SUPPORTING INFORMATION

Traceless Chemical Ligations at Ser- sites through O- to N- Acyl Shifts

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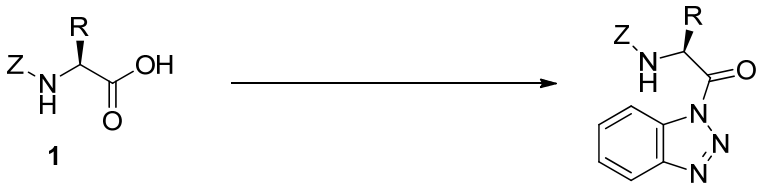
Experimental procedures and characterization of compounds

General

Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. The NMR spectra were recorded with TMS for ^{1}H (300 MHz) and ^{13}C (75 MHz) as an internal reference. Starting materials are available commercially. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (C18): (2 x 150 mm; 4 um) + C18 guard column (2 x 4 mm) using 0.2% acetic acid in H_2O/acetonitrile as mobile phases or 0.4 mM ammonium formate in H_2O/methanol; wavelength = 254 nm; flow rate 0.2 mL/min; and mass spectrometry was done with electro spray ionization (ESI).

General procedure for the preparation of *N*-(Z-α-aminoacyl)benzotriazoles



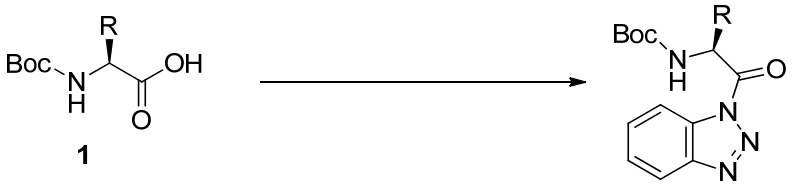
***N*-(Z-α-Aminoacyl)benzotriazoles.** Thionyl chloride (0.6 mL, 8.00 mmol, 1.2 equiv) was added to a solution of 1H-benzotriazole (3.17 g, 26.67 mmol, 4 equiv) in methylene chloride to give a clear yellow solution that was stirred for 15 min at room temperature. The amino acid **1** (6.67 mmol, 1 equiv) was then added to give a suspension which was stirred for 2.5 h at room temperature. The suspension was filtered, the filtrate evaporated, the residue dissolved in EtOAc and the solution was washed with a saturated solution of sodium carbonate. The organic portion was dried over anhyd $MgSO_4$, filtered, and dried to give the corresponding N-(Z-a-aminoacyl)benzotriazole.

(*S*)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (Z-L-Phe-Bt). White solid (90%); mp 150-152 ^{o}C (lit.[1] mp 149.0-150.0 ^{o}C); ^{1}H NMR ($CDCl_3$): δ 8.23

(d, J = 7.8 Hz, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.54 (t, J = 7.5 Hz, 1H), 7.32-7.23 (m, 7H), 7.14 (br s, 3H), 6.09 (d, J = 4.2 Hz, 1H), 5.57 (d, J = 6.6 Hz, 1H), 5.08 (s, 2H), 3.48 (d, J = 9.6 Hz, 1H), 3.24 (d, J = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 170.8, 155.7, 146.0, 135.9, 134.9, 131.0, 130.8, 129.2, 128.7, 128.5, 128.1, 127.4, 126.5, 120.4, 114.3, 67.2, 55.6, 38.8.

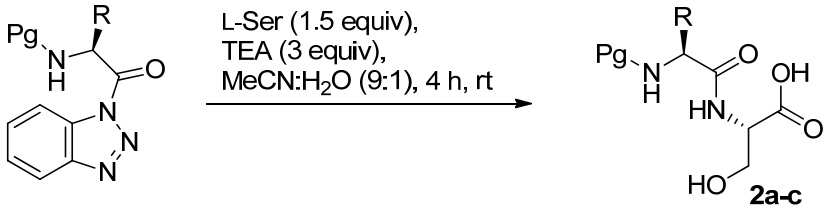
(*S*)-Benzyl (1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxopropan-2-yl)carbamate (Z-L-Ala-Bt). White solid (90%); mp 115 ^{o}C (lit.[2] mp 113-115 ^{o}C); ^{1}H NMR ($CDCl_3$): δ 8.16 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.40-7.03 (m, 6H), 5.80-5.60 (m, 2H), 5.10-4.99 (m, 1H), 1.59 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 172.2, 155.6, 145.9, 136.0, 131.0, 130.6, 128.4, 128.1, 126.4, 120.2, 114.3, 67.1, 50.5, 19.0.

General procedure for the preparation of *N*-(Boc-α-aminoacyl)benzotriazoles



***N*-(Boc-α-Aminoacyl)benzotriazoles.** Boc-protected amino acid **1** (0.03 mol) was added to a solution of DCC (1 equiv) in methylene chloride under an atmosphere of nitrogen. After 30 min., BtH (1 equiv) was added and this was stirred for 12 h.The suspension was filtered on a bed of silica and celite, the filtrate evaporated, and the residue dissolved in EtOAc, then filtered on a bed of silica and celite and washed with a solution of saturated sodium carbonate, then with water and brine. The organic portion was dried over anhyd $MgSO_4$, filtered on a bed of silica, and dried to give the corresponding *N*-(Boc-α-aminoacyl)benzotriazole. ^{1}H NMR and mp of Boc-L-Phe-Bt and Boc-Gly-Bt matched that reported in the literature.[3,4]

General Procedure for the Preparation of Serine-containing dipeptides 2a-c



N-(Pg-α-Aminoacyl)benzotriazoles (1.0 mmol) in MeCN (5 mL) was added dropwise to a solution of L-Ser (1.5 mmol) and Et_3N (3.0 mmol) in MeCN/H_2O (9:1, 15 mL) at room temperature and stirred for 4 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 3N HCl (5 x 50 mL). The organic portion was dried over anhyd. $NaSO_4$, filtered and concentrated to give **2a-c**.

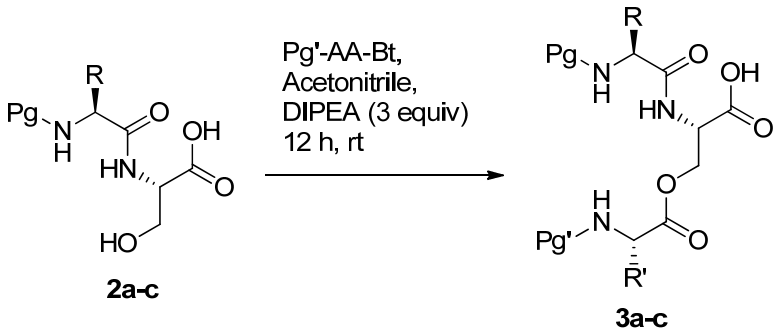
(*S*)-2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoic acid (2a). White solid (85%); mp 156-157 ^oC; ^1H NMR (CD_3OD) δ 8.16 (d, J = 7.8 Hz, 1H), 7.38-7.20 (m, 10 H), 5.05-4.80 (m, 2H), 4.52-4.42 (m, 2H), 3.95-3.80 (m, 2H), 3.23-3.16 (m, 1H), 2.90-2.81 (m, 1H); ^{13}C NMR (CD_3OD) δ 174.3, 173.2, 158.4, 138.7, 138.2, 130.5, 129.6, 129.0, 128.8, 127.8, 67.7, 63.0, 57.9, 56.2, 39.3; Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25; Found: C, 62.47; H, 5.82; N, 7.21.

(*S*)-2-((*S*)-2-((Tert-butoxycarbonyl)amino)-3-phenylpropanamido)-3-hydroxypropanoic acid (2b). White solid (81%); 63.0 - 65.0 ^oC; ^1H NMR ($CDCl_3$) δ 7.52 (br s, 1H), 7.26-7.17 (m, 5H), 6.98 (br s, 1H), 4.62-4.57 (m, 2H), 4.05-3.87 (m, 2H), 3.16-2.88 (m, 2H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.7, 172.1, 156.1, 136.3, 129.4, 128.5, 126.9, 80.8, 62.6, 55.5, 54.7, 38.7, 28.2, 28.0; Anal. Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.86; N, 7.95; Found: C, 57.83; H, 7.34; N, 7.47.

(*S*)-2-((*S*)-2-(((Benzyloxy)carbonyl)amino)propanamido)-3-hydroxypropanoic acid (2c). White solid (73%); 195.0 - 197.0 ^oC; (lit.[5] mp 192.0 - 194.0 ^oC); ^1H NMR (DMSO-d_6) δ 7.99 (d,

J = 7.8 Hz, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.36-7.29 (m, 5H), 5.02 (s, 2H), 4.29-4.23 (m, 1H), 4.17-4.10 (m, 1H), 3.72 (dd, *J*= 11, 5 Hz, 1H) 3.62 (dd, *J*= 11, 4 Hz, 1H), 1.21 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 172.5, 171.9, 155.6, 137.0, 128.3, 127.8, 127.7, 65.4, 61.3, 54.6, 49.8, 18.3.

General Procedure for the Preparation of *O*-Acyl Isopeptides 3a-c



Compound **2** (1.0 mmol) was added to a solution of Pg'-AA-Bt (1.0 mmol) and DIPEA (3.0 mmol) in MeCN (20 mL) at room temperature and stirred for 12 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 2N HCl (3 x 50 mL). The organic portion was dried over anhyd. $NaSO_4$, filtered and concentrated to give **3**.

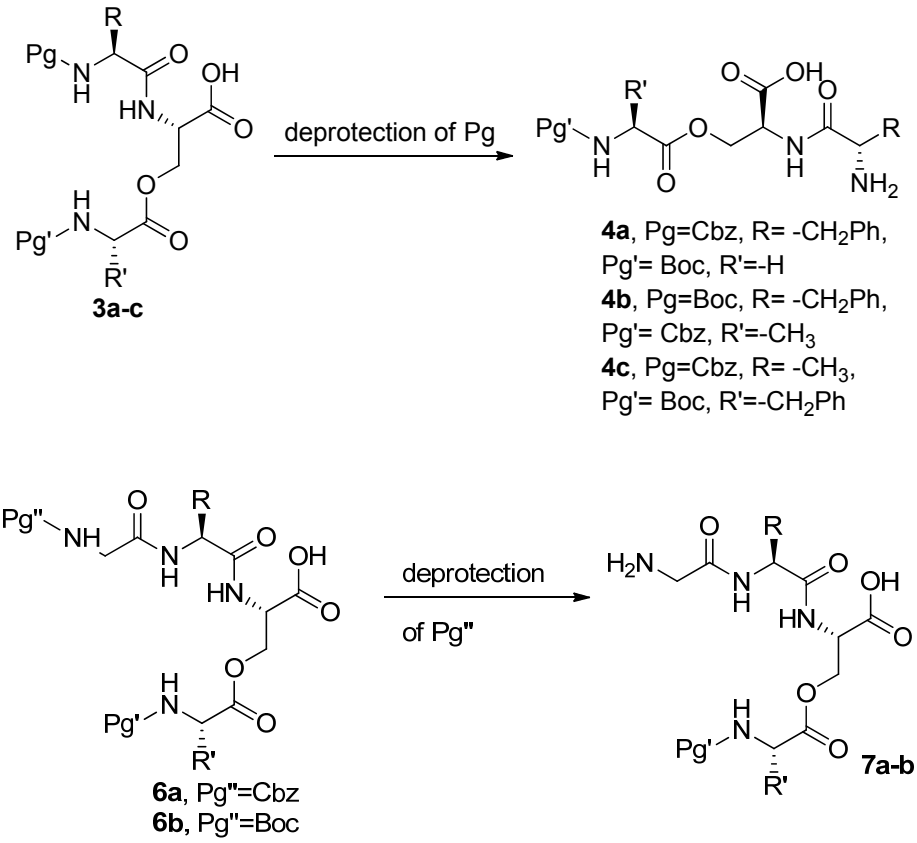
(*S*)-2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (3a). White solid. (86%); mp 86-90 ^{o}C; ^{1}H NMR (CD_3OD) δ 8.22 (d, J = 8.1Hz, 1H), 7.20-7.04 (m, 10 H), 4.88-4.85 (m, 2H), 4.64-4.60 (m, 1H), 4.44 (dd, J = 11.4, 3.6 Hz, 1H), 4.35-4.23 (m, 2H), 3.65 (s, 2H), 3.05 (dd, J = 13.8, 4.5 Hz,, 1H), 2.76-2.67 (m, 1H), 1.29 (s, 9H); ^{13}C NMR (CD_3OD) δ 174.3, 171.8, 158.6, 158.3, 138.6, 138.2, 130.5, 129.5, 129.0, 128.8, 127.8, 80.9, 67.7, 65.0, 57.8, 53.0, 43.0, 39.2, 28.9; Anal. Calcd for $C_{27}H_{33}N_3O_9$: C, 59.66; H, 6.12; N, 7.73; Found C, 59.62; H, 6.13; N, 6.96.

(*S*)-3-(((*S*)-2-(((Benzyloxy)carbonyl)amino)propanoyl)oxy)-2-((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoic acid (3b). White solid. (73%); mp 72.0 - 73.0 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 7.85 (br s, 2H), 7.36-7.16 (m, 10H), 5.65 (br s, 1H), 5.21-4.98 (m, 2H), 4.80-4.69 (m, 2H), 4.53-4.23 (m, 3H), 3.24-2.88 (m, 2H), 1.39-1.28 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 172.4, 172.1, 171.3, 156.4, 155.7, 136.7, 135.8, 129.3, 128.5, 128.2, 128.1,

126.7, 80.3, 67.3, 63.7, 55.8, 51.8, 49.9, 38.3, 28.2, 17.5; Anal. Calcd for $C_{28}H_{35}N_3O_9$: C, 60.31; H, 6.33; N, 7.54; Found C, 60.05; H, 6.77; N, 7.39.

(*S*)-2-((*S*)-2-(((Benzyloxy)carbonyl)amino)propanamido)-3-(((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanoyl)oxy)propanoic acid (3c). White solid. (70%); mp 66.0 - 68.0 ^{o}C; ^{1}H NMR ($CDCl_3$) δ 7.03-7.09 (m, 10H), 5.17-4.83 (m, 3H), 4.53-4.36 (m, 4H), 3.07-2.78 (m, 2H), 1.38 (d, J = 2.5 Hz, 3H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.3, 171.9, 171.6, 156.4, 155.9, 136.3, 136.0, 129.4, 128.9, 128.7, 128.4, 128.3, 127.3, 80.8, 67.3, 64.0, 54.7, 51.8, 50.7, 38.0, 28.4, 18.8; Anal. Calcd for $C_{28}H_{35}N_3O_9$: C, 60.31; H, 6.33; N, 7.54; Found C, 60.34; H, 6.74; N, 7.37.

General Procedure for the Preparation of *O*-Acyl Isopeptides 4a-c and 7a-b



For deprotection of the Cbz- protecting group. Compound **3 (6)** (1.0 mmol) was dissolved in anhydrous MeOH (30 mL) and stirred under an atmosphere of hydrogen in the presence of a catalytic amount of Pd/C for 4 h. Filtration through a bed of celite and evaporation afforded **4 (7)**.

For deprotection of the Boc- protecting group. Compounds **3 (6)** (1.0 mmol) was dissolved in either HCl-dioxane (4.0 M in dioxane) or freshly prepared HCl-MeOH (prepared by bubbling HCl in MeOH) (30 mL) and stirred for 1 h. Solvent is evaporated, and ether was added to the residue and stirred for 2h. Filtration gave a white solid **4 (7)** (when sticky solid resulted, decantation of ether several times was performed instead).

(*S*)-2-((*S*)-2-Amino-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (4a). White solid (80%);mp 170 ^{o}C; ^{1}H NMR (CD_3OD) δ 7.32-7.30 (m, 5H), 4.60-4.50 (m, 1H), 4.39 (s, 2H), 4.25-4.18 (m, 1H), 3.77 (s, 2H), 3.30-3.00 (m, 2H) , 1.38 (s, 9H); ^{13}C NMR (CD_3OD) δ 174.2, 172.8, 169.5, 158.8, 135.5, 130.7, 130.3, 129.0, 81.5, 66.5, 55.9, 55.6, 43.1, 38.2, 28.8; HRMS *m/z* for $C_{19}H_{28}N_3O_7$ $[M+H]^+$ calcd. 410.1922, found 410.1909.

(*S*)-2-((*S*)-2-Amino-3-phenylpropanamido)-3-(((*S*)-2-(((benzyloxy)carbonyl)amino)propanoyl)oxy)propanoic acid (4b). White microcrystals (79%);mp 103.0 - 104.0 ^{o}C; ^{1}H NMR (DMSO-d_6) δ 9.15 (d, J = 8.1 Hz, 1H), 8.37 (br s, 3H), 7.82 (d, J = 7.2 Hz, 1H), 7.39-7.23 (m, 10H), 4.99 (dd, J = 15.7, 12.6 Hz, 2H), 4.65-4.59 (m, 1H), 4.36 (dd, J = 11.3, 4.7 Hz, 1H), 4.27 (dd, J = 11.3, 5.9 Hz, 1H), 4.16-4.05 (m, 2H), 3.20 (dd, J = 14.3, 5.7 Hz, 1H), 3.03 (dd, J = 14.3, 7.5 Hz), 1.29 (d, J = 7.4 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 172.6, 170.0, 168.2, 155.9, 136.9, 134.8, 129.7, 128.5, 128.4, 127.8, 127.1, 66.4, 65.6, 53.2, 51.2, 49.3, 36.7, 16.9; Anal. Calcd for $C_{46}H_{58}N_6O_{15:}$ C, 54.93; H, 5.81; N, 8.35; Found C, 54.63; H, 6.27; N, 8.02.

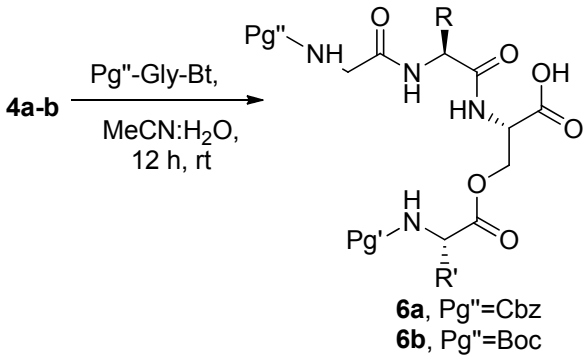
(*S*)-2-((*S*)-2-Aminopropanamido)-3-(((*S*)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanoyl)oxy)propanoic acid (4c). White solid (80%);mp 150.0-152.0 ^{o}C; ^{1}H NMR (CD_3OD) δ 7.26-7.19 (m, 5H), 4.53-4.33 (m, 4H), 3.18 (dd, J = 13.9, 4.7 Hz, 1H), 2.87 (dd, J = 13.9, 9.5 Hz, 1H), 1.53 (d, J = 6.7 Hz, 3H), 1.36 (s, 9H); ^{13}C NMR (CD_3OD) δ 174.2, 173.4, 170.8, 157.8, 138.5, 130.5, 130.3, 129.4, 127.7, 80.6, 66.5, 56.5, 55.6, 50.4, 38.4, 28.7, 17.5; Anal. Calcd for $C_{20}H_{29}N_3O_{7:}$ C, 56.73; H, 6.90; N, 9.92; Found C, 56.61; H, 7.33; N, 9.18.

(*S*)-2-((*S*)-2-(2-Aminoacetamido)-3-phenylpropanamido)-3-(2-((tert-butoxycarbonyl)amino)acetoxy)propanoic acid (7a). White solid (85%) yield; mp 168-173 ^{o}C;

^{1}H NMR (CD_3OD) δ 7.18-6.88 (m, 5H), 4.55-4.40 (m, 1H), 4.39-4.26 (m, 2H), 4.20-4.10 (m, 1H), 3.70-3.40 (m, 3H), 3.04-2.95 (m, 1H), 2.78-2.40 (m, 4H), 1.20 (s, 9H); ^{13}C NMR (CD_3OD) δ 174.9, 173.2, 172.2, 167.8, 158.7, 138.5, 130.5, 129.7, 128.0, 80.9, 66.0, 56.6, 54.7, 44.8, 43.1, 38.8, 28.9; HRMS *m/z* for $C_{21}H_{30}N_4O_8Na$ $[M+Na]^+$ calcd. 489.1956, found 489.1965.

(5S,9S,12S)-12-Benzyl-9-carboxy-5-methyl-3,6,11,14-tetraoxo-1-phenyl-2,7-dioxa-4,10,13-triazapentadecan-15-aminium chloride (7b). White solid (78%) yield; mp 93.0 - 94.0^{o}C; ^{1}H NMR (CD_3OD) δ 7.35-7.18 (m, 10H), 5.14-5.02 (m, 3H), 4.61 (dd, J = 10.6, 4 Hz, 1H), 4.39 (dd, J = 10.6, 5 Hz, 1H), 4.27-4.22 (m, 1H), 3.75-3.67 (m, 2H), 3.59-3.55 (m, 1H), 3.24 (dd, J =13.7, 5 Hz, 1H), 2.92 (dd, J =13.7, 9.2Hz, 1H), 1.38 (d, J = 6.7 Hz, 3H); ^{13}C NMR (CD_3OD) δ 174.5, 173.4, 172.0, 167.4, 158.8, 138.2, 138.0, 130.4, 129.6, 129.6, 129.2, 128.9, 128.0, 68.2, 68.0, 65.1, 56.2, 53.2, 41.6, 38.9, 17.6; HRMS *m/z* for $C_{25}H_{30}N_4O_8$ $[M+H]^+$ calcd. 515.2136, found 515.2137.

General Procedure for the Preparation of *O*-Acyl Isopeptides 6a-b



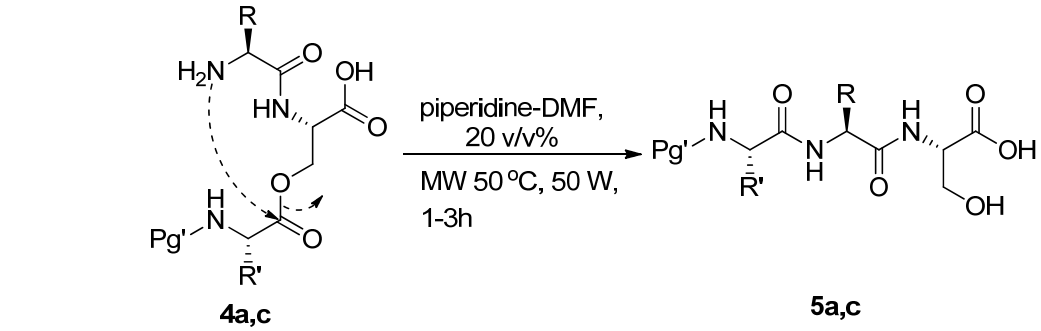
Pg"-Gly-Bt (1.0 mmol) was added to a solution of **4** (1.0 mmol) and DIPEA (3.0 mmol) in MeCN:H_2O (9.5:0.5, 20 mL) at room temperature and stirred for 12 h. MeCN was evaporated and the residue dissolved in EtOAc (50 mL) and washed with 2N HCl (3 x 50 mL). The organic portion was dried over anhyd. $NaSO_4$, filtered and concentrated to give **6**.

(8*S*,11*S*)-8-Benzyl-11-((2-((tert-butoxycarbonyl)amino)acetoxy)methyl)-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazadodecan-12-oic acid (6a). White solid (89%), converted to compound **7** after checking NMR.**6**: mp 180 ^{o}C (decomposed). ^{1}H NMR (CD_3OD) δ 7.20-7.03 (m, 10H), 4.89 (s, 2H), 4.56-4.44 (m, 2H), 4.38-4.30 (m, 1H), 4.24-4.18 (m, 1H), 3.60-3.47 (m, 4H), 3.11-2.87 (m, 1H), 2.78-2.58 (m, 4H), 1.23 (s, 9H); ^{13}C NMR (CD_3OD) δ 177.0, 173.6, 173.4, 172.1, 171.9, 171.8, 159.1, 138.3, 130.5, 129.6, 129.1, 129.0, 127.9, 127.2, 80.9, 68.0, 65.0, 55.7, 53.0, 44.0, 43.0, 38.7, 28.6.

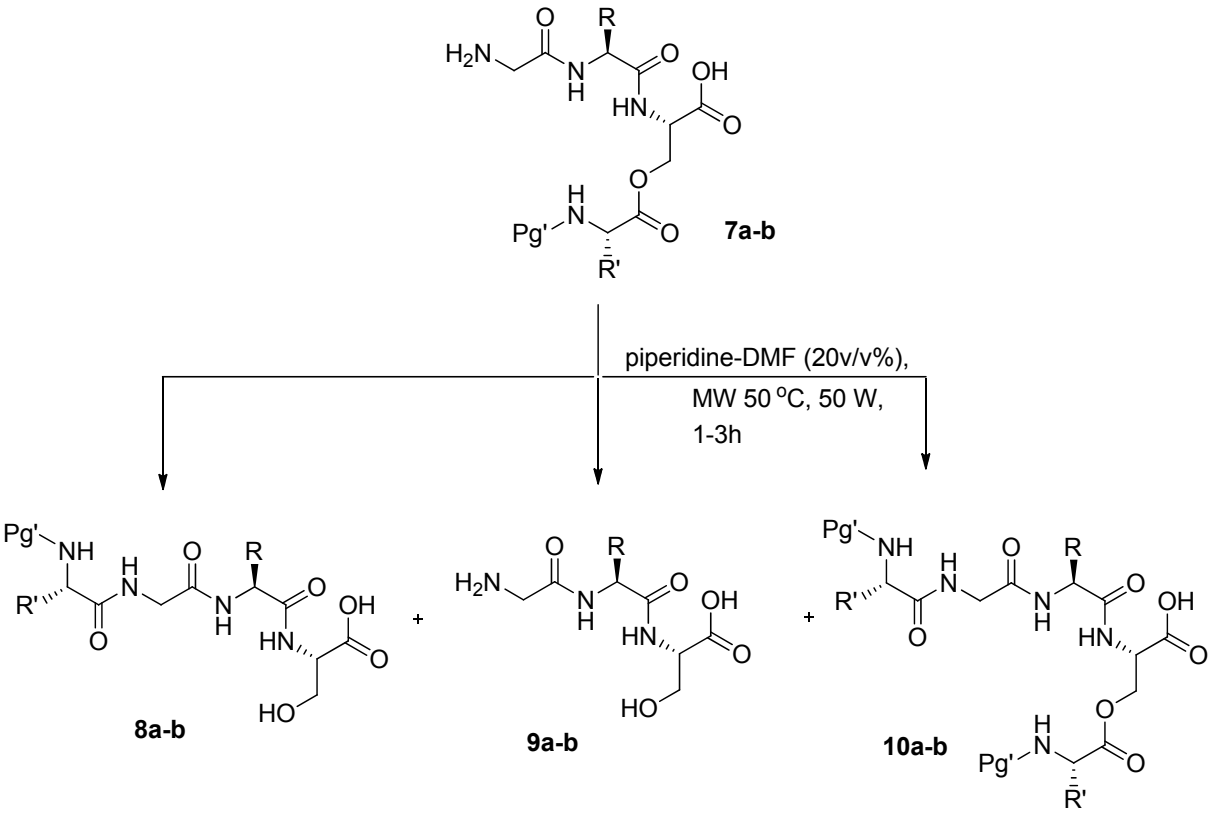
(9*S*,12*S*)-9-Benzyl-12-((((*S*)-2-(((benzyloxy)carbonyl)amino)propanoyl)oxy)methyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oic acid (6b). Colorless oil (89%); converted to compound 7 after checking NMR; ^{1}H NMR ($CDCl_3$) δ 9.70 (br s, 2H), 7.34 (d, J = 7.4 Hz, 1H), 7.25-7.07 (m, 10H), 6.03 (d, J = 7.4 Hz, 1H), 5.07-4.57 (m, 4H), 4.29-4.14 (m, 2H),

3.84-3.55 (m, 3H), 3.12-2.89 (m, 2H), 1.36 (d, J = 2.8 Hz, 3H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 155.5, 155.1, 135.1, 134.9, 128.2, 127.5, 127.4, 127.1, 127.0, 125.9, 124.4, 66.1, 62.6, 53.0, 50.9, 48.8, 42.8, 36.5, 29.3, 27.2, 16.4.

Procedure for Ligation



4a,c **5a,c**



7a-b **8a-b** **9a-b** **10a-b**

(9*S*,12*S*)-9-Benzyl-12-(hydroxymethyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatridecan-13-oic acid (5). Compound **4** (20 mg, 0.05 mmol) was dissolved in piperidine 20 v/v% in DMF (1 mL) and stirred at 50 °C and 50 W for 1h. The mixture was then evaporated and

purified by HPLC to give ligated product **5** (for example **5a**, 57%); The sample was analyzed via reverse phase gradient C18 HPLC/UV/(-)ESI-MSn to give a retention time of 23.07 min (for **5a**).

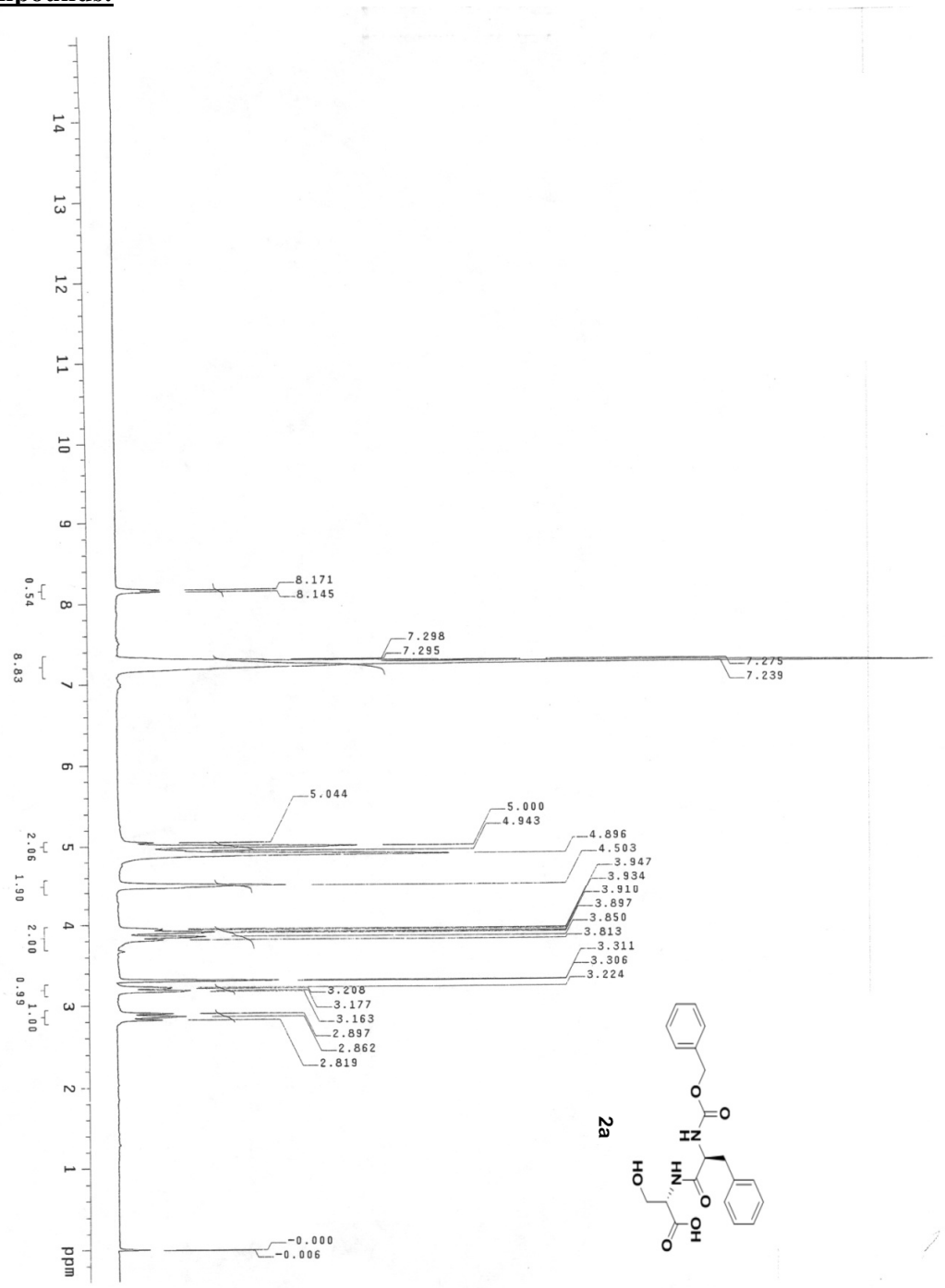
To confirm structure, HRMS for **5a** *m/z* for $C_{19}H_{26}N_3O_7$ $[M-H]^+$ calcd. 408.1776, found 408.1794.

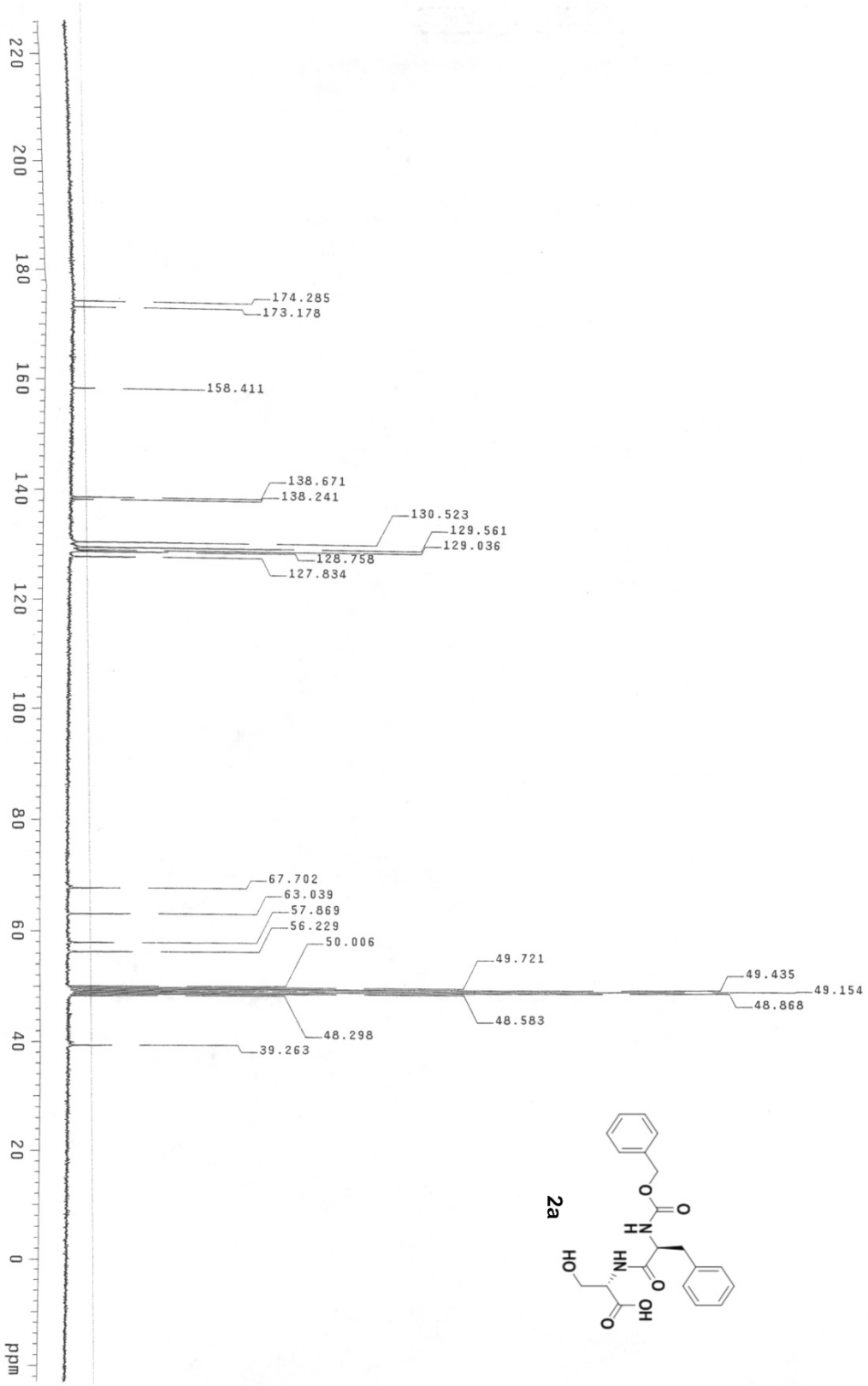
(12*S*,15*S*)-12-benzyl-15-(hydroxymethyl)-2,2-dimethyl-4,7,10,13-tetraoxo-3-oxa-5,8,11,14-tetraazahexadecan-16-oic acid (8). Compound **7** (20 mg, 0.04 mmol) was dissolved in piperidine 20 v/v% in DMF (1 mL) and stirred at 50 ^oC and 50 W for 1h (3h for **8b**). The mixture was then evaporated and purified by HPLC to give ligated product **8** (for example **8a**, 99.39%); The sample was analyzed via reverse phase gradient C18 HPLC/UV (254 nm/ESI-MSn to give a retention time of 21.67 min (for **8a**). To confirm structure, HRMS for **8a** *m/z* for $C_{21}H_{29}N_4O_8$ $[M-H]^+$ calcd. 465.2064, found 465.1992.

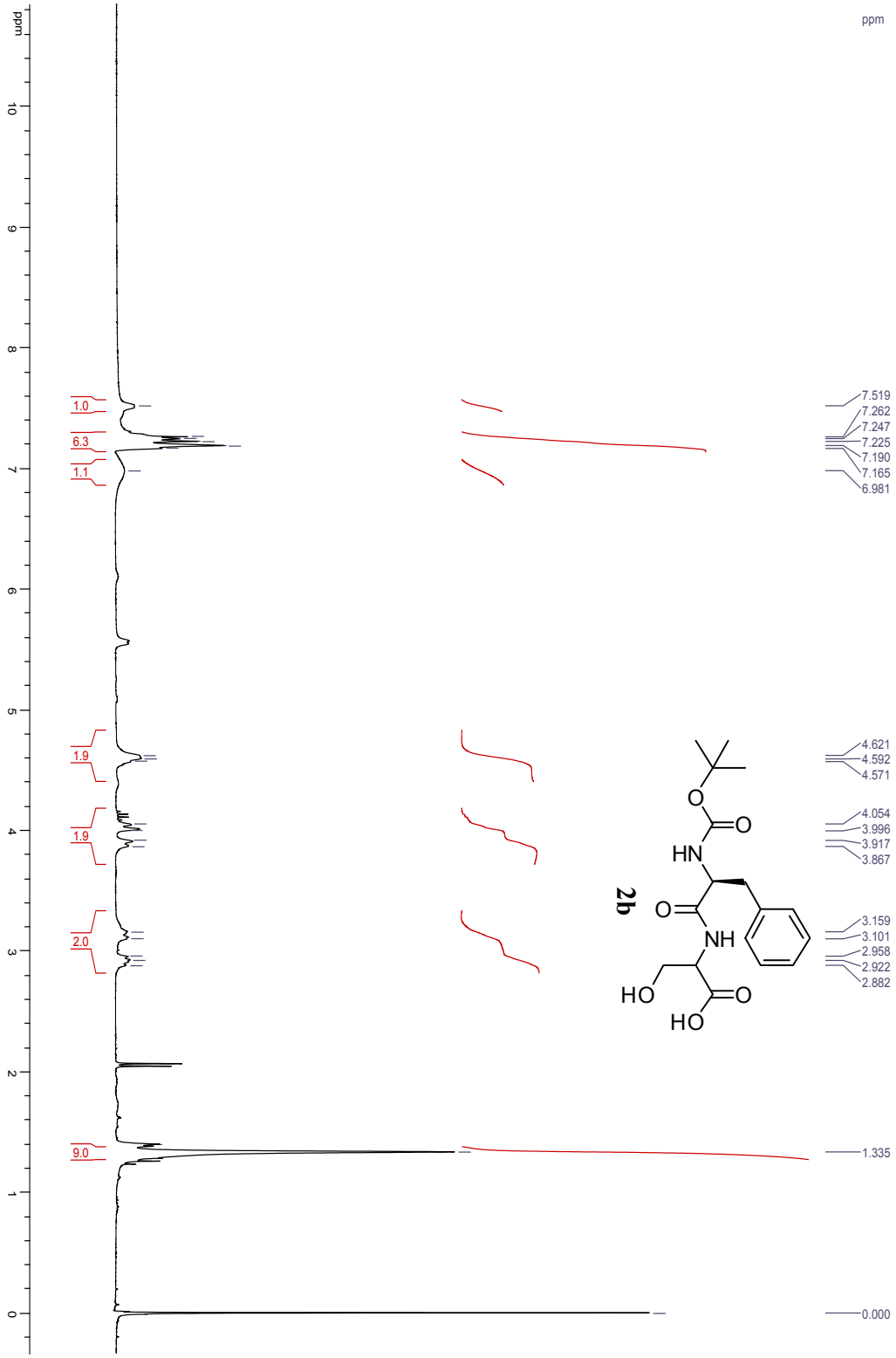
References

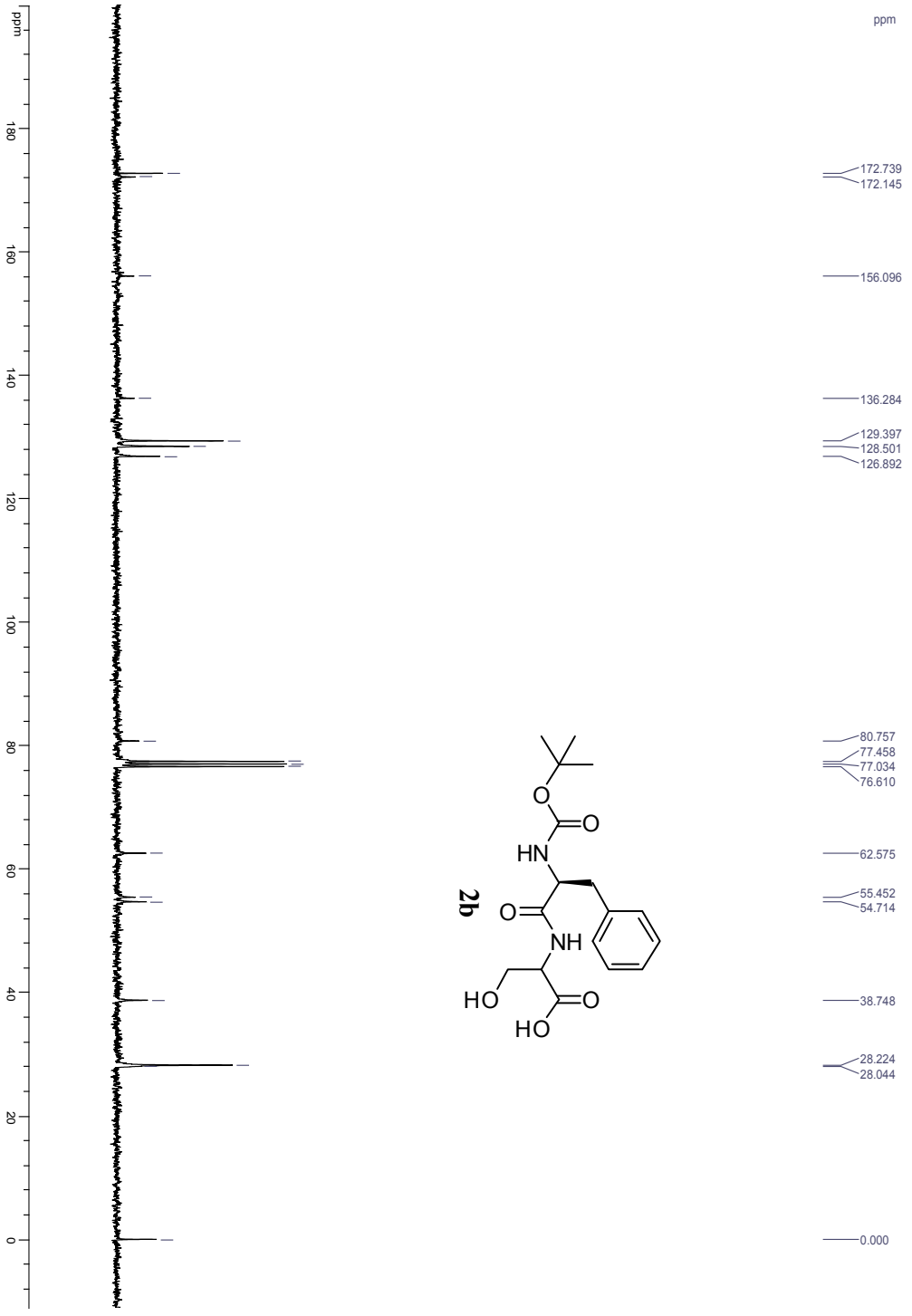
1 A. R. Katritzky, A. A. Shestopalov and K. Suzuki, *Arkivoc*, 2005, **vii**, 36.
2 A. R. Katritzky, S. R. Tala, N. E. Abo-Dya, T. S. Ibrahim, S. A. El-Feky, K. Gyanda and K. M. Pandya, *J. Org. Chem*., 2011, **76**, 85.
3 E. Masiukiewicz, B. Rzeszotarska, I. Wawrzycka-Gorczyca and E. Kolodziejczyk, *Synth. Commun*., 2007, **37**, 1917.
4 A. R. Katritzky, A. A. Shestopalov and K. Suzuki, *Synthesis*, 2004,1806.
5 A. R. Katritzky, K. Suzuki and K. Sandeep *Synthesis,* 2004, **16**, 2645.

