Supplementary Material

Chemical ligation from O-acyl isopeptides *via* 8- and 11-membered cyclic transition states

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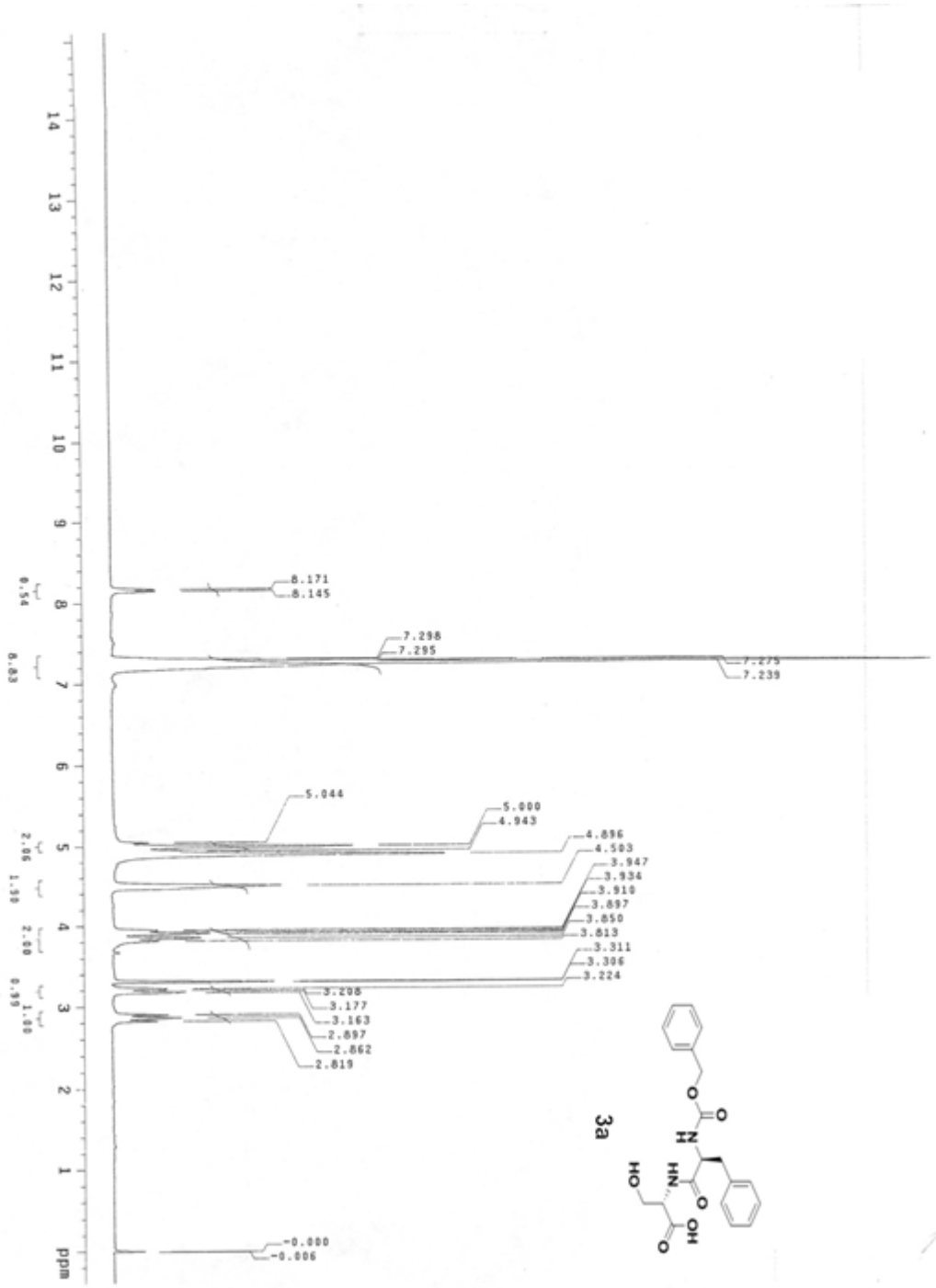

