Supporting Information

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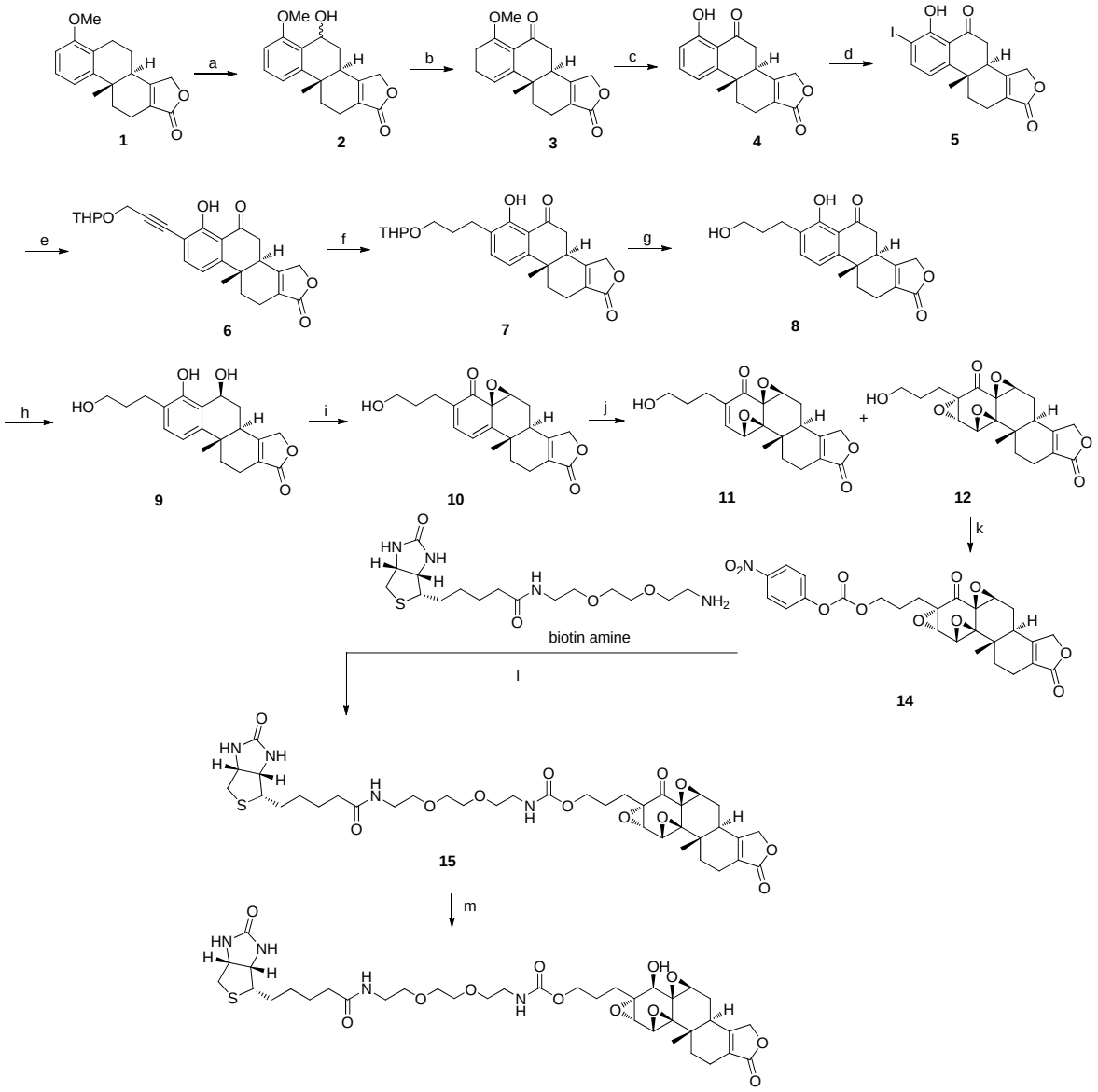
Table of Contents

Chemical Synthesis and Characterization of Triptolide Probes



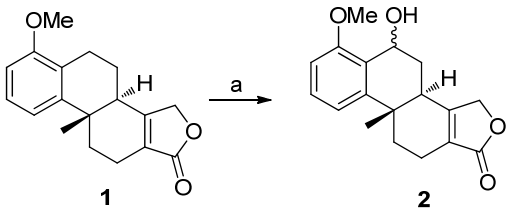
Scheme S1. Chemical synthesis of biotin-triptolide (Biotin-TL)

Reagents and conditions: a) cerium(VI) ammonium nitrate, MeCN, 0 °C, 99 %; b) IBX, acetone, reflux, 98 %; c) $AlCl_3$, MeCN, reflux, 100 %; d) $Sc(OTf)_3$, NIS, AcOH, rt, 82 %; e) $THPOCH_2C{\equiv}CH$, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 35 °C, 97 %; f) H_2, Pd/C, EtOH, 40-50 °C, 100 %; g) $TsOH{\cdot}H_2O$, MeOH, rt, 95 %; h) $NaBH_4$, EtOH, CH_2Cl_2, 0 °C, 99 %; i) $NaIO_4$, MeOH, H_2O, rt, 80 %; j) CF_3COCH_3, OXONE, $NaHCO_3$,

CH_3CN, $Na_2(EDTA)$, 0 °C; k) *p*-nitrophenylchloroformate, pyridine, CH_2Cl_2, rt, the yield of **14** was 13% from **10**; l) DMAP, Et_3N, CH_2Cl_2/MeOH, rt, 24 %; m) K-Selectride, THF, −40 °C, 39 %.

Biotin-amine used in step **l** was prepared according to procedure described in C. Gnaccarini *et al. Bioorg. Med. Chem.* **2009**, *17*, 6354-6359.

Preparation of Compound **2**



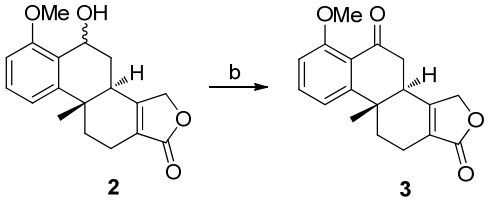
Compound **1** was synthesized in accordance with or by slight modification of the method disclosed in Gao, Q.; Xue, J.; Zheng, B.; Liu, R. (Shanghai Haoyuan Chemexpress Co., Ltd., P.R. China) Patent application: CN101638426 (2010) p. 13.

To a suspension of **1** (10.0 g, 35.2 mmol) in CH_3CN (250 mL) was added ceric ammonium nitrate (CAN) solution (40.5 g in 25 mL water) dropwise at 0 °C over a period of 30 min. The resulting mixture turned transparent red, and was stirred for 30 min and monitored by TLC (EtOAc: Hexane = 3:7). When the starting material was consumed and the mixture turned yellow, Na_2SO_3 solution (102 g Na_2SO_3 in 300 mL H_2O) was added. The mixture was stirred vigorously for 10 min and filtered through sand funnel. The filtrate was extracted with CH_2Cl_2 (300 mL × 3), and the solid was washed with CH_2Cl_2 (200 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and filtered via a short pad of silica gel. After the solvent was removed by evaporation, compound **2** was obtained as a yellow powder (10.5 g, 99 %), which was used directly in the next step without further purification.

Analytical TLC (silica gel 60), 40 % EtOAc in *n*-hexane, R_f = 0.16; ^{1}H NMR (300 MHz) δ 7.30 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 8.2 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 5.13 (t, J = 2.3 Hz, 1H), 4.80 (AB system, 2H), 3.88 (s, 3H), 3.17–3.12 (m, 1H), 2.54–2.40 (m, 3H), 2.11–2.05 (m, 2H), 1.74–1.72 (m, 1H), 1.00 (s, 3H);

^{13}C NMR (100 MHz) δ 174.0, 163.1, 157.9, 146.3, 129.0, 125.2, 125.1, 117.1, 108.3, 70.5, 62.2, 55.5, 36.8, 36.7, 32.6, 27.9, 21.9, 18.2; IR (CH_2Cl_2) 1757, 1682 cm^{-1}.

Preparation of Compound **3**



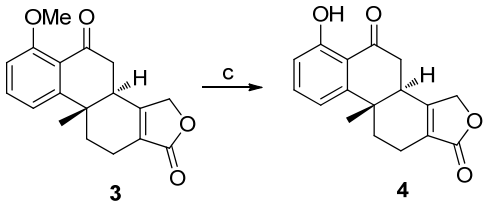
2 **3**

Compound **3** was prepared in accordance with or by slight modification of the method disclosed in Gao, Q.; Xue, J.; Zheng, B.; Liu, R. (Shanghai Haoyuan Chemexpress Co., Ltd., P.R. China), Patent application CN101638426 (2010), p. 13.

Compound **2** prepared in the previous step (10.5 g, 35.0 mmol, crude), 2-iodoxybenzoic acid (IBX, 14.8 g, 52.8 mmol) and acetone (120 mL) were heated under reflux. The reaction was monitored by TLC (EtOAc: Hexane = 3:7 twice). After 6 h, the mixture was cooled and the solid was removed by filtration and washed with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4. After solvent removal, crude compound **3** (10.2 g, 98 %) was obtained and was directly used for next step without further purification.

Analytical TLC (silica gel 60), *n*-Hexane:CH_2Cl_2:EtOAc = 3:1:1, R_f = 0.1; ^{1}H NMR (300 MHz) δ 7.51 (t, J = 8.2 Hz, 1H), 7.05 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 4.70 (AB system, 2H), 3.93 (s, 3H), 3.15–3.09 (m, 1H), 2.81 (dd, J = 6.0, 18.2 Hz, 1H), 2.63 (dd, J = 18.2, 13.7 Hz, 1H), 2.64–2.55 (m, 1H), 2.54–2.50 (m, 1H), 2.50–2.28 (m, 1H), 1.84 (td, J = 12.5, 7.3 Hz, 1H), 1.13 (s, 3H); ^{13}C NMR (75 MHz) δ 194.6, 173.4, 160.5, 160.1, 153.5, 134.8, 125.8, 121.1, 115.2, 110.9, 70.1, 56.2, 39.7, 37.8, 36.9, 32.0, 21.4, 17.8; IR (CH_2Cl_2) 1757, 1682 cm^{-1}; LRMS (EI, 20 eV) *m/z* 298 (M^+, 100), 265 (27), 175 (60); HRMS (EI) calcd. for $C_{18}H_{18}O_4$ (M^+) *m/z* 298.1205, found 298.1215.