1 **^{1}H NMR and ^{13}C NMR spectra for compounds:**









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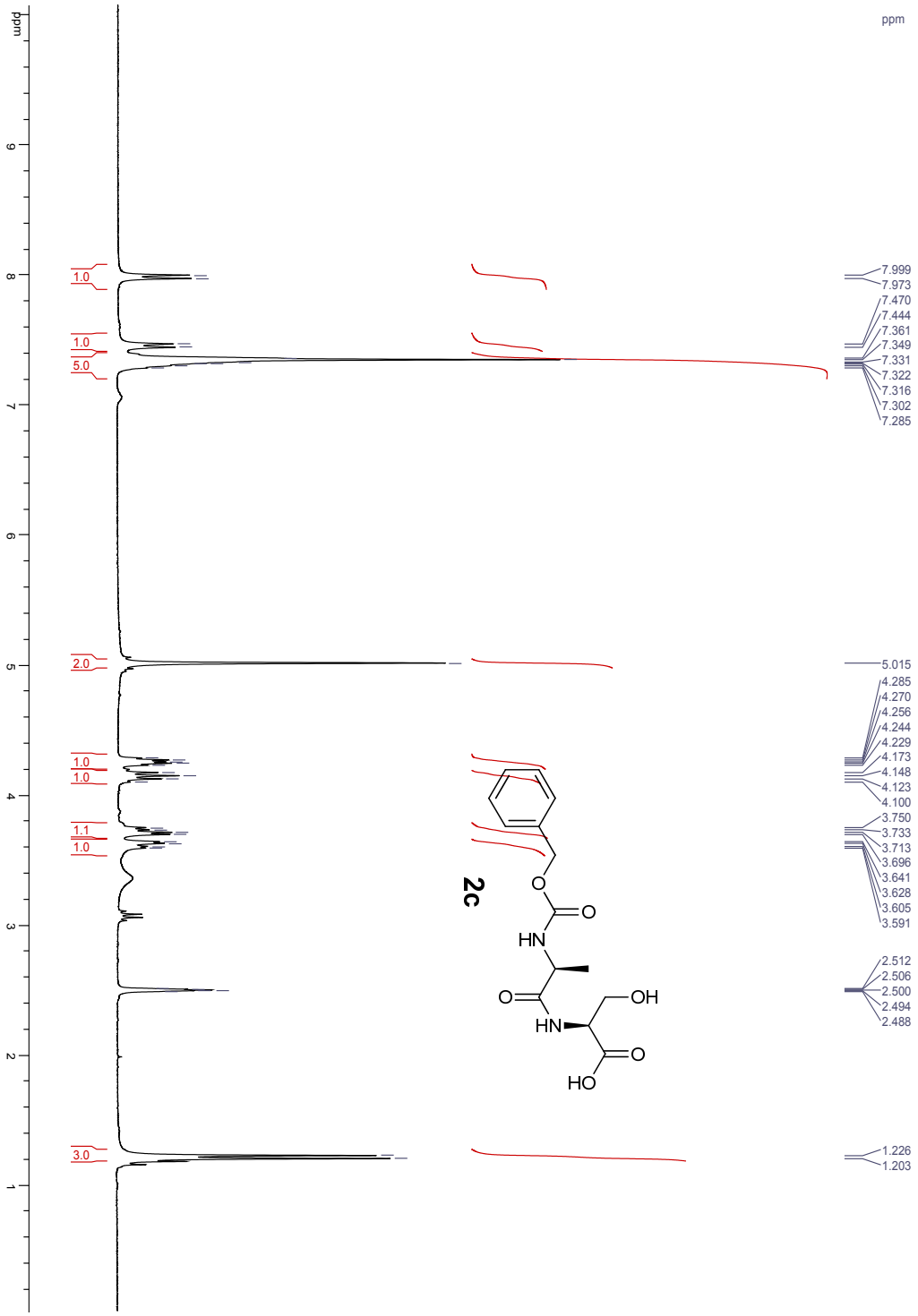
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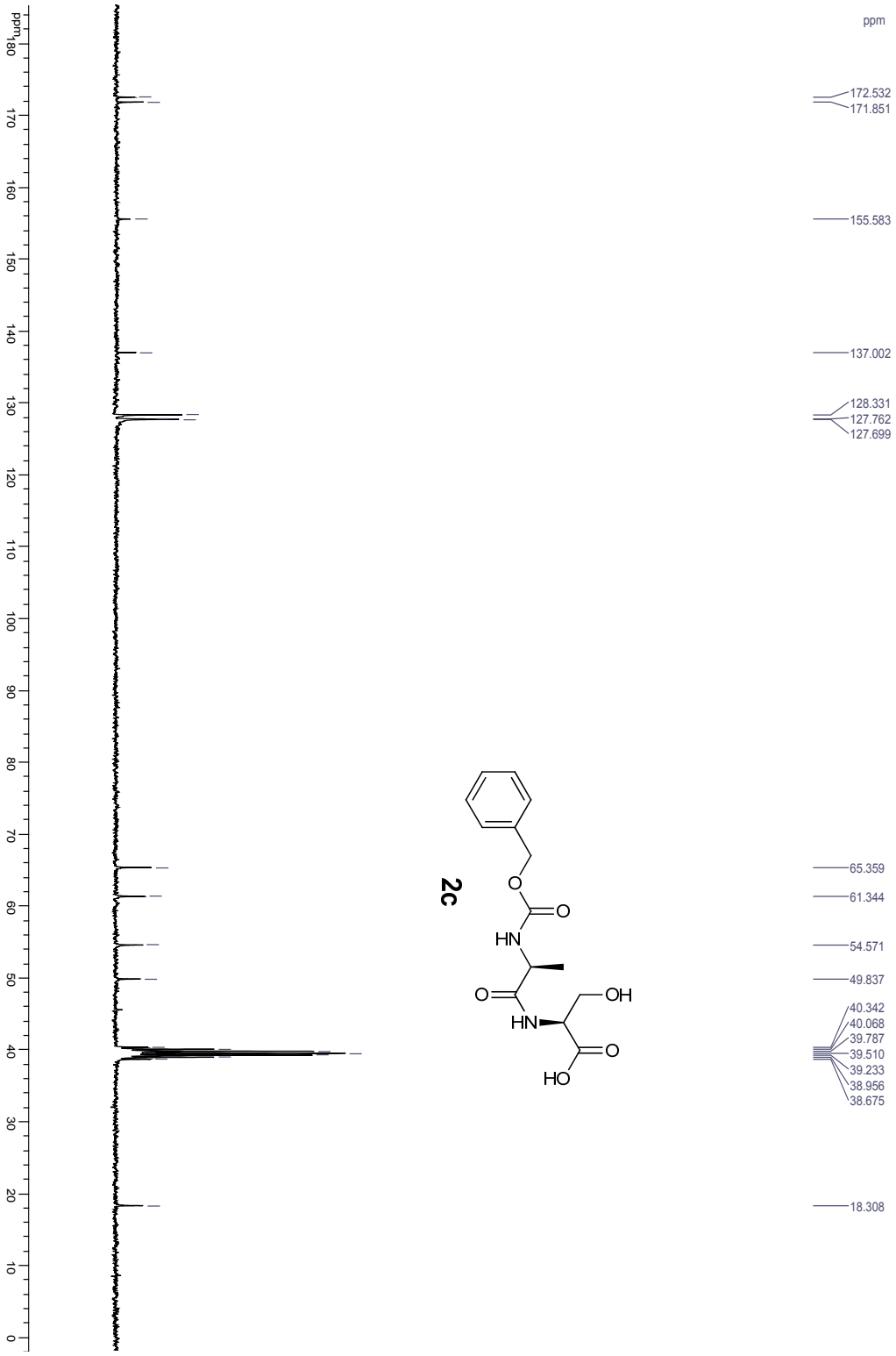

