

# Selective Detection of Lipophagy with a Highly Specific Lipid Droplet Probe

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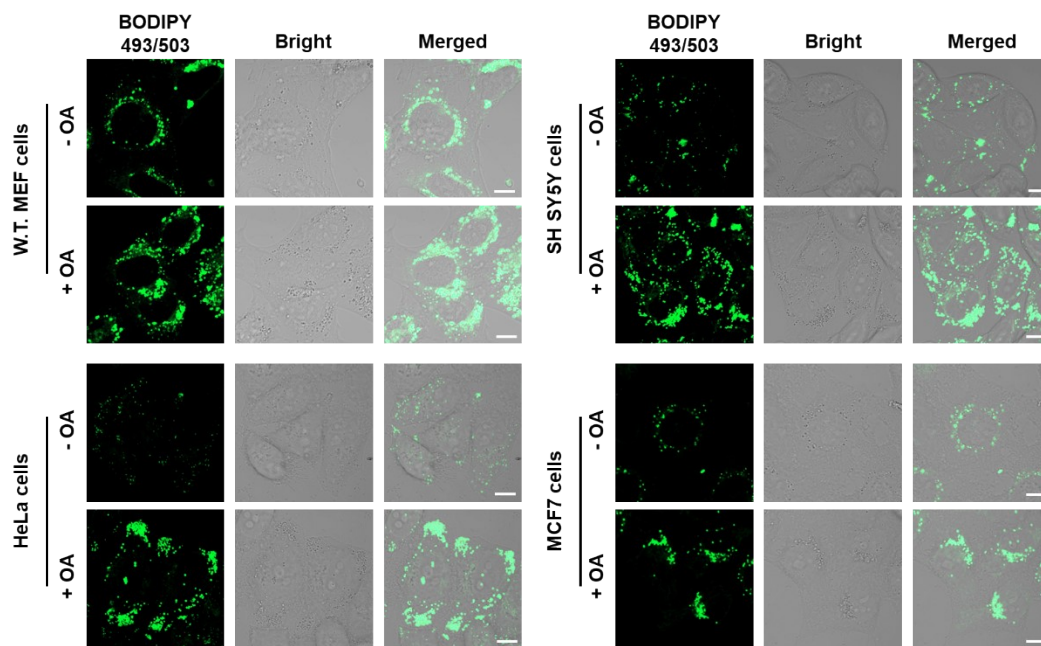
<sup>‡</sup>Both authors contributed equally to this work.

