SUPPORTING INFORMATION

Combination of chemotherapy and oxidative stress to enhance cancer cell apoptosis

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1. Supplementary Results.

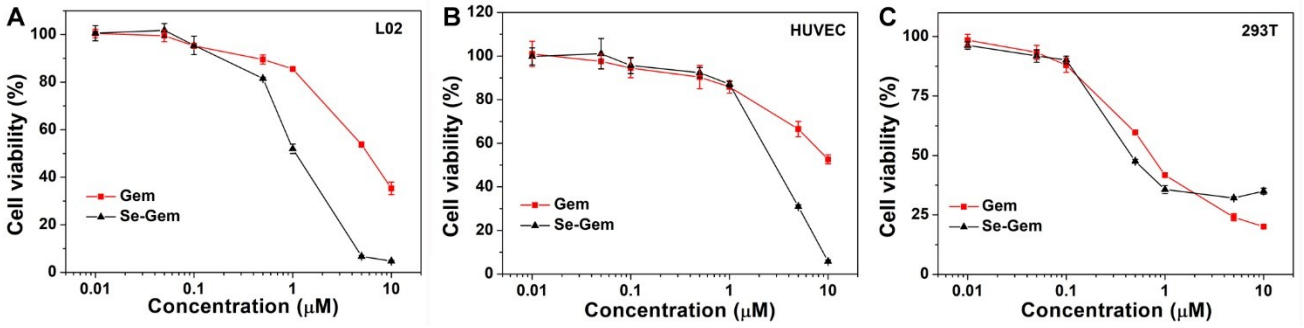


Figure S1. Cytotoxicity of Gem and Se-Gem on noncancerous cells. Compounds were incubated with cells for 96 h, and the cytotoxicity was determined by the MTT assay. Data are expressed as mean ± SE from triplicates, and all are expressed as the percentage of the control (cells treated with DMSO only). The IC_{50} values for Se-Gem to the three cell lines are 1.09, 2.93 and 0.45 μM, respectively.

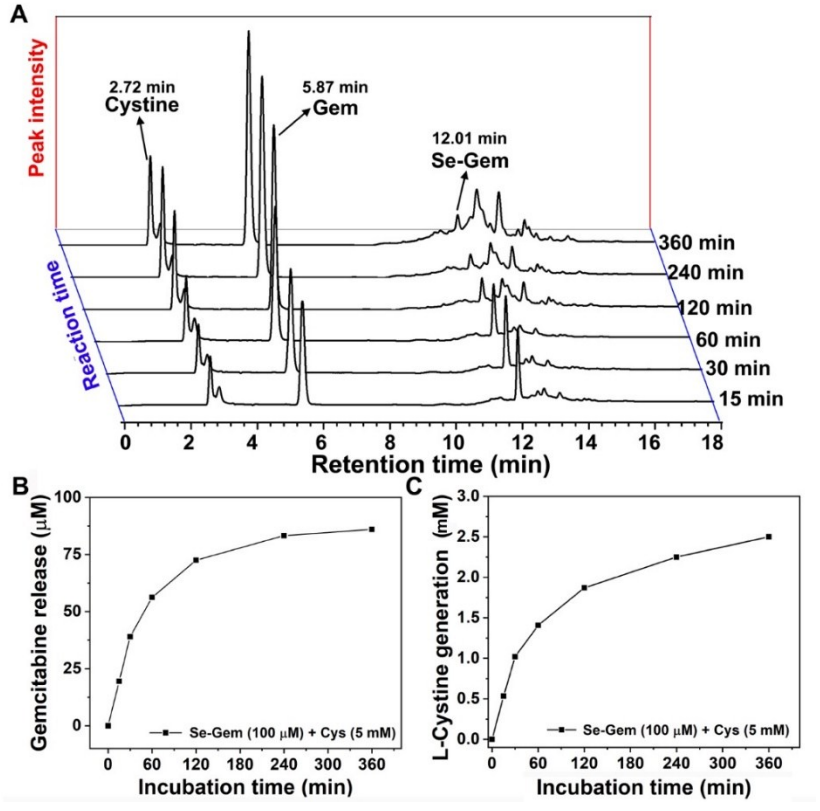


Figure S2. Cys-mediated Gem release from Se-Gem. (A) Se-Gem (100 μM) was incubated with Cys (5 mM) in TE buffer at 37 °C under air condition, and the reaction mixture was analyzed by HPLC at the indicated time points. Quantification of the time-dependent release of Gem and generation of cystine was shown in (B) and (C).

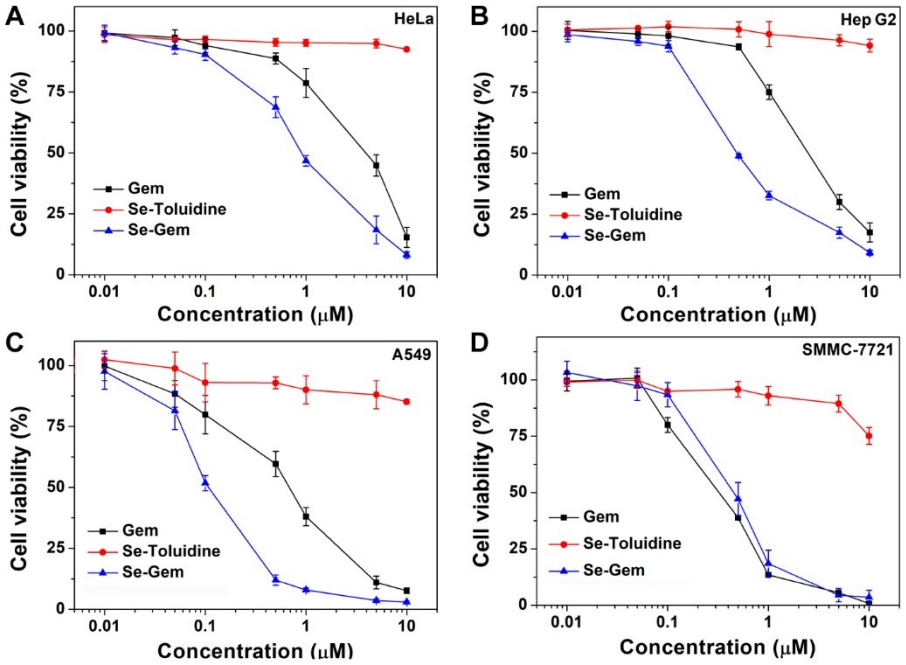


Figure S3. Cytotoxicity of Gem, Se-Gem and Se-Toluidine. Compounds were incubated with cells for 96 h, and the cytotoxicity was determined by the MTT assay. Data are expressed as mean ± SE from triplicates, and all are expressed as the percentage of the control (cells treated with DMSO only).

2. Experimental Section.

Materials and Instruments. The recombinant rat TrxR1 was a gift from Prof. Arne Holmgren at Karolinska Institute, Sweden. Dulbecco's modified Eagle's medium (DMEM), GSH, GSSG, dimethyl sulfoxide (DMSO) and yeast GR were obtained from Sigma-Aldrich (St. Louis, USA). NADPH was obtained from Roche (Mannheim, Germany). Fetal bovine serum (FBS) was obtained from Sijiqing (Hangzhou, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), penicillin, and streptomycin were obtained from Sangon (Shanghai, China). DCFH-DA and DHE were products of Santa Cruz Biotech (Santa Cruz, USA). Bovine Serum Albumin (BSA) and phenylmethylsulfonyl fluoride (PMSF) were obtained from Beyotime (Nantong, China). All other reagents were of analytical grade and were purchased from commercial supplies. Absorption spectra were recorded on an evolution 200 UV−vis spectrometer (Thermo Scientific). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 400, and tetramethylsilane (TMS) was used as a reference. MS spectra were recorded on Bruker Daltonics esquire 6000 mass spectrometer or Shimadzu LCMS-2020. HRMS was obtained on Orbitrap Elite (Thermo Scientific). HPLC analysis were performed on Shimadzu LCMS-2020 system with a Wondasil C18 Superb reversed-phase column (5 μm, 4.6 × 150 mm). The column was eluted with methanol/water, and the detailed information on the eluent composition was given in the section of compound characterization. The flow rate was set at 0.6 mL min^{-1}. A PDA detector was used to monitor the products at 270 nm. Gem and its derivatives were dissolved in DMSO to prepare stock solutions. The DMSO concentrations in all cell experiments and other experiments are 0.1% (v/v) and 0.5%, respectively.

Compounds Purity Analysis. All final compounds were analyzed by HPLC to determine their purity. The analyses were performed on Shimadzu LCMS-2020 system with a Wondasil C18 Superb reversed-phase column (5 μm, 4.6 × 150 mm) at room temperature. The column was eluted with methanol/water, and the flow rate was set at 0.6 mL min^{-1}. The tested compounds were dissolved in methanol, and the injection volume is 10 μL. The maximal absorbance at the range of 254-300 nm was used as the detection wavelength.

Cell Lines and Culture Conditions. The cancerous cell lines, *i.e.*, Hep G2 cells, HeLa cells, A549 cells and SMMC-7721 cells, and the non-cancerous cell lines, *i.e.*, 293T cells,

and L02 and HUVEC cells were obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were kept in DMEM with 10% FBS, 2 mM glutamine and 100 units/ml penicillin/streptomycin, and maintained in a humidified atmosphere of 5% CO_2 at 37 ^oC. The tested compounds were dissolved in DMSO to prepare stock solutions, and the final concentration of DMSO in all the cell experiments is 0.5 % (V/V).

Synthesis of Target Compounds.

Synthesis of 1,2-diselenolan-4-ol. This compound was synthesized according to our previous publication.[1]

Synthesis of Se-Toluidine. p-Tolyl isocyanate (666 mg, 5 mmol), pyridine (435 mg, 5.5 mmol) and 1,2-diselenolan-4-ol (1.09 g, 5 mmol) were dissolved in 50 mL of distilled toluene and stirred at 100 ^oC for 1 h. After cooled to room temperature, a large amount of yellow solid was precipitated, collected by filtration, and washed with toluene to give compound Se-Toluidine (1.55 g, 89% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 6.74 (s, 1H), 6.29 (m, 1H), 3.60-3.57 (m, 2H), 3.49-3.45 (m, 2H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.2 (C), 134.7 (C), 133.3 (C), 129.6 $(CH)_2$, 118.7 $(CH)_2$, 80.1 (CH), 36.2 $(CH_2)_2$, 20.7 (CH_3); HRMS (ESI) calculated for $[C_{11}H_{14}NO_2Se_2]^+$ $(M+H^+)$ requires m/z = 351.9349, found 351.9343; purity 96.4% ($MeOH/H_2O$ = 40/60, R_t = 6.149 min).

*Synthesis of Compound **7**.* This compound was synthesized according to the literature.[2] ^1H NMR (400 MHz, DMSO-d_6) δ 5.40 (d, J = 6.4 Hz, 2H), 4.26-4.23 (m, 2H), 4.13-4.09 (m, 2H), 3.80-3.76 (m, 2H), 3.18 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 71.7 $(CH)_2$, 69.1 $(CH_2)_2$, 37.3 $(CH_3)_2$; ESI-MS (m/z): $[M+H]^+$ 279.1.

Synthesis of DSTox.[3] Selenium powder (790 mg, 10 mmol) and naphthalene (1.28 g, 10 mmol) were dispersed in 50 mL of anhydrous tetrahydrofuran (THF). The resulting mixture was stirred at room temperature under argon. Freshly shaved sodium metal (230 mg, 10 mmol) was then added to the mixture under argon. The reaction mixture was allowed to stir for 2 h to enable consumption of all the sodium metal. Compound **7** (1.4 g, 5 mmol) was dissolved in 10 mL of anhydrous THF, and the resulting solution was added to the reaction mixture under argon. After 30 min, the reaction mixture was filtered, evaporated under reduced pressure. Chromatography of the residue on silica gel with a mixture of petroleum ether/ethyl acetate (1/1) gave DSTox as a yellow solid (134 mg, 11% yield). ^1H NMR (400

MHz, DMSO-d_6) δ 5.19 (d, *J* = 4.0 Hz, 2H), 3.39-3.35 (m, 4H), 3.06-3.00 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 74.4 $(CH)_2$, 31.6 $(CH_2)_2$; EI-MS m/z (%): 248 (M+, 90), 246 (82), 244 (52), 204 (40), 202 (35), 160 (83), 158 (76), 87 (80), 70 (82), 44 (56), 43 (100).

Synthesis of Bn-DSTox. DSTox (1.0 g, 4 mmol) and benzyl bromide (524 μL, 4.4 mmol) were dissolved in 25 mL of 2-methyl-THF and stirred at room temperature. A solution of KOH (5 M in water) (6 mL, 30 mmol) was added to the reaction mixture, followed by the addition of tetrabutylammonium hydrogen sulfate (TBAHS) (340 mg, 1 mmol). The reaction mixture was stirred at ambient temperature overnight. The resulting mixture was diluted with ethyl acetate (100 mL) and washed with brine (3×50 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ ethyl acetate = 10/1) to afford Bn-DSTox as a yellow solid (55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.31 (m, 5H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 3.83-3.77 (m, 1H), 3.58-3.53 (m, 1H), 3.48-3.42 (m, 2H), 3.34-3.20 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.4 (C), 128.6 $(CH)_2$, 128.1 (CH), 127.8 $(CH)_2$, 82.8 (CH), 73.3 (CH), 71.3 (CH_2), 30.3 (CH_2), 26.8 (CH_2); ESI-MS (m/z): $[M+Na]^+$ 360.9.

Synthesis of DTTox. This compound was synthesized according to previous publication.[1, 3]

Synthesis of Bn-DTTox. This compound was synthesized according to the literature.[4] DTTox (600 mg, 4 mmol) and benzyl bromide (524 μL, 4.4 mmol) were dissolved in 25 mL of 2-methyl-THF and stirred at room temperature. A solution of KOH (5 M in water) (6 mL, 30 mmol) was added to the reaction mixture, followed by the addition of tetrabutylammonium hydrogen sulfate (TBAHS) (340 mg, 1 mmol). The reaction mixture was stirred at ambient temperature overnight. The resulting mixture was diluted with ethyl acetate (100 mL) and washed with brine (3×50 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ ethyl acetate = 10/1) to afford Bn-DTTox as a light yellow solid (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.33 (m, 5H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.52 (d, *J* = 11.2 Hz, 1H), 3.78-3.73 (m, 1H), 3.53-3.47 (m, 1H), 3.18-3.10 (m, 2H), 3.02-3.00 (m, 2H), 2.96-2.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 129.3 (C), 128.6 $(CH)_2$, 128.1

$(CH)_2$, 127.9 (CH), 81.0 (CH), 72.5 (CH), 71.7 (CH_2), 40.9 (CH_2), 37.6 (CH_2); ESI-MS (m/z): $[M-H]^-$ 240.9.

Synthesis of TBSGem, S-Gem and C-Gem. These compounds were synthesized according to our previous publication.[5] The purity of S-Gem and C-Gem was determined to be 97.8% and 96.5%, respectively.

General Procedure for Synthesis of Se-TBSGem, C6-TBSGem, S6-TBSGem and Se6-TBSGem. TBSGem (490 mg, 1 mmol) and pyridine (320 μL, 4 mmol) were dissolved in 20 mL of distilled dichloromethane (DCM). The reaction mixture was cooled to 0 °C, then triphosgene (330 mg, 1.1 mmol) was added and stirred at 0 °C for another 3 h. The solvent was removed under reduced pressure and the residue was dissolved in 20 mL of distilled toluene. To the result solution was added compound 1,2-diselenolan-4-ol, cyclohexanol, Bn-DTTox or Bn-DSTox (1 mmol) and heated at 100 °C for 1 h. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with brine (3×100 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (petroleum ether/ ethyl acetate = 3/1) to afford the target molecules.

Se-TBSGem. a brownish-red solid, 45% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, *J* = 7.2 Hz, 1H), 7.18 (d, *J* = 6.8 Hz, 1H), 6.33 (d, *J* = 7.2 Hz, 1H), 6.10 (s, 1H), 4.37-4.29 (m, 1H), 4.02 (d, *J* = 11.2 Hz, 1H), 3.95 (d, *J* = 7.6 Hz, 1H), 3.81 (d, *J* = 11.2 Hz, 1H), 3.56-3.46 (m, 4H), 0.95 (s, 9H), 0.90 (s, 9H), 0.13 (s, 9H), 0.10 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2 (C), 154.2 (C), 151.9 (C), 143.8 (CH), 121.8 (t, J_{C-F} = 259.0, C), 95.3 (CH), 84.5 (t, J_{C-F} = 41.0, CH), 82.0 (CH), 81.3 (CH), 69.4 (t, J_{C-F} = 27.0, CH), 59.9 (CH_2), 34.8 (CH_2), 34.7 (CH_2), 25.7 $(CH_3)_3$, 25.4 $(CH_3)_3$, 18.2 (C), 17.9 (C), -4.85 (CH_3, $Si(CH_3)_2$), -5.40 (CH_3, $Si(CH_3)_2$), -5.56 (2 CH_3, $Si(CH_3)_2$); ESI-MS (m/z): $[M-H]^-$ 734.1.

C6-TBSGem. a white solid, 84% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.0 Hz, 1H), 7.53 (s, 1H), 7.23 (d, *J* = 8.0 Hz, 1H), 6.36-6.33 (m, 1H), 4.76 (t, *J* = 4.0 Hz, 1H), 4.35-4.29 (m, 1H), 4.03-4.00 (m, 1H), 3.96-3.94 (m, 1H), 3.82-3.79 (m, 1H), 1.92-1.89 (m, 2H), 1.76-1.73 (m, 2H), 1.56-1.25 (m, 6H), 0.95 (s, 9H), 0.90 (s, 9H), 0.13-0.96 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8 (C), 154.6 (C), 152.0 (C), 143.6 (CH), 121.9 (t, J_{C-F} = 259.0, C), 95.1 (CH), 84.5 (t, J_{C-F} = 41.0, CH), 81.3 (CH), 75.3 (CH), 69.4 (t, J_{C-F} = 27.0, CH), 59.9 (CH_2),

31.4 (CH_2)$_2$, 25.8 (CH_3)$_3$, 25.4 (CH_3)$_3$, 25.1 (CH_2), 23.6 (CH_2)$_2$, 18.2 (C), 17.9 (C), -4.86 (CH_3, Si(CH_3)$_2$), -5.38 (CH_3, Si(CH_3)$_2$), -5.54 (2 CH_3, Si(CH_3)$_2$); ESI-MS (m/z): $[M+Na]^+$ 640.6.

S6-TBSGem. a light yellow solid, 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.6 Hz, 1H), 7.89 (s, 1H), 7.31-7.25 (m, 5H), 7.19 (d, J = 7.6 Hz, 1H), 6.37-6.34 (m, 1H), 5.01-4.97 (m, 1H), 4.66 (m, 1H), 4.50 (m, 1H), 4.40-4.32 (m, 1H), 4.40-3.96 (m, 2H), 3.83-3.80 (m, 1H), 3.64-3.58 (m, 1H), 3.22-3.17 (m, 2H), 3.11-3.02 (m, 2H), 0.95 (s, 1H), 0.92 (s, 9H), 0.13 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0 (C), 154.3 (C), 151.7 (C), 143.8 (CH), 137.5 (C), 128.3 (CH)$_2$, 127.9 (CH), 127.7 (CH)$_2$, 122.0 (t, $J_{C\text{-}F}$ = 259.0, C), 95.1 (CH), 84.6 (t, $J_{C\text{-}F}$ = 40.0, CH), 81.4 (CH), 81.3 (CH), 72.4 (CH_2), 69.4 (t, $J_{C\text{-}F}$ = 27.0, CH), 59.9 (CH_2), 38.7 (CH_2), 38.4 (CH_2), 29.8 (CH_2), 25.8 (CH_3)$_3$, 25.4 (CH3)$_3$, 18.2 (C), 17.9 (C), -4.81 (CH_3, Si(CH_3)$_2$), -5.34 (CH_3, Si(CH_3)$_2$), -5.53 (2 CH_3, Si(CH_3)$_2$); ESI-MS (m/z): $[M+H]^+$ 760.4.

Se6-TBSGem. a yellow solid, 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.6 Hz, 1H), 8.06 (s, 1H), 7.34-7.24 (m, 5H), 7.20-7.17 (m, 1H), 6.36 (d, J = 10.4 Hz, 1H), 5.05 (m, 1H), 4.69-4.65 (m, 1H), 4.55-4.47 (m, 1H), 4.38-4.32 (m, 1H), 4.15-3.96 (m, 2H), 3.83-3.80 (m, 1H), 3.68-3.65 (m, 1H), 3.53-3.36 (m, 4H), 0.95 (s, 9H), 0.91 (s, 9H), 0.13 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0 (C), 154.3 (C), 151.6 (C), 143.8 (CH), 137.6 (C), 129.0 (CH)$_2$, 128.5 (CH), 127.9 (CH)$_2$, 121.9 (t, $J_{C\text{-}F}$ = 259.0, C), 95.1 (CH), 84.6 (t, $J_{C\text{-}F}$ = 40.0, CH), 81.4 (CH), 81.3 (CH_2), 72.4 (CH)$_2$, 69.4 (t, $J_{C\text{-}F}$ = 26.0, CH), 59.9 (CH_2), 28.9 (CH_2), 28.3 (CH_2), 25.8 (CH_3)$_3$, 25.5 (CH_3)$_3$, 18.3 (C), 17.9 (C), -4.80 (CH_3, Si(CH_3)$_2$), -5.34 (CH_3, Si(CH_3)$_2$), -5.52 (2 CH_3, Si(CH_3)$_2$); ESI-MS (m/z): $[M-H]^-$ 854.1.

General Procedure for Synthesis of Se-Gem, C6-Gem, S6-Gem and Se6-Gem. To a solution of Se-TBSGem, C6-TBSGem, S6-TBSGem or Se6-TBSGem (0.4 mmol) in 50 mL of distilled THF was added a 1 M solution of TBAF in THF (1.2 mL, 1.2 mmol). The solution was stirred at ambient temperature for 30 min. The solvent was removed under reduced pressure, the residue was dissolved in 100 mL of ethyl acetate and washed with brine (3×100 mL). The organic phase was separated, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH = 20/1) to afford the target molecules.

Se-Gem. a brownish-red solid, 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.9 (s, 1H), 8.20 (d, J = 7.6 Hz, 1H) 7.02 (d, J = 7.6 Hz, 1H), 6.37 (s, 1H), 6.15 (t, J = 7.2 Hz, 1H), 6.05-

6.03 (m, 1H), 5.35 (s, 1H), 4.20-4.15 (m, 1H), 3.89-3.87 (m, 1H), 3.81-3.78 (m, 1H), 3.66-3.63 (m, 1H), 3.51-3.50 (m,4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.8 (C), 154.5 (C), 152.9 (C), 144.8 (CH), 123.5 (t, J_{C-F} = 257.0, C), 95.9 (CH), 84.6 (t, J_{C-F} = 33.0, CH), 81.9 (CH), 81.5 (CH), 68.8 (t, J_{C-F} = 22.0, CH), 59.3 (CH_2), 35.9 $(CH_2)_2$; ESI-MS (m/z): $[M+H]^+$ 508.0; HRMS (ESI) calculated for $[C_{13}H_{15}F_2N_3O_6Se_2Na]^+$ ($M+Na^+$) requires *m/z* = 529.9152, found 529.9142; purity 96.6% (MeOH/H_2O = 40/60, R_t = 7.48 min).

