Supporting Information

Palladium-catalyzed in vivo bioorthogonal activation of N, N-dialkyl-α-hydroxymethyl acrylamide for efficient payload release

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General Information

Materials and Instruments

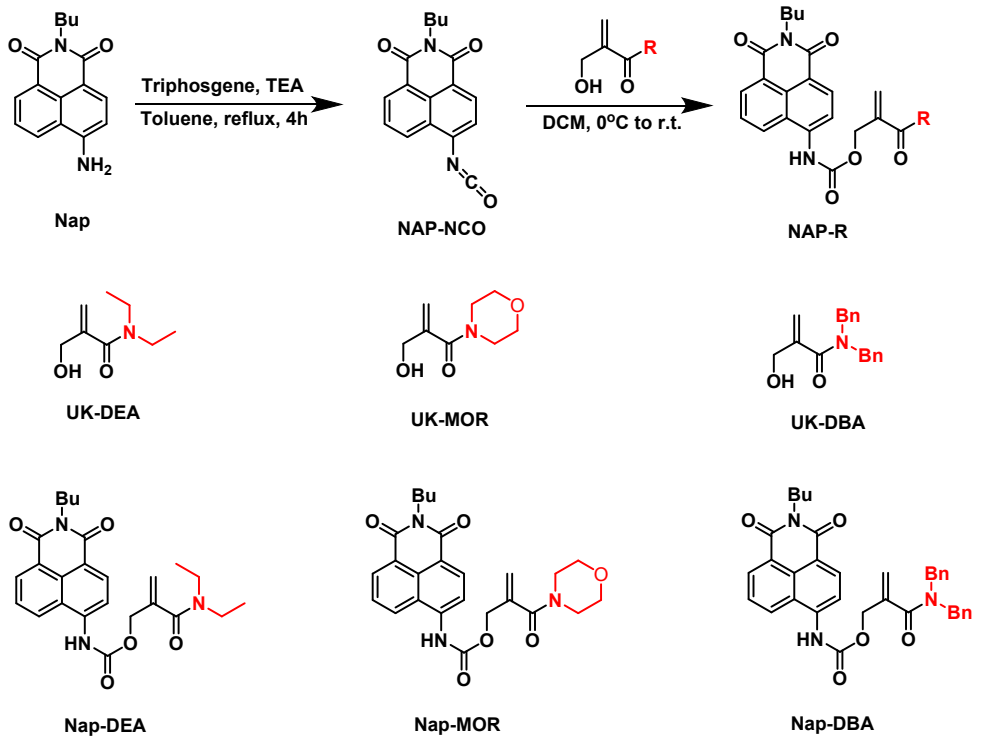
Chemicals were purchased as reagent grade and used without further purification except as indicated below. Solvents (CH_2Cl_2, $CHCl_3$, MeOH, DMF, THF, H_2O) were purchased from commercial suppliers. Thin-layer chromatography (TLC) was performed using silica gel pre-coated plastic sheets (Polygram SIL G/UV_{254}, 0.2 mm, with fluorescent indicator; Macherey-Nagel), which were visualized with a UV lamp (254 nm), or Potassium Permanganate stain (1.5g of $KMnO_4$, 10g K_2CO_3, and 1.25mL 10% NaOH in 200 mL H_2O). Silica gel column chromatography was carried out using silica Gel 60 (200−300 mesh). Analytical thin layer chromatography (TLC) was done using silica gel (silica gel 60 F254). ^1H (400 or 600 MHz) and ^{13}C (101 or 151 MHz) NMR spectra were recorded on a Bruker AMX spectrometer. MS spectra were recorded on Orbitrap Elite (Thermo Scientific) and Shimadzu LCMS-2020. HPLC was recorded with Shimadzu LCMS-2020 system with a Wondasil C18 Superb reversedphase column (5 μm, 4.6 × 150 mm). The luminescence and fluorescence intensity were monitored in a Multi-Mode Microplate Reader (SynergyTM 4, Bio-Tek,USA).

Cell culture

The HepG2 cells and 4T1 cells (adenocarcinomic human alveolar basal epithelial cell) were purchased from the Centre for Cell Resources, Institute for Biological Sciences of Shanghai, Chinese Academy of Sciences (CAS). The HepG2 cells and 4T1 cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum), All cell cultures were incubated at 37 ^oC under 5% CO_2.

1. Chemical Synthetic Methods

1.1. Chemical synthesis of amino-naphthalimide derivatives.



The compounds **Nap**, **UK-DEA**, **UK-MOR**, **UK-DBA**, **Nap-DEA**, **Nap-MOR** and **Nap-DBA** were synthesized according to our previous publication.

Synthesis of compound **Nap-NCO**. **Nap** (0.28 mmol, 1.0 eq) was dissolved in 10 mL of dry toluene. Triphosgene (0.14 mmol, 0.5 eq) and TEA (0.42 mmol, 1.5 eq) were then added. The reaction mixture was stirred at 110 °C for 4 h under nitrogen. After cooling to room temperature, concentrate the organic layer to obtain crude product **Nap-NCO**.

Synthesis of compound **Nap-DEA**. To 2 mL of crude product **Nap-NCO** (0.28 mmol, 1.0 eq) solution in dry DCM, **UK-DEA** (0.28 mmol, 1.0 eq) and TEA (0.34 mmol, 1.2 eq) were added at 0 °C. The reaction was stirring for 60 min. The residue was purified by flash chromatography on silica gel to yield yellow solid **Nap-DEA** (62%).

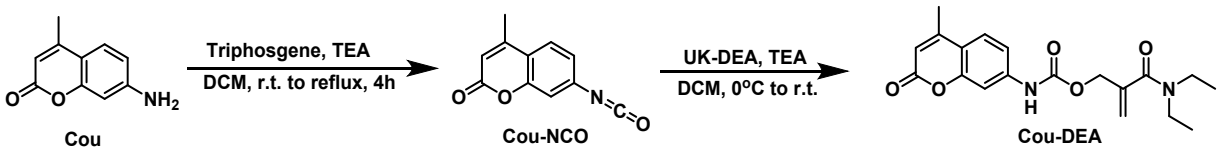
Synthesis of compound **Nap-MOR**. To 2 mL of crude product **Nap-NCO** (0.28 mmol, 1.0 eq) solution in dry DCM, **UK-MOR** (0.28 mmol, 1.0 eq) and TEA (0.34 mmol, 1.2 eq) were added at 0 °C. The reaction was stirring for 60 min. The residue was purified by flash chromatography on silica gel to yield yellow solid **Nap-MOR** (66%).

Synthesis of compound **Nap-DBA**. To 2 mL of crude product **Nap-NCO** (0.28 mmol, 1.0 eq) solution in dry DCM, **UK-DBA** (0.28 mmol, 1.0 eq) and TEA (0.34 mmol, 1.2 eq) were added at 0 °C. The reaction was stirring for 60 min. The residue was purified by flash chromatography on silica gel to yield yellow solid **Nap-DBA** (60%).

1.2. Chemical synthesis of $Pd(TPPTS)_4$.

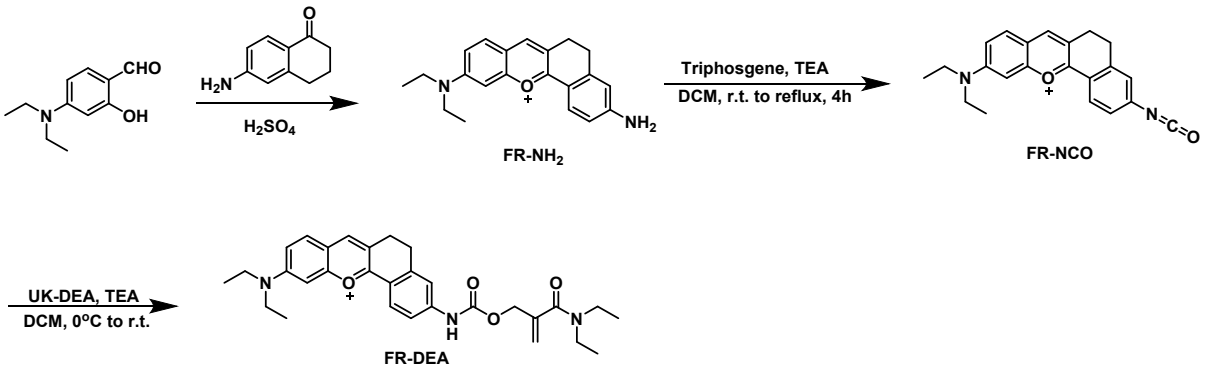
Synthesis of compound **$Pd(TPPTS)_4$.** Compound **$Pd(TPPTS)_4$** was synthesized according to the reported literature.[1] 3, 3', 3''-Phosphanetriyltris (benzenesulfonic acid) trisodium salt hydrate (TPPTS) and 30 mg Na_2PdCl_4 was dissolved in 3 mL 1 M HCl and then 100 μL hydrazine hydrate was added. After stirring for 30min, 10 mL ethanol was added to the yellow solution, the mixture was centrifuged and the precipitate was washed with ethanol for three times to obtain yellow powder (78%).

1.3. Chemical synthesis of other fluorescent scaffold.



Synthesis of compound **Cou-NCO**. **Cou** (0.28 mmol, 1.0 eq) was dissolved in 10 mL of dry DCM. Triphosgene (0.14 mmol, 0.5 eq) and TEA (0.34 mmol, 1.2 eq) were then added. The reaction mixture was stirred at 45 °C for 4 h under nitrogen1. After cooling to room temperature, the organic layer was concentrated to obtain crude product **Cou-NCO**.

Synthesis of compound **Cou-DEA**. To 2 mL of crude product **Cou-NCO** (0.28 mmol, 1.0 eq) solution in dry DCM, **UK-DEA** (0.28 mmol, 1.0 eq) and TEA (0.34 mmol, 1.2 eq) were then added at 0 °C. The reaction was stirring for 40 min. The residue was purified by flash chromatography on silica gel to yield yellow solid **Cou-DEA** (59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.46-7.36 (m, 1H), 6.24 (d, J = 1.4 Hz, 1H), 5.46 (d, J = 1.5 Hz, 1H), 5.27 (s, 1H), 4.81 (s, 2H), 3.34 (s, 4H), 2.38 (s, 3H), 1.07 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.47, 160.47, 154.27, 153.64, 153.29, 143.02, 140.97, 126.52, 116.14, 114.97, 114.85, 112.48, 105.09, 65.33, 18.45. HRMS: $[M+H]^+$ calculated for $C_{19}H_{22}N_2O_5$: 359.1601; found: 359.1612.

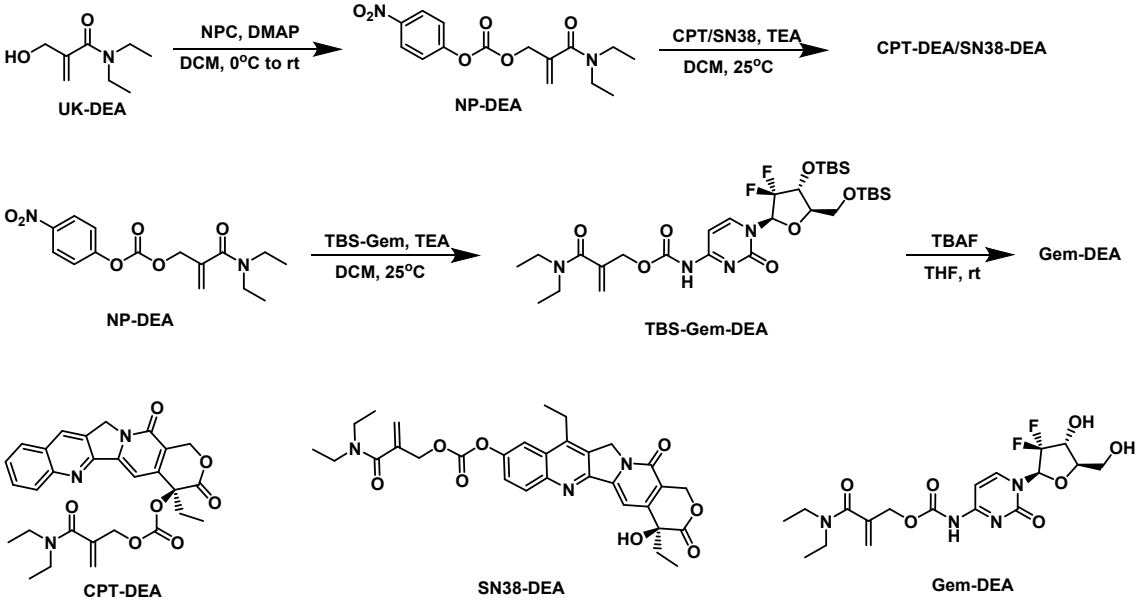


Synthesis of compound **FR-NH$_2$**. Compound **FR-NH$_2$** was synthesized according to the reported literature.[2] The mixture of 4-(Diethylamino) salicylaldehyde (1.03 mmol, 1.0 eq) and 6-Amino-3,4-dihydro-1(2 H)-naphthalenone (1.13 mmol, 1.1 eq) in methanesulfonic acid was stirred at 90 °C for 5 h. Subsequently, thereaction mixture cooled down to room temperature. And the reactionmixture poured it into ice water mixture (200 mL). Perchloric acid (70%; 7 mL) was then slowly added to the reaction mixture. Filter and obtain black solid after filtration product **FR-NH$_2$** (74%).

Synthesis of compound **FR-NCO**. **FR-NH$_2$** (0.28 mmol, 1.0 eq) was dissolved in 10 mL of dry DCM. Triphosgene (0.14 mmol, 0.5 eq) and TEA (0.34 mmol, 1.2 eq) were then added. The reaction mixture was stirred at 45 °C for 4 h under nitrogen1. After cooling to room temperature, The organic layer was concentrated to obtain crude product **FR-NCO**.

Synthesis of compound **FR-DEA**. To 2 mL of crude product **FR-NCO** (0.28 mmol, 1.0 eq) solution in dry DCM, **UK-DEA** (0.28 mmol, 1.0 eq) and TEA (0.34 mmol, 1.2 eq) were then added at 0 °C. The reaction was stirring for 30 min. The residue was purified by flash chromatography on silica gel to black solid **FR-DEA** (28%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.47 (s, 1H), 8.78 (t, J = 6.1 Hz, 1H), 8.66 (s, 1H), 8.20 (d, J = 8.7 Hz, 1H), 7.93 (d, J = 9.3 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.58 (s, 1H), 7.45 (dd, J = 9.4, 2.4 Hz, 1H), 7.34-7.21 (m, 6H), 6.03 (s, 1H), 5.75 (s, 1H), 4.89 (s, 2H), 4.37 (d, J = 6.0 Hz, 2H), 3.69 (q, J = 7.1 Hz, 4H), 3.04 (s, 4H), 1.25 (t, J = 7.2 Hz, 6H).^{13}C NMR (151 MHz, DMSO-d_6) δ 166.04, 163.19, 158.53, 155.63, 153.27, 148.70, 145.71, 144.31, 139.89, 139.70, 132.27, 128.76, 128.63, 127.88, 127.63, 127.24, 122.01, 121.17, 120.73, 118.50, 118.26, 117.73, 117.42, 96.12, 64.38, 45.86, 42.68, 27.11, 25.01. HRMS: $[M]^+$ calculated for $C_{30}H_{36}N_3O_4^+$: 502.2700; found: 502.3238.

1.4. Chemical synthesis of prodrugs.



Synthesis of compound **NP-DEA**. A solution of DCM containing **NPC** (0.6 mmol 1.5 eq) was added dropwise to a dry DCM solution containing **UK-DEA** (0.4 mmol 1.0 eq) and DMAP (0.6 mmol 1.5 eq) at 0 °C. The reaction was stirring for 0.5 h at room temperature, the organic layer was concentrated to obtain crude product **NP-DEA**.

Synthesis of compound **CPT-DEA**. To 2 mL of crude product **NP-DEA** (0.4 mmol, 1.0 eq) solution in dry DCM, **CPT** (0.4 mmol, 1.0 eq) and TEA (0.4 mmol, 1.0 eq) were added at 0 °C. The reaction was stirring for 24 h. The residue was purified by flash chromatography on silica gel to yield white solid **CPT-DEA** (32%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (s, 1H), 8.14 (dd, J = 18.2, 7.6 Hz, 2H), 7.86 (t, J = 7.7 Hz, 1H), 7.71 (t, J = 7.5 Hz, 1H), 7.35 (s, 1H), 6.54 (s, 1H), 5.43 (s, 2H), 5.28 (s, 2H), 3.17 (s, 2H), 2.50 (p, J = 1.8 Hz, 4H), 1.87 (p, J = 6.8 Hz, 2H), 1.22 (s, 7H), 0.89 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.94, 157.29, 153.02, 150.46, 148.40, 145.95, 132.01, 130.85, 130.28, 129.50, 128.97, 128.42, 128.11, 119.53, 97.17, 72.85, 65.72, 50.70, 49.06, 31.75, 30.76, 29.47, 29.16, 29.01, 22.56, 14.42, 8.24. HRMS: $[M+H]^+$ calculated for $C_{29}H_{29}N_3O_7$: 532.2038; found: 532.1520.

