Supporting Information to:

Host-guest systems based on collagen-like triple-helix hybridization

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TABLE OF CONTENTS

1. **Samples Preparation.**

Peptides solutions were prepared in a 10 mM phosphate buffer (pH 7.2) for all experiments. For the 1:1 host-guest mixtures, melting temperatures were obtained with 0.1 mM solutions of each partner. For the hosts alone or guests alone, 0.1 mM solutions were used whereas for the template-free peptides mixtures the final concentration of the guest was 0.1 mM and 0.2 mM for Ac6 or Ac7. For titrations, one sample for each ratio was prepared. The partners were mixed in such a way that the final concentration of the host was 21, 5, or 1 µM. Unfolding studies and titrations were performed with preheating: peptides were mixed in the desired ratios, heated 15 min at 85°C, slowly cooled to 20°C at 1°C min^{-1} and then incubated overnight at 20°C before performing the experiments.

2. Spectroscopy

Circular Dichroism Spectroscopy. CD measurements were performed with a Jasco-J-720W spectropolarimeter equipped with a Julabo temperature control system, using quartz cells with a path length of 1, 0.2, 0.1 or 0.05 cm depending on the signal saturation, a scan speed of 50 nm $min^{-1}$and a resolution of 0.5 nm. Thermal unfolding curves were obtained by monitoring the decrease in ellipticity at 225 or 226 nm at a heating rate of 10°C h^{-1} for all systems. The folded fraction was calculated for the melting curves according to the following equation: $F_T = ([\Theta]_T - [\Theta]_{unfolded})/([\Theta]_{folded} - [\Theta]_{unfolded})$, where F_T is the folded fraction at temperature T, $[\Theta]_T$ is the molar residue ellipticity (MRE) at T, $[\Theta]_{unfolded}$ and $[\Theta]_{folded}$ are the MRE of the folded and unfolded forms, respectively. KaleidaGraph 4.0 software was used to calculate the first derivative of the folded fraction versus temperature. The minimum of theses derivatives, which corresponds to the steepest slope, indicates the melting temperature (T_m). Melting temperatures were given with an estimated error of 2°C. When only CD was used to measure the binding constant, samples preparations and measurements were performed twice.

Fluorescence spectroscopy. Fluorescence measurements were performed with a Perkin-Elmer LS55 spectrometer equipped with a Julabo temperature control system set at 20°C, using quartz cell with a 1 cm path length. Titration curves were obtained by monitoring the increase in intensity at the wavelength of maximum emission intensity with a scan speed of 300 nm/min.

UV spectroscopy. UV measurements were performed with a Shimadzu UV-visible 2550 spectrometer equipped with a Julabo temperature control system, using quartz cells with a 1 cm path length. For anthracene-derived templates, the increase of the absorption at 250 nm was monitored at 20°C with a scan speed of 300 nm/min and a 0.5 nm resolution.

3. CD Studies

Template-free peptides. Since each peptide involved in our design possesses six triads, the free peptides themselves may be able to self assemble into triple helices. Ac6 and G6o and a 2:1 mixture of both were therefore characterized by CD studies at pH 7.2 using 10 mM phosphate buffer. Both CD spectra of AcD-6o and AcD-6o/AcR-6o show a large negative peak around 200 nm and a small positive peak around 225 nm (Figure S1a). These features mean that they adopt a polyproline II or a collagen-like triple-helical structure in solution. For AcR-6o, more surprisingly, whereas the substitution of O by R is supposed to have a smaller destabilizing effect on the helical conformation than the P/D substitution, the positive cotton effect at 225 nm is absent, suggesting that it is not folded in these conditions. In order to elucidate more in details the conformation of AcD-6o and AcD-6o/AcR-6o mixture, thermal denaturation studies monitored by CD were performed. Ac6 shows a linear decrease of the molar residue ellipticity (MRE) instead of a cooperative unfolding as observed for normal triple helix (Figure S1b). It means that above 5°C AcD-6o adopts a weak polyproline II conformation or a disordered structure. When AcD-6o and AcR-6o were mixed in a 2:1 ratio, followed by preheating, a cooperative denaturation curve was observed leading to a single melting temperature (T_m) of 20°C. Heterotrimeric species were therefore formed revealing that the positively charged (AcR-6o) and negatively charged (AcD-6o) peptides interact favorably by electrostatic interactions to allow the formation of a stable heterotrimeric triple helix (Figure S1b). This kind of stabilizing electrostatic interactions in AAB heterotrimeric species was already reported by Gauba & al with glutamic acid and arginines side chains salt bridges.[1]

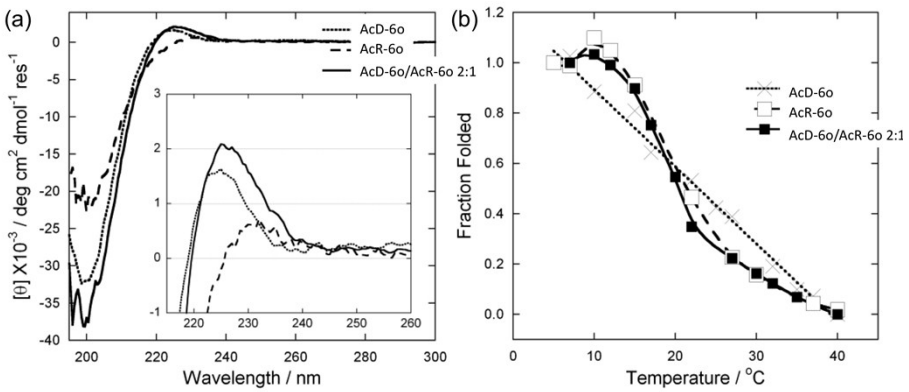


Figure S1. Circular dichroism of the non templated peptides. (a) CD spectra of AcD-6o (200 µM), AcR-6o (100 µM) and the 2:1 mixture AcD-6o/AcR-6o (200/100 µM) at 5°C. The inset is a zoom of the 215-260 nm region. (b) Thermal denaturations monitored by CD at 225 nm showing a linear decrease for AcD-6o and a single cooperative transition for the 2:1 mixture AcD-6o/AcR-6o. The inflection point corresponds to the melting temperature (T_m) of the triple helix.

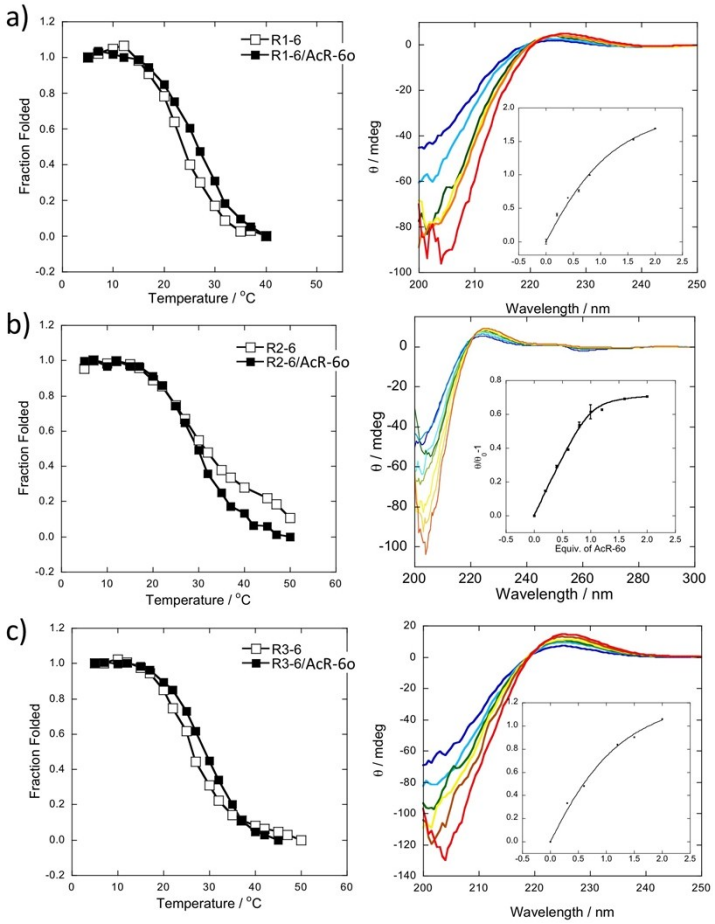


Figure S2. Thermal denaturation of Hosts alone and Host-Guest 1:1 mixtures (left) and titration experiments (right) for a) R1-6 b) R2-6 and c) R3-6 monitored by circular dichroism at 225 nm. Unfolding curves show in each case a single cooperative transition. The titrations were performed by CD spectroscopy at 20°C and the insets show the titration curves monitored at 225 nm for [R1-6] = 5 µM, [R2-6] = 5 µM and [R3-6] = 21 µM.

