Supporting Information For:

Intercepting triazine dendrimer synthesis with nucleophilic pharmacophores as a general strategy toward drug delivery vehicles

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TABLE OF CONTENTS

Materials and Instrumentation. All reagents were procured from Sigma-Aldrich (St. Louis, MO) and used as received without further purification. NHS-mPEG was purchased from NOF American Corporation. Amicon filters (YM-3: MWCO 3kDa) were purchased from Millipore. Size exclusion chromatography (SEC) was carried out using a Waters Delta 600 system and a Waters 2414 refractive index detector. A Suprema 10 micron GPC analytical column (1000 Å, 8 x 300mm) was used with 0.1 M $NaNO_3$ as the eluent and a flow rate of 1 mL/min. NMR spectra were recorded on a Varian Mercury 300 MHz spectrometer in $CDCl_3$, or DMSO-d6. All mass spectral analyses were carried out by the Laboratory for Biological Mass Spectrometry at Texas A&M University. MCF-7 (human breast cancer) cells were purchased from ATCC. Cells were maintained in phenol red free DMEM (Sigma) containing 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2 atmosphere. HT-29 (human colon cancer) cells were purchased from ATCC. Cells were maintained in phenol red free DMEM F-12 (Sigma) containing 10 % FBS and 1% 1M HEPES.

Synthesis of BOC-Inp-CPT(3): A solution of 1.40 g BOC-Inp (6.11 mmol) in 50 mL dichloromethane stirred as 3.55 g 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (18.5 mmol) and 0.78 g N,N-dimethylaminopyridine (6.38 mmol)were added. After stirring for 30 minutes, 1.50 g camptothecin (4.31 mmol) was added and stirring continued for 18 h. The solution was concentrated *in vacuo* and the residue was precipitated with methanol to yield a yellow solid. Yield: 2.34 g, (4.18 mmol), 97%. ESI MS: calcd. mass for $(C_{31}H_{33}N_3O_7)^+$ 560.2398, found 560.2537. ^{1}H NMR (300MHz, $CDCl_3$): 0.98 (t, 3H), 1.43 (s, 9H), 1.71 (m, 3H), 1.97 (t, 2H), 2.15 (m, 1H), 2.27 (m,1H), 2.68 (m, 1H), 2.94 (m,2H), 3.98 (m,2H), 5.28 (s, 2H), 5.40 (d,1H), 5.68 (d, 1H), 7.21 (s, 1H), 7.68 (t,1H),

7.84 (t, 1H), 7.95 (d, 1H), 8.24 (d, 1H), 8.41 (s,1H). ^{13}C NMR (75MHz, $CDCl_3$): 8.3, 28.0, 28.2, 28.7, 30.9, 51.0, 67.0, 76.4, 79.4, 95.2, 119.5, 128.4, 128.7, 129.3, 129.6, 130.5, 131.1, 132.3, 146.1, 146.8, 148.5, 153.0, 154.5, 157.2, 167.8, 173.6.

Synthesis of Inp-CPT (4): A solution of 1.01 g **3** (1.80 mmol) was stirred in 20 mL dichloromethane as 20 mL trifluoroacetic acid was added slowly. The reaction continued to stir for 3 h and was concentrated in vacuo to yield a yellow residue, which was precipitated with methanol to yield the TFA salt as a yellow solid. Yield: 0.82 g (1.47 mmol), 82%. ESI MS: calcd. mass for $(C_{26}H_{27}N_3O_5)^+$ 460.1873, found 460.1827. ^{1}H NMR (300MHz, DMSO-d_6): 0.95 (t, 3H), 1.79 (m, 2H), 2.17 (m, 4H), 3.05 (m, 3H), 3.28 (t, 2H), 5.30 (s, 2H), 5.52 (s, 2H), 7.06 (s, 1H), 7.72 (t, 1H), 7.87 (t, 1H), 8.14 (t, 2H), 8.70 (s, 1H). ^{13}C NMR (75MHz, DMSO-d_6): 8.3, 24.8, 25.2, 30.8, 37.7, 42.8, 51.0, 67.0, 76.8, 95.2, 119.4, 128.5, 128.7, 129.3, 129.6, 130.5, 131.1, 132.3, 146.0, 146.8, 148.5, 153.0, 157.2, 167.9, 172.9.

Synthesis of BOC-G2-CPT (5): A solution of 54 mg of **4** (0.12 mmol) in 1 mL of N,N-dimethylformamide stirred as 22 mg second generation chlorotriazine dendrimer **8** (5.7 µmol) was added. The solution continued to stir as 40 µL of N,N-diisopropylethylamine was added to the reaction. The solution then heated to 80 ^{o}C for 48 h and was cooled to rt. The reaction was then concentrated *in vacuo* and the residue was taken up in chloroform and passed through a Sephadex LH-20 size exclusion chromatography column. The purified material was dried *in vacuo*. MALDI-TOF MS: calcd. mass for $(C_{327}H_{444}N_{84}O_{54})^+$ 6399.36, found 6413.79. ^{1}H NMR (300MHz, DMSO-d_6): 0.99 (m, 18H), 1.10-2.50 (m, 245H), 2.75 (br m, 5H), 3.04 (br m, 30H), 3.34 (br m, 12H), 3.57 (br m, 30H), 3.76 (br m, 32H), 4.56 (br m, 12H), 5.27 (br m, 12H), 5.37 (br d,

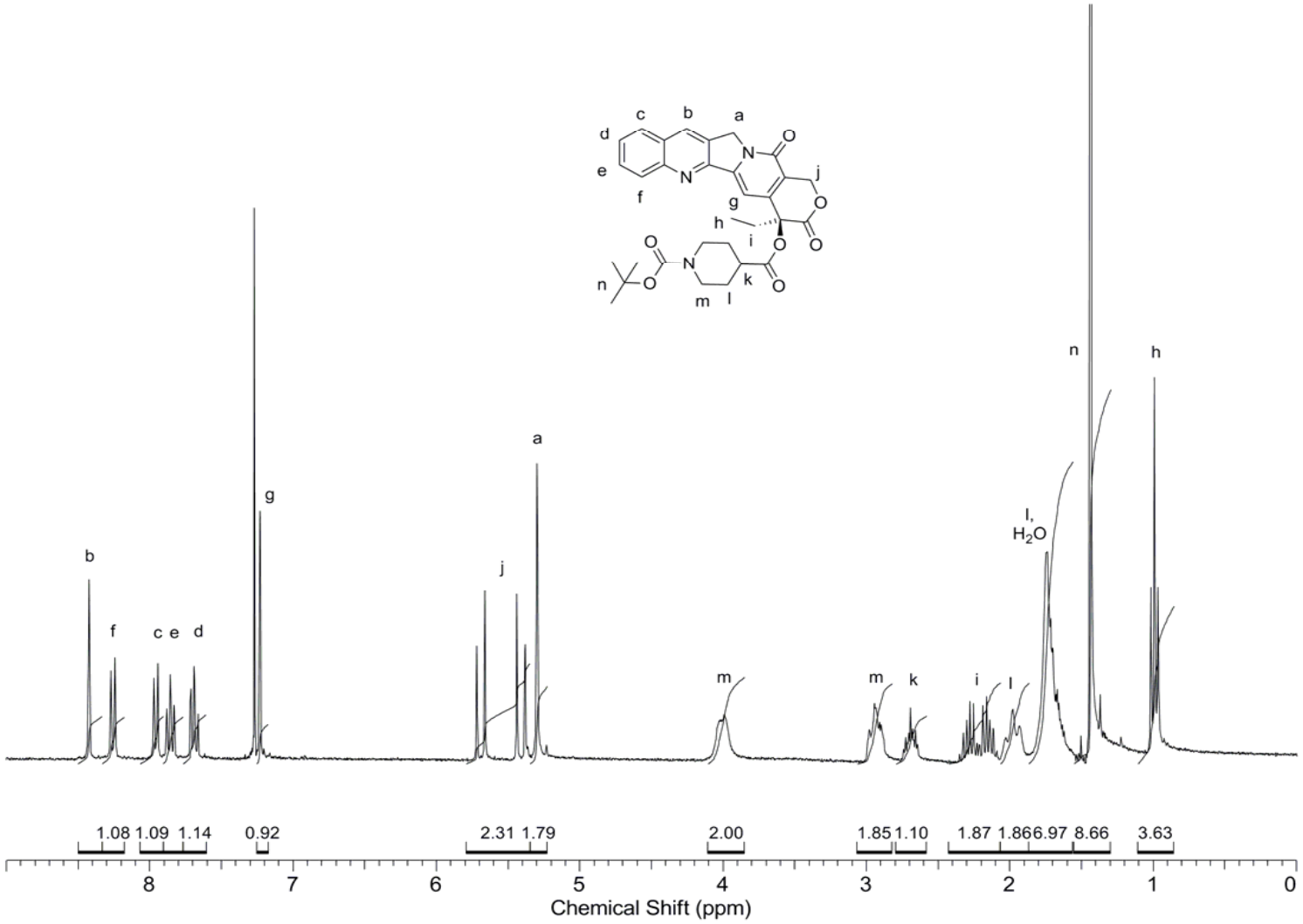
6H), 5.71 (br d, 6H), 7.19 (br m, 6H), 7.66 (br m, 6H), 7.82 (br m, 6H), 7.92 (br m, 6H), 8.20, (br m, 6H), 8.38 (br m, 6H). ^{13}C NMR (75MHz, DMSO-d_6): 7.6, 25.0, 25.8, 27.8, 28.5, 31.8, 37.2, 42.4, 42.7, 43.1 44.1, 49.9 67.0, 79.0, 95.7, 120.1, 128.1, 128.4, 129.5, 130.6, 131.2, 145.9, 146.3, 148.8, 152.2, 156.0, 157.3, 164.9, 165.2, 167.4.

