Electronic Supplementary Information

for

Dual-Targeting Delivery System for Selective Cancer Cell Death and Imaging

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Experimental Procedure

Synthesis of Fmoc-Lys(Boc)-PABOH (PABOH = *p*-aminobenzyl alcohol). To a stirred solution of Fmoc-Lys(Boc)-OH (984 mg, 2.10 mmol) and HBTU (756 mg, 1.99 mmol) in 30 mL DMF was added DIEA (867 μL, 5.25 mmol) at 0 ^{o}C. After 10 min, *p*-aminobenzyl alcohol (284.5 mg, 2.31 mmol) in 2 mL DMF was added to the above solution and then the mixture was warmed to room temperature. After 4 h, the mixture was diluted with EtOAc (200 mL), washed with water (50 mL × 3) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to 1 : 2) to give Fmoc-Lys(Boc)-PABOH in 82% yield as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 9.97 (s, 1 H), 7.88 (d, *J* = 7.6 Hz, 2 H), 7.75 (t, *J* = 6.8 Hz, 2 H), 7.61 (d, *J* = 8.0 Hz, 1 H), 7.57 (d, *J* = 7.6 Hz, 2 H), 7.42 (t, *J* = 7.2 Hz, 2 H), 7.35-7.31 (m, 2 H), 7.25 (d, *J* = 8.0 Hz, 2 H), 6.77 (m, 1 H), 5.10 (m, 1 H), 4.45 (s, 2 H), 4.28 (m, 2 H), 4.22 (m, 1 H), 4.13 (s, 1 H), 2.93 (m, 2 H), 1.65 (m, 2 H), 1.40 (m, 2H), 1.37 (m, 13 H); ^{13}C NMR (100 MHz, DMSO) δ 171.0, 156.1, 155.6, 143.9, 143.8,140.7, 137.6, 137.4, 127.6, 127.0, 126.8, 125.3, 120.0, 118.9, 79.3, 78.9, 78.6, 77.3, 65.6, 62.6, 55.4, 46.7, 31.6, 29.2, 28.3, 22.9; MALDI-TOF-MS, calcd for $C_{33}H_{39}N_3NaO_6^+$ $[M + Na]^+$ 596.2731, found 596.2644.

Synthesis of Fmoc-Phe-Lys(Boc)-PABOH. To a stirred solution of Fmoc-Lys(Boc)-PABOH (900 mg, 1.57 mmol) in 5 mL DMF was added 5 mL of 40% piperidine in DMF at room temperature. After 30 min, the mixture was diluted with EtOAc (200 mL), washed with water (50 mL x 3) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH : TEA = 10 : 1 : 0.1) to give H_2N-Lys(Boc)-PABOH in 92% yield.

To a stirred solution of Fmoc-Phe-OH (558 mg, 1.44 mmol) and HBTU (520 mg, 1.37 mmol) in 30 mL DMF was added DIEA (595 μL, 3.60 mmol) at 0 ^{o}C. After 10 min, H_2N-Lys(Boc)-PABOH (508 mg, 1.44 mmol) in 2 mL DMF was added to the above solution and then the mixture was warmed to room temperature. After 4 h, the mixture was diluted with EtOAc (200 mL), washed with water (50 mL × 3) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to 1 : 4) to give Fmoc-Phe-Lys(Boc)-PABOH in

80% yield as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 9.98 (s, 1 H), 8.18 (d, *J* = 8.0 Hz, 1 H), 7.85 (d, *J* = 7.6 Hz, 2 H), 7.63 (dd, *J* = 3.2, 8.4 Hz, 2 H), 7.57 (t, *J* = 8.4 Hz, 3 H), 7.39 (t, *J* = 7.2 Hz, 2 H), 7.27 (m, 4 H), 7.23 (m, 5 H), 7.14 (t, *J* = 7.2 Hz, 1 H), 6.70 (m, 1 H), 4.45 (m, 3 H), 4.35 (m, 1 H), 4.15 (m, 3 H), 3.05 (m, 1 H), 2.84 (m, 2 H), 2.81 (m, 1 H), 1.70-1.62 (m, 2 H), 1.37 (m, 13 H); ^{13}C NMR (100 MHz, DMSO) δ 171.5, 170.3, 155.7, 155.5, 143.7, 143.6, 140.6, 138.0, 137.5, 137.4, 129.2, 127.9, 127.5, 126.9, 126.8, 126.2, 125.3, 125.2, 119.9, 119.0, 79.3, 78.9, 78.6, 77.3, 65.6, 62.6, 56.0, 53.4, 46.6, 37.5, 32.0, 29.3, 28.2, 22.7; MALDI-TOF-MS, calcd for $C_{42}H_{49}N_4O_7^+$ $[M + H]^+$ 721.3596, found 721.4503.

Synthesis of Alkyne-Phe-Lys(Boc)-PABOH. To a stirred solution of Fmoc-Phe-Lys(Boc)-PABOH (780 mg, 1.08 mmol) in 5 mL DMF was added 5 mL of 40% piperidine in DMF at room temperature. After 30 min, the mixture was diluted with EtOAc (200 mL), washed with water (50 mL × 3) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH : TEA = 10 : 1 : 0.1) to give H_2N-Phe-Lys(Boc)-PABOH in 90% yield.

To a stirred solution of H_2N-Phe-Lys(Boc)-PABOH (496 mg, 0.97 mmol) in 20 mL DMF were added linker (392 mg, 1.07 mmol) and DIEA (442 μL, 2.67 mmol) at 0 ^{o}C. The mixture was warmed to room temperature. After 12 h, the mixture was diluted with EtOAc (100 mL), washed with water (40 mL x 3) and brine (40 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH = 20 : 1) to give Alkyne-Phe-Lys(Boc)-PABOH in 82% yield: ^{1}H NMR (400 MHz, DMSO) δ 9.98 (s, 1 H), 8.33 (d, *J* = 8.0 Hz, 1 H), 7.63 (d, *J* = 8.4 Hz, 1 H), 7.56 (d, *J* = 8.8 Hz, 2 H), 7.26-7.15 (m, 7 H), 6.77 (s, 1 H), 5.09 (t, *J* = 5.6 Hz, 1 H), 4.67 (m, 1 H), 4.44 (d, *J* = 5.6 Hz, 2 H), 4.39 (m, 1 H), 4.11 (d, *J* = 2.4 Hz, 2 H), 3.82 (dd, *J* = 15.2, 38.0 Hz, 2 H), 3.53-3.50 (m, 10 H), 3.46 (m, 1 H), 3.39 (m, 1 H), 3.09 (m, 1 H), 2.92-2.87 (m, 3 H), 1.73 (m, 1 H), 1.63 (m, 1 H), 1.38 (m, 13 H); ^{13}C NMR (100 MHz, DMSO) δ 170.7, 170.3, 168.9, 155.6, 137.6, 137.5, 137.4, 129.3, 127.9, 126.9, 126.3, 119.0, 80.3, 77.3, 77.1, 70.1, 69.8, 69.7, 69.6, 69.5, 68.5, 62.6, 57.5, 53.5, 53.0, 37.6, 31.9, 29.3, 28.3, 22.8; MALDI-TOF-MS, calcd for $C_{38}H_{54}N_4O_{10}Na^+$ $[M + Na]^+$ 749.3732, found 749.3784.

Synthesis of Alkyne-Phe-Lys(Boc)-PAB-Br. To a stirred solution of Alkyne-Phe-Lys(Boc)-PABOH (150 mg, 0.21 mmol) in 7 mL THF was added phosphorus tribromide (15.7 μL of 1 M solution in CH_2Cl_2) at 0 ^oC. After 30 min, the volatile material was removed under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to 1 : 10) to give Alkyne-Phe-Lys(Boc)-PAB-Br in 75% yield as a white solid: ^1H NMR (400 MHz, DMSO) δ 10.31 (s, 0.6 H), 10.13 (s, 0.4 H), 8.37 (t, *J* = 8.4 Hz, 1 H), 7.78 (d, *J* = 8.4 Hz, 1 H) 7.62 (t, *J* = 9.6 Hz, 2 H),7.44 (d, *J* = 8.4, Hz, 1 H), 7.40 (d, *J* = 8.4, Hz, 1 H), 7.22-7.17 (m, 5 H), 6.77 (d, *J* = 5.6 Hz, 1 H), 5.42 (s, 1 H), 4.68 (m, 2 H), 4.40 (m, 1 H), 4.12 (s, 2 H), 3.82 (dd, *J* = 15.2, 37.6 Hz, 2 H), 3.51 (m, 12 H), 3.42 (m, 2 H), 3.09 (s, 1 H), 2.88 (m, 3 H), 1.73 (m, 1 H), 1.64 (m, 1 H), 1.35 (m, 13 H); ^{13}C NMR (125 MHz, DMSO) δ 171.0, 170.9, 169.0, 155.6, 140.87, 137.4, 132.4, 129.9, 129.3, 128.0, 119.7, 119.3, 80.3, 77.4, 77.1, 70.1, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 57.5, 56.7, 53.7, 52.9, 37.6, 34.8, 31.7, 29.3, 28.3, 22.9; MALDI-TOF-MS, calcd for $C_{38}H_{53}BrN_4NaO_9$ $[M + Na]^+$ 811.2888 and 813.2873, found 811.2551 and 813.3003 (1 : 1).

Synthesis of TG-Alkyne. To a stirred solution of Tokyo green (37.5 mg, 0.12 mmol), Cs_2CO_3 (50.3 mg, 0.15 mmol) and 18-crown-6 (39.6 mg, 0.15 mmol) in 2 mL DMF was added Alkyne-Phe-Lys(Boc)-PAB-Br (80 mg, 0.103 mmol) at room temperature. After 6 h, the mixture was diluted with CH_2Cl_2 (50 mL), washed with water (20 mL x 3) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH = 10 : 1) to give Alkyne-Phe-Lys(Boc)-PAB-TG in 65% yield as a yellow solid: ^1H NMR (400 MHz, DMSO) δ 10.11 (s, 1 H), 8.35 (t, *J* = 8.0 Hz, 1 H), 7.65 (m, *J* = 8.4 Hz, 2 H), 7.57 -7.48 (m, 2 H), 7.43 (m, 2 H), 7.29 (m, 2 H), 7.21 (m, 5 H), 6.95 (m, 1 H), 6.85 (d, *J* = 10.0 Hz, 1 H), 6.76 (m, 1 H), 6.43(d, *J* = 9.6 Hz, 1 H), 6.25 (d, *J* = 1.2 Hz, 1 H), 5.24 (s, 2 H), 4.67 (m, 1 H), 4.42 (m, 2 H), 4.11 (s, 2 H), 3.82 (dd, *J* = 15.6, 37.2 Hz, 2 H), 3.51 (m, 10 H), 3.41 (m, 2 H), 3.07 (m, 1 H), 2.89 (m, 3 H), 2.02 (s, 3 H), 1.73 (m, 1 H), 1.63 (m, 1 H), 1.35 (m, 13 H); ^{13}C NMR (100 MHz, DMSO) δ 180.4, 170.8, 170.6, 169.0, 165.0, 158.9, 155.6, 139.1, 137.4, 135.6, 131.7, 131.4,130.6, 130.4, 130.1, 129.9, 129.3, 129.0, 128.8, 127.9, 127.2, 126.3, 126.2, 119.3, 117.3, 116.3, 114.7, 104.0, 101.5, 80.3, 77.3, 77.0, 70.1, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 59.8, 57.4, 53.6, 53.0, 37.8, 31.8, 29.3, 28.3, 22.8, 19.2; MALDI-TOF-MS, calcd for $C_{58}H_{66}N_4NaO_{12}^+$ $[M + Na]^+$ 1033.4564, found 1033.2854.

Alkyne-Phe-Lys(Boc)-PAB-TG (50 mg, 0.05 mmol) was dissolved in 0.3 mL TFA and 0.7 mL CH_2Cl_2 at room temperature. After stirring for 2 h, the volatile material was removed under reduced pressure. The residue was diluted with EtOAc (50 mL), washed with 5% $NaHCO_3$ (20 mL), water (20 mL x 2) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give TG-Alkyne in 95% yield as a yellow solid: ^{1}H NMR (500 MHz, DMSO) δ 10.18 (s, 1 H), 8.46 (d, *J* = 7.5 Hz, 1 H), 7.67 (d, *J* = 8.0 Hz, 2 H), 7.56-7.50 (m, 2 H), 7.45 (m, 3 H), 7.40 (s, 1 H), 7.29 (d, *J* = 7.5 Hz, 1 H), 7.24 -7.19 (m, 5 H), 7.14 (t, *J* = 7.0 Hz, 1 H), 7.06 (d, *J* = 8.0 Hz, 1 H), 6.99 (d, *J* = 9.0 Hz, 1 H), 6.96 (d, *J* = 9.5 Hz, 1 H), 6.57 (d, *J* = 10.0 Hz, 1 H), 6.43 (s, 1 H), 5.28 (s, 2 H), 4.66 (m, 1 H), 4.43 (m, 1 H), 4.11 (s, 2 H), 3.83 (dd, *J* = 15.5, 43.5 Hz, 2 H), 3.51 (m, 12 H), 3.41 (m, 2 H), 3.08 (m, 1 H), 2.89 (m, 1 H), 2.79 (m, 2 H), 2.02 (s, 3 H), 1.76 (m, 1 H), 1.66 (m, 1 H), 1.56 (m, 2 H), 1.39 (m, 2 H); ^{13}C NMR (125 MHz, DMSO) δ 182.5, 170.9, 170.5, 169.1, 164.0, 158.6, 158.4, 158.1, 154.7, 151.3, 138.9, 137.4, 135.6, 131.9, 130.9, 130.7, 130.6, 129.7, 129.6, 129.3, 129.1, 128.9, 128.6, 128.0, 126.3, 126.2, 119.3, 117.4, 115.3, 114.3, 104.5, 101.6, 80.3, 77.1, 70.3, 70.1, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 57.5, 53.3, 53.1, 39.0, 37.5, 31.5, 26.7, 22.5, 19.2; MALDI-TOF MS, calcd for $C_{53}H_{58}N_4NaO_7^+$ $[M + Na]^+$ 933.4045, found 933.2967.

