Supporting Information For:

Novel Far-Emission Lysosomes-Targeting Ratiometric Fluorescent Probes for Imaging in Live Cells

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Contents:

1. Calculation of Quantum Yield
2. Synthesis and Characterization of Fluorescent Probe
3. Supplementary Figures.

Figure S-1. The pH-dependent optical properties of MCDBI.
Figure S-2. The pH-dependent optical properties of MCDI.

Figure S-3. Selectivity studies of MCDI in the presence of various metal ions and bioactive species.

Figure S-4. Photostability and reversibility of MCDI.

Figure S-5. Cell cytotoxicity effect of MCDBI and MCDI on HepG2 cells.

Figure S-6. The co-localization images of MCDBI and MCDI.

Figure S-7. pH-depended images of MCDI.

Figure S-8. 1H NMR spectra of MCDBI and MCDI.

Figure S-9. 13C NMR spectra of MCDBI and MCDI.

Figure S-10. LC- MS spectra of MCDBI and MCDI.
1. Calculation of Quantum Yield

The quantum yield of BIMC was determined according to the following equation.\(^1\)

\[
\Phi_x = \Phi_{st} \left( \frac{D_x}{D_{st}} \right) \left( \frac{A_{st}}{A_x} \right) \left( \frac{\eta_x^2}{\eta_{st}^2} \right)
\]  

(1)

Where \(\Phi_{st}\) is the quantum yield of the standard, \(D\) is the area under the emission spectra, \(A\) is the absorbance at the excitation wavelength and \(\eta\) is the refractive index of the solvent used. \(x\) subscript denotes unknown, and \(st\) means standard. We chose Rhodamine B (\(\Phi = 0.69\) in MeOH) as the standard.

2. Synthesis and Characterization of Fluorescent Probes

Scheme S1. Synthetic scheme for MCDI and MCDBI

Synthesis of (2-methoxyethoxy) methyl 4-methylbenzenesulfonate (S1)

To a three-neck flask was added 2-(2-methoxyethoxy) methanol (6.01 g, 50 mmol), sodium hydroxide (7.00 g, 175 mmol), THF (35 mL), and water (35 mL). The solution mixture was stirred at 0 °C. To the solution mixture, THF solution (50 mL) of \(p\)-toluenesulfonyl chloride (11.4 g, 60 mmol) was added dropwise over 2 h, and the reaction mixture was stirred for 12 h at room
Supporting Information

temperature. The reaction mixture was poured into aqueous hydrochloric acid, and the product was extracted with dichloromethane three times. The organic layer was dried over anhydrous magnesium sulfate filtered and evaporated. The crude colorless oil product was used for the subsequent synthesis without purification.

\[ ^{1}H \text{ NMR (CDCl}_3, 400 \text{ MHz)} \delta 9.89 (s, 1H), 7.81 (d, J = 8.8 \text{ Hz}, 2H), 7.02 (d, J = 8.8 \text{ Hz}, 2H), 4.23 (t, J = 4.8 \text{ Hz}, 2H), 3.90 (t, J = 4.8 \text{ Hz}, 2H), 3.73 (dd, J = 2.0 \text{ Hz}, 4.8 \text{ Hz}, 2H), 3.59 (dd, J = 2.0 \text{ Hz}, 4.8 \text{ Hz}, 2H), 3.34 (s, 3H) \text{ ppm}; ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz)} \delta 190.4, 163.2, 131.6, 129.7, 114.6, 71.7, 70.6, 69.3, 67.2, 58.9 \text{ ppm.} \]

**Synthesis 9-(2-(2-methoxyethoxy) ethyl)-9H-carbazole (1)**

To a solution of carbazole (3.34 g, 20 mmol) in THF (50 mL) at 0 °C was added NaH (0.72 g, 30 mmol). The solution was stirred for 30 min, S1 (8.23 g, 30 mmol) was added with constant stirring. The resulting mixture was stirred at room temperature for 5 h. The solution was poured into ice water, and the product was extracted with ethyl acetate three times. The organic layer was washed with water and brine then dried over anhydrous sodium sulfate and the solvent removed. The crude product separated by silica gel column chromatography with \( n \)-hexane: ethyl acetate 4:1 to give the desired product 1 (4.30 g) in 80% yield as brown oil.

\[ ^{1}H \text{-NMR (CDCl}_3, 400 \text{ MHz)} \delta 8.09 (d, J = 6.4 \text{ Hz}, 2H), 7.46 (m, 4H), 7.23 (m, 2H), 4.51 (t, J = 6.4 \text{ Hz}, 2H), 3.86 (t, J = 6.4 \text{ Hz}, 2H), 3.52 (m, 2H), 3.42 (m, 2H), 3.31 (s, 3H) \text{ ppm.} ^{13}C \text{-NMR (CDCl}_3, 100 \text{ MHz)} \delta 140.5, 125.6, 122.8, 120.2, 118.9, 108.7, 71.8, 70.7, 69.1, 59.0, 43.0 \text{ ppm.} \]

