Supporting Information

A Hepatocyte Targeting Single Galactose-appended Naphthalimide: A Tool for Intracellular Thiol Imaging in Vivo

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Contents

Synthetic Materials and Methods

All reactions were carried out under a nitrogen atmosphere. Silica gel 60 (Merck, 0.063~0.2 mm) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). ^{1}H and ^{13}C NMR spectra were collected in $CDCl_3$, CD_3OD (Cambridge Isotope Laboratories, Cambridge, MA) on a Varian 300 and 400 MHz spectrometer. All chemical shifts are reported in ppm value using the peak of residual proton signals of TMS as an internal reference. ESI mass spectral analyses were carried out using LC/MS-2020 Series (Shimadzu). MALDI-TOF mass spectral analyses were carried out at Seoul national university national center for inter-university research facilities. HRMS data received directly from Korea Basic Science Institute.

Additional UV/Vis Absorption and and Fluorescence Studies

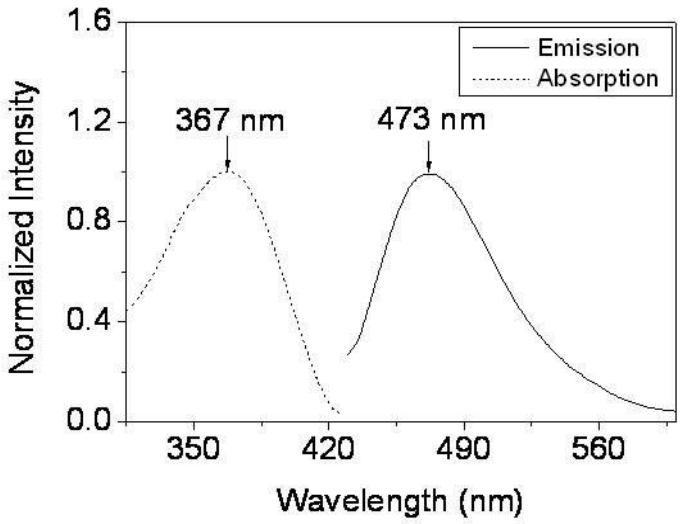

Figure S1. Normalized absorption and emission spectra of **1** recorded in PBS buffer (pH 7.4) at 37 ℃.

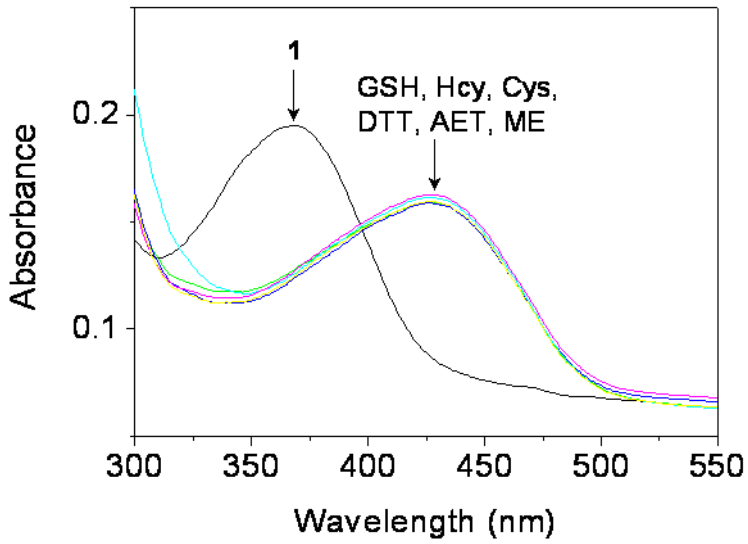

Figure S2. Absorption spectra of **1** (10.0 μM) recorded upon the addition of 5.0 mM of the indicated thiols, namely GSH, cystein (Cys), homocystein (Hcy), 2-aminoethanthiol (AET), 2-mercaptoethanol (ME), dithiothreitol (DTT). Each spectrum was acquired 1 h after the addition of the thiols except in the case of GSH, where a delay of 3 h was used. The spectra were recorded in PBS buffer (pH 7.4) at 37 ℃.

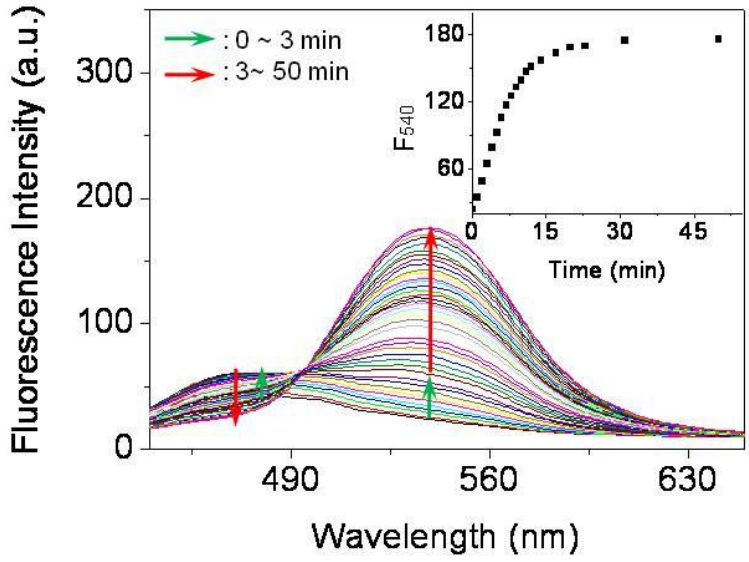

Figure S3. Fluorescence spectral changes as a function of time following the exposure of **1** (1.0 μM) to Cys (5.0 mM) in PBS buffer (pH 7.4) at 37 ℃ with λ_{ex} = 428 nm. The inset shows the fluorescence intensity changes at 540 nm (F_{540}) as a function of time.

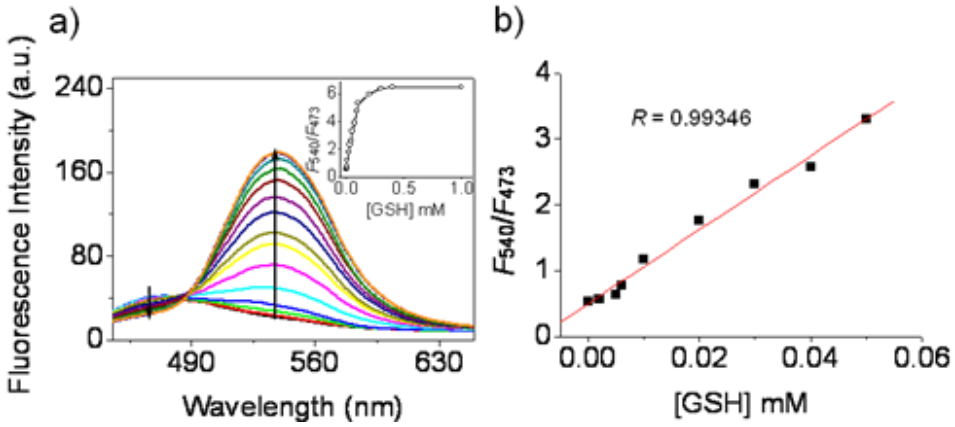

Figure S4. (a) Fluorescence changes observed for aqueous solutions **1** (1.0 μM in PBS buffer) in the presence of increasing concentrations of GSH (0–1.0 mM). All spectra were acquired 3 h after GSH addition at 37 ℃. Inset: Fluorescence intensity ratio (F_{540}/F_{473}) as a function of GSH concentration. (b) Plot showing the linear correlation between the fluorescence intensity ratio (F_{540}/F_{473}) and the GSH concentration (0-0.05 mM).

