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Supplementary Information

A novel anticancer theranostic pro-prodrug based on hypoxia and photo sequential control

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1. Experimental section

1.1 Materials

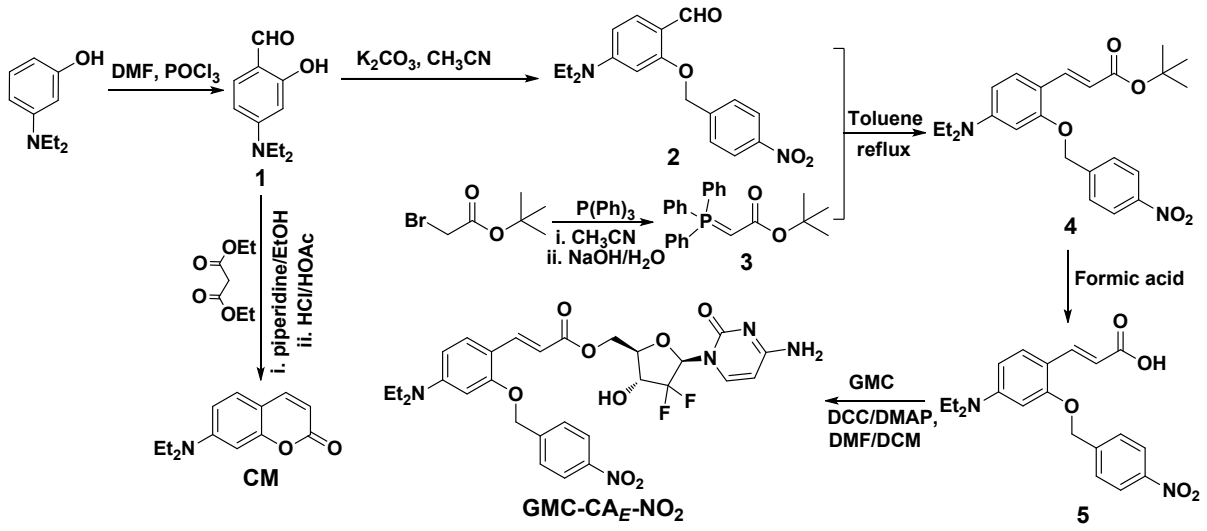
Anhydrous dimethyl sulfoxide (DMSO), gemcitabine (GMC), and bovine serum albumin (BSA) were purchased form Alfa-aesar. Nitroreductase (NTR) (≥100 units/mg) from *Escherichia coli*, and nicotinamide adenine dinucleotide (NADH) were purchased from Sigma-Aldrich. The lyophilized powder of NTR was dissolved in pure water, and the solution was divided into 20 parts as suitable amounts for daily experiments. All these enzyme solutions were frozen immediately at −20 °C for storage and allowed to thaw before use according to the known procedure[1], which results in no change of the enzyme activity. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin and phosphate-buffer saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) were obtained from Gibco (Grand Island, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), benzyl penicillin and streptomycin were obtained from Sigma. All other chemicals used were local products of analytical grade. A stock solution (1 mM) of **GMC-CA$_E$-NO$_2$** was prepared by dissolving an appropriate amount of **GMC-CA$_E$-NO$_2$** in DMSO. Ultrapure water (over 18 MΩ/cm) was used. Column chromatography was performed using silica gel 60 (70–230 mesh) as stationary phase.

1.2 Instrumentation and measurement

UV-irradiation (ZF-8 lamp, 365 nm filter, 10 mW/cm^2) was employed in corresponding photolysis tests. Melting points were determined on an X-4 microscopic melting-point spectrometer. The IR spectra were recorded using KBr pellets and a Thermo Scientific Nicolet iS10 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AVANCEIII 600 NMR spectrometer in CD_3Cl or DMSO-d_6. Electrospray ionization mass spectra (ESI-MS) were recorded in negative mode with a HRMS apex ultra 7.0T US+ and AB SCIEX 4000 Q TRAP MS system. Fluorescence measurements were performed on a Hitachi F-7000 luminescence spectrophotometer in 2.5×10 mm quartz cells. The optical density (*OD*) was measured on a microplate spectrophotometer. A liquid chromatography system from Shimadzu Technology (Shimadzu, Japan) was applied to all chromatography tests. The HPLC system was performed with a binary LC-20AT pump, a SPD-20A UV-vis detector, as well as a RF-10AXL detector. The data acquired and processed throughout a LC solution chromatographic workstation (Shimadzu, Japan). The analytical column was a Waters Symmetry C18 column (4.6 mm ×250 mm, 3.5 μm). Fluorescence imaging experiments were

performed on confocal microscope (Olympus, FV 1000, JPN) with excitation at 405 nm.

2. Synthesis and characterization



Scheme S1. Synthetic route for **GMC-CA$_E$-NO$_2$** and **CM**

4-Diethylaminosalicylaldehyde (1): According to a reported literature with some modification[2], compound **1** was synthesized. Phosphorous oxychloride (5.32 mL, 57.1 mmol) were slowly added to a cooled DMF (5.32 mL) in an ice bath and stirred for 15 min, followed by addition of a solution of diethyl-*m*-aminophenol (DEMAP, 5.0 g, 30.3 mmol) in DMF (5.0 mL). After stirring at room temperature for 0.5 h, the mixture was heated at 90-95 ^{o}C for 30 min. Then the reaction mixture was cooled to room temperature and poured into ice-cold water, diluted with EtOAc (70 mL x 3), washed with water (30 mL×3) and brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography (PE:EA = 200:1 to 30:1) to give compound **1** (2.7 g) as a white solid in 47% yield. Mp: 59.1-61.3 ^{o}C.

4-Nitrobenzyloxy-4-diethylaminobenzaldehyde (2): According to a reported method[3] with some modification, compound **2** was prepared. To a mixture of **1** (869.6 mg, 4.5 mmol) and K_2CO_3 (1.2 g, 2 mmol) in acetonitrile (5.8 mL), 4-nitrobenzyl bromide (991.6 mg, 4.6 mmol) was added and stirred at room temperature over night. Then the reaction mixture was poured into water and extracted with ethyl acetate (10 mL x 4). The combined organic layer was washed with brine and dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography (PE: EA = 5:1) to give compound **2** (1.2 g) as a yellow solid in 79% yield. Mp: 143.3-144.8 ^{o}C; ^{1}H NMR ($CDCl_3$, 600

MHz, ppm): δ 10.24 (s, 1 H), 8.27 (d, J = 8.4 Hz, 2 H), 7.75 (d, J = 9 Hz, 1 H), 7.65 (d, J = 8.4 Hz, 2 H), 6.35 (d, J = 9 Hz, 1 H), 6.05 (s,1 H), 5.28 (s , 2 H), 3.39 (q, J = 6.9 Hz, 4 H), 1.17 (t, J = 6.9 Hz, 6 H). ^{13}C NMR ($CDCl_3$, 150 MHz, ppm): δ 185.59, 161.42, 152.69, 146.65, 143.06, 130.20, 126.41, 122.92, 113.41, 104.01, 92.94, 67.83, 43.85, 11.50. MS (ESI), calcd for $C_{18}H_{21}N_2O_4$, *m/z*: 329.1 $[M+H]^+$; found 329.1.

***tert*–Butyl-(triphenylphosphoranylidene)acetate (3):** Compound **3** was prepared according to the literature with some modification[4]. A solution of triphenylphosphine (2.6 g, 10 mmol) and *tert*-butyl chloroacetate (3.9 g, 20 mmol) in of acetonitrile (10 mL) was refluxed for 7 h. The resulting phosphonium salt was collected by filtration, washed with acetonitrile (10 mL), and air-dried. Then 20% NaOH solution was added to a solution of the crude salt dissolved in 100 mL of water with vigorous stirring in an ice bath, and pH of the mixture solution was modulated to 8. The solid was collected by filtration, washed well with cold water, and air-dried to give a white solid **3** (2.5 g) in 66% yield.

(*E*)-*tert*-Butyl 3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylate (4): Compound **4** was prepared according to the literature with some modification[5]. Under N_2 atmosphere and protecting form light, a mixture of compound **2** (504.0 mg, 1.5 mmol) and **3** (1.2 g, 3.1 mmol) in toluene (15.4 mL) was stirred at 95 °C. After the reaction was almost completed (determined by TLC (PE: EA=3: 1)), the toluene was removed in a vacuum. The crude residue was purified by flash chromatography on silica gel (PE: EA= 100: 1 to 10: 1) to generate compound **4** (458.0 mg) as an orange solid in 70% yield. Mp: 113.7-118.3 ^{o}C; ^{1}H NMR ($CDCl_3$, 600 MHz, ppm): δ 8.25 (d, J = 8.4 Hz, 2 H), 7.93 (d, J = 16.2 Hz, 1 H), 7.63 (d, J = 8.4 Hz, 2 H), 7.41 (d, J = 8.4 Hz, 1 H), 6.29 (dd, J_1 = 8.7 Hz, J_2 = 1.5 Hz ,1 H), 6.23 (d, J = 15.6 Hz, 1 H), 6.03 (d, J = 1.2 Hz, 1 H), 5.23 (s, 2 H), 3.32 (q, J = 7.2 Hz, 4 H), 1.53 (s, 9 H), 1.12 (t, J = 6.9 Hz, 6 H). ^{13}C NMR ($CDCl_3$, 150 MHz, ppm): δ 167.72, 158.34, 150.39, 147.57, 144.65, 138.71, 130.02, 127.47, 123.89, 114.60, 111.53, 105.18, 95.62, 79.56, 69.06, 44.60, 28.34, 12.59. MS (ESI), calcd for $C_{24}H_{31}N_2O_5$, *m/z*: 427.2 $[M+H]^+$; found 427.3.