C6-Gem. a white solid, 88% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.8 (s, 1H), 8.21 (d, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 6.33 (d, *J* = 6.4 Hz, 1H), 6.16 (t, *J* = 7.2 Hz, 1H), 5.31 (t, *J* = 5.4 Hz, 1H), 4.68-4.12 (m, 1H), 3.90-3.86 (m, 1H), 3.79-3.78 (m, 1H), 3.67-3.63 (m, 1H), 3.17-3.16 (m, 1H), 1.86-1.83 (m, 2H), 1.71-1.69 (m, 2H), 1.51-1.23 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0 (C), 154.6 (C), 153.2 (C), 144.8 (CH), 123.5 (t, J_{C-F} = 257.0, C), 95.5 (CH), 84.6 (t, J_{C-F} = 31.0, CH), 81.5 (CH), 74.3 (CH), 68.9 (t, J_{C-F} = 23.0, CH), 59.3 (CH_2), 31.7 $(CH_2)_2$, 25.4 (CH_2), 23.7 $(CH_2)_2$; ESI-MS (m/z): $[2M-H]^-$ 777.2; HRMS (ESI) calculated for $[C_{16}H_{22}F_2N_3O_6]^+$ ($M+H^+$) requires *m/z* = 390.1471, found 390.1459; purity 99.8% (MeOH/H_2O = 40/60, R_t = 9.055 min).

S6-Gem. a light yellow solid, 67% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.28-7.22 (m, 5H), 7.07 (d, *J* = 7.6 Hz, 1H), 6.34-6.31 (m, 1H), 6.19-6.16 (m, 1H), 5.31-5.28 (m, 1H), 4.85-4.84 (m, 1H), 4.71-4.68 (m, 1H), 4.59-4.55 (m, 1H), 4.22-4.17 (m, 1H), 3.90-3.88 (m, 1H), 3.80 (m, 1H), 3.67-3.61 (m, 2H), 3.49-3.45 (m, 1H), 3.17-3.16 (m, 1H), 3.06-2.93 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.0 (C), 154.6 (C), 153.0 (C), 145.0 (CH), 138.7 (C), 128.8 $(CH)_2$, 128.2 $(CH)_3$, 123.6 (t, J_{C-F} = 256.0, C), 95.6 (CH), 84.8 (t, J_{C-F} = 31.0, CH), 81.6 (CH), 79.1 (CH_2), 72.1 (CH), 68.9 (t, J_{C-F} = 23.0, CH), 60.4 (CH), 59.3 (CH_2), 38.7 (CH_2), 21.4 (CH_2); ESI-MS (m/z): $[M+H]^+$ 532.2; HRMS (ESI) calculated for $[C_{21}H_{24}F_2N_3O_7S_2]^+$ ($M+H^+$) requires *m/z* = 532.1018, found 532.1006; purity 99.5% (MeOH/H_2O = 40/60, R_t = 4.525 min).

Se6-Gem. a yellow solid, 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.27-7.21 (m, 5H), 7.06 (d, *J* = 7.6 Hz, 1H), 6.34-6.31 (m, 1H), 6.20-6.16 (m, 1H), 5.32-5.30 (m, 1H), 4.93-4.86 (m, 1H), 4.70-4.67 (m, 1H), 4.57-4.53 (m, 1H), 4.22-4.17 (m, 1H), 3.91-3.88 (m, 1H), 3.82-3.74 (m, 2H), 3.68-3.58 (m, 3H), 3.30-3.19 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 163.9 (C), 154.5 (C), 152.8 (C), 144.8 (CH), 138.6 (C),

128.6 $(CH)_2$, 128.5 (CH), 128.1 $(CH)_2$, 123.4 (t, J_{C-F} = 257.0, C), 95.4 (CH), 84.3 (t, J_{C-F} = 31.0, CH), 81.4 (CH), 80.2 (CH_2), 76.5 (CH), 71.9 (CH), 68.7 (t, J_{C-F} = 21.0, CH), 59.2 (CH_2), 29.5 (CH_2), 26.3 (CH_2); ESI-MS (m/z): $[M-H]^-$ 626.0; HRMS (ESI) calculated for $[C_{21}H_{23}F_2N_3O_7Se_2Na]^+$ ($M+Na^+$) requires *m/z* = 649.9727, found 649.9725; purity 95.2% (MeOH/H_2O = 40/60, R_t = 4.06 min).

Reduction of Prodrugs by GSH/GR. GR (0.5 U/mL), GSH (5 mM) and NADPH (200 μM) were incubated in TE buffer (50mmM Tris/1 mM EDTA, pH 7.4) at 37 °C. The decrease of absorbance at 340 nm was recorded for the initial 5 minutes, followed by immediately adding the individual prodrug (100 μM). The decrease of absorbance at 340 nm, due to the oxidation of NADPH to $NADP^+$, was recorded for another 10 minutes. The tested prodrugs were dissolved in DMSO, and the final concentration of DMSO is 1 % (V/V) in all reaction mixtures.

Reduction of Se-Gem by GSH under Anaerobic and Aerobic Conditions. The anaerobic reaction between GSH and Se-Gem was performed in a cuvette equipped with a rubber stopper. To the cuvette was added 995 μL of TE buffer containing GR (0.5 U/mL), GSH (5 mM) and NADPH (200 μM). Then the mixture in the cuvette was bubbled with argon for 10 min to generate anaerobic atmosphere. The decrease of absorbance at 340 nm was recorded for the initial 5 minutes, followed by adding 5 μL of Se-Gem (20 mM, the final concentration was 100 μM) through a microsyringe. The decrease of absorbance at 340 nm was recorded. When the decrease of NADPH reached a platform, the stopper was removed and the mixture in the cuvette was bubbled with air. The decrease of absorbance at 340 nm was recorded.

Reduction of Se-Gem by TrxR/Trx. TrxR (50 or 100 nM)/NADPH (200 μM) or TrxR (100 nM)/Trx (10 μM)/NADPH (200 μM) were incubated in TE buffer at 37 °C. The decrease of absorbance at 340 nm was recorded for the initial 5 minutes, followed by immediately adding Se-Gem or S-Gem (100 μM). The decrease of absorbance at 340 nm was recorded for another hour. S-Gem, a substrate of TrxR, was used as a control molecule. The tested prodrugs were dissolved in DMSO, and the final concentration of DMSO is 1 % (V/V) in all reaction mixtures.

GSH-mediated Release of Gem from Se-Gem. Se-Gem (100 μM) was incubated with

GSH (5 mM) in TE buffer at 37 °C. The reaction crude was analyzed at 15, 30, 60, 120, 240 and 360 min by HPLC using PDA (270 nm) and MS detectors. Eluent A, MeOH; eluent B, water; 0-4 min, A/B = 7.5/92.5; 4-8 min, A/B = 7.5/92.5 to 95/5; 8-16 min, A/B = 95/5; 16-20 min, A/B = 95/5 to 7.5/92.5； Flow rate = 0.6 mL min^{-1}.

Cys-mediated Release of Gem from Se-Gem. Se-Gem (100 μM) was incubated with Cys (5 mM) in TE buffer at 37 °C. The reaction crude was analyzed at 15, 30, 60, 120, 240 and 360 min by HPLC using PDA (270 nm) detectors. Eluent A, MeOH; eluent B, water; 0-4 min, A/B = 7.5/92.5; 4-8 min, A/B = 7.5/92.5 to 95/5; 8-16 min, A/B = 95/5; 16-20 min, A/B = 95/5 to 7.5/92.5； Flow rate = 0.6 mL min^{-1}.

Determination of Selenolate Intermediate in Reaction Crude by Sel-green. Sel-green is a selenolate fluorescent probe developed by our group.[6] In brief, Se-Gem (100 μM) was incubated with GSH (5 mM) in TE buffer at 37 °C overnight. Then the reaction crude (250 μL) was incubated with TE buffer containing Sel-green (10 μM) and GSH (1 mM) at 37 °C (to a final volume of 500 μL). The fluorescence increment (λ_{ex}=370 nm; λ_{em}=517 nm) was determined for 4 min. SeW, a synthesized diselenide compound was used as a standard sample to quantify the concentration of selenolate in the reaction mixture.

Cell Viability Assay. The cell viability was measured by the MTT assay. Unless otherwise noted, 2.5×10^3 cells were seeded in 96-well plates and allowed to attach for 12 h. Cells were then treated with varying concentrations of prodrugs for 96 h. Then the medium was removed, and 100 μL of the same medium containing MTT (0.5 mg mL^{-1}) was added to each well and incubated for an additional 4 h at 37 °C. An extraction buffer (100 μL, 10% SDS, 5% isobutanol, 0.1% HCl) was added, and the cells were incubated overnight at 37 °C. The absorbance was measured at 570 nm using a microplate reader (Thermo Scientific Multiskan GO, Finland).

Induction of Superoxide by Se-Gem. The superoxide production was determined by the cytochrome c reduction assay.[7] Briefly, GSH (100 μM) and cytochrome c (1 mg mL^{-1}) were incubated in TE at 37 °C and the absorbance spectra from 480 to 650 nm were recorded every 2 min for 6 min, followed by adding 20 μM of Se-Gem or S-Gem. The absorbance spectra from 480 to 650 nm were recorded every 2 min for another 8 min. Then SOD was added to reach a final amount of 150 units. The inhibition of the increment of the absorbance

at 550 nm after addition of SOD indicates the production of superoxide.

Assessment of Intracellular ROS. To a 12-well plate was seeded 2.5×10^4 Hep G2 cells per well and allowed to adhere overnight. The cells were incubated with Gem, S-Gem or Se-Gem for the indicated time. After removal of the medium, the ROS indicator DCFH-DA (10 μM) or DHE (10 μM) in fresh FBS-free medium was added and incubated for an additional 30 min at 37 °C. The fluorescence images were acquired by a FLoid Cell Imaging Station.

Measurement of Intracellular Total Thiols. After treatment of Hep G2 cells (2×10^5) with increasing concentrations of Se-Gem for 72 h in 60-mm dishes, the cells were collected, and washed twice with PBS. Total cellular proteins were extracted by RIPA buffer, and were quantified using the Bradford procedure. Total cellular thiols were measured by DTNB-titration. Briefly, cell lysate (20 μL) was added to cuvettes containing DTNB (1 mM in 80 μL of 6 M guanidine hydrochloride, pH 8.0). After incubation for 5 min at room temperature, the absorbance at 412 nm was read on a microplate reader. Total thiols were calculated from a calibration curve using GSH as the standard.

Determination of Intracellular GSH and GSSG. Determination and quantification of total glutathione and GSSG was based on the enzyme recycling method.[7] Cells (1×10^6) were treated with indicated concentrations of Se-Gem for 72 h in 100 mm dishes, the cells were collected and resuspended using ice-cold extraction buffer containing 0.1% Triton X-100 and 0.6% sulfosalicyclic acid in 0.1 M potassium phosphate buffer with 5 mM EDTA, pH 7.5 (KPE buffer). After sonication of the suspension in ice water for 2-3 min with vortexing every 30 s, the solution was centrifuged at 3000 g for 4 min at 4 ^{o}C, and the supernatant was immediately collected. To assay the total glutathione, a solution (120 μL) containing 1.66 GR (units mL^{-1}) and 0.33 DTNB (mg mL^{-1}) was added to each sample (20 μL). Then NADPH (60 μL of 0.66 mg mL^{-1}) was added and the absorbance at 412 nm was immediately read every 10 s for 2 min. GSSG was determined after GSH derivatization by 2-vinylpyridine. Briefly, 2 μL of 2-vinylpyridine was added to 100 μL of cell supernatant and mixed, then the reaction was allowed to take place for 1 h at room temperature in a fume hood. Finally, 6 μL of triethanolamine was added to the supernatant and the solution was mixed. Assay of GSSG was performed as described above for total glutathione. The amount of GSSG was subtracted from the total glutathione to give the GSH content.

Apoptosis assay. To 6-well plates were seeded 1×10^5 Hep G2 cells/well and allowed to adhere overnight, and then the cells were further incubated with the indicated concentrations of Gem or Se-Gem for 48 h. The cells were harvested and washed twice with PBS. Apoptotic cells, necrotic cells and live cells were identified by the PI and Annexin V-FITC double staining assay according to the manufacturer's instructions. After staining, the cells were determined by a FACSCanto™ flow cytometer (BD Biosciences, USA), and the data were analyzed with the CellQuest software.

Statistics. Comparisons among multiple groups were assessed by the one-way analysis of variance (ANOVA), followed by a post hoc Scheffe test. Statistical differences between two groups were analyzed by the Student's t-test. $p < 0.05$ was considered as the criterion for statistical significance.

References

1. X. Li, B. Zhang, C. Yan, J. Li, S. Wang, X. Wei, X. Jiang, P. Zhou and J. Fang, *Nature communications*, 2019, **10**, 2745.
2. S. Park, C. Anderson, R. Loeber, M. Seetharaman, R. Jones and N. Tretyakova, *Journal of the American Chemical Society*, 2005, **127**, 14355-14365.
3. J. C. Lukesh, 3rd, B. Vanveller and R. T. Raines, *Angewandte Chemie*, 2013, **52**, 12901-12904.
4. G. Butora, N. Qi, W. Fu, T. Nguyen, H. C. Huang and I. W. Davies, *Angewandte Chemie*, 2014, **53**, 14046-14050.
5. X. Li, Y. Hou, X. Meng, C. Ge, H. Ma, J. Li and J. Fang, *Angewandte Chemie*, 2018, **57**, 6141-6145.
6. B. Zhang, C. Ge, J. Yao, Y. Liu, H. Xie and J. Fang, *Journal of the American Chemical Society*, 2015, **137**, 757-769.
7. T. Liu, J. Zhang, X. Han, J. Xu, Y. Wu and J. Fang, *Free radical biology & medicine*, 2019, **135**, 216-226.

3. Original Spectra (NMR, MS & HPLC).

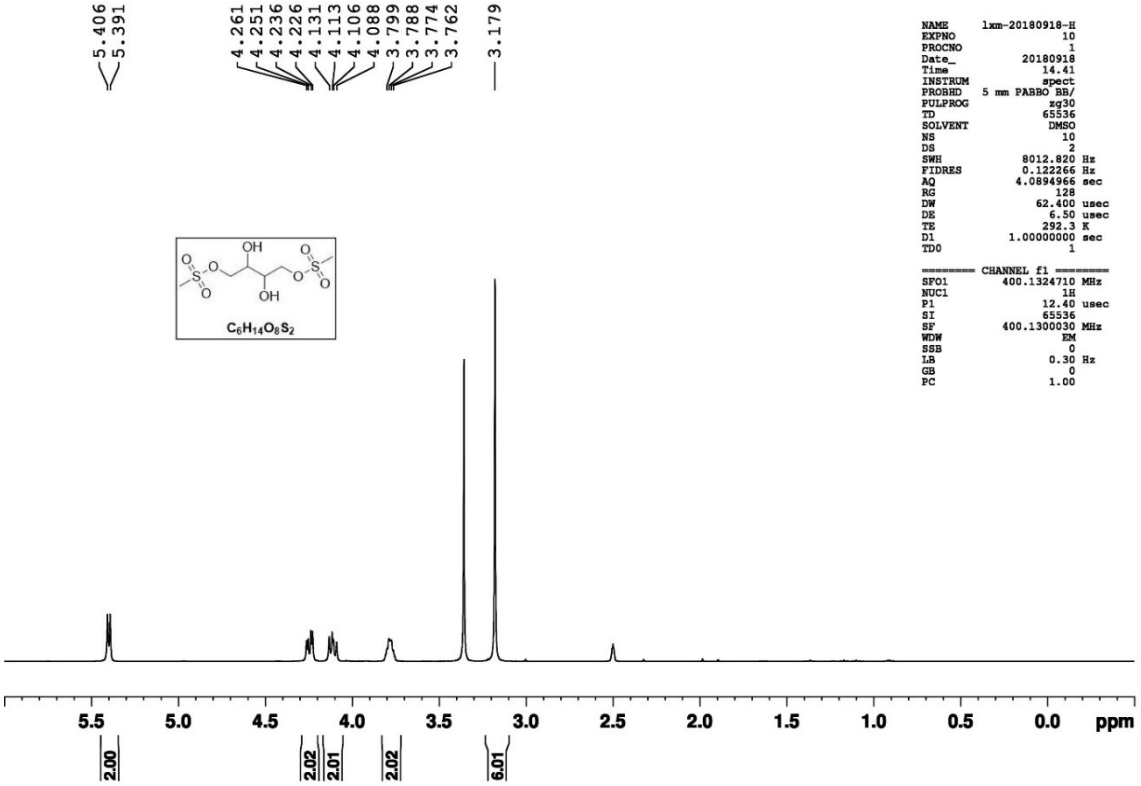


Figure S4. ^{1}H NMR Spectra of Compound **7** in DMSO-d_6 (400 MHz).

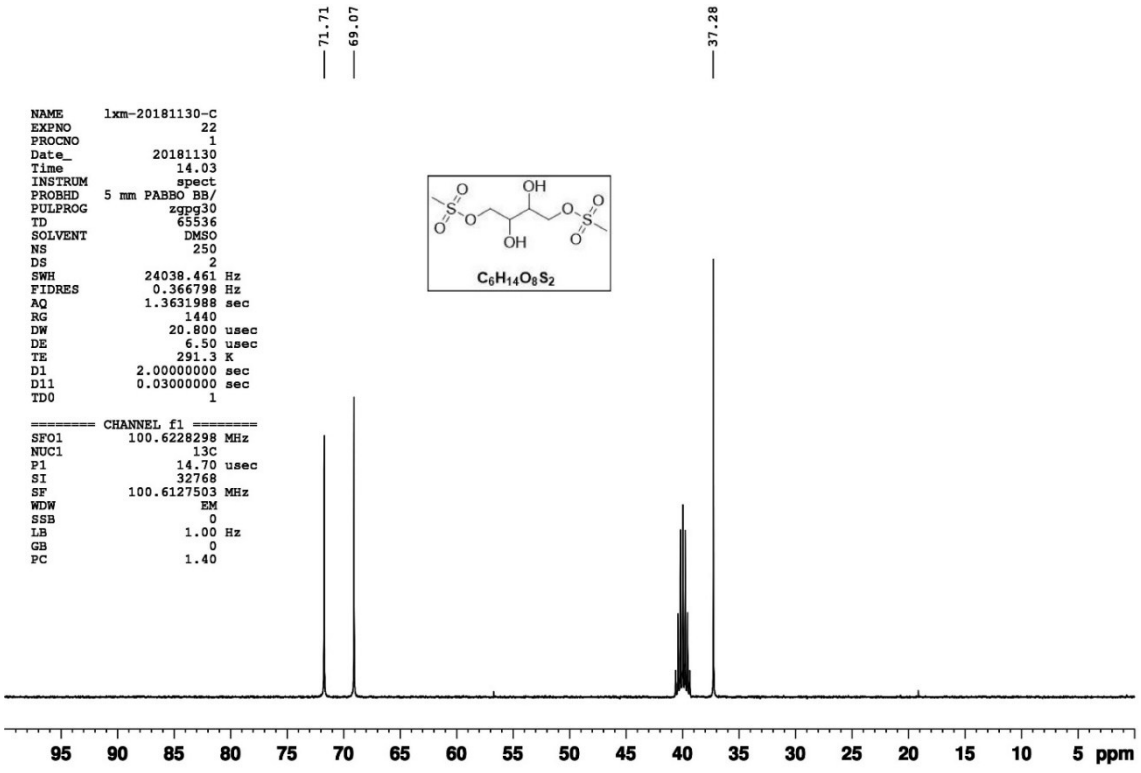


Figure S5. ^{13}C NMR Spectra of Compound **7** in DMSO-d_6 (100 MHz).

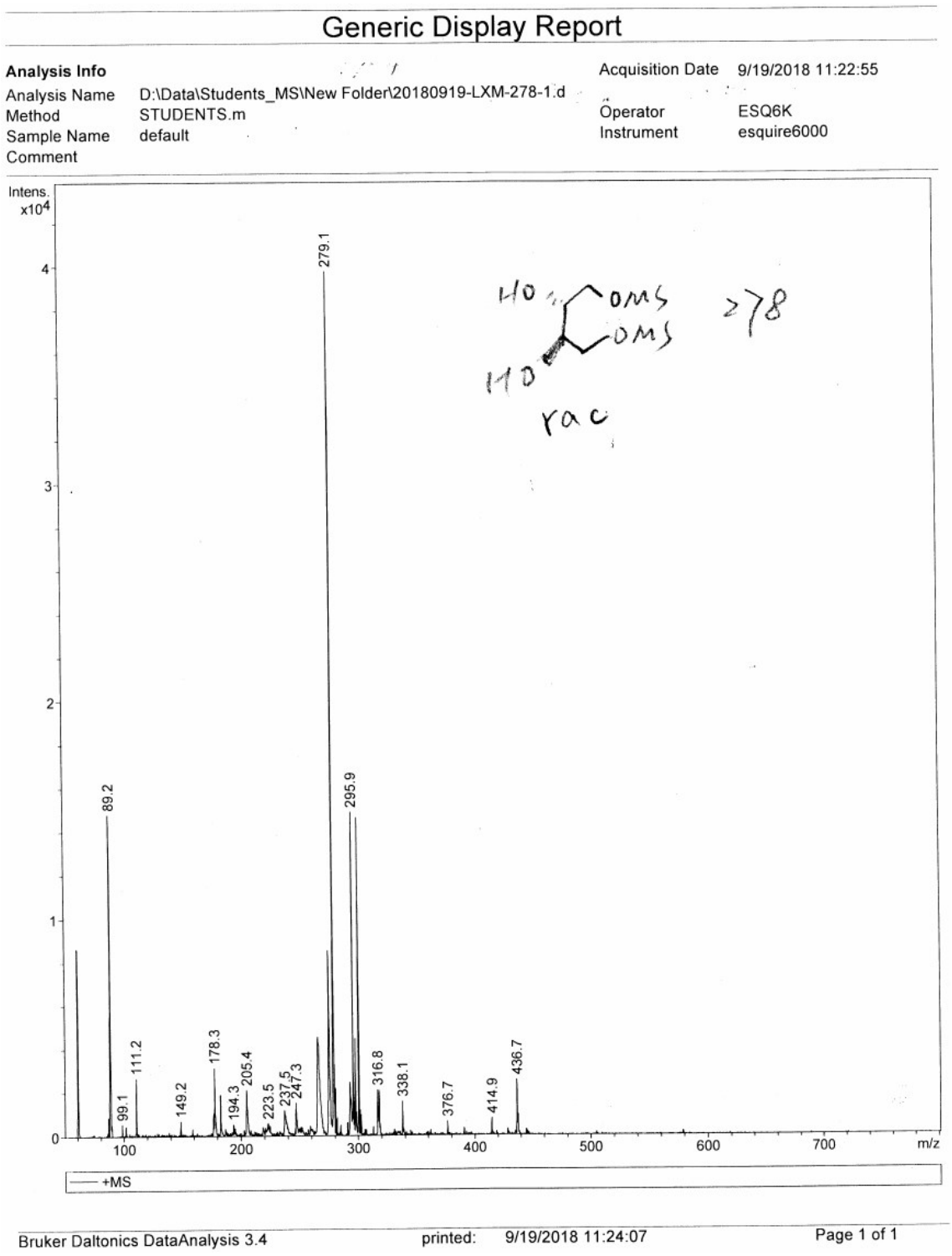


Figure S6. MS Spectra of Compound **7** (ESI).

