

## < Supporting Information >

### Seoul-Fluor-based Bioprobe for Lipid Droplet and its Application in Image-based High Throughput Screening

Eunha Kim,<sup>a,‡</sup> Sanghee Lee,<sup>a,‡</sup> and Seung Bum Park<sup>\*,a,b</sup>

<sup>a</sup>Department of Chemistry and <sup>b</sup>Department of Biophysics and Chemical Biology,

Seoul National University, Seoul 151-747, Korea

Fax: (+82)2-884-4025; E-mail: [sbpark@snu.ac.kr](mailto:sbpark@snu.ac.kr)

[‡] These authors have contributed equally to this work.

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## 2 | SUPPORTING INFORMATION

### I. General information

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker DRX-300 (Bruker Biospin, Germany) and Varian Inova-500 (Varian Assoc., Palo Alto, USA), and chemical shifts were measured in ppm downfield from internal tetramethylsilane (TMS) standard. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); br s (broad singlet), etc. Coupling constants were reported in Hz. Routine mass analyses were performed on LC/MS system equipped with a reverse phase column (C-18, 50 x 2.1 mm, 5  $\mu\text{m}$ ) and photodiode array detector using electron spray ionization (ESI) or atmospheric pressure chemical ionization (APCI). Triethylamine, 1-naphthaldehyde, 9-anthraldehyde, diisobutylaluminium hydride, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), sodium borohydride, bromoacetyl bromide, sodium hydride, pyridine analogs, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), acetic acid, anhydrous dimethyl formamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60 F<sub>254</sub> 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with anisaldehyde,  $\text{KMnO}_4$ , and phosphomolybdic acid followed by heating. All reactions were conducted in oven-dried glassware under dry argon atmosphere, unless otherwise specified. Toluene and THF were dried by distillation from sodium-benzophenone immediately prior to use.  $\text{CH}_2\text{Cl}_2$  was distilled from  $\text{CaH}_2$  and TEA was distilled over KOH. Other solvents and organic reagents were purchased from commercial vendors and used without further purification unless otherwise mentioned. Distilled water was polished by ion exchange and filtration. Biochemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ez-cytox kit was purchased from Daeil Co. (Korea) and was used for the cell viability test. Commercial dyes such as Lysotracker, Nile Red and BODIPY-fatty acids were purchased from Invitrogen. All antibodies for immunofluorescence were purchased from Abcam and Cell Signalling.

#### **Fluorescence microscope, HCS equipment, and analysis program for Bio-Imaging experiment.**

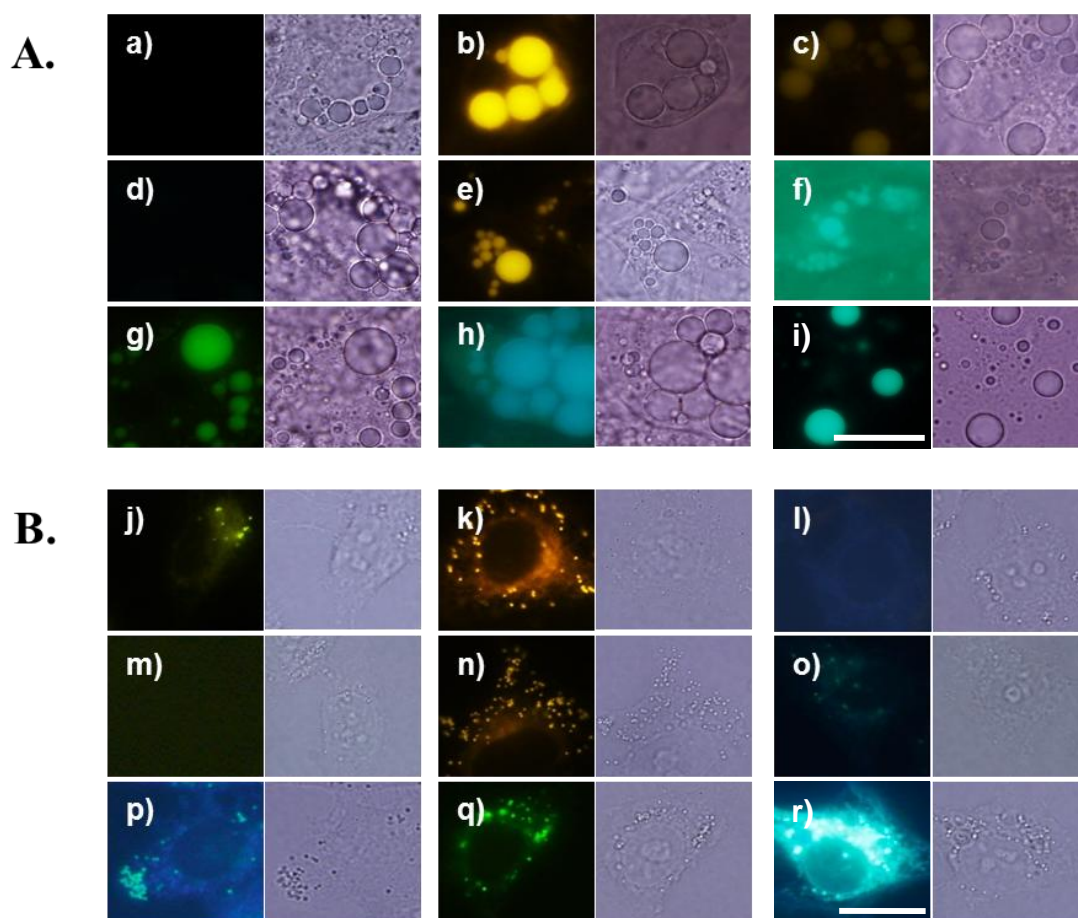
We carried out fluorescence microscopy studies with Olympus Inverted Microscope Model IX71, equipped for epi-illumination using a halogen bulb (Philips No. 7724). Emission signal of each experiments were observed at two spectral setting: green channel, using a 450–480 band pass exciter filter, a 500 nm center wavelength chromatic beam splitter, a 515 nm-long pass barrier filter (Olympus filter set U-MWB2); and red channel using a 510–550 band pass exciter filter, a 570 nm center wavelength chromatic beam splitter, a 590 nm-long pass barrier filter (Olympus filter set U-MWG2). Emission signal of each experiments were detected with 12.5M pixel recording digital color camera (Olympus, DP71) Quantification of fluorescence images was analyzed by Image-Pro Plus® 6.2 program and all graphs were figured by GraphPad Prism 5. The quantified data are the mean measurements of 40–50 cells from at least three different independent experiments and SEM. High contents screening was performed by InCell analyzer 2000 [GE Healthcare] and fluorescence images were analyzed by InCell analyzer 1000 workstation 3.6 program according to manufacturer's protocol

using granularity module. For the Ez-cytox-based cell cytotoxicity test, the absorbance of 96-well plate was measured by BioTek Synergy HT Microplate reader.

