Electronic Supplementary Material (ESI) for Integrative Biology

Supplemental Information

Chemical principles for a novel fluorescent probe with high cancer-targeting selectivity and sensitivity

Chi-Chih Kang,[a,b] Wei-Chun Huang,[a,b] Chiung-Wen Kouh,[a,c] Zi-Fu Wang,[a,d] Chih-Chien Cho,[a] Cheng-Chung Chang,[a,e] Chiung-Lin Wang,[a] Ta-Chau Chang,[*a,c,d] Joachim Seemann,[f] Lily Jun-shen Huang,[f]

Supplemental Experimental Procedures

1. Synthesis

General procedural A: Synthesis methods of BrMVC is similar to the synthesis of BMVC [1] through Heck reaction, **Supplementary Scheme 1**.

For BrMVC, the 3,6-Dibromocarbazole (10 mmole, Aldrich) was added into a high pressure bottle containing the mixture of Palladium(II) acetate (15 mg, Strem) and tri-*o*-tolyl phosphine (150 mg, Aldrich), then to which was added the solvent pair (triethylamine 15 ml / acetonitrile 45 ml) and 4-vinylpyridine (10 mmole, Merck). The bottle was sealed after bubbling 10 min with nitrogen. After keeping the system under ~ 105 ^{o}C for two days, the precipitant was collected and then extracted with H_2O/CH_2Cl_2 twice. The insoluble solids in CH_2Cl_2 layer were filtered and collected, washed with hot THF and then dried by $MgSO_4$. Crude powders, $\mathbf{A_2}$, the precursor of BrMVC, were purified by flash column chromatography with acetone/n-hexane as eluent gradient. The orange-red powders (BrMVC) were collected in very good yield after refluxing the compound $\mathbf{A_2}$ with excess CH_3I in DMF, and characterized by NMR and EA methods.

Data for : 3-(1-methyl-4-vinylpyridium iodide)-6-bromo-9*H*-carbazole (BrMVC)

The orange-red powder (Yield: 52%, mp > 300^{o}C) was collected. ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.78 (d, *J* = 5.6Hz, 2H), 8.59 (s, H), 8.36 (s, H), 8.16 (d, *J* = 5.2Hz, 2H), 8.15 (d, *J* = 16.0Hz, H), 7.83 (d, *J* = 8.4Hz, H), 7.58 (d, *J* = 8.4Hz, H), 7.55 (dd, *J* = 2.0,8.4Hz, H), 7.49 (d, *J* = 15.6Hz, H), 7.47 (d, *J* = 8.8Hz, H), 4.22 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 175.56, 153.38, 145.23, 142.62, 141.96, 139.43, 129.09, 127.35, 126.91, 124.73, 123.37, 123.29, 122.47, 121.93, 120.63, 113.89, 112.36, 111.85, 99.62, 47.11. EA ($C_{20}H_{16}BrIN_2$): calc. (obs. %) C: 48.91 (48.84), H: 3.28 (3.22), N: 5.70 (5.58).

General procedural B: The syntheses of 9-substituted BMVC derivatives were shown in **Supplementary Scheme 2**. Briefly; Compounds **B2** were synthesized from 3,6-dibromocarbazole

(**1,** 2 g, 6.15 mmole, Aldrich) through 9-position substituting by sodium hydride (0.295 g, 12.3 mmole, Aldrich) in DMF (20 ml) under nitrogen. Dibromo alkanes (Br-R-Br, 100 mmole) were then added and the mixture was refluxed for 12 hours. After cooling and quenching the waste sodium hydride with methanol, the solution was extracted with H_2O/ethyl acetate twice and the organic layer dried by $MgSO_4$. The products (**B2a-B2e**) were collected by flash column (silica, hexane/ethyl acetate. 2/1, v/v). The piperidine terminates compound **B3** was convenient obtain by way of refluxing **B2** (5.0 mmole) and piperidine (0.5 ml, Aldrich) in ethanol (20 ml) for 6 hours with trace of NaI. The solvent was evaporated in vacuum and the residue purified via column chromatography (silica, hexane/ethyl acetate. 1/2, v/v) to collect the yellow products (**B3a-B3e**). Then the reactants **B3** could couple with 4-vinylpyridine at mixed powders of Palladium (II) acetate/tri-*o*-tolylphosphine under the triethylamine/acetonitrile solvent pairs in high-pressure system. Compounds **B4** were obtained in good yield after extraction and crystallization (acetone) procedures followed the description in procedure A **(Supplementary Scheme 1)**. The orange-red powders (9-substituted BMVC derivatives) were collected in very good yield after refluxing the compound **B4** with excess CH_3I in DMF.

Data for 3,6-Dibromo-9-(1-bromobutyl) carbazole (B2a) (Yield: 58%, Mw: 462.02),^{1}H NMR ($CDCl_3$) δ: 8.07 (d, *J* = 1.8Hz, 2H), 7.55 (dd, *J* = 1.8,8.4Hz 2H), 7.21 (d, *J* = 8.4Hz, 2H), 4.20 (t, *J* = 6.2Hz, 2H), 3.35 (t, *J* = 6.6Hz, 2H), 1.95 (m, 2H), 1.84 (m, 2H).

3,6-Dibromo-9-(1-(piperidin-1-yl)butyl) carbazole (B3a) (Yield: 92%, Mw: 464.03), ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.86 (d, *J* = 1.89Hz, 2H), 7.35 (dd, *J* = 1.8,8.7Hz, 2H), 7.03 (d, *J* = 8.7Hz, 2H), 3.95 (t, *J* = 7.2Hz, 2H), 2.15 (m, 4H), 2.10 (t, *J* = 7.5Hz, 2H), 1.63 (t, *J* = 7.2Hz, 2H), 1.44 (m, 4H), 1.36 (m, 2H), 1.32 (m, 2H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)butyl) carbazole (B4a) (Yield: 71%, Mw: 512.69), ^{1}H NMR (300MHz, $CDCl_3$) δ: 8.59 (d, *J* = 4.5Hz, 4H), 8.29 (d, *J* = 1.2Hz, 2H), 7.70 (dd, *J* = 1.2,8.7Hz, 2H), 7.52 (d, *J* = 16.2Hz, 2H), 7.43 (d, *J* = 9.0Hz, 2H), 7.40 (d, *J* = 4.8Hz, 4H), 7.06 (d, *J* = 16.2Hz, 4H), 4.35 (t, *J* = 7.2Hz, 2H), 2.32 (m, 8H), 1.93 (t, *J* = 7.5Hz, 2H), 1.56 (m, 4H), 1.44

(m, 2H). EA ($C_{35}H_{36}N_4$): calc. (obs. %) C: 81.99 (81.84), H: 7.08 (7.06), N: 10.93 (10.90).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-piperidinium iodide) butyl) carbazole (BMVC-4C) (Yield: 92%), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.83 (d, *J* = 6Hz, 4H), 8.62 (s, 2H), 8.23 (d, *J* = 12.4Hz, 2H), 8.20 (d, *J* = 4.4Hz, 4H), 7.96 (d, *J* = 8.4Hz, 2H), 7.82 (d, *J* = 8.4Hz, 2H), 7.57 (d, *J* = 15.6Hz, 2H), 4.52 (t, 2H), 4.25 (s, 6H), 2.94 (s, 3H), 2.25 (t, 4H), 2.21 (t, 2H), 1.84 (m, 4H), 1.53 (m, 4H), 1.48 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.26, 145.18, 142.14, 127.63, 127.33, 123.30, 123.13, 121.66, 120.99, 111.09, 60.46, 47.17, 25.98, 20.99, 19.64, 19.16. EA ($C_{38}H_{45}I_3N_4 \cdot 0.5H_2O$): calc. (obs. %) C: 48.17 (48.03), H: 4.89 (4.91), N: 5.91 (5.86).

Data for 3,6-Dibromo-9-(1-bromopentyl) carbazole (B2b) (Yield: 55%, Mw: 474.03), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.10 (d, *J* = 2Hz, 2H), 7.53 (dd, *J* = 2.0,8.8Hz, 2H), 7.24 (d, *J* = 8.4Hz, 2H), 4.19 (t, *J* = 7.2Hz, 2H), 3.37 (t, *J* = 6.8Hz, 2H), 1.95 (m, 2H), 1.84 (m, 2H), 1.35 (m, 2H).

