Electronic Supplementary Material (ESI) for Chemical Communications

Exploiting peptide self-assembly driven by oxo-ester mediated native chemical ligation

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Contents

General Methods

All the chemicals and reagents were obtained commercially. Thin layer chromatography was done on pre-coated silica gel plates (Kieselgel 60F254, Merck). Column chromatographic purification was done with 100-200 mesh silica gel. NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on Bruker AV 400 MHz spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q) and multiplet (m) *etc*. Elemental analyses were performed on an elemental analyzer Thermo Scientific Flash 2000. FT-IR spectra were recorded using the KBr pellet technique on a bruker (Tensor 27) FT-IR spectrophotometer. Ionization (ESI) mass spectrometric measurements were done with Bruker's micrOTOF-Q II mass spectrometer.

Self-assembly

A compound **1** Nmoc-AV-*p*NP (20 mmol/L, 9.8 mg) was dissolved in 100 µl of methanol. A solution of cysteine (80 mmol/L, 9.6 mg) in phosphate buffer 900 µl (pH 8) was mixed together to homogeneous followed by incubation for 15 min at 80^0 C. Self-assembly of ligated product **1b** was observed after 6h of native chemical ligation reaction. Formation of Nmoc-AVC and Nmoc-AVC-CVA-Nmoc were confirmed by HPLC and ESI-MS. Similarly, compound **5** Nmoc-F-*p*NP (20 mmol/L, 9.4 mg) was ligated to cysteine at same reaction condition. Self-assembly of ligated product **5b** was observed after 3h of native chemical reatcion. Synthesized products Nmoc-FC **5a** and Nmoc-FC-CF-Nmoc **5b** were confirmed by HPLC and ESI-MS.

Gel- Sol Transition

Self-assembly and dis-assembly were observed for gelators **1b** and **5b** as described in the text. **1a** transforms into **1b** upon exposure to air resulting into the formation of self-supporting strong gel. Gel-sol transition occurred after 3h after addition of reductant Tris(2-carboxyethyl)phosphine (TCEP) 40 mmol/L to **1b** gel at pH 5.2. However, quick gel-sol transition was observed for compound **5b** (1.5 h) at pH 4.8. TCEP is an effective reductant which can cleave disulfide linkage.

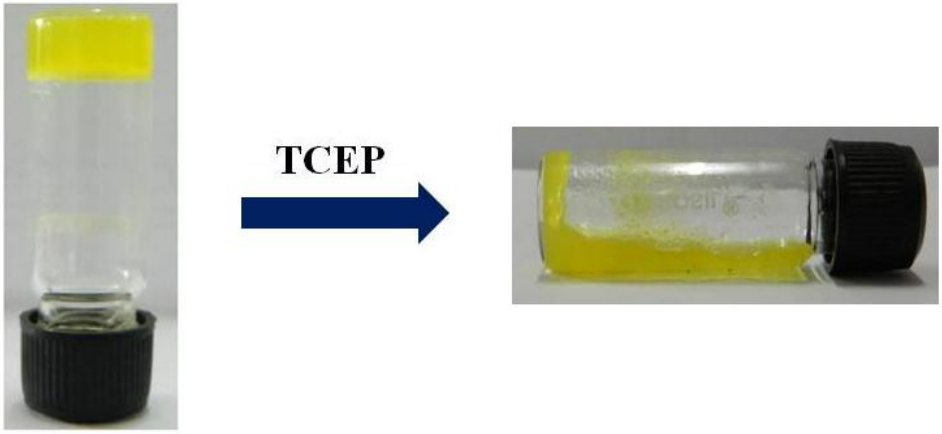


Fig. S1 Optical images showing gel transform into solution upon treatment with Tris(2-carboxyethyl)phosphine (TCEP) after 3h.

HPLC Analysis

A Dionex HPLC-Ultimate 3000 (High Performance Liquid Chromatography) pump was used to analyze native chemical ligated products. A 20 µL of sample was injected onto a Dionex Acclaim ® 120 C 18 column of 250 mm length with an internal diameter 4.6 mm and 5 µm fused silica particles at a flow rate of 1 mL min^{-1} (linear gradient of 40 % v/v) acetonitrile in water for 35 min, gradually rising to 100 % (v/v) acetonitrile in water at 35 min). This concentration was kept constant until 40 min when the gradient was decreased to 40 % (v/v) acetonitrile in water at 42 min. The sample preparation involved mixing 100 µL of gel/solution with acetonitrile-water (900 µL, 50: 50 mixture) containing 0.1 % trifluoacetic acid. The samples were then filtered through a 0.45 µm syringe filter (Whatman, 150 units, 13 mm diameter, 2.7 mm pore size) prior to injection. The native chemical ligated products were identified by using Ultimate 3000 RS Variable Wavelength Detector at 280 nm.

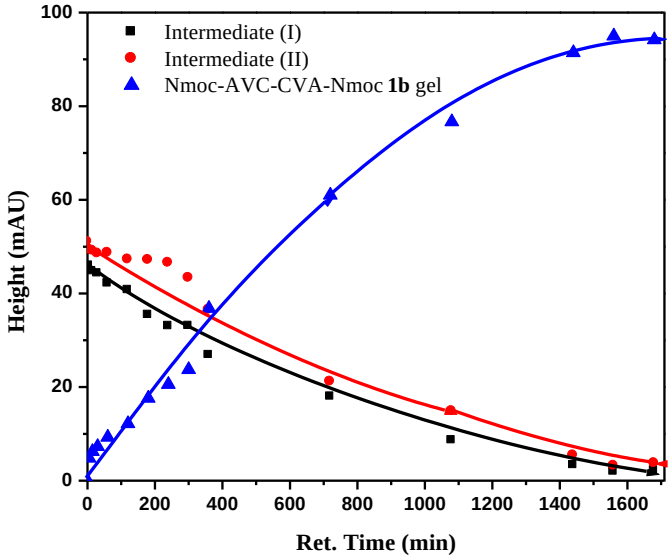


Fig. S2 HPLC analysis for product **1b** formation as function of time upon exposure to air (intermediates I and II Nmoc-AVC **1a**).

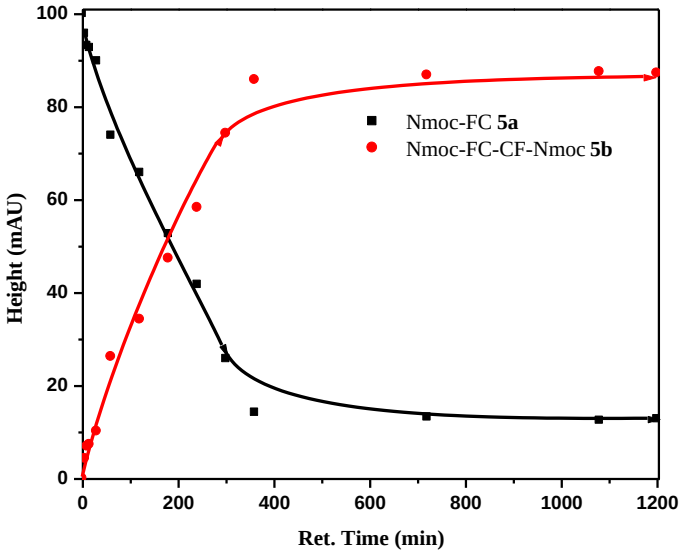


Fig. S3 HPLC analysis for product **5b** formation as function of time upon exposure to air.

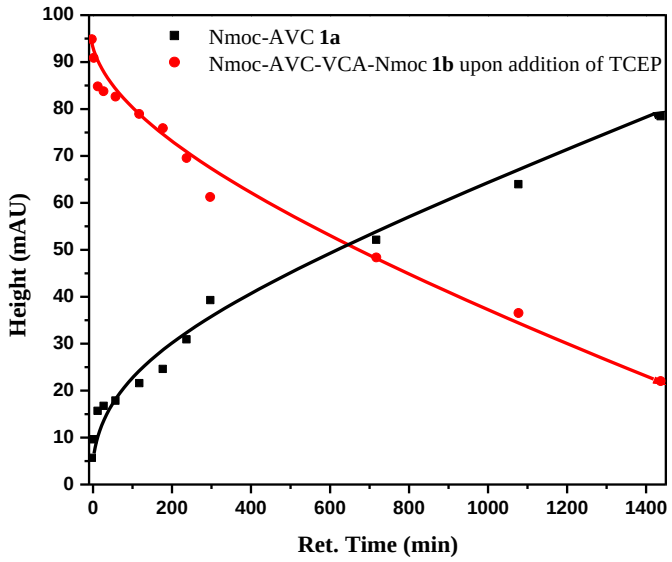


Fig. S4 HPLC analysis for product **1a** formation as function of time upon addition of TCEP 40 mmol/L to **1b.**

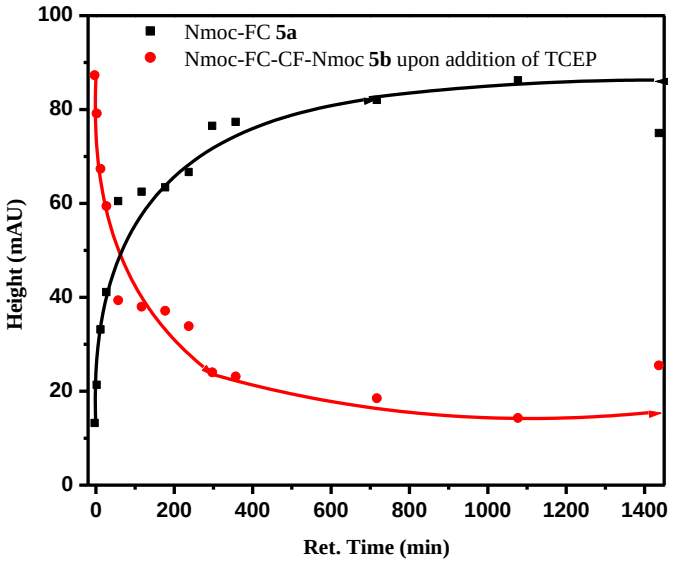


Fig. S5 HPLC analysis for product **5a** formation as function of time upon addition of TCEP 40 mmol/L to **5b.**

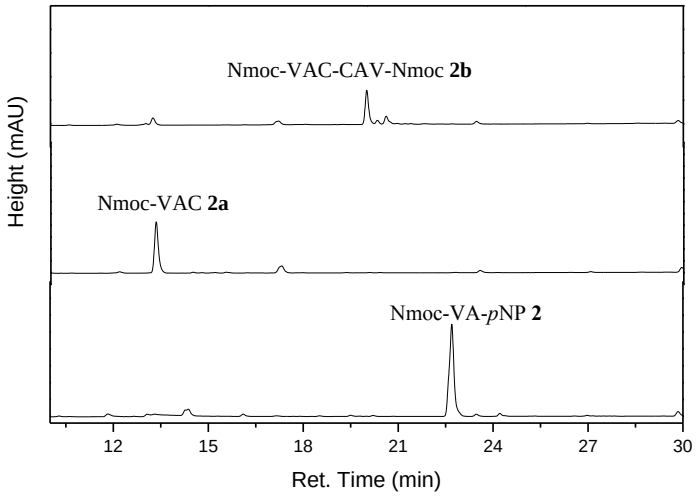


Fig. S6 Overlaid HPLC chromatographs for compound **2** showing peak **2a** after native chemical ligation and peak **2b** for its corresponding disulfide.

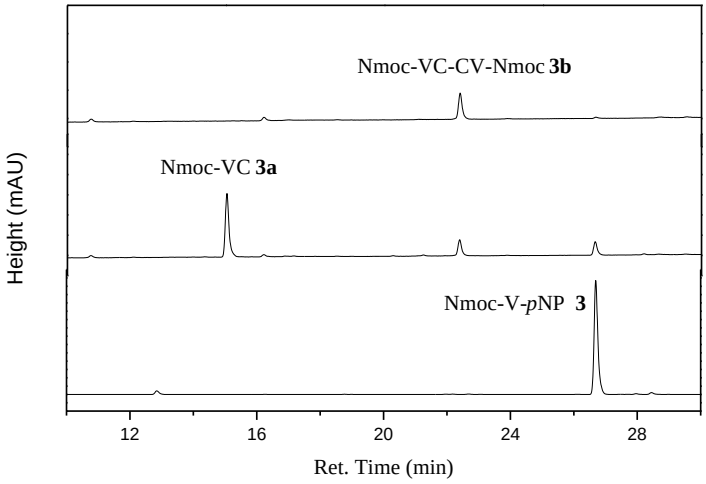


Fig. S7 Overlaid HPLC chromatographs for compound **3** showing peak **3a** after native chemical ligation and peak **3b** for its corresponding disulfide.

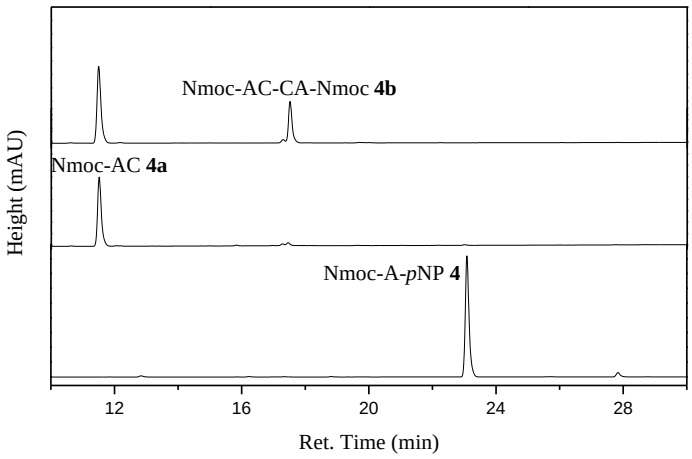


Fig. S8 Overlaid HPLC chromatographs for compound **4** showing peak **4a** after native chemical ligation and peak **4b** for its corresponding disulfide.

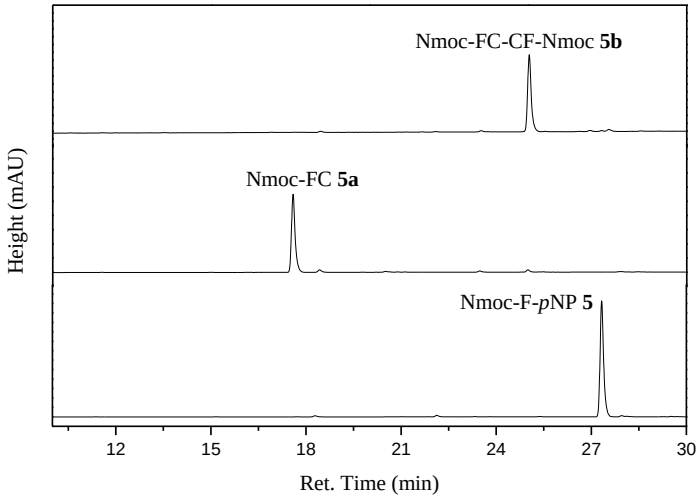


Fig. S9 Overlaid HPLC chromatographs for compound **5** showing peak **5a** after native chemical ligation and peak **5b** for its corresponding disulfide.

ESI-MS Analysis for Ligated products

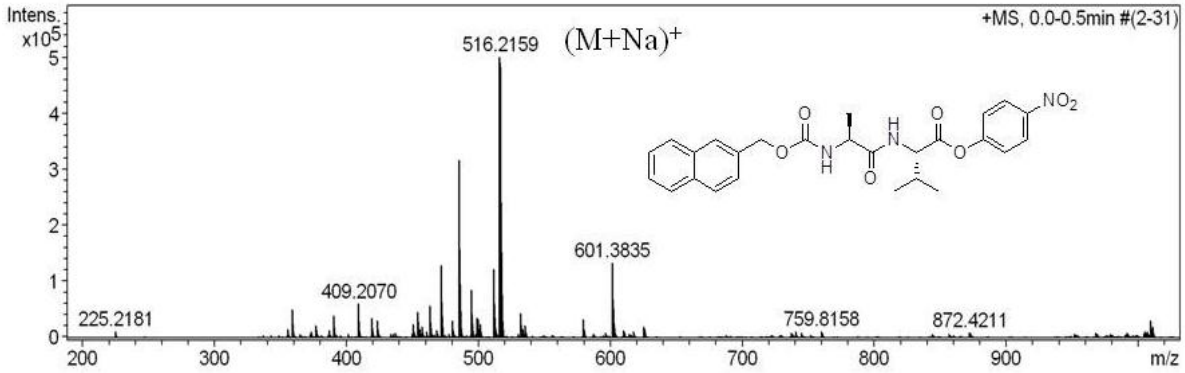


Fig. S10 ESI-MS spectra for compound **1**.

