Supporting Information for

"A Ratiometric Two-Photon Fluorescent Probe for Quantitative Detection of β-Galactosidase Activity in Senescent Cells"

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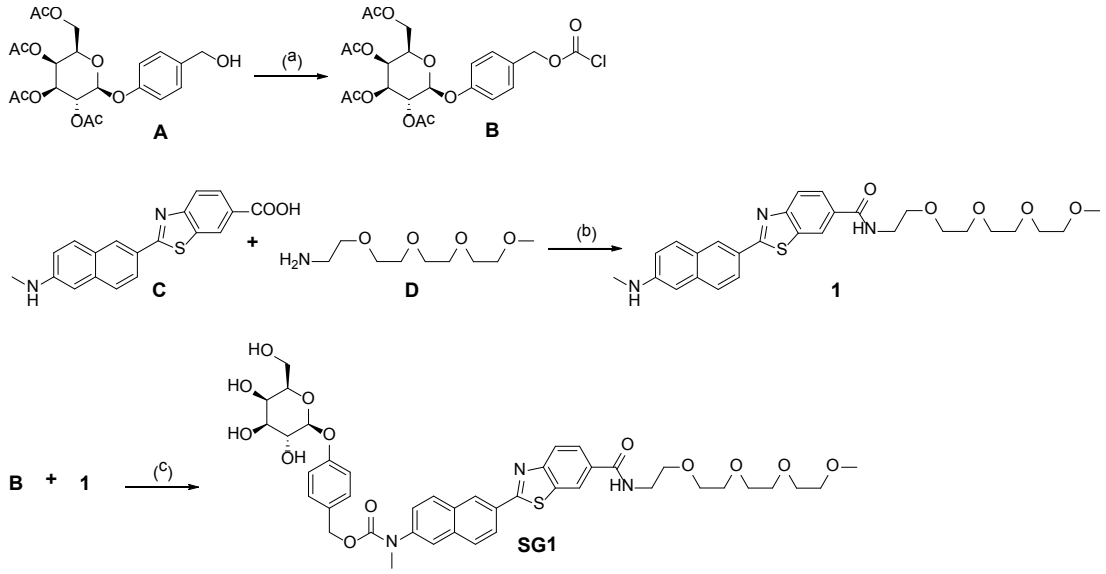
Synthesis of SG1. Compounds **A**, **C** and **D** were prepared by the literature methods[1-3] and synthesis of other compounds is described below.



Scheme S1. Synthesis of SG1. *Reagents and conditions:* (a) triphosgene, Na_2CO_3, THF, N_2, rt, 6h. (b) DCC, HOBt, DMF, N_2, rt, 10h. (c) i: pyridine, CH_2Cl_2, N_2, rt, 6h. ii: NaOMe, CH_3OH, 0 ^oC, 1h.

Synthesis of **B.** Na_2CO_3 (187 mg, 1.76 mmol) and triphosgene (131 mg, 0.44 mmol) were *vacuo* dried in a round-bottom flask. The flask was cooled in an ice bath and 5 mL of THF was added. After stirring under nitrogen in the dark for 30 min. at 0 °C, a solution of **A** (200 mg, 0.44 mmol) in dry THF (15 mL) was added dropwise and the stirring was continued for additional 6 h at room temperature. The solvent was removed under reduced pressure and the product was used without further purification for the next step; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.31 (d, *J* = 8.4 Hz, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 5.48-5.44 (m, 2H), 5.19 (s, 2H), 5.12-5.10 (m, 1H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.24-4.20 (m, 1H), 4.17-4.12 (m, 1H), 4.10-4.03 (m, 1H), 2.18 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H).

Synthesis of **1.** To a stirred solution of **C** (250 mg, 0.75 mmol) in dry DMF (10 mL), *N*,*N*'-dicyclohexylcarbodiimide (DCC, 232 mg, 1.12 mmol) and 1-hydroxybenzotriazole (HOBt, 152 mg, 1.12 mmol) were added and the reaction mixture was stirred for one hour at room temperature under nitrogen atmosphere. Then, 2,5,8,11-tetraoxatridecan-13-amine (**D**, 156 mg, 0.75 mmol) was added to the reaction mixture and further reaction was continued for 10 h. After completion of the reaction, solvent was evaporated and the crude solid was dissolved in CH_3CN. Precipitate dicyclohexylurea was removed by filtration and the filtrate was then concentrated under reduced pressure. The product was purified by column chromatography using 5 % CH_3OH in EtOAc as eluent to obtain **2** as a brown semi-solid (280 mg, 72 %); ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.40 (s, 1H), 8.34 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.90 (dd, *J* = 8.4, 2.0 Hz,

1H), 7.66 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.35 (brs, NH-amide, 1H), 6.87 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.72 (d, *J* = 2.0 Hz, 1H), 4.29 (brs, 1H), 3.70-3.61 (m, 12H), 3.58 (t, *J* = 4.8 Hz, 2H), 3.49 (t, *J* = 4.8 Hz, 2H), 3.31 (s, 3H), 2.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.4, 167.2, 156.2, 148.7, 137.3, 135.1, 131.1, 130.1, 128.0, 126.8, 126.7, 126.6, 125.3, 125.0, 122.4, 121.5, 118.9, 103.4, 72.1, 70.8, 70.7, 70.5, 70.2, 59.2, 40.4, 30.8; HRMS (FAB^+): m/z calculated for $[C_{28}H_{33}O_5N_3S]^+$: 524.2220, found: 524.2219.

Synthesis of **SG1**: (i) To a solution of compound **B** (150 mg, 0.29 mmol) and pyridine (0.1 mL, 1.2 mmol) in 5 mL of dry CH_2Cl_2, a solution of **1** (152 mg, 0.29 mmol) in CH_2Cl_2 was added dropwise and the reaction mixture was allowed to stand for 6 h at room temperature under nitrogen atmosphere. The solvent was evaporated and the crude product was purified by silica gel column chromatography using 6 % CH_3OH in $CHCl_3$ as eluent to afford the intermediate as a yellow solid (120 mg, 41 %); M.p. 63-65 ^oC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.55 (s, 1H), 8.51 (s, 1H), 8.20 (dd, *J* = 8.8, 2.0 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.97 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.70 (s, 1H), 7.50 (d, *J* = 8.8 Hz, 1H), 7.41 (brs, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 6.96 (d, *J* = 8.0 Hz, 2H), 5.48-5.44 (m, 2H), 5.15 (s, 2H), 5.12-5.10 (m, 1H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.24-4.20 (m, 1H), 4.17-4.12 (m, 1H), 4.10-4.03 (m, 1H), 3.72-3.63 (m, 12H), 3.62-3.60 (m, 2H), 3.52-3.50 (m, 2H), 3.44 (s, 3H), 3.32 (s, 3H), 2.18 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 170.1, 169.4, 165.4, 157.2, 156.8, 155.5, 150.5, 149.0, 142.1, 136.5, 134.6, 131.5, 131.3, 129.8, 129.4, 128.6, 126.8, 125.9, 125.0, 123.9, 122.9, 117.0, 116.5, 105.4, 99.7, 72.2, 71.3, 71.2, 71.1, 71.0, 70.9, 70.0, 68.9, 68.4, 67.3, 67.1, 61.6, 59.4, 38.2, 21.2, 21.1, 21.0.

(ii) To a suspension containing this intermediate (104 mg, 0.10 mmol) in 3 mL of dry CH_3OH, 30 µL of NaOMe solution was added and stirred the reaction mixture at 0 ^oC for 1h. After that, solvent was evaporated and washed with saturated NH_4Cl solution and then extracted with $CHCl_3$ (3×50 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated under *vacuo*. Finally the crude solid was purified by column chromatography using $CHCl_3$: CH_3OH (9:1) as eluent to obtain **SG1** as a light yellow solid (60 mg, 70 %); M.p. 174-176 ^oC; ^{1}H NMR (DMSO, 400 MHz): δ (ppm) 8.71 (s, 2H), 8.67 (s, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 8.15 (s, 1H), 8.13 (s, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.93 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 5.15 (d, *J* = 5.2 Hz, 1H), 5.10 (s, 2H), 4.87 (d, *J* = 5.6 Hz, 1H), 4.81 (d, *J* = 7.6 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 1H), 4.51 (d, *J* = 4.4 Hz, 1H), 3.69 (t, 1H), 3.58-3.48 (m, 21H), 3.38 (s, 3H), 3.21 (s, 3H); ^{13}C NMR (DMSO, 100 MHz): δ (ppm) 170.2, 166.2, 157.9, 157.8, 155.2, 143.0, 135.1, 132.1, 131.1, 130.4, 130.2, 130.0, 129.9, 129.4, 128.0, 126.6, 126.5, 125.0, 123.0, 122.4, 116.8, 101.6, 76.2, 74.1, 72.0, 71.1, 70.5, 70.4, 70.3, 69.6, 68.8, 67.4, 61.1, 58.8, 38.1.; HRMS (FAB^+): m/z calculated for $[C_{42}H_{49}O_{13}N_3S]^+$: 836.3065, found: 836.3064.

Spectroscopic measurements. Absorption spectra were recorded on a S-3100 UV-Vis spectrophotometer and fluorescence spectra were obtained with FluoroMate FS-2 fluorescence spectrophotometer with a 1 cm standard quartz cell. The fluorescence quantum yield was determined by using 9, 10-diphenylanthrancene (Φ = 0.93 in cyclohexane) as the reference by the literature method.[4, 5]

Water solubility. Small amount of dye was dissolved in DMSO to prepare the stock solutions (1.0×10^{-2} M). The solution was diluted to $1.0 \times 10^{-5} \sim 5.0 \times 10^{-8}$ M and added to a cuvette containing 3.0 mL of PBS buffer (10 mM, pH 7.4) by using a micro syringe. In all cases, the concentration of DMSO in buffer was maintained to be 0.1 %.[6] The plots of fluorescence intensity against the dye concentration were linear at low concentration and showed downward curvature at higher concentration (Figure S1). The maximum concentration in the linear region was taken as the solubility. The solubilities of SG1 and **1** in PBS buffer were ~ 4.0 and 3.0 μM, respectively.