#### **Cell culture**

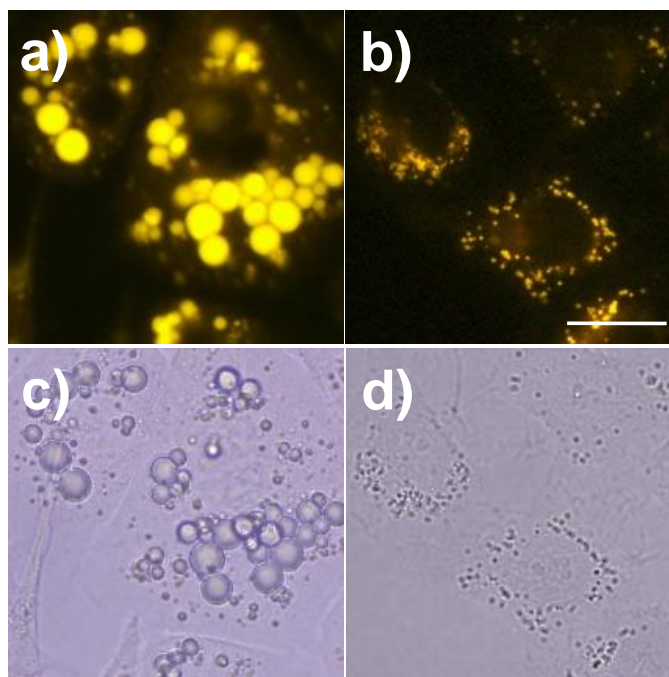
HeLa and 3T3-L1 cells were obtained from American Type Culture Collection [ATCC, Manassas, VA, USA]. HeLa cell lines were cultured in RPMI 1640 [GIBCO, Invitrogen] supplemented with heat-inactivated 10 % (v/v) fetal bovine serum [GIBCO, Invitrogen] and 1 % (v/v) antibiotic-antimycotic solution [GIBCO, Invitrogen]. 3T3-L1 cells were maintained in DMEM [GIBCO, Invitrogen] supplemented with heat-inactivated 10 % (v/v) calf serum [GIBCO, Invitrogen] and 1 % (v/v) antibiotic-antimycotic solution [GIBCO, Invitrogen]. Cells were maintained in a humidified atmosphere of 5 % CO<sub>2</sub> incubator at 37 °C, and cultured in 100 mm cell culture dish [CORNING].

## II. Screening results

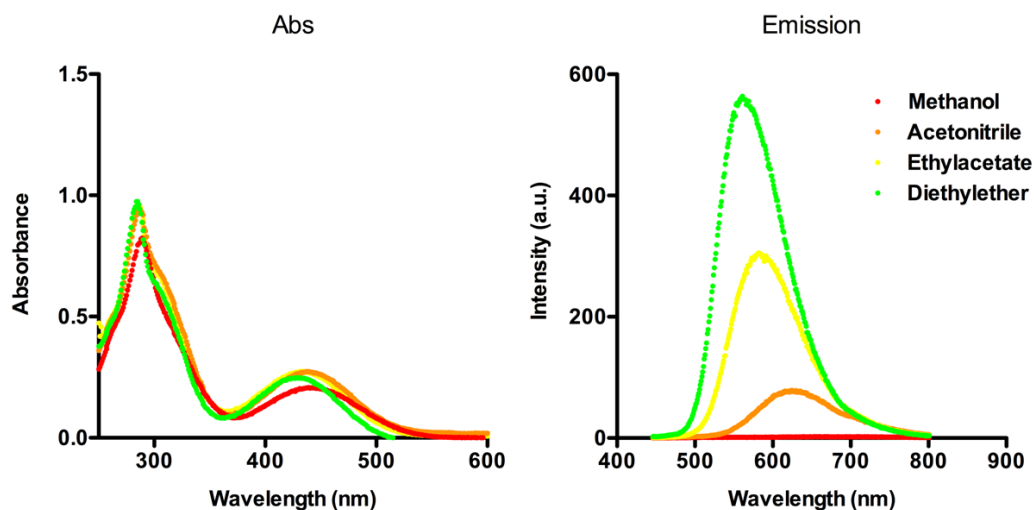


**Figure S1.** Image-based screening results of nine Seoul-Fluor (SF) compounds, against two different cell lines, differentiated 3T3-L1 adipocyte (A) and HeLa cervical cancer cells (B). (a, j: SF20; b, k: SF24; c, l: SF31; d, m: SF32; e, n: SF44; f, o: SF46; g, p: SF47; h, q: SF53; i, r: SF54). Individual pictures are composed of fluorescence microscopy image (left) and phase contrast images (right) of the given fluorescent compound, respectively. The scale bar represents 20 μm.

### III. Novel LD-specific fluorescent bioprobe, SF44, and solvatochromism study

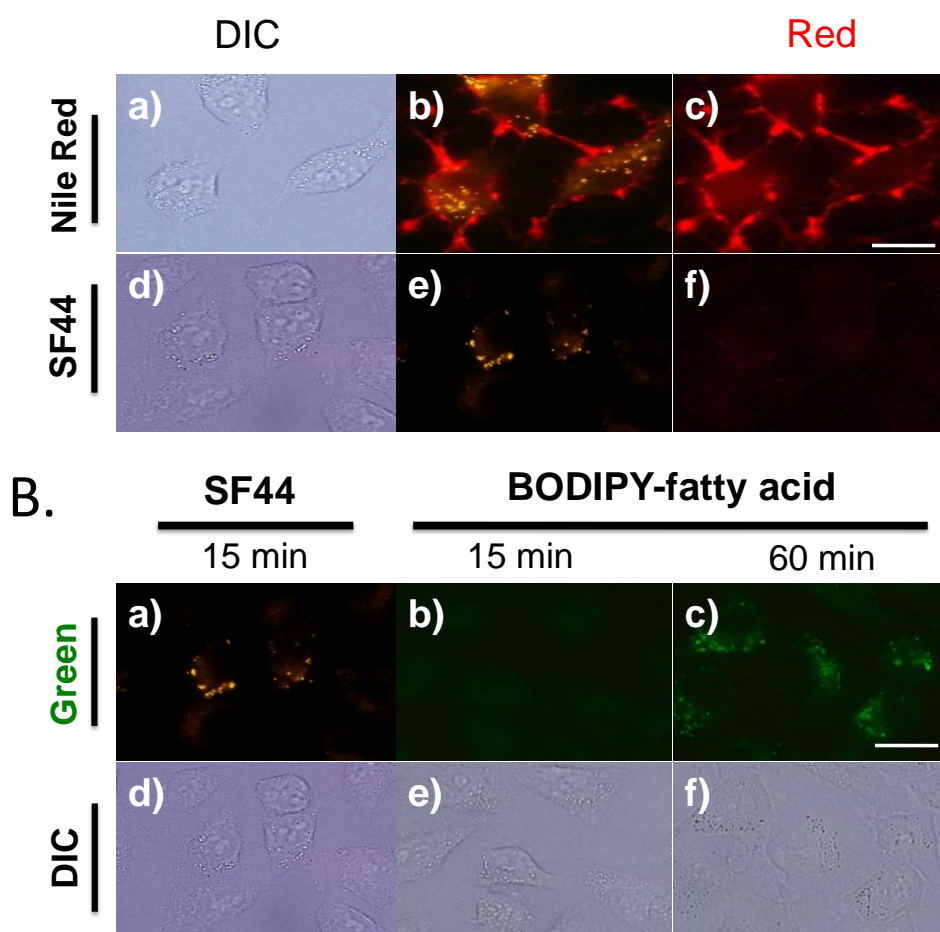


**Figure S2.** Novel LD-specific fluorescent bioprobe, SF44. Lipid droplets in differentiated 3T3-L1 adipocytes (a, c) and HeLa cells (b, d) were successfully stained with SF44. a, b) fluorescence microscopy images. c, d) phase contrast images. The scale bar represents 20  $\mu\text{m}$ .



