

## Supplementary Information

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### Target-selective photo-degradation of a sialyl Lewis A (sLe<sup>a</sup>) conjugate and photo-cytotoxicity against sLe<sup>a</sup> positive cancer cells using an anthraquinone-antibody hybrid

Daisuke Takahashi, Takashi Nagao, Shota Sotokawa, and Kazunobu Toshima\*

*Department of Applied Chemistry, Faculty of Science and Technology, Keio University,  
3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan*

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## General methods for chemical synthesis

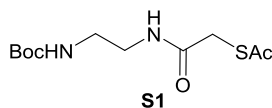
NMR spectra were recorded on a JEOL Lamda (300 MHz for  $^1\text{H}$ ) or a JEOL ECA-500 (125 MHz for  $^{13}\text{C}$ ) spectrometer in the indicated solvent.  $^1\text{H}$ -NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from tetramethylsilane (TMS) ( $\delta$  0.00),  $\text{CD}_3\text{OD}$  ( $\delta$  3.34), or  $\text{CDCl}_3$  ( $\delta$  7.26), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet) and coupling constants (Hz).  $^{13}\text{C}$ -NMR data are reported as follows; chemical shift in parts per million (ppm) downfield or upfield from  $\text{CD}_3\text{OD}$  ( $\delta$  49.70),  $\text{CDCl}_3$  ( $\delta$  77.16) or external standard acetone ( $\delta$  30.89). Melting points were determined on a micro hot-stage (Yanako MP-S3). ESI-TOF mass spectra were measured on a Waters LCT premier XE. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF MS) was conducted using a Bruker Ultraflex mass spectrometer with detection in linear mode. Sinapinic acid was used as the matrix, with positive ionization mode. UV-vis spectra were recorded on a JASCO V-650DS spectrophotometer. Silica gel TLC and column chromatography were performed using Merck TLC 60F-254 (0.25 mm) and Silica gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.), respectively. Reverse phase column chromatography separations were performed using Wakosil 25C18 (Wako pure chemical industries, Ltd.). Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon using oven-dried glass ware. In general, organic solvents were purified and dried using an appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

## Materials

Sulfo-SMCC (**13**) was purchased from Dojindo. Human serum albumin (HSA) was purchased from Funakoshi. Anti-sLe<sup>a</sup> mAb CA19-9-203 (**12**) was purchased from Abcam. HSA-sLe<sup>a</sup> conjugate **4** and HSA-Le<sup>a</sup> conjugate **5** were purchased from GlycoTech. Anti-KLH mAb **14** and anti-HSA mAb were purchased from R&D Systems. Anti-Le<sup>a</sup> mAb was purchased from SPRING. The human epidermoid squamous carcinoma cell line, A431 (RCB0202), the human cervix epidermoid carcinoma cell line, HeLa (RCB0007), and the human hepatocellular carcinoma cell line, HuH-7 (RCB1942) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The human colon adenocarcinoma cell lines, WiDr (JCRB0224) was purchased from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan).

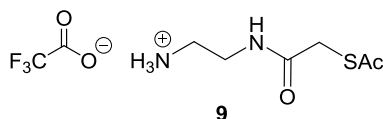
## Synthesis of AQ-mAb hybrids 6 and 7

### Compound S1



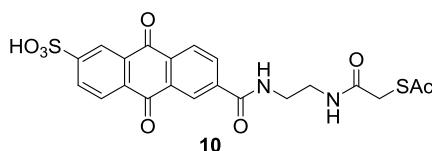
To a solution of **8**<sup>[1]</sup> (1.07 g, 3.81 mmol) in dry DMF (20.0 mL) was dropwisely added thioacetic acid potassium salt (KSAc) (522 mg, 4.57 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was added to water (50.0 mL). The aqueous layer was extracted with three portions of EtOAc (50.0 mL). The combined extracts were washed with brine (10.0 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in *vacuo*. The residue was subjected to silica gel column chromatography (40.0 g, hexane/EtOAc = 1/2 to 0/1) to give **S1** (1.01 g, 3.66 mmol, 96% yield) as a white solid. *R*<sub>f</sub> 0.48 (1/2 hexane/EtOAc); m.p. 103-105 °C; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>, TMS): δ 3.55 (2H, s), 3.33 (2H, t, *J* = 5.7 Hz), 3.26 (2H, t, *J* = 5.6 Hz), 2.42 (3H, s), 1.45 (9H, s); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>, TMS): δ 195.4, 168.6, 156.6, 79.6, 40.9, 40.1, 32.9, 30.3, 28.4; HRMS (ESI-TOF) *m/z* 299.1033 (299.1041 calcd for C<sub>11</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>SNa, [M+Na]<sup>+</sup>).

### Compound 9



To a solution of **S1** (579 mg, 2.09 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (17.0 mL) was dropwisely added TFA (17.0 mL) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was subjected to reverse phase column chromatography (H<sub>2</sub>O:MeOH = 1:0 to 0:1) to give **9** (339 mg, 1.17 mmol, 56% yield) as a light yellow oil. *R*<sub>f</sub> 0.33 (3/1 CHCl<sub>3</sub>/MeOH); <sup>1</sup>H-NMR (300 MHz, CD<sub>3</sub>OD, TMS): δ 3.56 (2H, s), 3.38 (2H, t, *J* = 6.0 Hz), 2.97 (2H, t, *J* = 5.9 Hz), 2.28 (3H, s); <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD, TMS): δ 196.32, 171.9, 40.6, 38.5, 33.6, 30.0; HRMS (ESI-TOF) *m/z* 177.0691 (177.0698 calcd for C<sub>6</sub>H<sub>13</sub>N<sub>2</sub>O<sub>2</sub>S, [M+H]<sup>+</sup>).