(*E*)-3-(4-Diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (5): Compound **3** was prepared according to the literature with some modification[6]. A mixture of compound **4** (350 mg, 0.8 mmol) and formic acid (4.3 mL) was stirred vigorously for 2 h. The mixture was diluted by adding water (10 mL), followed by extracting with DCM (10 mL x 3). The organic layer was concentrated under reduced pressure to offer product **5** as a red brown solid (303 mg) in 99% yield which was taken on without any purification. Mp: 172.6-174.5 ^{o}C. ^{1}H NMR (DMSO-d_6, 600 MHz, ppm): δ 11.82 (br s, 1 H), 8.29 (d, J = 8.4 Hz, 2 H), 7.80 (d, J =

15.6 Hz, 1 H), 7.73 (d, *J* = 8.4 Hz, 2 H), 7.47 (d, *J* = 9.0 Hz, 1 H), 6.30 (d, *J* = 9.0 Hz, 1 H), 6.21 (d, *J* = 16.2 Hz, 1 H), 6.19 (s, 1 H), 5.40 (s, 2 H), 3.34 (q, *J* = 6.6 Hz, 4 H), 1.03 (t, *J* = 6.9 Hz, 6 H). ^{13}C NMR (DMSO-d_6, 150 MHz, ppm): δ 168.58, 158.12, 150.37, 147.04, 145.13, 139.23, 130.05, 128.21, 123.68, 112.34, 109.98, 104.76, 95.53, 68.26, 43.85, 12.40. MS (ESI), calcd for $C_{20}H_{23}N_2O_5$, *m/z*: 371.2 $[M+H]^+$; found 371.2.

GMC-CA$_E$-NO$_2$: According to a reported protocol with some modification[7], **GMC-CA$_E$-NO$_2$** was synthesized. A solution of GMC (114.3 mg, 0.43 mmol) dissolved in dry DMF (2.7 mL) was added to a mixture of compound **5** (134.0 mg, 0.36 mol), DMAP (4.4 mg, 36.2 μmol), and dry DCM (5.3 mL). After stirred for 15 min, a solution of DCC (149.2 mg, 0.72 mmol) in pyridine (0.35 mL) was added and stirred at room temperature for 12 h. The reaction was monitored by TLC (DCM: MeOH = 20: 1). Then 30 mL water was added and extracted with DCM (10 mL x 3), the organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by flash chromatography on silica gel (DCM: MeOH= 10: 1) to give **GMC-CA$_E$-NO$_2$** (34.7 mg) as an orange solid in 16% yield. Mp: 133.4-136.3 ^{o}C; ^{1}H NMR (DMSO-d_6, 600 MHz, ppm): δ 8.29 (d, *J* = 8.4 Hz, 2 H), 7.98 (d, *J* = 16.2 Hz, 1 H), 7.74 (d, *J* = 8.4 Hz, 2 H), 7.71 (d, *J* = 7.2 Hz, 1 H), 7.57 (d, *J* = 9.0 Hz, 1 H), 7.47 (s, 1 H), 7.42 (s, 1 H), 6.39 (d, *J* = 15.6 Hz, 1 H), 6.32 (d, *J* = 9.0 Hz, 1 H), 6.28 (t, *J* = 8.7 Hz, 1 H), 6.19 (d, *J* = 1.8 Hz, 1 H), 5.84 (d, *J* = 7.2 Hz, 1 H), 5.44 (s, 2 H), 5.29 (br s, 1 H), 4.21-4.18 (m, 1 H), 4.12-4.11 (m, 1 H), 3.79-3.77 (m, 1 H), 3.67-3.65 (m, 1 H), 3.17 (d, *J* = 6.9 Hz, 4 H), 1.04 (t, *J* = 7.2 Hz, 6 H). ^{13}C NMR (DMSO-d_6, 150 MHz, ppm): δ 166.32, 166.16, 159.22, 154.97, 151.65, 147.54, 145.54, 142.61, 141.78, 131.34, 128.57, 124.18, 122.43 (t, $^1J_{FC}$ = 259.05 Hz), 110.04, 108.78, 105.43, 95.82, 95.28, 79.27, 69.86 (m), 68.80, 59.75, 49.06, 44.42, 12.90. MS (ESI), calcd for $C_{29}H_{32}F_2N_5O_8$, *m/z*: 616.2 $[M+H]^+$; found 616.3.

7-Diethylaminocoumarin (CM): According to a reported literature with some modification[8], **CM** was synthesized. A mixture of 4-(*N,N*-diethylamino)salicylaldehyde (3.1 g, 16 mmol), diethylmalonate (5.1 g, 32 mmol), piperidine (1.6 mL), and absolute ethanol (48 mL) was stirred under reflux condition for 4 h. After ethanol was evaporated under reduced pressure, a mixture of concentrated HCl (32 mL) and glacial acetic acid (32 mL) were added and stirred at 90 ^{o}C for 48 h. Then the solution was cooled to room temperature and poured into 200 mL of ice water. Next, the pH of the mixture solution was modulated to 5 by adding NaOH solution (20%), and extracted with ethyl acetate (100 mL x 4). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude

product was purified by column chromatography (PE: EA =200:1~30:1) to give **CM** as a yellow-green solid in 49 % yield (1.7 g). Mp: 87.5-89.5 °C. MS (ESI), calcd for $C_{13}H_{16}NO_2$, *m/z*: 218.1 $[M+H]^+$; found 218.2.

3. Fluorescence measurements of GMC-CA$_E$-NO$_2$ incubated with NTR and then illuminated by UV-light

DMSO stock solution of **GMC-CA$_E$-NO$_2$** (1 mM) were dilute into 100 μM by a mixture solvent of DMSO and PBS (*V*/*V* = 3/7). Then the solution was incubated with NTR (10 μg/mL) in presence of NADH (2 mM) under N_2 protection for different time (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 h) at 37 °C. Next, the mixture was illuminated by UV light (365 nm) for different time. All the fluorescence measurements were carried out on a Hitachi F-7000 luminescence spectrophotometer in 2.5×10 mm quartz cells. The solution was excited at 365 nm with 2.5/2.5 nm slit widths, and the emission was collected from 400 to 600 nm.

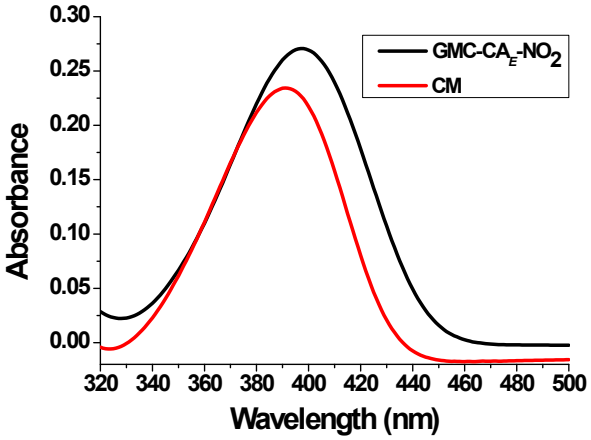
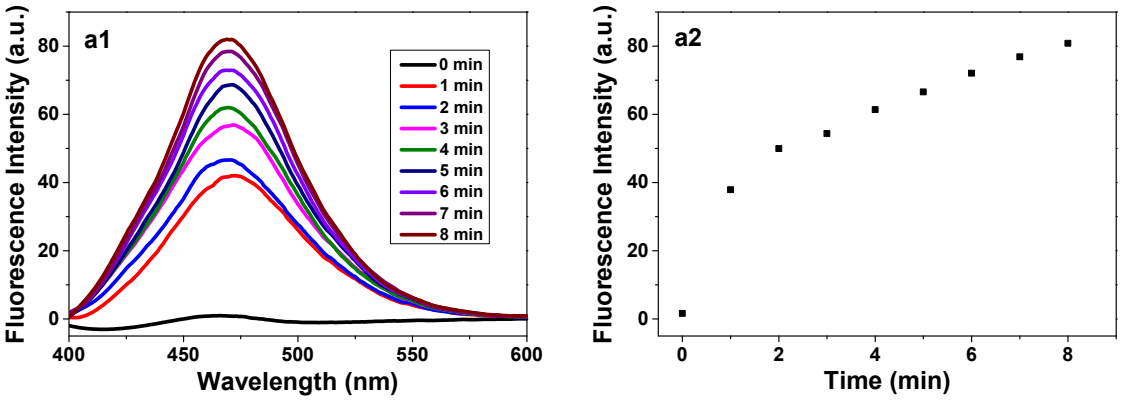
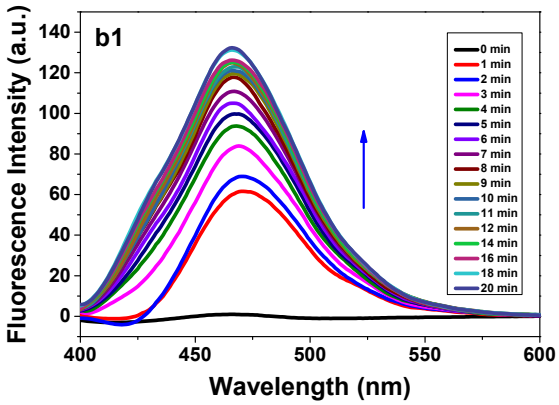


Figure S1. Absorbance of **GMC-CA$_E$-NO$_2$** and **CM** (10 μM, 30% DMSO, and 70% PBS buffer)

