Electronic Supplementary Material (ESI) for Chemical Science.

Electronic Supplementary Information

Phosphorescent soft salt for ratiometric and lifetime imaging of intracellular pH variations

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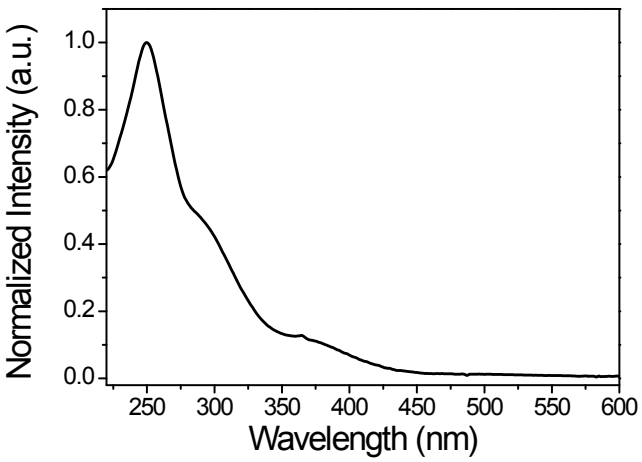

Fig. S1 Normalized absorption spectrum of **S1** in acetonitrile solution.

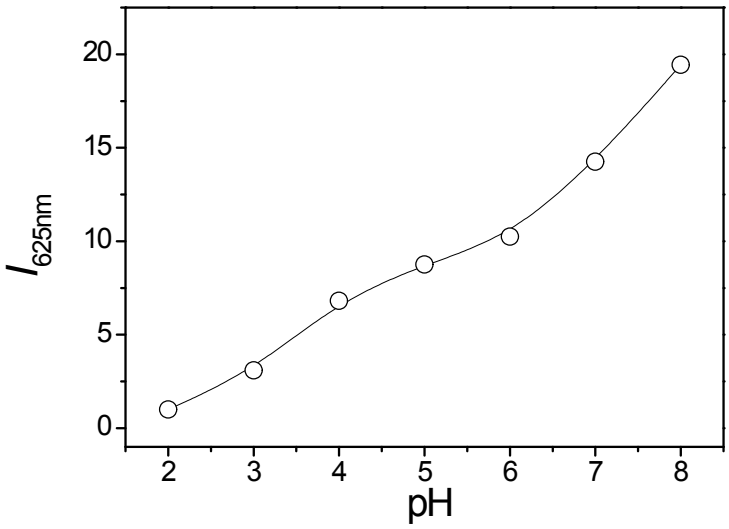

Fig. S2 Plot of I_{625nm} versus pH value.

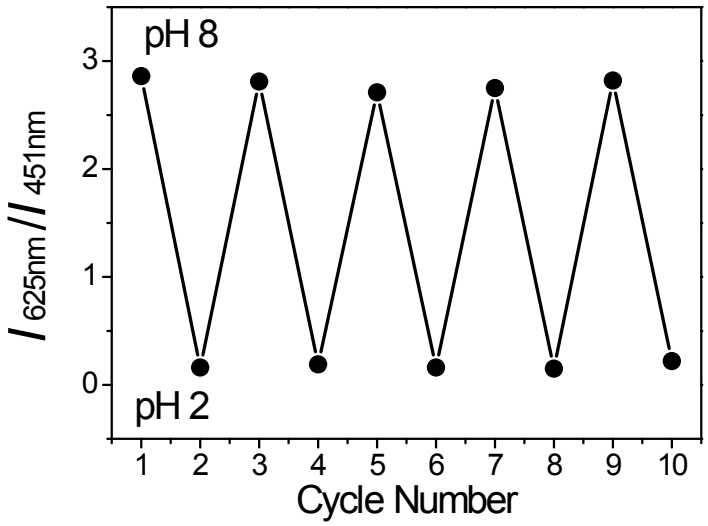

Fig. S3 pH reversibility study of **S1** between pH 2 and 8.

