and images (b) of SiHa cells stained with SP-8 of different concentrations ranging from 200 nM to 1 nM. $\lambda_{ex} = 473$ nm; $\lambda_{em} = 550-650$ nm. Bar = 20 $\mu$m.
Figure S16. LSCM imaging parameters (a) and images (b) of SiHa cells stained with SP-10 of different concentrations ranging from 200 nM to 1 nM. $\lambda_{\text{ex}} = 473$ nm; $\lambda_{\text{em}} = 550-650$ nm. Bar = 20 μm.

Figure S17. LSCM images of SiHa cells stained with 1 nM SPs for 30 min. $\lambda_{\text{ex}} = 473$ nm; $\lambda_{\text{em}} = 550-650$ nm. In the experiments, the image acquisition parameters were set to be consistent. Bar = 20 μm.
Figure S18. Time-dependent LSCM images (a) of SiHa cells stained with 1 nM SP-6, SP-8, and SP-10, as well as the corresponding normalized fluorescence intensity (b). $\lambda_{ex} = 473$ nm; $\lambda_{em} = 550-650$ nm. Bar = 20 μm.

Figure S19. Time-dependent LSCM images of SiHa cells stained with MTG of different concentrations (200 nM, 100 nM, 10 nM, and 1 nM). $\lambda_{ex} = 473$ nm; $\lambda_{em} = 500-600$ nm. Bar = 20 μm.
**Figure S20.** Time-dependent LSCM images of SiHa cells stained with MTR of different concentrations (200 nM, 100 nM, 10 nM, and 1 nM). $\lambda_{ex} = 543$ nm; $\lambda_{em} = 550$-650 nm. Bar = 20 $\mu$m.

**Figure S21.** LSCM images of different types of cells (HeLa and MSC) stained with 1 nM SP-6, SP-8, and SP-10 for 20 min. $\lambda_{ex} = 473$ nm; $\lambda_{em} = 550$-650 nm. Bar = 20 $\mu$m.
Figure S22. TPEF images of SiHa cells stained with 1 nM SP-6, SP-8, and SP-10. $\lambda_{\text{ex}} = 900$ nm; $\lambda_{\text{em}} = 570-630$ nm. Bar = 20 $\mu$m.
Figure S23. TPEF tomography images at different depths of rat skeletal muscle tissue stained with SP-1, SP-2, SP-3, SP-8, SP-10, SP-12, and SP-14 (10 μM, 30 min). $\lambda_{ex} = 900$ nm, $\lambda_{em} = 570-630$ nm. Bar = 20 μm.
**Figure S24.** TPEF images of mitochondria in zebrafish stained with SP-6 (10 μM, 30 min). (a) DIC image, (b) enlargement images of red box in (a), (c-i) tomography images at different depths from 0 μm to 86 μm. $\lambda_{ex} = 900$ nm, $\lambda_{em} = 570$-630 nm. Bar (a) = 500 μm, bar (b-i) = 100 μm.

**Figure S25.** TPEF images of mitochondria in zebrafish stained with SP-8 (10 μM, 30 min). (a) DIC image, (b) enlargement images of red box in (a), (c-i) tomography images at different depths from 0 μm to 70 μm. $\lambda_{ex} = 900$ nm, $\lambda_{em} = 570$-630 nm. Bar (a) = 500 μm, bar (b-i) = 100 μm.

**Figure S26.** TPEF images of mitochondria in zebrafish stained with SP-10 (10 μM, 30 min). (a) DIC image, (b) enlargement images of red box in (a), (c-i) tomography images at different depths from 0 μm to 66 μm. $\lambda_{ex} = 900$ nm, $\lambda_{em} = 570$-630 nm. Bar (a) = 500 μm, bar (b-i) = 100 μm.
### Table S2. Optical properties of 9E-BMVC derivatives (9E-BMVCs).