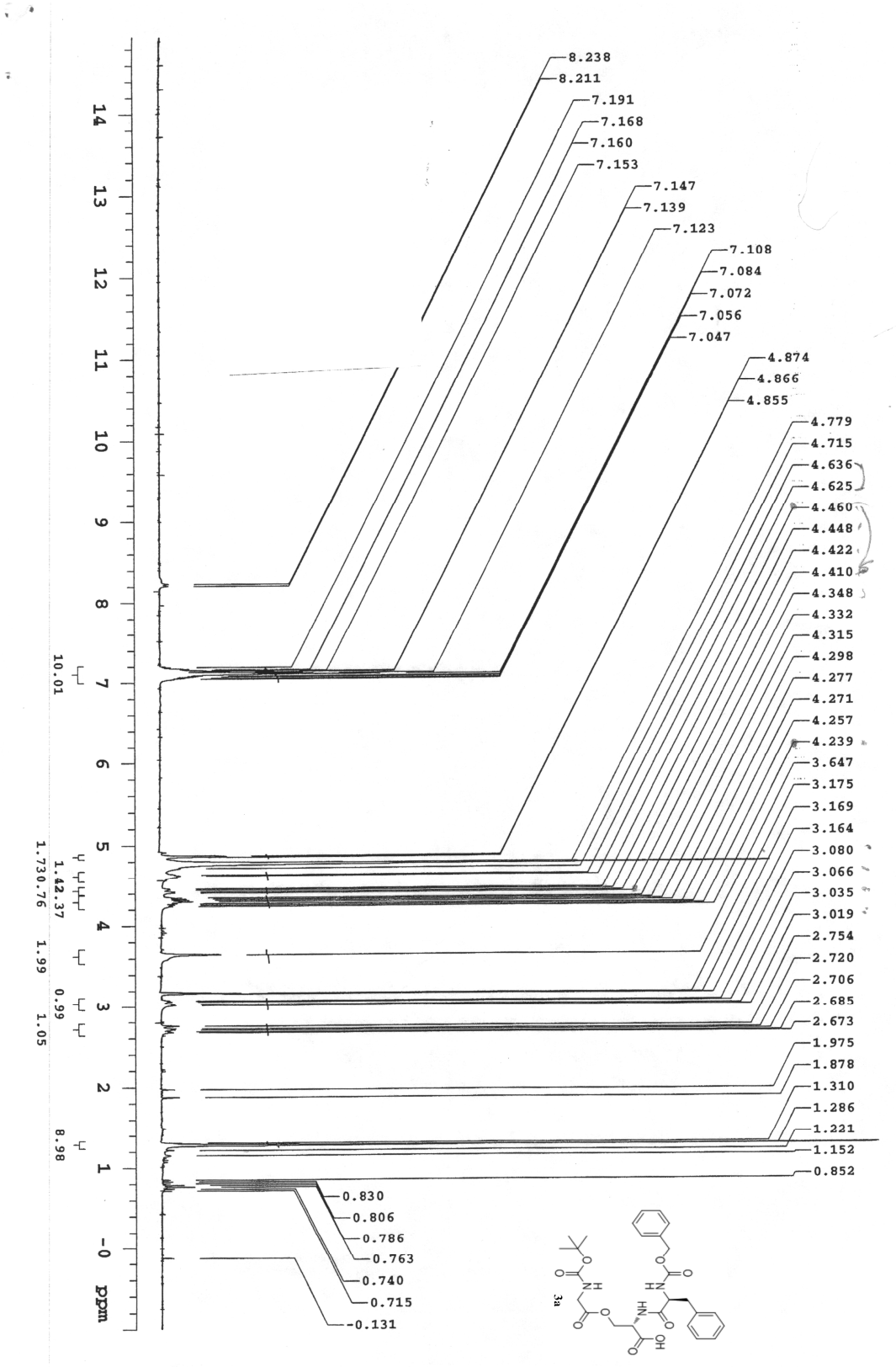

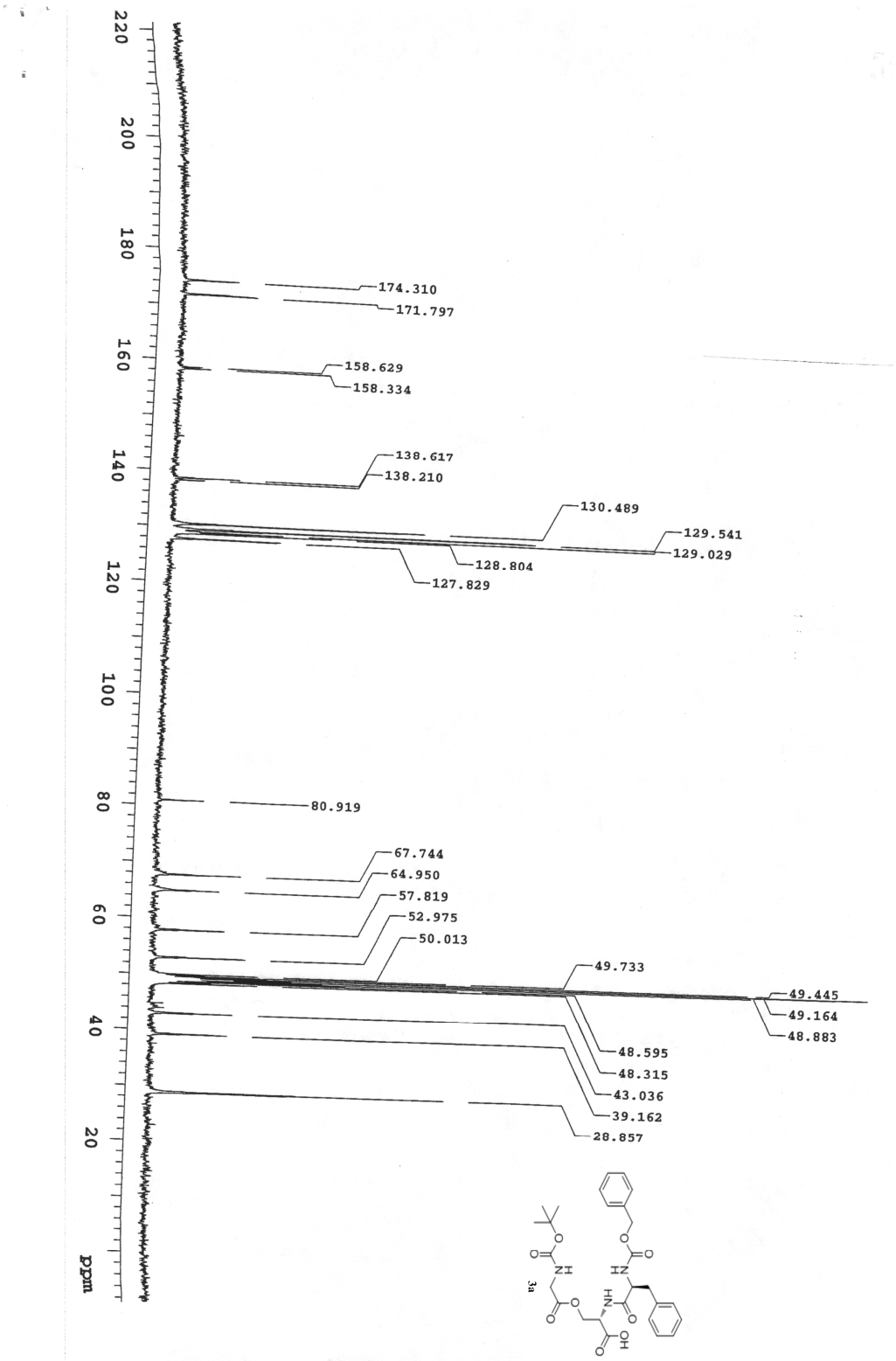

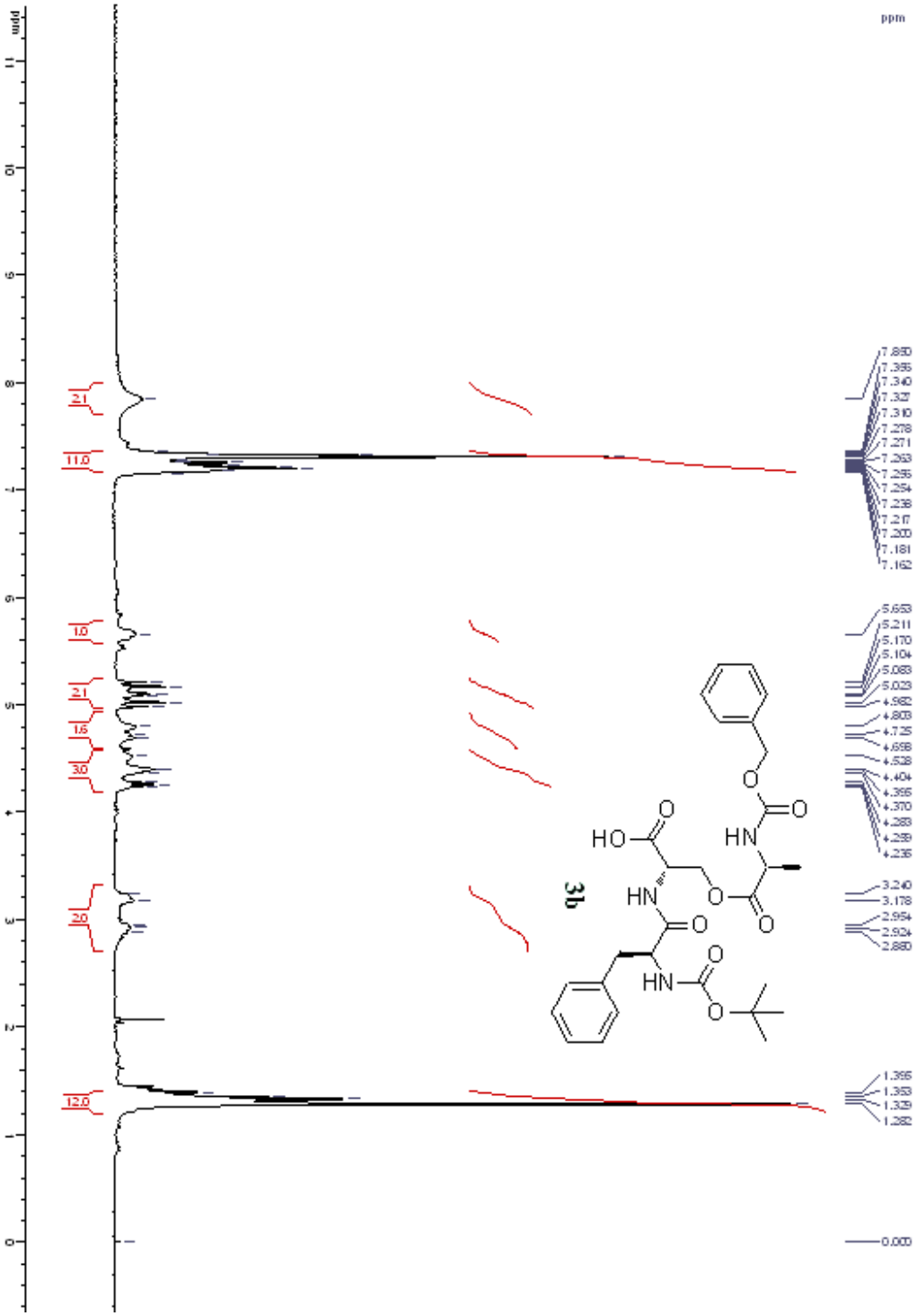

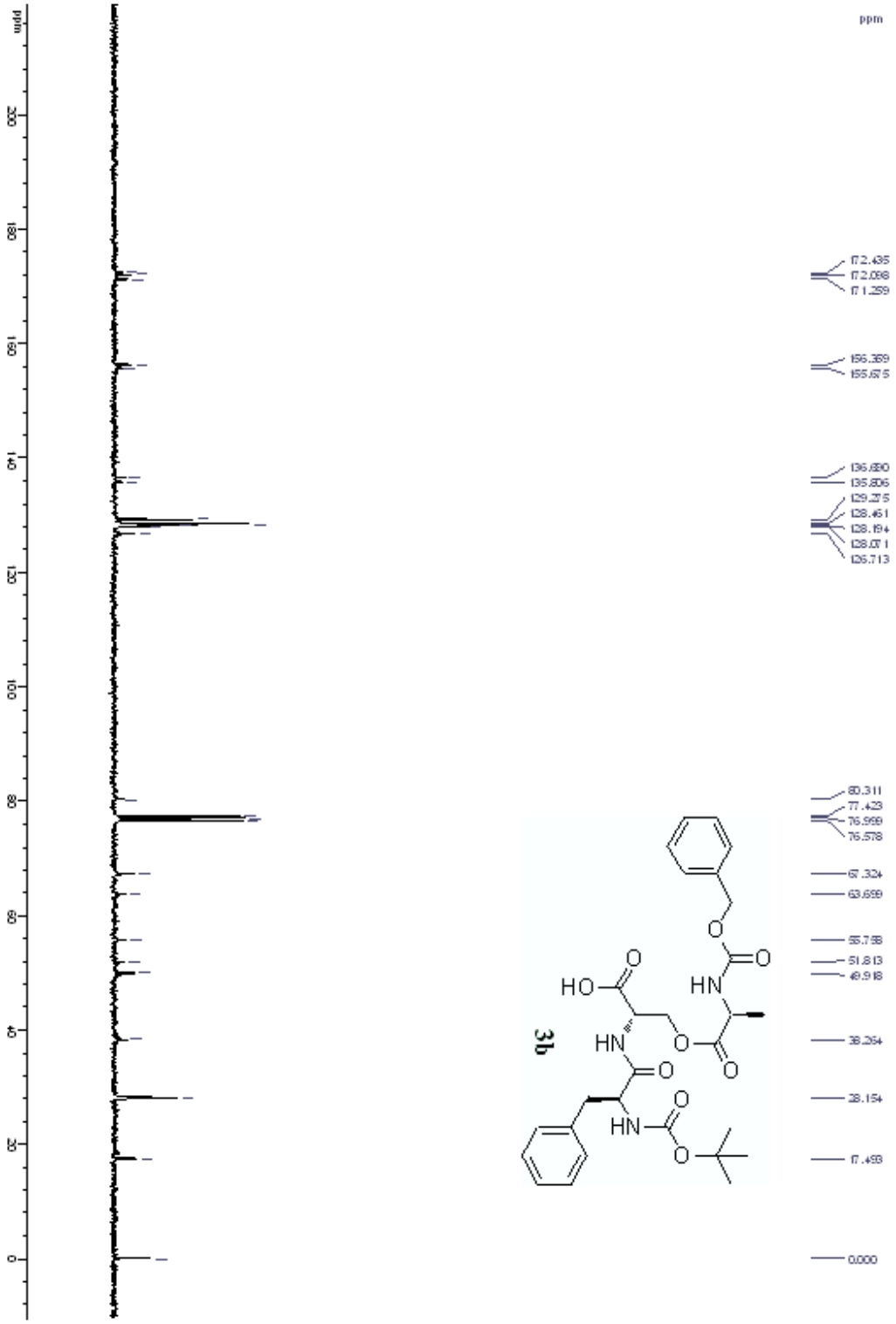

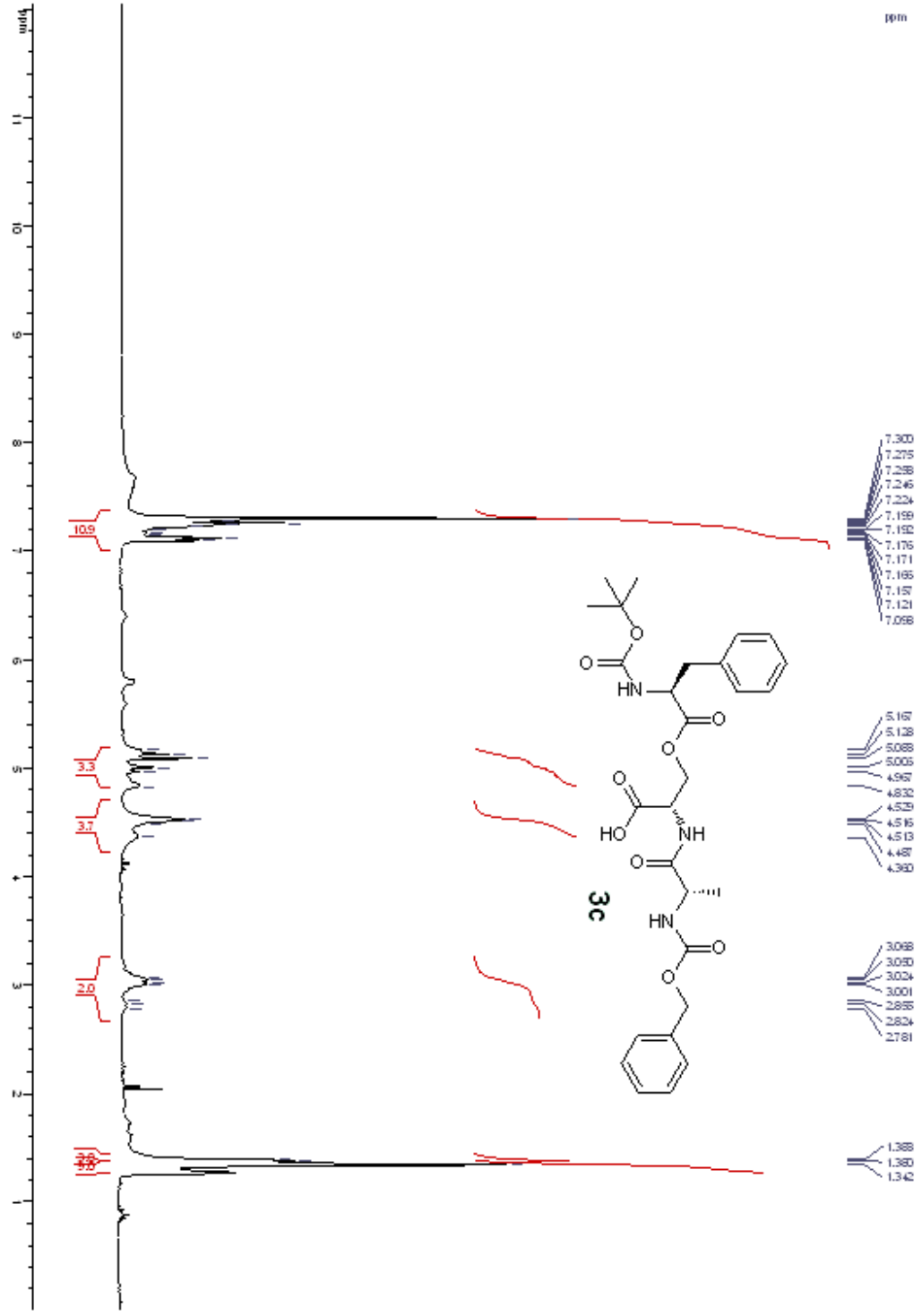

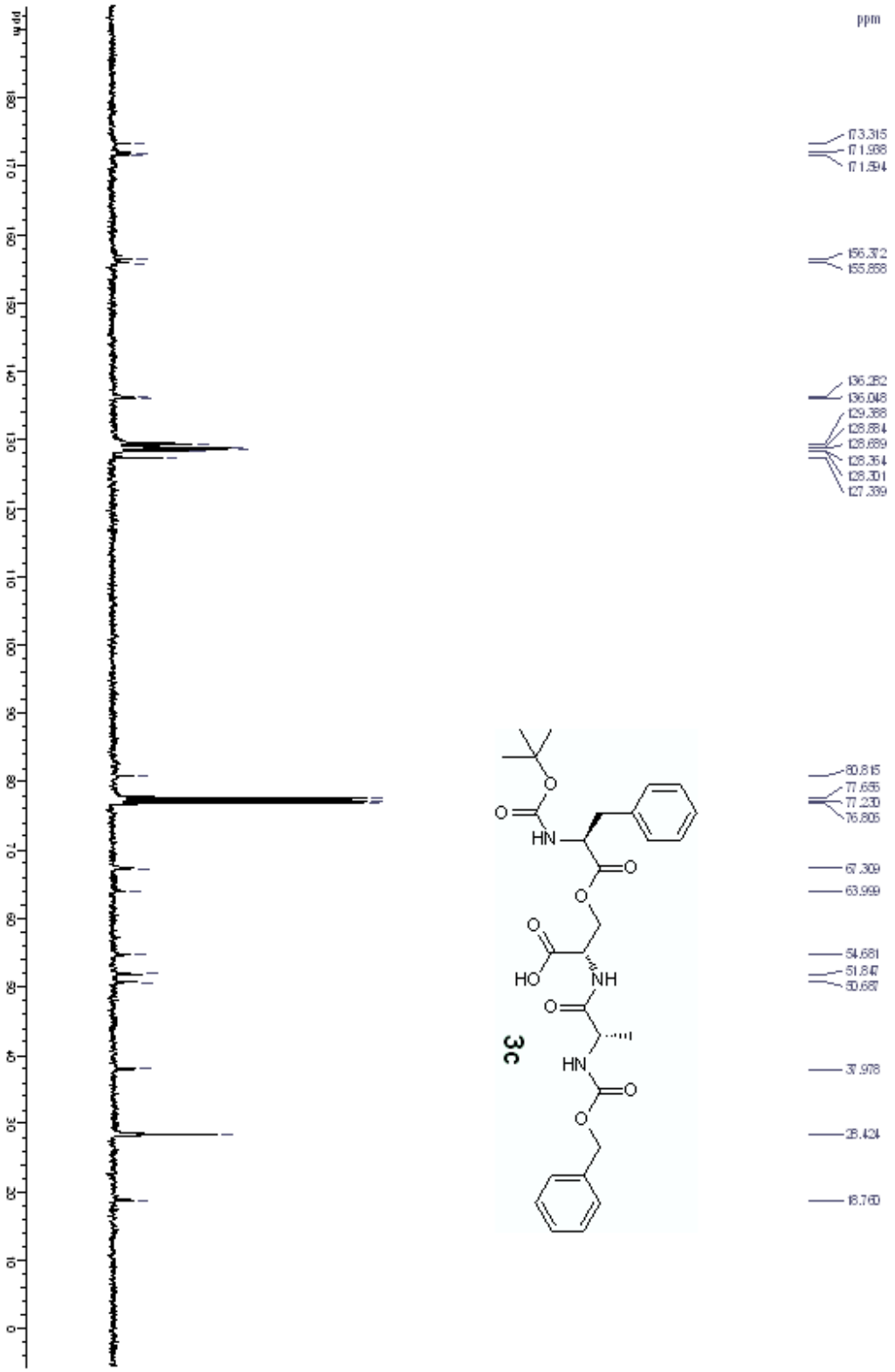

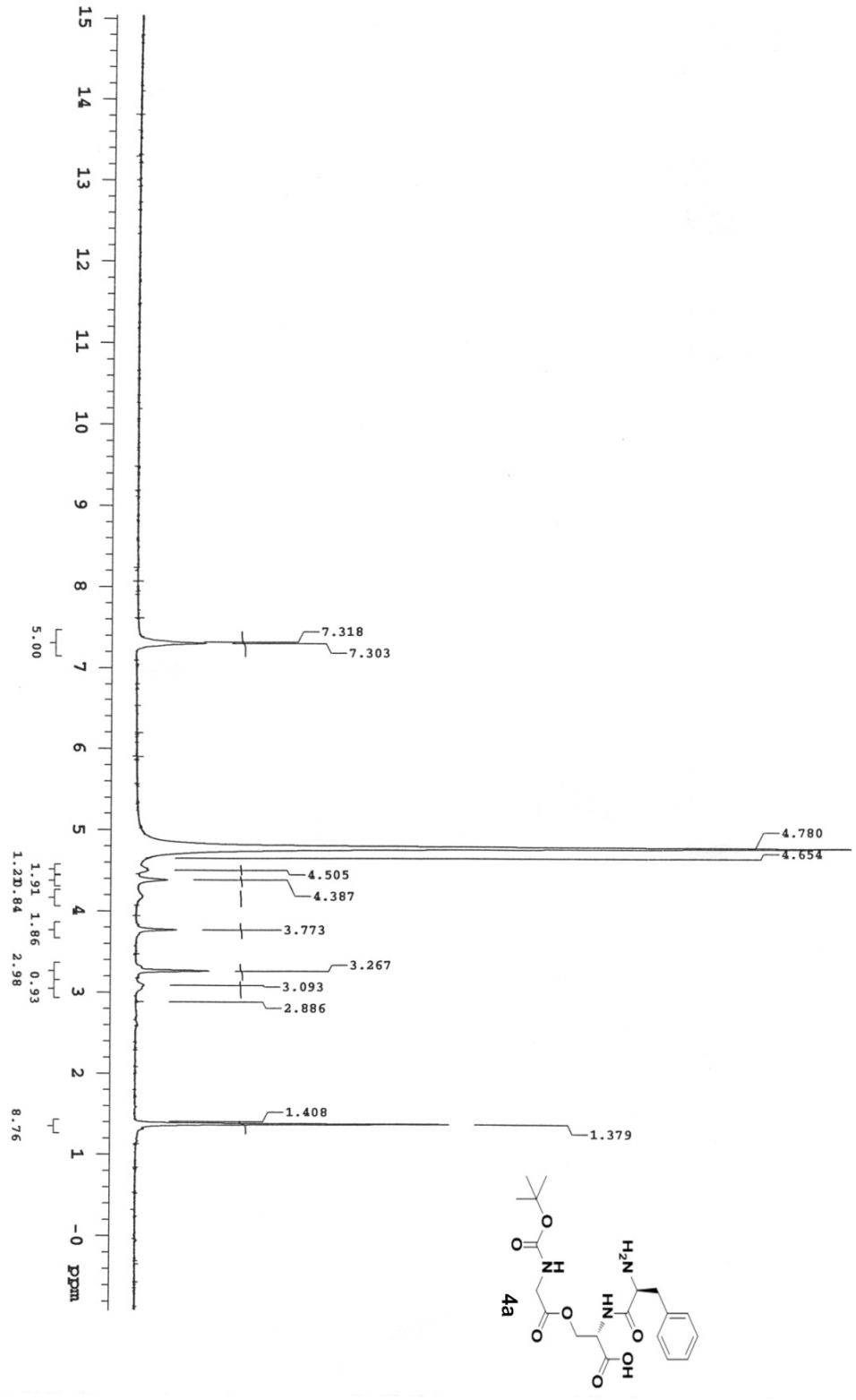

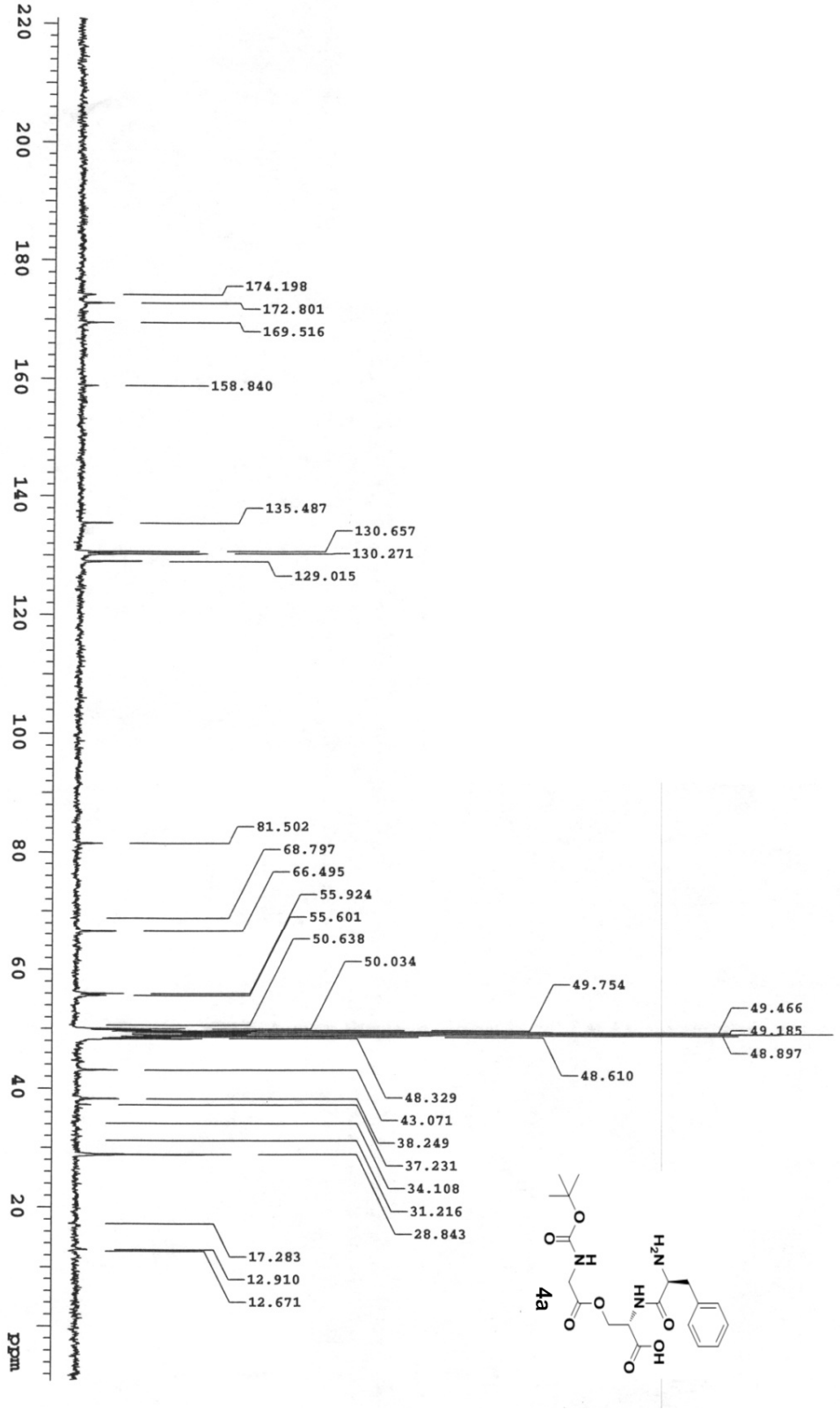

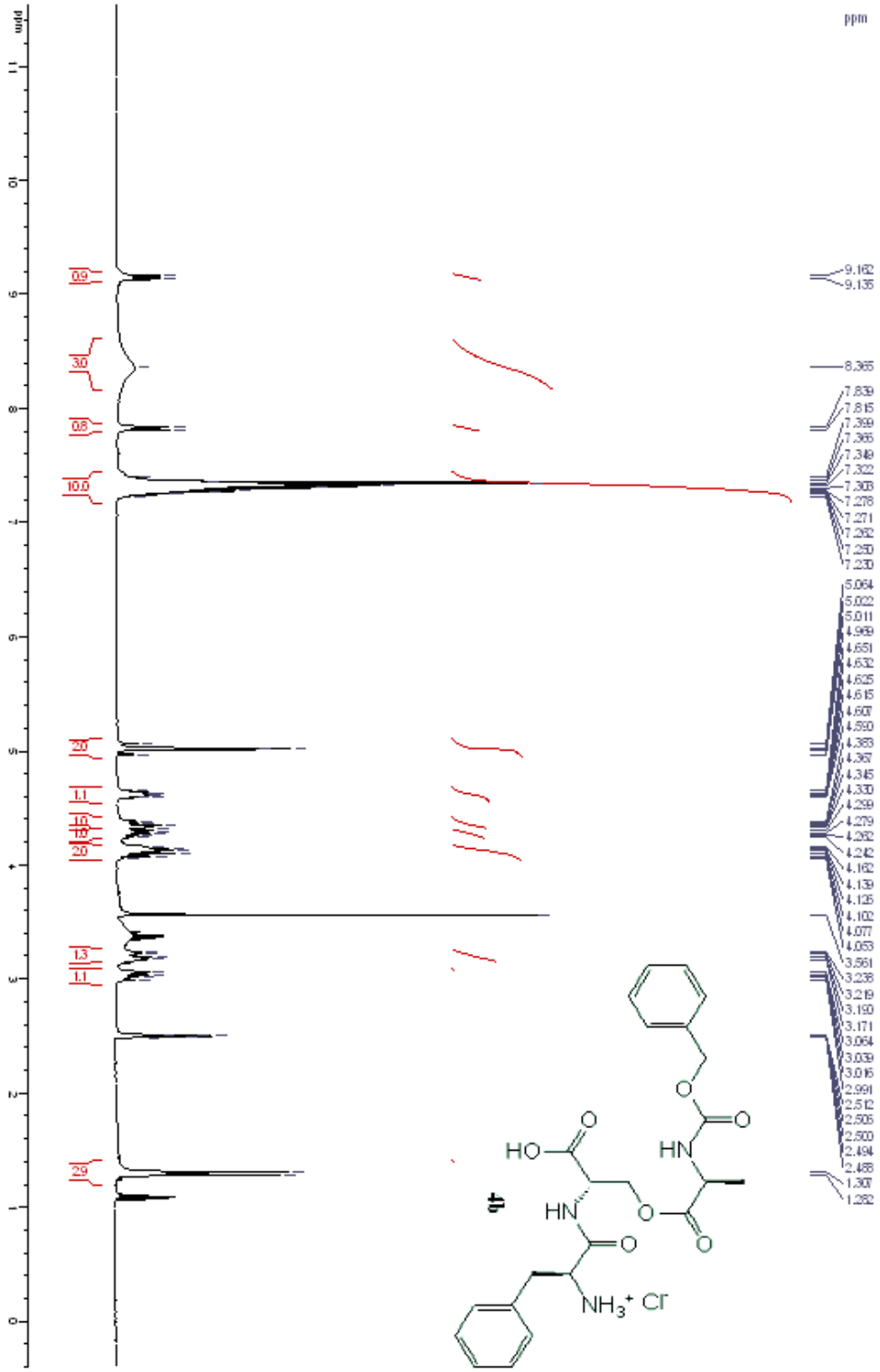

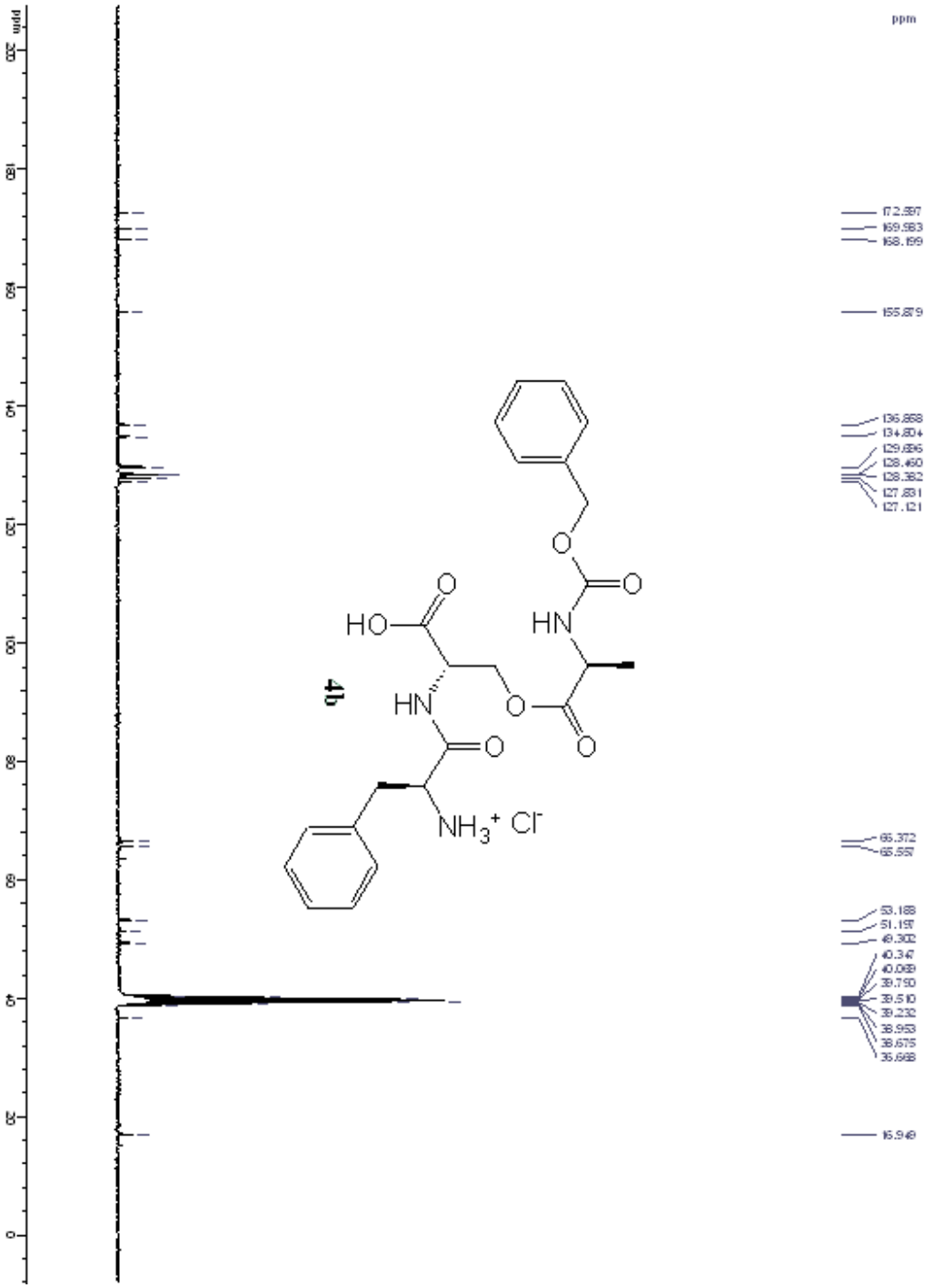
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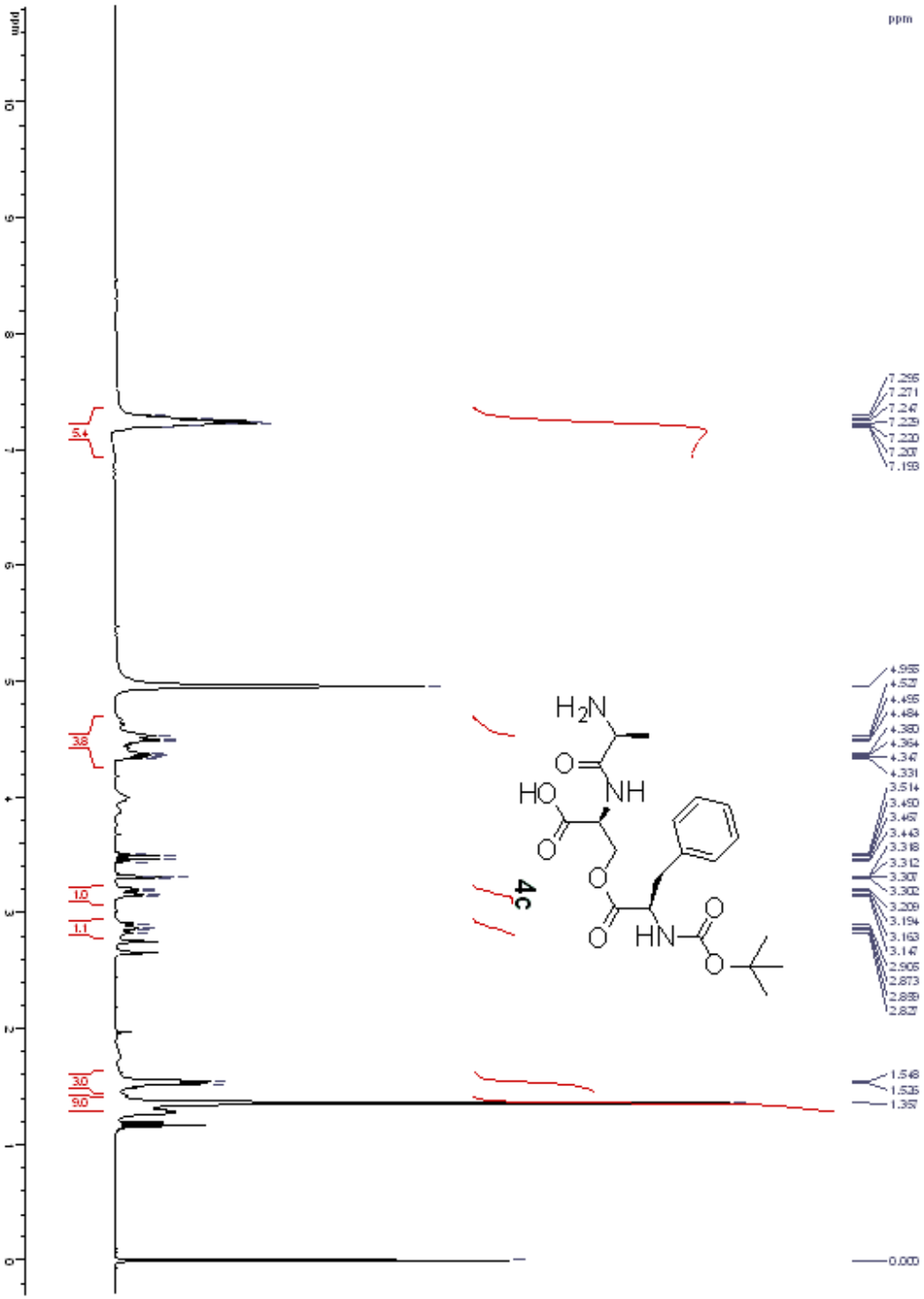
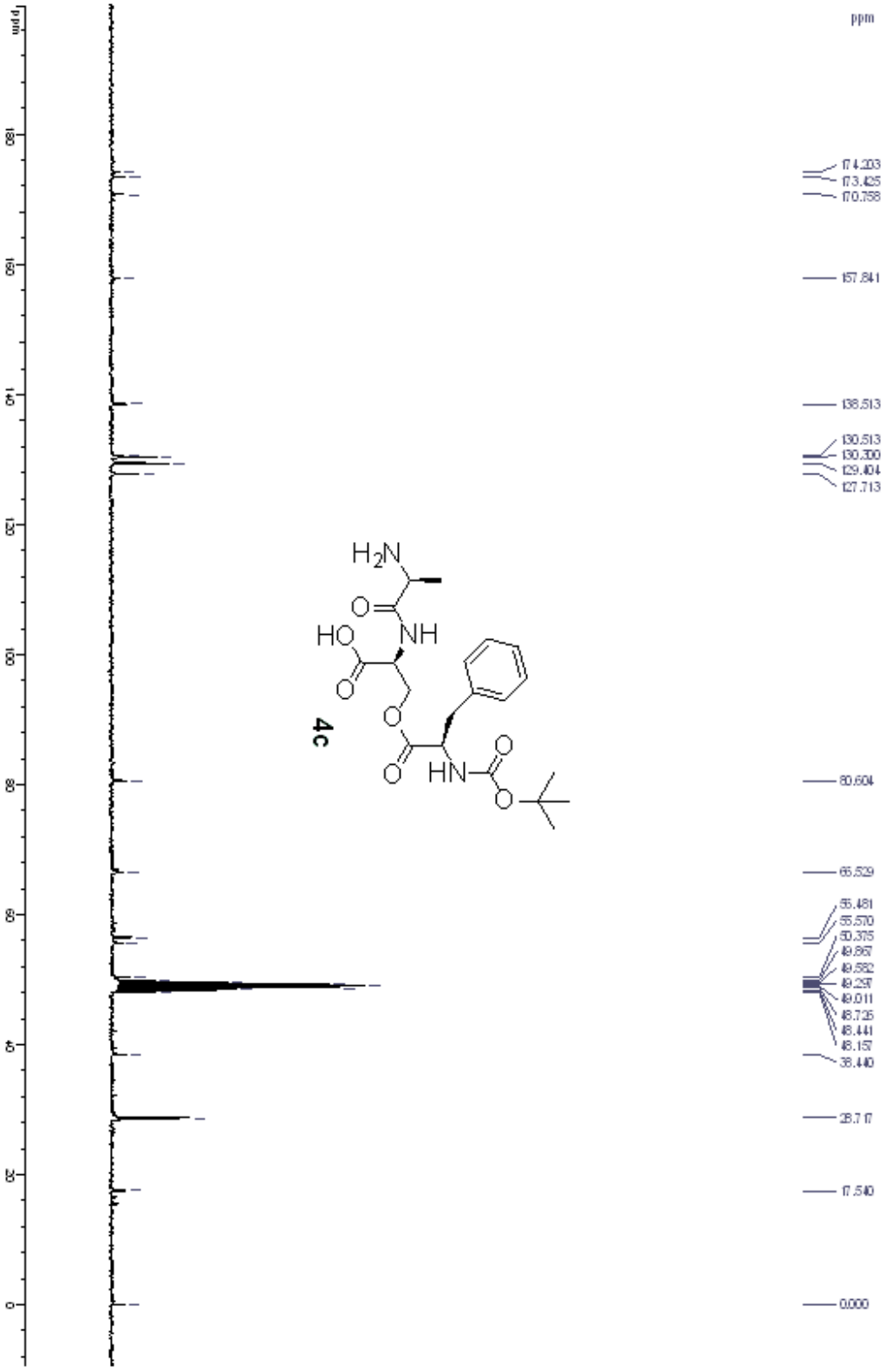
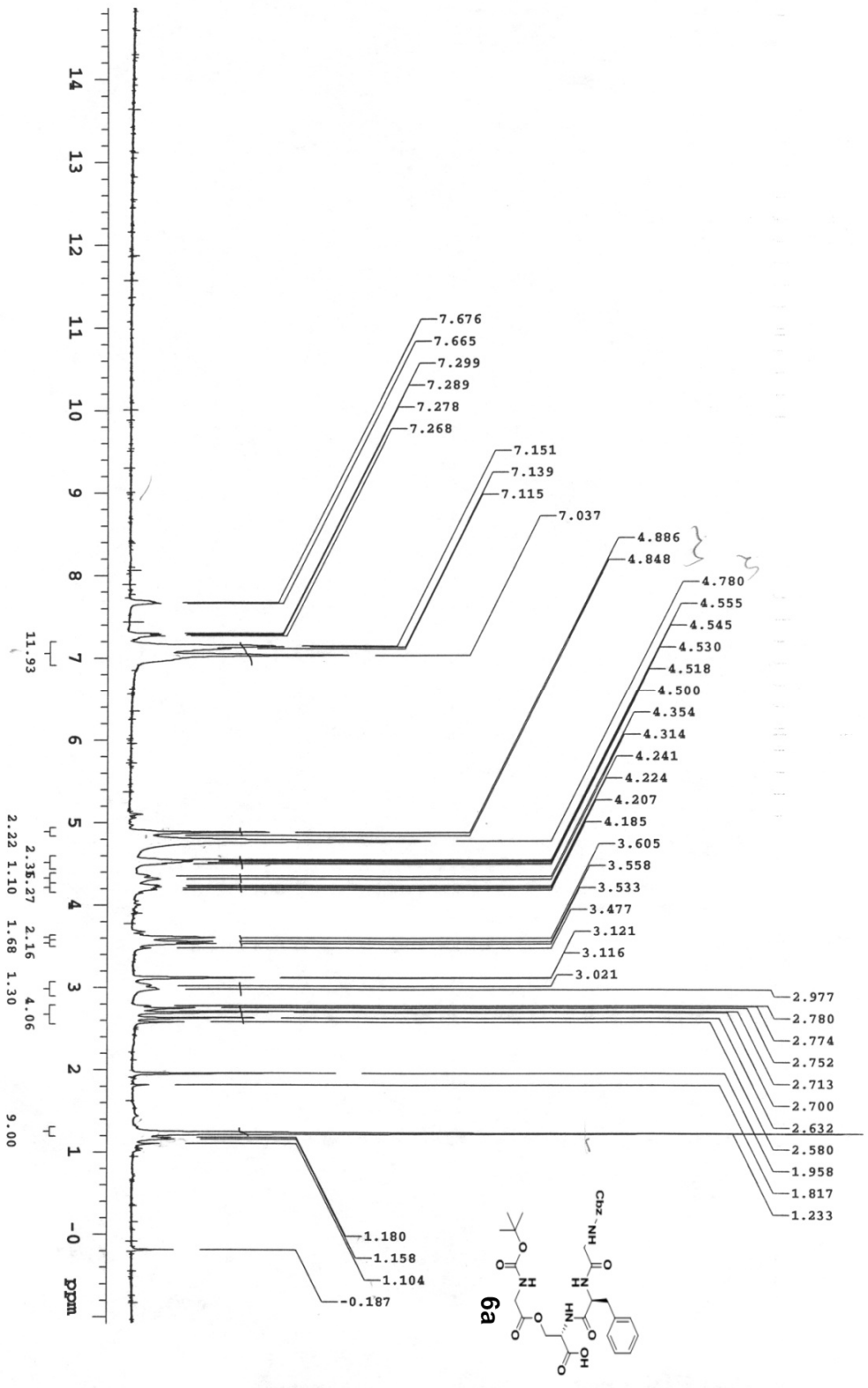
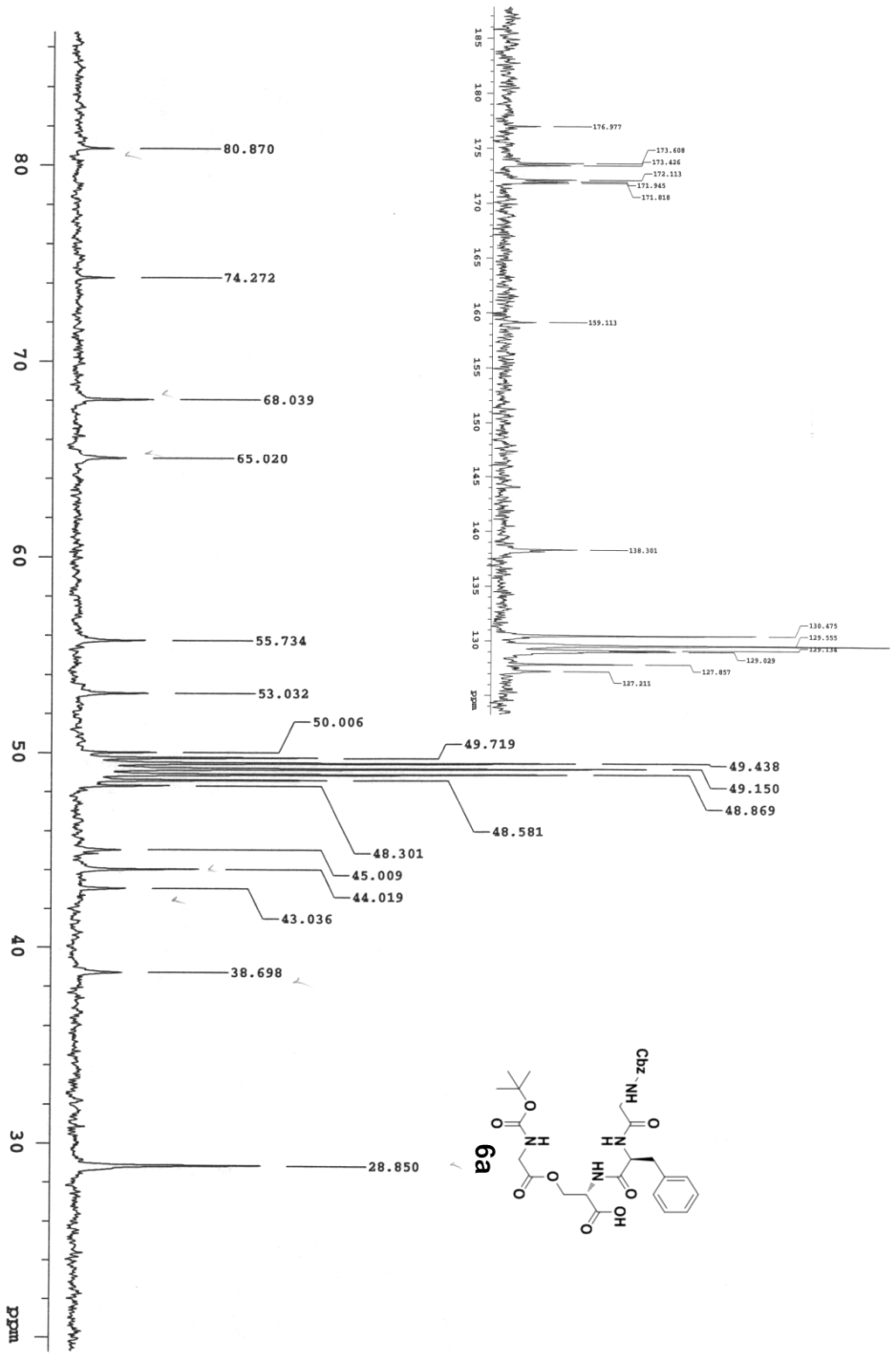
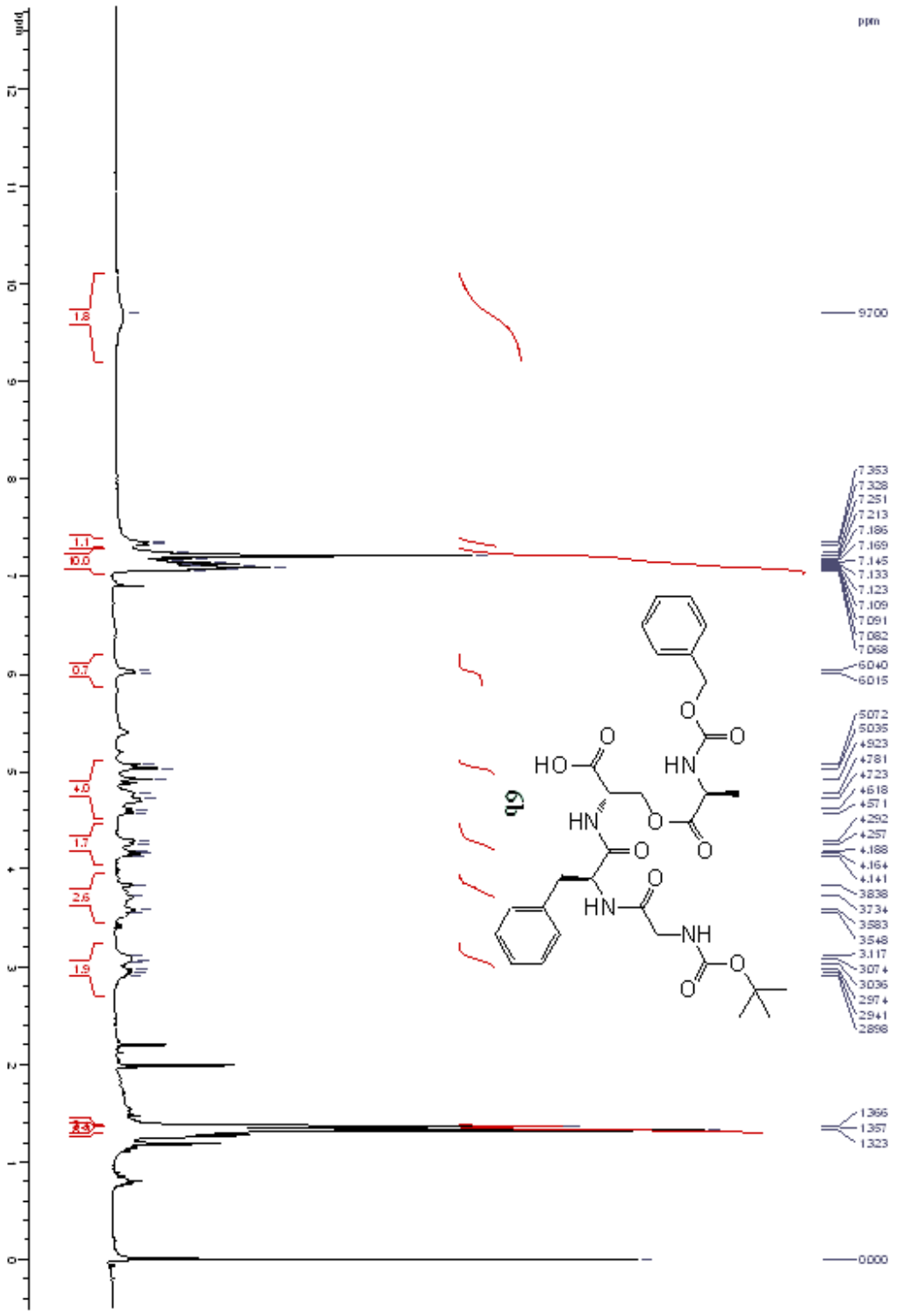
NH_3^+ Cl^-
4b

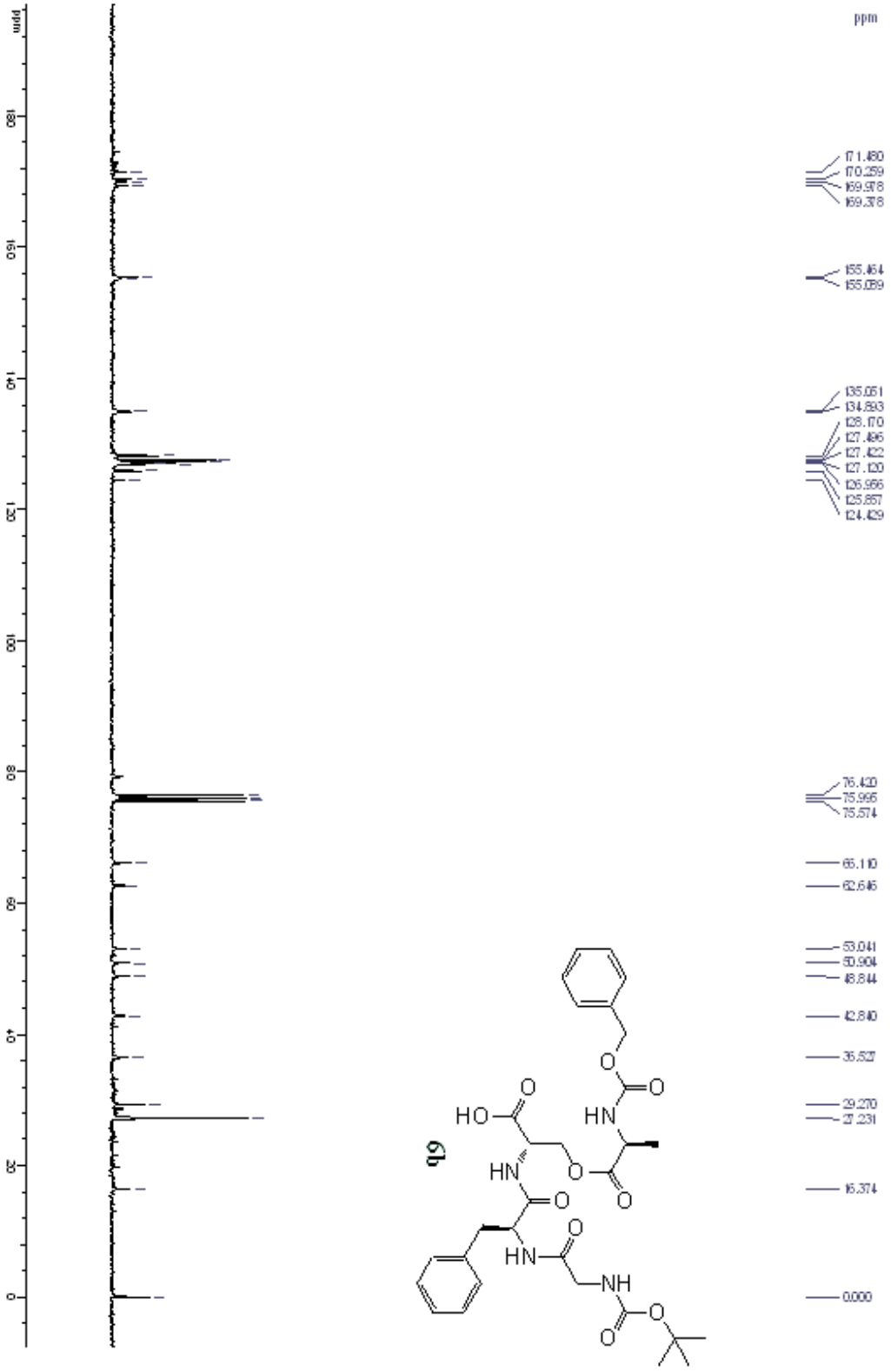








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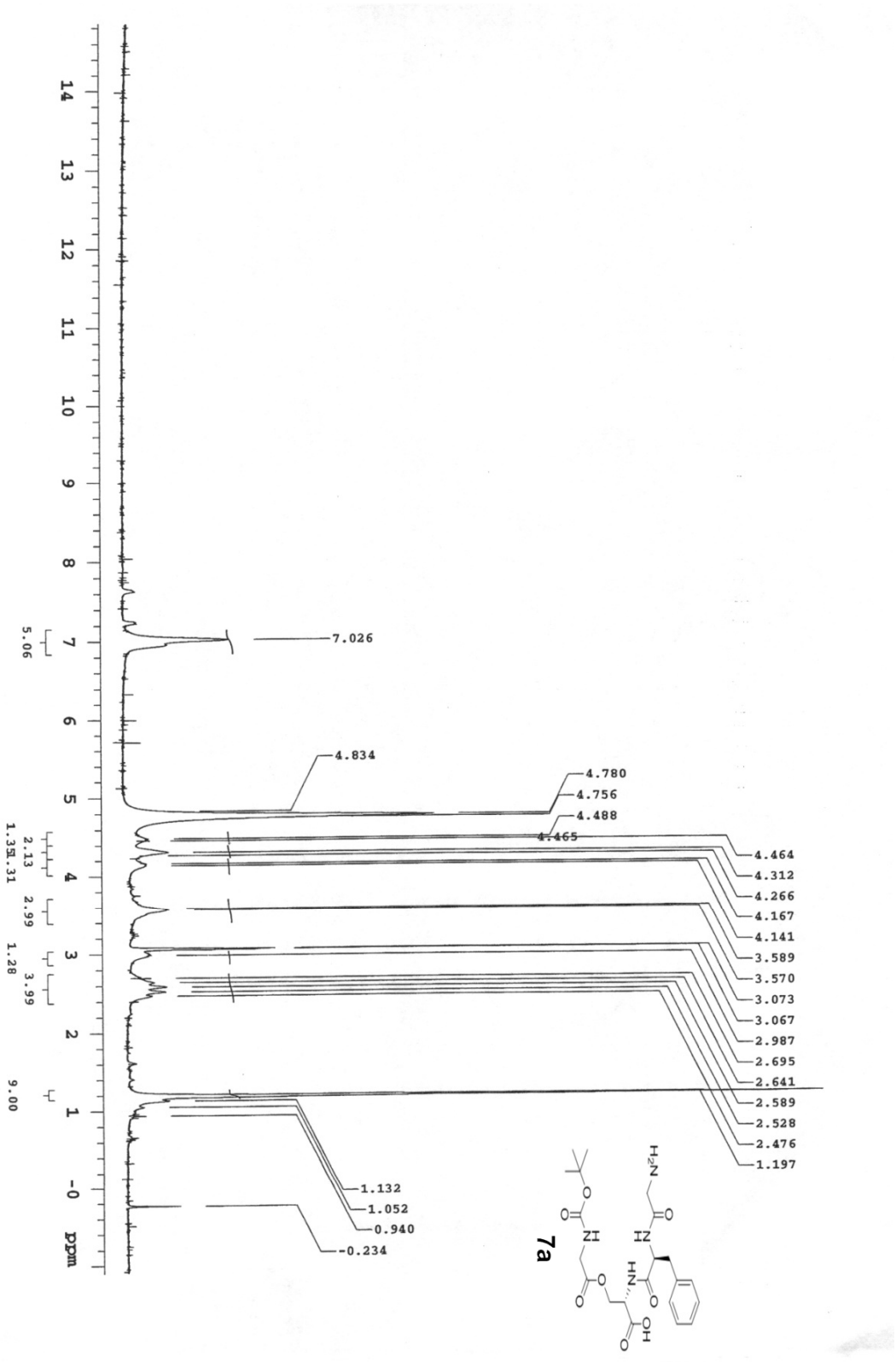
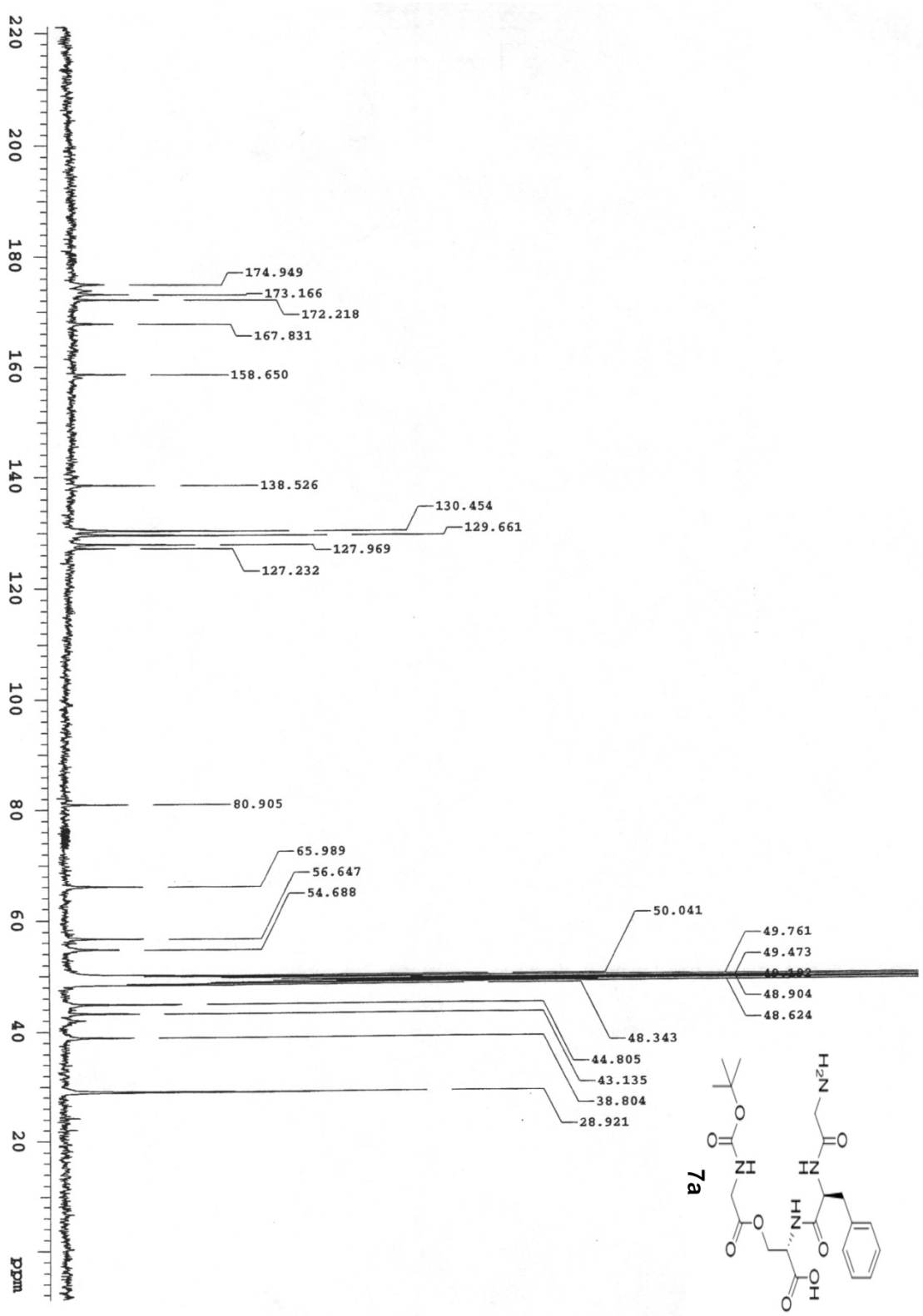
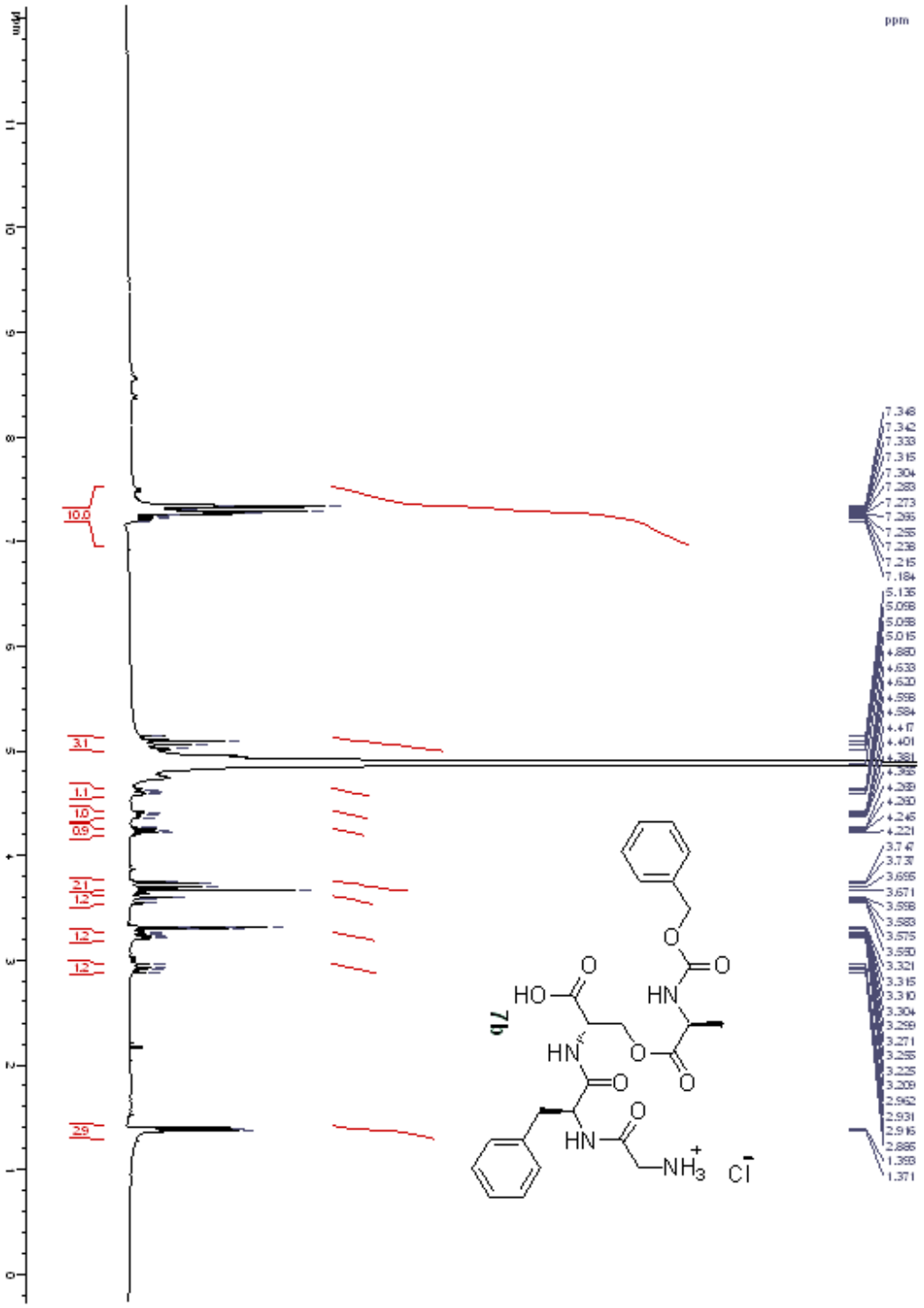
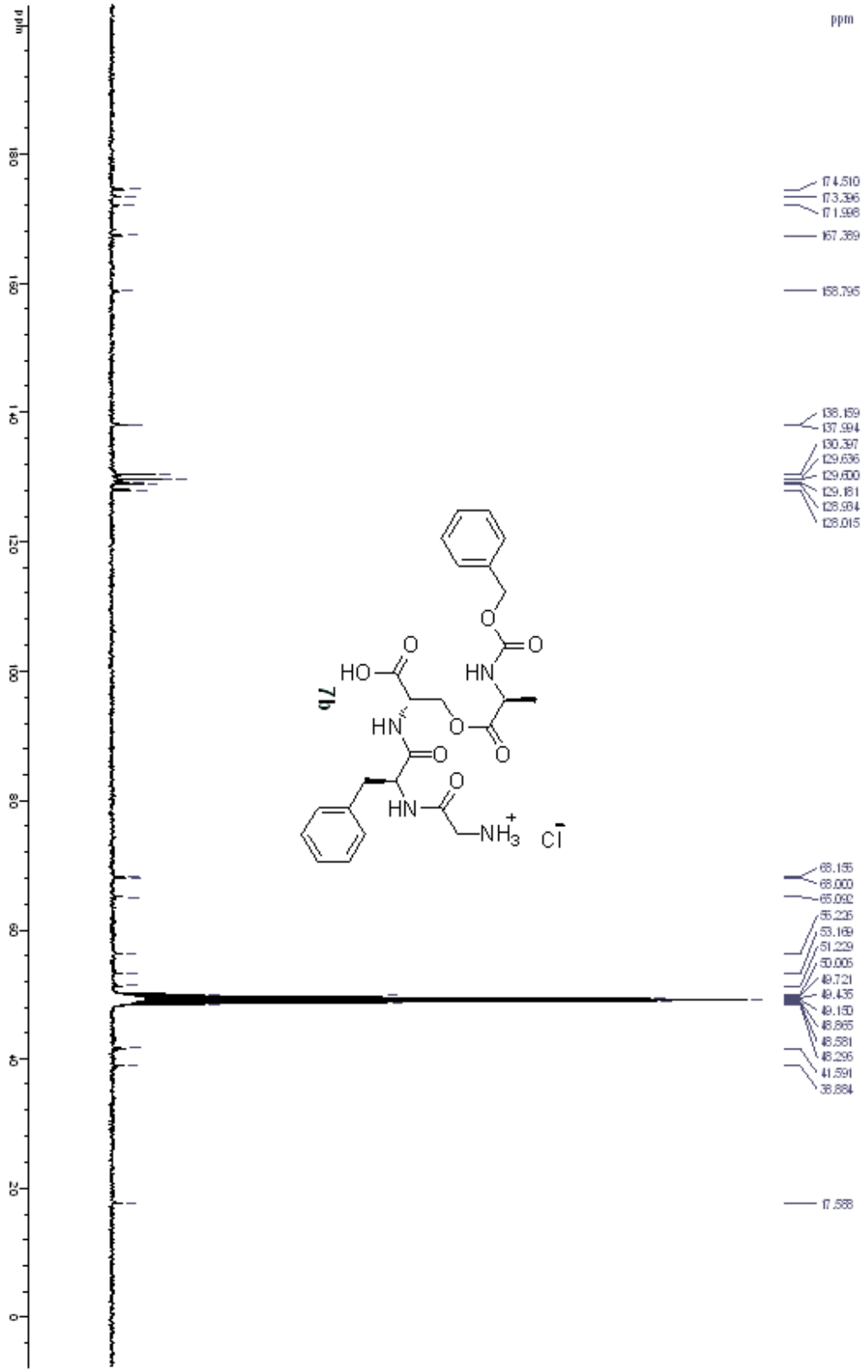
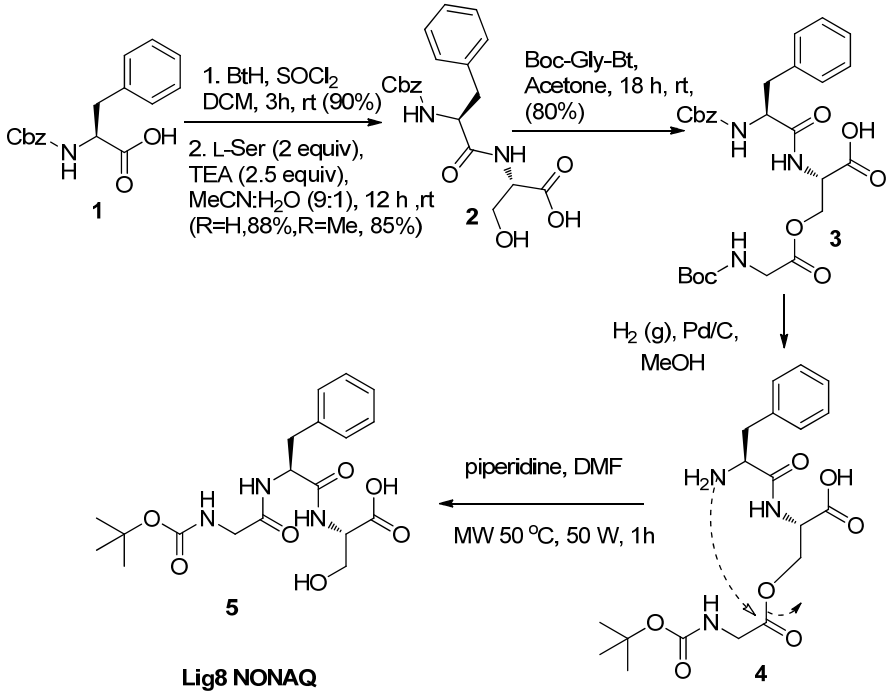