Synthesis of Oct-TG. TG-Alkyne (15 mg, 0.016 mmol) and Octrotide-N_3 (20.3 mg, 0.016 mmol) were dissolved in 0.4 mL of DMF/*t*-BuOH (1 : 3) and then ascorbic acid (2.82 mg, 0.016 mmol) and $CuSO_4$ (2.6 mg, 0.016 mmol) in water (100 μL) were added to the reaction mixture. After stirring for 4 h, the mixture was subjected to preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min to give Oct-TG: MALDI-TOF MS, calcd for $C_{110}H_{137}N_{17}NaO_{24}S_2^+$ $[M + Na]^+$ 2166.9356, found 2166.8711.

Solid-phase synthesis of octreotide-N_3. Octreotide-N_3 was synthesized by using a Fmoc/*t*-Bu strategy on H-threoninol(*t*-Bu)-2-chlorotrityl resin (0.45 mmol/g). Fmoc amino acid (4 equiv) was activated with HBTU (3.9 equiv) in DMF/CH_2Cl_2 (4:1) and coupled in the presence DIEA (10 equiv) at room temperature for 4 h with shaking. The Fmoc group was removed by treatment with 20% piperidine in DMF. After assembly of Fmoc-amino acid and N_3-linker-COOH on a solid support, a disulfide bond was formed using $Ti(tfa)_3$ (thallium(III) trifluoroacetate, 3 equiv) in DMF at room temperature for 2 h. Octreotide-N_3 was cleaved from the resin by treatment with TFA/triisopropylsilane (TIS)/H_2O (95:2.5:2.5) at 0 ^{o}C for 2 h.

The crude product was purified by preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min to give octreotide-N_3: MALDI-TOF-MS, $C_{57}H_{79}N_{13}O_{14}S_2^+$ $[M]^+$ 1233.5311, found 1233.7158.

Synthesis of Fmoc-Lys(Mtt)-PABOH (Mtt = 4-methyltrityl). To a stirred solution of Fmoc-Lys(Mtt)-OH (1.0 g, 1.60 mmol) and HBTU (576 mg, 1.52 mmol) in 30 mL DMF was added DIEA (661 mg, 4.0 mmol) at 0 °C. After 10 min, *p*-aminobenzyl alcohol (237 mg, 1.92 mmol) in 2 mL DMF was added to the solution, which was then wormed to room temperature. After 4 h, the mixture was diluted with EtOAc (200 mL), washed with water (50 mL x 3) and brine (50 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 4 : 1 to 1 : 1) to give Fmoc-Lys(Mtt)-PABOH in 80% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1 H), 7.69 (dd, *J* = 3.2, 7.6 Hz, 2 H), 7.47 (d, *J* = 7.6 Hz, 2 H), 7.41 (t, *J* = 7.2 Hz, 4 H), 7.36 (t, *J* =7.2 Hz, 2 H), 7.30 (m, 2 H), 7.26 (m, 4 H), 7.19 (m, 6 H), 7.12 (d, *J* = 8.0 Hz, 4 H), 7.02 (d, *J* = 2.0 Hz, 2 H), 5.72 (d, *J* = 7.6 Hz, 1 H), 4.52 (s, 2 H), 4.33 (m, 3H), 4.10 (m, 1 H), 2.25 (s, 3 H), 2.09 (m, 4 H), 1.85 m,1 H), 1.63 (m, 1 H), 1.46 (m, 2 H), 1.38 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 156.8, 146.4, 143.7, 143.7, 143.3, 141.4, 137.1, 137.0, 135.8, 128.7, 128.6, 127.8, 127.7, 127.2, 126.2, 125.0, 120.3, 120.0, 70.7, 67.3, 64.8, 55.8, 47.1, 43.5, 32.7, 30.7, 23.5, 21.0; MALDI-TOF-MS, calcd for $C_{48}H_{47}N_3NaO_4^+$ $[M + Na]^+$ 752.3459, found 752.3865.

Synthesis of Fmoc-Phe-Lys(Mtt)-PABOH. Fmoc-Phe-Lys(Mtt)-PABOH was prepared by using the procedure described above for Fmoc-Lys(Boc)-PABOH in 72% yield as a white solid: ^{1}H NMR (400 MHz, DMSO) δ 7.86 (d, *J* = 7.2 Hz, 2 H), 7.60-7.55 (m, 4 H), 7.38 (m, 6 H), 7.30 (m, 3 H), 7.23 (m, 11 H), 7.12 (m, 4 H), 7.02 (d, *J* = 7.6 Hz, 2 H), 5.11 (t, *J* = 5.6 Hz, 1 H), 4.46 (s, 2 H), 4.44 (s, 3 H), 4.32 (m, 1 H), 4.17-4.02 (m, 3 H), 3.02 (m, 1 H), 2.79 (m, 1 H), 2.19 (s, 3 H), 1.94 (m, 1 H), 1.68 (m, 1 H), 1.59 (m, 1 H), 1.40 (m, 2 H), 1.35 (m, 2 H); ^{13}C NMR (100 MHz, DMSO) δ 171.4, 170.3, 155.7, 146.4, 143.7, 143.2, 140.7, 140.6, 138.1, 137.5, 137.4, 137.3, 134.9, 129.2, 128.3, 128.0, 127.6, 127.0, 126.9, 126.2, 125.8, 125.3, 125.0, 120.1, 119.0, 118.9, 70.1, 65.6, 62.6, 55.9, 53.4, 46.5, 43.2, 37.4, 32.4, 29.9, 23.4, 20.5; MALDI-TOF-MS, calcd for $C_{57}H_{56}N_4NaO_5^+$ $[M + Na]^+$

889.4143, found 889.5206.

Synthesis of Alkyne-Phe-Lys(Mtt)-PABOH. Alkyne-Phe-Lys(Mtt)-PABOH was prepared by using the procedure described above for Alkyne-Phe-Lys(Boc)-PABOH in 85% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1 H), 7.43 (m, 8 H), 7.31 (d, J = 8.0 Hz, 2 H), 7.26-7.21 (m, 7 H), 7.16-7.11 (m, 8 H), 7.04 (d, J = 8.0 Hz, 2 H), 6.82 (d, J = 7.2 Hz, 2 H), 4.65 (m, 1 H), 4.60 (s, 2 H), 4.41 (dd, J = 7.2, 13.6 Hz, 1 H) 4.13 (s, 2 H), 3.90 (d, J = 2.8 Hz, 2 H), 3.63 (m, 8 H), 3.56 (m, 2 H), 3.52 (m,2 H), 3.18-3.03 (m, 2 H), 2.39 (s, 1 H), 2.28 (s, 3 H), 2.09 (m, 2 H), 2.01 (m, 2 H), 1.88 (m, 2 H), 1.57 (m, 1 H), 1.47 (m, 3 H), 1.27 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 171.0, 169.5, 146.5, 143.3, 137.4, 137.0, 136.3, 135.8, 129.3, 128.8, 128.7, 128.6, 127.9, 127.8, 127.3, 126.3, 120.3, 79.7, 74.9, 71.0, 70.8, 70.7, 70.6, 70.5, 69.2, 65.0, 58.5, 54.8, 54.3, 43.6, 37.5, 31.4, 30.7, 23.7, 21.0; MALDI-TOF-MS, calcd for $C_{53}H_{62}N_{40}NaO_8^+$ $[M + Na]^+$ 905.4460, found 905.5688.

Synthesis of Alkyne-Phe-Lys(Mtt)-PABC-PNP. To a stirred solution of Alkyne-Phe-Lys (Mtt)-PABOH (185.3 mg, 0.21 mmol) and bis(4-nitrophenyl) carbonate (64 mg, 0.42 mmol) in 5 mL CH_2Cl_2 was added TEA (54 μL, 0.42 mmol) at room temperature. After 2 days, the reaction mixture was concentrated under reduced pressure and The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 4 : 1 to 1 : 5) to give Alkyne-Phe-Lys(Mtt)-PABC-PNP in 90% yield as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1 H), 8.24 (d, J = 9.2 Hz, 2 H), 7.57 (t, J = 8.8 Hz, 2 H), 7.41 (d, J = 7.6 Hz, 4 H), 7.36 (m, 4 H), 7.29 (d, J = 8.4 Hz, 2 H), 7.25 (m, 5 H), 7.15 (m, 8 H), 7.04 (d, J = 8.0 Hz, 2 H), 6.97 (d, J = 7.6 Hz, 1 H), 5.24 (s, 2 H), 4.63 (m, 1 H), 4.45 (m, 1 H), 4.16 (m, 3 H), 3.94 (dd, J = 16.0, 24.0 Hz, 2 H), 3.63 (m, 8 H), 3.57 (m, 2 H), 3.53 (m, 2 H), 3.22-3.06 (m, 2 H), 2.40 (t, J = 2.4 Hz, 1 H), 2.24 (s, 3 H), 2.12 (d, J = 6.8 Hz, 2 H), 1.92 (m, 1 H), 1.61 (m, 1 H), 1.46 (m, 2 H), 1.32 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 171.6, 169.9, 163.2, 163.1, 155.5, 152.4, 146.0, 145.4, 142.9, 140.7, 138.2, 135.8, 135.7, 130.4, 129.6, 129.1, 128.8, 128.7 128.6, 128.5, 128.4, 128.0, 127.8, 127.4, 126.3, 125.3, 121.8, 120.4, 79.3, 74.9, 70.8, 70.6, 70.4, 70.3, 70.2, 70.1, 70.0, 69.0, 60.7, 58.4, 55.1, 54.6, 43.5, 37.3, 31.3, 30.4, 23.6, 20.9; MALDI-TOF-MS, calcd for $C_{60}H_{65}N_5O_{12}$ $[M]^+$ 1047.4630, found 1046.8608.

Synthesis of Alkyne-Phe-Lys(Mtt)-PABC-Dox. To a stirred solution of doxorubicin·HCl (66 mg, 0.114 mmol) in 2 mL of anhydrous DMF and TEA (15 μL, 0.114 mmol) was added Alkyne-Phe-Lys(Mtt)-PABC-PNP (80 mg, 0.076 mmol) in 3 mL of anhydrous DMF at room temperature. After 12 h, the mixture was diluted with EtOAc (50 mL), washed with wat er (25 mL x 3) and brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated unde r reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH = 10 : 1) to give Alkyne-Phe-Lys(Mtt)-PABC-Dox in 72% yield as a red solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.95 (s, 1 H), 13.20 (s, 1 H), 8.45 (s, 1 H), 8.00 (d, *J* = 7.6 Hz, 1 H), 7.76 (t, *J* = 8.0 Hz 1 H), 7.46-7.39 (m, 5 H), 7.36 (d, *J* = 8.8 Hz, 2 H), 7.30 (d, *J* = 8.0 Hz, 2 H), 7.22-7.19 (m, 6 H), 7.16-7.04 (m, 6 H), 6.68 (d, *J* = 7.2 Hz, 2 H), 5.48 (s, 1 H), 5.26 (s, 2 H), 4.94 (dd, *J* = 12.0, 18.4 Hz, 2 H), 4.76 (s, 2 H), 4.59 (m, 2 H), 4.39 (m, 1 H), 4.13 (m, 3 H), 4.05 (m, 4 H), 3.91 (d, *J* = 2.4 Hz, 1 H), 3.62 (m, 8 H), 3.56 (m, 2 H), 3.53 (m, 2 H), 3.27-2.95 (m, 3 H), 2.40 (t, *J* = 2.4 Hz, 1 H), 2.28 (s, 3 H), 1.89-1.79 (m, 2 H), 1.54-1.47 (m, 4 H), 1.27 (m, 2 H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 213.9, 186.9, 186.6, 171.3, 170.9, 169.5, 161.0, 156.2, 155.7, 155.6, 146.4, 143.3, 137.8, 136.2, 135.7, 135.4, 133.7, 129.2, 129.0, 128.8, 128.6, 128.5, 127.8, 127.2, 126.2, 120.7, 120.0, 119.8, 118.5, 111.5, 111.4, 100.9, 79.6, 74.8, 70.9, 70.6, 70.4, 70.3, 69.5, 69.1, 67.4, 65.6, 58.4, 56.7, 54.7, 54.3, 43.5, 30.7, 23.7, 21.0, 16.9; MALDI-TOF-MS, calcd for $C_{81}H_{89}N_5NaO_{20}$ $[M + Na]^+$ 1474.5993, found 1474.3779.