| Dye       | Solvent | $\lambda_{\text{abs}}$ | $\lambda_{\text{em}}$ | $\lg \varepsilon$ | $\Phi$  |
|-----------|---------|------------------------|------------------------|-------------------|--------|
| 9E-BMVC1  | DMSO    | 452                    | 557                    | 4.53              | 0.008  |
|           | MeOH    | 456                    | 558                    | 4.55              | 0.009  |
|           | Gly     | 460                    | 548                    | 4.55              | 0.068  |
| 9E-BMVC3  | DMSO    | 456                    | 560                    | 4.41              | 0.011  |
|           | MeOH    | 458                    | 560.5                  | 4.45              | 0.014  |
|           | Gly     | 462                    | 549.5                  | 4.48              | 0.064  |
| 9E-BMVC6  | DMSO    | 456                    | 563.5                  | 4.46              | 0.012  |
|           | MeOH    | 458                    | 557                    | 4.50              | 0.016  |
|           | Gly     | 464                    | 547.5                  | 4.49              | 0.069  |
| 9E-BMVC8  | DMSO    | 456                    | 564                    | 4.51              | 0.011  |
|           | MeOH    | 460                    | 559.5                  | 4.57              | 0.014  |
|           | Gly     | 464                    | 551                    | 4.48              | 0.079  |
| 9E-BMVC10 | DMSO    | 456                    | 560                    | 4.61              | 0.010  |
|           | MeOH    | 460                    | 560                    | 4.65              | 0.014  |
|           | Gly     | 464                    | 549.5                  | 4.59              | 0.065  |
| 9E-BMVC12 | DMSO    | 456                    | 561.5                  | 4.43              | 0.012  |
|           | MeOH    | 460                    | 557.5                  | 4.62              | 0.014  |
|           | Gly     | 464                    | 551                    | 4.59              | 0.077  |

$\lambda_{\text{abs}}$: the maximum emission wavelength, $\lambda_{\text{em}}$: the maximum emission wavelength (unit: nm); $\varepsilon$: the molar extinction coefficient; $\Phi$: the fluorescence quantum yield. The excitation wavelength: 473 nm.
**Figure S27.** LSCM images of SiHa cells stained with 5 μM 9E-BMVCs at different time (5-30 min). 

$\lambda_{\text{ex}} = 473$ nm, $\lambda_{\text{em}} = 500-600$ nm. Bar = 20 μm.
Figure S28. LSCM images of SiHa cells co-stained with 9E-BMVC6 and MTDR. (1): DIC, (2) 9E-BMVC6, (3) MTDR, (4) merged image of (2) and (3), (5) merged image of (1) - (3), (6) the normalized fluorescence profiles of 9E-BMVC6 and MTDR along the red line in (4). Bar = 20 μm.

Table S3. The ClogP values and the cell permeability of SP derivatives.

| Dye       | SP-1 | SP-2 | SP-3 | SP-6 | SP-8 | SP-10 | SP-12 | SP-14 | SP-15 | SP-16 | SP-18 | SP-22 |
|-----------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|
| ClogP     | -1.34| -0.81| -0.28| 1.30 | 2.38 | 3.42  | 4.48  | 5.53  | 6.06  | 6.59  | 7.65  | 9.77  |
| Cell permeability | ✓    | ✓    | ✓    | ✓    | ✓    | ✓     | ✓     | ✓     | ✓     | ✓     | ✓     | ✓     |

Table S4. The ClogP values and the cell permeability of 9E-BMVC derivatives.

| Dye       | 9E-BMVC1 | 9E-BMVC3 | 9E-BMVC6 | 9E-BMVC8 | 9E-BMVC10 | 9E-BMVC12 |
|-----------|----------|----------|----------|----------|-----------|-----------|
| ClogP     | -2.79    | -0.14    | 2.50     | 4.62     | 5.74      | 8.85      |
| Cell permeability | ×    | ✓        | ✓        | ×        | ×         | ×         |

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S22
The synthetic steps and structural characterization of SPs and 9E-BMVC variants.

Scheme S1 The synthesis routine of SPs.