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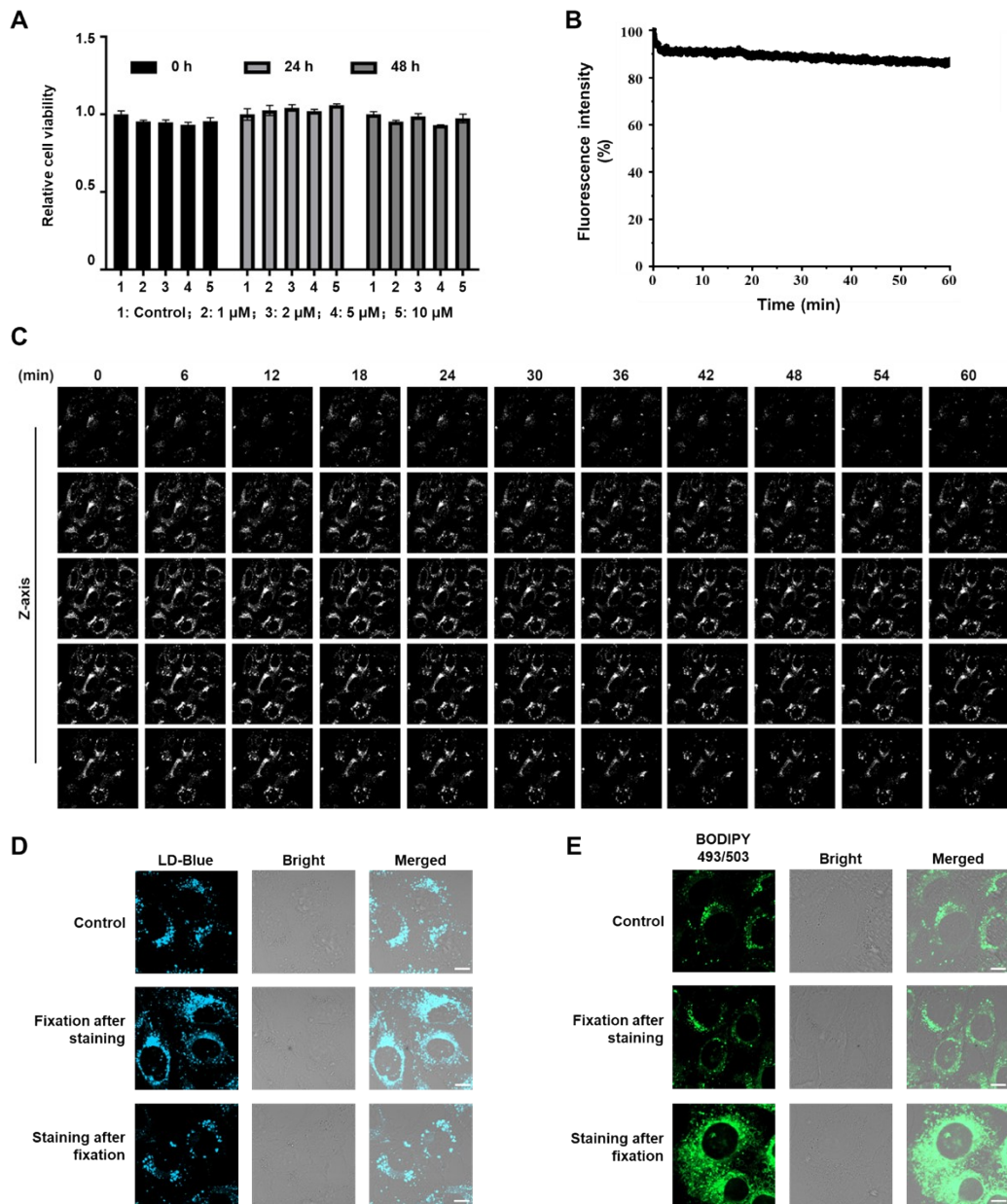
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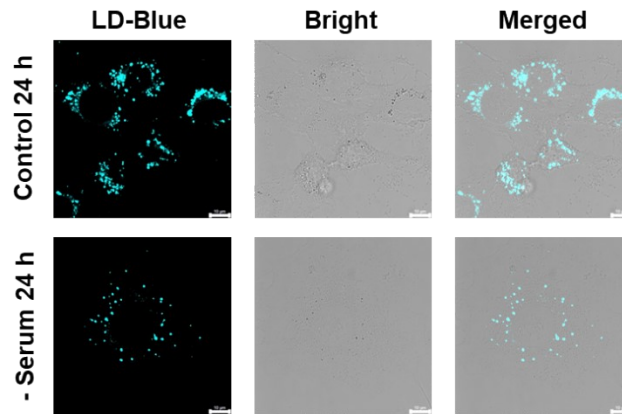
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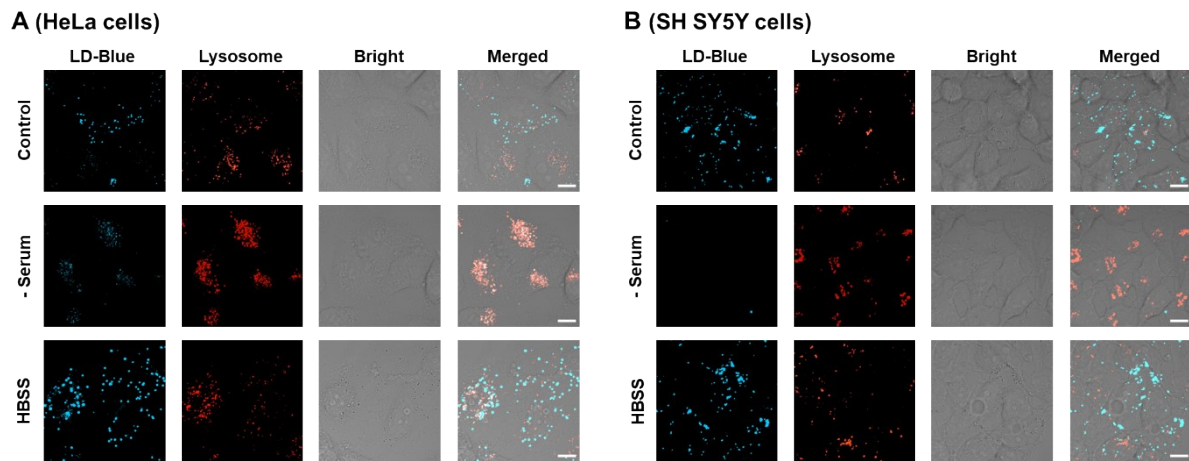
**Figure S1.** Labeling of lipid droplets in oleic acid-treated cells by the commercial BODIPY 493/503. Oleic acid-treated (200  $\mu$ M, 12 h) MEF cells, SH SY5Y cells, HeLa cells and MCF7 cells were stained with the commercial LD probe BODIPY 493/503 (2  $\mu$ M, 1 h), washed three times with PBS and maintained in fresh DMEM, then imaged by confocal microscopy. Scale bar: 10  $\mu$ m.