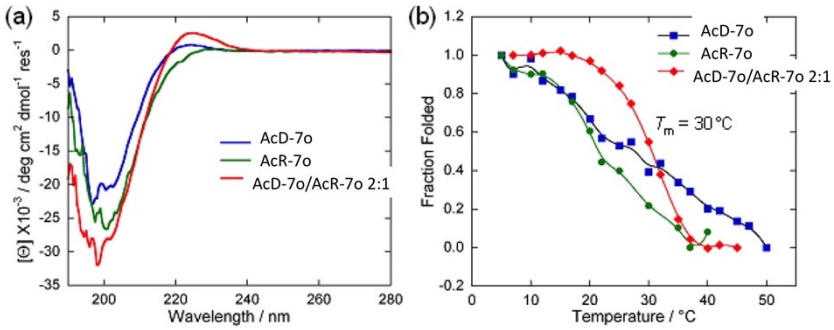


Figure S3. Circular dichroism data for the non templated peptides having 7 triads (a) CD spectra of AcD-7o (200 µM), AcR-7o (100 µM) and the 2:1 mixture AcD-7o/AcR-7o (200/100 µM) at 5°C. (b) Thermal denaturations monitored by CD at 225 nm showing a linear decrease for AcD-7 and G7o, and a single cooperative transition for the 2:1 mixture Ac7/G7o. The inflection point corresponds to the melting temperature (T_m) of the triple helix which is 30°C in this case.

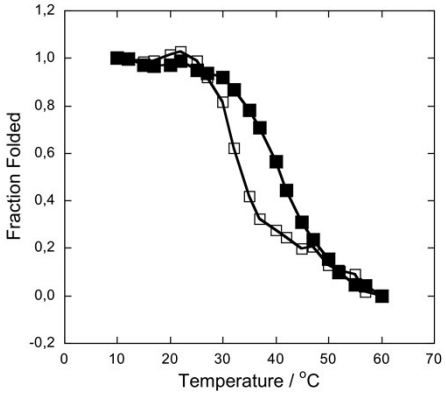


Figure S4. Thermal denaturations monitored by CD at 225 nm showing a single cooperative transition for R2-7 alone (open squares) and the 1:1 mixture R2-7/AcR-7o (filled squares). The inflection points correspond to the melting temperature (T_m) of the triple helix which are 30°C and 40°C for R2-7 alone and R2-7/AcR-7o 1:1 mixture respectively.

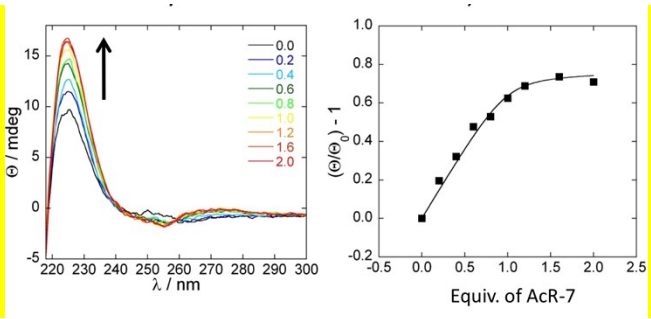


Figure S5. Titration curves obtained upon addition of AcR-7 to a 5 µM solution of R2-7 monitored by CD spectroscopy at 225 nm in phosphate buffer (10 mM, pH 7.2) at T = 20°C. The 1:1 fitting leads to an estimated apparent binding constant of 5.6 ± 4.3 10^6 M^{-1}.

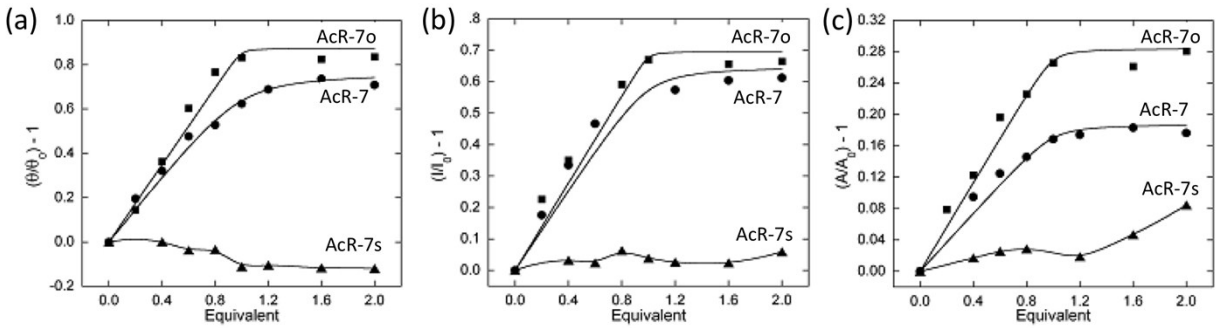


Figure S6. Titration curves obtained upon addition of AcR-7o, AcR-7 and AcR-7s to a 5 µM solution of R2-7 monitored by (a) CD spectroscopy (b) fluorescence spectroscopy (λ_{exc} = 354 nm, λ_{em}= 450 nm, $slit_{exc}$ = 10 nm, $slit_{em}$ = 15 nm) and (c) UV spectroscopy (250 nm). T = 20°C.

4. Fluorescence data

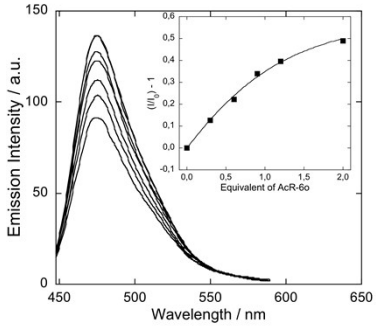
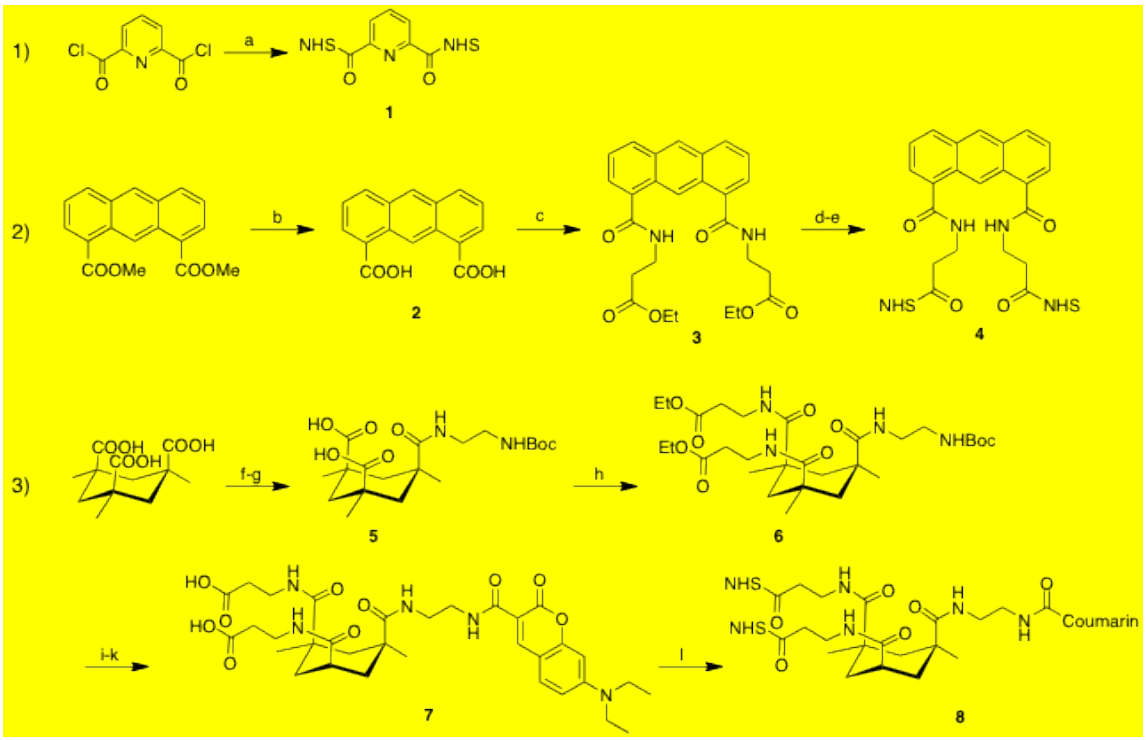


Figure S7. Titration of R3-6 (21 µM in PBS 20 mM, pH 7.2) with AcR-6o monitored by fluorescence at 20°C. λ_{exc} = 360 nm, λ_{em}= 474 nm, $slit_{exc}$ = $slit_{em}$ = 15 nm).

