Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information to

Synthesis of Peptide-Grafted Comb Polypeptides via Polymerisation of NCA-Peptides

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Experimental Section

Materials and methods

All amino acid derivatives were purchased from Iris Biotech. Other chemicals were purchased from commercial suppliers, Iris Biotech, Senn Chemicals, Merck, Carlo Erba, Fluka, Riedel-de Haën, Sigma Aldrich, Acros Organics, FluoroChem and VWR Prolabo. The Fmoc-Rink amide AM resin (100-200 Mesh, 0.45 mmol/g) was purchased from Iris Biotech and stored at 4 ºC. All compounds were analyzed under standard analytical HPLC conditions on a Beckman Gold apparatus composed of the 126 solvent module, the 168 detector, and the 32 Karat software, on a C18 reversed phase column (VWR chromolith column, 50 mm x 3.9 mm): 0% B to 100% B linear gradient over 3 min at a flow rate of 5 mL/min. Solvent A was 0.1% TFA aqueous solution. Solvent B was 0.1% TFA in acetonitrile. Detection and purity were performed and determined at 214 nm. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated DMSO on an AMX 400 Brucker spectrometer at 400 MHz and 100 MHz, respectively. The LC/MS analyses were performed on a Quatromicro (Micromass, Manchester, U.K.) triple-quadrupole mass spectrometer fitted with an electrospray interface. Solvent used for HPLC and LC/MS were HPLC grade. Molecular weight of the polymers was determined by size exclusion chromatography (SEC) using Waters Inc. equipment fitted with a 5 µm mixed C PLgel 5 µm MIXED-D (300×7.5 mm) and SHODEX RI 101 refractometric detector. The mobile phase was DMF at 0.8 mL/min flow. Typically, the polymer (10 mg) was dissolved in DMF (2 mL) and the resulting solution was filtered through a 0.45 µm Millipore filter before injection of 20 µL of a filtered solution. Mn and Mw were expressed according to calibration using poly(methyl methacrylate) standards.

General SPPS protocol

The synthesis of compound **2** was performed by conventional solid phase peptide synthesis[1] from Fmoc-Rink amide AM resin (0.45 mmol/g) (5.0 g). Peptide chain elongation was performed using Fmoc/tBu strategy with HBTU/DIEA as coupling reagent. The coupling step was carried out on the resin using N^{α}-Fmoc-amino acid (3 eq.) in DMF in the presence of HBTU (3 eq.) and DIEA (3 eq.) for 2 h at room temperature. The Fmoc deprotection step was performed by treatment with a solution of piperidine (20% of v/v in DMF) for 30 min. After each coupling and deprotection step, the resin was washed with DMF (three times), MeOH (one time) and DCM (three times). Completion of the reaction was checked by the standard TNBS test.[2]

The pendant peptide sequence anchored to the resin **2** was then used for the synthesis of compound **1** as described in the experimental section of the main text.

Synthesis of compound 1 and polymer P3

The synthesis of the resin-bound peptide 2 (Scheme 1) was performed by a conventional solid phase peptide synthesis from Fmoc-Rink amide AM resin (0.45 mmol/g). Then, 2 was reacted with Boc-Glu-OBzl (3 eq.) in DMF in the presence of HBTU (3 eq.) and DIEA (3 eq.) for 2 h at room temperature to yield resin-bound peptide 3. Then, 3 was treated with 2 M lithium hydroxide aqueous solution in THF (30/70) at room temperature for 3 h. The resin was washed with THF (3 times), DMF (3 times), methanol (3 times) and DMF (3 times) to give 4. The resin-bound peptide 4 (1.0 g, theoretical loading 0.40 mmol/g) was reacted with cyanuric chloride (0.37 g, 2.0 mmol) in anhydrous DCM (60 ml) at room temperature for 12 h. The resin was washed with anhydrous DCM (five times), anhydrous THF (three times), anhydrous DMF (three times) and anhydrous DCM (three times) to yield the NCA derivative 5 linked to the solid support. Then, 5 was treated with trifluoroacetic acid in anhydrous DCM (50 ml/50 ml) at room temperature for 1.5 h. The cleaved resin was removed by filtration and the filtrate was evaporated under reduced pressure. To the residue was added diethyl ether, which gave compound 1 as a white solid (yield: 0.17 g, yield 93 %, purity 90%). This material was dissolved in acetonitrile and a small amount of 4 M HCl in dioxane was added. After filtration, the filtrate was evaporated under reduced pressure and the residue dried under vacuum to afford the pure compound 1 in 56% yield (purity > 98%). Compound 1 was analyzed by HPLC, LC/MS and NMR (**Figures SI-1-SI-4**).