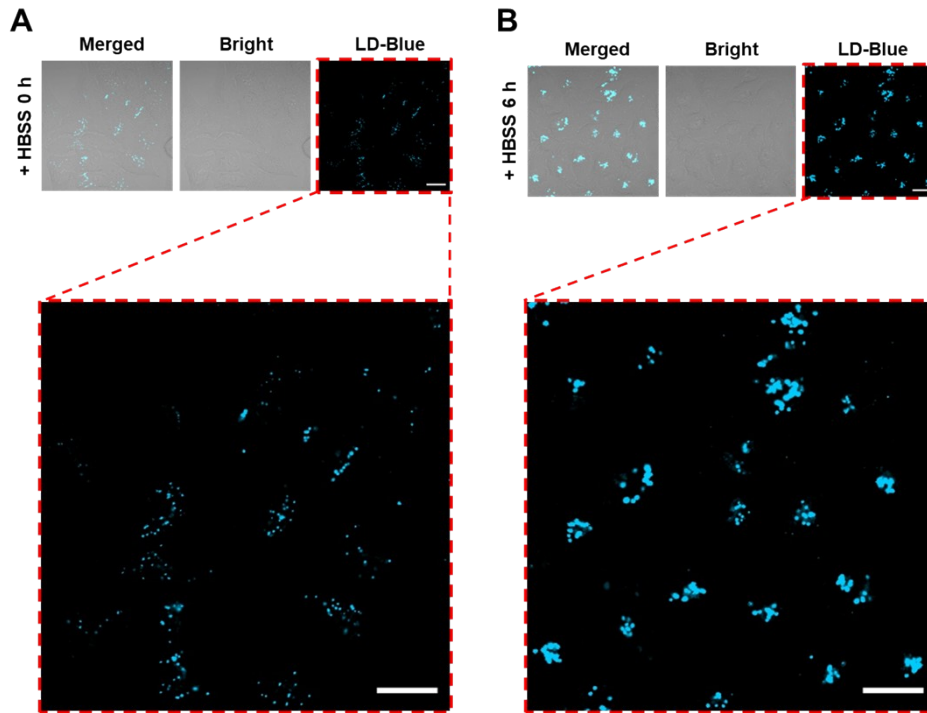
**Figure S2.** Cytotoxicity and stability of LD-Blue probe. (A) Cytotoxicity assay of LD-Blue probe. MEF cells were incubated with different concentrations of LD-Blue probe (0  $\mu$ M, 1  $\mu$ M, 2  $\mu$ M, 5  $\mu$ M, 10  $\mu$ M), washed three times with PBS, then maintained in fresh DMEM, and viability was measured using a CCK-8 kit at 0 h, 24 h, or 48 h to evaluate the cytotoxicity of LD-Blue probe. (B-C) Photostability assay of LD-Blue probe. The LD-Blue probe was irradiated extracellularly (B) or intracellularly (C) under 445 nm excitation for 60 min, and the fluorescence intensity of the probe was detected at the time points indicated. (D) Staining stability of the LD-Blue probe for paraformaldehyde fixation. MEF cells were stained with LD-Blue and then fixed with 4% paraformaldehyde, or after fixation with 4% paraformaldehyde, then stained with LD-Blue and imaged by confocal microscopy. (E) Effect of paraformaldehyde fixation on BODIPY 493/503 staining. MEF cells were stained with BODIPY 493/503 and then fixed with 4% paraformaldehyde, or stained with BODIPY 493/503 after 4% paraformaldehyde fixation, and then imaged by confocal microscopy. Scale bar: 10  $\mu$ m.