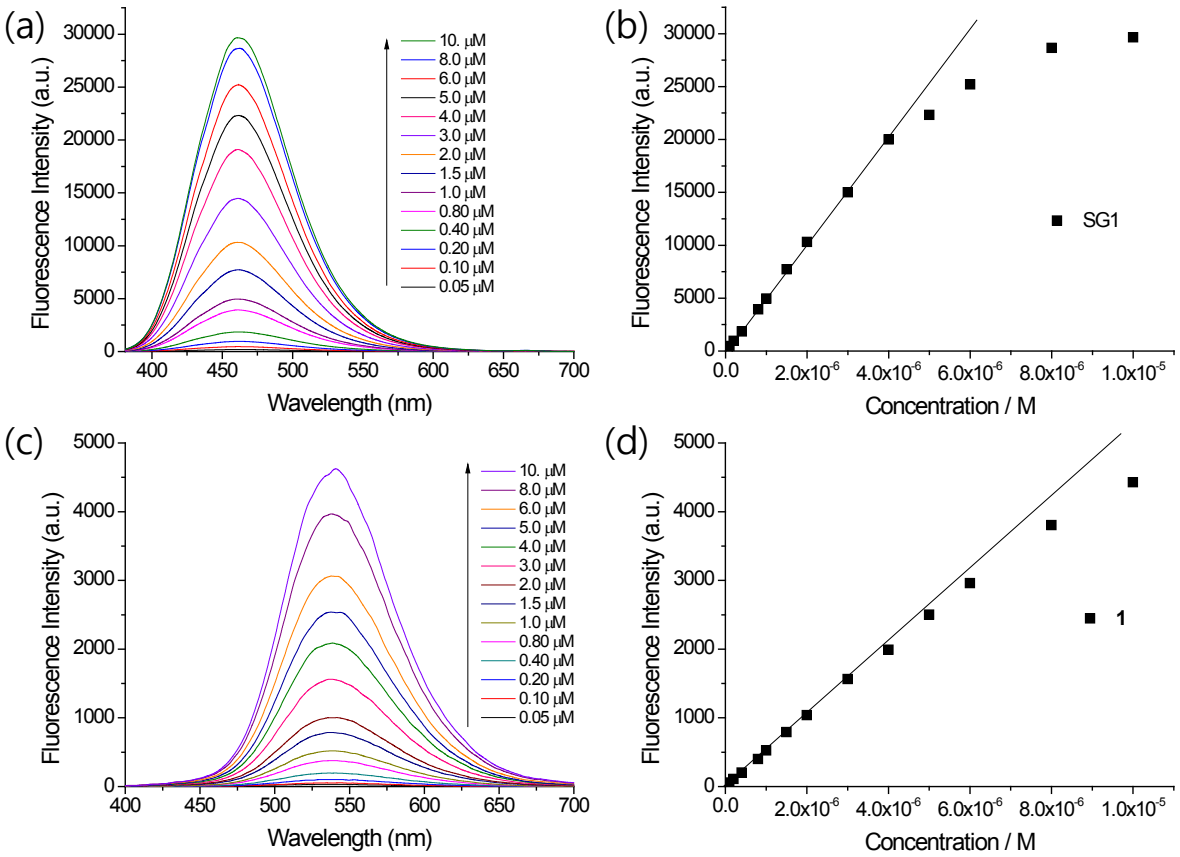


Figure S1. (a,c) One-photon fluorescence spectra and (b,d) plot of fluorescence intensity against the concentration of the dye for (a,b) SG1 and (c,d) **1** in PBS buffer (10 mM, pH 7.4). The excitation wavelengths were 334 and 378 nm for SG1 and **1**, respectively.

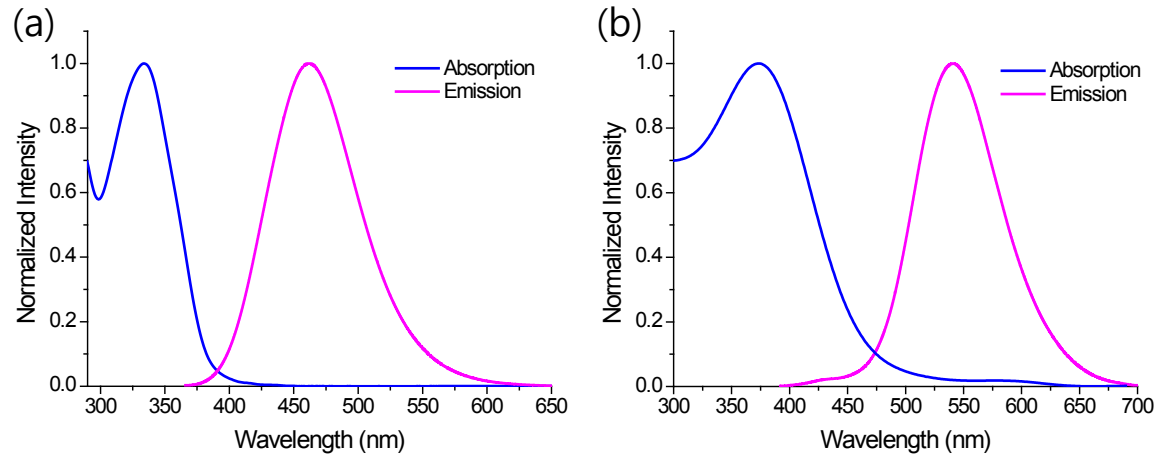


Figure S2. Normalized one-photon absorption and emission spectra of (a) SG1 and (b) **1** in PBS buffer (10 mM, pH 7.4).

Table S1. Photophysical data for SG1 and **1** in buffer.[a]

Compound	$\lambda_{max}^{(1)}$ (10^{-4} ε)[b]	λ_{max}^{fl} [c]	Φ[d]	R_{max}/R_{min}[e]	$\lambda_{max}^{(2)}$ [f]	$\Phi\delta_{max}$[g]
SG1	334 (2.70)	461	1.00	120	740	21
1	378 (2.50)	540	0.16		750	58

a) All data were measured in PBS buffer (10 mM, pH 7.4). b) λ_{max} of the one-photon absorption spectra in nm. The numbers in parentheses are molar extinction coefficients in $M^{-1}cm^{-1}$. c) λ_{max} of the one-photon emission spectra in nm. d) Fluorescence quantum yield, ±10%. e) Emission ratio ($F_{520\text{-}570}/F_{410\text{-}460}$) conversion factor, R_{max}/R_{min}, measured by one-photon processes before and 10 min after the addition of 1.0 unit of β-galactosidase. f) λ_{max} of the two-photon emission spectra in nm. g) The peak two-photon action cross sections in GM (1 GM = 10^{-50} cm^4 s $photon^{-1}$), ±15%.

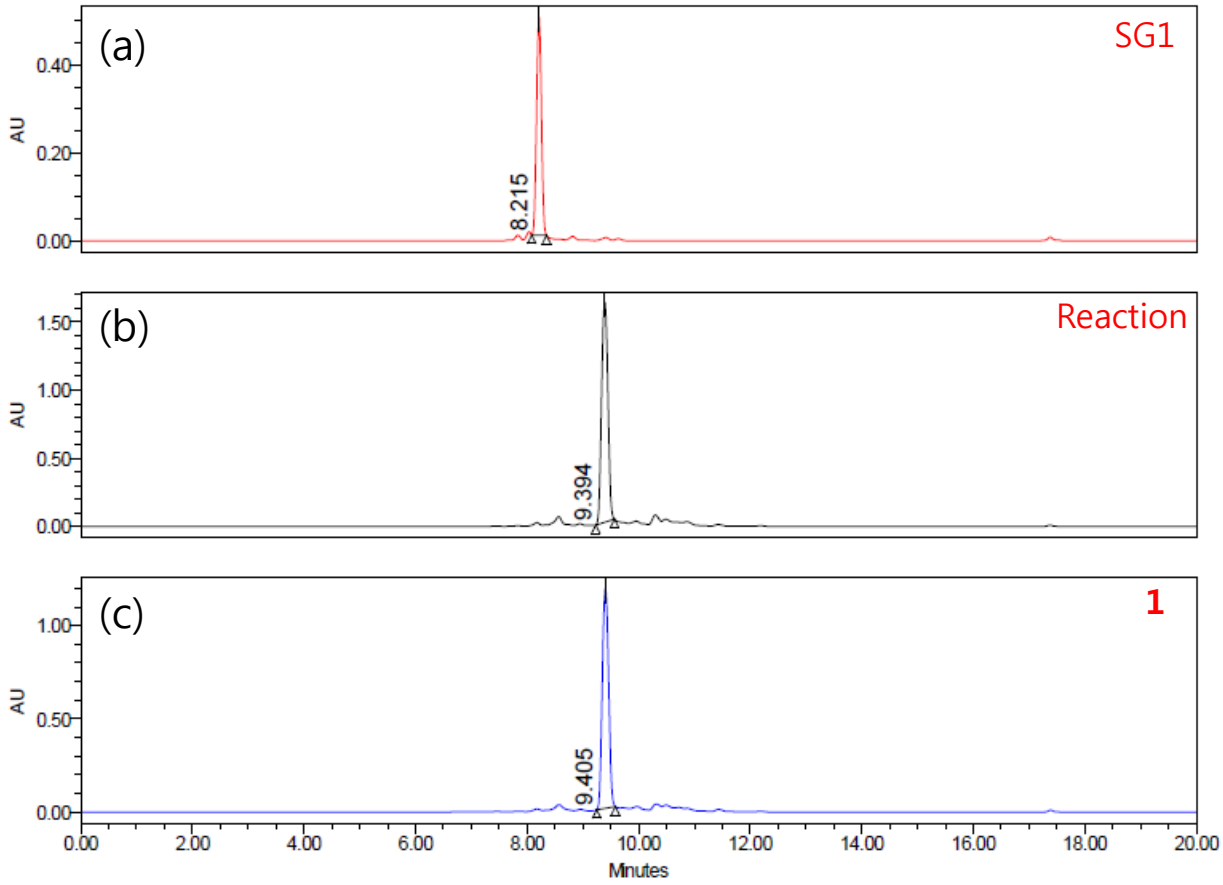


Figure S3. HPLC traces of (a) SG1, (b) the reaction product between SG1 and β-galactosidase, (c) **1**. HPLC conditions: 0.3 mL/min flow rate, 5% B to 100% B over 20 min, detected at 370 nm. Solvent A is water and solvent B is acetonitrile. Peaks at 8.2 min and 9.4 min correspond to SG1 and **1**, respectively.