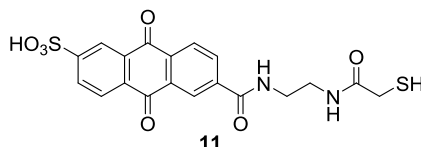
### Compound 10



To a solution of **3**<sup>[2]</sup> (140 mg, 0.421 mmol) in DMF (7.00 mL) were added TBTU (271 mg, 0.843 mmol), NEM (160 μL, 1.26 mmol) and **9** (89.0 mg, 0.505 mmol). After being stirred for 16 h at room temperature, the reaction mixture was concentrated in *vacuo*. The residue was subjected to reverse phase column chromatography (H<sub>2</sub>O:MeOH = 1:0 to 0:1). The resulting

solid was dissolved in water (5.00 mL) and recrystallized with brine (5.00 mL) to give 2/6-disubstituted **10** (80.5 mg, 0.164 mmol, 39% yield) as a brown solid.  $R_f$  0.59 (3/2 CHCl<sub>3</sub>/MeOH); m.p. >300 °C; <sup>1</sup>H-NMR (300 MHz, D<sub>2</sub>O):  $\delta$  7.78-8.12 (6H, m), 3.51 (2H, s), 3.37-3.39 (4H, m), 2.13 (3H, s); <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O, acetone):  $\delta$  199.8, 182.8, 182.6, 172.1, 168.3, 149.1, 139.5, 134.8, 134.4, 133.7, 133.4, 133.0, 132.2, 128.8, 128.3, 126.3, 124.7, 40.0, 39.6, 33.5, 30.1; HRMS (ESI-TOF)  $m/z$  489.0414 (489.0426 calcd for C<sub>21</sub>H<sub>17</sub>N<sub>2</sub>O<sub>8</sub>S<sub>2</sub>, [M-H]<sup>-</sup>).

### Compound 11



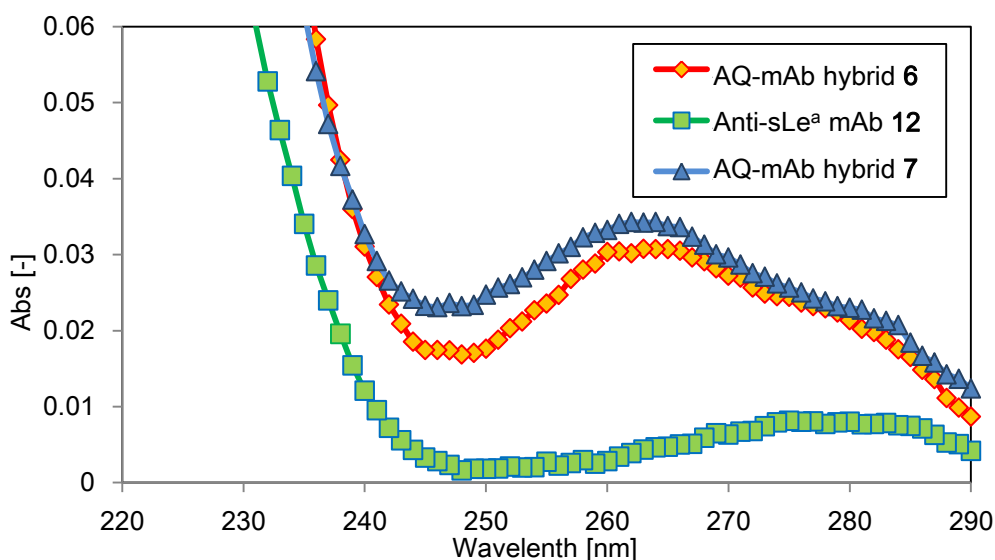
To a solution of **10** (16.4 mg, 33.4  $\mu$ mol) in dry MeOH (1.60 mL) was added 28% NaOMe in MeOH (7.03  $\mu$ L, 40.1  $\mu$ mol) at room temperature. After being stirred at room temperature for 20 min, the reaction mixture was filtered, and quenched with Amberlite<sup>®</sup>. The residue was filtered and concentrated in *vacuo* to give **11** (11.2 mg, 25.1  $\mu$ mol, 75% yield) as a brown oil.  $R_f$  0.72 (3/2 CHCl<sub>3</sub>/MeOH); <sup>1</sup>H-NMR (300 MHz, D<sub>2</sub>O):  $\delta$  7.81-8.10 (6H, m), 3.40 (4H, q,  $J$  = 7.8 Hz), 3.14 (2H, s); <sup>13</sup>C-NMR (125 MHz, D<sub>2</sub>O, acetone):  $\delta$  182.3, 182.0, 174.7, 167.9, 149.2, 139.3, 134.3, 133.9, 133.7, 133.0, 132.6, 132.2, 128.7, 128.2, 126.1, 124.6, 40.0, 39.6, 28.0; HRMS (ESI-TOF)  $m/z$  447.0308 (447.0321 calcd for C<sub>19</sub>H<sub>15</sub>N<sub>2</sub>O<sub>7</sub>S<sub>2</sub>, [M-H]<sup>-</sup>).

### AQ-mAb hybrid 6

To the column (Ab-Rapid SPiN Ex, Protenova) was added anti-sLe<sup>a</sup> mAb **12** (500  $\mu$ L, 0.2 mg/mL) at room temperature. After the suspension was gently shaken at room temperature for 90 min, the resulting gel was washed three times with 10 mM PBS (pH 7.4). And then, **12** was eluted with elution buffer (400  $\mu$ L, 0.1 M Glycine-HCl, pH 2.8) and promptly neutralized with neutralization buffer (9.0  $\mu$ L, 1 M Tris). To a solution of **12** (100  $\mu$ g, 685 pmol) in 10 mM PBS (pH 7.4, 130  $\mu$ L) was added sulfo-SMCC (**13**) (29.9  $\mu$ g, 68.5 nmol) in DMF (6.85  $\mu$ L) at room temperature. After the reaction mixture was incubated for 1 h at 25 °C, Amicon 3K centrifugal filter device (Millipore) was used to separate from excess **13** and to concentrate the sample solution. And then, to a solution of anti-sLe<sup>a</sup> mAb-SMCC **S2** in 10 mM PBS (pH 7.4, 100  $\mu$ L) was added **11** (30.7  $\mu$ g, 68.5 nmol) in 10 mM PBS (pH 7.4, 37.0  $\mu$ L) at room temperature. After the reaction mixture was incubated for 16 h at 4 °C, Amicon 3K centrifugal filter device (Millipore) was used to separate from excess **11** and to concentrate the sample solution to give AQ-mAb hybrid **6** (0.27 mg/mL, 100  $\mu$ L, 27% yield in 2 steps). The chemical yield of **6** was calculated based on the Bradford method.