Synthesis of Dox-Alkyne. To a stirred suspension of Alkyne-Phe-Lys(Mtt)-PABC-Dox (50 mg, 0.034 mmol) in 5 mL CH_2Cl_2 was dropwise added 0.4% TFA in 5 mL CH_2Cl_2 at 0 ^{o}C. After stirring for 4 h, the volatile material was removed under reduced pressure. The residue was subjected to preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min to give Dox-Alkyne in 48% yield as a red solid: ^{1}H NMR (400 MHz, DMSO) δ 14.05 (s, 1 H), 13.30 (s, 1 H), 10.09 (s, 1 H), 8.42 (d, *J* = 7.6 Hz, 1 H), 7.93 (d, *J* = 4.0 Hz, 2 H), 7.65 (m, 5 H), 7.57 (d, *J* = 8.0 Hz, 2 H), 7.27 (d, *J* = 8.4 Hz, 2 H), 7.21-7.14 (m, 6 H), 6.86 (d, *J* = 8.0 Hz, 2 H), 5.47 (s, 1 H), 5.22 (s, 2 H), 4.95 (s, 1 H), 4.91 (s, 2 H), 4.67 (s, 1 H), 4.64 (s, 2 H) 4.56 (s, 2 H), 4.39 (m, 1 H), 4.15 (m, 1 H), 4.11 (s, 2 H), 3.80 (dd, *J* = 19.6, 50.8 Hz, 2 H), 3.51 (m, 14 H), 3.27-2.95 (m, 3 H), 2.28 (s, 2 H), 1.89-1.79 (m, 2H), 1.54-1.47 (m, 4 H); ^{13}C NMR (100 MHz,

DMSO) δ 213.8, 186.5, 186.4, 170.9, 170.4, 169.1, 160.8, 158.3, 157.9, 156.1, 155.4, 154.5, 138.4, 137.4, 136.3, 135.6, 134.7, 134.1, 132.1, 129.3, 128.5, 128.0, 126.4, 125.4, 122.6, 120.0, 119.7, 119.2, 119.0, 110.8, 110.6, 100.3, 80.4, 77.1, 74.9, 70.2, 69.8, 69.7, 69.6, 69.5, 69.4, 68.5, 68.0, 66.7, 64.9, 63.7, 57.5, 56.6, 53.3, 53.1, 47.2, 37.5, 36.6, 32.1, 31.5, 29.8, 26.7, 22.4, 17.0; MALDI-TOF-MS, calcd for $C_{61}H_{73}N_5NaO_{20}^+$ $[M + Na]^+$ 1218.4741, found 1218.4771.

Synthesis of Oct-Dox. Dox-Alkyne (15 mg, 0.013 mmol) and Octreotide-N_3 (15.4 mg, 0.013 mmol) were dissolved in 0.4 mLof DMF/*t*-BuOH (1 : 3). Ascorbic acid (2.3 mg, 0.013 mmol) and $CuSO_4$ (2.0 mg, 0.013 mmol) in water (100 μL) were added to the above solution. After stirring for 4 h, the mixture was purified by preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min to give Oct-Dox: MALDI-TOF-MS, calcd for $C_{118}H_{152}N_{18}NaO_{34}S_2^+$ $[M + Na]^+$ 2452.0052, found 2452.1895.

Synthesis of noncleavable Oct-Dox. To a stirred solution of doxorubicin·HCl (132 mg, 0.228 mmol) in 5 mL of anhydrous DMF and TEA (30 μL, 0.228 mmol) was added linker (100.6 mg, 0.274 mmol) in 5 mL of anhydrous DMF at room temperature. After 12 h, the reaction mixture was diluted with EtOAc (50 mL), washed with water (20 mL x 3) and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH = 20 : 1) to give noncleavable alkyne-Dox in 89% yield: ^{1}H NMR (400 MHz, DMSO) δ 13.87 (s, 1 H), 13.09 (s, 1 H), 7.74 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 7.2 Hz, 1 H), 7.48 (d, *J* = 8.0 Hz , 1 H), 7.19 (d, *J* = 8.4 Hz , 1 H), 5.36 (s, 2 H), 5.21 (s, 1 H), 4.94 (d, *J* = 6.4 Hz , 1 H), 4.87 (t, *J* = 5.6 Hz ,1 H), 4.83 (m, 1 H), 4.59 (d, *J* = 5.2 Hz, 2 H), 4.20 (m, 1 H), 4.13 (d, *J* = 2.4 Hz, 2 H), 4.08 (m, 1 H), 3.90 (s, 2 H), 3.82 (dd, *J* = 15.2, 38.0 Hz, 2 H), 3.60-3.48 (m, 10 H), 3.38 (m, 1 H), 3.35(s, 2 H), 2.90 (m, 1 H), 2.79-2.68 (m, 2 H), 2.20 (m, 1 H), 2.06 (m, 1 H), 1.83 (m, 1 H), 1.53 (m, 1 H), 1.25 (m, 1 H), 1.14 (d, *J* = 6.4 Hz, 2 H); ^{13}C NMR (100 MHz, DMSO) δ 213.9, 186.0, 185.9, 168.1, 160.6, 156.0, 154.4, 135.9, 135.2, 134.2, 133.8, 119.5, 118.7, 110.4, 110.3, 100.4, 80.3, 77.9, 77.18, 74.8, 70.2, 69.8, 69.7, 69.4, 68.5, 68.2, 68.1, 67.98, 67.6, 66.6, 63.7, 57.8, 56.4, 44.3, 36.2, 31.9, 29.9, 16.7; MALDI-TOF-MS, calcd for $C_{38}H_{45}NO_{16}Na^+$ $[M + Na]^+$ 794.2738, found 794.2936.

Noncleavable alkyne-Dox (18.5 mg, 0.026 mmol) and octreotide-N_3 (30.8 mg, 0.026

mmol) were dissolved in 0.8 mL of DMF and *t*-BuOH (1 : 3). Ascorbic acid (4.7 mg, 0.026 mmol) and $CuSO_4$ (4.0 mg, 0.026 mmol) in water (200 μL) were added to the above solution. After stirring for 4 h, the reaction mixture was purified by preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min to give noncleavable Oct-Dox: MALDI-TOF-MS, calcd for $C_{95}H_{124}N_{14}NaO_{30}S_2^+$ $[M+Na]^+$ 2027.8049, found 2027.8154.

Synthesis of Rho-Oct. Rho-pip-succinic acid (4 equiv) was activated with HBTU (3.9 equiv) in DMF-CH_2Cl_2 (4:1) and added to an amino-terminal linear peptide assembled on a chlorotrityl resin (20 μmol, 1 equiv) in the presence of DIEA (10 equiv). After 4 h under shaking, disulfide bond was formed using $Ti(tfa)_3$ (thallium(III) trifluoroacetate, 3 equiv) in DMF for 2 h at room temperature. Rho-Oct was cleaved from the resin by treatment with TFA/TIS/H_2O (95 : 2.5 : 2.5) for 2 h at 0 ^oC. The crude product was purified by preparative RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 60 min: MALDI-TOF-MS, $C_{93}H_{122}N_{15}O_{18}S_2^+$ $[M + H]^+$, 1801.8528, found 1801.8136.

Synthesis of Cbz-Phe-Lys-AMC (AMC = 7-amino-4-methylcoumarin). To a stirred solution of 7-amino-4-methylcoumarin (50 mg, 0.285 mmol), Fmoc-Lys(Boc)-OH (160.4 mg, 0.342 mmol) and HATU (124.6 mg, 0.328 mmol) in 5 mL DMF was added DIEA (118 μL, 0.713 mmol) at 0 ^oC. The reaction mixture was warmed to room temperature. After 6 h, the reaction mixture was diluted with EtOAc (50 mL), washed with water (25 mL × 3) and brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to 1 : 2) to give Fmoc-Lys(Boc)-AMC in 82% yield as a pale yellow solid: ^1H NMR (400 MHz, DMSO) δ 9.18 (s, 1 H),7.74 (d, *J* = 7.2 Hz, 2 H), 7.70(s, 1 H), 7.58 (d, *J* = 7.2 Hz, 2 H), 7.46 (s, 2 H), 7.37 (t, *J* = 6.8 Hz, 2 H), 7.27 (m, 3 H), 6.17 (s, 1 H), 5.84 (s, 1 H), 4.41 (d, *J* = 6.8 Hz, 2 H), 4.38 (m, 1 H), 4.20 (t, *J* = 6.8 Hz, 2 H), 3.10 (m, 2 H), 2.38 (s, 3 H), 1.97 (m, 1 H), 1.79 (m, 1 H), 1.52 (m, 2H), 1.42 (m, 11 H); ^{13}C NMR (100 MHz, DMSO) δ 171.2, 161.4, 156.6, 154.2, 152.7, 143.8, 143.7, 141.5, 141.4, 127.9, 127.2, 125.3, 125.1, 120.1, 116.2, 115.9, 113.4, 107.4, 79.6, 67.5, 60.5, 55.8, 47.2, 39.6, 31.7, 29.5, 28.6, 22.6, 18.7; MALDI-TOF-MS, calcd for $C_{36}H_{39}N_3NaO_7^+$ $[M + Na]^+$ 648.2680, found 648.1620.

To a stirred solution of Fmoc-Lys(Boc)-AMC (140 mg, 0.224 mmol) in 1 mL DMF was added 1 mL of 40% piperidine in DMF at room temperature. After 30 min, the reaction mixture was diluted with EtOAc (50 mL), washed with water (25 mL × 3) and brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to CH_2Cl_2 : MeOH : TEA = 10 : 1 : 0.1) to give H_2N-Lys(Boc)-AMC in 95% yield.

To a stirred solution of H_2N-Lys(Boc)-AMC (86 mg, 0.213 mmol), Cbz-Phe-OH (76.5 mg, 0.256 mmol) and HBTU (92.2 mg, 0.243 mmol) in 5 mL DMF was added DIEA (88 μL, 0.533 mmol) at 0 ^{o}C. The reaction mixture was warmed to room temperature. After 4 h, the reaction mixture was diluted with EtOAc (50 mL), washed (25 mL×3) with water and brine (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2 : EtOAc = 2 : 1 to 1 : 4) to give Cbz-Phe-Lys(Boc)-AMC in 86% yield as a pale yellow solid: ^{1}H NMR (400 MHz, DMSO) δ 10.56 (br, 1 H), 8.34 (s, 1 H), 7.80 (s, 1 H), 7.72 (d, *J* = 8.4 Hz, 1 H), 7.51 (t, *J* = 7.2 Hz, 2 H), 7.33(m, 5 H), 7.25 (m, 5 H), 7.18 (d, *J* = 7.2 Hz, 1 H), 6.77 (m, 1 H), 6.26 (s, 1 H), 4.96 (s, 2 H), 4.44 (m, 1 H), 4.36 (m, 1 H), 3.05 (m, 1 H), 2.92 (m, 2 H), 2.73 (m, 1 H), 2.40 (s, 3 H), 1.75-1.66 (m, 2 H), 1.41-1.38 (m, 13 H); ^{13}C NMR (100 MHz, DMSO) δ 171.7, 171.4, 160.0, 155.8, 155.6, 153.6, 153.0, 142.2, 138.0, 137.0, 129.2, 128.3, 128.0, 127.7, 127.4, 126.2, 125.9, 115.3, 115.1, 105.7, 77.3, 65.2, 56.0, 53.7 ,52.0, 37.4, 31.7, 29.3, 28.4, 22.8, 17.9; MALDI-TOF-MS, calcd for $C_{38}H_{44}N_4NaO_8^+$ $[M + Na]^+$ 707.3051, found 707.3326.

Cbz-Phe-Lys(Boc)-AMC (100 mg, 0.146 mmol) was dissolved in 0.3 mL TFA and 0.7 mL CH_2Cl_2 at room temperature. After stirring for 2 h, the volatile material was removed under reduced pressure to give Cbz-Phe-Lys-AMC in 95% yield as a pale yellow solid: ^{1}H NMR (400 MHz, DMSO) δ 10.71 (s, 1 H) 8.44 (d, *J* = 7.6 Hz, 1 H), 7.80 (s, 1 H), 7.68 (d, *J* = 8.8 Hz, 1 H), 7.53 (d, *J* = 8.4 Hz, 1 H), 7.48 (d, *J* = 8.4 Hz 1 H), 7.28(m, 5 H), 7.25 (m, 5 H), 7.13 (t, *J* = 7.2 Hz, 1 H), 6.22 (s, 1 H), 4.91 (s, 2 H), 4.44 (m, 1 H), 4.29 (m, 1 H), 3.04 (m, 1 H), 2.73 (m, 4 H), 2.36 (s, 3 H), 1.75 (m, 1 H), 1.66 (m, 1 H), 1.56 (m, 2 H), 1.38 (m, 2 H); ^{13}C NMR (100 MHz, DMSO) δ 171.7, 171.4, 160.0, 155.8, 155.6, 153.6, 153.0, 142.2, 138.0, 137.0, 129.2, 128.3, 128.0, 127.7, 127.4, 126.2, 125.9, 115.3, 115.1, 105.7, 65.2, 56.1, 53.7 ,38.4, 37.4, 31.2, 26.5, 22.2, 18.0; MALDI-TOF-MS, calcd for $C_{33}H_{36}N_4NaO_6^+$ $[M + Na]^+$ 607.2522, found 607.2368.