**Figure S3.** Solvatochromism study: Absorbance and emission spectra of SF44 in various solvents with different polarities.

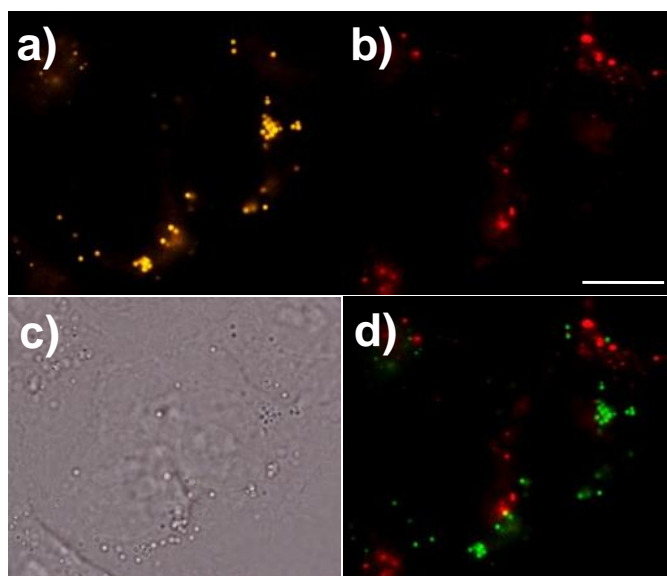
#### IV. Comparison of SF44 with Nile red and BODIPY-fatty acid



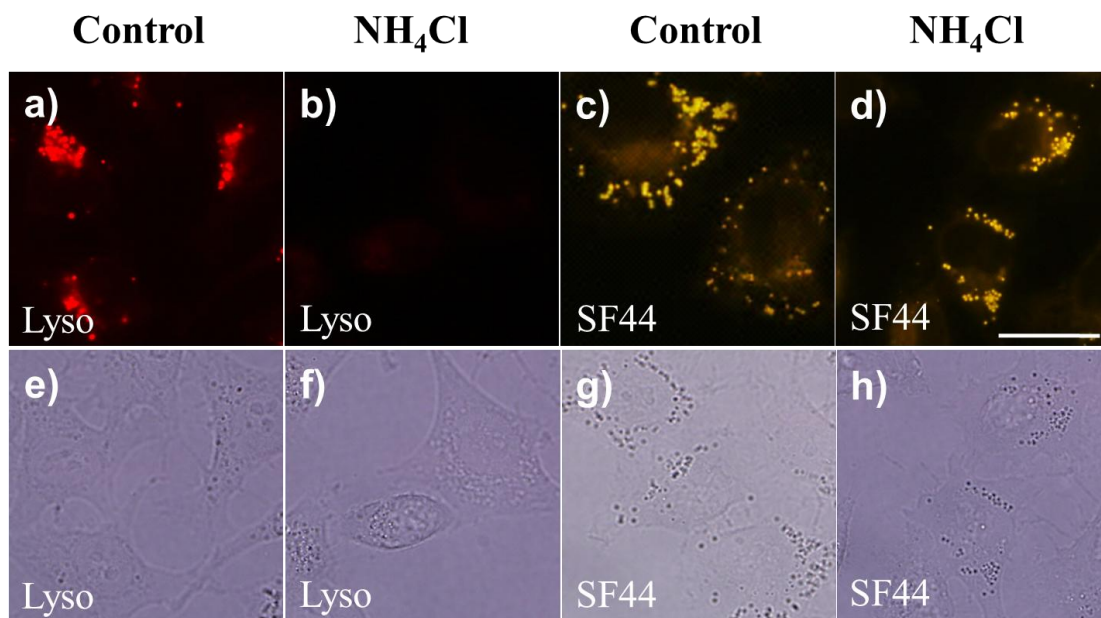
**Figure S4.** Comparison of staining patterns of Nile Red and BODIPY-fatty acids with SF44 in HeLa cells. A) Nile Red stains LDs in HeLa cells with golden yellow color under a green channel of our fluorescence microscopy system, but certain portions of the membrane are stained in red under both green and red channels (b and c); these images were captured using the fluorescence microscope equipped with long-pass color filters and a color charge-coupled device (CCD) camera setting (see the general information). On the other hand, SF44 selectively stains the neutral LDs in golden yellow under the green channel without any signals under the red channel (e and f). B) SF44 stains LDs more rapidly than the BODIPY-fatty acids do; SF44 stained LDs within 15 min (a and d), whereas the BODIPY-fatty acids required a longer incubation time and additional washing step, more than 1 h (b, c, e and f). The scale bar represents 20  $\mu\text{m}$ .



### V. pH-independent LD-specific staining pattern by SF44

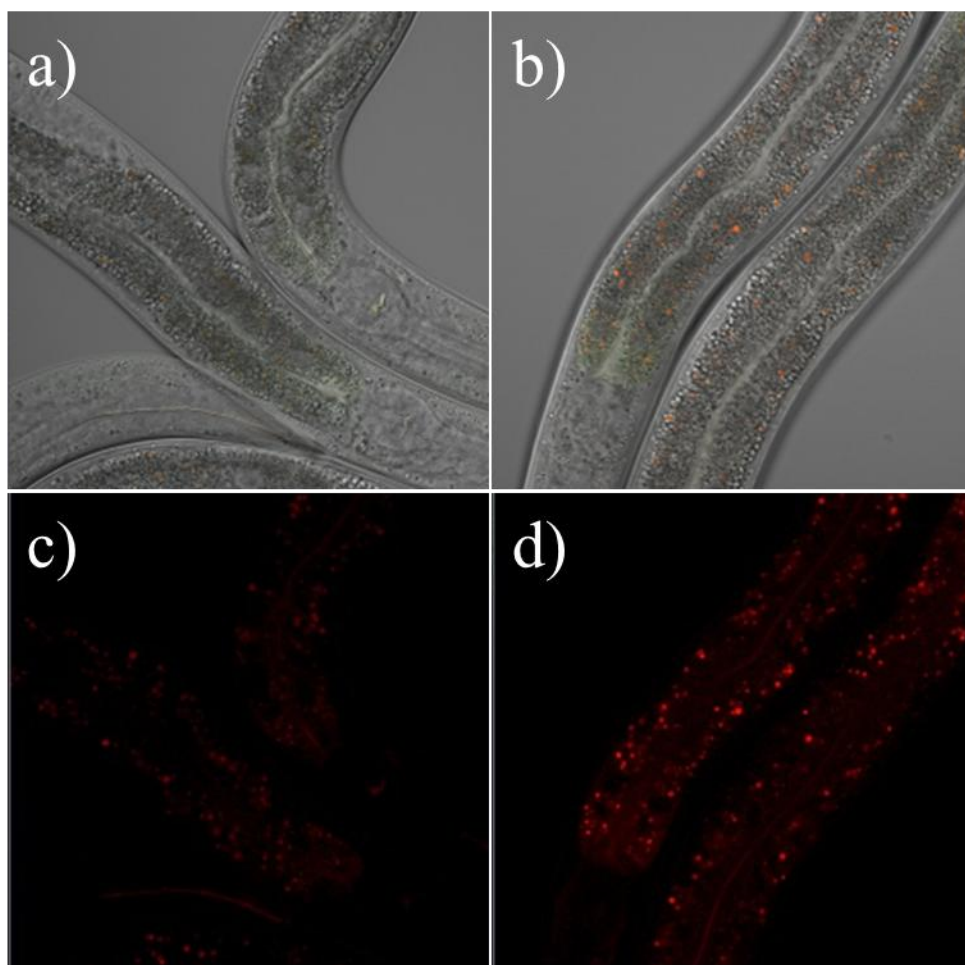


**Figure S5.** Staining pattern comparison of SF44 and LysoTracker™ Red in HeLa cells; a) fluorescence microscopy image of SF44 detected by green channel; b) fluorescence microscopy image of LysoTracker™ Red detected by red channel; c) phase contrast image; d) merged image between pseudo colored images of SF44 (green) and LysoTracker (red). The scale bar represents 20  $\mu\text{m}$ .