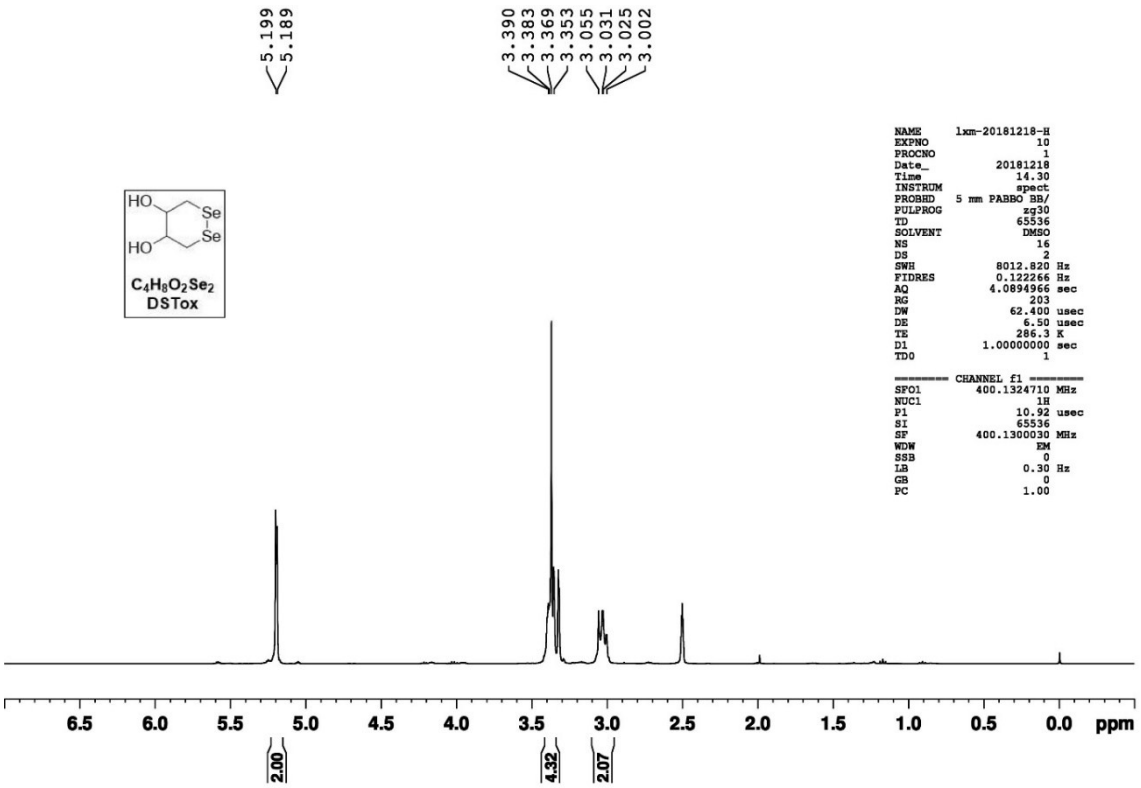


Figure S7. ^{1}H Spectra of **DSTox** in DMSO-d_6 (400 MHz).

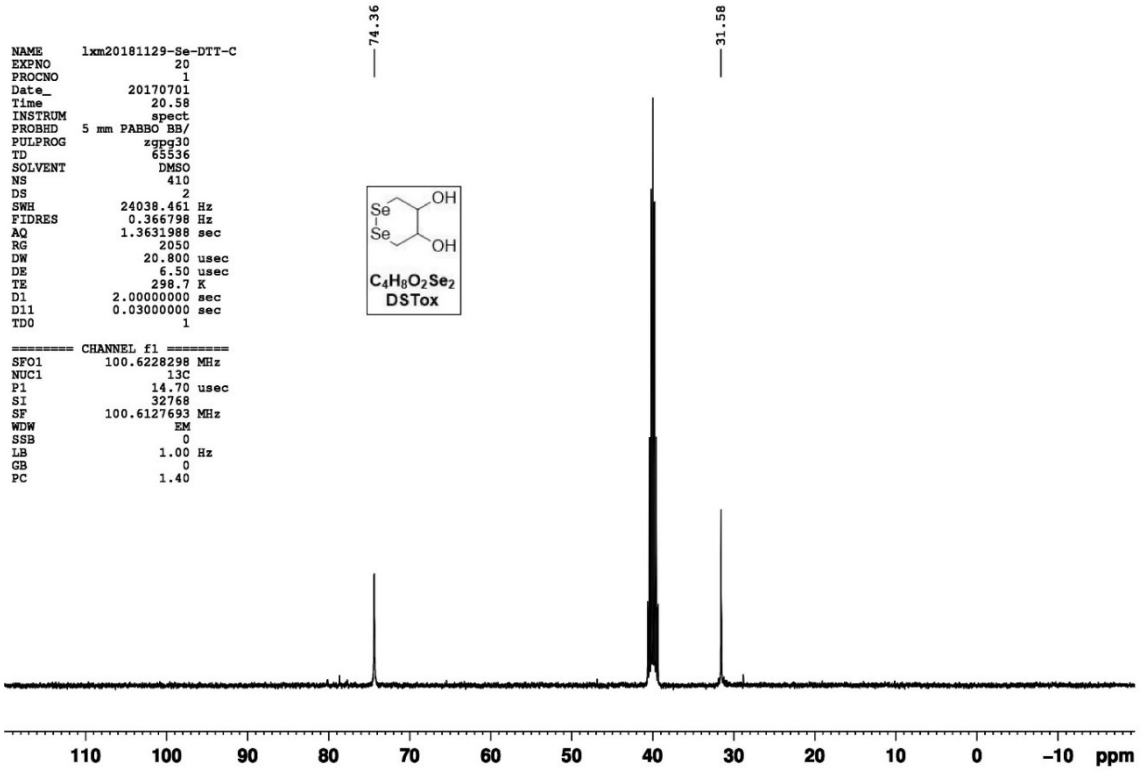


Figure S8. ^{13}C NMR of **DSTox** in DMSO-d_6 (100 MHz).

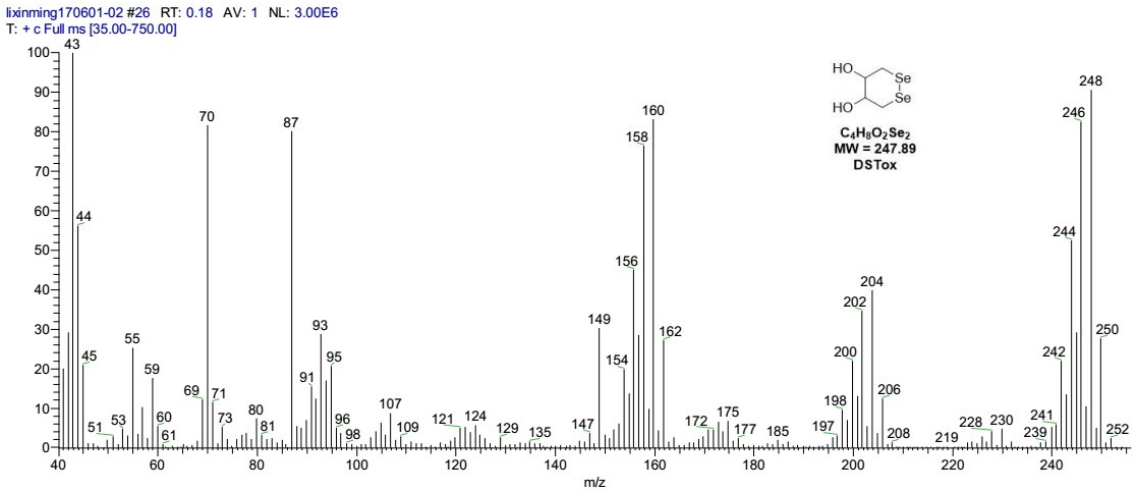


Figure S9. MS Spectra of **DSTox** (EI).

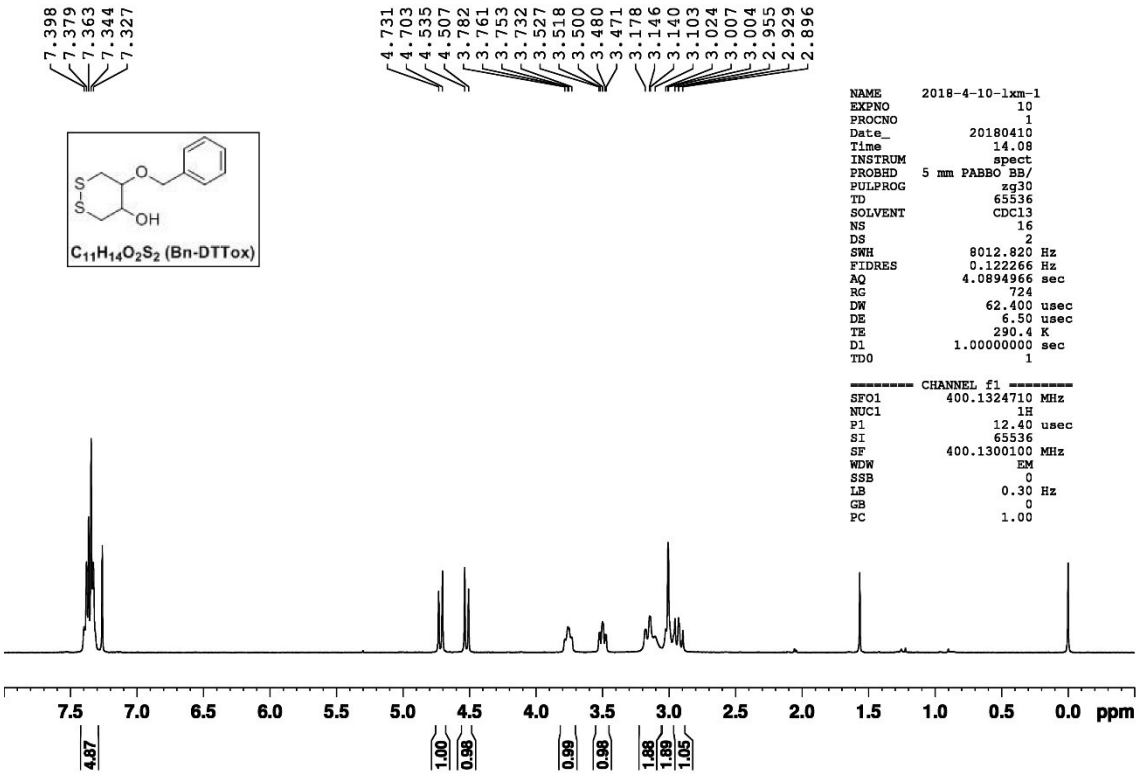


Figure S10. ^{1}H NMR Spectra of **Bn-DTTox** in $CDCl_3$ (400 MHz).

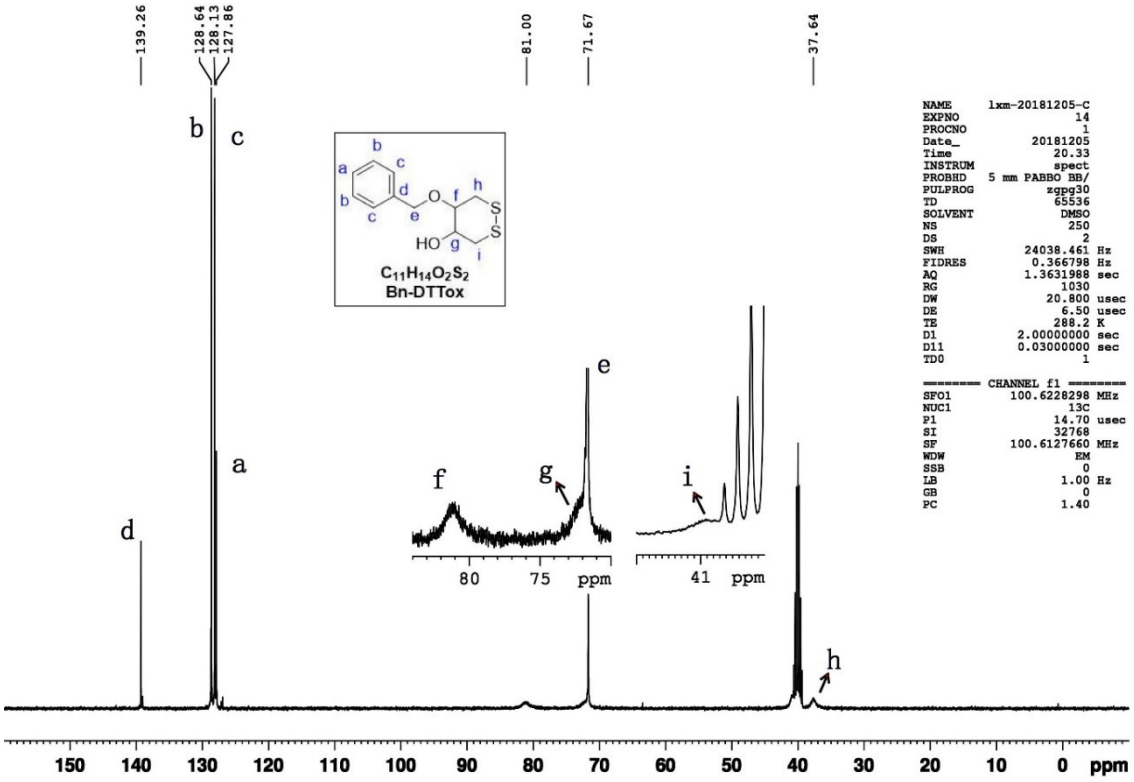


Figure S11. ^{13}C NMR Spectra of **Bn-DTTox** in DMSO-d_6 (100 MHz).

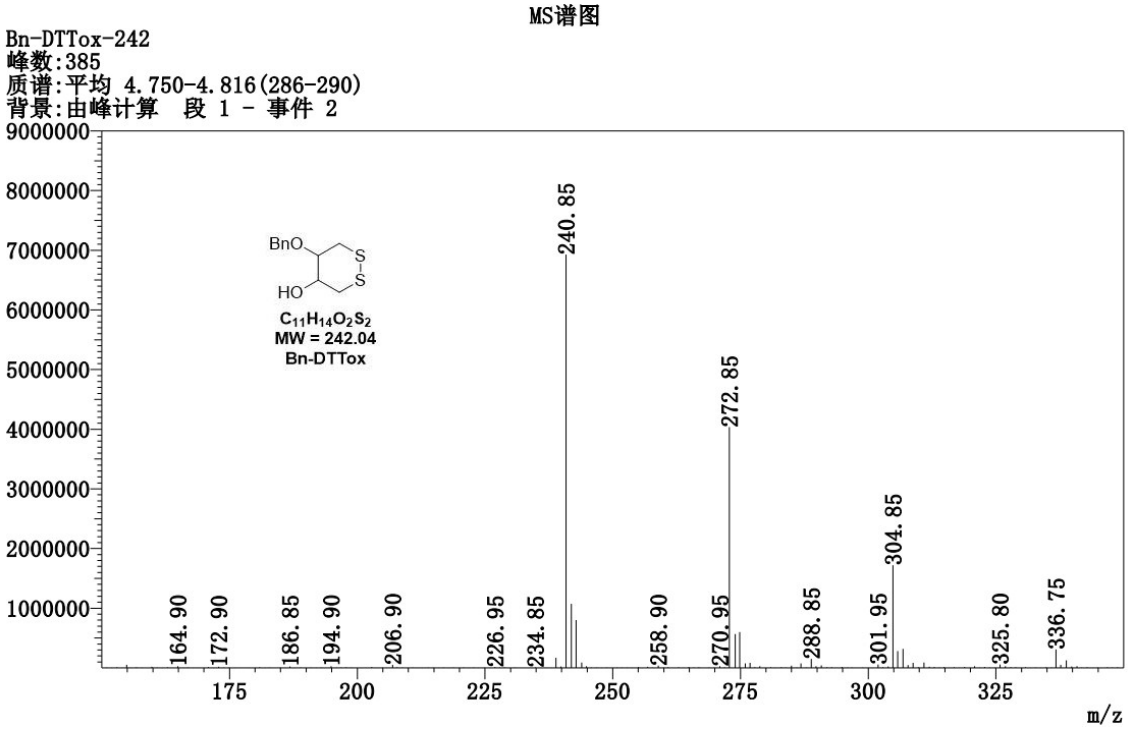


Figure S12. MS Spectra of **Bn-DTTox** (ESI).

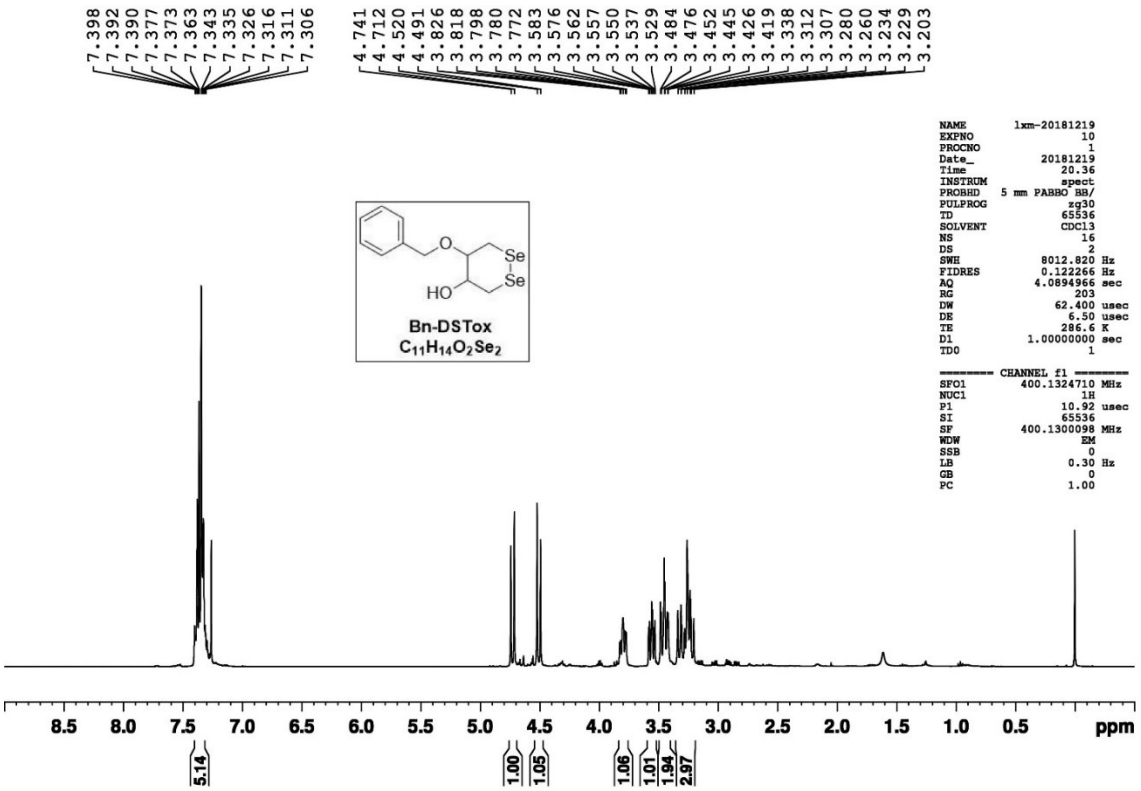


Figure S13. ^{1}H NMR Spectra of **Bn-DSTox** in $CDCl_3$ (400 MHz).

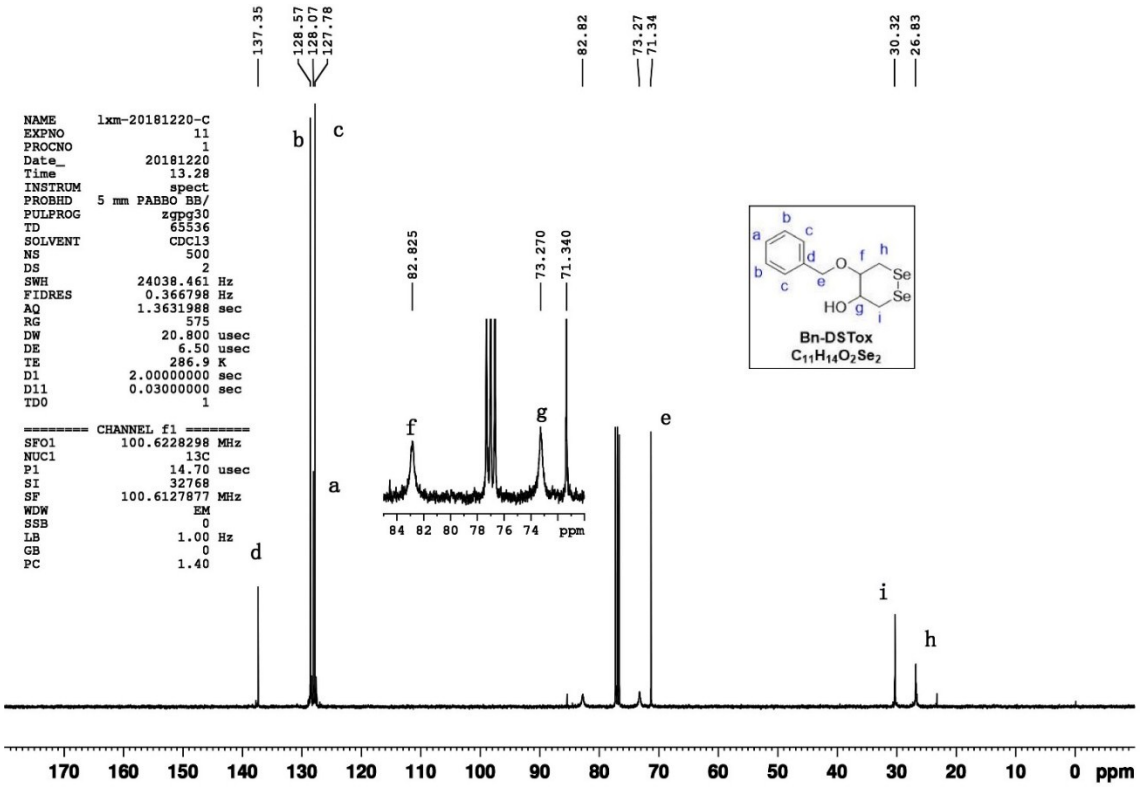


Figure S14. ^{13}C NMR Spectra of **Bn-DSTox** in $CDCl_3$ (100 MHz).