Cleavage of Oct-TG and Oct-Dox by recombinant cathepsin B. Cathepsin B (EC 3.4.22.1, 25 units) from bovine spleen was dissolved in 2 mL of 25 mM sodium acetate containing 1 mM EDTA (pH 5.0). A solution of cathepsin B (6 μL) was added to 12 μL of 30 mM DTT/15 mM EDTA at room temperature and activated for 15 min. In parallel, a solution of 0.5 μL of 10 mM Oct-TG or Oct-Dox was added to 32 μL of 25 mM acetate containing 1 mM EDTA (pH 5.0). The above solution was added to a solution (18 μL) of activated cathepsin B and then incubated at 37 ^{o}C for 1 h. The enzyme reaction was quenched by addition of cold MeOH (50 μL). After centrifugation, the supernatant was analyzed using analytical RP-HPLC with a gradient of 5–100% CH_3CN (0.1% TFA) in water (0.1% TFA) over 45 min.

Measurements of intracellular cathepsin B activity. HeLa (human cervix adenocarcinoma), HepG2 (human hepatocellular carcinoma), MCF-7 (human breast adenocarcinoma), U-87MG (human glioma) and CHO (Chinese hamster ovary) cells were seeded in a 96-well plate at a density of 2×10^3 cells per well in culture media (RPMI-1640 for HeLa and CHO cells, DMEM for HepG2, MCF-7 and U-87MG cells supplemented with 10% fetal bovine serum (FBS)). After 24 h, the growth media were removed and the cells were washed with 100 μL Dulbecco's phosphate buffered saline (DPBS, without calcium and magnesium ions). The cells were then incubated with 50 μL PAB (Hank's balanced salt solution lacking sodium bicarbonate and containing 0.6 mM $CaCl_2$, 0.6 mM $MgCl_2$, 2 mM L-cysteine, 25 mM PIPES, pH 7.0) at 37 ^{o}C for 30 min and then the buffer was replaced with fresh PAB containing Cbz-Phe-Lys-AMC (5, 10 μM) in the presence or absence of 20 μM CA074-Me and 0.1% Triton X-100. Release of AMC was detected using a microplate reader (Spectramax Gemini EM, Molecular Devices, Sunnyvale, CA, USA) at 37 ^{o}C with 365 nm excitation filter and 447 nm emission filter. The reaction was run for 3 h at 37 ^{o}C with readings taken every 5 min.

RT-PCR. Measurements of mRNA levels in HeLa, HepG2, MCF-7, U-87MG and CHO cells were performed using appropriate primers according to the procedure describe previously with minor modification.[1] Briefly, mRNA was isolated using easy-BLUE^{TM} Total RNA Extraction kit (iNtRON Biotechnology, Inc., Korea). RNA concentrations were determined using NanoDrop ND-1000 spectrophotometer (Nanodrop, Wilmington, DE). cDNA was generated from 2 μg of total RNA with Reverse Transcriptase (iNtRON Biotechnology) and

oligo-dT primers in 70 μL reactions. An aliquot of 2 μL of cDNA was subjected to 40 cycles of PCR in a 20 μL reaction mixture using following primers:

SSTR2 forward : 5'-AGAGCCGTACTATGACCTGA-3'
(human) reverse : 5'-AGCCCACTCGGATTCCAGAG-3'

SSTR2 forward : 5'-TCATTTATGTCATCCTCCGCTAT-3'
(hamster) reverse : 5'-CACCACAAAGTCAAACATGCC-3'

GAPDH forward : 5'-ACCACAGTCCATGCCATCAC-3'
(human) reverse : 5'-TCCACCACCCTGTTGCTGTA-3'

GAPDH forward : 5'-ACCACAGTCCATGCCATCAC-3'
(hamster) reverse : 5'-TCCACCACCCTGTTGCTGTA-3'

Thermal cycling conditions were as follows: 95 ^{o}C for 2 min, followed by 20 s at 95 ^{o}C, 20 s at an optimal temperature for each primer set (59 ^{o}C for human SSTR2, 55 ^{o}C for hamster SSTR2, 60 ^{o}C for human GAPDH, and 58 ^{o}C for hamster GAPDH) and 1 min at 72 ^{o}C for 40 cycles and then 72 ^{o}C for 5 min. PCR products were separated by electrophoresis on a 1.5% agarose gel with SYBR safe DNA gel stains (Invitrogen) and photographed using Gel Doc (Bio-Rad).

Cellular uptake of Rho-Oct. HeLa, MCF-7 and CHO cells were seeded on a cover slip in a 24-well plate at a density of 2×10^3 cells per well in culture media. After 24 h, the cells were incubated with 50 μM Rho-Oct in the culture media containing 0.5% DMSO (v/v) for 2 h at 37 ^{o}C. After washing twice with 400 μL Dulbecco's phosphate buffered saline (DPBS, without calcium and magnesium) to remove the remaining conjugate, the cells were fixed with 3.5% paraformaldehyde containing 1 μg/mL of Hoechst 33342. After washing twice with 400 μL DPBS, the cover slip seeded with cells was mounted upside down on a glass slide. The cells were imaged by using confocal microscopy (LSM 510 META, Carl Zeiss, Germany). For competition experiments, the cells were incubated with 3 mM octreotide in the culture media containing 1% DMSO (v/v) for 1 h at 37 °C. Without washing, the cells

were treated with 50 μM Rho-Oct for 2 h at 37 °C.

Incubation of cells with Oct-TG, Oct-Dox and noncleavable Oct-Dox. HeLa, HepG2, MCF-7, U-87MG and CHO cells were seeded on a cover slip in a 24-well plate at a density of 2×10^3 cells per well in culture media. After 24 h, the cells were incubated with 10 μM Oct-TG, Oct-Dox or noncleavable Oct-Dox in the culture media containing 0.1–1% (v/v) DMSO for 6 h at 37 ^{o}C. After washing twice with 400 μL Dulbecco's phosphate buffered saline (DPBS, without calcium and magnesium) to remove the remaining conjugate, the cells were fixed with 3.5% paraformaldehyde containing 1 μg/mL of Hoechst 33342. After washing twice with 400 μL DPBS, the cover slip seeded with cells was mounted upside down on a glass slide. The cells were imaged by using confocal microscopy. For competition experiments, the cells were pre-incubated with 3 mM octreotide in the culture media containing 1% (v/v) DMSO for 1 h at 37 °C. Without washing, the cells were further incubated with 10 μM Oct-TG or Oct-Dox for 6 h at 37 °C. For inhibition of cellular cathepsin B activity, the cells were pre-incubated with 20 μM CA-074-Me in the culture media containing 0.2% (v/v) DMSO for 24 h at 37 °C. Without washing, the cells were further incubated with 10 μM Oct-TG or Oct-Dox for 6 h at 37 °C.

Quantitation of fluorescence intensity of cells incubated with Oct-TG and Oct-Dox. HeLa, HepG2, MCF-7, U-87MG and CHO cells were seeded in a 96-well plate at a density of 2×10^3 cells per well in culture media. After 24 h, the cells were incubated with 10 μM Oct-TG in the culture media containing 1% (v/v) DMSO for 6 h at 37 ^{o}C. After washing twice with 100 μL DPBS without calcium and magnesium to remove the remaining conjugate, the cells were fixed with 3.5% paraformaldehyde containing 1 μg/mL Hoechst 33342 for 15 min, as an internal control for ArrayScan VTI HCS Reader. After washing twice with 100 μL DPBS, the cells were imaged by ArrayScan VTI HCS Reader with a 20X objective (Cellomics, USA). Target Activation BioApplication of ArrayScan VTI HCS Reader was used to acquire and analyze the images. Images of 1,000 cells for each probe were analyzed to obtain the average cell number per field, fluorescence area and intensity per cell.

For competition experiments, cells were pre-incubated with 3 mM octreotide for 1 h at 37 ^{o}C. Without washing, the cells were treated with 10 μM Oct-TG in the culture media

containing 1% DMSO (v/v) for 6 h at 37 ^{o}C. After washing twice with DPBS, the cells were fixed with 3.7% formaldehyde containing 1 μg/mL Hoechst 33342 for 15min. After washing twice with DPBS, the cells were imaged and evaluated by ArrayScan VTI HCS Reader with a 20X objective.

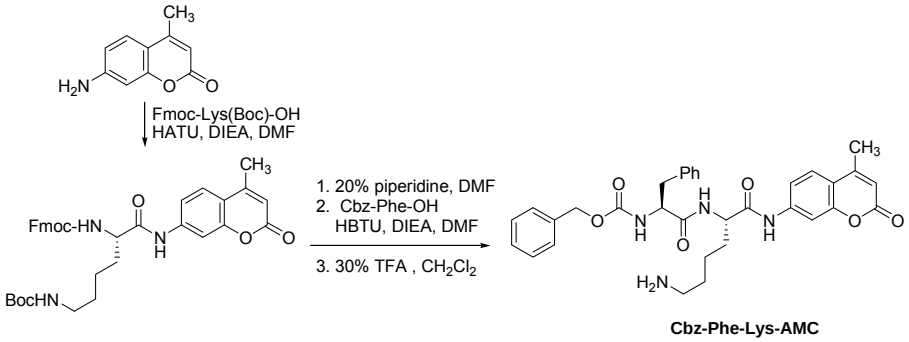
For inhibition of cathepsin B activity, the cells were pre-incubated with 20 μM CA-074-Me for 24 h containing 0.2% (v/v) DMSO at 37 ^{o}C. Without washing, the cells were incubated with 10 μM Oct-TG for 6 h at 37 ^{o}C. After washing twice with DPBS, the cells were fixed with 3.7% formaldehyde containing 1 μg/mL Hoechst 33342 for 15 min. After washing twice with DPBS, the cells were imaged and evaluated by ArrayScan VTI HCS Reader with a 20X objective.

To quantitation of fluorescence intensity of Dox in the nucleus, Image J (NIH, USA) software is used.

MTT assay. HeLa, HepG2, MCF-7, U-87MG, HT-29 (human colon adenocarcinoma), A549 (human lung adenocarcinoma epithelial cells), Capan-1 (human pancreas adenocarcinoma), MDA-MB-231 (human breast carcinoma) and CHO (Chinese hamster ovary) cells were seeded in a 96-well plate at a density of 1×10^4 cells per well in culture media (RPMI-1640 for A549, HeLa, HT29 and CHO cells, DMEM for Capan-1, HepG2, MCF-7, MDA-MB-231 and U-87MG cells supplemented with FBS). After 12 h, the growth media were removed and the cells were incubated with 60 μL growth media containing 0, 0.2, 0.5, 1, 2, 5, 10, 20 μM doxorubicin, Oct-Dox or noncleavable Oct-Dox for 36 h at 37 ^{o}C. The cells were then incubated with 12 μL of 5 mg/mL MTT solution for 5 h at 37 ^{o}C. The growth media were aspirated and 100 μL DMSO was added to dissolve insoluble purple formazan product. The absorbance of formazan product was recorded using a microplate reader (SpectraMax 340PC 384, Molecular Devices, Sunnyvale, CA, USA) on an endpoint mode at 560 nm.

Supplementary reference

1. S. B. Curtis, J. Hewitt, S. Yakubovitz, A. Anzarut, Y. N. Hsiang and A. M. J. Buchan, *Am. J. Physiol. Heart Circ. Physiol*., 2000, **278**, H1815–H1822.



Scheme S1 Preparation of a fluorogenic substrate Cbz-Phe-Lys-AMC for cathepsin B.

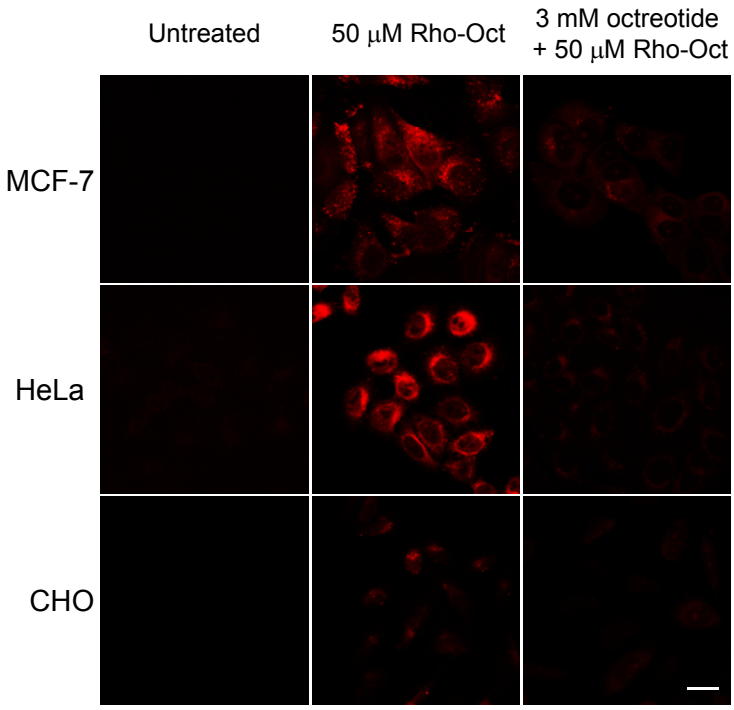


Fig. S1 Detection of internalization of Rho-Oct into cells. MCF-7, HeLa and CHO cells were treated with 50 μM Rho-Oct for 2 h at 37 ^{o}C in the absence or presence of 3 mM octreotide (scale bar = 20 μm).