Synthesis of G2-CPT (6): A solution of 25 mg of **5** (0.004 mmol) stirred in 2 mL dichloromethane as 1 mL trifluoroacetic acid was added. The solution stirred for 12 h and was concenctrated *in vacuo*. The residue was taken up in methanol and concentrated three times and used in the next step without further purification. MALDI-TOF MS: calcd. mass for $(C_{267}H_{336}N_{84}O_{30})^+$ 5202.07, found 5204.21. ^{1}H NMR (300MHz, $CDCl_3$): 0.95 (m, 18H), 1.10-2.50 (m, 245H), 2.88 (br m, 5H), 3.38 (br m, 12H), 3.61 (br m, 62H), 4.30 (br m, 12H), 5.24 (br m, 12H), 5.35 (br d, 6H), 5.57 (br d, 6H), 7.24 (br m, 6H), 7.64 (br m, 6H), 7.79 (br m, 6H), 7.93 (br m, 6H), 8.10, (br m, 6H), 8.43 (br m, 6H). ^{13}C NMR (75MHz, $CDCl_3$): 8.2, 26.0, 26.8, 28.0, 29.5, 30.8, 37.3, 42.4, 42.7, 43.0 44.1, 50.7 66.9, 76.5, 95.2, 119.4, 128.6, 129.3, 129.5, 131.0, 131.6, 132.7, 146.1, 146.7, 148.4, 152.8, 156.0, 157.3, 158.9, 159.3 165.3, 167.8, 173.6.

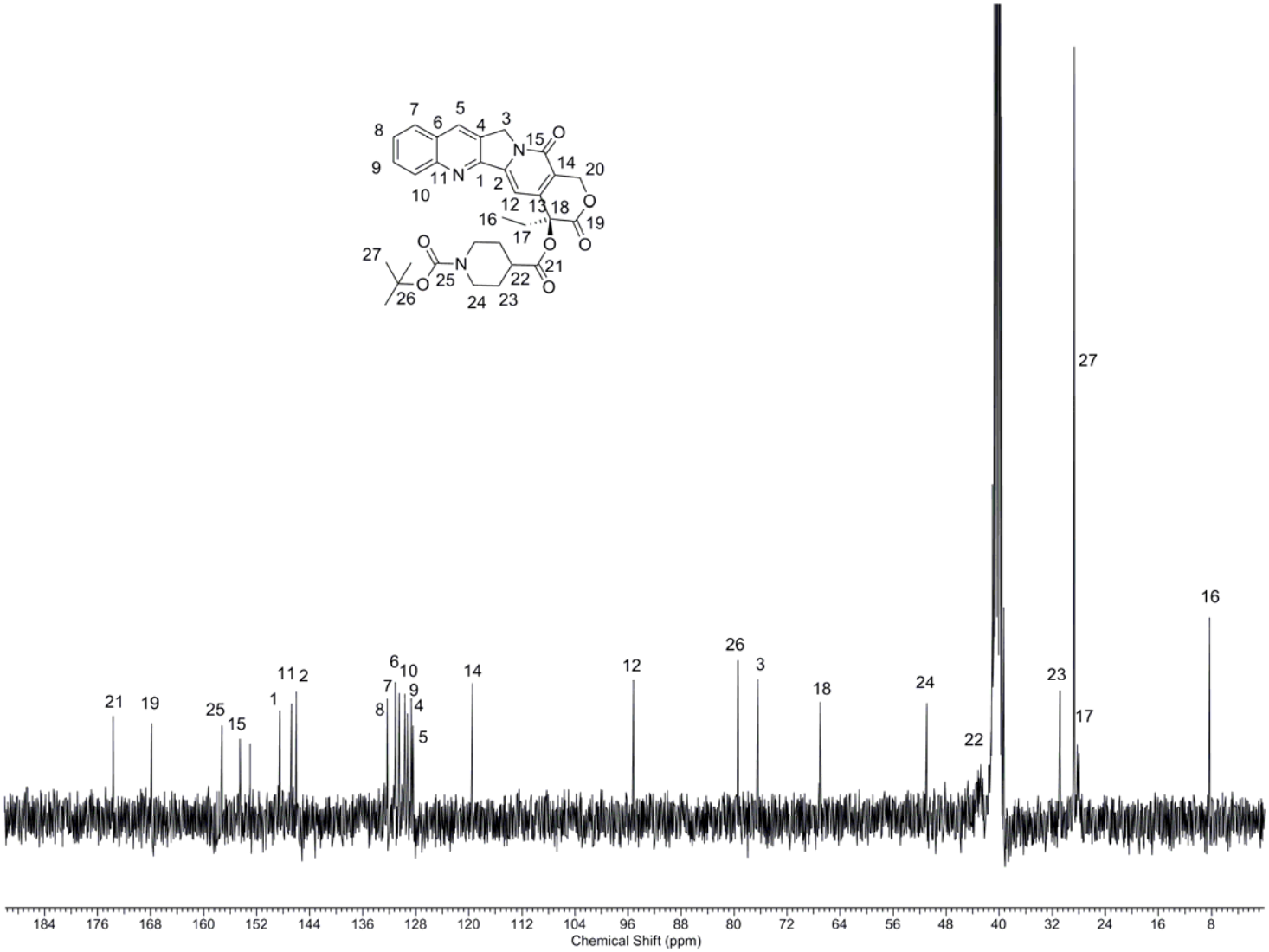
Synthesis of PEG2000-G2-CPT (7): A solution of 7.2 mg of **6** (0.002 mmol) in 4 mL dimethylformamide stirred as 445 mg NHS-mPEG_{2000} was added to the solution. Then, 20 µL of N,N-diisopropylethylamine was added to the solution and stirring continued for 48h and was concentrated in vacuo. The product was then purified with ultrafiltration using an Amicon stirred cell and YM3 regenerated cellulose membrane. Upon filtration of approximately 6 L of water, the retentate was collected and concentrated in vacuo to yield a yellow residue.

MTT Assay: *Cell Toxicity Assay.* Cells were plated at a density of 5,000 cells / well in 96 well plates. Plates were incubated for 24 hours at 37°C. Cells were treated with Control (DMSO), triton X-100 (all dead control), CPT, Irinotecan, G_2-CPT-PEG_{2000}, G_2-CPT, or $G2NH_2$ for 72 hours at 37°C. Treatment concentrations were as follows: 3% triton X-100 (all dead control), CPT (1µM-10mM), Irinotecan (1µM-50mM), G_2-CPT-PEG_{2000} (1µM-1mM), G_2-CPT (1µM-1mM), or $G2NH_2$ (1µM-1mM). Cell toxicity was determined using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay kit (Promega). For this analysis, 15 µl dye was added per well; plates were incubated for 4 hr at 37°C. Then a stop solution (100µl) was added and plates incubated for 1 hr at 37°C. Wells were mixed to create a uniform color and absorbance was read at 570 nm with a reference wavelength of 650 nm.

