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Lipase catalyzed inclusion of gastrodigenin for the evolution of blue light emitting peptide nanofibers

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General Methods.

All the chemicals and reagents were obtained commercially. All NMR spectra were recorded with 400 MHz Bruker AV 400 NMR. Compounds concentrations were in the range of 1-10 mmol in $(CD_3)_2SO$ and $CDCl_3$. Mass spectra were recorded on Bruker micrOTOF-Q II by positive and negative mode electrospray ionisations. All the reported FT-IR spectra were taken using Bruker (Tensor 27) FT-IR spectrophotometer. Specific rotations of the synthesized compounds were measured on an Autopol^R V automatic polarimeter (Rudolph research analytical). The cell (length = 100 mm, capacity = 2 mL) was used for this study at 25 °C.

HPLC analysis

A Dionex HPLC-Ultimate 3000 (High Performance Liquid Chromatography) pump was used to analyse products. 20 µL of sample was injected onto a Dionex Acclaim ® 120 C18 column of 250 mm length with an internal diameter of 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 of 100 µL gel in 900 µL acetonitrile-water (50: 50 mixture) solution containing 0.1 % trifluroacetic 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 products were identified by using Ultimate 3000 RS Variable Wavelength Detector at 280 nm.

Rheological study

Rheological study was performed to determine the mechanical properties of hydrogels. These properties were assessed using an Anton Paar Physica Rheometer (MCR 301, Austria) with cone plate geometry (20 mm in diameter, 50 µm gap and 1° angle) and temperature was controlled at 25 °C. The dynamic moduli of the hydrogel were measured as a function of frequency in the range of 0.1-100 rad s^{-1} with a constant strain value 0.01 %. To determine the exact strain for frequency sweep experiments, the linear viscoelastic (LVE) regime was performed at constant frequency of 10 rad s^{-1}. Experimental data was acquired in thrice and the average data is shown. 200 µL of gel was prepared in glass vial and transferred it over the plate using microspatula to

proceed for rheological measurements.

Circular Dichroism study

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. Samples were prepared at a concentration of 2 mmol L^{-1}. Experimental data were acquired in thrice and the average data is shown.

Time resolved study

2 mL gel 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

$$\text{Çú—Ç} = \sum_{i=1}^{n} a_i \text{ú—}_i$$

where τ_1 is the fluorescence lifetime of various fluorescent species and are the a_1 normalized pre-exponential factors. To gain the best fitting in all cases the χ^2 was kept near to unity.

UV-Vis spectroscopy

UV-vis absorption spectrum of the hydrogel **1** was recorded using a Varian Cary100 Bio UV-Vis spectrophotometer at a concentration of 2 mmol L^{-1}. Similarly, UV-vis absorption spectra of Nmoc-LW acids and *p*-hydroxybenzyl alcohol were recorded at concentration of 2 mmol L^{-1}.

Fluorescence spectroscopy

Fluorescence emission spectra of hydrogel (2 mmol L^{-1}) as well as solution of Nmoc-LW and *p*-hydroxy benzyl alcohol were recorded at different excitation wavelength of 280 nm and 365 nm with medium sensitivity on a Horiba Scientific Fluoromax-4 spectrophotometer. The slit width for the excitation and emission was set at 2 nm and a 1 nm data pitch. Samples were prepared in 1 cm^2 quartz cuvette at room temperature.

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 hydrogel was dried on carbon-coated copper grids (300 mesh) by slow evaporation in air and then allowed to dry separately in a vacuum at room temperature.

The morphology of gels was investigated using 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 milli-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: Smart SPM-1000).

Fluorescence Microscopy experiments were performed on a home-built epifluorescence microscopy setup. An air-cooled argon ion laser (Melles Griot, model 400-A03) with excitation wavelength at 457 nm was used to excite the hydrogel sample placed on an inverted microscope (Nikon, model Eclipse Ti-U). The images were analyzed with ImageJ (Version 1.46r) NIH. Dilute solution of the hydrogel was spin coated over glass cover slip before analysis.

Table S1. Lipase catalyzed formation of peptide esters in aqueous medium

Entry	Substrate [20 mmol L^{-1}]	HBA[a] [80 mmol L^{-1}]	Enzyme[b] [0.5 mg/mL]	Product Yield[c] [%]	Physical Characteristics[d]
1.	Nmoc-LW	HBA	CRL	91.29	G
2.	Nmoc-LY	HBA	CRL	12.48	S
3.	Nmoc-YW	HBA	CRL	19.66	S

[a]HBA = *p*-hydroxybenzyl alcohol, [b]Enzyme = Lipase from Candida Rugosa, [c]Analysed after 30 days, [d]G = gelation and S= solution

Table S2. Decay parameters for Nmoc-LW and Nmoc-LW-HBA hydrogel.

Entry	α_1	α_2	τ_1(ns)	τ_2(ns)	τ^a(ns)	χ^2
Nmoc-LW-OH solution	0.90	0.10	0.90	4.44	1.25	1.62
Nmoc-LW-HBA Hydrogel	0.81	0.19	0.84	3.77	1.39	1.49

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

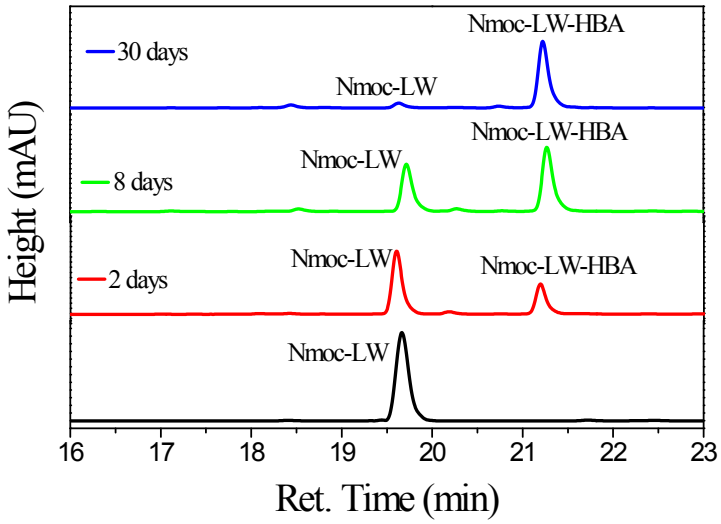


Fig. S1. HPLC chromatograms exihibit formation of Nmoc-LW-HBA from Nmoc-LW **1** upon lipase catalyzed inclusion of gastrodigenin at different time.

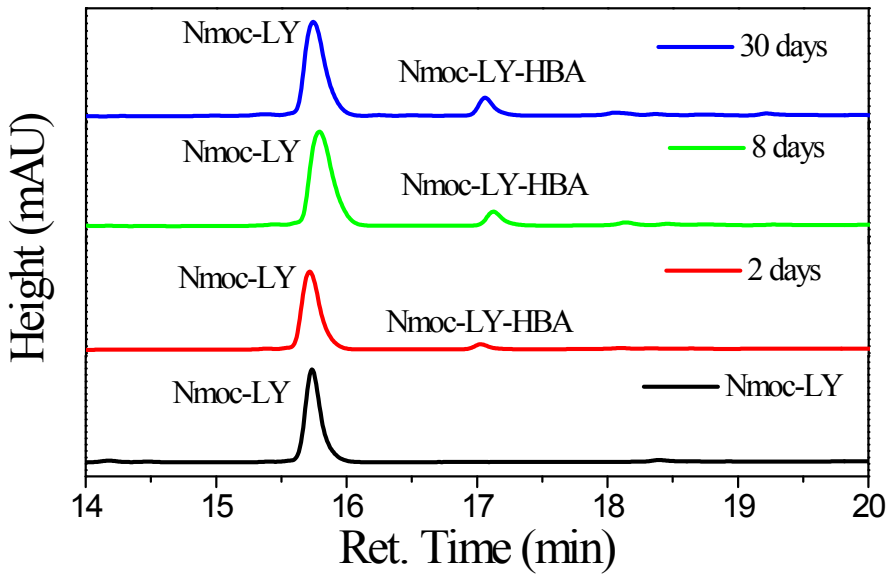


Fig. S2. HPLC chromatograms exihibit formation of Nmoc-LY-HBA from Nmoc-LY **2** upon lipase catalyzed inclusion of gastrodigenin at different time.

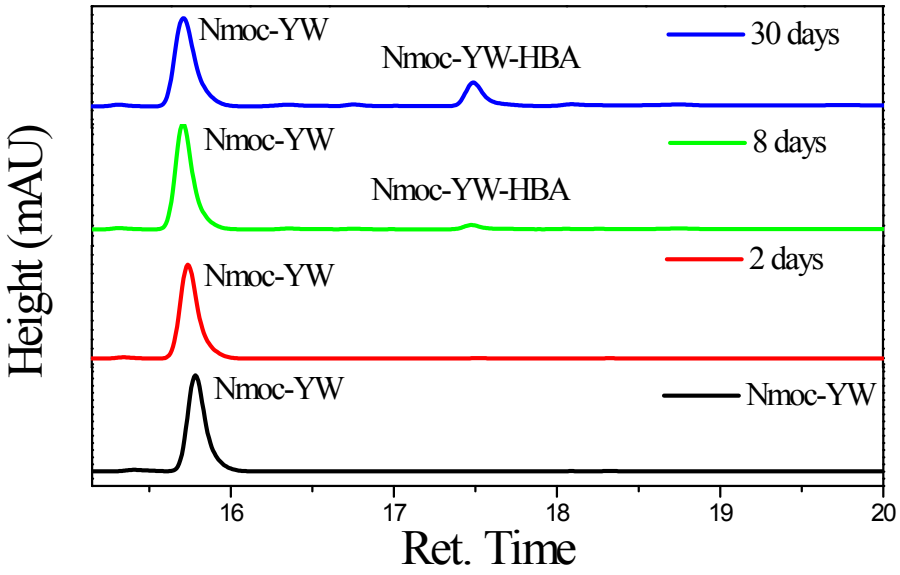


Fig. S3. HPLC chromatograms exihibit formation of Nmoc-YW-HBA from Nmoc-YW **3** upon lipase catalyzed inclusion of gastrodigenin at different time.

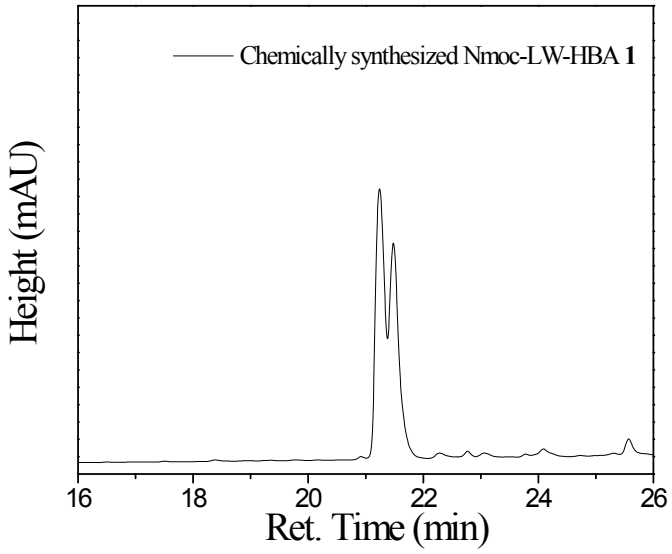


Figure S4. HPLC chromatogram exihibits mixture of two products obtained via chemically synthesized Nmoc-LW-HBA due to presence of two hydroxyl groups of HBA.

