Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplemental Information for "A general solid phase method for the synthesis of depsipeptides"

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Contents:

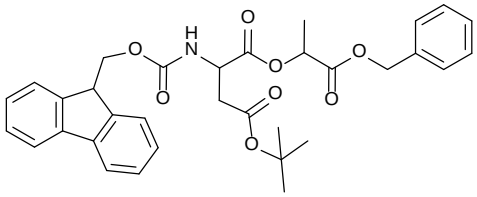
Experimental section

General experimental information

Abbreviations: Boc: tert-butyloxycarbonyl; OtBu: tert-butyl; Bn: benzyl; DCC, N,N'-Dicyclohexylcarbodiimide; DCM, dichloromethane; DMAP, 4-Dimethylaminopyridine; DMF, dimethylformamide; DIC, N,N'-Diisopropylcarbodiimide; DIPEA, N,N-diisopropylethylamine; Fmoc, 9-Fluorenylmethyloxycarbonyl; HPLC, high pressure liquid chromatography; Lac, lactic acid; Pd/C, palladium on carbon; Pfp, pentafluorophenol; TFA, trifluoroacetic acid. TFA, DCM, DMF, ethyl acetate, and diethyl ether were from Fisher Scientific. DIPEA, Pfp, and R-mandelic acid were from Acros Organics. Pd/C was purchased from Aldrich. Fmoc-amino acids, DMAP, and OxymaPure were purchased from EMD Biosciences. The side chain protecting groups were chosen as: Boc or Z for lysine and OtBu or OBzl for aspartic acid. Trityl chloride resin, Fmoc-Ala-Wang resin, and DIC were from Anaspec. Piperidine was from Alfa Aesar. L-lactic acid was from Biosynth.

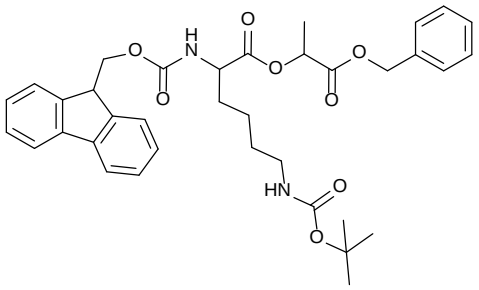
Analytical thin layer chromatography (TLC) was performed on Whatman aluminum backed silica plates ($UV_{254,}$ 250 µm). Spots were visualized with UV light or a permanganate potassium stain. Column chromatography was performed manually on silica gel (230-400 mesh size, grade 60). ^{1}H-NMR,^{13}C-NMR, and two dimensional spectra were obtained on a Varian 500 MHz and 500 MHz spectrometers. Chemical shifts are given in ppm with respect to internal standard TMS for ^{1}H-NMR and ^{13}C-NMR. Analytical HPLC was performed using an automatic HPLC system (Beckman System Gold) with an analytical reversed-phase column, an UV detector operating at 214 nm, at a flow rate of 1 mL/min. A Vydac C18 column (300 Å, 5 µm, 3.1 x 150 mm) was used. Preparative HPLC was performed using a GE Biosciences AKTA 10 system on a Biorad HiPore RP C18 column (300 Å, 5 µm, 3.1 x 150 mm), an UV detector operating at 214 nm. The depsipeptides were characterized using liquid chromatography mass spectroscopy (LC-MS) and electrospray mass spectrometry (ESI-MS). They were performed on an Aglient 1200 series HPLC, a Agilent 6130 single quadrupole mass spectrometer or a Gemini column (C18, 55 µm, 2.1 x 50 mm), operating in a positive or negative ionization mode.

Synthesis of Fmoc-Asp(OtBu)-Lac-Bn (3a)



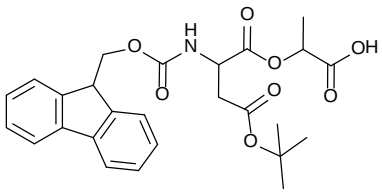
Fmoc-Asp(OtBu)-OH (1.2 mmol) and Lac-Bn[1] (1.0 mmol) was dissolved in DCM and placed on an ice bath. DIC (1.2 mmol) and DMAP (0.01 mmol) was added to the cold mixture and mixed for 1 hour. The reaction was stirred for 5 hours at room temperature and monitored by TLC. The solution was filtered and concentrated. The crude oil was purified on silica gel hexanes and ethyl acetate (67%). ^{1}H-NMR (500 MHz, d_6-DMSO) δ (ppm): 1.33-1.43 (m, 12H), 2.58 and 2.78 [dd, *J* = 4.76, 2H, $CH_2C\underline{H}_2CO$], 4.24 [t, *J* = 6.83, 1H, C<u>H</u>(Fmoc)], 4.34 [d, *J* = 6.66, 2H, $C\underline{H}_2$(Fmoc)], 4.54 [m, *J* = 4.76, 1H, $NHC\underline{H}CH_2$], 7.32-7.44 (m, 9H), 7.70 [q, *J* = 7.07, 2H, H_{Ar}(Fmoc)], 7.89 [d, *J* = 7.51, 2H, H_{Ar}(Fmoc)], 7.96 [d, *J* = 8.24, 1H, N<u>H</u>]; 13C-NMR (125 MHz, d_6-DMSO) δ (ppm): 170.77, 169.90, 168.75, 155.00, 143.74, 140.79, 135.50, 128.51, 128.26, 127.90, 127.87, 127.10, 125.18, 120.15, 80.63, 69.09, 66.44, 65.84, 50.43, 46.63, 36.81, 27.66, 16.59. MS (ESI+) calculated for $C_{33}H_{35}NO_8$ [M+ - OtBu - H]: 517.53; found: 518.00

Synthesis of Fmoc-Lys(Boc)-Lac-Bn (3b)



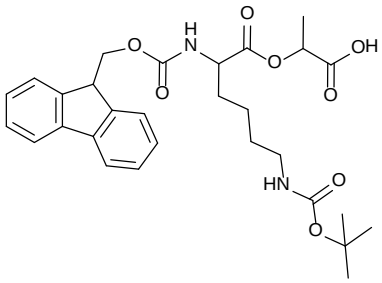
Fmoc-Lys(Boc)-OH (1.2 mmol) and Lac-Bn[1] (1.0 mmol) was dissolved in DCM and placed in an ice bath. DIC (1.2 mmol) and DMAP (0.01 mmol) was added to the cold mixture and mixed for 1 hour. The reaction was stirred for 5 hours at room temperature and monitored by TLC. Diisocarbohexylurea was removed via filtration, and the mixture was concentrated. The crude oil was purified on silica gel with 37.5% EtOAc in hexanes (82%). ^{1}H-NMR (500 MHz, d_6-DMSO) δ (ppm): 1.24-1.45 (m, 16H), 1.56-1.73 (m, 2H), 2.88 [t, *J* = 5.13, 2H, $CH_2C\underline{H}_2NH$], 4.04 [m, *J* = 7.07, 1H, $NHC\underline{H}CH_2CO$], 4.21-4.32 (m, 3H), 5.10-5.15 (m, 3H), 6.75 [t, *J* = 5.61, 1H, N<u>H</u>], 7.31-7.45 (m, 9H), 7.71 [d, *J* = 7.57, 2H, H_{Ar}(Fmoc)], 7.79 [d, *J* = 7.56, 1H, N<u>H</u>], 7.89 [d, *J* = 7.56, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (125 MHz, d_6-DMSO) δ (ppm): 172.06, 169.96, 156.12, 155.54, 143.73, 140.68, 135.44, 128.43, 128.17, 127.90, 127.61, 127.02, 125.19, 120.08, 77.32, 68.66, 66.31, 65.67, 53.58, 46.58, 40.09, 30.15, 28.99, 28.23, 22.77, 16.63. MS (ESI-) calculated for $C_{36}H_{42}N_2O_8$ [M^- - H]: 630.73; found: 629.8

Synthesis of Fmoc-Asp(OtBu)-Lac-OH (4c)



(3a) was dissolved in dry methanol with 0.1 equiv Pd/C under H_2 at 5 psi for 8 hours or as monitored with TLC. The Pd/C was filtered through a pad of celite and concentrated in vacuo. The sample was purified on silica gel with 1% MeOH in DCM to yield 4c as white crystals (33%). ^{1}H-NMR (500 MHz, d_6-DMSO) δ (ppm): 1.36-1.38 (m, 12H), 2.55 and 2.79 [dd, *J* = 4.64, 2H, $CH_2C\underline{H}_2CO$], 4.22 [t, *J* = 7.08, 1H, C<u>H</u>(Fmoc)], 4.30 [d, *J* = 7.07, 2H, C<u>H</u>$_2$(Fmoc)], 4.93 [q, *J* = 7.07, 1H, NHC<u>H</u>CH_2], 7.31 [t, *J* = 7.32, 2H, H_{Ar}(Fmoc)], 7.41 [t, *J* = 7.33, 2H, H_{Ar}(Fmoc)], 7.68 [t, *J* = 7.08, 2H, H_{Ar}(Fmoc)], 7.87-7.89 (d, 3H); ^{13}C-NMR (125 MHz, d_6-DMSO) δ (ppm): 171.44, 170.64, 168.74, 155.79, 143.69, 140.68, 127.60, 127.01, 125.12, 120.09, 80.49, 69.05, 65.70, 50.33, 46.52, 36.74, 27.61, 16.62 (shift at 54.86 ppm is residual DCM). MS (ESI-) calculated for $C_{26}H_{29}NO_8$ [M^- - H]: 483.51; found: 482.2

Synthesis of Fmoc-Lys(Boc)-Lac-OH (4d)



(3b) was dissolved in dry methanol with 0.1 equiv Pd/C under H_2 at 12 psi for 9 hours or as monitored with TLC. The Pd/C was filtered through a pad of celite and concentrated in vacuo. The sample was eluted with 1-6% MeOH in DCM to yield 4d as white crystals in a yield of (67%). ^{1}H-NMR (500 MHz, d_6-DMSO) δ (ppm): 1.34-1.47 (m, 16H), 1.60-1.79 (m, 2H), 2.90 [t, *J* = 5.96, 2H, $CH_2C\underline{H}_2NH$], 4.04 [m, *J* = 5.03, 1H, NHC<u>H</u>CH_2CO], 4.32-4.22 (m, 3H), 4.93 [q, *J* = 7.07, 1H, $CH_2C\underline{H}CH_3$], 6.75 [t, *J* = 5.53, 1H, N<u>H</u>], 7.33 [t, *J* = 7.49, 2H, H_{Ar}(Fmoc)]; 7.42 [t, *J* = 7.50, 2H, H_{Ar}(Fmoc)], 7.72 [t, *J* = 6.90, 2H, H_{Ar}(Fmoc)], 7.77 [d, *J* = 7.83, 1H, N<u>H</u>], 7.89 [d, *J* = 7.50, 2H, H_{Ar}(Fmoc)]; ^{13}C-NMR (125 MHz, d_6-DMSO) δ (ppm): 171.91, 171.84, 156.05, 155.47, 143.69, 140.62, 127.54, 126.96, 125.15, 120.01, 77.25, 68.59, 65.60, 53.52, 46.53, 30.12, 28.98, 28.18, 22.72, 16.63. MS (ESI-) calculated for $C_{29}H_{36}N_2O_8$ [M^- - H]: 540.60; found: 539.00

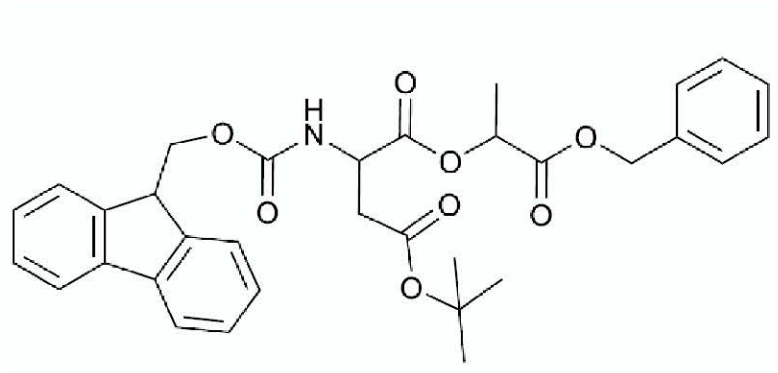
General procedure for the removal of protecting groups for solid phase synthesis

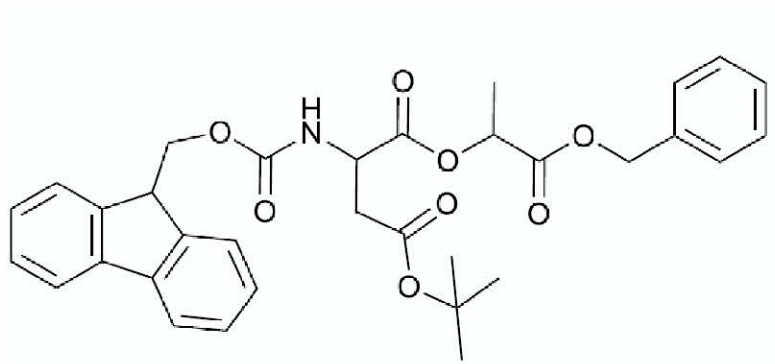
The Fmoc-protecting group was removed from the N-terminus with 20% piperidine in DMF. The solution was mixed for 5 minutes three times. The resin was washed with DMF. Cleavage of butyl-groups was investigated using the following cocktails: A: TFA/TIPS – 95/5; B: TFA/Water/TIPS – 95/2/3; C: TFA/DCM/TIPS – 95/2/3; D: TFA/DCM/TIPS – 50/48/2. The filtrate was precipitated into cold ether and purified with HPLC.

