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Synthesis of nisin AB dicarba analogs using ring-closing metathesis: Influence of sp^3 versus sp^2 hybridization of the α-carbon atom of residues dehydrobutyrine-2 and dehydroalanine-5 on lipid II binding affinity

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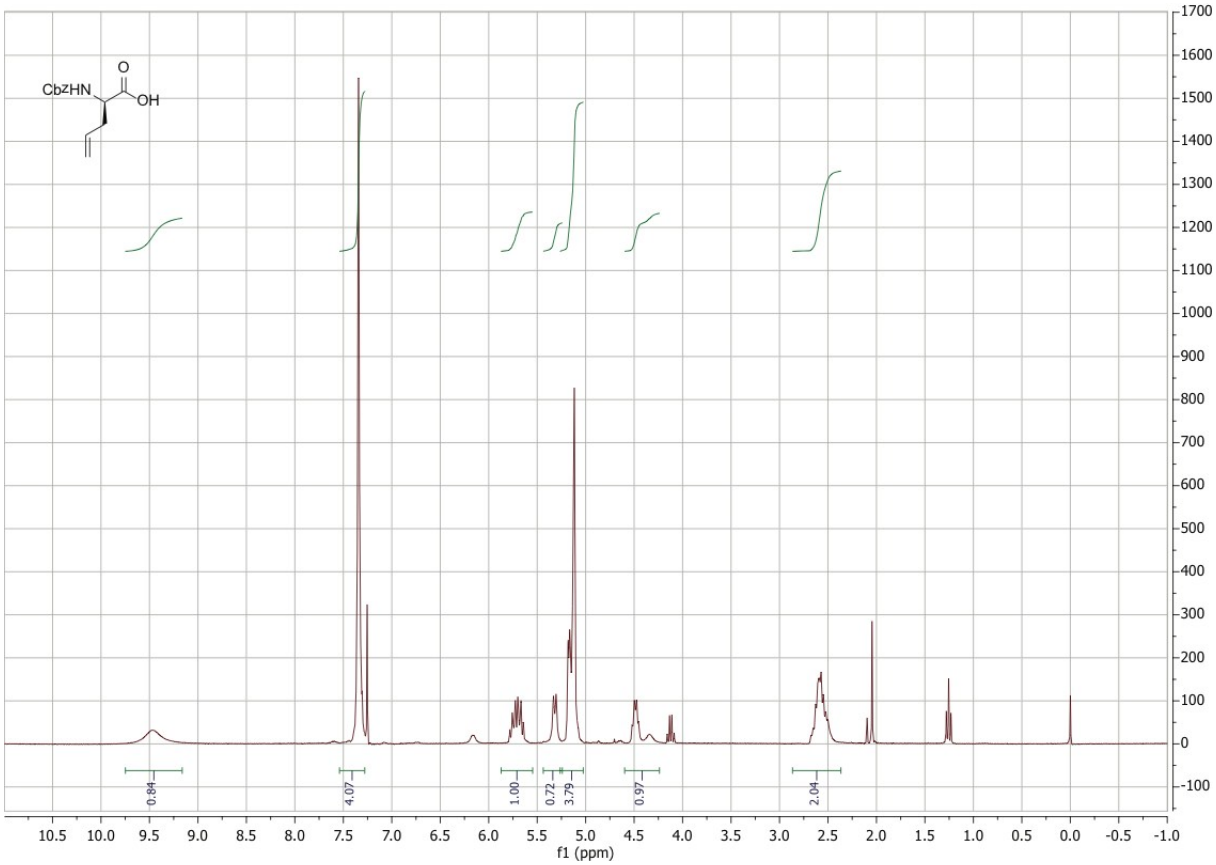
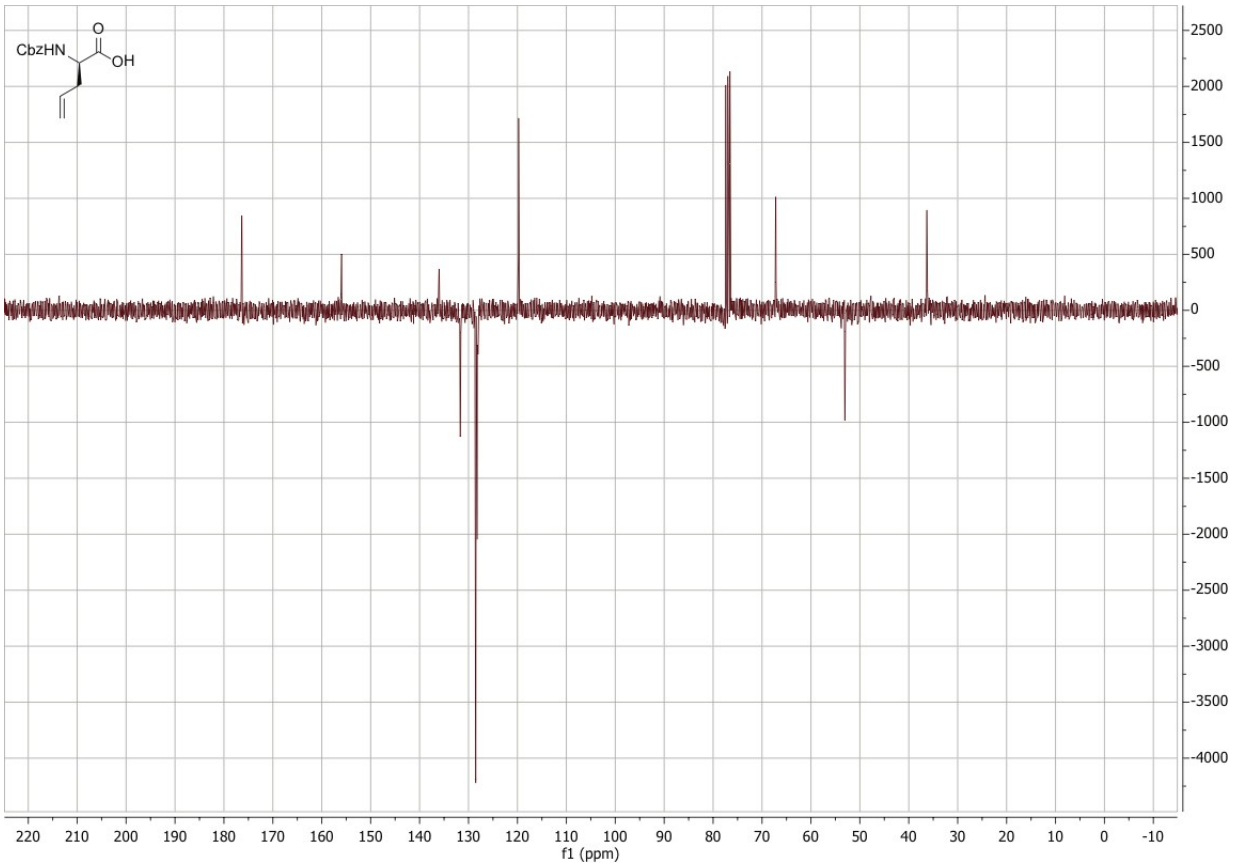
1. Synthesis and analysis of Cbz-D-Alg-OH

Synthesis of Cbz-D-Alg-OH. H-D-Alg-OH (1.13 g, 10 mmol) was dissolved in aq. 2M NaOH (5 mL) and the solution was vigorously stirred. Then, benzyl chloroformate (1.71 mL, 12 mmol) and aq. 2M NaOH (5.5 mL) were simultaneously added dropwise for 2 h to the reaction mixture and stirring was continued at room temperature for 16 h. Then, CH_2Cl_2 (15 mL) was added to the reaction mixture which was acidified with aq. 1N HCl until pH 1–2. The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), dried (Na_2SO_4) and concentrated *in vacuo* affording Cbz-D-Alg-OH as a colorless oil in a good yield of 79% (1.97 g). R_f 0.75 ($CHCl_3$/MeOH/AcOH 90:9:1 v/v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ = 2.34-2.76 (m, 2H, CβH$_2$), 4.49 (dd (J_{gem} = 13.0 Hz, J_{vic} 6.0 Hz), 1H, CαH), 5.03-5.23 (m, 4H, CδH$_2$ alkene / OCH$_2$ benzyl), 5.32 (d (J = 7.9 Hz), 1H, NH), 5.55-5.94 (m, 1H, CγH alkene), 7.33 (m, 5H, Ar-H benzyl), 9.47 (s, 1H, COOH); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 36.3, 53.1, 67.2, 119.8, 128.1, 128.3, 128.5, 132.7, 136.0, 156, 176.4.

^{1}H NMR spectrum of Cbz-D-Alg-OH (300 MHz, $CDCl_3$, T 298 K):

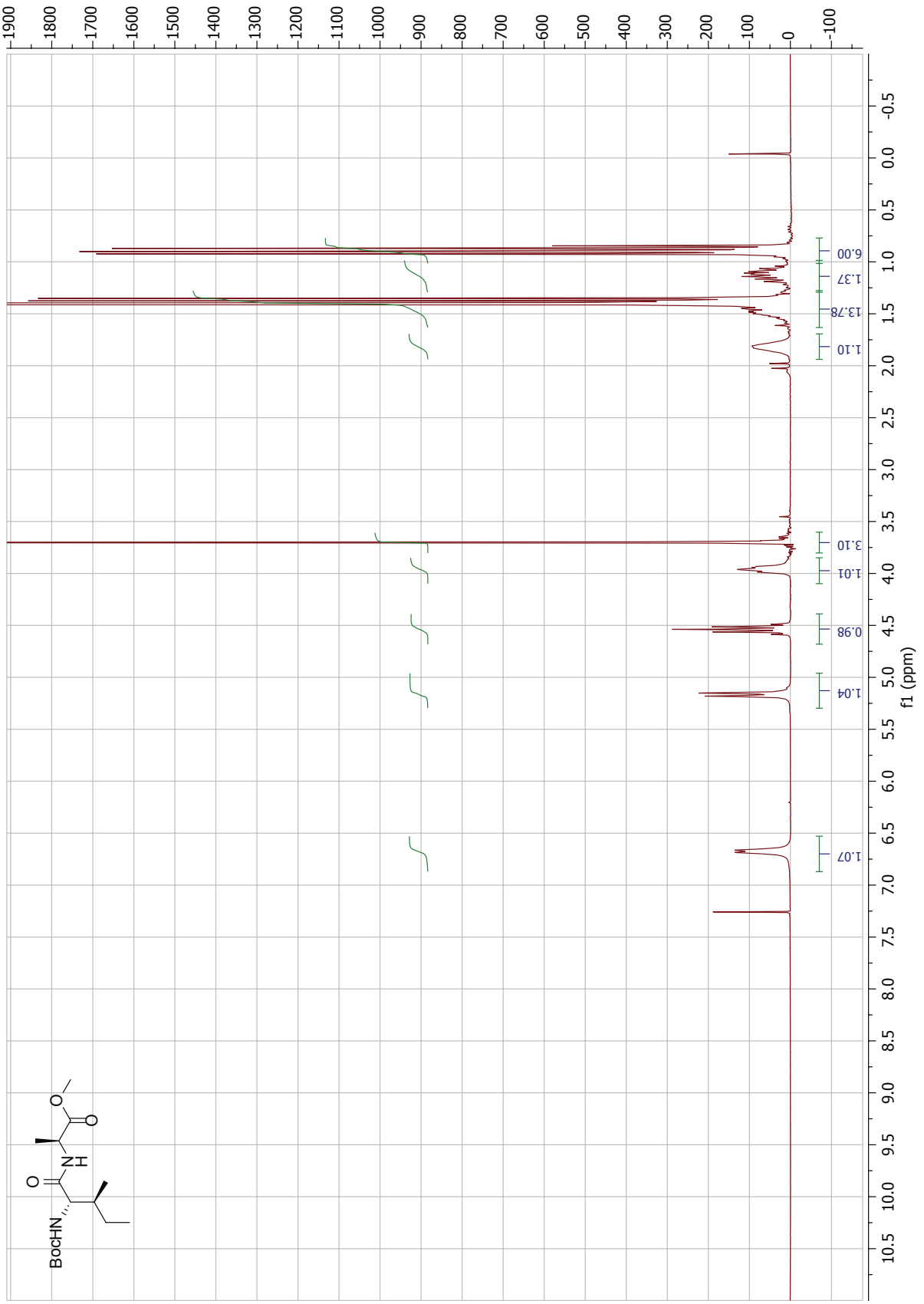


^{13}C NMR spectrum of Cbz-D-Alg-OH (75.5 MHz, $CDCl_3$, *T* 298 K):

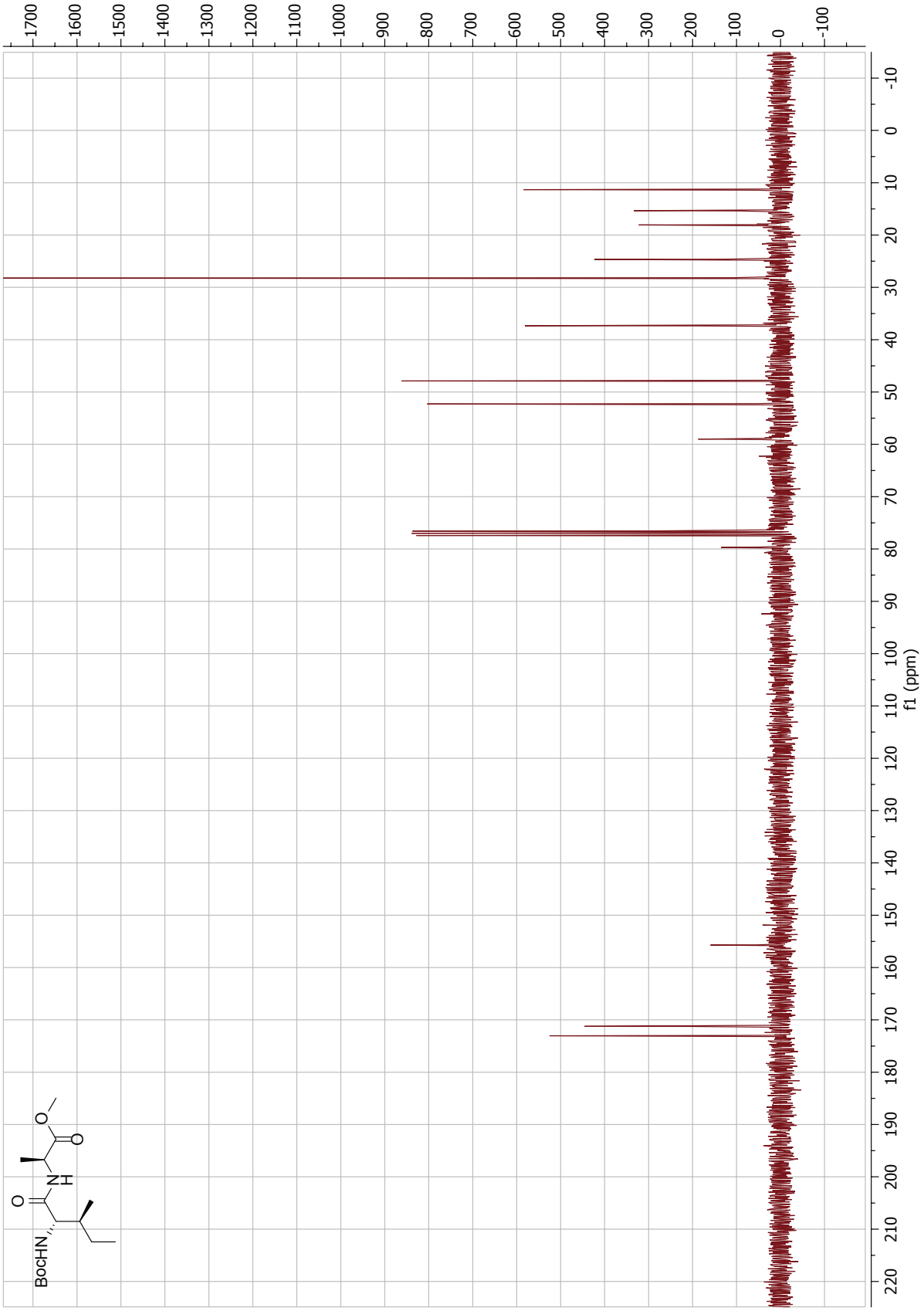


2. ^{1}H and ^{13}C NMR Spectra of Compounds 4-9.

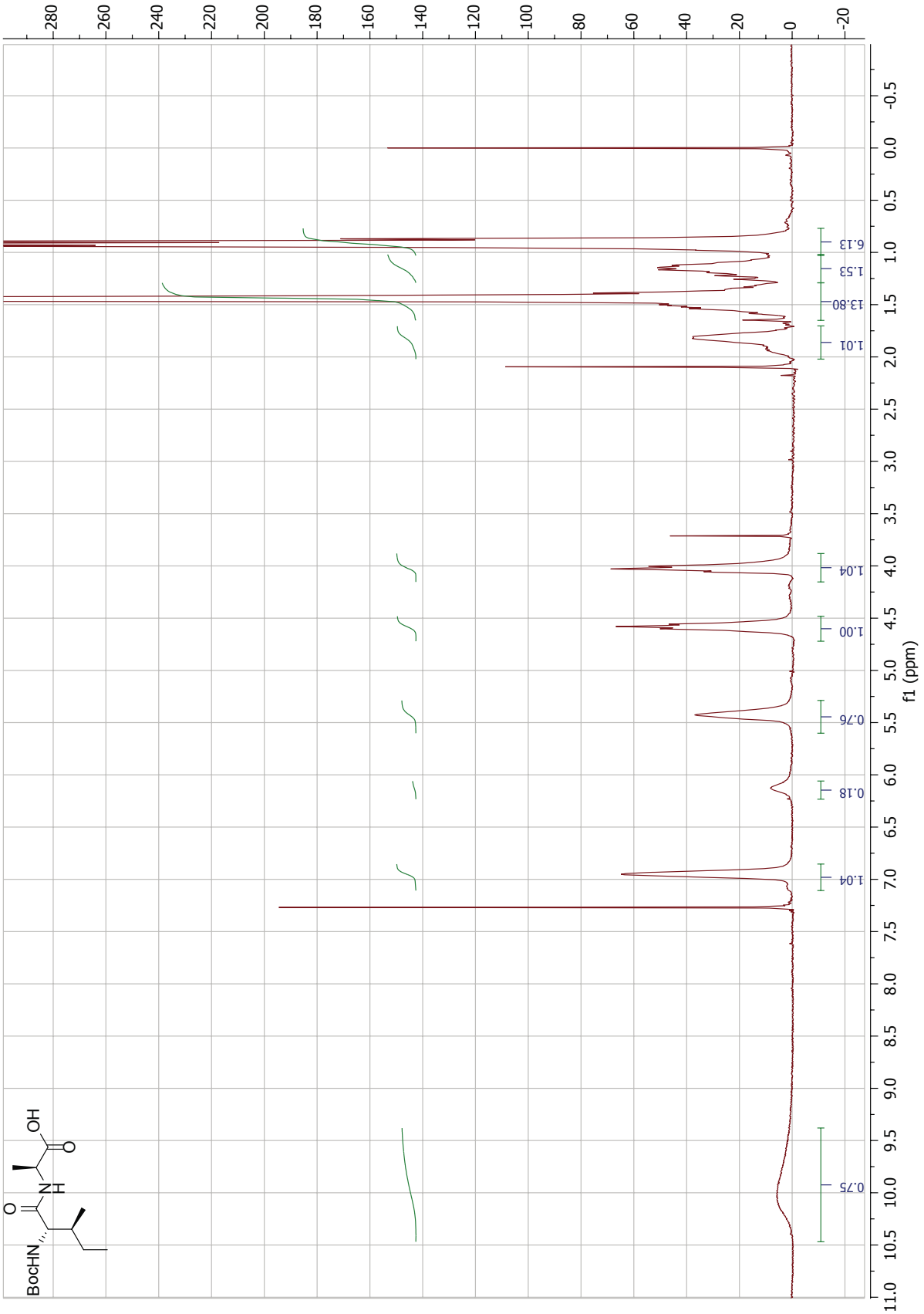
^{1}H NMR spectrum of compound **4** (300 MHz, $CDCl_3$, *T* 298 K):



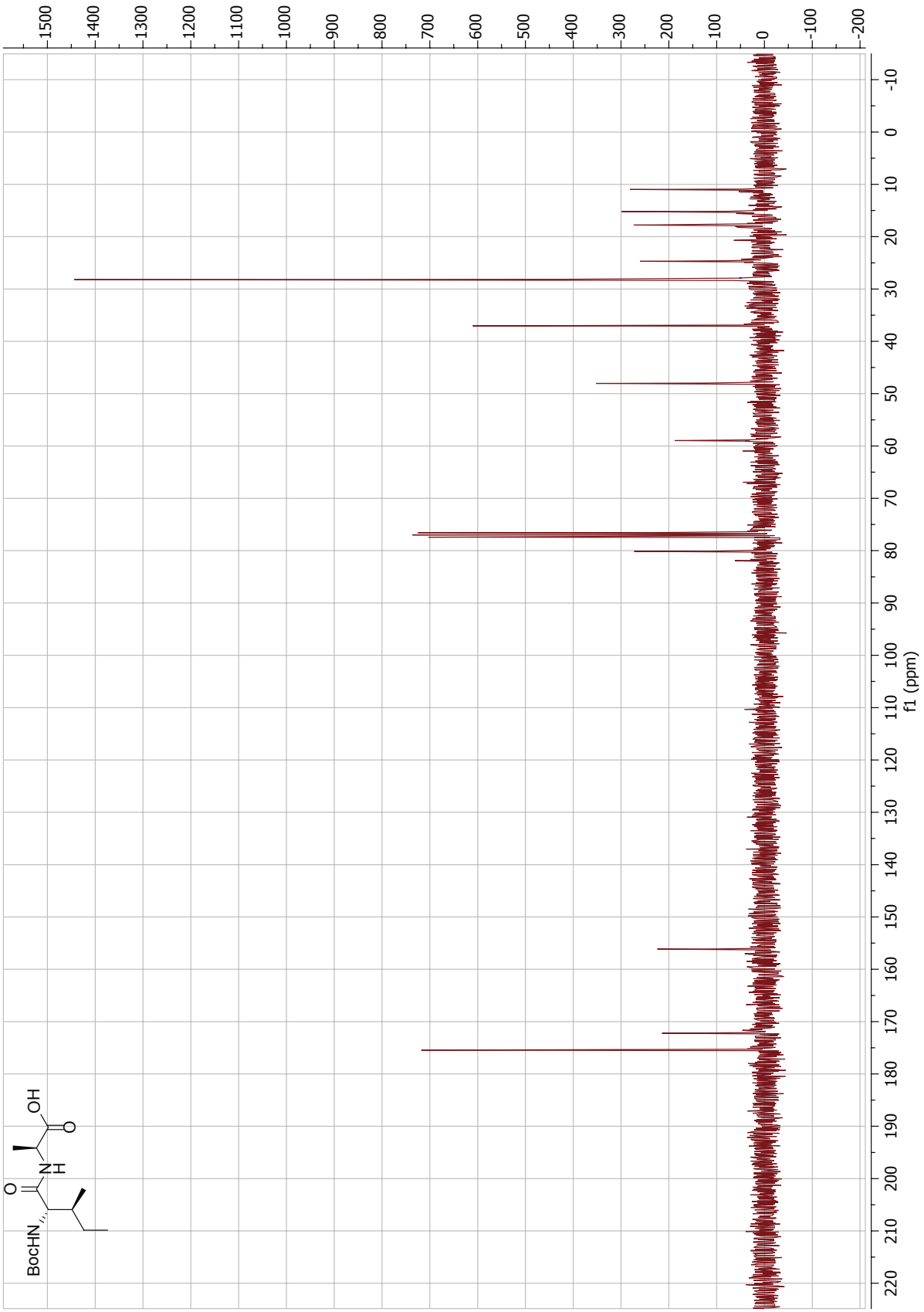
^{13}C NMR spectrum of compound **4** (75.5 MHz, $CDCl_3$, *T* 298K):