BOC-Inp-CPT: ^{1}H NMR



BOC-Inp-CPT: ^{13}C NMR



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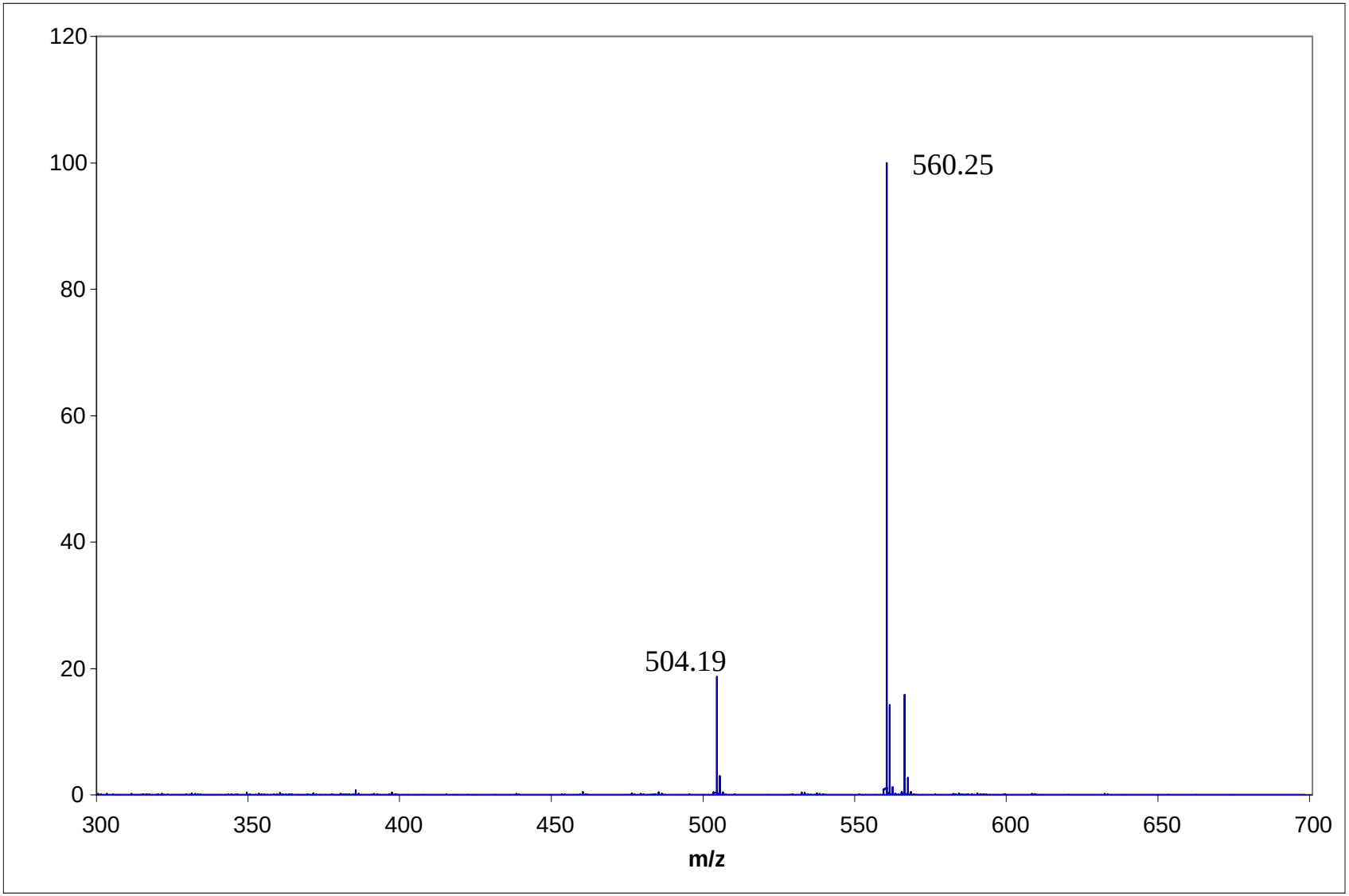

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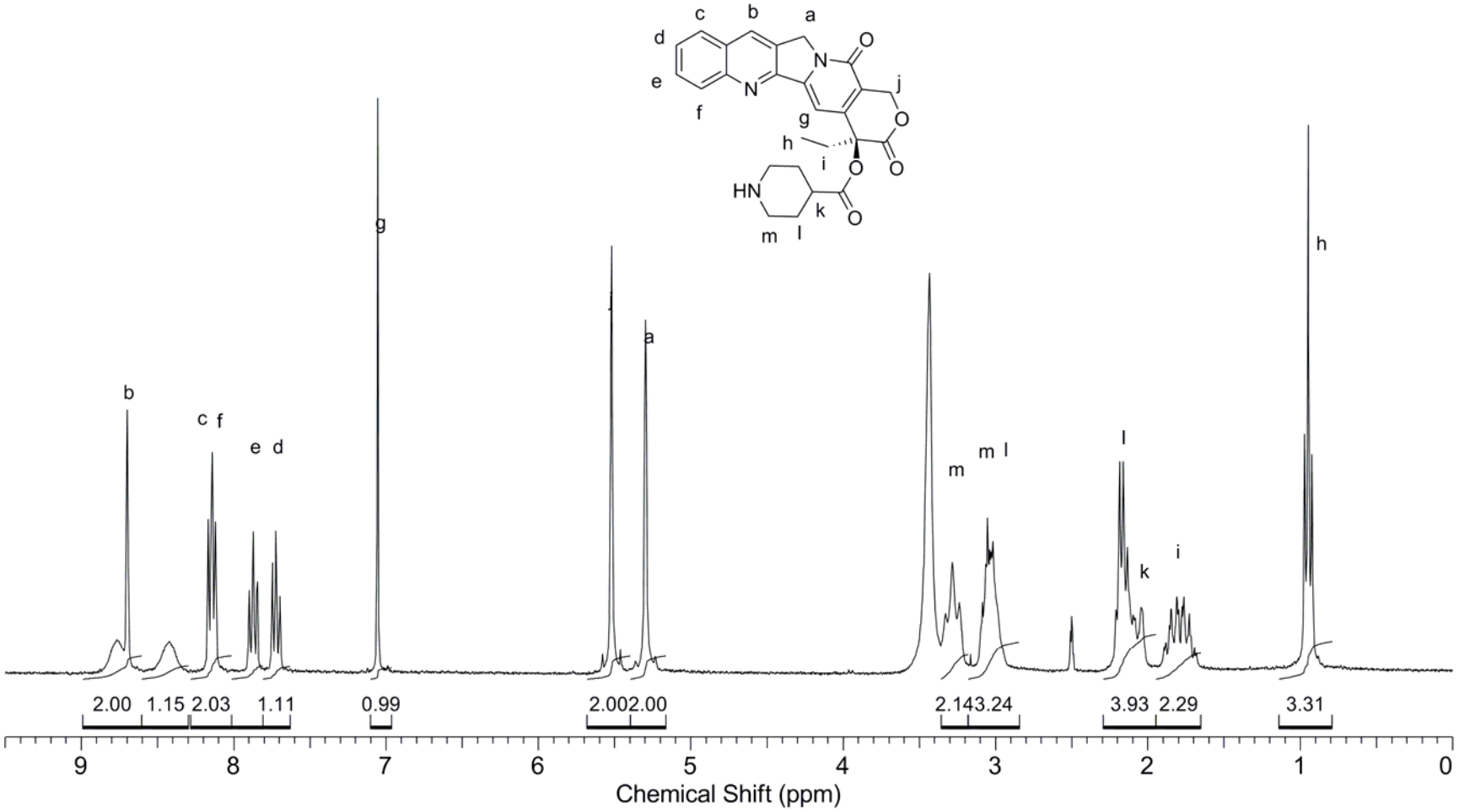

