Supporting Information

Emerging π-stacked dynamic nanostructured library

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Contents:

Time resolved study:

A 2 mL of gel sample was prepared in a 1 cm^2 quartz cuvette and Time resolved studies were done by a time correlated single photon counting (TCSPC) system from Horiba Yovin (Model: Fluorocube-01-NL). Samples were excited at 376 nm using a picosecond diode laser (Model: Pico Brite-375L). The signals were collected at magic angle (54.70) polarization using a photomultiplier tube (TBX-07C) as detector, which has a dark counts less than 20 cps. The instrument response function was typically 140 ps. The data analysis was performed using IBH DAS (version 6, HORIBA Scientific, Edison, NJ) decay analysis software.

The amplitude weighted lifetime was estimated by

$$\langle \tau \rangle = \sum_{i=1}^{n} a_i \, \tau_i$$

where τ_i is the fluorescence lifetime of various fluorescent species and a_i are the normalized pre-exponential factors. To gain the best fitting in all cases the χ^2 was kept near to unity.

Table S1. Decay parameters for hydrogel of Nmoc-V after enzyme reaction

Hydrogel	α_1	α_2	α_3	τ_1 (ns)	τ_2(ns)	τ_3(ns)	τ^a(ns)	χ^2
1 day	0.24	0.05	0.70	1.6119	7.1659	0.4615	1.1080	1.1488

τ^a The amplitude weighted average lifetime, Normalized amplitude of each component is given by α

Table S2. Decay parameters for a solution of Nmoc-VVV prior to enzyme addition

Nmoc-VVV	α_1	α_2	α_3	τ_1 (ns)	τ_2(ns)	τ_3(ns)	τ^a(ns)	χ^2
Prior to enzyme addition	0.21	0.05	0.74	1.2374	7.0116	0.24230	0.7755	1.3455

τ^a The amplitude weighted average lifetime, Normalized amplitude of each component is given by α

Table S3. Decay parameters for hydrogel of Nmoc-V after enzyme reaction at different time.

Hydrogel	α_1	α_2	α_3	τ_1 (ns)	τ_2(ns)	τ_3(ns)	τ^a(ns)	χ^2
6 h	0.27	0.06	0.67	1.4205	6.7926	0.4091	1.0436	1.1981
24 h	0.24	0.05	0.70	1.6119	7.1659	0.4615	1.1080	1.1488
36 h	0.26	0.06	0.68	1.5301	6.8341	0.4375	1.1140	1.2106
48 h	0.23	0.71	0.06	1.7070	0.4857	7.2150	1.1402	1.2374

τ^a The amplitude weighted average lifetime, Normalized amplitude of each component is given by α

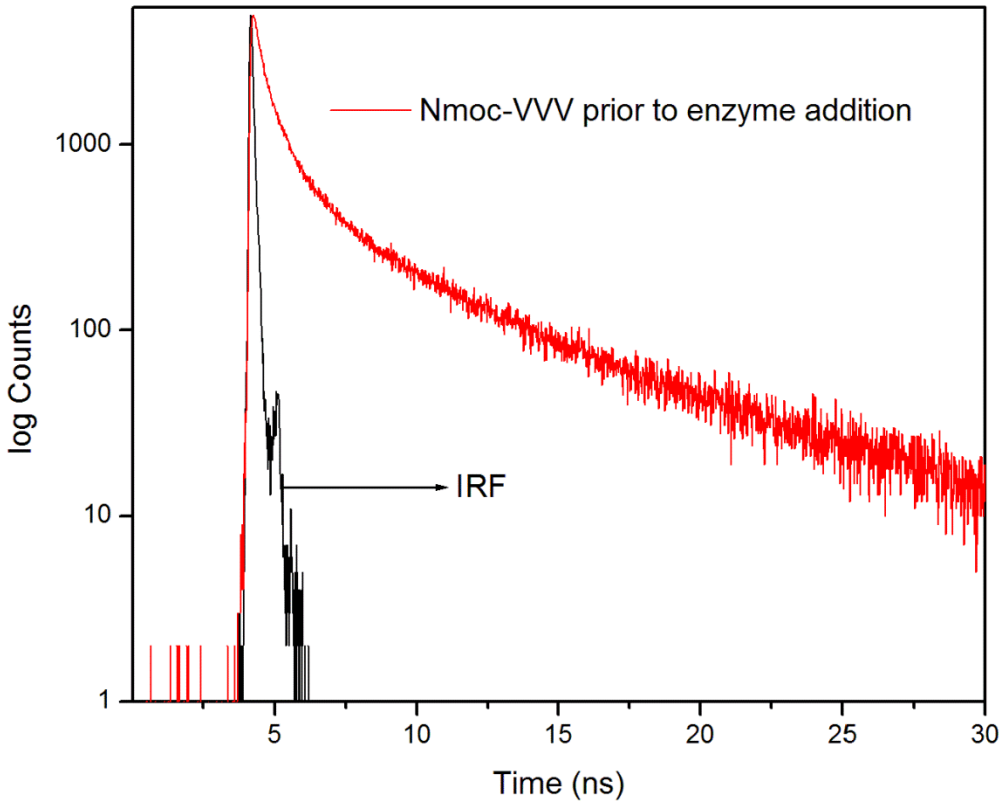


Figure S1. Emission decay curves for a solution of Nmoc-VVV prior to enzyme addition monitored at 463 nm (IRF: instrument response function).

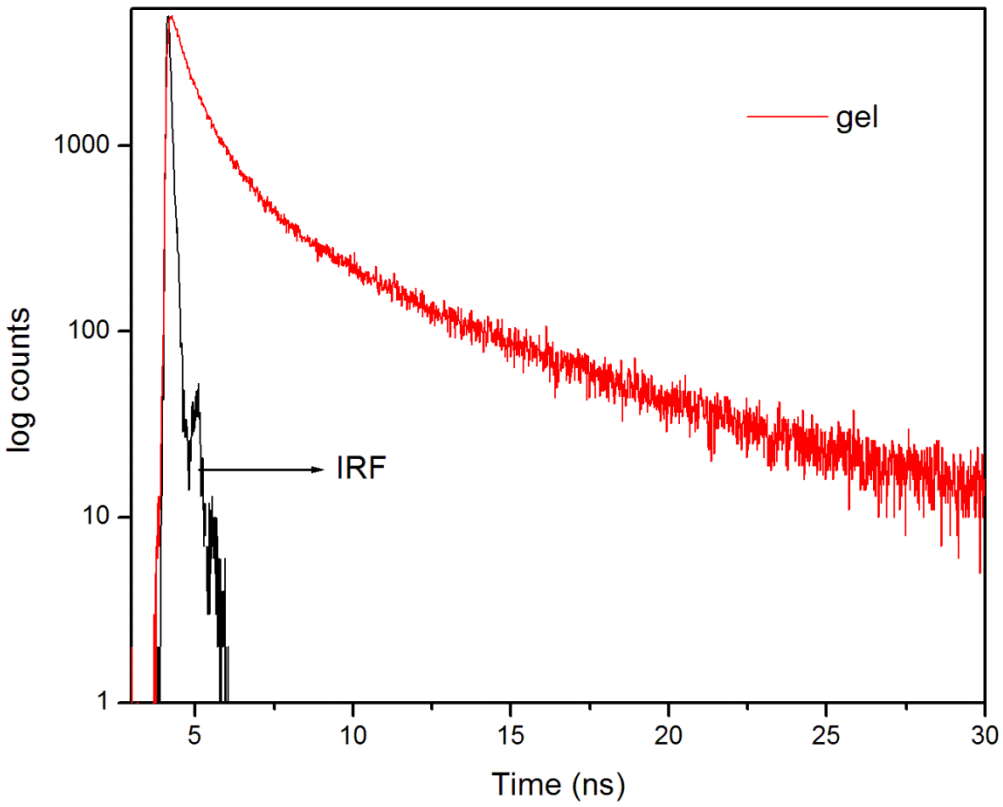


Figure S2. Emission decay curves for a hydrogel of Nmoc-V monitored at 463 nm at 6 h of enzyme reaction (IRF: instrument response function).

Fluorescence spectra:

Fluorescence spectra were recorded for a solution of Nmoc-VVV (20 mg/ml) prior to enzyme addition and hydrogel formed by Nmoc-V after addition of enzyme. Excitation wavelength was λ_{ex}=265 a small peak was observed at 463 nm indicate excimer formation in higher order state. To get more significant about peak at 463 nm we excited gel sample at 380 nm showed prominent peak at 463 nm.

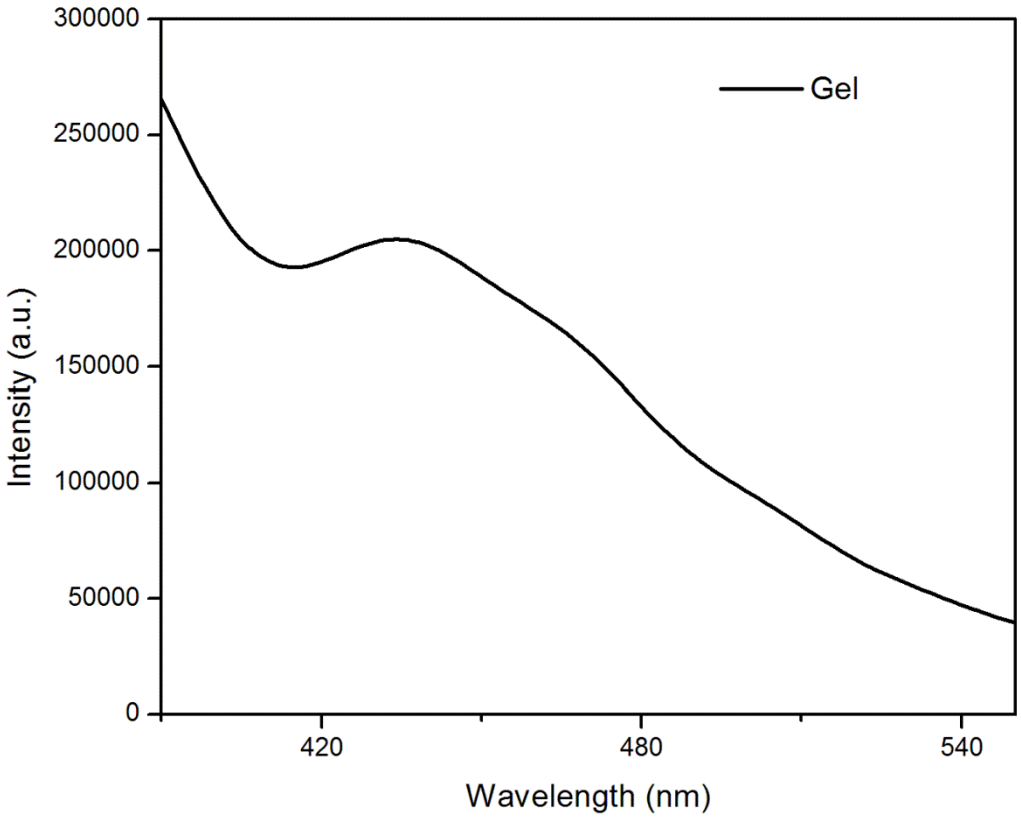


Figure S3. Emission spectra ($\lambda_{excitation}$ = 380 nm) of Nmoc-V hydrogel.