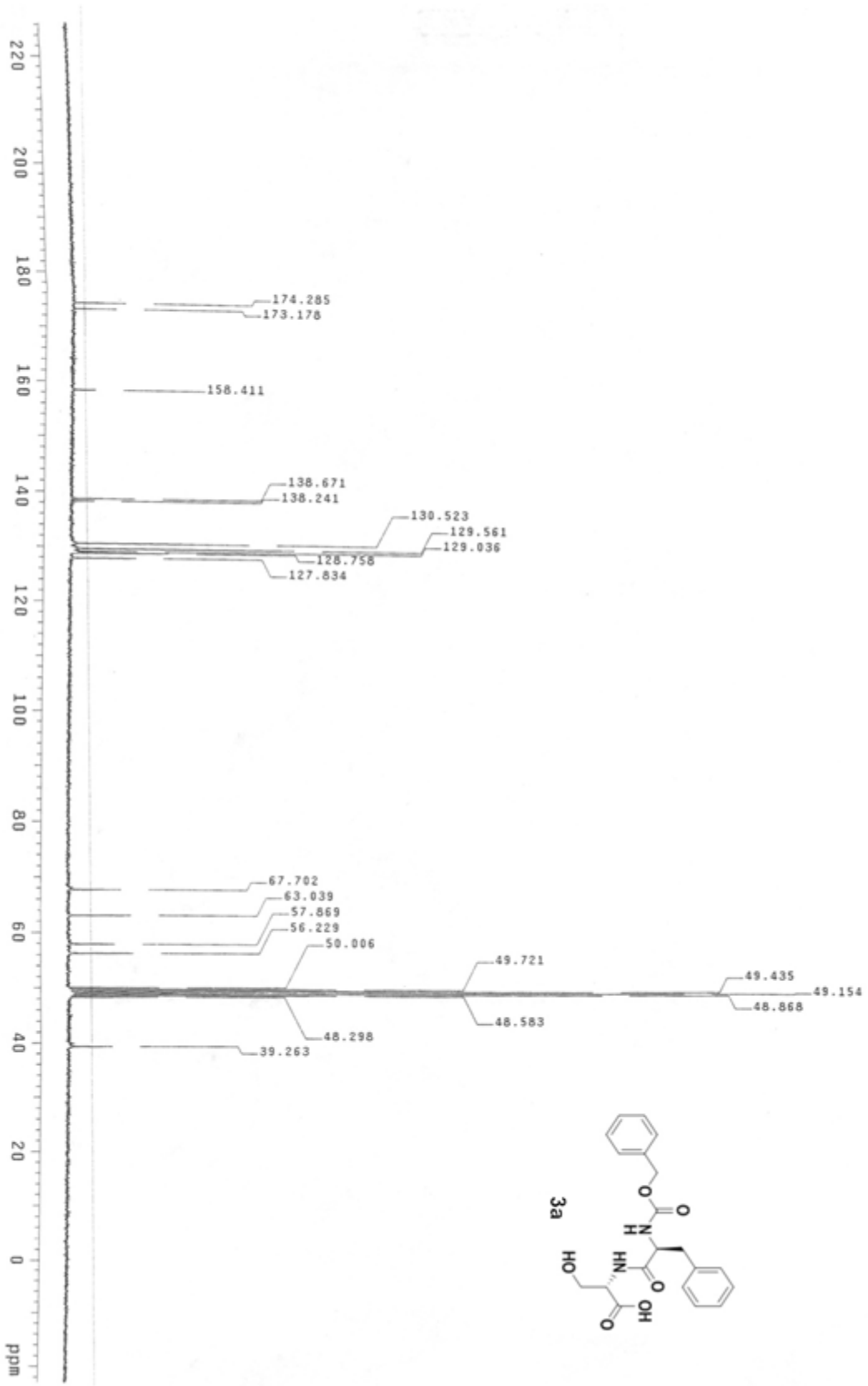

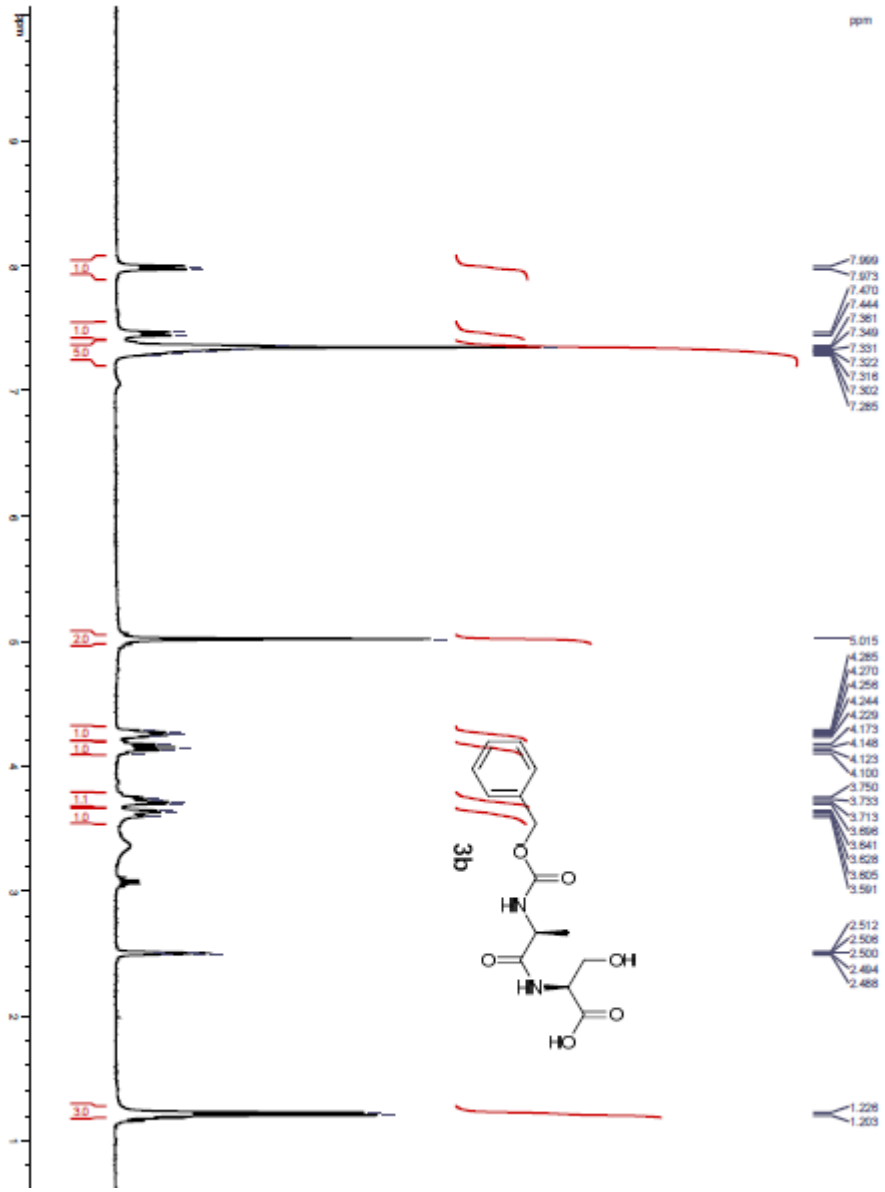

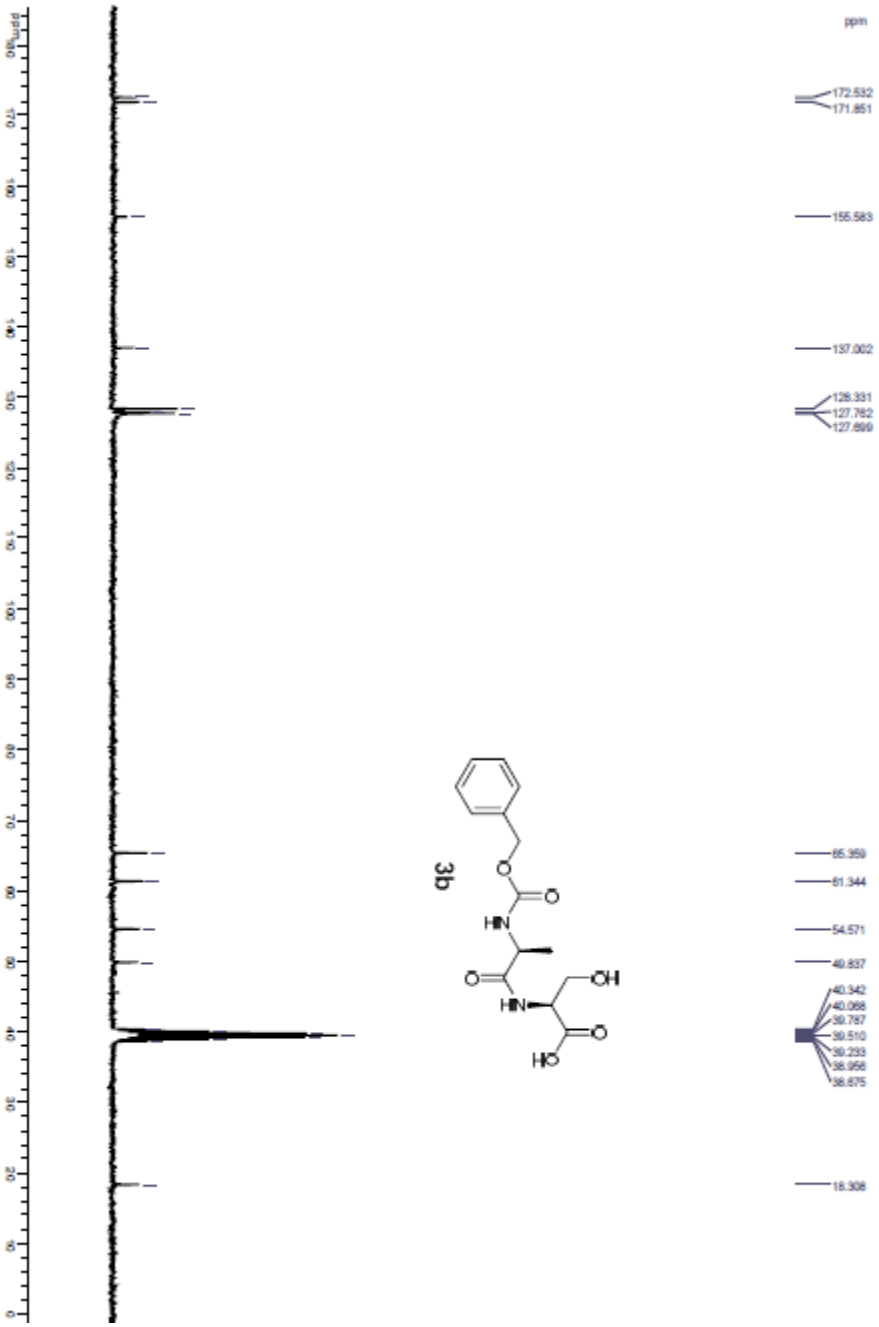

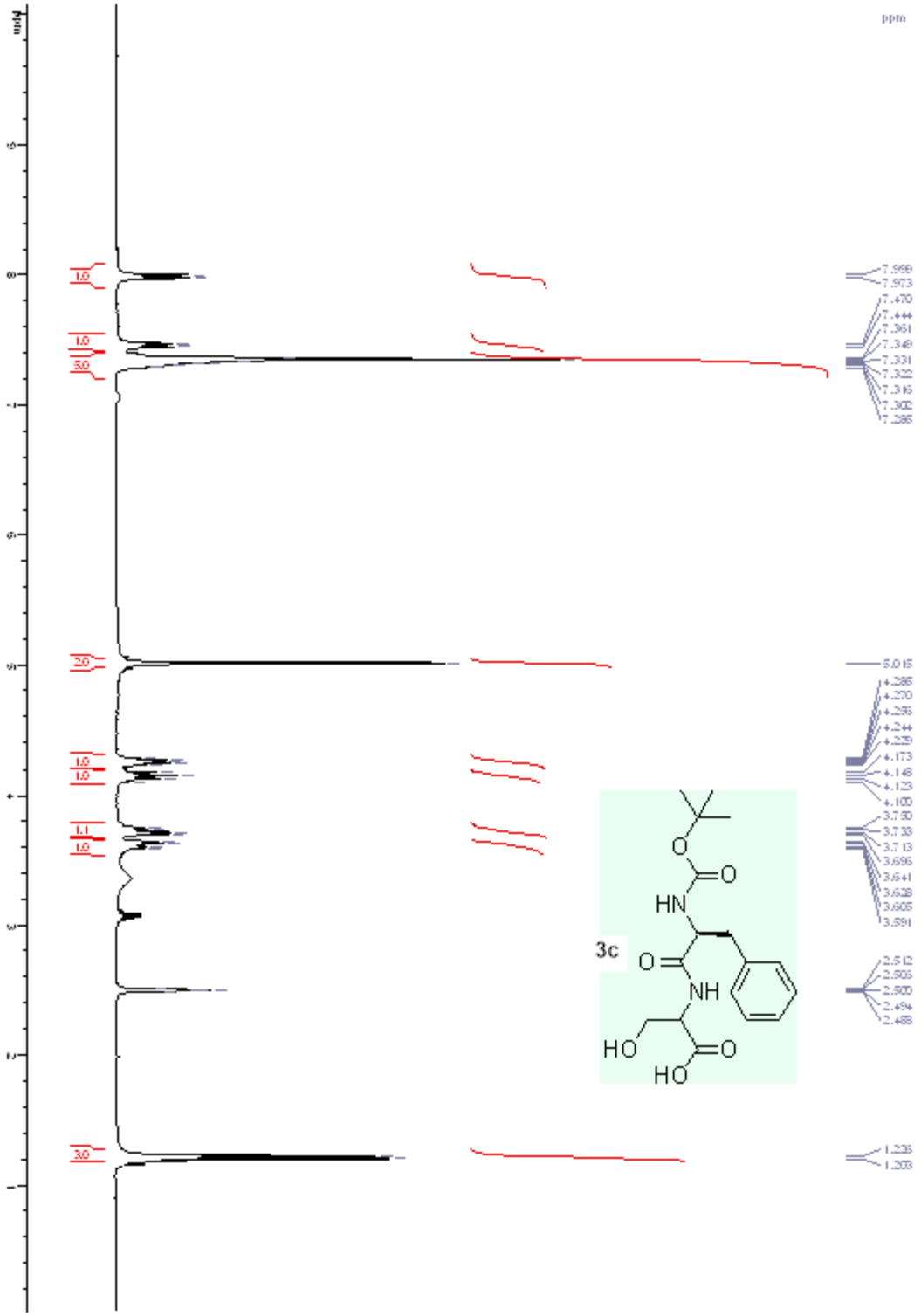

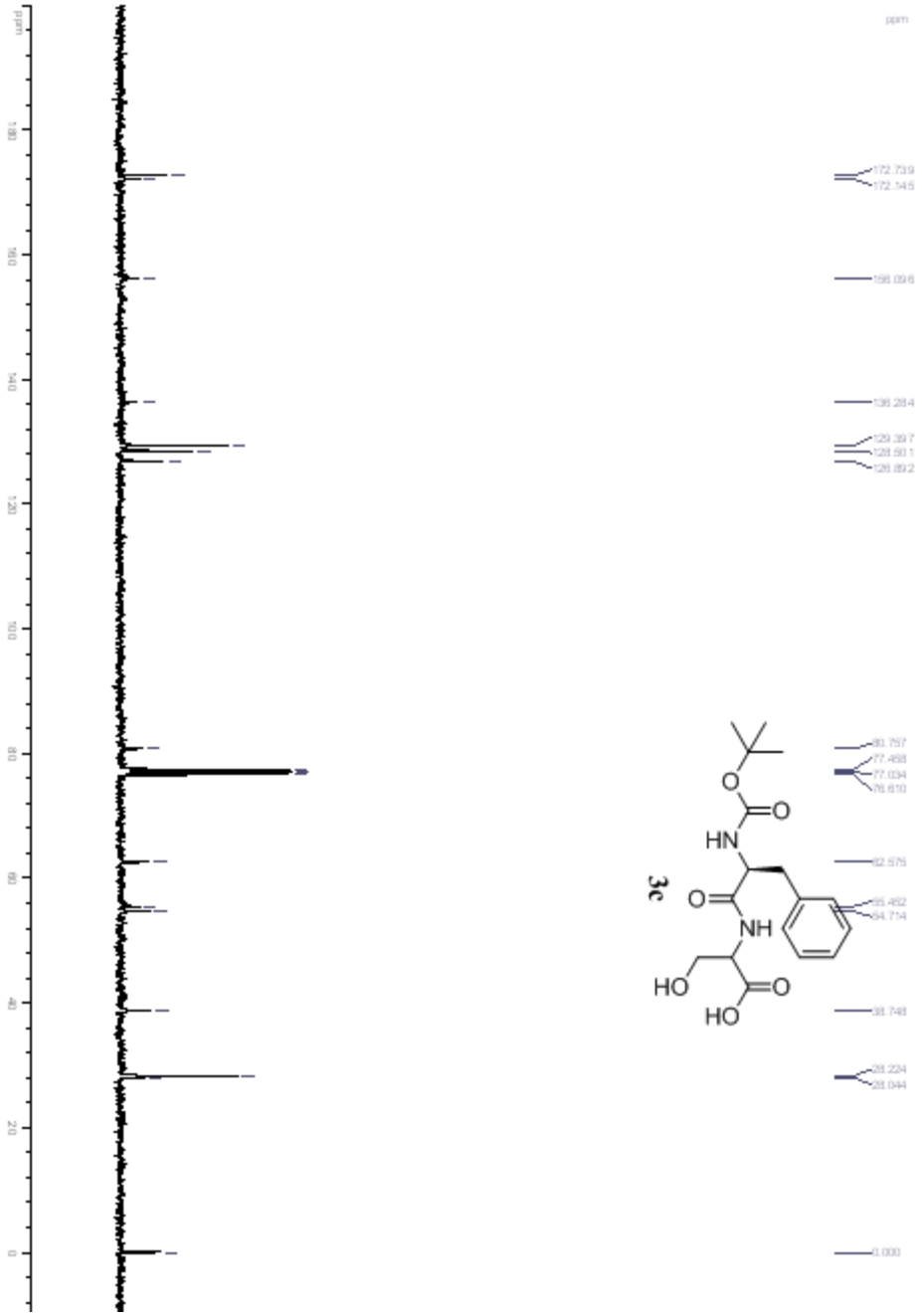

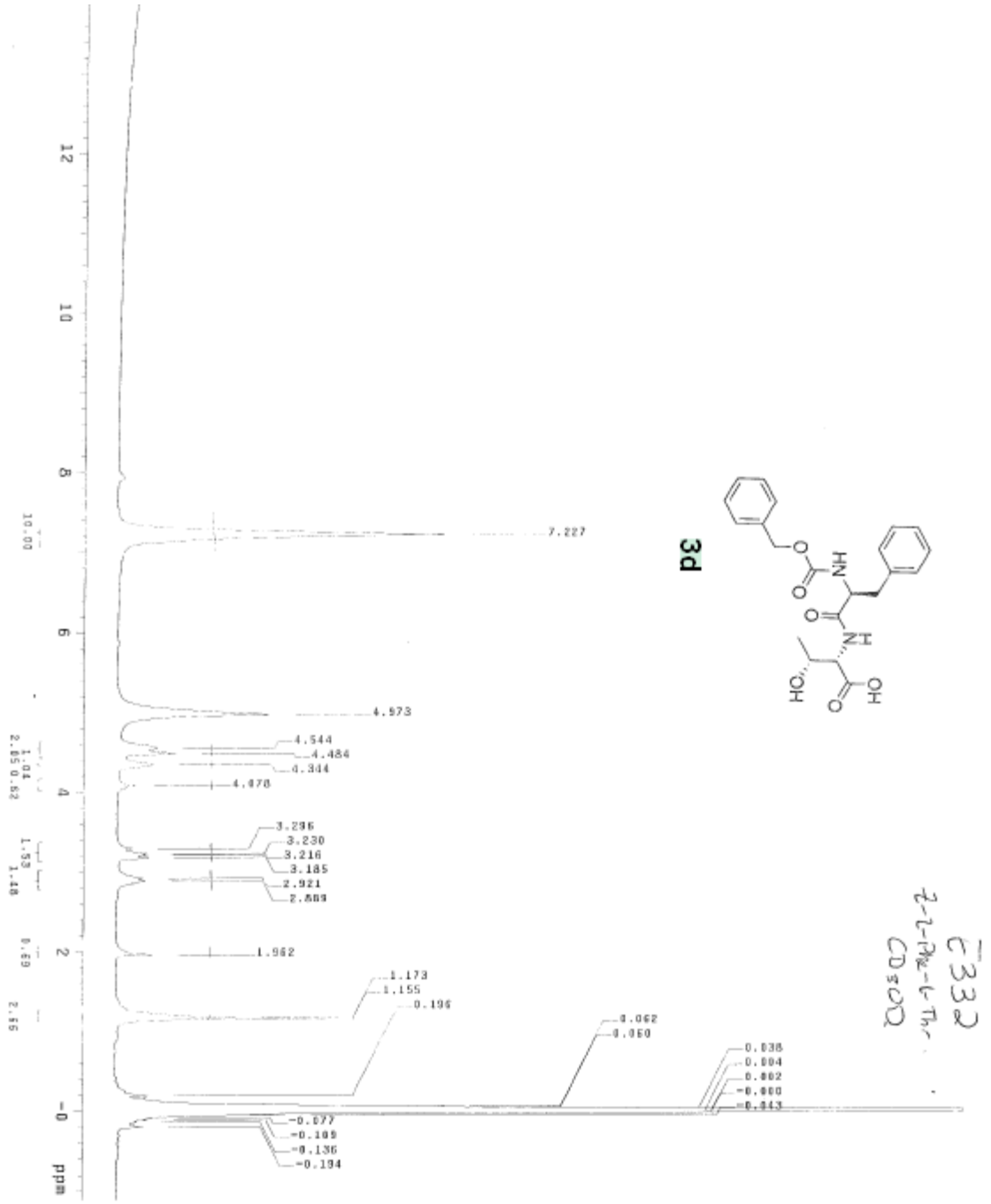

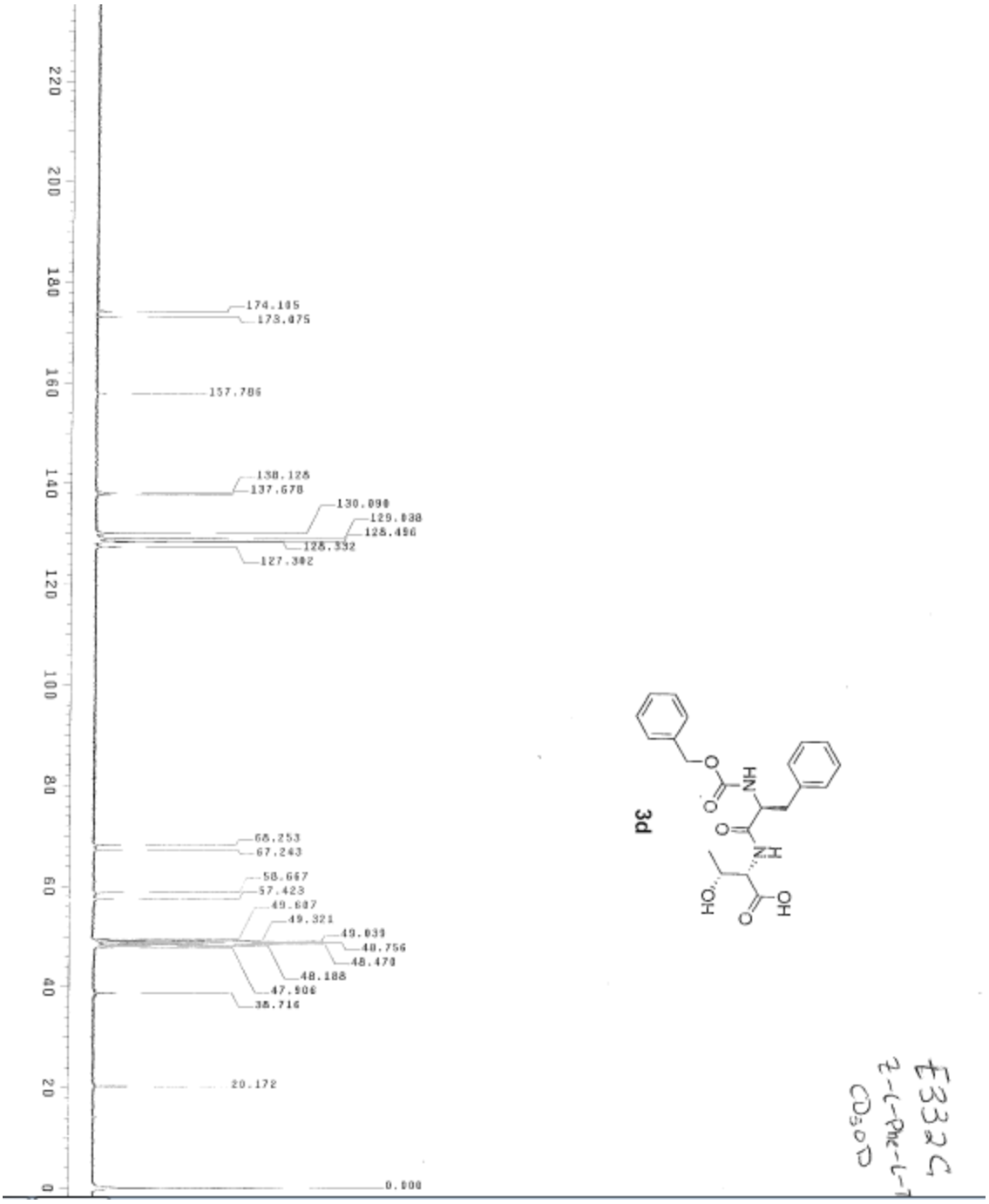

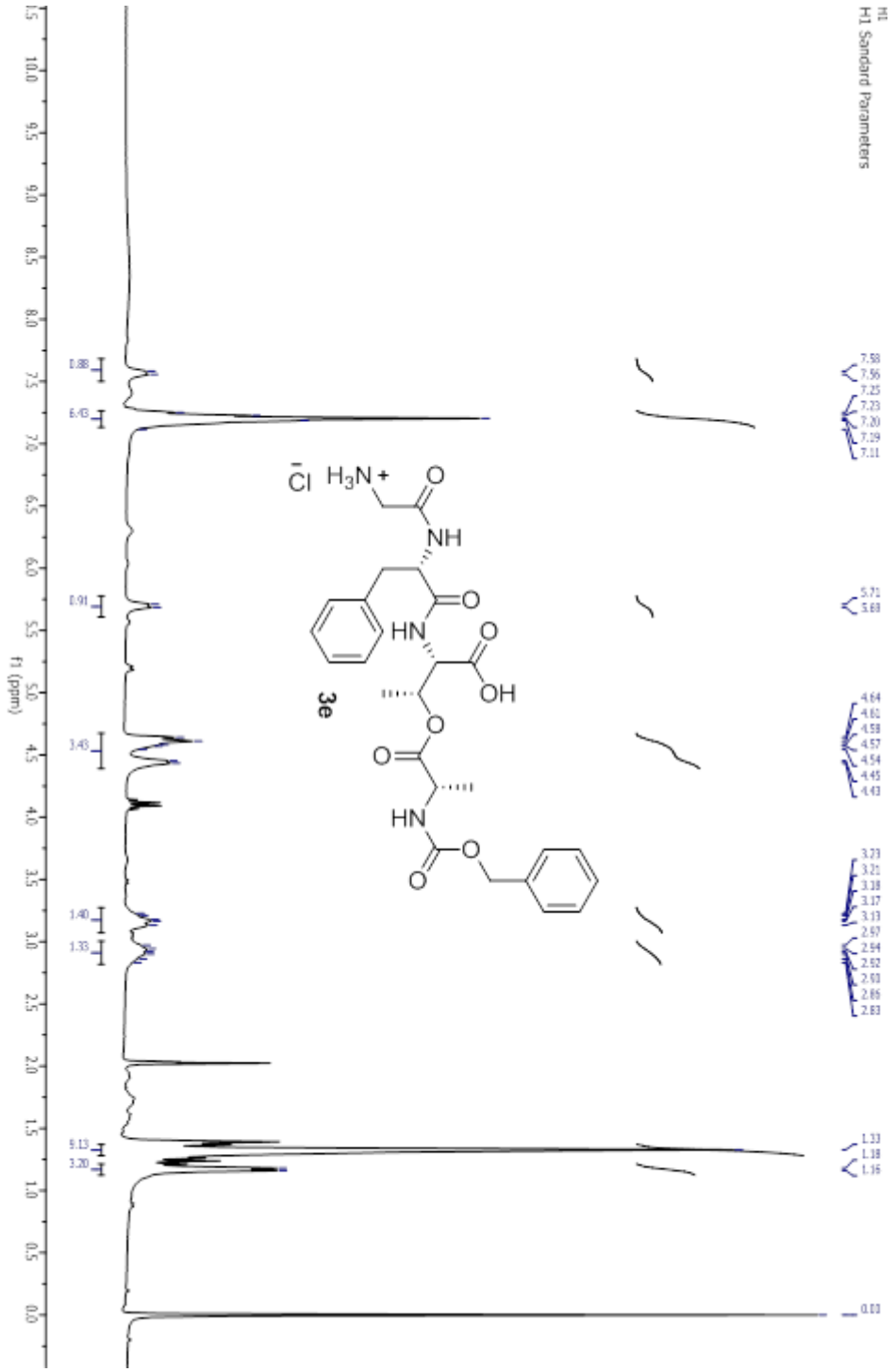

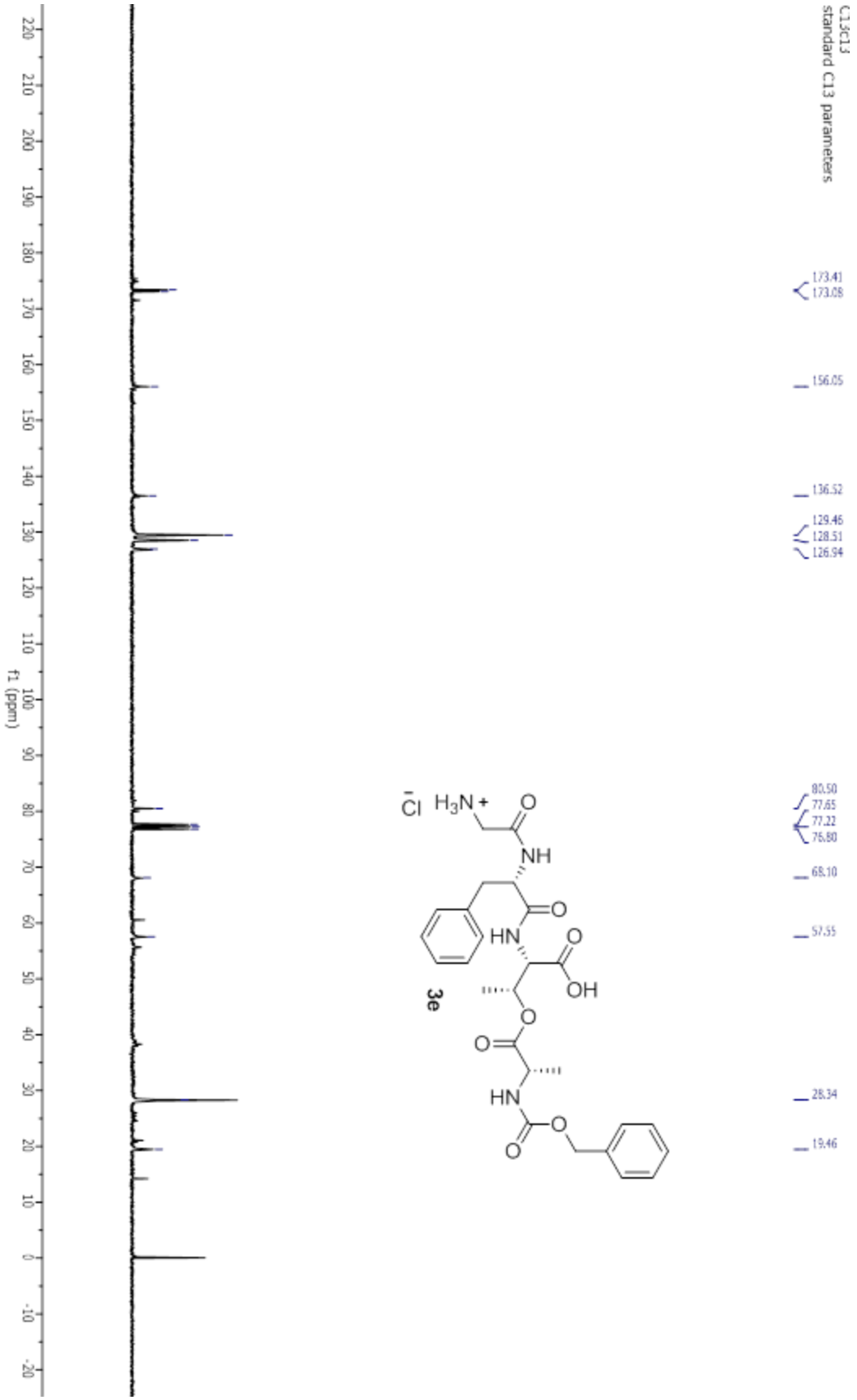

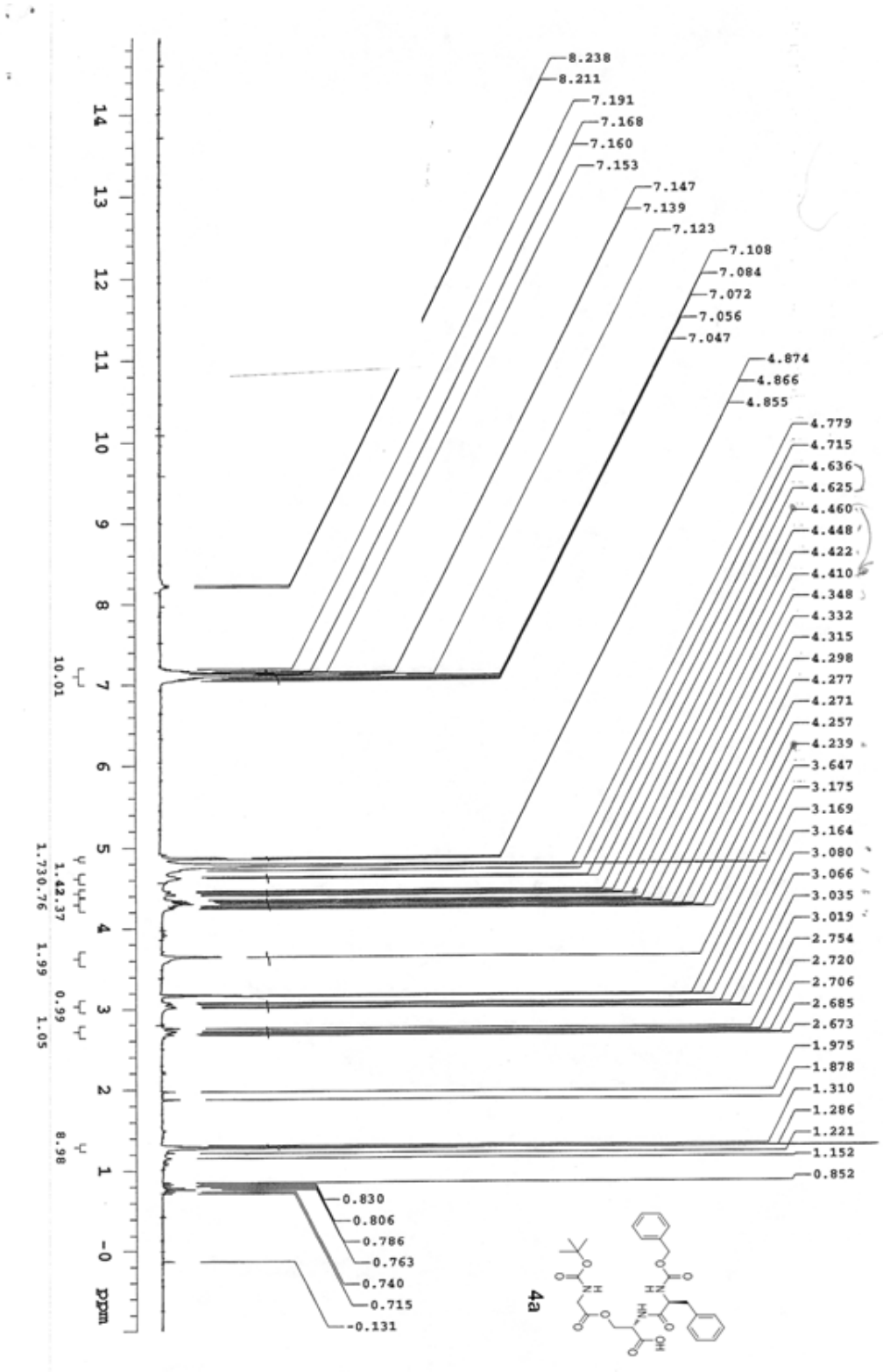

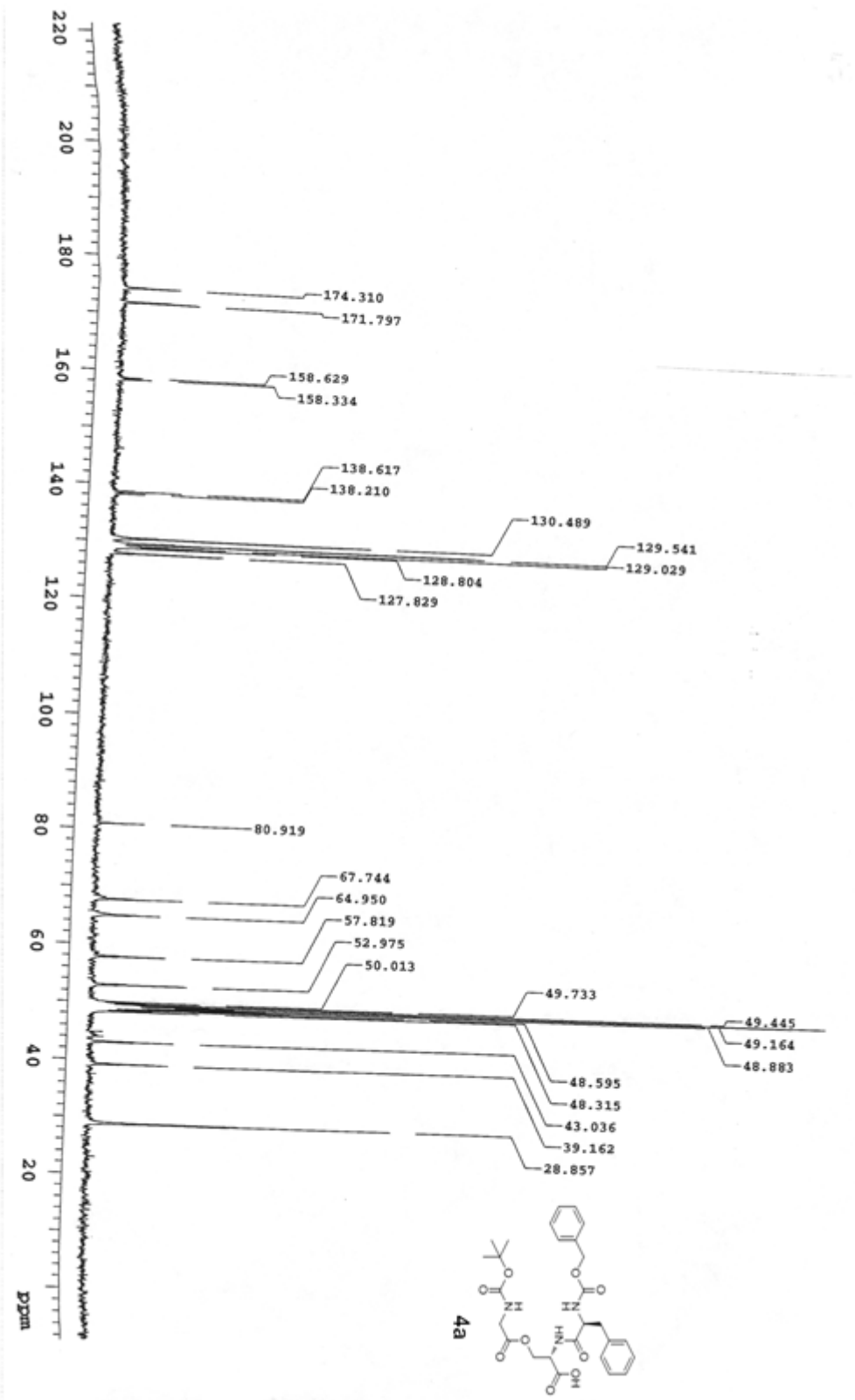

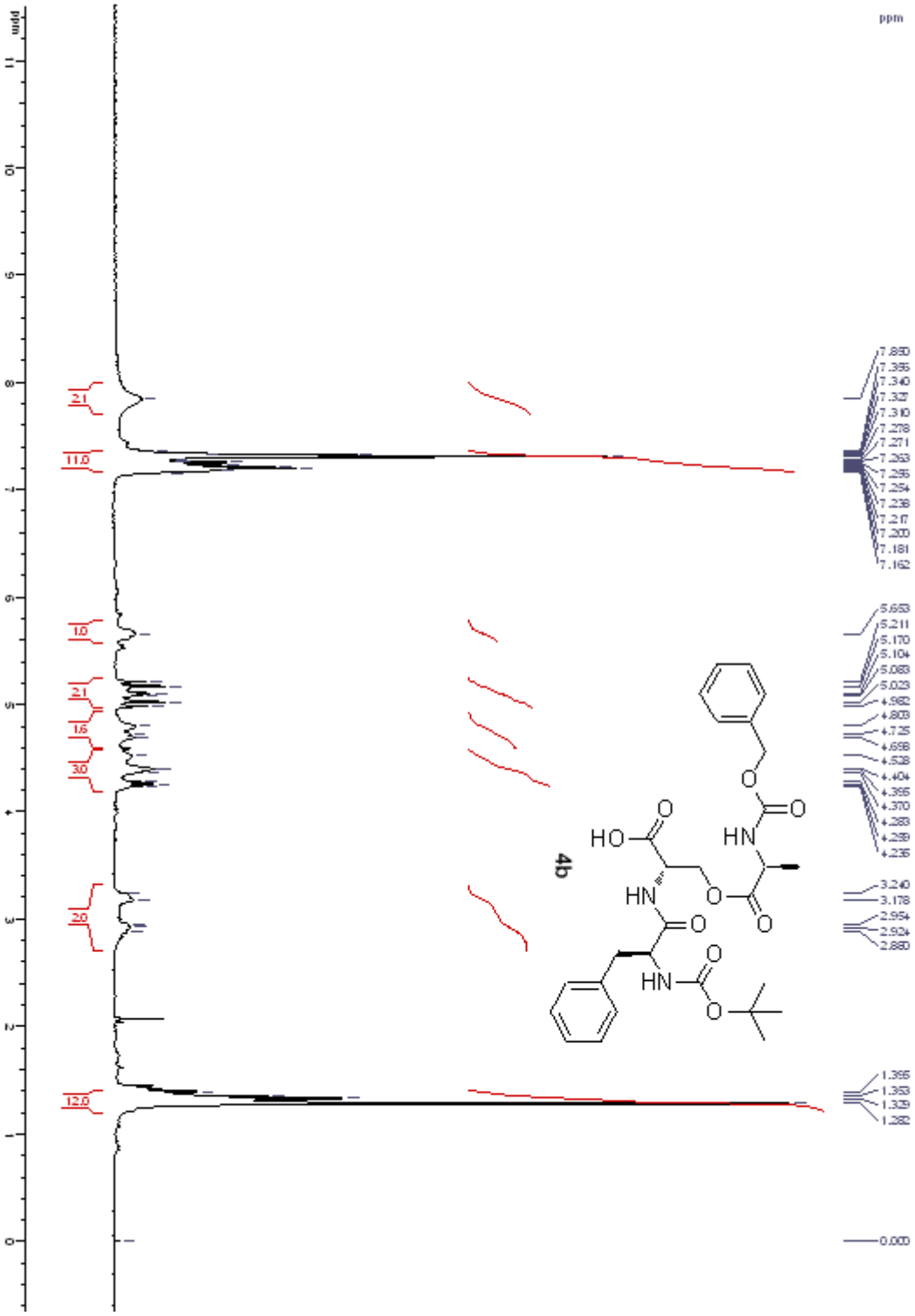

