

Supporting Information

Specific Imaging of Intracellular Lipid Droplets Using a Benzothiadiazole Derivative with Solvatochromic Properties

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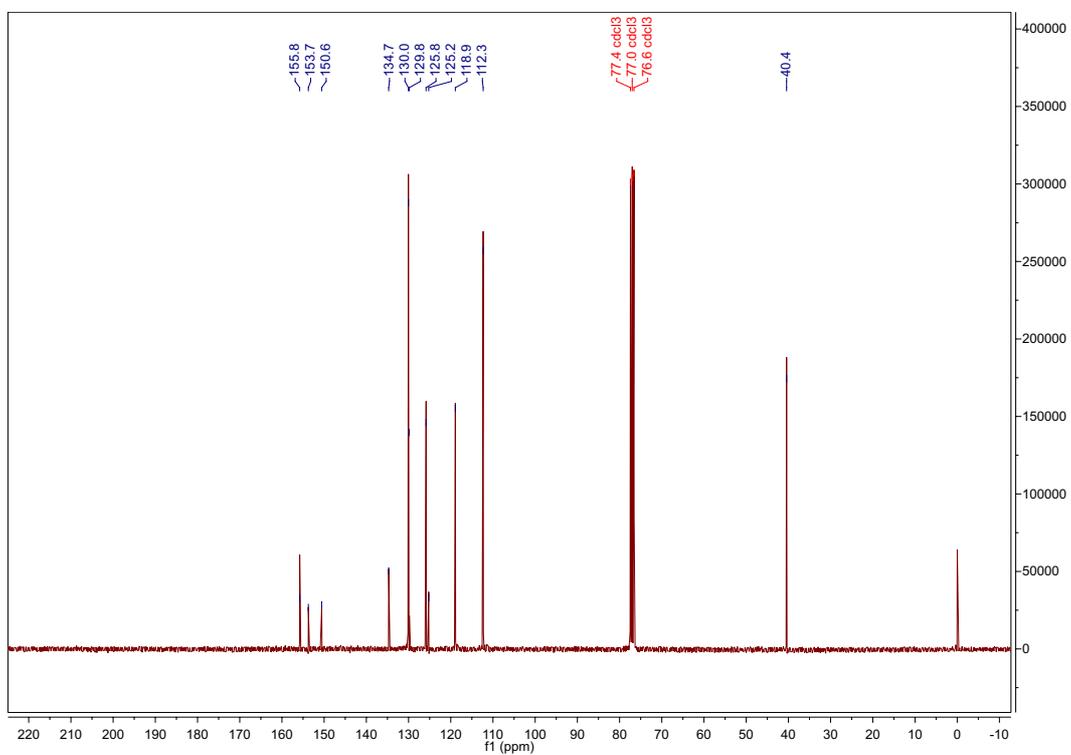
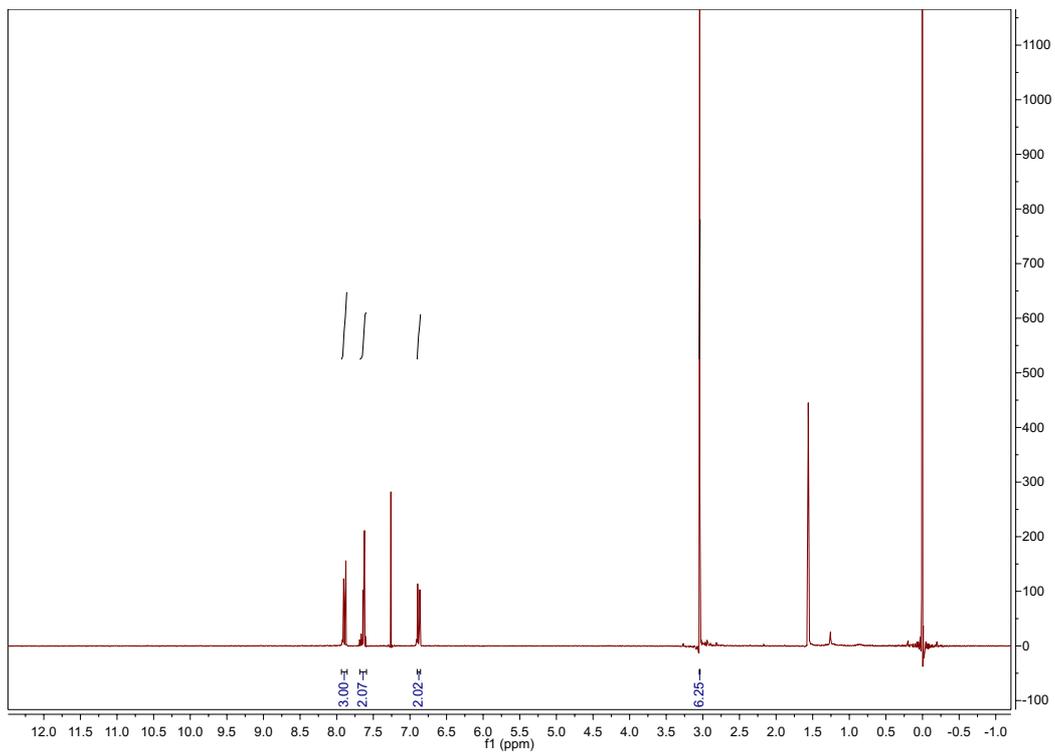
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$^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of **LD-BTD1** in CDCl_3 .



