Electronic Supplementary Material (ESI) for RSC Advances.


# Synthesis, self-assembly, and catalytic activity of histidine-based structured lipopeptides for hydrolysis reactions in water

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# SUPPORTING INFORMATION



## 1. Structural characterization of the lipopeptides

### Methods

NMR analyses were realized using a Bruker Avance 300 Ultra-Shield with precession frequencies of 300 MHz for proton and 75 MHz for carbon and using a Bruker Avance 500 Ultra-Shield with precession frequencies of 500 MHz for proton and 125 MHz for carbon. Chemical shifts (δ) are given in parts per million (ppm) downfield from tetramethylsilane (TMS).

FT-IR spectra were recorded using a Thermo Nicolet Nexus spectrometer.

ESI-MS spectra were recorded using a Waters Q-TOF Premier operating in positive or negative ion mode and for high resolution analysis or a Xevo™ G2 QTOF (Waters).

Melting points were measured on a Kofler bench from Wagner&Munz, using analytical standards for calibration.

Preparative high performance liquid chromatography was carried out with an Autopurif chromatograph from Waters for the purification of N1 ($C_{18}$ 150x30 mm column / eluent: $H_2O/CH_3CN$ 80:20, 30 $mL.min^{-1}$ / detection: ELSD).

Purity was checked by ultra-performance liquid chromatography, using an UPLC Acquity system from Waters ($C_{18}$ 50x2.1 mm, 1.7 µm column / eluent: $H_2O$-TFA/$CH_3CN$-TFA 80:20, 0.7 $mL.min^{-1}$ / detection: ELSD and UV).

**Spectroscopic and analytical data**

$C_8$-Gly-Gly-OH. Yield: 76 %. $^1H$ NMR (1 M NaOD in $D_2O$) δ: 0.83 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$), 1.26 (m, 8 H, $CH_2$ chain), 1.57 (m, 2 H, $^{\beta}CH_2$ chain), 2.30 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.74 (s, 2 H, $CH_2$ glycine), 3.91 (s, 2 H, $CH_2$ glycine). $^{13}C$ NMR *J-Mod* (1 M NaOD in $D_2O$) δ: 13.6 ($CH_3$), 22.2-35.8 ($CH_2$ chain), 43.2 ($CH_2$ glycine), 44.7 ($CH_2$ glycine), 171.3 (C=O amide), 176.6 (C=O amide), 177.0 (C=O acid). FTIR ($cm^{-1}$) ν: 1640 (C=O, amide), 1699 (C=O amide), 1726 (C=O acid), 2848-2953 (C-H chain), 3082 (N-H), 3308 (O-H). ESI-MS (positive mode) m/z : 259.17 $[M+H]^+$, 281.15 $[M+Na]^+$, 539.30 $[2M+Na]^+$. ESI-MS (negative mode) m/z: 257.15 $[M-H]^-$, 515.31 $[2M-H]^-$. HRMS calc. for $C_{12}H_{23}N_2O_4$: 259.1658, exp. 259.1659 (+0.4 ppm). Melting point: 180-182 °C (from ethyl acetate and methanol).

$C_{12}$-Gly-Gly-OH. Yield: 72 %. $^{1}$H NMR ($CD_3OD$) δ: 0.90 (m, 3 H, $CH_3$), 1.29 (m, 16 H, $CH_2$ chain), 1.63 (m, 2 H, $^{\beta}CH_2$ chain), 2.27 (m, 2 H, $^{\alpha}CH_2$ chain), 3.90 (m, 4 H, $CH_2$ glycines). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.5 ($CH_3$), 23.8-37.0 ($CH_2$ chain), 41.9 ($CH_2$ glycine), 43.4 ($CH_2$ glycine), 172.2 (C=O amide), 173.1 (C=O amide), 176.9 (C=O acid). FTIR ($cm^{-1}$) ν: 1642 (C=O, amide), 1699 (C=O amide), 1726 (C=O acid), 2850-2957 (C-H chain), 3082-3317 (N-H). ESI-MS (positive mode) m/z : 315.23 $[M+H]^+$, 337.21 $[M+Na]^+$. ESI-MS (negative mode) m/z: 313.21 $[M-H]^-$. HRMS calc. for $C_{16}H_{31}N_2O_4$: 315.2284, exp. 315.2278 (-1.9 ppm). HRMS calc. for $C_{16}H_{29}N_2O_4$: 313.2127, exp. 313.2131 (+1.9 ppm). Melting point: 182-183 °C (from petroleum ether).

$C_8$-Gly-Gly-His(1-Trt)-OMe. Yield: 86 %. $^{1}$H NMR ($CD_3OD$) δ: 0.90 (t, $^{3}J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.31 (m, 8 H, $CH_2$ chain), 1.63 (m, 2 H, $^{\beta}CH_2$ chain), 2.25 (m, 2 H, $^{\alpha}CH_2$ chain), 3.00 (m, 2H, $CH_2$ histidine), 3.65 (s, 3H, O-$CH_3$), 3.85 (m, 4 H, $CH_2$ glycines), 4.69 (m, 1H, CH histidine), 6.77 (s, 1 H, =CH imidazole), 7.16 (m, 6 H, 5 =CH trityl and 1 =CH imidazole), 7.38 (m, 10 H, =CH trityl). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.5 ($CH_3$ chain), 23.8-32.9 ($CH_2$ chain and $CH_2$ histidine), 36.9 ($^{\alpha}CH_2$ chain ), 43.2 ($CH_2$ glycine), 43.8 ($CH_2$ glycine), 52.8 (O-$CH_3$), 54.1 (CH histidine), 76.9 ($C_q$ trityl), 121.3 (=CH imidazole), 129.4 (=CH trityl), 130.9 (=CH trityl), 137.3 ($C_q$ histidine), 139.6 (=CH imidazole), 143.6 ($C_q$ trityl), 171.2 (C=O amide), 172.1 (C=O amide), 173.1 (C=O amide), 176.9 (C=O ester). FTIR ($cm^{-1}$) ν: 1627 (C=O, amide), 1686 (C=O amide), 1743 (C=O ester), 2848-2925 (C-H chain), 3060 (=C-H trityl), 3290 (N-H). ESI-MS (positive mode) m/z : 652.35 $[M+H]^+$, 675.33 $[M+Na]^+$. ESI-MS (negative mode) m/z: 650.33 $[M-H]^-$. HRMS calc. for $C_{38}H_{46}N_5O_5$: 652.3499, exp. 652.3500 (+0.2 ppm). Melting point: 101-103 °C (from TBME).

$C_{12}$-Gly-Gly-His(1-Trt)-OMe. Yield: 65 %. $^{1}$H NMR ($CD_3OD$) δ: 0.89 (t, $^{3}J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.28 (m, 16 H, $CH_2$ chain), 1.59 (m, 2 H, $^{\beta}CH_2$ chain), 2.23 (m, 2 H, $^{\alpha}CH_2$ chain), 3.00 (m, 2H, $CH_2$ histidine), 3.64 (s, 3H, O-$CH_3$), 3.83 (m, 4 H, $CH_2$ glycines), 4.68 (m, 1H,

CH histidine), 6.75 (m, 1 H, =CH imidazole), 7.14 (m, 6 H, 5 =CH trityl and 1 =CH imidazole), 7.37 (m, 10 H, =CH trityl). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.5 ($CH_3$ chain), 23.8-33.1 ($CH_2$ chain and $CH_2$ histidine), 36.9 ($^{\alpha}CH_2$ chain ), 43.2 ($CH_2$ glycine), 43.8 ($CH_2$ glycine), 52.8 (O-$CH_3$), 54.2 (CH histidine), 76.9 ($C_q$ trityl), 121.3 (=CH imidazole), 129.3 (=CH trityl), 130.9 (=CH trityl), 137.3 ($C_q$ histidine), 139.6 (=CH imidazole), 143.7 ($C_q$ trityl), 171.3 (C=O amide), 172.1 (C=O amide), 173.1 (C=O amide), 176.9 (C=O ester). FTIR ($cm^{-1}$) ν: 1631 (C=O, amide), 1670 (C=O amide), 1744 (C=O ester), 2856-2963 (C-H chain), 3065-3325 (N-H). ESI-MS (positive mode) m/z: 708.41 $[M+H]^+$. HRMS calc. for $C_{42}H_{54}N_5O_5$: 708.4125, exp. 708.4124 (-0.1 ppm). Melting point: 117-118°C (from TBME).

$C_8$-Gly-Gly-His-OMe (**C7**). Yield: 83 %. $^1$H NMR ($CD_3OD$) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.31 (m, 8 H, $CH_2$ chain), 1.62 (m, 2 H, $^{\beta}CH_2$ chain), 2.27 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.09 (m, 2H, $CH_2$ histidine), 3.70 (1s, 3H, O-$CH_3$), 3.87 (m, 4 H, $CH_2$ glycines), 4.66 (m, 1H, CH histidine), 6.88 (s, 1 H, =CH imidazole), 7.58 (s, 1 H, =CH imidazole). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.5 ($CH_3$ chain), 23.7-32.9 ($CH_2$ chain and $CH_2$ histidine), 36.9 ($^{\alpha}CH_2$ chain), 43.3 ($CH_2$ glycine), 43.8 ($CH_2$ glycine), 52.8 (O-$CH_3$), 54.2 (CH histidine), 136.4 (=CH imidazole), 171.5 (C=O amide), 172.4 (C=O amide), 173.2 (C=O amide), 177.1 (C=O ester). FTIR ($cm^{-1}$) ν: 1650 (C=O, amide), 1733 (C=O ester), 2851-2927 (C-H chain), 3078-3310 (N-H). ESI-MS (positive mode) m/z : 410.24 $[M+H]^+$, 432.25 $[M+Na]^+$. ESI-MS (negative mode) m/z: 408.2 $[M-H]^-$. HRMS calc. for $C_{19}H_{32}N_5O_5$: 410.2403, exp. 410.2417 (+3.4 ppm). Melting point: 200-202 °C (from DCM/MeOH).

$C_{12}$-Gly-Gly-His-OMe. Yield: 74 %. $^1$H NMR ($CD_3OD$) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.29 (m, 16 H, $CH_2$ chain), 1.62 (m, 2 H, $^{\beta}CH_2$ chain), 2.27 (t, $^3J_{HH}$ = 7.7 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.09 (m, 2H, $CH_2$ histidine), 3.35 and 3.70 (2s, 3H, O-$CH_3$), 3.87 (m, 4 H, $CH_2$ glycines), 4.66 (m, 1H, CH histidine), 6.88 (s, 1 H, =CH imidazole), 7.58 (s, 1 H, =CH imidazole). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.3 ($CH_3$ chain), 23.6-33.0 ($CH_2$ chain and $CH_2$ histidine), 36.9

($^{\alpha}CH_2$ chain ), 43.6 ($CH_2$ glycine), 44.2 ($CH_2$ glycine), 52.9 (O-$CH_3$), 53.0 (CH histidine), 118.9 (=CH imidazole), 130.8 (Cq imidazole), 171.9 (C=O amide), 172.0 (C=O amide), 173.0 (C=O amide), 177.9 (C=O ester). FTIR ($cm^{-1}$) ν: 1650 (C=O, amide), 1734 (C=O ester), 2851-2955 (C-H chain), 3075-3320 (N-H). ESI-MS (positive mode, MeOD/TFA) m/z: 469.32 $[M(3D)+H]^+$, 937.64 $[2M(3D)+H]^+$. ESI-MS (negative mode, MeOD/TFA) m/z: 581.30 $[M(3D)+TFA-H]^-$, 1049.61 $[2M(3D)+TFA-H]^-$. HRMS calc. for $C_{23}H_{37}D_3N_5O_5$: 469.3218, exp. 469.3215 (-0.6 ppm). HRMS calc. for $C_{25}H_{36}D_3F_3N_5O_7$: 581.2990, exp. 581.2991 (+0.2 ppm). Melting point: 173-174 °C (from EtOAc).

$C_8$-Gly-Gly-His-OH (**C1**). Yield: 61 %. $^1$H NMR (1 M NaOD in $D_2O$) δ: 0.85 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.28 (m, 8 H, $CH_2$ chain), 1.60 (m, 2 H, $^{\beta}CH_2$ chain), 2.33 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.22 (m, 2H, $CH_2$ histidine), 3.94 (m, 4 H, $CH_2$ glycines), 4.55 (m, 1H, CH histidine), 7.27 (s, 1 H, =CH imidazole), 8.60 (s, 1 H, =CH imidazole). $^{13}$C NMR *J-Mod* (1 M NaOD in $D_2O$) δ: 13.5 ($CH_3$ chain), 21.9-30.9 ($CH_2$ chain and $CH_2$ histidine), 35.5 ($^{\alpha}CH_2$ chain ), 42.4 ($CH_2$ glycine), 42.5 ($CH_2$ glycine), 53.6 (CH histidine), 116.8 (=CH imidazole), 129.4 (Cq imidazole), 170.7 (C=O amide), 172.3 (C=O amide), 175.6 (C=O amide), 178.2 (C=O acid). FTIR ($cm^{-1}$) ν: 1646 (C=O, amide), 2854-2955 (C-H chain), 3143-3296 (N-H). ESI-MS (positive mode) m/z : 396.22 $[M+H]^+$. ESI-MS (negative mode) m/z: 394.2 $[M-H]^-$. HRMS calc. for $C_{18}H_{30}N_5O_5$: 396.2247, exp. 396.2250 (+0.8 ppm). Melting point: 133-135 °C (from $CH_3CN$).

$C_{10}$-Gly-Gly-His-OH (**C2**). $^1$H NMR (1 M MeOD) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.28 (m, 12 H, $CH_2$ chain), 1.62 (m, 2 H, $^{\beta}CH_2$ chain), 2.29 (t, $^3J_{HH}$ = 7.7 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.27 (m, 2H, $CH_2$ histidine), 3.85 (m, 4 H, $CH_2$ glycines), 4.72 (m, 1H, CH histidine), 7.34 (s, 1 H, =CH imidazole), 8.77 (s, 1 H, =CH imidazole). $^{13}$C NMR *J-Mod* ($CD_3OD$) δ: 14.5 ($CH_3$ chain), 23.8-33.1 ($CH_2$ chain and $CH_2$ histidine), 36.8 ($^{\alpha}CH_2$ chain), 43.6 ($CH_2$ glycine), 44.0 ($CH_2$ glycine), 52.9 (CH histidine), 118.7 (=CH imidazole), 131.2 (Cq imidazole), 171.7 (C=O

amide), 172.8 (C=O amide), 173.2 (C=O amide), 177.4 (C=O acid). FTIR ($cm^{-1}$) ν: 1647 (C=O, amide), 2852-2955 (C-H chain), 3150-3298 (N-H). ESI-MS (positive mode) m/z : 424.26 $[M+H]^+$, 446.24 $[M+Na]^+$. ESI-MS (negative mode) m/z: 422.2 $[M-H]^-$. HRMS calc. for $C_{20}H_{34}N_5O_5$: 424.2560, exp. 424.2556 (- 0.9 ppm). Melting point: 105-106°C.

$C_{12}$-Gly-Gly-His-OH (**C3**). Yield: 44 %. $^1H$ NMR ($CD_3OD$) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz, 3 H, $CH_3$ chain), 1.29 (m, 16 H, $CH_2$ chain), 1.62 (m, 2 H, $^{\beta}CH_2$ chain), 2.29 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.21 (m, 2H, $CH_2$ histidine), 3.85 (m, 4 H, $CH_2$ glycines), 4.54 (m, 1H, CH histidine), 7.25 (s, 1 H, =CH imidazole), 8.59 (s, 1 H, =CH imidazole). $^{13}C$ NMR *J-Mod* ($CD_3OD$+TFA) δ: 14.5 ($CH_3$ chain), 23.7-33.0 ($CH_2$ chain and $CH_2$ histidine), 36.9 ($^{\alpha}CH_2$ chain), 43.7 ($CH_2$ glycine), 44.2 ($CH_2$ glycine), 53.0 (CH histidine), 118.8 (=CH imidazole), 131.0 (Cq imidazole), 172.0 (C=O amide), 173.1 (C=O amide), 173.3 (C=O amide), 177.9 (C=O acid). FTIR ($cm^{-1}$) ν: 1645 (C=O, amide), 1711 (C=O acid), 2851-2953 (C-H chain), 3138-3306 (N-H). ESI-MS (positive mode) m/z: 452.29 $[M+H]^+$. ESI-MS (negative mode) m/z: 450.27 $[M-H]^-$. HRMS calc. for $C_{22}H_{38}N_5O_5$: 452.2873, exp.: 452.2869 (-0.9 ppm). HRMS calc. for $C_{22}H_{36}N_5O_5$: 450.2717, exp.: 450.2717 (0 ppm). Melting point: 165-166 °C (from $CH_3CN$).

$C_8$-Gly-Gly-Gly-OH (**C4**). Yield: 87 % $^1H$ NMR (DMSO) δ: 0.86 (m, 3 H, $CH_3$ chain), 1.24 (m, 8 H, $CH_2$ chain), 1.48 (m, 2 H, $^{\beta}CH_2$ chain), 2.12 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.71 (m, 6 H, $CH_2$ glycines), 8.06 (m, 3 H, NH). $^{13}C$ NMR *J-Mod* (1 M NaOD in $D_2O$) δ: 13.4 ($CH_3$ chain), 21.9-35.7 ($CH_2$ chain), 43.1 ($CH_2$ glycine), 43.2 ($CH_2$ glycine), 44.7 ($CH_2$ glycine), 171.2 (C=O amide), 172.3 (C=O amide), 176.5 (C=O amide), 178.2 (C=O acid). FTIR ($cm^{-1}$) ν: 1637 (C=O, amide), 1651 (C=O, amide), 1705 (C=O, acide), 2849-2955 (C-H chain), 3085-3291 (N-H). ESI-MS (positive mode) m/z : 316.19 $[M+H]^+$, 338.17 $[M+Na]^+$. ESI-MS (negative mode) m/z: 314.2 $[M-H]^-$. HRMS calc. for $C_{14}H_{26}N_3O_5$: 316.1872, exp. 316.1871 (-0.3 ppm). Melting point: 233-234 °C (from acetone).

$C_{10}$-Gly-Gly-Gly-OH (**C5**). Yield: 82 % $^{1}$H NMR (DMSO) δ: 0.85 (t, $^{3}J_{HH}$ = 6 Hz 3 H, $CH_3$ chain), 1.24 (m, 10 H, $CH_2$ chain), 1.48 (m, 2 H, $^{\beta}CH_2$ chain), 2.12 (t, $^{3}J_{HH}$ = 7.4 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.71 (m, 6 H, $CH_2$ glycines), 8.11 (m, 3 H, NH). $^{13}$C NMR *J-Mod* (DMSO) δ: 13.9 ($CH_3$ chain), 22.0-35.1 ($CH_2$ chain), 40.5 ($CH_2$ glycine), 41.7 ($CH_2$ glycine), 42.0 ($CH_2$ glycine), 169.1 (C=O amide), 169.3 (C=O amide), 171.0 (C=O amide), 172.6 (C=O acid). FTIR ($cm^{-1}$) ν: 1651 (C=O, amide), 1705 (C=O, acid), 2850-2955 (C-H chain), 3085-3292 (N-H). ESI-MS (negative mode) m/z: 342.20 $[M-H]^-$, 685.41 $[2M-H]^-$. HRMS calc. for $C_{16}H_{28}N_3O_5$: 342.2029, exp. 342.2026 (-0.9 ppm). Melting point: 229-230°C (from acetone).

$C_{12}$-Gly-Gly-Gly-OH (**C6**). Yield: 56 % $^{1}$H NMR (DMSO) δ: 0.85 (t, $^{3}J_{HH}$ = 6.6 Hz 3 H, $CH_3$ chain), 1.24 (m, 10 H, $CH_2$ chain), 1.48 (m, 2 H, $^{\beta}CH_2$ chain), 2.12 (t, $^{3}J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.33 (br s, OH) 3.74 (m, 6 H, $CH_2$ glycines), 8.10 (m, 3 H, NH). $^{13}$C NMR *J-Mod* (DMSO) δ: 14.0 ($CH_3$ chain), 22.1-35.2 ($CH_2$ chain), 40.5 ($CH_2$ glycine), 41.7 ($CH_2$ glycine), 42.1 ($CH_2$ glycine), 169.2 (C=O amide), 169.4 (C=O amide), 171.0 (C=O amide), 172.8 (C=O acid). FTIR ($cm^{-1}$) ν: 1639 (C=O, amide), 1652 (C=O, amide), 1705 (C=O, acide), 2850-2956 (C-H chain), 3087-3292 (N-H). FAB-MS (positive mode) m/z: 372 $[M+H]^+$, 394 $[M+Na]^+$, ESI-MS (negative mode) m/z: 370.23 $[M-H]^-$. HRMS calc. for $C_{18}H_{32}N_3O_5$: 370.2342, exp. 370.2331 (-3.0 ppm) Melting point: 230-231°C (from acetone).

Boc-Gly-Gly-$C_8$: Yield: 90 % $^{1}$H NMR (MeOD) δ: 0.90 (t, $^{3}J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.31 (m, 10 H, $CH_2$ chain), 1.46 (m, 9 H, $CH_3$ Boc), 1.52 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^{3}J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.71 (s, 2 H, $CH_2$ glycine), 3.83 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.5 ($CH_2$ chain), 43.5 ($CH_2$ glycine), 45.1 ($CH_2$ glycine), 81.0 ($C_q$ $^{t}$Bu), 158.8 (C=O carbamate), 171.5 (C=O amide), 173.2 (C=O amide). FTIR ($cm^{-1}$) ν: 1654 (C=O, amide), 1686 (C=O, amide), 2852-2967 (C-H chain), 3089-3339 (N-H). ESI-MS (positive mode) m/z: 366.2 $[M+Na]^+$. HRMS calc. for $C_{17}H_{34}N_3O_4$: 344.2549, exp. 344.2539 (-2.9 ppm). Melting point: 71-72 °C (from petroleum ether).

Boc-Gly-Gly-$C_{12}$: Yield: 88 % $^{1}$H NMR (MeOD) δ: 0.90 (t, $^{3}J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.31 (m, 10 H, $CH_2$ chain), 1.46 (m, 9 H, $CH_3$ Boc), 1.52 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^{3}J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.71 (s, 2 H, $CH_2$ glycine), 3.83 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* MeOD) δ: 14.5 ($CH_3$ chain), 23.7-40.5 ($CH_2$ chain), 43.4 ($CH_2$ glycine), 45.1 ($CH_2$ glycine), 81.1 ($C_q$ $^{t}$Bu), 158.8 (C=O carbamate), 171.5 (C=O amide), 173.3 (C=O amide). FTIR ($cm^{-1}$) ν: 1645 (C=O, amide), 2851-2953 (C-H chain), 3096-3300 (N-H). ESI-MS (positive mode) m/z: 422,30 $[M+Na]^+$, 821,61 $[2M+Na]^+$. HRMS calc. for $C_{21}H_{41}N_3O_4Na$: 422.2995, exp. 422.2987 (-1.9 ppm). Melting point: 96-97°C (from petroleum ether).

H-Gly-Gly-$C_8$ : Yield: 88 % $^{1}$H NMR (MeOD) δ: 0.90 (t, $^{3}J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.31 (m, 10 H, $CH_2$ chain), 1.50 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^{3}J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.32 (s, 2 H, $CH_2$ glycine), 3.86 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.4 ($CH_3$ chain), 23.7-40.6 ($CH_2$ chain), 41.6 ($CH_2$ glycine), 43.3 ($CH_2$ glycine), 168.0 (C=O amide), 171.0 (C=O amide). FTIR ($cm^{-1}$) ν: 1654 (C=O, amide), 1685 (C=O, amide), 2850-2961 (C-H chain), 3087-3360 (N-H). ESI-MS (positive mode) m/z: 244.2 $[M+H]^+$, 266,2 $[M+Na]^+$. HRMS calc. for $C_{12}H_{26}N_3O_2$: 244.2025, exp. 244.2027 (+0.8 ppm). Melting point: 140-141°C (from ethyl acetate).

H-Gly-Gly-$C_{12}$ : Yield: 75 % $^{1}$H NMR (MeOD) δ: 0.90 (t, $^{3}J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.31 (m, 10 H, $CH_2$ chain), 1.50 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^{3}J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.32 (s, 2 H, $CH_2$ glycine), 3.86 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.5 ($CH_2$ chain), 43.4 ($CH_2$ glycine), 45.1 ($CH_2$ glycine), 171.5 (C=O amide), 175.8 (C=O amide). FTIR ($cm^{-1}$) ν: 1642 (C=O, amide), 1680 (C=O, amide), 2849-2956 (C-H chain), 3095-3368 (N-H). ESI-MS (positive mode) m/z: 300.27 $[M+H]^+$, 338,22 $[M+Na]^+$. HRMS calc. for $C_{16}H_{34}N_3O_2$: 300.2651, exp. 300.2654 (+1.0 ppm). Melting point: 143-144°C (from ethyl acetate).

Boc-His(1-Boc)-Gly-Gly-$C_8$: Yield: 75 % $^1$H NMR (MeOD) δ: 0.89 (t, $^3J_{HH}$ = 6.3 Hz 3 H, $CH_3$ chain), 1.29 (m, 10 H, $CH_2$ chain), 1.41 (m, 9 H, $CH_3$ Boc chain), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 1.62 (m, 9 H, $CH_3$ Boc imidazole), 2.99 (m, 2H, $CH_2$ histidine), 3.19 (t, $^3J_{HH}$ = 7.5 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.87 (m, 4 H, $CH_2$ glycines), 4.30 (m, 1 H, CH histidine), 7.34 (s, 1H, =CH imidazole), 8.12 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.6 ($CH_2$ chain), 43.4 ($CH_2$ glycine), 44.3 ($CH_2$ glycine), 55.9 (CH histidine), 81.0 ($C_q$ $^t$Bu chain), 87.2 ($C_q$ $^t$Bu imidazole), 116.6 (=CH imidazole), 139.7 (C=O carbamate imidazole), 148.1 (C=O carbamate chain), 171.4 (C=O amide), 172.2 (C=0 amide), 175.3 (C=O amide). FTIR ($cm^{-1}$) ν: 1659 (C=O, amide), 1753 (C=O carbamate), 2856-2972 (C-H chain), 3082-3306 (N-H). ESI-MS (positive mode) m/z: 581.4 $[M+H]^+$, 603.3 $[M+Na]^+$. ESI-MS (negative mode) m/z: 579.4 $[M-H]^-$. HRMS calc. for $C_{28}H_{49}N_6O_7$: 581.3663, exp. 581.3663 (+1.9 ppm) Melting point: 100-101°C (from ethyl acetate).

Boc-His(1-Boc)-Gly-Gly-$C_{12}$: Yield: 73 % $^1$H NMR (MeOD) (impurities such as BocHis(1-Boc)OSu or BocHis(1-Boc)OH are detectable on the spectrum) δ: 0.90 (m, 3 H, $CH_3$ chain), 1.28 (m, 10 H, $CH_2$ chain), 1.41 (m, 9 H, $CH_3$ Boc chain), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 1.62 (m, 9 H, $CH_3$ Boc imidazole), 3.02 (m, 2H, $CH_2$ histidine), 3.19 (m, 2 H, $^{\alpha}CH_2$ chain), 3.87 (m, 4 H, $CH_2$ glycines), 4.30 (m, 1 H, CH histidine), 7.34 (s, 1H, =CH imidazole), 8.12 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.6 ($CH_2$ chain), 43.4 ($CH_2$ glycine), 44.3 ($CH_2$ glycine), 55.9 (CH histidine), 81.1 ($C_q$ $^t$Bu chain), 87.2 ($C_q$ $^t$Bu imidazole), 116.6 (=CH imidazole), 139.7 (C=O carbamate imidazole), 148.1 (C=O carbamate chain), 171.5 (C=O amide), 172.2 (C=0 amide), 175.3 (C=O amide). FTIR ($cm^{-1}$) ν: 1660 (C=O, amide), 1696 (C=O, amide), 1753 (C=O, carbamate), 2853-2979 (C-H chain), 3082-3307 (N-H). ESI-MS (positive mode) m/z: 637.43 $[M+H]^+$. HRMS calc. for $C_{32}H_{57}N_6O_7$: 637.4289, exp. 637.4286 (-0.5 ppm). Melting point: 94-95°C (from petroleum ether).

H-His-Gly-Gly-$C_8$ (**N1**). Yield: 95 % $^1$H NMR (MeOD) δ: 0.85 (t, $^3J_{HH}$ = 7.2 Hz, 3 H, $CH_3$ chain), 1.26 (m, 10 H, $CH_2$ chain), 1.49 (m, 2 H, $^{\beta}CH_2$ chain), 3.20 (t, $^3J_{HH}$ = 6.9 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.44 (d, $^3J_{HH}$ = 6.3 Hz, 2 H, CH histidine), 3.92 (s, 2 H, $CH_2$ glycine), 4.06 (m, 2 H, $CH_2$ glycine), 4.39 (t, $^3J_{HH}$ = 6.3 Hz, 1 H, CH histidine), 7.47 (s, 1H, =CH imidazole), 8.70 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.7-40.6 ($CH_2$ chain), 43.2 ($CH_2$ glycine), 43.5 ($CH_2$ glycine), 53.2 (CH histidine), 120.2 (=CH imidazole), 127.8 ($C_q$ imidazole) 169.5 (C=O amide), 171.1 (C=0 amide), 172.4 (C=O amide). FTIR ($cm^{-1}$) ν: 1662 (C=O, amide), 2644-2929 (C-H chain), 3086-3290 (N-H). ESI-MS (positive mode) m/z: 381.26 $[M+H]^+$, 403.24 $[M+Na]^+$. HRMS calc. for $C_{18}H_{33}N_6O_3$: 381.2614, exp. 381.2603 (-2.9 ppm). Melting point: 90-91°C (from acetonitrile/water).

H-His-Gly-Gly-$C_{10}$ (**N2**). $^1$H NMR (MeOD) δ: 0.82 (t, $^3J_{HH}$ = 6.3 Hz, 3 H, $CH_3$ chain), 1.22 (m, 14 H, $CH_2$ chain), 1.47 (m, 2 H, $^{\beta}CH_2$ chain), 3.17 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.45 (d, $^3J_{HH}$ = 6.6 Hz, 2 H, CH histidine), 4.00 (m, 4 H, $CH_2$ glycines), 4.42 (t, $^3J_{HH}$ = 6.3 Hz, 1 H, CH histidine), 7.49 (s, 1H, =CH imidazole), 8.73 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* ($D_2O$) δ: 13.6 ($CH_3$ chain), 22.3-39.5 ($CH_2$ chain), 42.2 ($CH_2$ glycine), 42.5 ($CH_2$ glycine), 52.0 (CH histidine), 118.8 (=CH imidazole), 125.6 ($C_q$ imidazole), 168.7 (C=O amide), 170.4 (C=0 amide), 171.2 (C=O amide). FTIR ($cm^{-1}$) ν: 1666 (C=O, amide), 2643-2928 (C-H chain), 3088-3272 (N-H). ESI-MS (positive mode) m/z: 409.29 $[M+H]^+$, 431.28 $[M+Na]^+$. HRMS calc. for $C_{20}H_{37}N_6O_3$: 409.2927, exp. 409.2935 (+2.0 ppm) Melting point: 112-113°C.

H-His-Gly-Gly-$C_{12}$ (**N3**). Yield : 40% $^1$H NMR (MeOD) δ: 0.90 (t, $^3J_{HH}$ = 6.3 Hz, 3 H, $CH_3$ chain), 1.29 (m, 14 H, $CH_2$ chain), 1.50 (m, 2 H, $^{\beta}CH_2$ chain), 2.97 (t, $^3J_{HH}$ = 6 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.19 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $CH_2$ histidine), 3.61 (t, $^3J_{HH}$ = 6.0 Hz, 1 H, CH histidine), 3.88 (s, 4 H, $CH_2$ glycines), 6.61 (s, 1H, =CH imidazole), 7.59 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.5 ($CH_2$ chain), 43.5 ($CH_2$ glycine), 44.1 ($CH_2$ glycine), 56.0 (CH histidine), 136.6 (=CH imidazole), 171.2 (C=O amide), 172.5 (C=0

amide), 178.1 (C=O amide). FTIR ($cm^{-1}$) ν: 1648 (C=O, amide), 2849-2956 (C-H chain), 3082-3302 (N-H). ESI-MS (positive mode) m/z: 437.32 $[M+H]^+$, 459.30 $[M+Na]^+$. HRMS calc. for $C_{22}H_{41}N_6O_3$: 437.3240, exp. 437.3231 (-2.1 ppm). Melting point: 141-142°C.

Boc-Gly-Gly-Gly-$C_8$: Yield: 40 % $^1$H NMR (MeOD) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.31 (m, 10 H, $CH_2$ chain), 1.45 (m, 9 H, $CH_3$ Boc), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.74 (s, 2 H, $CH_2$ glycine), 3.84 (s, 2 H, $CH_2$ glycine), 3.89 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.6 ($CH_2$ chain), 28.8 ($CH_3$ Boc), 43.5 ($CH_2$ glycine), 43.9 ($CH_2$ glycine), 45.0 ($CH_2$ glycine), 81.0 ($C_q$ $^t$Bu Boc), 159.9 (C=O Boc), 171.3 (C=O amide), 172.2 (C=0 amide), 173.7 (C=O amide). FTIR ($cm^{-1}$) ν: 1635 (C=O, amide), 1666 (C=O, amide), 1707 (C=O carbamate), 2869-2968 (C-H chain), 3103-3339 (N-H). ESI-MS (positive mode) m/z: 423.26 $[M+Na]^+$. HRMS calc. for $C_{19}H_{36}N_3O_4Na$: 423.2583, exp. 423.2573 (-2.4 ppm). Melting point: 159-160 °C (from ethyl acetate).

Boc-Gly-Gly-Gly-$C_{10}$: Yield: 72 % $^1$H NMR (MeOD) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.30 (m, 14 H, $CH_2$ chain), 1.45 (m, 9 H, $CH_3$ Boc), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 3.18 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.74 (s, 2 H, $CH_2$ glycine), 3.84 (s, 2 H, $CH_2$ glycine), 3.89 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 15.3 ($CH_3$ chain), 23.7-40.1 ($CH_2$ chain), 29.5 ($CH_3$ Boc), 43.5 ($CH_2$ glycine), 43.8 ($CH_2$ glycine), 44.9 ($CH_2$ glycine), 80.0 ($C_q$ $^t$Bu Boc), 157.6 (C=O Boc), 170.2 (C=O amide), 170.9 (C=0 amide), 171.9 (C=O amide). FTIR ($cm^{-1}$) ν: 1635 (C=O, amide), 1671 (C=O, amide), 1708 (C=O carbamate), 2852-2921 (C-H chain), 3089-3331 (N-H). ESI-MS (positive mode) m/z: 451.29 $[M+Na]^+$, 879.59 $[2M+Na]^+$. HRMS calc. for $C_{21}H_{40}N_4O_5Na$: 451.2896, exp. 451.2891 (-1.1 ppm). Melting point: 177-178 °C (from ethyl acetate).

Boc-Gly-Gly-Gly-$C_{12}$: Yield: 56% $^1$H NMR (MeOD) δ: 0.90 (t, $^3J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.29 (m, 18 H, $CH_2$ chain), 1.45 (m, 9 H, $CH_3$ Boc), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^3J_{HH}$ =

7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.74 (s, 2 H, $CH_2$ glycine), 3.84 (s, 2 H, $CH_2$ glycine), 3.89 (s, 2 H, $CH_2$ glycine). $^{13}C$ NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.6-40.6 ($CH_2$ chain), 28.8 ($CH_3$ Boc), 43.5 ($CH_2$ glycine), 43.9 ($CH_2$ glycine), 45.0 ($CH_2$ glycine), 81.0 ($C_q$ $^{t}$Bu Boc), 158.8 (C=O Boc), 171.4 (C=O amide), 172.2 (C=0 amide), 173.7 (C=O amide). FTIR ($cm^{-1}$) ν: 1651 (C=O, amide), 1686 (C=O, amide), 2850-2955 (C-H chain), 3089-3293 (N-H). ESI-MS (positive mode) m/z: 457.34 $[M+H]^+$, 479.32 $[M+Na]^+$, 935.65 $[2M+Na]^+$. HRMS calc. for $C_{23}H_{45}N_4O_5$: 457.3390, exp. 457.3391 (+0.2 ppm). Melting point: 168-169 °C (from ethyl acetate).

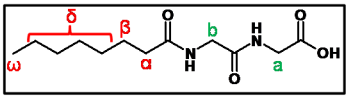
H-Gly-Gly-Gly-$C_8$ (**N4**): Yield: 32 % $^1H$ NMR (MeOD) δ: 0.89 (t, $^3J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.30 (m, 10 H, $CH_2$ chain), 1.50 (m, 2 H, $^{\beta}CH_2$ chain), 3.18 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.76 (s, 2 H, $CH_2$ glycine), 3.85 (s, 2 H, $CH_2$ glycine), 3.97 (s, 2 H, $CH_2$ glycine). $^{13}C$ NMR *J-Mod* (MeOD) δ: : 14.3 ($CH_3$ chain), 23.6-40.5 ($CH_2$ chain), 41.5 ($CH_2$ glycine), 43.3 ($CH_2$ glycine), 43.4 ($CH_2$ glycine), 168.2 (C=O amide), 171.2 (C=O amide), 171.7 (C=O amide) FTIR ($cm^{-1}$) ν: 1651 (C=O, amide), 2848-2955 (C-H chain), 3087-3368 (N-H). ESI-MS (positive mode) m/z: 301.2 $[M+H]^+$, 323.2 $[M+Na]^+$. HRMS calc. for $C_{14}H_{29}N_4O_3$: 301.2240, exp. 301.2239 (-0.3 ppm). Melting point: 201-202 °C (from methanol/water).

H-Gly-Gly-Gly-$C_{10}$ (**N5**): Yield: 85 % $^1H$ NMR (MeOD) δ: 0.90 (t, $^3J_{HH}$ = 7.2 Hz 3 H, $CH_3$ chain), 1.30 (m, 14 H, $CH_2$ chain), 1.51 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^3J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.75 (s, 2 H, $CH_2$ glycine), 3.85 (s, 2 H, $CH_2$ glycine), 3.98 (s, 2 H, $CH_2$ glycine). $^{13}C$ NMR *J-Mod* (MeOD) δ: 14.5 ($CH_3$ chain), 23.8-40.6 ($CH_2$ chain), 41.6 ($CH_2$ glycine), 43.4 ($CH_2$ glycine), 43.5 ($CH_2$ glycine), 168.3 (C=O amide), 171.3 (C=O amide), 171.8 (C=O amide). FTIR ($cm^{-1}$) ν: 1652 (C=O, amide), 2849-2959 (C-H chain), 3086-3366 (N-H). ESI-MS (positive mode) m/z: 329.25 $[M+H]^+$. HRMS calc. for $C_{16}H_{33}N_4O_3$: 329.2553, exp. 329.2552 (-0.3 ppm). Melting point: 204-205 °C (from methanol/water).

H-Gly-Gly-Gly-$C_{12}$ (**N6**): Yield: 63% $^{1}$H NMR (MeOD) δ: 0.89 (t, $^{3}J_{HH}$ = 6.9 Hz 3 H, $CH_3$ chain), 1.28 (m, 18 H, $CH_2$ chain), 1.52 (m, 2 H, $^{\beta}CH_2$ chain), 3.19 (t, $^{3}J_{HH}$ = 7.2 Hz, 2 H, $^{\alpha}CH_2$ chain), 3.75 (s, 2 H, $CH_2$ glycine), 3.85 (s, 2 H, $CH_2$ glycine), 3.98 (s, 2 H, $CH_2$ glycine). $^{13}$C NMR *J-Mod* (MeOD) δ: 24.0 ($CH_3$ chain), 32.3-41.5 ($CH_2$ chain), 50.2 ($CH_2$ glycine), 52.0 ($CH_2$ glycine), 52.1 ($CH_2$ glycine), 176.6 (C=O amide), 179.1 (C=O amide), 179.4 (C=O amide). FTIR ($cm^{-1}$) ν: 1651 (C=O, amide), 2850-2956 (C-H chain), 3093-3369 (N-H). ESI-MS (positive mode) m/z: 357.29 $[M+H]^+$, 380.27 $[M+Na]^+$, 714.57 $[2M+H]^+$. HRMS calc. for $C_{18}H_{37}N_4O_3$: 357.2866, exp. 357.2857 (-2.5 ppm). Melting point: 202-203 °C (from methanol/water).

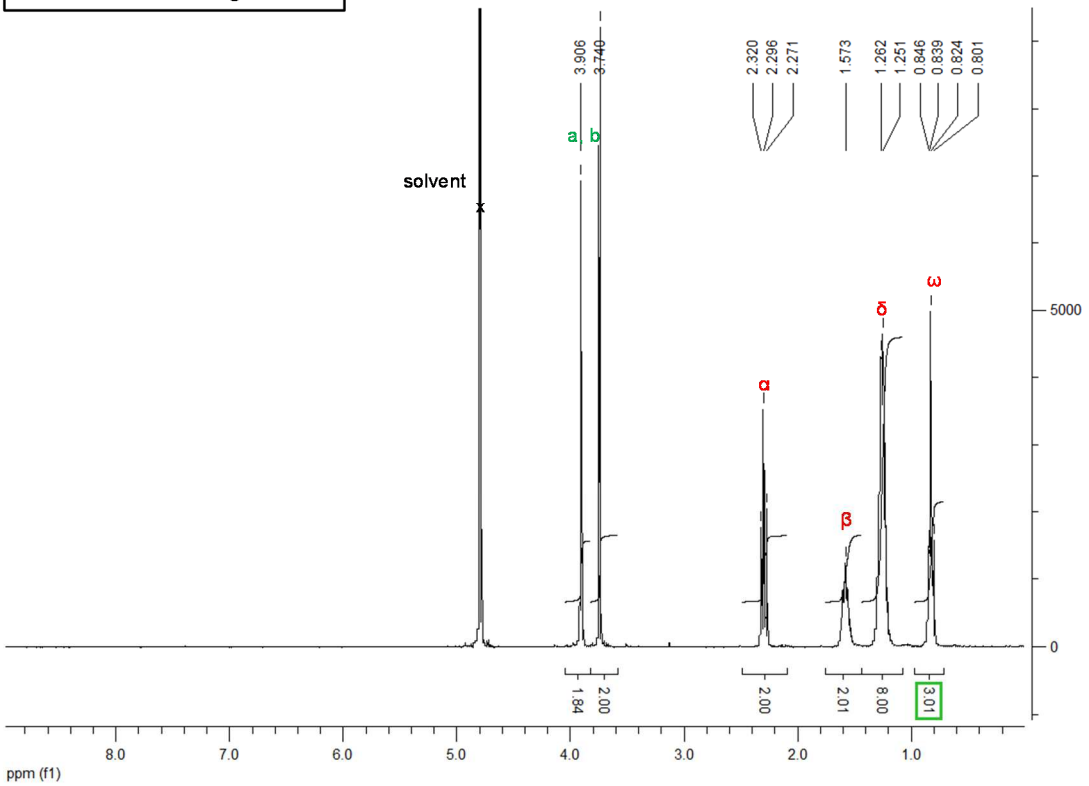
$C_{10}$-Gly-Gly-His-OH / H-His-Gly-Gly-$C_{10}$ (**C2N2**): $^{1}$H NMR ($D_2O$ + MeOD) δ: 0.85 (m, 6 H, $CH_3$ chain), 1.25 (m, 26 H, $CH_2$ chain), 1.49-1.59 (m, 4 H, $^{\beta}CH_2$ chain), 2.31 (m, 2 H, $^{\alpha}CH_2$ chain), 3.19 (m, 3 H, $^{\alpha'}CH_2$ chain + $CH_2$ histidine c), 3.45 (m, 2 H, $CH_2$ histidine c'), 3.99 (m, 8 H, $CH_2$ glycines), 4.37 (t, $^{3}J_{HH}$ = 6.3 Hz 1 H, CH histidine d'), 4.63 (m, 1 H, CH histidine d), 7.29 (s, 1H, =CH imidazole), 7.49 (s, 1H, =CH imidazole), 8.62 (s, 1H, =CH imidazole), 8.73 (s, 1H, =CH imidazole). $^{13}$C NMR *J-Mod* (MeOD) δ: 13.3 ($CH_3$ chain), 22.1-39.4 ($CH_2$ chain), 42.2 ($CH_2$ glycine), 42.5 ($CH_2$ glycine), 51.9 (CH histidine), 52.7 (CH histidine), 117.0 (=CH imidazole), 118.7 (=CH imidazole), 125.6 ($C_q$ imidazole), 129.2 ($C_q$ imidazole), 168.5 (C=O amide), 170.52 (C=O amide), 170.72 (C=O amide), 171.2 (C=O amide), 172.1 (C=O amide), 174.1 (C=O amide), 177.8 (C=O carboxylate). FTIR ($cm^{-1}$) ν: 1664-1665 (C=O, amide), 2854-2925 (C-H chain), 3084-3296 (N-H). Melting point: 111-112 °C (from water).

