

Supplementary Information for:
Structure-Based Design of a Cyclic Peptide Inhibitor of the SARS-CoV-2 Main Protease

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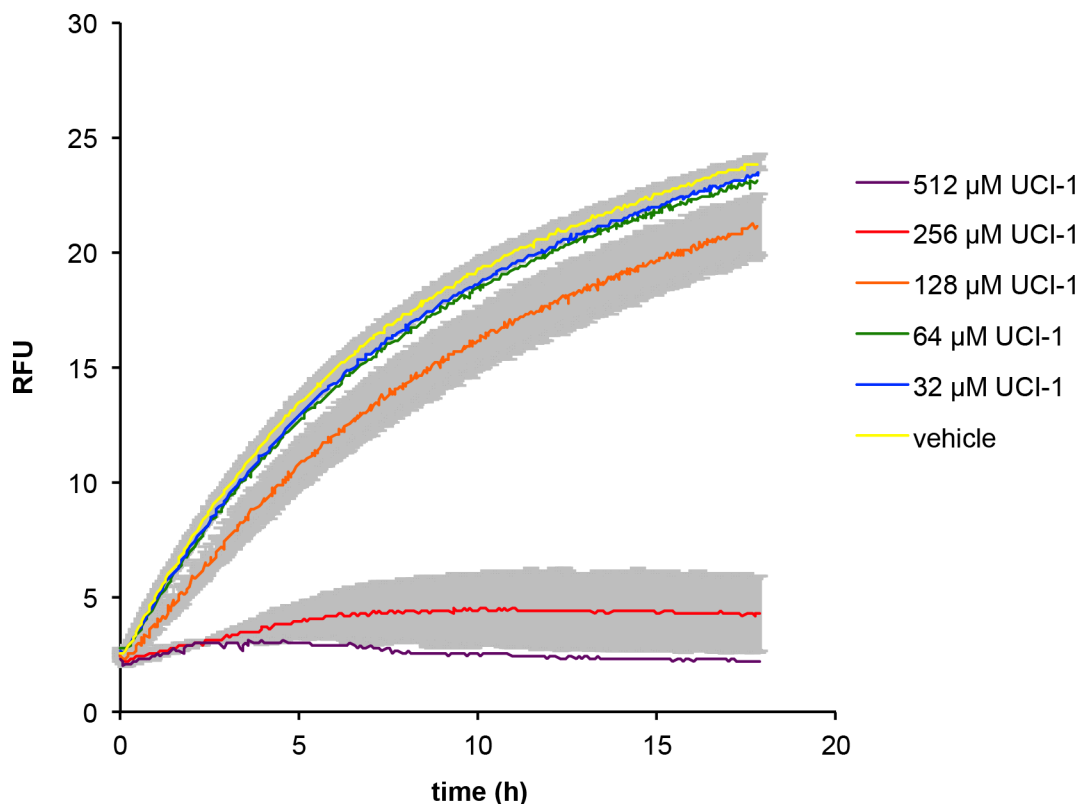
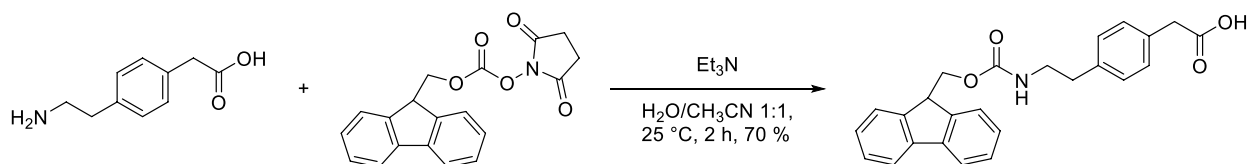


Figure S1. Continuous kinetic inhibition assay of UCI-1 against MBP-M^{pro}. For clarity, error bars (\pm s.d.) are only shown for 256 μ M UCI-1, 128 μ M UCI-1, and vehicle.

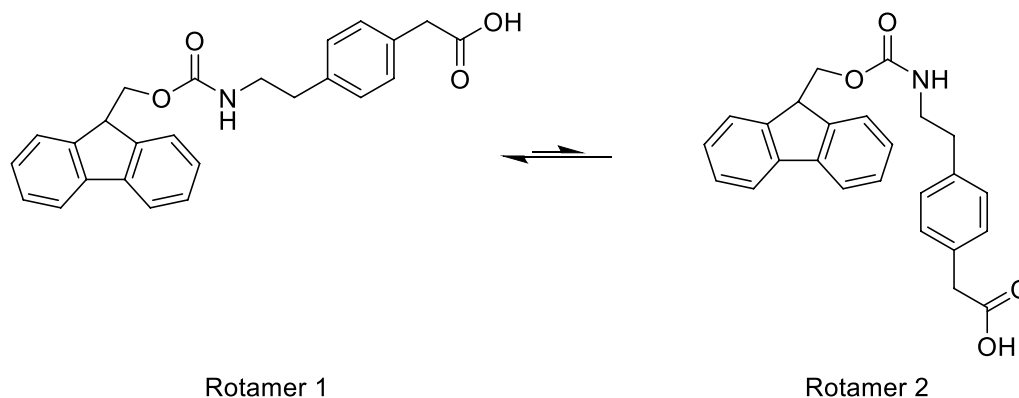
Supplementary Procedures

Synthesis of 2-(4-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)phenyl)acetic acid (Fmoc-AEPA)



A 50 mL round-bottom flask equipped with a magnetic stirring bar was charged with 100 mg (0.55 mmol, 1 equiv) of 2-(4-(2-aminoethyl)phenyl)acetic acid dissolved in 10 mL H₂O. 0.156 mL (1.10 mmol, 2 equiv) of Et₃N was added. 160 mg of Fmoc-OSu (0.50 mmol, 0.9 equiv) was dissolved in 10 mL CH₃CN and added to the reaction mixture. The reaction was run for 2 hours at room temperature. While it was running, the reaction was monitored by TLC (3:1

EtOAc/hexanes + 10 % MeOH, $R_f = 0.44$) to determine the consumption of starting material and ($R_f = 0.77$) and an appearance of fulvene ($R_f = 0.81$). 10 mL of EtOAc was then added to the reaction mixture and the organic layer was removed. The aqueous layer was acidified with 30 mL 1 M HCl, and then 10 mL of EtOAc was added. The organic layer was washed with water and brine, dried over $MgSO_4$, and solvent was evaporated *in vacuo* to afford a white powder (70 %, 0.140 g). The Fmoc-AEPA was used in solid-phase peptide synthesis without further purification. The product contains a minor contaminant (< 10 %) of Fmoc-AEPA-AEPA-OH, as detected by 1H NMR spectroscopy. HRMS (ESI-TOF) m/z : $[M+Na]^+$ calcd for $C_{25}H_{23}NO_4$ 424.1525 found 424.1507.