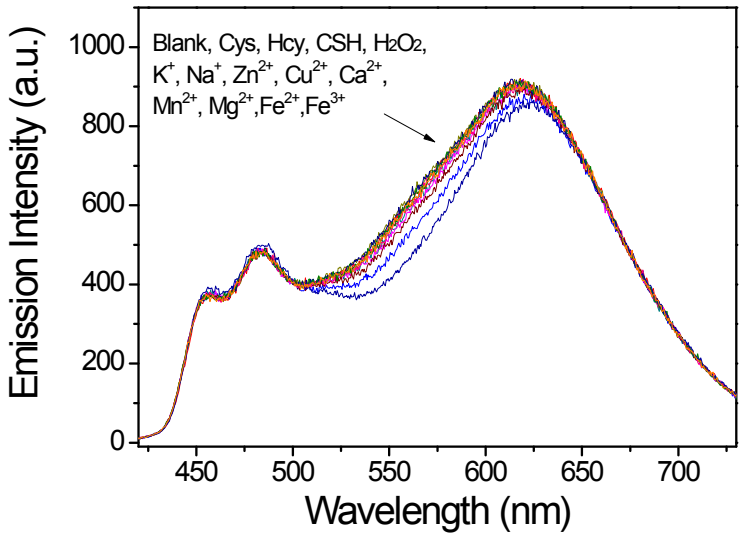

Fig. S4 Phosphorescence spectra of **S1** (2.0×10^{-5} M) in the presence of 1.0×10^{-4} M of oxidative-stress-associated redox chemicals and metal ions (Cys, Hcy, GSH, H_2O_2, K^+, Na^+, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}) in CH_3CN/buffer (1 : 9, v : v).

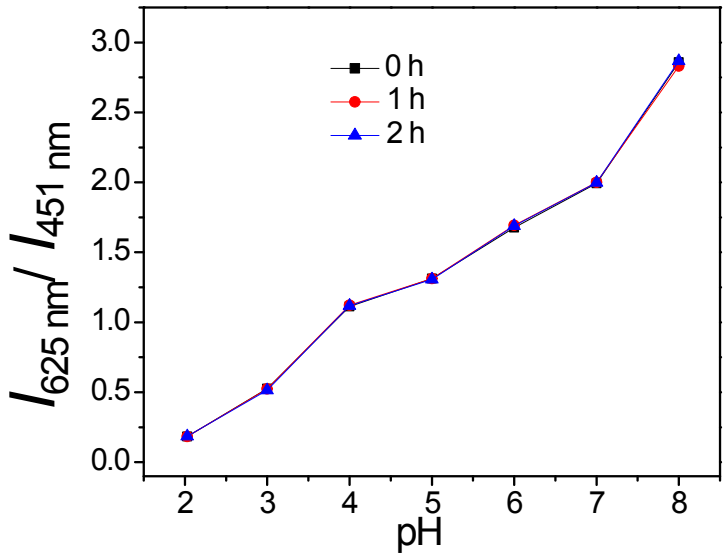

Fig. S5 Relative ratio of phosphorescence intensity (I_{625} nm/I_{451} nm) changes of **S1** in the pH range of 2–8 at 37 °C.

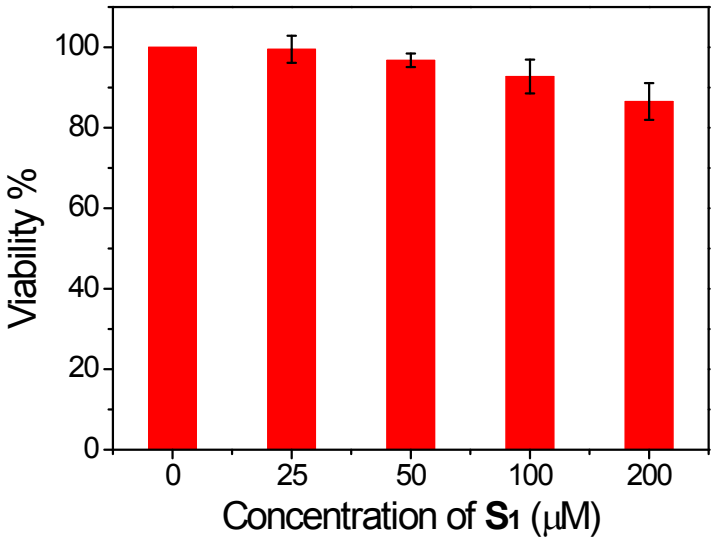

Fig. S6 Cell viability values (%) assessed using an MTT test versus incubation concentrations of **S1**. HepG-2 cells were cultured in the presence of 0–200 μM **S1** at 37 ^{o}C for 24 h.

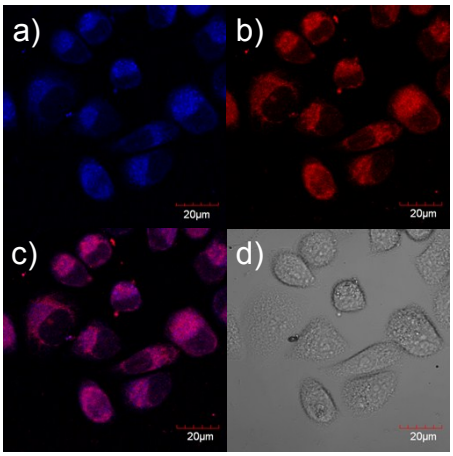

Fig. S7 (a, b) Confocal luminescence, (c) overlay images and (d) bright-field of living HepG-2 cells. HepG-2 cells were incubated with 10 μM **S1** for 1 h at 37 °C.