Self-assembly study of chemically synthesized Nmoc-LW-HBA: Compound Nmoc-LW-HBA (20 mml L^{-1}, 12.1 mg) was dispersed in 1 mL of water and vortexed to make it homogenous. Nmoc-LW-HBA was unable to dissolved in water. Then pH was increased to 9. Further the solution was vortexed and subjected to sonication. However, compound Nmoc-LW-HBA was remained insoluble in water. The mixture was incubated for 2 days at 37 °C. The solution still remained insoluble and precipitated out. Therefore, no self-assembly was observed for chemically synthesized Nmoc-LW-HBA in water.

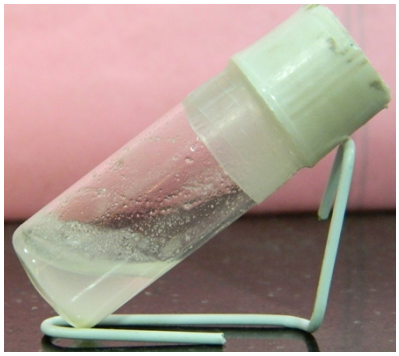


Fig. S5. Optical image shows solution of chemically synthesized Nmoc-LW-HBA, which was unable to self-assemble.

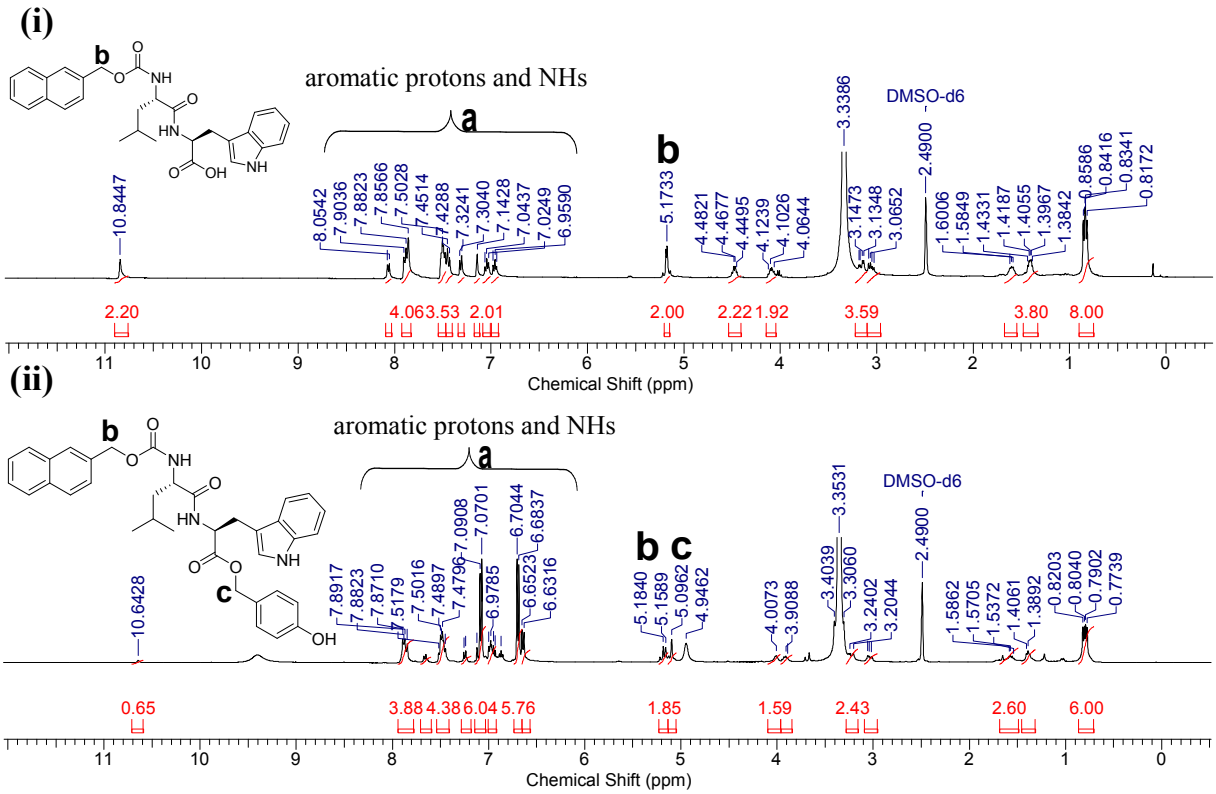


Fig. S6. ^{1}H NMR spectra of (i) Nmoc-LW-OH in DMSO-d_6 and (ii) dried hydrogel of Nmoc-LW-HBA **1** in DMSO-d_6. The hydrogel Nmoc-LW-HBA **1** was isolated by centrifugation and dried under vacuum.

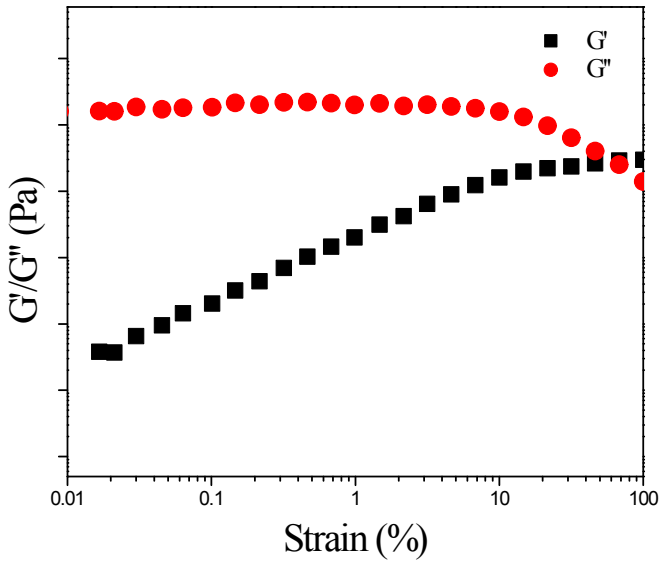


Fig. S7. Rheological measurement of LVE at constant frequency 10 rad s^{-1}.

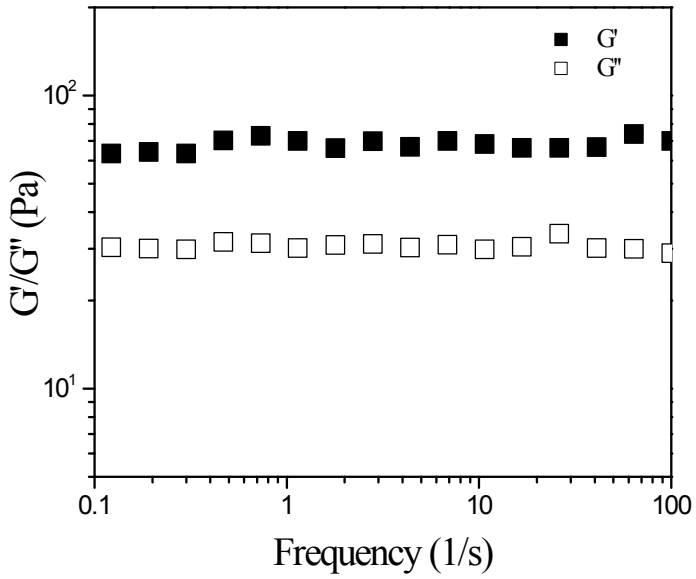


Fig. S8. Dynamic frequency sweep of Nmoc-LW-HBA hydrogel **1** at constant strain 0.01%.

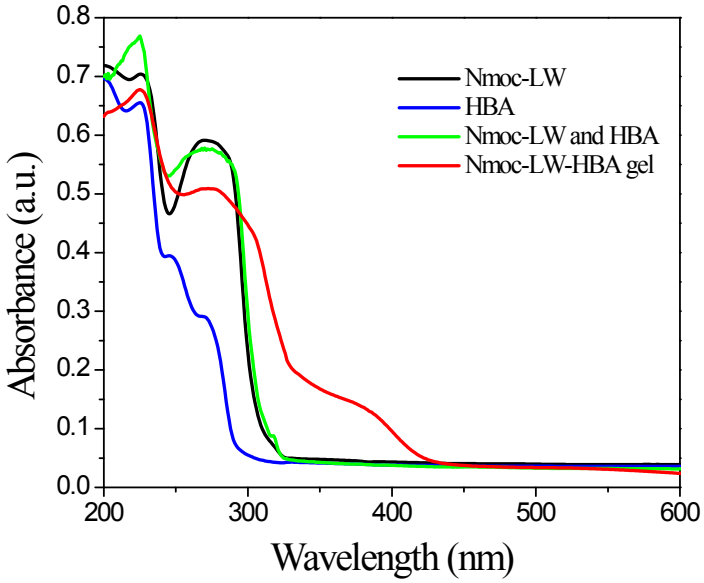


Fig. S9. Absorption spectra of Nmoc-LW and *p*-hydroxybenzyl alcohol (HBA), Nmoc-LW and HBA and hydrogel of Nmoc-LW-HBA **1** in aqueous medium.

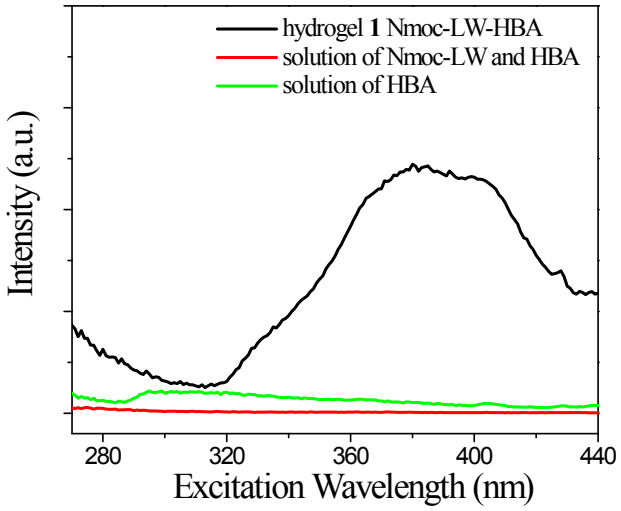


Fig. S10. Fluorescence excitation spectra of hydrogel of Nmoc-LW-HBA **1**, mixture of Nmoc-LW and *p*-hydroxybenzyl alcohol (HBA), and HBA in aqueous medium (emission wavelength 470 nm).

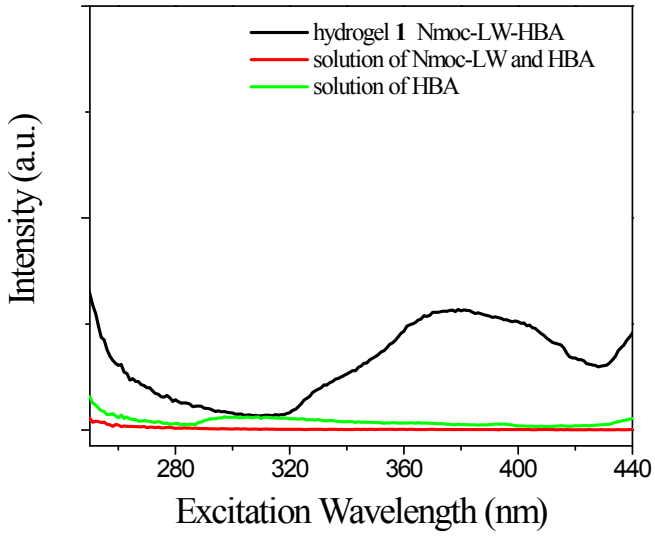


Fig. S11. Fluorescence excitation spectra of hydrogel of Nmoc-LW-HBA **1**, mixture of Nmoc-LW and *p*-hydroxybenzyl alcohol (HBA), and HBA in aqueous medium (emission wavelength 455 nm).

