

Supplementary

# A BODIPY-Based Fluorogenic Probe for Specific Imaging of Lipid Droplets

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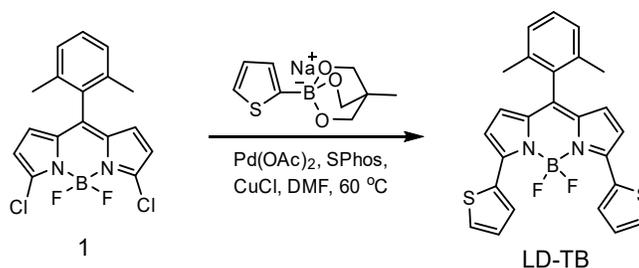
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## Synthesis of 8-(2,6-dimethylphenyl)-3,5-bis(2-thienyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (LD-TB)


 LD-TB was synthesized according to an earlier reported protocol<sup>1</sup>.

3,5-dichloroBODIPY **1** (91 mg, 0.25 mmol), (2-Thiophene)cyclic-triolborate Sodium Salt (175 mg, 0.75 mmol), Palladium(II) acetate [Pd(OAc)<sub>2</sub>] (6 mg, 0.027 mmol), 2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos) (12 mg, 0.029 mmol), and CuCl (0.10 mmol) were placed in a 10 ml two-necked round bottom flask. The flask was purged with N<sub>2</sub> gas three times, and then added *N,N*-dimethylformamide (DMF) 2.5 mL. After stirring at 60 °C for 20 h, the reaction mixture was cooled to room temperature, then extracted with ethyl acetate and washed with brine successively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, evaporated and the crude sample was purified by silica gel column chromatography using gradient eluent with eluent pair toluene/hexane, to give LD-TB (48 mg, 42%) as a dark red solid.

R<sub>f</sub> = 0.22 (toluene:hexane 1:2), M.p. 218–220 °C

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) = 8.23 (dd, *J* = 3.8 Hz, *J* = 0.8 Hz, 2H, thio-H), 7.48 (dd, *J* = 5.4 Hz, *J* = 0.8 Hz, 2H, thio-H), 7.30–7.25 (m, 1H, Ph-H), 7.2–7.19 (m, 2H, thio-H), 7.14 (d, *J* = 7.6 Hz, 2H, Ph-H), 6.75 (d, *J* = 4.3 Hz, 2H, Pyr-H), 6.53 (d, *J* = 4.3 Hz, 2H, Pyr-H), 2.19 (s, 6H, CH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 153.3, 140.1, 137.1, 136.6, 134.3, 133.2, 131.2 (t, *J*<sub>C-F</sub> = 8.2 Hz), 129.2, 129.1, 128.8, 128.6, 127.4, 120.7, 20.1.

MS (ESI) *m/z*: [M+H]<sup>+</sup> calcd for C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>BF<sub>2</sub>S<sub>2</sub> 461.11, found 461.12; [M+Na]<sup>+</sup> calcd for C<sub>25</sub>H<sub>19</sub>N<sub>2</sub>BF<sub>2</sub>S<sub>2</sub>Na 483.09, found 483.10.