3,6-Dibromo-9-(1-(piperidin-1-yl)pentyl) carbazole (B3b) (Yield: 85%, Mw: 478.26), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.06 (d, *J* = 2Hz, 2H), 7.51 (dd, *J* = 8.4,2.0Hz, 2H), 7.19 (d, *J* = 8.8Hz, 2H), 4.18 (t, *J* = 7.2Hz, 2H), 2.34 (m, 4H), 2.23 (t, *J* = 8.0Hz, 2H), 1.81 (t, *J* = 8.0Hz, 2H), 1.59 (m, 4H), 1.53 (m, 2H), 1.42 (m, 2H), 1.31 (m, 2H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)pentyl) carbazole (B4b) (Yield: 68%, Mw: 526.71), ^{1}H NMR (400MHz, DMSO-d6) δ: 8.53 (d, *J* =4.0Hz, 4H), 8.49 (d, *J* = 2Hz, 2H), 7.78 (dd, *J* = 2.0,8.8Hz, 2H), 7.69 (d, *J* = 16Hz, 2H), 7.64 (d, *J* = 8.4Hz, 2H), 7.55 (d, *J* = 4.8Hz, 4H), 7.24 (d, *J* = 16Hz, 4H), 4.42 (t, *J* = 7.2Hz, 2H), 2.32 (m, 4H), 1.79 (t, *J* = 7.2Hz, 2H), 1.47 (m, 8H), 1.36 (m, 2H) , 1.28 (m, 2H). EA ($C_{36}H_{38}N_4$): calc. (obs. %) C: 82.09 (82.11), H: 7.27 (7.24), N: 10.64 (10.56).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-piperidinium iodide) pentyl) carbazole (BMVC-5C) (Yield: 88%, mp > 300^{o}C, Mw: 952.53), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.80 (d, *J* = 6Hz, 4H), 8.62 (s, 2H), 8.23 (d, *J* = 16Hz, 2H), 8.20 (d, *J* = 4.4Hz, 4H), 7.93 (d, *J* = 8.8Hz, 2H), 7.80(d, *J* = 8.4Hz, 2H), 7.53 (d, *J* = 16Hz, 2H), 4.52 (t, 2H), 4.25 (s, 6H), 3.26 (m, 6H), 1.89 (m, 2H), 1.73 (m, 6H), 1.51 (m, 2H), 1.34 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ :

153.30, 145.23, 142.66, 142.26, 127.61, 127.19, 123.35, 123.12, 121.56, 121.03, 111.46, 70.46, 70.03, 69.86, 69.80, 69.23, 63.86, 61.22, 47.19, 20.87, 19.69. EA ($C_{39}H_{47}I_3N_4 \cdot 0.5H_2O$): calc. (obs. %) C: 48.72 (48.59), H: 5.03 (5.02), N: 5.83 (5.75).

Data for 3,6-Dibromo-9-(1-bromooctyl) carbazole (B2c) (Yield: 56%, Mw: 516.11), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 2.0Hz, 2H), 7.50 (dd, *J* = 2.0,8.8 Hz, 2H), 7.23 (d, *J* = 8.4Hz, 2H), 4.21 (t, *J* = 7.2Hz, 2H), 3.35 (t, *J* = 7.2Hz, 2H), 1.81 (m, 4H), 1.34 (m, 2H) , 1.27 (m, 2H), 1.22 (m, 4H).

3,6-Dibromo-9-(1-(piperidin-1-yl)octyl) carbazole (B3c) (Yield: 90%, Mw: 520.34), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.07 (d, *J* = 2.0Hz, 2H), 7.51 (dd, *J* = 2.0,8.4Hz, 2H), 7.19 (d, *J* = 8.4Hz, 2H), 4.18 (t, *J* = 7.6Hz, 2H), 2.34 (m, 4H), 2.25 (t, *J* = 8.4Hz, 2H), 1.80 (t, *J* = 8.4Hz, 2H), 1.62 (m, 4H), 1.51 (m, 4H), 1.42 (m, 4H), 1.27 (m, 4H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)octyl) carbazole (B4c) (Yield: 68%, Mw: 568.79), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.55 (d, *J* =4.4 Hz, 4H), 8.51 (d, *J* =2 Hz, 2H), 7.77 (dd, *J* = 2.0, 8.8 Hz, 2H), 7.68 (d, *J* = 16 Hz 2H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.57 (d, *J* =4.4 Hz, 4H), 7.23 (d, *J* =16 Hz, 4H), 4.41 (t, *J* = 7.2 Hz, 2H), 2.38 (m, 4H), 2.25(t, *J* = 7.6 Hz, 2H), 1.76 (t, *J* = 7.2 Hz, 2H), 1.40 (m, 4H), 1.32 (m, 4H) , 1.25 (m, 4H) , 1.13 (m, 4H). EA ($C_{39}H_{44}N_4$): calc. (obs. %) C: 82.35 (82.21), H: 7.80 (7.72), N: 9.85 (9.79).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-piperidinium iodide) octyl) carbazole (BMVC-8C) (Yield: 90%, mp > 300^{o}C), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.82 (d, *J* = 6.4Hz, 4H), 8.63 (s, 2H), 8.20 (d, *J* = 6.4Hz, 4H), 8.19 (d, *J* = 16Hz, 2H), 7.92 (d, *J* = 8.4Hz, 2H), 7.77 (d, *J* = 8.4Hz, 2H), 7.55 (d, *J* = 16Hz, 2H) 4.48 (t, 2H), 4.24 (s, 6H), 3.25 (m, 6H), 2.94 (s, 3H),1.80 (m, 2H), 1.73 (m, 4H), 1.59 (m, 2H), 1.50 (m, 2H), 1.30 (m, 4H), 1.24 (m, 4H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.29, 145.26, 142.35, 142.28, 127.53, 127.30, 123.34, 123.07, 121.66, 121.02, 111.12, 60.32, 47.18, 28.98, 28.84, 26.75, 26.16, 21.27, 21.07, 19.66. EA ($C_{42}H_{53}I_3N_4 \cdot H_2O$): calc. (obs. %) C: 49.82 (49.73), H: 5.47 (5.43), N: 5.53 (5.45).

Data for 3,6-Dibromo-9-(1-bromononyl) carbazole (B2d) (Yield: 57%, Mw: 530.13), ^{1}H NMR

(400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 2.0Hz, 2H), 7.51 (dd, *J* = 2.0,8.8 Hz, 2H), 7.24 (d, *J* = 8.4Hz, 2H), 4.20 (t, *J* = 7.2Hz, 2H), 3.35 (t, *J* = 7.2Hz, 2H), 1.80 (m, 4H), 1.35 (m, 2H), 1.28 (m, 4H), 1.22 (m, 4H).

3,6-Dibromo-9-(1-(piperidin-1-yl)nonyl) carbazole (B3d) (Yield: 90%, Mw: 520.34), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 2.0Hz, 2H), 7.51 (dd, *J* = 2.0,8.8 Hz, 2H), 7.20 (d, *J* = 8.8Hz, 2H), 4.18 (t, *J* = 7.2Hz, 2H), 2.36 (m, 4H), 2.27 (t, *J* = 8.0Hz, 2H), 1.81 (t, *J* = 8.0Hz, 2H), 1.62 (m, 4H), 1.52 (m, 4H), 1.42 (m, 4H), 1.29 (m, 6H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)nonyl) carbazole (B4d) (Yield: 64%, Mw: 582.82), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.54 (d, *J* = 4.0Hz, 4H), 8.49 (d, *J* = 2.0Hz, 2H), 7.78 (dd, *J* = 2.0,8.8Hz, 2H), 7.68 (d, *J* = 16Hz, 2H), 7.64 (d, *J* = 8.4Hz, 2H), 7.56 (d, *J* = 4.8Hz, 4H), 7.23 (d, *J* = 16Hz, 4H), 4.42 (t, *J* = 7.2Hz, 2H), 2.38 (m, 4H), 2.25(t, *J* = 7.6Hz, 2H), 1.77 (t, *J* = 7.2Hz, 2H), 1.42 (m, 4H), 1.32 (m, 4H) , 1.24 (m, 4H) , 1.14 (m, 6H). EA ($C_{40}H_{46}N_4$): calc. (obs. %) C: 82.43 (82.35), H: 7.96 (7.94), N: 9.61 (9.54).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-piperidiniumiodide)nonyl) carbazole (BMVC-9C) (Yield: 92%, mp > 300^{o}C), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.81 (d, *J* = 6.0Hz, 4H), 8.67 (s, 2H), 8.22 (d, *J* = 6.4Hz, 4H), 8.20 (d, *J* = 16Hz, 2H), 7.92 (d, *J* = 8.4Hz, 2H), 7.76 (d, *J* = 8.8Hz, 2H), 7.58 (d, *J* = 16Hz, 2H), 4.47 (t, 2H), 4.25 (s, 6H), 3.28 (m, 6H), 2.95 (s, 3H), 1.75 (m, 4H), 1.59 (m, 4H), 1.21 (m, 12H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.28, 145.27, 142.35, 142.29, 127.50, 127.19, 123.34, 123.04, 121.64, 121.02, 111.13, 60.33, 47.16, 28.99, 28.84, 26.76, 26.17, 21.26, 21.07, 19.63. EA ($C_{43}H_{55}I_3N_4 \cdot 1.5H_2O$): calc. (obs. %) C: 49.87 (49.79), H: 5.64 (5.62), N: 5.41 (5.35).