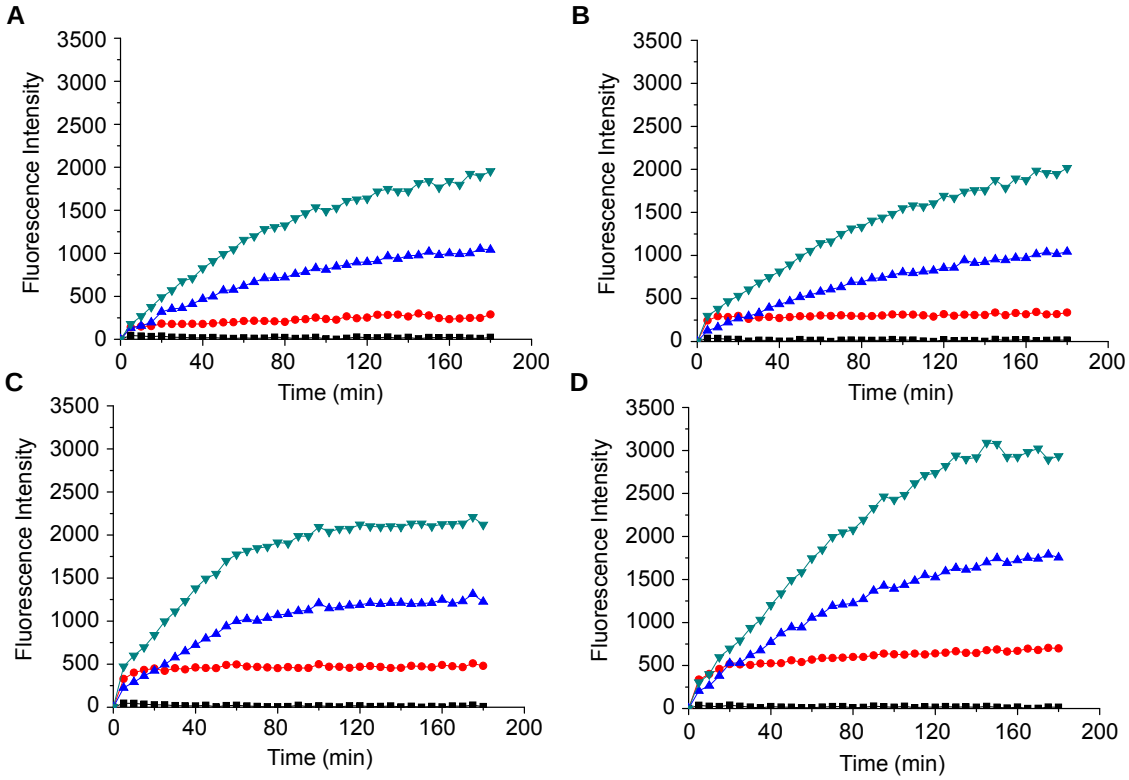
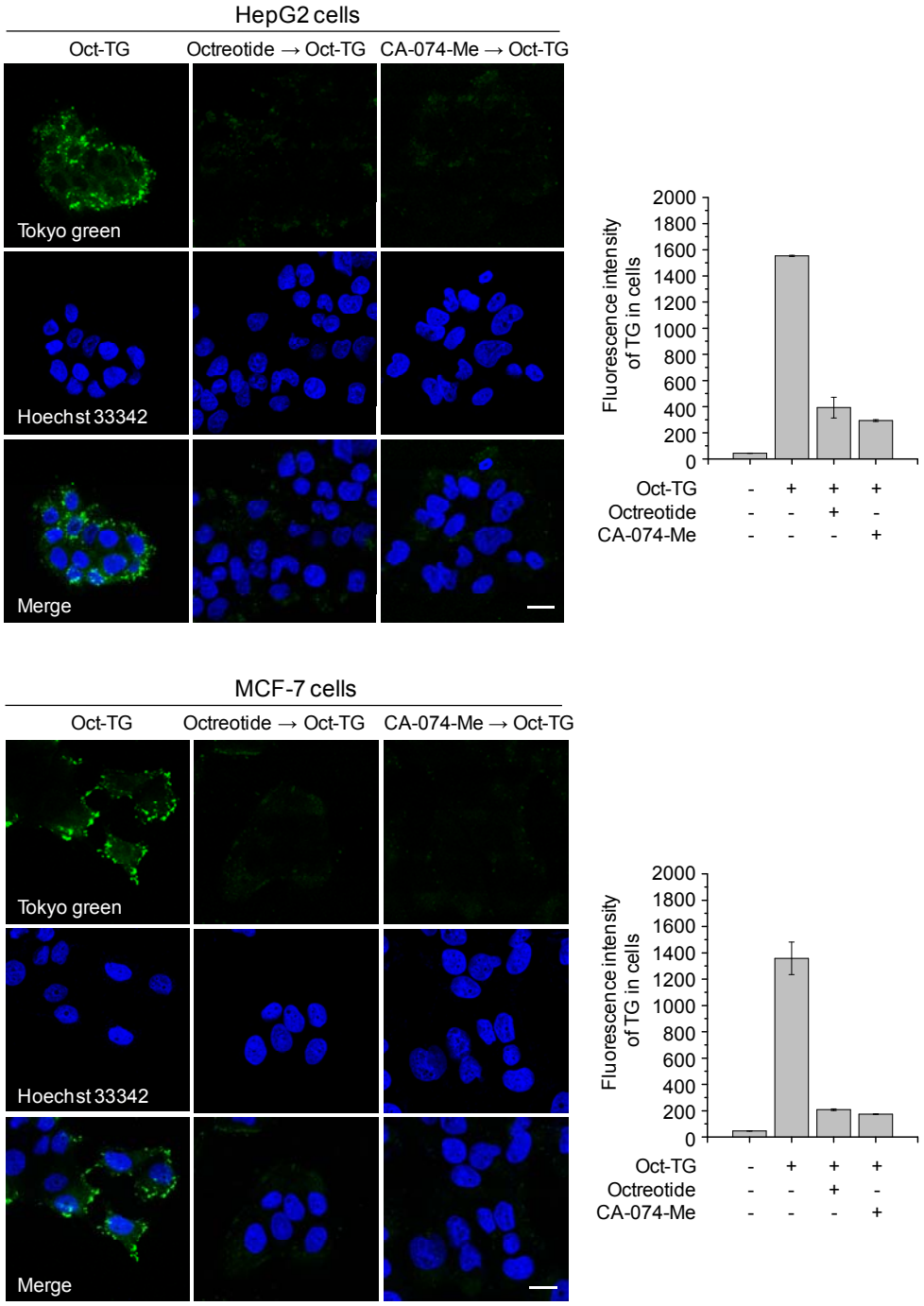


Fig. S2 Detection of intracellular cathepsin B activity using a fluorogenic substrate Cbz-Phe-Lys-AMC. Cellular cathepsin B activities were measured by incubating Cbz-Phe-Lys-AMC with lysates of A) HepG2, B) MCF-7, C) U-87MG and D) CHO cells for 3 h at 37 ^{o}C (λ_{ex} = 365 nm, λ_{em} = 447 nm). ▼: 10 μM substrate, ▲: 5 μM substrate, ●: 10 μM substrate and 20 μM CA074-Me, ■: no substrate.



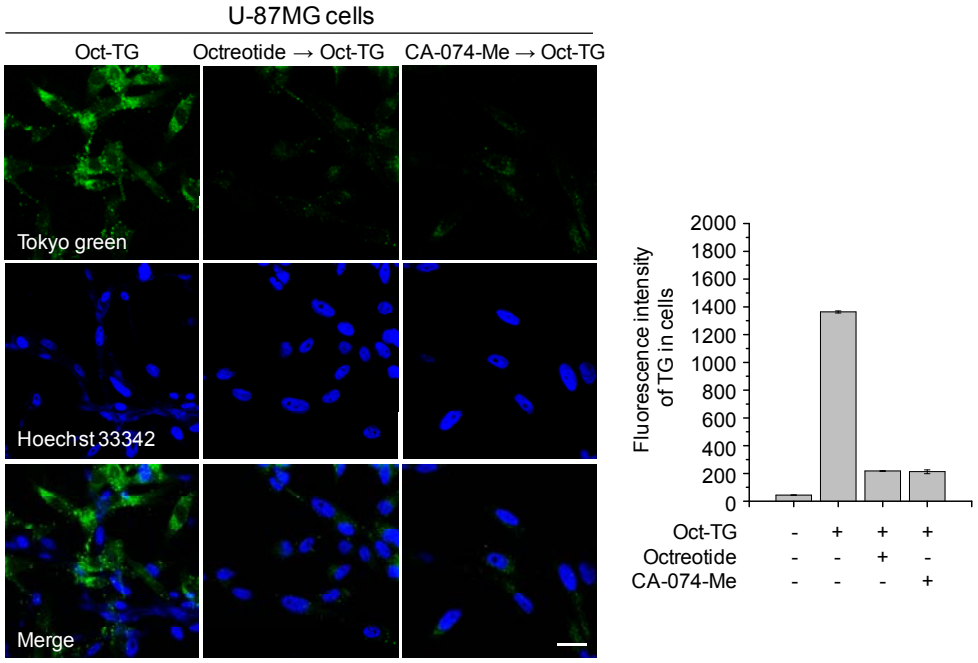
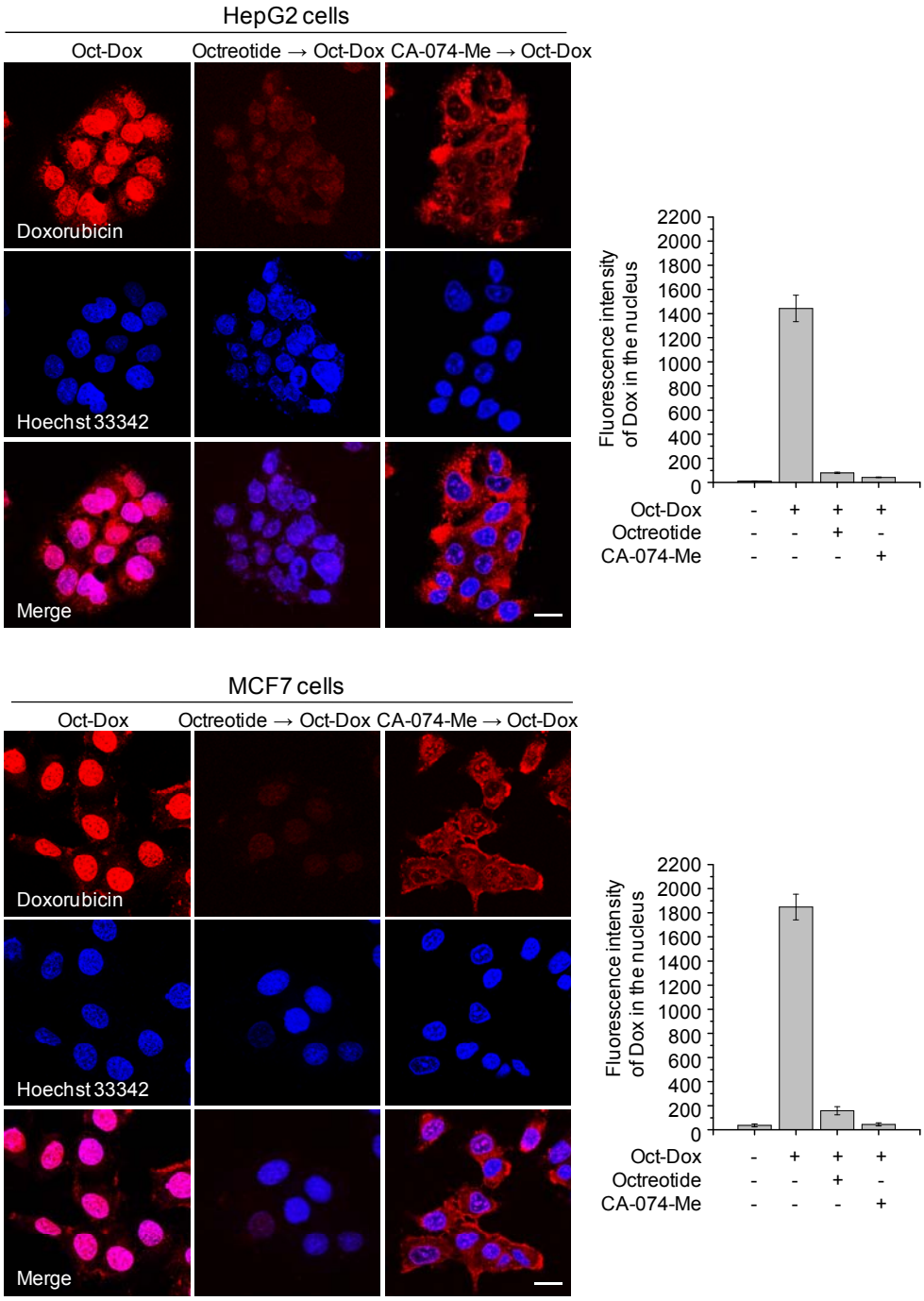


Fig. S3 Fluorescence imaging of cancer cells using Oct-TG. HepG2, MCF-7 and U-87MG cells were incubated with 10 μM Oct-TG for 6 h (left) or pretreated with either 3 mM octreotide for 1 h (middle) or 20 μM CA074-Me for 24 h (right) followed by incubation with 10 μM Oct-TG for 6 h. Hoechst 33342 was used to stain the nucleus (scale bar = 20 μm). Right graph: quantitative analysis of fluorescence intensity of cells incubated with Oct-TG.