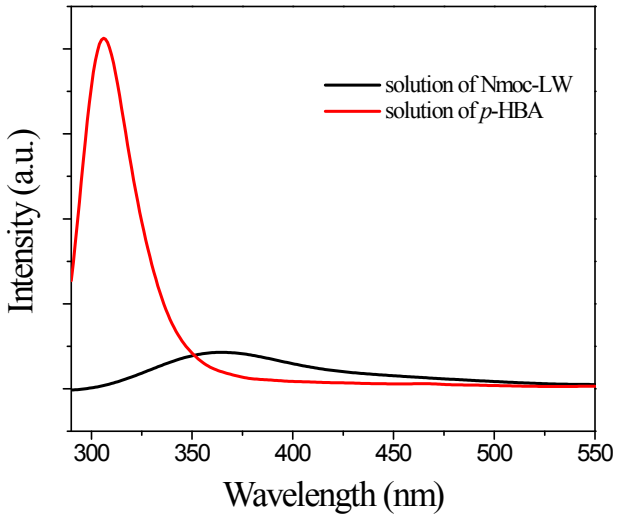


Fig. S12. Emission spectra of Nmoc-LW and *p*-hydroxybenzyl alcohol in aqueous medium (λ_{ex} = 280 nm).

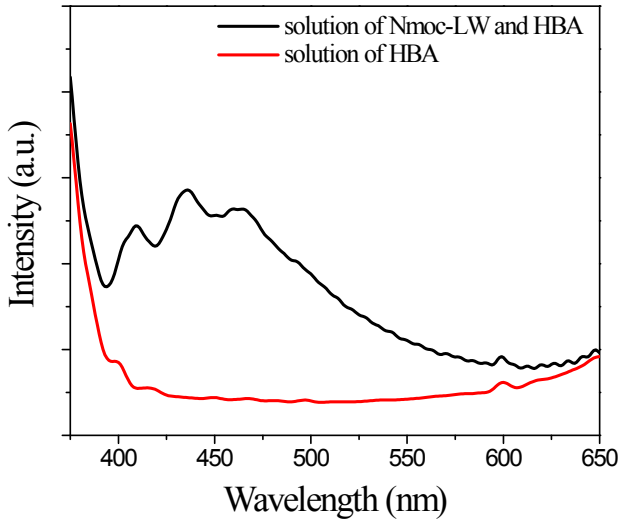


Fig. S13. Emission spectra of solution of Nmoc-LW and HBA and solution of *p*-hydroxybenzyl alcohol (HBA) in aqueous medium (λ_{ex} = 365 nm).

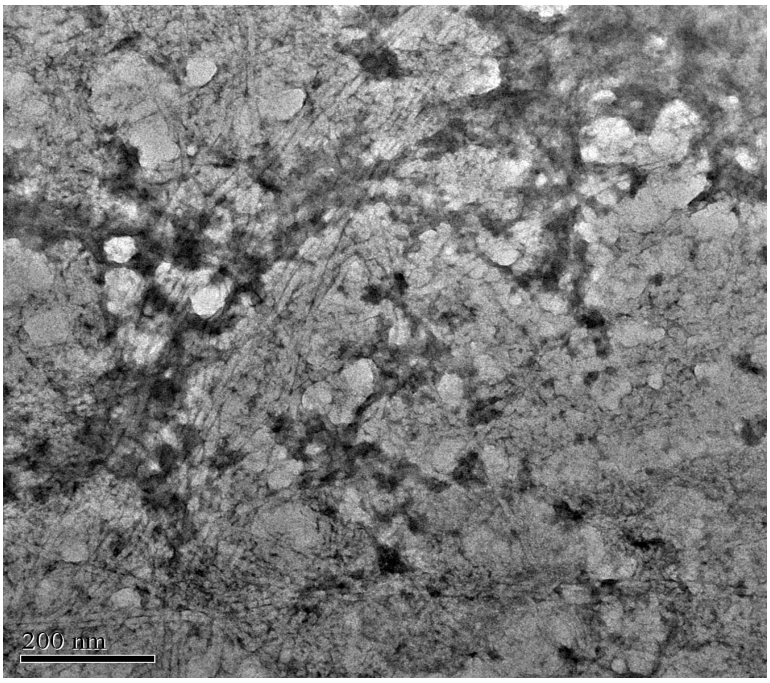


Fig. S14. TEM image of hydrogel of Nmoc-LW-HBA **1** indicating nanofibrillar network in gel phase medium.

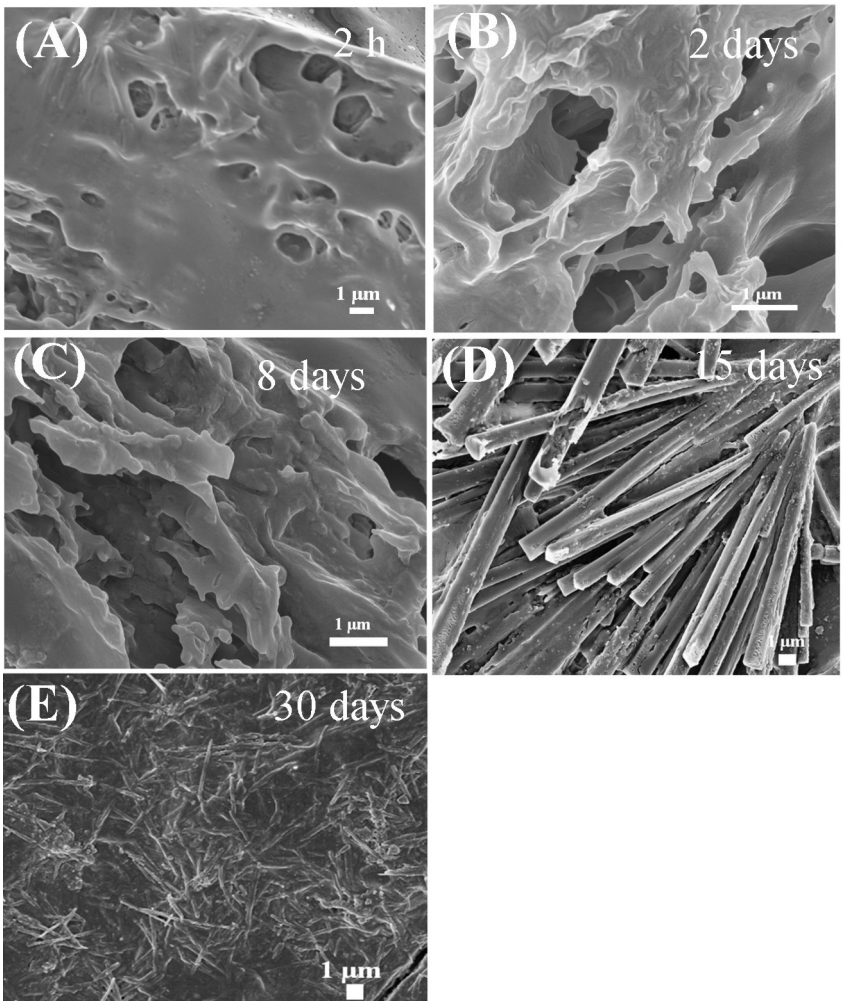


Fig. S15. SEM images during the formation of Nmoc-LW-HBA **1** hydrogel at (A) 2 h, (B) 2 days, (C) 8 days, (D) 15 days and (E) 30 days after enzyme addition. Image E shows nanofibrillar morphology for Nmoc-LW-HBA **1** hydrogel.

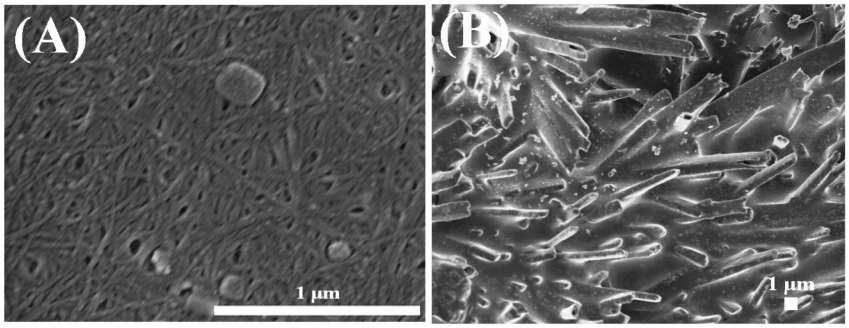
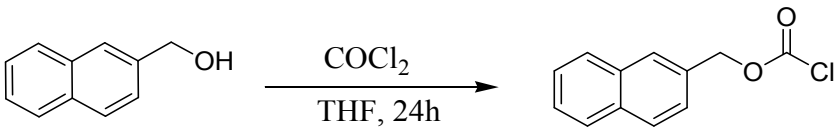


Fig. S16. SEM images of (A) isolated Nmoc-YW-HBA showing entangled nanofibers and (B) isolated Nmoc-LY-HBA showing long fibrillar structures.

Synthesis of precursors

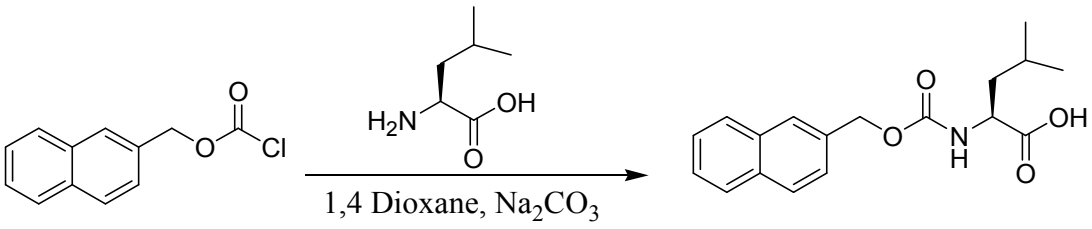
Synthesis of Naphthalene-2-methyloxy chloroformate (4)



To a stirred solution of naphthalene methanol (5 g, 31.6 mmol) in dry THF (140 mL), phosgene (39.2 mL, 75.5 mmol) was added at 0 °C. The stirring was continued at ambient temperature for 24 h. 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 **4** as crystalline product**.**

Yield = 6.8 g (30 mmol, 94.93 %). mp: 62 °C; FT-IR (KBr): ῡ 3066 (m), 1777 (s), 1601(ms), 1168 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (m, 4H), 7.52 (m, 3H), 5.45 (s, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.82, 125.7, 126.1, 126.6, 127.8, 128.1, 128.6, 130.6, 133.5, 140.9, 147.9, 150.7 ppm.