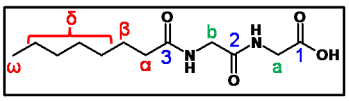
## 2. NMR spectra




**$C_8$-Gly-Gly-OH**

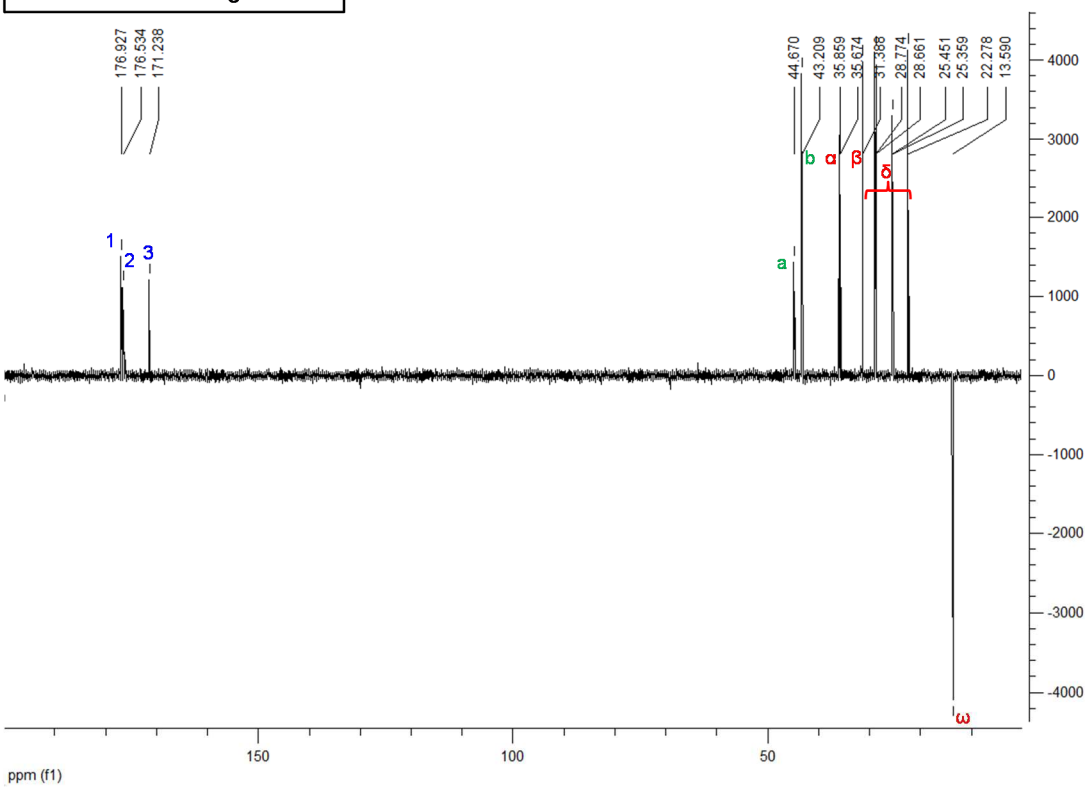
$^1$H NMR ($D_2O$ + NaOD), 300 MHz

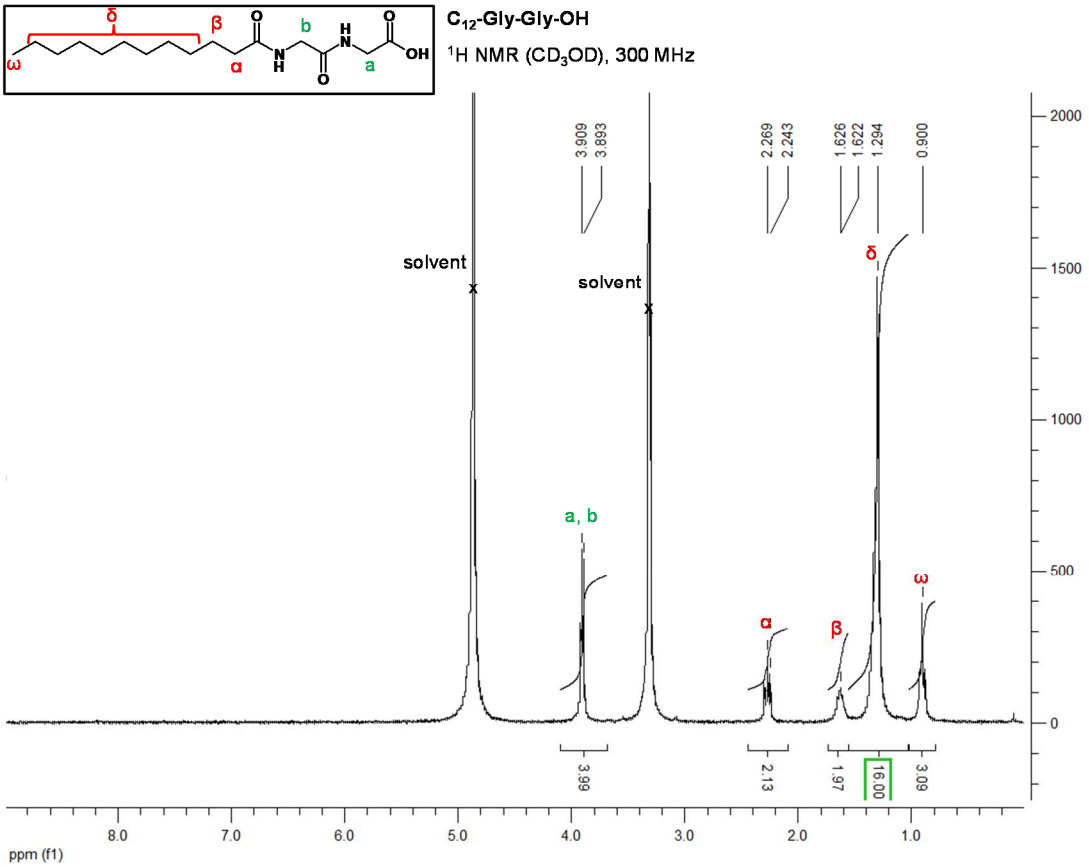


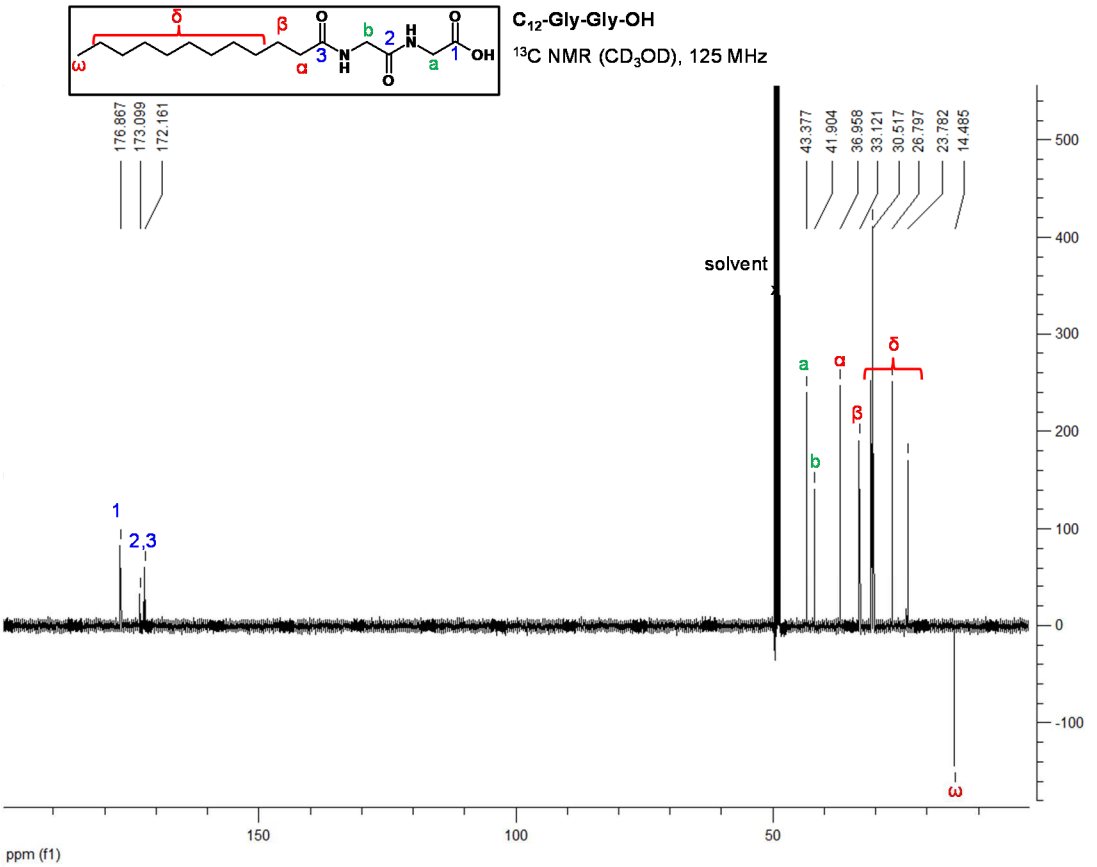




**$C_8$-Gly-Gly-OH**

$^{13}$C NMR ($D_2O$ + NaOD), 75 MHz




δ
β
b
ω
α
a
OH
C12-Gly-Gly-OH
1H NMR (CD3OD), 300 MHz
3.909
3.893
2.269
2.243
1.626
1.622
1.294
0.900
solvent
solvent
δ
a, b
α
β
ω
3.99
2.13
1.97
16.00
3.09
ppm (f1)


δ
β
b
2
ω
3
α
a
1
OH
C12-Gly-Gly-OH
13C NMR (CD3OD), 125 MHz
176.867
173.099
172.161
43.377
41.904
36.958
33.121
30.517
26.797
23.782
14.485
solvent
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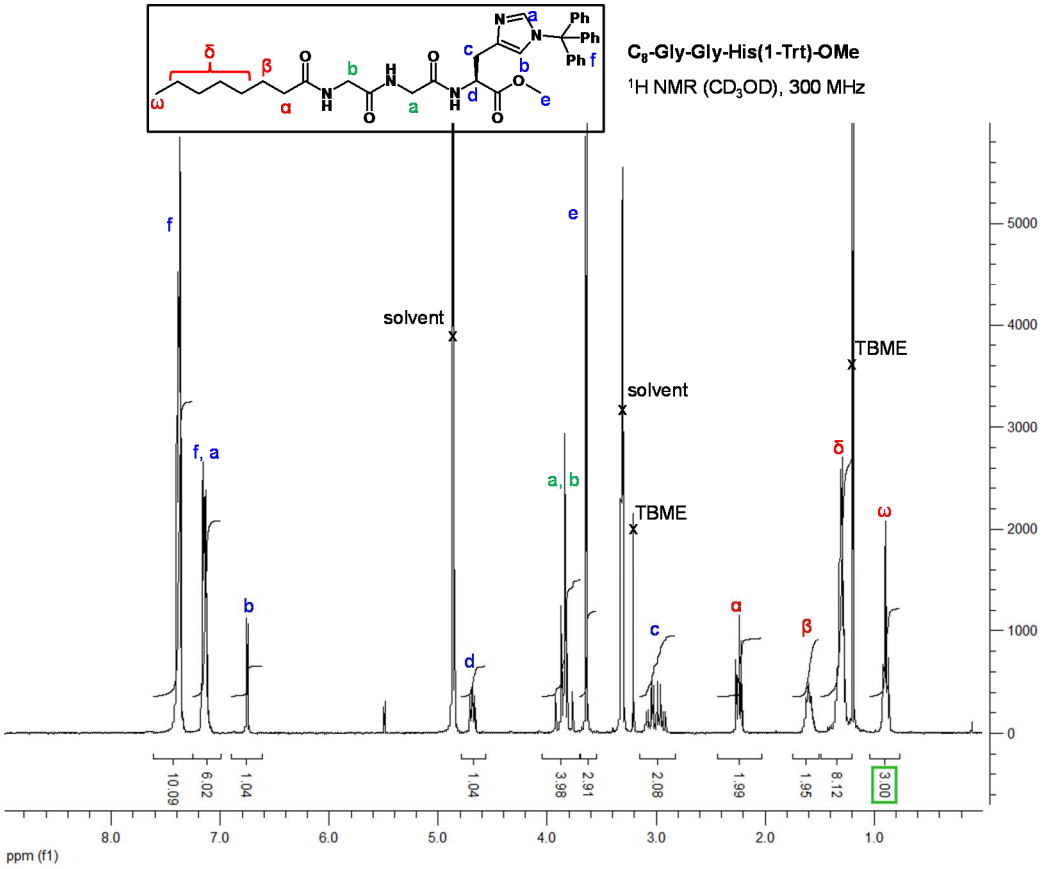
α
δ
β
b
1
2,3
ω
ppm (f1)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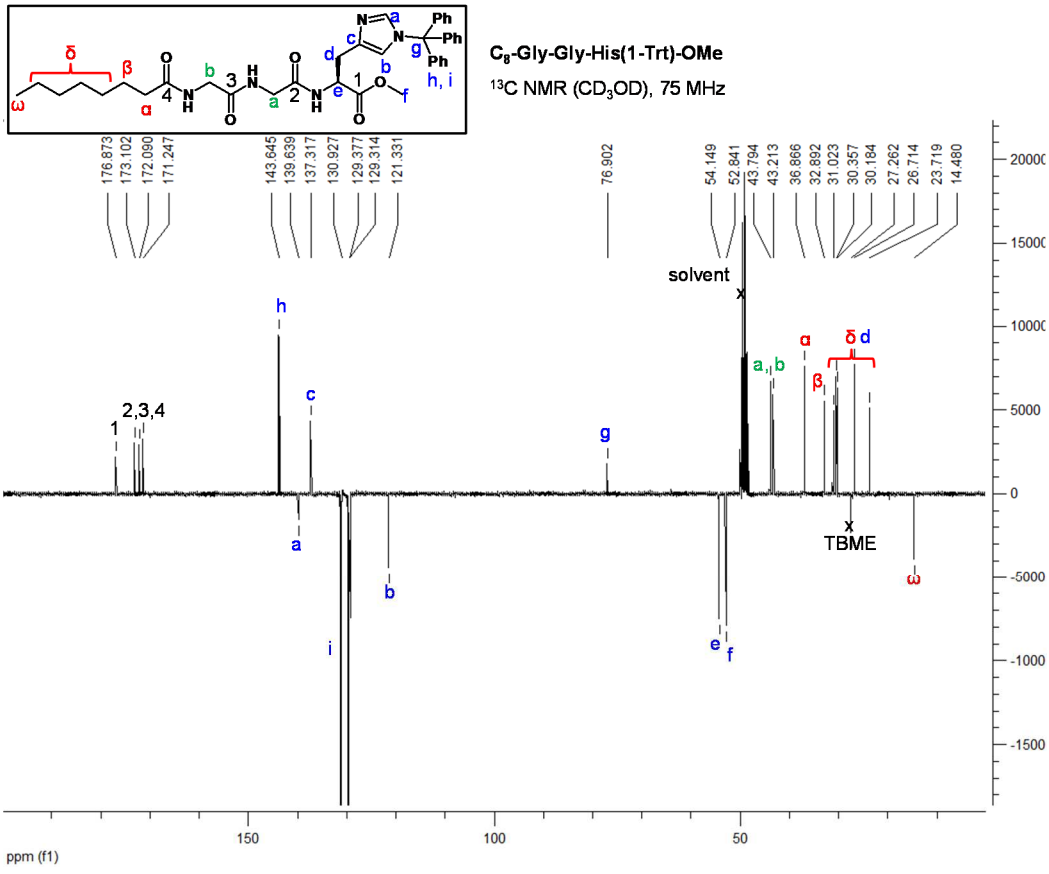
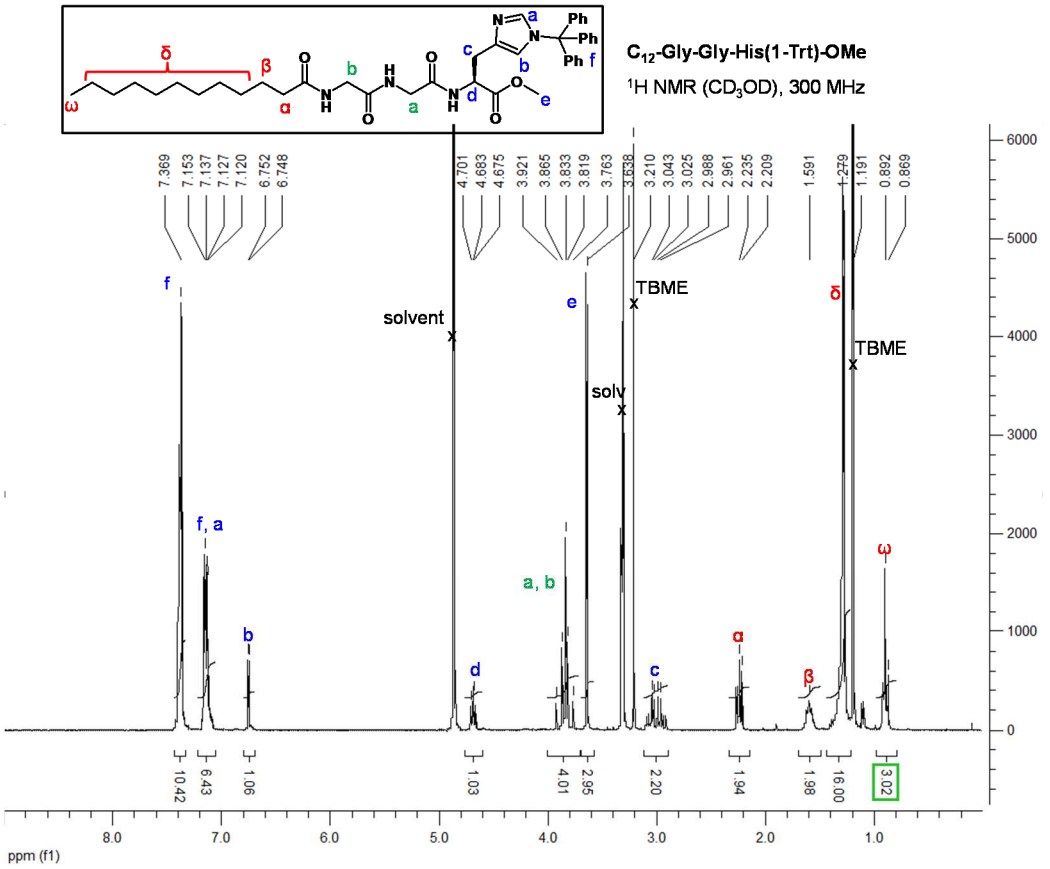
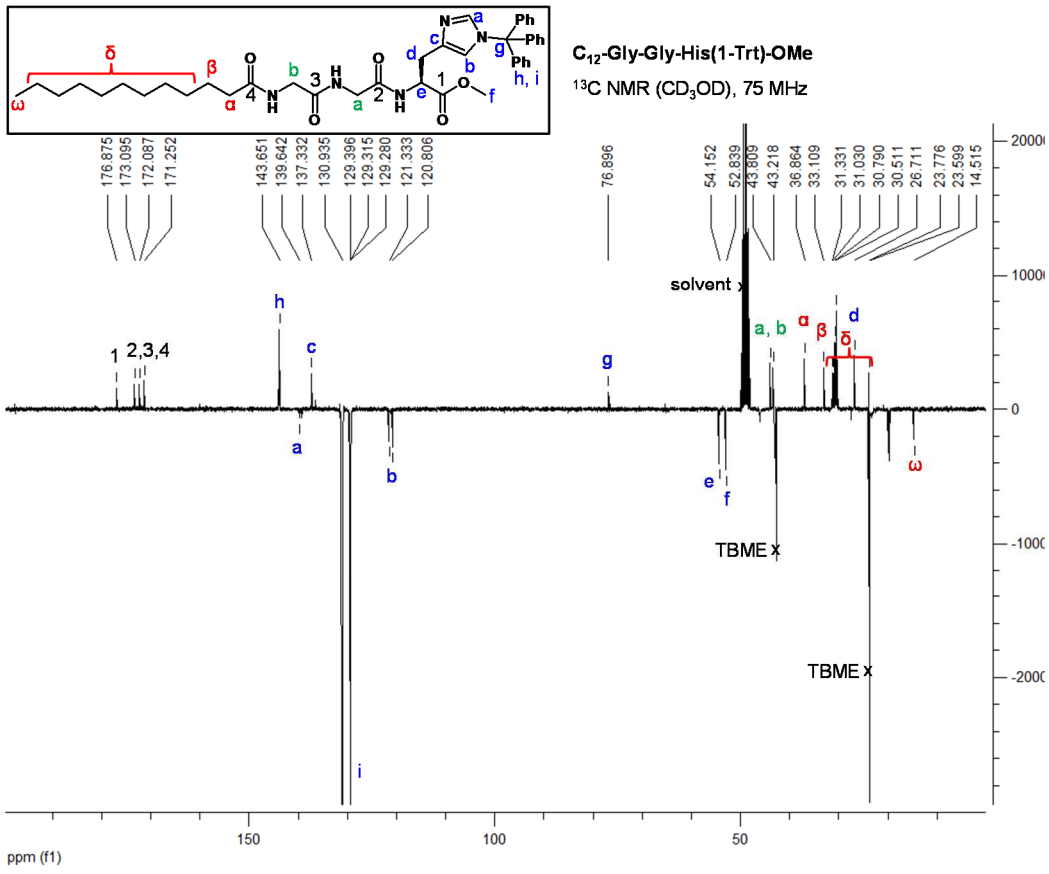
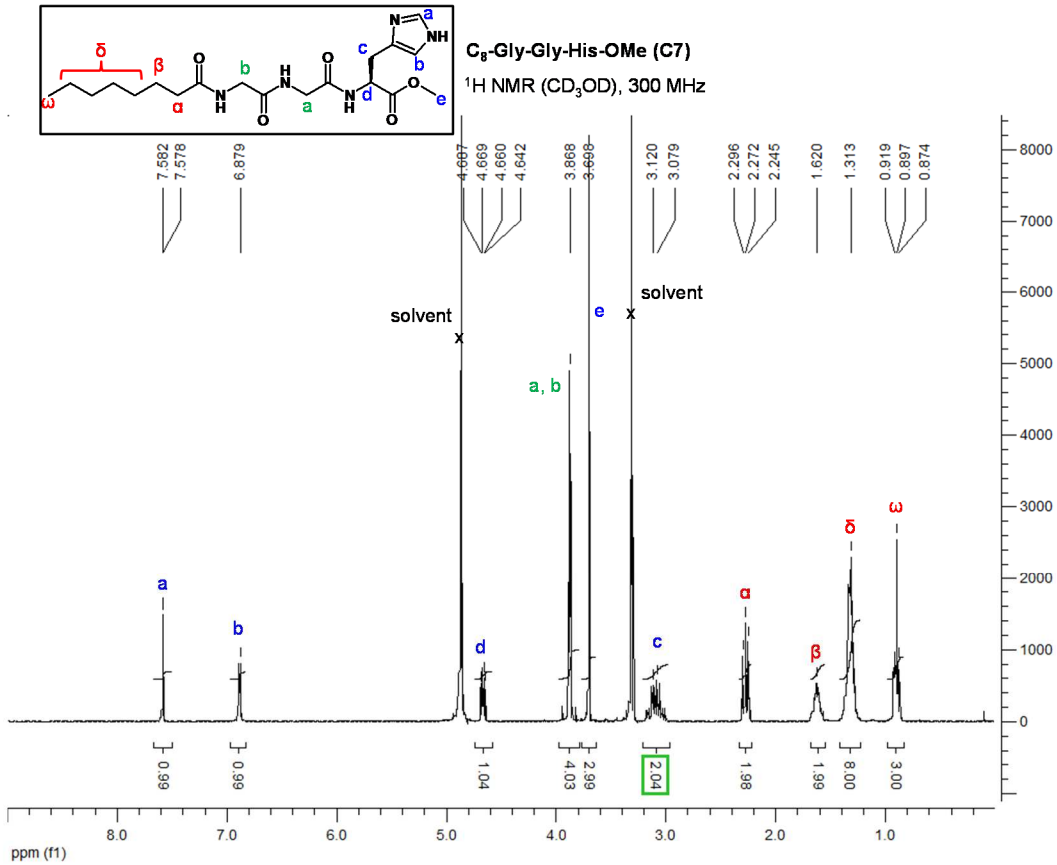
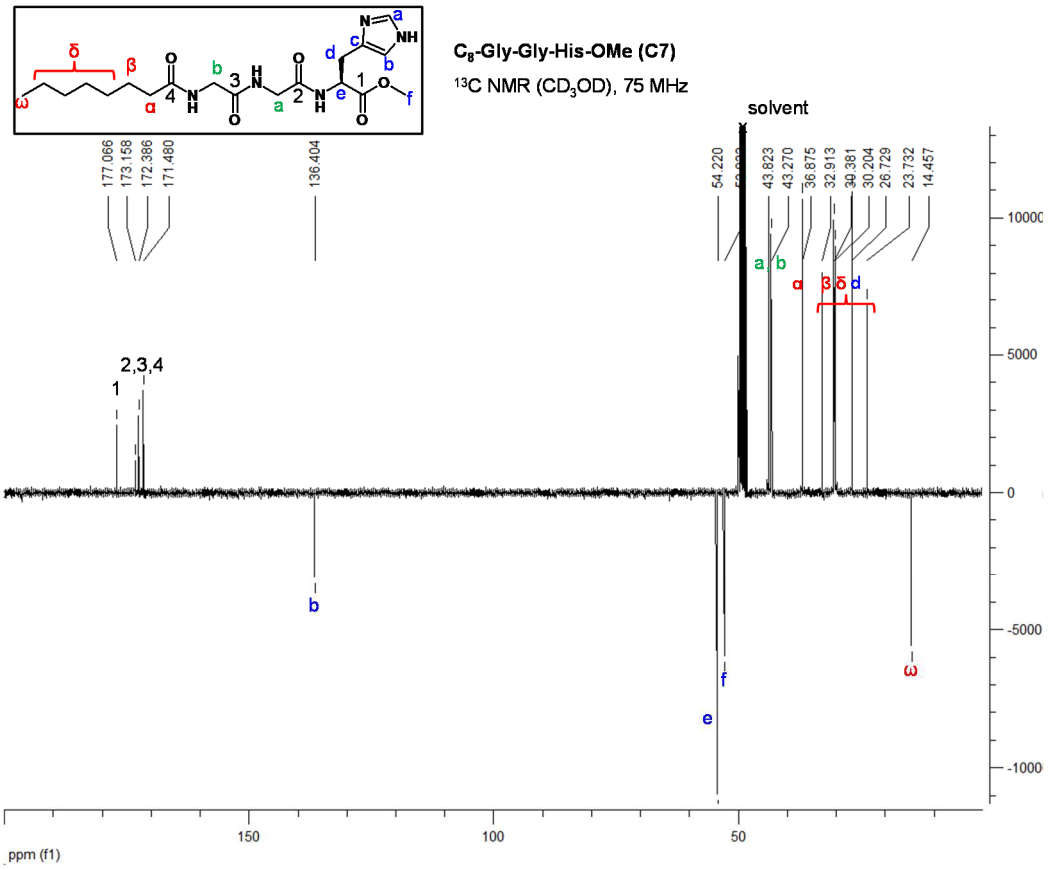
solvent
TBME
ppm (f1)

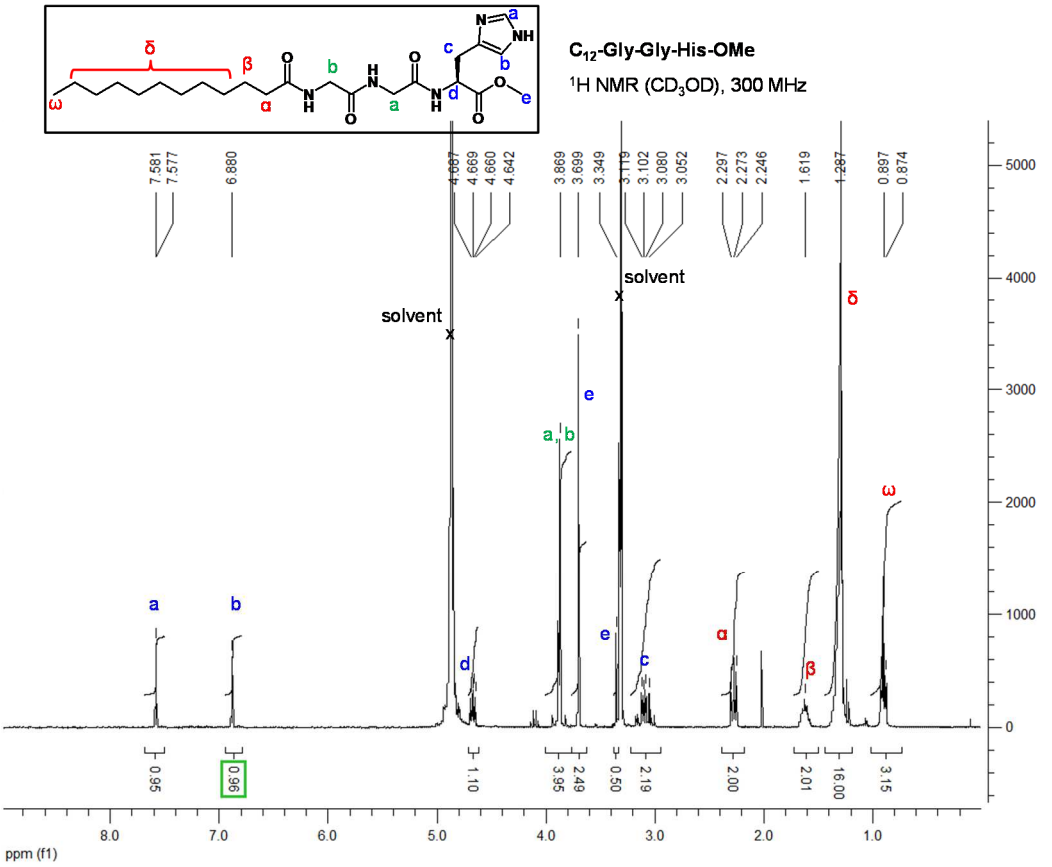

C8-Gly-Gly-His(1-Trt)-OMe
13C NMR (CD3OD), 75 MHz
solvent
TBME
ppm (f1)

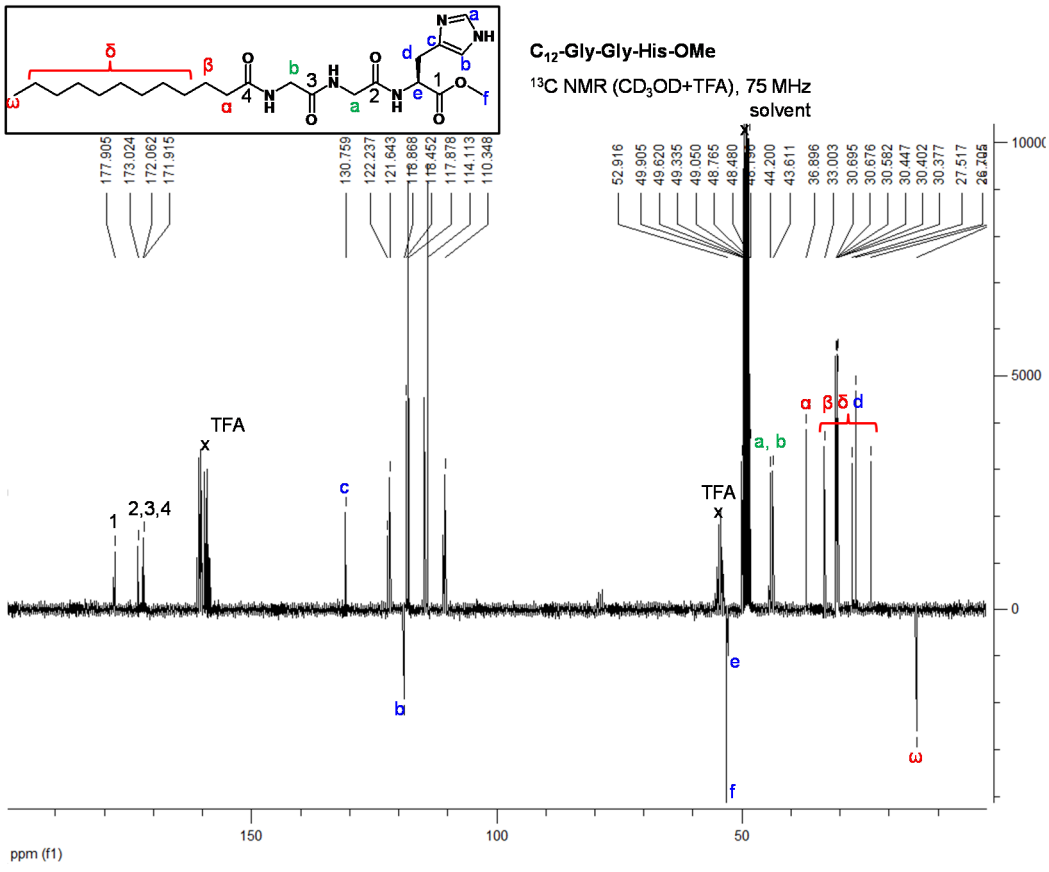

C12-Gly-Gly-His(1-Trt)-OMe
1H NMR (CD3OD), 300 MHz
solvent
TBME
solv
f, a
a, b
ppm (f1)

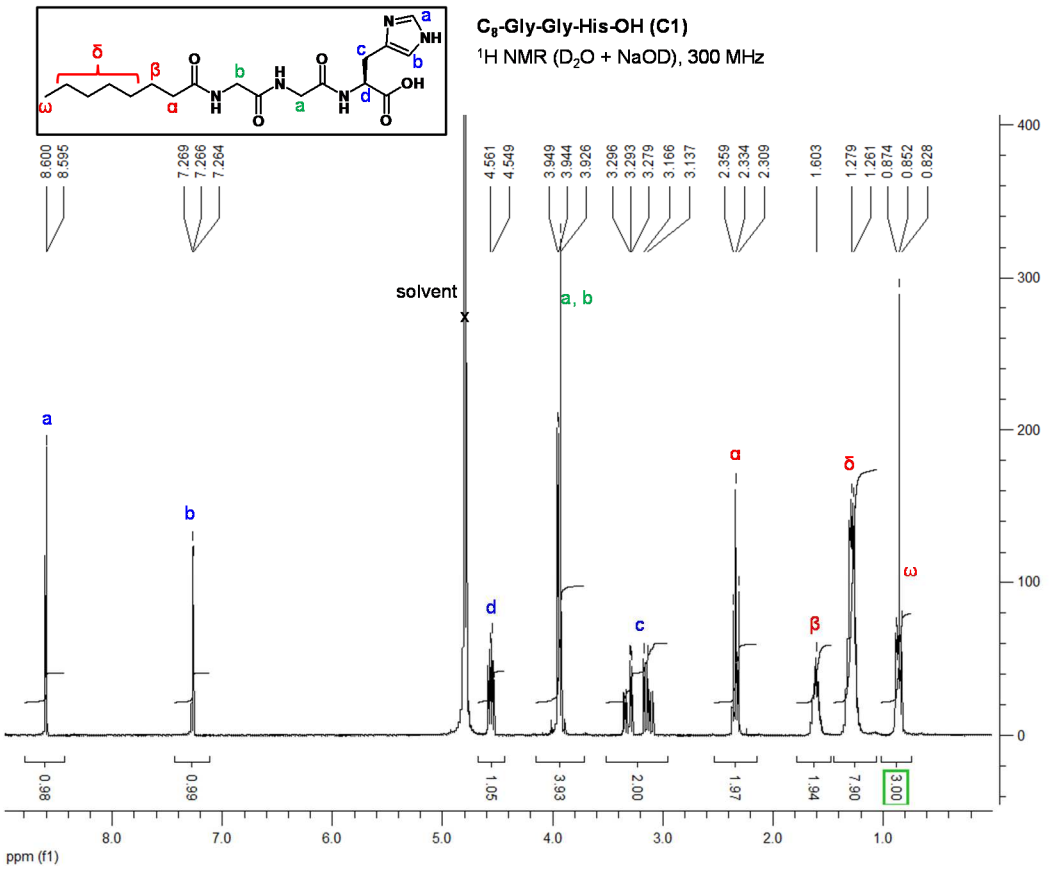

C12-Gly-Gly-His(1-Trt)-OMe
13C NMR (CD3OD), 75 MHz
solvent
TBME
2,3,4
a, b
ppm (f1)

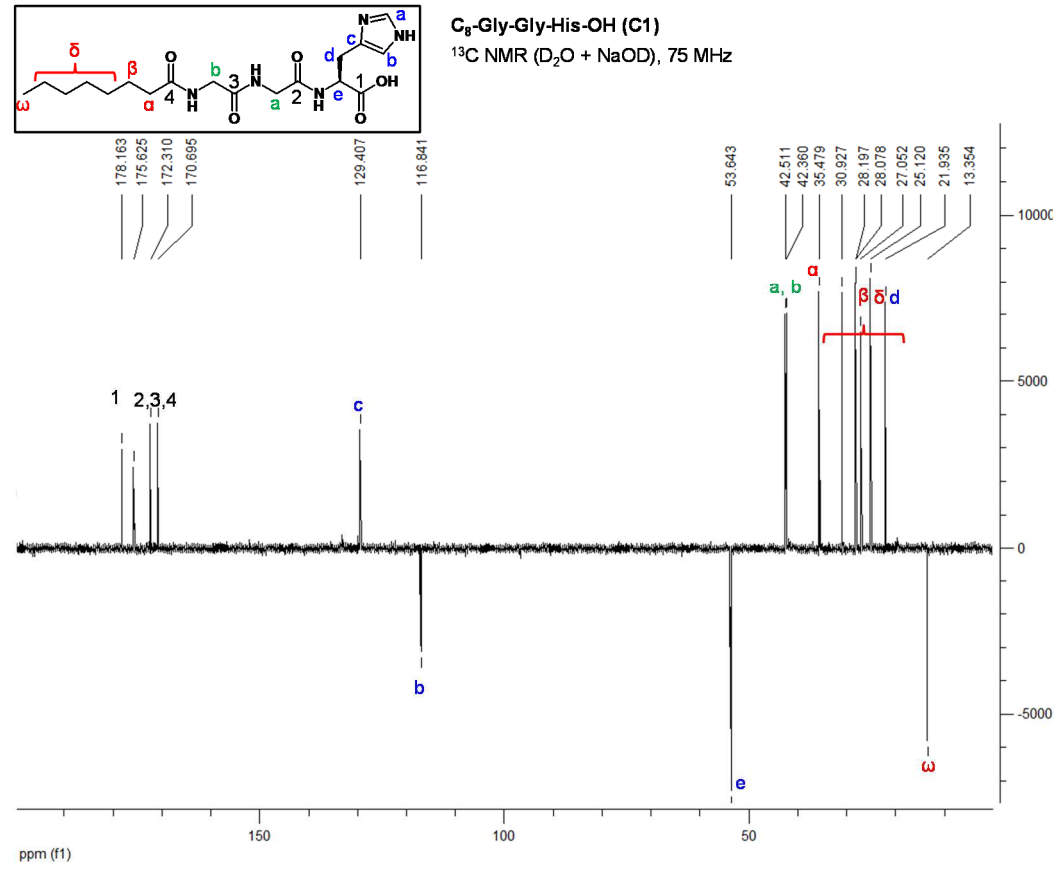

C8-Gly-Gly-His-OMe (C7)
1H NMR (CD3OD), 300 MHz
solvent
solvent
ppm (f1)

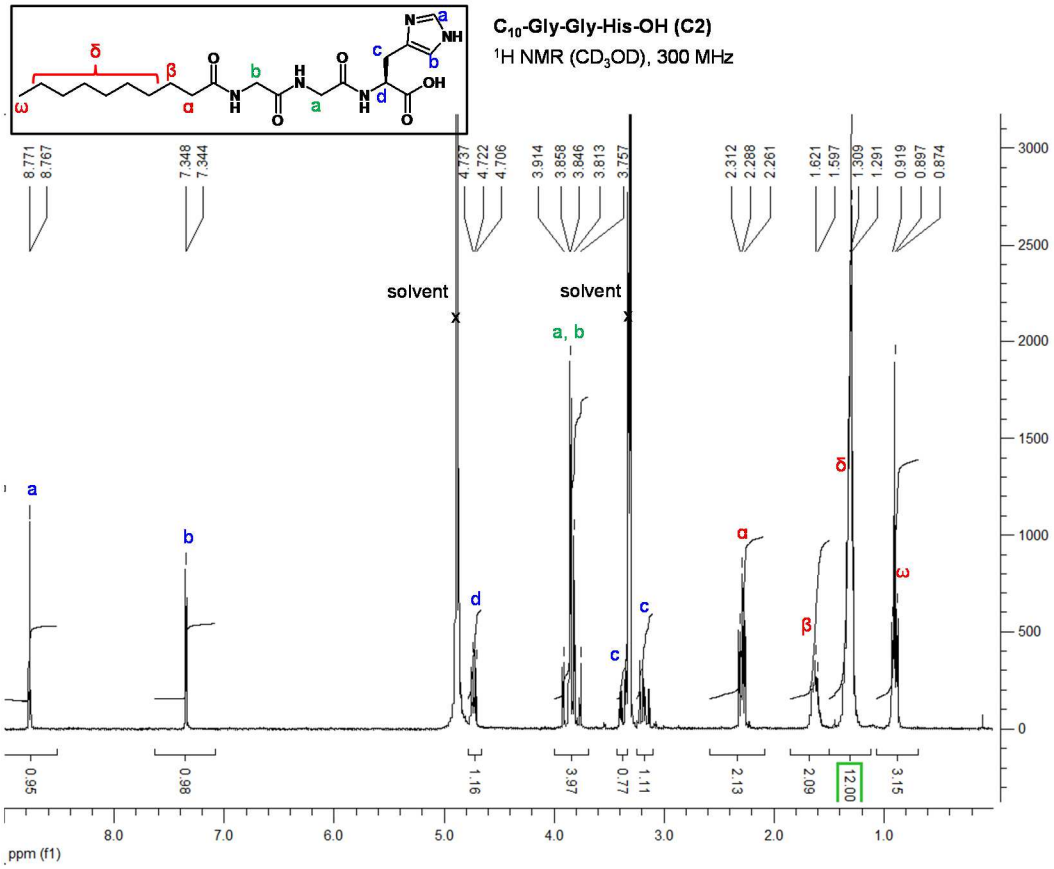

C8-Gly-Gly-His-OMe (C7)
13C NMR (CD3OD), 75 MHz
solvent
2,3,4
1
ppm (f1)

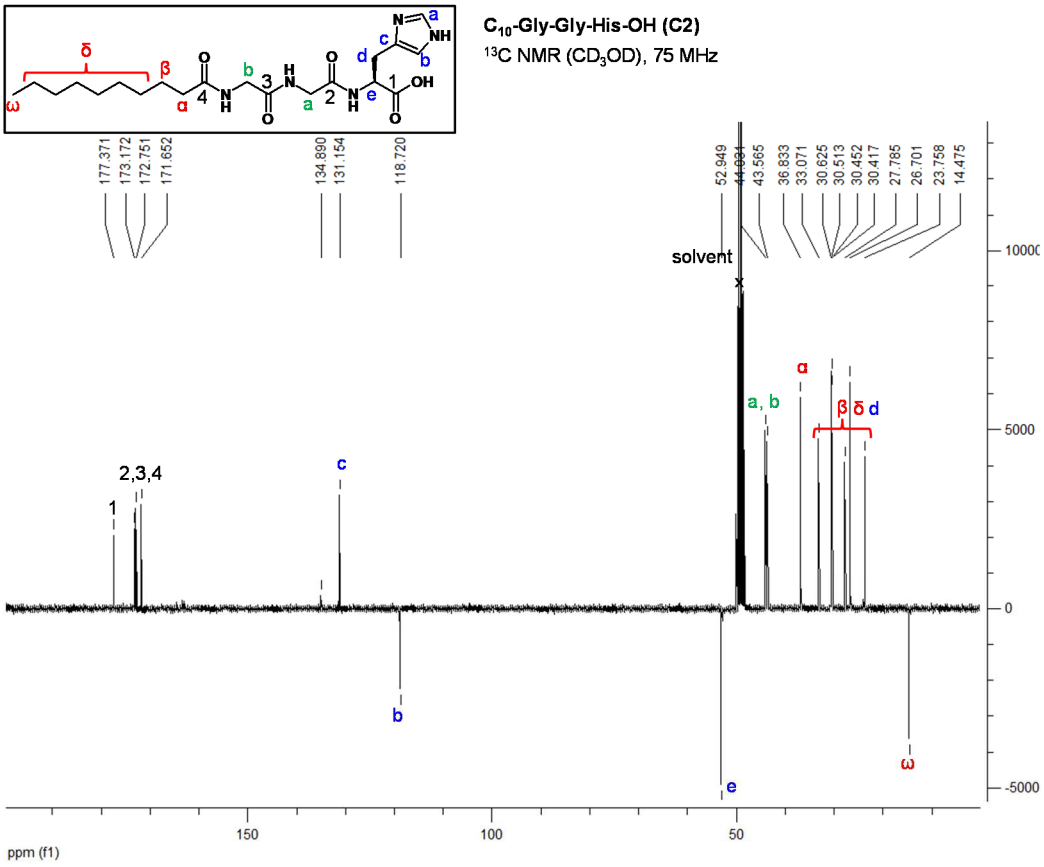

C12-Gly-Gly-His-OMe
1H NMR (CD3OD), 300 MHz
solvent
solvent
ppm (f1)