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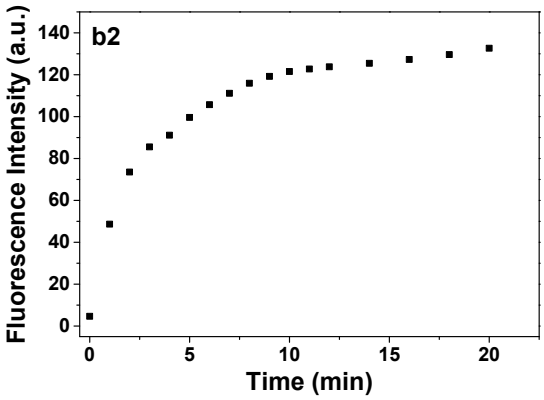
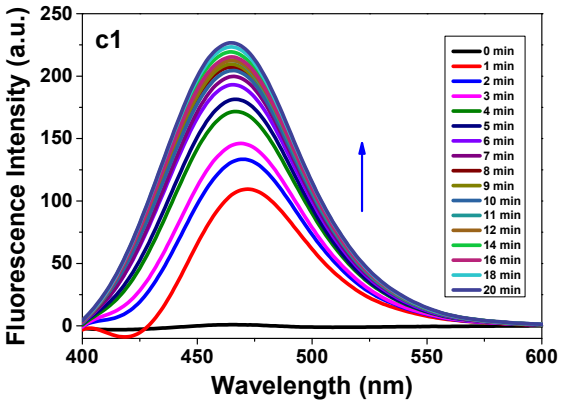
Fluorescence Intensity (a.u.)
Wavelength (nm)
0 min
1 min
2 min
3 min
4 min
5 min
6 min
7 min
8 min
9 min
10 min
11 min
12 min
14 min
16 min
18 min
20 min


b2
Fluorescence Intensity (a.u.)
Time (min)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Fluorescence Intensity (a.u.)
Wavelength (nm)
0 min
1 min
2 min
3 min
4 min
5 min
6 min
7 min
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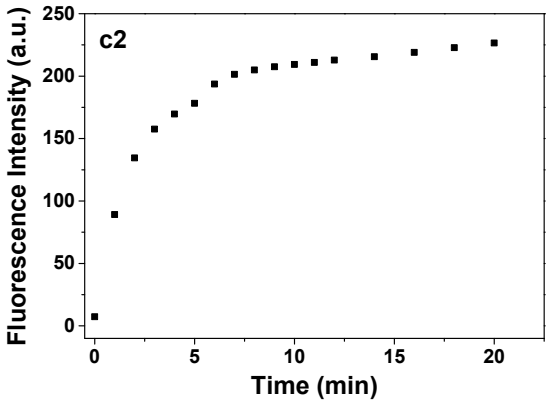
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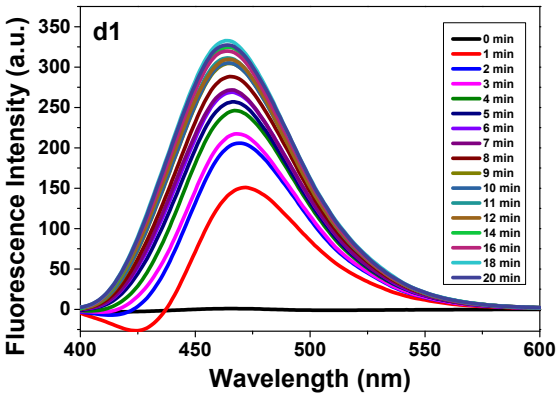
Fluorescence Intensity (a.u.)
Time (min)


d1
Fluorescence Intensity (a.u.)
Wavelength (nm)
0 min
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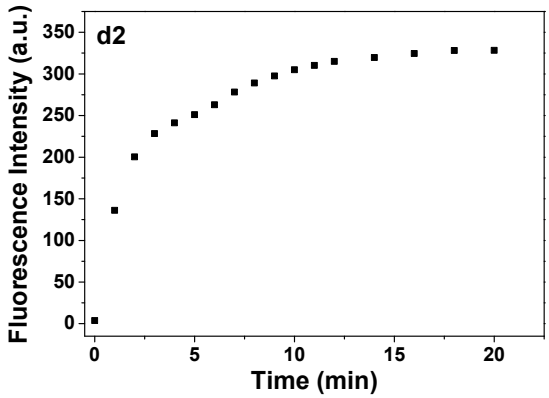
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Fluorescence Intensity (a.u.)
Time (min)

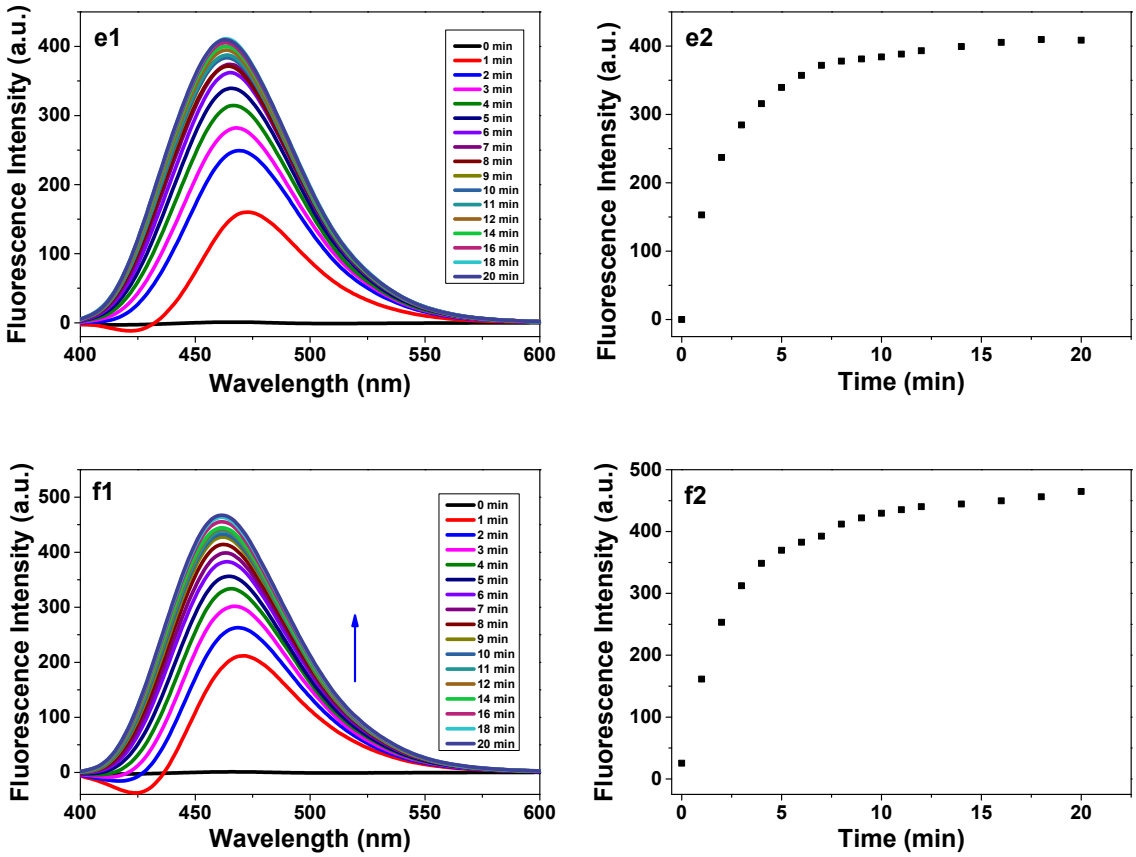

Figure S2. Fluorescence change of **GMC-CA$_E$-NO$_2$** (100 μM, in 30% DMSO and 70% PBS buffer) after incubated with NTR in presence of NADH under N_2 protection for different time (a: 0.5 h; b: 1 h; c:1.5 h; d: 2.5 h; e: 3 h, and f:3.5 h) at 37 ^oC and then irradiated by UV light (365 nm)

4. HPLC for GMC-CA$_E$-NO$_2$ incubated with $Na_2S_2O_4$ and then illuminated by UV-light

$Na_2S_2O_4$ (10 mg) was added to a solution of **GMC-CA$_E$-NO$_2$** (0.5 mM) in DMSO and H_2O (1:1, *V/V*), and stirred at 25 ^oC for 20 min. Then the mixture was illuminated by UV light (365 nm) for 10 min. The mixture solution was detected through HPLC.

A liquid chromatography system from Shimadzu Technology (Shimadzu, Japan) was applied. The HPLC system was performed with a binary LC-20AT pump, a SPD-20A UV-vis detector, as well as a RF-10AXL detector. The data acquired and processed throughout a LC solution chromatographic workstation (Shimadzu, Japan). The analytical column was a Waters Symmetry C18 column (4.6 mm ×250 mm, 3.5 μm). The solvent system was composed of two solutions: solution A (water containing 0.05% TFA) and solution B (acetonitrile: ACN). The 30 min gradient LC separation include 5 steps: The elution began with a 99:1

mixture of A and B, followed by a linear increase to 2.5% B over the course of 8.0 min; then 2.5-50% solvent B for 8-15 min (linear); 50-70% solvent B for 15-25 min; then returned to 1% B over the next 1 min, and at this elution to balance column; flow rate of 1.0 mL/min; detection under UV light at 254 nm.

5. The stability of GMC-CA$_E$-NO$_2$ in serum solution

DMSO stock solution of **GMC-CA$_E$-NO$_2$** (1 mM) were dilute into 25, 50, 100, 150, and 200 μM by a mixture solvent of DMSO and PBS (V_{DMSO}/V_{PBS} = 3/97). The standard curve between concentration and peak area was detected through HPLC.

The solution of **GMC-CA$_E$-NO$_2$** (1 mM) was dilute into 90 μM by a mixture of DMSO, PBS and serum ($V_{DMSO}/V_{PBS}/V_{serum}$ = 3/87/10, pH 7.4). Then the mixture was stirred at 37 °C for different time (0, 2, 4, 6, 8, 10, 14, 18, and 24 h), and the concentration of **GMC-CA$_E$-NO$_2$** was detected through HPLC by concentration-peak area standard curve.

A liquid chromatography system from Agilent Technologies was applied. The HPLC system was performed with a quaternary G1311C pump, a G1314F UV-vis detector. The data acquisition and processing was performed throughout a LC solution chromatographic workstation (Agilent, American). The analytic column was Agilent HC-C18 (250 mm × 4.6 mm, 5 μm). The solvent system was composed of two solutions: solution A (water containing 0.05% TFA) and solution B (ACN). The 12 min gradient LC separation include 2 steps: The elution began with a 90:10 mixture of A and B, followed by a linear increase to 70% B over the course of 8.0 min; then 70-10% solvent B for 8-12 min (linear); flow rate of 1.0 mL/min; detection under UV light at 254 nm.