Ring opening polymerization of **1** was performed under conventional conditions.

For polymer P3: **1** (35 mg, 7.8 x 10^{-5} mol) was dissolved in DMF (0.5 mL) in a Schlenk flask under inert atmosphere and stirring. Temperature was raised to 55°C and stock solution of diethylamine in DMF was added (1.56 x 10^{-6} mol). After 3 days, the reaction medium was poured into cold diethyl ether. The solid was filtered and dried under vacuum. Monomer conversion was calculated on the final crude reaction medium by comparison between the integration of the NMR resonance peak at 9.0 ppm corresponding to

the amide proton of the NCA cycle and the integration of the resonance peak at 1.2 corresponding to the methyl protons of the alanine residue in the monomer and the polymer (**Figure SI-3**).

[1] M. Amblard, J. A. Fehrentz, J. Martinez, G. Subra, *Mol. Biotechnol.* 2006, **33**, 239-254.

[2] W. S. Hancock, J. E. Battersby, D. R. K. Harding, *Anal. Biochem.* 1975, **69**, 497-503.

Compound 1 characterization

LC/MS monoisotopic mass calculated for $C_{20}H_{25}N_5O_7$, 447.5. Fouund: $[M+H]^+$, *m/z* 448.3.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.21 (d, *J* = 4.8 Hz, 3H), 1.77-1.78 (m, 1H), 1.82-1.84 (m, 1H), 2.16 (t, *J* = 5.1 Hz, 2H), 2.74 (dd, *J* = 9.2, 6.8 Hz, 1H), 3.04 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.68 (dd, *J* = 3.8, 11.0 Hz, 1H), 3.75 (dd, *J* = 3.8, 11.0 Hz, 1H), 4.18-4.22 (m, 2H),4.49-4.51 (m, 1H), 7.00 (s, 1H),7.17-7.18 (m, 1H), 7.24-7.27 (m, 5H), 7.85 (d, *J* = 5.1 Hz, 1H), 8.20 (d, *J* = 5.4 Hz, 1H), 8.30 (t, *J* = 3.8 Hz, 1H), 8.97 (s, 1H).

^{13}C NMR (DMSO-d_6, 100 MHz) δ 18.21, 27.01, 30.24, 37.36, 42.07, 47.95, 54.11, 56.34, 126.24, 127.99, 129.11, 137.95, 151.75, 168.17, 170.84, 171.27, 171.65, 174.09.