HepG2 cells
Oct-Dox
Octreotide → Oct-Dox
CA-074-Me → Oct-Dox
Doxorubicin
Hoechst 33342
Merge
Fluorescence intensity of Dox in the nucleus
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Oct-Dox - + + +
Octreotide - - + -
CA-074-Me - - - +
MCF7 cells
Oct-Dox
Octreotide → Oct-Dox
CA-074-Me → Oct-Dox
Doxorubicin
Hoechst 33342
Merge
Fluorescence intensity of Dox in the nucleus
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Oct-Dox - + + +
Octreotide - - + -
CA-074-Me - - - +

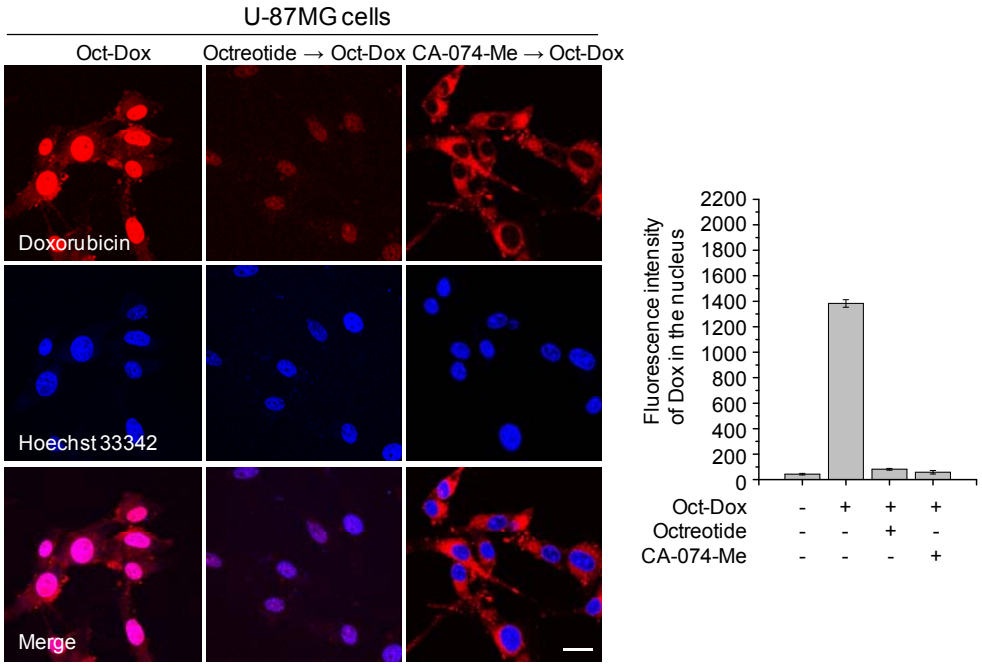


Fig. S4 Cleavage of doxorubicin from Oct-Dox in cancer cells. HepG2, MCF-7 and U-87MG cells were incubated with 10 μM Oct-Dox for 6 h (left) or pretreated with either 3 mM octreotide for 1 h (middle) or 20 μM CA-074-Me for 24 h (right) followed by incubation with 10 μM Oct-Dox for 6 h. Hoechst 33342 was used to stain the nucleus (scale bar = 20 μm). Right graph: Quantitative analysis of fluorescence intensity of Dox in the nucleus of cells incubated with Oct-Dox.

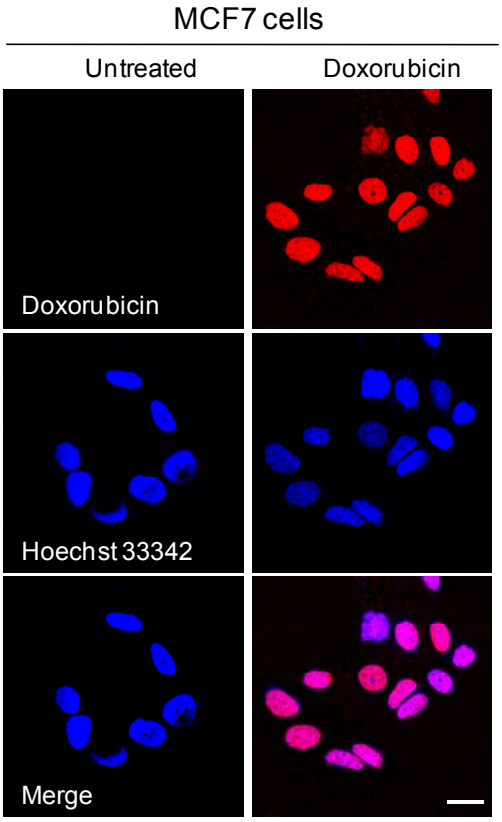


Fig. S5 Uptake of free Dox by MCF-7 cell. The cells were incubated with 10 μM Dox for 1 h. Hoechst 33342 was used to stain the nucleus (scale bar = 20 μm).

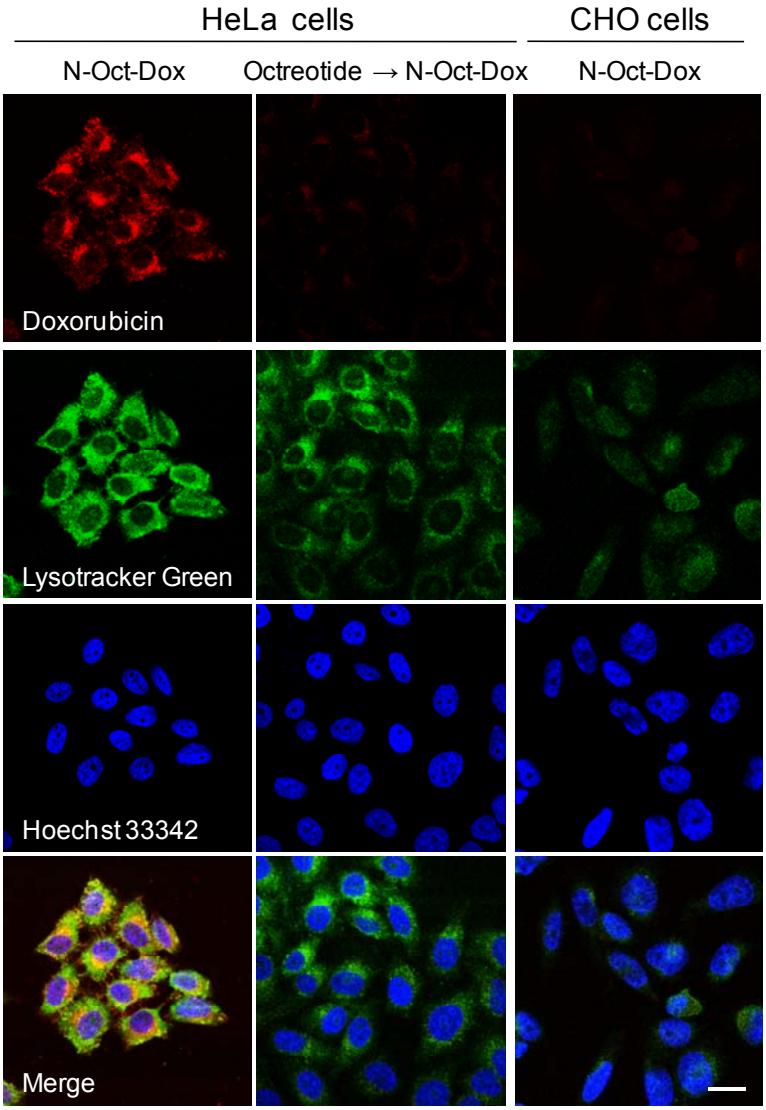


Fig. S6 Cellular distribution of noncleavable Oct-Dox in HeLa and CHO cell. HeLa cells were incubated with 10 μM noncleavable Oct-Dox (N-Oct-Dox) for 6 h (left) or pretreated with either 3 mM octreotide for 1 h (middle) or 20 μM CA074-Me for 24 h (right) followed by incubation with 10 μM N-Oct-Dox for 6 h. As a control, CHO cells were treated with 10 μM N-Oct-Dox for 6 h. Lysotracker Green and Hoechst 33342 were used to stain the lysosome and the nucleus, respectively (scale bar = 20 μm).