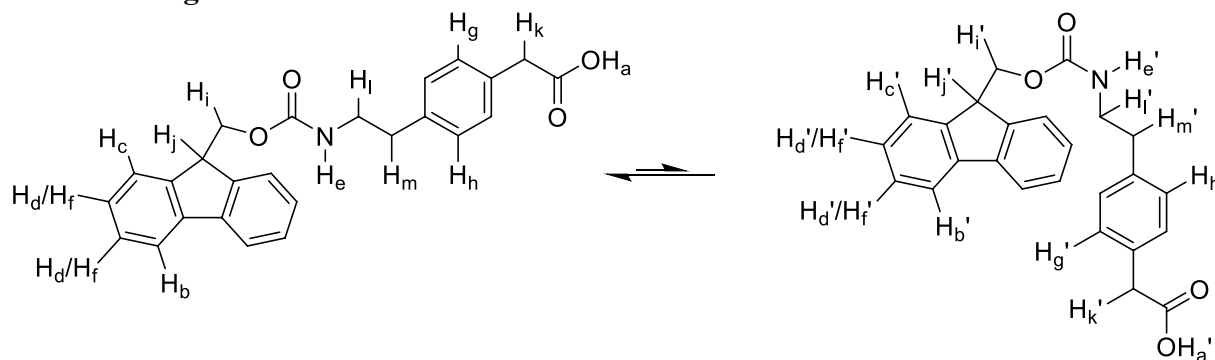


Rotamer 1: Rotamer 2 = ~ 5:1

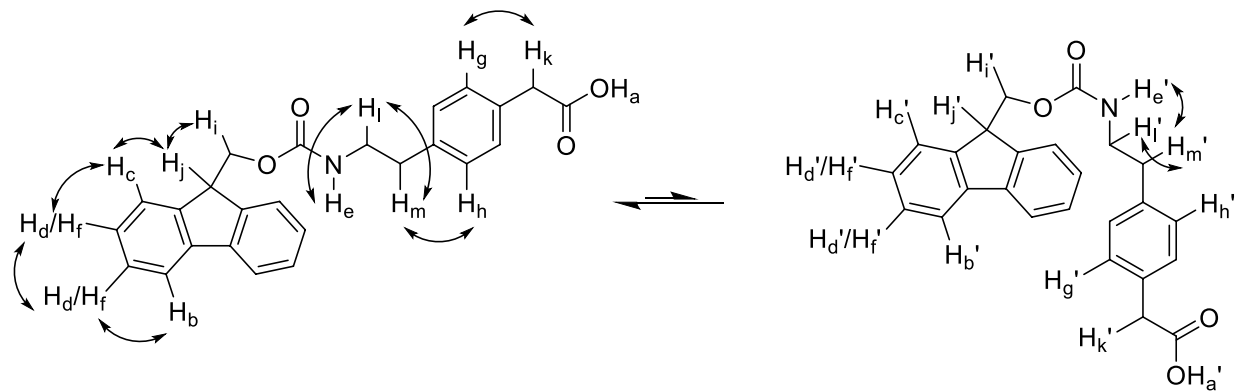
Rotamer 1: 1H NMR (600MHz, DMSO-*d*6): δ 12.17 (s, 1H), 7.89 (d, $J = 7.6$ Hz, 2H), 7.68 (d, $J = 7.3$ Hz, 2H), 7.41 (t, $J = 7.1$ Hz, 2H), 7.38 (t, $J = 5.3$ Hz, 1H), 7.33 (t, $J = 7.3$ Hz, 2H), 4.30 (d, $J = 3.2$ Hz, 2H), 4.20 (t, $J = 6.6$ Hz, 1H), 3.51 (s, 2H), 3.19 (q, $J = 6.3$ Hz, 2H), 2.69 (t, $J = 7.1$ Hz, 2H). ^{13}C NMR (150 MHz, DMSO-*d*6): δ 173.3, 144.4, 141.3, 138.1, 133.2, 129.8, 129.0, 128.1, 127.5, 125.7, 120.6, 65.7, 47.3, 42.3, 40.8, 40.5, 35.4.

Rotamer 2: 1H NMR (600MHz, DMSO-*d*6): δ 12.17 (s, 1H), 7.89 (d, $J = 7.6$ Hz, 2H), 7.68 (d, $J = 7.3$ Hz, 2H), 7.41 (t, $J = 7.1$ Hz, 2H), 7.33 (t, $J = 7.3$ Hz, 2H), 6.84 (t, $J = 5.6$ Hz, 1H), 4.30 (d, $J = 3.2$ Hz, 2H), 4.20 (t, $J = 6.6$ Hz, 1H), 3.51 (s, 2H), 2.92 (q, $J = 6.3$ ppm, 2H), 2.36 (t, $J = 7.1$ Hz, 2H).