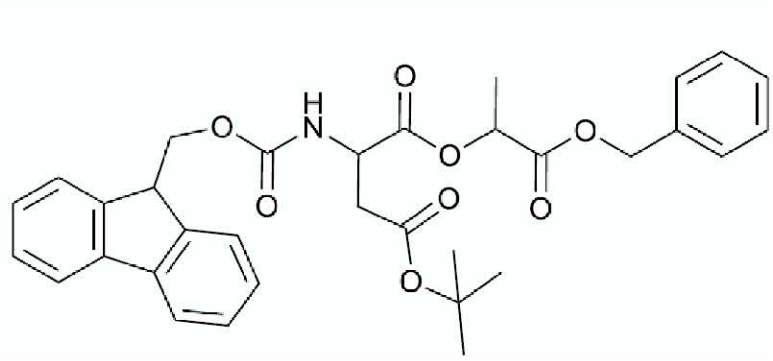
Preparation of peptides 5 – 8

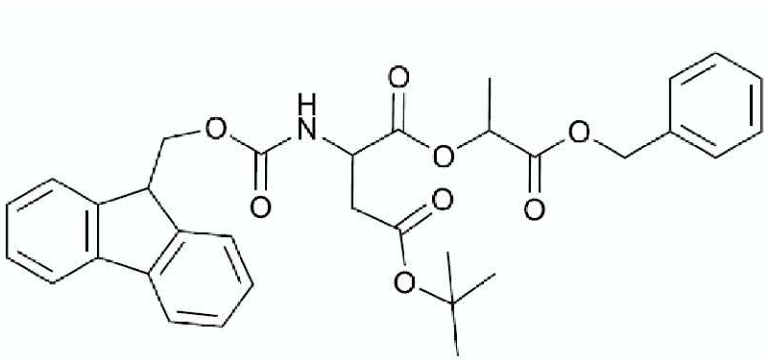
Functionalized peptides were added to the resin with DIC/DMAP as per standard Fmoc SPPS protocols. Fmoc-Ala-Wang resin (0.25 equiv) was swelled in DCM. The Fmoc group was removed with 20% pyridine in DMF and washed. Fmoc-peptide-OH (1 equiv) was dissolved in DCM and DMF and was added to the reaction vessel with DIC (0.75 equiv) and DMAP (0.05 equiv). After 2 hours the sample was washed with DCM and DMF. The Fmoc group was removed with pyridine in DMF and washed. Fmoc-depsidipeptide (1 equiv) was dissolved in DCM with Oxymapure (0.2 equiv) and was added to the reaction vessel upon a 2 minute preactivation time with DIC (0.75 equiv). After 2 hours the sample was washed with DCM and DMF. The Fmoc-removal, coupling, and washing steps were repeated with the Fmoc-depsidipeptide until the desired length was achieved. The oligodepsipeptide was removed from the resin accordingly and purified with HPLC.

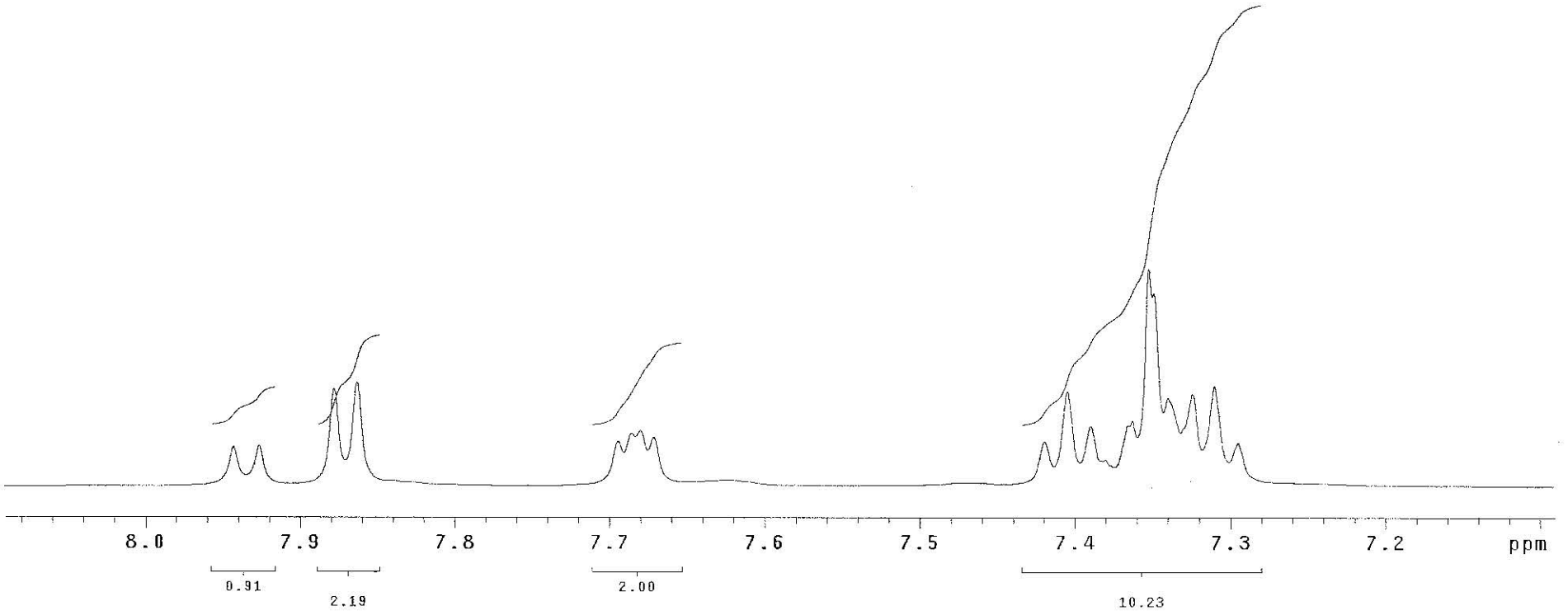
1. Fan, W., et al., *Enhanced Brain Targeting of Tegafur Using Novel Lactyl Cholesterol Liposome as a Carrier*. Letters in Drug Design & Discovery, 2009. **6**(7): p. 542-547.