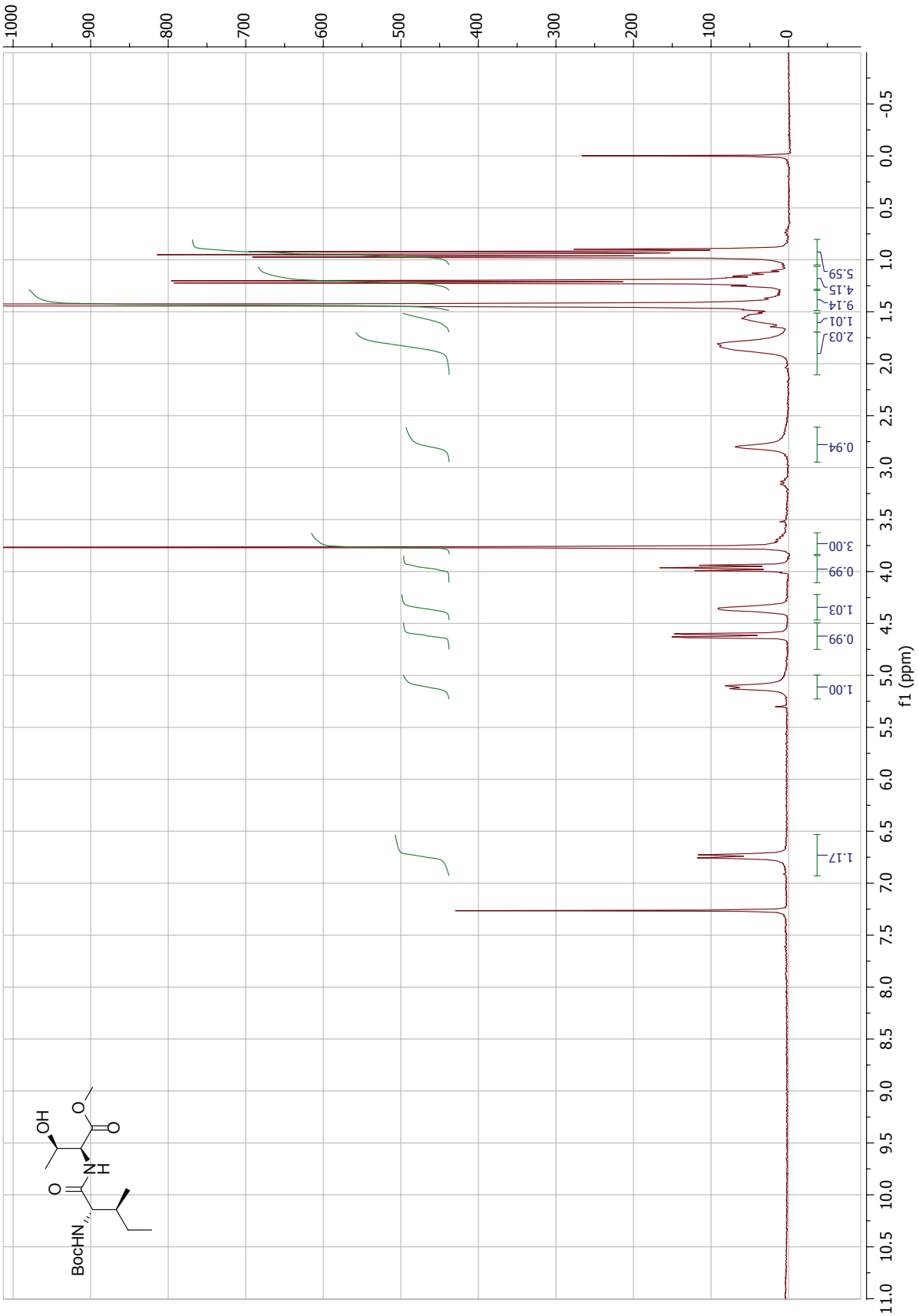
^{1}H NMR spectrum of compound **5** (300 MHz, $CDCl_3$, *T* 298K):



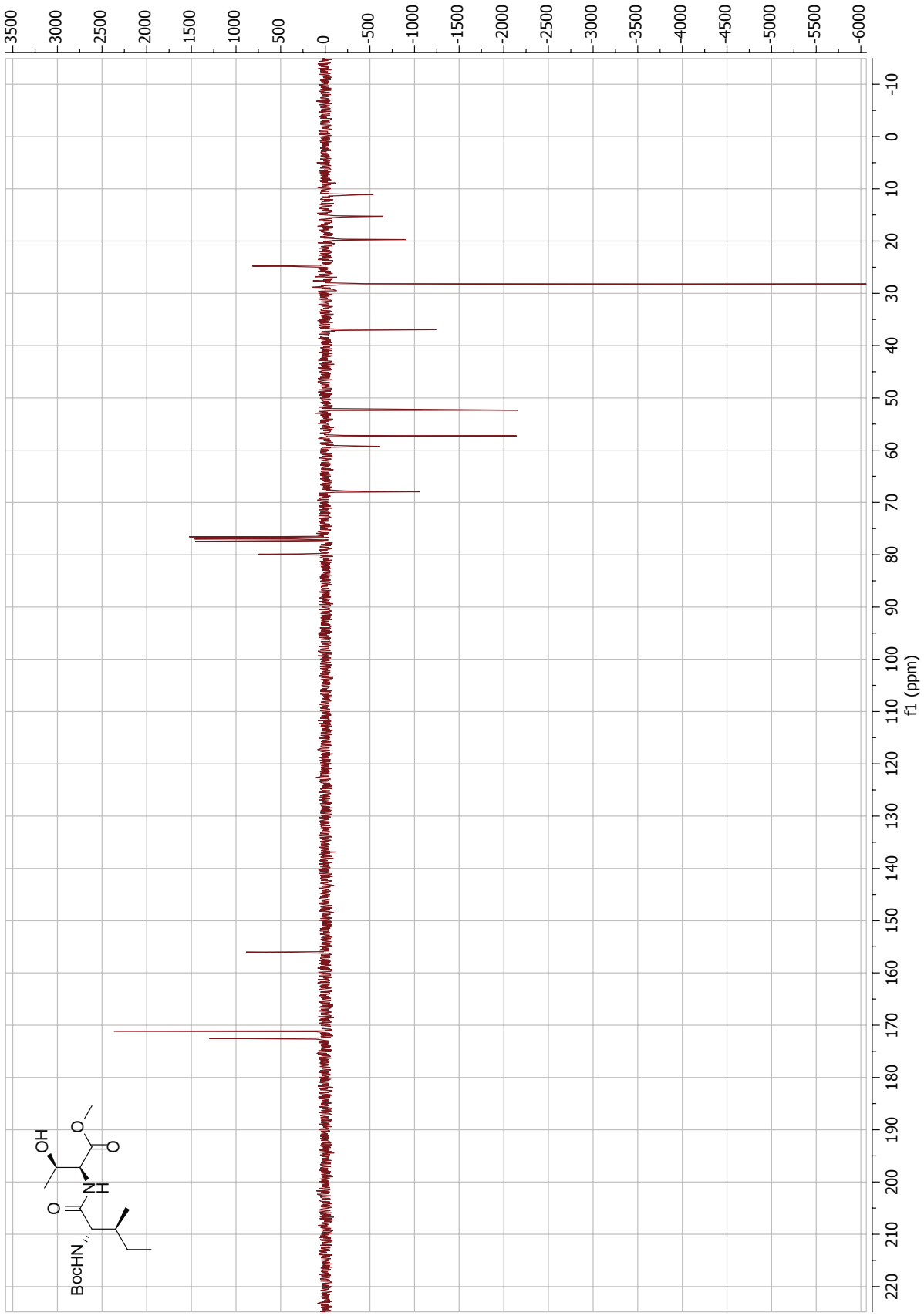
^{13}C NMR spectrum of compound **5** (75.5 MHz, $CDCl_3$, *T* 298K):



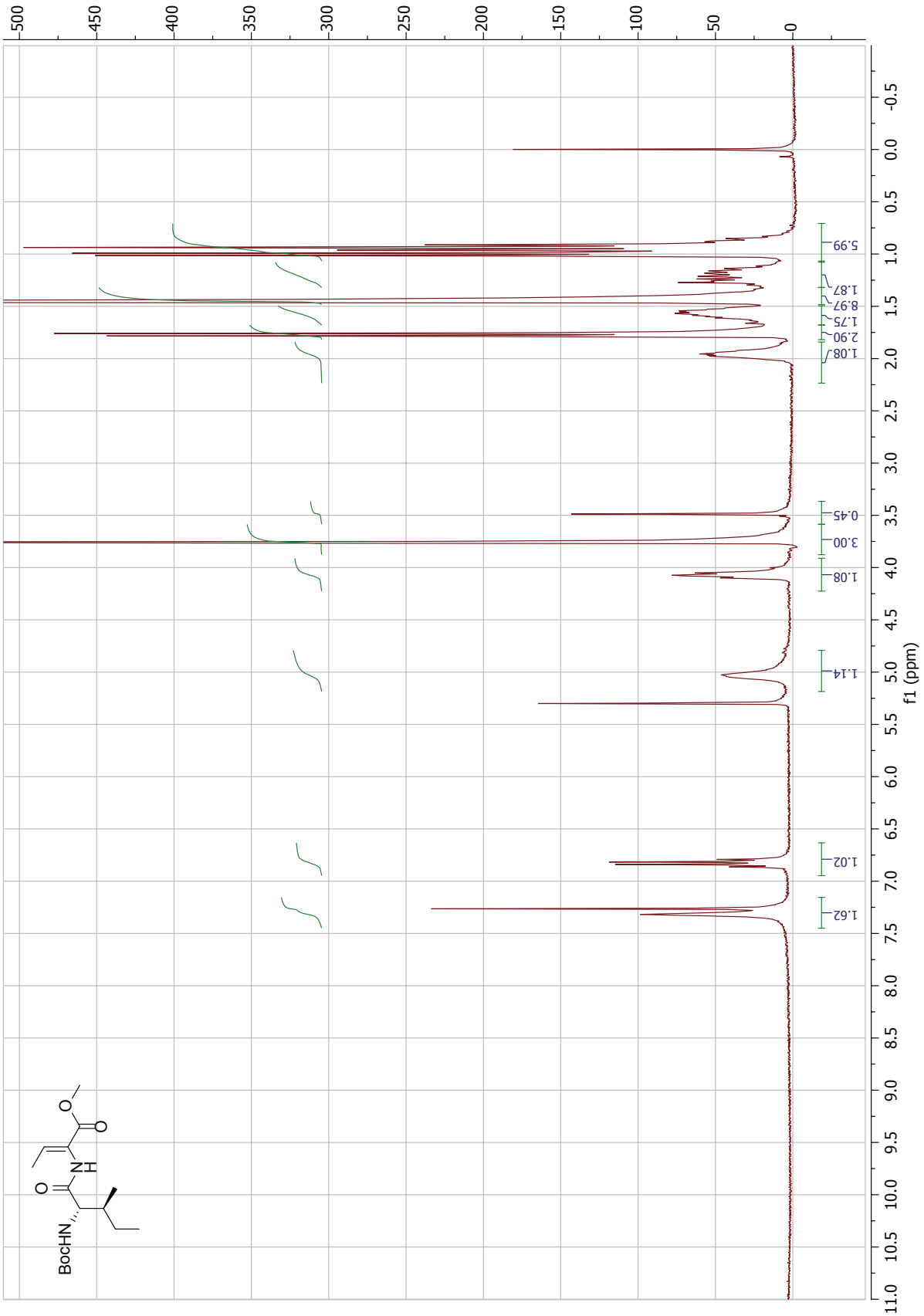
^{1}H spectrum of compound **6** (300 MHz, $CDCl_3$, *T* 298K):



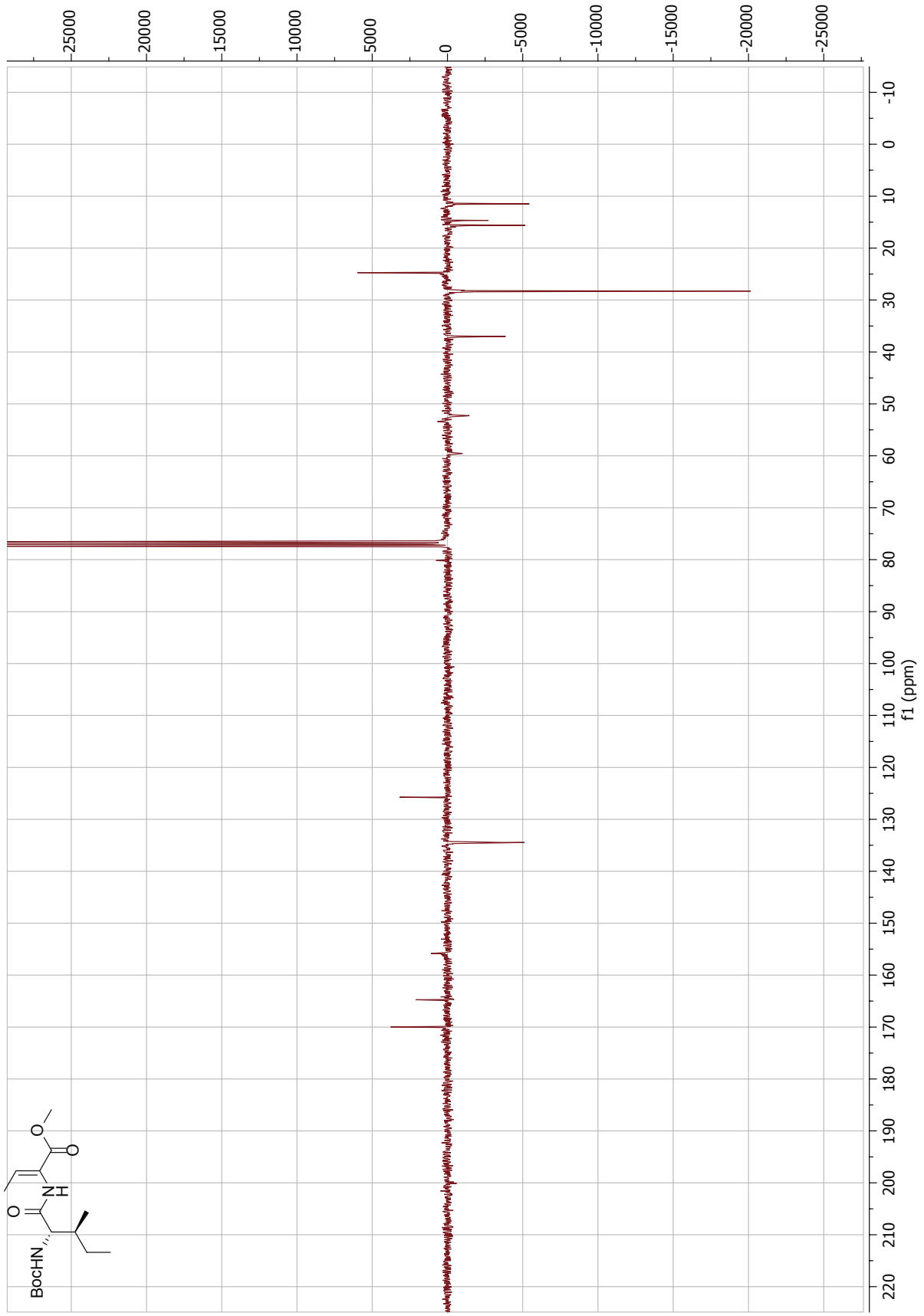
^{13}C NMR (APT) spectrum of compound **6** (75.5 MHz, $CDCl_3$, *T* 298K):



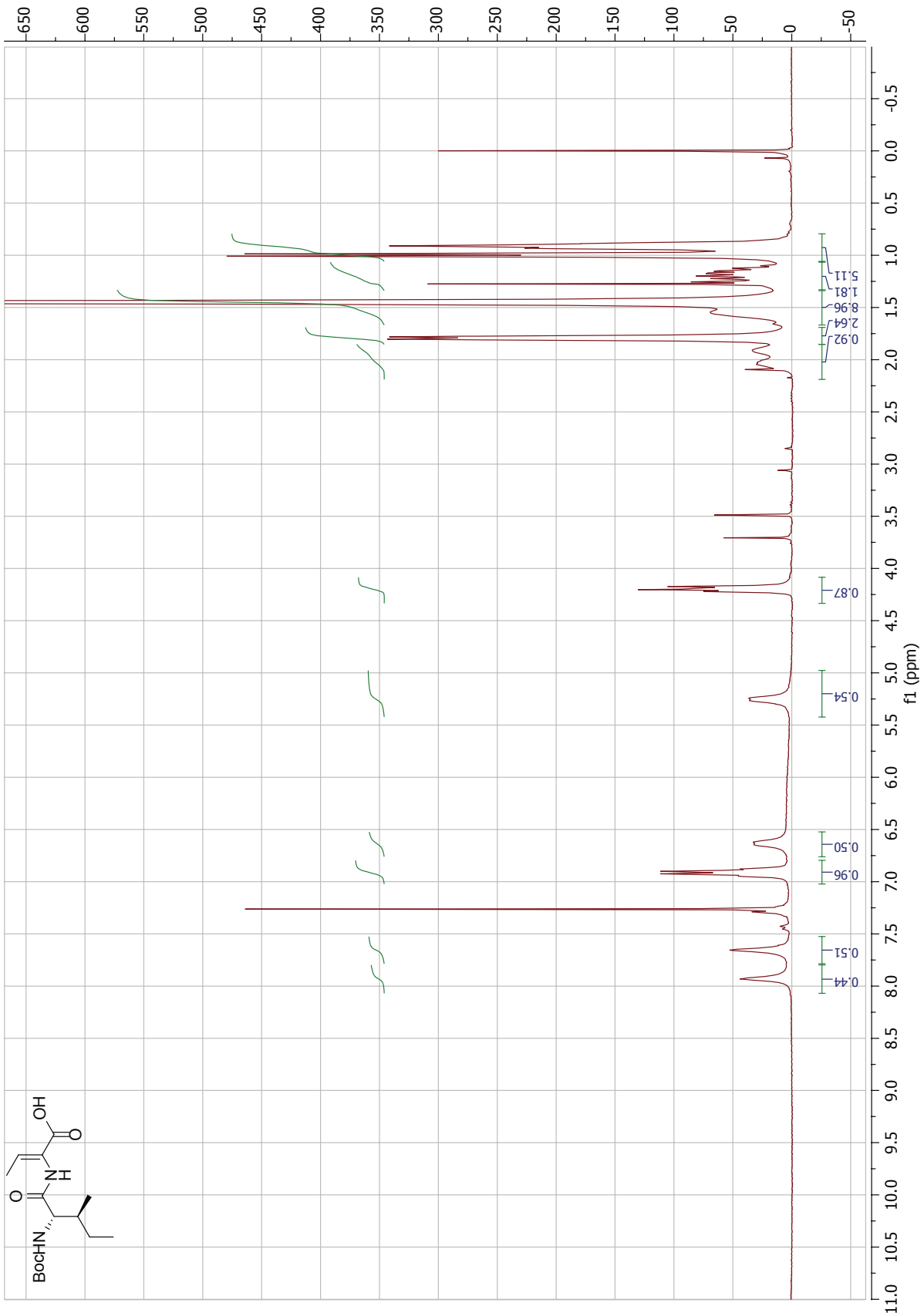
^{1}H NMR spectrum of compound **7** (300 MHz, $CDCl_3$, *T* 298K):



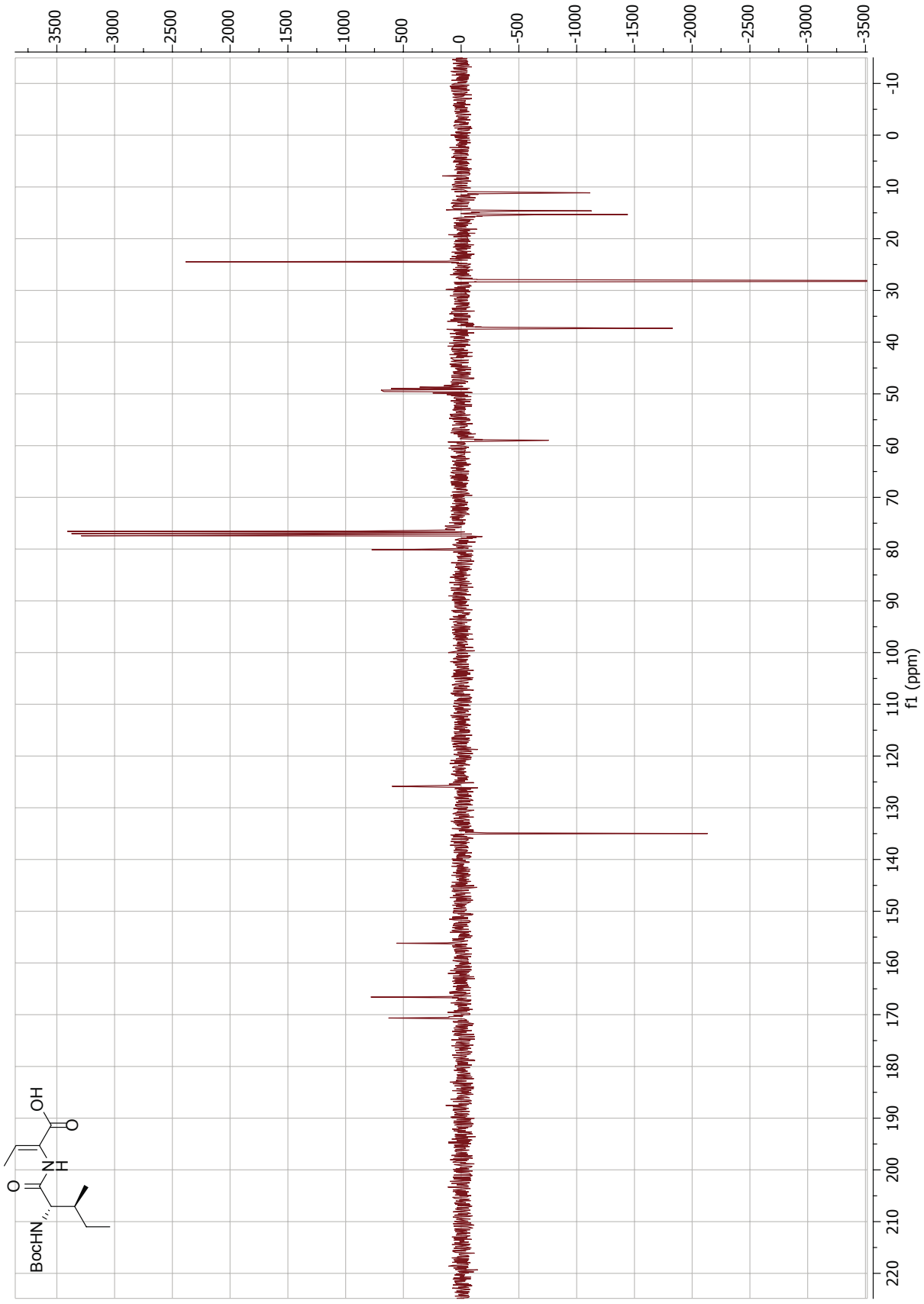
^{13}C NMR spectrum of compound **7** (75.5 MHz, $CDCl_3$, *T* 298K):



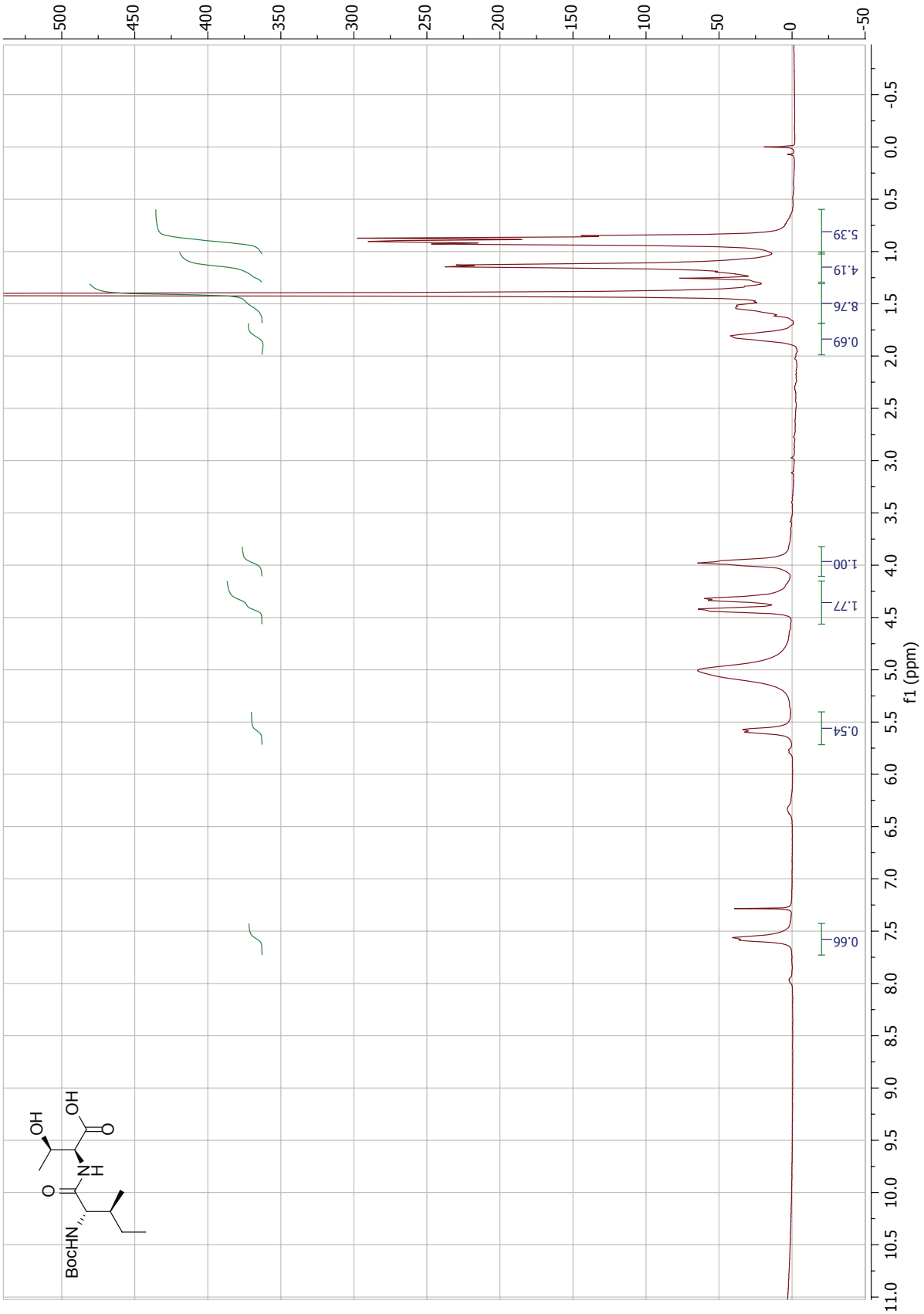
^{1}H NMR spectrum of compound **8** (300 MHz, $CDCl_3/CD_3OD$ 95:5 v/v, *T* 298K):