HPLC chromatograms and MS Spectra for 4a and 5a



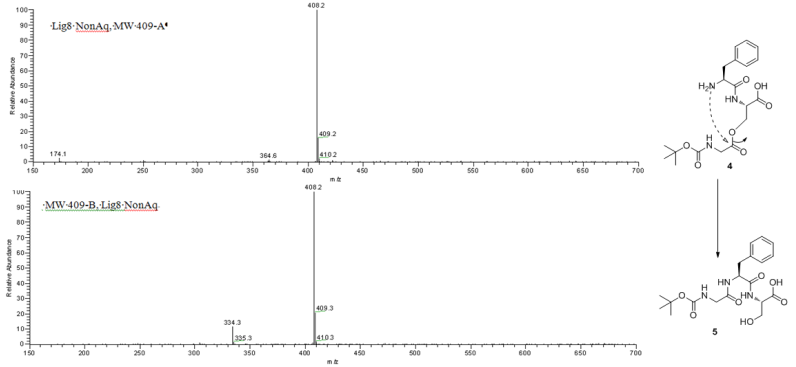


Figure S1. Lig8 NonAq, MW 409-A

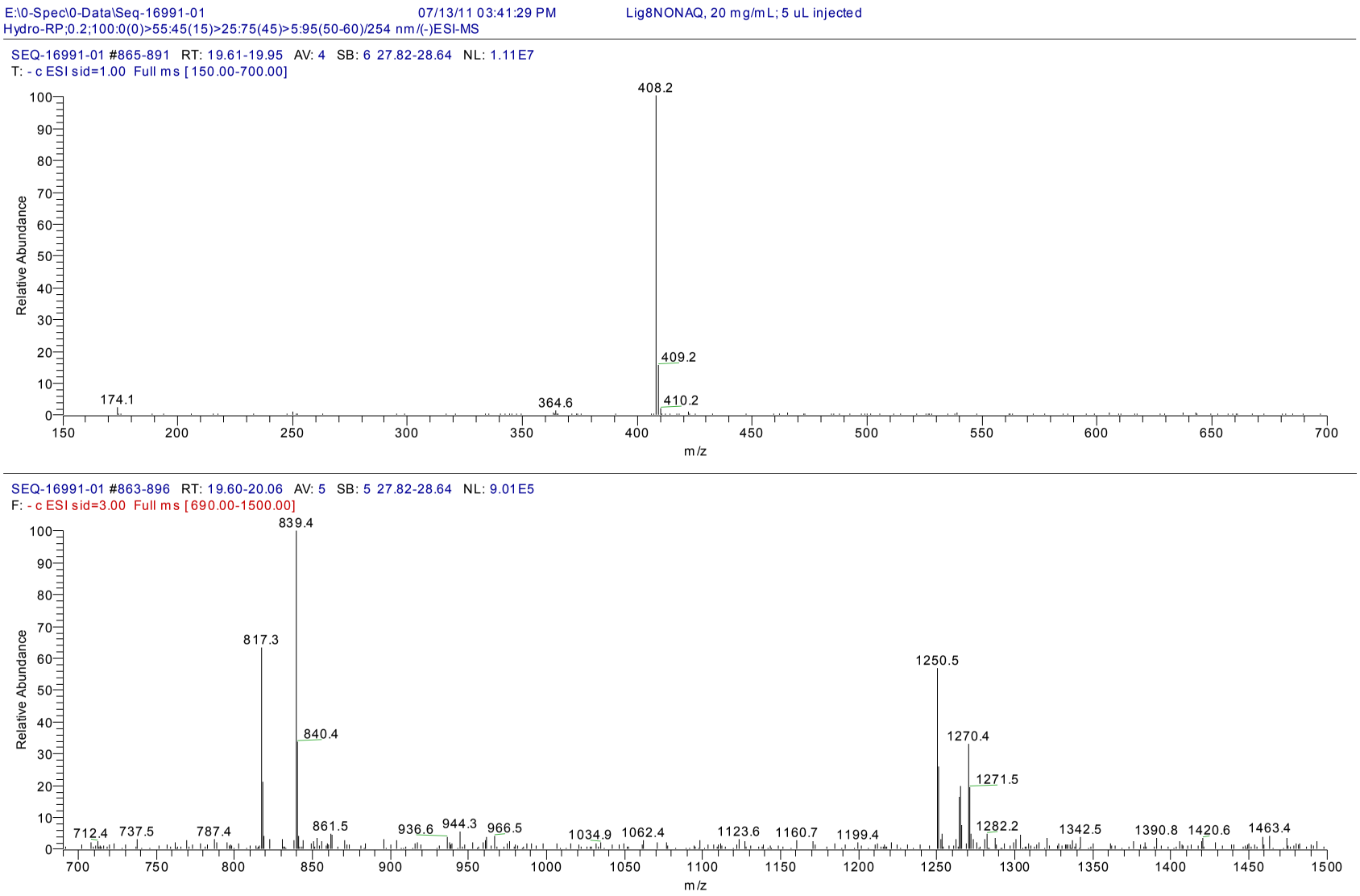


Figure S2. MW 409-B, Lig8 NonAq

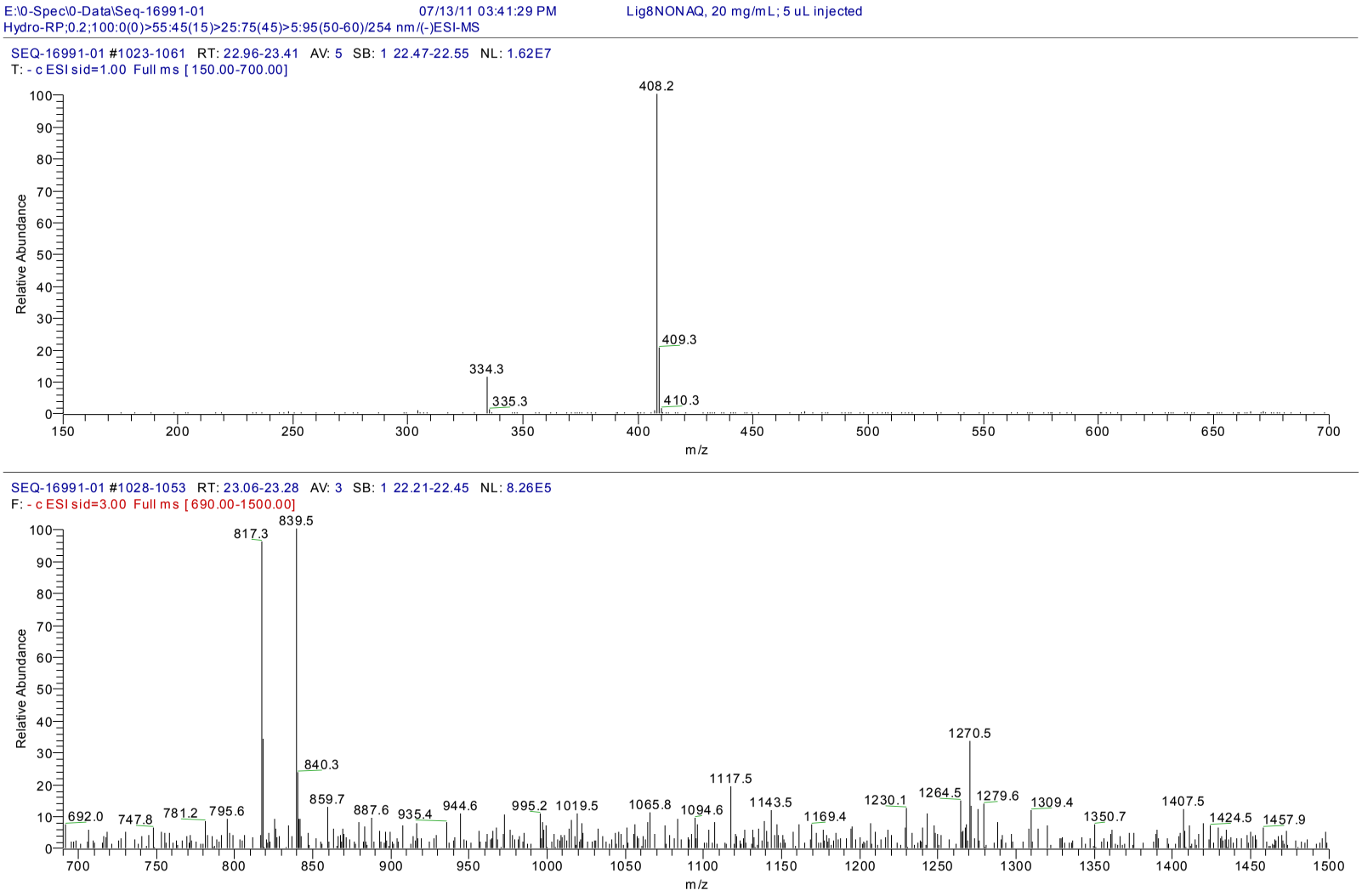


Figure S3. Lig8 NonAq: HPLC/UV/(-)ESI-MS and –MSn chromatograms for the MW 409 compounds

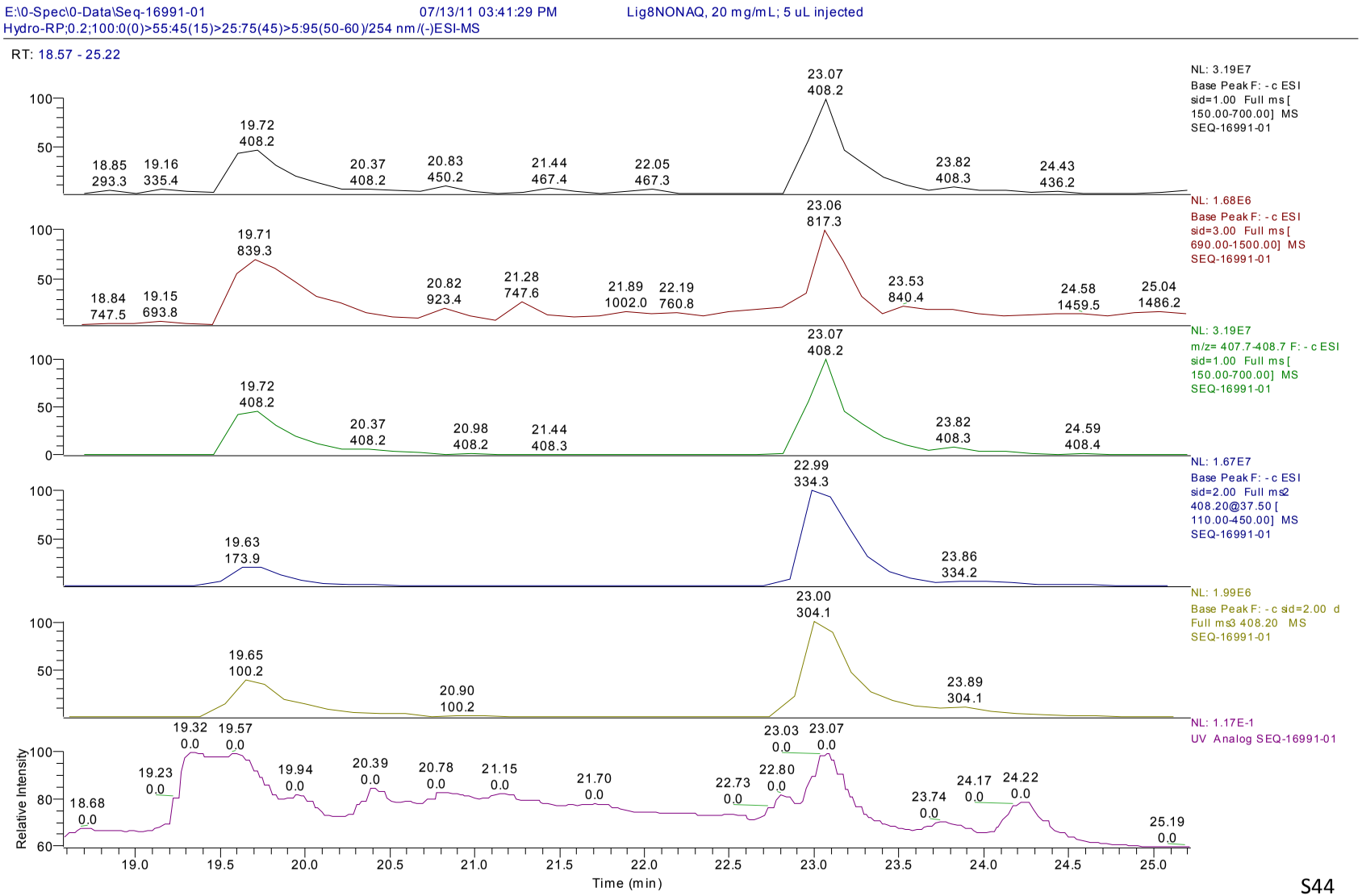


Figure S4. MW 409 compounds: (-)ESI-MS/MS (top and 3rd) and –MS/MS/MS (2nd and bottom) for the MW 409-A (RT 19.72 min) and MW 409-B (RT 23.07 min)

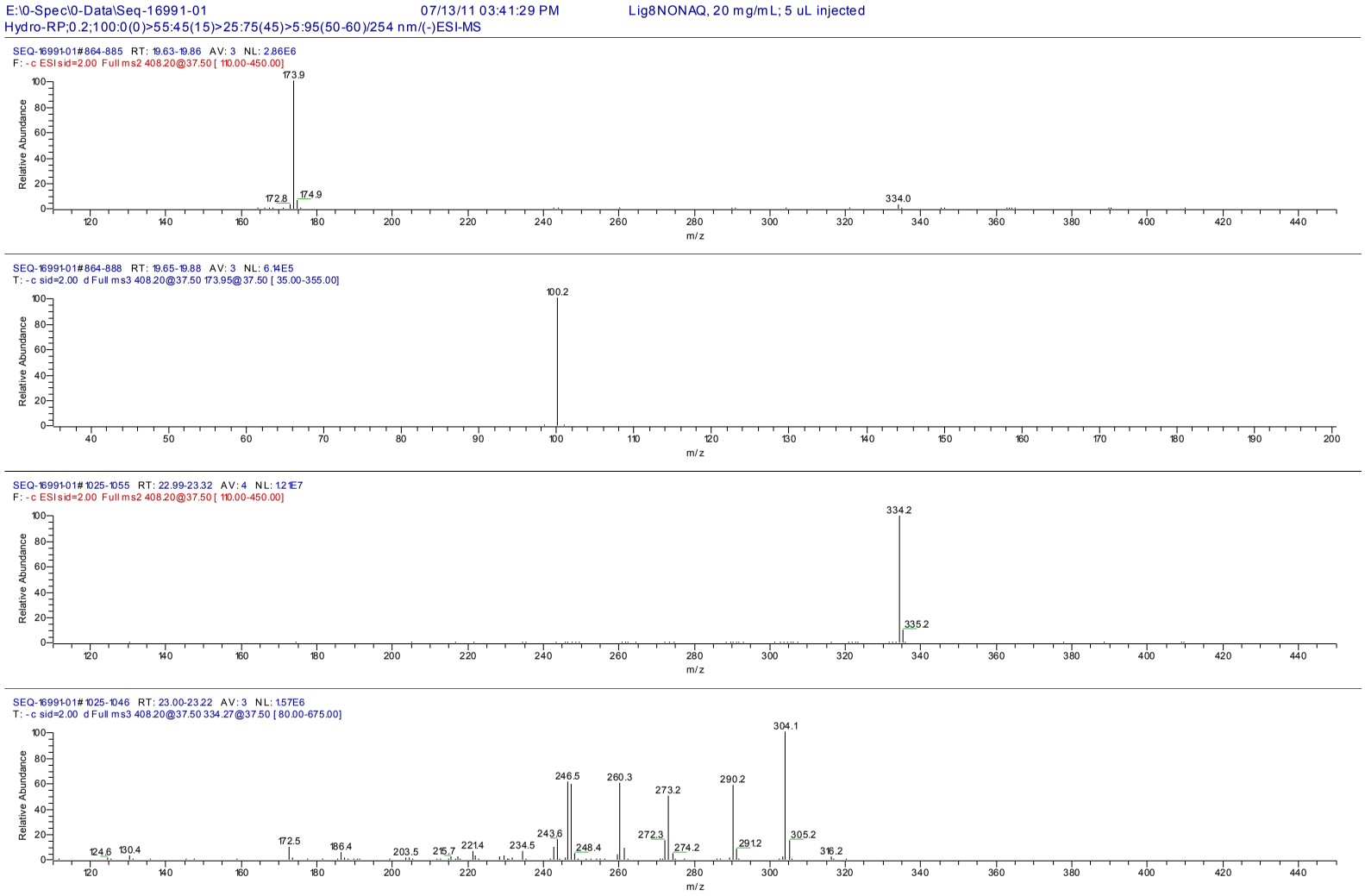
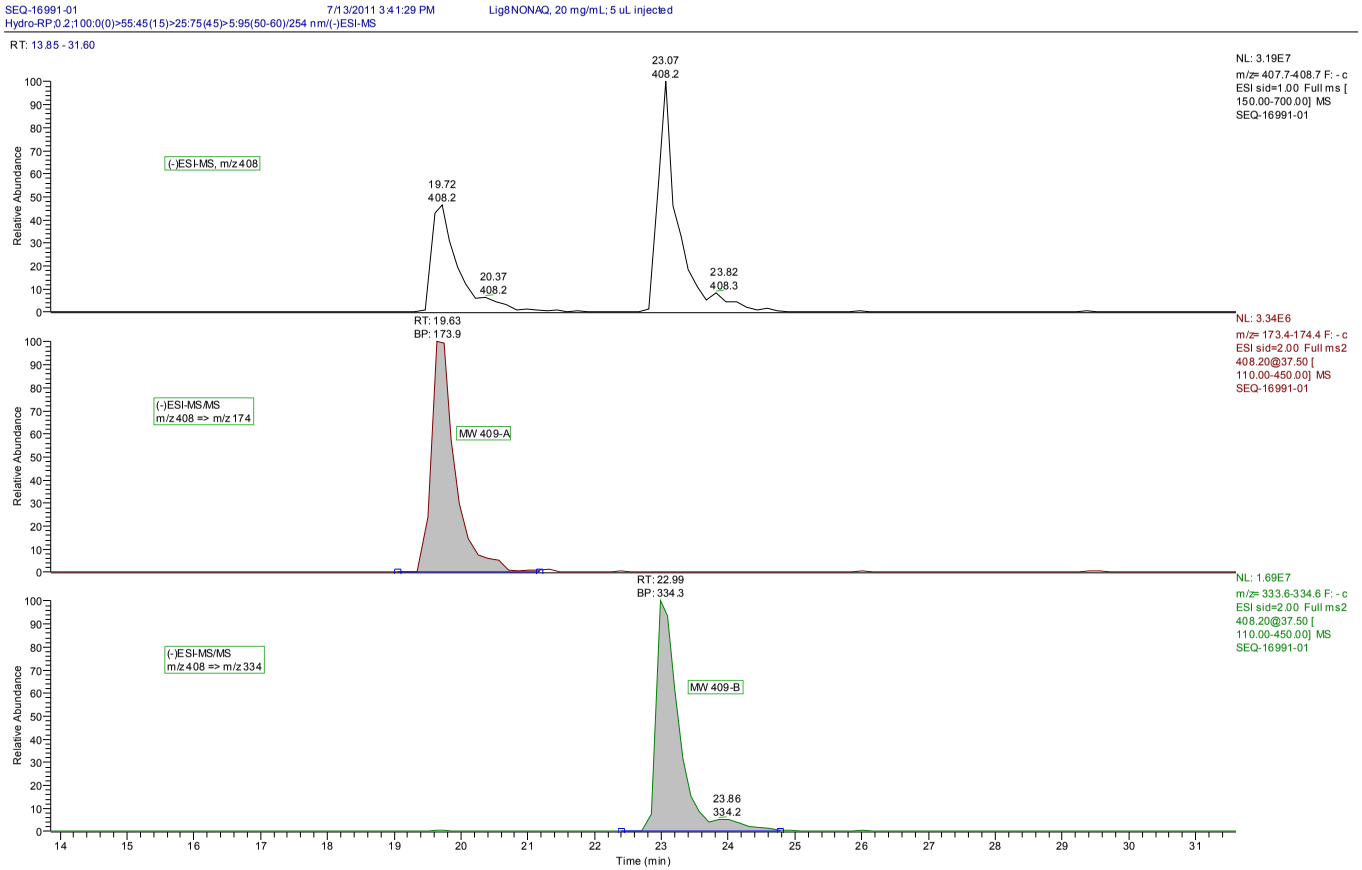


Figure S5. Lig8 NonAq: MW 409 isomers. The two MW 409 isomers were well separated and were characterized relatively uniquely by their (-)ESI-MS/MS product ions as shown below. The MW 409-A was the starting material while MW 409-B was



product. Scheme 1 shows the likely formation of the two product ions below

Scheme S1. The two MW 409 compounds are characterized by the formation of different major products ions from the (-)ESI-MS/MS collision-induced dissociation (CID) of their m/z 408 [M-H]- ions.

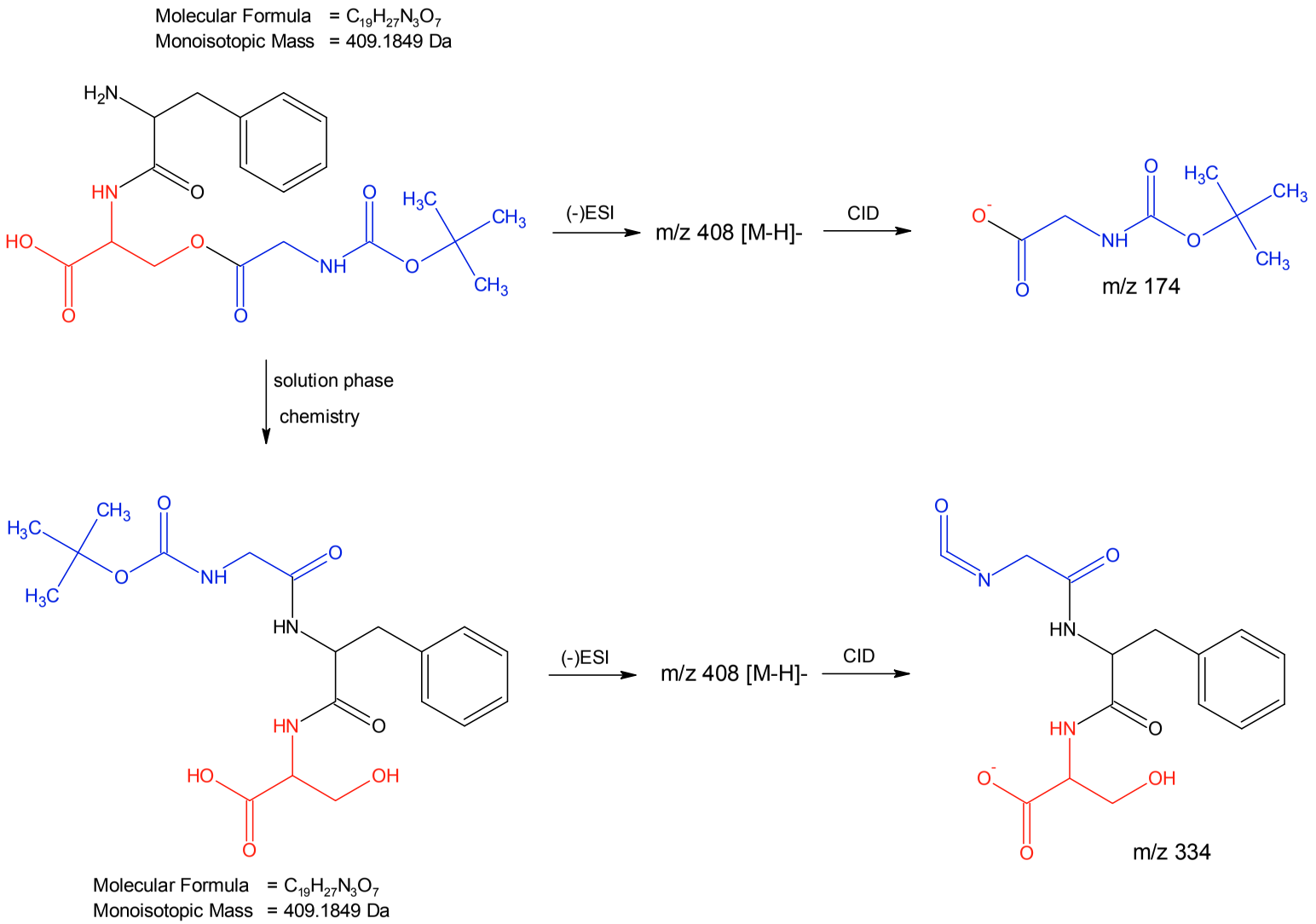
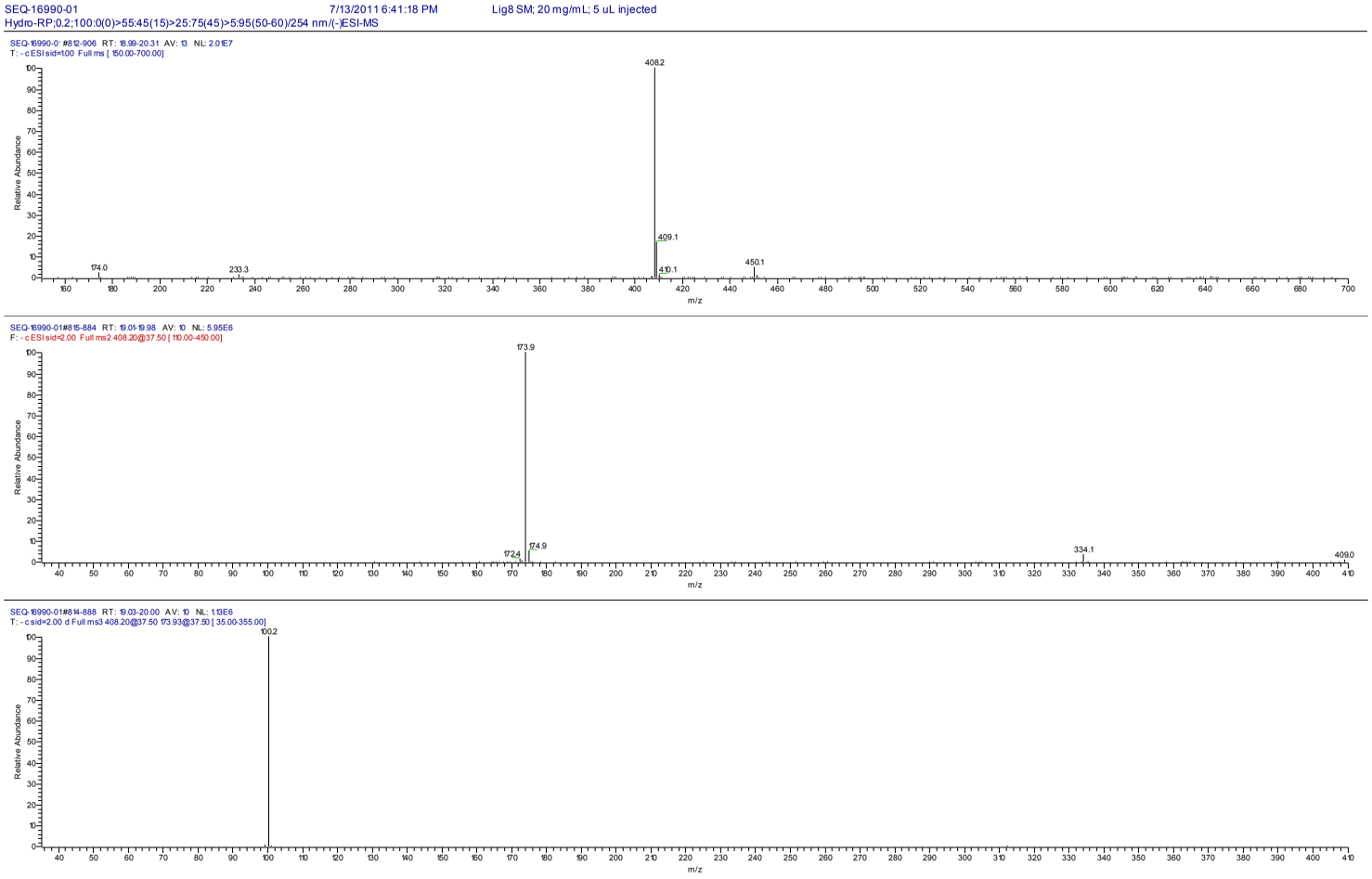


Figure S6. Lig8 SM, MW 409, RT 19.20 min: With (-)ESI-MS, the MW 409 produced an m/z 408 [M-H]- ion (top) which was dissociated to m/z 174 (middle) which was further dissociated to m/z 100 (bottom). The RT and MSn spectra match those of the MW 409-A compound of Lig8 NonAq



HPLC chromatograms and MS Spectra for 4c (ME36) and 5c (ME37).

Figure S7. MW 423, Rt 38.4 min: This was the most abundant MS and UV peaks.

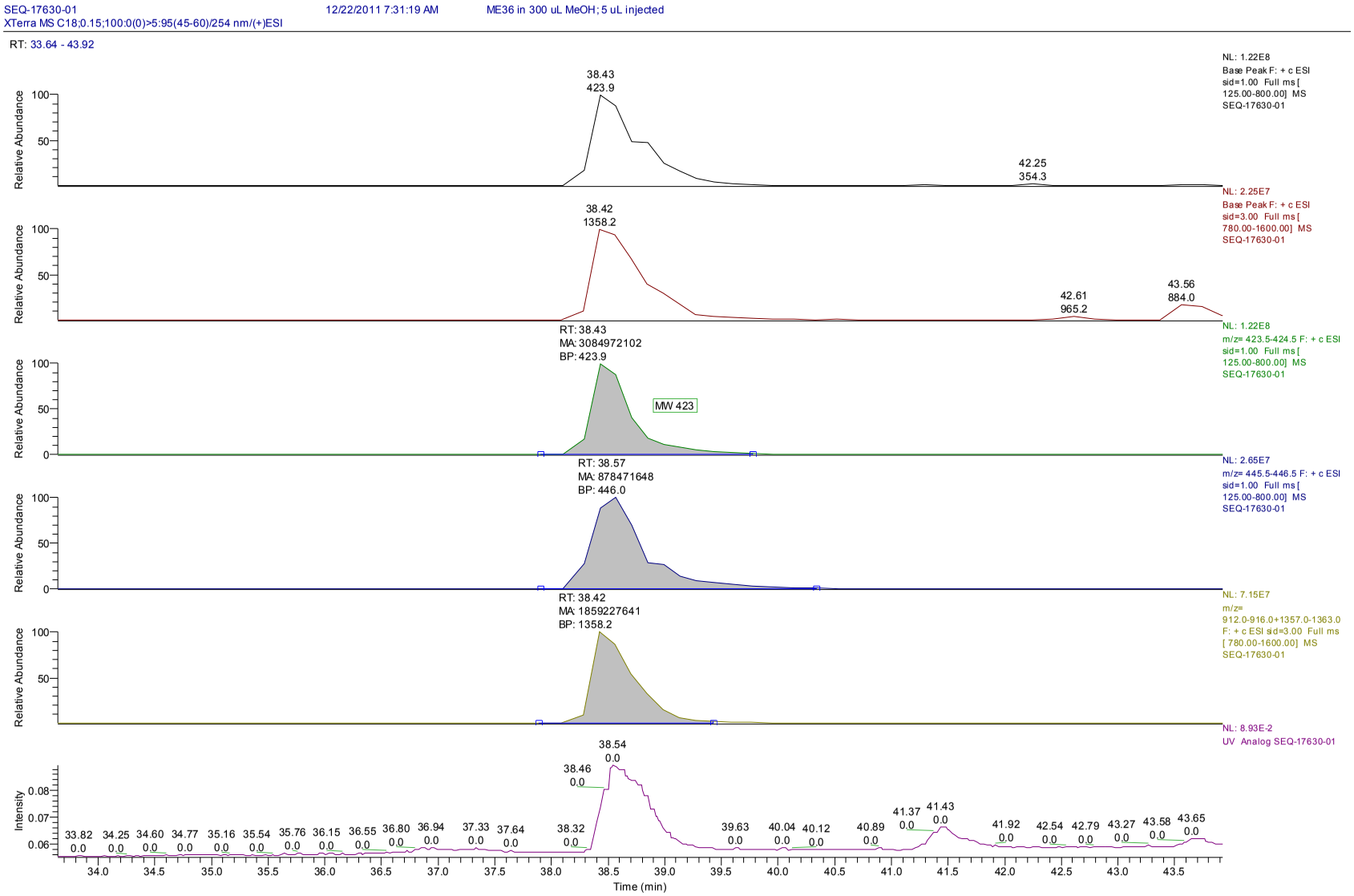


Figure S8. Sample ME36, MW 423. Due to the high concentration, self-adduct ions (m/z 890-958; m/z 1350-1410) were also formed in addition to the m/z 424 [M+H]+ and m/z 445 [M+Na]+. Note the presence of other compounds also, e.g. m/z 438.

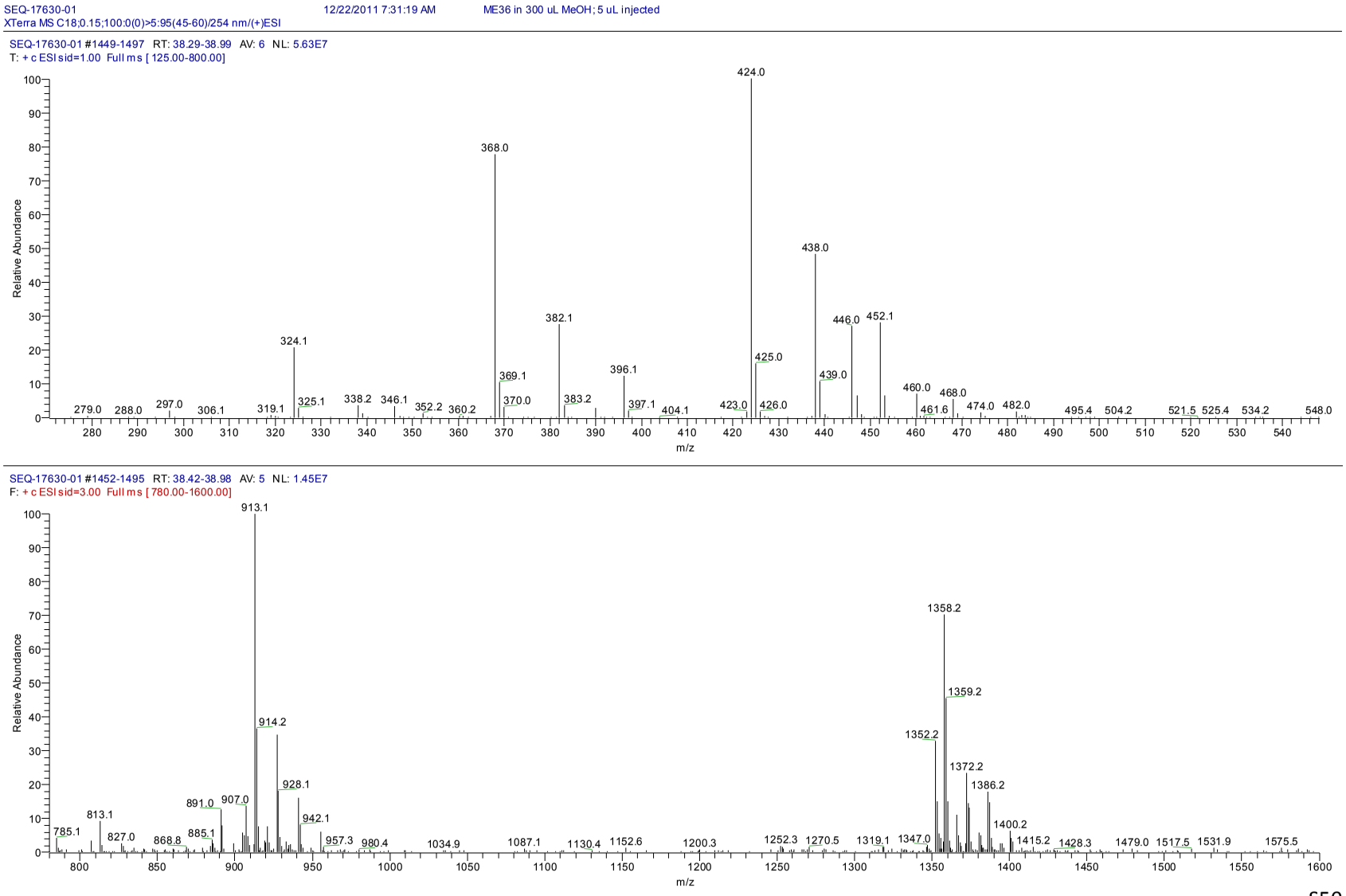


Figure S9. Sample ME36, MW 423. (+)ESI-MSn.