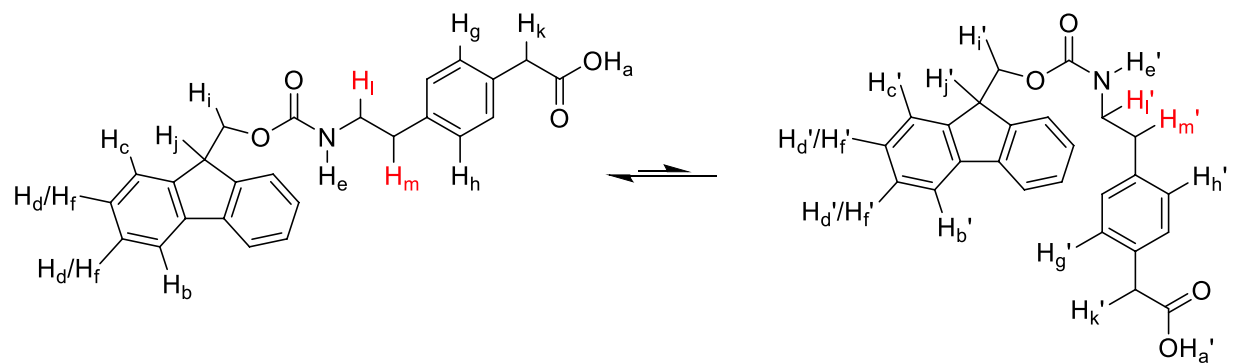
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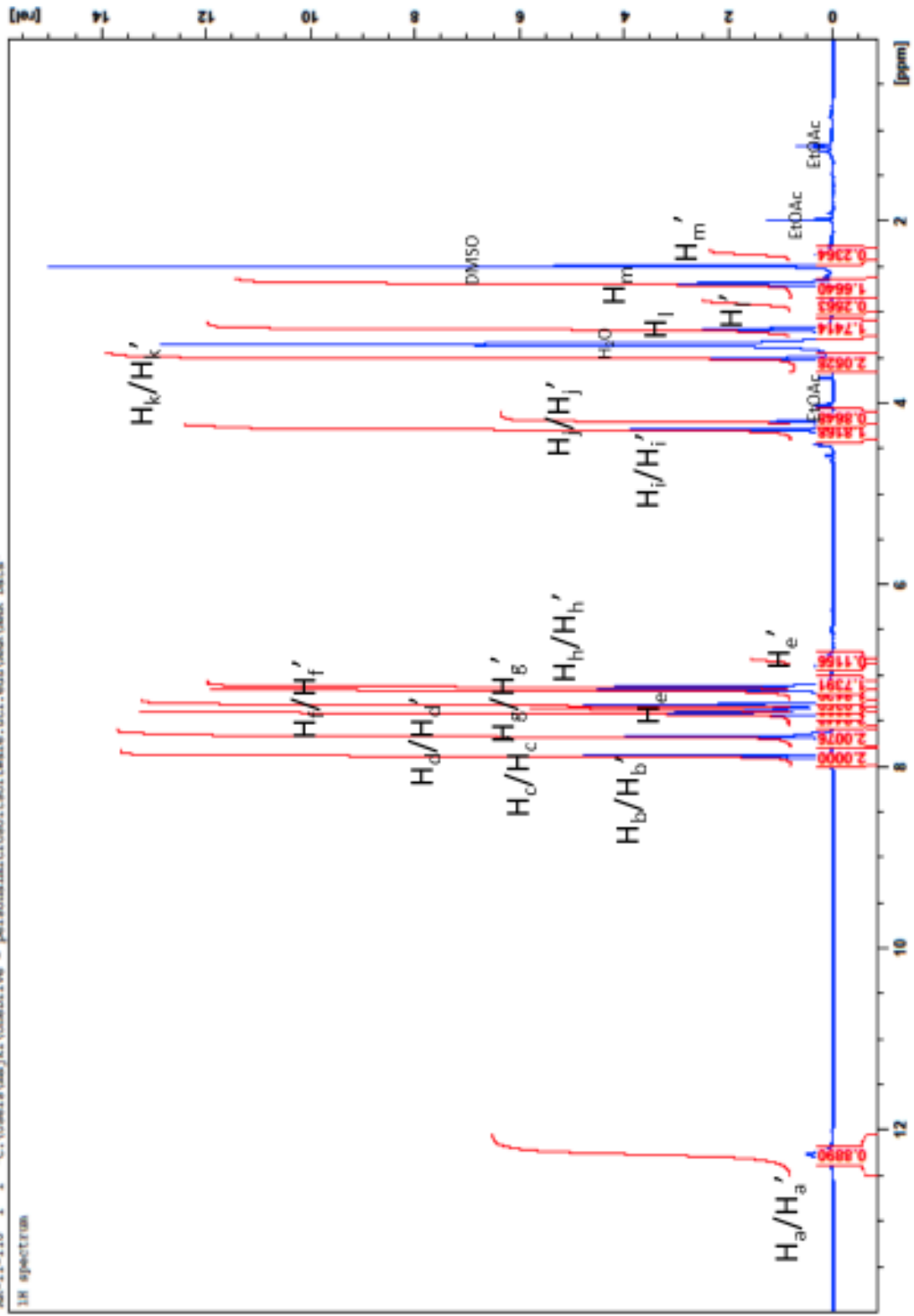
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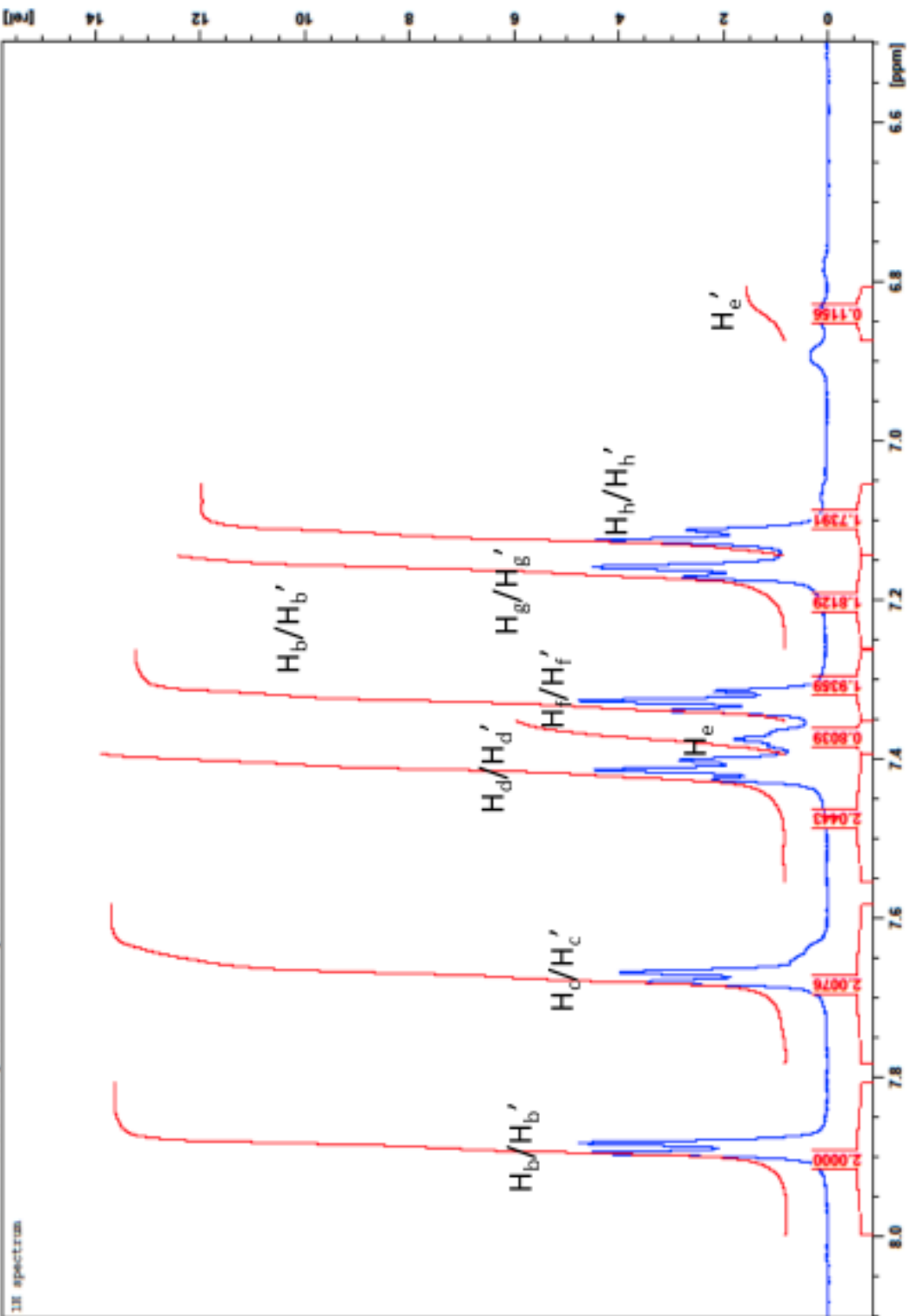
Rotamer indicative EXSY correlations:



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1H spectrum



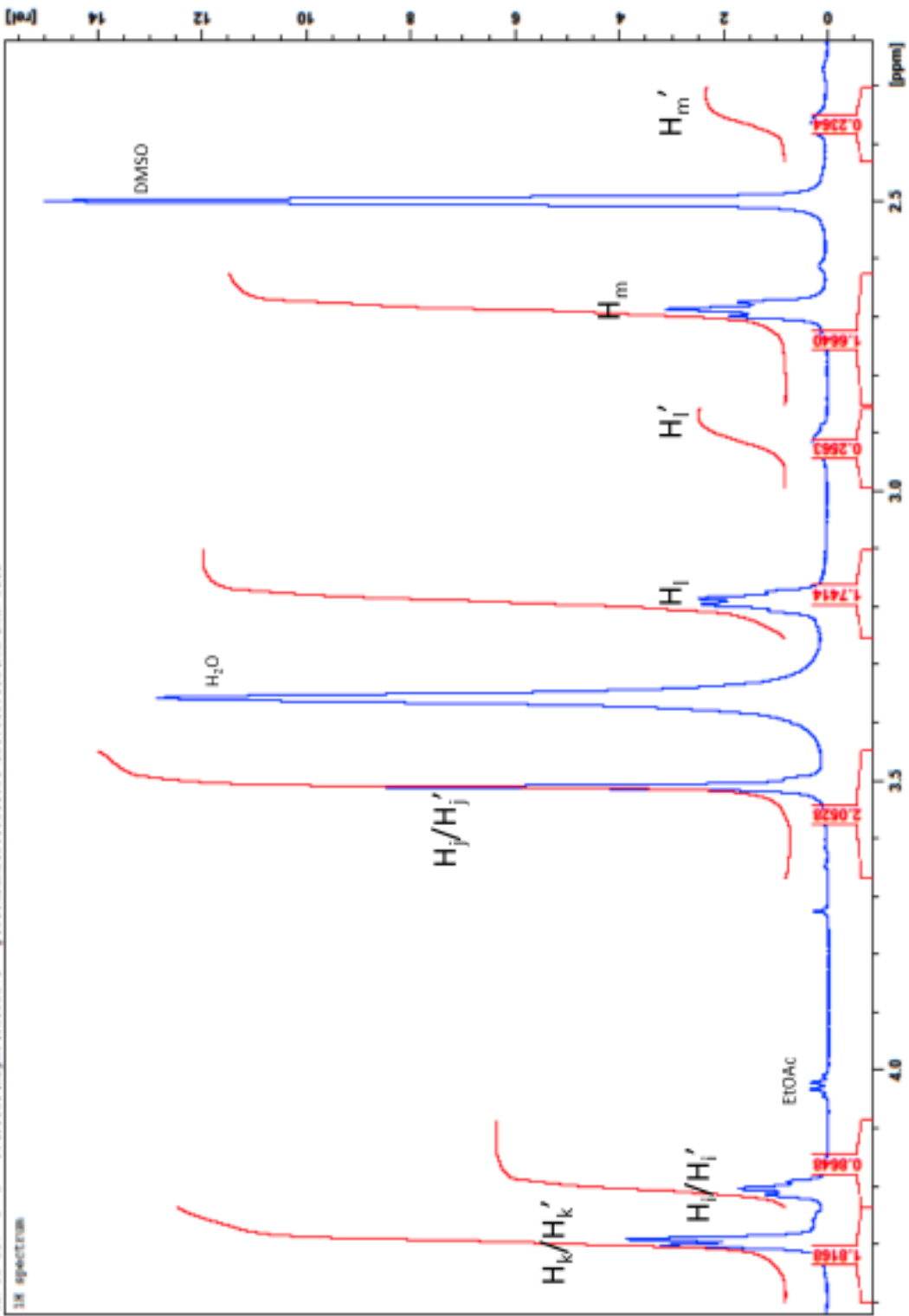
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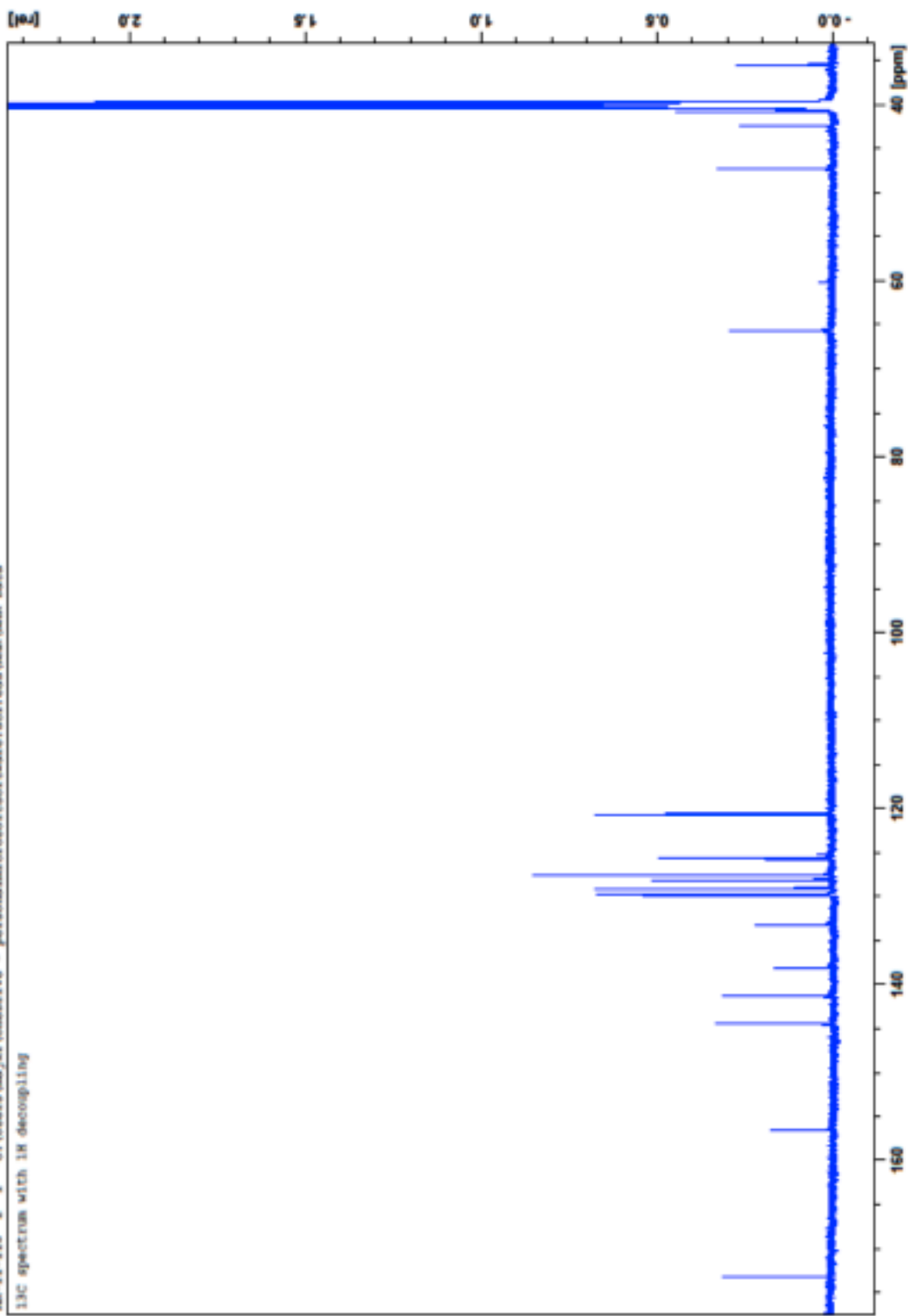
1H spectrum