HPLC analysis:

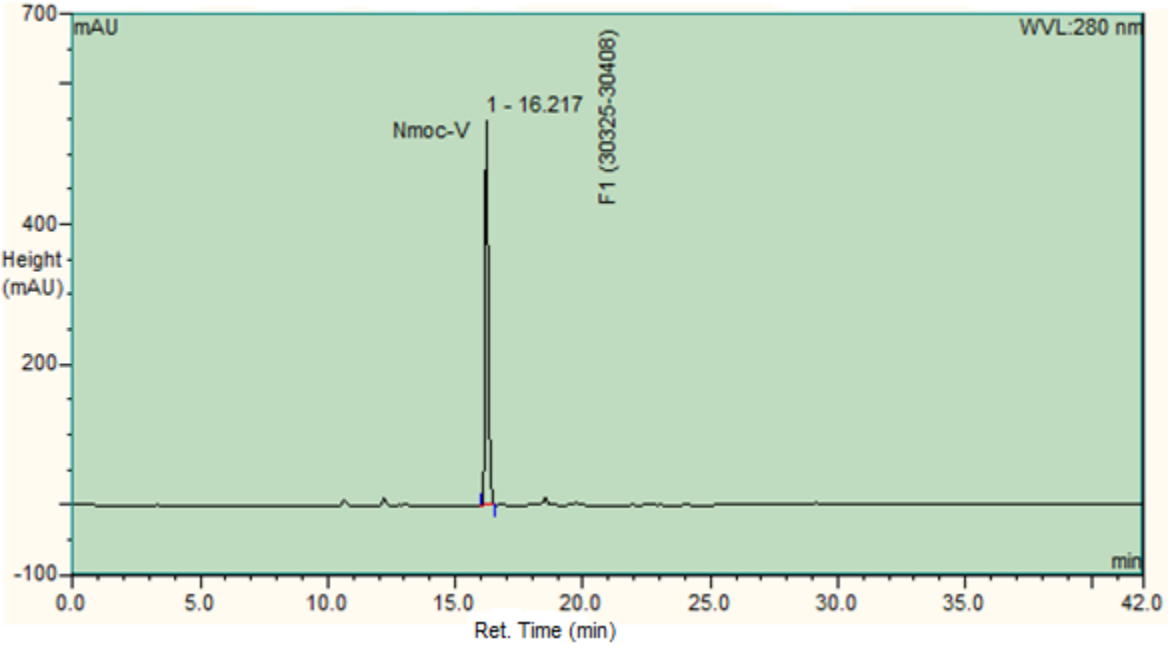


Figure S4. HPLC chromatogram showing peak for synthesized Nmoc-V compound (standard).

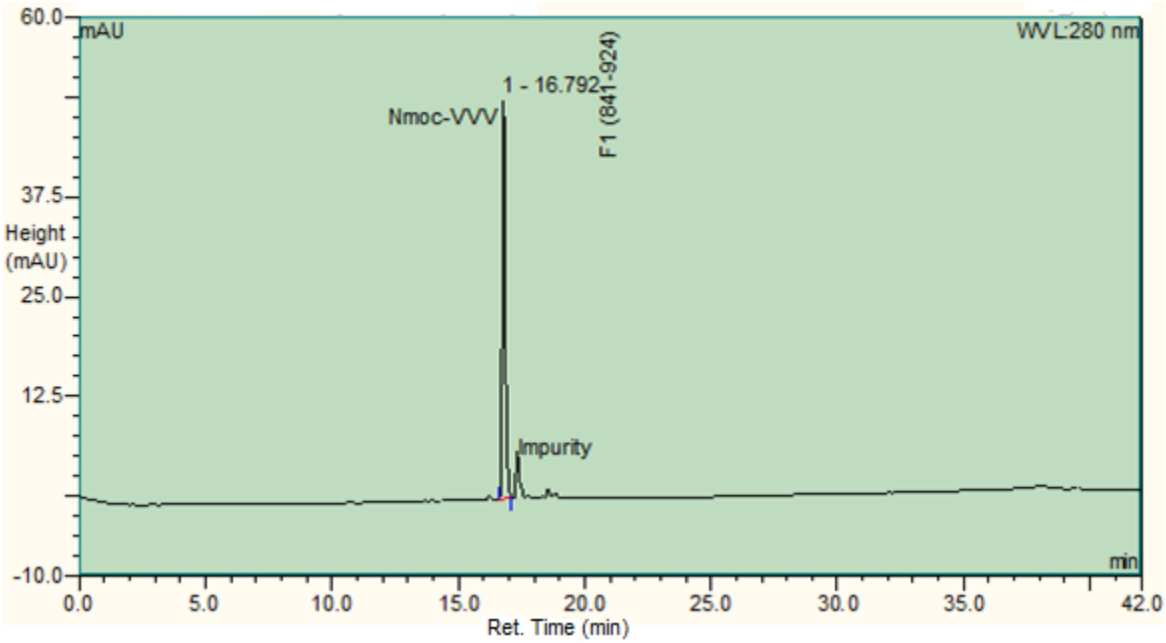
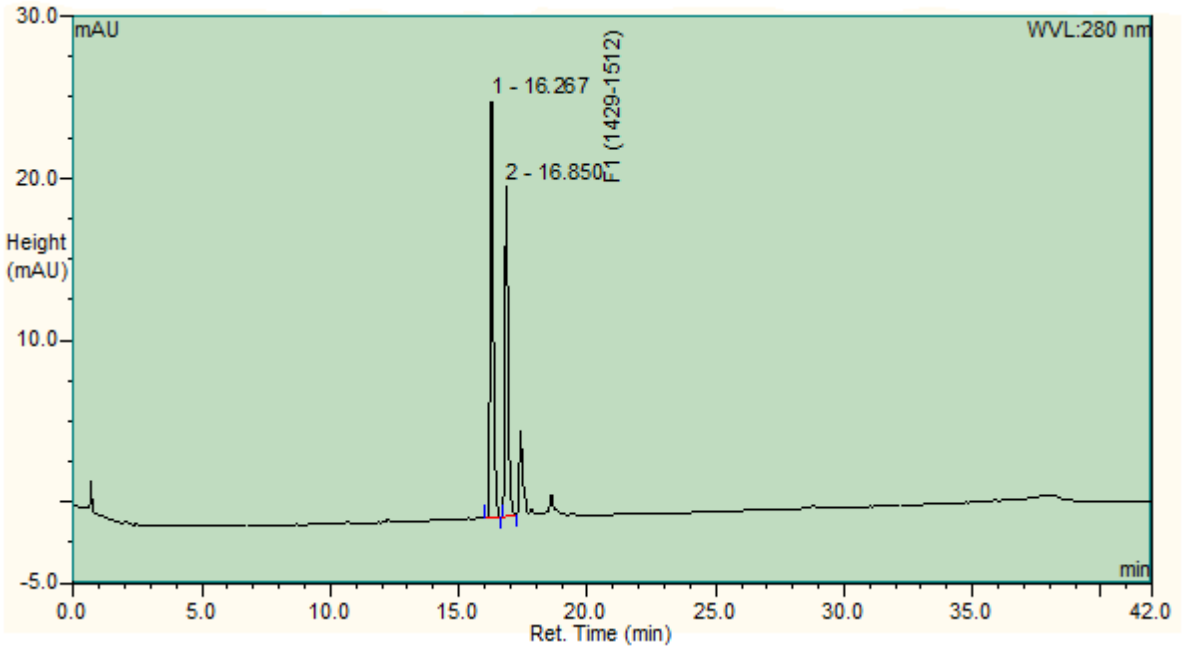


Figure S5. HPLC chromatogram showing peak for parent (standard) Nmoc-VVV molecule.



Fugure S 6. HPLC chromatogram showing 53 % conversion for Nmoc-V from Nmoc-VVV at 3h of enzyme reaction.

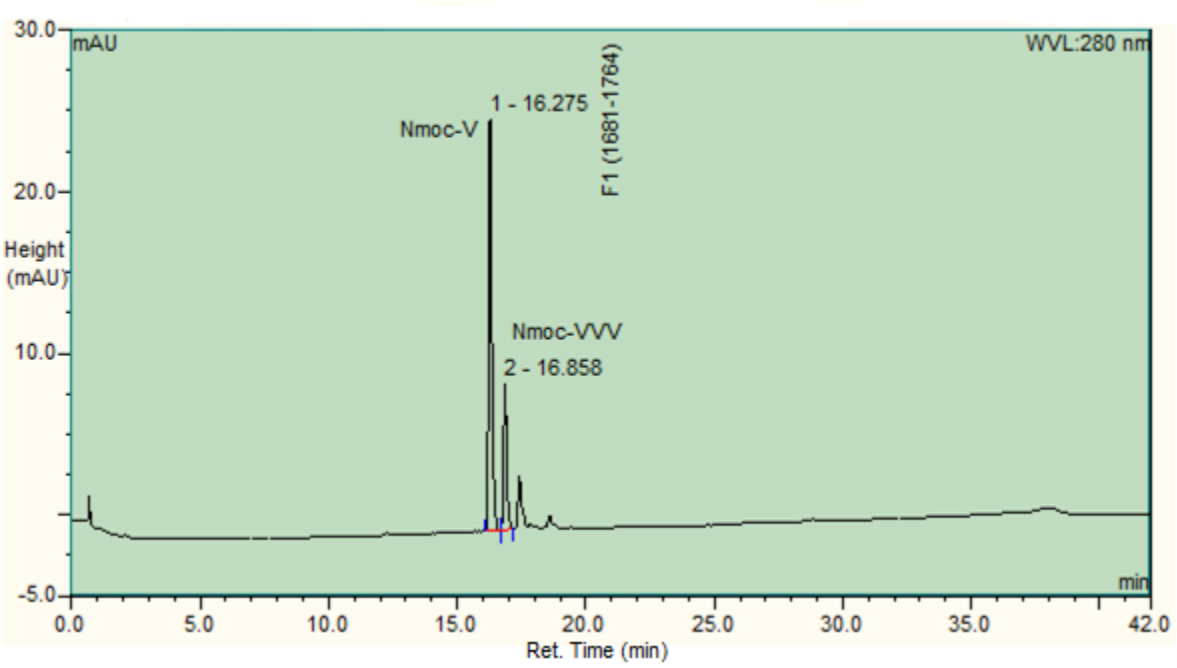


Figure S7. HPLC chromatogram showing 67 % conversion for Nmoc-V from Nmoc-VVV at 6 h of enzyme reaction.

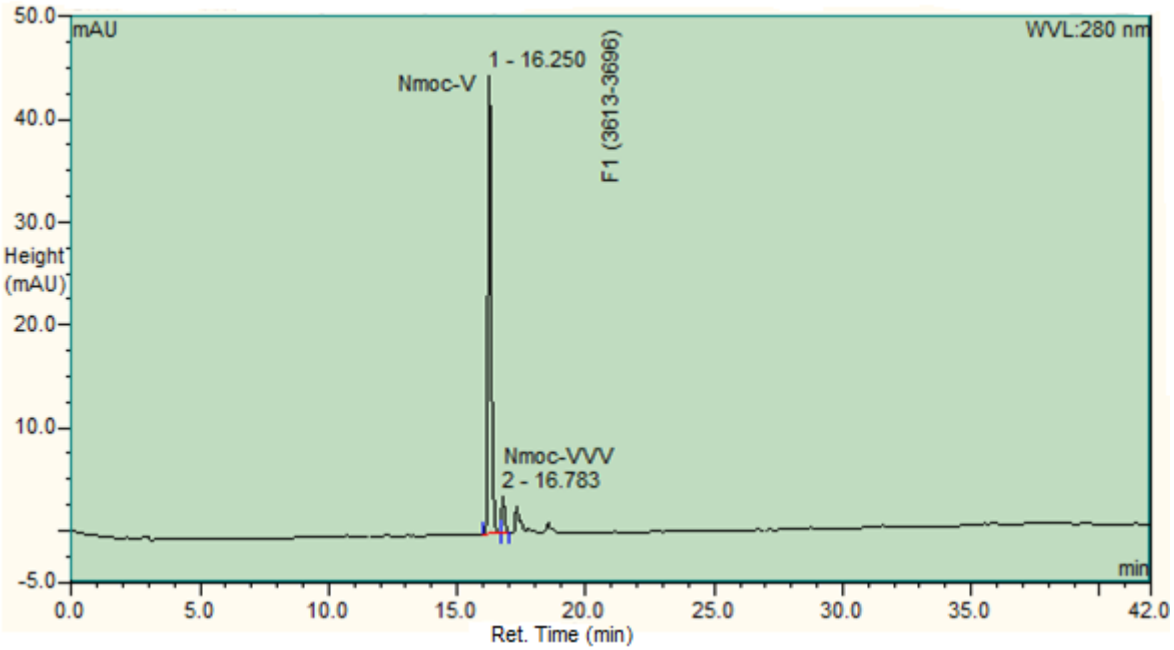


Figure S8. HPLC chromatogram shows conversion for Nmoc-V when a solution of Nmoc-VVV treated with thermolysin at 48 h.