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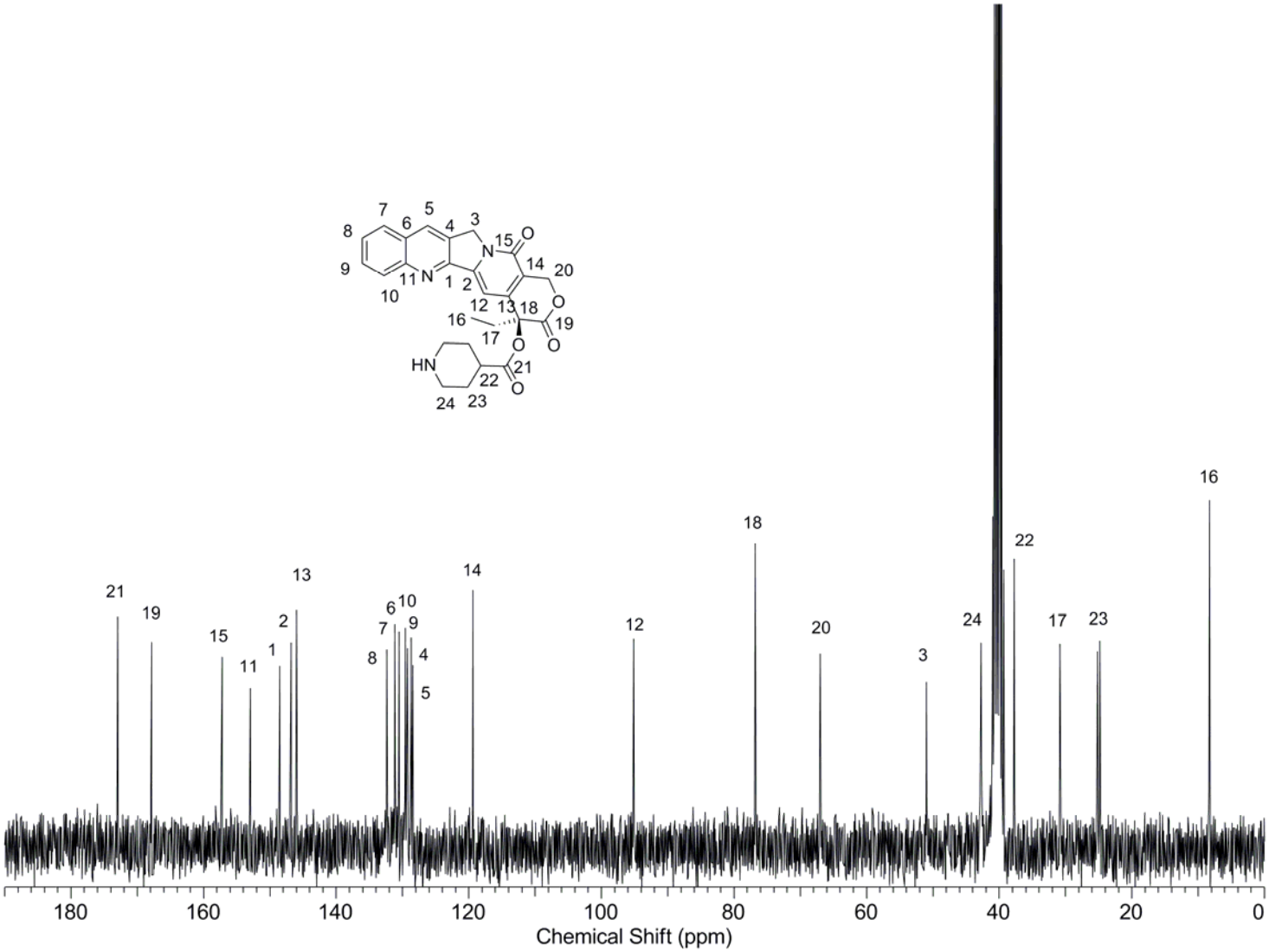
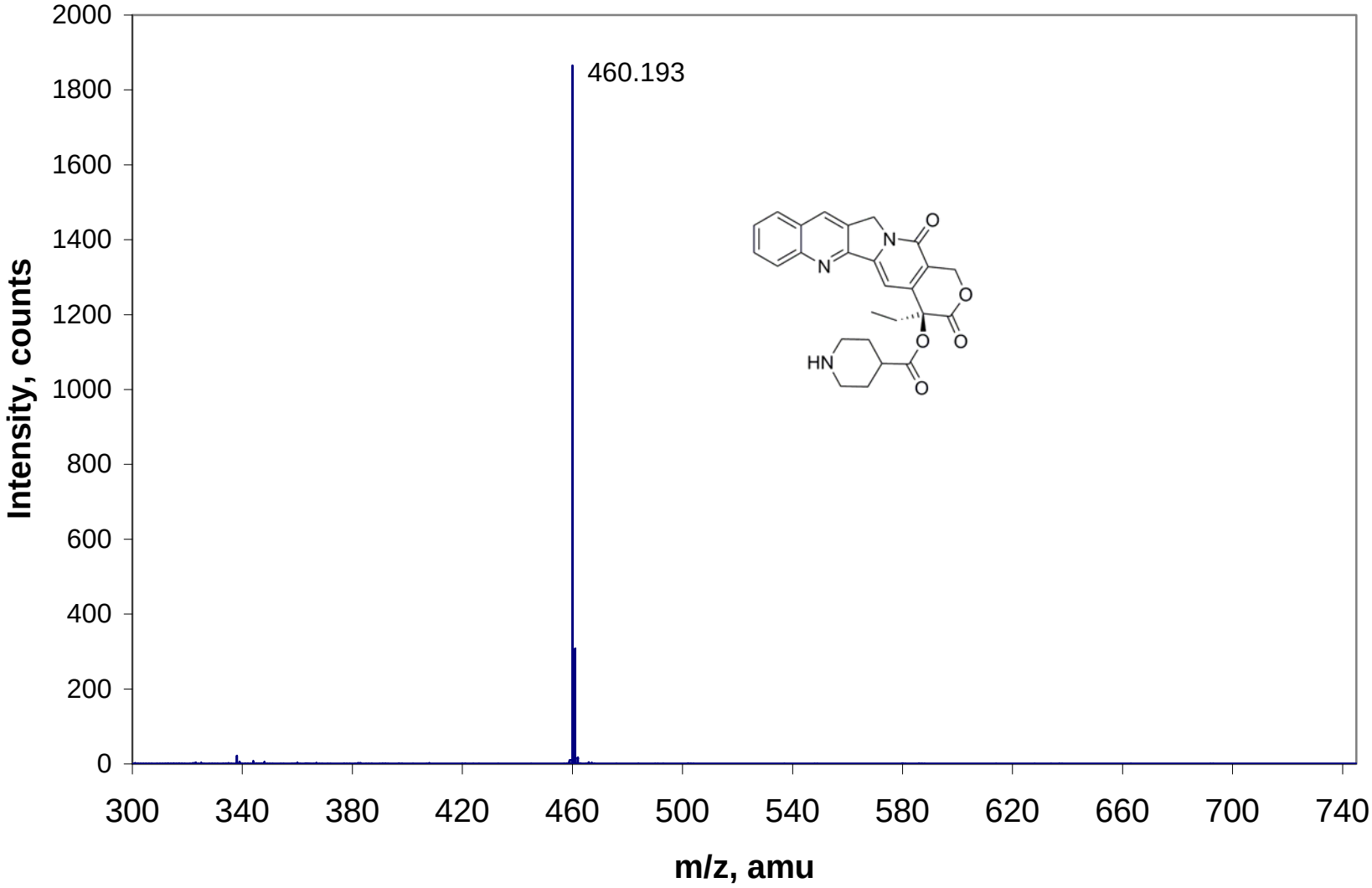
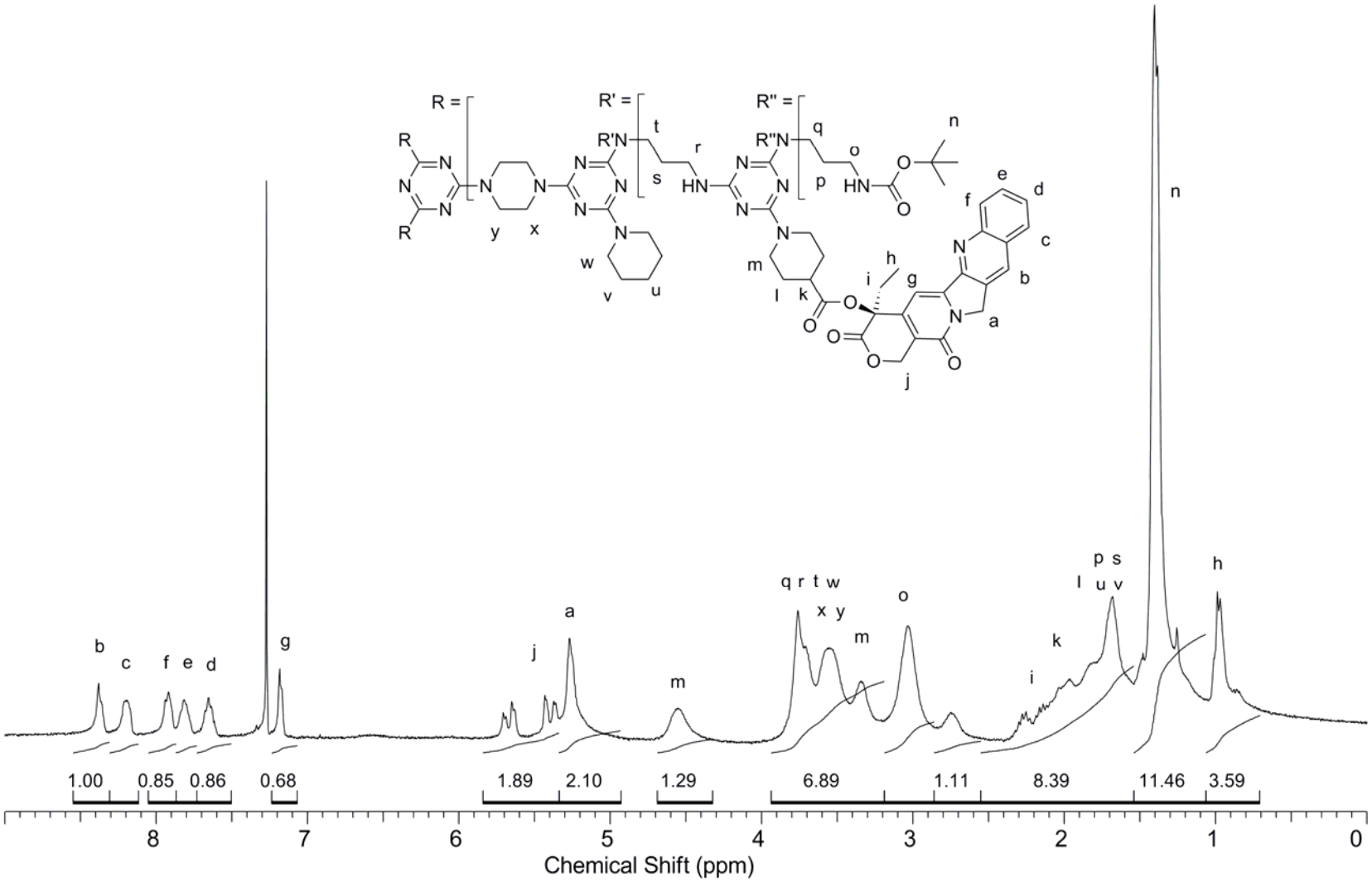