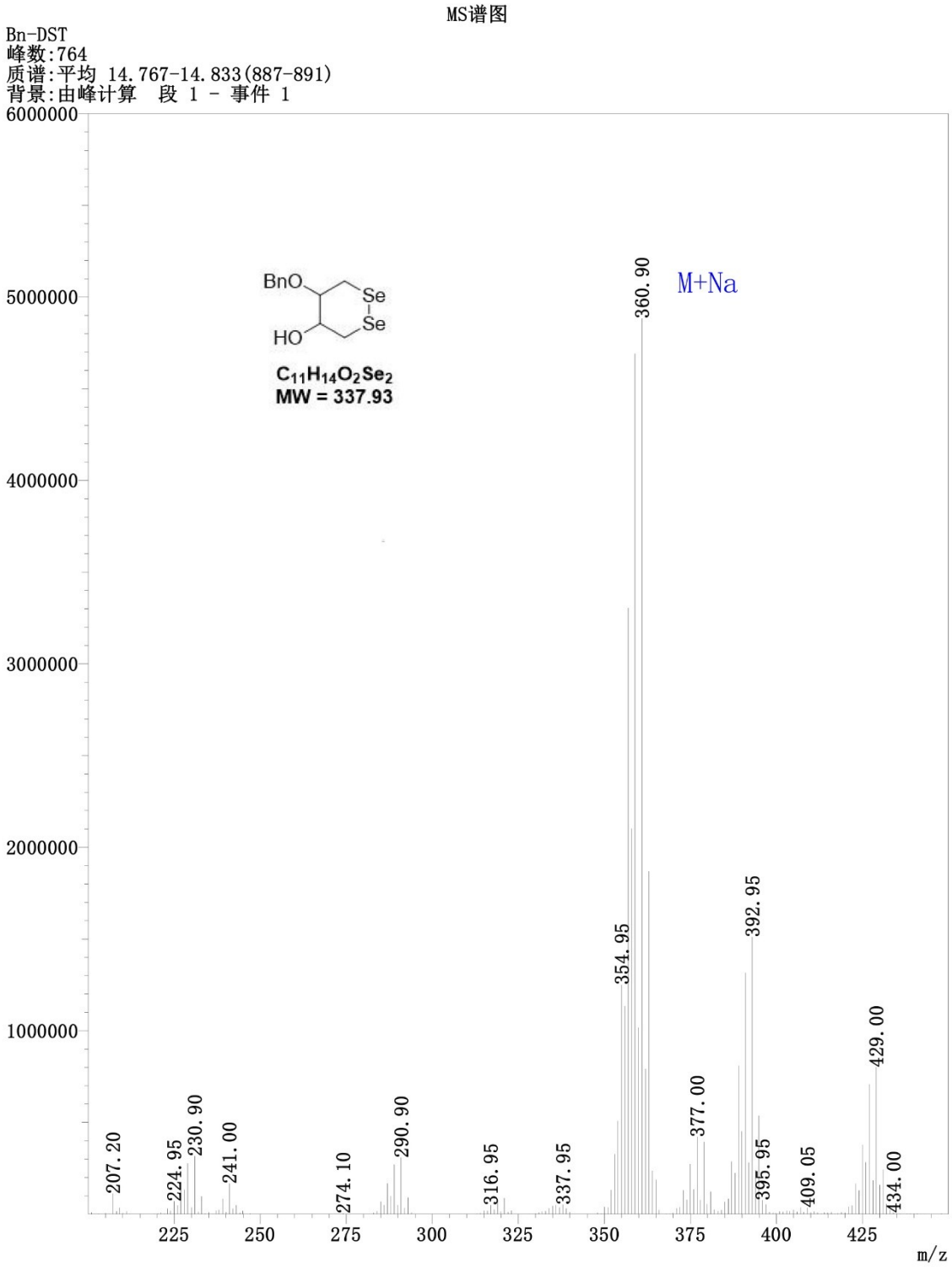


Figure S15. MS Spectra of **Bn-DSTox** (ESI).

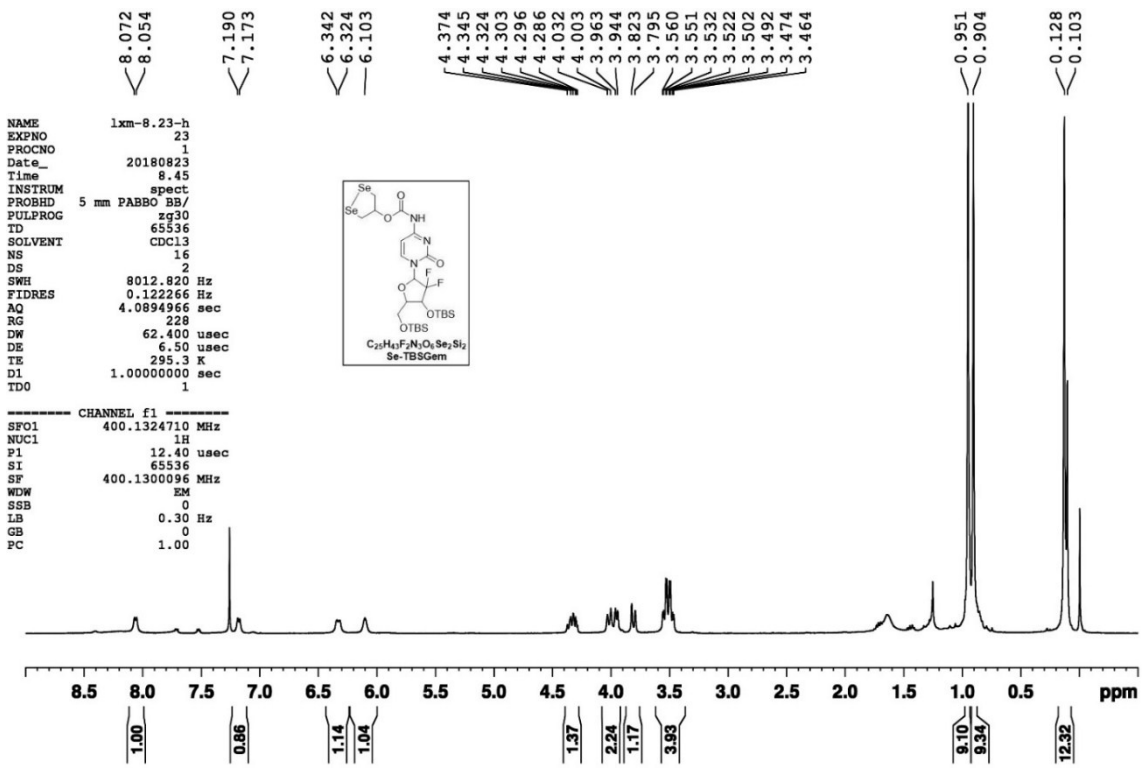


Figure S16. ^{1}H NMR Spectra of **Se-TBSGem** in $CDCl_3$ (400 MHz).

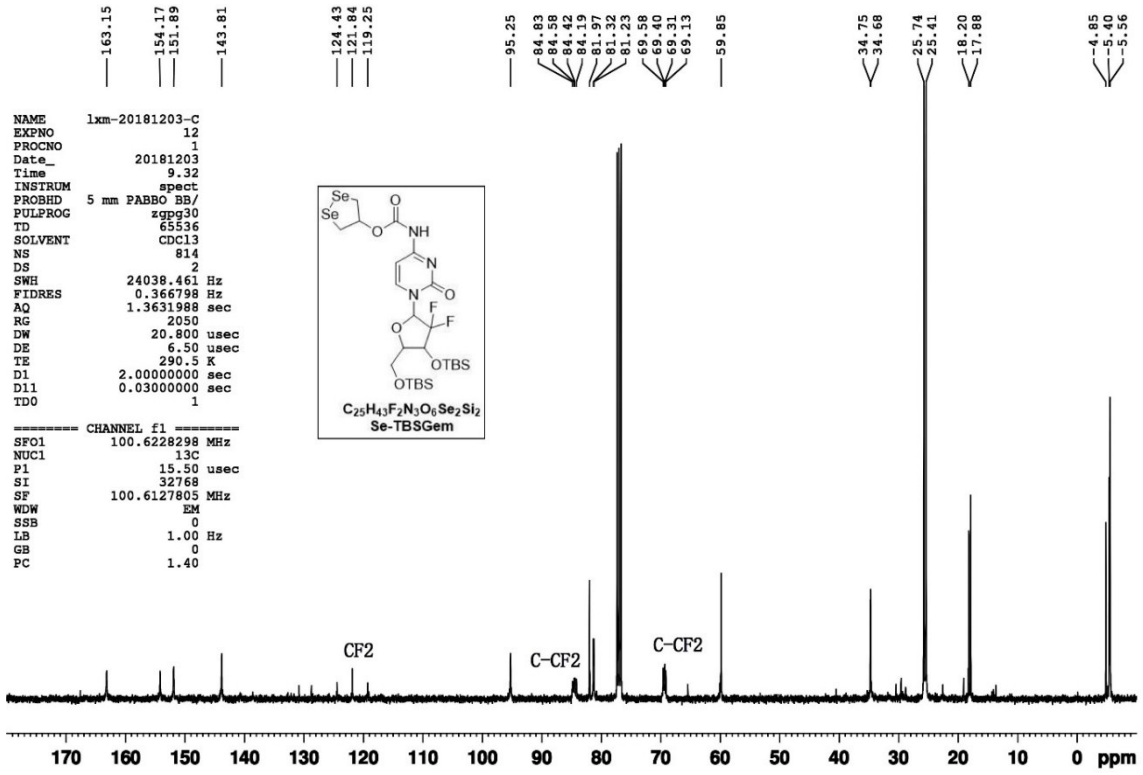


Figure S17. ^{13}C NMR Spectra of **Se-TBSGem** in $CDCl_3$ (100 MHz)

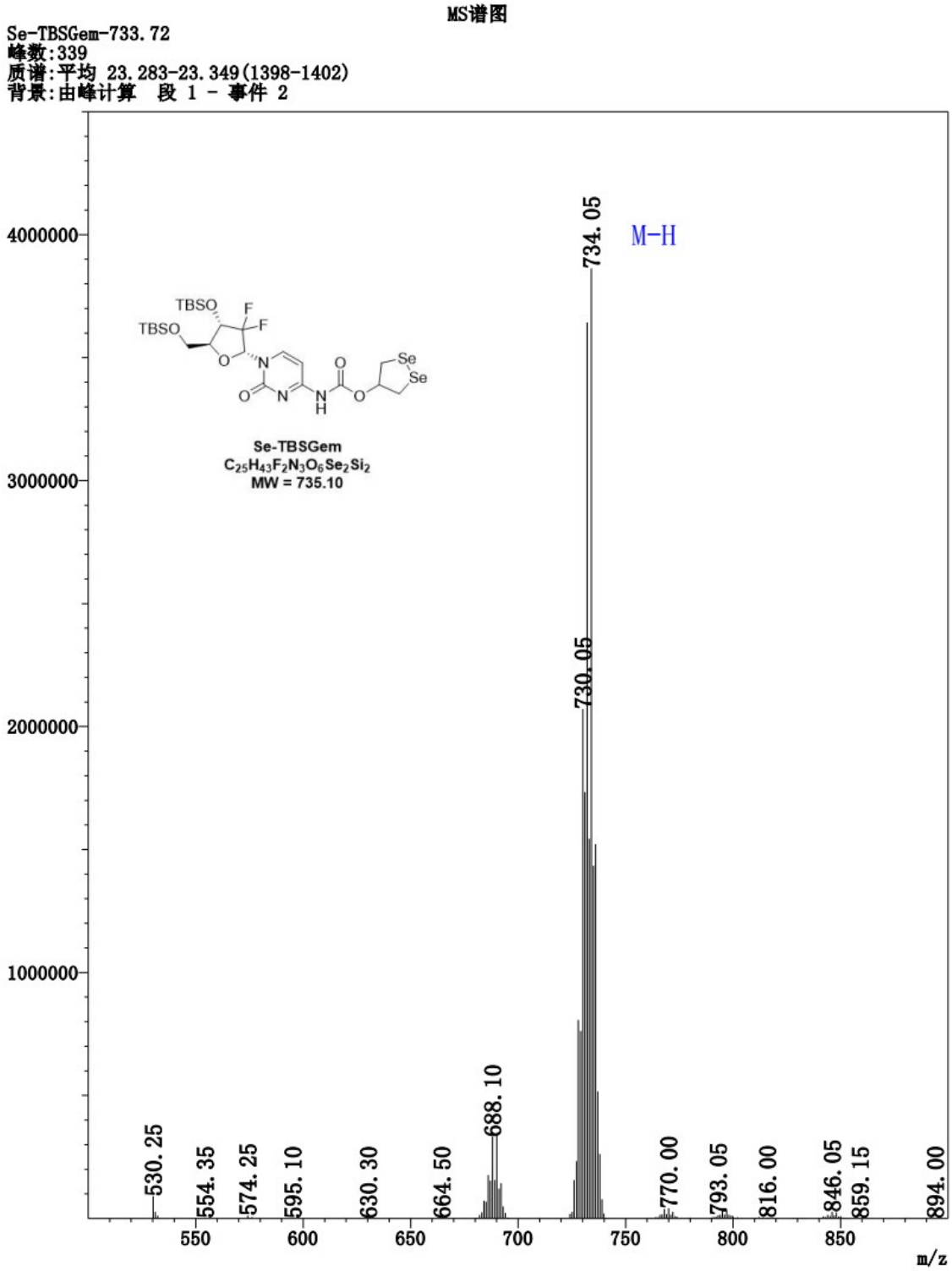


Figure S18. MS Spectra of **Se-TBSGem** (ESI).

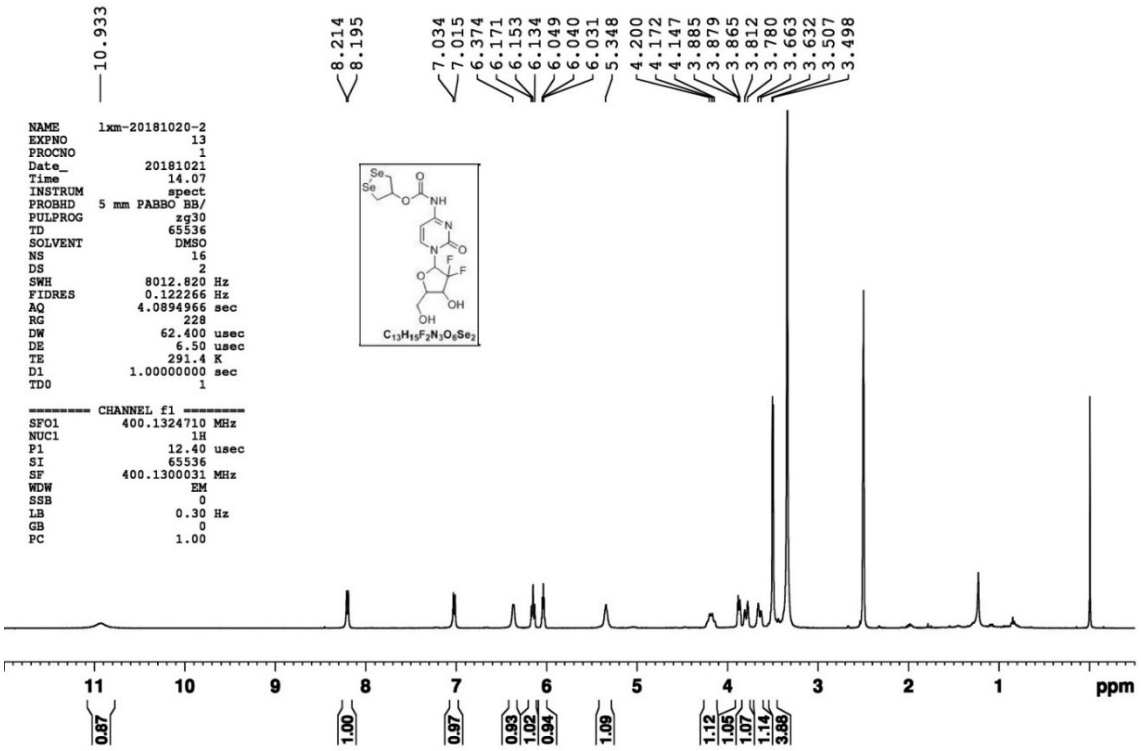


Figure S19. ^{1}H NMR Spectra of **Se-Gem** in DMSO-d_6 (400 MHz).

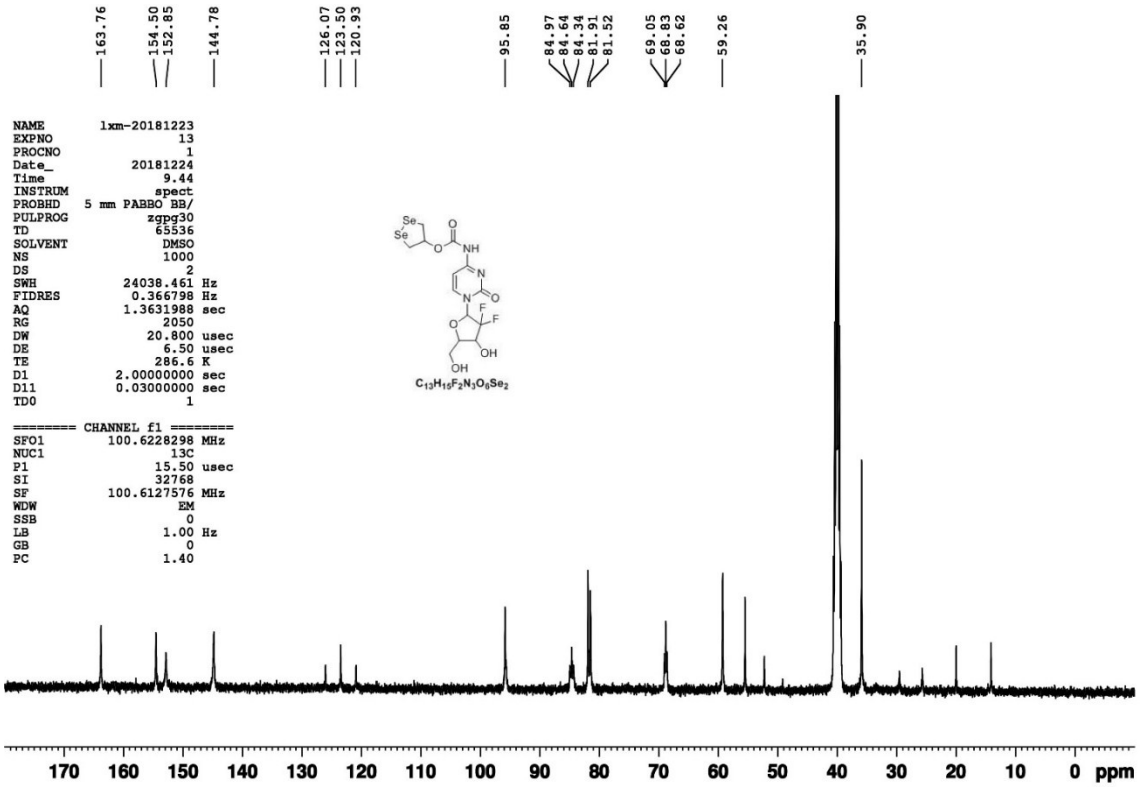


Figure S20. ^{13}C NMR Spectra of **Se-Gem** in DMSO-d_6 (100 MHz).

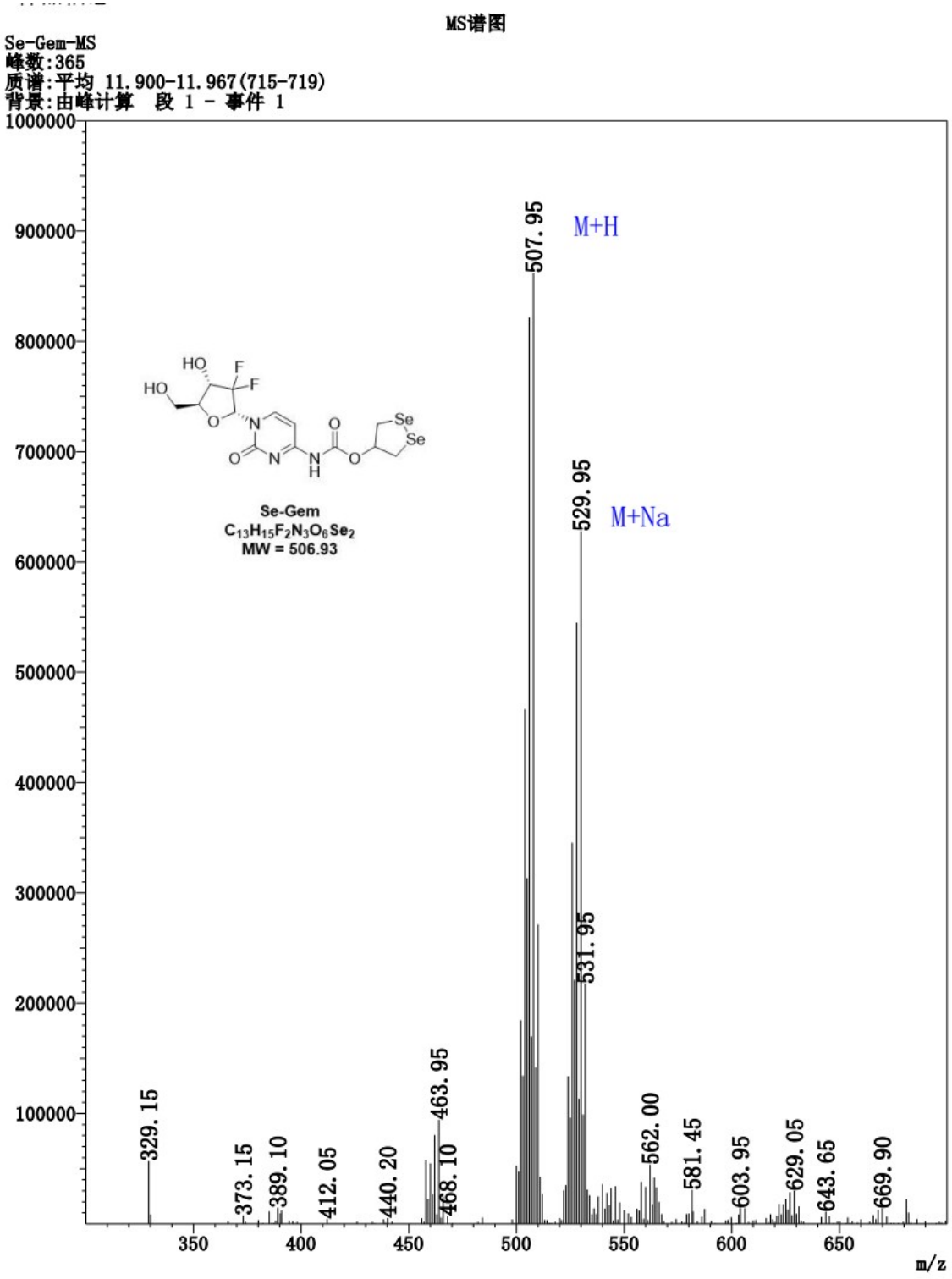


Figure S21. MS Spectra of **Se-Gem** (ESI).