Synthesis of compound **SN38-DEA**. To 2 mL of crude product **NP-DEA** (0.4 mmol, 1.0 eq) solution in dry DCM, **SN38** (0.4 mmol, 1.0 eq) and TEA (0.4 mmol, 1.0 eq) were added at 0 °C. The reaction was stirring for 24 h. The residue was purified by flash chromatography on silica gel to yield yellow solid **SN38-DEA** (29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 9.1 Hz, 1H), 7.91 (s, 1H), 7.72-7.55 (m, 2H), 5.73 (d, J = 16.5 Hz, 1H), 5.58 (s, 1H), 5.39 (s, 1H), 5.26 (d, J = 10.1 Hz, 2H), 5.01 (s, 2H), 4.30 (s, 1H), 3.97 (s, 1H), 3.48 (d, J = 7.1 Hz, 4H), 3.15 (d, J = 7.8 Hz, 2H), 1.89 (p, J = 7.2 Hz, 2H), 1.39 (t, J = 7.6 Hz, 3H), 1.17 (d, J = 7.9 Hz, 6H), 1.02 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.86, 168.61, 157.64, 153.01, 152.14, 150.24, 149.79, 147.48, 146.76, 145.44, 138.94, 132.31, 127.41, 124.62, 118.71, 117.47, 114.48, 114.15, 98.15, 72.79, 69.44, 66.32, 64.45, 49.39, 42.99, 39.12, 31.62, 29.70, 23.20, 13.99, 7.84. HRMS: $[M+Na]^+$ calculated for $C_{31}H_{33}N_3O_8$: 598.2160; found: 598.2170.

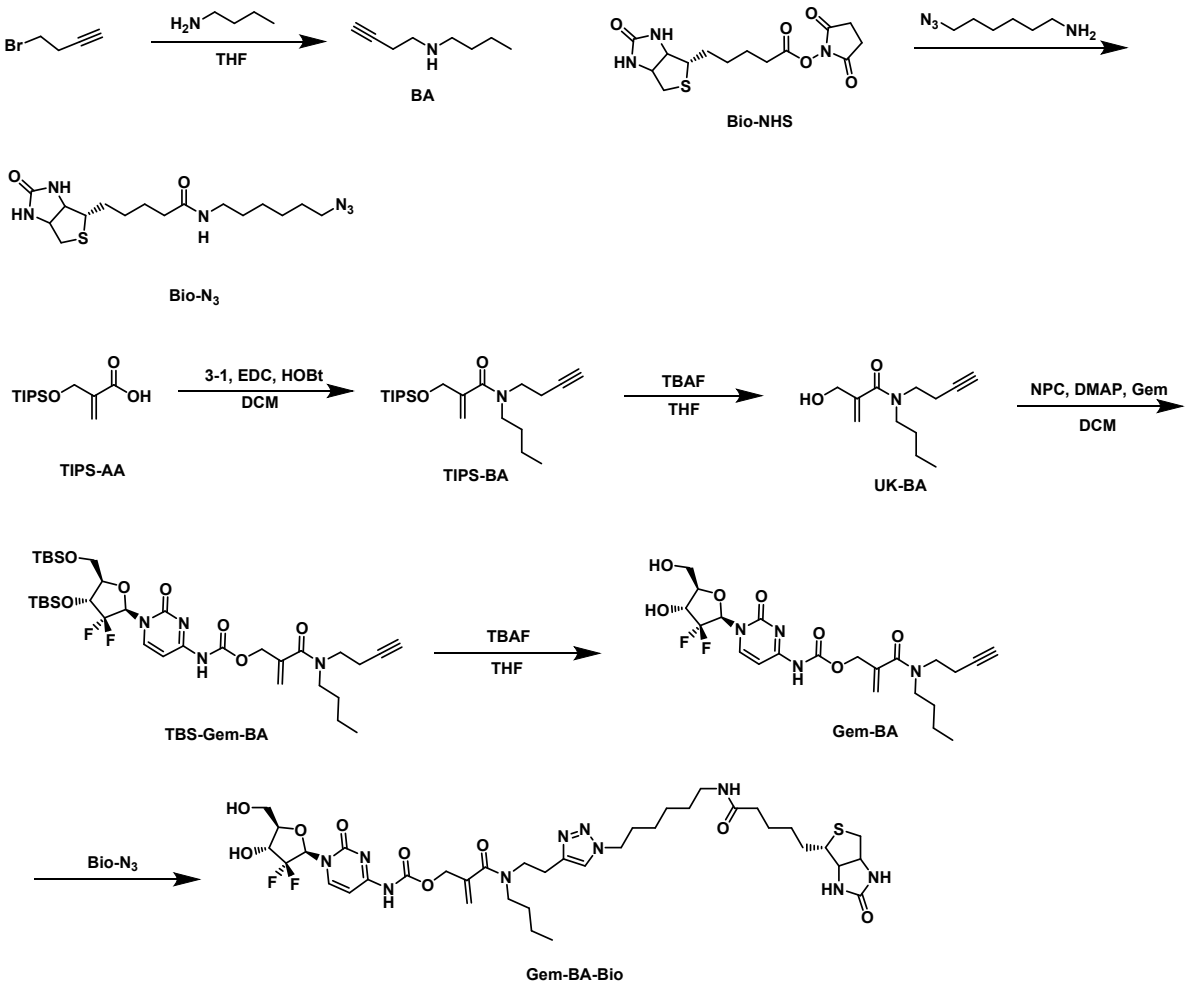
The compound **TBS-Gem** was synthesized according to our previous publication.[3]

Synthesis of compound **TBS-Gem-DEA**. To 2 mL of crude product **NP-DEA** (0.4 mmol, 1.0 eq) solution in dry DCM, **TBS-Gem** (0.4 mmol, 1.0 eq) and TEA (0.4 mmol, 1.0 eq) were added at 0 °C. The reaction was stirring for 12 h. The residue was purified by flash chromatography on silica gel to yield white solid **TBS-Gem-DEA** (38%). ^{1}H NMR (400 MHz,

$CDCl_3$) δ 11.47 (s, 1H), 8.04 (d, *J* = 7.7 Hz, 1H), 7.31 (s, 1H), 6.42 (d, *J* = 7.8 Hz, 1H), 4.39-4.28 (m, 1H), 4.03 (d, *J* = 11.9 Hz, 1H), 3.95 (d, *J* = 10.3 Hz, 1H), 3.80 (d, *J* = 12.0 Hz, 1H), 1.24 (s, 4H), 0.92 (d, *J* = 23.6 Hz, 24H), 0.24-0.00 (m, 15H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.58, 148.78, 127.39, 102.89, 86.80, 65.39, 37.40, 35.17, 34.84, 31.33, 30.98, 28.17, 23.79, 23.46, 19.61, 0.71, 0.10.

Synthesis of compound **Gem-DEA**. To a solution of **TBS-Gem-DEA** (0.4 mmol, 1.0 eq) in 10 mL of distilled THF was added a 1 M solution of TBAF in THF (1.2 mL, 1.2 mmol, 3.0 eq). The solution was stirred at ambient temperature for 30 min. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH = 20/1) to afford the compound **Gem-DEA** (85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.25 (d, *J* = 7.6 Hz, 1H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.35 (d, *J* = 6.5 Hz, 1H), 6.16 (t, *J* = 7.3 Hz, 1H), 5.46 (s, 1H), 5.34 (s, 1H), 5.26 (s, 1H), 4.78 (s, 2H), 4.19 (dt, *J* = 11.9, 5.3 Hz, 1H), 3.93-3.74 (m, 2H), 3.70-3.59 (m, 1H), 3.32 (d, *J* = 8.3 Hz, 4H), 1.07 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.60, 168.36, 163.80, 154.41, 153.16, 144.98, 140.24, 125.98, 123.41, 120.84, 116.29, 95.28, 84.55, 81.41, 68.95, 68.73, 68.51, 65.92, 59.18, 42.76, 38.78, 19.89, 14.58, 14.03, 13.50, 13.02. HRMS: $[M+H]^+$ calculated for $C_{18}H_{24}F_2N_4O_7$: 447.1686; found: 447.1452.

1.5. Chemical synthesis of Gem-BA-Bio.



Synthesis of compound **BA**. Compound **BA** was synthesized according to the reported literature.[4] Dissolve n-butylamine (156 mmol, 3.0 eq) in acetonitrile (100 mL), then dilute 4-bromobutyrgyl (52 mmol, 1.0 eq) with 50 mL acetonitrile and slowly add to the reaction system through a constant pressure drop funnel at room temperature. After stirring at 45 °C for 12 h, the solvent was removed under reduced pressure. The reaction mixture was diluted with dichloromethane and water, the organic phase was separated, washed with a saturated $NaHCO_3$ aqueous solution, dried with anhydrous Na_2SO_4, and concentrated to remove the solvent. Purify by chromatography (DCM/MeOH=20/1) to obtain yellow liquid **BA** (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.83 (t, J = 6.7 Hz, 2H), 2.71-2.63 (m, 2H), 2.45 (td, J = 6.7, 2.7 Hz, 2H), 2.01 (s, 1H), 1.53 (m, 2H), 1.36 (dq, J = 14.5, 7.3 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H).

Synthesis of compound **Bio-N_3**. Dissolve compound 6-azidohexylamine (0.7 mmol, 1.0 eq) and **Bio-NHS** (0.7 mmol, 1.0 eq) in DMF (2 mL) and stir at room temperature for 12 h. Then slowly pour 100 mL of ice water, filter and dry to obtain the white solid compound **Bio-N_3** (89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 48.2 Hz, 2H), 5.18 (s, 1H), 4.58-4.49 (m, 1H), 4.38-4.28 (m, 1H), 3.31-3.22 (m, 3H), 3.20-3.11 (m, 1H), 2.92 (dd, *J* = 12.9, 4.9 Hz, 1H), 2.82-2.66 (m, 2H), 2.21 (t, *J* = 8.4 Hz, 2H), 1.83-1.57 (m, 7H), 1.54-1.27 (m, 8H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.25, 163.16, 61.52, 59.65, 55.92, 51.05, 40.32, 38.72, 35.69, 29.51, 28.69, 28.67, 28.52, 26.41, 26.35, 25.81.

The compound **TIPS-AA** was synthesized according to our previous publication.[5]

Synthesis of compound **TIPS-BA**. **TIPS-AA** (6.0 mmol, 1.0 eq), EDCI (9.0 mmol, 1.5 eq) and HOBT (12.0 mmol, 2.0 eq) were dissolved in 10 mL dichloromethane, and the mixture was stirred at room temperature for 10 min. Then add compound **BA** (5.0 mmol, 0.8 eq) and stir at 45 °C for 12 h. Collect the product (PE/EA=6/1) by column chromatography to obtain yellow oily compound **TIBS-BA** (69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.45 (s, 1H), 5.15 (s, 1H), 4.41 (s, 2H), 3.62-3.36 (m, 5H), 2.57-2.39 (m, 3H), 2.02-1.94 (m, 1H), 1.53 (d, *J* = 7.0 Hz, 2H), 1.30 (dd, *J* = 16.1, 8.2 Hz, 2H), 1.09-1.01 (m, 21H), 0.93 (d, J = 7.1 Hz, 2H).

Synthesis of compound **UK-BA.** To a solution of **TIBS-BA** (0.4 mmol, 1.0 eq) in 10 mL of distilled THF was added a 1 M solution of TBAF in THF (1.2 mL, 1.2 mmol, 3.0 eq). The solution was stirred at ambient temperature for 30 min. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH = 100/1) to afford the compound **UK-BA** (88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (s, 1H), 5.24 (s, 1H), 4.32 (s, 2H), 3.57 (d, *J* = 19.6 Hz, 2H), 3.44 (s, 2H), 2.60-2.40 (m, 3H), 2.02 (d, *J* = 16.5 Hz, 1H), 1.56 (s, 2H), 1.29 (d, *J* = 6.5 Hz, 2H), 0.93 (dt, *J* = 8.3, 4.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.66, 120.99, 64.50, 53.43, 47.16, 43.68, 19.92, 17.38, 13.79.

Synthesis of compound **TBS-Gem-BA**. The compound **UK-BA** (0.48 mmol, 1.0 eq) was dissolved in 5 mL dichloromethane, and then DMAP (0.72 mmol, 1.5 eq) was added. NPC (0.57 mmol, 1.2 eq) was dissolved in 1 mL dichloromethane, added to the reaction solution at 0 ^{o}C, and stirred at room temperature for 1 h. Subsequently, **TBS-Gem** (0.3 mmol, 0.6 eq) and TEA (65 μL, 0.47 mmol) were added to the reaction solution and stirred at room temperature for 12 h. The product was collected by column chromatography (DCM/MeOH = 100/1) to obtain a transparent oil **TBS-Gem-BA**. (48 %)

Synthesis of compound **Gem-BA**. To a solution of **TBS-Gem-BA** (0.4 mmol, 1.0 eq) in 10 mL of distilled THF was added a 1 M solution of TBAF in THF (1.2 mL, 1.2 mmol, 3.0 eq). The solution was stirred at ambient temperature for 30 min. The solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH = 20/1) to afford the compound **Gem-BA** (62 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 7.6 Hz, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 6.21 (s, 1H), 5.55 (s, 1H), 5.35 (s, 1H), 4.87 (s, 2H), 4.54 (d, *J* = 10.5 Hz, 1H), 4.14-3.99 (m, 2H), 3.94 (d, *J* = 12.2 Hz, 1H), 3.54 (d, *J* = 23.6 Hz, 2H), 3.42 (s, 2H), 3.07-2.89 (m, 1H), 2.50 (s, 2H), 2.06 (d, *J* = 27.5 Hz, 2H), 1.74 (t, *J* = 16.1 Hz, 1H), 1.53 (p, *J* = 8.0 Hz, 2H), 1.38 (q, *J* = 7.4 Hz, 1H), 0.97 (d, *J* = 7.3 Hz, 1H), 0.89 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.72, 162.97, 155.41, 151.84, 145.07, 138.65, 124.09, 122.37, 120.65, 95.76, 81.40, 71.34, 70.06, 59.50, 49.62, 46.91, 44.44, 43.73, 31.93, 30.99, 29.70, 29.37, 22.70, 20.14, 19.88, 18.70, 17.11, 14.13, 13.76. ESI-MS: calculated for $C_{22}H_{28}F_2N_4O_7$ $[M+Na]^+$: 521.1818; found: 521.1816.

Synthesis of compound **Gem-BA-Bio**. **Gem-BA** (0.20 mmol, 1.0 eq) and **Bio-N_3** (0.48 mmol, 2.4 eq) were dissolved in a mixed solvent of DCM (10 mL) and methanol (5 mL), and Cs_2CO_3 (0.02 mmol, 0.1 eq) was added. CuI (0.02 mmol, 0.1 eq) was dissolved in acetonitrile solution (3 mL) and added to the reaction system. The reaction was carried out at room temperature for 30 min. The solvent was removed by vacuum distillation. Column chromatography purification (DCM/MeOH = 10/1) to get white solid **Gem-BA-Bio** (90 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.07 (s, 1H), 8.24 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.72 (t, J = 5.6 Hz, 1H), 7.07 (d, J = 7.6 Hz, 1H), 6.48-6.28 (m, 3H), 6.16 (t, J = 7.5 Hz, 1H), 5.47 (d, J = 10.7 Hz, 1H), 5.30 (t, J = 5.4 Hz, 1H), 5.19 (d, J = 38.1 Hz, 1H), 4.75 (d, J = 13.6 Hz, 2H), 4.29 (q, J = 7.5 Hz, 3H), 4.24-4.04 (m, 3H), 3.88 (d, J = 8.3 Hz, 1H), 3.79 (d, J = 14.4 Hz, 1H), 3.70-3.49 (m, 3H), 3.23 (d, J = 4.9 Hz, 2H), 3.16 (d, J = 5.2 Hz, 1H), 3.12-3.04 (m, 1H), 2.99 (q, J = 6.5 Hz, 2H), 2.93-2.75 (m, 3H), 2.57 (d, J = 12.4 Hz, 1H), 2.03 (t, J = 7.4 Hz, 2H), 1.75 (p, J = 7.2 Hz, 2H), 1.60 (dd, J = 13.8, 9.5 Hz, 1H), 1.54-1.39 (m, 5H), 1.33 (q, J = 6.5 Hz, 3H), 1.28 (d, J = 7.1 Hz, 4H), 1.17 (t, J = 7.1 Hz, 4H), 0.92-0.71 (m, 4H). 13C NMR (151 MHz, DMSO-d_6) δ 172.28, 168.92, 166.10, 163.83, 163.18, 154.44, 145.02, 144.40, 143.88, 140.03, 130.13, 125.11, 123.40, 122.89, 122.74, 121.69, 116.88, 95.26, 81.45, 68.95, 65.90, 61.51, 59.64, 59.24, 55.92, 49.61, 49.07, 38.70, 35.68, 30.20, 29.44, 28.69, 28.52, 26.27, 26.03, 25.81, 14.02. ESI-MS: calculated for $C_{38}H_{56}F_2N_{10}O_9S$ $[M+H]^+$: 867.3997; found: 867.3965.