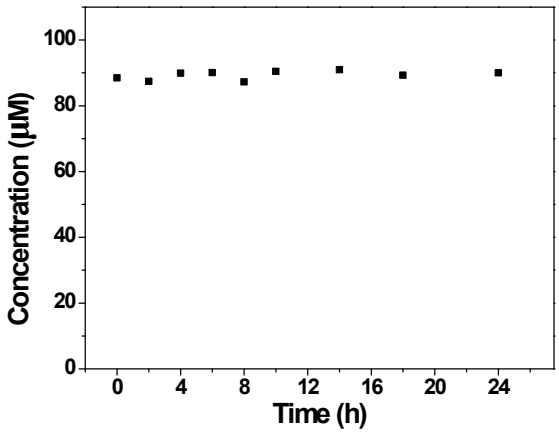

Figure S3. Concentration change of **GMC-CA$_E$-NO$_2$** in serum (90 μM, $V_{DMSO}/V_{PBS}/V_{serum}$ = 3/87/10, pH 7.4) after stirred for 0, 2, 4, 6, 8, 10, 14, 18, and 24 h at 37 °C

6. The measurements of released GMC and CM by GMC-CA_E-NO_2 incubated with NTR and subsequent illuminated by UV-light

DMSO stock solution of **GMC-CA_E-NO_2** (1 mM) were dilute into 100 μM by a mixture solvent of DMSO and PBS (*V*/*V* = 3/7). Then the solution was incubated with NTR (10 μg/mL) in presence of NADH (2 mM) under N_2 protection for different time (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 h) at 37 °C. Next, the mixture was illuminated by UV light (365 nm) for 10 min. The concentration of **GMC** was detected through HPLC (Agilent Technologies). The concentration of **CM** was detected through a Hitachi F-7000 luminescence spectrophotometer.

The solvent system was composed of two solutions: solution A (water containing 0.05% TFA) and solution B (ACN). The 30 min gradient LC separation include 5 steps: The elution began with a 99:1 mixture of A and B, followed by a linear increase to 2.5% B over the course of 8.0 min; then 2.5-50% solvent B for 8-15 min (linear); 50-70% solvent B for 15-25 min; then returned to 1% B over the next 1 min, and at this elution to balance column; flow rate of 1.0 mL/min; detection under UV light at 254 nm.

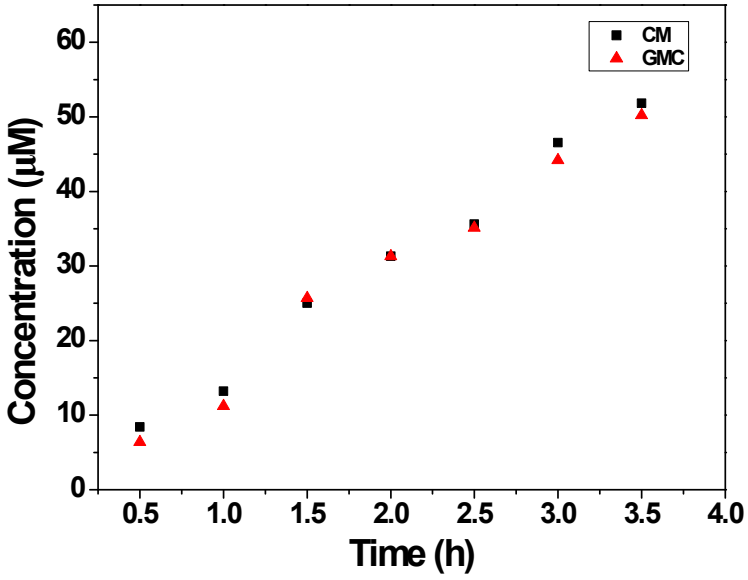

Figure S4. Concentration change of released **GMC** and **CM** by **GMC-CA_E-NO_2** (100 μM, in 30% DMSO and 70% PBS buffer) which was incubated with NTR in presence of NADH under N_2 protection for different time (0.5, 1, 1.5, 2, 2.5, 3, and 3.5 h) at 37 °C and subsequently irradiated by UV light (365 nm) for 10 min

7. Cell Culture

Michigan cancer foundation-7 (MCF-7) cells were obtained from American Type Culture Collection (ATCC). The cells were cultured in DMEM containing 10% fetal bovine serum and 1% penicillin

streptomycin in 5% CO_2 at 37 ^oC.

8. Confocal microscopy imaging

MCF-7 cells were seeded in confocal dish at 2×10^4 cells·mL^{-1} and incubated for 12 h under the cell culture condition (5% CO_2 at 37 ^oC) in a humidified incubator (SANYO, MCO-20AIC, JPN). Then, the medium was replaced with 10 μM **GMC-CA_E-NO_2** in fresh culture medium. After incubation with **GMC-CA_E-NO_2** under normoxic condition (20%O_2, 5% CO_2, and 75%N_2, at 37 ^oC) or different hypoxic conditions (1%O_2, 5% CO_2, and 94%N_2; 5%O_2, 5% CO_2, and 90%N_2; 10%O_2, 5% CO_2, and 85%N_2; 15%O_2, 5% CO_2, and 80%N_2; at 37 ^oC) in a humidified incubator (Heal Force, HF 100, CHN) for 6 h. Then the cells were irradiated with UV (365 nm) light for 10 min. Next, the medium was removed and the cells were washed with PBS twice. The fluorescence imaging experiments were performed on a confocal laser scanning microscope (Olympus, FV1000-IX81, JPN) with FV5-LD405-2 for excitation at 405 nm and variable band pass emission filter set to 445-495 nm through a 60×1.42 N.A. objective. Optical section was acquired at 0.8 um. The software of FV10-ASW3.0 was also used to determine the semi-quantitative fluorescence intensity of generated **CM**.

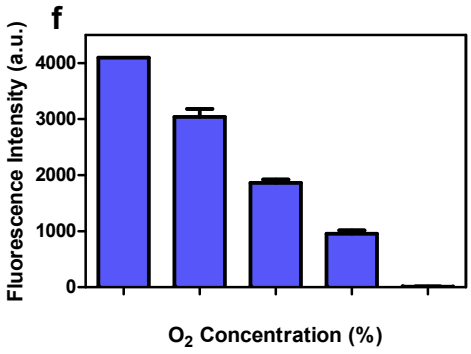

Figure S5. The semi-quantitative fluorescence intensity of MCF-7 cells, which was incubated with **GMC-CA_E-NO_2** under normoxic (20% O_2) and different hypoxic (15%, 10%, 5%, and 1% O_2) conditions for 6 h and then irradiated at 365 nm for 10 min

9. MTT assay

The effect of the compounds on cells viability was determined using the MTT method. In brief, the MCF-7 cells harvested at subconfluent stage were seeded in a 96-well plates at an intensity of 2×10^4 cells·mL^{-1} and incubated under the cell culture condition (5% CO_2 at 37 ^oC) for 12 h. After being incubated

with **GMC**, **GMC-CA$_E$-NO$_2$** and **CM** on different concentrations (0.01, 0.1, 1, 5, and 10 μM) under normoxic or hypoxic condition for 6 h, the cells were irradiated by ZF-8 lamp (365 nm filter, 10 mW/cm^2) for 10 min, and then further incubated for 42 h. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. Subsequently, the cells were washed twice with PBS buffer, and 10 μL of freshly prepared MTT solution (0.5 mg/mL in PBS buffer) was added into each well and the cells were incubated for an additional 4 h at 37 °C. Next, 100 μL of DMSO was added into each well at 37 °C for fully dissolve the MTT formazan. The optical density (OD) of formazan solutions produced was recorded on a microplate spectrophotometer at 570 nm. The cell viability (%) was presented as the fold over the control group: $(OD_{sample}\text{-}OD_{blank})/(OD_{control}\text{-}OD_{blank})\times 100$.

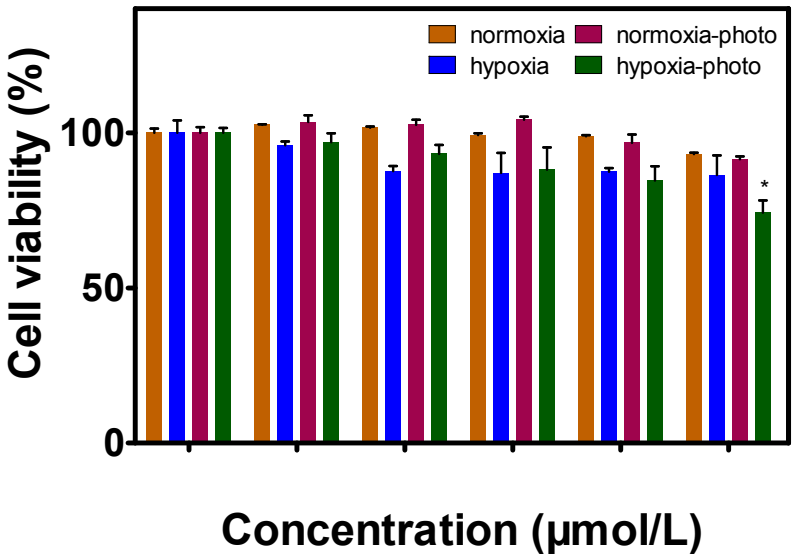

Figure S6. Cell viability of MCF-7 cells incubated with **CM** under different conditions (normoxia (6 h), normoxia (6 h) combining UV-irradiation (10 min), hypoxia (6 h), and hypoxia (6 h) combining UV-irradiation condition (10 min))