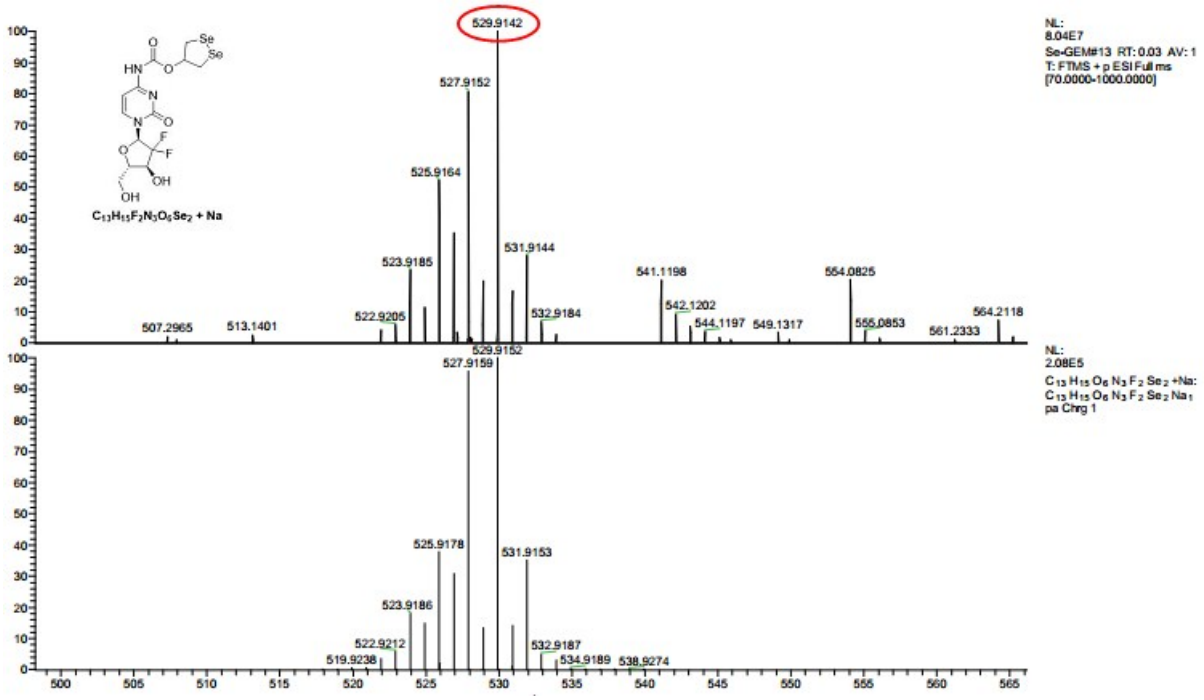


Figure S22. HRMS Spectra of **Se-Gem** (ESI).

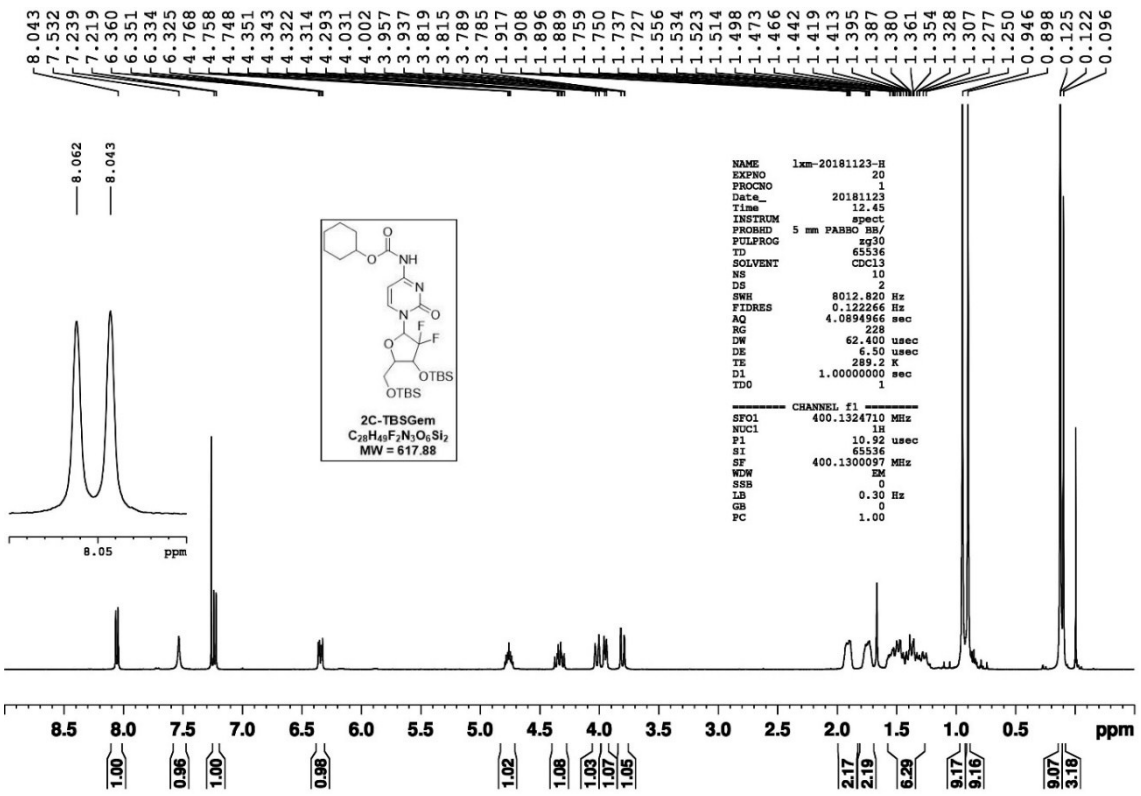


Figure S23. ^{1}H NMR Spectra of **C6-TBSGem** in $CDCl_3$ (400 MHz).

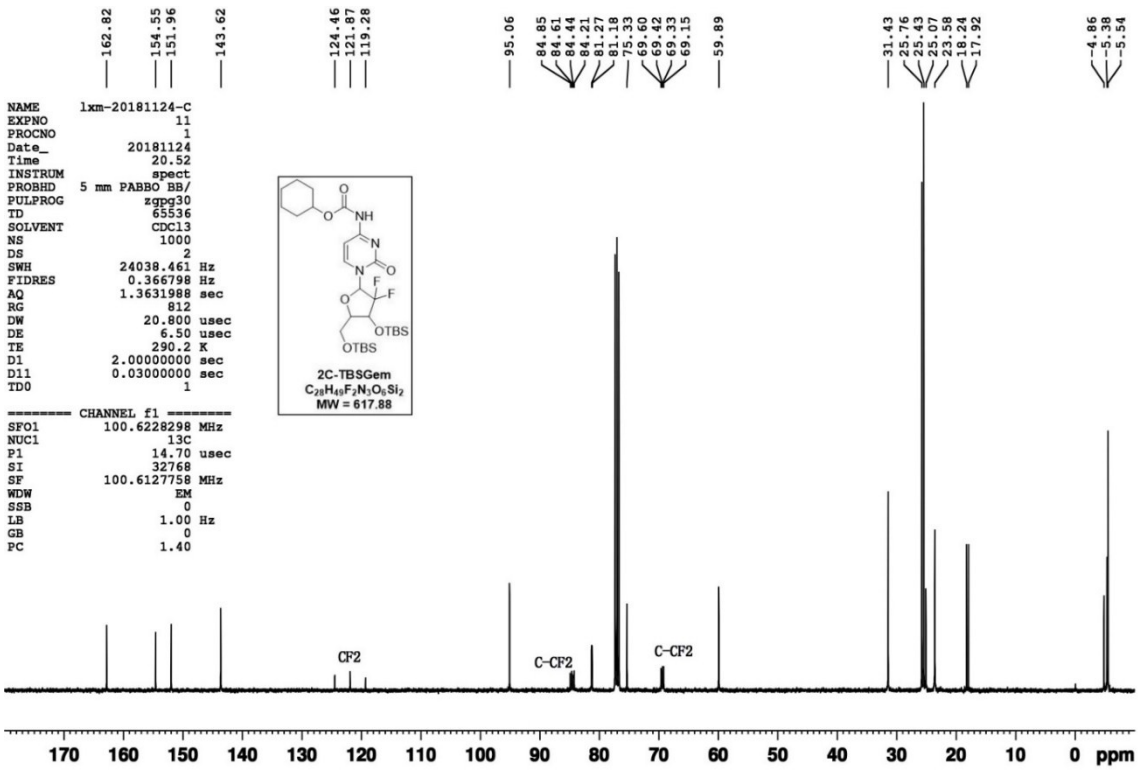


Figure S24. ^{13}C NMR Spectra of **C6-TBSGem** in $CDCl_3$ (100 MHz).

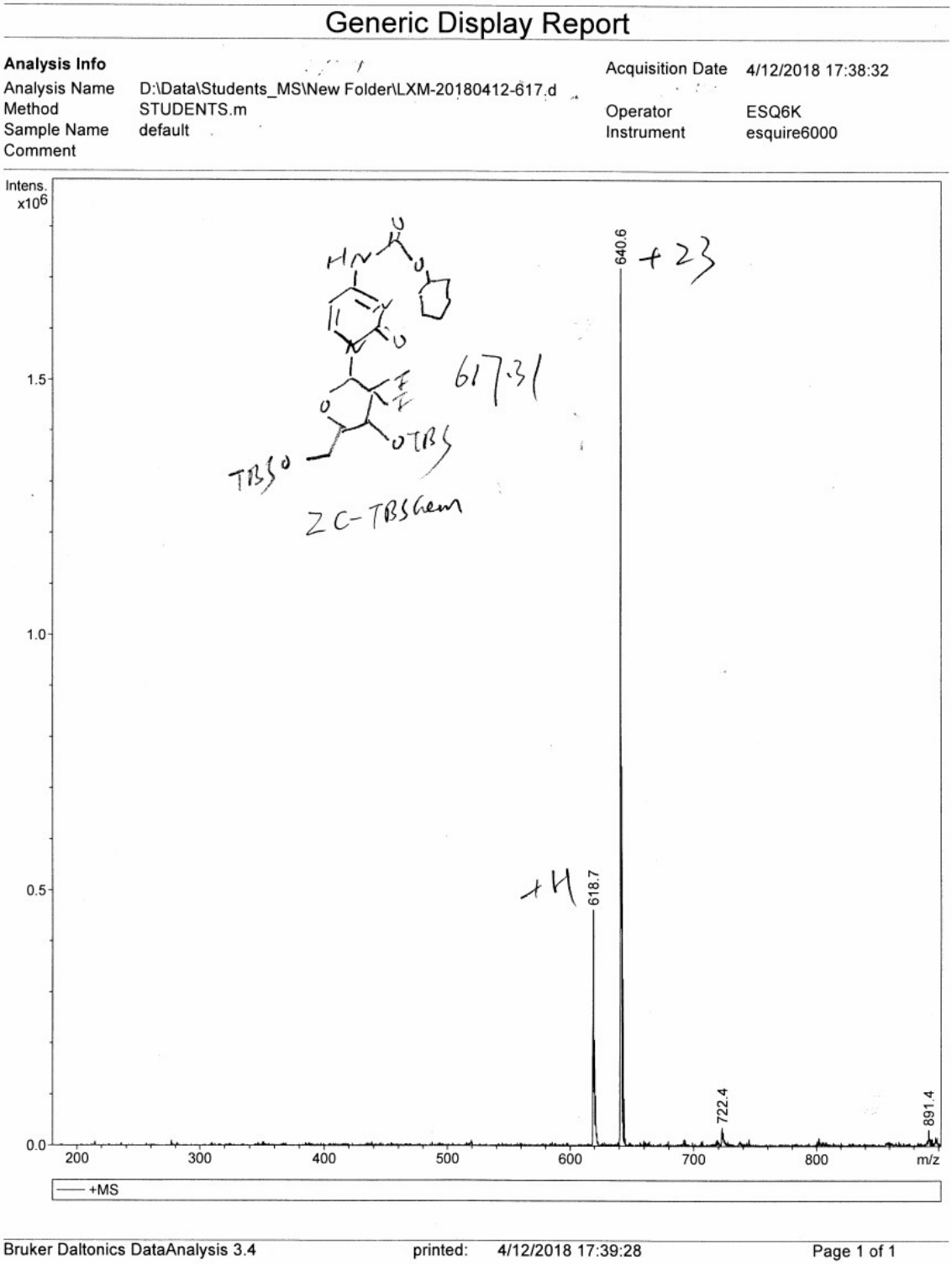


Figure S25. MS Spectra of **C6-TBSGem** (ESI).

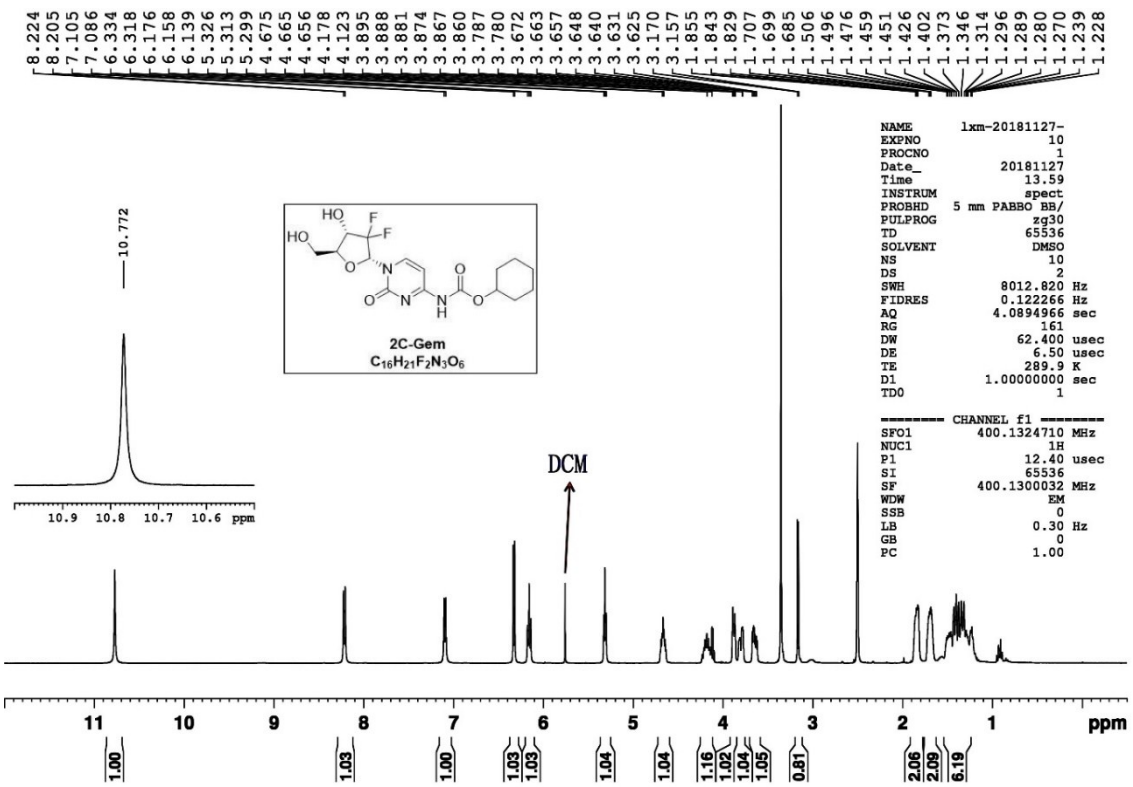


Figure S26. ^{1}H Spectra of **C6-Gem** in DMSO-d_6 (400 MHz)

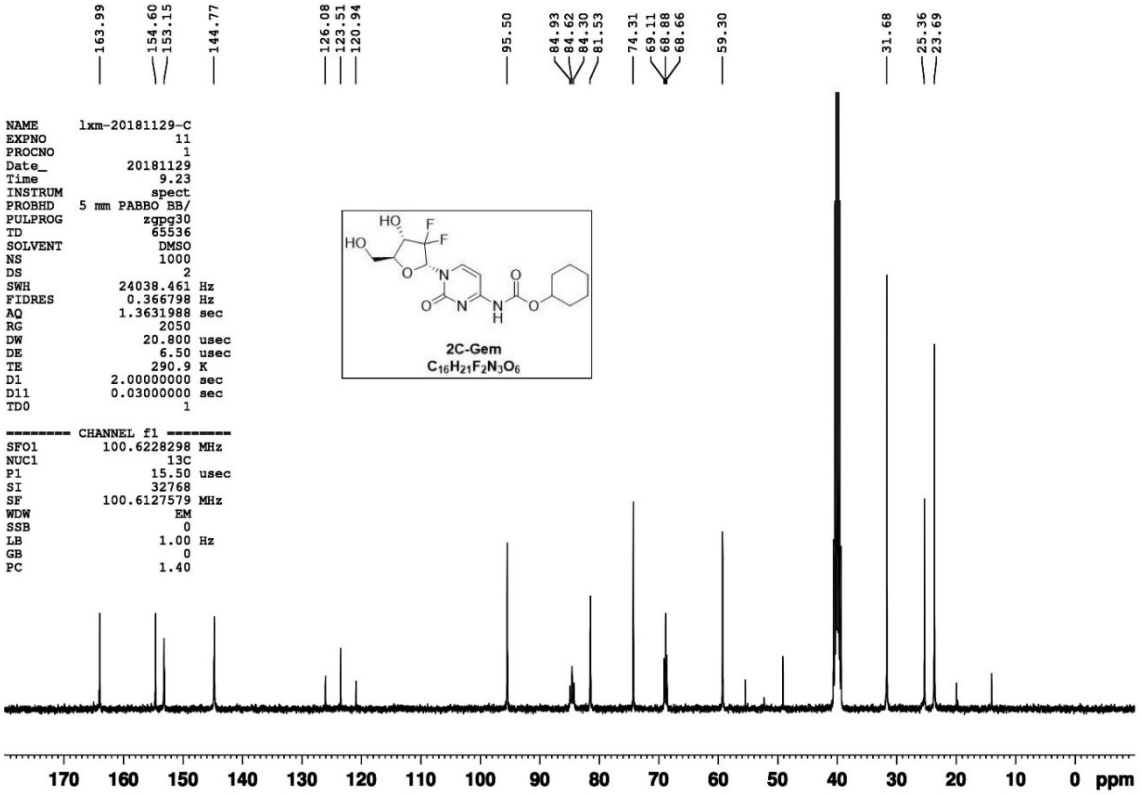


Figure S27. ^{13}C NMR of **C6-Gem** in DMSO-d_6 (100 MHz).

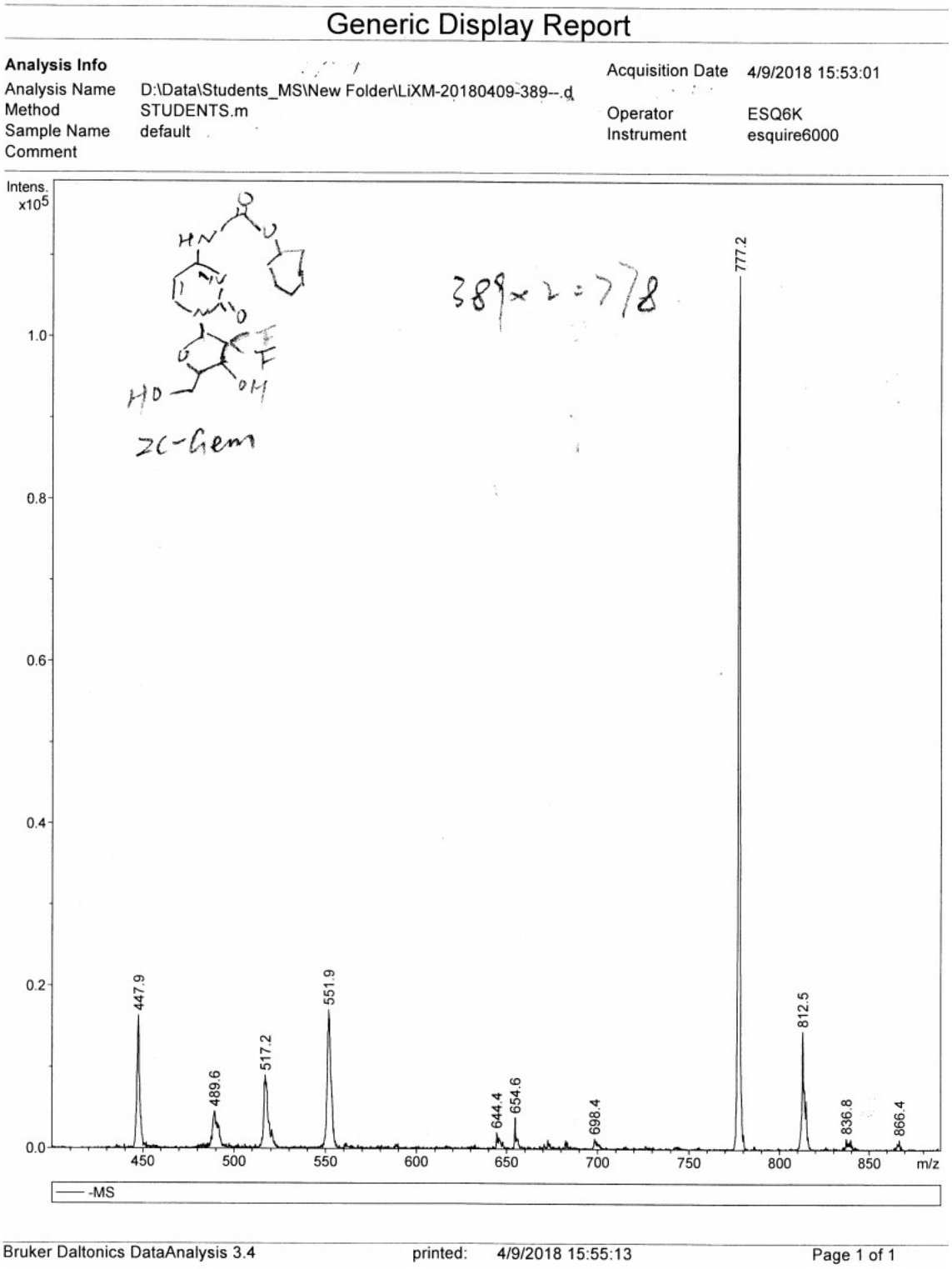


Figure S28. MS Spectra of **C6-Gem** (ESI).

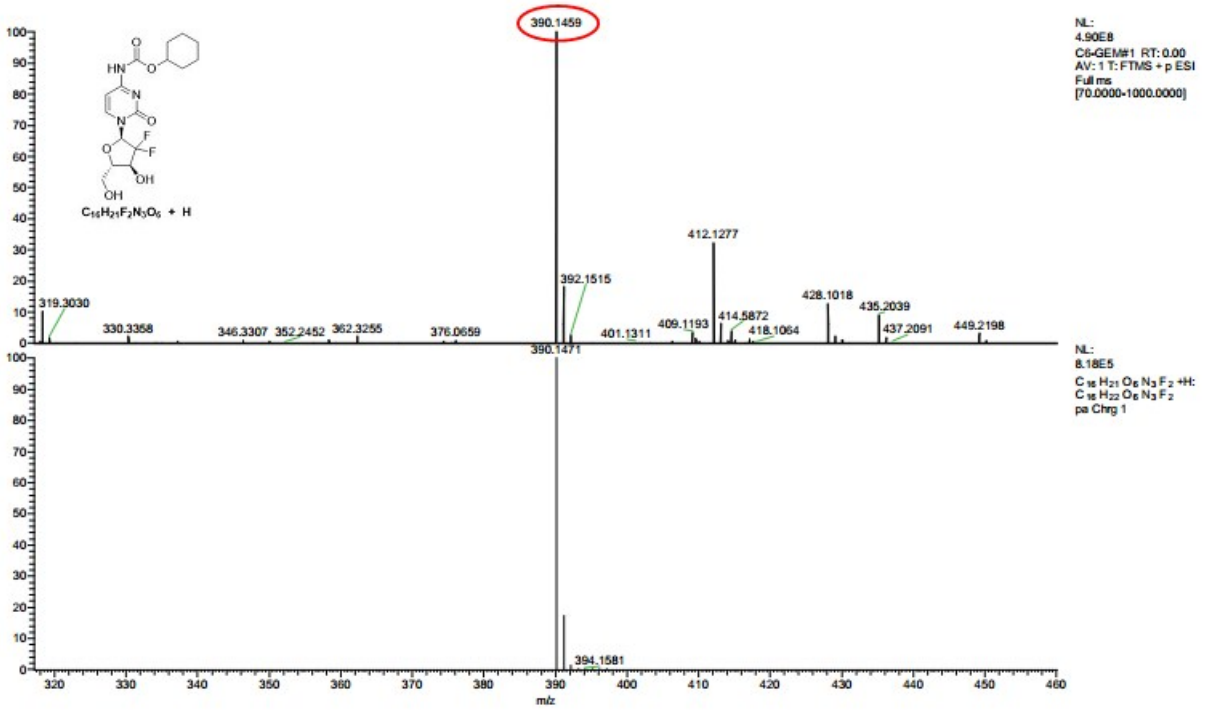


Figure S29. HRMS Spectra of **C6-Gem** (ESI).