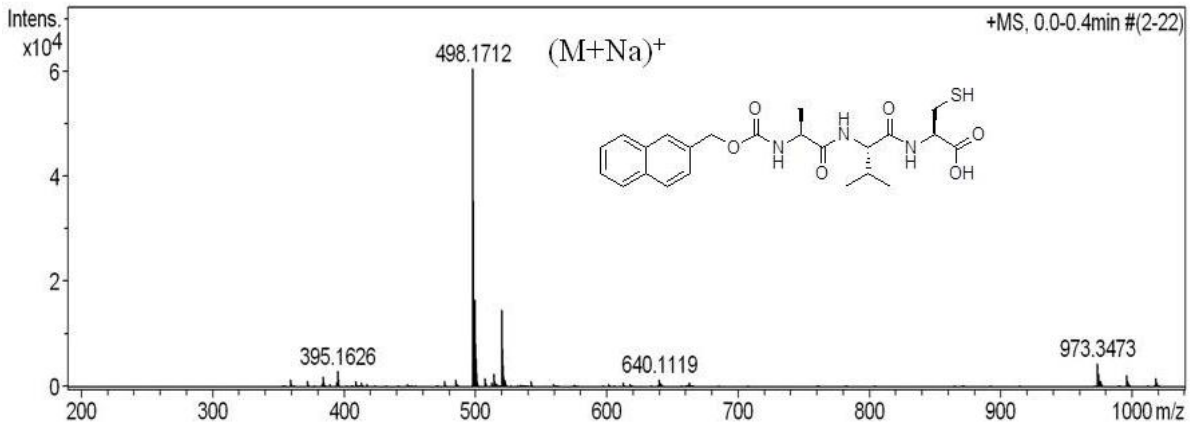


Fig. S11 ESI-MS spectra for compound **1a**.

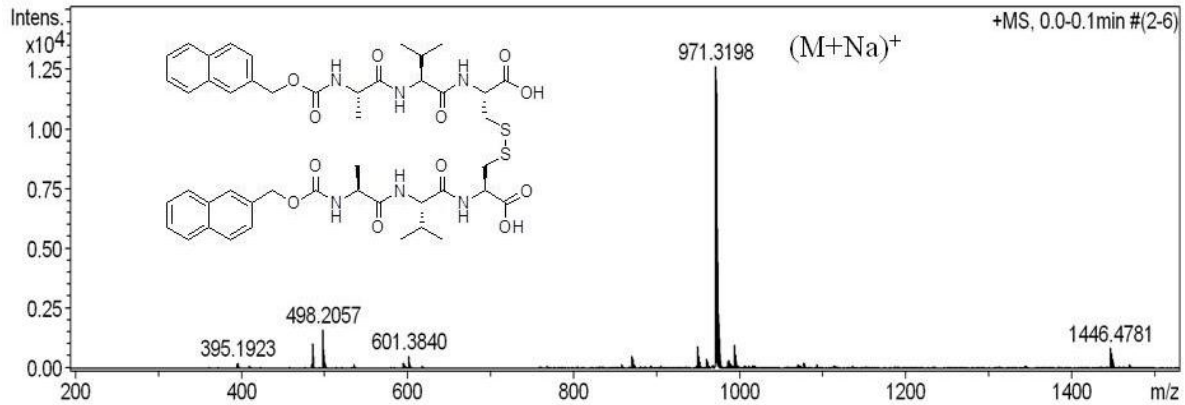


Fig. S12 ESI-MS spectra for compound **1b**.

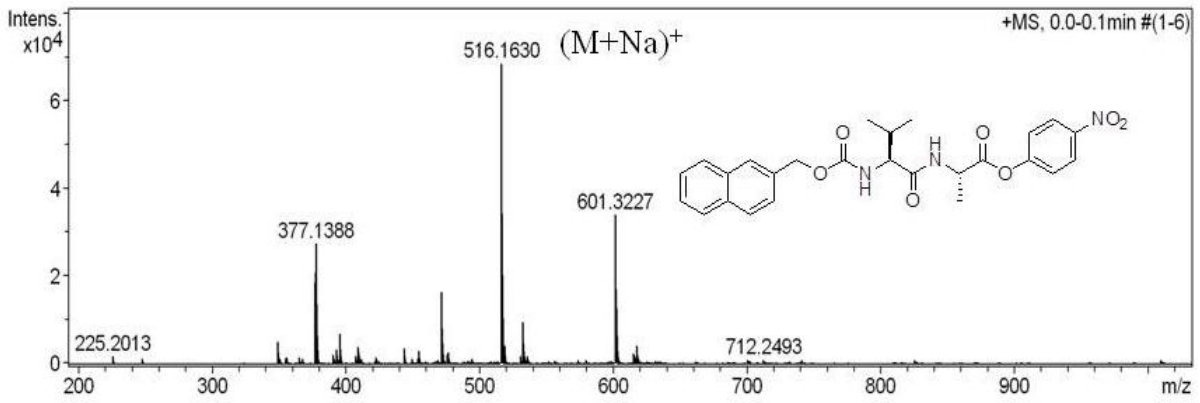


Fig. S13 ESI-MS spectra for compound **2**.

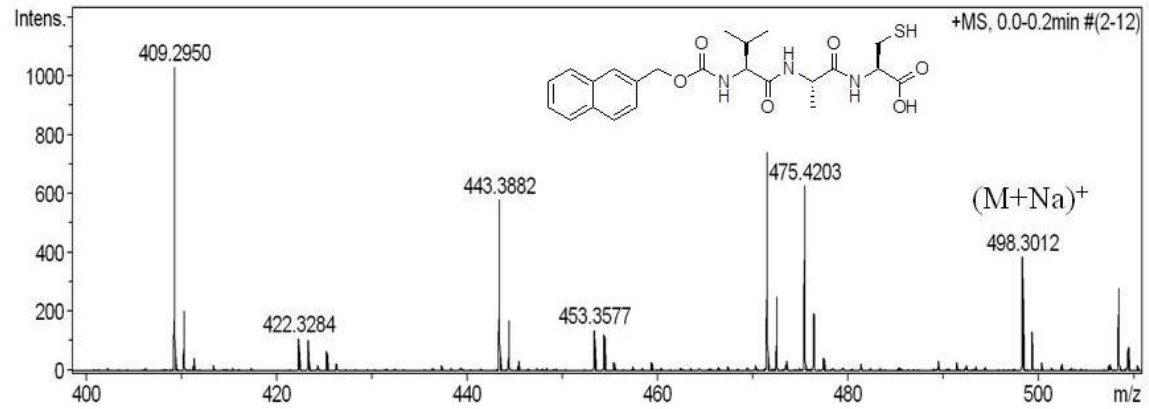


Fig. S14 ESI-MS spectra for compound **2a**.

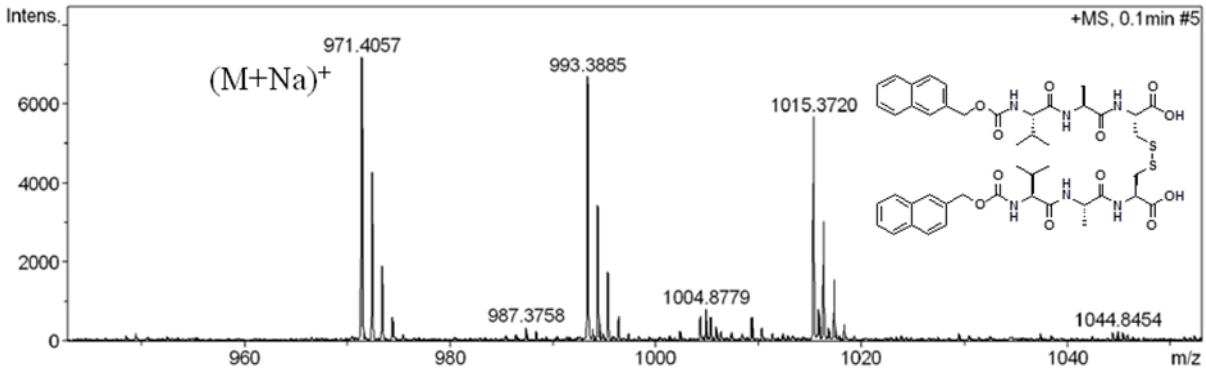


Fig. S15 ESI-MS spectra for compound **2b**.

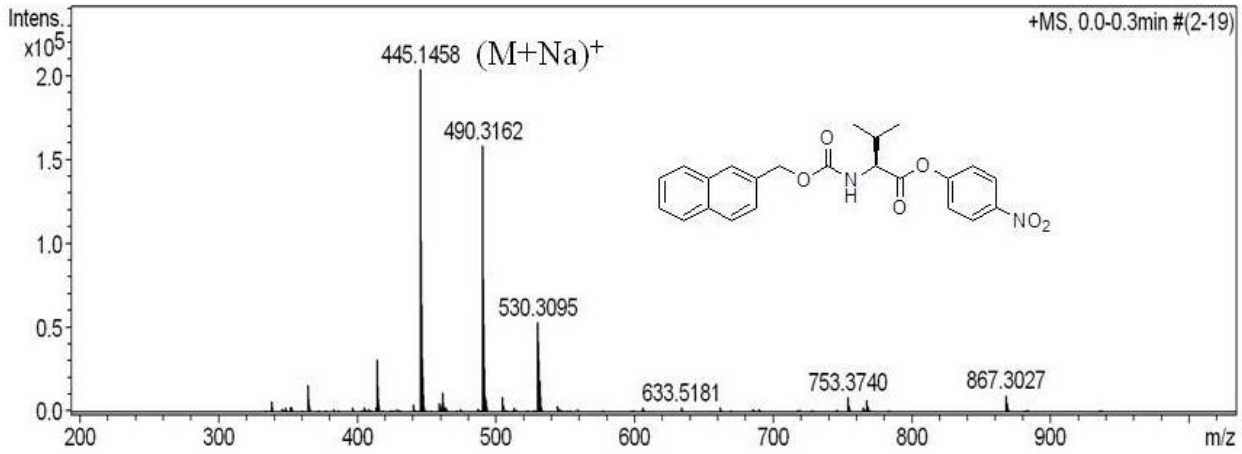


Fig. S16 ESI-MS spectra for compound **3**.

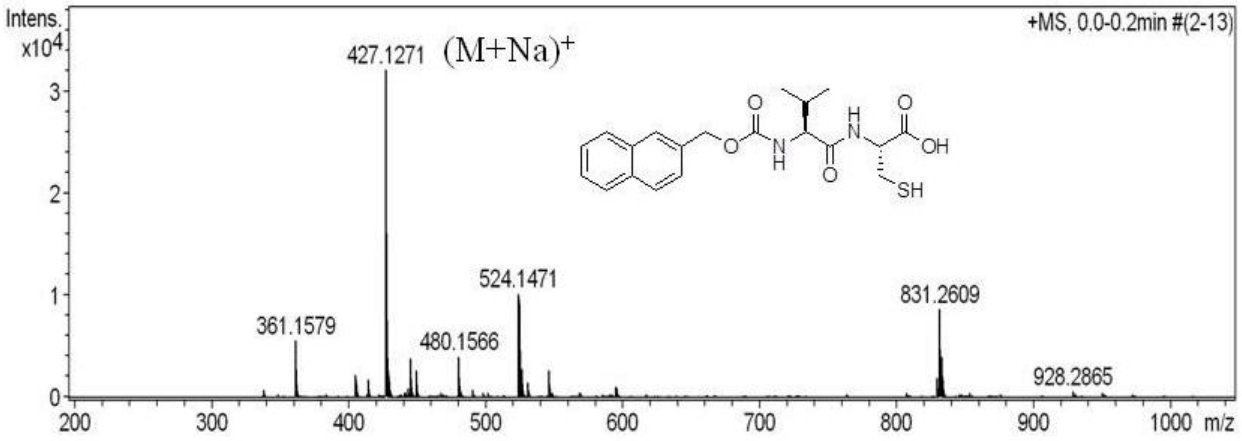


Fig. S17 ESI-MS spectra for compound **3a**.

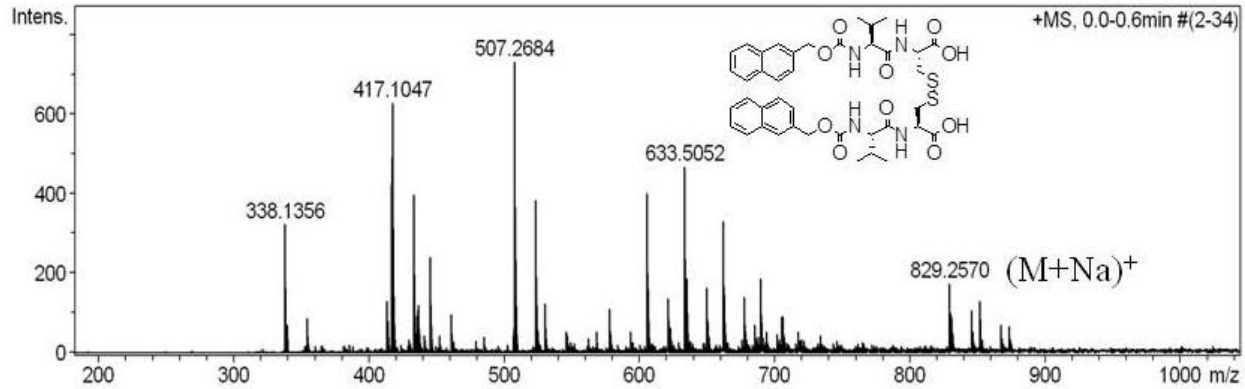


Fig. S18 ESI-MS spectra for compound **3b**.

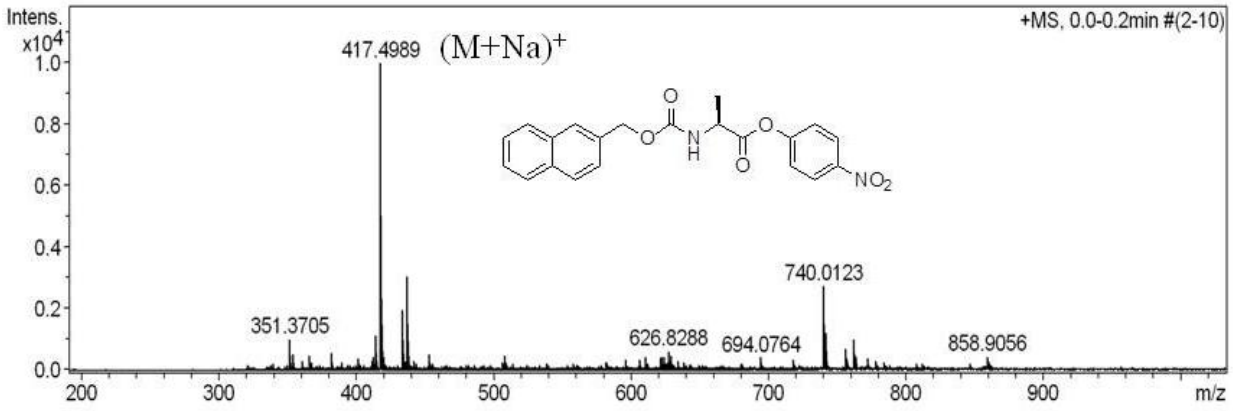


Fig. S19 ESI-MS spectra for compound **4**.

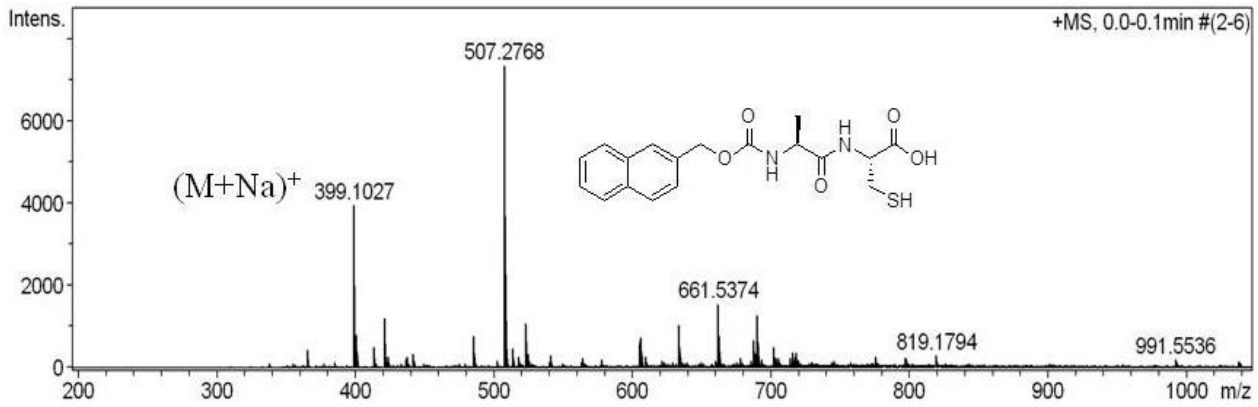


Fig. S20 ESI-MS spectra for compound **4a**.

