Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Supporting Information

An experimental evidence for the key role of diphenylalanine in fibril formation

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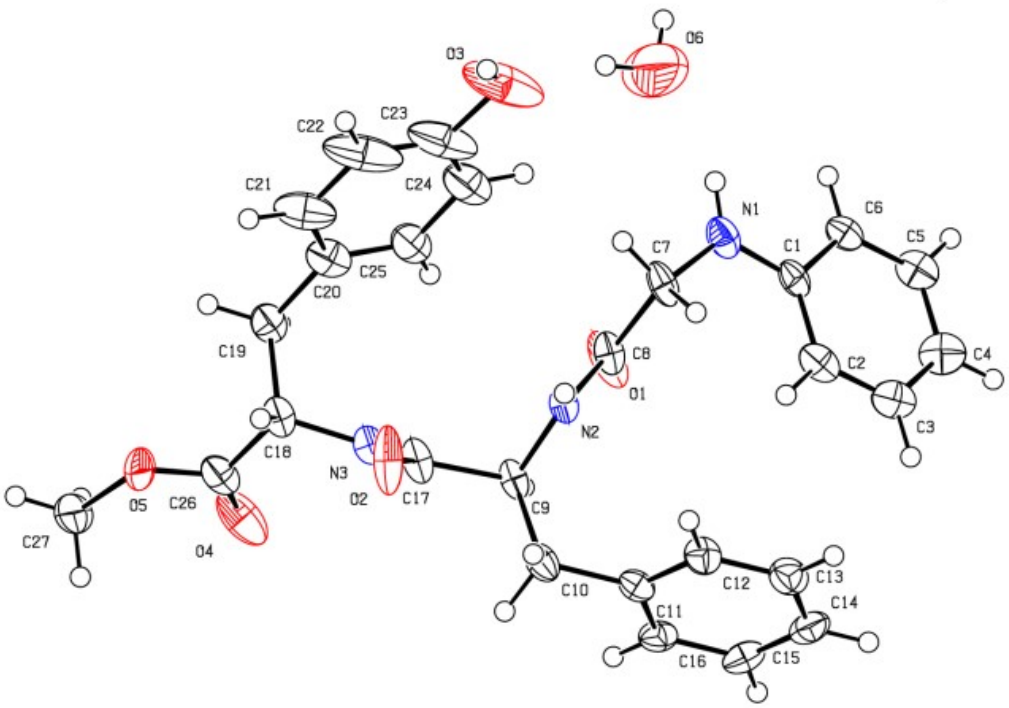

Fig. S1: The ORTEP diagram of peptide **2** including the atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

ESI Table S1: Crystal data and structure refinement for peptide **2**.

Identification code	Npt_xylene_a
Empirical formula	$C_{27}H_{29}N_3O_5$
Formula weight	493.55
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	P21
a/Å	4.9730(3)
b/Å	26.2917(17)
c/Å	9.3394(6)
α/°	90
β/°	90.444(2)
γ/°	90
Volume/Å^3	1221.08(13)
Z	2
ρ_{calc}g/cm^3	1.342
μ/mm^{-1}	0.096
F(000)	524.0

Crystal size/mm^3	0.20× 0.20× 0.20
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	2.3 to 28.7
Index ranges	-6 ≤ h ≤ 6, -35 ≤ k ≤ 35, -12 ≤ l ≤ 12
Reflections collected	35544
Independent reflections	4381
Goodness-of-fit on F^2	1.063
Largest diff. peak/hole / e Å^{-3}	0.44/-0.63
Flack parameter	0.8(7)
R	0.0646
WR2	0.1683

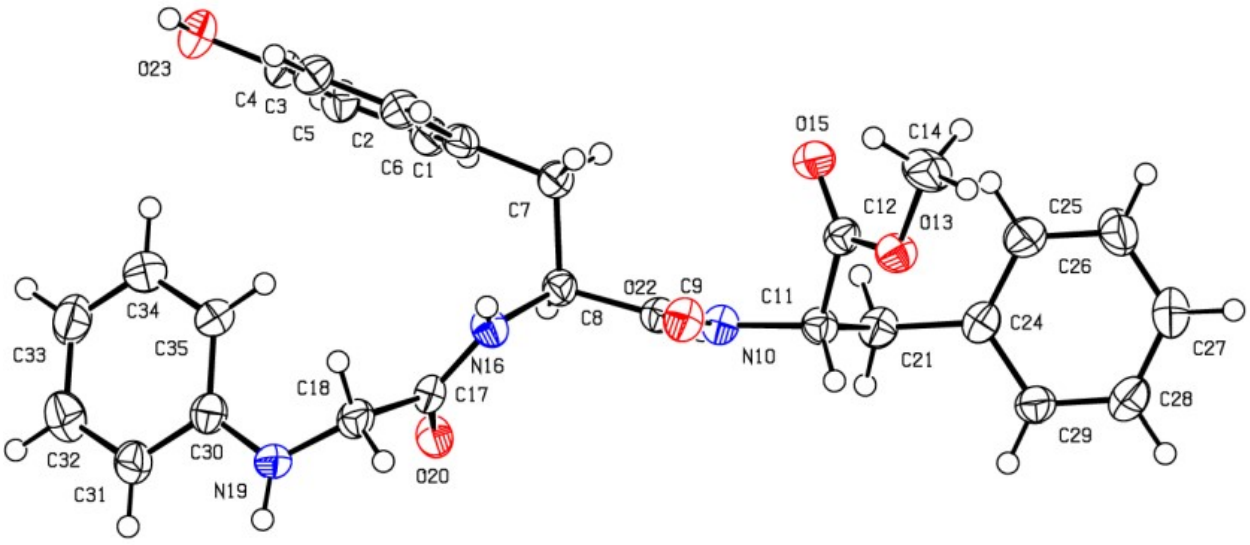

Fig. S2: The ORTEP diagram of peptide **3** including the atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

ESI Table S2: Crystal data and structure refinement for Peptide **3**.