10. NMR, IR and MS spectra

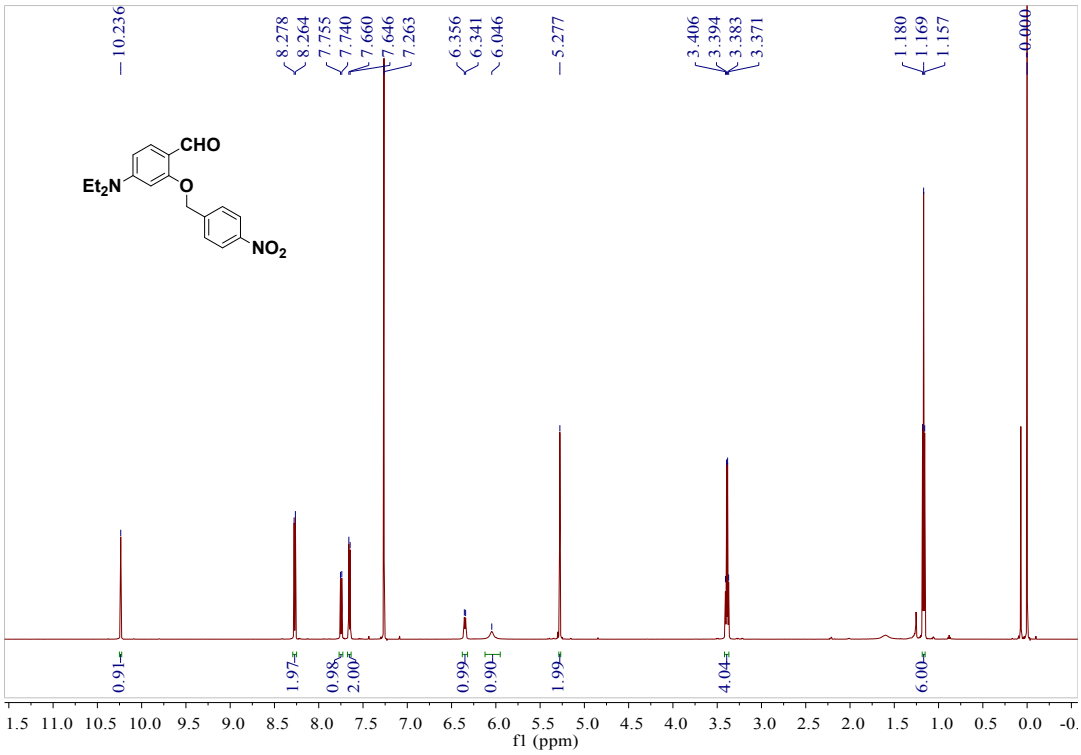

Figure S7. ^{1}H NMR of 4-nitrobenzyloxy-4-diethylaminobenzaldehyde (**2**)

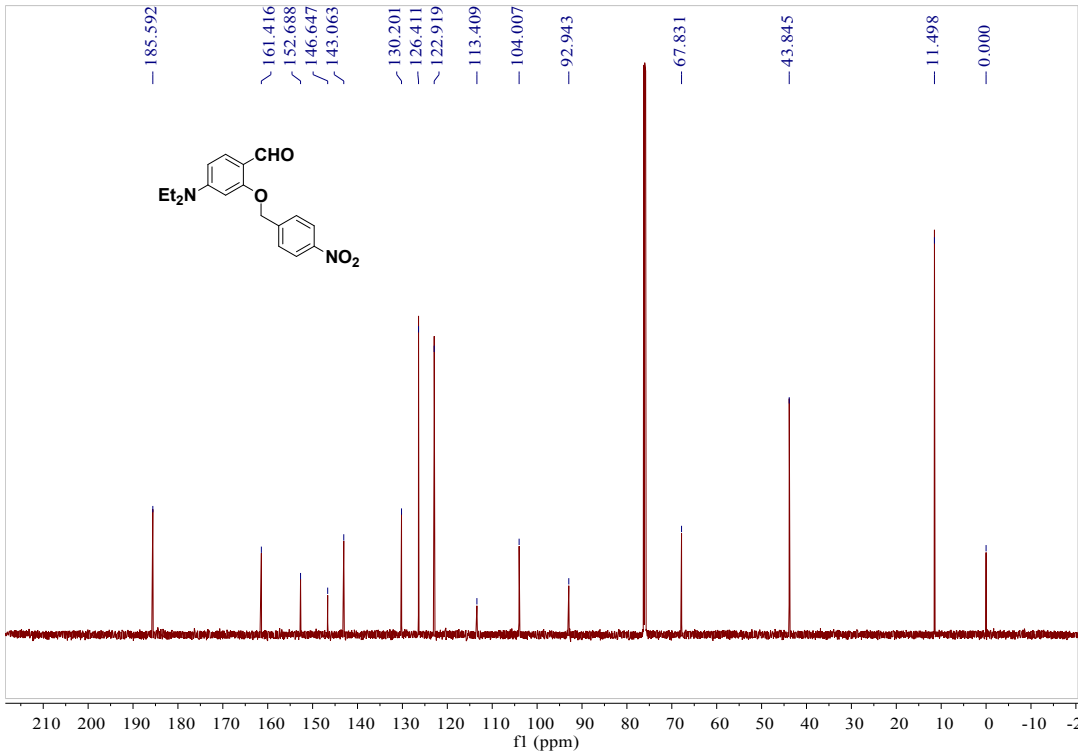

Figure S8. ^{13}C NMR of 4-nitrobenzyloxy-4-diethylaminobenzaldehyde (**2**)

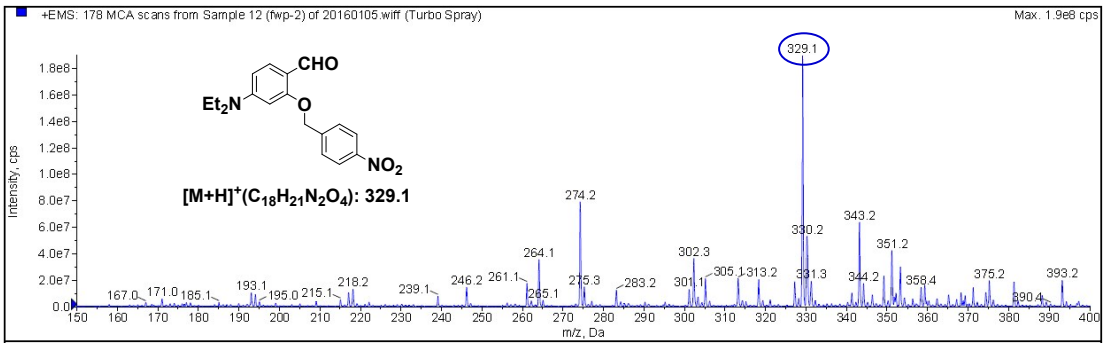

Figure S9. MS of 4-nitrobenzyloxy-4-diethylaminobenzaldehyde (**2**)

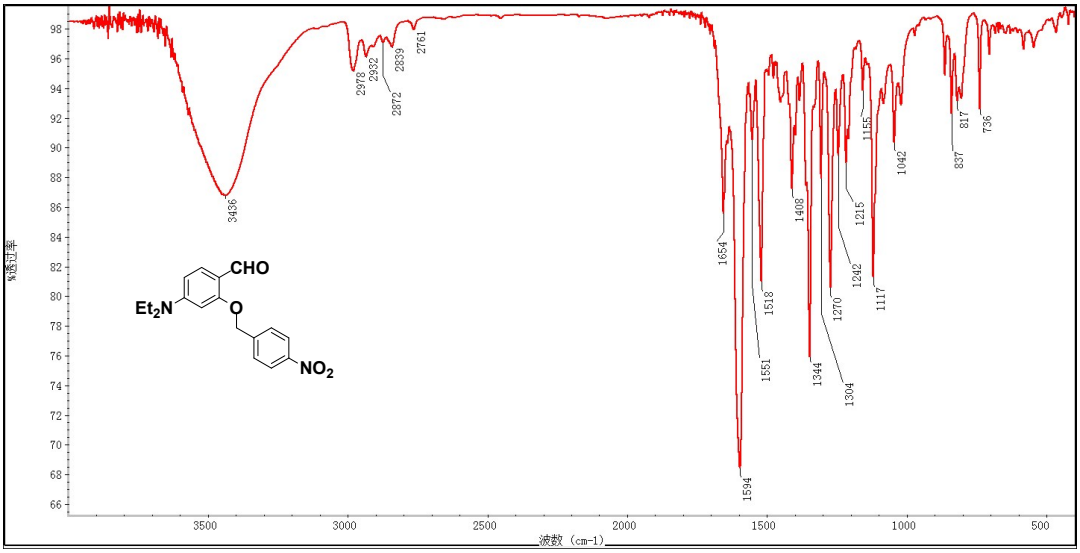

Figure S10. IR of 4-nitrobenzyloxy-4-diethylaminobenzaldehyde (**2**)

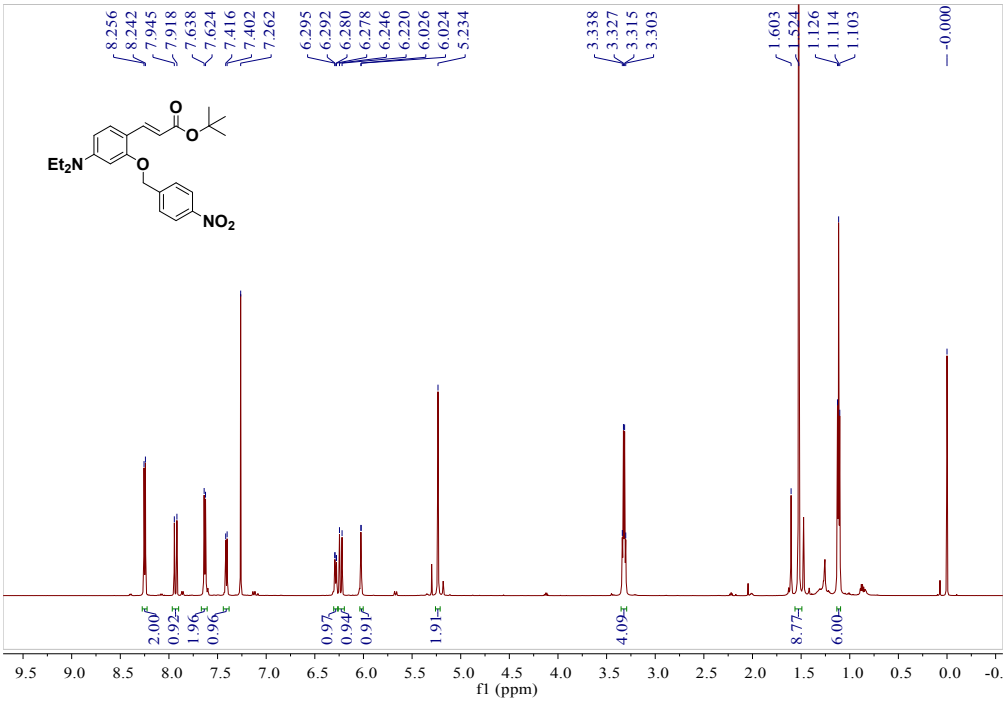

Figure S11. ^{1}H NMR of (*E*)-*tert*-butyl 3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylate (**4**)

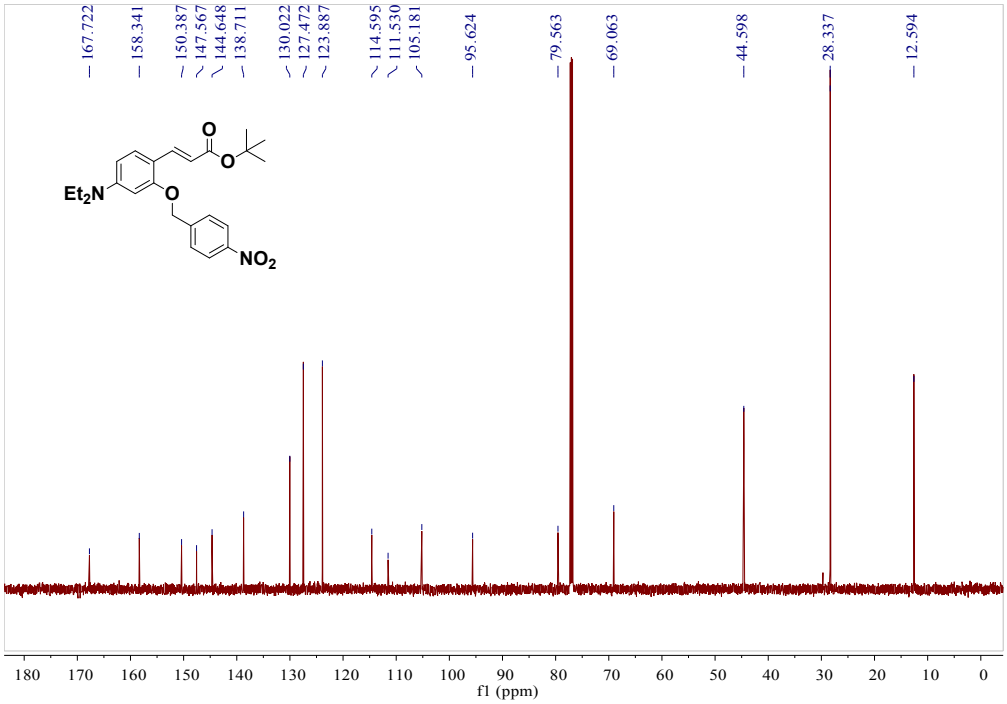

Figure S12. ^{13}C NMR of (*E*)-*tert*-butyl 3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylate (**4**)

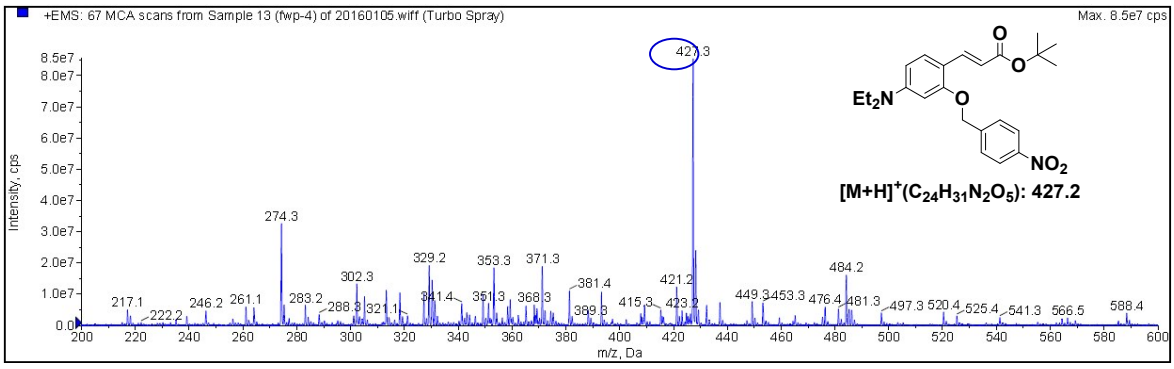

Figure S13. MS of (*E*)-*tert*-butyl 3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylate (**4**)

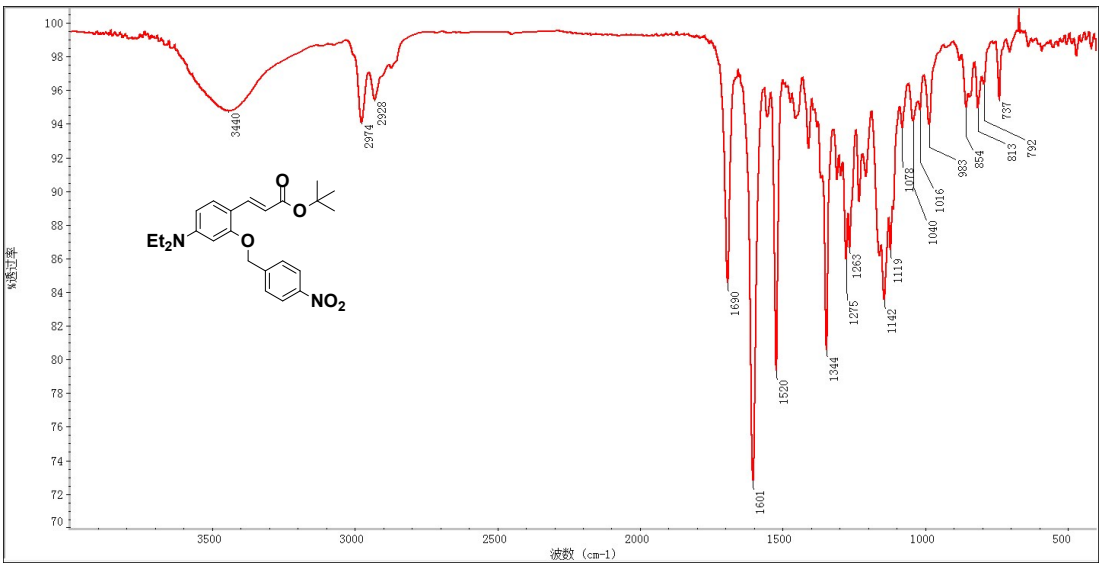

Figure S14. IR of (*E*)-*tert*-butyl 3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylate (**4**)

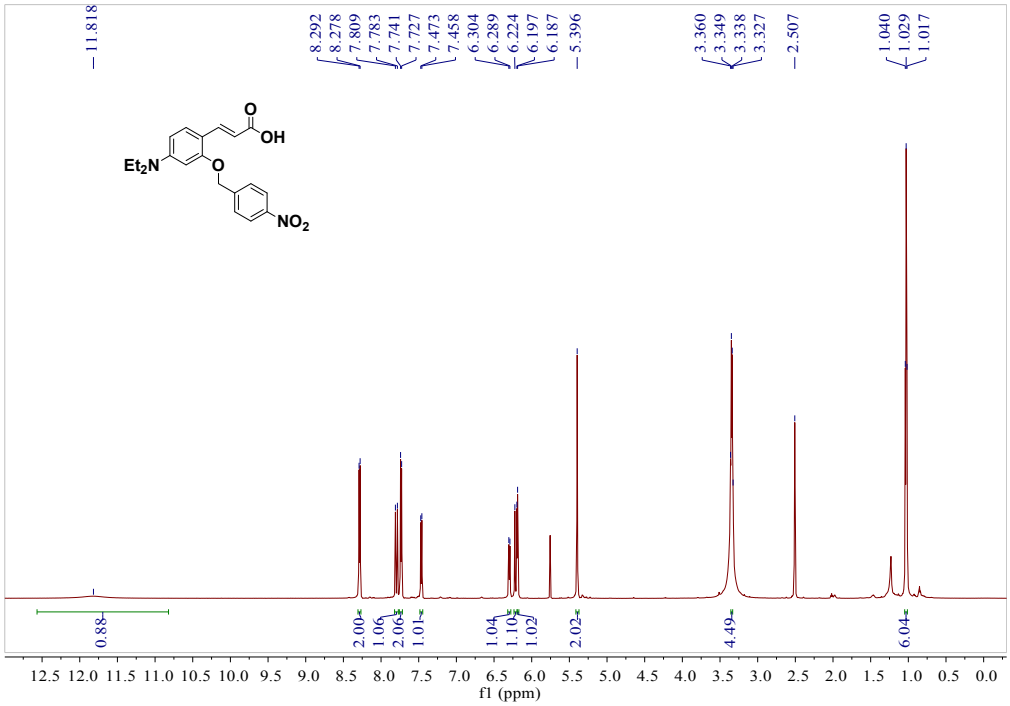

Figure S15. ^{1}H NMR of (*E*)-3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (**5**)

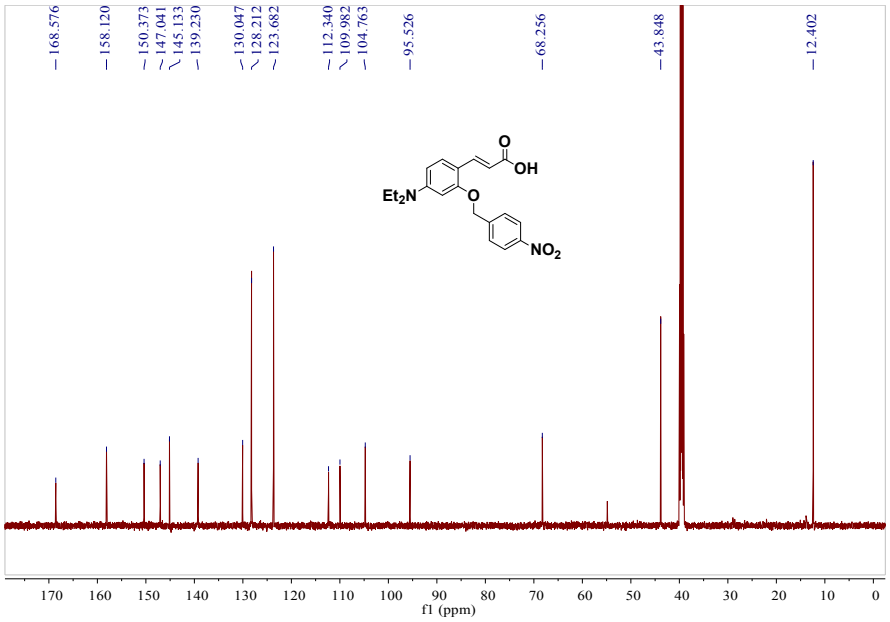

Figure S16. ^{13}C NMR of (*E*)-3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (**5**)

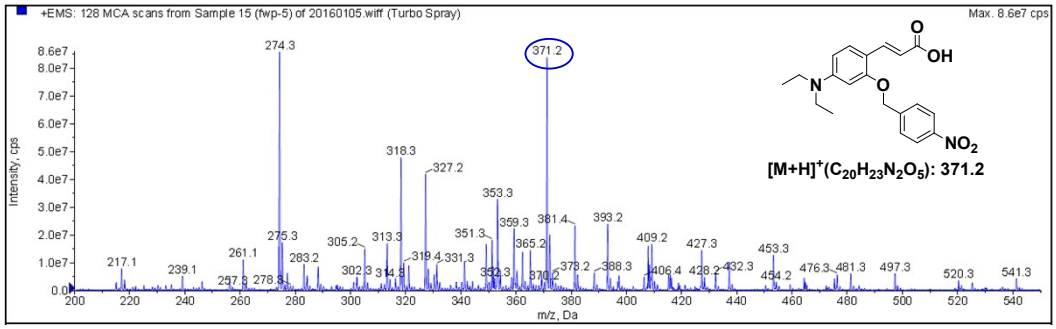

Figure S17. MS of (*E*)-3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (**5**)

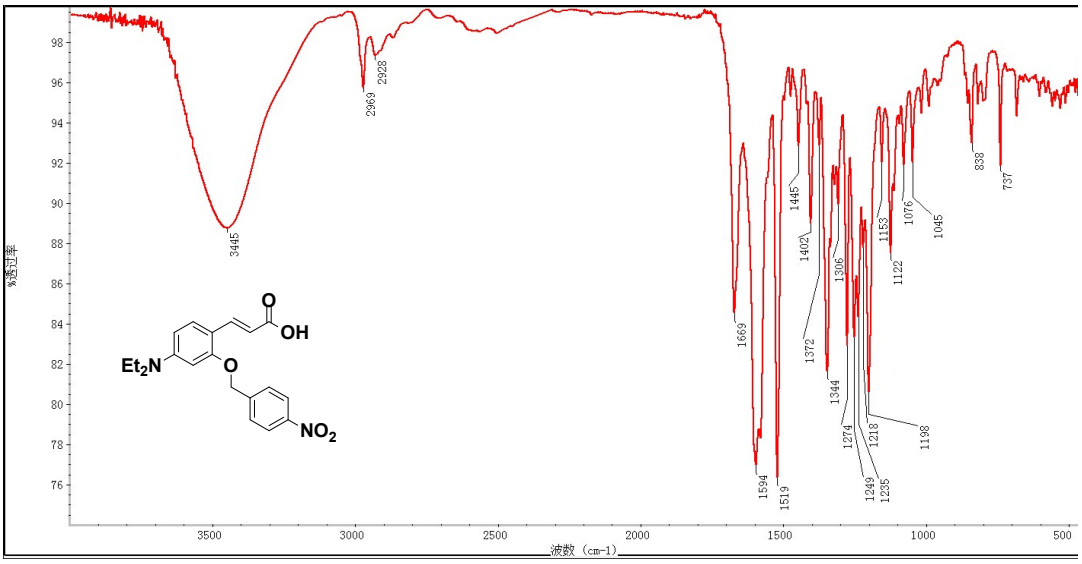

Figure S18. IR of (*E*)-3-(4-diethylamino-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (**5**)