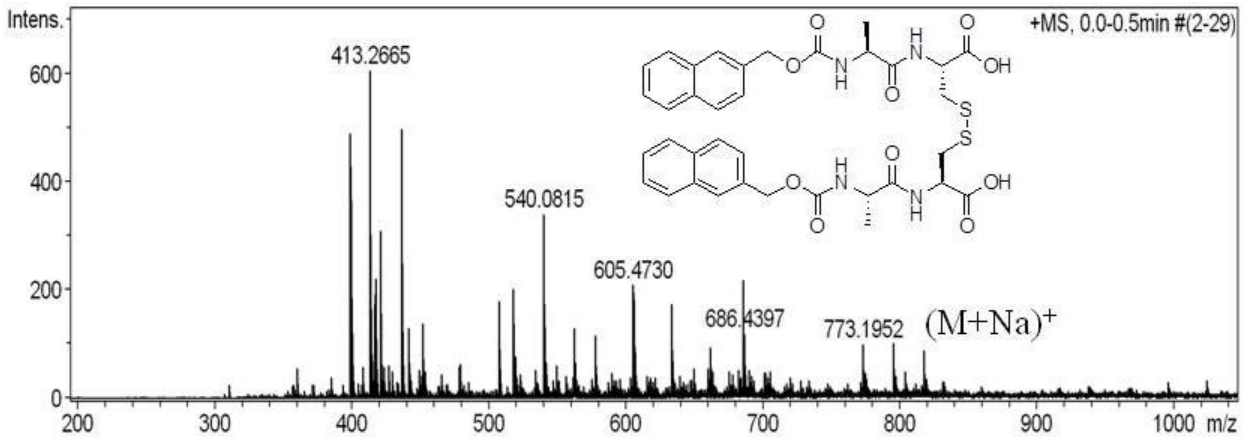


Fig. S21 ESI-MS spectra for compound **4b**.

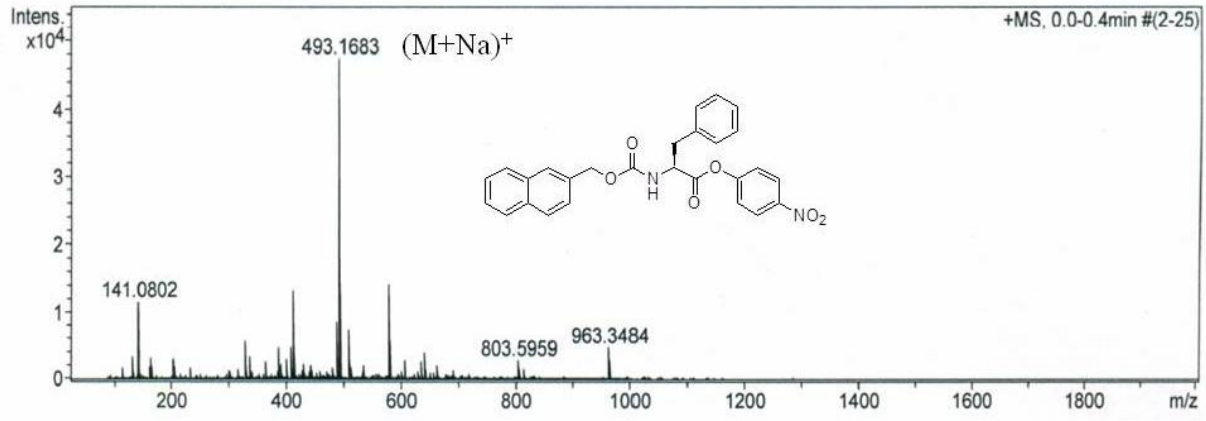


Fig. S22 ESI-MS spectra for compound **5**.

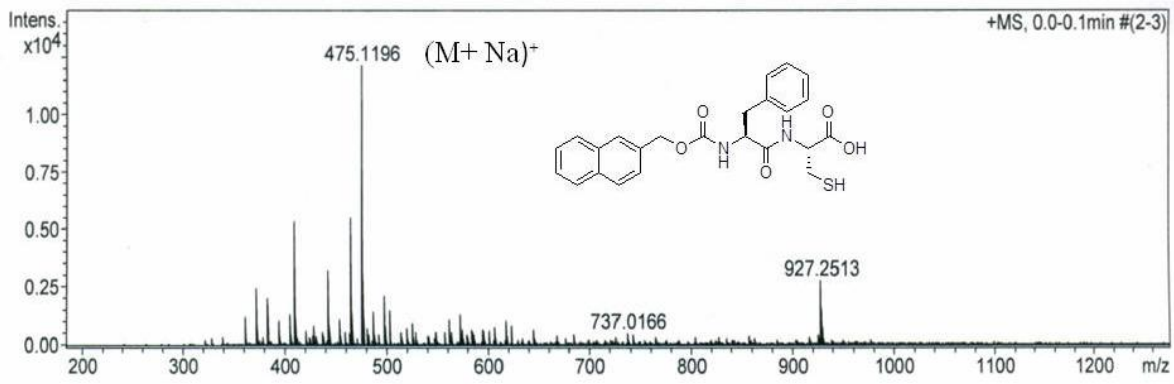


Fig. S23 ESI-MS spectra for compound **5a**.

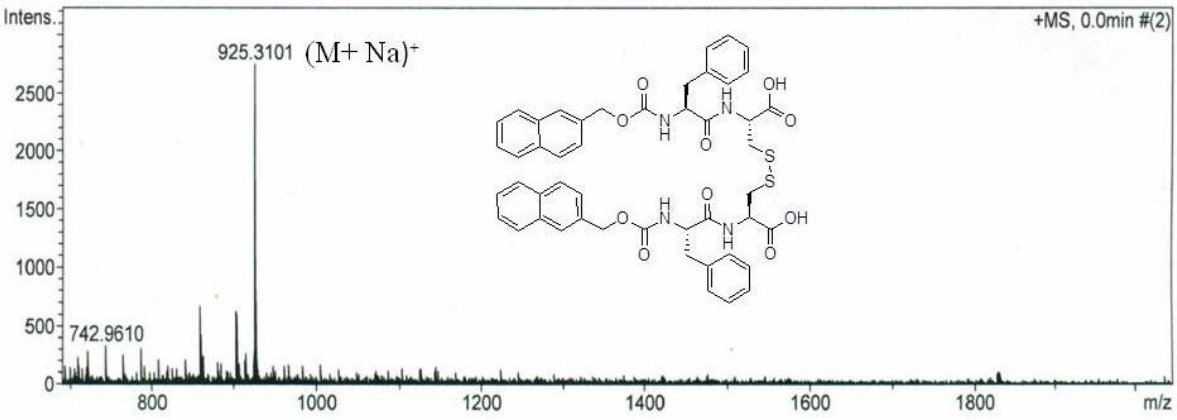


Fig. S24 ESI-MS spectra for compound **5b**.

Rheology

Rheological measurement was carried out using an Anton Paar Physica MCR 301 rheometer with parallel plate of geometry (25 mm in diameter, 0.200 µm gap). 200 µl of **1b** and **5b** gels were prepared in glass vials and transfered onto the plate of the instrument using microspatulla. The temperature was kept at 25^0 C by using an integrated temperature controller. Then dynamic frequency sweep of the gel Nmoc-AVC- CVA-Nmoc and Nmoc-FC-CF-Nmoc were measured as function of frequency in the range of 0.05-100 rad s^{-1} with constant strain value 0.05%. The time sweep was measured at constant strain of 0.05%. To determine the exact strain for frequency sweep and time sweep experiments the linear viscoelastic (LVE) regime were performed at constant frequency of 10 rad s^{-1}. The stiffness of gel determined when the value storage modulus G' exceed over the loss modulus G''. The value of G' for gel **1b** and **5b** is almost 6 times higher than the value G'' which indicates the formation of strong gel. The Kinetcs of gel formation of Nmoc-AVC-CVA-Nmoc is carried out by rheology. A solution of Nmoc-AVC (immediately after NCL reaction) was poured onto the instrument plate and time sweep measurement was done at constant strain of 0.05%. The gelation point was observed after 32 min. where the G' (storage modulus) and G'' (loss moduls) start to separate and G' rises above the G''. Nmoc-AVC converts to Nmoc-AVC-CVA-Nmoc **1b** that results the gel formation. However, time sweep measurement of gel Nmoc-FC-CF-Nmoc **5b** shows gelation point at 22 min. Similarly, a solution of Nmoc-FC (immediately after NCL reaction) poured on instrument plate and time sweep measurement was done for gel **5b**. Kinetics of the gel reversal was performed by adding Tris(2-carboxyethyl)phosphine (TCEP) (40 mmol/L, 10.6 µl) to a gel formed by **1b** (20 mmol/L) and **5b** (20 mmol/L). The gels were transferred onto plate of rheometer by using micro-spatula immediately after addition of TCEP. The time sweep measurement was done at constant strain 0.05%. As time sweep experiment shows the gel **1b** start to break at 110 min, where the G' (storage modulus) and G'' (loss modulus) start to intermix with each other. Similarly, gel **5b** shows G' higher than G'' then after 20 min it start to break and G' and G'' goes down and finely after 80 min G' and G'' intermix with each other (figure S30 and S31).

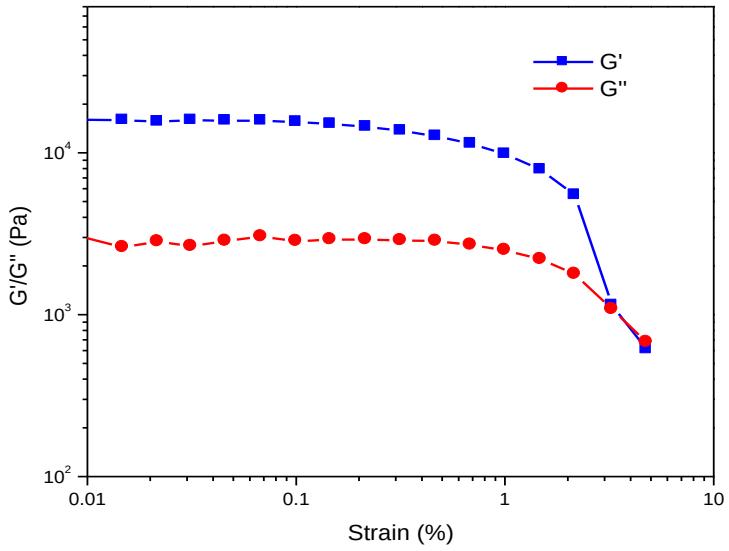


Fig. S25 Rheological measurement of LVE at constant frequency 10 rad s^{-1} for gel **1b**.

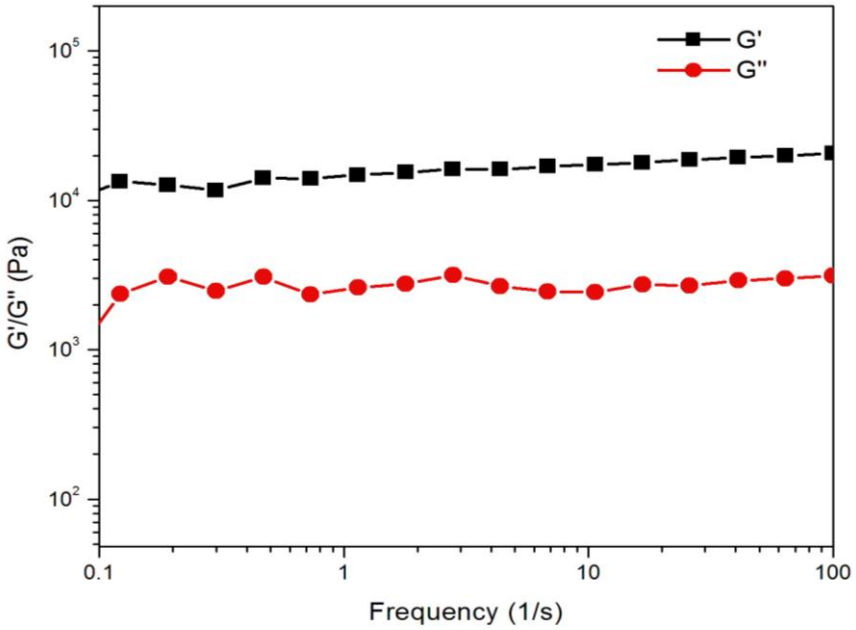


Fig. S26 Dynamic frequency sweep of Nmoc-AVC-CVA-Nmoc **1b** gel at contant strain 0.05%.

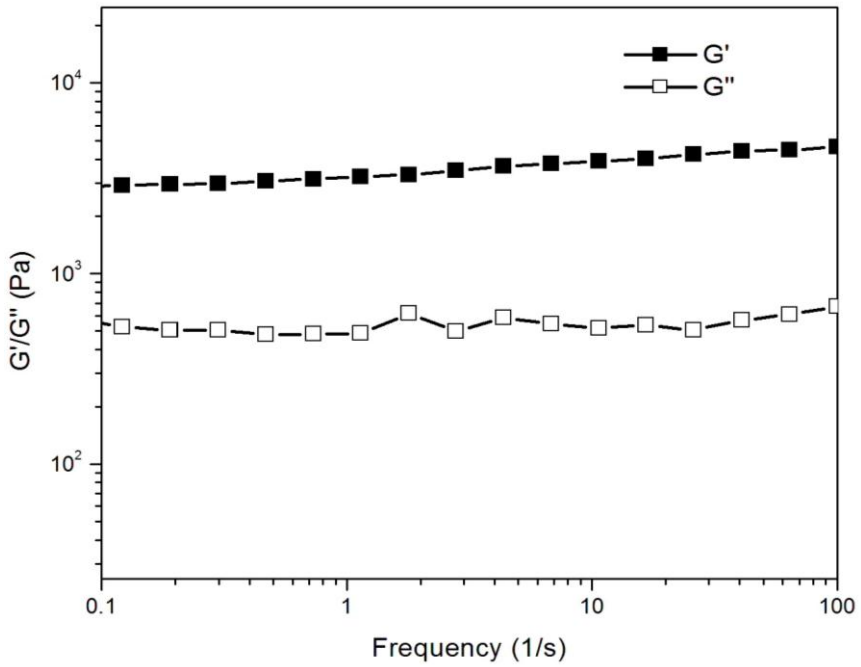


Fig. S27 Dynamic frequency sweep of Nmoc-FC-CF-Nmoc **5b** gel at contant strain 0.05%.

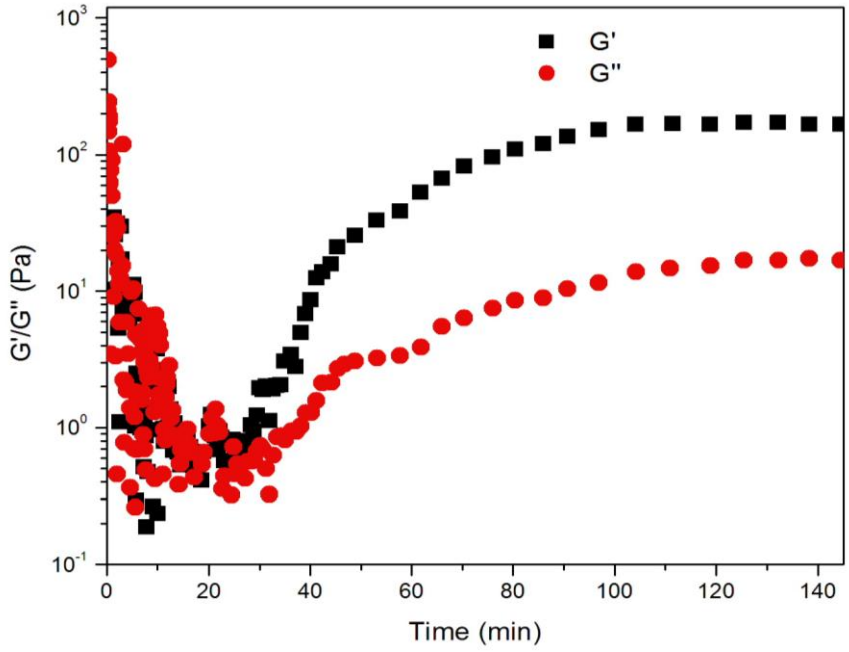


Fig. S28 Oscillatory rheology of a solution containing 20 mmol of **1b** at 25^0 C.

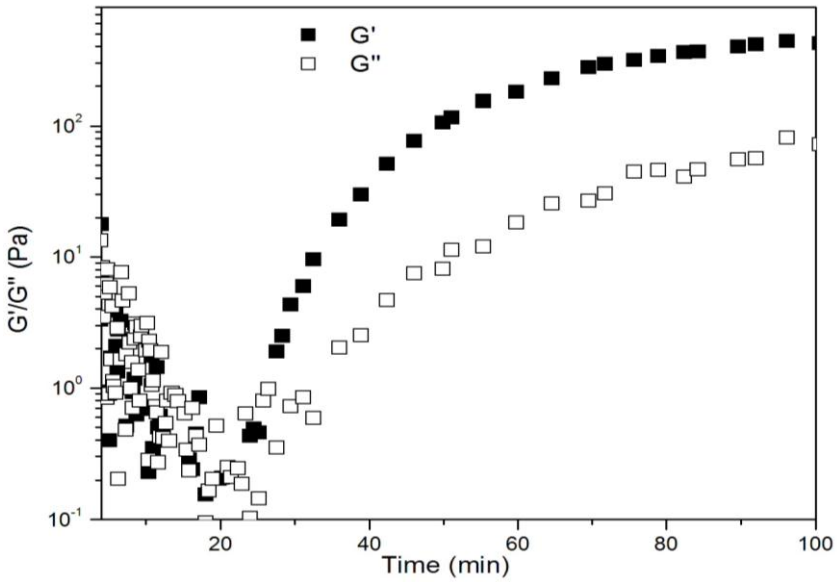


Fig. S29 Oscillatory rheology of a solution containing 20 mmol of **5b** at 25^0 C.