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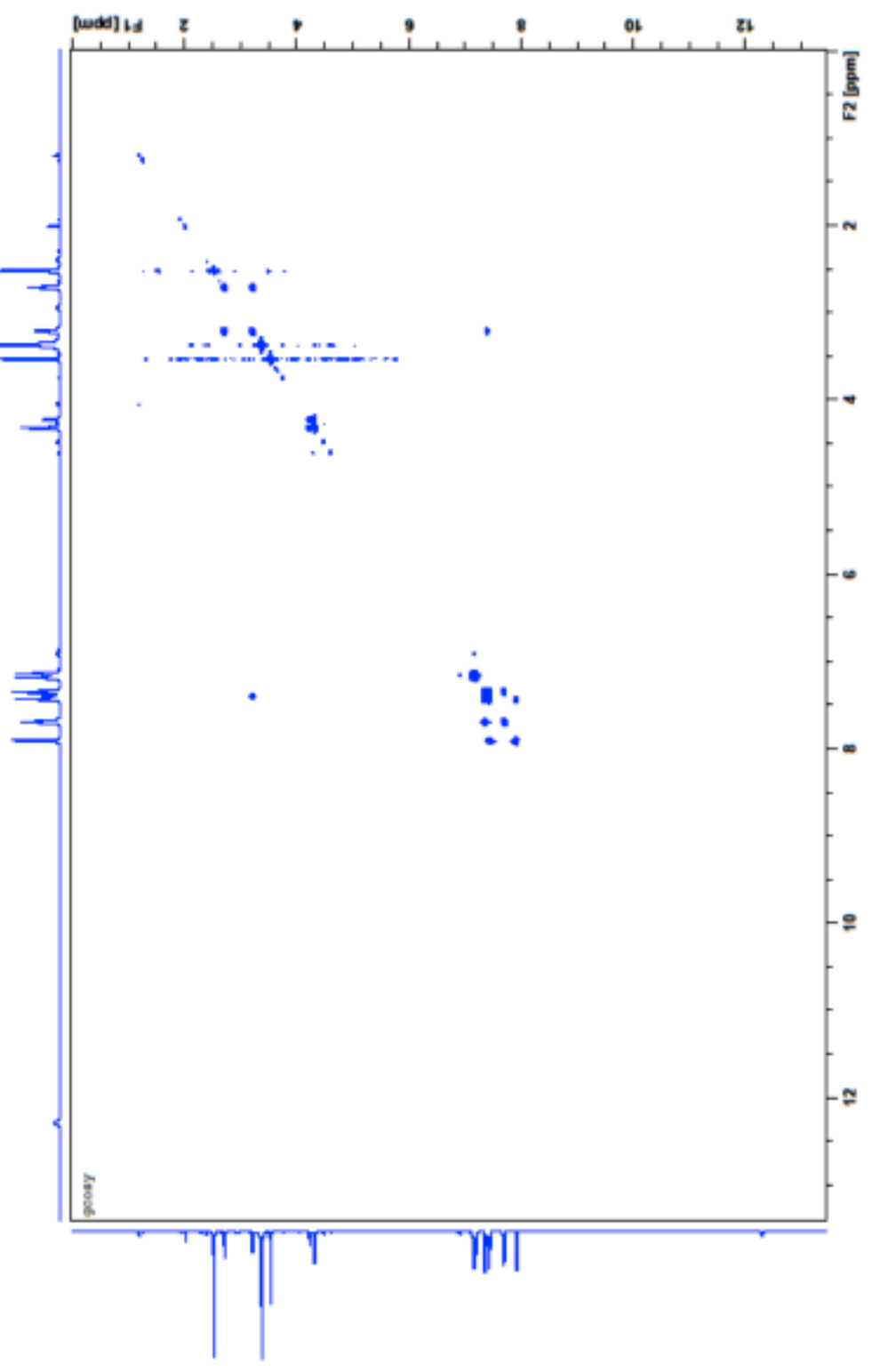
1H spectrum



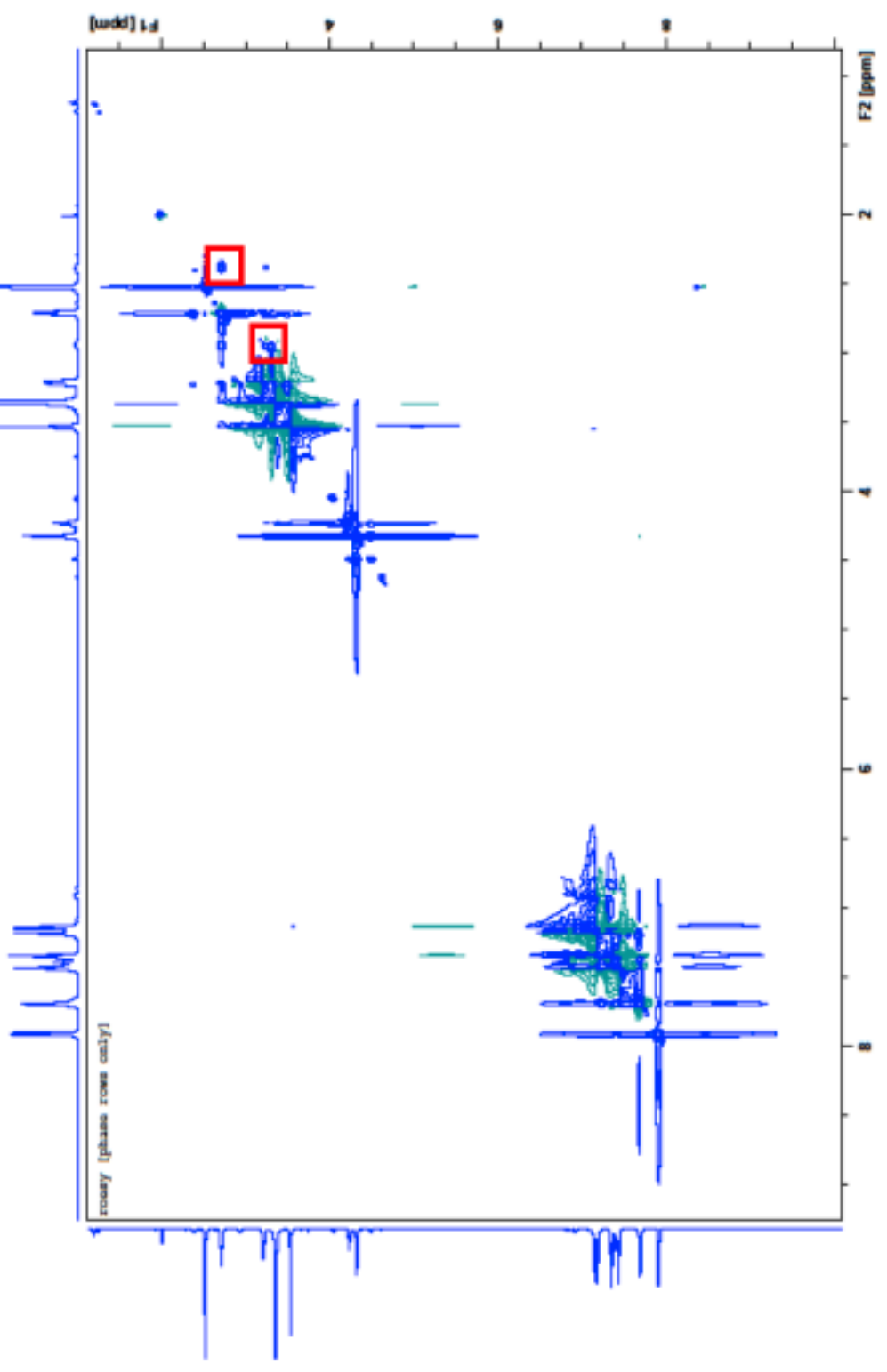
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13C spectrum with 1H decoupling