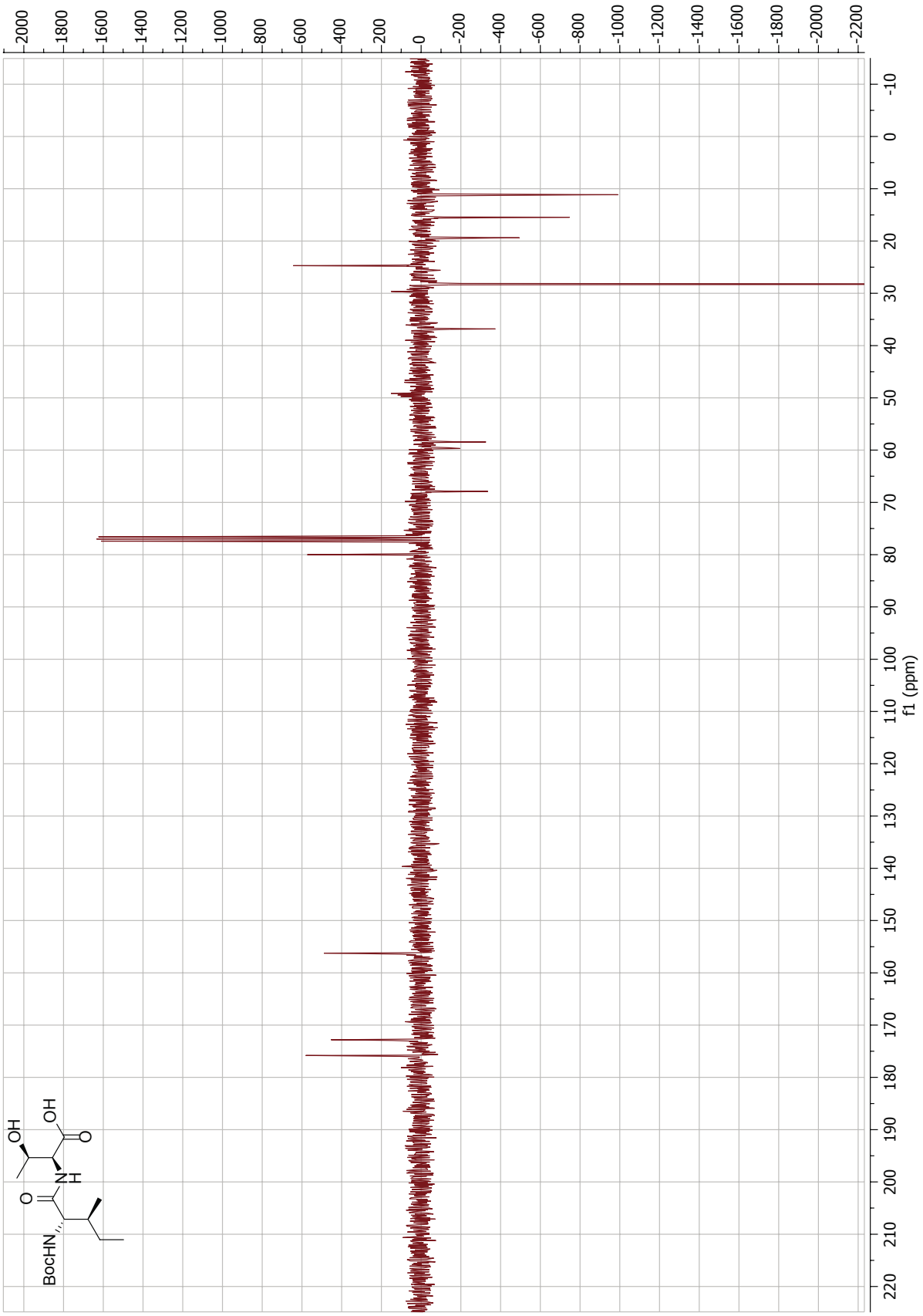
^{13}C NMR spectrum of compound **8** (75.5 MHz, $CDCl_3/CD_3OD$ 95:5 v/v, *T* 298K):



^{1}H NMR spectrum of compound **9** (300 MHz, $CDCl_3/CD_3OD$ 99:1 v/v, *T* 298K):



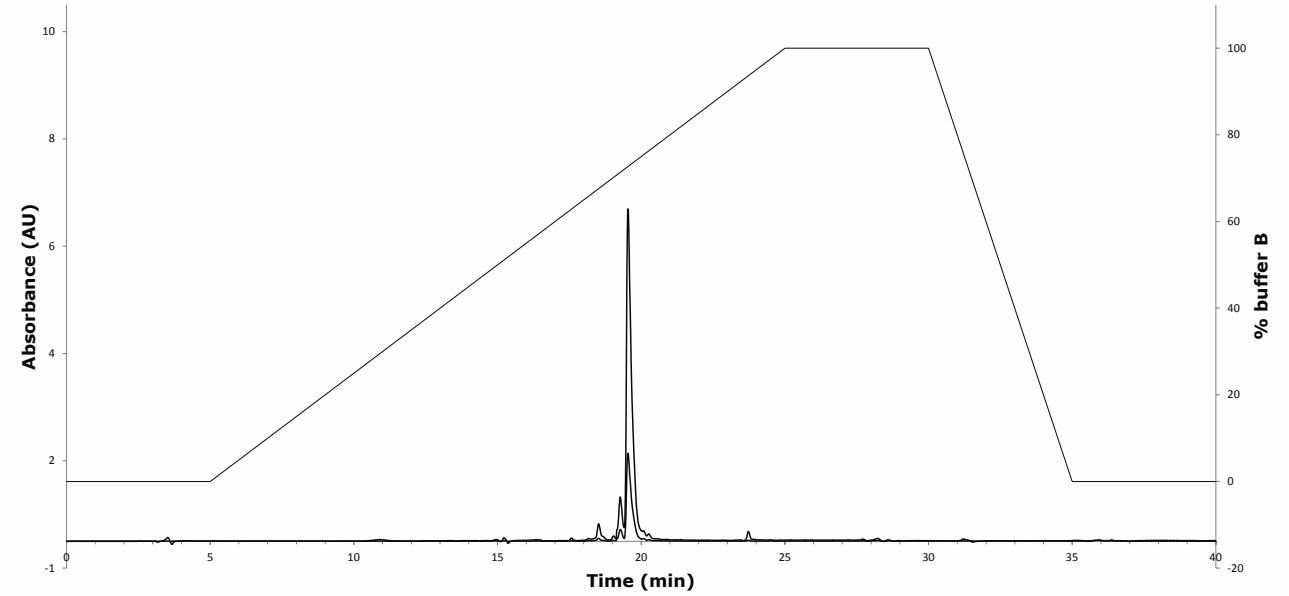
^{13}C NMR spectrum of compound **9** (75.5 MHz, $CDCl_3/CD_3OD$ 99:1 v/v, *T* 298K):



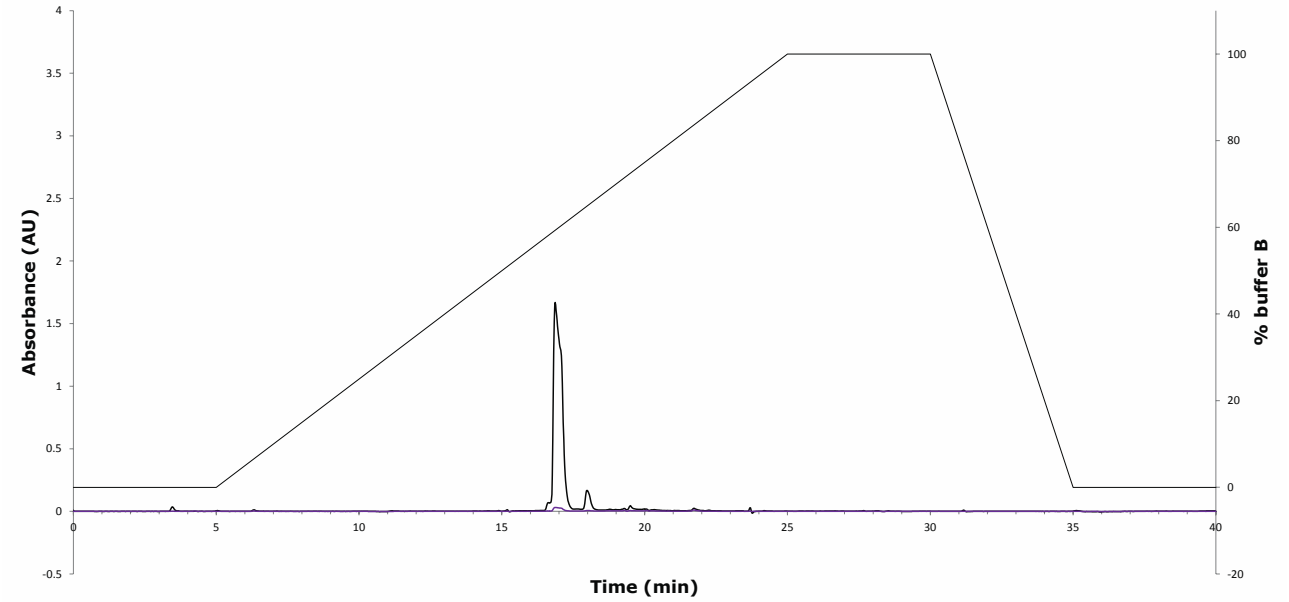
3. HPLC Chromatograms of Compounds 1-3, 10-12, 16-21, and 23-25.

All chromatograms were run on an automated Shimadzu HPLC system equipped with a UV/vis detector operating at 220/254 nm using an Alltech Prosphere C4 column (pore size: 300 Å, particle size: 5 μm, 250 × 4.6 mm) at a flow rate of 1 mL/min (from 100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 40 min.

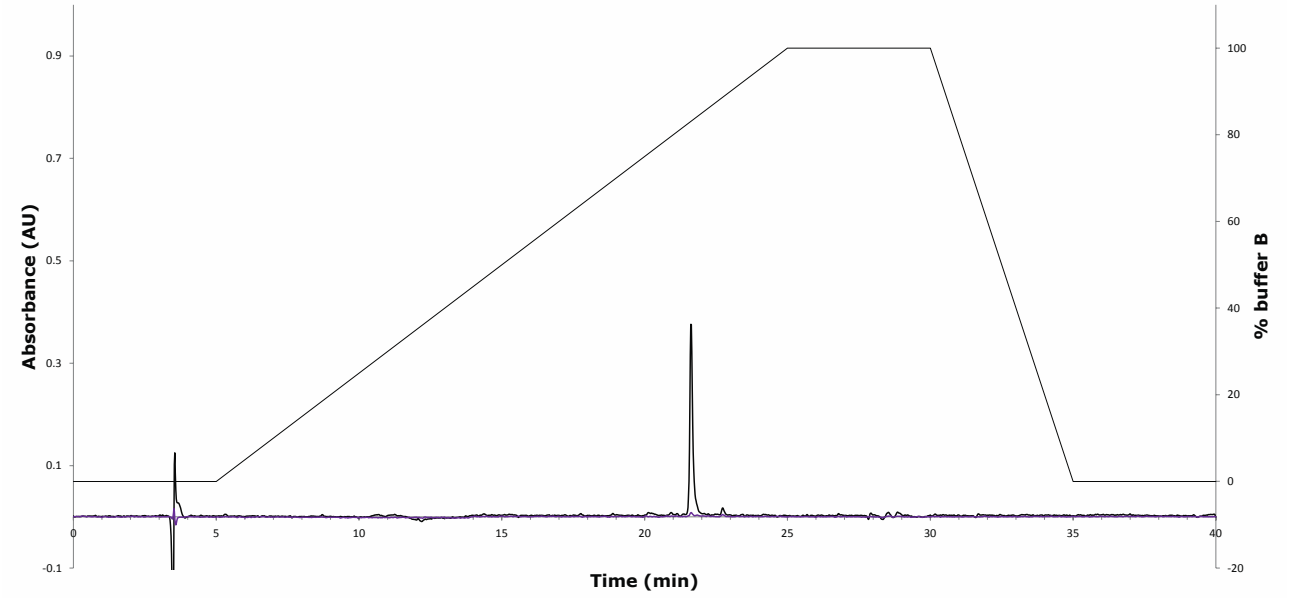
Compound **1**:



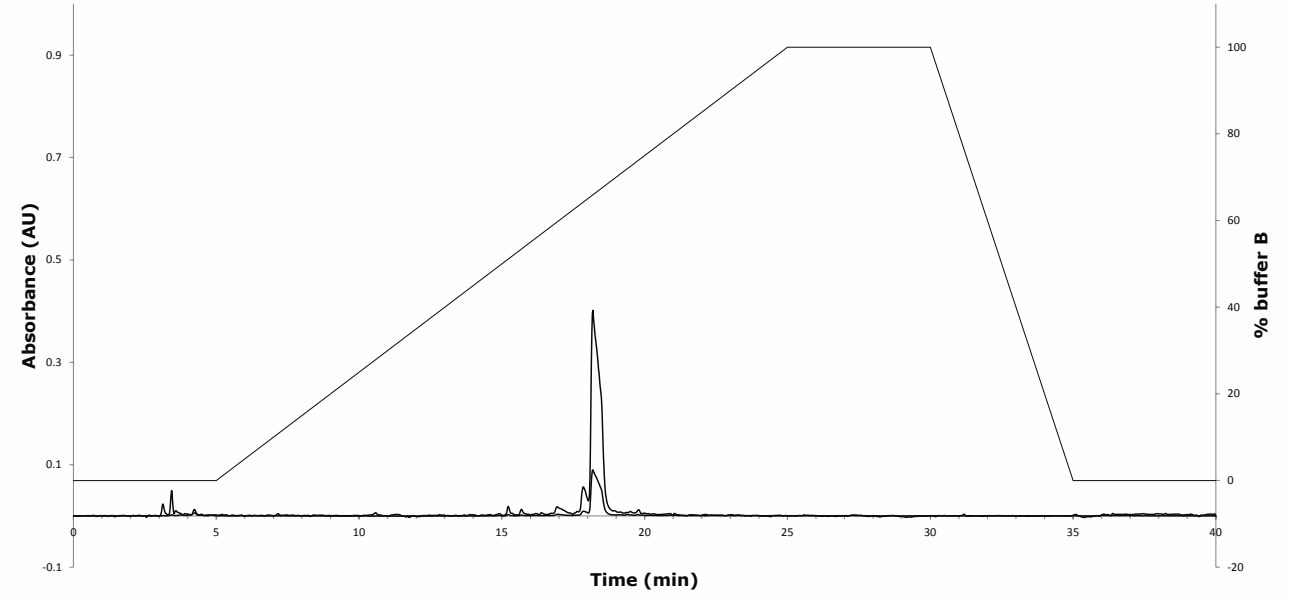
Compound **2**:



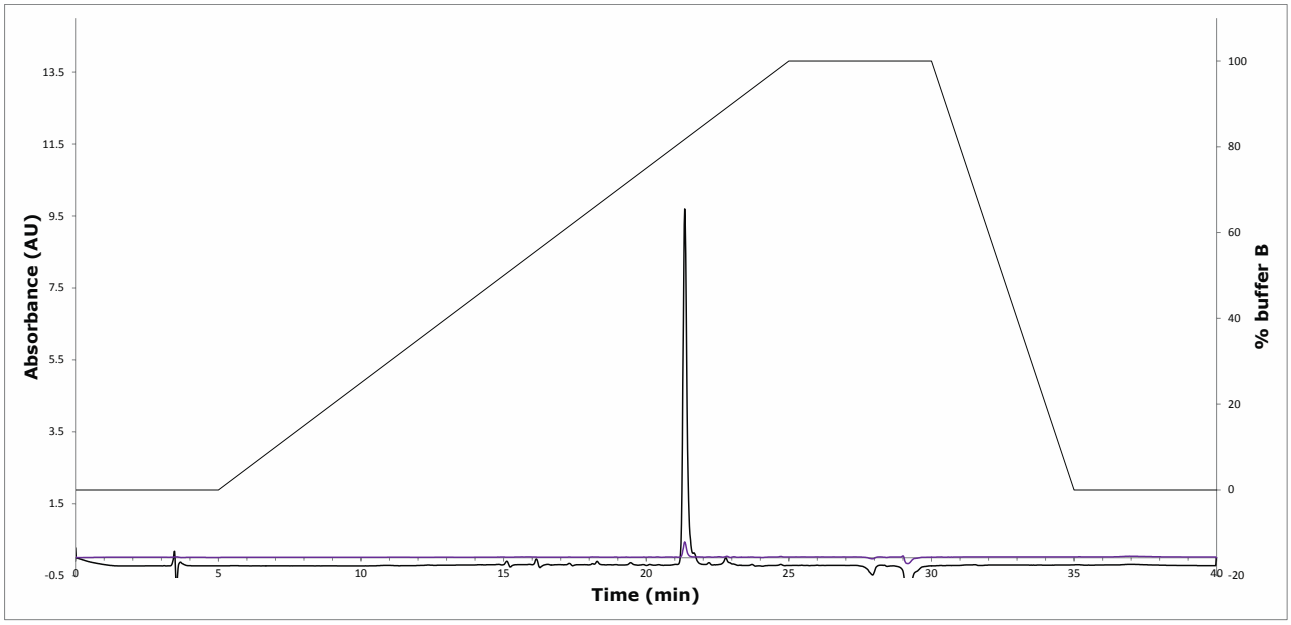
Boc-protected precursor of Compound **2**:



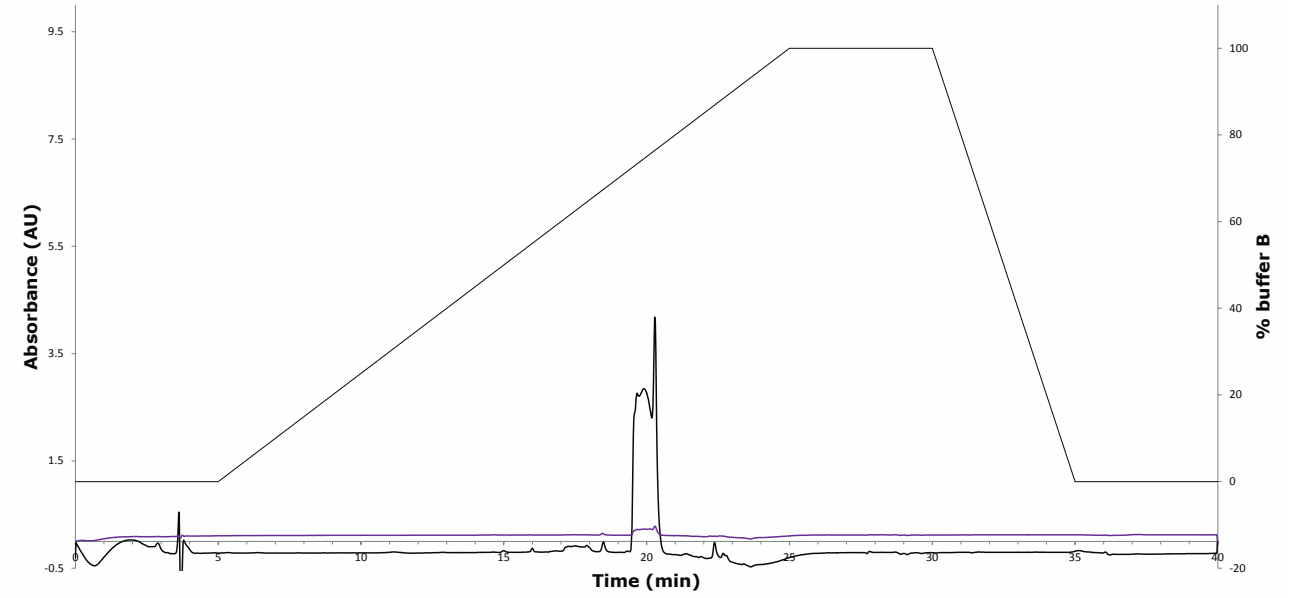
Compound **3**:



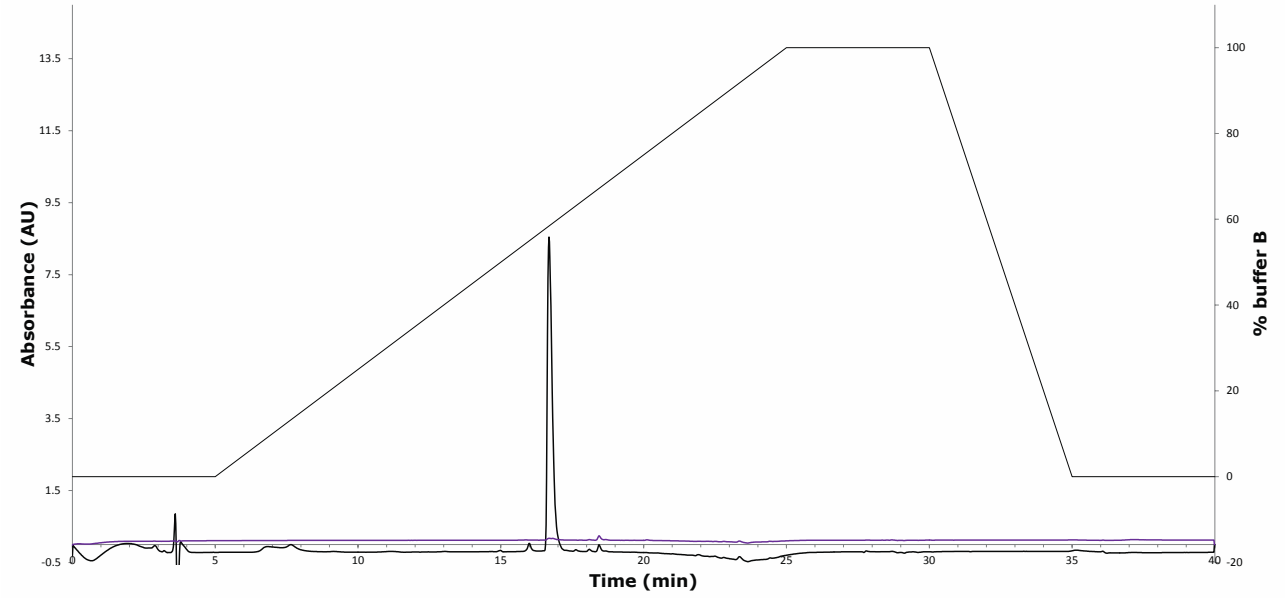
Compound **10**:



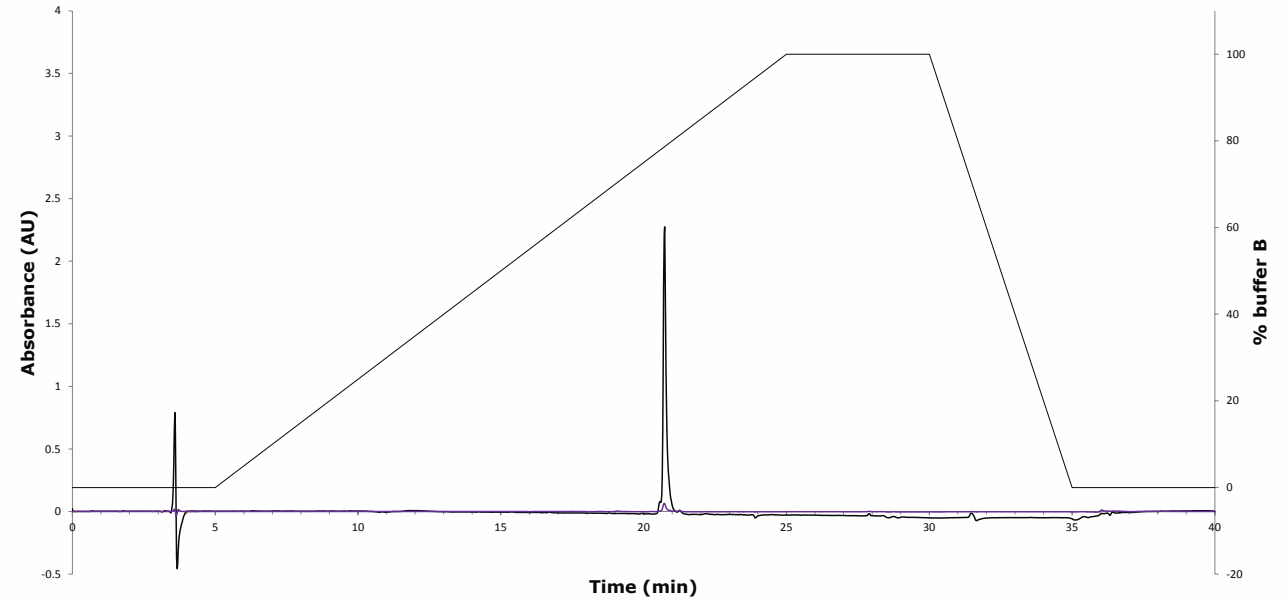
Compound **11**:



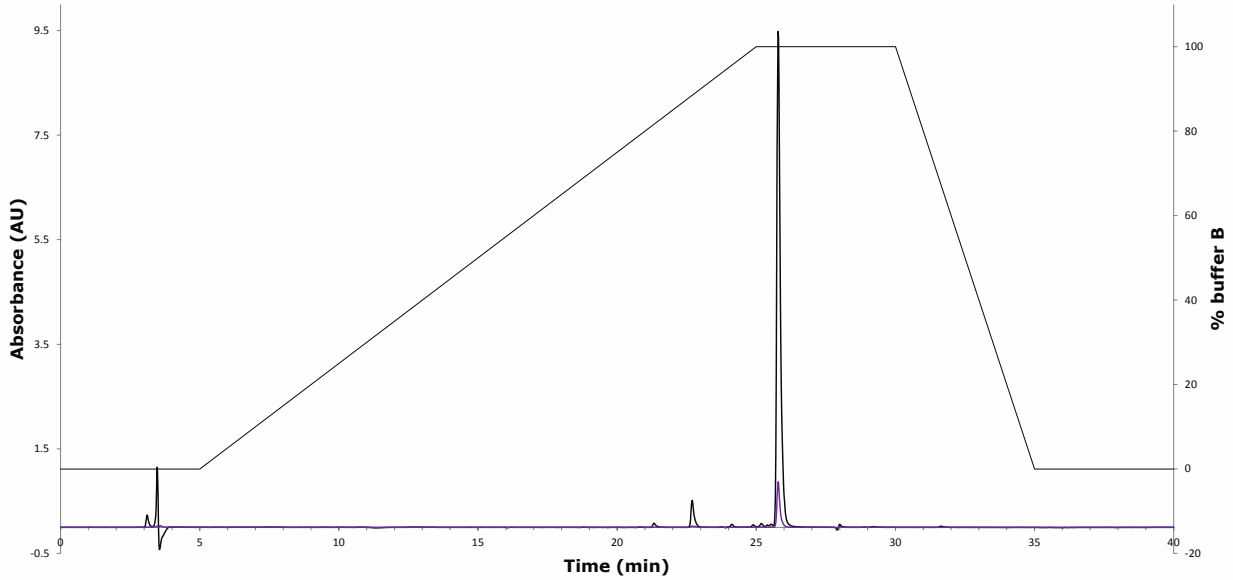
Compound **12**:



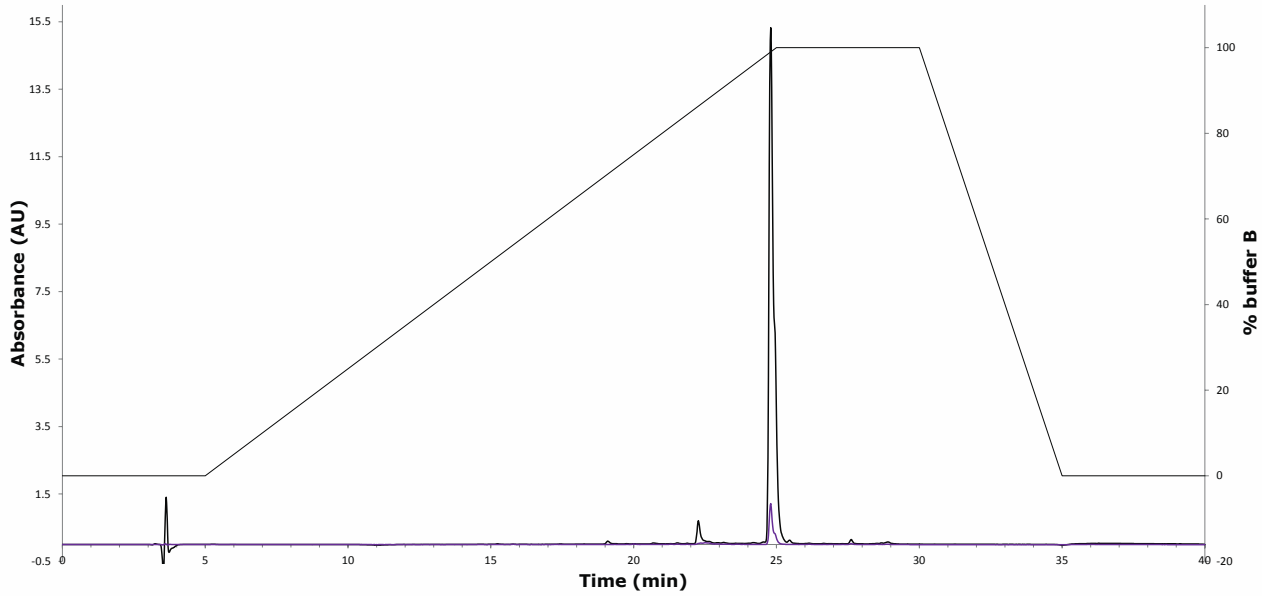
Compound **16**:



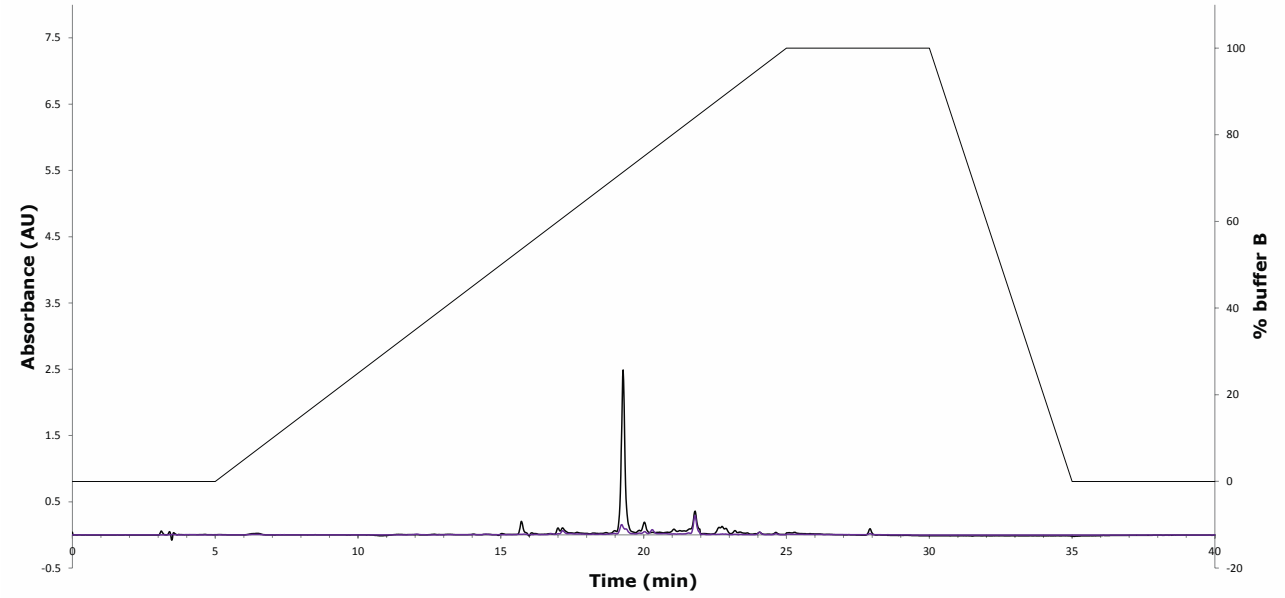
Compound **17**:



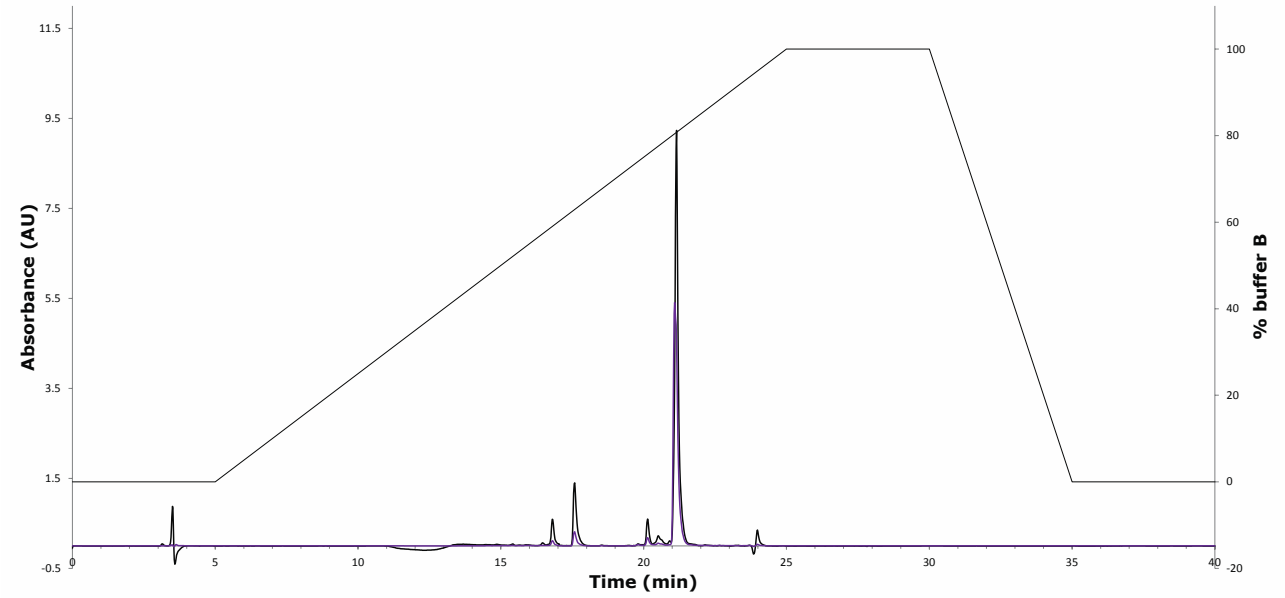
Compound **18**:



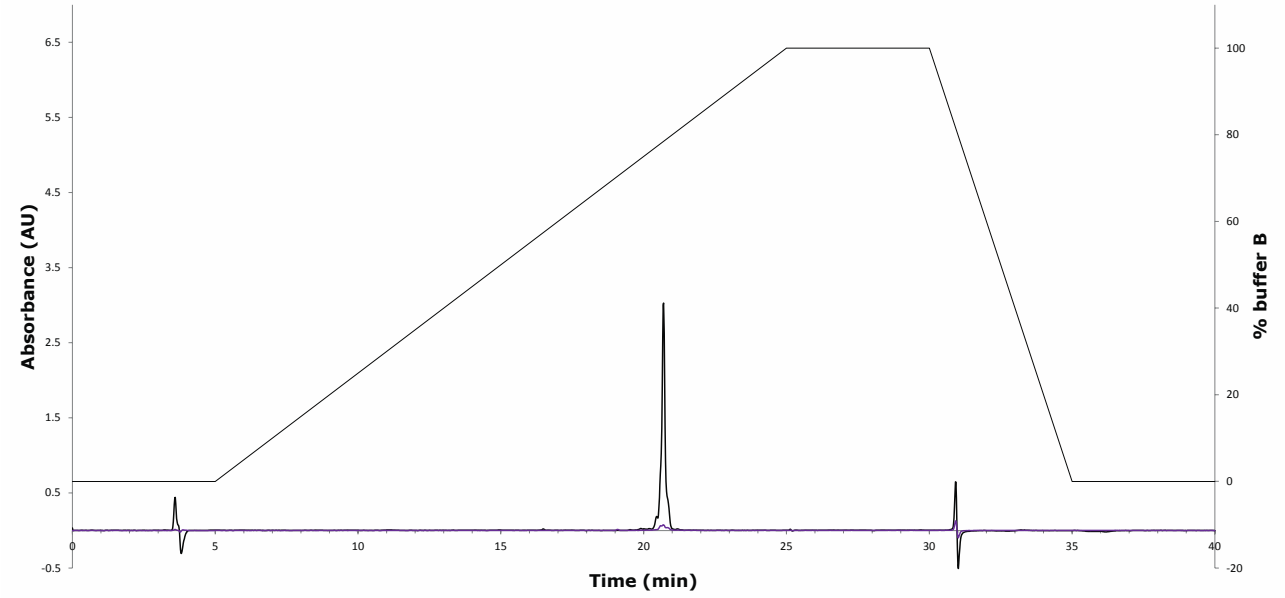
Compound **19**:



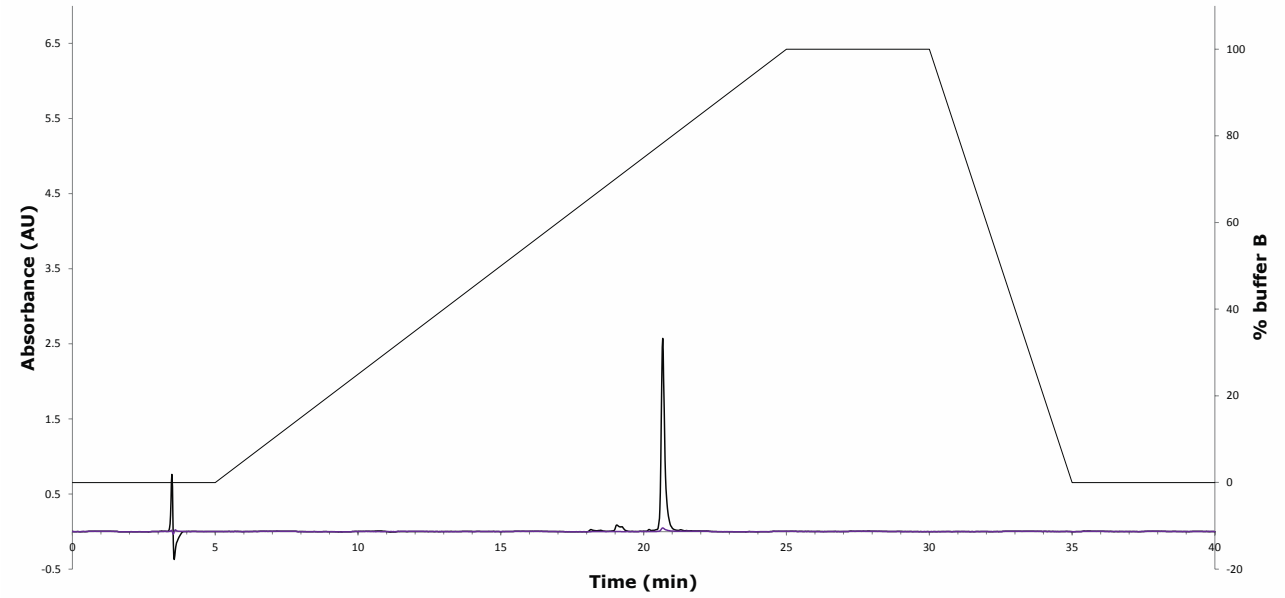
Compound **20**:



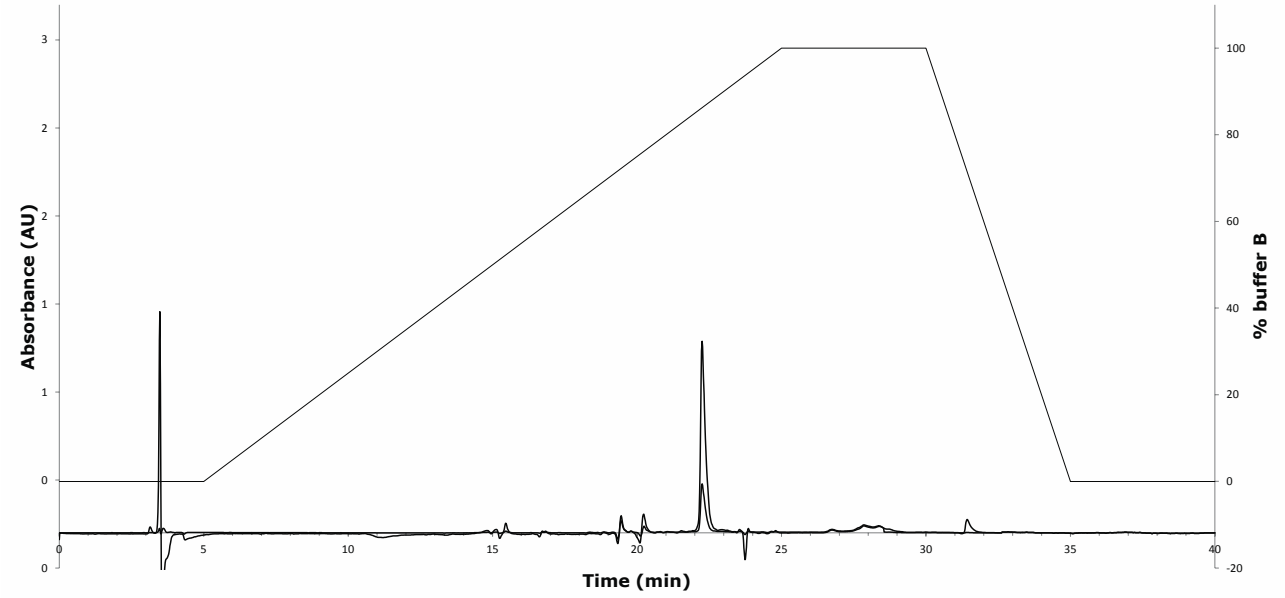
Compound **21**:



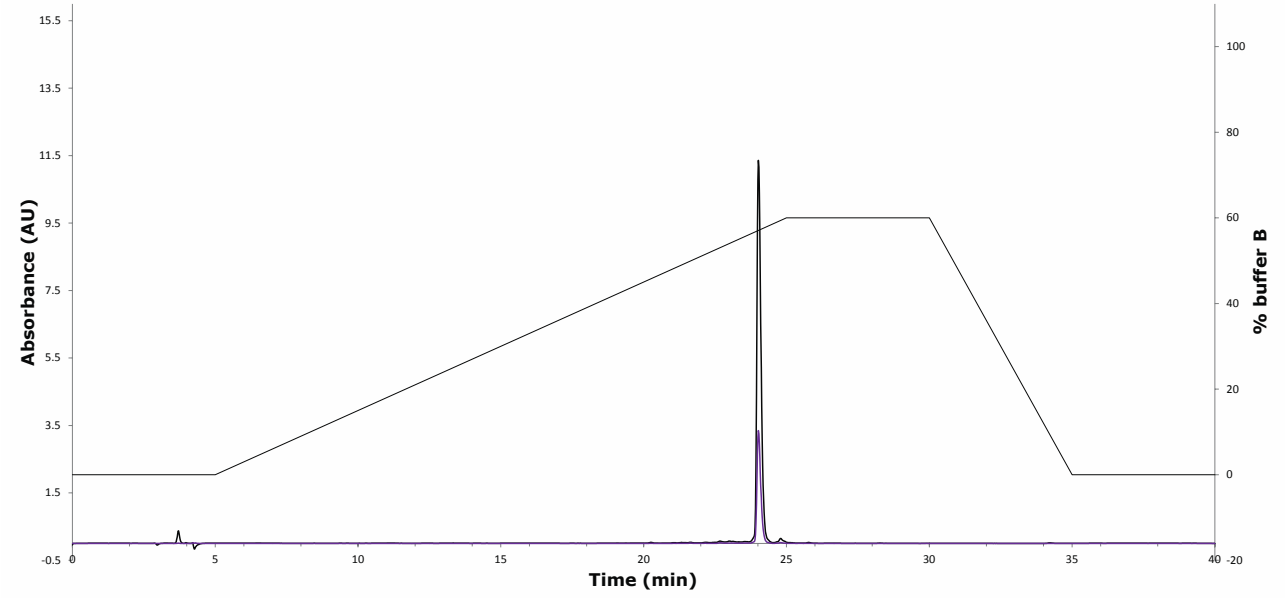
Compound **23**:



Compound **24**:

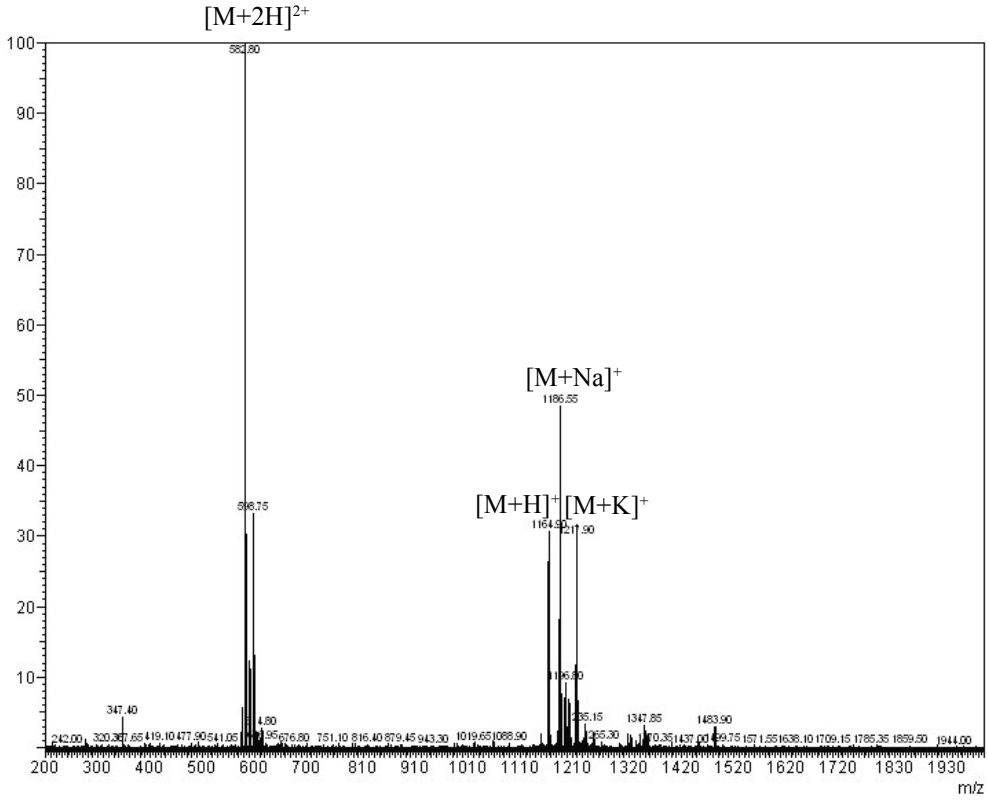


Compound **25**:

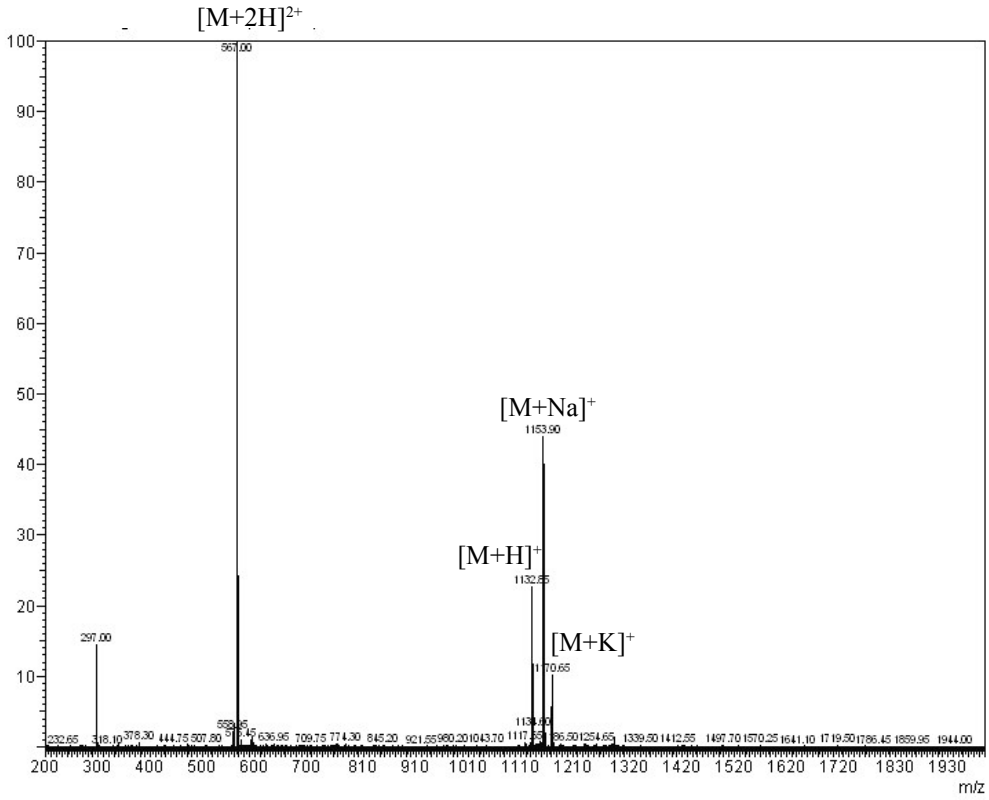


4. ESI-MS Spectra of Compounds 1-6, 8-12, 16-21, and 23-25.

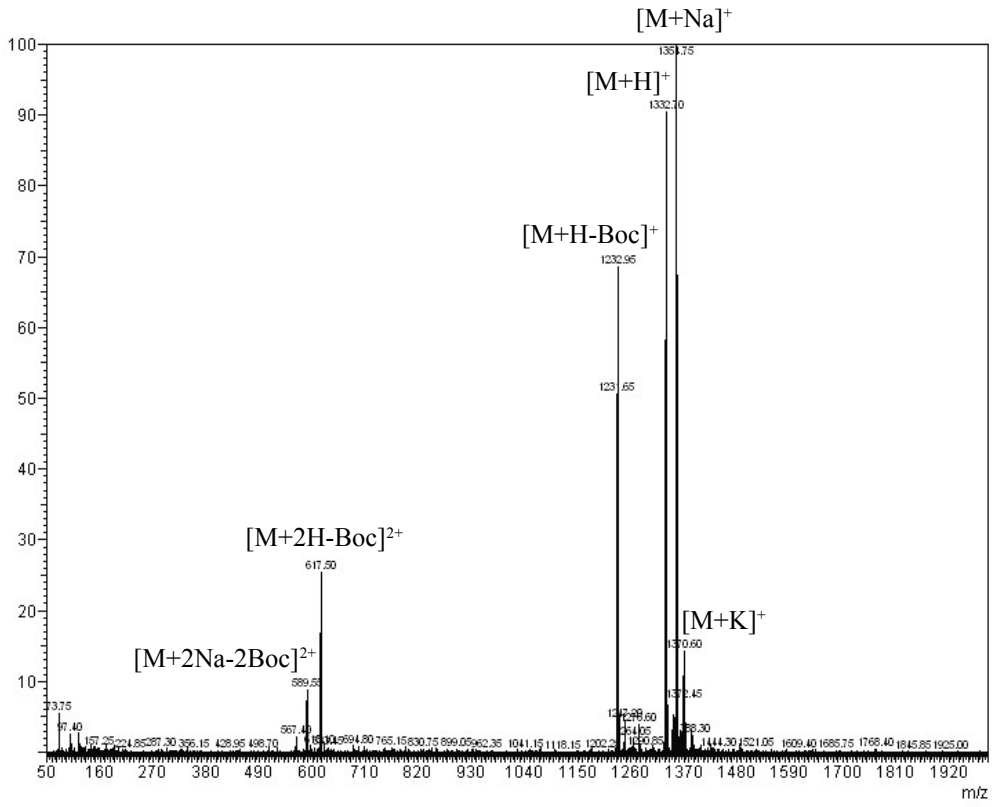
Compound **1**:



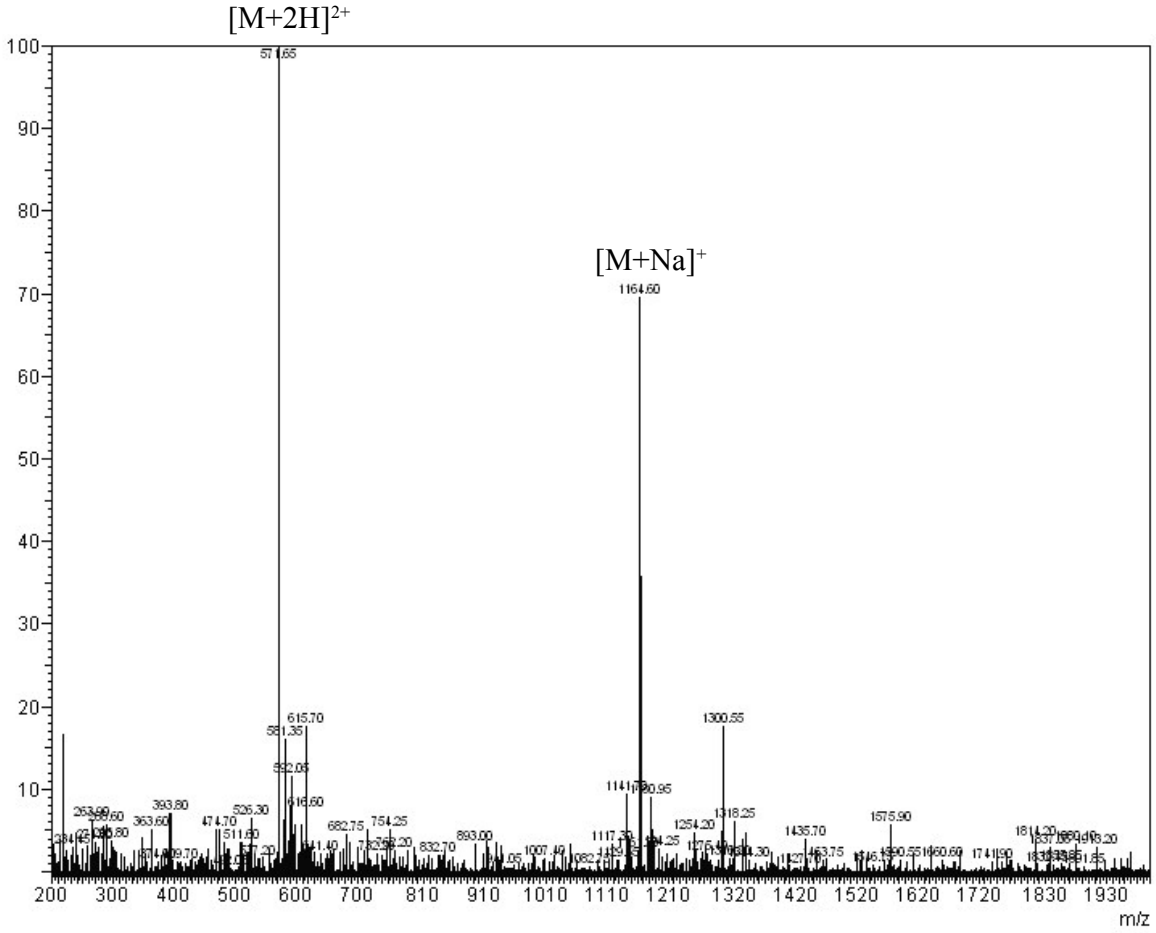
Compound **2**:



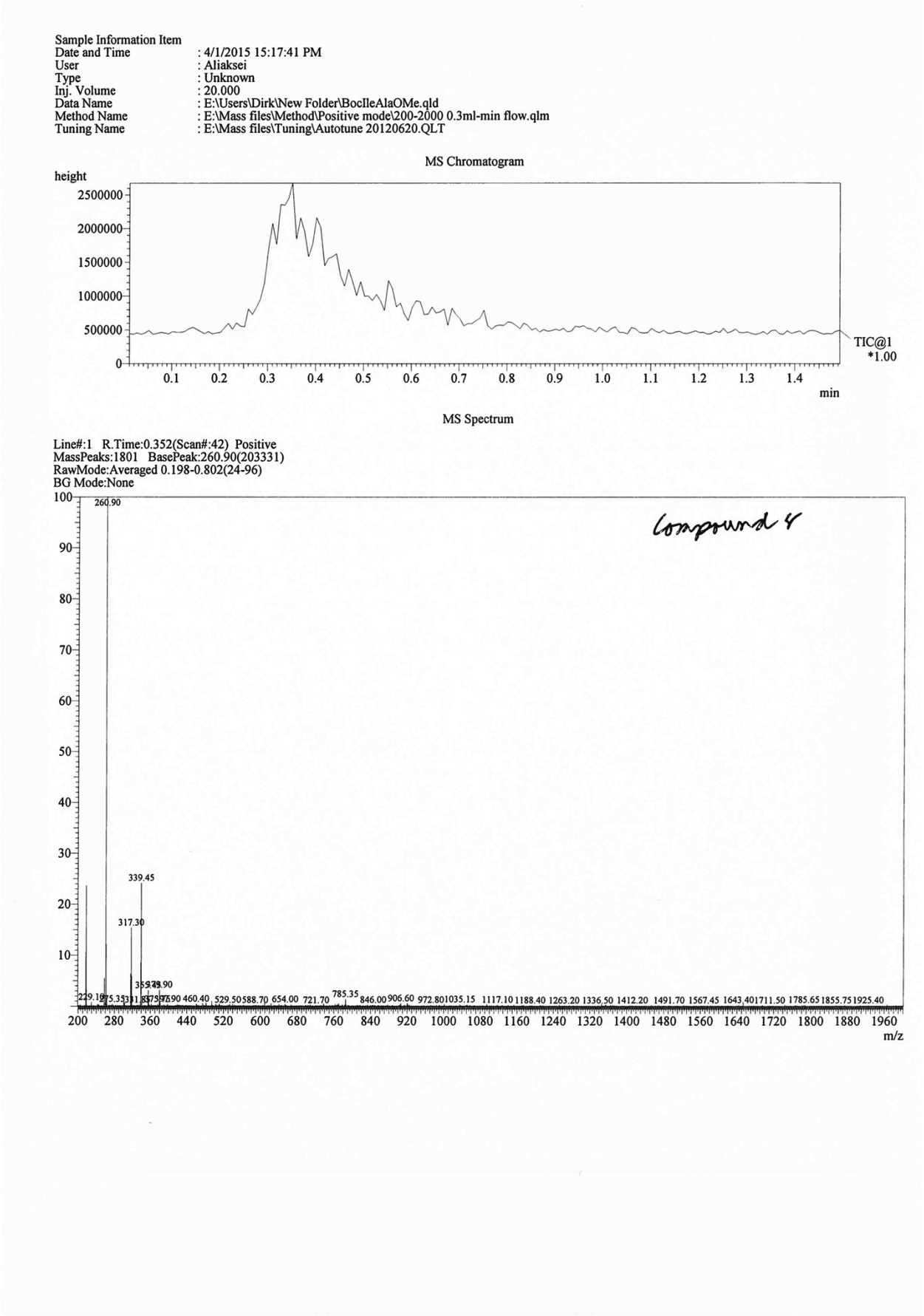
Boc-protected precursor of Compound **2**:



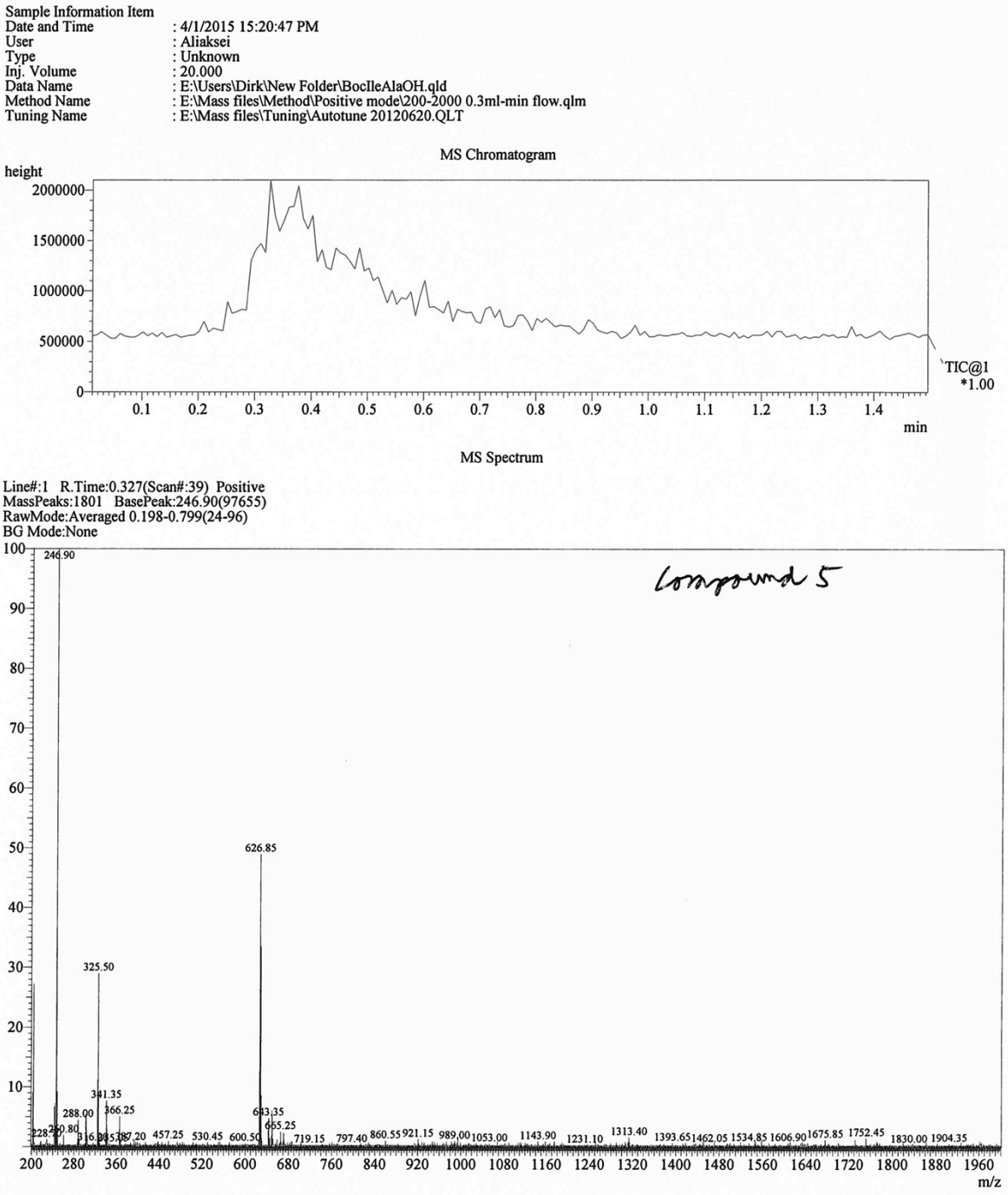
Compound **3**:



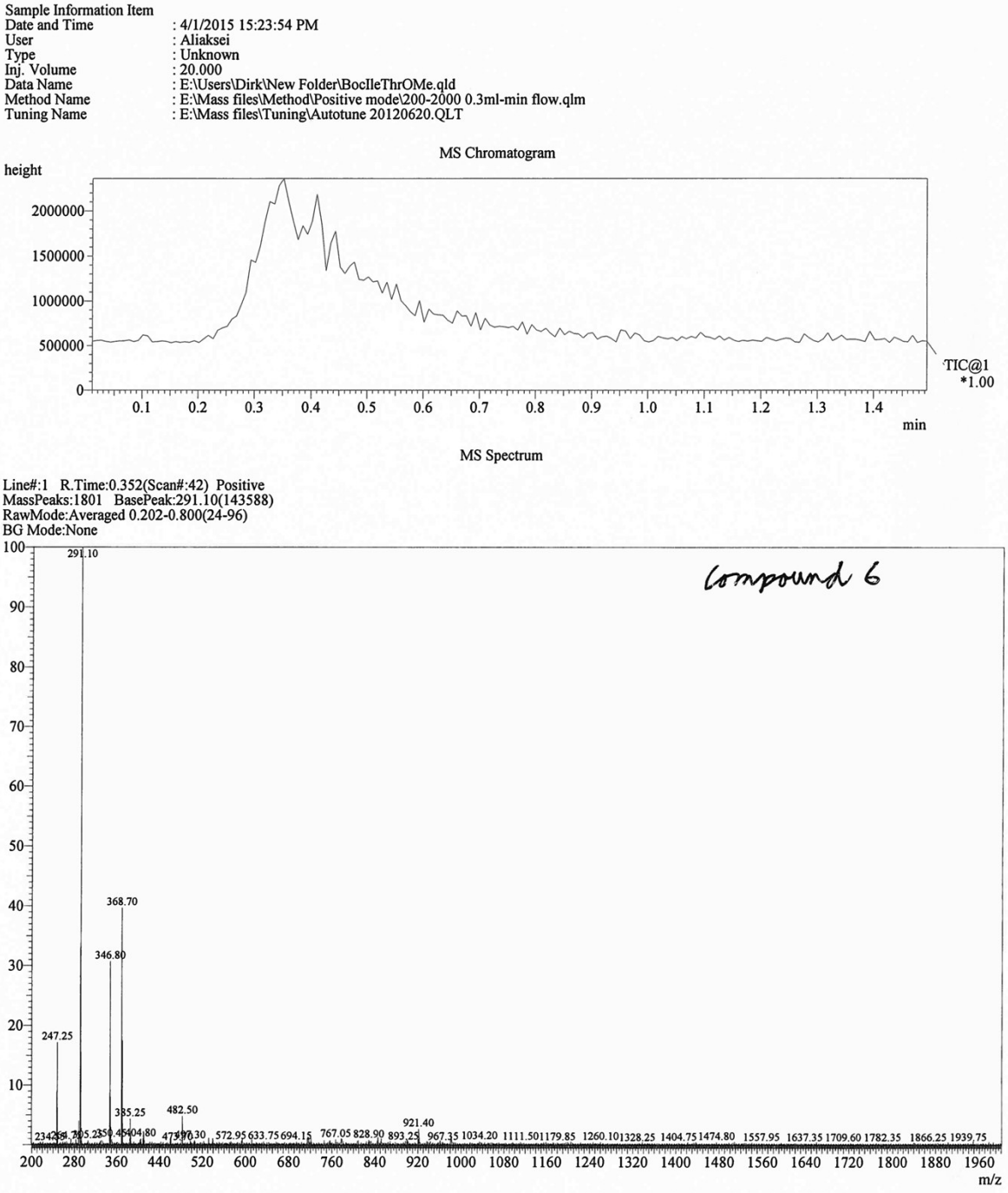
Compound **4**: ESI-MS calcd for $C_{15}H_{28}N_2O_5$ 316.20, found *m/z* $[M+H]^+$ 317.30, $[M+Na]^+$ 339.45, $[(M-{}^t\text{Bu})+H]^+$ 260.90, $[(M\text{-Boc})+H]^+$ 217.00.



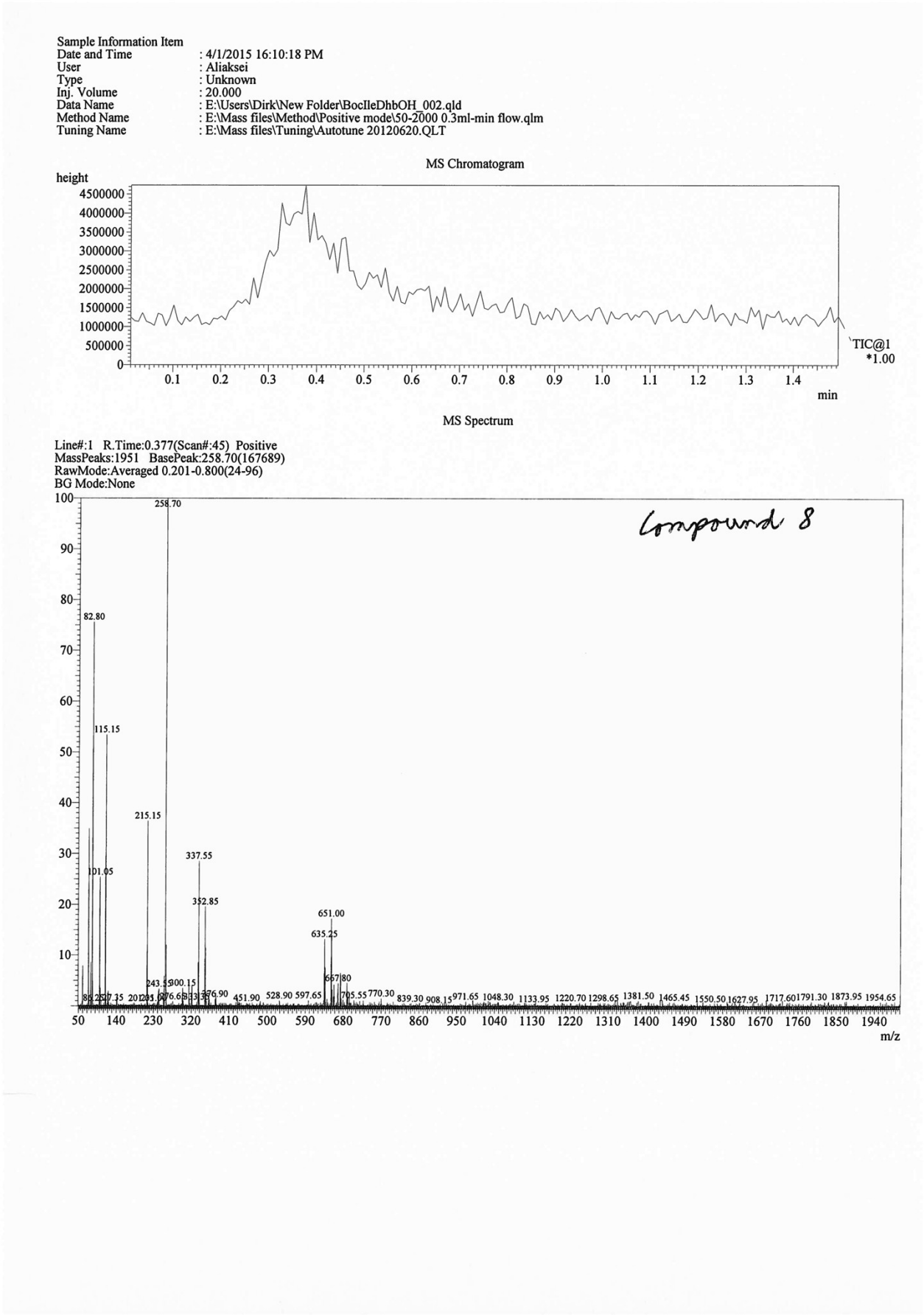
Compound **5**: ESI-MS calcd for $C_{14}H_{26}N_2O_5$ 302.18, found *m/z* $[M+Na]^+$ 325.50, $[2M+Na]^+$ 626.85, $[(M-{}^t\text{Bu})+H]^+$ 260.90, $[(M\text{-Boc})+H]^+$ 203.00.



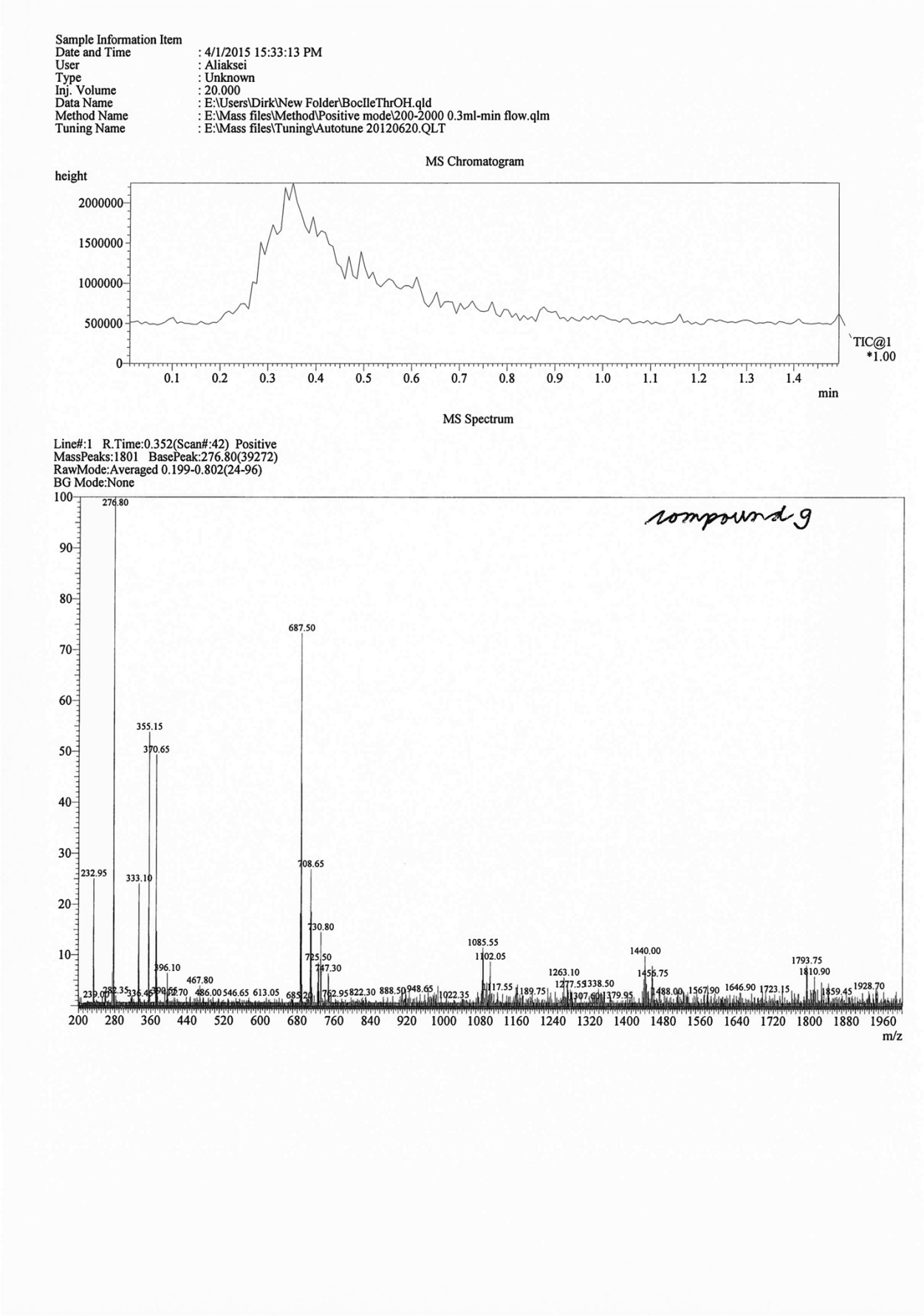
Compound **6**: ESI-MS calcd for $C_{16}H_{30}N_2O_6$ 346.21, found *m/z* $[M+H]^+$ 346.80, $[M+Na]^+$ 368.70, $[(M-{}^t\text{Bu})+H]^+$ 291.10, $[(M\text{-Boc})+H]^+$ 247.25.