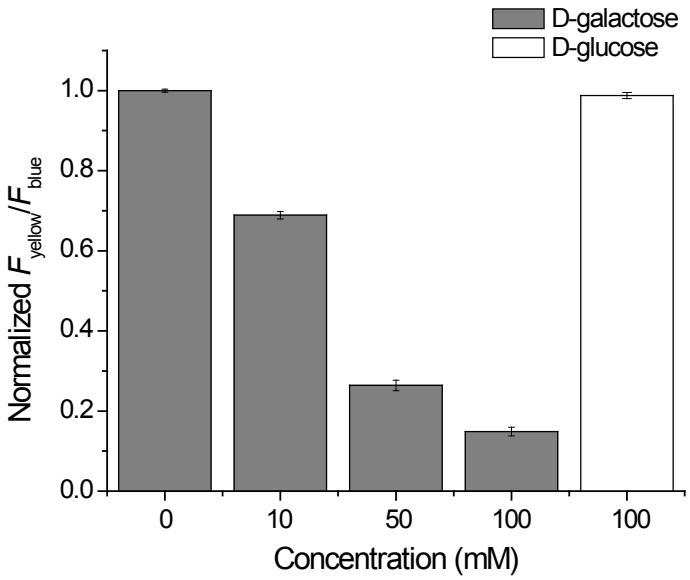


Figure S4. Relative fluorescence intensity ratios (F_{yellow}/F_{blue}) of SG1 at 10 min after addition of 1 unit of β-galactosidase in PBS buffer (10 mM, pH 7.4, 37 °C), in the presence of 0, 10, 50, 100 mM D-galactose (gray bars) and 100 mM D-glucose (white bar). The excitation wavelength was 376 nm (n = 3).

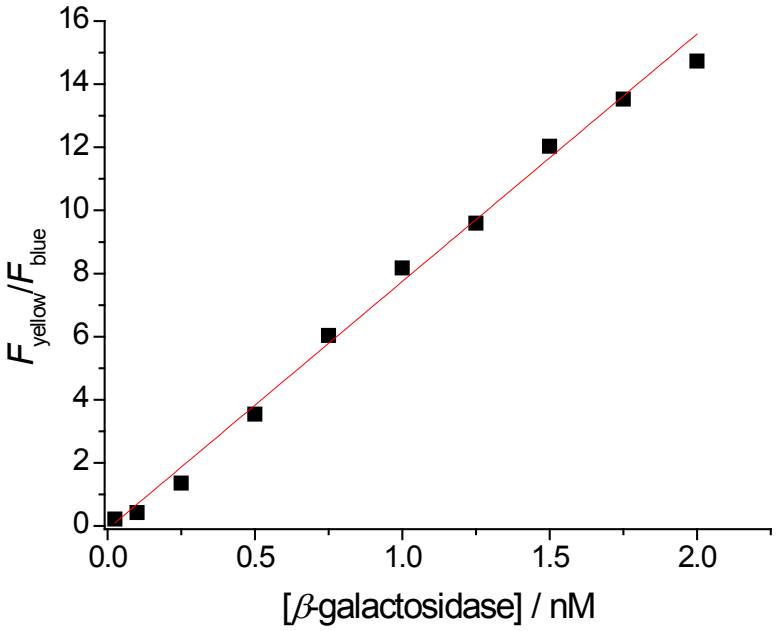


Figure S5. Plot of the F_{yellow}/F_{blue} ratios for SG1 vs [β-galactosidase] in PBS buffer (10 mM, pH 7.4). Each data was acquired 10 min after β-galactosidase addition at 37 °C.

Enzymatic Kinetics Assays. Enzymatic kinetic experiments were performed by using FluoroMate FS-2 fluorescence spectrophotometer with a 1 cm standard quartz cell. Various concentrations of SG1 (0-40 μM) and FDG (0-100 μM) were prepared in PBS buffer solution (10 mM, pH = 7.4). β-galactosidase enzyme was added to a final concentration of 57.7 μg/L, the fluorescence intensity was collected at 460 nm (λ_{ex} = 334 nm) for SG1, 514 nm (λ_{ex} = 490 nm) for FDG with 40 second intervals from 0 to 30 min at 37 ºC. The kinetic parameters of Michaelis-Menten equation were calculated with hyperbolic function by the nonlinear fitting algorithm (OriginPro 8.0). Three independent experiments were performed.

Table S2. Kinetic parameters for SG1 and FDG with β-galactosidase.

Compound	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (μM^{-1} s^{-1})	V_{max} (nmol mg^{-1} s^{-1})
SG1	1.73 ± 0.15	7.56 ± 0.17	4.37 ± 0.48	16.3 ± 0.3
FDG	10.2 ± 1.2	0.156 ± 0.01	0.0157 ± 0.002	0.33 ± 0.2

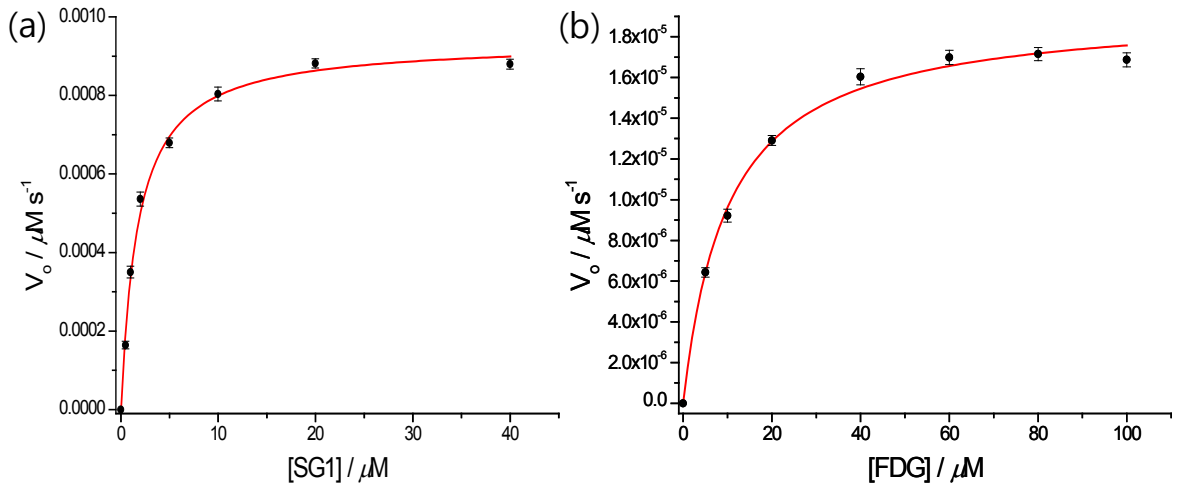


Figure S6. Kinetic studies of β-galactosidase (57.7 μg/L) with various concentrations of (a) SG1 and (b) FDG in PBS buffer (10 mM, pH 7.4) at 37 °C (n = 3).

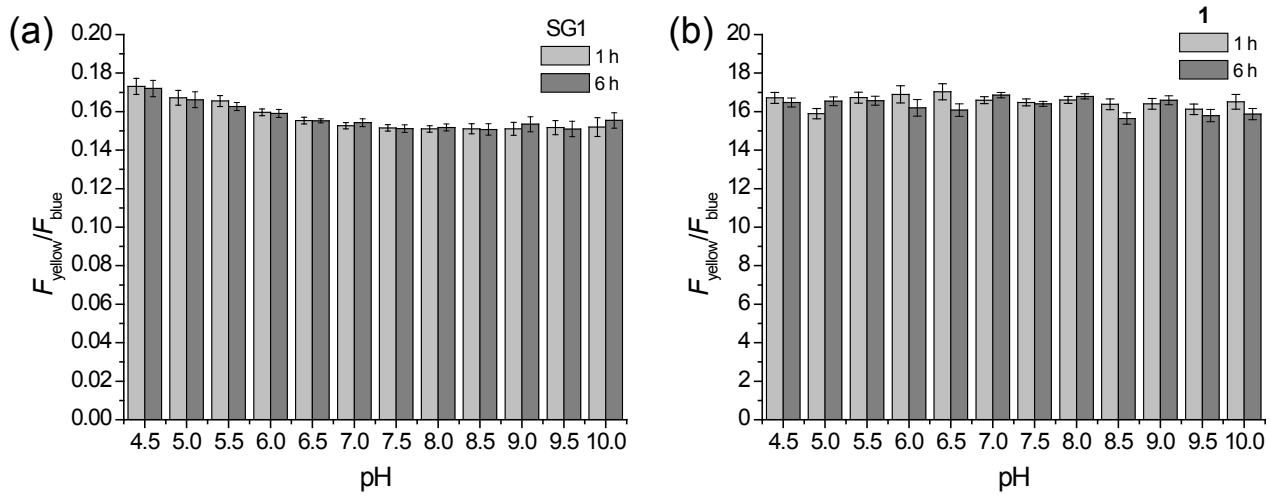


Figure S7. Effect of pH on the fluorescence intensity ratios (F_{yellow}/F_{blue}) for (a) SG1 and (b) **1** in universal buffer (0.1 M citric acid, 0.1 M KH_2PO_4, 0.1 M $Na_2B_4O_7$, 0.1 M Tris, 0.1 M KCl) at 25 °C. The excitation wavelength was 376 nm (n=3).