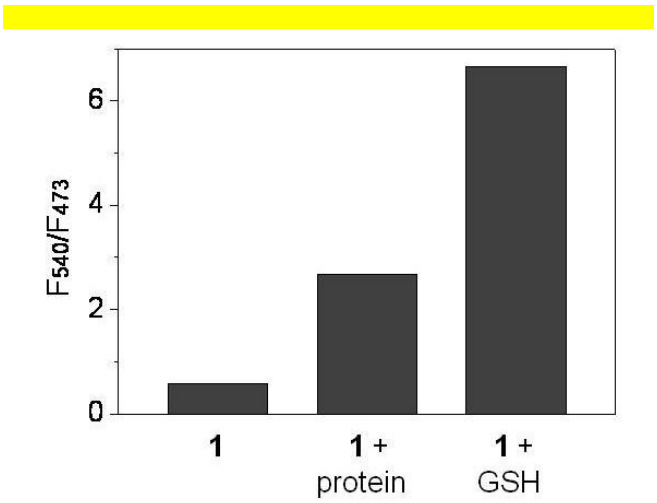

Figure S5. Fluorescence responses of **1** (1.0 μM) in macro-protein mixture (10.0 mg/ml) and GSH (1.0 mM). All data were acquired 3 h in PBS buffer (pH 7.4) at 37 ℃.

MALDI-TOF MS Spectroscopic Analysis

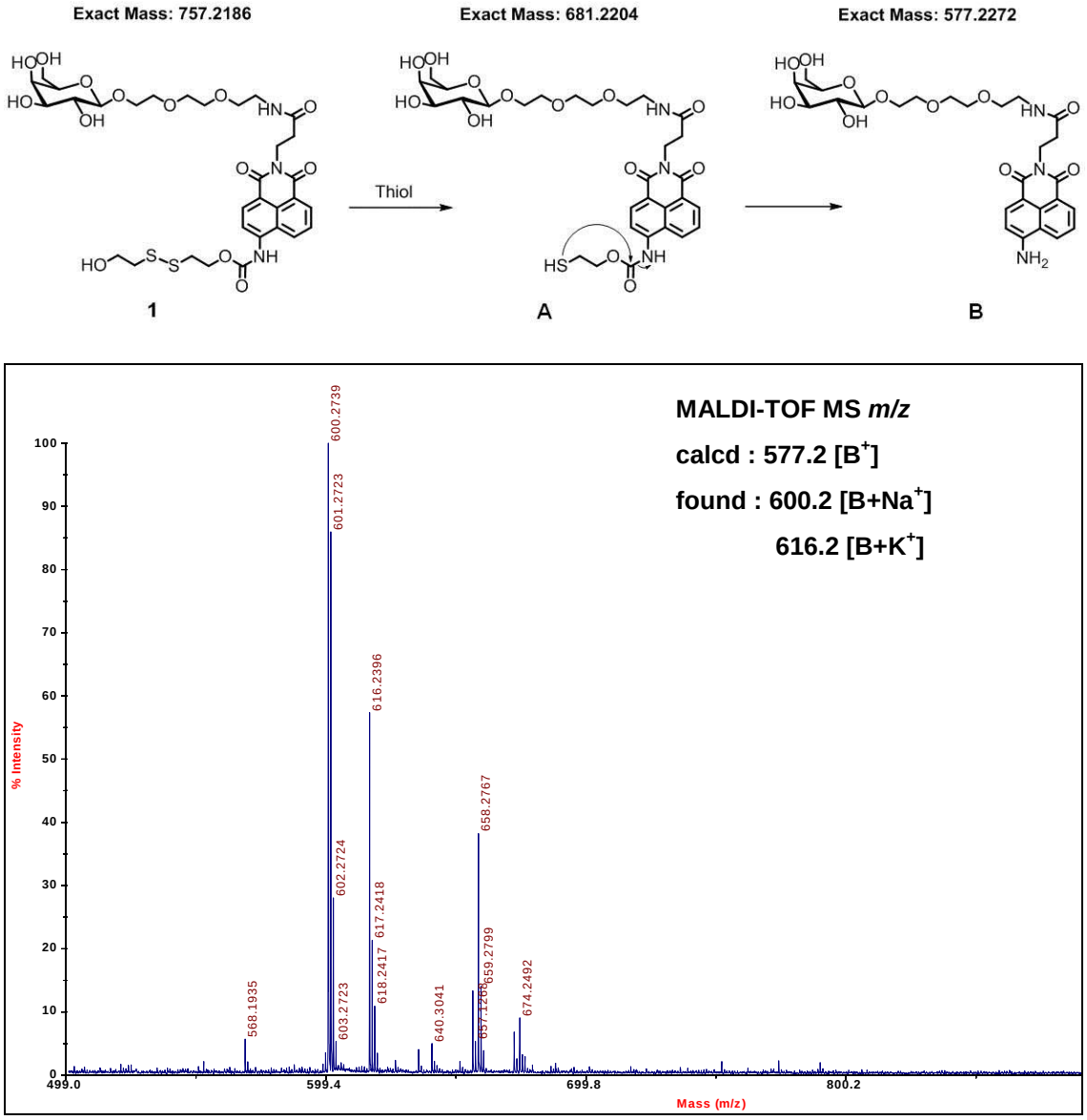

Figure S6. MALDI-TOF MS spectrum of **1** recorded upon the addition of GSH. The spectrum was acquired 3 h after the addition of GSH in PBS buffer (pH 7.4) at 37 °C.