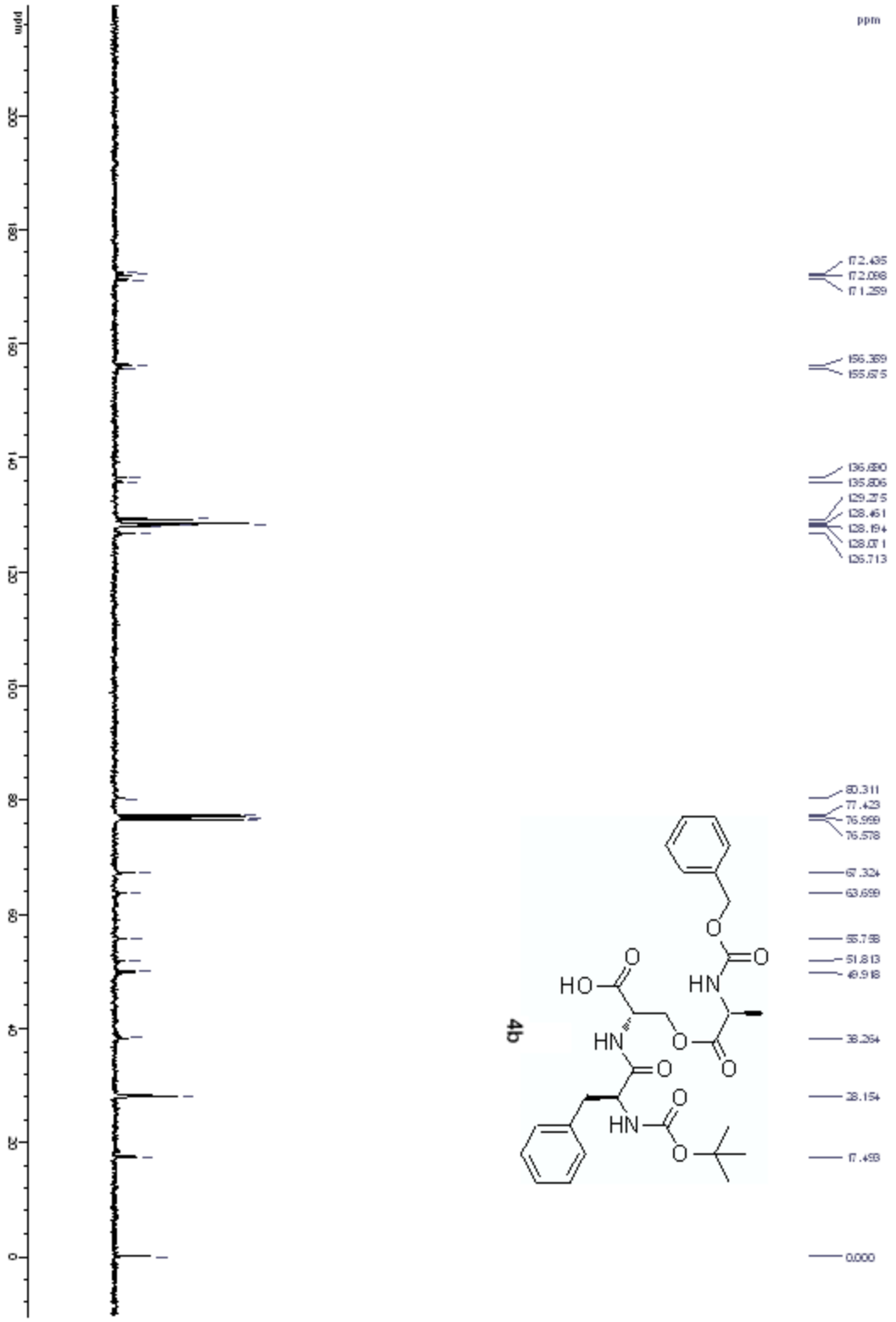

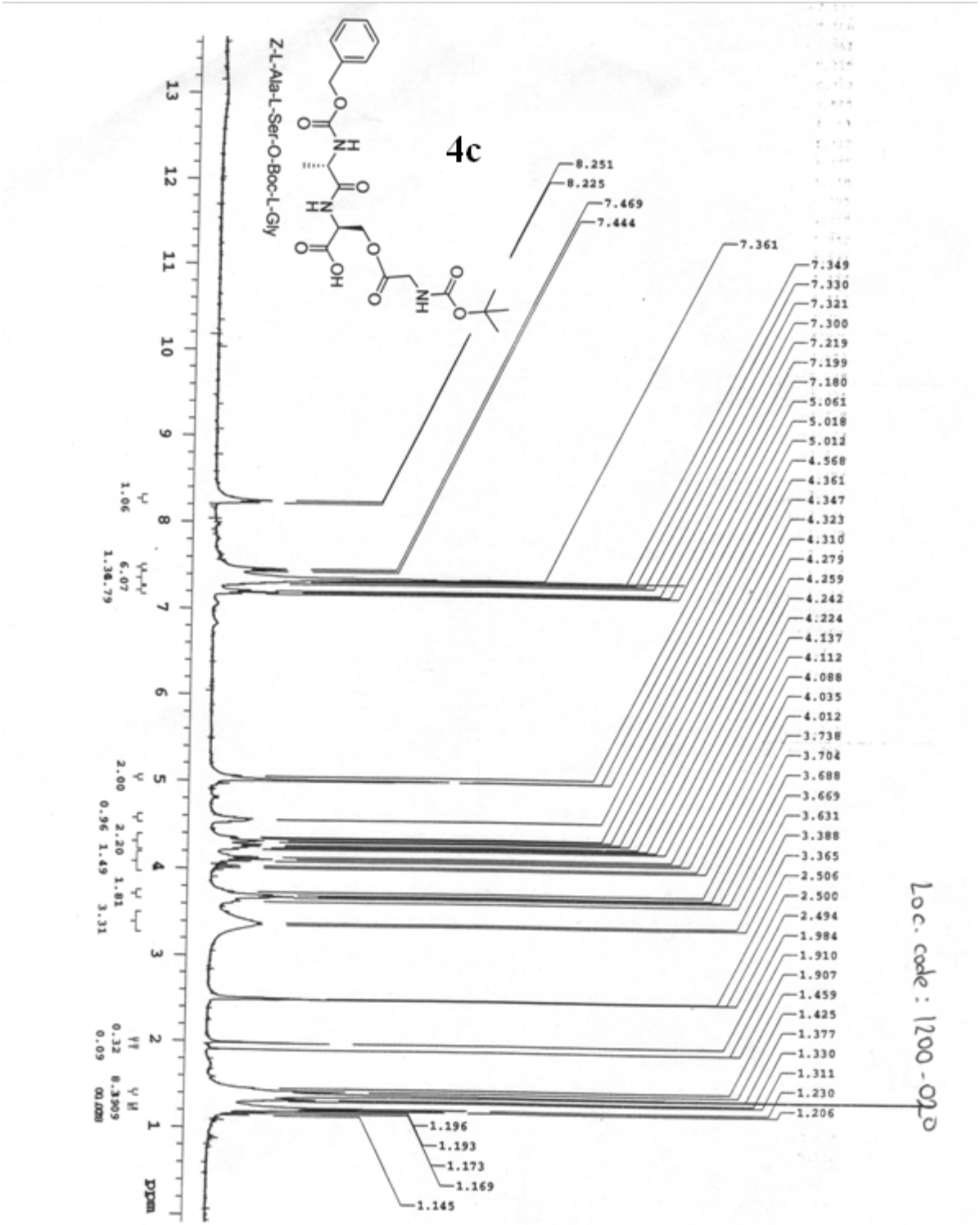

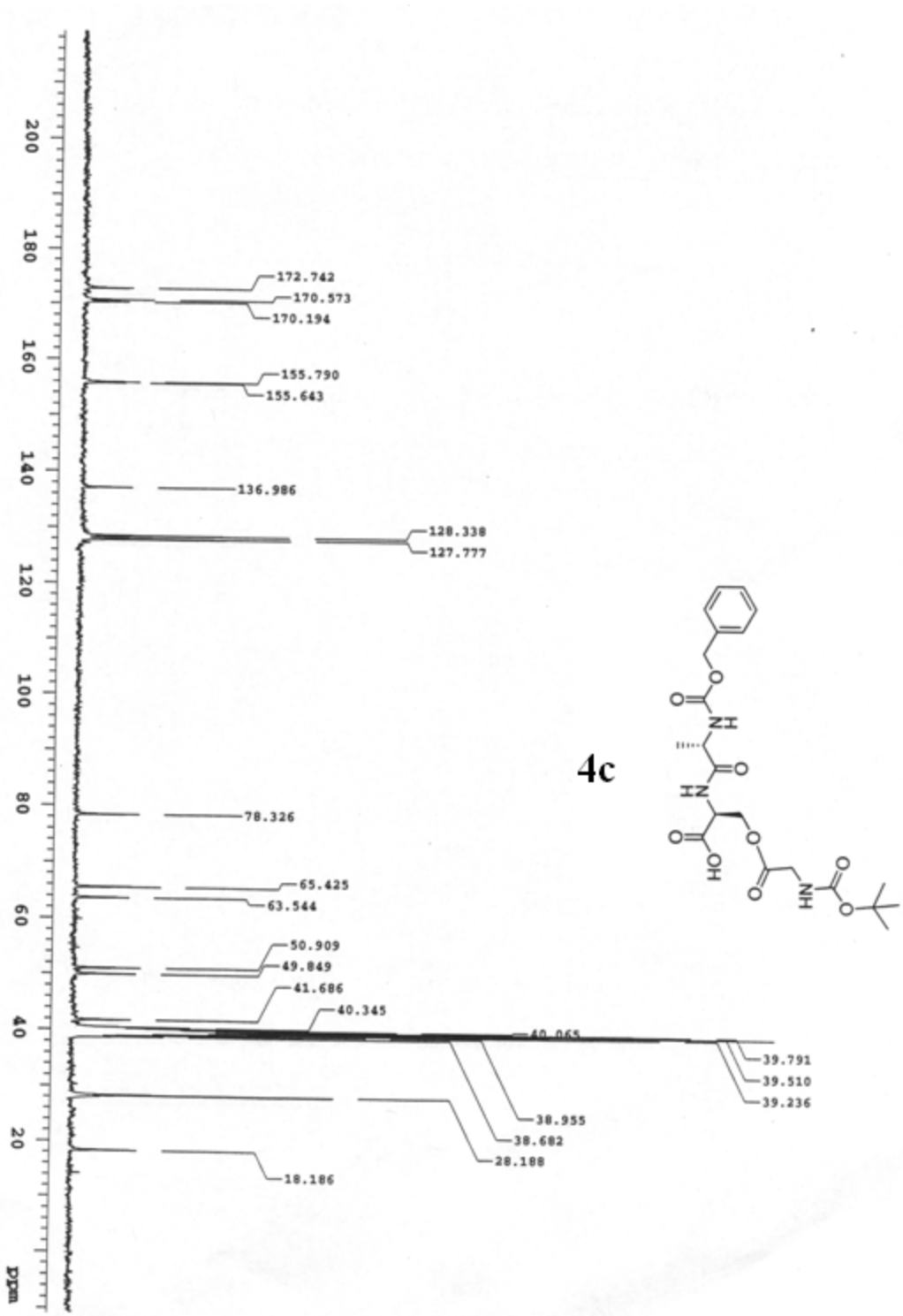

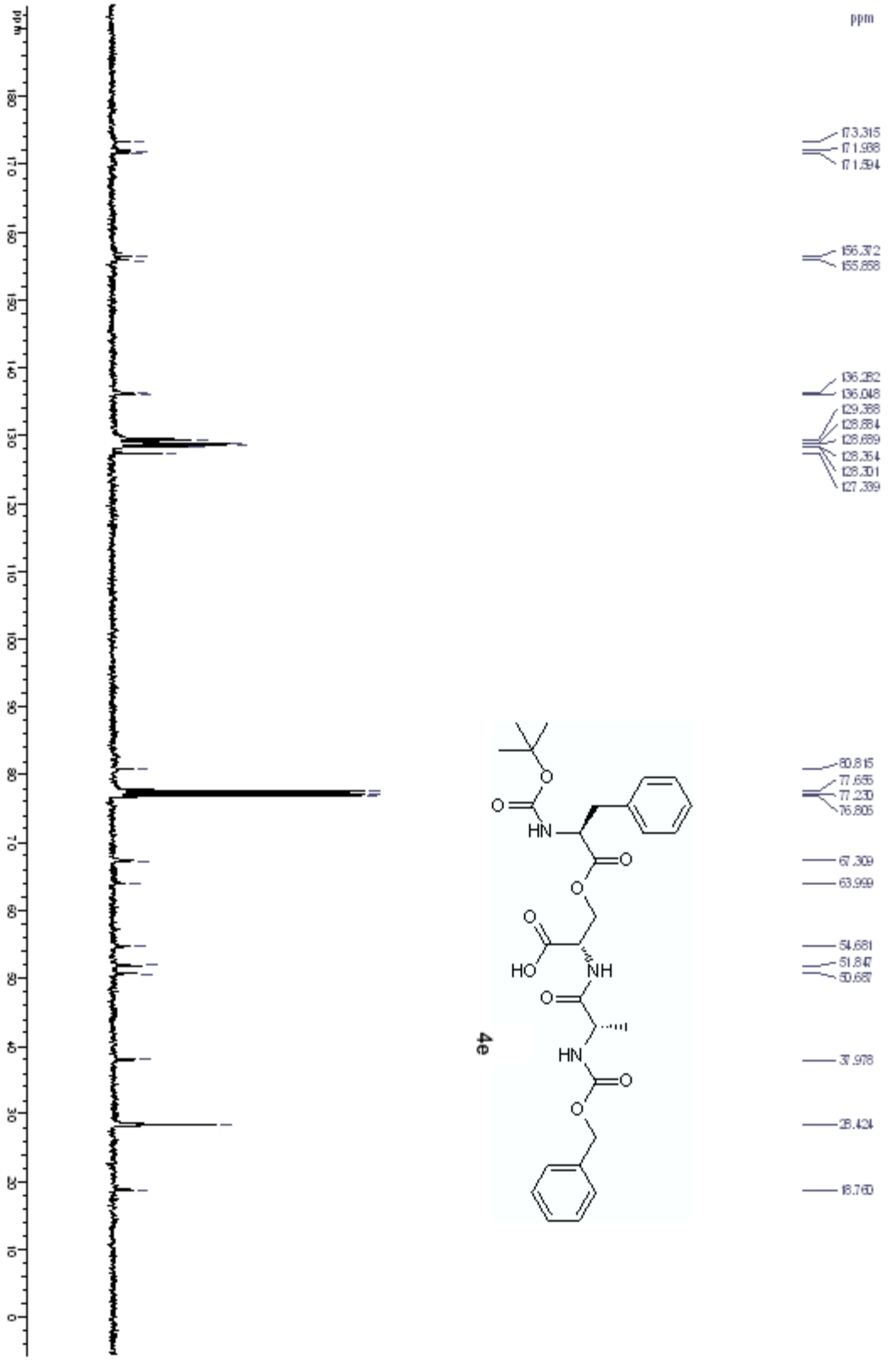

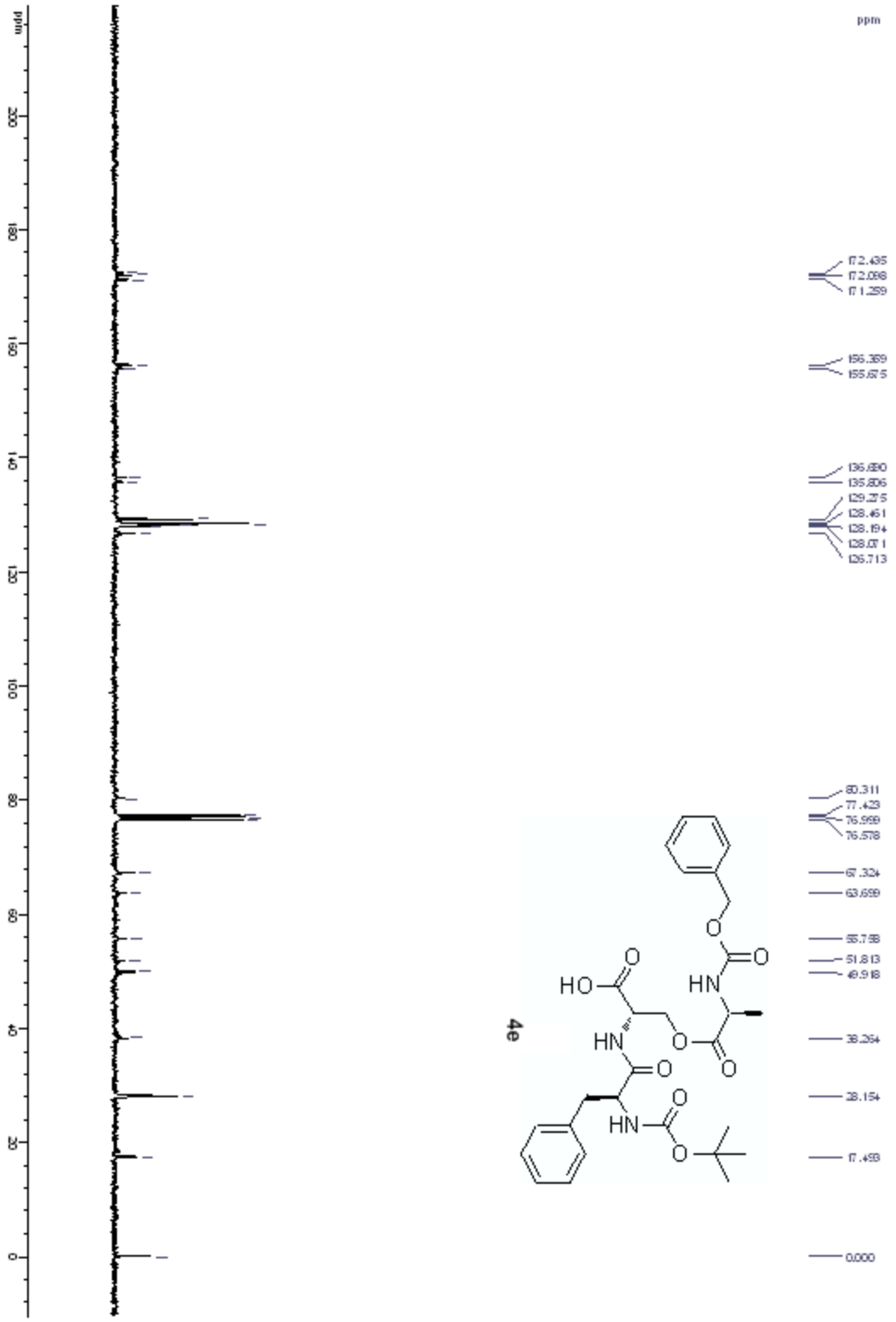

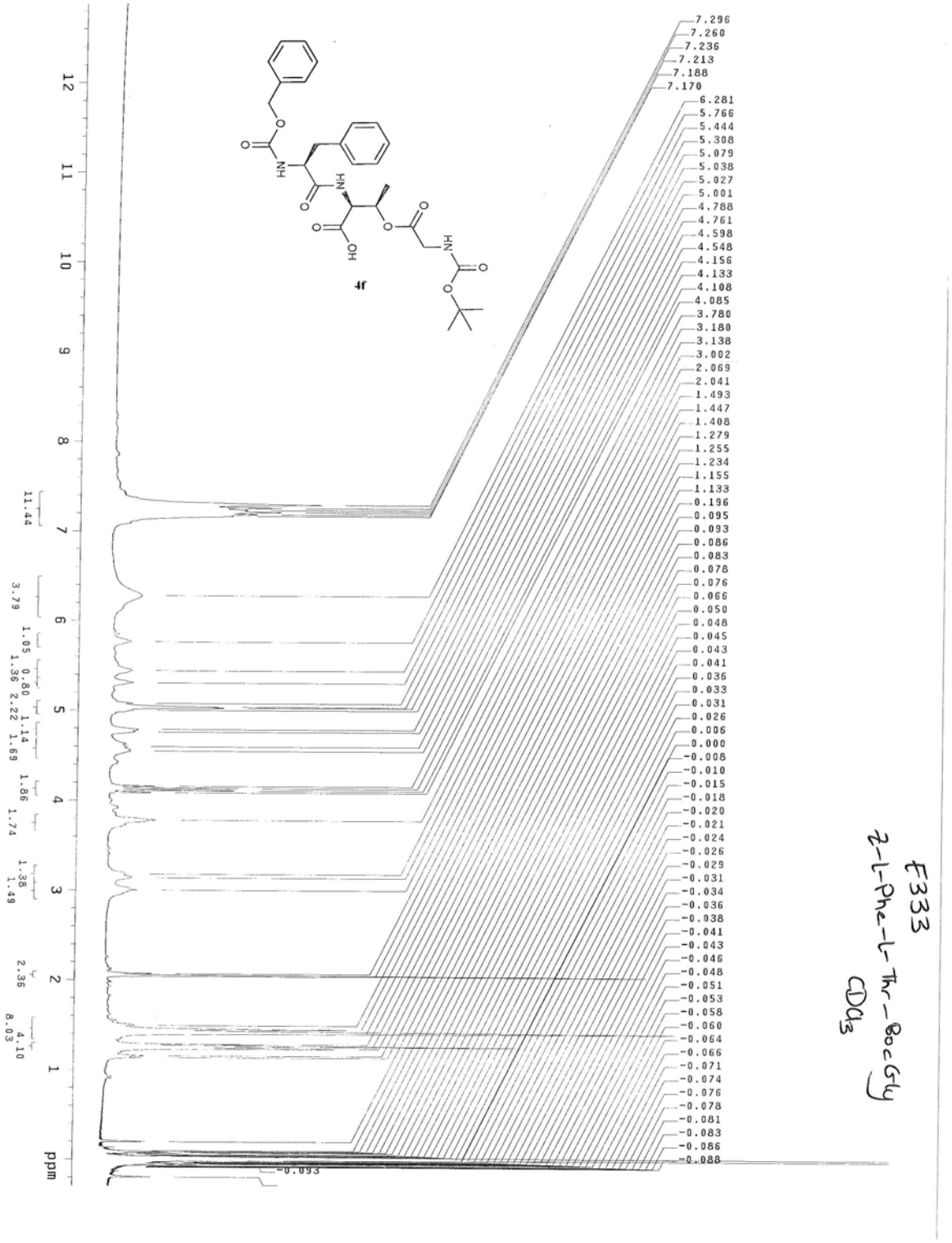

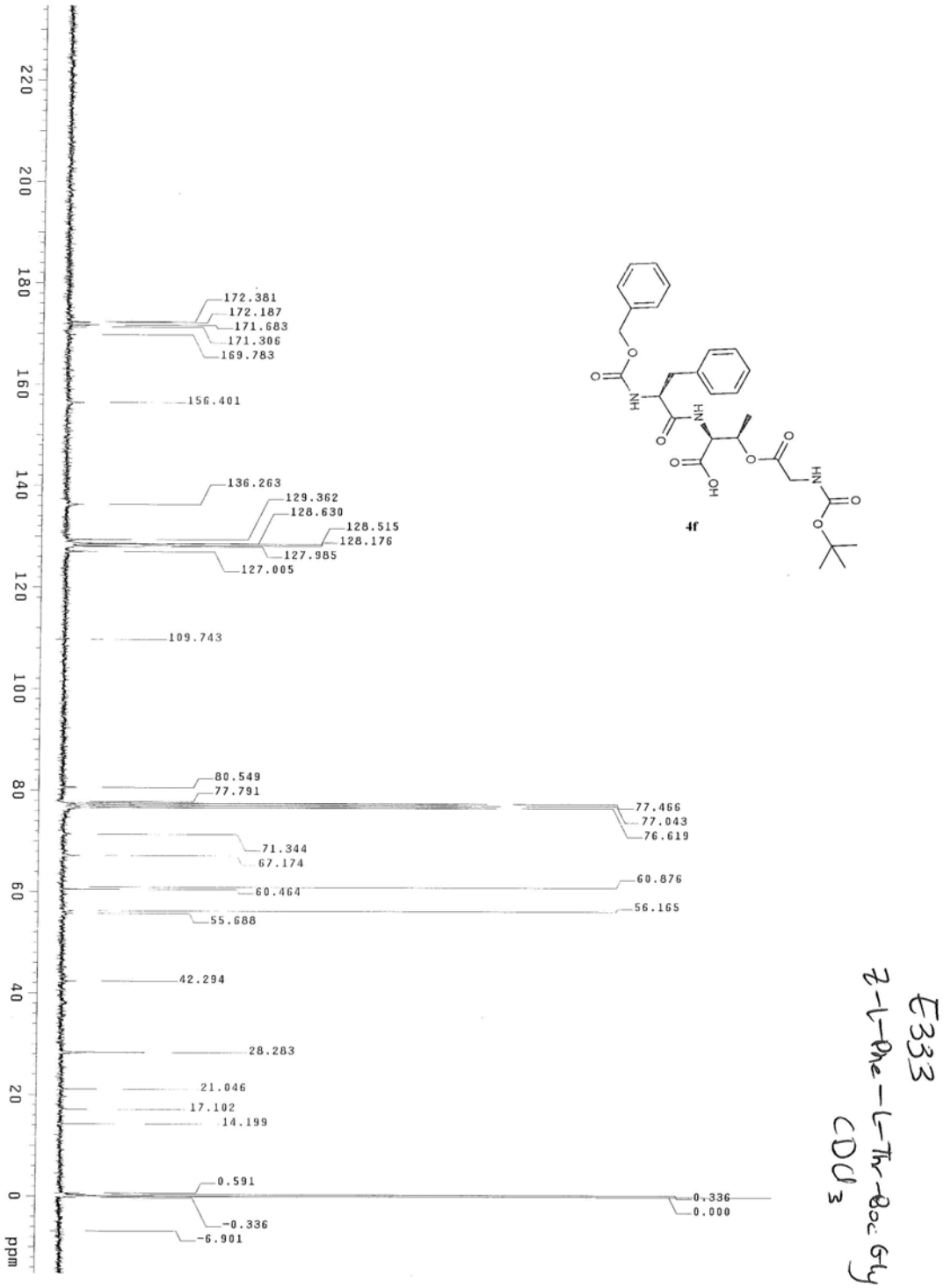

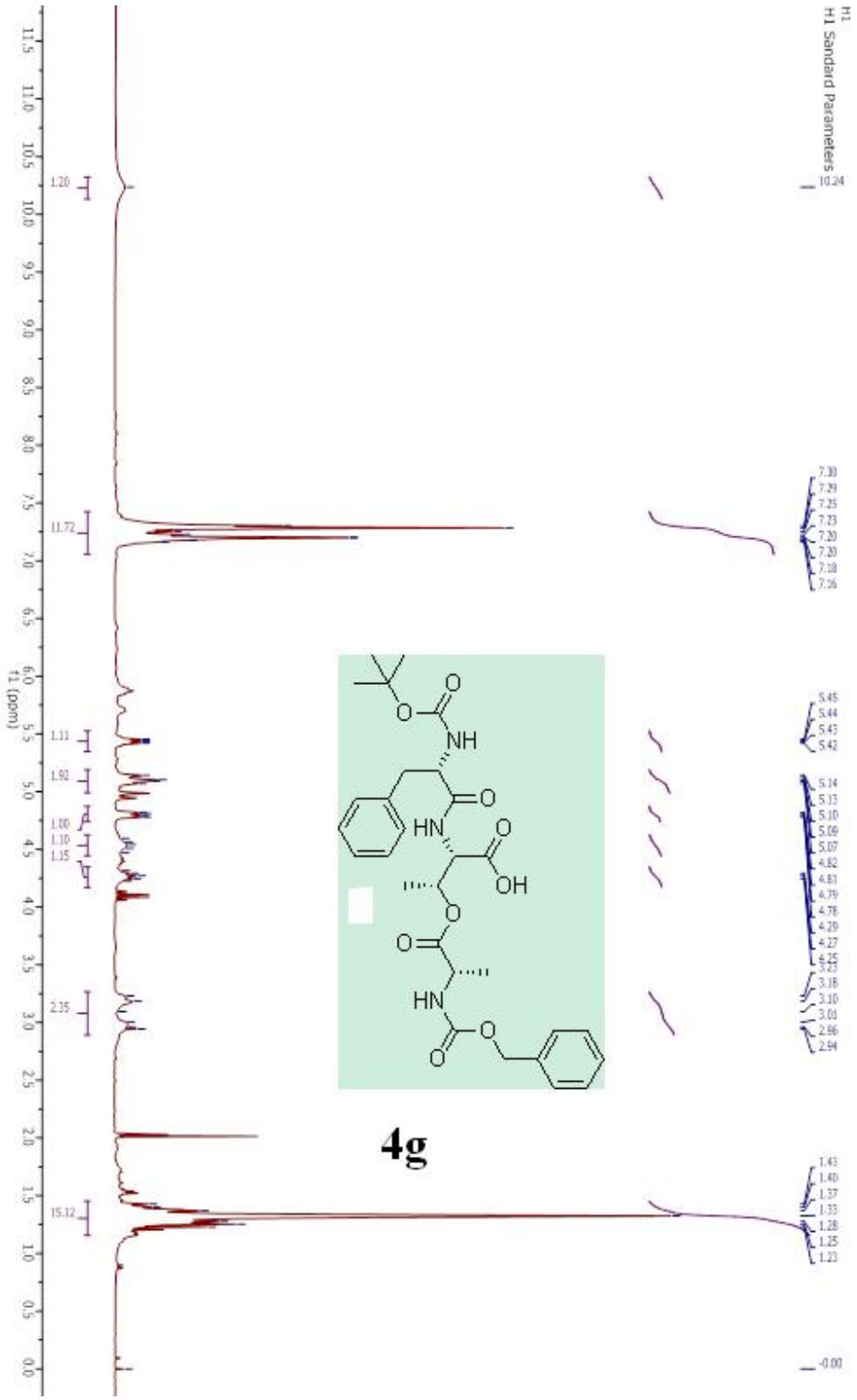

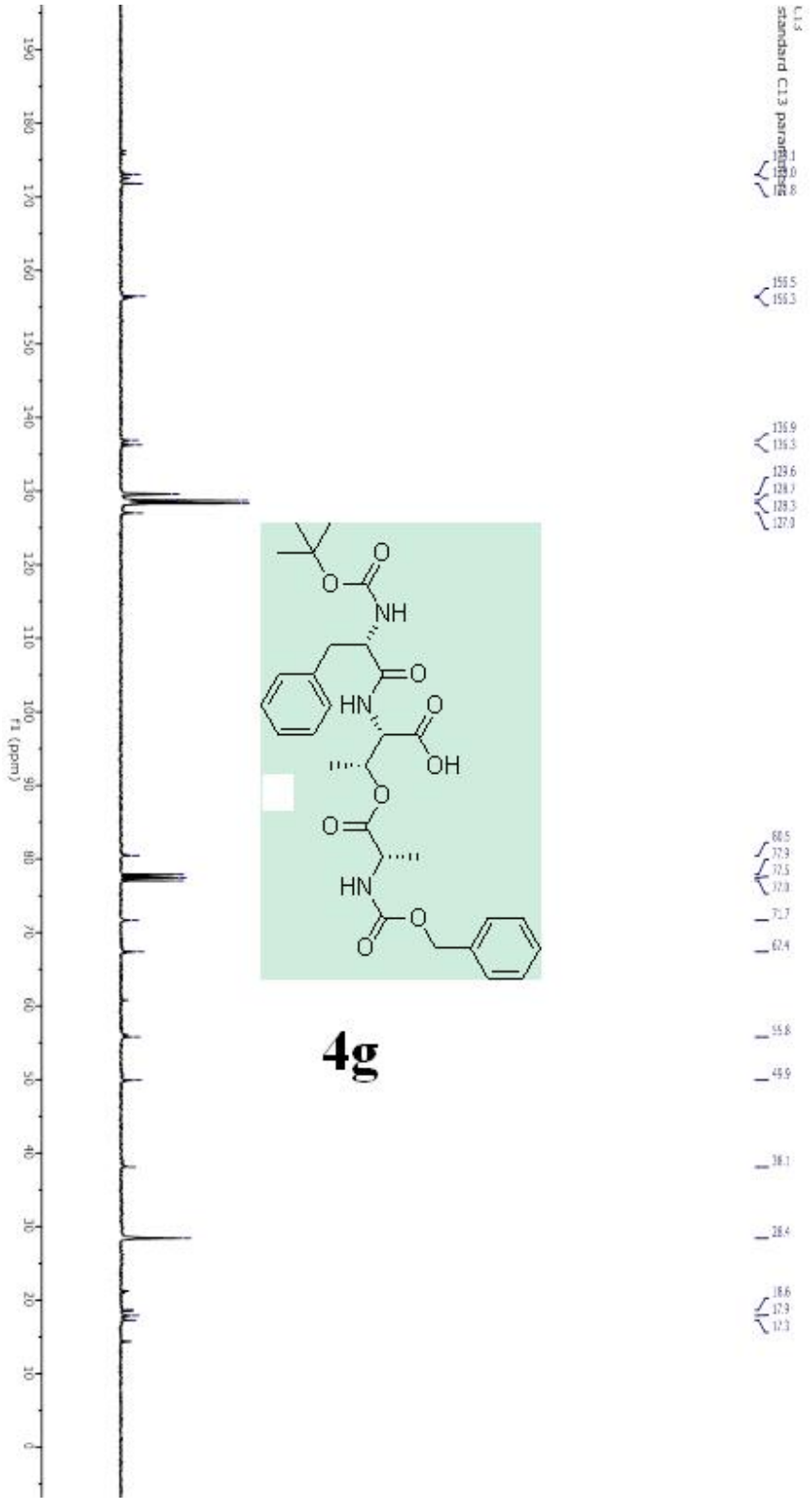

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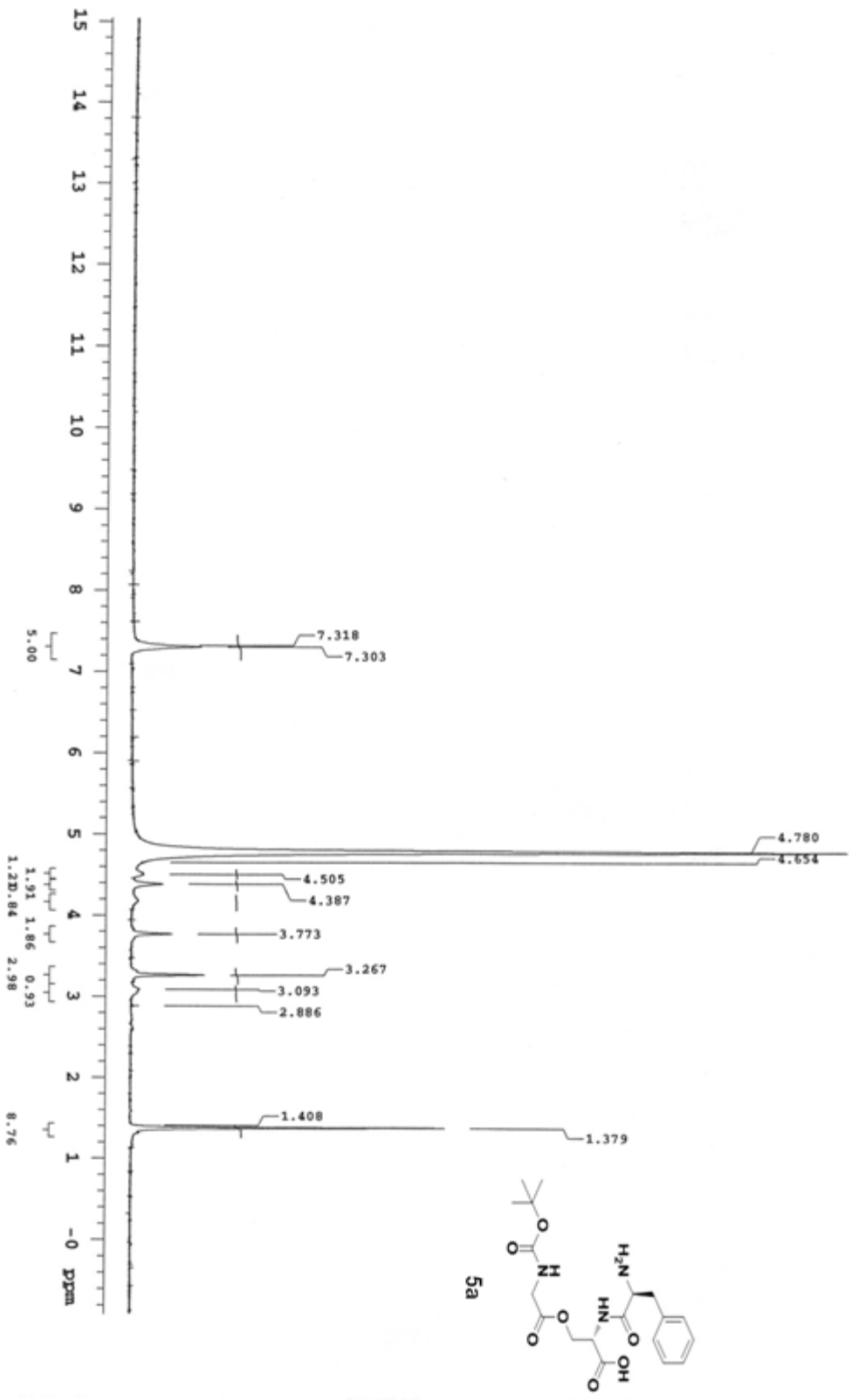