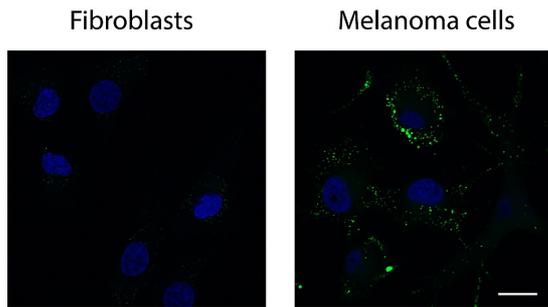


Figure S1. Human fibroblasts and melanoma cells stained with **LD-BTD1** (seen in green; ex 458 nm, em 465-682 nm) after cell fixation (500 nM, 30 min, RT). Cell nuclei are stained with DAPI (seen in blue; ex 405 nm, em 410-453 nm). Scale bar 10 μ m.

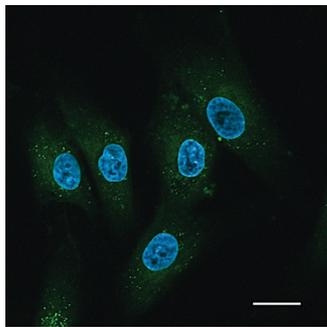


Figure S2. Human fibroblasts stained with **LD-BTD1** (seen in green; ex 458 nm, em 465-682 nm) at 10 μ M for 24 h. Images were taken with higher gain compared to Figure 1A. Cell nuclei are stained with DAPI (seen in blue; ex 405 nm, em 410-453 nm). Scale bar 10 μ m. Living cells were stained with **LD-BTD1**, fixated after 24h, and mounted prior to microscopy analysis.

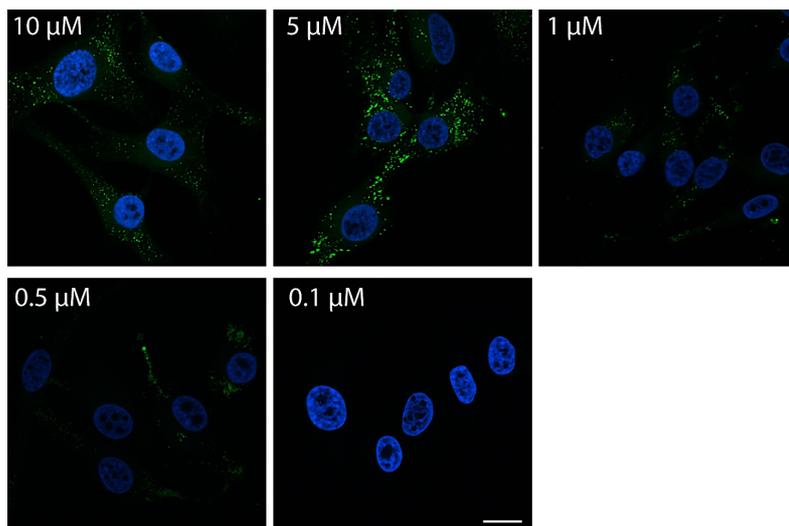


Figure S3. Melanoma cells stained with **LD-BTD1** (seen in green, ex 458 nm, em 465-682 nm) using different concentrations (0.1, 0.5, 1, 5, and 10 μ M). Cell nuclei are stained with DAPI (seen in blue; ex 405 nm, em 410-453 nm). Scale bar 10 μ m. **LD-BTD1** staining was performed on living cells, which were fixated after 24 h incubation (prior to the microscopy analysis).