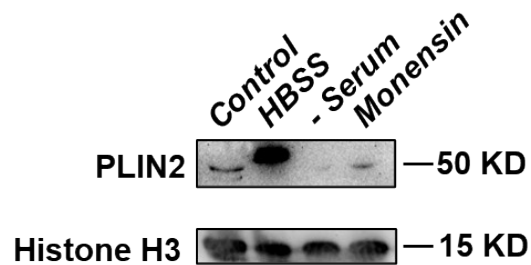
**Figure S3.** Serum deprivation leads to a decrease in lipid droplet fluorescence. MEF cells were maintained in serum-depleted DMEM or complete DMEM for 24 h respectively and then stained with LD-Blue (2  $\mu$ M, 1 h). All the cells were analyzed by confocal laser microscopy.



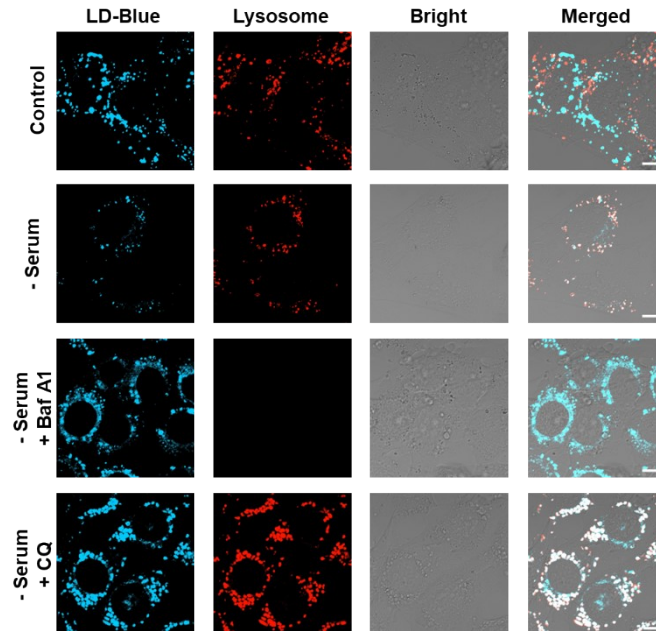
**Figure S4.** LD-Blue-based lipophagy imaging in HeLa and SH SY5Y cells. Lysosome-prestained HeLa (A) or SH SY5Y (B) cells were labelled with LD-Blue and subjected to either serum starvation or complete nutrient deprivation (HBSS), with non-starved cells serving as controls. Cellular imaging was performed by confocal microscopy. Scale bar: 10  $\mu$ m.



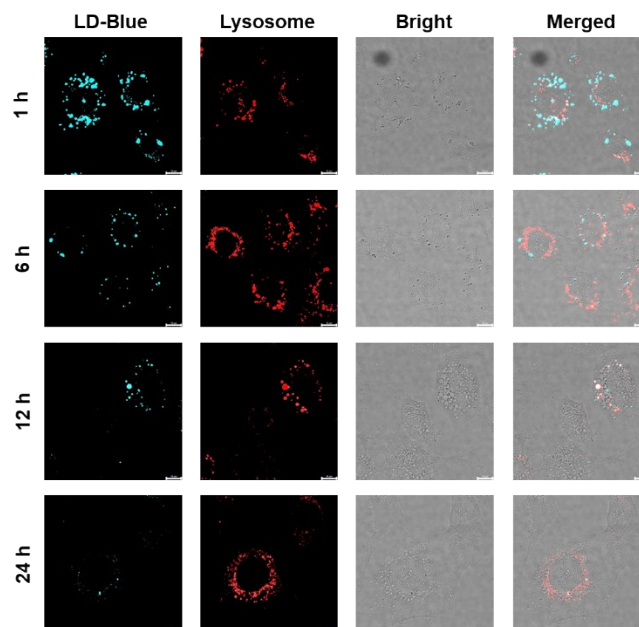
**Figure S5.** Complete nutrient deficiency leads to enhanced LD-Blue probe signal. MCF7 cells were treated with HBSS for 0 h (A) or 6 h (B) and stained with an LD-Blue (2  $\mu$ M, 1 h), then washed three times with PBS and maintained in fresh DMEM. These cells were analyzed by confocal microscopy. Scale bar: 10  $\mu$ m.