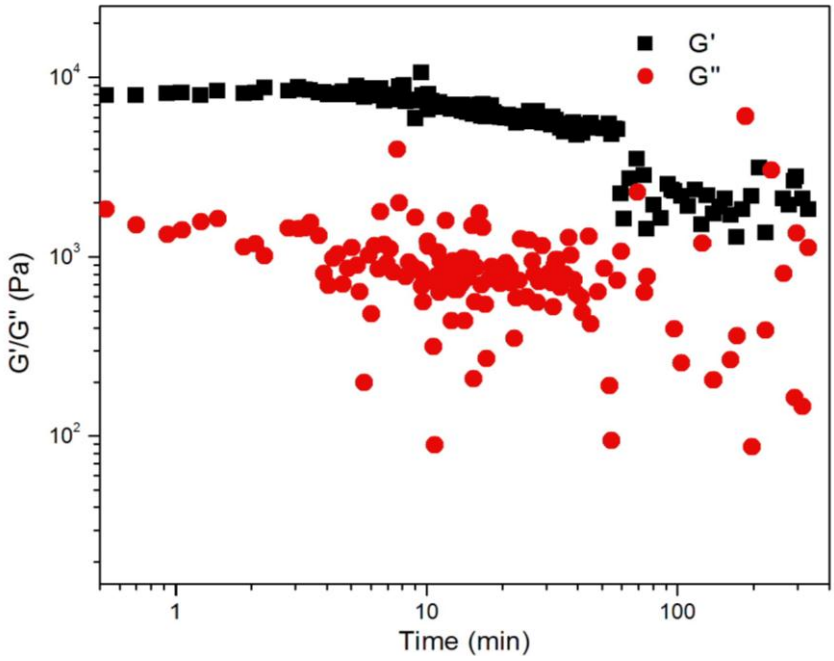


Fig. S30 Oscillatory rheology of a gel containing 20 mmol of **1b** and solution of TCEP 40 mmol at 25^0 C.

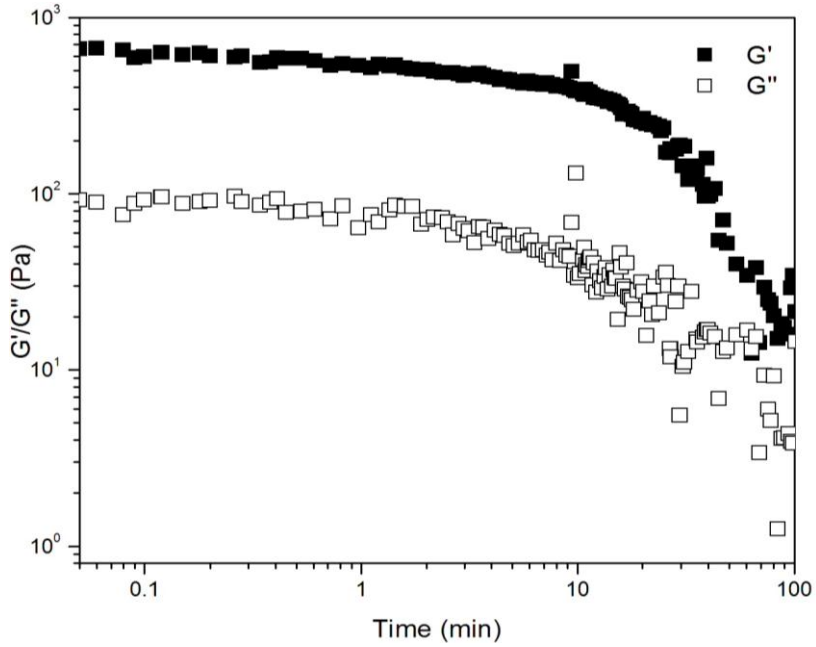


Fig. S31 Oscillatory rheology of a gel containing 20 mmol of **5b** and solution of TCEP 40 mmol at 25^0 C.

FT-IR Study

Fourier transform infrared (FTIR) spectra were recorded using a bruker (Tensor 27) FTIR spectrophotometer for wet gel material by using ZnSe windows. The gel samples were placed between crystal Zn-Se windows and scanned between 400 and 4000 cm^{-1} over 64 scans at a resolution of 4 cm^{-1} and an interval of 1 cm^{-1}.

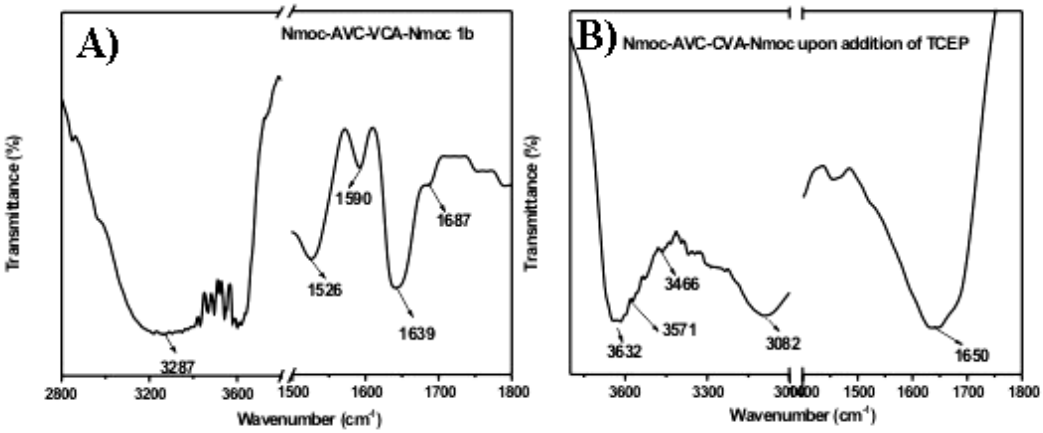


Fig. S32 FT-IR spectra of **A)** Nmoc-AVC-CVA-Nmoc **1b** gel and **B)** a solution of Nmoc-AVC formed after addition of TCEP.

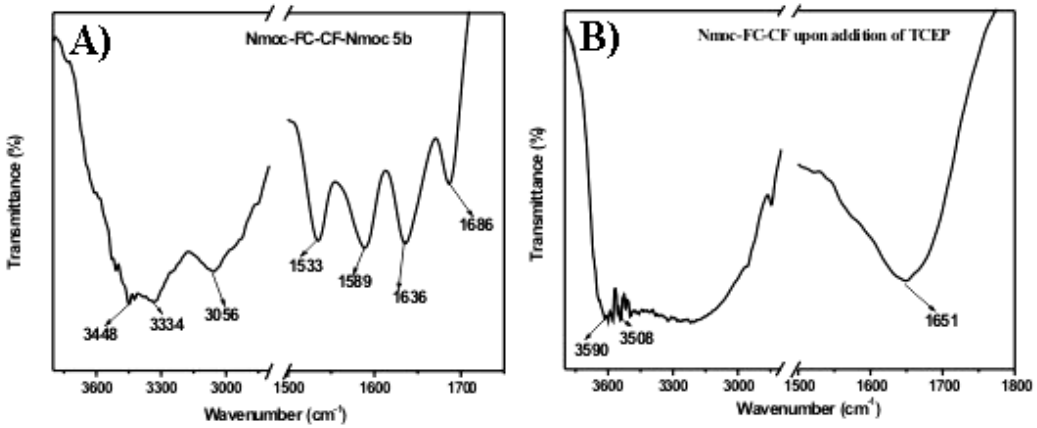


Fig. S33 FT-IR spectra of **A)** Nmoc-FC-CF-Nmoc **5b** gel and **B)** a solution of Nmoc-FC formed after addition of TCEP.

Circular dichroism

Circular dichroism (CD) spectra were measured at 25^0C on a Jasco J-815 spectropolarimeter. Spectra were measured between 300 and 190 nm with a data pitch of 0.1 nm. The bandwidth was set to 1 nm with a scanning speed of 20 nm min^{-1} and a response time of 1 s. The path length was 1 mm quartz cell (Starna Scientific Ltd. Hainault, UK). Samples were prepared at concentration of 20 mmol/L. Experimental data were acquired in thrice and the average data is shown.

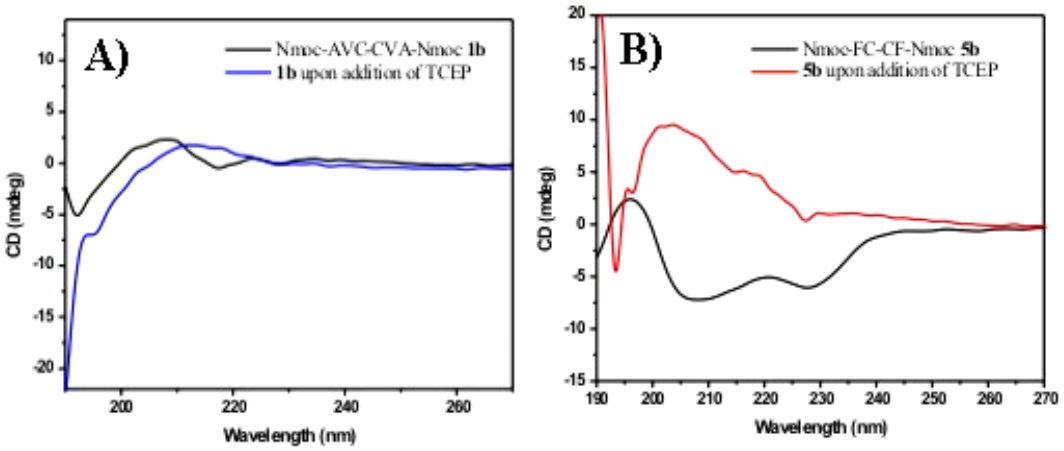


Fig. S34 CD spectra of **A)** Nmoc-AVC-AVC-Nmoc **1b** gel and Nmoc-AVC solution formed after addition of TCEP. **B)** Nmoc-FC-FC-Nmoc **5b** gel and Nmoc-FC solution formed after addition of TCEP.

Fluorescence Spectra

Fluorescence spectra of gel **1b** as well as solution **1** were recorded from 275–650 nm, exciting at 265 nm, using slit settings of 2 nm, with medium sensitivity on a Horiba Scientific Fluoromax-4 spectrophotometer. Samples were prepared in path length 1 cm quartz cuvettes at room temperature.

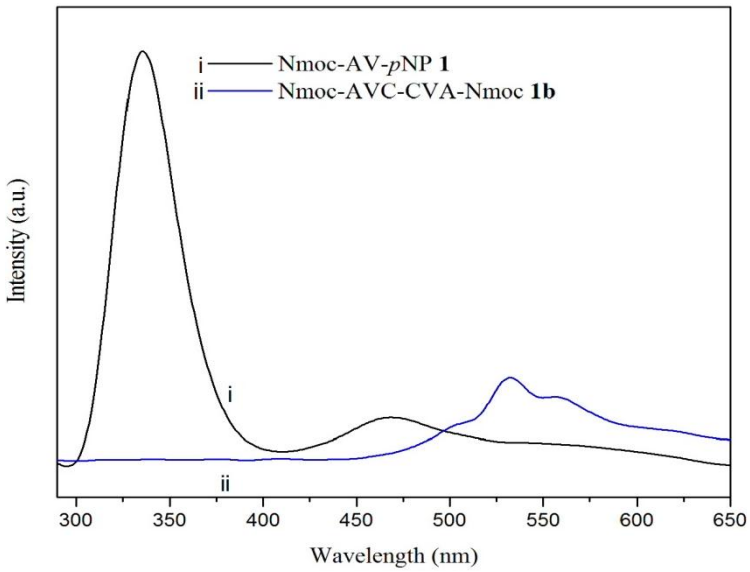


Fig. S35 Fluorescence emission spectra of Nmoc-AV-*p*NP **1** before and Nmoc-AVC-CVA-Nmoc **1b** gel after NCL reaction ($\lambda_{ex.}$= 265 nm).

Morphological study

Transmission electron microscopic images were taken using a PHILIPS electron microscope (model: CM 200), operated at an accelerating voltage of 200 kV. Dilute solution of the gel was dried on carbon-coated copper grids (300 mesh) by slow evaporation in air, then allowed to dry separately in a vacuum at room temperature.

Field-emission Gun-scanning electron microscopic study was done by using Jeol Scanning Microscope-JSM-7600F. The gel samples were dried on a glass cover slip and coated with platinum. Finally the morphology of gels were investigated using a tapping- mode atomic force microscope (AFM). AFM study was done by placing very dilute solution of gel (200 µl of gel was dissolved in 800 µl of milii-Q water) on mica and allowed it dry in air for 2 days at room temperature. Images were recorded by using scanning probe microscope AIST-NT instrument (model no. smart SPM-1000).

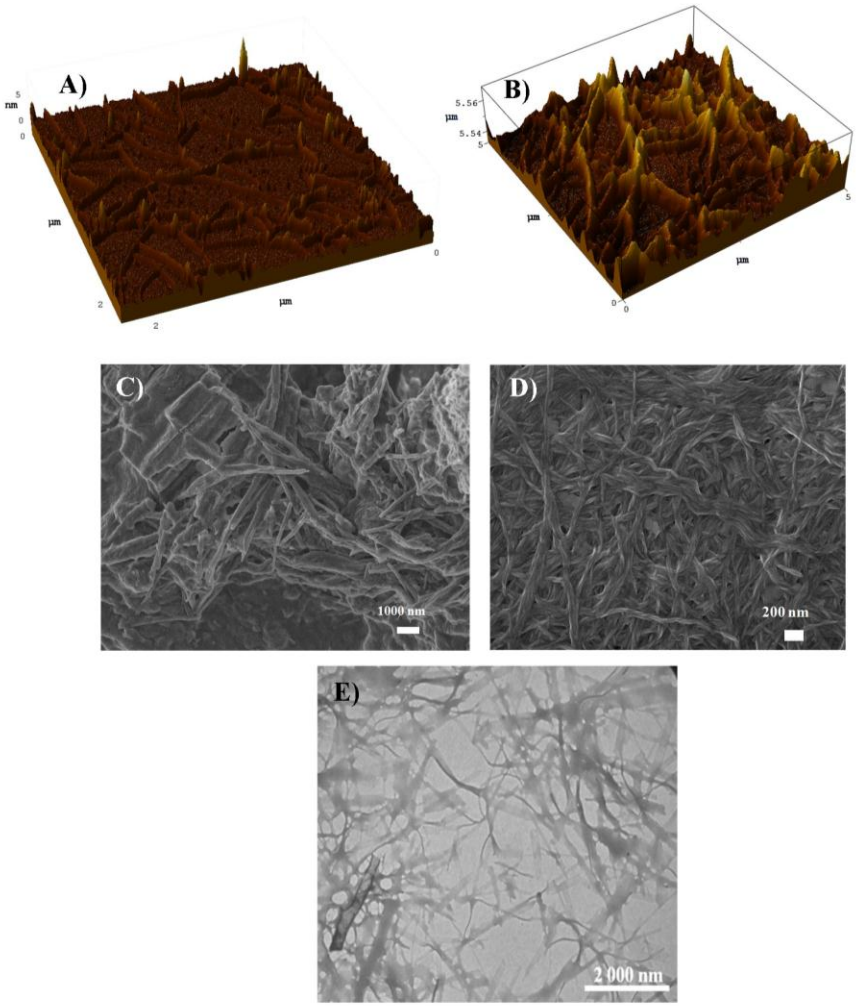
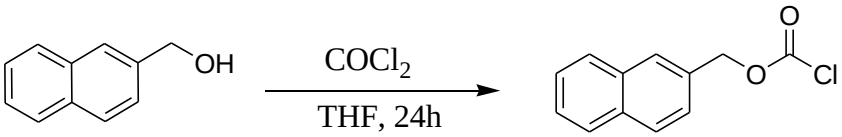


Fig. S36 A) and B) AFM height images for **1b** and **5b** gels. C) and D) SEM images for **1b** and **5b** gels. E) TEM image of **1b** gel.

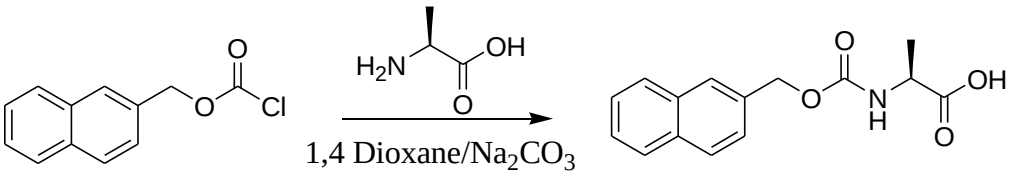
Synthetic Procedure

Synthesis of Naphthalene-2-methyloxy chloroformate 6



To a stirred solution of naphthalene methanol (5g, 31.6 mmol) in dry THF (143.3 ml), phosgene (39.2 ml, 75.5 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 to get crystalline product **6.** Yield = 6.8 g (30 mmol, 94.93 %). 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.