### AQ-mAb hybrid 7

To a solution of anti-KLH mAb **14** (100  $\mu\text{g}$ , 685 pmol) in 10 mM PBS (pH 7.4, 130  $\mu\text{L}$ ) was added sulfo-SMCC (**13**) (29.9  $\mu\text{g}$ , 68.5 nmol) in DMF (6.85  $\mu\text{L}$ ) at room temperature. After the reaction mixture was incubated for 1 h at 25  $^{\circ}\text{C}$ , Amicon 3K centrifugal filter device (Millipore) was used to separate from excess **13** and to concentrate the sample solution. And then, to a solution of anti-KLH mAb-SMCC **S3** in 10 mM PBS (pH 7.4, 100  $\mu\text{L}$ ) was added **11** (30.7  $\mu\text{g}$ , 68.5 nmol) in 10 mM PBS (pH 7.4, 37.0  $\mu\text{L}$ ) at room temperature. After the reaction mixture was incubated for 16 h at 4  $^{\circ}\text{C}$ , Amicon 3K centrifugal filter device (Millipore) was used to separate from excess **11** and to concentrate the sample solution to give AQ-mAb hybrid **7** (0.54 mg/mL, 100  $\mu\text{L}$ , 55% yield in 2 steps). The chemical yield of **7** was calculated based on the Bradford method.

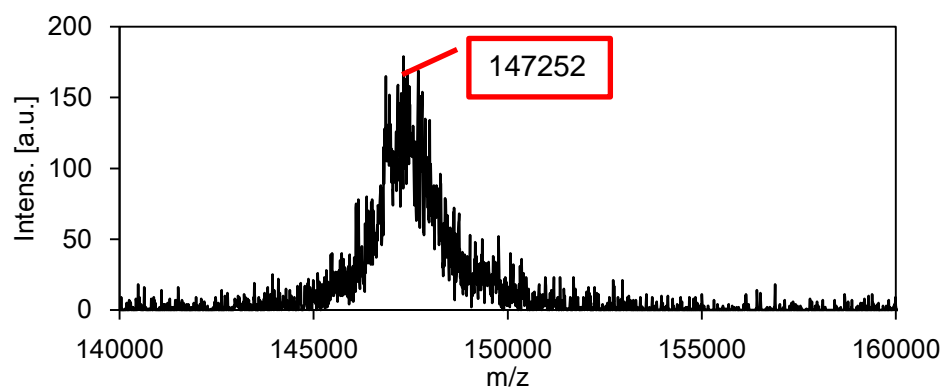


**Figure S1.** UV spectra of **6**, **12**, and **7**. These compounds (1.2  $\mu\text{M}$ ) were dissolved in PBS (10 mM, pH 7.4).

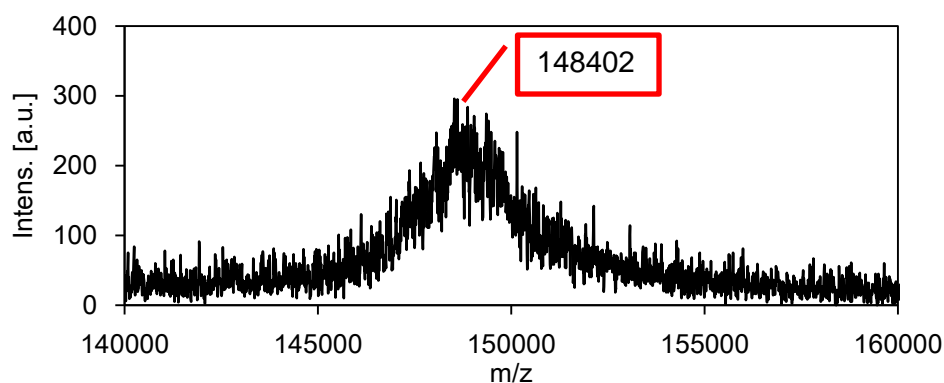
### MALDI TOF MS analysis

The sample (1.00  $\mu\text{L}$ ) was mixed with sinapinic acid (1.00  $\mu\text{L}$  in 0.1% TFA in water: acetonitrile = 50:50) matrix. Analyses by MALDI TOF MS were performed in the positive ion mode on Ultra flex (Bruker).