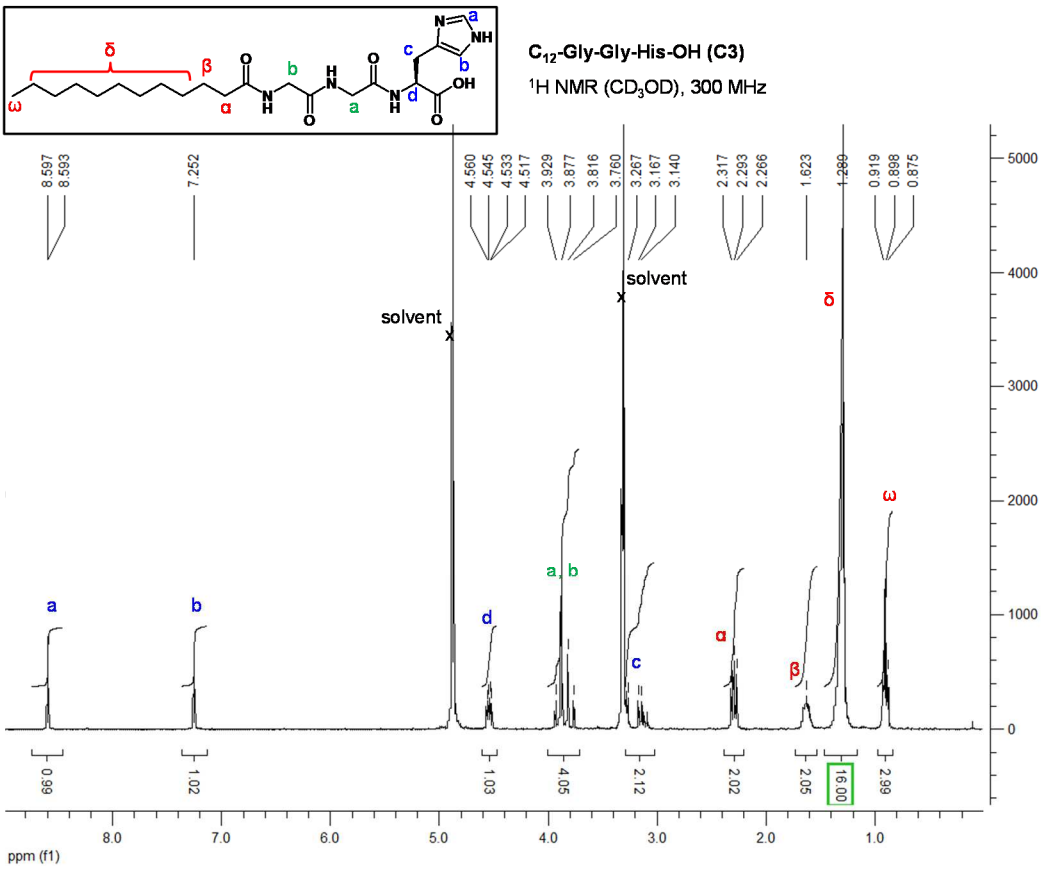

C12-Gly-Gly-His-OMe
13C NMR (CD3OD+TFA), 75 MHz
solvent
TFA
TFA
ppm (f1)

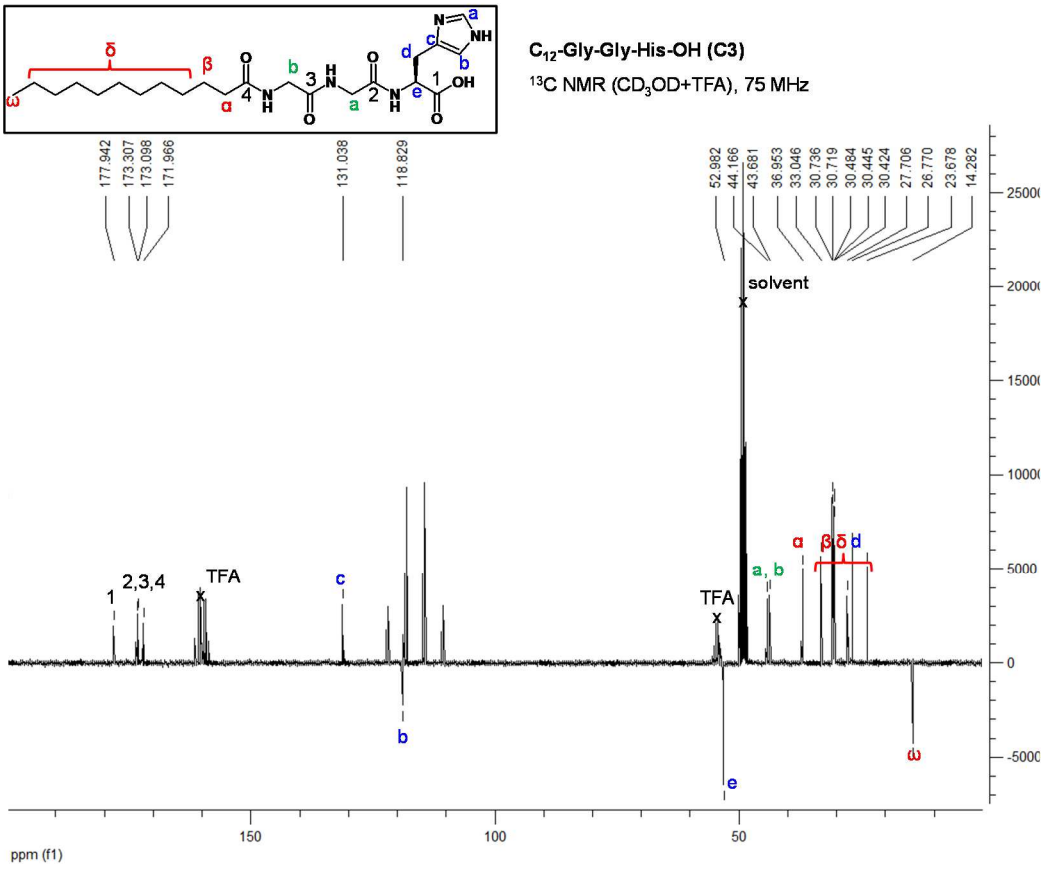

C8-Gly-Gly-His-OH (C1)
1H NMR (D2O + NaOD), 300 MHz
solvent
ppm (f1)

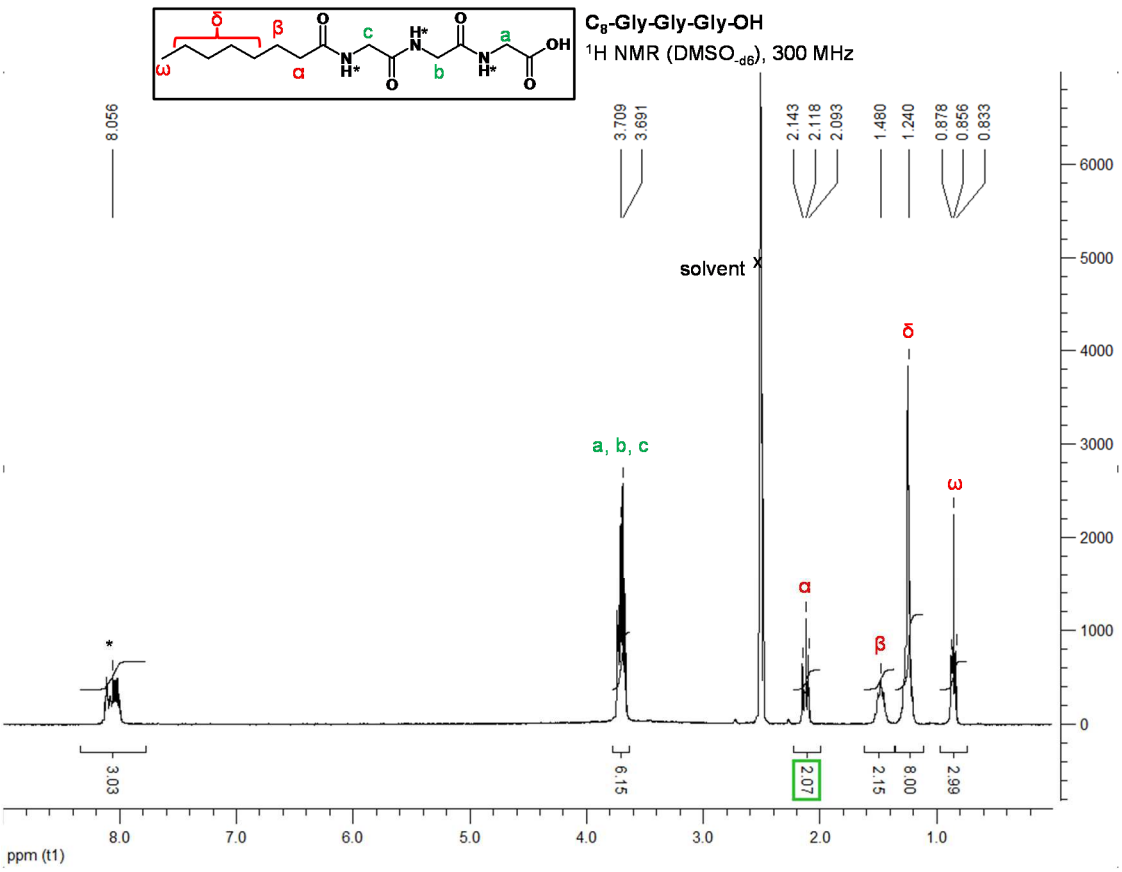

C8-Gly-Gly-His-OH (C1)
13C NMR (D2O + NaOD), 75 MHz
ppm (f1)

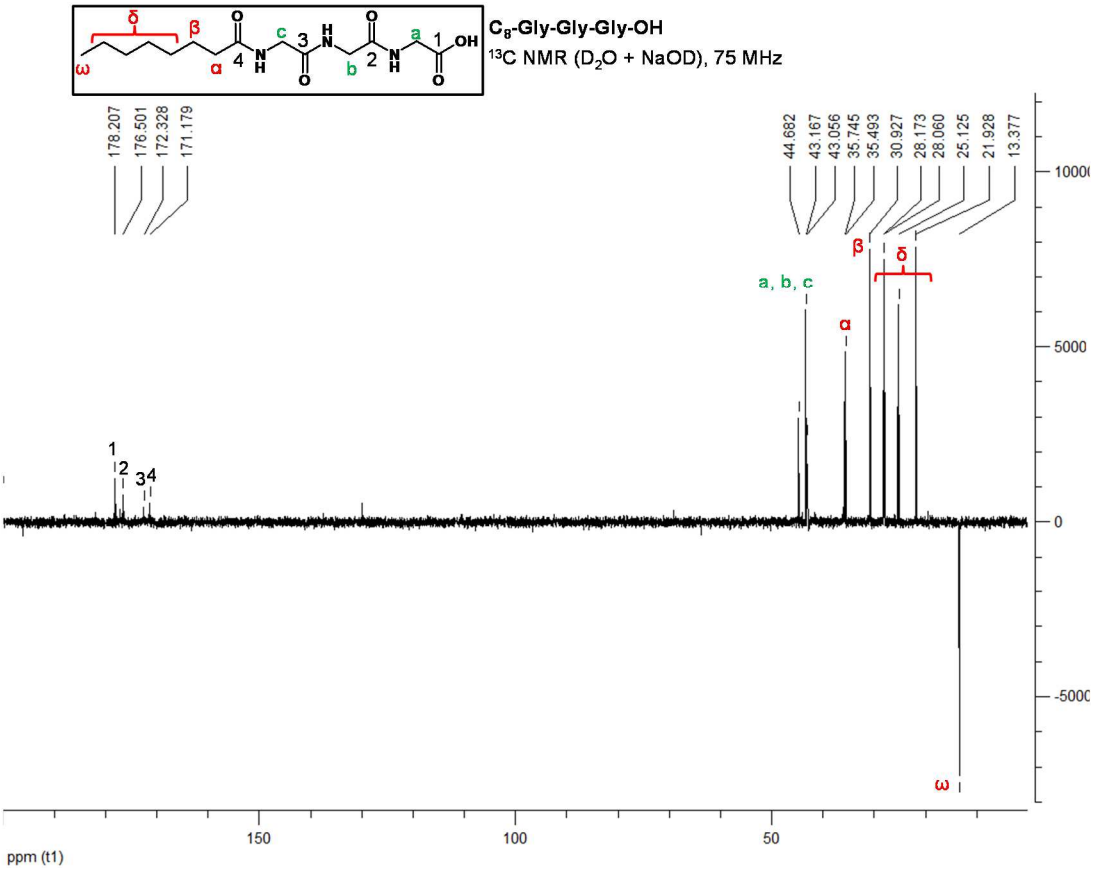

C10-Gly-Gly-His-OH (C2)
1H NMR (CD3OD), 300 MHz
solvent
solvent
ppm (f1)

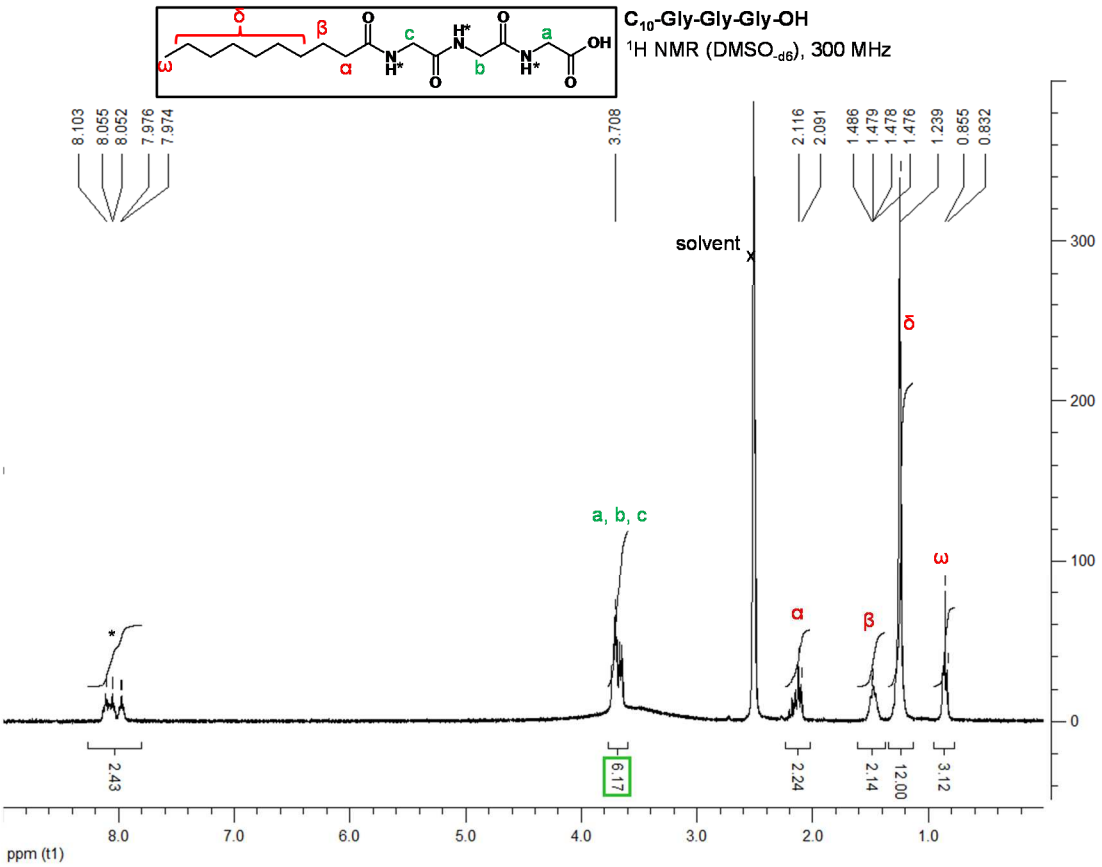

C10-Gly-Gly-His-OH (C2)
13C NMR (CD3OD), 75 MHz
solvent
ppm (f1)

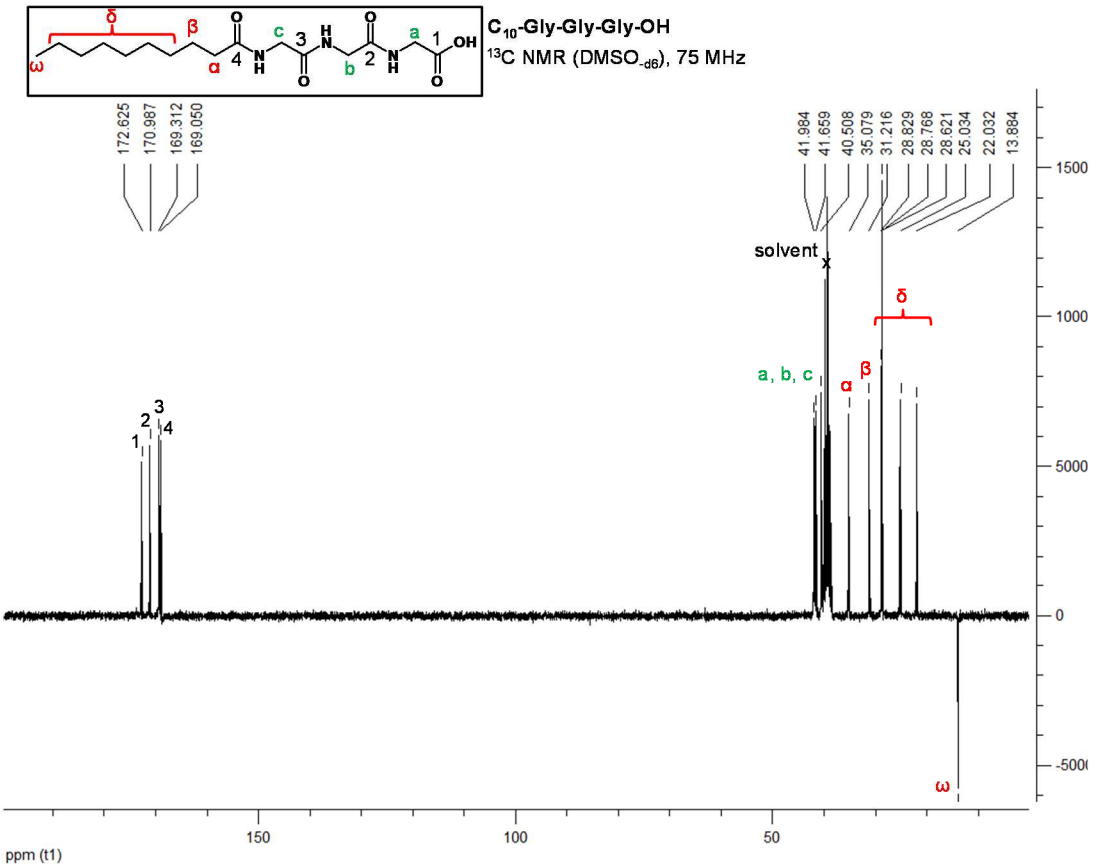

C12-Gly-Gly-His-OH (C3)
1H NMR (CD3OD), 300 MHz
solvent
solvent
ppm (f1)

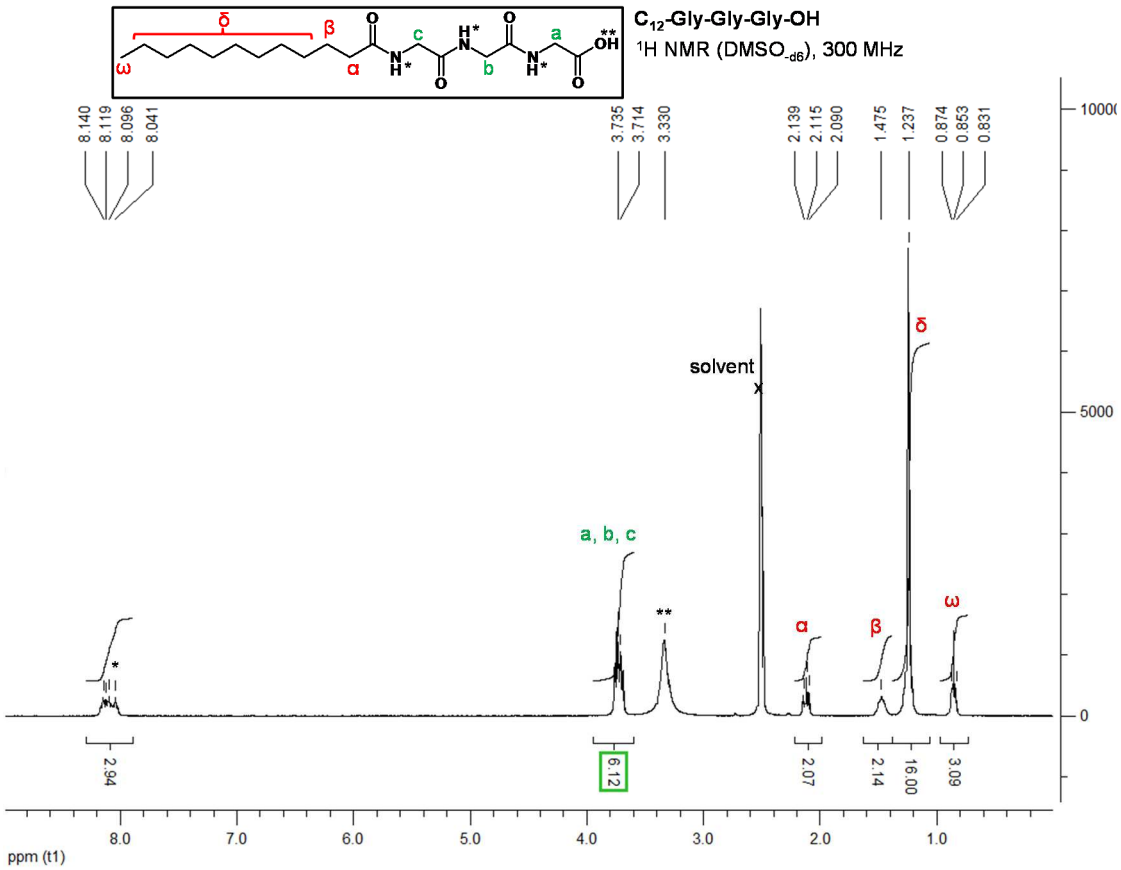

C12-Gly-Gly-His-OH (C3)
13C NMR (CD3OD+TFA), 75 MHz
solvent
TFA
TFA
ppm (f1)

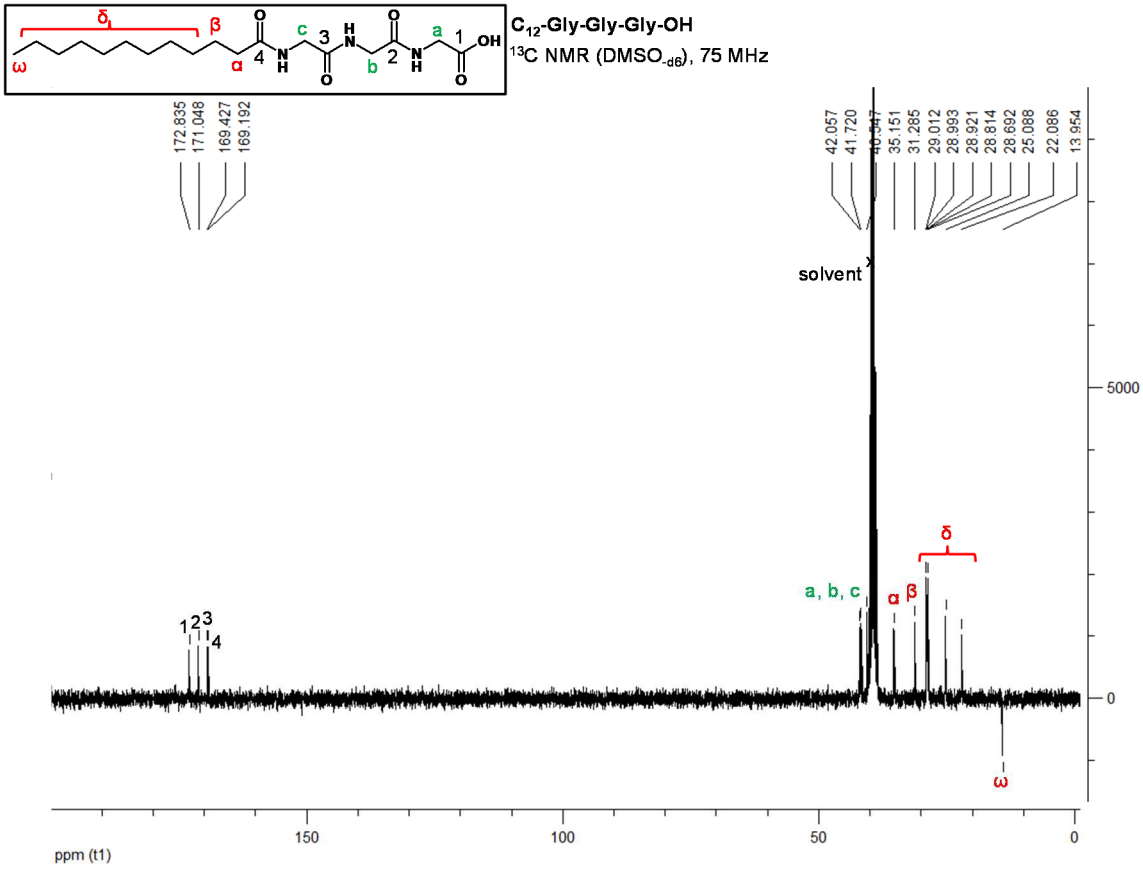

C8-Gly-Gly-Gly-OH
1H NMR (DMSO-d6), 300 MHz
8.056
3.709
3.691
2.143
2.118
2.093
1.480
1.240
0.878
0.856
0.833
solvent
a, b, c
δ
ω
α
β
*
3.03
6.15
2.07
2.15
8.00
2.99
ppm (t1)

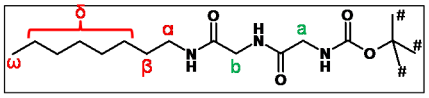

C8-Gly-Gly-Gly-OH
13C NMR (D2O + NaOD), 75 MHz
178.207
176.501
172.328
171.179
44.682
43.167
43.056
35.745
35.493
30.927
28.173
28.060
25.125
21.928
13.377
a, b, c
α
β
δ
ω
1
2
3
4
ppm (t1)


C10-Gly-Gly-Gly-OH
1H NMR (DMSO-d6), 300 MHz
8.103
8.055
8.052
7.976
7.974
3.708
2.116
2.091
1.486
1.479
1.478
1.476
1.239
0.855
0.832
solvent
a, b, c
2.43
6.17
2.24
2.14
12.00
3.12
ppm (t1)


C10-Gly-Gly-Gly-OH
13C NMR (DMSO-d6), 75 MHz
172.625
170.987
169.312
169.050
41.984
41.659
40.508
35.079
31.216
28.829
28.768
28.621
25.034
22.032
13.884
solvent
a, b, c
ppm (t1)

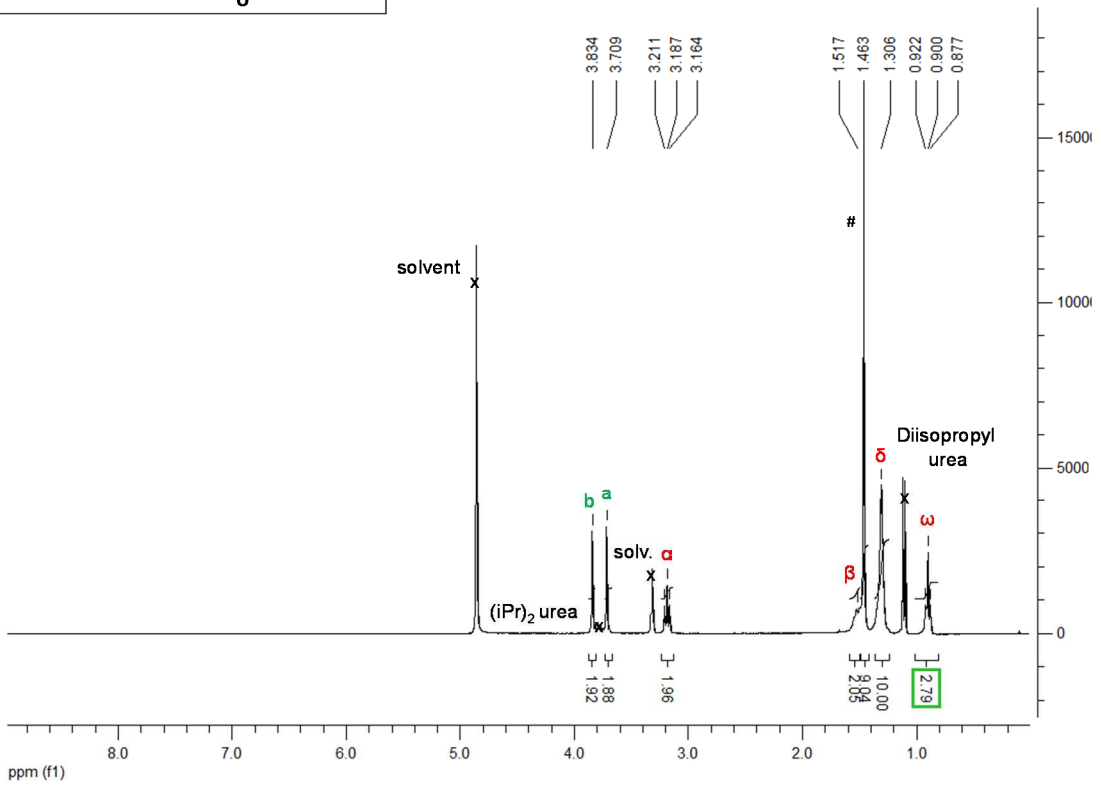

δ
β
ω
α
c
b
a
OH
C12-Gly-Gly-Gly-OH
1H NMR (DMSO-d6), 300 MHz
8.140
8.119
8.096
8.041
3.735
3.714
3.330
2.139
2.115
2.090
1.475
1.237
0.874
0.853
0.831
solvent
a, b, c
**
*
α
β
δ
ω
2.94
6.12
2.07
2.14
16.00
3.09
ppm (t1)

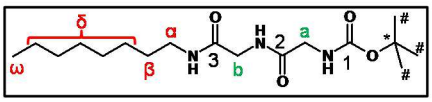

δ
β
ω
α
c
b
a
OH
C12-Gly-Gly-Gly-OH
13C NMR (DMSO-d6), 75 MHz
172.835
171.048
169.427
169.192
42.057
41.720
40.047
35.151
31.285
29.012
28.993
28.921
28.814
28.692
25.088
22.086
13.954
solvent
a, b, c
α
β
δ
ω
1
2
3
4
ppm (t1)



**Boc-Gly-Gly-$C_8$**

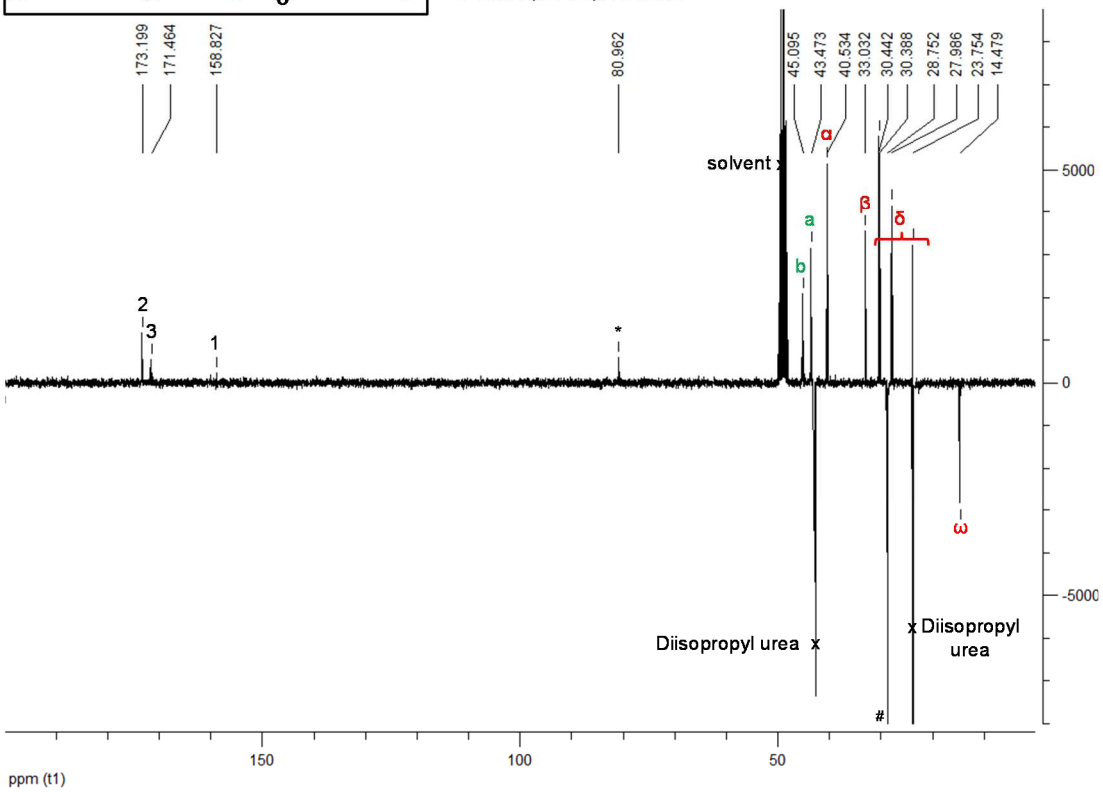
$^1$H NMR (MeOD), 300 MHz

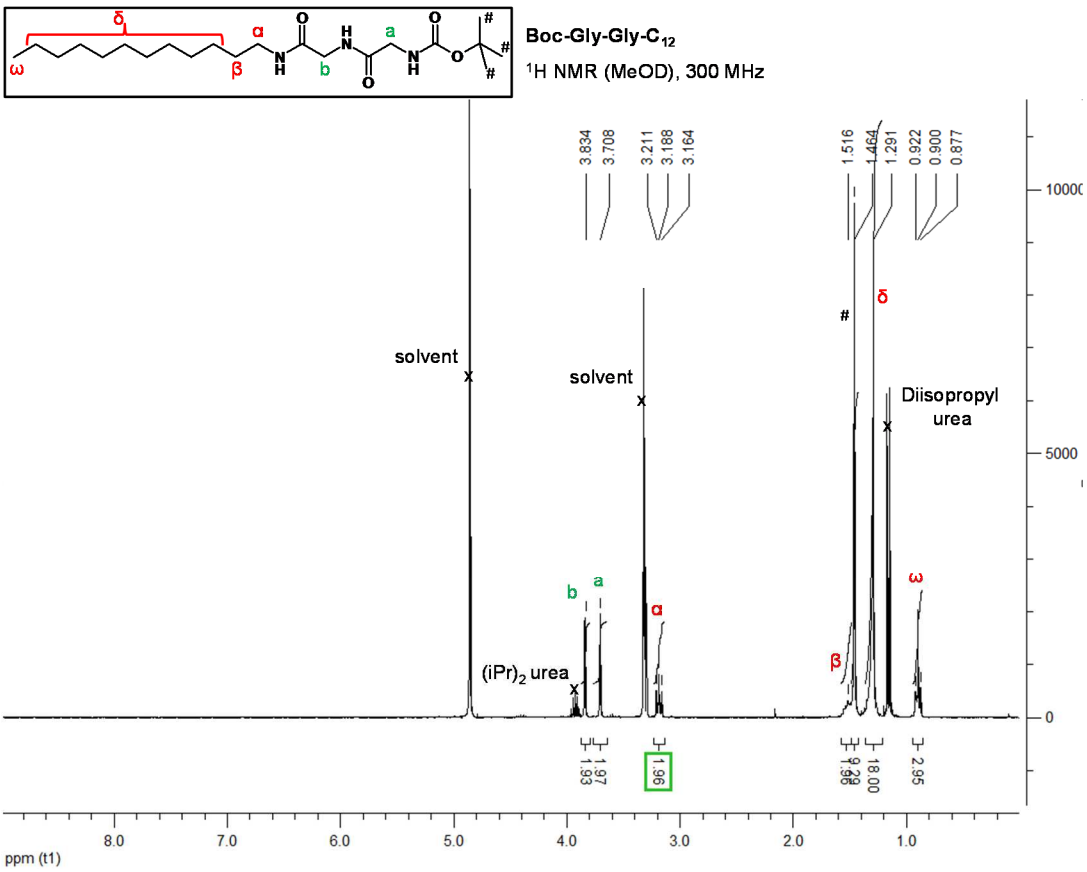


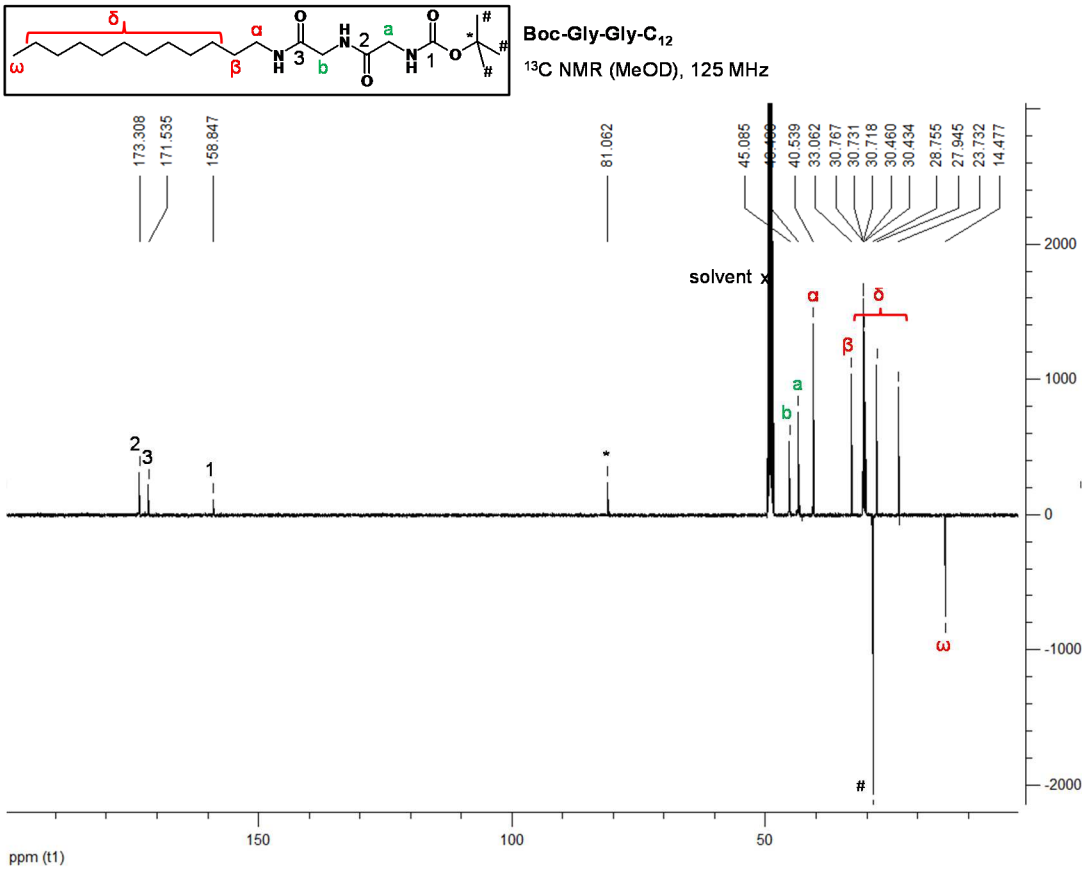



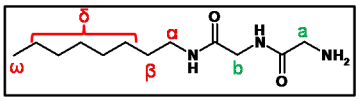
**Boc-Gly-Gly-$C_8$**

$^{13}$C NMR (MeOD), 75 MHz



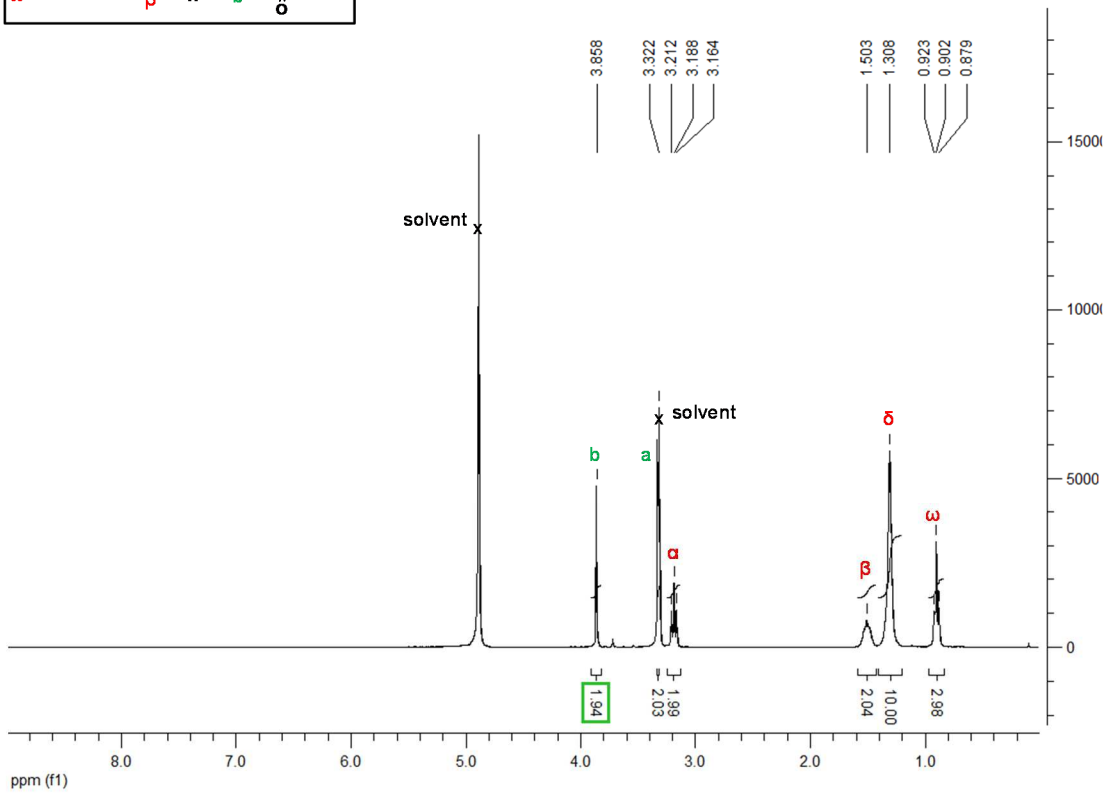

Boc-Gly-Gly-C12
1H NMR (MeOD), 300 MHz
solvent
solvent
(iPr)2 urea
Diisopropyl urea
3.834
3.708
3.211
3.188
3.164
1.516
1.464
1.291
0.922
0.900
0.877
1.97
1.93
1.96
9.58
18.00
2.95
ppm (t1)

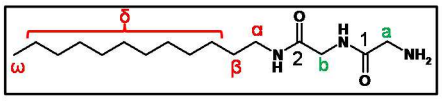

Boc-Gly-Gly-C12
13C NMR (MeOD), 125 MHz
173.308
171.535
158.847
81.062
45.085
40.539
33.062
30.767
30.731
30.718
30.460
30.434
28.755
27.945
23.732
14.477
solvent
ppm (t1)