![Reaction Scheme](attachment:image.png)

The mixture comprised of 4-methylpyridine (1 mL, 11 mmol) and iodoalkane or bromoalkane (11 mmol) was added into a flask, with ethyl alcohol (5 mL) as the solvent. Next, the reaction system was stirred for 24 h at 85 °C. After that, adding 4-(dimethylamino)benzaldehyde (1.53 ml, 11 mmol) and 200uL piperidine into this mixture with stirring at 85 °C for 24 h. After being cooled to room temperature, the precipitate was washed with little ethyl alcohol two times and then petroleum ether three times. Red power product was obtained after the residue was recrystallized from ethyl alcohol, with a yield of 80%.

For SP-1, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.68 (d, $J = 6.8$ Hz, 2H), 8.04 (d, $J = 6.8$ Hz, 2H), 7.90 (d, $J = 16$ Hz, 1H), 7.59 (d, $J = 8.8$ Hz, 2H), 7.17 (d, $J = 16$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.17 (s, 3H), 3.02 (s, 6H). HR-MS calculated for C$_{16}$H$_{19}$N$_2^+$ m/z 239.34, found 239.15.

For SP-2, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.79 (d, $J = 6.8$ Hz, 2H), 8.06 (d, $J = 6.8$ Hz, 2H), 7.93 (d, $J = 16$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.18 (d, $J = 16$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.51 (t, $J = 2.4$ Hz, 2H), 3.02 (s, 6H), 1.50 (t, $J = 7.4$ Hz, 3H). HR-MS calculated for C$_{17}$H$_{21}$N$_2^+$ m/z 253.37, found 253.17.

For SP-3, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.77 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 7.2$ Hz, 2H), 7.93 (d, $J = 16$ Hz, 1H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.18 (d, $J = 16$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.39 (t, $J = 7.4$ Hz, 2H), 3.02 (s, 6H), 1.90 (q, $J = 7.3$ Hz, 2H), 0.89 (t, $J = 7.2$ Hz, 3H). HR-MS calculated for C$_{18}$H$_{23}$N$_2^+$ m/z 267.40, found 267.19.

For SP-6, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.78 (d, $J = 6.8$ Hz, 2H), 8.08 (d, $J = 6.8$ Hz, 2H), 7.95 (d, $J = 16.0$ Hz, 1H), 7.61 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 16.4$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.42 (t, $J = 7.2$ Hz, 2H), 3.03 (s, 6H), 1.88 (t, $J = 6.6$ Hz, 2H), 1.28 (s, 6H), 0.86 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for
C$_{21}$H$_{29}$N$_2^+$ m/z 309.48, found 309.23.

For **SP-8**, $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 8.78 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.94 (d, $J = 16.0$ Hz, 1H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.19 (d, $J = 16.4$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.41 (t, $J = 7.2$ Hz, 2H), 3.03 (s, 6H), 1.89 (t, $J = 6.4$ Hz, 2H), 1.26 (s, 10H), 0.86 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for C$_{23}$H$_{33}$N$_2^+$ m/z 337.53, found 337.27.

For **SP-10**, $^1$H NMR (300 MHz, DMSO-$d_6$): δ (ppm) 8.78 (d, $J = 6.9$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.94 (d, $J = 16.0$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 16.0$ Hz, 1H), 6.79 (d, $J = 9.2$ Hz, 2H), 4.41 (t, $J = 7.4$ Hz, 2H), 3.03 (s, 6H), 1.88 (t, $J = 6.6$ Hz, 2H), 1.27 (s, 14H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for C$_{25}$H$_{37}$N$_2^+$ m/z 365.58, found 365.30.

For **SP-12**, $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 8.78 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.94 (d, $J = 16.0$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.18 (d, $J = 16.0$ Hz, 1H), 6.80 (d, $J = 9.2$ Hz, 2H), 4.41 (t, $J = 7.4$ Hz, 2H), 3.03 (s, 6H), 1.88 (t, $J = 6.6$ Hz, 2H), 1.25 (s, 18H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for C$_{27}$H$_{41}$N$_2^+$ m/z 393.64, found 393.33.