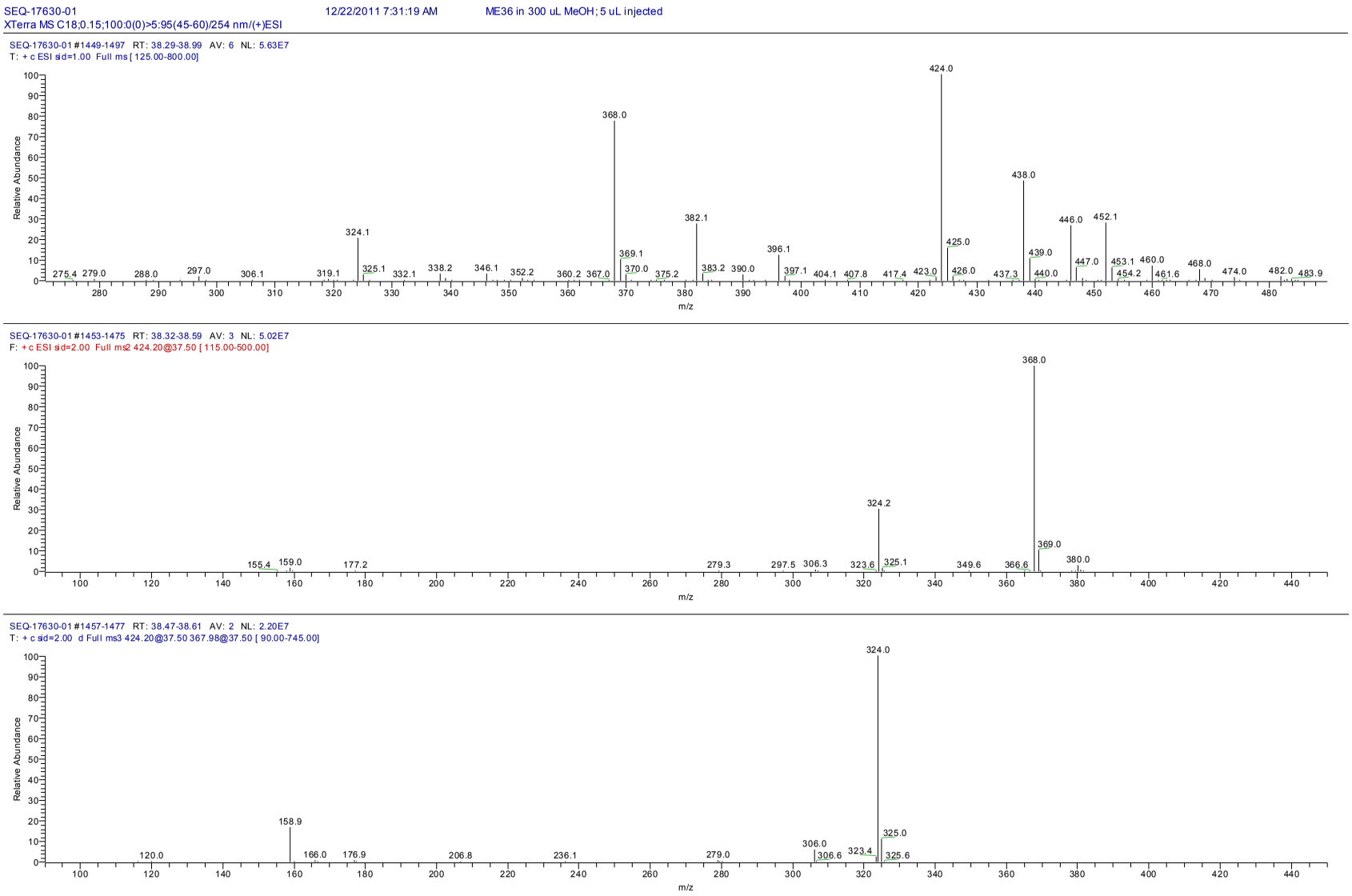


Figure S10. ME37, MW 423 compounds: (+)ESI mass spectra of RT 38.6 min (top) and RT 39.5 min (bottom) The m/z 324 and 368 are fragment ions.

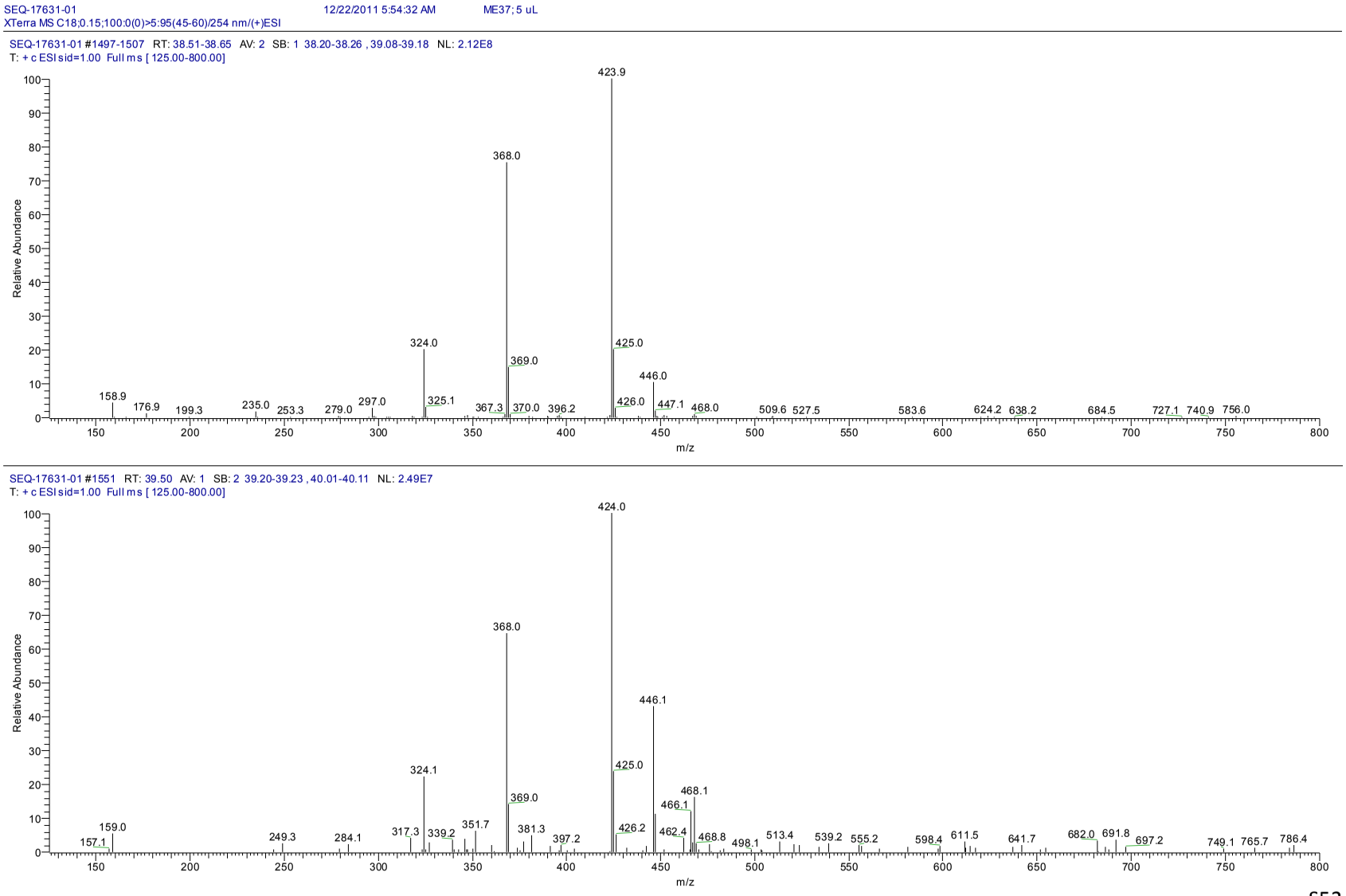


Figure S11. ME37, MW 423 compounds: (+)ESI mass spectra (m/z 780-1600) of RT 38.6 min (top) and RT 39.5 min (bottom)..

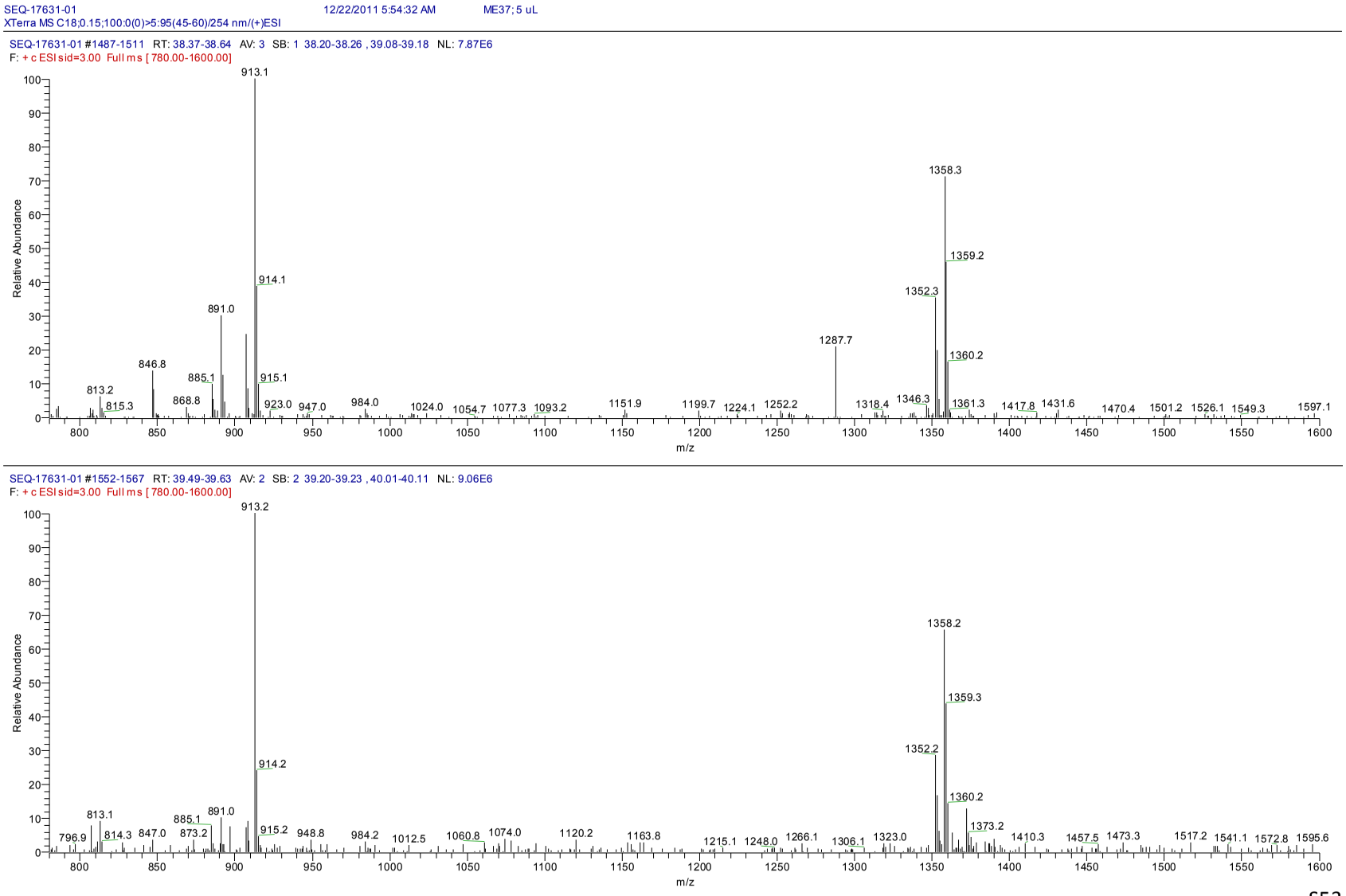


Figure S12. MW 423 RT 38.5 min: (+)ESI-MS/MS of the m/z 424 [M+H]+ ions from ME37 (top) and ME 36 (bottom) were identical.

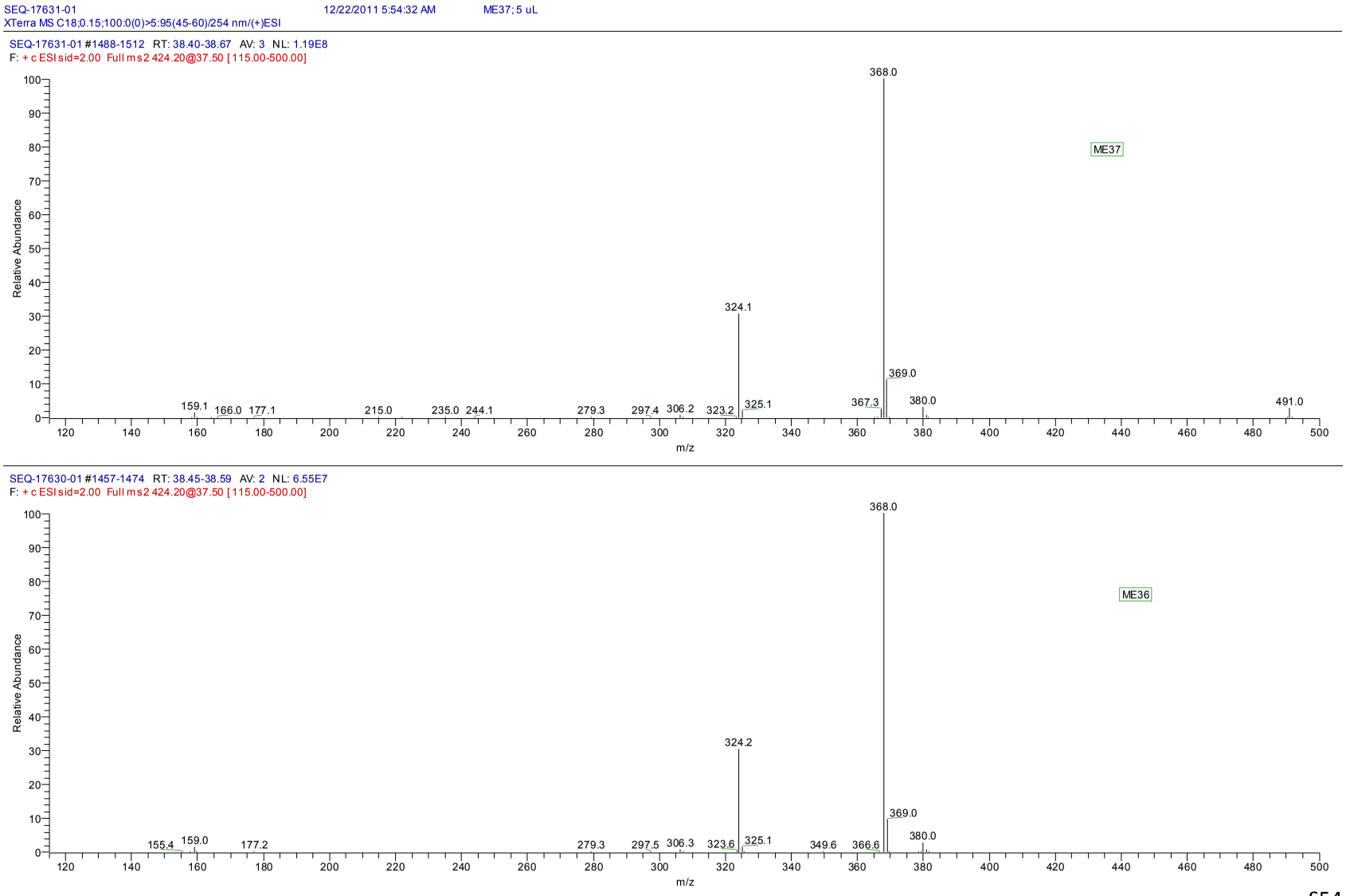


Figure S13. MW 423 compounds in ME37. (+)ESI-MS/MS (top and 3rd) and –MS/MS/MS (2nd and 4th) for the 38.6 min (top 2) and RT 39.5 min (bottom 2) MW 423 compounds. The corresponding spectra are near identical.

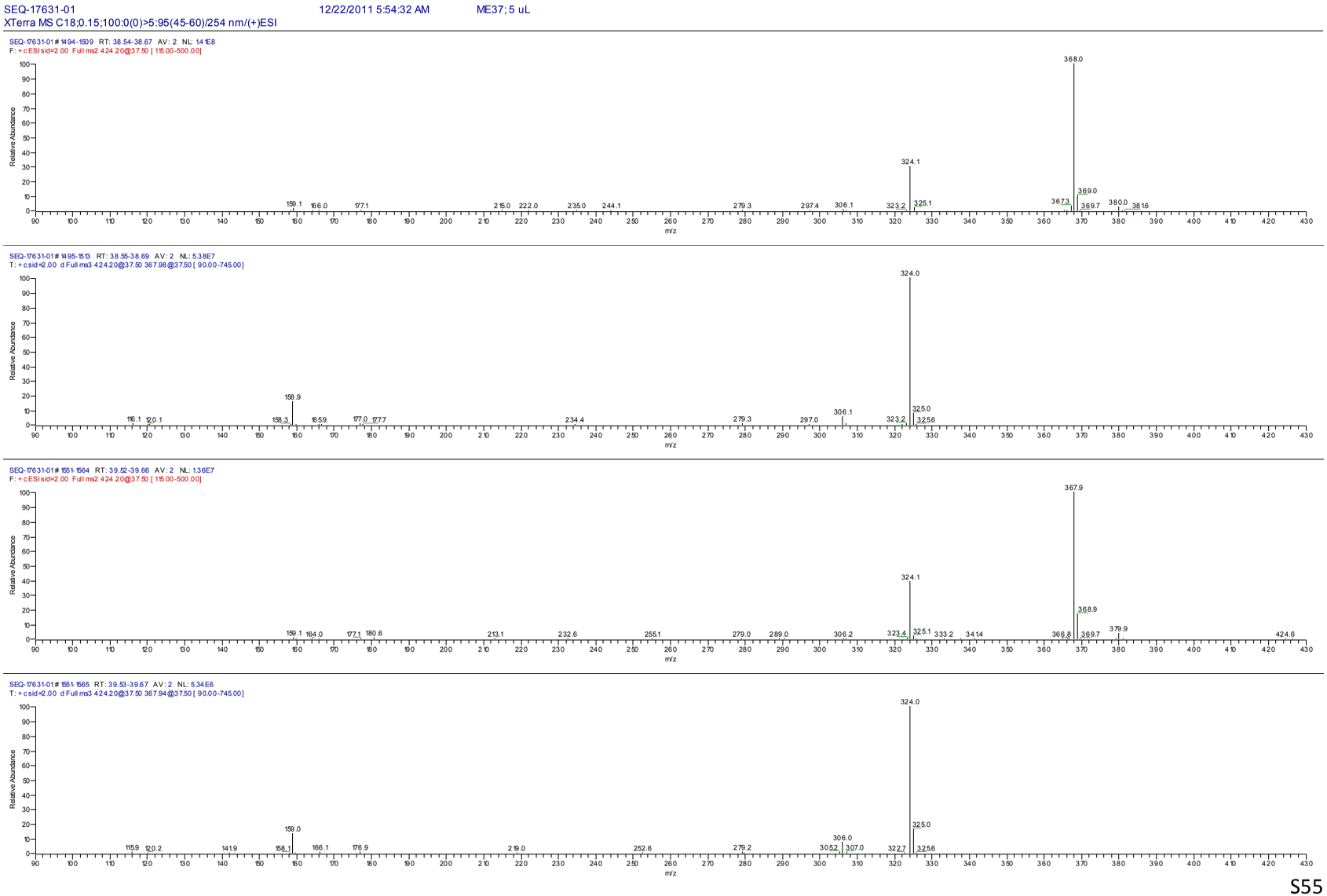


Figure S14. MW 423 compounds, integrated peak areas. The corresponding areas should be summed.

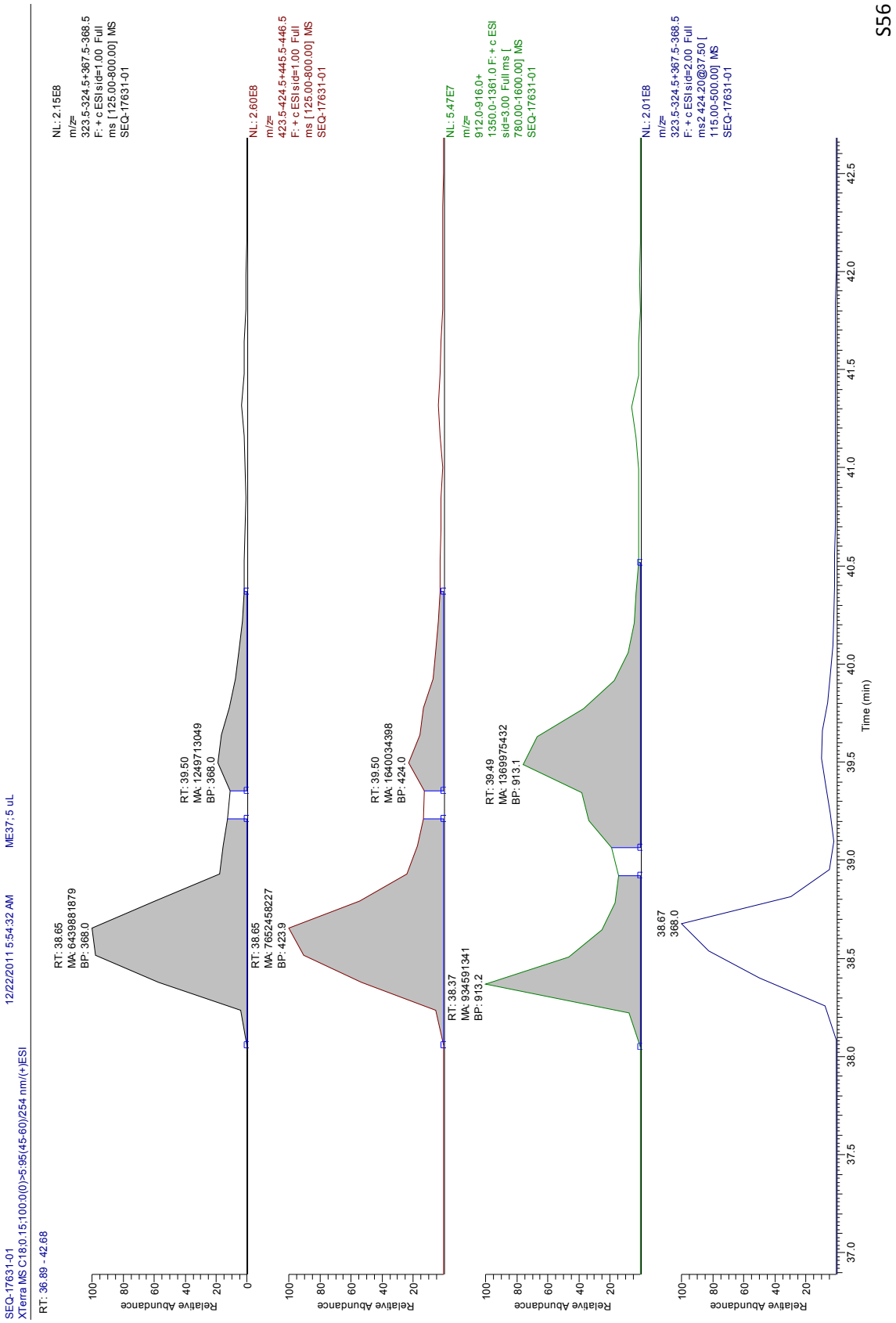


Figure S15. MW 176: This compound was not retained on the HPLC column and eluted at RT 2.8 min. Due to its ion-chemistry and abundance, it produced mostly self-adduct ions. Note also that it produced m/z 221 [M-H+Na+Na]+ and the neutral for adduction was the MW 198 $[M-H+Na]^0$. There also appeared to be CH2 homolog at m/z 235. The adduct ions were likely homo (all MW 198 or all MW 212) and hetero(mixture of the 2 ions).

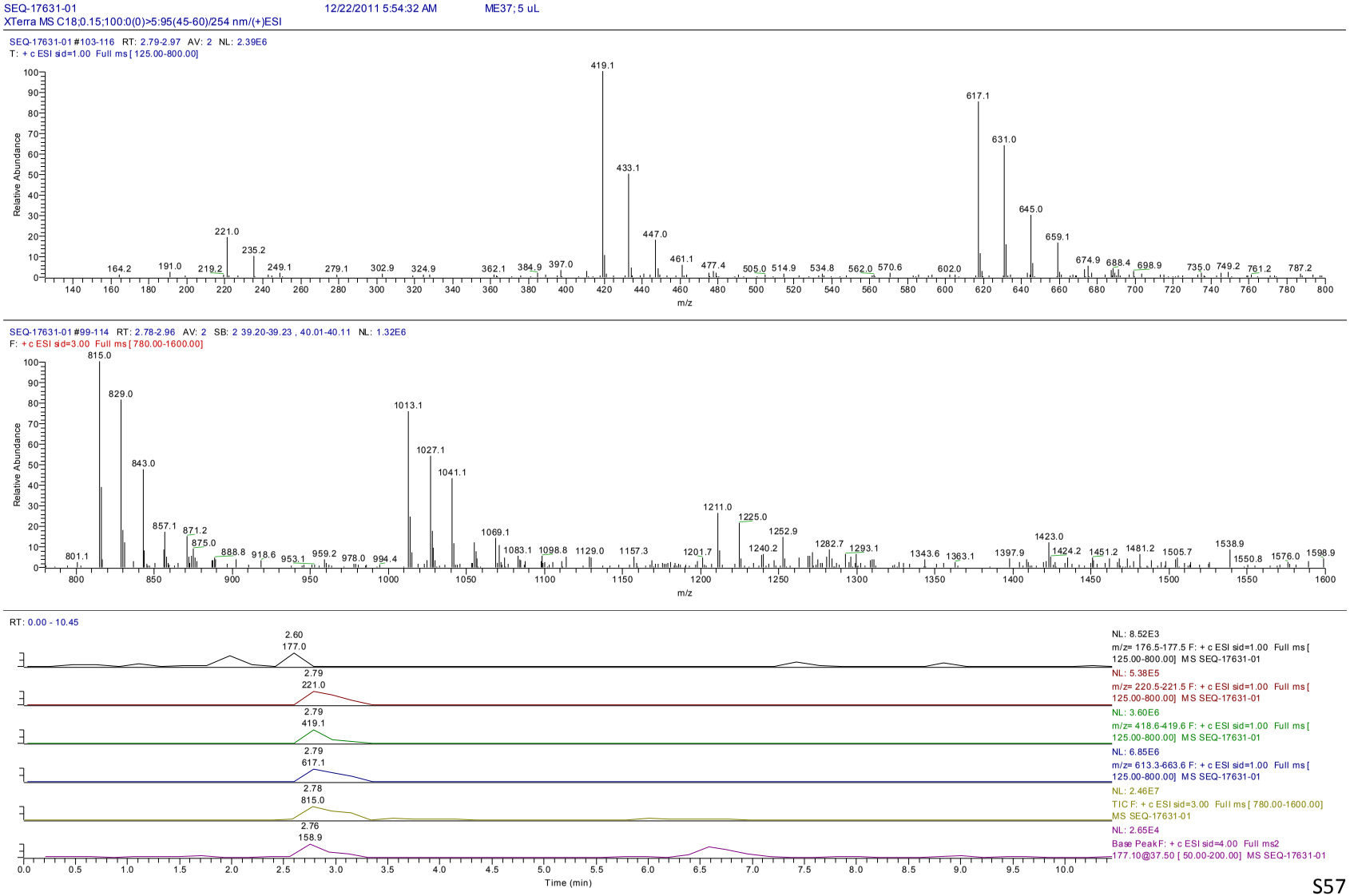


Figure S16. MW 176: (+)ESI-MS (top) and –MS/MS Of m/z 177 (very weak; middle) and of m/z 419 [2(M-H+Na)+Na]+ (bottom).

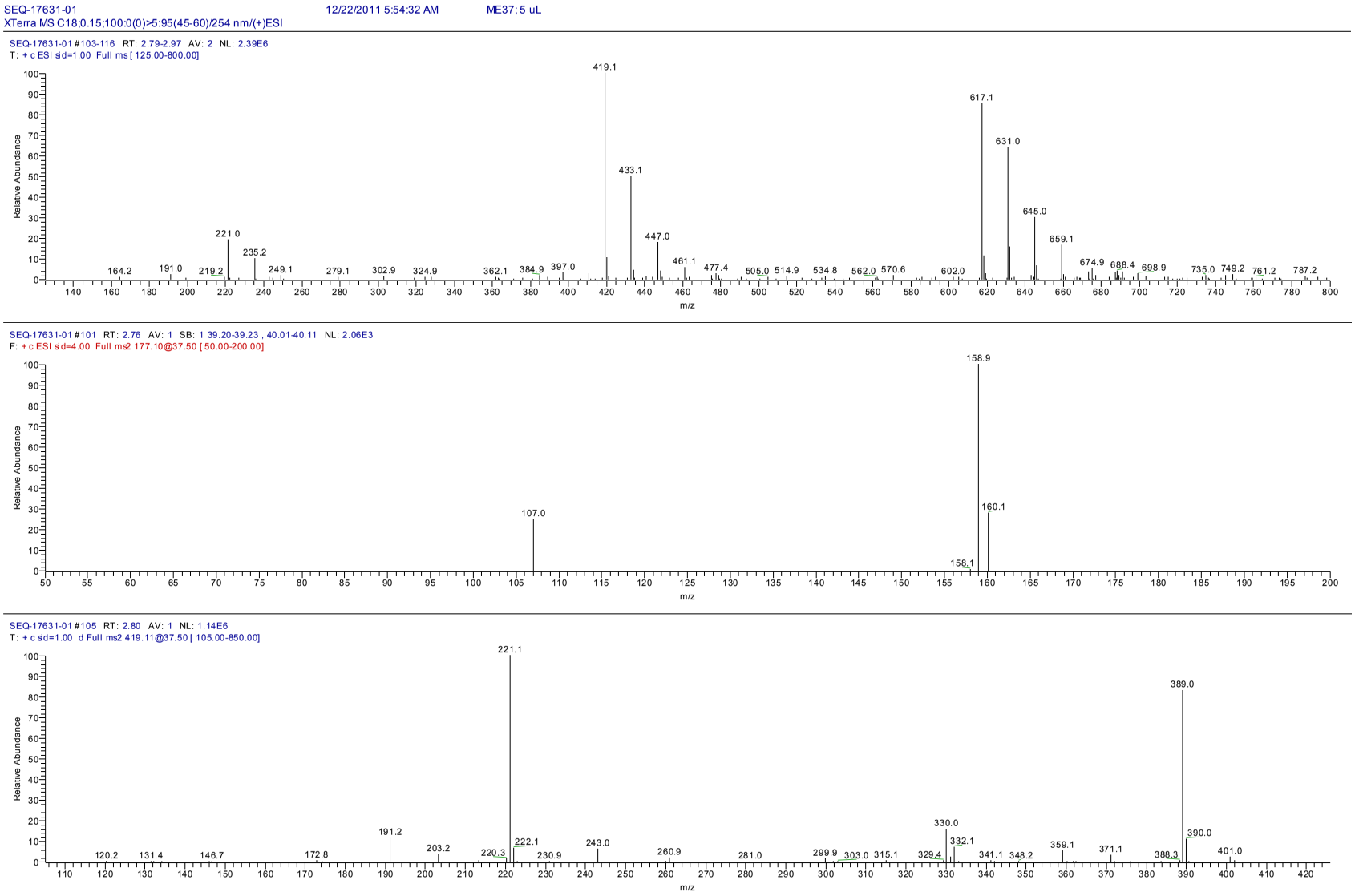


Figure S17. MW 176: HPLC/(+)ESI-MS ion-peaks integrated.

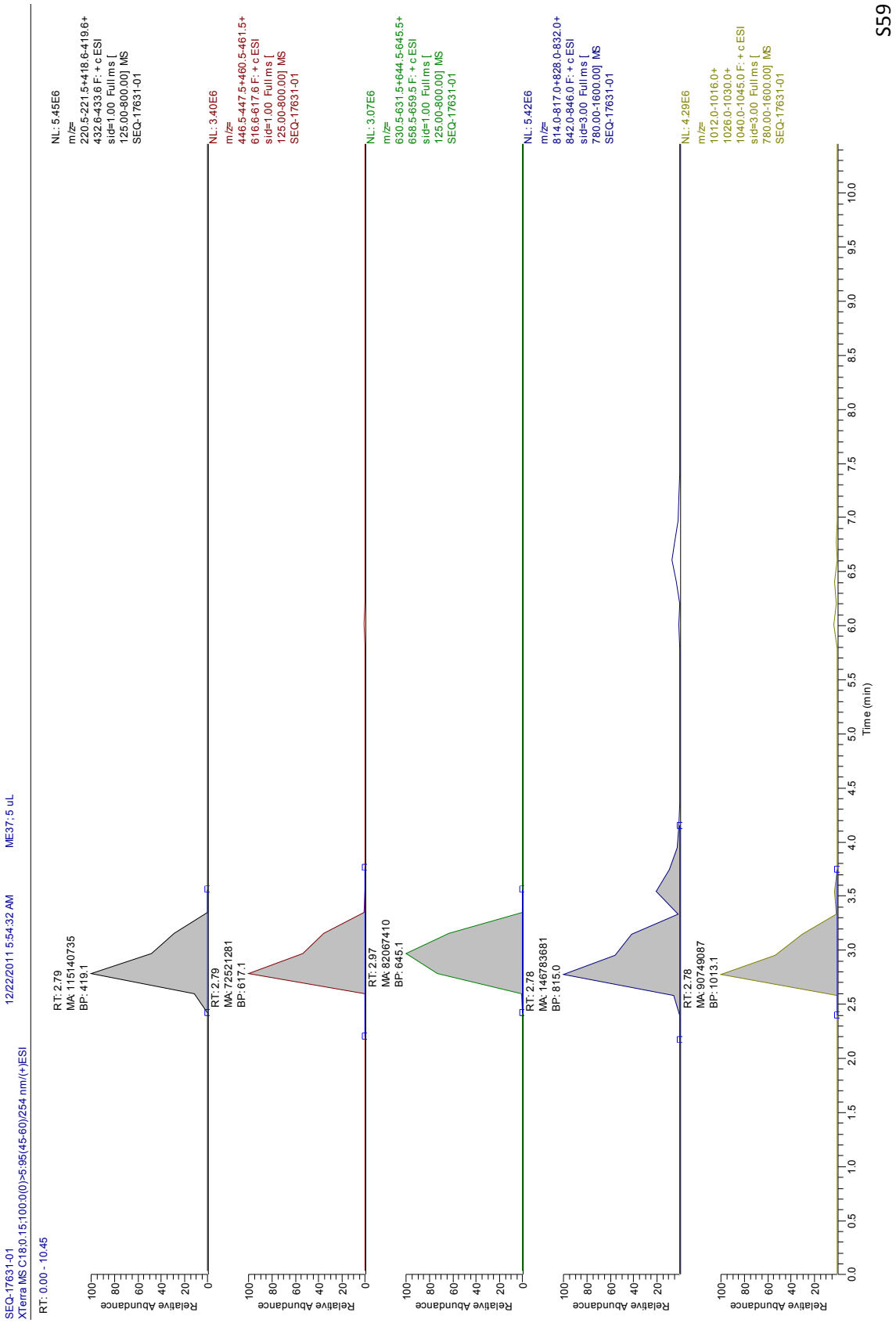
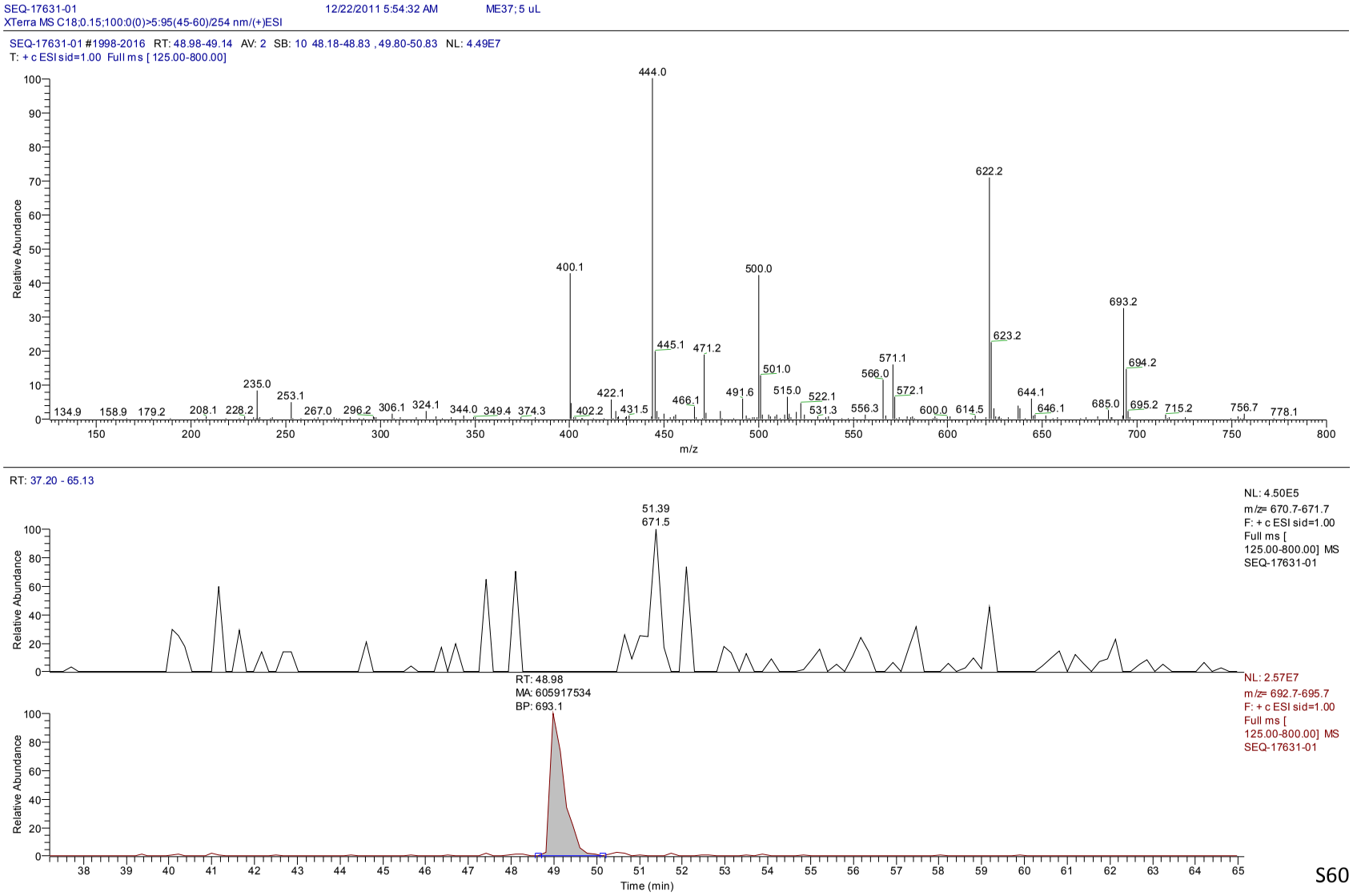
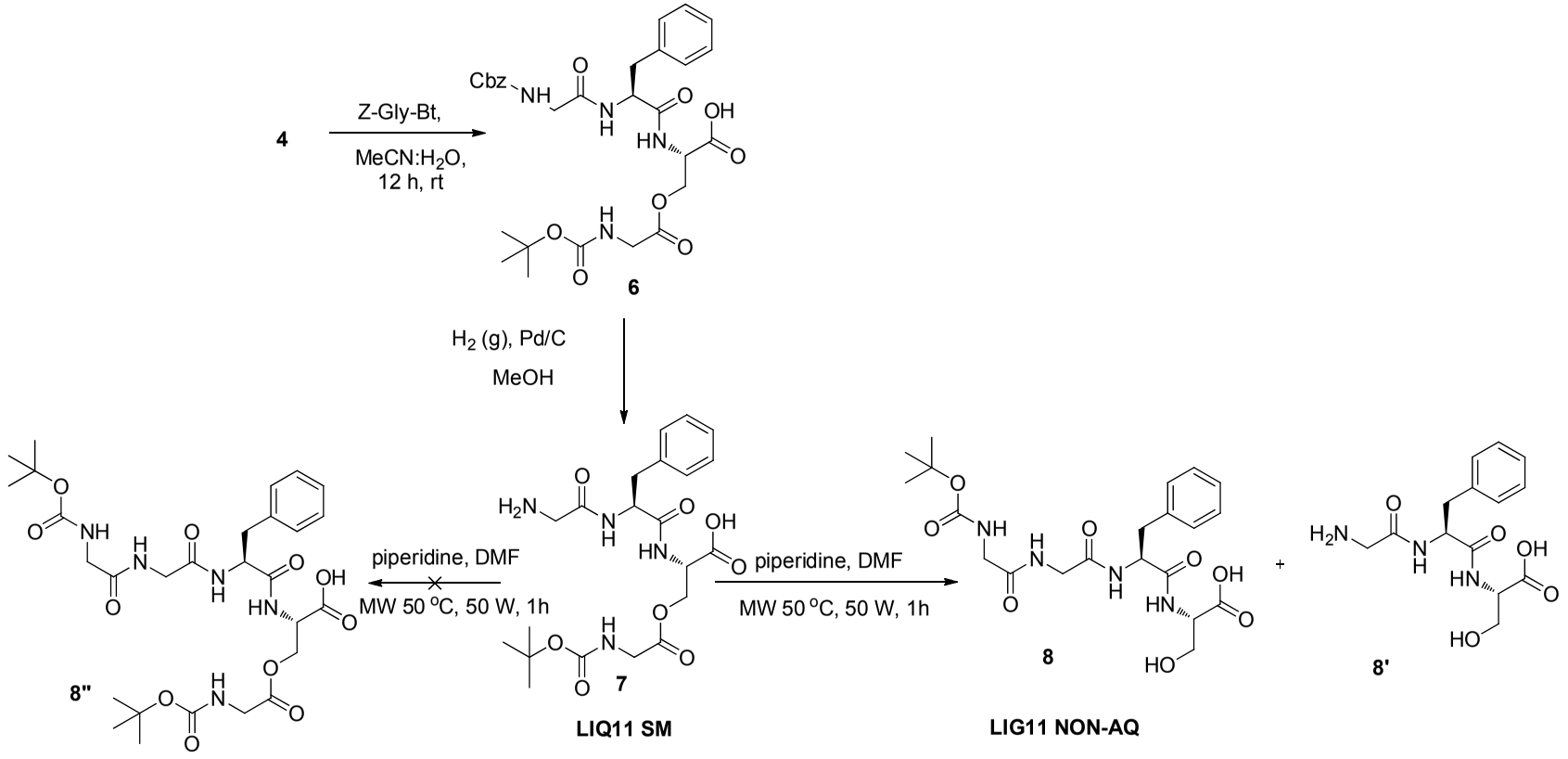


Figure S18. MW 670? The MW 670 should have produced an m/z 671 [M+H]+ and m/z 693 [M+Na]+ ions. There were no correlating ion-peaks for these two ions. There was a very small m/z 671 ion-peak at RT 51.39 but the signal was too low to be conclusive. The (+)ESI mass spectra for the relataively abundant m/z 693 ion-peak at Rt 48.98 does not indicate an m/z 671. The presence of an m/z 715 suggests this is a MW 692 compound. I have integrated the m/z 693 ion-peak.