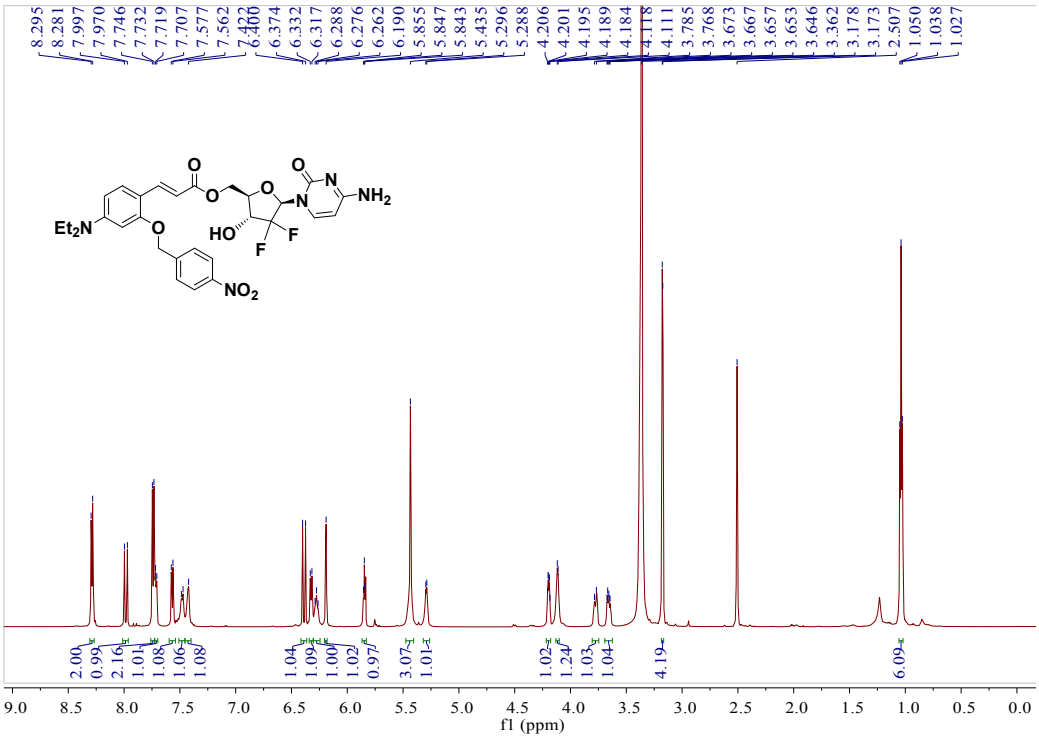

Figure S19. ^{1}H NMR of **GMC-CA$_{E}$-NO$_2$**

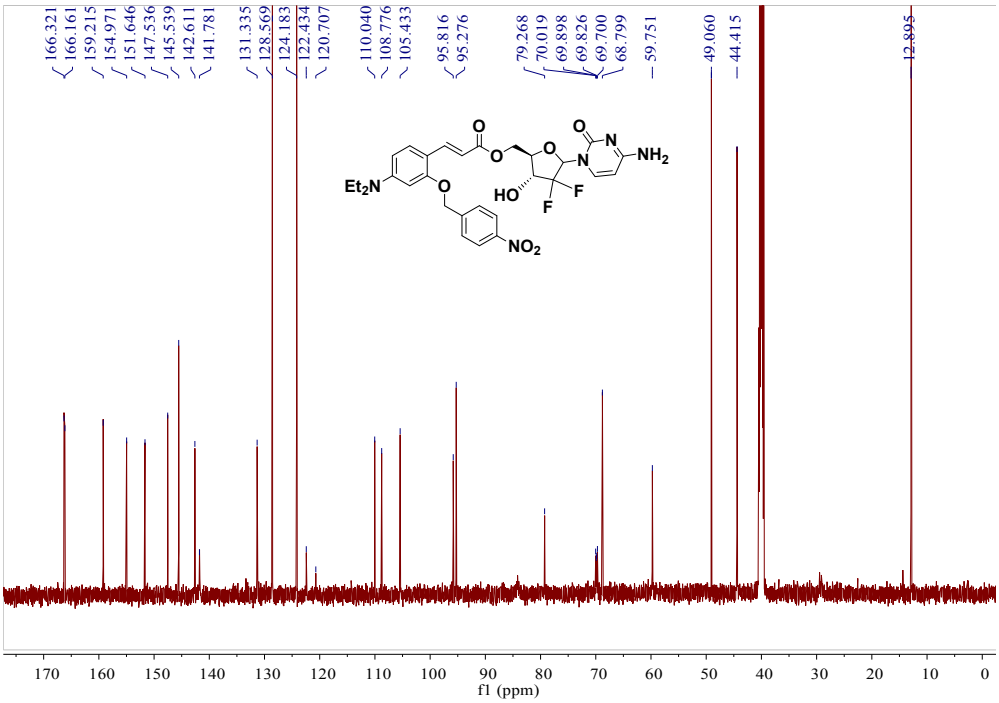

Figure S20. ^{13}C NMR of **GMC-CA$_E$-NO$_2$**

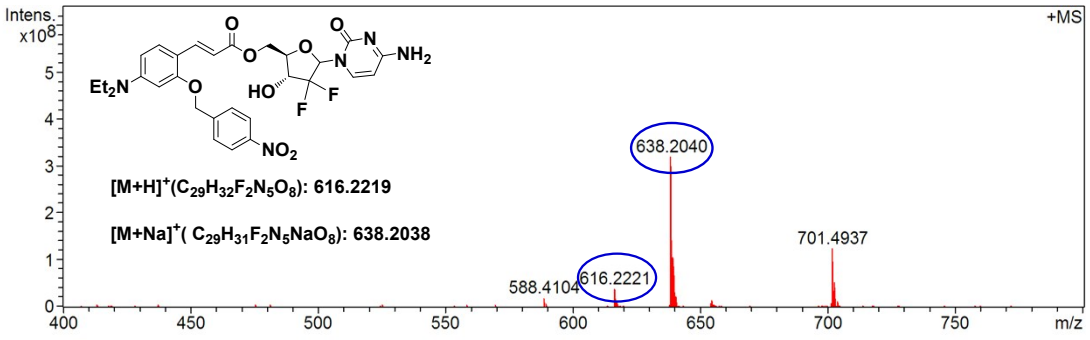

Figure S21. High Resolution Mass Spectrum of **GMC-CA$_E$-NO$_2$**

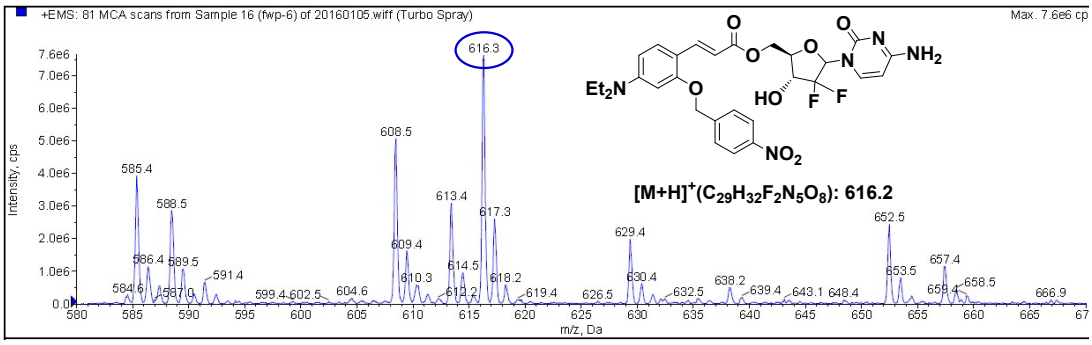

Figure S22. MS of **GMC-CA$_E$-NO$_2$**

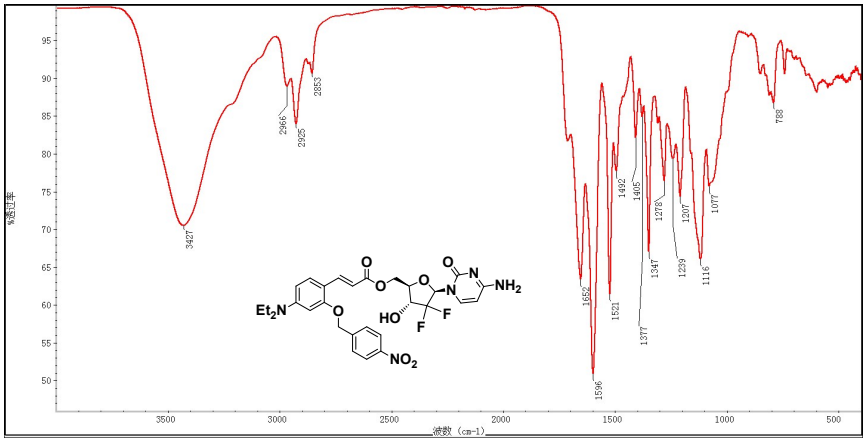

Figure S23. IR of **GMC-CA$_E$-NO$_2$**

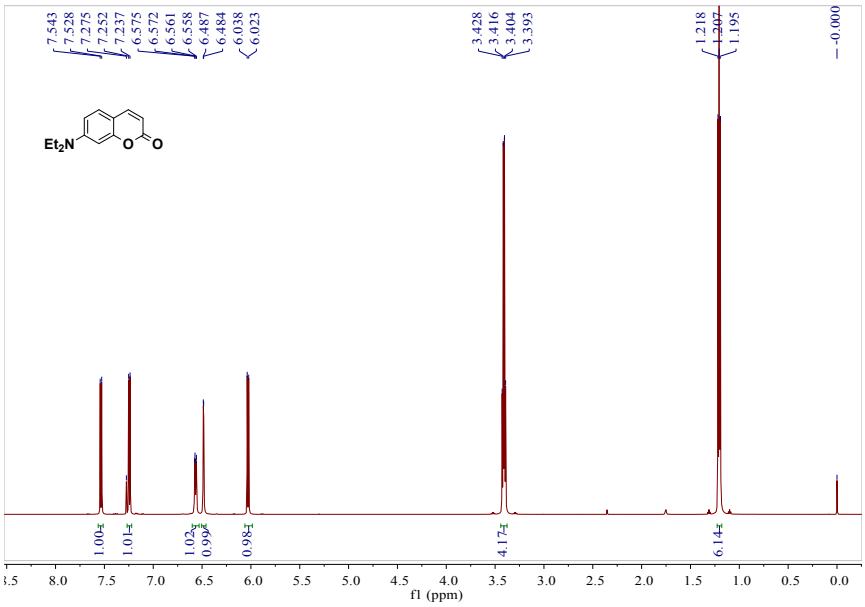

Figure S24. ^{1}H NMR of 7-diethylaminocoumarin (**CM**)

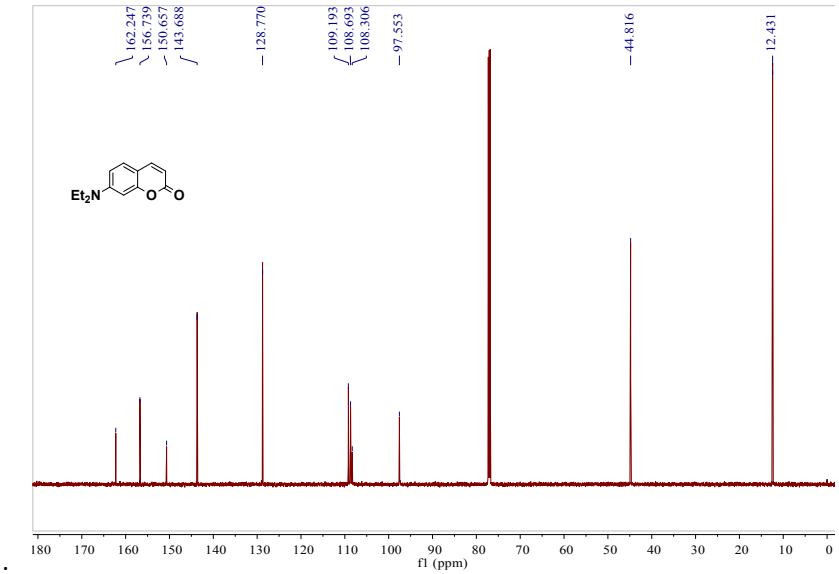

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Figure S25. ^{13}C NMR of 7-diethylaminocoumarin (**CM**)

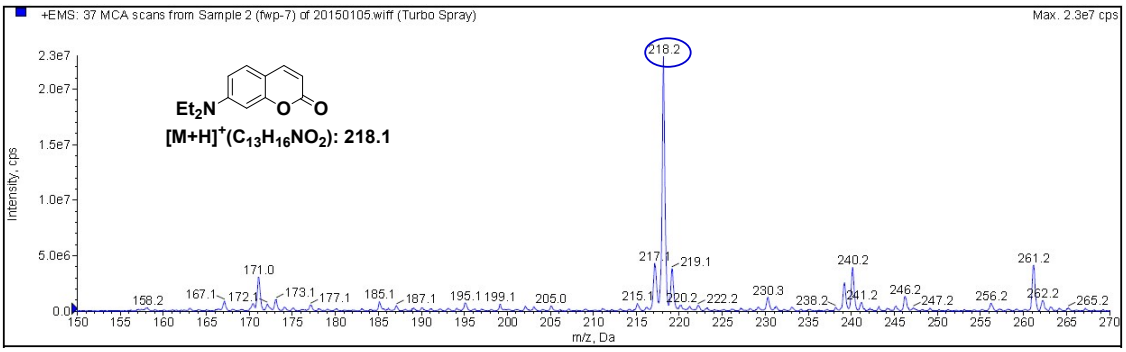

Figure S26. MS of 7-diethylaminocoumarin (**CM**)

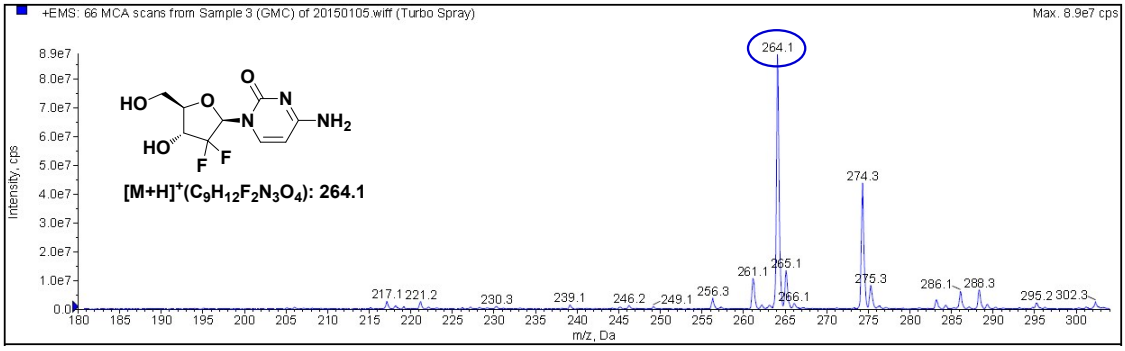

Figure S27. MS of GMC

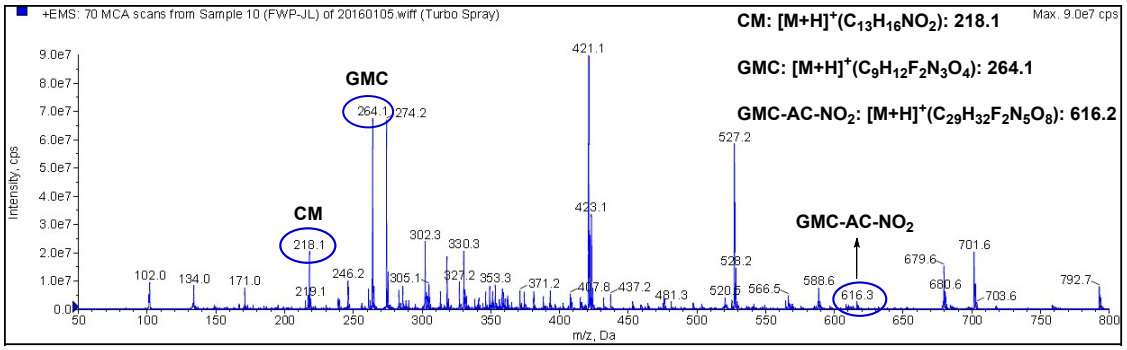

Figure S28. MS of the solution of **GMC-CA$_E$-NO$_2$** incubated with $Na_2S_2O_4$ and then illuminated by UV-light

References

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