ROS Dependency. Reactive oxygen species (200 μM unless otherwise stated) were administered to SG1 in PBS buffer (10 mM, pH 7.4) as follows. H_2O_2, *tert*-butylhydroperoxide (TBHP), and sodium hypochlorite (NaOCl) were from 30%, 70%, 5% aqueous solutions, respectively. Hydroxyl radical (·OH), and *tert*-butoxy radical (·O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 200 μM H_2O_2 or TBHP, respectively. Nitric oxide (NO) was used from stock solution (1.9 mM), prepared by purging PBS buffer (10 mM, pH 7.4) with N_2 gas for 30 min, followed by NO (99.5%) for 30 min. Superoxide (O_2^-) was delivered from KO_2. Peroxynitrite was used from stock solution 10 mM in 0.3 M NaOH. Three independent experiments were performed.

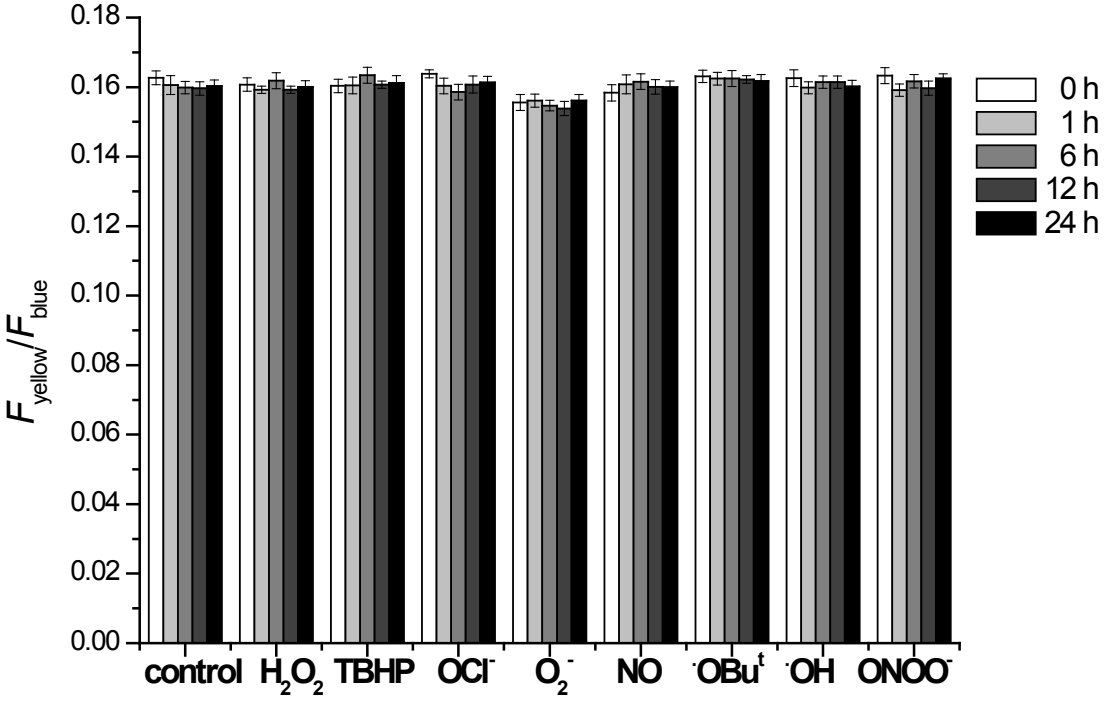


Figure S8. Fluorescence responses of 1 μM SG1 to various reactive oxygen and nitrogen species (200 μM). Bars represent the integrated fluorescence ratios F_{yellow}/F_{blue} at 0 to 24 hours after the addition of each reactive species. Data were acquired at 25 °C in PBS buffer (10 mM, pH 7.4). The excitation wavelength was 376 nm (n=3).

Measurement of Two-Photon Cross Section. The two-photon cross section (δ) was determined by using femto second (fs) fluorescence measurement technique as described.[7] Probe (1.0×10^{-6} M) was dissolved in PBS buffer (10 mM, pH = 7.4) and the two-photon induced fluorescence intensity was measured at 720−880 nm by using rhodamine 6G as the reference, whose two-photon property has been well characterized in the literature.[8] The intensities of the two-photon induced fluorescence spectra of the reference and sample emitted at the same excitation wavelength were determined. The TPA cross section was calculated by using $\delta = \delta_r(S_s\Phi_r\phi_r c_r)/(S_r\Phi_s\phi_s c_s)$: where the subscripts *s* and *r* stand for the sample and reference molecules. The intensity of the signal collected by a CCD detector was denoted as *S*. Φ is the fluorescence quantum yield. ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as *c*. δ_r is the TPA cross section of the reference molecule.

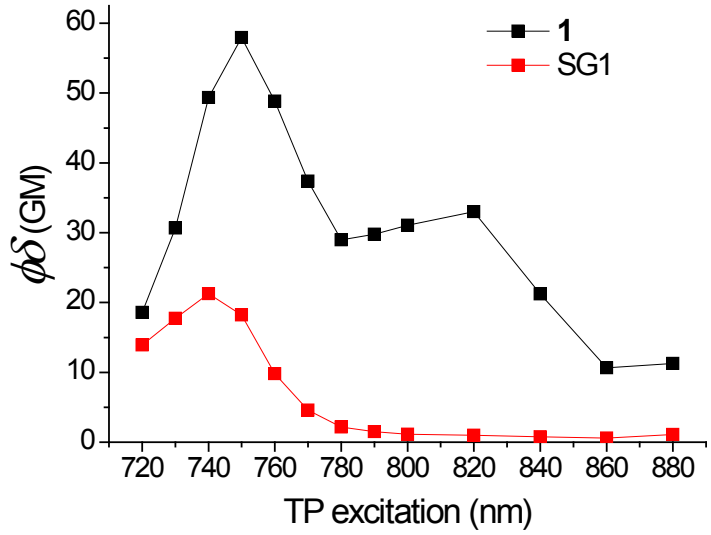


Figure S9. Two-photon action spectra of SG1 and **1** in PBS buffer (10mM, pH 7.4). The estimated uncertainties for the two-photon action cross section values ($\delta\Phi$) are ± 15 %.

Development of replicative senescence of primary HDF. Primary HDFs isolated from the foreskin of a 5-year-old boy according to the method described previously.[9] Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY) and antibiotics at 37°C in a humidified incubator with 5% CO_2.

To develop replicative senescence, confluent HDFs were evenly transferred into two new dishes and the cells were cultured until getting confluent again to generate one population doubling. The numbers of population doublings (PD, times), the doubling time (DT, days), and total culturing period were continuously monitored (Table S3). DT determined for this study includes the cells' adaptation time after seeding freshly because confluent grown cells were divided into two new plates at every passage.

Table S3. DT and PD of the cells in the process of replicative senescence of HDFs.

Total culture period (days)	54	78	86	126	143	232	272	238
DT (days)	2	2	2	3	5	12	15	20
PD (number)	26	39	42	52	58	69	72	76

SA-β-gal assay by X-gal cytostaining. SA-β-gal activity was assayed at pH 6.0 as described by Dimri *et al.*[10] with a slight modification. Briefly, cells were washed twice with PBS (phosphate buffered saline), fixed to plates by 3.5% formaldehyde for 5 min, and then incubated overnight in freshly prepared staining solution [40 mM citrate-phosphate buffer, pH 6.0, containing 1 mg/ml of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; Sigma, St. Louis, MO, USA), 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, and 2 mM $MgCl_2$]. The stain was visible 12 h after incubation at 37 ^oC. By counting the numbers of the blue-colored and total cells under using Image J software (NIH, Bethesda, MA, USA), percentage of the cells stained blue was estimated to compare the degree of senescence-associated cells.

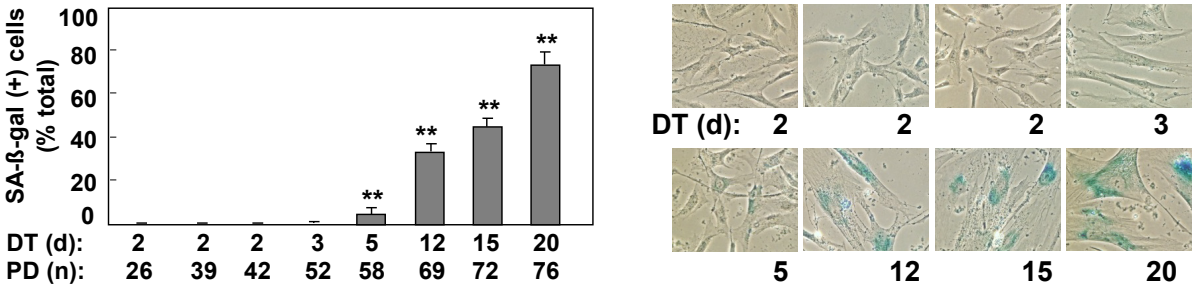


Figure S10. Establishment of replicative senescence of HDFs replicative senescence. Primary HDFs were continuously subcultured in DMEM medium containing 10 % FBS and their PD and DT were recorded. Cells at various time points were subjected to SA-β-gal assay and SA-β-gal positive cell populations were counted.

Two-Photon Fluorescence Microscopy. Two-photon fluorescence microscopy images of probe-labeled cells and tissues were obtained with spectral confocal and multiphoton microscopes (Leica TCS SP8 MP) with ×10 dry, ×40 oil, and ×100 oil objectives, numerical aperture (NA) = 0.30, 1.30, and 1.30, respectively. The two-photon fluorescence microscopy images were obtained with a DMI6000B Microscope (Leica) by exciting the probes with a mode-locked titanium-sapphire laser source (Mai Tai HP; Spectra Physics, 80 MHz pulse frequency, 100 fs pulse width) set at wavelength 750 nm and output power 2679 mW, which corresponded to approximately 2.2 mW average power in the focal plane. To obtain images at 410-460 nm (F_{blue}) and 520-570 nm (F_{yellow}) range, internal PMTs were used to collect the signals in an 8 bit unsigned 512×512 and 1024×1024 pixels at 400 and 200 Hz scan speed, respectively. Ratiometric image processing and analysis was carried out using MetaMorph software.