HPLC chromatograms and MS Spectra for 7a and 8a



LIG11 AQ7.65

The ligation, under aq conditions:
pH 7.6, 1 M buffer strength, MW 50 °C, 50 W, 1 h

Scheme S2. Submitted reaction.

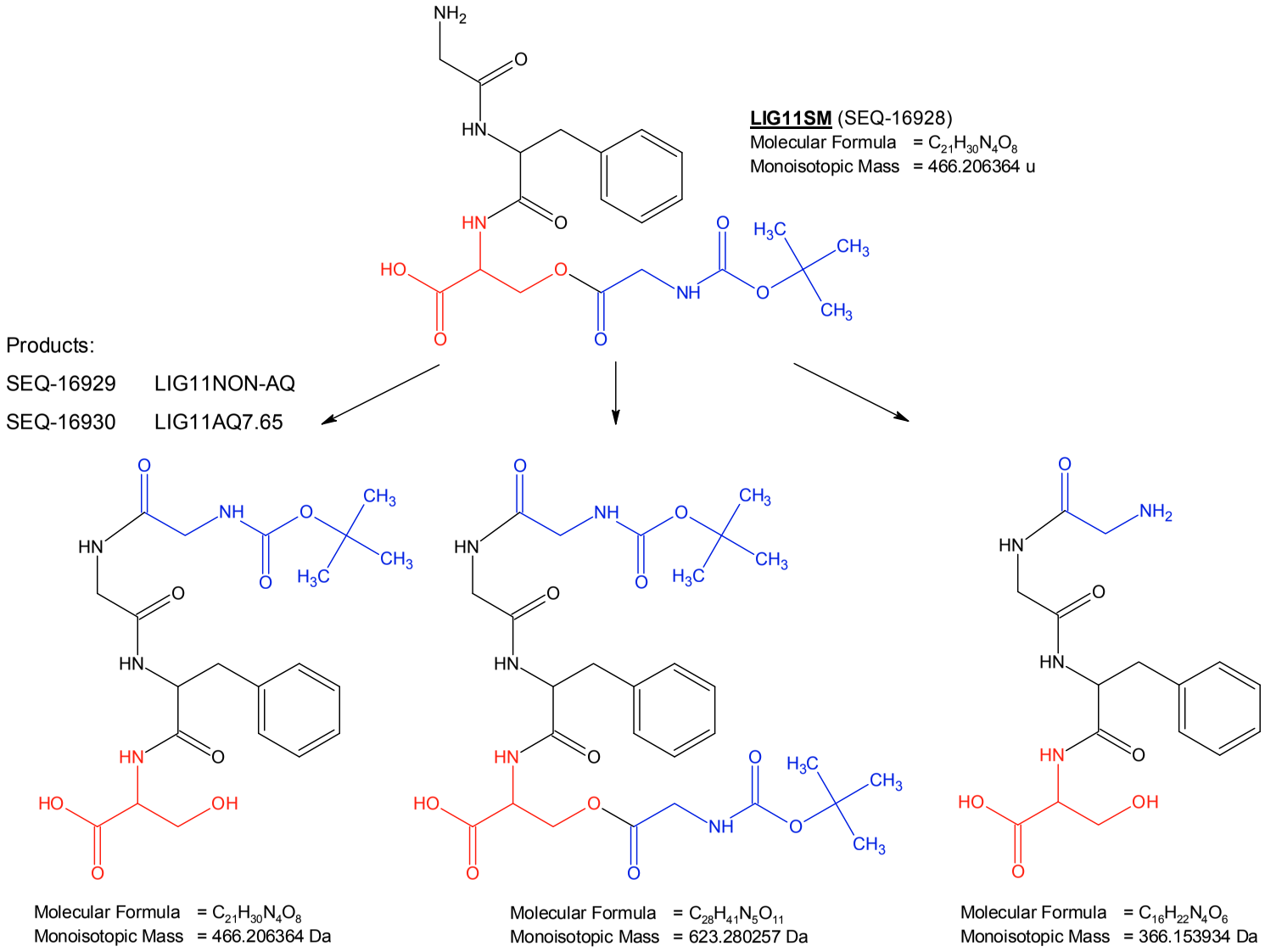


Figure S19. LIG11SM, MW 466, RT 25.46 min. The MW 466 produced m/z 467 [M+H]+, m/z 489 [M+Na]+ and m/z 511 [(M-H+Na)+Na]+ ions (top). The latter is characteristic of compounds with acidic protons, e.g. RCOOH

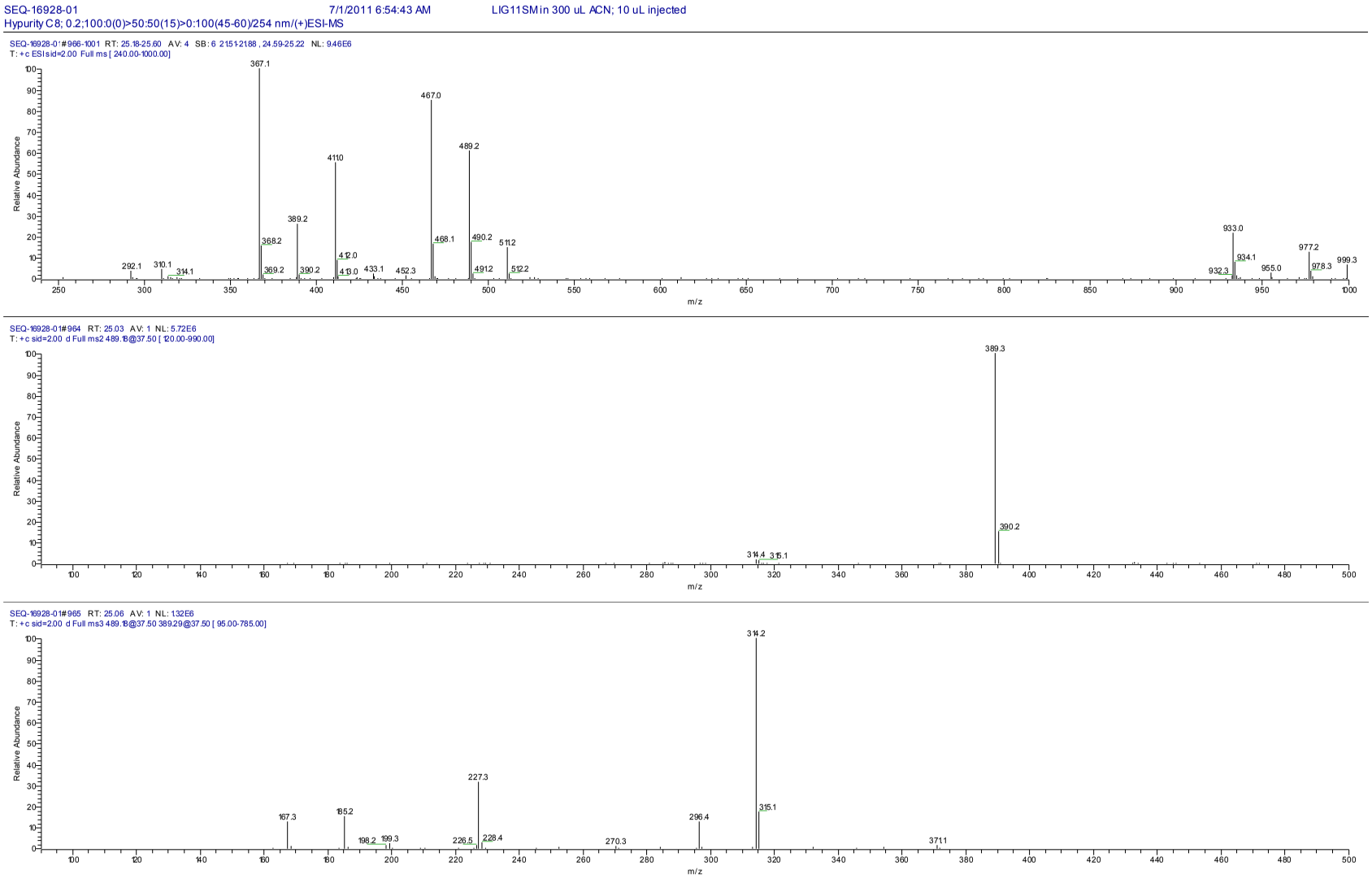


Figure S20. LIG11NONAQ, MW 466, RT 21.67 min. The MW 466 product was expected to produce m/z 467 [M+H]+ and m/z 489 [M+Na]+ ions. The mass chromatograms show only one potential MW 466 compound at RT 21.67.

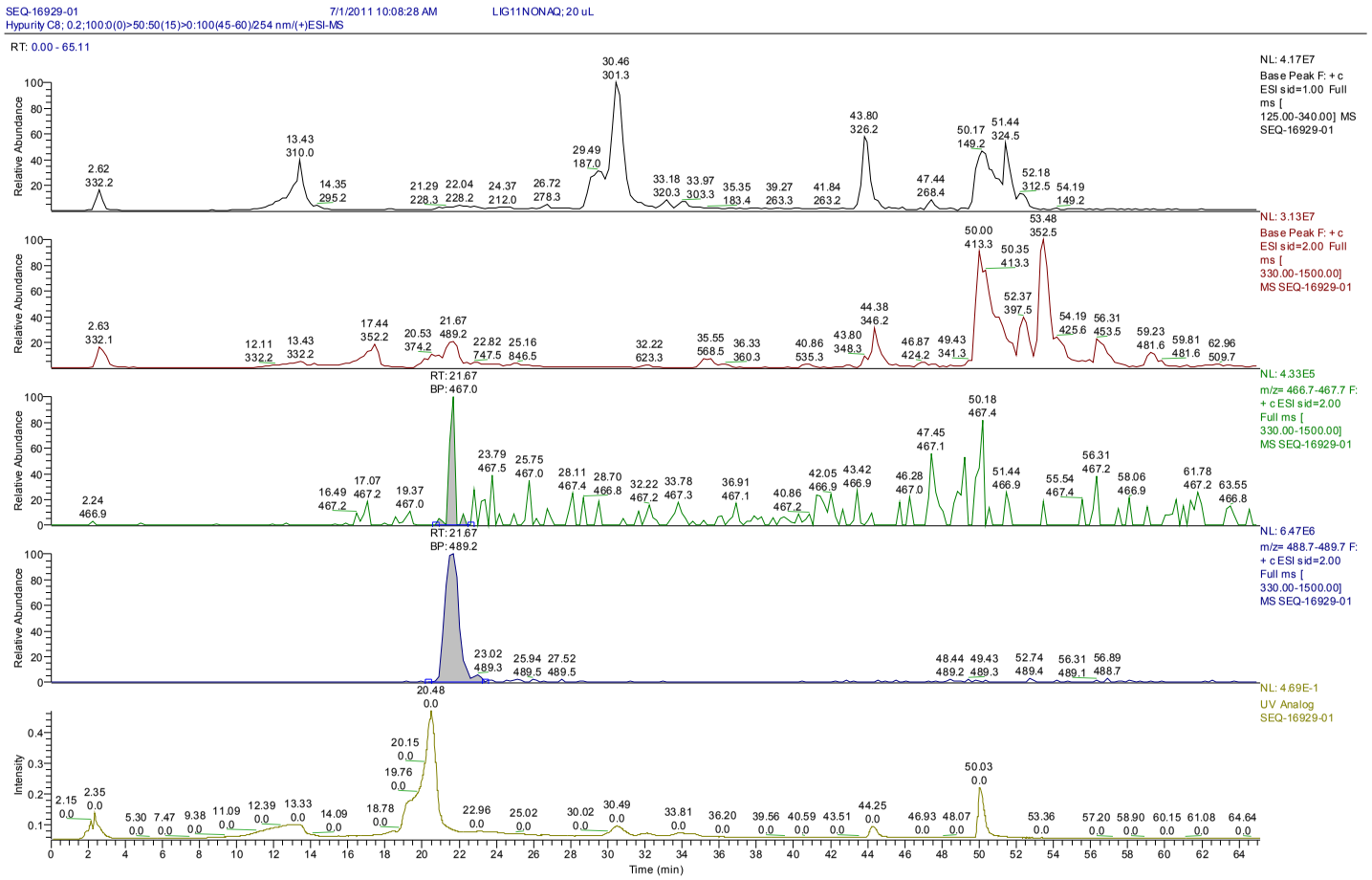


Figure S21. LIG11 NONAQ via HPLC/UV/(-)ESI-MSn, MW 466, RT 21.67 min. The tentative MW 466 detected via (+)ESI-MS above produced predominantly an m/z 465 [M-H]- ion under (-)ESI-MS conditions and thus confirmed it as being a MW 466 compound

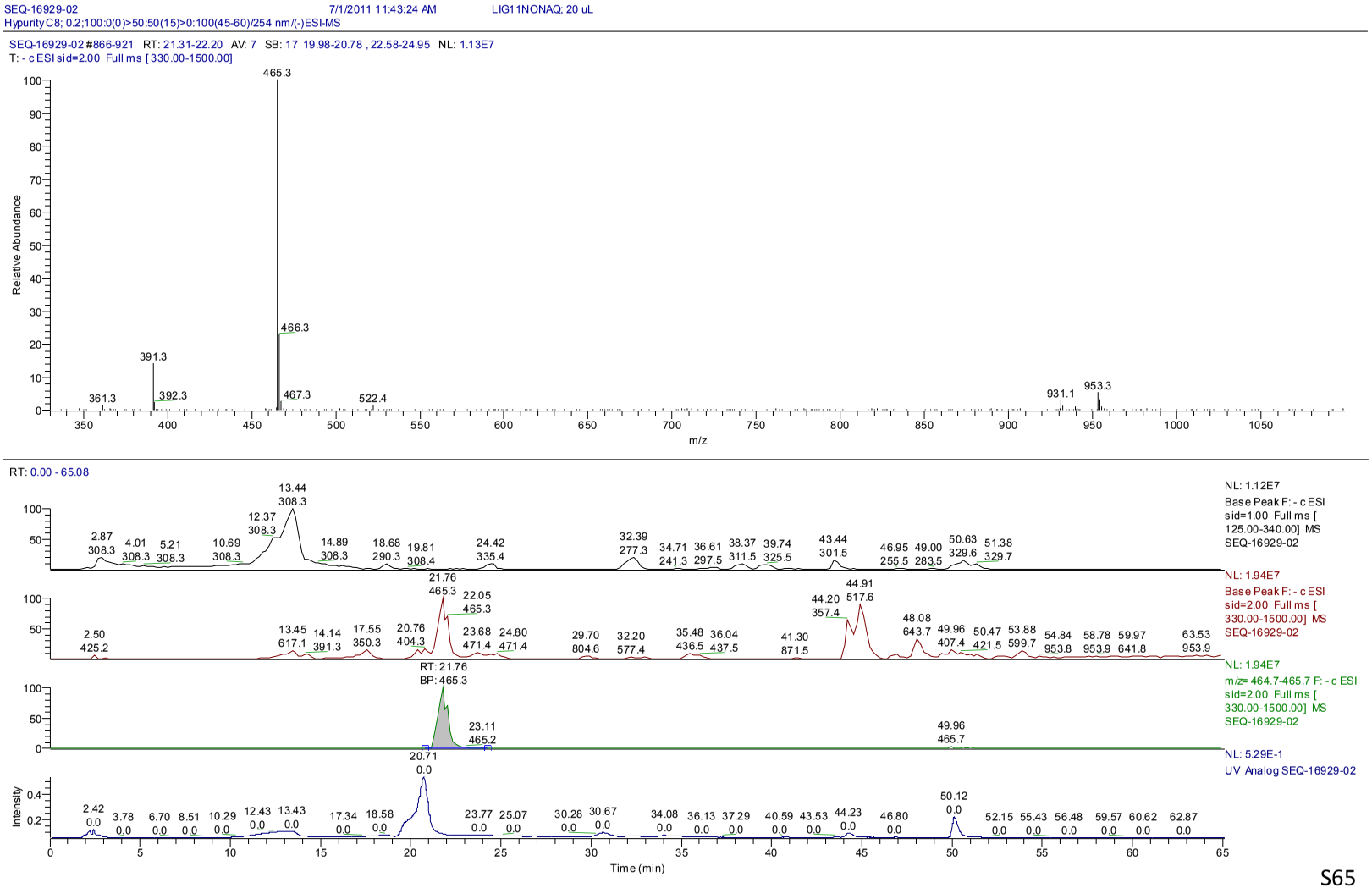


Figure S22. HPLC/(-)ESI-MS mass chromatograms of m/z 465. MW 466 compounds in (top to bottom): LIG11SM, LIG11NONAQ and LIG11 AQ 7.65. Only a very minor amount of MW 466 was detected in the LIG11 AQ7.65 sample. Note the products eluted prior to the starting material.

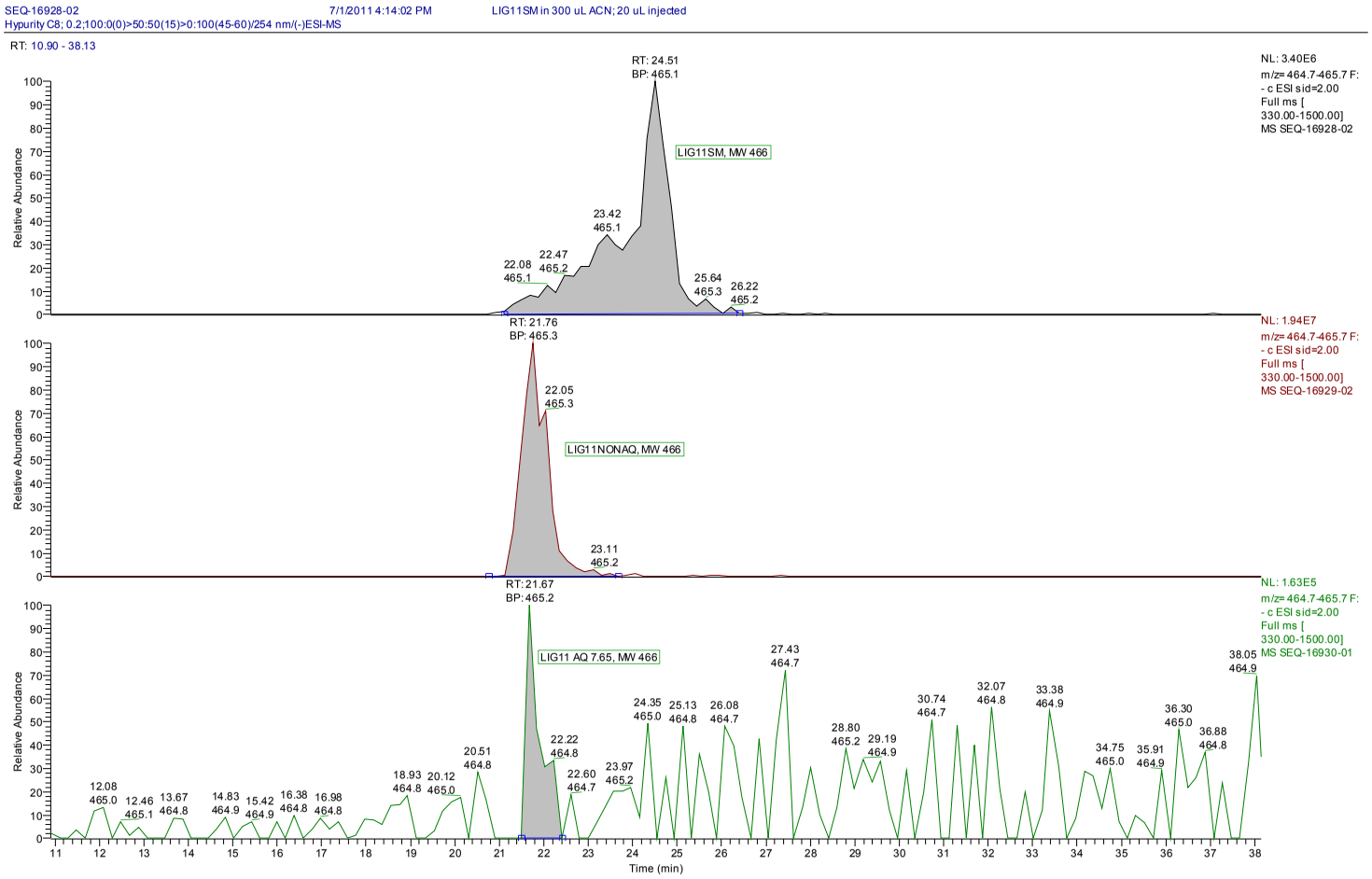


Figure S23. (-)ESI-MS/MS of the m/z 465 [M-H]- ions of the MW 466 compounds in (top to bottom): LIG11SM, LIG11NONAQ and LIG11 AQ 7.65. While it was expected that the starting material and products were different, the two MW 466 products also produced different dissociation ions.

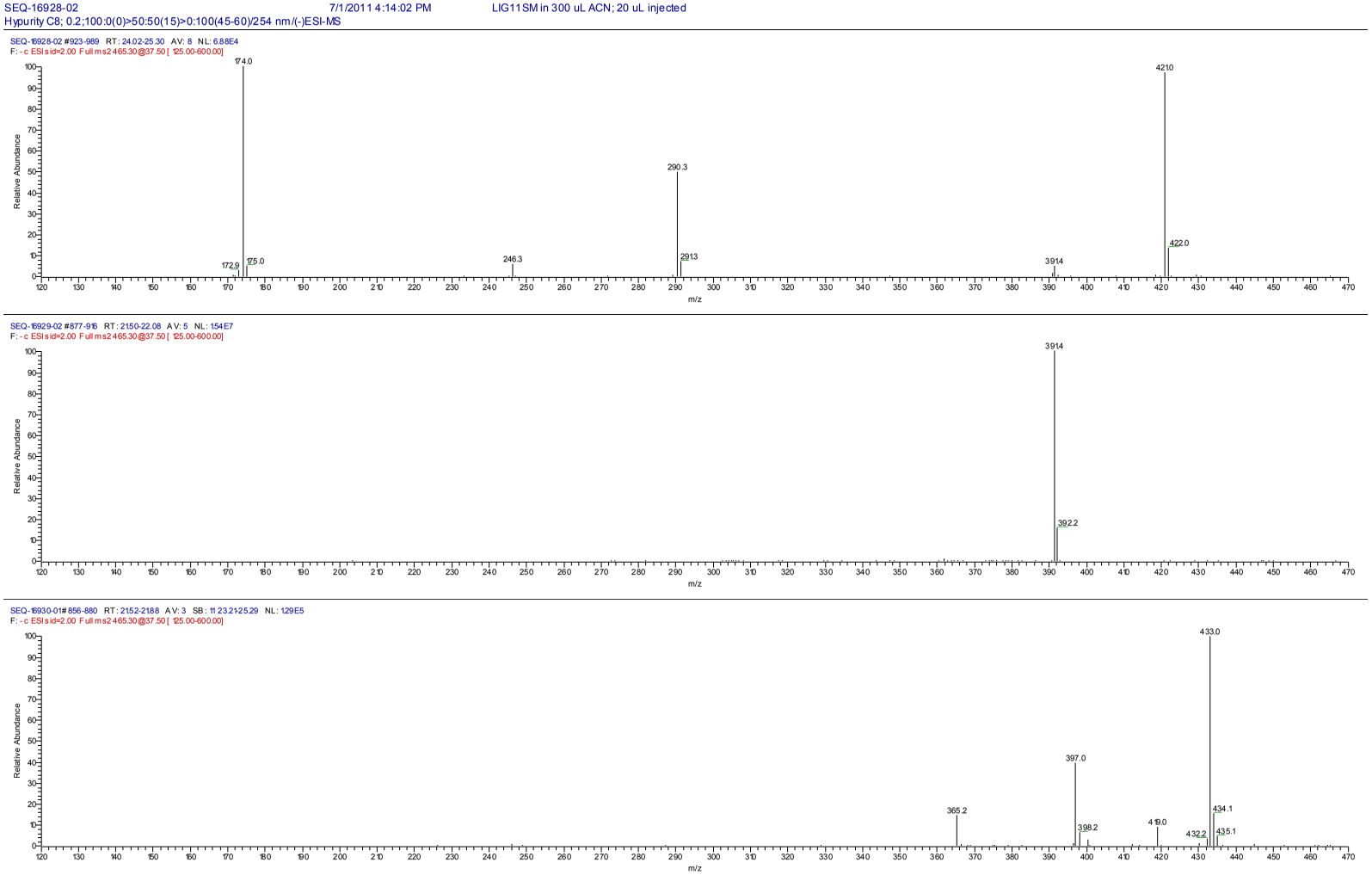


Figure S24. LIG11SM, MW 466, (-)ESI-MSn of the m/z 465 [M-H]- ion.

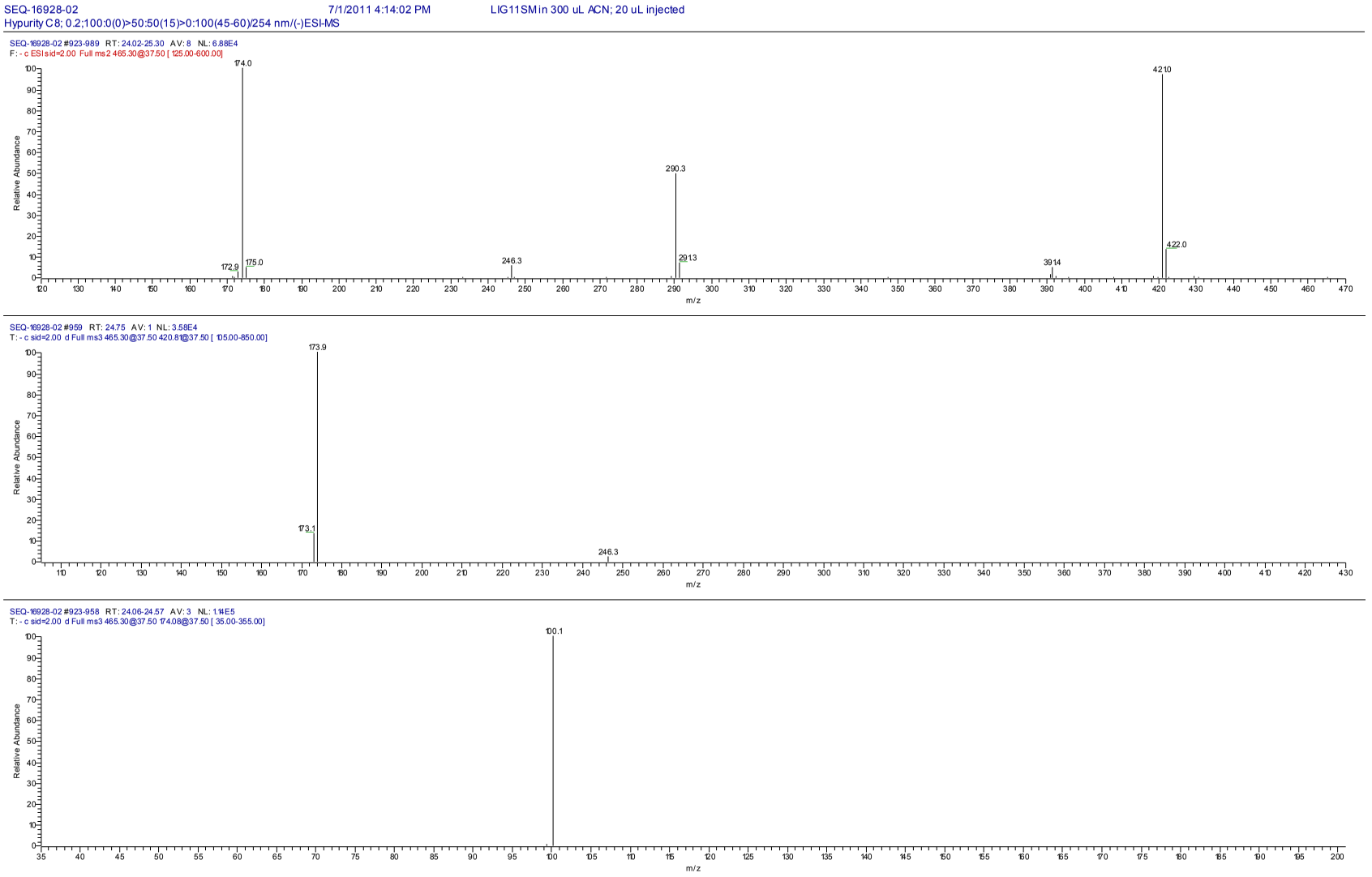


Figure S25. LIG11 NON-AQ, MW 466, RT 21.67 min: (-)ESI-MS/MS (top) and –MS/MS/MS (bottom) of the m/z 465 [M-H]- ion.

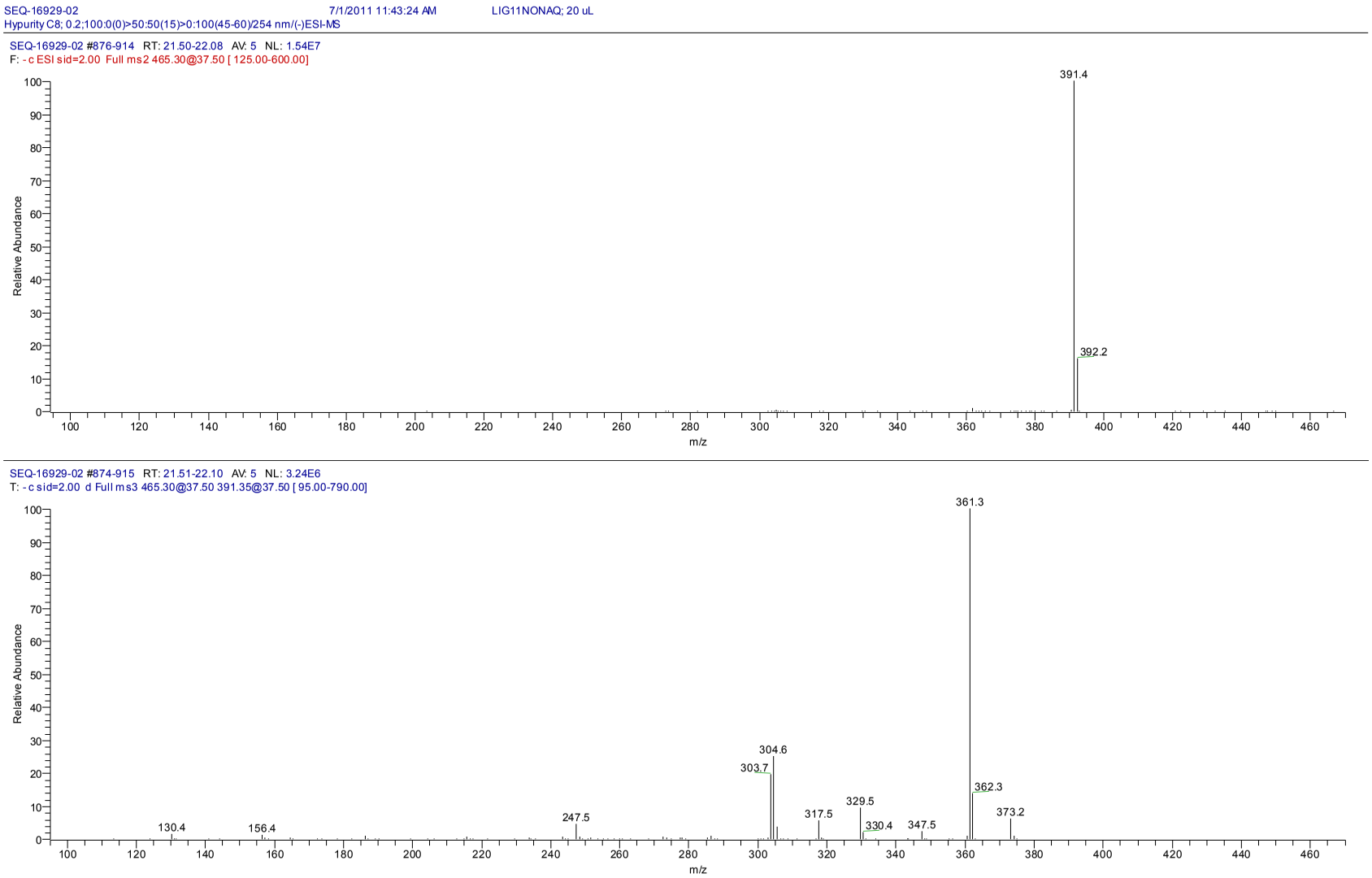
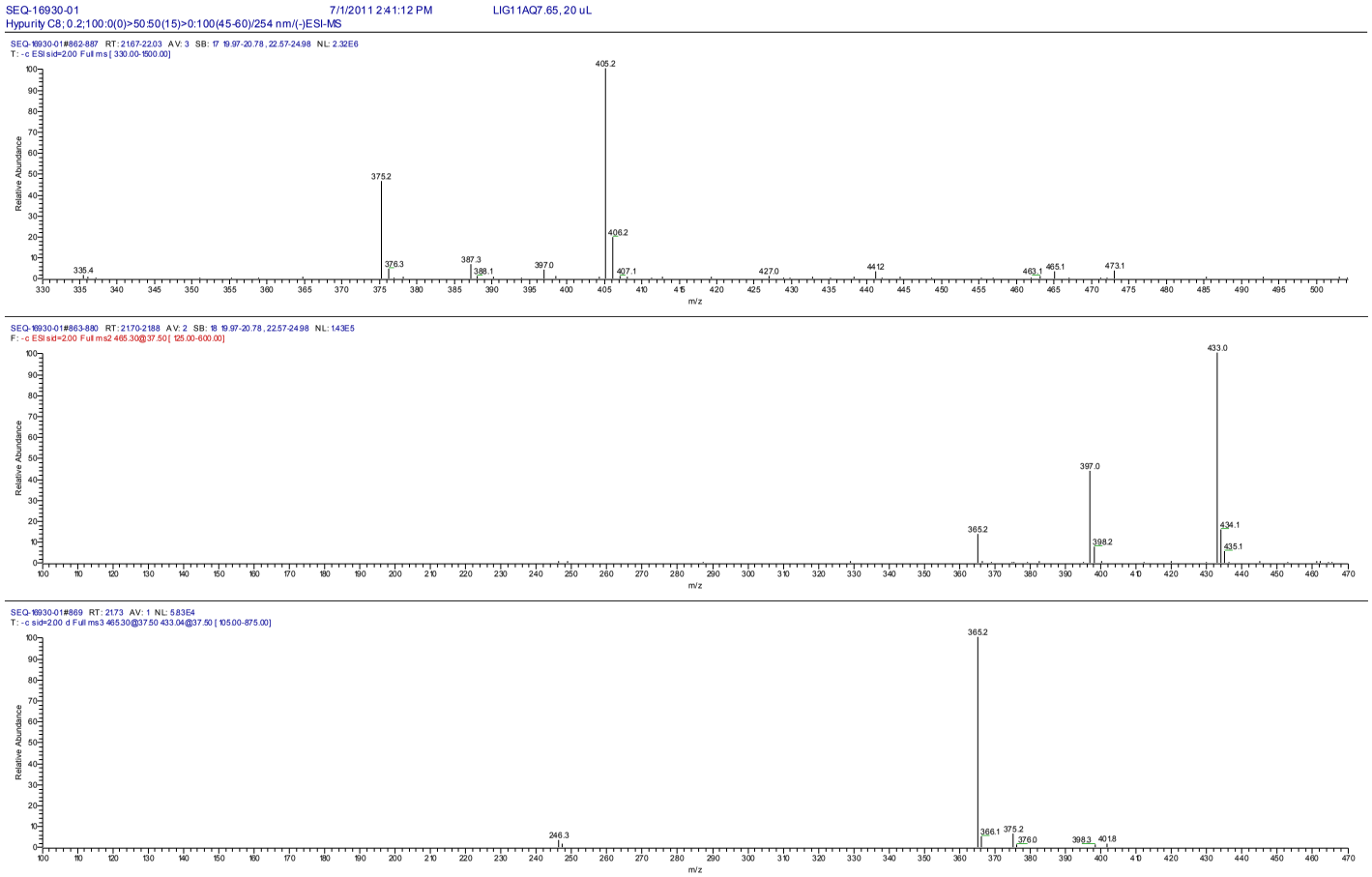


Figure S26. LIG11 AQ7.65, MW 466, RT 21.67 min. The m/z 465 [M-H]- was very minor in its (-)ESI-MS (top). The dominant ion was m/z 405. It is not uncommon to see [M+59]- ions where 59 is the acetate anion (from acetic acid often used in the mobile phase). Might the m/z 465 be an [M+59]- ion of a MW 406 compound? The m/z 465 was dissociated to m/z 433, 397 and 365 (m/z 397 may be background). The m/z 433 was dissociated further to m/z 365 and m/z 246 (bottom).



HPLC chromatograms and MS Spectra for 7b(ME41) and 8b(ME43A)

Scheme S3. Conversion of the MW 514 starting material to MW 515 product (taken from the submitted reaction).

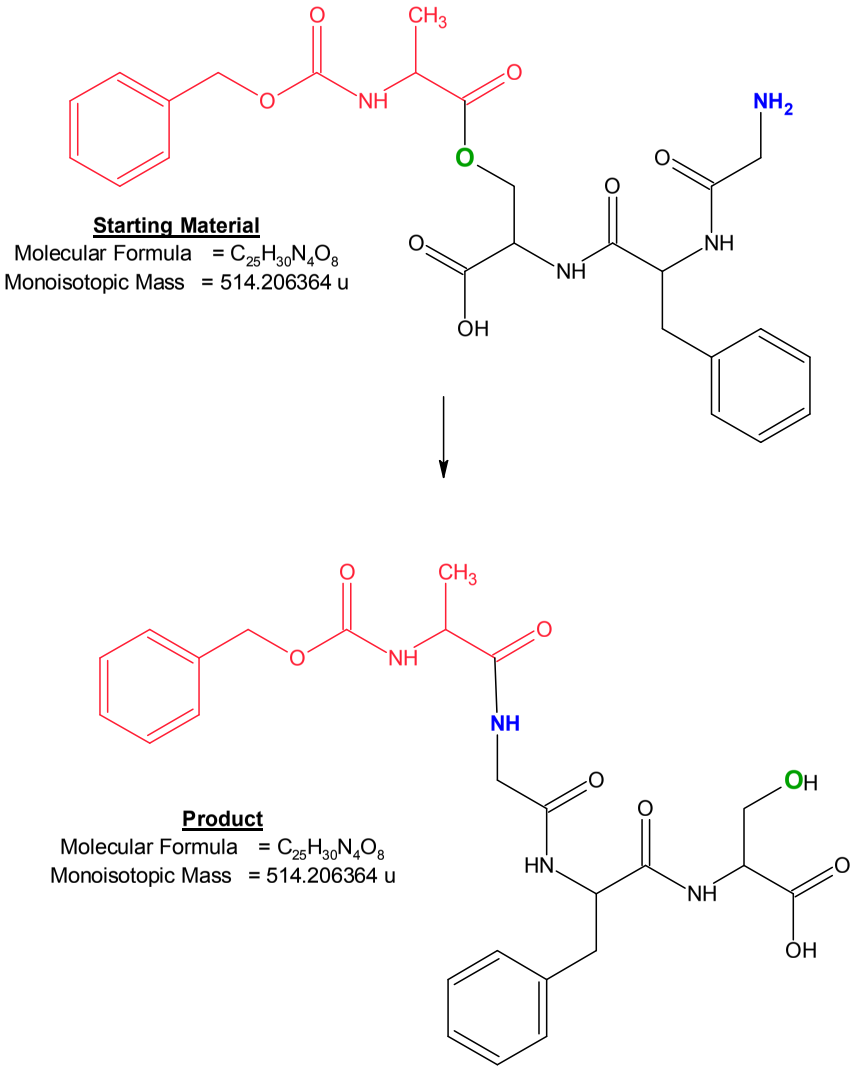


Figure S27. ME43A via HPLC/254 nm UV/(+)ESI-MS. Summary chromatogram s of the major (+)ESI-MS ions characteristic of the compounds of interest. The shaded area have been integrated and were used to determine the relative amounts.