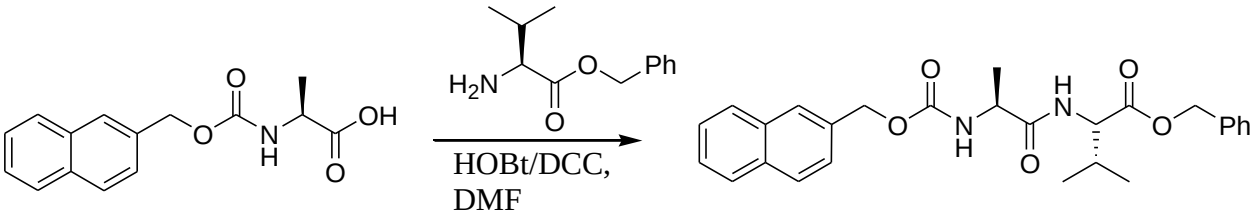
Synthesis of Nmoc-A-OH 7



A solution of L-alanine (0.445 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. Naphthalene-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 **7** as colorless oil. Yield= 0.890 mg (3.2 mmol, 64%)

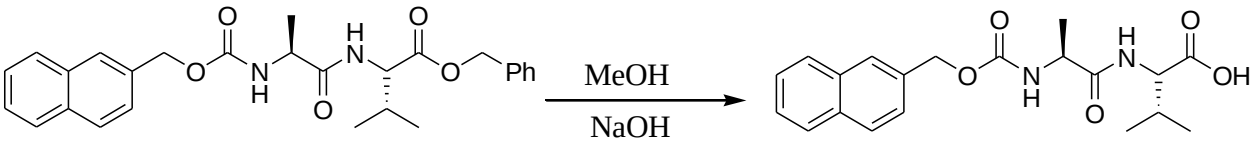
[α] $^{25}_D$ -13° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): 8.14, (d, *J* = 7.2 Hz, 1H, NH), 7.98 (t, 4H, Nph), 7.65 (d, *J* = 7.6 Hz, 1H), 7.53 (t, 2H, Nph), 5.15 (s, 2H), 4.09 (m, 1H, C^α of Ala), 1.34 (d, *J*=7.6 Hz, 3H, C^β of Ala). HRMS (ESI) m/z for $C_{15}H_{15}NO_4$ $(M+Na)^+$ calcd.: 296.0893, found: 296.0911. Elemental Analysis calculated for $C_{15}H_{15}NO_4$: C, 65.95; H, 5.53; N, 5.13; Found: C, 65.82; H, 5.52; N, 5.11.

Synthesis of Nmoc-AV-OBn 8



A solution of Nmoc-Ala-OH (3.1 mmol, 0.846 g) and HOBt (3.1 mmol, 0.418 g) was stirred in 2 ml of DMF. A neutralized solution of valine benzyl ester (6.2 mmol, 2.349 g) was extracted from its corresponding *p*-toluene sulfonate salt and concentrated to add to the reaction mixture followed by dicyclohexylcarbodiimde (3.2 mmol, 0.659 g) 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 **8** as white powder. Yield = 1.219 g (2.6 mmol, 83.87 %).
[α] $^{25}_D$ -30° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): 7.83 (d, 4H, Nph), 7.51 (m, 3H, Nph), 7.37 (m,5H, ph), 5.29 (s, 2H), 4.73 (q, 2H), 4.61 (m, 1H, C^α of Ala), 4.31 (t,1H, C^α of Val), 2.20 (m, 1H, C^β of Val), 1.41 (d, 3H, C^β of Ala), 0.88 (d, *J*=6.8 Hz, 6H, C^γ of Val). HRMS (ESI) m/z for $C_{27}H_{30}N_2O_5$ $(M+Na)^+$ calcd.: 485.2047, found: 485.2123. Elemental Analysis calculated for $C_{27}H_{30}N_2O_5$: C, 70.11; H, 6.54; N, 6.06; Found: C, 70.14; H, 6.51; N, 6.04.

Synthesis of Nmoc-AV-OH 9



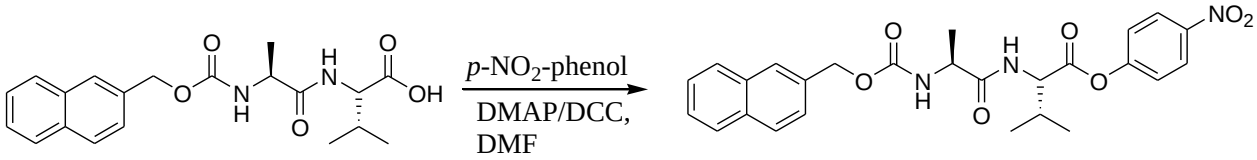
A solution of Nmoc-Ala-Val-OBn (1.180 g, 2.5 mmol) in 20 ml of dry MeOH was allowed to react with a solution of 10 ml 2M NaOH solution. The progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred upto 10 h. Then, methanol

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 adjusted 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 **9** as white powder and used further without purification.

Yield = 0.716 g (1.9 mmol, 76 %).

[α] $^{25}_{D}$ -14° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): 7.98 (t, 4H, Nph), 7.48 (d, *J* = 6.8 Hz, 3H), 7.92 (d, 1H, NH), 7.51 (d, 1H, NH), 5.19 (s, 2H), 4.18 (m, 1H, C^{α} of Ala), 4.76 (t, 1H, C^{α} of Val), 2.06 (m, 1H, C^{β} of Val), 1.23(d, *J* = 7.2 Hz, 3H, C^{β} of Ala), 1.1 (d, 6H C^{β} of Val). HRMS (ESI) m/z for $C_{20}H_{24}N_2O_5$ $(M+Na)^+$ calcd.: 395.1577, found: 395.1821. Elemental Analysis calculated for $C_{20}H_{24}N_2O_5$: C, 64.50; H, 6.50; N, 7.52; Found: C, 64.74; H, 6.53; N, 7.51.

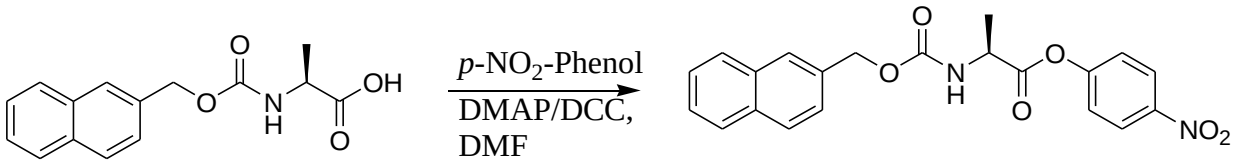
Synthesis of Nmpc-AV-*p*-nitrophenol 1



A solution of Nmoc-Ala-Val-OH (0.690 g, 1.8 mmol) and 4-dimethyl amino pyridine (DMAP) (10 Mol %, 21.9 mg) was stirred in 2 ml of DMF. A *p*-Nitrophenol (0.264 g, 1.9 mmol) was added to the reaction mixture followed by DCC (2 mmol, 0.412 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 0.1M HCL (2 x 30 ml), 1M $NaHCO_3$ (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **1** as yellow solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent. Yield= 751 g (1.5 mmol, 83.33 %).

[α] $^{25}_{D}$ -12° (*c* = 1, MeOH); FT-IR (KBr): ῡ = 3306(s), 3062 (m), 2965 (m), 1765 (s), 1694 (w), 1654 (m), 1525(s), 1451(w), 1344 (s), 1245(s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 8.23 (d, 1H, NH), 8.13 (d, 1H NH), 7.82 (d, 4H, NO_2-Ph), 7.51 (d, 2H, Nph), 7.45 (t,2H, Nph), 7.28 (s, 1H, Nph), 7.24 (d, 2H, Nph), 5.31 (s, 2H), 4.72 (m, 1H, C^{α} of Val), 4.39 (m, 1H, C^{α} of Ala), 2.33 (m, 1H, C^{β} of Val), 1.45 (d, 3H, C^{β} of Ala), 0.99 (d,6H, C^{γ} of Val).^{13}C NMR (400 MHz, $CDCl_3$) δ 172.87, 169.59, 156.33, 154.95, 145.56, 133.16, 128.40, 126.34, 125.27, 122.27, 115.65, 67.42, 57.55, 50.52, 30.91, 19.05, 17.76. HRMS (ESI) m/z for $C_{26}H_{27}N_3O_7$ $(M+Na)^+$ calcd.: 516.1741, found: 516.2159. Elemental Analysis calculated for $C_{26}H_{27}N_3O_7$: C, 63.28; H, 5.51; N, 8.51; Found: C, 63.42; H, 5.53; N, 8.55.

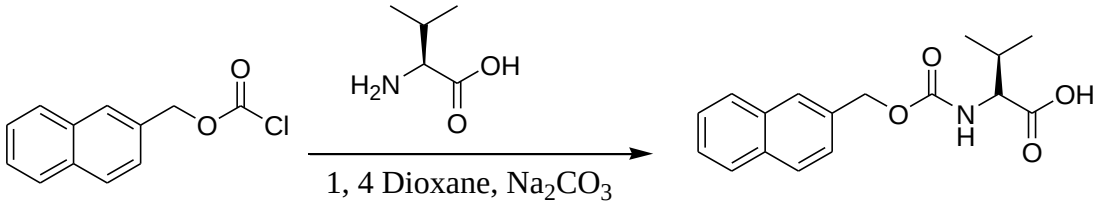
Synthesis of Nmoc-A-*p*-nitrophenol 4



A solution of Nmoc-Ala-OH (0.529 g, 1.9 mmol) and 4-Dimethyl amino pyridine (DMAP) (5 Mol %, 11.5 mg) was stirred in 2 ml of DMF. A *p*-Nitrophenol (0.278 g, 2 mmol) was added to the reaction mixture followed by DCC (2 mmol, 0.412 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 0.1M HCL (2 x 30 ml), 1M $NaHCO_3$ (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **4** as yellow solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent. Yield = 0.618 g (1.5 mmol, 78.94%).

[α] $^{25}_{D}$ -17° (*c* = 1, MeOH); FT-IR (KBr): ῡ = 3381(s), 3056 (m), 2920 (m), 1763 (s), 1617 (m), 1592(m), 1513(m), 1345 (s), 1291(m) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 8.24 (d,1H, Nph), 8.16 (d, 1H, Nph), 7.85 (d, 4H, ph), 7.51(t, 2H, Nph), 7.28 (t, 2H, Nph), 6.91(d, 1H, Nph), 5.33 (s, 2H, CH_2), 4.65 (m, 1H C^{α} of Ala), 1.63 (d, 3H, C^{β} of Ala).^{13}C NMR (400 MHz, $CDCl_3$) δ 170.86, 155.80, 155.00, 145.54, 133.40, 133.14, 128.41, 127.96, 127.69, 126.36, 125.21, 122.18, 115.58, 67.45, 50.01, 18.02. HRMS (ESI) m/z for $C_{21}H_{18}N_2O_6$ $(M+Na)^+$ calcd.:417.1057, found: 417.4989. Elemental Analysis calculated for $C_{21}H_{18}N_2O_6$: C, 63.96; H, 4.60; N, 7.01; Found: C, 64.13; H, 4.62; N, 6.98.

Synthesis of Nmoc-V-OH 10

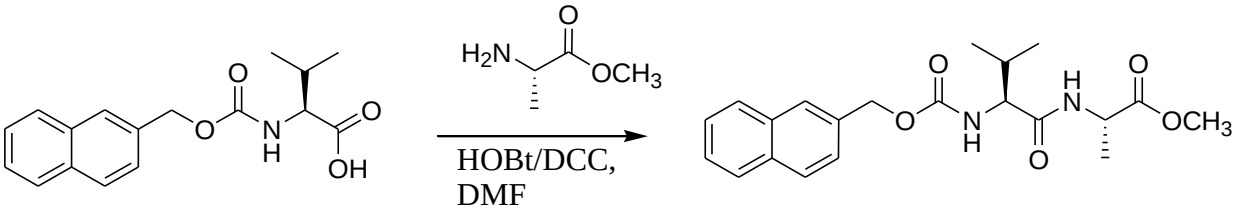


A solution of valine (0.468 g, 4 mmol) in a mixture of 1, 4 dioxane (10 ml) and 2M sodium carbonate (13 ml) was stirred and cooled in an ice-water bath. Naphthalene-2-methoxychloroformate (0.882 g, 4 mmol) was added and stirring was continued at room temperature for 12 h. Reaction mixture was diluted 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 concentrated in vacuo to give **10** as colorless oil. Yield=0.863 g (2.8 mmol, 70 %).

$[\alpha]^{25}_D$ -5° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): 7.90 (t, 4H, Nph), 7.54 (d, 3H, Nph), 5.22 (s, 2H, CH_2), 3.88 (t,1H, C^{α} of Val), 2.08 (m, 1H, C^{β} of Val), 0.92(d, 6H, C^{γ} of Val). HRMS (ESI) m/z for $C_{17}H_{19}NO_4$ $(M+Na)^+$ calcd.: 324.1206, found: 324.1228. Elemental Analysis calculated for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65; Found: C, 67.65; H, 6.38, N, 4.63.

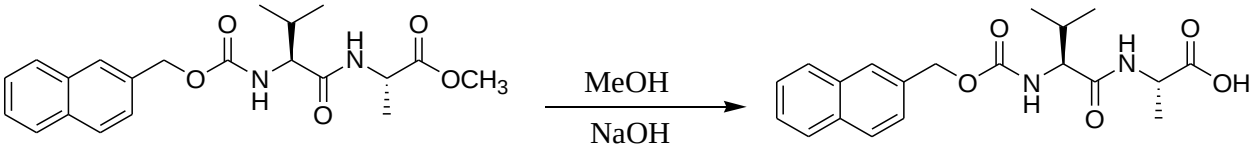
Synthesis of Nmoc-VA-methyl ester 11



A solution of Nmoc-Val-OH (0.602 g, 2.2 mmol) and HOBt (0.297 g, 2.2 mmol) was stirred in 2 ml of DMF. A neutralized solution of Alanine methyl ester (1.223 g, 8.8 mmol) was extracted from its corresponding hydrochloride salt and concentrated to add to the reaction mixture followed by dicyclohexylcarbodiimde (0.474 g, 2.2 mmol) 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 **11** as white powder. Yield= 0.676 g, (1.6 mmol, 72.72 %).

$[\alpha]^{25}_D$ -41° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): 7.84 (d, 5H, *J*=10.28 Hz, Nap), 7.46 (t, 2H, Nap), 6.40(d, 1H, *J*=8.28 Hz, NH), 5.4 (d, 1H, *J*=9.28, NH), 5.27 (s, CH_2), 4.57 (t, 1H, C^{α} of Val), 4.03 (m,1H, C^{α} of Ala), 3.73 (s, 3H, OCH_3), 1.6 (m, 1H, C^{β} of Val), 1.4 (d, 3H, *J*=7.8 Hz, C^{β} of ala), 0.97 (d, 6H, *J*=7.28 Hz). HRMS (ESI) m/z for $C_{21}H_{26}N_2O_5$ $(M+Na)^+$ calcd.: 409.1734, found: 409.1694. Elemental Analysis calculated for $C_{21}H_{26}N_2O_5$: C, 65.27; H, 6.78; N, 7.25; Found: C, 65.08; H, 6.37; N, 7.49.

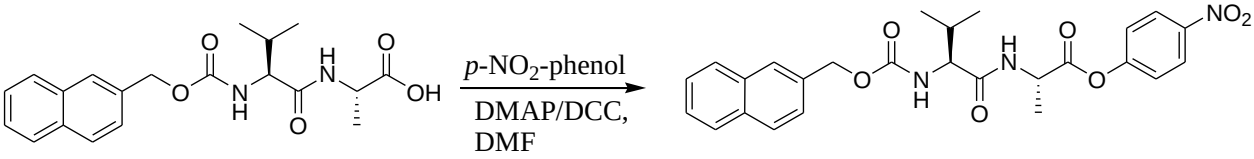
Synthesis of Nmoc-VA-OH 12



A solution of Nmoc-Val-Ala methyl ester (0.630 g, 1.5 mmol) in 20 ml of dry MeOH was allowed to react with a solution of 15 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 adjusted 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 **12** as white powder and used further without purification. Yield= 0.538 g (1.4 mmol, 93.33 %)

$[\alpha]$ $^{25}_{D}$ -36° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): 8.22 (d, 1H, *J*=7.76 Hz, Nph), 7.93 (t, 3H, Nph), 7.54 (t, 2H, Nph), 7.31(d, 1H, Nph), 5.20 (s, CH_2), 4.22 (t, 1H, C^{α} of Val), 3.92 (m, 1H, C^{α} of Ala), 1.91 (m, 1H, C^{β} of Val), 1.26 (d, 3H C^{β} of Ala), 0.91 (d, 6H, *J*=7.52 Hz). HRMS (ESI) m/z for $C_{20}H_{24}N_2O_5$ $(M+Na)^+$ calcd.: 395.1611, found: 395.1845. Elemental analysis calculated for $C_{20}H_{24}N_2O_5$: C, 64.50; H, 6.50; N, 7.52; Found: C, 64.36; H, 6.53; N, 7.53.

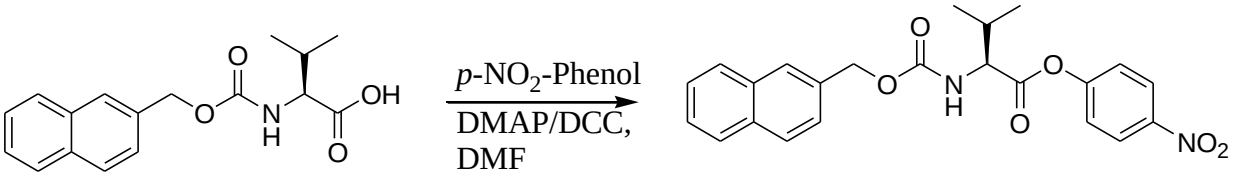
Synthesis of Nmoc-VA-*p*-nitrophenol 2



A solution of Nmoc-Val-Ala-OH (0.508 g, 1.3 mmol) and 4-Dimethyl amino pyridine (DMAP) (5 Mol %, 8 mg) was stirred in 2 ml of DMF. A *p*-Nitrophenol (0.217 g, 1.5 mmol) was added to the reaction mixture followed by DCC (0.288 g, 1.4 mmol) 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 0.1M HCL (2 x 30 ml), 1M $NaHCO_3$ (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **2** as yellow solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent. Yield = 0.545 g (1.1 mmol, 84.61 %).