Figure SI-1. HPLC of crude (**A**) and purified (**B**) compound **1.**

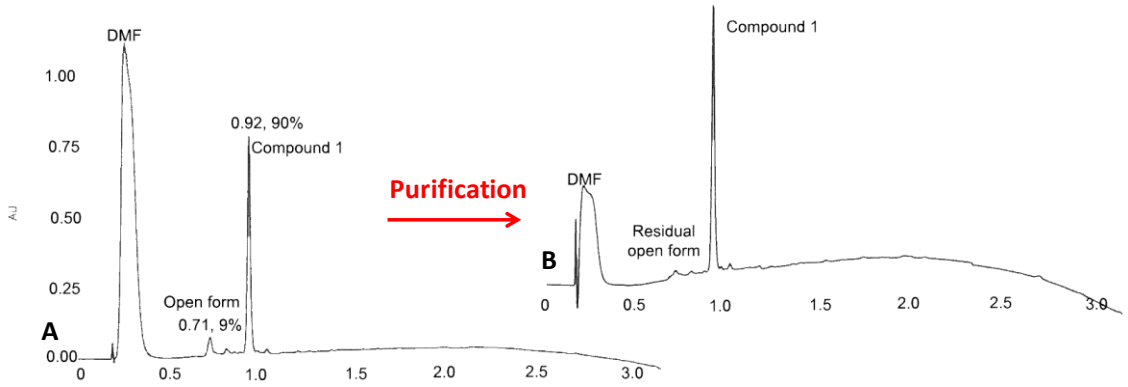
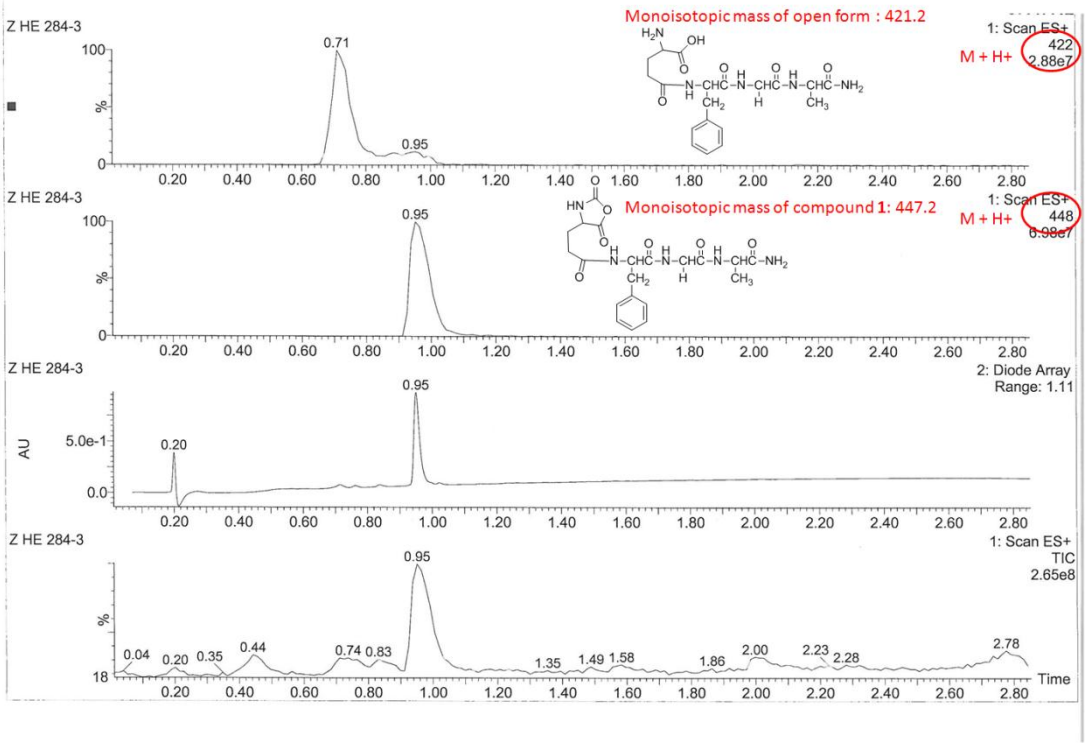