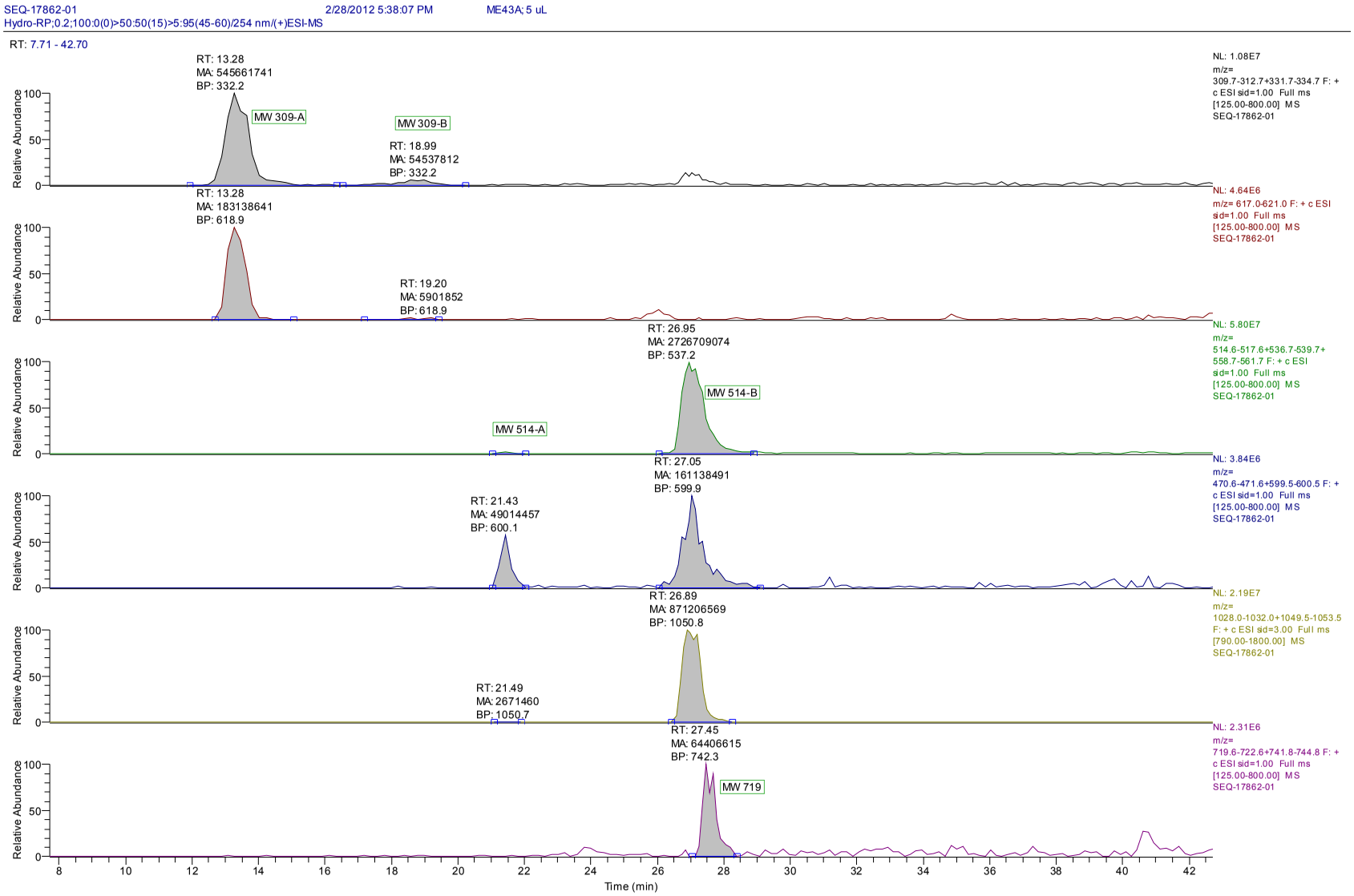
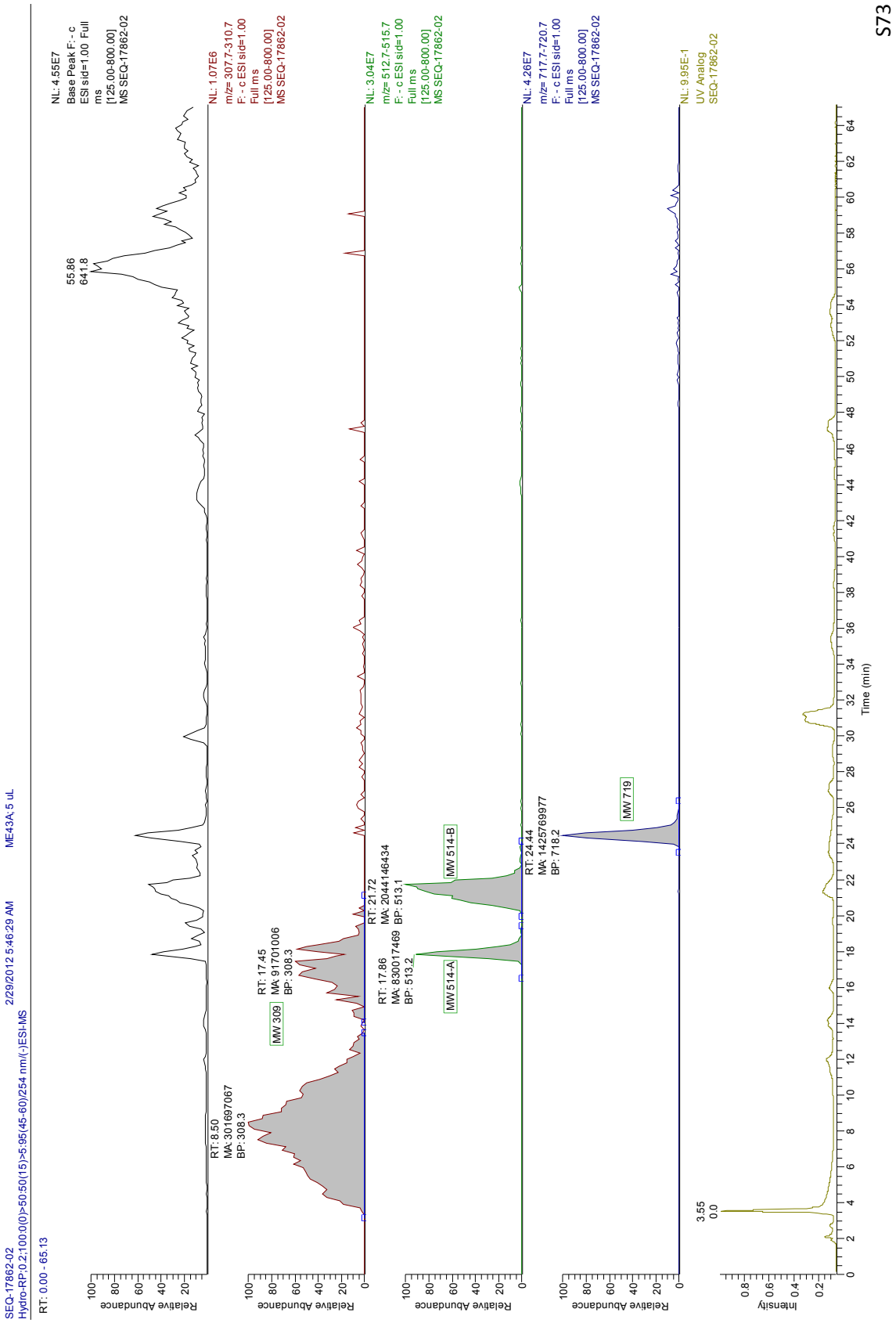


Figure S28. ME43A: via HPLC/254 nm UV/(-)ESI-MS. Summary chromatogram s of the (-)ESI-MS [M-H]- ions characteristic of the compounds of interest. The shaded area have been integrated and were used to determine the relative amounts.



Retention time(RT), characteristic ions used for integrating, and areas from these integrations. Taken from the previous chromatograms.

MW	RT	Ions-1	Ions-2	Ions-3	Area 1	Area-2	Area-3	Sum of Areas-i	%Total
MW 309	13.28	292.1, 309.9, 332.2	618.9		545,661,741	183,138,641	0	728,800,382	15.49
MW 309 (maybe)	18.99	292.1, 309.9, 332.2	618.9		54,537,812	5,901,852	0	60,439,664	1.28
MW 514-A	21.43	515.1, 537.2, 559.2	471.1, 600	1028-1032, 1049.5-1053	39,336,966	49,014,457	2,671,460	91,022,884	1.94
MW 514-B	26.95	515.1, 537.2, 559.2	471.1, 600	1028-1032, 1049.5-1053	2,726,709,074	161,138,491	871,206,569	3,759,054,134	79.92
MW 719	27.45	720.1, 742.3			64,406,615			64,406,615	1.37
Total Areas								4,703,723,679	

MW	RT	Ions-1	Area	%Total
MW 309	8.5	308.2	301697066.9	6.43
MW 309 (maybe)	17.45	308.2	91701006.14	1.95
MW 514-A	17.86	513.2	830017468.6	17.69
MW 514-B	21.72	513.2	2044146434	43.55
MW 719	24.44	718.2	1425769977	30.38
Total Area			4693331953	

Figure S29. ME43A via C18 HPLC/254 nm UV/(+)ESI-MS. There were two MW 514 compounds indicated by the shaded peaks. I have labeled them as MW 514-A (RT 21.43 min) and MW 514-B (RT 26.95 min).

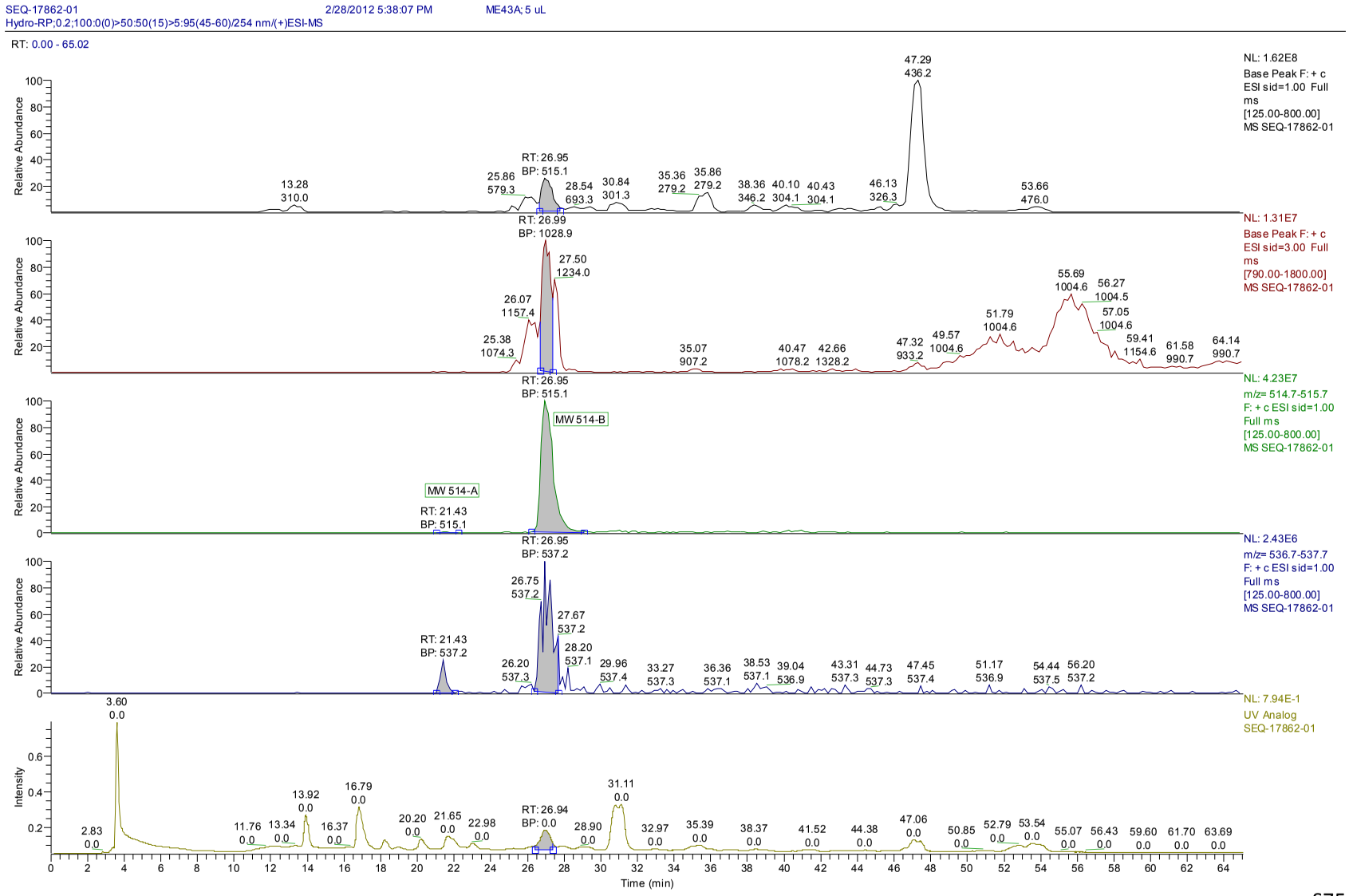


Figure S30. ME43A via C18 HPLC/254 nm UV/(-)ESI-MS. The m/z 513 [M-H]- ion-peaks of the two MW 514 compounds are shaded. The retention times of these and the other compounds are significantly different from the (+)ESI-MS analysis . The (+)ESI-MS analysis was the last run of one day while the (-)ESI-MS analysis was the first analysis fun the 2nd day. Often the first analysis after the instrument has been off overnight exhibits significant differences from previous and subsequent analyses.

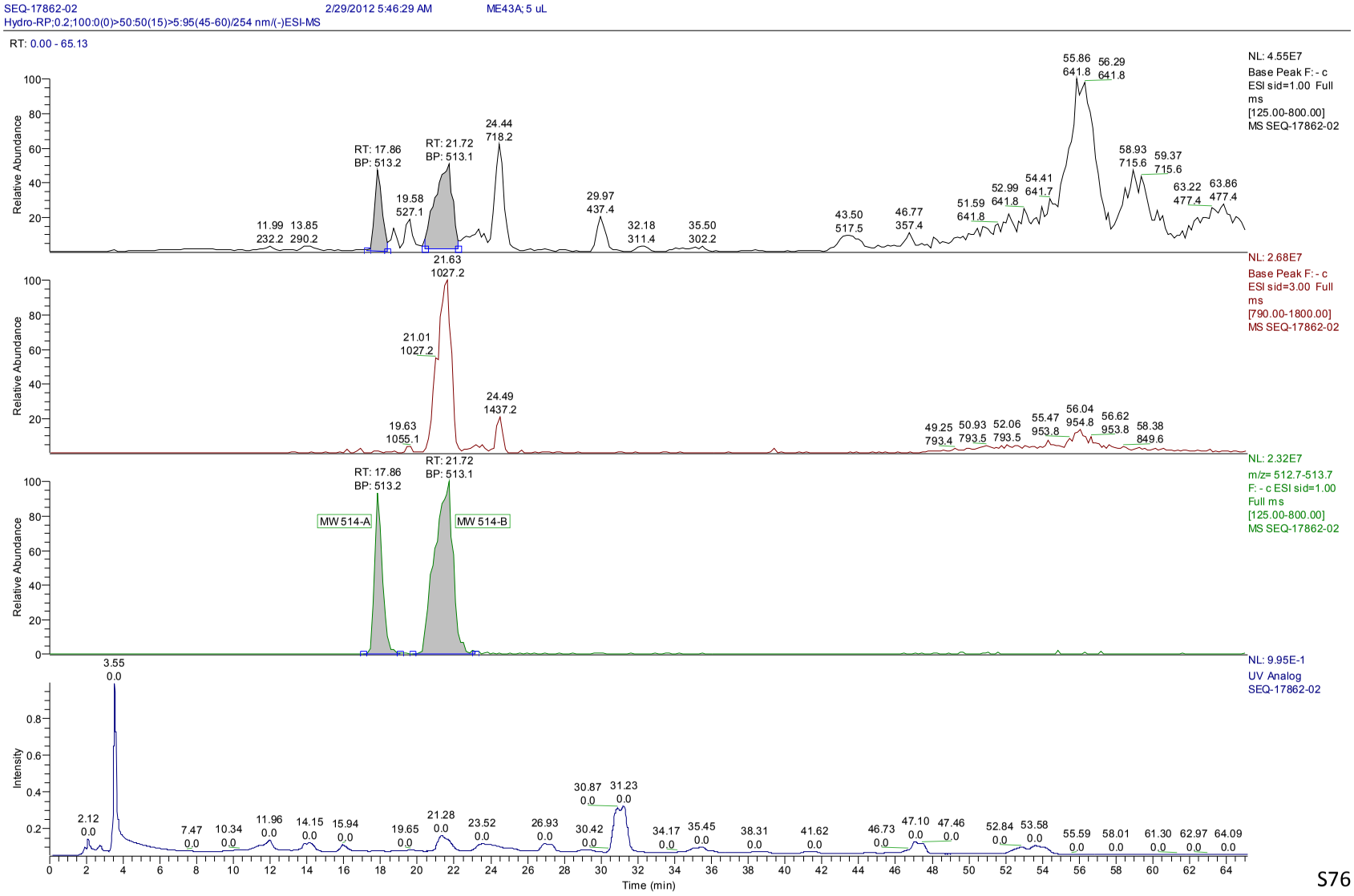


Figure S31. MW 514-A (RT 21.43 min): The MW 514-A produced m/z 515 [M+H]+, m/z 537 [M+Na]+ and m/z 559 [(M-H+Na)+Na]+ ions (top and chromatograms on bottom). It was thought initially that the m/z 600 ion was from some other compound which just happens to co-elute with the MW 514-A. However,

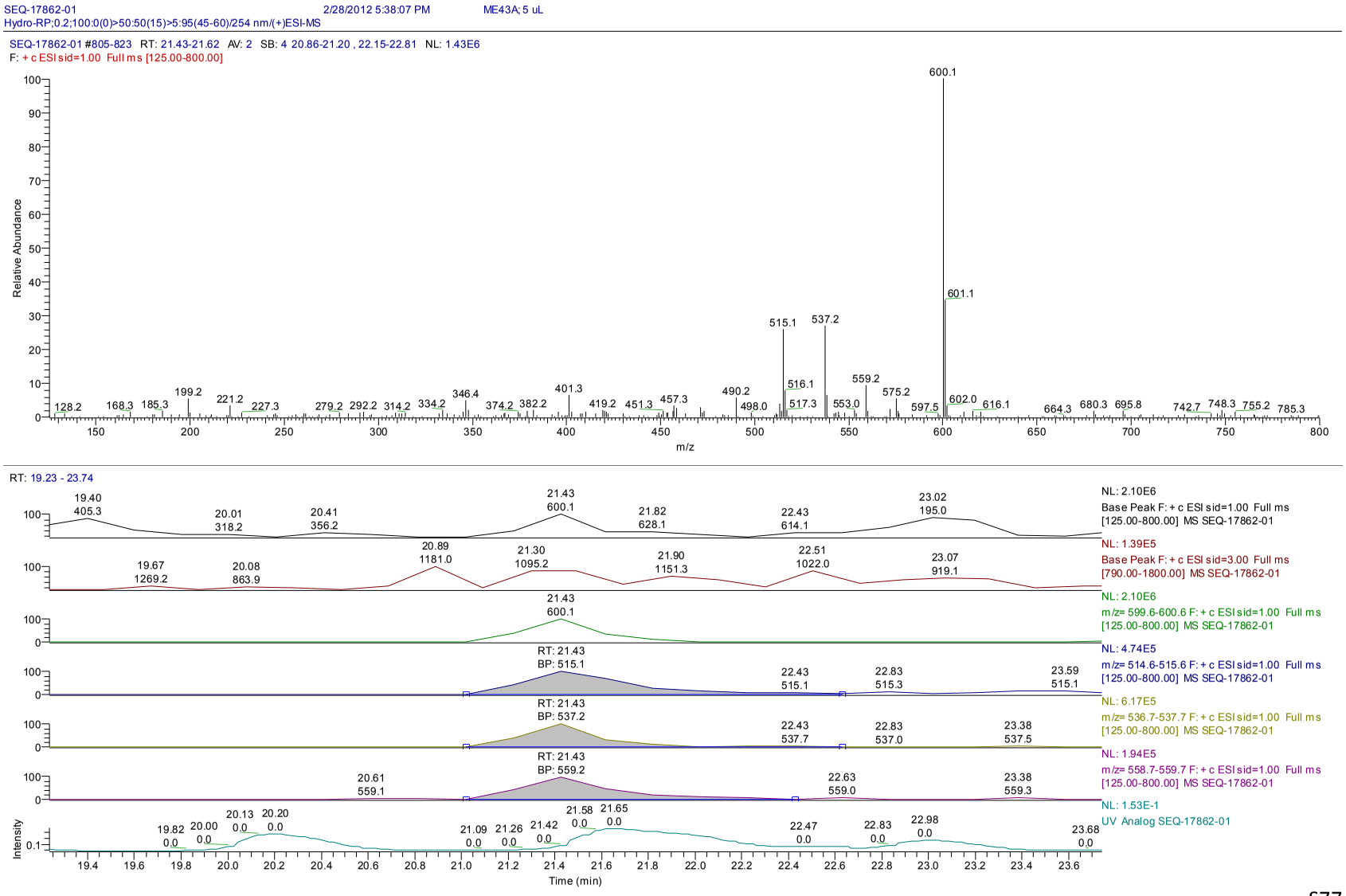


Figure S32. MW 514-A: The abundant m/z 600 ion in the (+)ESI-MS spectrum (top) was dissociated to produce m/z 515, 497, 471, 410 etc (bottom). The m/z 600 => m/z 515 occurred via loss of 85 u. An analysis of the HPLC mobile phase was done after these samples. There is an abundant m/z 86 ion (MW 85 u) present in the mobile phase at high organic content. It is possible that this formed an [85 u + H + 514 u]+ adduct ion during the (+)ESI process. There were no obvious sodiated versions of the m/z 600 and thus I do not think it is another compound.

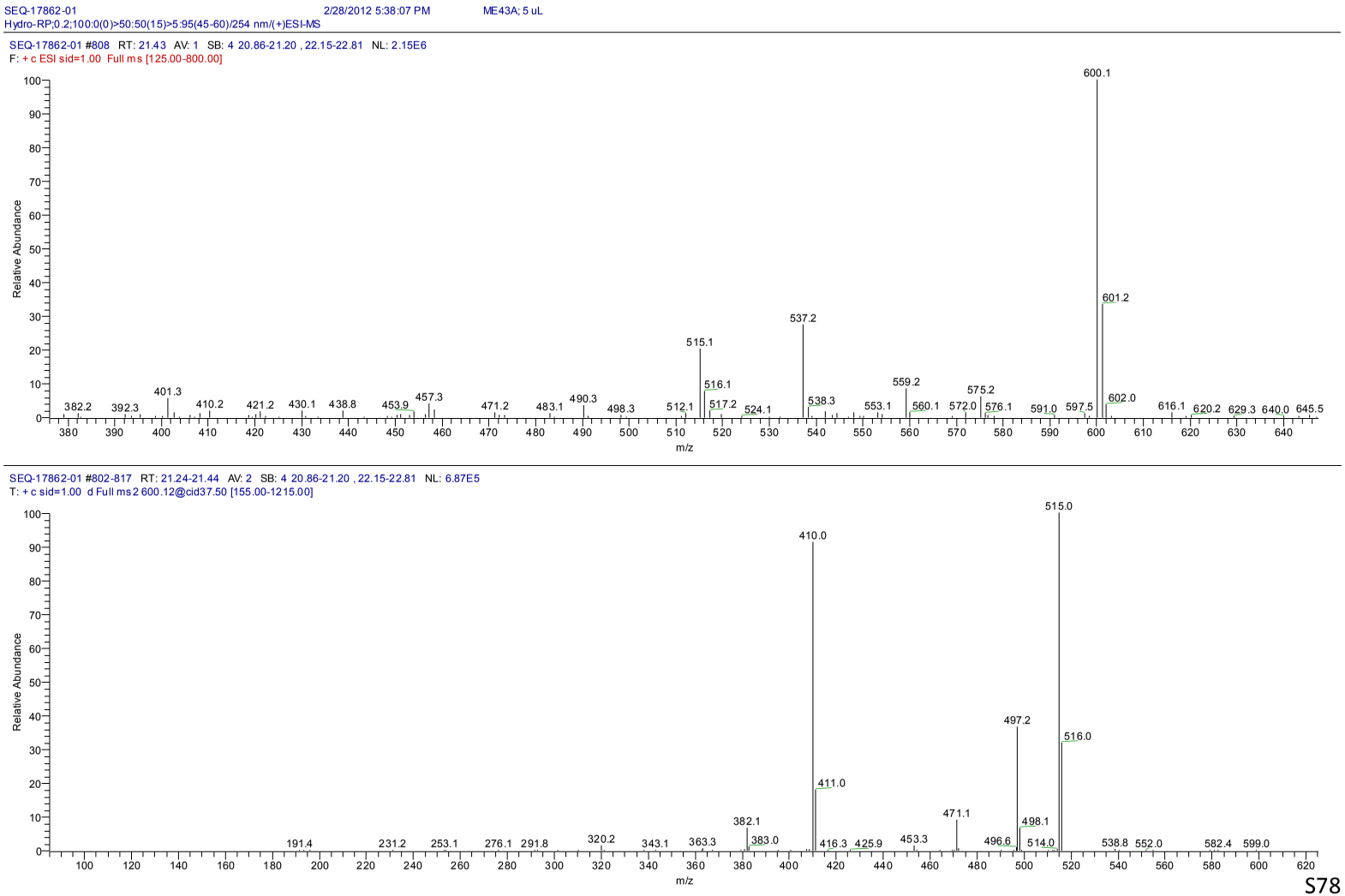


Figure S33. MW 514-A, (-)ESI-MS. With (-)ESI-MS, the MW 514-A produced predominantly the m/z 513 [M-H]- ion; there was no indication of an m/z 598 [M-H]- ion of a MW 599 compound (if the m/z 600(+) ion was an [M+H]+ ion).

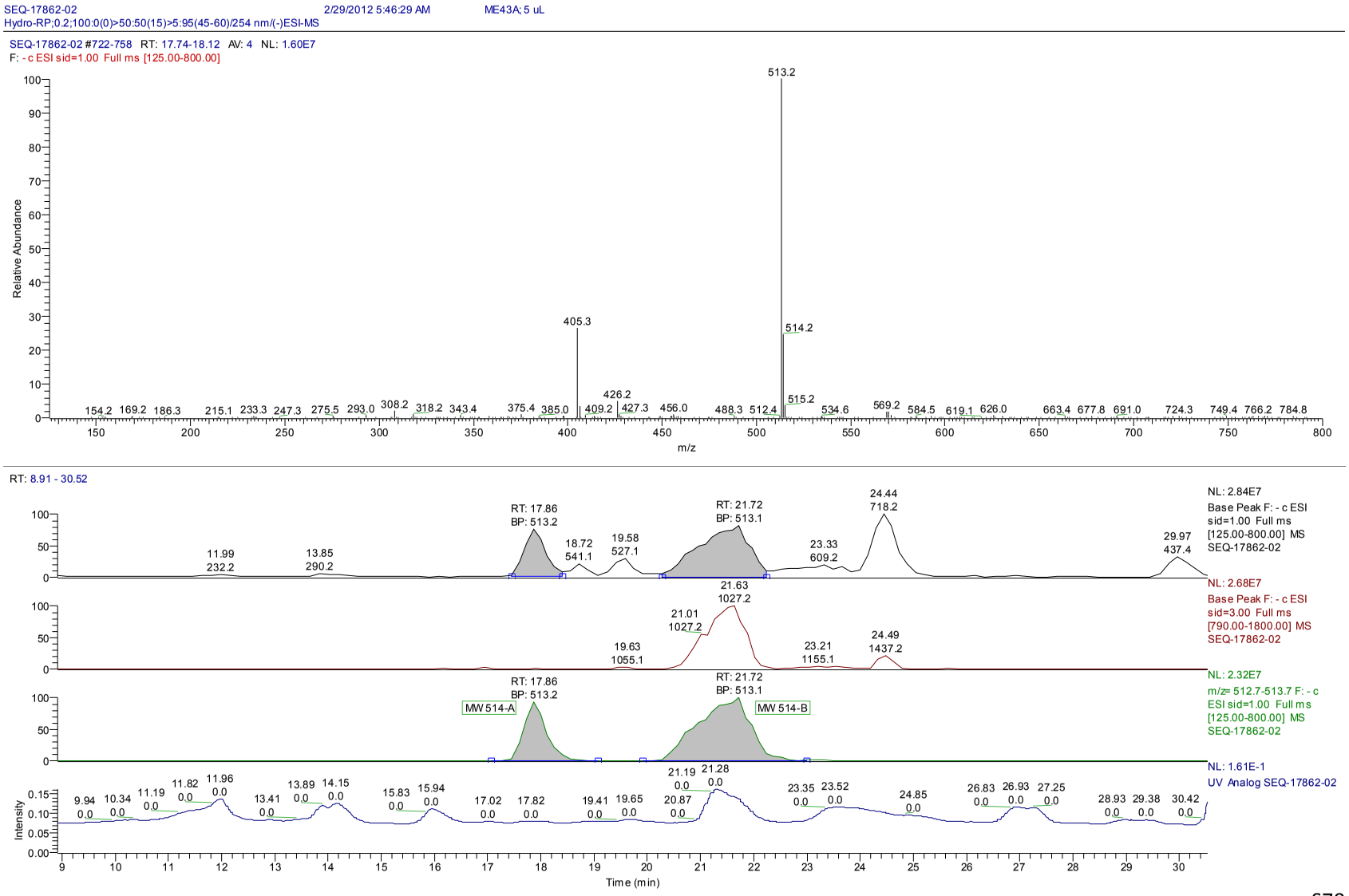
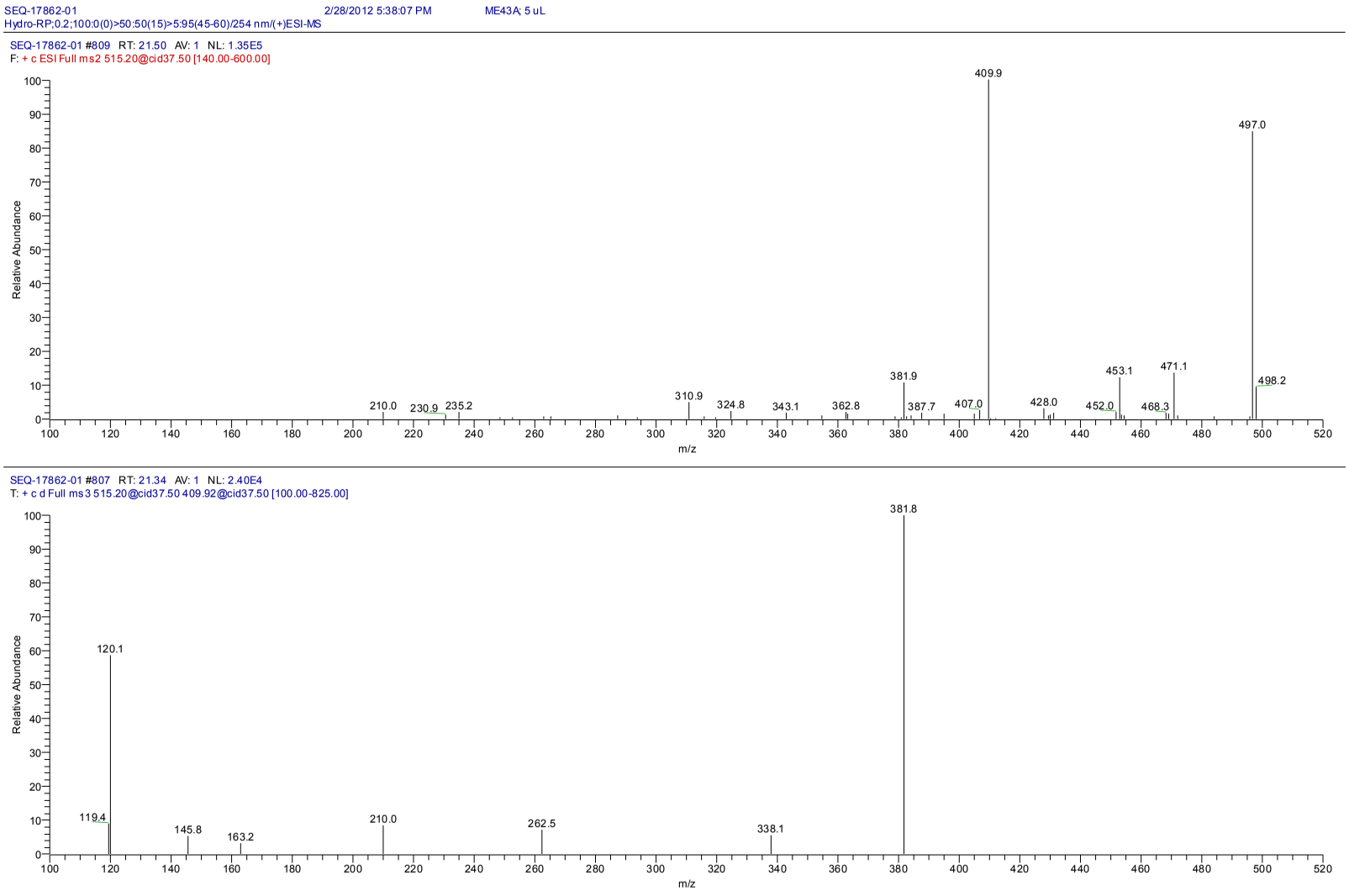


Figure S34. MW 514-A (RT 21.43 min): (+)ESI-MS/MS (top) and –MS/MS/MS (bottom) of the m/z 515 [M+H]+ ion. These product spectra are much different than those obtained from the MW 514-B compound.



Scheme MW 514-A-1. Probable (+)ESI-MSn of the m/z 515 [M+H]+ ion is consistent with the structure submitted.

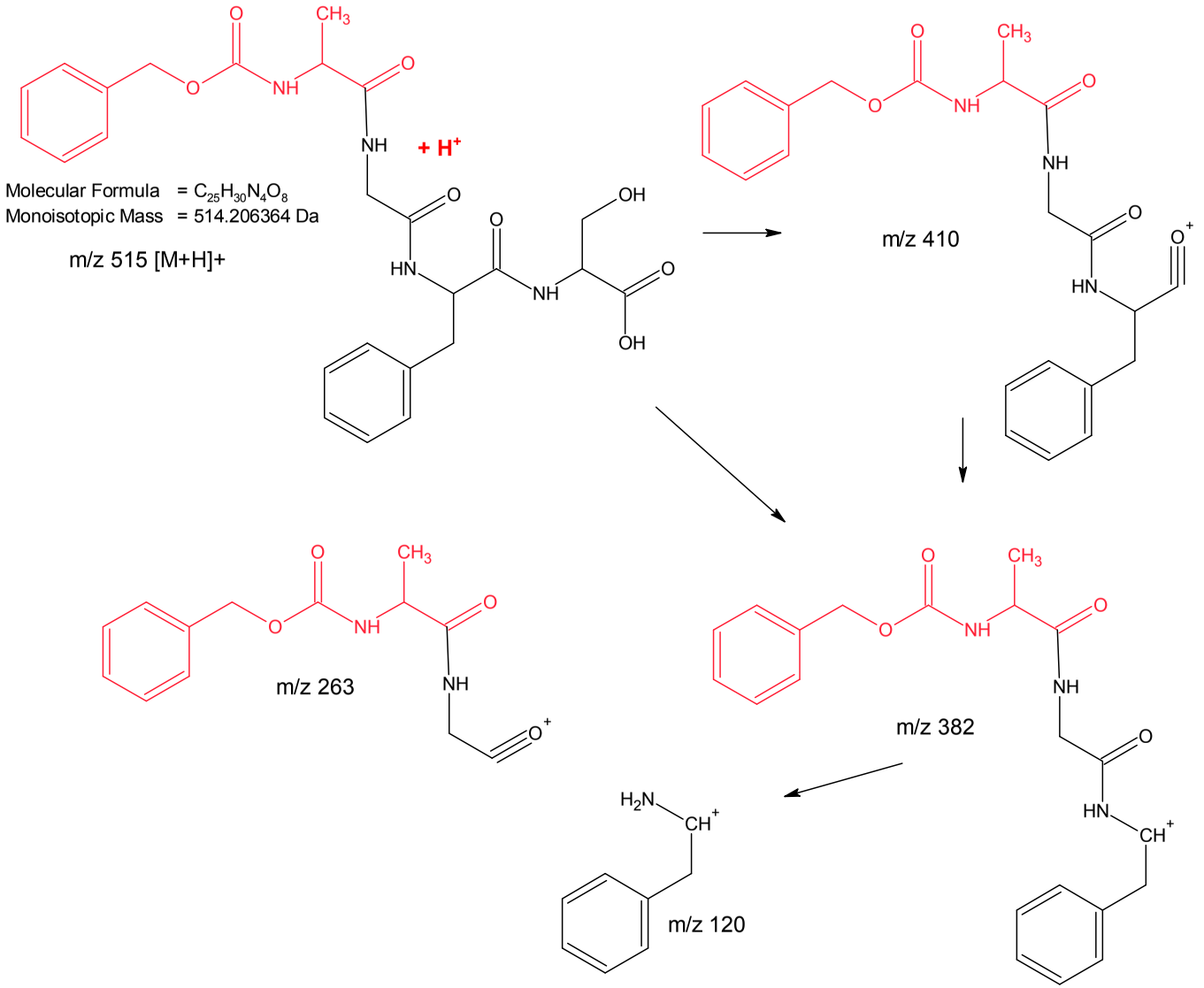


Figure S35. MW 514-A: (-)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 513 [M-H]- ion.

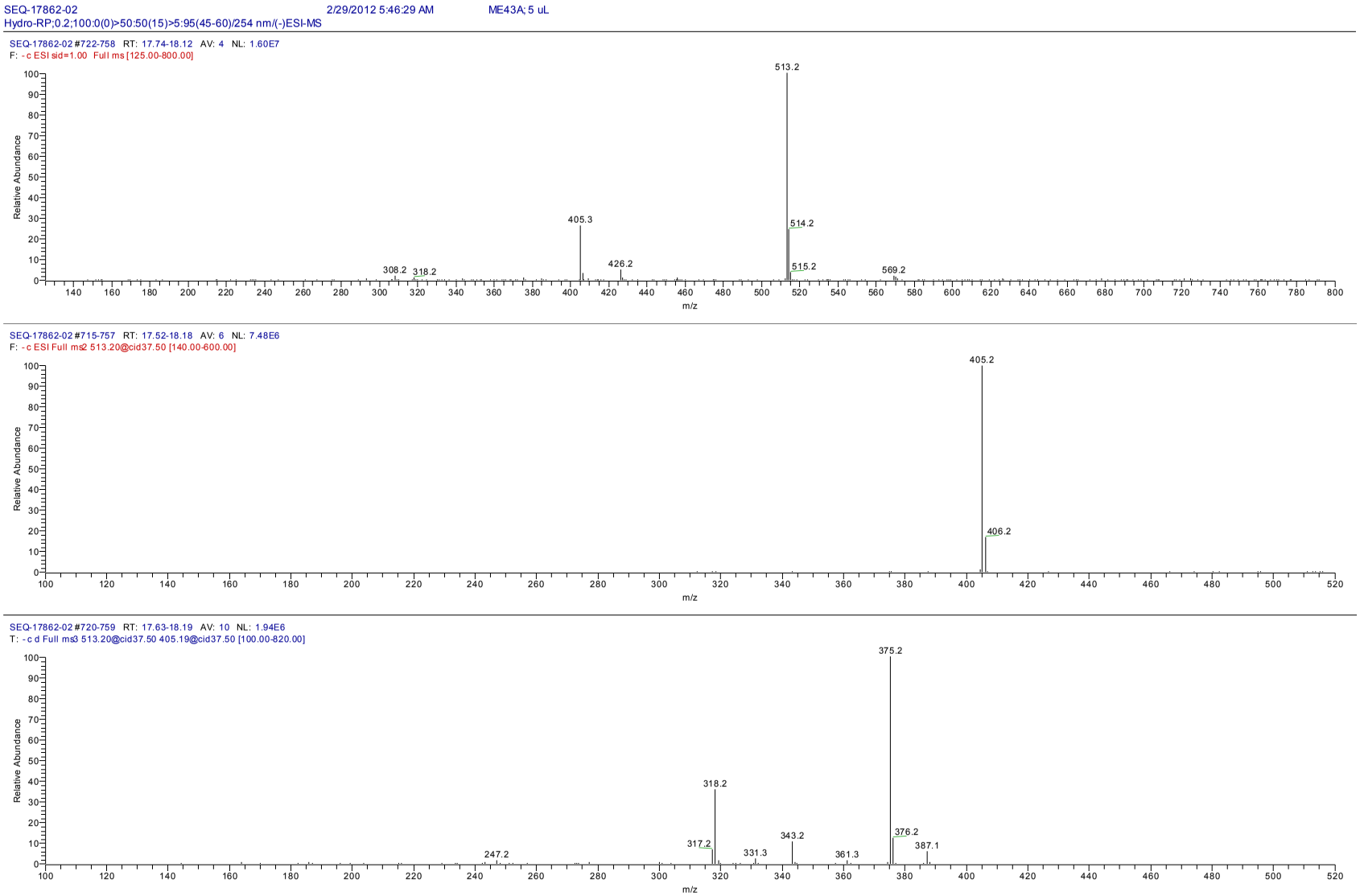


Figure S36. MW 514-B (RT 26.95 min): (+)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 515 [M+H]+ ion. These spectra match well those of the MW 514 compound of ME41 and ME43 (see SEQ-17677-n-17678-Bajaj(Elagawany)-n-Katritzky.doc). This is likely the MW 514 starting material.

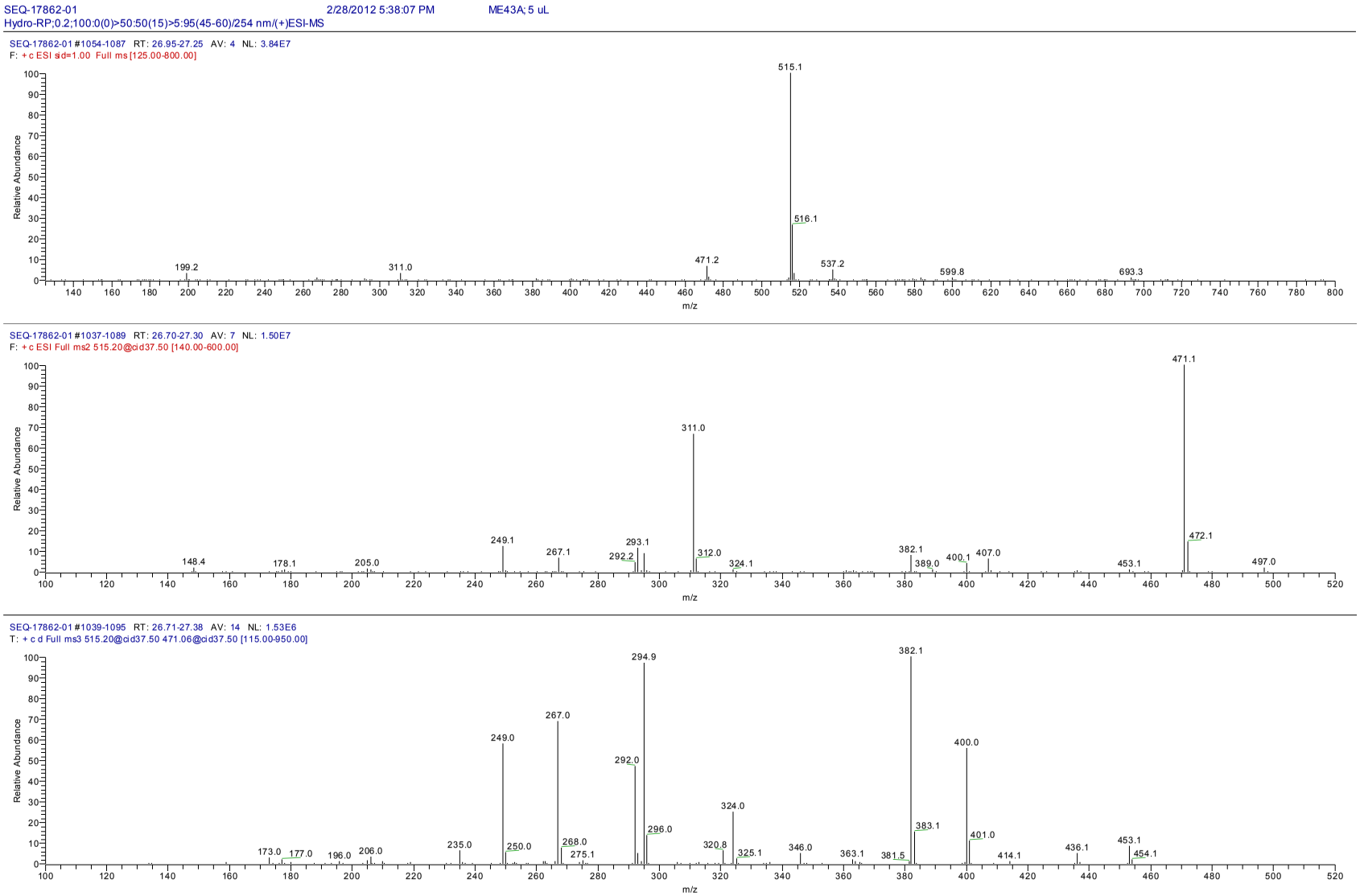


Figure S37. MW 514-B: (-)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 513 [M-H]- ion.

