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

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Contents

General Methods

Table S1 showing yield of products

Self-assembly

Gel-sol transition

HPLC analysis

ESI-MS analysis for ligated products

Rheology

FT-IR study

Circular dichroism

Fluorescence spectra

Morphological study

Synthetic procedure

$^1$H and $^{13}$C NMR Spectra
**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-pNP (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°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-pNP (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 reaction. 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 transferred onto the plate of the instrument using microspatulilla. 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 Kinetics 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 modulus) 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 microspatula 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 constant strain 0.05%.
Fig. S27 Dynamic frequency sweep of Nmoc-FC-CF-Nmoc 5b gel at constant strain 0.05%.

Fig. S28 Oscillatory rheology of a solution containing 20 mmol of 1b at 25°C.
Fig. S29 Oscillatory rheology of a solution containing 20 mmol of 5b at 25°C.

Fig. S30 Oscillatory rheology of a gel containing 20 mmol of 1b and solution of TCEP 40 mmol at 25°C.
**Fig. S31** Oscillatory rheology of a gel containing 20 mmol of 5b and solution of TCEP 40 mmol at 25°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°C 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-pNP 1 before and Nmoc-AVC-CVA-Nmoc 1b gel after NCL reaction (λ_{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**

\[ \text{Synthesis of Naphthalene-2-methyloxy chloroformate 6} \]

\[
\begin{align*}
\text{C}_7\text{H}_8\text{O}_3 \rightarrow \text{C}_7\text{H}_8\text{O}_3\text{Cl} \\
\text{Nap}h\text{thalene} \quad \text{COCl}_2 \quad \text{THF, 24h} \\
\end{align*}
\]

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°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): \( \tilde{\nu} = 3066 \text{ (m), 1777 \text{ (s), 1601 \text{ (m), 1168 \text{ (s) cm}^{-1}}}} \); \(^1\)H NMR (400 MHz, CDCl\(_3\)): 7.89 (m, 4H), 7.56 (m, 3H), 5.48 (s, 2H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)) \( \delta 71.82, 125.7, 126.1, 126.6, 126.8, 127.8, 128.1, 128.6, 130.6,133.0, 133.5, 140.9, 147.9, 150.7 \text{ ppm.} \)

**Synthesis of Nmoc-A-OH 7**

\[
\begin{align*}
\text{Synthesis of Nmoc-A-OH 7} \quad \text{H}_2\text{NCOOH} \rightarrow \text{H}_2\text{NCOOH} \\
\text{Nap}h\text{thalene} \quad 1,4 \text{ Dioxane/Na}_2\text{CO}_3 \\
\end{align*}
\]

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 vaccu. 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 \( \text{Na}_2\text{SO}_4 \) and concentrated in vacuo to give 7 as colorless oil. Yield= 0.890 mg (3.2 mmol, 64%)

\([\alpha]^{25}_D -13^\circ \text{ (c = 1, MeOH); } \quad \text{H NMR (400 MHz, DMSO-d}_6\text{): 8.14, (d, } J = 7.2 \text{ Hz, 1H, NH), 7.98 (t, 4H, Nph), 7.65 (d, } J = 7.6 \text{ Hz, 1H), 7.53 (t, 2H, Nph), 5.15 (s, 2H), 4.09 (m, 1H, C}_\alpha \text{ of Ala), 1.34 (d, } J=7.6 \text{ Hz, 3H, C}_\beta \text{ of Ala). } \text{HRMS (ESI) m/z for C}_{15}\text{H}_{15}\text{N}_4\text{ (M+Na)}^+ \text{ calcd.: 296.0893, found: 296.0911. Elemental Analysis calculated for C}_{15}\text{H}_{15}\text{N}_4\text{: C, 65.95; H, 5.53; N, 5.13; Found: C, 65.82; H, 5.52; N, 5.11.} \)
Synthesis of Nmoc-AV-OH 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 dicyclohexylcarbodiimide (3.2 mmol, 0.659 g) at 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 Na₂CO₃ (3 x 30 ml) and brine solution ethyl acetate layer was dried over Na₂SO₄ and evaporated under vacuum to yield 8 as white powder. Yield = 1.219 g (2.6 mmol, 83.87%).