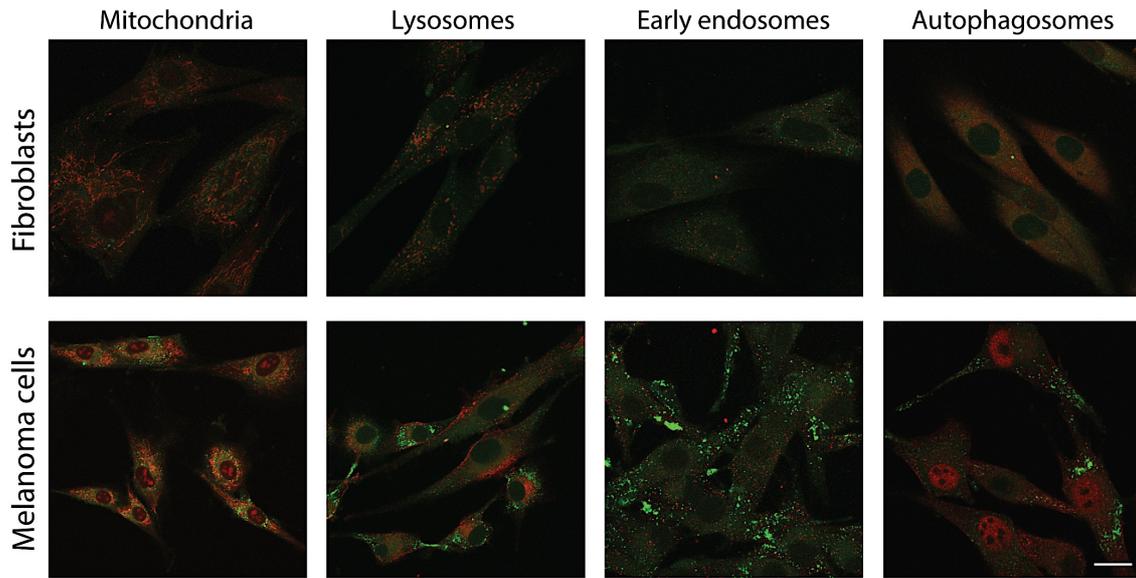


Figure S4. Co-staining of **LD-BTD1** (10 μ M, 24h) seen in green (ex 458 nm, em 484-707)* and markers (seen in red) for mitochondria (Mitotracker Orange CMTMRos; ex 550 nm, em 555-750 nm), lysosomes (LAMP-2), early endosomes (EEA1) and autophagosomes (LC3B). Secondary antibodies for LAMP-2, EEA1 and LC3B were conjugated to Alexa Fluor 594 (ex 595 nm, em 600-734). Scale bar 10 μ m. **LD-BTD1** staining was performed on living cells, which were fixated directly after the incubation (prior to the microscopy analysis). *The emission range for **LD-BTD1** in the co-localization experiment with mitochondria were: em 466-545 nm.

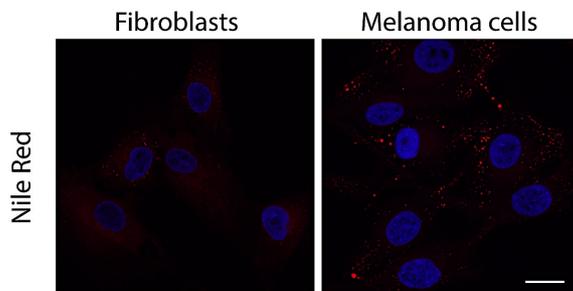


Figure S5. Fibroblasts and melanoma cells stained with Nile Red (0.1 μ g/ml in 150 mM NaCl, 10 min) seen in red (ex 458 nm, em 465-682nm). Cell nuclei are stained with DAPI (seen in blue; ex 405 nm, em 410-453 nm). Scalebar 10 μ m. Nile Red staining was performed on fixed cells.