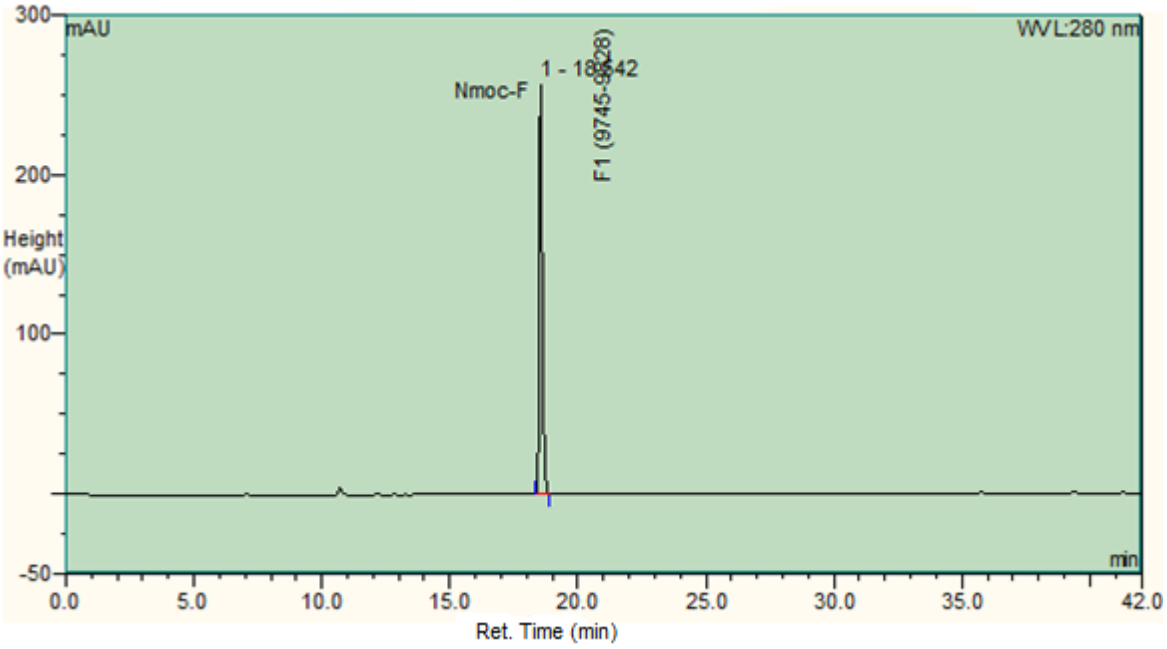


Figure S9: HPLC chromatogram showing peak for synthesized standard Nmoc-F compound (standard).

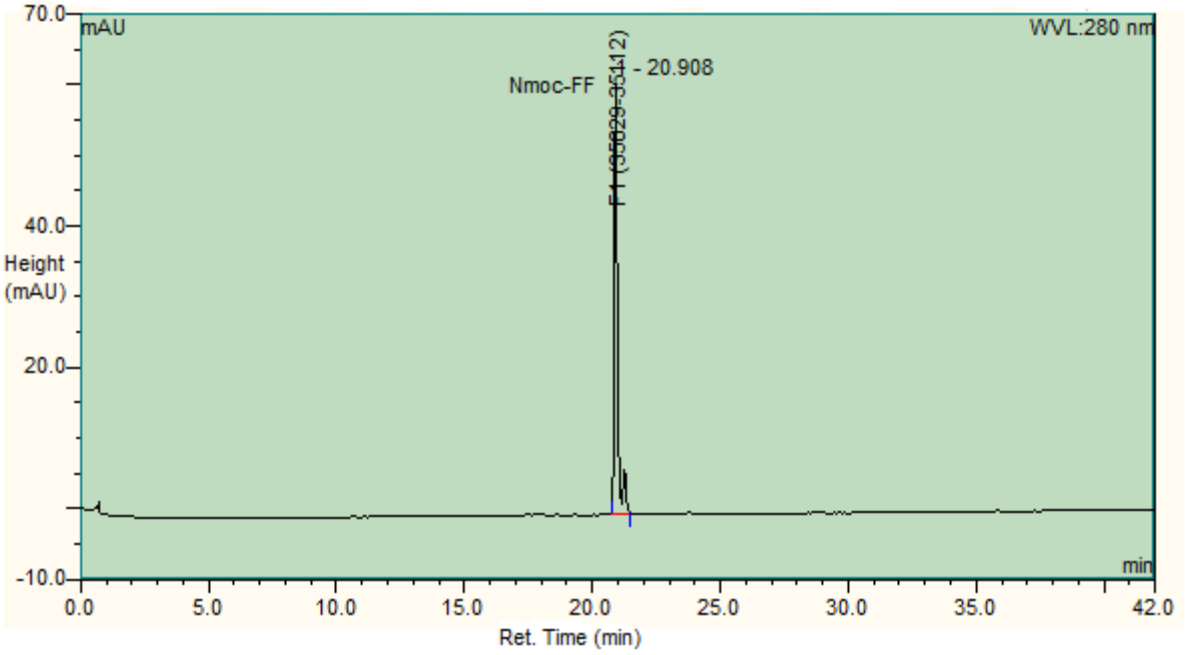


Figure S10. HPLC chromatogram showing peak for synthesized Nmoc-FF molecule (standard).

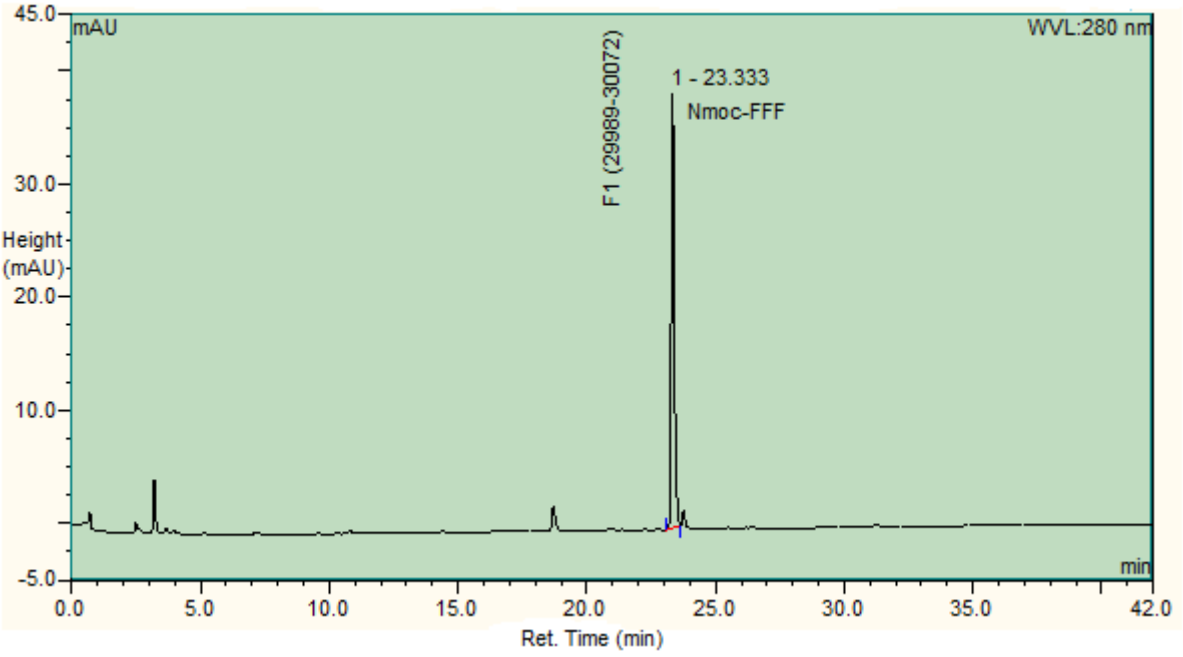


Figure S11. HPLC chromatogram showing peak for parent (standard) Nmoc-FFF molecule.

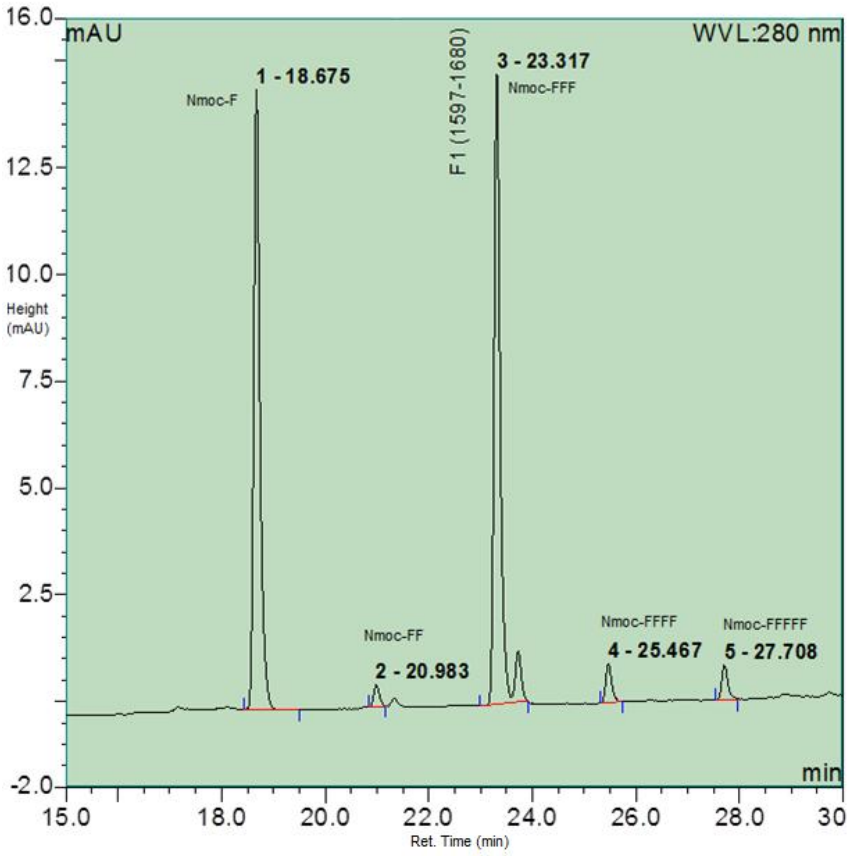


Figure S12. HPLC chromatogram showing the library of components generated when Nmoc-FFF treated with thermolysin (Nmoc-F, Nmoc-FF, Nmoc-FFF, Nmoc-FFFF and Nmoc-FFFFF).

Reverse hydrolysis

A compound Nmoc-F (13.9 mg, 20 mmol/L) and NH_2-FF-COOH (49.9 mg, 80 mmol/L) were dispersed in 2 ml water pH was increased to 10 by using 0.5 M NaOH and vertex for 5 min. After dissolving completely pH was adjusted to 8 using 0.1 M HCL, then 1mg (~40 U/mg) of thermolysin was added to the reaction vial and allowed to remain undisturbed at room temperature strong self-supporting hydrogel was observed after 1 h of reaction time and conversion was checked by HPLC and product formation was confirmed by ESI-MS (Figure S14 and S16 respectively).

