Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Genetic incorporation of 4-fluorohistidine into peptides enables selective affinity purification

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1. Supplementary Figures

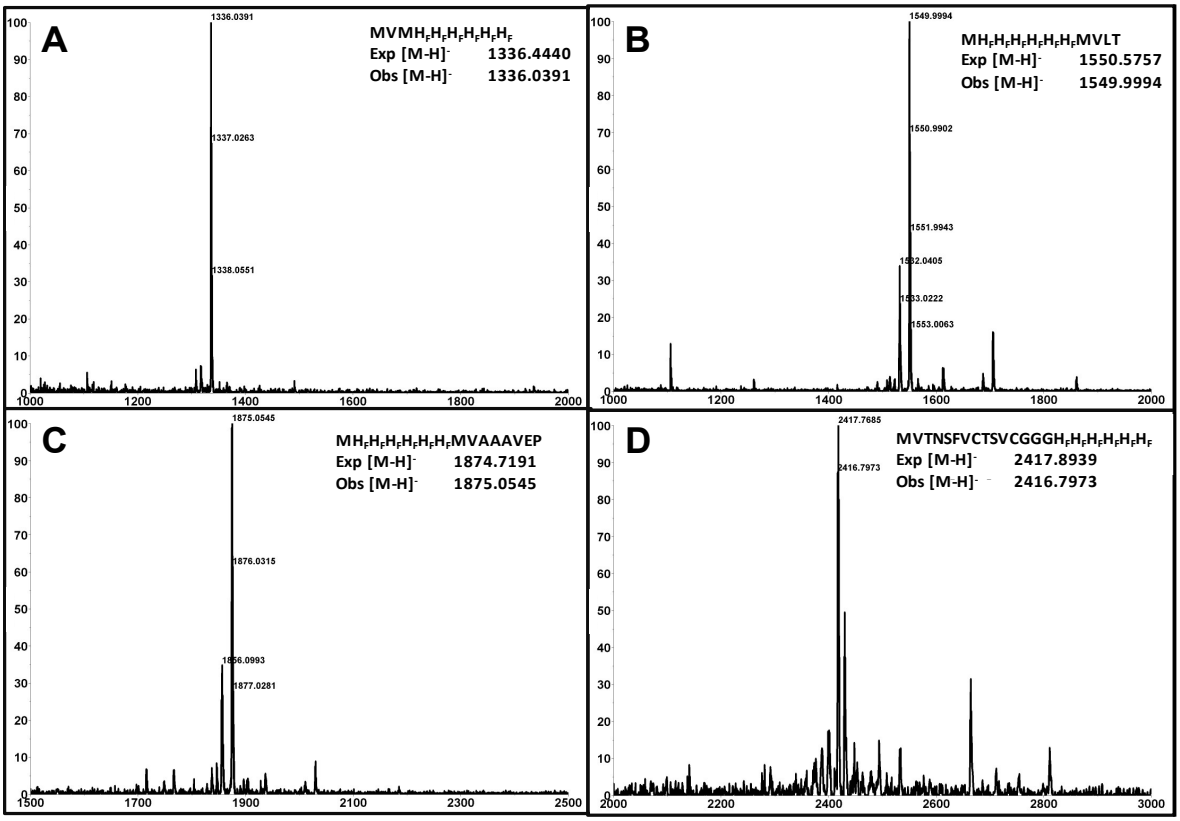


Figure S1. MALDI-TOF MS of in vitro translations using mRNAs encoding a Hexa-4-fluoro-histidine tag. Expected and observed masses are shown for each figure.

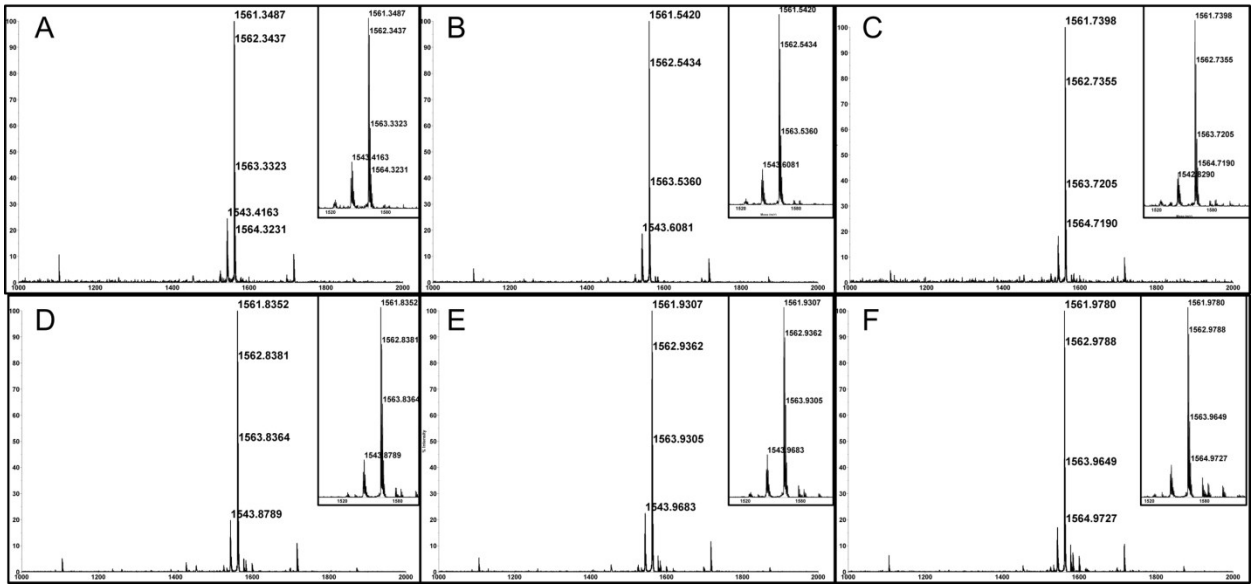
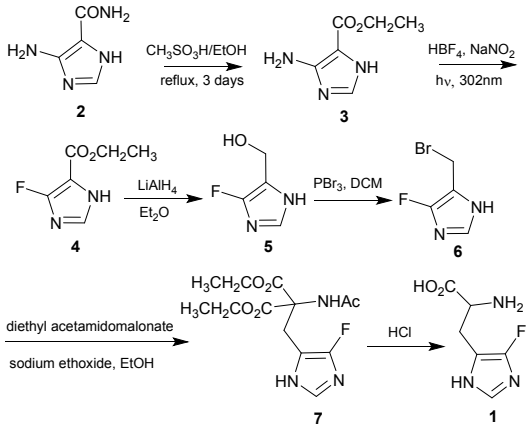


Figure S2. Binding/Wash Buffer pH does not degrade peptide. In vitro translation reactions with mRNAs encoding for the peptide MHF$_6$MVEP were captured onto Ni-NTA resin in buffers with pH adjusted to:

(A) pH 8.0, (B) pH 7.0, (C) pH 6.0, (D) pH 5.0, (E) pH 4.0, and (F) pH 3.0. Peptides were eluted with 1% TFA and analyzed by MALDI-TOF MS.