[α] $^{25}_{D}$ -33° (*c* = 1, MeOH); FT-IR (KBr): ῡ = 3321(s), 3058 (w), 2930 (s), 2851(m), 1760 (s), 1688 (s), 1627 (s), 1528(s), 1450(m), 1381 (s), 1245(m) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, 1H, Nph), 7.83 (d, 4H, *J*= 4.76 Hz, Nph), 7.49 (d, 2H, *J*= 3.48 Hz, Nph), 5.27 (s, 2H), 4.10 (m, 1H, C^{α} of Ala), 3.48 (m, 1H, C^{α} of val), 1.9 (m, 1H, C^{β} of Val), 1.01 (d, 3H, C^{β} of Ala), 0.95 (d, 6H, C^{α} *J*=5.28 Hz, of val).^{13}C NMR (400 MHz, $CDCl_3$) δ 171.32, 170.44, 156.75, 155.03, 133.16, 128.38, 127.95, 127.12, 126.31, 125.66, 125.27, 122.22, 67.35, 49.18, 48.43, 33.92, 30.99, 25.58, 24.92, 19.13, 17.57. HRMS (ESI) m/z for $C_{26}H_{27}N_3O_7$ $(M+Na)^+$ calcd.: 516.1741, found: 516.1630. Elemental analysis calculated for $C_{26}H_{27}N_3O_7$: C, 63.28; H, 5.51; N, 8.51; Found: C, 63.55; H, 5.54; N, 8.50.

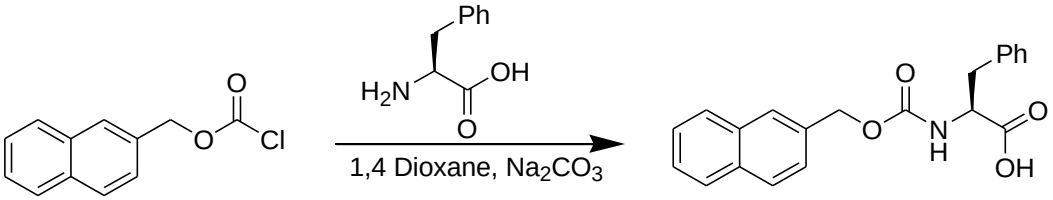
Synthesis of Nmoc-V-*p*-nitrophenol 3



A solution of Nmoc-Val-OH (0.820 g, 2.7 mmol) and 4-Dimethyl amino pyridine (DMAP) (5 Mol %, 15.8 mg) was stirred in 2 ml of DMF. A *p*-Nitrophenol (0.393 g, 2.83 mmol) was added to the reaction mixture followed by DCC (0.598 g, 2.9 mmol) 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 0.1M HCL (2 x 30 ml), 1M $NaHCO_3$ (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield **3** as yellow solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent. Yield= 0.832 g (1.9 mmol, 70.37 %).

[α] $^{25}_{D}$ -11° (*c* = 1, MeOH); FT-IR (KBr): ῡ = 3299(s), 3055 (m), 2966 (s), 2893(sm), 1762 (s), 1685 (w), 1614 (m), 1520(s), 1488(m), 1341 (s), 1245(w) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): 8.20 (d, 2H, ph-NO_2), 7.84 (d, 1H, Nph), 7.78(d, 3H,Nph), 7.45 (d, 3H, Nph), 7.32 (d, 2H, ph-NO_2), 5.39 (s, 2H), 4.68 (t, 1H, C^{α} of Val), 1.48 (m, 1H, C^{β} of Val), 1.18 (d, 6H, Val).^{13}C NMR (400 MHz, $CDCl_3$) δ 170.05, 156.36, 154.97, 145.56, 133.17, 128.43, 72.12, 67.53, 59.46, 31.67, 19.14, 17.76. HRMS (ESI) m/z for $C_{23}H_{22}N_2O_6$ $(M+Na)^+$ calcd.: 445.1370, found: 445.1458. Elemental Analysis calculated for $C_{23}H_{22}N_2O_6$: C, 65.39; H, 5.25; N, 6.63; Found: C, 65.59; H, 5.26; N, 6.60.

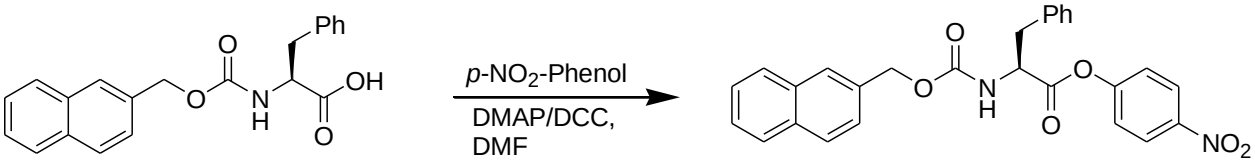
Synthesis of Nmoc-F 13



A solution of L-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 with Na_2SO_4 and concentrated in vacuo to give 1**3** as colorless oil. Yield= 1.46 g, (4.1 mmol, 82 %)

$[\alpha]^{25}_{D}$: -7° (*c* = 1, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6): 7.91 (d, 2H, *J*=9.01 Hz, Naph), 7. 81 (s, 1H, Naph), 7.73 (d, 1H, *J*=8.04Hz, Naph), 7.53 (t, 2H, Nph), 7.42 (d, 1H, *J*= 8.28 Hz, Naph), 7.27 (m, 5H, Phenyl ring), 6.53 (d, 1H, *J*= 7.52 Hz, NH) 5.16 (s, 2H), 4.21 (q, 1H, C^{α} of Phe), 3.11 (d, *J*=11.2 Hz, 1H, C^{β} of Phe), 2.86 (t, 1H, C^{β} of Phe); ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.2, 156.0, 137.8, 132.6, 129.0, 128.1, 127.8, 127.6, 127.5, 126.3,126.0, 125.5, 65.3, 55.2; HRMS (ESI) m/z for $C_{21}H_{19}NO_4$ $(M+Na)^+$ calcd.: 372.1212, found: 372.1206; Elemental Analysis calculated for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01; Found: C, 72.21; H, 5.53; N, 4.11.

Synthesis of Nmoc-F-*p*-nitrophenol 5



A solution of Nmoc-F-OH (1.25 g, 3.5 mmol) and 4-Dimethyl amino pyridine (DMAP) (10 Mol %, 42.7 mg) was stirred in 2 ml of DMF. A *p*-Nitrophenol (0.264 g, 1.9 mmol) was added to the reaction mixture followed by DCC (4.2 mmol, 0.583 g) at 0^0C. 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 0.1M HCL (2 x 30 ml), 1M $NaHCO_3$ (3 x 30 ml) and brine

solution. The ethyl acetate layer was dried over Na_2SO_4 and evaporated under vacuum to yield as yellow solid. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene as eluent Yield= 1.50 g (3.1 mmol, 85.57 %).

$[\alpha]^{25}_D$: -40° (*c* = 1, MeOH); FT-IR (KBr): ῡ = 3322 (s), 3057 (m), 2924 (m), 2857 (m), 1763 (s), 1686 (s), 1594 (s), 1510 (m), 1453 (m), 1347 (s), 1301 (m), 1252(s); ^{1}H NMR (400 MHz, $CDCl_3$): 8.17 (d, 1H, *J*= 8.28 Hz, *p*-NP), 8.08 (d, 1H, *J*= 8.76 Hz, *p*-NP), 7.79 (m, 5H, Phenyl ring), 7.49 (d, 2H, *J*= 3.52 Hz, Naph), 7.44 (d, 1H, *J*=8.52 Hz, Naph), 7.21 (d, 2H, *J*= 6.52 Hz, Naph), 7.03 (d, 2H, *J*= 8.28 Hz, Naph), 6.84 (d, 1H, *J*= 8.8 Hz, NH), 5.28 (s, 2H), 4.88 (t, 1H, C^{α} of Phe), 3.24 (d, 2H, C^{β} of Phe); ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.6, 161.9, 155.9, 154.7, 145.5, 135.0, 133.1, 129.2, 128.9, 127.9, 127.7, 126.3, 125.2, 122.1, 115.5, 67.5, 55.2, 38.0, 29.7, HRMS (ESI) m/z for $C_{27}H_{22}N_2O_6$ $(M+Na)^+$ calcd.: 493.1376, found: 493.1683. Elemental Analysis calculated for $C_{27}H_{22}N_2O_6$: C, 68.93; H, 4.71; N, 5.95; Found: C, 67.97; H, 4.84; N, 6.07.

NMR Spectra

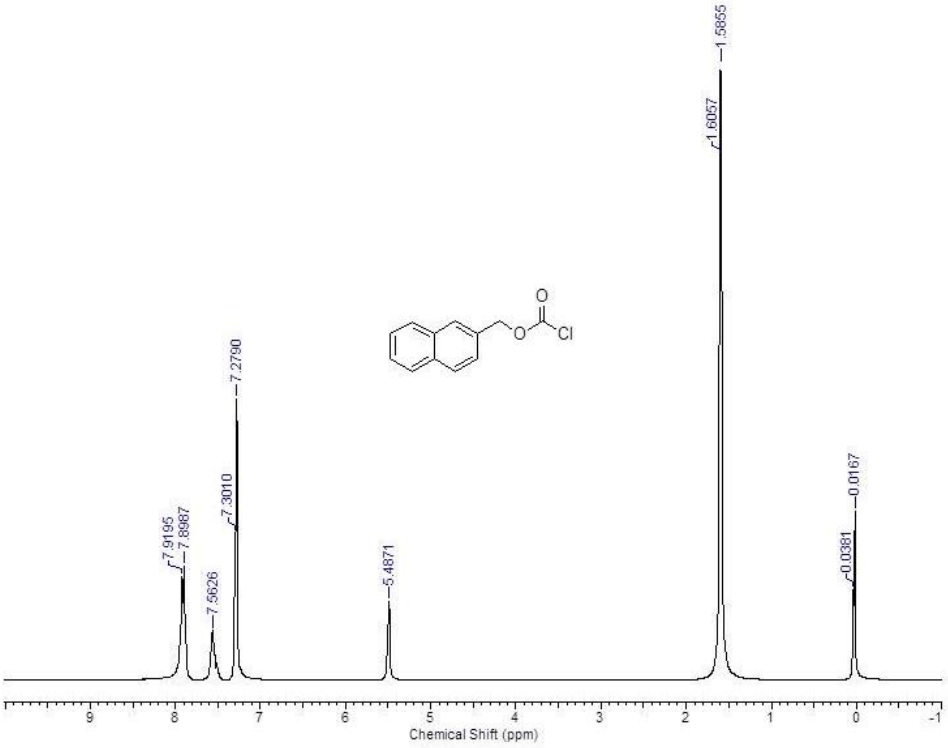


Fig. S37 ^{1}H NMR spectrum of 2-naphthalenemethoxychloroforamate **6**.

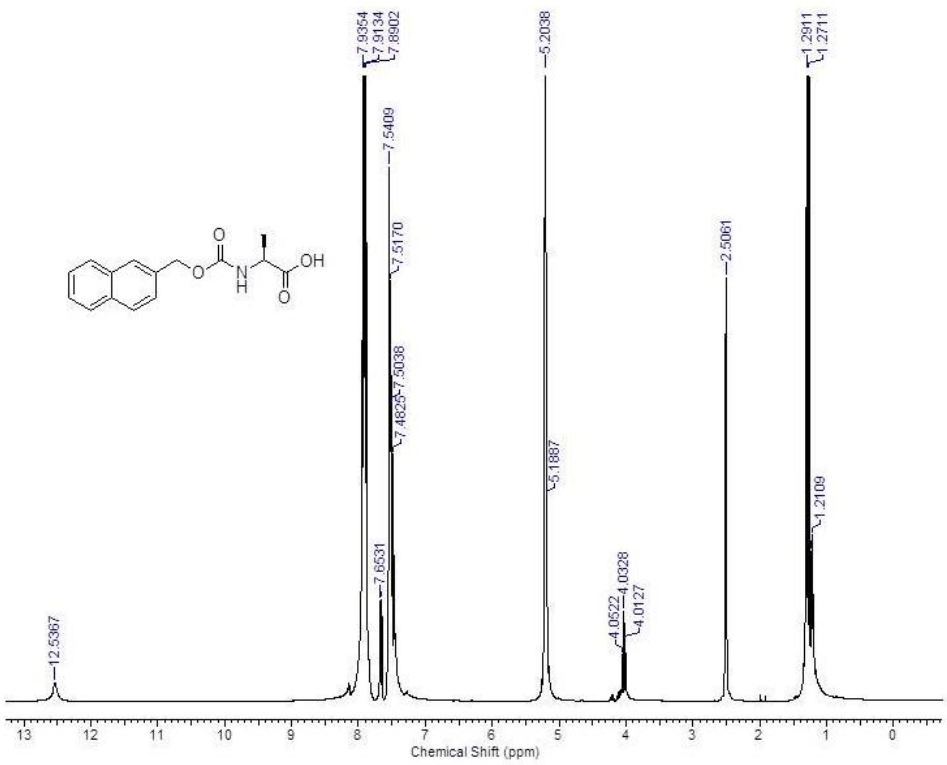


Fig. S38 ^{1}H NMR spectrum of Nmoc-A **7**.

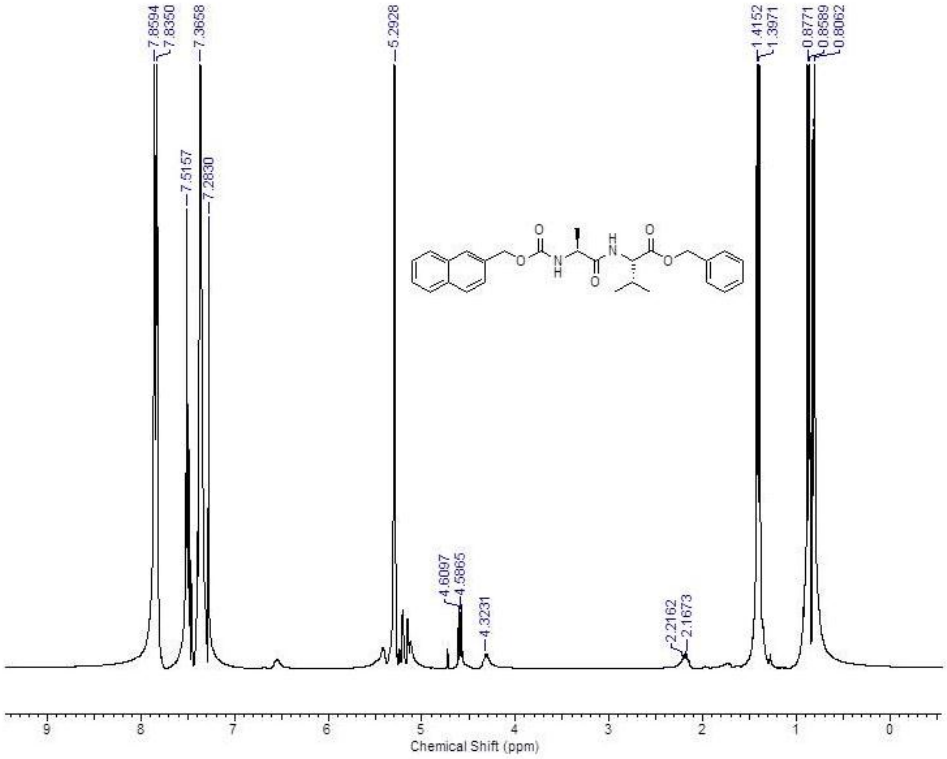


Fig. S39 ^{1}H Nmoc-AV-benzyl ester **8**.