5. Templates Synthesis

The templates bearing linkers were synthesized in solution as activated ester (Scheme 1). The pyridine-derived template **1** was prepared by adding N-hydroxysuccidimyl (NHS) to a solution of 2,6-pyridine dicarbonyl dichloride under basic conditions as already described. For the anthracene-derived template the synthesis starts from the commercially available 1,8-dimethylanthracene dicarboxylate. The corresponding diacid 2 was obtained by saponification with lithium hydroxide (LiOH) to which the β-alanine amino ester (βAla-OEt) was condensed with PyBop and excess of DIEA to yield **3** with good yields. After purification, the diester was saponified with LiOH and the expected activated ester **4** was obtained by using WSC•HCl as activating reagent with an excess of NHS. For the Kemp's triacid-derived template, the Boc protected amino ethylene linker was added by first forming the KTA anhydride by sublimation which was then opened by condensing the mono Boc protected ethylene diamine in presence of DIEA. To this acid **5**, the β-alanine amino ester (βAla-OEt) was condensed using HBTU/HOBt and excess of DIEA leading to the compound **6**. The diacid was obtained by saponification with LiOH and the Boc group was removed by treatment with TFA. The free amine was acylated with N-diethyl amino coumarin succidimylester to yield after purification **7**. The NHS succidimyl diester **8** was finally obtained from **7** by using WSC•HCl as activating reagent with an excess of NHS.



Scheme S1. Synthesis of the ready-to-use templates. a) NHS, DIEA, THF, 12h, r.t., 84%. b) LiOH, THF/MeOH/H_2O, r.t., 4h, 90%. c) H-βAla-OEt, PyBOP, DIEA, DMA, 12h, r.t., 88%. d) LiOH, THF/MeOH/H_2O, r.t., 4h, 90%. e) NHS, WSCI•HCl, DIEA, DMF, r.t., 12h, 68%. f) Sublimation, 90%. g) $H_2N(CH_2)_2NHBoc$, DIEA, DMF, 6h, r.t., 88%. h) H-βAla-OEt, HBTU, HOBt, DIEA, DMF, 12h, r.t., 56%. i) LiOH, THF/H_2O 1:1, r.t., 1h, 98%. j) TFA/DCM 1:1, r.t., 30 min, 82%. k) DMACA-SE, DIEA, DMF, r.t., 12h, 45%. l) NHS, WSCI•HCl, DIEA, DMF, r.t., 12h, 84%.

General remarks. All chemical reagents and solvents were obtained from commercial suppliers (Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries, Acros Organics, Sasaki Chemical, or Watanabe Chemical Industries) and used without further purification. Thin layer chromatography (TLC) was performed on silica gel 60 F254 precoated aluminium sheets (Merck) and visualized by fluorescence quenching or ninhydrin staining. Chromatographic purification was conducted by flash column chromatography on silica gel 60N (neutral, 40–50 m, Kanto Chemical). ^{1}H NMR spectra were recorded in deuterated solvents on a Varian Mercury 400 (400 MHz) spectrometer and calibrated to the residual solvent peak or tetramethylsilane (= 0 ppm). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = doublet of doublet, br s = broad singlet. Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) was measured by an Autoflex II instrument (Bruker Daltonics) using α-cyano-4-hydroxycinnamic acid (CHCA) as the matrix. FAB spectra were recorded using QP5050A (Shimadzu).

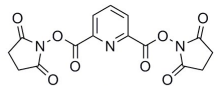
General procedure for couplings with PyBop or HBTU. The diacid (1 equiv.), amino ester (from 1.5 to 4 equiv.), PyBOP or HBTU (3 equiv.) and HOBt (2 equiv.) were placed in a round-bottom flask under argon. Dried DMA or DMF was added followed by DIEA (6 equiv.). The reaction mixture was stirred overnight at room temperature. Solvent was removed and the crude dissolved in $CHCl_3$. The organic phase was washed successively with a saturated $NaHCO_3$ solution, with citric acid (10%), water and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was purified by precipitation or silica-gel chromatography.

General procedure for synthesis of activated esters. DIEA was added (6 to 10 equiv.) to a solution of the diacid (1 equiv.), with WSC•HCl (3 equiv.) and N hydroxysuccimidyl (2.5 equiv.) in DMF. The reaction mixture was stirred at room temperature overnight. DMF was removed before $CHCl_3$ and water addition. The aqueous phase was extracted three times and the combined organic phase was dried over Na_2SO_4, filtered and concentrated. The crude was used for the final coupling without further purification.

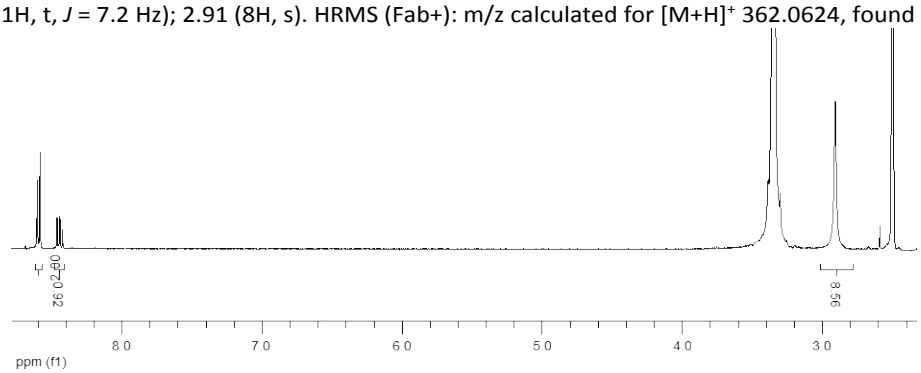
General procedure for coupling with activated esters. The starting peptide (2.2 equiv.) was dissolved in a DMF/H_2O mixture. DIEA (6 equiv.) was added followed by a solution of activated ester (1 equiv.). The mixture was stirred

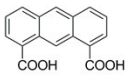
overnight at room temperature, DMF was removed, H_2O containing 0.1% of TFA was added and the crude purified by HPLC.

Experimental procedures

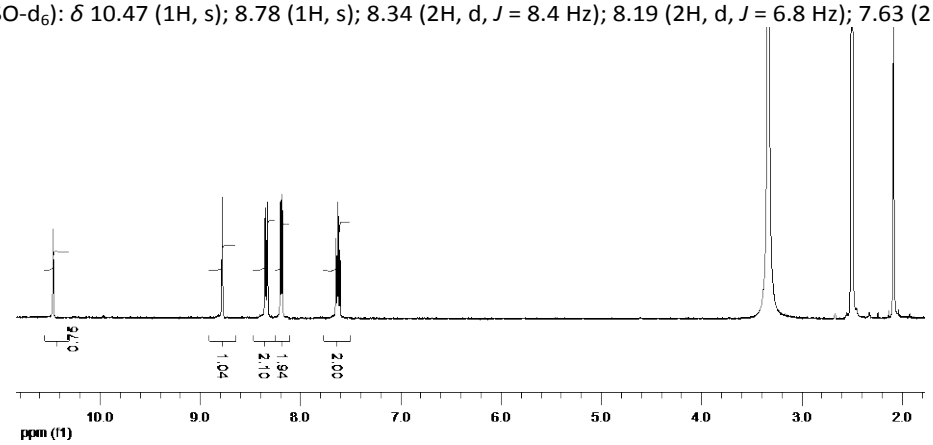


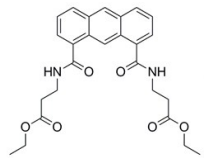
1. To a mixture of 2,6-pyridine dicarbonyl dichloride (204 mg, 1 mmol, 1 equiv.) and N-hydroxysuccimidyl (253 mg, 2.2 mmol, 2.2 equiv.) in THF (5 mL) was slowly added diisopropylethylamine (1.04 mL, 6 mmol, 6 equiv.) at room temperature. The reaction mixture was vigorously stirred at room temperature for 12h since a white precipitate appeared. The solvent was removed under vacuum and 2-propanol was added. The solid was filtered off, washed 3 times with 2-propanol and dried in vacuum to afford 259 mg of a white powder (84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (2H, d, J = 8.0 Hz); 8.45 (1H, t, J = 7.2 Hz); 2.91 (8H, s). HRMS (Fab+): m/z calculated for $[M+H]^+$ 362.0624, found 362.0641.