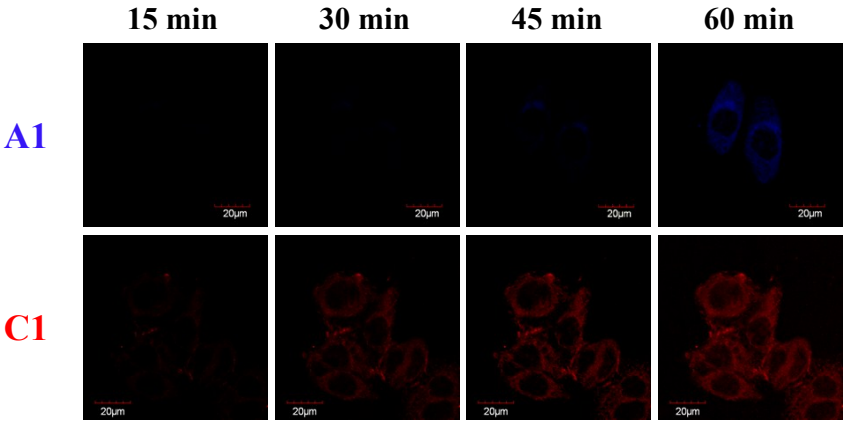

Fig. S8 Real-time monitoring of live cells stained with **A1** and **C1**, respectively.

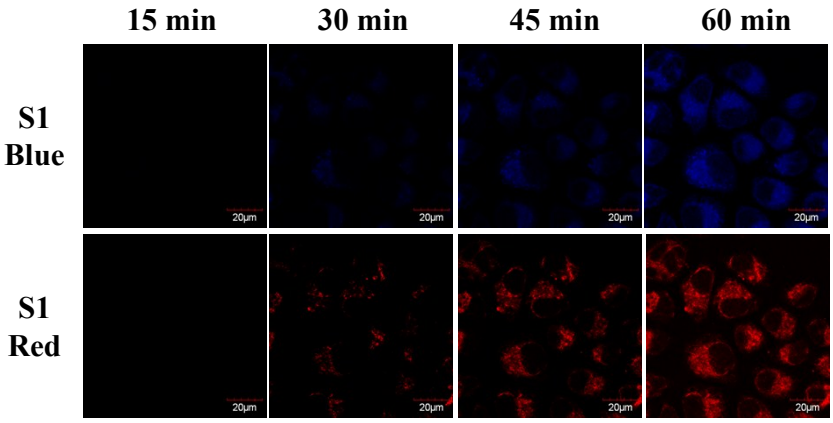

Fig. S9 Real-time monitoring of live cells stained with **S1**.

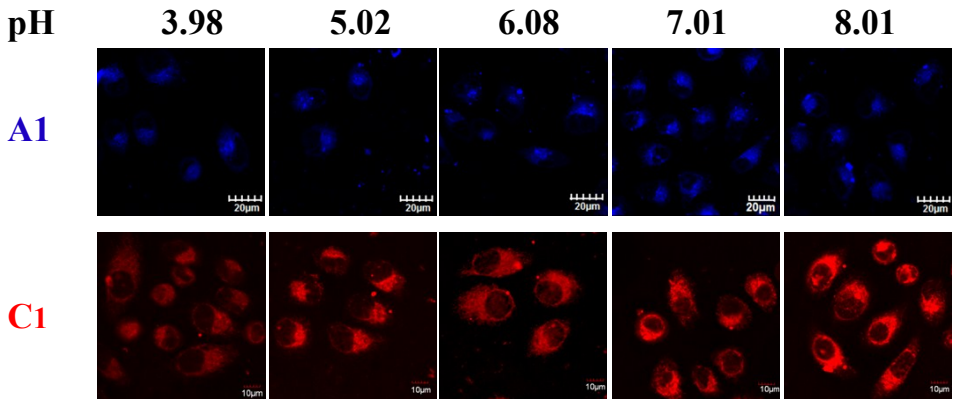

Fig. S10 Phosphorescence images of **A1** and **C1** in HepG-2 cells clamped at pH 3.98, 5.02, 6.08, 7.01 and 8.0, respectively.