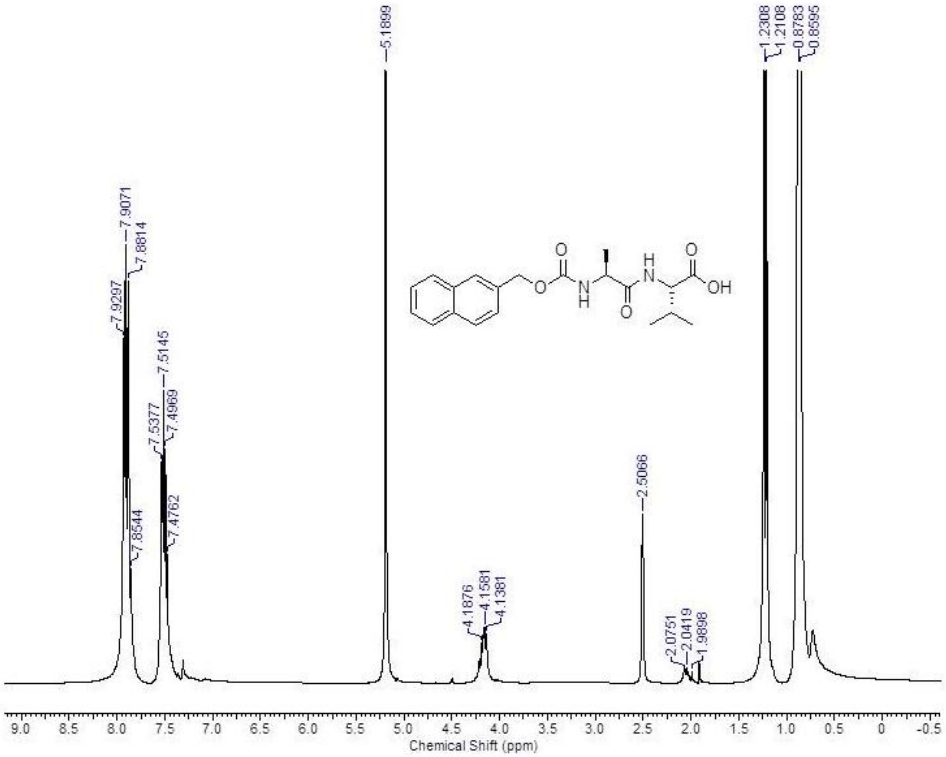


Fig. S40 ^{1}H NMR spectrum of Nmoc-AV **9**.

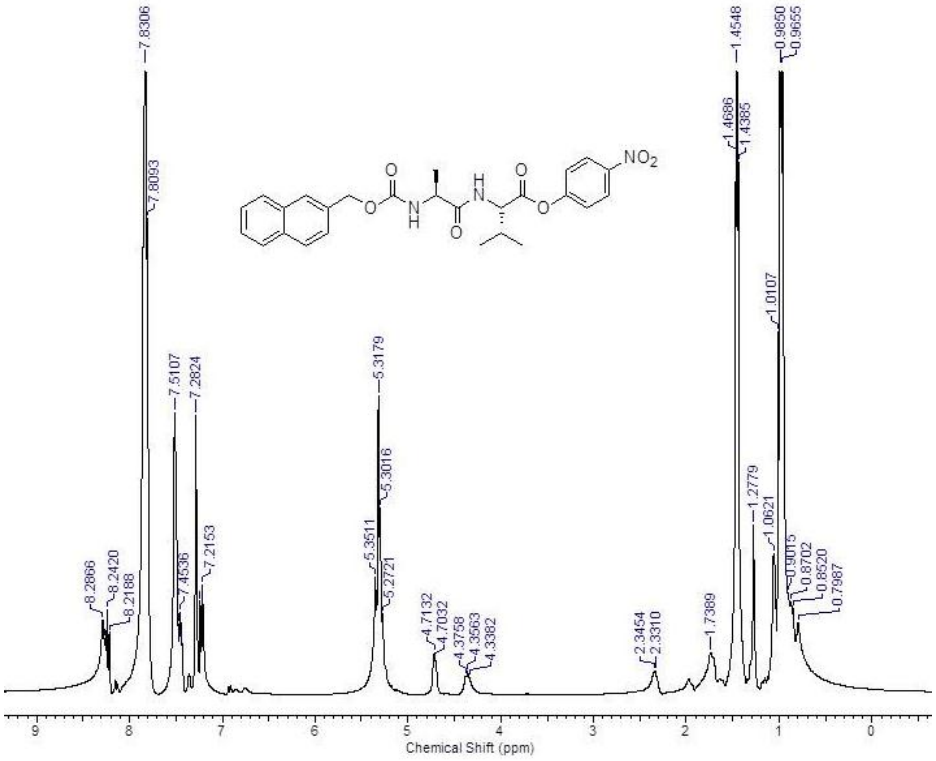


Fig. S41 ^{1}H NMR spectrum of Nmo-AV-*p*NP **1**.

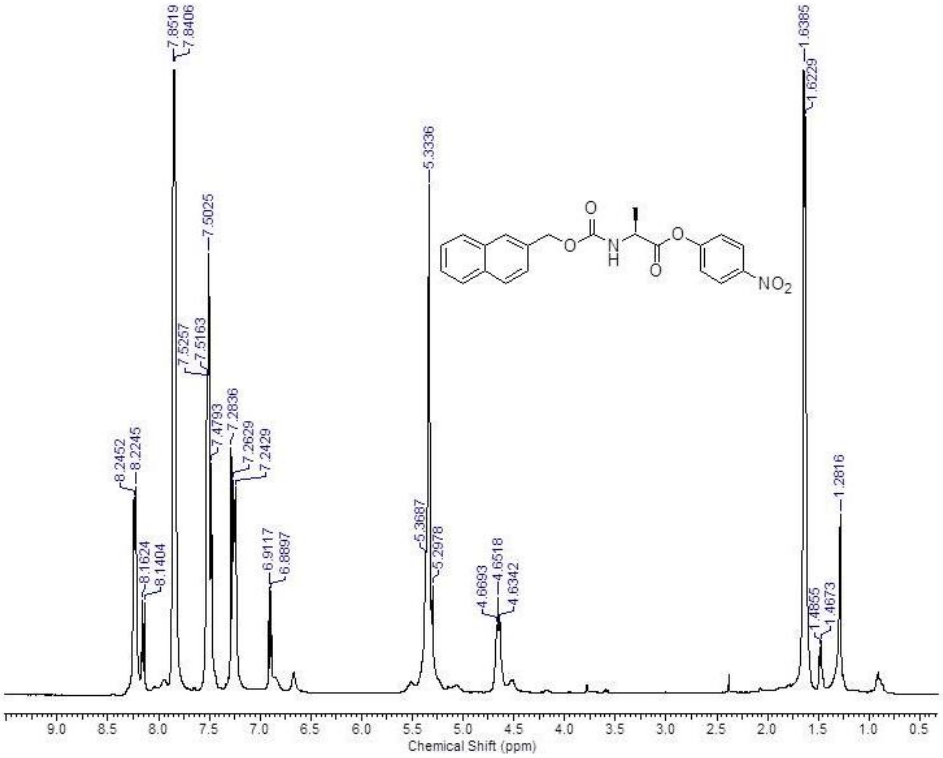


Fig. S42 ^{1}H NMR spectrum of Nmoc-A-*p*NP **4**.

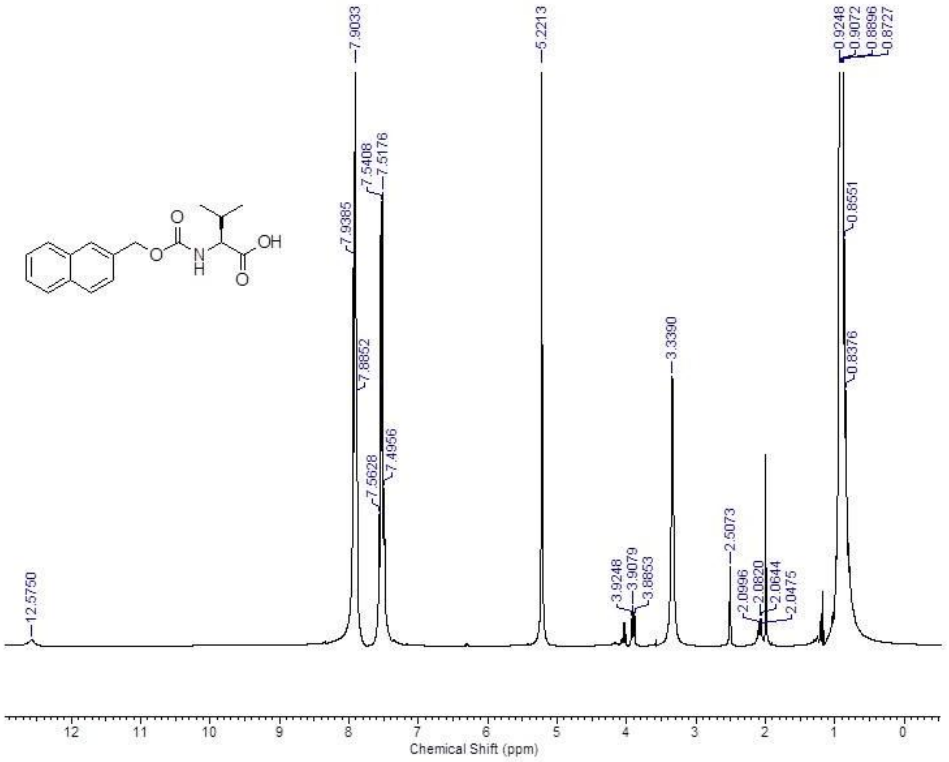


Fig. S43 ^{1}H NMR spectrum of Nmoc-V-OH **10**.

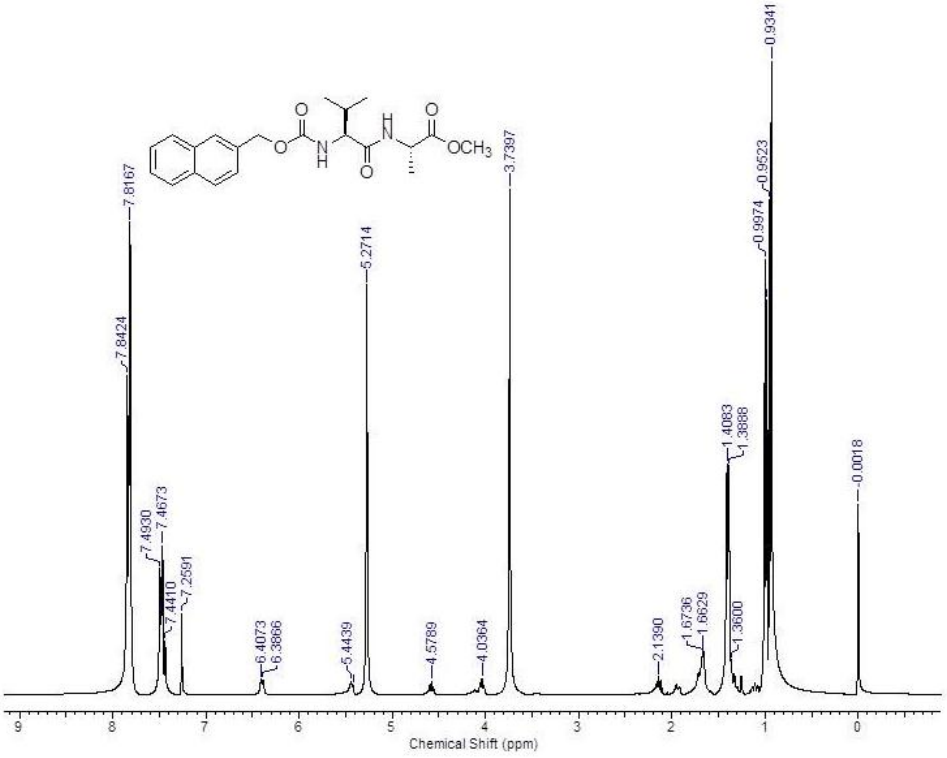


Fig. S44 ^{1}H NMR spectrum of Nmoc-VA-methyl ester **11**.

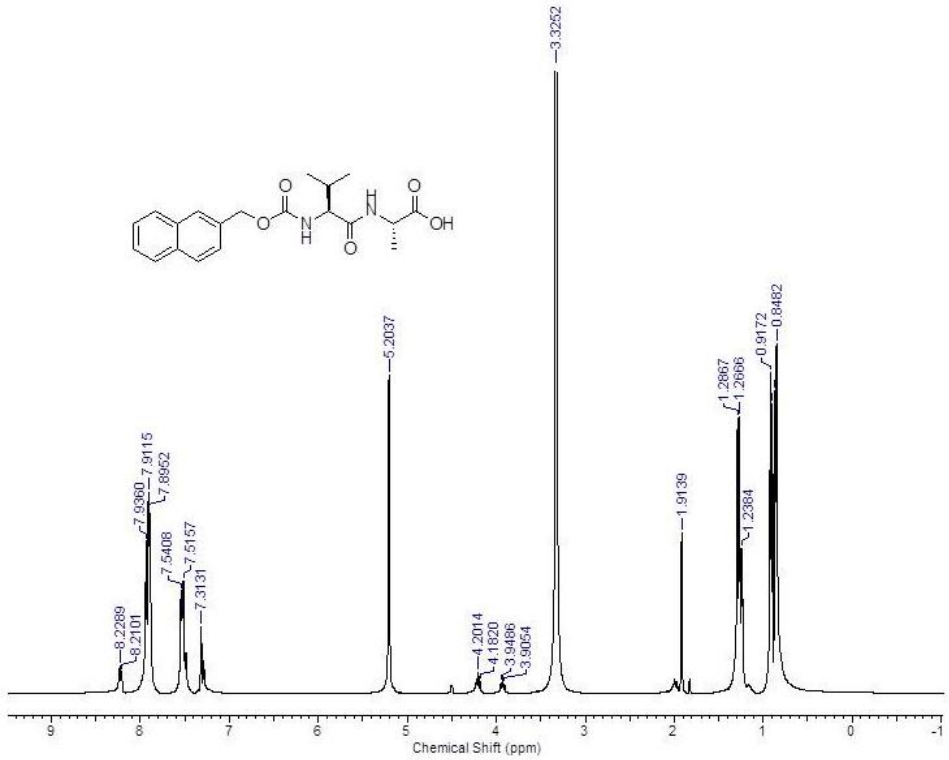


Fig. S45 ^{1}H NMR spectrum of Nmoc-VA **12**.

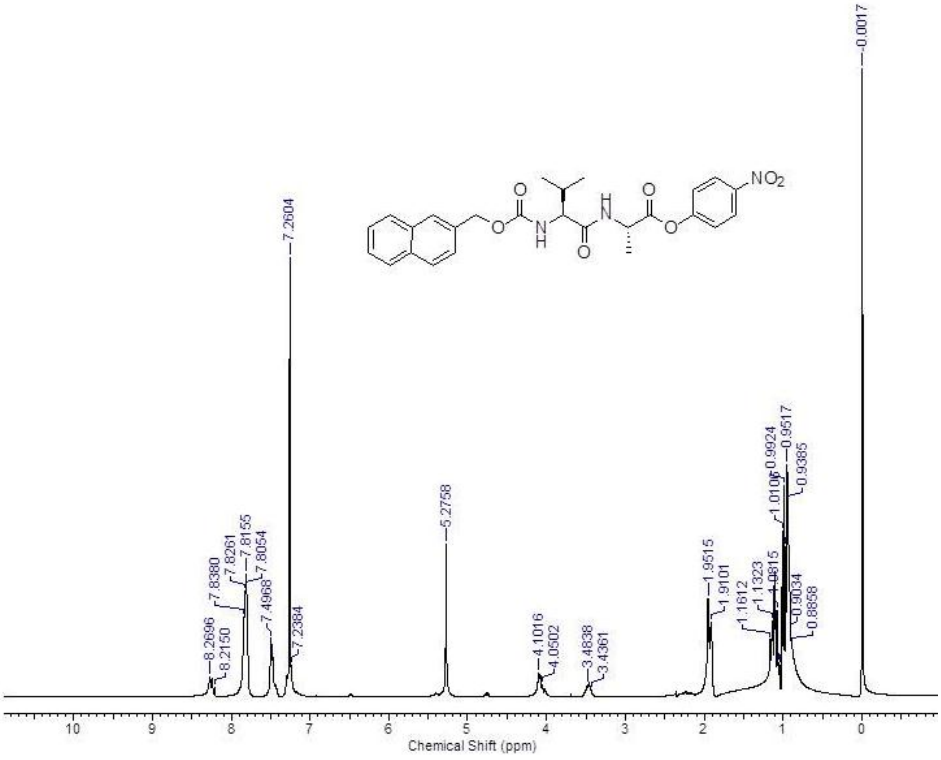


Fig. S46 ^{1}H NMR spectrum of Nmoc-VA-*p*NP **2**.

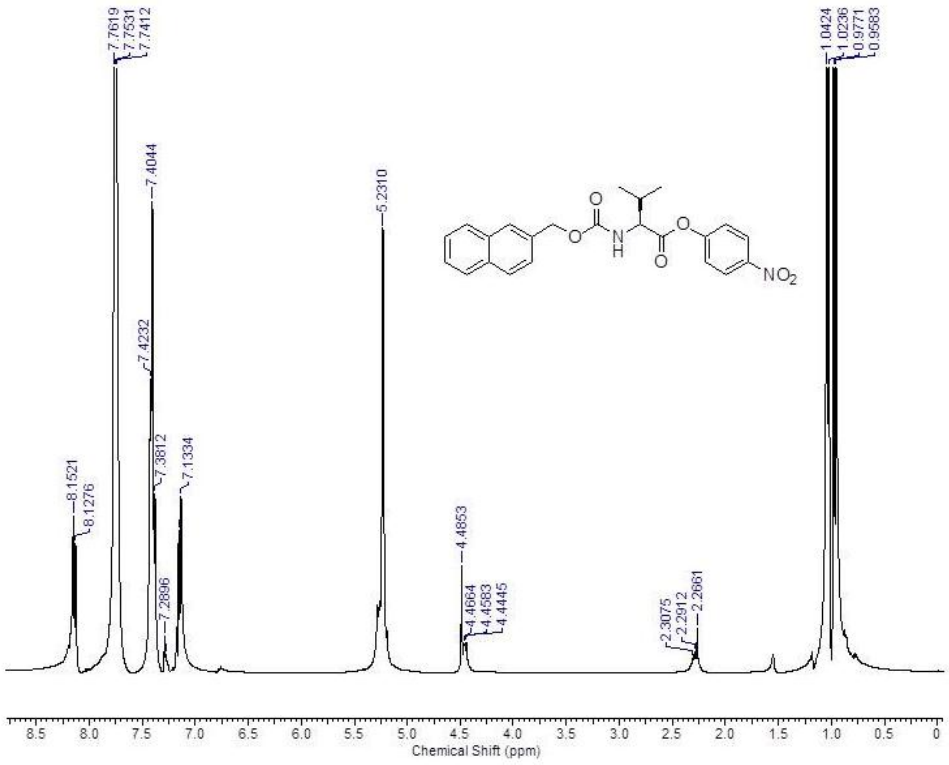


Fig. S47 ^{1}H NMR spectrum of Nmoc-V-*p*NP **3**.