[α]²⁵D -30° (c = 1, MeOH); ¹H NMR (400 MHz, CDCl₃): 7.83 (d, 4H, Nph), 7.51 (m, 3H, Nph), 7.37 (m, 5H, ph), 5.29 (s, 2H), 4.72 (q, 2H), 4.61 (m, 1H, Cα of Ala), 4.31 (t, 1H, Cα of Val), 2.20 (m, 1H, Cβ of Val), 1.41 (d, 3H, Cβ of Ala), 0.88 (d, J=6.8 Hz, 6H, Cγ of Val). HRMS (ESI) m/z for C₂₇H₃₀N₂O₅ (M+Na)⁺ calcld.: 485.2047, found: 485.2123. Elemental Analysis calculated for C₂₇H₃₀N₂O₅: 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 %).

\[ \alpha \] \text{D}^\text{25} -14° (c = 1, MeOH); \text{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\text{α} of Ala), 4.76 (t, 1H, C\text{α} of Val), 2.06 (m, 1H, C\text{β} of Val), 1.72 (d, J = 6.8 Hz, 3H, C\text{β} of Ala), 1.1 (d, 6H C\text{β} of Val).

HRMS (ESI) m/z for C_{20}H_{24}N_{2}O_{5}(M+Na)^{+} calcd.: 395.1577, found: 395.1821.

Elemental Analysis calculated for C_{20}H_{24}N_{2}O_{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°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 %).

\[ \alpha \] \text{D}^\text{25} -12° (c = 1, MeOH); FT-IR (KBr): \tilde{\nu} = 3306(s), 3062 (m), 2965 (m), 1765 (s), 1694 (w), 1654 (m), 1525(s), 1451( w), 1344 (s), 1245(s) cm\(^{-1}\); \text{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\text{α} of Val), 4.39 (m, 1H, C\text{α} of Ala), 2.33 (m, 1H, C\text{β} of Val), 1.45 (d, 3H, C\text{β} of Ala), 0.99 (d,6H, C\text{γ} of Val).\text{ C NMR} (400 MHz, CDCl_3) \delta 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_{3}O_{7}(M+Na)^{+} calcd.: 516.1741, found: 516.2159. Elemental Analysis calculated for C_{26}H_{27}N_{3}O_{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°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 0.1 M HCl (2 x 30 ml), 1M NaHCO₃ (3 x 30 ml) and brine solution. The ethyl acetate layer was dried over Na₂SO₄ 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]²⁵D -17° (c = 1, MeOH); FT-IR (KBr): δ = 3381(s), 3056 (m), 2920 (m), 1763 (s), 1617 (m), 1592(m), 1513( m), 1345 (s), 1291(m) cm⁻¹; ¹H NMR (400 MHz, CDCl₃): 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, CH₂), 4.65 (m, 1H Cα of Ala), 1.63 (d, 3H, Cβ of Ala). ¹³C NMR (400 MHz, CDCl₃) 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 C₂₁H₁₈N₂O₆ (M+Na)⁺ calcd.:417.1057, found: 417.4989. Elemental Analysis calculated for C₂₁H₁₈N₂O₆: 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 vacum. 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 Na₂SO₄ concentrated in vacuo to give 10 as colorless oil. Yield=0.863 g (2.8 mmol, 70%).

[α]₂⁵°D =-5° (c = 1, MeOH); H NMR (400 MHz, CDCl₃): 7.90 (t, 4H, Nph), 7.54 (d, 3H, Nph), 5.22 (s, 2H, CH₂), 3.88 (t, 1H, Cα of Val), 2.08 (m, 1H, Cβ of Val), 0.92 (d, 6H, Cγ of Val).

HRMS (ESI) m/z for C₁₇H₁₉NO₄ (M+Na)⁺ calcd.: 324.1206, found: 324.1228. Elemental Analysis calculated for C₁₇H₁₉NO₄: 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°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 Na₂CO₃ (3 x 30 ml) and brine solution ethyl acetate layer was dried over Na₂SO₄ and evaporated under vacuum to yield 11 as white powder. Yield= 0.676 g, (1.6 mmol, 72.72%).