Preparation of Compound **4**

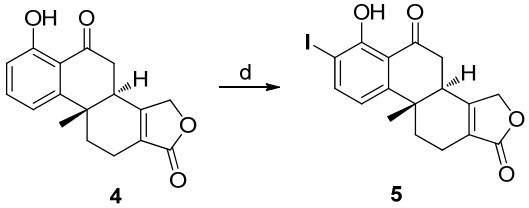


Compound **4** was synthesized in accordance with or by slight modification of the method disclosed in Gao, Q.; Xue, J.; Zheng, B.; Liu, R.; (Shanghai Haoyuan Chemexpress Co., Ltd., PR. China), Patent application CN101638426 (2010), p. 13.

To a solution of compound **3** (10.2 g, 34.2 mmol, crude) in redistilled CH_3CN (60 mL) was added anhydrous $AlCl_3$ (14.8 g, 105.6 mmol). The mixture was heated to reflux, and the reaction was monitored by TLC (EtOAc:Hexane = 2:3). After 12 h, 1 M HCl (50 mL) solution was added to the reaction mixture while stirring. The mixture was extracted with CH_2Cl_2 (300 mL × 3), and the organic layer was filtered *via* short pad of silica gel. After solvent removal by rotary evaporation, yellow solid **4** (8.9 g, 100 %) was obtained, which was used in the next step without further purification.

Analytical TLC (silica gel 60), *n*-Hexane:EtOAc:CH_2Cl_2 = 1:1:1, R_f = 0.5; ^{1}H NMR (300 MHz) δ 12.61 (s, 1H), 7.49 (t, *J* = 8.1 Hz, 1H), 6.92 (t, *J* = 7.6 Hz, 2H), 4.78 (AB system, 2H), 3.25–3.21 (m, 1H), 2.81 (d, *J* = 2.7 Hz, 1H), 2.79 (s, 1H), 2.75–2.52 (m, 3H), 1.84 (td, *J* = 12.5, 7.3 Hz, 1H), 1.16 (s, 3H); ^{13}C NMR (75 MHz) δ 201.9, 173.2, 164.1, 159.5, 152.1, 137.4, 126.2, 117.1, 115.4, 114.2, 69.9, 40.3, 36.7, 36.3, 31.7, 21.8, 17.8; IR (CH_2Cl_2) 1763, 1265 cm^{-1}; LRMS (EI, 20 eV) *m/z* 284 (M^+, 100), 269 (17), 187 (35); HRMS (EI) calcd. for $C_{17}H_{16}O_4$ (M^+) *m/z* 284.1049, found 284.1034.

Preparation of Compound **5**

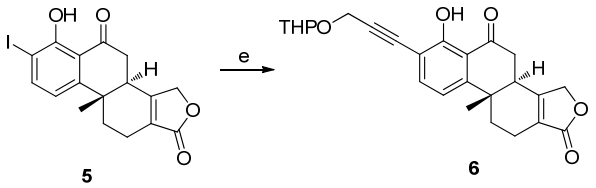


To a solution of **4** (8.9 g, 31.1 mmol) in HOAc (100 mL) were added *N*-iodosuccinimide (7.5 g, 34.4 mmol) and scandium (III) triflate (1.5 g, 3.1 mmol). The resulting suspension was stirred in the absence of light at

room temperature, and the reaction was monitored by TLC (Hexane: EtOAc: CH_2Cl_2 = 1:1:1). After stirring overnight, the reaction mixture was diluted with Na_2SO_3 solution (10 g Na_2SO_3 in 1000 mL water) and mixed at room temperature for 1 h. The product was collected by filtration, washed with water (200 mL × 5) and EtOH/H_2O (1:1 v/v, 200 mL × 5), dried under vacuum to give product **5** (10.8 g, 82 %) as a light yellow solid.

Analytical TLC (silica gel 60), EtOAc: Hexane: CH_2Cl_2 =1:1:1, R_f = 0.50; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.5 (s, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 4.77 (AB system, 2H), 3.14 (d, J = 13.6 Hz, 1H), 2.83 (dd, J = 6.0, 18.3 Hz, 1H), 2.72–2.58 (m, 2H), 2.57–2.40 (m, 2H), 1.88–1.78 (m, 1H), 1.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.5, 173.1, 160.8, 159.5, 153.1, 144.1, 126.4, 125.9, 120.9, 69.9, 62.4, 39.5, 37.4, 36.9, 31.9, 21.4, 17.7; HRMS (EI) calcd. for $C_{17}H_{15}IO_4$ (M^+) *m/z* 410.0015; found 410.0007.

Preparation of Compound **6**

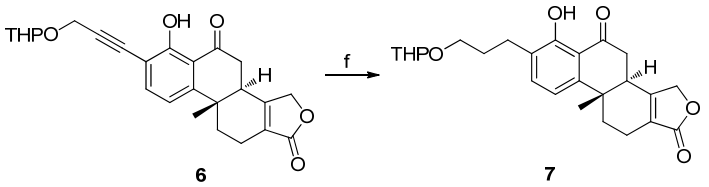


$Pd(PPh_3)_2Cl_2$ (685 mg, 0.97 mmol), CuI (372 mg, 1.95 mmol) and alkyne (1.02 g, 7.31 mmol) were added to a 250 mL round-bottom flask, evacuated by vacuum and filled with argon. Dry THF (100 mL) was injected to a round-bottom flask under argon atmosphere. Compound **5** (2.0 g, 4.88 mmol) was added to the above solution, and the distilled triethylamine (3.5 mL) was finally added. The resulting mixture was stirred at 35 °C until compound **5** was consumed as determined by TLC (EtOAc: Hexane = 1:4). Upon completion, the mixture was allowed to cool to room temperature, filtered through silica gel to desalt, and washed with EtOAc/CH_2Cl_2 (1:1, v/v, 250 mL). Removal of solvent under reduced pressure afforded a residue, which was purified by flash chromatography to afford arylated alkyne **6** as a solid (2.0 g, 97 %).

Analytical TLC (silica gel 60), EtOAc: Hexane = 1:4, R_f = 0.25; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.15 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 4.94 (t, J = 3.4 Hz, 1H), 4.77 (AB system, 2H), 4.55 (t, J = 5.3 Hz, 2H), 3.89–3.72 (m, 1H), 3.62–3.58 (m, 1H), 3.25–3.21 (m, 1H), 2.83 (d, J = 9.1 Hz, 2H),

2.75–2.52 (m, 3H), 1.90–1.45 (m, 7H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.8, 173.0, 164.5, 159.1, 152.4, 140.6, 126.2, 115.1, 114.0, 111.8, 96.9, 90.8, 80.1, 69.9, 62.0, 54.9, 40.1, 36.9, 36.3, 31.5, 30.3, 25.4, 21.6, 19.0, 17.7; HRMS (EI) calcd. for $C_{25}H_{26}O_6$ (M^+) *m/z* 422.1729; found 422.1729.

Preparation of Compound **7**

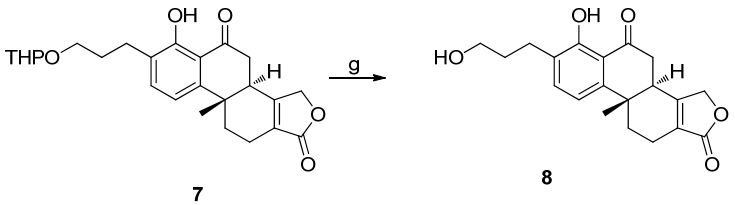


6 7

Compound **6** (400 mg, 0.95 mmol) in ethanol (50 mL) was hydrogenolyzed in the presence of triethylamine (0.6 mL, 4.29 mmol) and 5% Pd/C (400 mg) using a balloon filled with H_2 gas at room temperature. After 12 h, the mixture was heated to 45–50 °C for 2 h. The mixture was filtered through silica gel, washed with EtOAc:CH_2Cl_2 (1:1, v/v, 100 mL × 2), and the filtrate was concentrated to give product **7** without further purification (400 mg, 100 %).

Analytical TLC (silica gel 60), EtOAc: Hexane = 1:4, R_f = 0.30; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.93 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 4.77 (AB system, 2H), 4.59 (t, *J* = 4.2 Hz, 1H), 3.89–3.72 (m, 2H), 3.56–3.38 (m, 2H), 3.17–3.13 (m, 1H), 2.82–2.72 (m, 4H), 2.60–2.30 (m, 3H), 1.95–1.62 (m, 6H), 1.59–1.52 (m, 3H), 1.45 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.1, 162.2, 159.6, 149.5, 137.2, 129.6, 126.1, 115.2, 113.4, 112.0, 98.9, 69.9, 67.0, 62.3, 40.3, 36.4, 36.3, 31.6, 30.7, 29.0, 26.0, 25.4, 21.7, 19.6, 17.7; HRMS (EI) calcd. for $C_{25}H_{30}O_6$ (M^+) *m/z* 426.2042; found 426.2042.

Preparation of Compound **8**



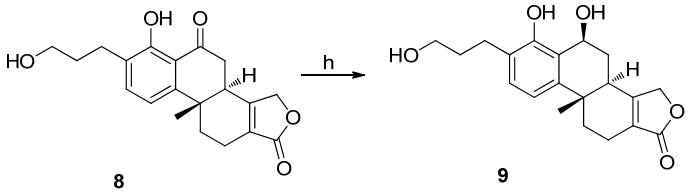
7 8

To a solution of compound **7** (400 mg, 0.95 mmol) in methanol (50 mL) was added TsOH·H_2O (72 mg, 0.38 mmol). The reaction mixture was stirred at room temperature and monitored by TLC (EtOAc:Hexane

= 1:4). After 2 h, the solvent was evaporated under vacuum. The residue was dissolved in 100 mL of dichloromethane, washed with brine (30 mL × 3), and dried over anhydrous Na_2SO_4. The solvent was removed by evaporation, and product **8** (308 mg, 95%) was obtained as a yellow powder, which was purified by flash column chromatography.

Analytical TLC (silica gel 60), EtOAc: Hexane = 1:4, R_f = 0.15; ^{1}H NMR (300 MHz, $CDCl_3$) δ 15.05 (s, 1H), 7.39 (d, J = 7.8 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 4.80–4.76 (m, 2H), 3.66 (t, J = 6.0 Hz, 2H), 3.20–3.16 (m, 1H), 2.82–2.73 (m, 4H), 2.60–2.40 (m, 3H), 1.92–1.83 (m, 4H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.2, 162.1, 159.5, 149.8, 137.6, 129.3, 126.1, 118.2, 114.8, 113.8, 69.9, 61.7, 40.4, 36.5, 36.3, 32.4, 31.6, 25.1, 21.7, 17.7; HRMS (EI) calcd. for $C_{20}H_{22}O_5$ (M^+) *m/z* 342.1467; found 342.1462.