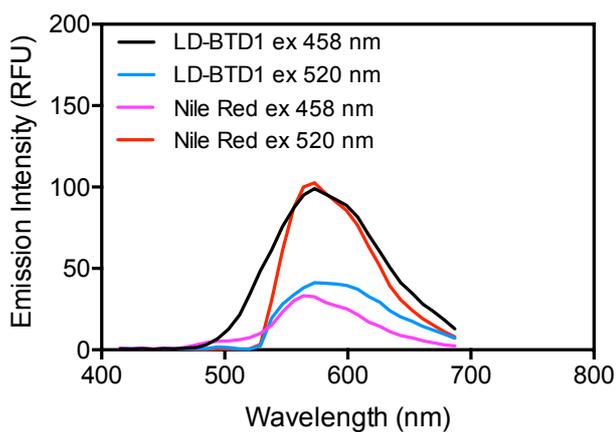


Figure S6. Emission spectra from the confocal microscopy study of melanoma cells stained with **LD-BTD1** or Nile Red using two different excitation wavelengths (458 and 520 nm).

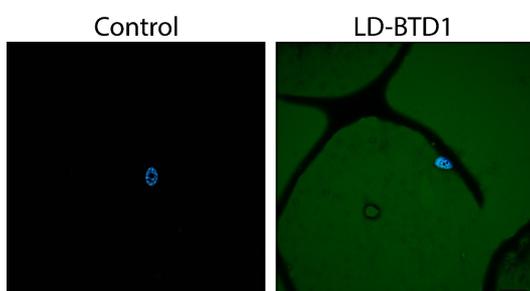


Figure S7. Isolated human adipocytes stained with **LD-BTD1** (seen in green; ex 458 nm, em 465-682 nm) at 10 μ M for 24 h. Cell nuclei are stained with DAPI (seen in blue; ex 405 nm, em 410-453 nm). Scalebar 20 μ m. **LD-BTD1** staining was performed on living cells, which were fixated directly after the incubation (prior to the microscopy analysis).

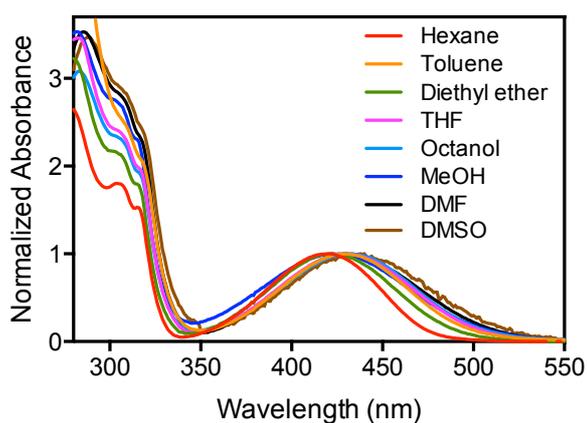


Figure S8. Normalized absorbance spectra of **LD-BTD1** in solvents of different polarity.

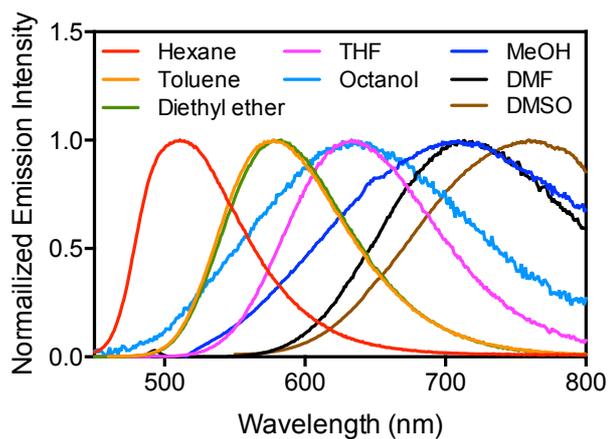


Figure S9. Normalized emission spectra of **LD-BTD1** in solvents of different polarity.

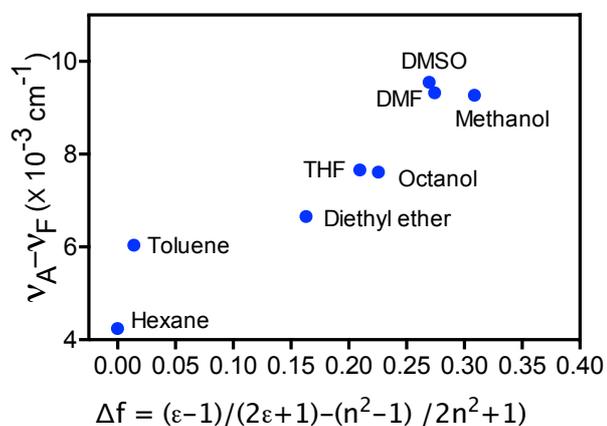


Figure S10. Lippert plot for **LD-BTD1** in solvents of different polarity. The orientation polarizability Δf is dependent on the refractive index (n) and the dielectric constant (ϵ) of the solvent.