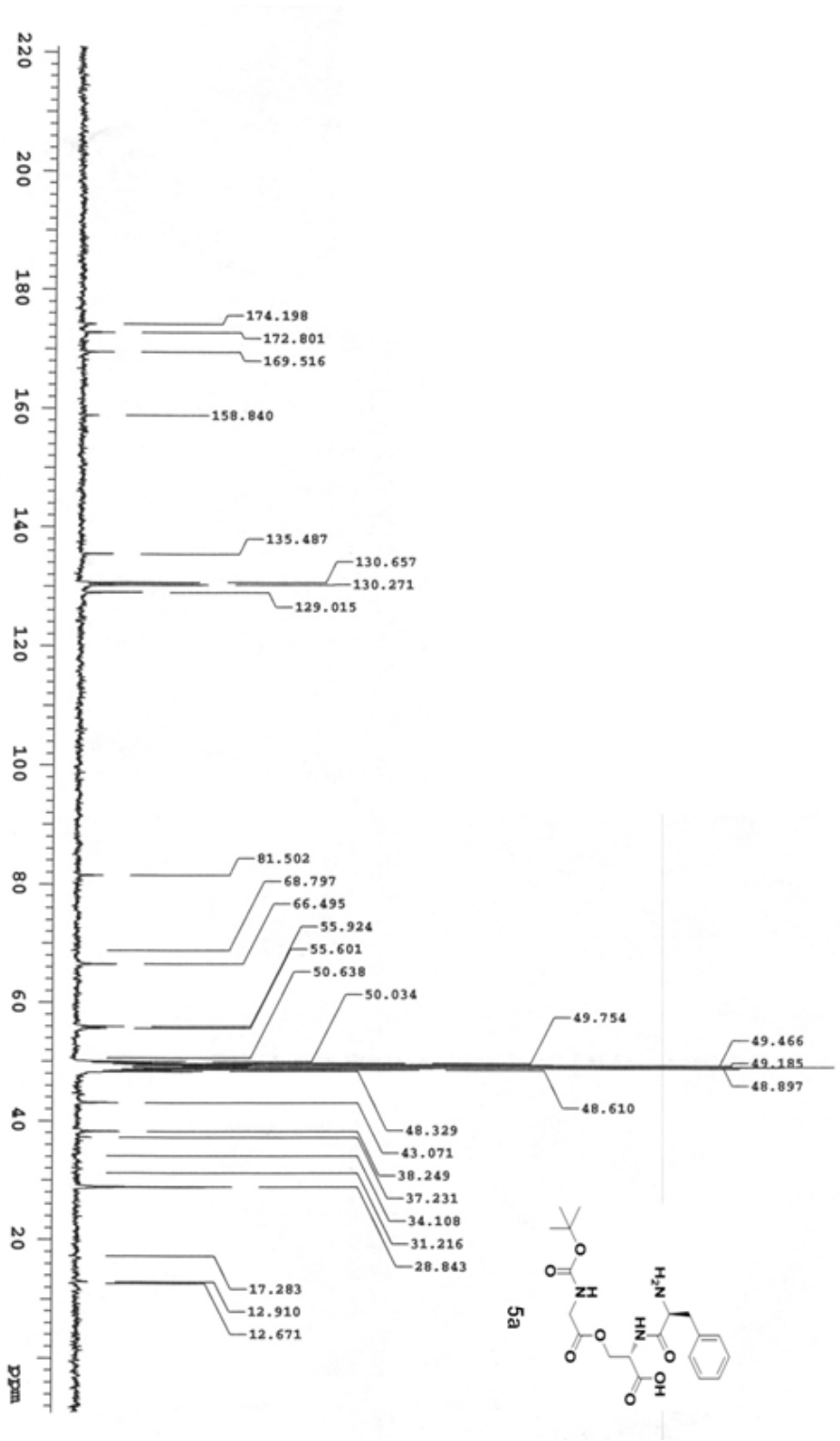

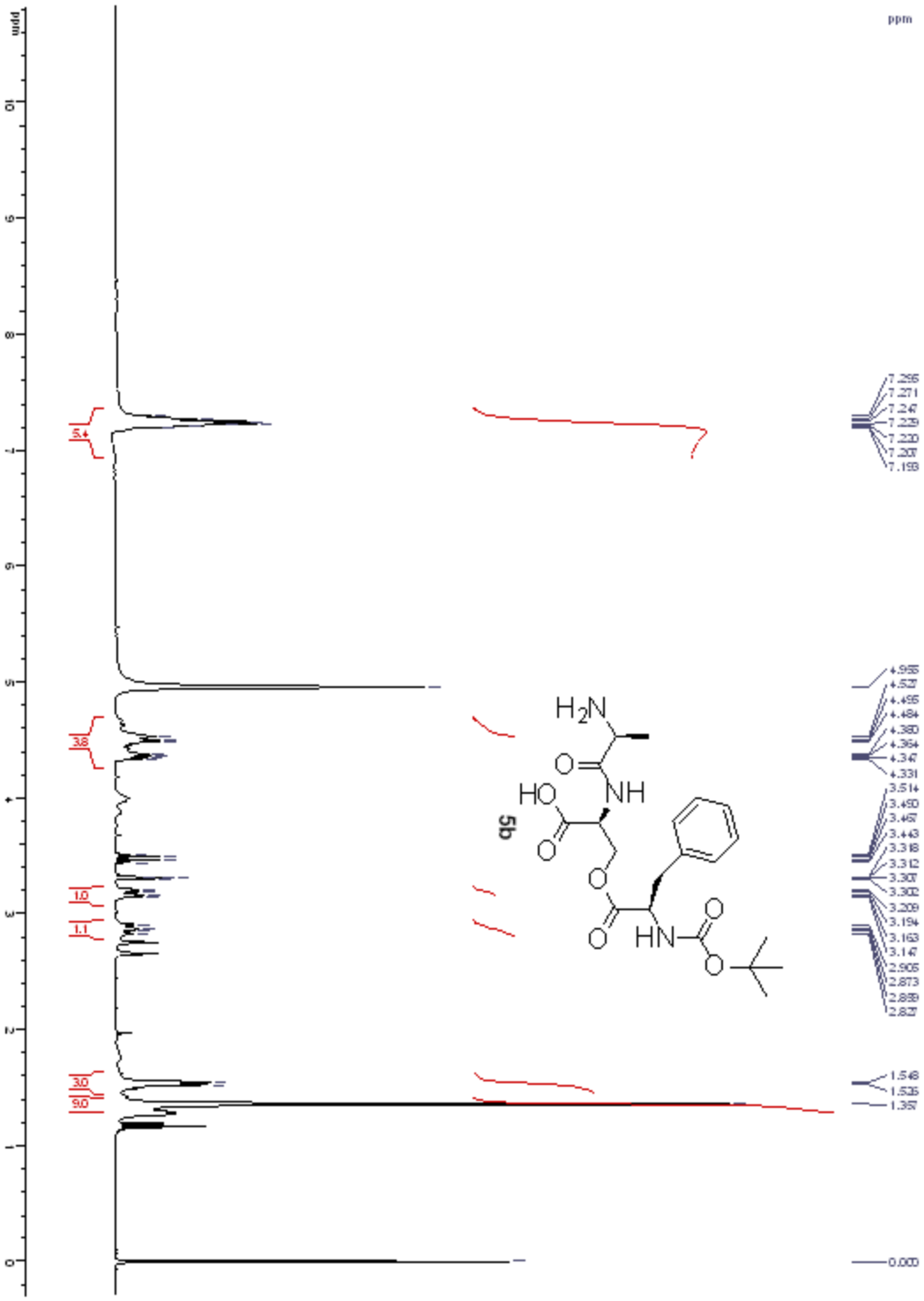

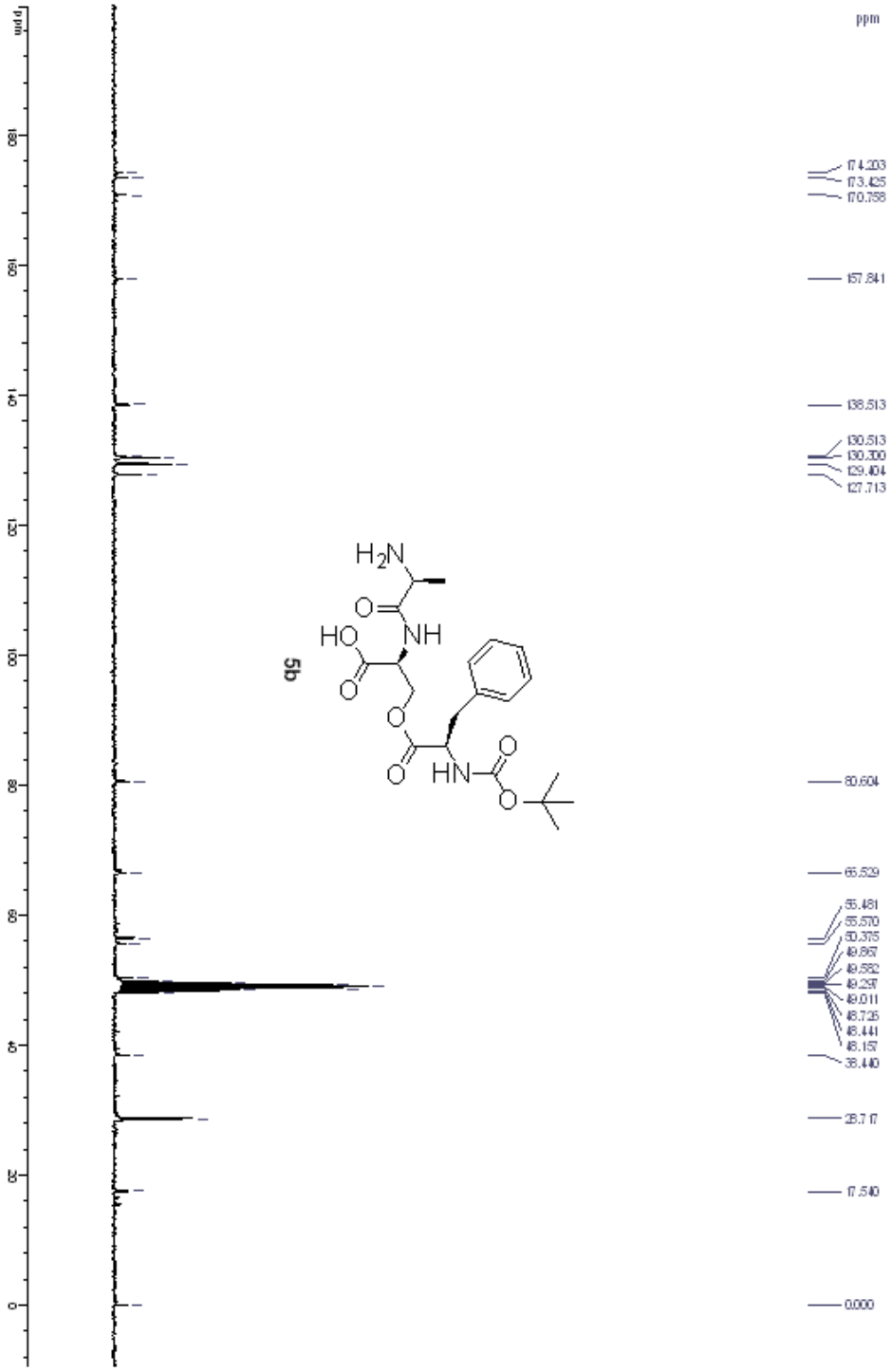

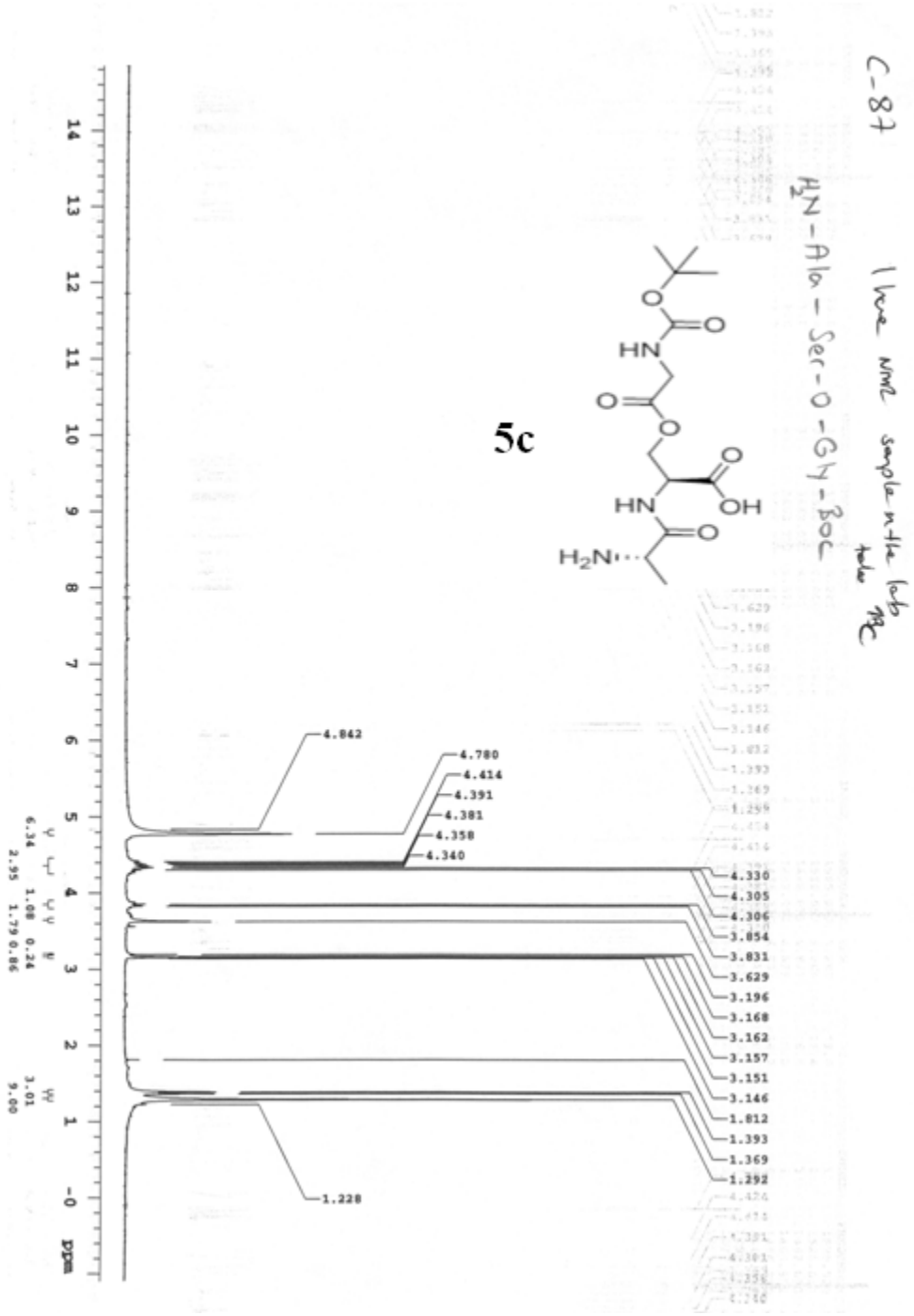

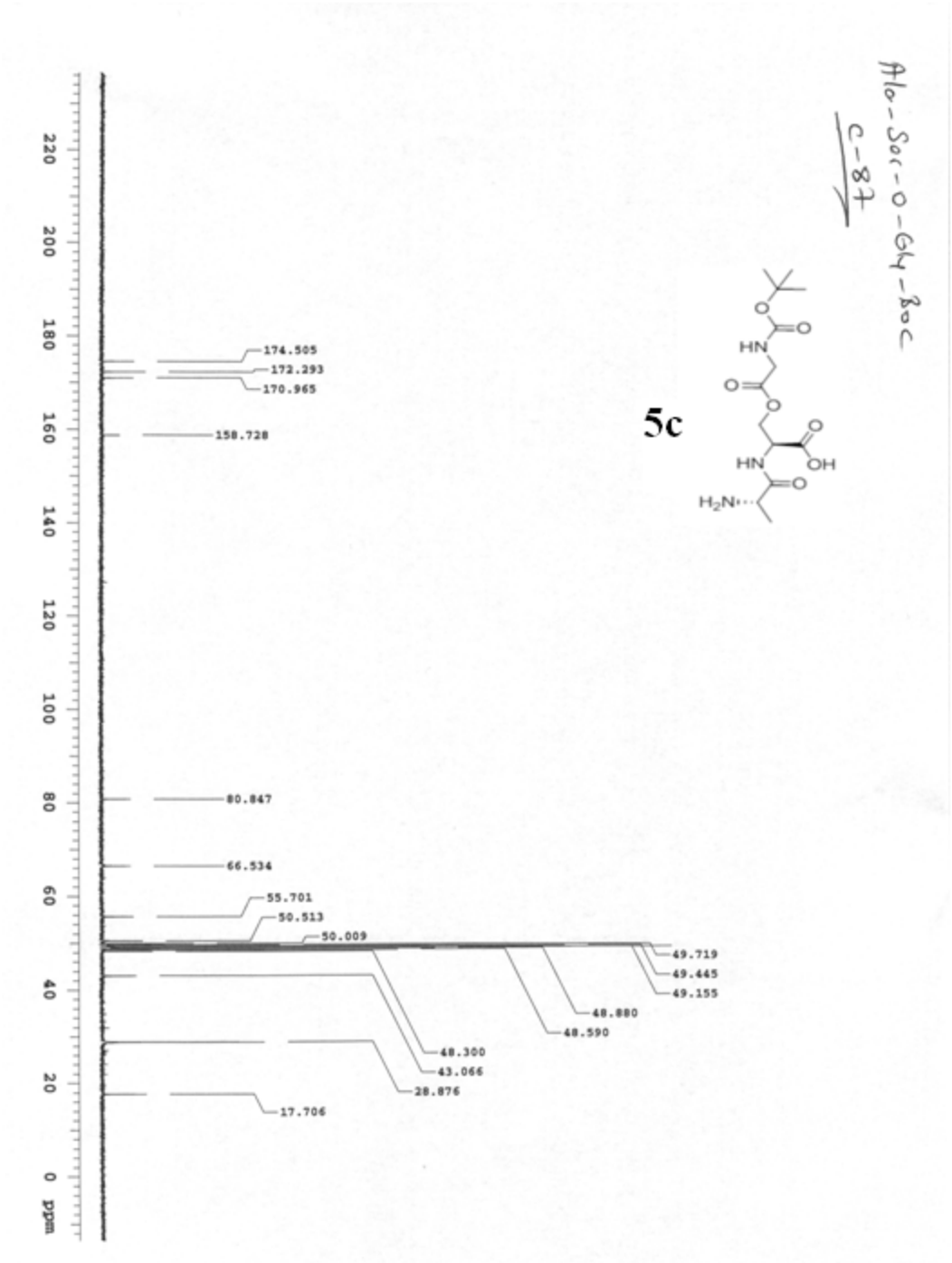

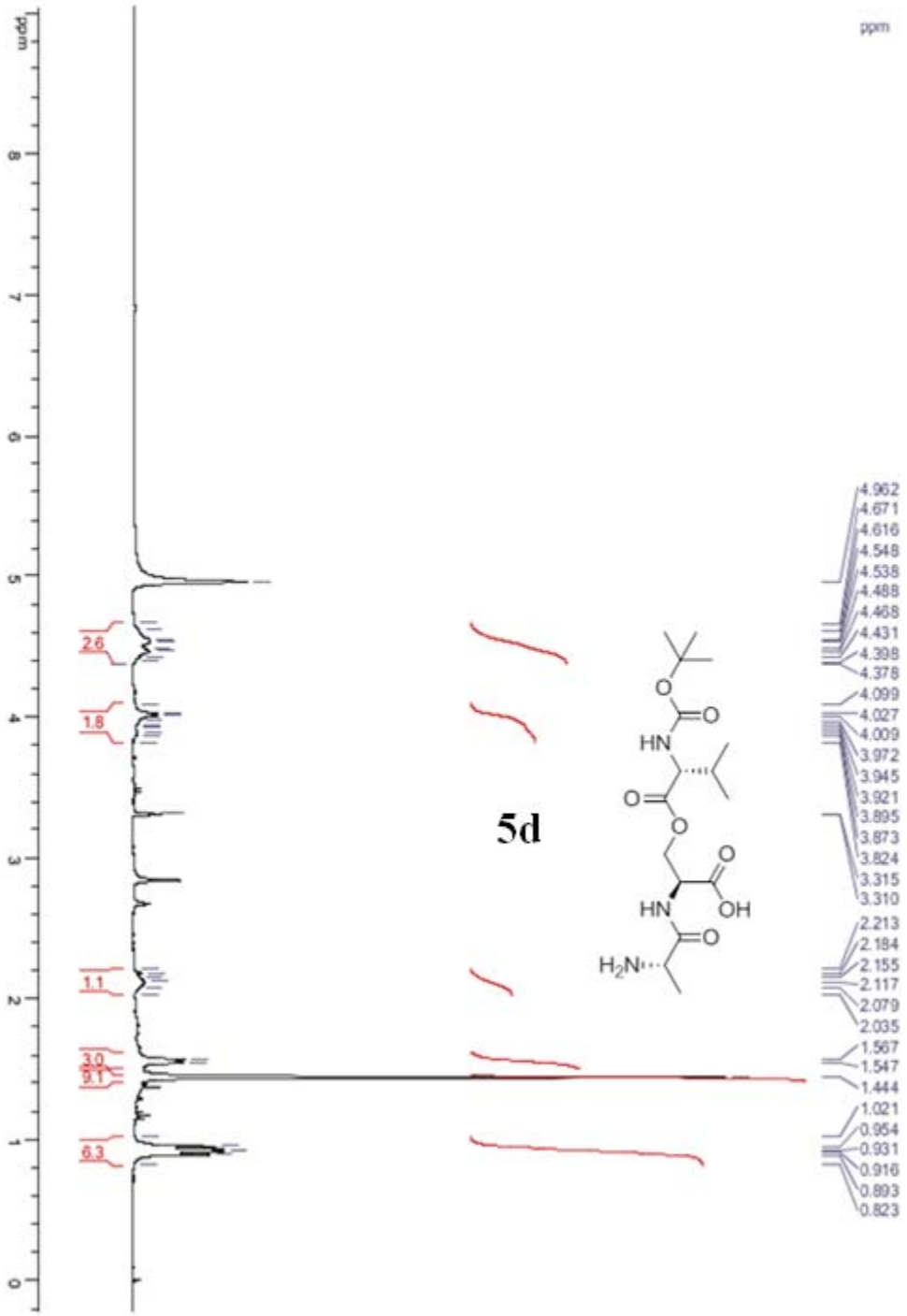

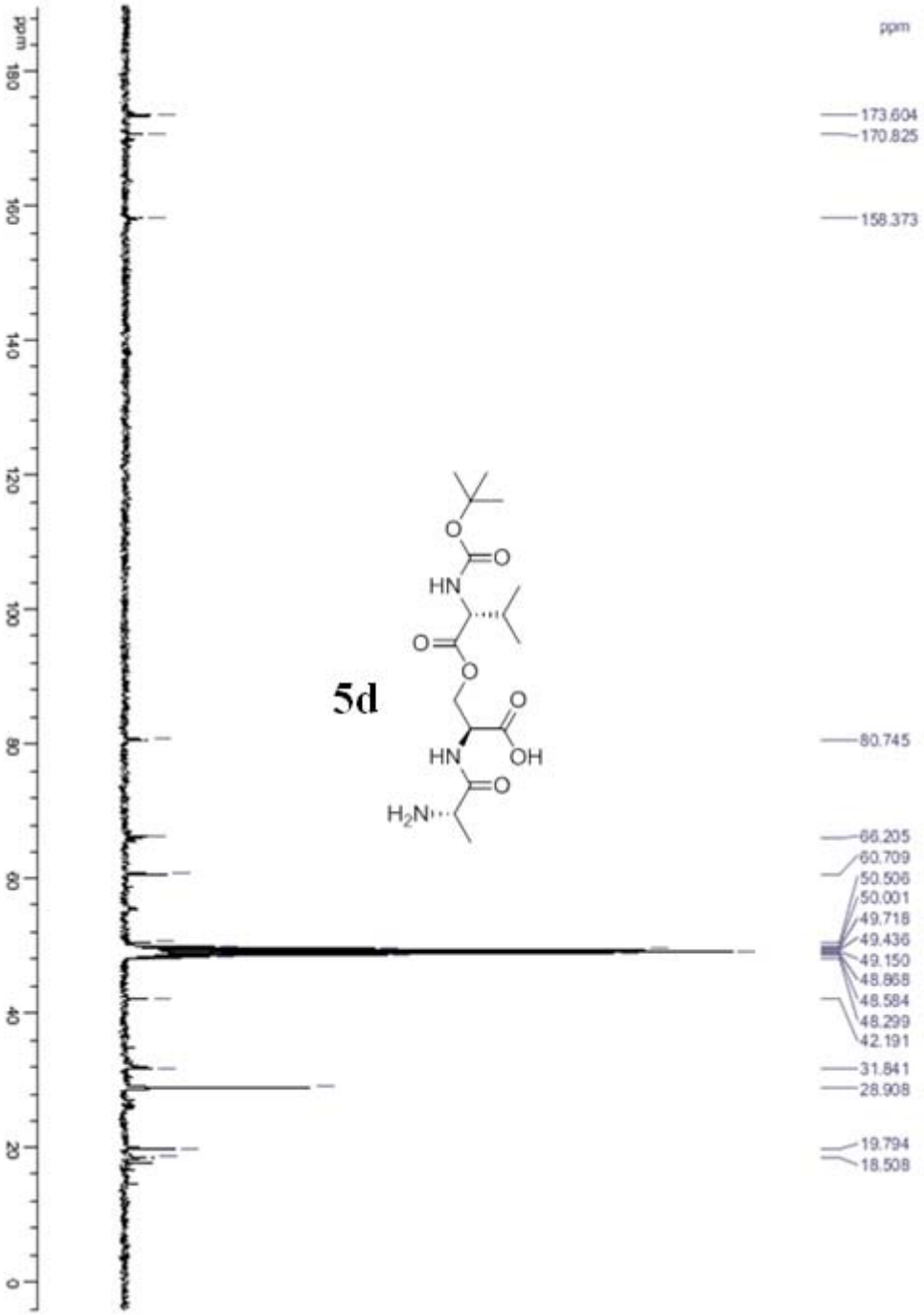

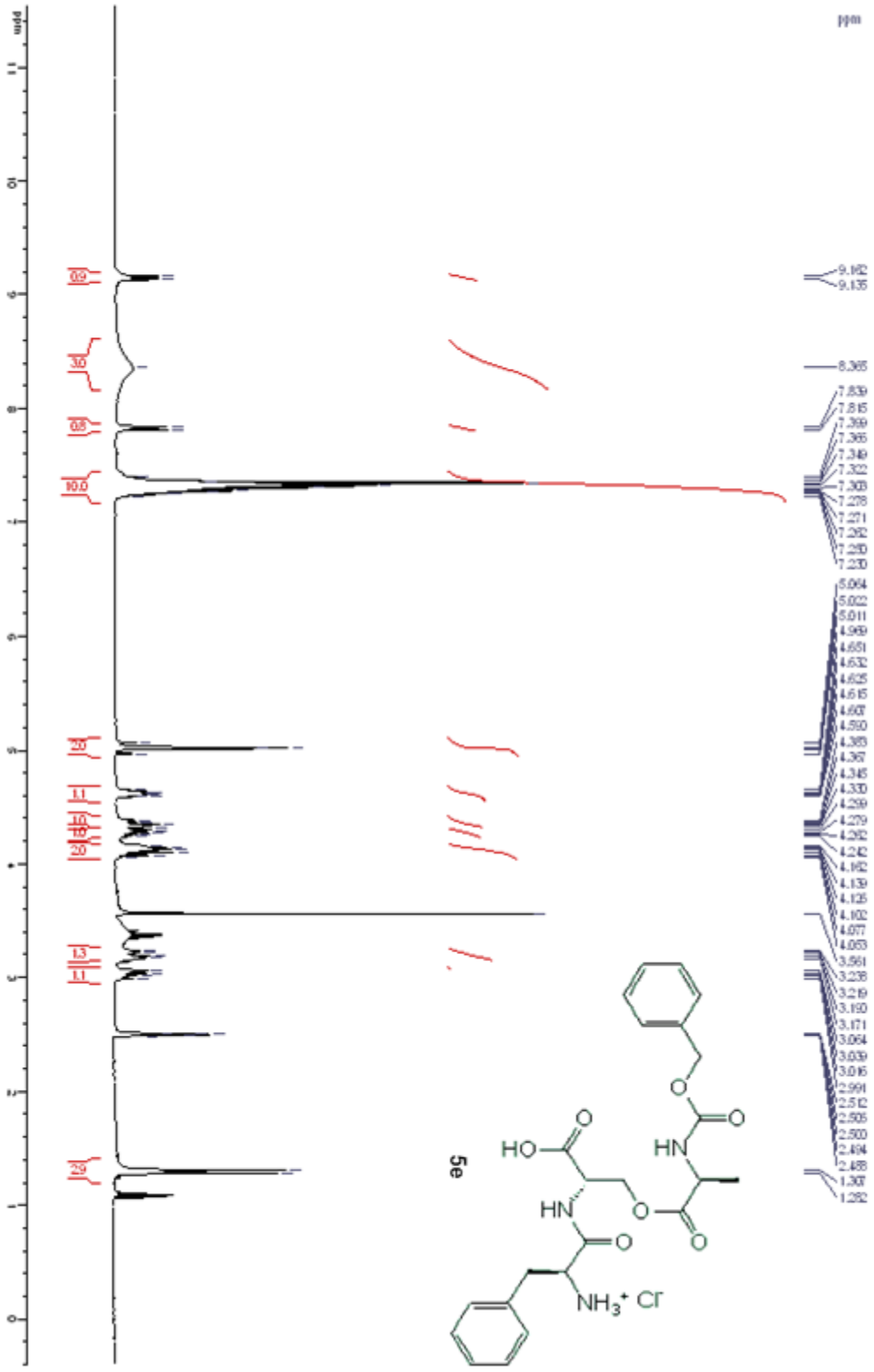

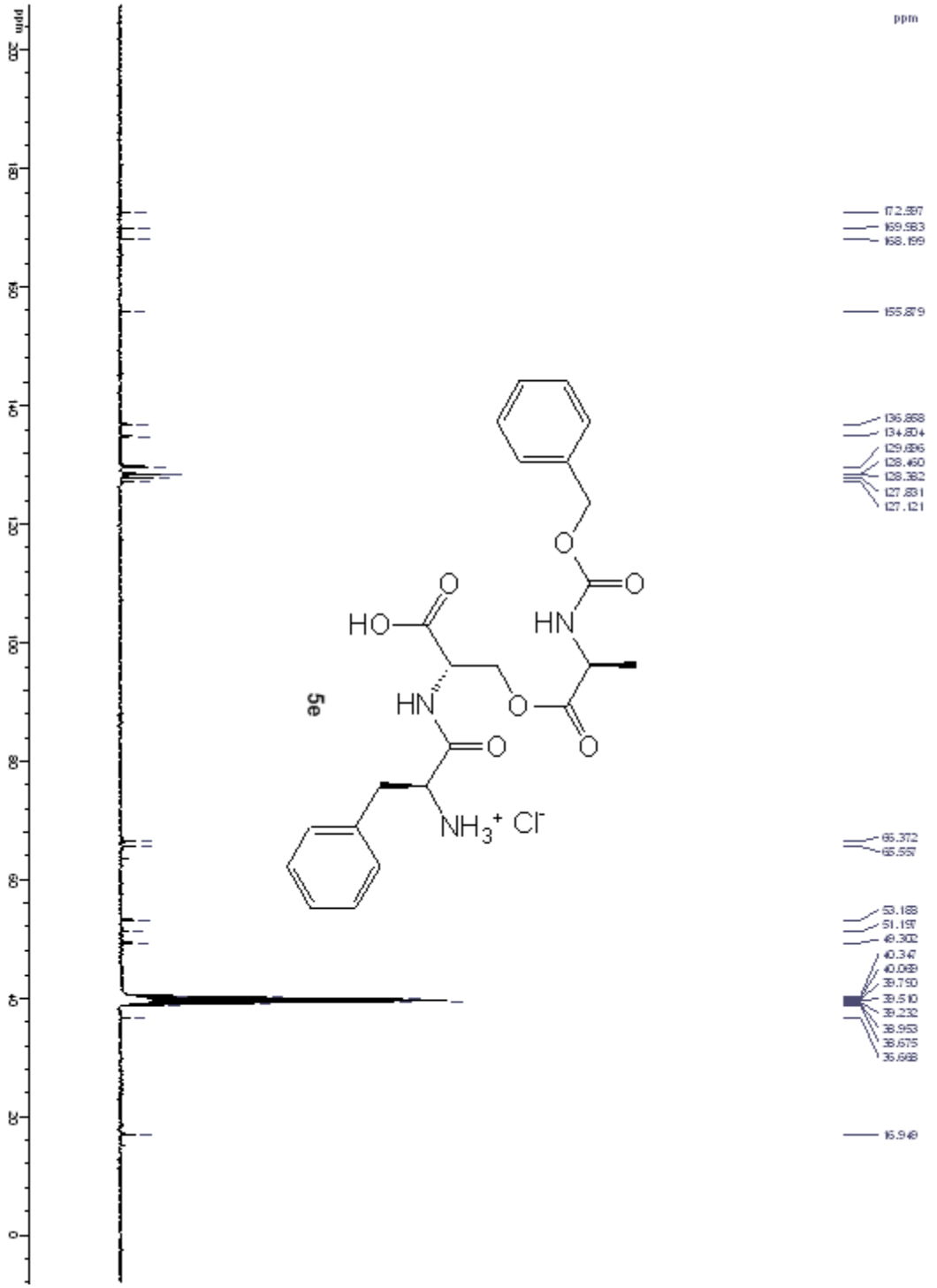

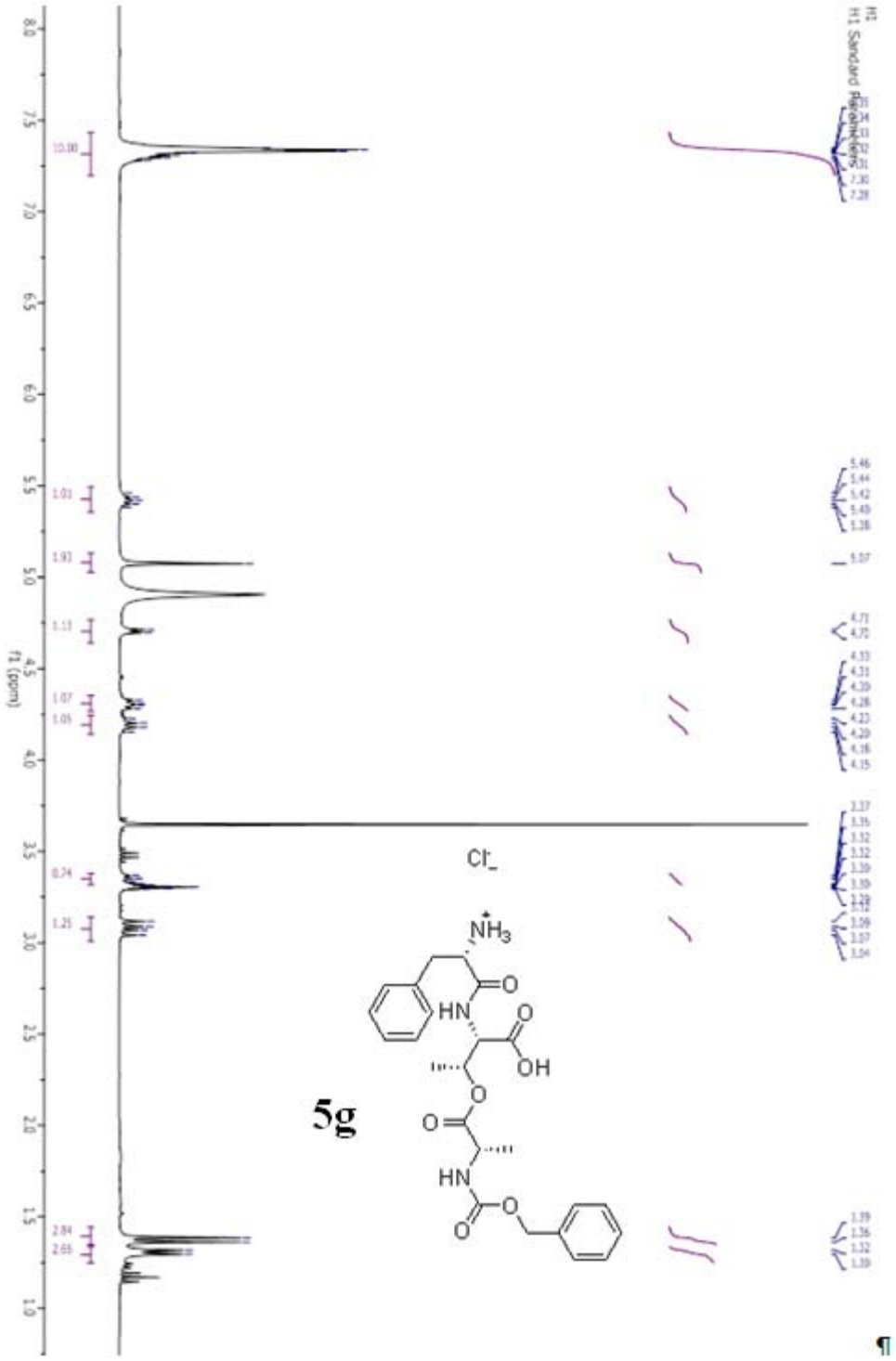

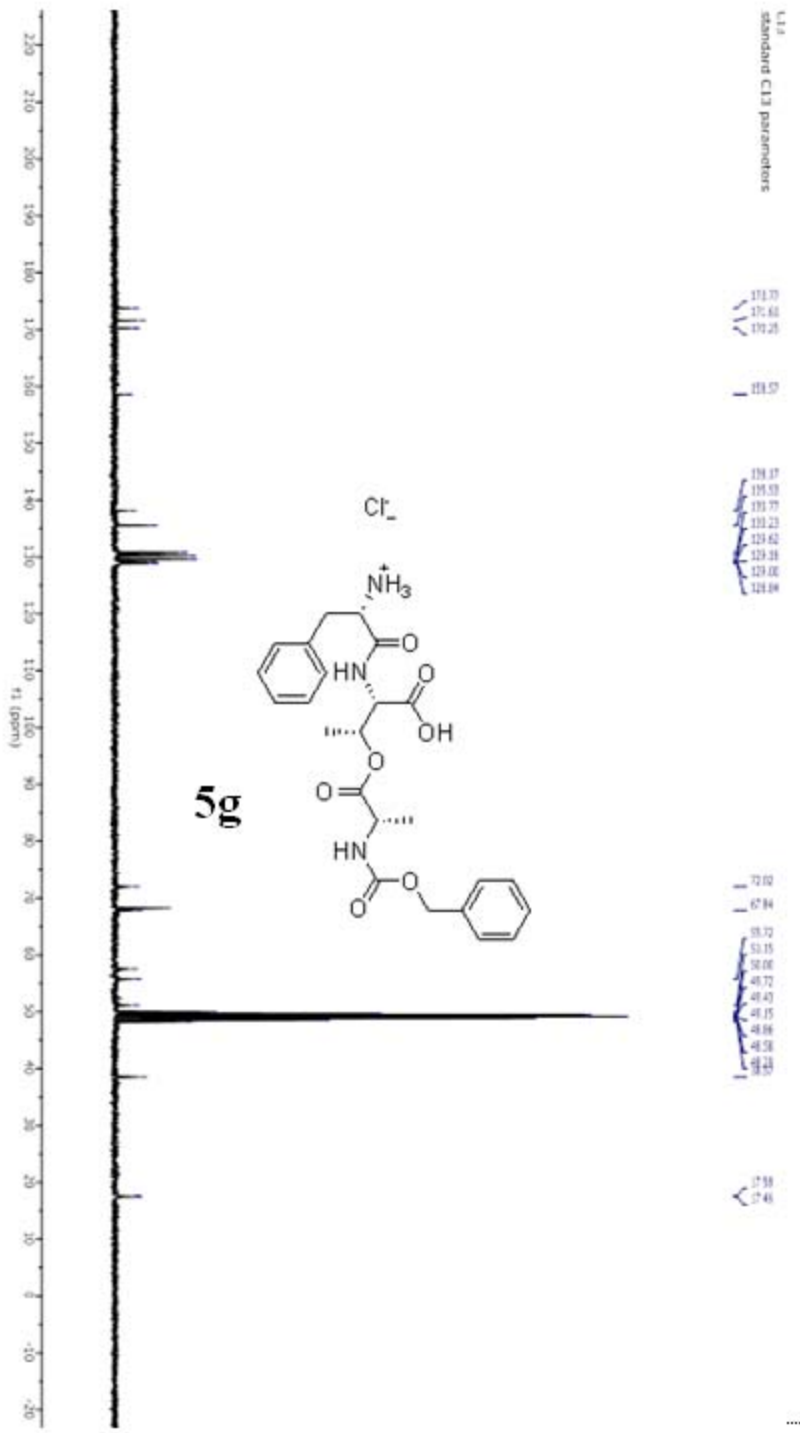

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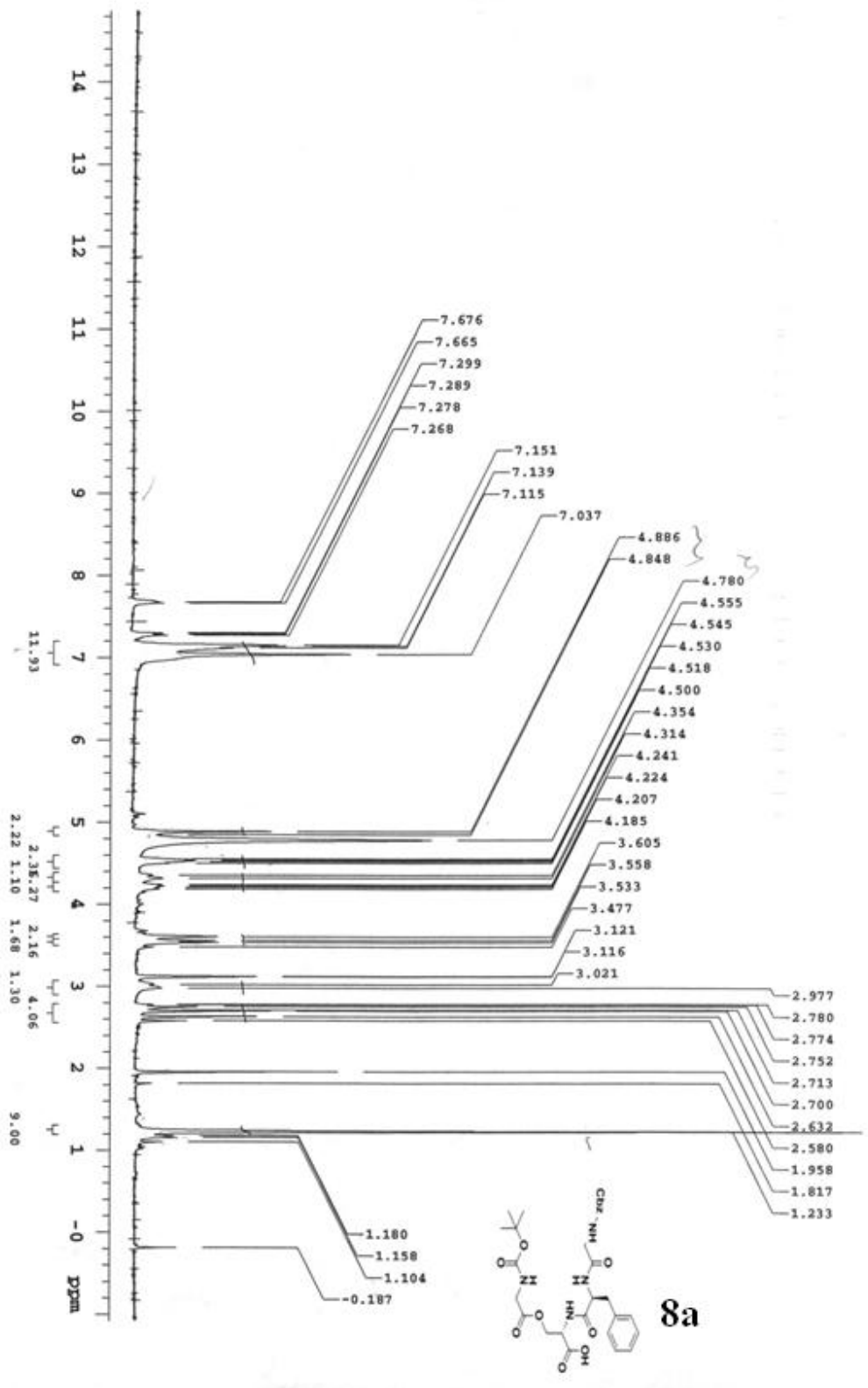
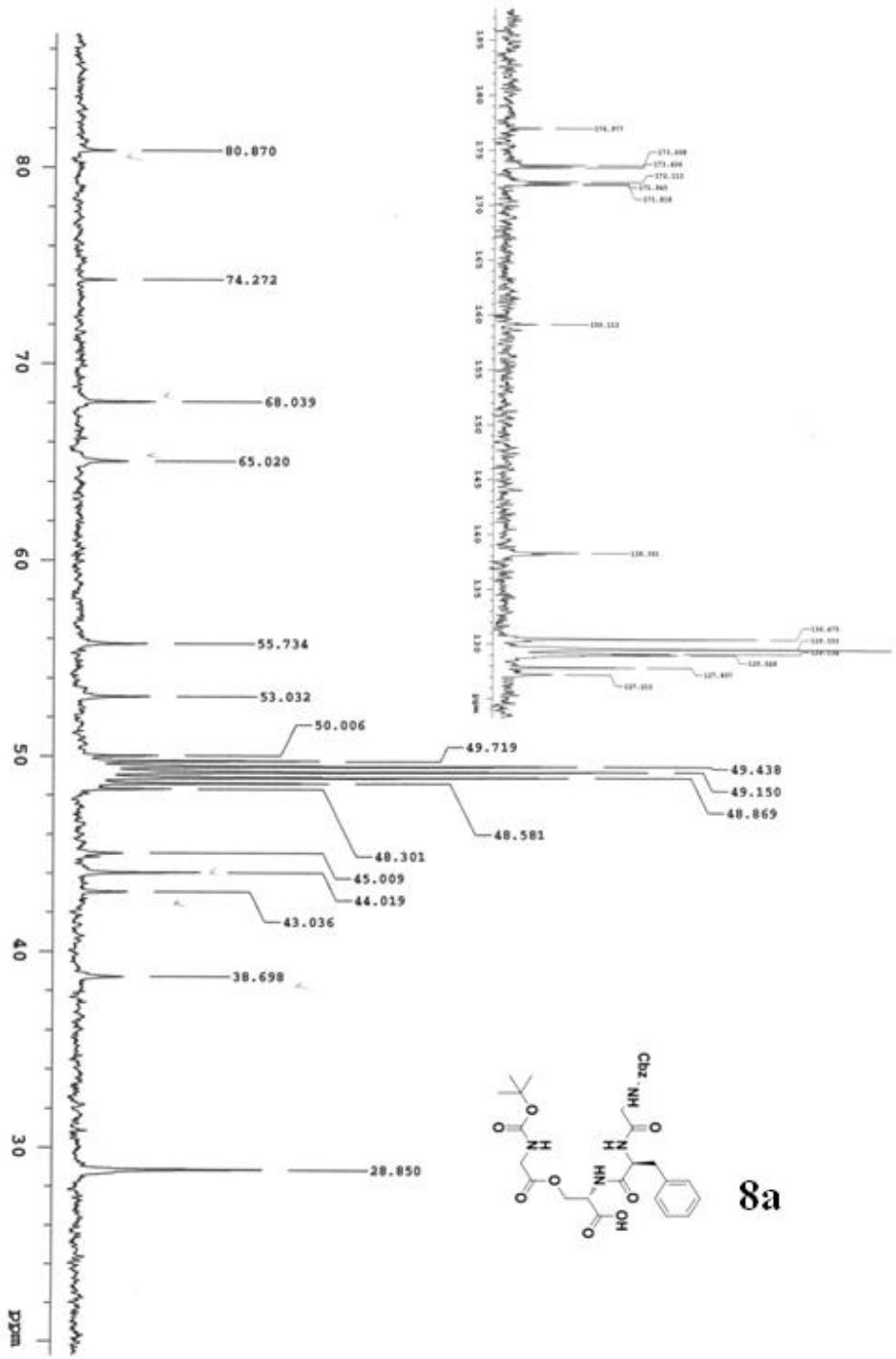
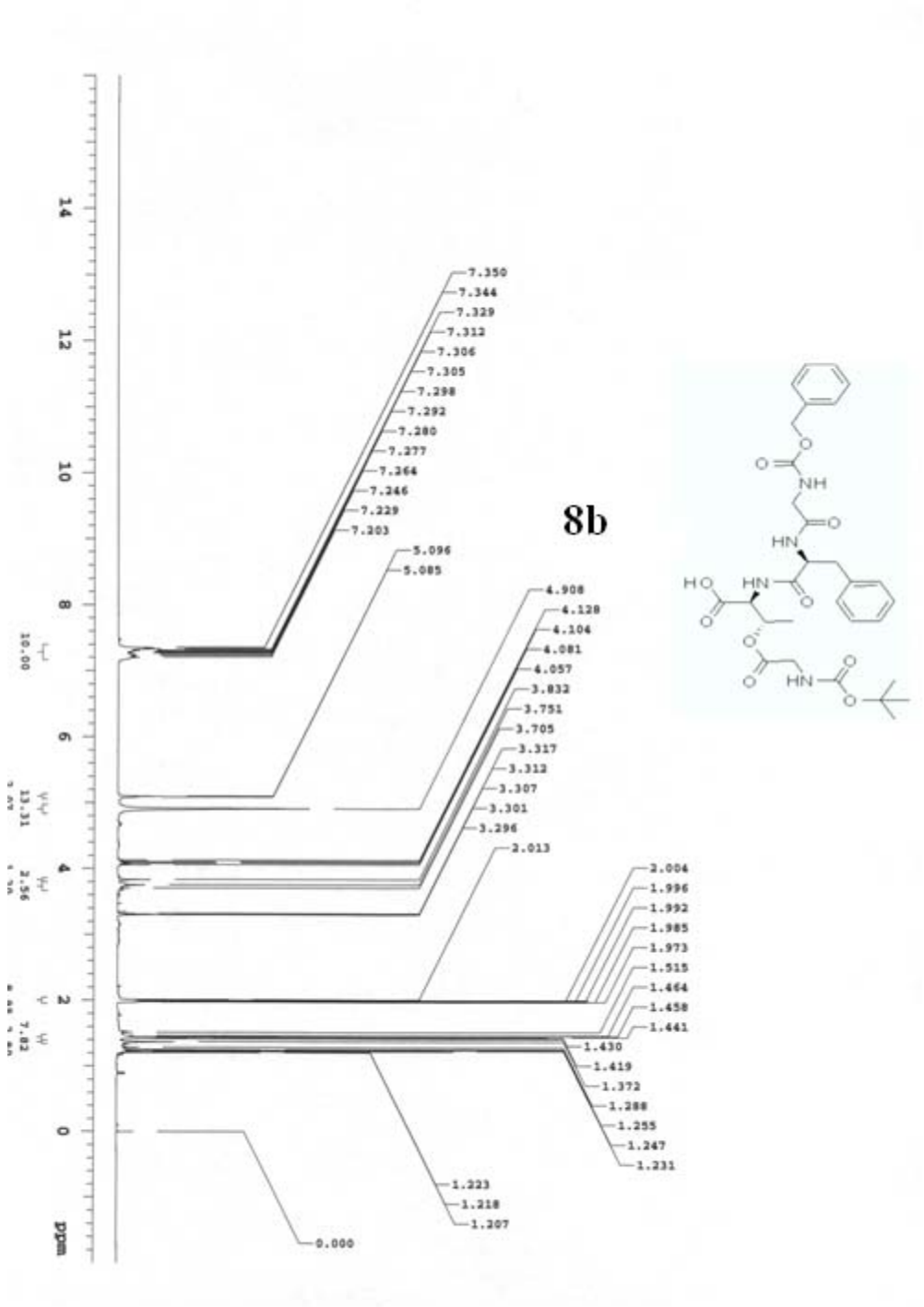