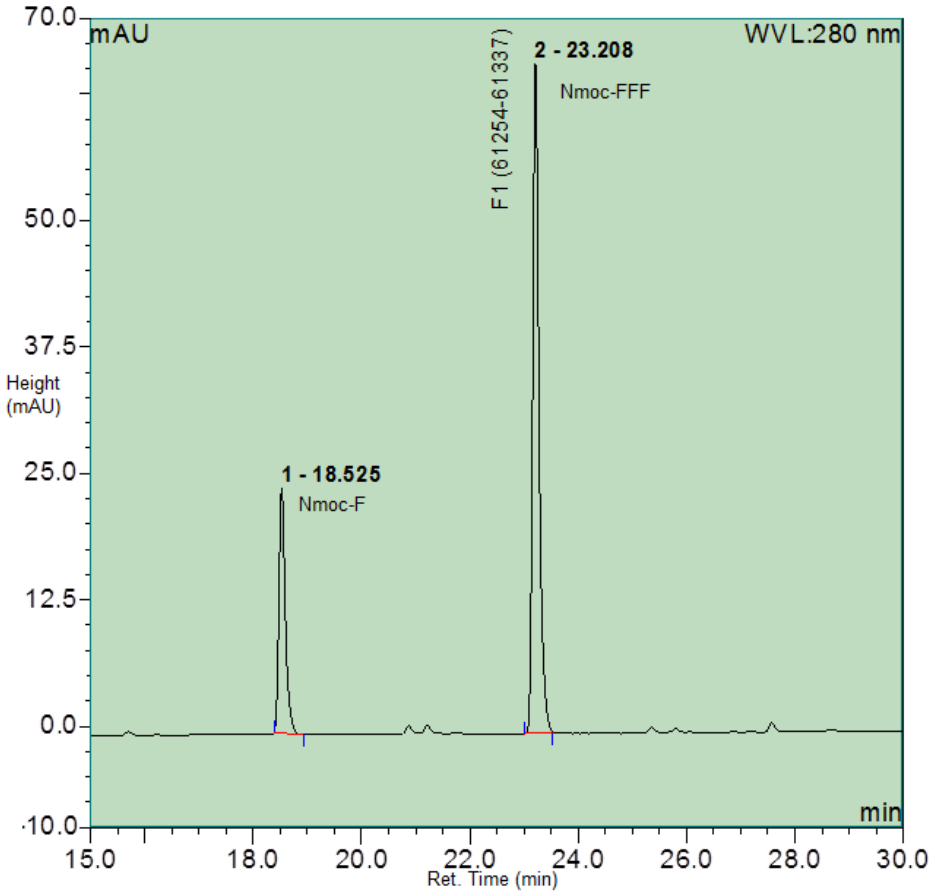


Figure S13. HPLC chromatogram showing reverse hydrolysis for Nmoc-F and FF to obtain Nmoc-FFF.

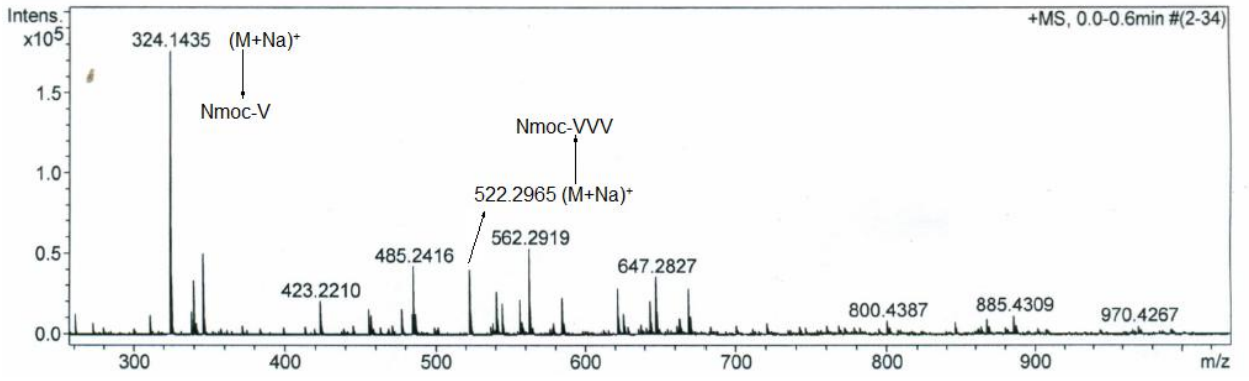


Figure S14. ESI-MS spectrum confirm the formation of library members Nmoc-V and Nmoc-VVV

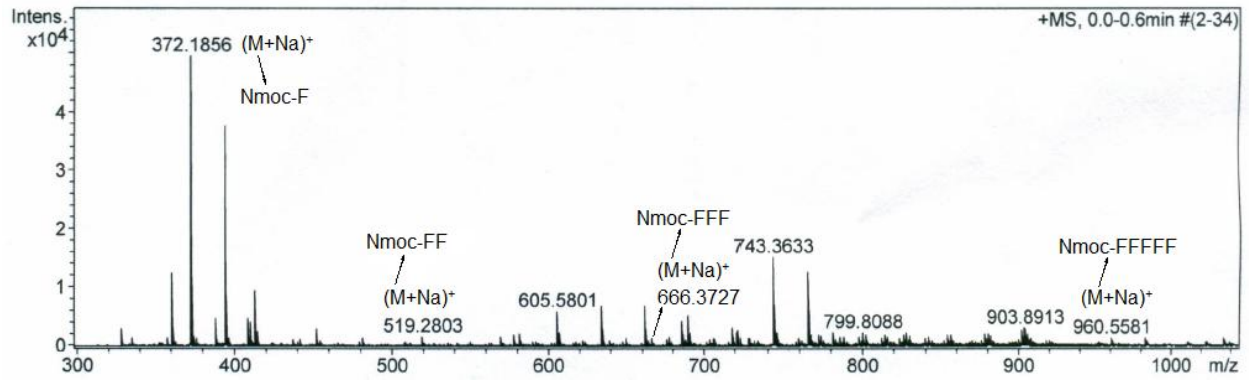


Figure S 15. ESI-MS spectrum confirms the generation library of components Nmoc-F , Nmoc-FF, Nmoc-FFF and Nmoc-FFFFF.

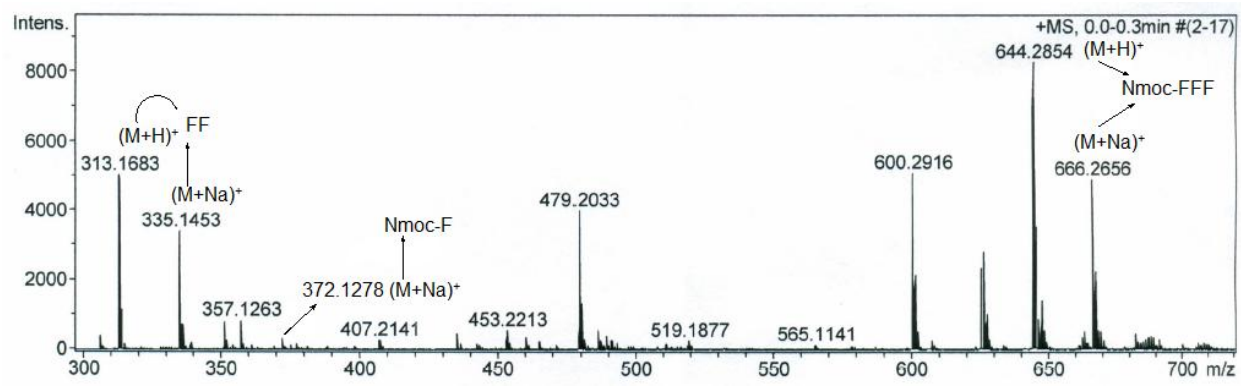


Figure S 16. ESI-MS spectrum confirms the formation Nmoc-FFF from Nmoc-F and FF upon enzymatic reverse hydrolysis.

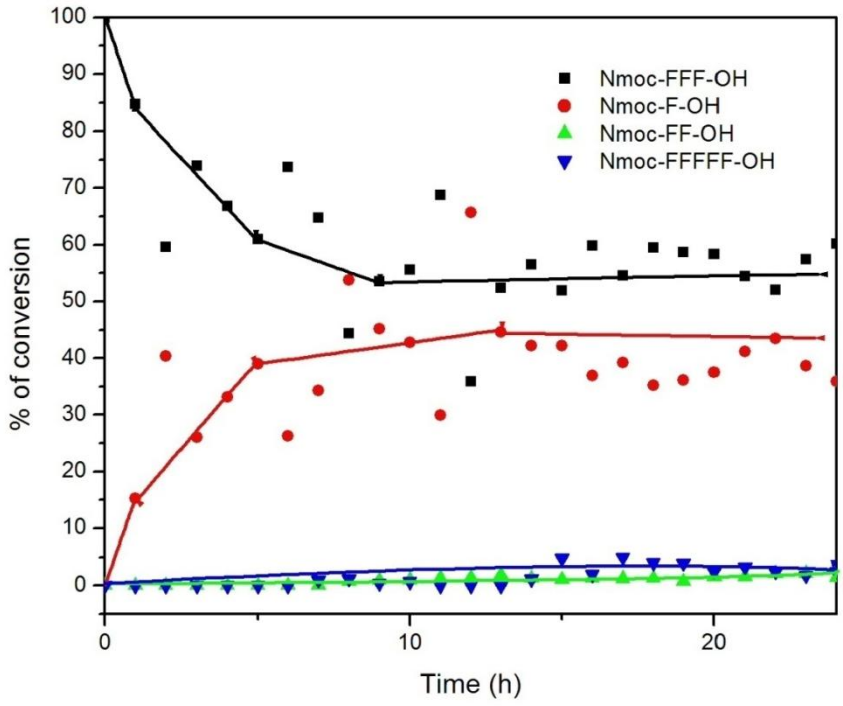


Figure S17. Percentage of conversion for Nmoc-F, Nmoc-FF and Nmoc-FFFFF as function of time followed by HPLC. It was achieved from Nmoc-FFF after treatment with enzyme thermolysin.

Rheology:

Rheological measurement was carried out using an Anton Paar Physica MCR 301 rheometer with cone plate of geometry (25 mm in diameter, 50 µm gap and 1^0 cone). Nmoc-V hydrogel 200 µl poured onto the plate of the instrument, which was kept at 25^0 C by using an integrated temperature controller. Then dynamic frequency sweep of the hydrogel Nmoc-V were measured as function of frequency in the range of 0.1- 100 rad s^{-1} with constant strain value. The stiffness of gel determined when the value storage modulus G' exceed over the loss modulus G''. The value of G' for hydrogel is abmost 20 times more than value G'' indicating very strong hydrogel.

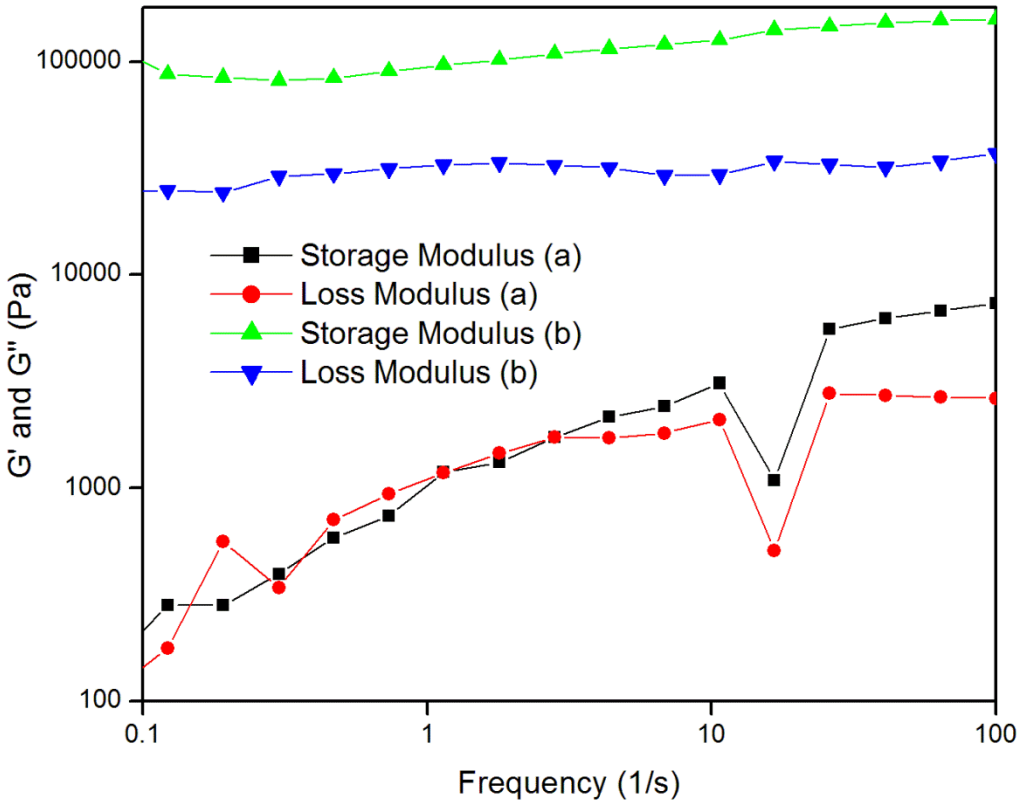


Figure S18. Dynamic frequency sweep showing viscoelastic nature of Nmoc-V hydrogel **a)** a solution of Nmoc-VVV prior enzyme addition and **b)** hydrogel of Nmoc-V 5 min after addition of enzyme.