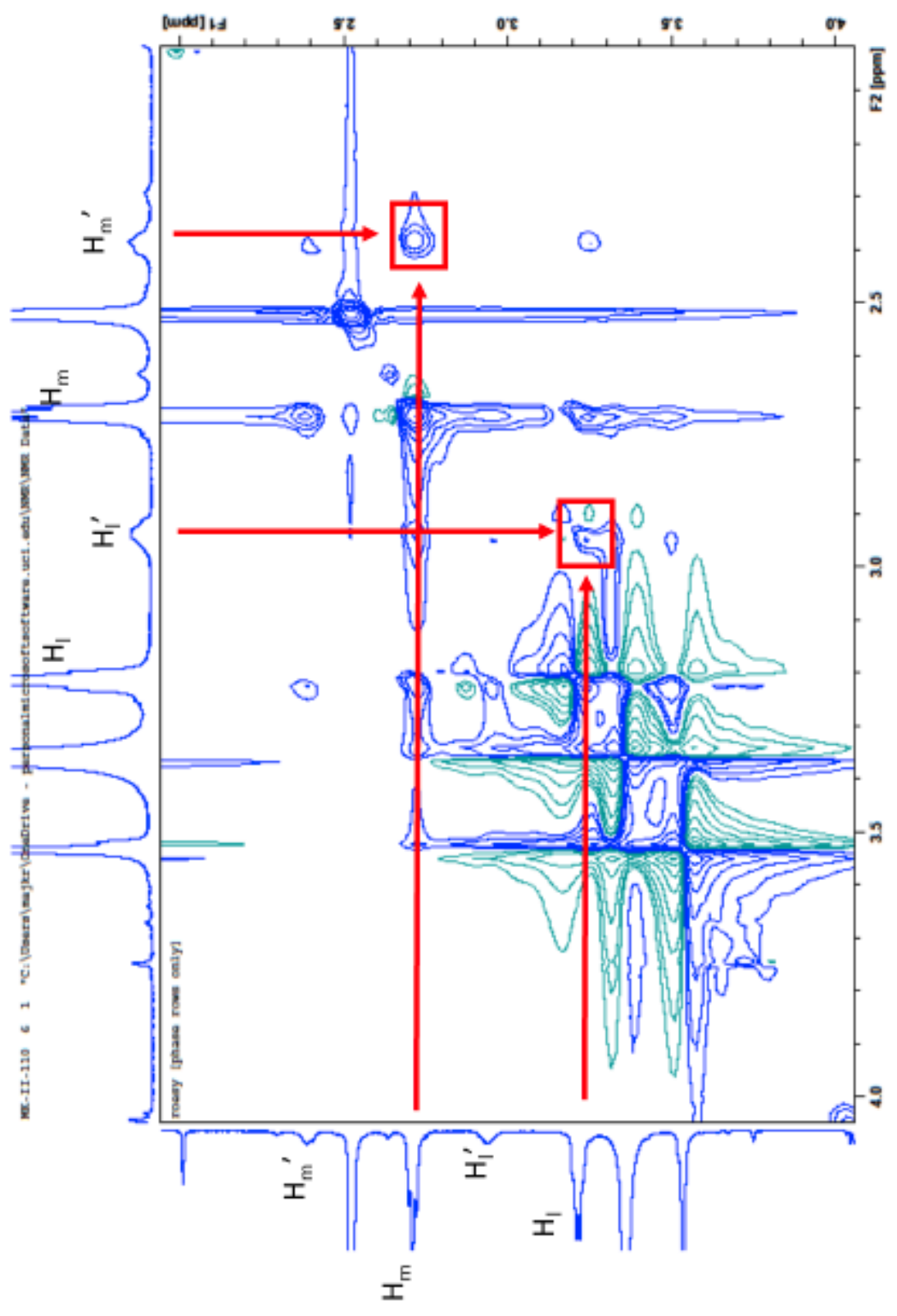


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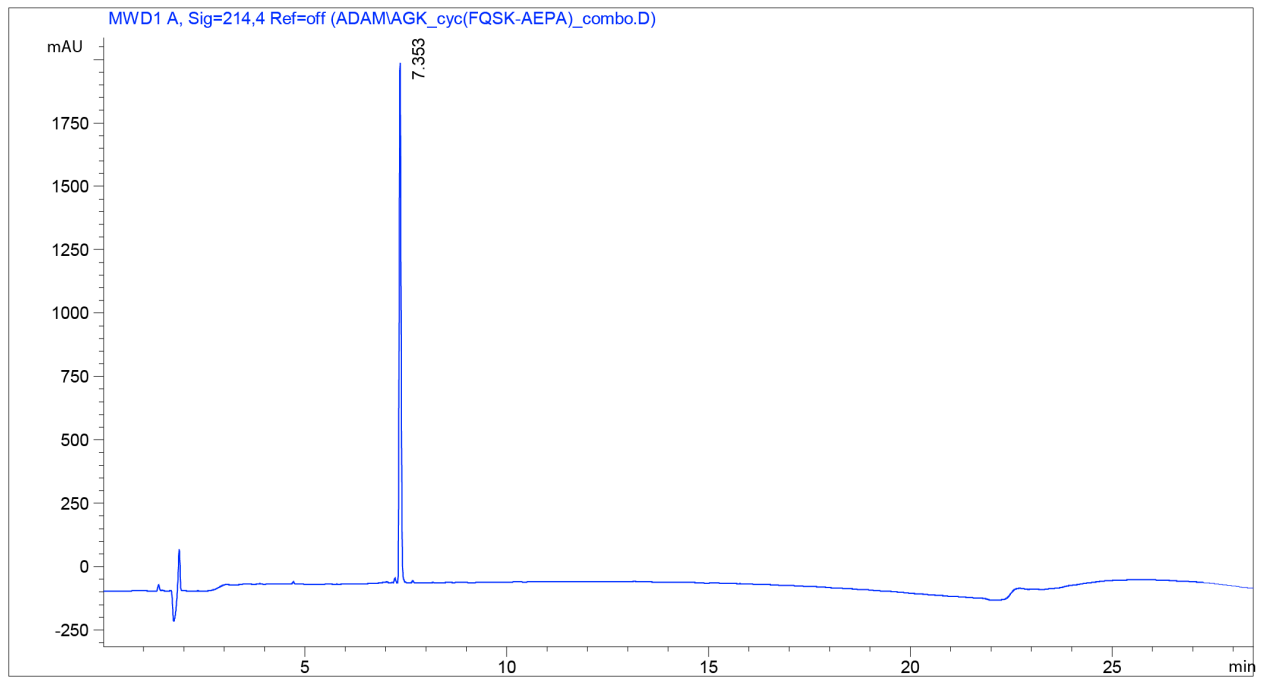