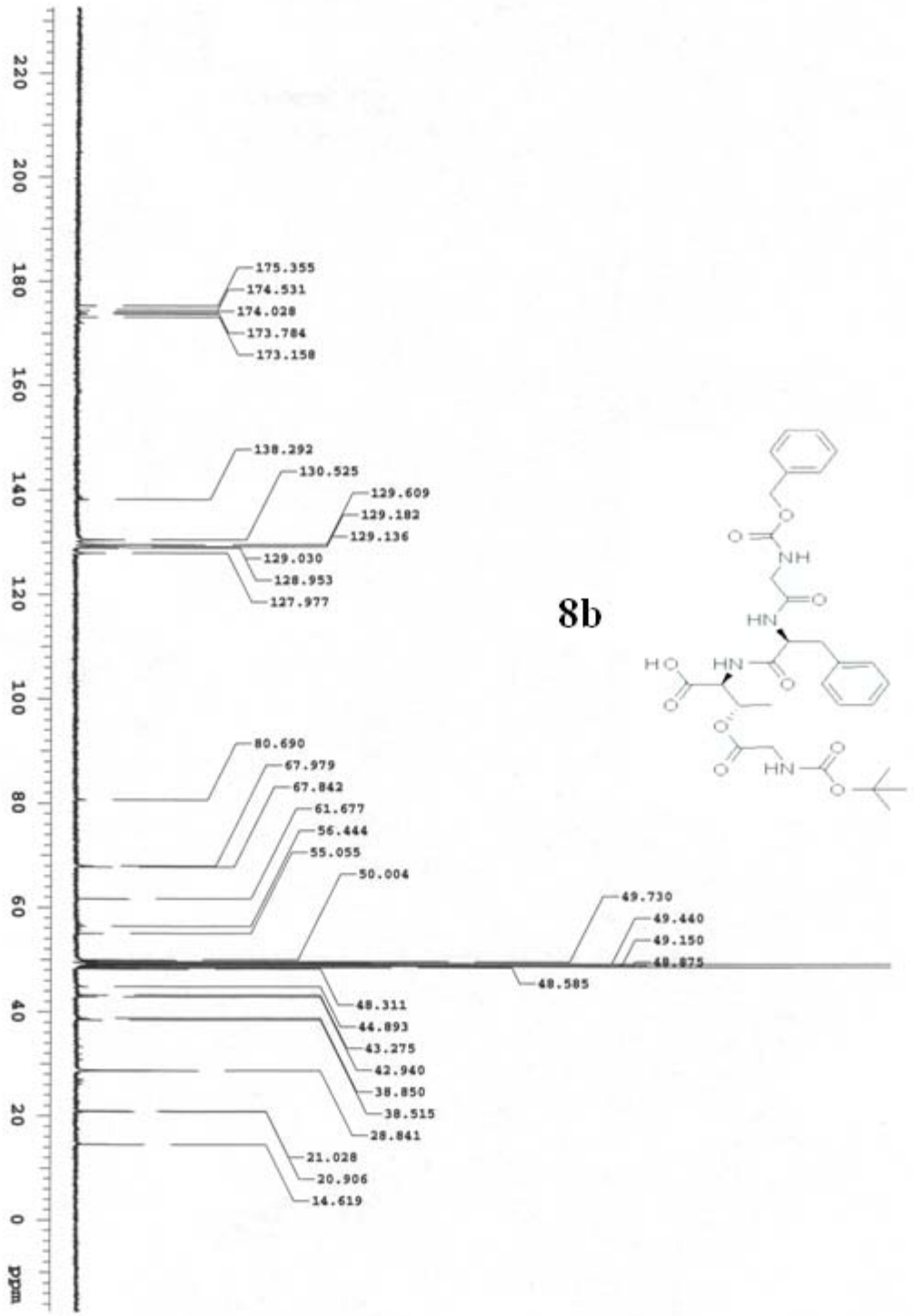
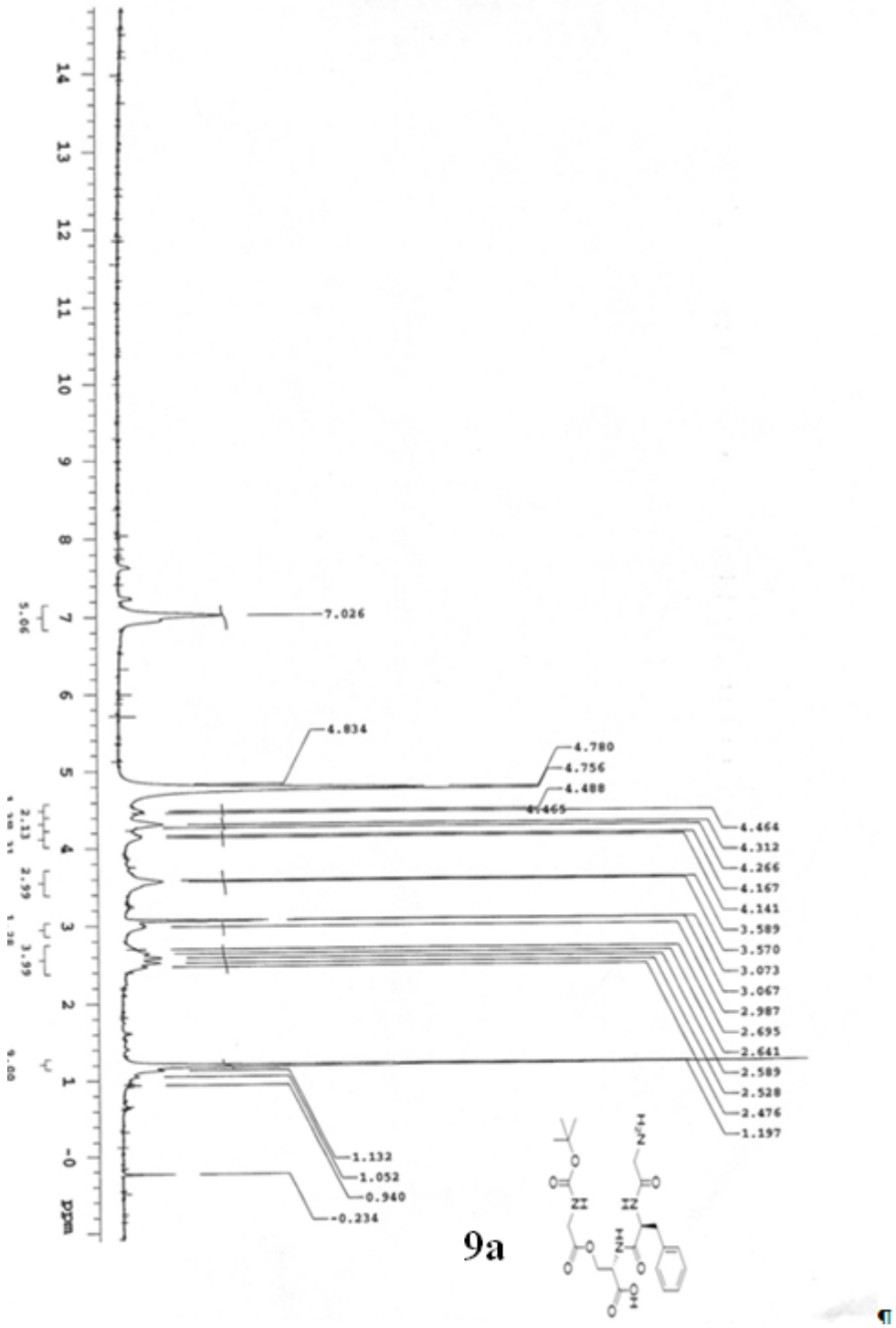
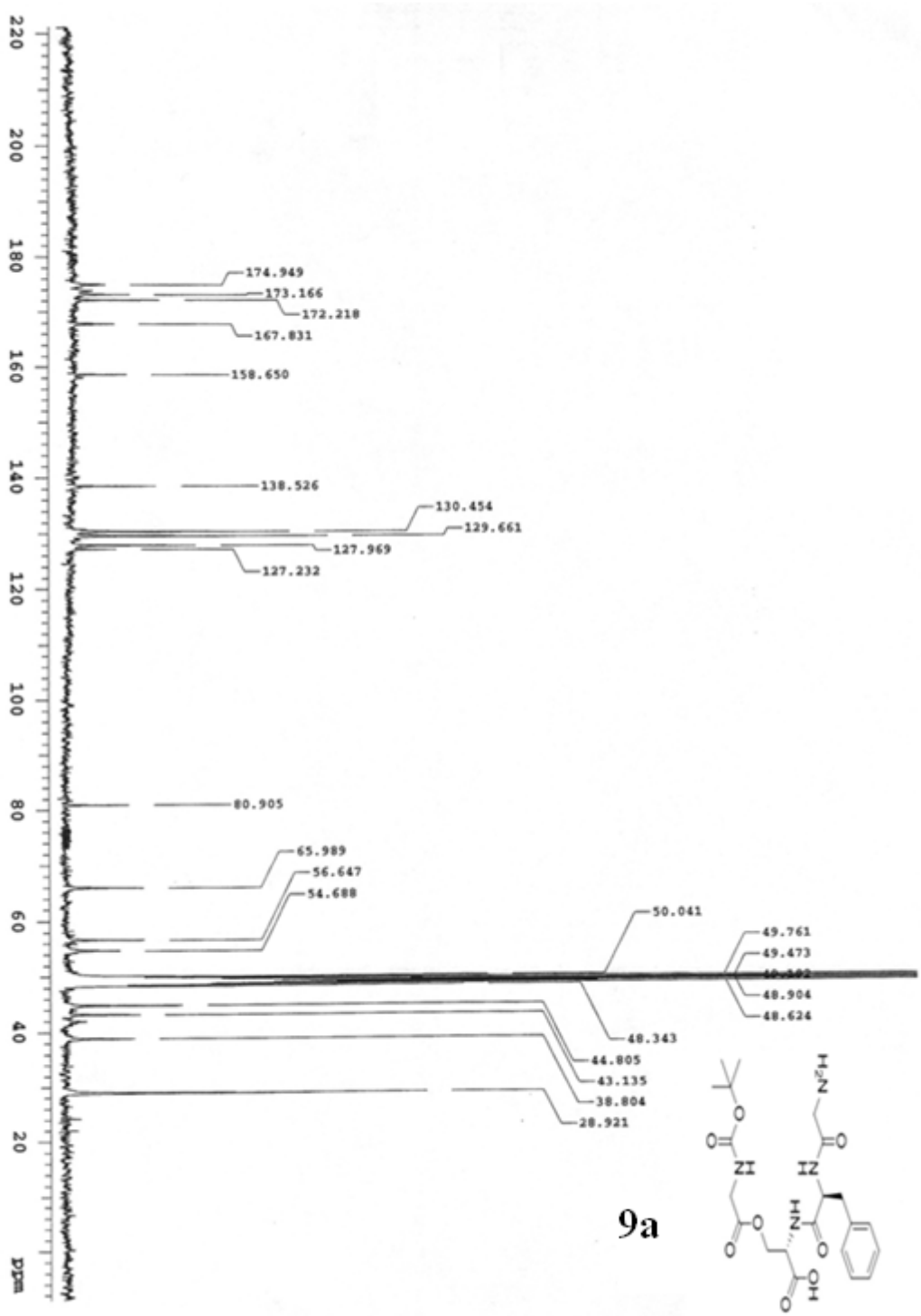












HPLC chromatograms and MS Spectra for 5a and 6a

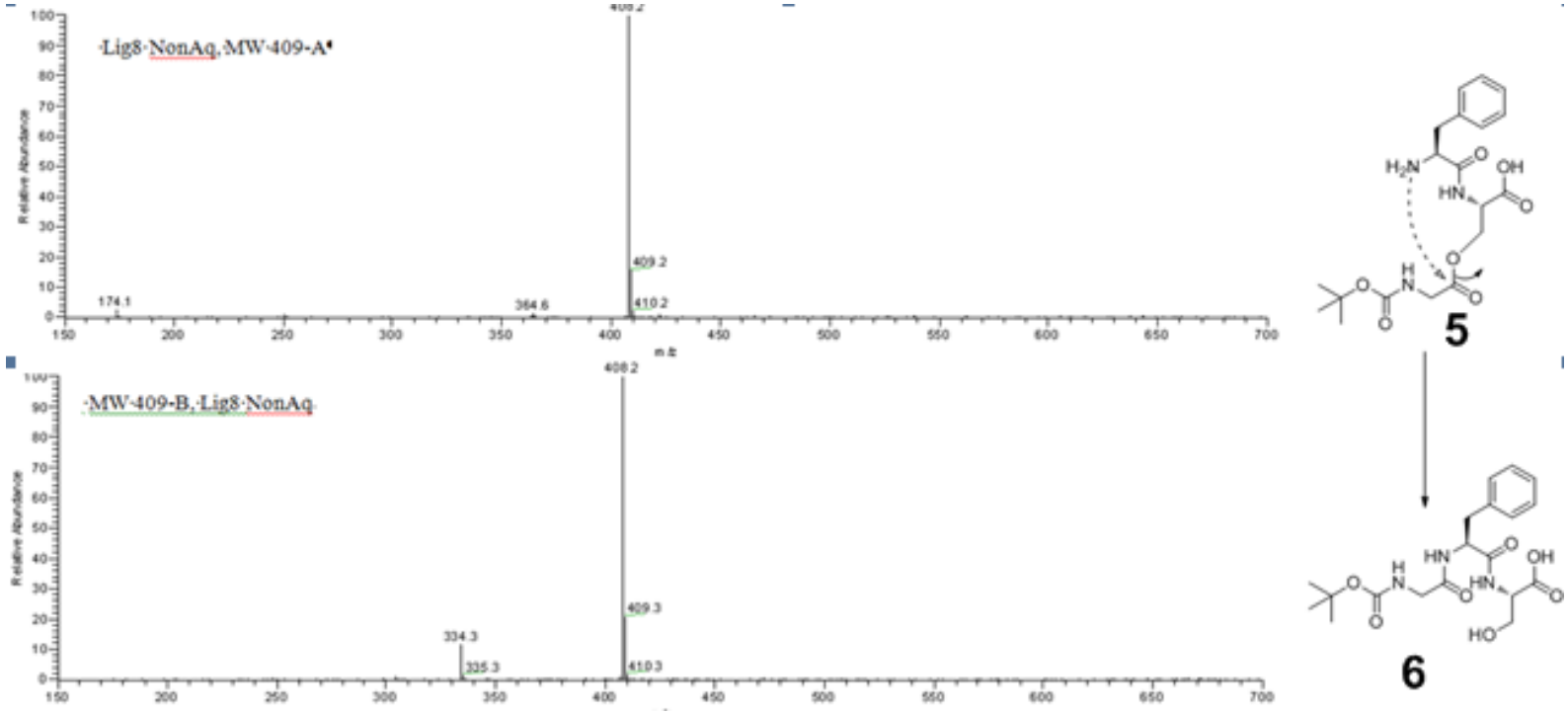


Figure S1. Lig8 NonAq, MW 409-A

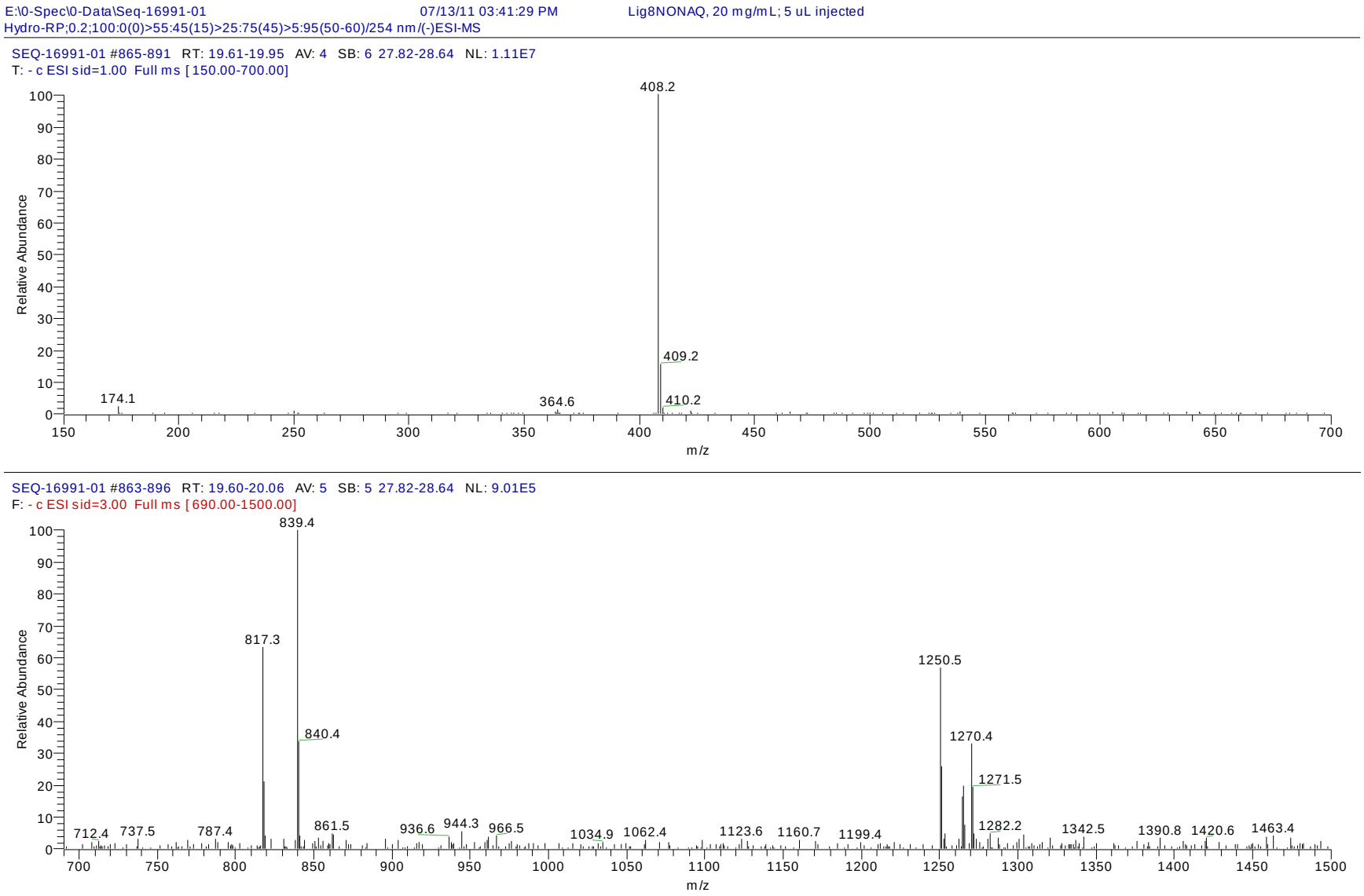


Figure S2. MW 409-B, Lig8 NonAq

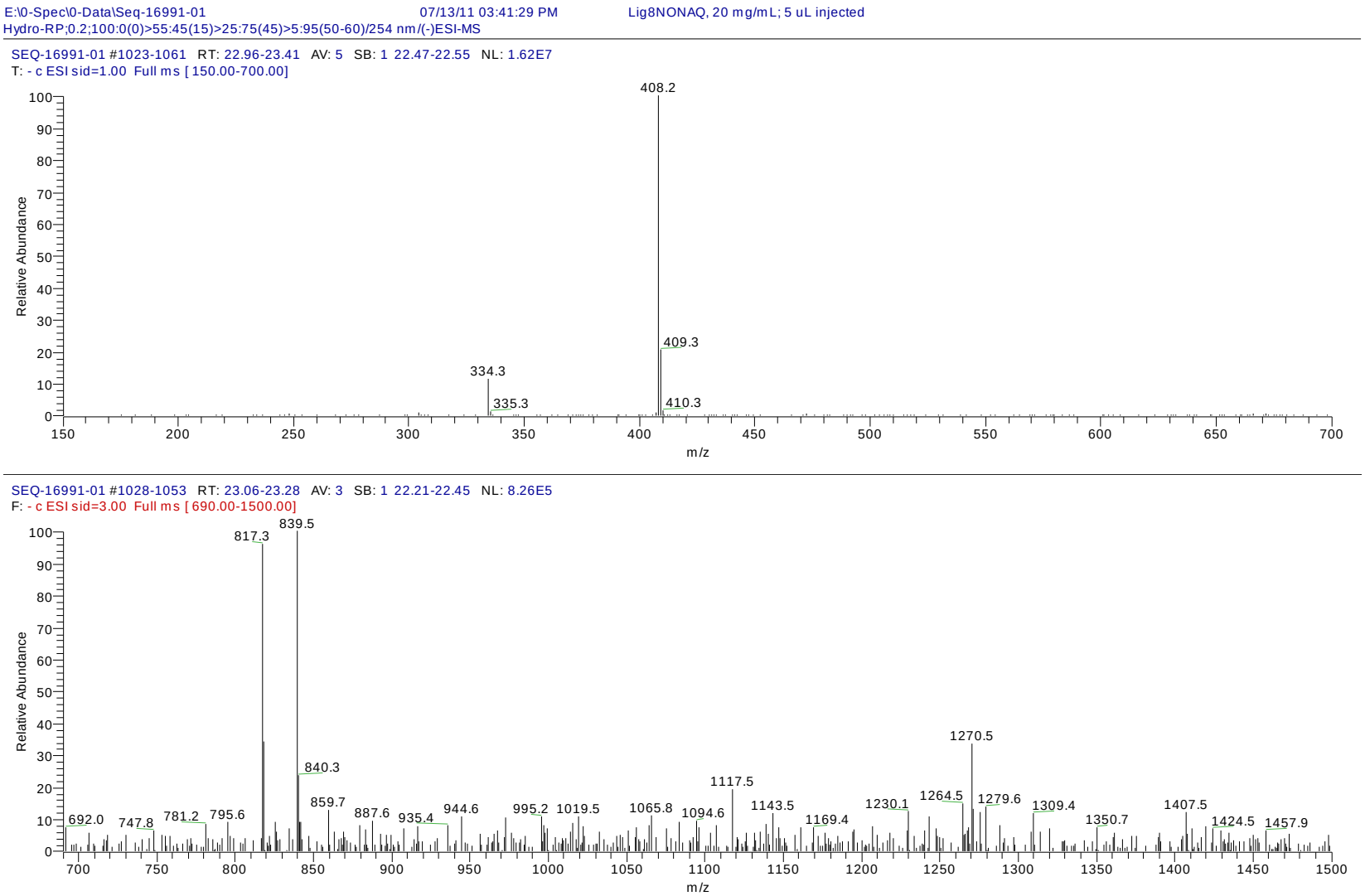


Figure S3. Lig8 NonAq: HPLC/UV/(-)ESI-MS and –MSn chromatograms for the MW 409 compounds

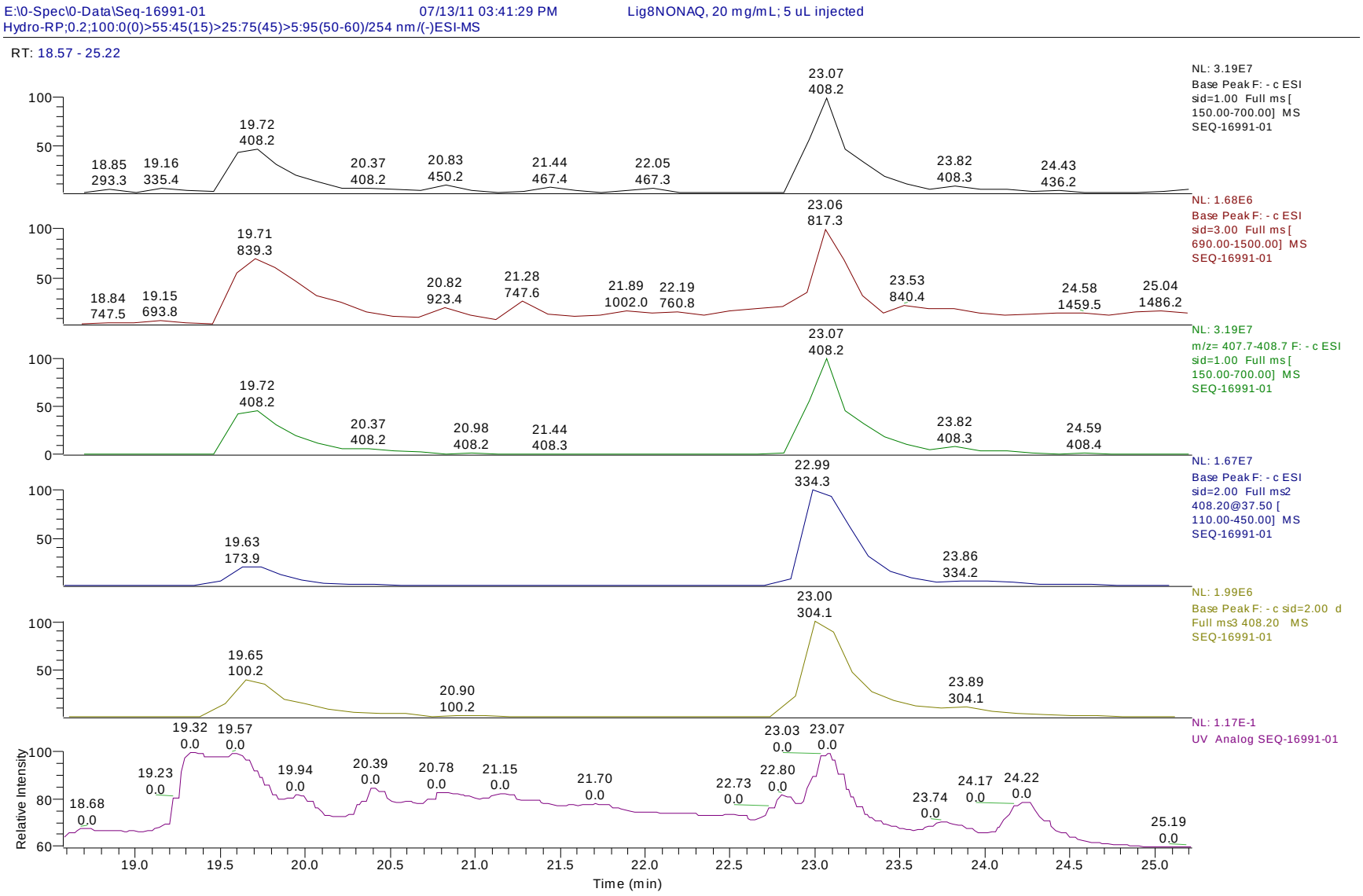


Figure S4. MW 409 compounds: (-)ESI-MS/MS (top and 3rd) and –MS/MS/MS (2nd and bottom) for the MW 409-A (RT 19.72 min) and MW 409-B (RT 23.07 min)