Data for 3,6-Dibromo-9-(1-bromododecyl) carbazole (B2e) (Yield: 56%, Mw: 572.21), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.12 (d, *J* = 2.0Hz, 2H), 7.55 (dd, *J* = 2.0,8.8 Hz, 2H), 7.24 (d, *J* = 8.4Hz, 2H), 4.21 (t, *J* = 7.2Hz, 2H), 3.39 (t, *J* = 6.8Hz, 2H), 1.82 (m, 4H), 1.39 (m, 2H), 1.25 (m, 14H).

3,6-Dibromo-9-(1-(piperidin-1-yl)dodecyl) carbazole (B3e) (Yield: 85%, Mw: 576.45), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.06 (d, *J* = 2.0Hz, 2H), 7.50 (dd, *J* = 2.0,8.4 Hz, 2H), 7.21 (d, *J* = 8.8Hz, 2H),

4.19 (t, *J* = 8.0Hz, 2H), 2.37 (m, 4H), 2.25 (t, *J* = 8.4Hz, 2H), 1.80 (t, *J* = 8.0Hz, 2H), 1.63 (m, 4H), 1.51 (m, 4H), 1.42 (m, 4H), 1.24 (m, 12H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)dodecyl) carbazole (B4e) (Yield: 70%, Mw: 624.9), ^{1}H NMR (400MHz, DMSO-d6) δ: 8.52 (d, *J* = 4.4Hz, 4H), 8.48 (d, *J* = 1.6Hz, 2H), 7.77 (d, *J* = 8.8Hz, 2H), 7.68 (d, *J* = 16Hz, 2H), 7.63 (d, *J* = 8.4Hz, 2H), 7.55 (d, *J* = 4.0Hz, 4H), 7.23 (d, *J* = 16Hz, 4H), 4.41 (t, *J* = 7.2Hz, 2H), 2.23 (m, 4H), 2.18 (t, *J* = 7.2Hz, 2H), 1.77 (m, 2H), 1.41 (m, 4H), 1.32 (m, 4H), 1.24 (m, 4H), 1.13 (m, 12H). EA ($C_{43}H_{52}N_4$): calc. (obs. %) C: 82.65 (82.53), H: 8.39 (8.38), N: 8.97 (8.92).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-Piperidinium iodide) dodecyl) carbazole (BMVC-12C) (Yield: 92%, mp > 300^{o}C), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.81 (d, *J* = 6.8Hz, 4H), 8.65 (s, 2H), 8.24 (d, *J* = 16Hz, 2H), 8.20 (d, *J* = 7.2Hz, 4H), 7.92 (d, *J* = 8.8Hz, 2H), 7.76 (d, *J* = 8.8Hz, 2H), 7.56 (d, *J* = 16Hz, 2H), 4.45 (t, 2H), 4.29 (s, 6H), 3.25 (m, 6H), 2.95 (s, 3H), 1.75 (m, 6H), 1.61 (m, 2H), 1.52 (m, 2H),1.20 (m, 16H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.30, 145.25, 142.24, 142.21, 127.68, 127.35, 123.37, 123.17, 121.71, 121.09, 111.13, 61.98, 60.52, 52.57, 47.84, 47.20, 42.75, 26.02, 22.87, 21.03, 19.67, 19.18. EA ($C_{46}H_{61}I_3N_4 \cdot H_2O$): calc. (obs. %) C: 51.70 (51.61), H: 5.94 (5.91), N: 5.24 (5.17).

The synthesis of 3, 6, 9-trioxaundecane-1,11-dibromide was according to the Soma De, et. al. [2]

Data for 3,6-Dibromo-9-(1-bromo-3,6,9-trioxaundecane) carbazole (B2f) (Yield: 51%, Mw: 564.11), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.04 (d, *J* = 1.6 Hz, 2H), 7.46 (dd, *J* = 2.0,8.4Hz, 2H), 7.24 (d, *J* = 8.4Hz, 2H), 4.36 (t, *J* = 6.4Hz, 2H), 3.78 (m, 4H), 3.69 (t, *J* = 6.0Hz, 2H), 3.46 (m, 4H), 3.40 (m, 4H).

3,6-Dibromo-9-(1-(piperidin-1-yl)-3,6,9-trioxaundecane) carbazole (B3f) (Yield: 88%, Mw: 568.34), ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.95 (d, *J* = 2.4Hz, 2H), 7.41 (dd, *J* = 1.6,8.8Hz, 2H), 7.22 (d, *J* = 8.8Hz, 2H), 4.30 (t, *J* = 5.6Hz, 2H), 3.73 (t, *J* = 5.6Hz, 2H), 3.58 (t, *J* = 5.6Hz, 2H), 3.36 (m, 10H), 2.62 (t, *J* = 5.6Hz, 2H), 2.56 (m, 4H), 1.63 (m, 4H), 1.40 (m, 2H).

3,6-Bis-(4-vinylpyridine)-9-(1-(piperidin-1-yl)-3,6,9-trioxaundecane) carbazole (B4f) (Yield:

62%, Mw: 616.79), ^{1}H NMR (400MHz, DMSO-d6) δ: 8.53 (d, *J* = 4.4 Hz, 4H), 8.50 (d, *J* = 2Hz, 2H), 7.78 (dd, *J* = 2.0,8.8Hz, 2H), 7.68 (d, *J* = 16Hz, 2H), 7.65 (d, *J* = 8.0Hz, 2H), 7.56 (d, *J* =4.8Hz, 4H), 7.25 (d, *J* =16Hz, 4H), 4.33 (t, *J* = 5.6 Hz, 2H), 3.75 (t, *J* = 5.6Hz, 2H), 3.58 (t, *J* = 5.6Hz, 2H), 3.37 (m, 10H), 2.61 (t, *J* = 5.6Hz, 2H), 2.56 (m, 4H), 1.64 (m, 4H), 1.38 (m, 2H). EA ($C_{39}H_{44}N_4O_3$): calc. (obs. %) C: 75.94 (75.87), H: 7.19 (7.14), N: 9.08 (8.97).

3,6-Bis(1-methyl-4-vinylpyridium iodide)-9-(1-(1-methyl-piperidinium iodide)-3, 6,9-trioxaundecane) carbazole (BMVC-8C3O): (Yield: 86%, mp > 300^{o}C), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.80 (d, *J* = 6Hz, 4H), 8.68 (s, 2H), 8.23 (d, *J* = 16Hz, 2H), 8.20 (d, *J* = 7.2Hz, 4H), 7.90 (d, *J* = 8.8Hz, 2H), 7.76 (d, *J* = 8.4Hz, 2H), 7.59 (d, *J* = 16Hz, 2H), 4.64 (t, 2H), 4.24 (s, 6H), 3.82 (t, 2H), 3.71 (t, 2H), 3.47 (m, 4H), 3.38 (m, 10H), 2.97 (s, 3H), 1.67 (m, 4H), 1.43 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.32, 145.24, 142.68, 142.28, 127.63, 127.21, 123.37, 123.14, 121.58, 121.04, 111.47, 70.48, 70.05, 69.87, 69.83, 63.87, 61.24, 47.21, 20.88, 19.71. EA ($C_{42}H_{53}I_3N_4O_3\cdot 2H_2O$): calc. (obs. %) C: 46.77 (46.68), H: 5.33 (5.31), N: 5.19 (5.12).

General procedural C: The synthesis of **$(MVC)_2$-8C** was shown in **Supplementary Scheme 3**. Briefly; Compound **C1** was synthesized from carbazole (**1,** 3.48 g, 20 mmole, Aldrich) through brominating by N-bromosuccinimide (NBS, 3.6 g, 20 mmole, Aldrich) in dty MeCN (30 ml) under nitrogen. And then to a solution of **C1** (10 mmole) in 10 ml of dry DMF were slowly added 0.48 g of NaH (20 mmole), after stirring for 5 minutes , the dibromo precursors (Br-R-Br, 5 mmole) were added and the mixture was refluxed for 12 hours. After cooling and quenching the waste sodium hydride with methanol, the solution was extracted with H_2O/ethyl acetate twice and the organic layer dried by $MgSO_4$. The product (**C2**) was collected by flash column (silica, hexane/ethyl acetate. 2/1, v/v). Then the reactants **C2** could couple with 4-vinylpyridine at mixed powders of Palladium (II) acetate/tri-*o*-tolylphosphine under the triethylamine/acetonitrile solvent pairs in high-pressure system. Compounds **C3** were obtained in good yield after extraction and crystallization (acetone) procedures followed the description in procedure A (**Supplementary**

Scheme 1). The orange-red powders (**(MVC)$_2$-8C**) were collected in very good yield after refluxing the compound **C3** with excess CH_3I in DMF.