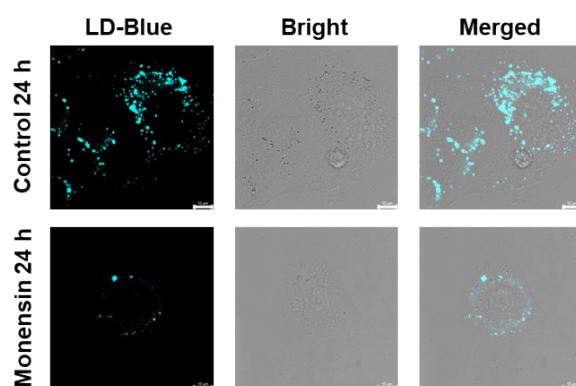
**Figure S6.** Changes of PLIN2 protein levels during lipophagy and LD generation. MEF cells were subjected to serum starvation, complete nutrient deprivation (HBSS) or monensin treatment. Cell lysates from the aforementioned treatments were analyzed by western blot with PLIN2 antibody and Histone H3 antibody.



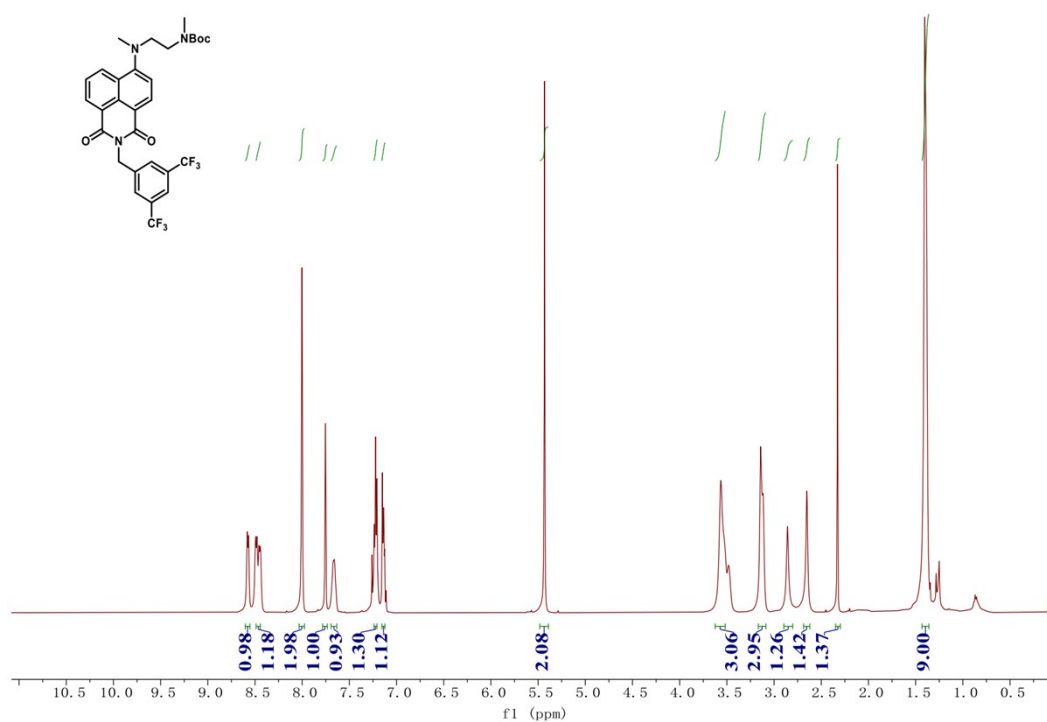
**Figure S7. Autophagy inhibitors block lipophagy.** Lysosome-prestained MEF cells were labelled with LD-Blue and then maintained in serum deficient DMEM with or without Baf-A1 (25 nM) or CQ (10  $\mu$ M) for 24 h. These cells were analyzed by confocal microscopy. Scale bar: 10  $\mu$ m.



**Figure S8. Time-dependent co-localization increased in monensin-treated MEF cells.** LD-Blue<sup>+</sup> MEF cells were maintained in DMEM supplemented with or without 10  $\mu$ M monensin for 1, 6, 12 and 24 h, respectively, and then stained with lysoTracker Red (1  $\mu$ M, 1 h). All the cells were analyzed by confocal laser microscopy.