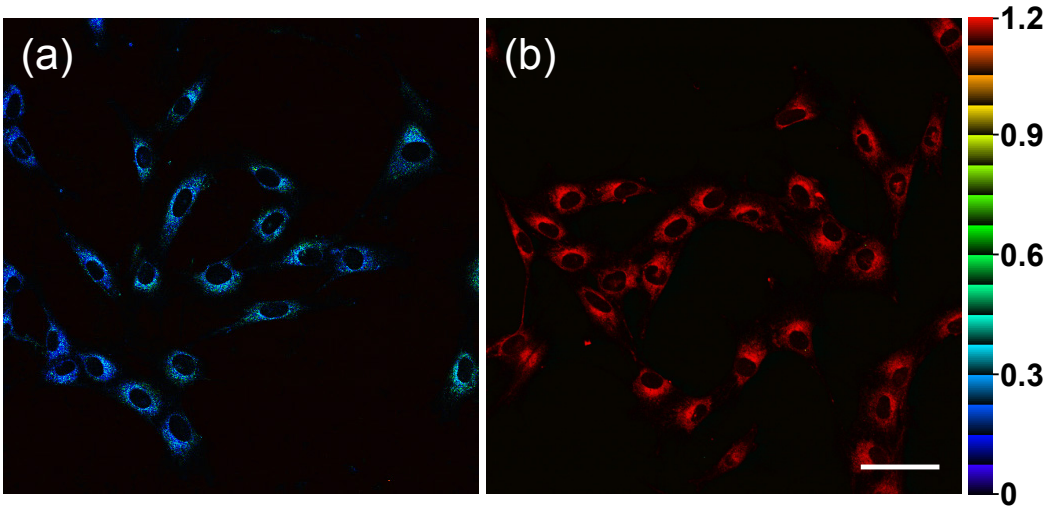


Figure S11. Pseudocolored ratiometric TPM images of primary young HDFs (DT = 2 and PD = 26, Table S3) incubated with (a) SG1 and (b) **1**. Average F_{yellow}/F_{blue} intensity ratios were 0.30 for SG1 and 1.21 for **1** in the TPM images, respectively. Images were acquired using 750 nm excitation and fluorescent emission windows of 410-460 nm (blue) and 520-570 nm (yellow). Scale bar: 65 μm. Cells shown are representative images from replicate experiments (n = 6).

Photostability. Photostability of SG1 was determined by monitoring the changes in TPEF intensity with time at three designated positions of SG1-labeled (2.0 μM) HDFs chosen without bias (Figure S12). The TPEF intensity remained nearly the same for one hour, indicating high photostability.

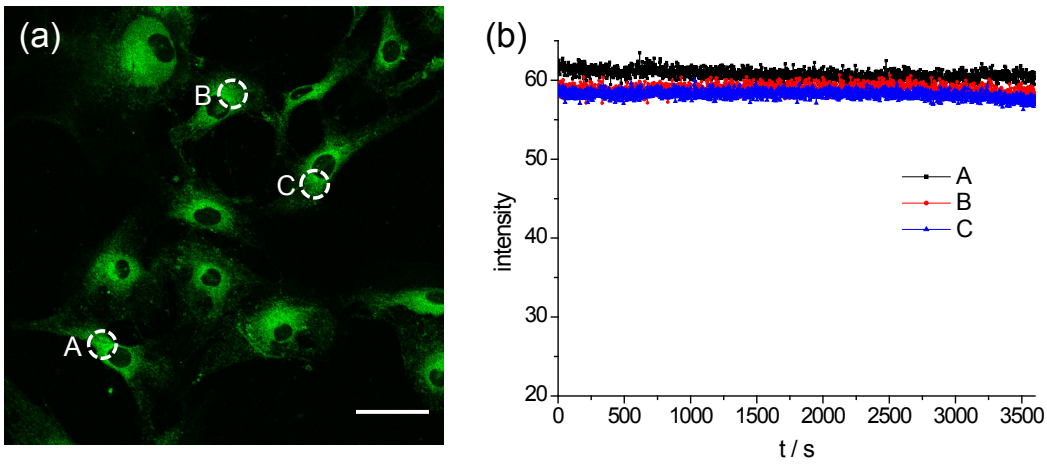


Figure S12. (a) TPM images of HDFs labeled with SG1. (b) The relative TPEF intensity from A-C in Figure (a) as a function of time. The digitized intensity was recorded with 2.00 sec intervals for the duration of one hour using *xyt* mode. The TPEF intensities were collected at 410-570 nm upon excitation at 750 nm with femto-second pulses. Cells shown are representative images from replicate experiments (n = 3).

Cell viability. To confirm that the probe couldn't affect the viability of HDFs in our incubation condition, MTS assay (Cell Titer 96H; Promega, Maidson, WI, USA) was used according to the manufacture's protocol. The results are shown in Figure S13.

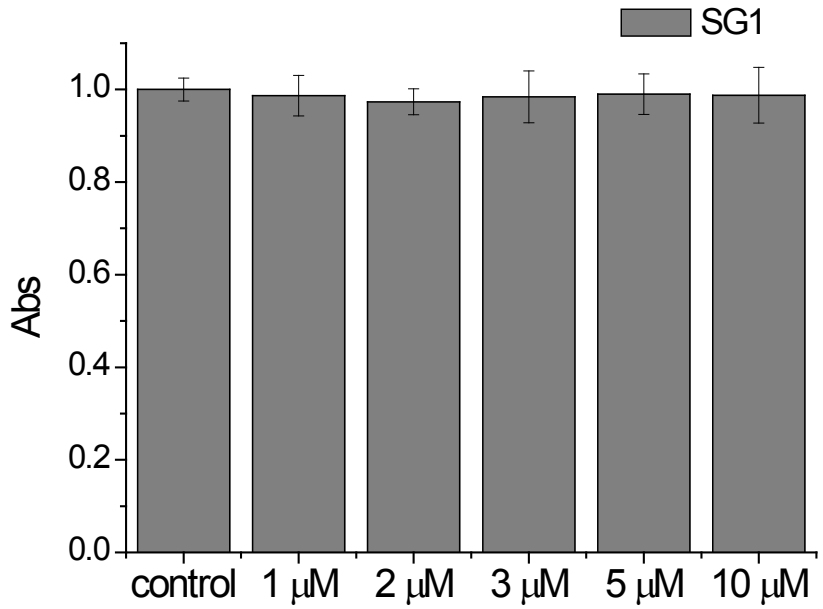


Figure S13. Viability of HDFs in the presence of SG1 as measured by using MTS assay. The cells were incubated with probe for 24 h. Six independent experiments are performed.

Colocalization experiment. Co-localization experiments were conducted by co-staining the HDFs with appropriate combinations of SG1 (2.0 μM) and Lysotracker Red (LTR, 1.0 μM) for 30 min. TPM and OPM images were obtained by collecting the emissions at 520-570 nm (SG1, λ_{ex} = 750 nm) and 600-650 nm (LTR, λ_{ex} = 514 nm), respectively. The background images were corrected, and the distribution of pixels in the TPM and OPM images acquired in the green and red channels, respectively, was compared by using scatter gram. The Pearson's colocalization coefficient (A) was calculated using LAS AF software.

Oxidative stress-induced senescence of HDFs by treatment of exogenous H_2O_2. For induction of oxidative stress, the cells were incubated with 150 μM H_2O_2 in the medium twice with 12 hour interval and continuously cultured for 1, 3, 6, 12, 24, 48, and 72 hours. For labeling, these cells were treated and incubated with 2.0 μM probe for 30 min each time intervals. The cells were washed three times with phosphate buffered saline (PBS; Gibco) and then imaged after further incubation in colorless serum-free medium for 30 min. Control cells were incubated under identical conditions without H_2O_2 treatment in the medium. To determine intracellular level of ROS, we used DCFH-DA fluorogenic probe (Molecular probe, Eugene, OR, USA). Cells were incubated in media containing 10 μM DCFH-DA for 15 min at 37 ^oC. Stained cells were washed, resuspended in PBS, and analyzed by flow cytometry (FACS Vantage, Becton Dickinson Corp.). Mean values of arbitrary fluorescence unit of 10,000 cells were presented and H_2O_2-treated cells were used as positive control.

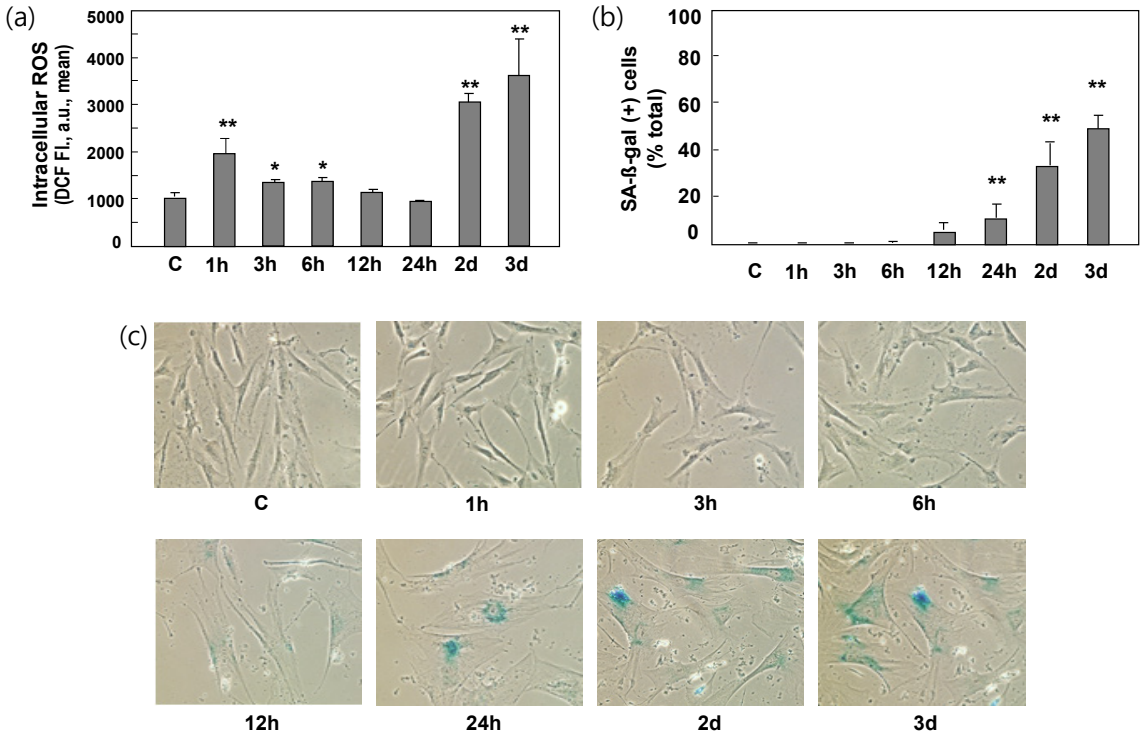


Figure S14. Primary young HDFs were treated with 150 μM H_2O_2 for 12h to monitor early intracellular ROS increase and were exposed again to additional treatment of 150 μM H_2O_2 to stably induce senescence. The cells were continuously cultured with fresh DMEM without H_2O_2 for 3 days. (a) Intracellular ROS levels of H_2O_2-treated HDFs were monitored by flow cytometric analysis after staining cells with DCFH-DA. After the second treatment, additional immediate peak of intracellular ROS was not observed, delayed progressive ROS were accumulated (data not shown). (b,c) Cells at various time points were subjected to SA-β-gal assay and SA-β-gal positive cell populations were counted. Three independent experiments are performed. *, <0.05; **, <0.01 vs control by student t-test.