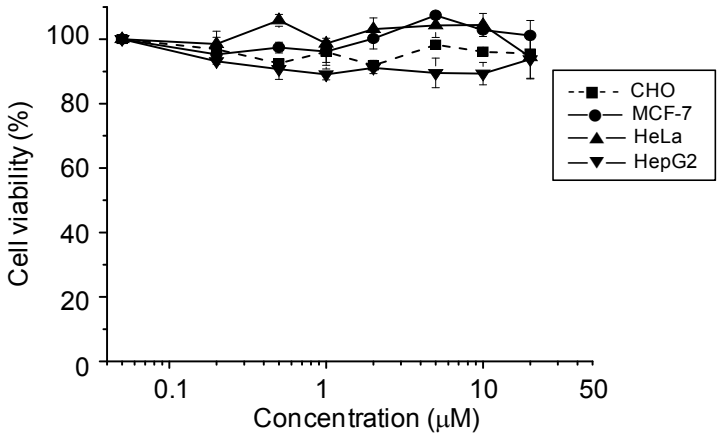
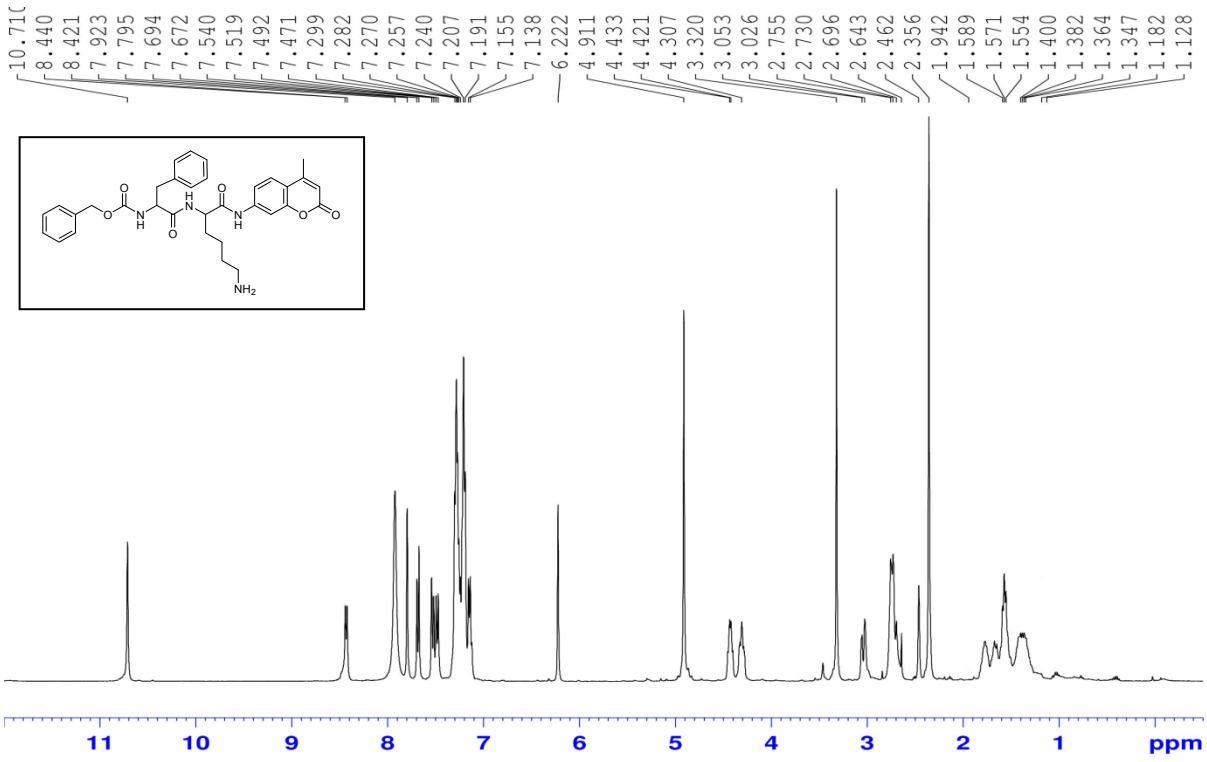
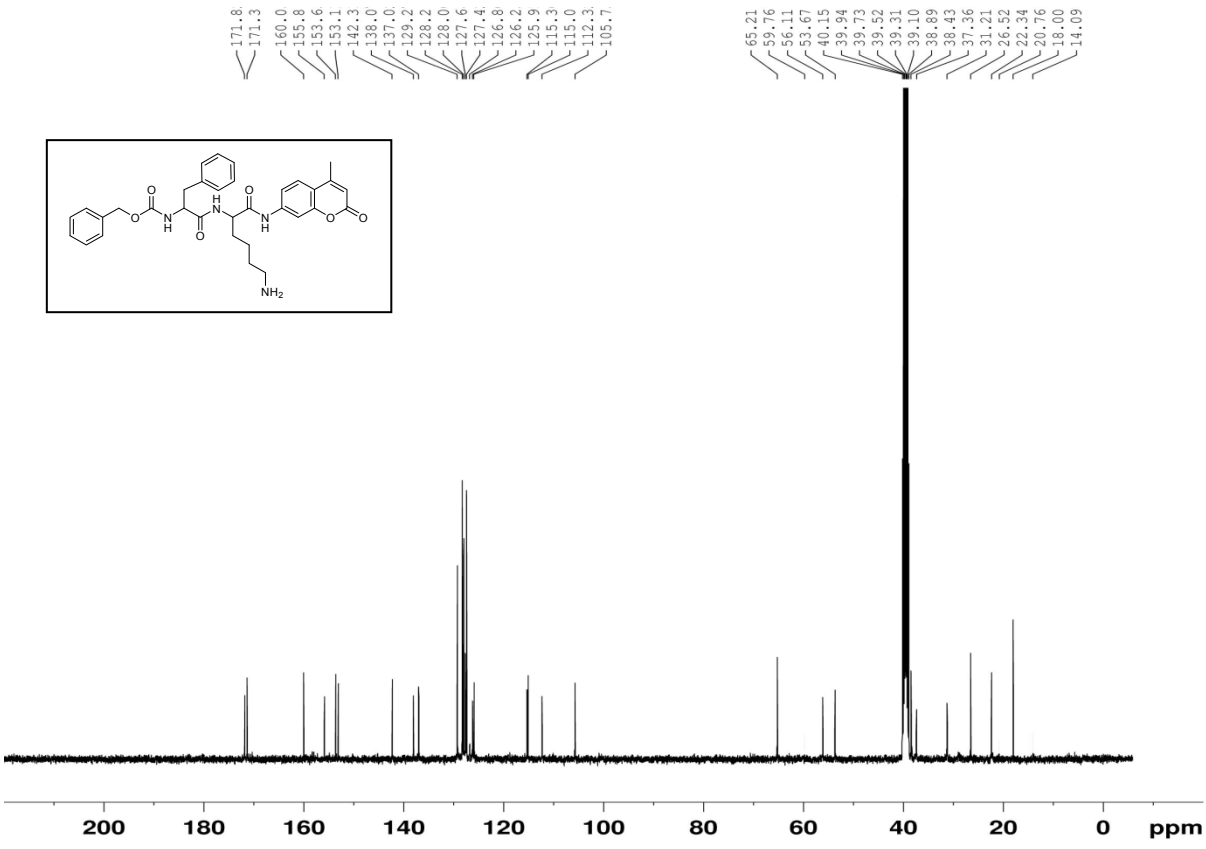
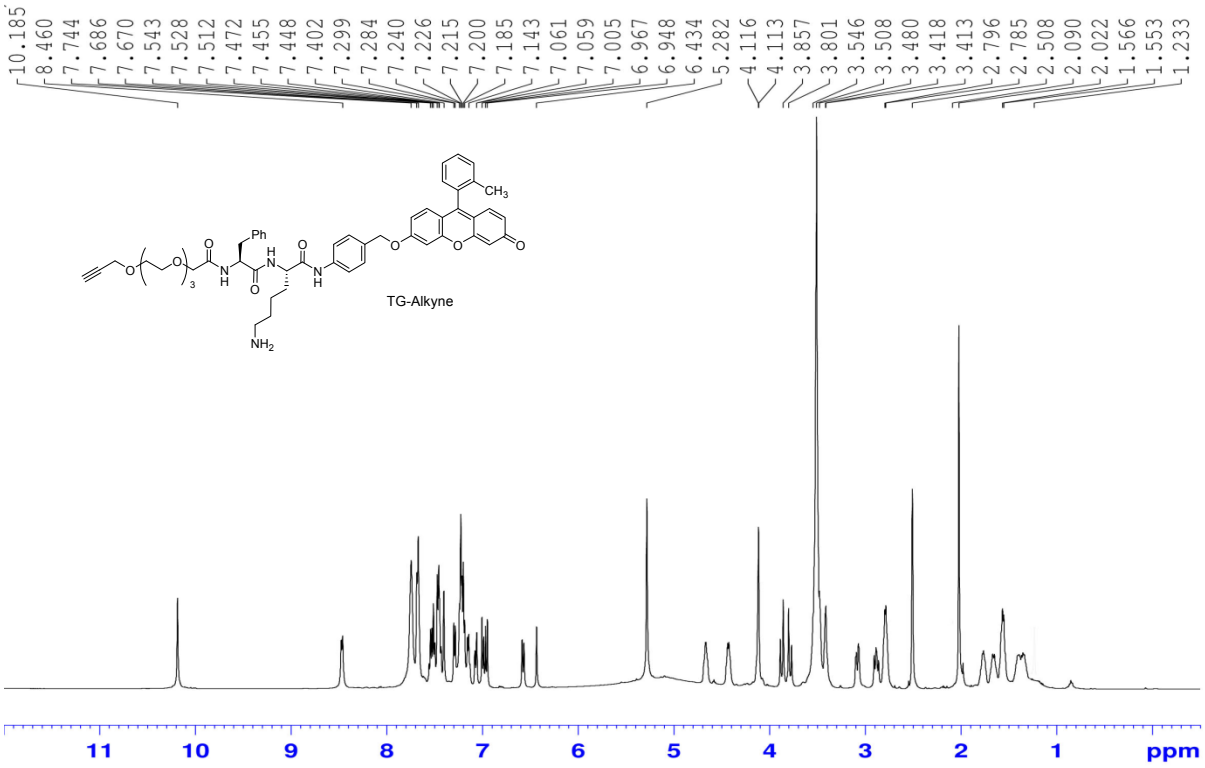


Fig. S7 Cell death effects of noncleavable Oct-Dox. Cancer cells and CHO cells were incubated with various concentrations of noncleavable Oct-Dox for 36 h and then an MTT assay was performed to measure the cell viability.

NMR spectra of compounds



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7.284
7.240
7.226
7.215
7.200
7.185
7.143
7.061
7.059
7.005
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6.948
6.434
5.282
4.116
4.113
3.857
3.801
3.546
3.508
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2.785
2.508
2.090
2.022
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1.233
TG-Alkyne
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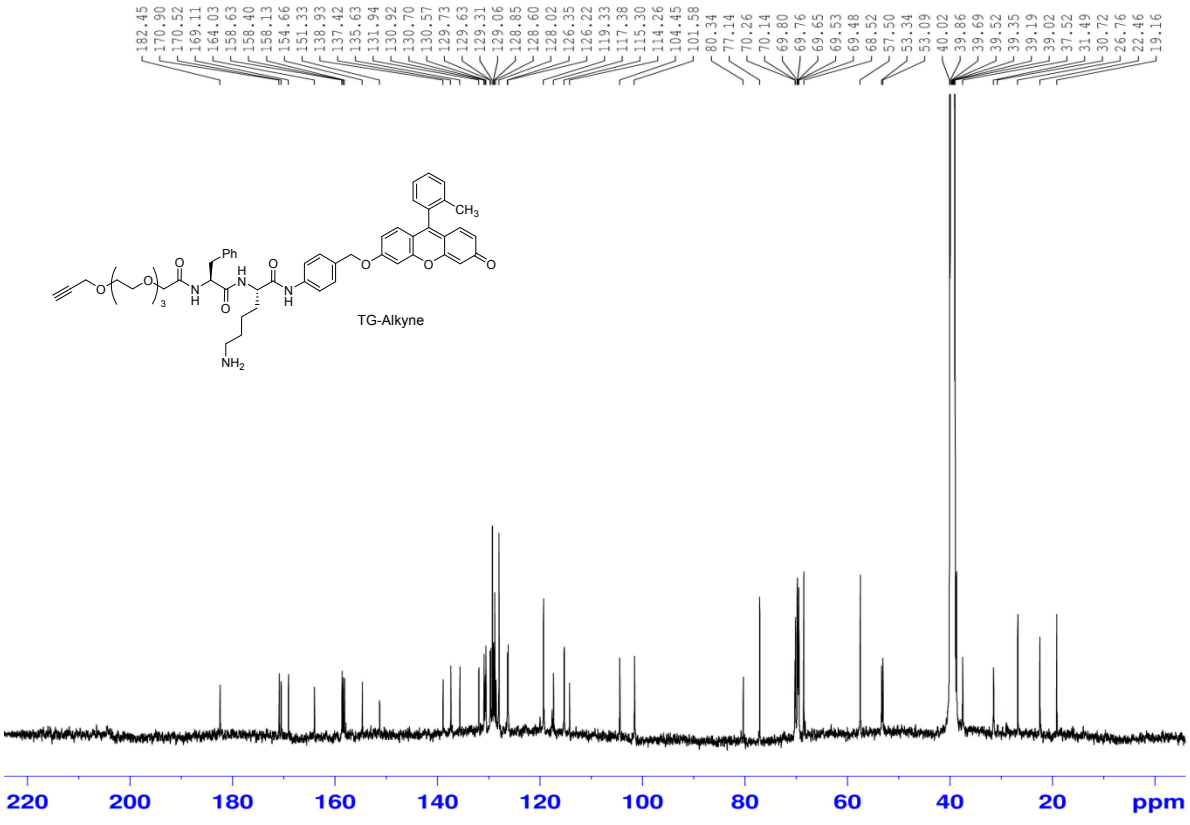
7
6
5
4
3
2
1
ppm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129.63
129.31
129.06
128.85
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128.02
126.35
126.22
119.33
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70.14
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57.50
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31.49
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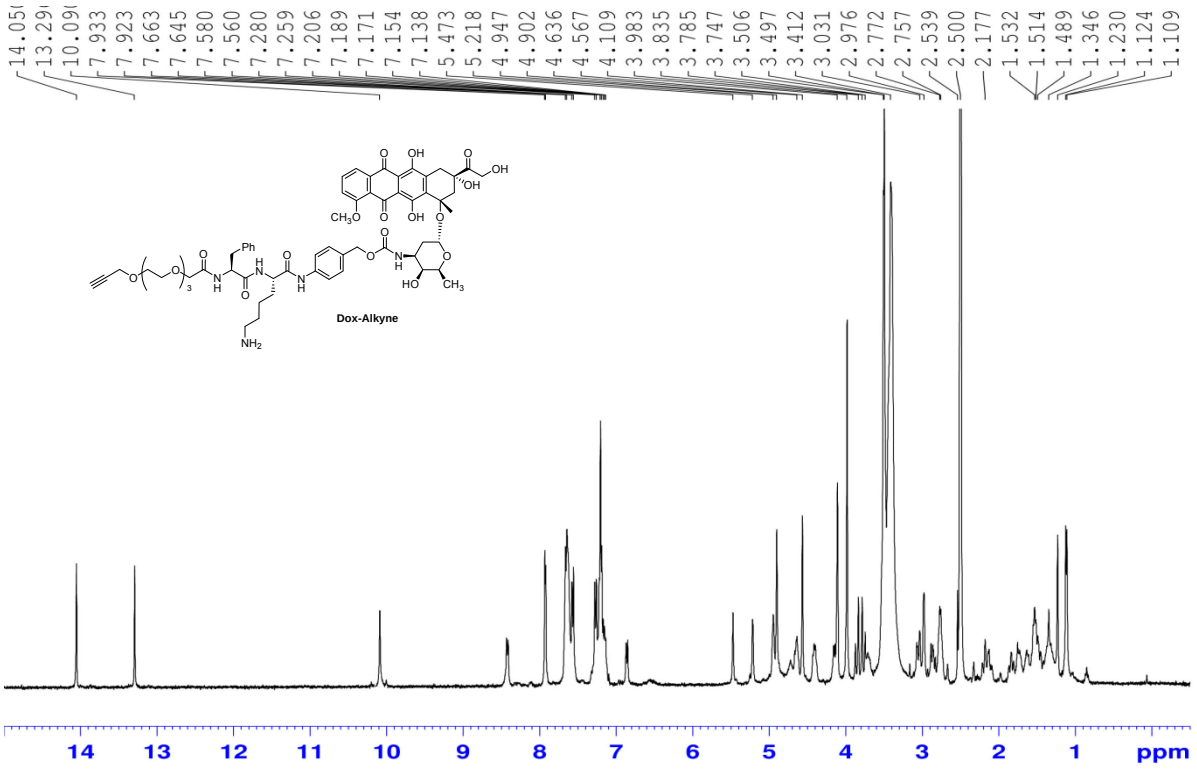
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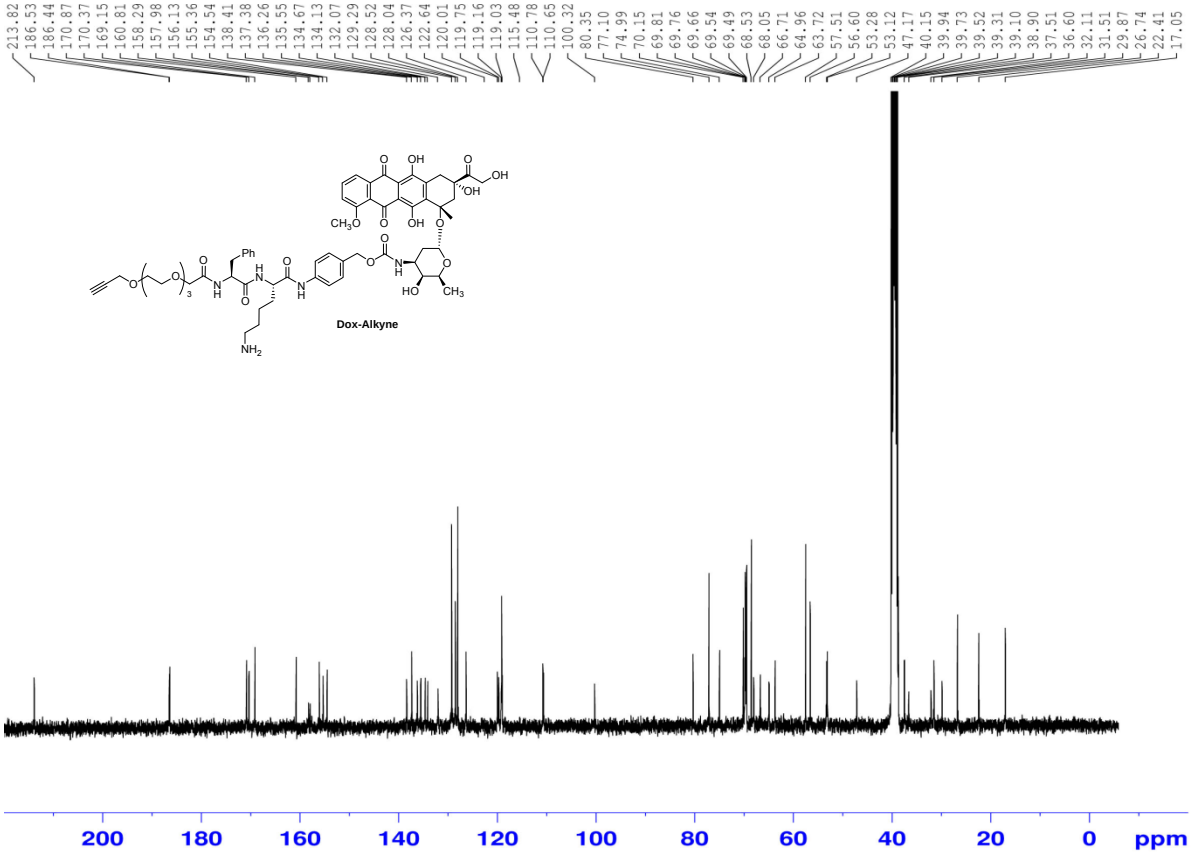
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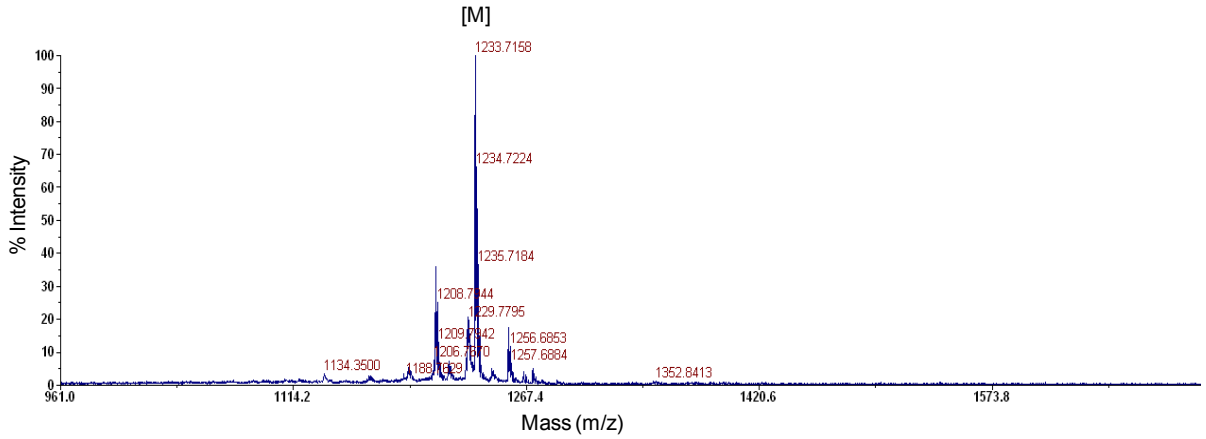

