Supplementary Material (ESI) for Chemical Science

End-stapled homo and hetero collagen triple helices: A click chemistry approach

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General chemistry

Chemicals and solvents were purchased from standard suppliers and used without further purification. Silica Gel 60 Å, 40–63 μm, for flash chromatography was supplied by Fluorochem and anhydrous solvents were purchased from Fluka or Aldrich. All other solvents were used as supplied (analytical, HPLC or peptide grade), without prior purification. Milli-Q water was used for chemical reactions. Deuterated solvents were purchased from Goss or Sigma Aldrich. All reactions requiring anhydrous conditions were performed using flame- or oven-dried apparatus under an atmosphere of nitrogen. Reactions were monitored by analytical thin-layer chromatography on commercially available precoated aluminium packed plates (Merck Kieselgel 60 F_{254}) or using analytical RP-HPLC. Visualization of the silica plates was achieved using a UV lamp (λ_{max} = 254 nm) and/or potassium permanganate or ninhydrin staining.

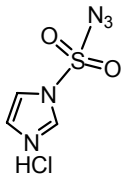
Melting points were recorded on a Stuart Scientific melting point apparatus (SMP3). Mass spectra (ES-TOF) were recorded on a Waters 2795 separation module/Micromass LCT^{TM} platform. MALDI-TOF spectra were recorded at the Biopolymer Synthesis and Analysis Unit, QMC Nottingham, using sinapinic acid or α-cyano-4-hydroxycinnamic acid as the matrix.

Proton nuclear magnetic resonance (δ_H) and carbon nuclear magnetic resonance (δ_C) spectra were recorded at 20 °C on a Bruker AV400 operating at 400.13 MHz and 101.62 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm), referenced to either $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm), [D_6]-DMSO (^{1}H, 2.50 ppm; ^{13}C, 39.51 ppm) or CD_3OD (^{1}H, 3.31 ppm; ^{13}C, 77.23 ppm). Coupling constants (J) are recorded in Hz and significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m) or doublet of doublets (dd). Spectra were assigned using appropriate COSY, DEPT and HSQC sequences.

RP-HPLC was performed using a Waters setup, comprised of two 510 pumps, a 486 detector and Millenium^{TM} software. The systems outlined below were used for purification and to confirm purity.
Analytical RP-HPLC was performed using Phenomenex Onyx Monolithic reversed-phase C18 column (4.6 x 100 mm), using Solvent A: 0.06% TFA in water and solvent B: 0.06% TFA in CH_3CN:H_2O (90:10), at flow rate of 3.0 mL min^{-1} and UV detection at 216 nm.
System 1a: Linear gradient: 3–30% solvent B in 12 min; also used for purification of 'clicked' peptide products from reaction mixture.
System 1b: Linear gradient: 30–80% solvent B in 12 min.
Semi-preparative RP-HPLC was performed using a Hichrom Kromasil^{TM} 5 Å C18 column (10 x 100 mm) with a flow rate of 4 mL min^{-1} using the gradient indicated.

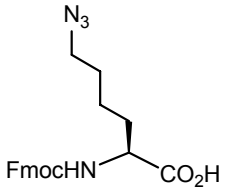
Experimental procedures

Imidazole-1-sulfonyl azide hydrochloride[1]



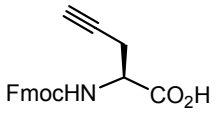
Imidazole-1-sulfonyl azide hydrochloride was synthesized using a slight modification to the procedure outlined by Goddard-Borger and Stick.[1]
Sulfuryl chloride (1.67 g, 1.0 mL, 12.4 mmol) was added to a stirred, ice-cooled suspension of NaN_3 (804 mg, 12.4 mmol) in CH_3CN (20 mL). The solution was allowed to return to room temperature and then stirred overnight. Imidazole (1.52 g, 12.4 mmol) was added and the solution stirred for a further 3 h. EtOAc (50 mL) was added to the solution and the solution washed with water (2 x 50 mL) and satd. $NaHCO_3$ (50 mL). The organic extract was dried over $MgSO_4$ and the solvent removed *in vacuo* to reveal a white solid. An ethanolic solution of HCl (made by addition of acetyl chloride (1.06 mL) to ethanol (5mL)) was added dropwise and the precipitated product was filtered, washed with ice-cold ethanol and dried *in vacuo* to yield the title compound (1.0 g, 40 %).
δ_H (400 MHz, D_2O): 9.36 (1H, dd, J = 1.3, 2.2), 8.04 (1H, dd, J = 1.6, 2.2), 7.63 (1H, dd, J = 1.3, 1.6);
δ_C (100 MHz, D_2O): 137.6, 122.4, 118.9.

N-(9-Fluorenylmethoxycarbonyl)-2-(*S*)-amino-6-azidohexanoic acid (Fmoc-Lys(N_3)-OH; 2)[2]