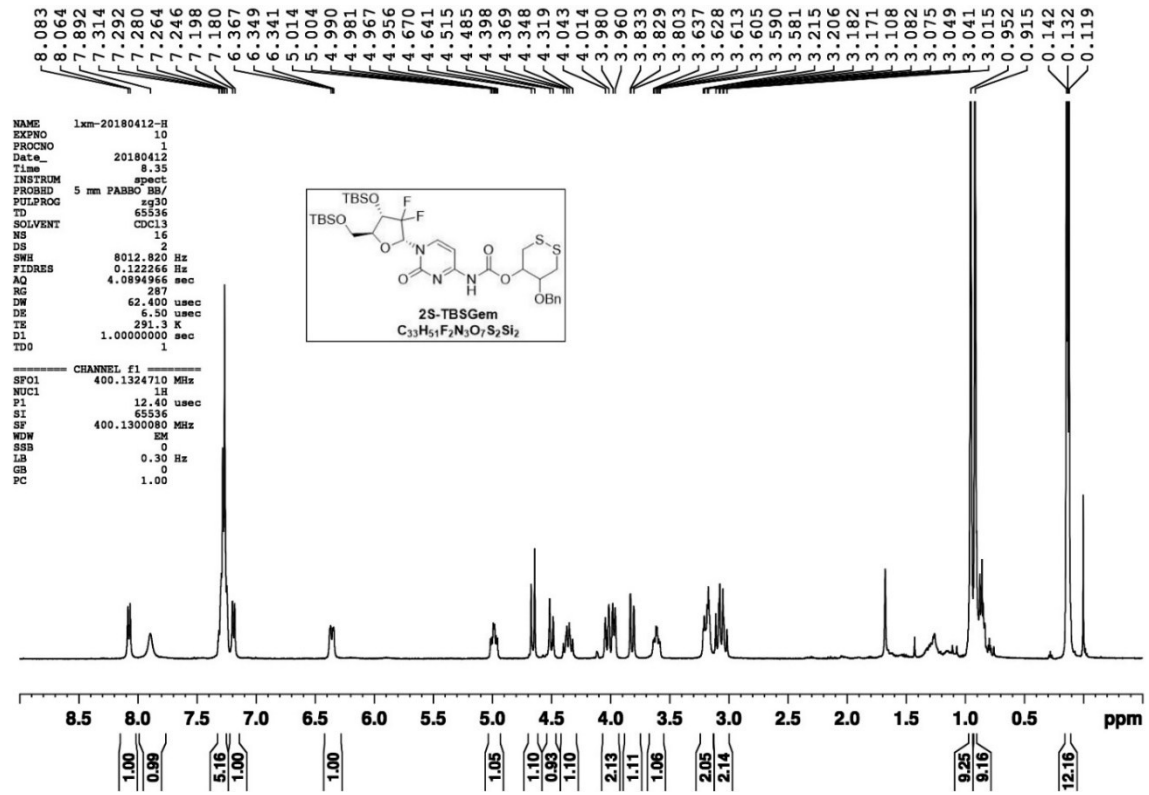


Figure S30. ^{1}H NMR Spectra of **S6-TBSGem** in $CDCl_3$ (400 MHz).

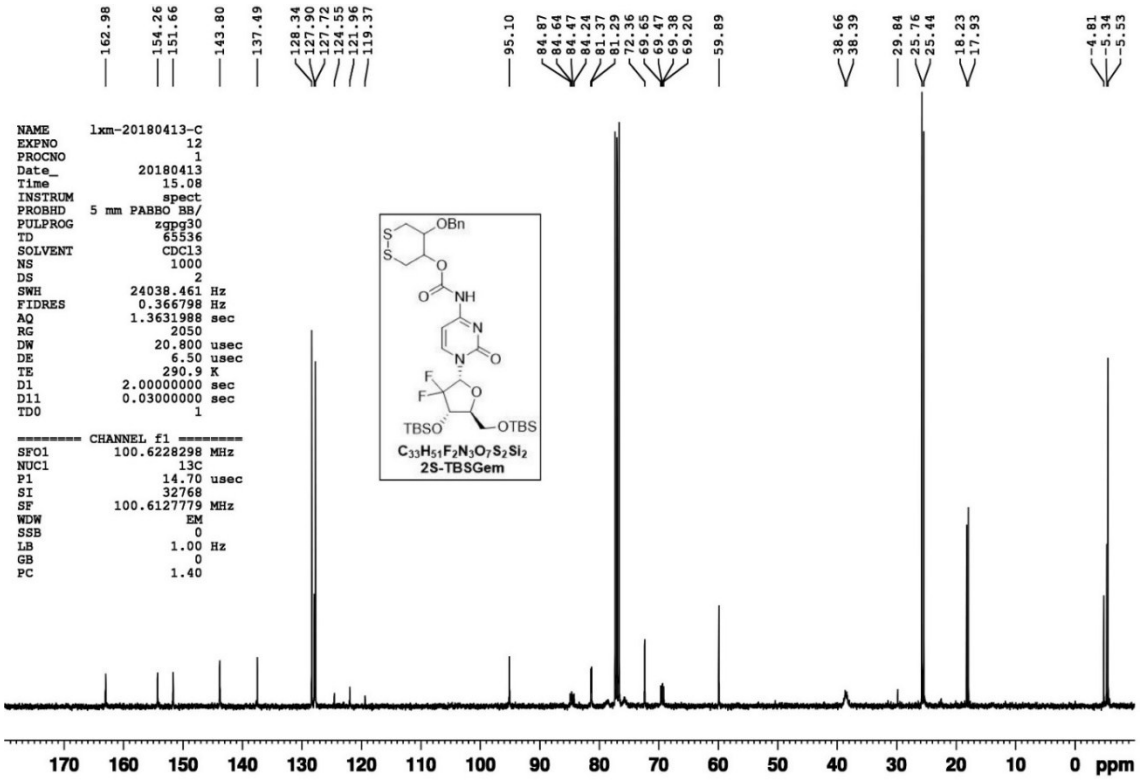


Figure S31. ^{13}C NMR Spectra of **S6-TBSGem** in $CDCl_3$ (100 MHz).

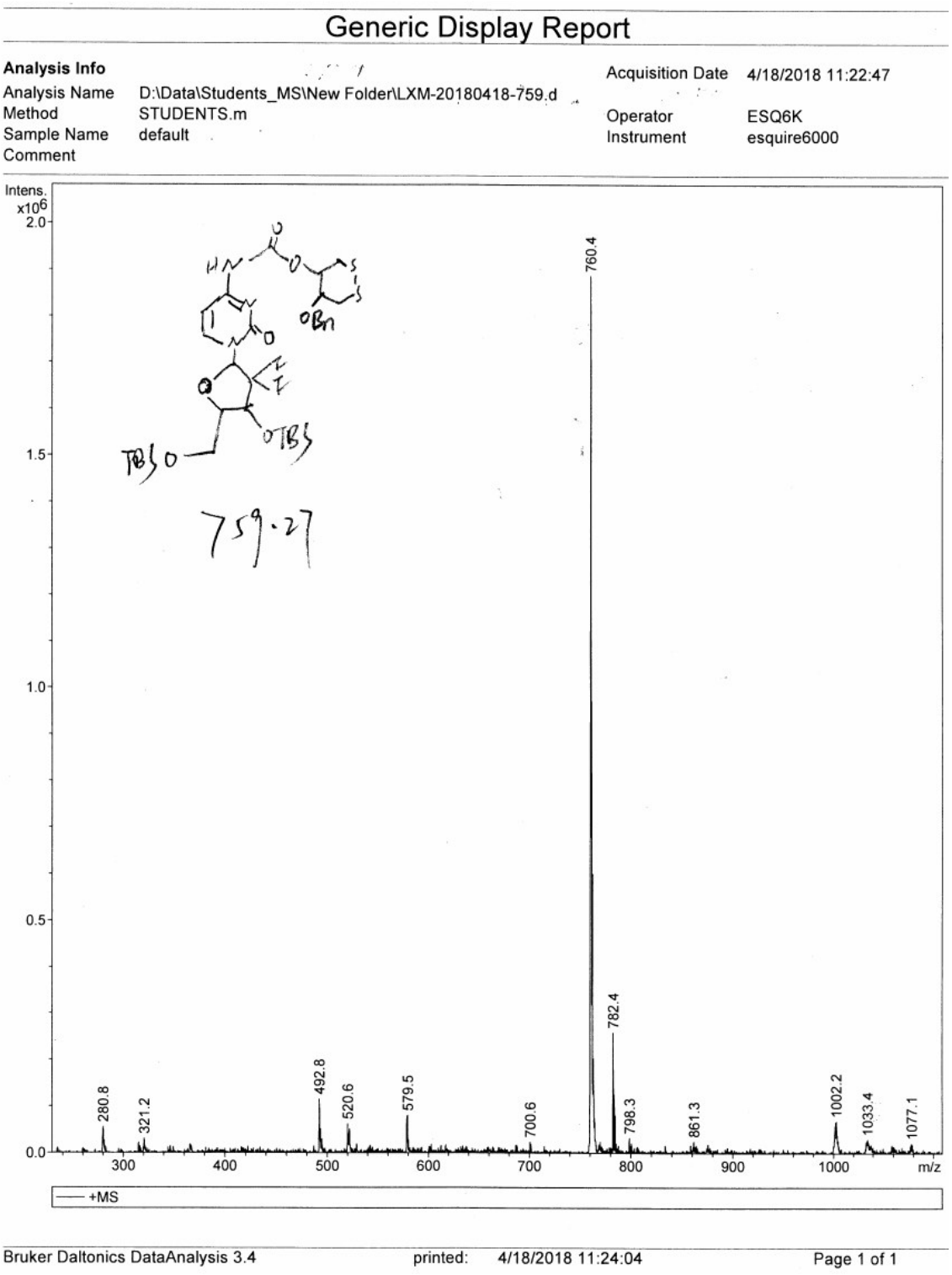


Figure S32. MS Spectra of **S6-TBSGem** (ESI).

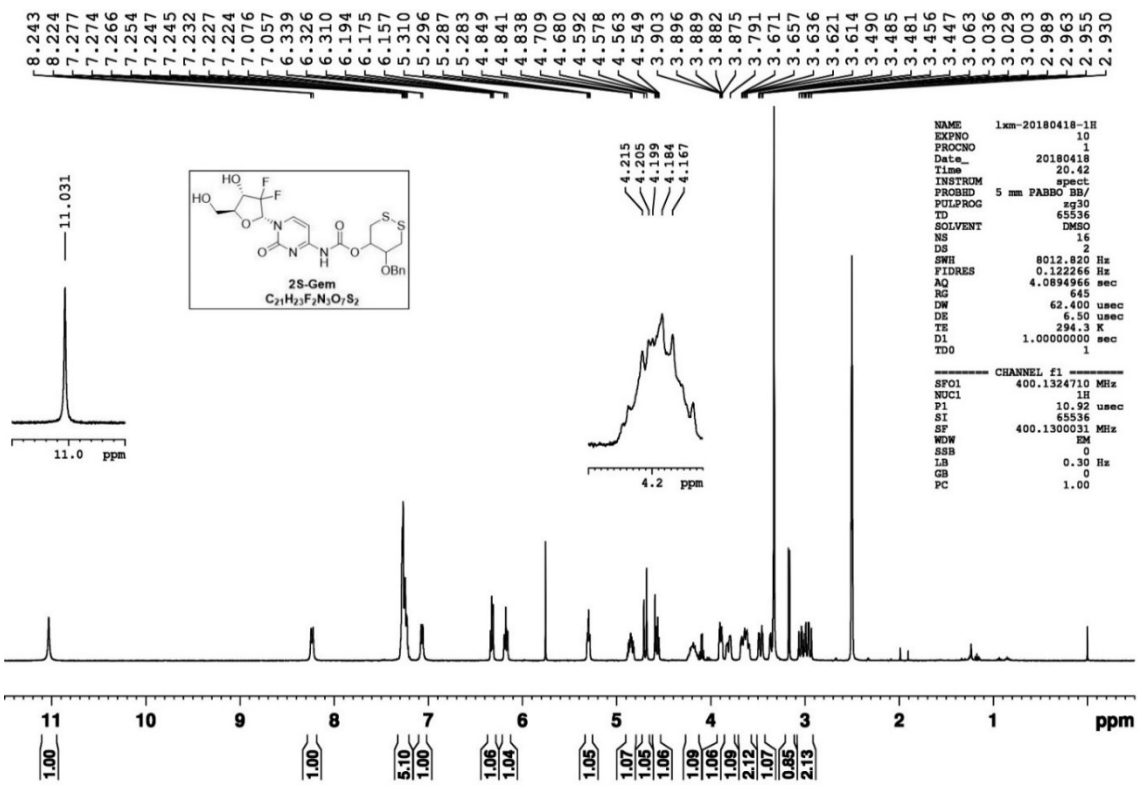


Figure S33. ^{1}H NMR Spectra of **S6-Gem** in DMSO-d_6 (400 MHz).

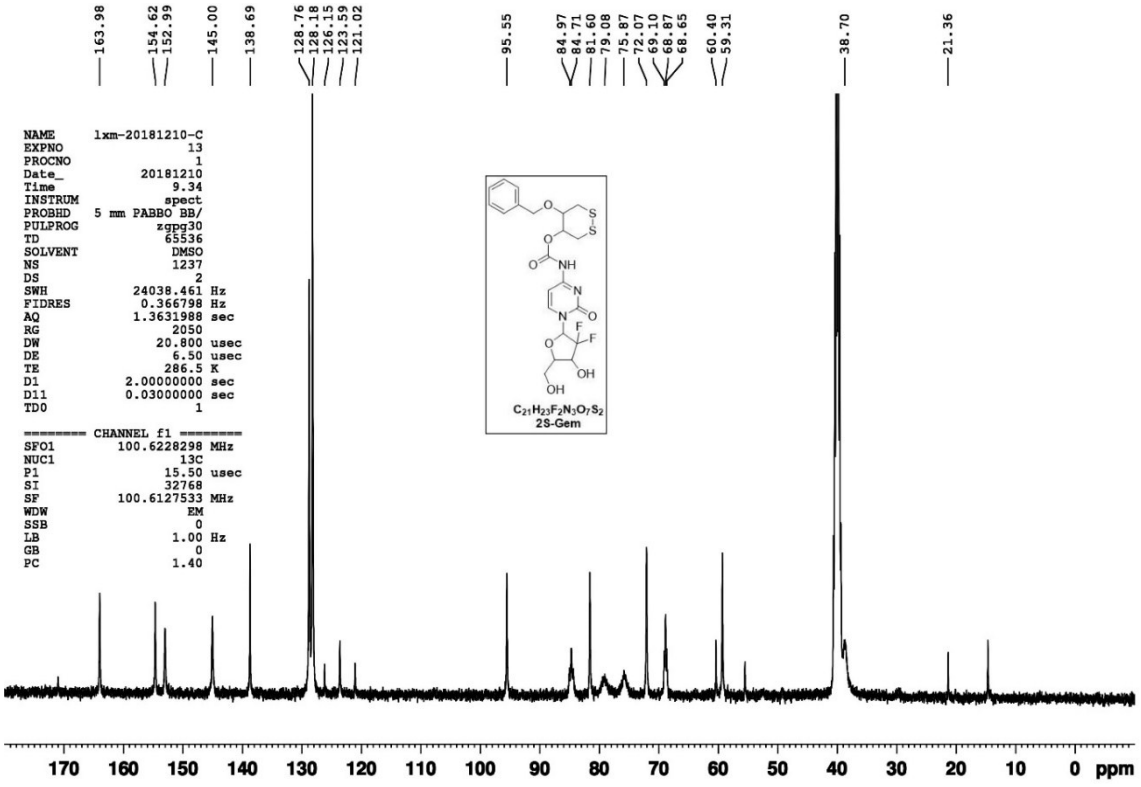


Figure S34. ^{13}C NMR Spectra of **S6-Gem** in DMSO-d_6 (100 MHz).

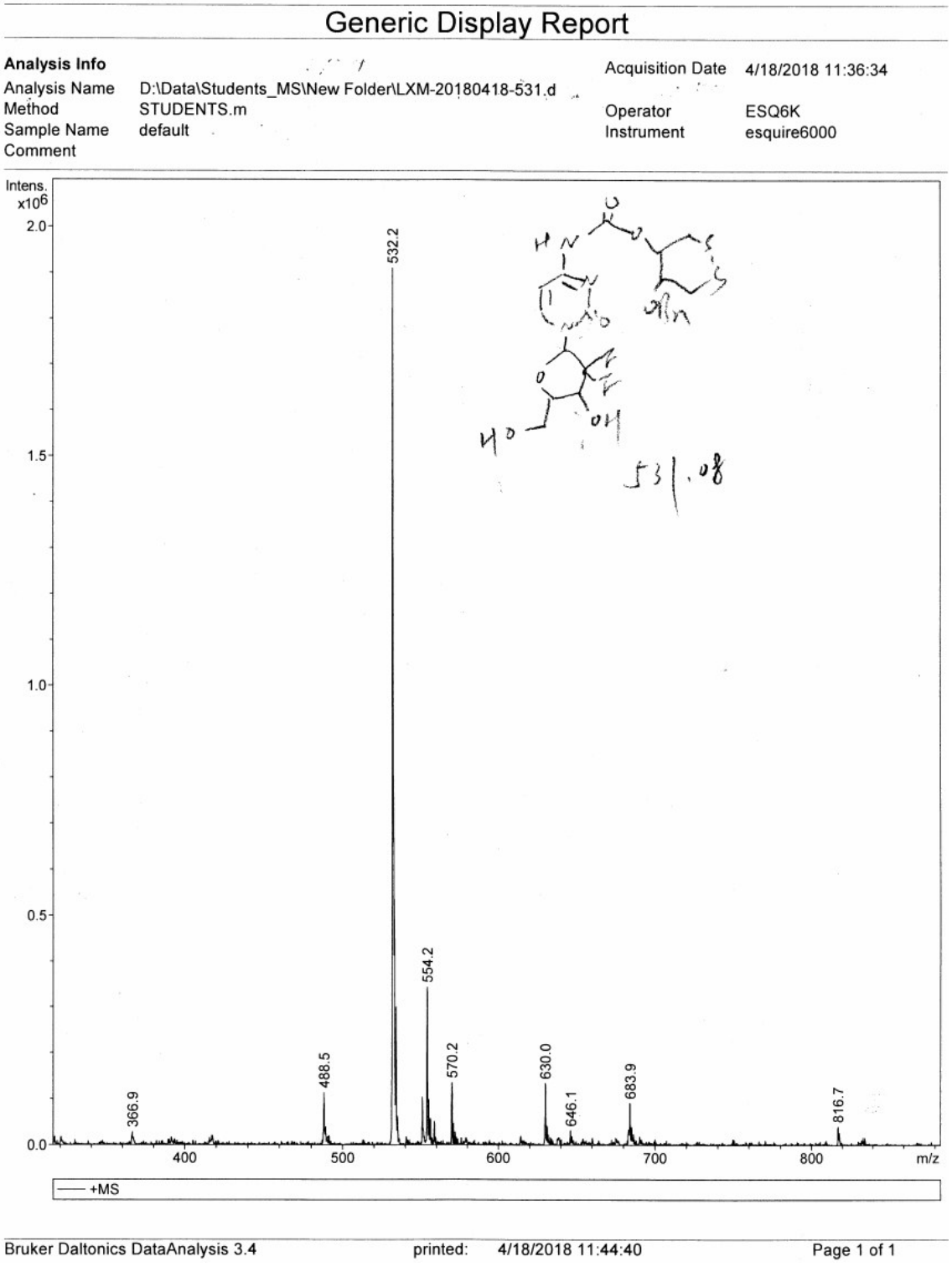


Figure S35. MS Spectra of **S6-Gem** (ESI).

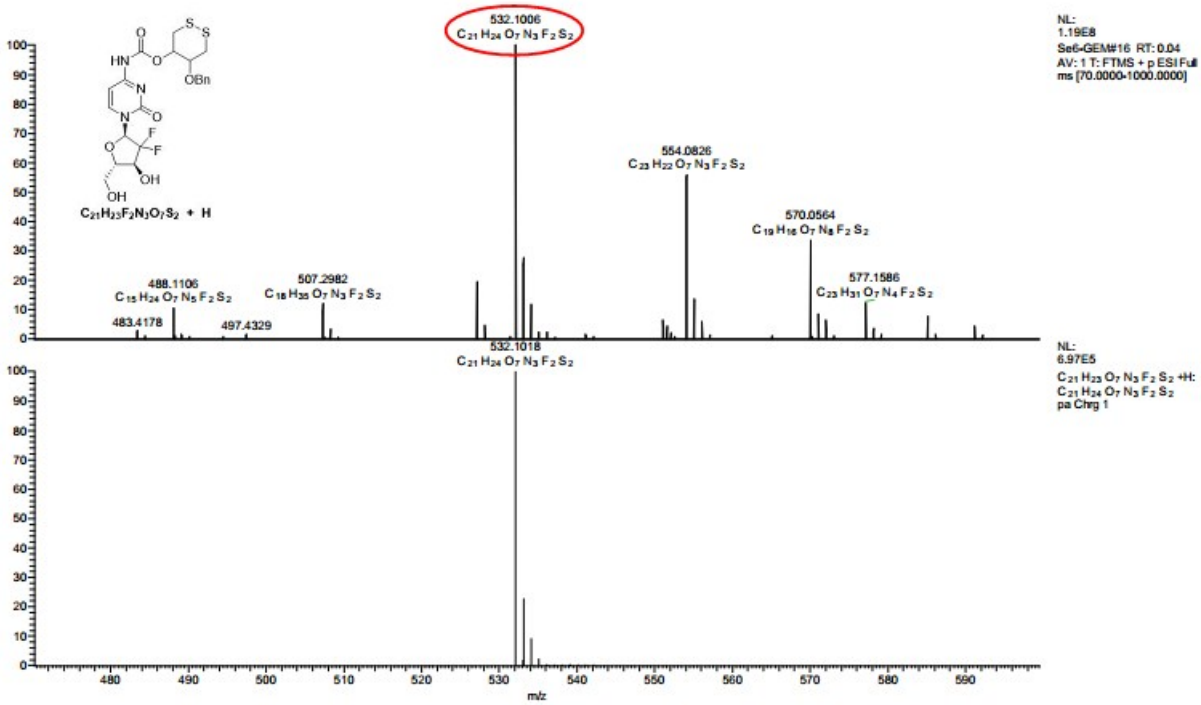


Figure S36. HRMS Spectra of **S6-Gem** (ESI).