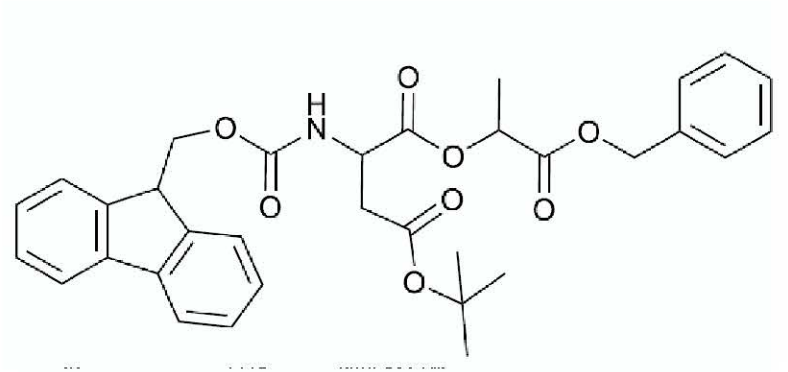


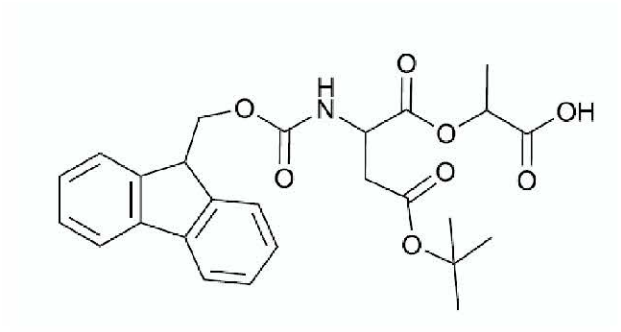


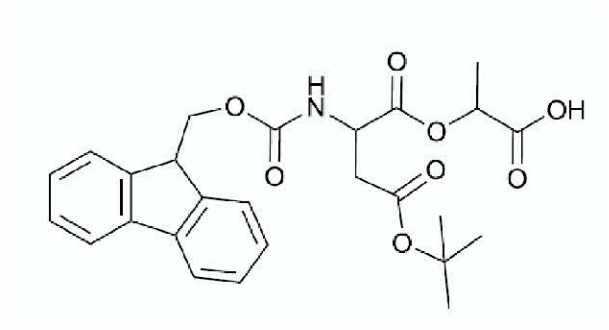


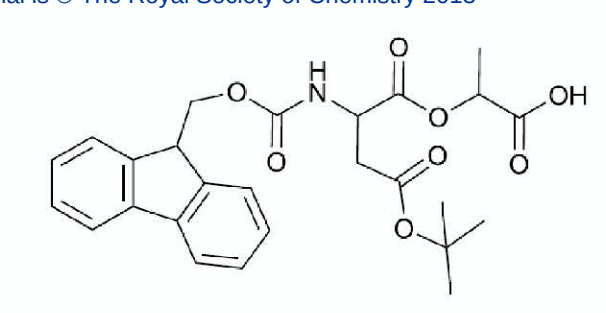


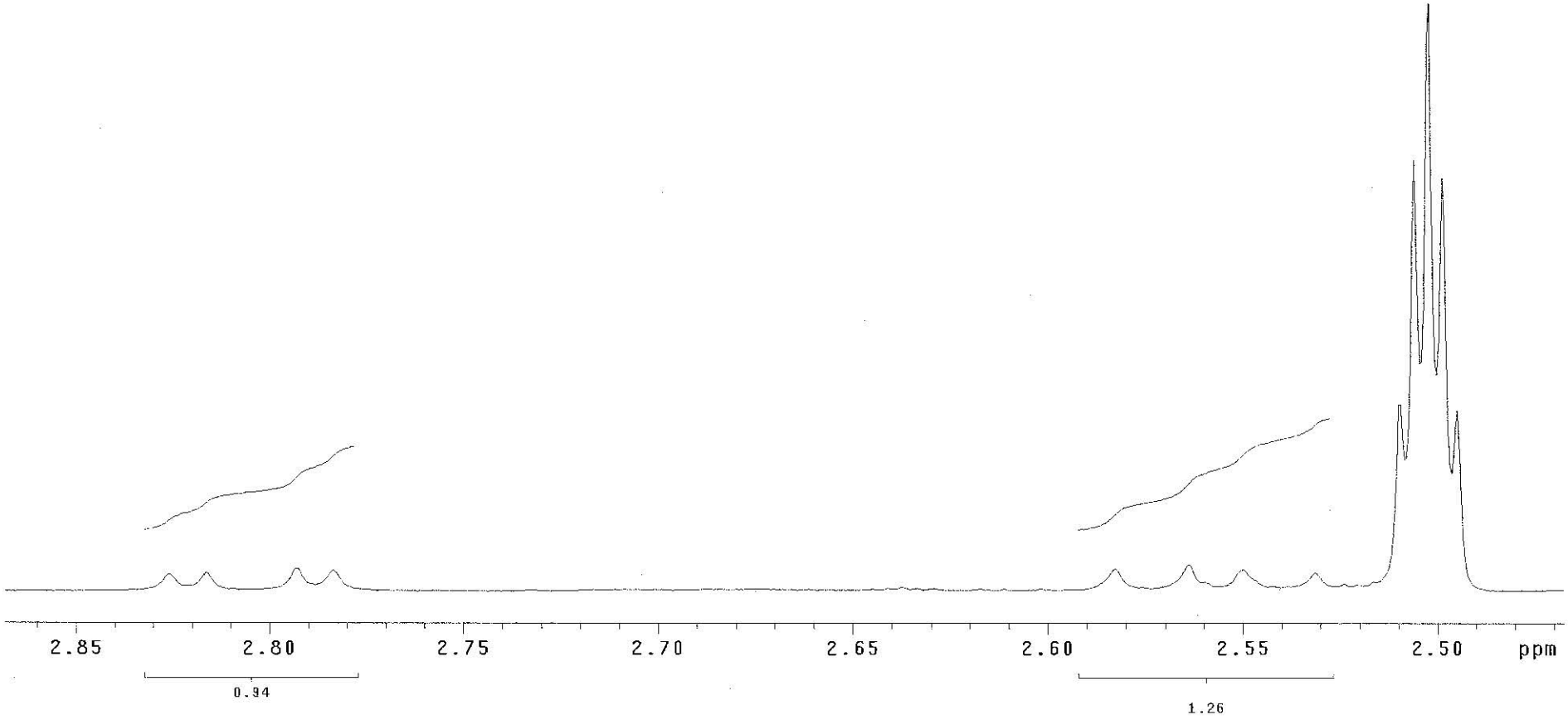


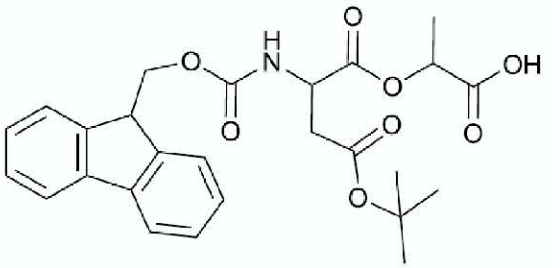


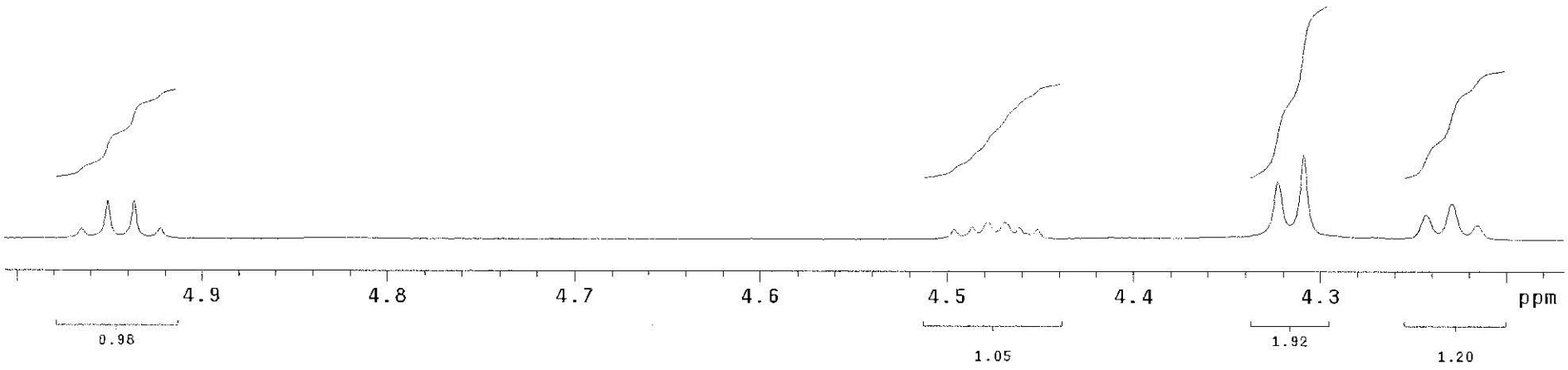


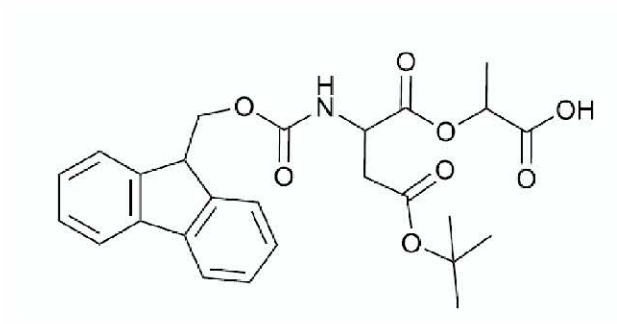