**Figure S6.** Comparison of staining patterns by LysoTracker™ Red (a,b) and SF44 (c,d) with the neutralization of lysosome using ammonium chloride (NH<sub>4</sub>Cl) in HeLa cells. a–d) fluorescence microscopy images; e–h) phase-contrast images. Each image was captured before (a, c, e, and g) and after (b, d, f, and h) the treatment with NH<sub>4</sub>Cl (20 mM) for 20 min. The scale bar represents 20  $\mu\text{m}$ .

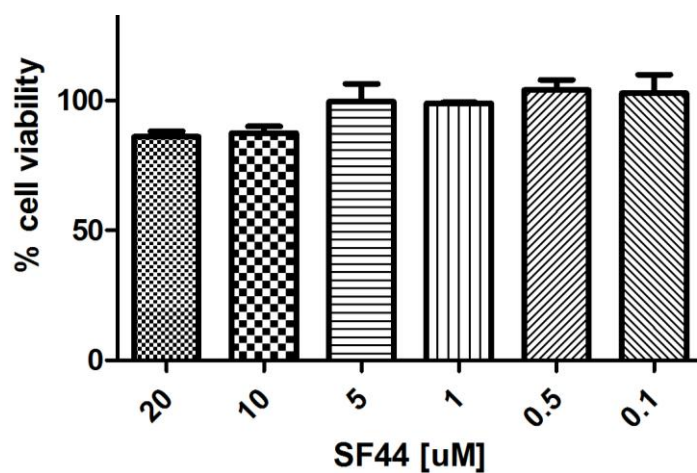
## VI. *In vivo* application of SF44



**Figure S7.** Fluorescent staining of LD in *C. elegans* with SF44; a, b) phase contrast images; c, d) confocal laser scanning microscopy images. 100  $\mu$ M of SF44 (b, d) or none (a, c) in media were treated to the embryo state of *C. elegans*. After the treatment of SF44 for 48 h, L4 state of *C. elegans* were fixed with levamisole (2.5 mM) on agar pad and imaged with confocal laser scanning microscopy (CLSM) instrument.

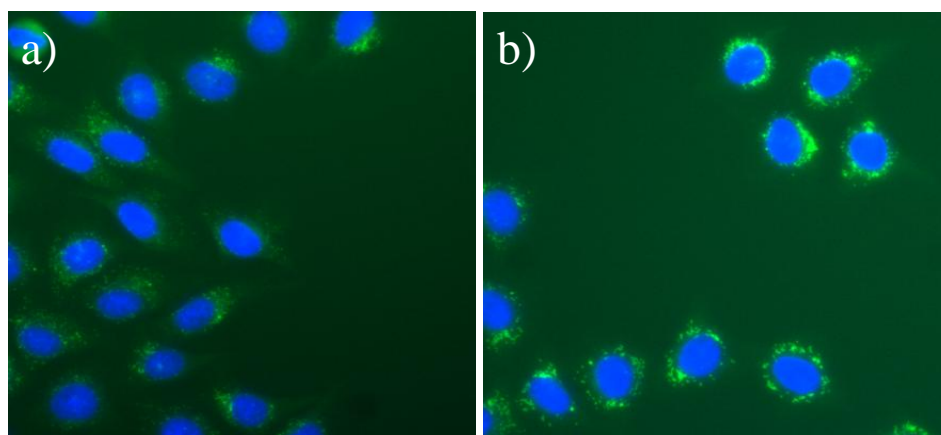


### VII. *In vitro* cytotoxicity test of SF44

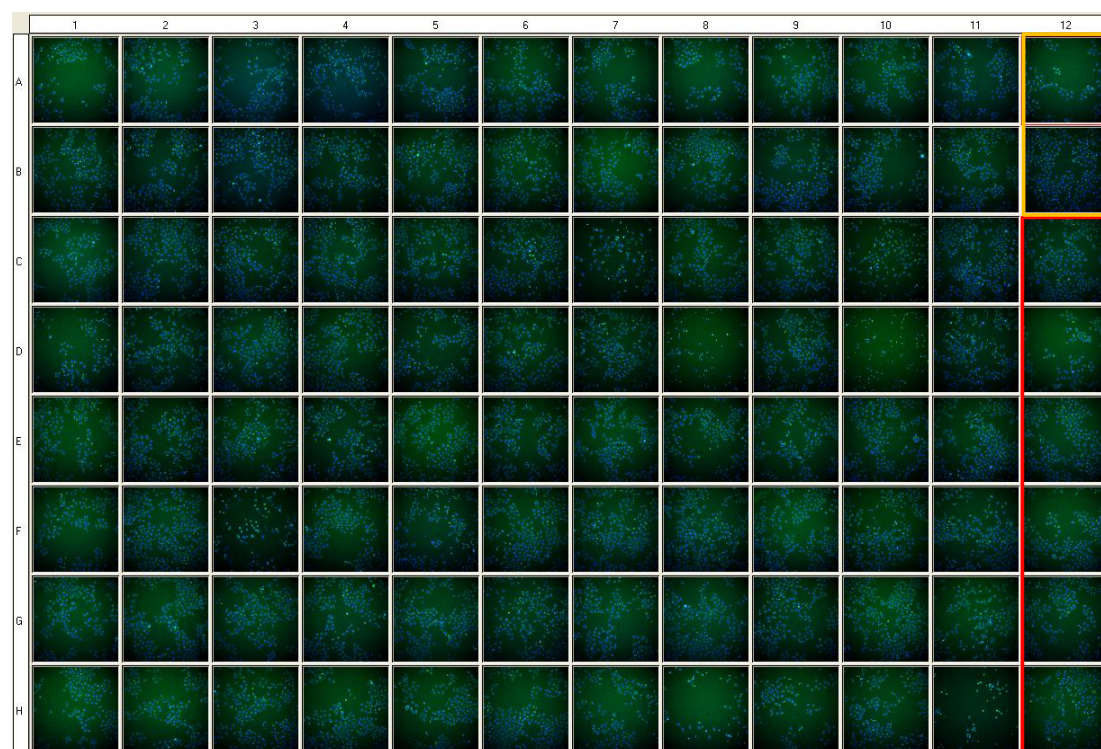


**Figure S8.** Cell viability test of SF44 against HeLa cells. Various concentration of SF44 was treated to HeLa cells for 12 h. Cell viability was normalized for DMSO control as 100 %. The graph shows average of triplicate experiment and SEM.