Identification code	NTPOMe
Empirical formula	$C_{27}H_{29}N_3O_5$
Formula weight	475.53
Temperature/K	100.00(10)
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a/Å	8.3717(2)
b/Å	15.2576(4)
c/Å	19.7373(4)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2521.09(10)
Z	4
ρ_{calc}g/cm^3	1.253
μ/mm^{-1}	0.712
F(000)	1008
Crystal size/mm^3	0.22× 0.24× 0.27
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	3.7 to 68.4
Index ranges	$-10 \leq h \leq 10$, $-18 \leq k \leq 18$, $-17 \leq l \leq 17$
Reflections collected	13781
Independent reflections	3741
Goodness-of-fit on F^2	1.037
Largest diff. peak/hole / e Å^{-3}	1.50/-0.26
Flack parameter	0.02(15)
R	0.0674
WR2	0.1897

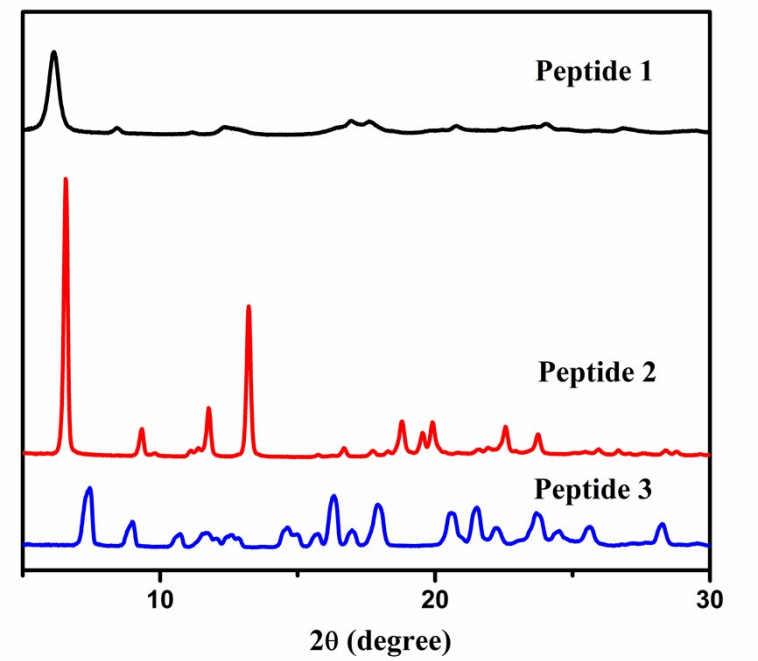

Fig. S3. WAXS spectra of peptides **1-3.**). Peptides **2** and **3** polydisperse microspheres show sharp reflections in the 5–25° 2θ range whereas only a very broad feature was observed for peptide 1 fibers.

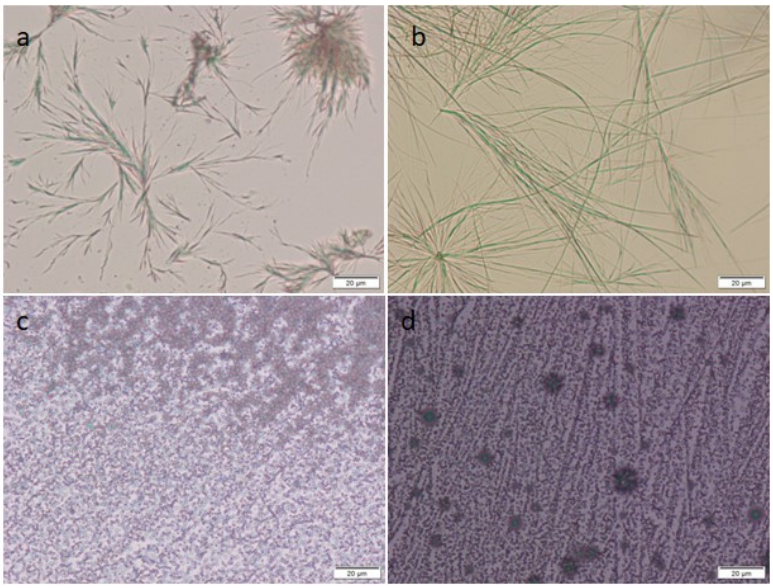

Fig. S4. POM images of peptides 1 showing fibers formation in fresh solution (a) and elongated fibers after 24 h incubation. Peptides 2 (b) and 3 (c) shows no change in polydisperse microspheres on 24 h incubation.

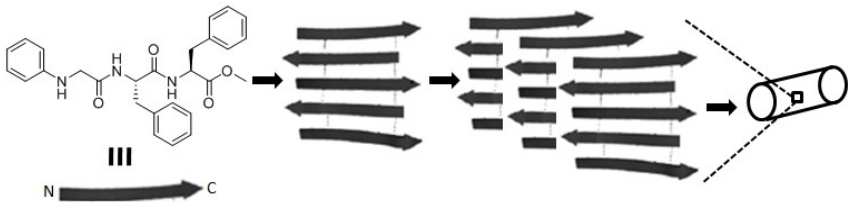

Fig. S5. Schematic presentation showing the mechanism for fibril formation of peptide **1.**

Experimental

Synthesis of NPG-Phe-OMe :

N-phenylglycine-OH (1.51 g, 10 mmol) was dissolved in 50 mL dry DCM in an ice-cold water bath. H_2N-Phe- OMe (1.81 g, 11 mmol) was dissolved in 10 mL DCM. It was then added to the reaction mixture, followed by immediate addition of 2.26 g (11 mmol) dicyclohexylcarbodiimide (DCC) and 1.48 g (11 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stir for 48 hrs. After that, DCM was evaporated, and the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulfate.The products were purified by column chromatography using silica (60-120-mesh size) gel as stationary phase and n-hexane-ethyl acetate mixture as eluent.Yield: 2.05 g (6.5 mmol, 65 %).