For **SP-14**, $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 8.79 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.93 (d, $J = 16$ Hz, 1H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.18 (d, $J = 16$ Hz, 1H), 6.80 (d, $J = 8.8$ Hz, 2H), 4.41 (t, $J = 7.2$ Hz, 2H), 3.03 (s, 6H), 1.87 (t, $J = 6.4$ Hz, 2H), 1.25 (s, 22H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for C$_{29}$H$_{45}$N$_2^+$ m/z 421.69, found 421.36.

For **SP-15**, $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 8.78 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.93 (d, $J = 16.4$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.18 (d, $J = 16$ Hz, 1H), 6.79 (d, $J = 9.2$ Hz, 2H), 4.41 (t, $J = 7.4$ Hz, 2H), 3.03 (s, 6H), 1.87 (t, $J = 6.4$ Hz, 2H), 1.22 (s, 24H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for C$_{30}$H$_{47}$N$_2^+$ m/z 435.72, found 435.37.

For **SP-16**, $^1$H NMR (400 MHz, DMSO-$d_6$): δ (ppm) 8.78 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 6.8$ Hz, 2H), 7.94 (d, $J = 16.0$ Hz, 1H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.18 (d, $J = 16.0$ Hz, 1H), 6.80 (d, $J = 9.2$ Hz, 2H), 4.41 (t, $J = 7.2$ Hz, 2H), 3.03 (s, 6H), 1.88 (t, $J = 6.6$ Hz, 2H), 1.24 (s, 26H), 0.85 (t, $J = 7.0$ Hz, 3H). HR-MS calculated for
$C_{30}H_{47}N_2^+$ m/z 449.75, found 449.39.

For SP-18, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.77 (d, $J = 6.8$ Hz, 2H), 8.06 (d, $J = 7.2$ Hz, 2H), 7.92 (d, $J = 16.4$ Hz, 1H), 7.60 (d, $J = 9.2$ Hz, 2H), 7.17 (d, $J = 16.0$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.40 (t, 2H), 3.04 (s, 6H), 1.88 (t, $J = 6.4$ Hz, 2H), 1.25 (s, 32H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for $C_{33}H_{53}N_2^+$ m/z 477.80, found 477.42.

For SP-22, $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm) 8.77 (d, $J = 6.8$ Hz, 2H), 8.06 (d, $J = 6.8$ Hz, 2H), 7.92 (d, $J = 16.0$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 2H), 7.17 (d, $J = 16.4$ Hz, 1H), 6.79 (d, $J = 8.8$ Hz, 2H), 4.40 (t, $J = 14.4$ Hz, 2H), 3.04 (s, 6H), 1.87 (t, $J = 6.8$ Hz, 2H), 1.25 (s, 40H), 0.85 (t, $J = 6.8$ Hz, 3H). HR-MS calculated for $C_{37}H_{61}N_2^+$ m/z 533.91, found 533.48.

**Scheme S2** The synthesis routine of 9E-BMVCs.

3,6-dibromo-9-(2-ethyl)-carbazole (1): 3 g KOH was initially added into a 250 mL flask, then 30 mL DMF was slowly poured in. After that the mixture has been stirred up to 10 min, 2 g 3,6- Dibromo-9H-carbazole was carefully added. Then the mixture was stirred for 30 min, and 1.38 mL (12.3 mmol) bromoethane was subsequently added. The resulting system was then stirred for over 18 h to finish the reaction at room temperature. After that, the reaction solution was poured into 400 mL water and then extracted with 400 mL dichloromethane. The organic compartment was then dried with 4 g anhydrous $Na_2SO_4$. Further removing of the
solvents could give the final products of white powder, and the yield is 83%. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.48 (dd, $J = 1.8$, 0.7 Hz, 2H), 7.62 (m, 4H), 4.44 (q, $J = 7.1$ Hz, 2H), 1.28 (t, $J = 7.1$ Hz, 3H).