^{1}H-NMR, ^{13}C-NMR and ESI-MS Analyses

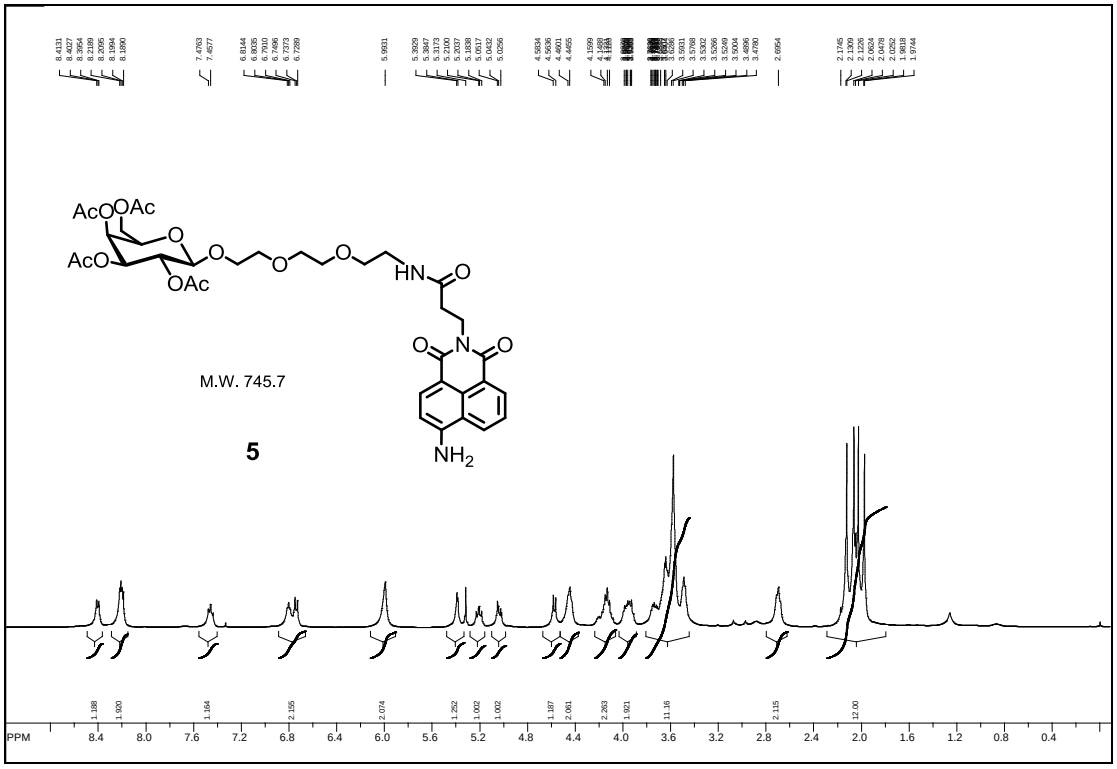

Figure S7. ^{1}H NMR spectrum of **5** recorded in $CDCl_3$.

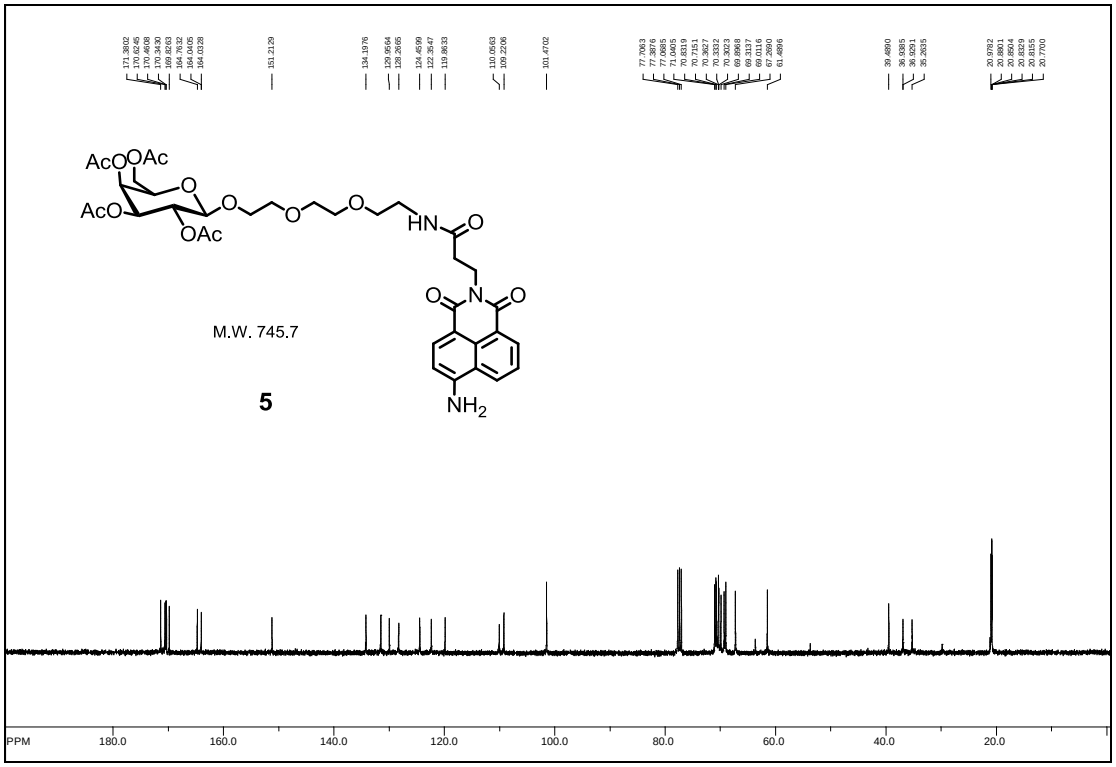

Figure S8. ^{13}C NMR spectrum of **5** recorded in $CDCl_3$.

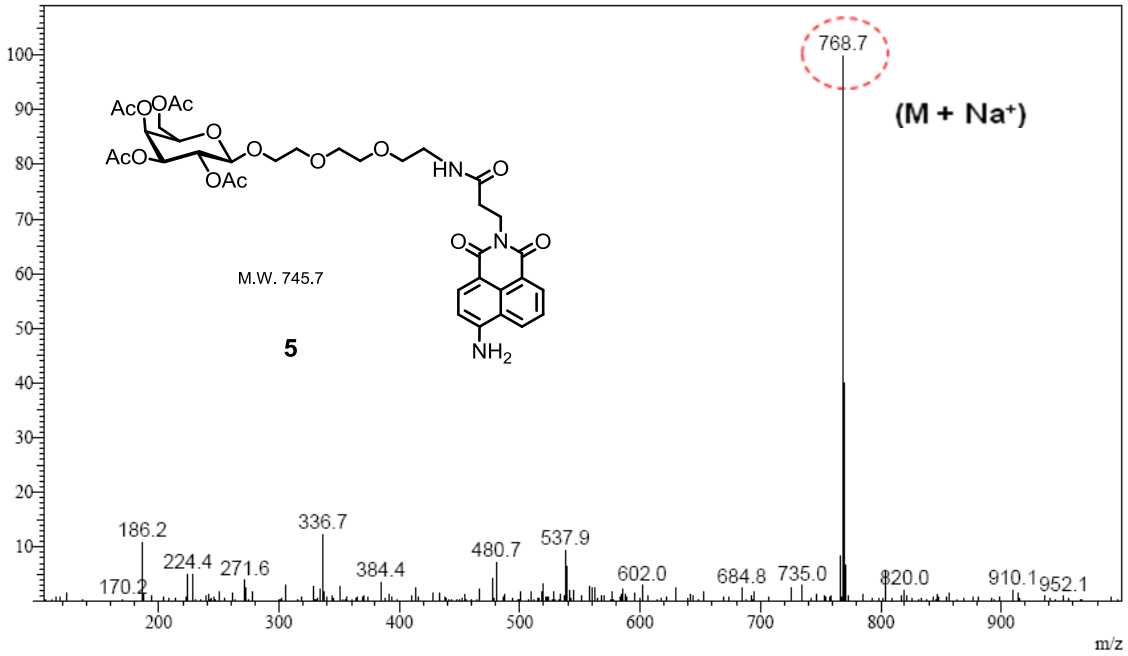

Figure S9. ESI-MS spectrum of **5**.