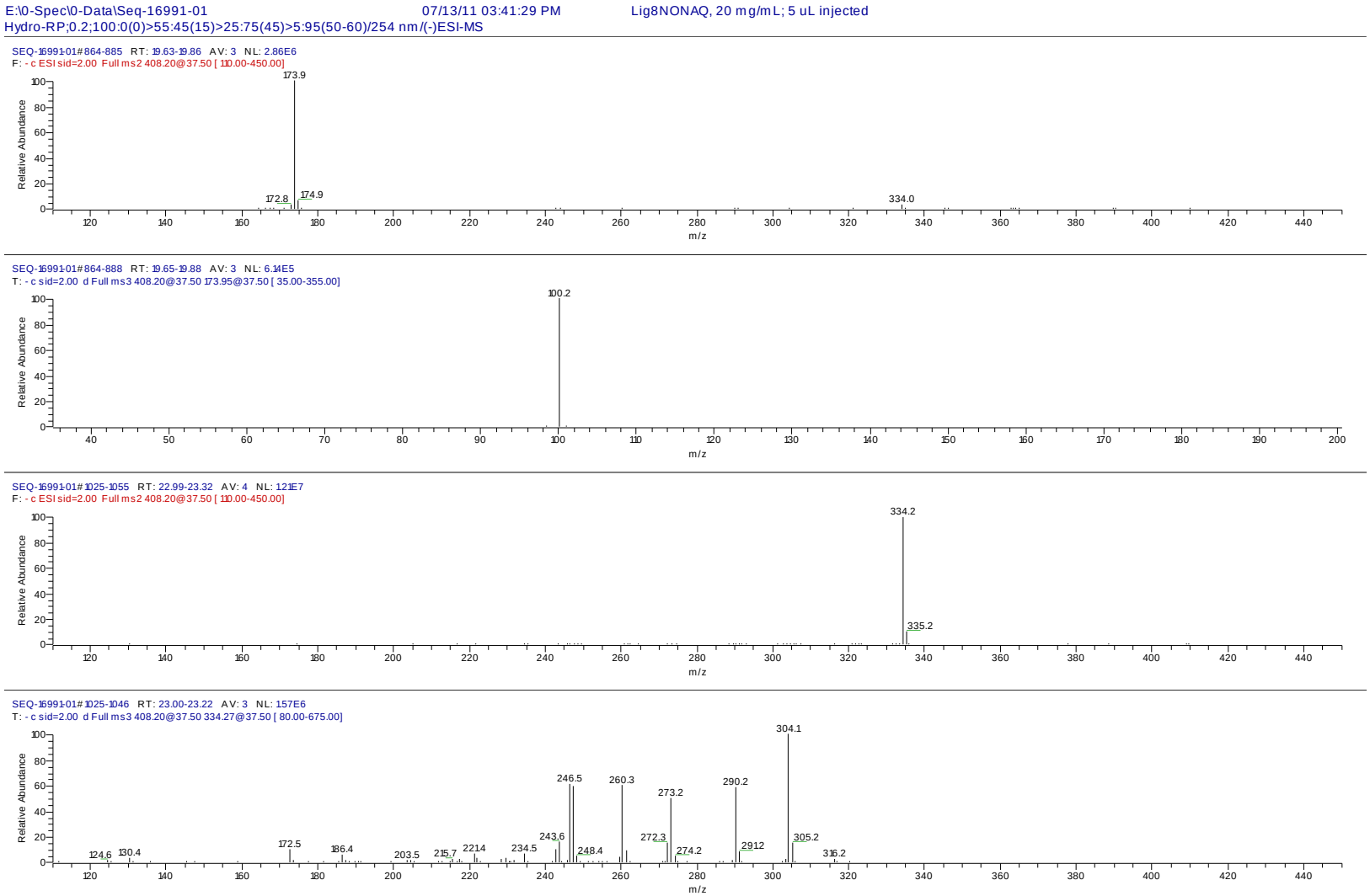
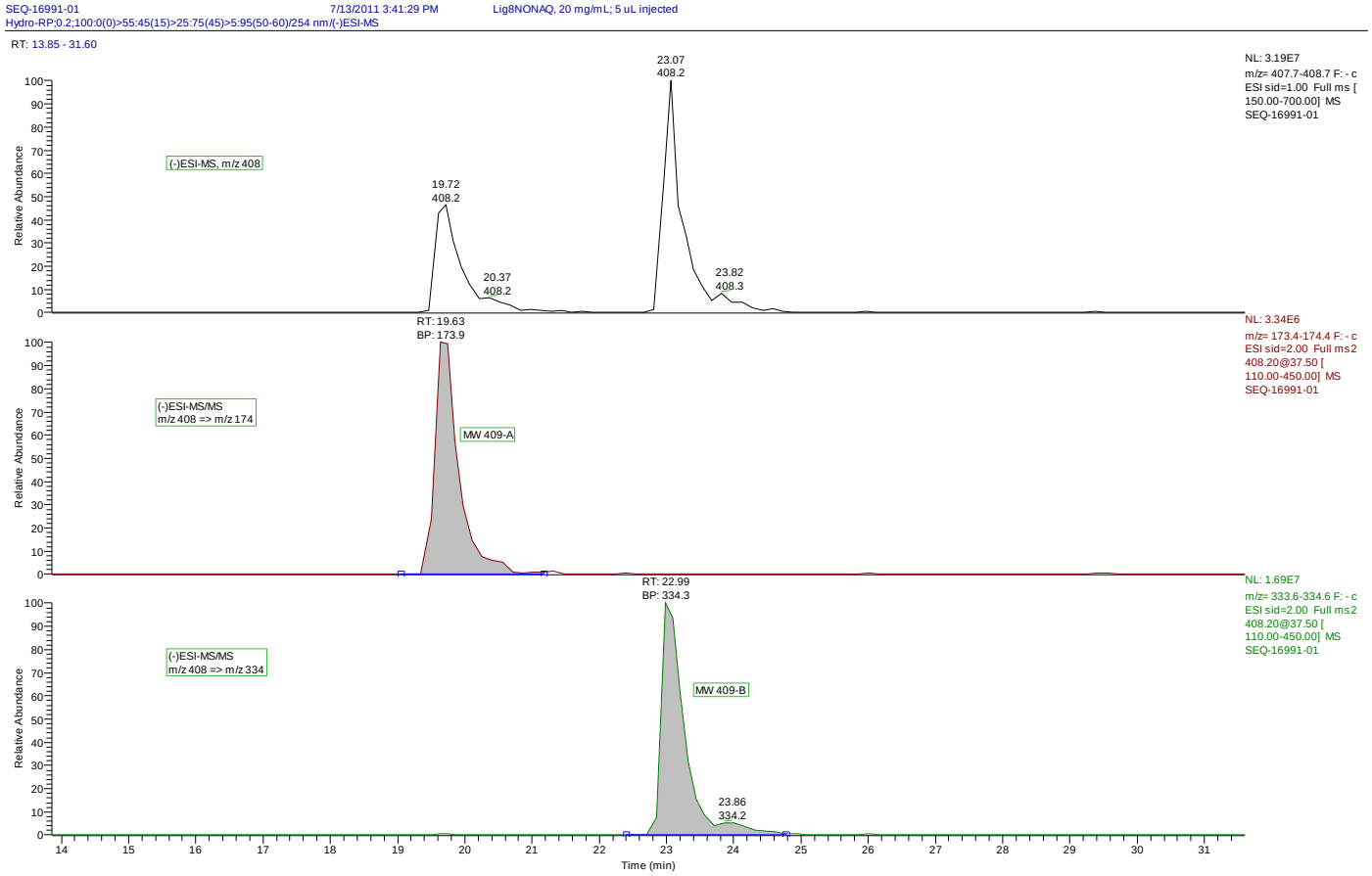


Figure S5. Lig8 NonAq: MW 409 isomers. The two MW 409 isomers were well separated and were characterized relatively uniquely by their (-)ESI-MS/MS product ions as shown below. The MW 409-A was the starting material while MW 409-B was



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.

1.

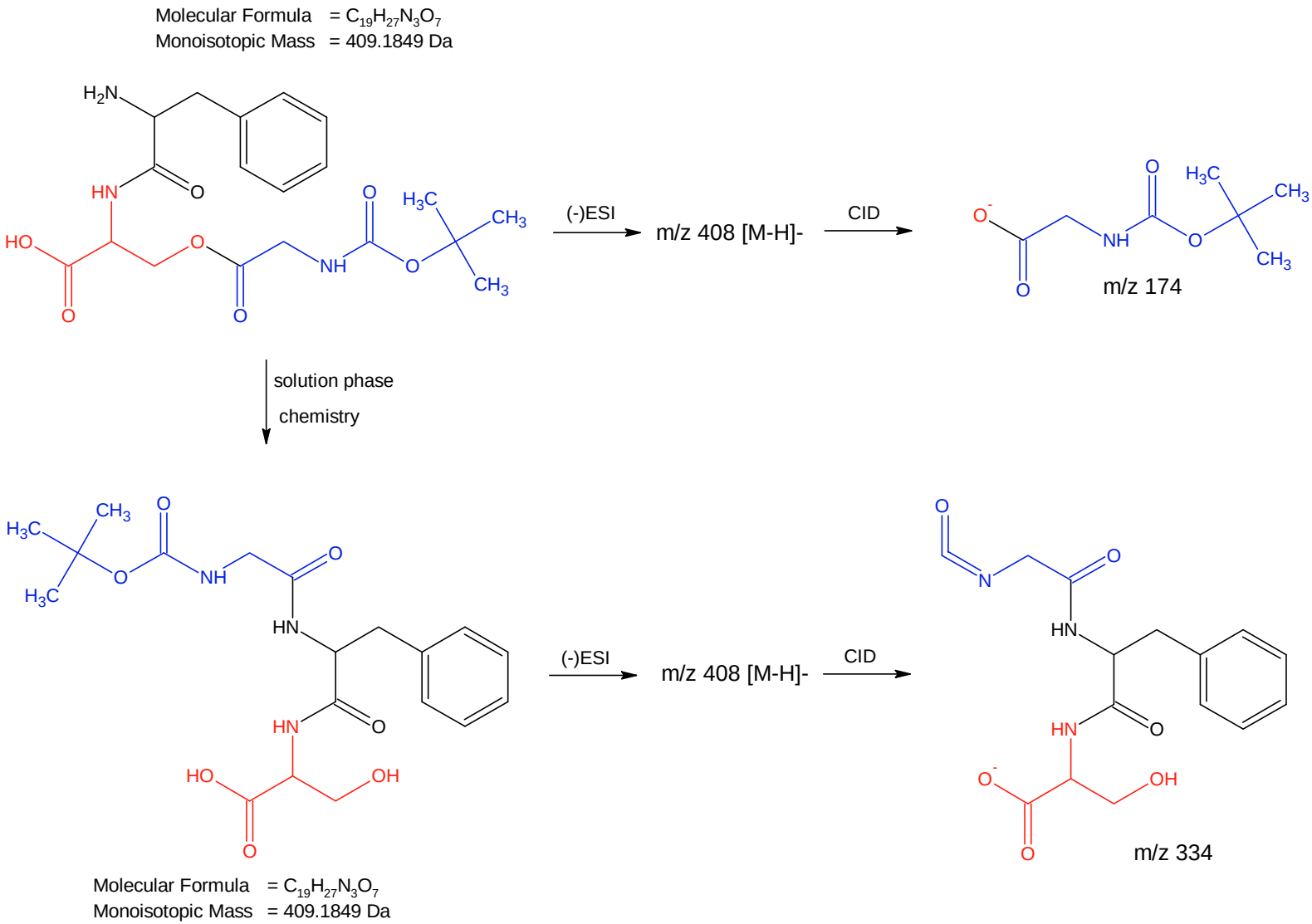
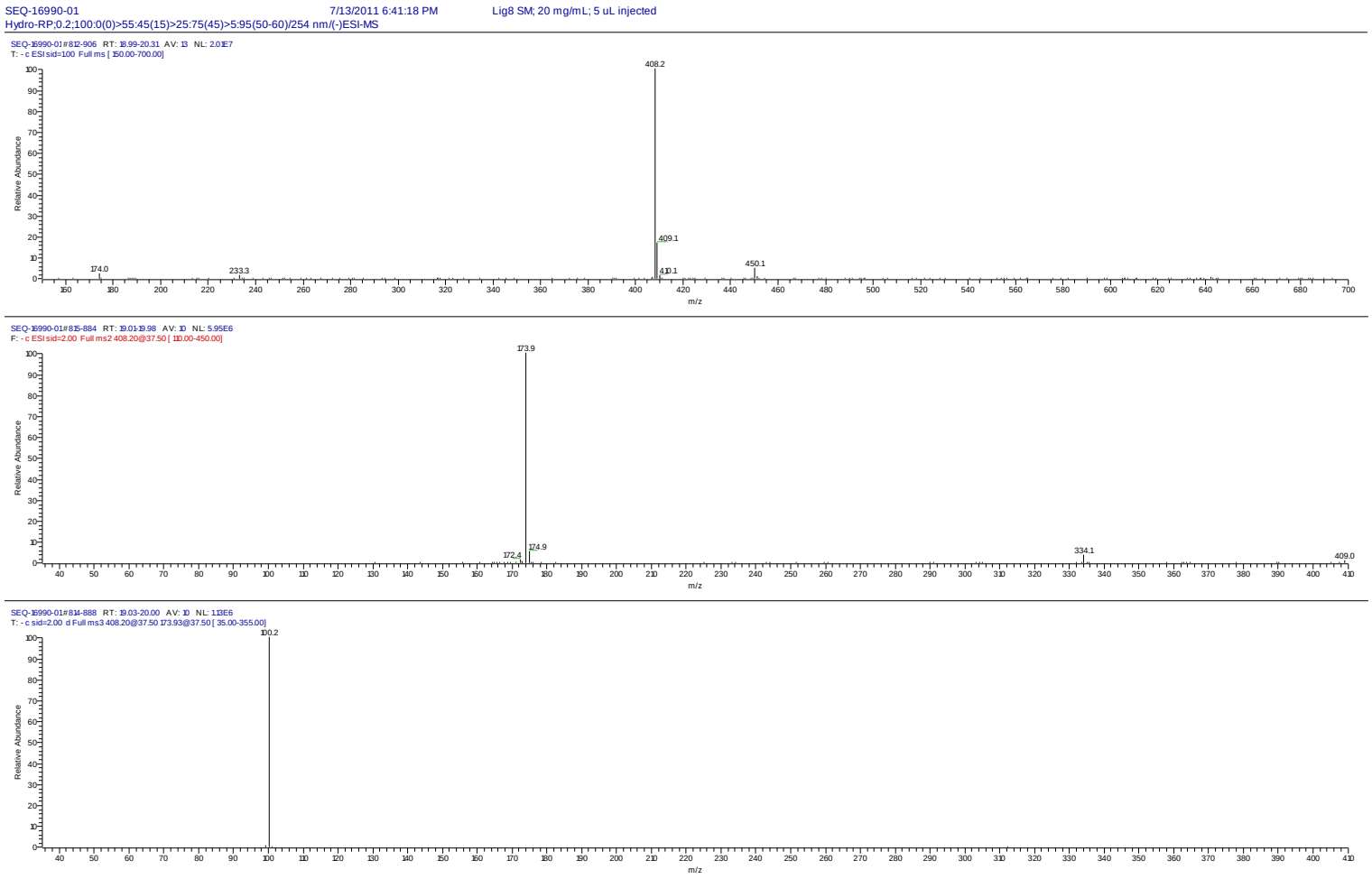


Figure S6. Lig8 SM, MW 409, RT 19.20 min: With (-)ESI-MS, the MW 409 produced an m/z 408 [M-H]- ion (top) which was dissociated to m/z 174 (middle) which was further dissociated to m/z 100 (bottom). The RT and MSn spectra match those of the MW 409-A compound of Lig8 NonAq



HPLC chromatograms and MS Spectra for 5f and 6f.

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

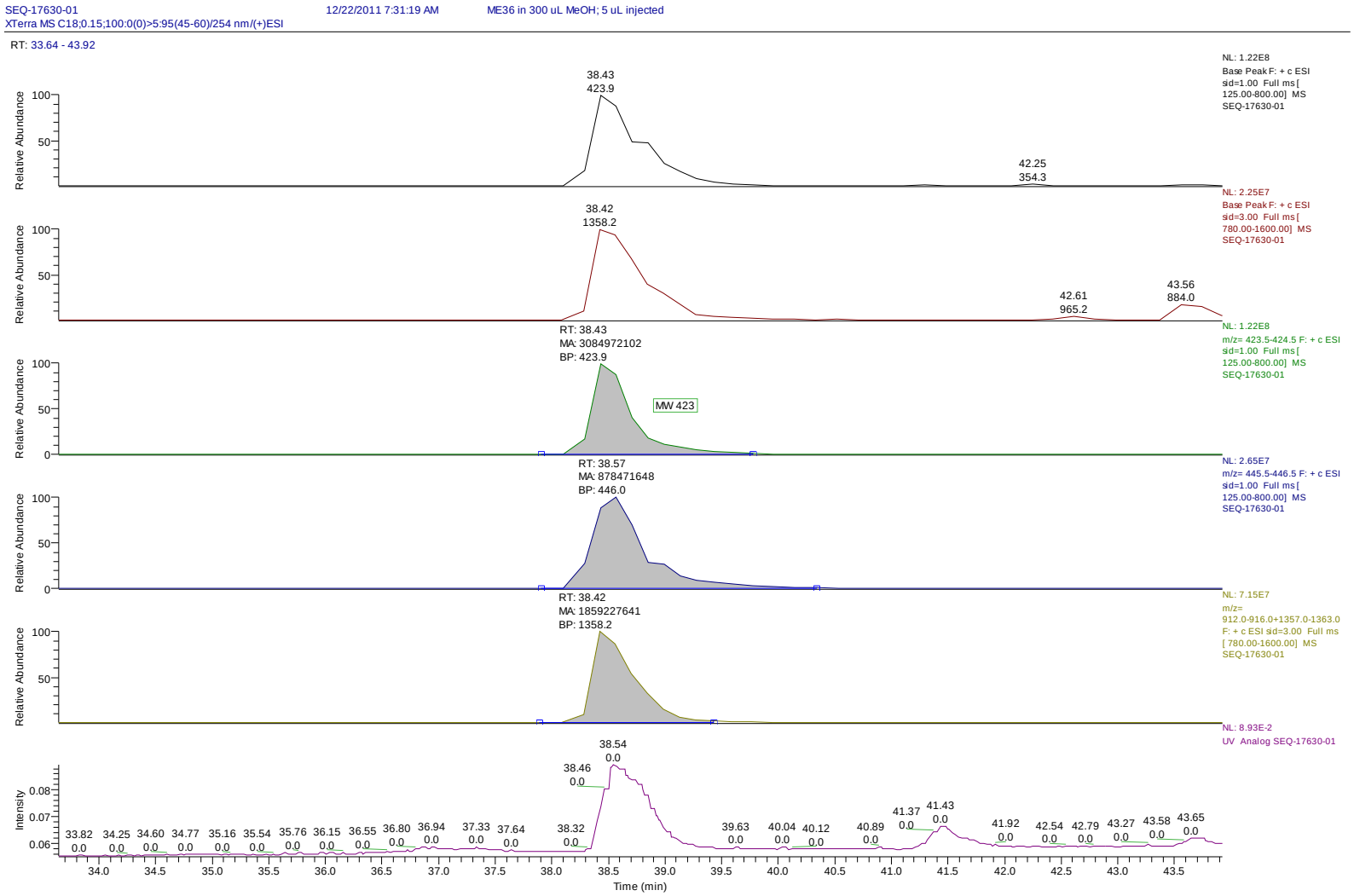


Figure S8. Sample ME36, MW 423. Due to the high concentration, self-adduct ions (m/z 890-958; m/z 1350-1410) were also formed in addition to the m/z 424 [M+H]+ and m/z 445 [M+Na]+. Note the presence of other compounds also, e.g. m/z 438.

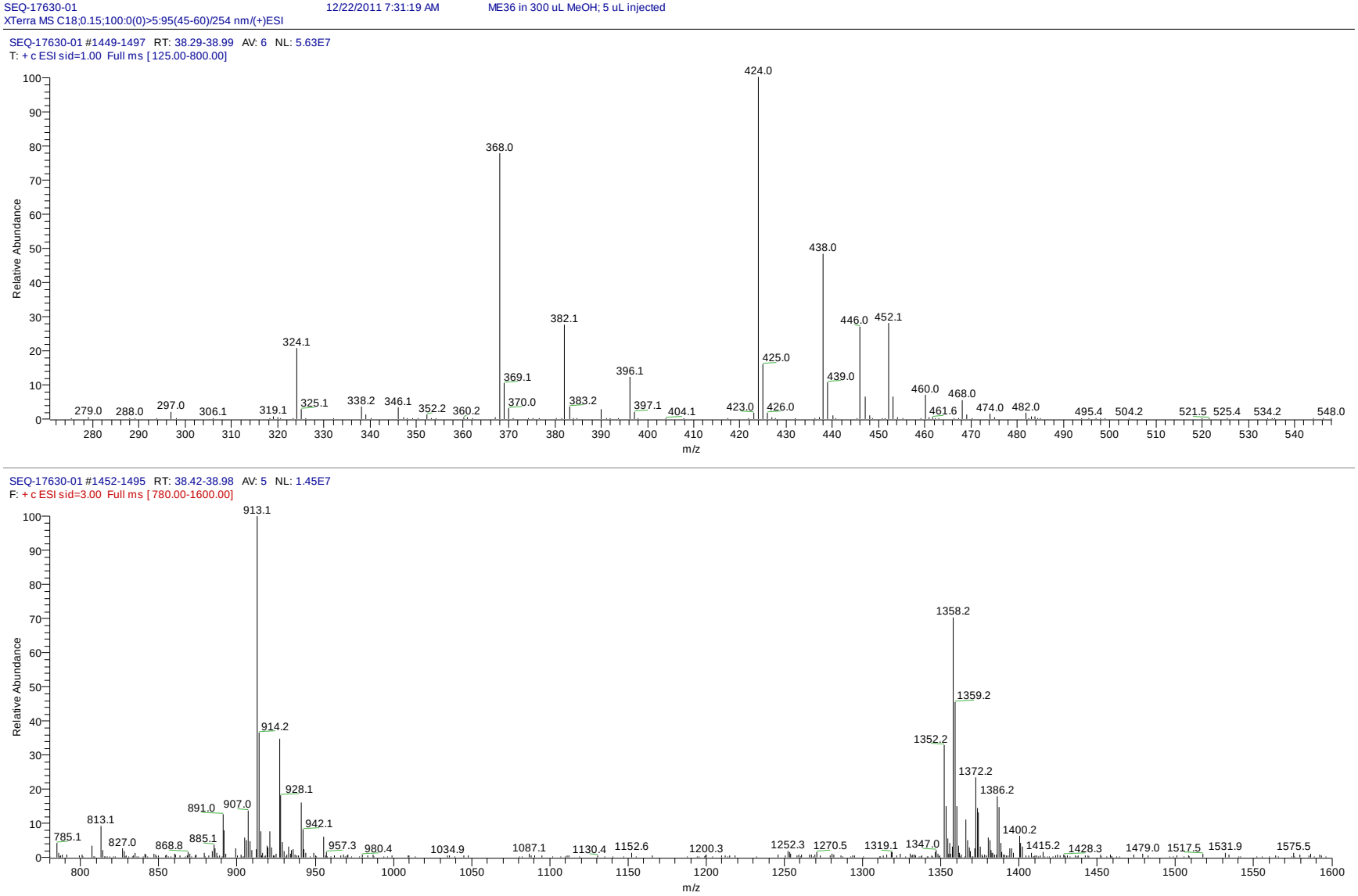


Figure S9. Sample ME36, MW 423. (+)ESI-MSn.

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.

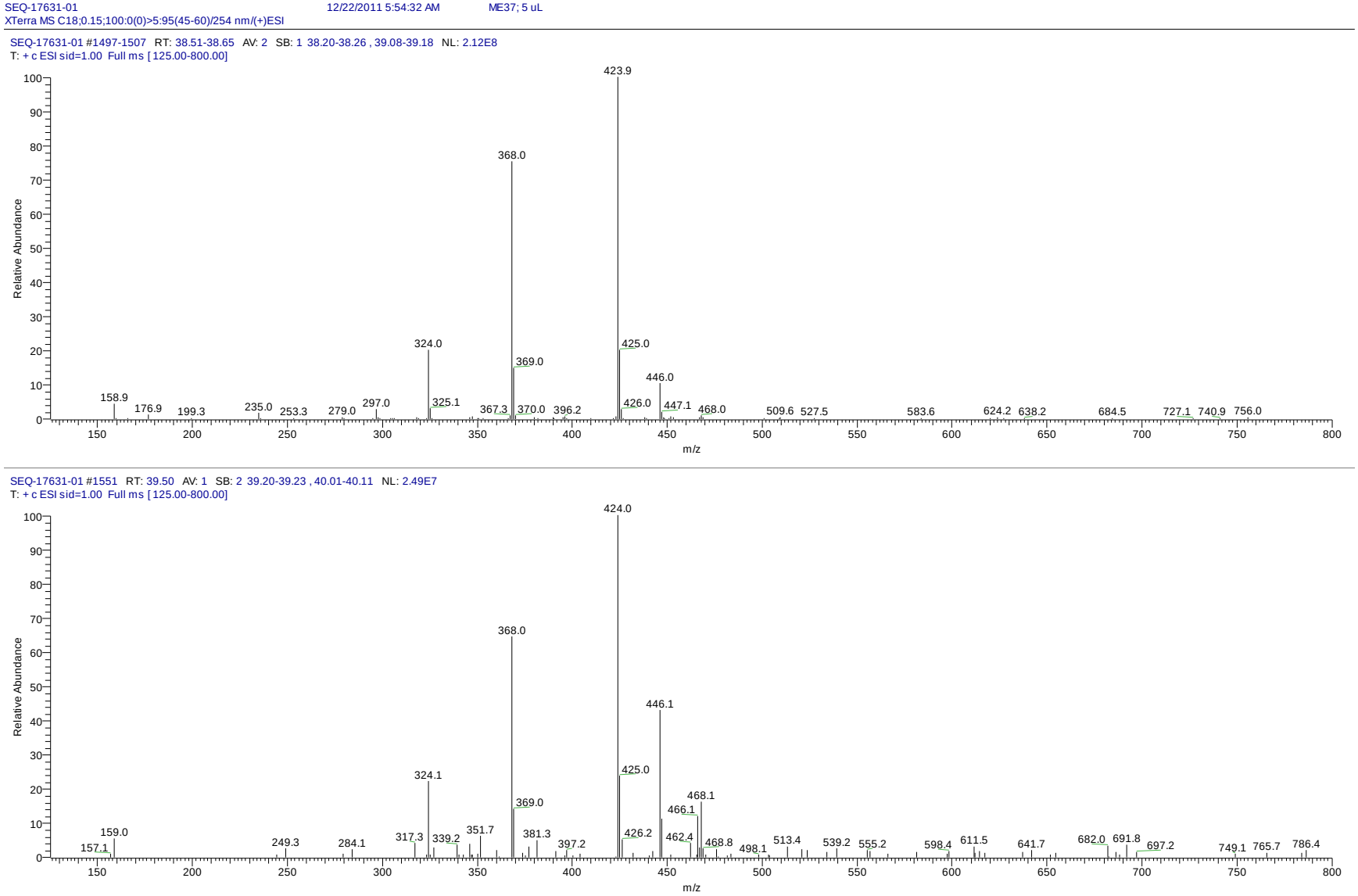


Figure S11. ME37, MW 423 compounds: (+)ESI mass spectra (m/z 780-1600) of RT 38.6 min (top) and RT 39.5 min (bottom)..

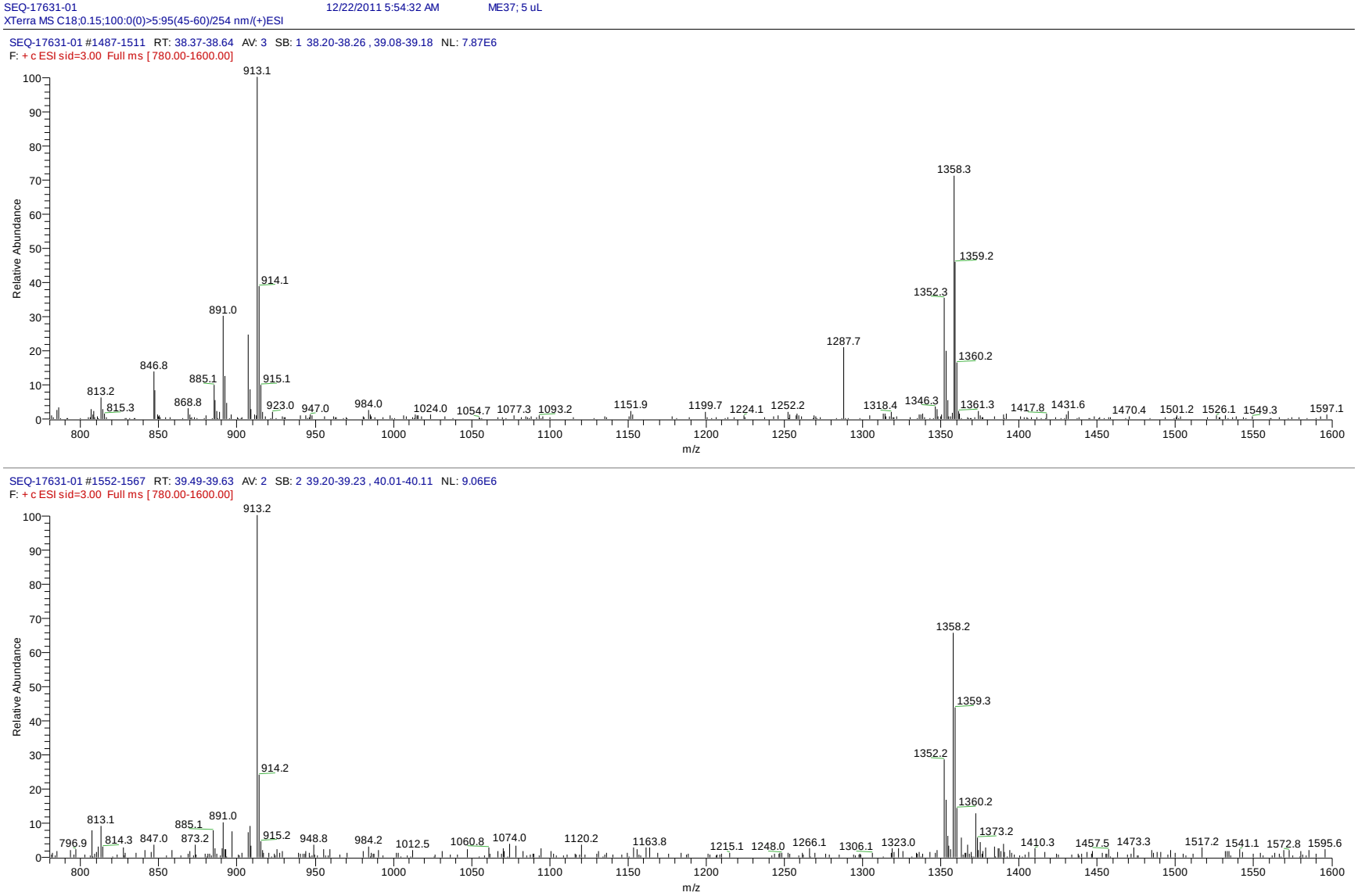


Figure S12. MW 423 RT 38.5 min: (+)ESI-MS/MS of the m/z 424 [M+H]+ ions from ME37 (top) and ME 36 (bottom) were identical.

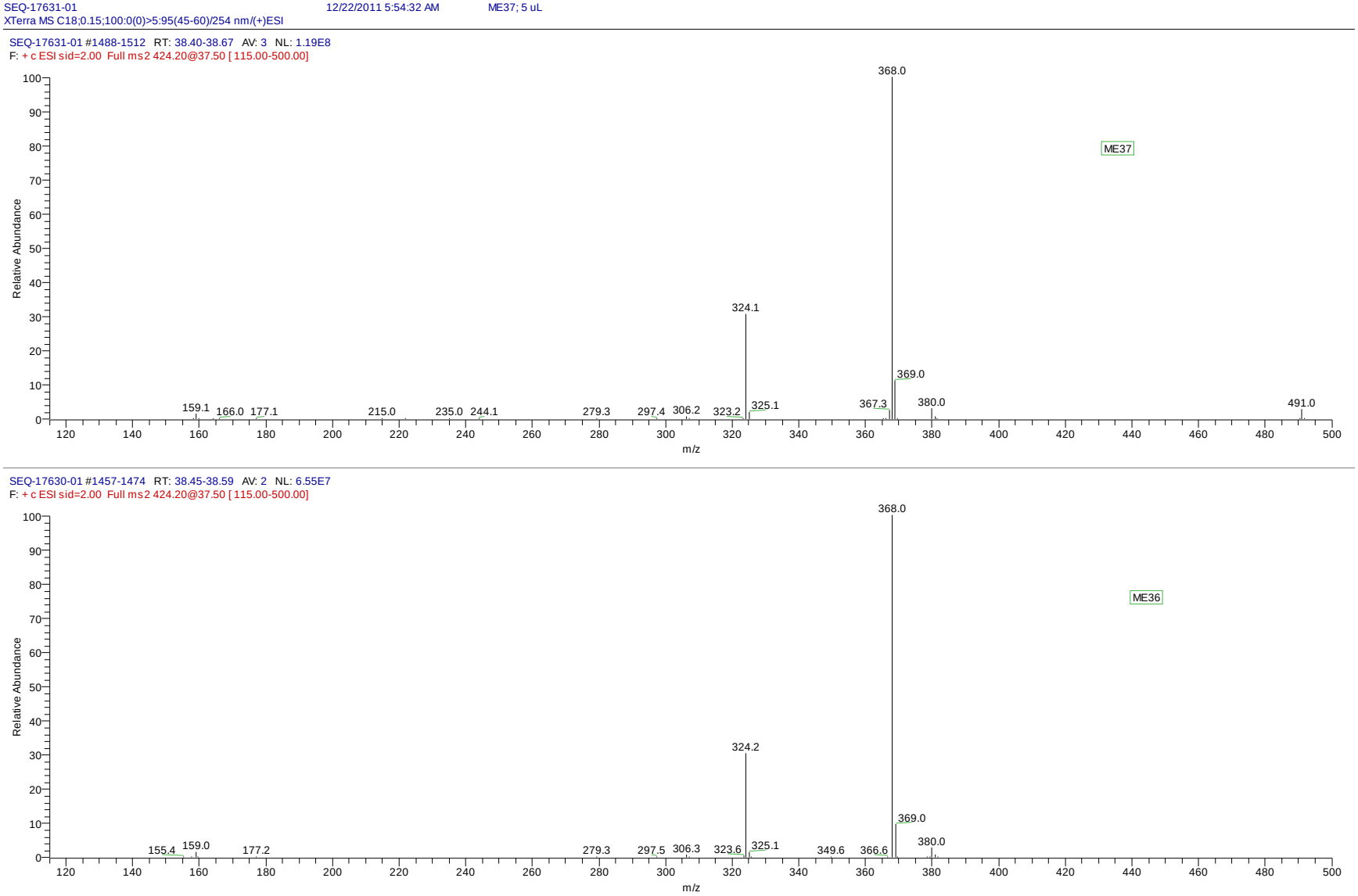


Figure S13. MW 423 compounds in ME37. (+)ESI-MS/MS (top and 3rd) and –MS/MS/MS (2nd and 4th) for the 38.6 min (top 2) and RT 39.5 min (bottom 2) MW 423 compounds. The corresponding spectra are near identical.

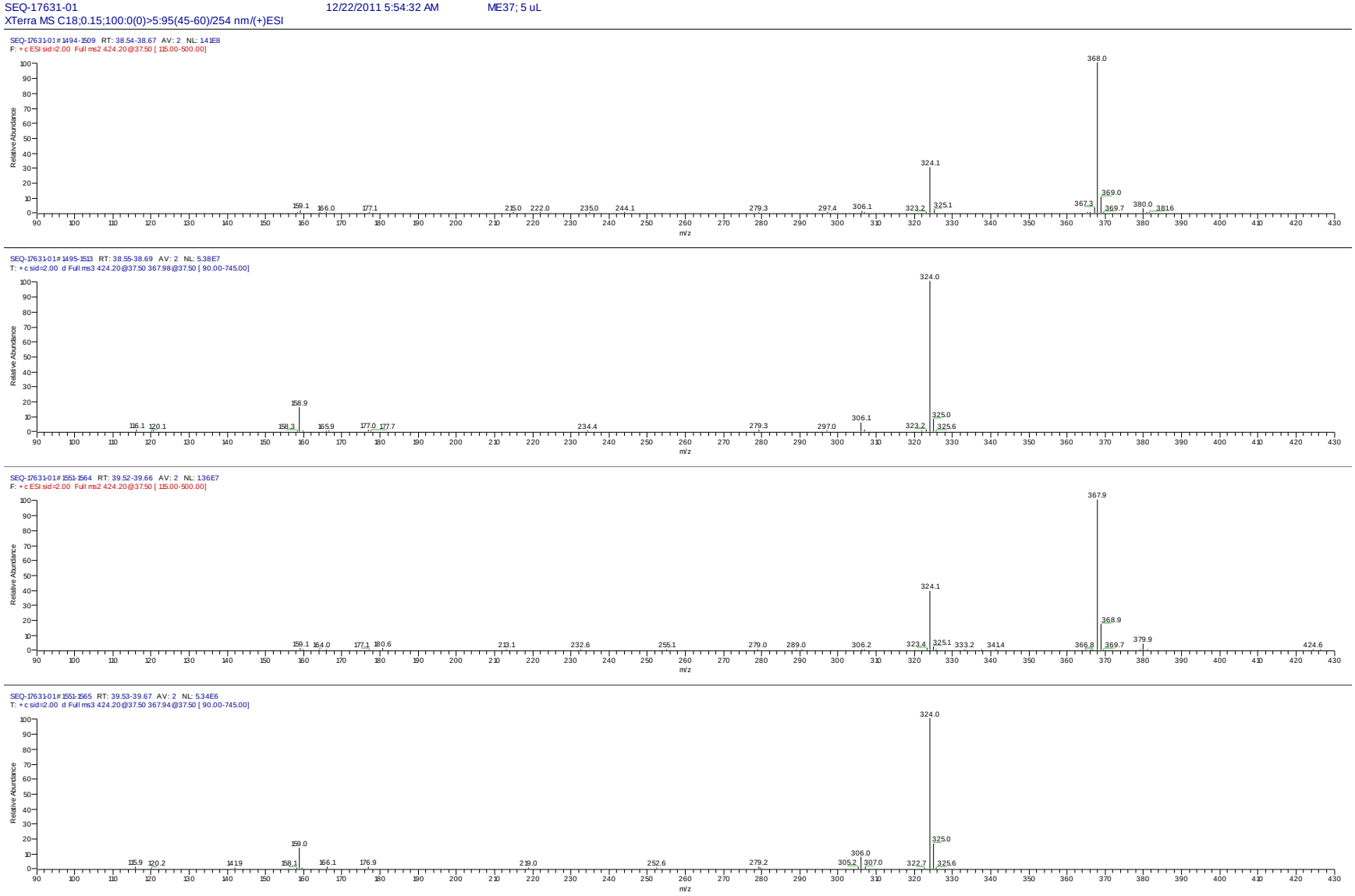


Figure S14. MW 423 compounds, integrated peak areas. The corresponding areas should be summed.

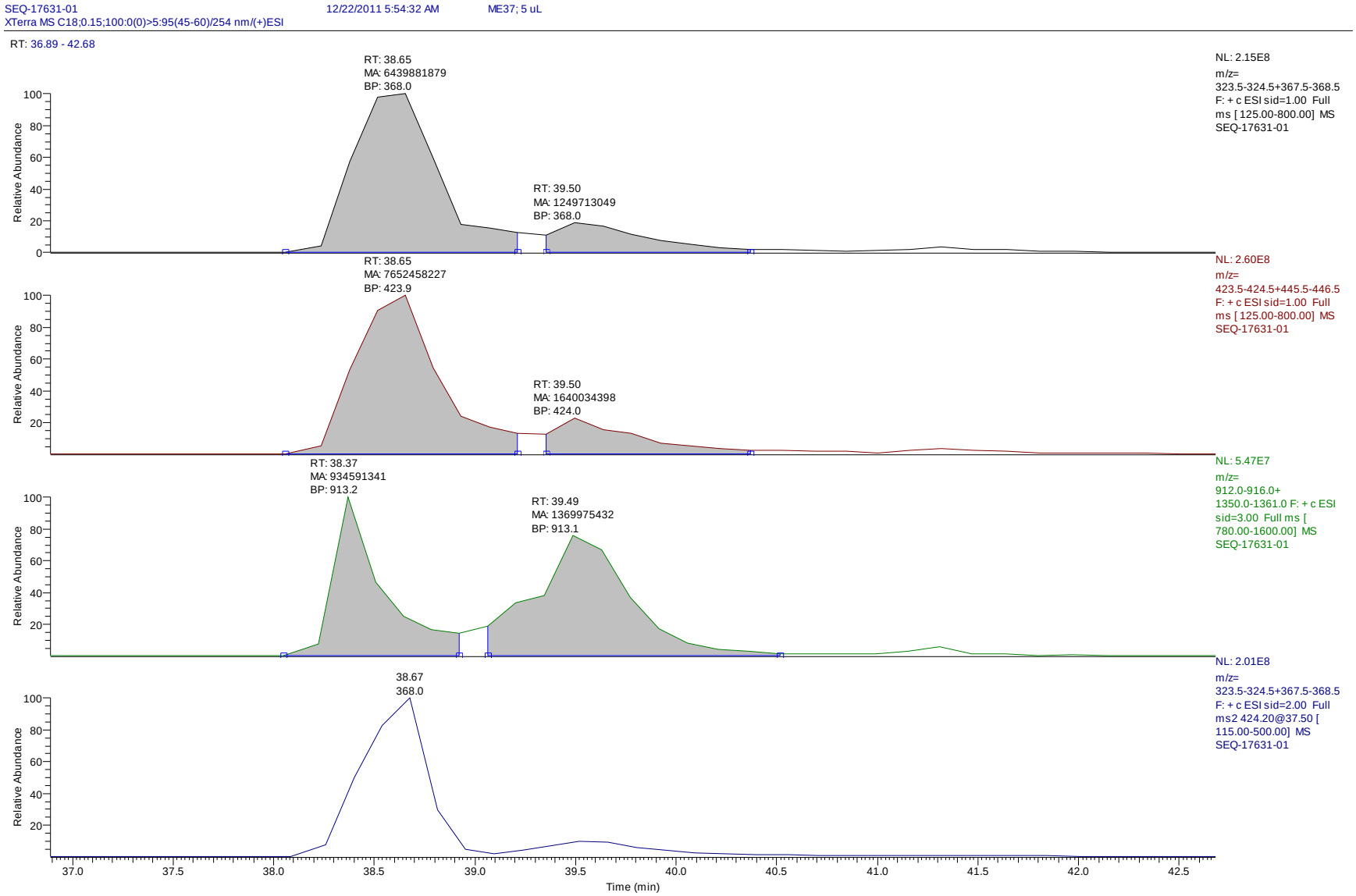


Figure S15. MW 176: This compound was not retained on the HPLC column and eluted at RT 2.8 min. Due to its ion-chemistry and abundance, it produced mostly self-adduct ions. Note also that it produced m/z 221 [M-H+Na+Na]+ and the neutral for adduction was the MW 198 $[M-H+Na]^0$. There also appeared to 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).

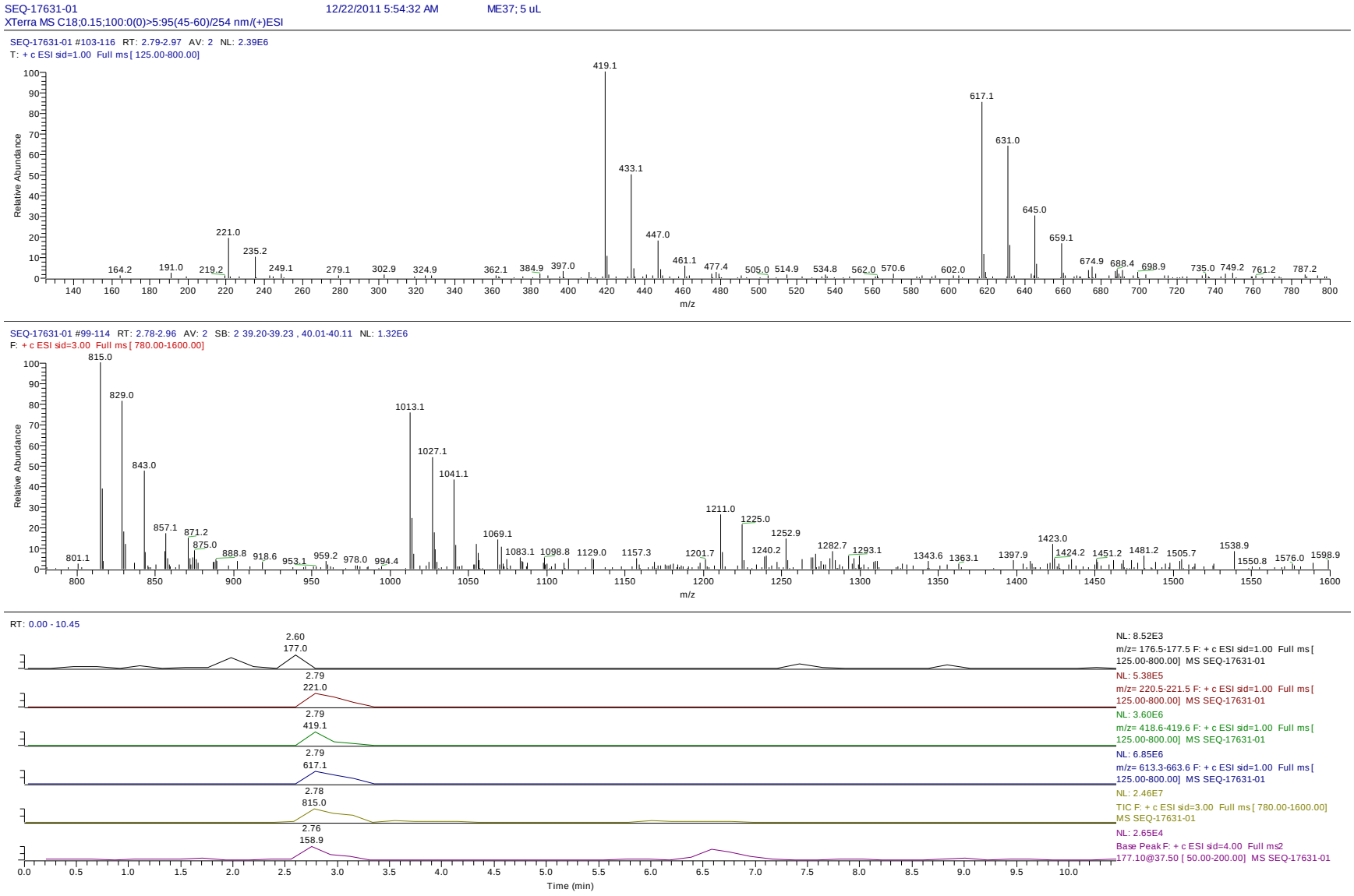


Figure S16. MW 176: (+)ESI-MS (top) and –MS/MS Of m/z 177 (very weak; middle) and of m/z 419 [2(M-H+Na)+Na]+ (bottom).

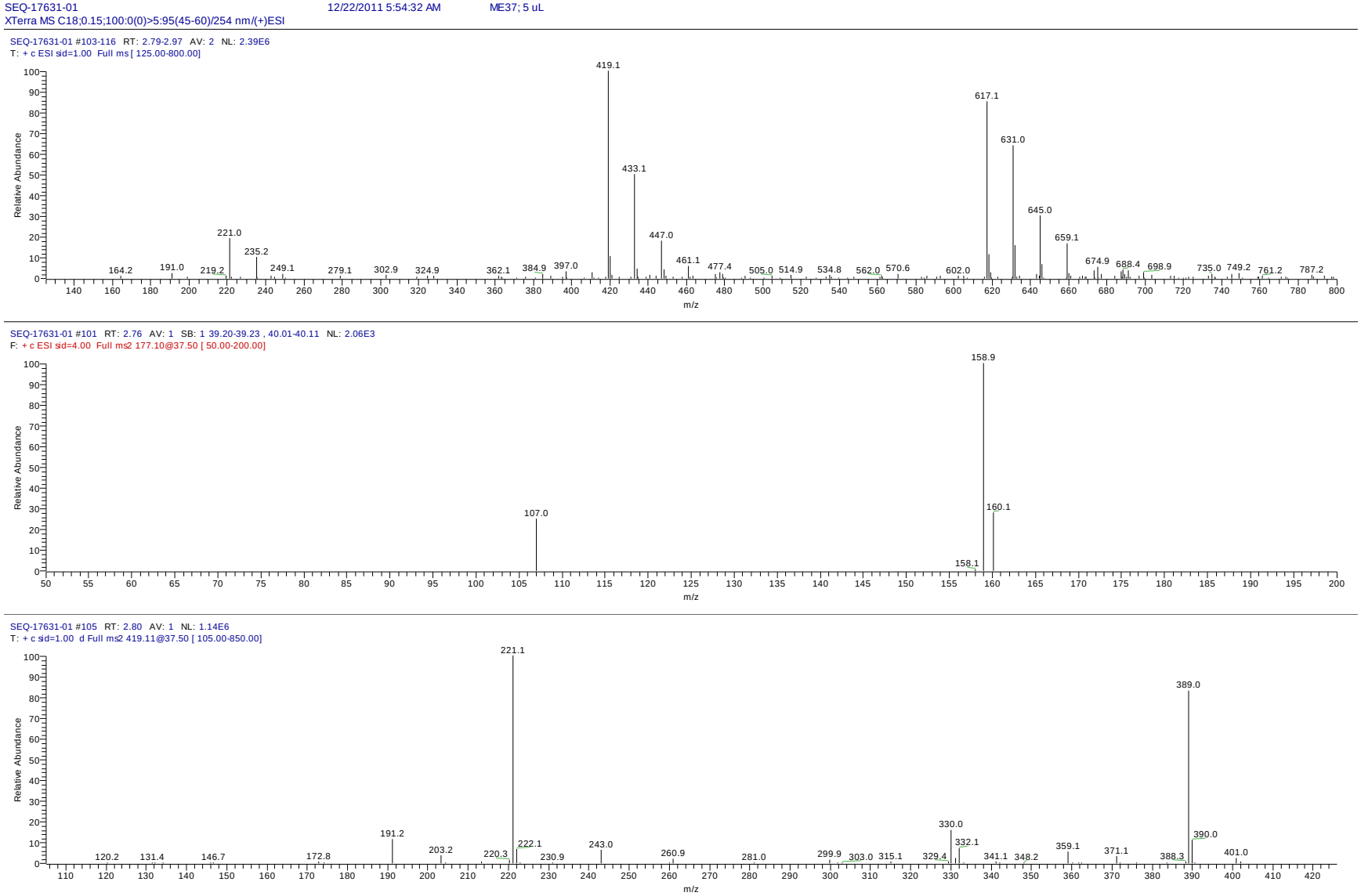


Figure S17. MW 176: HPLC/(+)ESI-MS ion-peaks integrated.

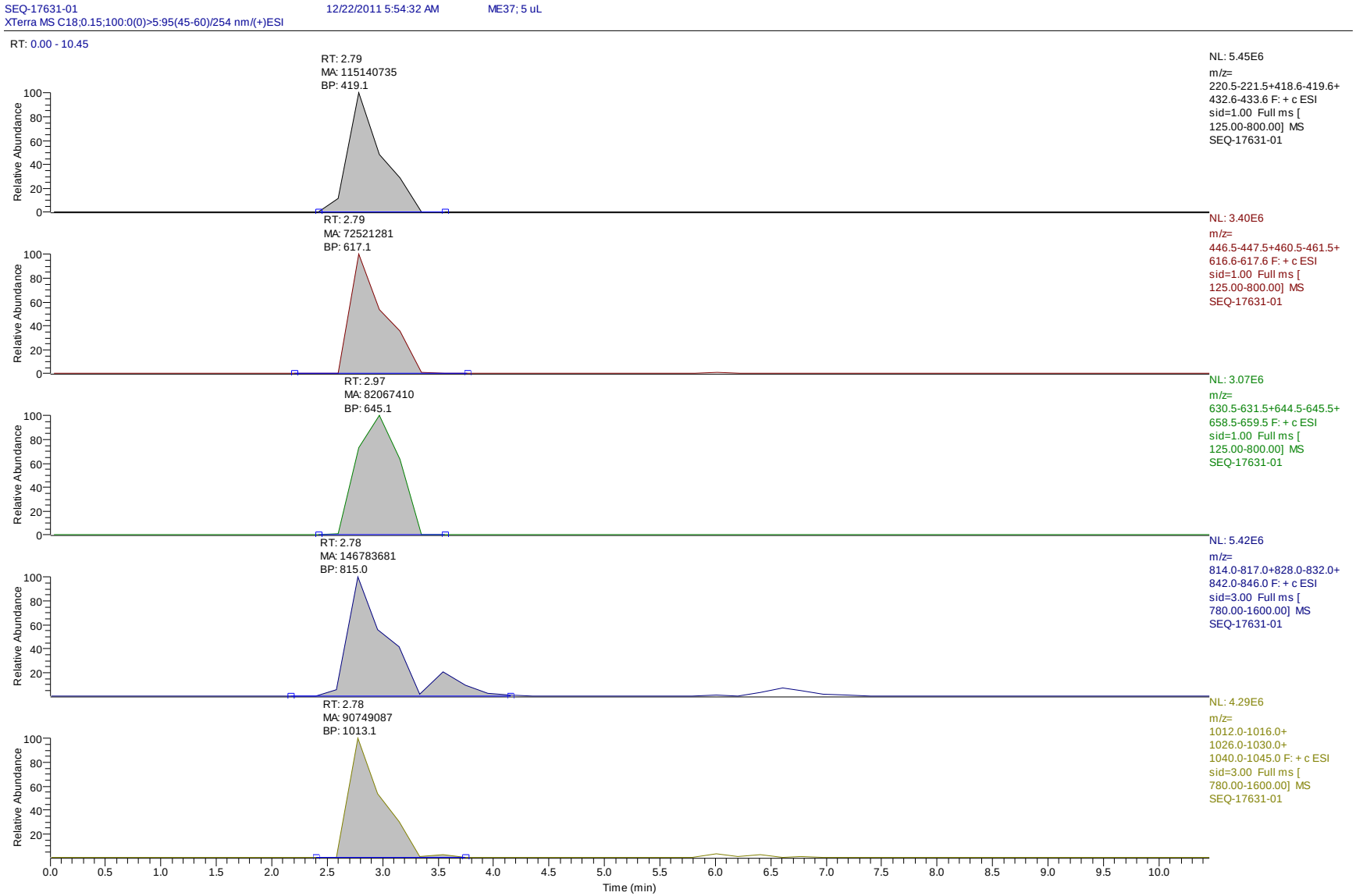
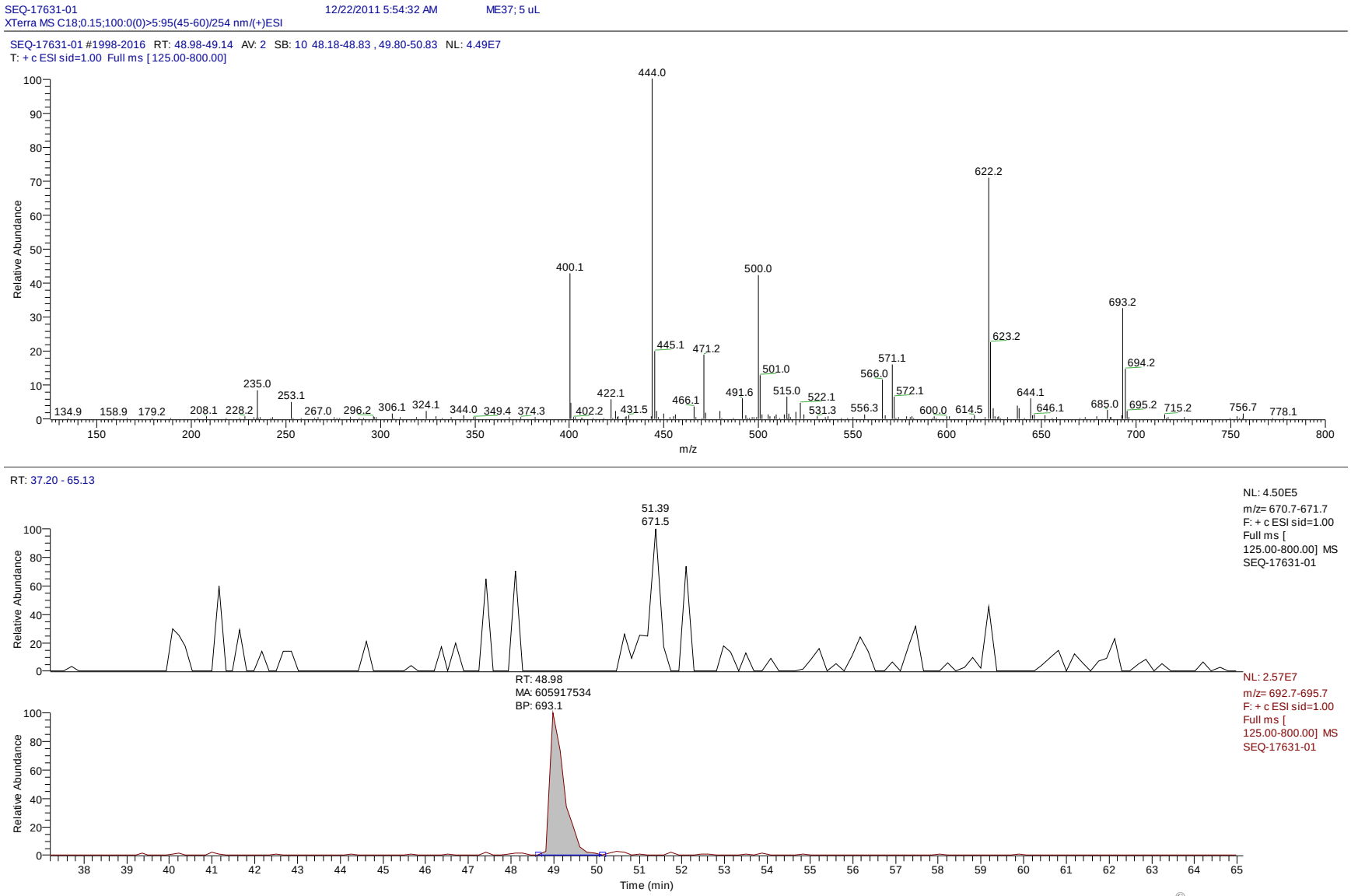


Figure S18. MW 670? The MW 670 should have produced an m/z 671 [M+H]+ and m/z 693 [M+Na]+ ions. There were no correlating ion-peaks for these two ions. There was a very small m/z 671 ion-peak at RT 51.39 but the signal was too low to be conclusive. The (+)ESI mass spectra for the relataively abundant 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 5c and 6c

Figure S19. MW 333, RT 18.01 min. The MW 333 compound formed m/z 334 [M+H]+, m/z 356 [M+Na]+ and m/z 378 [M-H+2Na]+ ions (top) with the latter being indicative of an acidic proton. The m/z 334 was readily dissociated to m/z 278 (middle) which was dissociated further to m/z 234, 216 159 and several other secondary product ions (bottom).

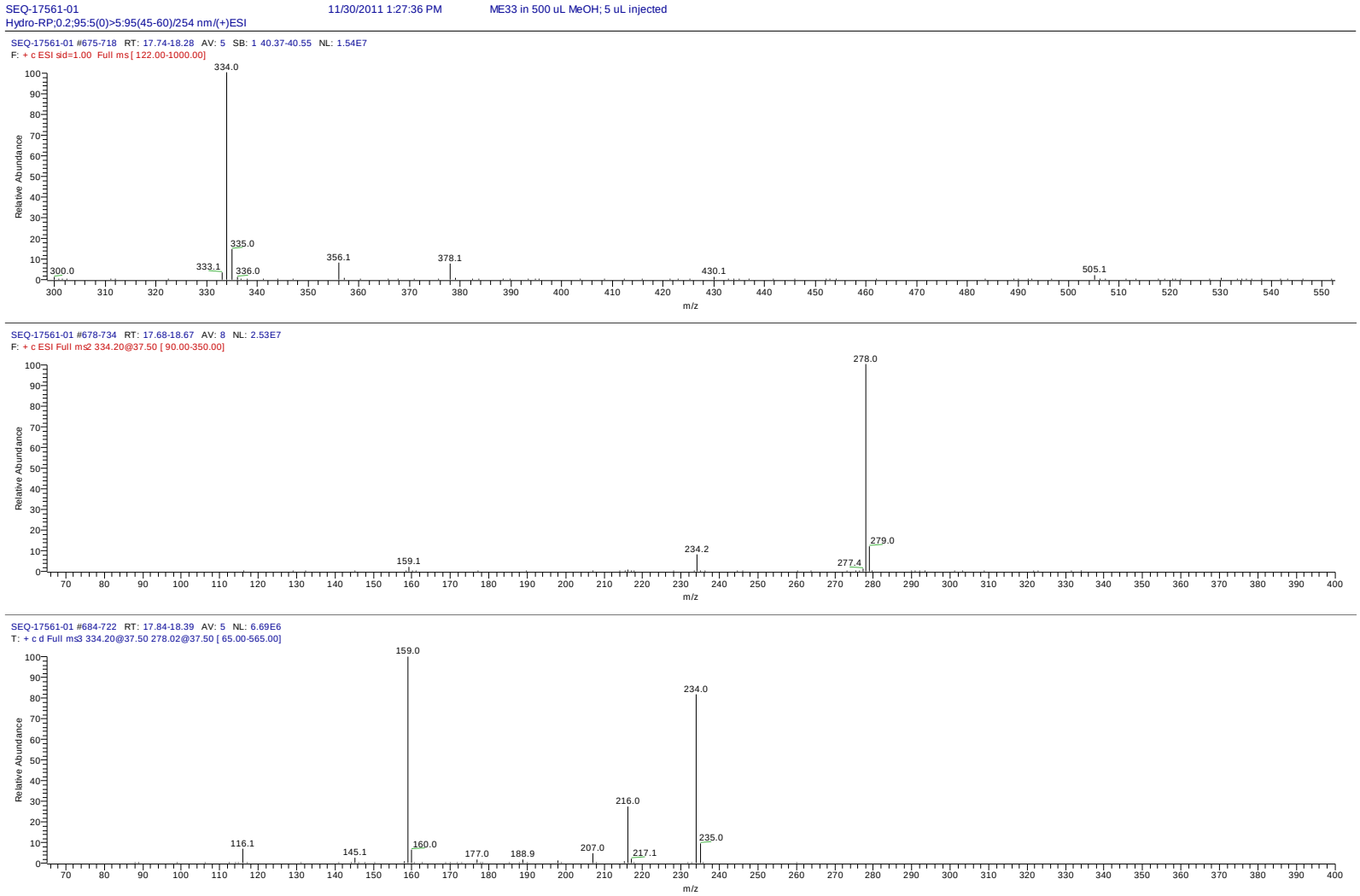
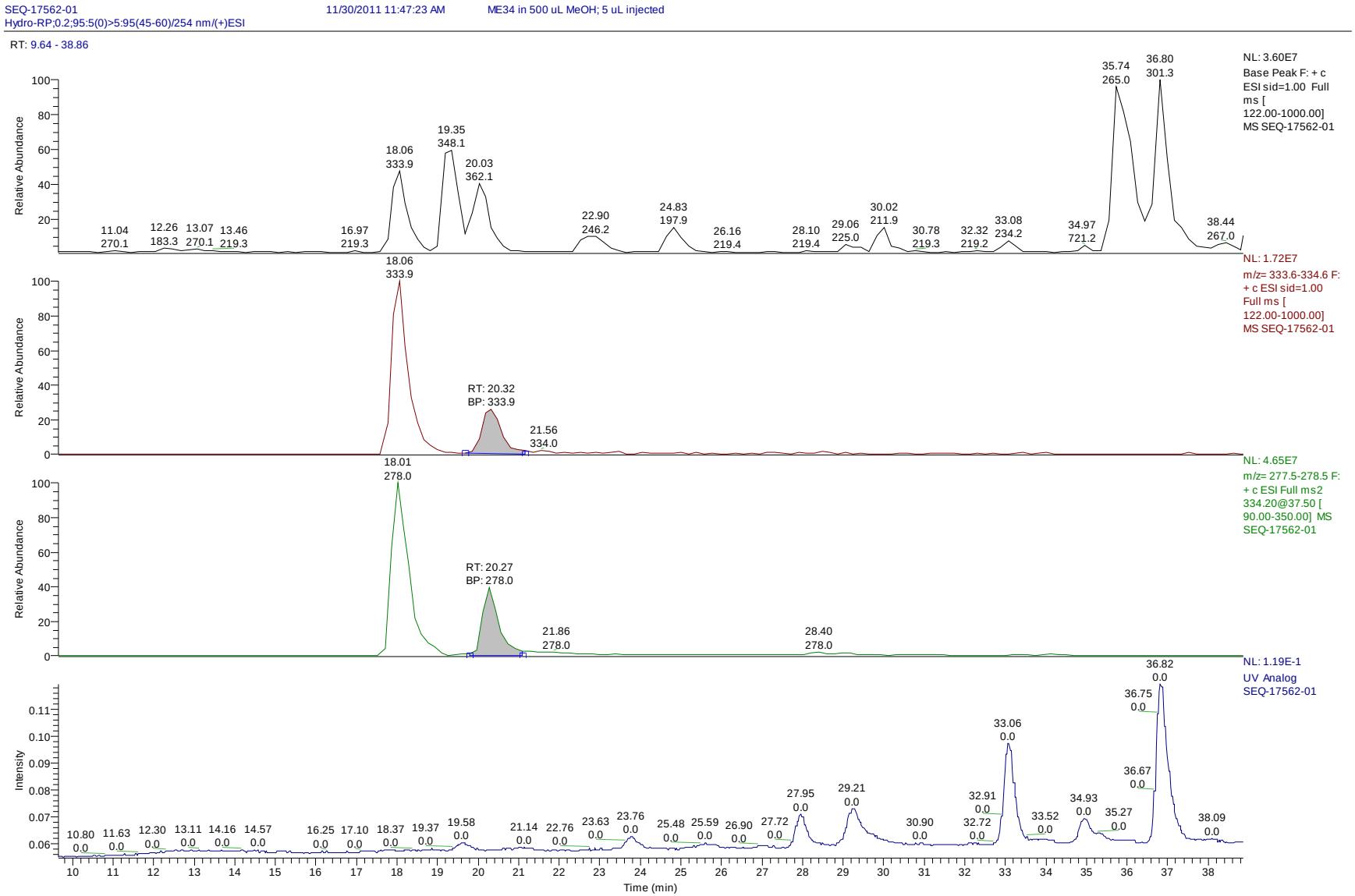