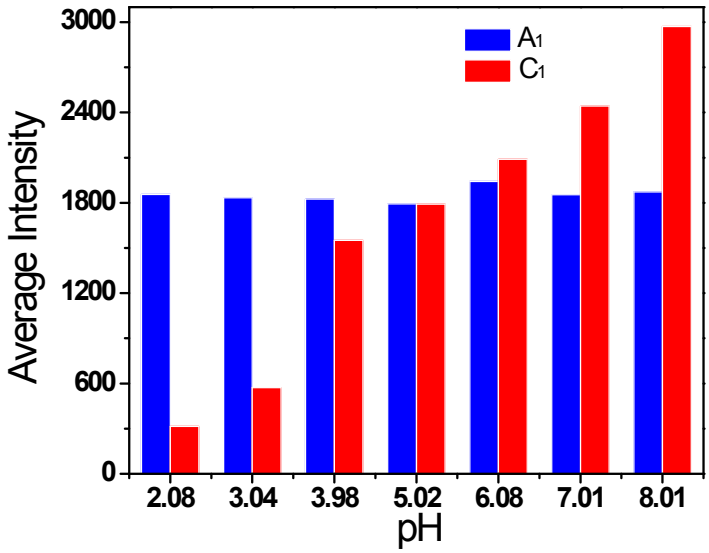

Fig. S11 Average intracellular emission intensity of **A1** and **C1** at different pH values.

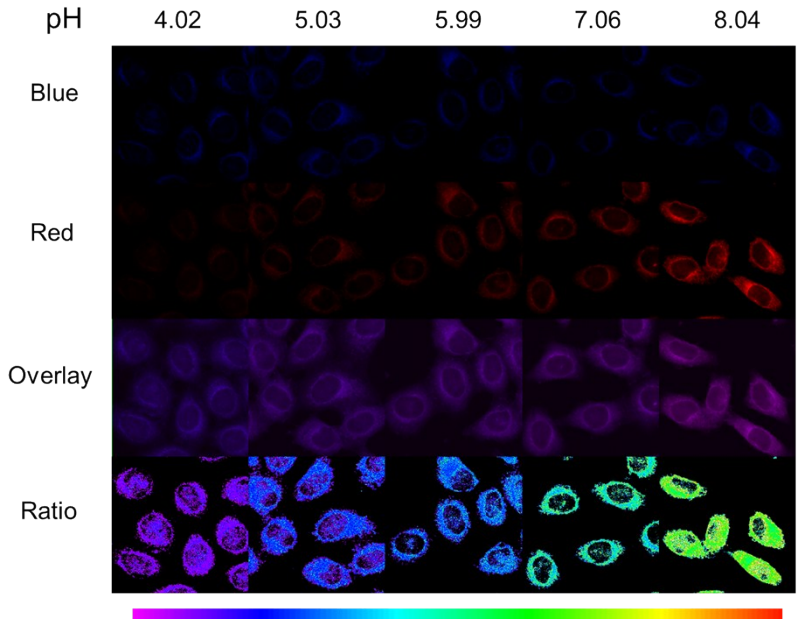

Fig. S12 Phosphorescence images of **S1** (1 μM) in living cells clamped at pH 4.02, 5.03, 5.99, 7.06 and 8.04, respectively. The excitation wavelength was 405 nm and the images of the first row (blue channel) and second row (red channel) were collected in the ranges of 430–480 nm and 600–700 nm, respectively. Overlay images (third row) and ratio images obtained from the red and blue channels (fourth row).

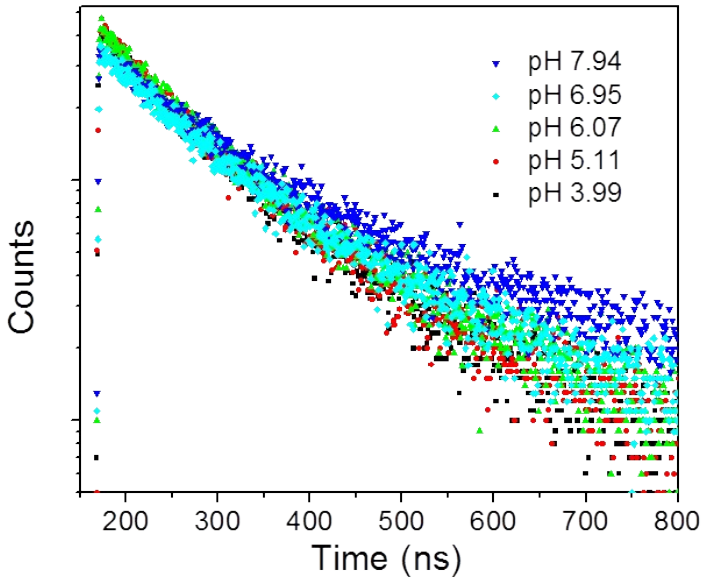

Fig. S13 Phosphorescence decay curves of **S1** (10 μM) at 625 nm at different pH values.