**H-Gly-Gly-C$_8$**

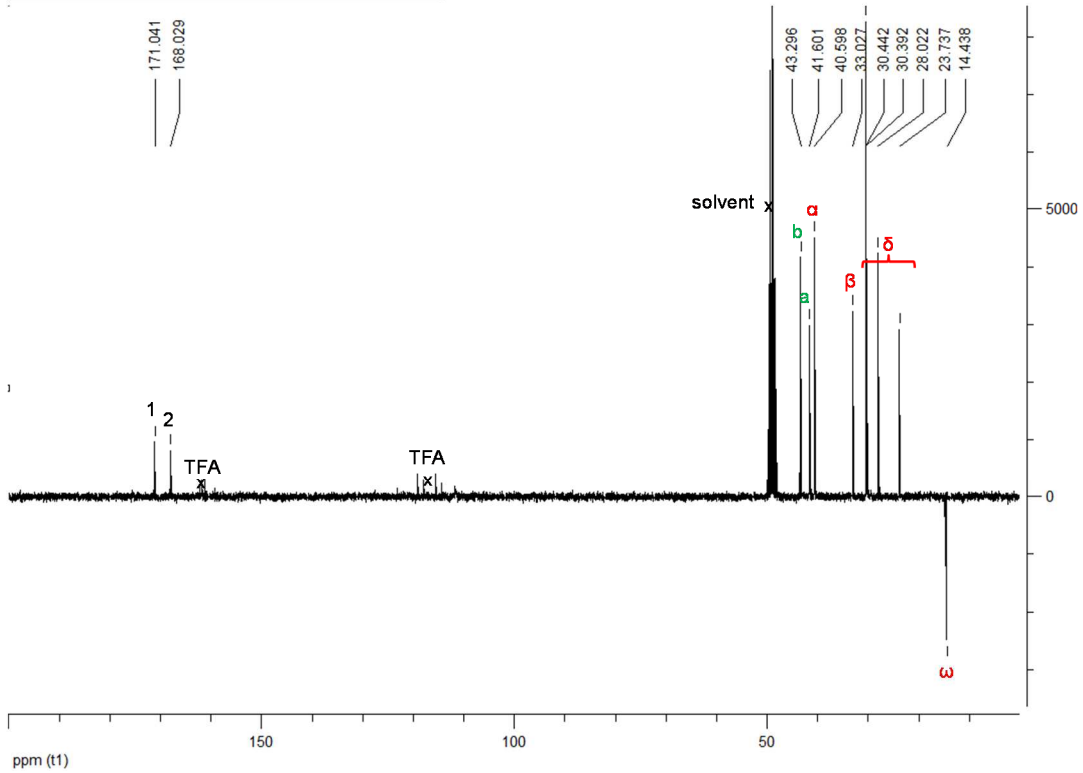
$^1$H NMR (MeOD), 300 MHz

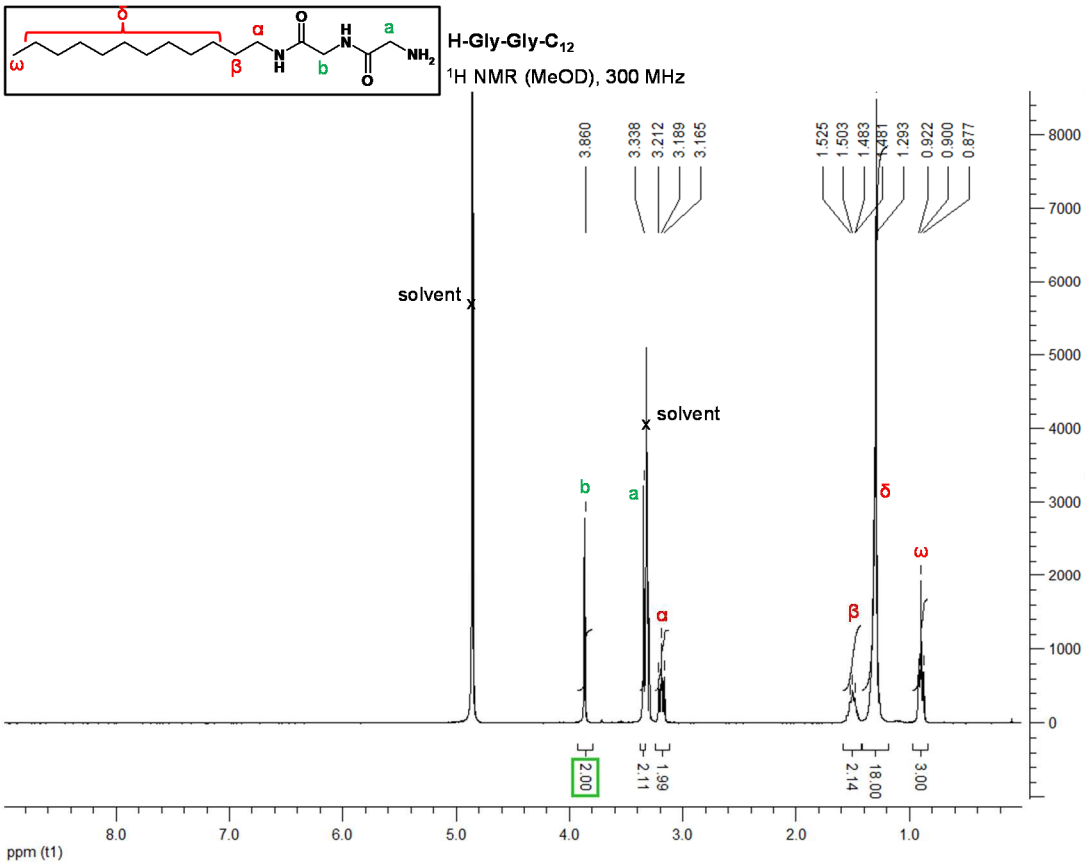


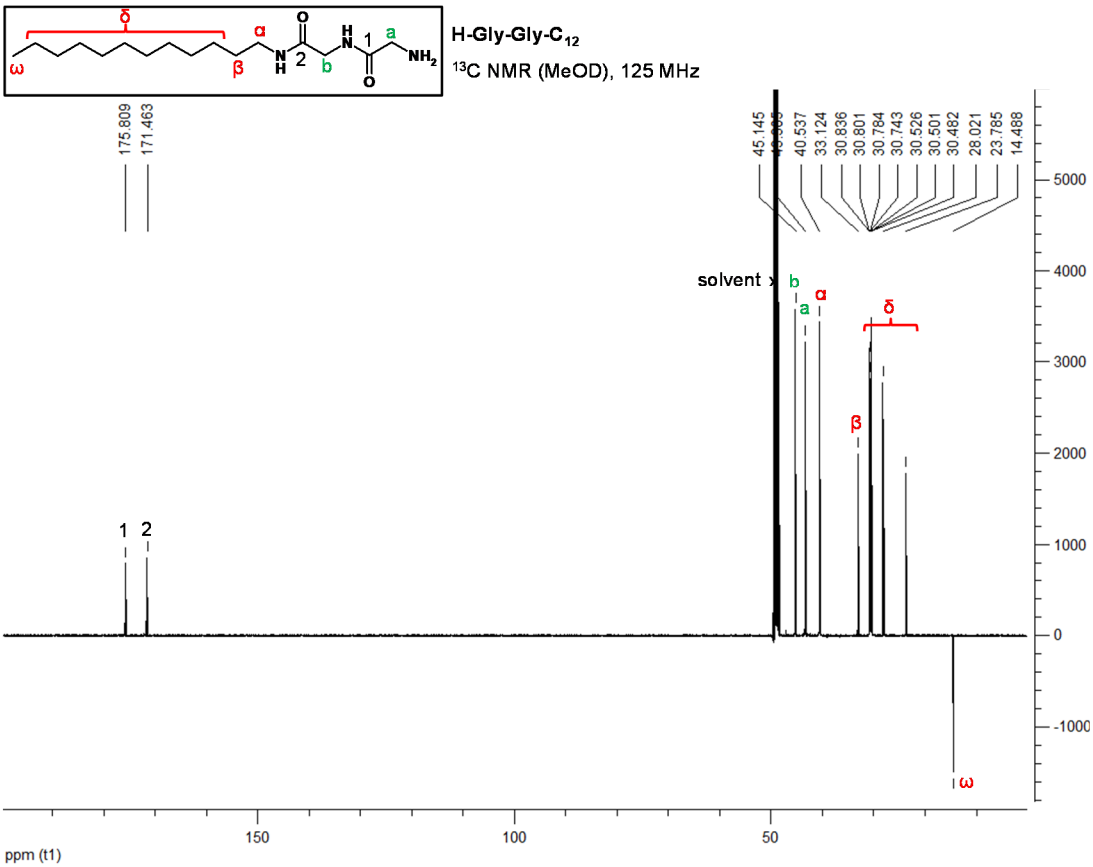



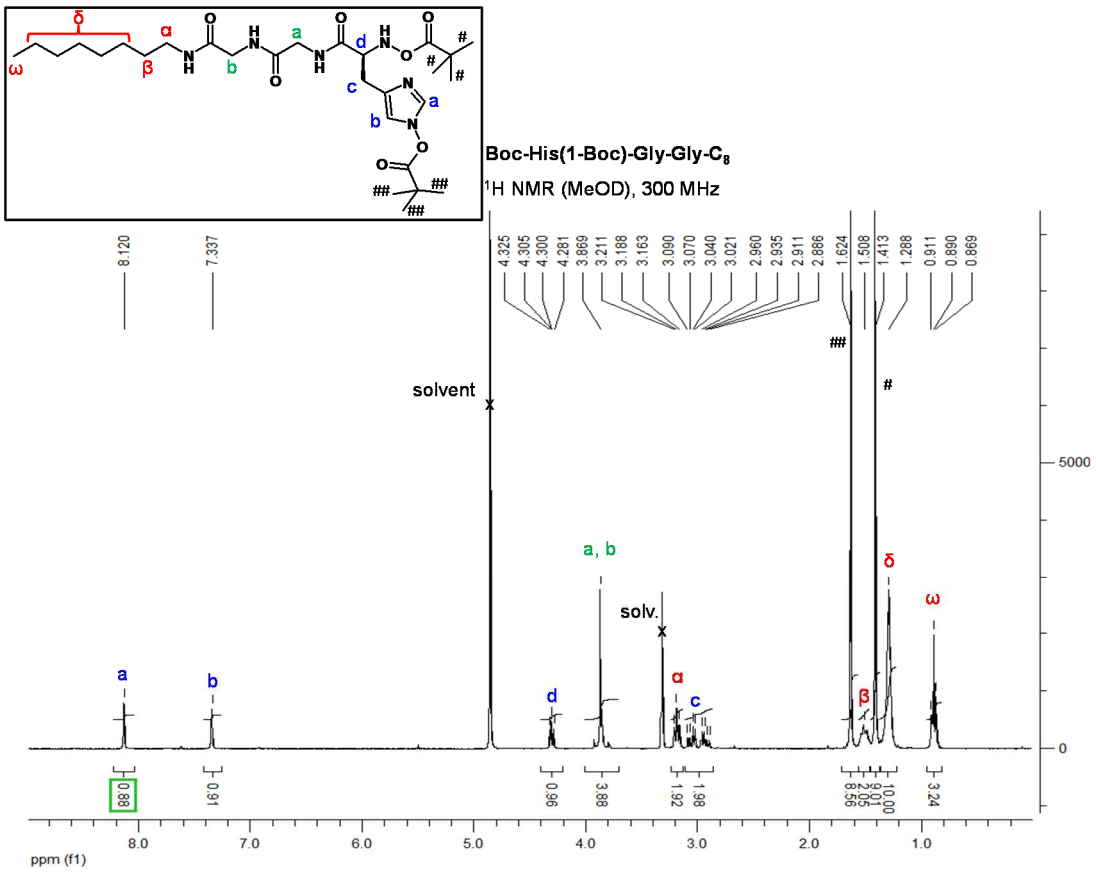

**H-Gly-Gly-C$_8$**

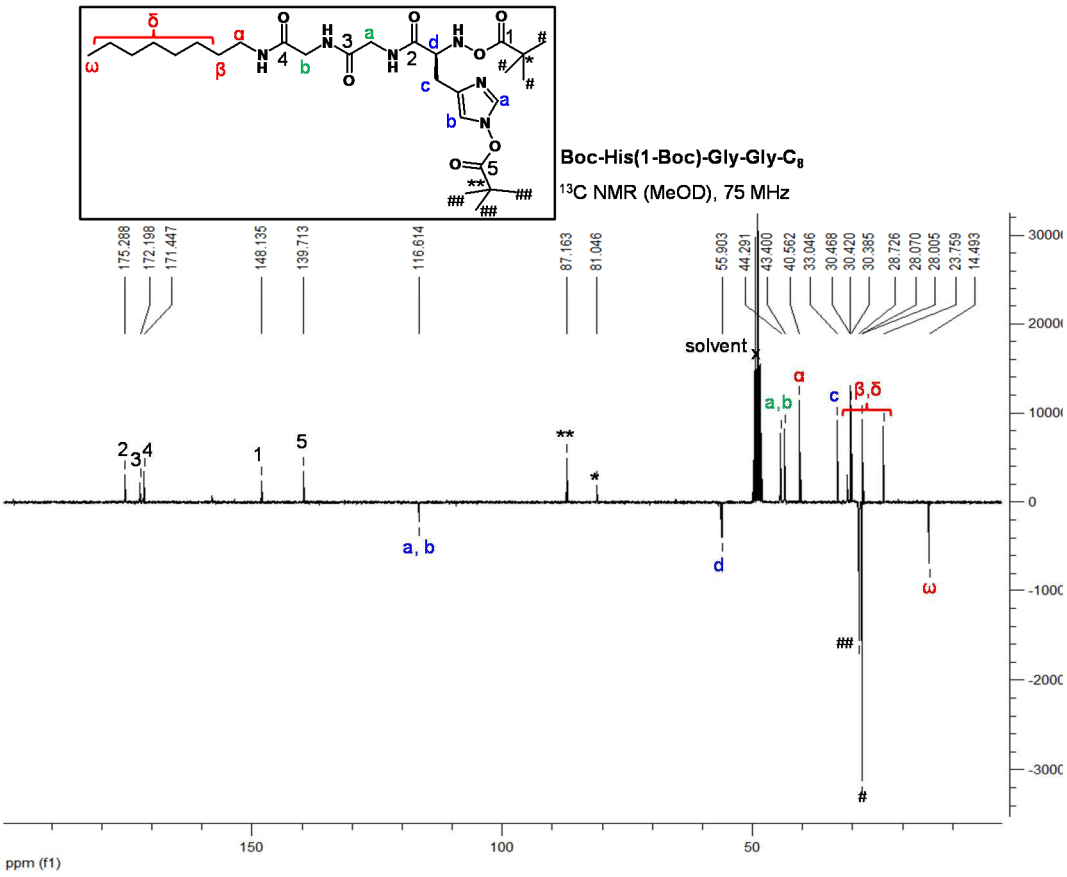
$^{13}$C NMR (MeOD), 75 MHz

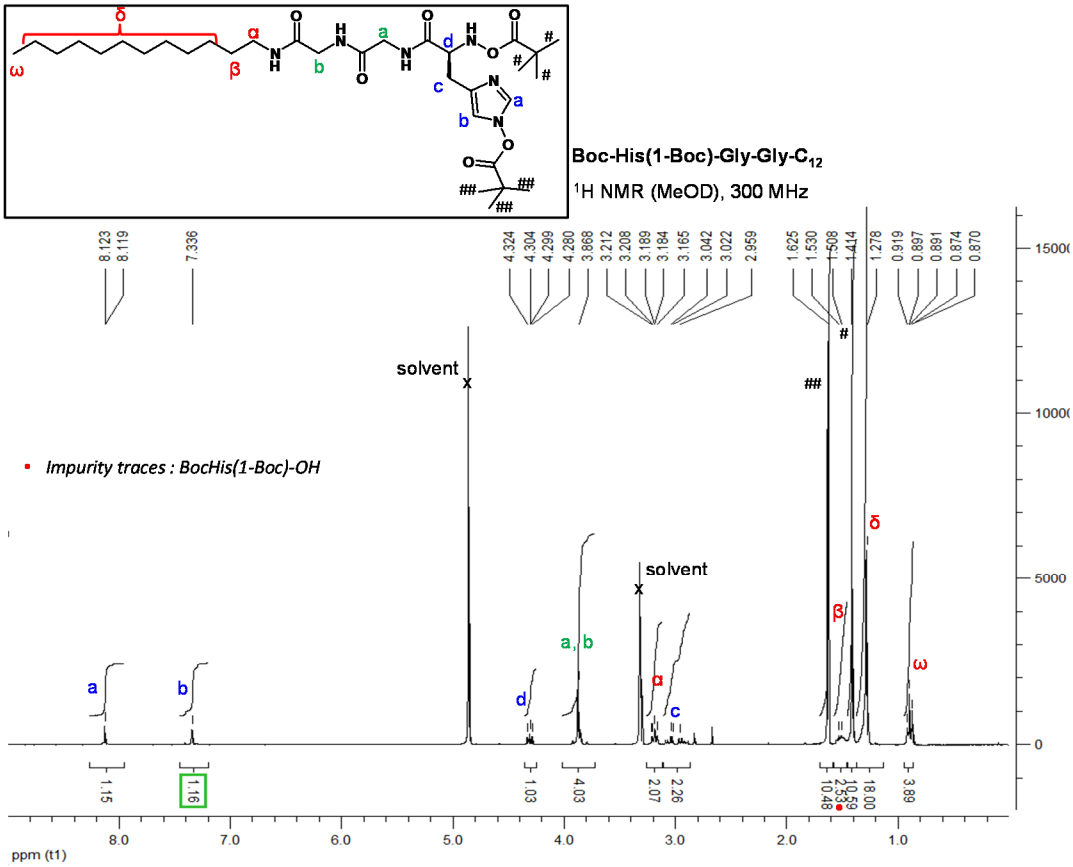


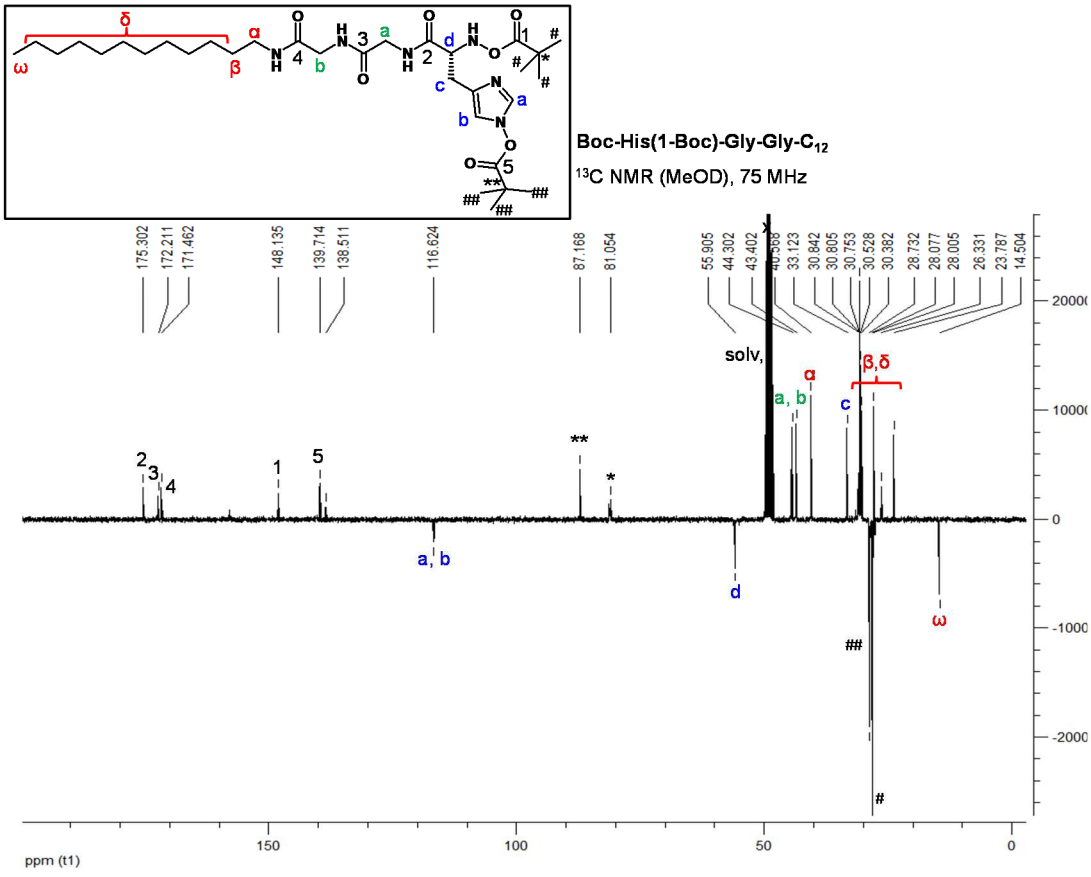

H-Gly-Gly-C12
1H NMR (MeOD), 300 MHz
solvent
solvent
3.860
3.338
3.212
3.189
3.165
1.525
1.503
1.483
1.481
1.293
0.922
0.900
0.877
2.00
1.99
2.11
2.14
18.00
3.00
ppm (t1)

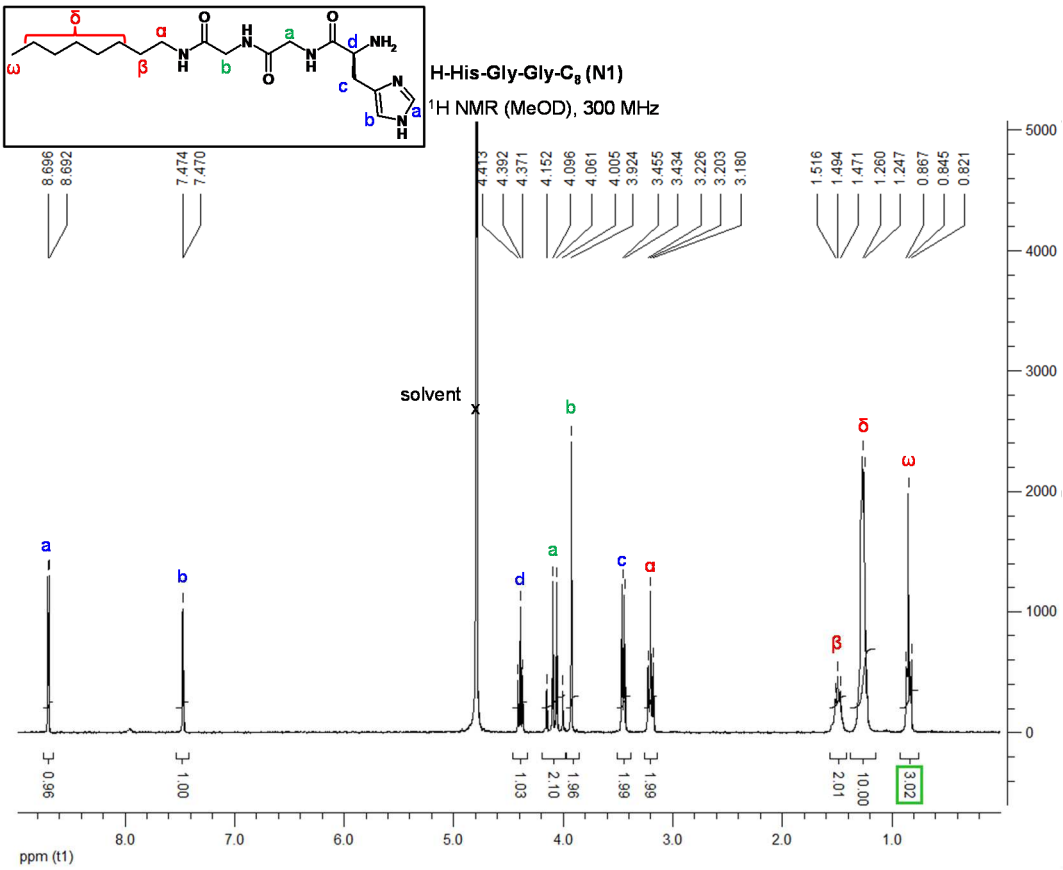

H-Gly-Gly-C12
13C NMR (MeOD), 125 MHz
175.809
171.463
45.145
40.537
33.124
30.836
30.801
30.784
30.743
30.526
30.501
30.482
28.021
23.785
14.488
solvent
ppm (t1)

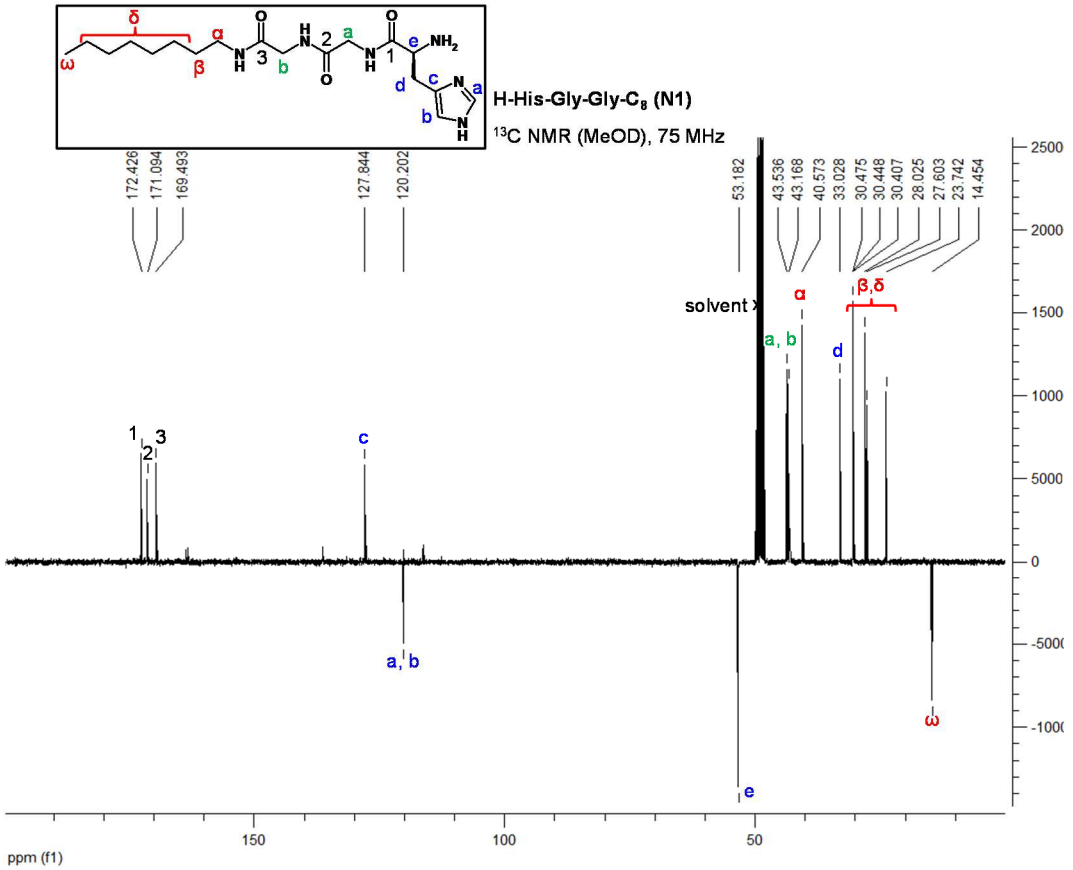

Boc-His(1-Boc)-Gly-Gly-C8
1H NMR (MeOD), 300 MHz
solvent
solv.
a, b
ppm (f1)

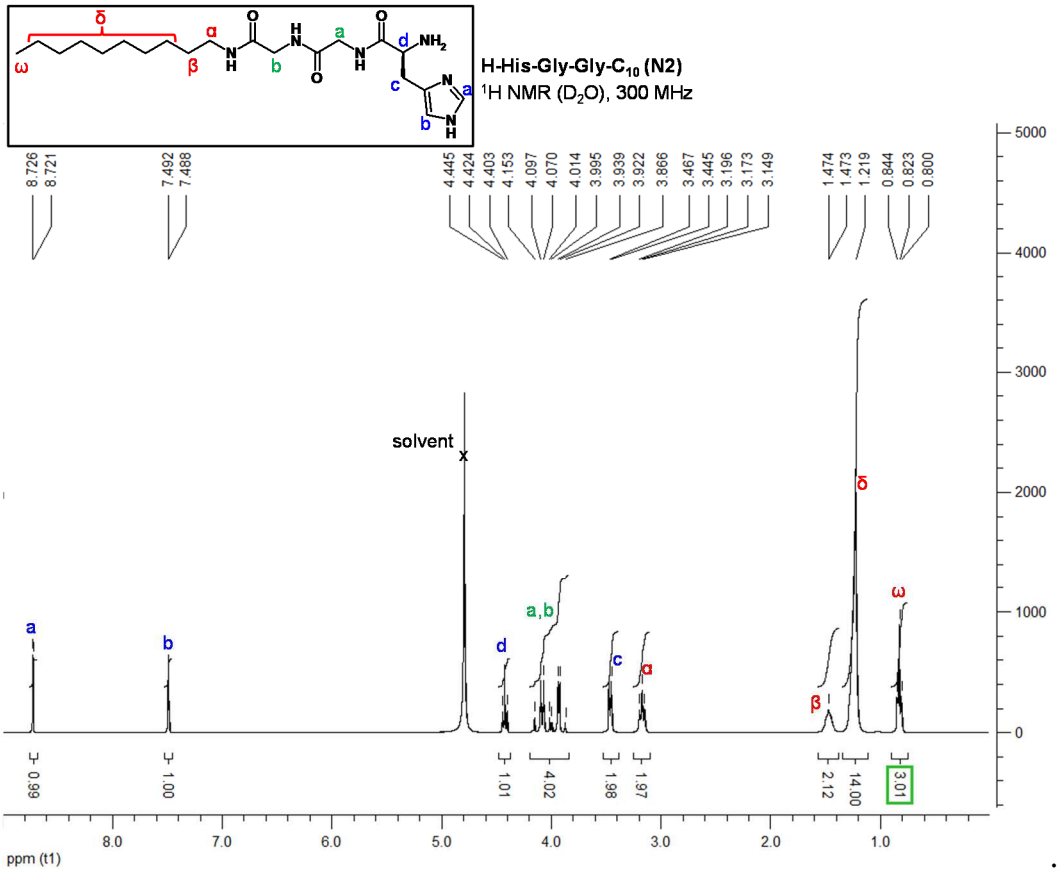

Boc-His(1-Boc)-Gly-Gly-C8
13C NMR (MeOD), 75 MHz
solvent
a, b
d
ω
ppm (f1)

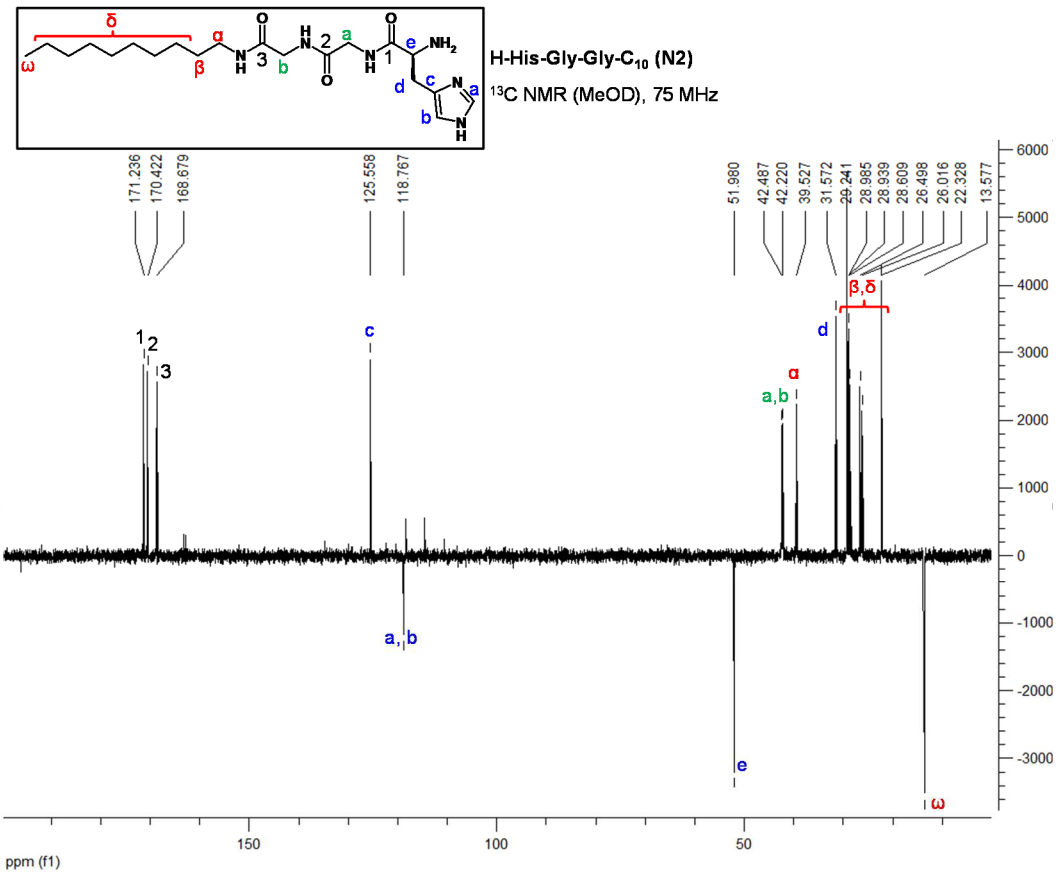

Boc-His(1-Boc)-Gly-Gly-C12
1H NMR (MeOD), 300 MHz
solvent
solvent
Impurity traces : BocHis(1-Boc)-OH
ppm (t1)

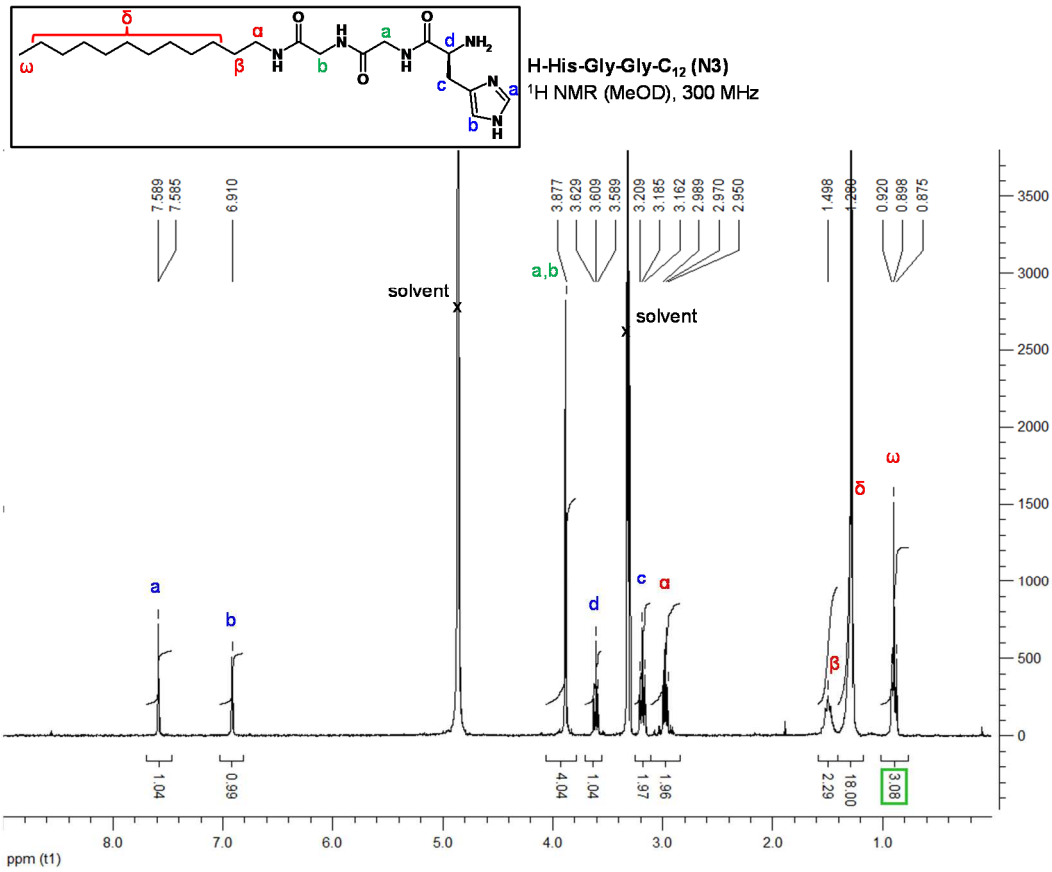

Boc-His(1-Boc)-Gly-Gly-C12
13C NMR (MeOD), 75 MHz
solv,
ppm (t1)

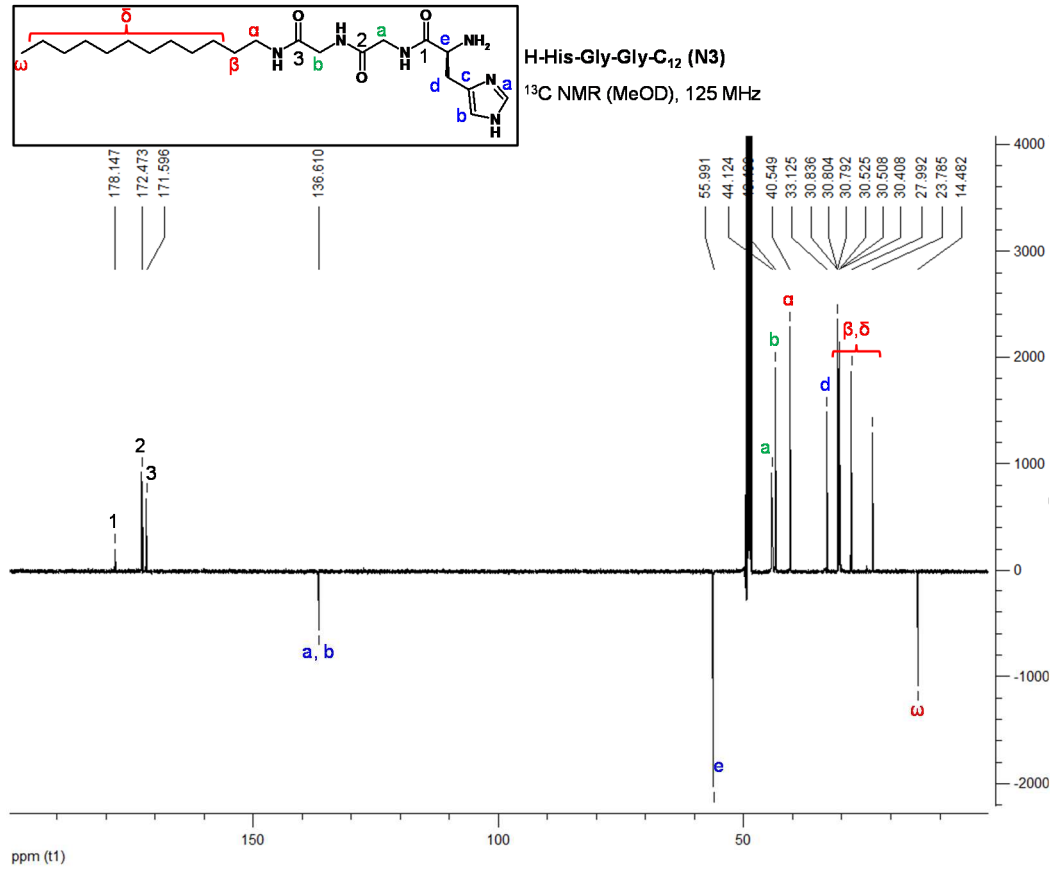

H-His-Gly-Gly-C8 (N1)
1H NMR (MeOD), 300 MHz
solvent
ppm (t1)

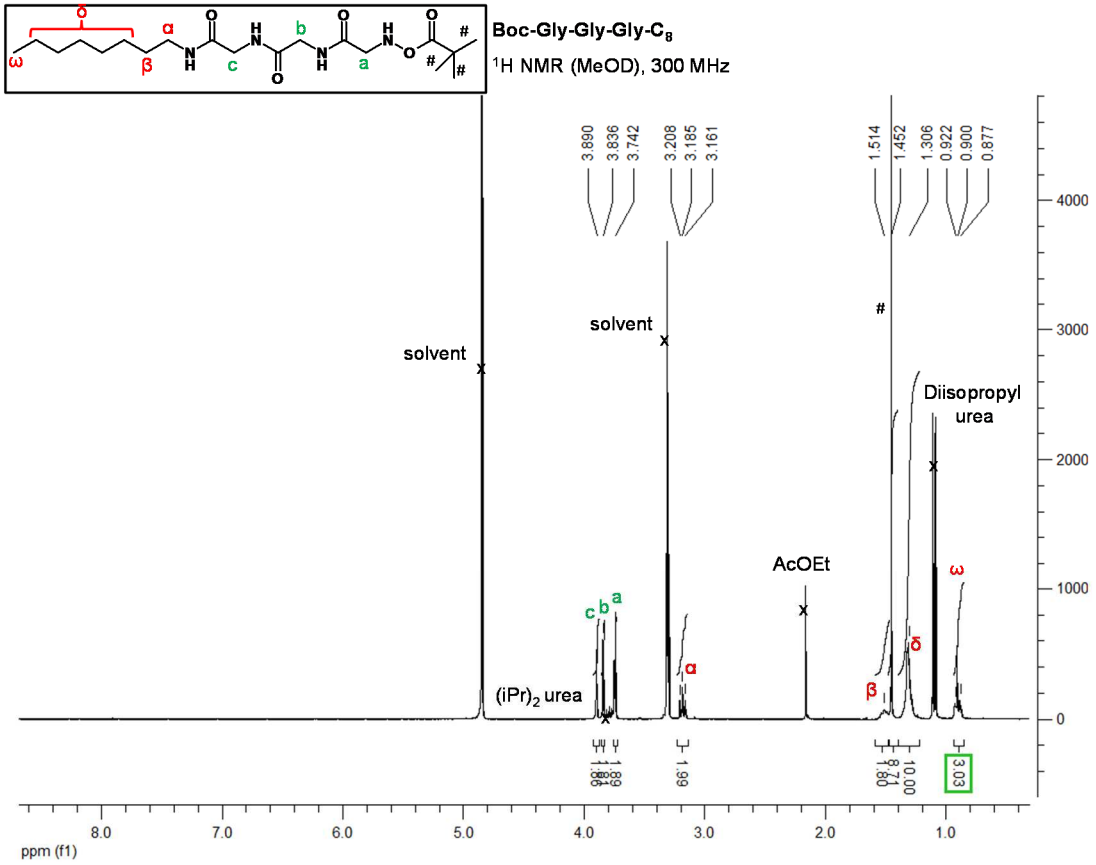

H-His-Gly-Gly-C8 (N1)
13C NMR (MeOD), 75 MHz
solvent
ppm (f1)

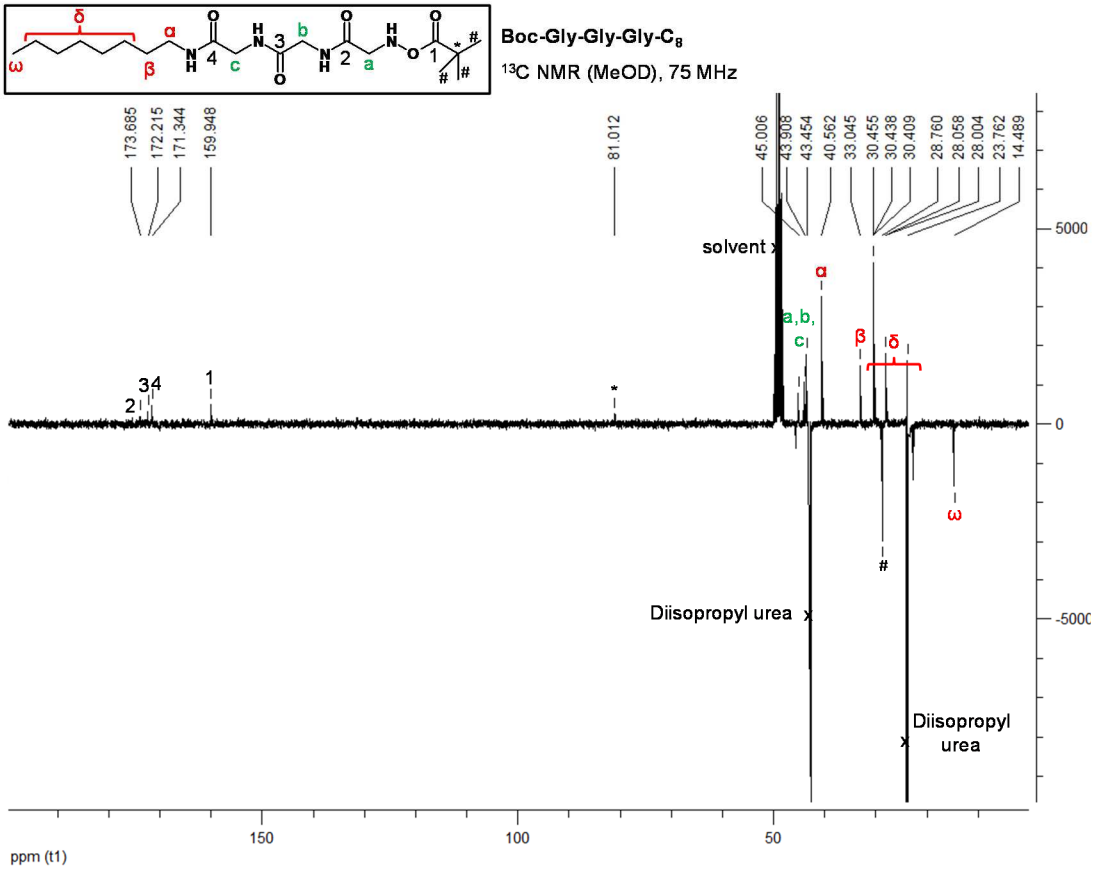

H-His-Gly-Gly-C10 (N2)
1H NMR (D2O), 300 MHz
8.726
8.721
7.492
7.488
4.445
4.424
4.403
4.153
4.097
4.070
4.014
3.995
3.939
3.922
3.866
3.467
3.445
3.196
3.173
3.149
1.474
1.473
1.219
0.844
0.823
0.800
solvent
a
b
d
a,b
c
α
β
δ
ω
0.99
1.00
1.01
4.02
1.98
1.97
2.12
14.00
3.01
ppm (t1)

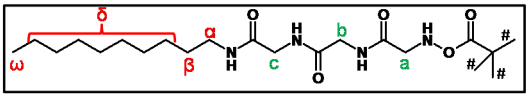

H-His-Gly-Gly-C10 (N2)
13C NMR (MeOD), 75 MHz
171.236
170.422
168.679
125.558
118.767
51.980
42.487
42.220
39.527
31.572
29.241
28.985
28.939
28.609
26.498
26.016
22.328
13.577
1
2
3
c
a,b
α
d
β,δ
e
ω
ppm (f1)


H-His-Gly-Gly-C12 (N3)
1H NMR (MeOD), 300 MHz
solvent
solvent
ppm (t1)


H-His-Gly-Gly-C12 (N3)
13C NMR (MeOD), 125 MHz
ppm (t1)

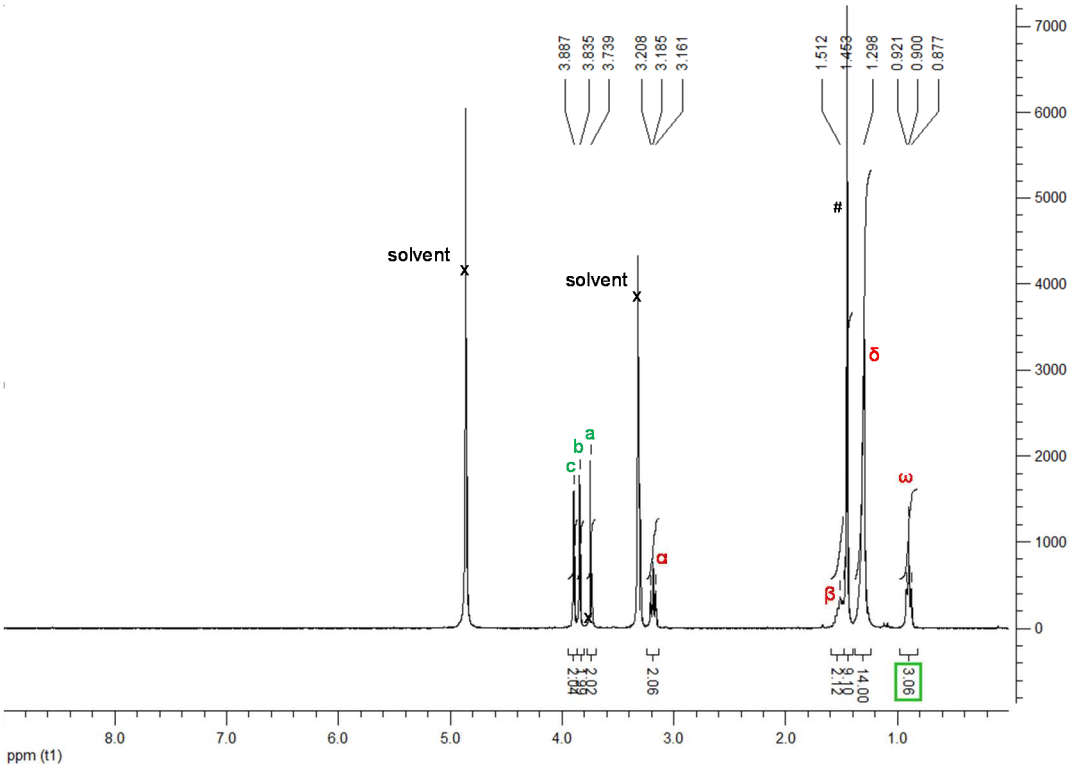

Boc-Gly-Gly-Gly-C8
1H NMR (MeOD), 300 MHz
solvent
solvent
(iPr)2 urea
AcOEt
Diisopropyl urea
ppm (f1)

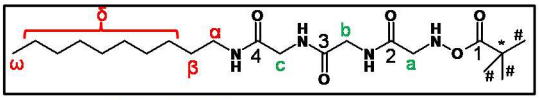

Boc-Gly-Gly-Gly-C8
13C NMR (MeOD), 75 MHz
solvent
Diisopropyl urea
Diisopropyl urea
ppm (t1)




**Boc-Gly-Gly-Gly-C$_{10}$**

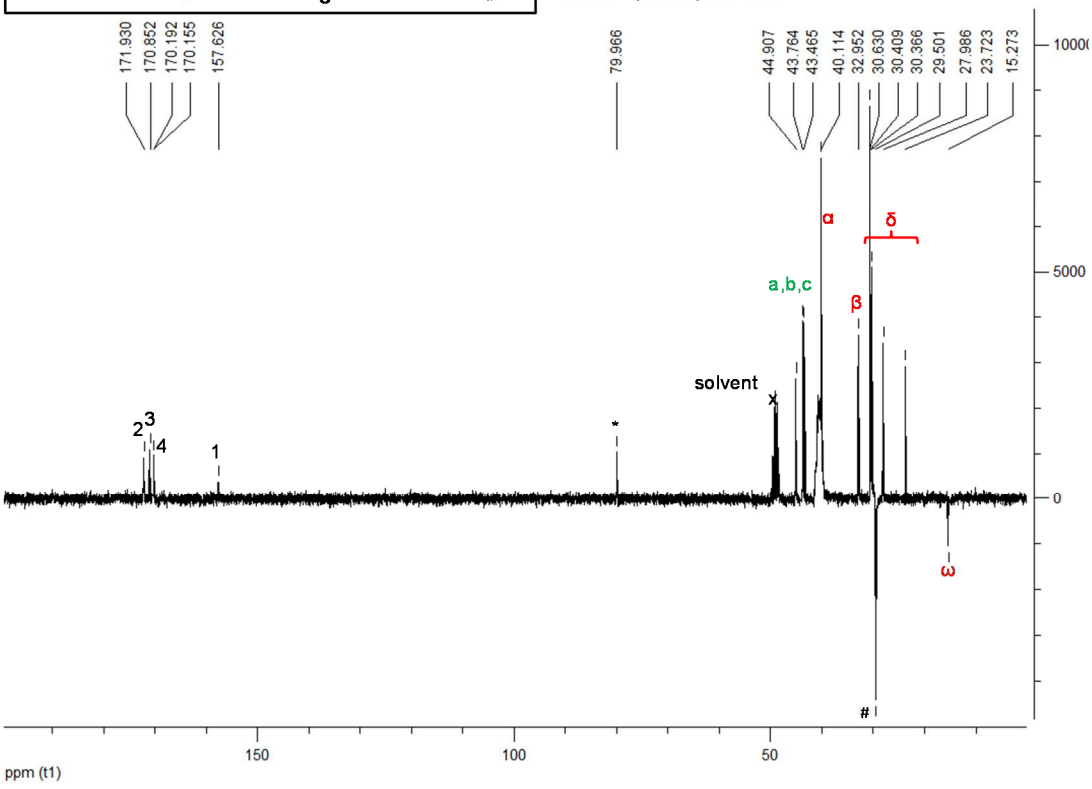
$^{1}$H NMR (MeOD), 300 MHz

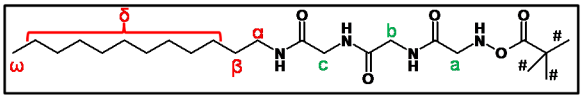



**Boc-Gly-Gly-Gly-C$_{10}$**

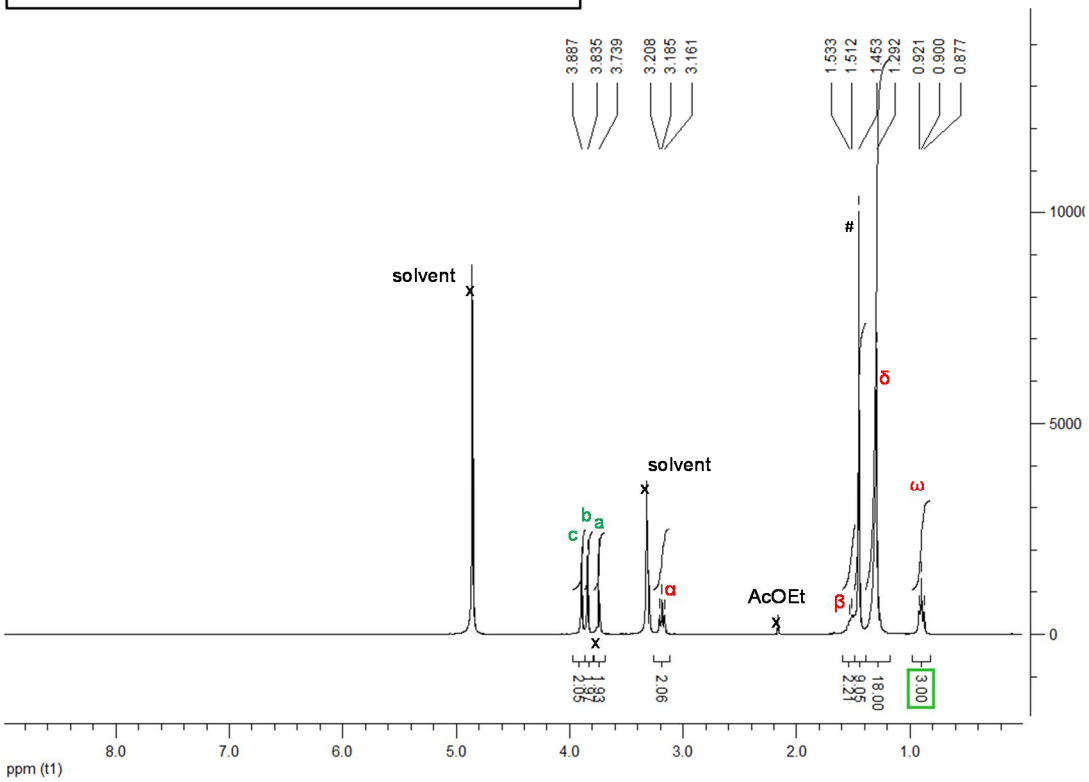
$^{13}$C NMR (MeOD), 75 MHz

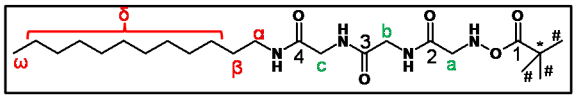





**Boc-Gly-Gly-Gly-C$_{12}$**

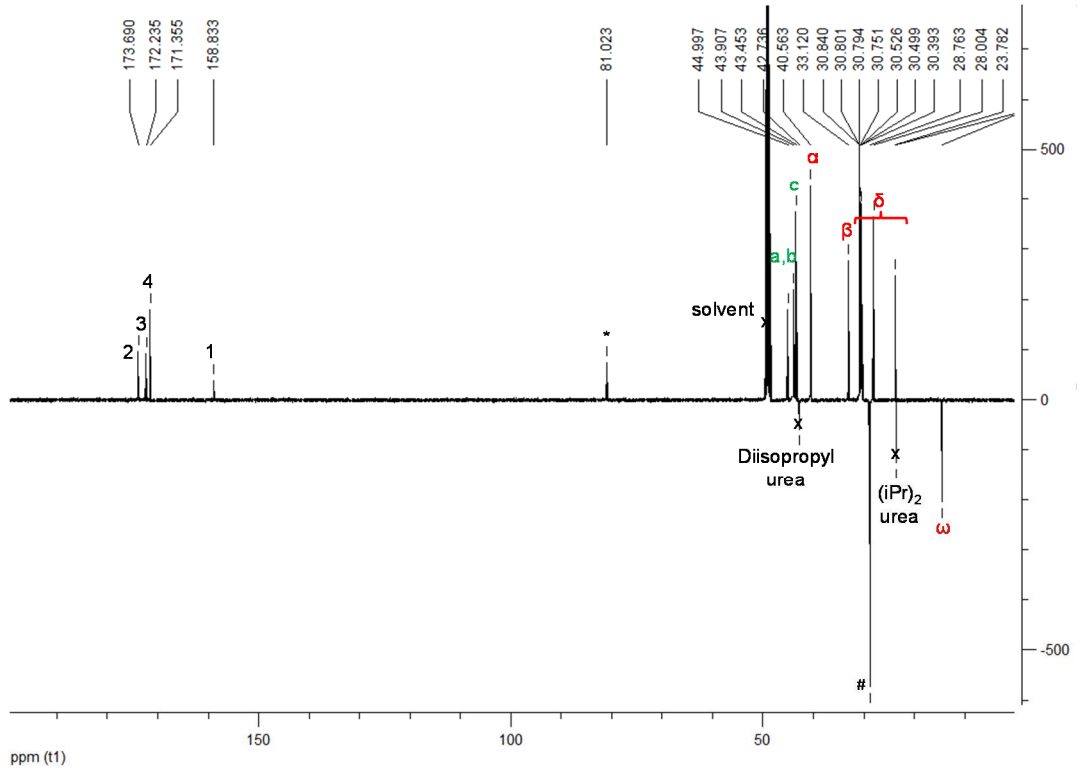
$^{1}$H NMR (MeOD), 300 MHz

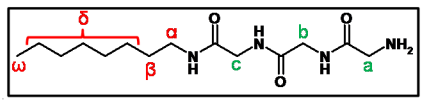



**Boc-Gly-Gly-Gly-C$_{12}$**

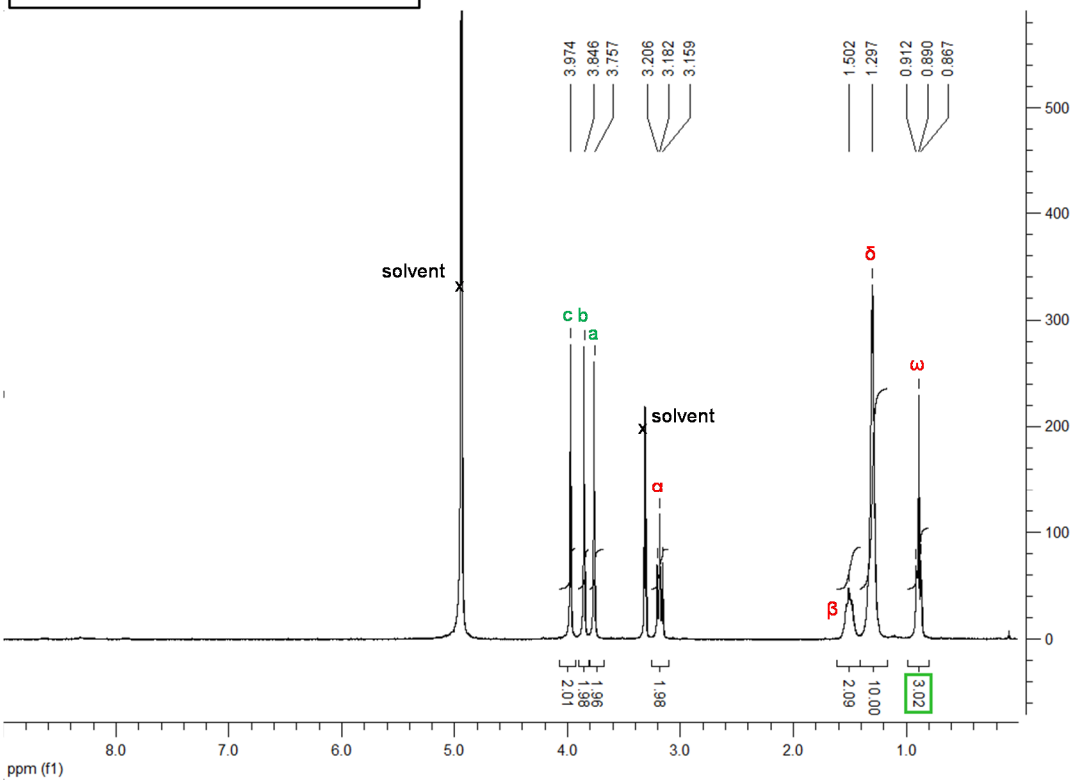
$^{13}$C NMR (MeOD), 125 MHz

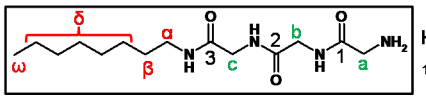





**H-Gly-Gly-Gly-$C_8$ (N4)**

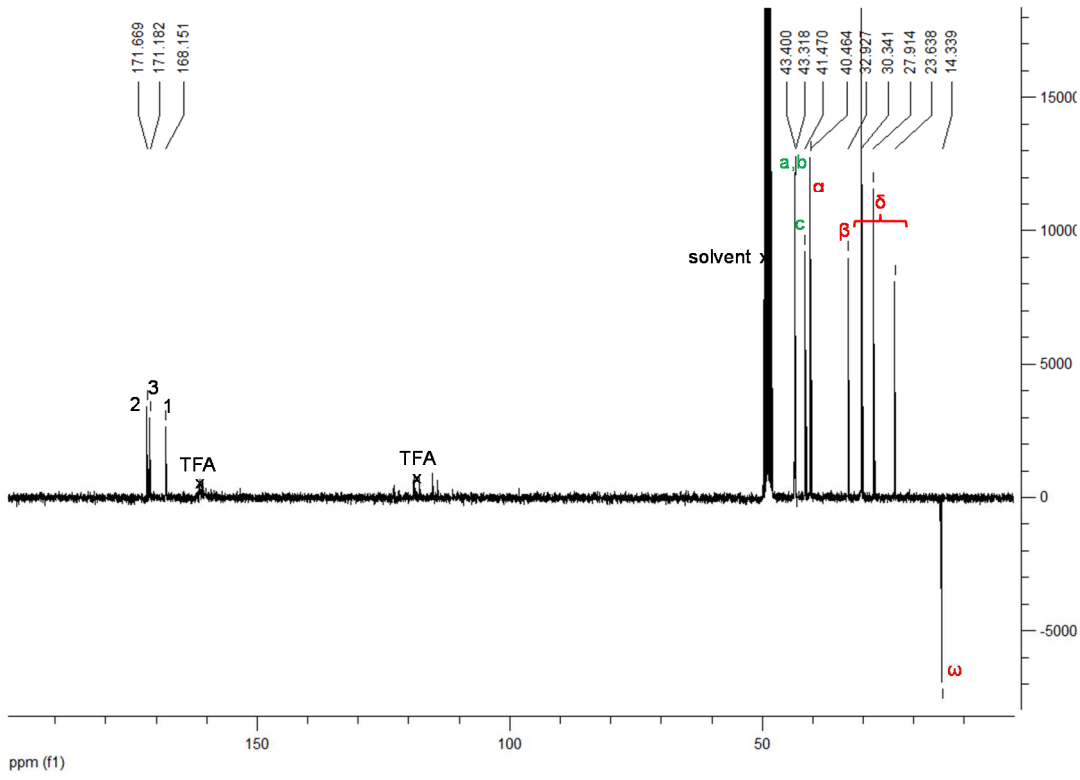
$^1$H NMR (MeOD), 300 MHz

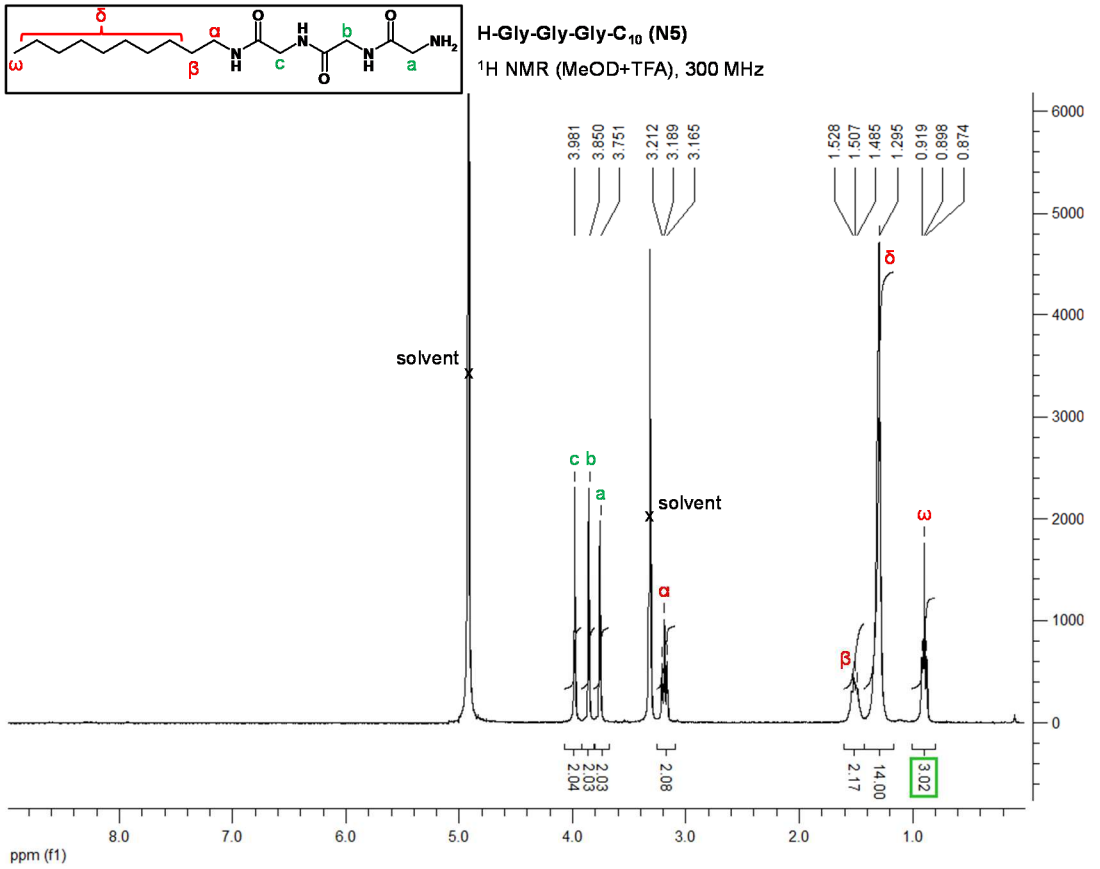


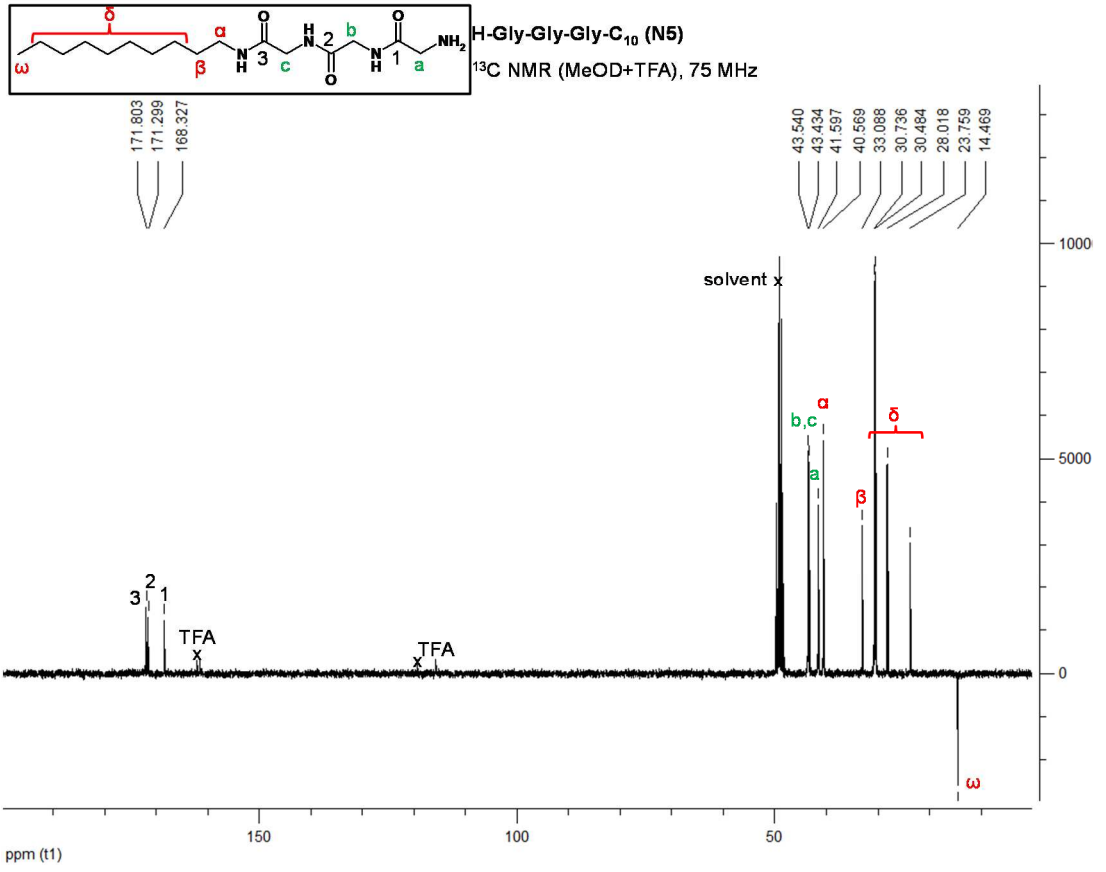



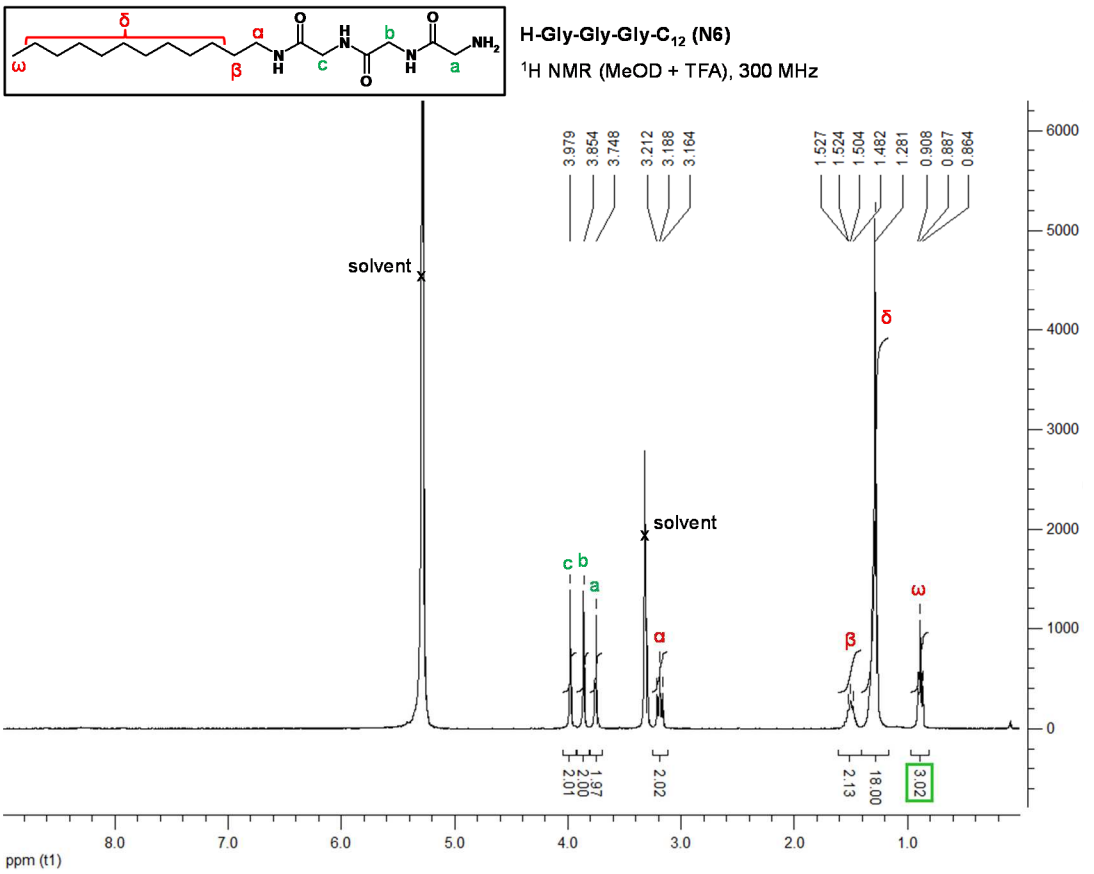

**H-Gly-Gly-Gly-$C_8$ (N4)**

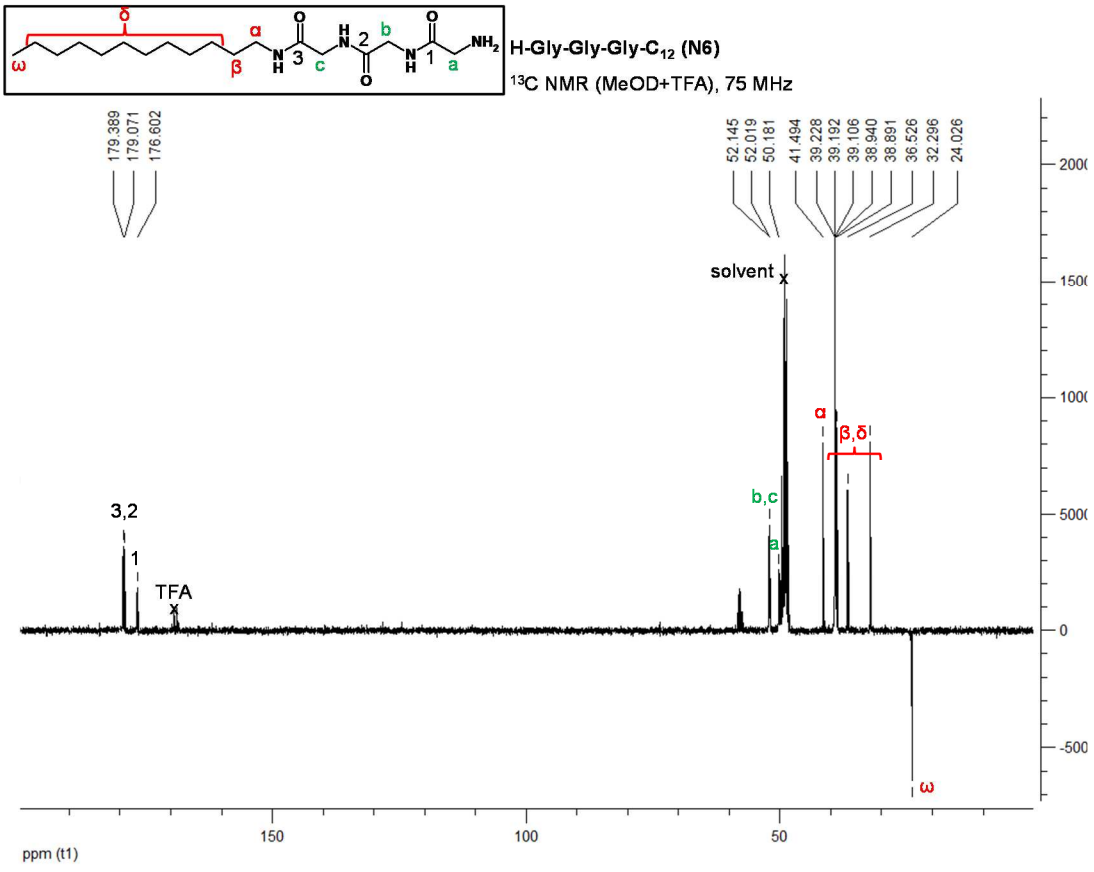
$^{13}$C NMR (MeOD + TFA), 75 MHz

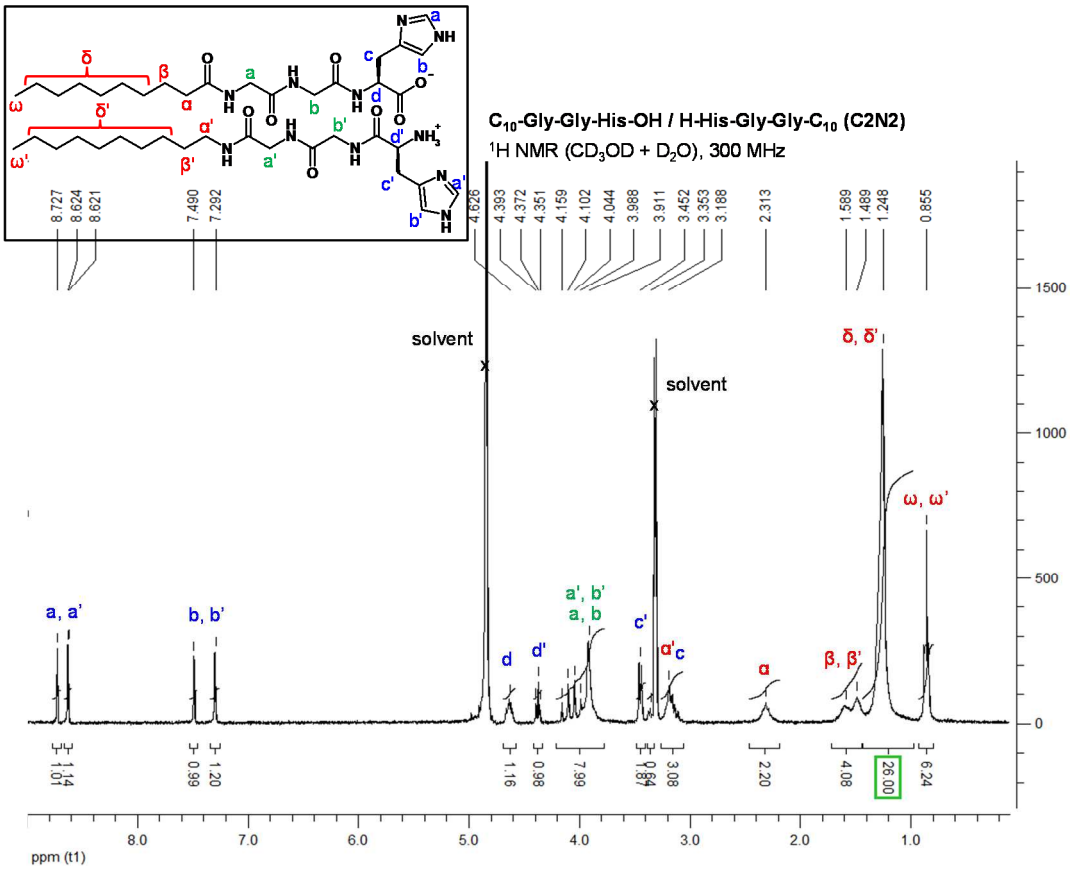


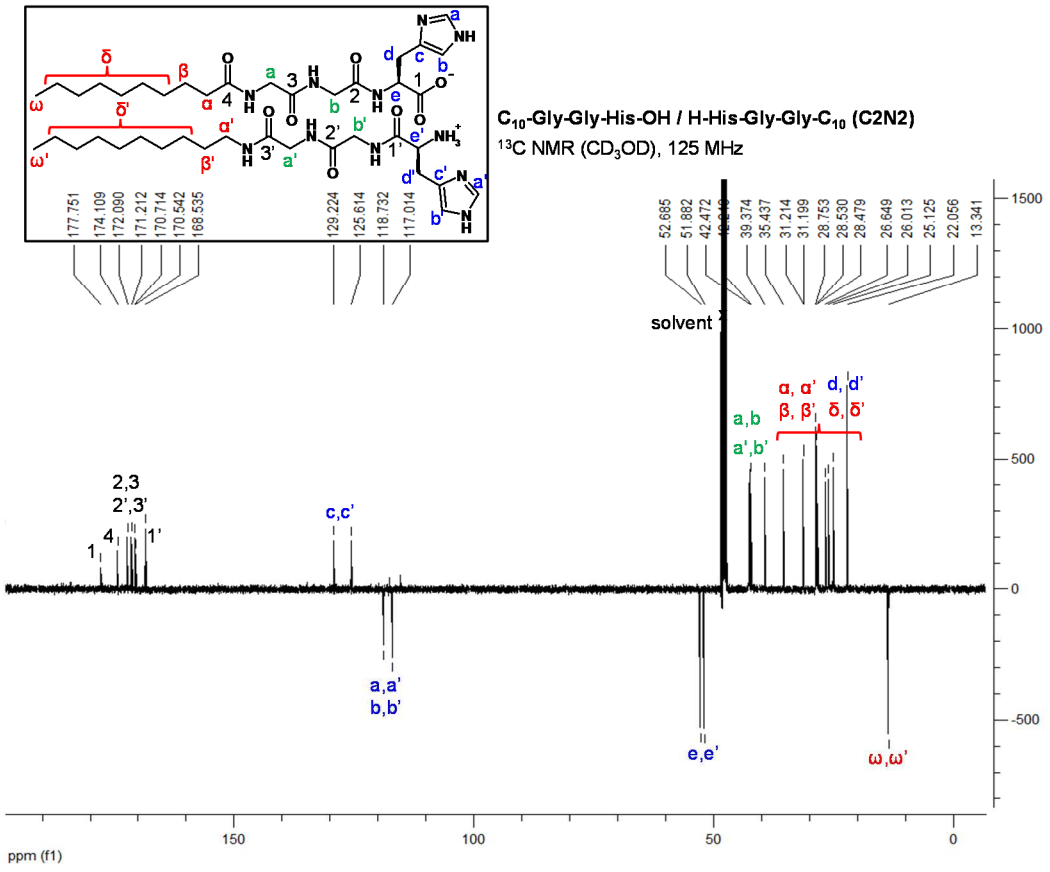

H-Gly-Gly-Gly-C10 (N5)
1H NMR (MeOD+TFA), 300 MHz
solvent
solvent
ppm (f1)


H-Gly-Gly-Gly-C10 (N5)
13C NMR (MeOD+TFA), 75 MHz
solvent
TFA
TFA
ppm (t1)

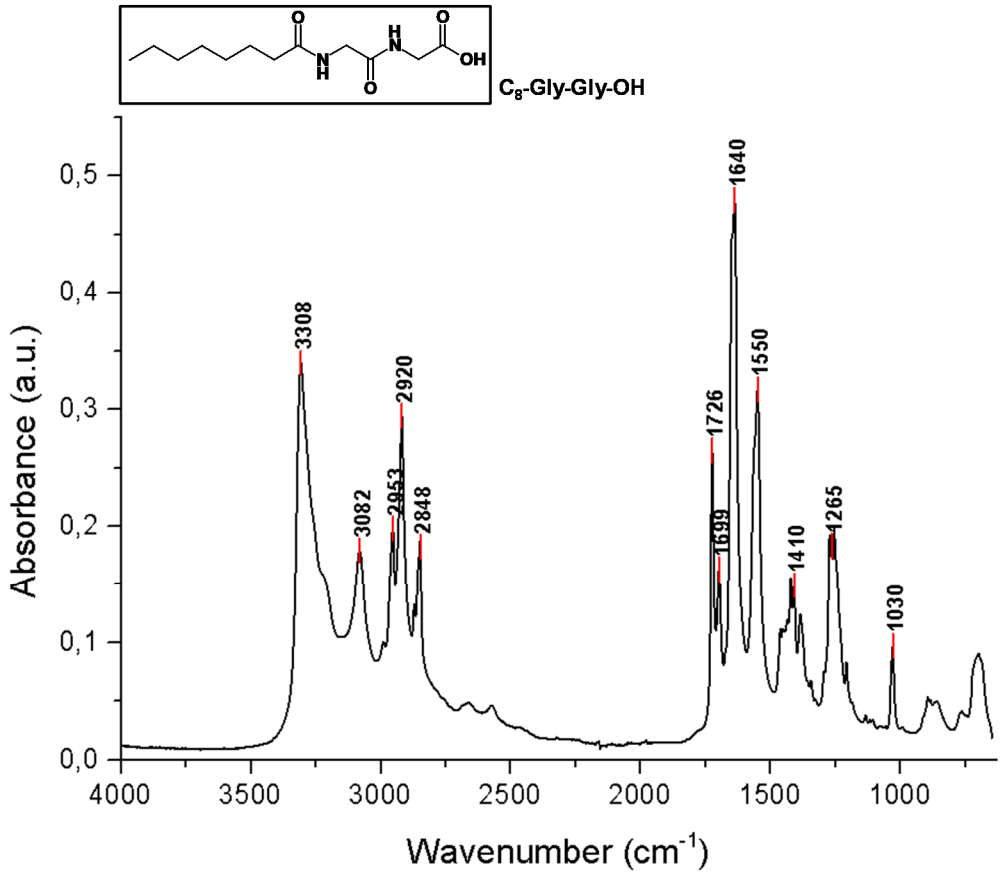

δ
α
b
ω
β
c
a
NH2
H-Gly-Gly-Gly-C12 (N6)
1H NMR (MeOD + TFA), 300 MHz
3.979
3.854
3.748
3.212
3.188
3.164
1.527
1.524
1.504
1.482
1.281
0.908
0.887
0.864
solvent
solvent
δ
c b
a
α
β
ω
2.01
1.97
2.00
2.02
2.13
18.00
3.02
ppm (t1)

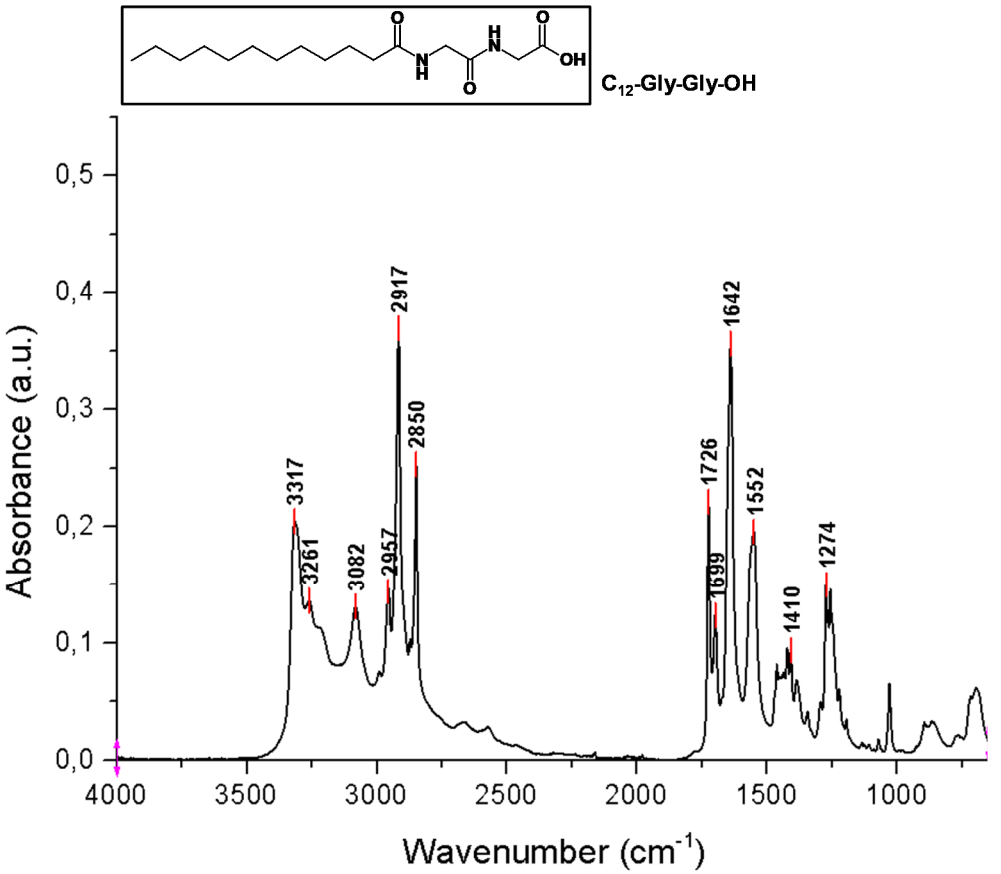

δ
α
b
ω
β
c
a
NH2
H-Gly-Gly-Gly-C12 (N6)
13C NMR (MeOD+TFA), 75 MHz
179.389
179.071
176.602
52.145
52.019
50.181
41.494
39.228
39.192
39.106
38.940
38.891
36.526
32.296
24.026
solvent
α
β,δ
b,c
a
3,2
1
TFA
ω
ppm (t1)

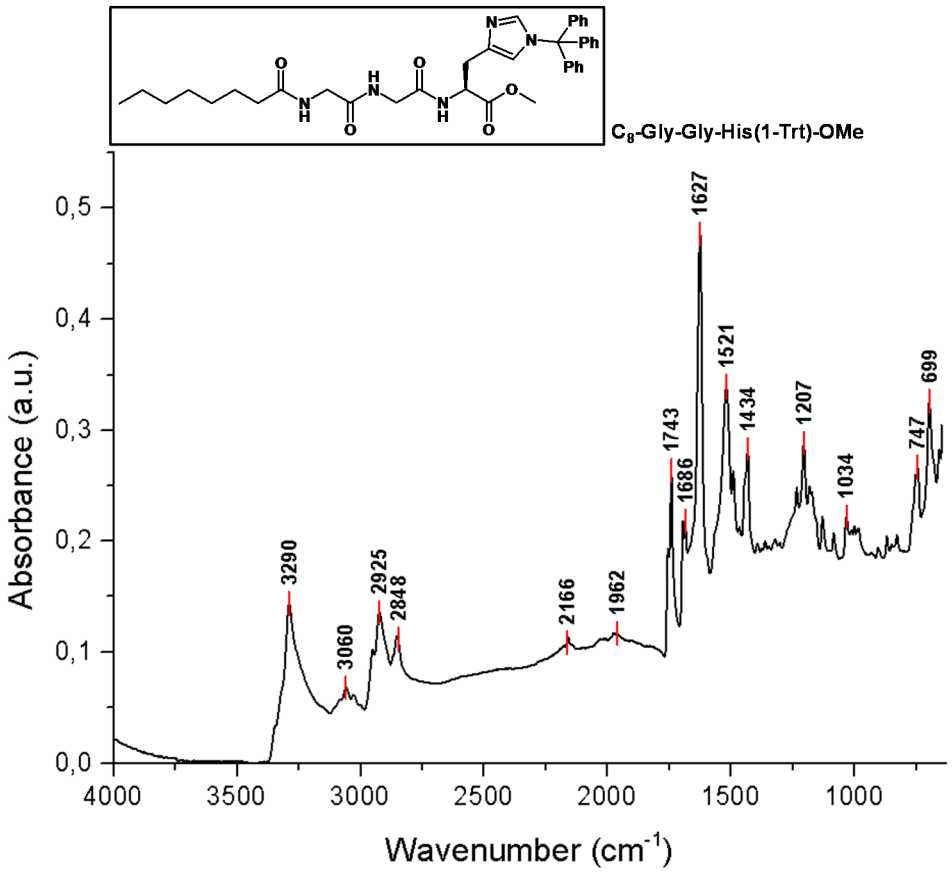

C10-Gly-Gly-His-OH / H-His-Gly-Gly-C10 (C2N2)
1H NMR (CD3OD + D2O), 300 MHz
solvent
solvent
δ, δ'
ω, ω'
a, a'
b, b'
a', b'
a, b
c'
α'c
α
β, β'
d
d'
ppm (t1)

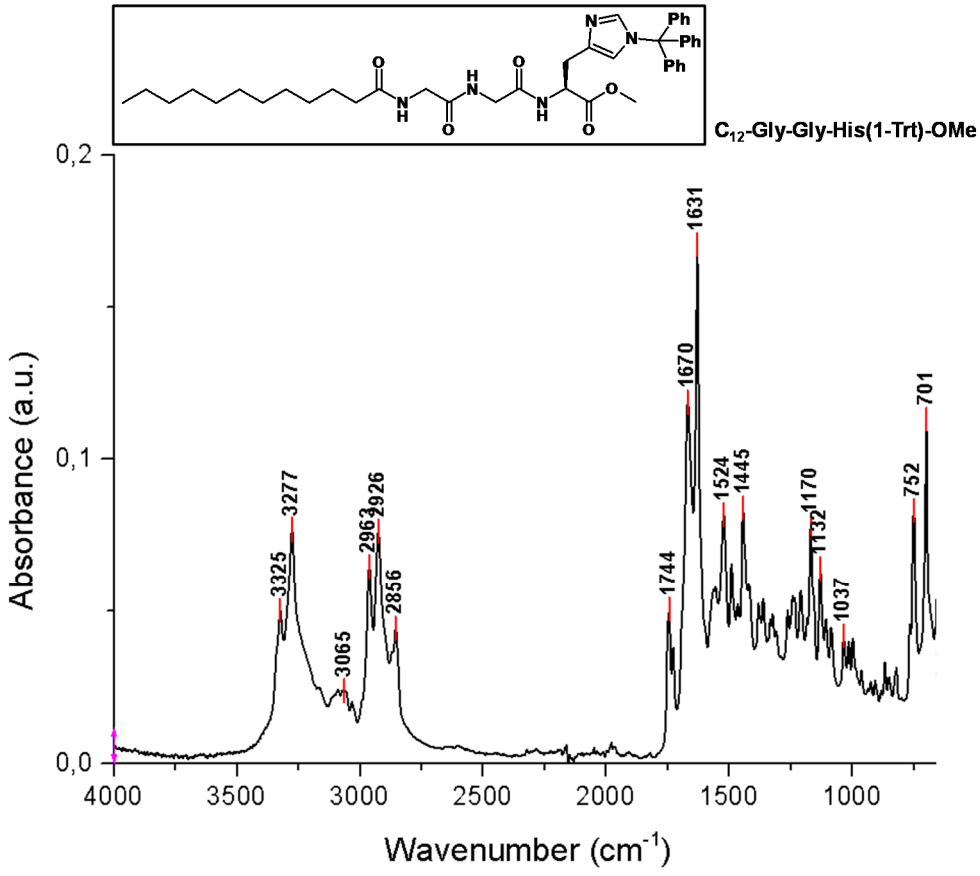

C10-Gly-Gly-His-OH / H-His-Gly-Gly-C10 (C2N2)
13C NMR (CD3OD), 125 MHz
solvent
α, α'
d, d'
β, β'
δ, δ'
a,b
a',b'
2,3
2',3'
1
4
1'
c,c'
a,a'
b,b'
e,e'
ω,ω'
ppm (f1)