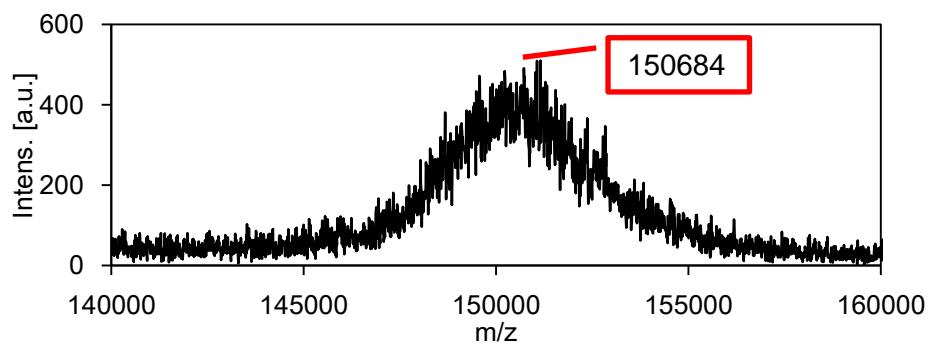
(a) Anti-sLe<sup>a</sup> mAb**12**



(b) Anti-sLe<sup>a</sup> mAb-SMCC **S2**

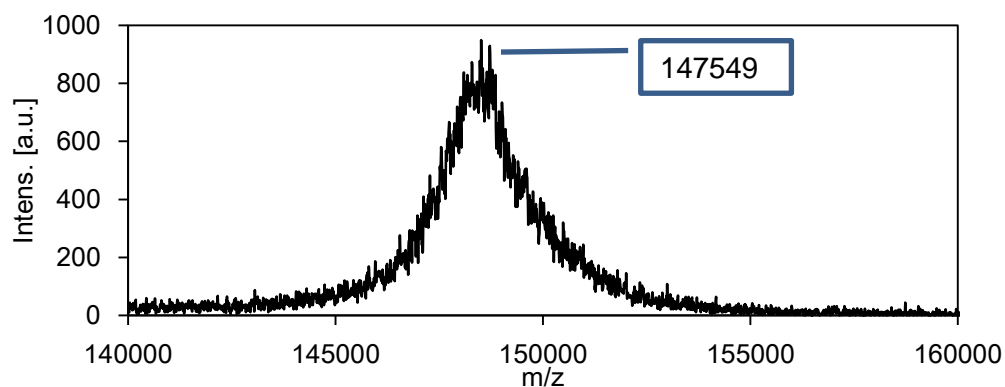


(c) AQ-mAb hybrid **6**

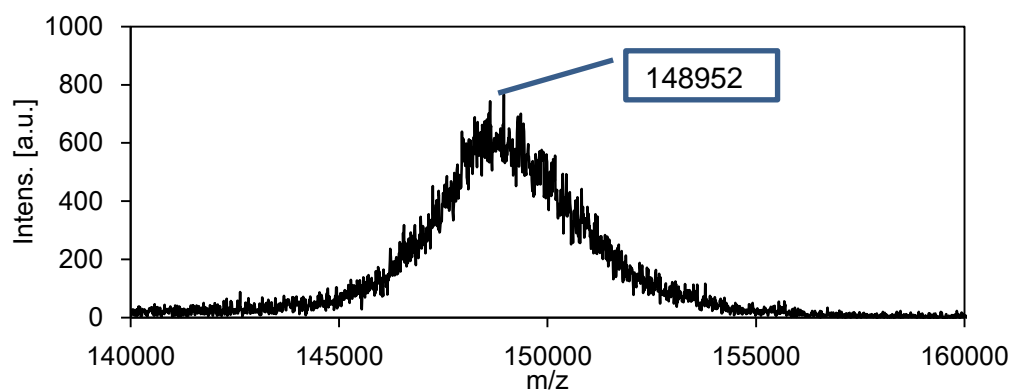


**Figure S2.** MALDI-TOF MS spectra of (a) anti-sLe<sup>a</sup> mAb **12**, (b) anti-sLe<sup>a</sup> mAb-SMCC **S2**, and (c) AQ-mAb hybrid **6**.

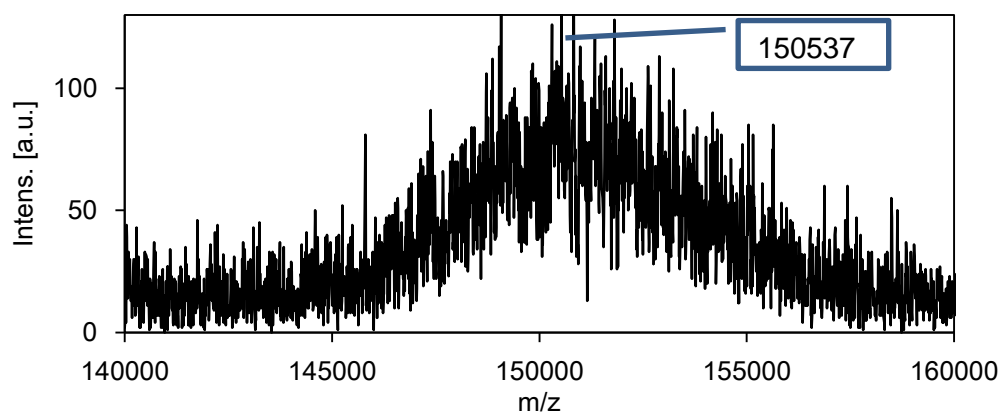
(a) Anti-KLH mAb **14**



(b) Anti-KLH mAb-SMCC **S3**

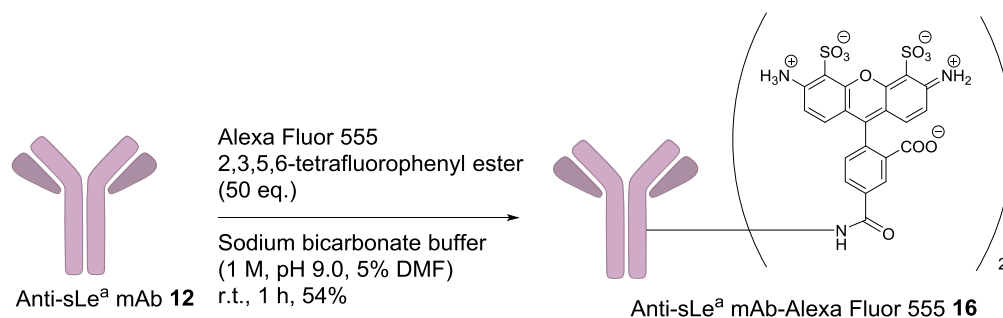


(c) AQ-mAb hybrid **7**



**Figure S3.** MALDI-TOF MS spectra of (a) anti-KLH mAb **14**, (b) anti-KLH mAb-SMCC **S3**, and (c) AQ-mAb hybrid **7**.

## Synthesis of anti-sLe<sup>a</sup> mAb-Alexa fluor 555 conjugate **16**

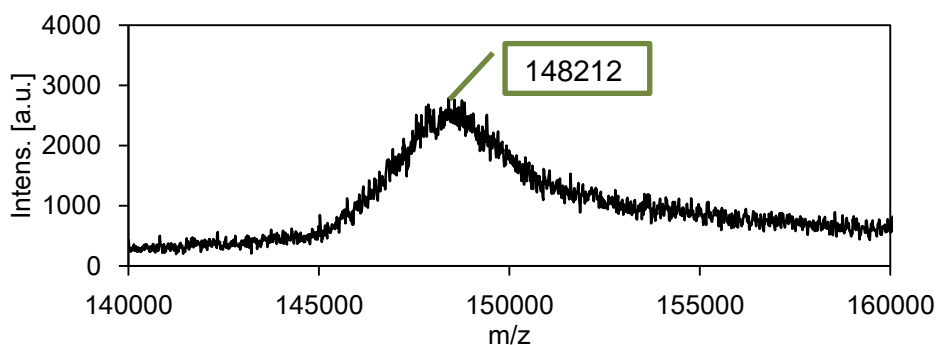


**Scheme S1.** Synthetic scheme of anti-sLe<sup>a</sup> mAb-AF555 **16**.

### Preparation of anti-sLe<sup>a</sup> mAb-AF555 **16**

To a solution of anti-sLe<sup>a</sup> mAb **12** (100 µg, 685 pmol) in 1 M sodium bicarbonate buffer (pH 9.0) was added Alexa Fluor 555 2,3,5,6-tetrafluorophenyl ester (29.9 µg, 34.3 nmol) in DMF (6.85 µL) at room temperature. After the reaction mixture was incubated for 1 h at 25 °C, Amicon 3K centrifugal filter device (Millipore) was used to separate from excess AF555 and to concentrate the sample solution to give anti-sLe<sup>a</sup> mAb-AF555 **16** (0.54 mg/mL, 100 µL, 54% yield). The chemical yield of **16** was calculated based on the Bradford method.