Figure SI-2. LC/MS of purified compound **1.**



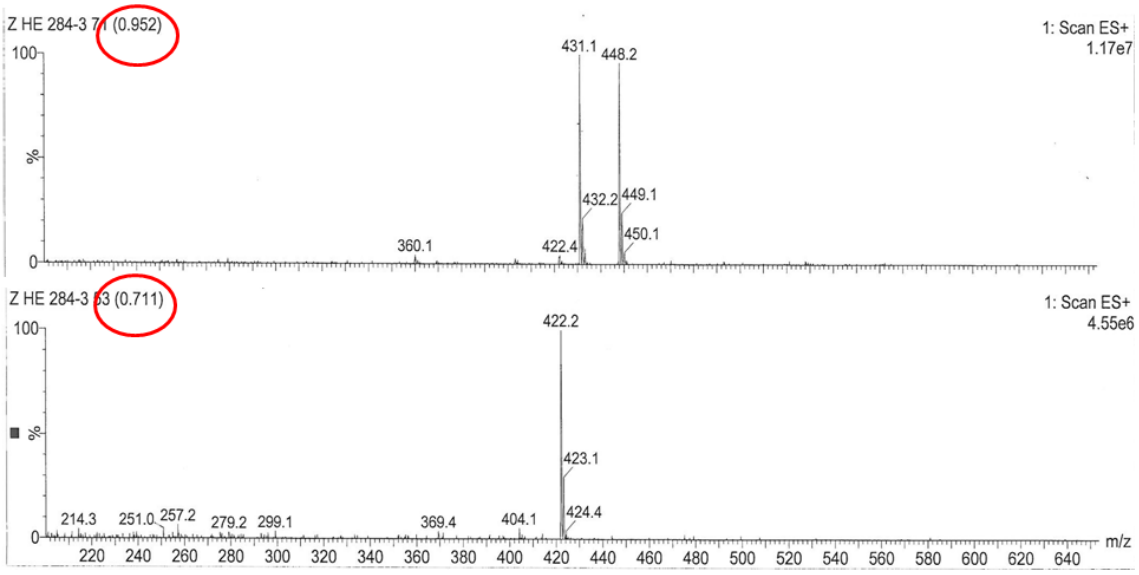


Figure SI-3. ^{1}H NMR of compound **1** (blue) and **P3**.(red) **(X** correspond to residual solvent peaks, DMF, Et_2O).

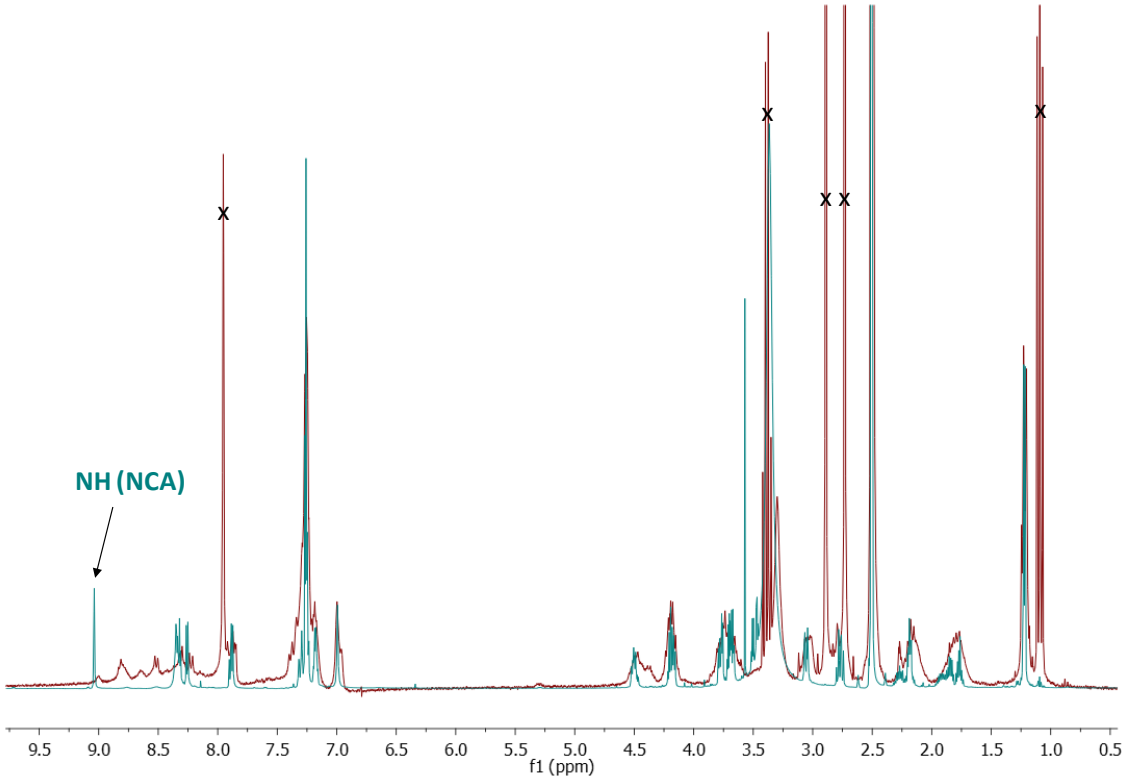


Figure SI-4. MS/MS spectra of **P2**.