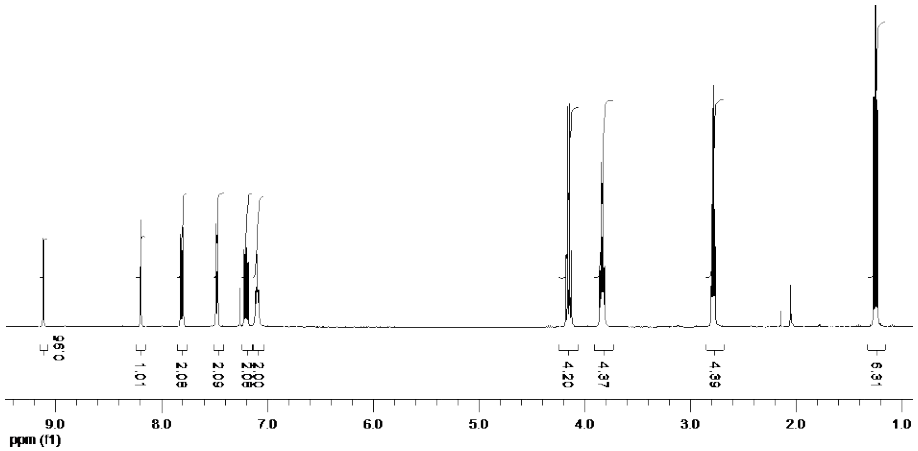


2. [4]To a solution of 1,8-dimethylanthracene dicarboxylate (200 mg, 0.68 mmol, 1 equiv.) dissolved in a mixture THF/MeOH/H_2O 2:1:1 (4/2/2 mL) was added LiOH (114 mg, 2.72 mmol, 4 equiv.). The reaction mixture was stirred for 4 hours at room temperature. Acetic acid (1 mL) was added and the organic solvents were removed. A yellow precipitate appeared which was filtered off, washed 3 times with water and dried under vacuum. It affords 240 mg of the expected compound (90%) used without further purification. ^{1}H NMR (400MHz, DMSO-d_6): δ 10.47 (1H, s); 8.78 (1H, s); 8.34 (2H, d, J = 8.4 Hz); 8.19 (2H, d, J = 6.8 Hz); 7.63 (2H, t J = 7.6 Hz).

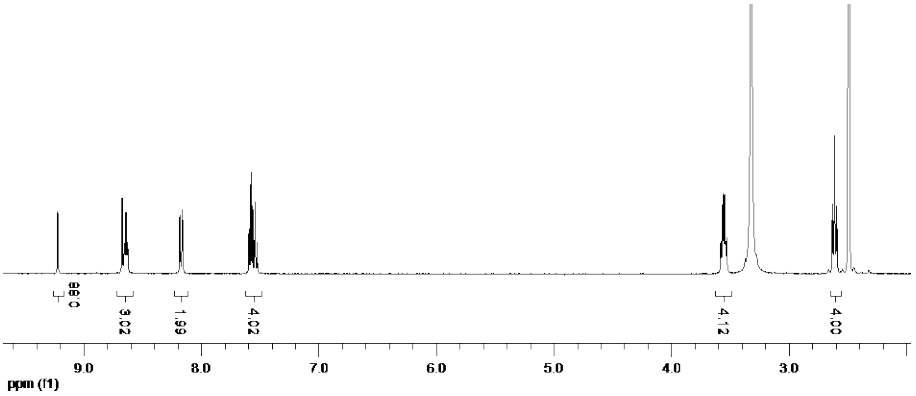


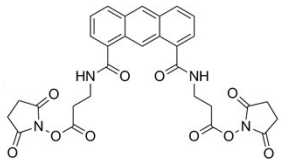


3. According to the general procedure, 1,8-anthracene dicarboxylic acid (236 mg, 0.89 mmol, 1 equiv.), β alanine amino ester (783 mg, 3.56 mmol, 4 equiv.), PyBOP (1.38 g, 2.66 mmol, 3 equiv.) and DIEA (0.92 mL, 5.34 mmol, 6 equiv.) were mixed in DMF (9.5 mL). After work-up, the expected compound was precipitated in dethylether and chromatographed on silica-gel eluted with $CHCl_3$/MeOH 99/1 v:v). It was obtained 361 mg of the expected compound (88%). ^{1}H NMR (400MHz, $CDCl_3$): δ 9.12 (1H, s); 8.20 (1H, s); 7.81 (2H, d, J = 8.4 Hz), 7.48 (2H, d, J = 8.4 Hz); 7.20 (2H, m), 7.10 (2H, bt, J = 6.0 Hz); 4.15 (4H, q, J = 7.2 Hz); 3.83 (4H, q, J = 6.0 Hz); 2.79 (4H, t, J = 6.4 Hz); 1.25 (6H, t, J = 7.2 Hz). LRMS (FAB+): m/z $[M+H]^+$ 465, $[M+Na]^+$ 487.

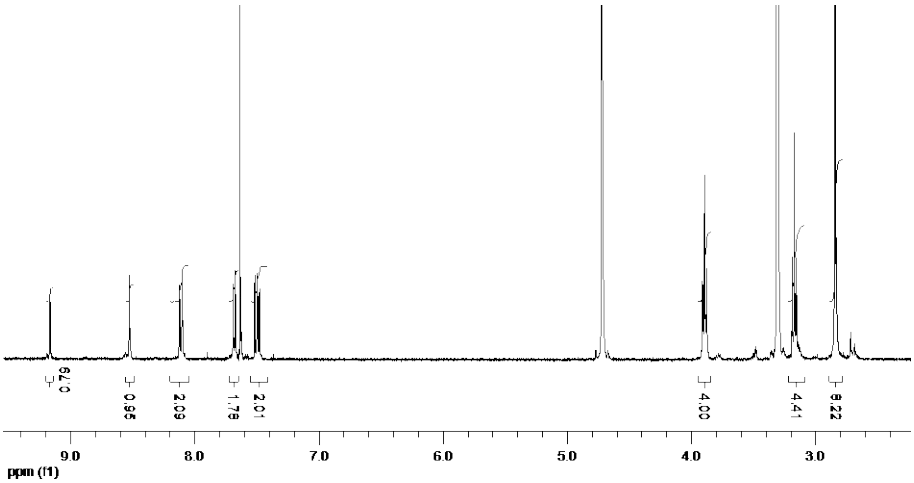


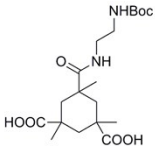
3.1. To a solution of **3** (350 mg, 0.75 mmol, 1 equiv.) dissolved in a mixture THF/MeOH/H_2O (8/5/3 mL) was added LiOH (127 mg, 3.01 mmol, 4 equiv.). The reaction mixture was stirred for 4 hours at room temperature. HCl 1M was added and the organic solvents were removed. A pale yellow precipitate appeared which was filtered off, washed 3 times with water, once with Et_2O, dried under vacuum to afford 275 mg of the expected compound (90%) used without further purification. ^{1}H NMR (400 MHz, DMSO d_6): δ 9.23 (1H, s); 8.68 (1H, s); 8.65 (bt, 2H, J = 5.2 Hz); 8.18 (2H, d, J = 8.0 Hz); 7.56 (4H, m); 3.56 (4H, q, J = 7.2 Hz); 2.62 (4H, t, J = 7.2 Hz). ^{13}C NMR (100 MHz, DMSO d_6): δ 172.9; 168.5; 135.1; 131.0; 129.9; 128.1; 127.0; 125.3; 124.8; 122.8; 35.5; 33.9. HRMS (Fab+): m/z calculated for $[M]^+$ 408.1321, found 408.1302.



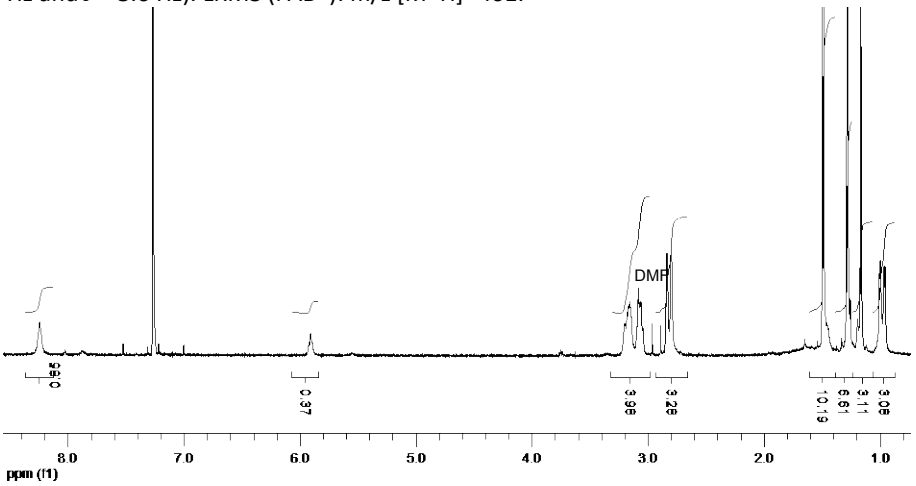


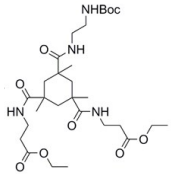
4. According to the general procedure **3.1** (50 mg, 0.012 mmol, 1 equiv.), with WSC•HCl (71 mg, 0.37 mol, 3 equiv.), N hydroxysuccimidyl (35 mg, 0.31 mmol, 2.5 equiv.) and DIEA (0.13 mL, 0.73 mmol, 6 equiv.) were mixed in DMF (2.5 mL). After completion and workup, the crude was triturated with isopropanol, filtered off, washed 3 times with isopropanol, once with Et_2O and dried under vacuum to afford a pale yellow solid (51 mg, 68%) used for the next step without further purification. ^{1}H NMR (400 MHz, MeOD/$CDCl_3$): δ 9.16 (1H, s); 8.52 (1H, s); 8.11 (2H, d, J = 8.4 Hz); 7.68 (2H, d, J = 6.8 Hz); 7.49 (2H, m); 3.89 (4H, t, J = 6.8 Hz); 3.17 (4H, t, J = 6.8 Hz); 2.84 (8H, s). LRMS (FAB+): m/z $[M+H]^+$ 603.



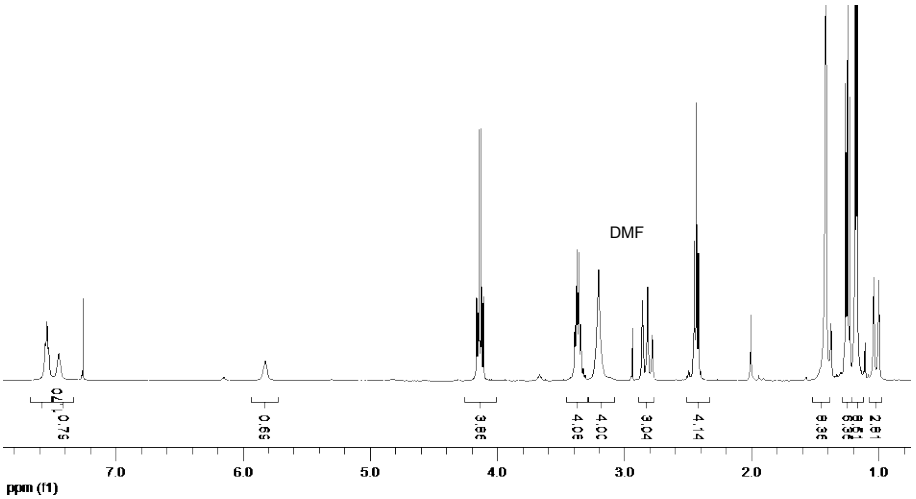


5.[5b] The starting anhydride was prepared by sublimation according to the literature procedure.[5a] Kemp's anhydride (170 mg, 0.71 mmol, 1 equiv.) and Boc amino ethylene amine hydrochloride salt (209 mg, 1.06 mmol, 1.5 equiv.) were dissolved in DMF (11.3 mL) under Argon. DIEA (0.61 mL, 3.54 mmol, 5 equiv.) was slowly added and the reaction mixture was stirred for 6 hours at room temperature. DMF was removed and $CHCl_3$ (23 mL) was added. The organic phase was washed with a 0.1 M citric acid solution (3×14 mL), dried over Na_2SO_4, filtered and concentrated. The crude product (255 mg, 90%) was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (1H, bt, J = 2.8 Hz); 5.90 (1H, bt, J = 6.0 Hz); 3.17-3.05 (4H, m); 2.81 (3H, d, J = 14.8 Hz); 1.49 (9H, s); 1.28 (6H, s); 1.16 (3H, s); 0.98 (3H, dd, J = 14.4 Hz and J = 3.6 Hz). LRMS (FAB+): m/z $[M+H]^+$ 401.

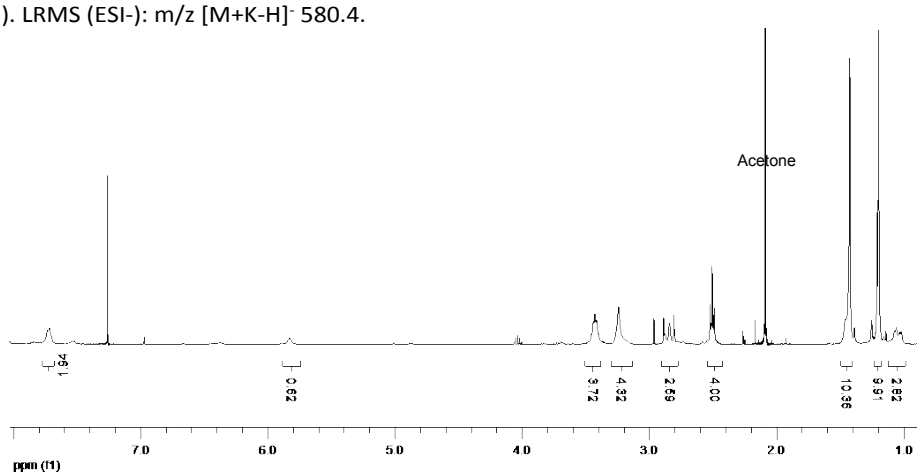


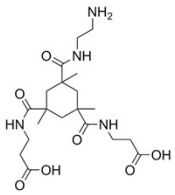


6. According to the general coupling procedure, **5** (145 mg, 0.36 mmol, 1 equiv.), NH_2-βAla-OEt hydrochloride salt (167 mg, 1.09 mmol, 3 equiv.), HBTU (410 mg, 1.09 mmol, 3 equiv.), HOBt (111 mg, 0.72 mmol, 2 equiv.) and DIEA (0.37 mL, 2.16 mmol, 6 equiv.) were dissolved in dried DMF (5 mL) under argon. After workup, the crude mixture was purified by silica-gel chromatography ($CHCl_3$/MeOH 99/1 v:v) to afford 121 mg of a colorless oil (56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (2H, bt, J = 5.2 Hz),7.45 (1H, bs), 5.83 (1H, bs), 4.14 (4H, q, J = 7.2 Hz), 3.37 (4H, q, J = 6.4 Hz), 3.20 (4H, bs), 2.82 (3H, t, J = 14.8 Hz), 2.44 (4H,t, J = 6.8 Hz), 1.43 (9H, s), 1.25 (6H, t, J = 7.2 Hz), 1.19 (3H, s), 1.17 (6H, s), 1.02 (3H, d, J = 15.6 Hz). LRMS (FAB+): m/z $[M+H]^+$ 599.

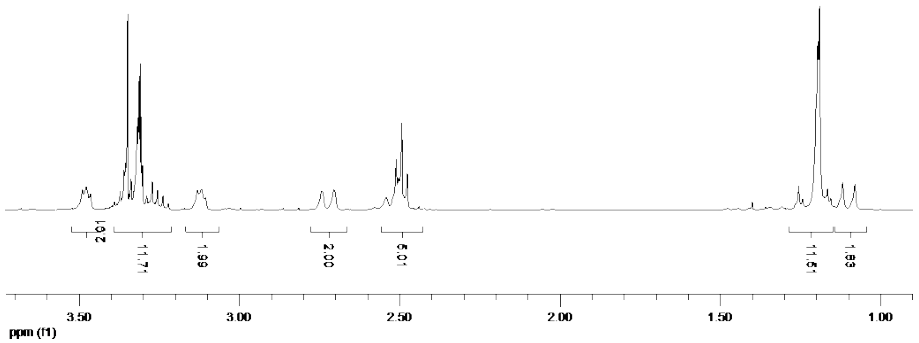


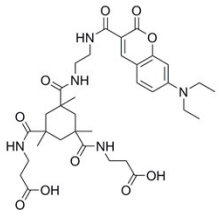
6.1. To a solution of **6** (105 mg, 0.17 mmol, 1 equiv.) dissolved in a mixture THF/H_2O (2/2 mL) was added LiOH (22 mg, 0.53 mmol, 3 equiv.). The reaction mixture was stirred for 1 hour at room temperature. The completion was monitored by TLC. Acetic acid was added and THF removed. $CHCl_3$ was added and the aqueous phase extracted 4 times. The organic phase was dried over Na_2SO_4, filtered and concentrated to afford 95 mg (98%) of the expected compound used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (2H, bs), 5.83 (1H, bs), 3.43 (4H, bt, J = 5.2 Hz); 3.24 (4H, bs), 2.84 (3H, m), 2.51 (4H, t, J = 6.4 Hz), 1.42 (9H, s), 1.21 (3H, s), 1.20 (6H, s), 1.035 (3H, m). LRMS (ESI-): m/z $[M+K-H]^-$ 580.4.



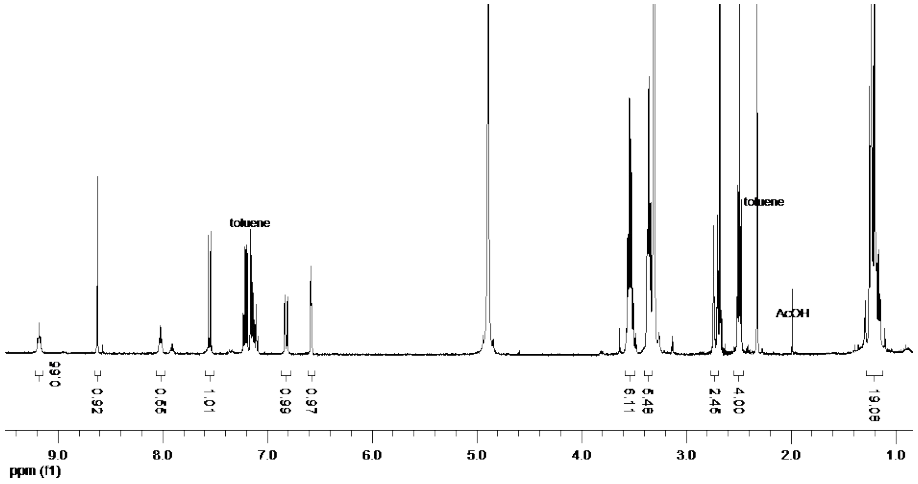


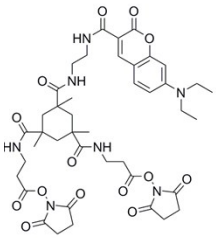
6.2. According to the general procedure for Boc deprotection, **6.1** (95 mg, 0.175 mmol, 1 equiv.) was dissolved in a mixture DCM/TFA (1/1 mL). After completion and evaporation of the solvents, the expected amine was precipitated in cold MTBE. The white solid was recovered by centrifugation, washed 3 times with MTBE and dried under vacuum to afford 80 mg of the free amine as TFA salt (82%). ^{1}H NMR (400 MHz, MeOD): δ 3.49 (2H, m), 3.35 (4H + CD_2HOD, m), 3.12 (2H, m), 2.73 (2H, d, J = 15.2 Hz), 2.52 (1H, d, J = 15.2 Hz), 2.49 (4H, t, J = 6.8 Hz), 1.24 (1H, d, J = 15.2 Hz), 1.19 (9H, s), 1.10 (2H, d, J = 15.2 Hz). LRMS (FAB+): m/z $[M+H]^+$ 443.



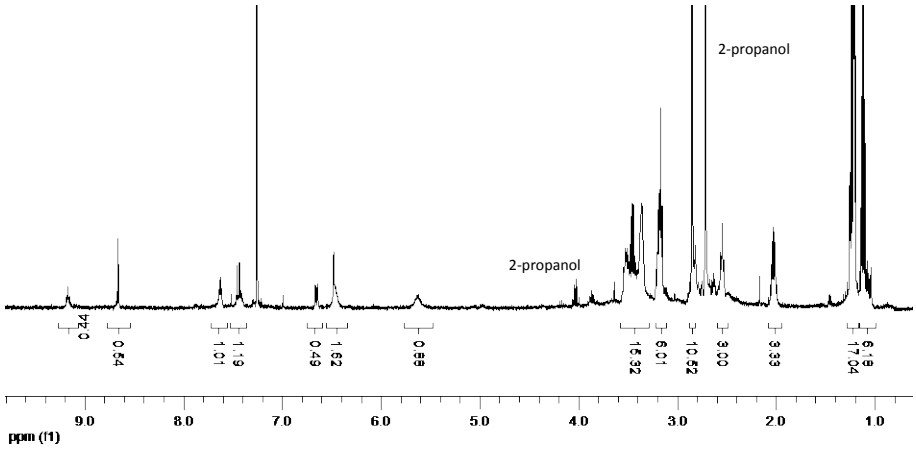


7. According to the general procedure for coupling with coumarin, **6.2** (38 mg, 0.063 mmol, 1.1 equiv.), coumarin activated ester (18.7 mg, 0.052 mmol, 1 equiv.) and DIEA (36 µL, 0.21 mmol, 4 equiv.) were mixed in DMF (1 mL). After workup, the crude mixture was purified by chromatography on silica-gel eluting with a gradient AcOH/MeOH/$CHCl_3$ (from 1/5/94, v:v:v to 1/10/89) to afford 15 mg (45%) of the expected compound. ^{1}H NMR (400 MHz, MeOD): δ 8.62 (1H, s), 7.53 (1H, d, J = 8.8 Hz), 6.82 (1H, dd, J = 2.8 Hz and J = 9.2 Hz), 6.58 (1H, d, J = 2.4 Hz), 3.54 (6H, m), 3.36 (6H, m), 2.72 (3H, d, J = 14.8 Hz), 2.49 (4H, t, J = 7.2 Hz), 1.24 (6H, t, J = 7.2 Hz), 1.22 (3H, m), 1.20 (9H, s). HRMS (Fab+): m/z calculated for $[M+H]^+$ 686.3401, found 686.3400





8. According to the general procedure **7** (14 mg, 0.02 mmol, 1 equiv.), WSC•HCl (12 mg, 0.06 mol, 3 equiv.), N hydroxysuccimidyl (6 mg, 0.05 mmol, 2.5 equiv.) and DIEA (21 µL, 0.12 mmol, 5 equiv.) were mixed in DMF (1 mL). After completion and workup, the crude was triturated in Et_2O and dried under vacuum to afford a yellow solid (15 mg, 84%) used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.17 (1H, bs), 8.66 (1H, s); 7.63(2H, bt, J = 8.8 Hz); 7.45 (1H, d, J = 9.2 Hz); 6.65 (1H, dd, J = 9.2 Hz and J = 2.4 Hz); 6.48 (1H, d, J = 2.0 Hz); 5.63 (1H, bs); 3.53-3.36 (10H, m); 3.17 (6H, m); 2.85 (8H, s); 2.55 (3H, t, J = 6.0 Hz), 2.03 (3H, m); 1.24-1.22 (9H, m +2-propanol); 1.12 (6H, t, J = 7.2 Hz).



6. Peptide synthesis

General procedure for peptide synthesis. A Fmoc (fluorenylmethyloxycarbonyl) strategy was employed and 3 equivalents of each amino acid were used with 3 equivalents of the coupling reagents 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and N-hydroxybenzatriazole (HOBt) in presence of 6 equivalents of diisopropylethylamine (DIEA). Each coupling reaction was allowed to proceed for 30 minutes at room temperature and reaction completion was monitored by Kaiser and chloranil test for primary and secondary amines, respectively. A 20 % piperidine solution in N-methylpyrrolidinone (NMP) was used for Fmoc removal. After synthesis of the sequence, in desired cases, acetylation of the N-terminus by treatment with acetic anhydride was performed. The peptides were removed from the resin by using trifluoroacetic acid (TFA)/ triisopropylsilane/H_2O cocktails. After precipitation in cold methyl tert-butyl ether (MTBE), the peptides were purified by reverse phase HPLC on C18 column and analyzed by mass spectrometry.

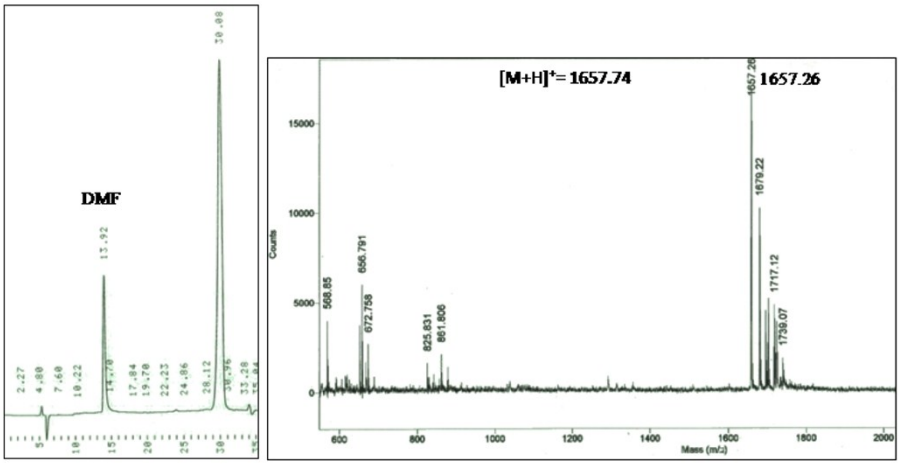
Peptide characterizations. For peptide purification and analysis, both solvents (water and acetonitrile) contained 0.1% of TFA

D-6. NH_2-GPOGPOGDOGPOGDOGPO-NH_2 (71%). Maldi (positive mode): MS calculated for $[M+H]^+$ 1657.6 found 1657.3.

HPLC conditions:

Semi-preparative column: 0 to 15% of CH_3CN over 30 min

Preparative column: 0 to 15% of CH_3CN over 40 min

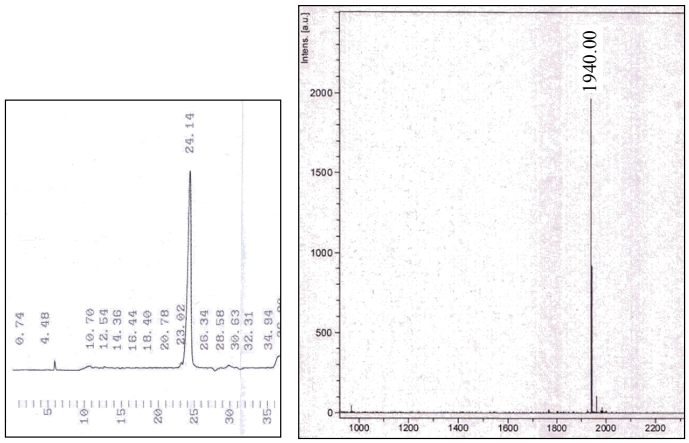


D-7. NH_2-GDOGPOGPOGDOGPOGDOGPO-NH_2 (64%). Maldi (negative mode): MS calculated for $[M-H]^-$ 1940.9 found 1940.0.

