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Supporting Information

A Thioacetal Photocage Designed for Dual Release: Application in the Quantitation of Therapeutic Release by Synchronous Reporter Decaging

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SUPPORTING INFORMATION

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I. Materials and Analytical Methods

Materials. All reagents and solvents were purchased from commercial suppliers and used as received including paclitaxel (taxol; purity 99%, AvaChem Scientific LLC), doxorubicin (purity 99%, AvaChem Scientific LLC), fluorescein (purity 95%, Sigma-Aldrich), 6-nitroverataldehyde (Sigma-Aldrich) and 4-methyl-7-coumarinol (Sigma-Aldrich). Deuterium-labeled NMR solvents including $CDCl_3$, DMSO-d_6 and CD_3OD were purchased from Sigma-Aldrich. Thin layer chromatography was performed using Merck® TLC plates (250 μm thick). Flash column chromatography was performed using silica gel (200–400 mesh).

Analytical Methods. Structural characterization of photocaged compounds and their synthetic intermediates was performed by standard analytical methods based on mass spectrometry (ESI), high resolution mass spectrometry and NMR (^{1}H, ^{13}C nuclei) spectroscopy. NMR spectroscopy was performed with a Varian nuclear magnetic resonance spectrometer at 500 MHz for ^{1}H NMR spectral acquisition and at 100 MHz for ^{13}C NMR spectral acquisition. Chemical shift values for ^{1}H NMR spectra are reported in ppm with a reference to an internal standard such as tetramethylsilane (TMS) or 4,4-dimethyl-4-silapentane-1-sulfonic acid-d_6 sodium salt (DSS), each at $\delta = 0.00$ ppm, or to residual proton signals from the NMR solvent used. All NMR experiments were performed at 297.3 K using standard default pulse sequences. Mass spectrometric analysis was performed by electrospray ionization mass spectrometry (ESIMS) with a Micromass AutoSpec Ultima Magnetic sector mass spectrometer. Measurements of exact mass were performed with a high resolution VG 70-250-S mass spectrometer by the EI or FAB mode. UV–vis absorption spectra were recorded on a Perkin Elmer Lamda 20 spectrophotometer. Fluorescence emission spectra were acquired with a fluorimeter (ISA-Spex Fluoro Max 2 spectrofluorometer).

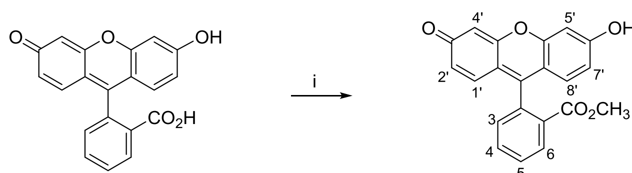
Compound homogeneity was determined by ultrahigh performance liquid chromatography (UPLC) with a Waters Acquity System equipped with a photodiode array detector. Each sample was run through a C4 BEH column (100 × 2.1 mm, 300 Å) at a flow rate of 0.2 mL min^{-1}. The elution method was based on a linear gradient beginning with 99:1 (v/v) water/acetonitrile with TFA (0.1% v/v) (eluent A and B respectively). This initial mobile phase 1% B (0–1.4 min) was linearly increased to 80% B (1.4–13.4 min) followed by a decrease to 50% B (13.4–13.8 min), a

decrease to 1% B (13.8–14.4 min) and isocratic elution at 1% B (14.4–18 min). This UPLC analysis was also performed to determine the kinetics of drug and dye release.

II. Synthesis of TNB linkers (1, 2) and its conjugates (3–5)

Fluorescein Methyl Ester. To a solution of fluorescein carboxylic acid (1.21 g, 3.63 mmol) dissolved in DMSO (5 mL) was added methyl methanesulfonate (400 mg, 3.63 mmol), sodium iodide (0.55 g, 3.63 mmol) and potassium carbonate (1.0 g, 7.26 mmol). The mixture was stirred overnight at room temp, and poured into water (50 mL), which resulted in the formation of yellow precipitant. The material was collected and washed with water. This crude product was purified by flash column chromatography eluting with 6% methanol in dichloromethane. The desired product (fluorescein methyl ester) was obtained as a yellow solid (397 mg, 32%). R_f (2% MeOH/EtOAc) = 0.31. MS (ESI) *m/z* (relative intensity, %) = 347.0 (100) $[M+H]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ 8.29–8.28 (d, J = 5 Hz, 1H, H6), 7.85–7.82 (t, J = 5 Hz, 1H, H4), 7.78–7.75 (t, J = 5 Hz, 1H, H5), 7.42–7.41 (d, J = 5 Hz, 1H, H3), 7.00–6.98 (m, 3H, H1', H8', H5'), 6.74 (s, 1H, H4'), 6.69–6.66 (m, 2H, H2', H7'), 3.61 (s, 3H, CO_2CH_3).

Scheme S1. Synthesis of fluorescein methyl ester ("fluorescein").

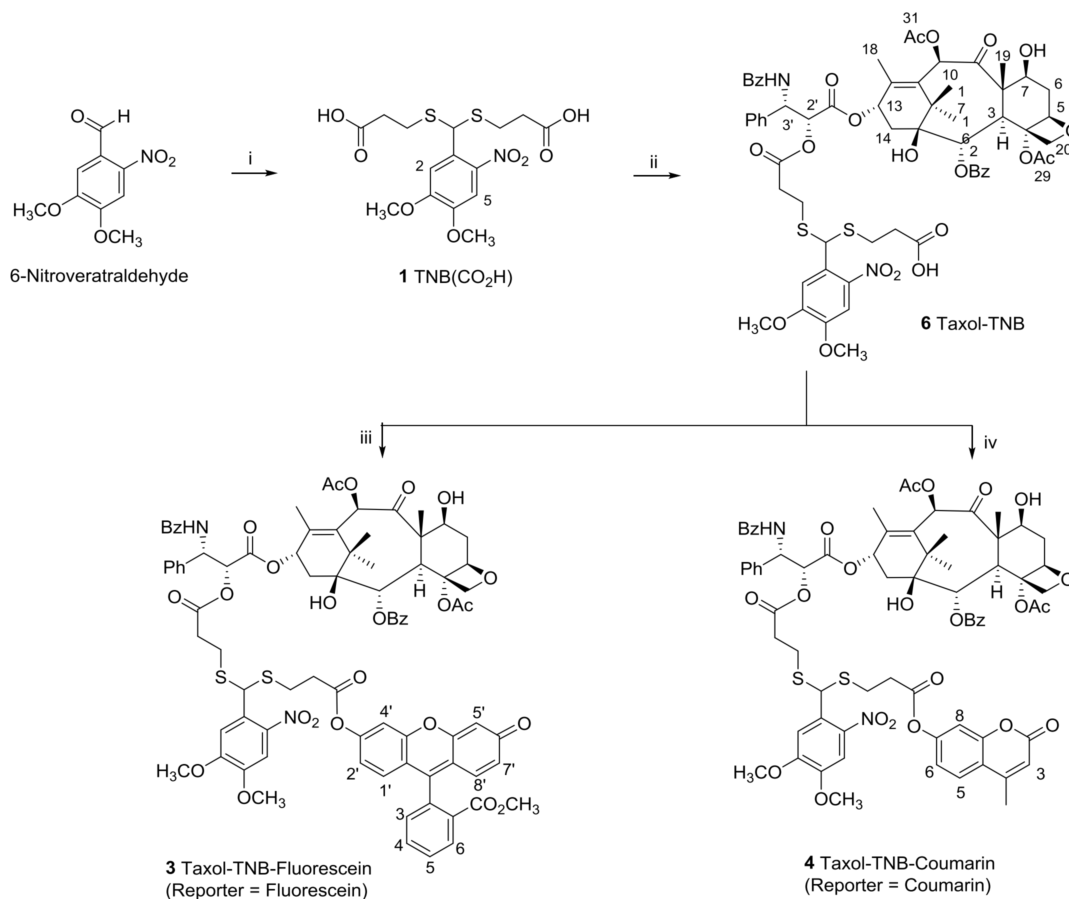


reagents and conditions: i) methyl methanesulfonate, NaI, K2CO3, DMSO, room temp.

1 TNB(CO_2H). To a solution of 4,5-dimethoxy-2-nitrobenzaldehyde (6-nitroveratraldehyde; 350 mg, 1.657 mmol) dissolved in dichloromethane (10 mL) cooled with an ice bath was added 3-mercaptopropionic acid (347 μL, 3.98 mmol), acetic acid (227 μL, 3.98 mmol) and $BF_3 \cdot Et_2O$ (245 μL, 1.99 mmol). The mixture was stirred at 5°C for 1 h and then warmed to room temp. A second portion of 3-mercaptopropionic acid (347 μL, 3.977 mmol) and $BF_3 \cdot Et_2O$ (245 μL, 1.99 mmol) was added and stirred for 2 h. A saturated $NaHCO_3$ solution (10 mL) was added and the mixture was stirred for 2 h. The solution was then acidified with 0.1M HCl (10 mL), resulting in precipitation of a white solid material. It was collected by filtration through filter paper and washed with 0.01 M HCl and followed by water. After drying *in vacuo*, the desired product **1**

was obtained as white solid (596 mg, 89%). R_f (5% methanol/ethyl acetate) = 0.2. MS (ESI) *m/z* (relative intensity, %) = 428.0 (15) $[M+Na]^+$, 300.0 (100). ^{1}H NMR (500 MHz, CD_3OD): δ 7.57 (s, 1H, H5), 7.49 (s, 1H, H2), 6.02 (s, 1H, ArCH), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 2.91–2.86 (m, 2H, SCH_2), 2.81–2.76 (m, 2H, SCH_2), 2.63–2.54 (m, 4H, CH_2CO_2H) ppm. UV–vis (1% aq MeOH): λ_{max} = 346 (ε = 5,950 $M^{-1}cm^{-1}$), 248 (ε = 16,343 $M^{-1}cm^{-1}$) nm.

Scheme S2. Synthesis of TNB(CO_2H) **1** and its conjugates **3**–**4**.



reagents and conditions: i) 3-Mercaptopropionic acid, $BF_3{\cdot}Et_2O$, cat. AcOH, CH_2Cl_2, 0 °C to room temp; ii) Taxol, DCC, DPTS, CH_2Cl_2, 0 °C to room temp; iii) Fluorescein methyl ester, DCC, DPTS, CH_2Cl_2, 0 °C to room temp ; iv) 4-Methyl-7-hydroxycoumarin, DCC, DPTS, CH_2Cl_2, 0 °C to room temp.