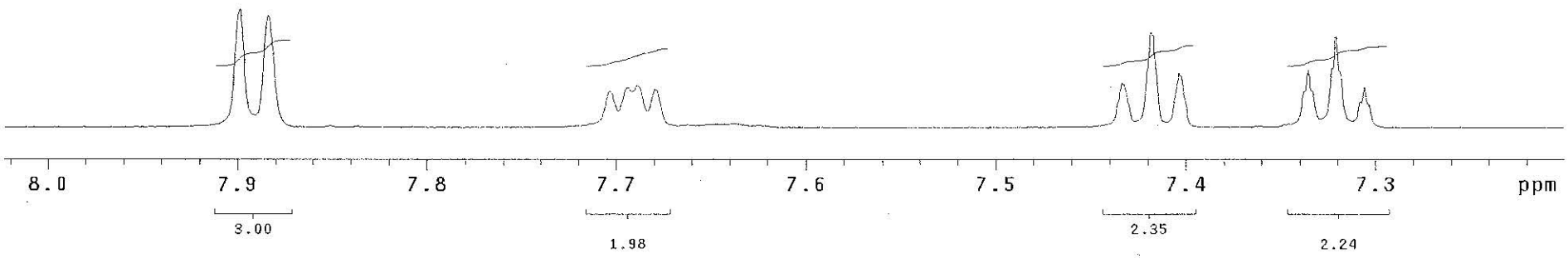


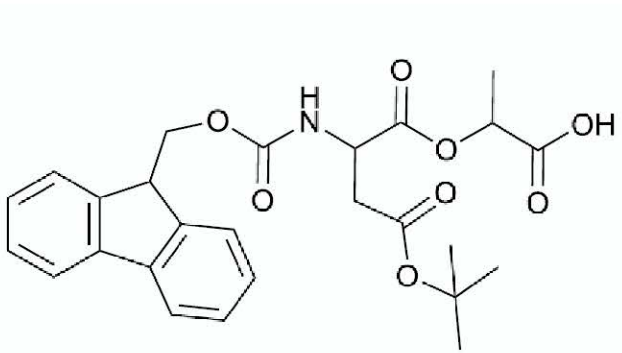


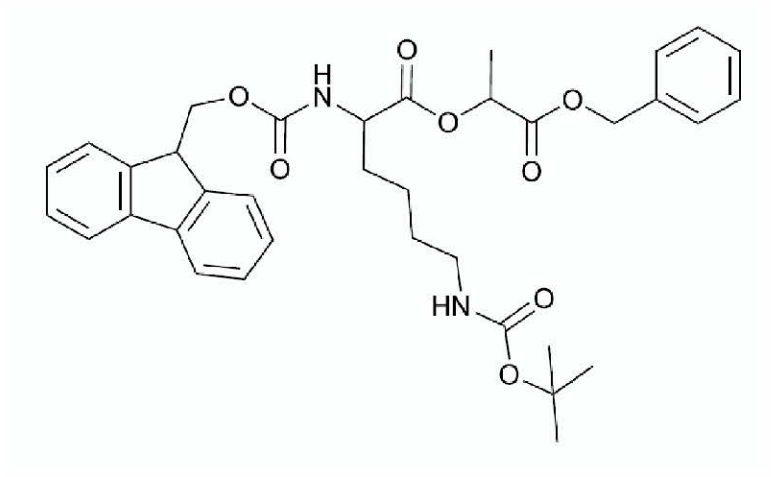


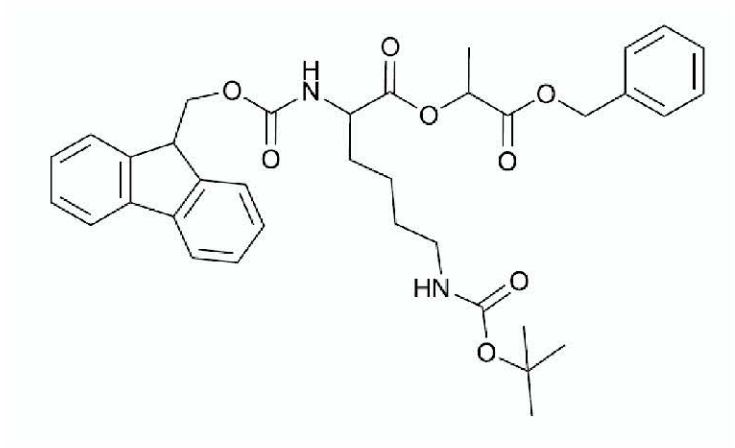


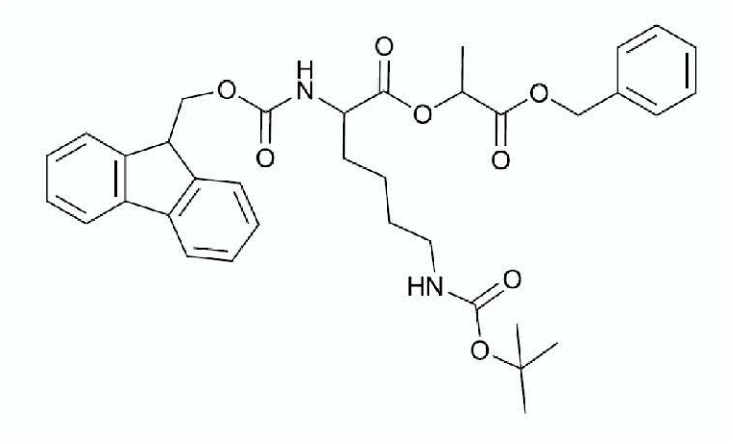


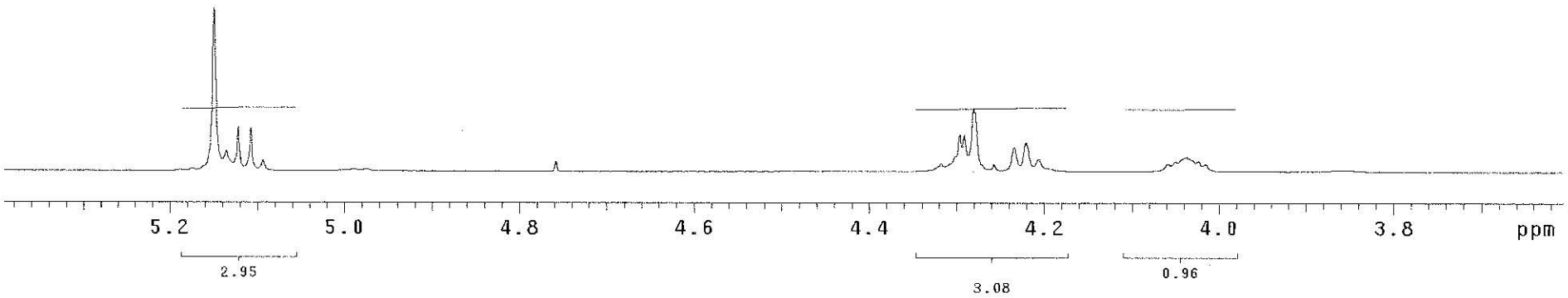


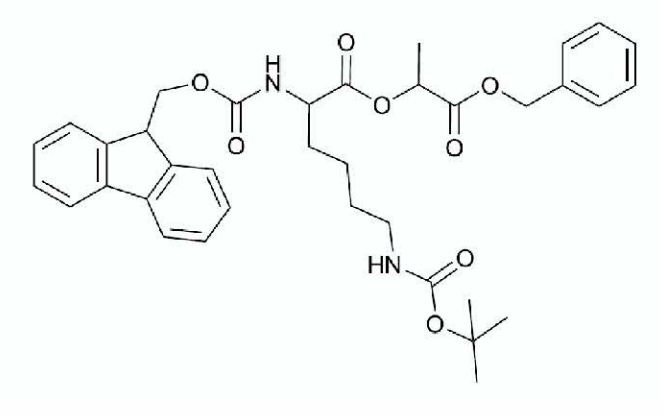


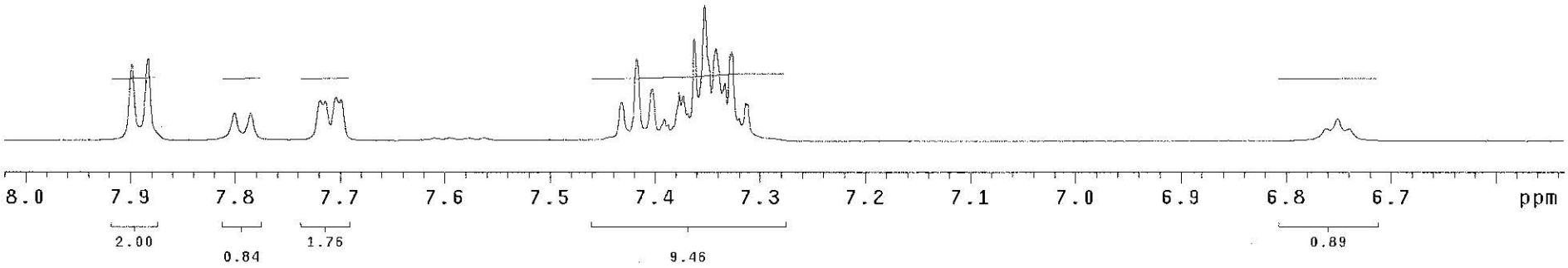


