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Synthesis of self assembling cyclic peptide-polymer conjugates using click chemistry

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Materials: Fmoc-L-Lysine, Fmoc-D-Leucine, 1-hydroxy-l-azabenzotriazoleuronium (HBTU) and *O*-benzotriazole-l-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HOBt) were obtained from Novabiochem and used as supplied. Butyl-trithiocarbonate propanoic acid (BTCPA), was obtained from Dulux, and used as supplied and azo-iso-butyronitrile (AIBN) was obtained from Aldrich, and precipitated from methanol prior to use. Hünig's base [di(isopropyl)ethylamine, DIPEA], trifluoroacetic acid (TFA), trifluoroethanol (TFE), 2,2' pentamethyldiethylenetriamine (PMDETA), sulphuryl chloride, and all solvents were ordered through Sigma-Aldrich and used as received. CuBr, used in the click reactions, was obtained from Aldrich and purified by suspending it in acetic acid (6 g in 50 ml) for 20 min.[1] Pure CuBr was filtered off, washed with cold EtOH (100%, 2 x 20 ml), dried under vacuum and stored under nitrogen. The monomer, hydroxyl ethyl acrylate (HEA) was purified as described in literature.[2]

Azido Fmoc-L-Lysine (1): Azido Fmoc-L-Lysine was using Stick's diazo transfer agent,[3] using a method adapted from his procedure for similar compounds. Sulfuryl chloride (8.05 ml, 100 mmol) was added dropwise to an ice cold suspension of sodium azide (6.50 g, 100 mml) in acetonitrile (100 ml) and stirred overnight at room temperature. The mixture was then recooled to 0°C and imidazole (12.95g, 190 mmol) was added over 20 min. After stirring for a further 3h at room temperature, the solution was diluted with EtOAc (200 ml), washed with water (2 x 200 ml) and

saturated $NaHCO_3$ (aq) (2 x 200 ml), and then dried over $MgSO_4$. A solution of HCl in ethanol was generated by dropwise addition of AcCl (10.65 g, 150 mmol) to EtOH (37.5 ml), and this was added dropwise to the dried EtOAc solution to generate the HCl salt of the product. After cooling in an ice bath, the colourless needles were collected by filtration and washed with EtOAc (3 x 50 ml) yielding imidazole-1-sulfonyl azide hydrochloride (6.92 g, 33 mmol, 33%). m.p. 102 – 104°C (lit 100 – 102°C),[3] IR ν_{max}. (KBr) 2173, 1429 & 1162 cm^{-1}. ^{1}H-NMR (300 MHz, D_2O) δ ppm: 7.47 (m, 1H), 7.89 (m, 1H), 8.95-9.15 (m, 1H).

Fmoc-L-Lysine-OH (5.50 g, 14.93 mmol) was ground, dissolved in water (15 ml) with 1 equivalent of HCl, and diluted with a water / methanol mixture (1:2, 65 ml). Imidazole-1-sulfonyl azide hydrochloride (3.75g, 17.9 mmol), $NaHCO_3$ (8.11 g, 96.54 mmol) and $CuSO_4.5H_2O$ (37.3 mg, 0.149 mmol) in water (1 ml) were added and the mixture stirred for 16 h at room temperature. After completion of the reaction, the mixture was concentrated to 50 ml then diluted with more water (150 ml), and acidified to pH 2 by dropwise addition of 5M HCl (aq). The aqueous phase was extracted with EtOAc (3 x 50 ml), dried over $MgSO_4$, and purified over a silica column (Toluene / EtOAc / Acetic acid 85:10:5). Concentration of the appropriate fractions gave azido Fmoc-L-lysine **1** as a white powder (3.83 g, 9.72 mmol, 72%). IR ν_{max}. (ATR) cm^{-1}: 2096 (N_3), 1714 (C=O), ^{1}H-NMR (300MHz, $CDCl_3$) δ ppm: 1.10-2.05 (m, 6H, 3 x CH_2), 3.22 (m, 2H, N_3- CH_2), 4.16 (t, *J* = 6.7 Hz, 1H, Fmoc CH), 4.30-4.55 (m, 3H, α-H & Fmoc O-CH_2), 5.18 (d, *J* = 8.0 Hz, N-H), 7.10-7.40 (4H, 4 x Fmoc CH), 7.52 (d, *J* = 7.1 Hz, 2H, Fmoc CH), 7.70 (d, *J* = 7.3 Hz, 2H, Fmoc CH). ^{13}C-NMR (300 MHz, $CDCl_3$) δ ppm: 175.8 (COOH), 156.1 (CONH), 143.8, 143.6, and 141.3 (fluorenyl C-4a, C-4b, C-8a, and C-9a), 127.8, 127.1, and 125.0 (fluorenyl C-2 to C-7), 120.0 (fluorenyl C-1 and C-8), 67.1 (CH2-O), 53.4 (C-α), 51.1 (C-ε), 47.2 (fluorenyl C-9), 31.8 (CH_2), 28.4 (CH_2), 22.4 (CH_2). Data are in good agreement with literature values.[4]