^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm):7.23-7.14 [5H aromatic & 1H NH proton], 7.16-7.15[b,1H NH proton] 6.93-6.91[2H aromatic], 6.83[1H aromatic], 6.57-6.53[2H aromatic], 4.95-4.93 [m, C^{β}1H], 3.77--3.75 [b, 2H], 3.69 [s, 3H, OCH_3], 3.06-3.05 [t, Cα 2H]. ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm):172.03, 170.68, 147.81, 129.74, 129.48, 128.91, 127.40, 119.50, 113.61, 52.87, 52.76, 48.95, 38.23. Mass spectra, found m/z: 335.1495 [M+Na]+, calculated for $C_{18}H_{20}N_2O_3Na$ 335.1407.

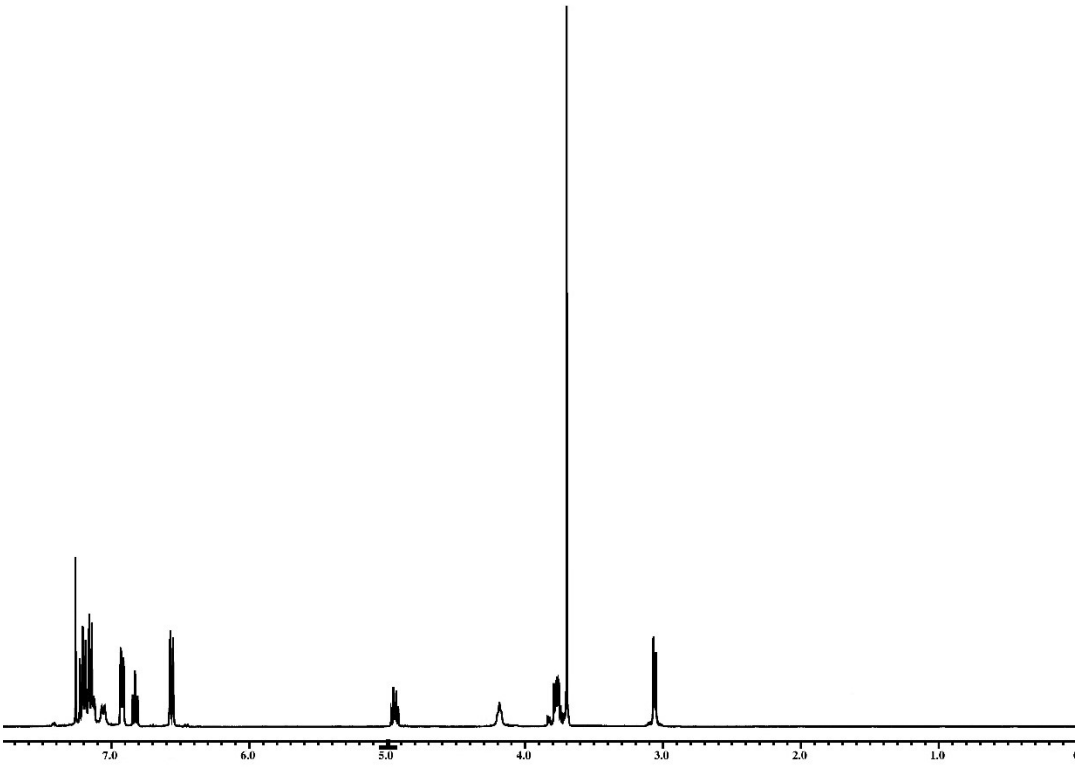

Fig. S3: ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-OMe.

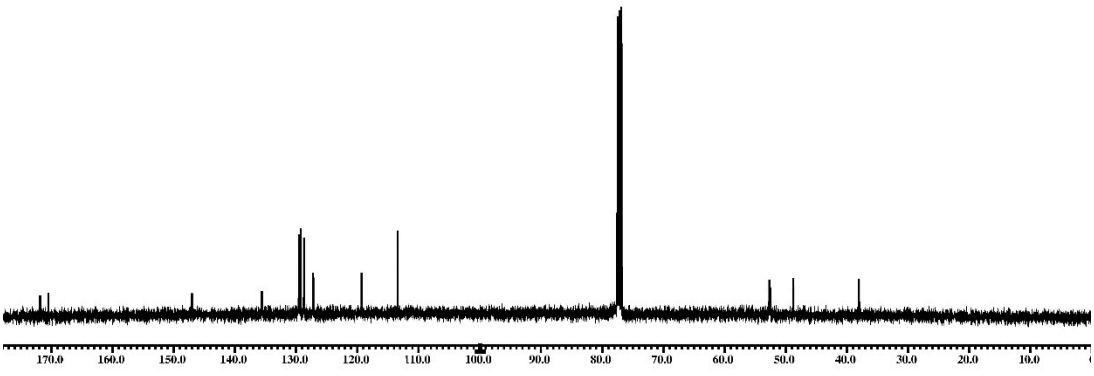

Fig. S4: ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-OMe.