2. Experimental procedures for synthesis of 4-fluorohistidine.



General Information

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra for compounds **3** and **4** were recorded on a Varian Mercury spectrometer. For compounds **5**, **7**, and **1** ^{1}H (400 MHz) and ^{13}C (101 MHz) were recorded on a Bruker NanoBay Avance III spectrometer. For ^{1}H NMR spectra the solvent resonance was employed as an internal standard (DMSO-D δ=2.50, $CDCl_3$ δ=7.26, D_2O δ=4.79). ^{13}C NMR spectra were recorded with complete proton decoupling and solvent resonance was employed as an internal standard (DMSO-D, δ=39.52, $CDCl_3$ δ=77.16). All chemical shifts are expressed in parts per million (ppm) and coupling constant (J) is given in Hz. HRMS experiments for compounds **3** and **4** were performed on JEOL AccuTOF DART Mass Spectrometer. HRMS experiments for compounds **5**, **7**, and **1** were performed on a ThermoFisher Scientific LTQ Orbitrap Velos. All commercially-available reagents were used as received. Reactions were magnetically stirred. Formation of compounds **3**, **4**, **5**, and **7** were monitored by TLC on silica gel (60 F_{254} precoated aluminum sheets). Visualization was accomplished by irradiation with a UV lamp and/or staining with Pauly Reagent. Column

chromatography was performed using silica gel (60 Å). Irradiation in the formation of compound **4** performed using UVP UVM-57 lamp, 302 nm.

Ethyl 4-amino-1H-imidazole-5-carboxylate (3)

32.31 g (198.7 mmol) 4-amino-1H-imidazole-5-carboxamide hydrochloride **(2)** was suspended in 360 mL of dry EtOH. 102 mL methane sulfonic acid was added and the reaction was stirred at reflux for 3 days. Reaction progress was monitored by TLC (mobile phase = 4:1:1 N-butanol:acetic acid: water, R_f 0.64). The solvent was removed *in vacuo* and the residual syrup taken up in approximately 200 mL water. The solution was neutralized with cold, saturated NaOH and extracted with EtOAc (3 x 100mL), dried ($MgSO_4$) and the solvent removed to yield 10.48 g (33.9%) as a grey-green powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ: 12.05 (s, 1H), 7,36 (s, 1H), 5.46 (s, 1H), 4.22-4.15 (q, 2H, J=7.0 Hz), 1.28-1.23 (t, 3H, J=7.0 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ: 160.98, 152.55, 135.22, 101.11, 58.76, 14.58. HRMS, (DART-TOF): Calculated for $C_6H_9N_3O_2$ $[M+H]^+$: 156.0695. Found: 156.0657.

Ethyl 4-fluoro-1H-imidazole-5-carboxylate (4)

0.584 g (3.764 mmol) of **3** was dissolved in 20 mL 50% HBF_4 in water. The solution was cooled to -10º C. 1.2 g of sodium nitrite was dissolved in approximately 1.5 mL of water and then added dropwise to the chilled solution of **3**. The solution turned blue-green. The reaction mixture was then transferred to a shallow glass dish and irradiated overnight with a handheld 302 nm UV light (6 watt handheld lamp, 5 cm distance). Irradiation occurred 12-16 hours, until the solution turned a pale yellow and nitrogen bubbles ceased. To monitor TLC, small aliquots of the reaction mixture were removed and neutralized before running in 3:1 EtOAc: hexanes (product R_f 0.56). The solution was stirred on ice and neutralized with cold,

concentrated NaOH, extracted with EtOAc (3 x 50mL), and dried ($MgSO_4$). The solvent was removed to yield **4** (0.597 g, 99 %). The material was used for the subsequent reaction without further purification. ^{1}H NMR (300 MHz, DMSO-d_6): δ: 13.25 (s, 1H), 7.64 (s, 1H), 4.27 (q, 2H, J=7.1 Hz), 1.27 (t, 3H, J=7.1 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ: 161.44, 158.30 (d, $J_{C,F}$ = 249 Hz), 133.15, 110.58, 61.13, 15.08. HRMS (DART-TOF): Calculated for $C_6H_7FN_2O_2$ $[M+H]^+$: 159.0492. Found 159.0340.

(4-fluoro-1H-imidazol-5-yl) methanol (5)

354.9 mg $LiAlH_4$ (9.352 mmol) was dissolved in 10 mL cold Et_2O. 211.3 mg (1.336 mmol) of **4** was slowly added in 50 mg portions. Disappearance of the starting material was observed immediately by TLC (mobile phase=3:1 EtOAc:hexanes, starting material R_f 0.56, product R_f 0 (baseline). Product was also visualized with Pauly's reagent. Upon confirmation of the disappearance of compound starting material, a second TLC was run in 5:1:1 EtOAc:methanol:NH_3 (aq). ($R_{f\ product}$ 0.62) The reaction was allowed to stir on ice for an additional ten minutes. The reaction was quenched by the very slow addition of cold water. The precipitate was removed by vacuum filtration and the filtrate was reserved. The solid was boiled in methanol and vacuum filtered again. Both filtrates were combined and solvent was removed *in vacuo*. EtOAc was added to this crude solid to dissolve the product leaving inorganic impurities behind. Removal of the solvent *in vacuo* yielded **5** (68.9 mg, 44%). This dissolution in EtOAc was not necessary to continue on to the next step, although it was necessary for characterization and yield calculations. ^{1}H NMR: (400 MHz, DMSO-d_6) δ: 12.16 (s, 1H), 7.25 (s, 1H), 5.08 (t, 1H, J=5.3 Hz) 4.33 (d, 2H, J=5.0 Hz). ^{13}C NMR: (101 MHz,

DMSO-d_6) δ:153.08 (d, $J_{C,F}$ = 229 Hz) 154.21, 151.94, 128.30, 107.93 (d, $J_{C,F}$ = 35.35 Hz) , 51.05

HRMS (ESI): Calculated for $C_4H_5FN_2O$ $[M+H]^+$: 117.0386. Found 117.0446.

5-bromomethyl-4-fluoro-1H-imidazole (6)

358.3 mg (3.088 mmol) **5** was dissolved in 50 mL DCM. 880 μL (9.263 mmol) of PBr_3 was added and the solution was stirred for eight hours at room temperature. The solvent and excess PBr_3 was removed *in vacuo* to yield a yellow, glassy film (Note: solid sodium bicarbonate was added to the collection flask of the rotary evaporator prior to evaporation. This allowed for neutralization of the acidic solution). Due to the reactivity of the product, no attempt at purification or characterization was made, and the material used immediately for the next step.