Peptide Characterization Data

Characterization of UCI-1

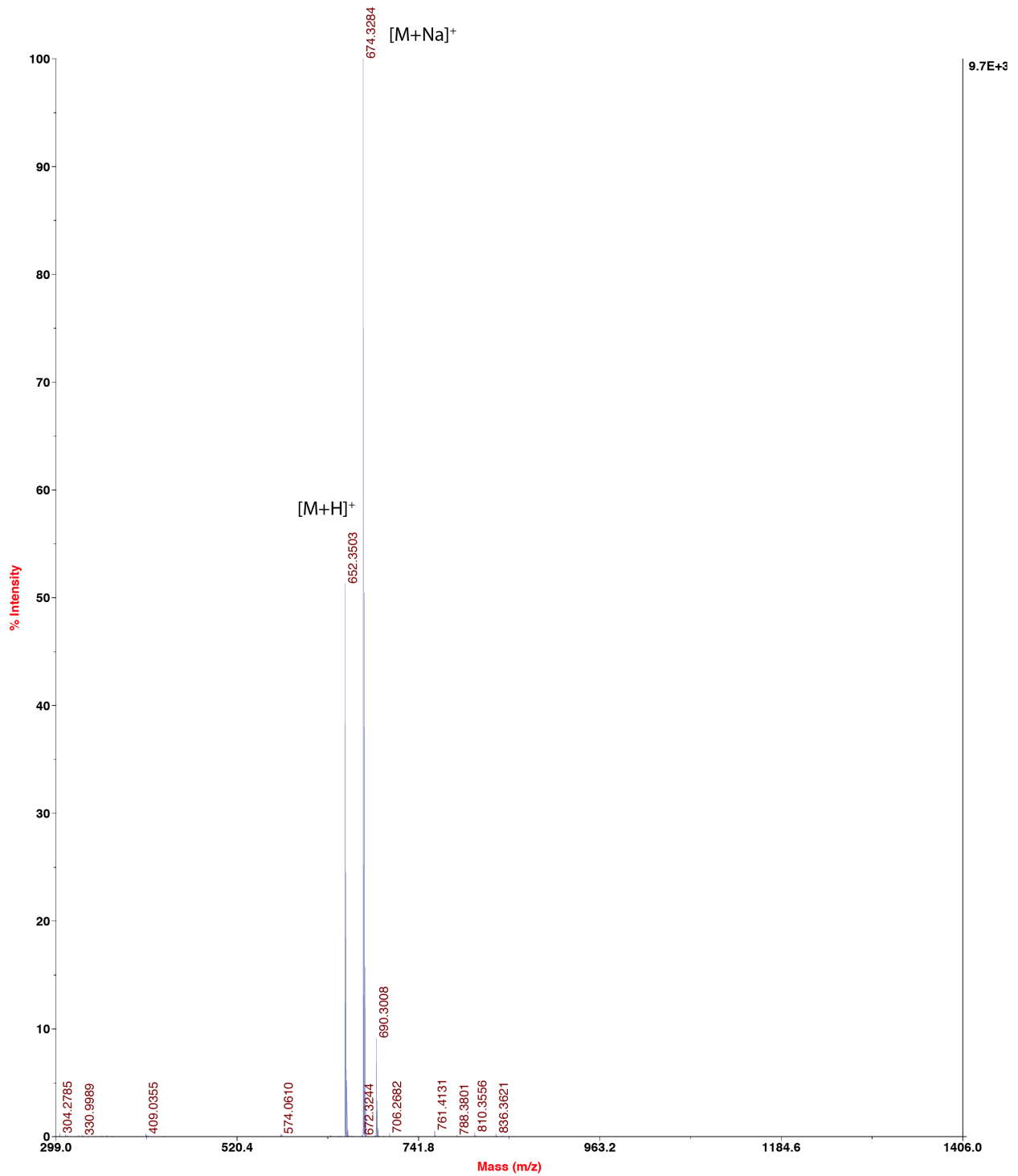
Analytical HPLC trace of UCI-1.



Mass spectrum of UCI-1.

Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 674.3, 9693]

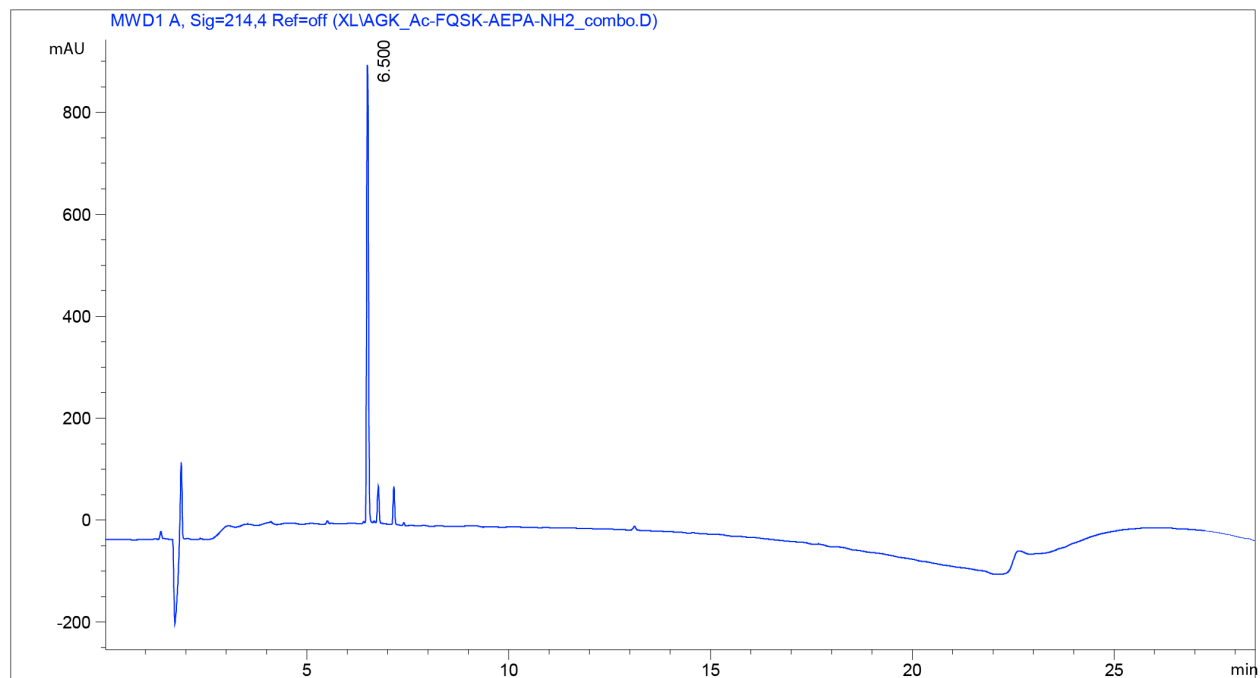


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Characterization of peptide-1a