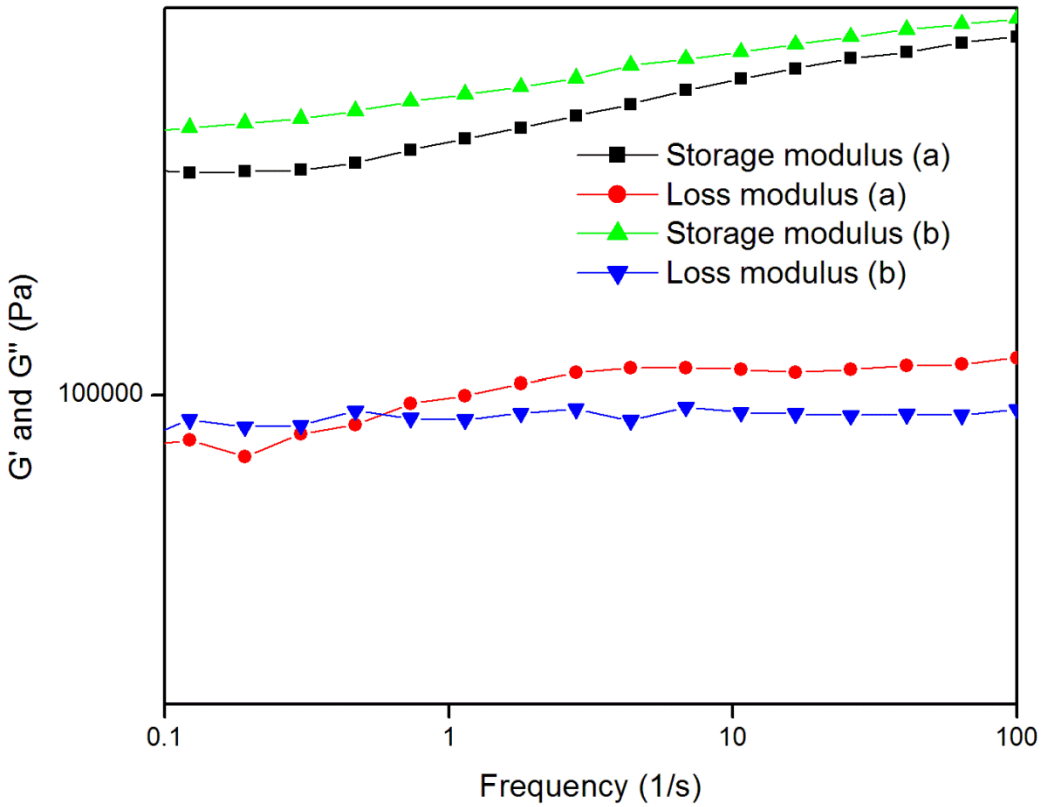


Figure S19. Dynamic frequency sweep showing viscoelastic nature of Nmoc-V hydrogel **a)** Nmoc-V hydrogel after 1 h of enzyme reaction and **b)** Nmoc-V hydrogel after 6 h of enzyme reaction.

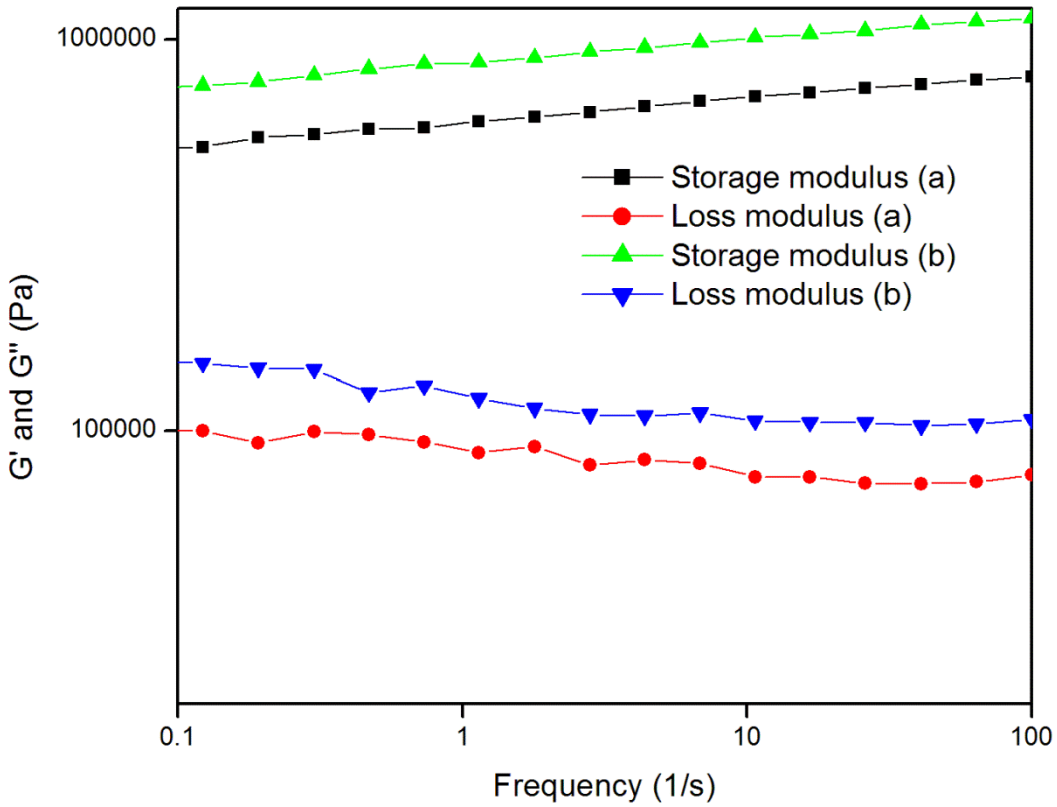


Figure S20. Dynamic frequency sweep showing viscoelastic nature of Nmoc-V hydrogel **a)** Nmoc-V hydrogel 1 day after enzyme reaction and **b)** Nmoc-V 2 days after enzyme reaction.

Experimental part:

A compound Nmoc-VVV (20 mg, 20 mmol/L) dispersed in 2 ml water. The pH was increased to 10 by using 0.5 M NaOH and vertex for 5 min. After dissolving completely pH was adjusted to 6.5-7 using 0.1 M HCL, but compound Nmoc-VVV was unable to form gel. 1mg (~40 U/mg) of thermolysin was added to the reaction vial and allowed to remain undisturbed at room temperature. Opaque self-supporting hydrogel was observed after 1 h of reaction time. Gel was exceptionally stable for two months at room temperature.

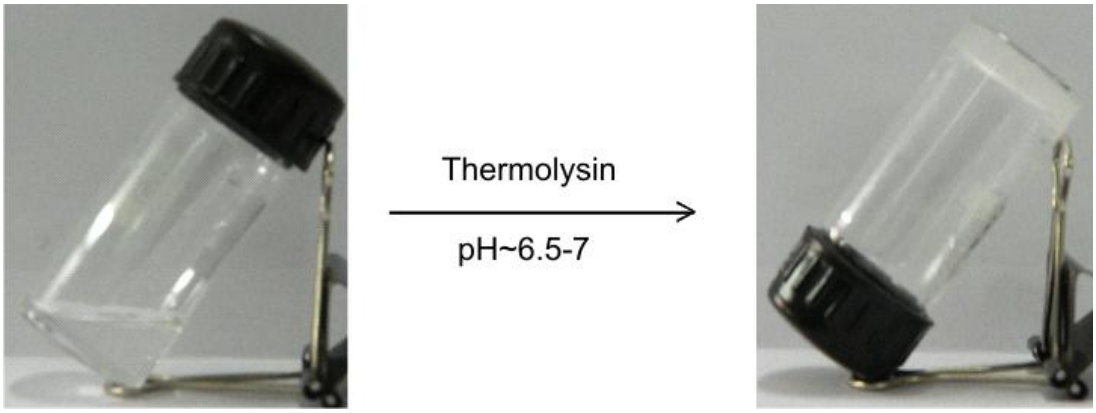
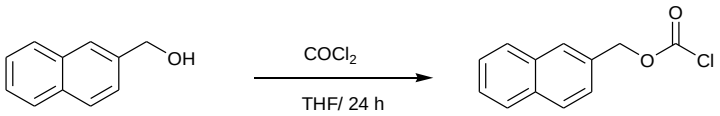


Figure S21. Optical image showing a solution of Nmoc-VVV turned to hydrogel upon treatment with thermolysin (Nmoc-V is responsible moiety for hydrogelation).

Enzyme units:

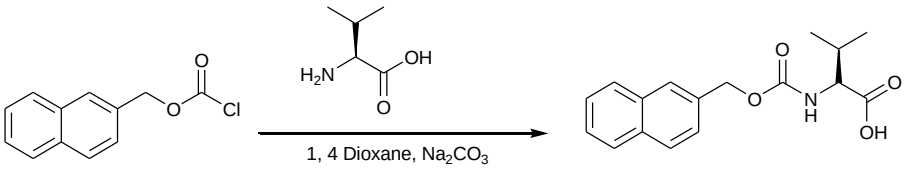
One unit will hydrolyze casein to produce color equivalent to 1.0 μmole (181 μg) of tyrosine per min at pH 7.5 at 37 °C (color by Folin-Ciocalteu reagent).

Synthesis of Naphthalene-2-methyloxychloroformate.



To a stirred solution of naphthalene methanol (10 g, 63.2 mmol) in dry THF (287.2ml), phosgene (78.5 ml, 20 mmol) was added at 0^0 C. The stirring was continued at ambient temperature for 24h. The reaction was monitored by thin layer chromatography (TLC). After completion of reaction, excess phosgene was removed under low vacuum and trapped with aqueous NaOH. Reaction mixture was concentrated and oily product was obtained. Then it was dissolved in hot hexane and kept overnight to yield crystalline product **1** (13 g, 58.9 mmol, 93.19 %). FT-IR (KBr): ῡ = 3066(m), 1777 (s), 1601 (m), 1168 (s) cm^{-1} ; ^{1}H NMR (400 MHz, $CDCl_3$): 7.89 (m, 4H), 7.56 (m, 3H), 5.48 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 71.82, 125.7, 126.1, 126.6, 126.8, 127.8, 128.1, 128.6, 130.6,133.0, 133.5, 140.9, 147.9, 150.7 ppm.

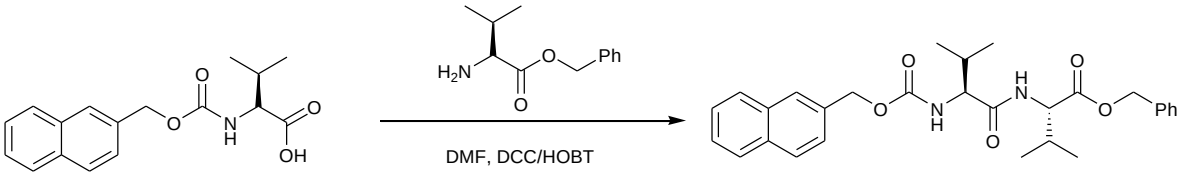
Synthesis of Nmoc-V-OH (2)



A solution of valine (0.585 g, 5 mmol) in a mixture of 1, 4 dioxane (10 ml) and 2M sodium carbonate (13 ml) was stirred and cooled in an ice-water bath. Napthalene-2-methyloxychloroformate (1.102 g, 5 mmol) was added and stirring was continued at room temperature for 12 h. Reaction mixture was diluted with 200 ml of water and dioxane was evaporated under vaccum. Aqueous layer was washed with diethyl ether and the pH of aqueous layer was adjusted to 2 with 2N hydrochloric acid. The aqueous phase was extracted with ethyl acetate (3 x 50 ml) and dried with Na_2SO_4 and concentrated in vacuo to give **2** as colorless oil.