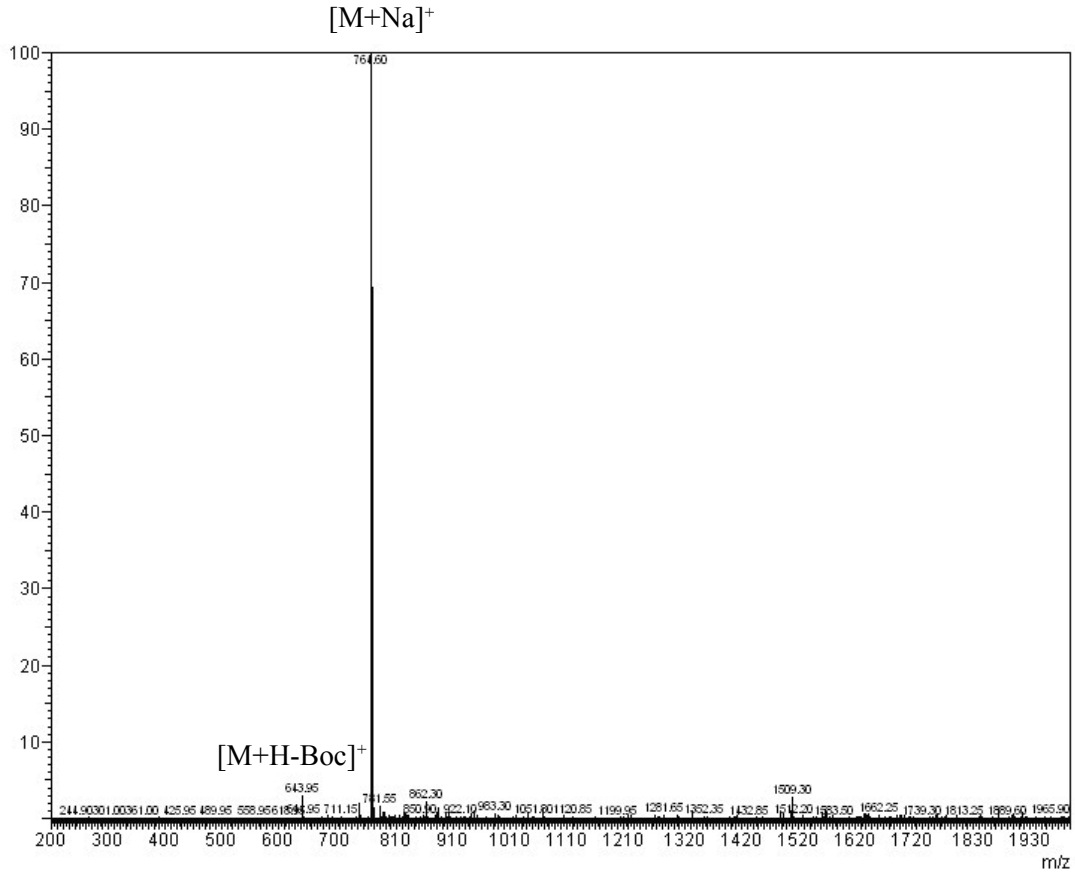
Compound **8**: ESI-MS calcd for $C_{15}H_{26}N_2O_5$ 314.18, found *m/z* $[M+Na]^+$ 337.55, $[M+K]^+$ 352.85, $[2M+Na]^+$ 651.00, $[(M-^tBu)+H]^+$ 258.70, $[(M-Boc)+H]^+$ 215.15.



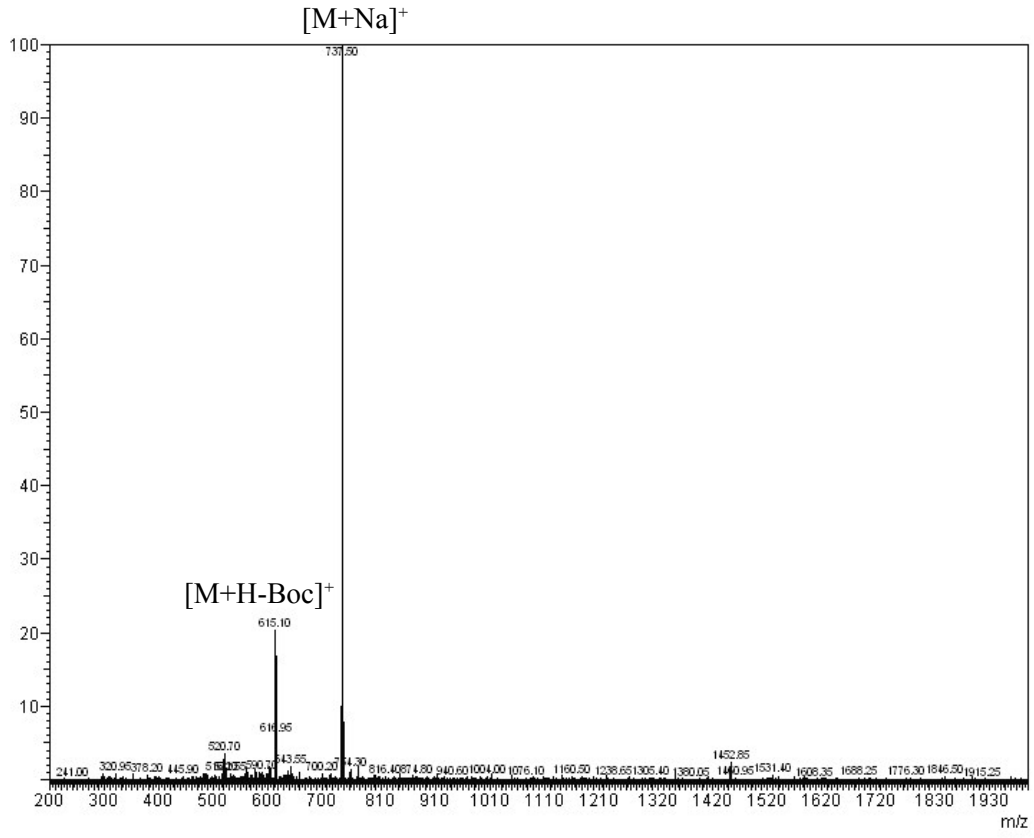
Compound **9**: ESI-MS calcd for $C_{15}H_{28}N_2O_6$ 332.19, found *m/z* $[M+H]^+$ 333.10, $[M+Na]^+$ 355.15, $[2M+Na]^+$ 687.50, $[(M-{}^t\text{Bu})+H]^+$ 276.80, $[(M\text{-Boc})+H]^+$ 232.95.



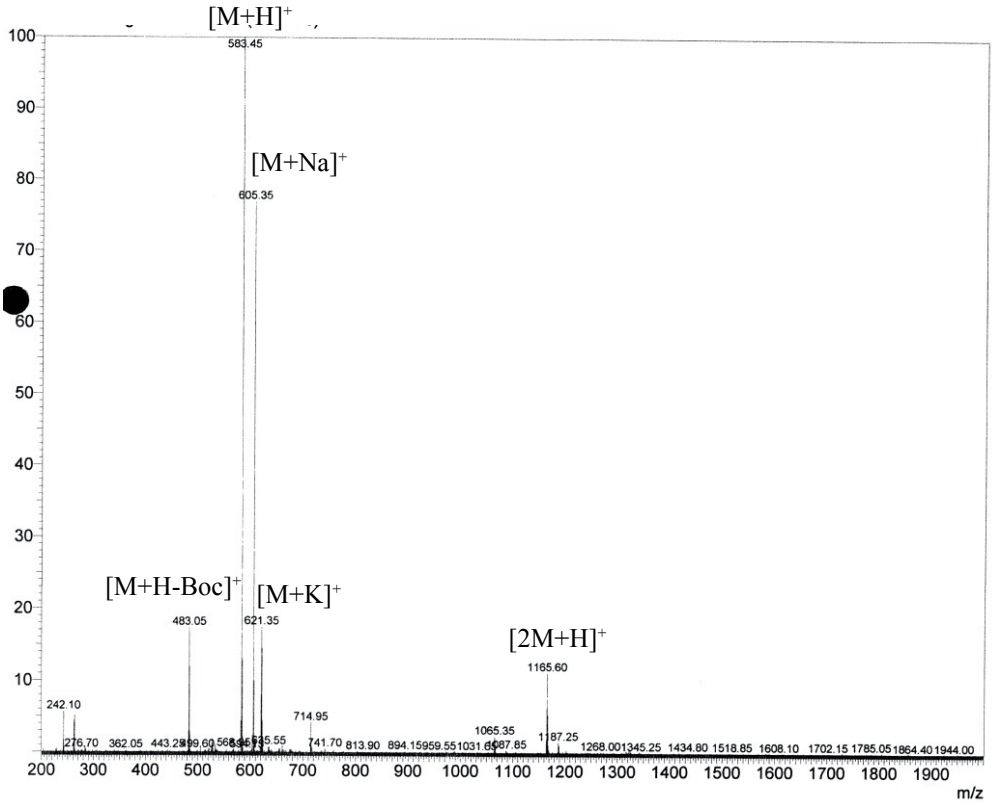
Compound **10**:



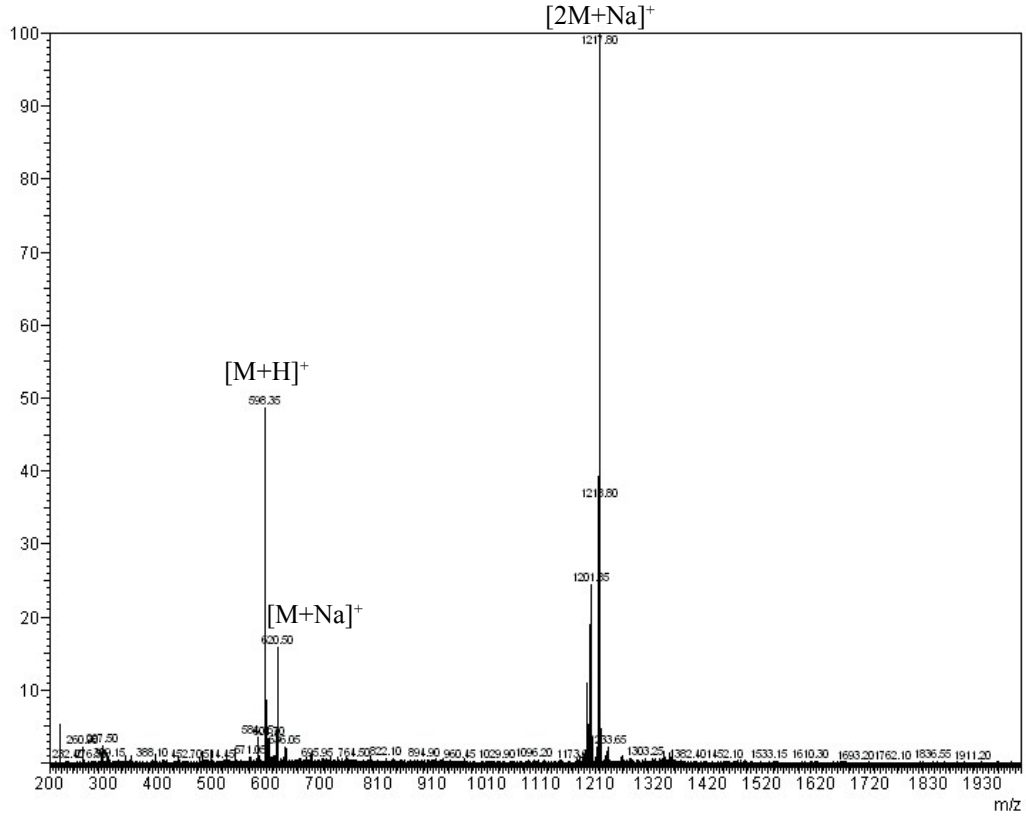
Compound **11**:



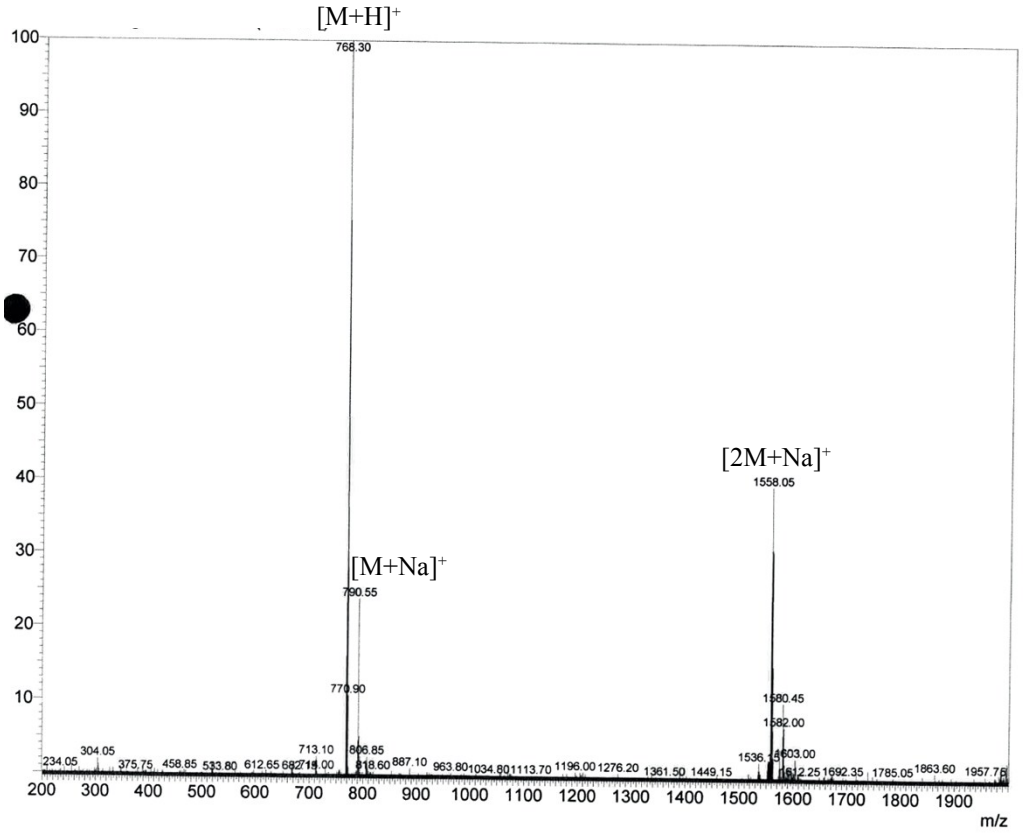
Compound **12**:



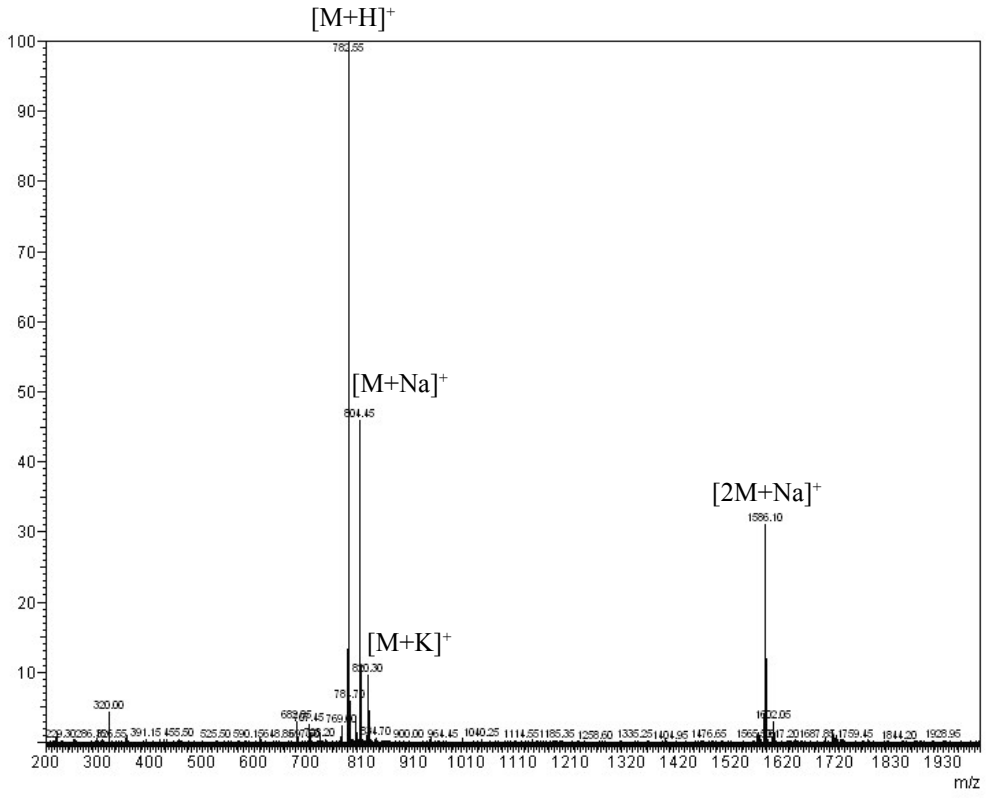
Compound **15**:



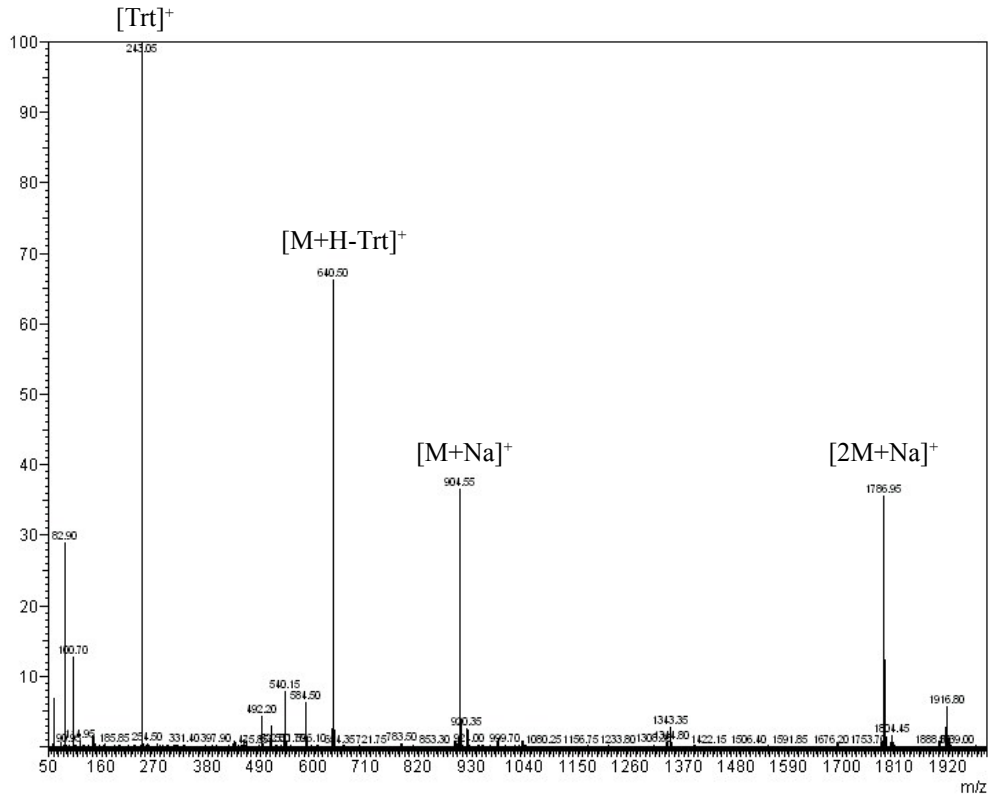
Compound **16**:



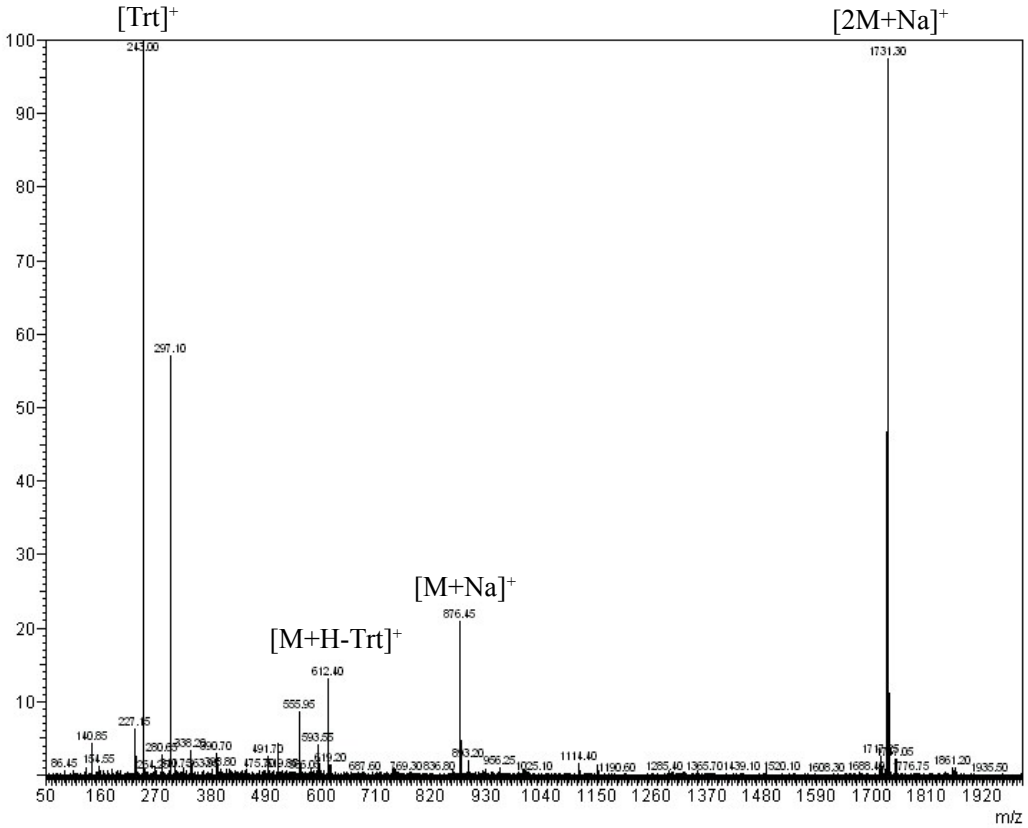
Precursor of Compound **16**:



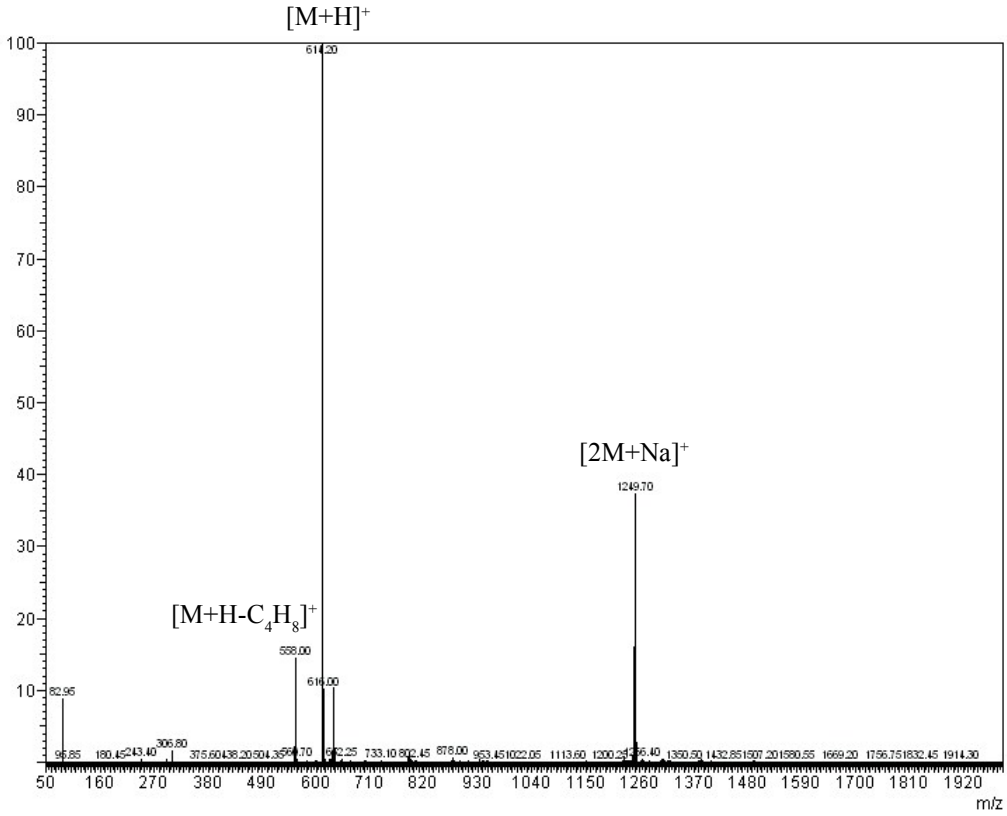
Compound **17**:



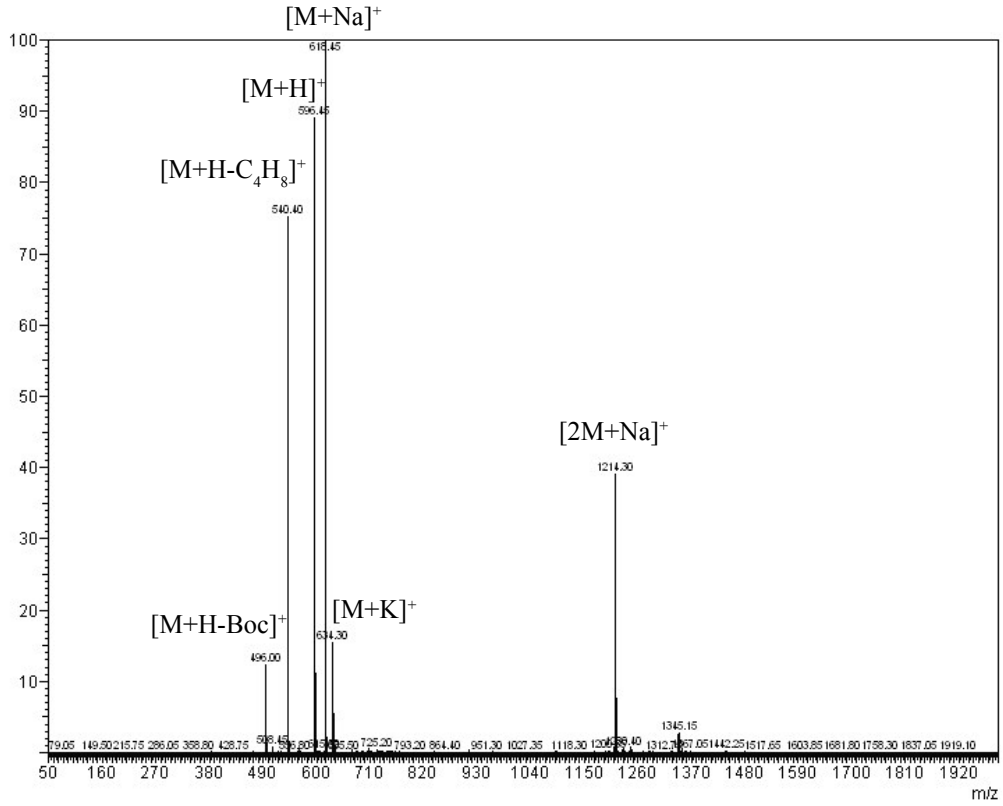
Compound **18**:



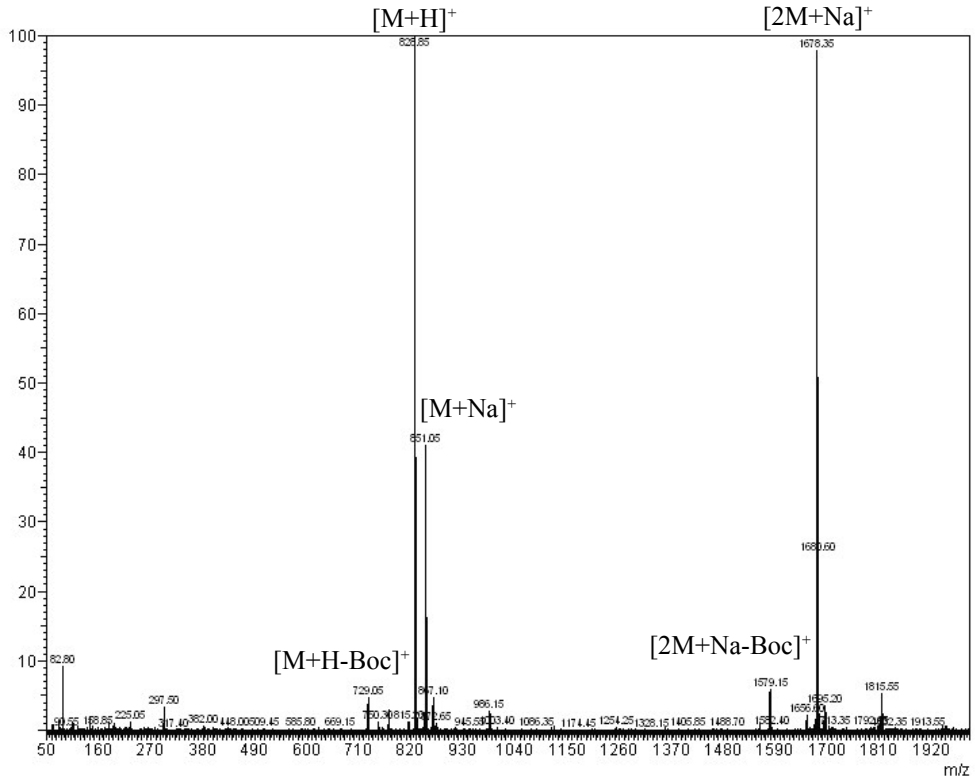
Compound **19**:



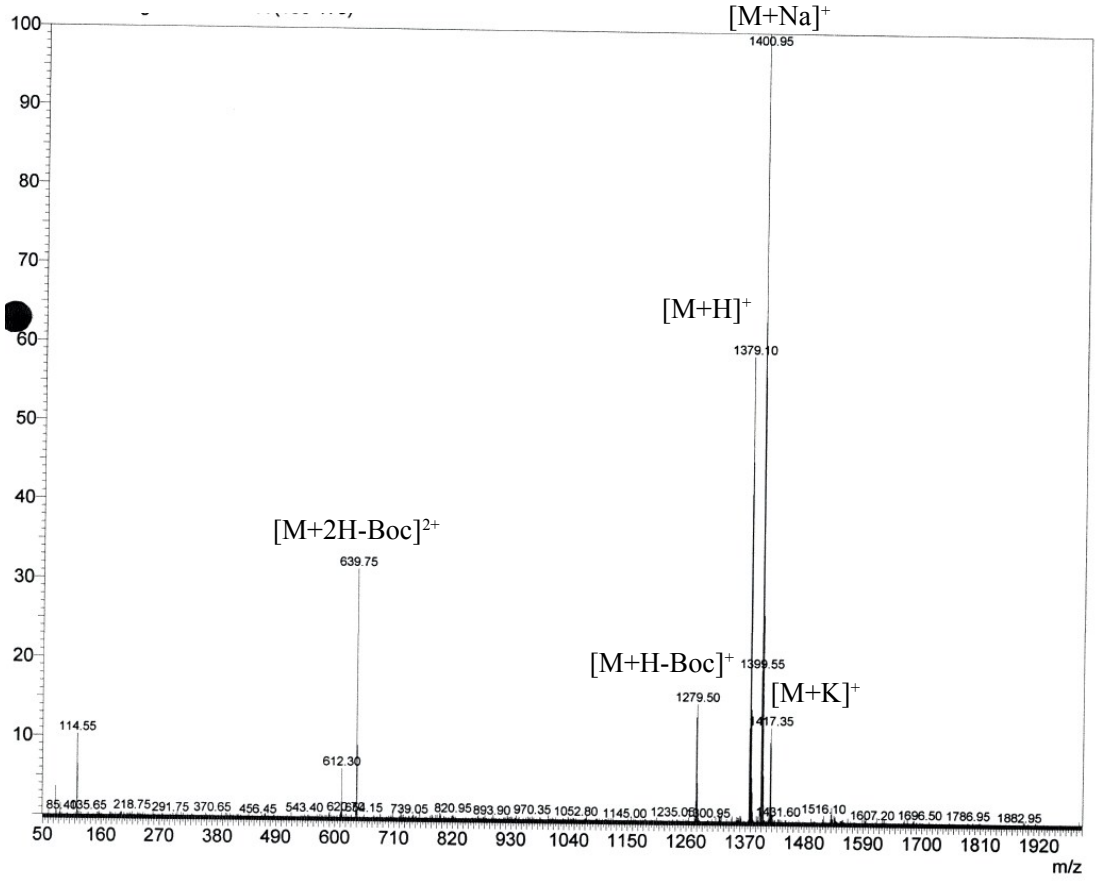
Compound **20**:



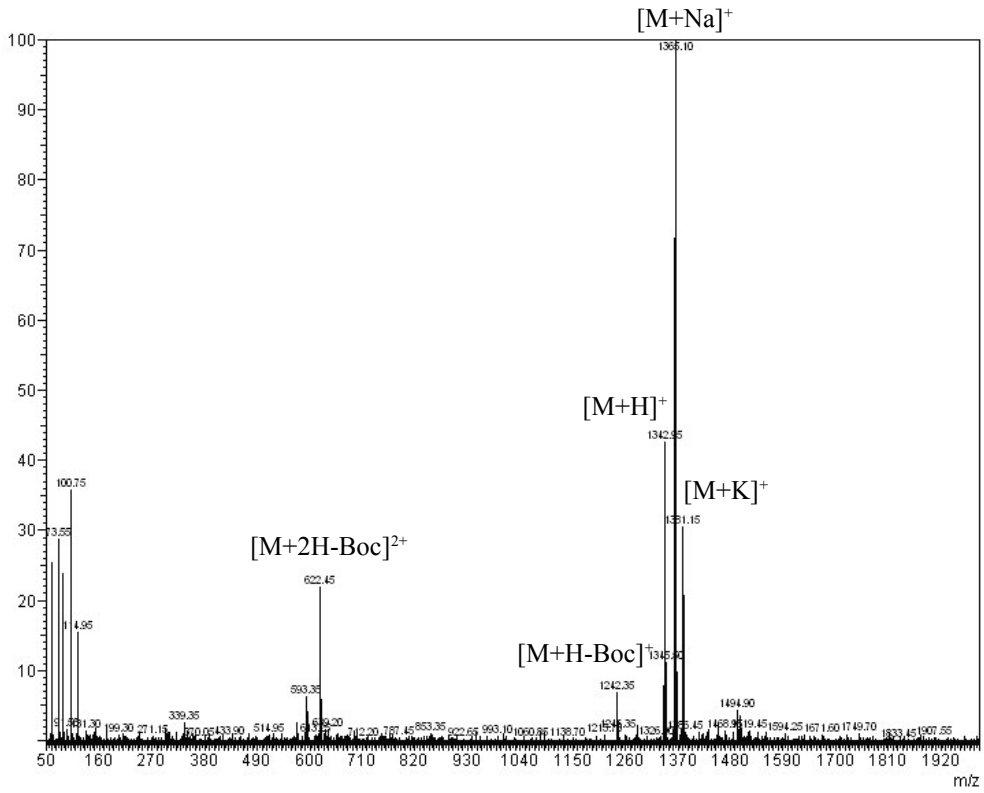
Compound **21**:



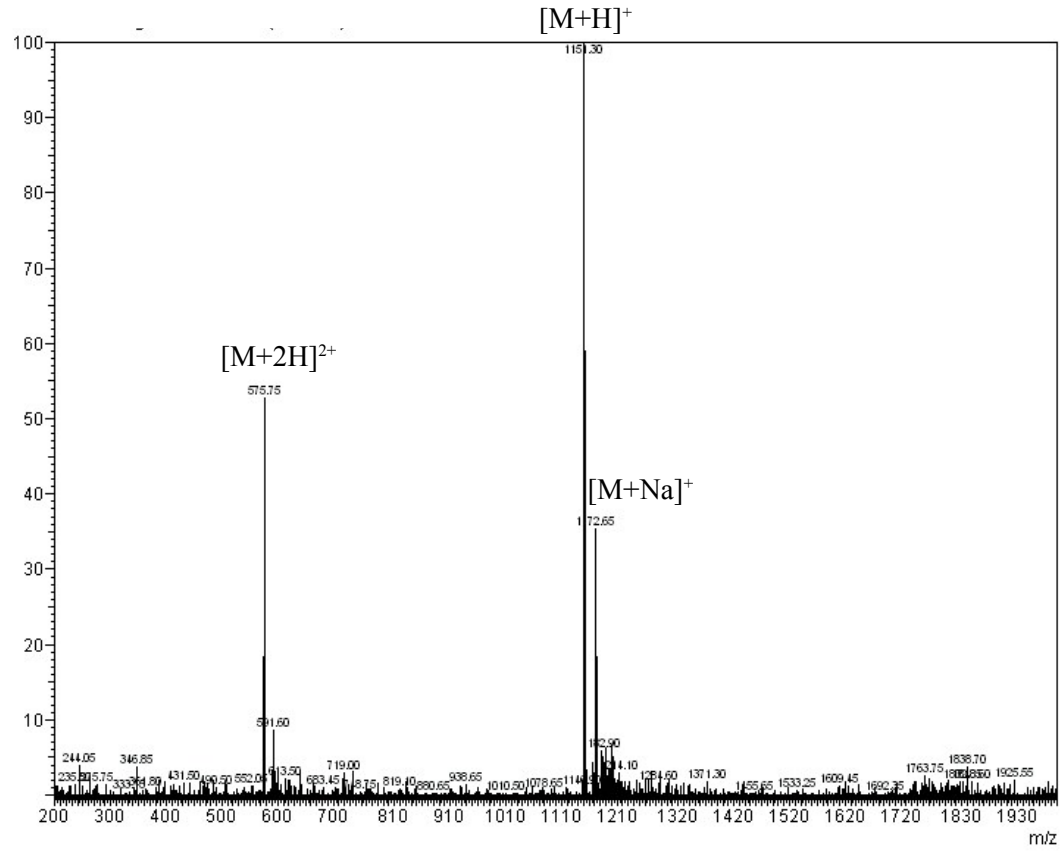
Compound **23**:



Compound **24**:

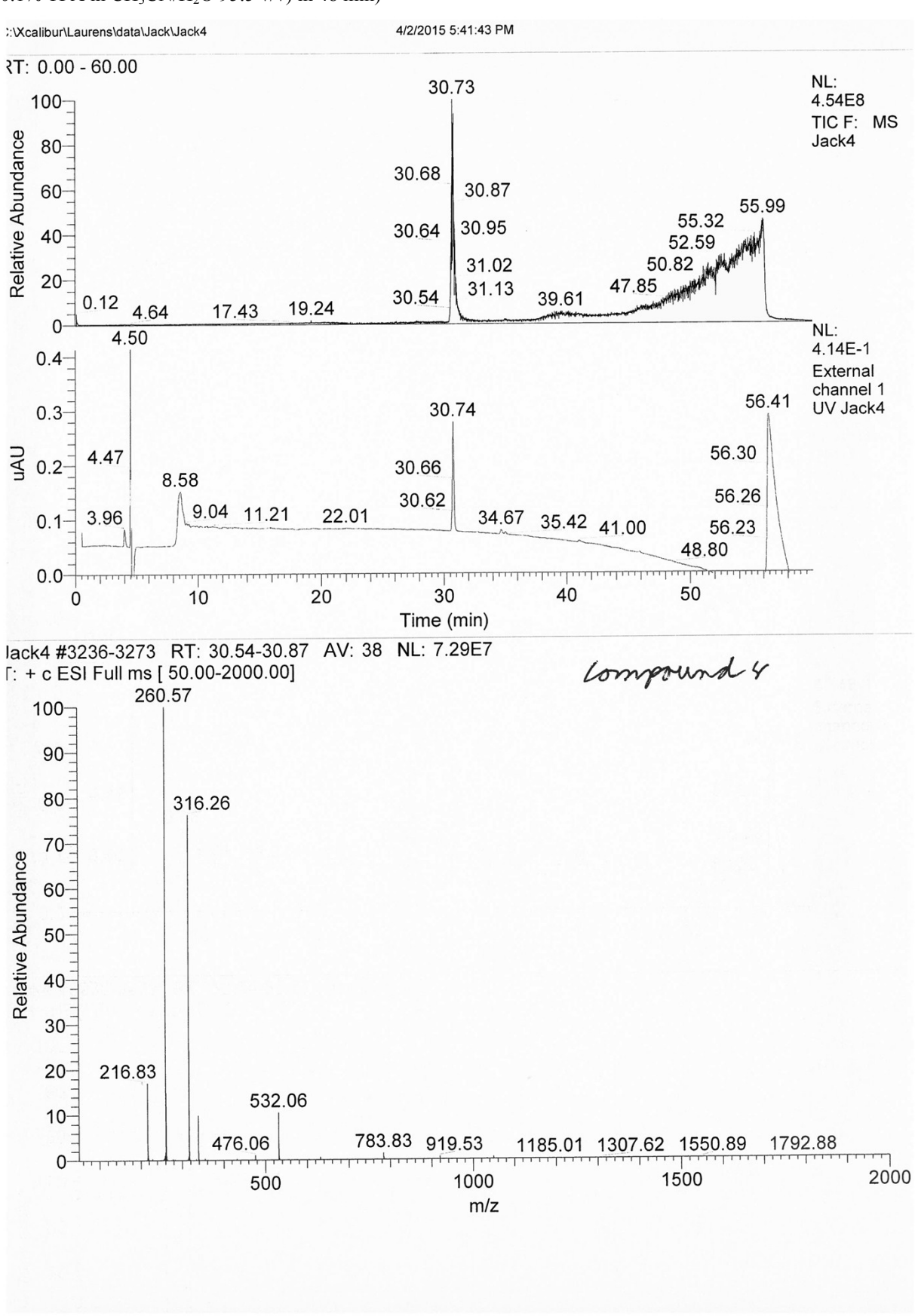


Compound **25**:

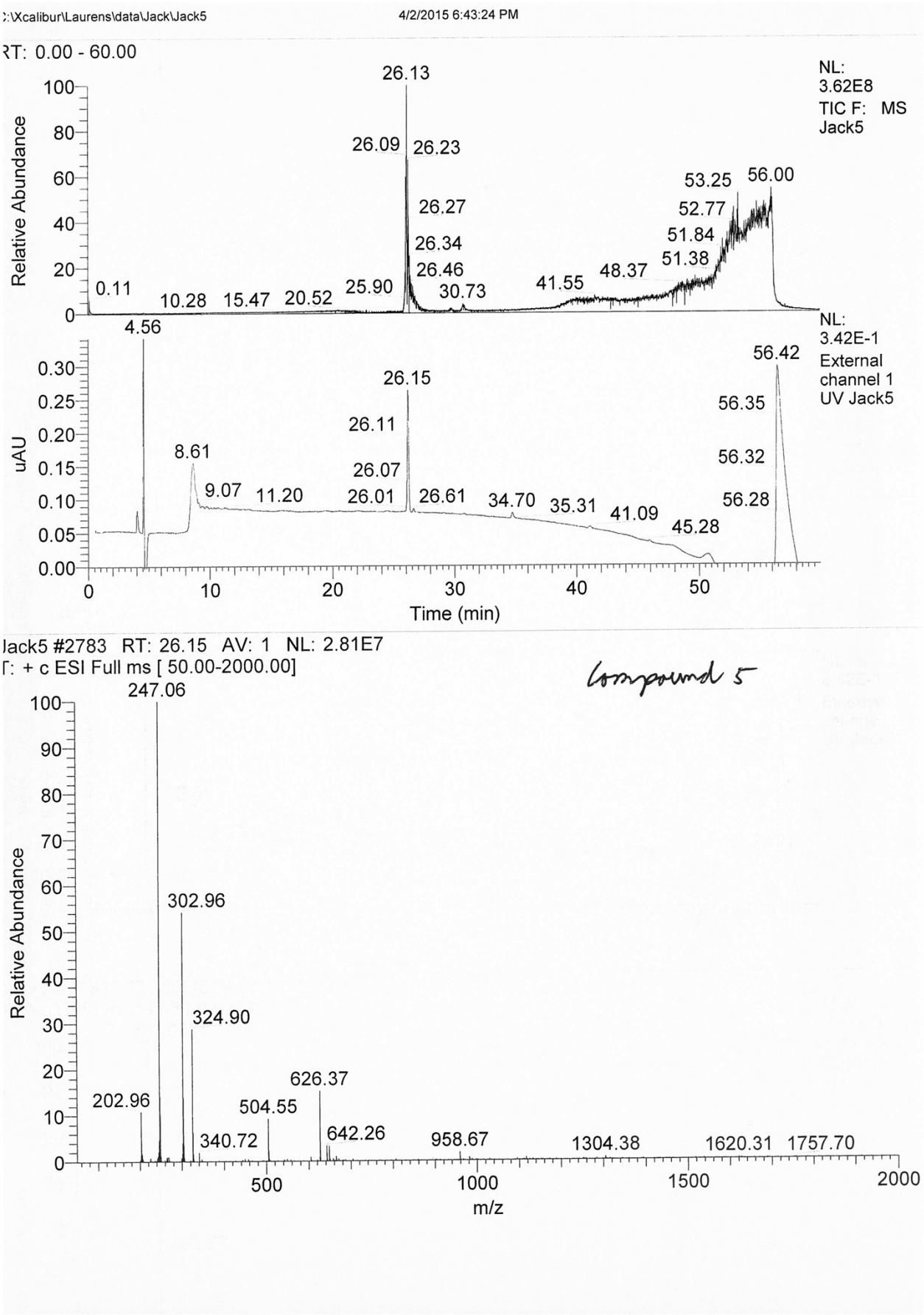


5. LCMS Spectra of Compounds 4-6, 8, and 9.

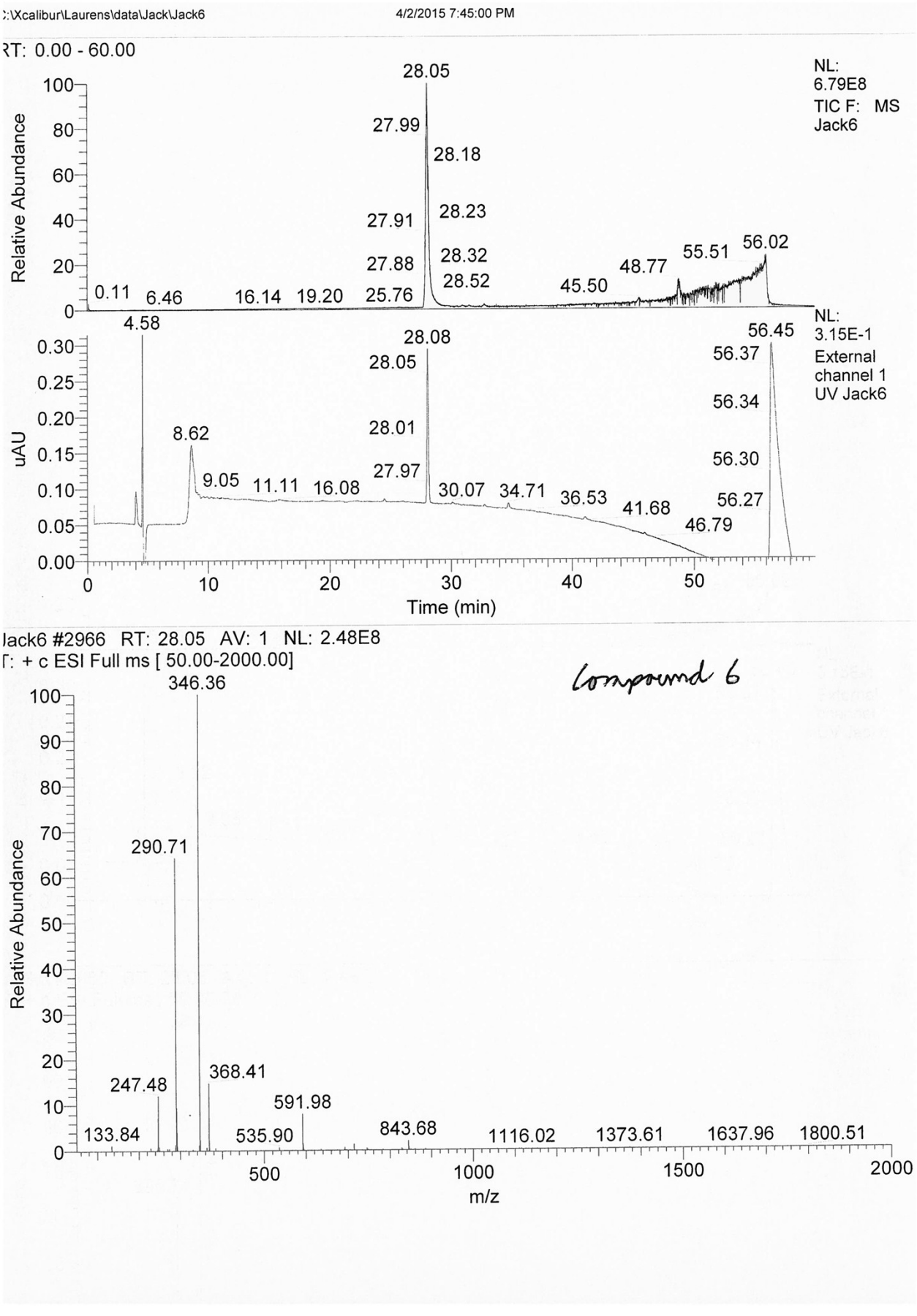
Compound **4**: R_t = 30.74 min (on a Maisch ReproSil 120 C18-AQ column (pore size: 120 Å, particle size: 5 µm, 250 × 4.6 mm) at a flow rate of 1 mL/min (from 100% buffer A (0.1% TFA in CH_3CN/H_2O 5:95 v/v) to 100% buffer B (0.1% TFA in CH_3CN/H_2O 95:5 v/v) in 48 min)