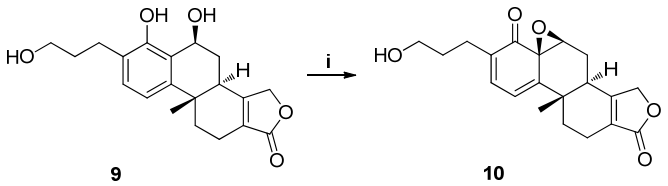
Preparation of Compound **9**



Compound **8** (500 mg, 1.46 mmol) was stirred in the mixture of ethanol (20 mL) and CH_2Cl_2 (5 mL), and treated with $NaBH_4$ (110 mg, 2.92 mmol). After 2 h, the reaction mixture was concentrated, dissolved in 1 M HCl (5 mL) and extracted with CH_2Cl_2 (30 mL × 3), then the organic layer was washed with saturated NaCl solution (30 mL × 1), dried over $NaSO_4$, filtered and concentrated to give colorless crude product **9** (500 mg, 99 %), which has high purity for next step.

Analytical TLC (silica gel 60), EtOAc: Hexane= 4:1, R_f = 0.40; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.50 (br s, 1H), 6.98 (d, J = 7.9 Hz, 1H), 6.81 (d, J = 8.0 Hz, 1H), 6.50 (br, s, 1H), 5.00 (t, J = 7.9 Hz, 1H), 4.89 (AB system, 2H), 4.40 (br s, 1H), 3.41 (t, J = 6.6 Hz, 2H), 2.81 (d, J = 13.1 Hz, 1H), 2.60–2.40 (m, 3H), 2.38–2.12 (m, 3H), 1.95–1.80 (m, 1H), 1.71–1.59 (m, 2H), 1.54 (td, J = 11.4, 6.8 Hz, 1H), 1.02 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.2, 163.4, 154.6, 143.7, 128.5, 126.3, 123.0, 122.2, 114.7, 70.4, 67.9, 60.5, 36.0 (2C), 32.5, 32.1, 29.3, 25.7, 22.8, 17.7; HRMS (EI) calcd. for $C_{20}H_{22}O_4$ (M^+-H_2O) *m/z* 326.1518; found 326.1522.

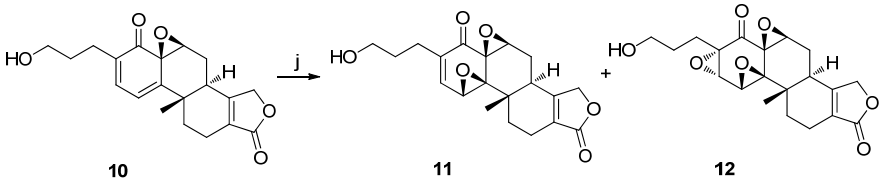
Preparation of Compound **10**



To a solution of compound **9** (100 mg, 0.29 mmol) in methanol (5 mL) at 0°C was added a solution of $NaIO_4$ (93 mg, 0.43 mmol, in 1.0 mL distilled water). The reaction flask was covered with aluminum foil and the reaction was stirred at room temperature and monitored by TLC (EtOAc: Hexane = 4:1). A yellow solution with white precipitate was obtained. After 2 h, the precipitate was filtered by a short silica gel column and rinsed with EtOAc:CH_2Cl_2 (1:1, v/v, 200 mL). The combined filtrates were concentrated, and the residue was separated with flash chromatography to give compound **10** as a solid (80 mg, 80 %).

Analytical TLC (silica gel 60), EtOAc: Hexane= 4:1, R_f= 0.30; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (d, J = 6.6 Hz, 1H), 6.40 (d, J = 6.6 Hz, 1H), 4.70 (AB system, 2H), 4.08 (d, J = 5.3 Hz, 1H), 3.61 (t, J = 6.3 Hz, 2H), 2.62–2.57 (m, 1H), 2.50–2.40 (m, 3H), 2.40–2.24 (m, 2H), 2.24–2.06 (m, 2H), 1.90–1.72 (m, 4H), 1.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.9, 172.9, 159.6, 151.0, 138.5, 135.8, 125.3, 120.7, 69.8, 66.9, 61.5, 57.0, 43.7, 38.3, 32.8, 31.6, 29.6, 24.8, 24.2, 17.5; HRMS (EI) calcd. for $C_{20}H_{22}O_5$ (M^+) *m/z* 342.1467; found 342.1462.

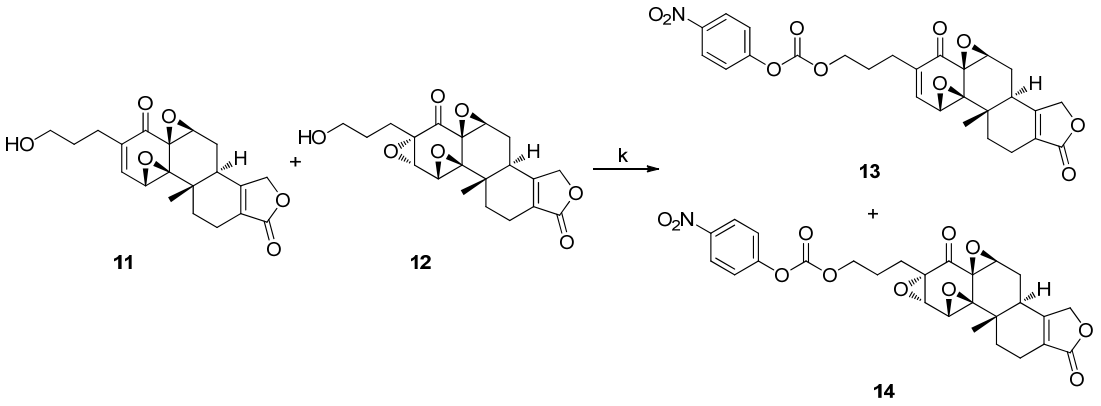
Preparation of Compounds **11** and **12**



To a solution of crude compound **10** (240 mg, 0.701 mmol) in acetonitrile (15 mL) was added an aqueous Na_2(EDTA) solution (4×10^{-4} mol/L, 10 mL). The resulting homogeneous solution was cooled to 0–1°C, followed by the addition of 1,1,1-trifluoroacetone (4.0 mL) in a pre-cooled syringe. To this homogeneous solution was added in portions a mixture of sodium bicarbonate (765 mg, 7.22 mmol) and OXONE (1.73 g, 5.63 mmol) over the period of 1 h (pH 7.0–7.4). The reaction was monitored by TLC (EtOAc: Hexane

= 1:1, v/v). When the reactant was consumed, the mixture was poured into water (30 mL), and extracted with dichloromethane (60 mL × 3). The organic extracts were dried with anhydrous Na_2SO_4, filtered, and concentrated to give a yellow oil for the following step without further purification. Due to the similar polarity of two products **11** and **12** on TLC (80% EtOAc in Hexane as developing solution, R_f = 0.2) and the instability of product **12** under basic conditions, products **11** and **12** could be directly used for next step without further purification.

Preparation of Compounds **13** and **14**



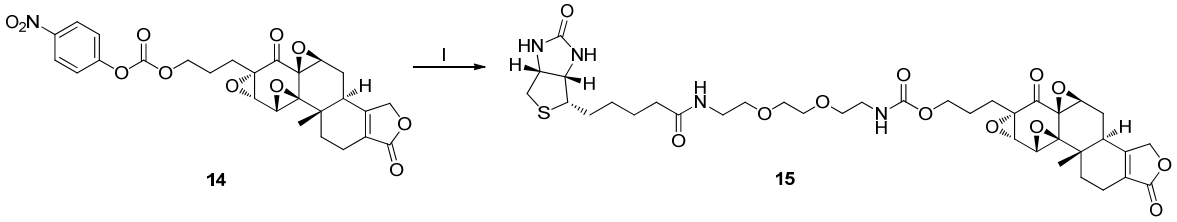
To a solution of the mixture of crude compounds **11** and **12** obtained in the previous procedure in dry THF (10 mL) were added 4-nitrophenyl chloroformate (283 mg, 1.402 mmol) and 113 μL of pyridine. The reaction mixture was stirred under argon at room temperature and monitored by TLC (EtOAc: Hexane = 1:1, v/v). When the reactant was consumed, the solvent was evaporated under vacuum and the residue was dissolved in 100 mL dichloromethane, washed with saturated $NaHCO_3$ solution (30 mL × 3) and brine (30 mL × 3). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was then removed. The crude product was purified by flash chromatography. Evaporation of solvents yielded compounds **13** (140 mg, 39% yield for 2 steps) and **14** (50 mg, 13% yield for 2 steps) as a colorless solid.

Compound **13**: analytical TLC (silica gel 60), EtOAc: Hexane=5:5, R_f = 0.125; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 9.1 Hz, 2H), 7.39 (d, J = 9.1 Hz, 2H), 7.08 (d, J = 4.6 Hz, 1H), 4.69 (AB system, 2H), 4.28 (t, J = 6.4 Hz, 2H), 3.83 (d, J = 4.7 Hz, 1H), 3.62 (d, J = 5.7 Hz, 1H), 2.75 (d, J = 13.5 Hz, 1H), 2.42–2.40 (m, 1H), 2.39–2.25 (m, 2H), 2.22–2.10 (m, 2H), 2.08–1.84 (m, 3H), 1.68 (dd, J = 4.5, 12.5 Hz, 1H), 1.27

(dt, J = 6.0, 12.1Hz, 1H), 1.12 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.8, 173.1, 159.5, 155.4, 152.3, 145.4, 142.9, 140.0, 125.7 (2C), 125.3, 121.7 (2C), 69.9, 68.4, 64.2, 62.3, 60.9, 51.4, 40.8, 35.0, 30.0, 26.1, 25.9, 23.4, 17.0, 13.7; HRMS (EI) calcd. for $C_{20}H_{22}O_6$ (M^+-$NO_2C_6H_5OCO$+H) *m/z* 358.1411, found 358.1416.

