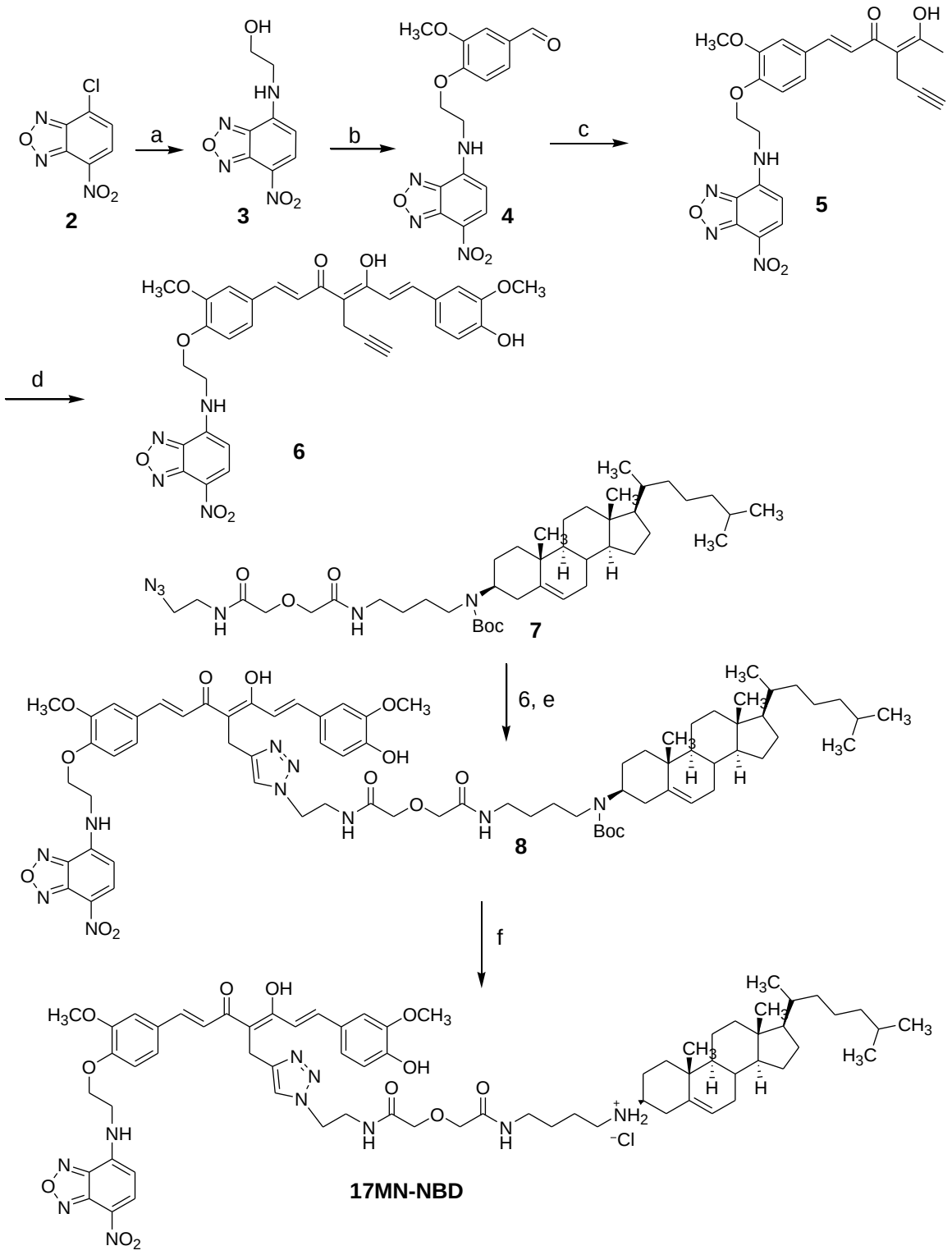


Reagents and conditions: a) Hydroxyethylamine, ACN; b) Vanillin, DIAD, Ph_3P, THF; c) 3-propargylacetylacetone, B_2O_3, $B(OBu)_3$, $BuNH_2$, EA; d) B_2O_3, $B(OBu)_3$, piperidine, EA; e) $CuSO_4$, sodium ascorbate, THF/H_2O 1/1; f) HCl-dioxane, THF.