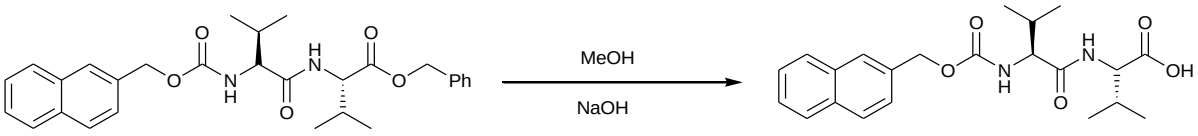
Yield= 1.05 g, (3.5 mmol, 70 %) ^{1}H NMR (400 MHz, $CDCl_3$): 7.93 (m, 4H, Nph-5, 6, 7, 8 Hs), 7.54 (m, 3H, Nph-1, 3, 4 Hs), 7.35 (d, 1H, NH Carbamoyl), 5.22 (s, 2H, CH_2), 4.45 (t, 1H, $C^{\alpha}H$ of Val), 2.18 (m, 1H, C^{β} H of Val), 1.01(d, 6H, C^{γ} H of Val) ppm. HRMS (ESI) m/z for $C_{17}H_{19}NO_4$ $(M+Na)^+$ calcd.: 324.1212, found: 324.1238

Synthesis of Nmoc-Val^1-Val^2-OBn (3)



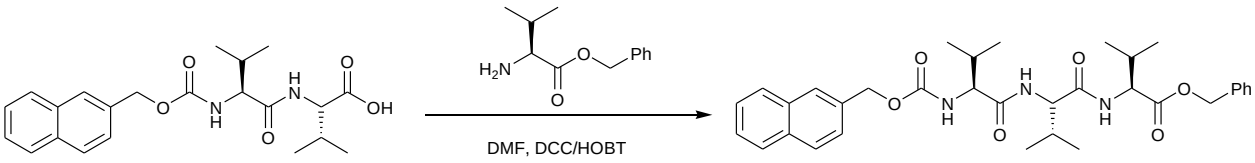
A solution of Nmoc-Val^1-OH (3.2 mmol, 0.980 g) and HOBT (3.2 mmol, 0.432 g) was stirred in 2 ml of DMF. A neutralized solution of valine benzyl ester (6.4 mmol, 2.425 g) was extracted from its corresponding p- toluene sulfonate salt and concentrated to add to the reaction mixture followed by diisopropylcarbodiimde (3.2 mmol, 0.403g) at 0^0 C and allowed to stirred at room temperature for 12 hours. The mixture was diluted with ethyl acetate and organic layer was washed with 1M HCL (2 x 30 ml), brine solution, 1M Na_2CO_3 (3 x 30 ml) and brine solution ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **3** as white powder Yield= 1.56 g, (3.1 mmol, 96.87%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.86 (d, 1H, NH of carbamoyl), 7.46 (m, 4H, Nph-5, 6, 7, 8 Hs), 7.33 (m, 3H, Nph-1, 3, 4 Hs), 7.28, (m, 5H, Ph), 6.27 (d,1H, NH of Val^2), 5.37 (s, 2H, CH_2), 4.61 (t, 1H, $C^{\alpha}H$ of Val^1), 4.06 (t, 1H, $C^{\alpha}H$ of Val^2), 2.20 (m, 1H, $C^{\beta}H$ of Val^1), 2.18 (m,1H, $C^{\beta}H$ of Val^2), 0.99 (d, 12H, Me) ppm. HRMS (ESI) m/z for $C_{29}H_{34}N_2O_5$ $(M+Na)^+$ calcd.: 513.2366, found: 513.2360

Synthesis of Nmoc-Val^1-Val^2-OH (4)



A solution of Nmoc-Val^1-Val^2-OBn(1.46 g, 2.9 mmol) in 20 ml of dry MeOH was allowed to react with a solution of 17 ml 2M NaOH solution. The progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred upto 10 h. Then, methanol was removed under vacuum, residue was taken in 100 ml of water and washed with diethyl ether (2 x 20 ml). Then the pH of aqueous layer was adusted to 2 using 2 M HCL and it was extracted with ethyl acetate (3 x 30 ml) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **4** as white powder and used further without purification. Yield = 1.10 g, (2.7 mmol, 93.1 %). ^{1}H NMR (400 MHz, DMSO-d_6): 7.92 (m, 4H, Nph-5, 6, 7, 8 Hs), 7.53 (m, 3H, Nph-1, 3, 4 Hs), 7.36 (d, 1H, *J*= 8Hz, NH of Val^1), 7.31 (d, 1H, *J*= 8Hz, NH of Val^2), 5.20 (s, 2H, CH_2), 4.14 (t, 1H, C$^{\alpha}$H of Val^1), 4.00 (t, 1H, C$^{\alpha}$H of Val^2), 1.99 (m, 6H, CH_3 of Val^1), 0.90 (m, 6H, CH_3 of Val^2), 12.6 (s, 1H, COOH) ppm. HRMS (ESI) m/z for $C_{22}H_{28}N_2O_5$ $(M+Na)^+$ calcd.: 423.1998, found: 423.1911

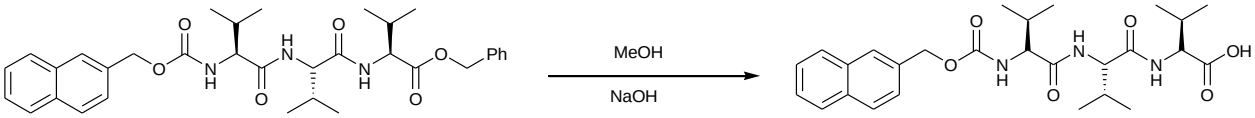
Synthesis of Nmoc-Val^1-Val^2-Val^3-OBn (5)



A solution of Nmoc-Val^1-Val^2-OH (1.00 g, 2.5 mmol) and HOBT (2.5 mmol, 0.337 g) was stirred in 2 ml of DMF. A neutralized solution of valine benzyl ester was extracted from its

corresponding p-toluene sulfonate salt and concentrated to add to the reaction mixture followed by DIC (2.5 mmol, 0.315 g) at 0 ^{0}C. The mixture was allowed to stir at room temperature for 12 h. The mixture was diluted with ethyl acetate and organic layer was washed with 1M HCL (2 x 30 ml), brine solution, 1M Na_2CO_3 (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **5** as white solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent. Yield= 1.12 g, (1.9 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.84 (d,1H, *J*= 8 Hz NH of Val^1), 7.50 (m, 4H, Nph-5, 6, 7, 8 Hs), 7.35 (m, 3H, Nph-1, 3, 4 Hs), 5.28 (s, 2H, CH_2), 5.24 (d, 1H, *J*=8 Hz NH of Val^2), 5.14 (d, 1H, NH of Val^3), 4.63 (t, 1H, C$^{\alpha}$H of Val^1), 4.36 (t, 1H, C$^{\alpha}$H of Val^2), 4.07 (t, 1H, C$^{\alpha}$H of Val^3), 2.19 (m, 1H, C$^{\beta}$H), 2.06 (m, 1H, C$^{\beta}$H), 1.27 (m, 1H, C$^{\beta}$H), 1.5 (d, 6H, CH_3), 0.93 (d, 12H, CH_3) ppm. HRMS (ESI) m/z for $C_{34}H_{43}N_3O_6$ $(M+Na)^+$ calcd.: 612.3152, found: 612.3127

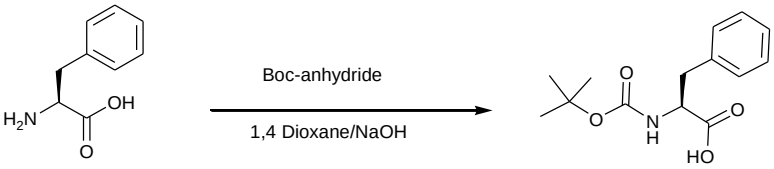
Synthesis of Nmoc-Val^1-Val^2-Val^3-OH (6)



A solution of Nmoc-Val^1-Val^2-Val^3-OBn(1.02g, 1.7 mmol) in 50 ml of dry MeOH allowed to react with a solution of 25 ml 1(M) NaOH solution. The progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred upto 12 h. Then, methanol was removed under vacuum, residue was taken in 100 ml of water and washed with diethyl ether (2 x 20 ml). Then the pH of aqueous layer was adusted to 2 using 2 M HCL and it was extracted with ethyl acetate (3 x 30 ml) and the ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **6** as white solid and used further without purification.

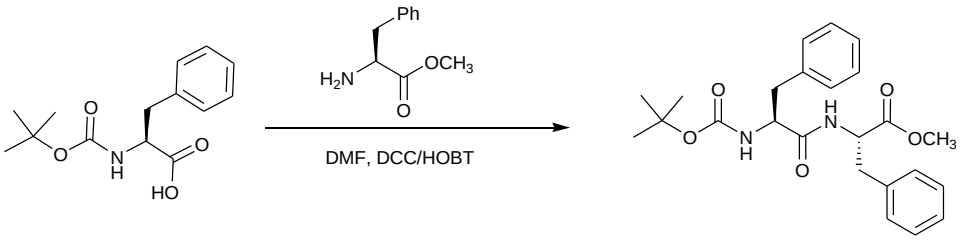
Yield= 0.618 g (1.2 mmol, 70.58 %). FT-IR (KBr): ῡ = 3388 (s), 3065(m), 1711 (ms), 1640 (s), 1542 (s), 1462 (m) cm^{-1} ; ^{1}H NMR (400 MHz, DMSO-d_6): 7.92 (m, 4H, Nph-5, 6, 7, 8 Hs), 7.77 (d, 1H, *J*= 8 Hz, NH of Val^1) 7.54 (m, 3H, Nph-1, 3, 4 Hs), 5.75 (s, 2H, CH_2), 5.48 (d, 1H, *J*= 8Hz, NH of Val^2), 5.24 (d, 1H, *J*=8Hz, NH of Val^3), 4.32 (t,1H, $C^{\alpha}H$ of Val^1), 4.12(t, 1H, $C^{\alpha}H$ of Val^2), 3.95 (t, 1H, $C^{\alpha}H$ of Val^3), 2,09 (m, 1H,$C^{\beta}H$ of Val^1), 2.00 (m, 1H,$C^{\beta}H$ of Val^2), 1.99 (m, 1H, $C^{\beta}H$ of Val^3), 1.00 (d, 18H, Me); ^{13}C NMR (400MHz, DMSO-d_6) δ 17.4, 17.9, 18.1, 18.9, 19.0, 19.1, 29.6, 30.1, 30.7, 60.3, 62.8, 65.3, 125.5, 125.9, 126.3, 126.5, 127.5, 127.9, 132.4, 132.7, 134.7, 142.4, 156.0, 171.0, 172.0, 172.6, 174.0 ppm. HRMS (ESI) m/z for $C_{27}H_{37}N_3O_6$ $(M+Na)^+$ calcd.: 522.2580, found: 522.2559.

Synthesis of Boc-Phe-OH (7)



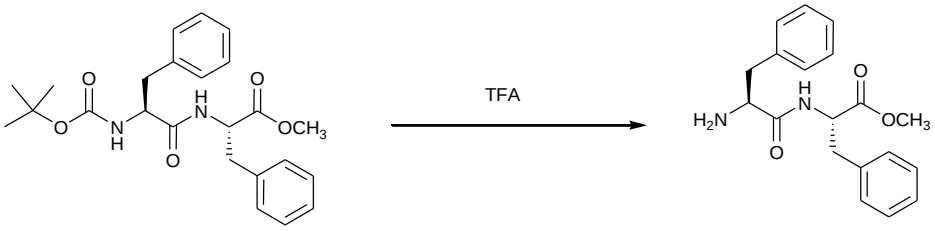
A solution of Phenylalanine (3.3 g, 20 mmol) in a mixture of 1, 4 dioxane (40 ml), 1N sodium hydroxide (20 ml) and dd water (20 ml) was stirred and cooled in an ice-water bath.Boc-anhydride (4.8ml, 21 mmol) was added and stirring was continued at room temperature for 12 h. Reaction mixture was diluted with 200ml of water and dioxane was evaporated under vaccum. Aqueous layer was washed with diethyl ether and the pH of aqueous layer was adjusted to 2 with 2N hydrochloric acid. The aqueous phase was extracted with ethyl acetate (3 x 50 ml) and dried with Na_2SO_4 and concentrated in vacuo to obtain product **7** as colorless oil. Yield= 5.105 g (19.2 mmol, 96 %) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.28 (m, 5H), 7.11 (d, 1H, NH), 4.09 (q, 1H C^{α} of Phe), 3.00 (d, 2H, C^{β} of Phe), 1.32 (s, 9H CH_3), 12.66 (s, 1H) ppm. HRMS (ESI) m/z for $C_{14}H_{19}NO4$ $(M+Na)^+$ calcd.: 288.1212, found: 288.1223

Synthesis of Boc-Phe^1-Phe^2-OMe (8)



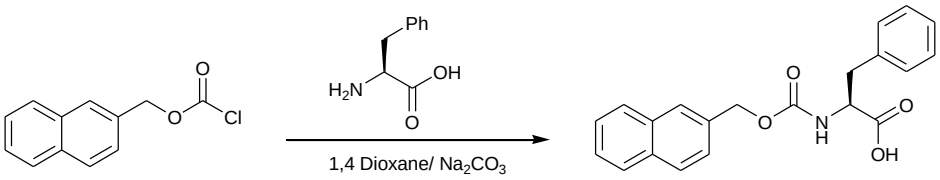
A solution of Boc-Phe-OH (1.32 g, 5 mmol) and HOBT (5 mmol, 0.677 g) was stirred in 2 ml of DMF. A neutralized solution of phenyalanine methyl ester was extracted from its corresponding hydrochloride salt and concentrated to add to the reaction mixture followed by DCC (5.1 mmol, 1.052 g) at 0 ^{0}C. The mixture is allowed to stir at room temperature for 12 h. The mixture was diluted with ethyl acetate and organic layer was washed with 1M HCL (2 x 30 ml), brine solution, 1M Na_2CO_3 (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield white solid product **8**. Purification was done by silica gel column (100-200 mesh) using ethyl acetate- toluene as eluent. Yield= 1.85g, (4.6 mmol, 92 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 7.54 (m, 10H), 7.00 (d, 1H NH of Phe^{1}), 6.28 (d, 1H NH of Phe^{2}), 4.81(q, 1H C^{α} H of Phe^{1}), 4.34 (q, 1H of C^{α} of F^{2}), 3.12 (d, 4H CH_2), 1.47 (s, 9H CH_3) ppm. HRMS (ESI) m/z for $C_{24}H_{30}N_2O_5$ $(M+Na)^{+}$ calcd.: 449.2052, found: 449.2086

Synthesis of NH_2-Phe^1-Phe^2-OMe (9)



A solution of Boc-NH-Phe-Phe-OMe (1.6 g, 3.7 mmol) in TFA stirred for 12 h under argon at room temperature. The excess TFA removed under vaccum oily residue was taken in 100 ml of water and washed with diethyl ether (2 x 20 ml) white product **9** was obtained after lypholization and used further for the reactions. Yield= 1.18 g (3.6 mmol, 97.29 %) ^{1}H NMR (400 MHz, DMSO-d_6): δ= 9.03 (d, 1H NH of Phe^2), 7.35 (m, 10H), 4.60 (q, 1H C^{α} H of Phe^2),4.03 (q, 1H C^{α} H of Phe^1), 3.66 (s, 3H OCH_3), 3.12 (d,2H C^{β} H of Phe^2), 3.05 (d, 2H C^{β} H of Phe^1) ppm. HRMS (ESI) m/z for $C_{19}H_{23}N_2O_3$ $(M+H)^+$ calcd.: 327.1709, found: 327.1717

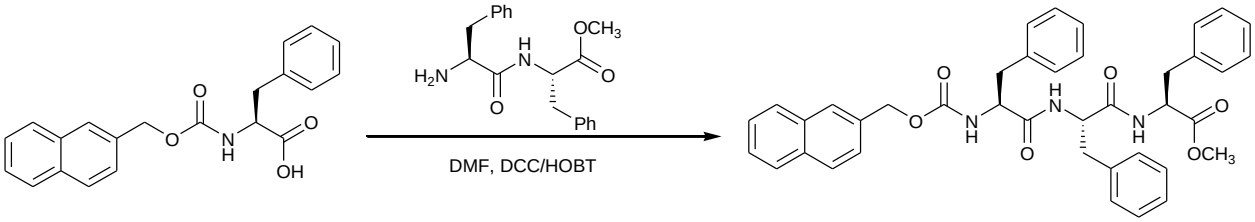
Synthesis of Nmoc-Phe-OH (10)



A solution of Phenylalanine (0.825 g, 5 mmol) in a mixture of 1,4 dioxane (10 ml) and 2M sodium carbonate (13 ml) was stirred and cooled in an ice-water bath. Napthalene-2-methyloxychloroformate (1.102 g, 5 mmol) was added and stirring was continued at room temperature for 12 h. Reaction mixture was diluted with 200 ml of water and dioxane was evaporated under vaccum. Aqueous layer was washed with diethyl ether and the pH of aqueous

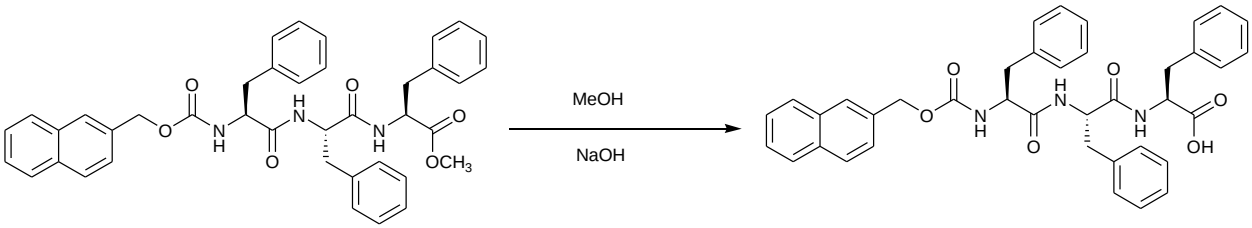
layer was adjusted to 2 with 2N hydrochloric acid. The aqueous phase was extracted with ethyl acetate (3 x 50 ml) and dried over Na_2SO_4 and concentrated in vacuo to give product **10** as a colorless oil. Yield= 1.568 (4.4 mmol, 88 %) ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.76 (d, 2H, *J*=8Hz), 7.53 (t, 2H), 7.41 (d, 1H, *J*= 8Hz), 7.23 (m, 5H), 6.39 (d, 1H, NH), 5.15 (s, 2H), 4.19 (t, 1H, C^{α} H of Phe), 3.07 (d, 2H, C^{β} H of Phe), 12.8 (s, 1H) ppm. HRMS (ESI) m/z for $C_{21}H_{19}NO_4$ $(M+Na)^+$ calcd.: 372.1212, found: 372.1206

Synthesis of Nmoc-Phe^1-Phe^2-Phe^3-OMe (11)



A solution of Nmoc-Phe-OH (1.42 g, 4.1 mmol) and HOBT (0.555 g, 4.1mmol) was stirred in 2 ml of DMF. A neutralized solution of NH_2-Phe^1-Phe^2-OMe was extracted from its corresponding trifluroacetate salt and concentrated to add to the reaction mixture followed by DCC (0.866 g, 4.2 mmol) at 0 ^{0}C. The mixture is allowed to stirred at room temperature for 12 h. The reaction mixture was diluted with ethyl acetate and organic layer was washed with 1M HCL (2 x 30 ml), brine solution, 1M Na_2CO_3 (3 x 30 ml) and brine solution, dried over Na_2SO_4 and evaporated under vacuum to yield white solid product **11**. Purification was done by silica gel column (100-200 mesh) using ethyl acetate- toluene as eluent. Yield= 1.54 g (2.3 mmol, 56.09 %) ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (d, 1H NH of Phe^1) 7.79 (s, 1H), 7.54 (m, 2H), 7.43 (d, 2H), 7.32), 7.15 (d, 1H), 7.07 (d, 1H), 6.37 (d, 1H NH of Phe^2), 5.79 (d, 1H NH of Phe^3), 5.22 (s, 2H), 4.75 (q, 1H C^{α} H of Phe^1), 4.57 (q, 1H C^{α} H of Phe^2), 4.39 (q, C^{α} H of Phe^3), 3.74 (s, 3H), 3.13 (d, 2H $^{\beta}CH_2$ of PhePhe^1), 3.09 (d, 2H $^{\beta}CH_2$ of Phe^2), 3.06 (d, 2H $^{\beta}CH_2$ of Phe^3) ppm. HRMS (ESI) m/z for $C_{40}H_{39}N_3O_6$ $(M+Na)^+$ calcd.: 680.2737, found: 680.2733

Synthesis of Nmoc-Phe^1-Phe^2-Phe^3-OH (12)



A solution Nmoc-Phe-Phe-Phe-Ome (0.201g, 0.3mmol) in 100 ml of dry MeOH allowed to react with a solution of 2M NaOH. The progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture stirred upto 12 h. Then, methanol was removed under vacuum, residue was taken in 100 ml of water and washed with diethyl ether (2 x 20 ml). Then the pH of aqueous layer was adjusted to 2 using 2M HCL and it was extracted with ethyl acetate (3 x 30 ml) and ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuo to yield **6** as white solid and used further without purification. Yield= 0.18g (0.27 mmol, 90 %) FT-IR (KBr): ῡ = 3292 (s), 3061(m), 1707 (ms), 1647 (s), 1541 (s), 1444 (m) cm^{-1} ; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.57 (d, 1H NH of Phe^1), 8.61 (d, 1H NH of Phe^2), 8.35 (d, 1H NH of Phe^3), 8.06 (d, 2H), 7.89 (m, 5H), 7.75 (d, 2H), 7.51 (m, 5H), 7.37 (m, 3H), 7.22(m, 5H), 5.09 (s, 2H), 4.59 (q,1H C^{α} H of Phe^1), 4.46 (q, 1H C^{α} H of Phe^2), 4.23 (q, 1H C^{α} H of Phe^3), 2.94 (m, 6H), 12.8 (s,1H broad) ; ^{13}C NMR (400 MHz, DMSO-d_6) δ 22.0, 28.9, 36.6, 53.4, 56.0, 65.2, 125.4, 126.1, 127.5, 128.1, 129.4, 132.3, 134.5, 137.4, 138.0, 155.6, 156.7, 170.8, 171.5, 172.6, 173.6 ppm. HRMS (ESI) m/z for $C_{39}H_{37}N_3O_6$ $(M+Na)^+$ calcd.: 666.2580, found: 666.2580

NMR Charaterization

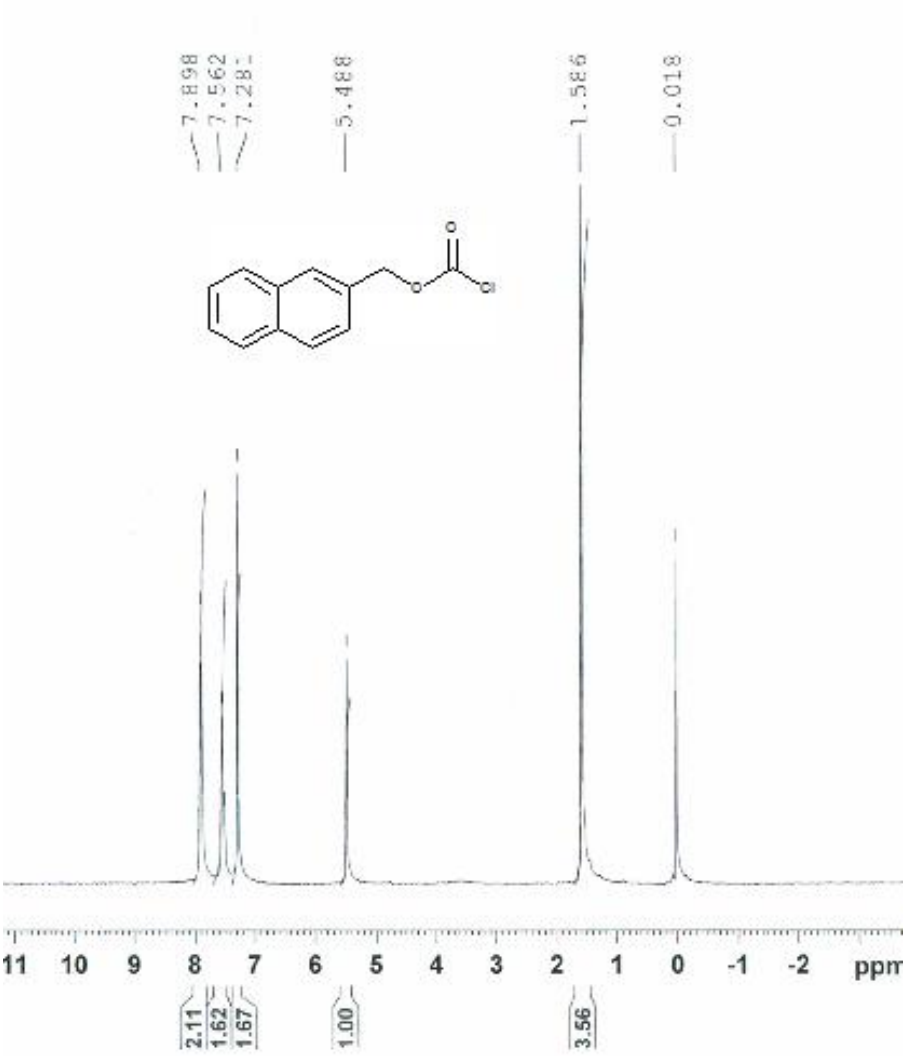


Figure S22. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Napthalene-2-methyloxychloroformate **1**.