Synthesis of Nmoc-Leu-OH (5)

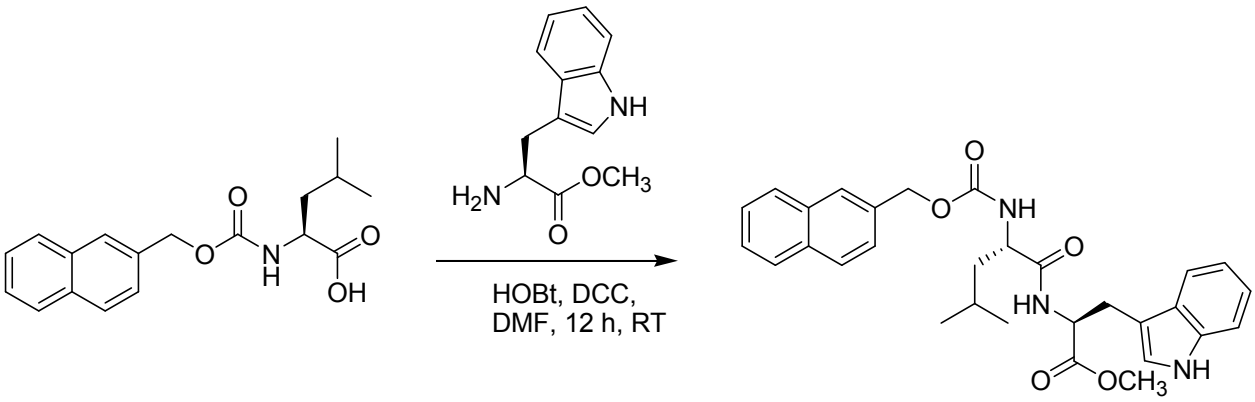


A solution of leucine (1.048 g, 8 mmol) in a mixture of 1, 4 dioxane (20 mL) and 2M sodium carbonate (26 mL) was stirred and cooled in an ice-water bath. Naphthalene-2-methoxychloroformate **4** (1.764 g, 8 mmol) was added and stirring was continued at room temperature for 12 h. Reaction mixture was 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 2M hydrochloric acid. The aqueous phase was extracted with ethyl

acetate (3 x 50 mL) and dried over Na_2SO_4. The organic layer was concentrated under vacuum to give **5** as white solid.

Yield =1.962 g (6.2 mmol, 77.5 %). mp: 79 °C; $[\alpha]_D^{25}$ = -12 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3418 (br), 3380 (br), 3053 (ms), 2960 (s), 2874 (ms), 1700 (s), 1604 (ms), 1517 (s), 1462 (ms), 1363 (ms), 1321 (ms), 1239 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.91 (t, 4H, *J* = 12.2 Hz, Nph), 7.64 (d, 1H, *J* = 7.84 Hz, NH), 7.53 (m, 3H), 5.20 (s, 2H, CH_2), 4.00 (m, 1H, $C^{\alpha}H$ of Leu), 1.47 (m, 2H, $C^{\beta}Hs$ of Leu), 1.17 (m, 1H, $C^{\gamma}H$ of Leu) 0.87 (m, 6H, $C^{\delta}Hs$ of Leu) ppm; HRMS (ESI) m/z for $C_{18}H_{21}NO_4$ $(M+Na)^+$ calcd.: 338.1363, found: 338.1386.

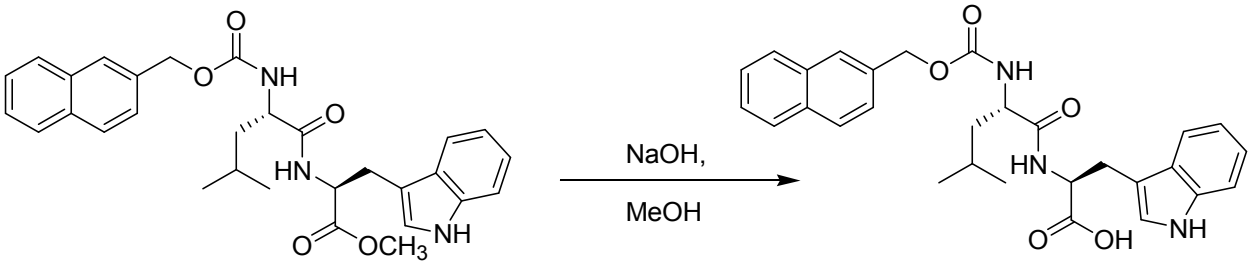
Synthesis of Nmoc-Leu-Trp-OCH_3 (6)



A solution of Nmoc-L (0.9 g, 2.8 mmol) and HOBt (0. 378 g, 2.8 mmol) was stirred in 2 mL of DMF. A neutralized solution of tryptophan methyl ester (1.42 g, 5.6 mmol) was extracted from its corresponding hydrochloride salt and concentrated to add 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 1 M HCl (2 x 30 mL), brine solution, 1 M 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 **6**. Purification was done by silica gel column (100-200 mesh) using chloroform-methanol (98:2, v/v) as eluent.

Yield =1.23 g (2.38 mmol, 82.14 %); $[\alpha]_D^{25}$ = -16 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3315 (br), 3055(ms), 2954 (s), 2869 (ms), 1738 (ms), 1709 (ms), 1659 (s), 1528 (s), 1439 (ms), 1358 (ms), 1219 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (m, 4H, Nph), 7.48 (m, 3H, Nph), 7.43 (s, 1H, NH), 7.22 (d, 1H, *J* = 8.28 Hz, NH), 7.12 (t, 1H, *J* = 8.28 Hz, Trp), 7.06 (t, 1H, *J* = 7.28 Hz, Trp), 6.90 (s, 2H, Trp), 5.21 (s, 2H, CH_2 of Nph), 4.87 (m, 1H, C^{α} H of Trp), 4.16 (m, 1H, C^{α} H of Leu), 3.65 (s, 3H, OCH_3), 3.27 (d, 2H, *J* = 4.76 Hz, C^{β} Hs of Trp), 1.93 (m, 1H, C^{β} H of Leu), 1.76 (m, 1H, C^{β} H of Leu), 1.44 (m, 1H, C^{γ} H of Leu), 0.88 (d, 6H, *J* = 5.28 Hz, C^{δ}Hs of Leu); HRMS (ESI) m/z for $C_{30}H_{33}N_3O_5$ $(M+K)^+$ calcd.: 554.2052, found: 554.2057.

Synthesis of Nmoc-Leu-Trp-OH (1)

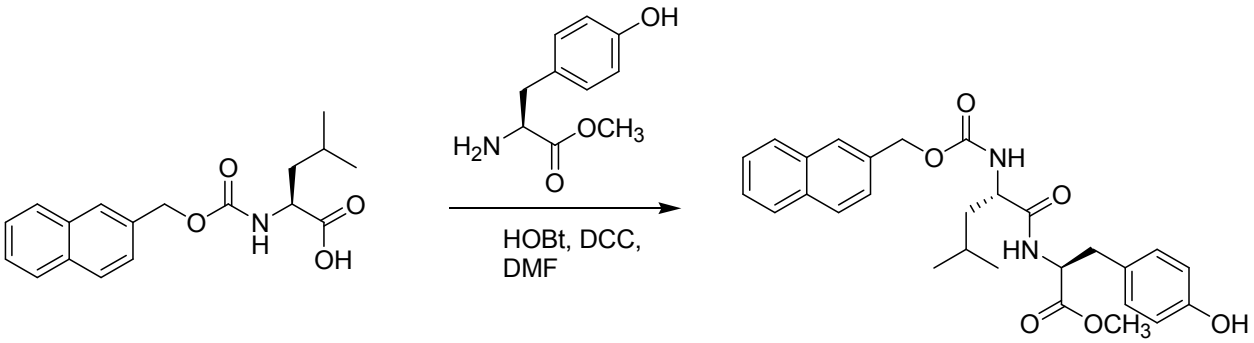


A solution of Nmoc-LW-OCH_3 **6** (0.980 g, 1.9 mmol) in 100 mL of dry MeOH was allowed to react with 10 mL 2 M NaOH solution. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for 12 h. Then, methanol was removed under vacuum. The residue was taken in 100 mL of water and washed with diethyl ether (2 x 20 mL). The pH of aqueous layer was adjusted to 2 using 2 M HCl and it was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuum to yield **1** as white solid.

Yield =0.711 g (1.4 mmol, 73.68 %); $[\alpha]_D^{25}$ = -7 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3321(br), 3056(ms), 2957 (s), 2870 (ms), 1712 (ms), 1660 (s), 1526 (s), 1457 (ms), 1337 (ms), 1255 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.84 (s, 1H, NH of Trp ring), 8.06 (d, 1H, *J* = 7.52 Hz, NH), 7.88 (t, 4H, Nph), 7.50 (m, 3H, Nph), 7.45 (d, 2H, *J* = 9.04 Hz, Trp), 7.31 (d, 1H, *J* = 8.04 Hz, NH), 7.14 (s, 1H, Trp), 7.04 (t, 1H, *J* = 7.51 Hz, Trp), 6.95 (t, 1H, *J* = 7.52 Hz, Trp), 5.17 (s, 2H, CH_2 of Nph), 4.46 (m, 1H, C^{α}H of Trp), 4.10 (m, 1H, C^{α}H of Leu), 3.15 (dd, 1H, *J* = 5.24, 5 Hz, C^{β} H of Trp), 3.05 (dd, 1H, *J* = 8, 7.76 Hz, C^{β} H of Trp), 1.60 (m, 1H, C^{γ}H of Leu),

1.40 (m, 2H, C^{β} Hs of Leu), 0.83 (m, 6H, C^{δ}Hs of Leu); ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.1, 172.2, 170.3, 155.8, 135.9, 134.6, 132.6, 132.4, 127.9, 127.6, 127.2, 126.2, 126.03, 125.6, 123.5, 120.8, 118.3, 118.1, 111.2, 109.5, 65.44, 59.71, 53.00, 33.30, 26.90, 24.11, 23.02, 21.35, 20.72; HRMS (ESI) m/z for $C_{29}H_{31}N_3O_5$ $(M+H)^+$ calcd.: 502.2336, found: 502.2361.

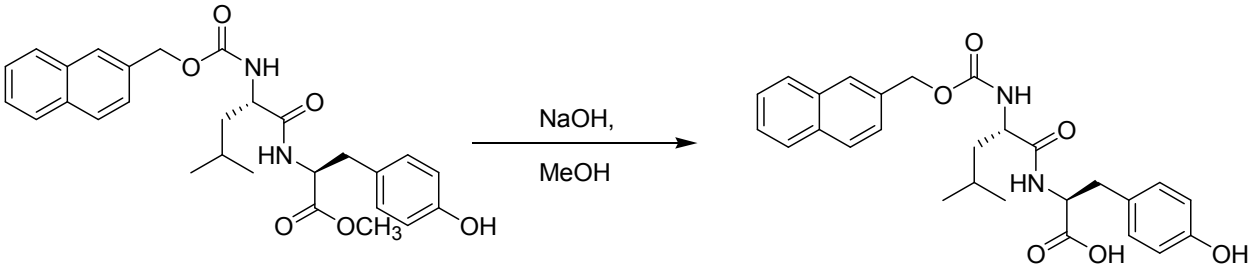
Synthesis of Nmoc-Leu-Tyr-OCH_3 (7)



A solution of Nmoc-L **5** (0.958 g, 3 mmol) and HOBt (0.405 g, 3 mmol) was stirred in 2 mL of DMF. A neutralized solution of tyrosine methyl ester (1.17 g, 6 mmol) was extracted from its corresponding hydrochloride salt and concentrated to add to the reaction mixture followed by DCC (0.639 g, 3.1 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 1 M HCl (2 x 30 mL), brine solution, 1 M 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. Purification was done by silica gel column (100-200 mesh) using chloroform- methanol (98:5, v/v) **7** as eluent.

Yield = 1.39 g (2.8 mmol, 93.33 %); $[\alpha]_D^{25}$ = -13 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3313 (br), 3057(ms), 2955 (s), 2869 (ms), 17 39 (ms), 1706 (ms), 1658 (s), 1616 (ms), 1515 (s), 1443 (ms), 1365 (ms), 1227 (s) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (m, 4H, Nph), 7.27 (m, 3H, Nph), 7.22 (s, 1H, NH), 7.05 (s, 1H, NH), 6.69 (d, 2H, *J* = 7.28 Hz, Tyr), 6.44 (d, 2H, *J* = 8.04 Hz, Tyr), 5.04 (s, 2H, CH_2 of Nph), 4.60 (m, 1H, C^{α} H of Tyr), 3.99 (m, 1H, C^{α} H of Leu), 3.50 (s, 3H, OCH_3), 2.84 (dd, 1H, *J* = 5.24, 5 Hz, C^{β}H of Tyr), 2.76 (m, 1H, C^{β} H of Tyr), 1.26 (m, 1H, C^{γ} H of Leu), 1.11-1.04 (m, 2H, C^{β} Hs of Leu), 0.69 (d, 6H, *J* = 5.04 Hz, C^{δ} Hs of Leu); HRMS (ESI) m/z for $C_{28}H_{32}N_2O_6$ $(M+Na)^+$ calcd.: 515.2153, found: 515.2158.