2. Experimental Methods

2.1 Screening of Nap-DEA, Nap-MOR and Nap-DBA with various palladium complexes.

All compounds were prepared as 10 mM stocks in DMSO, except for **Pd(TPPTS)$_4$**, which was prepared in PBS. Unless otherwise stated, all materials were freshly prepared. Amino-naphthalimide derivatives (10 μM) were incubated with palladium complexes (10 μM) in PBS (with 5% DMSO) at 37 °C for 30 min. Fluorescence intensity was measured using a fluorescence spectrophotometer. Relative fluorescence was calculated as the ratio of the fluorescence intensity of the reaction system to that of the fluorophore at the same concentration. (Ex = 428 nm, Em = 545 nm).

2.2 Spectral Response of amino-naphthalimide derivatives with Pd(TPPTS)$_4$.

To acquire the reaction efficiency of amino-naphthalimide derivatives with varying concentrations of **Pd(TPPTS)$_4$**, **Nap-DEA, Nap-MOR** and **Nap-DBA** (10 μM) were incubated with increasing concentrations of **Pd(TPPTS)$_4$** (1 μM, 2 μM, 5 μM and 10 μM) at 37 °C for 15 min, respectively. The emission spectra were scanned every minute, and fluorescence change at 545 nm were recorded. Relative fluorescence was calculated as the ratio of the fluorescence intensity of the reaction system to that of the fluorophore at the same concentration. (Ex = 428 nm, Em = 545 nm)

2.3 Fluorescence imaging of amino-naphthalimide derivatives with Pd(TPPTS)$_4$ in HepG2 cells.

HepG2 cells were seeded at a density of 10,000 cells per well in a 24-well glass chamber with 500 μL of DMEM cell culture medium and incubated at 37 °C with 5% CO2 for 24 h. Cells were treated with **Nap-DEA** (5 μM), **Nap-DEA/Pd(TPPTS)$_4$** (5 μM/2.5 μM), **Nap-MOR** (5 μM), **Nap-MOR/Pd(TPPTS)$_4$** (5 μM/2.5 μM), **Nap-DBA** (5 μM), **Nap-DBA/Pd(TPPTS)$_4$** (5 μM/2.5 μM) for 60 min. After treatment, cells were washed three times with PBS.

Fluorescence images were acquired using a Floid cell imaging station microscope (Life Technologies).

2.4 Spectral response of Cou-DEA and FR-DEA to $Pd(TPPTS)_4$, and nucleophilic molecules.

Cou-DEA (10 μM) and **FR-DEA** (10 μM) were incubated with **$Pd(TPPTS)_4$** (5 μM) and various nucleophilic molecules (GSH: 1 mM, 1,2-Ethanedithiol: 1 mM, DTT: 1 mM, rBSA: 5 μM, Thiophenol: 1 mM, Benzyl selenol: 1 mM) in PBS (with 5% DMSO) at 37 °C, respectively. Incubate for 30 min, monitor every 2 min. Relative fluorescence was calculated as the ratio of the fluorescence intensity of the reaction system to that of the fluorophore at the same concentration. (**Cou**: Ex = 350 nm, Em = 440 nm; **FR**: Ex = 580 nm, Em = 625 nm).

2.5 Evaluation of the prodrug stability and drug release of prodrug *in vitro*.

Stability experiment of prodrug**. CPT-DEA** (100 μM), **SN38-DEA** (100 μM) and **Gem-DEA** (100 μM) were incubated in cell lysis buffer at 37 °C for 24 h. Cell lysis buffer was prepared as follows: Cells were cultured until reaching approximately 90% confluency, washed three times with ice-cold PBS, collected, and resuspended in RIPA lysis buffer. Cell lysis was performed on ice for 30 min with vortexing for 10 sec at 10-minute intervals. The lysate was centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was collected for use. After incubation, 10 μL of each sample was subjected to HPLC analysis. HPLC analysis was carried out on an C18 Superb reversed-phase column (5 μm, 4.6×150 mm) with Phase A/Phase B gradients [Phase A: MeOH, Phase B: H_2O with 0.1% HCOOH]. **CPT-DEA**: A/B = 65/35 (v/v); **SN38-DEA** and **Gem-DEA**: A/B = 66/34 (v/v). The flow rate was set at 0.4 mL min^{-1}. The column temperature was maintained at 40 °C.

Drug release of prodrug. Produrg (10 μM) were incubated with **$Pd(TPPTS)_4$** (5 μM) in PBS (with 5% MeOH) at 37 °C. At designated time points, 10 μL of the incubation mixture was

analyzed by HPLC. Drug release efficiency was calculated by comparing the peak area to standard curves of the prodrugs. HPLC conditions were identical to those described above, with the following gradient conditions: **CPT-DEA**: A/B = 65/35 (v/v); **SN38-DEA** and **Gem-DEA**:: A/B = 66/34 (v/v); **Gem-BA-Bio**: A/B = 60/40 (v/v).

2.6 Cytotoxicity testing of $Pd(TPPTS)_4$ with prodrug.

The cytotoxicity of drug, prodrug and **$Pd(TPPTS)_4$** was evaluated in HepG2 cell by CCK-8 assay. Cells were seeded at a density of 5,000 cells per well in a 96-well plate and treated with varying concentrations of **$Pd(TPPTS)_4$**, drugs, or prodrugs (1 μM, 2.5 μM, 5 μM, 10 μM, and 25 μM) in a final volume of 100 μL per well. Cells treated with 0.5% DMSO served as the control. After 72 h of incubation, cells were washed three times with PBS to remove extracellular drugs, and the CCK-8 assay was performed.

Cytotoxicity of **$Pd(TPPTS)_4$** activated prodrug. HepG2 cells were seeded at 5,000 cells per well in a 96-well plate and pre-incubated for 24 h. Cells were treated with the following prodrug/**$Pd(TPPTS)_4$** combinations (in μM): 1/0.5, 2.5/1.25, 5/2.5, 10/5, and 25/12.5. After 72 h of incubation, cells were washed three times with PBS, and the CCK-8 assay was performed.

2.7 *In vivo* anti-tumor testing.

KM mice were obtained from Jackson Laboratory and housed in the animal facilities of the School of Basic Medical Sciences at Lanzhou University.

To establish the 4T1 subcutaneous breast cancer model, 5×10^6 4T1 cells were injected into the right leg muscle of each mouse, and the 4T1 tumor-bearing mice were randomly divided into five groups (four mice per group). When the tumor volume reached approximately 50 mm^3, mice were intravenously injected at Day 0, 2, 4, 6, and 8 with PBS, **Gem** (20 mg/kg), **Gem-BA-Bio** (20 mg/kg), **Gem-BA-Bio** (20 mg/kg) + **$Pd(TPPTS)_4$** (10 mg/kg) and **$Pd(TPPTS)_4$** (10 mg/kg) respectively. During the 15-day observation period, the tumor size

was measured with a digital caliper, and the tumor volume was calculated according to the following formula: volume = width^2 × length/2. At the same time, the weight of the mice was weighed and recorded. Then the mice were euthanized, and tumors were dissected for analysis.

Reference

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(2) Shang, H. M.; Chen, H.; Tang, Y. H.; Ma, Y. Y.; Lin, W. Y. Development of a two-photon fluorescent turn-on probe with far-red emission for thiophenols and its bioimaging application in living tissues. *Biosensors & Bioelectronics*, **2017**, *95*, 81-86.

(3) Li, X.; Hou, Y.; Meng, X.; Ge, C.; Ma, H.; Li, J.; Fang, J. Selective Activation of a Prodrug by Thioredoxin Reductase Providing a Strategy to Target Cancer Cells. *Angew Chem Int Ed Engl*, **2018**, *57* (21), 6141-6145.

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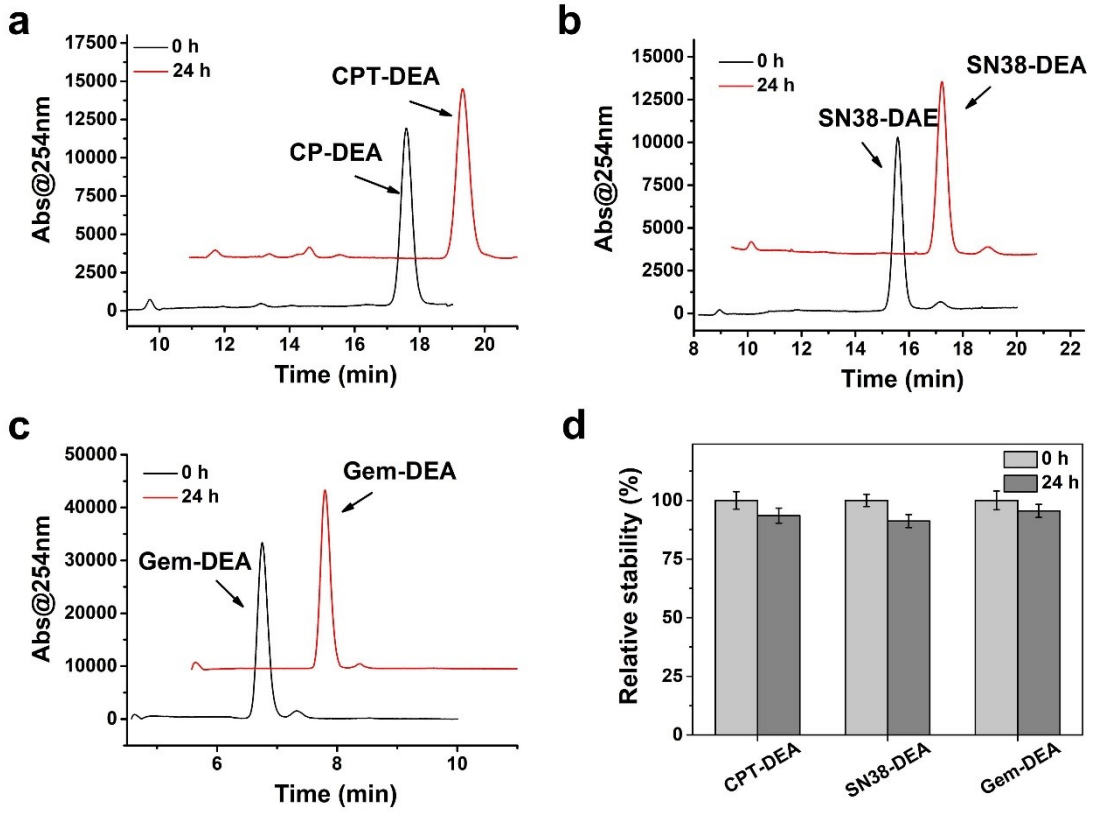
(5) Shen, R. P.; Zhang, B. X.; Zhao, L. N.; Chang, B. B.; Zhang, F.; Chen, Y. T.; Fang, J. G. A tunable stimuli-responsive module based on an α-hydroxymethyl-α,β-unsaturated carbonyl scaffold. *J. Mater. Chem. B*, **2025**.



3. Supplementary Figures

Figure S1. Stability testing of prodrug. All incubations were performed in Cell lysate (with 0.1% DMSO) under open air at 37 °C, and samples of the reaction mixture were taken out after 24 h and examined by HPLC. Data are expressed as means ± SD (n = 3).

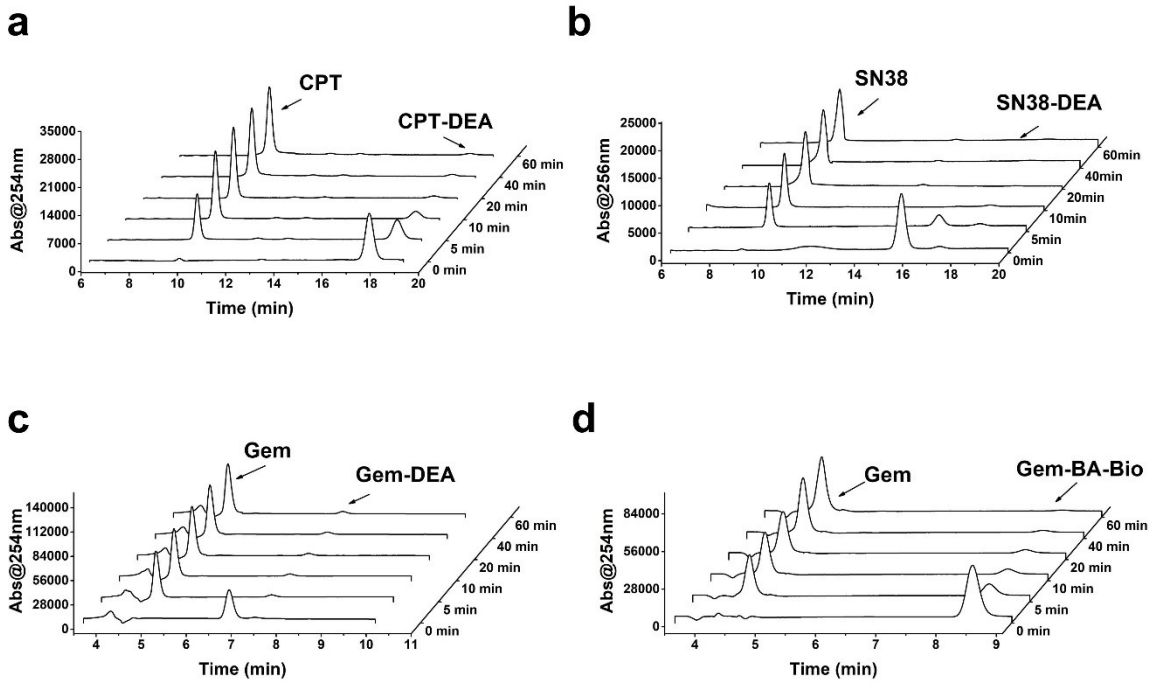
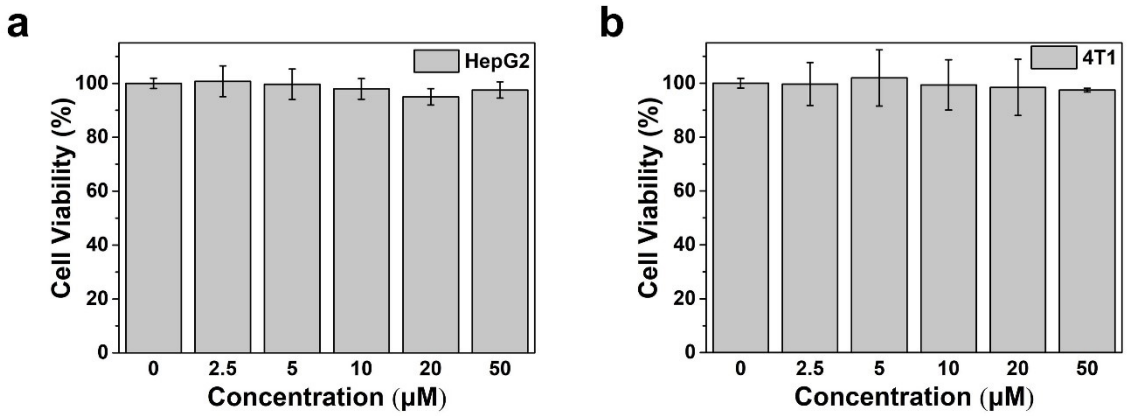
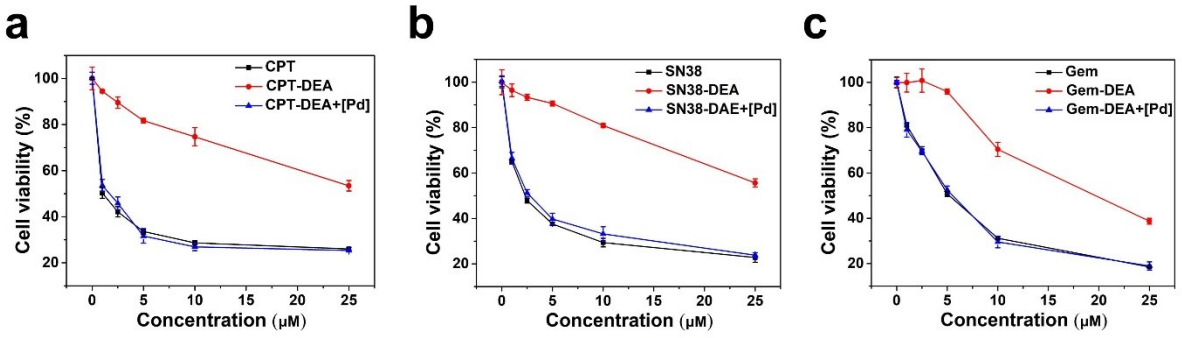


Figure S2. HPLC traces of prodrugs activation by Pd(TPPTS)$_4$. The release of drugs from **CPT-DEA** (a), **SN38-DEA** (b), **Gem-DEA** (**c**) and **Gem-BA-Bio** (**d**) by **Pd(TPPTS)$_4$** activation. Prodrugs (10 μM) were incubated with **Pd(TPPTS)$_4$** (2 μM) in PBS buffer (with 5% MeOH) at 37 °C. Samples of the reaction mixture were taken out at indicated time points and examined by HPLC. HPLC eluent conditions: MeOH/H_2O (0.1% HCOOH) = 65/35 for (**a**); MeOH/H_2O (0.1% HCOOH) = 66/34 for (**b**); MeOH/H_2O (0.1% HCOOH) = 66/34 for (**c**);



MeOH/H_2O (0.1% HCOOH) = 66/34 for (**d**). The flow rate is 0.4 mL/min.