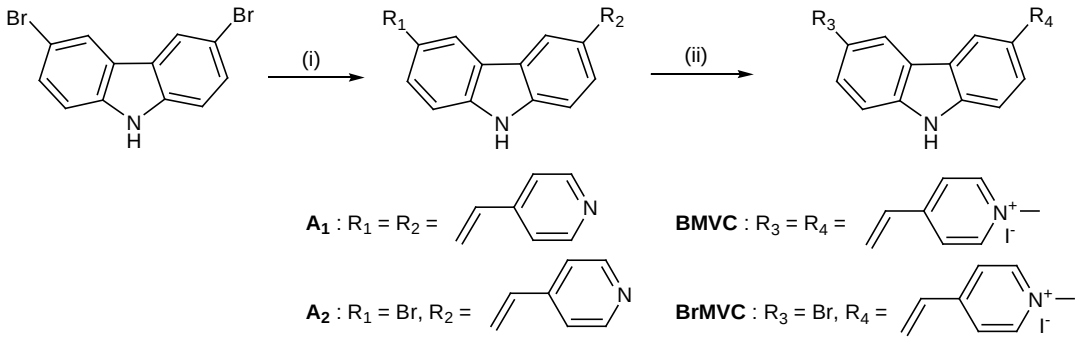
Data for **3-bromocarbazole (C1)** (Yield: 48%, Mw: 246.10), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.18 (d, *J* = 2.0Hz, 1H), 8.01 (d, *J* = 7.6Hz, 1H), 7.50 (dd, *J* = 2.0,8.8Hz, 1H), 7.44 (m, 2H), 7.27 (m, 2H).

1,8-bis(3-bromocarbazol-9-yl)octane (C2) (Yield: 45%, Mw: 602.40), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.19 (d, *J* = 2.0Hz, 2H), 8.03 (d, *J* = 7.6Hz, 2H), 7.52 (dd, *J* = 2.0,8.8Hz, 2H), 7.46 (m, 2H), 7.37 (m, 2H), 7.27 (m, 4H), 4.24 (t, *J* = 7.2Hz, 4H), 1.81(m, 4H), 1.26(m, 8H).

1,8-bis(3-(4-vinylpyridine)carbazol-9-yl)octane (C3) (Yield: 58%, Mw: 650.85), ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.55 (d, *J* = 6.4Hz, 4H), 8.24 (d, *J* = 1.6Hz, 2H), 8.12 (d, *J* = 7.2Hz, 2H), 7.68 (dd, *J* = 2.0,8.8Hz, 2H), 7.56 (d, *J* = 16.0Hz, 2H), 7.48 (m, 2H), 7.46 (d, *J* = 6.4Hz, 4H), 7.39 (t, *J* = 8.0Hz, 4H), 7.28 (m, 2H), 7.05 (d, *J* = 16.0Hz, 2H), 4.28 (t, *J* = 7.2Hz, 4H), 1.84 (m, 4H), 1.29 (m, 8H).

1,8-bis(3-(1-methyl-4-vinylpyridium iodide)carbazol-9-yl)octane ((MVC)$_2$-8C) (Yield: 85%, mp > 300^{o}C), ^{1}H NMR (400 MHz, DMSO-d6) δ: 8.78 (d, *J* = 6.4Hz, 4H), 8.54 (s, 2H), 8.18 (d, *J* = 16Hz, 2H), 8.16 (d, *J* = 9.2Hz, 2H), 8.14 (d, *J* = 6.4Hz, 4H), 7.85 (d, *J* = 7.6Hz, 2H), 7.68 (d, *J* = 9.2Hz, 2H), 7.61 (d, *J* = 8.0Hz, 2H), 7.50 (d, *J* = 16.4Hz, 2H), 7.46 (t, *J* = 7.2Hz, 2H), 7.26 (t, *J* = 7.2Hz, 2H), 4.37 (t, *J* = 6.8Hz, 4H), 4.21 (s, 6H), 1.72 (m, 4H), 1.21 (m, 8H). ^{13}C NMR (400 MHz, DMSO-d6) δ : 153.46, 145.17, 142.71, 141.79, 140.99, 126.83, 126.62, 123.17, 123.01, 122.41, 121.50, 120.84, 120.33, 120.05, 110.44, 110.28, 47.07, 42.86, 28.96, 28.76, 26.67. EA ($C_{48}H_{48}I_2N_4 \cdot H_2O$): calc. (obs. %) C: 60.51 (60.46), H: 5.29 (5.23), N: 5.88 (5.79).

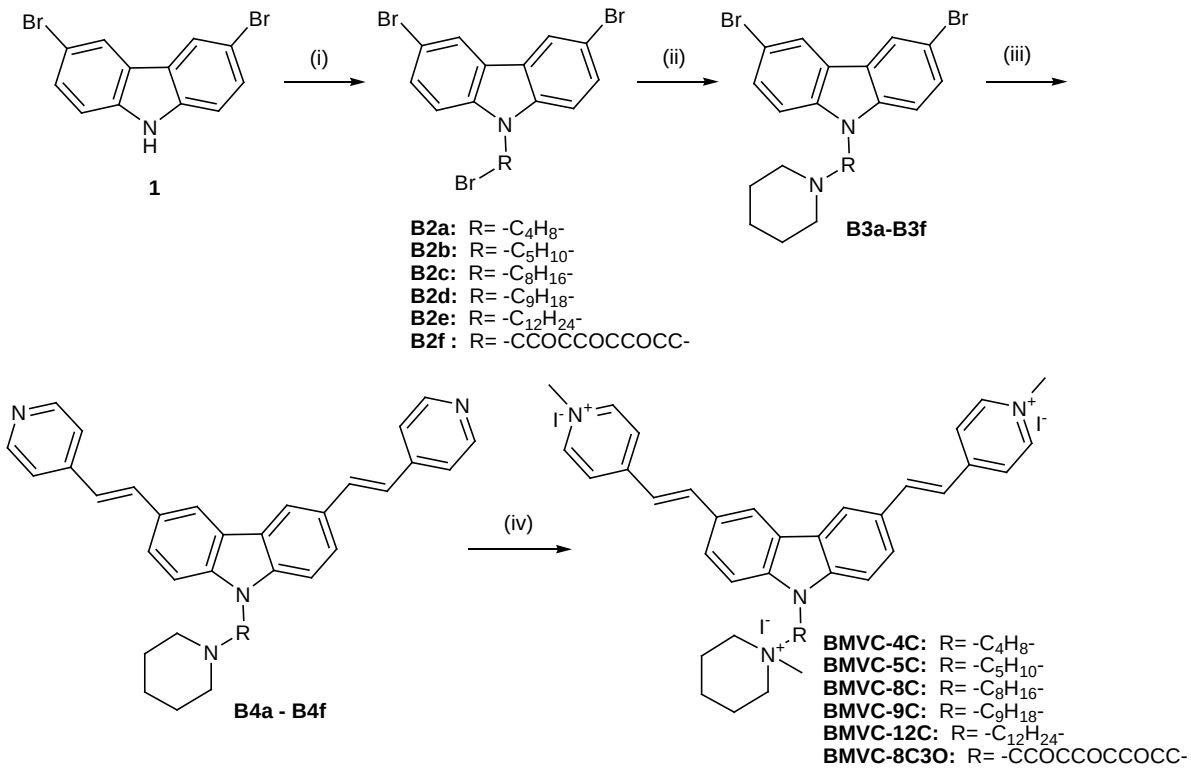
Supplementary Scheme 1



(i): $Pd(OAc)_2$ / (*o*-tol)$_3$P, 4-vinylpyridine,Et_3N / MeCN, N_2. (ii): MeI / DMF