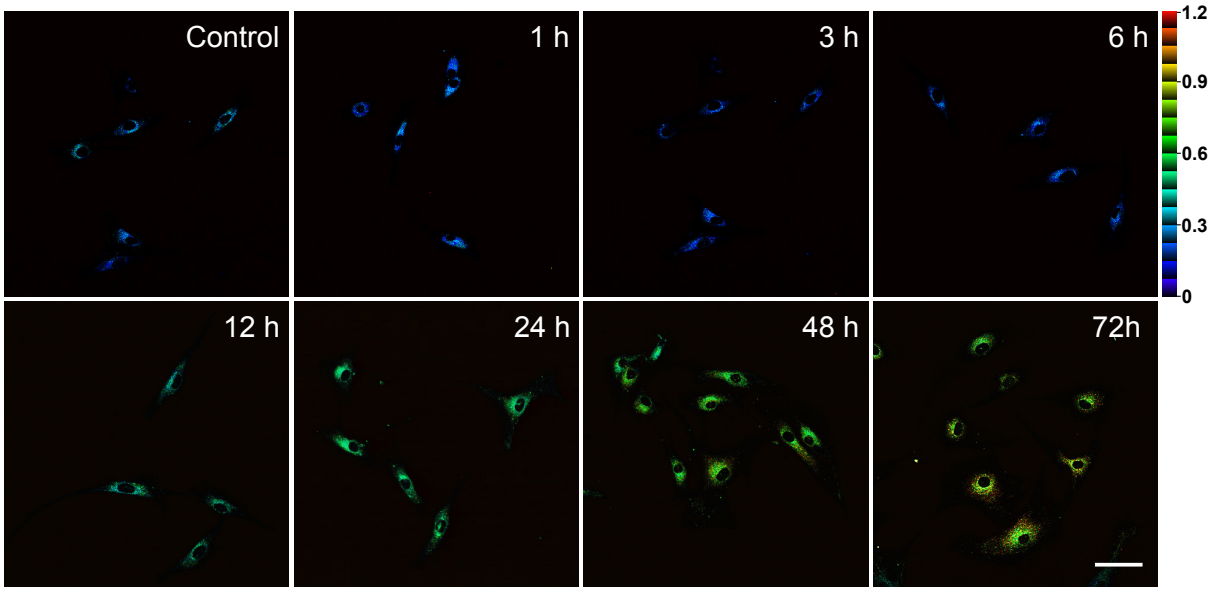


Figure S15. Hydrogen peroxide induced SA-βG activity in HDFs. HDFs were incubated with 150 μM H_2O_2 in the medium twice with 12 hour interval and continuously cultured for 1, 3, 6, 12, 24, 48, and 72 hours. Pseudocolored ratiometric TPM images of HDFs incubated with 2.0 μM SG1. HDFs of the same passage of 150 μM H_2O_2 showed an increase of SA-βG activity after 12 hours. Excitation wavelength was 750 nm. Scale bar: 65 μm. Cells shown are representative images from replicate experiments (n = 3).

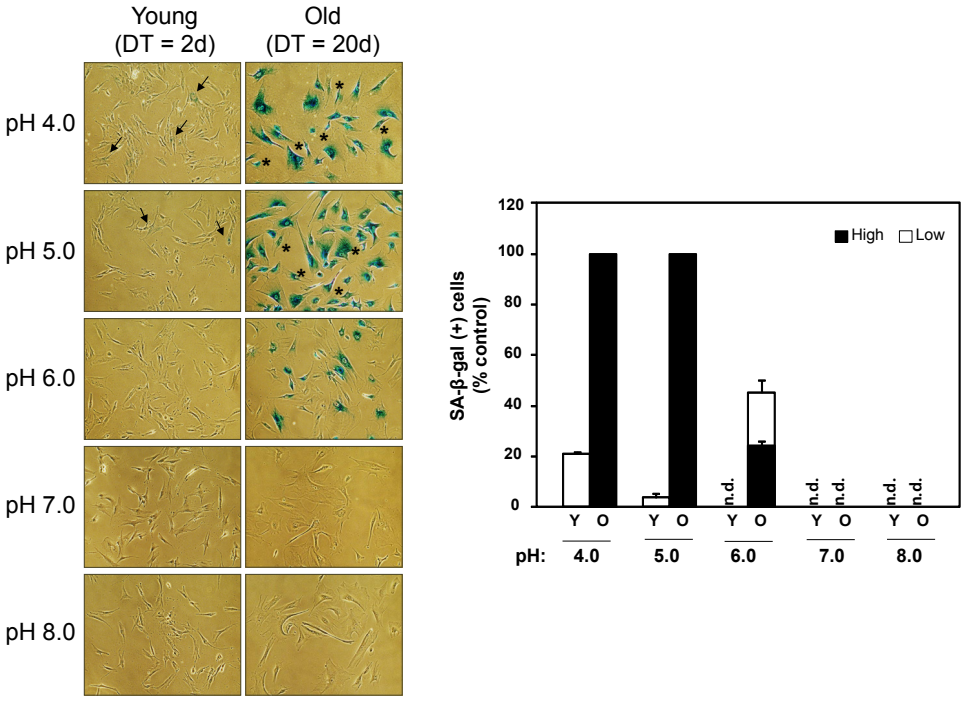


Figure S16. Young and old HDFs were subjected to conventional SA-β gal assay with various pH conditions (pH 4.0-8.0). SA-β-gal positive cell populations with low intensity (open bar) and high intensity (closed bar) were counted, respectively. At pH 4.0 and 5.0, young cell (DT = 2d) showed low SA-β-gal positive cells (arrows →) and old cell (DT = 20d) also showed SA-β-gal positive cells with young-like small morphology (asterisks *), implying non-specific staining.

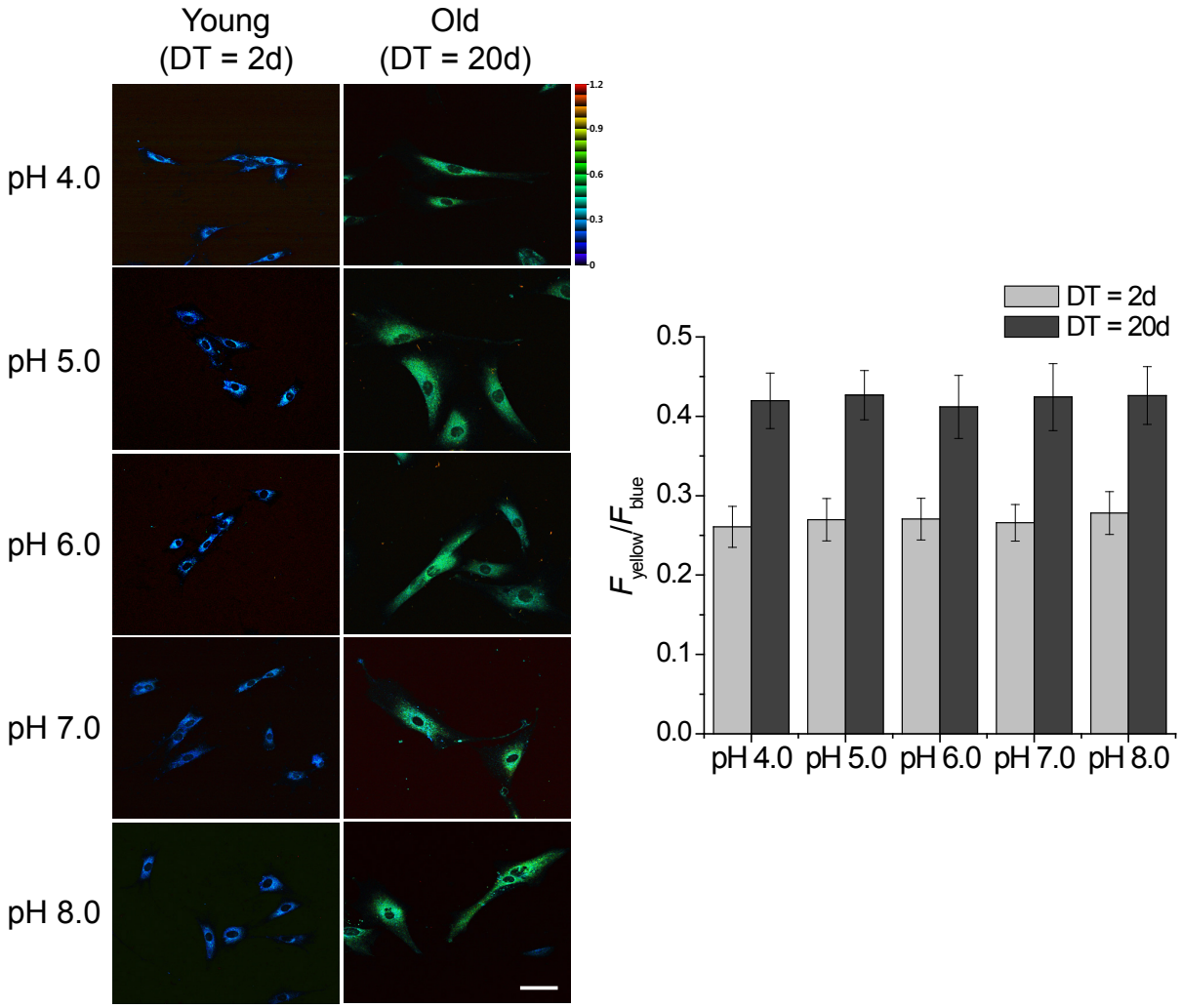


Figure S17. (*left*) Pseudocolored ratiometric TPM images of SG1-labeled HDFs at the young (DT = 2d) and old (20d) stages of replicative senescence with various pH conditions (pH 4.0-8.0). Cells were fixed to plates by 3.5 % formaldehyde for 5 min, and then incubated with 2 μM SG1 at various pH values (4.0-8.0) for 1h. Scale bar: 65 μm. (*right*) Corresponding average F_{yellow}/F_{blue} ratios of TPM images (*left*). The error bars represent the standard deviation of the ratios from 100 TPM images. Cells shown are representative images from replicate experiments (n = 3).