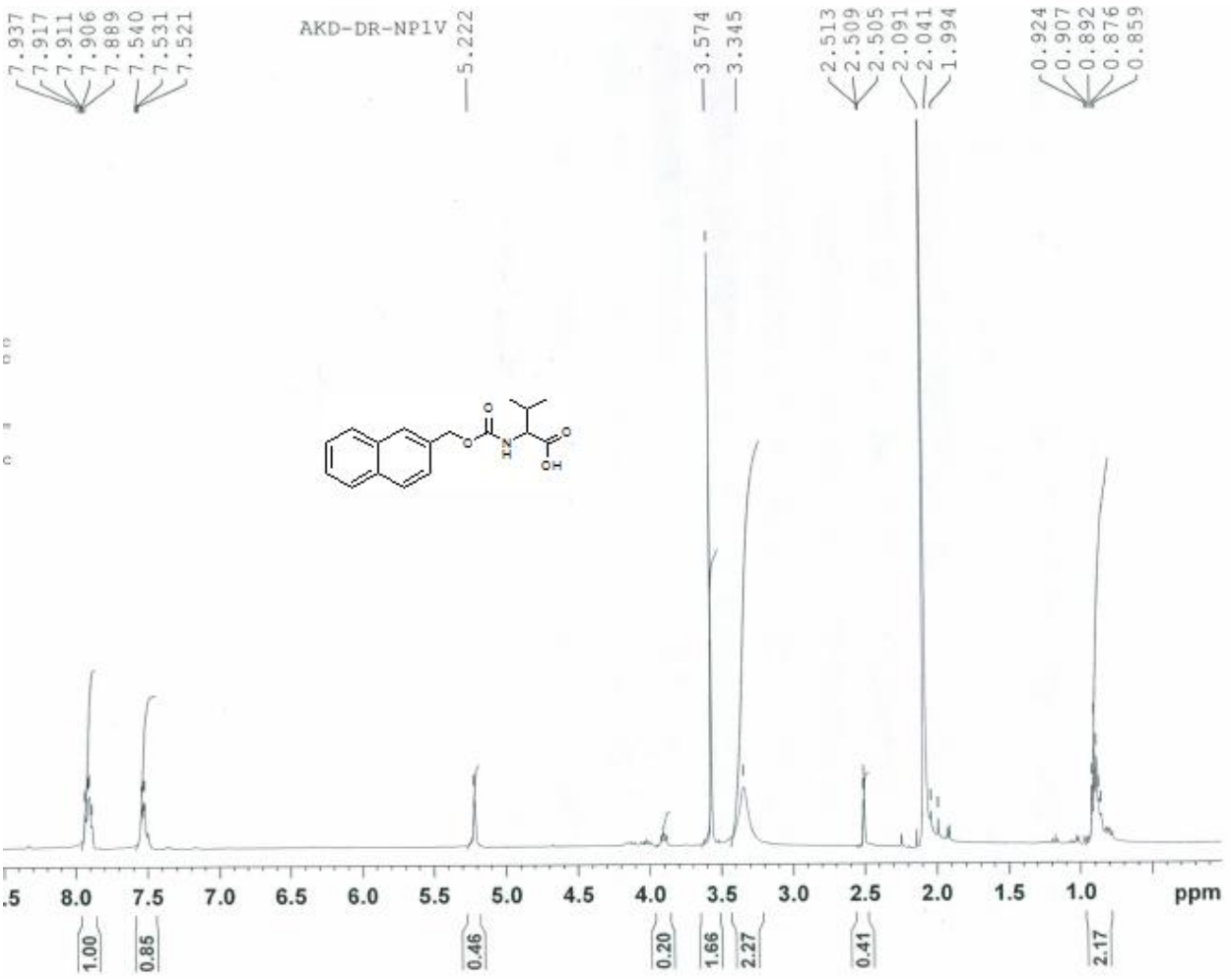


Figure S23. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Nmoc-V-OH **2**.

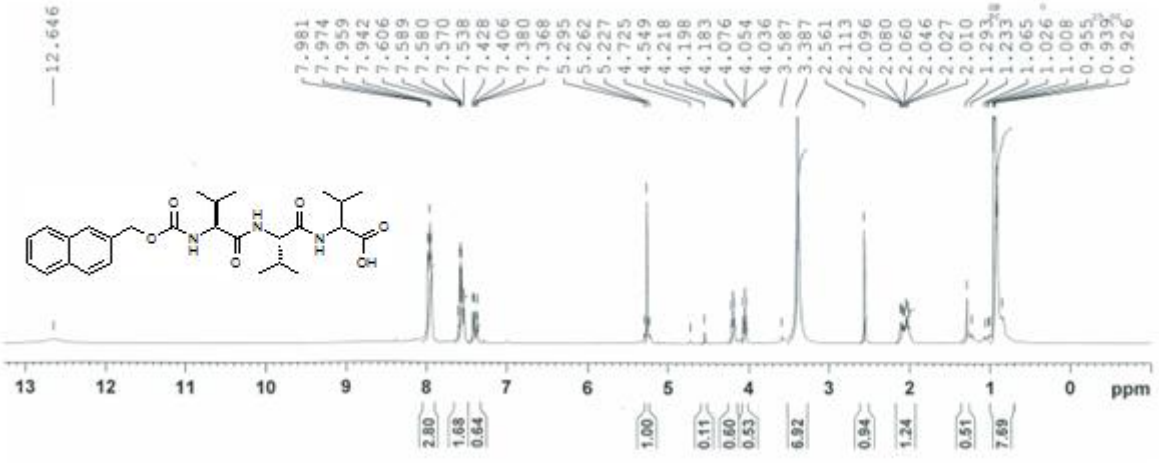


Figure S24. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Nmoc-VVV-OH **3**.

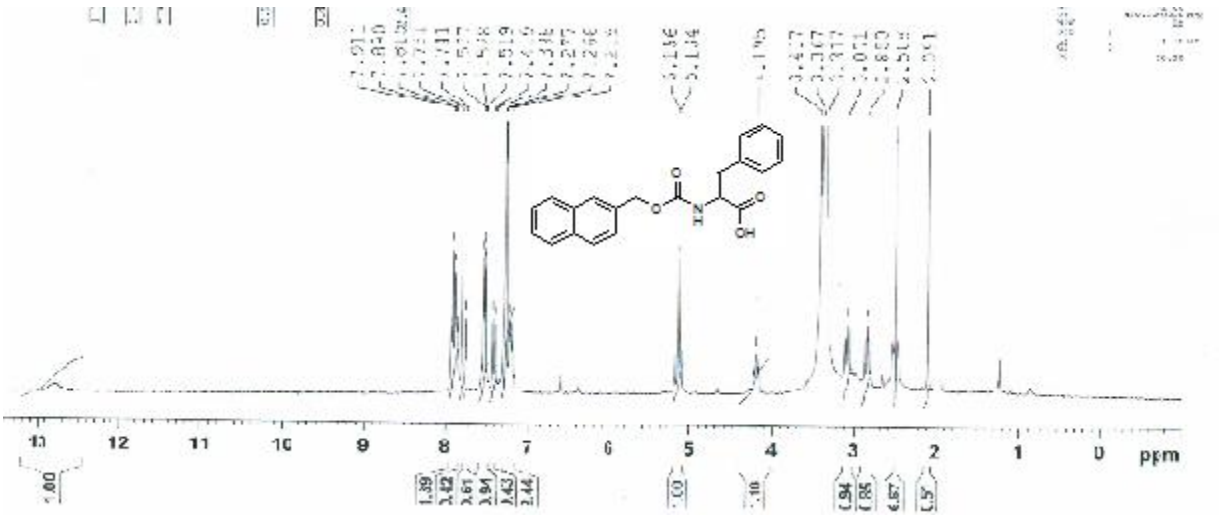


Figure S 25. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) of Nmoc-F-OH **10**.

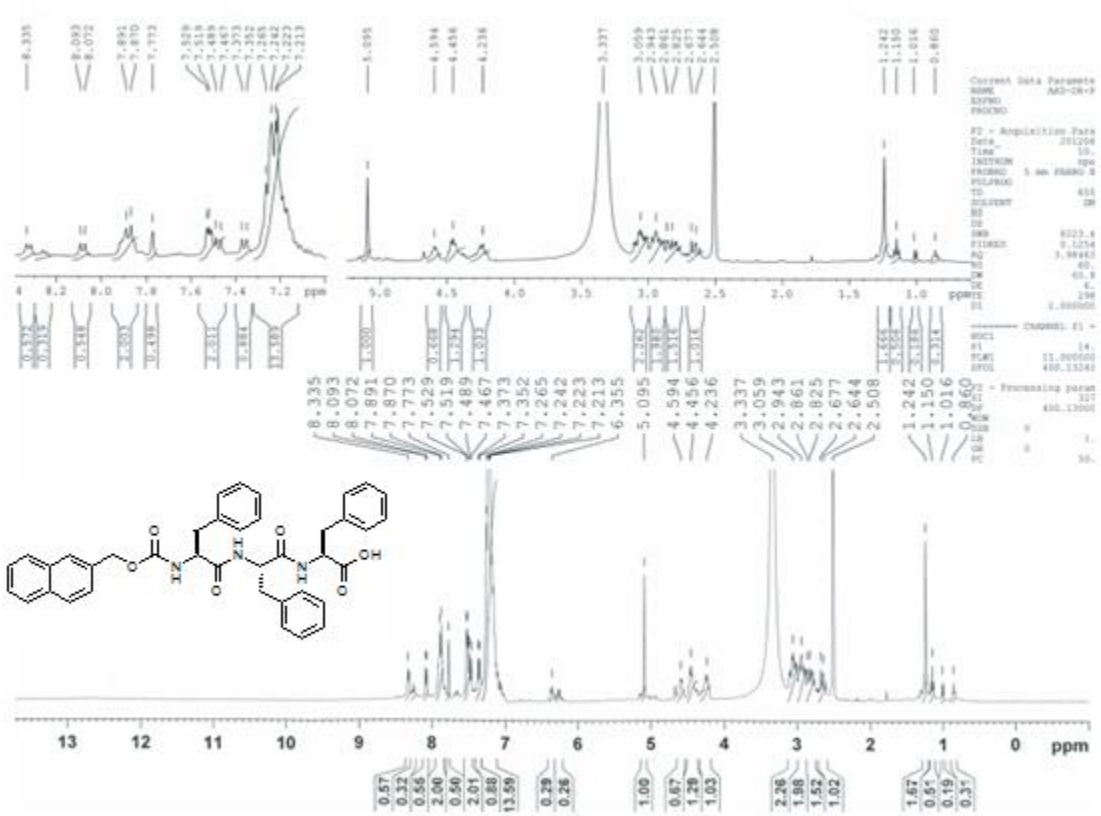


Figure S26. ^{1}H NMR spectrum (400 MHz, DMSO-d_6) for Nmoc-FFF-OH **12**.