Enzymatic synthesis of γ-glutamyl dipeptides catalysed by mutant E. coli γ-glutamyltransferases

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supporting information

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Docking simulation

Docking simulation was carried out using Autodock 4.2.5; Chimera 1.14.0 and Autodock Tools (ADT) 1.5.6 were used for both file setup and results analyses. The structure of *E. coli* GGT - γ-glutamyl enzyme intermediate was downloaded from RCSB Protein Data Bank, accession code 2DBW. The file contains an assembly of two GGT heterodimeric molecules. Chains A and B were retained for calculations, while chains C and D, water and glycerol molecules were removed using Chimera. Hydrogens were added using the "Dock Prep" function in Chimera. As the amino group of the catalytically active *N*-terminal threonine residue of the small subunit (Thr391) is believed to be in non-protonated form in order to act as a base during catalysis, a hydrogen atom of the protonated amino group was manually removed. The *N*-terminal Thr391 residue and the esterified γ-glutamyl moiety were treated as non-standard residues, for which Gasteiger partial charges were calculated and then manually corrected in the pdbqt file generated by ADT. The atom type for the *N*-terminal nitrogen atom of Thr391 was changed to NA (hydrogen bond-acceptor nitrogen atom) in the final pdbqt file. The side chain of residue Tyr444 was treated as flexible using the corresponding function in Autodock.

Acceptor amino acids were built with Avogadro; hydrogens were added for pH 10, in order to have the free amino group able to act as the nucleophile in the