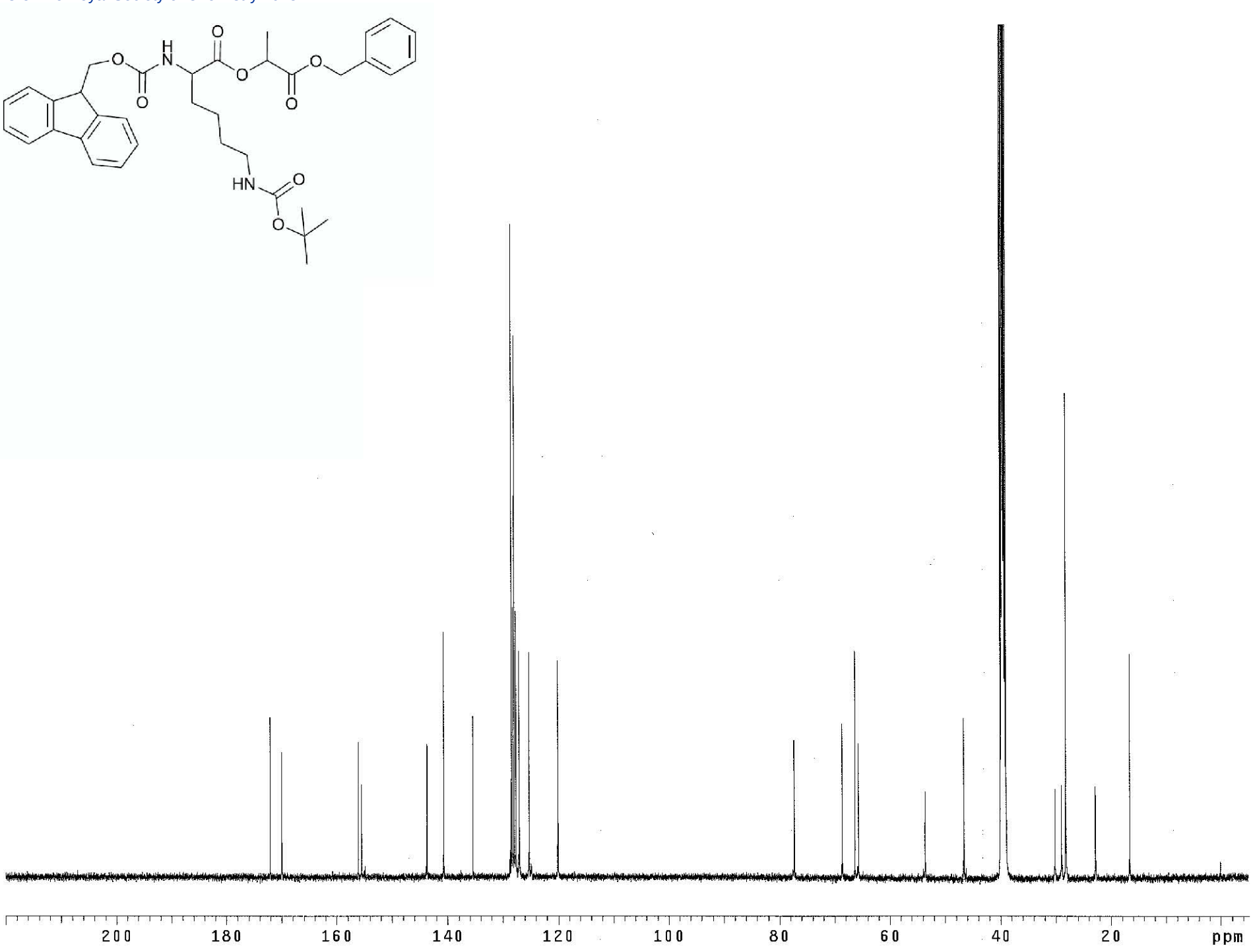


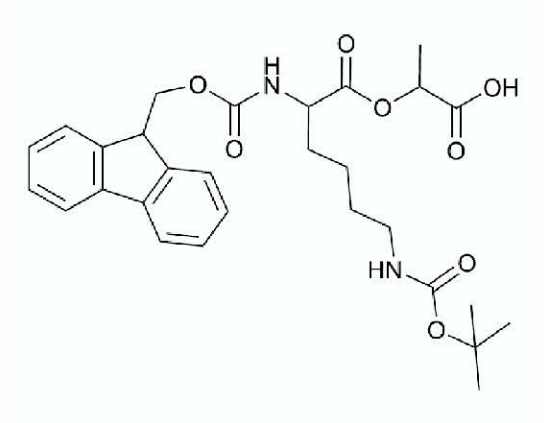


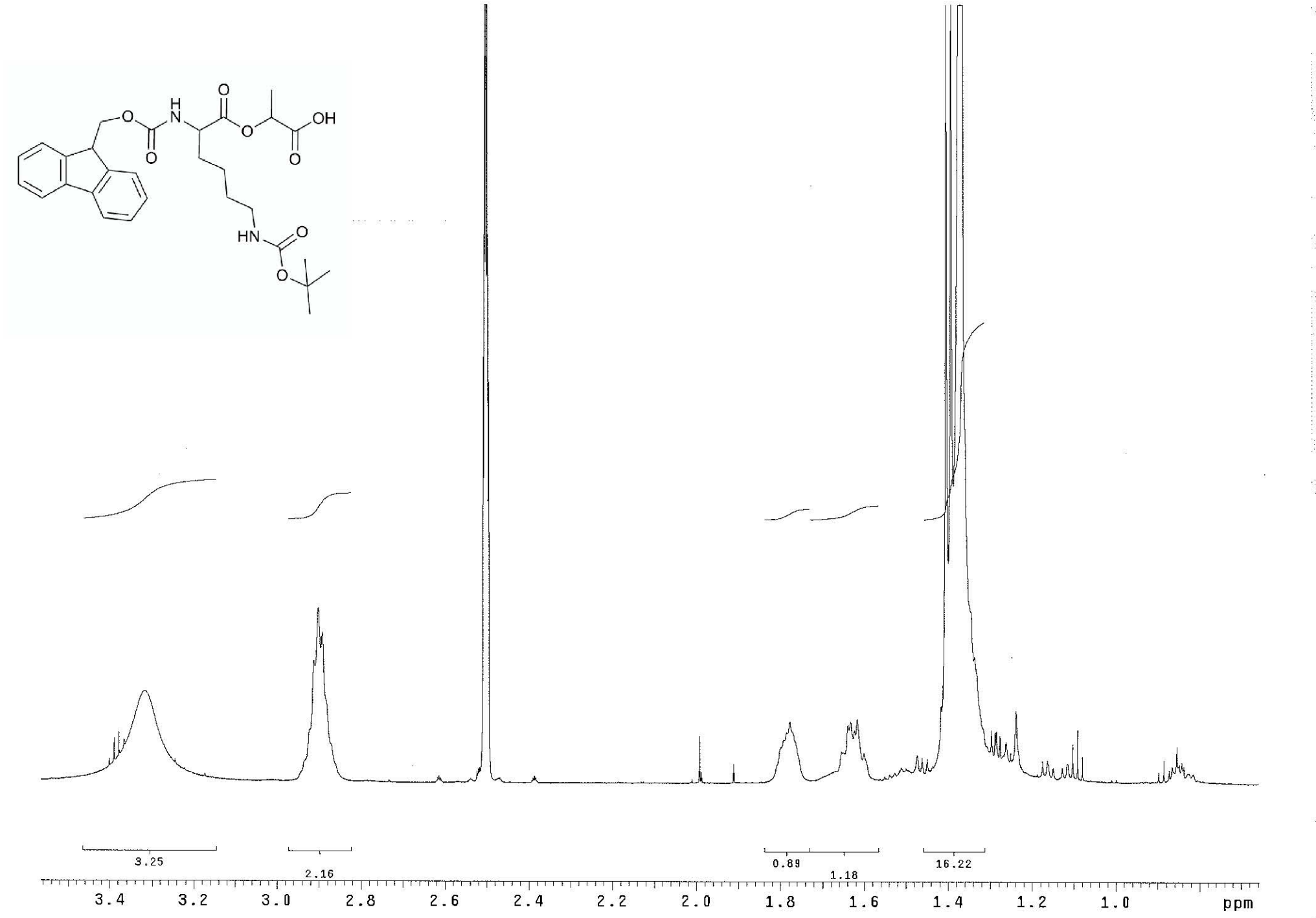


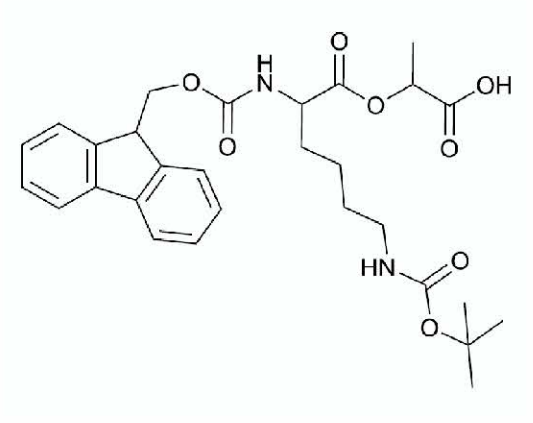


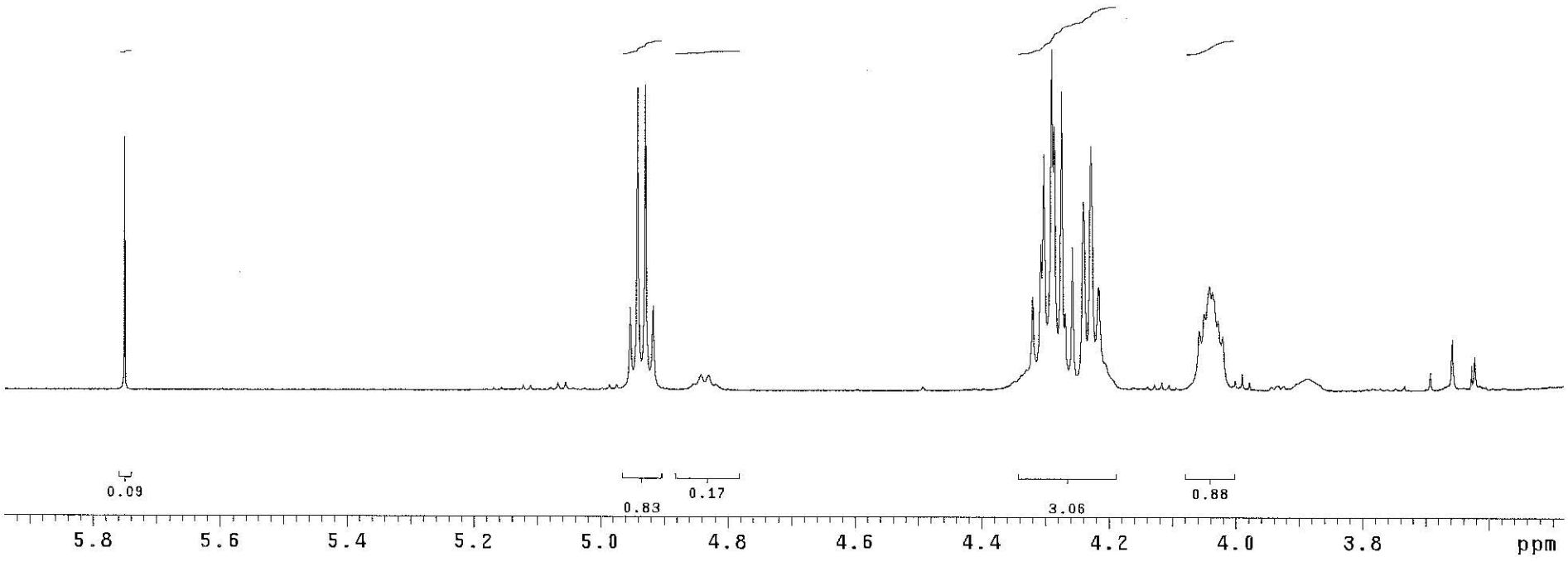


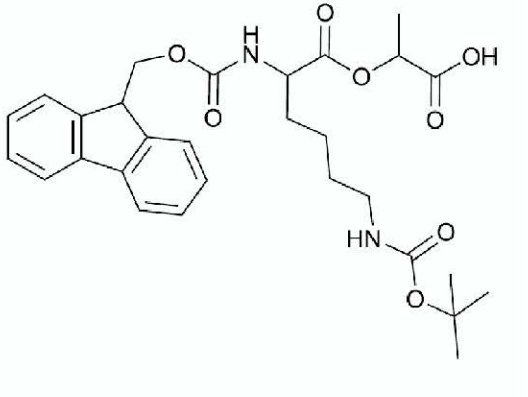


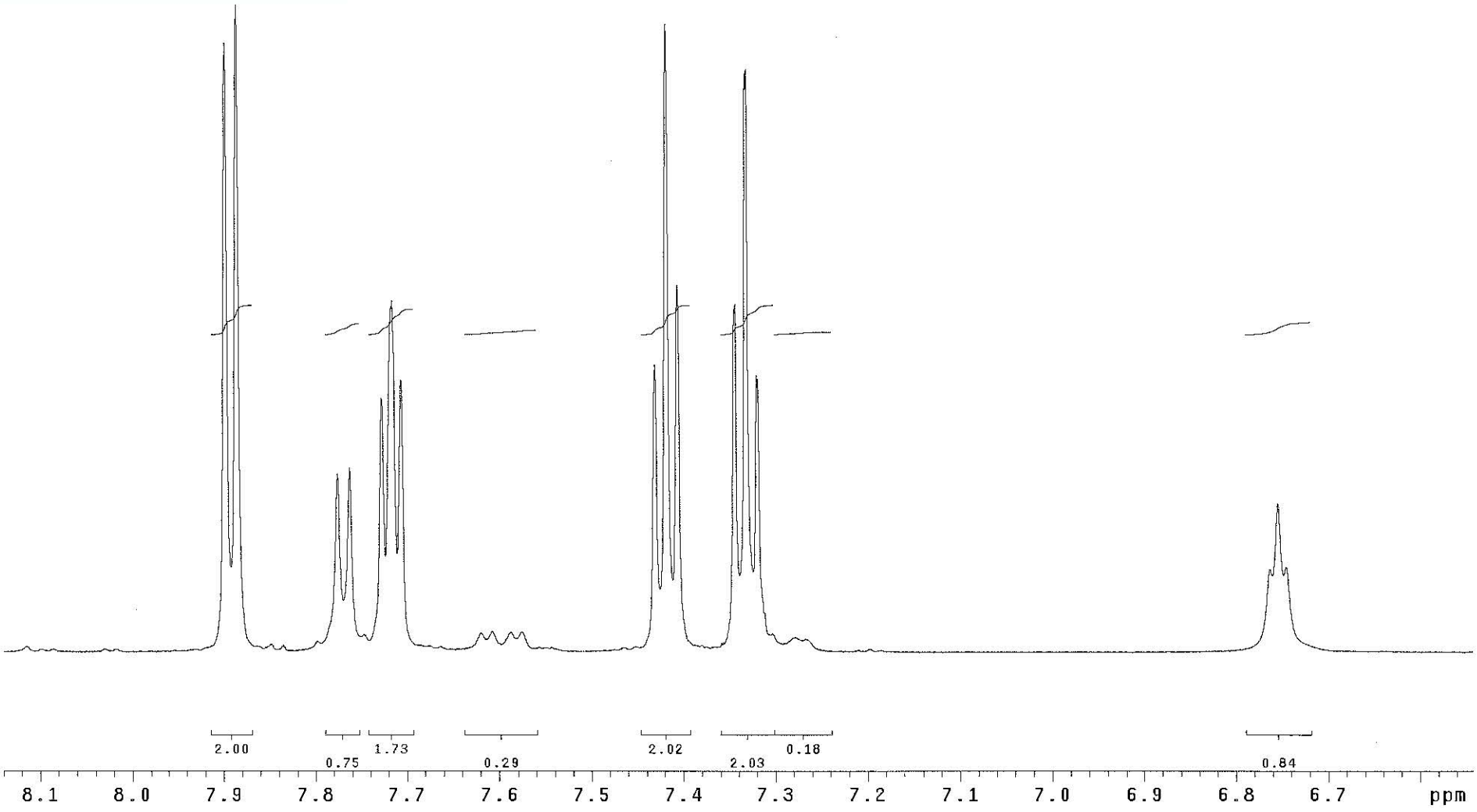


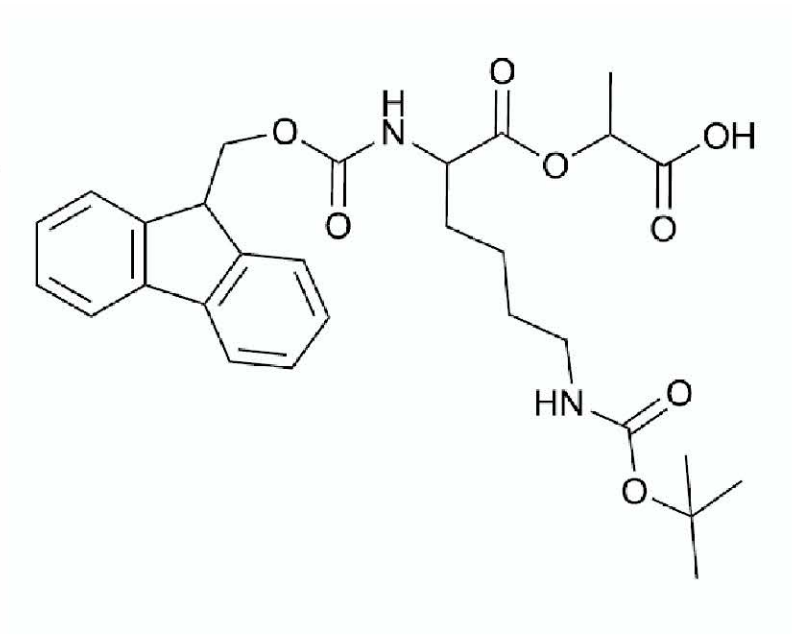


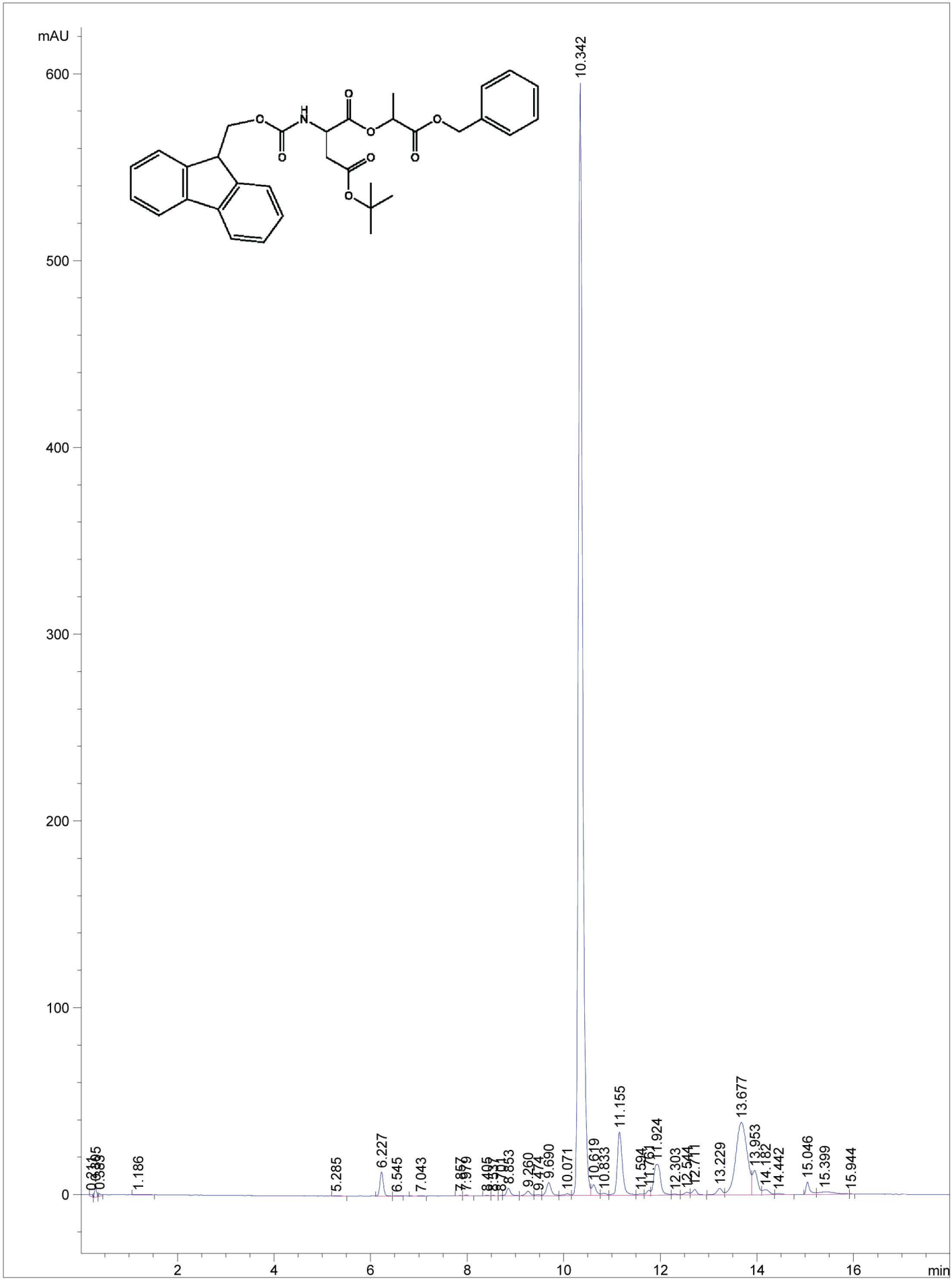


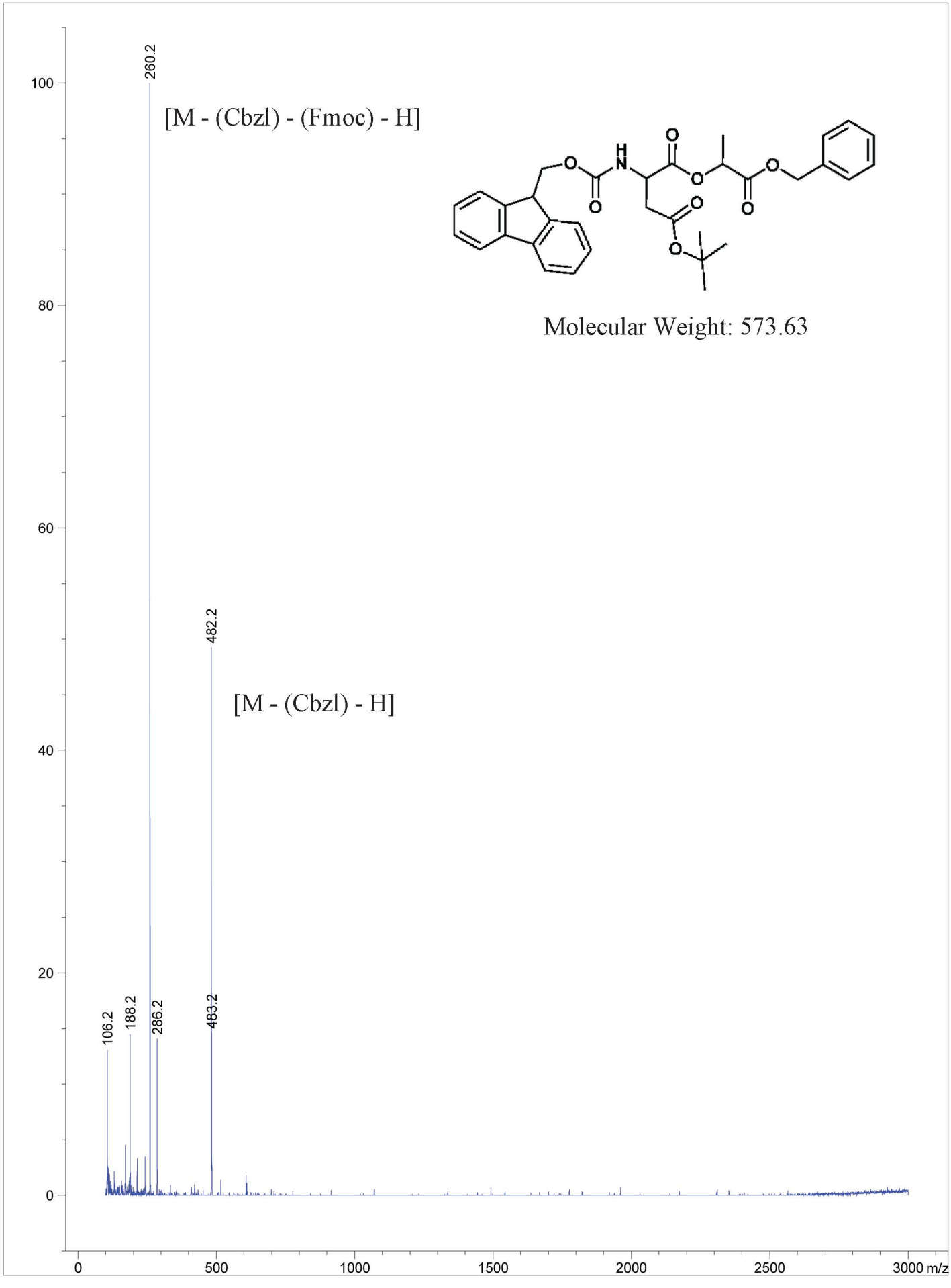


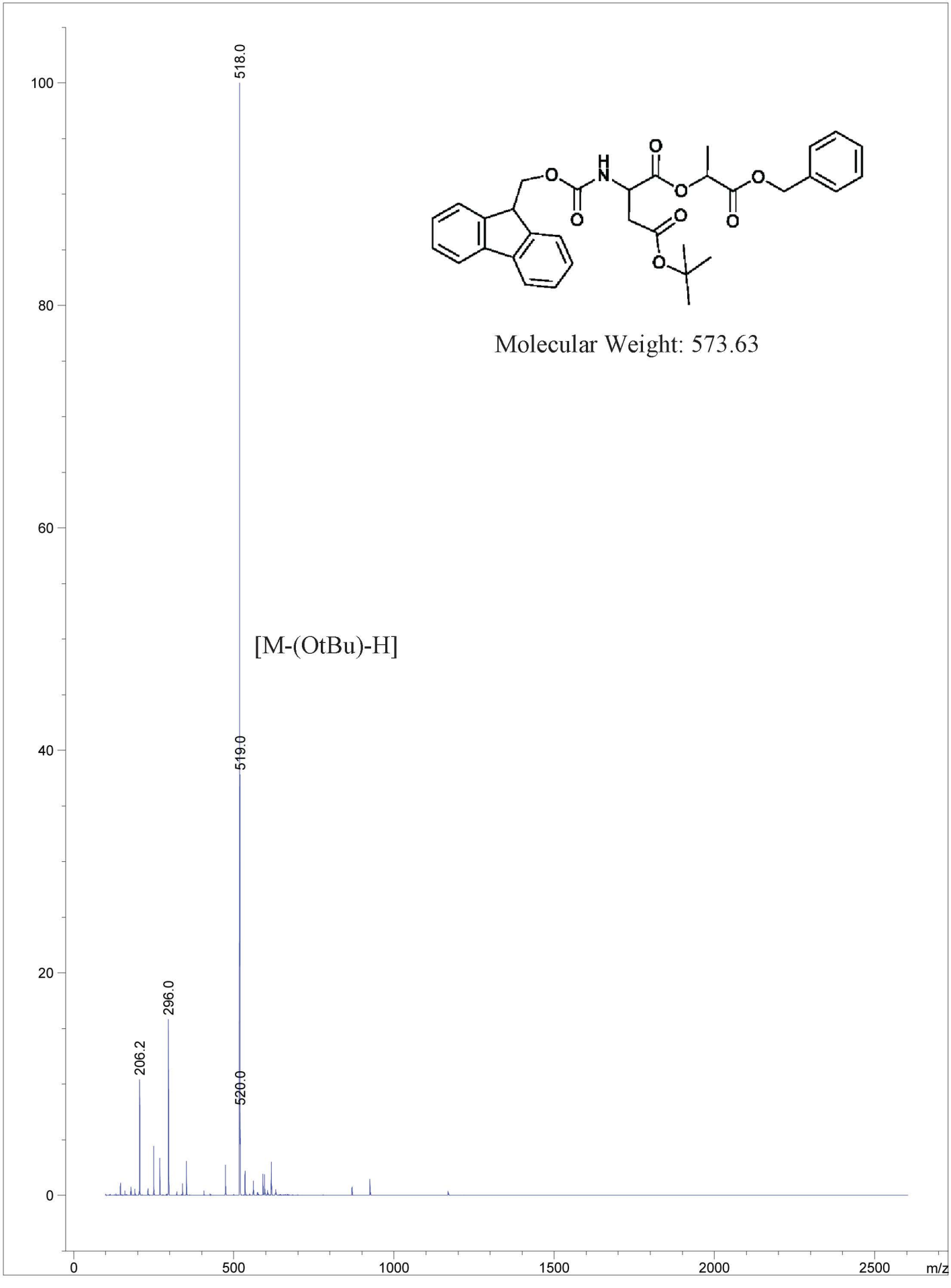


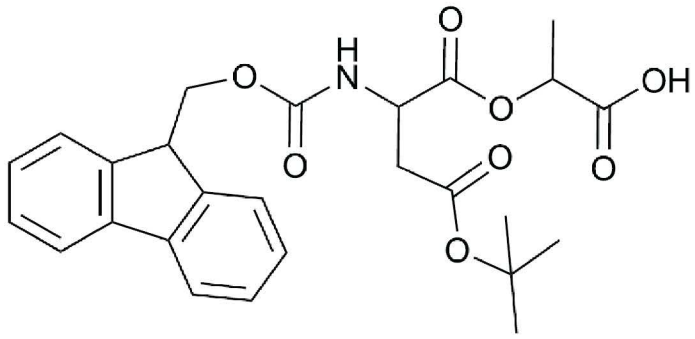


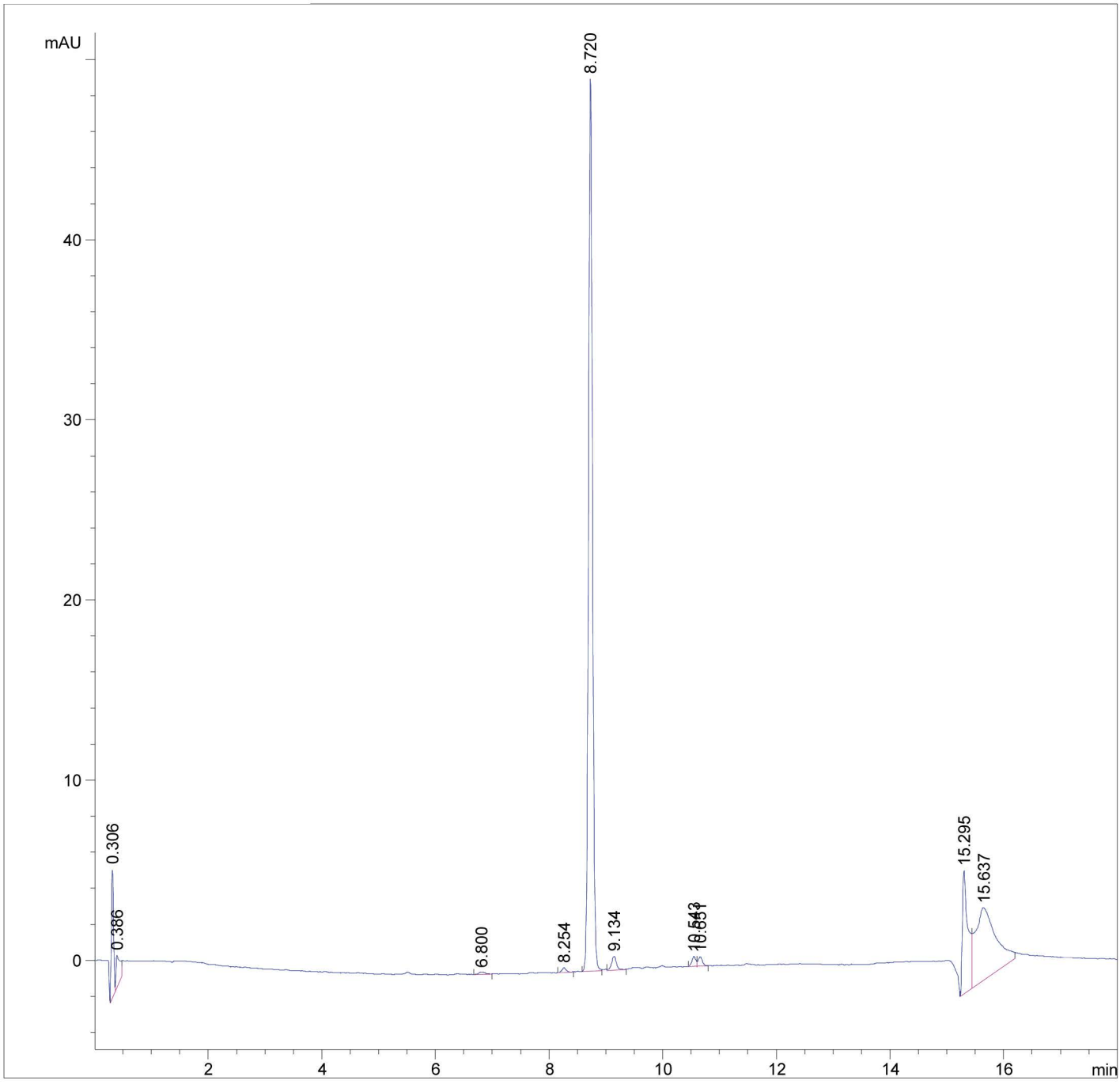


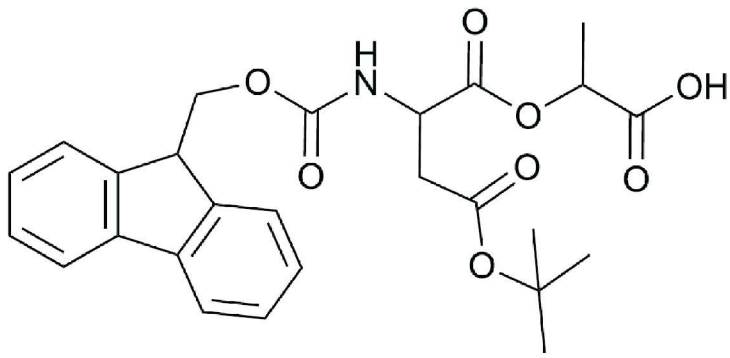




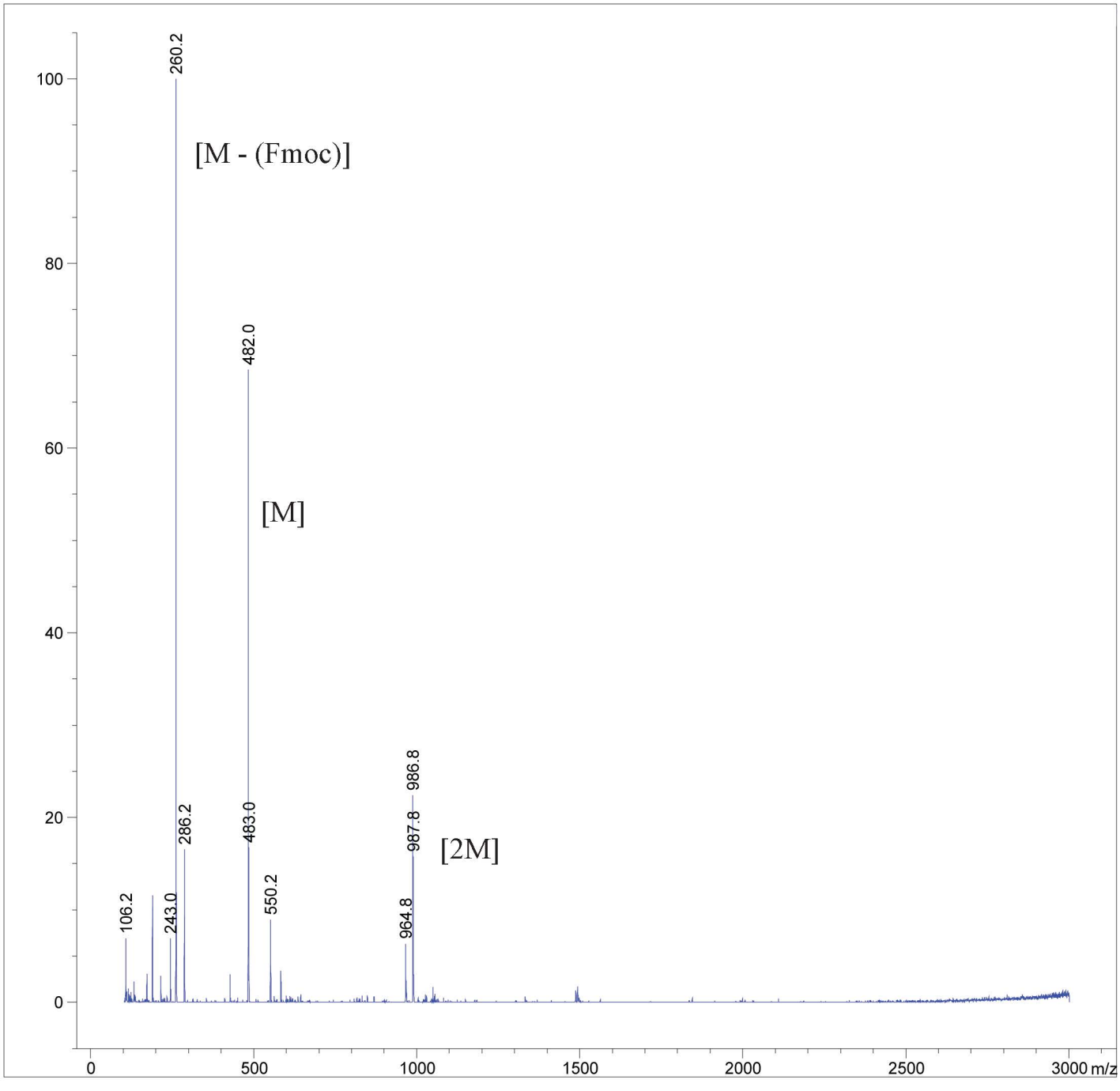