Diethyl-2-acetamido-2-((4-fluoro-1H-imidazol-5-yl)-methyl)-malonate (7)

1.14 g (16.70 mmol) sodium ethoxide was dissolved in cold, dry ethanol. Diethyl-2-acetomidomalonate (1.2 g, 5.525 mol) was added. The solution was poured into a flask containing the crude material containing compound **6** from above and stirred for four hours at room temperature. The solvent was removed *in vacuo*, and the reaction mixture was taken up in approximately 200 mL water and extracted with EtOAc (3 x 100mL). The target compound (R_f 0.26, 5% methanol in EtOAC) was purified by flash column chromatography silica gel, first by washing with 100% EtOAc and then eluting with 5% methanol in EtOAc, yielding **7** (290.6 mg, 29.8% over two steps from **5**). ^{1}H NMR: (400 MHz, $CDCl_3$) δ: 10.58 (s, 1H), 7.10 (s, 1H), 6.91 (s, 1H), 4.18-4.22 (m, 4H), 3.56 (s, 2H), 1.22-1.20 (m, 9H). ^{13}C NMR: (101 MHz,

$CDCl_3$) δ: 170.43, 167.62, 154.16 (d, $J_{C,F}$ = 238 Hz), 128.55, 101.21, 66.08, 63.17, 27.51, 23.00, 13.93.

HRMS (ESI): Calculated for $C_{13}H_{18}FN_3O_5$ $[M+H]^+$: 316.1231. Found 316.1259.

4-fluoro-histidine (1)

0.291 g (0.9217 mmol) of **7** was dissolved in 20 mL 6N HCl. The solution was heated at reflux for eight hours, after which the solvent was removed *in vacuo* to yield **1** as a white solid (146.5 mg, 91.8%). ^{1}H NMR: (400 MHz, D_2O) δ: 8.12 (s, 1H), 4.29 (t, 1H, J=6.5 Hz), 3.35 (d, 2H, J=6.4 Hz). ^{13}C NMR: (101 MHz, D_2O) δ: 170.62, 147.99 (d, $J_{C,F}$ = 255 Hz), 128.59, 51.83, 23.00. HRMS (ESI): Calculated for $C_6H_8FN_3O_2$ $[M+H]^+$=174.0601, found 174.0647

3. Experimental methods for the tRNA charging assay and in vitro translation reactions.

General Experimental details. An Applied Biosystems Voyager DE-Pro instrument was used for matrix-assisted laser desorption/ionization analysis. All mass spectrums were obtained using delayed extraction in reflectron negative mode. Peptide yield was measured by scintillation counting on a Beckman Coulter LS 6500 liquid scintillation counter. Phosphate binding/wash buffers were made to 100 mM with 300 mM NaCl and pH adjusted using H_3PO_4 and KOH to desired pH (pH 3.0 – 8.0).

Aminoacyl-tRNA synthetase MALDI assay. The aminoacyl-tRNA synthetase charging assay was performed exactly as described previously[1] on a 50 µL scale using HisRS (1.14 µM) and with and without 4-Fluorohistidine (1 mM).

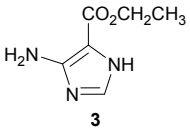
PURE In Vitro Translations. Translation mixtures (50 µL) consisted of HEPES-KOH pH=7.6 (50 mM), spermidine (2 mM), potassium acetate (10 mM), magnesium acetate (6 mM),

dithiothreitrol (1 mM), creatine kinase (40 μg/mL), inorganic pyrophosphatase (1 μM), (6R,S)-5,10-formyl-5,6,7,8-tetrahydrofolic acid (100 μM), creatine phosphate (30 mM), ATP (1.5 mM), GTP (1.5 mM), *E. coli* total tRNA (2.4 mg/mL), EF-G (0.52 μM), EF-Ts (8 μM), EF-Tu (10 μM), RF-1 (0.3 μM), RR-F (0.5 μM), RF-3 (0.17 μM), IF-1 (2.7 μM), IF-2 (0.4 μM), IF-3 (1.5 μM), ribosomes (1.2 μM), MTF (0.6 uM), Methionine (10 μM for radiolabeled samples, 100 μM for non-radiolabeled samples), 0.3 μM ^{35}S-Methionine (15 μCi, radiolabeled samples only), necessary amino acids for translation (100 μM each with the exception of 4-fluoro-histidine (600 μM)) and mRNA template (1 μM). Each reaction also contained only the essential aminoacyl-tRNA synthetase enzymes required for translation of that templated at concentrations ranging from 0.1 to 0.8 μM). Translations were initiated by addition of mRNA template and incubated at 37°C for 1 h. Reactions were quenched with addition of 10x volume (500 μL) of binding/wash buffer at desired pH (100 mM phosphate, 300 mM NaCl, 5mM BME) and bound to 50 μL Ni-NTA resin (McLab, NINTA-300) for 1 h in a centrifugal filter tube. After 1 h, reactions were washed 3x with 500 μL of binding/wash buffer with five minutes of rotation in buffer between washes. The bound peptide was eluted from resin using 1% trifluoroacetic acid (TFA) (50 μL). For reactions labeled with ^{35}S-Met, the yield was determined by scintillation counting of 47 μL of the elution. Non-radiolabeled reactions at binding/wash buffer pH 8.0 were concentrated and desalted via Zip-Tip C18 chromatography into 5 μL CHCA matrix (α-cyano-4-hydroxycinnamic acid in 1:1 MeCN:0.1% TFA) and analyzed via MALDI-TOF.

Bradford Assay. A standard Bradford Assay was performed according to BioRad Quick Start Bradford Assay protocol. BSA standards (2.0 mg/mL) were obtained from Thermo Fisher Scientific.

4. **Spectra of synthetic compounds.**

^{1}H-NMR Ethyl 4-amino-1H-imidazole-5-carboxylate (3)