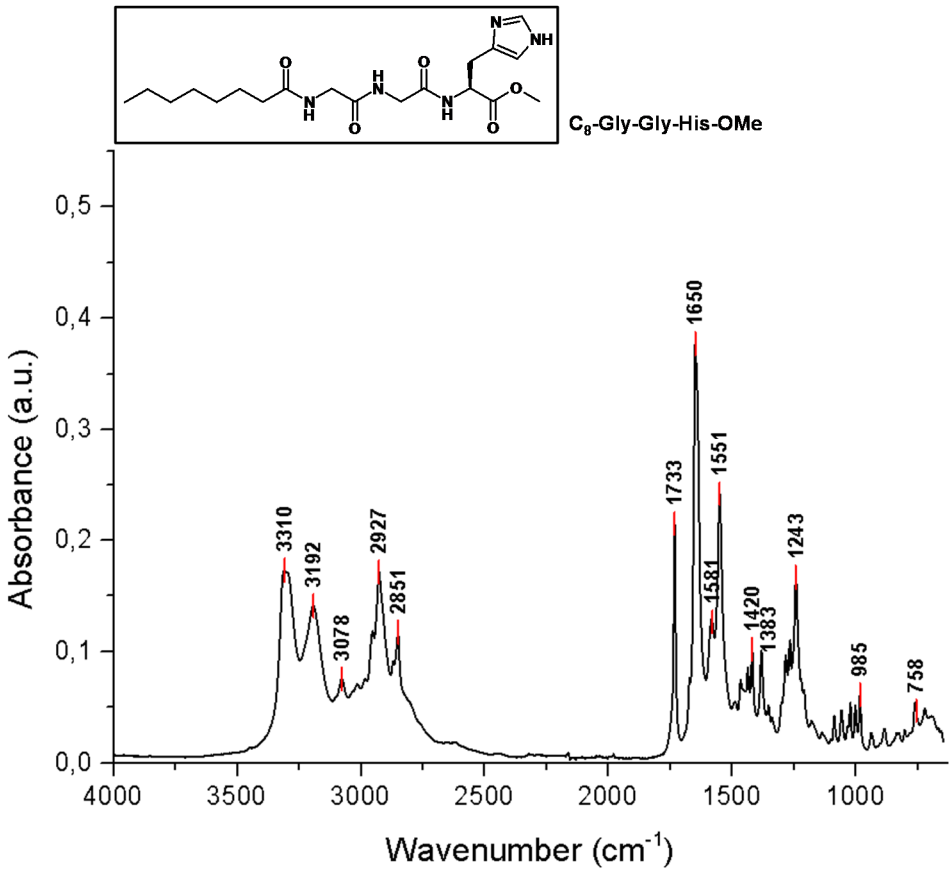
## 3. FTIR spectra

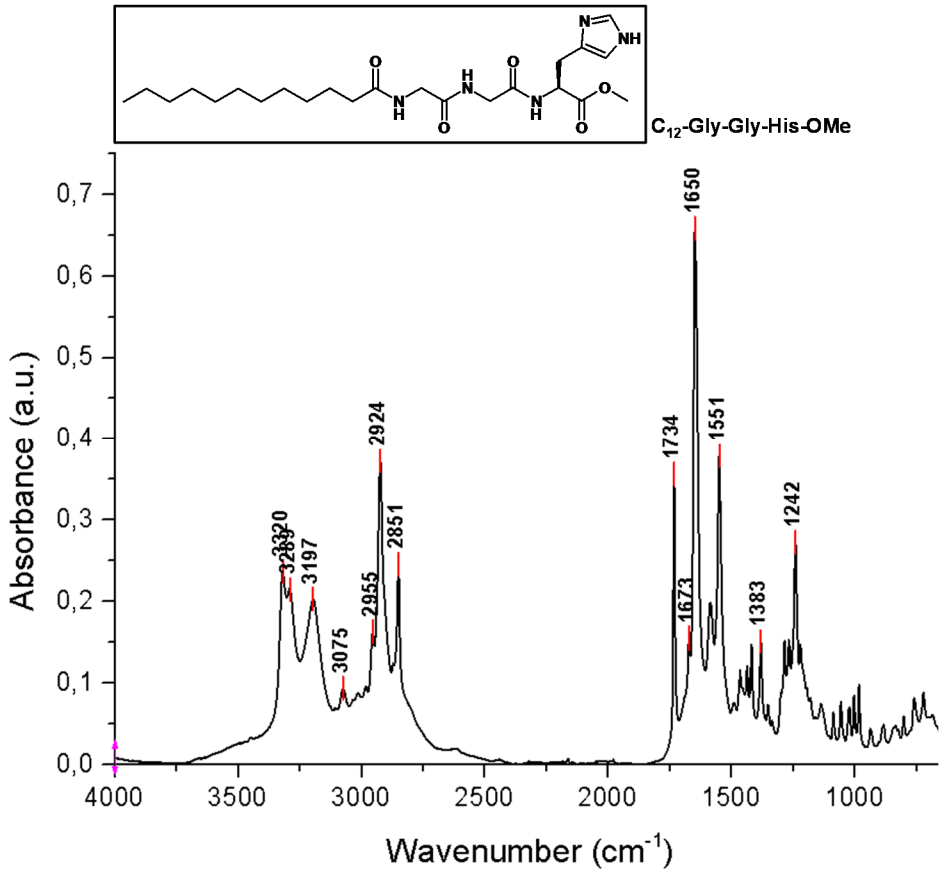


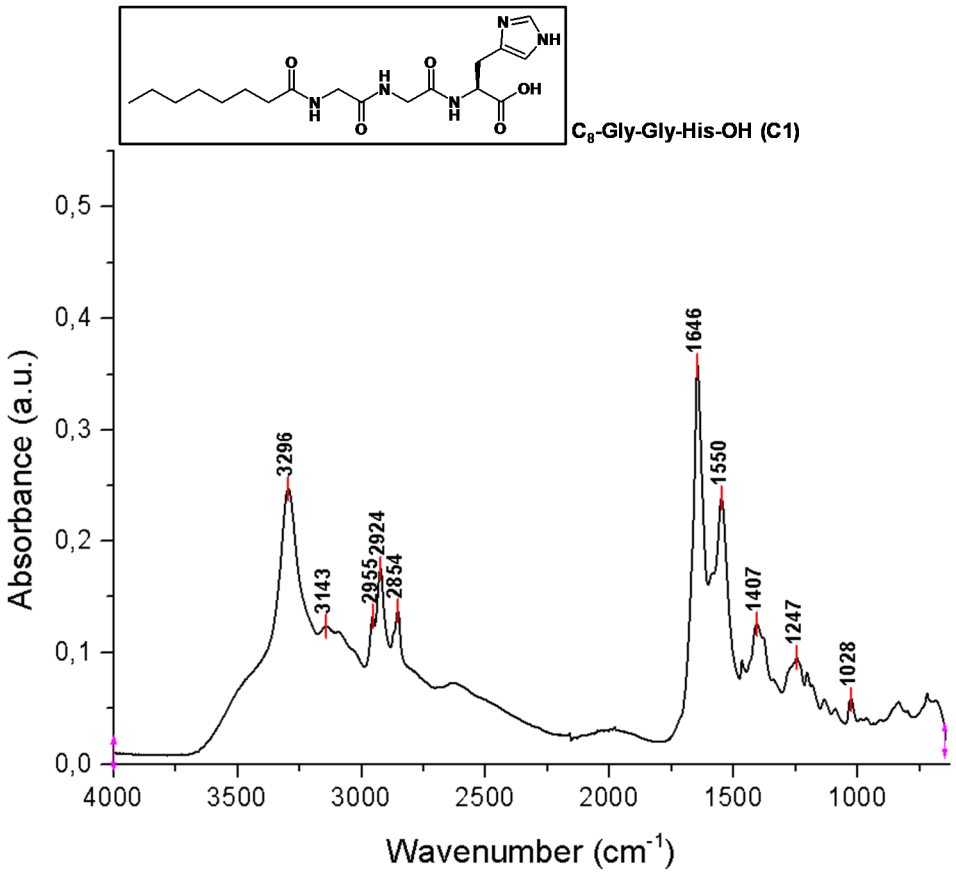



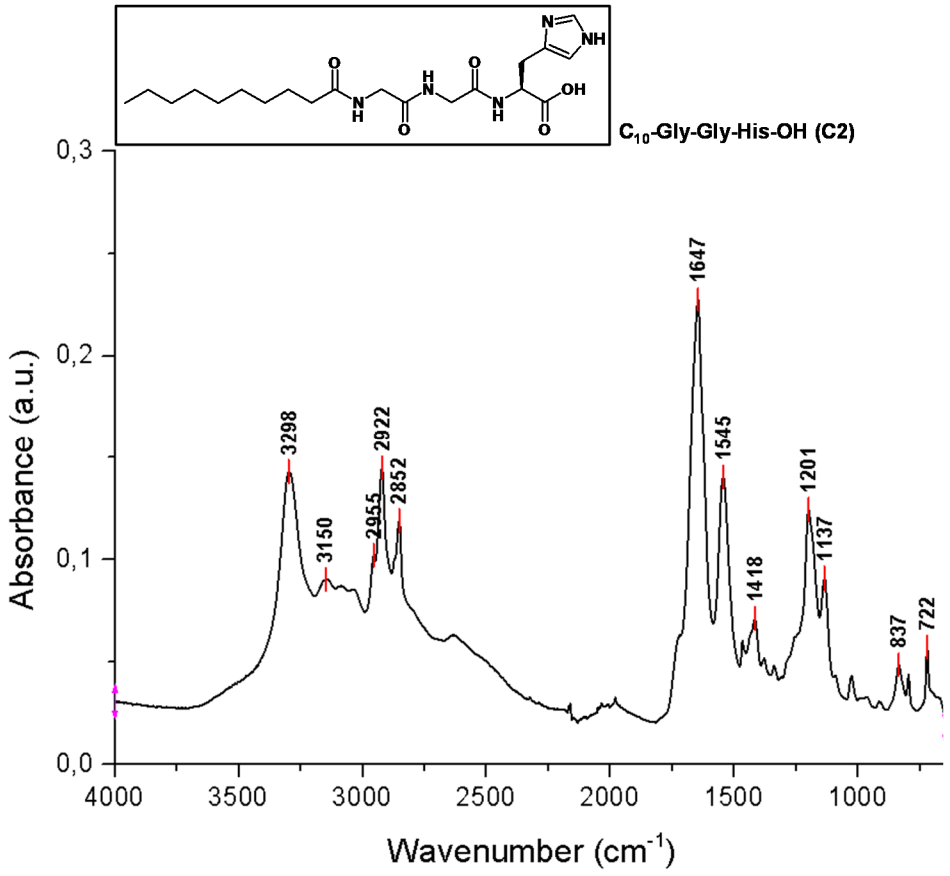

C8-Gly-Gly-His(1-Trt)-OMe
Absorbance (a.u.)
Wavenumber (cm-1)
3290
3060
2925
2848
2166
1962
1743
1686
1627
1521
1434
1207
1034
747
699

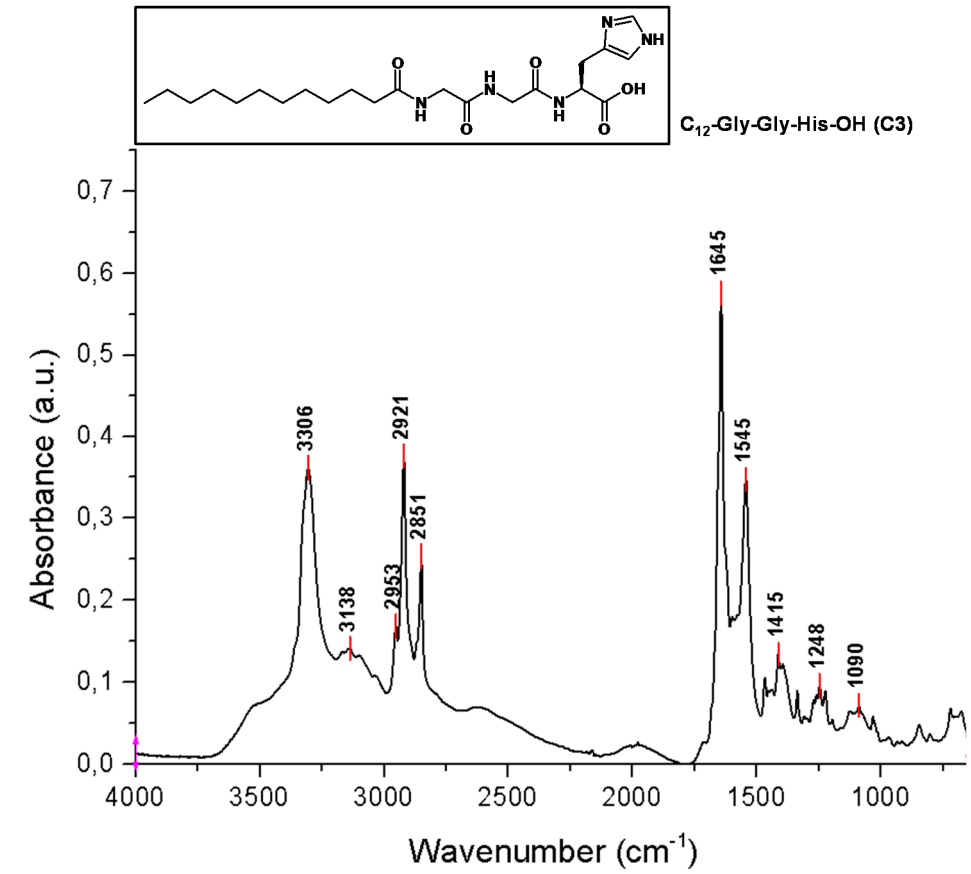

C12-Gly-Gly-His(1-Trt)-OMe
Absorbance (a.u.)
Wavenumber (cm-1)
3325
3277
3065
2963
2926
2856
1744
1670
1631
1524
1445
1170
1132
1037
752
701

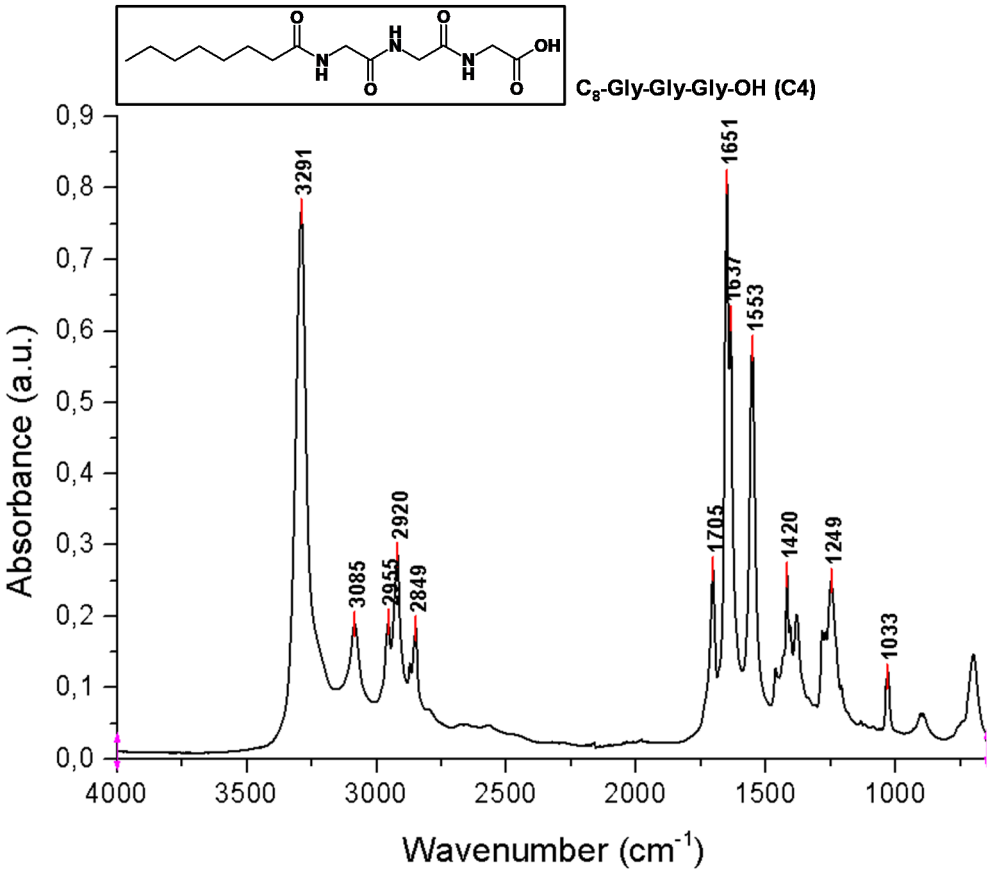

C8-Gly-Gly-His-OMe
Absorbance (a.u.)
Wavenumber (cm-1)
3310
3192
3078
2927
2851
1733
1650
1581
1551
1420
1383
1243
985
758

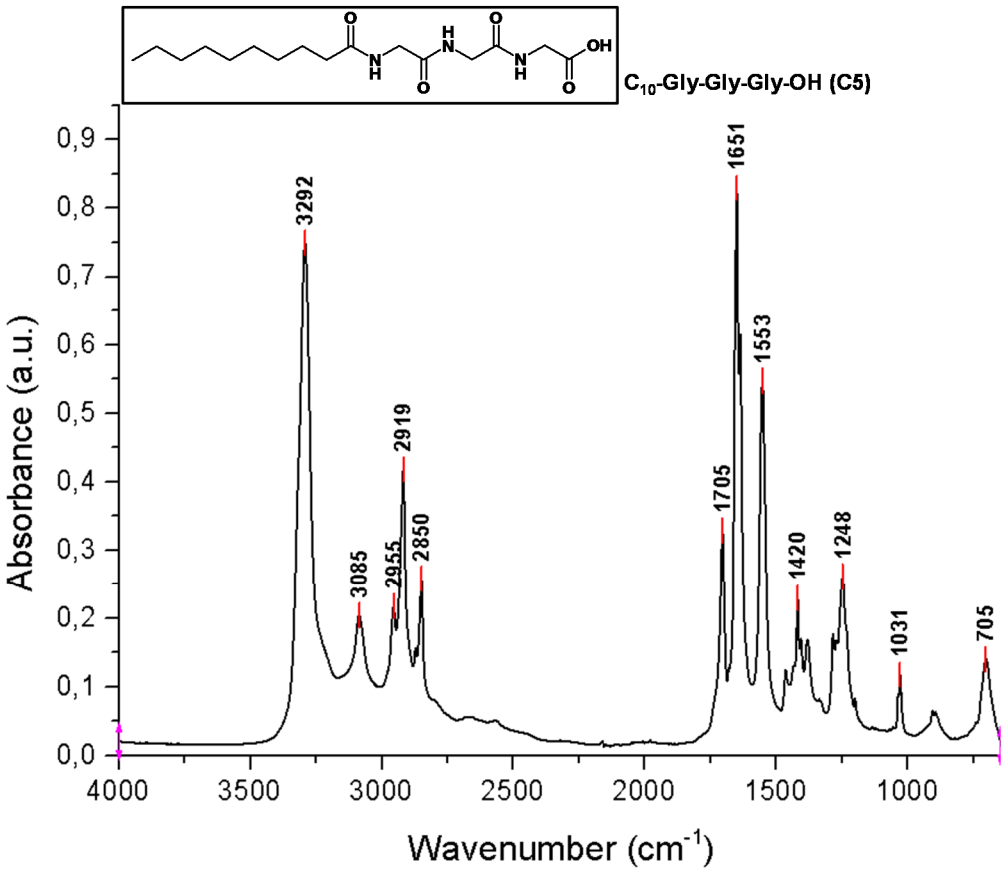

C12-Gly-Gly-His-OMe
Absorbance (a.u.)
Wavenumber (cm-1)
3320
3269
3197
3075
2955
2924
2851
1734
1673
1650
1551
1383
1242

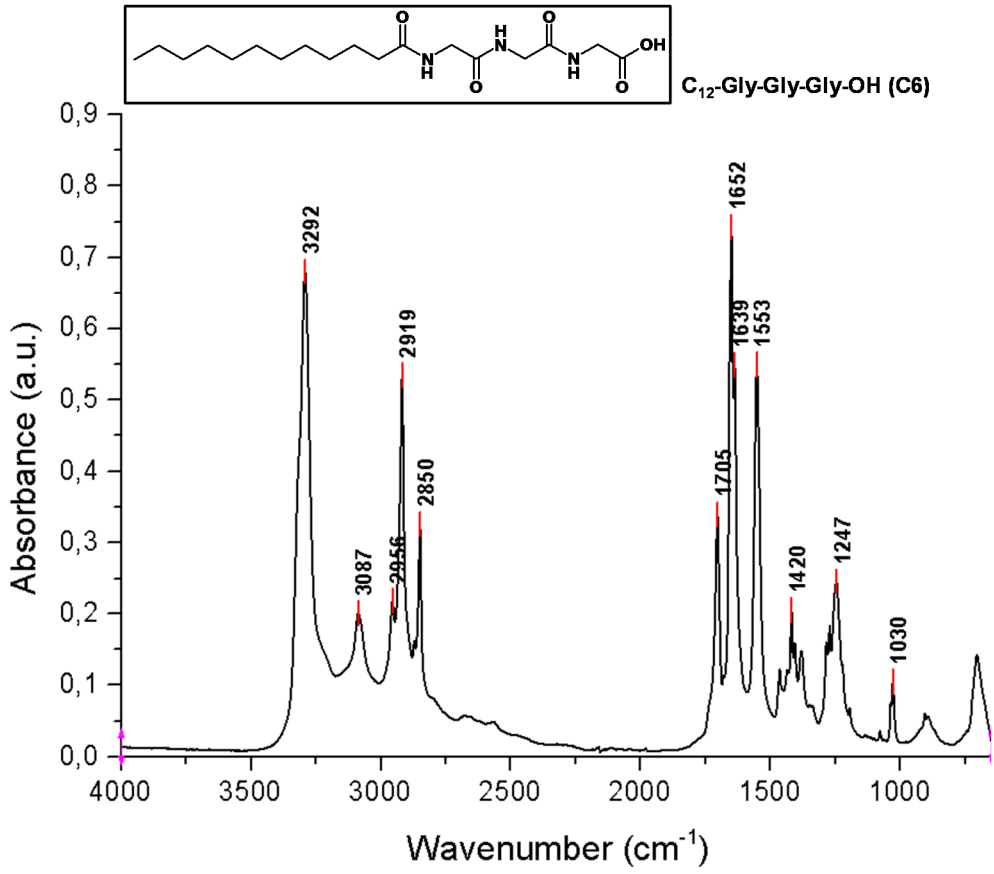

C8-Gly-Gly-His-OH (C1)
Absorbance (a.u.)
Wavenumber (cm-1)
3296
3143
2955
2924
2854
1646
1550
1407
1247
1028

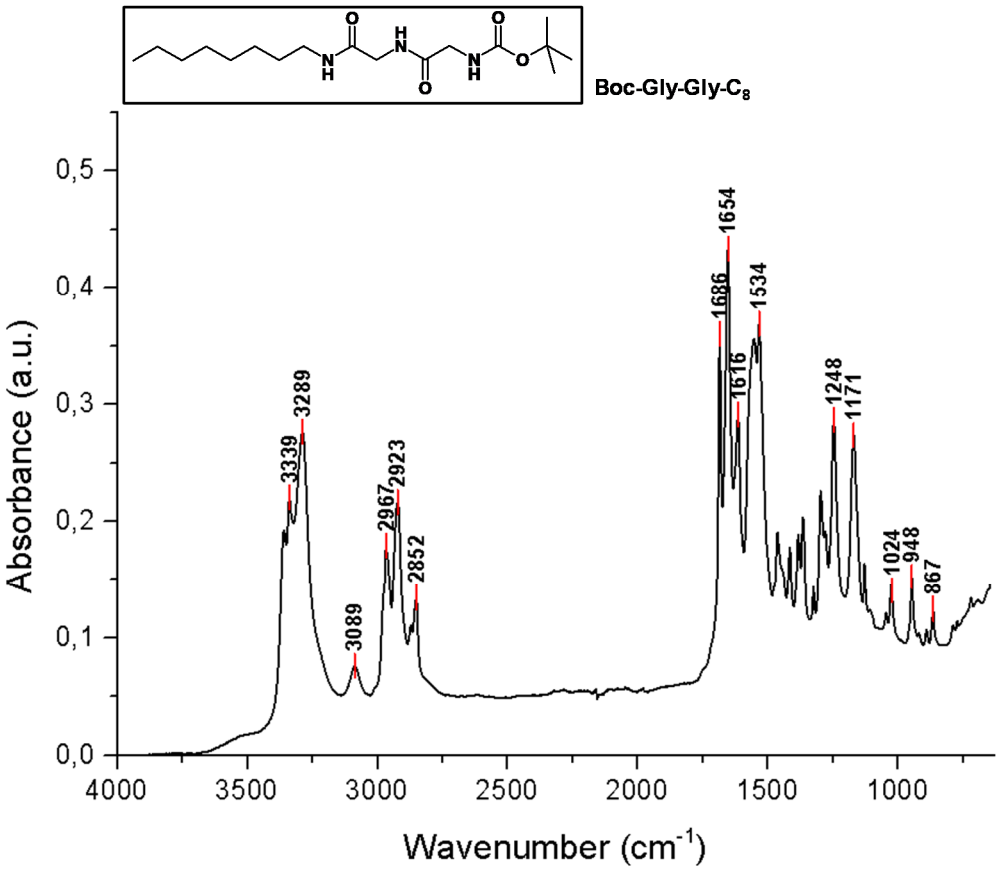

C10-Gly-Gly-His-OH (C2)
Absorbance (a.u.)
Wavenumber (cm-1)
3298
3150
2955
2922
2852
1647
1545
1418
1201
1137
837
722

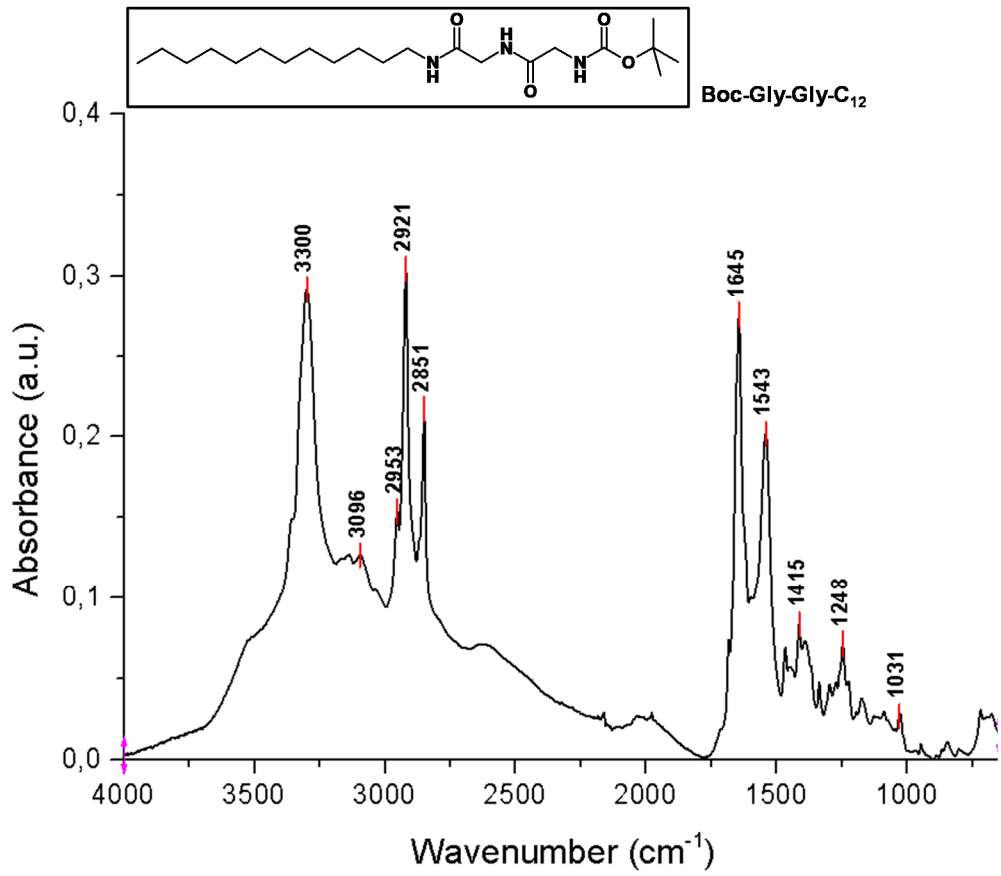

C12-Gly-Gly-His-OH (C3)
Absorbance (a.u.)
Wavenumber (cm-1)
3306
3138
2953
2921
2851
1645
1545
1415
1248
1090

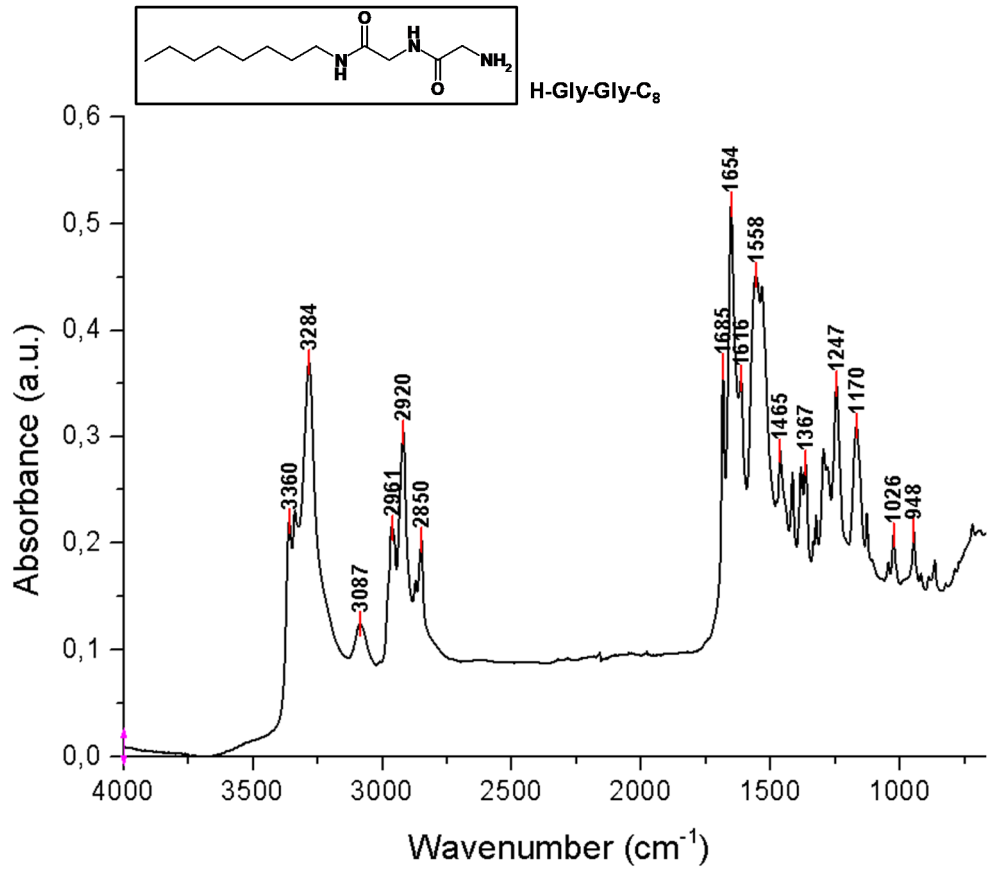

C8-Gly-Gly-Gly-OH (C4)
Absorbance (a.u.)
Wavenumber (cm-1)
3291
3085
2955
2920
2849
1705
1651
1637
1553
1420
1249
1033

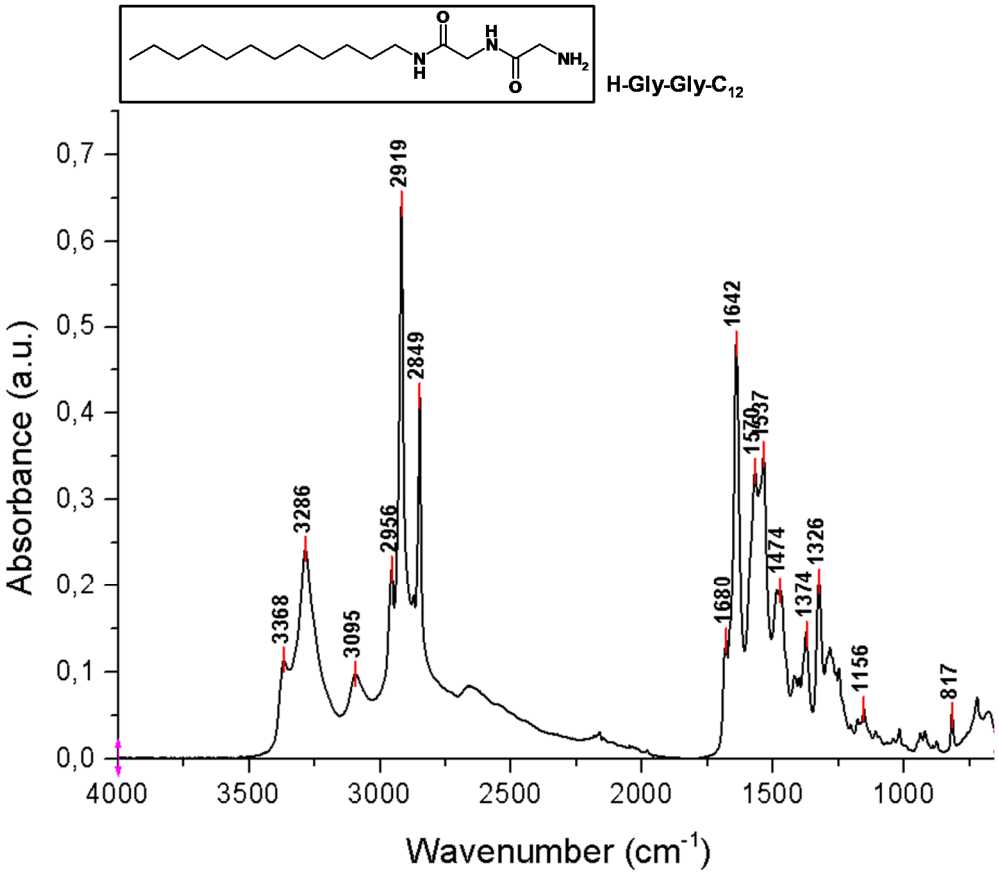

C10-Gly-Gly-Gly-OH (C5)
Absorbance (a.u.)
Wavenumber (cm-1)
3292
3085
2955
2919
2850
1705
1651
1553
1420
1248
1031
705

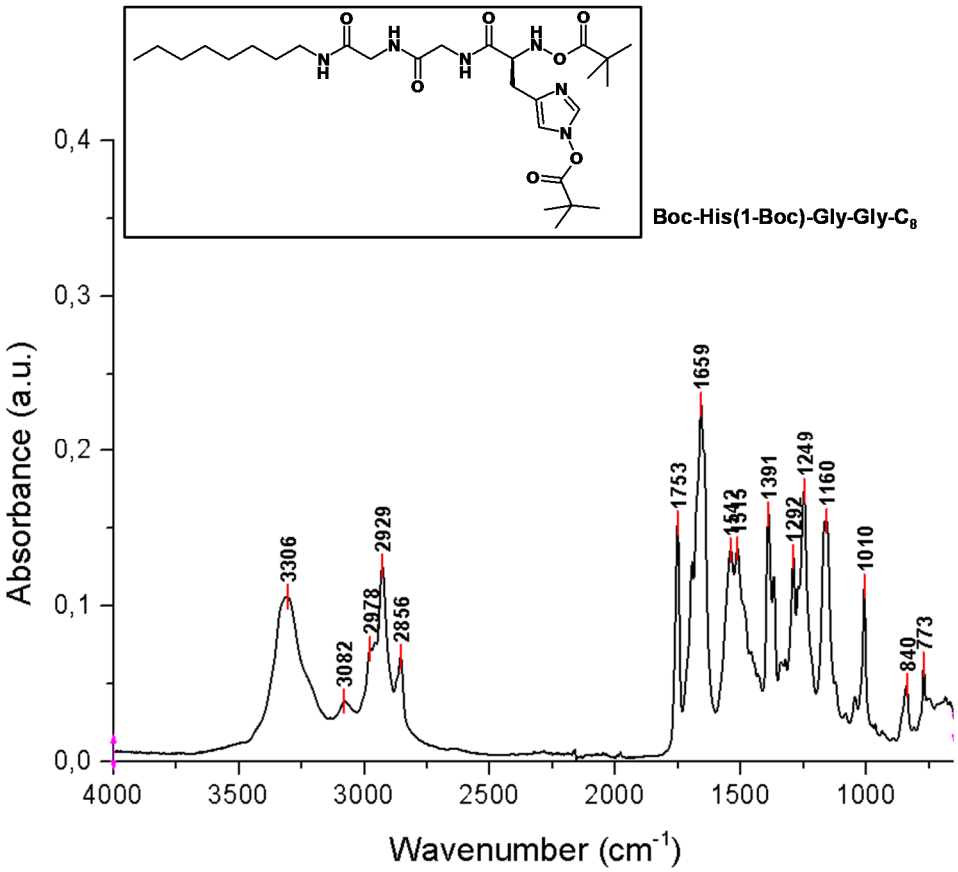

C12-Gly-Gly-Gly-OH (C6)
Absorbance (a.u.)
Wavenumber (cm-1)
3292
3087
2956
2919
2850
1705
1652
1639
1553
1420
1247
1030

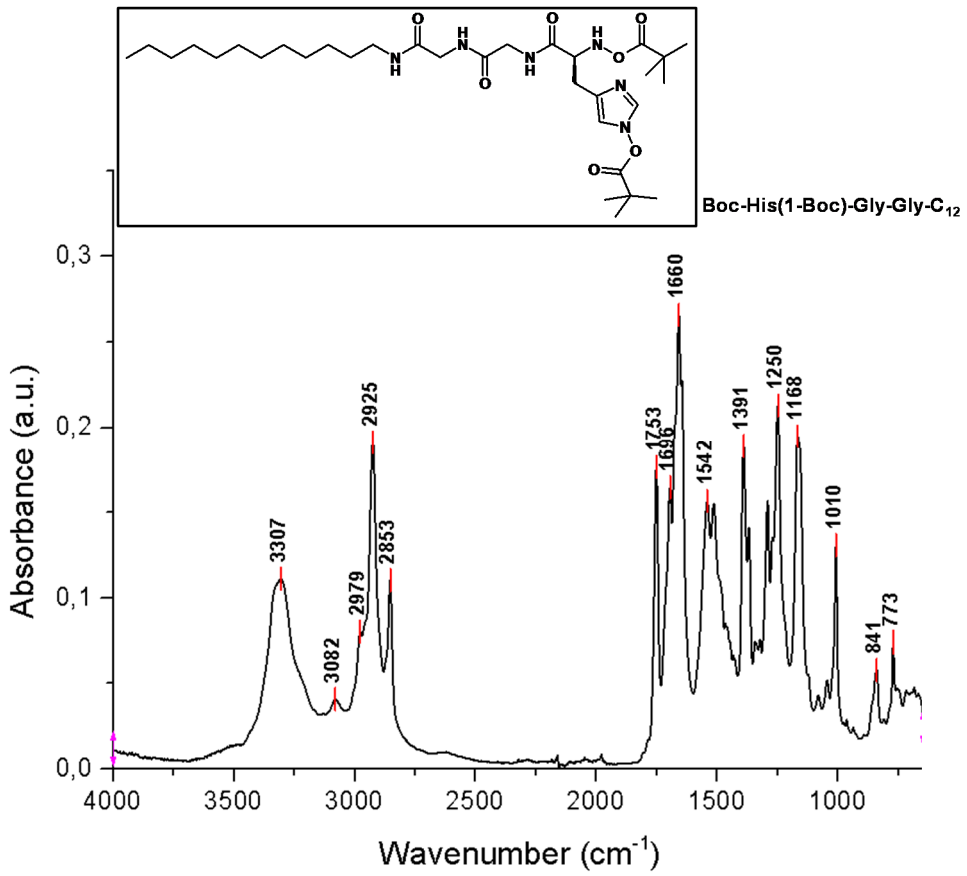

Boc-Gly-Gly-C8
Absorbance (a.u.)
Wavenumber (cm-1)
0,0
0,1
0,2
0,3
0,4
0,5
4000
3500
3000
2500
2000
1500
1000
3289
3339
3089
2967
2923
2852
1654
1686
1616
1534
1248
1171
1024
948
867

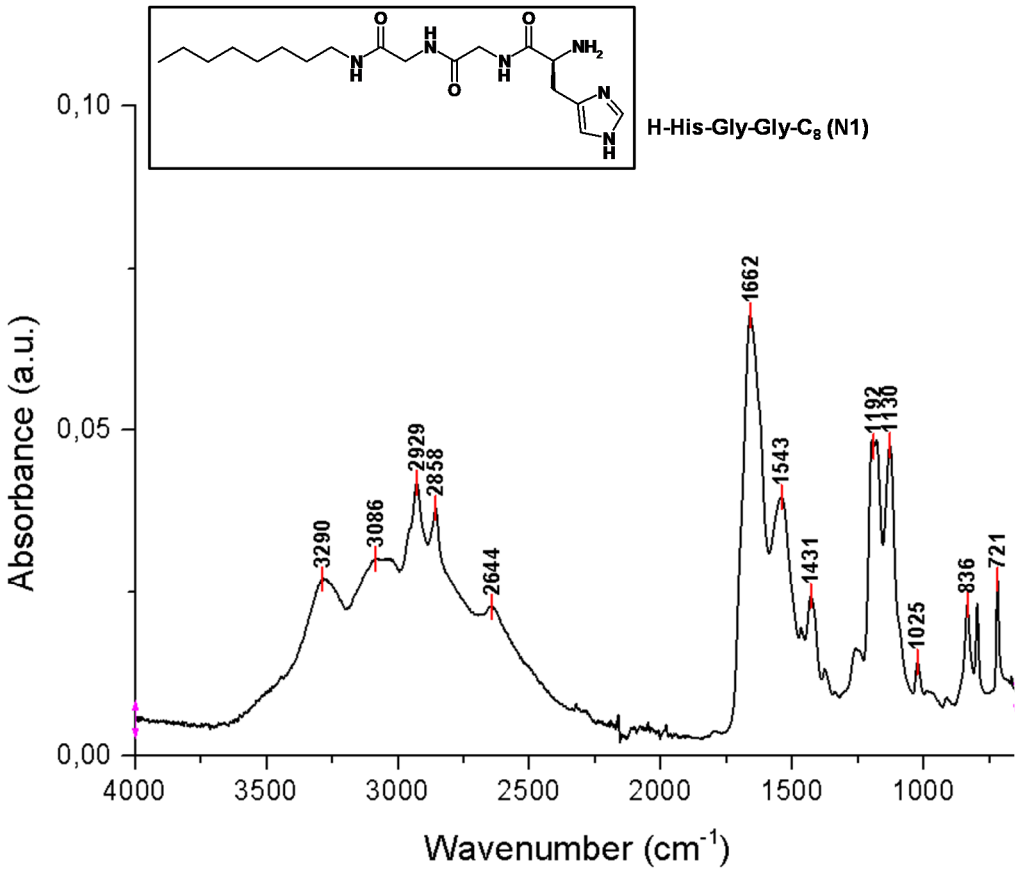

Boc-Gly-Gly-C12
Absorbance (a.u.)
Wavenumber (cm-1)
0,0
0,1
0,2
0,3
0,4
4000
3500
3000
2500
2000
1500
1000
3300
3096
2953
2921
2851
1645
1543
1415
1248
1031

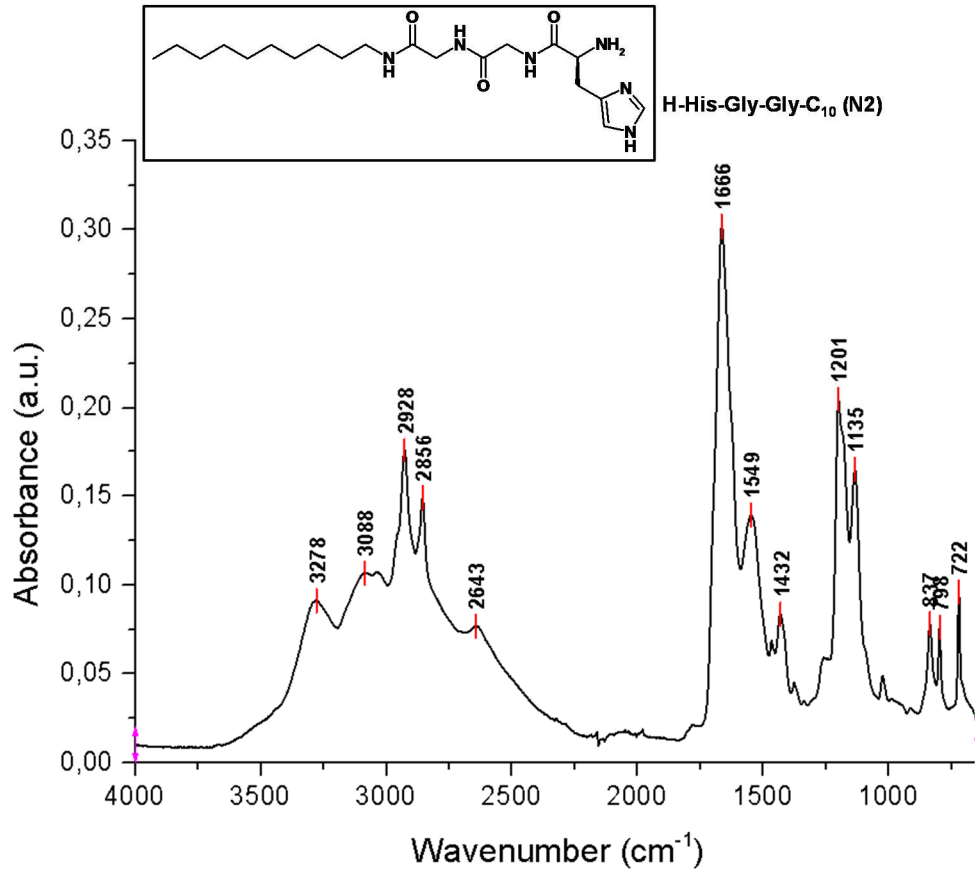

H-Gly-Gly-C8
Absorbance (a.u.)
Wavenumber (cm-1)
3360
3284
3087
2961
2920
2850
1685
1654
1616
1558
1465
1367
1247
1170
1026
948

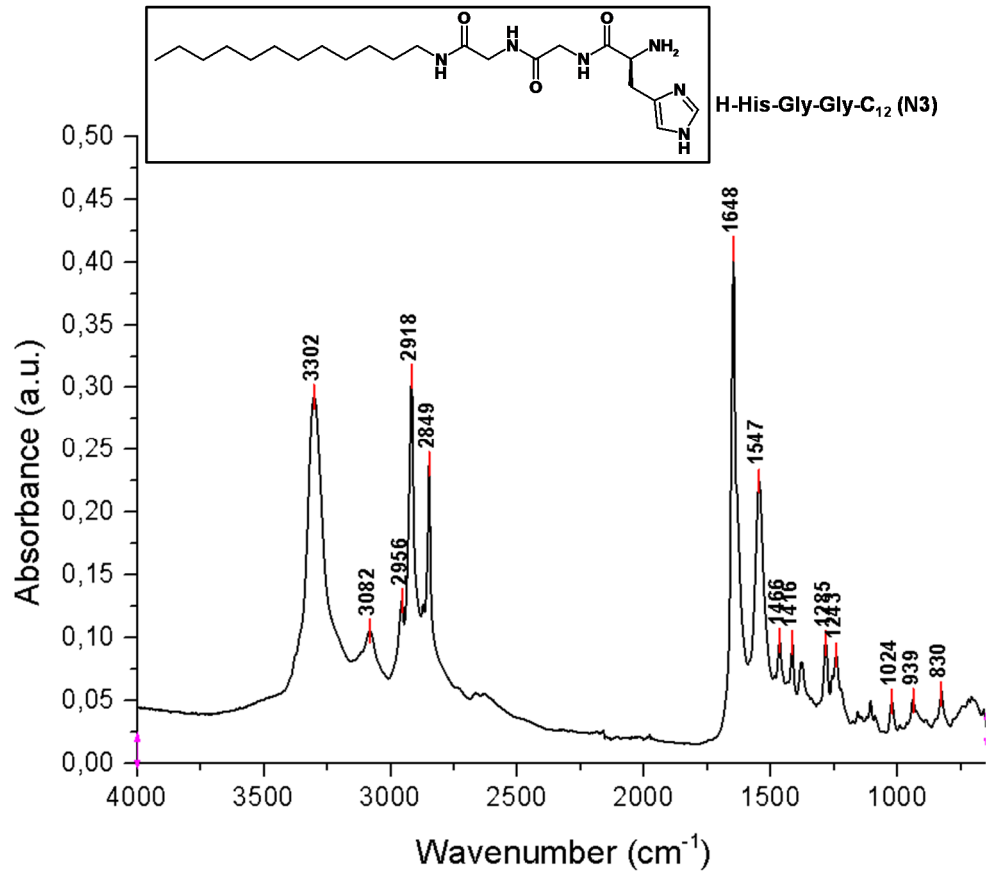

H-Gly-Gly-C12
Absorbance (a.u.)
Wavenumber (cm-1)
3368
3286
3095
2956
2919
2849
1680
1642
1570
1537
1474
1374
1326
1156
817

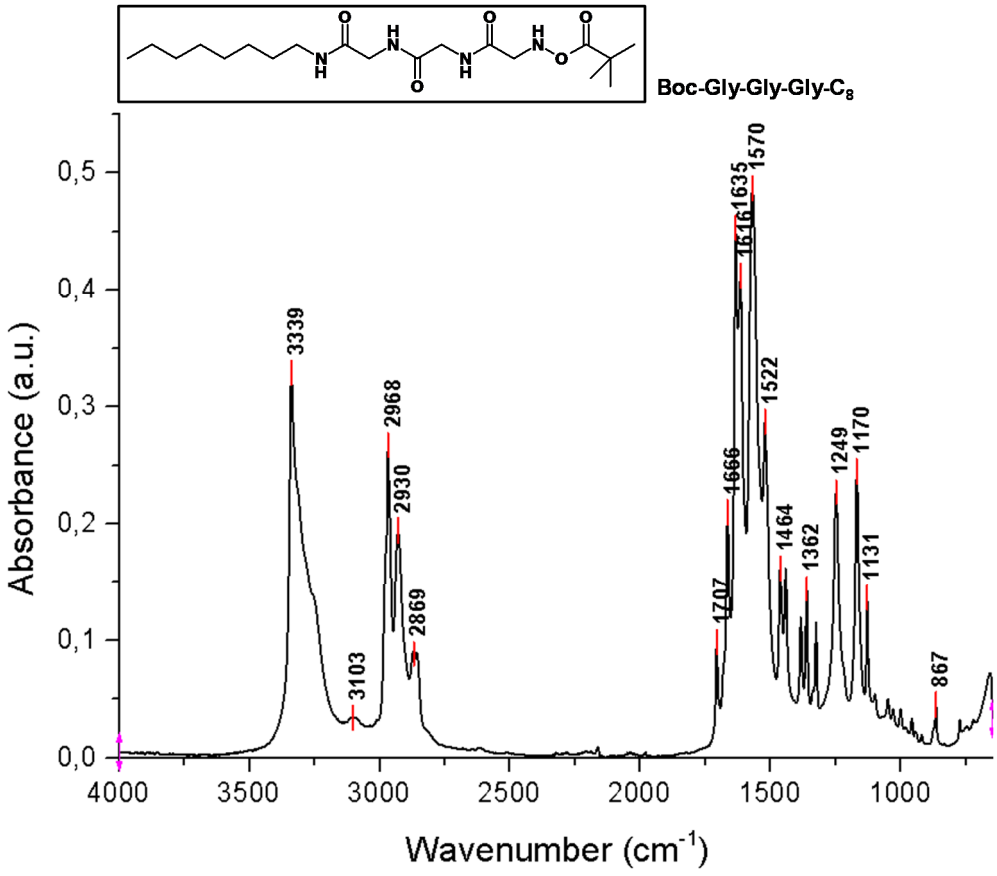

Boc-His(1-Boc)-Gly-Gly-C8
Absorbance (a.u.)
Wavenumber (cm-1)
3306
3082
2978
2929
2856
1753
1659
1543
1391
1292
1249
1160
1010
840
773

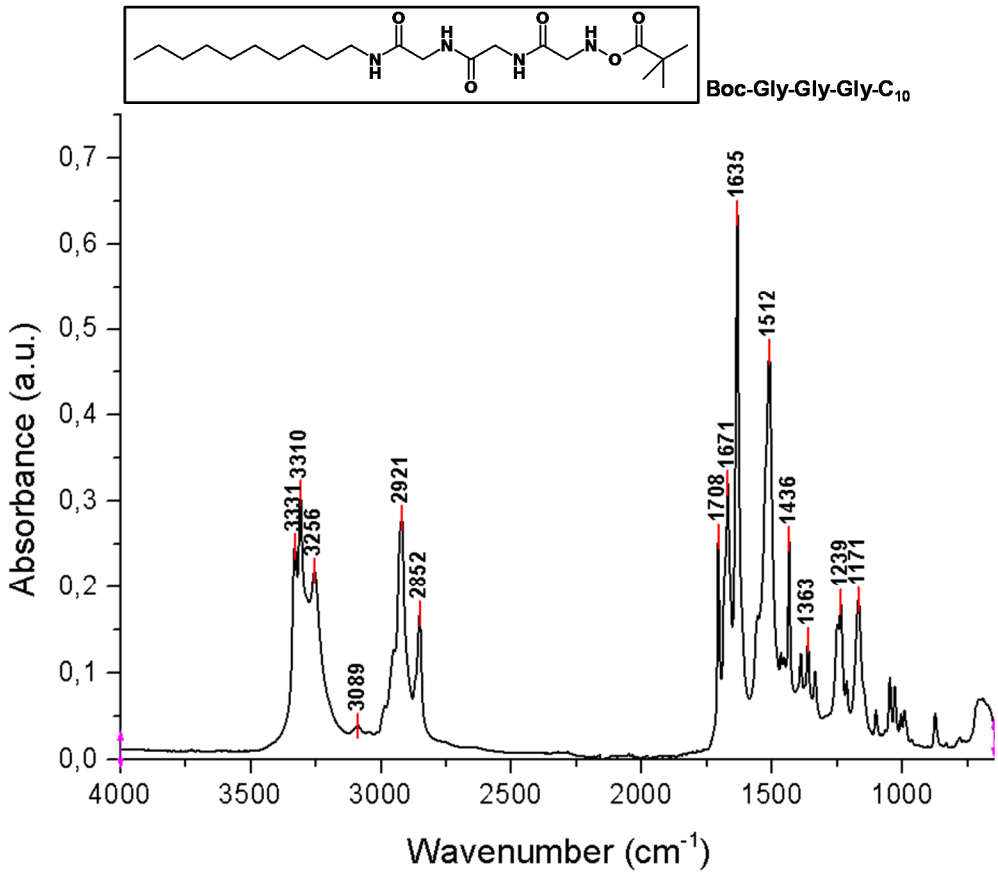

Boc-His(1-Boc)-Gly-Gly-C12
Absorbance (a.u.)
Wavenumber (cm-1)
3307
3082
2979
2925
2853
1753
1696
1660
1542
1391
1250
1168
1010
841
773

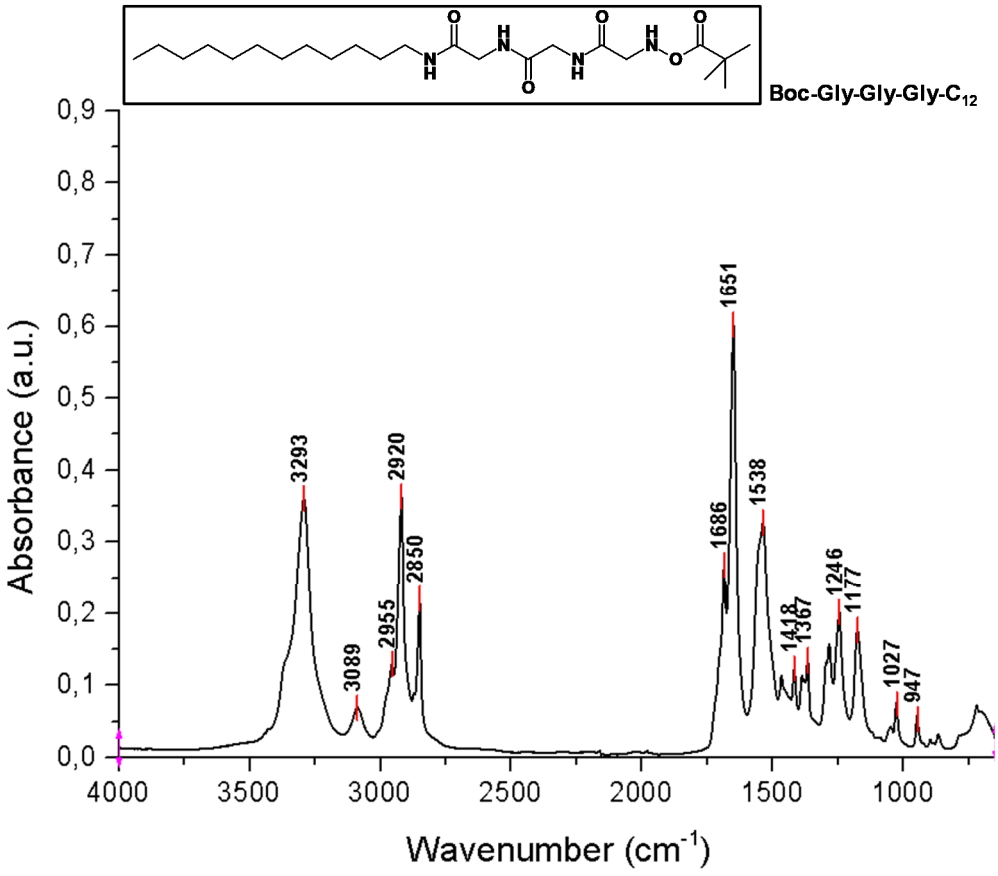

H-His-Gly-Gly-C8 (N1)
Absorbance (a.u.)
Wavenumber (cm-1)
0,00
0,05
0,10
4000
3500
3000
2500
2000
1500
1000
3290
3086
2929
2858
2644
1662
1543
1431
1192
1130
1025
836
721

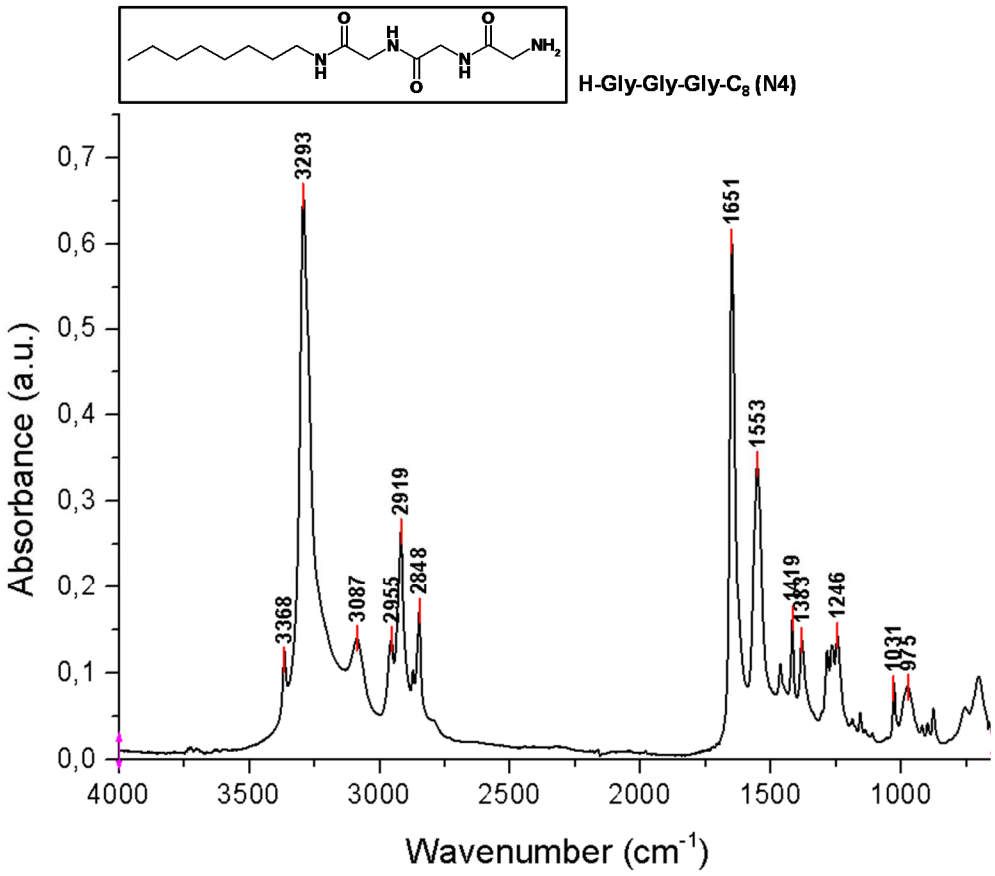

H-His-Gly-Gly-C10 (N2)
Absorbance (a.u.)
Wavenumber (cm-1)
0,00
0,05
0,10
0,15
0,20
0,25
0,30
0,35
4000
3500
3000
2500
2000
1500
1000
3278
3088
2928
2856
2643
1666
1549
1432
1201
1135
837
798
722

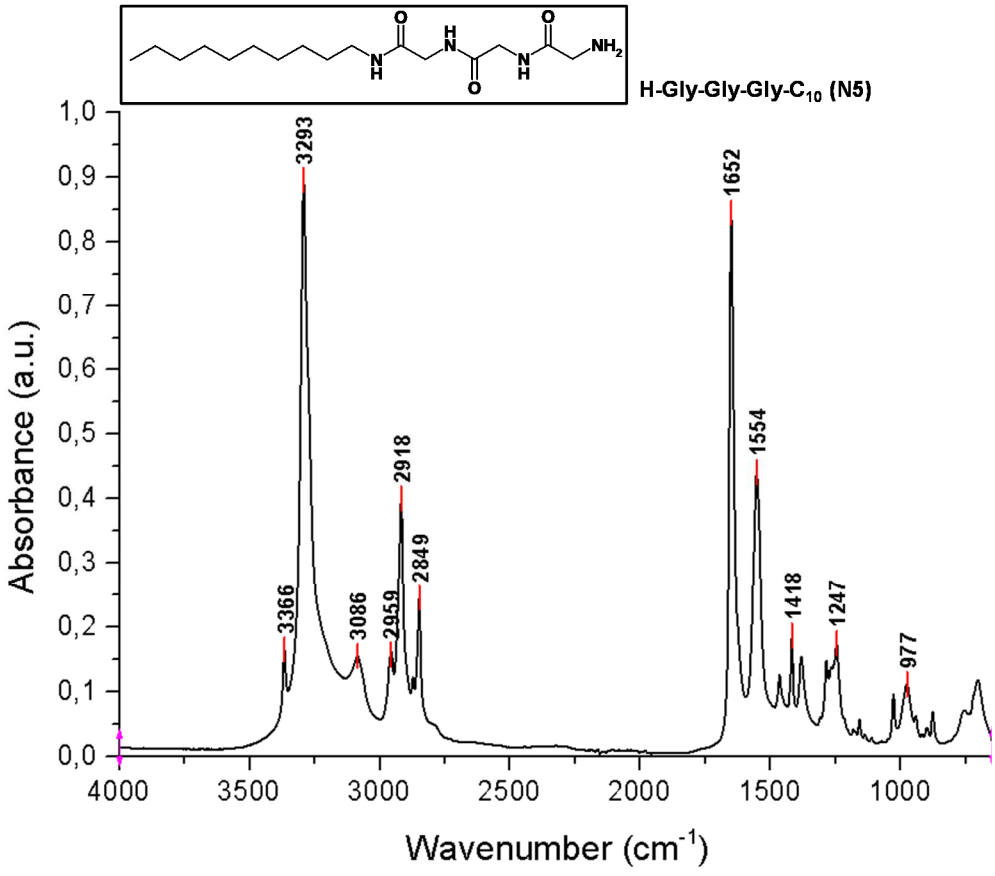

H-His-Gly-Gly-C12 (N3)
Absorbance (a.u.)
Wavenumber (cm-1)
3302
3082
2956
2918
2849
1648
1547
1466
1416
1285
1243
1024
939
830

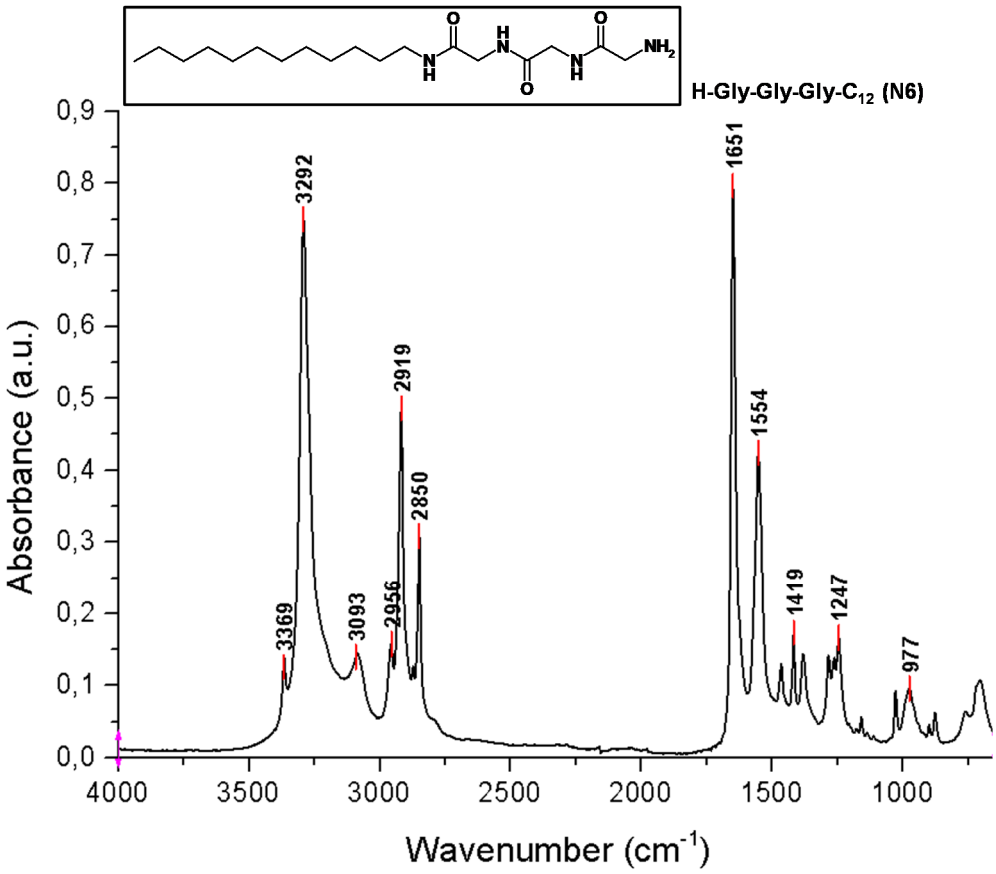

Boc-Gly-Gly-Gly-C8
Absorbance (a.u.)
Wavenumber (cm-1)
3339
3103
2968
2930
2869
1707
1666
1635
1616
1570
1522
1464
1362
1249
1170
1131
867

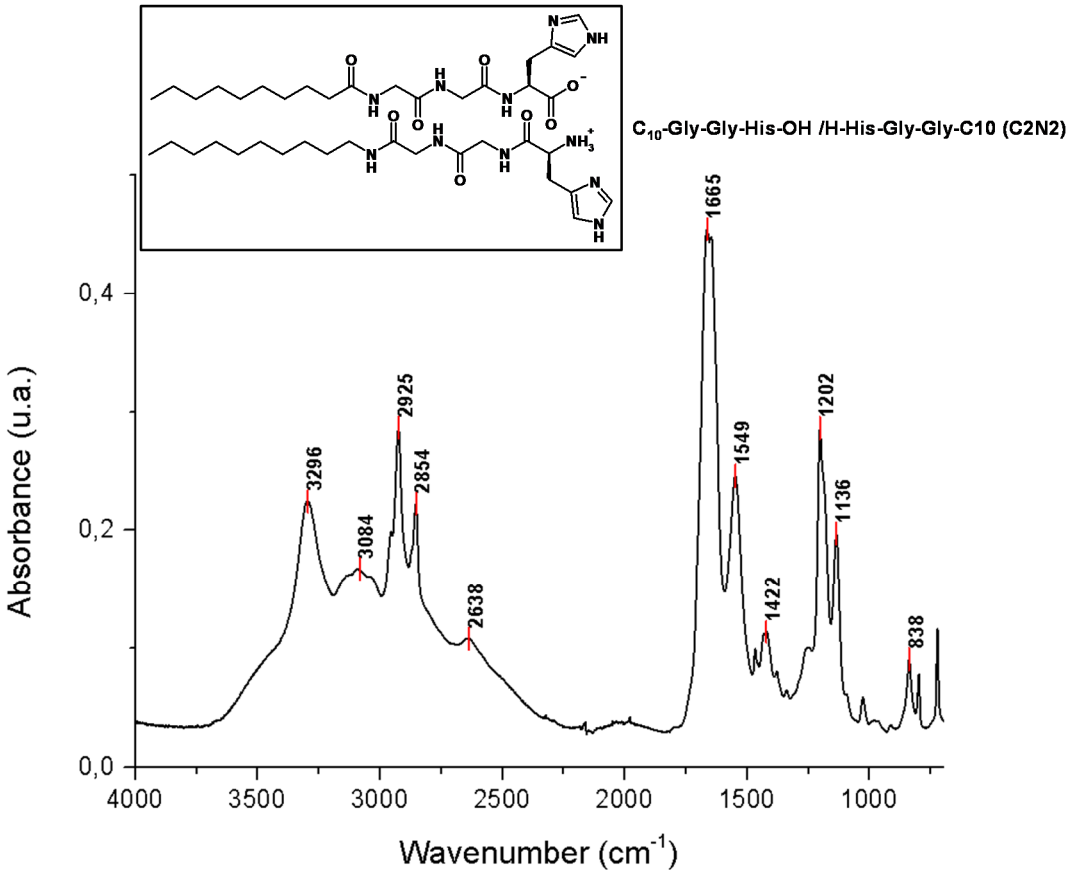

Boc-Gly-Gly-Gly-C10
Absorbance (a.u.)
Wavenumber (cm-1)
3331
3310
3256
3089
2921
2852
1708
1671
1635
1512
1436
1363
1239
1171


Boc-Gly-Gly-Gly-C12
Absorbance (a.u.)
Wavenumber (cm-1)
3293
3089
2955
2920
2850
1686
1651
1538
1418
1367
1246
1177
1027
947


H-Gly-Gly-Gly-C8 (N4)
Absorbance (a.u.)
Wavenumber (cm-1)
3293
3368
3087
2955
2919
2848
1651
1553
1419
1383
1246
1031
975


H-Gly-Gly-Gly-C10 (N5)
Absorbance (a.u.)
Wavenumber (cm-1)
3293
3366
3086
2959
2918
2849
1652
1554
1418
1247
977

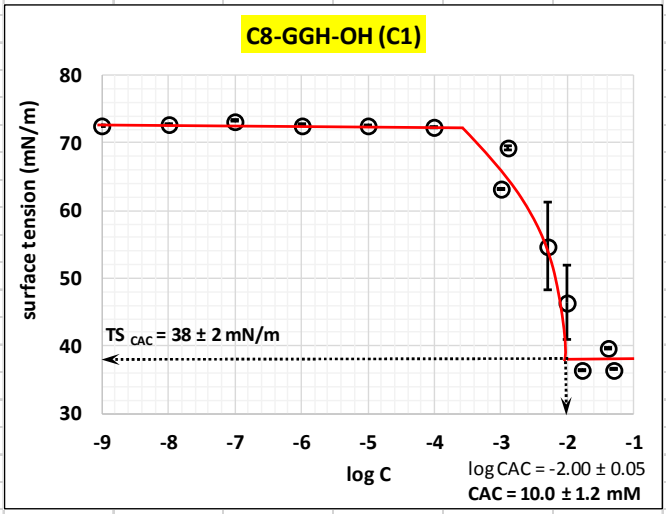

H-Gly-Gly-Gly-C12 (N6)
Absorbance (a.u.)
Wavenumber (cm-1)
3369
3292
3093
2956
2919
2850
1651
1554
1419
1247
977


C10-Gly-Gly-His-OH /H-His-Gly-Gly-C10 (C2N2)
Absorbance (u.a.)
Wavenumber (cm-1)
3296
3084
2925
2854
2638
1665
1549
1422
1202
1136
838

## 4. Tensiometry measurements

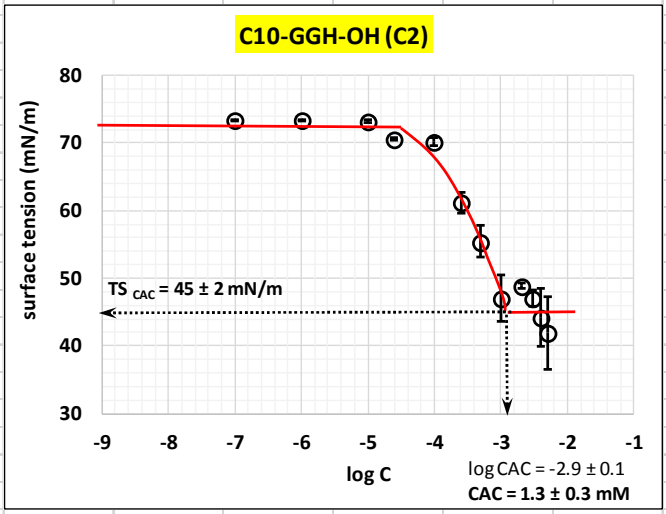
**C8-GGH-OH (C1)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -9 | 72,6 | 0,1 |
| -8 | 72,8 | 0,1 |
| -7 | 73,3 | 0,1 |
| -6 | 72,67 | 0,1 |
| -5 | 72,56 | 0,1 |
| -4 | 72,4 | 0,1 |
| -3 | 63,3 | 0,1 |
| -2,90309 | 69,3 | 0,3 |
| -2,30103 | 54,7 | 6,5 |
| -2 | 46,5 | 5,5 |
| -1,78516 | 36,5 | 0,1 |
| -1,39794 | 39,7 | 0,1 |
| -1,30103 | 36,6 | 0,1 |



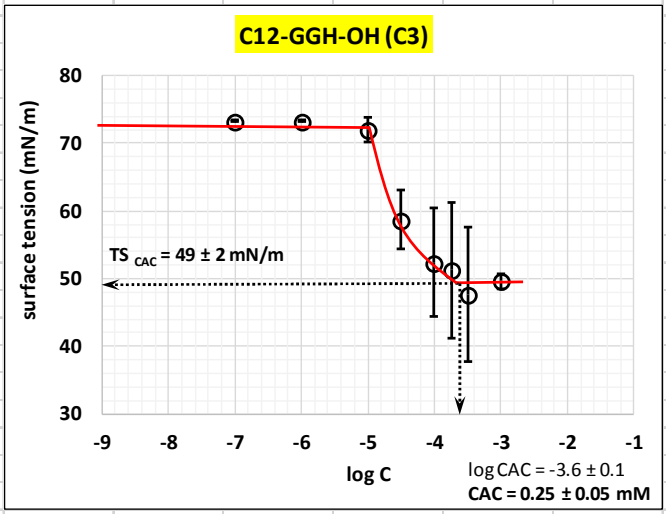

**C10-GGH-OH (C2)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,35 | 0,2 |
| -6 | 73,35 | 0,2 |
| -5 | 73,3 | 0,2 |
| -4,60206 | 70,6 | 0,2 |
| -4 | 70,2 | 0,7 |
| -3,60206 | 61,2 | 1,5 |
| -3,30103 | 55,45 | 2,3 |
| -3 | 47,1 | 3,5 |
| -2,69897 | 48,9 | 0,4 |
| -2,52288 | 47,05 | 1,3 |
| -2,39794 | 44,2 | 4,3 |
| -2,30103 | 41,95 | 5,4 |



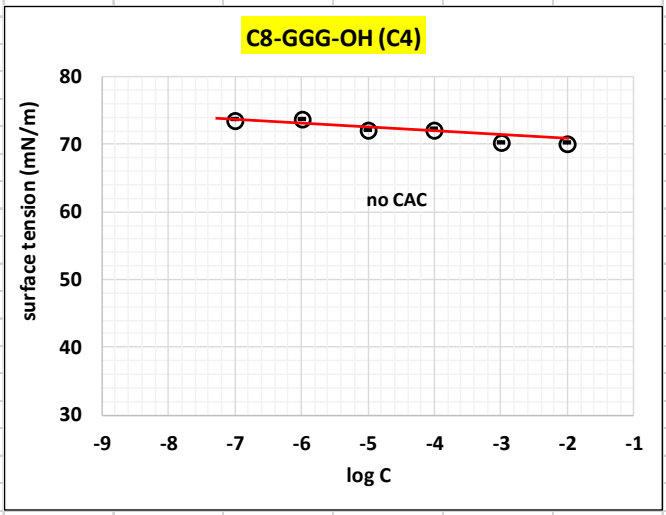
**C12-GGH-OH (C3)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,3 | 0,1 |
| -6 | 73,3 | 0,1 |
| -5 | 72,03333 | 1,9 |
| -4,5 | 58,7 | 4,4 |
| -4 | 52,43333 | 8 |
| -3,75 | 51,23333 | 10 |
| -3,5 | 47,66667 | 10 |
| -3 | 49,63333 | 1,1 |



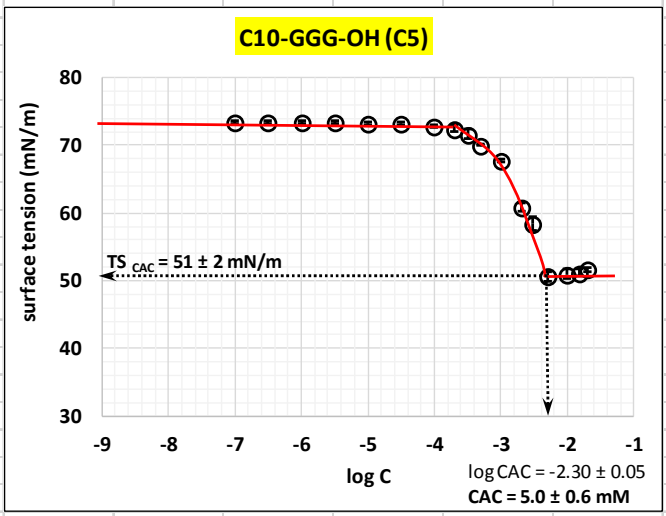

**C8-GGG-OH (C4)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,7 | 0,2 |
| -6 | 73,8 | 0,2 |
| -5 | 72,2 | 0,2 |
| -4 | 72,3 | 0,2 |
| -3 | 70,4 | 0,2 |
| -2 | 70,3 | 0,2 |



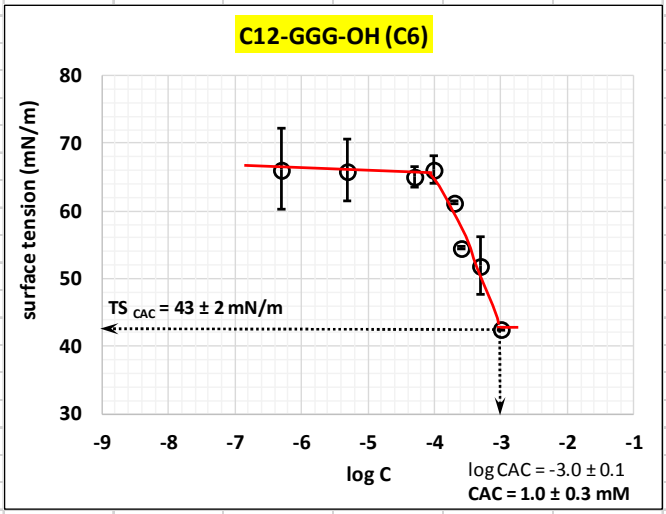

**C10-GGG-OH (C5)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,4 | 0,2 |
| -6,5 | 73,5 | 0,2 |
| -6 | 73,4 | 0,2 |
| -5,5 | 73,4 | 0,2 |
| -5 | 73,3 | 0,2 |
| -4,5 | 73,2 | 0,2 |
| -4 | 72,75 | 0,2 |
| -3,7 | 72,45 | 0,5 |
| -3,5 | 71,65 | 0,7 |
| -3,3 | 70,1 | 0,2 |
| -3 | 67,7 | 0,2 |
| -2,69897 | 60,9 | 0,6 |
| -2,52288 | 58,4 | 1 |
| -2,30103 | 50,65 | 0,7 |
| -2 | 50,95 | 0,7 |
| -1,82391 | 51,15 | 0,5 |
| -1,69897 | 51,7 | 0,3 |



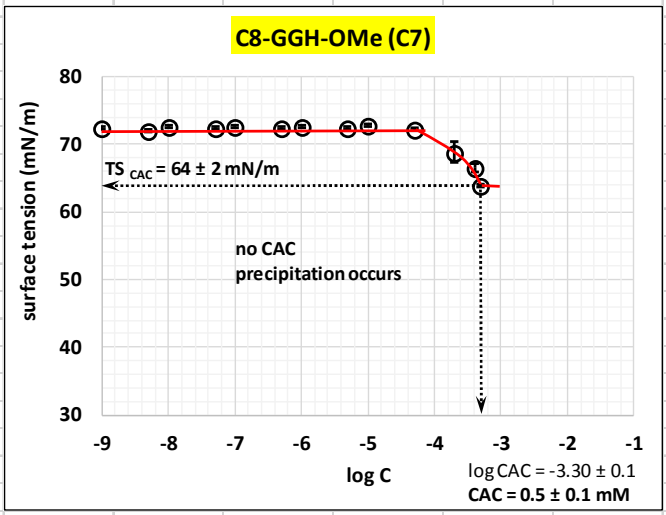
**C12-GGG-OH (C6)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -6,30103 | 66,23333 | 6 |
| -5,30103 | 66,03333 | 4,6 |
| -4,30103 | 65,1 | 1,5 |
| -4 | 66,2 | 2 |
| -3,6 | 54,6 | 0,2 |
| -3,30103 | 51,93333 | 4,2 |
| -3 | 42,7 | 0,2 |
| -3,69897 | 61,3 | 0,2 |



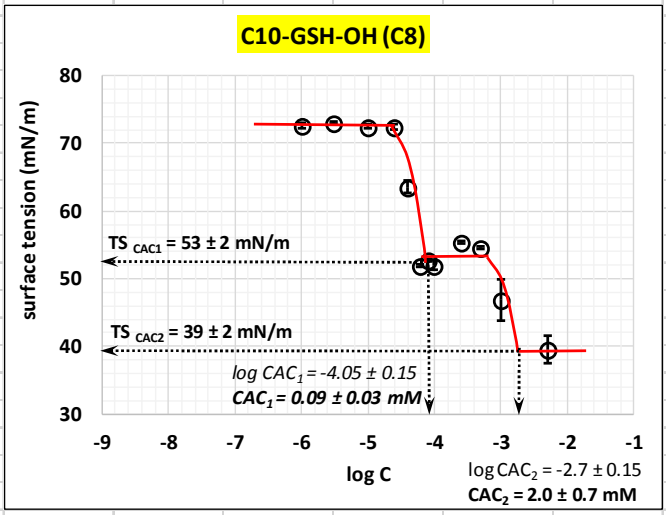

**C8-GGH-OMe (C7)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -9 | 72,5 | 0,2 |
| -8,30103 | 72,1 | 0,2 |
| -8 | 72,6 | 0,2 |
| -7,30103 | 72,5 | 0,2 |
| -7 | 72,65 | 0,2 |
| -6,30103 | 72,5 | 0,2 |
| -6 | 72,6 | 0,2 |
| -5,30103 | 72,5 | 0,2 |
| -5 | 72,8 | 0,2 |
| -4,30103 | 72,2 | 0,2 |
| -3,69897 | 68,83 | 1,5 |
| -3,398 | 66,5 | 0,6 |
| -3,301 | 63,9 | 0,2 |



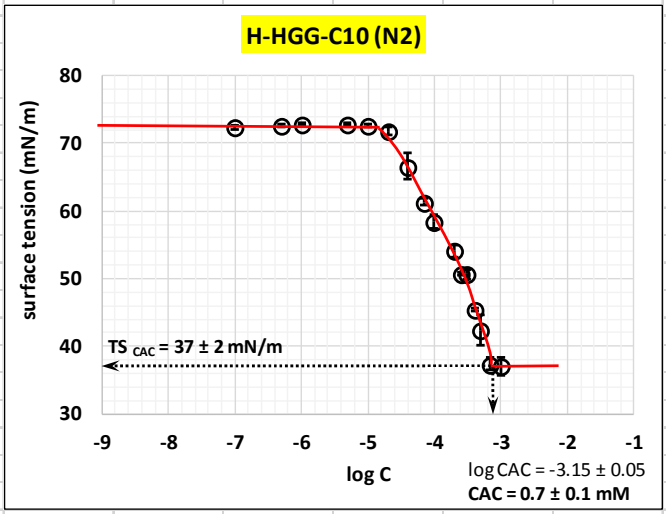

**C10-GSH-OH (C8)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -6 | 72,7 | 0,4 |
| -5,52288 | 73 | 0,2 |
| -5 | 72,53 | 0,3 |
| -4,60206 | 72,53 | 0,4 |
| -4,39794 | 63,55 | 0,9 |
| -4,22185 | 51,9 | 0,2 |
| -4,09691 | 52,8 | 0,2 |
| -4 | 52,03 | 0,7 |
| -3,60206 | 55,3 | 0,2 |
| -3,30103 | 54,5 | 0,2 |
| -3 | 46,9 | 3 |
| -2,30103 | 39,6 | 2 |



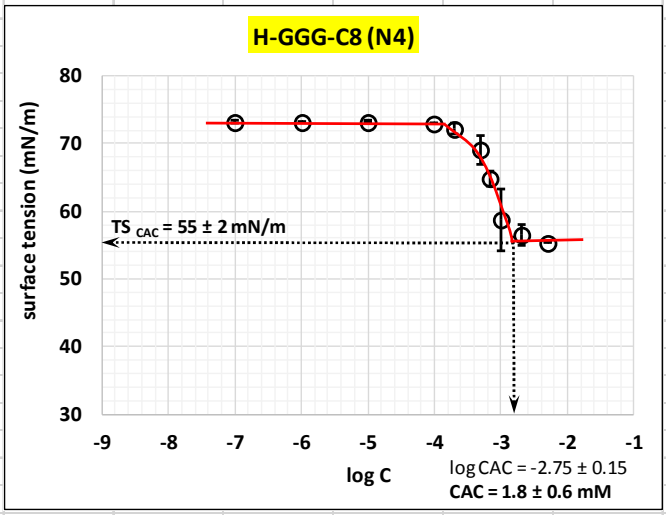
**H-HGG-C10 (N2)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 72,4 | 0,3 |
| -6,30103 | 72,65 | 0,2 |
| -6 | 72,8 | 0,2 |
| -5,30103 | 72,8 | 0,2 |
| -5 | 72,7 | 0,2 |
| -4,69897 | 71,8 | 0,6 |
| -4,39794001 | 66,6 | 1,9 |
| -4,15490196 | 61,3 | 0,5 |
| -4 | 58,47 | 1 |
| -3,69897 | 54,1 | 0,9 |
| -3,60205999 | 50,8 | 0,2 |
| -3,52287875 | 50,7 | 0,7 |
| -3,39794001 | 45,5 | 0,2 |
| -3,30103 | 42,43 | 2,2 |
| -3,15490196 | 37,35 | 0,9 |
| -3 | 37,04 | 1,3 |



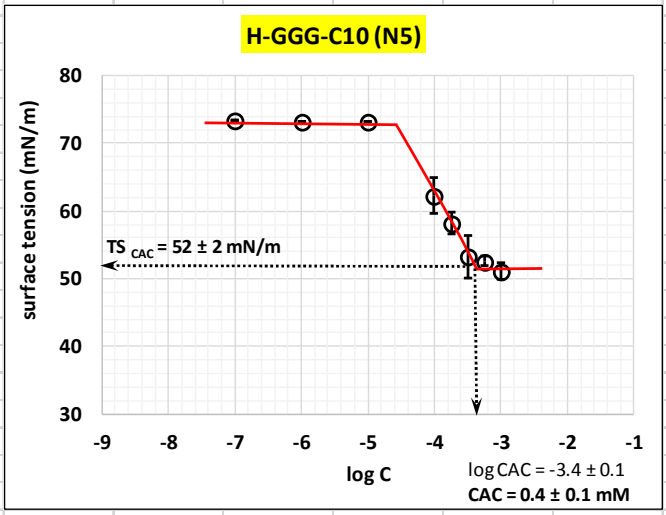

**H-GGG-C8 (N4)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,3 | 0,2 |
| -6 | 73,2 | 0,1 |
| -5 | 73,3 | 0,1 |
| -4 | 73,0 | 0,1 |
| -3,69897 | 72,2 | 0,9 |
| -3,30103 | 69,1 | 2,1 |
| -3,15490196 | 64,8 | 1,0 |
| -3 | 58,8 | 4,6 |
| -2,69897 | 56,6 | 1,5 |
| -2,30103 | 55,5 | 0,1 |



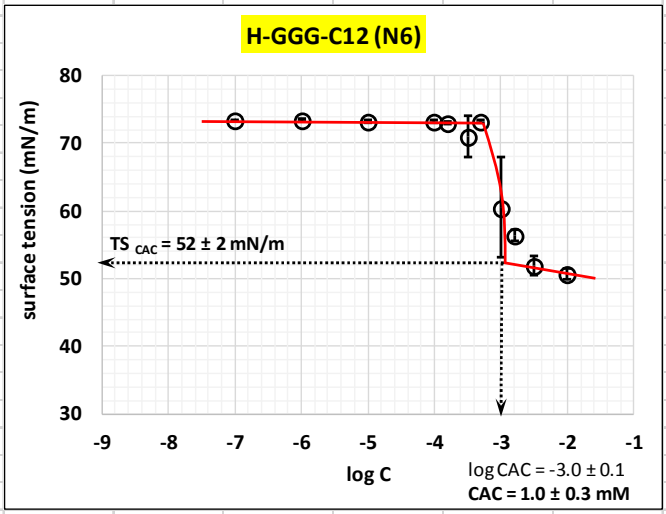

**H-GGG-C10 (N5)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,4 | 0,1 |
| -6 | 73,3 | 0,0 |
| -5 | 73,25 | 0,1 |
| -4 | 62,35 | 2,6 |
| -3,75 | 58,2 | 1,6 |
| -3,5 | 53,3 | 3,1 |
| -3,25 | 52,6 | 0,7 |
| -3 | 51,15 | 1,2 |



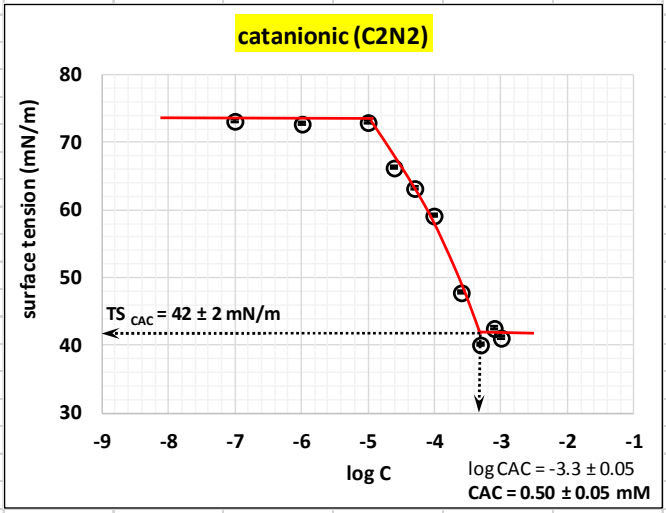
**H-GGG-C12 (N6)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,35 | 0,2 |
| -6 | 73,4 | 0,2 |
| -5 | 73,2 | 0,2 |
| -4 | 73,3 | 0,2 |
| -3,8 | 73,1 | 0,2 |
| -3,5 | 71 | 3,0 |
| -3,3 | 73,3 | 0,2 |
| -3 | 60,5 | 7,4 |
| -2,8 | 56,4 | 0,8 |
| -2,5 | 51,95 | 1,5 |
| -2 | 50,7 | 0,8 |




**catanionic (C2N2)**

| log c | ST (mN/m) | Δ ST |
|---|---|---|
| -7 | 73,2 | 0,2 |
| -6 | 72,9 | 0,2 |
| -5 | 73 | 0,2 |
| -4,60205999 | 66,3 | 0,2 |
| -4,30103 | 63,3 | 0,2 |
| -4 | 59,3 | 0,2 |
| -3,60205999 | 47,9 | 0,2 |
| -3,30103 | 40,2 | 0,2 |
| -3,09691001 | 42,7 | 0,2 |
| -3 | 41,2 | 0,2 |




## 5. Pyrene fluorescence measurements

CACs were estimated from the plots of the $I_1/I_3$ pyrene fluorescence ratios as a function of the concentrations of lipopeptides. The profiles showed a sharp decrease when the aggregates formed. The curves were adjusted with the following Sigmoid-Boltzmann equation:

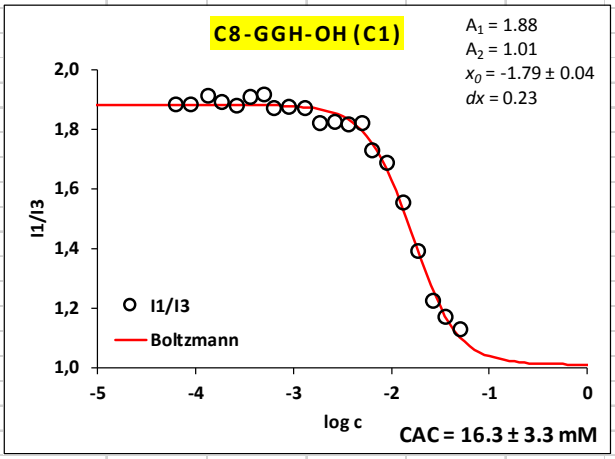
$$y = \frac{A_1 - A_2}{1 + e^{[\frac{x-x0}{dx}]}} + A_2$$

where y is the $I_1/I_3$ ratio, x is related to the lipopeptide concentration (x = log c), and $A_1$ and $A_2$ are the upper and lower limits of the sigmoid.