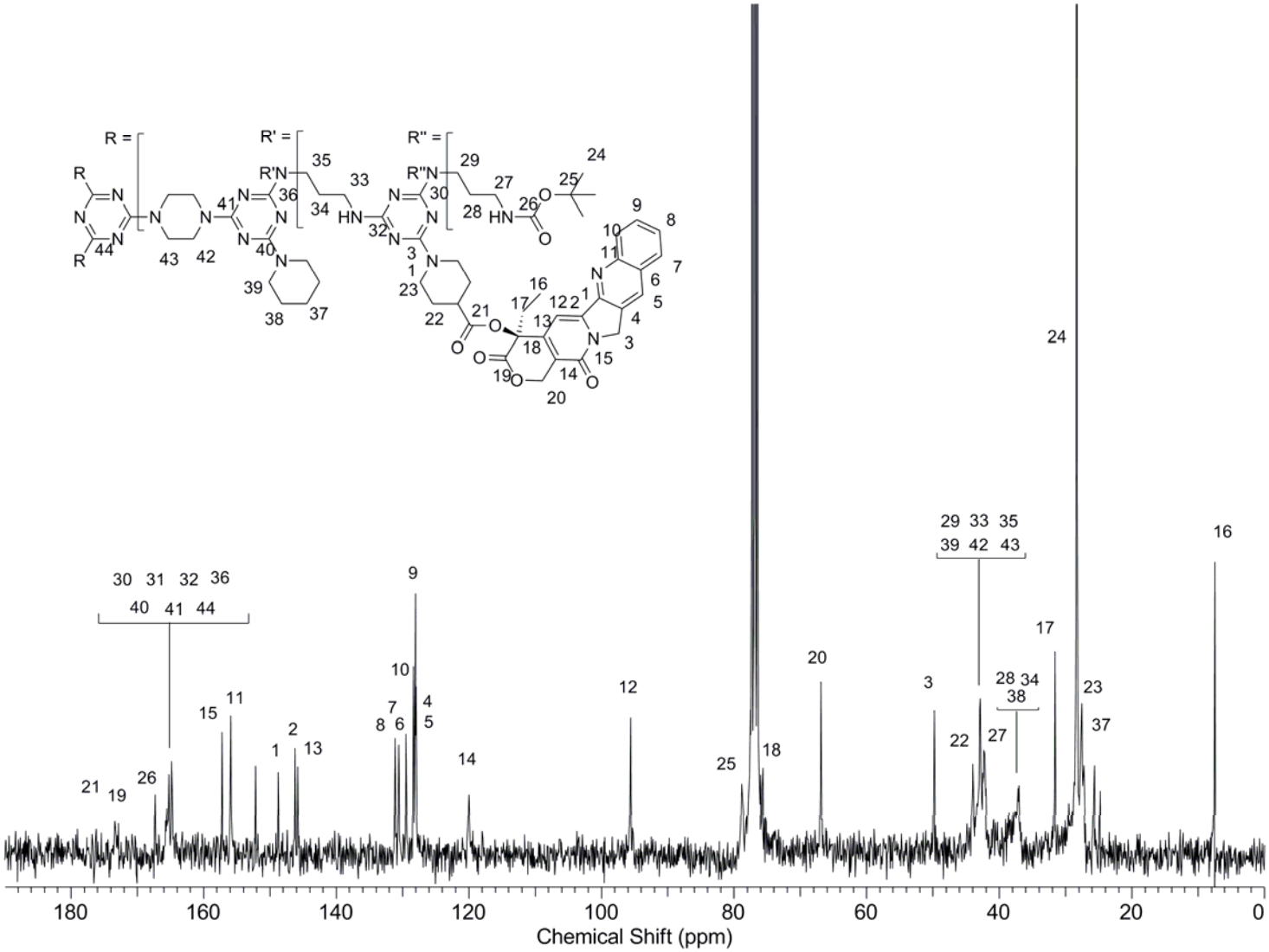
Inp-CPT: ESI MS



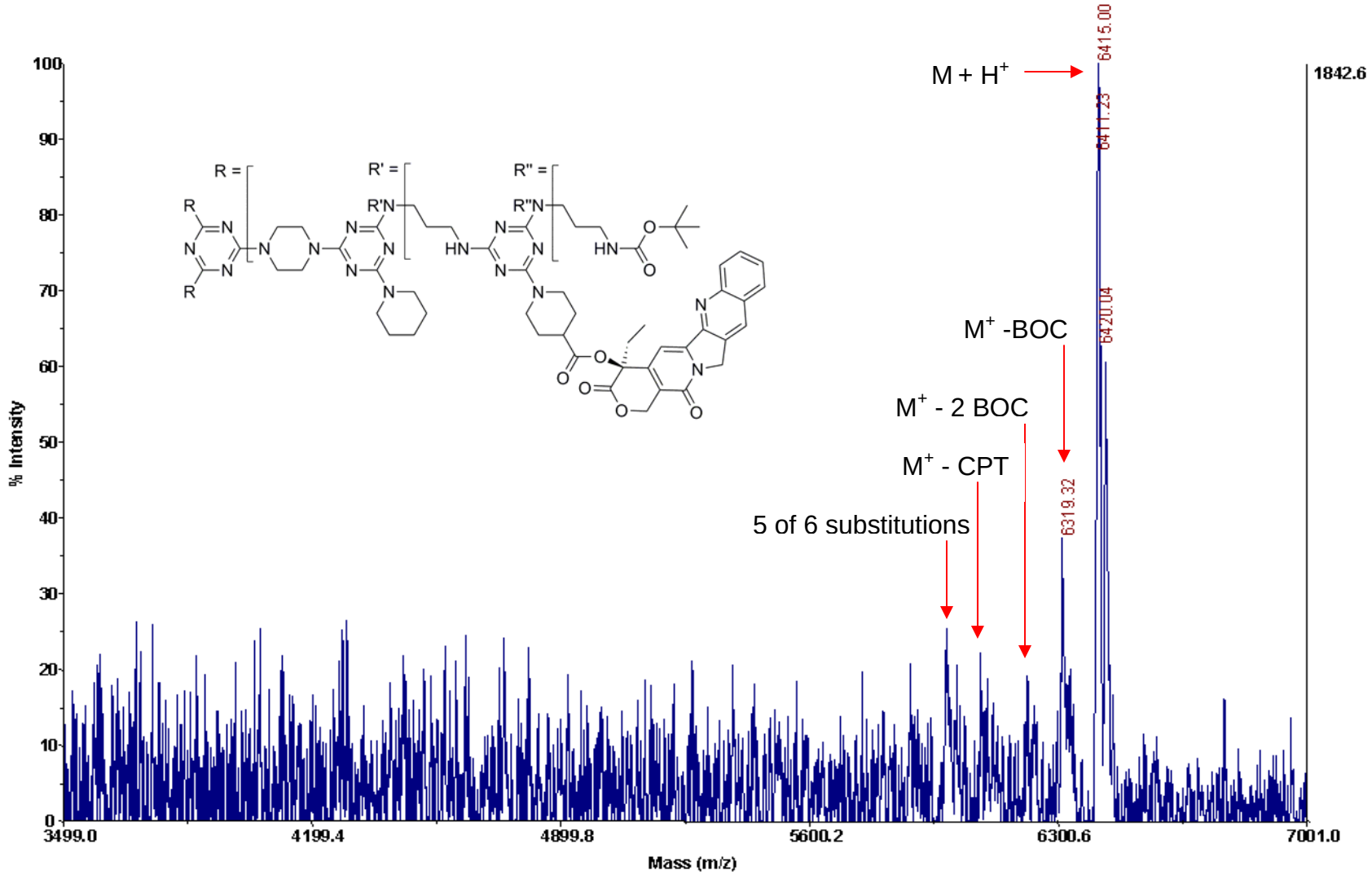
BOC-G2 -CPT: ^{1}H NMR



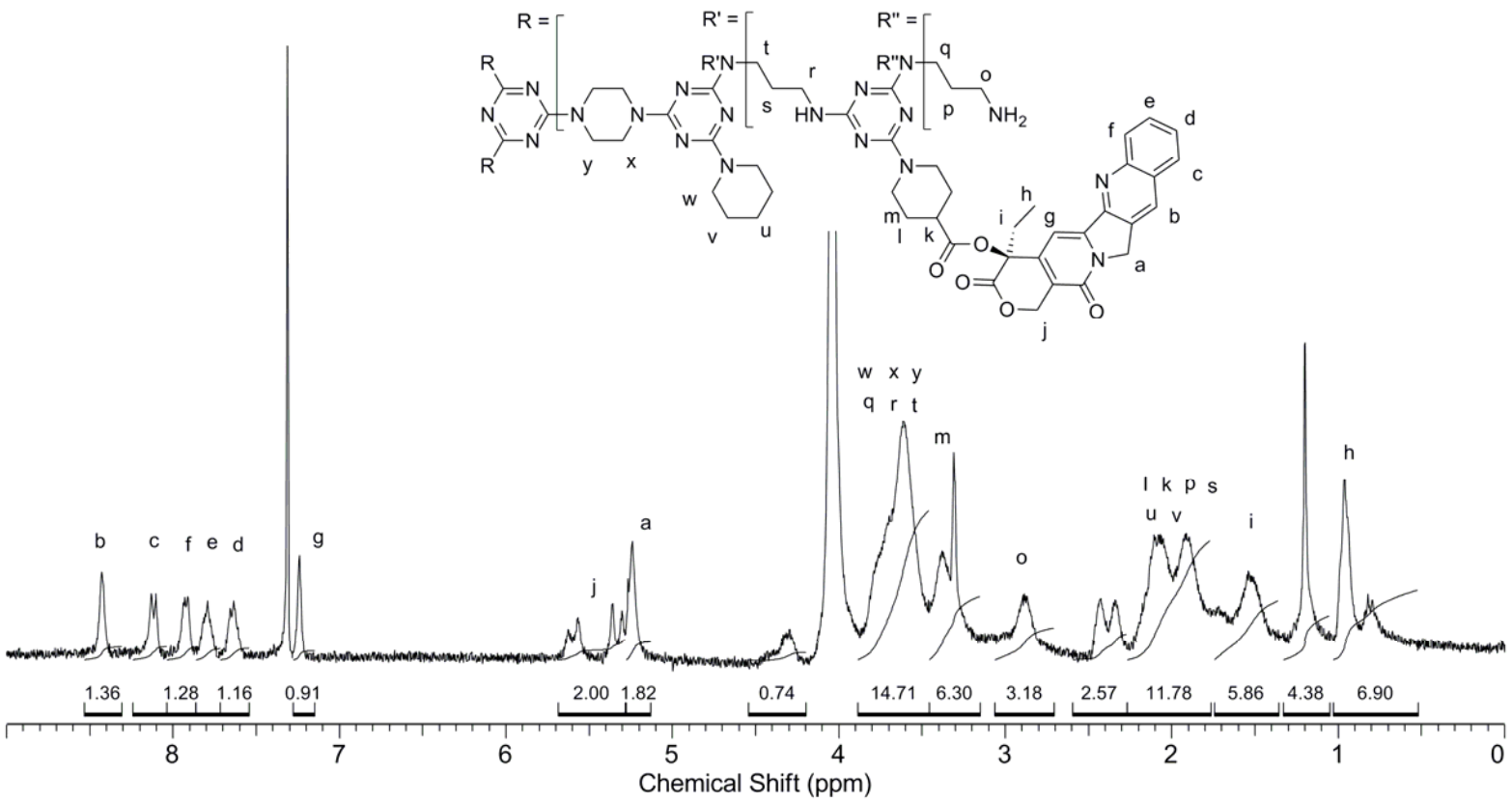
BOC-G2-CPT: ^{13}C NMR



BOC-G2 -CPT: MALDI TOF MS



G2-CPT: ^{1}H NMR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R" =