6 Taxol-TNB. To a solution of **1** TNB(CO_2H) (14.3 mg, 0.0353 mmol) and taxol (20 mg, 0.0232 mmol) in a mixture of dichloromethane (3 mL) and DMF (1 mL) cooled with an ice bath was added 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS; 3.5 mg, 0.0119 mmol) and then dicyclohexyldiimide (DCC; 28 μL; 1 M in CH_2Cl_2). The reaction mixture was gradually warmed to room temp and stirred for 3 h. Analysis by thin layer chromatography (TLC)

indicated formation of a product (R_f = 0.31 in 5% MeOH/CH_2Cl_2; R_f = 0.31 in 5% MeOH/EtOAc). After concentration *in vacuo*, the product was isolated by flash column chromatography by elution with 2% methanol/dichloromethane, yielding the product as a pale yellow solid (20 mg, 69%). R_f (5% MeOH/CH_2Cl_2) = 0.31. MS (ESI) *m/z* (relative intensity, %) = 1264.0 (40) $[M+Na]^+$**,** 553.7 (100). HRMS (ESI) calcd for $C_{62}H_{68}N_2O_{21}S_2$ $[M+H]^+$ 1241.3829, found 1241.3783. ^1H NMR (500 MHz, $CDCl_3$): δ 8.16–8.13 (m, 2H, ArH (taxol)), 7.79–7.75 (m, 2H, 2ArH (taxol)), 7.63–7.60 (m, 1H, ArH (taxol)), 7.55–7.38 (m, 10H, 8ArH (taxol) and H2, H5 (TNB)), 7.35–7.28 (m, 2H, ArH (taxol)), 6.30 and 6.29 (s, 1H, H10 (taxol)), 6.13 and 6.11 (s, 1H, CHS (TNB)), 6.05–5.96 (m, 1H, H13 (taxol)), 5.70–5.62 (m, 2H, H3 and H2 (taxol)), 5.00–4.96 (m, 1H, H5 (taxol)), 4.45–4.17 (m, 2H, H2 and H7 (taxol)), 4.00 and 3.98 (s, 2H, H20 (taxol)), 3.94 (s, 3H, OCH_3 (TNB)), 3.94 (s, 3H, OCH_3 (TNB)), 3.80–3.77 (m, 1H, H3 (taxol)), 3.16–2.55 (m, 10H, H14 (taxol), two SCH_2 and two CH_2CO), 2.52 and 2.51 (s, 3H, H29 (taxol)), 2.39–2.34 (m, 1H, 0.5 H6 (taxol)), 2.24 and 2.23 (s, 3H, H31 (taxol)), 2.02–1.97 (m, 1H, 0.5 H6 (taxol)), 1.94 and 1.91 (s, 3H, H18 (taxol)), 1.69 and 1.67 (s, 3H, H19 (taxol)), 1.24 and 1.21 (s, 3H, H16 (taxol)), 1.14 and 1.12 (s, 3H, H17 (taxol)) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): 203.68, 187.96, 174.44, 171.24, 170.93, 170.39, 168.58, 167.46, 166.90, 153.50, 148.35, 142.51, 140.10, 136.64, 133.68, 133.53, 133.41, 132.77, 131.86, 130.18, 129.84, 129.12, 128.98, 128.68, 128.61, 128.53, 127.25, 127.22, 126.82, 126.62, 111.64, 107.65, 84.36, 81.18, 81.06, 78.95, 76.32, 75.57, 74.97, 74.38, 74.05, 72.00, 71.90, 58.42, 58.36, 56.54, 56.38, 53.41, 53.10, 47.48, 45.60, 43.11, 43.07, 35.46, 35.32, 34.48, 34.09, 33.96, 27.80, 27.58, 27.38, 26.72, 22.67, 22.56, 21.99, 20.81, 19.28, 14.78, 12.72, 9.56 ppm.

3 Taxol-TNB-Fluorescein. To a solution of fluorescein methyl ester (fluorescein; 2.4 mg, 7.01 μmol) and **6** Taxol-TNB (5.8 mg, 4.67 μmol) dissolved in a mixture of dichloromethane (1 mL) and DMF (0.5 mL) was added 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS; 0.5 mg, 1.70 μmol) and DCC (7 μL; 1 M in CH_2Cl_2). The reaction mixture was stirred for 6 h at room temp. The mixture was concentrated *in vacuo*, and purified by flash column by elution with MeOH/CH_2Cl_2 (2 to 5%). The isolated product was further purified by a second round of flash column chromatography by elution with 1% methanol/ethyl acetate. The product was obtained as a yellow solid (6.5 mg, 89%). R_f = 0.74 (2% MeOH/EtOAc). MS (ESI) *m/z* (relative intensity, %) = 1569.6 (84) $[M+H]^+$**,** 347.1 (100). HRMS (ESI) calcd for $C_{83}H_{80}N_2O_{25}S_2$ $[M+H]^+$

1569.4564, found 1569.4542. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28–8.26 (m, 1H, H6 (fluorescein), 8.14–8.13 (m, 2H, ArH (taxol)), 7.80–7.77 (m, 1H, H4 (fluorescein), 7.76–7.74 (m, 2H, 2ArH (taxol)), 7.72–7.68 (m, 1H, H5 (fluorescein), 7.63–7.60 (m, 1H, ArH (taxol)), 7.53–7.28 (m, 13H, 10ArH (taxol), H2, H5 (TNB) and H3 (fluorescein)), 7.16–6.90 (m, 4H, H1', H8', H4', H5' (fluorescein)), 6.72–6.56 (m, 2H, H2', H7' (fluorescein)), 6.30–6.27 (m, 1H, H10 (taxol)), 6.12 and 6.10 (s, 1H, CHS), 5.99–5.94 (m, 1H, H13 (taxol)), 5.68–5.66 (m, 1H, H3), 5.54–5.52 (m, 1H, H2 (taxol)), 4.96–4.94 (m, 1H, H5 (taxol)), 4.43–4.40 (m, 1H, H7 (taxol)), 4.31–4.29 (m, 1H, H2 (taxol)), 3.98–3.92 (m, 8H, H20 (taxol) and two OCH_3 (TNB)), 3.79–3.78 (m, 1H, H3 (taxol)), 3.65 (s, 3H, CO_2CH_3 (fluorescein)), 3.06–2.47 (m, 10H, H14 (taxol), two SCH_2 and two CH_2CO), 2.44–2.41 (m, 3H, H29 (taxol)), 2.36–2.26 (m, 1H, 0.5 H6 (taxol)), 2.23–2.21 (m, 3H, H31 (taxol)), 1.92–1.91 (m, 3H, H18 (taxol)), 1.89–1.84 (m, 1H, 0.5 H6 (taxol)), 1.69–1.67 (m, 3H, H19 (taxol)), 1.22–1.20 (m, 3H, H16 (taxol)), 1.14–1.12 (m, 3H, H17 (taxol)) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 203.79, 171.23, 169.26, 167.91, 167.06, 142.75, 136.92, 133.70, 132.91, 132.75, 131.92, 131.30, 130.51, 130.23, 130.06, 129.68, 129.18, 129.06, 129.00, 128.73, 128.64, 128.49, 127.25, 127.19, 126.69, 119.52, 119.07, 111.48, 111.42, 107.81, 107.73, 105.92, 84.39, 81.00, 79.15, 76.42, 75.57, 75.06, 74.45, 72.10, 71.82, 60.38, 58.49, 56.61, 56.45, 52.53, 47.71, 45.56, 43.15, 35.51, 34.68, 34.29, 27.86, 26.78, 22.72, 22.11, 21.04, 20.82, 14.80, 14.19, 9.58 ppm. UV–vis (20% aq MeOH): λ_{max} = 456 (ε = 19,793 $M^{-1}cm^{-1}$), 353 (ε = 17,700 $M^{-1}cm^{-1}$), 230 (ε = 82,289 $M^{-1}cm^{-1}$) nm.

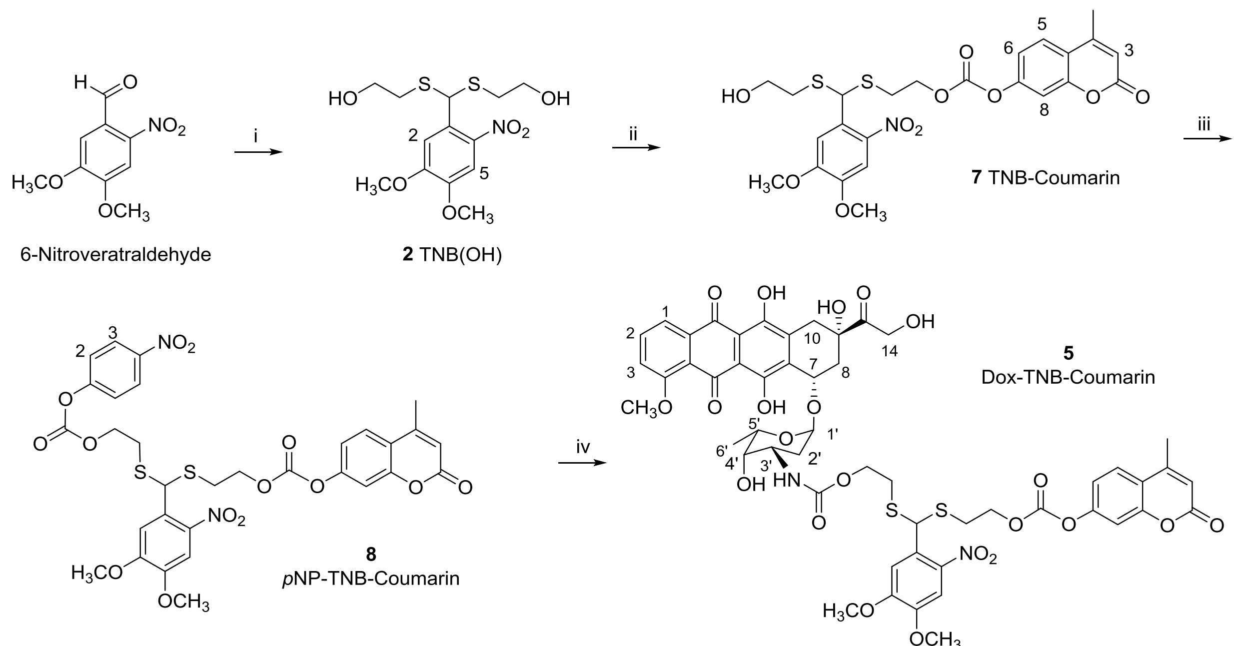
4 Taxol-TNB-Coumarin. To a solution of **6** Taxol-TNB (20 mg, 0.0161 mmol) and 4-methyl-7-hydroxycoumarin (3.4 mg, 0.0193 mmol) in a mixture of dichloromethane (1.5 mL) and DMF (0.5 mL) cooled with an ice bath was added 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS; 2.4 mg, 0.00815 mmol) and then dicyclohexylcarbodiimide (DCC; 19 μL; 1 M in CH_2Cl_2). The reaction mixture was warmed to room temp and stirred overnight. The mixture was concentrated *in vacuo*, and treated with ethyl acetate (3 mL), resulting in precipitation. Precipitate was filtered and washed with ethyl acetate (2 mL). The filtrate was concentrated and the residue was purified by flash column chromatography (8 g of silica gel) by elution with ethyl acetate/hexane (1/1 to 3/2). The desired product **4** was isolated as a pale yellow solid (11.5 mg, 51%). R_f (2:1 ethyl acetate/hexane) = 0.34. MS (ESI) *m/z* (relative intensity, %) = 1400.0 (100) $[M+H]^+$. HRMS (ESI) calcd for $C_{72}H_{74}N_2O_{23}S_2$ $[M+H]^+$ 1399.4203, found 1399.4145. ^{1}H NMR

(500 MHz, $CDCl_3$): δ 8.15–8.13 (m, 2H, ArH (taxol)), 7.78–7.72 (m, 2H, 2ArH (taxol)), 7.63–7.60 (m, 1H, ArH (taxol)), 7.57–7.28 (m, 13H, 10ArH (taxol), H2, H5 (TNB) and H5 (coumarin)), 7.12–7.07 (m, 1H, H8 (coumarin)), 7.05–7.02 (m, 1H, H6 (coumarin)), 6.28–6.22 (m, 2H, H3 (coumarin) and H10 (taxol)), 6.13 and 6.10 (s, 1H, SCH (TNB)), 6.00–5.94 (m, 1H, H13 (taxol)), 5.69–5.66 (m, 1H, H3' (taxol)), 5.53 and 5.52 (s, 1H, H2 (taxol)), 4.97–4.95 (m, 1H, H5 (taxol)), 4.32–4.30 (m, 1H, H2' (taxol)), 4.21–4.18 (m, 1H, H7 (taxol)), 3.97 and 3.93 (s, 2H, H20 (taxol)), 3.96 (s, 3H, OCH_3 (TNB)), 3.94 (s, 3H, OCH_3 (TNB)), 3.80–3.78 (m, 1H, H3 (taxol)), 3.03–2.72 (m, 8H, H14 (taxol), two SCH_2 and CH_2CO), 2.58–2.46 (m, 2H, CH_2CO), 2.44–2.40 (m, 6H, H29 (taxol) and CH_3 (coumarin)), 2.39–2.30 (m, 1H, 0.5 H6 (taxol)), 2.23 and 2.22 (s, 3H, H31 (taxol)), 2.20–2.06 (m, 1H, 0.5 H6 (taxol)), 1.92 (s, 3H, H18 (taxol)), 1.68 and 1.67 (s, 3H, H19 (taxol)), 1.22–1.20 (m, 3H, H16 (taxol)), 1.13–1.12 (m, 3H, H17 (taxol)) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 220.75, 216.57, 203.82, 174.45, 169.81, 151.92, 144.13, 142.78, 136.89, 133.43, 132.73, 130.22, 128.98, 128.71, 128.60, 128.39, 127.20, 127.15, 126.63, 125.46, 117.89, 114.57, 111.38, 110.31, 107.78, 84.41, 81.00, 79.11, 75.57, 75.04, 74.15, 72.11, 58.47, 56.58, 56.43, 47.17, 45.51, 43.15, 37.26, 34.57, 33.21, 30.02, 27.80, 26.76, 22.65, 22.13, 20.82, 19.30, 19.29, 18.70, 14.81, 9.57 ppm. UV–vis (20% aq MeOH): λ_{max} = 360 (ε = 3,546 $M^{-1}cm^{-1}$), 313 (ε = 8,067 $M^{-1}cm^{-1}$) nm.