Figure S3. Evaluation of Pd(TPPTS)$_4$ Cytotoxicity. HepG2 cells (**a**) or 4T1 cells (**b**) were seeded in a 96-well plate format with a density of 5000 cells per well and incubated for 24 h before treatment. **Pd(TPPTS)$_4$** (0, 2.5, 5, 10, 20 and 50 μM) with DMSO (0.5%, v/v) was



added to the cells and incubated for another 72 h. The cells were then washed with PBS for 3 times followed by a CCK8 assay. The data are expressed as mean ± s.d. (n=3).

Figure S4. Cytotoxicity of different molecules. HepG2 cells were seeded in a 96-well plate format with a density of 5000 cells per well and incubated for 24 h before treatment. Following prodrug/**Pd(TPPTS)$_4$** combinations (in μM) were chosen to treat cells: 1/0.5, 2.5/1.25, 5/2.5, 10/5 and 25/12.5 for **CPT-DEA/Pd(TPPTS)$_4$**, **SN38-DEA/(TPPTS)$_4$** and **Gem-DEA/Pd(TPPTS)$_4$**. All groups contain 0.5% DMSO (v/v). After 72 h, the cells were washed with PBS for 3 times followed by a CCK8 assay.

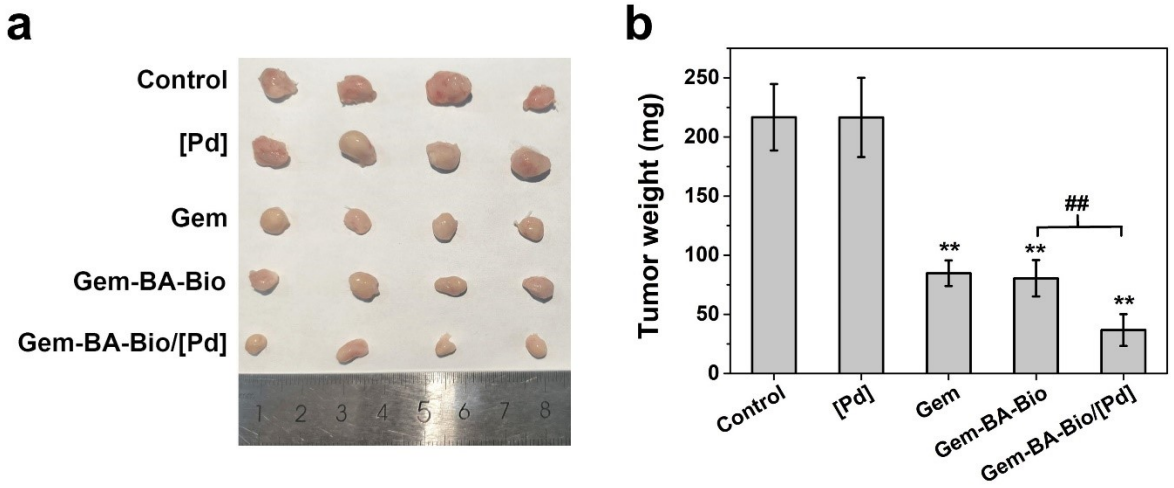
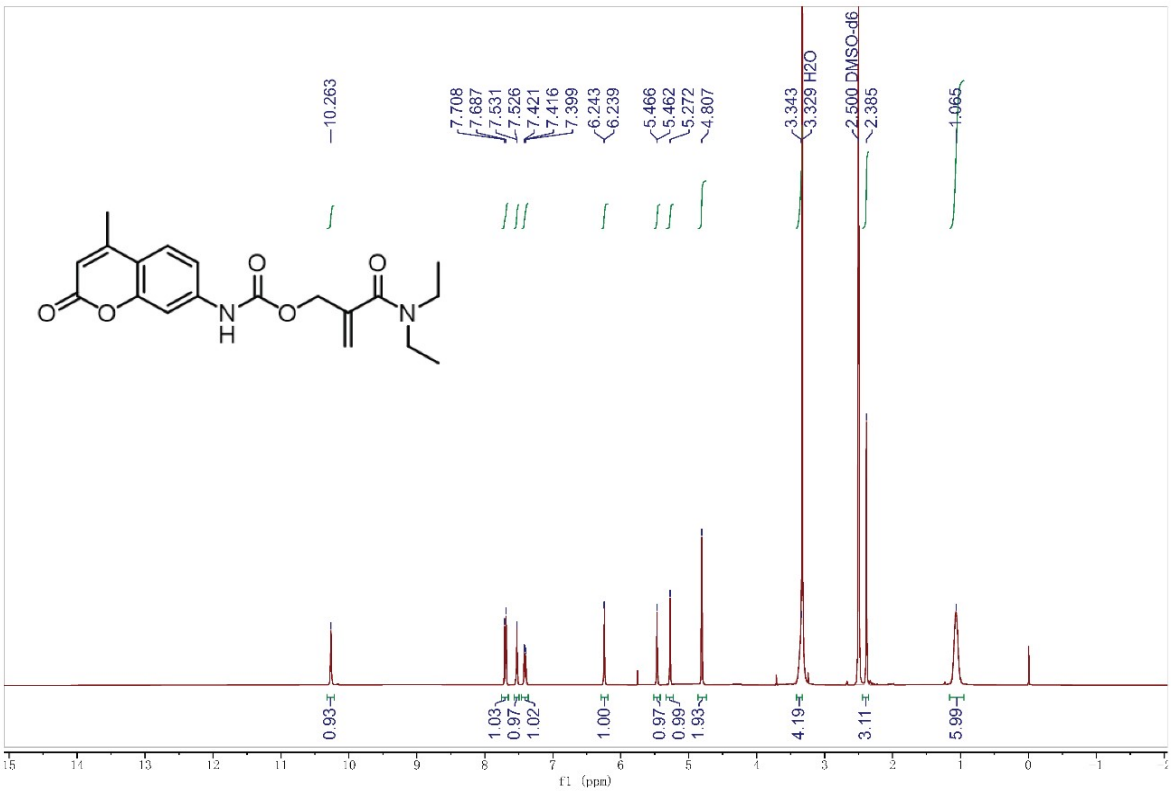
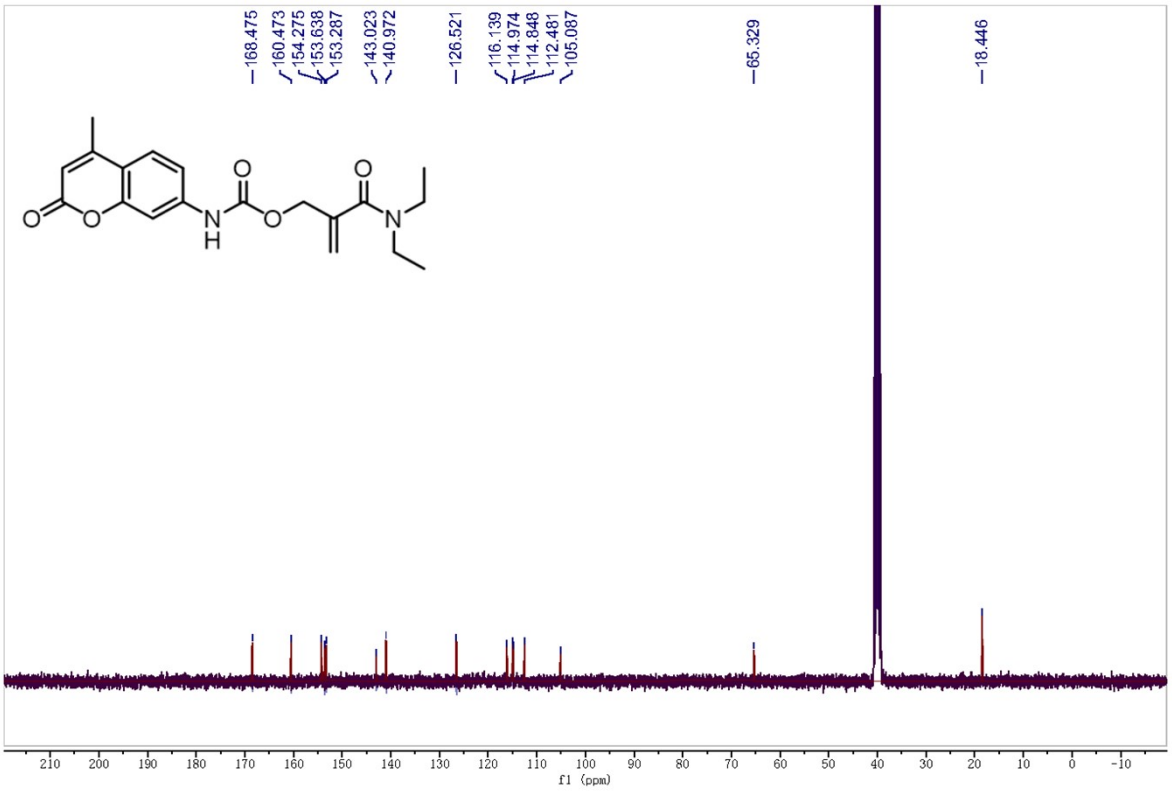


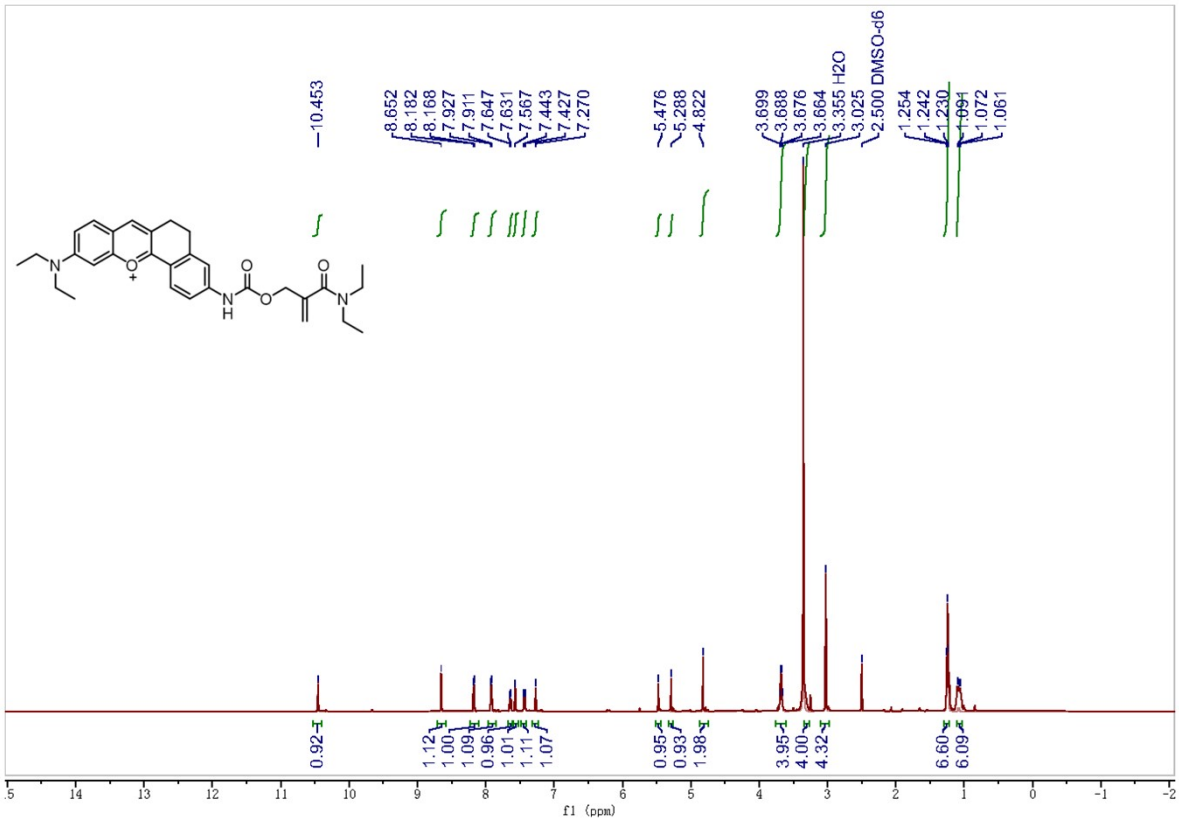
Figure S5. Mouse tumor model. (a) Photographs of the tumor excised from mice sacrificed after 14 days. (b) Tumor weights were measured when the mice were sacrificed. Data are expressed as means ± SD (n = 3). **P < 0.01 compared to the control group and ##P < 0.01 compared to the **Gem-BA-Bio** group.