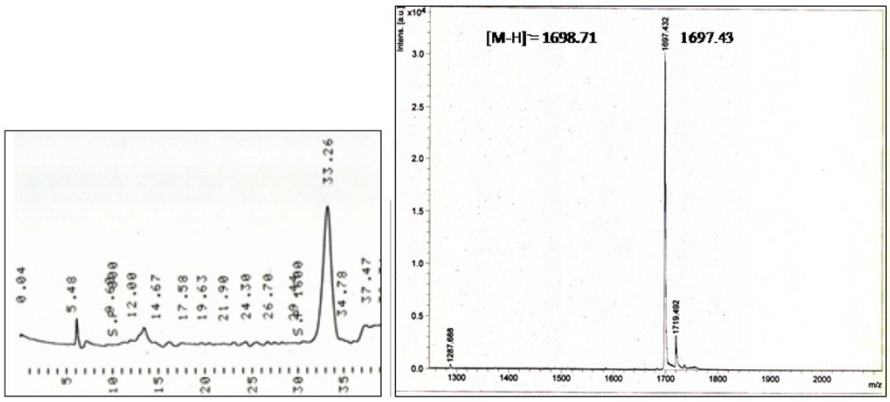
HPLC conditions:

Semi-preparative column: 0 to 20% of CH_3CN over 30 min

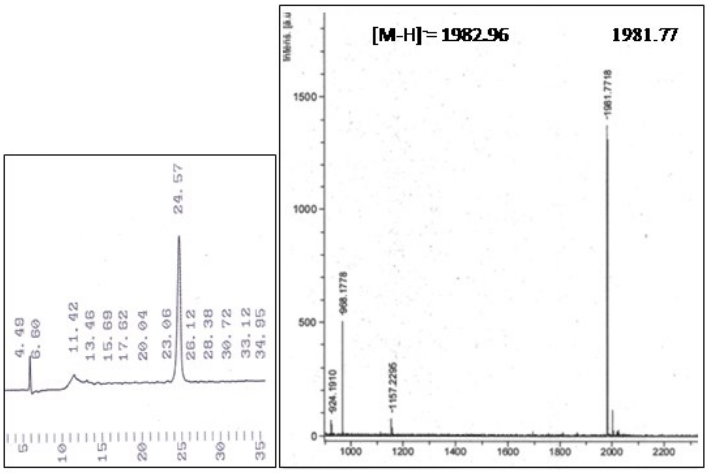
Preparative column: 0 to 20% of CH_3CN over 40 min



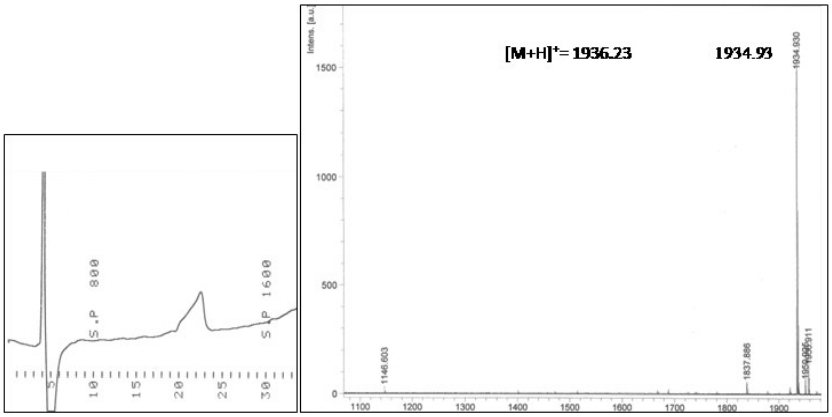
AcD-6. Ac-GPOGPOGDOGPOGDOGPO-NH_2(55%) Maldi (negative mode): MS calculated for $[M-H]^-$ 1697.7 found 1697.4.
HPLC conditions:
Semi-preparative column: 0 to 15% of CH_3CN over 30 min
Preparative column: 0 to 15% of CH_3CN over 40 min



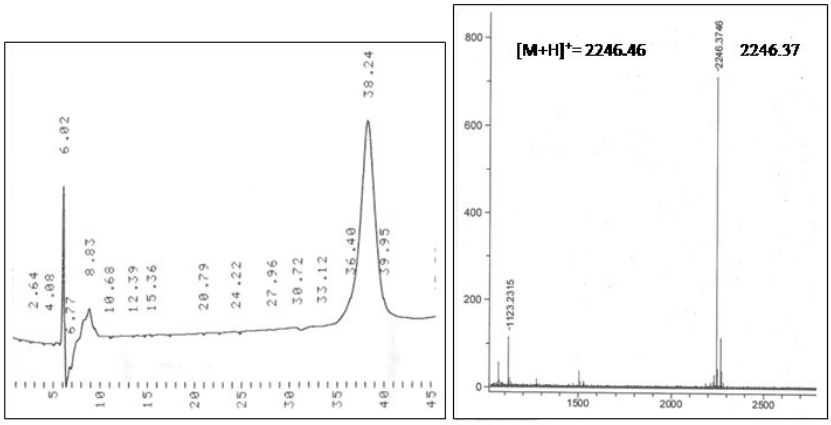
AcD-7. Ac-GDOGPOGPOGDOGPOGDOGPO-NH_2(66%) Maldi (negative mode): MS calculated for $[M-H]^-$ 1982.9 found 1981.8.
HPLC conditions:
Semi-preparative column: 0 to 20% of CH_3CN over 30 min
Preparative column: 0 to 20% of CH_3CN over 40 min



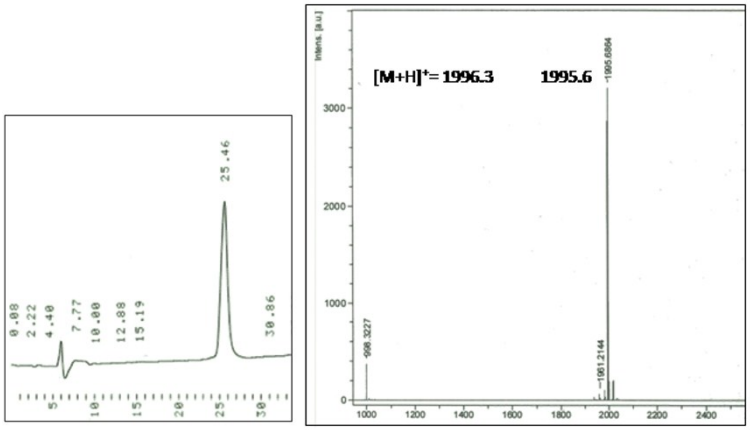
AcR-6o. Ac-$(GPO)_2$-GPR-GPO-GPR-GPO-W-NH_2(62%) Maldi (positive mode): MS calculated for $[M+H]^+$ 1936.1 found 1934.9.
HPLC conditions:
Semi-preparative column: 0 to 30% of CH_3CN over 30 min
Preparative column: 0 to 30% of CH_3CN over 40 min



AcR-7o. Ac-GPR-$(GPO)_2$-GPR-GPO-GPR-GPO-NH_2 (22%). Maldi (positive mode): MS calculated $[M+H]^+$ 2246.5 found 2246.4.
HPLC conditions:
Semi-preparative column: 5 to 30% of CH_3CN over 40 min
Preparative column: 5 to 30% of CH_3CN over 50 min



AcR-7. Ac-GPR-$(GPP)_2$-GPR-GPP-GPR-GPP-NH_2 (65%). Maldi (positive mode): MS calculated $[M+H]^+$ 1996.3 found 1995.6.
HPLC conditions:
Semi-preparative column: 5 to 40% of CH_3CN over 30 min
Preparative column: 5 to 40% of CH_3CN over 40 min