Synthesis of H_2N-(L-Lys(N_3)-D-Leu)$_4$-OH (2): 2-Chlorotrityl chloride resin (1.0 g, 1.01 mmol/g) was suspended in DCM (10 ml) in a fritted syringe and shaken for 5 min. After the DCM was filtered off, a solution of Fmoc-D-Leucine-OH (2.02 mmol, 0.71 g) and DIPEA (8.1 mmol, 1.04 g) in DCM (10 ml) was added to the resin, and the suspension was shaken for 2 h. The resin was then washed with 17:2:1 solution of

DCM:MeOH:DIPEA (3 x 10 ml) to cap any unreacted sites, and then with DCM (3 x 10 ml), DMF (3 x 10 ml), and DCM (3 x 10 ml). The resin was dried under vacuum and used for further solid phase peptide synthesis. Loading was calculated to be ~0.96 mmol / g original resin by UV-Vis spectroscopy.[5]

The dried resin (0.20 g) was swollen with DCM in a 2 ml fritted syringe by shaking for 30 min. The DCM was removed, and the chain was deprotected by the addition of 20% piperidine in DMF for 3 min (x 2) and then washed with DMF (x5), DCM (x3) and DMF (x2) to remove any piperidine. For a lysine addition, Fmoc-L-Lys(N_3)-OH (0.175 g, 0.287 mmol), HBTU (0.143 g, 0.383 mmol), and DIPEA (0.158 g, 0.958 mmol) in DMF were drawn into the syringe and the mixture was shaken overnight. For a leucine addition, Fmoc-D-Leu-OH (0.203 g, 0.575 mmol), HBTU (0.218 g, 0.575 mmol) and DIPEA (0.190 g, 1.149 mmol) were drawn into the syringe and the reaction was shaken for 3h. After reaction, the resin was washed with DMF (x5) then the deprotection step was repeated and the next amino acid added. After addition and deprotection of the last amino acid, the chain was cleaved from the resin by addition of a cocktail of TFA / thioanisole / triisopropylsilane / water (88:5:2:5), which was allowed to react for 3h. The resin was then filtered off, and the solution concentrated to near dryness under reduced pressure, redissolved in a small amount of MeOH and precipitated by the addition of ice cold diethyl ether to yield the linear peptide **2** as a white powder (0.16 g, 75%). ^{1}H-NMR (300 MHz, *d*-TFA) δ ppm: 0.95 (br, 24H, 8 Leu CH_3), 1.35-2.20 (m, 36H, 12 Lys CH_2 & 4 Leu CH & 4 Leu CH_2), 3.35 (br, 8H, 4 N_3-CH_2), 4.24-4.40 (m, 1H, 1 α-CH), 4.53-4.96 (m, 7H, 7 α-CH). *m/z* (ESI) 1087.87 ($[M+H]^+$, calc = 1087.70).

Synthesis of (prop-2-ynyl propanoate)yl butyltrithiocarbonate (PPBTC, 4): PPBTC was prepared according to previous work in our group.[6] Butyl-trithiocarbonate propanoic acid (BTCPA) (1.03 g, 4.30 mmol) was dissolved in DCM (50 ml) and cooled in an ice bath. To this was added propargyl alcohol (1.21 g, 21.51 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 1.65 g, 8.61 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 0.54 g, 4.42 mmol). After reaction for 2 h at 0°C and then for 16 h at room temperature, the excess reagents were removed by washing with water (5 x 20 ml). The organic fraction was dried over $MgSO_4$, concentrated, and passed over a silica pad with Toluene / EtOAc (9:1). The

solvent was evaporated to yield the product **4** as a yellow oil (1.08 g, 3.09 mmol, 72%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm: 0.86 (t, *J* = 7.2 Hz, 3H), 1.36 (sext., *J* = 7.0 Hz, 2H), 1.49-1.72 (m, 5H), 2.42 (t, *J* = 2.5 Hz, 1H), 3.29 (t, 2H), 4.66 (d, *J* = 10.8 Hz, 2H), 4.78 (q, *J* = 7.4 Hz, 1H).