Preparation of compound **3**

Hydroxyethylamine (0.36 mL) was added to a solution of compound **2** (0.6 g, 3 mmol) in 20 mL of ACN and was stirred at room temperature overnight. Solvent was then removed under vacuum. The

residue was purified by flash column chromatography (CH_2Cl_2/acetone: 10/1) to give compound **3** as an orange solid (342 mg, 51% yield). ^{1}H NMR (400 MHz, DMSO) δ 9.39 (s, 1H), 8.52 (d, J = 8.7 Hz, 1H), 6.47 (d, J = 8.7 Hz, 1H), 4.93 (s, 1H), 3.69 (t, J = 5.6 Hz, 2H), 3.56 (s, 2H).

Preparation of compound **4**

Compound **3** (0.342 g, 1.5 mmol), vanillin (0.47 g, 3 mmol), and triphenylphosphine (0.81 g, 3 mmol) were added together in 20 mL of THF. To this solution DIAD (0.604 mL) was added dropwise, and the solution was stirred at room temperature overnight. Water was then added to quench. The product was extracted into ethyl acetate and washed with aqueous $NaHCO_3$ followed by brine. The organic layer was collected and dried over anhydrous $NaSO_4$. Solvent was then removed under vacuum. The residue was purified by flash column chromatography (CH_2Cl_2/ethyl acetate: 10/1) to give compound **4** as an orange solid (60 mg, 31% yield). ^{1}H NMR (400 MHz, DMSO) δ 9.84 (s, 1H), 9.57 (s, 1H), 8.55 (d, J = 8.8 Hz, 1H), 7.54 (dd, J = 8.2, 1.8 Hz, 1H), 7.38 (s, 1H), 7.22 (d, J = 8.3 Hz, 1H), 6.58 (d, J = 8.9 Hz, 1H), 4.41 (t, J = 5.2 Hz, 2H), 3.94 (s, 2H), 3.78 (s, 3H).

Preparation of compound **5**

Boric anhydride (0.07 g, 1 mmol) was added to a solution of 3-propargylacetylacetone (0.138 g, 1 mmol) in 5 mL of ethyl acetate and stirred at 80 °C for 30 min. Tributylborate (0.135 mL, 0.5 mmol) was then added followed by compound **4** (0.073 g, 0.2 mmol) and stirred at 80 °C for 30 min. *n*-Butylamine (0.05 mL, 0.5 mmol) was added and the solution was refluxed for 2 h. After the solution was cooled to 50 °C, 4 mL of 0.5 M HCl was added and the solution was stirred at 50 °C for 30 min. The reaction was then filtered, and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography (CH_2Cl_2/ethyl acetate: 10/1) to afford compound **5** as a yellow solid (0.048 g, 50% yield). ^{1}H NMR (400 MHz, CDCl3) δ 8.51 (d, J = 8.6 Hz, 1H), 7.65 (d, J = 15.7 Hz, 1H), 7.17 (dd, J = 8.3, 1.7 Hz, 1H), 7.12 (d, J = 1.7 Hz, 1H), 7.08 - 6.99 (m, 2H), 6.74 (d, J = 15.9 Hz, 1H), 6.29 (d, J = 8.6 Hz, 1H), 4.39 (t, J = 5.0 Hz, 2H), 4.15 (t, J = 7.5 Hz, 1H), 4.00 (s, 3H), 3.93 (m, 2H), 3.49 (s, 1H), 2.81 (m, 2H), 2.25 (s, 3H), 2.03 (t, J = 2.6 Hz, 1H).

Preparation of compound **6**

To a solution of compound **5** (0.048 g, 0.1 mmol) in 2 mL ethyl acetate was added boric anhydride (0.02 g, 0.28 mmol), and the solution was stirred at 80 °C for 30 min. Tributylborate (0.135 mL, 0.5 mmol) was added followed by vanillin (0.03 g, 0.2 mmol), and the reaction was refluxed for 30 min. Piperidine (0.02 mL, 0.2 mmol) was added and the solution was then refluxed for 1 h. After the solution was cooled to 50 °C, 2 mL of 0.5 M HCl was added and the solution was stirred at 50 °C for 30 min. The reaction was filtered, and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography (CH_2Cl_2/ethyl acetate: 5/1) to afford compound **6** as a yellow solid (0.035 g, 60% yield). ^{1}H NMR (400 MHz, CDCl3) δ 8.51 (m, 1H), 7.74 (m, 1H), 7.66 (m, 1H), 7.21 (m, 1H), 7.17 – 6.89 (m, 5H), 6.73 (m, 1H), 6.30 (m, 1H), 5.90 (m, 1H), 4.38 (m, 2H), 4.04 – 3.92 (m, 6H), 3.89 (m, 2H), 3.49 (s, 3H), 3.45 (d, J = 2.6 Hz, 1H), 2.91 (m, 1H), 2.02 (t, J = 2.6 Hz, 1H).