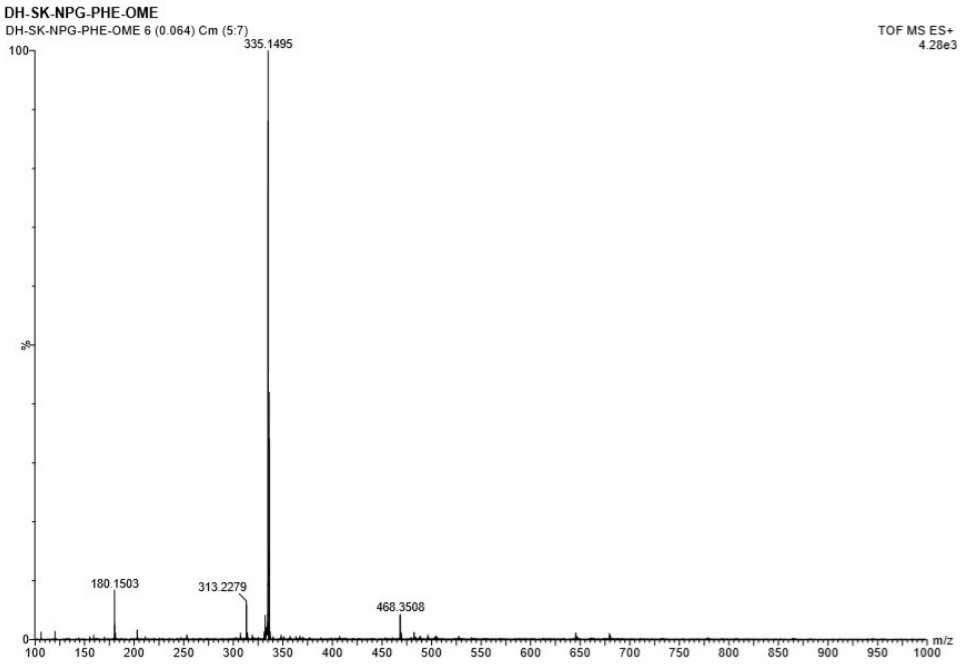

Fig. S5: Mass Spectra of NPG-Phe-OMe.

Synthesis of NPG-Phe-Phe-OMe 1 :

N-phenylglycine-Phe-OH (1.49 g, 5 mmol) was dissolved in 50 mL dry DCM in an ice-cold water bath. H_2N-Phe- OMe (1.15 g, 7 mmol) was dissolved in 10 mL DCM. It was then added to the reaction mixture, followed by immediate addition of 1.64 g (8 mmol) dicyclohexylcarbodiimide (DCC) and 1.08 g (8 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stir for 48 hrs. After that, DCM was evaporated, and the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulphate.The products were purified by column chromatography using silica (100-200-mesh size) gel as stationary phase and n-hexane-ethyl acetate mixture as eluent. Yield: 1.6 g (3.5 mmol, 70 %).

^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.25-7.13[m, 9H, aromatic proton], 7.13-7.06[1H, aromatic proton], 7.05-7.00[m, 2H, aromatic proton], 6.99-6.93[m, 2H, aromatic proton], 6.84-6.78[b, 1H, aromatic proton], 6.54-6.48[2H, b, NH] 6.46-6.40[1H, NH], 4.78-4.74[m, 1H, methine C^{α} Phe], 4.73-4.67[m, 1H, Methine C^{α} Phe], 3.65-3.69[5H, methylene protons of NPG & 3H-OMe], 3.08-3.00[4H, methylene protons of Phe]. ^{13}C-

NMR (100 MHz, $CDCl_3$, δ ppm): 171.64, 171.56, 177.47, 147.05, 136.2, 135.96, 129.78, 129.54, 129.48, 128.85, 128.74, 127.23, 127.17, 119.34, 113.05, 53.96, 53.43, 52.46, 48.75, 38.05, 37.76. FT-IR (cm^{-1}): 3374, 2955, 1646, 1604. Mass spectra, found m/z: 482.8907 $[M+Na]^+$, calculated for $C_{27}H_{29}N_3O_4$ 459.5369.

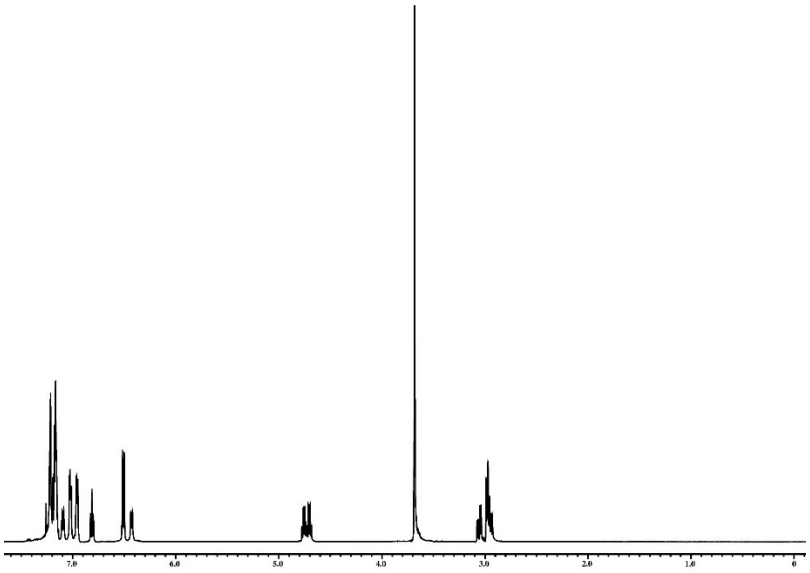

Fig. S6: ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-Phe-OMe **1.**

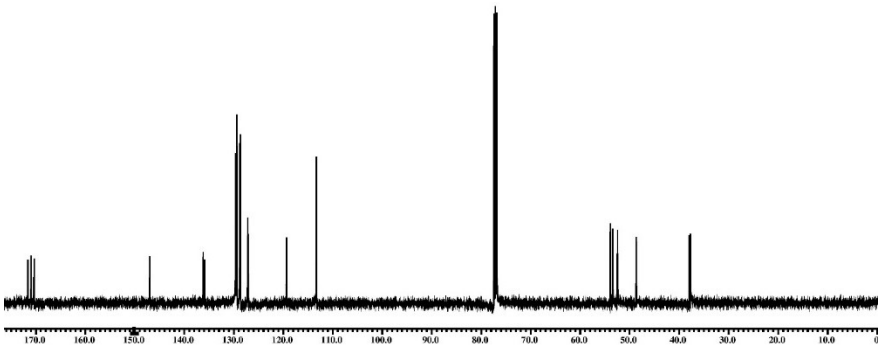

Fig. S7: ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-Phe-OMe **1**.

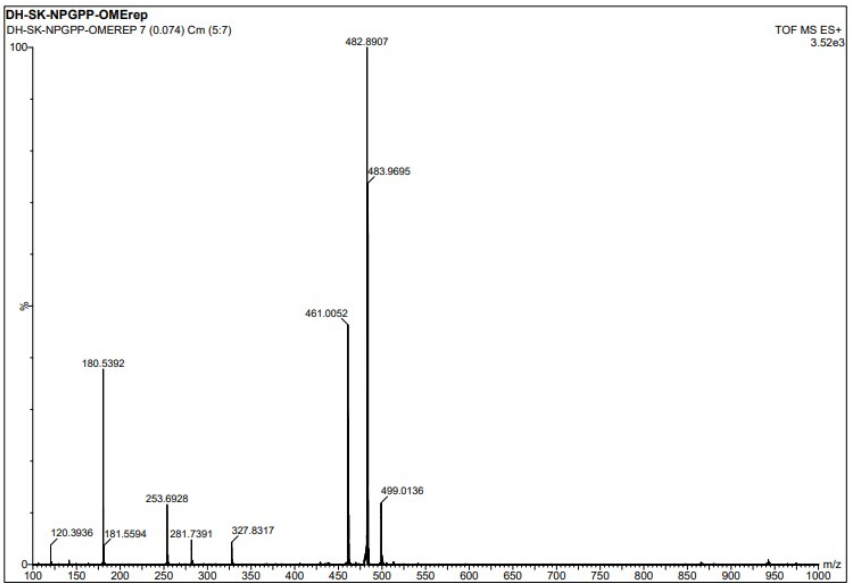

Fig. S8: Mass Spectra of NPG-Phe-Phe-OMe **1**.

Synthesis of NPG-Phe-Tyr-OMe 2 :

N-phenylglycine-Phe-OH (1.49 g, 5 mmol) was dissolved in 50 mL dry DCM in an ice-cold water bath. H_2N-Tyr- OMe 1.36 g, 7 mmol) was dissolved in 10 mL DCM. It was then added to the reaction mixture, followed by immediate addition of 1.64 g (8 mmol) dicyclohexylcarbodiimide (DCC) and 1.08 g (8 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stir for 48 hrs. After that, DCM was evaporated, and the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulphate.The products were purified by column chromatography using silica (100-200-mesh size) gel as stationary phase and n-hexane-ethyl acetate mixture as eluent. Yield: 1.5 g (3.2 mmol, 64 %).

^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.95-7.85[b, 1H, NH], 7.17-7.09[m, 5H, aromatic proton], 7.00-6.90[m, 3H, aromatic proton], 6.85-6.74[m, 3H, aromatic proton], 6.70-6.64[m, 3H, aromatic proton], 6.48-6.41[b, 2H, NH], 4.88-4.81[m, 1H, methine C^{α} Phe], 4.80-4.74[m, 1H, Methine C^{α}-Tyr], 4.25[b, 1H, Tyr-OH], 3.68[s, 3H, OMe], 3.57[s, 2H, methylene protons of NPG] 3.04-2.84[m, 4H, methylene protons of Phe & Tyr]. ^{13}C-

NMR (100 MHz, $CDCl_3$, δ ppm): 171.60, 170.72, 155.45, 146.74, 135.71, 130.41, 129.39, 129.21, 129.21, 128.50, 126.86, 119.06, 115.48, 113.13, 53.63, 53.46, 52.38, 48.35, 37.92, 37.01. FT-IR (cm^{-1}): 3374, 1740, 1654, 1606. Mass spectra, found m/z: 498.7579 $[M+Na]^+$, calculated for $C_{27}H_{29}N_3O_4Na$ 498.2005.

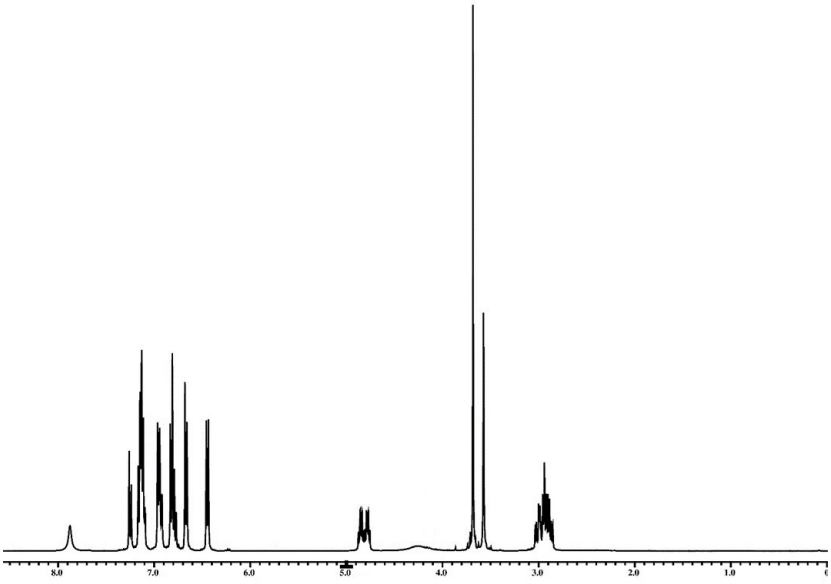

Fig. S9: ^1H NMR (400 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-Tyr-OMe **2.**

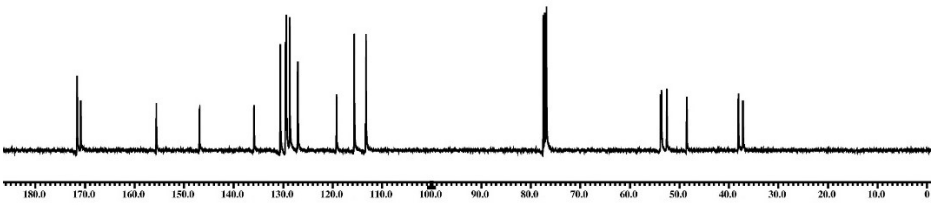

Fig. S10: ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Phe-Tyr-OMe **2**.