Compound **14**: analytical TLC (silica gel 60), EtOAc: Hexane=5:5, R_f= 0.25; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 9.2 Hz, 2H), 7.39 (d, J = 9.2 Hz, 2H), 4.71 (AB system, 2H), 4.30 (t, J = 6.3 Hz, 2H), 4.08 (d, J = 2.7 Hz,1H), 3.91 (d, J = 2.7 Hz, 1H), 3.45 (d, J = 5.4 Hz, 1H), 2.82 (d, J = 13.2 Hz, 1H), 2.40–2.30 (m, 1H), 2.21–2.01 (m, 3H), 1.99–1.92 (m, 2H), 1.90–1.85 (m, 2H), 1.62 (dd, J = 4.5, 12.5 Hz, 1H), 1.27 (dt, J = 6.0, 12.1Hz, 1H), 1.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.1, 173.0, 159.3, 155.4, 152.4, 145.5, 125.8, 125.3 (2C), 121.8 (2C), 69.9, 68.5, 65.3, 63.0, 61.4, 60.7, 55.9, 40.6, 35.4, 30.6, 29.7, 25.3, 23.6, 23.2, 17.1, 13.8; LRMS (ESI) *m/z* 562.1 (M^++Na).

Preparation of Compound **15**

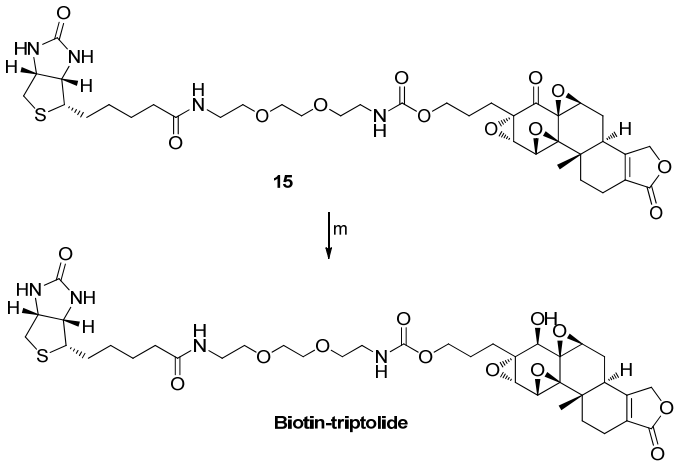


To a solution of biotin-amine (30 mg, 0.0802 mmol) in CH_2Cl_2 (4 mL)/CH_3OH (1 mL) was added compound **14** (20 mg, 0.037 mmol), followed by Et_3N (11 μL, 0.0802 mmol) and DMAP (1 mg, 0.00802 mmol). The mixture was stirred at rt overnight. After that, the mixture was concentrated and purified by column chromatography to give compound **15** as a white solid (10 mg, 24 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.41 (br s, 1H), 5.81 (br s, 1H), 5.54 (br s, 1H), 5.17 (br s, 1H), 4.98–4.69 (m, 2H), 4.49 (t, J = 4.9 Hz, 1H), 4.33 (t, J = 4.7 Hz, 1H), 4.10–4.08 (m, 2H), 3.92 (d, J = 2.6 Hz, 1H), 3.62–3.55 (m, 8H), 3.49–3.43 (m, 3H), 3.42–3.36 (m, 2H), 3.19–3.14 (m, 1H), 2.92 (dd, J = 4.9, 12.9 Hz, 1H), 2.85–2.81 (m, 1H), 2.73 (d, J = 12.9 Hz, 1H), 2.39–2.35 (m, 1H), 2.27–2.08 (m, 4H), 2.04–1.95 (m, 2H), 1.77–1.51 (m, 8H), 1.49–1.39 (m, 3H), 1.37–1.29 (m, 1H), 1.07 (s, 3H); ^{13}C NMR (125 MHz) δ 197.3,

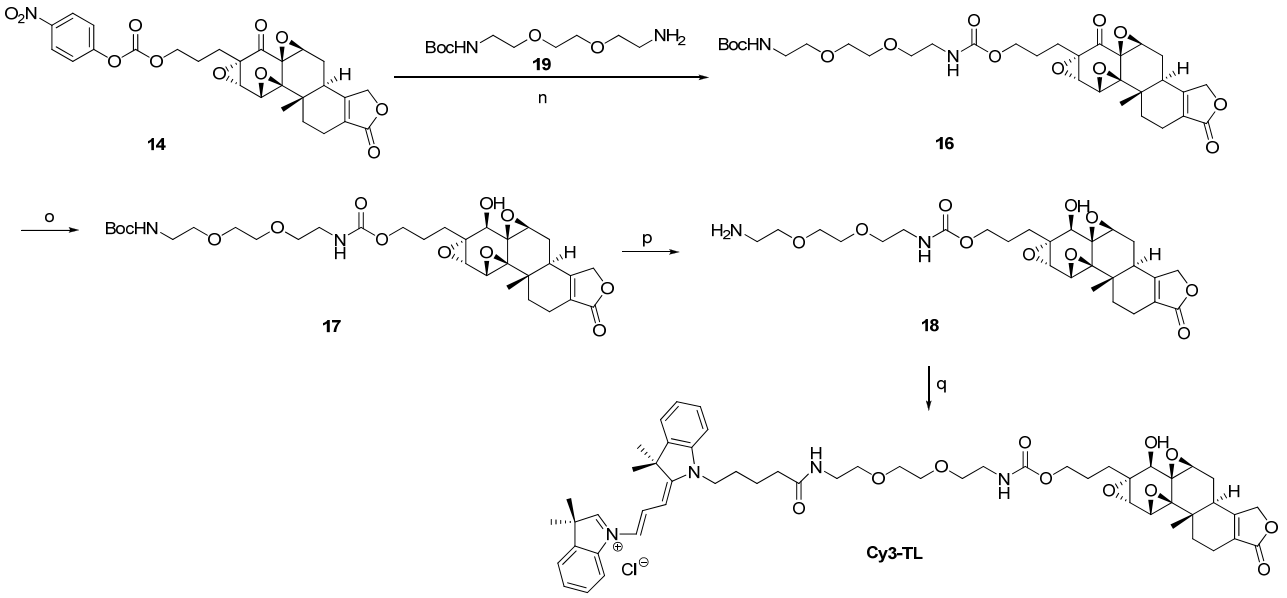
173.1, 173.0, 163.3, 159.4, 156.6, 125.8, 70.2, 70.0(4), 69.9(8), 69.9(1), 65.4, 64.1, 63.3, 61.8, 61.1, 60.8, 60.1, 56.2, 55.3, 55.3, 40.8, 40.6, 40.5, 39.2, 35.9, 35.4, 30.6, 29.7, 28.5, 28.1, 25.7, 25.5, 24.1, 23.2, 17.1, 13.9; LRMS (ESI) *m/z* 797.1 (M^++Na).

Preparation of Biotin-triptolide (Biotin-TL)



To a solution of compound **15** (10 mg, 0.0129 mmol) in THF (2.5 mL) was added K-Selectride solution (1 M in THF, 50 μL, 0.05 mmol) at –40 °C. The reaction was stirred for 3 h at –40 °C. After completion of reaction, the mixture was concentrated and purified by column chromatography to give biotin-triptolide as a white solid (4 mg, 39 %).

Analytical TLC (silica gel 60), CH_2Cl_2: CH_3OH= 9:1, R_f= 0.15; ^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD) δ 4.75 (AB system, 2H), 4.51 (dd, J = 4.9, 7.8 Hz, 1H), 4.32 (dd, J = 4.6, 8.3 Hz, 1H), 4.14–4.08 (m, 1H), 4.07–4.02 (m, 1H), 3.65–3.62 (m, 5H), 3.61–3.52 (m, 4H), 3.44–3.38 (m, 3H), 3.37–3.34 (m, 3H), 3.20–3.15 (m, 1H), 2.93 (dd, J = 5.0, 12.9 Hz, 1H), 2.74 (d, J = 12.8 Hz, 2H), 2.33–2.29 (m, 1H), 2.26–2.20 (m, 3H), 2.19–2.13 (m, 1H), 2.00–1.90 (m, 1H), 1.76–1.53 (m, 9H), 1.48–1.33 (m, 3H), 1.30–1.23 (m, 1H), 1.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD) δ 174.3, 164.3, 161.7, 161.2, 157.4, 125.4, 73.5, 70.5, 70.3, 70.0, 69.0, 66.5, 65.7, 64.8, 63.5, 62.4, 62.1, 60.6, 60.3, 56.5, 55.8, 55.3, 40.6, 40.5, 39.7, 39.2, 36.0, 35.9, 30.3, 29.8, 28.6, 28.3, 25.6, 24.3, 23.7, 17.2, 13.7; HRMS (ESI) calcd. for $C_{37}H_{53}N_4O_{12}S$ (M^++H) *m/z* 777.3375, found 777.3380.

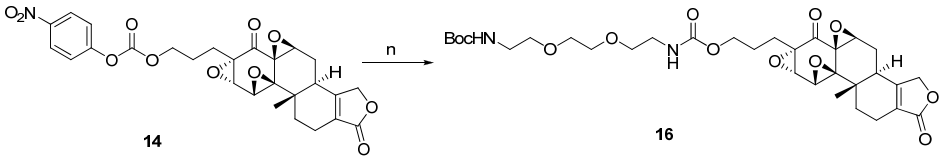


Scheme S2. Chemical synthesis of cyanine 3-triptolide (Cy3-TL)

Reagents and conditions: n) DMAP, Et_3N, CH_2Cl_2, rt, 85 %; o) K-Selectride, anhydrous THF, −78 °C; p) 30 % TFA in CH_2Cl_2 (v/v), rt; q) Cy3-NHS, CH_2Cl_2, DMAP, rt, 42 %.

Compound **19** was synthesized according to procedure described in C. Gnaccarini *et al. Bioorg. Med. Chem.* 2009, *17*, 6354–6359.

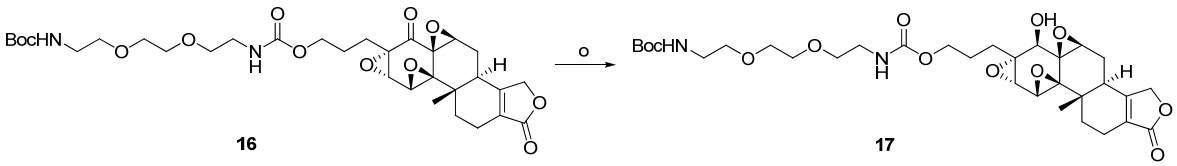
Preparation of compound **16**



To a mixture of **14** (50 mg, 0.093 mmol) and compound **19** (28 mg, 0.111 mmol) in CH_2Cl_2 (5 mL) was added one small crystal of DMAP. The reaction mixture was stirred at room temperature overnight under argon, after which time TLC analysis (CH_2Cl_2:CH_3OH = 15:1) indicated the reaction was complete. CH_2Cl_2 (45 mL) was added to the crude mixture, and the mixture was washed with $NaHCO_3$ solution until the water layer was colorless. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was then removed to give a crude product, which was purified by flash column chromatography to afford product **16** as a white solid (51 mg, 85% yield).