Supplementary Scheme 1. Asymmetric synthesis process of BrMVC

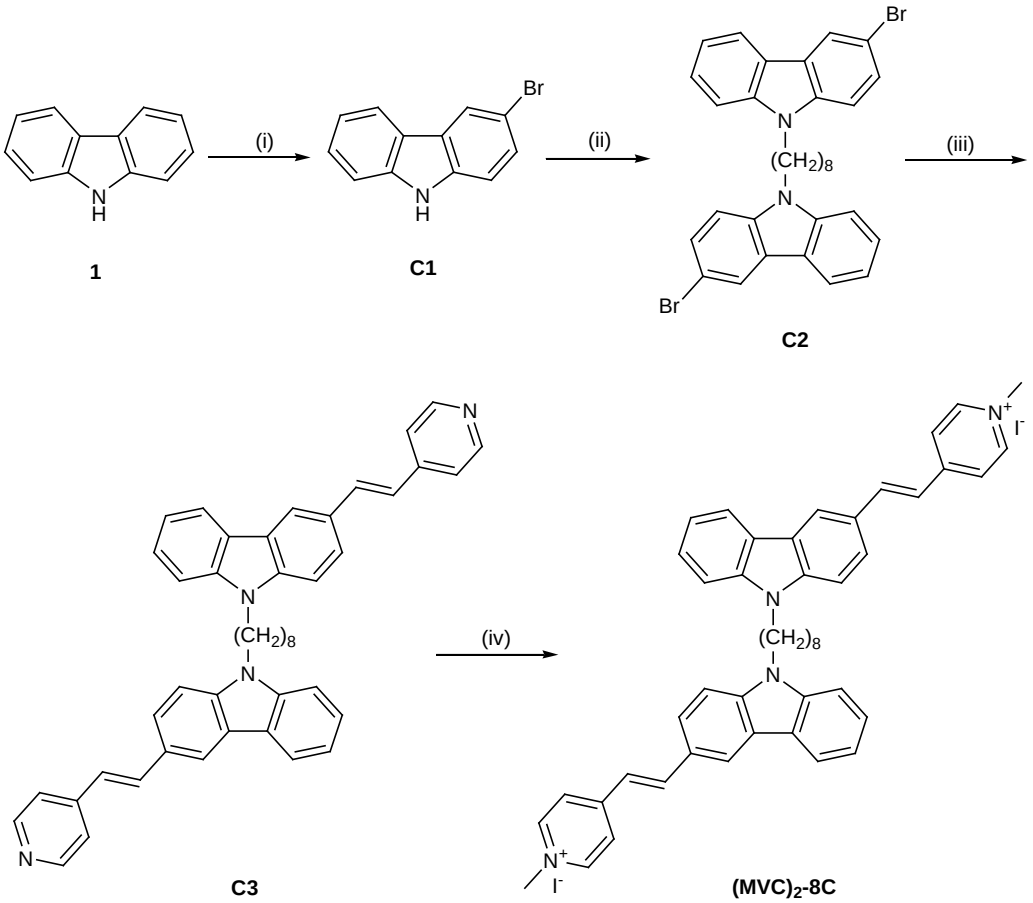
Supplementary Scheme 2:



(i) NaH/THF and BrRBr, N_2. (ii) NaI/EtOH, piperidin, refluxes under ethanol.
(iii) $Pd(OAc)_2$ / (*o*-tol)$_3$P, 4-vinylpyridine,Et_3N / MeCN, N_2. (iv) MeI / DMF

Supplementary Scheme 2. The syntheses of 9-substituted BMVC derivatives

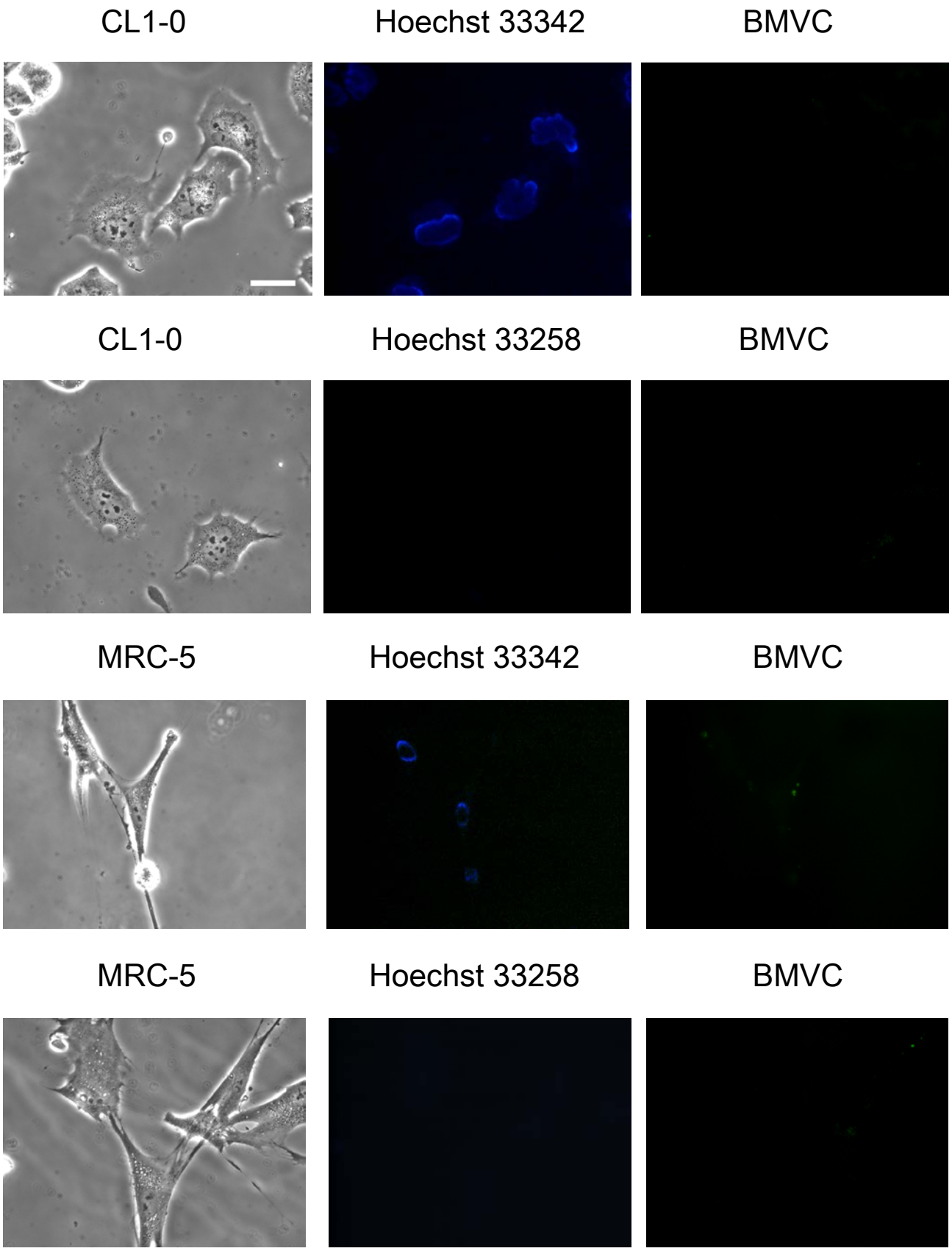
Supplementary Scheme 3:



(i): NBS / CH_3CN. (ii): NaH / DMF, 1,8-dibromooctane, N_2. (iii): $Pd(OAc)_2$ / (*o*-tol)$_3$P, 4-vinylpyridine,Et_3N / MeCN, N_2. (iv): MeI / DMF

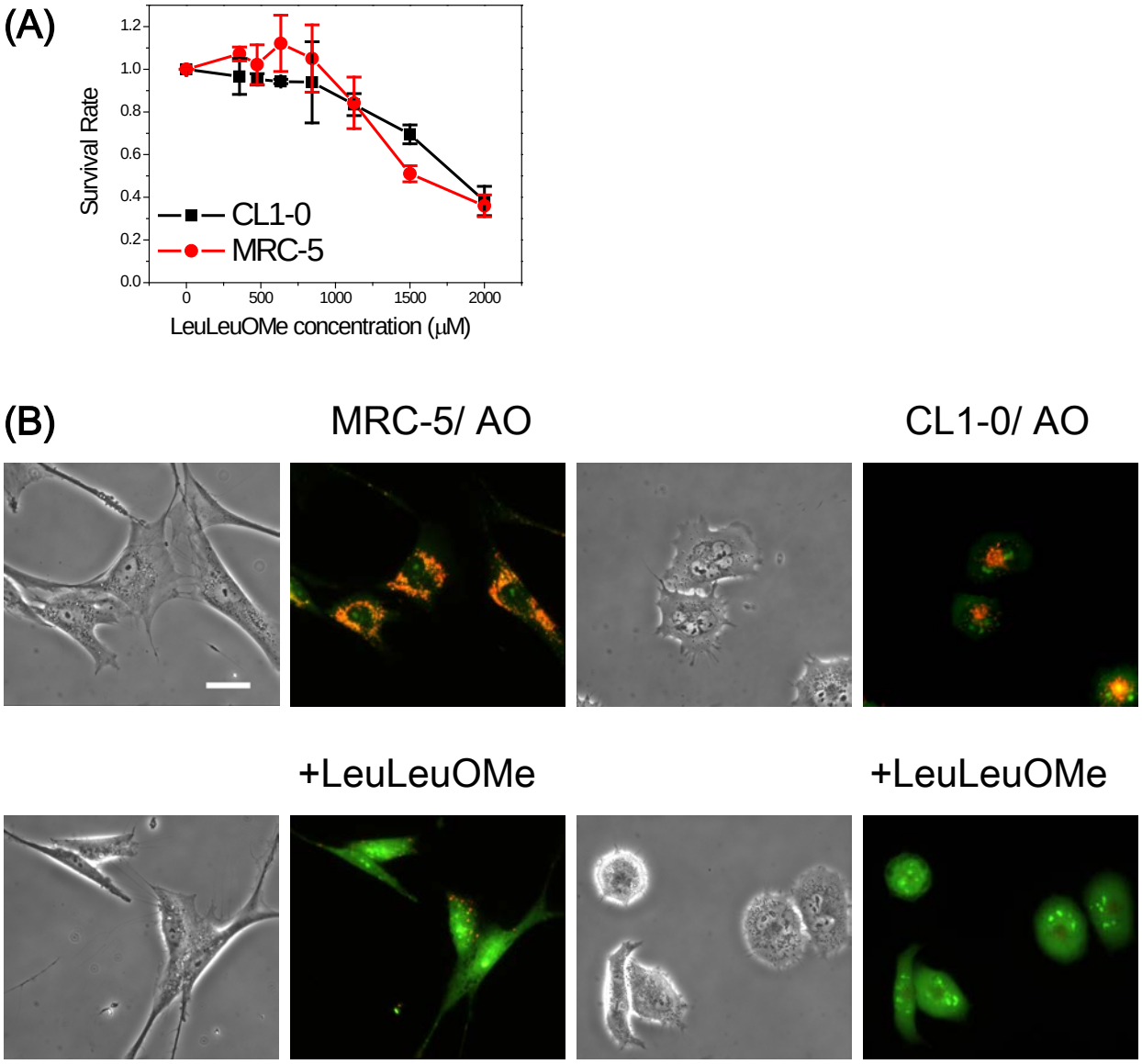
Supplementary Scheme 3. Synthesis of **$(MVC)_2$-8C**