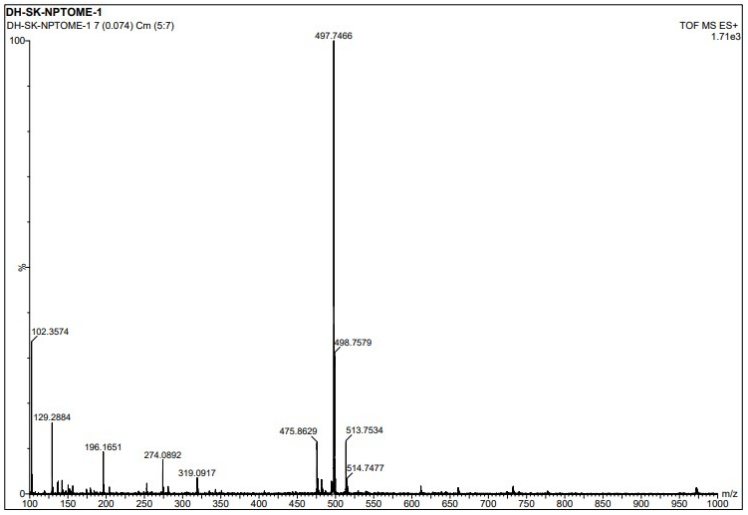

Fig. S11: Mass Spectra of NPG-Phe-Tyr-OMe **2**.

Synthesis of NPG-Tyr-OMe :

N-phenylglycine-OH (1.51 g, 10 mmol) was dissolved in 50 mL dry DCM in an ice-cold water bath. H_2N-Tyr- OMe (2.14 g, 11 mmol) was dissolved in 10 mL DCM. It was then added to the reaction mixture, followed by immediate addition of 2.26 g (11 mmol) dicyclohexyl carbodiimide (DCC) and 1.48 g (11 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stir for 48 hrs. After that, DCM was evaporated, and the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulfate.The products were purified by column chromatography using silica (60-120-mesh size) gel as stationary phase and n-hexane-ethyl acetate mixture as eluent. Yield: 2.23 g (6.7 mmol, 67 %).

^{1}H-NMR (400MHz, $CDCl_3$, δ ppm):7.22-7.12[m,3H (aromatic)], 6.84-6.80[t, 1H aromatic], 6.76-6.74[d,2H aromatic], 6.59-6.57[d,2H aromatic], 6.54-6.52[d, 2H aromatic],6.4-6.3[b,1H NH proton] 4.93-4.91[m,1H], 4.24-4.15[b,1H],3.74-3.73[b,2H, $^{\alpha}CH_2$ NPG], 3.71[s,3H,OMe], 2.99-2.97[t,2H, $^{\alpha}$CH Tyr]. ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 172.32, 171.12, 155.57, 147.14, 130.59, 129.75, 127.21, 119.53,115.90, 113.61, 53.01, 52.77, 48.88, 37.47. Mass spectra, found m/z: 351.1856 $[M+Na]^+$, calculated for $C_{18}H_{20}N_2O_4Na$ 351.1768.

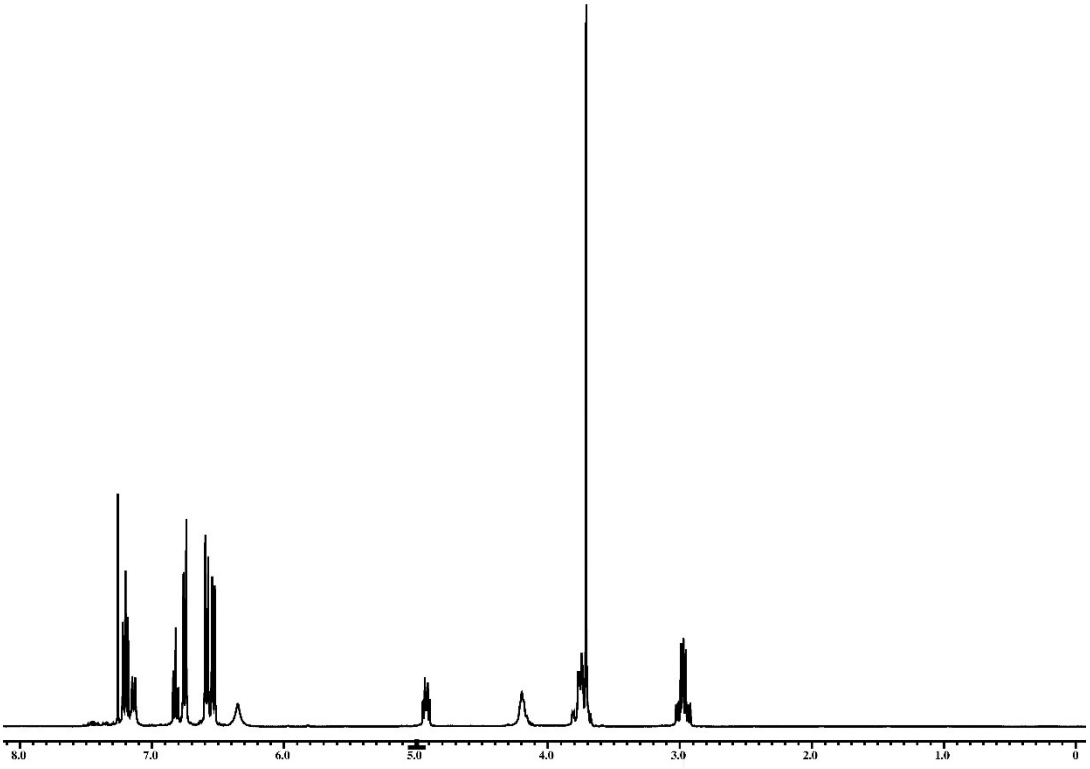

Fig. S12: ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Tyr-OMe.