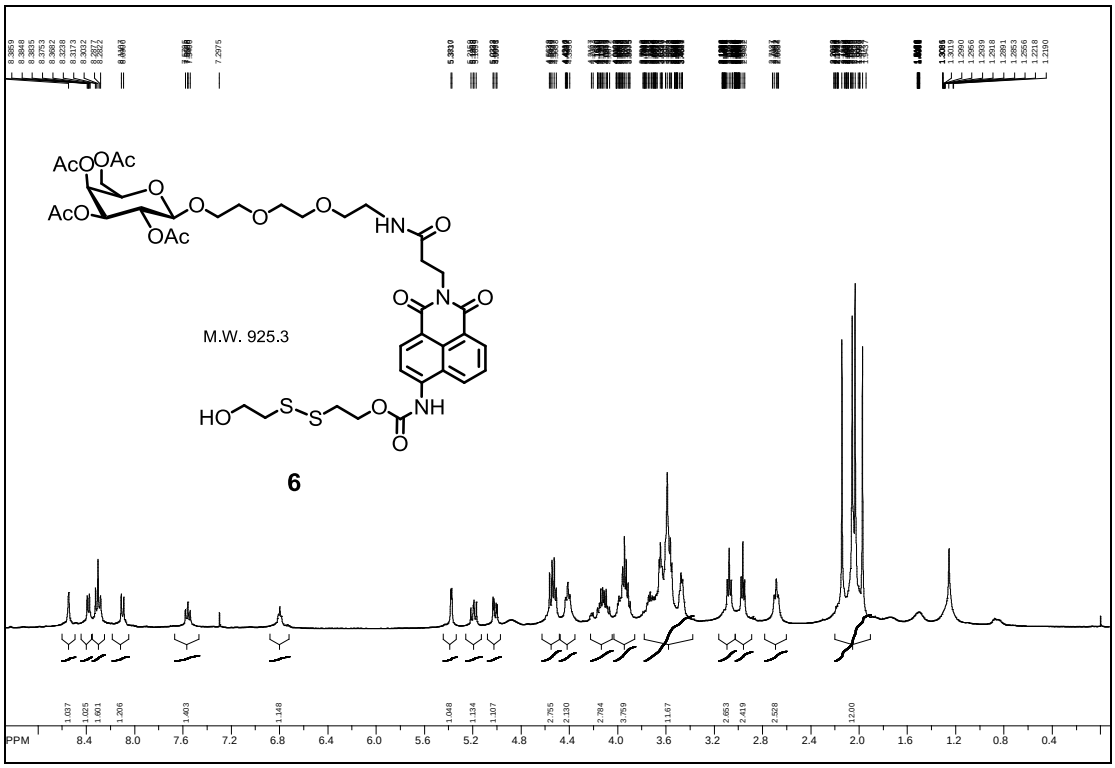

Figure S10. ^{1}H NMR spectrum of **6** recorded in $CDCl_3$.

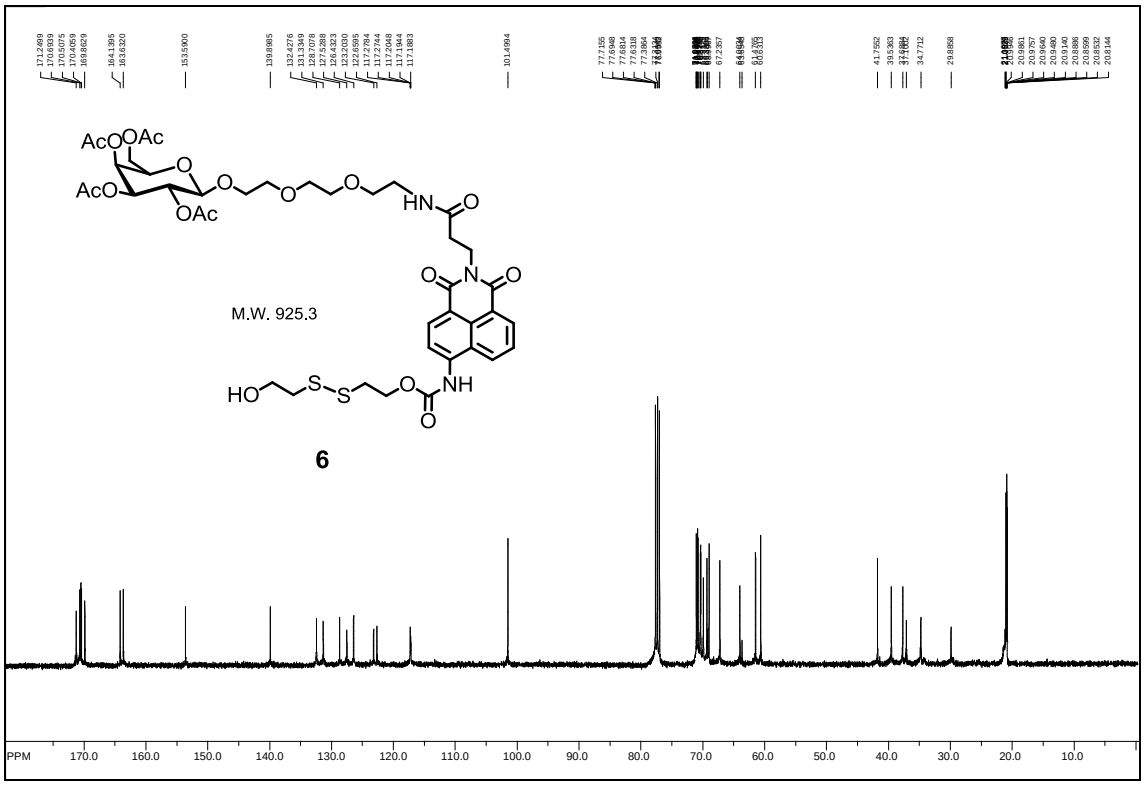

Figure S11. ^{13}C NMR spectrum of **6** recorded in $CDCl_3$.

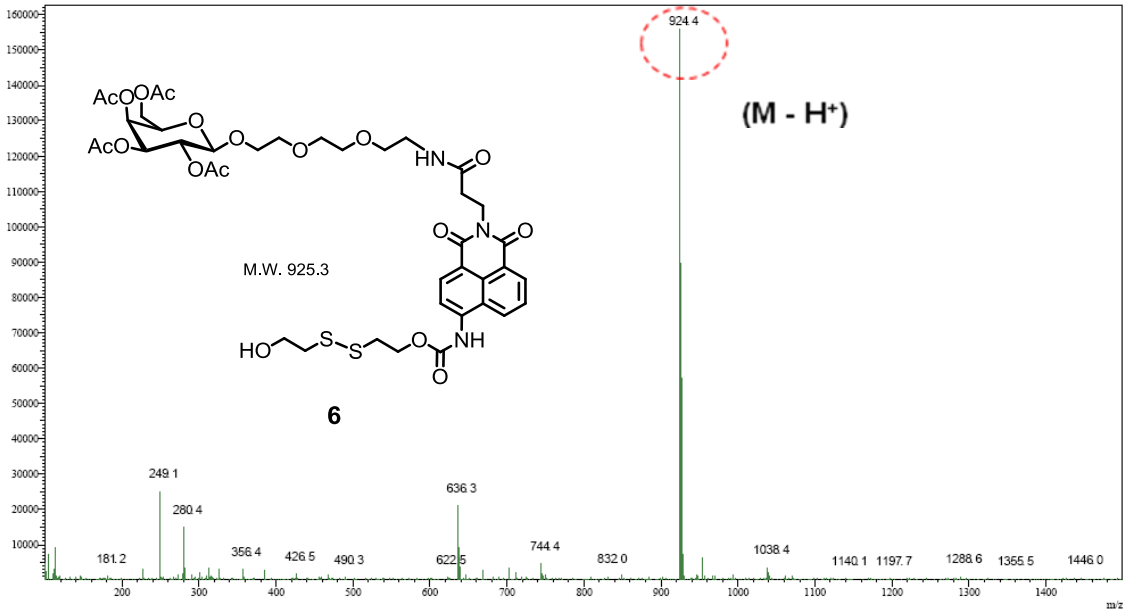

Figure S12. ESI-MS spectrum of **6**.