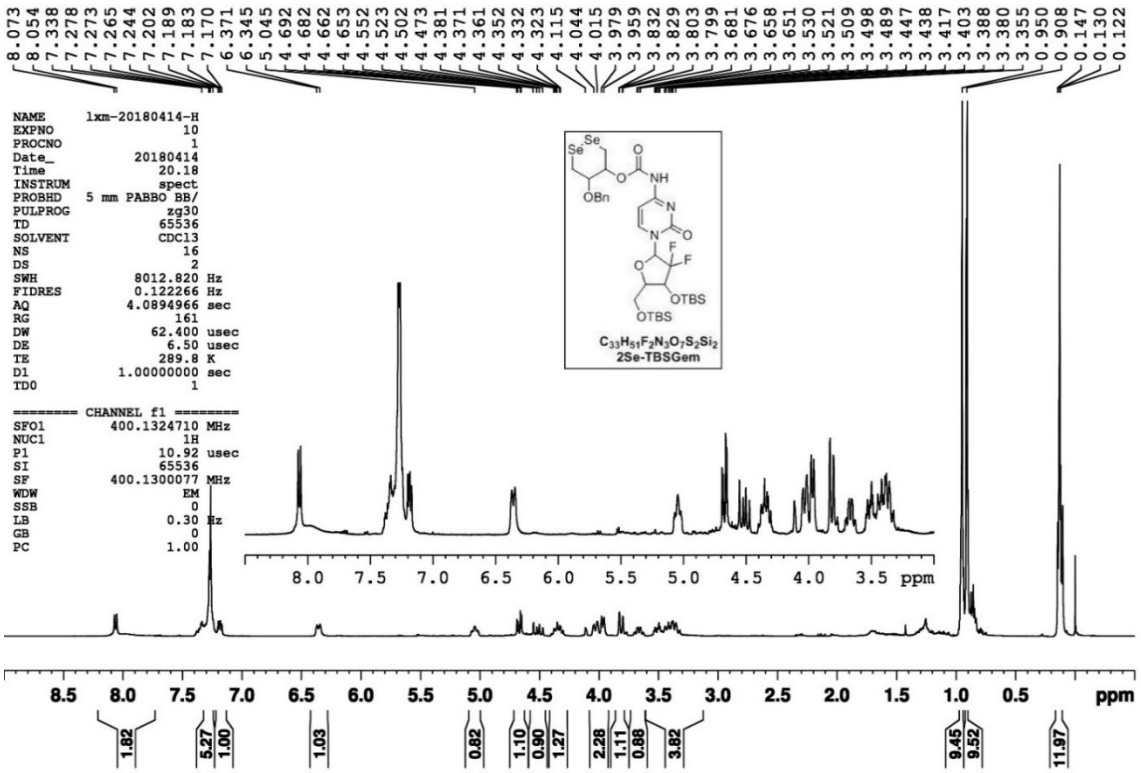


Figure S37. ^{1}H NMR Spectra of **Se6-TBSGem** in $CDCl_3$ (400 MHz).

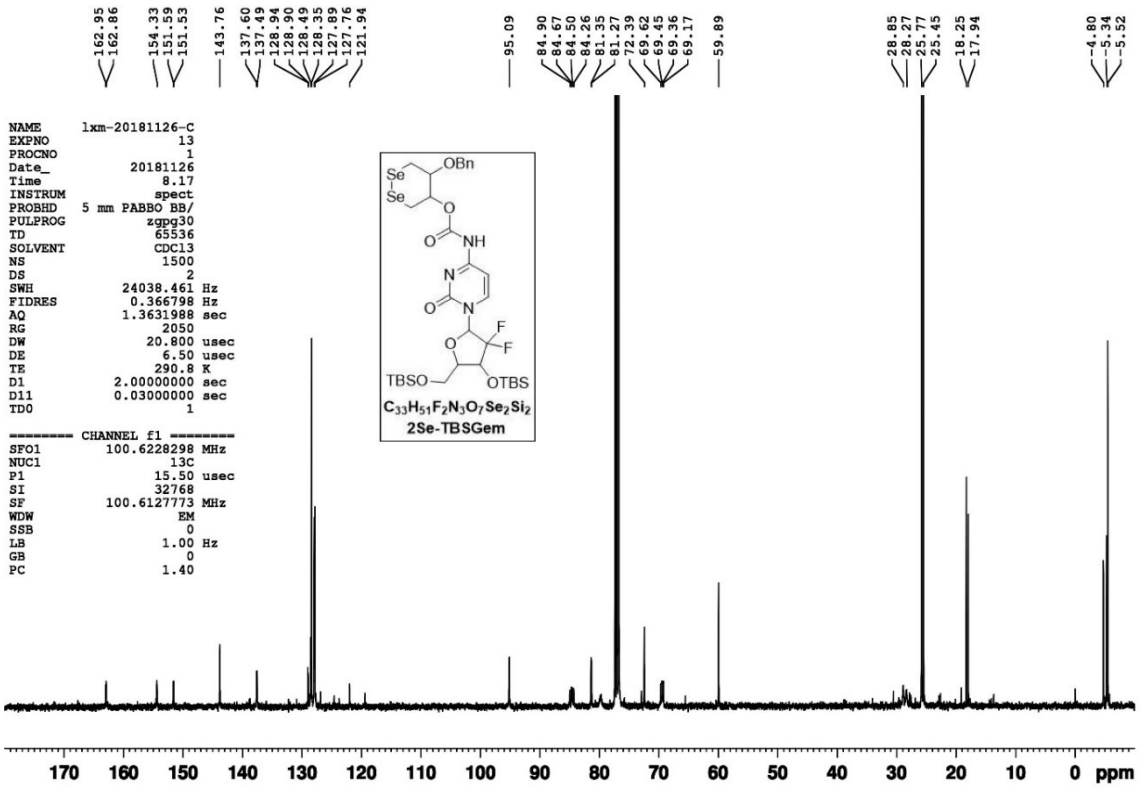


Figure S38. ^{13}C NMR Spectra of **Se6-TBSGem** in $CDCl_3$ (100 MHz).

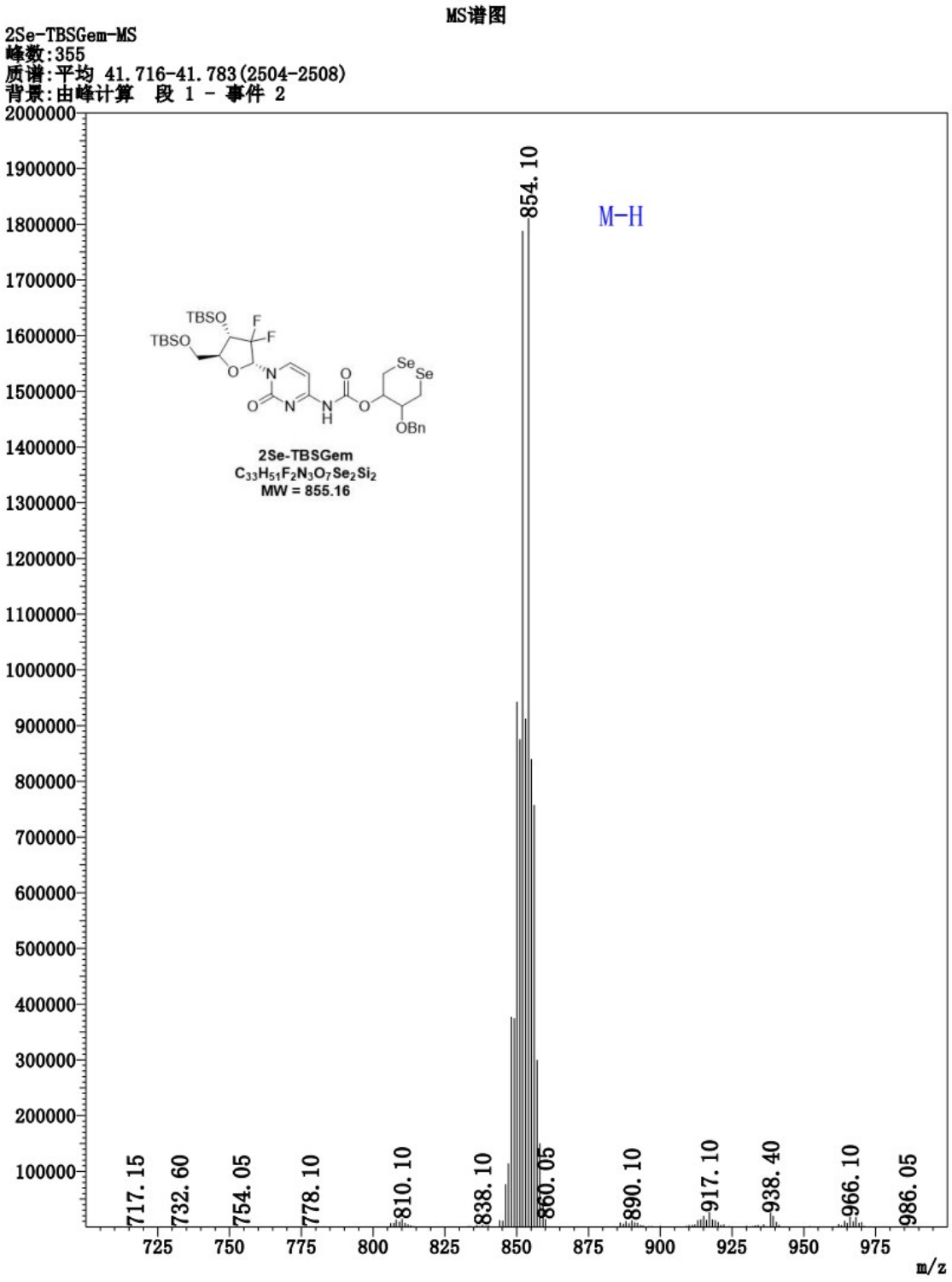


Figure S39. MS Spectra of **Se6-TBSGem** (ESI).

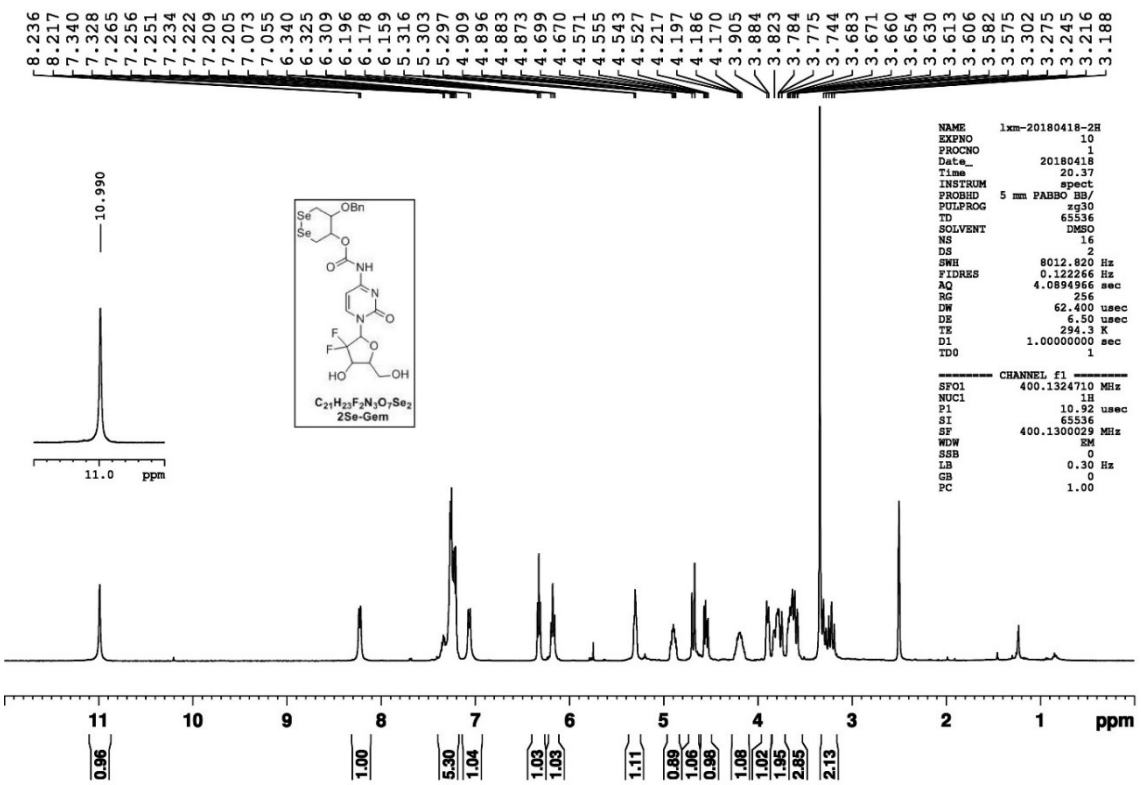


Figure S40. ^{1}H NMR Spectra of **Se6-Gem** in DMSO-d_6 (400 MHz).

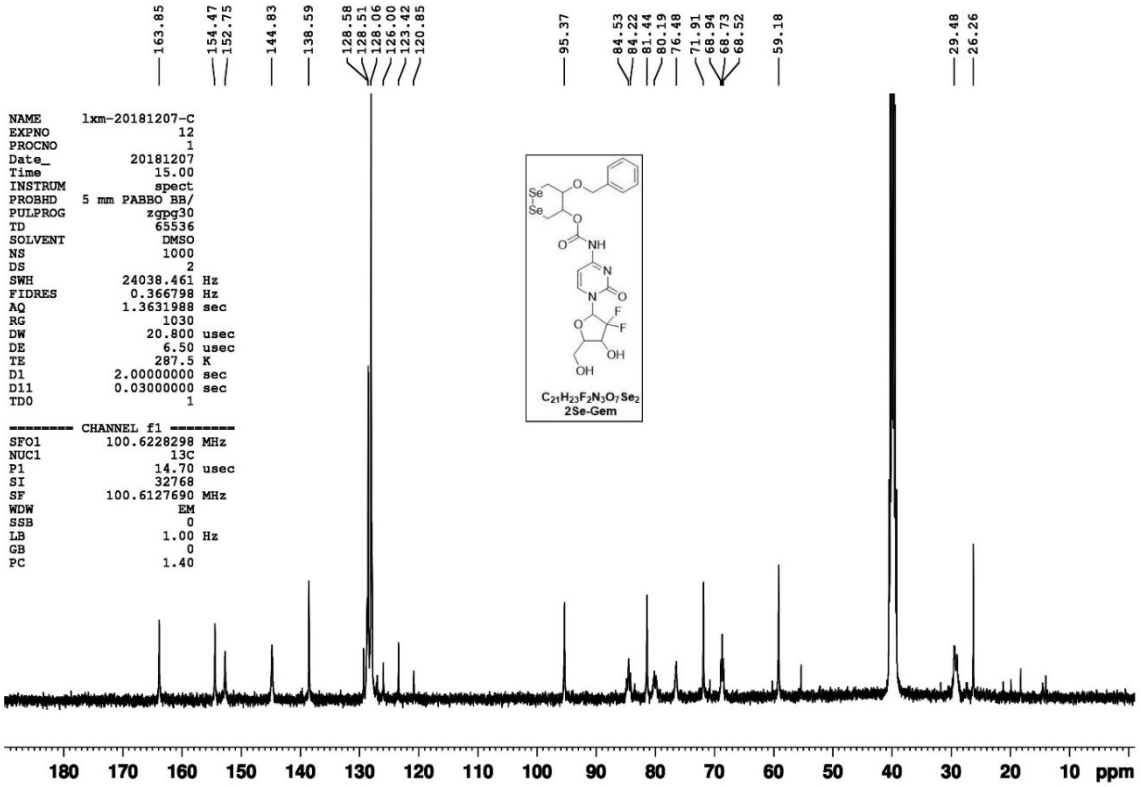


Figure S41. ^{13}C NMR Spectra of **Se6-Gem** in DMSO-d_6 (100 MHz).

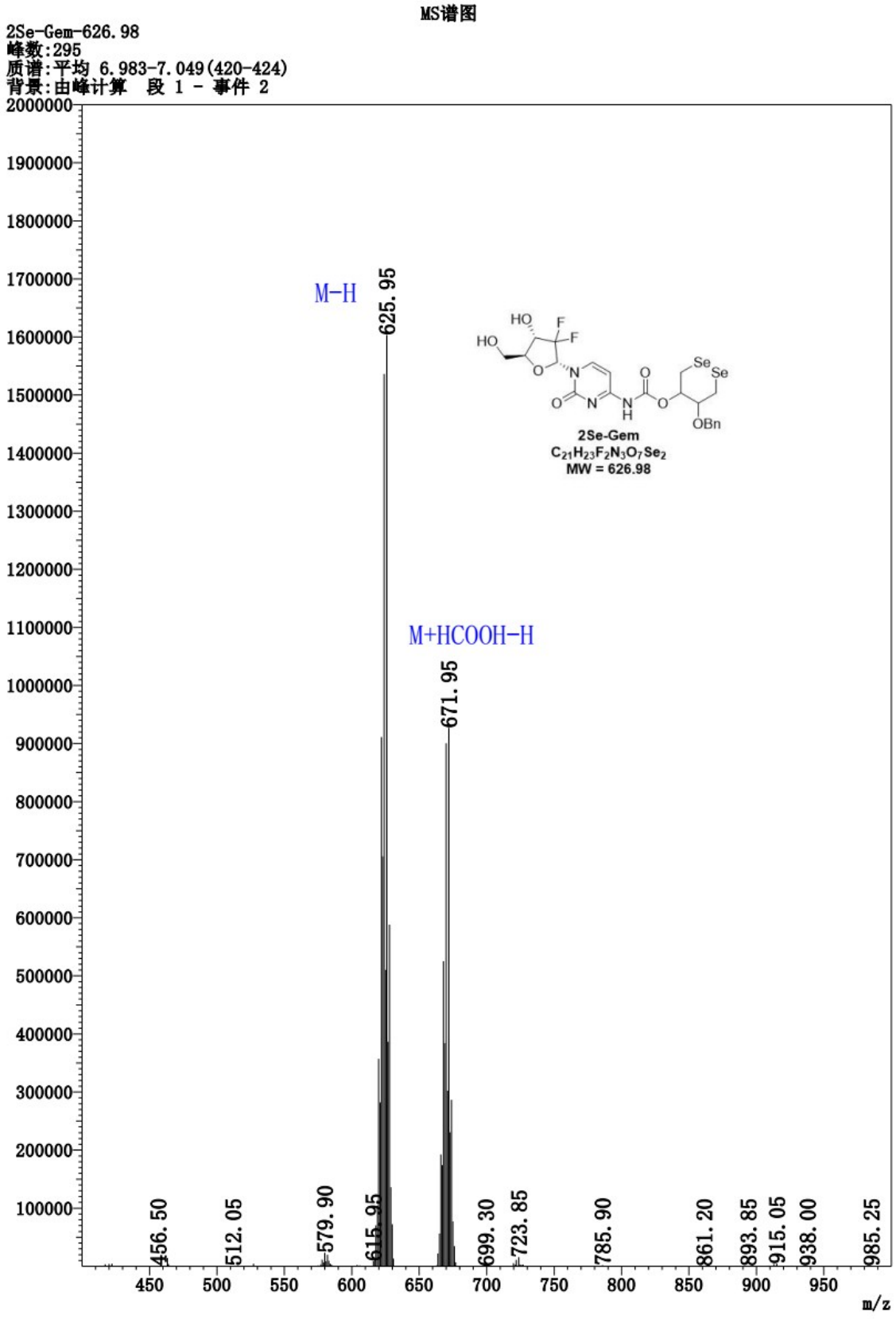


Figure S42. MS Spectra of **Se6-Gem** (ESI).

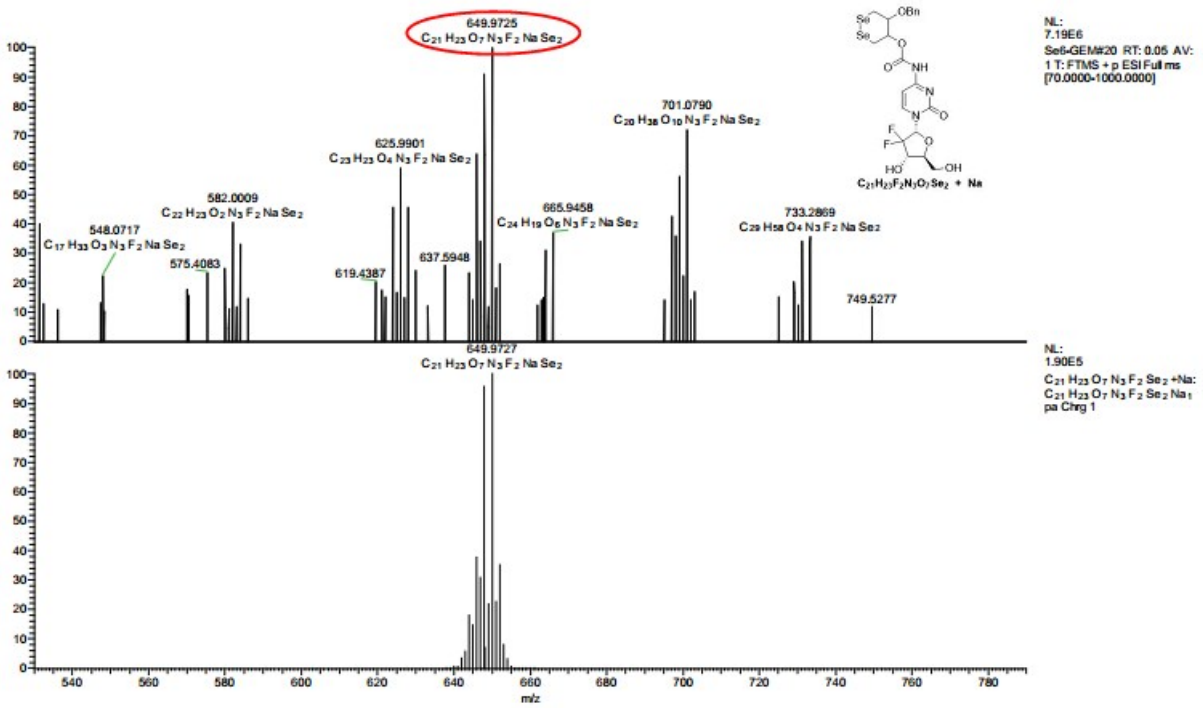


Figure S43. HRMS Spectra of **Se6-Gem** (ESI).