[α]₂⁵°D = -41° (c = 1, MeOH); H NMR (400 MHz, CDCl₃): 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, CH₂), 4.57 (t, 1H, Cα of Val), 4.03 (m, 1H, Cα of Ala), 3.73 (s, 3H, OCH₃), 1.6 (m, 1H, Cβ of Val), 1.4 (d, 3H, J=7.8 Hz, Cβ of ala), 0.97 (d, 6H, J=7.28 Hz). HRMS (ESI) m/z for C₂₁H₂₆N₂O₅ (M+Na)⁺ calcd.: 409.1734, found: 409.1694. Elemental Analysis calculated for C₂₁H₂₆N₂O₅: 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 up to 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 %)

$\left[\alpha\right]_{D}^{25} -36^\circ (c = 1, \text{MeOH})$; $^1$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$\alpha$ of Val), 3.92 (m, 1H, C$\alpha$ of Ala), 1.91 (m, 1H, C$\beta$ of Val), 1.26 (d, 3H C$\beta$ of Ala), 0.91 (d, 6H, $J=7.52$ Hz). HRMS (ESI) m/z for C$_{20}$H$_{24}$N$_2$O$_5$ (M+Na)$^+$ calcd.: 395.1611, found: 395.1845. Elemental analysis calculated for C$_{20}$H$_{24}$N$_2$O$_5$: 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$^\circ$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$_2$SO$_4$ 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]$_D$ -33° (c = 1, MeOH); FT-IR (KBr): $\tilde{\nu}$ = 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$_3$): $\delta$ 8.26 (d, 1H, N$_p$H), 7.83 (d, 4H, $J$= 4.76 Hz, N$_p$H), 7.49 (d, 2H, $J$= 3.48 Hz, N$_p$H), 5.27 (s, 2H), 4.10 (m, 1H, C$_\alpha$ of Ala), 3.48 (m, 1H, C$_\alpha$ of val), 1.9 (m, 1H, C$_\beta$ of Val), 1.01 (d, 3H, C$_\beta$ of Ala), 0.95 (d, 6H, C$_\alpha$ of val). $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 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, 33.92, 30.99, 25.58, 24.92, 19.13, 17.57. HRMS (ESI) m/z for C$_{26}$H$_{27}$N$_3$O$_7$(M+Na)$^+$ calcd.: 516.1741, found: 516.1630. Elemental analysis calculated for C$_{26}$H$_{27}$N$_3$O$_7$: 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°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$_2$SO$_4$ 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]$_D$ -11° (c = 1, MeOH); FT-IR (KBr): $\tilde{\nu}$ = 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$_\alpha$ of Val), 1.48 (m, 1H, C$_\beta$ of Val), 1.18 (d, 6H, Val ).$^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 170.05, 156.36, 154.97, 145.56, 133.17, 128.43, 72.12, 67.53, 59.46, 31.67, 19.14, 17.57. HRMS (ESI) m/z for C$_{26}$H$_{27}$N$_3$O$_7$(M+Na)$^+$ calcd.: 445.1370, found: 445.1458. Elemental Analysis calculated for C$_{26}$H$_{27}$N$_3$O$_7$: 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. Naphthalene-2-methoxychloroformate (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 Na₂SO₄ and concentrated in vacuo to give 13 as colorless oil. Yield= 1.46 g, (4.1 mmol, 82 %)

[α] D: -7° (c = 1, MeOH); ¹H NMR (400 MHz, DMSO-d₆): 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, Cα of Phe), 3.11 (d, J=11.2 Hz, 1H, Cβ of Phe), 2.86 (t, 1H, Cβ of Phe); ¹³C NMR (400 MHz, CDCl₃) 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 C₂₁H₁₉NO₄ (M+Na)⁺ calcd.: 372.1212, found: 372.1206; Elemental Analysis calculated for C₂₁H₁₉NO₄: 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°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 NaHCO₃ (3 x 30 ml) and brine.
solution. The ethyl acetate layer was dried over Na$_2$SO$_4$ 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): $\tilde{\nu}$ = 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$_\alpha$ of Phe), 3.24 (d, 2H, C$_\beta$ of Phe); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 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$_2$O$_6$ (M+Na)$^+$ calcd.: 493.1376, found: 493.1683. Elemental Analysis calculated for C$_{27}$H$_{22}$N$_2$O$_6$: 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-naphthalenemethoxychloroformate 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-\textit{p}NP 1.
Fig. S42 $^1$H NMR spectrum of Nmoc-A-pNP 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-pNP 3.
Fig. S48 $^1$H NMR spectrum of Nmoc-F 13.
Fig. S49 $^1$H NMR spectrum of Nmoc-F-pNP 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.