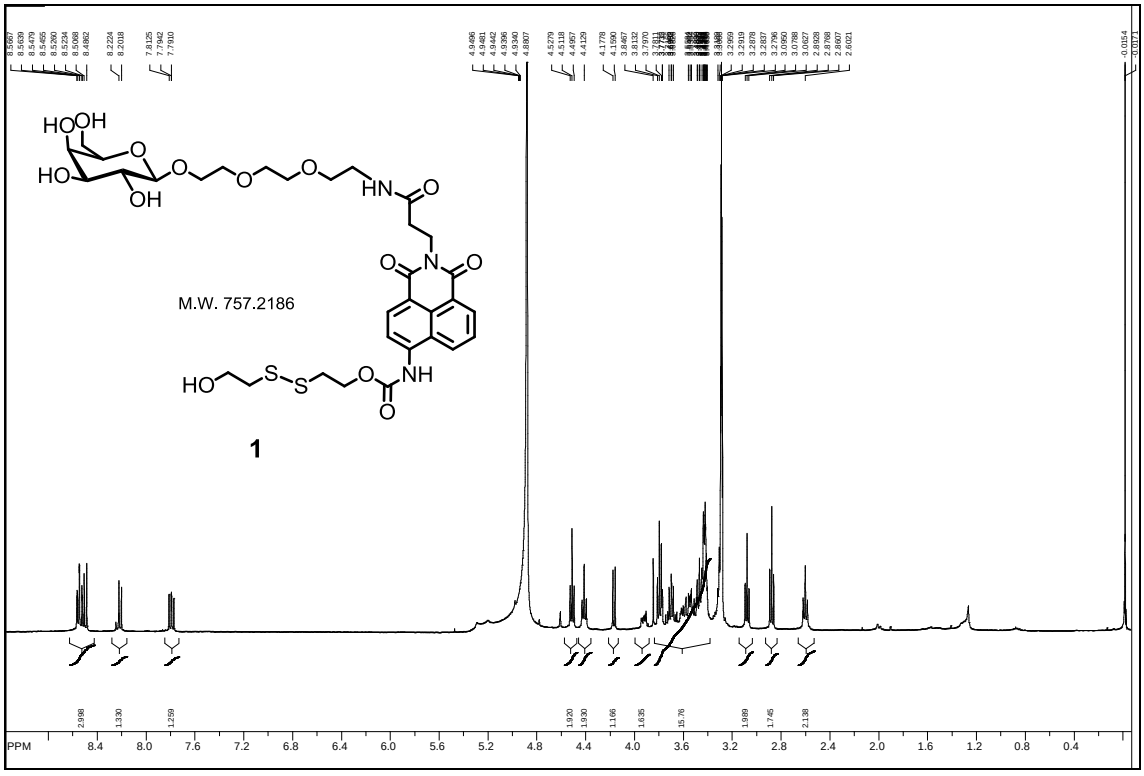

Figure S13. ^{1}H NMR spectrum of **1** recorded in CD_3OD.

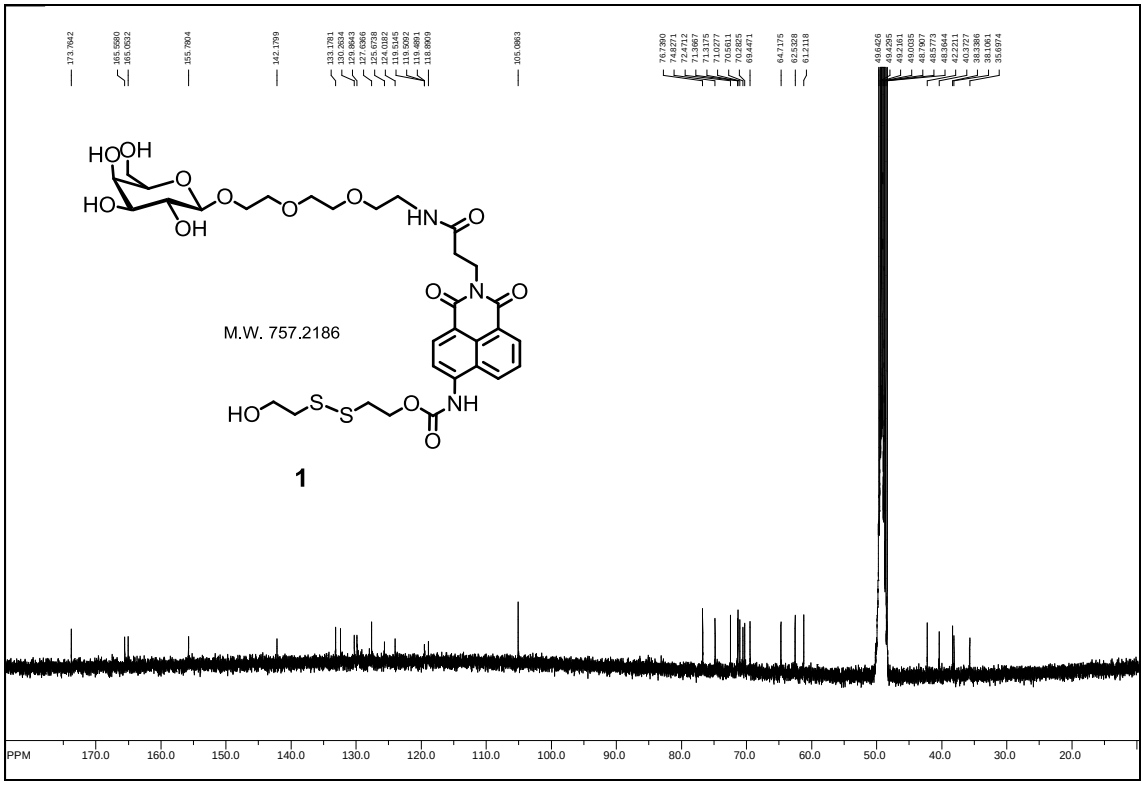

Figure S14. ^{13}C NMR spectrum of **1** recorded in CD_3OD.