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m
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h
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1.28
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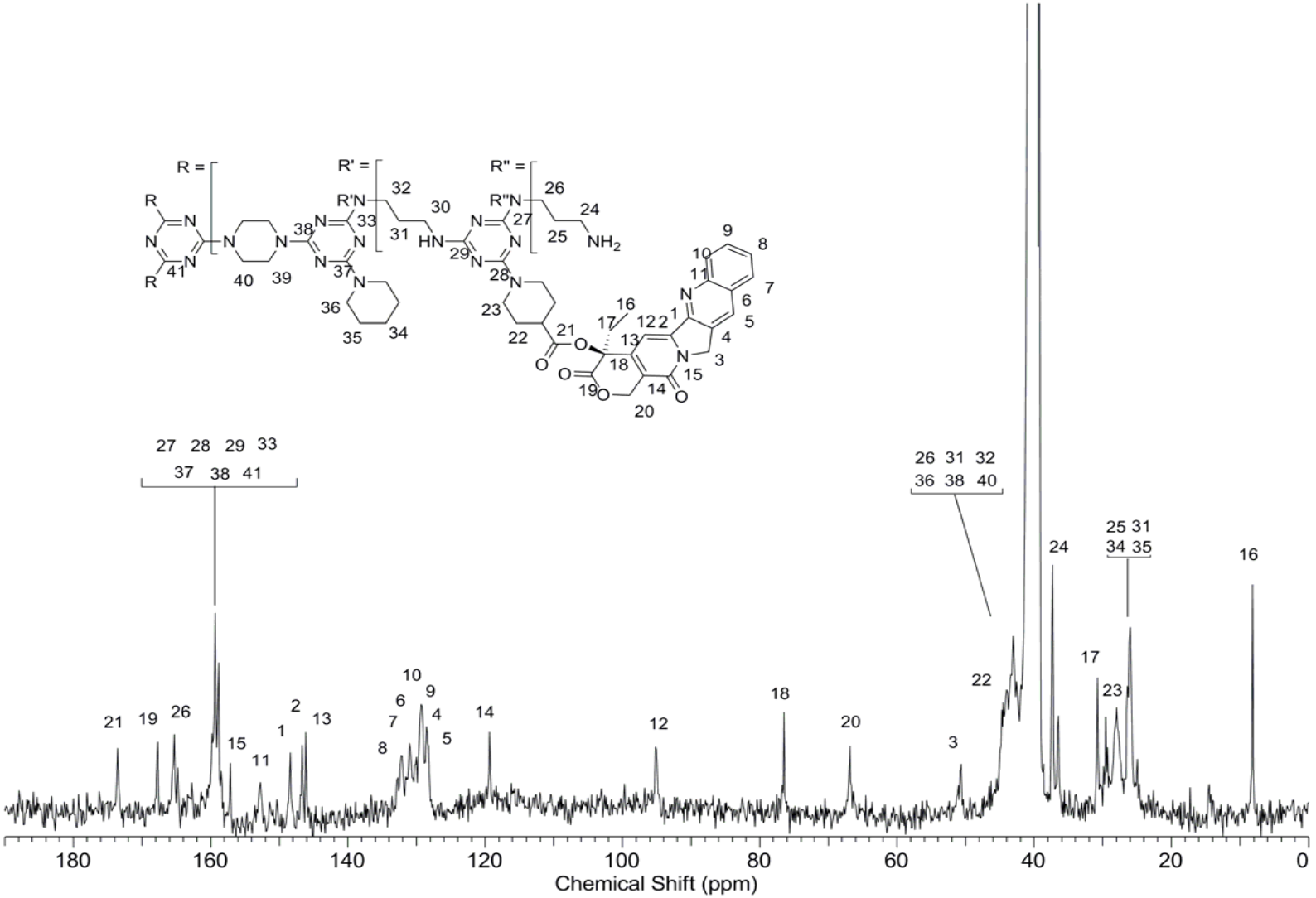
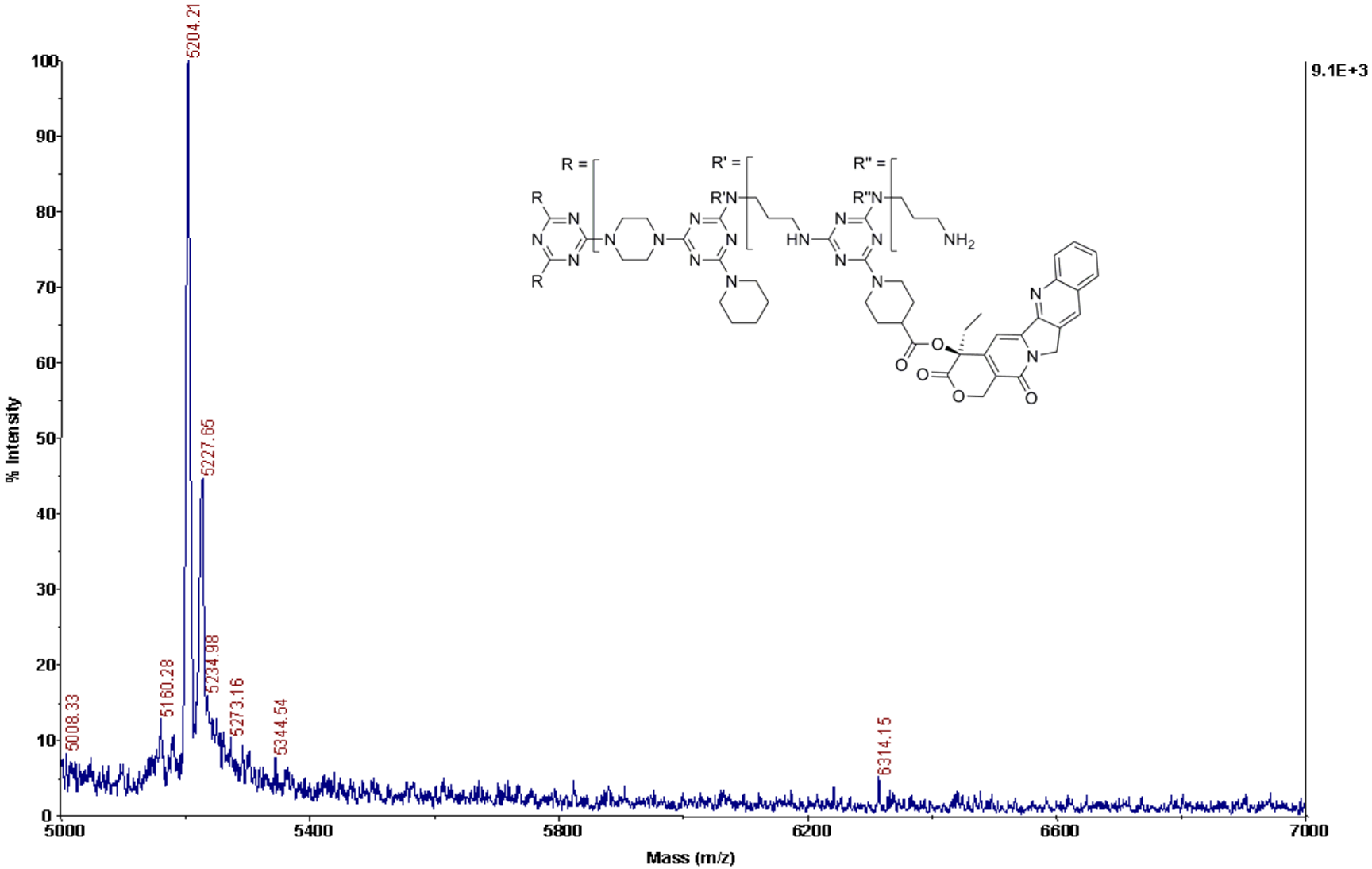
Chemical Shift (ppm)