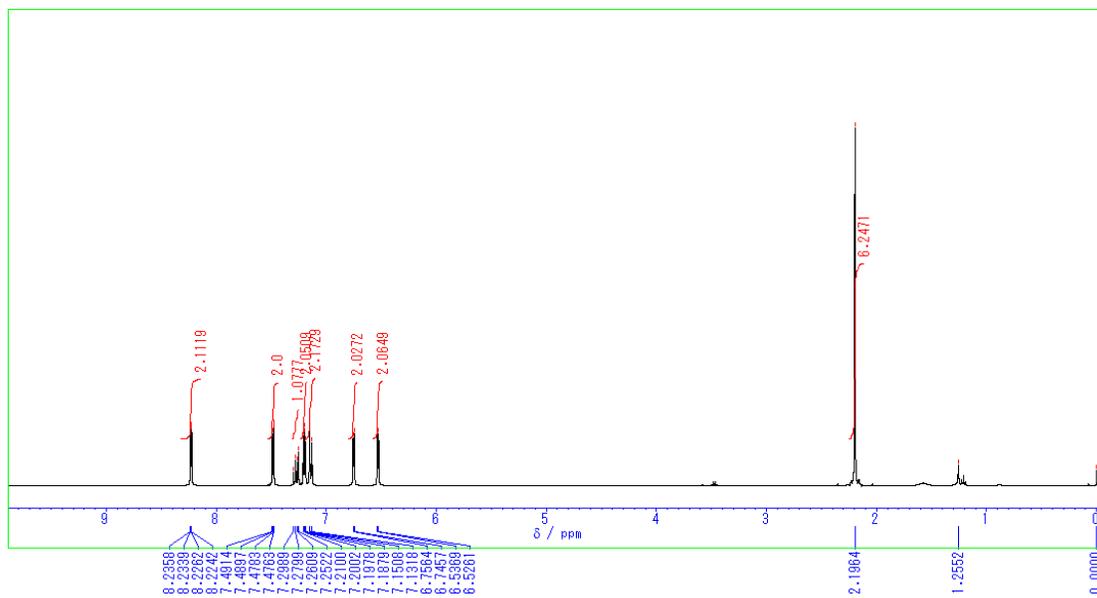


Figure S1. <sup>1</sup>H NMR spectrum of LD-TB (400 MHz, CDCl<sub>3</sub>) at room temperature.

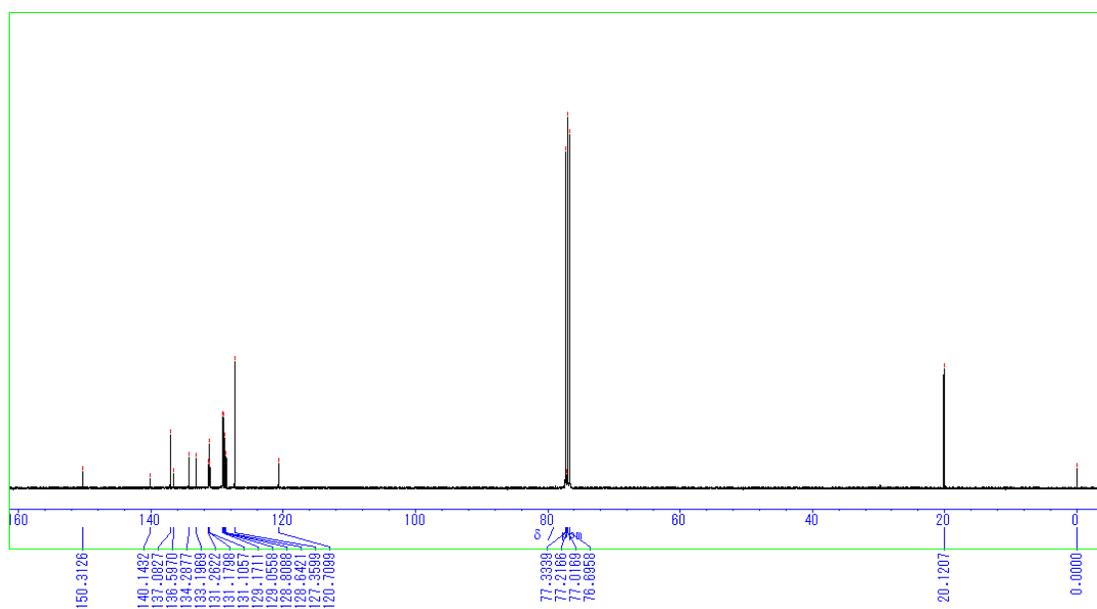
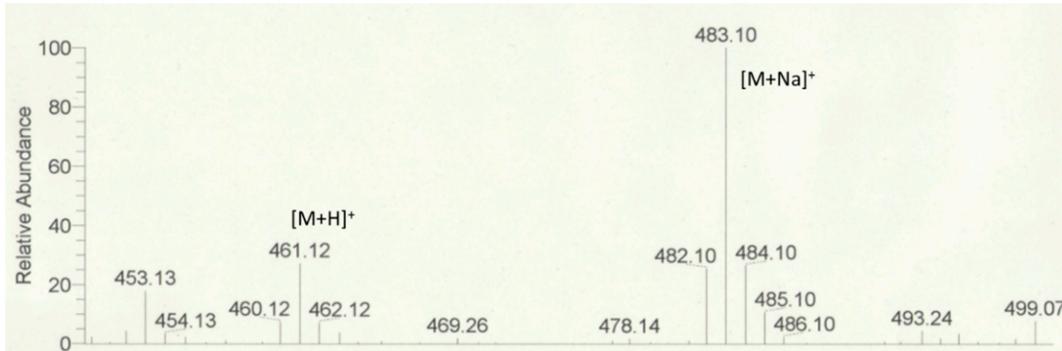
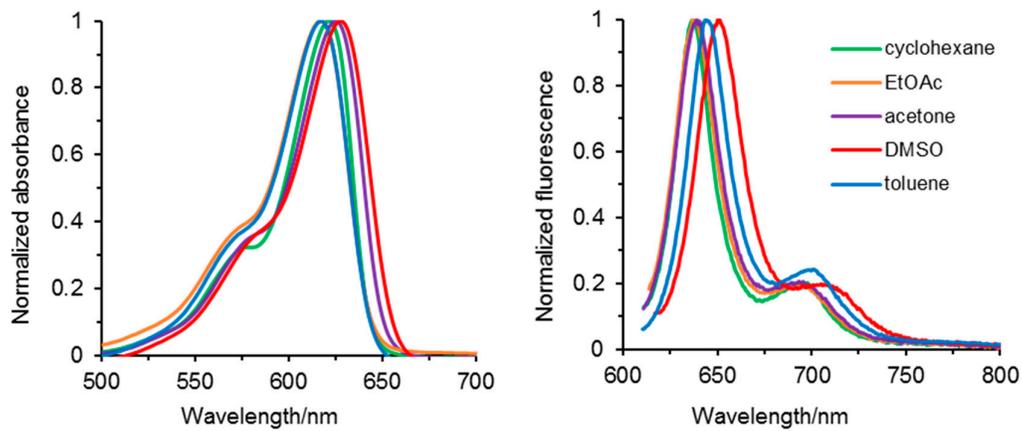