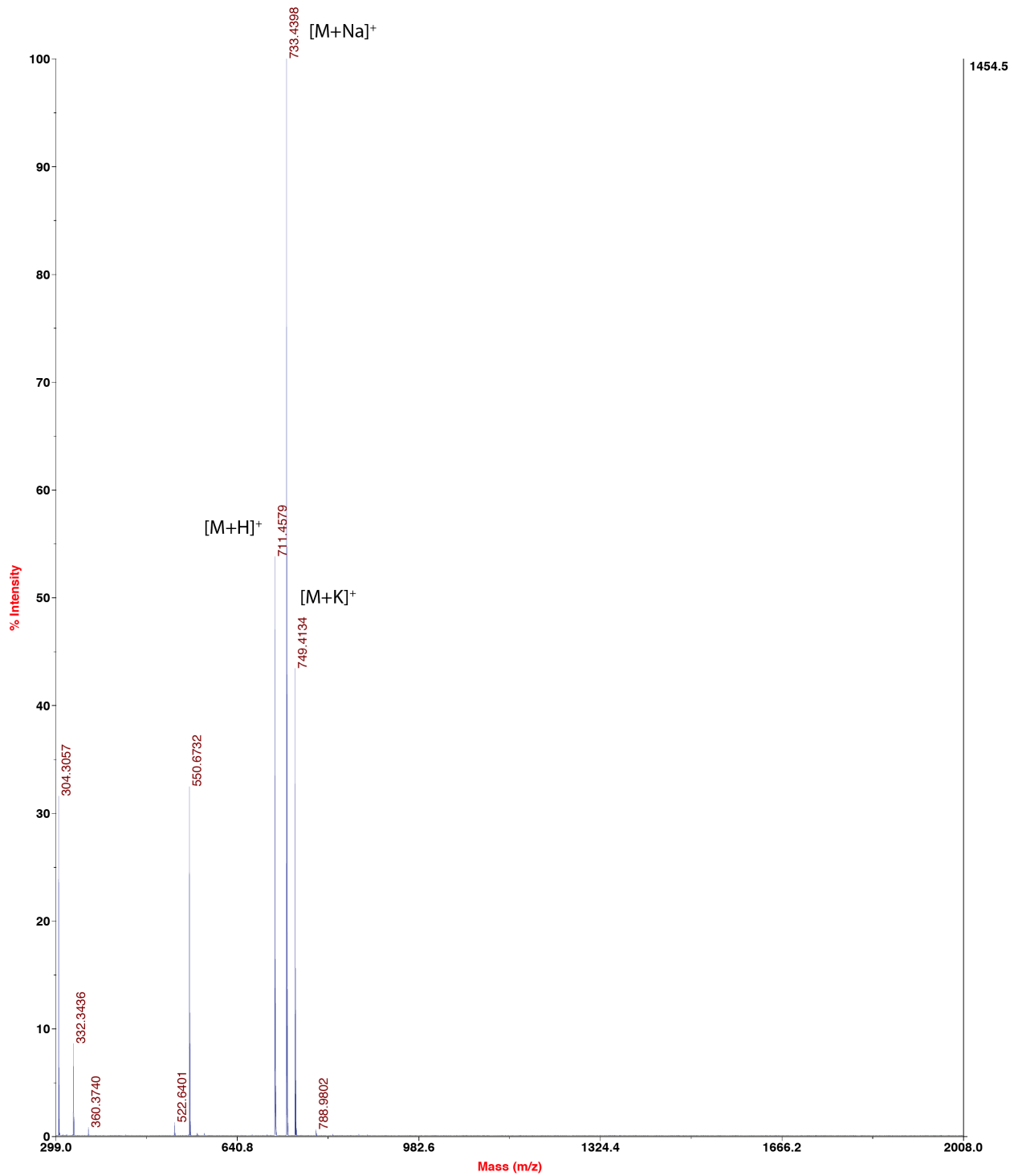
Analytical HPLC trace of peptide-1a.



Mass spectrum of peptide-1a.

Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72039

TOF/TOF™ Reflector Spec #1[BP = 733.4, 1454]

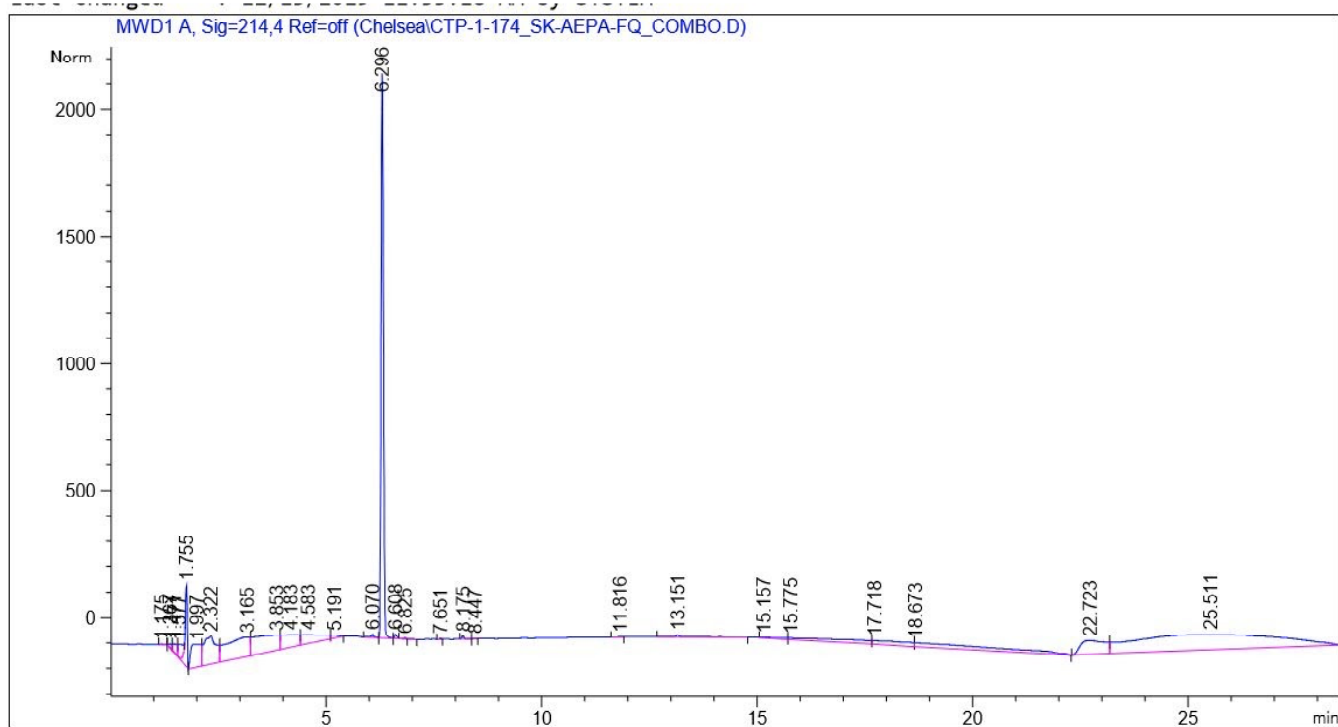


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Printed: 11:17, July 16, 2020

Characterization of peptide-1b

Analytical HPLC trace of peptide-1b.



Mass spectrum of peptide-1b.