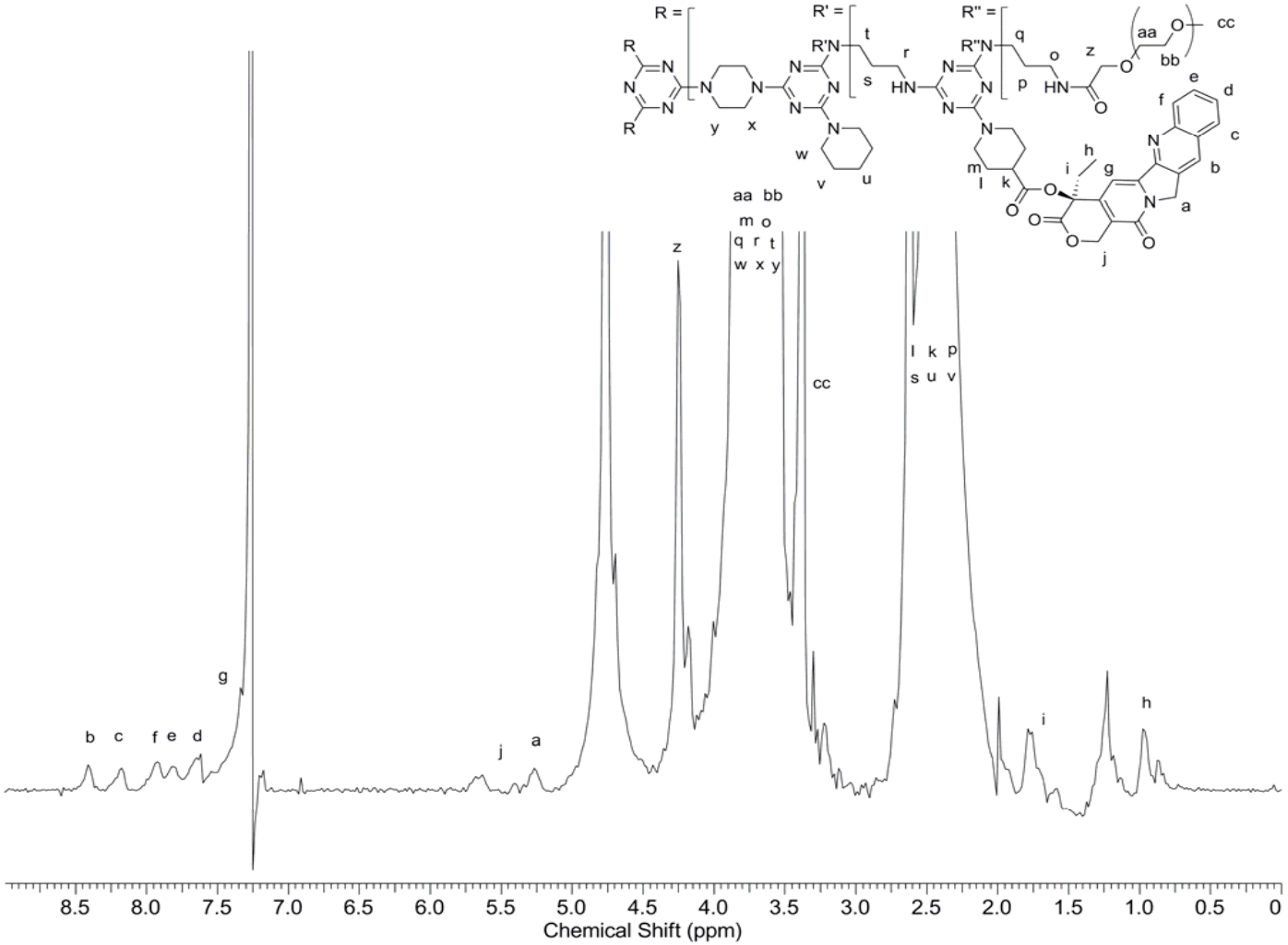
G2-CPT: ^{13}C NMR



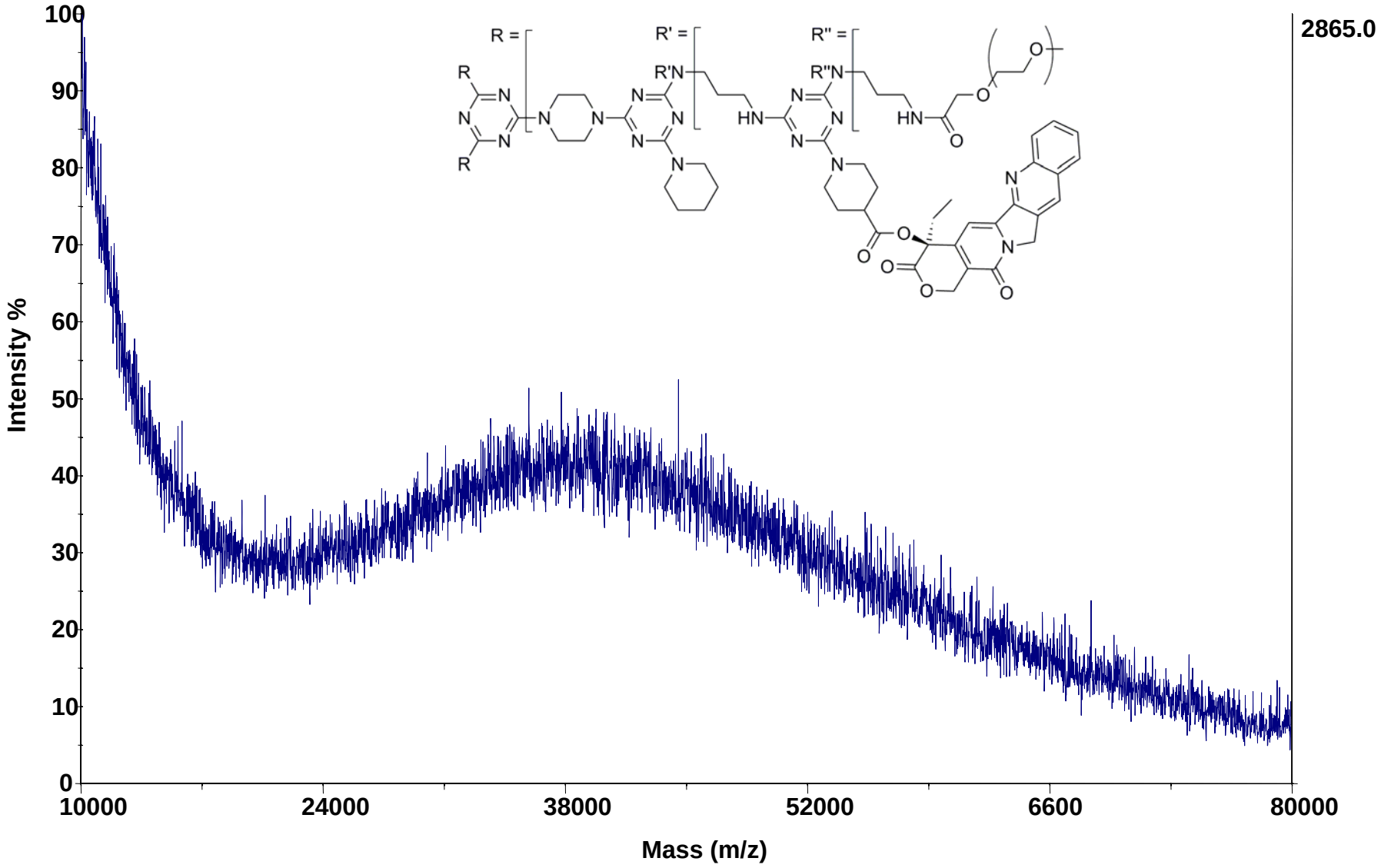
G2-CPT: MALDI TOF MS



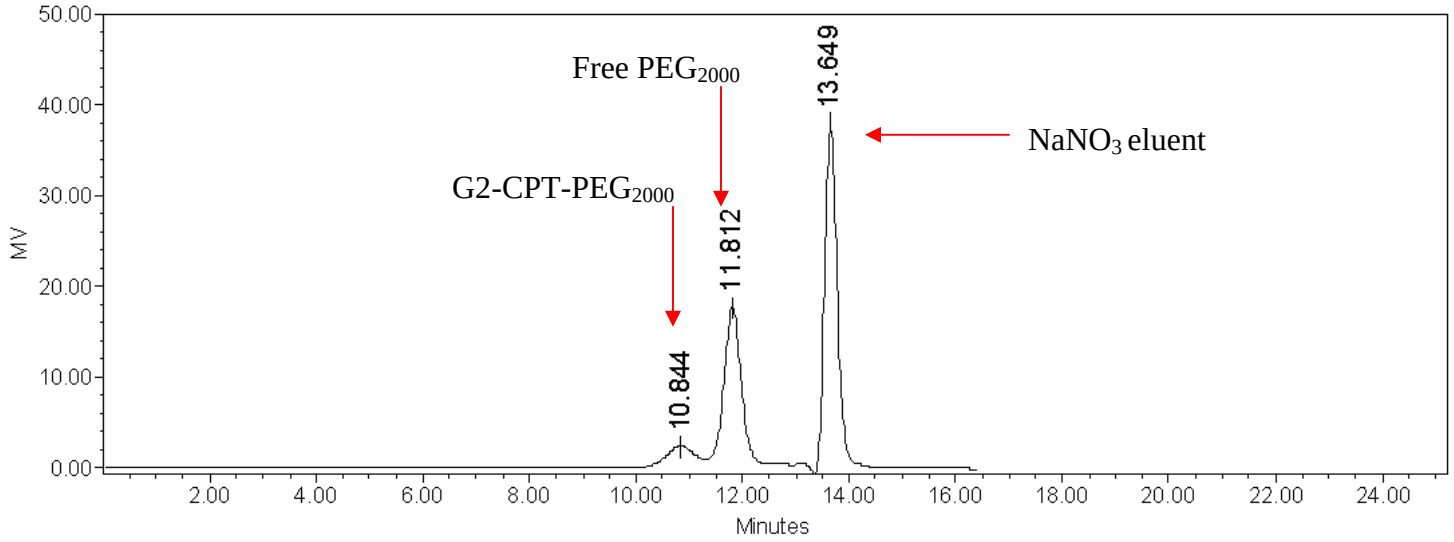
PEG2000-G2-CPT: ^{1}H NMR



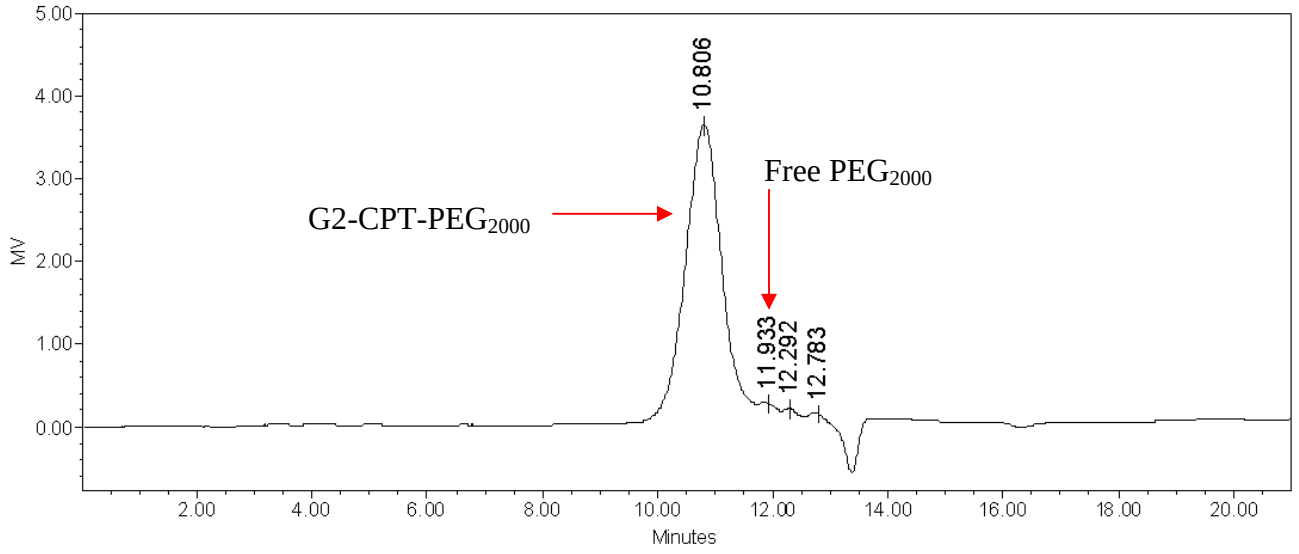
PEG2000-G2-CPT: MALDI-TOF MS: This trace is representative and no significant conclusions on the degree of PEGylation can be drawn.



PEG2000-G2-CPT: HPLC (before ultrafiltration)



PEG2000-G2-CPT: HPLC (after ultrafiltration)



320