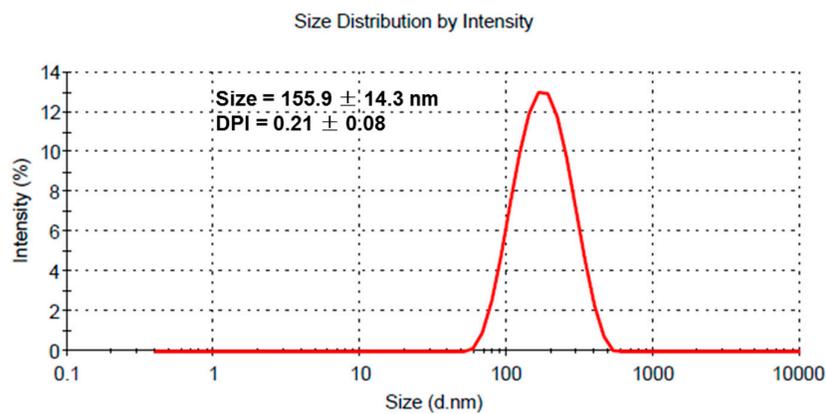
Figure S2. <sup>13</sup>C NMR spectrum of LD-TB (100 MHz, CDCl<sub>3</sub>) at room temperature.



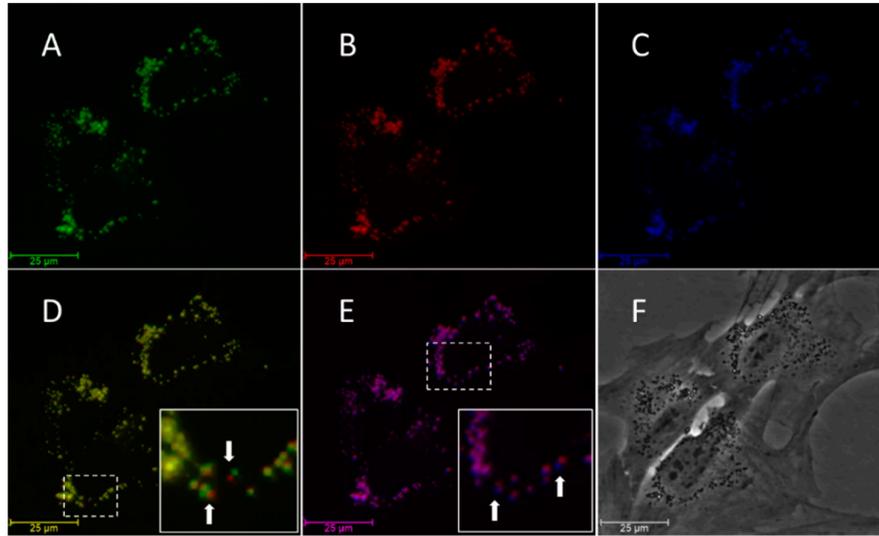
**Figure S3.** Mass spectrum of LD-TB.