Supplementary Figure 1



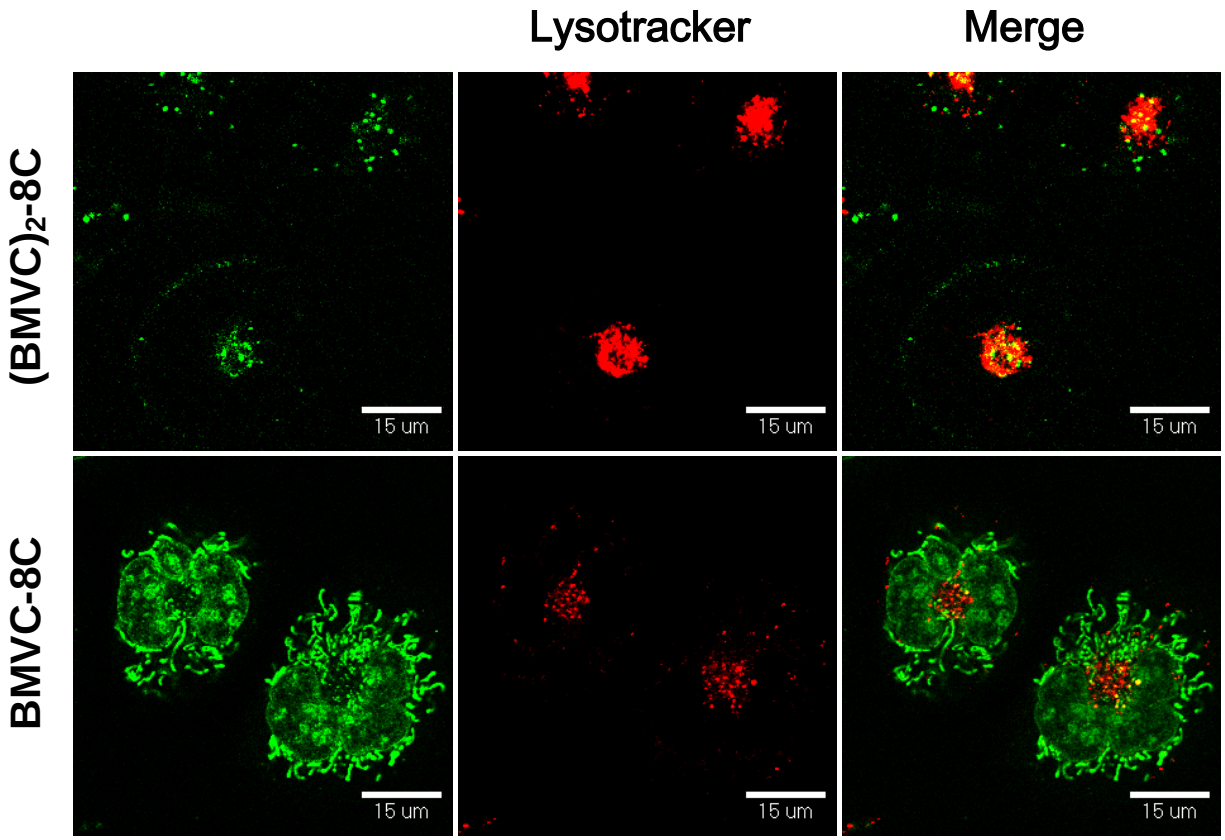
Supplementary Figure 1. BMVC likely enters cells through endocytosis. CL1-0 and MRC-5 cells were stained by 1 μM BMVC for 1 h under low temperature and observed by epi-fluorescence microscopy. Hoechst 33258 and Hoechst 33342 are two closely related DNA staining dyes. The major difference is that Hoechst 33342 is more lipophilic than Hoechst 33258 and therefore Hoechst 33342 is more membrane permeable than Hoechst 33258. Accordingly, by comparing to the negative control, Hoechst 33342 and the positive control, Hoechst 33258, we determined that BMVC mainly enters cells through endocytosis pathway. The scale bar is 25 μm.

Supplementary Figure 2



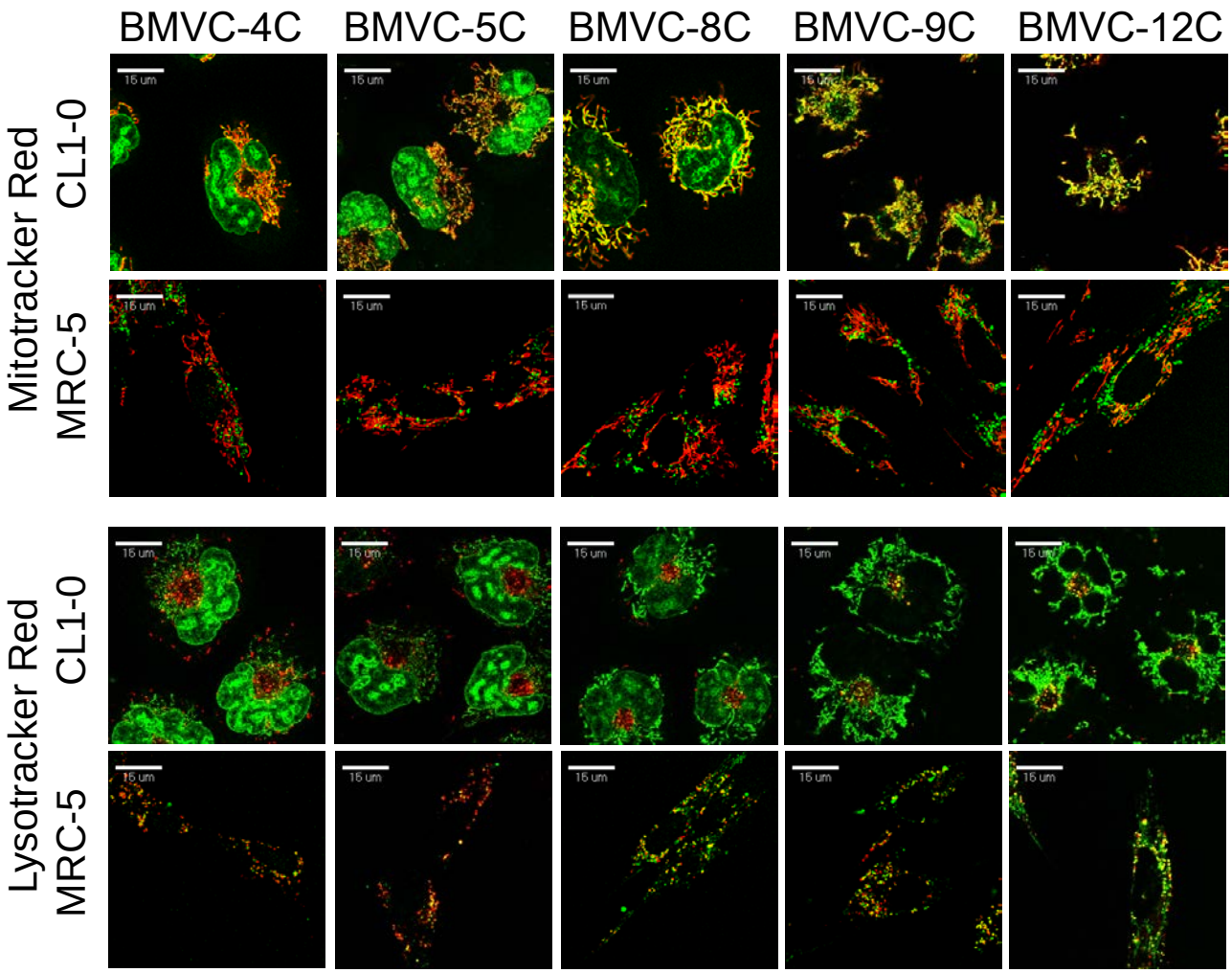
Supplementary Figure 2. The lysosomal membrane permeabilization effect of L-Leucy-L-Leucine methyl ester (LeuLeuOMe) on CL1-0 and MRC-5 cells. (a) The cytotoxicity of LeuLeuOMe in CL1-0 and MRC-5 cells were measured by MTT assay. (b) Loss of lysosomal membrane integrity by LeuLeuOMe is monitored by AO (acridine orange). The orange to red AO staining corresponds to acid granules, such as lysosomes in cells while the entire green AO staining appears due to ruptured lysosome caused by LeuLeuOMe. 1 μM AO stains MRC-5 (UL) and CL1-0 cells (UR) for 1 h and 1 μM AO stains with LeuLeuOMe 0.5 mM in MRC-5 (LL) and CL1-0 cells (LR) for 1 h. The scale bar is 25 μm.