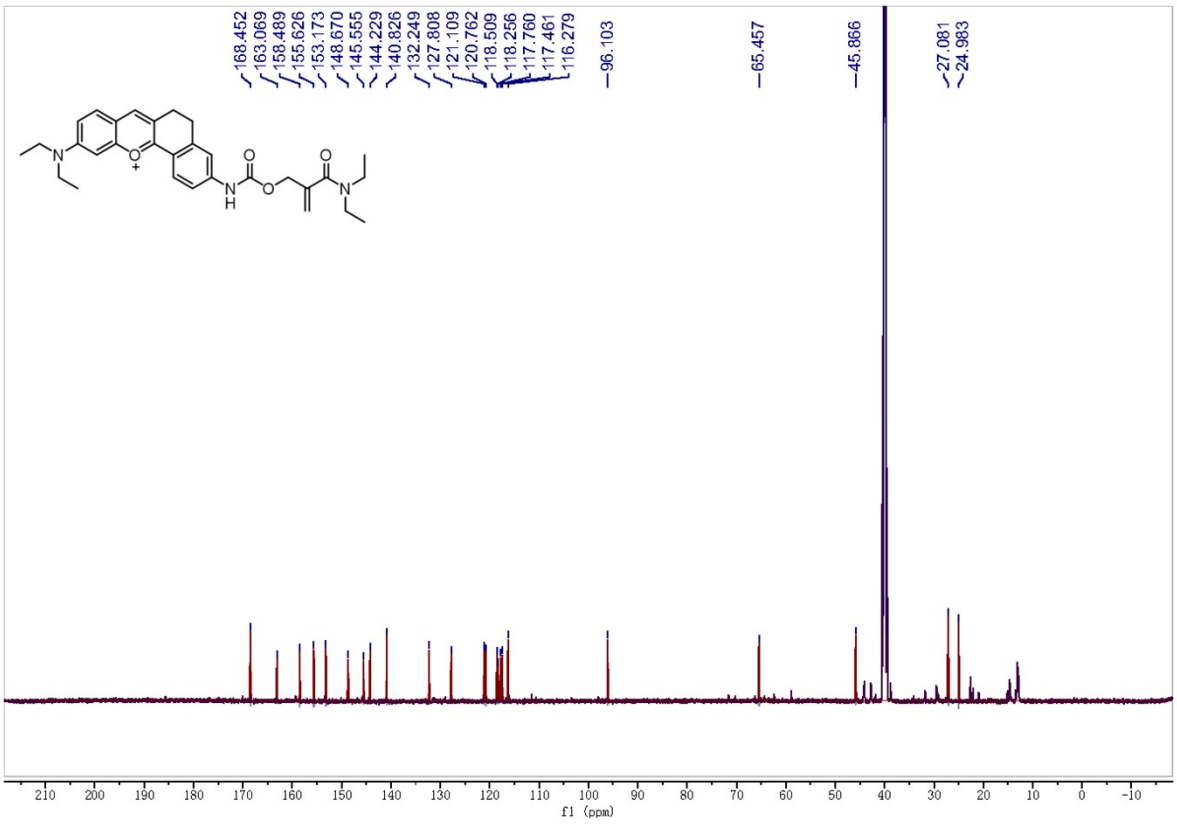




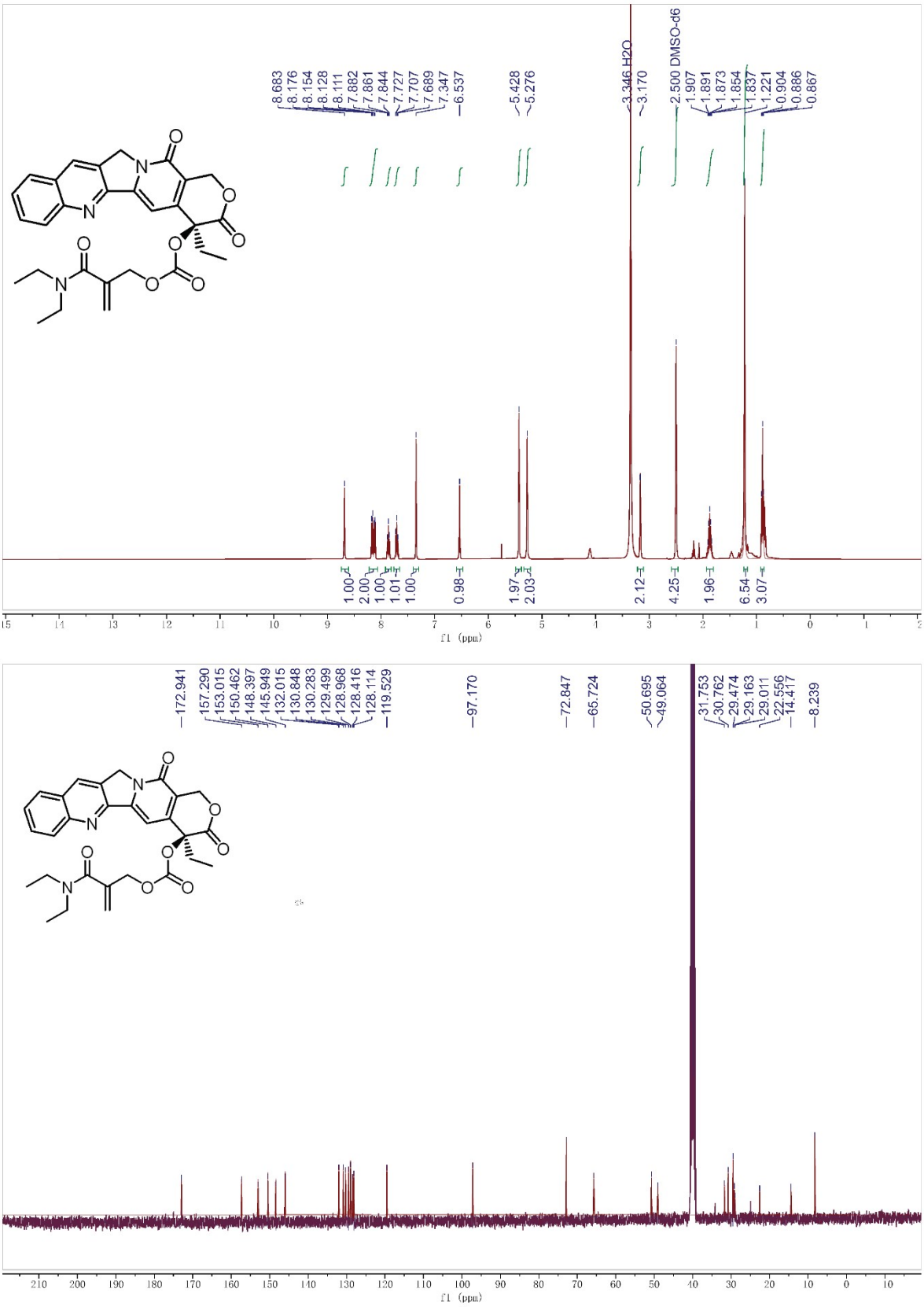
NMR spectra of the newly synthesized compounds

^{1}H NMR (400 MHz, DMSO- d_6) and ^{13}C NMR (101 MHz, DMSO- d_6) of compound **Cou-DEA**.





^{1}H NMR (600 MHz, DMSO- d_6) and ^{13}C NMR (151 MHz, DMSO- d_6) of compound **FR-DEA**.



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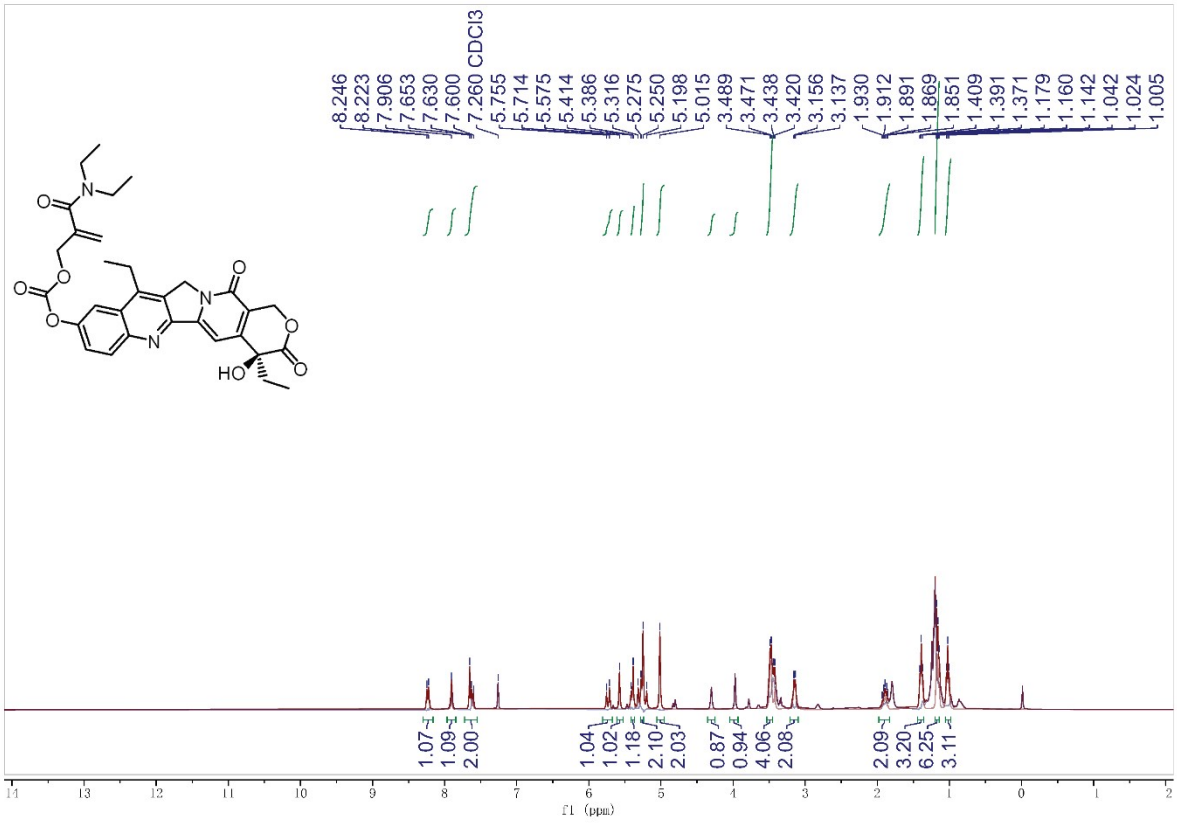
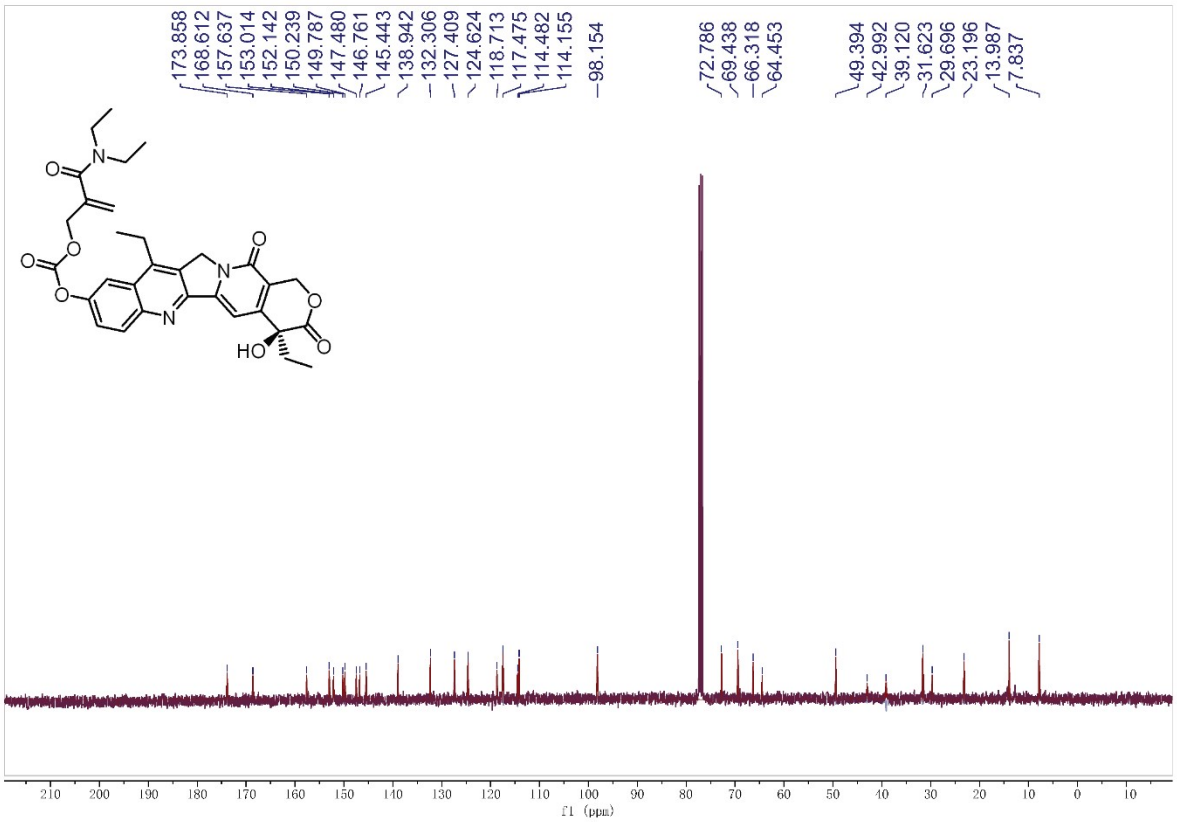
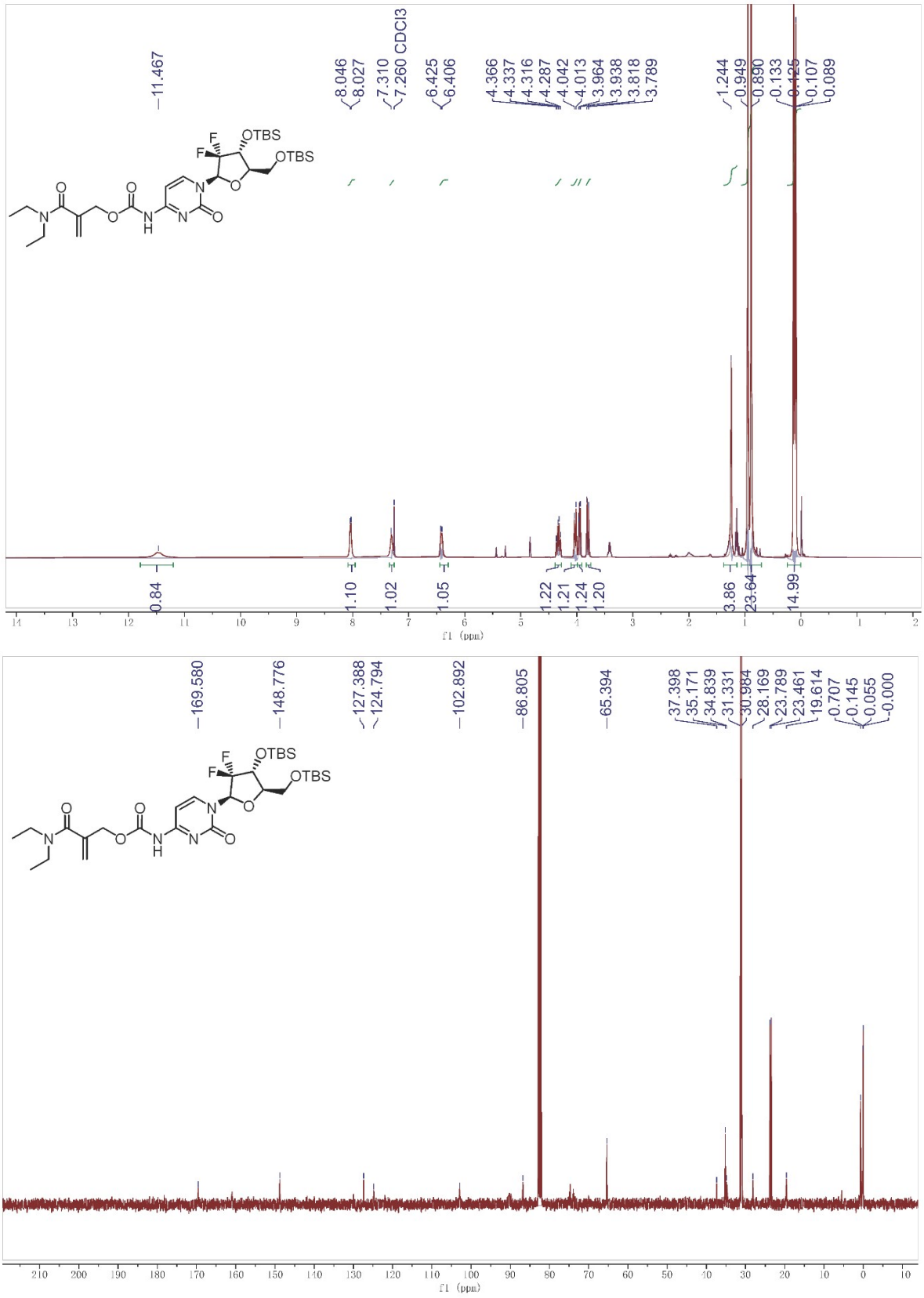
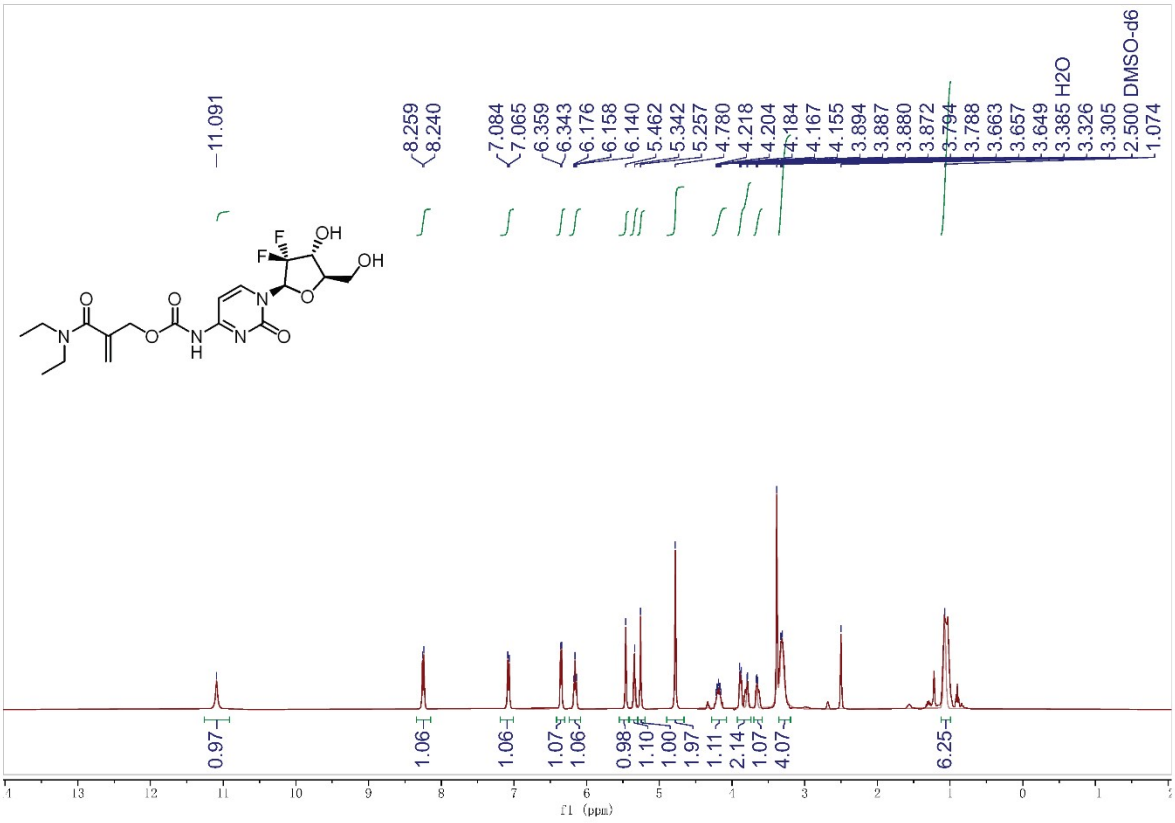