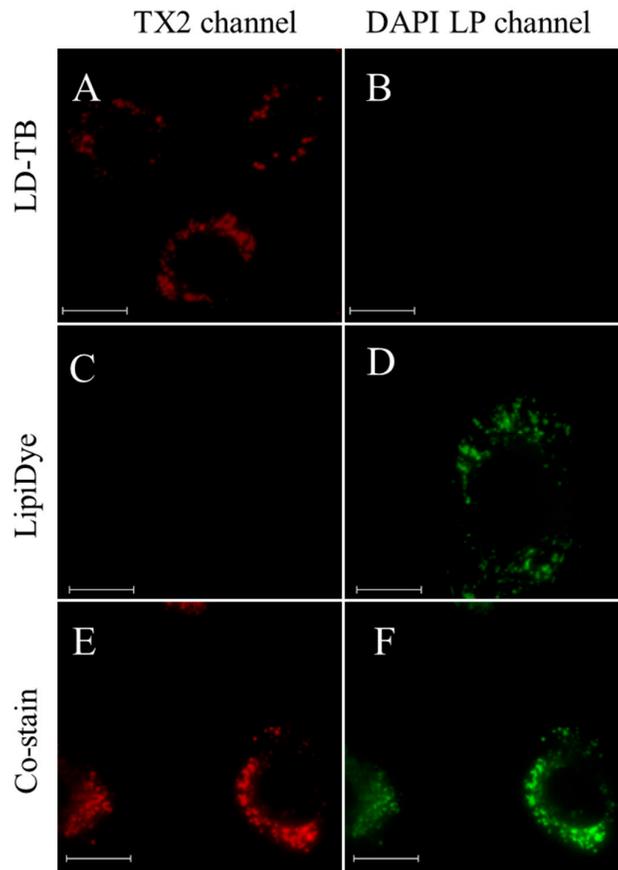
**Figure S4.** The normalized absorption spectra (left) and normalized emission spectra (right) of LD-TB (1  $\mu$ M) in various organic solvents.



**Figure S5.** DLS measurements of LD-TB in 0.5% DMSO/H<sub>2</sub>O at 0.05  $\mu$ M.

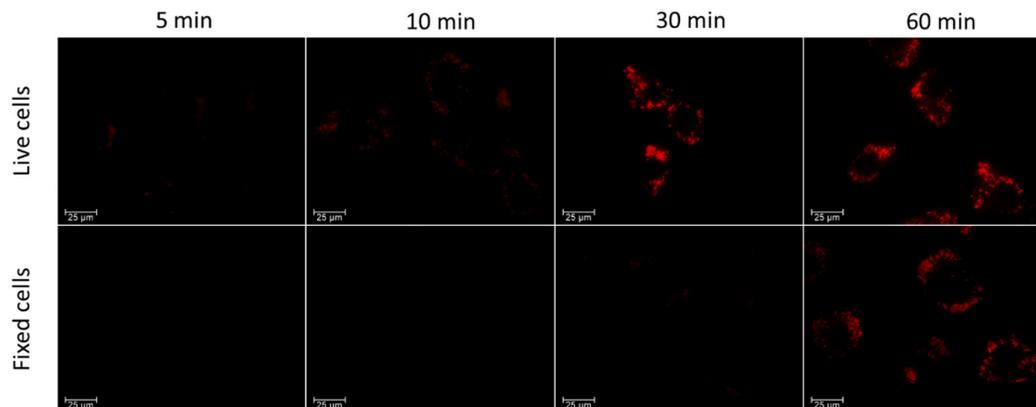


**Figure S6.** Fluorescence images of bovine cumulus cells stained with  $0.05 \mu\text{M}$  LD-TB 30 min. Different false color (green, red, blue) are used to present the images captured at different times of 0, 1, 5 min (A–C). Merged images at different times, 0 and 1 min (D), 1 and 5 min (E), respectively. Bright field (F). Movement of the stained vesicles can be easily observed in the merged images. Scale bar  $25 \mu\text{m}$ .