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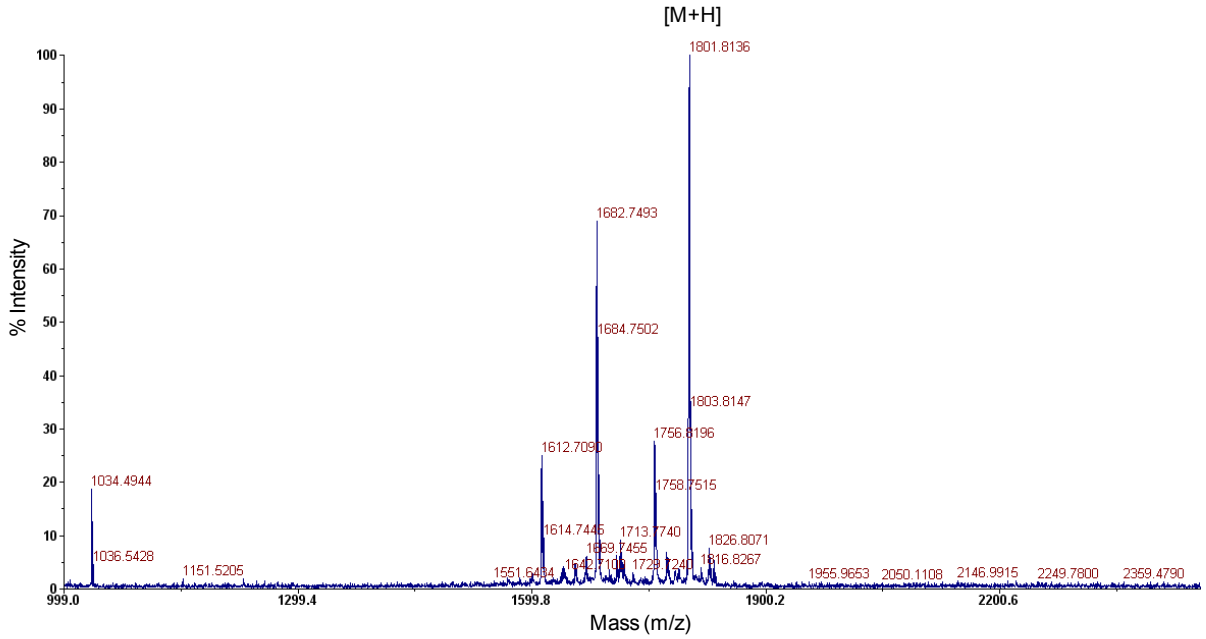

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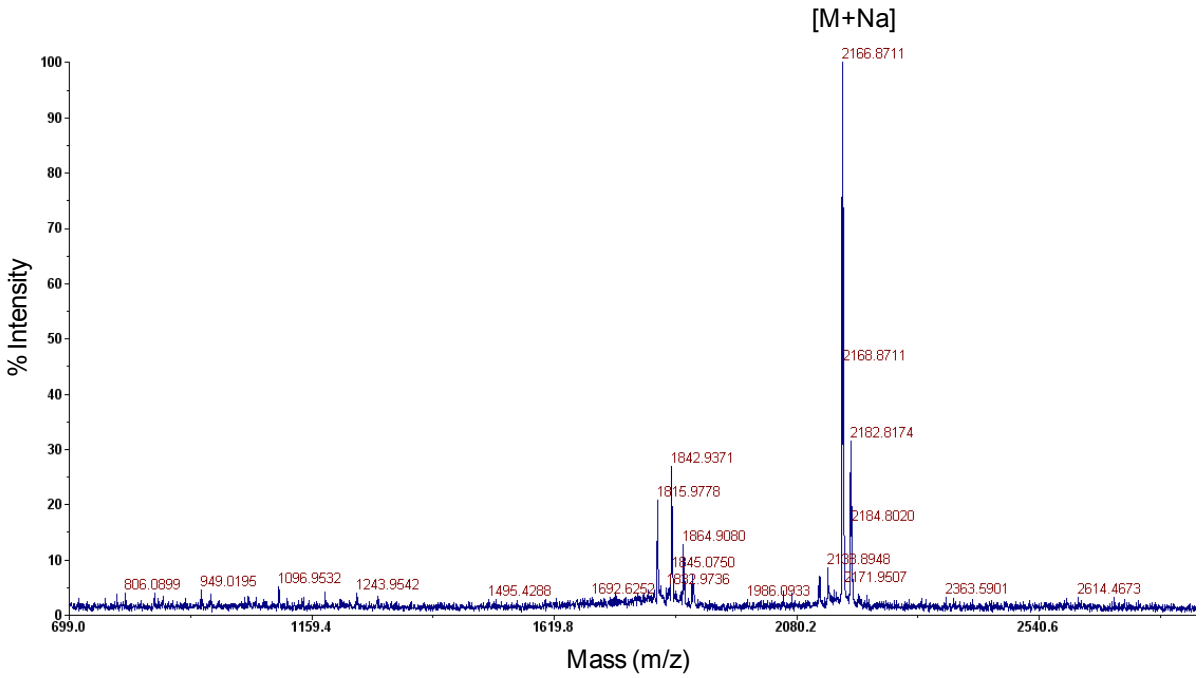

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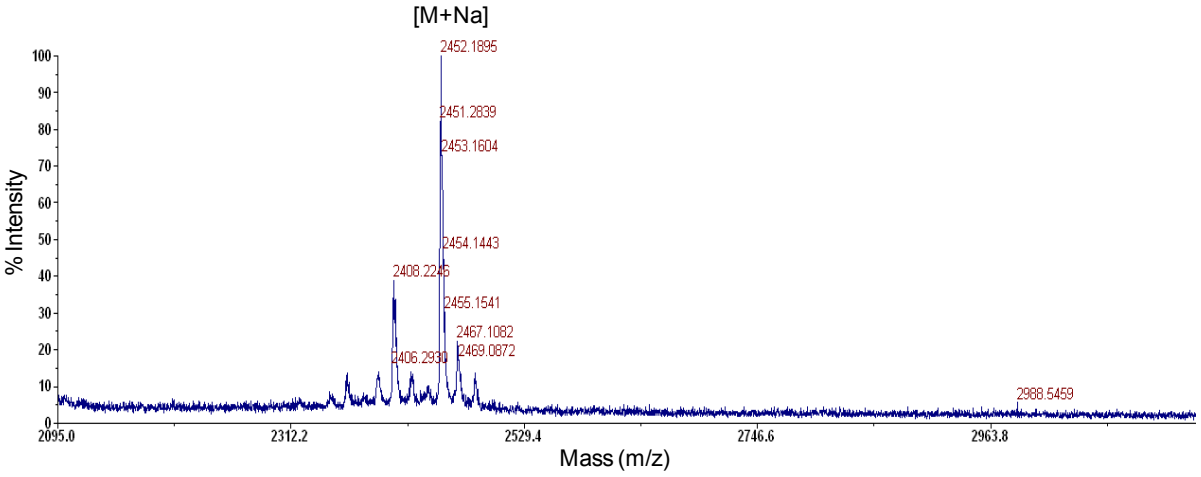

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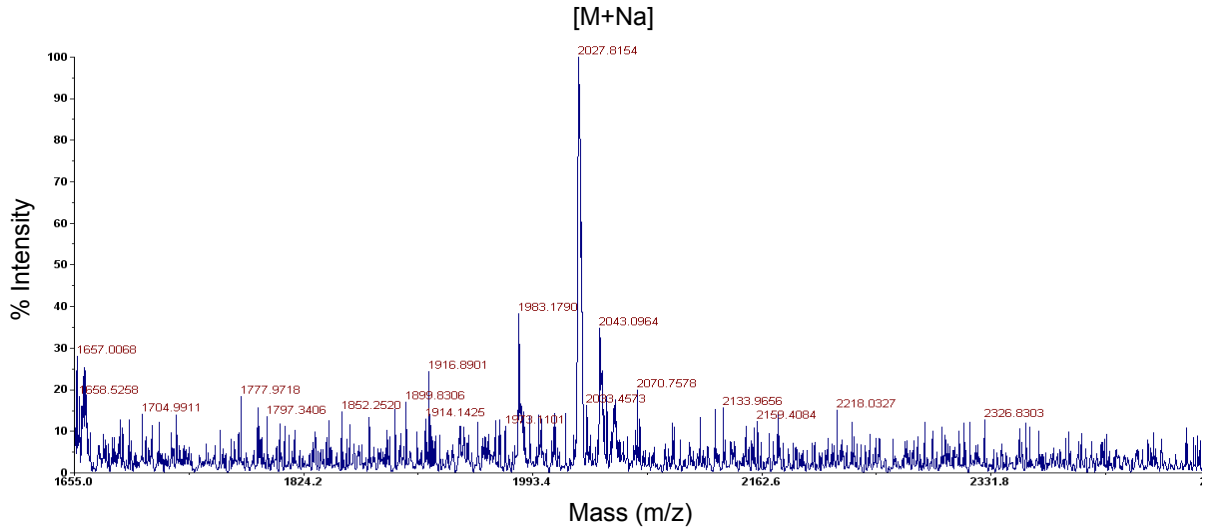

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