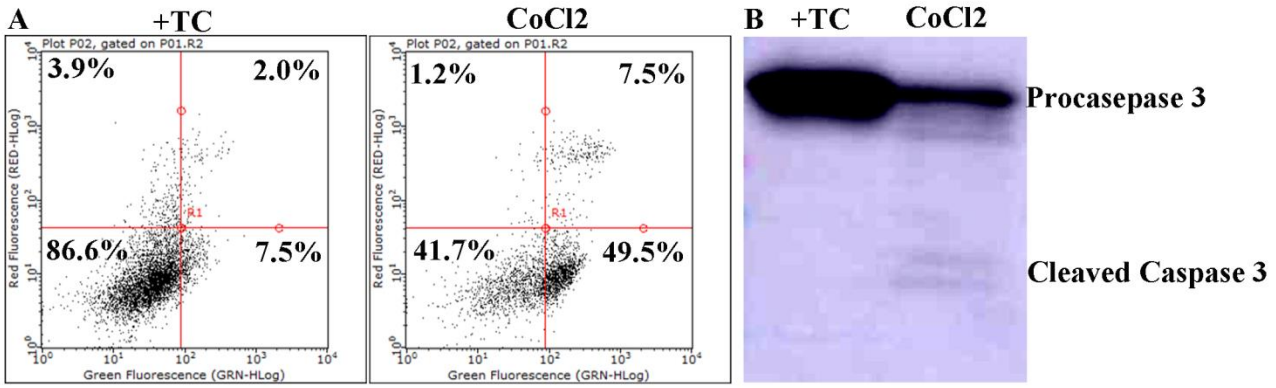
Preparation of compound **8**

Compound **6** (0.035 g, 0.06 mmol) and compound **7** (0.042 g, 0.06 mmol), previously synthesized in our lab,[13] were added together in 6 mL THF/H_2O (1:1) and to this sodium ascorbate (0.009 g, 0.05 mmol) and $CuSO_4$ (0.0009 g, 0.004 mmol) were added. The reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and then CH_2Cl_2 was added. The organic phase was washed with water and brine, and then dried over anhydrous Na_2SO_4. After filtration and removal of the solvent under reduced pressure, the crude residue was purified by flash column chromatography (CH_2Cl_2/Methanol: 20/1) to give compound **8** as an orange solid (0.0012 g, 15% yield). ^{1}H NMR (400 MHz, CDCl3) δ 8.51 (d, J = 8.6 Hz, 1H), 7.76 – 7.56 (m, 2H), 7.40 (s,