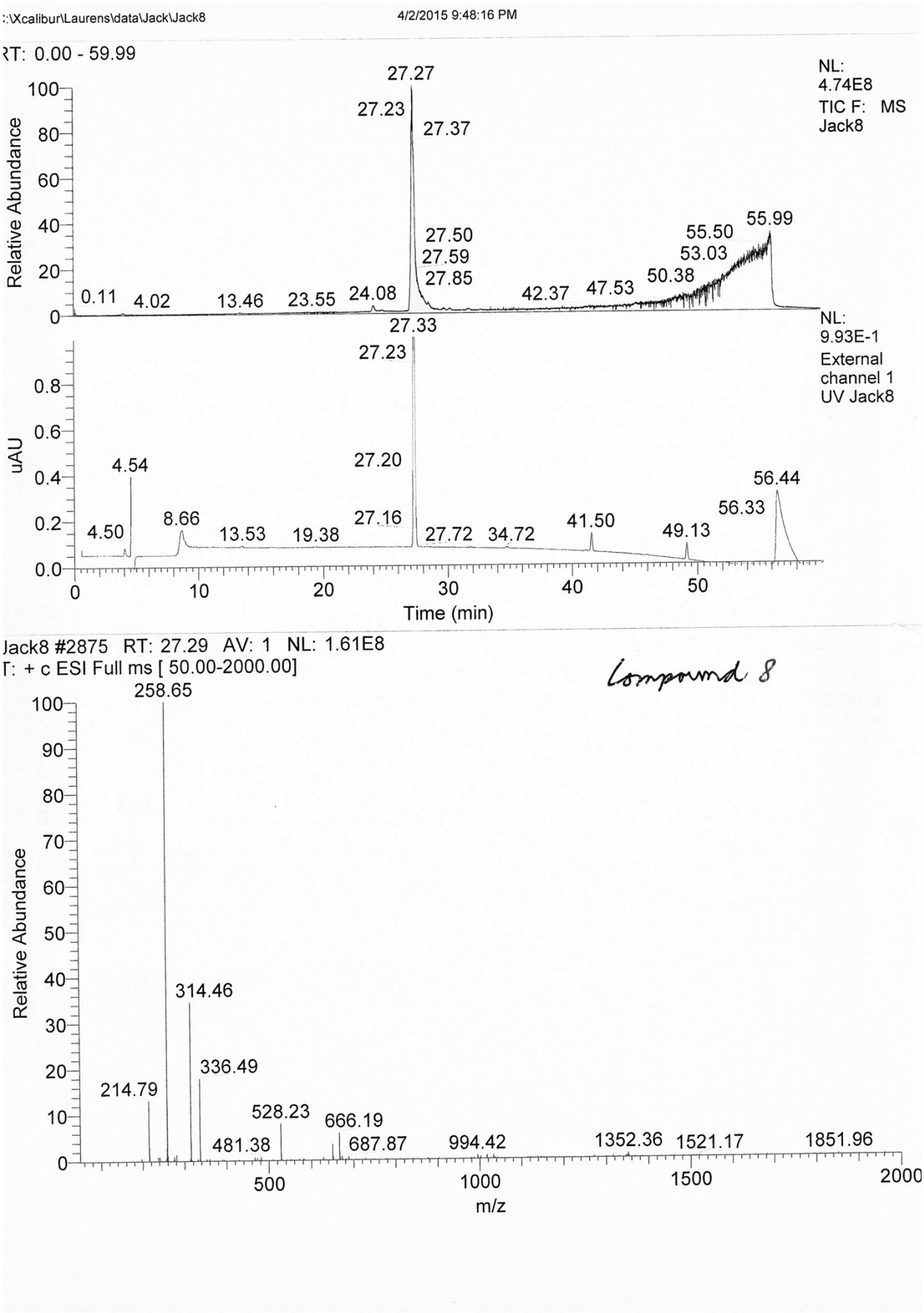
Compound **5**: R_t = 26.15 min



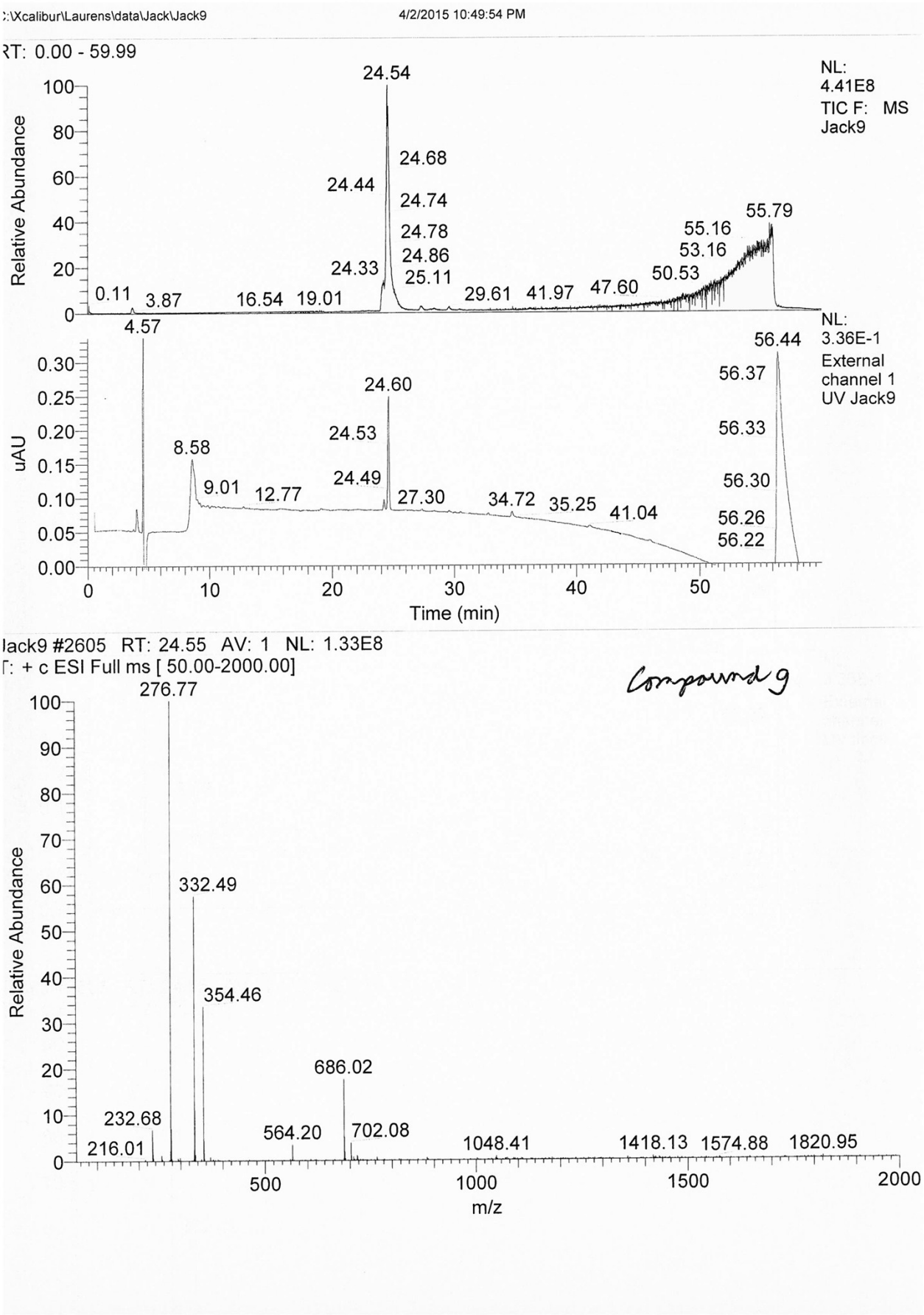
Compound **6**: R_t = 28.08 min



Compound **8**: R_t = 27.23 min

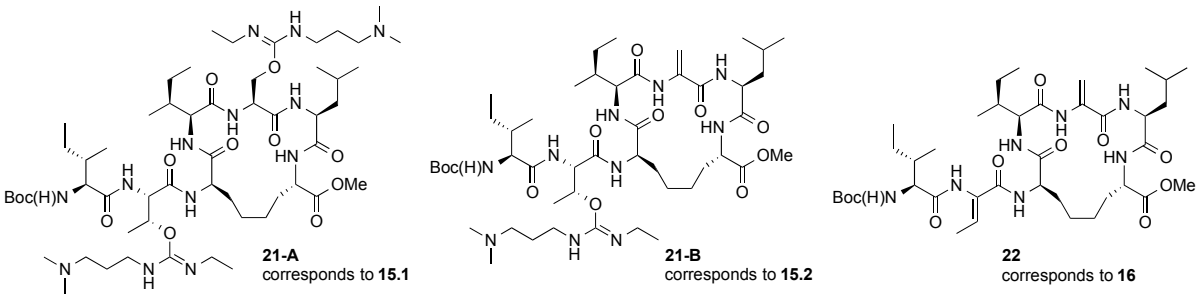


Compound **9**: R_t = 24.60 min

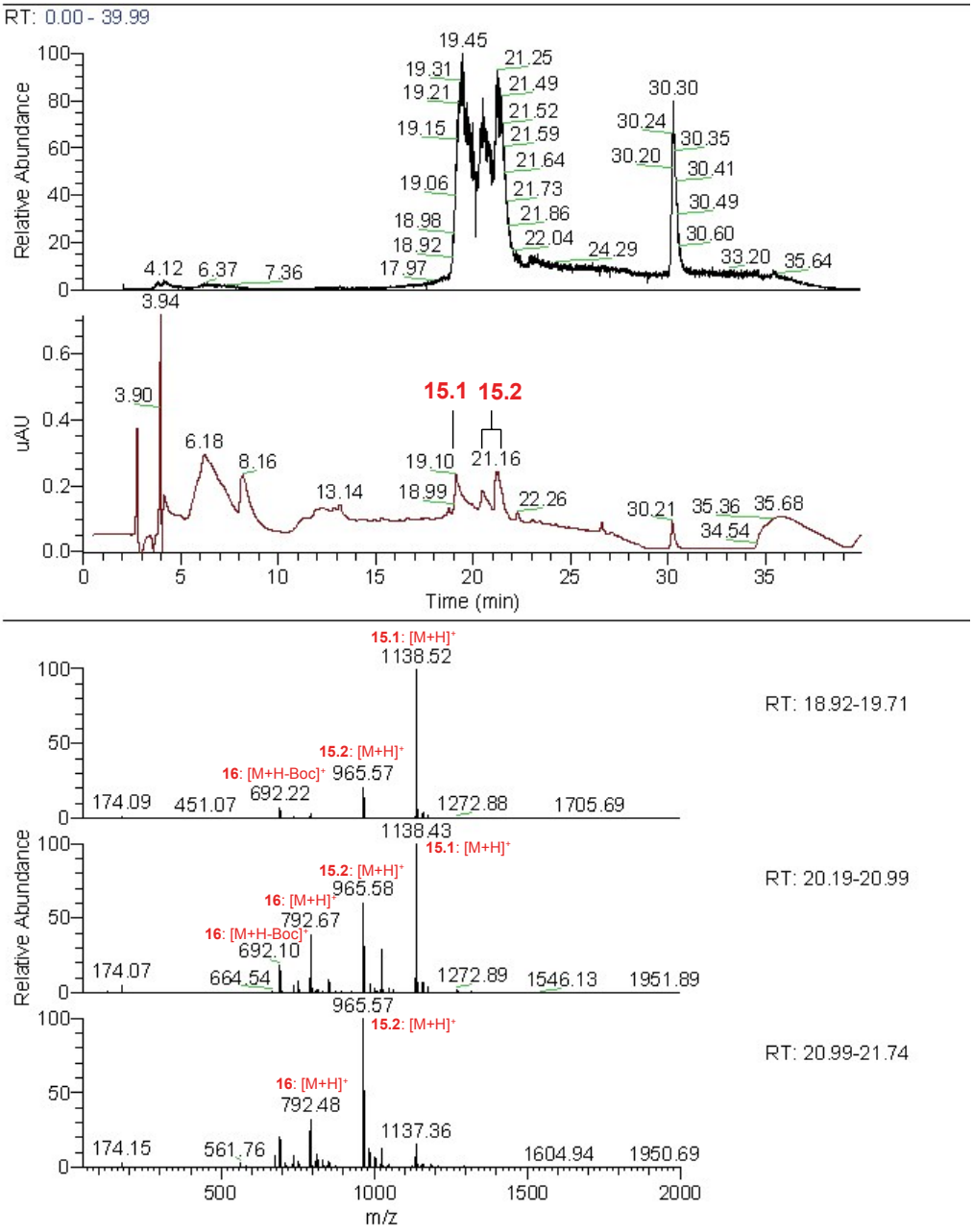


6. LCMS Spectra of Compounds 21A-B to monitor the dehydration reactions.

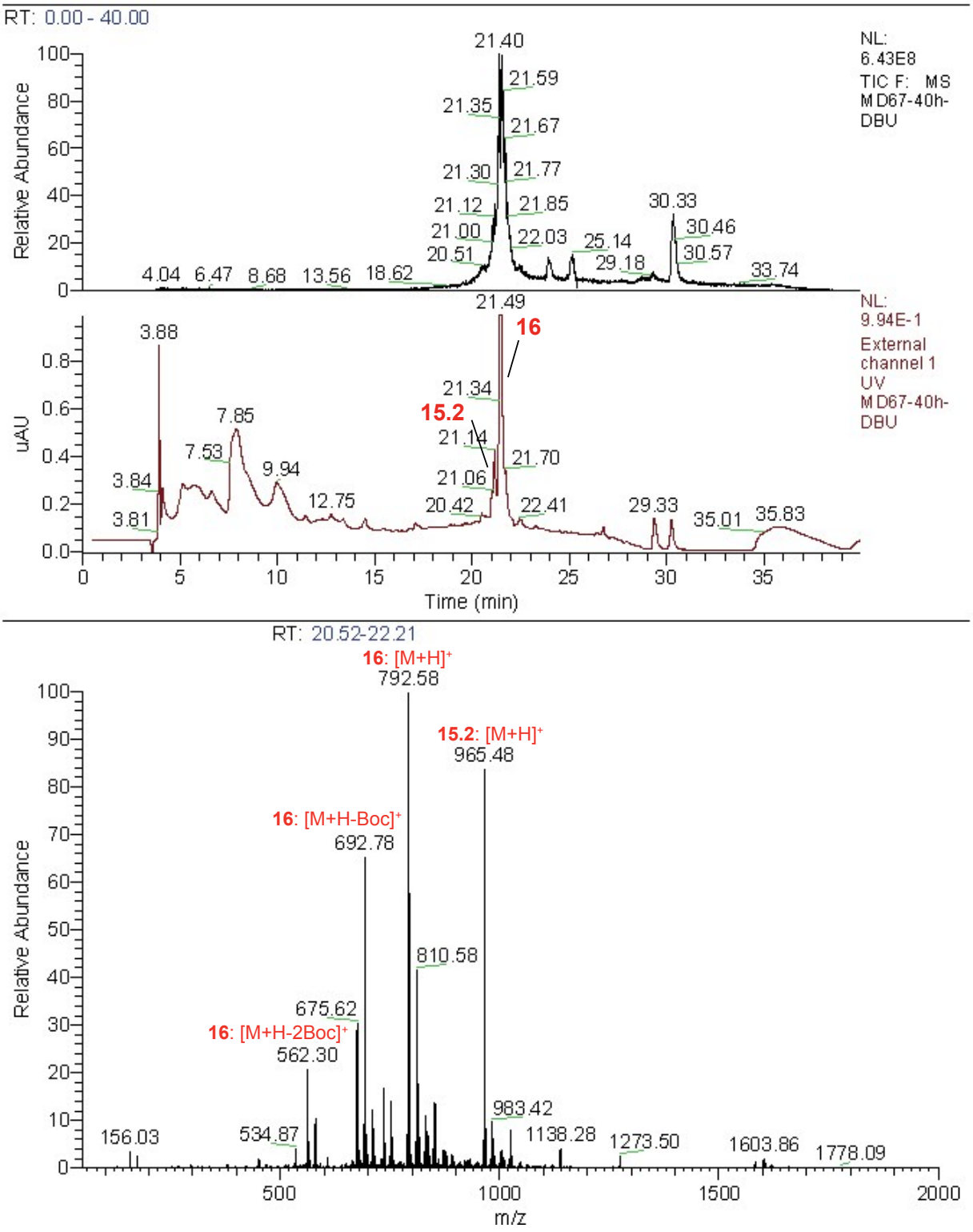
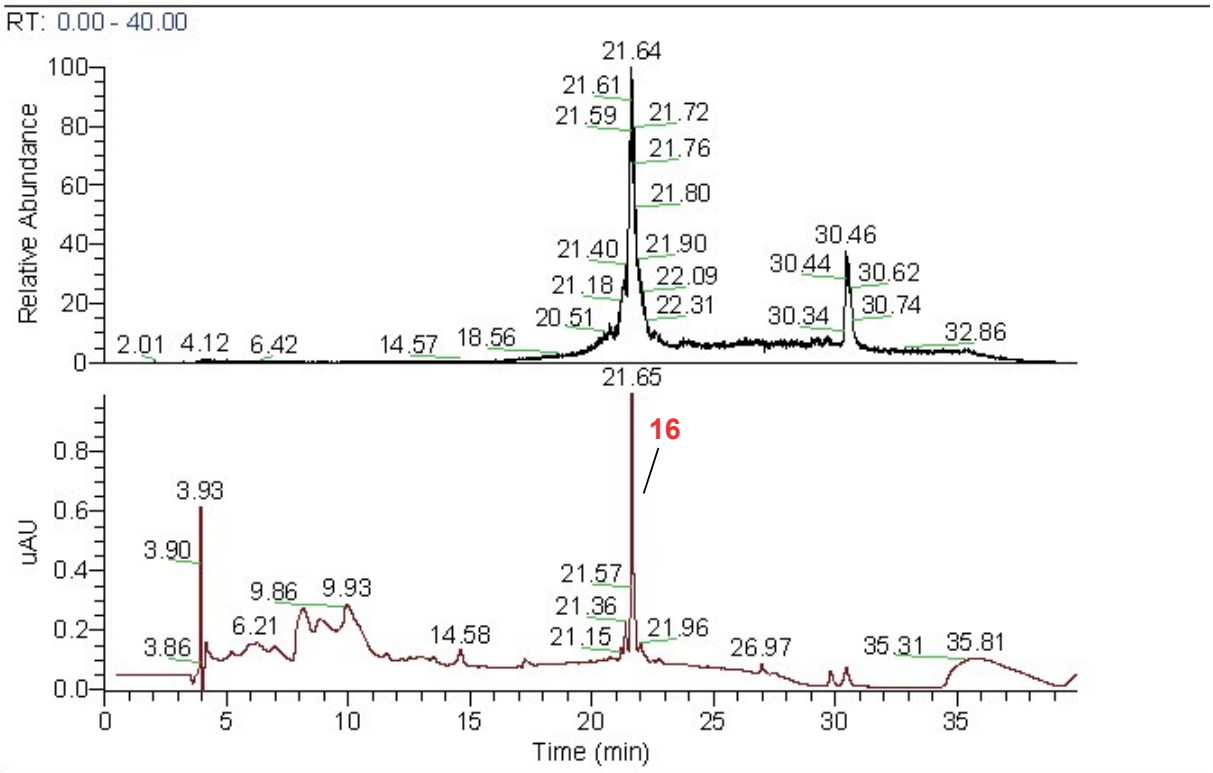
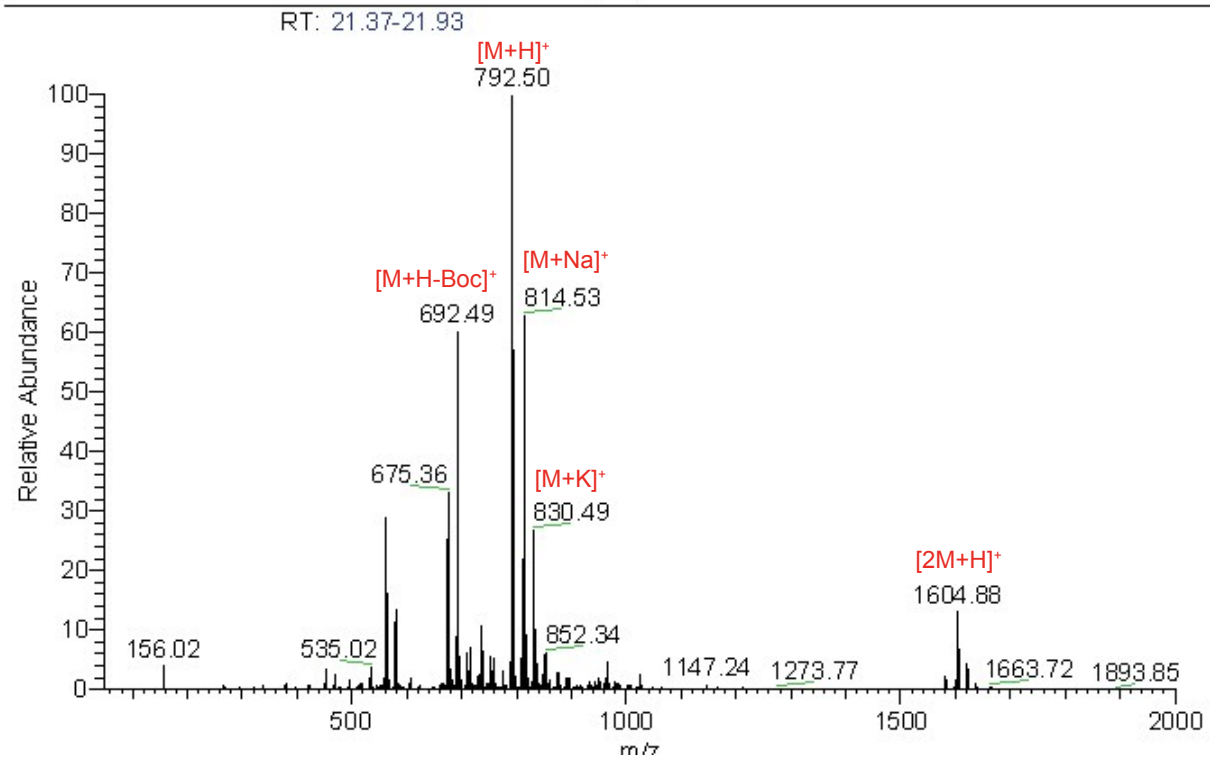
Reaction scheme:



LCMS after 16 h of reaction:



LCMS after 40 h of reaction:



LCMS after 64 h of reaction: