# Supplementary Information for

# The Microenvironment and p$K_a$ Perturbation of Aminoacyl-tRNA Guided the Selection of Cationic Amino Acids

Bibhas Hazra, Mahesh Prasad, Rajat Roy and Pradip K. Tarafdar*

*Corresponding author: Dr. Pradip Kumar Tarafdar

Email: tarafdar@iiserkol.ac.in, srpradip@gmail.com

## Table of Contents

Experimental procedures 2

General procedure for Boc-protection of amino acid (**GP1**) 2

General procedure for the formation of ester bond (**GP2**) 2

General procedure for Boc-deprotection of amino acid (**GP3**) 2

General procedure for Fmoc-deprotection of amino acid (**GP4**) 3

General procedure for the synthesis of reference model peptide (**GP5**) 3

Procedure for synthesis of aa-tRNA mimics and reference model peptides (**Figure S1-S43**) 4

UV-spectrophotometric analysis 25

UV-kinetics regarding crowding effect and effect of tris-HCl (**Figure S44**) 25

UV-kinetics regarding role of hydrophobic interaction and effect of organic solvent (**Figure S45**) 26

Time dependent $^1$H-NMR measurements of the cyclopentyl and octyl mimic (**Figure S46-S48**) 27

**Table S1**: Obtained initial rate from the kinetic data of degradation of cyclopentyl and octyl mimic vs. time (h) 28

Supporting evidence of lactam formation under physiological condition (**Figure S49**) 29

Procedures of the model peptide forming reaction 30

Determination of the critical aggregate concentration (CAC) (**Figure S50**) 30

Model peptide formation in phosphate buffer (**Figure S51**) 31

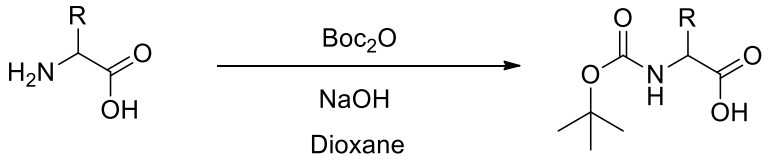
Model peptide forming reaction in tris-HCl and phosphate buffer for Dab and Dpr mimic (**Figure S52**) 32

$^1$H and $^{31}$P-NMR experiments to understand the role of phosphate (**Figure S53)** 32

IR spectra of Lys octyl mimic with $Na_2HPO_4$ (**Figure S54**) 33

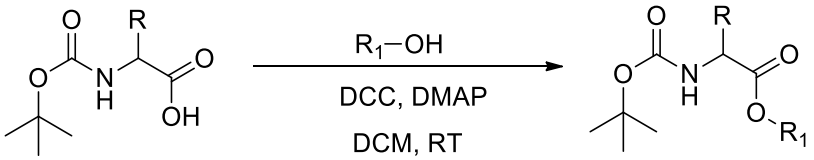
**Experimental Procedures:**

**General procedure for Boc-protection of amino acid (GP1):**



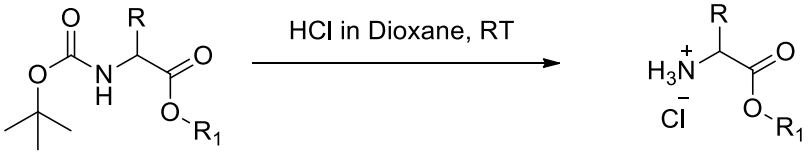

A solution of L-amino acid (1.0 eq.) in a mixture of dioxane (10 mL), water (10 mL) and NaOH (2.2 eq. for lysine and its lower analogues and 1.2 eq. for leucine) was stirred in an ice-water bath. Di-*tert*-butylpyrocarbonate (2.2 equiv) was added very slowly and stirring was continued at room temperature for 12hrs. Then, the solution was concentrated in vacuum to remove the dioxane, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2–3. The aqueous phase was extracted with ethyl acetate three times. The ethyl acetate extracts were pooled, washed with brine solution, and dried over anhydrous $Na_2SO_4$ and evaporated in a rotary evaporator under reduced pressure to obtain the product.

**General procedure for the formation of ester bond (GP2):**




Free Carboxylic acid containing compound (1 eq.) was dissolved in 20 mL of dry DCM in an ice-water bath. Dicyclohexylcarbodiimide (DCC) (1.2 eq.) and DMAP (0.2 eq.) was added to the reaction mixture followed by the corresponding alcohol (1.2 eq.). The reaction mixture was stirred for 20 hrs at room temperature under nitrogen atmosphere. After completion, first DCM was evaporated, cold ethyl acetate (around 50 mL) was added to the residue and dicyclohexyl urea was filtered off. The filtrate was evaporated in a rotary evaporator under reduced pressure to yield desired compound. The product was purified in silica gel (60-120 or 100-200 mesh) using *n*-hexane-ethyl acetate as eluent.

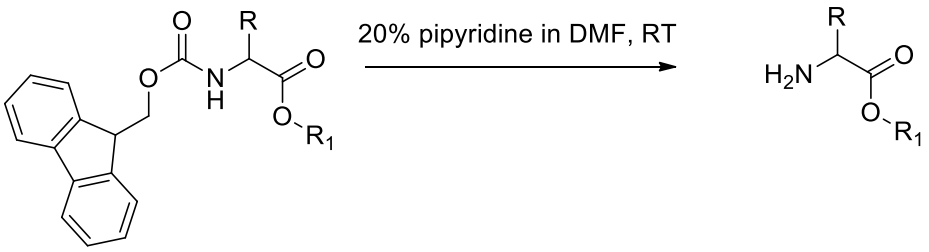
**General procedure for Boc-deprotection of amino acid (GP3):**




To Boc-protected compound (1.0 eq.), 5 mL of HCl in dioxane (~4M) was added, and the removal of Boc-group was monitored by thin layer chromatography (TLC). After ~6hrs, the

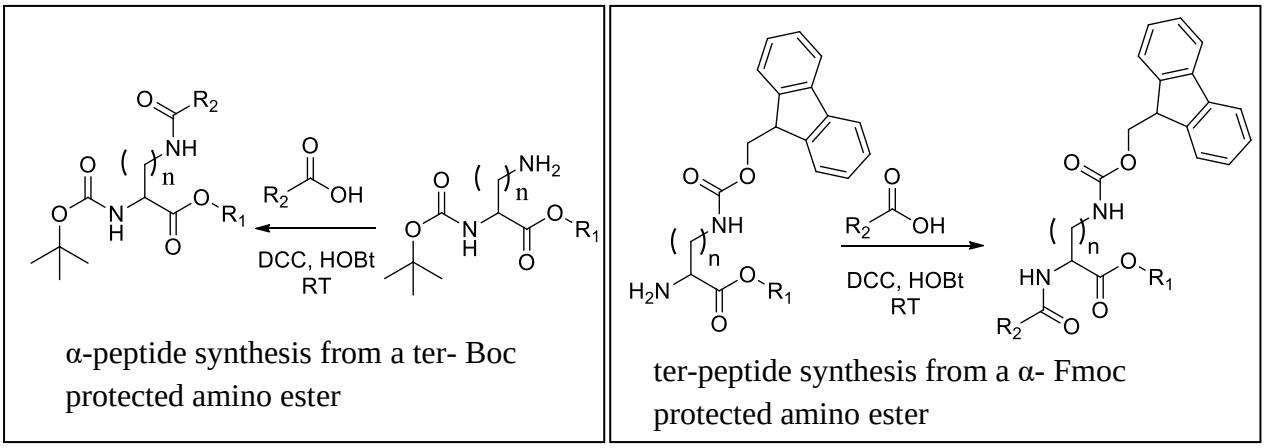
white precipitate was filtered with Whatmann 1 filter paper, washed with diethyl ether and finally dried under vacuum to get the pure deprotected product as a white solid.

**General procedure for Fmoc-deprotection of amino acid (GP4):**




To Fmoc protected compound (1.0 eq.), 2 mL of piperidine and 8 mL of dry DMF were added and the removal of the Fmoc group was monitored by TLC. After ~10 hrs the reaction mixture was taken out and around 50 mL of ethyl acetate was added to it. The DMF was removed after washing the organic layer with ice cold water. The organic layer was finally washed with brine solution, dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure to obtain the crude product. The obtained product was purified through column chromatography using basic alumina as the stationary phase and *n*-hexane-ethyl acetate as the eluent.

**General procedure for the synthesis of reference model peptide (GP5):**




α-peptide synthesis from a ter- Boc protected amino ester

ter-peptide synthesis from a α- Fmoc protected amino ester

Free Carboxylic acid containing compound (1 eq.) was dissolved in 20 mL of dry DCM in an ice-water bath. Corresponding ester protected amino acid (1.2 eq.) was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.2 eq.) and HOBt (1.5 eq.) and $Et_3N$ (1.2 eq.). The reaction mixture was stirred for 48 hrs at room temperature under nitrogen atmosphere. DCM was evaporated, ethyl acetate (60 mL) was added to the residue and dicyclohexyl urea was filtered off. The organic layer was washed with 2M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulphate; and evaporated in a rotary evaporator under reduced pressure to yield the crude product. The product was purified in silica gel (100-200 mesh) using *n*-hexane-ethyl acetate as eluent.

**Procedure for synthesis of Dab phenyl mimic:**

The aa-tRNA mimic was synthesized by following **GP1** (Yield: 1.53 g, 98%), **GP2** (Yield: 467 mg, 74%), **GP3** (Yield: 120 mg, 90%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 9.06 (s, 3H), 8.37 (s, 3H), 7.43 (t, *J* = 7.8 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.23 (d, *J* = 7.9 Hz, 2H), 4.48 (t, *J* = 6.2 Hz, 1H), 3.18 – 2.91 (t, 2H), 2.44 – 2.14 (m, 2H)**. ESI-MS:** m/z 195.1165 $[M+H]^{+}$; $M_{calcd}$: 195.1128.

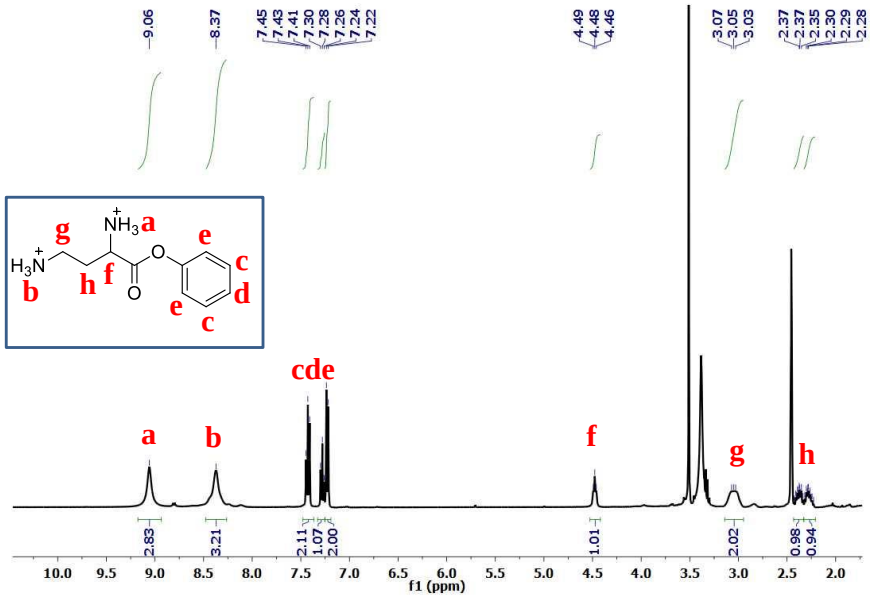



**Figure S1**: $^{1}$H-NMR of Dab phenyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 168.07, 150.26, 130.24, 127.13, 122.06, 50.31, 35.74, 28.24.

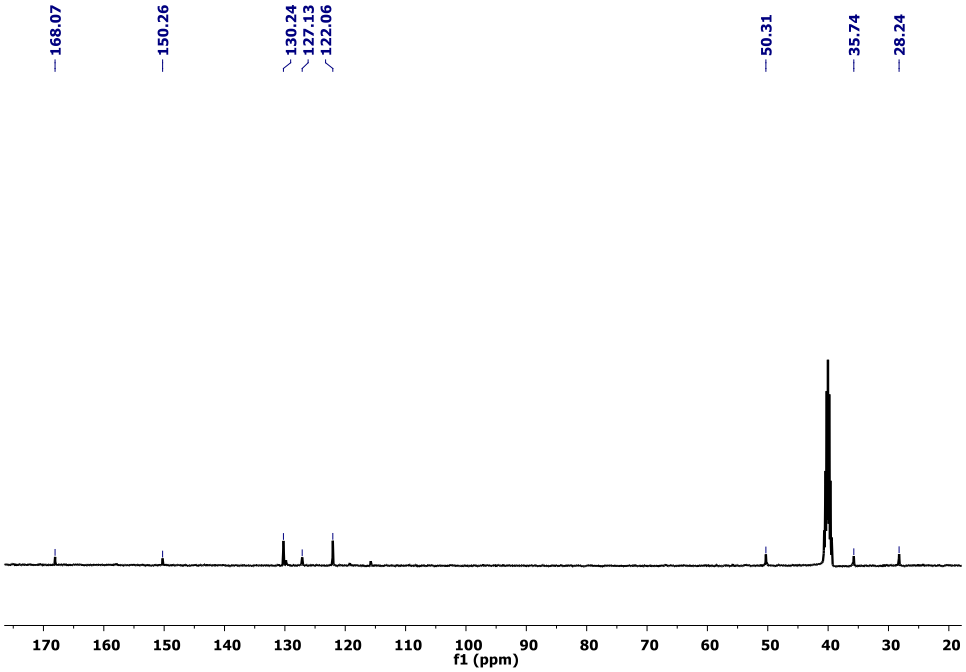



**Figure S2**: $^{13}$C-NMR of Dab phenyl mimic.

**Procedure for synthesis of Orn phenyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.92 g, 96%), **GP2** (Yield: 359 mg, 72%), **GP3** (Yield: 132 mg, 94%)sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.92 (s, 3H), 8.17 (s, 3H), 7.47 – 7.41 (t, 2H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 7.8 Hz, 2H), 4.28 (t, 1H), 2.82 (t, *J* = 5.7 Hz, 2H), 2.01 (m, 2H), 1.91 – 1.63 (m, 2H). **ESI-MS:** m/z 209.1295 $[M+H]^{+}$; $M_{calcd}$: 209.1285.

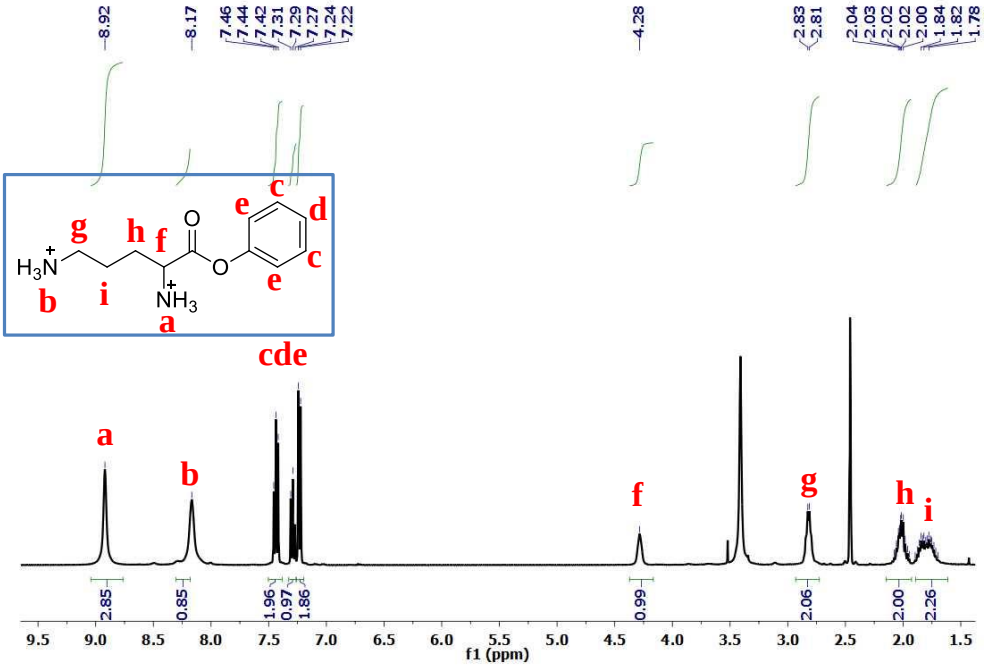



**Figure S3**: $^{1}$H-NMR of Orn phenyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 168.64, 150.28, 130.19, 127.08, 122.18, 52.07, 38.59, 27.65, 23.24.

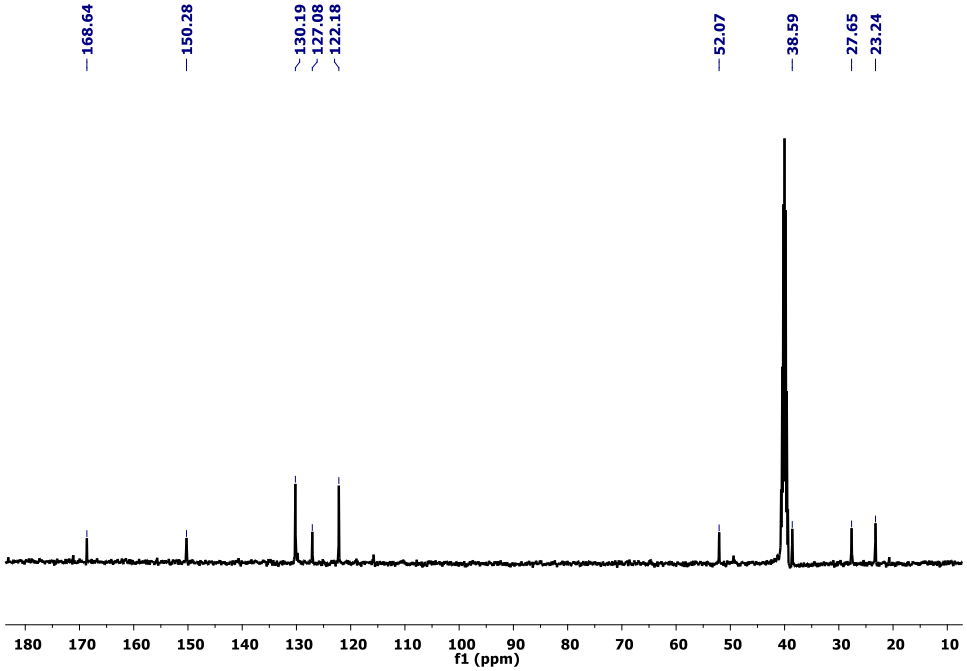



**Figure S4**: $^{13}$C-NMR of Orn phenyl mimic.

**Procedure for synthesis of Lys phenyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.76 g, 95%), **GP2** (Yield: 263 mg, 78%), **GP3** (Yield: 122 mg, 95%)sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.92 (s, 3H), 8.17 (s, 3H), 7.47 – 7.41 (t, 2H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 7.8 Hz, 2H), 4.28 (t, 1H), 2.82 (t, *J* = 5.7 Hz, 2H), 2.01 (m, 2H), 1.91 – 1.63 (m, 4H). **ESI-MS:** m/z 223.1462 $[M+H]^{+}$; $M_{calcd}$: 223.1441.

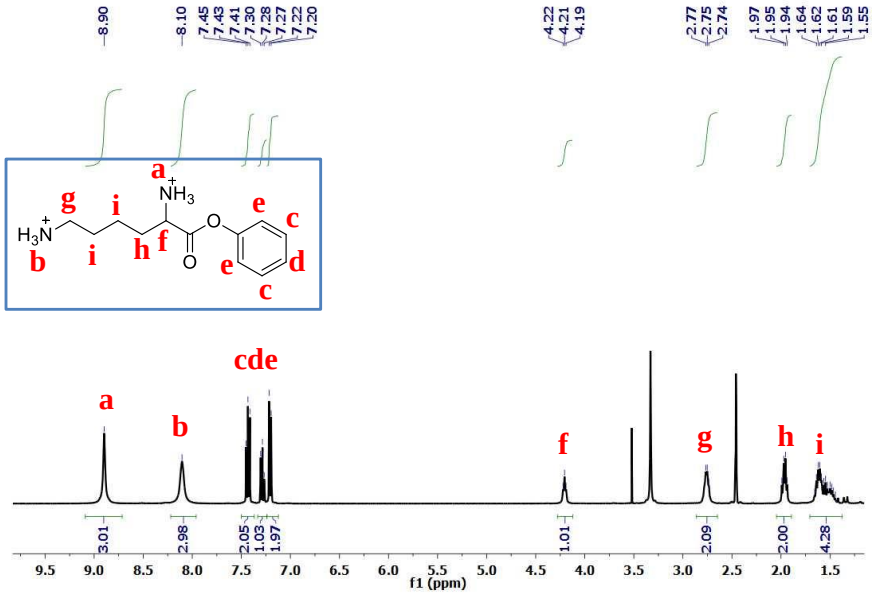



**Figure S5**: $^{1}$H-NMR of Lys phenyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 168.74, 150.30, 130.21, 127.04, 122.12, 52.37, 38.66, 29.79, 26.78, 21.88.

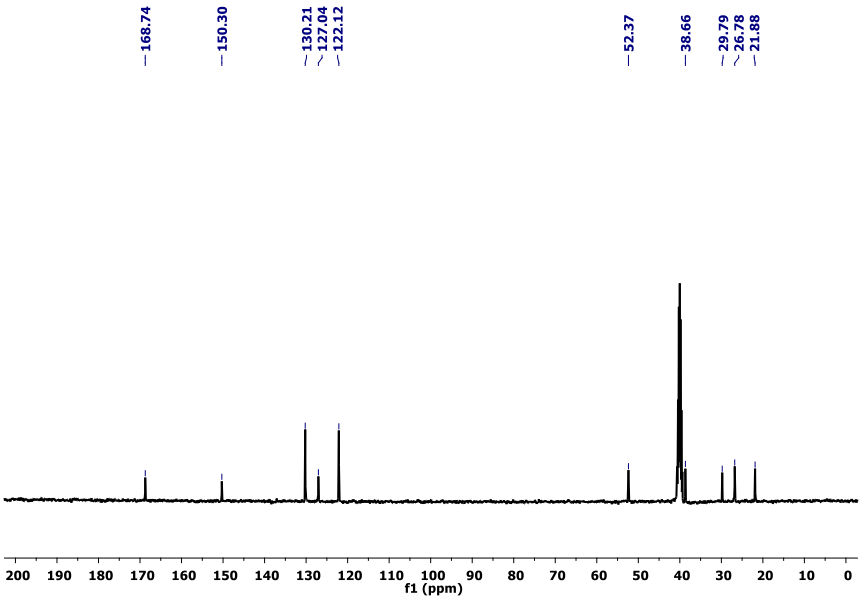



**Figure S6**: $^{13}$C-NMR of Lys phenyl mimic.

**Procedure for synthesis of Leu phenyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.85 g, 98%), **GP2** (Yield: 440 mg. 85%), **GP3** (Yield: 194 mg, 94%)sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.66 (s, 3H), 7.47 – 7.41 (t, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.9 Hz, 2H), 4.20 (t, *J*= 6.8 Hz, 1H), 1.88 – 1.72 (m, 3H), 0.95 – 0.91 (m, 6H).**ESI-MS:** m/z 208.1341 $[M+H]^{+}$; $M_{calcd}$: 208.1333.

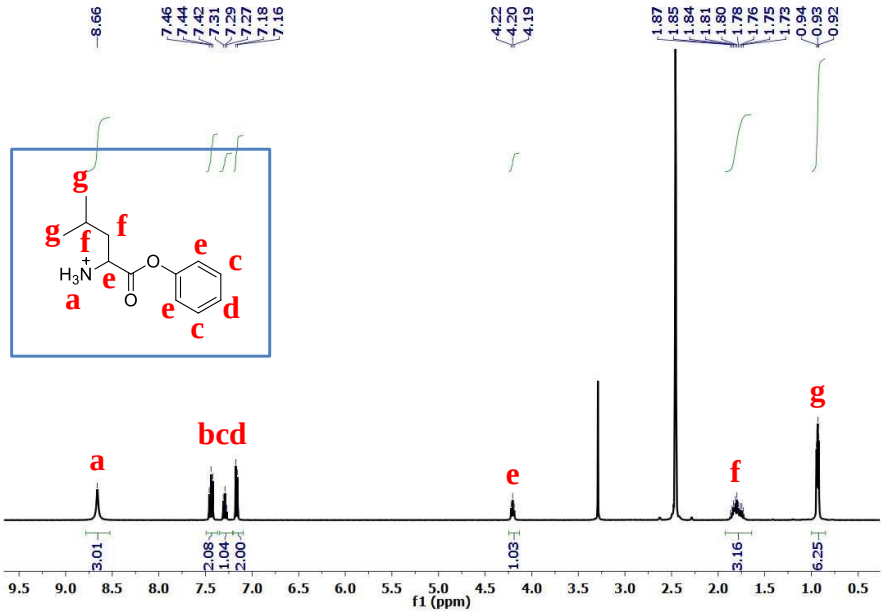



**Figure S7**: $^{1}$H-NMR of Leu phenyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.22, 150.29, 130.29, 127.07, 121.95, 51.24, 24.44, 22.70.

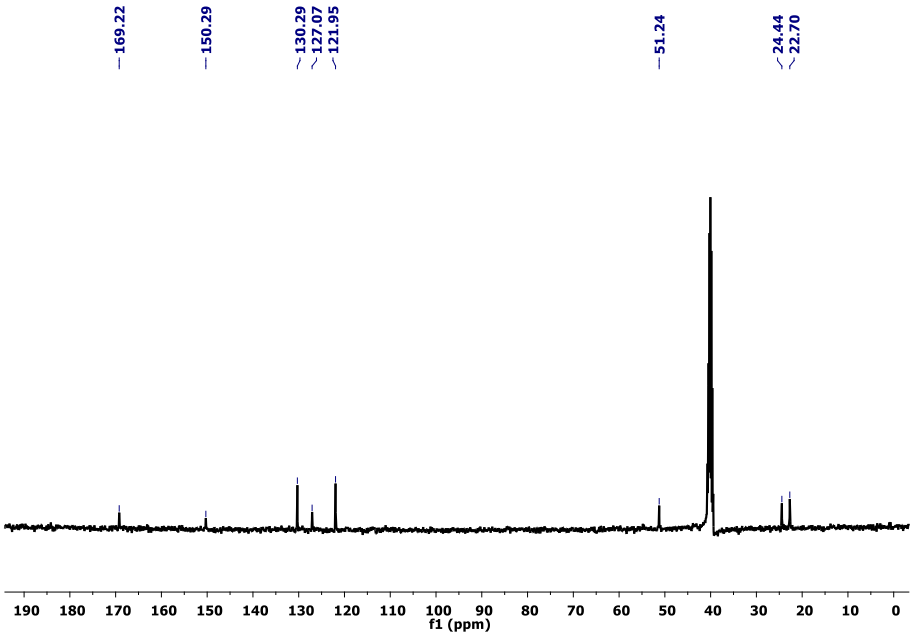



**Figure S8**: $^{13}$C-NMR of Leu phenyl mimic.

**Procedure for synthesis of Dpr cyclopentyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.6 g, 95%), **GP2** (Yield: 425 mg, 90%), **GP3** (Yield: 155 mg, 96%) sequence. $^{1}$H NMR (400 MHz, DMSO-D6) δ 8.81 (s, 6H), 5.15 (m, 1H), 4.41 – 4.19 (t, 1H), 3.41 – 3.19 (m, 2H), 1.87 – 1.59 (m, 6H), 1.60 – 1.43 (m, 2H). **ESI-MS:** m/z 173.1283 $[M+H]^{+}$; $M_{calcd}$: 173.1285.

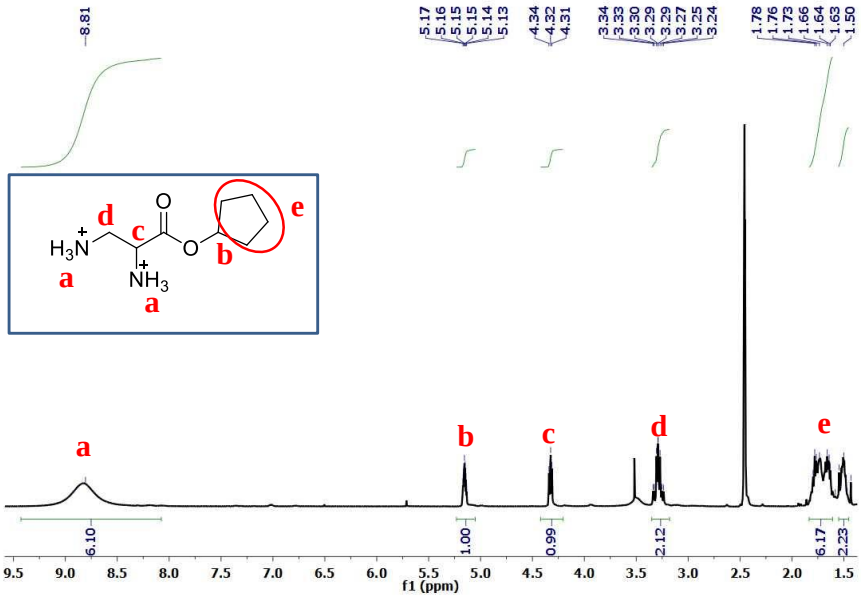



**Figure S9**: $^{1}$H-NMR of Dpr cyclopentyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 166.49, 80.32, 50.76, 38.85, 32.53, 23.80.

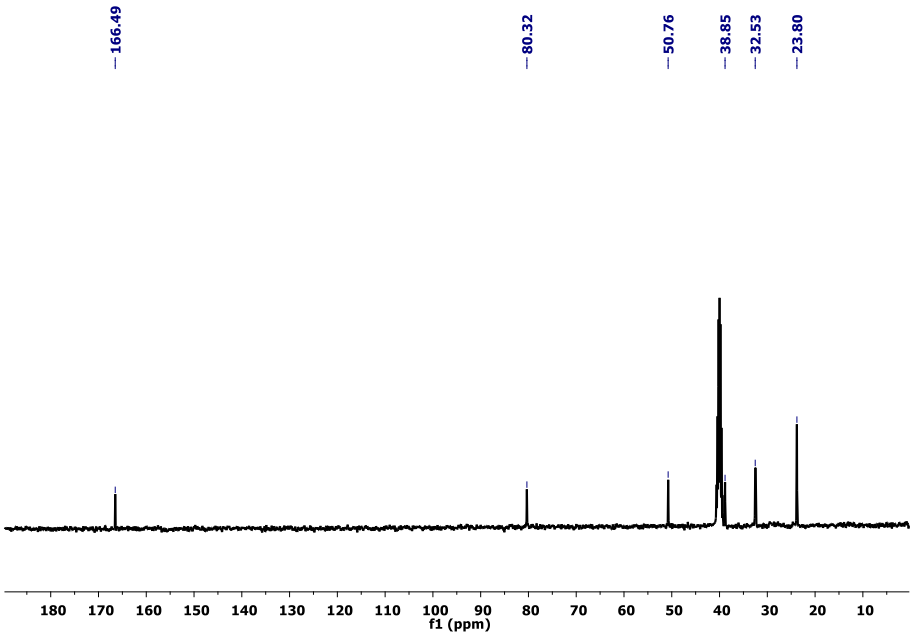



**Figure S10**: $^{13}$C-NMR of Dpr cyclopentyl mimic.

**Procedure for synthesis of Dab cyclopentyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.53 g, 98%), **GP2** (Yield: 369 mg, 92%), **GP3** (Yield: 123 mg, 93%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.68 (s, 3H), 8.34 (s, 3H), 5.15 (t, *J* = 5.5 Hz, 1H), 4.11 (t, *J*= 6.7 Hz, 1H), 3.13 – 2.75 (m, 2H), 2.24 – 1.91 (m, 2H), 1.79 (dd, *J* = 10.8, 5.7 Hz, 2H), 1.70 – 1.58 (m, 4H), 1.62 – 1.40 (m, 2H). **ESI-MS:** m/z 187.1438 $[M+H]^{+}$; $M_{calcd}$: 187.1441.

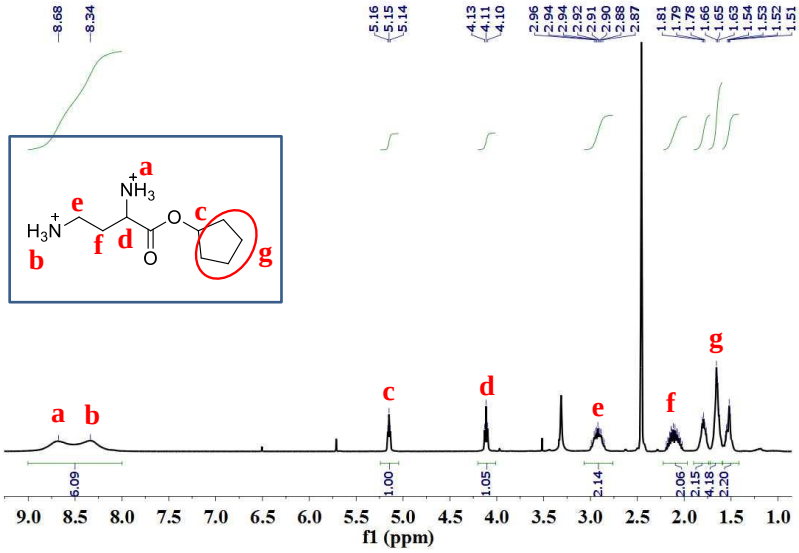



**Figure S11**: $^{1}$H-NMR of Dab cyclopentyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 168.81, 79.53, 50.17, 35.71, 32.61, 28.28, 23.75.

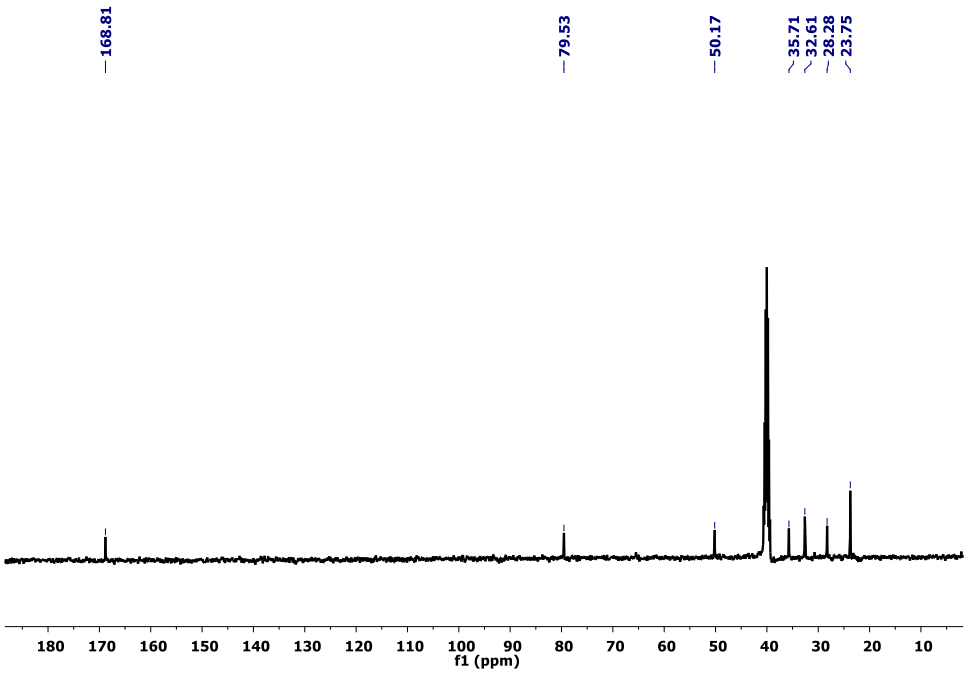



**Figure S12**: $^{13}$C-NMR of Dab cyclopentyl mimic.

**Procedure for synthesis of Orn cyclopentyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.92 g, 96%), **GP2** (Yield: 351 mg, 85%), **GP3** (Yield: 135 mg, 94%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.65 (s, 3H), 8.16 (s, 3H), 5.13 (t, *J* = 5.7 Hz, 1H), 3.90 (t, *J* = 6.0 Hz, 1H), 2.73 (t, 2H), 1.79 (m, 4H), 1.73 – 1.57 (m, 6H), 1.52 (m, 2H). **ESI-MS:** m/z 201.1599 $[M+H]^{+}$; $M_{calcd}$: 201.1598.

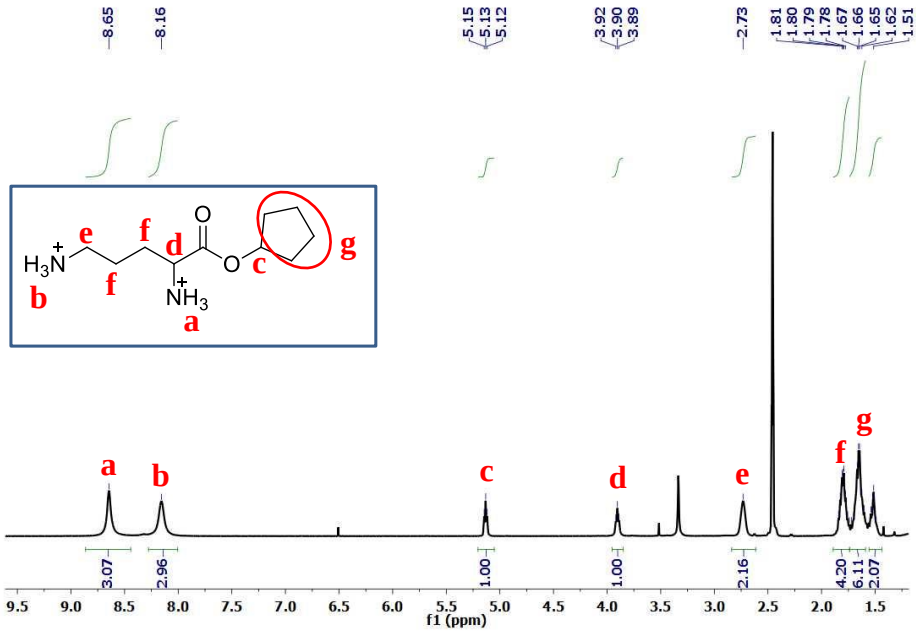



**Figure S13**: $^{1}$H-NMR of Orn cyclopentyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.38, 79.26, 51.88, 38.50, 32.57, 27.63, 23.80, 23.21.

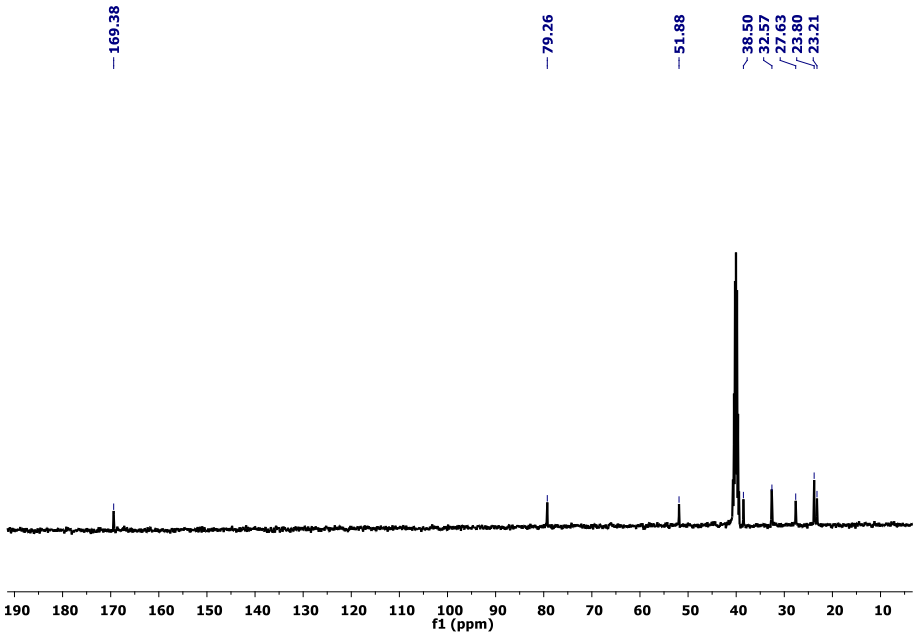



**Figure S14**: $^{13}$C-NMR of Orn cyclopentyl mimic.

**Procedure for synthesis of Lys cyclopentyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.76 g, 95%), **GP2** (Yield: 315 mg, 91%), **GP3** (Yield: 134 mg, 95%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.63 (s, 3H), 8.14 (s, 3H), 5.13 (t, *J* = 5.6 Hz, 1H), 3.83 (t, *J*= 6.1 Hz, 1H), 2.87 – 2.52 (m, 2H), 1.78 (m, 4H), 1.62 (m, 4H), 1.58 – 1.48 (m, 4H), 1.47 – 1.37 (m, 1H), 1.37 – 1.25 (m, 1H). **ESI-MS:** m/z 215.1765 $[M+H]^{+}$; $M_{calcd}$: 215.1754.

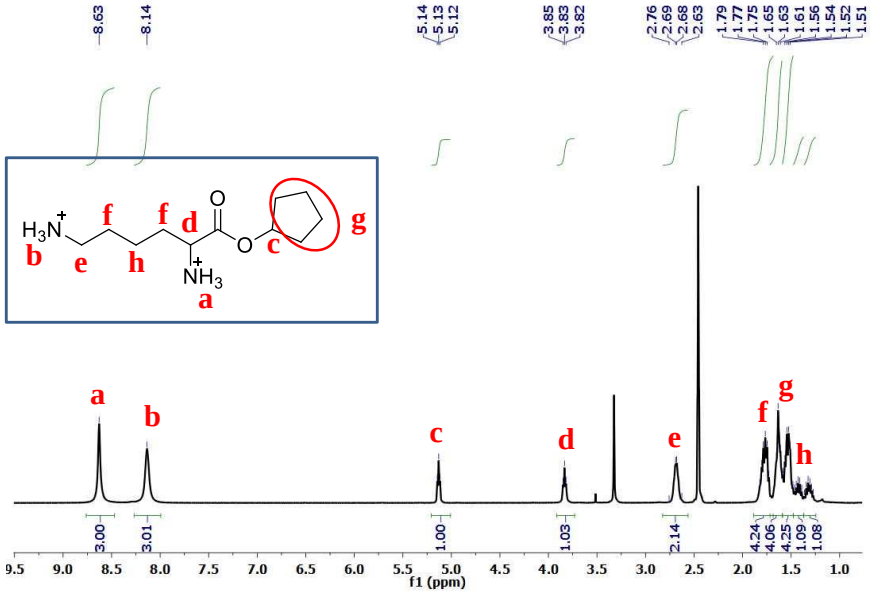



**Figure S15**: $^{1}$H-NMR of Lys cyclopentyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.45, 79.14, 52.15, 38.62, 32.53, 29.73, 26.62, 23.75, 21.75.

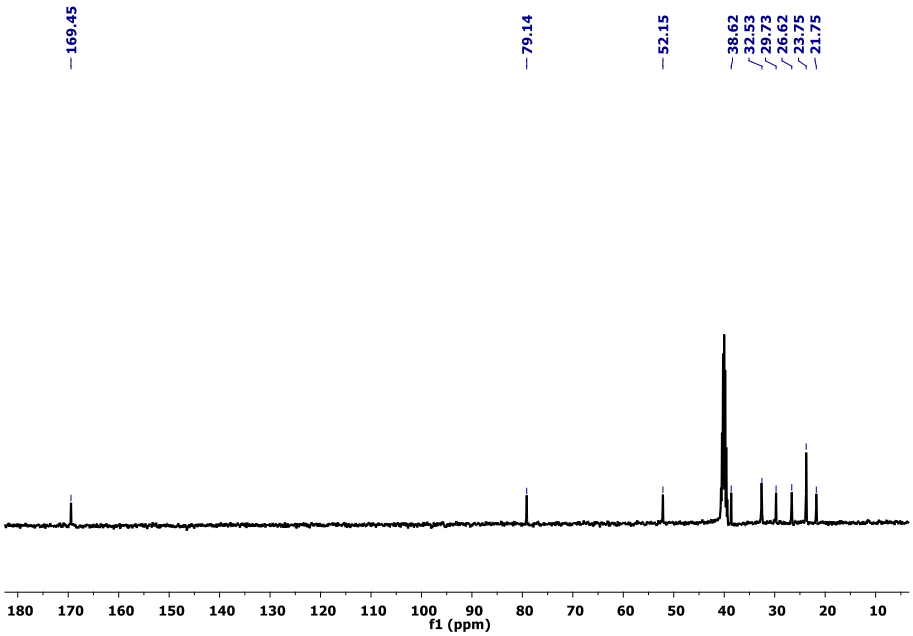



**Figure S16**: $^{13}$C-NMR of Lys cyclopentyl mimic.

**Procedure for synthesis of Leu cyclopentyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.85 g, 98%), **GP2** (Yield: 476 mg, 88%), **GP3** (Yield: 190 mg, 91%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.53 (s, 3H), 5.13 (t, *J* = 5.8 Hz, 1H), 3.80 (t, *J*= 7.1 Hz, 1H), 1.92 – 1.71 (m, 2H), 1.72 – 1.46 (m, 9H), 0.85 (dd, *J* = 6.5, 2.7 Hz, 6H). **ESI-MS:** m/z 200.1648 $[M+H]^{+}$; $M_{calcd}$: 200.1645.

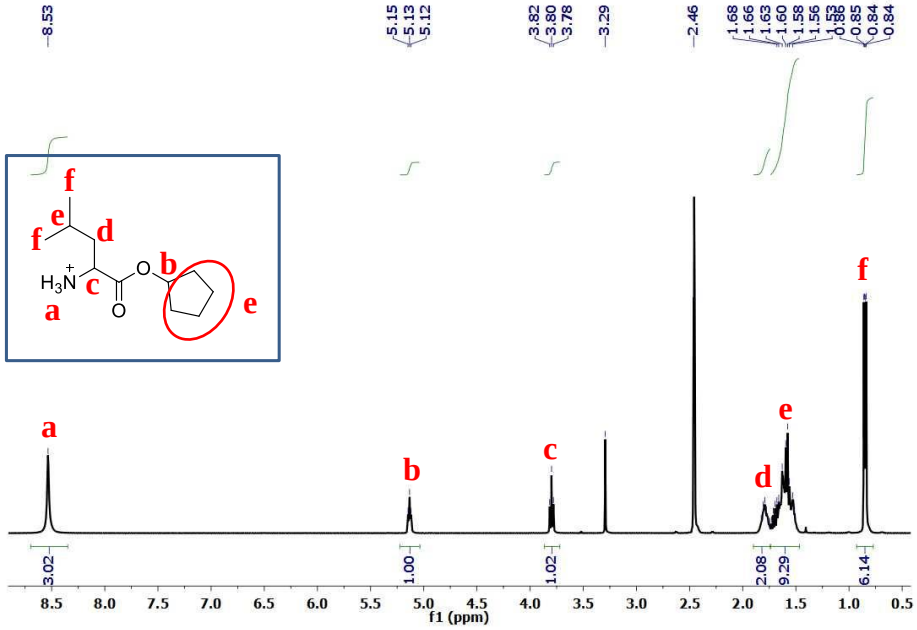



**Figure S17**: $^{1}$H-NMR of Leu cyclopentyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.94, 79.06, 51.07, 32.58, 24.41, 23.73, 22.83, 22.36.

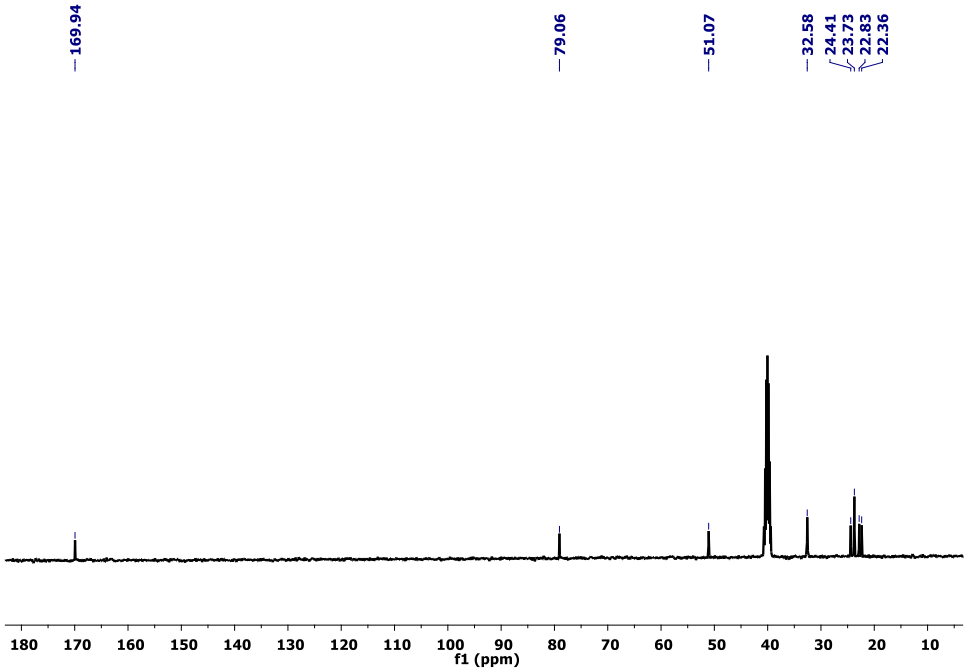



**Figure S18**: $^{13}$C-NMR of Leu cyclopentyl mimic.

**Procedure for synthesis of Dpr octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.6 g, 95%), **GP2** (Yield: 525 mg, 86%), **GP3** (Yield: 210 mg, 94%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.89 (s, 6H), 4.39 (t, *J*= 5.9 Hz, 1H), 4.25 – 3.94 (m, 2H), 3.40 – 3.13 (m, 2H), 1.69 – 1.47 (m, 2H), 1.42 – 1.06 (m, 10H), 0.81 (t, *J* = 6.8 Hz, 3H). **ESI-MS:** m/z 217.1929 $[M+H]^{+}$; $M_{calcd}$: 217.1911.

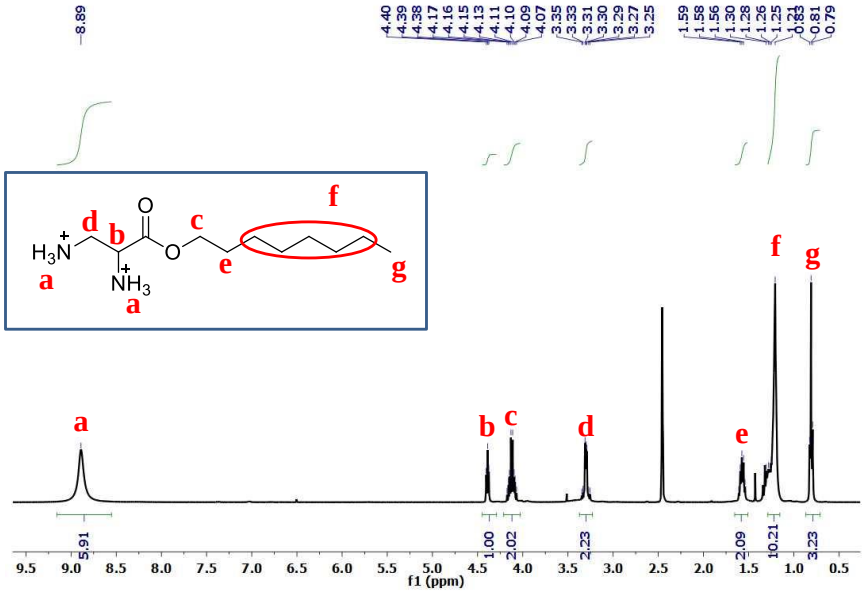



**Figure S19**: $^{1}$H-NMR of Dpr octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 166.98, 66.97, 50.64, 38.89, 31.75, 29.12, 28.29, 25.66, 22.61, 14.47.

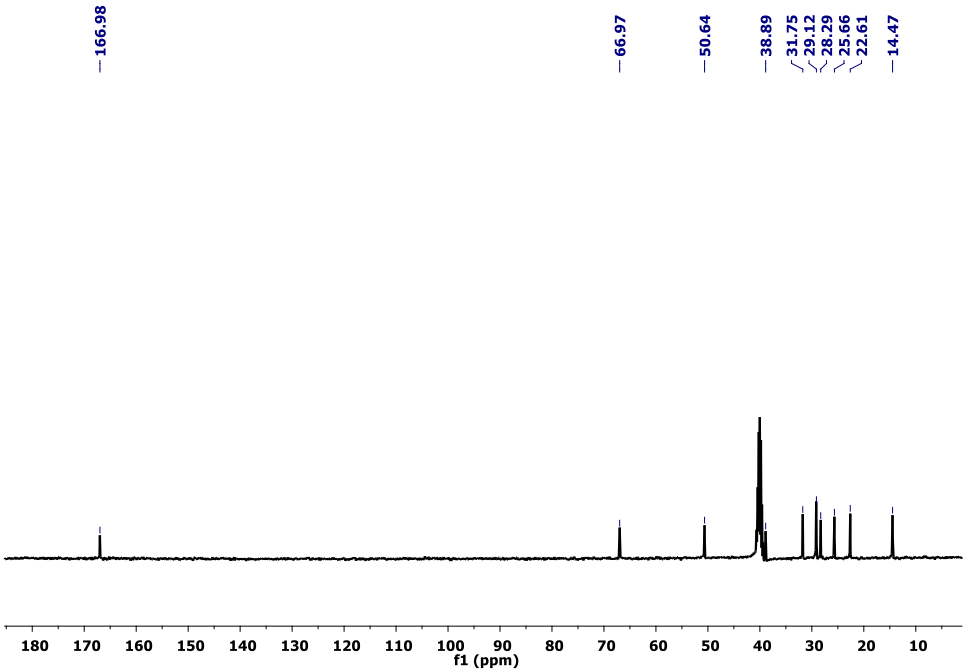



**Figure S20**: $^{13}$C-NMR of Dpr octyl mimic.

**Procedure for synthesis of Dab octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.53 g, 98%), **GP2** (Yield: 342 mg, 90%), **GP3** (Yield: 167 mg, 92%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.80 (s, 3H), 8.35 (s, 3H), 4.18 (t, 1H), 4.17 – 4.00 (m, 2H), 2.94 (t, 2H), 2.27 – 1.98 (m, 2H), 1.70 – 1.44 (m, 2H), 1.38 – 1.09 (m, 10H), 0.81 (t, *J* = 6.8 Hz, 3H).**ESI-MS:** m/z 231.2065 $[M+H]^{+}$; $M_{calcd}$: 231.2067.

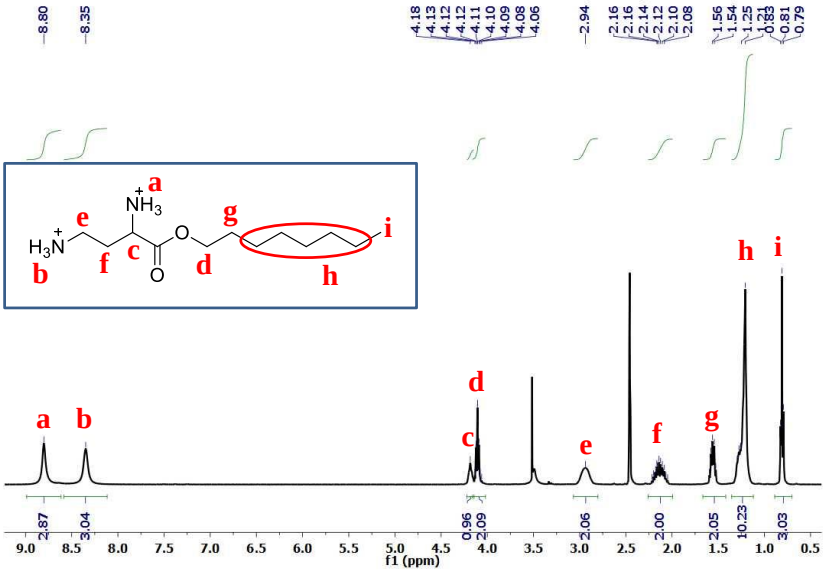



**Figure S21**: $^{1}$H-NMR of Dab octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.26, 66.38, 50.13, 35.67, 31.76, 29.10, 28.46, 28.32, 25.69, 22.60, 14.47.

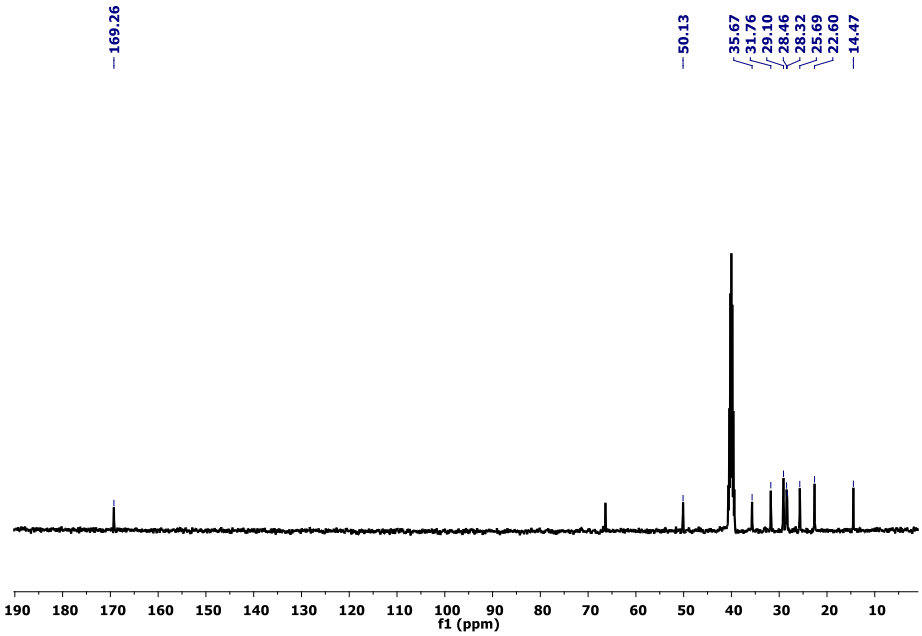



**Figure S22**: $^{13}$C-NMR of Dab octyl mimic.

**Procedure for synthesis of Orn octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.92 g, 96%), **GP2** (Yield: 431 mg, 88%), **GP3** (Yield: 178 mg, 95%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.72 (s, 3H), 8.22 (s, 3H), 4.09 (t, *J* = 6.7 Hz, 2H), 3.96 (t, *J* = 6.1 Hz, 1H), 2.73 (t, 2H), 1.89 – 1.78 (m, 2H), 1.77 – 1.51 (m, 4H), 1.34 – 1.09 (m, 10H), 0.81 (t, *J* = 6.7 Hz, 3H)**. ESI-MS:** m/z 245.2216 $[M+H]^{+}$; $M_{calcd}$: 245.2224.

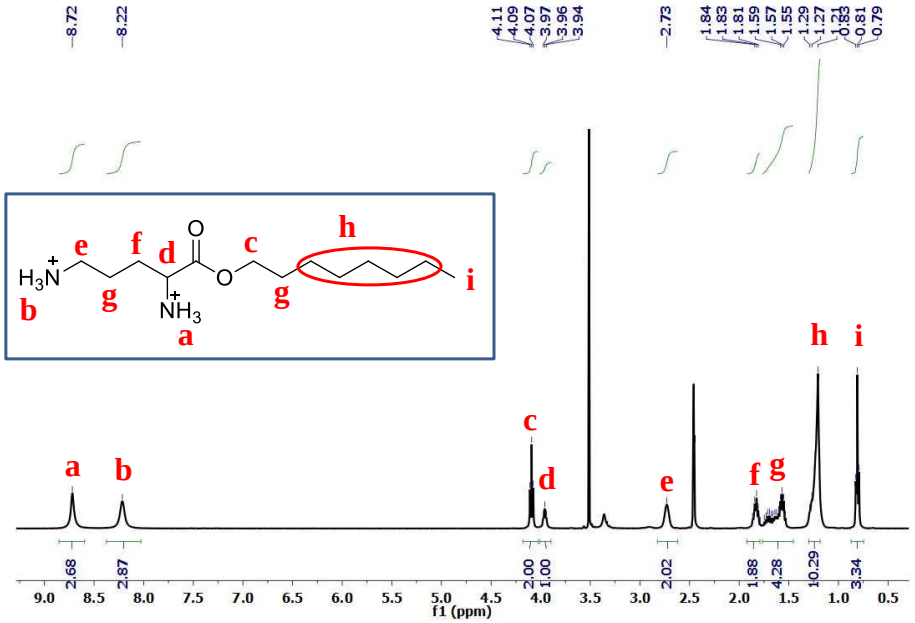



**Figure S23**: $^{1}$H-NMR of Orn octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.76, 66.18, 51.80, 38.43, 31.75, 29.12, 28.44, 27.61, 25.70, 23.16, 22.60, 14.48.

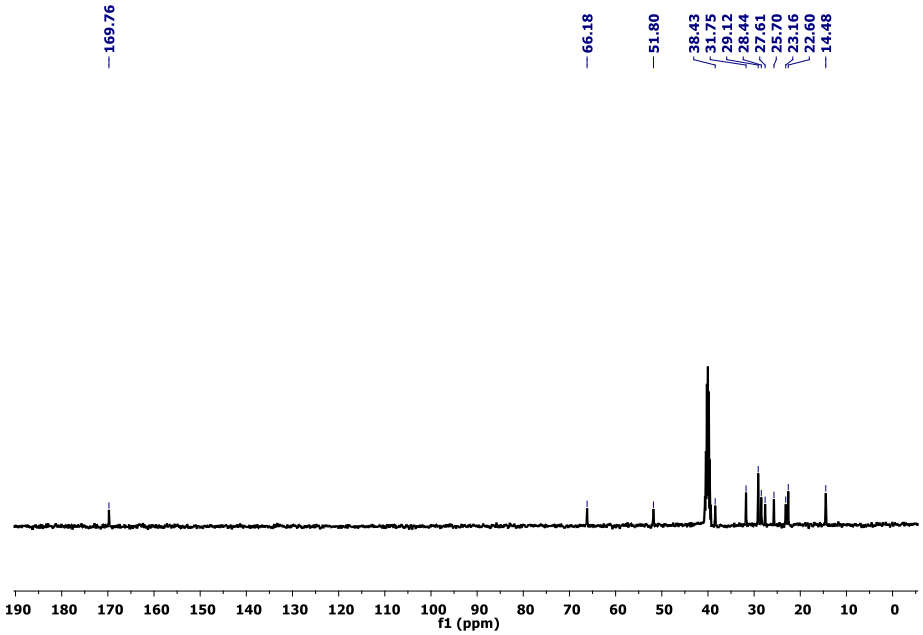



**Figure S24**: $^{13}$C-NMR of Orn octyl mimic.

**Procedure for synthesis of Lys octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.76 g, 95%), **GP2** (Yield: 527 mg, 90%), **GP3** (Yield: 213 mg, 91%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.69 (s, 3H), 8.18 (s, 3H), 4.09 (m, 2H), 3.89 (t, 1H), 2.68 (m, 2H), 1.84 – 1.71 (m, 2H), 1.61 – 1.49 (m, 4H), 1.50 – 1.29 (m, 2H), 1.29 – 1.14 (m, 10H), 0.81 (t, *J* = 6.8 Hz, 3H)**. ESI-MS:** m/z 259.2383 $[M+H]^{+}$; $M_{calcd}$: 259.2380.

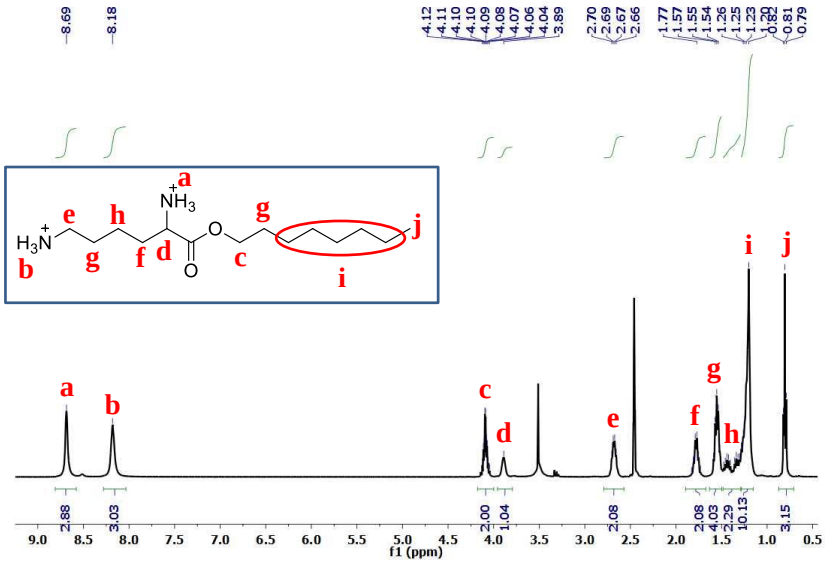



**Figure S25**: $^{1}$H-NMR of Lys octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.91, 66.10, 52.11, 38.60, 31.73, 29.76, 29.09, 28.44, 26.64, 25.71, 22.59, 21.73, 14.49.

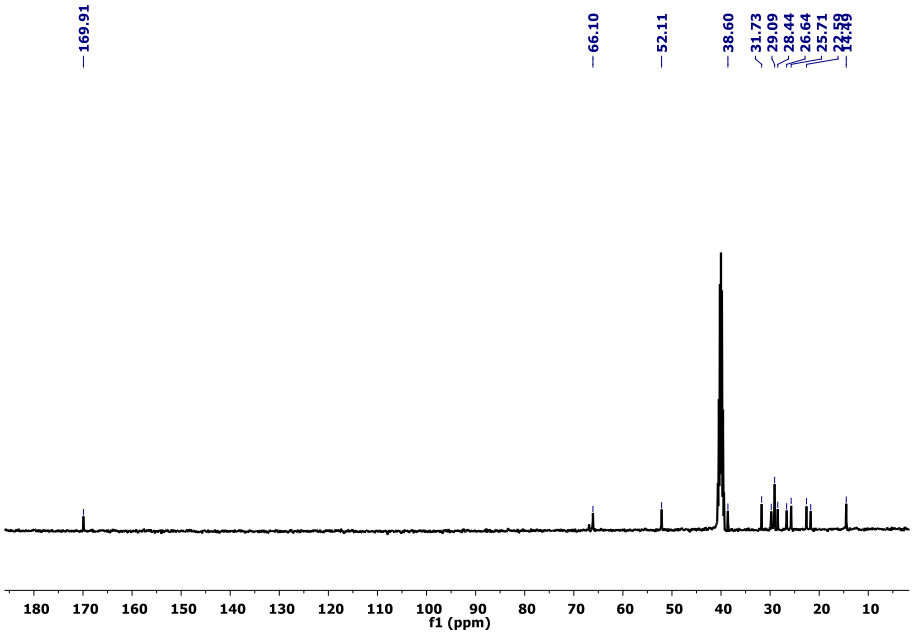



**Figure S26**: $^{13}$C-NMR of Lys octyl mimic.

**Procedure for synthesis of Leu octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.85 g, 98%), **GP2** (Yield: 525 mg, 88%), **GP3** (Yield: 180 mg, 94%) sequence.$^{1}$H NMR (400 MHz, CHLOROFORM-D) δ 8.79 (s, 3H), 4.23 – 4.09 (m, 2H), 4.06 (t, *J* = 29.5 Hz, 1H), 2.09 – 1.73 (m, 3H), 1.64 (m, 2H), 1.37 – 1.16 (m, 10H), 0.97 (d, *J* = 5.0 Hz, 6H), 0.86 (t, *J* = 6.8 Hz, 3H). **ESI-MS:** m/z 259.2383 $[M+H]^{+}$; $M_{calcd}$: 259.2380.

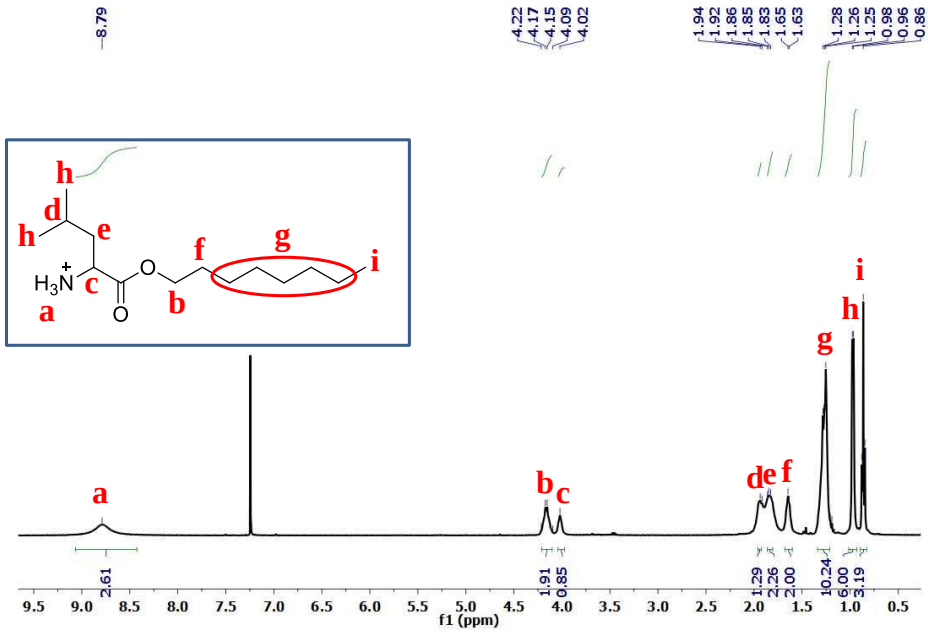



**Figure S27**: $^{1}$H-NMR of Leu octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 170.35, 65.96, 51.02, 31.70, 29.10, 28.41, 25.74, 24.33, 22.79, 22.60, 22.37, 14.43.

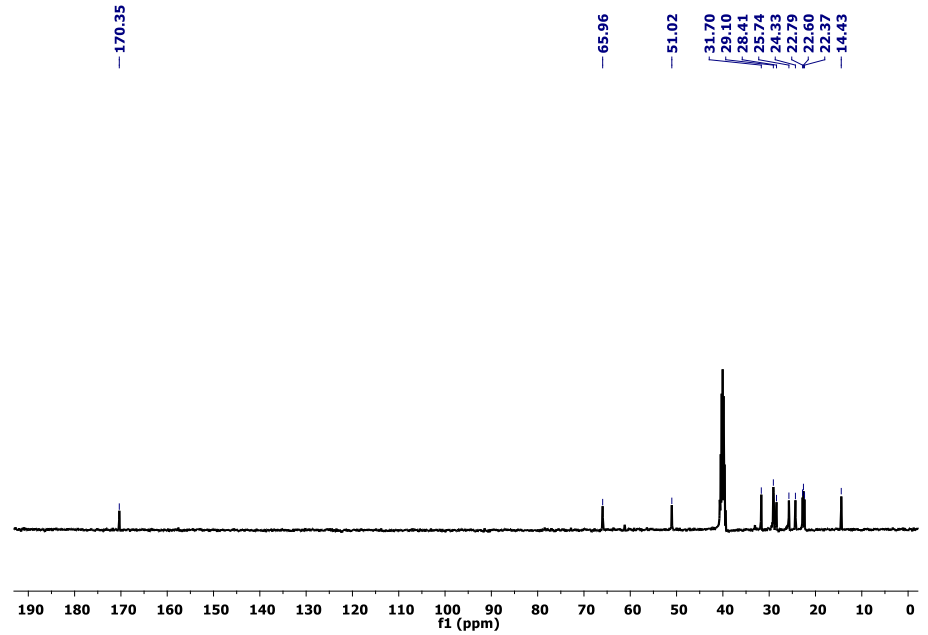



**Figure S28**: $^{13}$C-NMR of Leu octyl mimic.

**Procedure for synthesis of Orn butyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.92 g, 96%), **GP2** (Yield: 289 mg, 86%), **GP3** (Yield: 104 mg, 93%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.69 (s, 3H), 8.20 (s, 3H), 4.09 (t, *J* = 6.6 Hz, 2H), 3.94 (t, *J* = 6.2 Hz, 1H), 2.72 (t, *J* = 7.1 Hz, 2H), 1.82 (dd, *J* = 15.2, 8.0 Hz, 2H), 1.76 – 1.48 (m, 4H), 1.38 – 1.22 (m, 2H), 0.84 (t, *J* = 7.3 Hz, 3H). **ESI-MS:** m/z 189.1588 $[M+H]^{+}$; $M_{calcd}$: 189.1598.

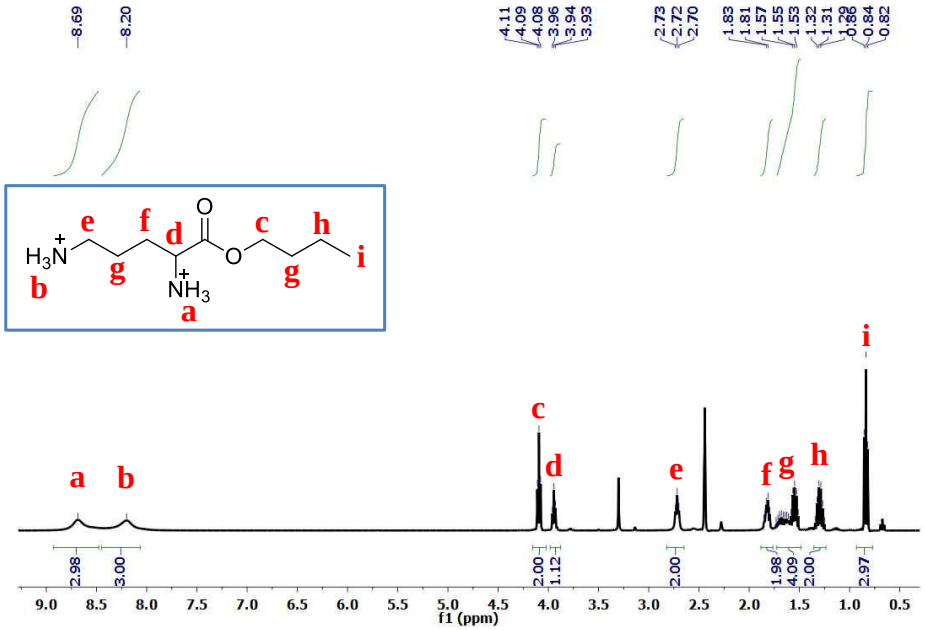



**Figure S29**: $^{1}$H-NMR of Orn butyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.78, 65.91, 51.83, 38.46, 30.48, 27.63, 23.17, 19.00, 14.08.

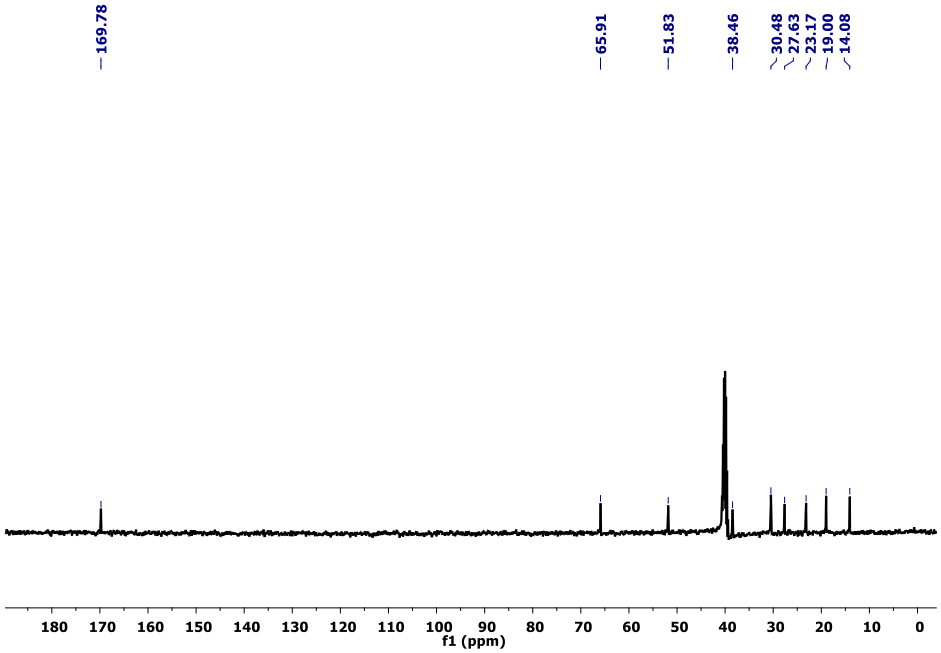



**Figure S30**: $^{13}$C-NMR of Orn butyl mimic.

**Procedure for synthesis of Lys butyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.76 g, 95%), **GP2** (Yield: 356 mg, 84%), **GP3** (Yield: 113 mg, 95%) sequence.$^{1}$H NMR (400 MHz, DMSO-D6) δ 8.48 (s, 3H), 7.88 (s, 3H), 4.07 (t, *J* = 6.5 Hz, 2H), 3.91 (t, *J* = 6.3 Hz, 1H), 2.68 (t, 2H), 1.70 (dd, *J* = 14.7, 7.5 Hz, 2H), 1.58 – 1.42 (m, 4H), 1.39 – 1.20 (m, 4H), 0.81 (t, *J* = 7.3 Hz, 3H**). ESI-MS:** m/z 203.1748 $[M+H]^{+}$; $M_{calcd}$: 203.1755.

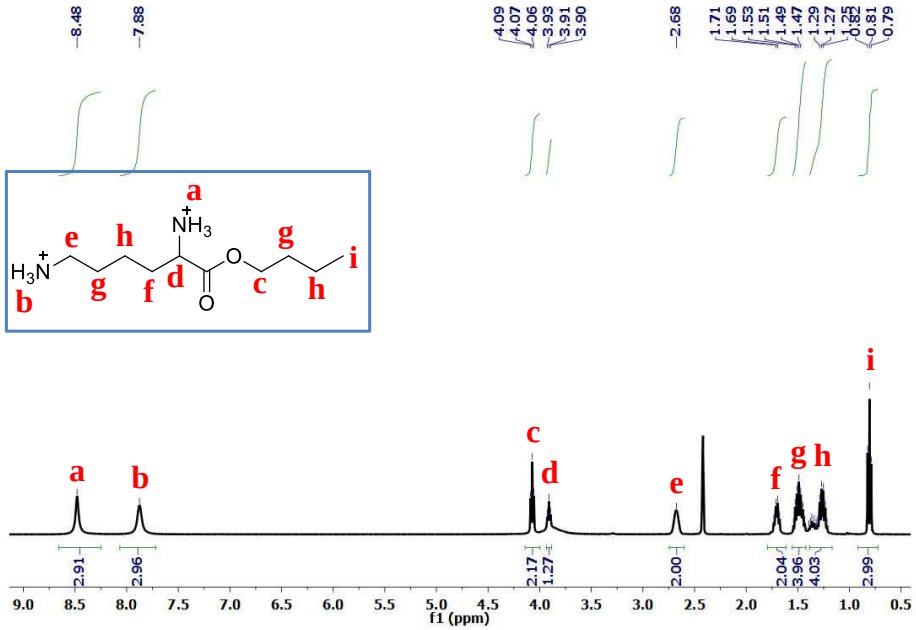



**Figure S31**: $^{1}$H-NMR of Lys butyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 169.94, 65.79, 52.23, 38.84, 30.42, 29.97, 26.85, 21.75, 18.93, 13.88.

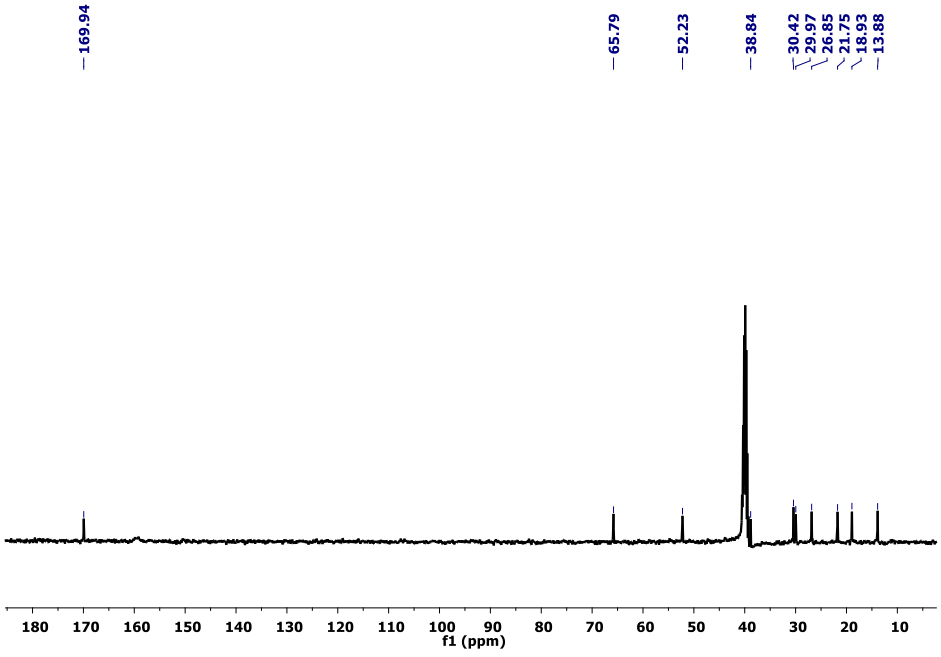



**Figure S32**: $^{13}$C-NMR of Lys butyl mimic.

**Procedure for synthesis of Arg octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.2 g, 90%), **GP2** (Yield: 610 mg, 84%), **GP3** (Yield: 225 mg, 86%) sequence.[1]H NMR (400 MHz, DMSO-D6) δ 8.55 (s, 3H), 7.86 (t, *J*= 5.7 Hz, 1H), 4.13 – 4.01 (m, 2H), 3.93 (s, 1H), 3.11 – 2.98 (m, 2H), 1.82 – 1.66 (m, 2H), 1.59 – 1.43 (m, 4H), 1.41 (s, 1H), 1.16 (s, 10H), 0.76 (t, *J* = 6.6 Hz, 3H). **ESI-MS:** m/z 287.2435 $[M+H]^+$; $M_{calcd}$: 287.2440.

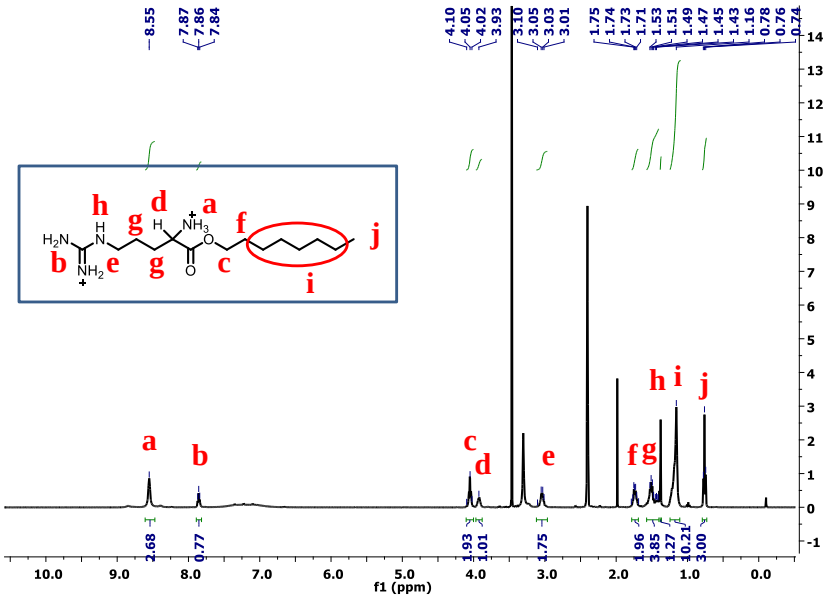



**Figure S33**: [1]H-NMR of Arg octyl mimic.

**Procedure for synthesis of His octyl mimic:**

The mimic was synthesized by following **GP1** (Yield: 1.35 g, 92%), **GP2** (Yield: 560 mg, 80%), **GP3** (Yield: 240 mg, 92%) sequence. [1]H NMR (400 MHz, CHLOROFORM-D) δ 8.85 (s, 1H), 8.69 (s, 1H), 7.52 (s, 1H), 4.63 (dt, *J* = 14.8, 7.3 Hz, 1H), 4.37 – 4.13 (m, 2H), 3.56 – 3.39 (m, 2H), 1.75 – 1.57 (m, 2H), 1.23 (s, 10H), 0.83 (t, *J* = 6.7 Hz, 3H). **ESI-MS:** m/z 268.2031 $[M+H]^+$; $M_{calcd}$: 268.2020.

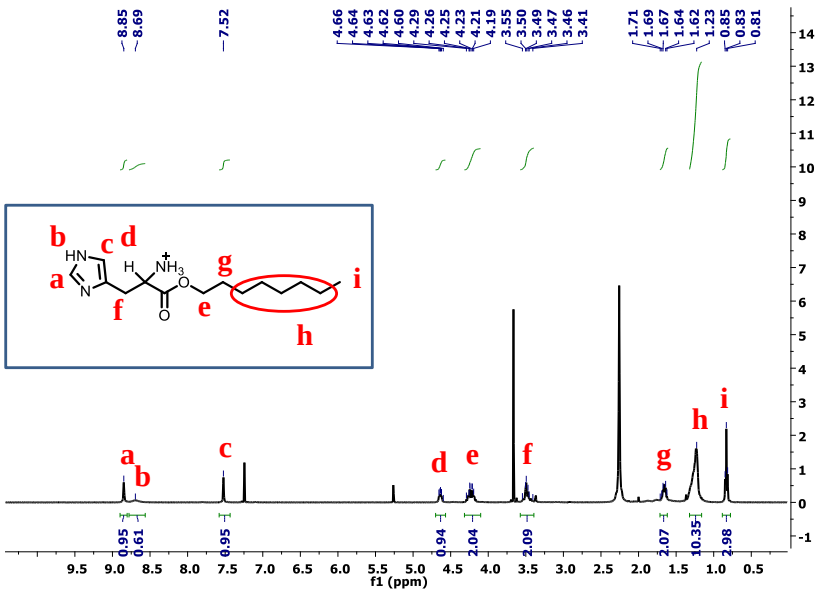



**Figure S34**: $^{1}$H-NMR of His octyl mimic.

**Procedure for synthesis of *p*-nitrophenolic ester of *n*-butyric acid:**

The said ester was synthesized by following **GP2** (Yield: 760 mg, 92%). $^{1}$H NMR (400 MHz, CHLOROFORM-D) δ 8.25 (d, *J* = 9.0 Hz, 2H), 7.26 (d, *J* = 9.1 Hz, 2H), 2.57 (t, *J* = 7.5 Hz, 2H), 1.78 (h, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). **ESI-MS:** m/z 209.0691 $[M+H]^{+}$; $M_{calcd}$: 209.0688.

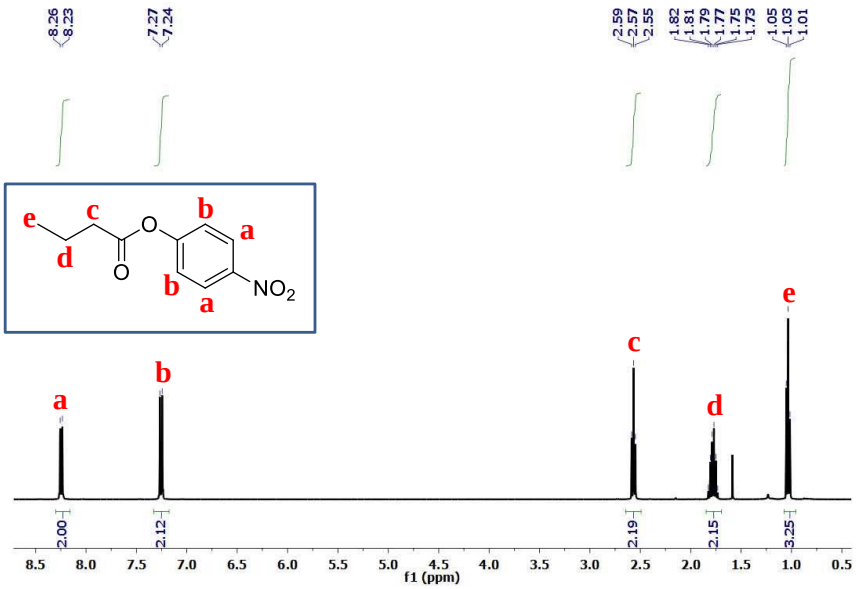



**Figure S35**: $^{1}$H-NMR of *p*-nitrophenolic ester of *n*-butyric acid (*p*-n).

**Procedure for synthesis of Orn α-butyl octyl mimic:**

The *N*-butyl mimic was synthesized by following **GP2** (Yield: 1.4 g, 82%), **GP4** (Yield: 540 mg, 88%), **GP5** (Yield: 460 mg, 78%), and **GP3** (Yield: 150 mg, 94%) sequence. $^{1}$H NMR (400 MHz, DMSO-D6) δ 8.21 (d, *J*= 7.6 Hz, 1H), 7.91 (s, 3H), 4.21 – 4.08 (m, 1H), 3.97 (m, 2H), 2.72 (m, 2H), 2.05 (t, *J* = 7.3 Hz, 2H), 1.71 (dd, *J* = 10.0, 5.0 Hz, 1H), 1.64 – 1.39 (m,

7H), 1.21 (m, 10H), 0.81 (t, *J* = 7.3 Hz, 6H). **ESI-MS:** m/z 315.2648 $[M+H]^+$; $M_{calcd}$: 315.2642.

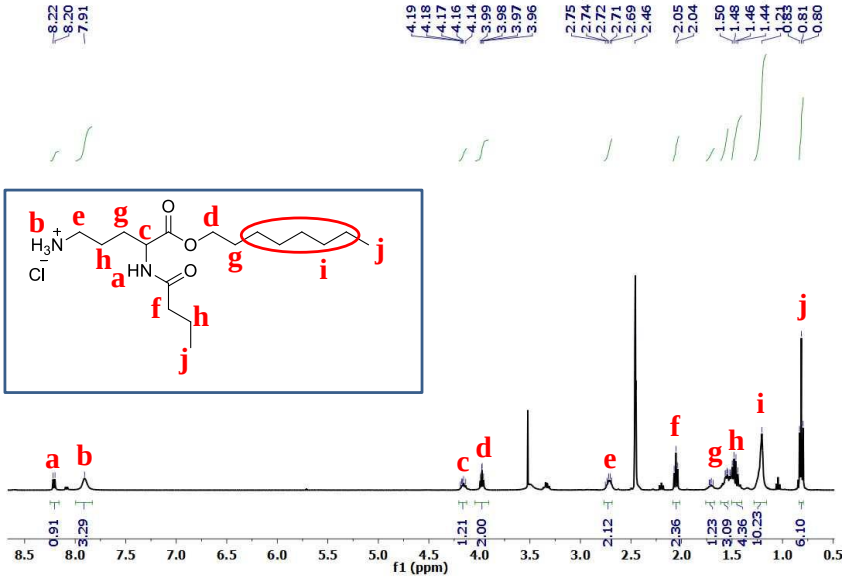



**Figure S36**: $^1$H-NMR of Orn α- butyl octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 172.70, 64.89, 52.09, 38.74, 37.41, 31.72, 29.12, 28.62, 28.20, 25.79, 24.28, 22.60, 19.17, 14.46, 14.08.

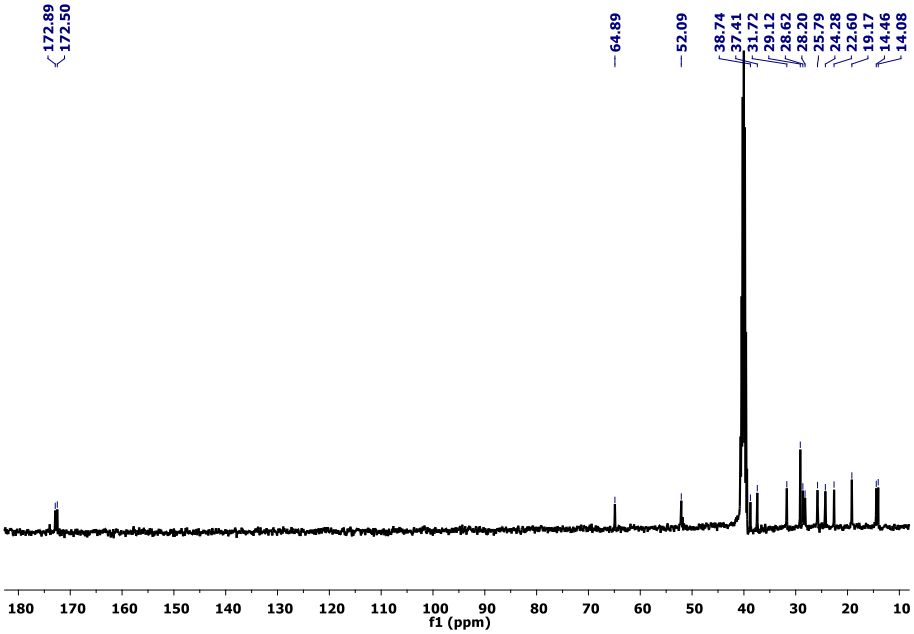



**Figure S37**: $^{13}$C-NMR of Orn α- butyl octyl mimic.

**Procedure for synthesis of Orn δ-butyl octyl mimic:**

The *N*-butyl mimic was synthesized by following **GP2** (Yield: 1.4 g, 82%), **GP3** (Yield: 468 mg, 94%), **GP5** (Yield: 356 mg, 75%), **GP4** (Yield: 105 mg, 88%), and **GP3** (Yield: 95%) sequence. $^1$H NMR (400 MHz, CHLOROFORM-D) δ 5.88 (s, 1H), 4.08 (t, *J* = 6.7 Hz, 2H), 3.42 (s, 1H), 3.36 – 3.20 (m, 2H), 2.19 – 2.06 (m, 2H), 1.84 – 1.68 (m, 2H), 1.61 (m, 6H), 1.26 (m, 10H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.86 (t, *J* = 6.7 Hz, 3H). **ESI-MS:** m/z 315.2654 $[M+H]^+$; $M_{calcd}$: 315.2642.

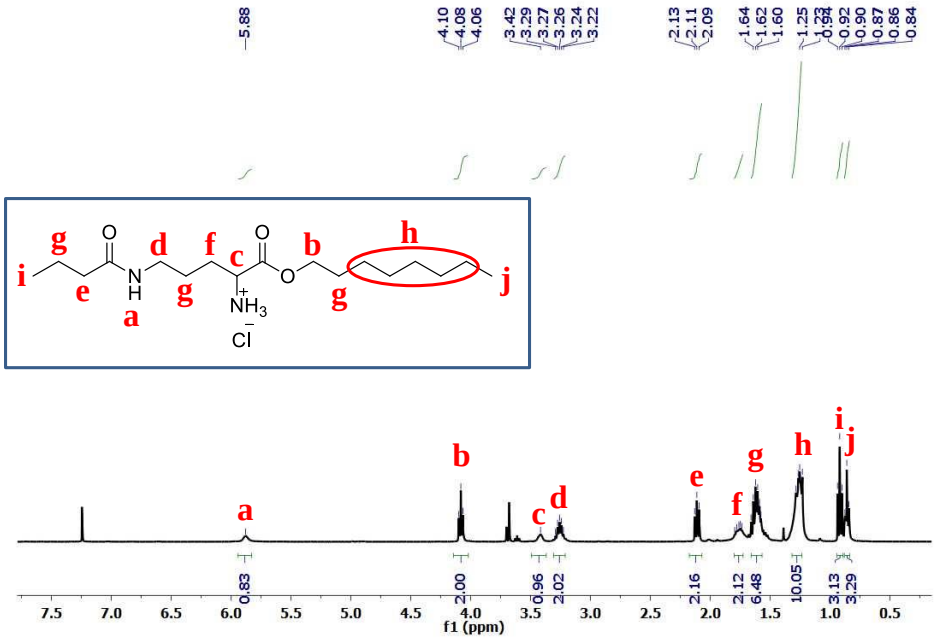



**Figure S38**: $^{1}$H-NMR of Orn δ-butyl octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 172.45, 170.89, 65.95, 52.47, 38.15, 37.90, 31.74, 29.10, 28.64, 28.50, 25.76, 25.42, 22.60, 19.23, 14.47, 14.15.

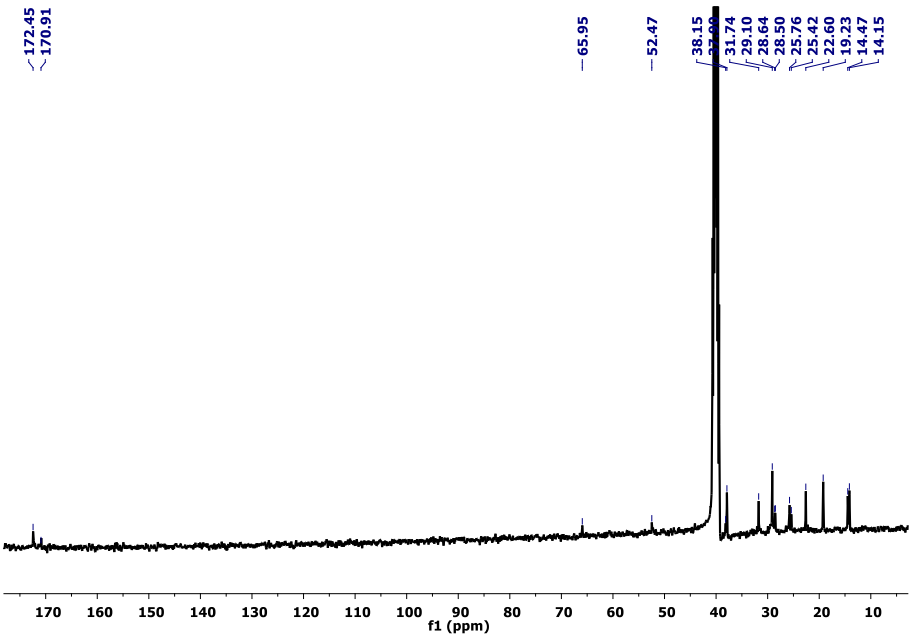



**Figure S39**: $^{13}$C-NMR of Orn δ-butyl octyl mimic.

**Procedure for synthesis of Lys α-butyl octyl mimic:**

The *N*-butyl mimic was synthesized by following **GP2** (Yield: 1.7 g, 80%), **GP4** (Yield: 650 mg, 85%), **GP5** (Yield: 535 mg, 76%), and **GP3** (Yield: 175 mg, 90%) sequence. $^{1}$H NMR (400 MHz, DMSO-D6) δ 8.17 (d, *J* = 7.4 Hz, 1H), 7.94 (s, 3H), 4.17 – 4.08 (m, 1H), 4.02 – 3.91 (m, 2H), 2.77 – 2.61 (m, 2H), 2.05 (t, *J* = 7.3 Hz, 2H), 1.68 – 1.39 (m, 10H), 1.32 – 1.17 (m, 10H), 0.88 – 0.75 (t, *J*= 7.4 Hz 6H). **ESI-MS:** m/z 329.2804 $[M+H]^{+}$; $M_{calcd}$: 329.2799.

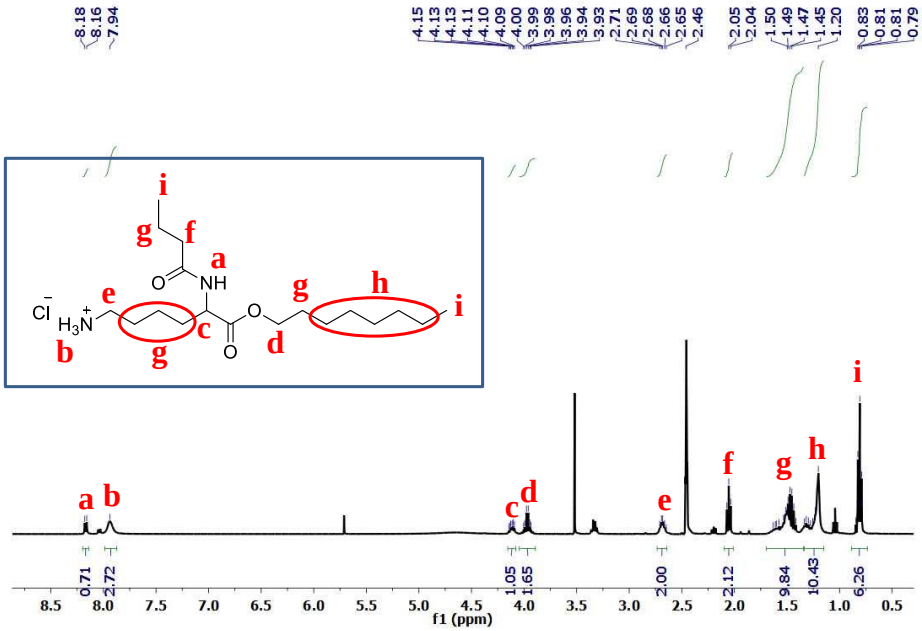



**Figure S40**: $^{1}$H-NMR of Lys α-butyl octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 174.24, 172.84, 64.78, 52.13, 38.87, 37.45, 31.70, 30.81, 29.09, 28.61, 26.95, 25.86, 22.97, 22.59, 19.20, 14.46, 14.07.

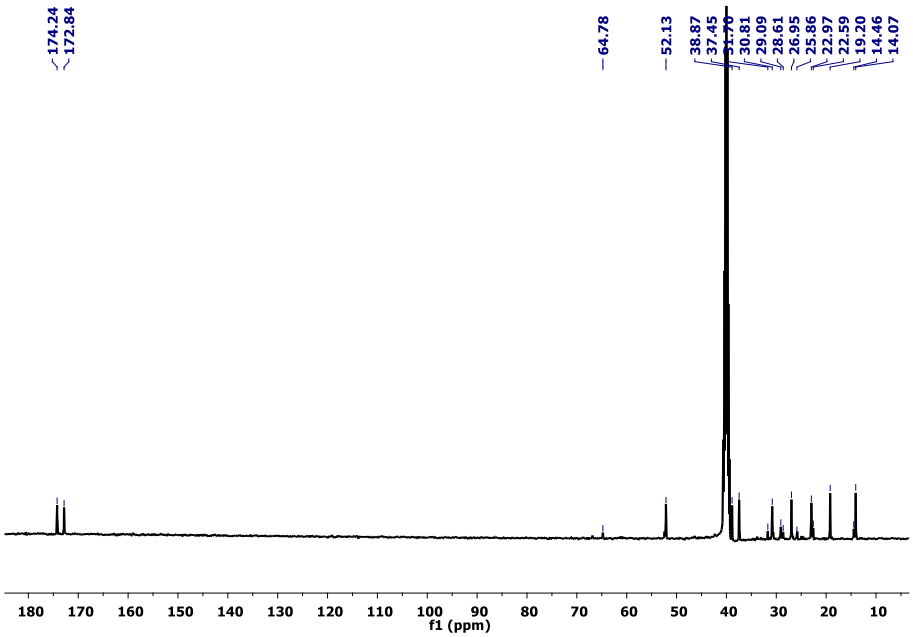



**Figure S41**: $^{13}$C-NMR of Lys α-butyl octyl mimic.

**Procedure for synthesis of Lys ε-butyl octyl mimic:**

The *N*-butyl mimic was synthesized by following **GP2** (Yield: 1.7g, 80%), **GP3** (Yield: 530 mg, 95%), **GP5** (Yield: 415 mg, 74%), **GP4** (Yield: 135 mg, 85%), and **GP3** (Yield: 93%) sequence. $^{1}$H NMR (400 MHz, CHLOROFORM-D) δ 5.59 (s, 1H), 4.07 (t, *J* = 6.8 Hz, 2H), 3.39 (dd, *J* = 7.4, 5.4 Hz, 1H), 3.22 (dd, *J* = 13.0, 6.8 Hz, 2H), 2.18 – 2.06 (m, 2H), 1.75 – 1.58 (m, 6H), 1.51 (m, 2H), 1.45 – 1.35 (m, 2H), 1.26 (m, 10H), 0.91 (t, *J* = 7.4 Hz, 3H), 0.85 (t, *J* = 6.8 Hz, 3H). **ESI-MS:** m/z 329.2799 $[M+H]^{+}$; $M_{calcd}$: 329.2799.

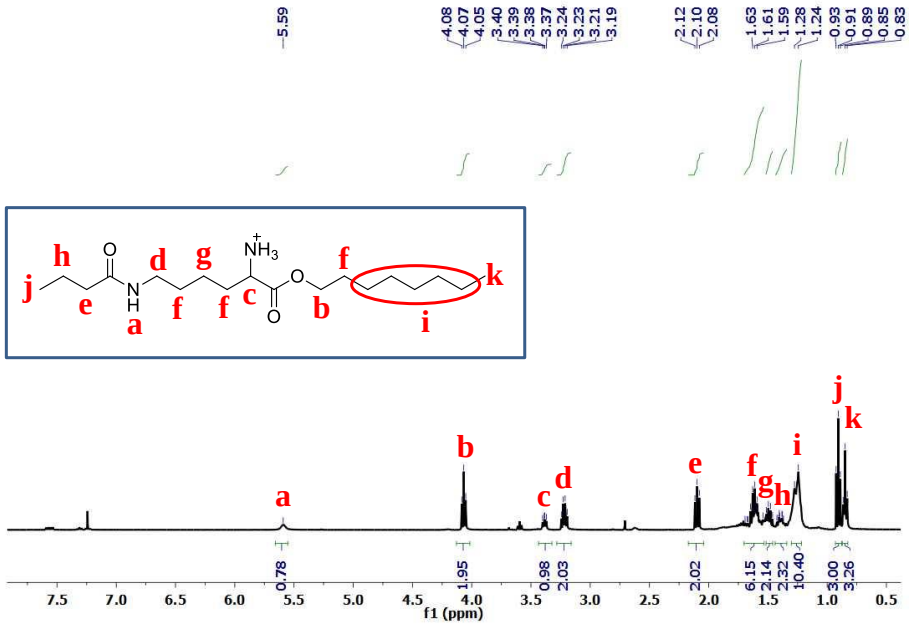



**Figure S42**: $^{1}$H-NMR of Lys ε-butyl octyl mimic.

$^{13}$C NMR (101 MHz, DMSO-D6) δ 172.26, 61.24, 48.02, 38.56, 37.90, 33.87, 33.07, 31.73, 29.10, 28.55, 25.80, 24.98, 22.60, 19.23, 14.45, 14.13.

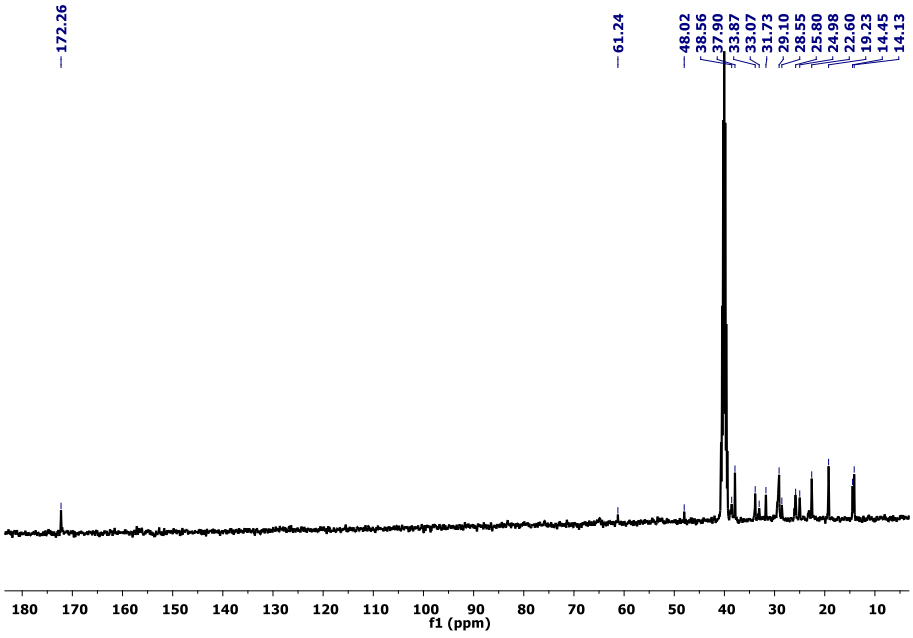



**Figure S43**: $^{13}$C-NMR of Lys ε-butyl octyl mimic.

**UV-spectrophotometric analysis**: UV- spectrophotometric analysis of aa-tRNA phenyl mimics were performed using an Epoch 2, BioTek UV-spectrophotometer. All the stock solutions & buffers were prepared using milli-Q water. Kinetic experiments at 0.2 mM concentration were carried (37 °C, 100 mM phosphate buffer pH 6.0 and 7.0) by measuring the OD at 270 nm (characteristic wavelength maximum of released phenol). All the kinetic

measurements were monitored for a period of 15 minutes. The percentage degradation was calculated from the reference standard plot of phenol (data not shown).

**UV-kinetics regarding crowding effect and effect of tris-HCl:** To rule out the effect of tris-HCl (primary amine) we performed the UV-kinetics at 37 °C by mixing 0.5 mM *p*-n in 100 mM tris without the octyl mimic (blank). Only a bit of background hydrolysis was observed in case of the blank experiment. Then to rule out the effect of molecular crowding we performed the similar kinetics by mixing 2 mM cyclopentyl mimic with 0.5 mM of *p*-n in 10% PEG-9000 (dissolved in 100 mM buffer). We found no amide formation even in presence of 10% PEG-9000, indicating no effect of molecular crowding.

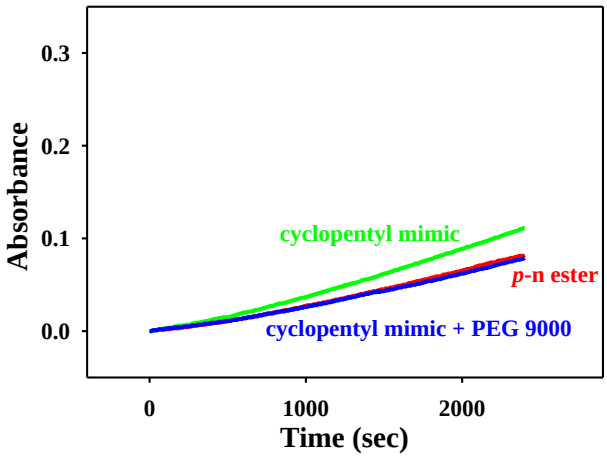
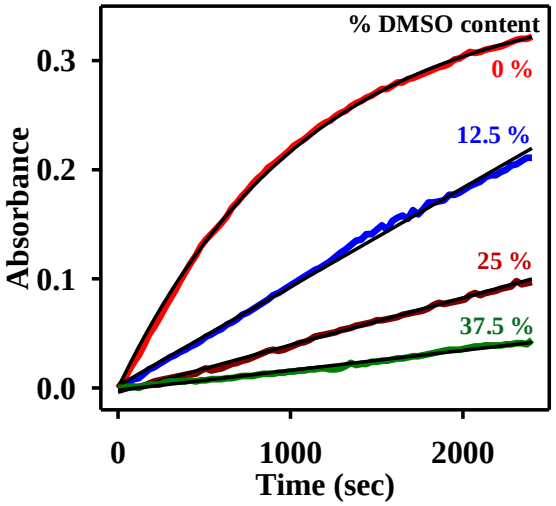



**Figure S44**: Stability of the *p*-n at experimental condition (Tris-HCl buffer). Effect of molecular crowding on the acylation of cyclopentyl mimic.

**UV-kinetics regarding role of hydrophobic interaction and effect of organic solvent:**




| % DMSO content | IRx10$^4$(s$^{-1}$) |
|---|---|
| 0 | 3.2 |
| 12.5 | 0.97 |
| 25 | 0.43 |
| 37.5 | 0.19 |

**Figure S45**: Effect of organic solvent (DMSO) in the model peptide forming reaction. IR means initial rate. The data was fitted to exponential (y = y0 + a*(1-exp$^{(-b*x)}$), IR = a * b) or linear (y = m*x, IR = m) equation to calculate IR.

**Time dependent $^{1}$H-NMR measurements of the cyclopentyl and octyl mimic:** 20mM compound was dissolved in 100mM phosphate buffer, pH 7.0 and incubated at 37°C. In the NMR tube few drops of $D_2O$ was added to lock the machine. $^{1}$H-NMR spectra were collected at different time intervals (0 h, 6 h, 18 h and 24 h). The detailed analysis was discussed in the results part of the main manuscript. The % degradation was the average of three different set of experiments and the error bars indicate the standard deviation. Initial rate (IR) was calculated by fitted the data into linear (y = m*x, IR = m) equation.

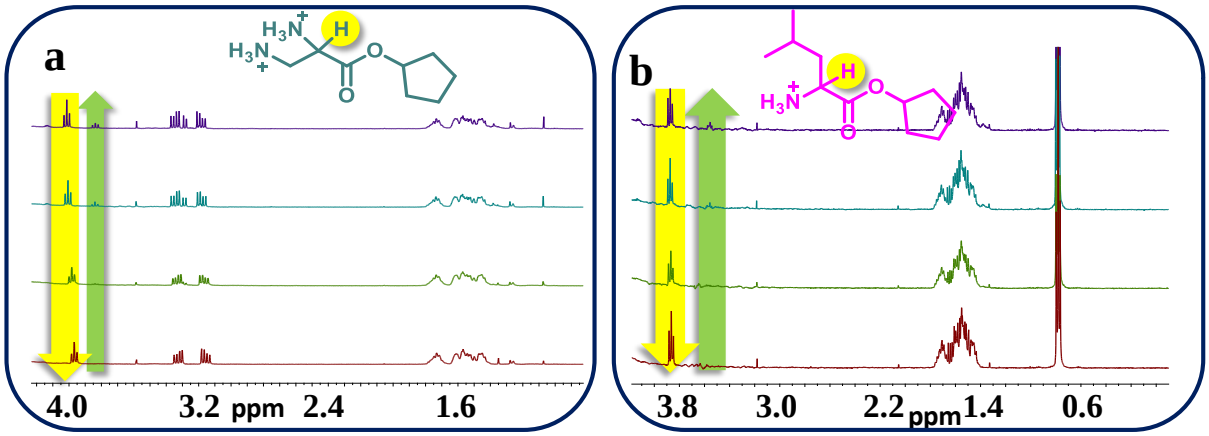



**Figure S46**: $^{1}$H-NMR experiments of the Dpr and Leu cyclopentyl mimic at different time intervals (0 h: red; 6 h: green; 18 h: cyan; and 24 h: violet).

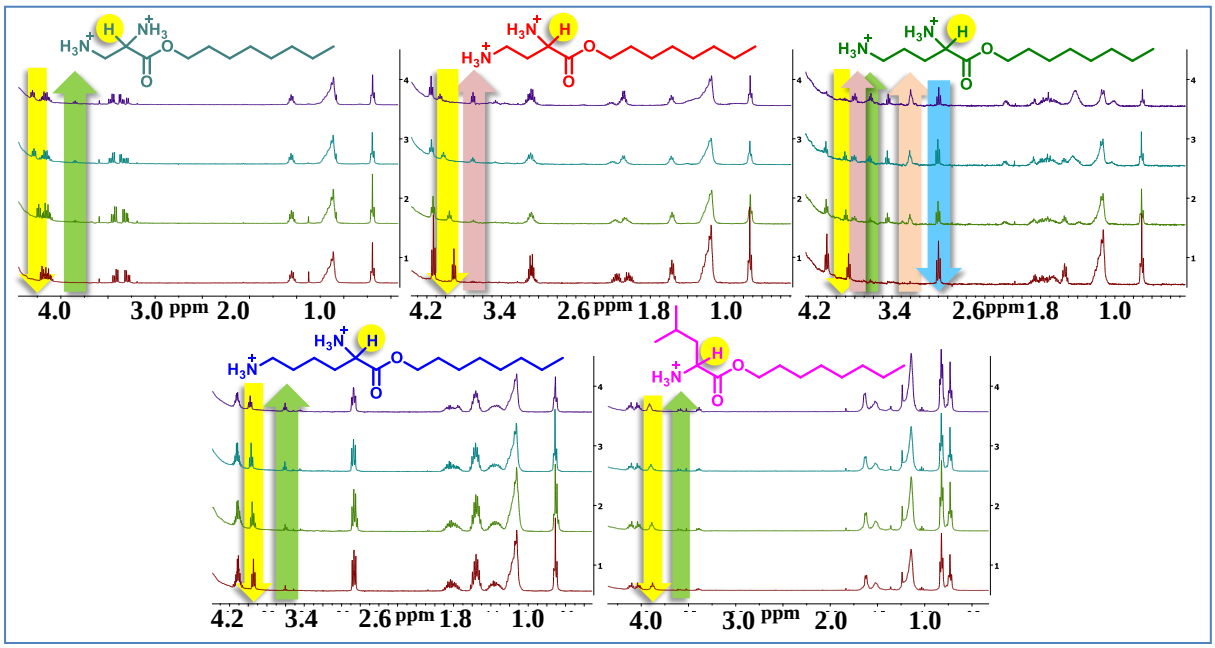



**Figure S47**: $^{1}$H-NMR experiments of the octyl mimics at different time intervals (0 h: red; 6 h: green; 18 h: cyan; and 24 h: violet).

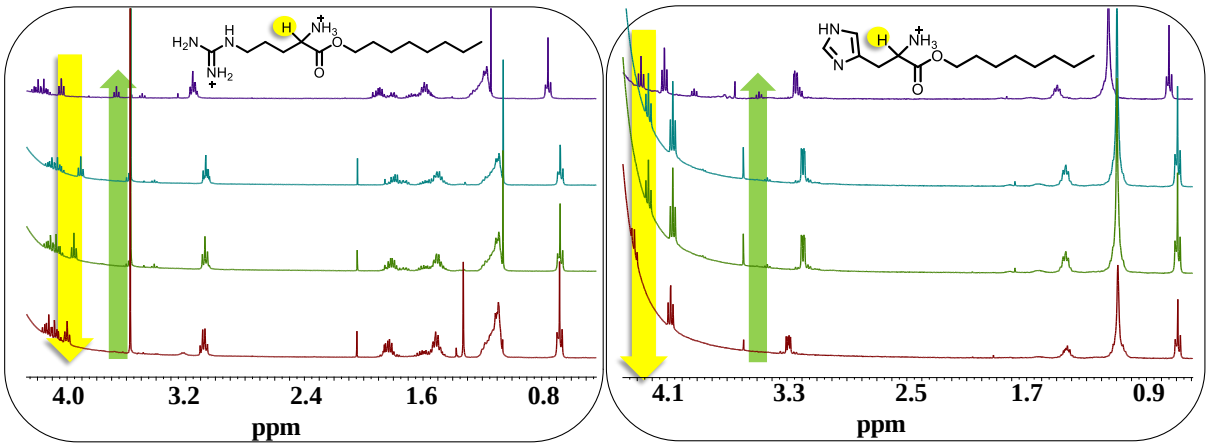



**Figure S48**: $^{1}$H-NMR experiments of the octyl mimics of Arg and His.

**Table S1:** Initial rate and % degradation of cyclopentyl and octyl mimic. * after 24 h.

| Molecules | Initial rate ($h^{-1}$) | % Degradation* |
|---|---|---|
| Dpr cyclopentyl mimic | 0.48 | 10 ± 3 |
| Dab cyclopentyl mimic | 1.67 | 42 ± 4 |
| Orn cyclopentyl mimic | 1.82 | 43 ± 3 |
| Lys cyclopentyl mimic | 0.37 | 10 ± 2 |
| Leu cyclopentyl mimic | 0.35 | 9 ± 2 |
| Dpr octyl mimic | 0.72 | 13 ± 4 |
| Dab octyl mimic | 3.42 | 85 ± 4 |
| Orn octyl mimic | 3.43 | 83 ± 5 |
| Lys octyl mimic | 0.72 | 18 ± 2 |
| Arg octyl mimic | 0.80 | 20 ± 4 |
| His octyl mimic | 0.78 | 15 ± 5 |
| Leu octyl mimic | 0.48 | 12 ± 3 |

**Supporting evidence in favour of lactam formation under physiological condition:** We synthesized the reference lactam (3-aminopiperidine-2-one, from Orn) by standard literature protocol. Briefly 1 part in weight of Orn and 4 parts in weight of alumina were mixed and refluxed in 25 parts in volume of toluene using a Dean-Stark trap to collect the water formed in the reaction. After 24 hours the mixture was filtered and the solid was repeatedly washed with a mixture of methylene chloride: methanol (9:1 v/v) to completely desorb the lactam. Evaporation of the filtrate and washings afforded the pure lactam. Then we recorded the 1H-NMR of the lactam in pH 7.0 buffer and compared with degraded aa-tRNA mimic of Orn to confirm the formation of lactam. The two newly appeared peak at 3.75 ppm and 3.2 ppm corresponds to lactam. The lactam formation was further characterized by Mass spectrometry.

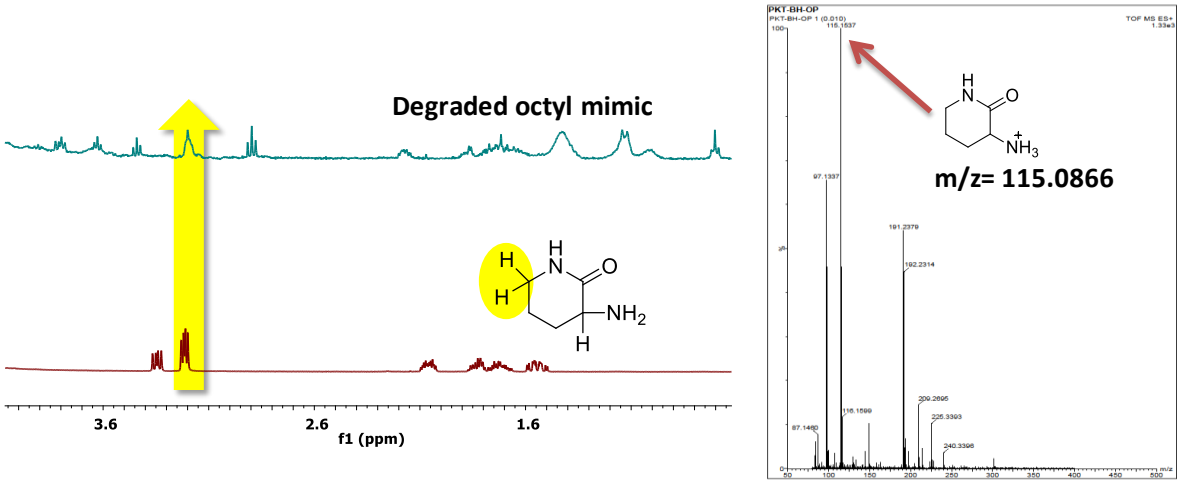



**Figure S49**: $^{1}$H-NMR and Mass data in support of lactam formation at physiological pH for Orn.

**Procedures for the model peptide forming reaction:**

2 mM octyl mimics and 2 mM4-nitrophenyl butyrate **(*p*-n,** acylating agent**)** were mixed at 100 mM tris buffer (pH 7.0) and the reaction mixture was kept at 37 °C for 2 hrs. The reaction mixture was lyophilised and the $^{1}$H-NMRs were recorded using $D_2O$ as solvent. Exactly same experiment was repeated with 100 mM phosphate buffer (pH 7.0) instead of tris buffer. All the experiments were performed three times and the ratio of terminal:α linkage were calculated for all four amino acids. The $^{1}$H-NMR spectra of the reference compounds were used to assign the peak positions.

The integration of peak at ~ 2.9 ppm (terminal-$CH_2$) of reaction mixture was assigned for unreacted species and α-amidation. The newly appeared peak at ~ 3.0 ppm (terminal $CH_2$, shifted) was assigned to terminal amidation. On the other hand, integration at newly appeared peak at ~ 4.0 ppm was assigned to α-amidation. This was subtracted (doubling the integration value of the newly appeared peak at 4.0 ppm) from the integration at ~ 2.9 ppm peak, to get the contribution from unreacted species. Now from the known integration values of terminal amidation, α-amidation and the unreacted species, we have calculated the individual percentages and total conversion (α + terminal). For Dab and Orn mimics, we have calculated the lactamization from the integration of the peak at 3.65 and 3.75 ppm respectively (Table 1). The yield of peptide formation reaction in most cases was increased when the reaction was carried out in phosphate buffer (Table 1). Model peptide forming reactions with Dpr and Dab were shown in Fig. S52. For Dab 4.0 ppm peak (shifted from 3.9 ppm) corresponds to α-amidation, 3.2 ppm peak (shifted from 3.0 ppm) corresponds to terminal amidation in both tris-HCl and phosphate buffer. In case of Dpr the 4.0 ppm peak corresponds to α-amidation (shifted from 3.9 ppm). In tris-HCl 3.1 ppm peak (shifted from 2.9 ppm) and in phosphate

buffer 3.4 ppm peak (shifted from 3.2 ppm) correspond to terminal amidation. By integrating the mentioned peak, we calculated the % of amidation and summarised in Table 1.

**Determination of critical aggregate concentration (CAC):**

The CAC of octyl mimics were determined by fluorescence spectroscopic measurements using pyrene as a probe (λex= 335 nm) in 100 mM tris-HCl buffer of pH 7.0. The pyrene emission was recorded within a range from 350-550 nm. From that, $I_1/I_3$ (characteristic first and third emission peak of pyrene) were calculated and plotted against the concentration of the aa-tRNA mimics (mM) to get the CAC. The typical pyrene concentration was 1 μM for all the measurements.

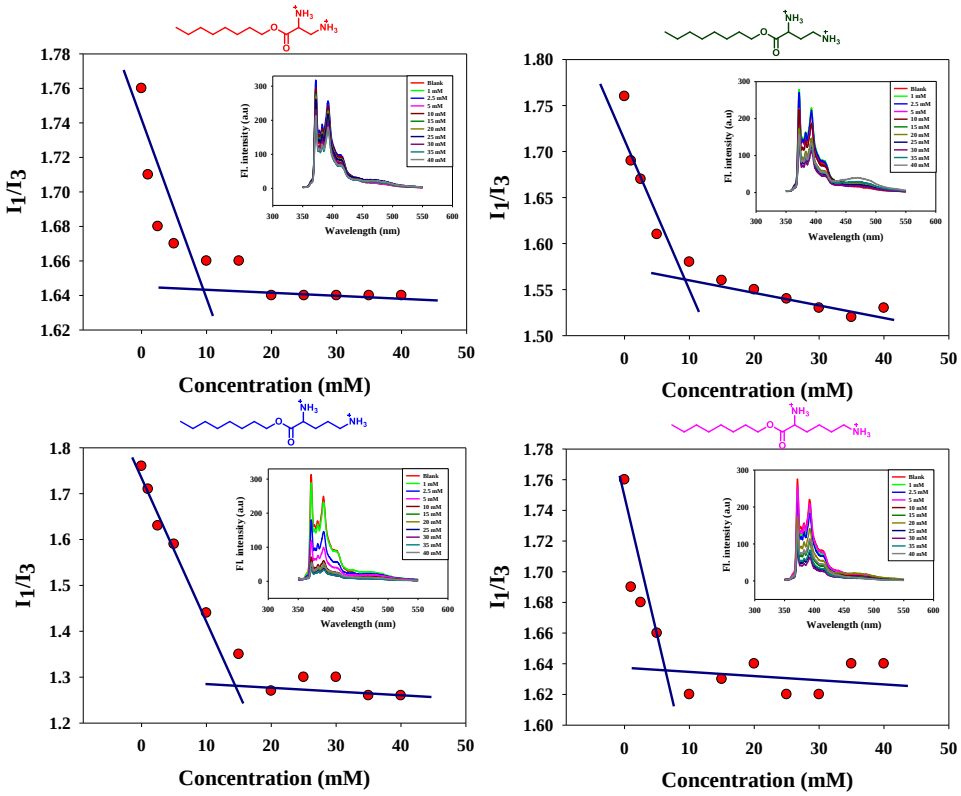



**Figure S50**: Critical aggregate concentration of octyl mimics. Inset is the stacked fluorescence spectrum for pyrene emission in each case.

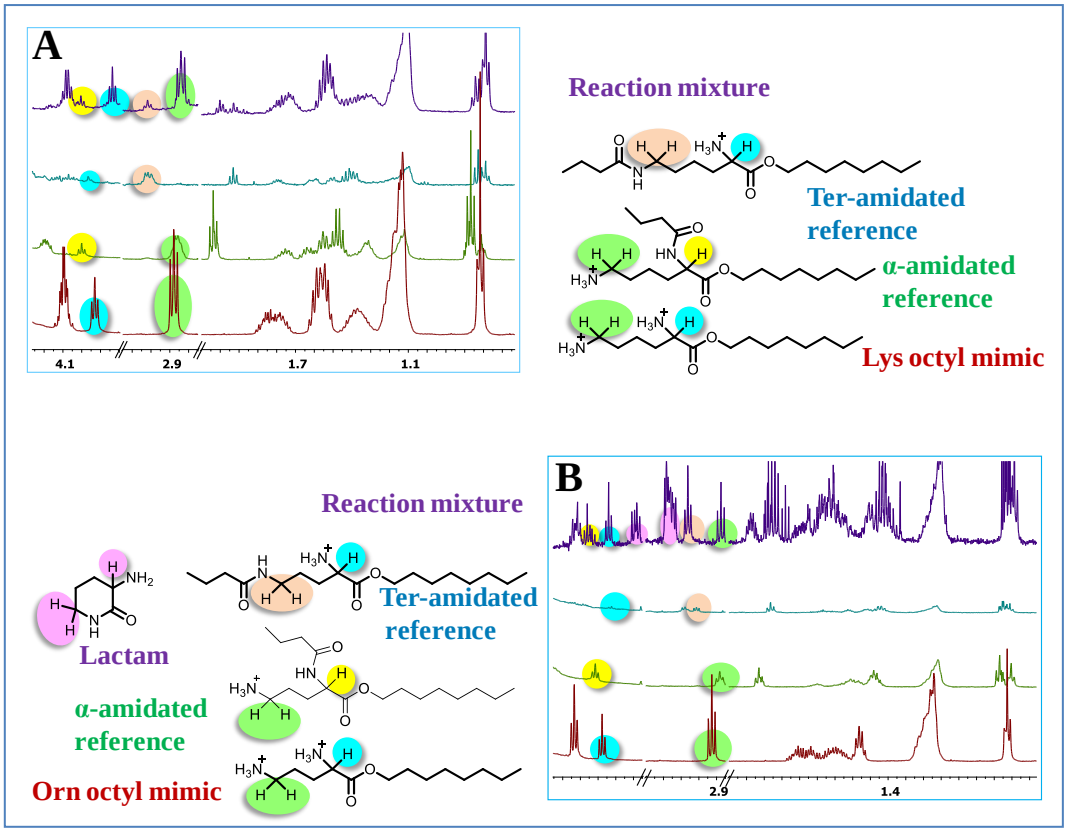



**Figure S51**: Model peptide formation reaction of **A)** Lys and **B)** Orn octyl mimic in phosphate buffer.

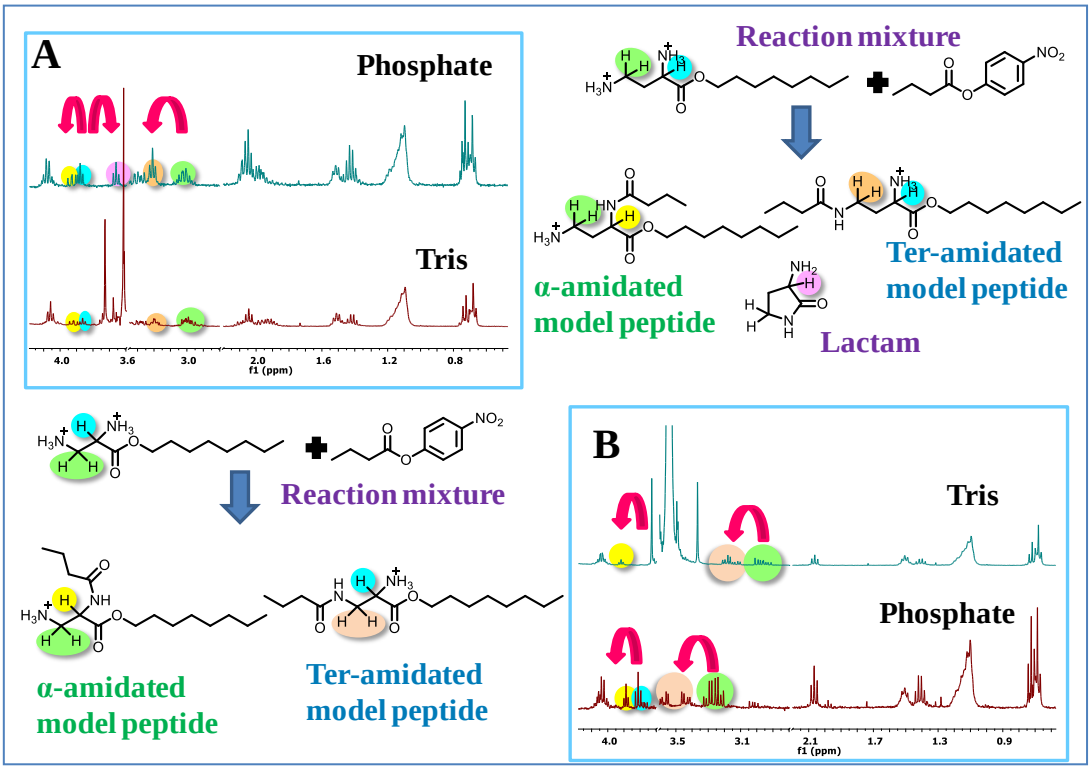



**Figure S52**: Model peptide formation reaction of **A)** Dab and **B)** Dpr octyl mimic in tris and phosphate buffer.

**$^{1}$H and $^{31}$P-NMR experiments to understand the role of phosphate:**

To understand the role of phosphate, we have performed $^{1}$H and $^{31}$P NMR with Lys octyl mimic along with blank. The concentration of the compound was maintained ~20 mM. It was dissolved in 100 mM tris at pH 7.0 (absence of phosphate) and 100 mM $K_2HPO_4$ + 100 mM tris at pH 7.0 (presence of phosphate). For $^{31}$P NMR we measured the extent of phosphorus chemical shift.

**$^{31}$P-NMR of $NaH_2PO_4$, $Na_2HPO_4$ and $Na_3PO_4$ references:**

Proton decoupled $^{31}$P-NMR of $NaH_2PO_4$, $Na_2HPO_4$ and $Na_3PO_4$(~20 mM) in $D_2O$ revealed that protonation shifted the chemical shift to a upfield region. To crosscheck our result, we have also recorded $^{31}$P-NMR of pH 6.0, 7.0 and 8.0 phosphate buffer. Similar results were recorded (data not shown).

We also recorded $^{1}$H decoupled $^{31}$P-NMR in the presence of phosphate (pH 7.0) and found the phosphorous peak slightly shifted to the upfield region after the addition of Lys mimic (Fig. S53B). It appears that the phosphate $O^-$ likely forms a hydrogen bond with the N-H of amine (Fig. 5A). In a control experiment with $NaH_2PO_4$, $Na_2HPO_4$ and $Na_3PO_4$, we found that protonation shifted the phosphorous NMR peak to an upfield region (Fig. S53C). This validates our probable model of α-amine and phosphate interaction or ion-pair formation (Fig. 5A).

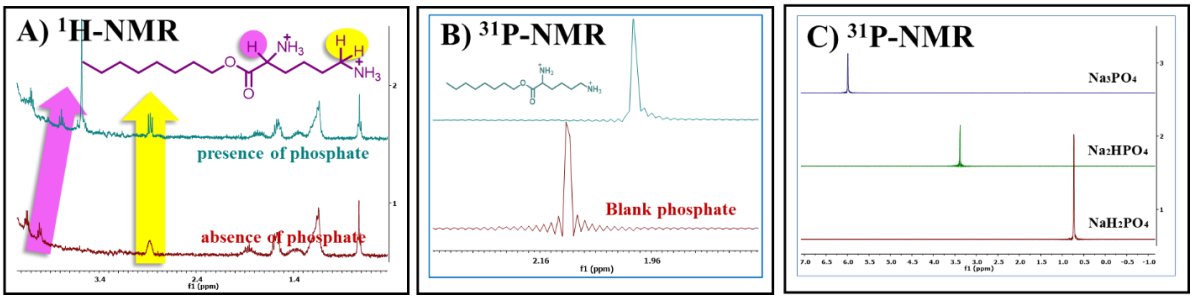



**Figure S53**: A) $^{1}$H-NMR spectra and B) $^{31}$P-NMR of Lys octyl mimic in the presence and absence of phosphate; C) $^{31}$P NMR of $NaH_2PO_4$, $Na_2HPO_4$ and $Na_3PO_4$references.

**IR studies with Lys octyl mimic with $Na_2HPO_4$:**

We have proposed that the phosphate –OH probably makes a H-bonding with the carbonyl oxygen (**Fig. 5A**). Therefore, we have recorded IR spectra of octyl mimic and 1:1 mixture of the octyl mimic and $Na_2HPO_4$. The carbonyl stretching frequency was shifted from 1742 to 1733 $cm^{-1}$, which suggest possibility of H-bond formation.

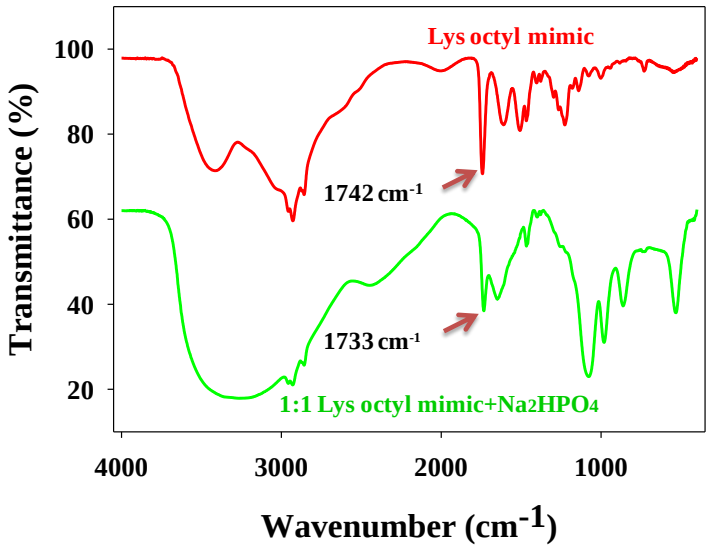



**Figure S54:** IR spectra of Lys octyl mimic and 1:1 mixture of the same with $Na_2HPO_4$.