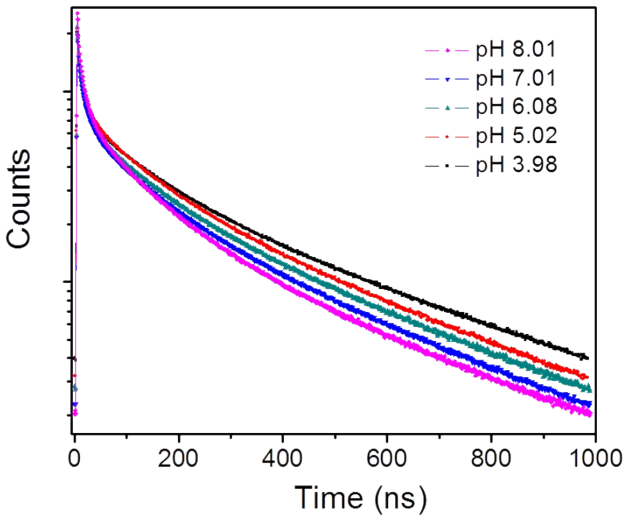

Fig. S14 Phosphorescence decay curves in cells stained with **S1** (10 μM) at different pH values, obtained by confocal TCSPC-PLIM.

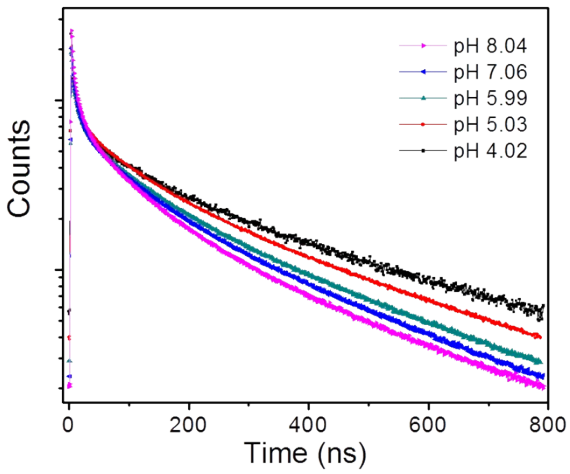

Fig. S15 Phosphorescence decay curves of **S1** (10 μM) in RPMI 1640 at different pH values, obtained by confocal TCSPC-PLIM.

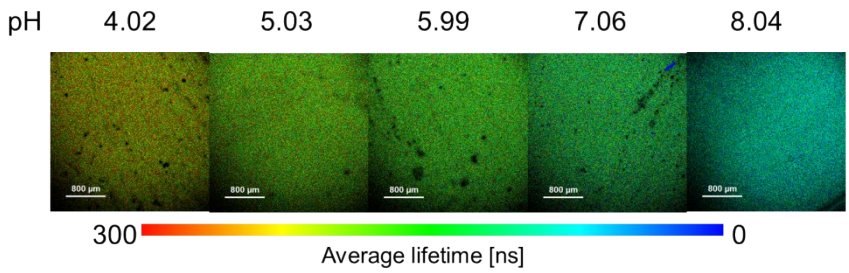

Fig. S16 Phosphorescence lifetime images of **S1** (10 µM) in RPMI 1640 at different pH values.

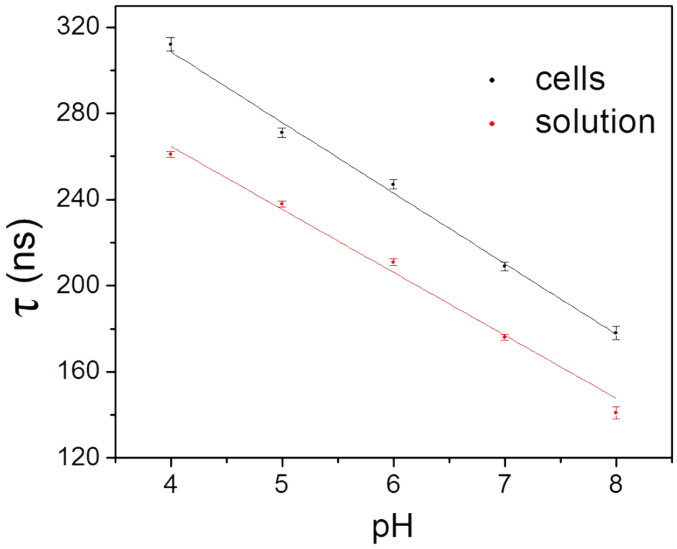

Fig. S17 Emission lifetime changes of **S1** (10 μM) at different pH values in the cells and RPMI 1640, respectively, obtained by confocal TCSPC-PLIM.