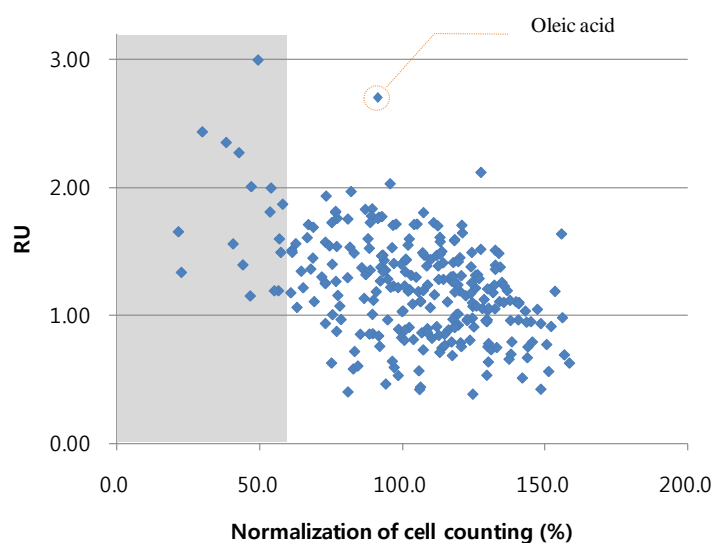
### VIII. Image based high throughput screening (HTS) application



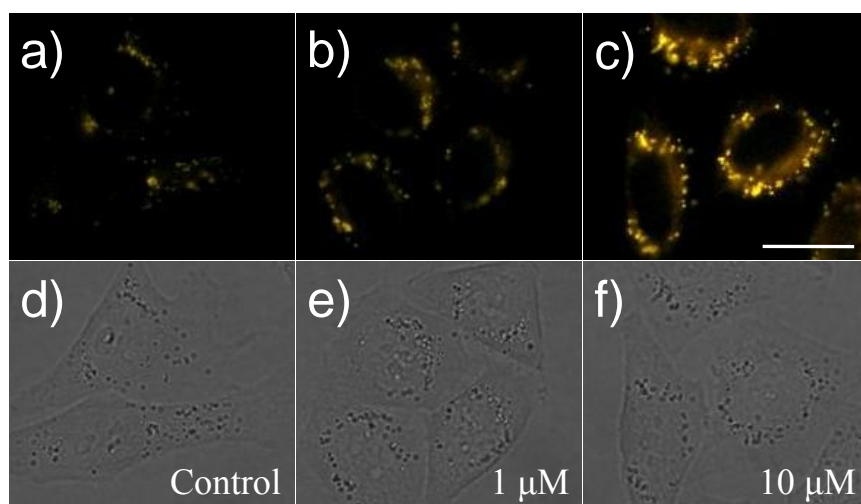
**Figure S9.** Application of SF44 to the image based high throughput screening system in live cells. a) DMSO as control, b) oleic acid 10  $\mu$ M. Each image is the merged images of lipid droplet staining (by SF44, pseudo color as green) and nucleus staining (by Hoechst 33342, pseudo color as blue). Fluorescence images were taken without any washing step and 20x magnification.



**Figure S10.** Representative images from the high throughput screening result of SF44 with pilot compound library in the HeLa cells. Each image is the merged images of lipid droplet staining (by SF44, pseudo color as green) and nucleus staining (by Hoechst 33342, pseudo color as blue). Each well was treated with the 10  $\mu$ M of each compound from pilot library. Yellow box represents the fluorescent images of 10  $\mu$ M of oleic acid treated wells and red box represents the fluorescent images of control wells, treated with DMSO.



**Figure S11.** Scatter plot of HTS result of pilot library. Average data of independent duplicate experiment was plotted. X-axis is normalized number of cells and y-axis represents relative unit (RU) for quantification of lipid droplet, corresponding to the organelles count  $\times$  mean area of organelle  $\times$  intensity of organelle value. The compounds, having cell counting value under 60 % (gray area on graph), were excluded from analysis because of their cytotoxic effect.



**Figure S12.** Monitoring of lipid droplet formation upon treatment of DMSO as control (a, d) and P8B05 at 1  $\mu$ M (b, e) and 10  $\mu$ M (c, f) in HeLa cells by staining of LD with SF44. a-c) fluorescence microscopy images in green channel; d-f) phase contrast images; The scale bar represents 20  $\mu$ m.

## IX. Experimental procedure for biological assay

### Differentiation of 3T3-L1 cell lines.

3T3-L1 cells were cultured to seeded on cover glass bottom dish and incubated with DMEM, (Dulbecco's modified Eagle's medium) supplemented with heat inactivated 10% (v/v) calf serum and 1% (v/v) antibiotic-antimycotic solution, for 2 days. At 2 days post-confluence (designated day 0), cells were induced to differentiate with DMEM supplemented with 10% (v/v) FBS (fetal bovine serum), 1  $\mu$ M dexamethasone, 10  $\mu$ M rosiglitazone, 5  $\mu$ g/ml insulin. 2 days later, replaced the media to the DMEM supplemented with 10% (v/v) FBS, and 5  $\mu$ g/ml insulin and refreshed the media with same condition every 2 day until the Seoul-Fluor compound treatment.

### Image-based screening of nine different Seoul-Fluor derivatives with differentiated 3T3-L1 cells and HeLa cells.

20  $\mu$ M solutions of series of Seoul-Fluor compounds in DMEM, or RPMI media, supplemented with 10% (v/v) FBS and 1% (v/v) antibiotic-antimycotic solution, were treated to the differentiated 3T3-L1 cells on day 7~8, or to the HeLa cells for 15 min. After washing with PBS for 5 min twice, fluorescent signals of the stained cells in normal culture media were measured with fluorescence microscopy.

### Immunohistochemistry with anti-ADRP and anti-perilipin antibodies.

3T3-L1 cells were seeded on cover glass cottom dish and differentiated by protocol as stated above. When the differentiation was completed, cells were fixed with 3.7 % paraformaldehyde in PBS for 15 min at r.t., and washed with icecold PBS for twice, followed by the incubation with 4% BSA in PBST for 4 h at r.t. BSA solution was decanted from glass bottom dish. Fixed cells on dish were introduced with diluted primary antibody solution (1:200) in PBST with 1% BSA, and incubated at 4 °C for overnight. Primary antibody was decanted and washed with PBS for 3 times. Diluted secondary antibody, conjugated Texas Red fluorescent dye, solution (1:100) was added, followed by the incubation at ambient temperature in dark for 1 h. After washing by PBS 3 times, SF44 (5  $\mu$ M) was treated to the cell in PBS for 15 min at r.t., followed by PBS washing for 3 min twice. Fluorescence images were taken by fluorescence microscopy under PBS condition.

### Lysosome neutralization with aqueous ammonium chloride.

HeLa cells were seeded on cover glass bottom petri dish and incubated at 5 % CO<sub>2</sub>, 37 °C incubator for overnight. LysoTracker<sup>TM</sup> Red (1  $\mu$ M) and SF44 (5  $\mu$ M) were treated for 1 hr and 15 min, respectively. After the treatment, dyes were washed with PBS for 5 min twice, and then NH<sub>4</sub>Cl (20 mM) solution was treated for 20 min. After PBS washing, fluorescent signals of the stained cells in normal culture media were measured with fluorescence microscopy.