Supplementary Figure 3.



Supplementary Figure 3. $(BMVC)_2$-8C mainly localizes with lysotracker in CL1-0 cells while BMVC-8C rarely does. The confocal fluorescence images of 1 μM $(BMVC)_2$-8C and BMVC-8C incubated with CL1-0 cells for 24 h and overlay with 3 μM LysoTracker Blue for 5 min and 200 nM LysoTracker Red for 10 min, respectively. Again, we use pseudocolor for the images of $(BMVC)_2$-8C. The green color represents $(BMVC)_2$-8C and red color represents LysoTracker Blue. In all panels, the scale bar is 15 μm.

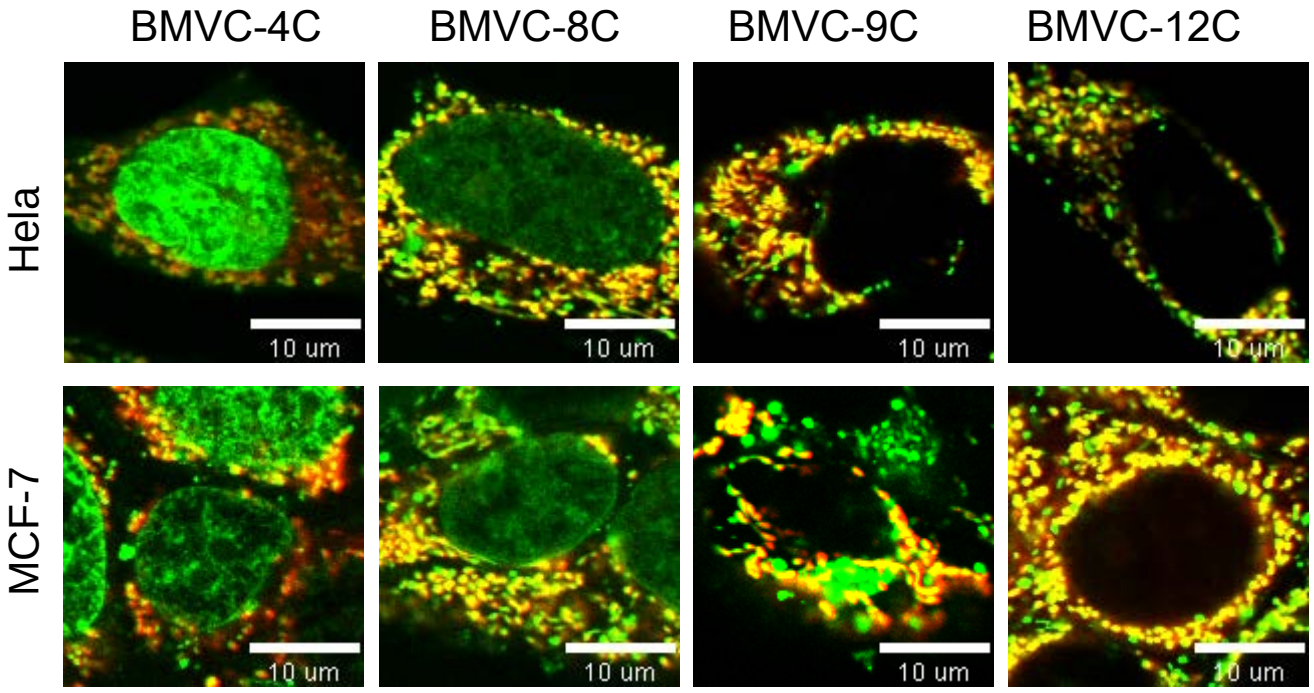
Supplementary Figure 4.



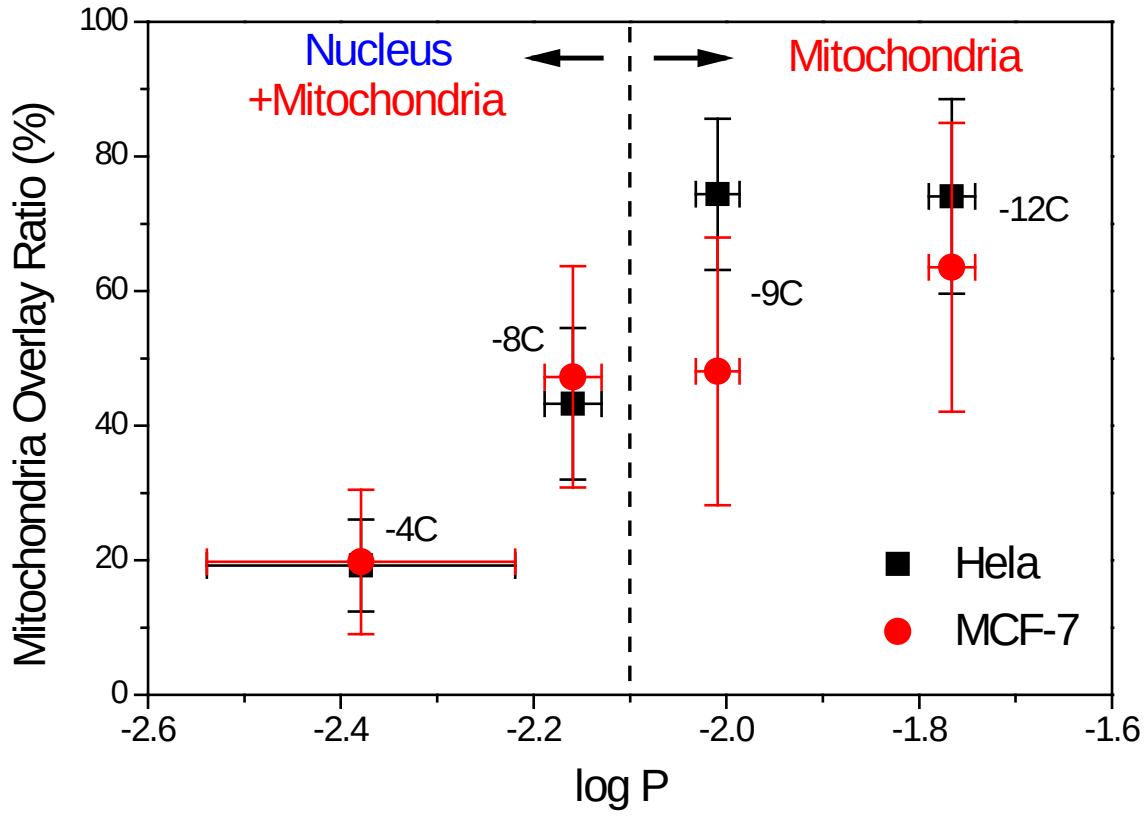
Supplementary Figure 4. BMVC derivatives mainly localize in mitochondria and/or nucleus of cancer cells while localize in lysosome of normal cells. The confocal fluorescence images of 1 μM BMVC derivatives overlay with 40 nM MitoTracker Red for 30 min in CL1-0 cells and MRC-5 cells and 200 nM LysoTracker Red for 10 min in CL1-0 cells and MRC-5 cells. The co-localization of BMVC and MitoTracker Red or LysoTracker Red appears yellow color in the overlay images. In all panels, the scale bar is 15 μm.

Supplementary Figure 5.