transpeptidation reaction. The structures were minimized with the MMFF94s force field implemented in Avogadro using the steepest descendent algorithm. Non-polar hydrogens were merged and Gasteiger partial charges were added using ADT. The atom type of the nitrogen atom of the alpha-amino group was manually changed in NA (hydrogen bond-acceptor nitrogen atom) in the final pdbqt file. Docking calculations were carried out using a grid consisting of 42, 40 and 60 points in x, y and z dimension, respectively. Points spacing inside the grid was 0.375 Å and the grid was placed in such a way to encompass completely the putative acceptor binding site (grid center at x = 17.994, y = –34.881 and z = –34.007). Three independent docking calculations were executed for each ligand, in each of them 100 docked poses were calculated (total poses for each ligand = 300) using the genetic algorithm and the lamarckian output. Initial populations size was 300 individuals; the number of energy evaluations was set on the basis of the number of rotatable bonds in the ligand (number of energy evaluations = 2,500,000 times the number of rotatable bonds in the ligand). Maximum number of generations was 27,000; for the other docking parameters, the default values in Autodock were accepted. The resulting docked poses for each ligand were clustered in ADT at 1.5 and 2.0 Å RMSD.

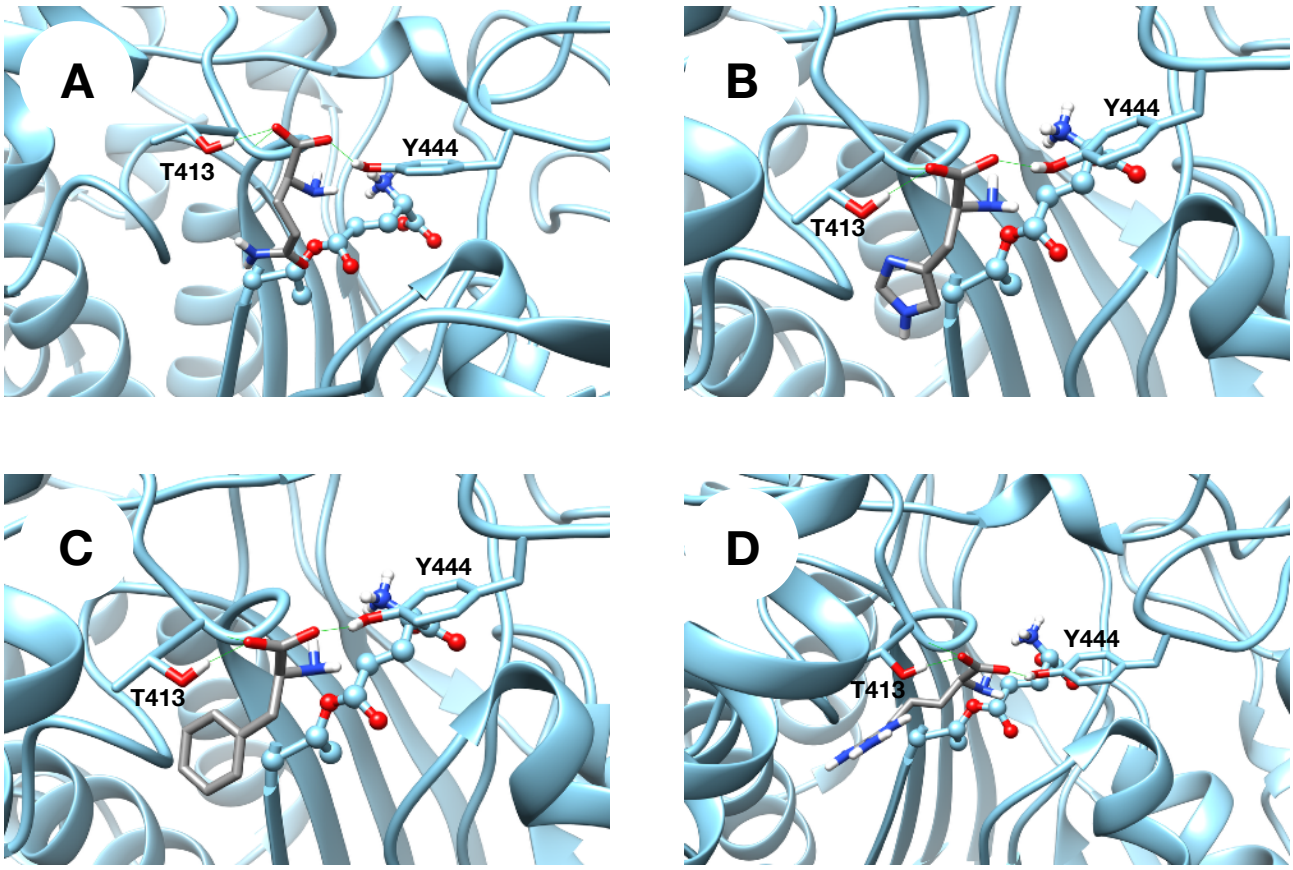
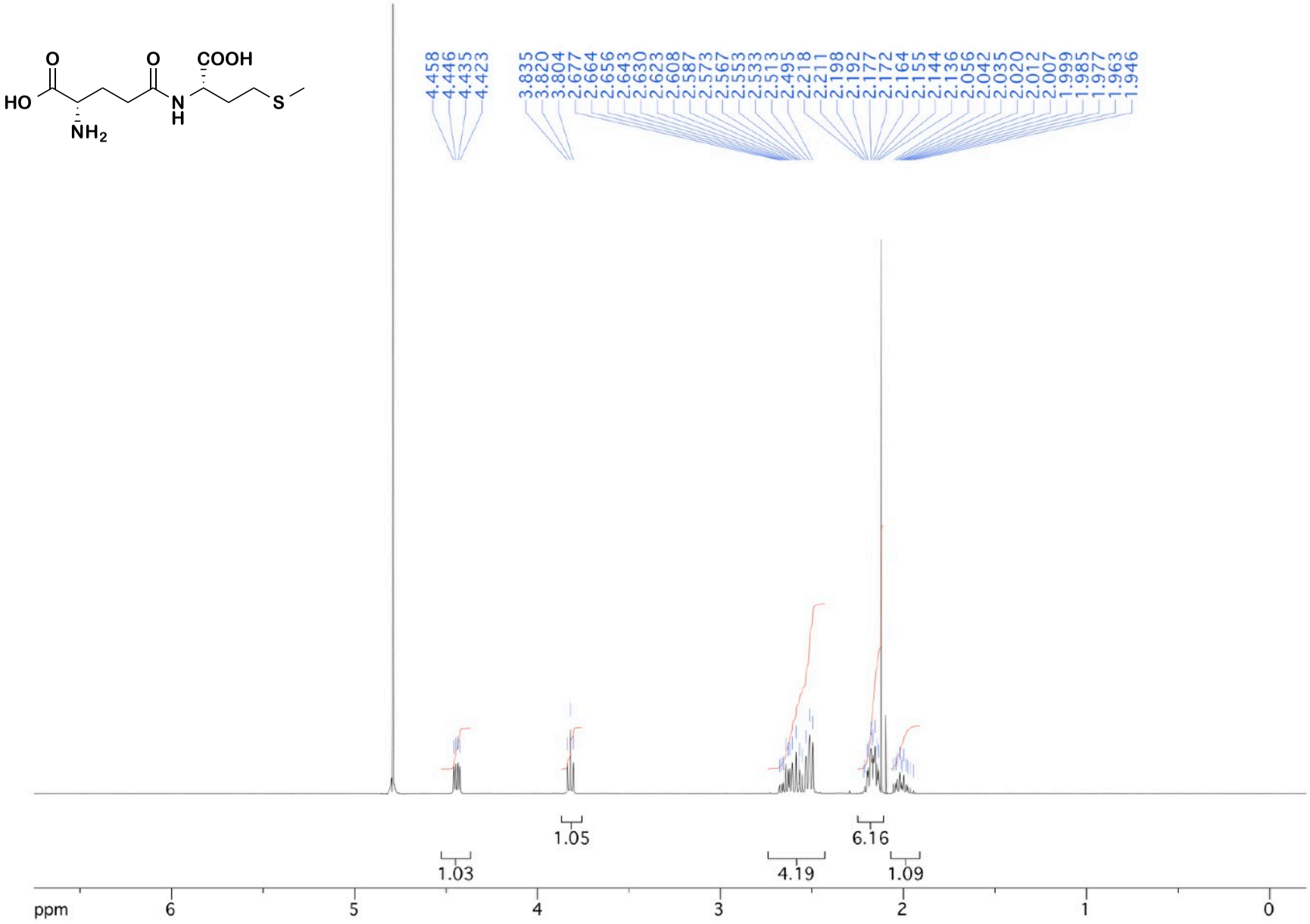


Fig. S1 Docking results showing interactions with Y444 and T413 of the productive conformation of selected acceptor amino acids inside the active site γ-glutamyl-enzyme intermediate of *E. coli* GGT. Three independent