Figure S20. MW 333 compounds. A new MW 333 compound eluted at RT 20.32 min (shaded peak). The unshaded m/z 334 and m/z 334 => m/z 279 peaks are due to SM compounds



HPLC chromatograms and MS Spectra for 9a and 10a
Scheme S2. taken from the submitted reaction

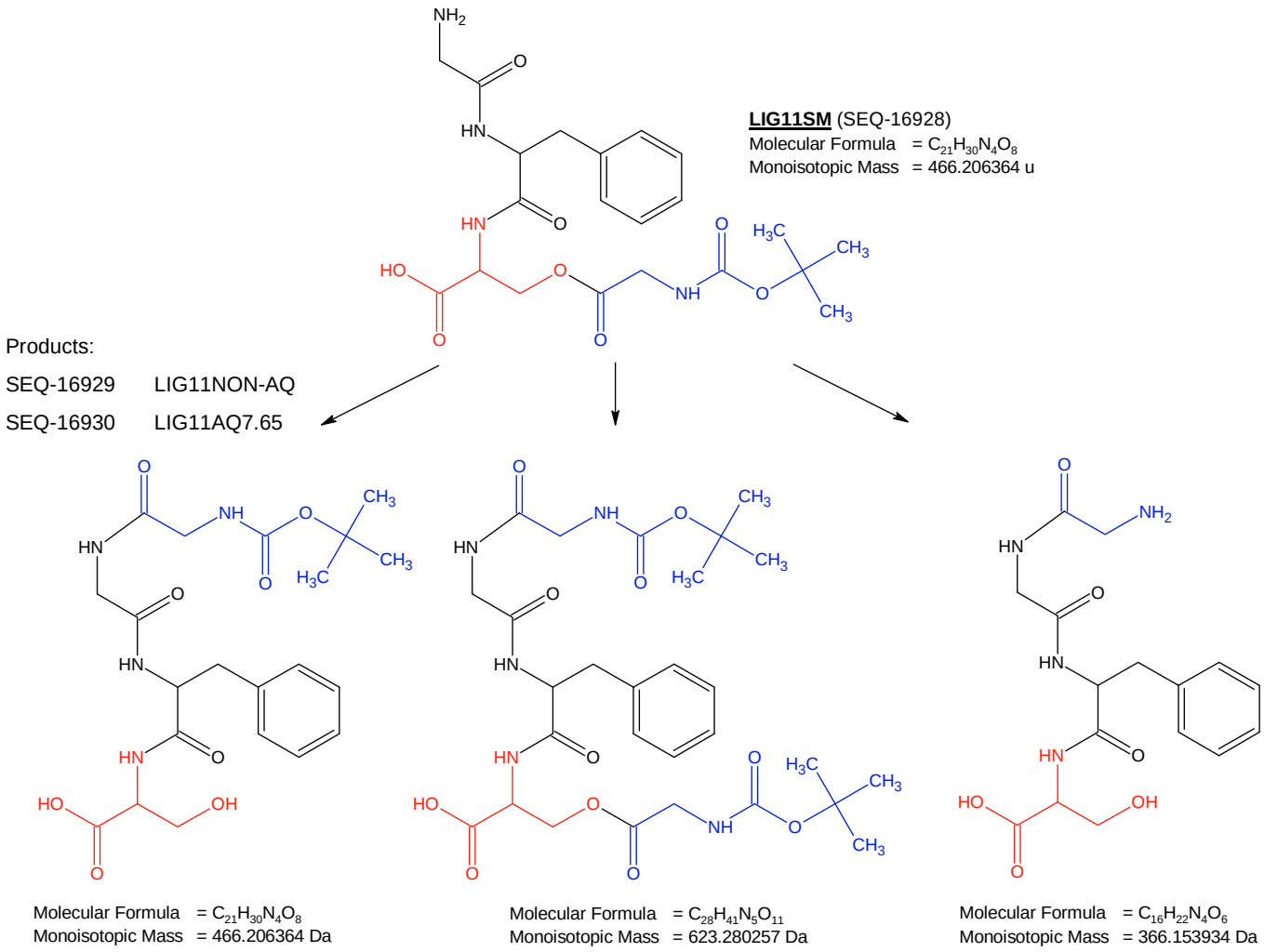


Figure S21. LIG11SM, MW 466, RT 25.46 min. The MW 466 produced m/z 467 [M+H]+, m/z 489 [M+Na]+ and m/z 511 [(M-H+Na)+Na]+ ions (top). The latter is characteristic of compounds with acidic protons, e.g. RCOOH

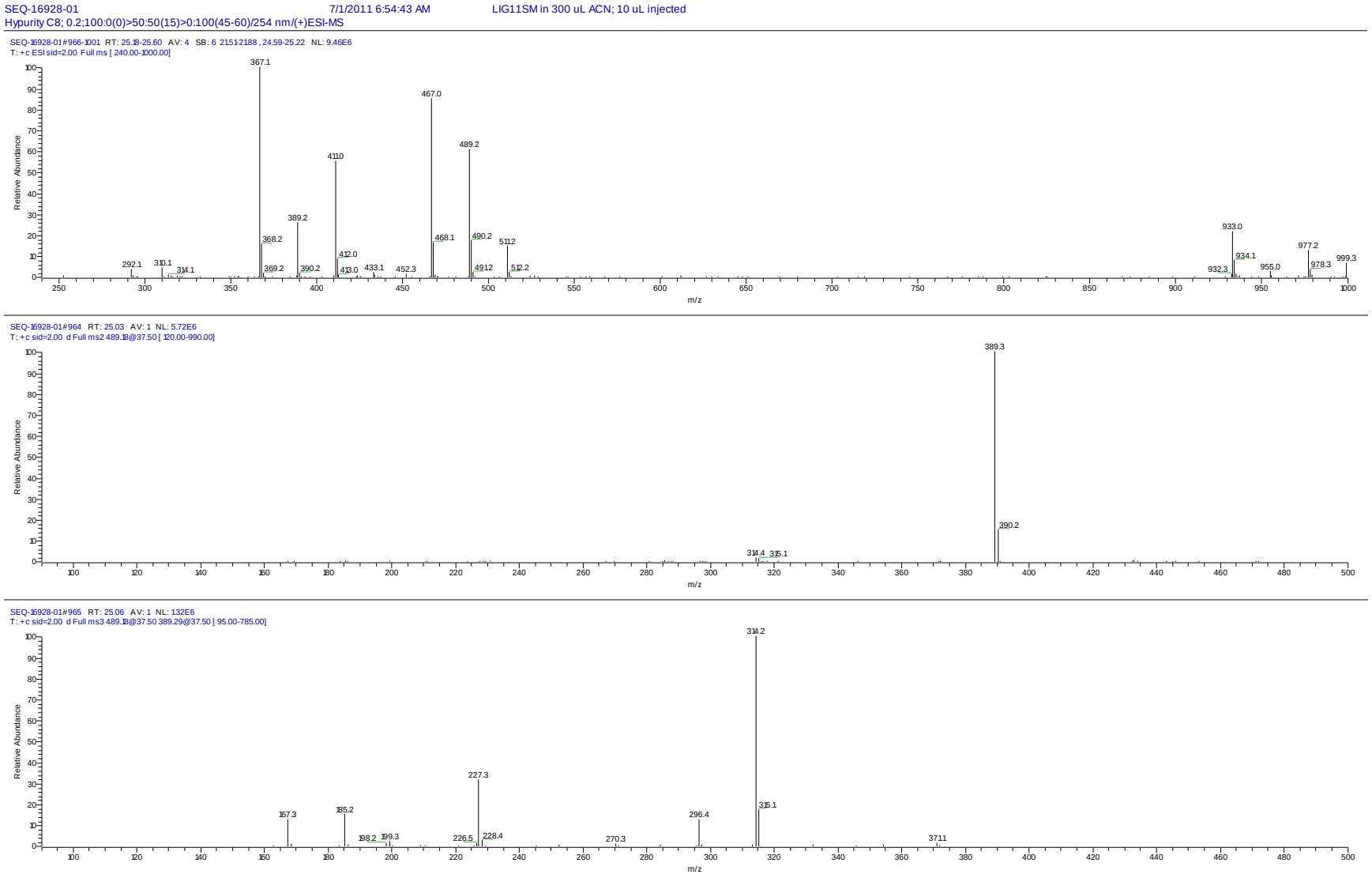


Figure S22. LIG11NONAQ, MW 466, RT 21.67 min. The MW 466 product was expected to produce m/z 467 [M+H]+ and m/z 489 [M+Na]+ ions. The mass chromatograms show only one potential MW 466 compound at RT 21.67.

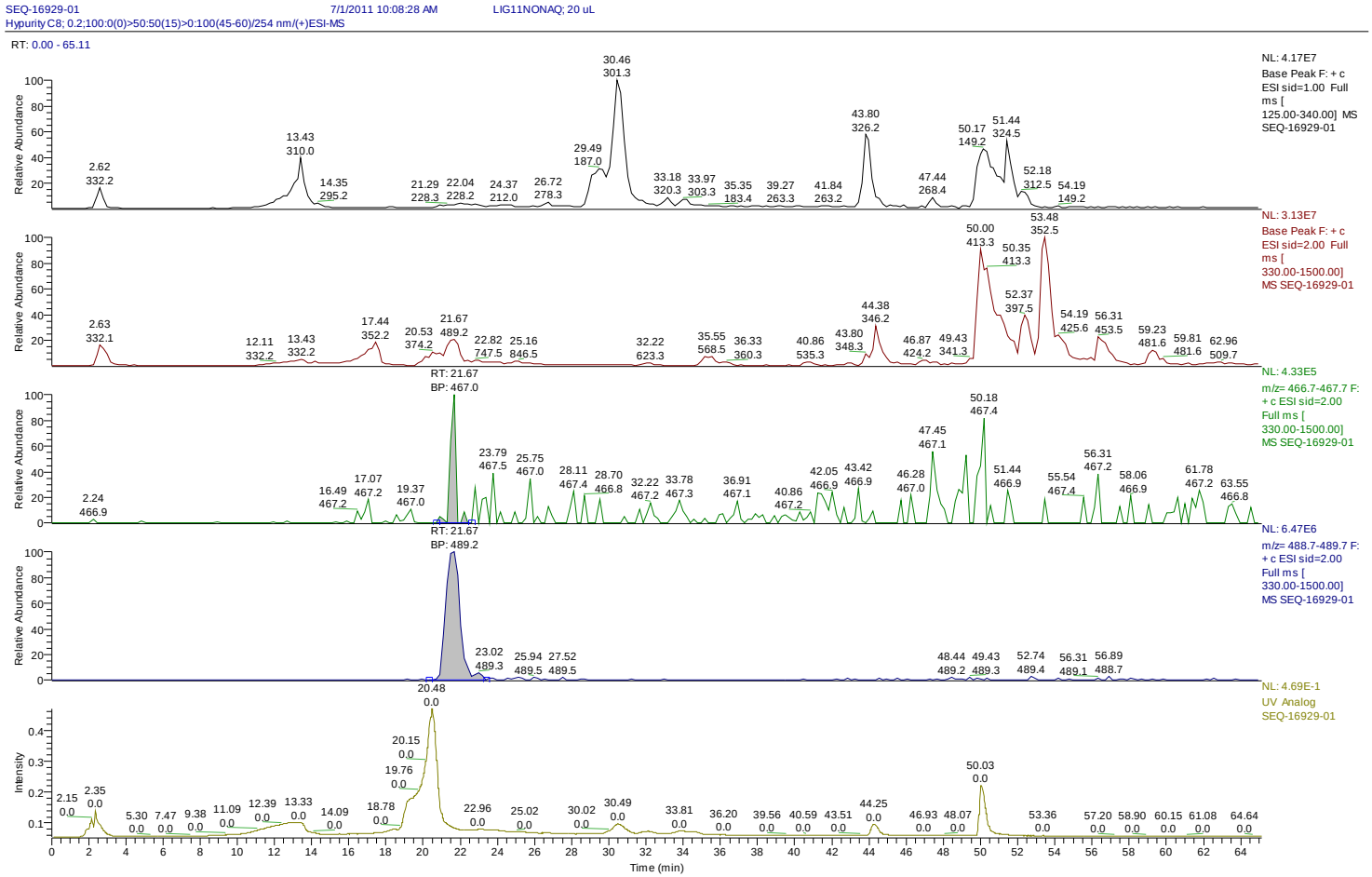


Figure S23. LIG11 NONAQ via HPLC/UV/(-)ESI-MSn, MW 466, RT 21.67 min. The tentative MW 466 detected via (+)ESI-MS above produced predominantly an m/z 465 [M-H]- ion under (-)ESI-MS conditions and thus confirmed it as being a MW 466 compound

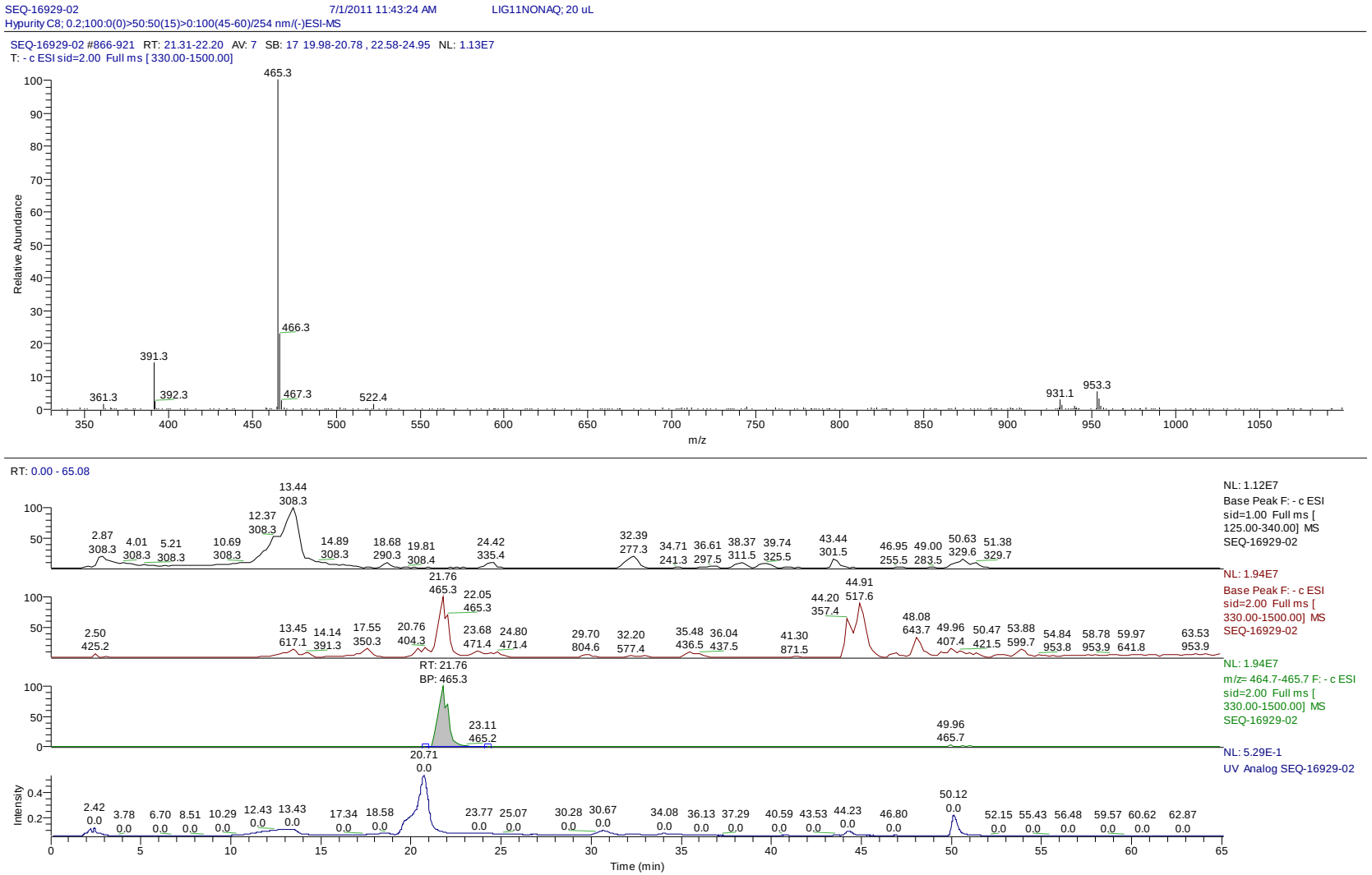


Figure S24. HPLC/(-)ESI-MS mass chromatograms of m/z 465. MW 466 compounds in (top to bottom): LIG11SM, LIG11NONAQ and LIG11 AQ 7.65. Only a very minor amount of MW 466 was detected in the LIG11 AQ7.65 sample. Note the products eluted prior to the starting material.

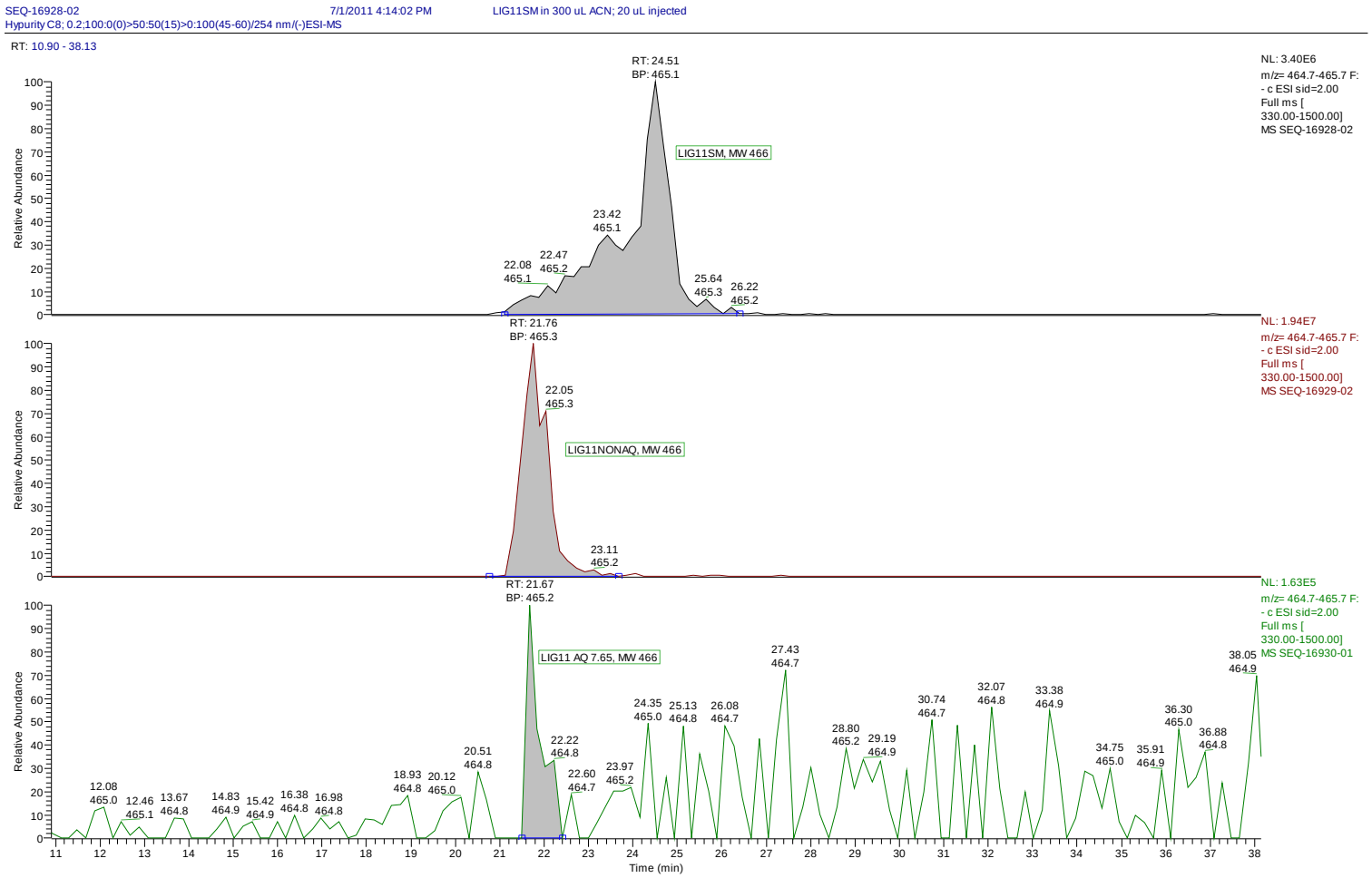


Figure S25. (-)ESI-MS/MS of the m/z 465 [M-H]- ions of the MW 466 compounds in (top to bottom): LIG11SM, LIG11NONAQ and LIG11 AQ 7.65. While it was expected that the starting material and products were different, the two MW 466 products also produced different dissociation ions.

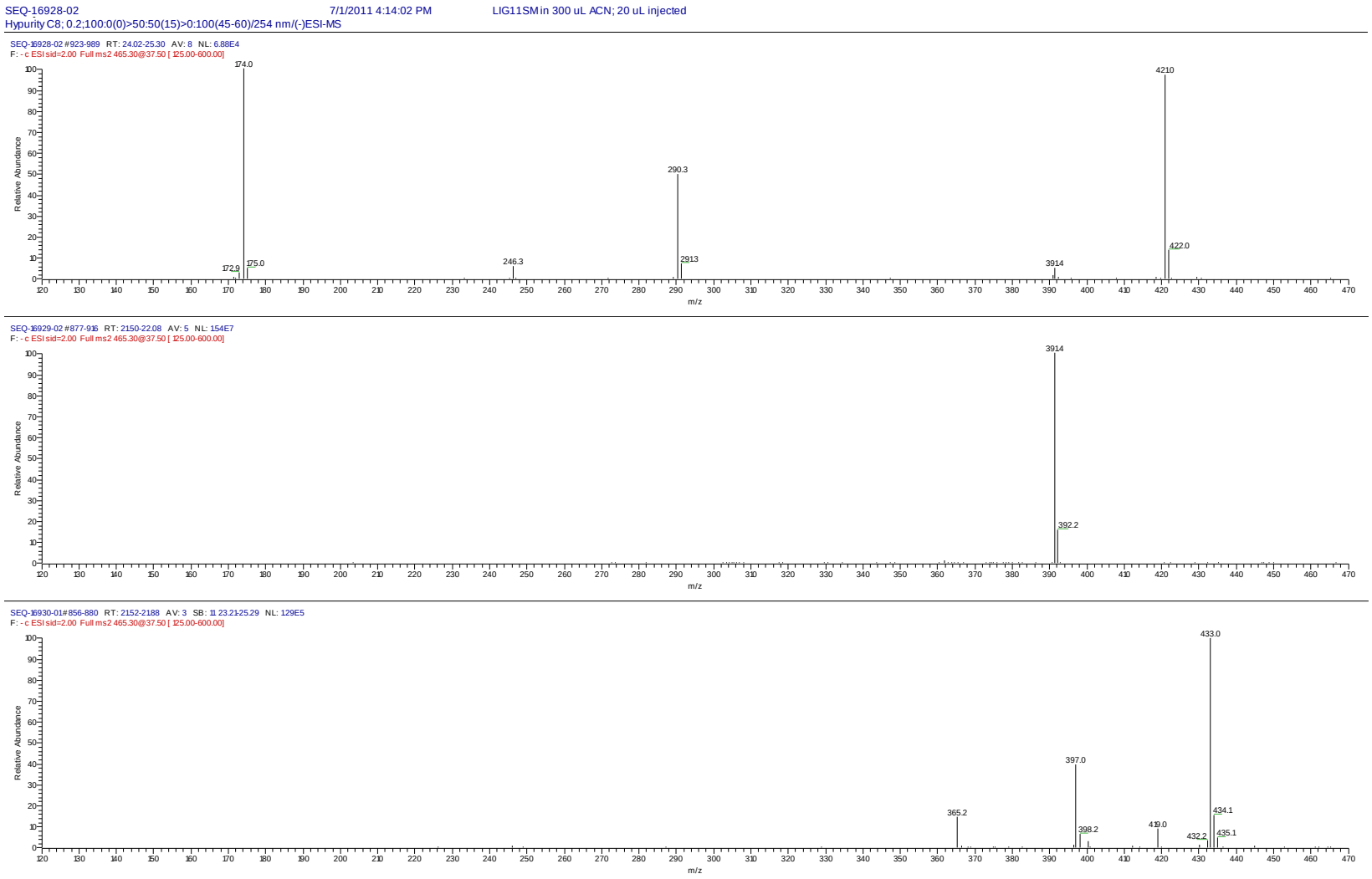


Figure S26. LIG11SM, MW 466, (-)ESI-MSn of the m/z 465 [M-H]- ion.

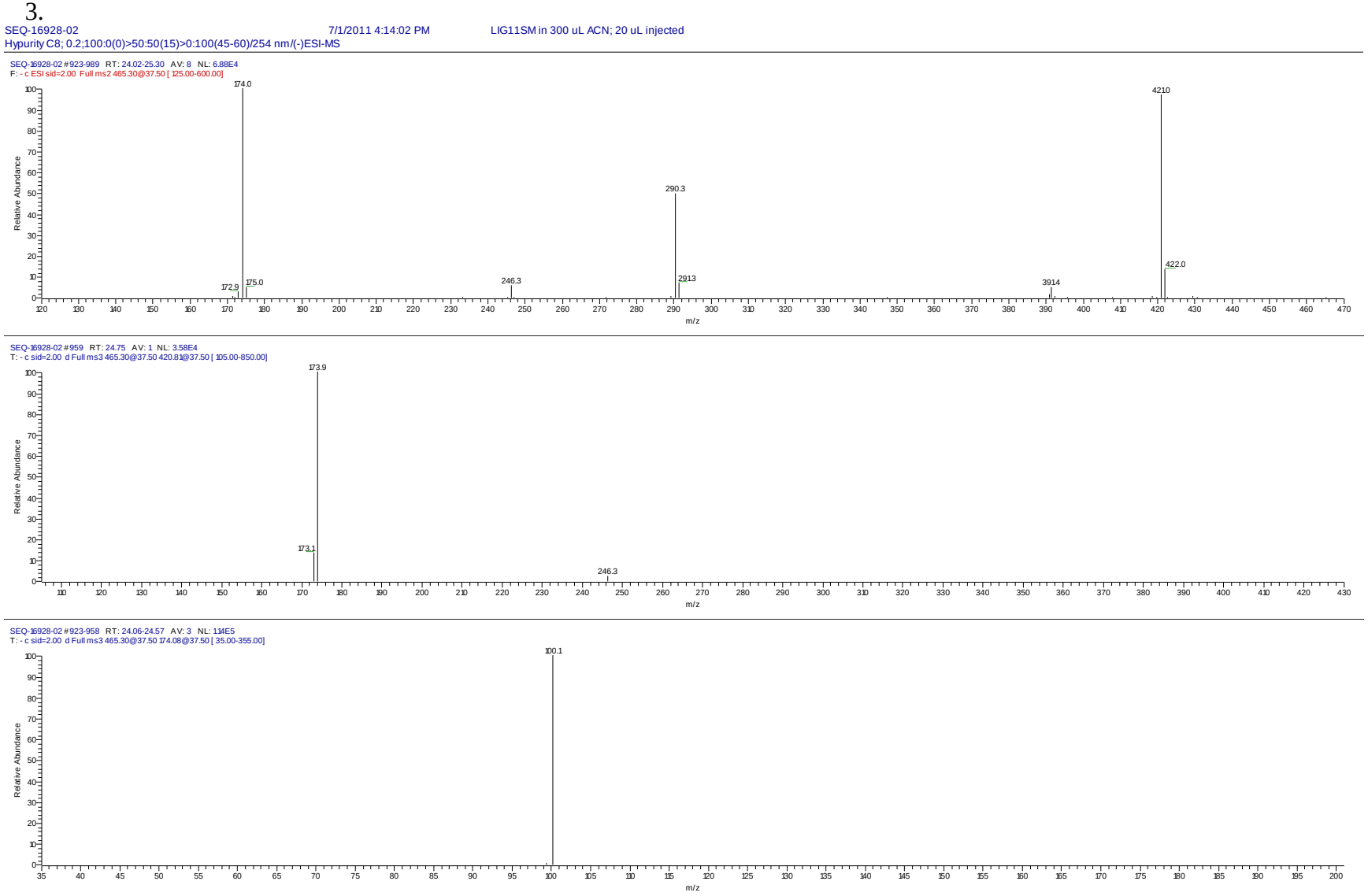


Figure S27. LIG11 NON-AQ, MW 466, RT 21.67 min: (-)ESI-MS/MS (top) and –MS/MS/MS (bottom) of the m/z 465 [M-H]- ion.

4.

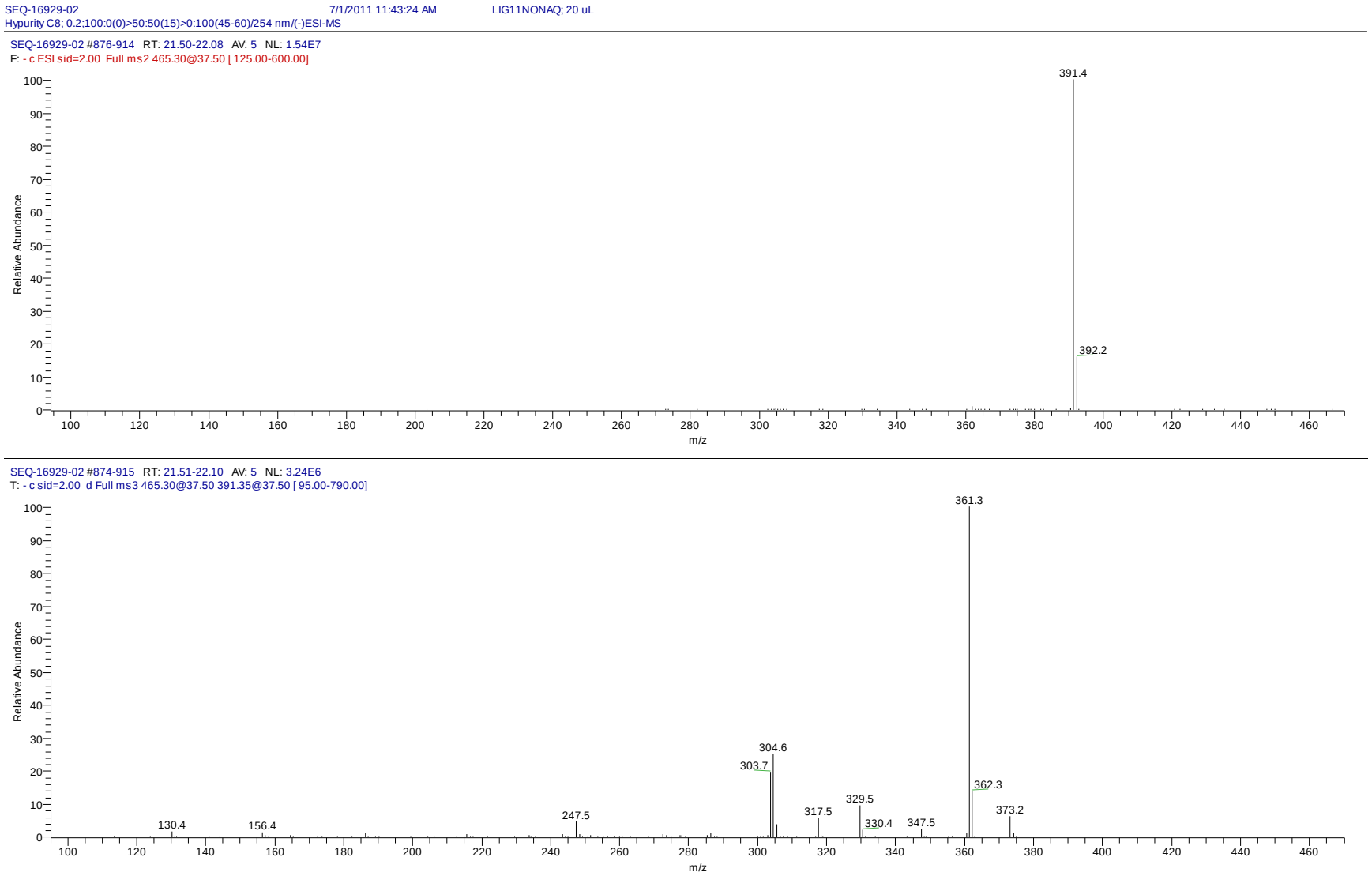


Figure S28. LIG11 AQ7.65, MW 466, RT 21.67 min. The m/z 465 [M-H]- was very minor in its (-)ESI-MS (top). The dominant ion was m/z 405. It is not uncommon to see [M+59]- ions where 59 is the acetate anion (from acetic acid often used in the mobile phase). Might the m/z 465 be an [M+59]- ion of a MW 406 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).