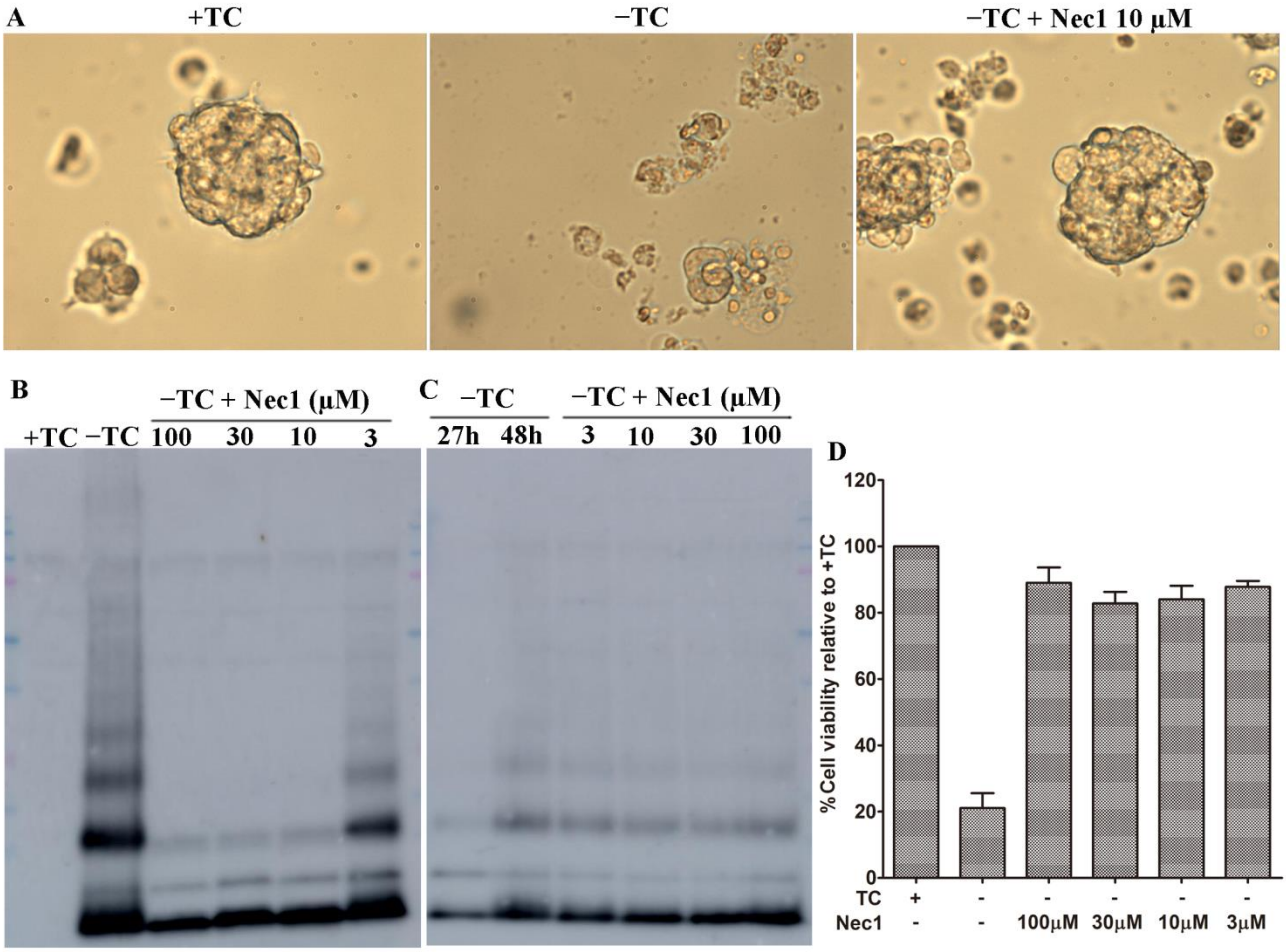
1H), 7.18 – 7.00 (m, 5H), 7.00 – 6.83 (m, 3H), 6.27 (dd, J = 8.6, 1.9 Hz, 1H), 5.30 (s, 1H), 4.51 – 4.32 (m, 4H), 4.12 – 3.80 (m, 10H), 3.74 (m, 2H), 3.49 (s, 6H), 3.44 – 3.26 (m, 4H), 3.13 (m, 2H), 2.00 (m, 4H), 1.92 – 0.74 (m, 46H), 0.66 (s, 3H). HRMS (m/z) $[M+Na]^+$: calc.d for $C_{74}H_{100}N_{10}O_{14}Na$ 1375.7313, found 1375.7323.

Preparation of 17MN-NBD

To a solution of compound **8** (0.0012 g, 0.009 mmol) in 1 mL THF, 1 mL of 4 M HCl-dioxane was added, and the solution was then stirred at room temperature overnight. After completion of the reaction, as monitored by TLC, solvent was removed and ether was added to precipitate the product. The precipitant was collected via filtration and washed again with ether to afford 17MN-NBD as an orange-red solid (0.009 g, yield 79%). HRMS (m/z) $[M+H]^+$: calc.d for $C_{69}H_{93}N_{10}O_{12}$ 1253.6969, found 1253.6968.



Supplementary Figure 1. MC65 cells under +TC condition were treated with $CoCl_2$ (500 µM) for 24 h. A) Apoptosis of cells was analyzed by flow cytometry using PI and Annexin V-FITC double stain. B) Lysates from cultures were analyzed by western blotting using a caspase 3 antibody.



Supplementary Figure 2. Effects of Nec-1 on MC65 cell death. A) MC65 cells were treated under indicated conditions for 48 h, and then the morphology was recorded on an Olympus IX71 microscope. B) MC65 cells were incubated with Nec-1 at indicated concentrations without TC for 48 h. Cell lysates were analyzed by western blotting using a 6E10 antibody. C) MC65 cells were incubated under -TC conditions for 27 h, and then Nec-1 at indicated concentrations was added and incubated for another 21 h. Lysates from cultures were analyzed by western blotting using a 6E10

antibody. D) MC65 cells were incubated under -TC conditions for 27 h, and then Nec-1 at indicated concentrations was added and incubated for another 45 h. Cell viability was assessed by MTT assay.

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