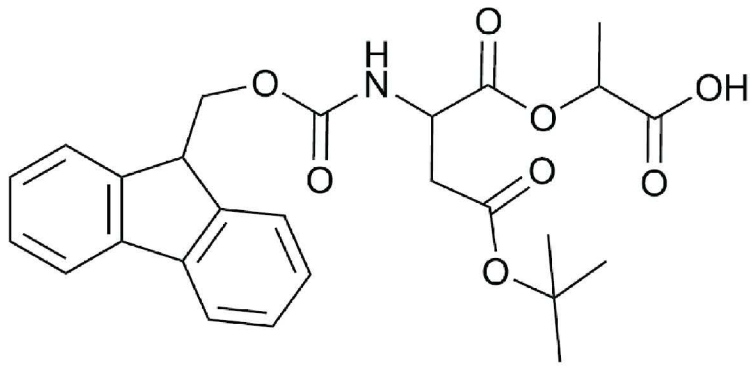






Molecular Weight: 481.53





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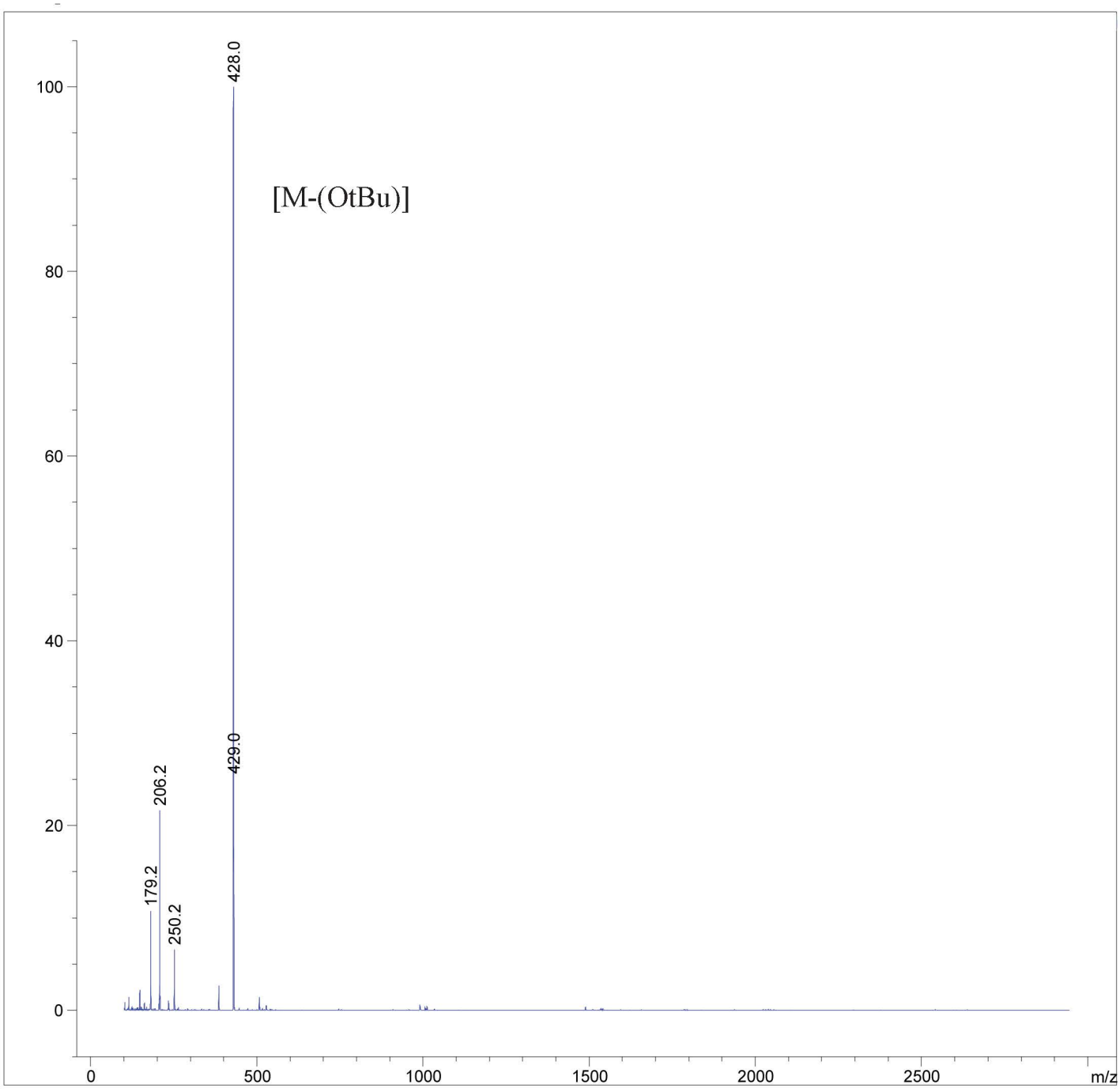
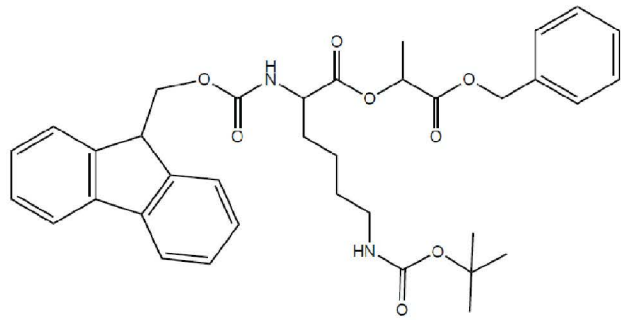
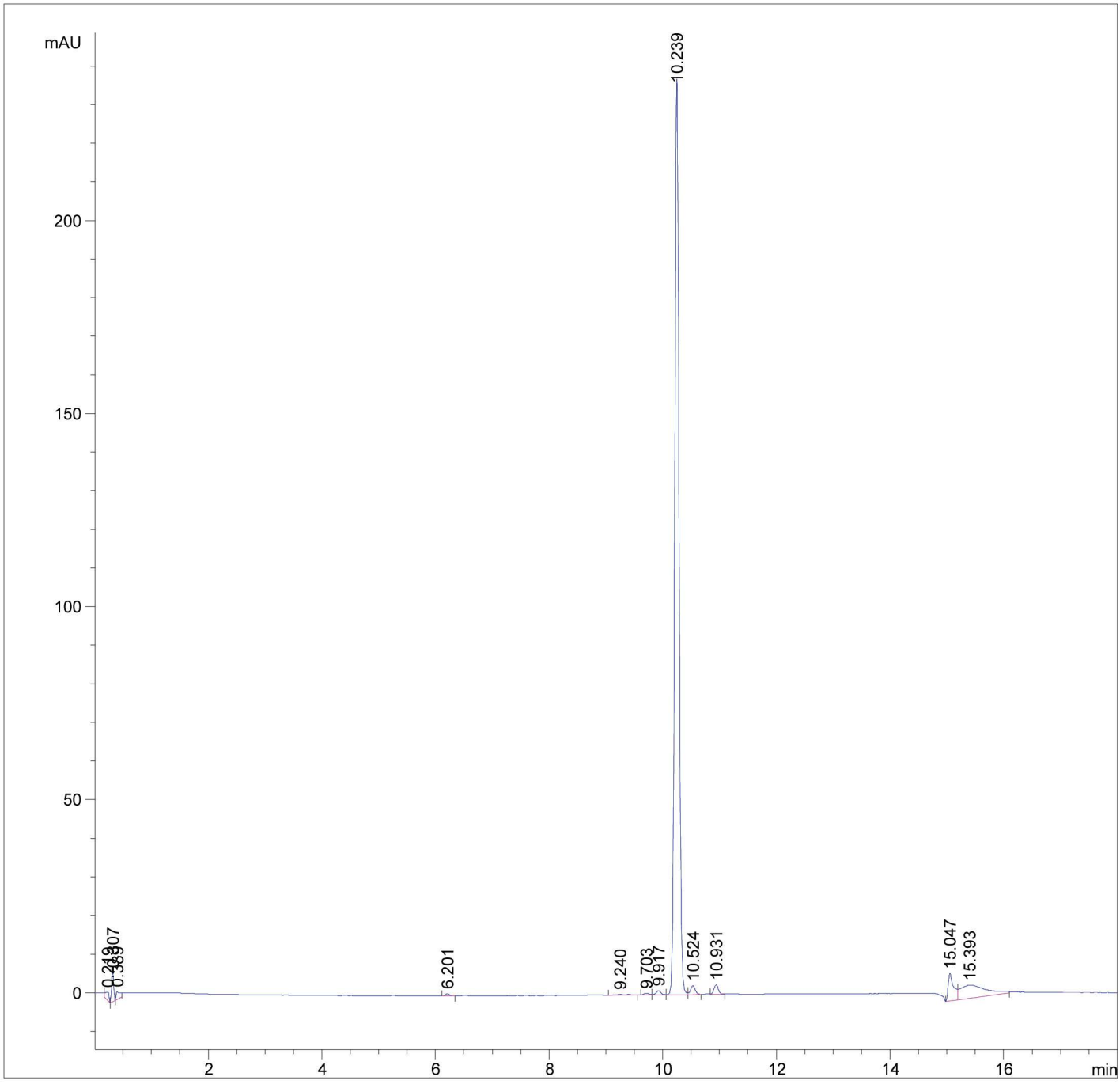
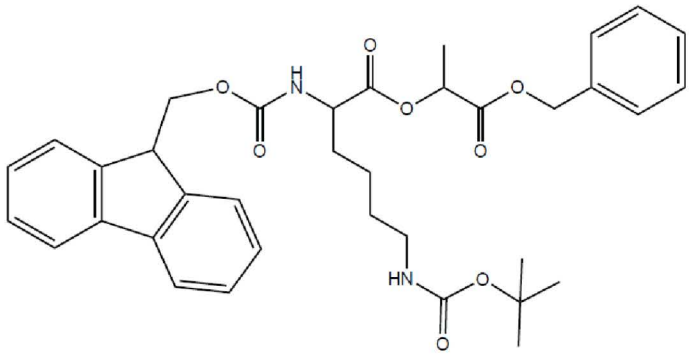
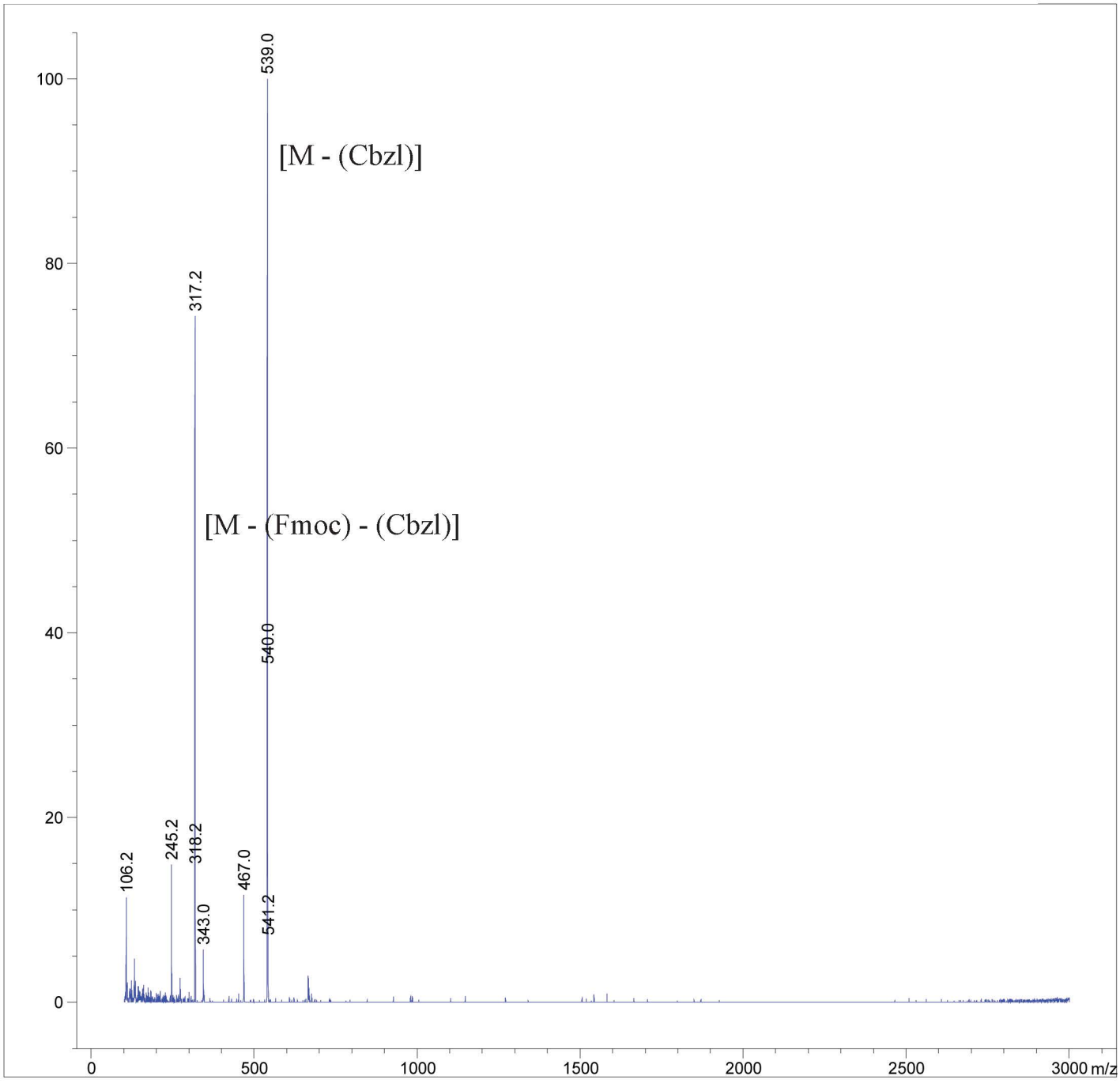