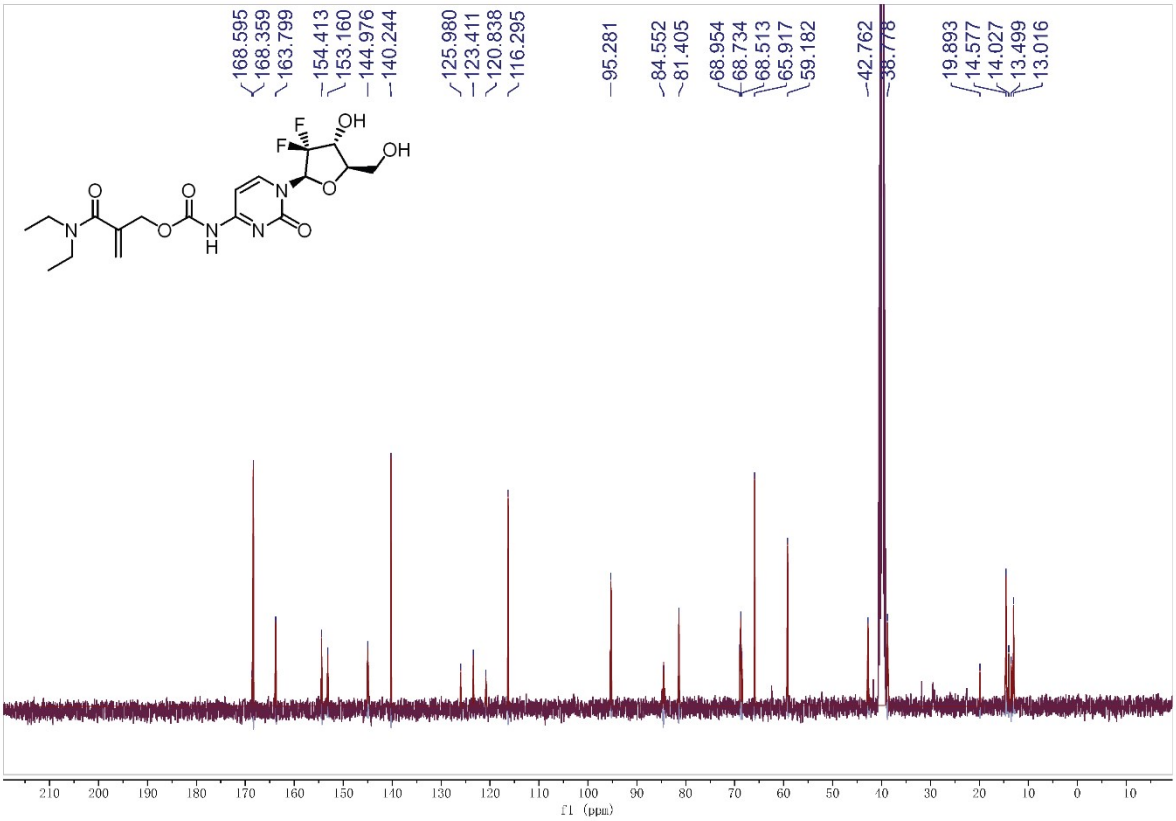


^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) of compound **SN38-DEA**.

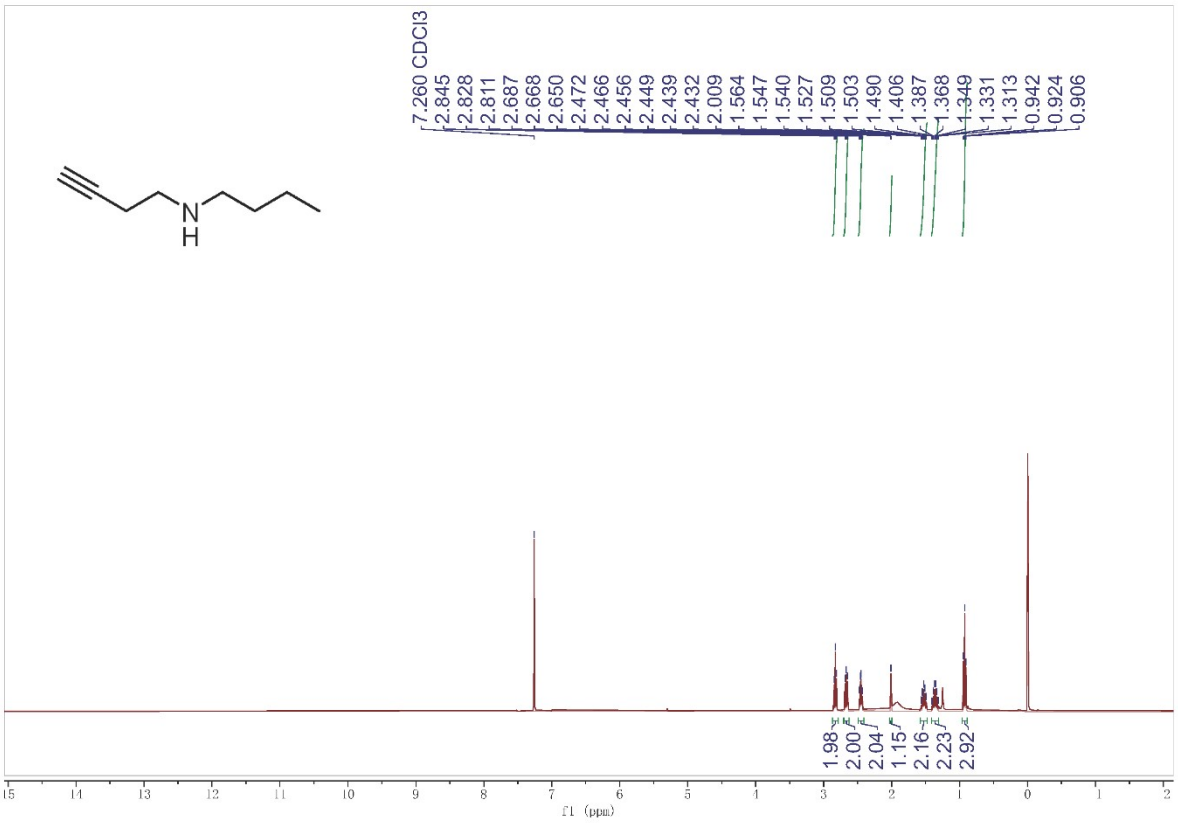


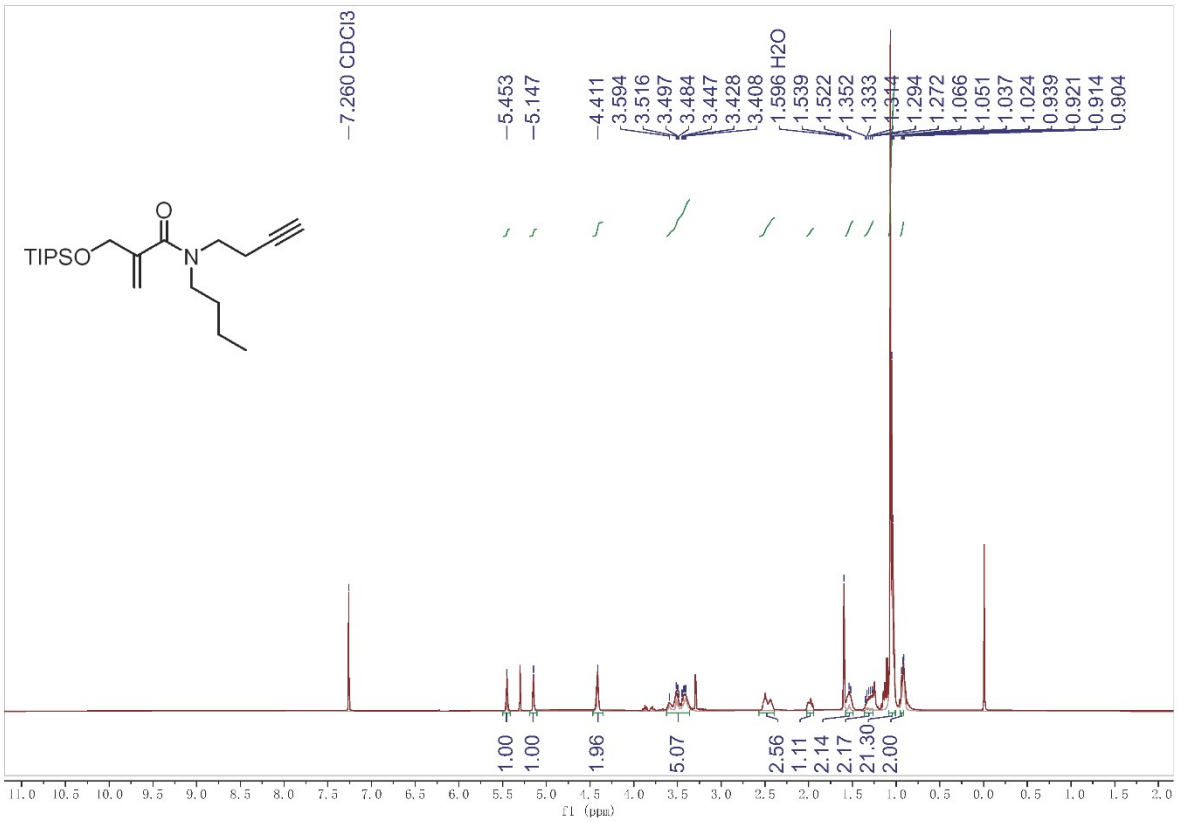
^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (101 MHz, $CDCl_3$) of compound **TBA-Gem-DEA**.



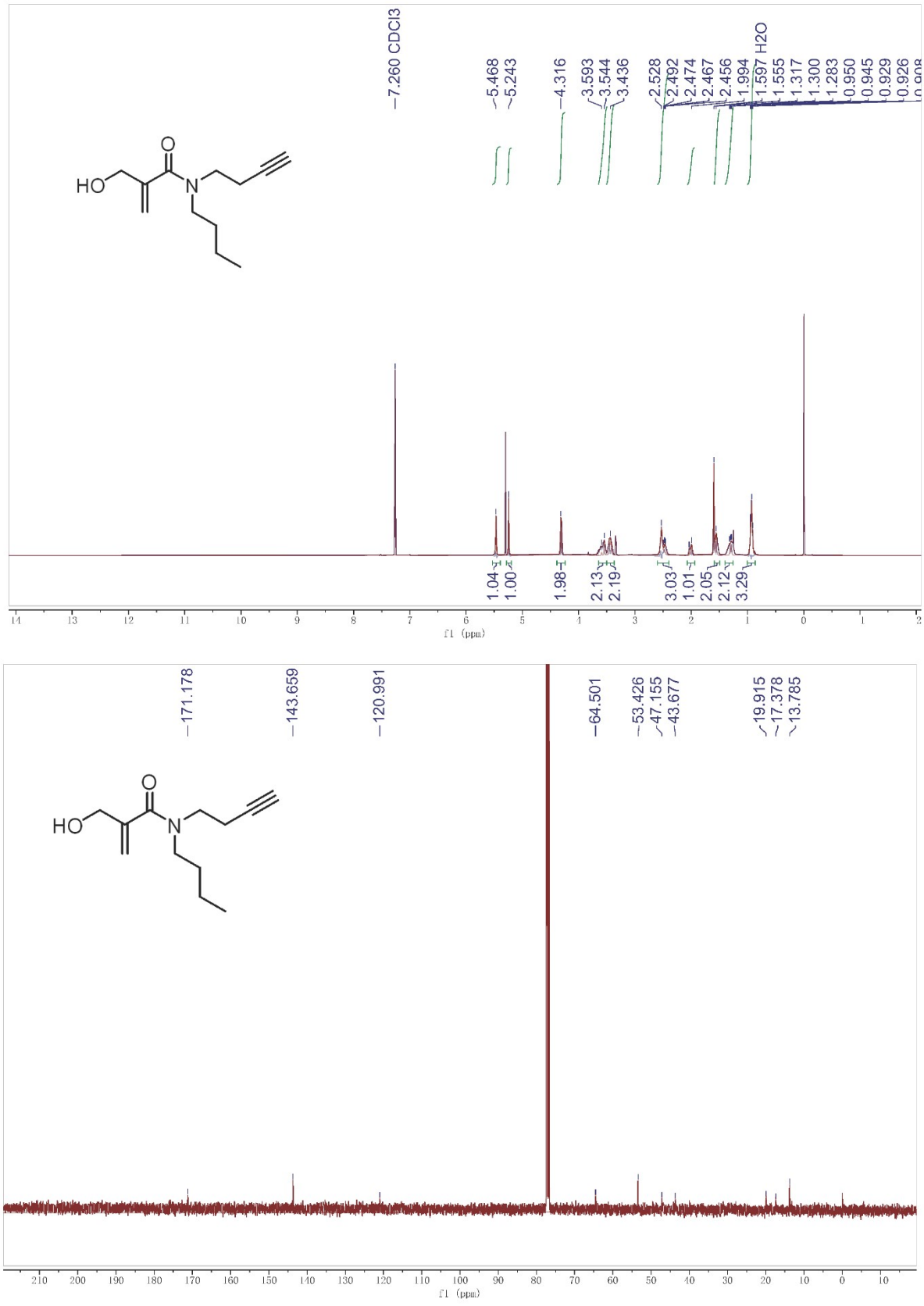


^{1}H NMR (400 MHz, DMSO- d_6) and ^{13}C NMR (151 MHz, DMSO- d_6) of compound **Gem-DEA**.





^{1}H NMR (400 MHz, $CDCl_3$) of compounds **BA** and **TIPS-BA**.