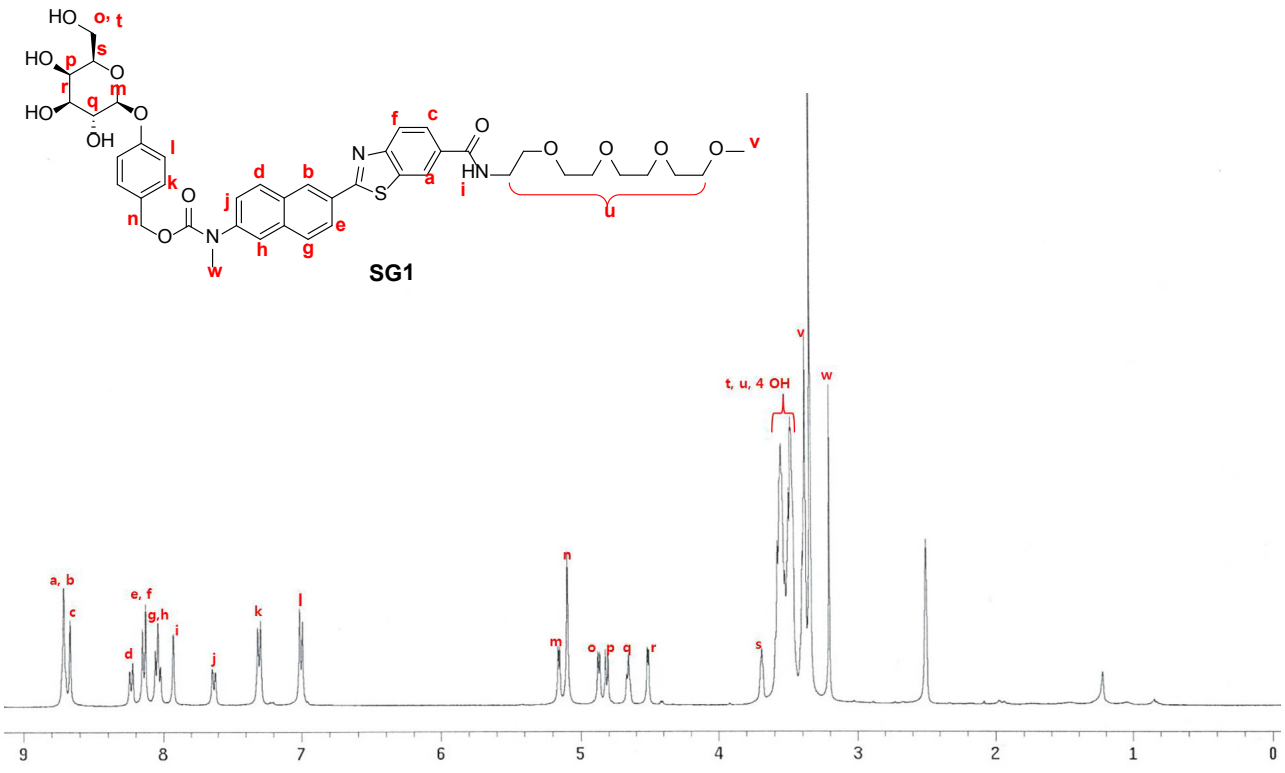


Figure S18. ^{1}H-NMR spectrum (400 MHz) of SG1 in DMSO

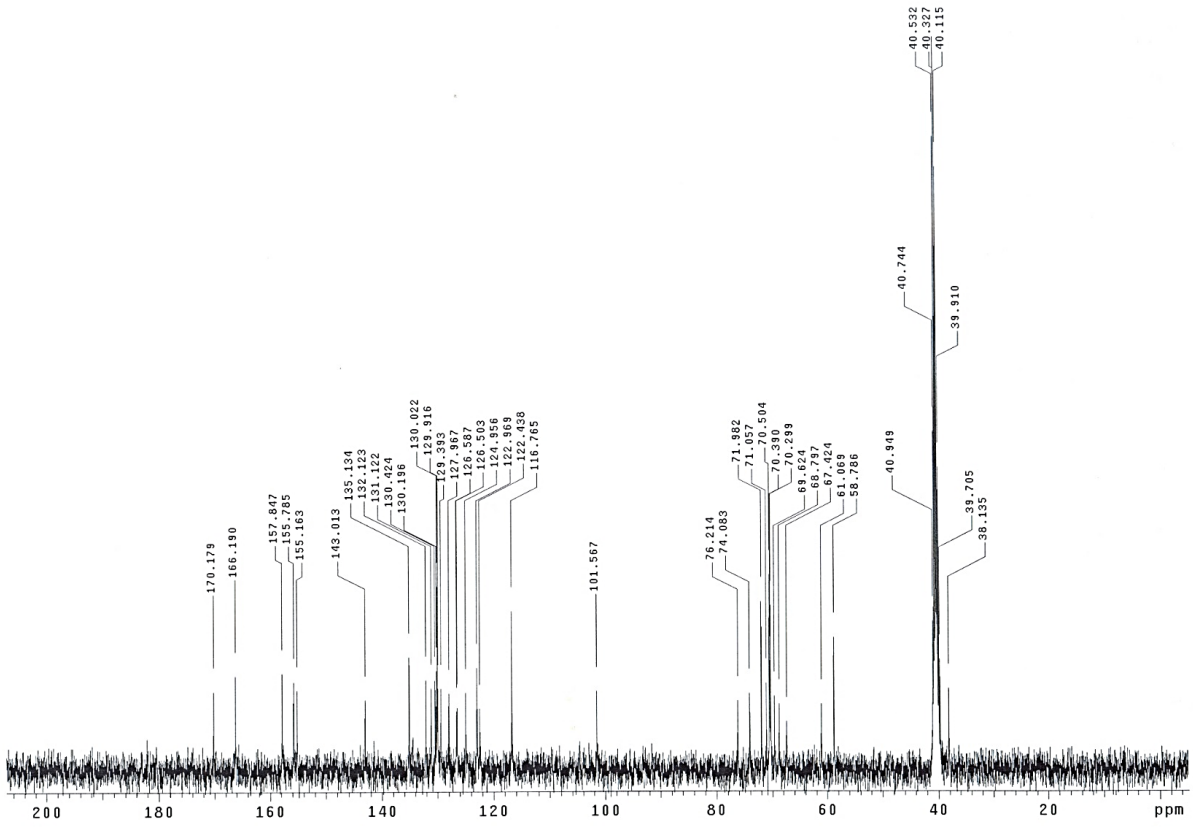


Figure S19. ^{13}C-NMR spectrum (100 MHz) of SG1 in DMSO

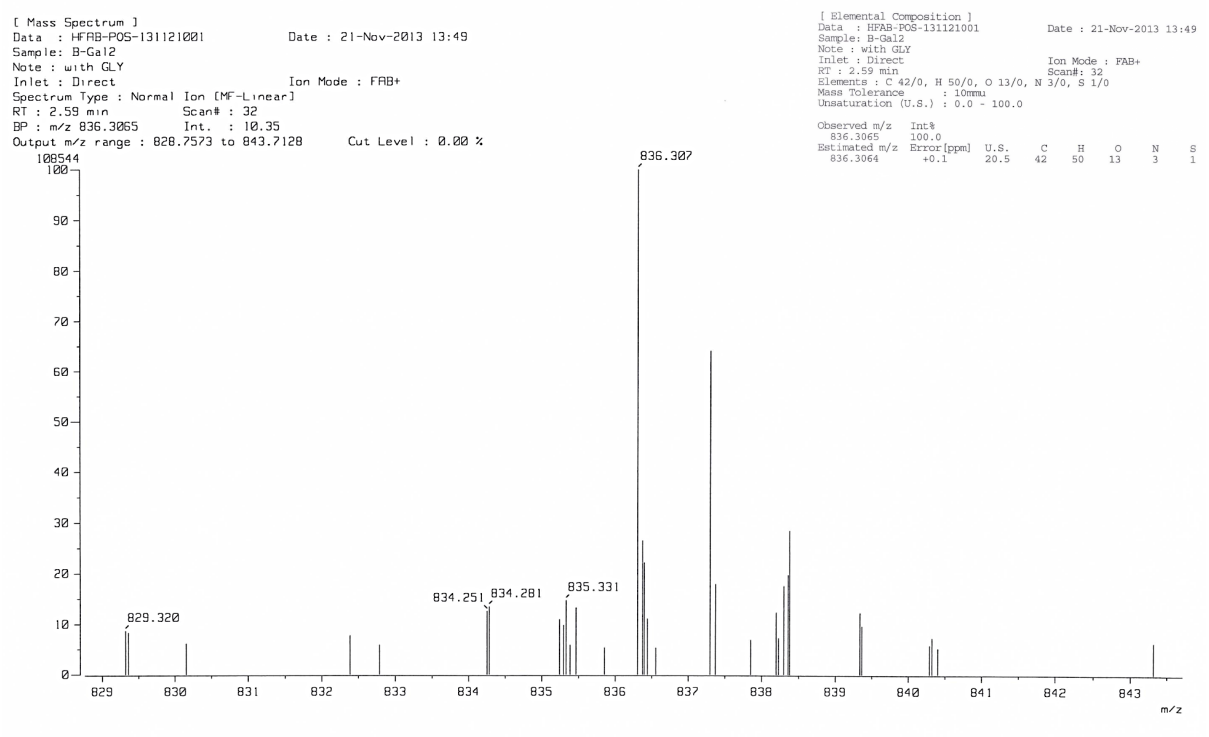


Figure S20. HRMS spectrum of SG1

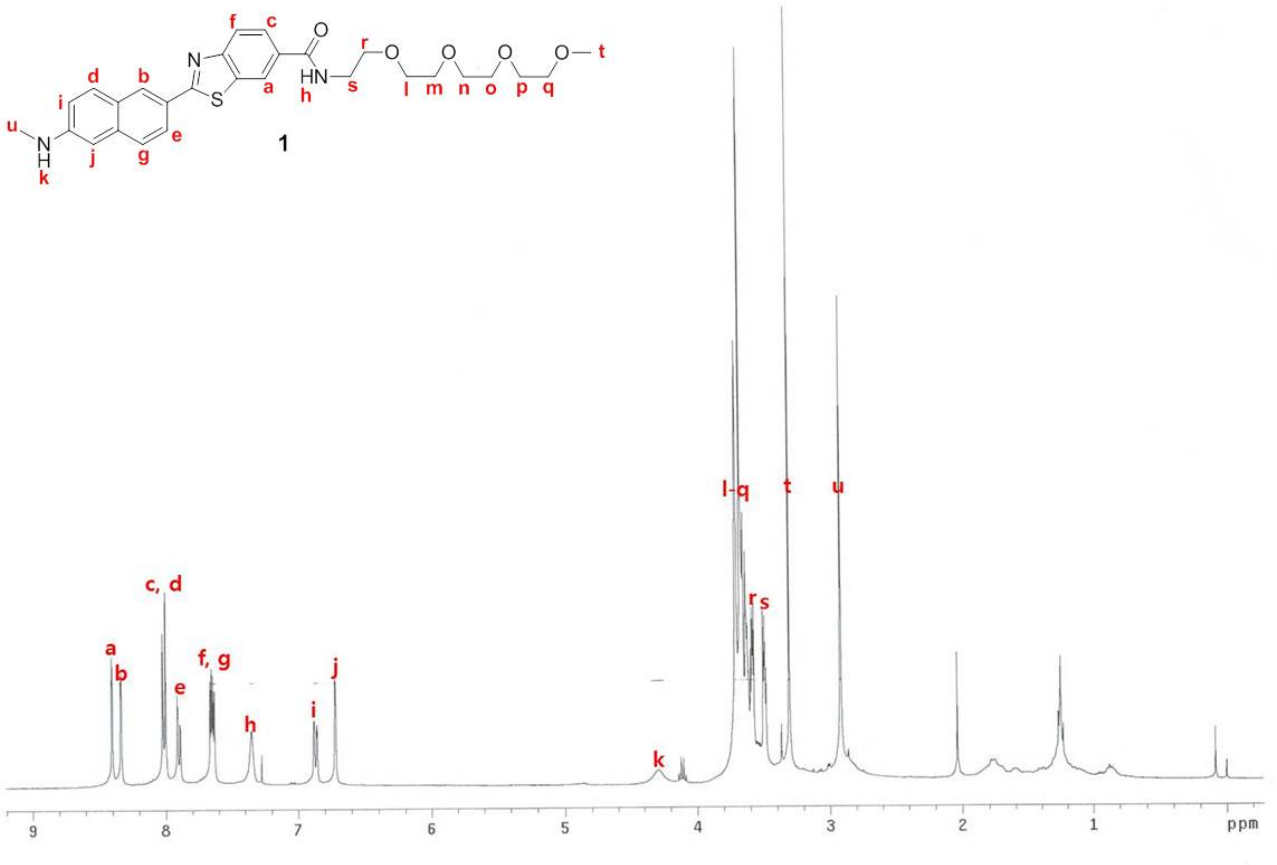