### Co-staining experiment with SF44 and LysoTracker™ Red

HeLa cells were seeded on cover glass bottom dish and incubated at 5 % CO<sub>2</sub>, 37 °C for overnight. LysoTracker™ Red (2 μM) treated in media for 1 h. After PBS washing, cells were treated by SF44 (5 μM) in media for 15 min. After the treatment, dyes were washed with PBS for 3 min twice and then fluorescence images were taken by fluorescence microscopy under media.

### LD staining procedure with Nile Red

HeLa cells were seeded on cover glass bottom petri dish and incubated at 5 % CO<sub>2</sub>, 37 °C incubator for overnight. Nile Red solution (250 μg/mL of stock solution in acetone was diluted with PBS and final concentration was 0.1 μg/mL according to recommended protocol) and SF44 (5 μM) were treated for 30 and 15 min, respectively. After PBS washing, fluorescent signals of the stained cells in normal culture media were measured with fluorescence microscopy.

### LD staining procedure with BODIPY-fatty acids

HeLa cells were seeded on cover glass bottom petri dish and incubated at 5 % CO<sub>2</sub>, 37 °C incubator for overnight. BODIPY-Fatty acid (1 mg/mL stock solution in DMSO was diluted with PBS and final concentration was 0.2 μg/mL according to recommended protocol) and SF44 (5 μM) were treated for 15 min and 1 h. After PBS washing, fluorescent signals of the stained cells in normal culture media were measured with fluorescence microscopy.

### Culture condition for *C. Elegans* and *in vivo* treatment condition of SF44

Wild type *C. elegans* (Bristol strain, N2) was provided by the Caenorhabditis Genetics Center. Worms were routinely grown at 20 or 25 °C on 50 mm diameter nematode growth medium (NGM) agar plates containing *Escherichia coli* (OP50) as a food source. SF44 compounds in media (20 mM DMSO stock solution was diluted with media and final concentration was 200 μM), and synchronized embryos, bleaching the wild strain (N2) with sodium hypochlorite, were seeded to the medium. After 48 hr incubation at 20 °C worms were observed with fixation using levamisole (2.5mM, diluted with M9).

### *In Vitro* Cytotoxicity Test

Cell viability was measured by the EZ-cytox assay kit, and the experimental procedure was based on the manufacturer's manual. Cells were cultured into 96-well plates at a density of  $3 \times 10^3$  cells/well for 24 h, followed by the treatment of compounds in various concentrations. After 12 h of incubation with increasing concentration, 10 μL of WST-1 solution (2-(4-nitrophenyl)-5-(2-sulfophenyl)-3-[4-(4-sulfophenylazo)-2-sulfophenyl]-2H-tetrazolium disodium salt, was added to each well, and plates were incubated for an additional 1 hr at 37 °C. Absorbance in 455 nm was measured by microplate reader. The percentage of cell viability was calculated by following formula: % cell viability = (mean absorbance in test wells)/(mean absorbance in control well) × 100. Each experiment was performed in triplicate experiments.

### **Oleic acid treatment**

HeLa cells were seeded on cover glass bottom petri dish and incubated at 5 % CO<sub>2</sub>, 37 °C incubator for overnight. Oleic acid (200 mM stock solution in isopropanol was diluted with normal growth media and final concentration was 200 μM) were treated for 6 h. After washing with PBS for 5 min twice, cells were treated with SF44 (5 μM) for 15 min in normal growth media. After PBS washing, fluorescent signals of the stained cells in normal culture media were measured with fluorescence microscopy.

### **High contents screening (HCS) using In Cell Analyzer 2000**

HeLa cells were seeded on black well and clear bottom 96 plate (2×10<sup>3</sup> cells / well) and incubated at 5 % CO<sub>2</sub>, 37 °C for overnight. Various kinds of chemicals from our in house chemical library, oleic acid and DMSO as vehicle were treated to the cell with pin tool as 10 μM of final concentration for 24 h. SF44 (5 μM) and Hoechst 33342 (2 μg/ml) was added to the cell. After 30 min incubation, fluorescence images of the plate were taken automatically by In cell analyzer 2000 without any washing step. (SF44: Excitation filter: 430/24x, Emission filter: 605/64m, Hoechst 33342: Excitation filter: 355/50x, Emission filter: 450/50m)

### **Fluorescence image of P8B05 treatment in HeLa cell**

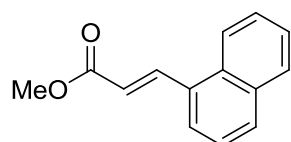
HeLa cells were seeded on cover bottom dish and incubated at 5 % CO<sub>2</sub>, 37 °C incubator for overnight. Various dose of P8B05 treated to the cells in media for 12 h and then followed washing by PBS twice. Cells were treated with SF44 (5 μM) for 15 min in normal growth media. After PBS washing, Fluorescence signal were observed by fluorescence microscopy under regular media.



## X. General synthetic procedure and compound characterization

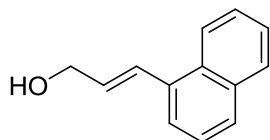
General procedure for preparing  $\alpha,\beta$ -unsaturated aldehydes and Seoul-Fluor (SF53, SF54) is same as the procedure described in the previous report.<sup>1</sup>

### (E)-Methyl 3-(naphthalen-1-yl)acrylate



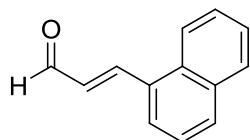
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.52 (d,  $J$  = 16.0 Hz, 1H), 8.19 (d,  $J$  = 8.5 Hz, 1H), 7.88 (t,  $J$  = 8.8 Hz, 2H), 7.75 (d,  $J$  = 7.0 Hz, 1H), 7.57 (t,  $J$  = 7.8 Hz, 1H), 7.53 (t,  $J$  = 7.5 Hz, 1H), 7.48 (t,  $J$  = 7.5 Hz, 1H), 6.53 (d,  $J$  = 15.5 Hz, 1H), 3.86 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  167.4, 142.0, 133.7, 131.8, 131.5, 130.6, 128.8, 128.8, 127.0, 126.3, 125.5, 125.1, 123.4, 120.5, 51.9; LRMS (EI):  $m/z$  calcd for C<sub>14</sub>H<sub>12</sub>O<sub>2</sub> [M] 212.08, found 212.10.

### (E)-3-(Naphthalen-1-yl)prop-2-en-1-ol



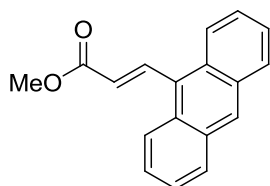
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.12 (d,  $J$  = 8.0 Hz, 1H), 7.85 (d,  $J$  = 8.5 Hz, 1H), 7.78 (d,  $J$  = 8.0 Hz, 1H), 7.60 (d,  $J$  = 7.0 Hz, 1H), 7.52–7.49 (m, 2H), 7.45 (t,  $J$  = 7.8 Hz, 1H), 7.38 (d,  $J$  = 15.5 Hz, 1H); <sup>13</sup>C (75 MHz, CDCl<sub>3</sub>)  $\delta$  134.5, 133.7, 131.9, 131.2, 128.6, 128.2, 128.1, 126.1, 125.9, 125.7, 124.0, 123.8, 63.9; LRMS (EI):  $m/z$  calcd for C<sub>13</sub>H<sub>12</sub>O [M] 184.09, found 184.10.