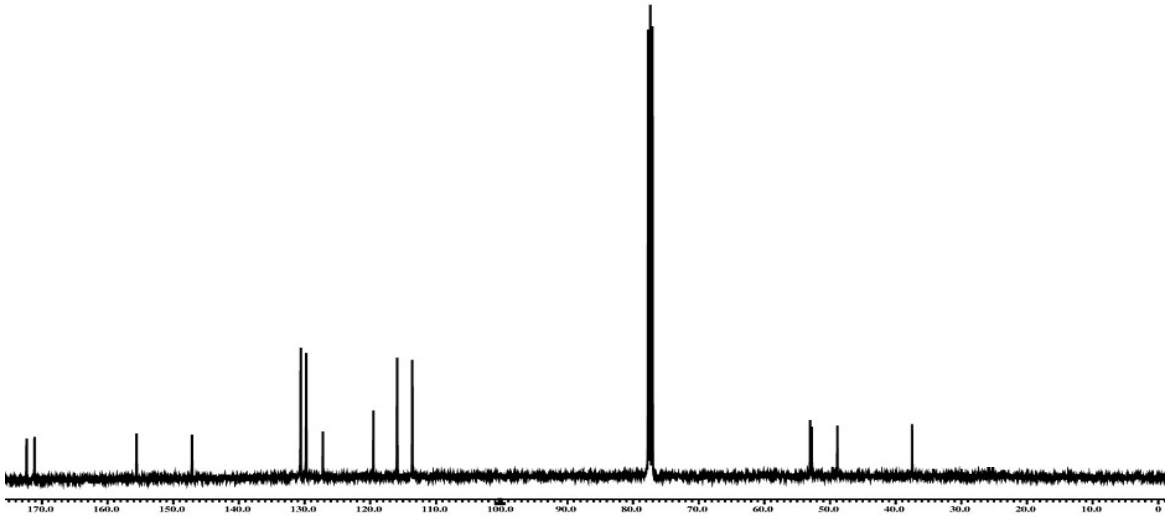

Fig. S13: ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm, 298K) spectra NPG-Tyr-OMe.

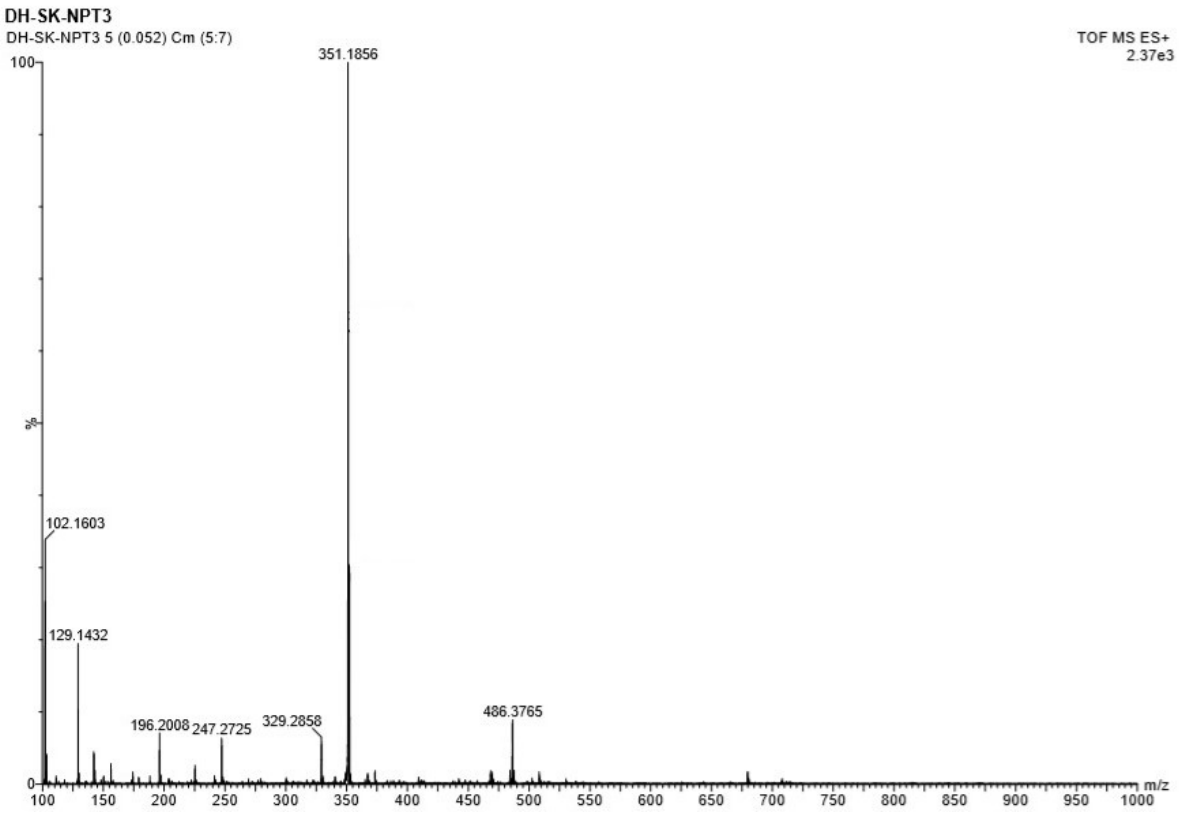

Fig. S14: Mass Spectra of NPG-Tyr-OMe.

Synthesis of NPG-Tyr-Phe-OMe 3 :

N-phenylglycine-Tyr-OH (1.57 g, 5 mmol) was dissolved in 50 mL dry DCM in an ice-cold water bath. H_2N-Phe- OMe (1.15 g, 7 mmol) was dissolved in 10 mL DCM. It was then added to the reaction mixture, followed by immediate addition of 1.64 g (8 mmol) dicyclohexylcarbodiimide (DCC) and 1.08 g (8 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stir for 48 hrs. After that, DCM was evaporated, and the residue was dissolved in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 (M) HCl (3 ×50 mL), brine (2 × 50 mL), 1(M) sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) and dried over anhydrous sodium sulphate.The products were purified by column chromatography using silica (100-200-mesh size) gel as stationary phase and n-hexane-ethyl acetate mixture as eluent. Yield: 1.6 g (3.4 mmol, 68 %).

