Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Materials and Methods

All reagents were purchased from Sigma-Aldrich, anhydrous and used without further purification unless otherwise noted. BODIPY FL NHS ester was purchased from ThermoFisher Scientific. ^{1}H nuclear magnetic resonance spectra were recorded on a Bruker Ascend 400 MHz spectrometer. Silica Gel 60 (40-63 µm) was used for flash column purification. High performance liquid chromatography-mass spectrometry analysis (HPLC-MS) was performed using a Waters instrument equipped with a Waters 2424 ELS Detector, Waters 2998 UV-Vis Diode array Detector, Waters 2475 Multi-wavelength Fluorescence Detector, Waters 3100 Mass Detector and a Terra MS C18 5 µm 4.6x50 mm column. Fluorescence data for quantum yield determination was acquired with a QuantaMaster 400 fluorimeter (PTI, New Jersey, USA) equipped with a K-Sphere "Petite" Integrating Sphere (Quantum yield measurements). Fluorescence measurements for characterization of the partition coefficient were acquired with a Horiba Dual FL fluorimeter (Horiba Scientific, New Jersey, USA).

Fluorescence Data

To obtain the absorption and emission fluorescence spectra of probes **2a**/**2b**, **3a**/**3b**, and **4a**/**4b**, a 10 mM DMSO stock solution was diluted to 1 µM using PBS and subsequently scanned. Quantum yield measurements were obtained by diluting 10 mM DMSO stocks of probes **2a**/**2b** and **3a**/**3b** to between 0.5-2.5 µM in EtOH and 10 mM DMSO stocks of **4a**/**4b** to between 0.5-2.5 µM in EtOH/1 mM HCl. Excitation- and emission-corrected spectra were obtained with the integrating sphere and a neutral density filter (ThorLabs, New Jersey, USA) was used to attenuate the beam for quantification of the excitation band. Spectra were collected at an excitation wavelength at 470 nm for probes **2a**/**2b, 3a**/**3b,** and the commercial vinblastine BODIPY (ThermoFisher Scientific, USA) and at an excitation wavelength of 640 nm for probes **4a**/**4b**. Quantum yields were calculated in accordance with the procedure of Porres et al (*J. Fluorescence*, 2006, **16,** 267-275) and calibrated against a fluorescein reference solution (NIST traceable Standard, ThermoFisher Scientific, USA), quantum yield 0.925 (Magde

et al., *Photochem. Photobiol*, 2002, **75**, 327-224).

Measurement of Partition Coefficient

A 50 mL falcon tube was charged with 25 mL of 1-octanol and 25 mL of deionized water and shaken for 3 minutes. The biphasic mixture was allowed to equilibrate for 24 hours. To six glass vials filled with 400 µl of 1-octanol/water (1:1) was added 1 µl of a 10 mM DMSO stock solution of probes **2a**/**2b**, **3a**/**3b**, and **4a**/**4b**. The resulting biphasic mixtures were mixed vigorously. Once the biphasic solution had separated entirely, an aliquot of the 1-octanol or water layer was removed and further diluted in EtOH for optimal fluorimetric measurement. For the octanol fraction, this corresponded to 500 to 5000-fold dilution; for water, 20 to 50-fold dilution was employed. Samples were scanned on the fluorimeter to determine the dilution-corrected Emm_{max}. LogP was calculated with the formula $logP = log(Emm_{oct}/Emm_{water})$ where Emm_{oct} = peak fluorescence emission intensity of 1-octanol aliquot diluted into EtOH and Emm_{water} = peak fluorescence emission maximum of water aliquot diluted in EtOH. Note that for analogues **4a**/**4b** 0.1 % (v/v) of a 1 M HCl solution was added to the EtOH dilution to fully convert the SiR to the open form.

HPLC traces

Time (min)	Flow (mL/min)	%A	%B
0	5	95	5
1.50	5	0	100
1.99	5	0	100
2.00	5	95	5

Tubulin Polymerization Assay

Microtubule polymerization was determined using the commercially available tubulin polymerization assay provided by Cytoskeleton Inc (Cat. #BK011P). Fluorescence intensity in the assay was analyzed on a TECAN Safire^{2}TM plate reader following the procedures and settings described, and using the tubulin reaction mix: “Volumes for inhibitor detection”. The 10x stock solutions were prepared by diluting a 2 mM solution (DMSO) using UltraPure^{TM} DNase/RNase-Free Distilled Water (Cat. #10977023), which was also the control for this experiment. Tubulin binding assays were analyzed by first fitting curves to a one-site total binding curve with the equation of Y=Max*X/(Kd+X), where Max is the maximum tubulin binding in fluorescent units, Kd is the binding constant (i.e. the time needed to achieve a half-maximum binding in units of seconds. Kd values were then averaged across two independent experiments and one-way ANOVA with multiple comparisons was conducted using Sidak’s multiple comparisons test. Values were deemed significant when $p<0.05$ relative to either control samples or Vinblastine alone.

In Vitro Imaging

Microscope: Multichannel images were collected on an Olympus *Fluoview* FV1000 confocal laser microscope (Figure 3, manuscript) or a DeltaVision microscope (Figure 4, manuscript) (Applied precision). BODIPY based probes **2a**/**2b** and **3a**/**3b** utilized the “FITC” excitation and emission filter (Ex = 473 nm and Emm = 490-540 nm) with RFP-tubulin utilizing the “TRITC” excitation and emission filter (Ex = 559 nm and Emm = 575-675 nm). SiR based probes **4a**/**4b** utilized the “Cy5” excitation and emission filter (Ex = 635 nm and Emm = 655-755), and RFP-tubulin utilized the “TRITC” excitation and emission filter (Ex = 559 nm and Emm = 575-620 nm).

Cell culture: OVCA429 cells (A kind gift from Dr. Micheal Birrer, Massachusetts General Hospital) and HT1080 cells (ATCC) were cultivated in RPMI-1640 and DMEM respectively, supplemented with 10 % fetal bovine serum and 1 % Penicillin/Streptomycin solution under standard culture conditions. For imaging experiments cells were plated in 96-well plates (Thermo Fisher Scientific) for imaging using a DeltaVision microscope or glass slides (Millipore) for imaging on

the Olympus *Fluoview* FV1000.

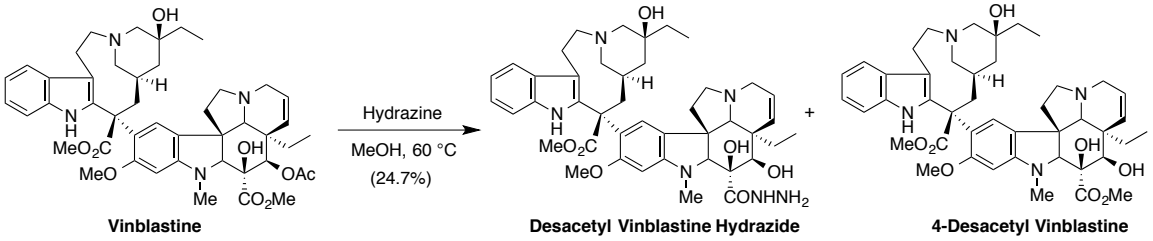
Image acquisition: Each sample was washed 1 time with either media (DeltaVision) or PBS (Olympus *Fluoview* FV1000) and imaged.

Photostability Analysis

OVCA429 cells (A kind gift from Dr. Micheal Birrer, Massachusetts General Hospital) were cultivated in RPMI-1640 and DMEM respectively, supplemented with 10 % fetal bovine serum and 1 % Penicillin/Streptomycin solution under standard culture conditions. Cells were plated on glass slides (Millipore) for imaging on the Olympus *Fluoview* FV1000 and then fixed for 15 minutes with paraformaldehyde, washed with PBS, after which a 10 μM solution of probe **2a** in PBS was added and incubated at 37 °C for 15 minutes then washed once with PBS (200 μl) and imaged. The “FITC” excitation laser power used (4 %) was ~10x the laser power used for the same concentration of probe **2a** for figure 3b in the manuscript.

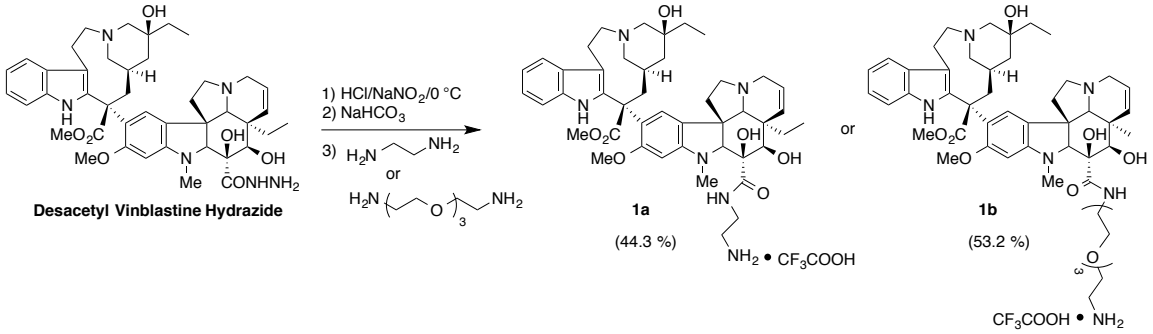
Synthesis and Characterization Data

Preparation of **desacetyl vinblastine hydrazide**:



Vinblastine (400 mg, 0.49 mmol) was dissolved in 1.20 mL of hydrazine and 1.20 mL of MeOH. The reaction mixture was heated at 60 °C for 2 hours after which it was concentrated using a rotary evaporator and purified using flash chromatography (methylene chloride:methanol gradient, 10:1 to 10:2) to give Desacetyl Vinblastine Hydrazide (93.9 mg, 0.12 mmol, 24.7%) and 4-Desacetyl Vinblastine (196.5 mg, 0.25 mmol). Desacetyl Vinblastine Hydrazide (*J. Med. Chem.* **2010**, 53, 7767-7777) and 4-Desacetyl Vinblastine (*J. Am. Chem. Soc*. **2009**, 131(13), 4904-4916).

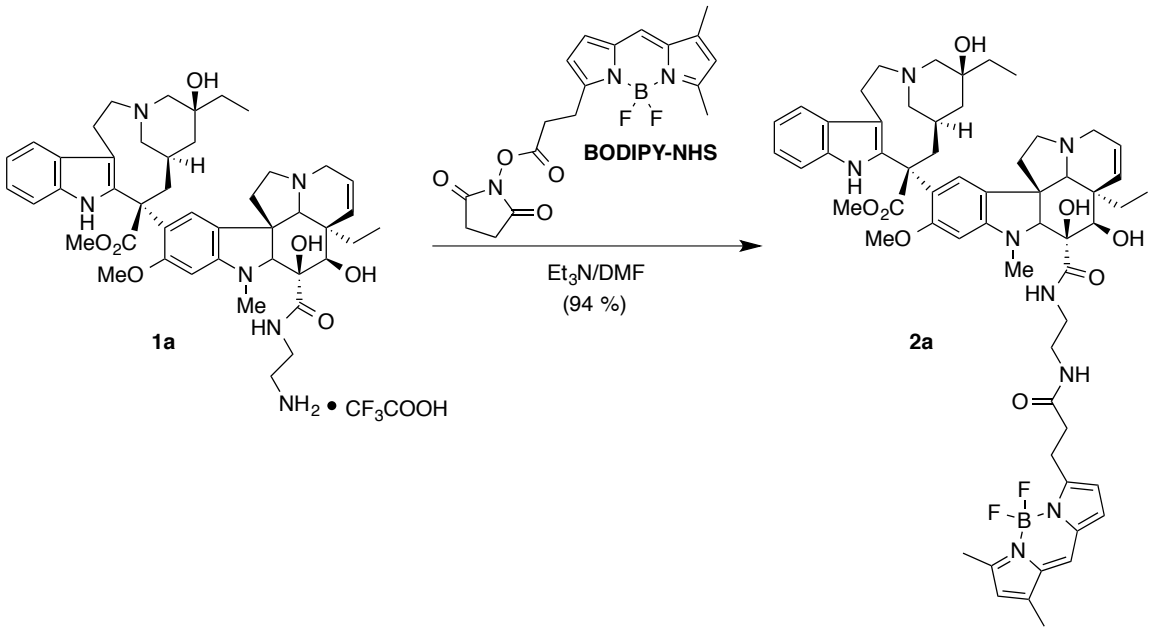
Preparation of **1a** and **1b**:



To **Desacetyl Vinblastine Hydrazide** (93.9 mg, 0.12 mmol .061) in 5.4 mL of 1 M HCl at 0 °C was added $NaNO_2$ (16.8 mg, 0.24 mmol) dissolved in 0.5 mL of H_2O. The mixture was allowed to stir at 0 °C for 10 minutes after which saturated $NaHCO_3$ was added until pH ~ 8.5. The solution was then extracted 3 times with methylene chloride (150 mL total) and concentrated on a rotary evaporator. The crude mixture was dissolved in 10 mL of methylene chloride and split in two batches. One portion (reaction A) received ethylenediamine (0.24 mmol, 16.3 µL) neat, the other portion (reaction B) received 1,11-Diamino-3,6,9-trioxaundecane (0.24 mmol, 46.9 mg) and the reactions were allowed to stir at room temperature for 3 hours after which they were concentrated on a rotary evaporator. Both reactions A and B were purified using a 10 g C_{18} Sep Pak (gradient from 100 % H_2O (0.1 % TFA) to 11:1 H_2O:MeCN (0.1 % TFA)) to give **1a** (24.6 mg, .027 mmol, 44.3 %) and **1b** (33.4 mg, .032 mmol, 53.2 %) as the TFA salts. Compound **1a**: ESIMS $[M+H]^+$ calcd for $C_{45}H_{61}N_6O_7$ 797.46, found

797.76. Compound **1b**: ESIMS $[M+H]^+$ calcd for $C_{51}H_{73}N_6O_{10}$ 929.54, found 929.82.

Preparation of **2a**:



To **1a** (4.0 mg, .0043 mmol) in 0.5 mL of DMF was added triethylamine (1.39 µL, .01 mmol) followed by **BODIPY-NHS** (2.34 mg, .0060 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.2 to 10:1 methylene chloride:methanol) to give **2a** as an orange solid (4.32 mg, 94.0 %).

^{1}H NMR (400 MHz, MeOD) δ 7.48 (d, J = 8.0 Hz, 1H), 7.42 (s, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 7.00 (d, J = 3.6 Hz, 1H), 6.46 (s, 1H), 6.34 (d, J = 4.0 Hz, 1H), 6.24 (s, 1H), 6.20 (s, 1H), 5.80 (dd, J = 10.8, 4.4 Hz, 1), 5.70 (m, 1H), 4.32 (m, 1H), 4.03 (s, 1H), 3.97 (t, J = 14.0 Hz, 1H), 3.79 (s, 3H), 3.69 (m, 1H), 3.66 (s, 3H), 3.48 (m, 2H), 3.43 (s, 1H), 3.42-3.35 (m, 4H), 3.23 (t, J = 7.6 Hz, 4H), 2.99 (q, J = 9.6 Hz, 2H), 2.77 (s, 3H), 2.73 (m, 1H), 2.62 (m, 4H), 2.46 (s, 3H), 2.38 (dt, J = 11.2, 4.4 Hz, 2H), 2.27 (s, 3H), 2.05 (ddd, J = 22.4, 8.6, 5.6 Hz, 1H), 1.74 (td, J =12.4, 5.6 Hz, 1H), 1.66 (dd, J = 13.6, 7.2 Hz, 1H), 1.57 (m, 1H), 1.48 (m, 1H), 1.41 (q, J = 7.6 Hz, 2H), 1.33 (dd, J = 14.0, 7.2 Hz, 1H), 1.29 (s, 1H), 1.18 (m, 1H), 0.93 (t, J = 7.2 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H). ESIMS $[M+H]^+$ calcd for $C_{59}H_{74}BF_2N_8O_8$ 1071.57, found 1071.72.

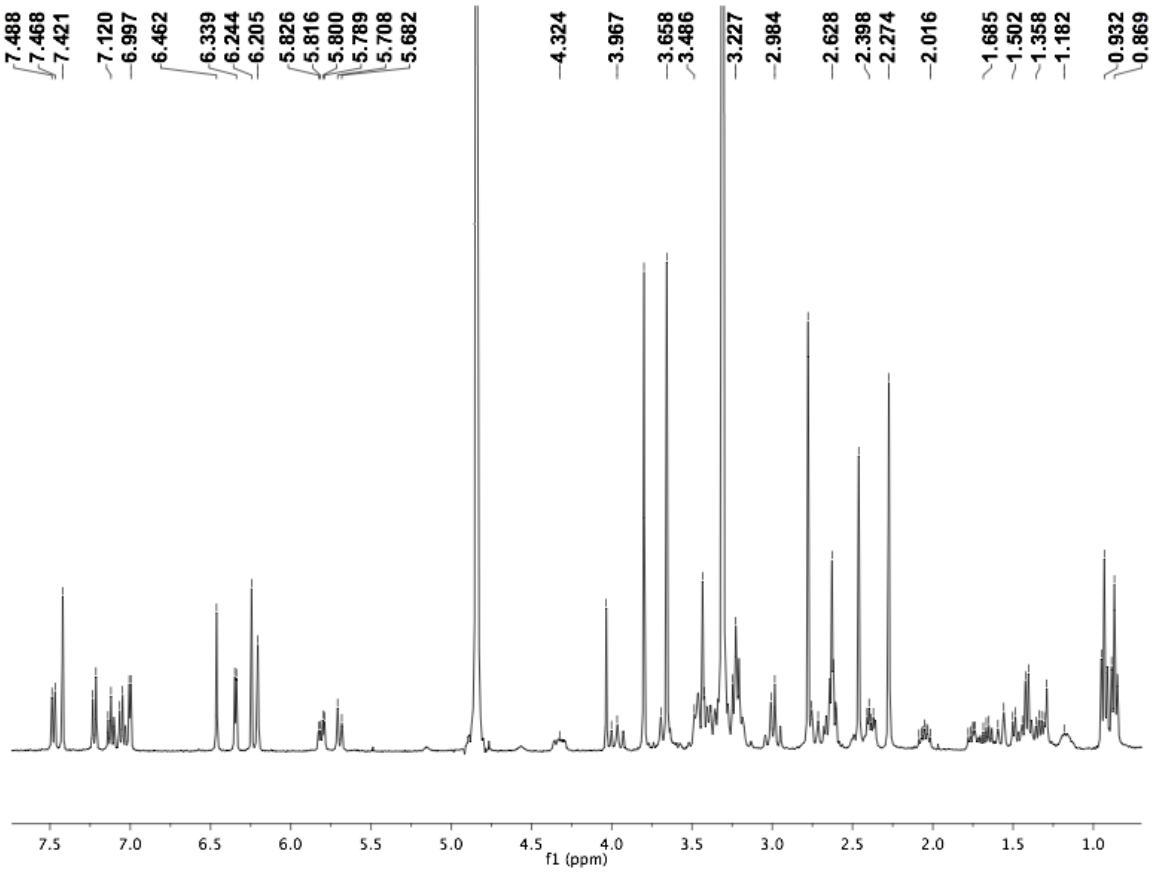
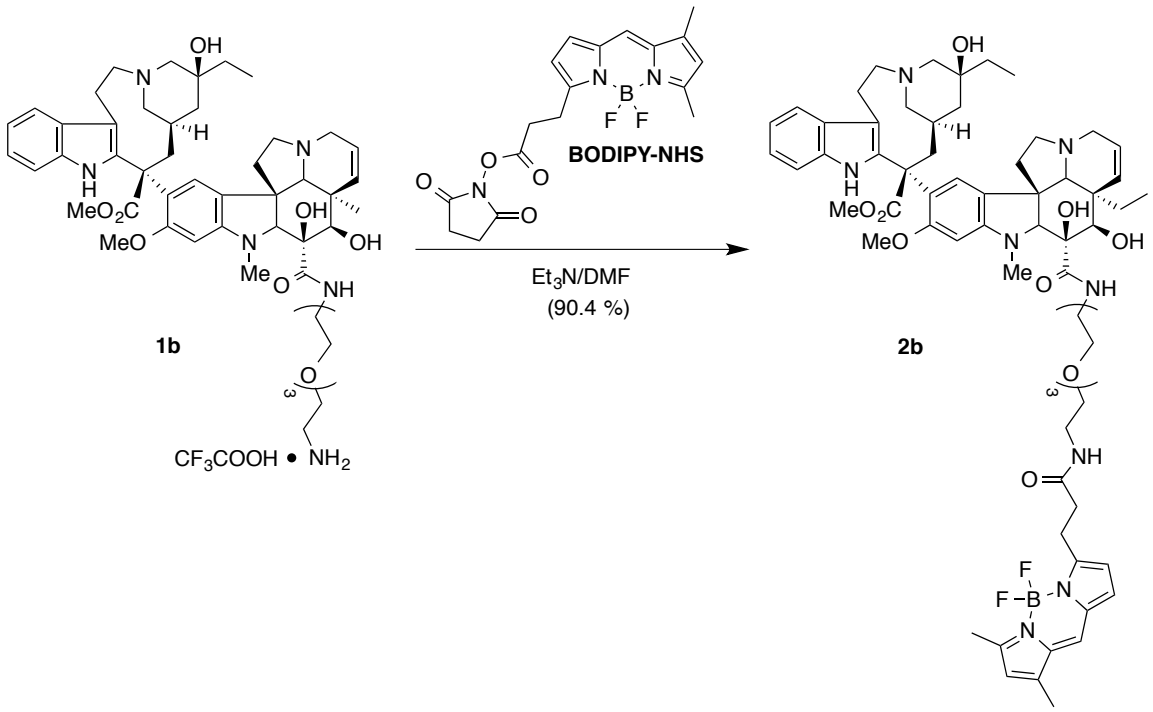


Figure S1 ^{1}H spectra of **2a** recorded in MeOD at 400 MHz.

Preparation of **2b**:



To **1b** (3.3 mg, .0032 mmol) in 0.5 mL of DMF was added triethylamine (1.0 µL, .0070 mmol) followed by **BODIPY-NHS** (1.63 mg, .0042 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.25 to 10:1.5 methylene chloride:methanol) to give **2b** as an orange solid (3.48 mg, .0029 mmol, 90.4 %).

^{1}H NMR (400 MHz, MeOD) δ 7.44 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 7.6 Hz, 1H), 7.00 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 4.0 Hz, 1H), 6.55 (s, 1H), 6.33 (d, J = 3.6 Hz, 1H), 6.22 (s, 1H), 6.20 (s, 1H), 5.80 (dd, J = 10.4, 4.0 Hz, 1H), 5.66 (m, 1H), 4.08-4.01 (m, 1H), 4.05 (s, 2H), 3.96 (dd, J = 14.8, 10.4 Hz, 1H), 3.79 (s, 3H), 3.63 (s, 3H), 3.62-3.57 (m, 8H), 3.52 (t, J = 5.6 Hz, 2H), 3.44-3.34 (m, 10H), 3.24-3.13 (m, 4H), 3.04 (dd, J = 15.2, 5.6 Hz, 1H), 2.76 (s, 3H), 2.73-2.70 (m, 1H), 2.62 (t, J = 7.6 Hz, 2H), 2.59 (s, 1H), 2.49 (s, 3H), 2.43 (dd, J = 14.4, 4.4 Hz, 1H), 2.35 (q, J = 4.4 Hz, 1H), 2.30 (m, 1H), 2.27 (s, 3H), 2.00 (ddd, J = 13.2, 8.4, 6 Hz, 1H), 1.77 (td, J = 13.2, 4.8 Hz, 1H), 1.64 (dd, J = 14.0, 7.6 Hz, 1H), 1.50 (m, 1H), 1.38 (dd, J = 14.0, 6.4 Hz, 1H), 1.30 (m, 6H), 0.89 (t, J = 7.2 Hz, 3H), 0.88 (t, J = 7.2 Hz, 3H). ESIMS $[M+H]^+$ calcd for $C_{65}H_{86}BF_2N_8O_{11}$ 1203.65, found 1203.85.

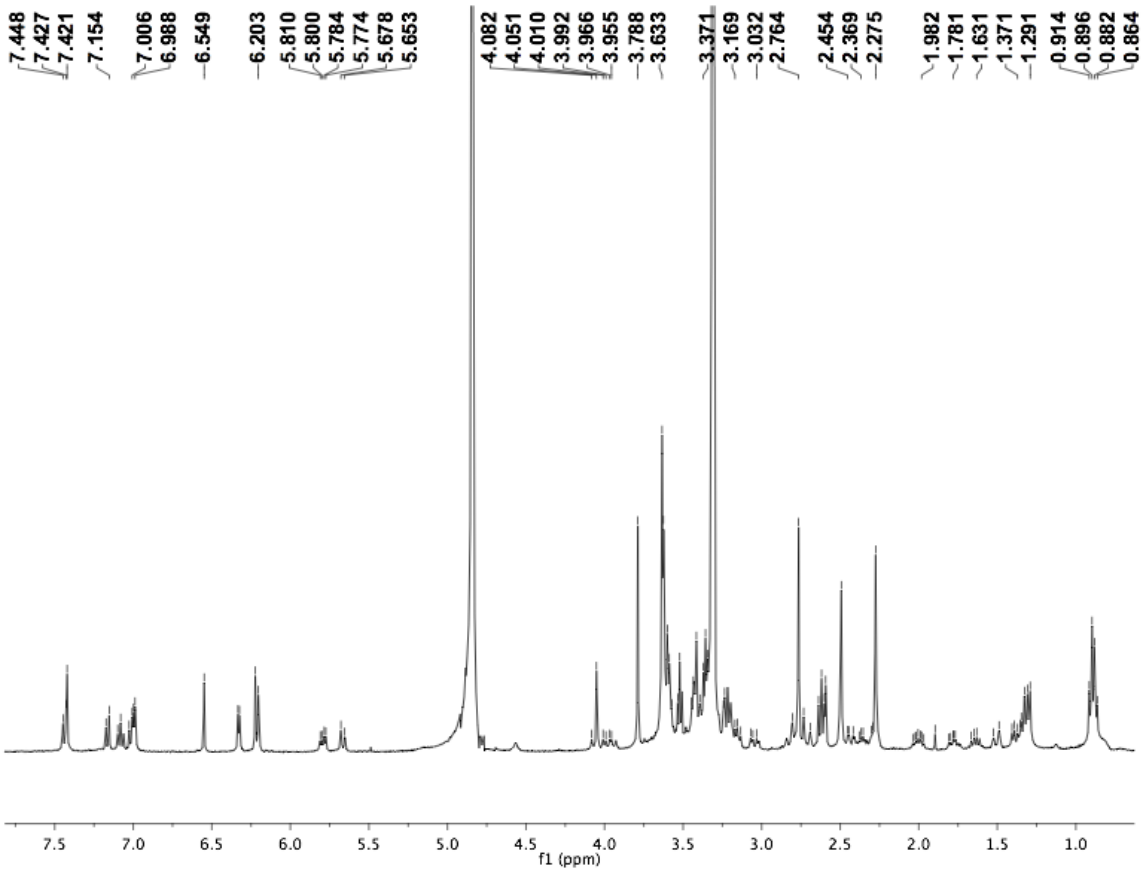
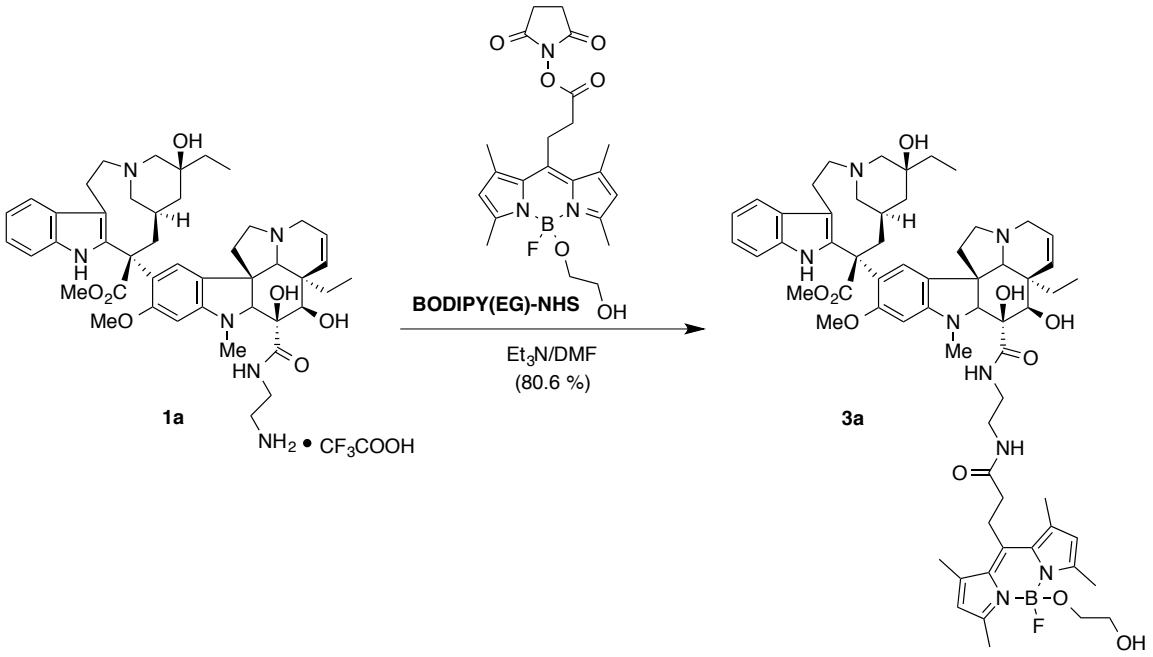


Figure S2 ^{1}H spectra of **2b** recorded in MeOD at 400 MHz.

Preparation of **3a**:



To **1a** (4.55 mg, .0050 mmol) in 0.5 mL of DMF was added triethylamine (1.39 µL, .010 mmol) followed by **BODIPY(EG)-NHS** (2.76 mg, .0060 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.2 to 10:1 methylene chloride:methanol) to give **3a** as an orange solid (4.6 mg, .0040 mmol, 80.6 %).

Compound **3a**: ^{1}H NMR (600 MHz, MeOD) δ 7.44 (d, J = 7.8 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 7.08 (t, J = 7.2 Hz, 1H), 7.01 (t, J = 7.2 Hz, 1H), 6.55 (s, 1H), 6.23 (s, 1H), 6.15 (s, 2H), 5.80 (dd, J = 10.8, 5.4 Hz, 1H), 5.68 (d, J = 9.6 Hz, 1H), 4.04 (s, 1H), 3.99 (m, 1H), 3.79 (s, 3H), 3.64 (s, 3H), 3.49 (dd, J = 12.6, 5.4 Hz, 1H), 3.44-3.38 (m, 8H), 3.28 (m, 1H), 3.21 (m, 4H), 3.06 (dd, J = 14.4, 6 Hz, 1H), 2.93 (t, J = 6.0 Hz, 2H), 2.85-2.78 (m, 2H), 2.75 (s, 3H), 2.61 (s, 1H), 2.55-2.52 (m, 3H), 2.49 (s, 6H), 2.48 (s, 6H), 2.46-2.43 (m, 1H), 2.39 (m, 1H), 2.29 (m, 1H), 2.01 (m, 1H), 1.92 (s, 1H), 1.78 (m, 1H), 1.65 (m, 1H), 1.52 (d, J = 14.4 Hz, 1H), 1.41 (dd, J = 14.4, 7.8 Hz, 1H), 1.33 (q, J = 7.2 Hz, 2H), 1.29 (s, 2H), 0.91 (t, J = 7.8 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H). ESIMS $[M+H]^+$ calcd for $C_{63}H_{83}BFN_8O_{10}$ 1141.63, found 1141.88.

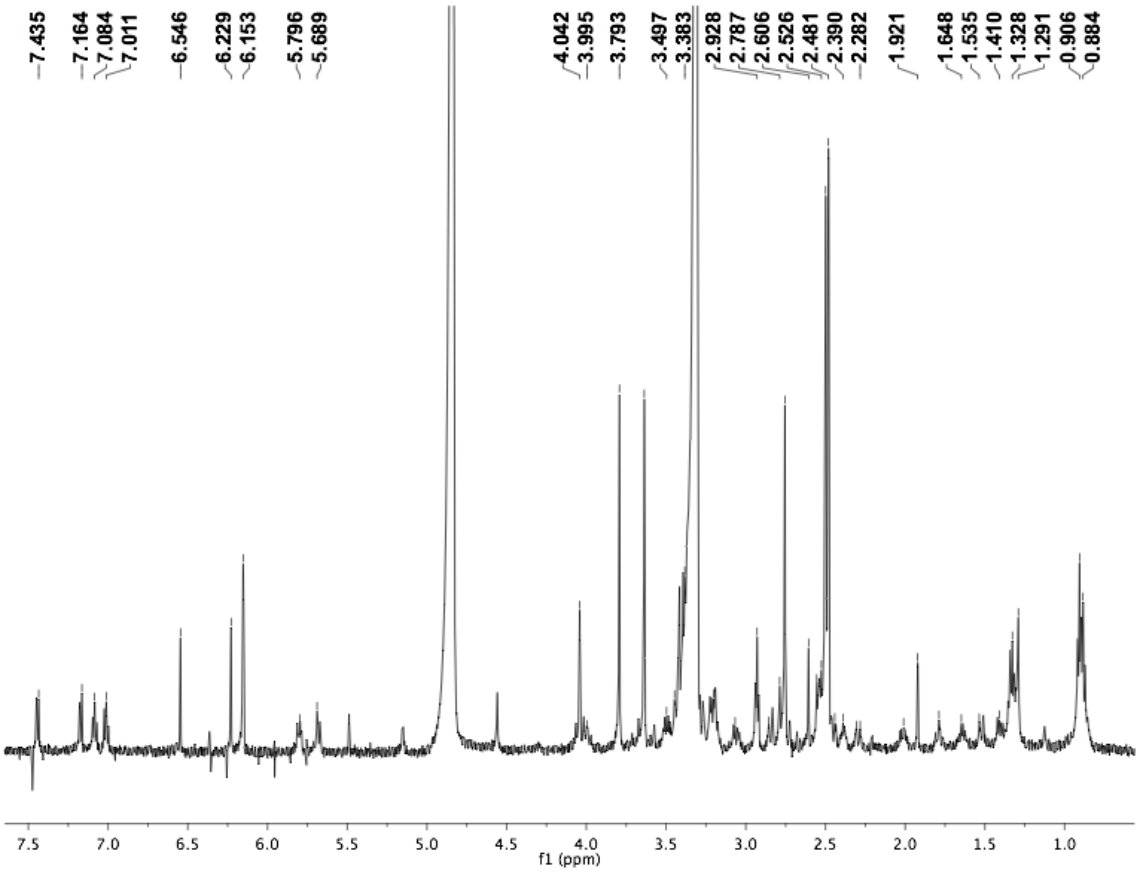
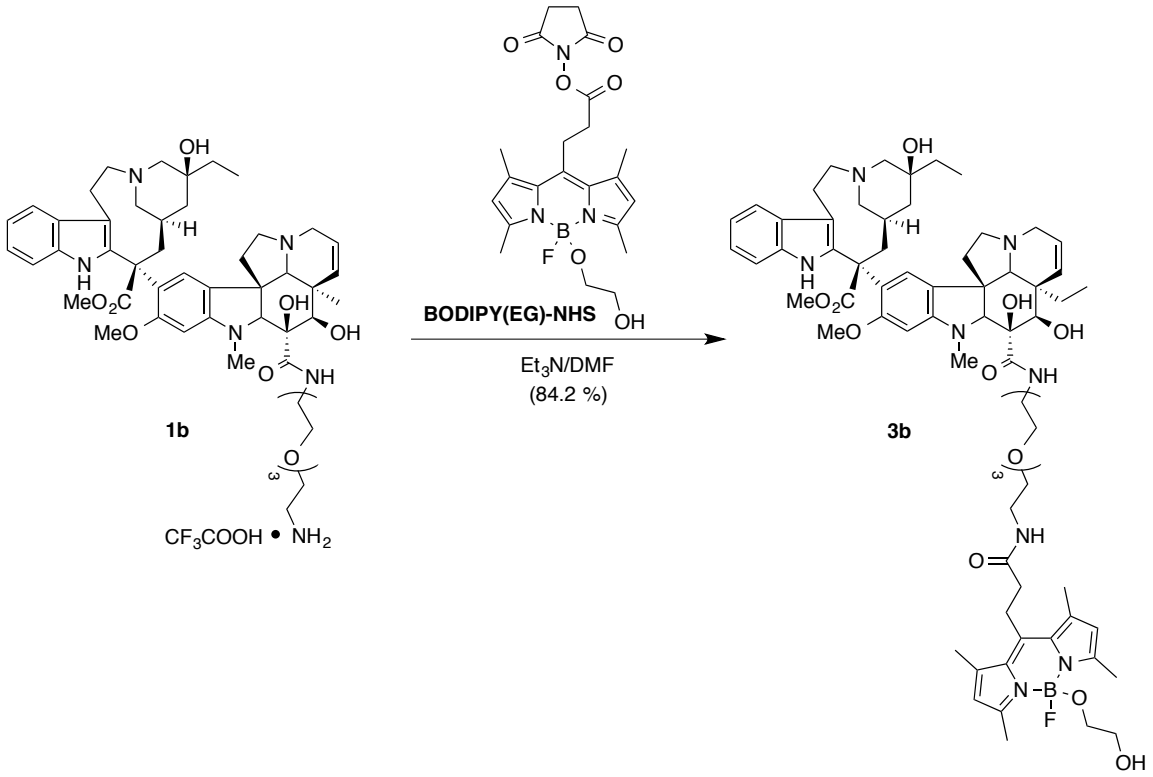


Figure S3 ^{1}H spectra of **3a** recorded in MeOD at 600 MHz.

Preparation of **3b**:



To **1b** (3.3 mg, .0032 mmol) in 0.5 mL of DMF was added triethylamine (1.0 µL, .0070 mmol) followed by **BODIPY(EG)-NHS** (1.95 mg, .0042 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.2 to 10:2 methylene chloride:methanol) to give **3b** as an orange solid (3.43 mg, .0027 mmol, 84.2 %).

Compound **3b**: ^{1}H NMR (600 MHz, MeOD) δ 7.45 (d, J = 7.8 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.09 (t, J = 7.2 Hz, 1H), 7.02 (t, J = 7.2 Hz, 1H), 6.52 (s, 1H), 6.23 (s, 1H), 6.16 (s, 2H), 5.79 (dd, J = 10.8, 6.6 Hz, 1H), 5.68 (m, 1H), 4.09 (m, 1H), 4.04 (s, 1H), 4.01 (m, 1H), 3.81 (m, 1H), 3.79 (s, 3H), 3.64 (s, 3H), 3.62-3.55 (m, 12H), 3.52 (t, J = 10.8 Hz, 2H), 3.43-3.36 (m, 10H), 3.18 (m, 1H), 3.10 (dd, J = 11.4, 4.8 Hz, 1H), 2.93 (t, J = 7.8 Hz, 2H), 2.88-2.82 (m, 2H), 2.76 (s, 3H), 2.74-2.71 (m, 1H), 2.60 (s, 1H), 2.55-2.53 (m, 3H), 2.49 (s, 6H), 2.48 (s, 6H), 2.37 (m, 1H), 2.32 (dd, J = 12.0, 3.0 Hz, 1H), 2.01 (m, 1H), 1.77 (td, J = 12.6, 5.4 Hz, 1H), 1.65 (dd, J = 13.8, 7.8 Hz, 1H), 1.54 (m, 1H), 1.44 (dd, J = 13.8, 5.4 Hz, 1H), 1.35 (q, J = 7.8 Hz, 2H), 1.33-1.29 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H), 0.88 (t, J = 7.8 Hz, 3H). ESIMS $[M+H]^+$ calcd for $C_{69}H_{95}BFN_8O_{13}$ 1273.71, found 1274.15.

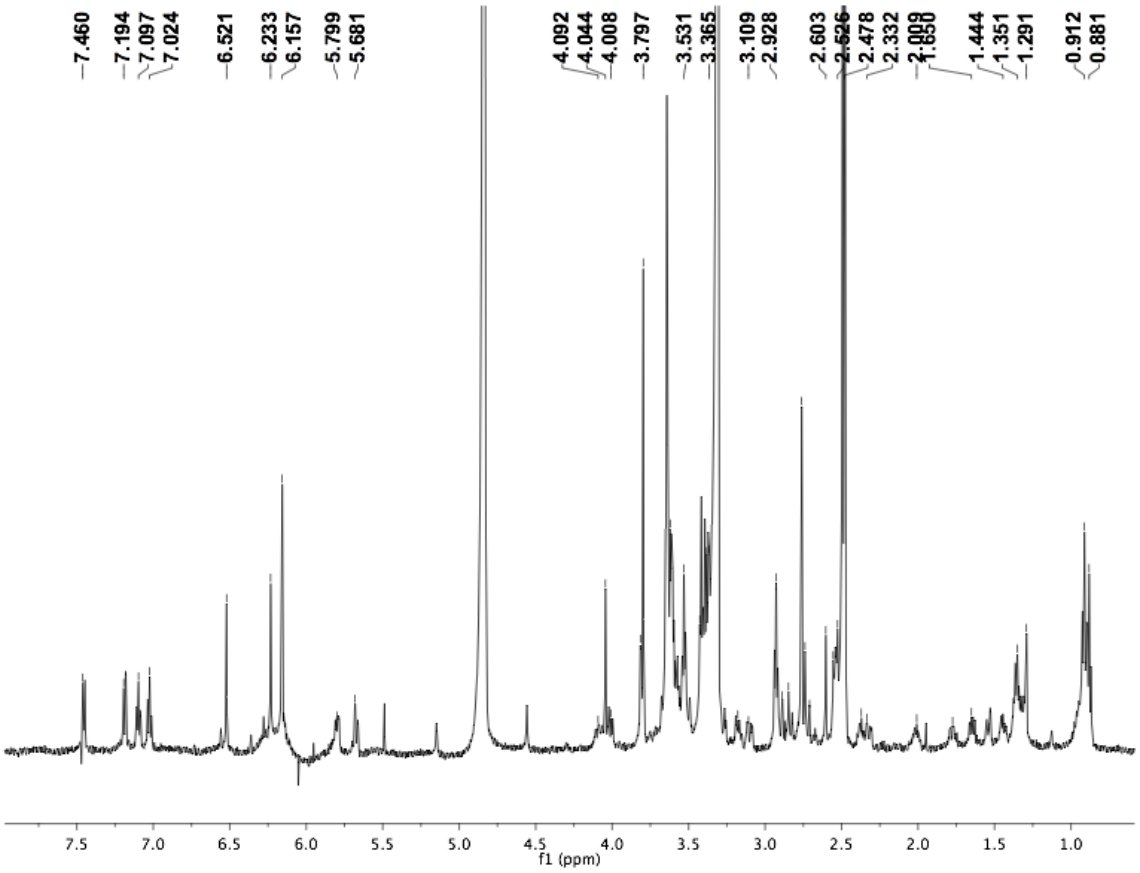
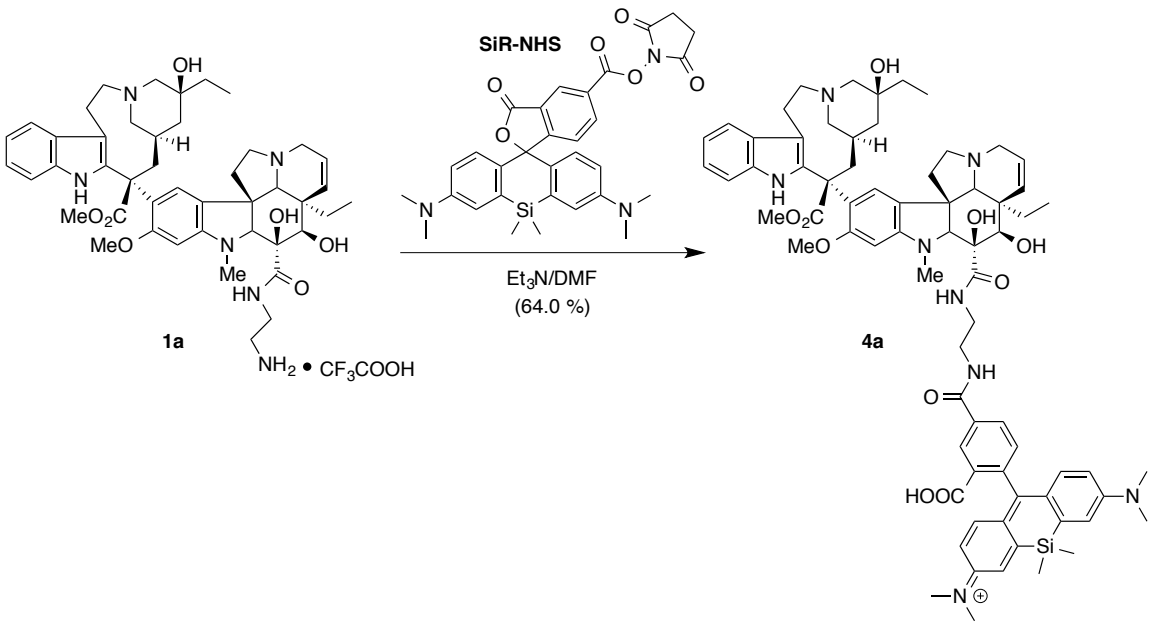


Figure S4 ^{1}H spectra of **3b** recorded in MeOD at 600 MHz.

Preparation of **4a**:



To **1a** (4.0 mg, .0044 mmol) in 0.5 mL of DMF was added triethylamine (1.39 µL, .010 mmol) followed by **SiR-NHS** (3.4 mg, .0060 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.3 to 10:0.8 methylene chloride:methanol) to give **4a** as a white solid (3.53 mg, .0028 mmol, 64.0 %).

Compound **4a**: ^{1}H NMR (400 MHz, MeOD) δ 8.07 (dd, J = 8.0, 1.2 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.76 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 6.8 Hz, 1H), 7.03-6.99 (m, 3H), 6.61 (d, J = 6.8 Hz, 1H), 6.58 (d, J = 6.8 Hz, 1H), 6.52 (dd, J = 8.0, 4.8 Hz, 1H), 6.51 (dd, J = 4.8, 2.8 Hz, 1H), 6.53 (s, 1H), 6.18 (s, 1H), 5.66 (dd, J = 10.4, 4.8 Hz, 1H), 5.53 (m, 1H), 4.56 (bs, 1H), 4.04 (m, 1H), 3.97 (s, 1H), 3.77 (s, 3H), 3.63 (s, 3H), 3.61-3.56 (m, 1H), 3.52 (q, J = 4.8 Hz, 2H), 3.35 (s, 1H), 3.24-3.11 (m, 4H), 3.05 (td, J = 14.0, 5.2 Hz, 2H), 3.05 (m, 1H), 2.957 (s, 6H), 2.950 (s, 6H), 2.84-2.73 (m, 2H), 2.69 (s, 3H), 2.68-2.62 (m, 1H), 2.61 (s, 1H), 2.56 (s, 1H), 2.41 (dd, J = 14.0, 3.2 Hz, 1H), 2.30 (m, 1H), 1.89 (m, 1H), 1.72 (td, J = 18.0, 5.2 Hz, 1H), 1.59-1.49 (m, 2H), 1.39 (dd, J = 13.6, 6.0 Hz, 1H), 1.34-1.24 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H), 0.84 (t, J = 7.2 Hz, 3H), 0.64 (s, 3H), 0.55 (s, 3H). ESIMS $[M+H]^+$ calcd for $C_{72}H_{88}N_8O_{10}Si$ 1252.64, found 1252.11.

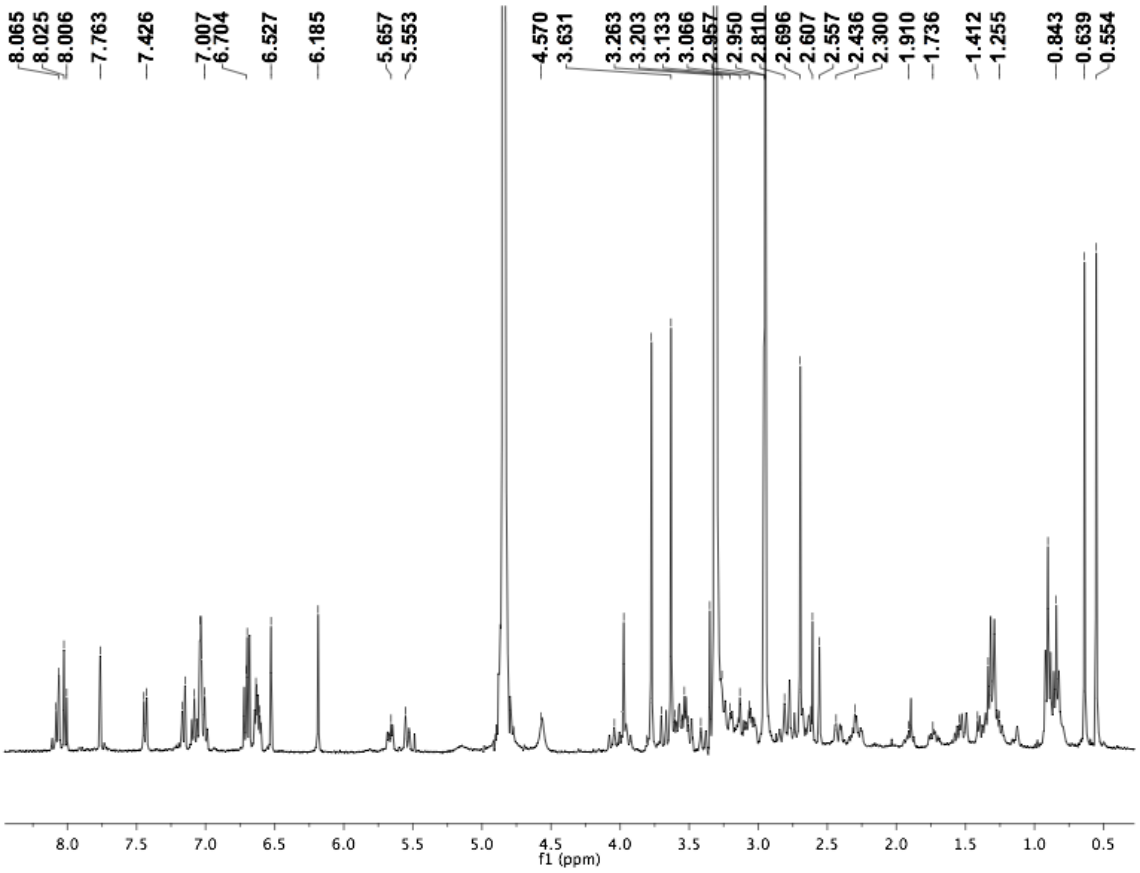
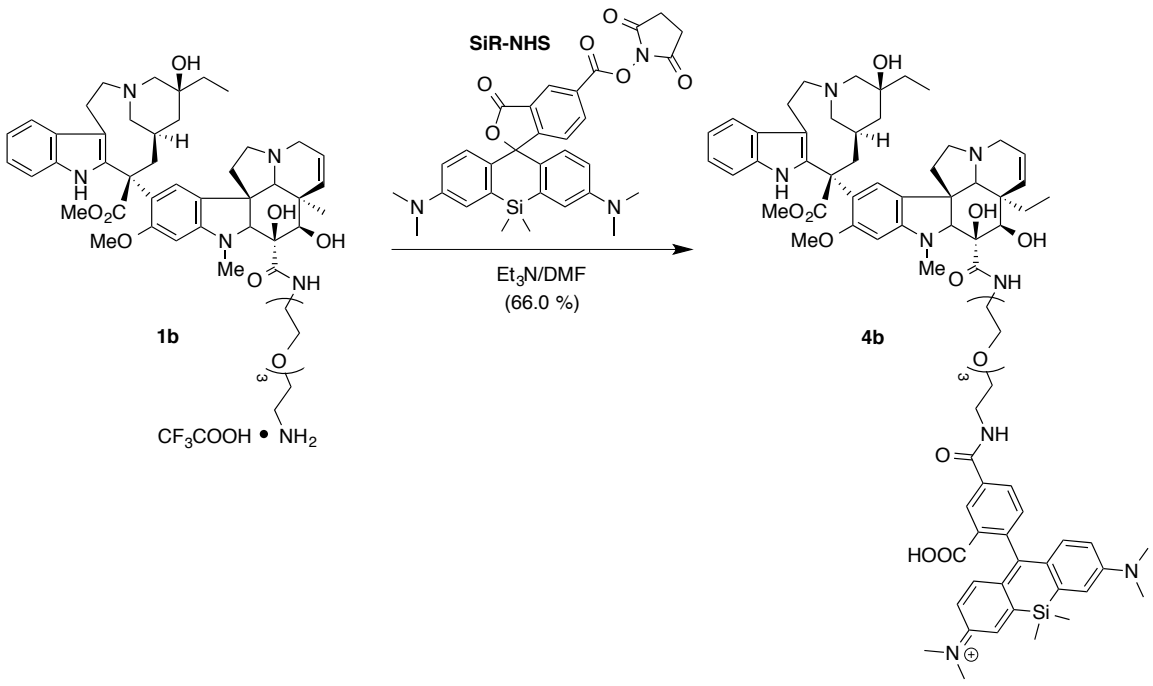


Figure S5 ^{1}H spectra of **4a** recorded in MeOD at 400 MHz.

Preparation of **4b**:



To **1b** (3.3 mg, .0032 mmol) in 0.5 mL of DMF was added triethylamine (1.0 µL, .0070 mmol) followed by **SiR-NHS** (2.39 mg, .0042 mmol) dissolved in 250 µL of DMF and allowed to stir at room temperature for 60 minutes. The crude mixture was concentrated on rotary evaporator and purified using flash chromatography (gradient from 10:0.3 to 10:0.8 methylene chloride:methanol) to give **4b** as a white solid (2.84 mg, .0020 mmol, 64.0 %).

Compound **4b**: ^{1}H NMR (400 MHz, MeOD) δ 8.03 (dd, J = 8.0, 1.6 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.66 (s, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.08 (t, J = 6.8 Hz, 1H), 7.01 (m, 3H), 6.72 (s, 1H), 6.69 (s, 1H), 6.37 (t, J = 3.2 Hz, 1H), 6.61 (t, J = 3.2 Hz, 1H), 6.55 (s, 1H), 6.22 (s, 1H), 5.74 (dd, J = 10.4, 4.0 Hz, 1H), 5.63 (m, 1H), 4.56 (bs, 1H), 4.05 (m, 1H), 4.01 (s, 1H), 3.94 (m, 1H), 3.78 (s, 3H), 3.72-3.60 (m, 8H), 3.62 (s, 3H), 3.58-3.35 (m, 10H), 3.25-3.02 (m, 4H), 2.94 (s, 6H), 2.92 (s, 6H), 2.84-2.77 (m, 2H), 2.75 (s, 3H), 2.71-2.60 (m, 2H), 2.56 (s, 1H), 2.41 (dd, J = 14.0, 3.6 Hz, 1H), 2.27 (m, 1H), 1.93 (ddd, J = 22.2, 8.6, 5.6 Hz, 1H), 1.71 (td, J = 12.2, 5.2 Hz, 1H), 1.62 (dd, J = 14.0, 7.6 Hz, 1H), 1.50 (m, 1H), 1.37 (dd, J = 14.8, 6.8 Hz, 1H), 1.32-1.27 (m, 4H), 0.89 (t, J = 7.2 Hz, 3H), 0.86 (t, J = 7.2 Hz, 3H), 0.64 (s, 3H), 0.55 (s, 3H). ESIMS $[M+H]^+$ calcd for $C_{78}H_{100}N_8O_{13}Si$ 1384.72, found 1384.35.

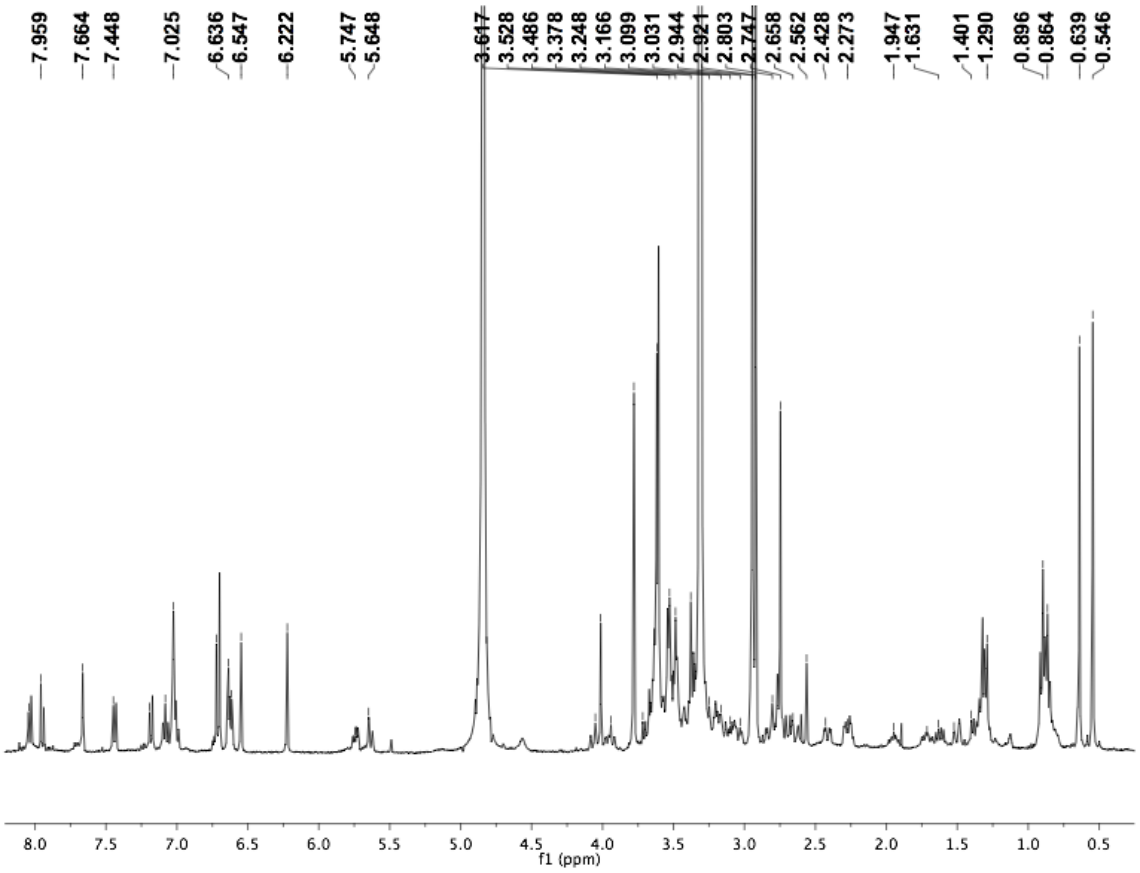


Figure S6 ^{1}H spectra of **4b** recorded in MeOD at 400 MHz.

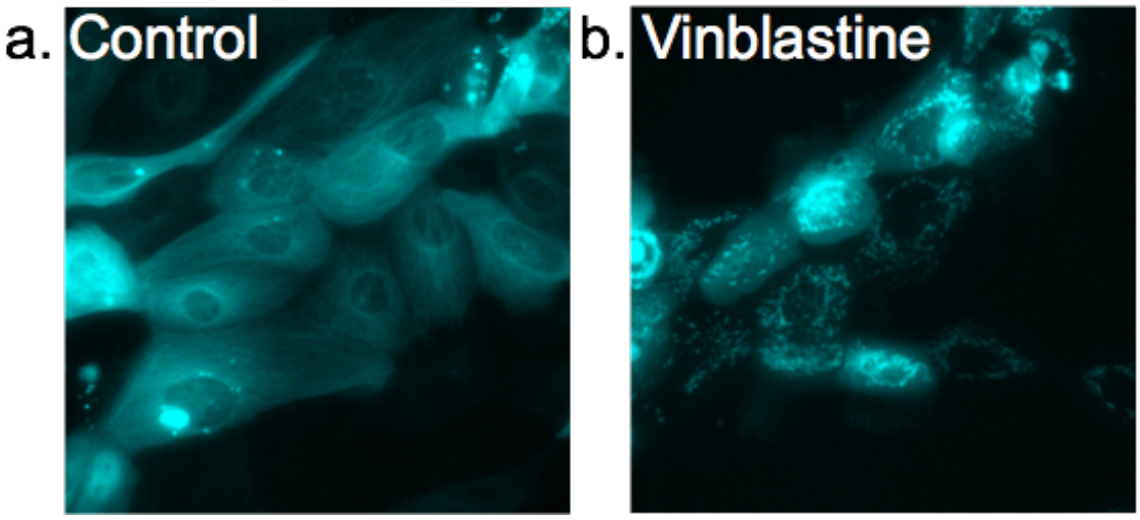


Figure S7 OVCA429 cells transfected with CellLight RFP tubulin. (a) Without agonist. (b) Incubated after 3.5 hours incubation with 100 μM of Vinblastine in media.

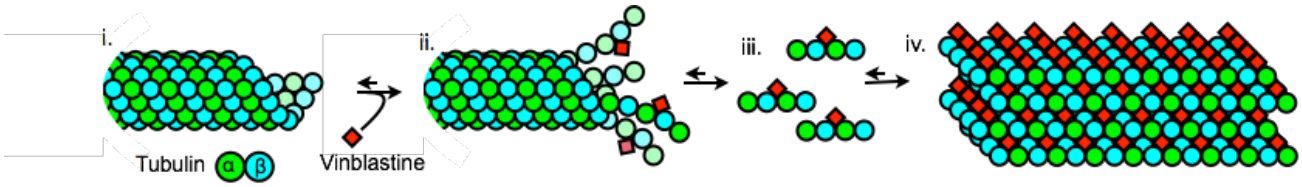


Figure S8 The effect of vinblastine on microtubules and subsequent tubulin reorganization. i) Microtubule ii) Vinblastine binding to a microtubule tip and halting microtubule polymerization iii) At higher concentrations there is depolymerization of the microtubule into vinca bound αβ heterodimers iv) Higher concentrations of vinblastine induce the formation of vinblastine-tubulin paracrystalline aggregates.

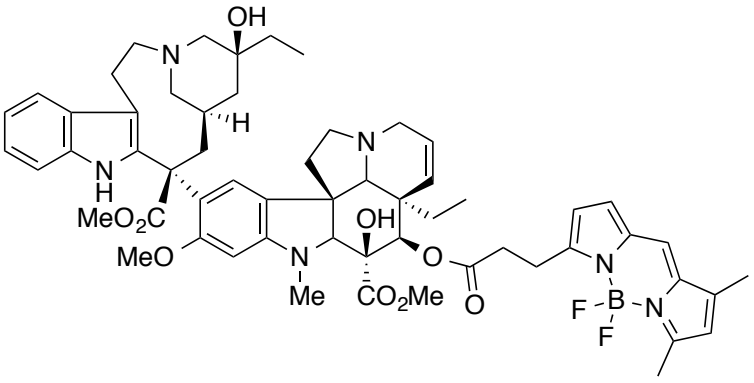


Figure S9 Commercially available vinblastine BODIPY (VBLS)

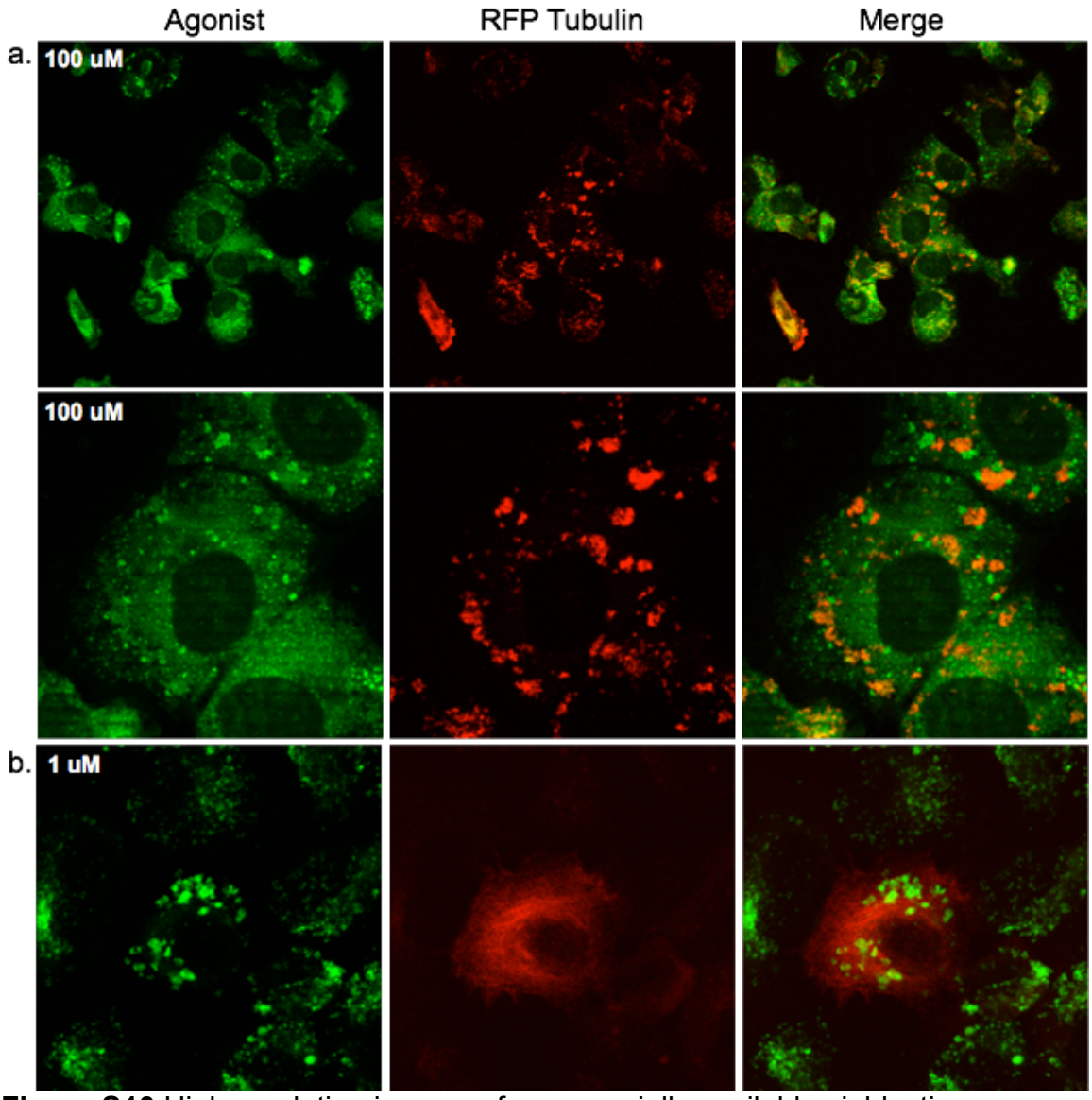


Figure S10 High resolution images of commercially available vinblastine BODIPY (VBLS) in HT1080 cells expressing tubulin RFP. Cells were incubated for 3 h at (a) 100 μM and (b) 1 h at 1 μM agonist concentration. Cells were washed once with PBS then imaged immediately.

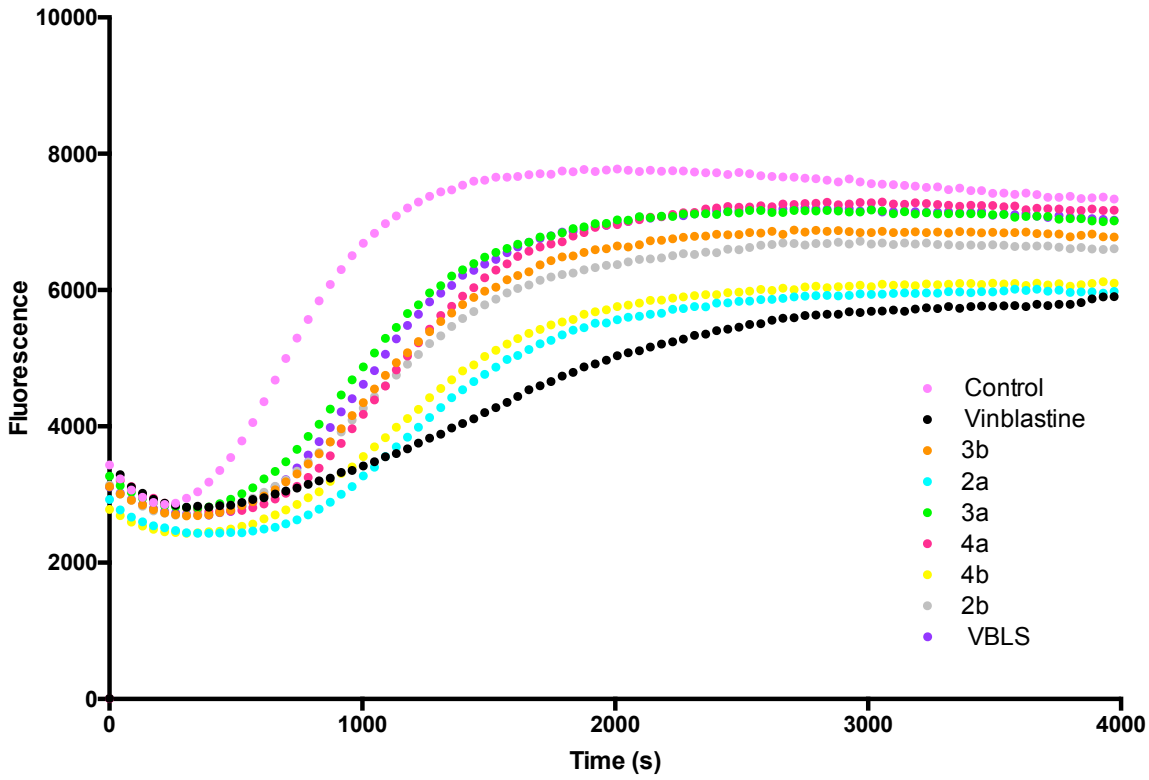


Figure S11 Representative experiment of tubulin polymerization assay for control, vinblastine, fluorescent conjugates **2a**/**2b**, **3a**/**3b**, **4a**/**4b** and commercially available vinblastine BODIPY (VBLS) all at 2 µM concentration.

Table1 Physical properties of probes

Probe	logP	QY
2a	1.8	0.14
2b	1.7	0.11
3a	2.2	0.28
3b	1.7	0.25
4a	3.5	0.40
4b	2.7	0.40
VBLS	ND	0.007

HPLC traces

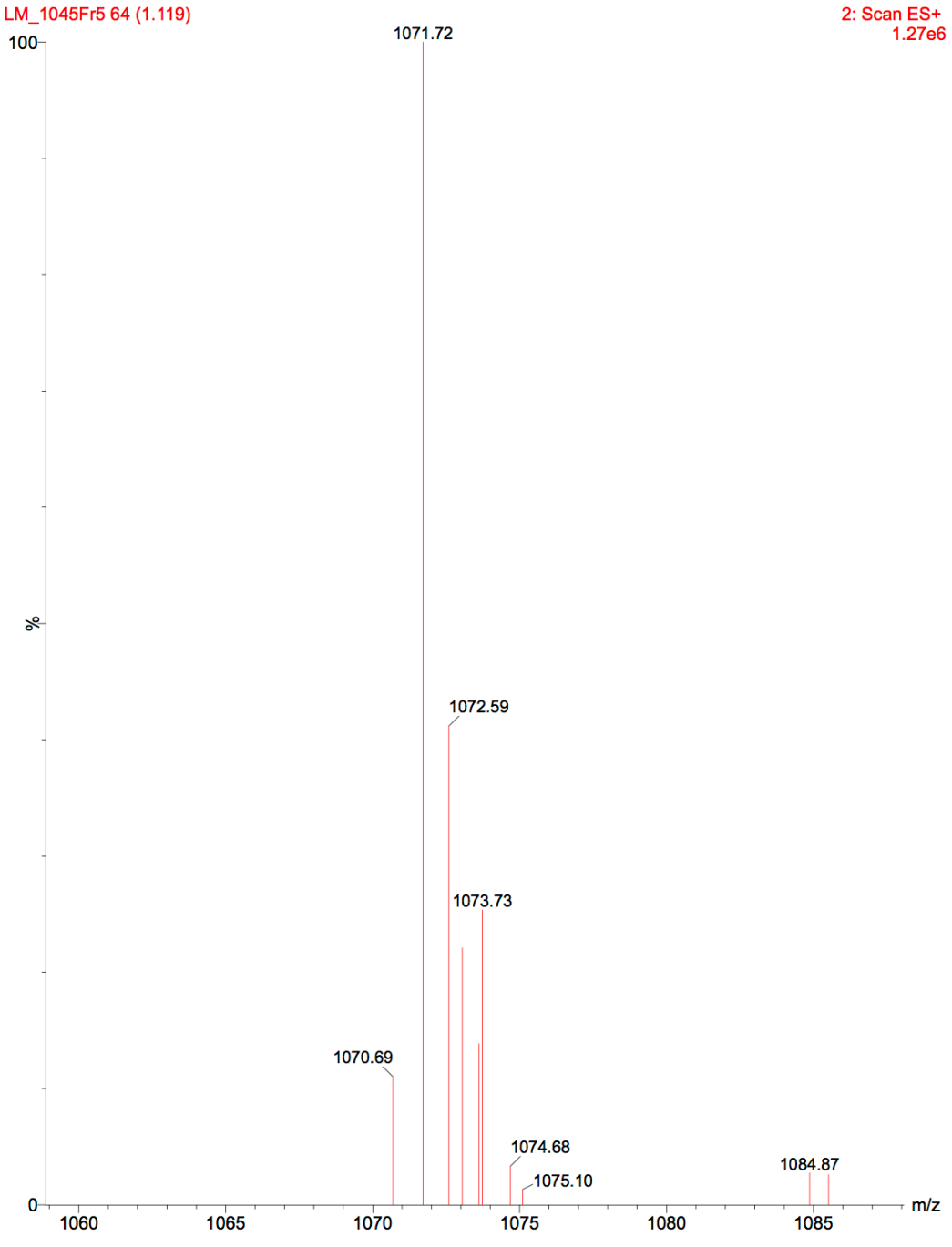


Figure S12 Mass trace of **2a**.

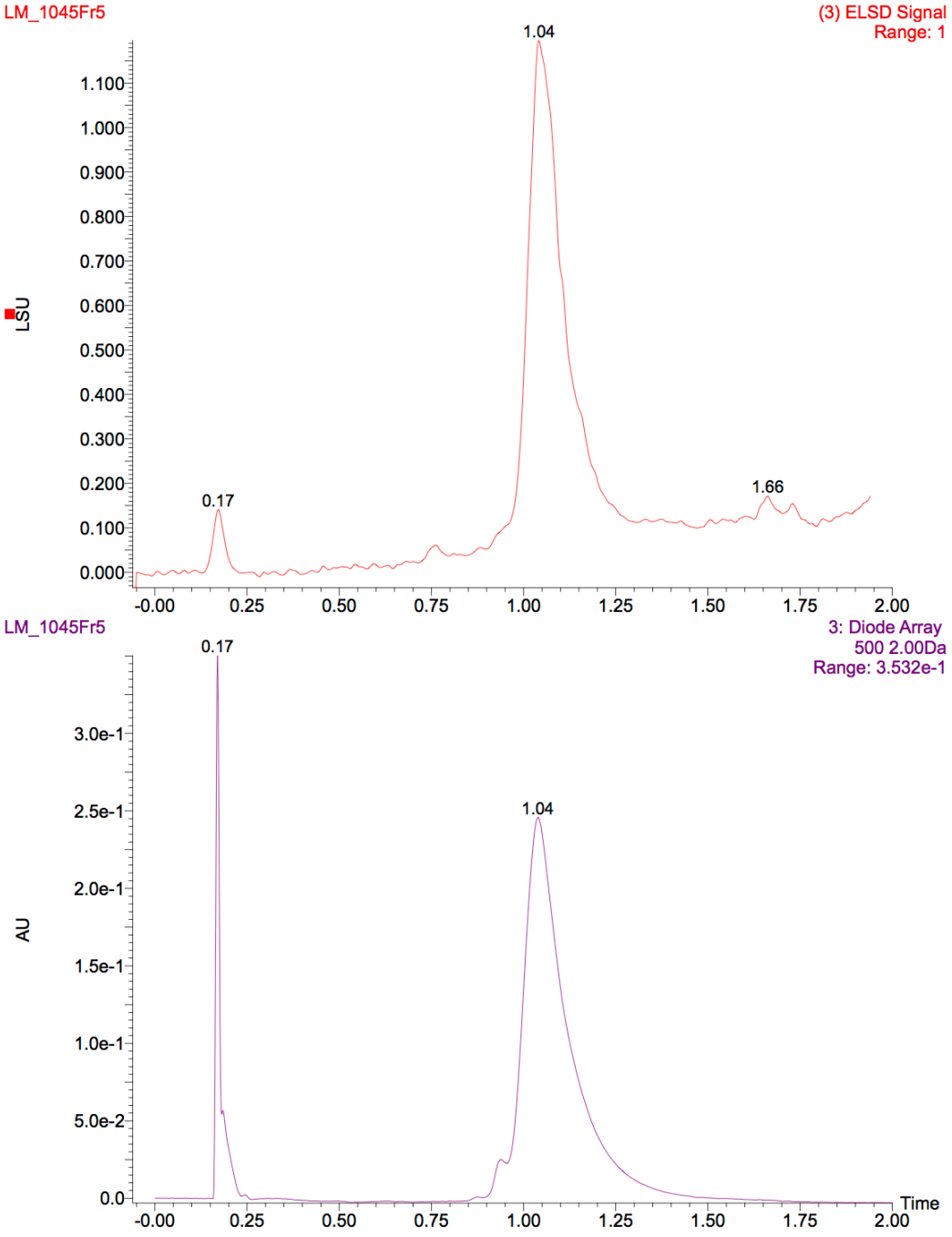


Figure S13 UV/ELSD trace of **2a**.

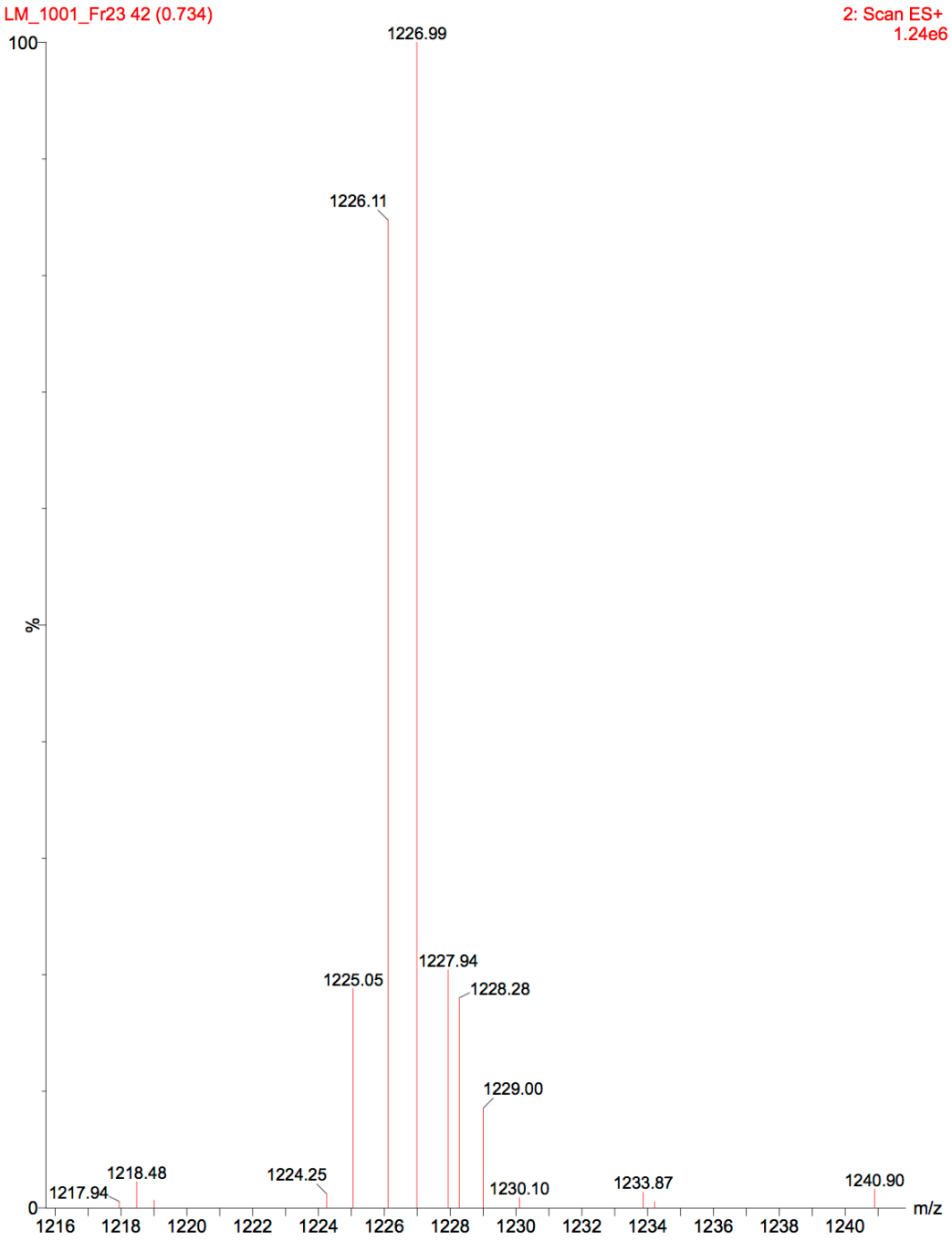


Figure S14 Mass trace of **2b**.

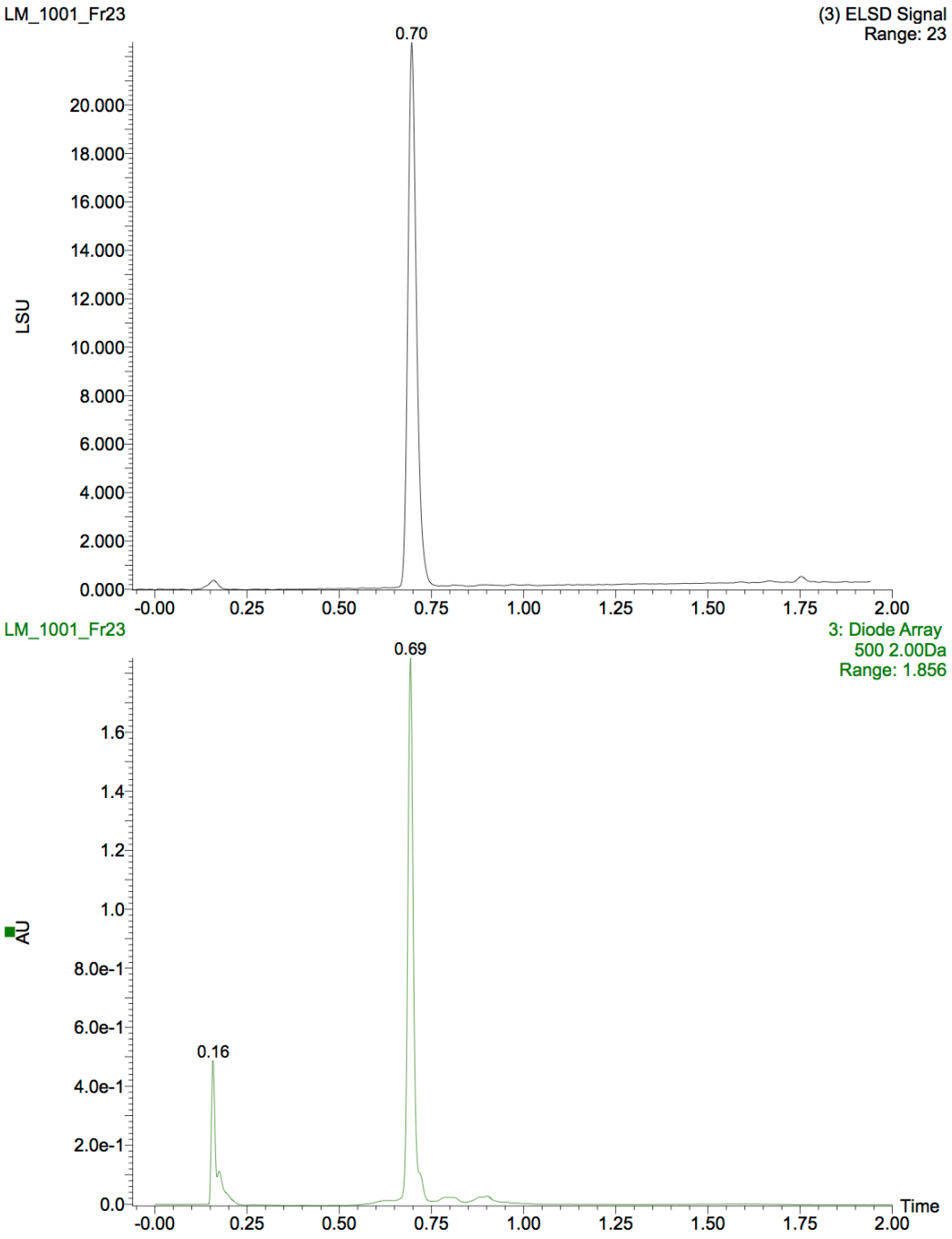


Figure S15 UV/ELSD trace of **2b**.

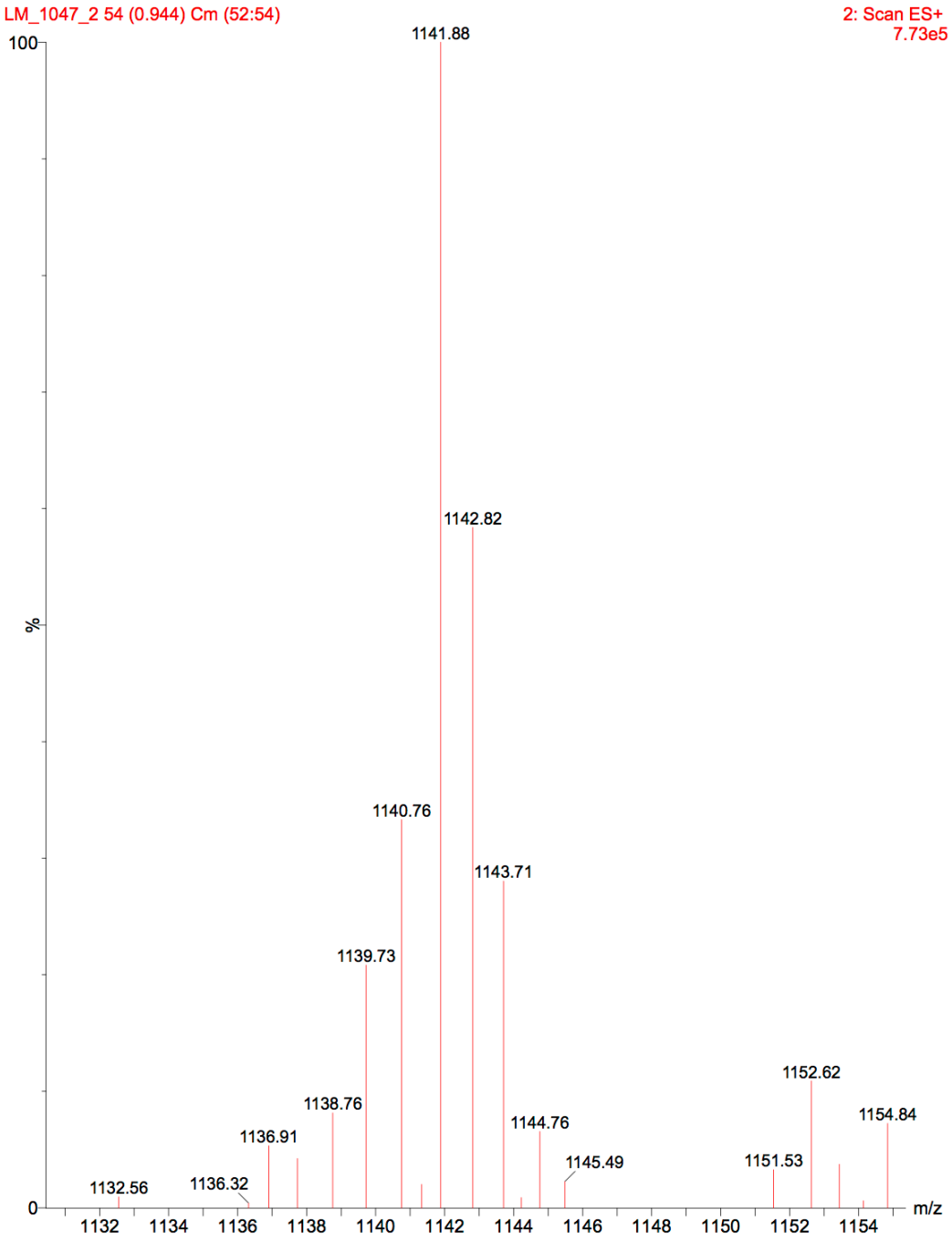


Figure S16 Mass trace of **3a**.

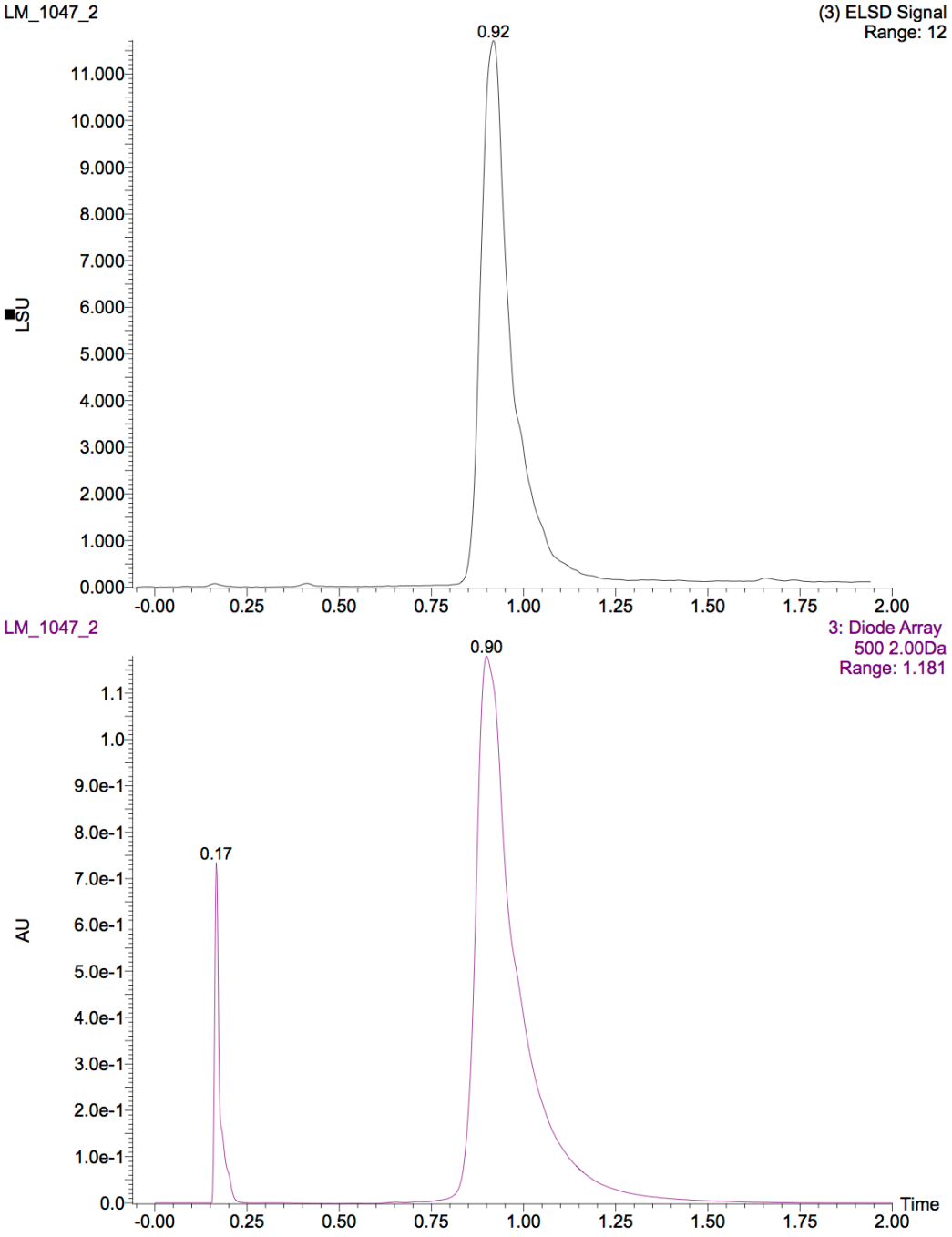


Figure S17 UV/ELSD trace of **3a**.

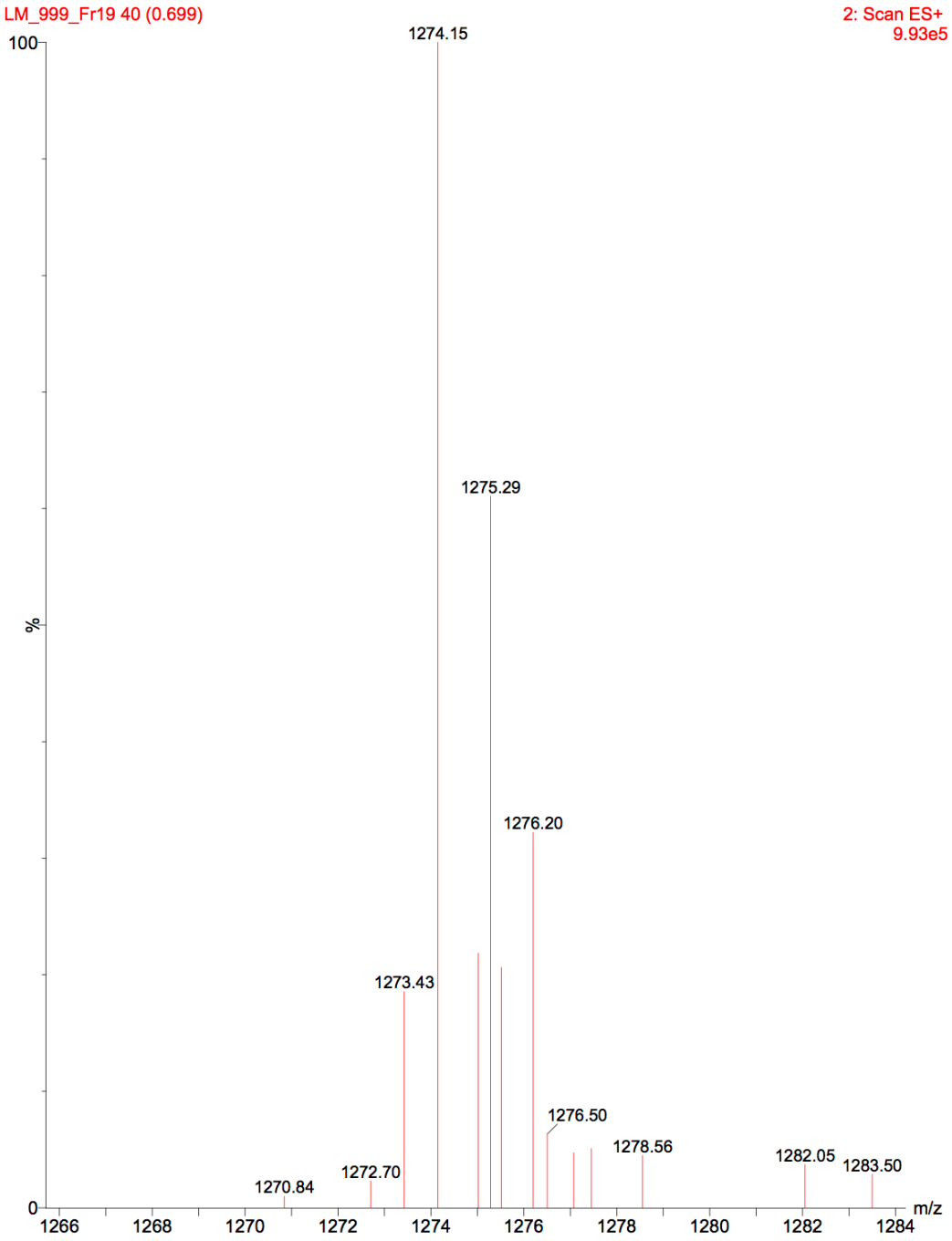


Figure S18 Mass trace of **3b**.

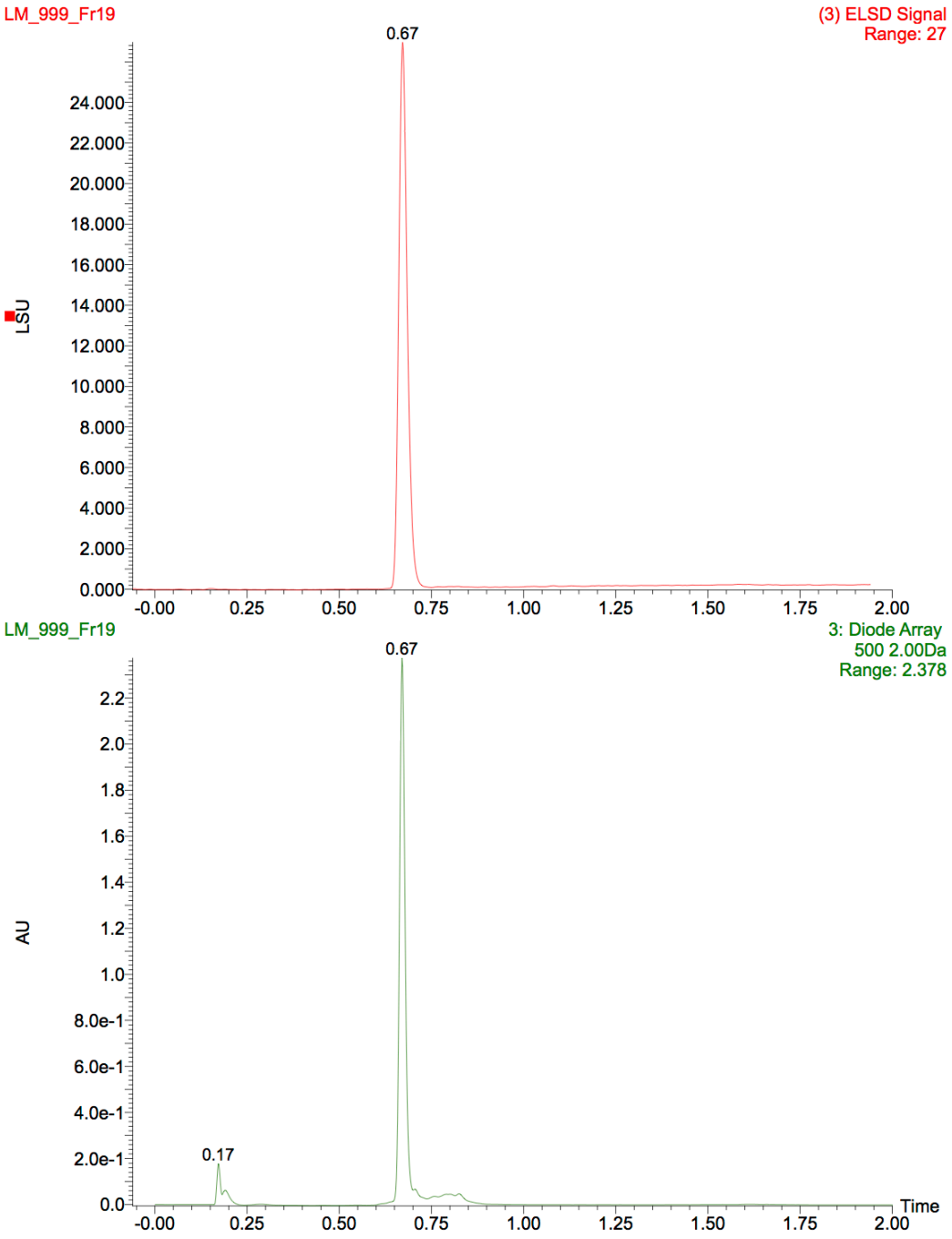


Figure S19 UV/ELSD trace of **3b**.

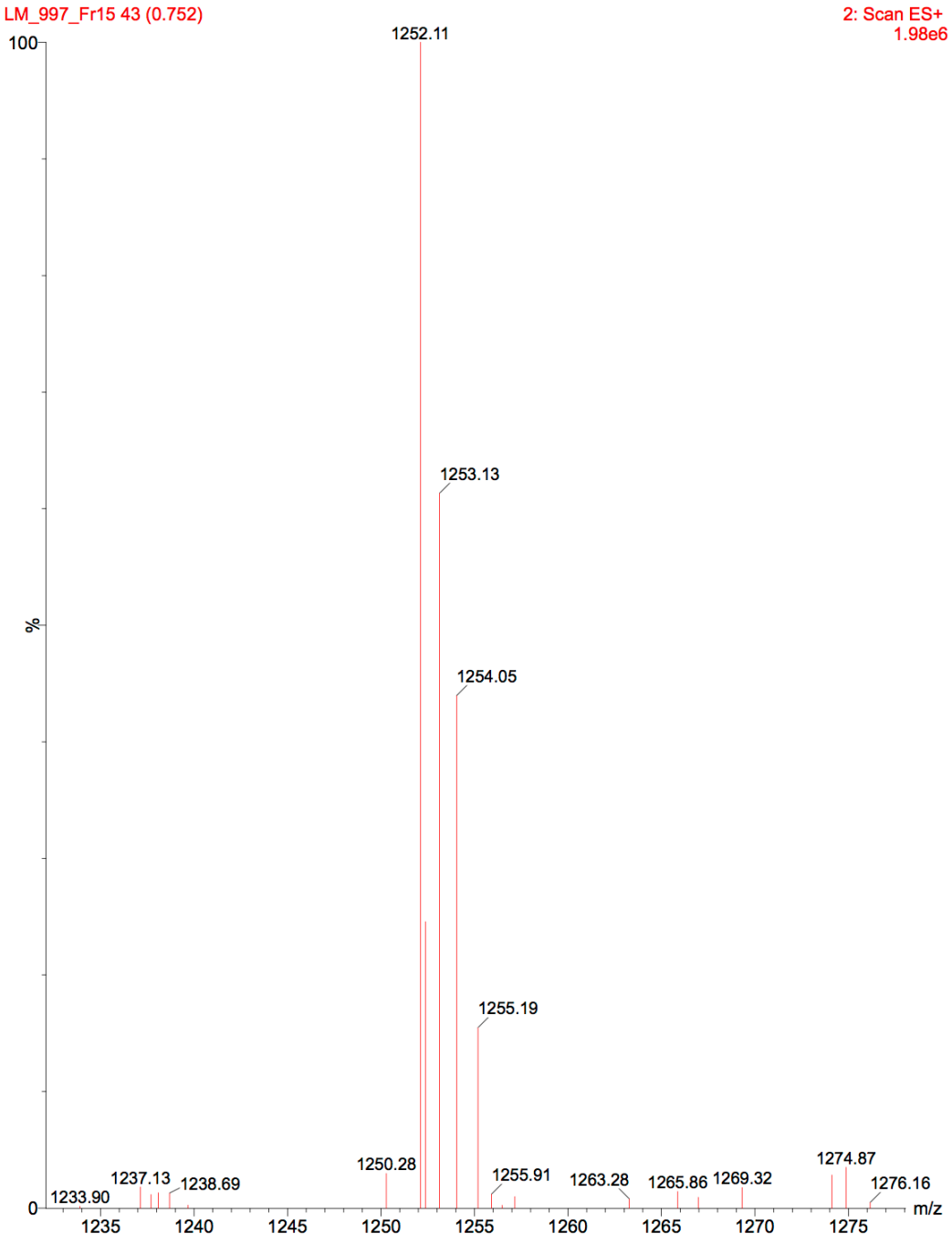


Figure S20 Mass trace of **4a**.

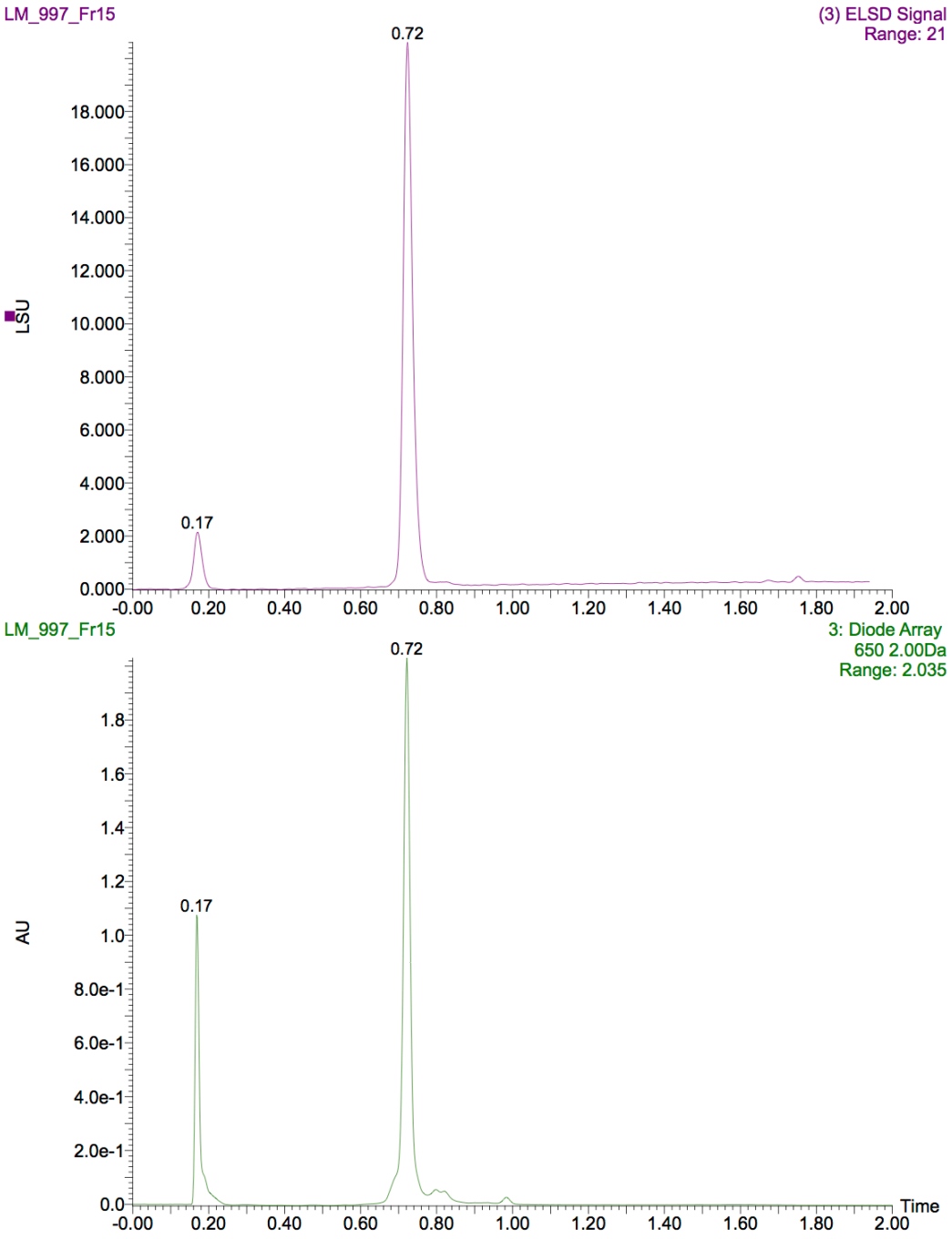


Figure S21 UV/ELSD trace of **4a**.

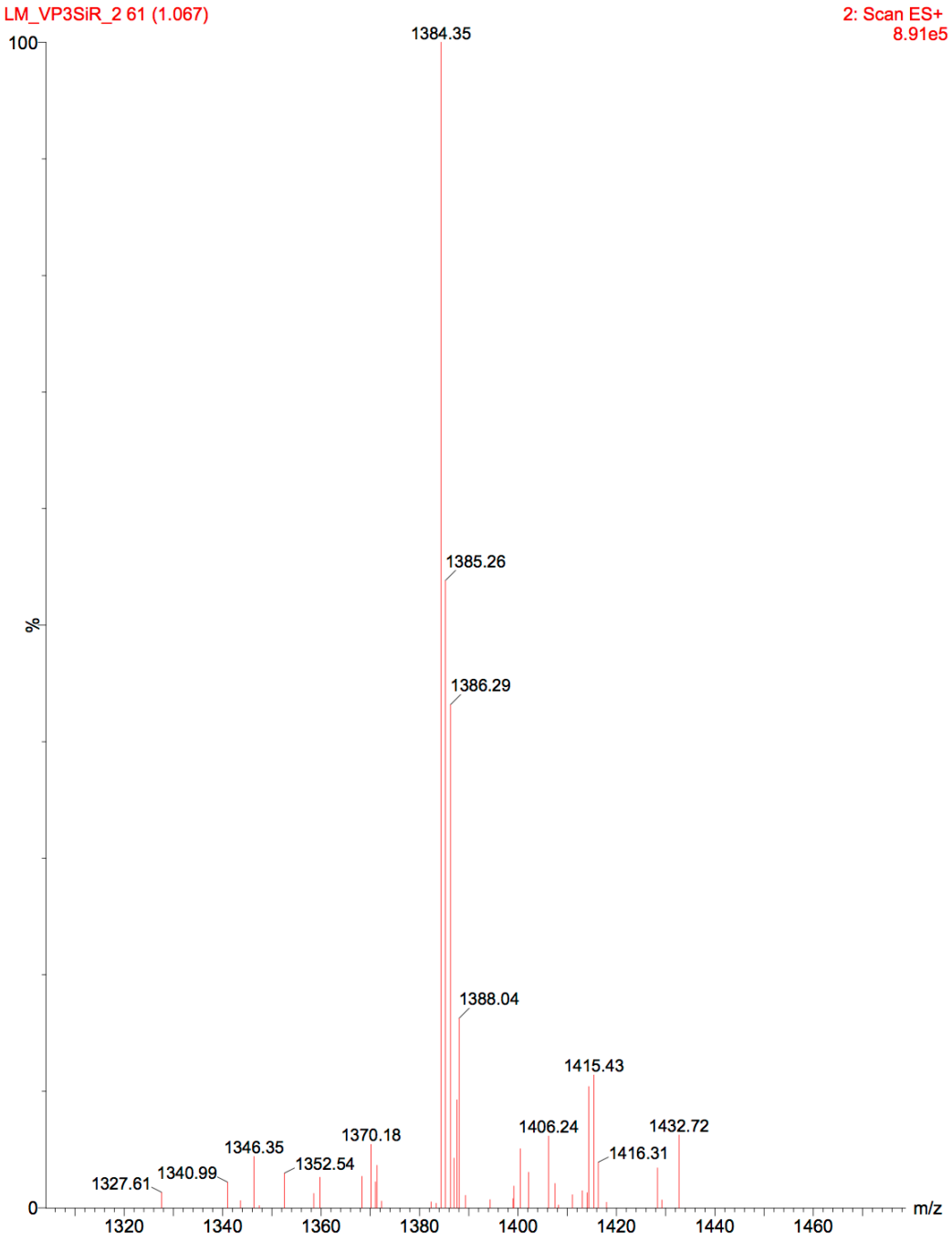


Figure S22 Mass trace of **4b**.

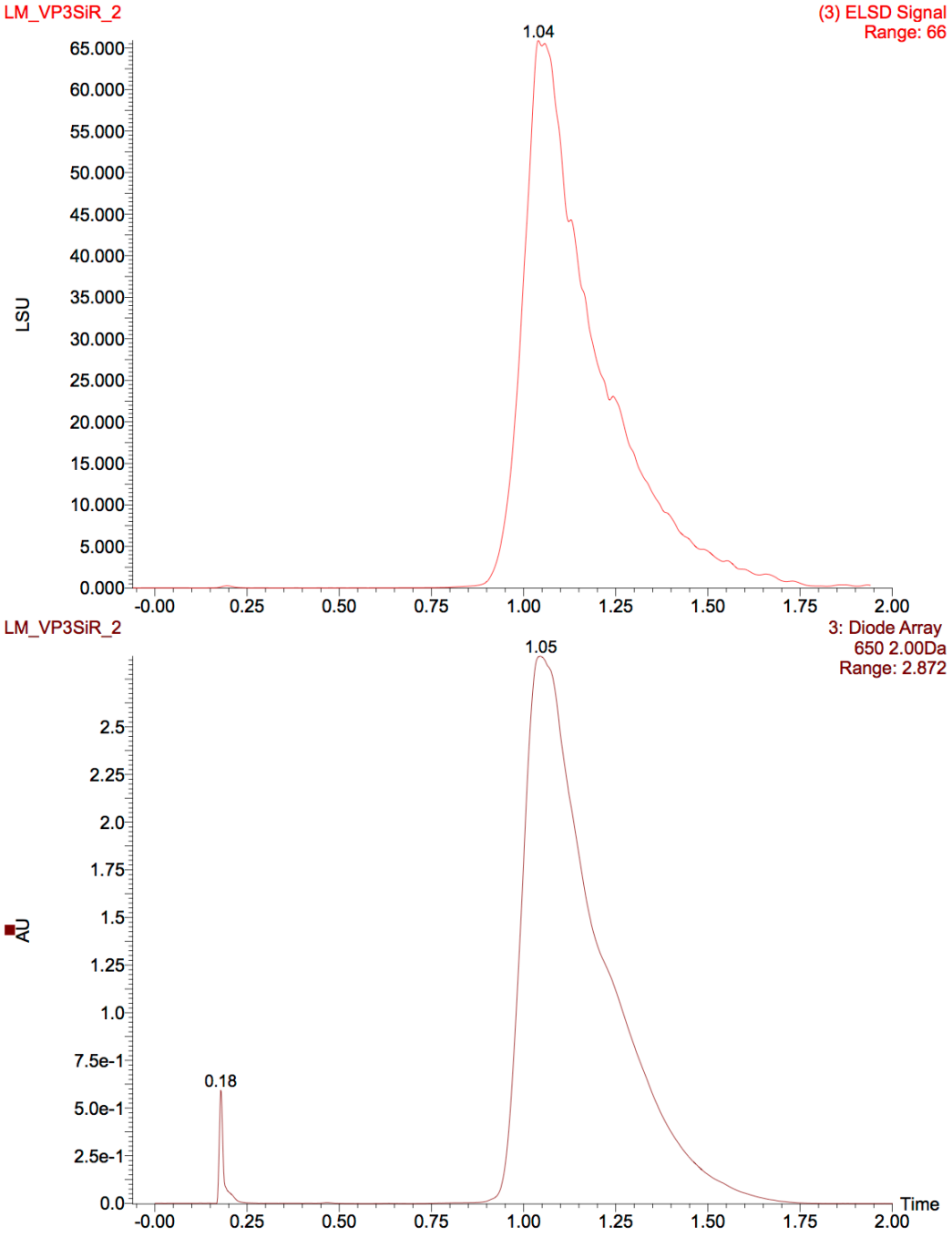


Figure S23 UV/ELSD trace of **4b**.

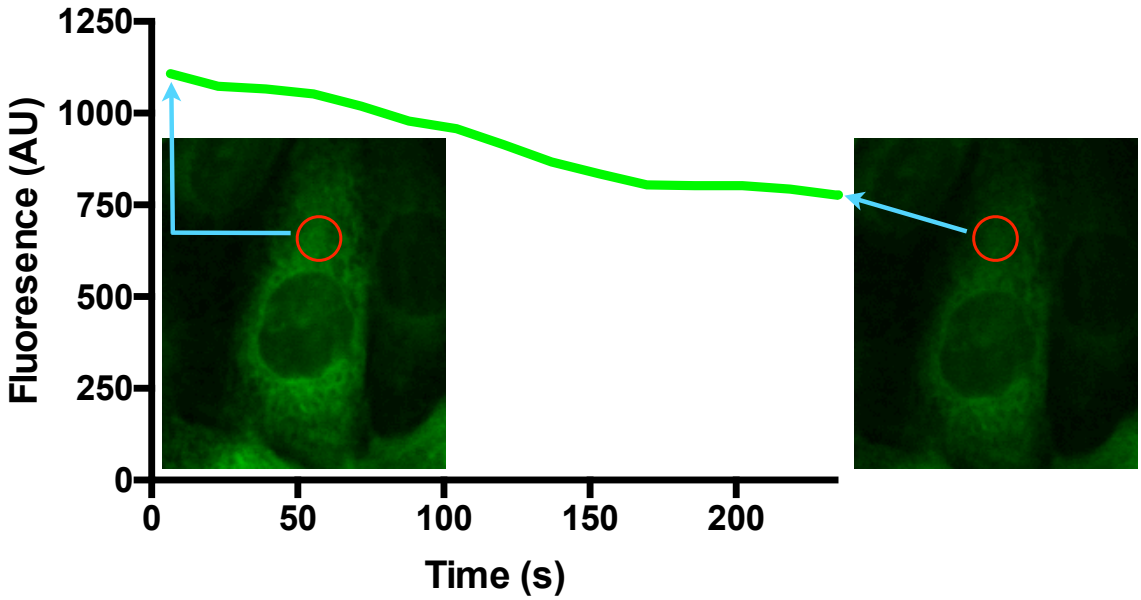
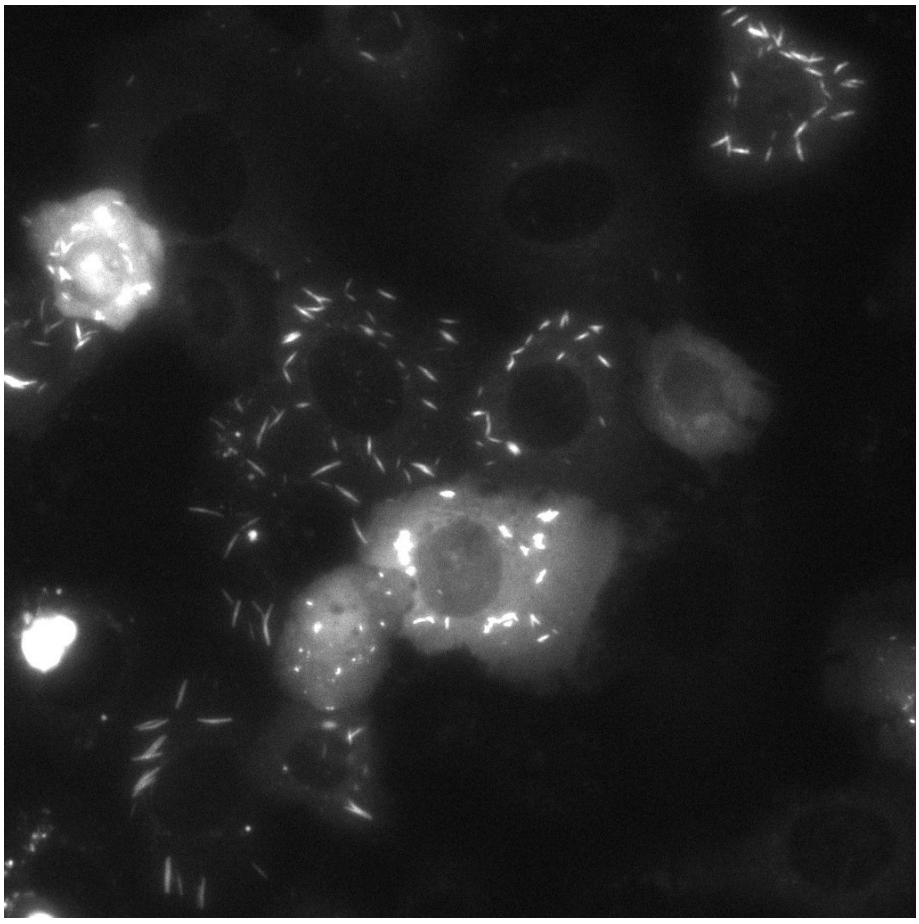


Figure S24 Photostability of probe **2a** in PBS and fixed OVCA429 cells. The ROI fluorescence intensity (red circle) was tracked over time.

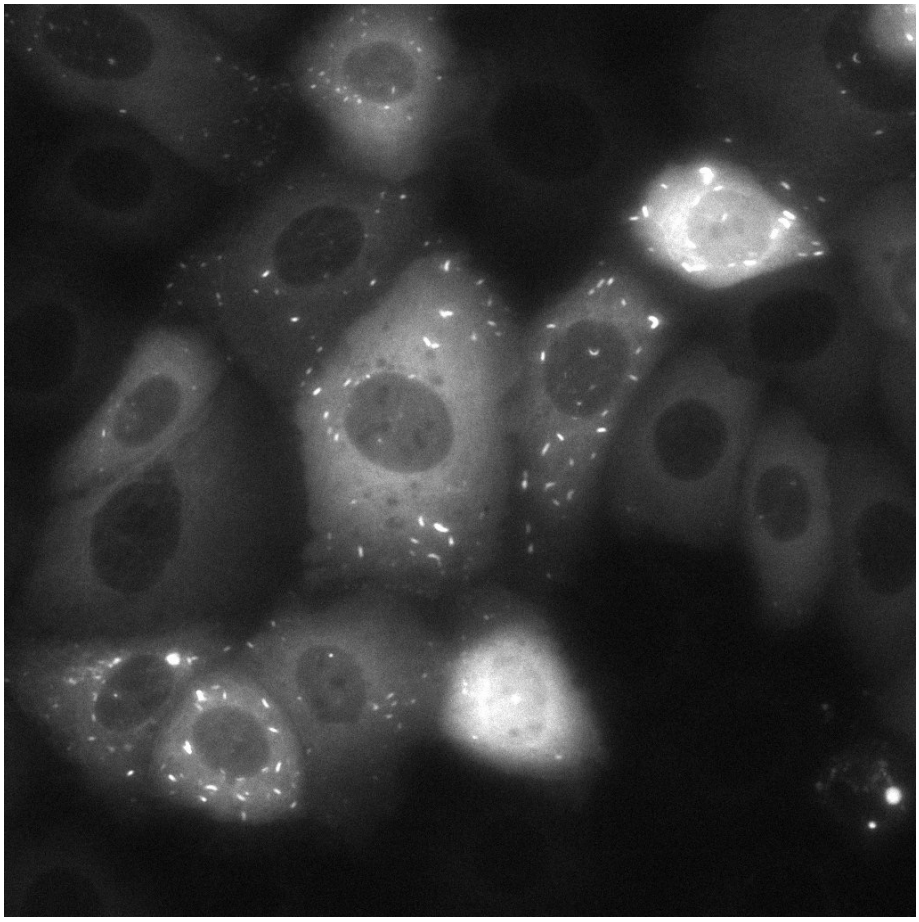
Vinblastine Conjugate Crystal Measurements

Compound 2b:



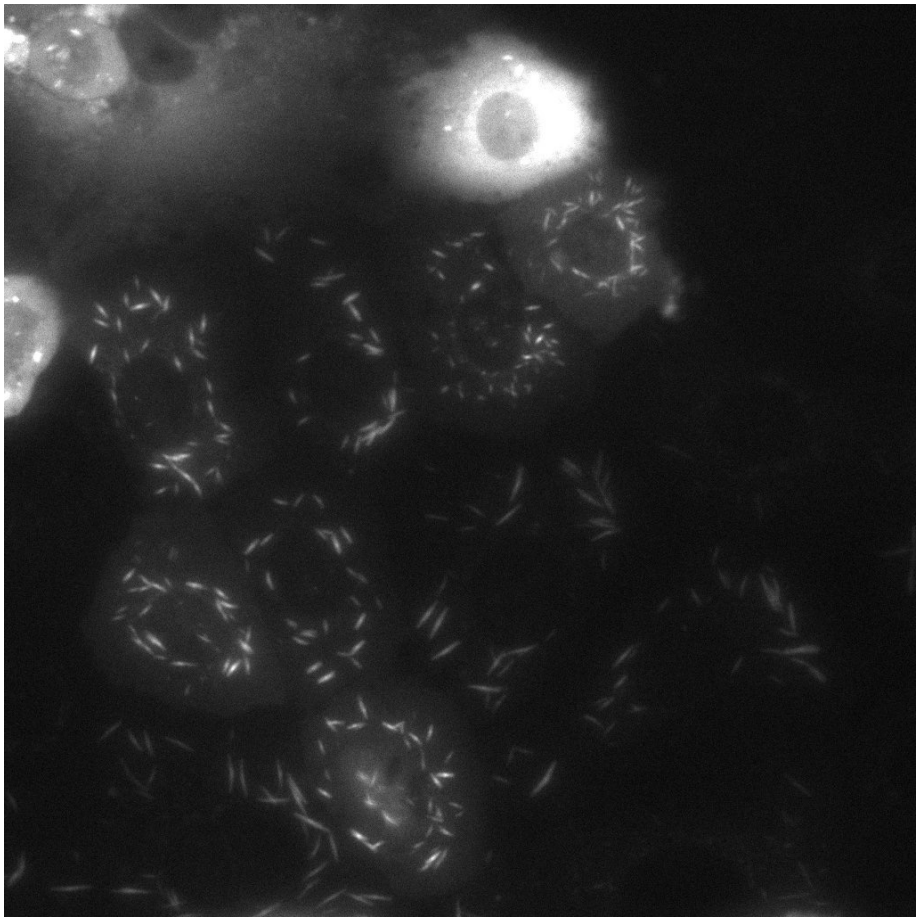
Average paracrystal length: 8.41 μm

Compound 3a:



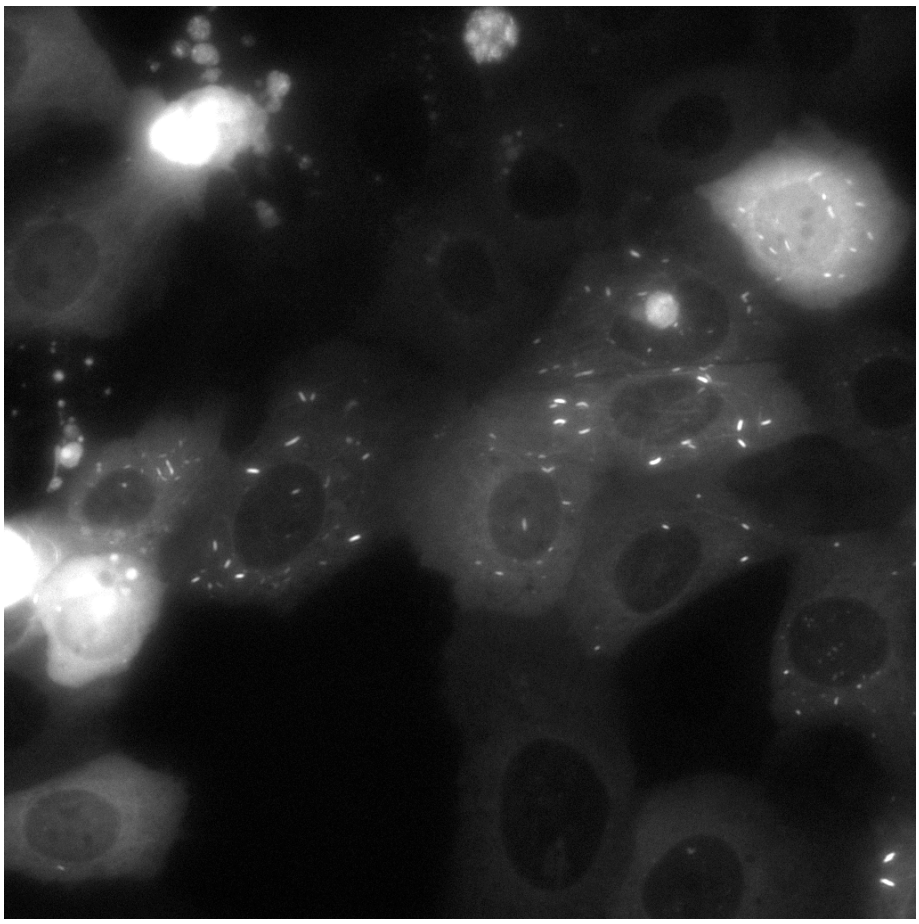
Average paracrystal length: 4.55 μm

Compound 3b:



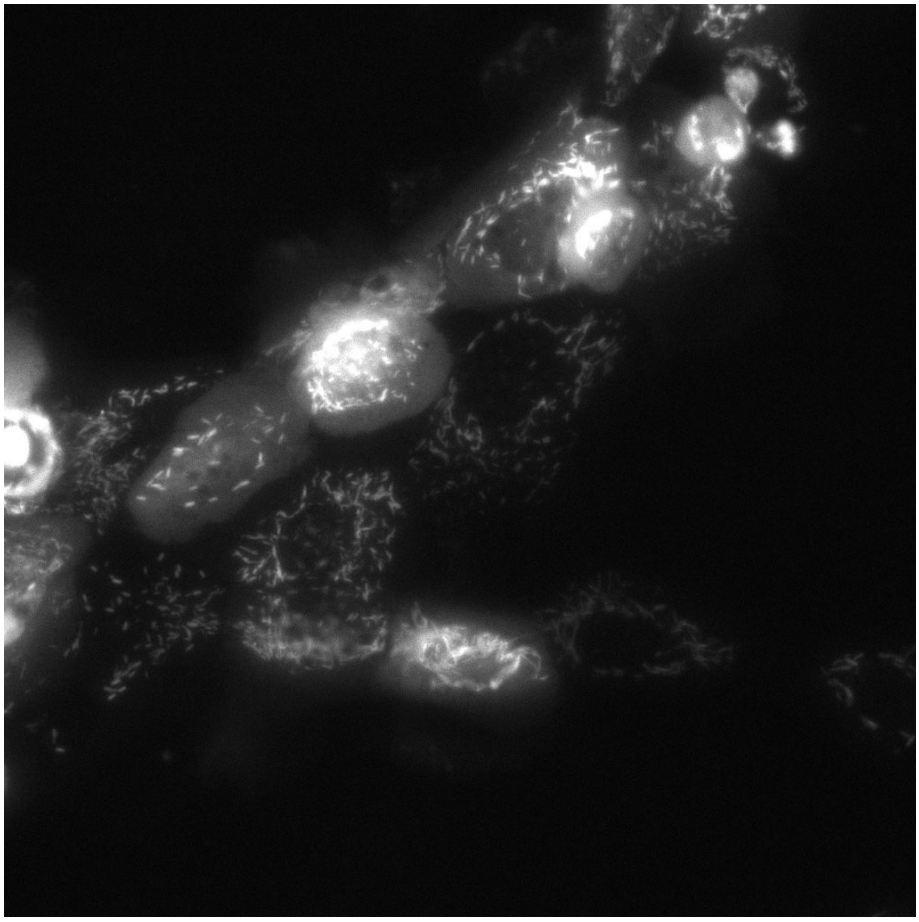
Average paracrystal length: 9.66 μm

Compound 4b:



Average paracrystal length: 4.64 μm

Vinblastine:



Average paracrystal length: 8.44 μM