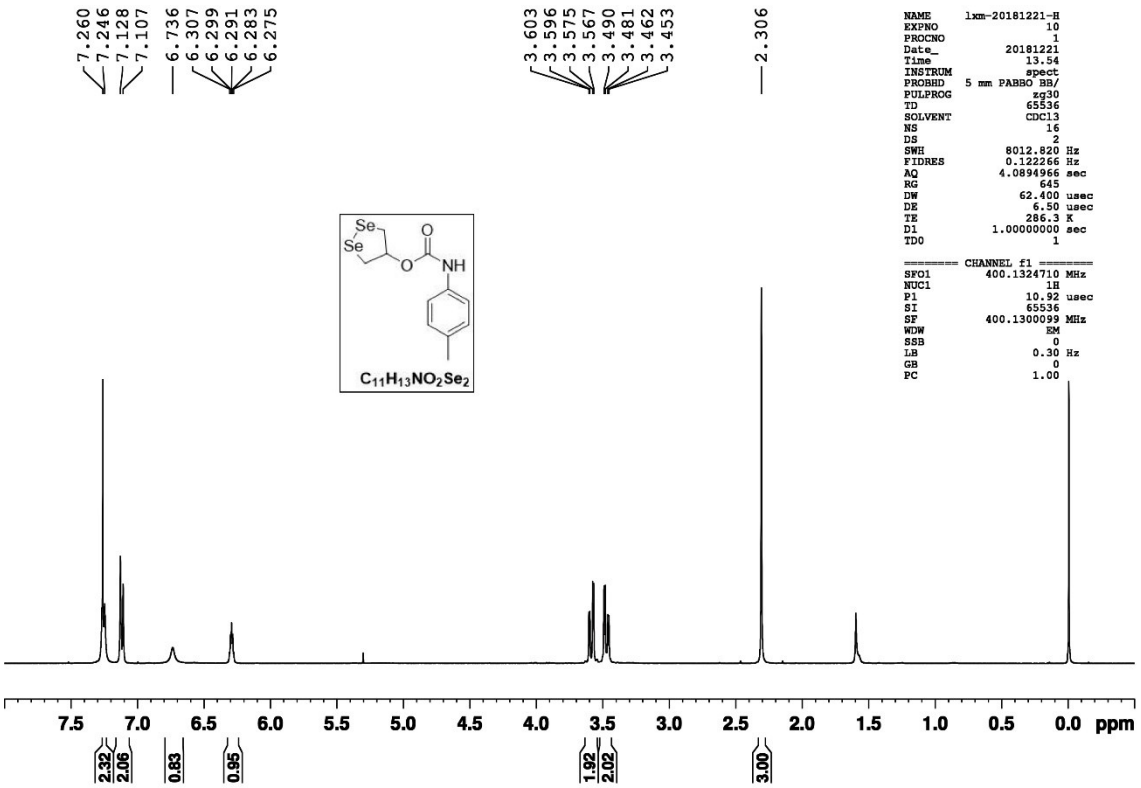


Figure S44. ^{1}H NMR Spectra of **Se-Toluidine** in $CDCl_3$ (400 MHz).

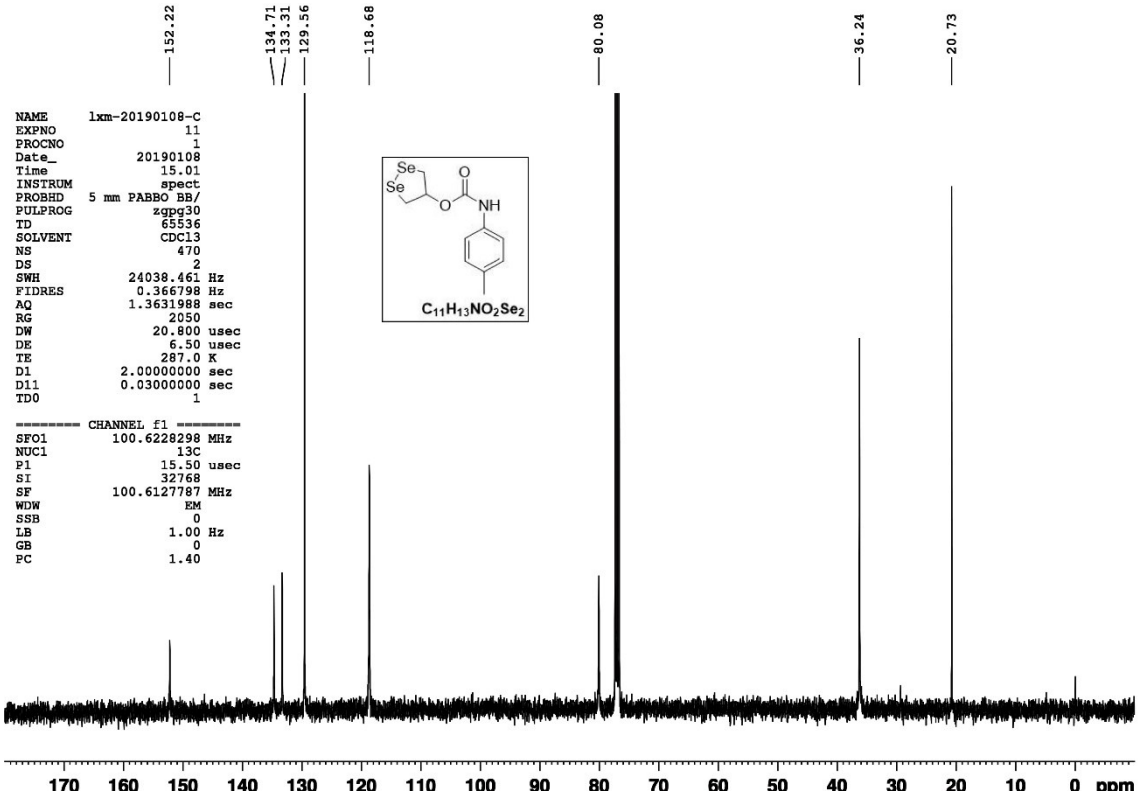


Figure S45. ^{13}C NMR Spectra of **Se-Toluidine** in $CDCl_3$ (100 MHz).

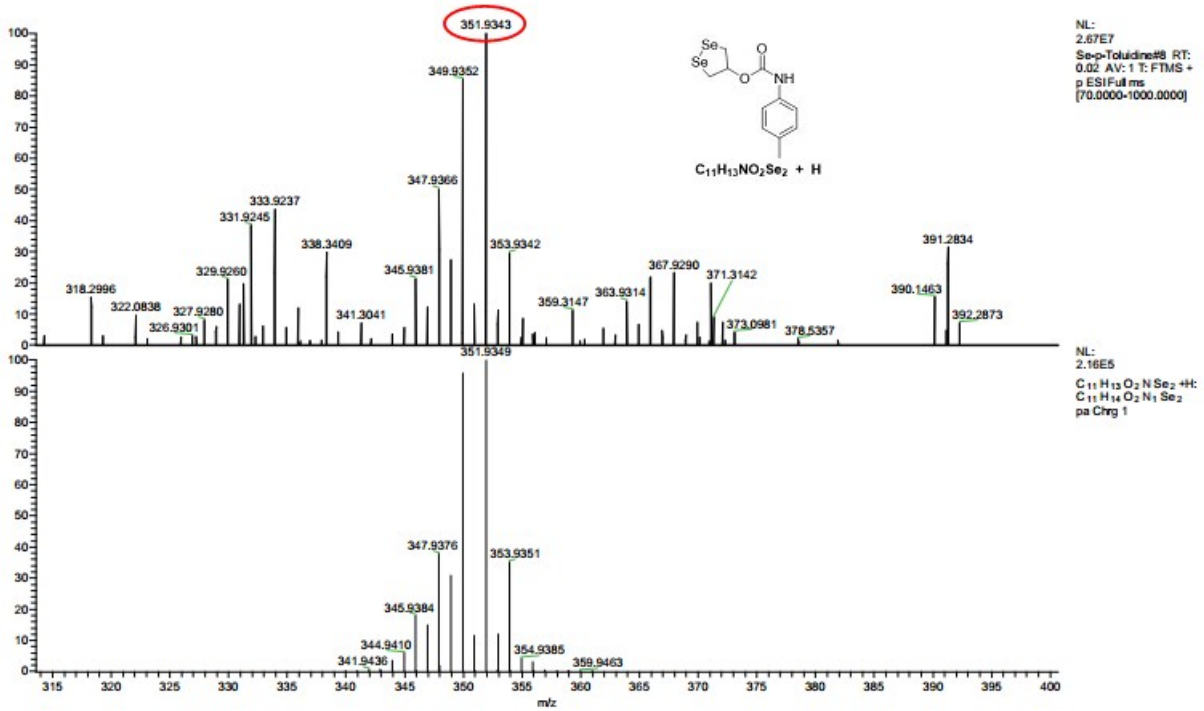


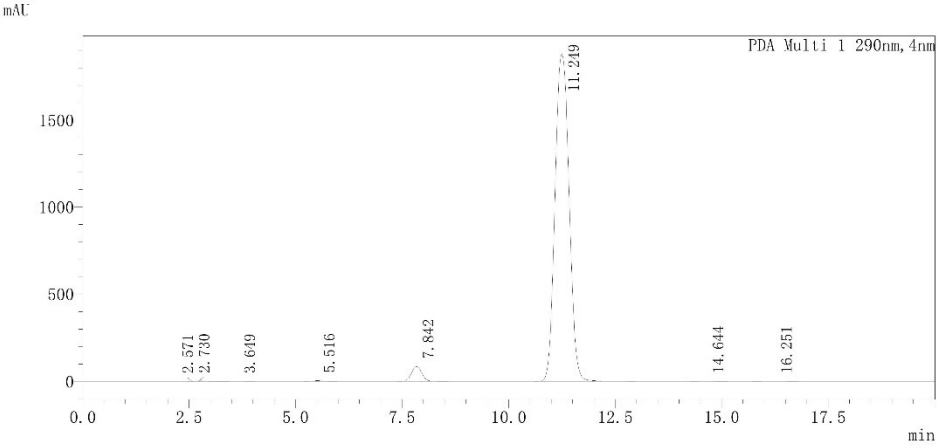
Figure S46. HRMS Spectra of **Se-Toluidine** (ESI).

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: C-Gem		
样品ID	: 20200114		
数据文件	: c-gem1-.lcd		
方法文件	: hou.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/14 21:34:27	分析者	: System Administrator
刷新日期	: 2020/1/14 21:54:29	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 290nm

峰号	保留时间	面积	浓度
1	2.571	1122	0.003
2	2.730	36332	0.081
3	3.649	17331	0.039
4	5.516	51453	0.115
5	7.842	1437236	3.206
6	11.249	43258074	96.492
7	14.644	9292	0.021
8	16.251	19929	0.044
总计		44830768	

C:\Users\Administrator\Desktop\lxm\c-gem1-.lcd

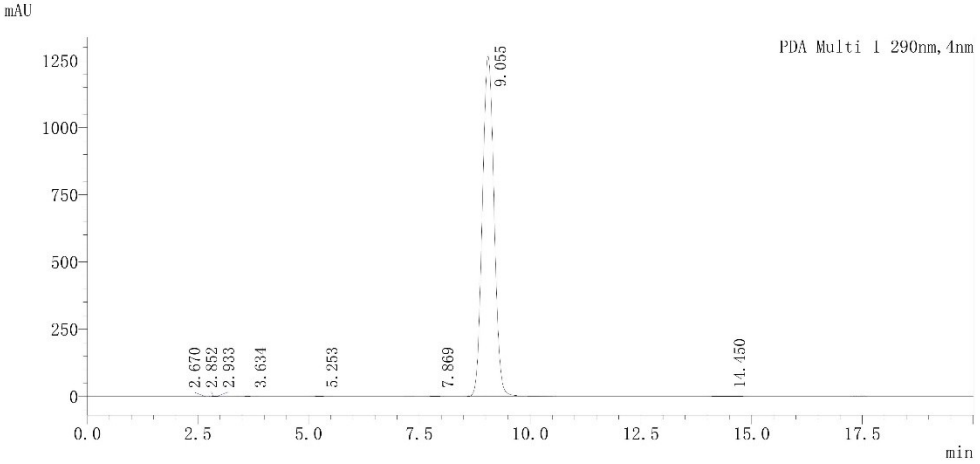
Figure S47. HPLC analysis of the purity of C-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: C6-Gem		
样品ID	: 20200114		
数据文件	: c6-gem2.lcd		
方法文件	: 1111.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/15 23:16:30	分析者	: System Administrator
刷新日期	: 2020/1/15 23:36:32	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 290nm

峰号	保留时间	面积	浓度
1	2.670	2096	0.009
2	2.852	4528	0.019
3	2.933	1884	0.008
4	3.634	3396	0.015
5	5.253	5204	0.022
6	7.869	1664	0.007
7	9.055	23264385	99.849
8	14.450	16383	0.070
总计		23299541	

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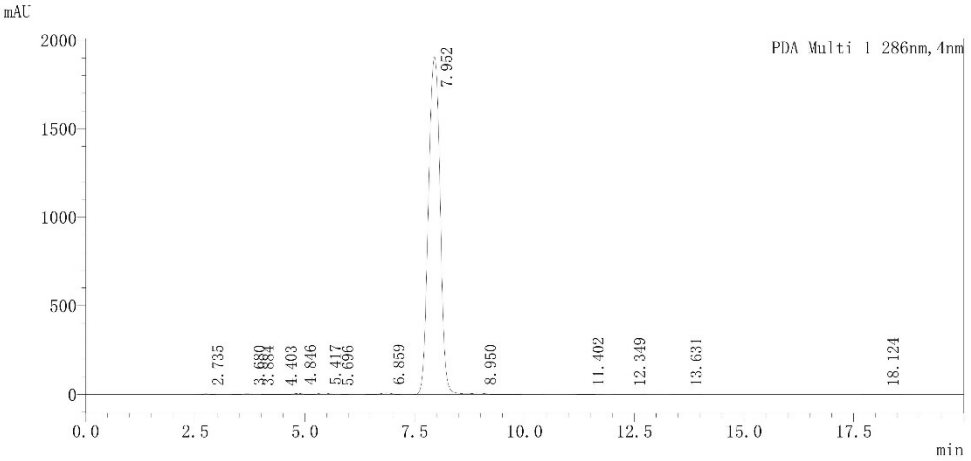
Figure S48. HPLC analysis of the purity of C6-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: S-Gem		
样品ID	: 20200114		
数据文件	: s-gem3.lcd		
方法文件	: 1111.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/15 21:31:53	分析者	: System Administrator
刷新日期	: 2020/1/15 21:51:55	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 286nm

峰号	保留时间	面积	浓度
1	2.735	38149	0.106
2	3.680	58625	0.163
3	3.884	40582	0.113
4	4.403	11444	0.032
5	4.846	88179	0.245
6	5.417	140773	0.390
7	5.696	34995	0.097
8	6.859	156383	0.434
9	7.952	35258162	97.779
10	8.950	191046	0.530
11	11.402	20502	0.057
12	12.349	5405	0.015
13	13.631	5634	0.016
14	18.124	9098	0.025
总计		36058978	

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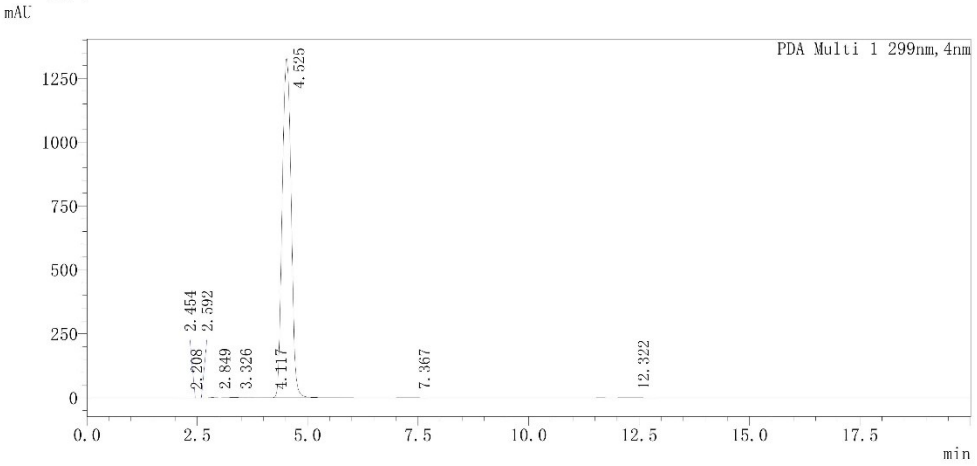
Figure S49. HPLC analysis of the purity of S-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: S6-Gem		
样品ID	: 20200114		
数据文件	: s6-gem6.lcd		
方法文件	: segem.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/18 0:32:02	分析者	: System Administrator
刷新日期	: 2020/1/18 0:52:04	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 299nm

峰号	保留时间	面积	浓度
1	2.208	1798	0.010
2	2.454	1576	0.008
3	2.592	3472	0.018
4	2.849	13829	0.074
5	3.326	34973	0.186
6	4.117	3546	0.019
7	4.525	18726540	99.548
8	7.367	13753	0.073
9	12.322	12111	0.064
总计		18811599	

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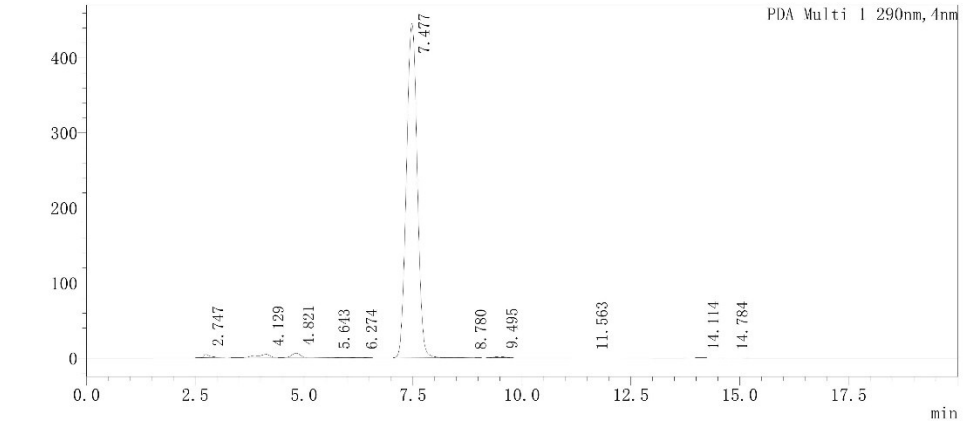
Figure S50. HPLC analysis of the purity of S6-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: Se-Gem		
样品ID	: 20200114		
数据文件	:		
方法文件	: hou.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/14 19:56:26	分析者	: System Administrator
刷新日期	: 2020/1/14 20:46:46	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 290nm

峰号	保留时间	面积	浓度
1	2.747	44682	0.561
2	4.129	84058	1.055
3	4.821	79947	1.004
4	5.643	6162	0.077
5	6.274	10348	0.130
6	7.477	7693594	96.582
7	8.780	8817	0.111
8	9.495	25755	0.323
9	11.563	3460	0.043
10	14.114	6107	0.077
11	14.784	2936	0.037
总计		7965866	

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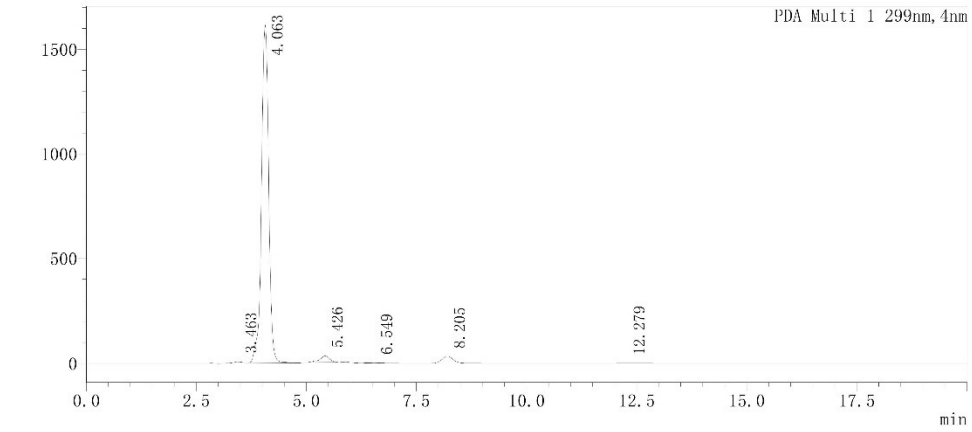
Figure S51. HPLC analysis of the purity of Se-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: Se6-Gem		
样品ID	: 20200114		
数据文件	: se6-gem14.lcd		
方法文件	: segem.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/18 1:44:35	分析者	: System Administrator
刷新日期	: 2020/1/18 2:04:38	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 299nm

峰号	保留时间	面积	浓度
1	3.463	25213	0.131
2	4.063	18335203	95.183
3	5.426	412932	2.144
4	6.549	11094	0.058
5	8.205	471258	2.446
6	12.279	7458	0.039
总计		19263159	

C:\Users\Administrator\Desktop\lxm\se6-gem14.lcd

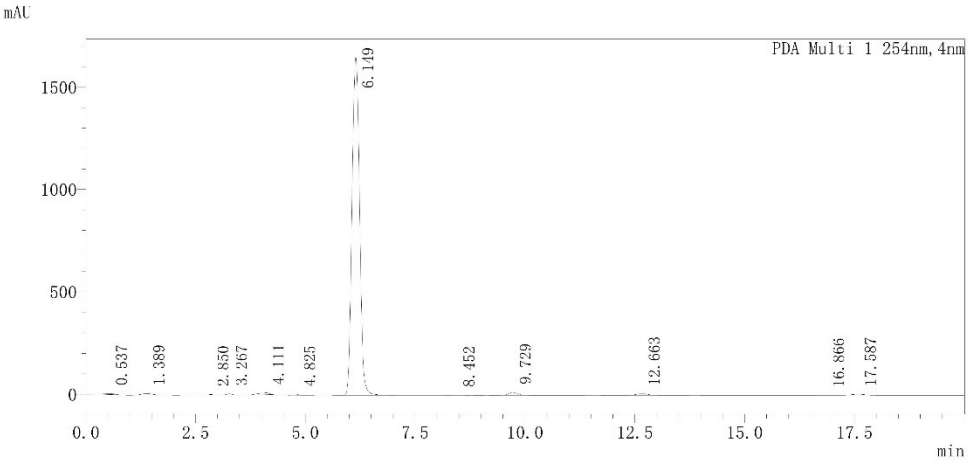
Figure S52. HPLC analysis of the purity of Se6-Gem.

SHIMADZU LabSolutions 分析报告

〈样品信息〉

样品名	: Se-p-Toluidine		
样品ID	: 20200114		
数据文件	: se-p-t2.lcd		
方法文件	: 1111.lcm		
批处理文件	:		
样品瓶号	: 0-0	样品类型	: 未知
进样体积	: 10 uL		
分析日期/时间	: 2020/1/16 0:11:28	分析者	: System Administrator
刷新日期	: 2020/1/16 0:31:30	处理者	: System Administrator

〈色谱图〉



峰表

PDA Ch1 254nm

峰号	保留时间	面积	浓度
1	0.537	77452	0.376
2	1.389	74057	0.360
3	2.850	5589	0.027
4	3.267	33542	0.163
5	4.111	95152	0.462
6	4.825	22122	0.108
7	6.149	19831317	96.378
8	8.452	26814	0.130
9	9.729	182150	0.885
10	12.663	152754	0.742
11	16.866	15937	0.077
12	17.587	59693	0.290
总计		20576580	

C:\Users\Administrator\Desktop\lxm\se-p-t2.lcd

Figure S53. HPLC analysis of the purity of Se-Toluidine.