(A)

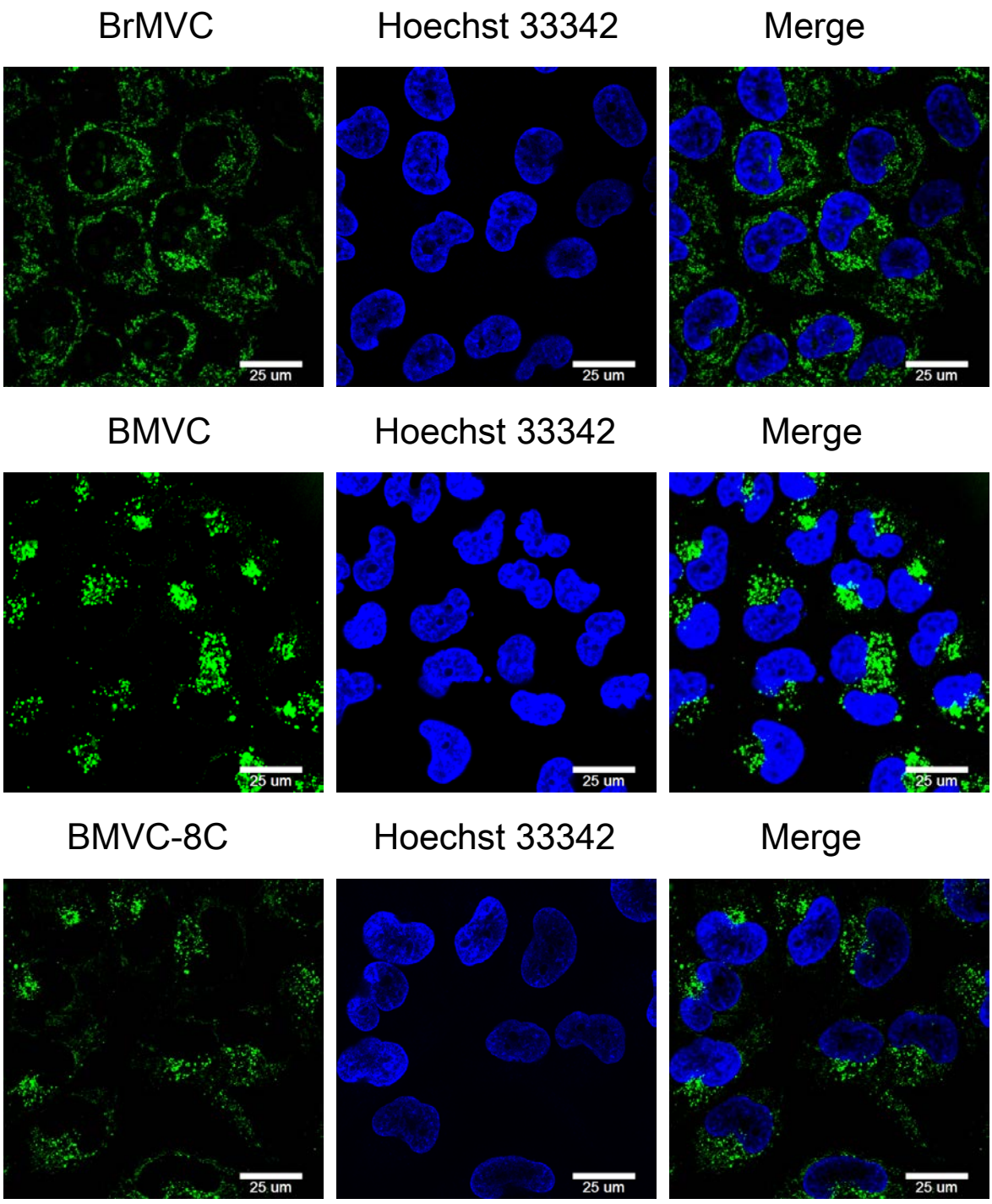


(B)



Supplementary Figure 5. Lipophilicity is a key determinant of mitochondrial vs. nuclear localization in Hela and MCF-7 cancer cells. (**A**) Confocal fluorescence images of Hela cervical cancer cells (upper panels) and MCF-7 breast cancer cells (lower panels) incubated with 5 μM BMVC-4C, BMVC-8C, BMVC-9C or BMVC-12C and co-stained with MitoTracker red. (**B**) BMVC derivatives with lipophilicities (log P) below -2.0 preferentially localized to the nucleus, whereas those above -2.0 can only be detected in the mitochondria. Structures of all compounds are in **Fig.7.** In all panels, the scale bar is 10 μm.

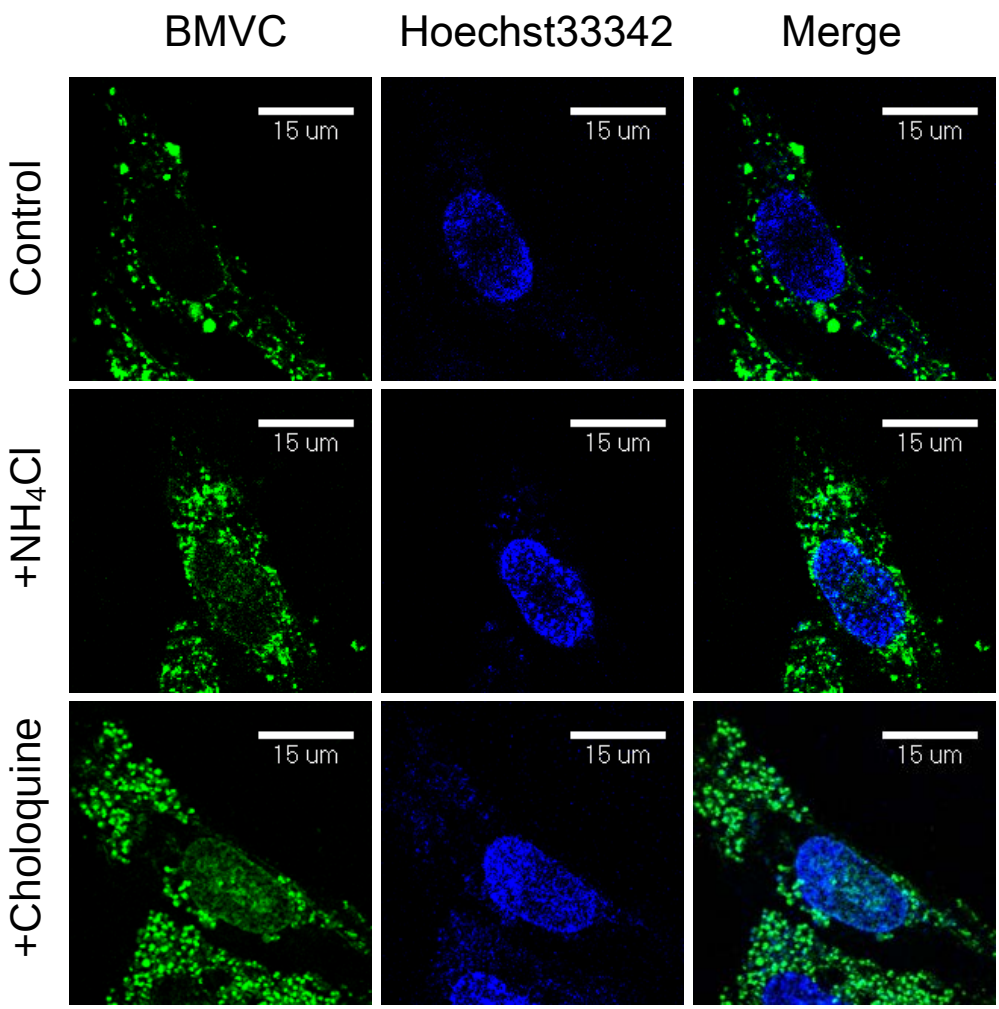
Supplementary Figure 6.



Supplementary Figure 6. Hydrogen bonding capacity (HBC) regulates lysosomal retention of BMVC derivatives in MCF-7/ADR cells. Confocal fluorescence images of MCF-7/ADR cells incubated with 5 μM BrMVC (monocation), BMVC (dication), or BMVC-8C (trication), and

co-stained with 4.5 μM Hoechst 33342 10 min for nuclear staining. In all panels, the scale bar is 25 μm.

Supplementary Figure 7.



Supplementary Figure 7. Confocal fluorescence images of MRC-5 cells incubated with 5 μM BMVC for 24 h, or with 5 μM BMVC followed by 100 mM NH_4Cl for 1 h or 100 μM chloroquine for 3 h. Cells were co-stained with 100 nM Hoechst 33342 for 10 min. The scale bar is 15 μm.

Supplemental References

1. C. C. Chang, J. Y. Wu, T. C. Chang, A carbazole derivative synthesis for stabilizing the quadruplex structure. *Journal of the Chinese Chemical Society* 2003, *50*. 185-188.

2. S. M. De, V. K. Aswal, P. S. Goyal, S. Bhattacharya, Novel gemini micelles from dimeric surfactants with oxyethylene spacer chain. Small angle neutron scattering and fluorescence studies. *Journal of Physical Chemistry B* 1998, *102*. 6152-6160.