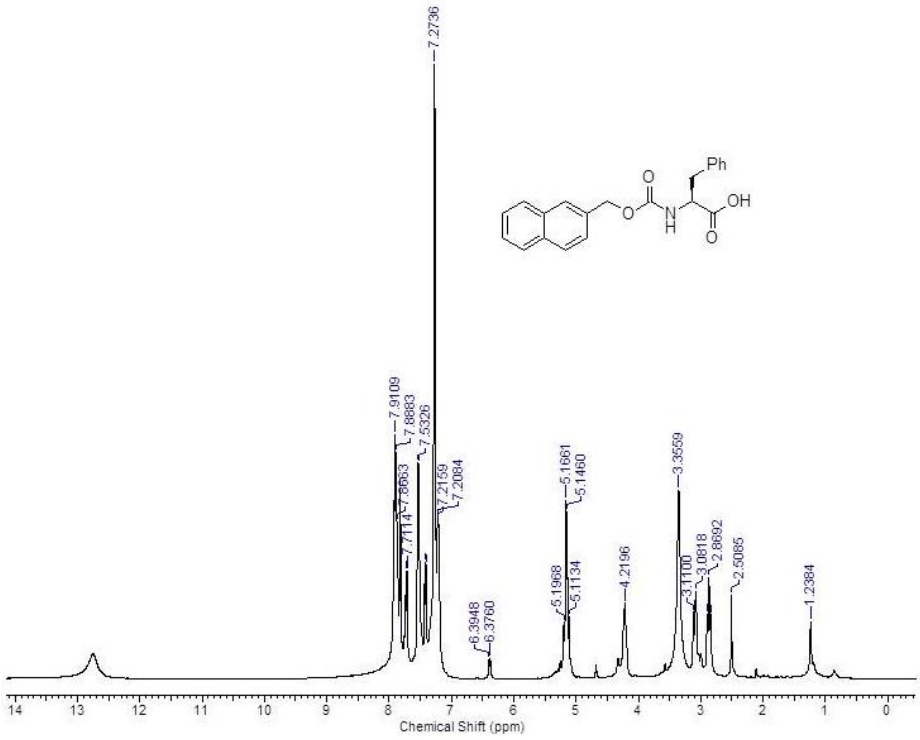


Fig. S48 ^{1}H NMR spectrum of Nmoc-F **13**.

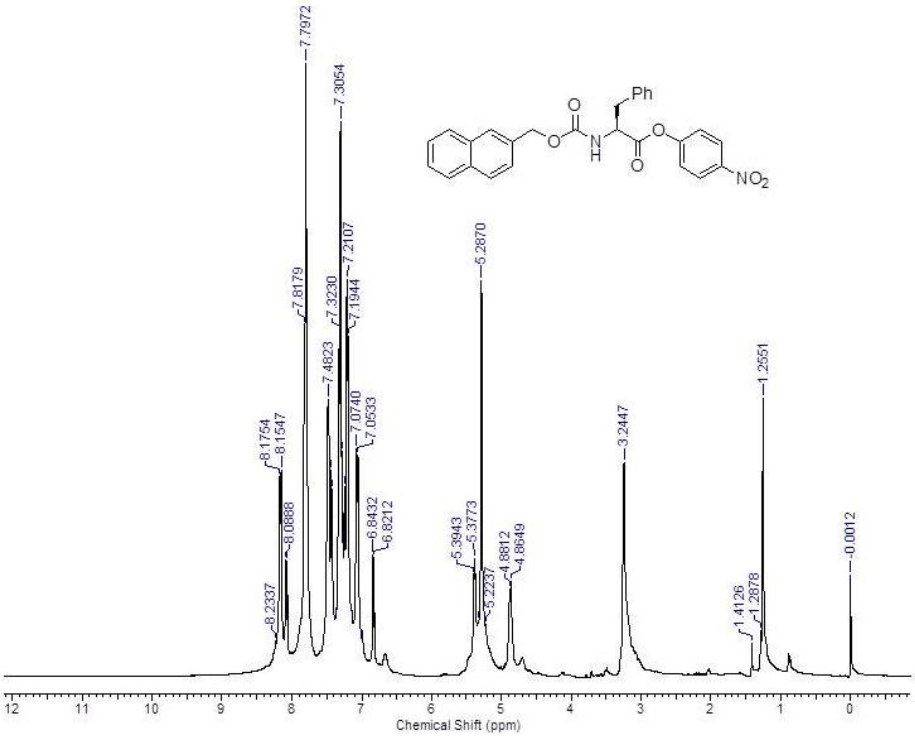


Fig. S49 ^{1}H NMR spectrum of Nmoc-F-*p*NP **5.**

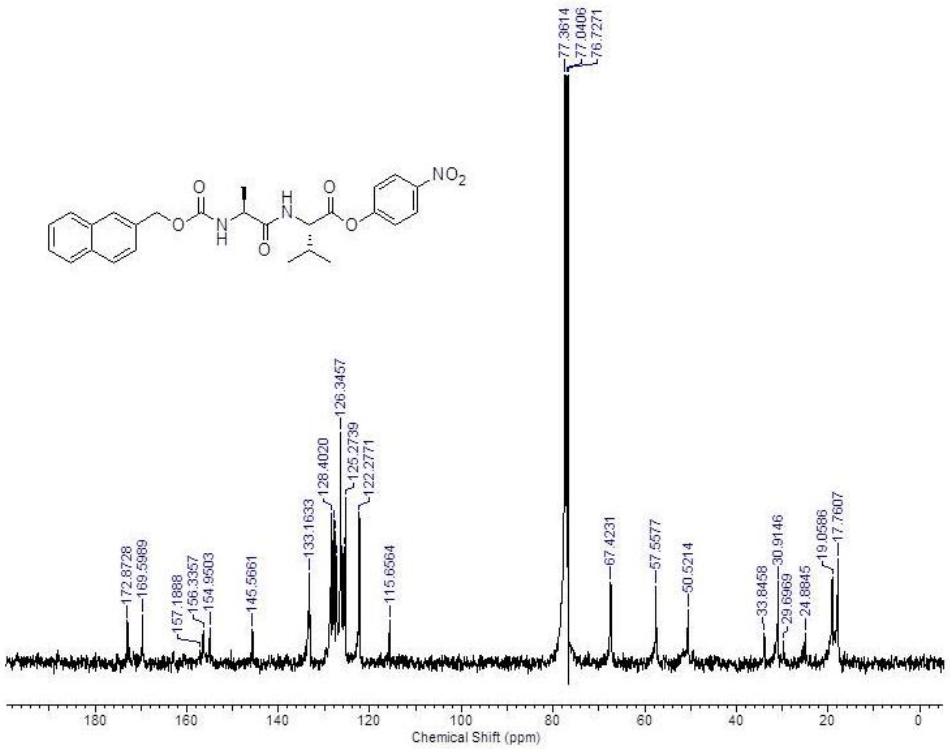


Fig. S50 ^{13}C NMR spectrum of Nmoc-AV-*p*NP **1**.

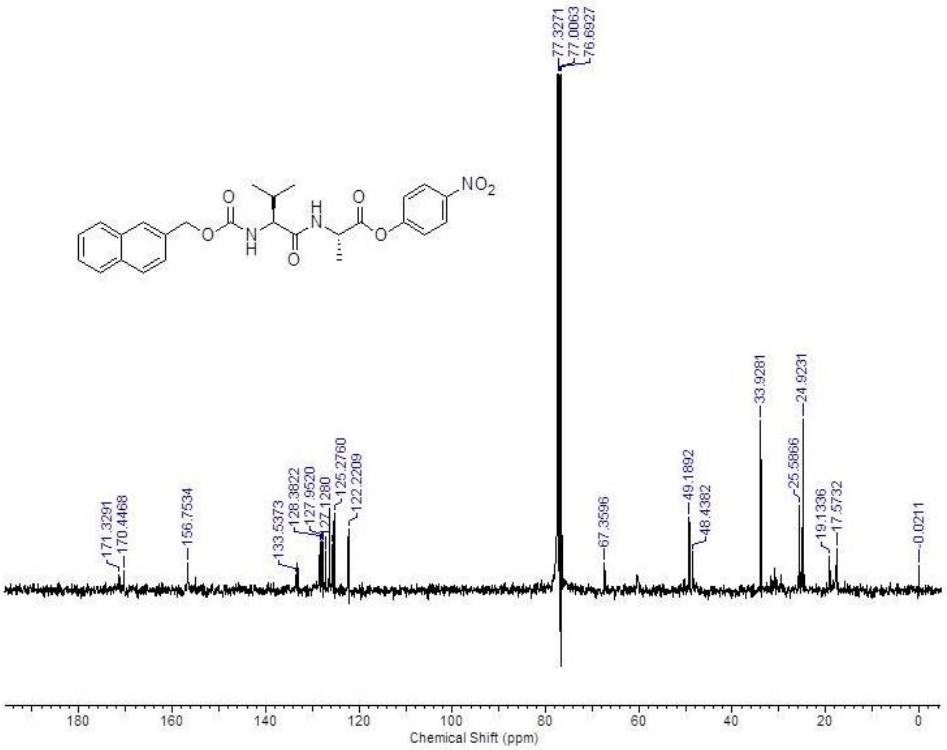


Fig. S51 ^{13}C NMR spectrum of Nmoc-VA-*p*NP **2.**

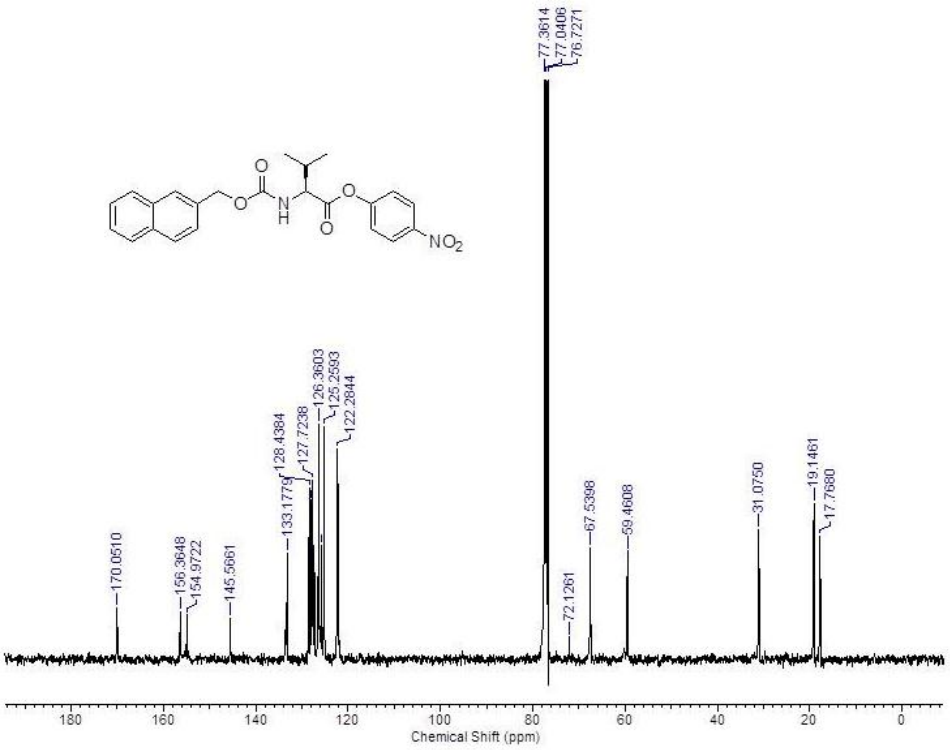


Fig. S52 ^{13}C NMR spectrum of Nmoc-V-*p*NP **3.**

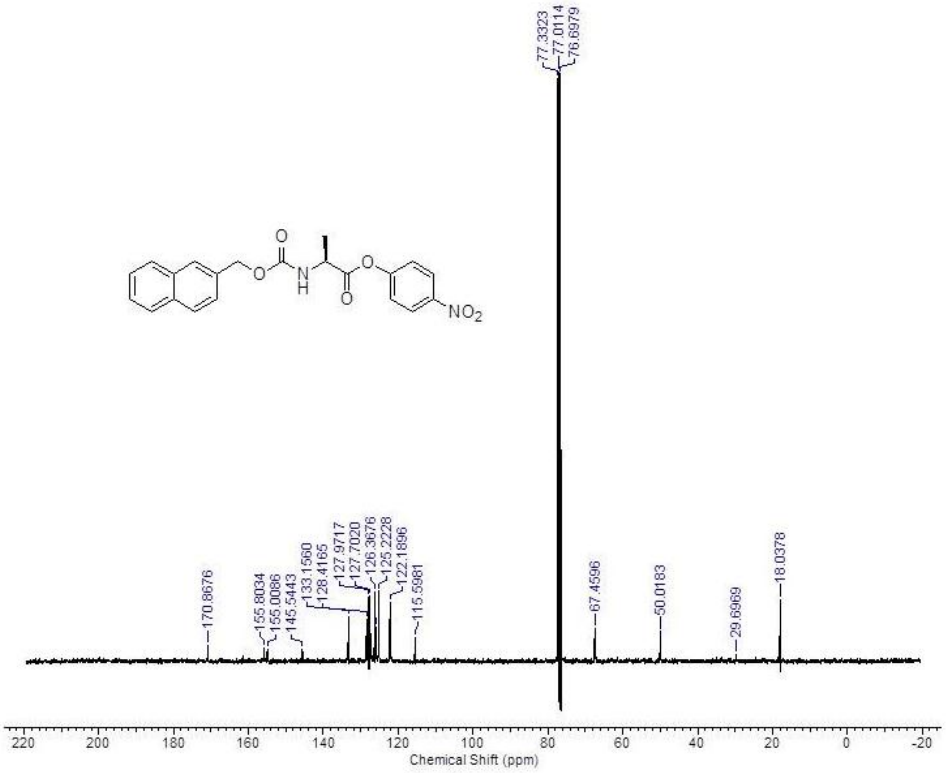


Fig. S53 ^{13}C NMR spectrum of Nmoc-A-*p*NP **4.**

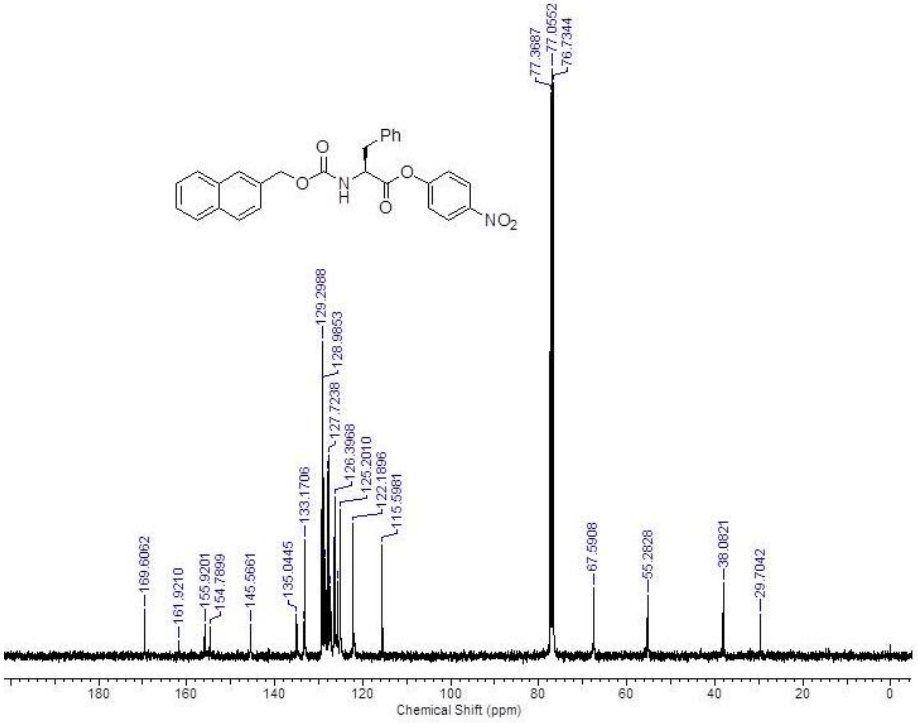


Fig. S54 ^{13}C NMR spectrum of Nmoc-F-*p*NP **5**.