2 TNB(OH). To a solution of 4,5-dimethoxy-2-nitrobenzaldehyde (6-nitroveratraldehyde; 1.4 g, 6.63 mmol) dissolved in dichloromethane (40 mL) cooled with an ice bath was added 2-mercaptoethanol (1.12 mL, 15.91 mmol), acetic acid (0.91 ml, 15.91 mmol) and $BF_3·Et_2O$ (0.98 mL, 7.96 mmol). The mixture was stirred at 5 °C for 1 h and warmed to room temp. A second portion of 2-mercaptoethanol (1.12 mL, 15.91 mmol) and $BF_3·Et_2O$ (0.98 mL, 7.96 mmol) was added and stirred for 2 h. The mixture was diluted with dichloromethane (40 mL), and a saturated $NaHCO_3$ solution (40 mL) was added. After stirring for 1 h, the organic layer was separated and saved. The aqueous layer was extracted with dichloromethane (40 mL × 2). The organic layers were combined, washed with brine (40 mL), and dried over Na_2SO_4. After concentration *in vacuo*, the residue was purified by flash column chromatography eluting with 3% methanol/dichloromethane. The desired product **2** was obtained as a pale yellow solid (1.76, 76%). R_f (5% MeOH/CH_2Cl_2) = 0.38. MS (ESI) *m/z* (relative intensity, %) = 372.0 (10) $[M+Na]^+$**,** 212.0 (100). ^1H NMR (500 MHz, CD_3OD): δ 7.55 (s, 1H, H5), 7.50 (s, 1H, H2), 6.09

(s, 1H, CHS), 3.95 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.68–3.66 (t, J = 7 Hz, 4H, $2CH_2OH$), 2.83–2.78 (m, 2H, SCH_2), 2.70–2.65 (m, 2H, SCH_2) ppm. UV–vis (1% aq MeOH): λ_{max} = 346 (ε = 4,292 $M^{-1}cm^{-1}$), 248 (ε = 11,560 $M^{-1}cm^{-1}$) nm.

Scheme S3. Synthesis of TNB(OH) **2** and Dox-TNB-Coumarin **5**



reagents and conditions: i) 2-Mercaptoethanol, $BF_3 \cdot Et_2O$, cat. AcOH, CH_2Cl_2, 0 °C to room temp; ii) 7-Methyl-4-coumarinol, triphosgene, Et_3N, CH_2Cl_2; iii) 4-Nitrophenyl chloroformate, DIPEA, CH_2Cl_2; iv) Doxorubicin HCl, DIPEA, DMF.

7 TNB-Coumarin. To a solution of dichloromethane (2 ml) containing 4-methyl-7-coumarinol (50.0 mg, 0.284 mmol) and DIPEA (247 μL, 1.42 mmol) cooled with an ice bath was added triphosgene (28 mg, 0.09460 mmol). The mixture was stirred at 5 °C for 10 min, and at room temp for 3 h. A solution of **2** TNB(OH) (99.0 mg, 0.284 mmol) was then prepared in a separate glass container in dichloromethane (3 mL) containing Et_3N (198 μL, 1.42 mmol). To this TNB(OH) solution was added the triphosgene-treated coumarinol. The final mixture was stirred at room temp overnight. The mixture was diluted by adding 5 mL of dichloromethane, and the solution was washed with water, 0.5 M HCl, and a saturated $NaHCO_3$ solution, and brine. After drying over Na_2SO_4, the solution was evaporated *in vacuo* and the residue was purified by flash column chromatography by eluting with 1% MeOH in dichloromethane. The product **7** was obtained as a pale yellow foam (43.0 mg, 28%). R_f (3% $MeOH/CH_2Cl_2$) = 0.36. MS (ESI) m/z (relative intensity, %) = 573.9 (100) $[M+Na]^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 7.63–7.61 (d, J =

10 Hz, 1H, H5 (coumarin)),7.52 (s, 1H, H5 (TNB)), 7.47 (s, 1H, H2 (TNB)), 7.24–7.23 (d, *J* = 5 Hz, 1H, H8 (coumarin)), 7.20–7.18 (dd, *J* = 5 Hz, *J* = 10 Hz, 1H, H6 (coumarin)), 6.29–6.28 (d, *J* = 5 Hz, 1H, H3 (coumarin)), 6.23 (s, 1H, SCH (TNB)), 4.49–4.42 (m, 2H, CH_2O), 4.02 (s, 3H, OCH_3 (TNB)), 3.95 (s, 3H, OCH_3 (TNB)), 3.84–3.80 (m, 2H, CH_2O), 3.08–3.02 (m, 1H, CH_2S), 2.95–2.86 (m, 2H, CH_2S), 2.80–2.74 (m, 1H, CH_2S), 2.44 (s, 3H, CH_3 (coumarin)) ppm.

8 *p*NP-TNB-Coumarin. To a solution of 4-nitrophenyl chloroformate (12.6 mg, 0.0627 mmol) in THF (1.5 mL) was added a solution of **7** TNB-Coumarin (33.0 mg, 0.0599 mmol) dissolved in a mixture of $CHCl_3$ (1.5 mL) and *N,N*-diisopropylethylamine (22 μL, 0.126 mmol). The mixture was stirred at room temp overnight, followed by addition of a second portion of 4-nitropheyl chloroformate (12.6 mg, 0.0627 mmol) and 4-dimethylaminopyridine (DMAP; 7.7 mg, 0.0627 mmol). The mixture was stirred for 4 h, concentrated *in vacuo*, and purified by flash column chromatography by eluting with 0.2% methanol/dichloromethane. The product **8** was obtained as pale yellow wax (34.0 mg, 79%). R_f (2% MeOH/CH_2Cl_2) = 0.42. MS (ESI) *m/z* (relative intensity, %) = 739.1 (100) $[M+Na]^+$, 636.1 (22), 413.2 (22). ^1H NMR (500 MHz, $CDCl_3$): δ 8.25–8.23 (d, *J* = 10 Hz , 2H, H3 (*p*NP), 7.63–7.61 (d, *J* = 10 Hz, 1H, H5 (coumarin)), 7.54 (s, 1H, ArH (TNB)), 7.46 (s, 1H, H5 (TNB)), 7.39–7.37 (d, *J* = 10 Hz , 2H, H2 (*p*NP)), 7.23–8.22 (d, *J* = 5 Hz, 1H, H8 (coumarin)), 7.18–7.16 (dd, *J* = 5 Hz, *J* = 10 Hz, 1H, H6 (coumarin)), 6.31 (s, 1H, H3 (coumarin)), 6.30 (s, 1H, SCH), 4.51–4.42 (m, 4H, two CH_2O), 4.02 (s, 3H, OCH_3 (TNB)), 3.95 (s, 3H, OCH_3 (TNB)), 3.12–3.06 (m, 2H, SCH_2), 2.96–2.90 (m, 2H, SCH_2), 2.45 (s, 3H, CH_3 (coumarin)) ppm.

5 Dox-TNB-Coumarin. To a solution of **8** *p*NP-TNB-Coumarin (10.0 mg, 0.0140 mmol; Supporting Information) dissolved in DMF (0.4 mL) was added doxorubicin HCl (8.1 mg, 0.0140 mmol) and *N,N*-diisopropylethylamine (1.2 μL, 0.0307 mmol). The mixture was stirred at room temp in the dark for 3 h. It was concentrated to 0.1 mL, and diluted with 6 mL of dichloromethane. This organic solution was washed with 0.5 M HCl, a saturated $NaHCO_3$ solution and a brine solution. The organic solution was dried over Na_2SO_4, concentrated *in vacuo* and purified by flash column chromatography by eluting with 1% methanol in dichloromethane with a gradual increase in methanol Composition to 3%. The product **5** was obtained as a pale red solid (3.2 mg, 21%). R_f (10% MeOH/CH_2Cl_2) = 0.58. MS (ESI) *m/z* (relative intensity, %) = 1143.3 (8.3) $[M+Na]^+$, 474.1 (100). HRMS (ESI) calcd for $C_{52}H_{52}N_2O_{22}S_2$ $[M+NH_4]^+$

1138.2791, found 1138.2777. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05–8.03 (m, 1H, H1 (Dox)), 7.81–7.78 (t, J = 10 Hz, 1H, H2 (Dox)), 7.63–7.59 (m, 1H, H5 (coumarin)), 7.47 (s, 1H, H5 (TNB), 7.41–7.38 (m, 2H, H2 (TNB) and H3 (Dox)), 7.23–7.16 (m, 2H, H6, H8 (coumarin)), 6.28–6.26 (m, 2H, H3 (coumarin) and SCH), 5.51–5.30 (m, 2H, H1', H7 (Dox)), 4.76 (m, 2H, H14' (Dox)), 4.50–4.45 (m, 2H, $CH_2OC(=O)$), 4.34–4.11 (m, 2H, H3', H5' (Dox)), 4.09 (s, 3H, OCH_3 (Dox)), 4.00 (s, 3H, OCH_3 (TNB)), 3.93 (s, 3H, OCH_3 (TNB)), 3.89–3.68 (m, 2H), 3.30–3.28 (m, 1H, 0.5 H10 (Dox)), 3.13–3.07 (m, 1H, 0.5 SCH_2 (TNB)), 3.07–3.03 (m, 1H, 0.5 H10 (Dox)), 2.96–2.82 (m, 2H, SCH_2), 2.60–2.69 (m, 1H, 0.5 SCH_2 (TNB)), 2.45–2.43 (m, 3H, CH_3 (coumarin)), 2.39–2.33 (m, 1H, 0.5 H8 (Dox)), 2.18–2.14 (m, 1H, 0.5 H8 (Dox)), 1.88–1.80 (m, 2H, H2' (Dox)), 1.31–1.28 (m, 3H, H6' (Dox)) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ 213.89, 187.08, 186.70, 161.05, 160.42, 156.20, 155.64, 155.09, 154.06, 153.58, 153.09, 152.67, 151.86, 148.53, 140.24, 135.73, 135.47, 133.67, 129.79, 125.52, 120.88, 119.82, 118.45, 118.03, 117.34, 114.69, 111.54, 111.41, 109.90, 107.63, 100.76, 76.60, 69.51, 69.17, 67.45, 67.30, 65.54, 65.40, 64.90, 56.67, 56.43, 47.44, 47.35, 47.09, 35.60, 33.99, 31.89, 31.22, 29.92, 18.72, 16.90, 16.84 ppm. UV–vis (20% aq MeOH): λ_{max} = 490 (Dox; ε = 3,630 $M^{-1}cm^{-1}$), 360 (ε = 3,564 $M^{-1}cm^{-1}$) nm.