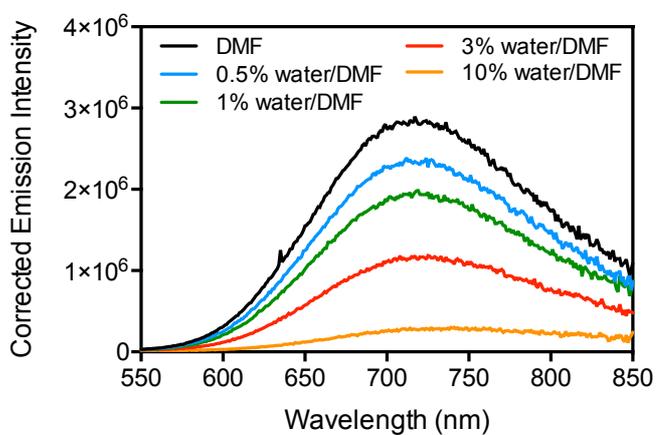


Figure S11. Emission spectra (corrected by sample absorbance change upon addition of water) of **LD-BTD1** in DMF upon increasing concentration of water in the solution.

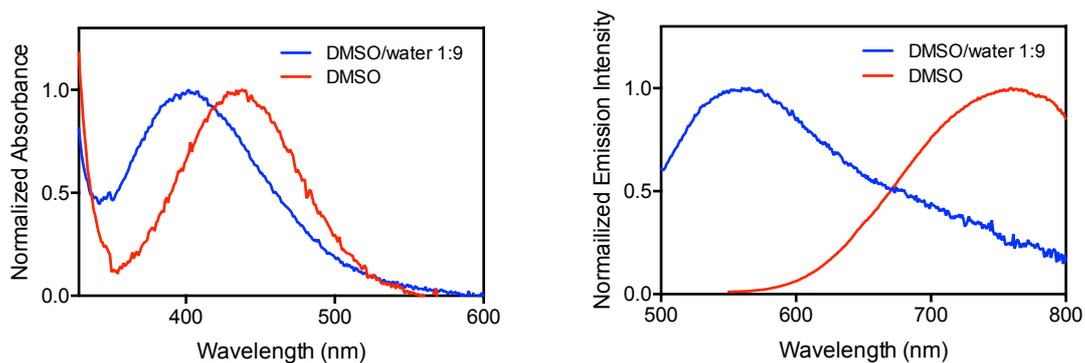


Figure S12. Normalized absorbance (left) and emission spectra (right) of **LD-BTD1** in DMSO and DMSO/water 1:9.

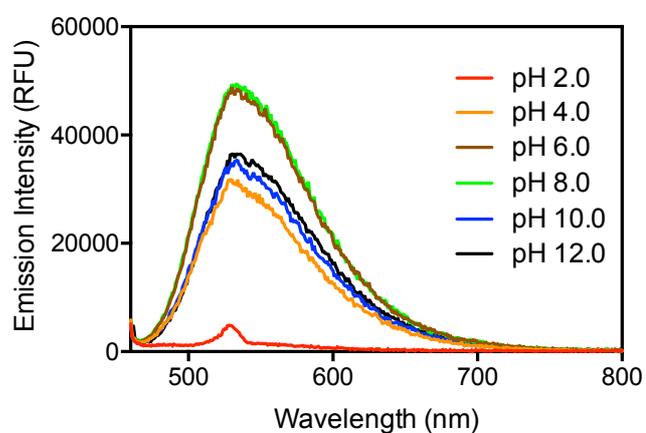


Figure S13. Emission spectra of **LD-BTD1** (5% DMSO in buffer) at different pH (2-12). pH 2 and 4: Glycine HCl buffer (50 mM); pH 6 and 8: phosphate buffer (25 mM); and pH 10 and 12: Glycine OH buffer (50 mM).