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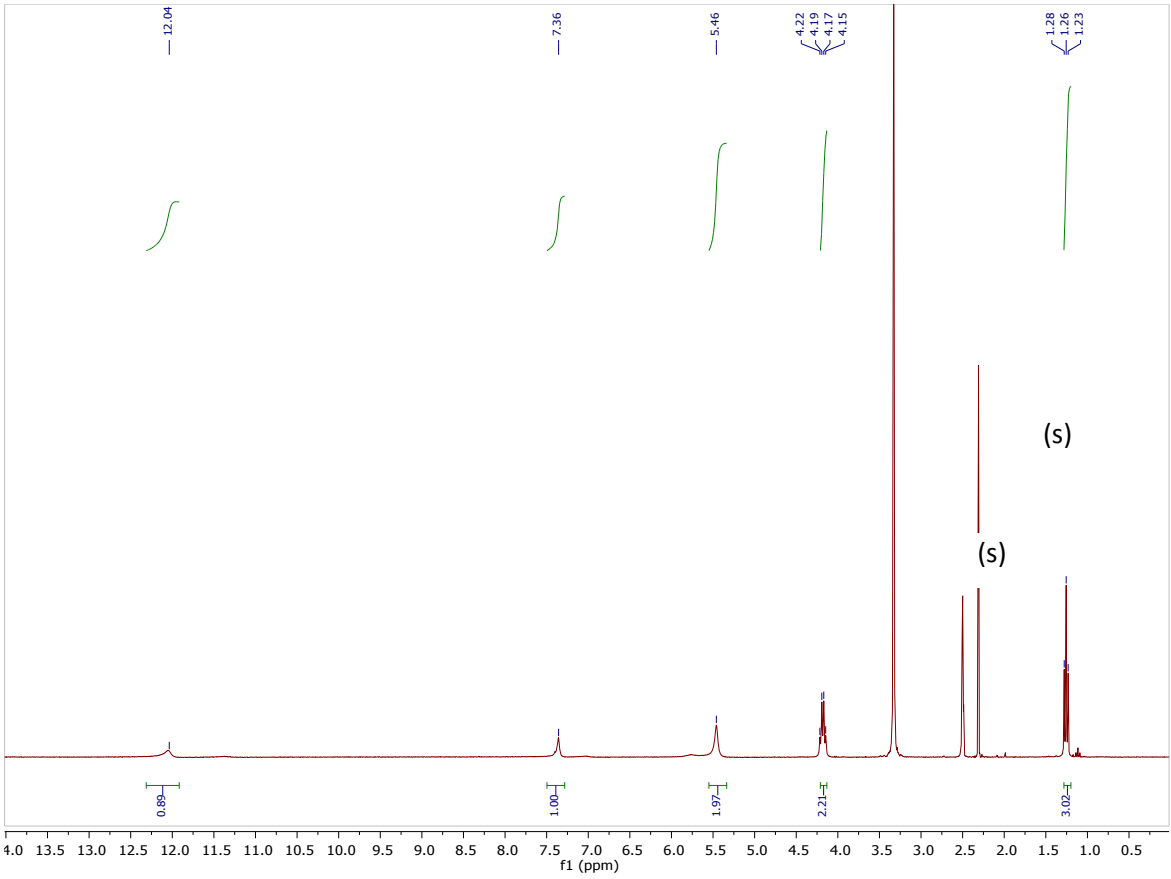

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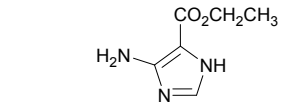

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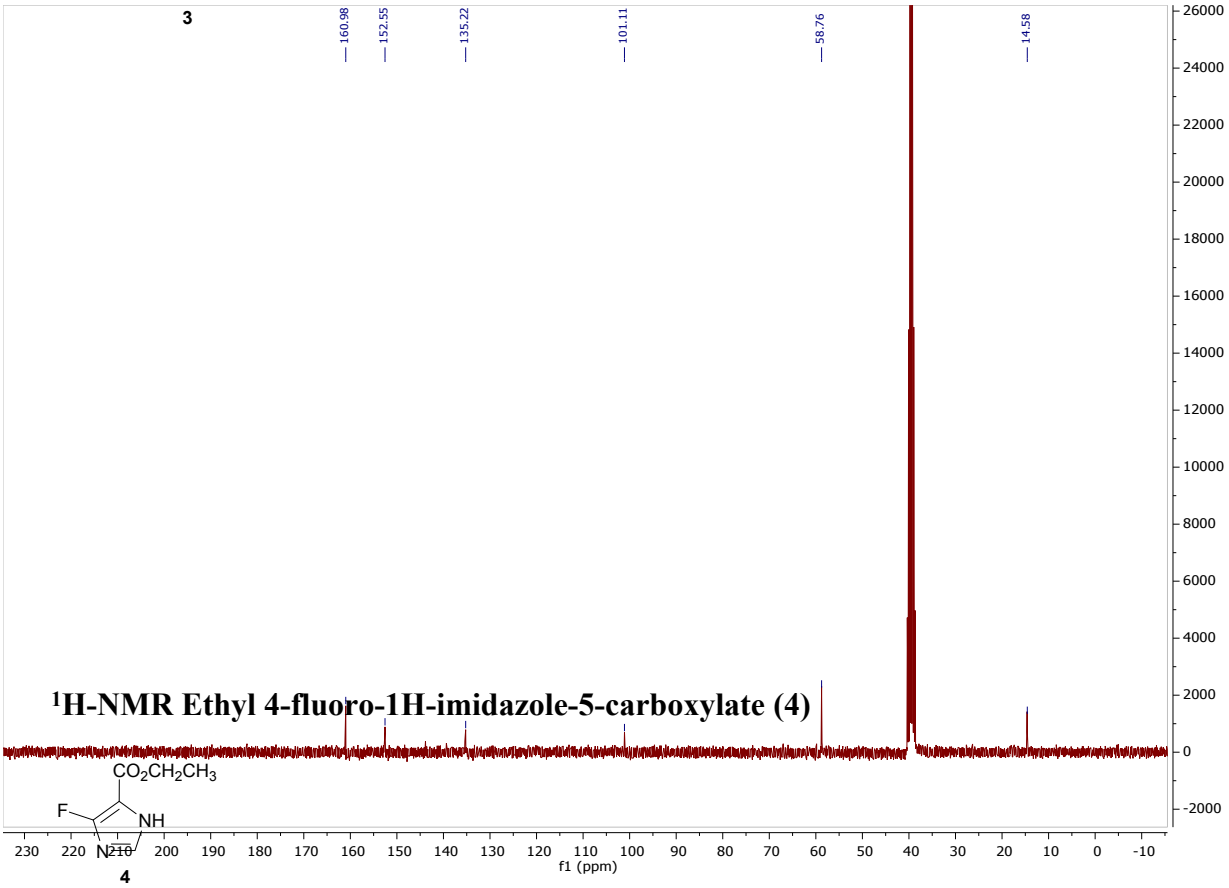

^{1}H-NMR Ethyl 4-fluoro-1H-imidazole-5-carboxylate (4)