**Synthesis 9-(2-(2-methoxyethoxy) ethyl)-9H-carbazole-3-carbaldehyde (2)**

A 100 mL three-neck flask containing DMF (10.0 g, 140 mmol) was cooled to 0 °C and then POCl\(_3\) (4.00 g, 25 mmol) was added dropwise. The solution mixture was warmed to room temperature and stirred for 1 h. To this reaction mixture, 1 (4.00 g, 15 mmol) in dichloromethane (15 mL) slowly was added. The reaction temperature was raised to 75 °C and kept for 8 h. After cooling to room temperature the solution was poured into ice water and extracted with dichloromethane. The organic phase was washed with water and brine. Then, the organic layer was dried over anhydrous sodium sulfate and the solvent was removed. The crude product was purified by silica gel column chromatography \( n \)-hexane: ethyl acetate 3: 1 to afford 2 (2.44 g) in 65% yield as a yellow solid.
$^1$H-NMR (CDCl$_3$, 400 MHz) $\delta$ 10.07 (s, 1H), 8.58 (d, $J$ = 0.8 Hz, 1H), 8.13 (d, $J$ = 8.0 Hz, 1H), 7.98 (dd, $J$ = 8.8 Hz, 0.8 Hz, 1H), 7.51 (m, 3H), 7.30 (m, 1H), 4.53 (t, $J$ = 6.0 Hz, 2H), 3.87 (t, $J$ = 6.0 Hz, 2H), 3.49 (m, 2H), 3.38 (m, 2H), 3.26 (s, 3H) ppm. $^{13}$C-NMR (CDCl$_3$, 100 MHz) $\delta$ 191.8, 144.3, 141.1, 128.5, 127.1, 126.6, 123.7, 123.0, 122.9, 120.6, 120.4, 109.4, 109.3, 71.8, 70.8, 69.1, 59.0, 43.4 ppm.

**Synthesis (E)-2-(2-(9-(2-(2-methoxyethoxy)ethyl)-9H-carbazol-3-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indole (MCDBI)**

To the solution of 1,1,2-trimethyl-1H-benzo[e]indole (0.63 g, 3 mmol) and 3 (0.745 g, 2.5 mmol) in ethanol (20 mL) in a three-necked flask, 1 mL piperidine was added. The resulting mixture was refluxed for 48 h. After cooling down to room temperature, water was added. The aqueous solution was extracted with dichloromethane three times. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After removing the solvent, the crude product was purified by silica gel column chromatography using $n$-hexane and ethyl acetate 1:1 as eluent to afford the desired product (0.696 g) in 57% yield as an orange solid. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ (ppm): 8.38 (s, 1H), 8.16 (d, $J$ = 7.8 Hz, 1H), 8.10 (m, 2H), 7.98 (d, $J$ = 8.4 Hz, 1H) 7.90 (m, 2H), 7.81 (d, $J$ = 7.8 Hz, 1H), 7.59 (t, $J$ = 7.8 Hz, 1H), 7.47 (m, 4H), 7.31 (d, $J$ = 7.8 Hz, 1H), 7.22 (d, $J$ = 16.2 Hz, 1H), 4.56 (t, $J$ = 6.6 Hz, 1H), 3.92 (t, $J$ = 6 Hz, 1H), 3.55 (m, 2H), 3.45 (m, 2H), 3.34 (s, 3H), 1.78 (s, 6H) ppm. $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 180.78, 146.85, 136.75, 136.34, 134.60, 134.17, 127.68, 124.99, 124.36, 121.57, 121.47, 120.76, 119.61, 18.74, 118.18, 118.02, 115.74, 115.59, 115.35, 114.95, 111.60, 104.72, 104.48, 67.13, 66.53, 64.55, 54.66, 49.62, 38.77, 26.86 ppm. MS (LC-MS) m/z 489.25360 for [M + H]$^+$. 

**Synthesis (E)-3-(2-(3,3-dimethyl-3H-indol-2-yl)vinyl)-9-(2-(2-methoxyethoxy)ethyl)-9H-carbazole (MCDI)**

To the solution of 2,3,3-trimethyl-3H-indole (0.477 g, 3 mmol) and 3 (0.745 g, 2.5 mmol) in ethanol (20 mL) in a three-necked flask, 1 mL piperidine was added. The resulting mixture was refluxed overnight. After cooling down to room temperature, water was added. The aqueous solution was extracted with dichloromethane three times. The combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After removing the solvent, the crude product was purified by silica gel column chromatography using $n$-hexane and ethyl acetate 1:1 as eluent
to afford the desired product (0.713 g) in 65% yield as a yellow solid. $^1$H NMR (600 MHz, CDCl$_3$) d (ppm): 8.34 (s, 1H), 8.15 (d, $J = 7.8$ Hz, 1H), 7.97 (d, $J = 16.2$ Hz, 1H), 7.79 (d, $J = 9$ Hz, 1H), 7.66 (d, $J = 7.8$ Hz, 1H), 7.51 (m, 2H), 7.37 (m, 2H), 7.32 (m, 1H), 7.25 (t, $J = 7.8$ Hz, 1H), 7.16 (d, $J = 16.2$ Hz, 1H), 4.55 (t, $J = 6$ Hz, 2H), 3.91 (t, $J = 6$ Hz, 2H), 3.54 (m, 2H), 3.45 (m, 2H), 3.34 (s, 3H), 1.55 (m, 6H) ppm. $^{13}$C NMR (151 MHz, CDCl$_3$) 179.04, 141.82, 136.77, 136.34, 134.65, 123.07, 122.74, 121.45, 120.64, 120.54, 118.74, 118.74, 116.29, 115.71, 115.66, 115.41, 114.96, 112.36, 104.72, 104.47, 67.44, 66.02, 64.87, 54.36, 47.85, 38.24, 25.39 ppm. MS (LC-MS) m/z 439.23788 for [M + H]$^+$. 