### (E)-3-(Naphthalen-1-yl)acrylaldehyde



<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  9.86 (d,  $J$  = 7.5 Hz, 1H), 8.34 (d,  $J$  = 15.5 Hz, 1H), 8.20 (d,  $J$  = 8.5 Hz, 1H), 7.96 (d,  $J$  = 8.5 Hz, 1H), 7.92 (d,  $J$  = 8.0 Hz, 1H), 7.83 (d,  $J$  = 7.5 Hz, 1H), 7.64–7.62 (m, 1H), 7.59–7.52 (m, 2H), 6.85 (q,  $J$  = 8.0 Hz, 1H); <sup>13</sup>C (75 MHz, CDCl<sub>3</sub>)  $\delta$  193.8, 149.4, 133.8, 131.7, 131.3, 131.0, 131.0, 129.1, 127.4, 126.5, 125.8, 125.6, 122.9; LRMS (EI):  $m/z$  calcd for C<sub>13</sub>H<sub>10</sub>O [M] 182.07, found 182.10.

### (E)-Methyl 3-(anthracen-9-yl)acrylate



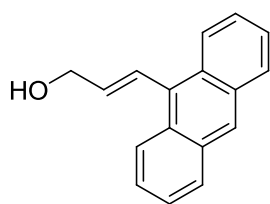
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.65 (d,  $J$  = 16.0 Hz, 1H), 8.45 (s, 1H), 8.23 (d,  $J$  = 8.0 Hz, 2H), 8.01 (d,  $J$  = 9.0 Hz, 2H), 7.52–7.47 (m, 2H), 6.44 (d,  $J$  = 16.5 Hz, 1H), 3.92 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  166.9, 142.3, 131.3, 129.4, 129.3, 128.9, 128.3, 126.8, 126.4, 125.4, 125.2, 52.0; LRMS

<sup>1</sup> a) E.Kim, M. Koh, R. J. Ryu, S. B. Park, *J. Am. Chem. Soc.* **2008**, *130*, 12206–12207; b) E. Kim, M. Koh, B. J. Lim, S. B. Park, *J. Am. Chem. Soc.* **2011**, *133*, 6642–6649.

## 16 | SUPPORTING INFORMATION

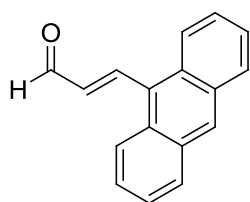
(EI):  $m/z$  calcd for  $C_{18}H_{14}O_2$  [M] 262.10, found 262.10.

### (E)-3-(Anthracen-9-yl)prop-2-en-1-ol



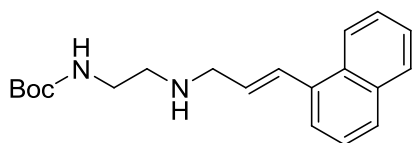
$^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.37 (s, 1H), 8.29–8.27 (m, 2H), 7.99–7.97 (m, 2H), 7.47–7.44 (m, 4H), 7.38 (d,  $J$  = 16.0 Hz, 1H), 6.22 (dt,  $J$  = 16.0, 5.5 Hz, 1H), 4.59 (d,  $J$  = 4.5 Hz, 2H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  137.3, 131.5, 129.6, 128.8, 126.8, 126.5, 126.0, 125.5, 125.2, 64.0; LRMS (EI):  $m/z$  calcd for  $C_{17}H_{14}O$  [M] $^+$  235.10, found 235.10.

### (E)-3-(Anthracen-9-yl)acrylaldehyde



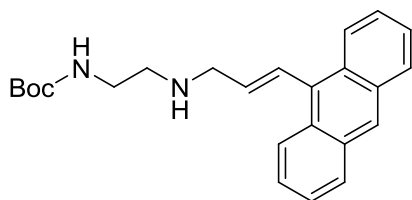
$^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  10.03 (d,  $J$  = 7.5 Hz, 1H), 8.52 (d,  $J$  = 6.5 Hz, 1H), 8.49 (s, 1H), 8.21 (d,  $J$  = 9.0 Hz, 1H), 8.05 (d,  $J$  = 7.5 Hz, 1H), 7.57–7.51 (m, 4H), 6.77 (q,  $J$  = 8.0 Hz, 1H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  193.6, 150.0, 137.6, 131.4, 129.6, 129.3, 129.2, 128.4, 127.0, 125.7, 129.8; LRMS (EI):  $m/z$  calcd for  $C_{17}H_{12}O$  [M] 232.09, found 232.10.

### (E)-tert-Butyl 2-(3-(naphthalen-1-yl)allylamino)ethylcarbamate



$^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$  8.05 (d,  $J$  = 7.7 Hz, 1H), 7.84–7.81 (m, 1H), 7.73 (d,  $J$  = 8.2 Hz, 1H), 7.53 (d,  $J$  = 7.1 Hz, 1H), 7.54–7.39 (m, 3H), 7.29 (d,  $J$  = 15.6 Hz, 1H), 6.23 (dt,  $J$  = 15.6, 6.5 Hz, 1H), 5.26 (br s, 1H), 3.58 (dd,  $J$  = 6.6, 1.3 Hz, 2H), 3.35–3.29 (m, 2H), 2.89 (t,  $J$  = 5.7 Hz, 2H), 1.43 (s, 9H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  156.4, 135.9, 132.4, 131.5, 129.6, 128.8, 128.1, 126.5, 126.0, 125.9, 125.8, 125.6, 125.2, 79.5, 51.8, 48.8, 40.2, 28.6; LRMS (EI):  $m/z$  calcd for  $C_{20}H_{26}N_2O_2$  [M+H] $^+$  327.20, found 327.30.

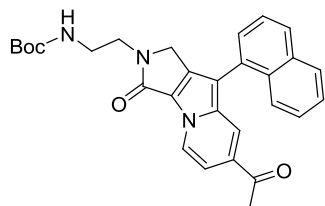
### (E)-tert-Butyl 2-(3-(anthracen-9-yl)allylamino)ethylcarbamate



$^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.34 (s, 1H), 8.28–8.25 (m, 2H), 7.98–7.95 (m, 2H), 7.46–7.43 (m, 4H), 7.25 (d,  $J$  = 16.0 Hz, 1H), 6.08 (dt,  $J$  = 16.0, 6.0 Hz, 1H), 3.66 (d,  $J$  = 6.0 Hz, 2H), 3.37–3.33 (m, 2H), 2.92 (t,  $J$  = 5.8 Hz, 2H), 1.45 (s, 9H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$  156.4, 135.9, 132.4, 131.5, 129.6, 128.8, 128.1, 126.5, 126.0, 125.9, 125.8, 125.6, 125.2, 79.5, 51.8, 48.8, 40.2, 28.6; LRMS (EI):  $m/z$  calcd for  $C_{24}H_{28}N_2O_2$  [M+H] $^+$  377.22, found 377.27.