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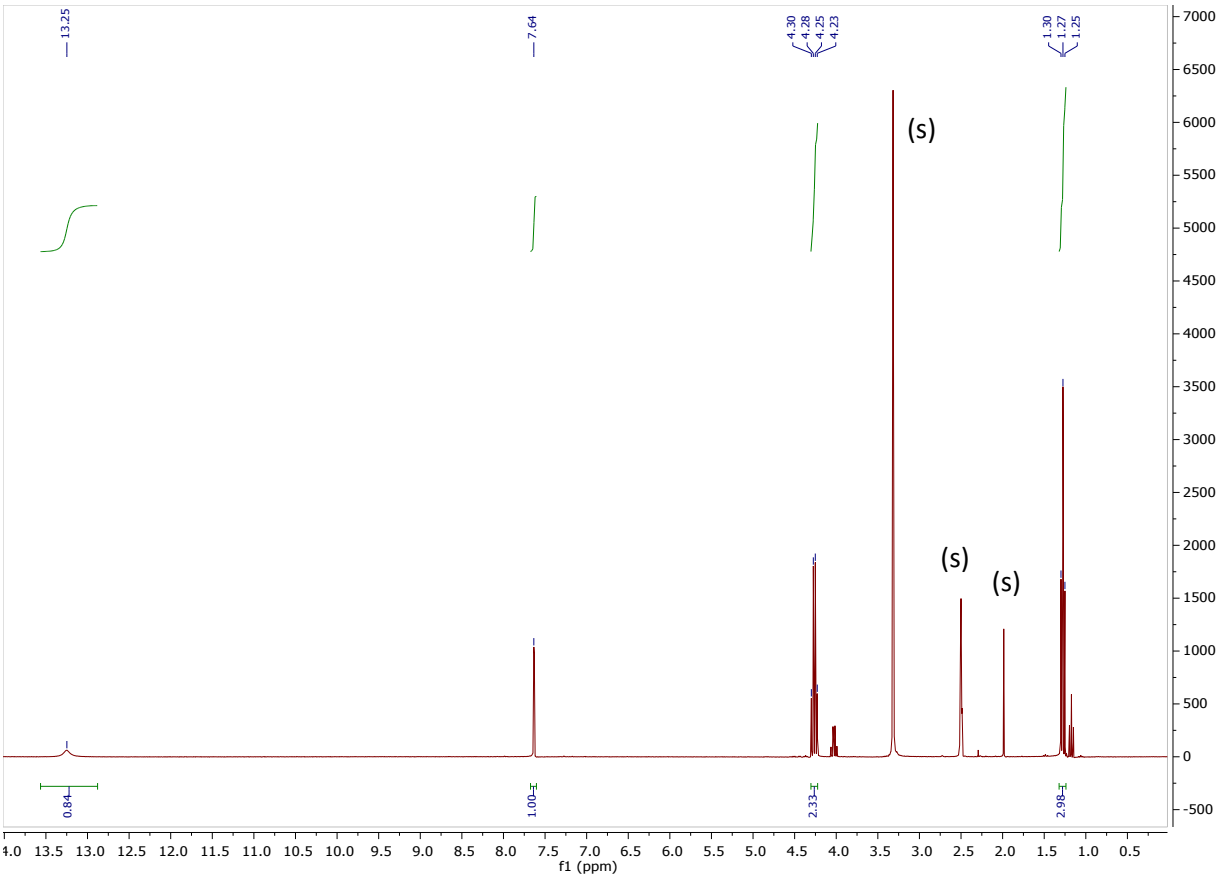

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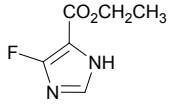

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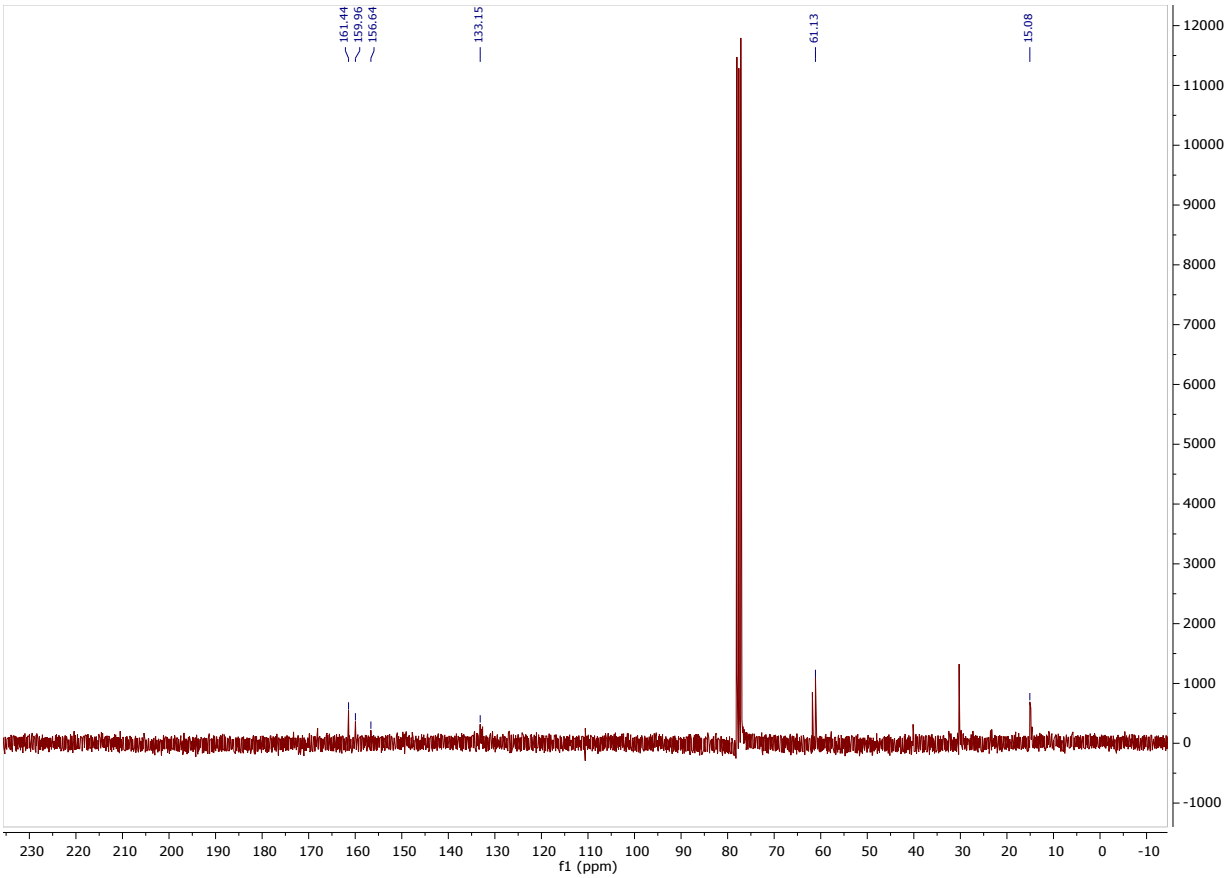
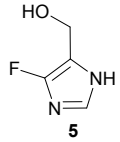


^{1}H-NMR (4-fluoro-1H-imidazol-5-yl) methanol (5)