9-(2-ethyl)-3,6-bis((E)-2-(pyridin-4-yl)vinyl)-carbazole (2): 1 g (2.83 mmol) of compound 1, 0.0283 g (0.126 mmol) of palladium acetate and 0.115 g (0.378 mmol) of tri(o-tolyl)phosphine were firstly added into a three-necked flask. Then, 20 mL DMF was added to the flask, and the system was subsequently stirred for 5 min before the addition of 5 mL trimethylamine and 1.08 mL (10.1 mmol) 4-vinyl pyridine. The solution was then stirred and bubbled with nitrogen for more than 30 min. The system was consequently heated to 95°C and stirred for at least 48 h under the protection of nitrogen to complete the reaction. The reaction solution was then poured into 400 mL water and then extracted with 400 mL dichloromethane. The organic compartment was then dried with 4 g anhydrous Na$_2$SO$_4$. The final products were purified with column chromatography separation method as yellow powder, and the yield is 60%. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.55 (d, $J = 6.0$ Hz, 4H), 8.52 (s, 2H), 7.84 (d, $J = 1.5$ Hz, 2H), 7.76 (m, 4H), 7.59 (t, $J = 6.0$ Hz, 4H), 7.28 (d, $J = 16.5$ Hz, 2H), 4.50 (q, $J = 7.0$ Hz, 2H), 1.35 (t, $J = 6.9$ Hz, 3H).

9-(2-ethyl)-3,7-bis(1-methyl-4-pyridinium) carbazole diiodide (9E-BHVC1): 0.2 g (0.499 mmol) of compound 2 was firstly added into a three-necked flask with 20 mL ethanol. The mixture was then stirred evenly before the addition of 2.5 mmol RI. The solution was then stirred for more than 10 min and heated to 80 °C. The reaction was finished for at least 36 h when red powder was precipitated. Then the solution was cooled down to room temperature and filtered, the solids was washed with ethanol for three times as crude product. The final product was further purified by recrystallization in ethanol as red powder, and the yield was 82%. The synthesis routes of other 9E-BHVCs were similar.

For 9E-BMVC1, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.84 (d, $J = 6.6$ Hz, 4H), 8.63 (s, 2H), 8.23 (m, 6H), 7.97 (dd, $J = 8.6$, 1.7 Hz, 2H), 7.82 (d, $J = 8.7$ Hz, 2H), 7.58 (d, $J = 16.2$ Hz, 2H), 4.55 (q, $J = 7.0$ Hz, 2H), 4.26 (s, 6H), 1.38 (t, $J = 7.1$ Hz, 3H). HR-MS calculated for C$_{30}$H$_{29}$N$_3^{2+}$ m/z 215.79, found 215.62.
For **9E-BMVC3**, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.93 (d, $J = 6.7$ Hz, 4H), 8.63 (s, 2H), 8.26 (m, 6H), 7.98 (dd, $J = 8.7$, 1.7 Hz, 2H), 7.84 (d, $J = 8.6$ Hz, 2H), 7.59 (d, $J = 16.2$ Hz, 2H), 4.60 (m, 2H), 4.47 (d, $J = 7.3$ Hz, 4H), 1.96 (t, $J = 7.3$ Hz, 4H), 1.39 (t, $J = 7.1$ Hz, 3H), 0.93 (t, $J = 7.4$ Hz, 6H). HR-MS calculated for C$_{34}$H$_{37}$N$_3$$_{2}^{+}$ m/z 243.84, found 243.65.

For **9E-BMVC6**, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.93 (d, $J = 6.7$ Hz, 4H), 8.63 (s, 2H), 8.26 (m, 6H), 7.98 (dd, $J = 8.7$, 1.7 Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H), 7.58 (d, $J = 16.4$ Hz, 2H), 4.50 (m, 6H), 1.93 (t, $J = 7.3$ Hz, 4H), 1.39 (t, $J = 7.1$ Hz, 3H), 1.31 (s, 12H), 0.87 (m, 6H). HR-MS calculated for C$_{40}$H$_{49}$N$_3$$_{2}^{+}$ m/z 285.92, found 285.71.

For **9E-BMVC8**, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.94 (d, $J = 6.6$ Hz, 4H), 8.64 (s, 2H), 8.25 (m, 6H), 7.98 (dd, $J = 8.7$, 1.7 Hz, 2H), 7.83 (d, $J = 8.7$ Hz, 2H), 7.59 (d, $J = 16.2$ Hz, 4H), 4.50 (m, 6H), 1.92 (t, $J = 7.2$ Hz, 4H), 1.38 (t, $J = 7.1$ Hz, 3H), 1.29 (m, 20H), 0.88 (m, 6H). HR-MS calculated for C$_{44}$H$_{57}$N$_3$$_{2}^{+}$ m/z 313.98, found 313.99.