Figure S21. ^{1}H-NMR spectrum (400 MHz) of **1** in $CDCl_3$

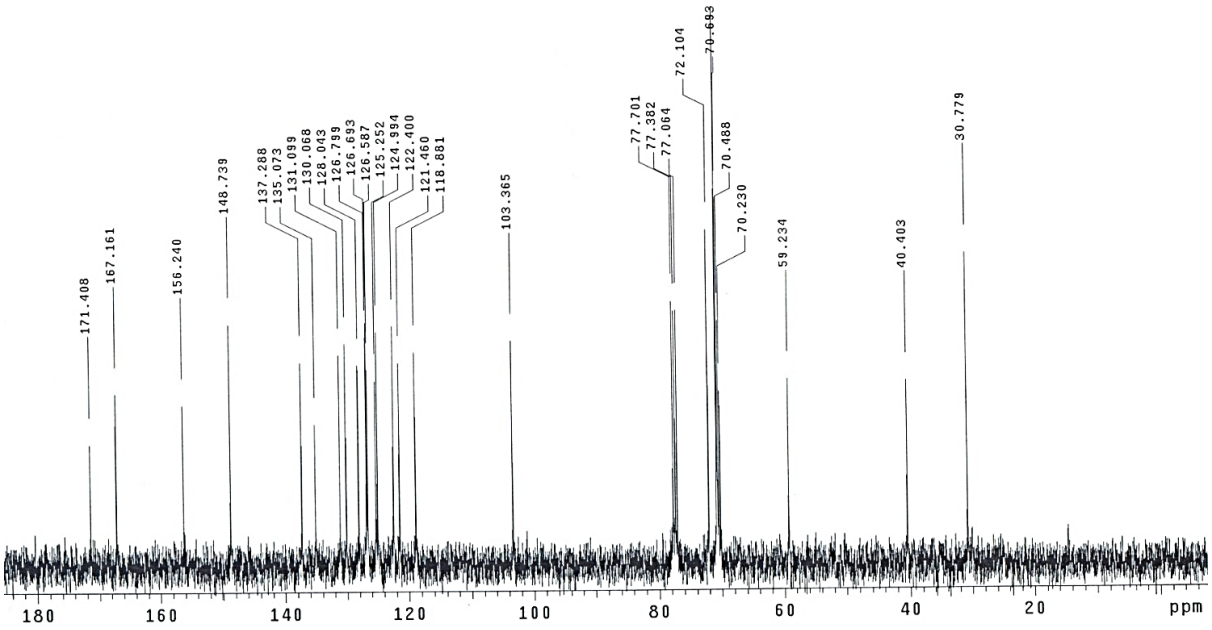


Figure S22. ^{13}C-NMR spectrum (100 MHz) of **1** in $CDCl_3$

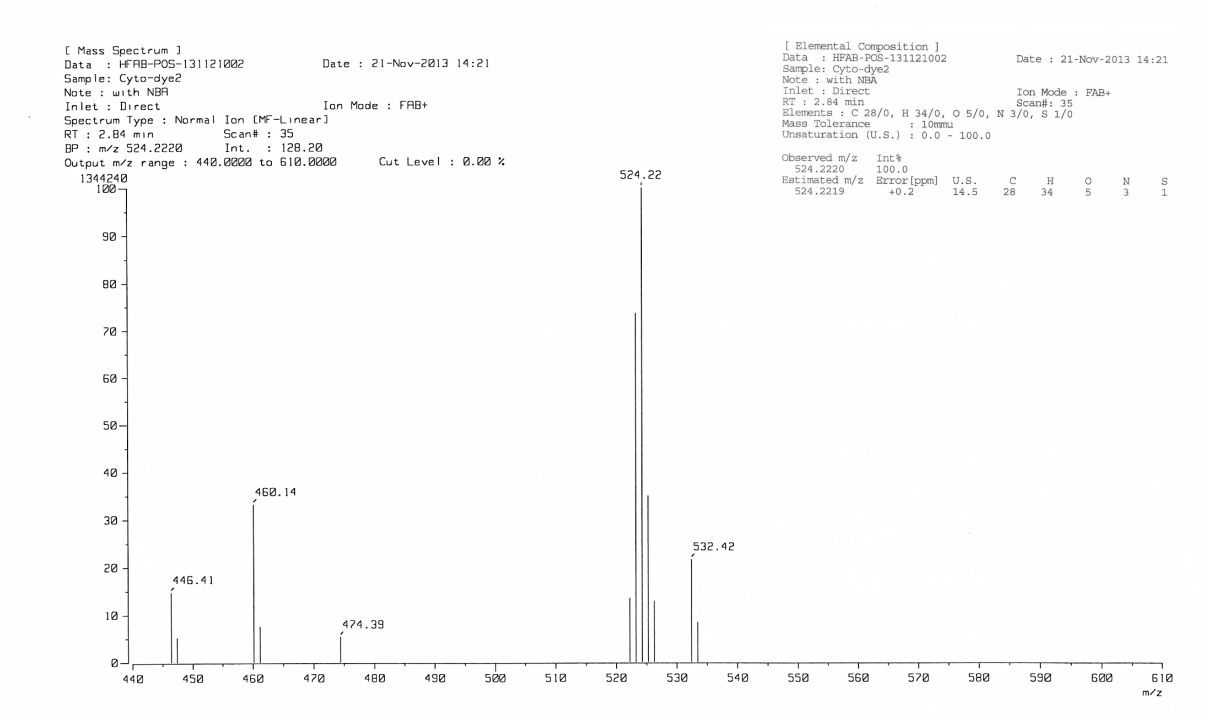


Figure S23. HRMS spectrum of **1**

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