Analytical TLC (silica gel 60), 10% MeOH in CH_2Cl_2,, R_f = 0.46; ^{1}H NMR (300 MHz, CD_3OD) δ 4.85 (AB system, 2H), 4.25 (d, *J* = 2.9 Hz, 1H), 4.09–4.02 (m, 2H), 3.61 (apparent s, 5H), 3.52 (q, *J* = 6.1 Hz, 5H), 3.43 (d, *J* = 5.4 Hz, 1H), 3.30–3.26 (m, 1H), 3.23 (t, *J* = 5.6 Hz, 2H), 2.94–2.90 (m, 1H), 2.38–2.26 (m, 2H), 2.14–2.11 (m, 1H), 1.97–1.89 (m, 2H), 1.82–1.68 (m, 3H), 1.57–1.49 (m, 2H), 1.44 (s, 9H), 1.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.1, 173.0,159.4, 156.4, 155.9, 125.6, 79.2, 70.2 (2C), 70.1, 70.0, 69.9 (2C), 65.2, 64.0, 63.2, 61.0, 60.6(2), 60.5(6), 40.7, 40.5, 40.3, 35.3, 30.5, 28.4, 25.5, 24.0, 23.2, 17.0, 13.8 (Boc); LRMS (ESI) *m/z* 671.2 (M^++Na).

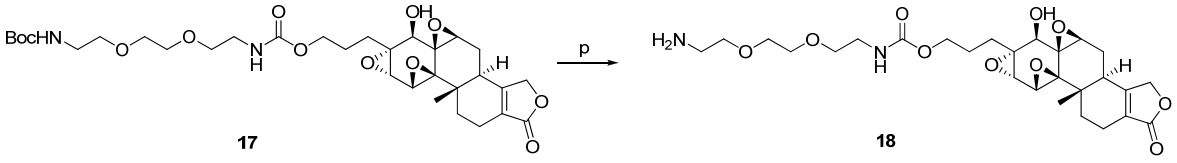
Preparation of Compound **17**



16 17

To a solution of compound **16** (51 mg, 0. 0787 mmol) in THF (2 mL) at −78°C under argon atmosphere was added K-Selectride. The reaction was stirred for 2 h, and then the reaction was quenched by saturated NH_4Cl aqueous solution. The mixture was extracted with dichloromethane (50 mL × 3) and washed with brine (20 mL × 3). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated, and separated by flash column chromatography to afford **17** as a white powder (50 mg, 100%), which was used directly in the following step without further purification.

Preparation of Compound **18**

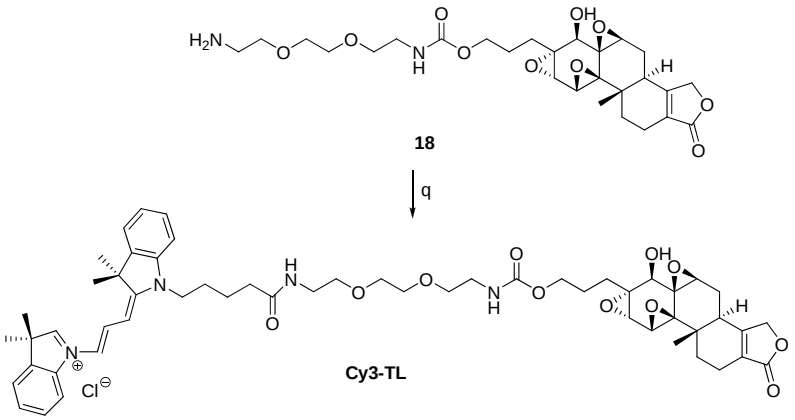


17 18

To a solution of compound **17** (50 mg, 0.0768 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (0.3 mL). The reaction mixture was stirred at room temperature for 1 h. Saturated $NaHCO_3$ solution (5 mL) was added to the mixture and stirred for another 30 min. The resulting mixture was extracted with

dichloromethane, then the organic layer was dried over anhydrous $NaSO_4$, filtered, concentrated to give crude product **18** as a yellow oil (50 mg), which was used for the next step without further purification.

Preparation of Cyanine 3-TL (Cy3-TL)



To a solution of compound **18** (15 mg, 0.027 mmol) in CH_2Cl_2 (1 mL) were added Cy3-NHS (15 mg, 0.023 mmol) and one small crystal of DMAP. The reaction mixture was stirred at room temperature overnight, followed by concentration *in vacuo* and purification by flash column chromatography to afford Cy3-TL as a red solid (10 mg, 42 %).

Analytical TLC (silica gel 60), CH_2Cl_2: CH_3OH = 9:1, R_f = 0.43; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (br s, 1H), 8.40 (t, J = 13.5 Hz, 1H), 7.50–7.30 (m, 5H), 7.32–7.20 (m, 2H), 7.18–7.11 (m, 3H), 6.02 (br s, 1H), 4.68 (AB system, 2H), 4.20–4.10 (m, 2H), 3.90–3.85 (m, 2H), 3.84 (dd, J = 4.8 Hz, 1H), 3.82–3.79 (m, 3H), 3.75–3.71 (m, 1H), 3.64–3.50 (m, 8H), 3.49–3.44 (m, 3H), 3.40–3.33 (m, 4H), 1.69–1.64 (m, 1H), 2.61 (s, 2H), 2.36–2.27 (m, 1H), 2.26–2.05 (m, 2H), 2.00–1.80 (m, 6H), 1.73–1.71 (m, 14H), 1.65–1.58 (m, 1H), 1.53 (dd, J = 4.5, 12.0 Hz, 1H), 1.30–1.24 (m, 1H), 1.10–1.07 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 173.8, 173.7, 173.3, 160.2, 157.0, 151.0, 142.8, 141.9, 140.6, 140.5, 129.0, 128.9, 125.5, 125.4, 125.3, 122.0, 122.0, 111.2, 110.8, 105.2, 104.6, 73.5, 70.6, 70.3, 70.1(3) (2C), 70.0(8), 70.0, 69.8 (2C), 65.8, 64.0, 62.4, 60.6, 60.2, 56.5, 55.4, 49.0, 48.8, 44.1, 40.8, 40.4, 39.0, 35.8, 35.4, 32.1, 29.8, 28.2, 28.1, 27.1, 23.7, 23.3, 22.6, 17.1, 13.6; HRMS (ESI) calcd. for $C_{56}H_{71}N_4O_{11}$ (M^+–Cl) *m/z* 975.5114, found 975.5091.

General Methods and Materials

Cell Culture. MDCK, HeLa, Hela S3 and HEK293T cells were cultured in Dulbecco's modified Eagle's media (Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin. HCT116 cells were cultured in McCoy's 5A media (Gibco) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were maintained in a humidified 37 °C incubator with 5% CO_2.

Target Pull-down Experiment. Cells were washed with PBS and harvested by trypsinization. After centrifuging, the cell pellets were resuspended in ice-cold lysis buffer (0.1% Triton X-100, 150 mM NaCl, 10% Glycerol, 0.2 U Benzonase, Roche EDTA-free protease inhibitor cocktail, 20 mM Tris-HCl, pH 8.0). The supernatant was filtered through 0.45 μm membrane to remove insoluble fractions. Lysate was then incubated with 5 μM Biotin-TL overnight at 4 °C with rotation. Strep-Tactin Superflow agarose beads (Qiagen) were washed and incubated with the mixture for 2 h at 4 °C. The beads were then washed intensively with lysis buffer. Following elution by incubating with 1 mM biotin, the bound proteins were resolved in SDS-PAGE (10% tris-glycine gel) and visualized by silver stain.

Expression and Purification of Recombinant Protein. Full length human Prx I coding sequence was cloned into pTT5SH8Q2 vector for mammalian expression and pET52b for *Escherichia coli* expression, respectively. Human embryonic kidney 293T (HEK293T) cell line was transiently transfected with plasmids using polyethyleneimine (PEI, Polysciences). Cells was harvested 40 h after transfection by centrifugation at 150x g for 5 min. Transfected cells were lysed with lysis buffer (0.1% Triton X-100 in 150 mM NaCl, protease inhibitor cocktail from Roche, 20 mM Tris-HCl, pH 8.0). Proteins for gel filtration and chaperone activity assay were expressed in *E. coli* host cell BL21/DE3 over night at 20 ºC in the ZYM-5052 auto-induction medium. After cell lysis and centrifugation, the streptag II-fusion proteins were purified by Strep-Tactin Superflow agarose (Qiagen) followed by tag cleavage using PreScission protease. Then the tag removed recombinant Prx I protein was further purified using anion exchange (Mono Q, GE Healthcare) and gel-filtration chromatography (Superdex 200, GE Healthcare).

In-gel Fluorescence Scan. 10 μM recombinant Prx I protein was incubated with 4 μM Cy3-TL in the absence or presence of triptolide at indicated concentration at 4 °C. The mixture was then resuspended in LDS sample loading buffer (Invitrogen) and resolved on 10% SDS-PAGE. Fluorescence was visualized by scanning the gel using Typhoon 9410 variable mode imager (excitation 546 nm, emission filter 580 nm).

Western Blot. 1 μM recombinant Prx I protein or its mutant was incubated with 100 nM Biotin-TL at 4 °C for 3 h before SDS-PAGE separation. The resolved proteins were transferred onto a PVDF membrane. After blocking in membrane blocking solution (Invitrogen), proteins were blotted with streptavidin-HRP (Abcam) for detection of biotin-TL or primary antibody rabbit anti-Prx I (Abcam ab41906) and goat anti-rabbit-HRP secondary antibody (Santa Cruz, sc-2030) for detection of Prx I, respectively. The bands were visualized with ECL reagent (Thermo). Western blot intensities were measured using ImageJ. Quantification was performed on band intensities normalized with loading control.