III. Photochemical Study

Photolytic experiments of TNB linkers (**1**, **2**), Taxol-TNB (**6**), and the dual release studies of drug conjugates (**3**–**5**) were performed by using a Spectroline® UV lamp device (XX-15A; emission wavelength with a maximal intensity at 365 nm).[1-3] As a representative procedure, a solution of **3** Taxol-TNB-Fluorescein was prepared in 50% aq methanol solution (0.1 mg mL^{-1}; 64 μM), and exposed to the long wavelength UV light at a distance of 5 cm. Multiple aliquots were taken as a function of exposure time, and each aliquot was analyzed by UPLC, UV–vis spectrometry, fluorescence spectroscopy and/or HPLC-MS spectrometry to determine the progress of the payload release and to identify the released products as illustrated in Figure 4 and Figures S1–S5.

IV. Cell Study

Cell Culture. The KB carcinoma cell line (ATCC) was used for all *in vitro* cell based studies. Cells were grown and maintained in RPMI 1640 medium with no folic acid (FA) (Life

Technologies) with 10% heat-inactivated fetal bovine serum (FBS), 100 IU penicillin, and 100 mg mL^{-1} streptomycin. All studies were performed in this media lacking FA.

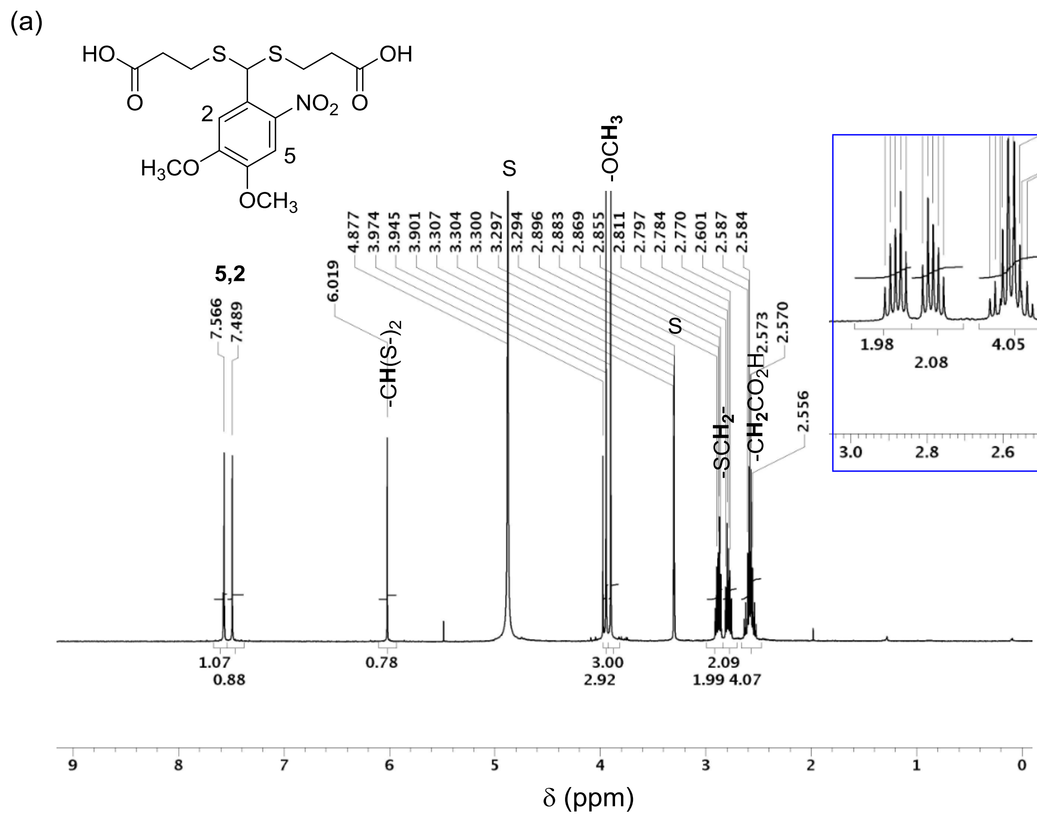
Flow Cytometry. KB cells/well (2×10^5) were seeded in a 24 well tissue culture plate in FA free media and grown overnight at 37 °C. Growth media was removed, and replaced with 280 μL of 0.5 μM or 1.5 μM **3** Taxol-TNB-Fluorescein in media. Cells were incubated at 37 °C for 0–24 hr time periods, and then washed 3 × with PBS to remove free extracellular conjugate. Fresh media was added, and the cells were placed under a UV lamp (365 nm) for 2 min of exposure. Cells were immediately washed with PBS and detached from the plate by addition of 200 μL of 0.25% trypsin to each well. 300 μL of media was added to each well, and the cells were transferred to flow cytometry tubes. Cells were washed with FACS buffer (PBS, 0.1% BSA, 0.1% sodium azide), and analyzed by flow cytometry on a BD Accuri C6 flow cytometer on the FL1 channel (λ_{ex} 488 nm, λ_{ex} 533/30 nm). 100,000 events were collected, and mean fluorescence intensities were calculated based on gating for intact cells.

Photorelease and Cell Viability Assessment *In Vitro*. 3,000 KB cells/well were seeded on a 96-well tissue culture plate in FA free media and grown for 2 days at 37 °C. Growth media was removed, and replaced with dilutions of each construct, **3–5**, in 100 μL of FA free media over a concentration range of 0–3 μM for taxol conjugates, and 0–24 μM for Dox conjugates. A baseline measurement of the cellular fluorescence was taken in a microplate reader (for a fluorescein conjugate **3** at λ_{ex} 485 nm, λ_{em} 528 nm; for coumarin conjugates **4**, **5** at λ_{ex} 360 nm, λ_{em} 460 nm). Plates for UV exposure were placed directly under a UV lamp for 2 min, while unexposed plates were placed in the dark during the time period. The fluorescence was measured again immediately at the same wavelengths, and the background was subtracted from these values. The cells were then incubated for 24 hr at 37 °C. Cell viability was assessed by removing the culture media, and performing an XTT assay (Roche) according to the manufacturer's protocol. Percent viability is expressed relative to cells incubated in media alone. Two min of UV exposure did not alter the viability of untreated cells. To assess the contribution of metabolic TNB cleavage to reporter release, another set of treated cells were exposed to UV for 0 or 2 min, and the fluorescence was measured immediately in the plate reader, and followed over the course of 24 hr of further incubation at 37 °C.

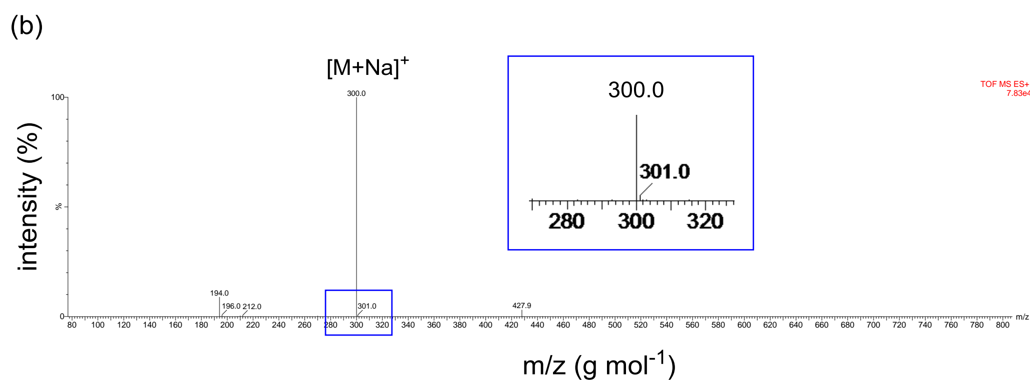
V. Copies of Spectral Data

1 TNB(CO_2H): ^1H NMR (CD_3OD) (a) and ESI mass (b). S = residual peaks from CD_3OD

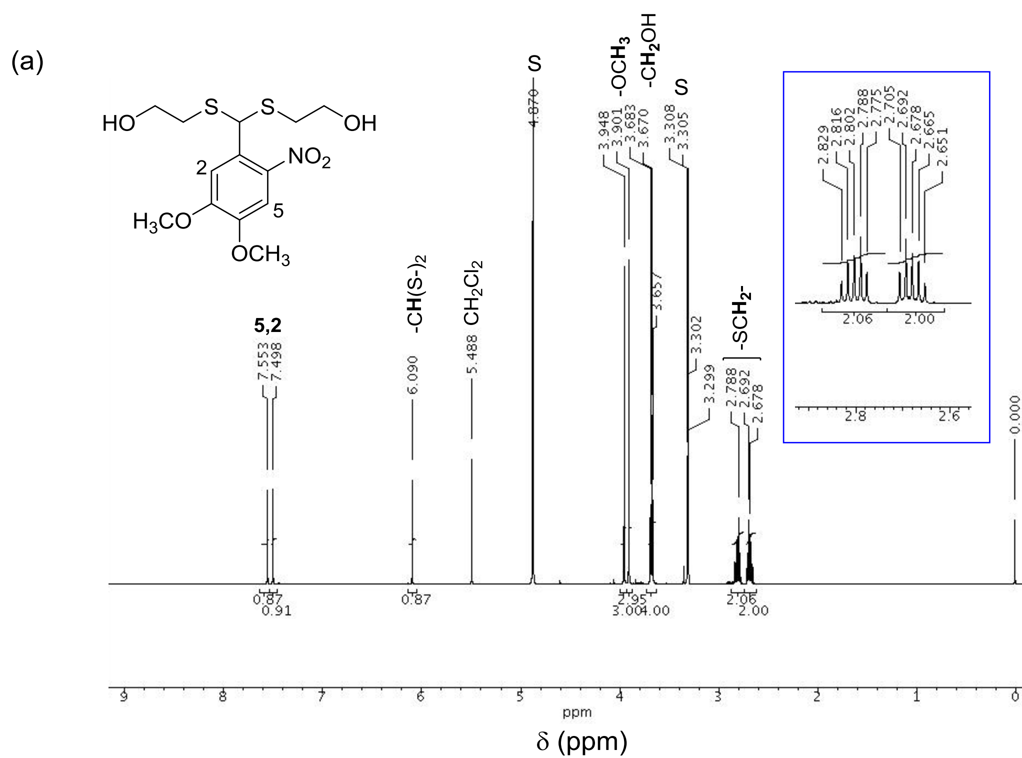
(a)

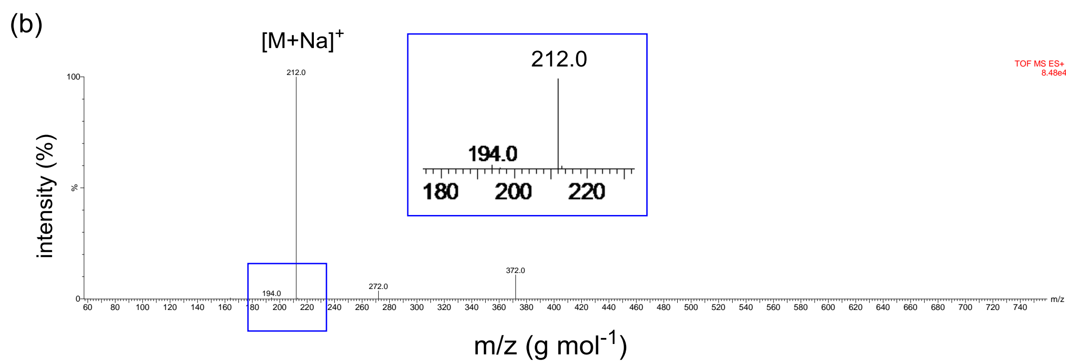


(b)