^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 7.25-7.13[m, 6H, aromatic proton], 7.02-6.95[m, 2H, aromatic proton], 6.86-6.75[m, 3H, aromatic proton], 6.62-6.53[m, 3H, aromatic proton], 6.52-6.46[b, 2H, NH proton], 4.80-4.72[m, 1H, methine C^{α} Phe], 4.71-4.63[m, 1H, Methine C^{α}-Tyr], 3.70-3.65[m, 5H, OMe proton and methylene protons of NPG], 3.10-2.92[m, 4H, methylene protons of Phe], 2.90-2.80[m, 2H, methylene protons of Tyr]. ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 171.66, 171.32, 170.74, 153.36, 146.78, 135.76, 130.53, 129.64, 129.36, 128.72, 127.39, 127.25, 119.45, 115.73, 113.36, 54.10, 53.56, 52.58, 48.65, 37.97, 37.15. FT-IR (cm^{-1}): 3382, 1650, 1602. Mass spectra, found m/z: 498.1987 $[M+Na]^+$, calculated for $C_{27}H_{29}N_3O_4Na$ 498.2005.

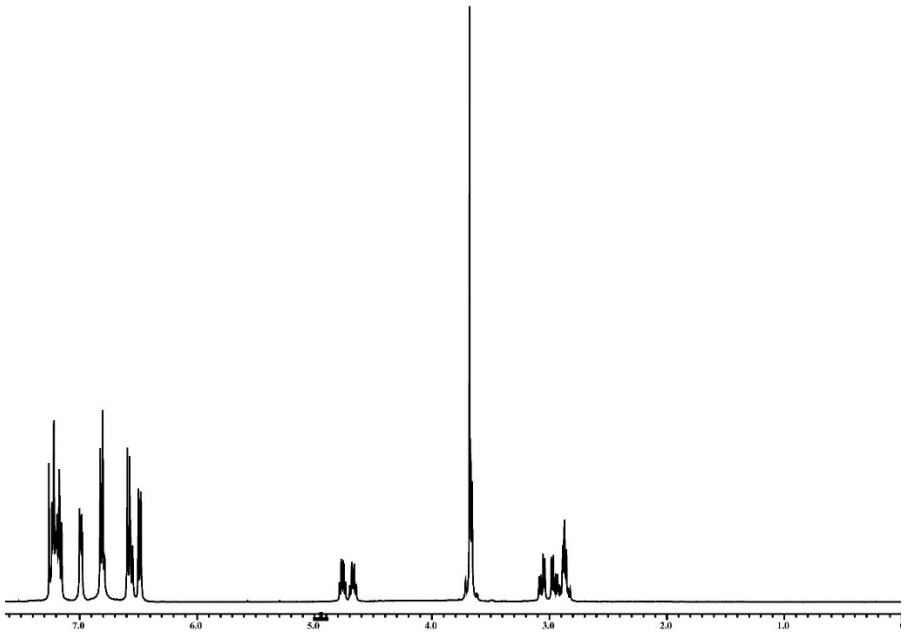

Fig. S15: ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Tyr-Phe-OMe **3.**

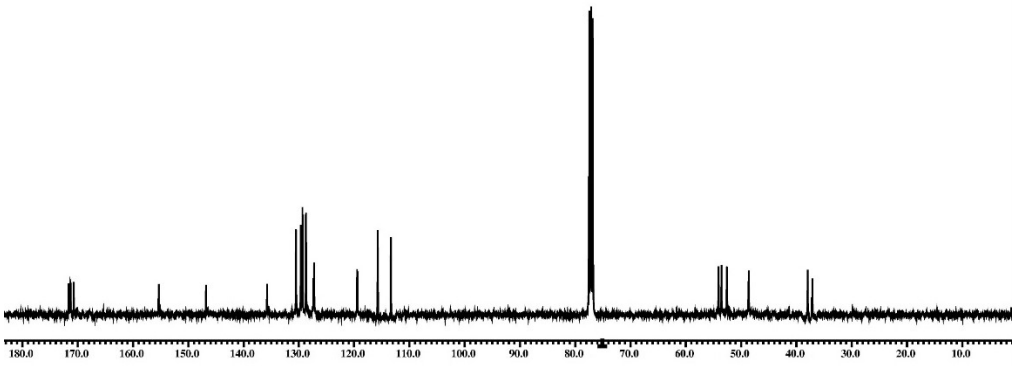

Fig. S16: ^{13}C NMR (100 MHz, $CDCl_3$, δ in ppm, 298K) spectra of NPG-Tyr-Phe-OMe **3.**

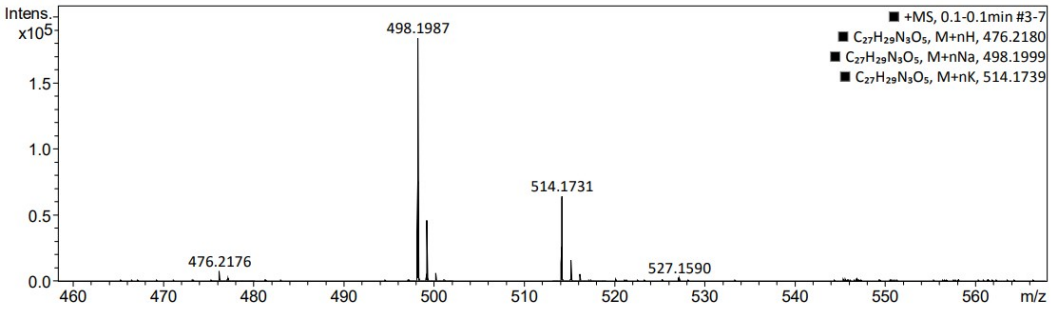

Fig. S17: Mass Spectra of NPG-Tyr-Phe-OMe **3**.