According to Aguiar *et al.* [J. Aguiar, P. Carpena, J. A. Molina-Bolivar, C. Carnero Ruiz, *J. Coll. Int. Sci.* **2003**, *258*, 116-122], the CACs corresponded to the $x_0$ values.

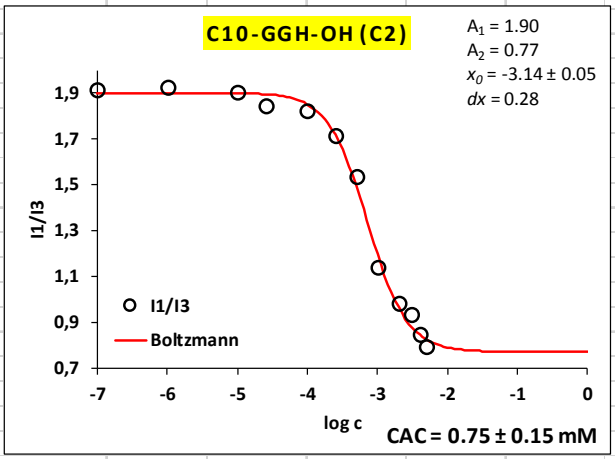
**C8-GGH-OH (C1)**

| log c | I1/I3 |
|---|---|
| -1,305 | 1,128 |
| -1,450 | 1,173 |
| -1,582 | 1,226 |
| -1,733 | 1,391 |
| -1,883 | 1,556 |
| -2,050 | 1,688 |
| -2,205 | 1,728 |
| -2,305 | 1,823 |
| -2,450 | 1,815 |
| -2,582 | 1,825 |
| -2,733 | 1,820 |
| -2,883 | 1,873 |
| -3,050 | 1,876 |
| -3,205 | 1,873 |
| -3,305 | 1,916 |
| -3,450 | 1,911 |
| -3,582 | 1,881 |
| -3,733 | 1,893 |
| -3,883 | 1,912 |
| -4,050 | 1,883 |
| -4,205 | 1,883 |



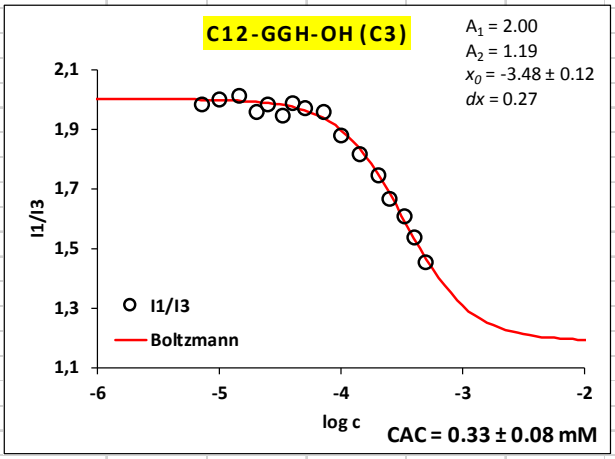

**C10-GGH-OH (C2)**

| log c | I1/I3 |
|---|---|
| -7,000 | 1,916 |
| -6,000 | 1,926 |
| -5,000 | 1,901 |
| -4,602 | 1,843 |
| -4,000 | 1,821 |
| -3,602 | 1,712 |
| -3,301 | 1,537 |
| -3,000 | 1,141 |
| -2,699 | 0,981 |
| -2,523 | 0,932 |
| -2,398 | 0,845 |
| -2,301 | 0,793 |



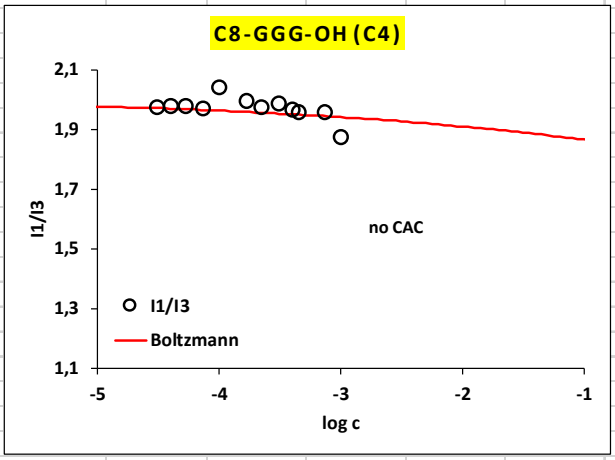
**C12-GGH-OH (C3)**

| log c | I1/I3 |
|---|---|
| -3,305 | 1,453 |
| -3,401 | 1,536 |
| -3,480 | 1,607 |
| -3,604 | 1,668 |
| -3,701 | 1,746 |
| -3,846 | 1,818 |
| -4,001 | 1,881 |
| -4,147 | 1,961 |
| -4,305 | 1,970 |
| -4,401 | 1,989 |
| -4,480 | 1,948 |
| -4,604 | 1,986 |
| -4,701 | 1,960 |
| -4,846 | 2,015 |
| -5,001 | 2,002 |
| -5,147 | 1,983 |



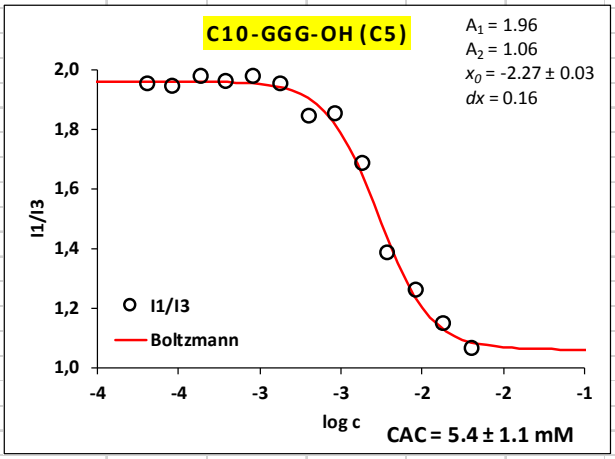

**C8-GGG-OH (C4)**

| log c | I1/I3 |
|---|---|
| -3,004 | 1,876 |
| -3,134 | 1,958 |
| -3,355 | 1,959 |
| -3,400 | 1,966 |
| -3,520 | 1,986 |
| -3,654 | 1,977 |
| -3,779 | 1,996 |
| -4,004 | 2,042 |
| -4,134 | 1,972 |
| -4,281 | 1,981 |
| -4,400 | 1,979 |
| -4,520 | 1,976 |



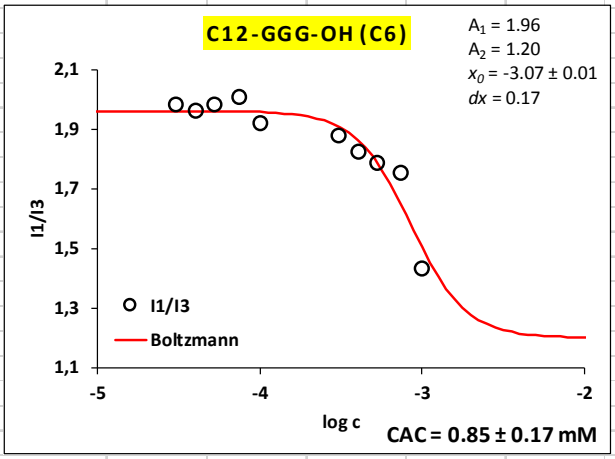

**C10-GGG-OH (C5)**

| log c | I1/I3 |
|---|---|
| -1,703 | 1,067 |
| -1,878 | 1,150 |
| -2,043 | 1,265 |
| -2,219 | 1,387 |
| -2,372 | 1,688 |
| -2,545 | 1,853 |
| -2,699 | 1,846 |
| -2,878 | 1,956 |
| -3,043 | 1,981 |
| -3,219 | 1,962 |
| -3,372 | 1,978 |
| -3,545 | 1,947 |
| -3,699 | 1,957 |



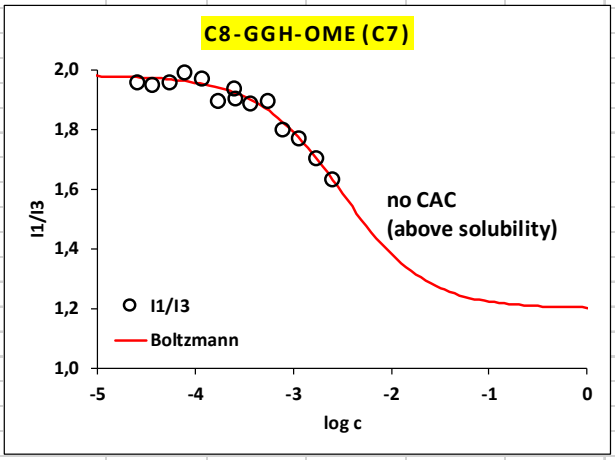
**C12-GGG-OH (C6)**

| log c | I1/I3 |
|---|---|
| -3,004 | 1,435 |
| -3,134 | 1,756 |
| -3,281 | 1,787 |
| -3,400 | 1,825 |
| -3,520 | 1,878 |
| -4,004 | 1,920 |
| -4,134 | 2,009 |
| -4,281 | 1,982 |
| -4,400 | 1,961 |
| -4,520 | 1,985 |



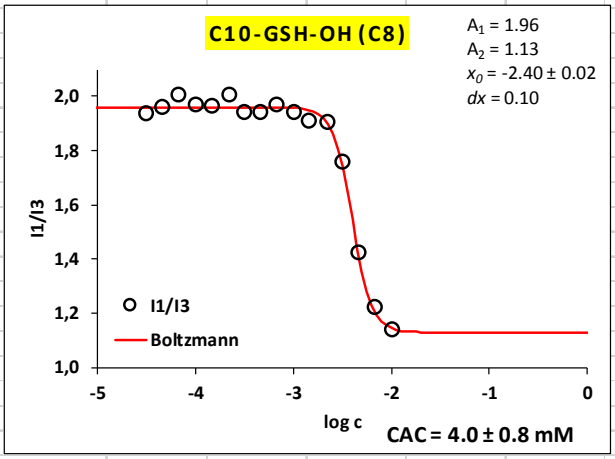

**C8-GGH-OMe (C7)**

| log c | I1/I3 |
|---|---|
| -2,606 | 1,636 |
| -2,781 | 1,707 |
| -2,946 | 1,770 |
| -3,122 | 1,802 |
| -3,275 | 1,897 |
| -3,448 | 1,887 |
| -3,602 | 1,905 |
| -3,606 | 1,937 |
| -3,781 | 1,898 |
| -3,946 | 1,971 |
| -4,122 | 1,992 |
| -4,275 | 1,958 |
| -4,448 | 1,950 |
| -4,602 | 1,959 |



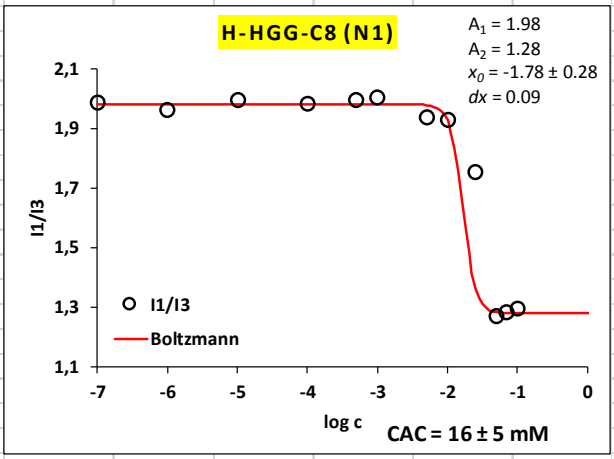

**C10-GSH-OH (C8)**

| log c | I1/I3 |
|---|---|
| -2,004 | 1,144 |
| -2,179 | 1,223 |
| -2,344 | 1,428 |
| -2,507 | 1,761 |
| -2,664 | 1,907 |
| -2,846 | 1,911 |
| -3,004 | 1,944 |
| -3,179 | 1,974 |
| -3,344 | 1,943 |
| -3,507 | 1,943 |
| -3,664 | 2,008 |
| -3,846 | 1,970 |
| -4,004 | 1,970 |
| -4,179 | 2,011 |
| -4,344 | 1,964 |
| -4,507 | 1,940 |



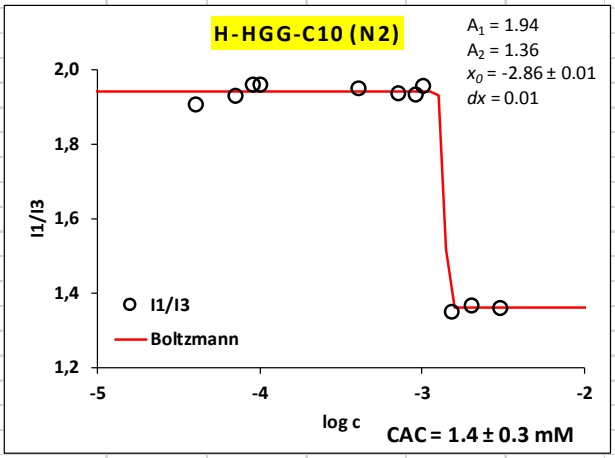
**H-HGG-C8 (N1)**

| log c | I1/I3 |
|---|---|
| -1 | 1,296 |
| -1,155 | 1,285 |
| -1,301 | 1,273 |
| -1,602 | 1,755000 |
| -2 | 1,93 |
| -2,301 | 1,937 |
| -3 | 2,006 |
| -3,301 | 1,996000 |
| -4 | 1,983 |
| -5 | 1,998 |
| -6 | 1,965 |
| -7 | 1,99 |



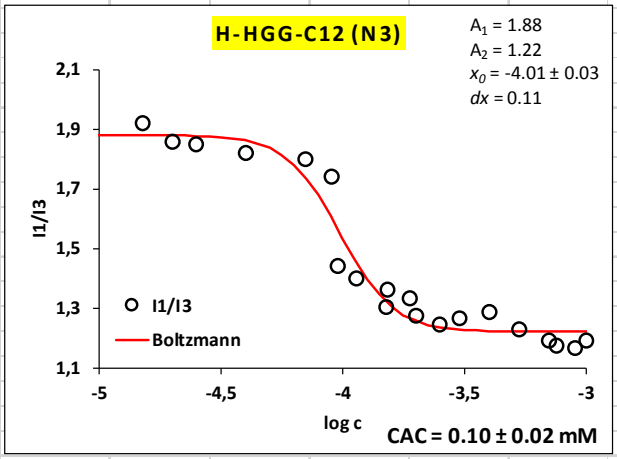

**H-HGG-C10 (N2)**

| log c | I1/I3 |
|---|---|
| -2,523 | 1,361 |
| -2,699 | 1,366 |
| -2,824 | 1,350 |
| -3,000 | 1,958 |
| -3,046 | 1,933 |
| -3,155 | 1,938 |
| -3,398 | 1,950 |
| -4,000 | 1,961 |
| -4,046 | 1,959 |
| -4,155 | 1,930 |
| -4,398 | 1,906 |



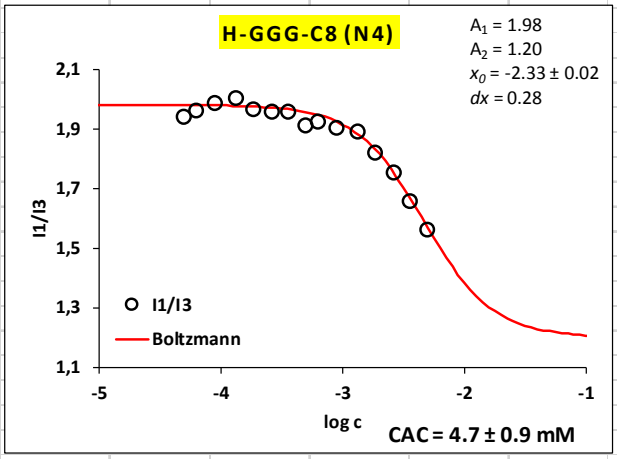

**H-HGG-C12 (N3)**

| log c | I1/I3 |
|---|---|
| -4,824 | 1,921 |
| -4,699 | 1,859 |
| -4,602 | 1,852000 |
| -4,398 | 1,822 |
| -4,155 | 1,8 |
| -4,046 | 1,742 |
| -4,024 | 1,444000 |
| -3,945 | 1,399 |
| -3,824 | 1,304 |
| -3,82 | 1,364 |
| -3,724 | 1,334 |
| -3,699 | 1,276 |
| -3,602 | 1,245 |
| -3,52 | 1,267 |
| -3,398 | 1,287 |
| -3,279 | 1,231 |
| -3,155 | 1,192 |
| -3,125 | 1,175 |
| -3,046 | 1,166 |
| -3 | 1,194 |



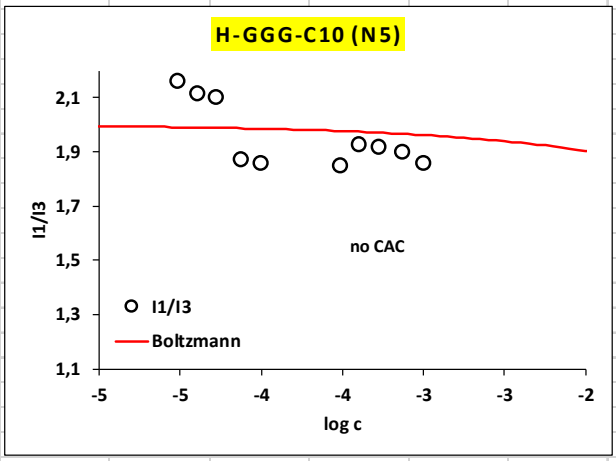
**H-GGG-C8 (N4)**

| log c | I1/I3 |
|---|---|
| -2,305 | 1,564 |
| -2,450 | 1,660 |
| -2,582 | 1,756 |
| -2,734 | 1,820 |
| -2,882 | 1,893 |
| -3,050 | 1,907 |
| -3,205 | 1,926 |
| -3,305 | 1,913 |
| -3,450 | 1,957 |
| -3,582 | 1,957 |
| -3,734 | 1,969 |
| -3,882 | 2,003 |
| -4,050 | 1,987 |
| -4,205 | 1,961 |
| -4,305 | 1,941 |



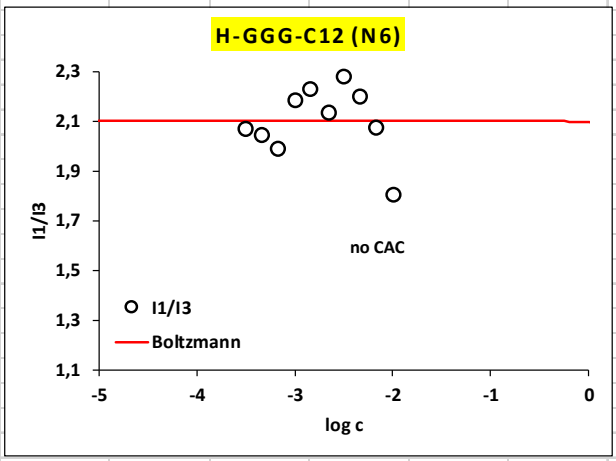

**H-GGG-C10 (N5)**

| log c | I1/I3 |
|---|---|
| -3,004 | 1,860 |
| -3,134 | 1,903 |
| -3,281 | 1,922 |
| -3,400 | 1,931 |
| -3,520 | 1,852 |
| -4,004 | 1,861 |
| -4,134 | 1,877 |
| -4,281 | 2,104 |
| -4,400 | 2,117 |
| -4,520 | 2,166 |



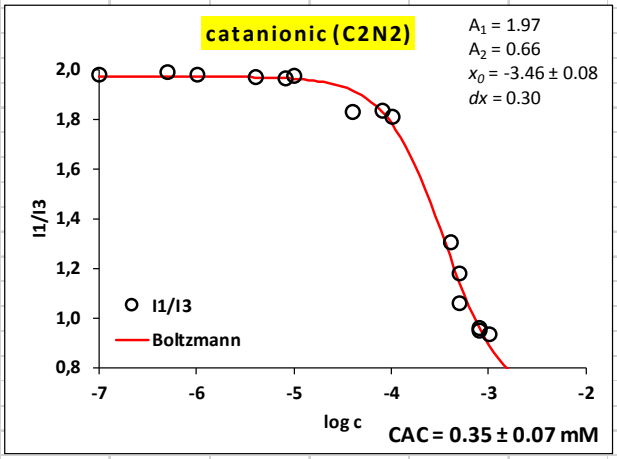

**H-GGG-C12 (N6)**

| log c | I1/I3 |
|---|---|
| -2,004 | 1,806 |
| -2,179 | 2,073 |
| -2,344 | 2,199 |
| -2,507 | 2,281 |
| -2,664 | 2,135 |
| -2,846 | 2,230 |
| -3,004 | 2,184 |
| -3,179 | 1,991 |
| -3,344 | 2,047 |
| -3,507 | 2,068 |
| -3,664 | 1,911 |
| -3,846 | 1,863 |
| -4,004 | 1,861 |



catanionic (C2N2)

| log c | I1/I3 |
|---|---|
| -3,000 | 0,935 |
| -3,097 | 0,958 |
| -3,097 | 0,950 |
| -3,301 | 1,179 |
| -3,301 | 1,059 |
| -3,398 | 1,308 |
| -4,000 | 1,810 |
| -4,097 | 1,838 |
| -4,398 | 1,829 |
| -5,000 | 1,976 |
| -5,097 | 1,964 |
| -5,398 | 1,973 |
| -6,000 | 1,980 |
| -6,301 | 1,991 |
| -7,000 | 1,982 |




## 6. pKa determinations

pKa determinations of six peptides were carried out by acid-base titrations. The concentrations of the peptides were fixed at 2 mM to be consistent with the kinetics experiments. In these conditions peptides $C_{10}$GGHOH and H-HGG-$C_{10}$ were aggregated (their respective CACs are lower than 2 mM) whereas peptides $C_8$GGHOH and H-HGG-$C_8$ were present as monomers (their respective CACs are higher than 2 mM) like the tripeptides AcGGHOH and H-HGG-NHMe. Ionic strength coming from the buffer was taken into account by adding proper amount of KCl. Second-derivative method was chosen to determine the pKa of the imidazole groups, which were found to be close to 7 for all six experiments performed. Therefore at pH = 7, half of the imidazole rings were protonated while half remained uncharged. The pKas of COOH and $NH_3^+$ functions could be estimated to be about 2 and 11 respectively. This indicates that, at pH = 7, C-terminal lipopeptides were present as carboxylates while N-terminal lipopeptides were present as ammoniums. Moreover the aggregation did not seem to have an influence on the pKa values, which were found to be the same for aggregates and monomers.

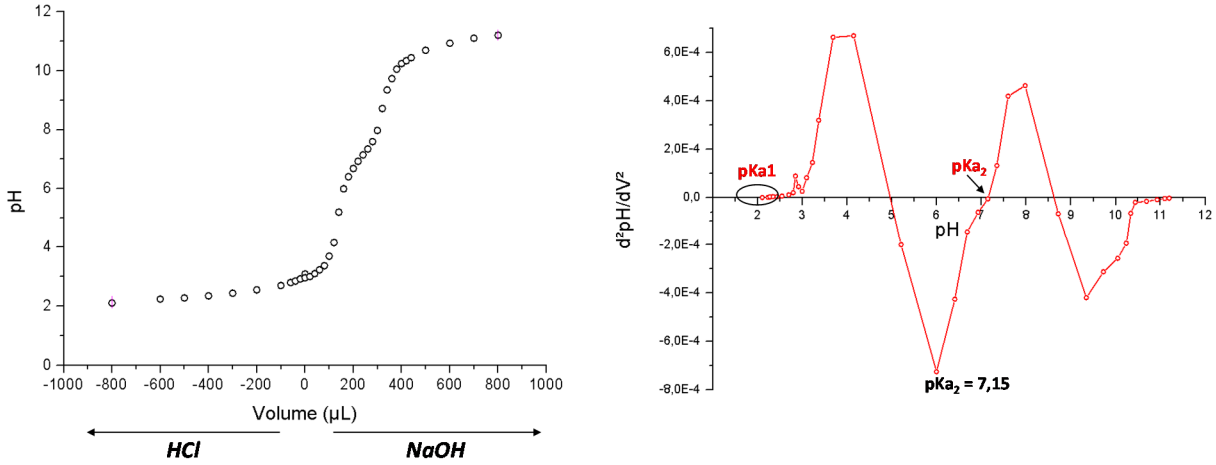



*Figure 1: titration curve and second-derivative plot for AcGGHOH (MONOMERS)*

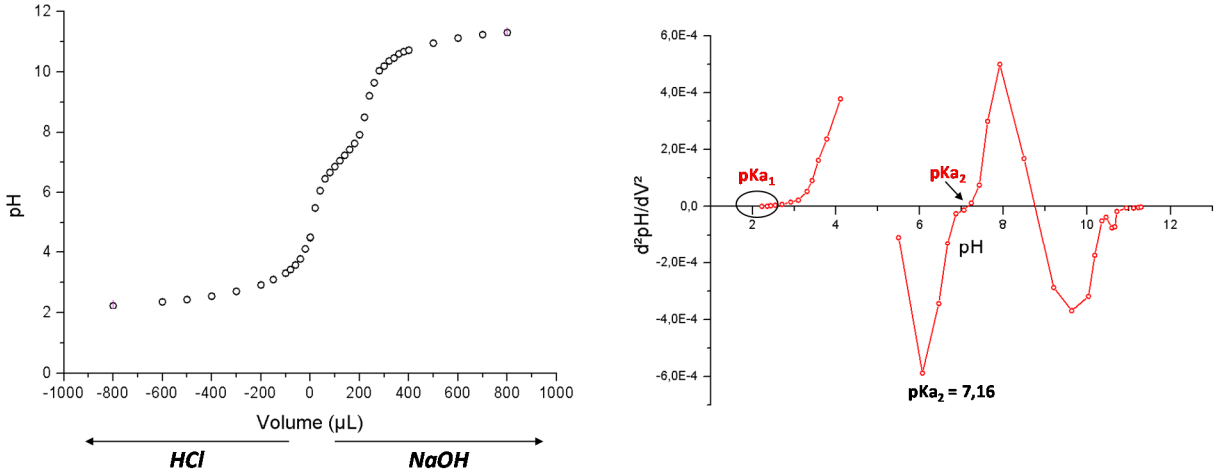



*Figure 2: Titration curve and second-derivative plot for $C_8$GGHOH (MONOMERS)*

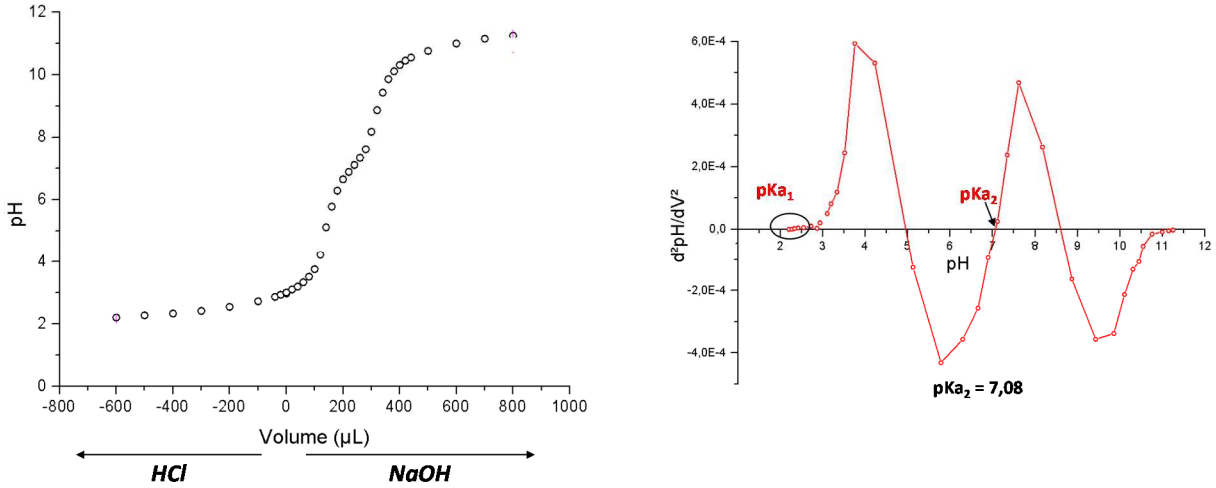



*Figure 3: Titration curve and second-derivative plot for $C_{10}$GGHOH (AGGREGATES)*

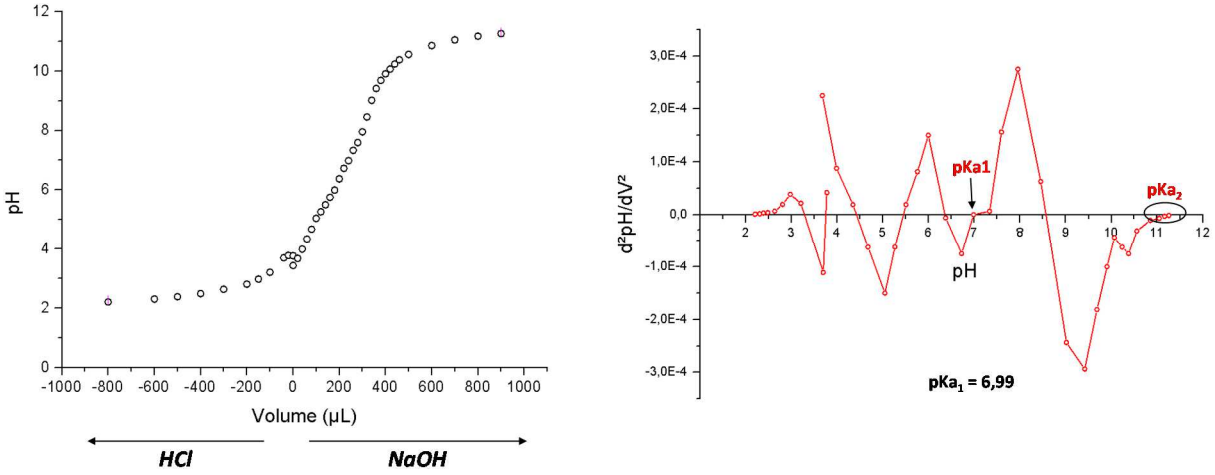



*Figure 4: Titration curve and second-derivative plot for H-HGG-NHMe (MONOMERS)*

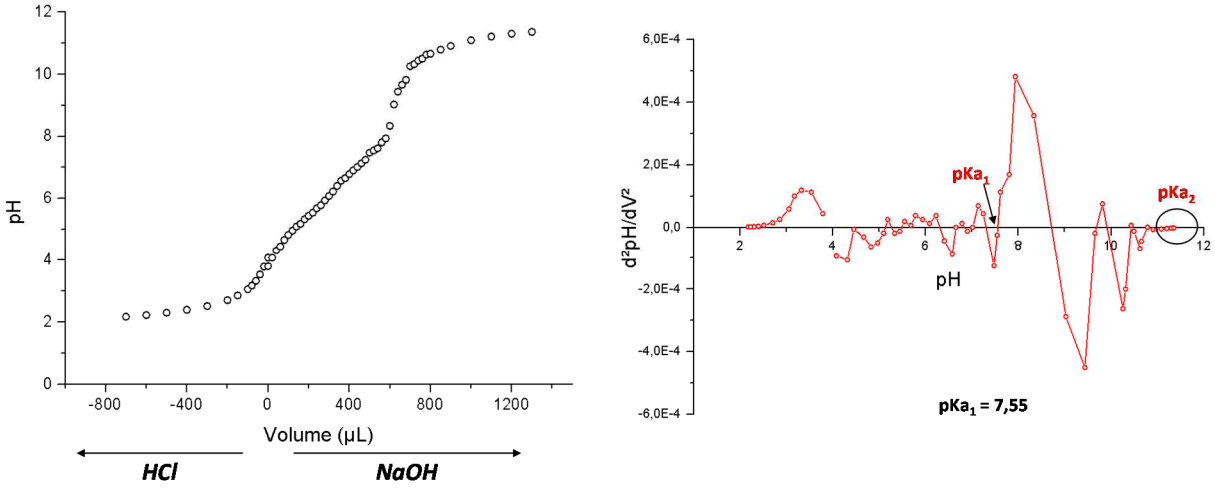



*Figure 5: Titration curve and second-derivative plot for H-HGG-$C_8$ (MONOMERS)*

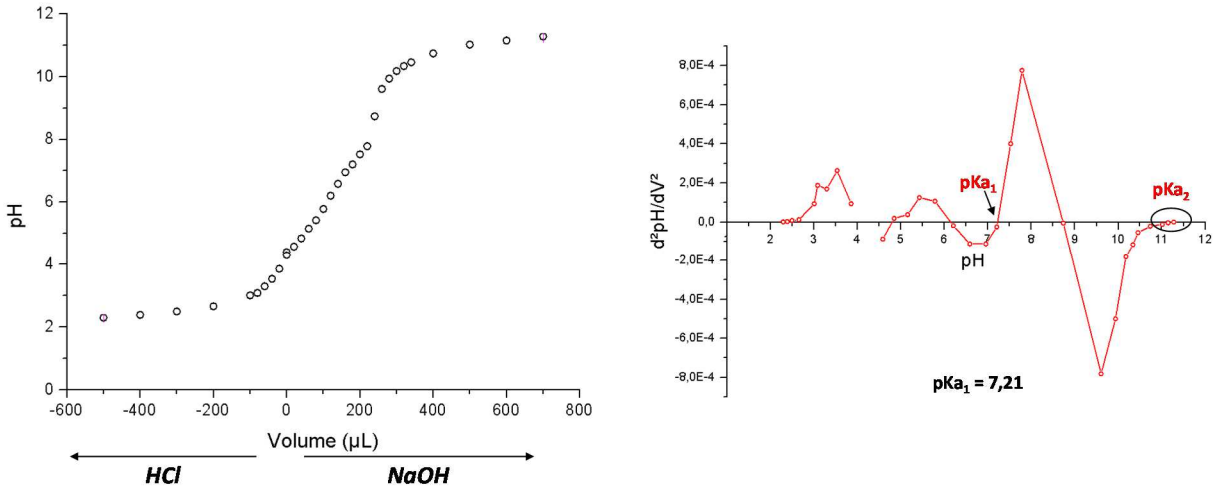
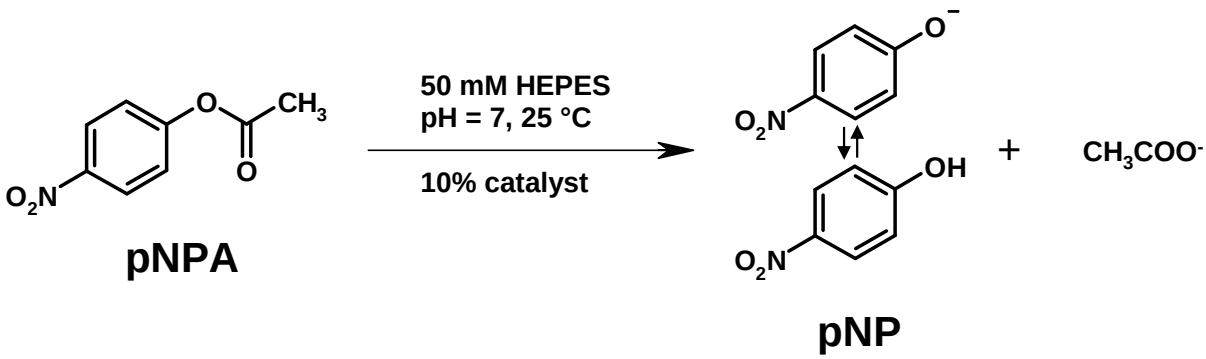



*Figure 6: Titration curve and second-derivative plot for H-HGG-$C_{10}$ (AGGREGATES)*

## 7. Determination of hydrolysis mechanism in buffer alone




$$v = k_{obs} \cdot [pNPA]^{\alpha} = -\frac{d[pNPA]}{dt} = \frac{d[pNP]}{dt}$$

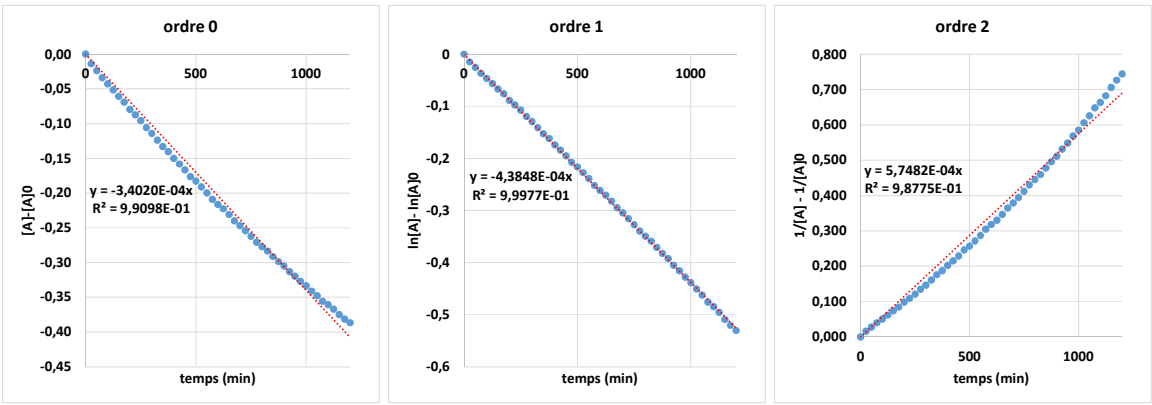
- <u>Determination of the reaction order α with respect to pNPA</u>

If α =0 then: $[pNPA] = [pNPA]_0 - k_{obs}.t$

If α =1 then: $\ln([pNPA]) = \ln([pNPA]_0) - k_{obs}.t$

If α =2 then: $1/[pNPA] = [pNPA]_0 - k_{obs}.t$

We recorded the disappearance of pNPA by UV-Vis at $\lambda_{max}$ = 273 nm:



Clearly we got the best correlation coefficient for the case **α = 1**, which corresponds to literature data.

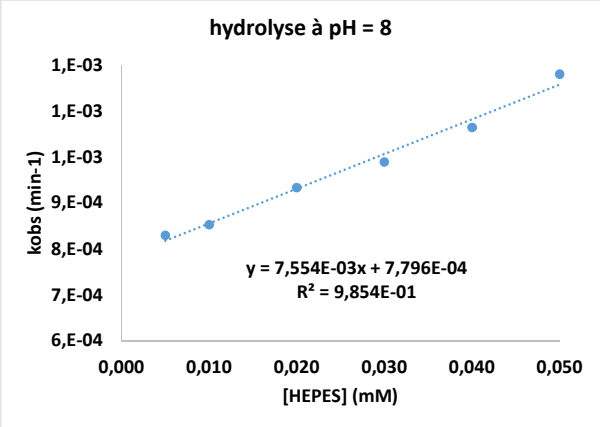
Therefore $v = k_{obs}.[pNPA]$ with $\mathbf{k_{obs} = 4.4 \cdot 10^{-4}\ min^{-1} = 7.3 \cdot 10^{-6}\ s^{-1}}$

- <u>Revealing an acid-base catalysis</u>

In the general case: $k_{obs} = k_0 + k_{H+}.[H^+] + k_{OH-}.[OH^-] + k_{HA}.[HA]\ k_{A-}.[A^-]$ with AH and $A^-$ being all the conjugated acids and bases (here, only the buffer species).

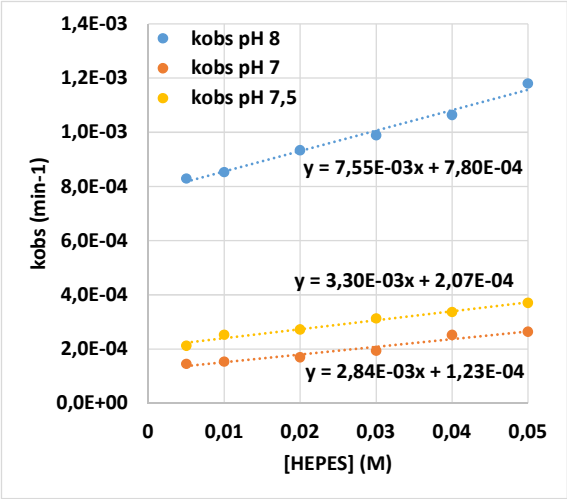
At constant pH: $[H^+]$ et $[OH^-]$ remain constant, as well as the ratio $[HA]/[A^-]$. By contrast, $[HA]$ and $[A^-]$ depend on the total buffer concentration $C_{tp} = [HA]+[A^-]$.

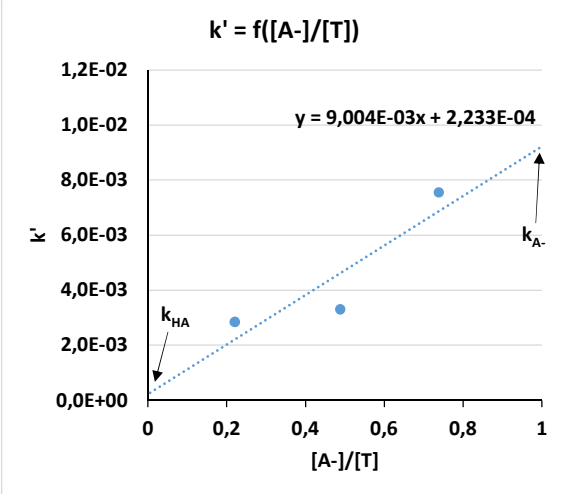
If $k_{obs}$ does not vary as a function of the buffer concentration then $k_{HA} = k_{A-} = 0$ and the catalysis is specific; otherwise the catalysis is general.




$k_{obs}$ varies as a function of the buffer concentration therefore we observe a **general catalysis**.

The determination of $k_{HA}$ and $k_{A-}$ can be realized by varying the buffer concentration at different pH values.




This reveals a **general acid and base catalysis** with $\mathbf{k_{A^-} = 9.20 \cdot 10^{-3}\ min^{-1}.mol^{-1}.L}$ and $\mathbf{k_{AH} = 0.22 \cdot 10^{-3}\ min^{-1}.mol^{-1}.L}$.

There are two simultaneous catalysis but the weak bases seem more efficient than the weak acids.