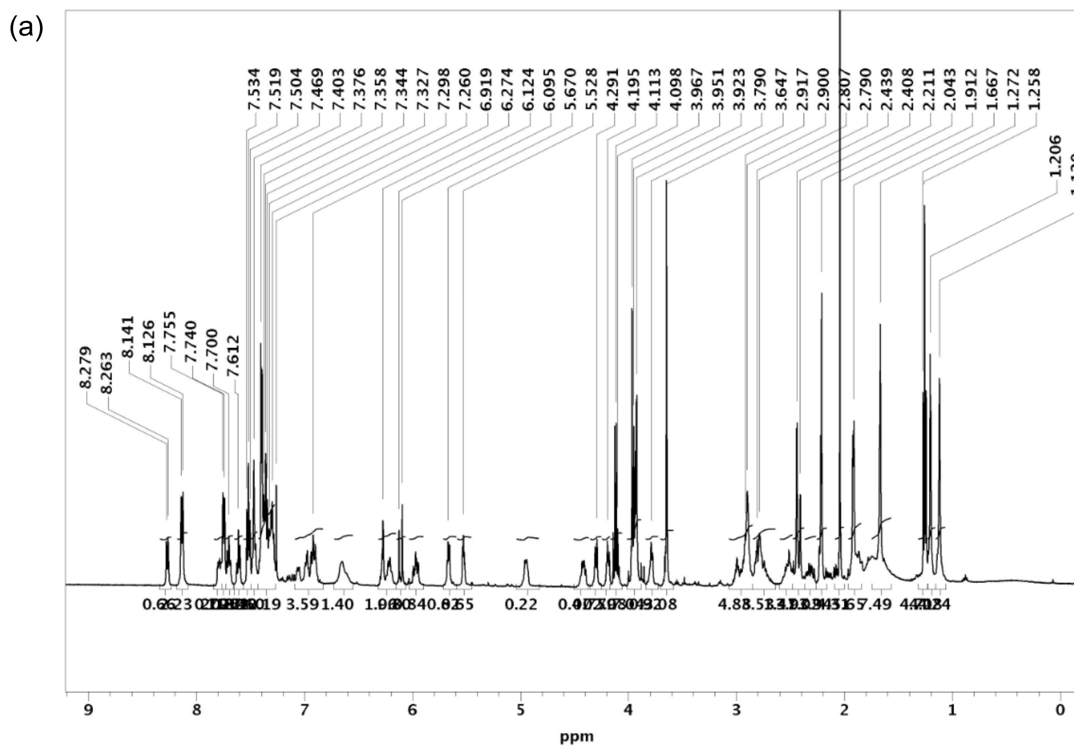
2 TNB(OH): ^{1}H NMR (CD_3OD) (a) and ESI mass (b). S = residual peaks from CD_3OD



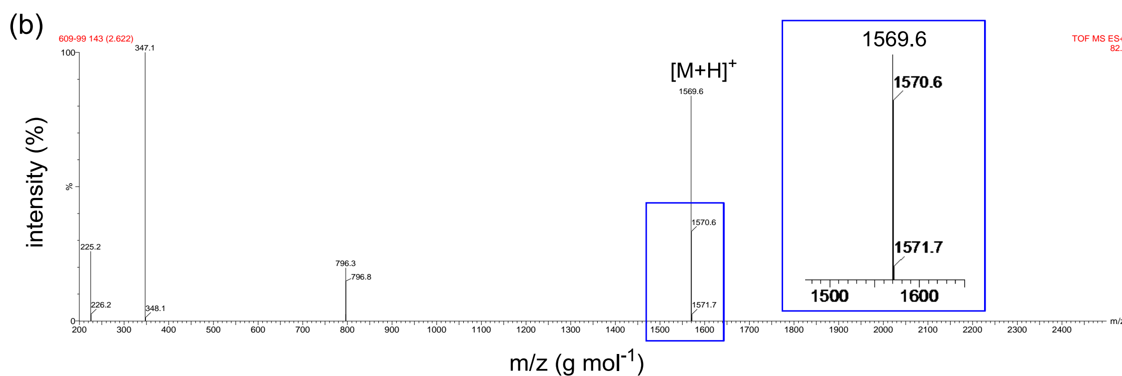


3 Taxol-TNB-Fluorescein: ^{1}H NMR ($CDCl_3$) (a), ESI (b) and high resolution (c) mass spectra

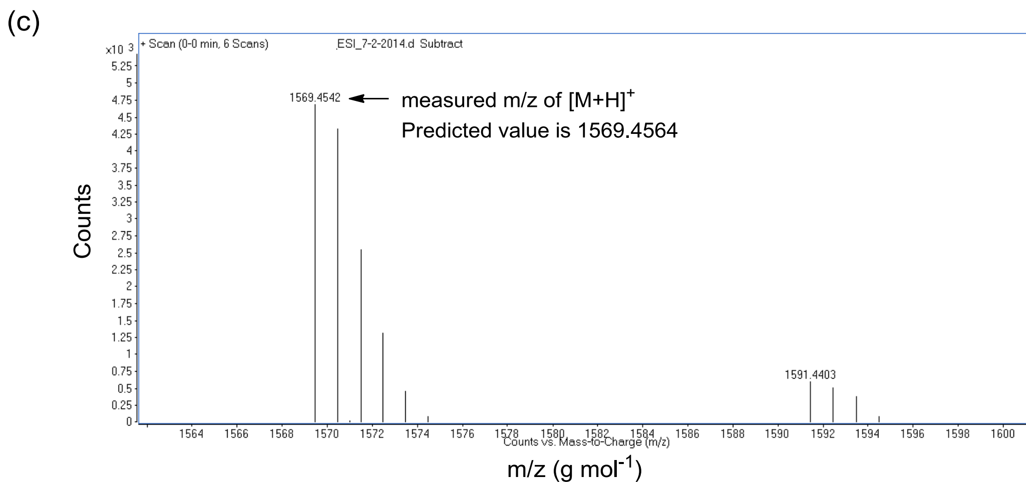
(a)



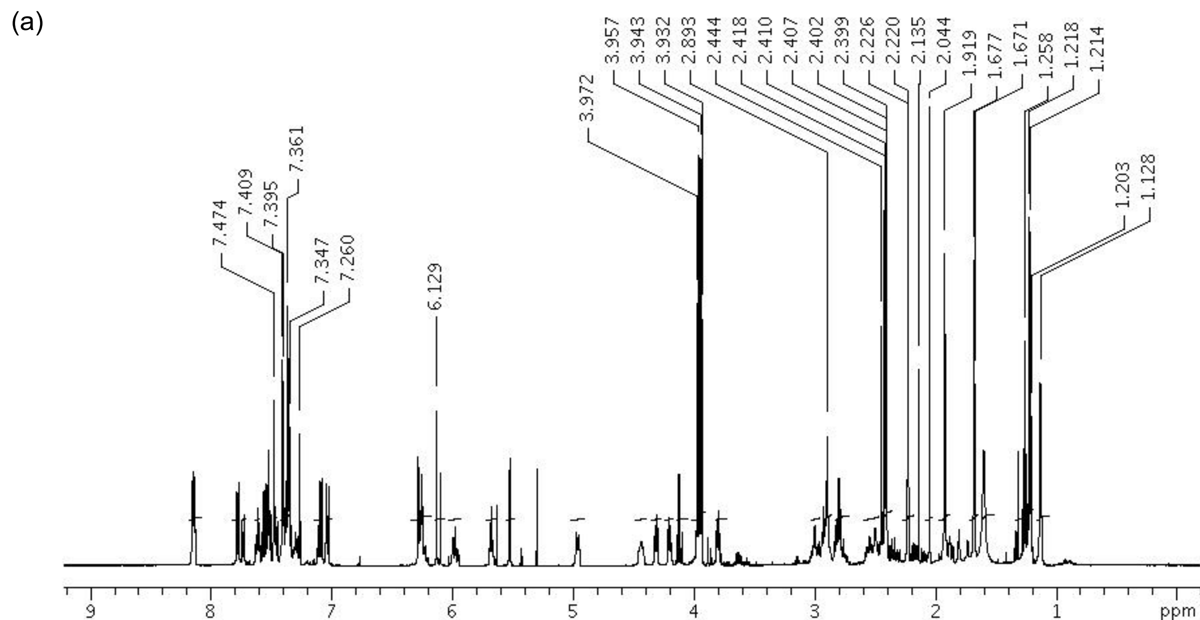
(b)

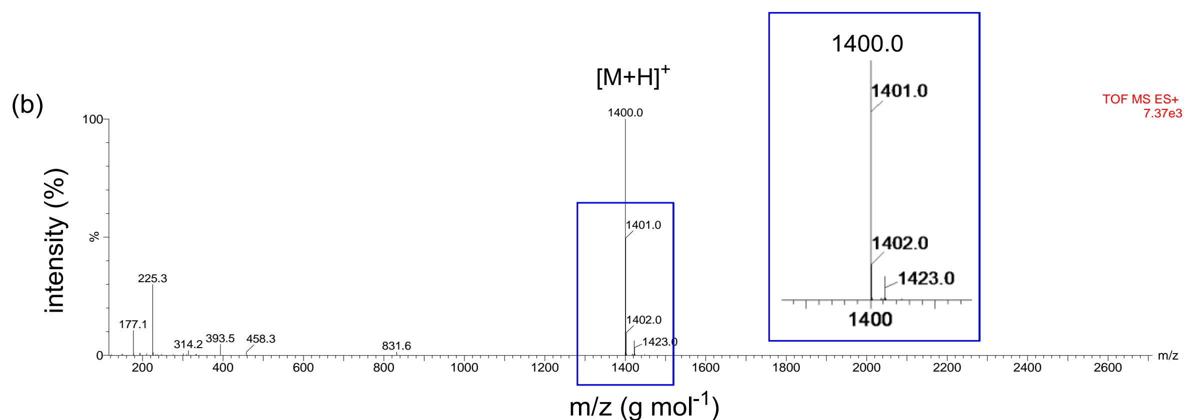


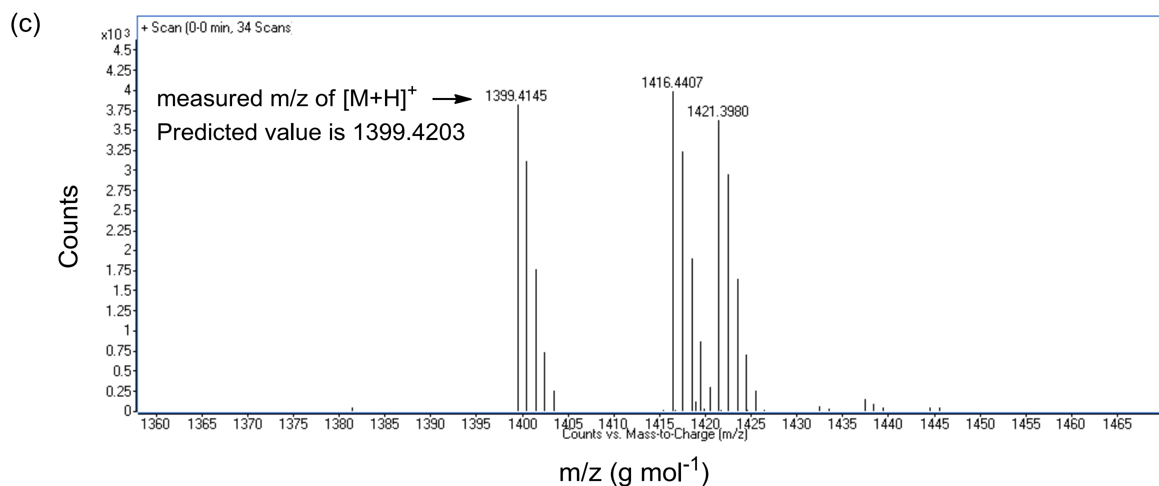
(c)



4 Taxol-TNB-Coumarin: ^{1}H NMR ($CDCl_3$) (a), ESI (b) and high resolution (c) mass spectra

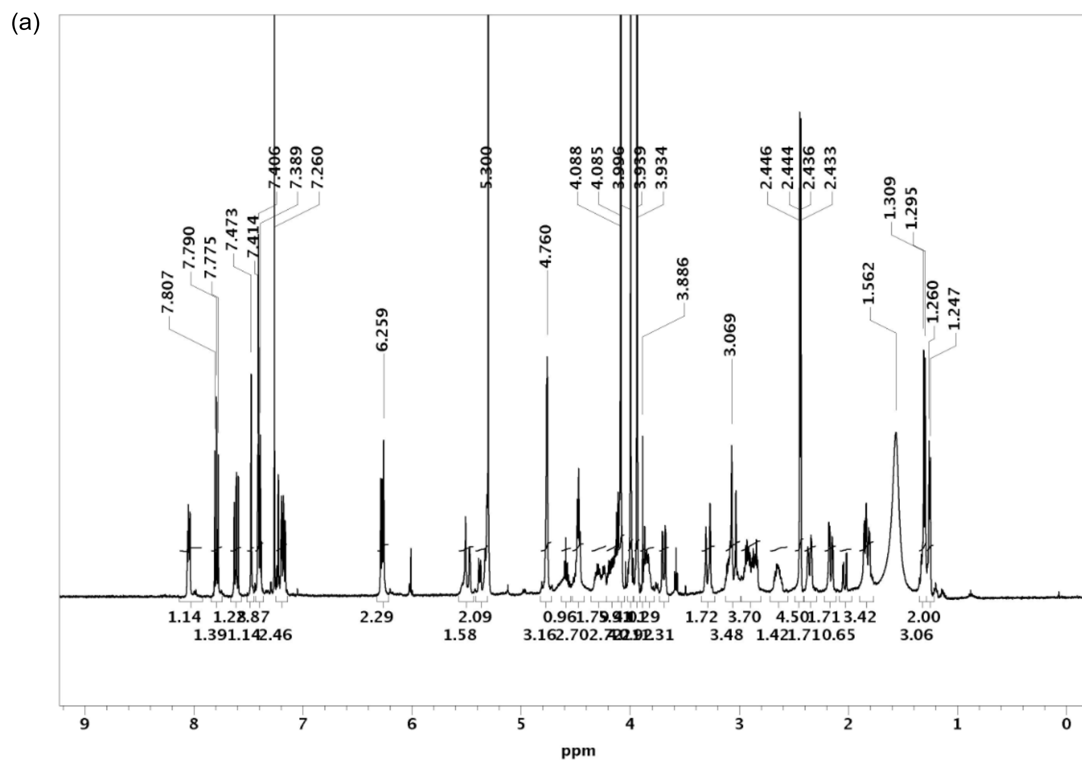




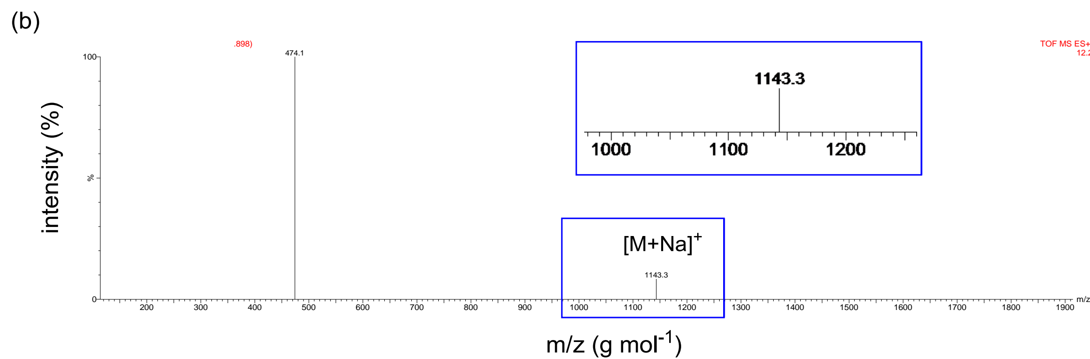


5 Dox-TNB-Coumarin: ^{1}H NMR ($CDCl_3$) (a), ESI (b) and high resolution (c) mass spectra

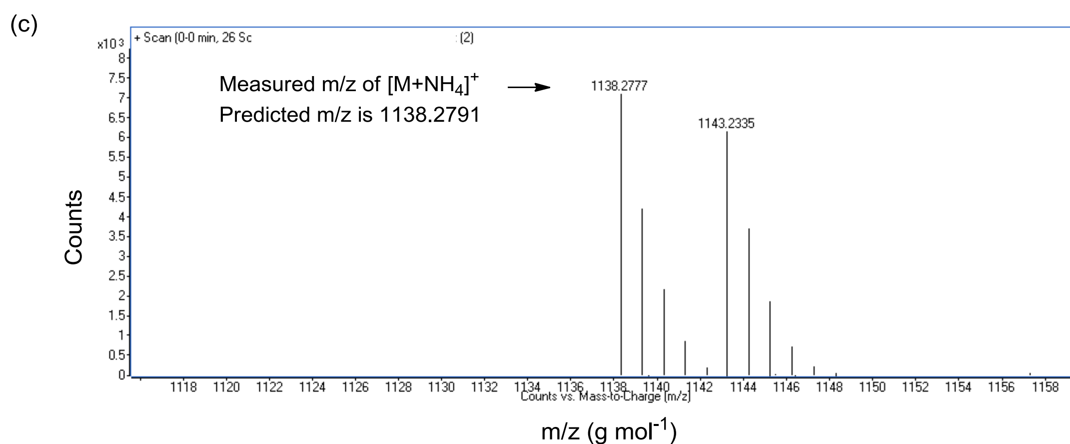
(a)



(b)

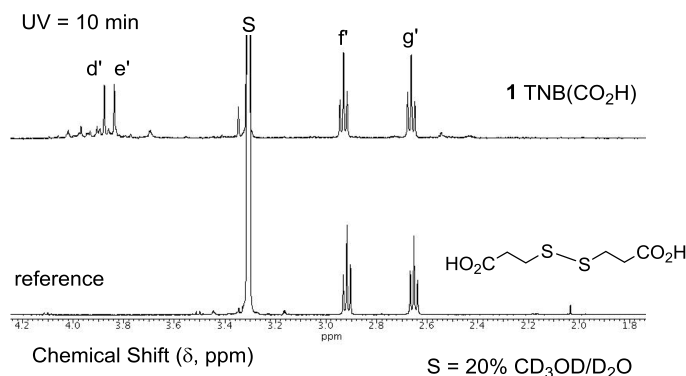


(c)



VI. Figures S1-S5

(a) ^{1}H NMR reference



(b) UPLC traces

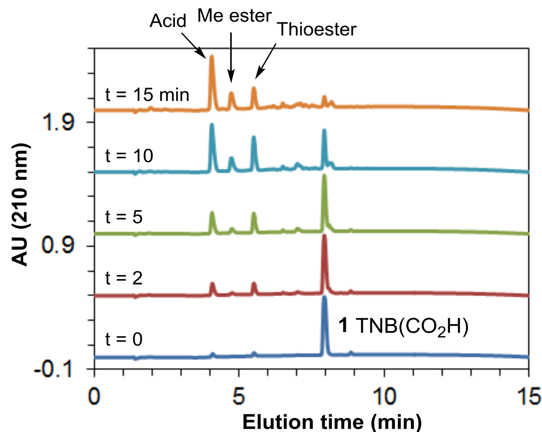


Figure S1. (a) A ^{1}H NMR trace (500 MHz) acquired after the photolysis (10 min) of **1** (2.5 mM) and the reference spectrum of 3,3'-dithiopropionic acid mono sodium salt (Sigma-Aldrich), supportive of the identity of this disulfide generated. S = residual solvent peak (OH). (b) UPLC traces acquired after the photolysis of **1** (0.25 mM in 20% aqueous methanol) overlaid against exposure time. Proposed assignment of peaks to products (acid, methyl ester, thioester).

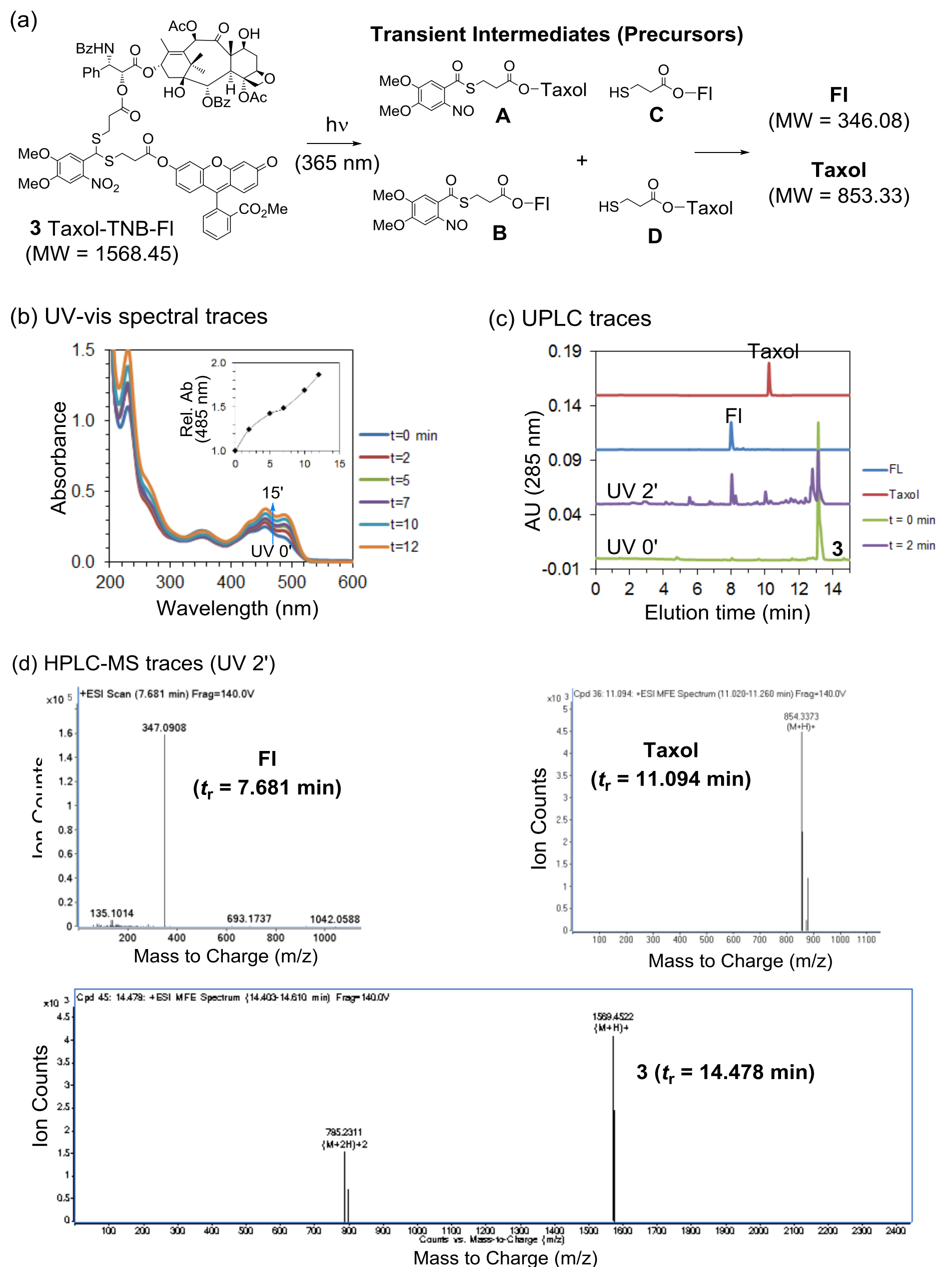
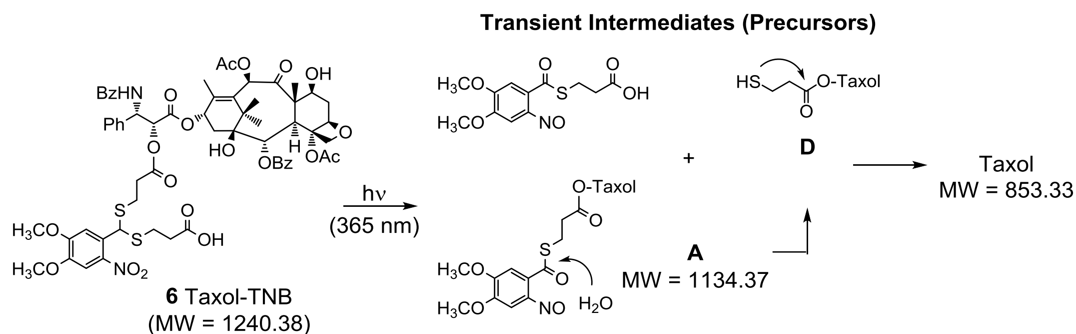


Figure S2. A proposed mechanism for photolysis of **3** Taxol-TNB-Fl (a); UV–vis spectral traces acquired after the photolysis of **3** (12.8 μM in 20% aq methanol) overlaid against exposure time (b) and selected UPLC traces (c); HPLC-MS spectrometric detection of fluorescein (Fl), taxol and a parent conjugate **3** following 2 min of irradiation (d).