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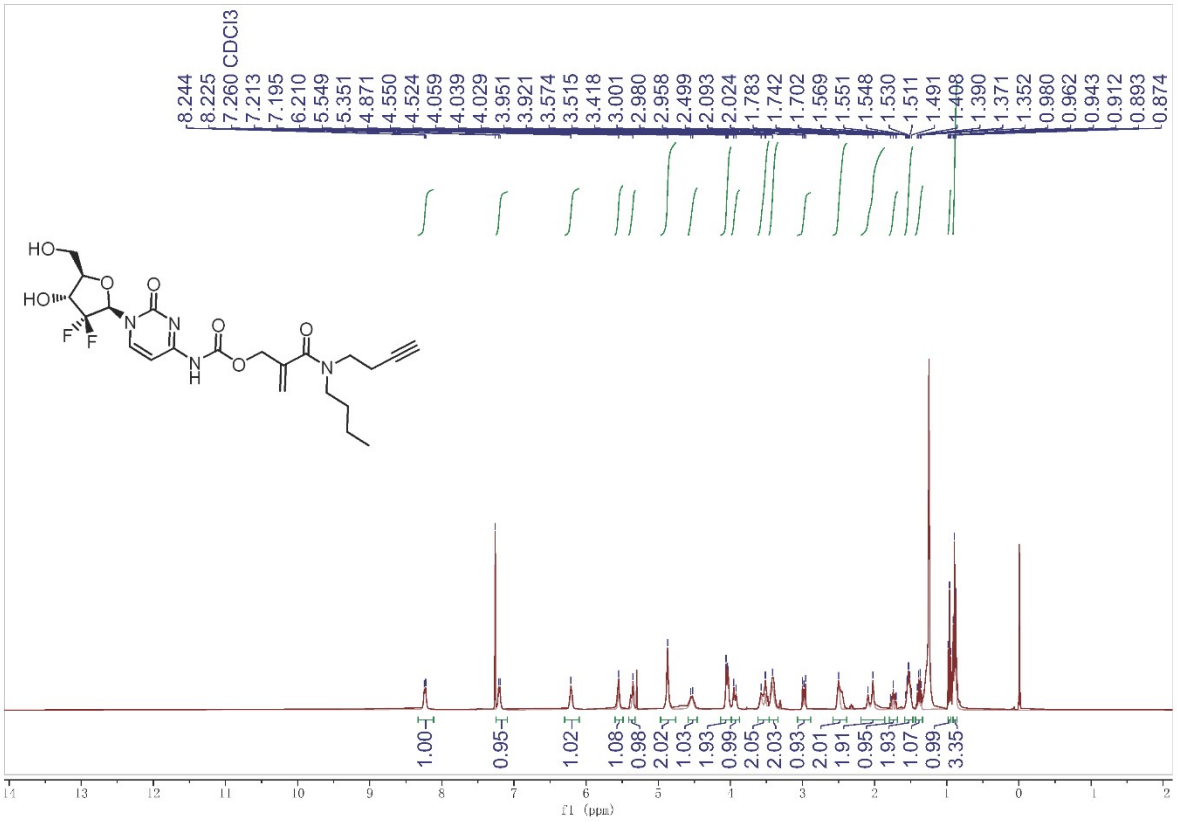

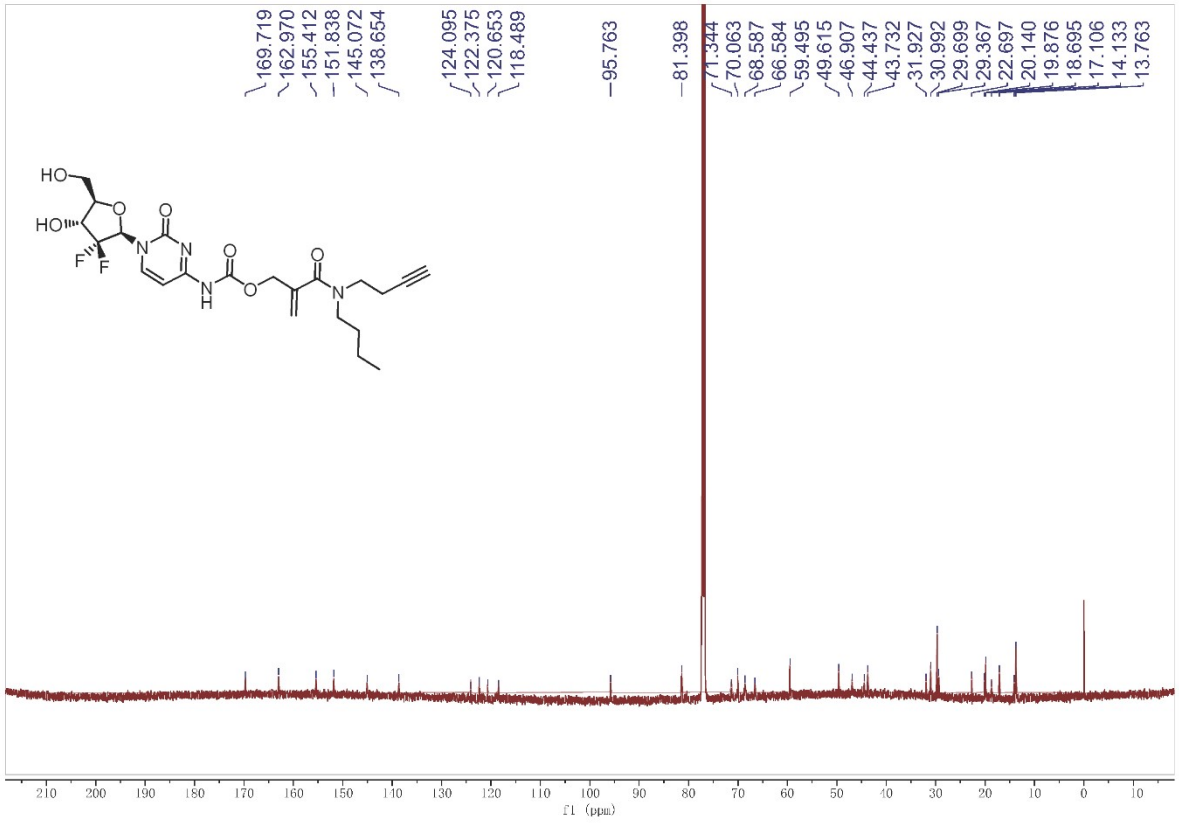

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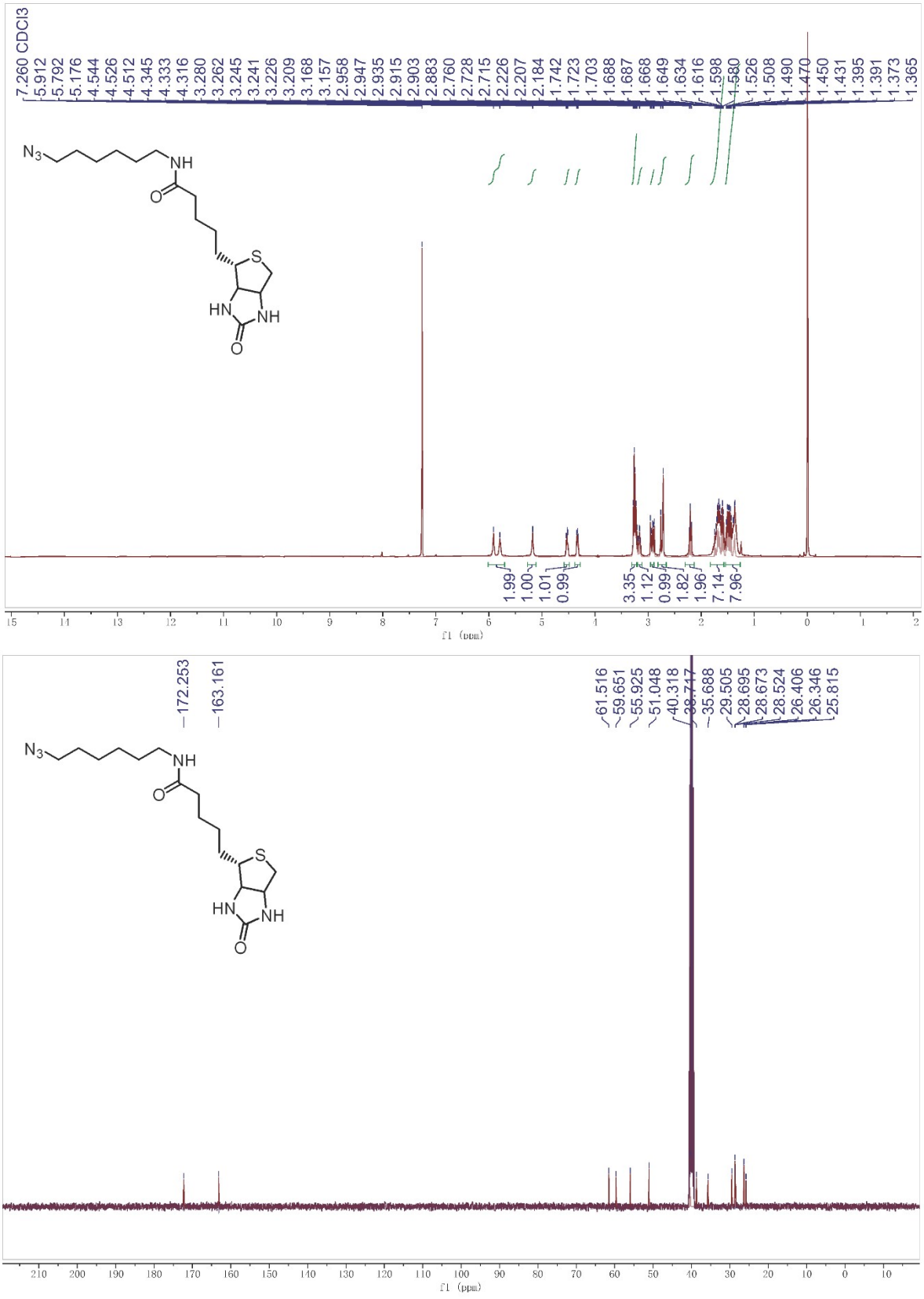
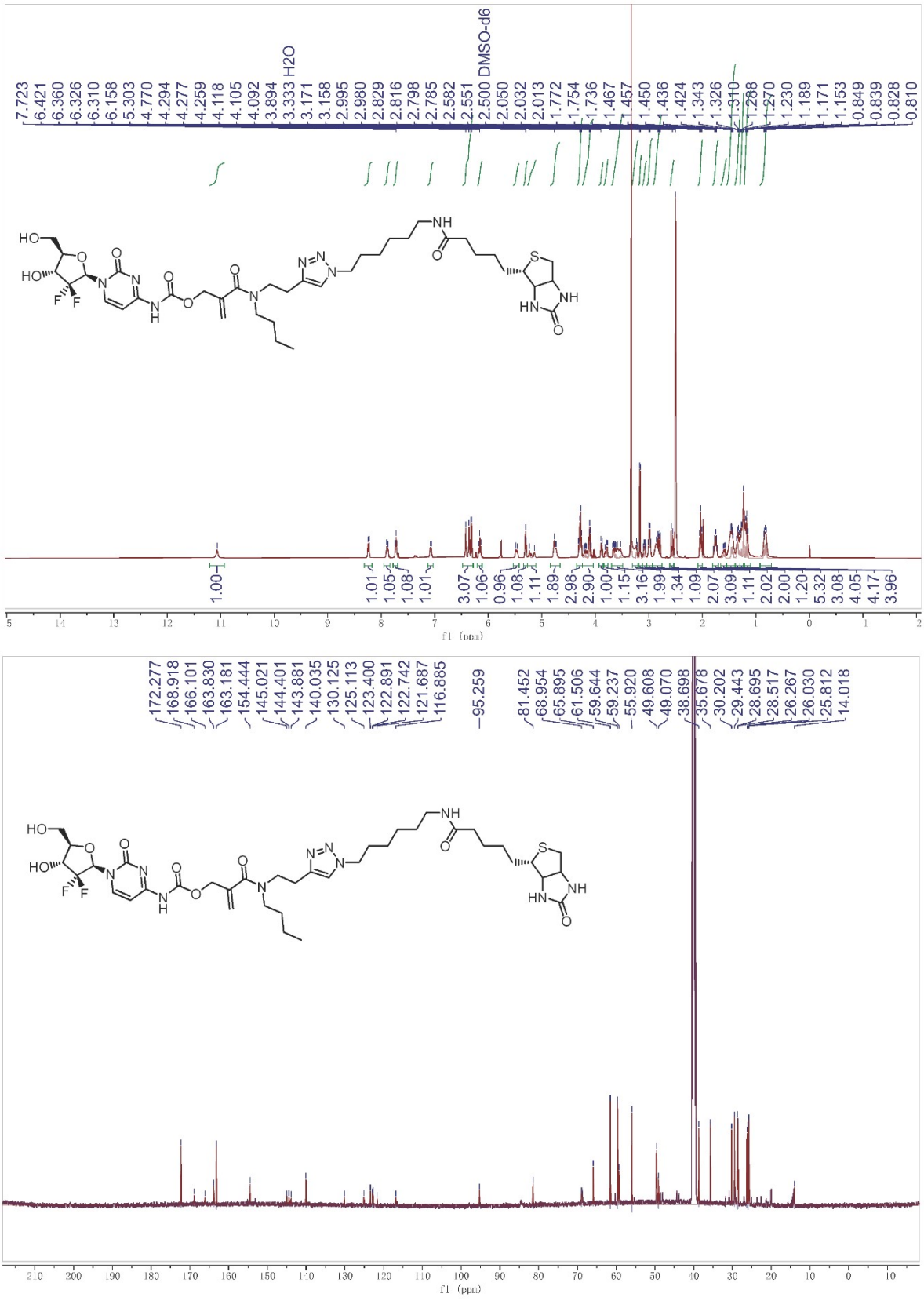


^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, DMSO- d_6) of compound **Bio-NHS**.



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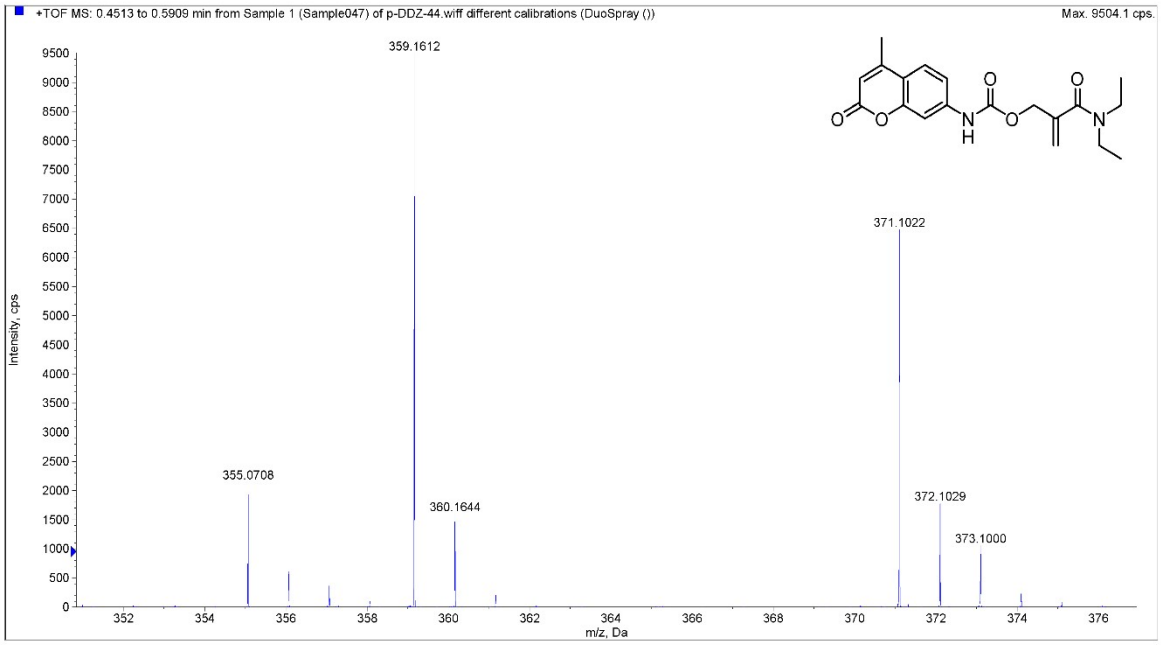
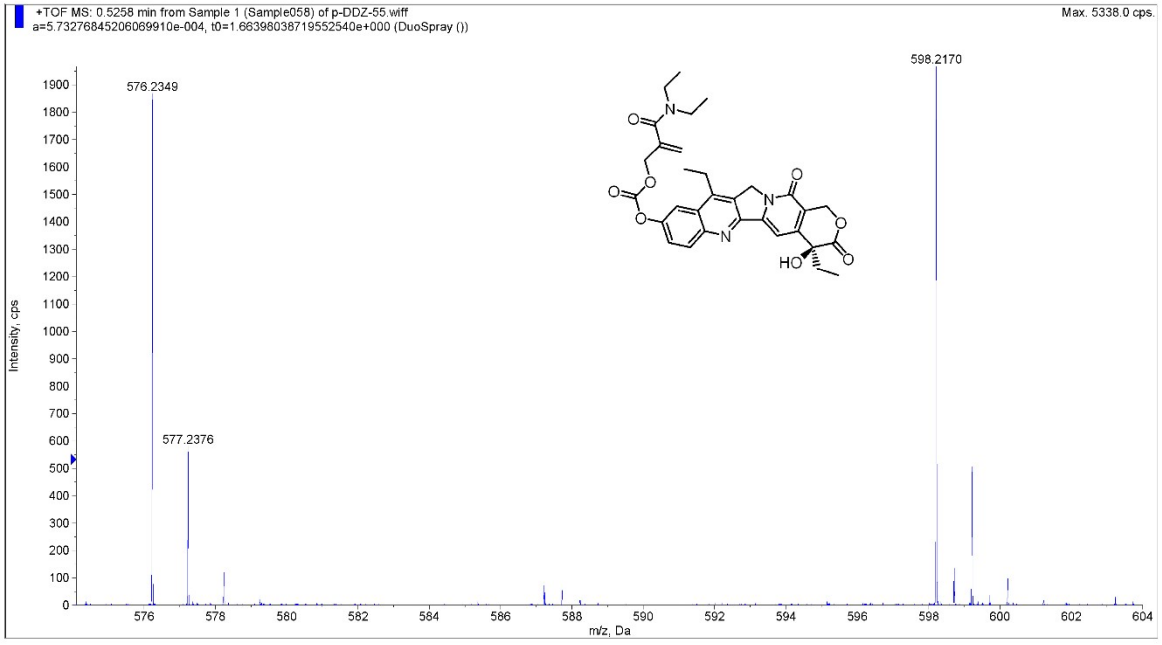
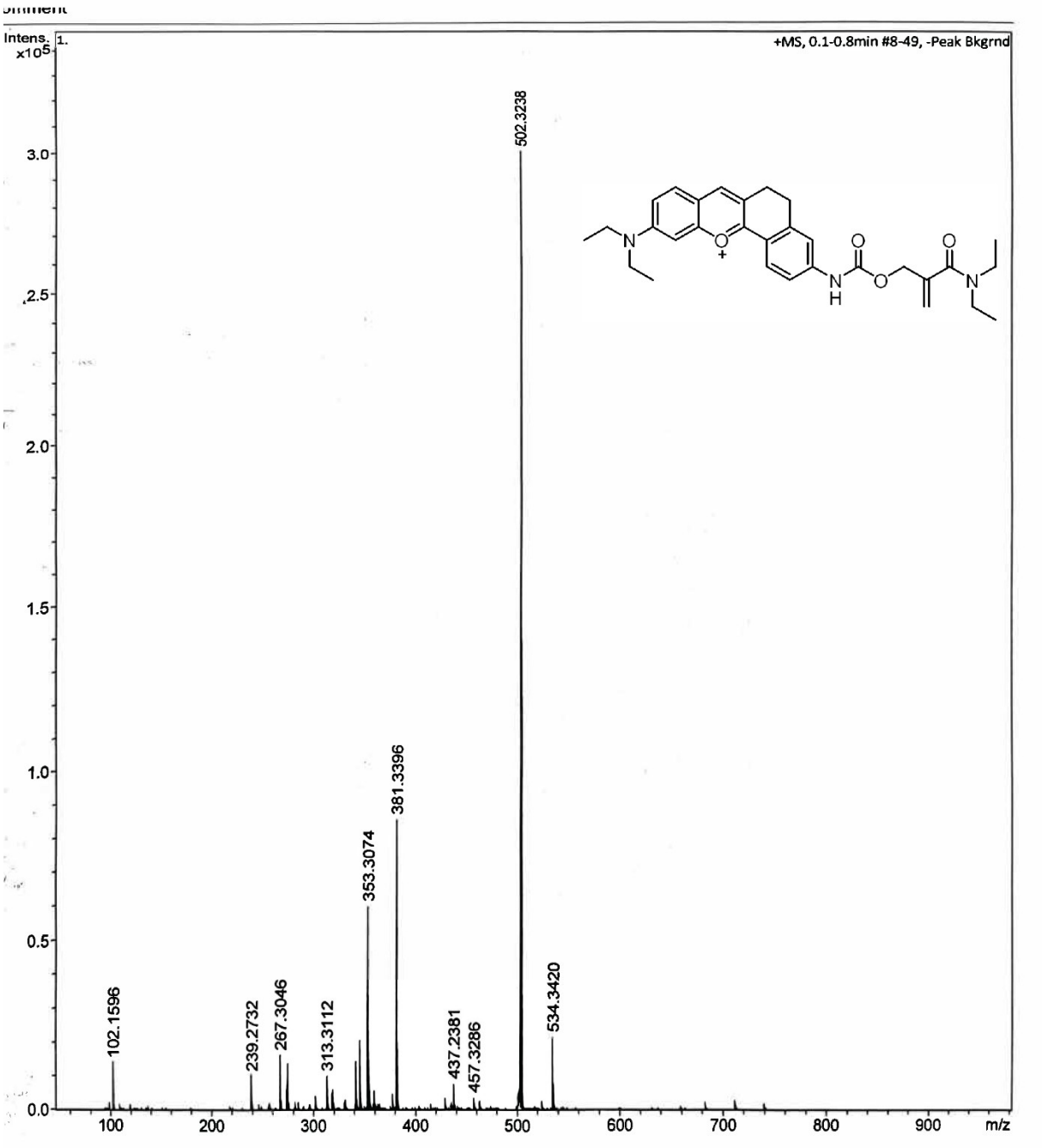