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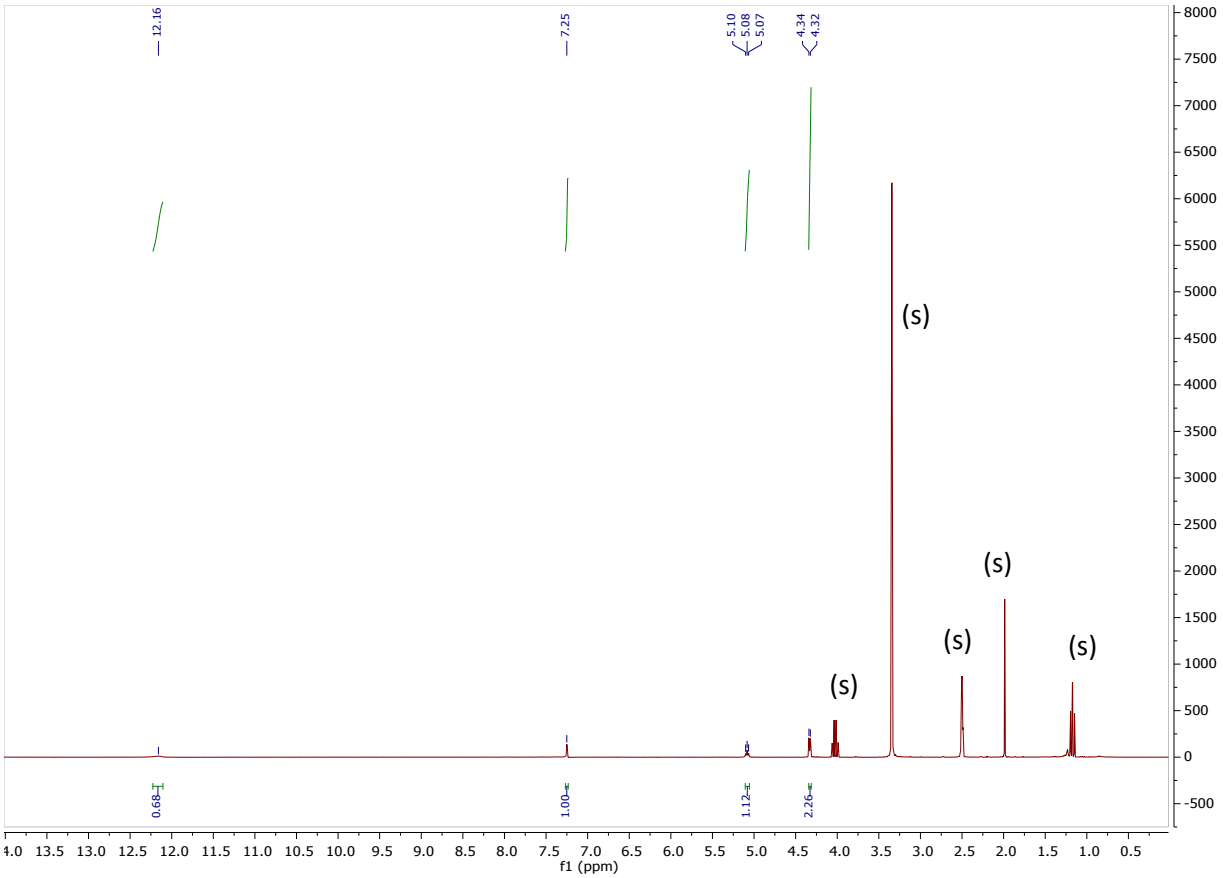

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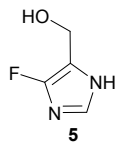
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F
NH
N

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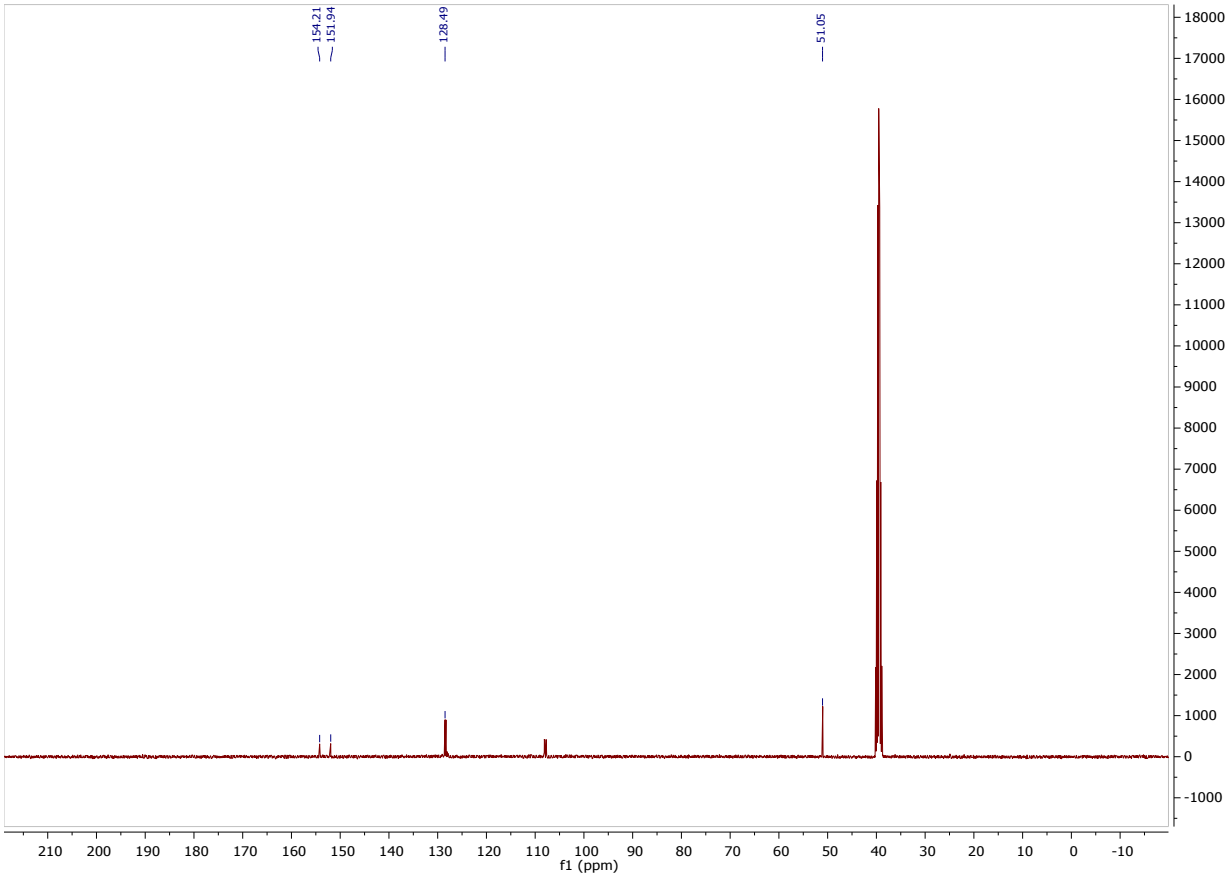
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180
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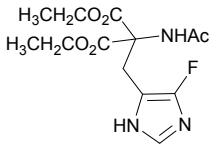
f1 (ppm)

^{1}H-NMR Diethyl-2-acetamido-2-((4-fluoro-1H-imidazol-5-yl)-methyl)-malonate (7)