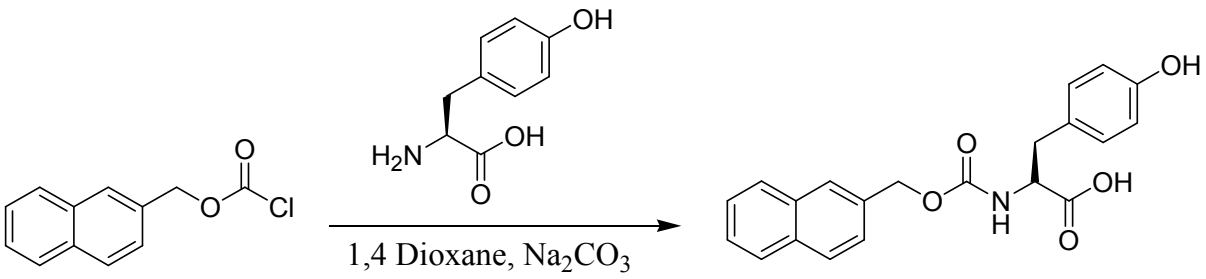
Synthesis of Nmoc-Leu-Tyr-OH (2)



A solution of Nmoc-LW-OCH_3 **7** (0.950 g, 1.9 mmol) in 100 mL of dry MeOH was allowed to react with (10 mL) solution of 2M NaOH. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for 12 h. Then, methanol was removed under vacuum. The residue was taken in 100 mL of water and washed with diethyl ether (2 x 20 mL). The pH of aqueous layer was adjusted to 2 using 2 M HCl and it was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuum to yield **2** as white solid.

Yield =0.788 g (1.6 mmol, 84.21 %); $[\alpha]_D^{25}$ = -3 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3318 (br), 3058(ms), 2958 (s), 1711 (s), 1658 (s), 1515 (s), 1445 (ms), 1364 (ms), 1336 (ms), 1235 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 9.19 (s, 1H, O<u>H</u> of Tyr ring), 7.97 (d, 1H, J = 6.76 Hz), 7.89 (m, 4H, Nph), 7.50 (m, 3H, Nph), 7.42 (d, 1H, *J* = 8.04 Hz, NH), 6.99 (d, 2H, *J* = 7 Hz, Tyr), 6.64 (d, 2H, *J* = 5.76 Hz, Tyr), 5.18 (s, 2H, CH_2 of Nph), 4.34 (m, 1H, C^{α} H of Tyr), 4.06 (m, 1H, C^{α} H of Leu), 2.89 (m, 1H, C^{β} H of Tyr), 2.80 (m, 1H, C^{β} H of Tyr), 1.58 (m, 1H, C^{γ} H of Leu), 1.38 (m, 2H, C^{β} Hs of Leu), 0.84 (m, 6H, C^{δ} Hs of Leu); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.8, 172.1, 155.8, 134.6, 132.6, 132.4, 130.0, 127.9, 127.6, 127.5, 127.3, 126.2, 126.1, 126.0, 125.6, 114.9, 65.45, 53.57, 53.03, 35.86, 24.10, 22.96, 21.39; HRMS (ESI) m/z for $C_{27}H_{30}N_2O_6$ $(M+Na)^+$ calcd.: 501.1996, found: 501.1996.

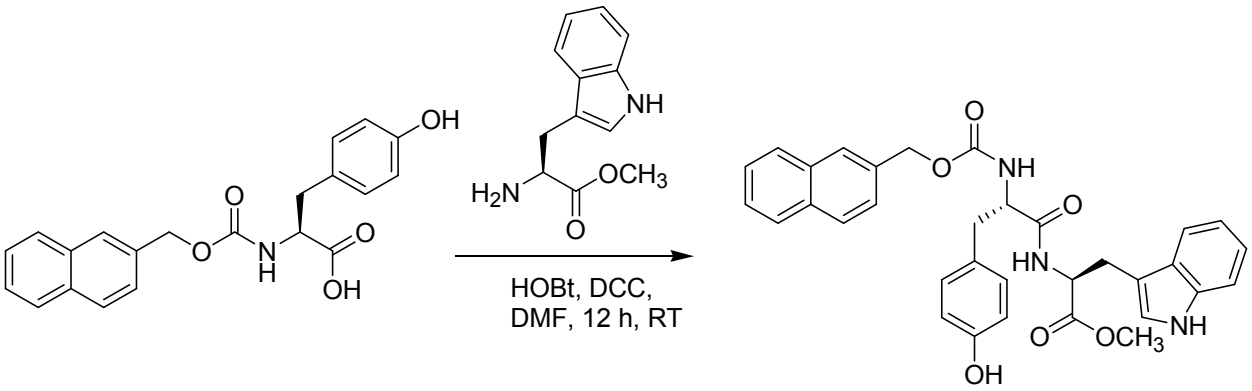
Synthesis of Nmoc-Tyr-OH (8)



A solution of Tyrosine (0.724 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 **4** (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. The organic layer was concentrated under vacuum to give **8** as colorless oil.

Yield= 1.124 g (3 mmol, 75 %). $[\alpha]_D^{25}$ = -7 (c = 1, MeOH); FT-IR (KBr): ῡ 3331 (br), 3055 (br), 2928 (ms), 1709 (s), 1612 (ms), 1513 (s), 1445 (s), 1367 (ms), 1336 (ms), 1225 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (m, 4H), 7.38 (m, 3H, Nph), 7.32 (s, 1H, NH), 6.86 (d, 2H, *J* = 8.04 Hz, Tyr), 6.57 (d, 2H, *J*= 8.04 Hz, Tyr), 5.17 (s, 2H, CH_2 of Nph), 4.57 (m, 1H, C^{α} H of Tyr), 2.95 (dd, 1H, *J* = 4.52, 4 Hz, C^{β} Hs of Tyr), 2.86 (dd, 1H, *J* = 5, 5 Hz, C^{β} Hs of Tyr) ppm; HRMS (ESI) m/z for $C_{21}H_{19}NO_5$ $(M+Na)^+$ calcd.: 388.1155 found: 388.1174.

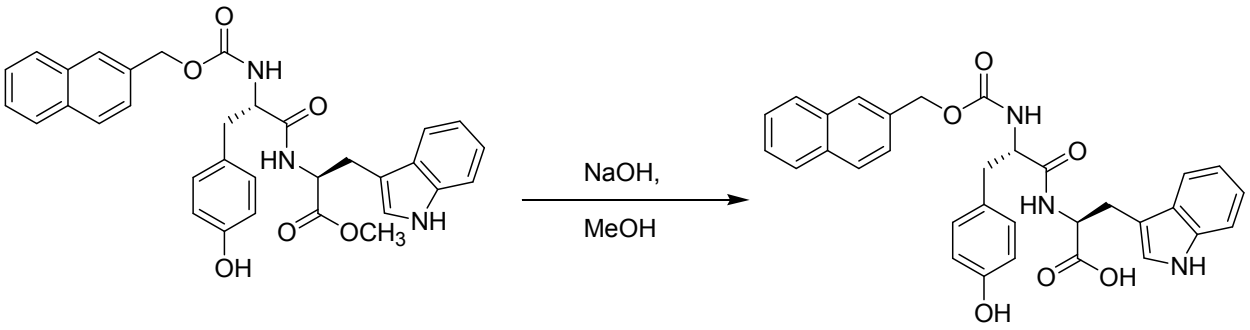
Synthesis of Nmoc-Tyr-Trp-OCH_3 (9)



A solution of Nmoc-Y **8** (0.642 g, 1.75 mmol) and HOBt (0. 236 g, 1.75 mmol) was stirred in 2 mL of DMF. A neutralized solution of tryptophan methyl ester (0.889 g, 3.5 mmol) was extracted from its corresponding hydrochloride salt and concentrated to add to the reaction mixture followed by DCC (0.371 g, 1.77 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 1 M HCl (2 x 30 mL), brine solution, 1 M 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 **9** as white solid product. Purification was done by silica gel column (100-200 mesh) using chloroform- methanol (98:5, v/v) as eluent.

Yield = 0.500 g (0.88 mmol, 50.28 %). $[\alpha]_D^{25}$ = -18 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3345(br), 3055(ms), 2951(s), 2852 (ms), 1729 (s), 1661 (s), 1515 (s), 1440 (ms), 1358 (ms), 1223 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 1H), 8.87 (s, 1H), 7.75 (m, 4H, Nph), 7.69 (s, 1H, Nph), 7.42 (m, 2H, Nph), 7.31(d, 1H, *J* = 8 Hz), 7.16 (d, 2H, *J*= 9.04 Hz), 7.05 (t, 1H, *J* = 7.52 Hz, Trp), 6.96 (t, 1H, *J* = 7.56 Hz, Trp), 6.90 (d, 2H, *J*= 7.28 Hz), 6.68 (s, 1H, Trp), 6.16 (d, 2H, *J*= 7.52 Hz), 5.12 (s, 2H, CH_2 of Nph), 4.75 (m, 1H, $C^{\alpha}H$ of Trp), 4.27 (m, 1H, $C^{\alpha}H$ of Tyr), 3.54 (s, 3H, OCH_3), 3.15 (d, 2H, *J* = 3.76 Hz, $C^{\beta}Hs$ of Trp), 2.85 (m, 2H, $C^{\beta}Hs$ of Tyr). HRMS (ESI) m/z for $C_{33}H_{31}N_3O_6$ $(M+Na)^+$ calcd.: 588.2105, found: 588.2131.

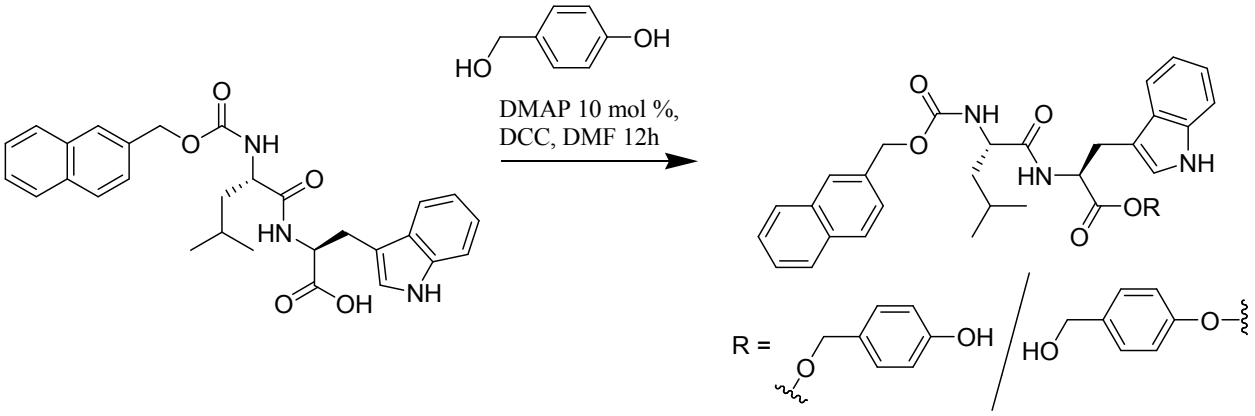
Synthesis of Nmoc-Tyr-Trp-OH (3)



A solution of Nmoc-YW-OCH_3 (0.450 g, 0.79 mmol) in 100 mL of dry MeOH was allowed to react with 10 mL 2 M NaOH solution. The progress of the reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for 12 h. Then, methanol was removed under vacuum. The residue was taken in 100 mL of water and washed with diethyl ether (2 x 20 mL). The pH of aqueous layer was adjusted to 2 using 2 M HCl and it was extracted with ethyl acetate (3 x 30 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated in vacuum to yield **3** as white solid.

Yield = 0.401 g (0.72 mmol, 91.13 %). $[\alpha]_D^{25}$ = - 6 (*c* = 1, MeOH); FT-IR (KBr): ῡ 3390 (br), 3056(ms), 2926 (s), 2854 (m), 1710 (s), 1659 (s), 1515 (s), 1441 (ms), 1358 (ms), 1339 (ms), 1234 (s) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 1H, NH of Trp ring), 9.16 (s, 1H, O<u>H</u> of Tyr), 8.22 (d, 1H, *J* = 7.52 Hz), 7.85 (m, 4H, Nph), 7.76 (s, 1H, Nph), 7.49 (m, 2H, Nph), 7.42 (d, 1H, *J* =8.56 Hz, NH), 7.35 (m, 2H, *J* = 8.56 Hz, Trp), 7.16 (s, 1H, Trp), 7.04 (d, 2H, *J* = 8.28 Hz, Tyr), 6.97 (t, 1H, *J*= 7.52 Hz, Trp), 6.81 (d, 1H, *J* = 8.56 Hz, Trp), 6.61 (d, 2H, *J* = 8.28 Hz, Tyr), 5.09 (s, 2H, CH_2 of Nph), 4.50 (m, 1H, $C^{\alpha}H$ of Trp), 4.22 (m, 1H, $C^{\alpha}H$ of Tyr), 3.19 (dd, 1H, *J* = 5.52, 5 Hz, $C^{\beta}H$ of Trp), 3.07 (dd, 1H, *J*= 7.8, 6.76 Hz, $C^{\beta}H$ of Trp), 2.87 (dd, 1H, *J*= 3.24, 2.76 Hz, $C^{\beta}H$ of Tyr), 2.59 (m, 1H, *J*= 3.24, 2.76 Hz, $C^{\beta}Hs$ of Tyr); ^{13}C NMR(100 MHz, DMSO-d_6): δ 173.1, 172.2, 155.8, 135.9, 134.6, 132.6, 132.4, 127.9, 127.6, 127.5, 127.2, 126.2, 126.0, 126.06, 125.6, 123.5, 120.8, 118.3, 118.1, 111.1, 109.5, 65.4, 59.7, 53.0, 52.7, 33.3, 26.9, 24.1, 23.02, 21.3. 20.7; HRMS (ESI) m/z for $C_{32}H_{29}N_3O_6$ $(M+H)^+$ calcd.: 552.2129, found: 552.2125.

Synthesis of Nmoc-Leu-Trp-HBA (10)



A solution of Nmoc-LW (0.419 g, 0.83 mmol) and DMAP (0.0102 g, 10 mol%) was stirred in 2 mL of DMF. *p*-hydroxybenzyl alcohol (0.153 g, 1.24 mmol) was added to the reaction mixture followed by DCC (0.173 g, 0.84 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 1 M HCl (2 x 30 mL), brine solution, 1 M 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 **10** as white solid product. Purification was done by silica gel column (100-200 mesh) using chloroform-methanol (98:2, v/v) as eluent.

Yield = 0.289 g (0.47 mmol, 56.62 %); FT-IR (KBr): ῡ 3318 (br), 3056(s), 2926 (s), 2854 (ms), 1713 (s), 1662 (s), 1511 (s), 1457 (ms), 1341 (ms), 1231 (ms), 1167 (ms) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 10.9 (s, 1H, NH of Trp ring), 10.8 (s, 1H, NH of Trp ring), 8.38 (d, 1H, *J*= 7.04 Hz), 7.87 (m, 8H, Nph), 7.55 (d, 1H, *J*= 5.76 Hz), 7.49 (m, 6H, Nph), 7.34 (s, 2H, Trp), 7.24 (d, 2H, *J*= 7.04 Hz, Trp), 7.13 (d, 2H, *J*= 8.56 Hz, Trp), 7.07 (t, 2H, *J*= 7.80 Hz, Trp), 6.98 (t, 2H, *J*= 7 Hz, Trp), 6.88 (d, 2H, *J*= 7.52 Hz, HBA), 6.76 (d, 2H, *J*= 7.8 Hz, HBA), 5.18 (s, 4H, CH_2 of Nph), 5.16 (s, 2H, CH_2 of HBA), 4.68 (m, 1H, $C^{\alpha}H$ of Trp), 4.55 (m, 1H, $C^{\alpha}H$ of Trp), 4.45 (m, 1H, $C^{\alpha}H$ of Leu), 4.16 (m, 1H, $C^{\alpha}H$ of Leu), 3.20 (m, 4H, $C^{\beta}Hs$ of Trp), 1.58 (m, 2H, $C^{\gamma}Hs$ of Leu), 1.41 (m, 4H, $C^{\beta}Hs$ of Leu), 0.81 (m, 12H, $C^{\delta}Hs$ of Leu); HRMS (ESI) m/z for $C_{36}H_{38}N_3O_6$ $(M+H)^+$ calcd.:608.2755, found: 608.2750.

^{1}H NMR, ^{13}C NMR and HRMS spectra

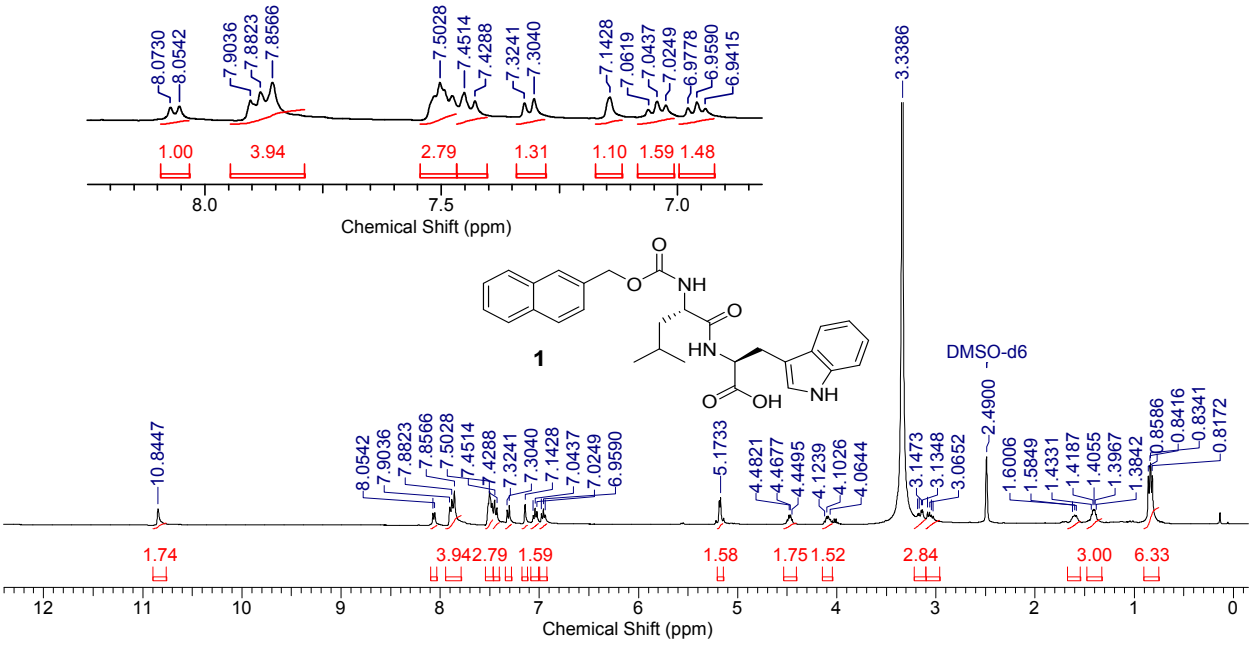


Fig. S17. ^{1}H NMR spectrum of Nmoc-LW **1** in DMSO-d_6.

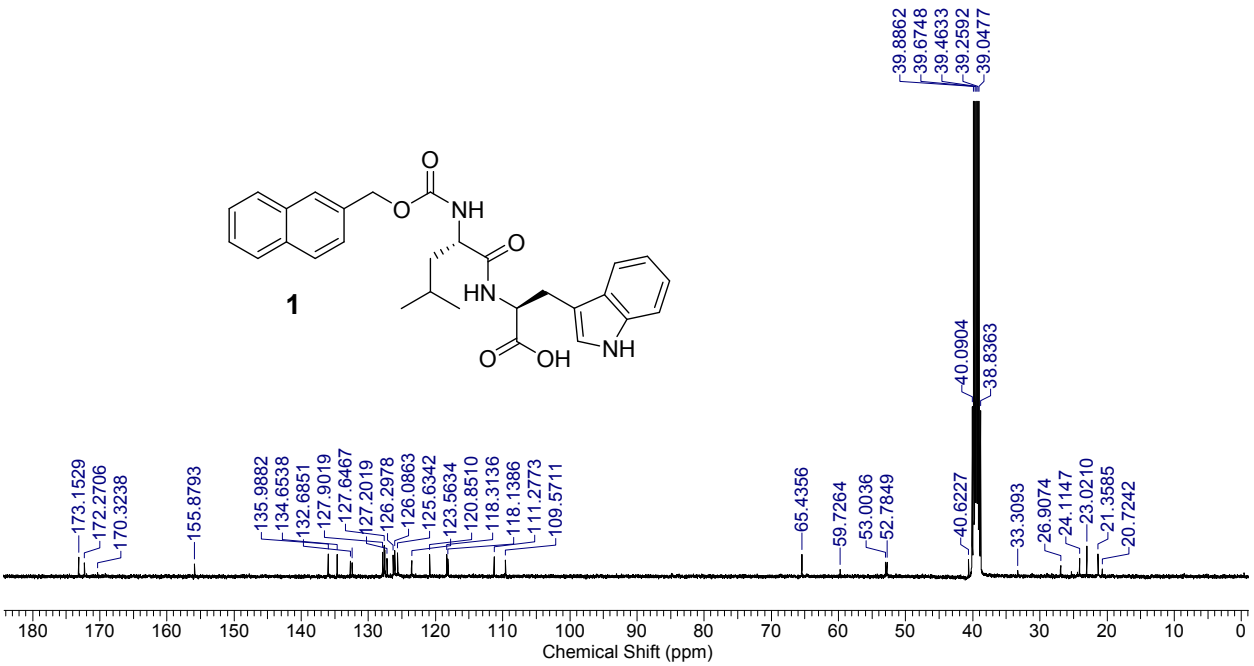


Fig. S18. ^{13}C NMR spectrum of Nmoc-LW **1** in DMSO-d$_6$.