![Fig. S1](image1.png)

**Fig. S1** The good linearity of MEDBI in the pH range of 3.33-4.67.

![Fig. S2](image2.png)

**Fig. S2** (a) Change of the absorption spectra of MCDI with decreasing pH from 7.1 to 3.3. Inset: sigmoidal fitting of the pH-dependent ratiometric absorbance ($A_{385\text{ nm}}/A_{486\text{ nm}}$); the color of the solution changed from yellow to orange with decreasing pH. (b) Change of the absorption spectra of MCDI with decreasing pH from 7.1 to 3.3. Inset: sigmoidal fitting of the pH-dependent ratiometric emission ($F_{516\text{ nm}}/F_{592\text{ nm}}$); the fluorescence color of the solution changed from yellow to orange with decreasing pH.
Fig. S3. Fluorescence intensity of 5 μM MCDI in ethanol/water (1/4 v/v) at different pH in the presence of various species: (1) blank; (2) 30 mM Na⁺; (3) 30 mM K⁺; (4) 20 mM Mg²⁺; (5) 20 mM Ca²⁺; (6) 20 mM Ba²⁺; (7) 0.1 mM Fe³⁺; (8) 1 mM Fe³⁺; (9) 1.2 mM Al³⁺; (10) 3 mM Cu²⁺; (11) 20 mM Zn²⁺; (12) 20 mM Mn²⁺; (13) 15 mM Ni²⁺; (14) 1 mM Co²⁺; (15) 0.1 mM Cr³⁺; (16) 0.5 mM Pb²⁺; (17) 5 mM Cd²⁺; (18) 5 mM Hg²⁺; (19) 0.5 mM Ag⁺; (20) 2 mM glucose; (21) 2 mM leucine; (22) 2 mM DL-methionine; (23) 2 mM valine; (24) 2 mM L-threonine; (25) 2 mM cysteine; (26) 2 mM glycine; (27) 2 mM arginine; (28) 2 mM serine; (29) 2 mM histidine; (30) 5 mM vitamin C. λ<sub>ex</sub> = 408 nm

Fig. S4. (a) Changes in the fluorescence emission ratio (F<sub>516 nm</sub>/F<sub>592 nm</sub>) of 5 μM MCDI with times at pH 3.30,
(b) Change in the fluorescence emission ratio ($F_{516\text{ nm}}/F_{592\text{ nm}}$) of 5 μM MCDI between pH 7.10 and 3.30 ($\lambda_{\text{ex}} = 408$ nm).

**Fig. S5.** Cell cytotoxicity effect of MCDBI and MCDI on HepG2 cells. 1, control; 2, 0.1 μM; 3, 1 μM; 4, 10 μM; 5, 100 μM; 6, 200 μM. Data are expressed as mean values ± standard error of the mean of six independent experiments, each performed in three triplicate.
Fig. S6. **MCDBI** in HeLa (A), SSMC-7721(B), A549 cells (C) and **MCDI** in HepG2 (D), HeLa (E), SSMC-7721(F) and A549 cells (G) colocalizes experiments to the lysosomes. Cells were stained with (a) Probe (5.0 μM, λex= 405 nm) for 30 min at 37°C and (b) LysoTracker Green DND-26 (1.0 μM, λex= 488 nm) for 15 min at 37°C. (c) Bright-field images. (d) Overlay of (a) and (b). (e) Intensity scatter plot of the channel of probes and Lyso Tracker Green.

Fig. S7. Images of 5 μM **MCDI** in HepG2 cells clamped at pH 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5, respectively. The first and second column were collected in yellow channel (Ex = 405 nm, Em = 450 -550 nm) and red channel (Ex = 488 nm, Em = 550 -650 nm). The third line shows the corresponding bright-field transmission images. The overlay images obtained from the yellow and red channels (fourth column).
Fig. S8 ¹H NMR spectra of MCDBI

¹H NMR spectra of MCDI
Fig. S9. $^{13}$C NMR spectra of MCDBI

$^{13}$C NMR spectra of MCDI
Fig. S10. LC-MS spectra of MCDBI

LC-MS spectra of MCDI

References