***tert*-Butyl  
yl)ethylcarbamate**

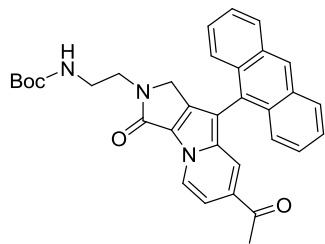
**2-(7-acetyl-9-(naphthalen-1-yl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-**



<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.62 (d, *J* = 7.0 Hz, 1H), 8.01 (s, 1H), 7.96 (dd, *J* = 8.0, 15.0 Hz, 2H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.61–7.54 (m, 3H), 7.52–7.49 (m, 1H), 7.33 (d, *J* = 7.5 Hz, 1H), 5.00 (br s, 1H), 4.39 (AB q, *J*<sub>AB</sub> = 16.8 Hz, 2H), 3.71 (m, 2H), 3.45–3.39 (m, 2H), 2.48 (s, 3H), 1.31 (s, 9H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 195.7, 162.3, 156.3, 137.1, 136.2, 134.2, 132.0, 130.5, 129.2, 128.9, 128.4, 128.1, 126.7, 126.4, 125.8, 125.6, 124.6, 122.6, 121.6, 112.6, 109.6, 79.5, 47.3, 43.1, 39.8, 28.4, 26.1; LRMS (EI): *m/z* calcd for C<sub>29</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub> [M+H]<sup>+</sup> 484.22, found 484.28.

***tert*-Butyl  
yl)ethylcarbamate**

**2-(7-acetyl-9-(anthracen-9-yl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-**

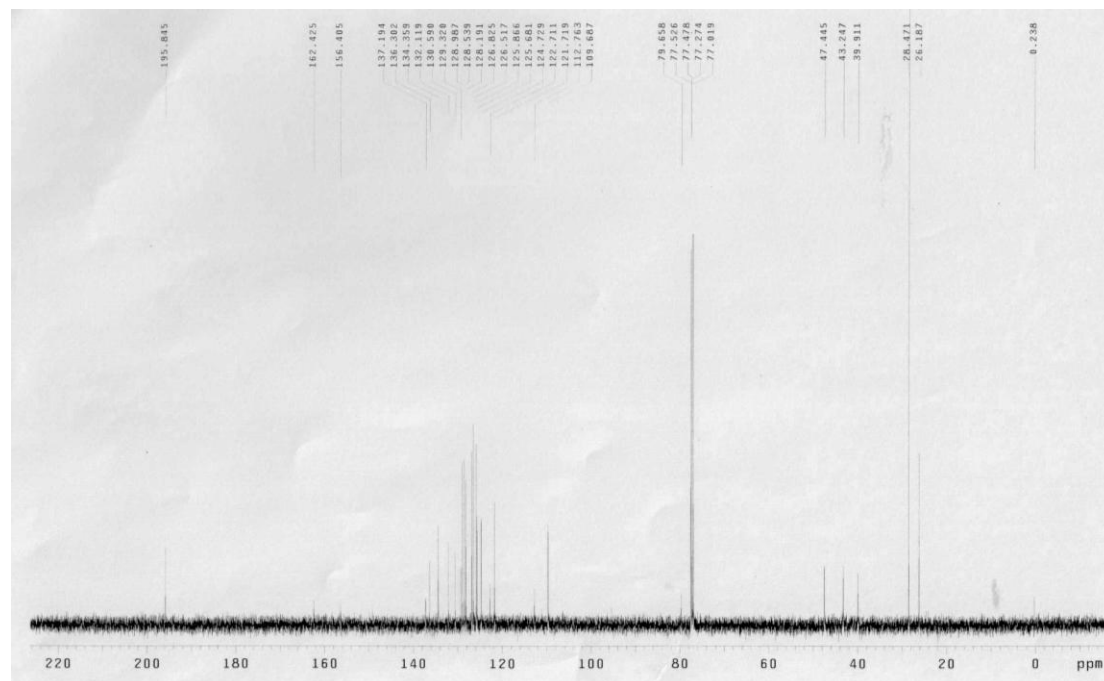
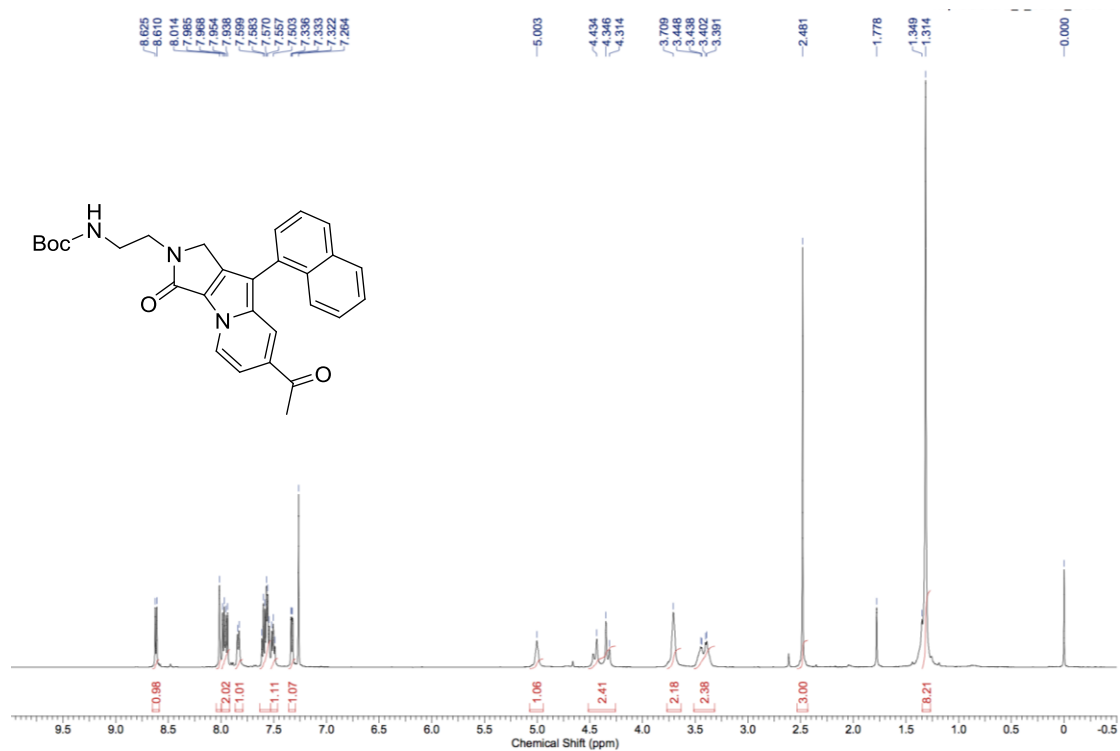


<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.71 (d, *J* = 7.0 Hz, 1H), 8.60 (s, 1H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.71 (d, *J* = 9.0 Hz, 2H), 7.55 (s, 1H), 7.52–7.49 (m, 2H), 7.42–7.37 (m, 3H), 4.99 (br s, 1H), 4.28 (s, 2H), 3.69 (t, *J* = 5.75 Hz, 2H), 3.41–3.37 (m, 2H), 2.32 (s, 3H), 1.32 (s, 9H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 195.8, 162.4, 156.3, 138.7, 136.9, 131.7, 131.3, 129.3, 129.0, 128.0, 126.4, 126.4, 126.2, 125.6, 124.9, 122.8, 121.6, 109.8, 109.6, 79.5, 47.2, 43.3, 39.8, 28.4, 26.0; LRMS (EI): *m/z* calcd for C<sub>33</sub>H<sub>31</sub>N<sub>3</sub>O<sub>4</sub> [M+H]<sup>+</sup> 534.23, found 534.39.

# 18 | SUPPORTING INFORMATION

## XI. Copies of $^1\text{H}$ and $^{13}\text{C}$ NMR Spectra of SF53 and SF54.

### SF53



SF54