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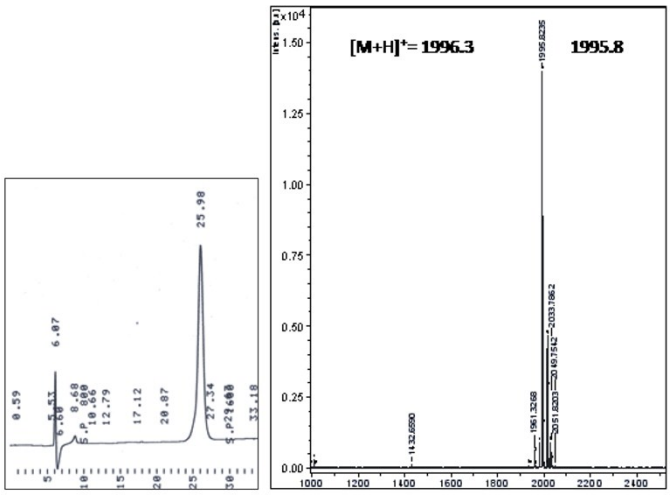
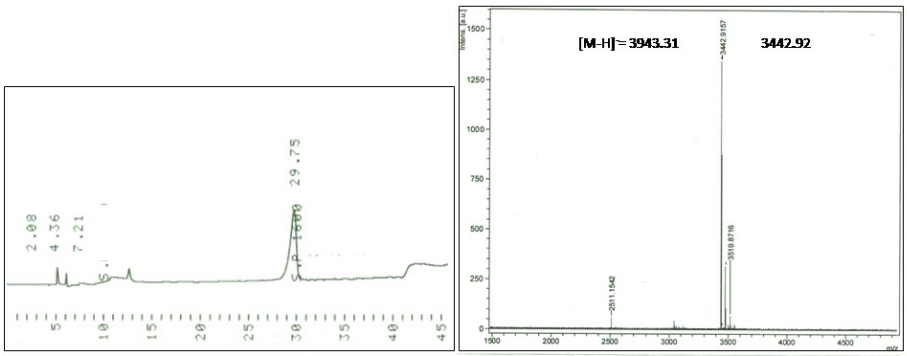
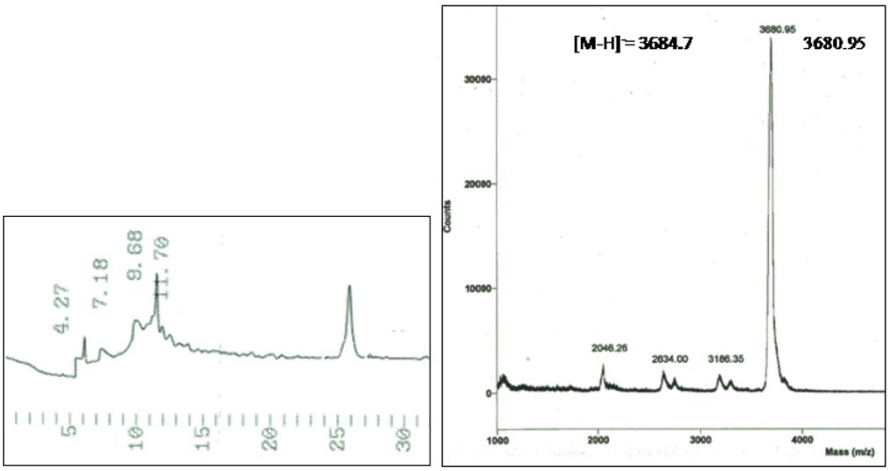
AcR-7s. Ac-PGG**R**PGPGPPGP**R**PGPP**R**PPG-NH_2. Maldi (positive mode): MS calculated $[M+H]^+$ 1996.3 found 1995.8.
HPLC conditions:
Semi-preparative column: 5 to 40% of CH_3CN over 30 min
Preparative column: 5 to 40% of CH_3CN over 40 min



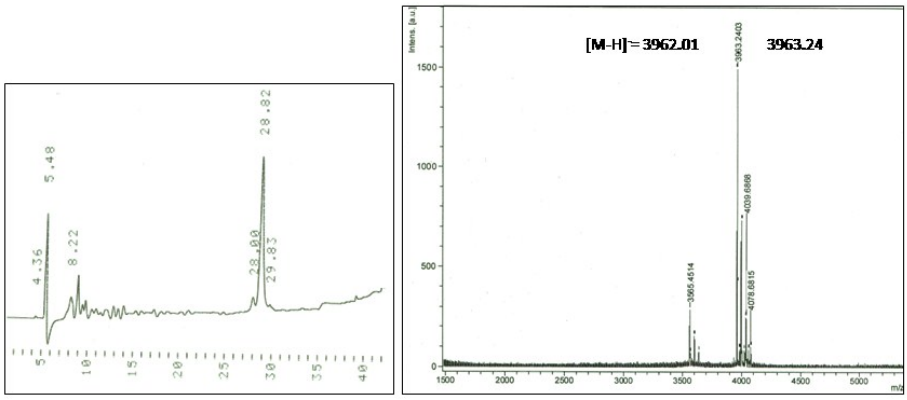
R1-6. According to the general coupling procedure, the starting peptide (7.6 mg, 4.23 µmol, 2.2 equiv.), DIEA (2.1 µL, 11.7 µmol, 6 equiv.) and a solution of activated ester (140.8 µL, 13.85 mM solution in DMF, 1 equiv.) were mixed in a mixture DMF/H_2O (500/40 µL). The crude was purified by HPLC to afford 4.8 mg of the expected compound (71%). Maldi (negative mode): MS calculated for $[M-H]^-$ 3943.31 found 3442.92.
HPLC conditions:
Semi-preparative column: 0 to 30% of CH_3CN over 35 min
Preparative column: 0 to 13% of CH_3CN over 35 min



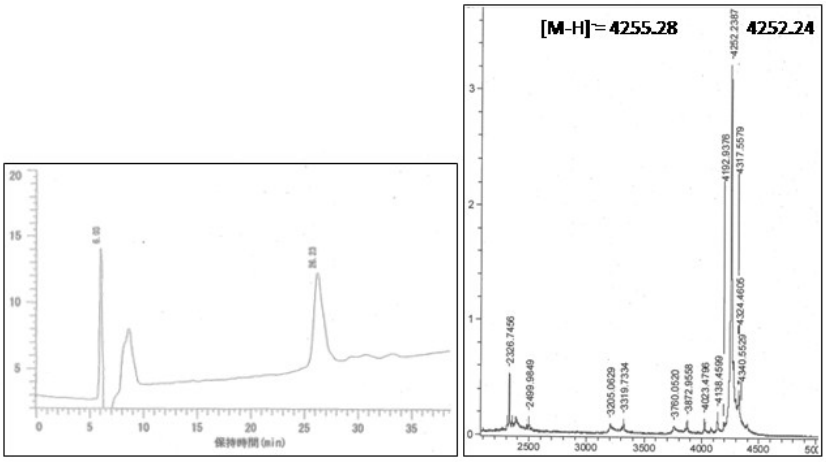
R2-6. According to the general coupling procedure, the starting peptide (4.2 mg, 2.26 µmol, 2.2 equiv.) DIEA (1.2 µL, 6.4 µmol, 6 equiv.) and a solution of activated ester (115 µL, 9.29 mM solution in DMF, 1 equiv.) were mixed in a mixture DMF/H_2O (0.3/0.1 mL). The crude was purified by HPLC to afford 3.5 mg of the expected compound (89%). Maldi (negative mode): MS calculated for $[M-H]^-$ 3684.7 found 3681.0.
HPLC conditions:
Semi-preparative column: 0 to 30% of CH_3CN over 30 min
Preparative column: 0 to 30% of CH_3CN over 40 min



R3-6. According to the general coupling procedure, the starting peptide (6.9 mg, 3.89 µmol, 2.2 equiv.) DIEA (1.9 µL, 11.6 µmol, 6 equiv.) and a solution of activated ester (54.3 µL, 34 mM solution in $CHCl_3$, 1 equiv.) were mixed in a mixture DMF/H_2O (0.5/0.2 mL). The crude was purified by HPLC to afford 1.5 mg of the expected compound (22%). Maldi (positive mode): MS calculated for $[M+Na]^+$ 3986.0 found 3985.2.
HPLC conditions:
Semi-preparative column: 0 to 40% of CH_3CN over 30 min
Preparative column: 0 to 40% of CH_3CN over 40 min



R2-7. According to the general coupling procedure, the starting peptide (6.0 mg, 2.92 µmol, 2.2 equiv.), DIEA (1.45 µL, 8.34 µmol, 6 equiv.) and a solution of activated ester (143 µL, 9.29 mM solution in DMF, 1 equiv.) were dissolved in a mixture DMF/H_2O (0.39/0.13 mL). The crude was purified by HPLC to afford 1.5 mg of the expected compound (25%). Maldi (negative mode): MS calculated for $[M-H]^-$ 4255.2 found 4252.2.
HPLC conditions:
Semi-preparative column: 5 to 35% of CH_3CN over 35 min
Preparative column: 5 to 35% of CH_3CN over 45 min



7. References

[1] (a) Gauba, V.; Hartgerink, J. D.; *J. Am. Chem. Soc.* **2007**, *129*, 2683-2690. (b) Gauba, V.; Hartgerink, J. D.; *J. Am. Chem. Soc.* **2007**, *129*, 15034-15041. (c) Persikov, A. V.; Ramshaw, J. A. M., Brodsky, B. *J. Biol. Chem.* **2005**, *280*, 19343.

[2] Delsuc, N.; Godde, F.; Kauffmann, B.; Léger, J.-M.; Huc, I.*; J. Am. Chem. Soc.* **2007**, *129*, 11348-11349.

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