Anti-sLe<sup>a</sup> mAb-AF555 **16**



**Figure S4.** MALDI-TOF MS spectra of anti-sLe<sup>a</sup> mAb-AF555 **16**.

### Bradford assay

To a sample (10.0 µL) in test tube, a volume of 300 µL Bradford assay reagent (Thermo Scientific) was added, and then the resulting mixture was blended by gentle vortex mixing. After 5 min, absorbance at 595 nm was measured in plastic 96-well microplate against a reagent blank using SpectraMax i3 (Molecular Devices). The calibration curve was built using BSA samples (0.100-0.500 µg).

## ELISA assay

HSA-sLe<sup>a</sup> **4** (1.00 µg/mL) in 10 mM PBS (pH 7.4) was coated overnight at 4 °C on a 96-well ELISA plate (Sumitomo Bakelite Co., Ltd.). The solution was then removed, and 300 µL of the blocking buffer (10 mM PBS, 1% nonfat dry milk) was added to each well and incubated at room temperature for 2 h. The wells were then washed with 300 µL of 10 mM PBS-T (pH 7.4, 0.05% Tween-20). Hybrid **6** was prepared in 10 mM PBS (pH 7.4) at a range of concentrations, and was added (100 µL) to each well. The plate was then incubated for 2 h at room temperature. The sample solutions were then removed, and the wells washed three times with PBS-T. To detect the sample, 100 µL of anti-mouse IgG-peroxidase conjugate (1:800 dilution) (GE healthcare) was added into each well and incubated for 1 h at room temperature. The solutions were then removed and the plate was washed with PBS-T three times. OPD (3.7 mM, 100 µL/well) in citrate buffer (citric acid 26.5 mM, sodium hydrogen phosphate 57.8 mM and H<sub>2</sub>O<sub>2</sub> 3.82 µM) was then added into each well. After approximately 15 min, when the yellow color was sufficiently visible, 50.0 µL of 4 N H<sub>2</sub>SO<sub>4</sub> solution was added into each well to stop the enzymatic reaction as indicated by the solution turning brown. The absorbance was read using a microplate reader at 490 nm within 30 min.

## Photo-degradation of HSA-sLe<sup>a</sup> **4** using hybrid **6**

To a solution of HSA-sLe<sup>a</sup> **4** (5.00 µL, 1 pmol) in 10 mM PBS (pH 7.4) was added a solution of hybrid **6** (5.00 µL, 0.3-10 pmol) in 10 mM PBS. After being incubated at room temperature for 30 min, the mixture was incubated at 25 °C for 2 h with or without photo-irradiation using a UV lamp (365 nm, 100 W, Blak-ray (B-100A), UVP. Inc.) placed 10 cm from the sample. And then, 5.00 µL of electrophoresis buffer consisted of SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol) was added to the photo-irradiated samples. The photo-degradation products were separated by SDS-PAGE in 7% polyacrylamide gels and electrotransferred onto Amersham Hybond ECL Nitrocellulose Membrane (GE Healthcare). The membrane was blocked with 10 mM Tris-buffered saline-0.1% Tween 20 (TBS-T) containing 5% nonfat dry milk for 2 h at room temperature. After a short wash in TBS-T, the membrane was incubated in a 1:1000 dilution of a primary antibody (abcam) in TBS-T for 14 h at 4 °C followed by washing with TBS-T for 30 min. The bound antibody was then detected with horseradish peroxidase-conjugated secondary antibody anti-mouse IgG (GE healthcare) diluted at 1:3000 in TBS-T and 2% (wt/vol) nonfat dry milk by incubation with it for 2 h at 4 °C. After having been washed for 30 min in TBS-T, the immunocomplexes were detected by using ECL reagent, Immobilon Western (Millipore, Billerica, MA). Exposure to RX-U films (Fuji Film, Kanagawa, Japan) was carried out for 1 min to 10 min.

## Cell Culture

### <A431, WiDr>

The A431 or WiDr cell line was routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycine. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### <HuH-7>

The HuH-7 cell line was routinely grown in RPMI medium supplemented with 5% (v/v) Fetal bovine serum, 0.5% (v/v) penicillin and kanamycine. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### <HeLa>

The HeLa cell line was routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% (v/v) Bovine Calf Serum, 0.5% (v/v) penicillin and kanamycine. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

## MTT assay

1×10<sup>3</sup> cells in 90.0 µL of medium were cultured in 96-well microplates, and then incubated for 24 h at 37 °C prior to the addition of experimental sample. To each well was added 10.0 µL of hybrid **6** (10.0 pmol/well) in medium and the culture was incubated for 24 h. The plate was irradiated with a UV lamp (368 nm, 30 W, FL15BLB-368, Sankyo Denki Co., Ltd.) from 20 cm for 30 min, and then incubated for 72 h at 37 °C. Cell viability was evaluated using the MTT assay. 10.0 µL of 5.00 mg/mL MTT dissolved in PBS was added to each well. After incubation for 3 h at 37 °C, medium was aspirated and 50.0 µL of DMSO was added to each well, and color intensity was measured using SpectraMax i3 (Molecular Devices) micro plate reader at 540 nm.

## Fluorescence microscopy analysis

Cancer cells were plated in 96-well plate (1×10<sup>3</sup> cells/well) and incubated overnight at 37 °C. Cells were then treated with anti-sLe<sup>a</sup> mAb-AF555 **16** for 1 h at 4 °C or at 37 °C. Cells were washed with PBS (3 × 100 µL) followed by PBS + DAPI (1 µg/mL) and imaged using a fluorescence microscopy EVOS AMF-4302.

## References

- 1) H. Y. Kim, S. Rhim, J. J. Park and J. Kang, *J. Incl. Phenom. Macrocycl. Chem.*, 2012, **74**, 317.
- 2) Y. Imai, S. Hirono, H. Matsuba, T. Suzuki, Y. Kobayashi, H. Kawagishi, D. Takahashi and

