Electronic Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Biomimetic Peptide Bond Formation In Water with Aminoacyl Phosphate Esters

Electronic Supporting Information (Organic and Biomolecular Chemistry)

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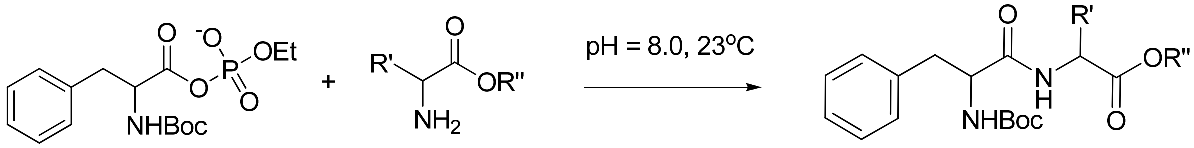
General Methods and Materials:

NMR spectra were recorded at 300 MHz for ^{1}H, 75 MHz for ^{13}C, and 121.5 MHz for ^{31}P. Electrospray Mass spectrometry analysis was performed at the AIMS facility at the University of Toronto. Amino acids and amino acid esters were purchased from commercial suppliers (Sigma-Aldrich, Alfa Aesar and AAPTEC) and used without further purification. N-t-Boc phenylalanine ethyl phosphate and phenylalanine ethyl phosphate were prepared by previously reported procedures.[1] All reactions with N-t-Boc phenylalanine ethyl phosphate were conducted in 0.25 M phosphate buffer (pH = 8.0 at 23°C). Reactions with phenylalanine ethyl phosphate were conducted in 0.25 M phosphate buffer (pH = 8.0 at 3°C). When reactions were monitored by ^{31}P-NMR, HEPES buffer was used in place of phosphate buffer. Melting points were determined with use of a Fisher-Johns apparatus and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer 100 FT-IR spectrometer; samples were prepared as a nujol mull on KBr plates.

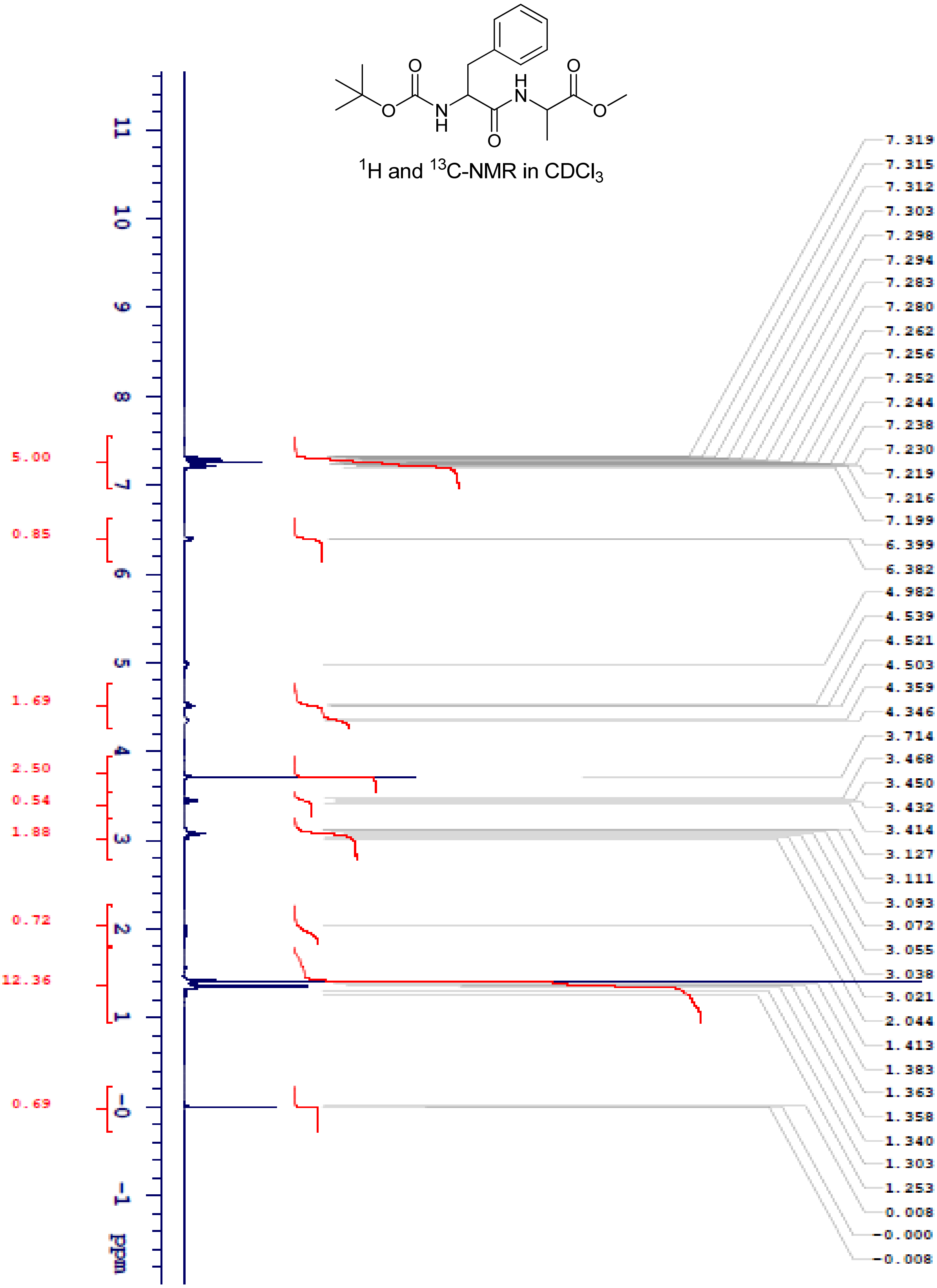
Reactions of N-t-Boc phenylalanine ethyl phosphate:



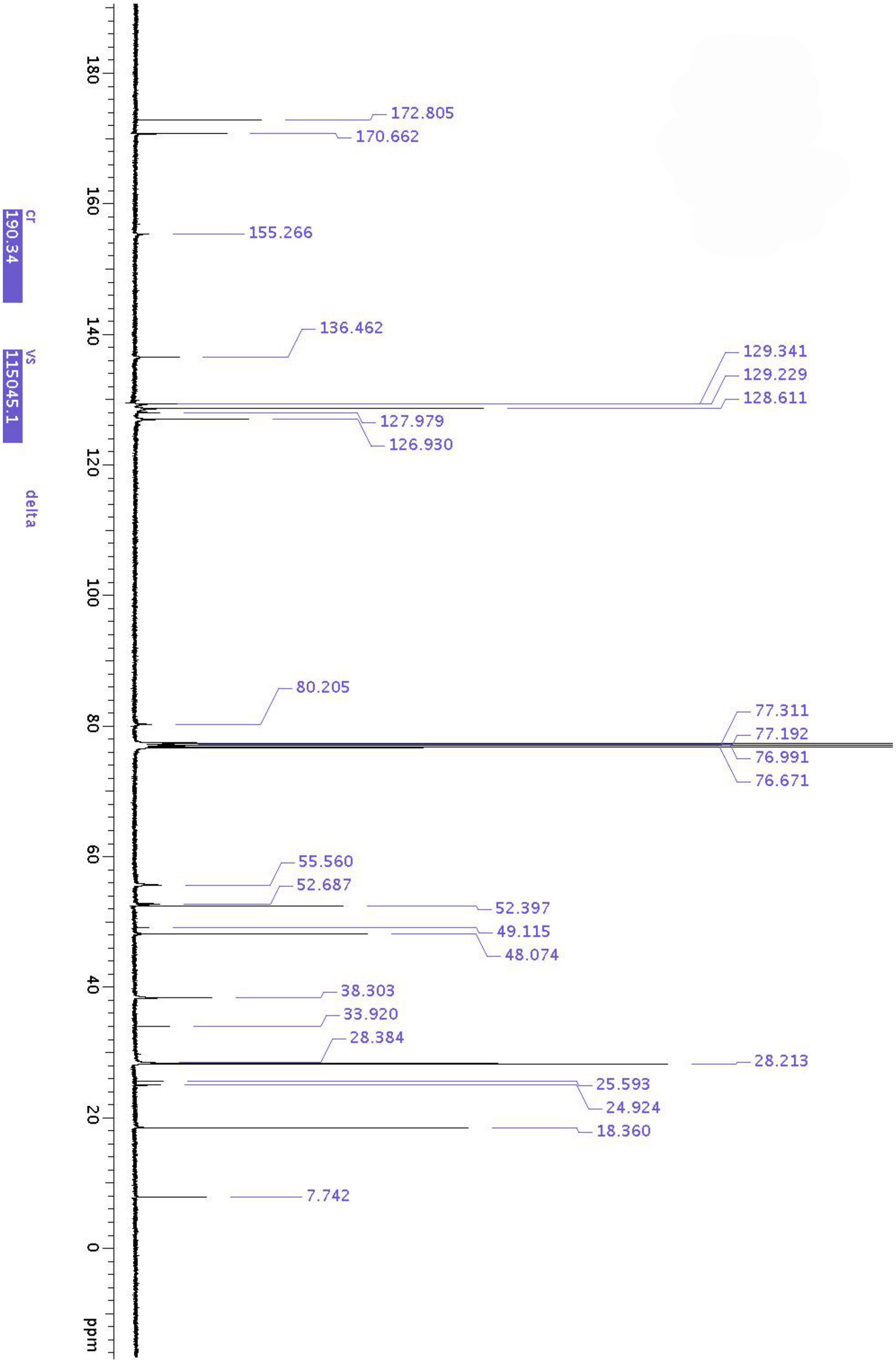
To a clear solution of N-t-Boc phenylalanine ethyl phosphate (1.2 mmol) in 10 mL of buffer was added 1 mmol of amino acid ester. The mixture was allowed to stir and the reaction was monitored by ^{31}P-NMR (observing change in chemical shift from -6.52 to 1.12 ppm in D_2O). After 1 hour the resulting precipitate was collected by vacuum filtration. The remaining product was isolated by passing the aqueous filtrate through an ENVI-Carb Graphite cartridge followed by elution with methanol. The organic eluant was collected and then concentrated to a residue under reduced pressure which was determined to be the remainder of the dipeptide product. Each dipeptide product was analyzed by ESI-MS, FT-IR, melting point, ^{1}H-NMR and ^{13}C-NMR; copies of NMR spectra are provided below and the data matches with literature.[2-5]

Table 1. ESI-MS, melting point and IR data for dipeptide products from reactions with BocPheEP.

Dipeptide	**ESI-MS Data**	**Melting Point (^{o}C)**	**IR (cm^{-1})**
BocPheAlaOMe	Calc. Mass: 350.1 ESI-MS: 351.1 (M+H^{+})	104-105	3583, 3330, 1753, 1694, 1658, 1525, 1377
BocPheGlyOEt	Calc. Mass: 350.1 ESI-MS: 351.1 (M+H^{+})	87-89	3583, 3334, 1747, 1692, 1660, 1521, 1376
BocPheValOMe	Calc. Mass: 378.1 ESI-MS: 379.1 (M+H^{+})	110-112	3583, 3311, 1745, 1681, 1650, 1536, 1376
BocPheLeuOMe	Calc. Mass: 392.2 ESI-MS: 393.2 (M+H^{+})	137-140	3583, 3332, 1750, 1684, 1653, 1526, 1376
BocPheSerOEt	Calc. Mass: 380.1 ESI-MS: 381.1 (M+H^{+})	112-114	3583, 3326, 1740, 1695, 1652, 1527, 1376
BocPheCysOEt	Calc. Mass: 396.1 ESI-MS: 397.1 (M+H^{+})	145-147	3583, 3331, 1731, 1687, 1655, 1526, 1377