Polymerisations: The RAFT agent **4**, AIBN and HEA (20:1:0.1 for the short polymer, and 100:1:0.1 for the long polymer) were mixed with 70% (wt/wt) of *t*-butanol in a schlenk tube and sonicated for 1 min to aid dissolution. 4 – 6 freeze pump thaw cycles were used to degass the samples and polymerisations were conducted at 60°C in a silicon oil bath, under N_2 at 1 atm. After reaction, a sample of the crude was retained for determination of conversion by ^{1}H-NMR, and the remainder was purified by precipitation and subsequent washing from ice-cold diethyl ether. Molecular weights and polydispersities were determined by SEC (DMF, 0.3% LiBr, 60°C) taking DMSO as a flowrate marker, using a DRI detector and by ^{1}H-NMR (*d6*-DMSO).

Table S1: Polymer characterisation data

Polymer	**[M]:[RAFT]**[a]	**Conversion (X)**	**M_n** [b,c] **(SEC)**	**M_n** [b,d] **(^{1}H-NMR)**	**Degree of Polymerisation**	**PDI**
Short	20 : 1	60%	2760	1698	12	1.10
Long	100 : 1	35%	4700	3965	30	1.13

Notes: (a) [M] = [Monomer], (b) M_n displayed as g.mol^{-1}, (c) SEC molecular weights relative to Styrene standards. No correction for the molecular weights of HEA was used, (d) ^{1}H-NMR M_n calculated by integration of the RAFT relative to the monomer peaks

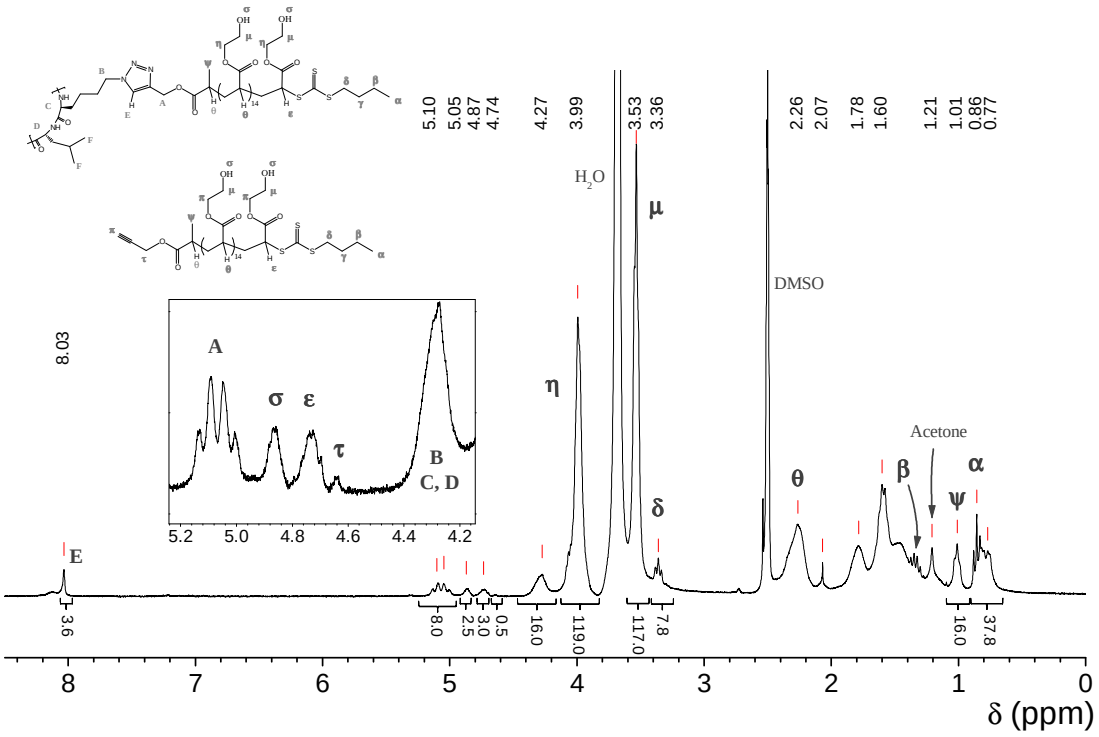

Figure S1: Example of a ^{1}H-NMR (*d6*-DMSO with D_2O) used to determine the conversion from the conjugation of the DP12 pHEA to the 4 arom cyclic peptide

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