K. Toshima, *Chem. Asian J.*, 2012, **7**, 97.

**$^1\text{H}$  and  $^{13}\text{C}$  NMR**

**spectrum charts**

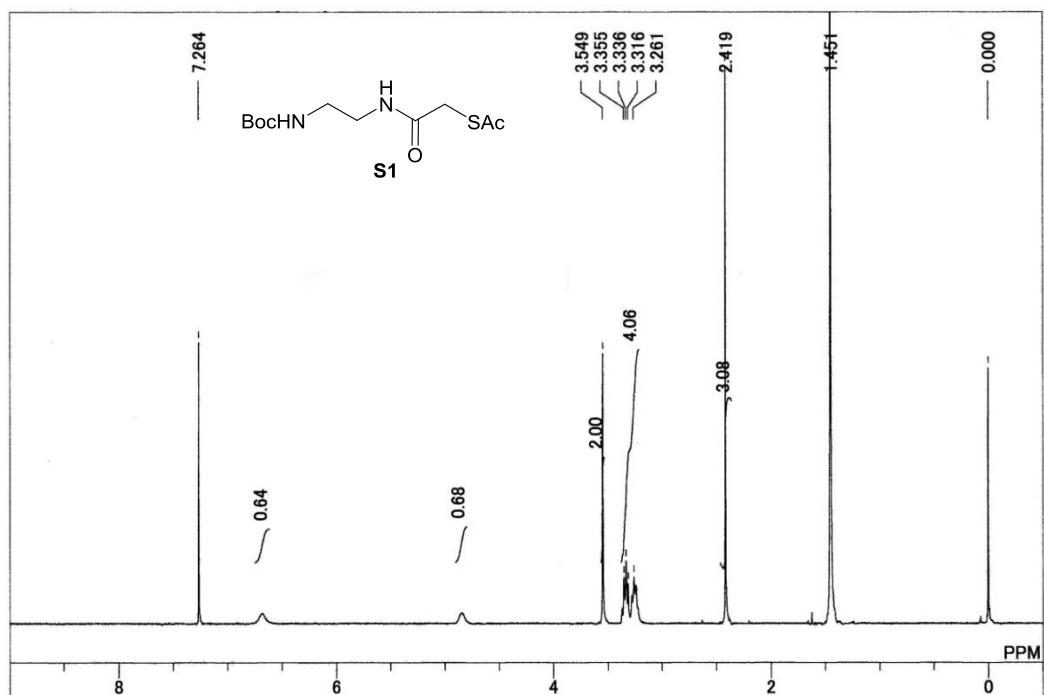


Figure S5. <sup>1</sup>H-NMR spectrum of S1

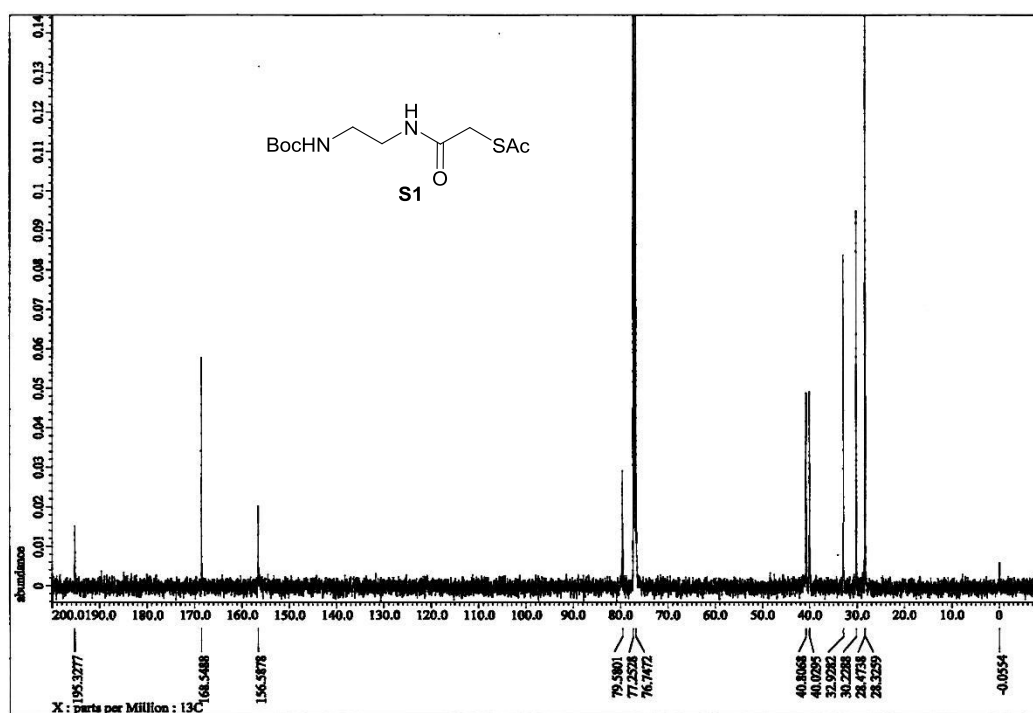
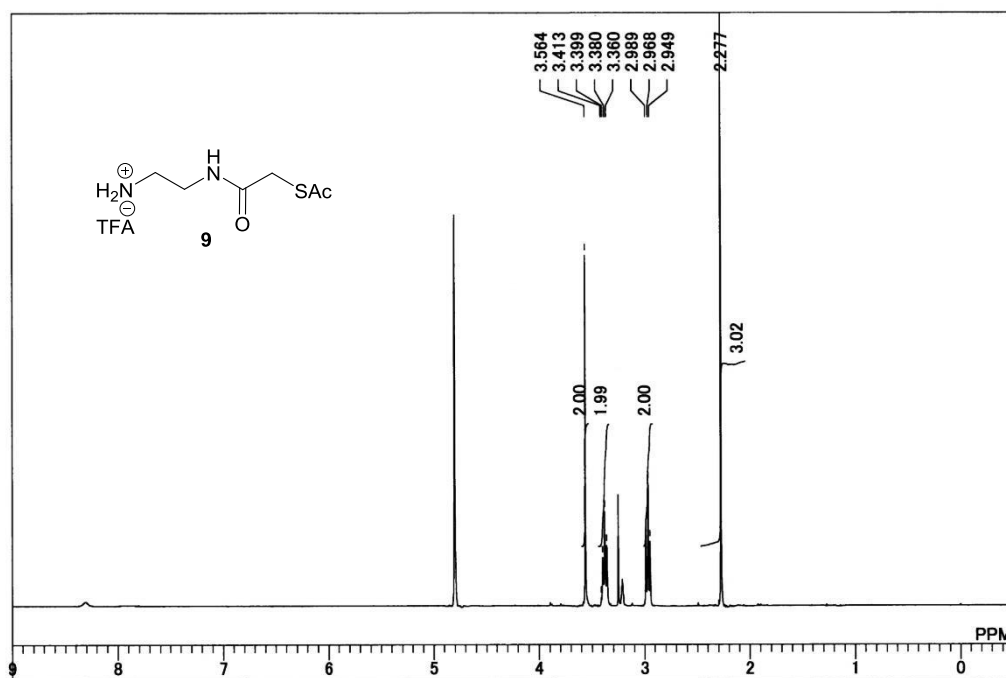
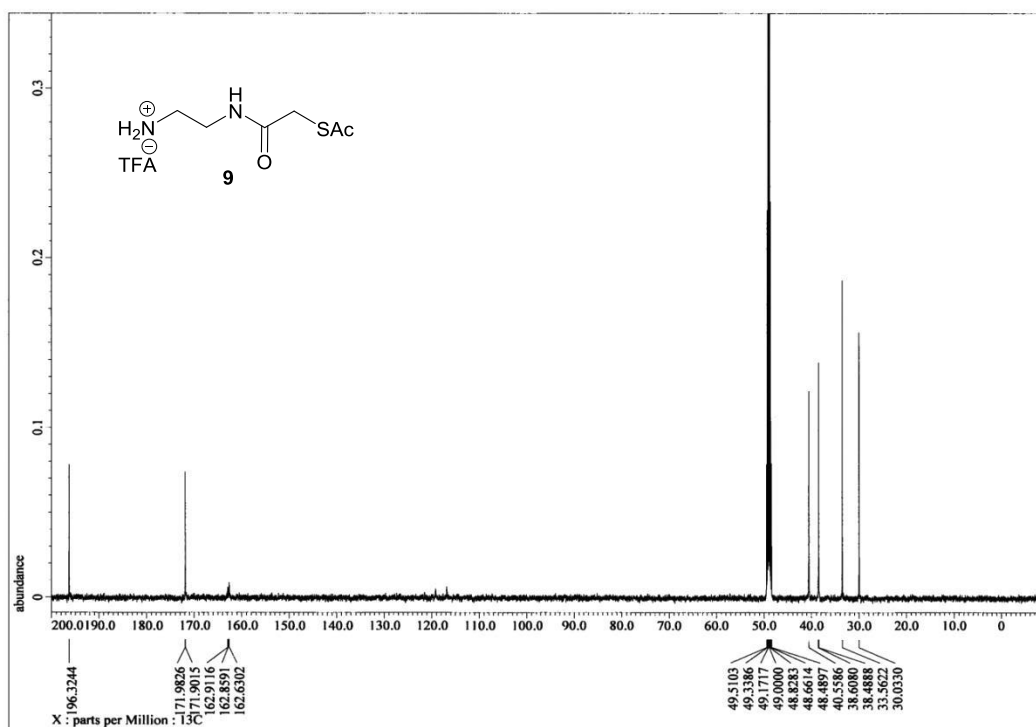