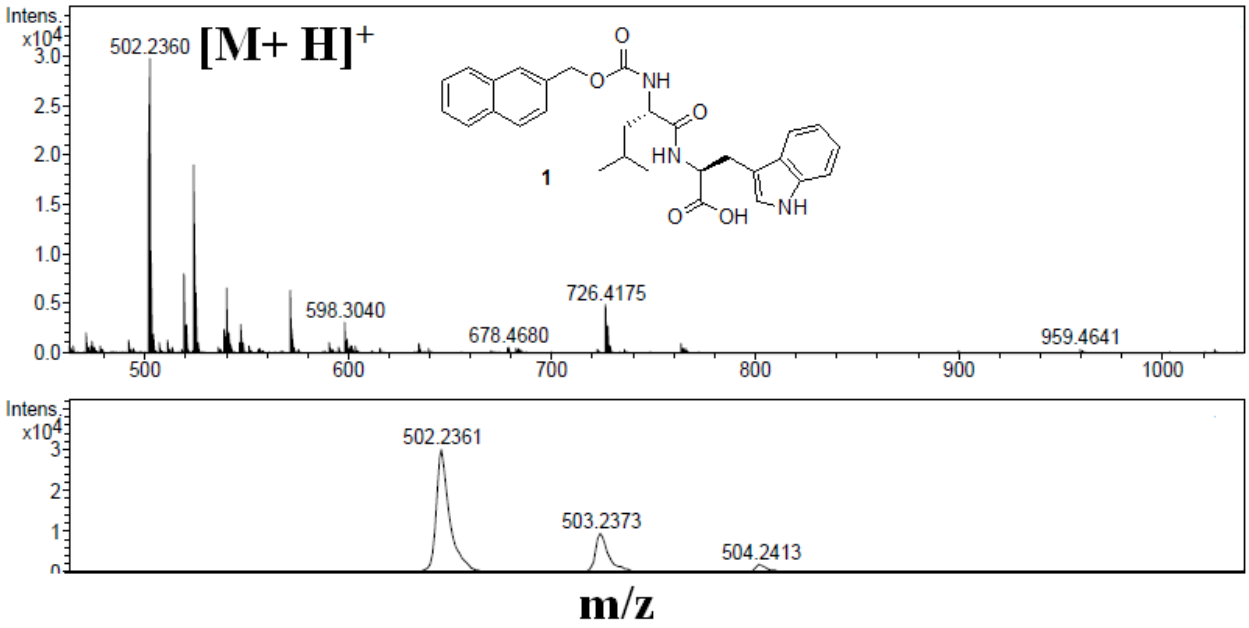


Fig. S19. ESI-MS spectra of Nmoc-LW **1**.

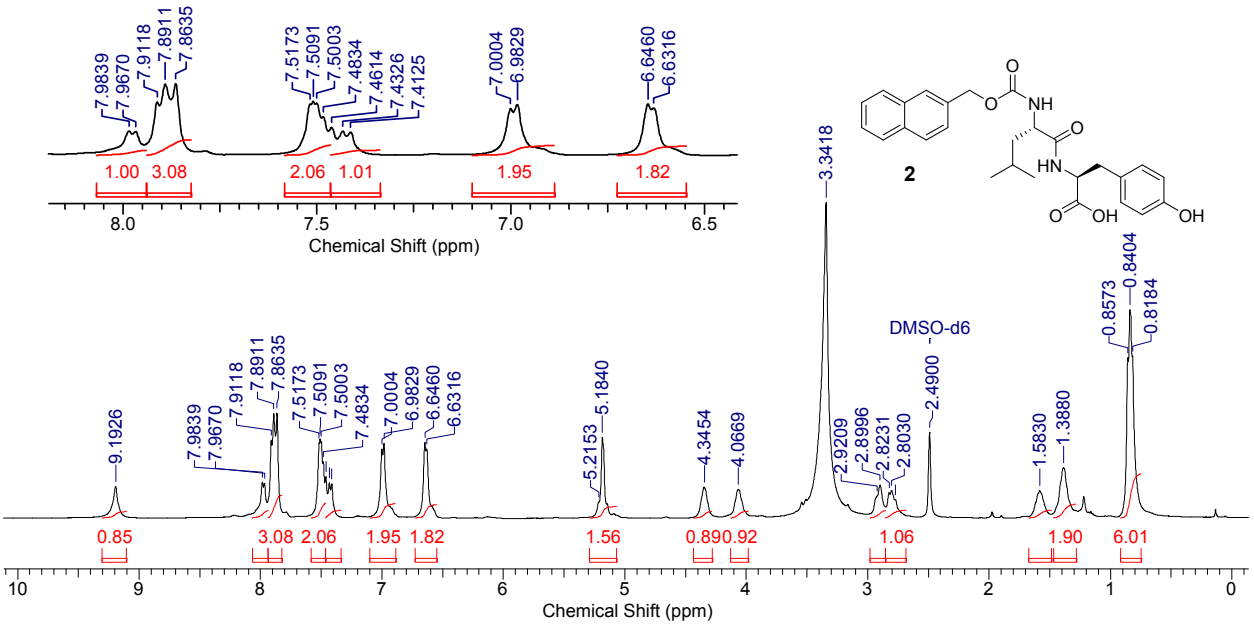


Fig. S20. ^{1}H NMR spectrum of Nmoc-LY **2** in DMSO-d_6.

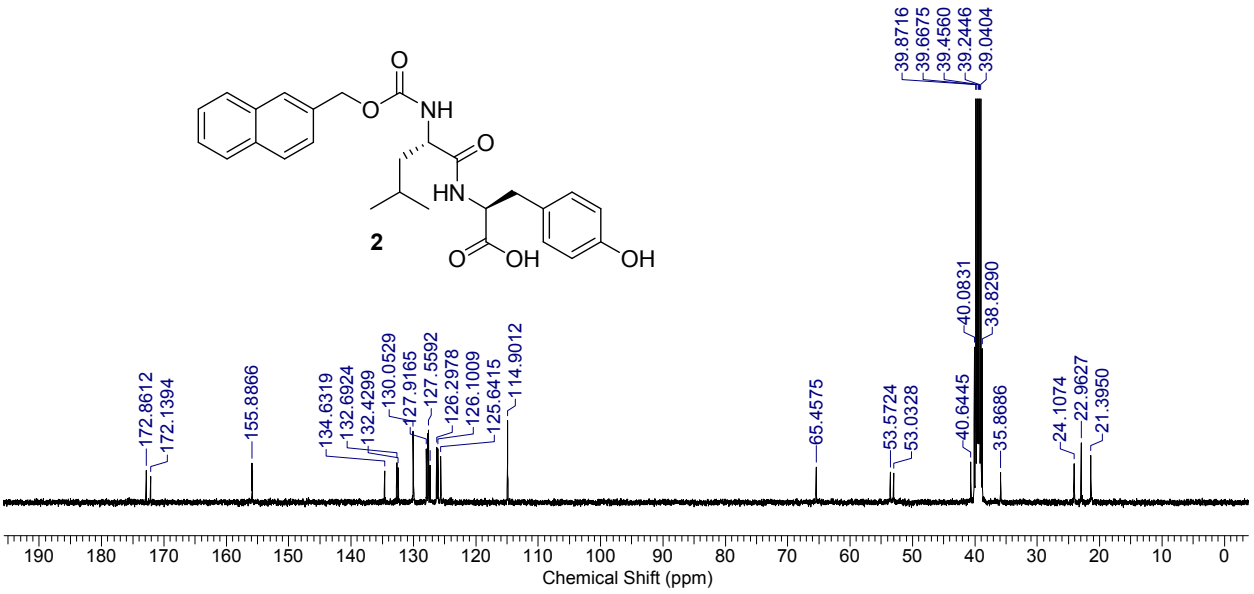


Fig. S21. ^{13}C NMR spectrum of Nmoc-LY **2** in DMSO-d_6.

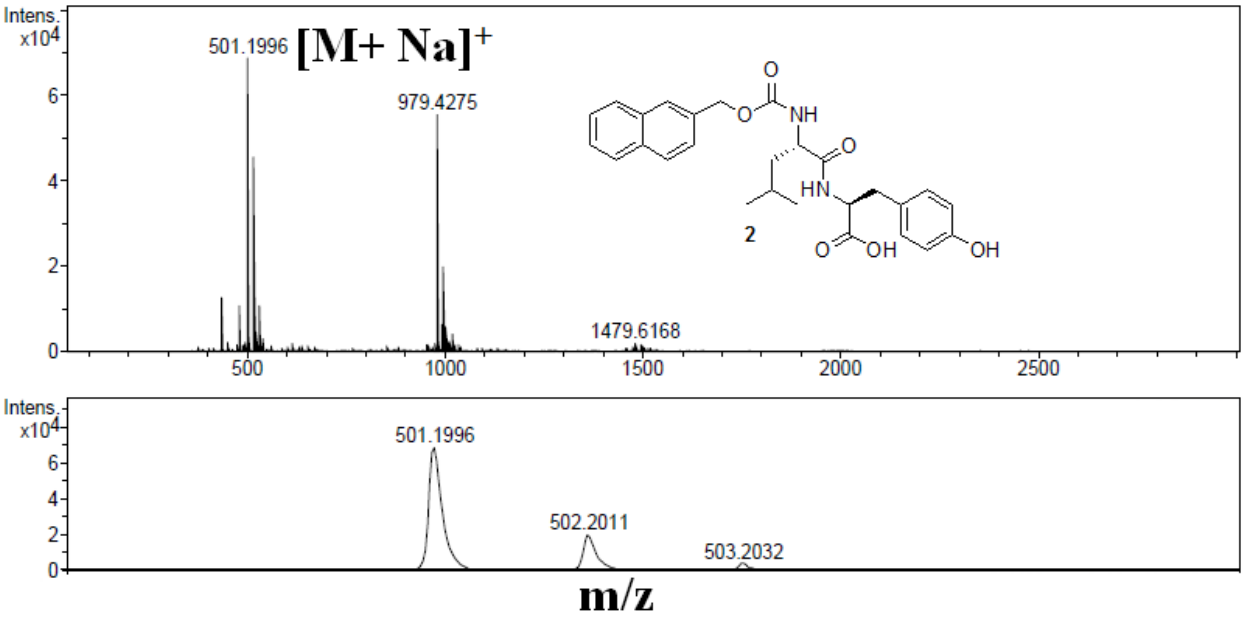


Fig. S22. ESI-MS spectra of Nmoc-LY **2**.

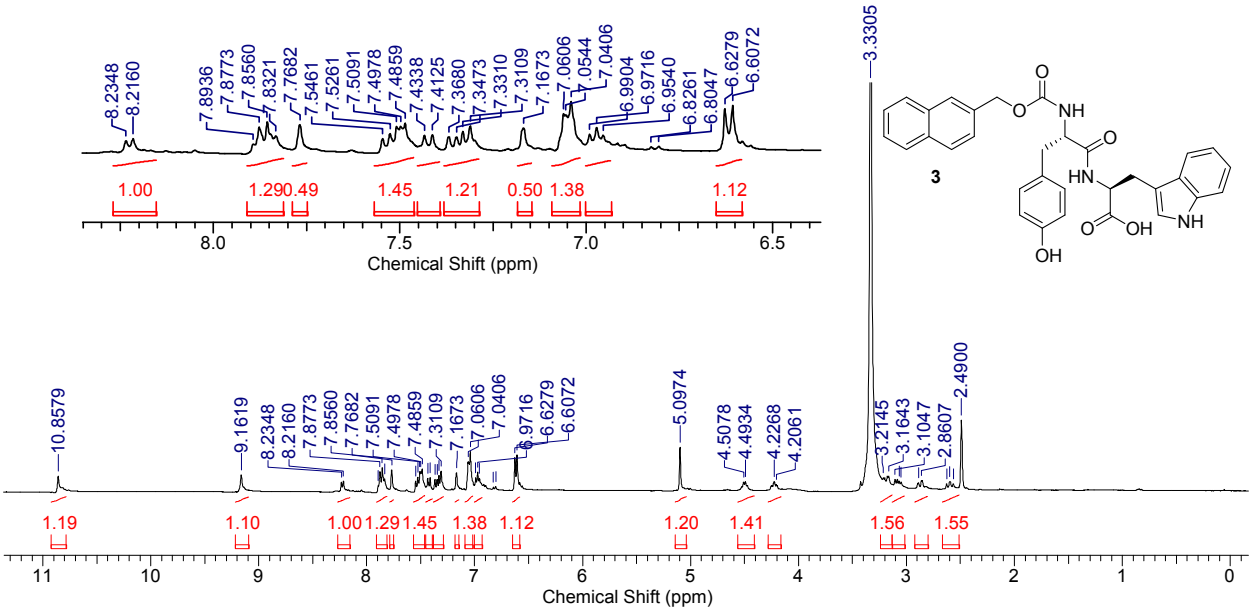


Fig. S23. ^{1}H NMR spectrum of Nmoc-YW **3** in DMSO-d_6.