runs were carried out for each acceptor, for a total of 300 docked conformations. Docked poses were clustered at 1.5 Å rmsd. The figures represent the minimum energy conformations of the minimum energy and most populated cluster for each amico acid. A) glutamine; B) histidine; C) phenylalanine; D) arginine. Catalytically active *N*-terminal threonine 391 and γ-glutamyl moiety forming the γ-glutamyl-enzyme intermediate are in ball and stick. Predicted hydrogen bonds are represented as green lines.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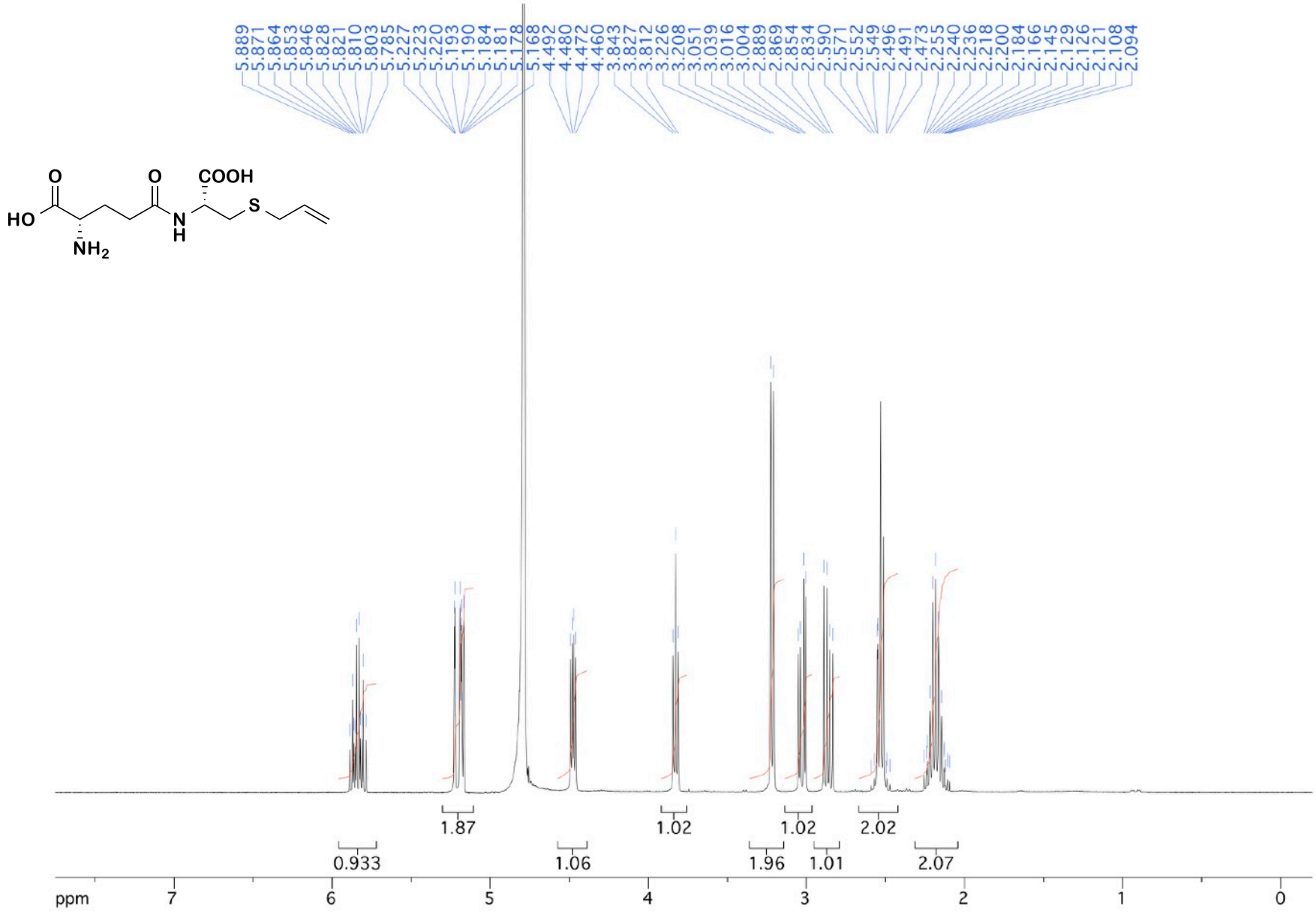
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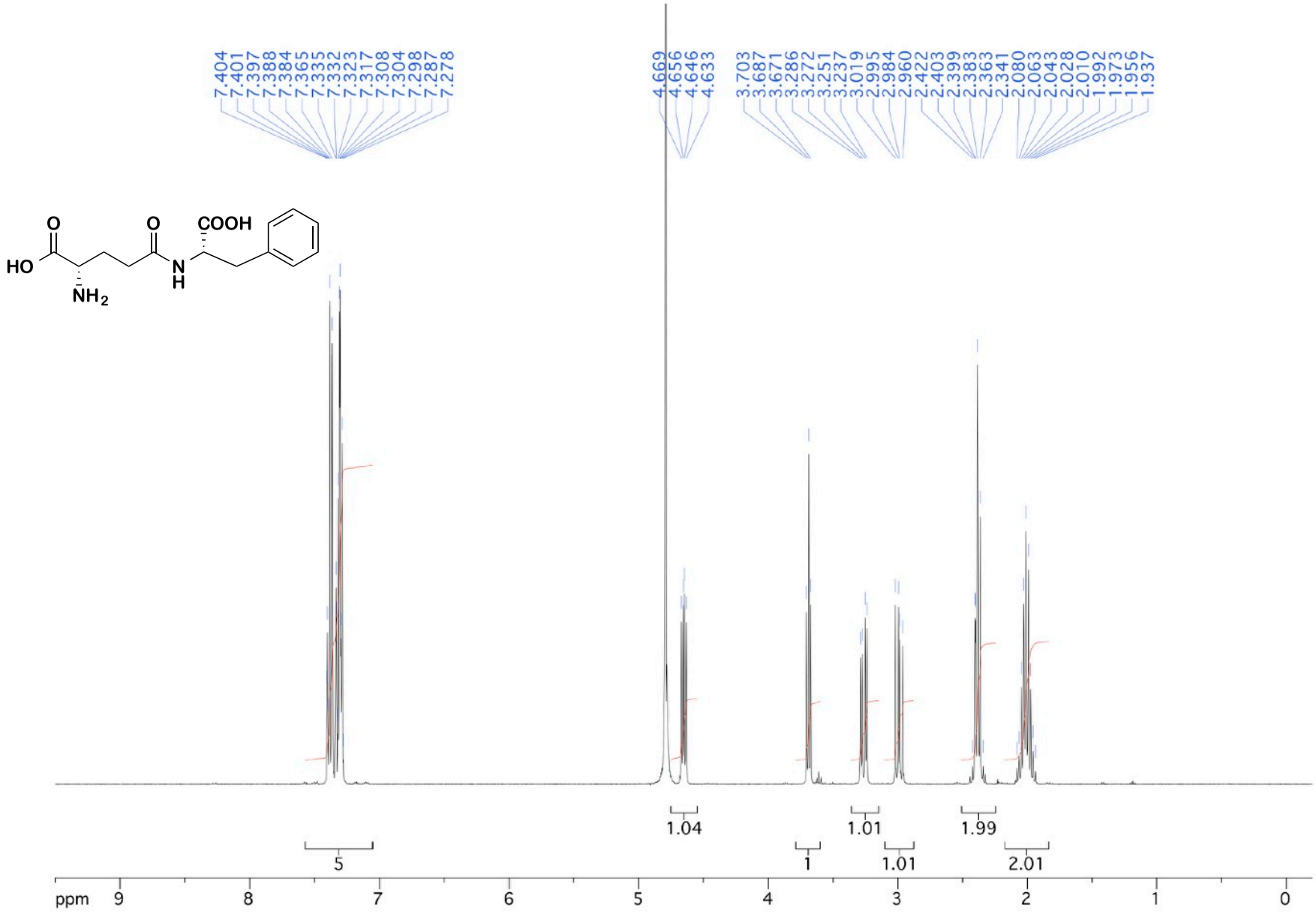
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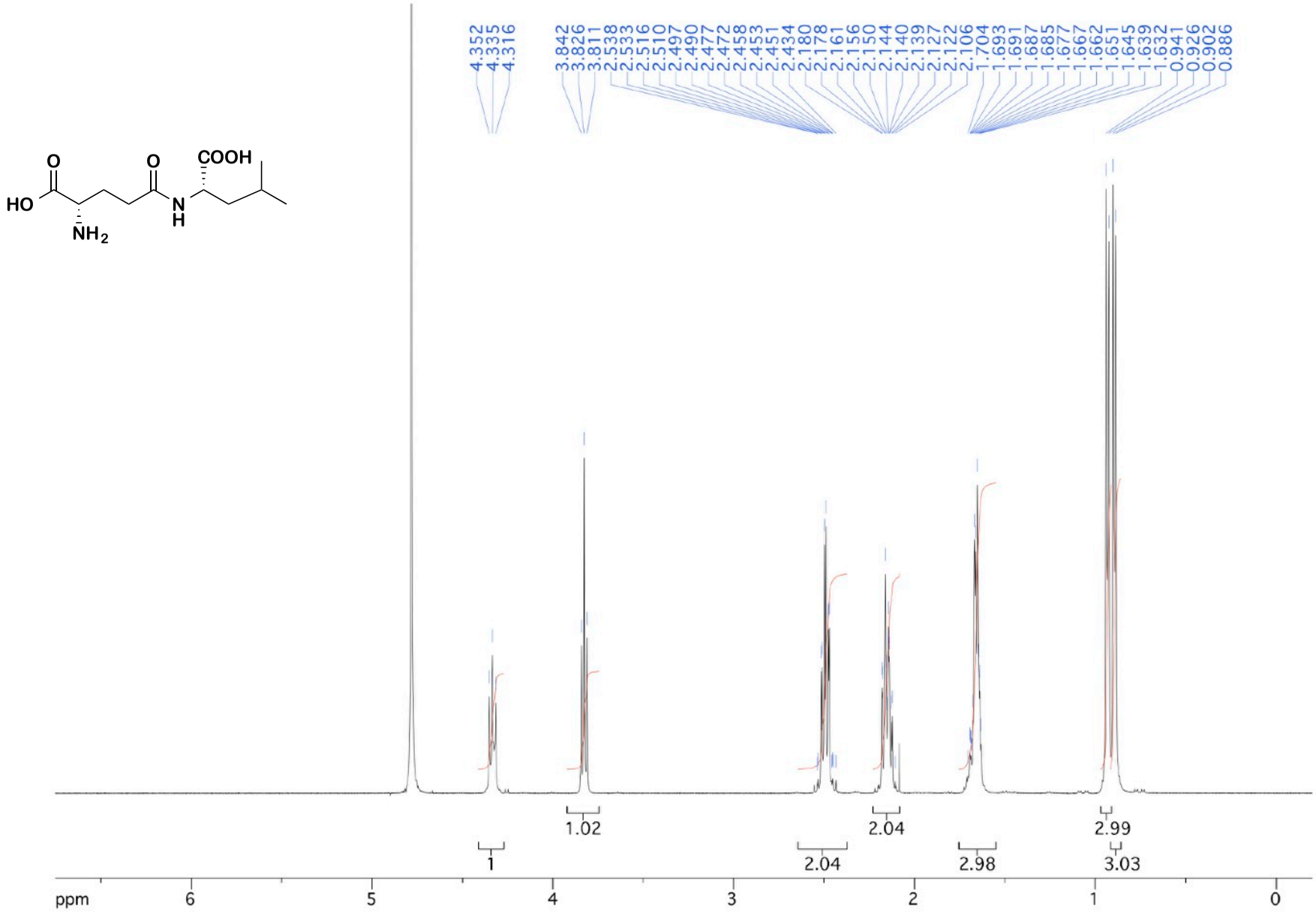
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