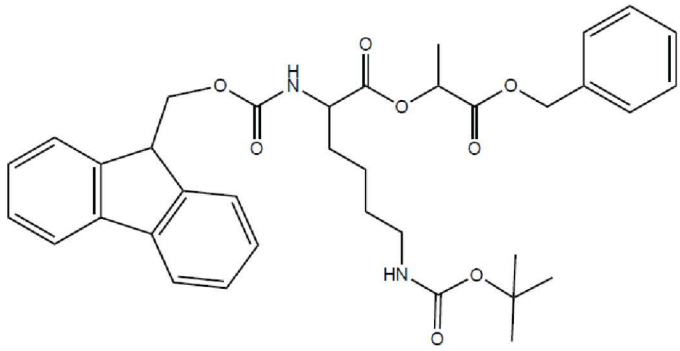




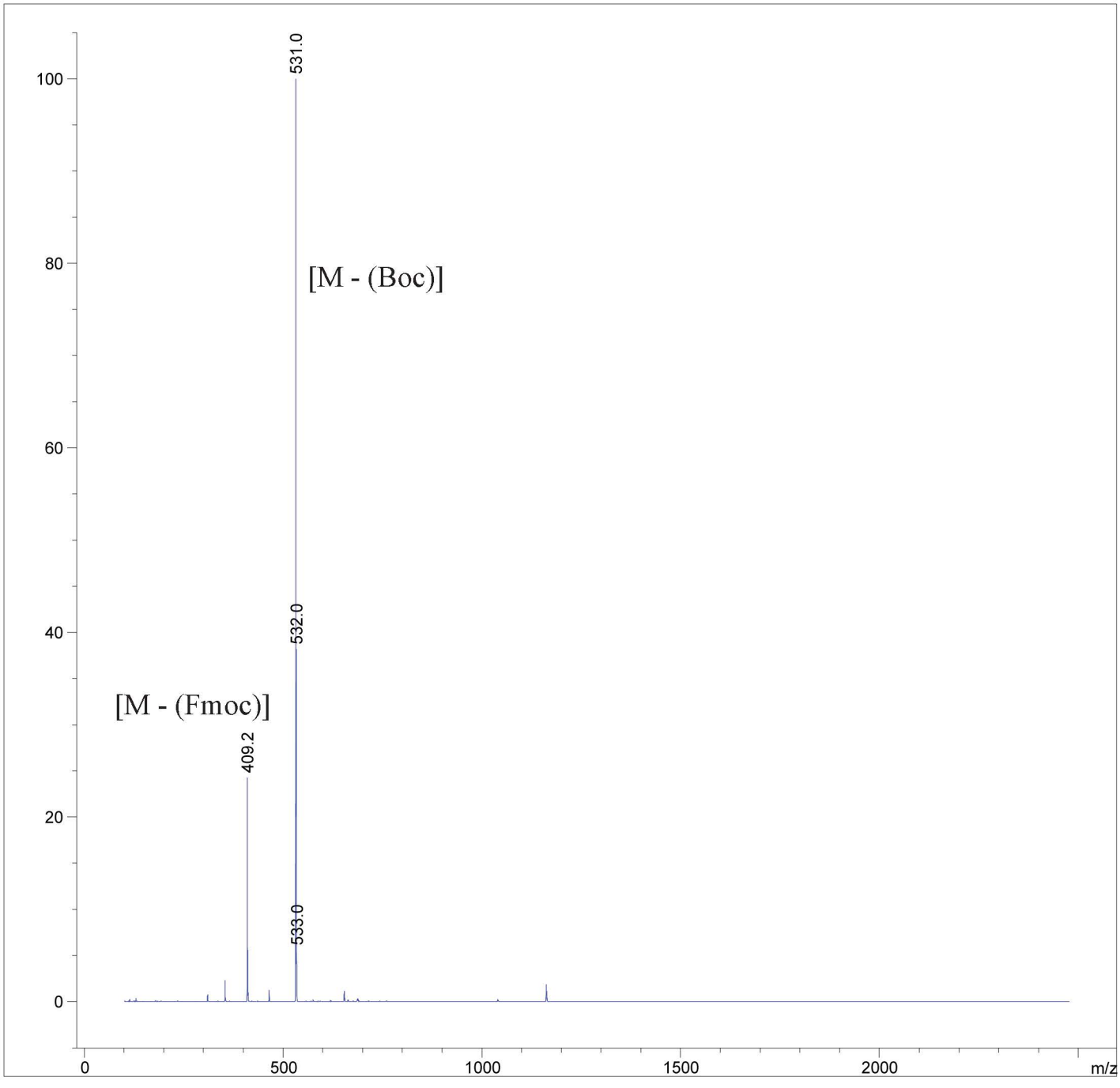


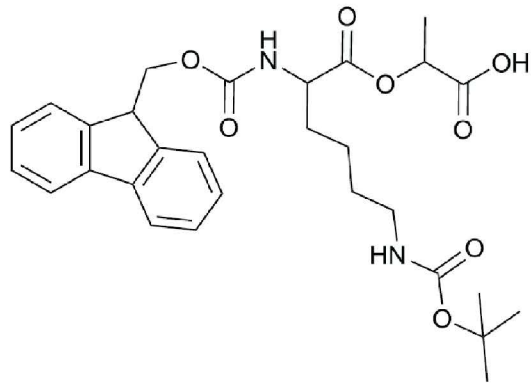
Molecular Weight: 630.73

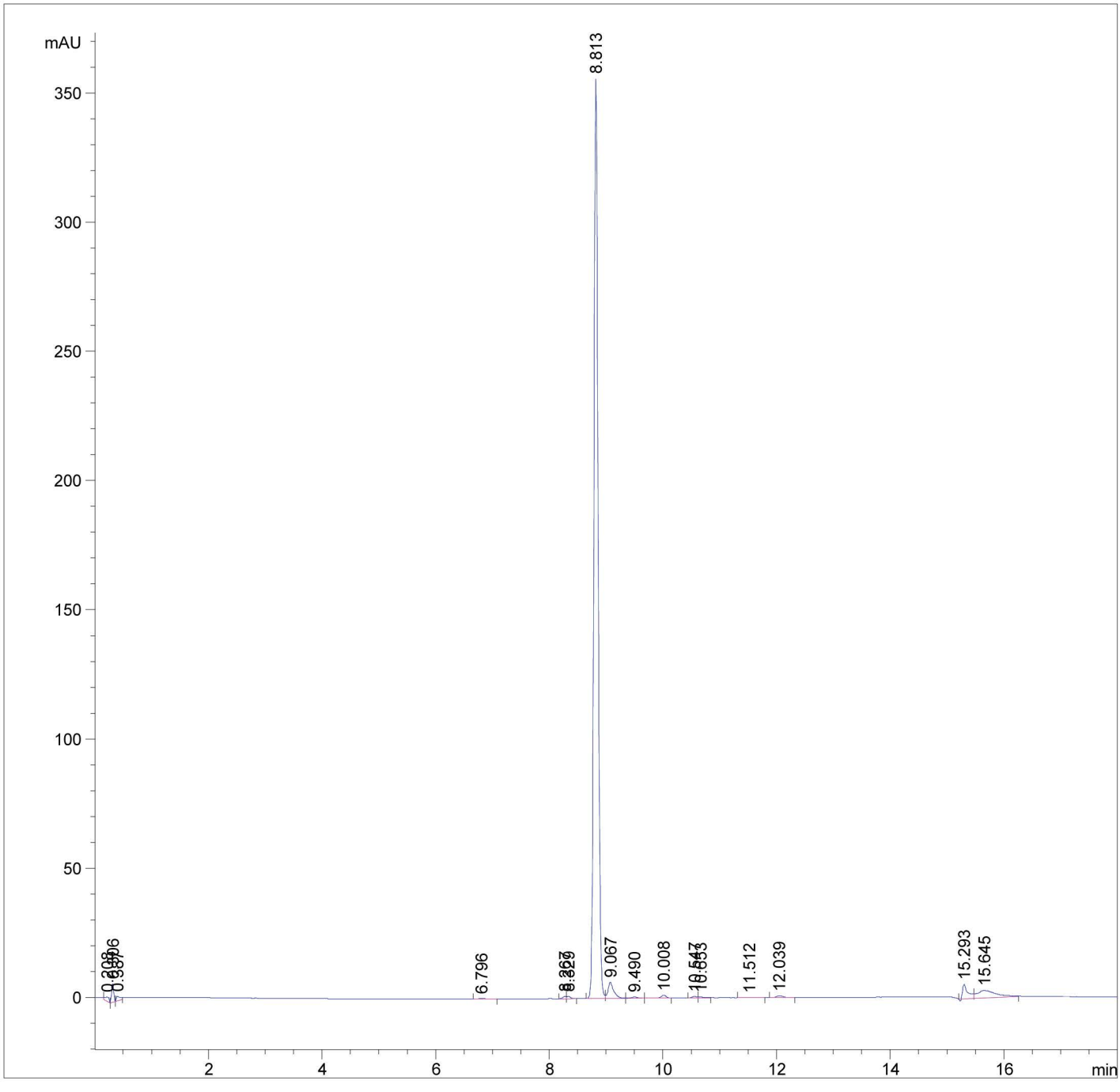


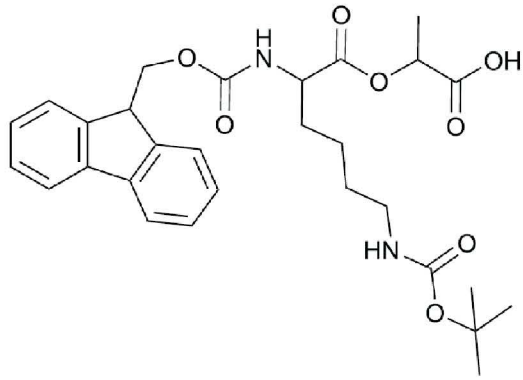


Molecular Weight: 630.73

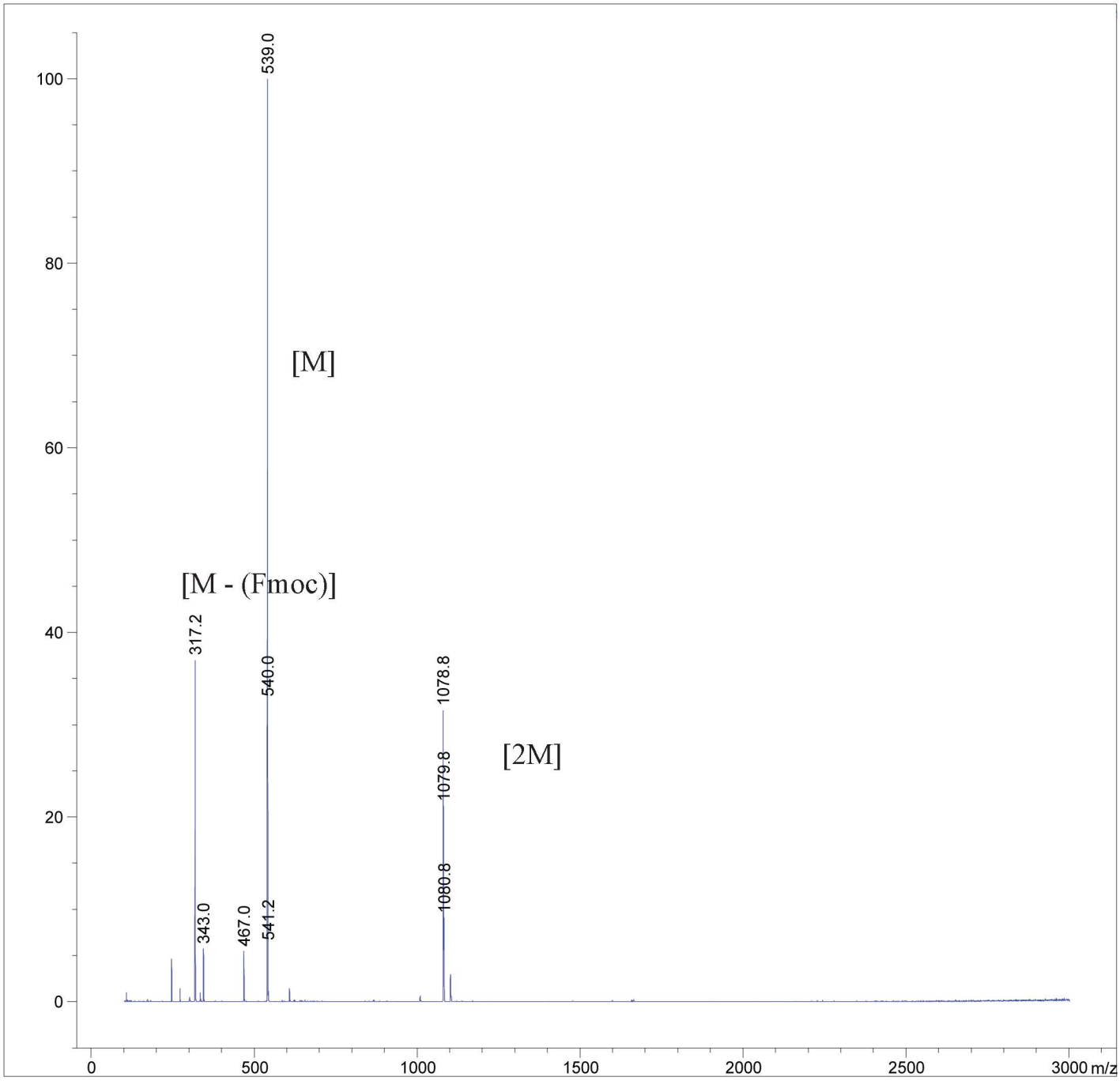


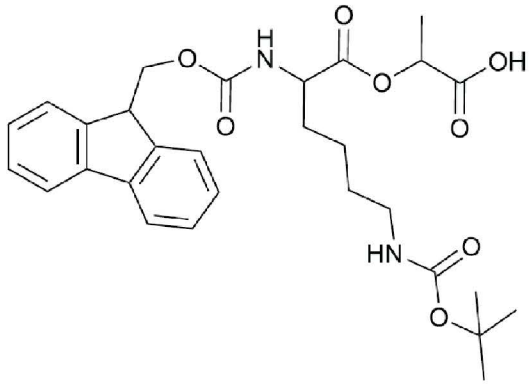






Molecular Weight: 540.60





Molecular Weight: 540.60