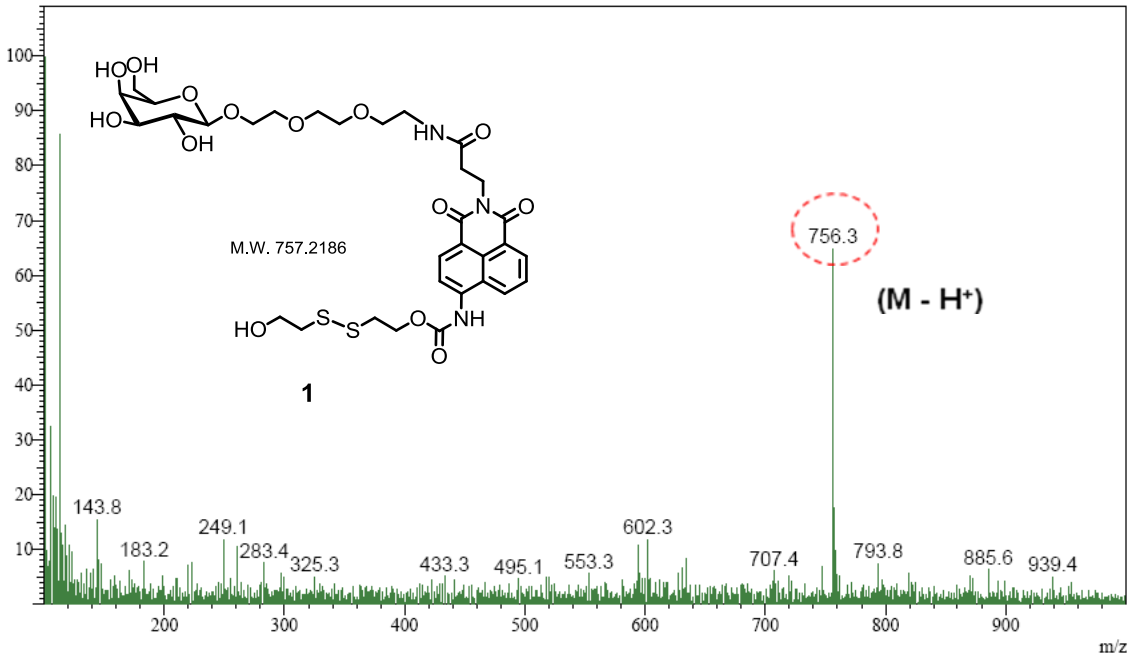

Figure S15. ESI-MS spectrum of **1**.

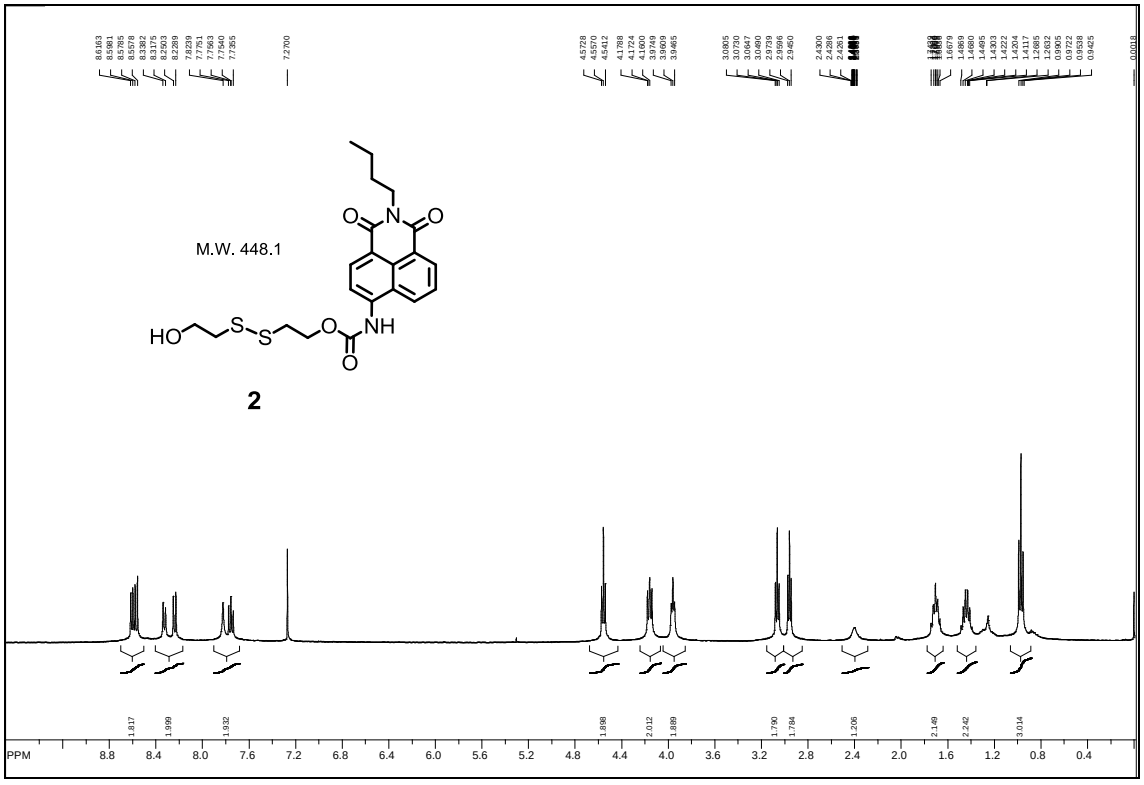

Figure S16. ^{1}H NMR spectrum of **2** recorded in $CDCl_3$.

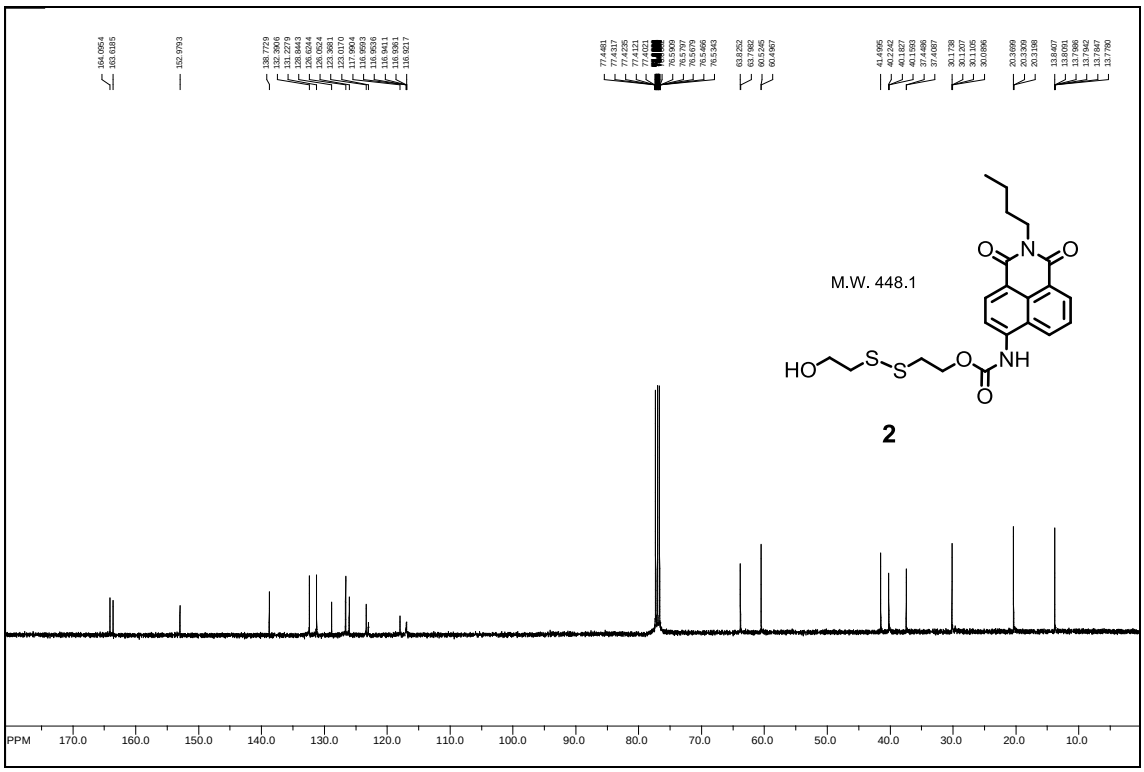

Figure S17. ^{13}C NMR spectrum of **2** recorded in $CDCl_3$.