MALDI-TOF/TOF Analysis. Gel plugs were destained, washed and then reduced with 10 mM DTT and treated with 55 mM iodoacetamide in the dark to alkylate all reduced cysteines. Then the mixture was diluted with 10 mM ammonium biocarbonate before digestion. 12.5 ng/μL of sequencing grade trypsin (Promega) was added to cover gel plugs and incubated at 4 °C. After 30 minutes, trypsin was removed and replaced with 20 μL of 10 mM ammonium bicarbonate and incubated at 37 ℃ overnight. Digestion was stopped by adding 2 fractions of 5% formic acid/50% acetonitrile and dehydrated with 100% acetonitrile. The extracted peptide mixtures were dried down by SpeedVac and then resuspended in 0.1% formic acid and followed by μC18 ZipTip.

α-Cyano-4-hydroxycinnamic acid (CHCA) was used as the MALDI matrix at a concentration of 10 mg/mL in 50% water/acetonitrile and 0.1% formic Acid. Samples were spotted and dried before applying matrix. All mass spectra were acquired on a 4800 MALDI TOF/TOF Analyzer (ABSciex, Framingham, MA) in positive ion reflector mode. Typical spectra were obtained by averaging 500 acquisitions in Reflector mode and 100-160 acquisitions in MSMS mode with the minimum possible laser energy in order to maintain the best resolution. Precursor ions with a charge state of 1+ were fragmented via post source decay (PSD). Peak

list was generated by Data Explorer (ABSciex, Framingham, MA) and searched using Mascot (version 2.1.0, Matrix Science, London, UK). Database search settings were as follows: +1 monoisptopic peaks were searched with a mass tolerance of 75 ppm for precursor masses; ±0.2 Da for MS/MS. Trypsin was selected as the enzyme while allowing one missed cleavage, cysteine carbamidomethylation as fixed modification and methionine oxidation as variable modification. Human taxonomy filter was applied when searching against SwissProt database downloaded on 6th January, 2010.

LC-MS/MS Mass Spectrometry Analysis. 1 μg recombinant Prx I protein incubated with 10 μM TL at 4 ℃ overnight was subjected to in-solution digestion. Following denaturing in 8 M urea, the protein complex was heated, reduced with 4 mM DTT and treated with 8 mM iodoacetamide in dark to alkylate all reduced cysteines. Then the mixture was diluted with 50 mM ammonium biocarbonate to reach final 0.5 M urea before digestion. 50 ng sequencing grade trypsin (Promega) was incubated with the mixture at 37 ℃ overnight in oven. Digestion was stopped by adding 1% formic acid. The resulting peptides were enriched with OASIS C18 plate (Waters) and eluted in 50% acetonitrile and 5% formic acid. The samples were dried down by SpeedVac and then resuspended in 0.1% formic acid for analysis by LC-MS/MS.

Resultant peptides were analyzed on an ETD equipped Orbitrap Velos instrument (Thermo Fisher Scientific, Bremen) connected to an LC system. The LTQ-Orbitrap Velos instrument was operated in the data-dependent mode to automatically switch between MS and MS/MS. Precursor ions with a charge state of 2+ or higher were fragmented by HCD or ETD under control of an in-house developed Data Dependent Decision Tree[1]. All generated peak lists were searched against IPI human database (ipi. HUMAN.v.3.68.fasta) using Maxquant (version 1.2.2.5). The database search was performed with the following parameters: a mass tolerance of 6 ppm for precursor masses; ±0.5 Da for MS/MS, allowing two missed cleavages, cysteine carbamidomethylation as fixed modification and methionine oxidation, triptolide addition (360.1573 Da) as variable modifications. The enzyme was specified as trypsin. A careful manual verification was used to ensure correct peptide identification.

Native Mass Spectrometry Analysis. As described previously[2], aliquots of approximately 2 μL of samples

were loaded into gold-plated borosilicate capillaries made in-house (Sutter P-97 puller, Sutter Instruments Co., Novato, CA an Edwards Scancoat six sputter-coater, Edwards Laboratories, Milpitas, CA) for analysis on a LCT 1 mass spectrometer (Waters Corp., Milford, MA). A capillary voltage of 1200 V was applied to allow optimal electrospray, in conjunction with a sampling cone voltage around 125 V. The source backing pressure was elevated in order to promote collisional cooling to approximately 7.5 mbar. A 300 to 10,000 m/z range was scanned to record the spectra. CsI clusters were used to calibrate spectra every day. Data was analyzed by MassLynx 4.1 and further processed with Origin 8.

Size-exclusion Chromatography. Size-exclusion chromatography was performed at 4 ºC using the fast protein liquid chromatography system (Bio-rad) with a Superdex 200 10/300 GL column (GE). Ribonuclease (13.7 kDa), carbonic anhydrase (29 kDa), ovalbumin (44 kDa), conalbumin (75 kDa), aldolase (158 kDa), ferritin (440 kDa) were used as molecular mass standard markers for calibration of the column. Column was equilibrated at a flow rate of 0.5 ml/min at 4 °C with 20 mM Tris-HCl, pH 8.0 containing 150 mM NaCl. Elution of the recombinant Prx I with or without TL treatment was monitored at 280 nm at a flow rate of 0.5 ml/min.

Peroxidase Activity Assay. Peroxidase activity of the Prx I with indicated treatment was measured as described previously.[3] The yTrx and yTrxR recombinat proteins were purchased from Abfrontier. 8 μM Recomibiant mamalian cell expressed hPrx I was first incubated with indicated compounds at 4 degree for overnight. To measure enzyme activity, 1 μg was mixed with 2.25 μM yTrx, and 1 μM yTrxR in 100 μL 50 mM Hepes (pH 7.0) containing 200 μM NADPH in 96-well plate. The reaction mixture was incubated at 30 °C for 5 min, followed by the addition of 100 μM H_2O_2 to start the reaction. NADPH oxidation was monitored for the next 10 min by a decrease in absorbance at 340 nm measured with the plate reader (Beckman Coulter DTX880). Activity measured without Prx I was subtracted as background activity.

Chaperone Activity Assay. Molecular chaperone activity was determined by assessing the ability of the recombinant Prx I to inhibit the thermal aggregation of substrate protein MDH. 10 μM of MDH was mixed with 2.5 μM Prx I in absence or presence of test compound, in a degassed buffer (100 mM Tris, 150 mM

KCl, 20 mM $Mg(OAc)_2$, pH 7.5) as previously described[4]. The reaction mixture was incubated at 45 °C for 30 min, and the increase of light scattering as a result of thermal aggregation of substrate proteins was monitored by using a DynaPro DLS Plate Reader (Wyatt Technology, Santa Barbara, CA, USA). For data analysis, curves were plotted and fitted using GraphPad Prism software.

Supplementary Data

Figure S1. Silver stained gels of pull-down products of Biotin-TL using HeLa, HCT116 and HEK293T cells. Arrows indicate protein bands confirmed as Prx I by MALDI-TOF MS analysis.

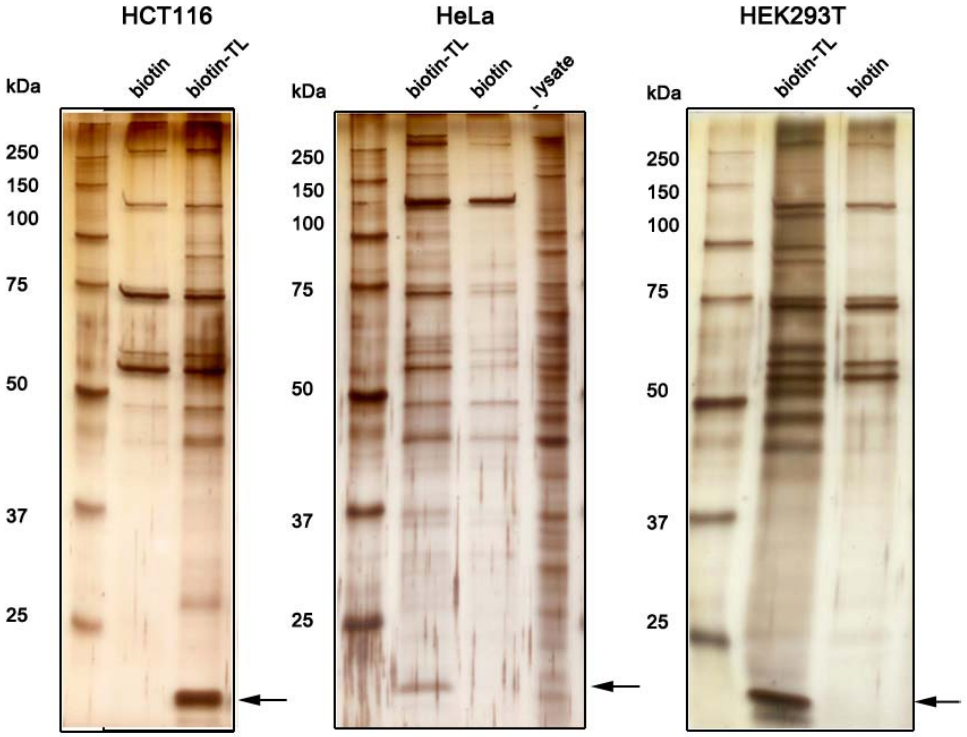


Figure S2. A silver stained gel of pull-down products of Biotin-TL in the absence or presence of TL. HeLa cell lysate was incubated with 5 μM Biotin-TL in the absence or presence of 500 μM TL. Proteins were then purified using streptavidin-conjugated agarose beads, followed by SDS-PAGE analysis. The arrow indicates a band confirmed as Prx I.

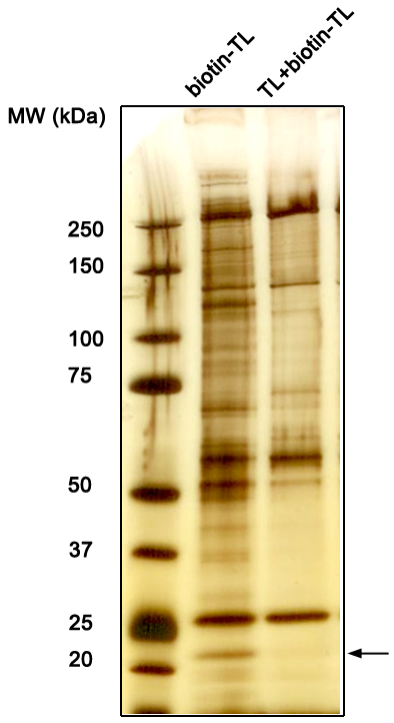


Figure S3. MALDI-TOF/MS analysis confirms the identity of 23-kDa protein as Prx I.

The identified peptides are highlighted in red. Mascot protein score= 121, Protein Score C. I. % = 100.