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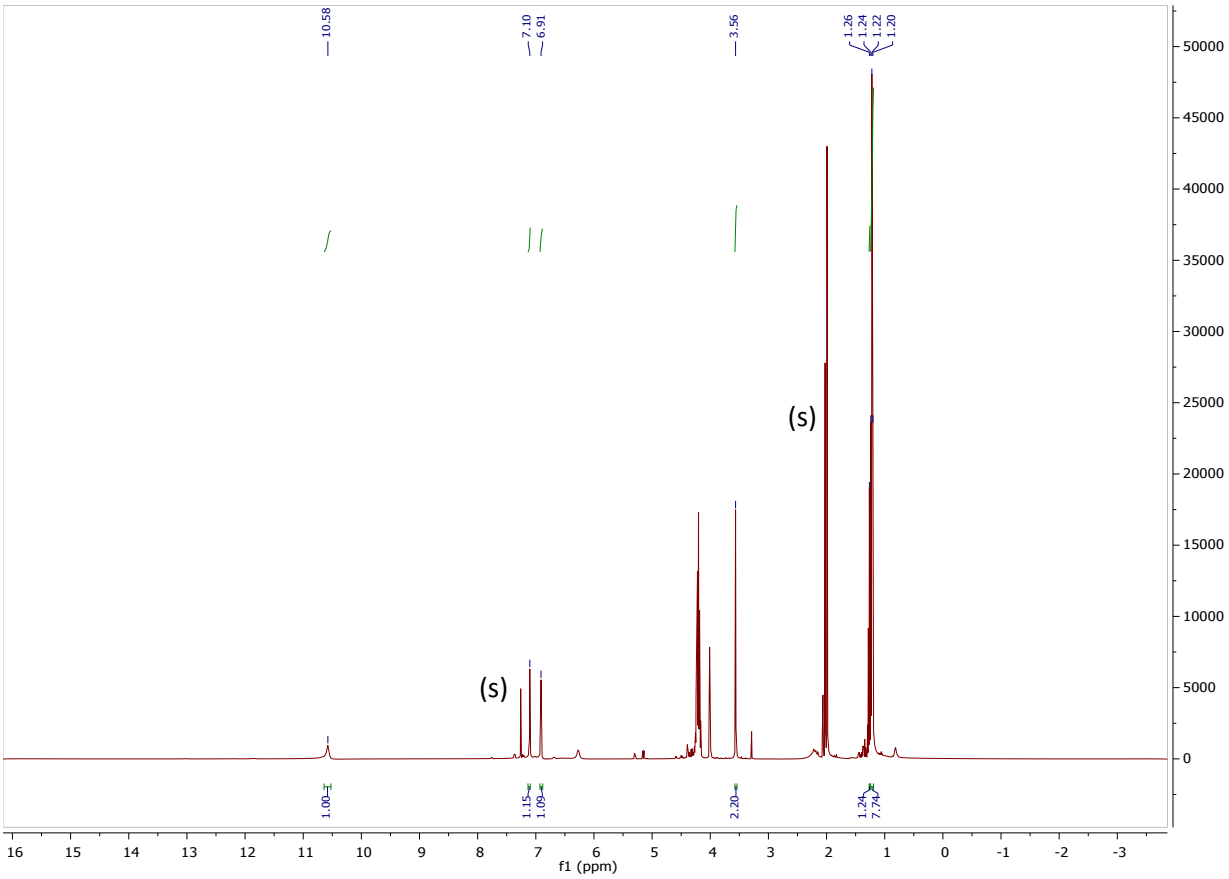

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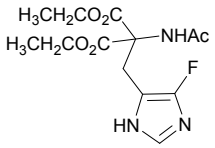

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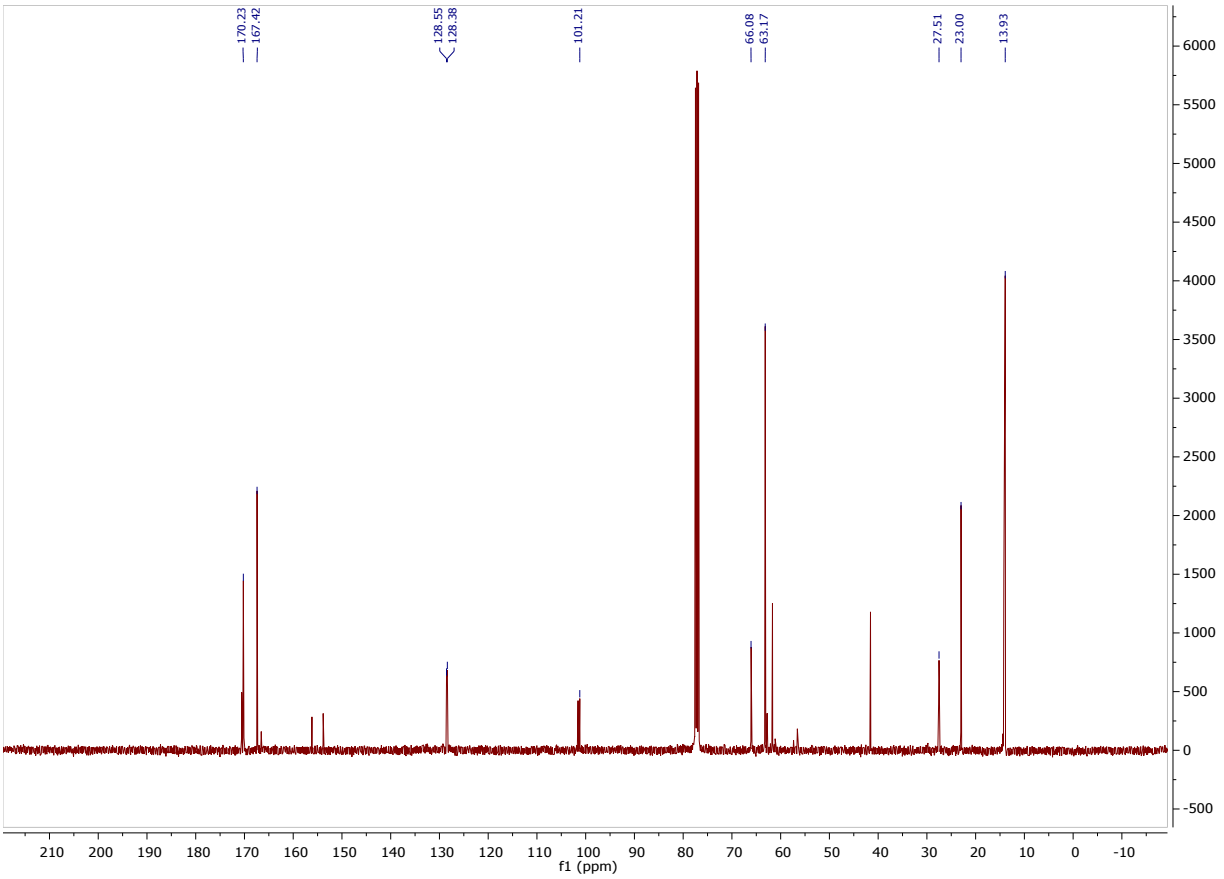
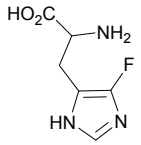


^{1}H-NMR 4-fluoro-histidine (1)