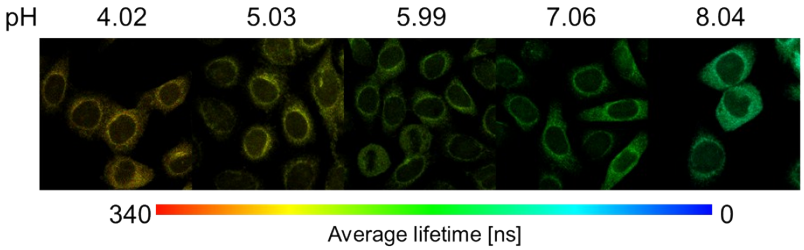

Fig. S18 Phosphorescence lifetime images of **S1** (1 μM) in living cells at different pH values.

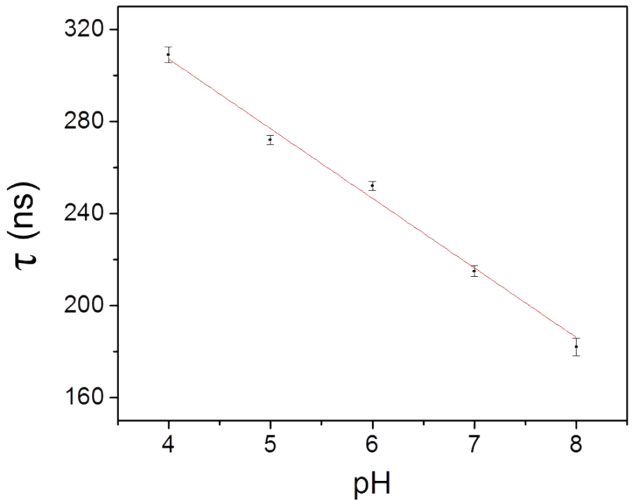

Fig. S19 Emission lifetime changes of **S1** (1 μM) at different pH values in the cells, obtained by confocal TCSPC-PLIM.

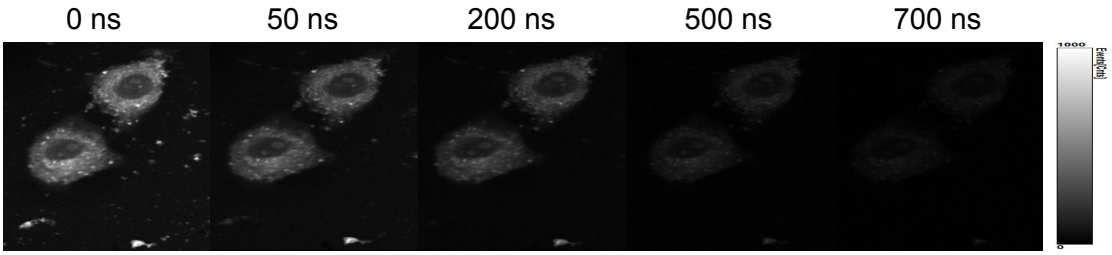

Fig. S20 Phosphorescence intensity images collected at different time ranges (0-700 ns).