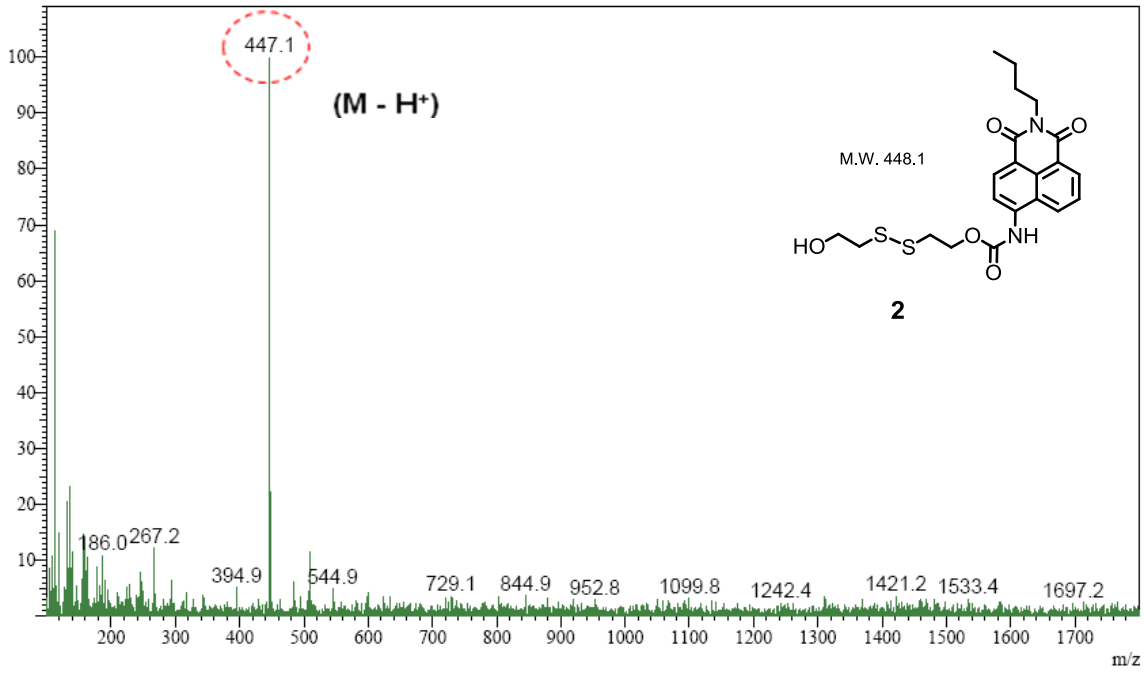

Figure S18. ESI-MS spectrum of **2**.

HRMS results of **1**, **2**, **5**, and **6**

Compound **1**

```
[ Elemental Composition ]
Data  : HFAB-POS-111207003              Date : 07-Dec-2011 16:44
Sample: 01
Note : with GLY
Inlet : Direct                          Ion Mode : FAB+
RT : 3.67 min                           Scan#: 45
Elements : C 32/0, H 44/0, O 14/0, N 3/0, S 2/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  758.2267     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     S
  758.2265       +0.3      14.5    32    44    14     3     2
```

Compound **2**

```
[ Elemental Composition ]
Data  : HFAB-POS-111207004              Date : 07-Dec-2011 17:08
Sample: 02
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 3.59 min                           Scan#: 44
Elements : C 21/0, H 25/0, O 5/0, N 2/0, S 2/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  449.1204     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     S
  449.1205       -0.3      12.5    21    25     5     2     2
```

Compound **5**

```
[ Elemental Composition ]
Data  : HFAB-POS-111207005              Date : 07-Dec-2011 18:11
Sample: 03
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 6.84 min                           Scan#: 83
Elements : C 35/0, H 44/0, O 15/0, N 3/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  746.2770     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N
  746.2772       -0.4      15.5    35    44    15     3
```

Compound **6**

```
[ Elemental Composition ]
Data  : HFAB-POS-111207007              Date : 07-Dec-2011 19:13
Sample: 04
Note : with NBA
Inlet : Direct                          Ion Mode : FAB+
RT : 6.59 min                           Scan#: 80
Elements : C 40/0, H 52/0, O 18/0, N 3/0, S 2/0
Mass Tolerance       : 10mmu
Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z   Int%
  926.2690     100.0
Estimated m/z  Error[ppm]  U.S.     C     H     O     N     S
  926.2687       +0.3      18.5    40    52    18     3     2
```

Figure S19. HRMS results of **1**, **2**, **5**, and **6**.

Additional cell imaging data

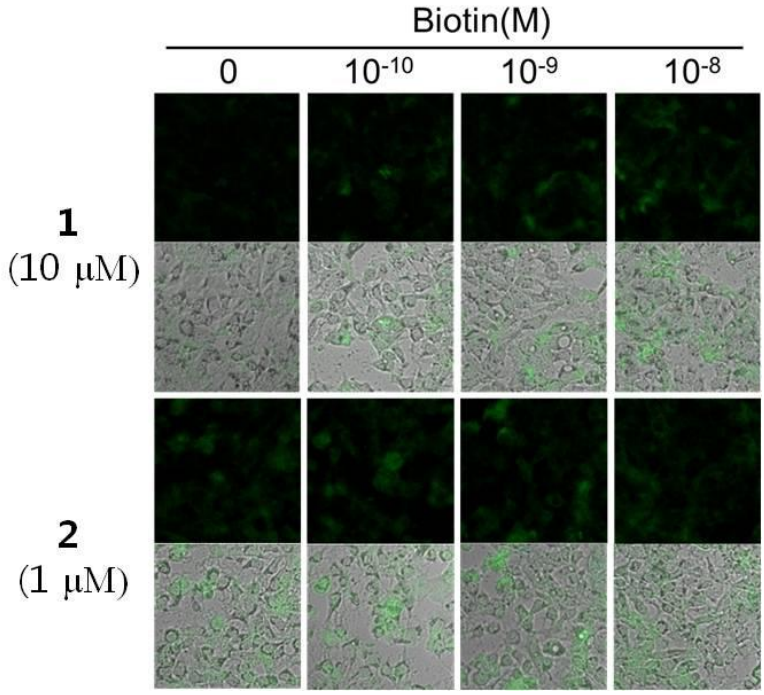

Figure S20. Biotin-dependent fluorescence changes of **1** and **2** in HepG2 cells.