**Figure S9.** Monensin treatment leads to a decrease in lipid droplet fluorescence. MEF cells were maintained in DMEM spiked with or without 10  $\mu$ M monensin for 1 h, 6 h, 12 h and 24 h respectively respectively and then stained with LD-Blue (2  $\mu$ M, 1 h). All the cells were analyzed by confocal laser microscopy.



**Figure S10.**  $^1\text{H-NMR}$  spectrum of LD-Blue.

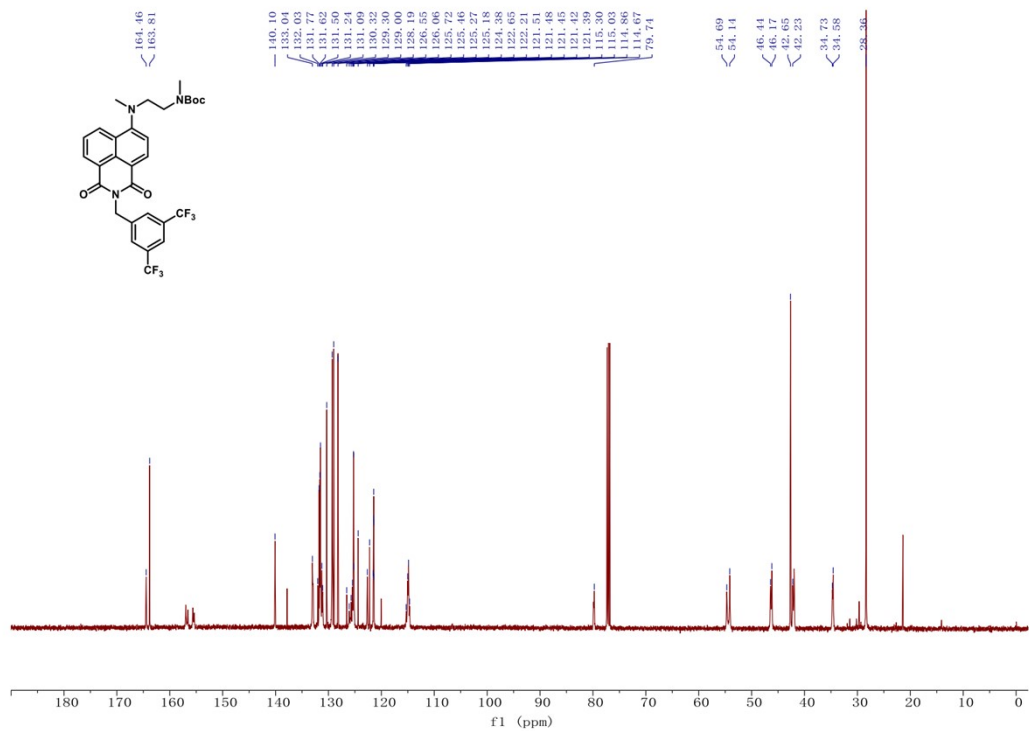


Figure S11. <sup>13</sup>C-NMR spectrum of LD-Blue.