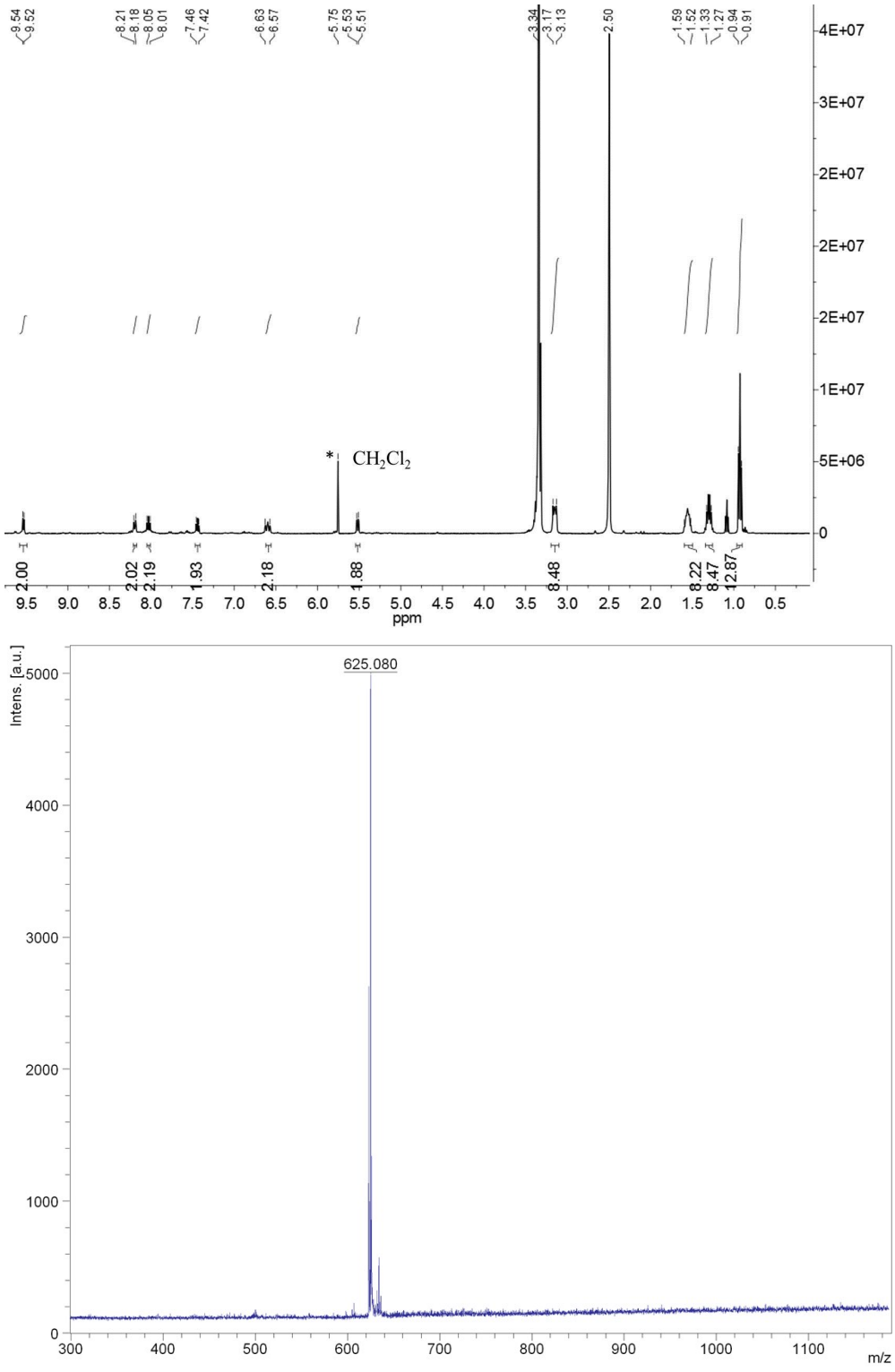

Fig. S21 ^{1}H NMR (400 MHz, DMSO-d_6) and mass spectra of **A1**.