Figure S6. <sup>13</sup>C-NMR spectrum of S1



**Figure S7.** <sup>1</sup>H-NMR spectrum of **9**



**Figure S8.** <sup>13</sup>C-NMR spectrum of **9**

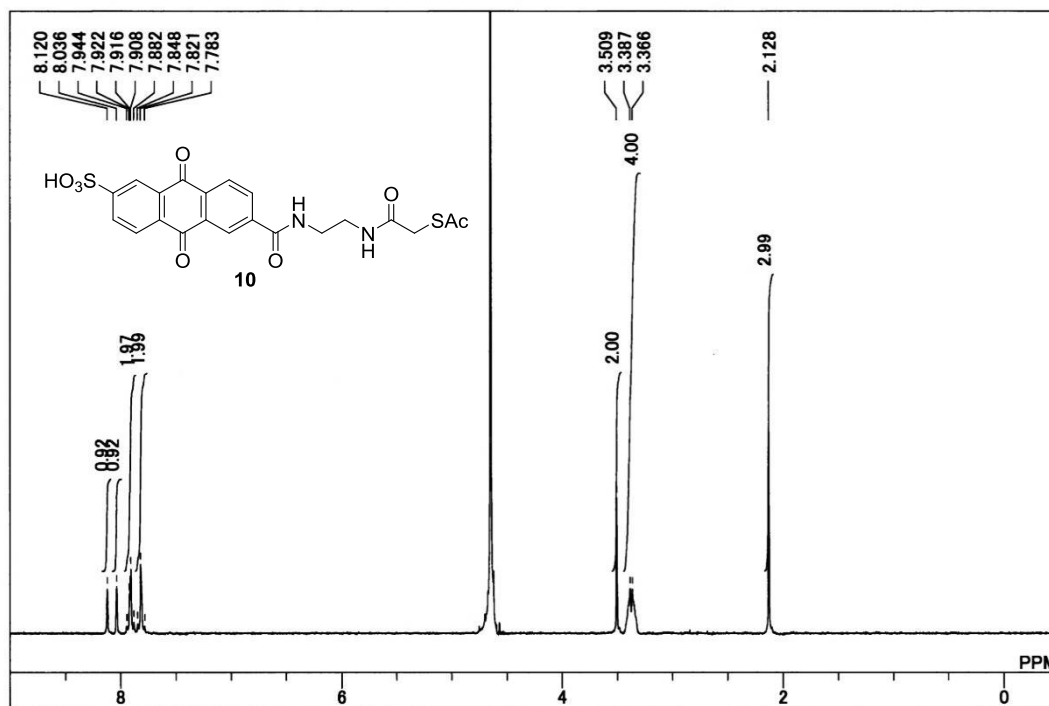


Figure S9.  $^1\text{H}$ -NMR spectrum of **10**

