Fluorescent macrocyclic probes with pendant functional groups as markers of acidic organelles within live cells

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Figure S-1. A) ¹H NMR spectrum of compound **8** in DMSO- d_6 . **B)** ¹³C NMR spectrum of compound **8** in DMSO- d_6 . **C)** ESI-TOF spectrum of compound **8** and simulated pattern for $[M+H]^+$.



Figure S-2. A) ¹H NMR spectrum of compound **9** in DMSO- d_6 . B) ¹³C NMR spectrum of compound **9** in CDCl₃. C) ESI-TOF spectrum of compound **9** and simulated pattern for [M+H]⁺.



Figure S-3. A) ¹H NMR spectrum of compound **10** in CDCl₃. **B)** ¹³C NMR spectrum of compound **10** in CDCl₃. **C)** ESI-TOF spectrum of compound **10** and simulated pattern for $[M+H]^+$.



Figure S-4. A) ¹H NMR spectrum of compound **1** in DMSO- d_6 . B) ¹³C NMR spectrum of compound **1** in DMSO- d_6 . C) ESI-TOF spectrum of compound **1** and simulated pattern for $[M+H]^+$.



Figure S-5. A) ¹H NMR spectrum of compound **2** in DMSO- d_{δ} . **B)** ¹³C NMR spectrum of compound **2** in DMSO- d_{δ} . **C)** ESI-TOF spectrum of compound **2** and simulated pattern for [M+H]⁺.



Figure S-6. A) ¹H NMR spectrum of compound **16** in DMSO- d_6 . B) ¹³C NMR spectrum of compound **16** in DMSO- d_6 . C) ESI-TOF spectrum of compound **16** and simulated pattern for [M+H]⁺.



Figure S-7. A) ¹H NMR spectrum of compound **17** in DMSO- d_6 . **B)** ¹³C NMR spectrum of compound **17** in CDCl₃. **C)** ESI-TOF spectrum of compound **17** and simulated pattern for [M+H]⁺.



Figure S-8. A) ¹H NMR spectrum of compound **18** in DMSO- d_6 . B) ¹³C NMR spectrum of compound **18** in DMSO- d_6 . C) ESI-TOF spectrum of compound **18** and simulated pattern for [M+H]⁺.



Figure S-9. A) ¹H NMR spectrum of compound **3** in CD₃CN. **B)** ¹³C NMR spectrum of compound **3** in CD₃CN. **C)** ESI-TOF spectrum of compound **3** and simulated pattern for $[M+H]^+$.



Figure S-10. A) ¹H NMR spectrum of compound **4** in DMSO- d_6 . B) ¹³C NMR spectrum of compound **4** in DMSO- d_6 . C) ESI-TOF spectrum of compound **4** and simulated pattern for $[M+H]^+$.



Figure S-11. A) Normalized absorption spectra of the anthracenophanes **1-4** in H₂O (0.2% DMSO) at pH 1.7, probe concentration 2 μ M. **B**) Normalized emission spectra of the anthracenophanes **1-4** in H₂O (0.2% DMSO) at pH 1.7, probe concentration 2 μ M, $\lambda_{exc} = 374$ nm.



Figure S-12. Fluorescence decay traces of the anthracenophanes; A) 1 and 2, and B) 3 and 4 in H₂O (0.2% DMSO) at pH 1.7 (λ_{exc} = 372 nm, λ_{em} = 420 nm). Probe concentration 2 μ M.



Figure S-13. Distribution and colocalization experiments with probe **2** and LysoSensor Green DND-189. **A**) Fluorescence images of the compound **2**, collected with a confocal laser scanning microscope in the blue channel **B**) Fluorescence images of the DND-189 probe collected with a confocal laser scanning microscope in the green channel. **C**) Differential interference contrast (DIC) images collected with a confocal laser scanning microscope in the DIC mode. **D**) Composite images of blue, green and DIC channels.



Figure S-14. Distribution and colocalization experiments with probe **1** and LysosSensor Green DND-189. **A**) Fluorescence image of the compound **1**, collected with a confocal laser scanning microscope in the blue channel. **B**) Fluorescence image of the DND-189 probe collected with a confocal laser scanning microscope in the green channel. **C**) Merged image of the blue and the green channels. **D**) DIC image collected with a confocal laser scanning microscope in the DIC mode. **E**) Composite image of blue, green and DIC channels.



Figure S-15. Distribution and colocalization experiments with probe **1** and LysoSensor Green DND-189. **A**) Fluorescence images of the compound **1**, collected with a confocal laser scanning microscope in the blue channel. **B**) Fluorescence images of the DND-189 probe collected with a confocal laser scanning microscope in the green channel. **C**) DIC images collected with a confocal laser scanning microscope in the DIC mode. **D**) Composite images of blue, green and DIC channels.



Figure S-16. Fluorescence emission spectra of probe 2 from within the intracellular environment.



Figure S-17. Intracellular emission spectra of probe 1. A) DIC image was collected with a confocal microscope in the DIC mode. B) Fluorescence image of 1 was collected with a confocal microscope in the blue channel. C) Composite image of blue and DIC channels.
D) Fluorescence spectra of compound 1 inside the cell selecting different areas within the cell.



Figure S-18. Fluorescence emission spectra of probe **1** from within the intracellular environment.