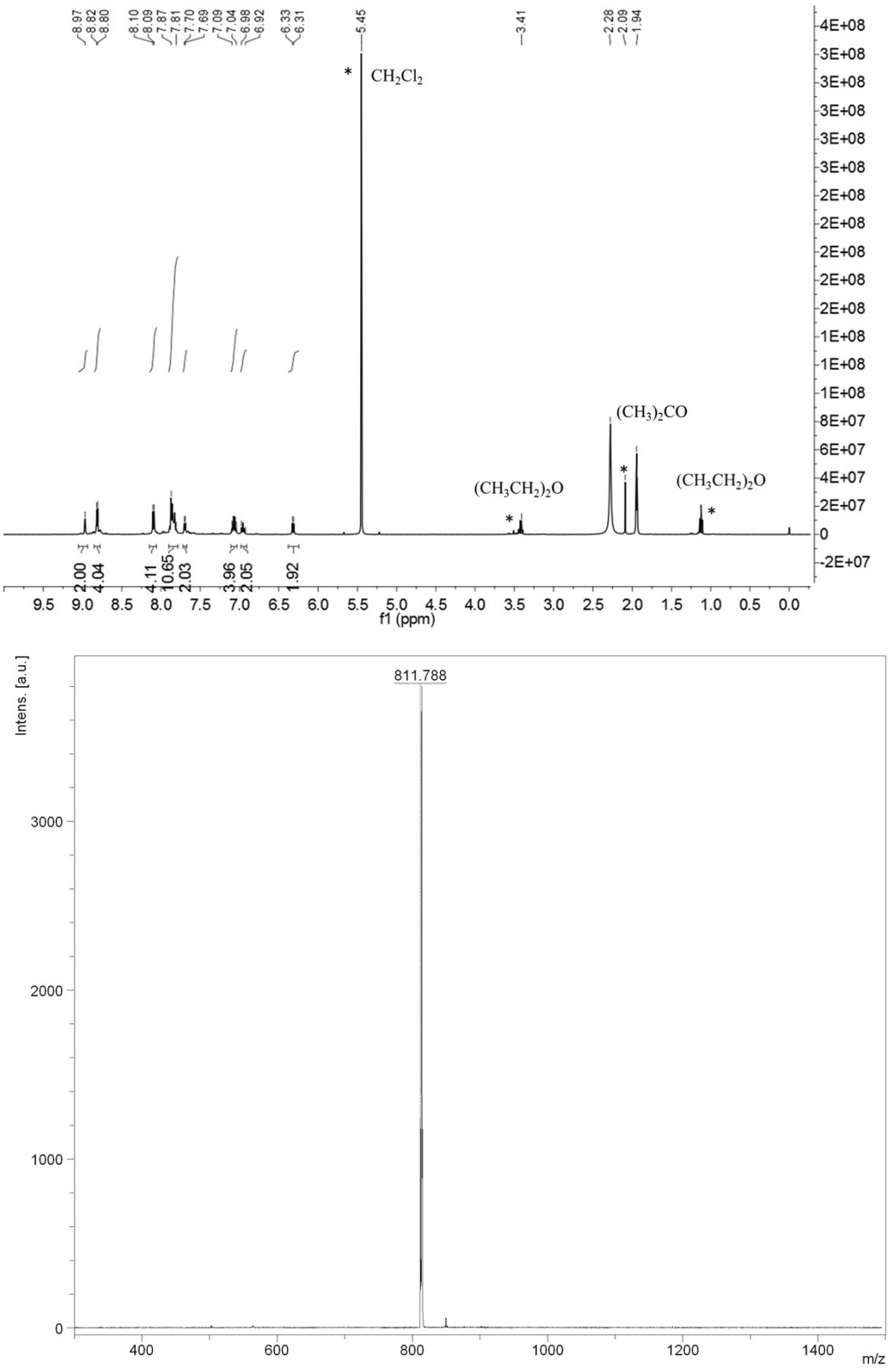

Fig. S22 ^{1}H NMR (400 MHz, acetonitrile-d_3) and mass spectra of **C1**.

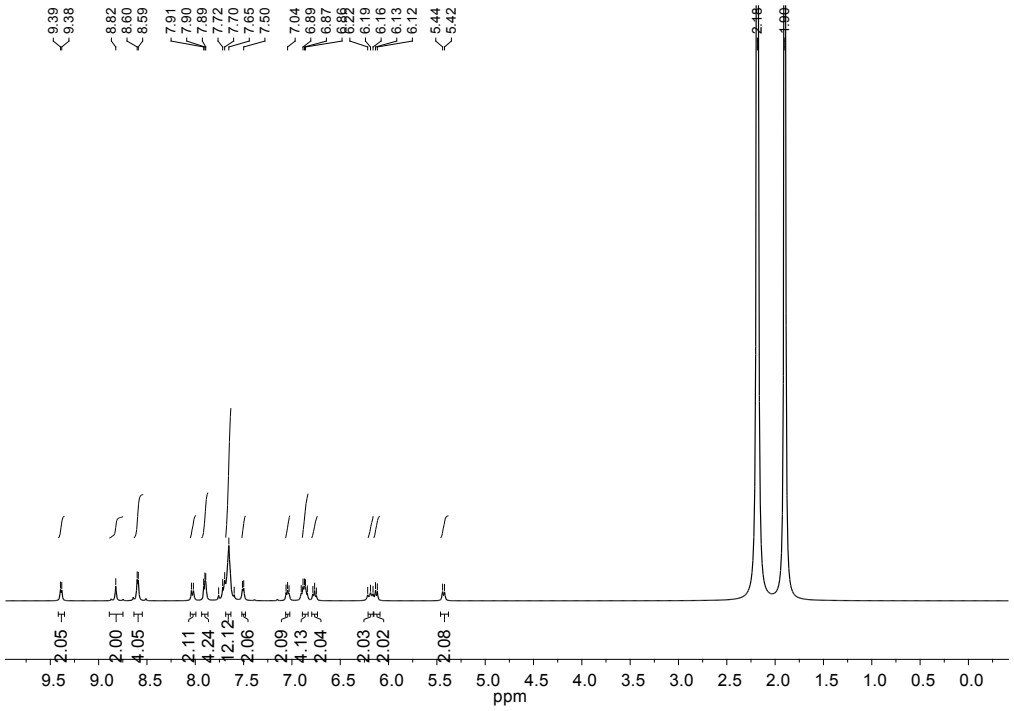

Fig. S23 ^{1}H NMR (400 MHz, acetonitrile-d_3) spectrum of **S1**.

Table S1 Photophysical properties of complexes **A1, C1** and **S1**

Complexes	Solvent	λ_{abs}, nm (logε)	λ_{PL}, nm	τ, ns	Φ_{em}
A1	CH_3CN	251 (5.21) 293(4.83) 364(4.31)	451, 475	1329	0.62
C1	CH_3CN	249 (4.99) 291 (4.73) 386 (4.10)	633	413	0.18
S1	CH_3CN	249 (5.21) 289 (4.90) 364 (4.34)	453, 480, 633	819/398	0.22