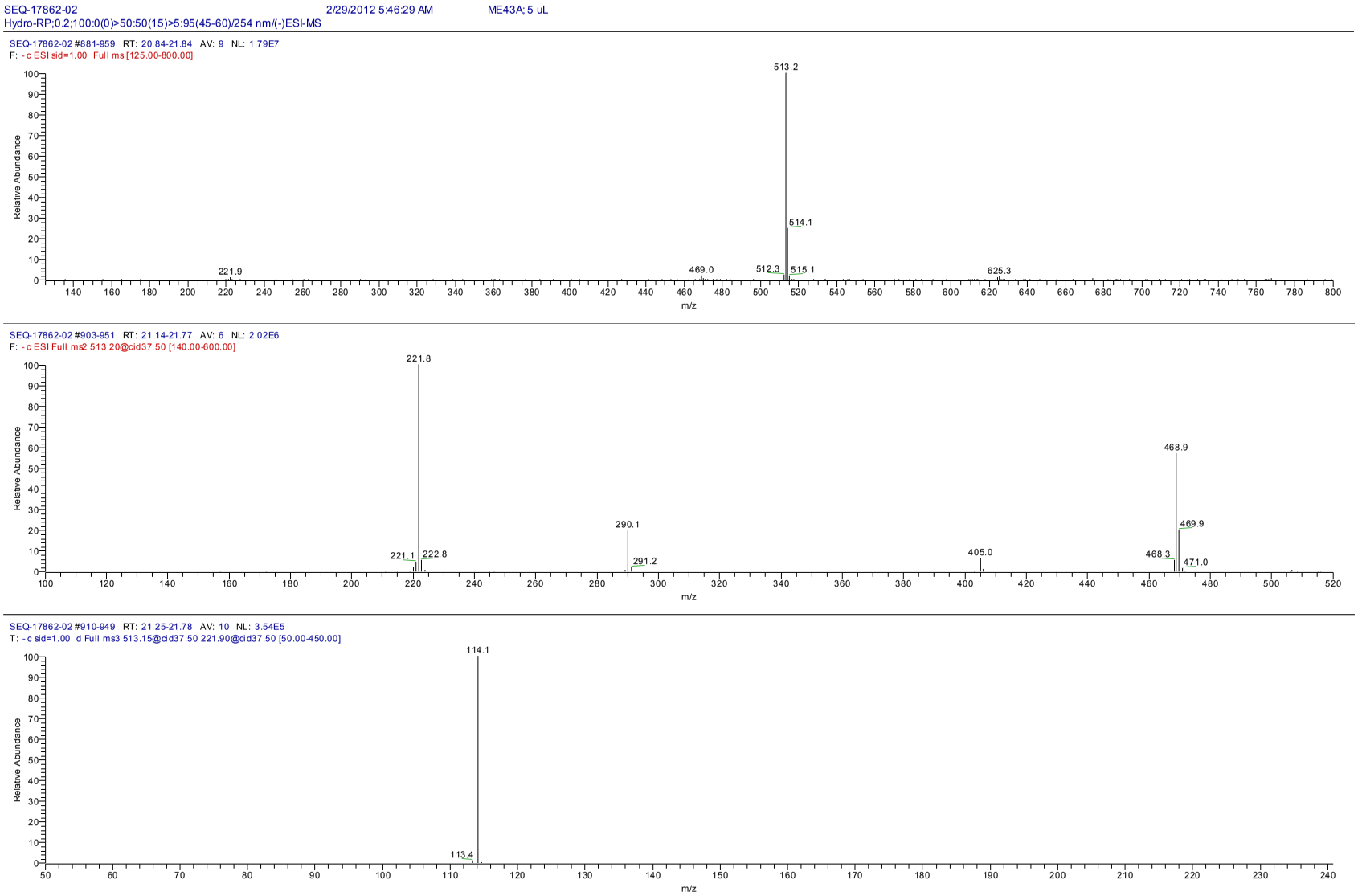


Figure S38. MW 514 compounds both produced m/z 537 [M+Na]+ ions. The product spectra of the MW 514-A (top) was much different than that of MW 514-B (bottom).

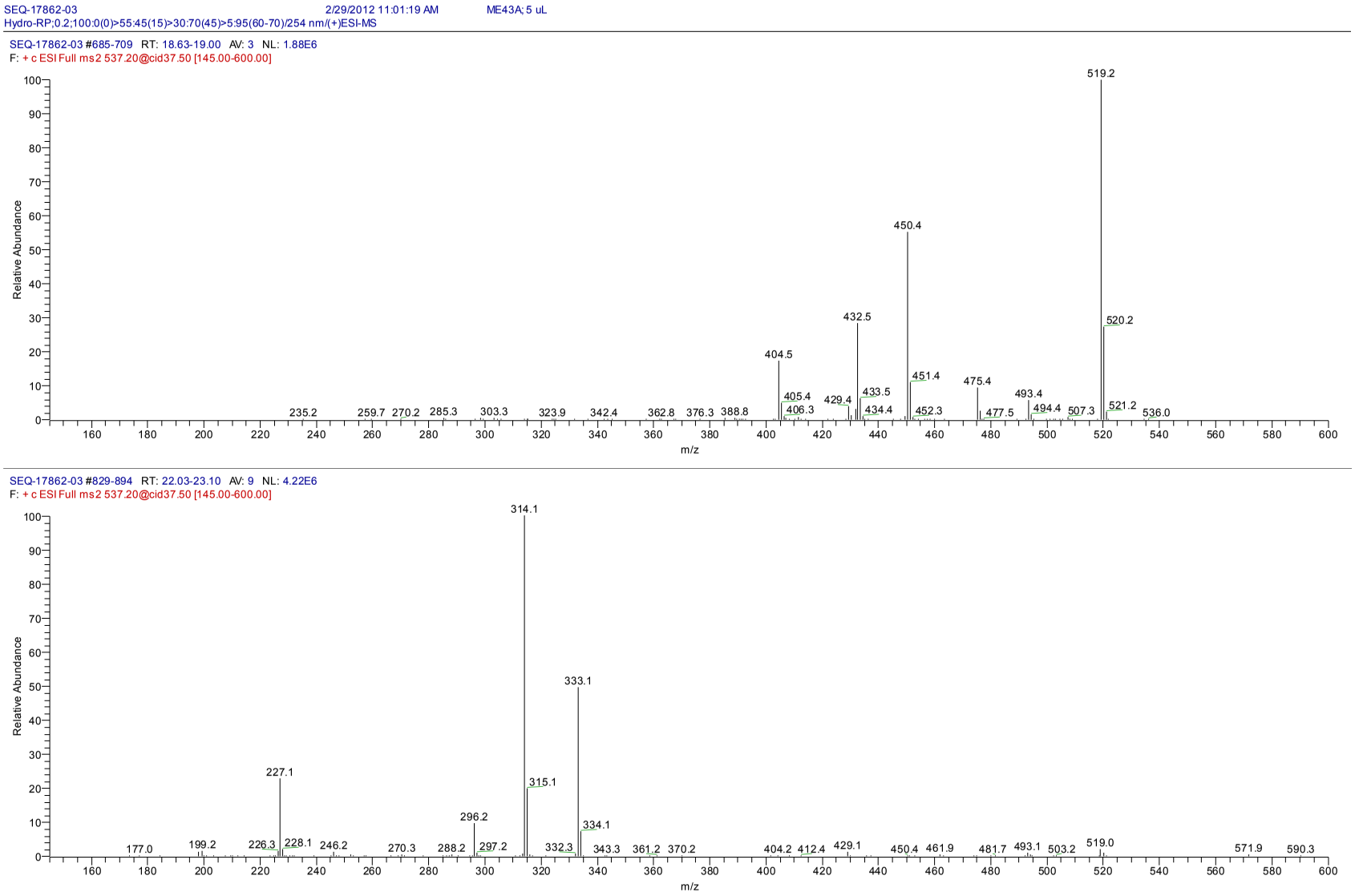


Figure S39. MW 309, RT 13.28 min: The HPLC/(+)ESI-MS mass chromatogram of the m/z 310 [M+H]+ ion and m/z 332 [M+Na]+ ion produced two pairs of ion-peaks (shaded on bottom). However, the (+)ESI mass spectra of these show that the RT 13.28 min peak is likely MW 309 while the 2nd peak may be due to fragment ions from some higher MW compound. Without more analyses it is unclear at this point.

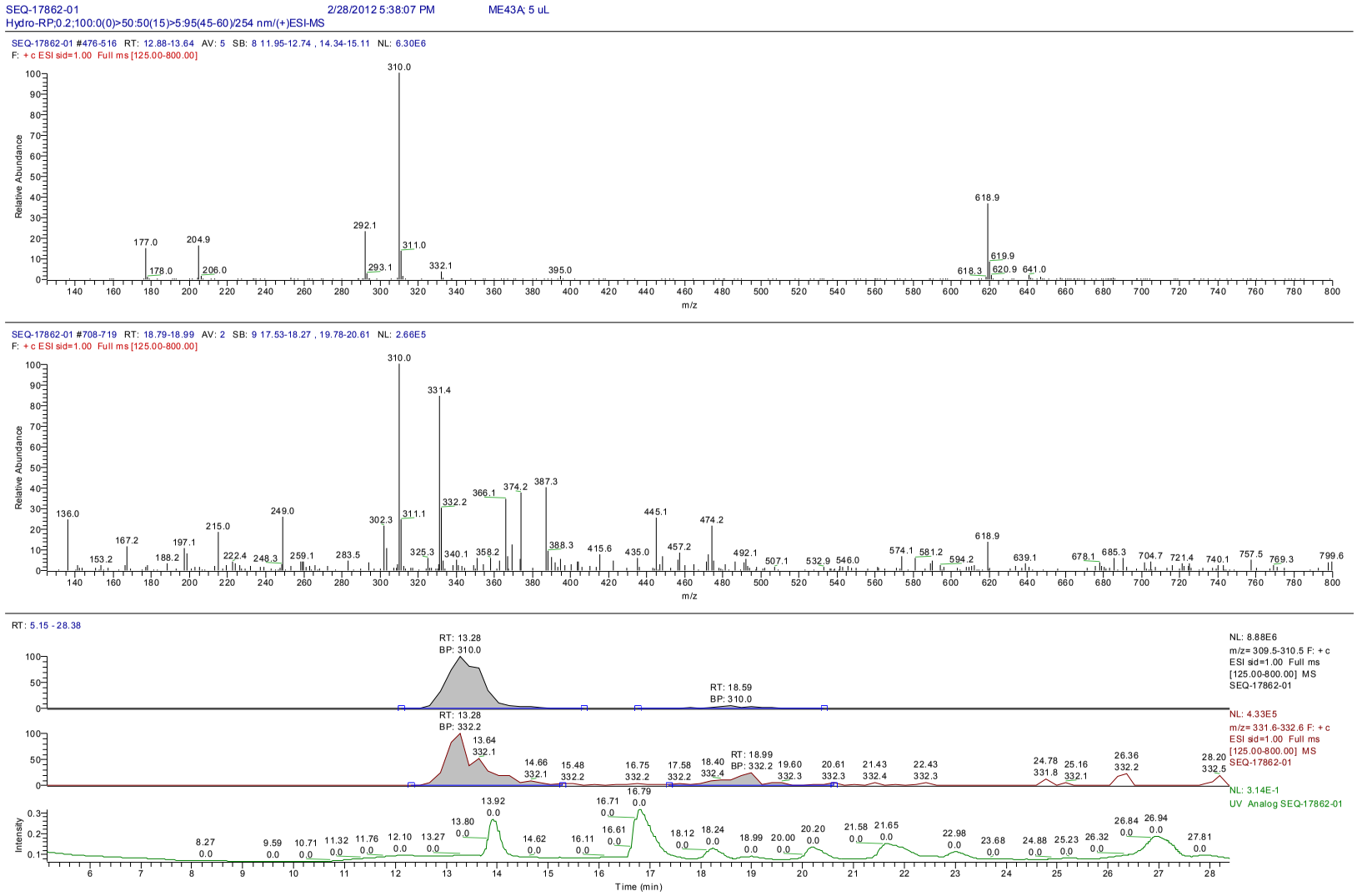


Figure S40. MW 309, RT 13.28 min: (+)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 310 [M+H]+ ion.

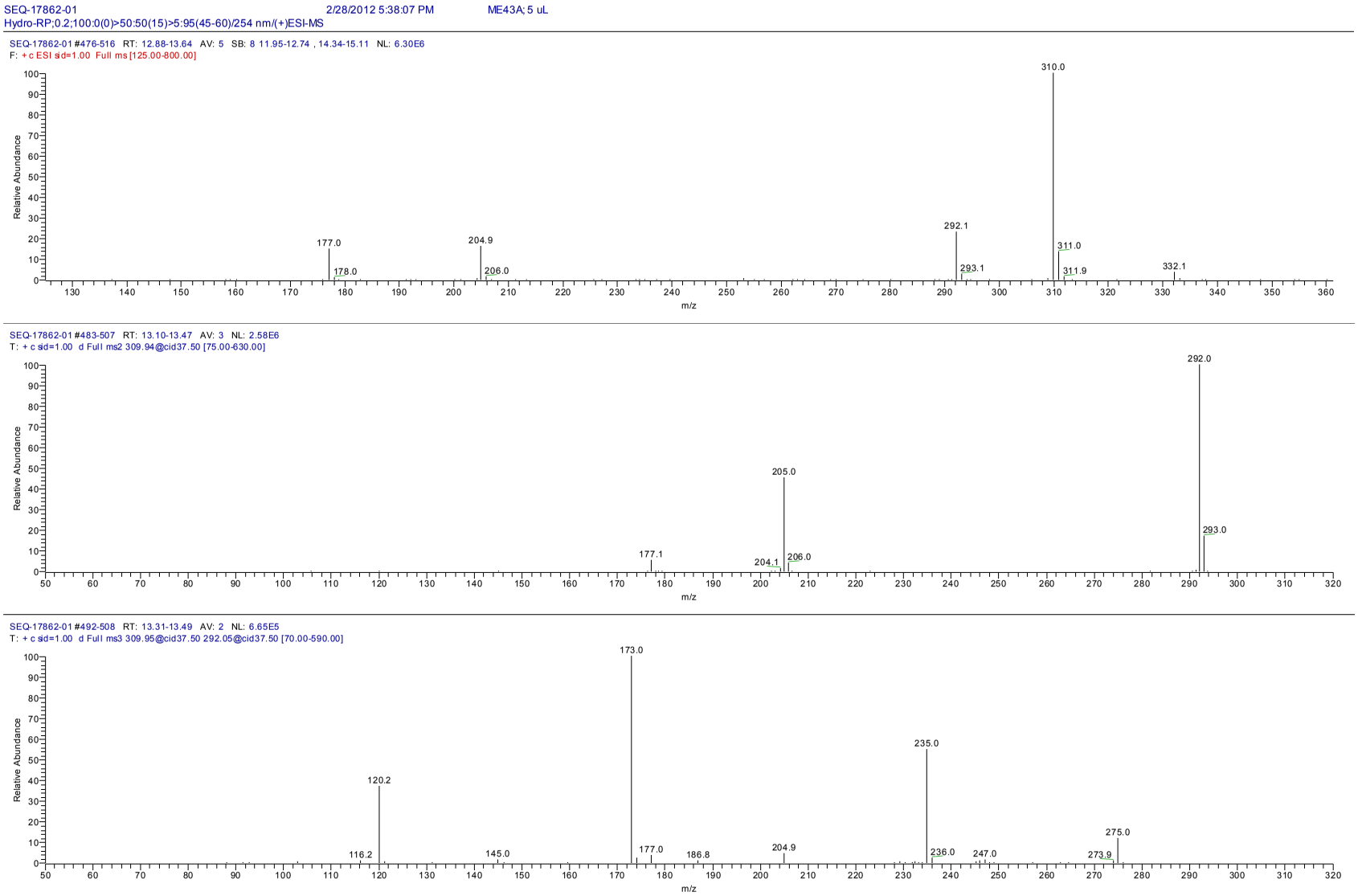


Figure S41. m/z 310, RT 18.6 min: (+)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 310 [M+H]+ ion. Note that the two CID spectra are almost identical to those of the m/z 310 from the MW 309, RT 13.28 min in the previous spectra. The m/z 310 is likely the same or very similar structure, even if it is a fragment ion from a higher MW compound.

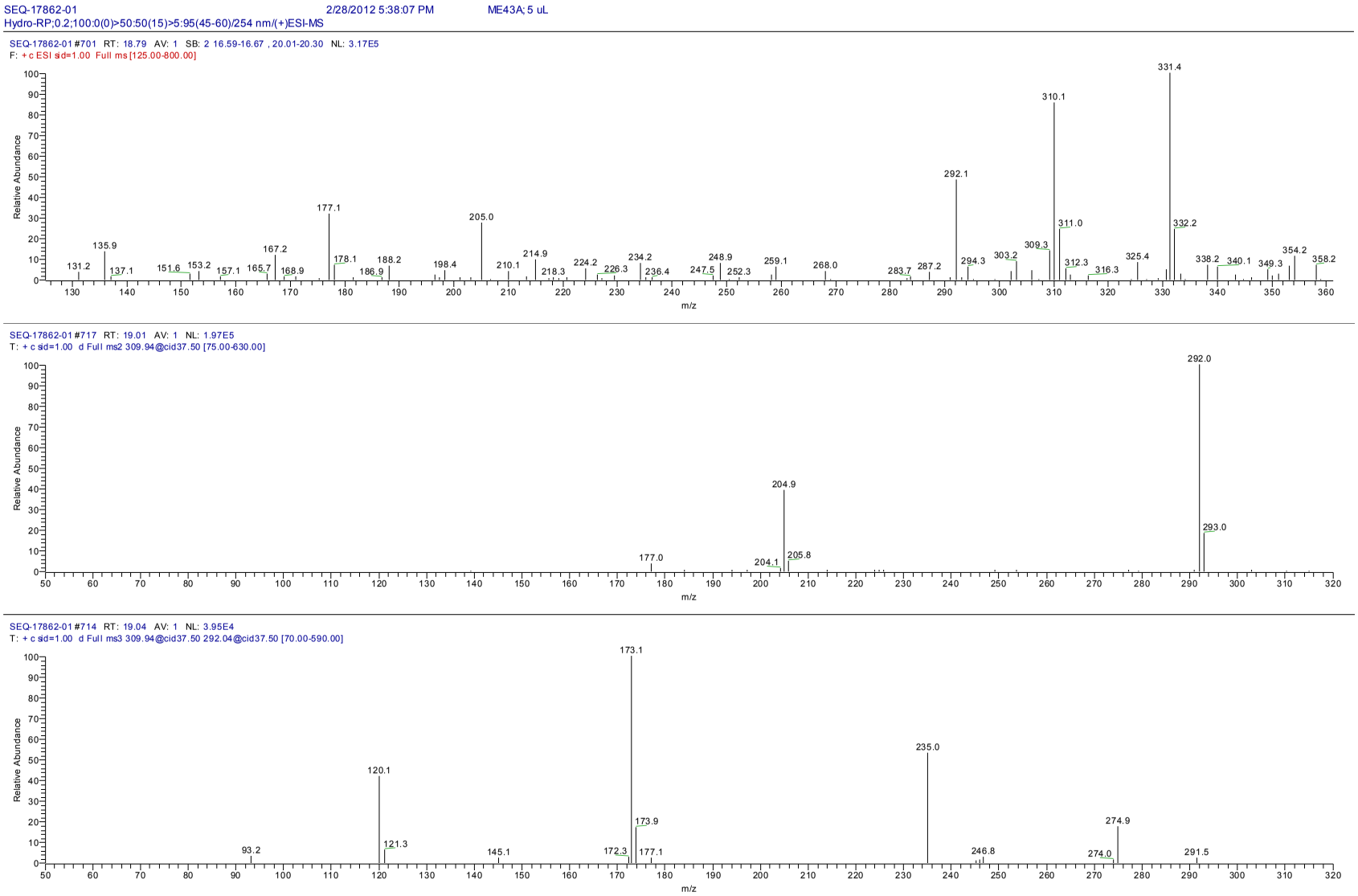


Figure S42. MW 309, (-)ESI-MS: With (-)ESI-MS, there were two m/z 308 ion-peaks.

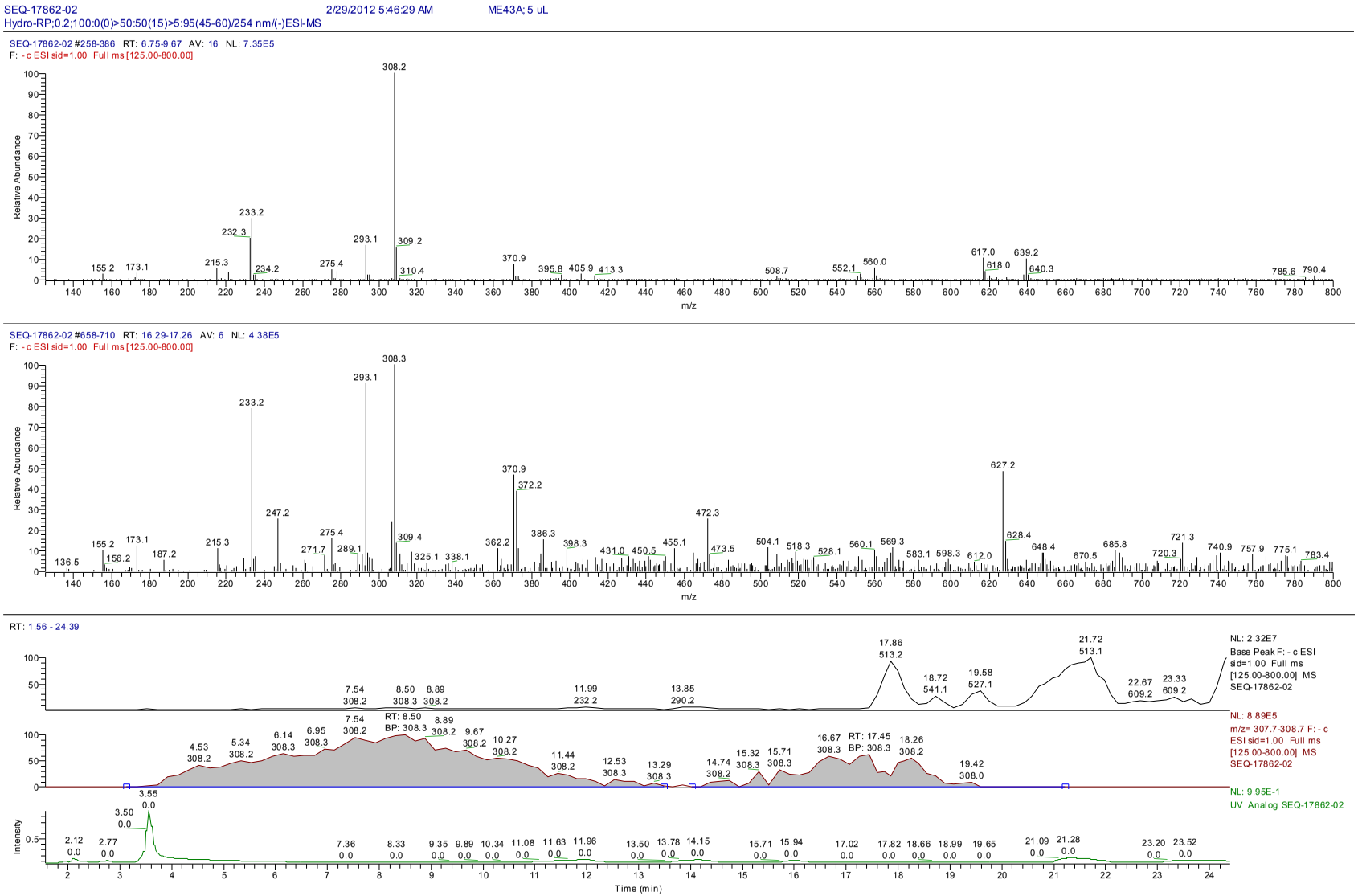


Figure S43. MW 309 compounds. (-)ESI-MS/MS (top and 3rd) and –MS/MS/MS (2nd and bottom) of the m/z 308 [M-H]- ions of the RT 8.5 min (top two) and RT 17.5 min (bottom two) m/z 308 ion-peaks.

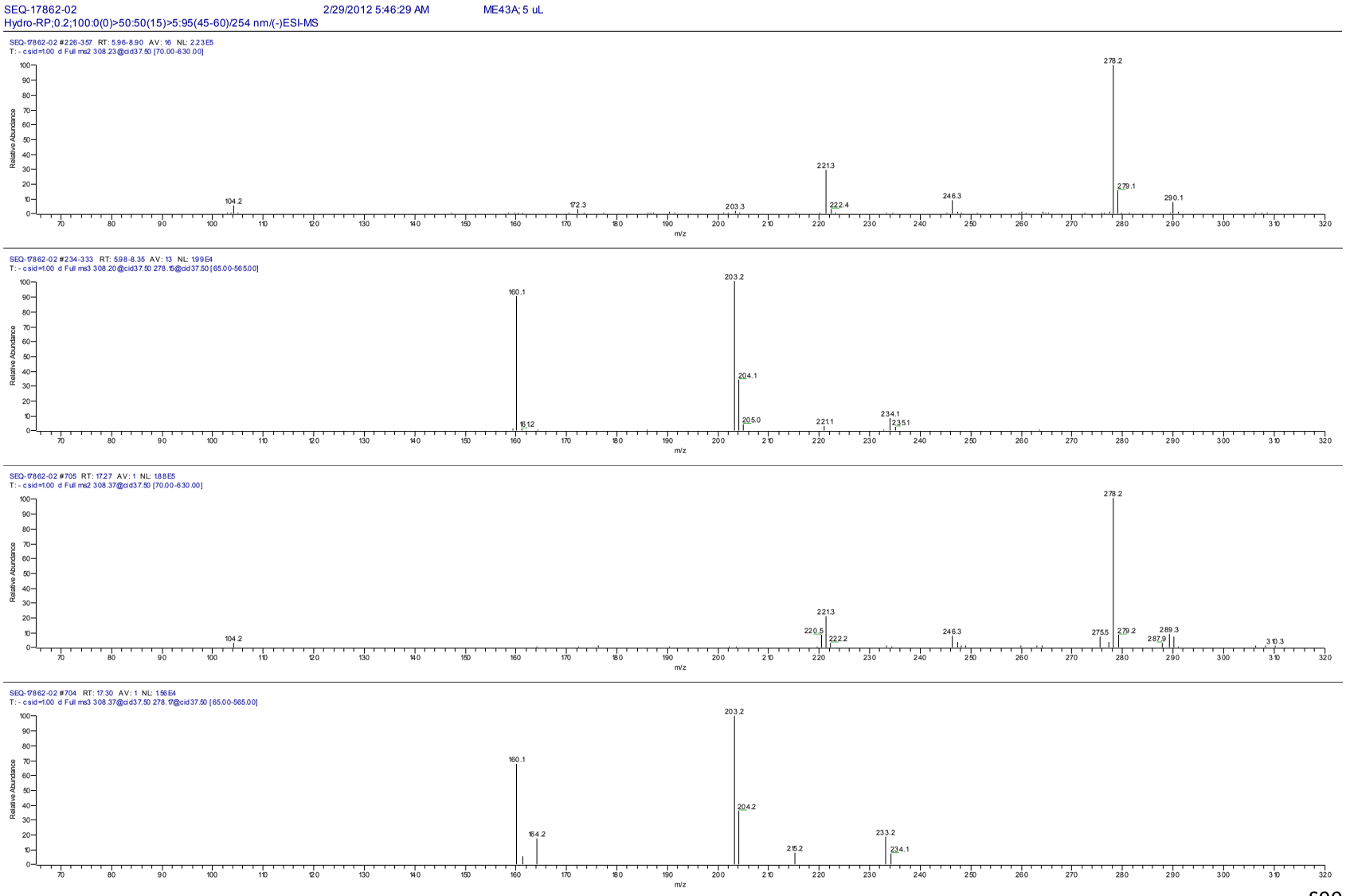


Figure S44. MW 719, RT 27.56: The MW 719 formed m/z 720 [M+H]+ and m/z 742 [M+Na]+ ions (spectrum on top and chromatograms on bottom). However, it co-eluted with other more abundant compounds and neither of the two molecular-type ions were chosen for CID scans in this analysis. A third analysis was done to obtain the MSn spectra of the MW 719 (see next Figure).

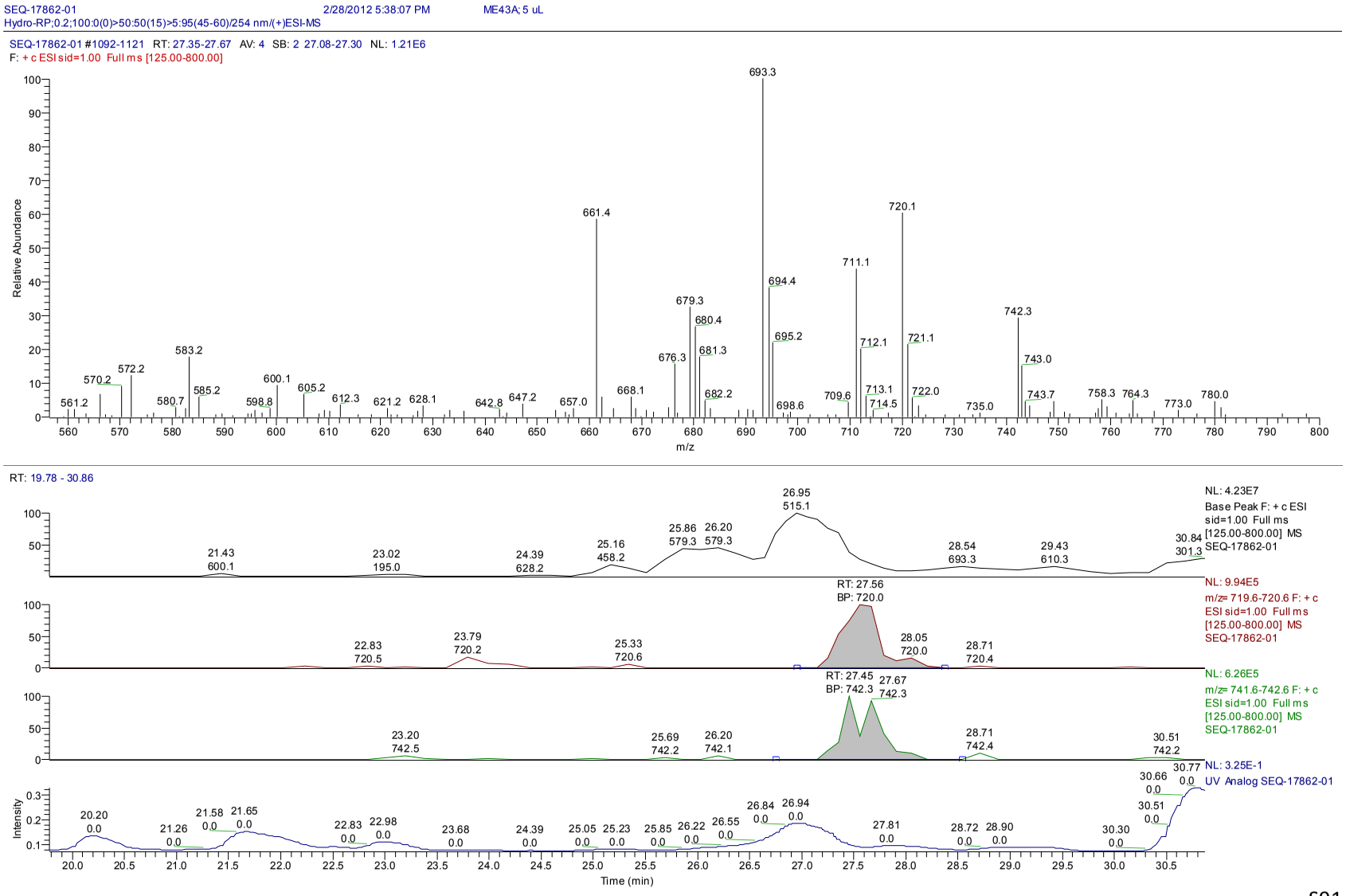


Figure S45. MW 719 from SEQ-17862-03 analysis. (+)ESI-MS (top) and –MS/MS (middle) and –MS/MS/MS (bottom) of the m/z 720 [M+H]+ ion.

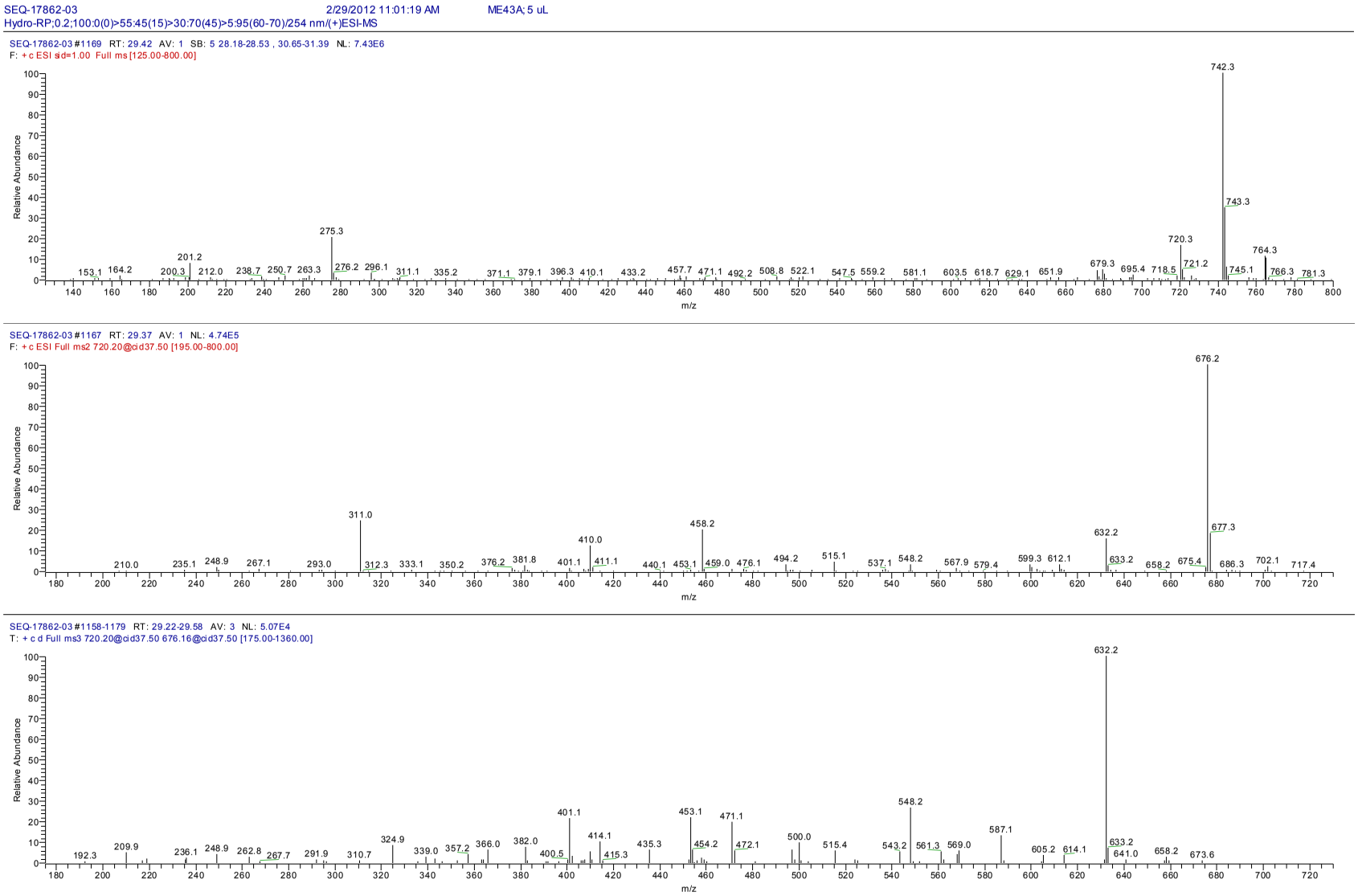


Figure S46. MW 719 from SEQ-17862-03 analysis. (+)ESI-MS (top) and –MS/MS of the m/z 742 [M+Na]+ ion (middle) with chromatograms on bottom.

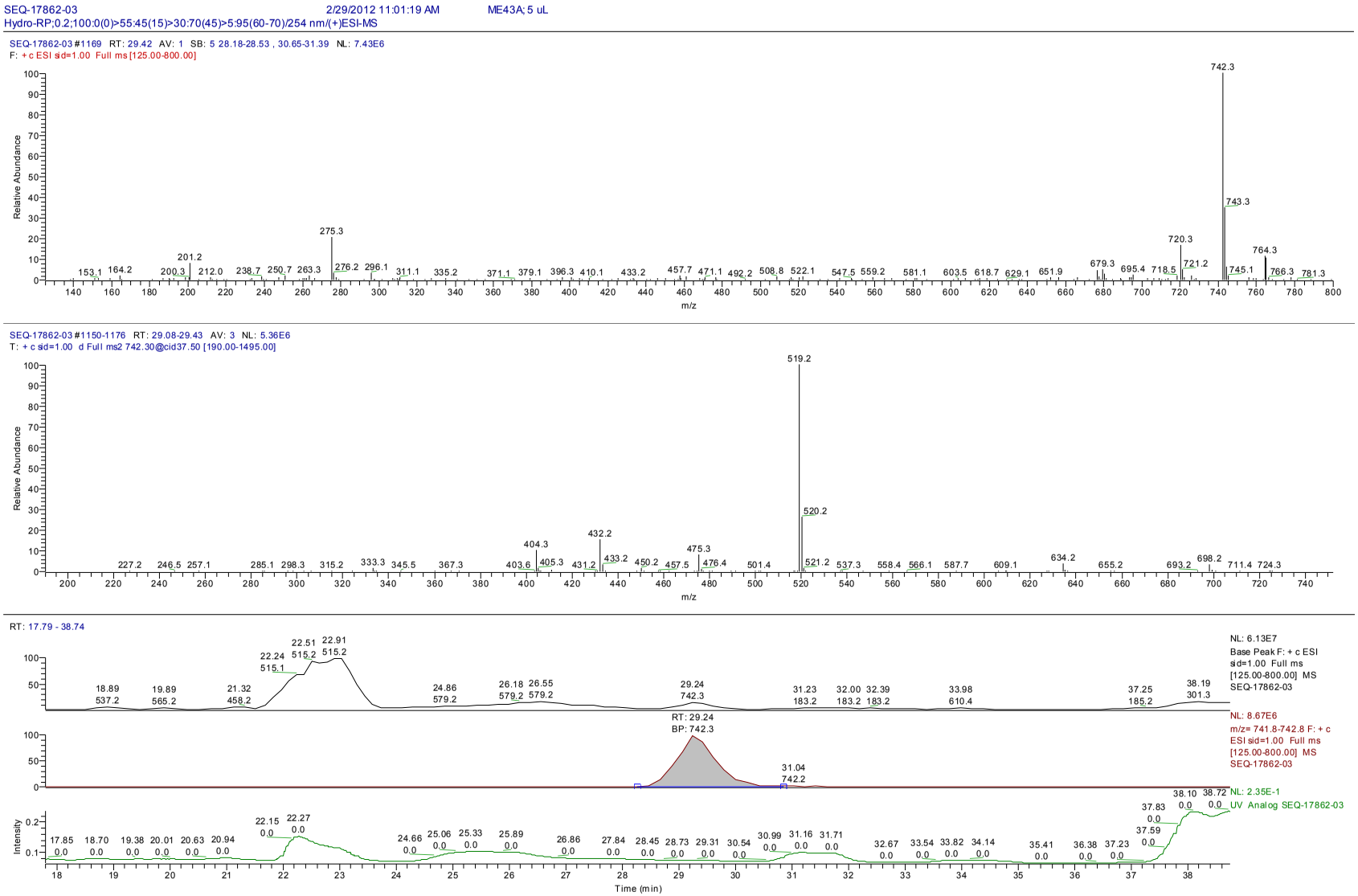


Figure S47. MW 719: produced an abundant m/z 718 [M-H]- ion (top) which was readily dissociated to a number of primary (middle) and secondary product ions (bottom).