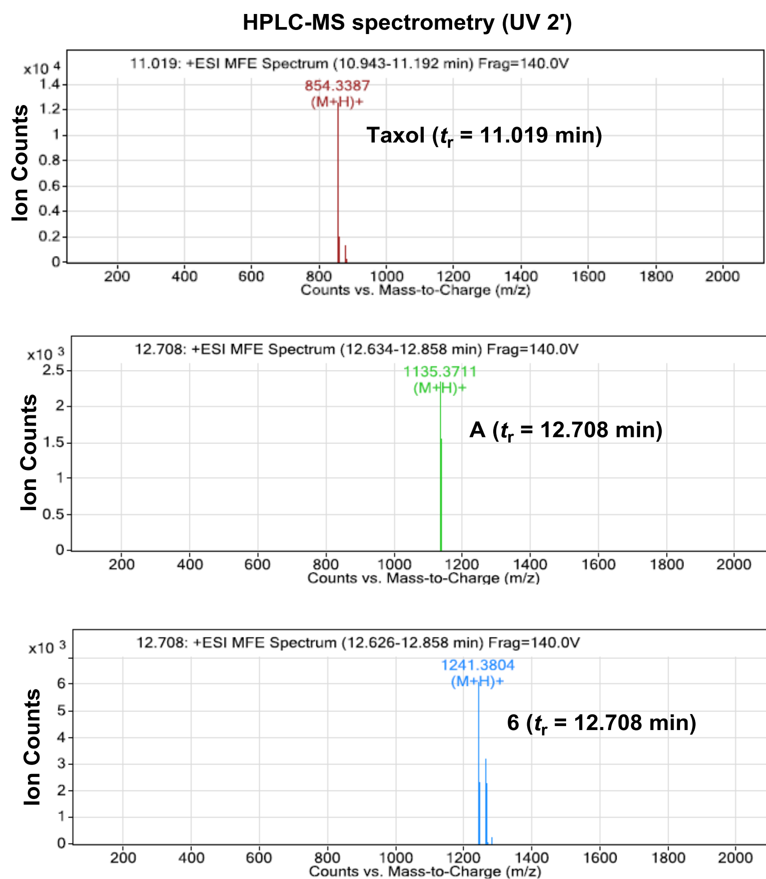
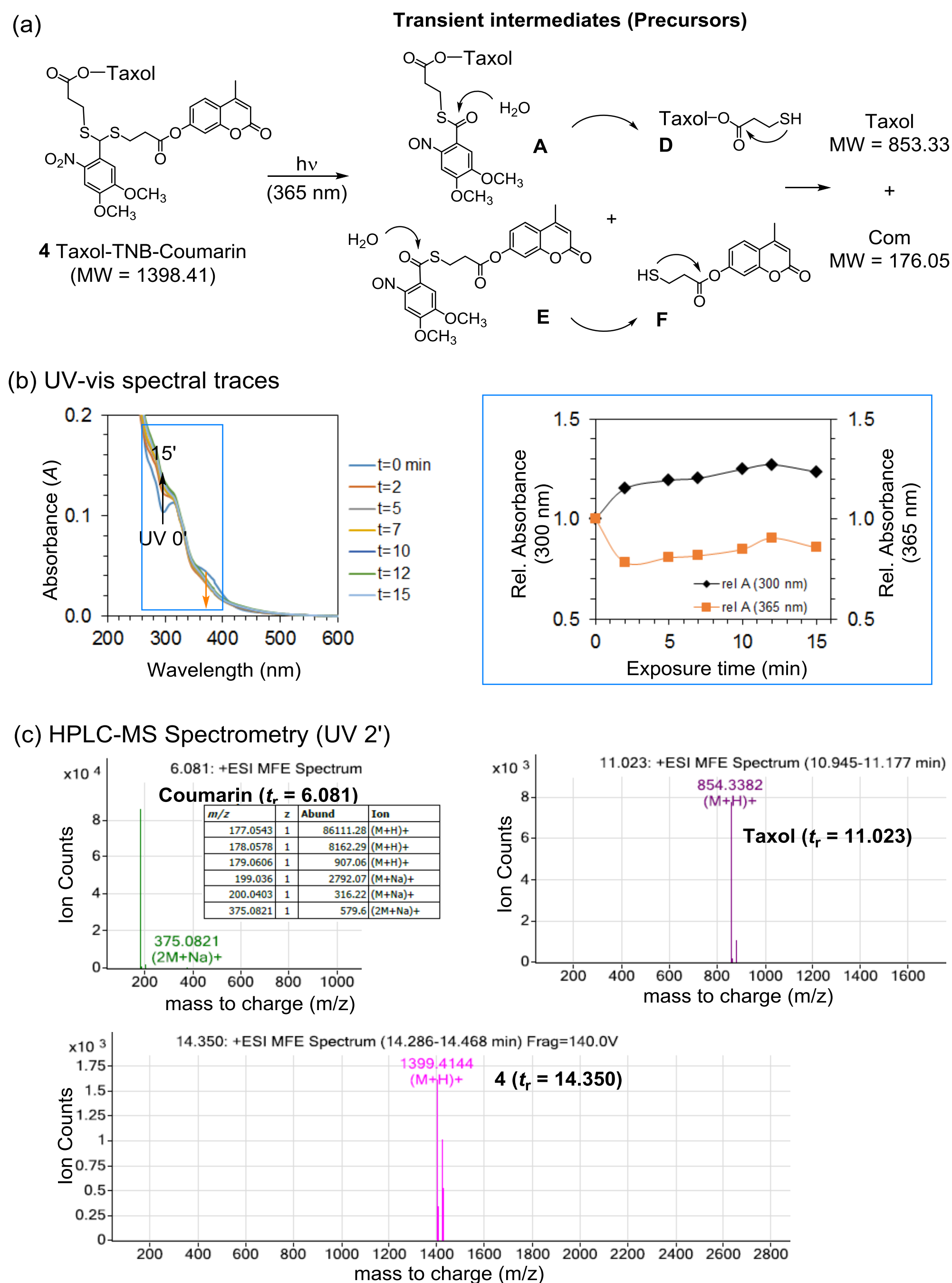
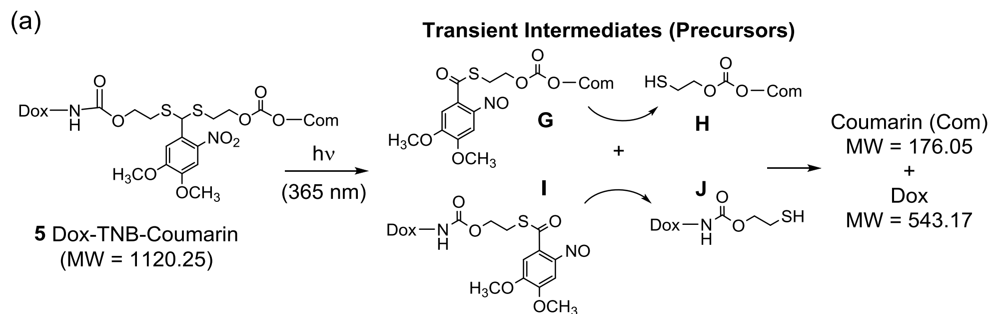


Figure S3. A proposed mechanism for the photolysis of **6** Taxol-TNB (top); HPLC-MS spectrometric detection of taxol and its precursor A following 2 min of irradiation (bottom).

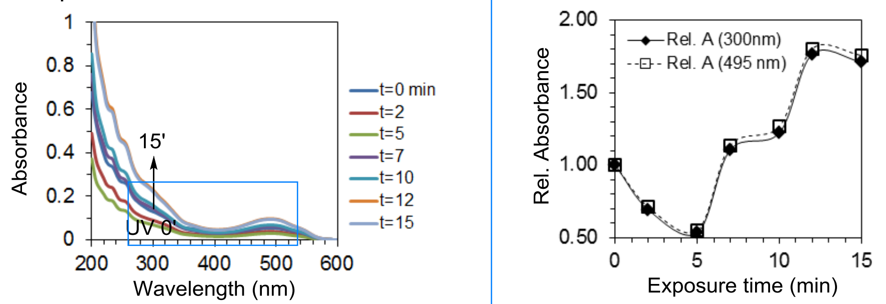


m/z	z	Abund	Ion
177.0543	1	86111.28	(M+H)+
178.0578	1	8162.29	(M+H)+
179.0606	1	907.06	(M+H)+
199.036	1	2792.07	(M+Na)+
200.0403	1	316.22	(M+Na)+
375.0821	1	579.6	(2M+Na)+

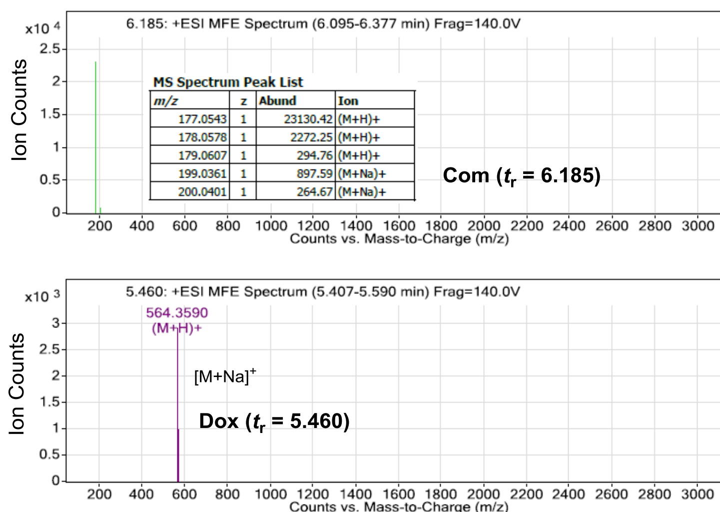
Figure S4. A proposed mechanism for the photolysis of **4** Taxol-TNB-Coumarin (top); UV–vis spectral traces acquired after the photolysis of **4** (14.3 μM in 20% aq methanol) overlaid against exposure time (b); HPLC-MS spectrometric detection of coumarin and taxol following 2 min of irradiation (c).



(b) UV-vis spectral traces



(c) HPLC-MS Traces (UV 2')



MS Spectrum Peak List

m/z	z	Abund	Ion
177.0543	1	23130.42	(M+H)+
178.0578	1	2272.25	(M+H)+
179.0607	1	294.76	(M+H)+
199.0361	1	897.59	(M+Na)+
200.0401	1	264.67	(M+Na)+

Figure S5. (a) A proposed mechanism for the photolysis of **5** Dox-TNB-Coumarin; UV–vis spectral traces acquired after the photolysis of **5** (17.8 µM in 20% aq methanol) overlaid against exposure time (b); HPLC-MS spectrometric detection of coumarin (Com) and doxorubicine following 2 min of irradiation (c).

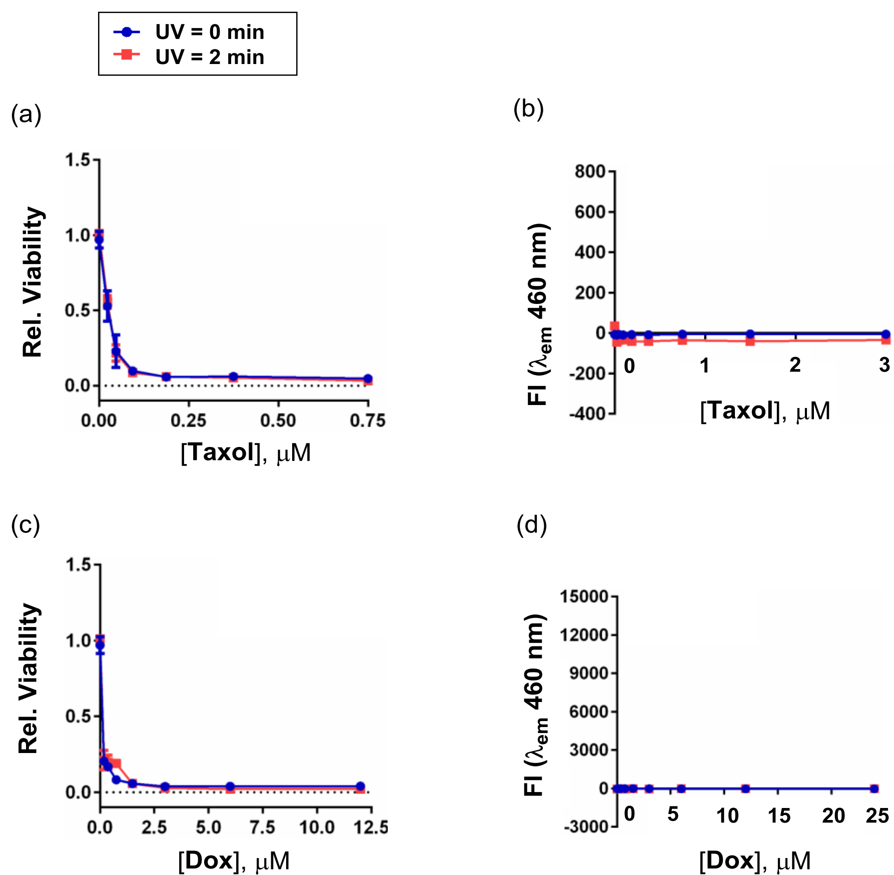
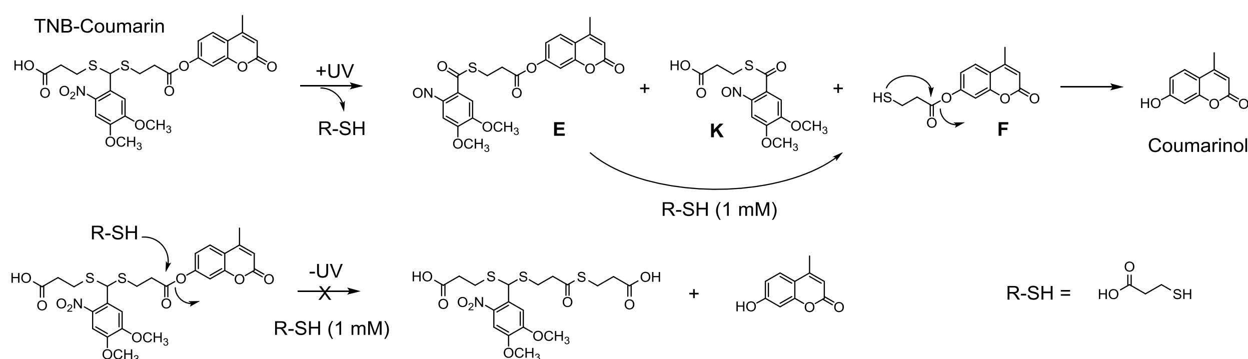
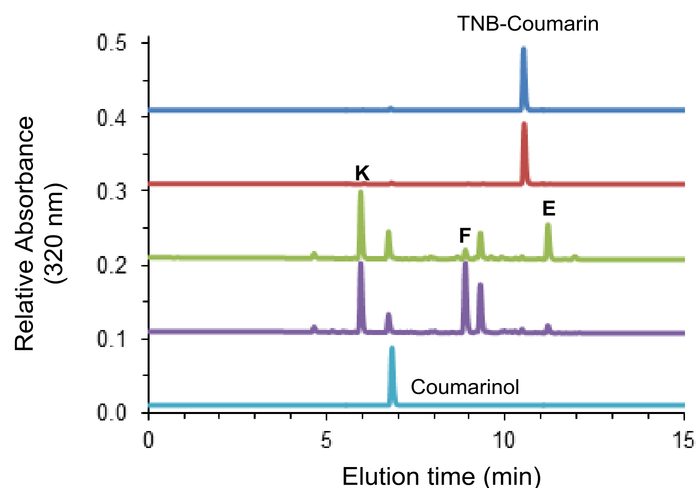


Figure S6. No effect of light exposure on KB cell viability (left) or fluorescence intensity (right) was observed for either free taxol (a, b) or doxorubicin (c, d), both of which were highly cytotoxic at even low nM concentrations.

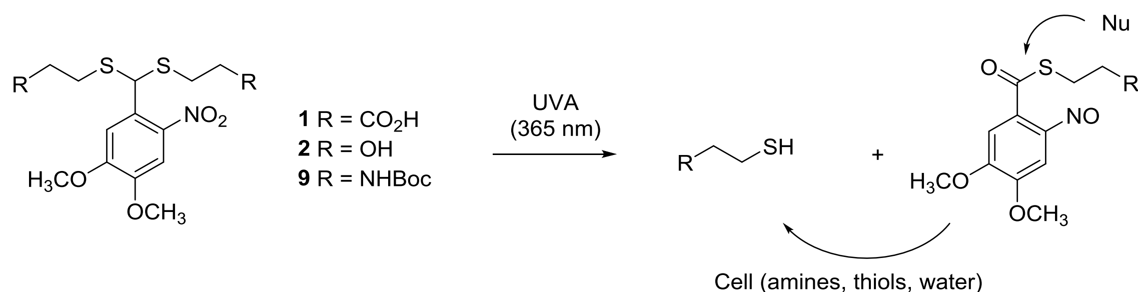




Condition	TNB-Coumarin [a]	Thiol (±) [b]	UVA (±)	UPLC acquired at
a	0.177 mM	0	0	19 hr
b	0.177 mM	1.0 mM	0	19 hr
c	0.177 mM	0	5 min	30–45 min
d	0.177 mM	1.0 mM	5 min	45–60 min

Figure S7. Effect of thiol on thiolytic stability and photouncaging of TNB-Coumarin under specific conditions marked a–d. (Top) A proposed mechanism for the photouncaging of TNB-Coumarin. (Bottom) Overlay of UPLC traces acquired under each of four representative reaction conditions. Assignments of peaks are proposed tentatively relative to TNB-Coumarin and coumarinol, each of which serves as a reference peak. [a] Each of the solution was prepared in 30% aq MeCN. [b] Thiol = 3-mercaptopropionic acid

VII. Table S1. Summary of quantum efficiency of uncaging ($\Phi_{uncaging}$) for selected TNB-caged thiols and their potential use in biology



Caged thiols	Thiols released	$\Phi_{uncaging}$ [a]	Conc [b]	Potential area of applications
1 TNB(CO_2H)	$HS(CH_2)_2CO_2H$	0.20	1.0 mM	• Modulation of redox-responsive ion channels[4, 5]
2 TNB(OH)	$HS(CH_2)_2OH$	0.19	1.0 mM	• Enzyme inhibition (captopril, cysteamine)[6-8]
9 TNB(NHBoc)	$HS(CH_2)_2NHBoc$	0.08	1.0 mM	• Redox biology[9]

[a] $\Phi_{uncaging}$ = [# of cage molecules consumed]/[# of photons absorbed] = $[dc/dt]_{initial}/[q_{n,p}(1-10^{-A})]$ where $q_{n.p}$ refers to photon flux ($q_p/N_A = 11.65 \times 10^{-8}$ mol s^{-1}) determined by ferrioxalate actinometry;[10] A refers to the absorbance at the irradiation wavelength (365 nm); and dc/dt is the initial rate of consumption (mol s^{-1}) before reaching a plateau.[11]

[b] 30% aqueous methanol

VIII. References

1. Choi, S. K.; Thomas, T.; Li, M.; Kotlyar, A.; Desai, A.; Baker Jr, J. R. Light-Controlled Release of Caged Doxorubicin from Folate Receptor-Targeting PAMAM Dendrimer Nanoconjugate. *Chem. Commun. (Cambridge, U. K.)* **2010**, 46, 2632–2634.

2. Choi, S. K.; Thomas, T. P.; Li, M.-H.; Desai, A.; Kotlyar, A.; Baker, J. R. Photochemical release of methotrexate from folate receptor-targeting PAMAM dendrimer nanoconjugate. *Photochem. Photobiol. Sci.* **2012**, 11, 653–660.

3. Wong, P. T.; Chen, D.; Tang, S.; Yanik, S.; Payne, M.; Mukherjee, J.; Coulter, A.; Tang, K.; Tao, K.; Sun, K.; Baker Jr, J. R.; Choi, S. K. Modular Integration of Upconversion Nanocrystal-Dendrimer Composites for Folate Receptor-Specific Near Infrared Imaging and Light Triggered Drug Release. *Small* **2015**, 11, 6078–6090.

4. Sun, J.; Xu, L.; Eu, J. P.; Stamler, J. S.; Meissner, G. Classes of Thiols That Influence the Activity of the Skeletal Muscle Calcium Release Channel. *J. Biol. Chem.* **2001**, 276, 15625–15630.

5. Alansary, D.; Schmidt, B.; Dörr, K.; Bogeski, I.; Rieger, H.; Kless, A.; Niemeyer, B. A. Thiol dependent intramolecular locking of Orai1 channels. *Sci. Rep.* **2016**, 6, 33347.

6. Reynolds, C. H. Kinetics of inhibition of angiotensin converting enzyme by captopril and by enalapril diacid. *Biochem. Pharmacol. (Amsterdam, Neth.)* **1984**, 33, 1273–1276.

7. Qi, J. H.; Zhang, L.; Wang, J.; Lu, M.; Wang, X. M.; Jin, Z. J. Effect of captopril or verapamil on intracellular sodium in cultured vascular smooth muscle cells1. *Cell Res.* **1996**, 6, 47–53.

8. Okamura, D. M.; Bahrami, N. M.; Ren, S.; Pasichnyk, K.; Williams, J. M.; Gangoiti, J. A.; Lopez-Guisa, J. M.; Yamaguchi, I.; Barshop, B. A.; Duffield, J. S.; Eddy, A. A. Cysteamine Modulates Oxidative Stress and Blocks Myofibroblast Activity in CKD. *J. Am. Soc. Nephrol.* **2014**, 25, 43–54.

9. Poole, L. B. The basics of thiols and cysteines in redox biology and chemistry. *Free Radical Biol. Med.* **2015**, 80, 148-157.

10. Hatchard, C. G.; Parker, C. A. A New Sensitive Chemical Actinometer. II. Potassium Ferrioxalate as a Standard Chemical Actinometer. *Proc. R. Soc. London, Ser. A* **1956**, 235, 518–536.

11. Braslavsky, S. E. Glossary of terms used in photochemistry, 3rd edition (IUPAC Recommendations 2006). *Pure Appl. Chem.* **2009**, 79, 293–465.