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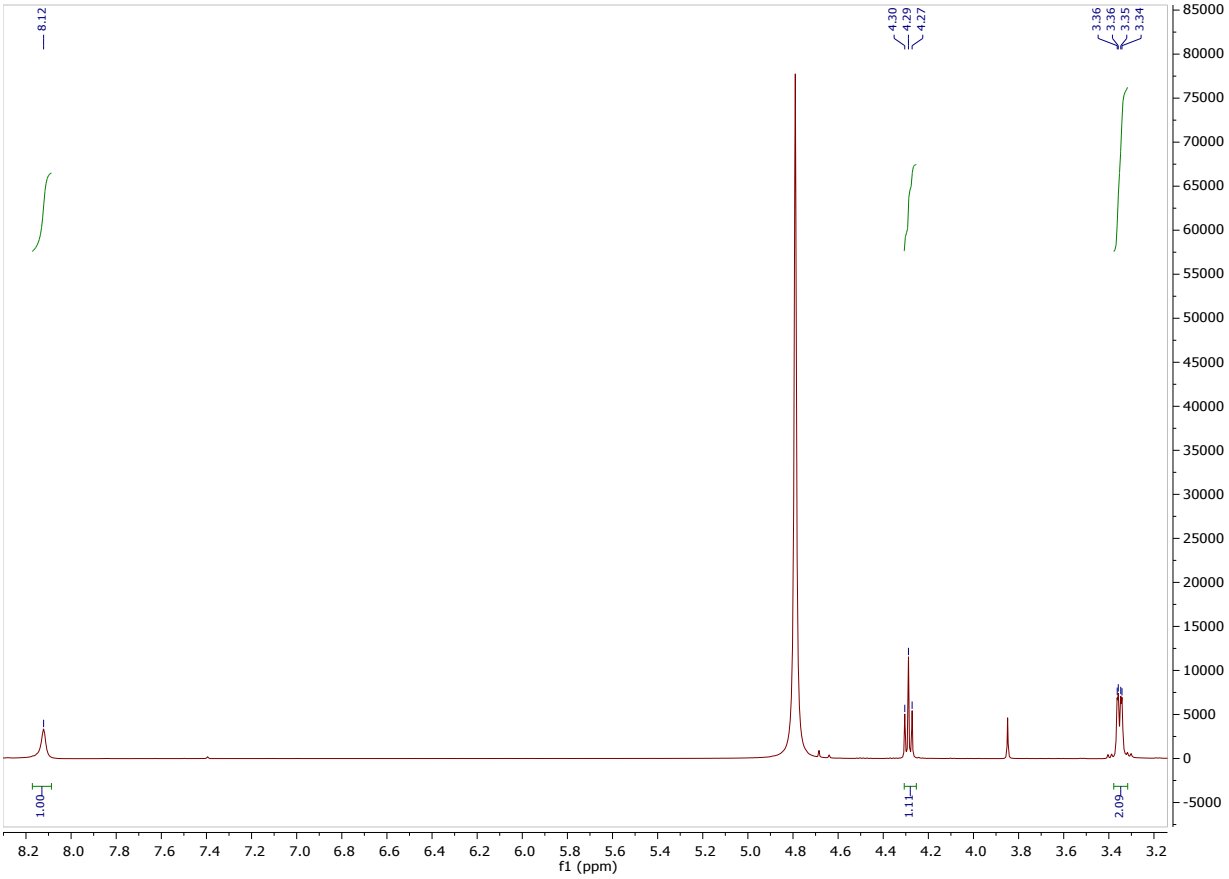

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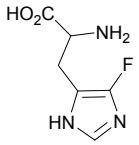

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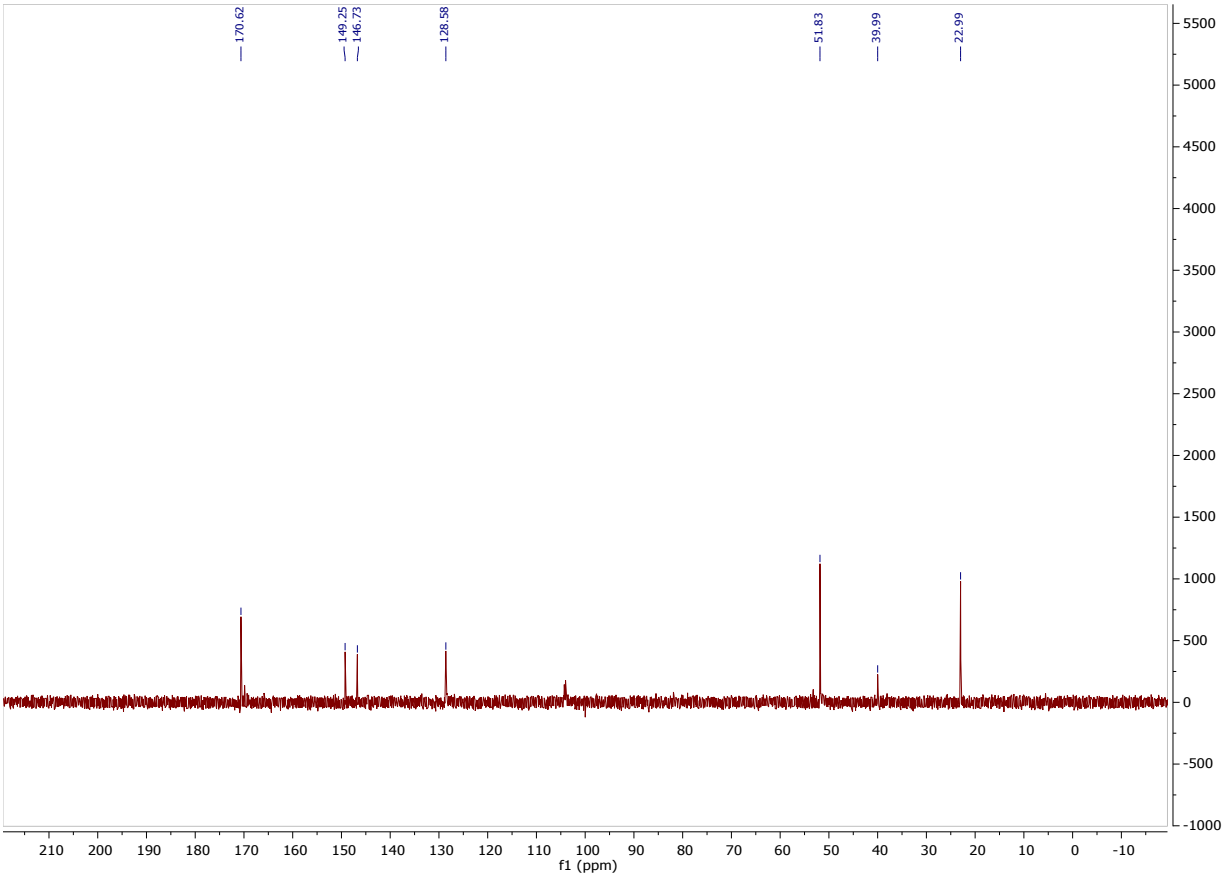

5. References

1. M. C. Hartman, K. Josephson and J. W. Szostak, *Proc. Natl. Acad. Sci. U. S. A.,* 2006, 103, 4356-4361.