1 MSSGNAK**IGH PAPNFKATAV MPDGQFKDIS LSDYK**GKYVV FFFYPLDFTF

51 VCPTEIIAFS DRAEEFKKLN CQVIGASVDS HFCHLAWVNT PK**KQGGLGPM**

101 **NIPLVSDPKR TIAQDYGVLK ADEGISFRGL FIIDDKGILR QITVNDLPVG**

151 **R**SVDETLR**LV QAFQFTDK**HG EVCPAGWKPG SDTIKPDVQK SKEYFSKQK

Figure S4. SDS-PAGE analysis of recombinant thioredoxin incubated with Cy3-TL. 400 ng Prx I, thioredoxin (Trx) and thioredoxin reductase (TrxR) were incubated with 1 μM Cy3-TL at 4 °C for 1 h before SDS-PAGE.

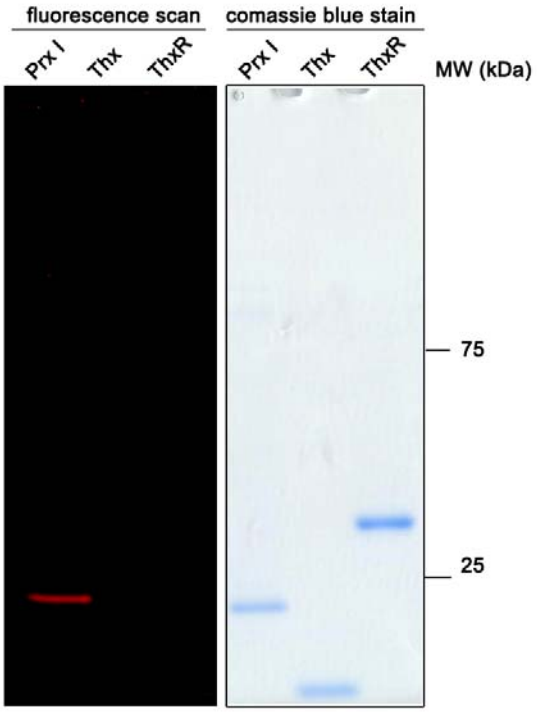


Figure S5. Triplicate western blot analyses of recombinant hPrx I and mutants after incubation with Biotin-TL.

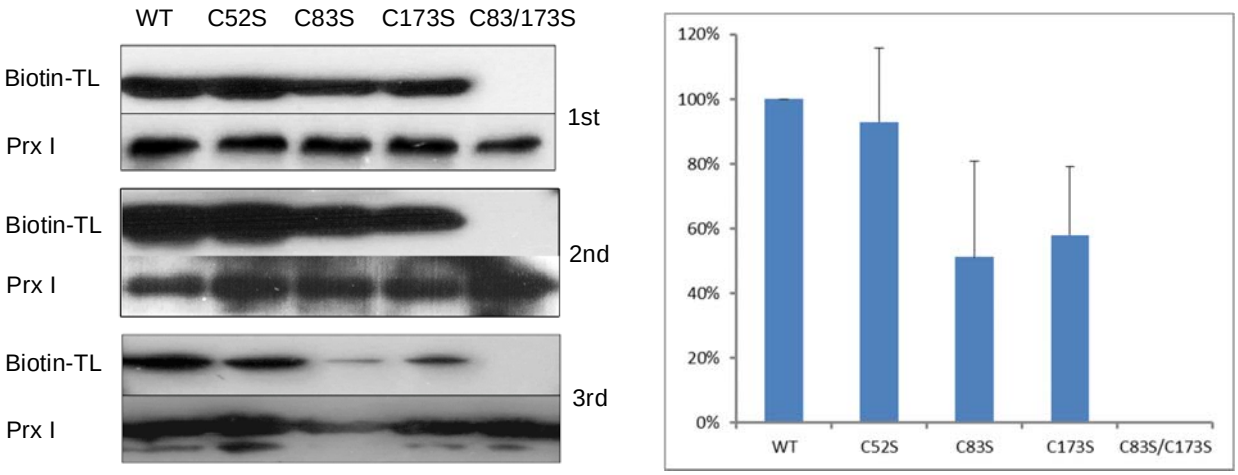


Figure S6. Kinetics curves show enzymatic activity of recombinant Prx I, in the presence or absence of TL or adenanthin.

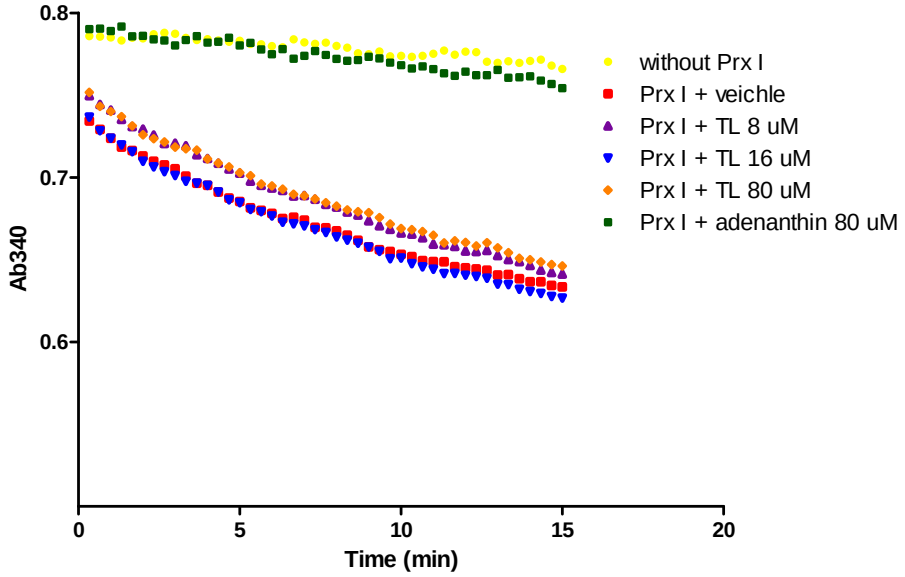


Figure S7. Native mass spectrometry analysis on intact wildtype Prx I or mutants in absence or presence of Cel and WA

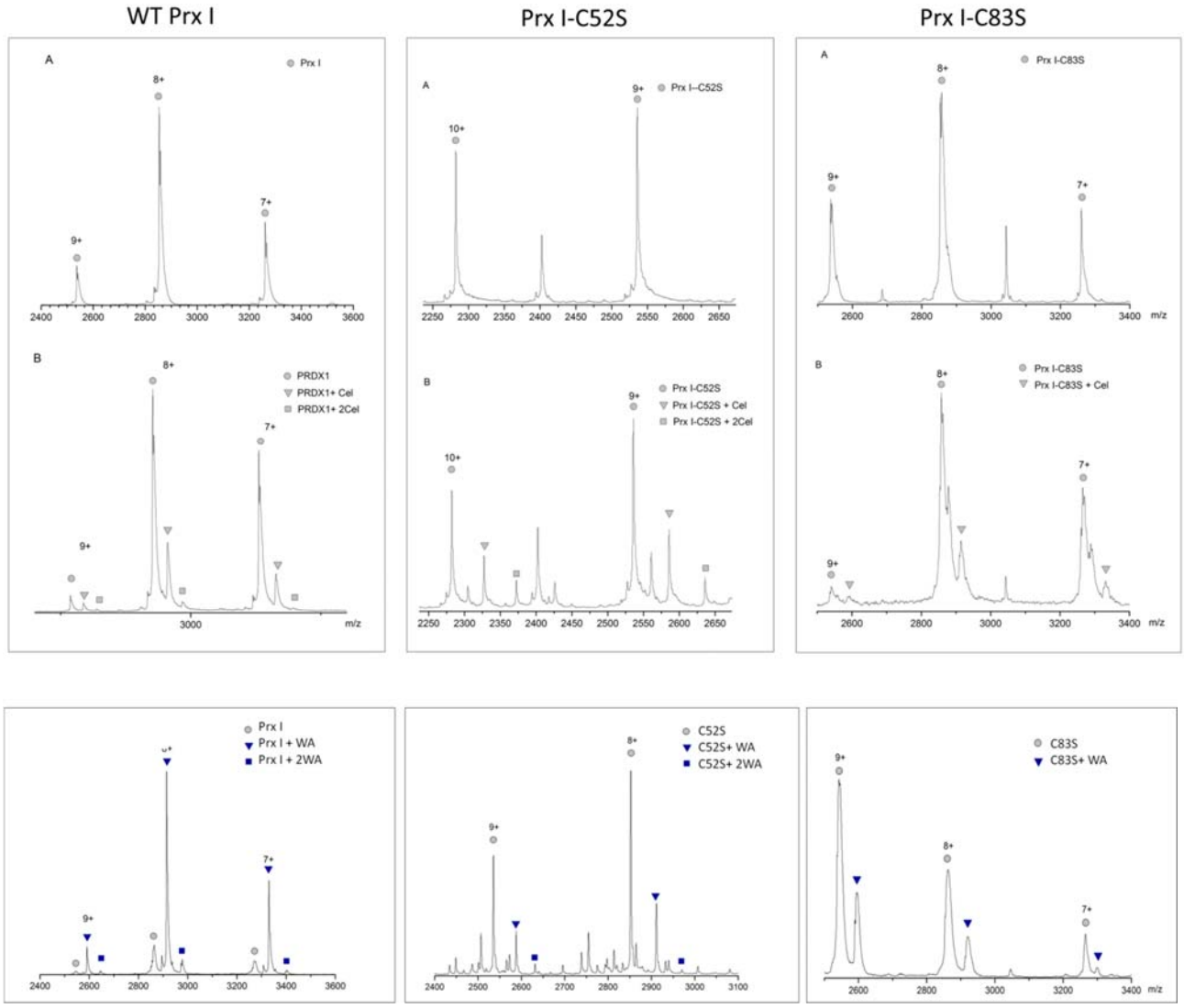
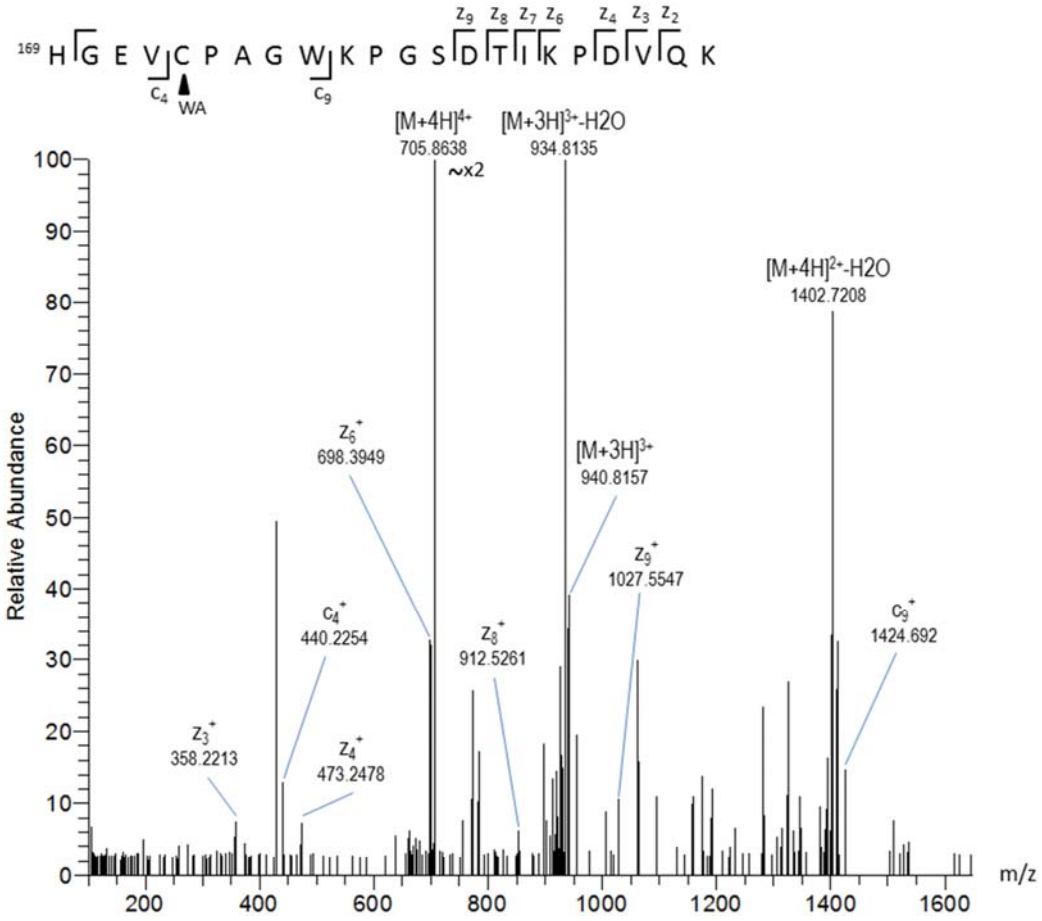