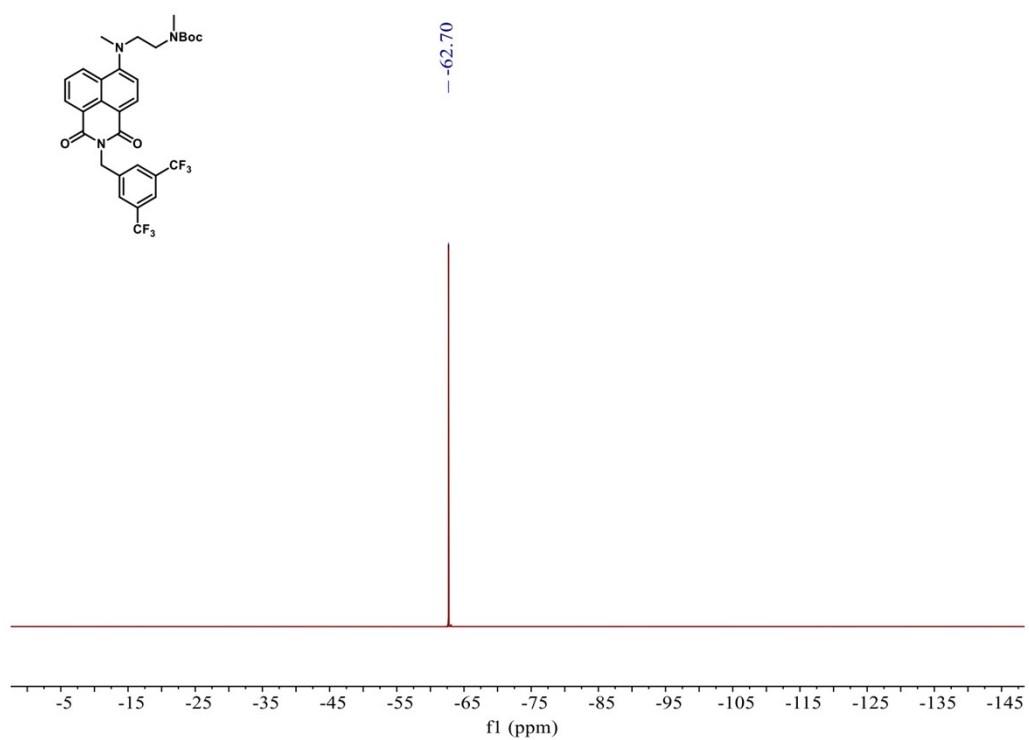


Figure S12. <sup>19</sup>F-NMR spectrum of LD-Blue.

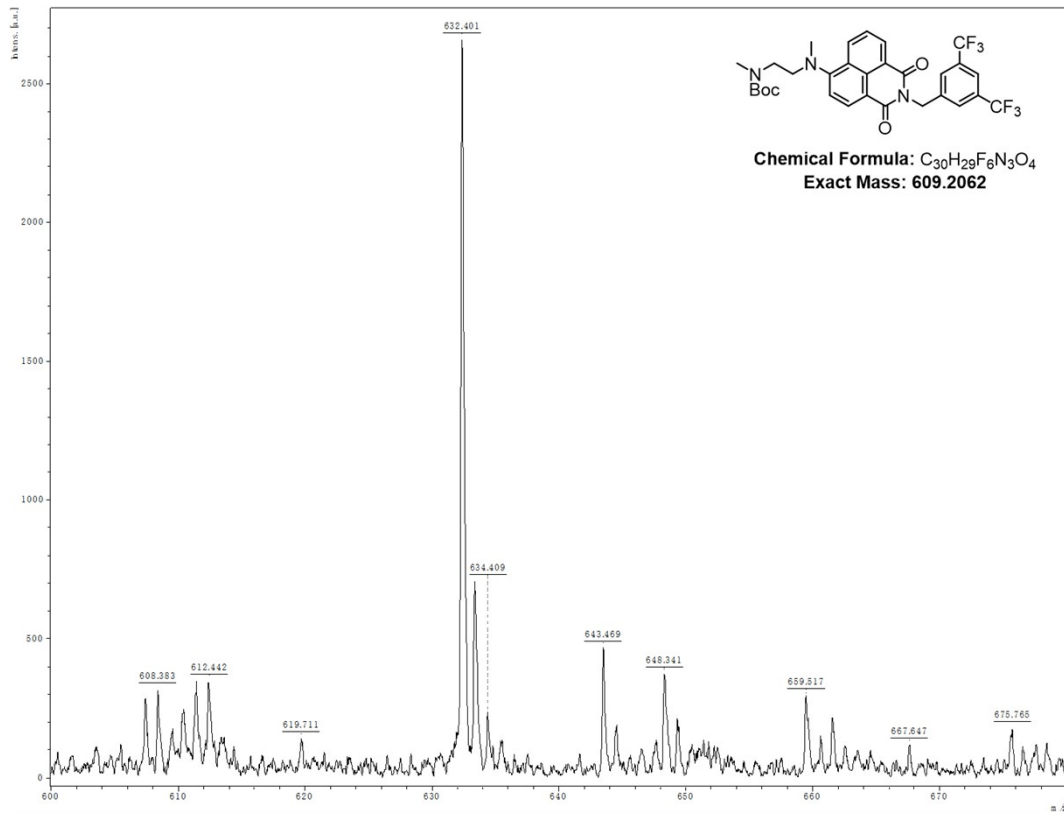


Figure S13. MALDI-TOF MS of LD-Blue.