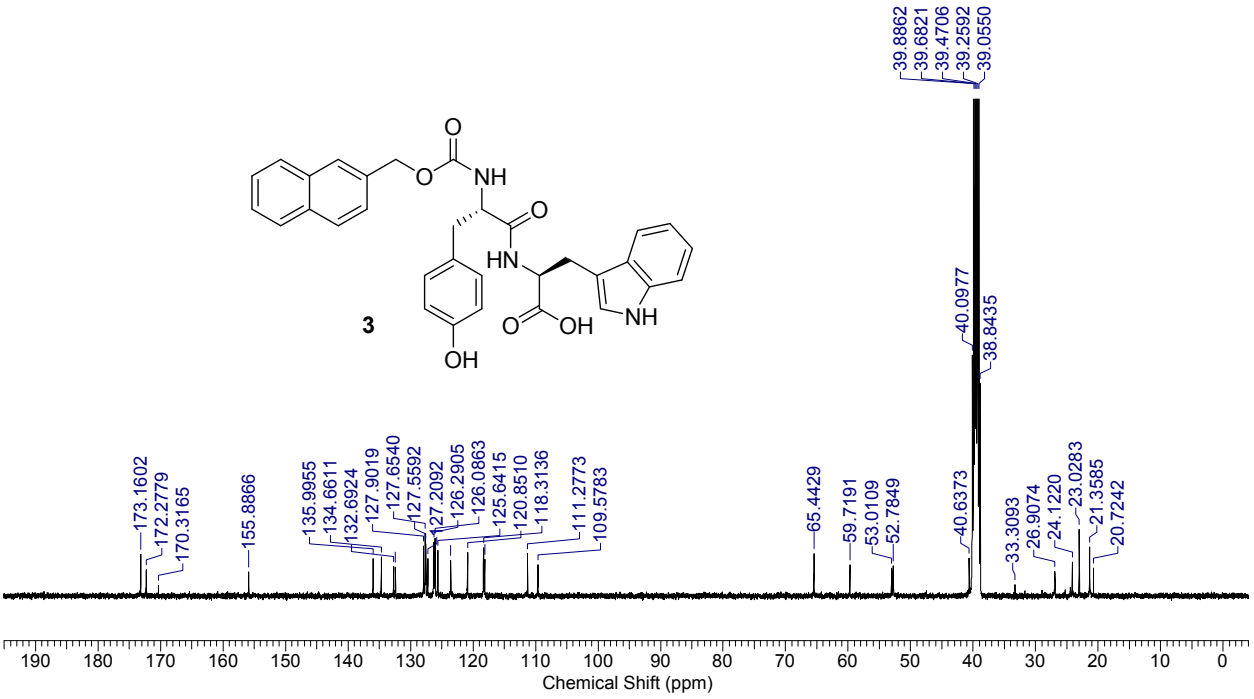


Fig. S24. ^{13}C NMR spectrum of Nmoc-YW **3** in DMSO-d_6.

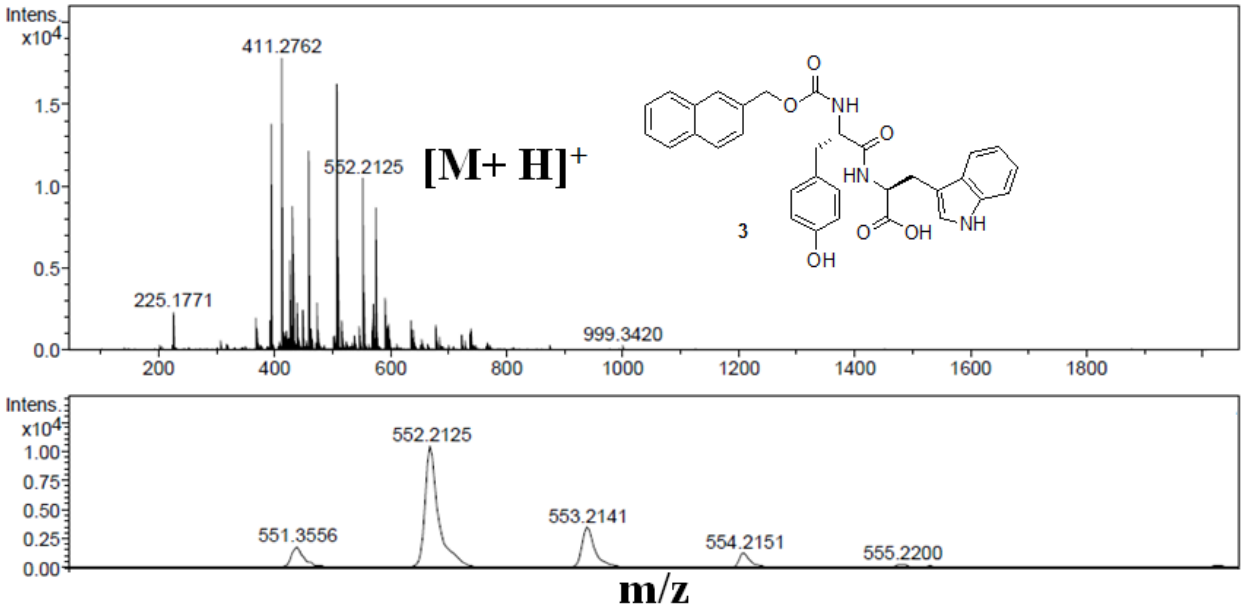


Fig. S25. ESI-MS spectra of Nmoc-YW **3**.

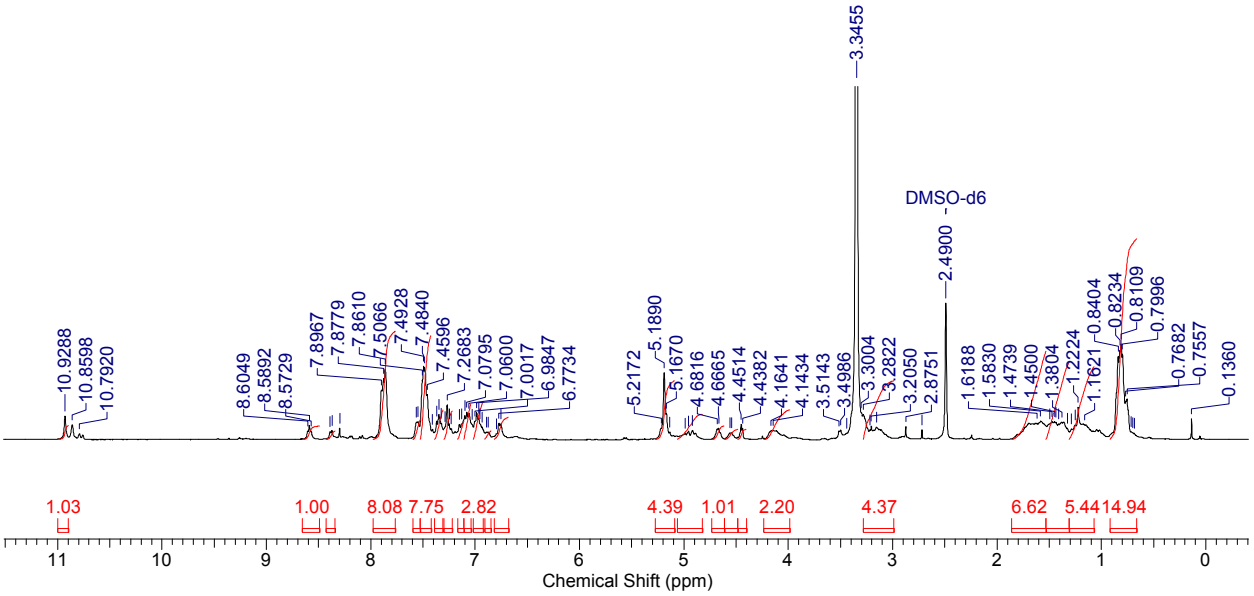


Fig. S26. ^{1}H NMR spectrum of chemically synthesized Nmoc-LW-HBA in DMSO-d_6.

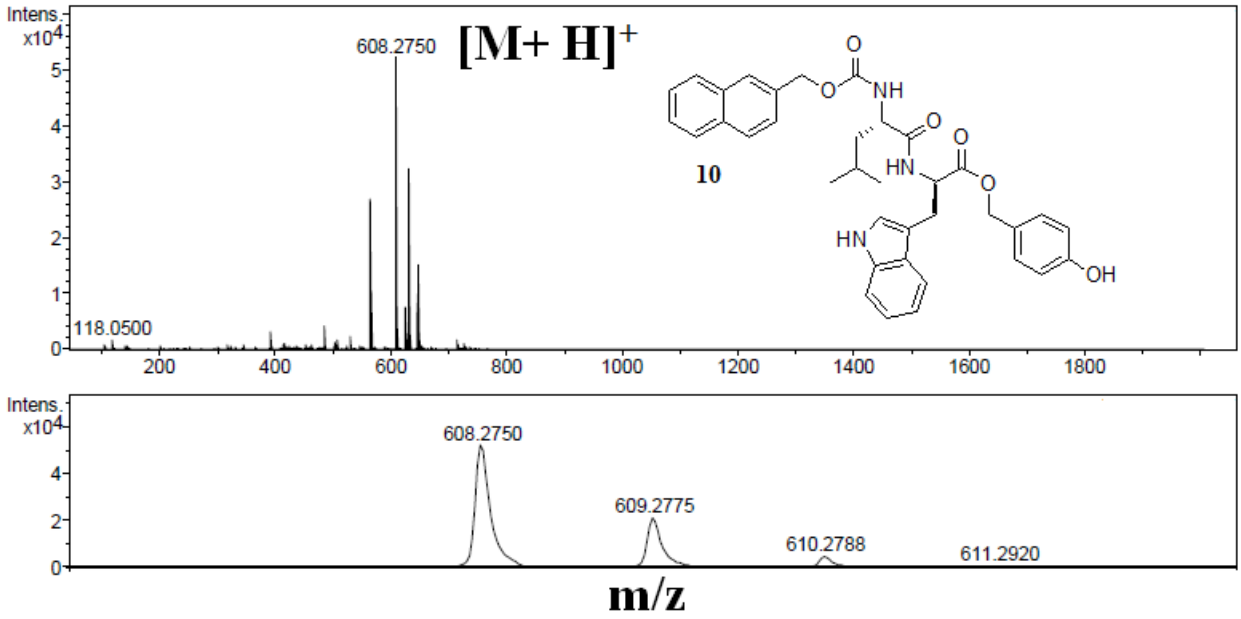


Fig. S27. ESI-MS spectrum of chemically synthesized Nmoc-LW-HBA **10**.