^{1}H and ^{13}C-NMR in $CDCl_3$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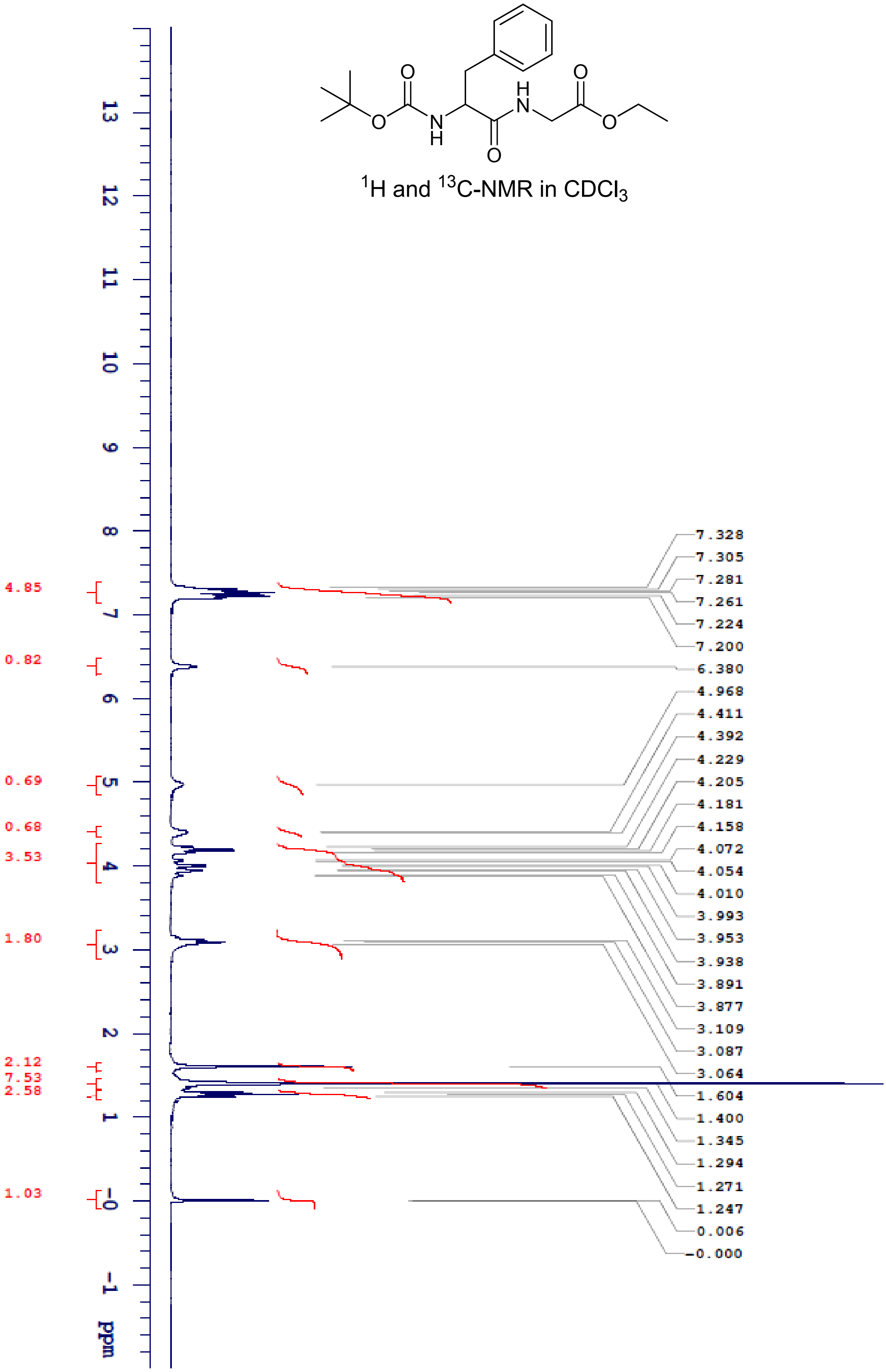
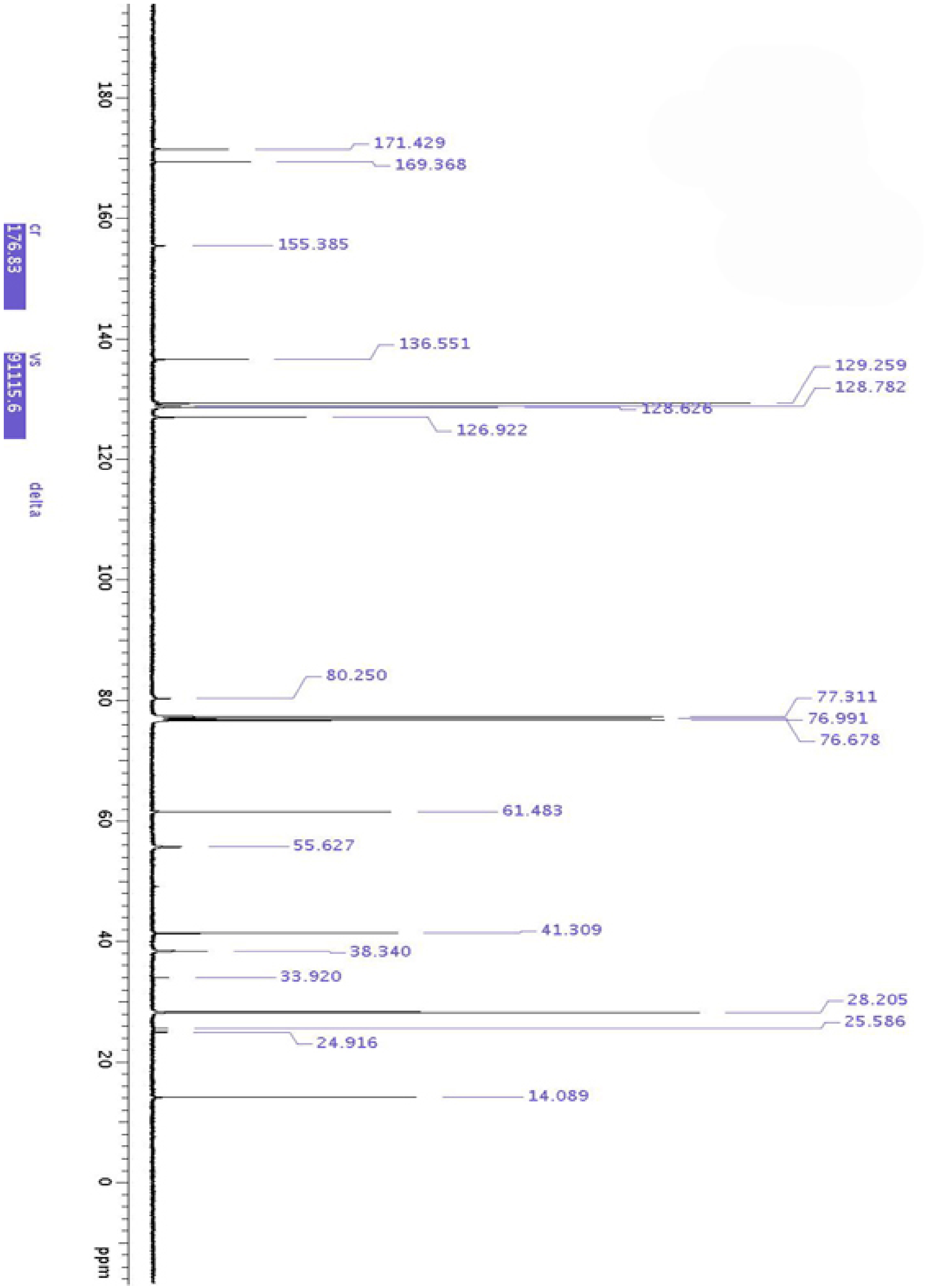


^{1}H and ^{13}C-NMR in $CDCl_3$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41.309
38.340
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ppm
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vs
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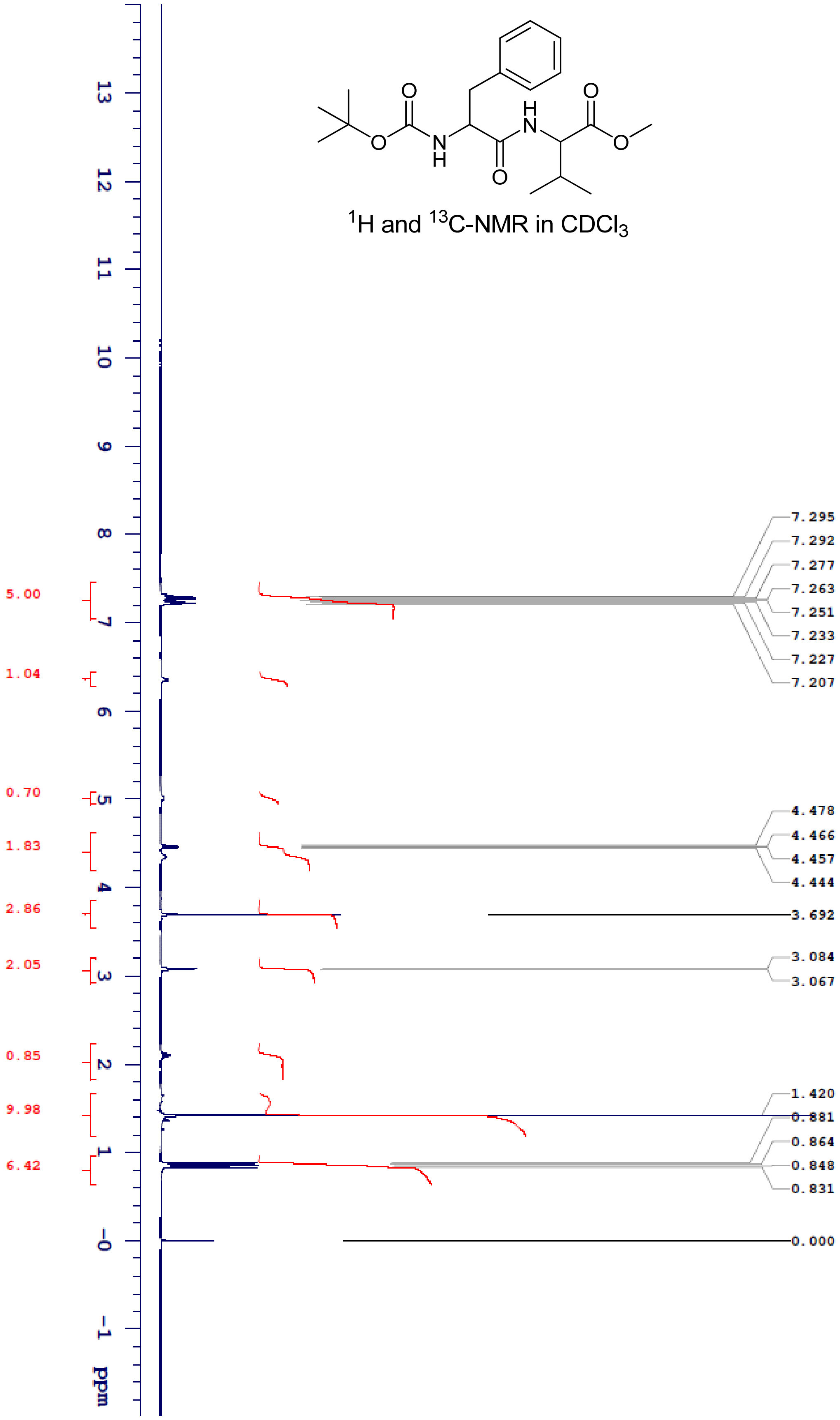
delta



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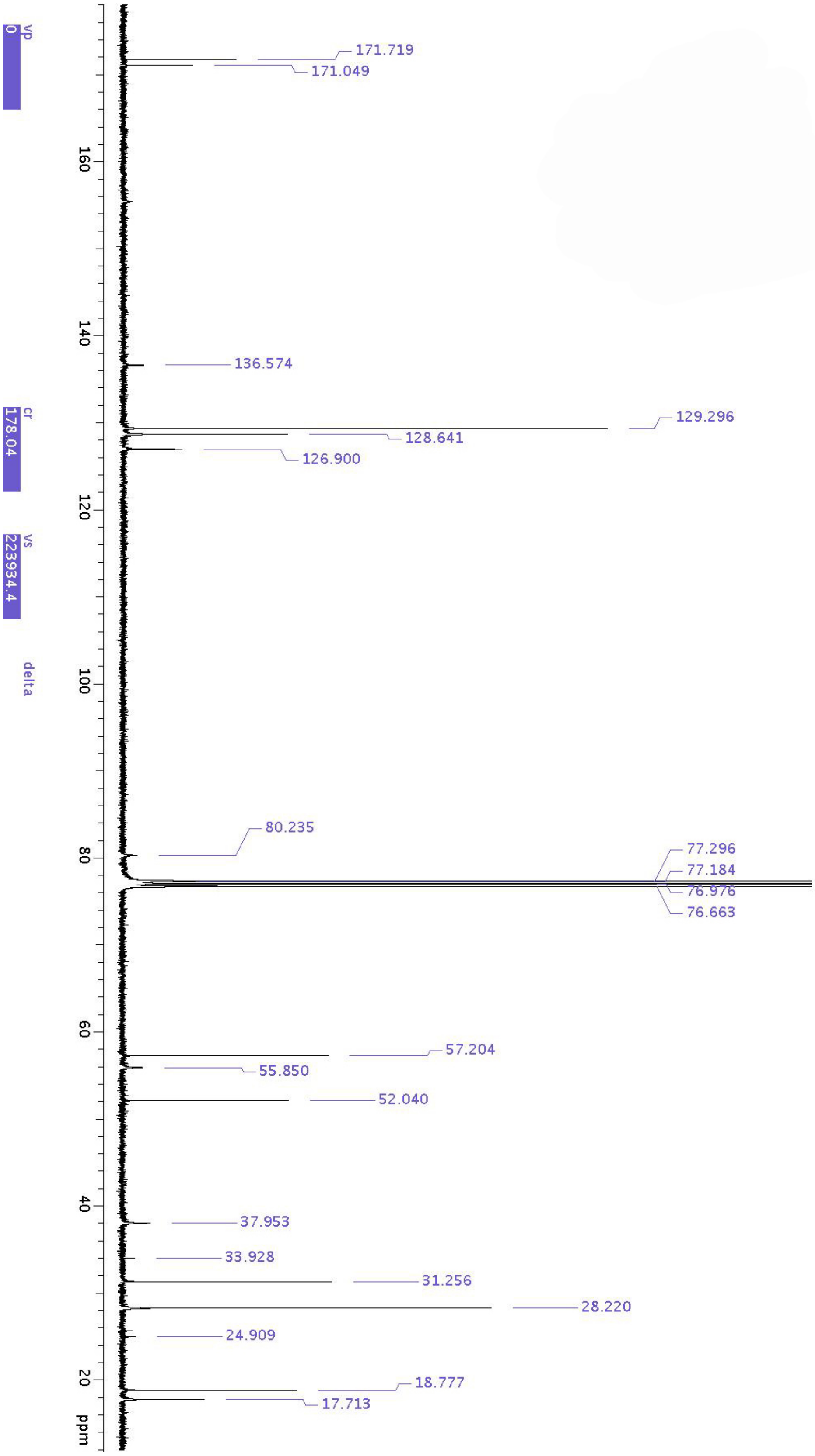
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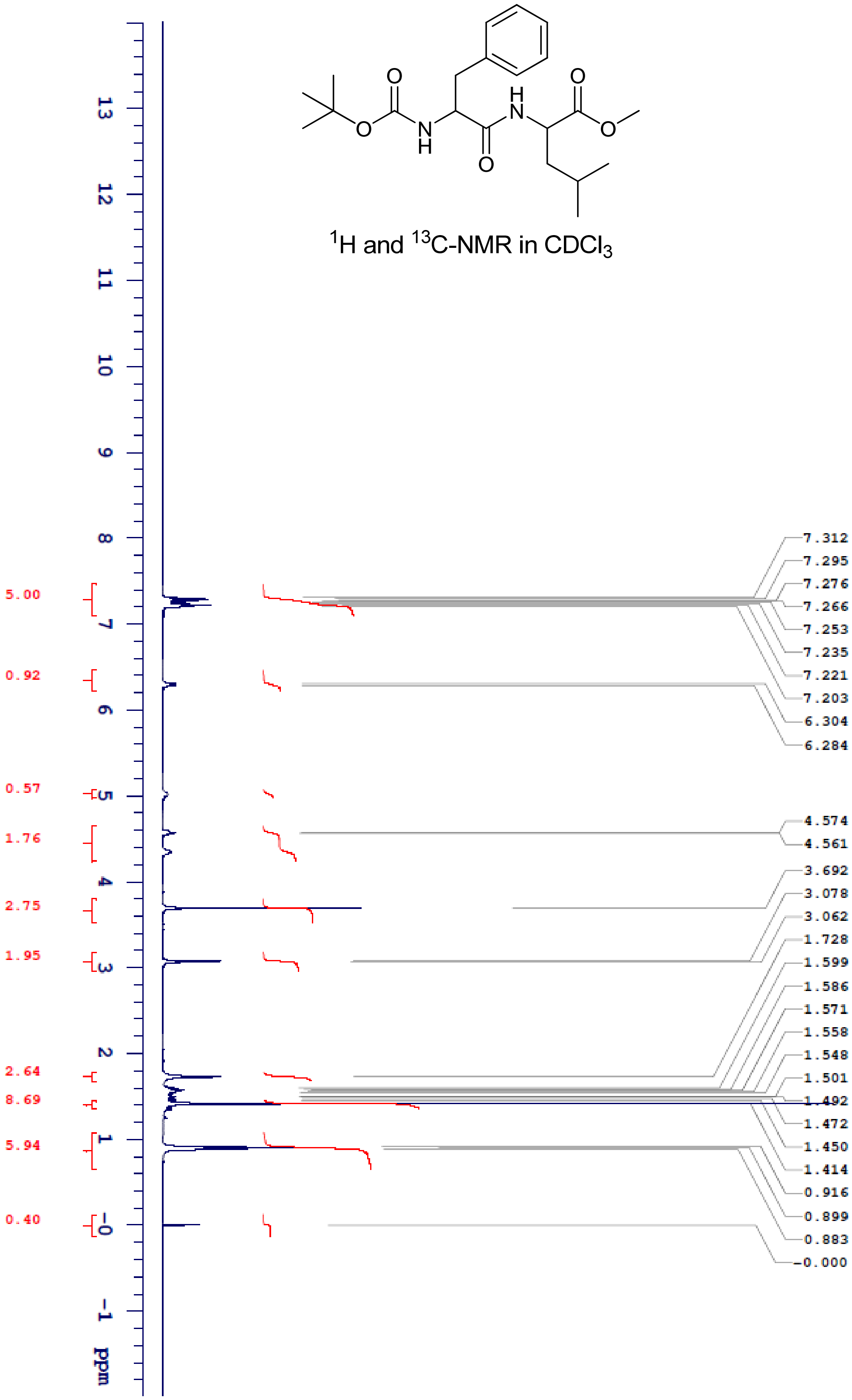

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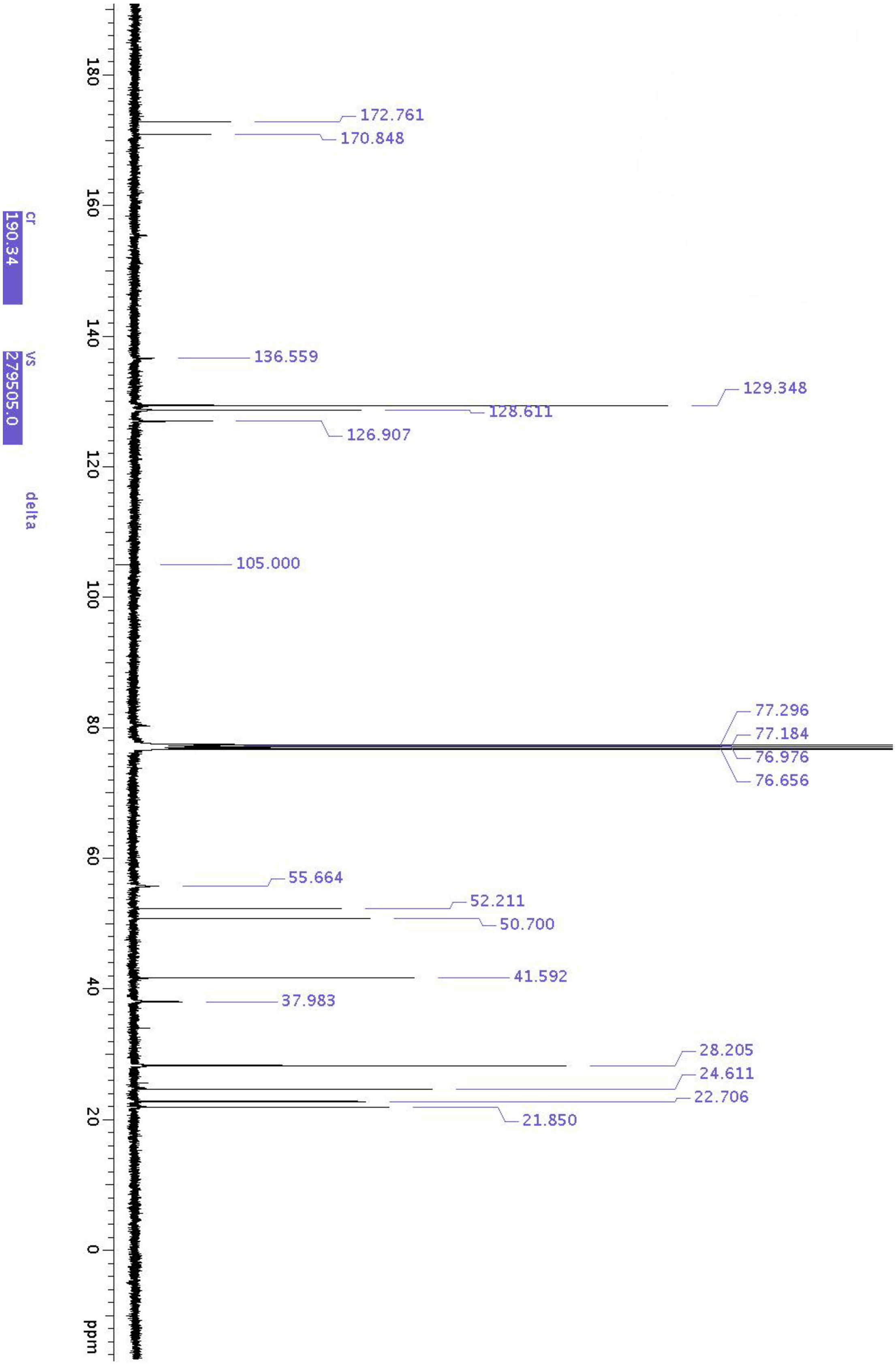
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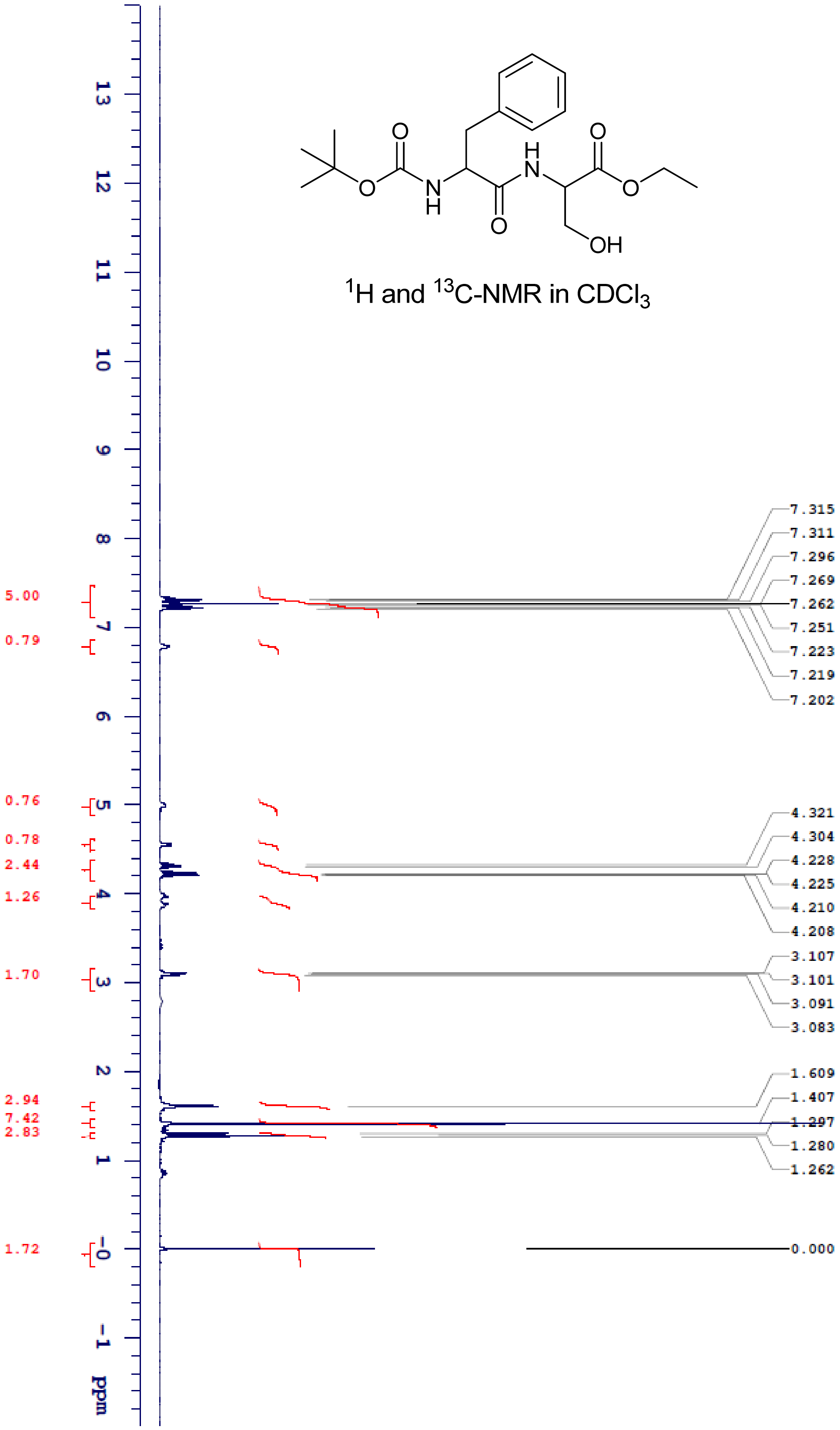

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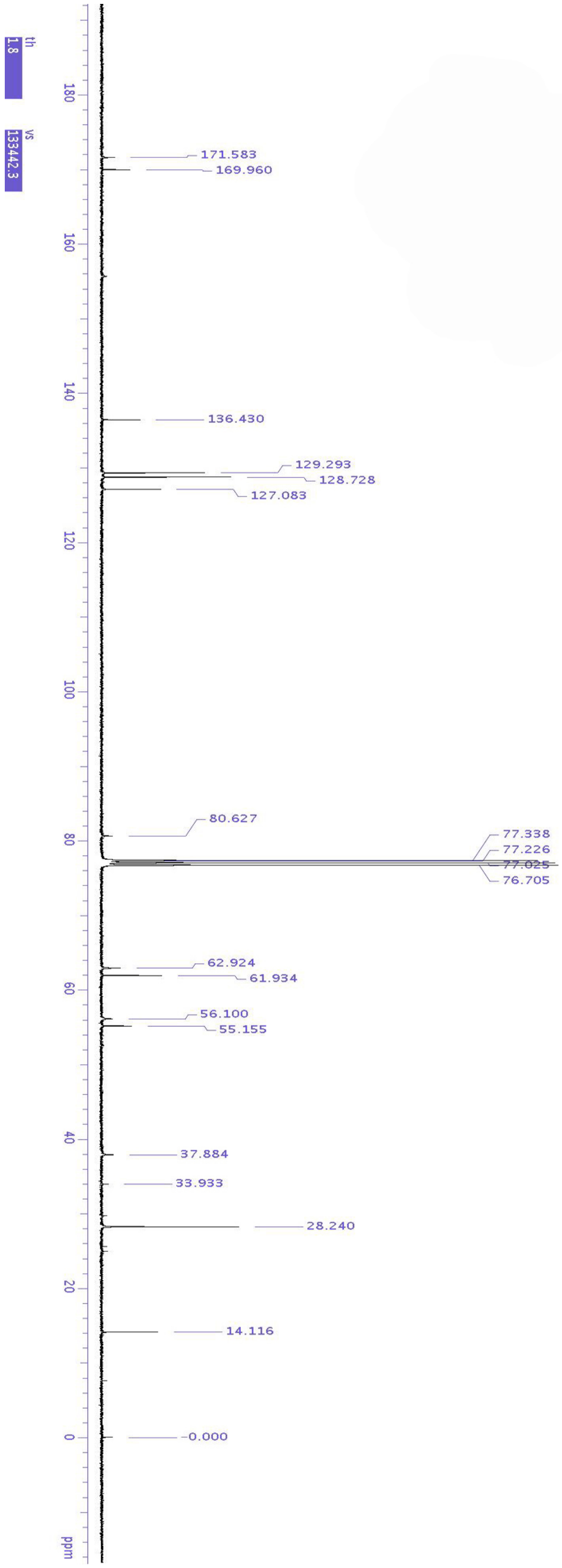
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40
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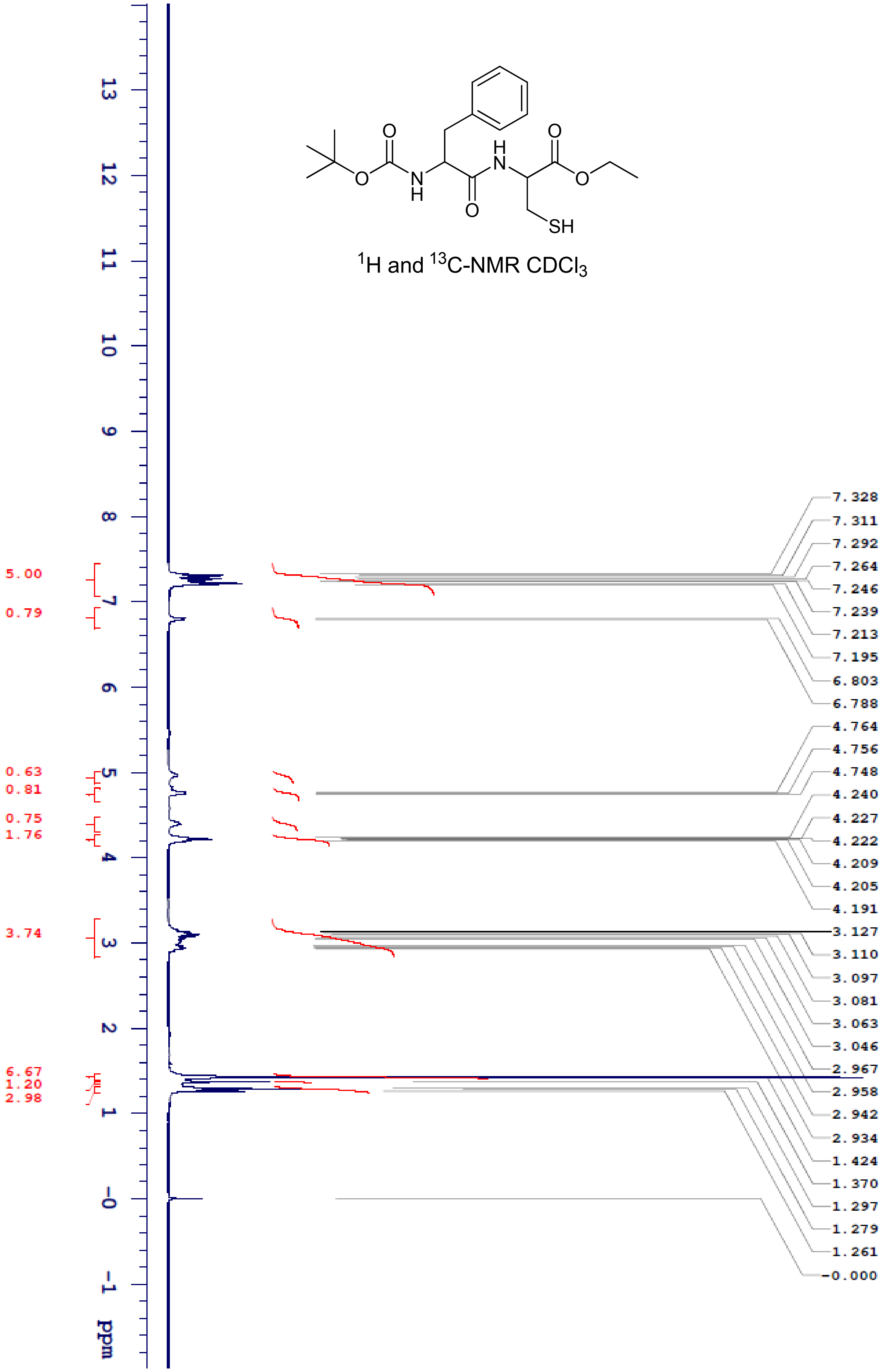
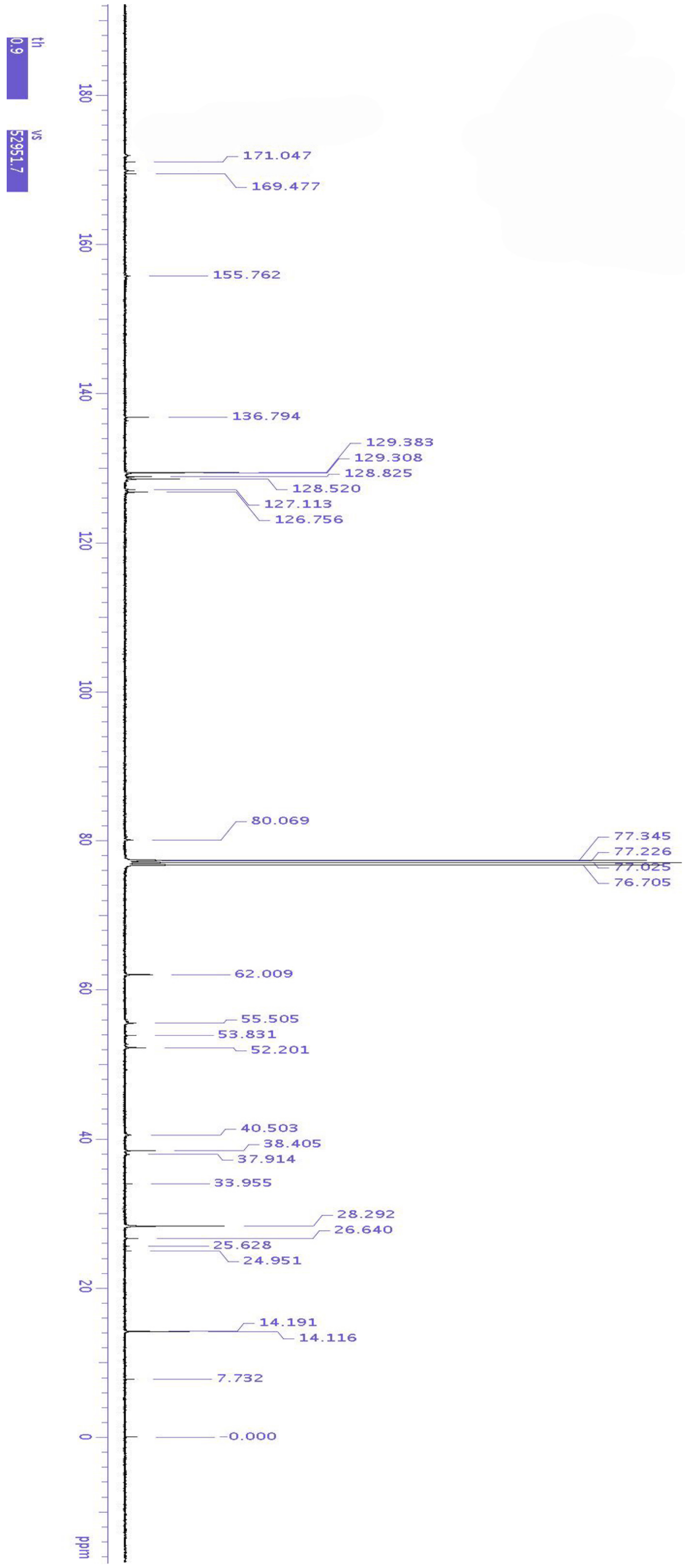
0
ppm



^{1}H and ^{13}C-NMR CDCl$_3$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128.825
128.520
127.113
126.756
80.069
77.345
77.226
77.025
76.705
62.009
55.505
53.831
52.201
40.503
38.405
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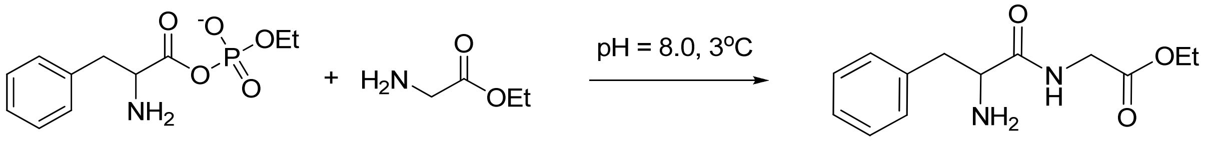
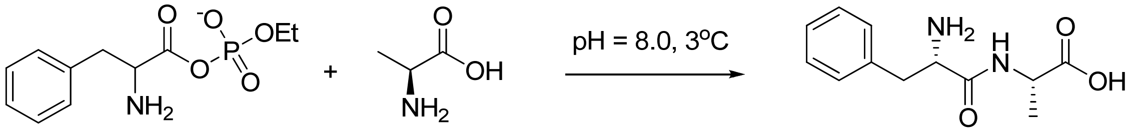
24.951
14.191
14.116
7.732
-0.000

Reactions of phenylalanine ethyl phosphate:



To a clear solution of phenylalanine ethyl phosphate (1.0 mmol) in 10 mL of buffer was added 1.0 mmol (or 1.5 mmol) of glycine ethyl ester hydrochloride. The mixture was allowed to stir and the reaction was monitored by ^{31}P-NMR (observing change in chemical shift from -6.52 to 1.12 ppm in D_2O). After 30 minutes the reaction was terminated by acidification to pH 5 (pH paper) by dropwise addition of 1 M HCl solution. The reaction was diluted with water and extracted three times with CH_2Cl_2, the extracts were dried (Na_2SO_4) and concentrated to give a residue which was analyzed by ESI-MS.



To a clear solution of phenylalanine ethyl phosphate (1.0 mmol) in 10 mL of buffer was added 1.0 mmol (or 1.5 mmol) of D-alanine. The mixture was allowed to stir and the reaction was monitored by ^{31}P-NMR (observing change in chemical shift from -6.52 to 1.12 ppm in D_2O). After 30 minutes the reaction was terminated by acidification to pH 5 (pH paper) by dropwise addition of 1 M HCl solution. The reaction was diluted with water and extracted three times with CH_2Cl_2, the extracts were dried (Na_2SO_4) and concentrated to give a residue which was analyzed by ESI-MS.

Table 2. ESI-MS analysis of reactions between PheEP and alanine.

Reagents	Ratio	Products	ESI-MS Data
PheEP + Ala	1:1.5	Phe-Ala	237.1
PheEP + Ala	1:1	Diketopiperizine Phe-Phe Phe-Phe-Phe Phe-Phe-Phe-Phe	295.1 313.2 460.1 607.1

References:

1. R. Kluger, X. F. Li and R. W. Loo, *Can. J. Chem,* 1996, **74**, 2395-2400.
2. V. Declerck, P. Nun, J. Martinez and F. Lamaty, *Angew. Chem. Int. Ed.*, 2009, **48**, 9318-9321.
3. M. Ueda and H. Oikawa, *J. Org. Chem.*, 1985, **50**, 760-763.
4. G. Priem, L. Rocheblave, C. De Michelis, J. Courcambeck and J. L. Kraus, *J. Chem. Soc., Perkin Trans. 1*, 2000, **5**, 819-824.
5. A. Meissner, W. Haehnel and H. Vahrenkamp, *Chem.–Eur. J.*, 1997, **3**, 261-267.