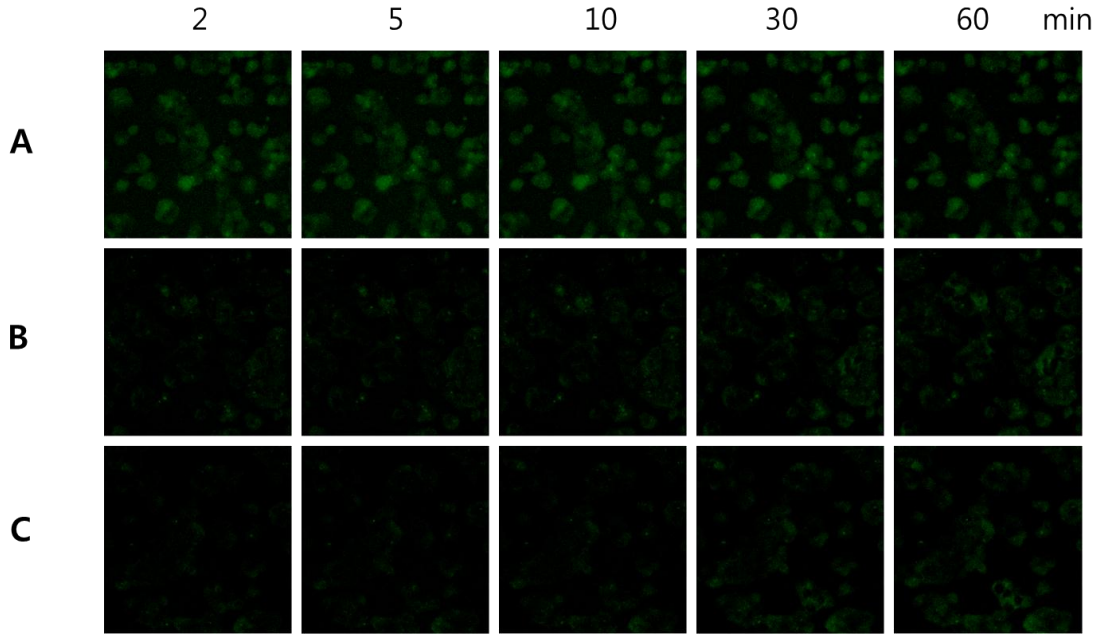

Figure S21. Okadaic acid-dependent fluorescence changes of **1** in HepG2 cells. Confocal microscopic analysis of HepG2 cells treated with **1** (10 μM) for 1h (A). For

the okadaic acid-treated samples, the cells were incubated with media containing okadaic acid at 15 nM (B) and 30 nM (C) for 30 min at 37 ℃ before the media were finally replaced with PBS containing **1**. Images of the cells were obtained using excitation at 458 nm and a band-path (530-600 nm) emission filter.

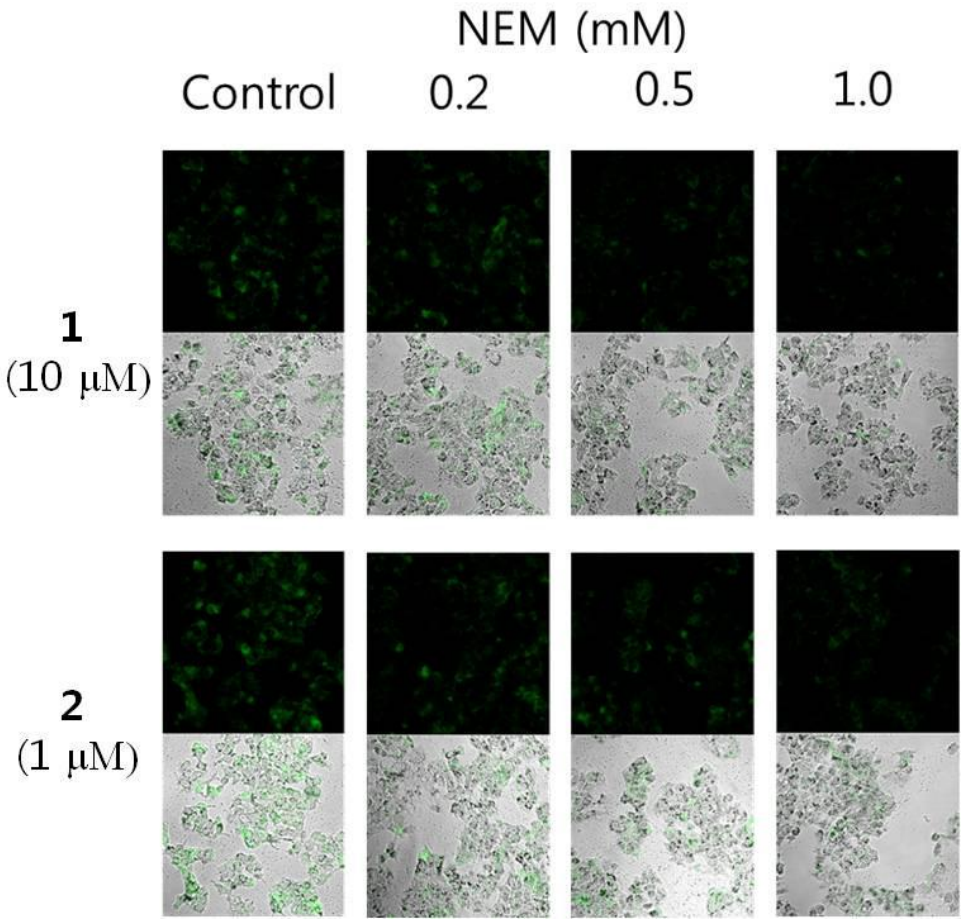

Figure S22. NEM-dependent fluorescence changes of **1** (10 μM) and **2** (1 μM) in HepG2 cells. Before treatment with **1** (10 μM), the cells were incubated with media containing NEM at various concentrations for 1 h at 37 °C (to allow reaction with the cellular thiols; see main text). The cells were then washed briefly with 1 ml of an aqueous PBS solution and then treated with a 10 μM solution of **1** in PBS. After 10 min incubation, free probe **1** was removed by washing three times with PBS before the cells were placed in 1 ml of PBS solution. Images of the cells were obtained using excitation at 458 nm and a band-path (530-600 nm) emission filter.

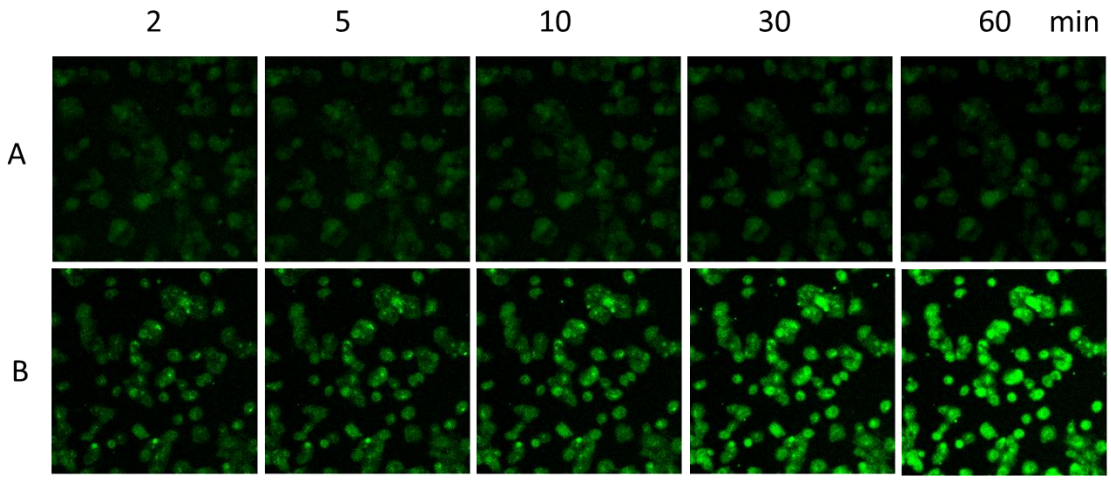

Figure S23. Confocal microscopic analysis of HepG2 cells treated with **1** (10 μM, B) and **2** (1 μM, A). Images of the cells were obtained using excitation at 458 and 488nm and a band-path(530-600nm) emission filter. The media were replaced with PBS containing the Butyl-1 prior to the image acquisition. The cells were then washed briefly with 1 ml of an aqueous PBS solution and then treated with solutions of **1** and **2** in PBS, respectively. After 10 min incubation, free probes **1** and **2** were removed by washing three times with PBS before the cells were placed in 1 ml of PBS solution. Images of the cells were obtained using excitation at 458 nm and a band-path (530-600 nm) emission filter.