HRMS of the synthesized compounds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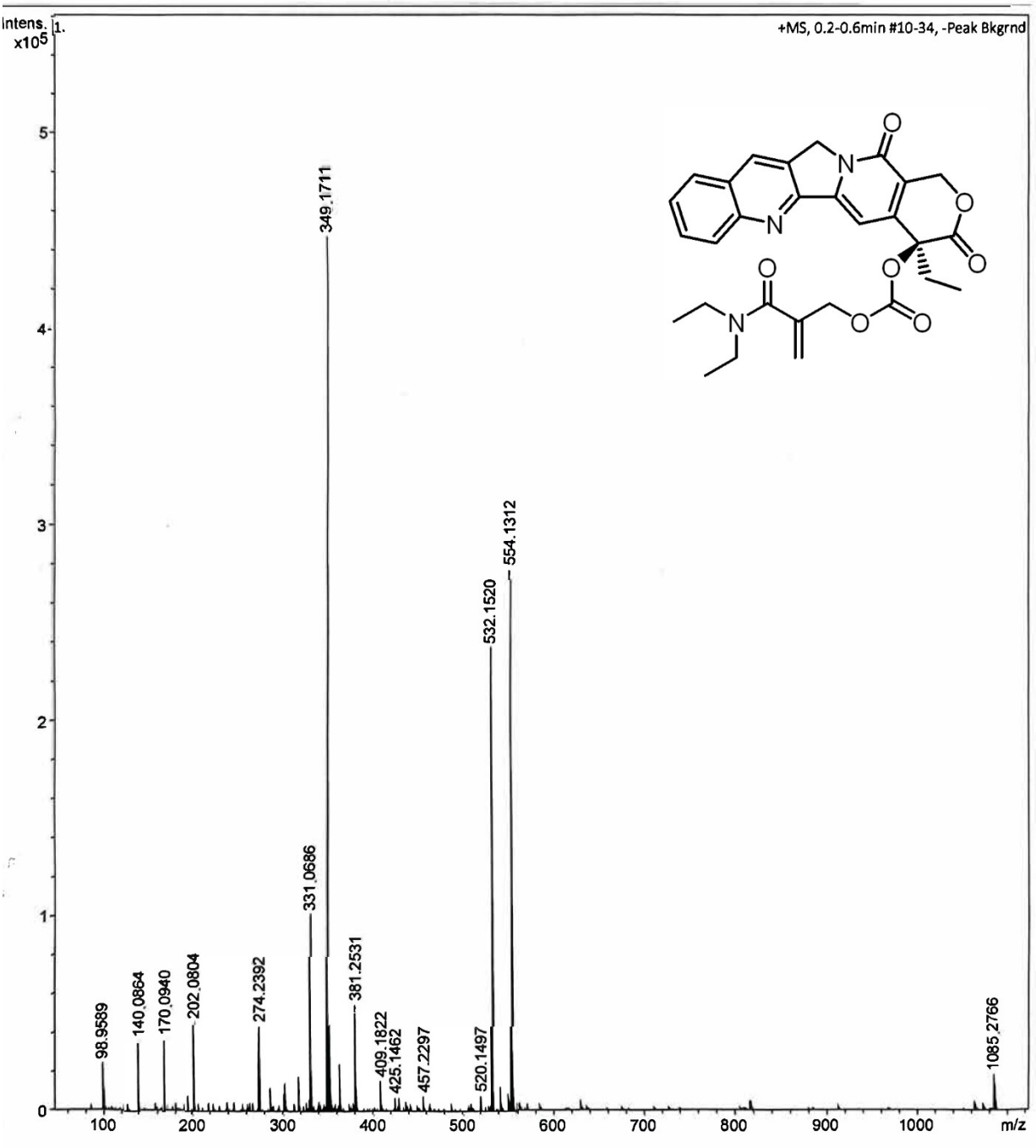
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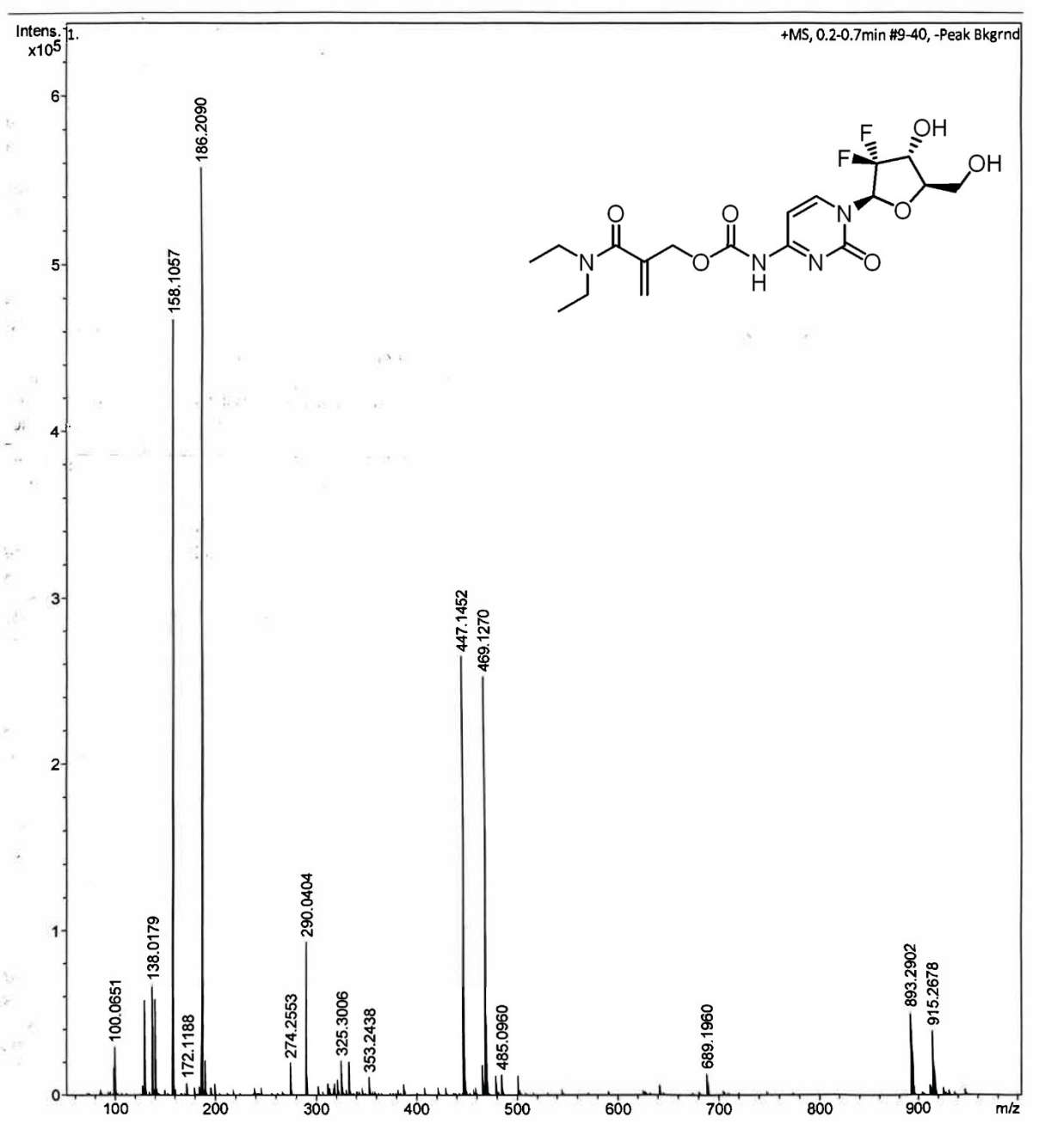
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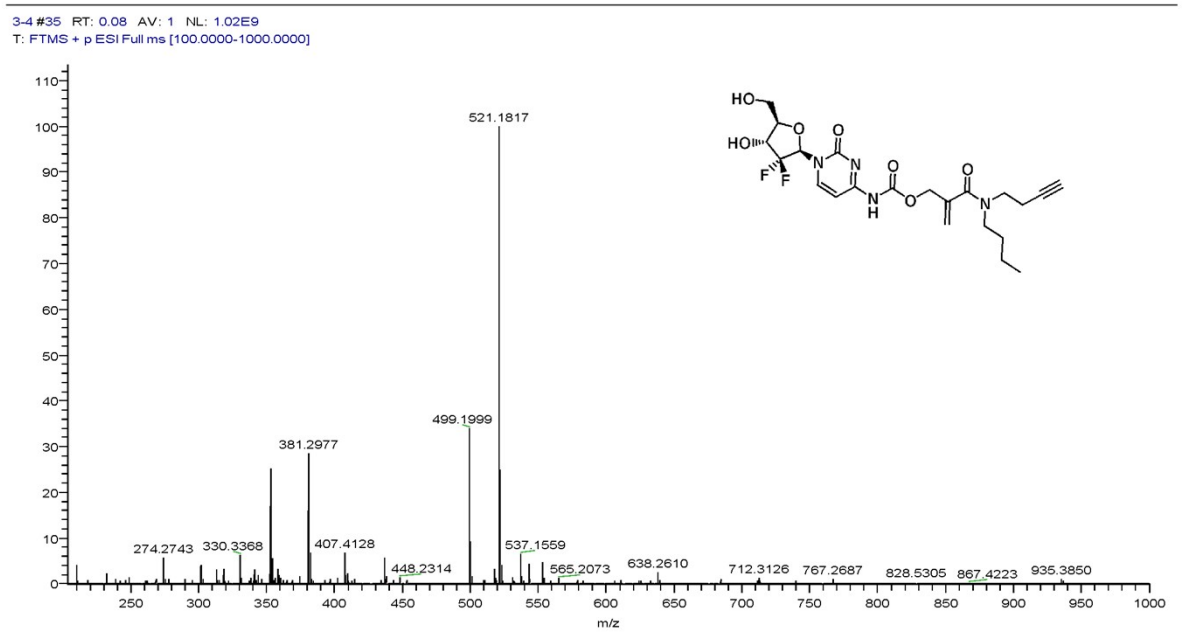
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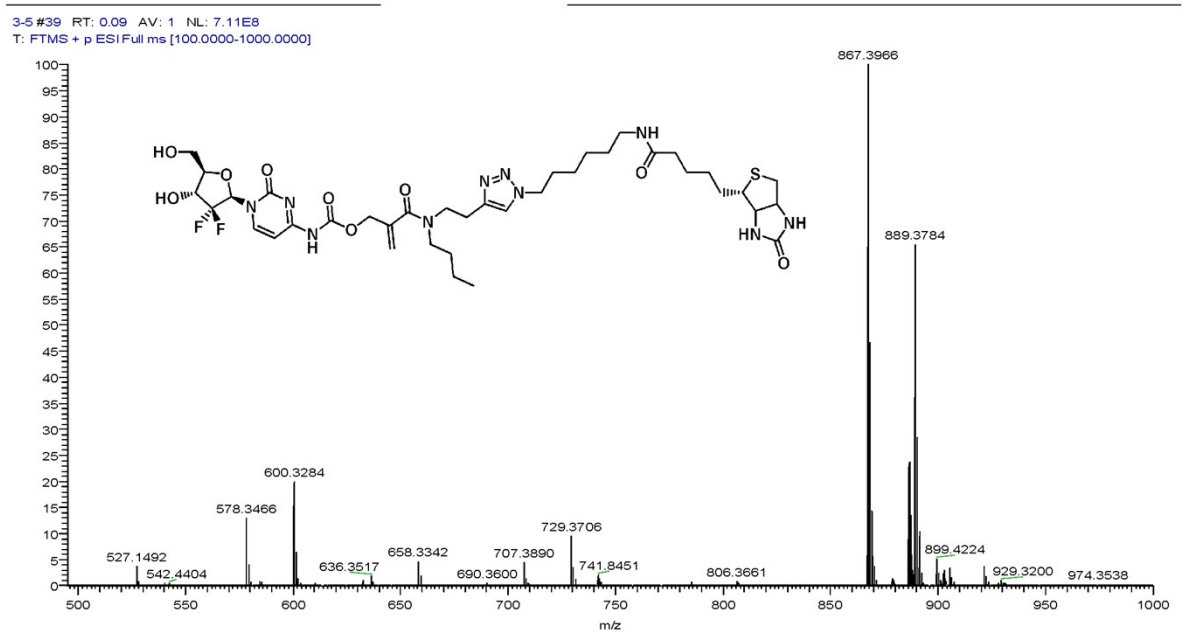
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