Figure S8. LC-MS/MS analysis of tryptic peptide ^{169}HGEVC^{WA}PAGWKPGSDTIKPDVQK^{190} from WA-treated Prx I. The ETD fragment ions are manually checked and annotated.



Assignment	[M+H]$^{1+}$		
	Exact m/z	Experimental m/z	Error (mmu)
z2	259.1527	259.1525	-0.2
z3	358.2211	358.2213	0.2
c4	440.2252	440.2254	0.2
z4	473.2480	473.2478	-0.2
z6	698.3957	698.3949	-0.8
z7	811.4798	811.4764	-3.4
z8	912.5275	912.5261	-1.4
z9	1027.5544	1027.5547	0.3
c9	1424.6919	1424.6920	0.1
[M+3H]$^{3+}$	940.4748	940.4810	6.2
[M+4H]$^{4+}$	705.6062	705.6129	6.7

Figure S9. Aggregation of MDH in absence of Prx I and in presence of TL, Cel and WA as monitored by light scattering.

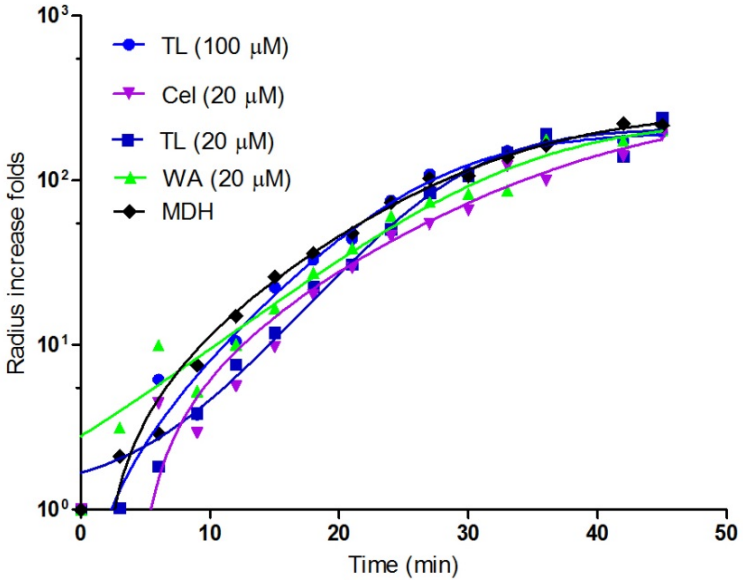


Figure S10. Monitoring aggregation of Prx I in absence of MDH and in presence of TL, Cel and WA by light scattering.

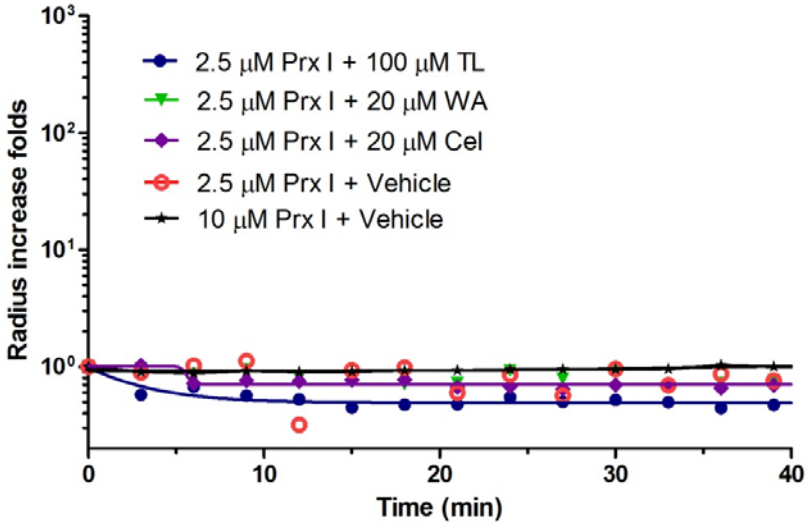


Table S1. Observed species in native mass spectrometry analysis of intact Prx I monomer and dimer in absence or presence of TL. hPrx I recombinant protein (10 μM) was incubated with DMSO or 40 μM TL at 4 °C overnight. Unbound small molecules were removed using desalting column before MS analysis.

Complex Assignment	Measured Mass
Prx I (monomer)	22823.95 ± 3.32
Prx I + TL (monomer)	23183.41 ± 4.93
Prx I + 2TL (monomer)	23544.55 ± 7.55
Prx I (dimer)	45685.39 ± 8.95
Prx I + TL (dimer)	46052.46 ± 4.22
Prx I + 2TL (dimer)	46402.91 ± 6.64

Table S2. HCD MS/MS analysis: a summary of the assigned fragment ion peaks in Figure 2a of a quaternary charged tryptic peptide, ^{169}HGEVCTLPAGWKPGSDTIKPDVQK^{190}, from the TL-treated Prx I.

Assignment	$[M+H]^{1+}$			$[M+2H]^{2+}$		
	Exact m/z	Experimental m/z	Error (mmu)	Exact m/z	Experimental m/z	Error (mmu)
b2	195.0877	195.0880	0.3			
b3	324.1302	324.1299	0.3			
b4	423.1987	423.1981	-0.6			
b5	886.3651	886.3621	-3.0			
b6				492.209	492.2197	10.7
b7	1054.4550	1054.4514	-3.6			
b8	1111.4765	1111.4723	-4.2			
b9	1297.5558	1297.5548	-1.0			
b10	1425.6507	1425.6293	-21.4			
b14				891.3920	891.3969	5.0
b15-H2O				932.9106	932.9089	-1.6
b16-H2O				989.4526	989.4588	6.3
b16				998.4579	998.4583	0.5
y2	275.1714	275.1701	-1.3			
y3	374.2398	374.2397	-0.1			
y5	586.3195	586.3205	1.0			
y5-H2O	568.3089	568.3098	0.9			
y6	714.4145	714.4135	-1.0			
y7	827.4985	827.5103	11.8			
y8				464.7731	464.776	2.9
y12	1284.6794	1284.6689	-10.5	642.8397	642.8427	3.0
y12-H2O				633.8344	633.8408	6.4
y13				706.8872	706.8895	2.3
y14				799.9269	799.928	1.2
y16				863.9562	863.9618	5.7
y16-NH3				855.4429	855.4129	-29.9
y17				912.4825	912.4859	3.4
$[M+4H]^{4+}$	678.0807	678.0865	5.8			

Table S3. HCD MS/MS analysis: summary of the assigned fragment ion peaks in Figure 4d of a tryptic peptides, ^{79}DSHFC^{WA}HLAWVNTPK^{92}, from the WA-treated Prx I.

Assignment	$[M+H]^{1+}$			$[M+2H]^{2+}$		
	Exact m/z	Experimental m/z	Error (mmu)	Exact m/z	Experimental m/z	Error (mmu)
y2	244.1656	244.165543	-0.1	122.5864		
y3	345.2132	345.212891	-0.3	173.1102		
y4	459.2562	459.256042	-0.2	230.1317		
y5	558.3246	558.32489	0.3	279.6659		
y6	744.4039	744.403564	-0.3	372.70555		
y7	815.4410	815.440308	-0.7	408.2241		
y8	928.5251	928.523926	-1.2	464.76615	464.765594	-0.6
b6-H2O	1179.5179			590.2626	590.262329	-0.2
b6	1197.5285	1197.526001	-2.5	599.2679	599.267761	-0.1
b7	1310.6126			655.8099	655.809387	-0.5
b8	1381.6497			691.3285	691.327881	-0.6
y10	1638.8600			819.9336	819.93512	1.5
b12	1881.8880			941.4476	941.444153	-3.4
y12	1922.9873			961.99725	961.994507	-2.7
y13-H2O	1992.0088	1992.011353	2.6	996.508		
[y13]$^{3+}$	670.6779	670.677124	-0.8			
[M+2H]$^{2+}$	1063.0268	1062.969604	-57.1			
[M+3H]$^{3+}$-H2O	703.0167	703.015503	-1.2			
[M+3H]$^{3+}$	709.02023	709.019836	-0.4			

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