For **9E-BMVC10**, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.94 (d, $J = 6.6$ Hz, 4H), 8.63 (s, 2H), 8.26 (m, 6H), 7.98 (dd, $J = 8.7$, 1.6 Hz, 1H), 7.84 (d, $J = 8.7$ Hz, 2H), 7.59 (d, $J = 16.2$ Hz, 2H), 4.52 (m, 6H), 1.93 (d, $J = 8.0$ Hz, 4H), 1.39 (t, $J = 7.1$ Hz, 3H), 1.30 (m, 28H), 0.8 (m, 6H). HR-MS calculated for C$_{48}$H$_{65}$N$_3$$_{2}^{+}$ m/z 342.03, found 341.78.

For **9E-BMVC12**, $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 8.93 (d, $J = 6.6$ Hz, 4H), 8.63 (s, 2H), 8.23 (m, 6H), 7.97 (dd, $J = 8.7$, 1.6 Hz, 2H), 7.83 (d, $J = 8.7$ Hz, 2H), 7.59 (d, $J = 16.1$ Hz, 2H), 4.50 (m, 6H), 1.92 (t, $J = 7.1$ Hz, 4H), 1.38 (t, $J = 7.2$ Hz, 3H), 1.29 (m, 36H), 0.84 (m, 6H). HR-MS calculated for C$_{52}$H$_{73}$N$_3$$_{2}^{+}$ m/z 370.09, found 369.83.
$^1$H NMR spectrum of SP-1 in DMSO-$d_6$.

HRMS spectrum of SP-1 in MeOH.
$^1$H NMR spectrum of SP-2 in DMSO-$d_6$.

HRMS spectrum of SP-2 in MeOH.
$^1$H NMR spectrum of **SP-3** in DMSO-$d_6$.

HRMS spectrum of **SP-3** in MeOH.
$^1$H NMR spectrum of SP-6 in DMSO-$d_6$.

HRMS spectrum of SP-6 in MeOH.
$^1$H NMR spectrum of \textbf{SP-8} in DMSO-$d_6$.

HRMS spectrum of \textbf{SP-6} in MeOH.
$^1$H NMR spectrum of **SP-10** in DMSO-$d_6$.

HRMS spectrum of **SP-10** in MeOH.
\(^1\)H NMR spectrum of SP-12 in DMSO-\(d_6\).

HRMS spectrum of SP-12 in MeOH.
$^1$H NMR spectrum of **SP-14** in DMSO-$d_6$.

HRMS spectrum of **SP-14** in MeOH.
$^1$H NMR spectrum of SP-15 in DMSO-$d_6$.

HRMS spectrum of SP-15 in MeOH.
$^1$H NMR spectrum of SP-16 in DMSO-$d_6$.

HRMS spectrum of SP-16 in MeOH.
$^1$H NMR spectrum of SP-18 in DMSO-$d_6$.

HRMS spectrum of SP-18 in MeOH.
$^1$H NMR spectrum of SP-22 in DMSO-$d_6$.

HRMS spectrum of SP-22 in MeOH.
$^1$H NMR spectrum of 1 in DMSO-$d_6$.

$^1$H NMR spectrum of 2 in DMSO-$d_6$. 
$^1$H NMR spectrum of 9E-BMVC1 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC1 in MeOH.
$^1$H NMR spectrum of 9E-BMVC3 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC3 in MeOH.
$^1$H NMR spectrum of 9E-BMVC6 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC6 in MeOH.
$^1$H NMR spectrum of 9E-BMVC8 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC8 in MeOH.
$^1$H NMR spectrum of 9E-BMVC10 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC10 in MeOH.
$^1$H NMR spectrum of 9E-BMVC12 in DMSO-$d_6$.

HRMS spectrum of 9E-BMVC12 in MeOH.