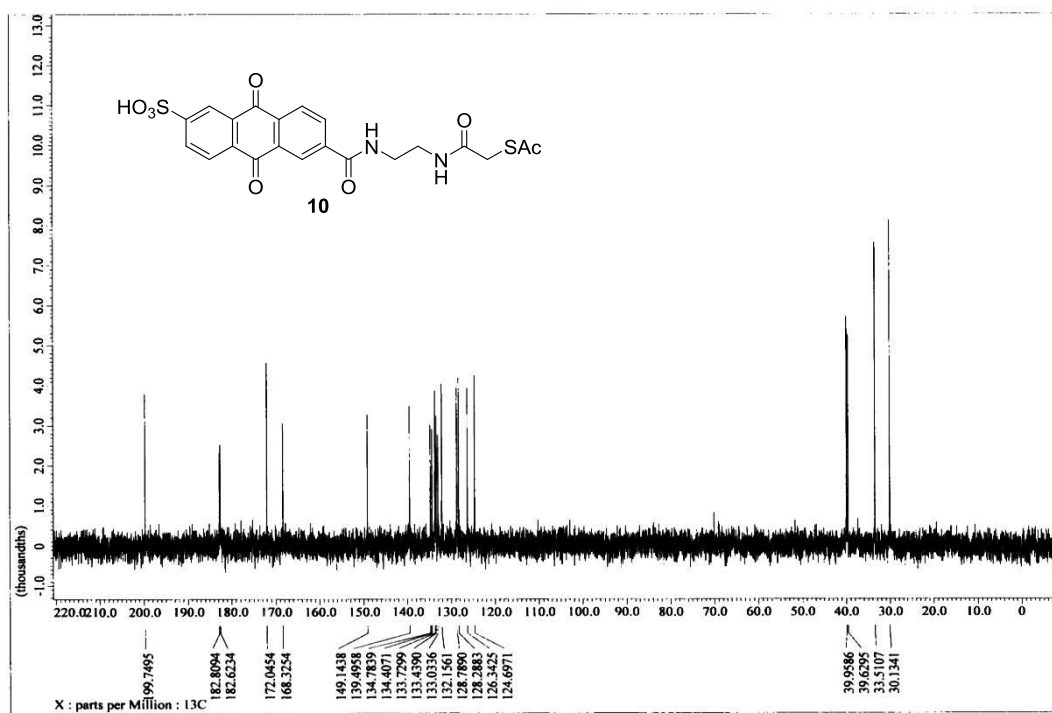


Figure S10.  $^{13}\text{C}$ -NMR spectrum of **10**

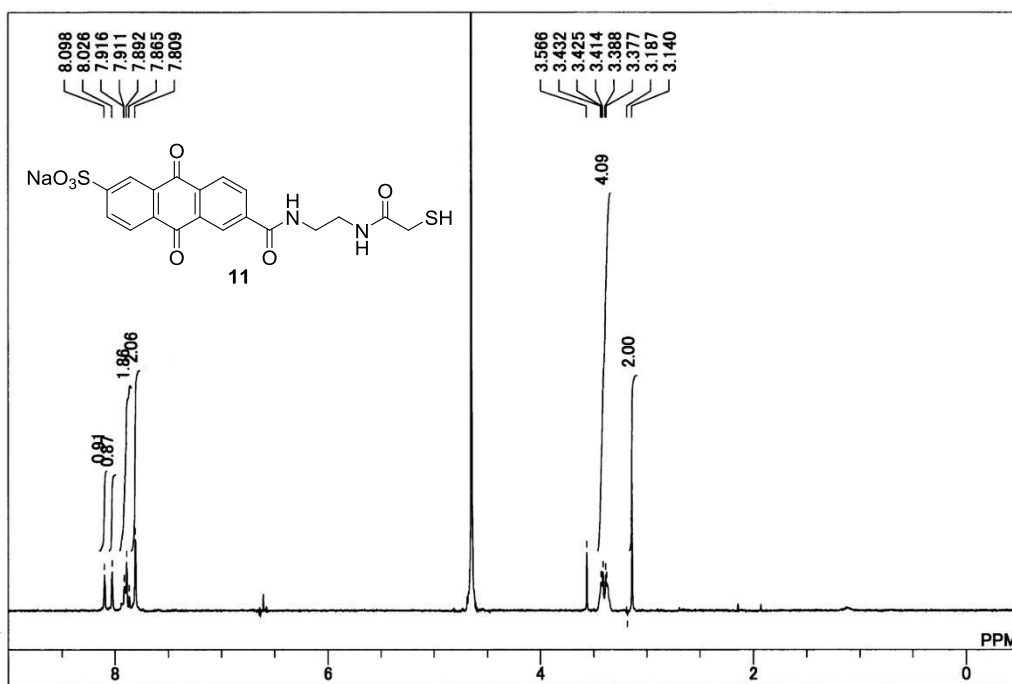


Figure S11. <sup>1</sup>H-NMR spectrum of **11**

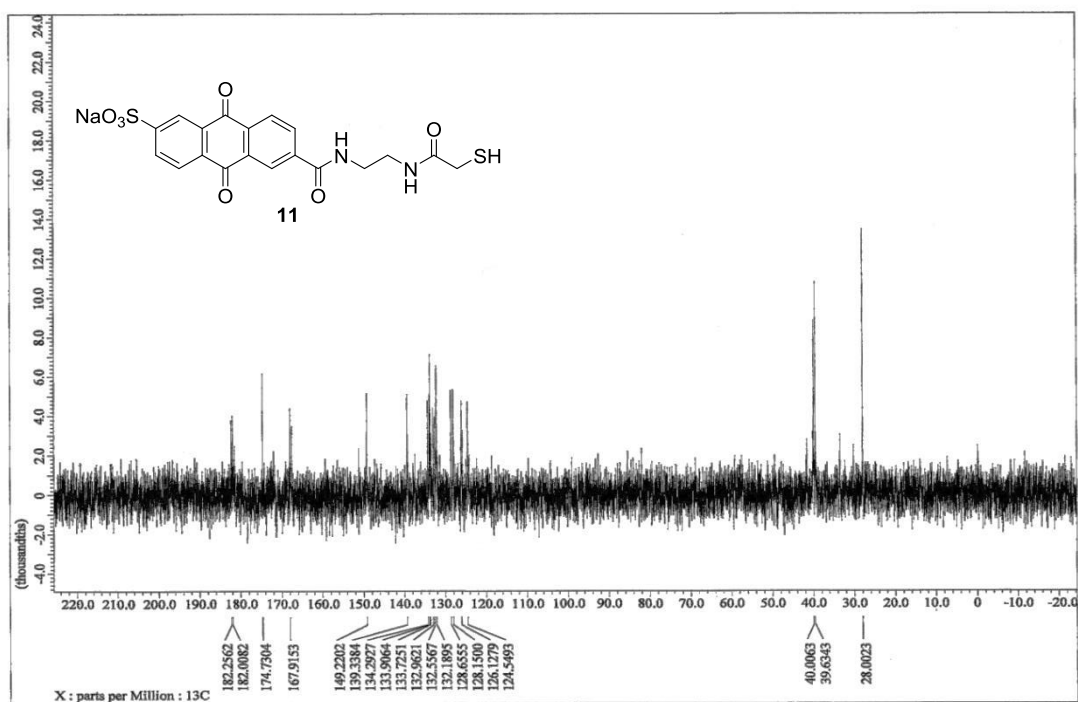


Figure S12. <sup>13</sup>C-NMR spectrum of **11**