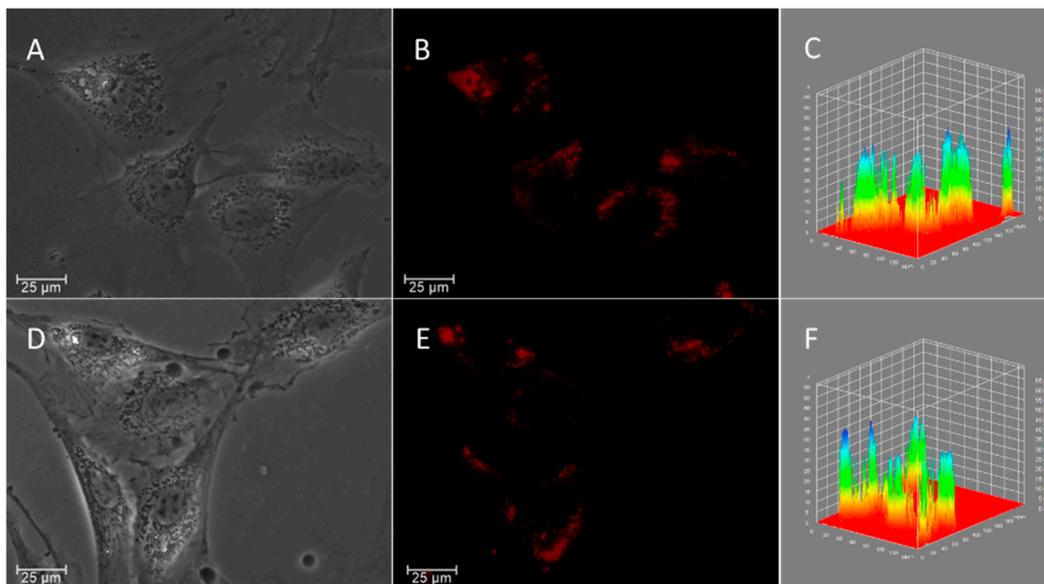


**Figure S7.** Fluorescence images of bovine cumulus cells: (A and B) stained with  $0.05 \mu\text{M}$  LD-TB 30 min, (C and D) stained with  $3 \mu\text{M}$  LipidDye 2 h, (E and F) stained with both dyes, firstly  $3 \mu\text{M}$  LipidDye

1.5 h and then co-stain with 0.05  $\mu\text{M}$  LD-TB for further 30 min. No cross-talk was observed in the colocalization experiments. Scale bar: 20  $\mu\text{m}$ .



**Figure S8.** Images of live and fixed bovine cumulus cells stained with LD-TB (0.05  $\mu\text{M}$ ) for different period of time. For live cells, they were incubated at 38.5  $^{\circ}\text{C}$ . For fixed cell, they were fixed, washed and then incubated with LD-TB at room temperature. Conditions for images recording and post-processing were identical. Scale bar: 25  $\mu\text{m}$ .



**Figure S9.** Images of bovine cumulus cells incubated with LD-TB (0.05  $\mu\text{M}$ ) for 30 min of live cells (A–C), and the images of cells incubated with LD-TB (0.05  $\mu\text{M}$ ) for 30 min of live cells and then fixed with PFA (D–F). (A and D) Bright field images. (B and E) Fluorescence images. (C and F) Image J 3D surface plot analysis. Conditions for images recording and post-processing were identical. No fluorescence quenching caused by fixation process was observed. Scale bar: 25  $\mu\text{m}$ .

## Reference

- Li, G.; Otsuka, Y.; Matsumiya, T.; Suzuki, T.; Li, J.; Takahashi, M.; Yamada, K. A Straightforward Substitution Strategy to Tune BODIPY Dyes Spanning the Near-Infrared Region via Suzuki–Miyaura Cross-Coupling. *Materials* **2018**, *11*, 1297.



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