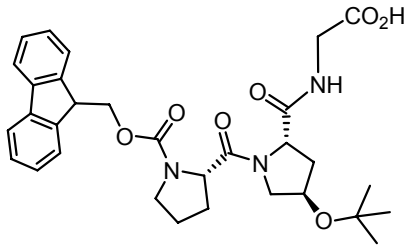
A mixture of Fmoc-L-Lys-OH (1.47 g, 3.98 mmol), K_2CO_3 (1.16 g, 8.36 mmol), imidazole-1-sulfonyl azide hydrochloride (1.0 g, 4.78 mmol) and $CuSO_4 \bullet 5H_2O$ (10 mg, 0.04 mmol) were dissolved in MeOH (50 mL). A few drops of water were added to aid dissolution. The solution was stirred for 5 h and then the solvent removed *in vacuo*. The crude material was partitioned between water (50 mL) and EtOAc (50 mL), and acidified to pH 4 by dropwise addition of 0.1 M aq HCl. The aqueous phase was removed, and the organic extract was then washed with water (50 mL), brine (50 mL), and dried over Na_2SO_4.
The solvent removed *in vacuo* to reveal a pale yellow oil that solidified to an off-white waxy solid on standing (1.42 g, 90 %).
δ_H (400 MHz, $CDCl_3$) 7.76 (2H, d, J = 7.5, Fmoc Ar*H*), 7.59 (2H, d, J = 7.0, Fmoc Ar*H*), 7.40 (2H, m, Fmoc Ar*H*), 7.31 (2H, m, Fmoc Ar*H*), 5.32 (1H, d, J = 8.0, N*H*), 4.53 (3H, m, Fmoc CHCH_2O, C$^{\alpha}H$), 4.22 (1H, t, J = 6.8, Fmoc C*H*CH_2CO), 3.28 (2H, t, J = 6.8, CH_2N_3), 1.92, 1.74 (2 x 1H, m, C$^{\beta}H_2$), 1.61 (2H, m, C$^{\delta}H_2$), 1.48 (2H, m, C$^{\gamma}H_2$);
δ_C (100 MHz, $CDCl_3$) 176.6, 156.3 (*C*O), 143.9, 143.7, 141.43, 127.9, 125.15, 120.15, 67.3 (Fmoc *C*H_2), 53.6 (*C*$^{\alpha}$H), 51.1 (*CH*$_2$N$_3$), 47.2 (Fmoc *C*H), 31.9 (*C*$^{\beta}H_2$), 28.4 (*C*$^{\delta}H_2$), 22.6 (*C*$^{\gamma}H_2$);
m/z (ES-TOF$^+$) calcd for $C_{21}H_{23}N_4O_4^+$ (MH^+) 395.1714, found 395.1729.

N-(9-Fluorenylmethoxycarbonyl)-2-(*S*)-amino-4-pentynoic acid (Fmoc-L-Pra-OH)[3]



To a stirred solution of L-propargylglycine (0.50 g, 4.42 mmol) and $NaHCO_3$ (0.93 g, 11.1 mmol) in water (25 mL) was added a solution of Fmoc succinimide (1.49 g, 4.42 mmol) in THF (25 mL). The clear solution was stirred overnight at ambient temperature. The mixture was then evaporated to dryness *in vacuo* and the residual material was dissolved in water (50 mL). This aqueous solution was extracted with ether (2 x 30 mL), added EtOAc (50 mL) and acidified to pH 1.5 using $KHSO_4$. The organic extract was removed, and the aqueous phase was extracted with EtOAc (2 x 30 mL). The EtOAc extracts were combined and washed with water (50 mL), brine (60 mL), dried over Na_2SO_4 and the solvent removed *in vacuo* to afford the crude product (1.26 g). The crude material was recrystallised from EtOAc to yield the title compound as a white crystalline solid (962 mg, 65 %).
m.p. 175–177 °C (Lit 175 °C)[3];
δ_H (400 MHz, [D^6]-DMSO) 12.95 (1H, br s, CO_2H), 7.90 (2H, d, J = 7.40, Fmoc Ar*H*), 7.75 (3H, m , N*H* and Fmoc Ar*H*), 7.42 (2H, t, J = 7.30, Fmoc Ar*H*), 7.34 (2H, t, J = 7.30, Fmoc Ar*H*), 4.29 (2H, d, J = 7.25, CHCH_2O) 4.25 (1H, t, J = 7.25, C*H*CH$_2$O), 4.15 (1H, m C$^{\alpha}H$), 2.89 (1H, s, C*H*), 2.60 (2H, m, C$^{\beta}H_2$);
δ_C (100 MHz, [D^6]-DMSO) 172.0 (*C*O), 155.9 (*C*O), 143.8, 140.7, 127.7, 127.1, 125.3, 125.3, 120.1, 80.7, 72.9, 65.8, 52.9, 46.6, 21.1;
HRMS (ES-TOF$^-$) calcd for $C_{20}H_{16}NO_4$ (M-H) 334.1079, found 334.1090.

***N*-(9-Fluorenylmethoxycarbonyl)-L-prolinyl-L-4-(*tert*-butoxy)prolinyl-glycine (Fmoc-Pro-Hyp(*t*Bu)-Gly-OH; 4)**[4]



The 2-chlorotrityl polystyrene N^{α}-Fmoc-glycinoate (14.0 g, 9.24 mmol, 0.66 mmol g^{-1}) was swollen in DMF (25 mL) for 30 min and then washed with DMF (5 x 25 mL). The DMF was removed and the resin was treated with 20 % piperidine in DMF (20 mL) for 10 min with agitation by nitrogen bubbling. The 20 % piperidine in DMF was then removed and the resin was subjected to a second treatment with 20 % piperidine in DMF (20 mL 10 min). The resin was then washed with DMF (10 x 20 mL).
A mixture of Fmoc-Hyp(*t*Bu)-OH (5.68 g, 13.86 mmol), HATU (5.08 g, 13.40 mmol) and DIEA (4.82 mL, 27.7 mmol) was dissolved in DMF (10 mL) and added to the resin. The suspension was agitated for 1 h and then washed with DMF (5 x 20 mL). The coupling was repeated using fresh reagents, and the resin product was then washed with DMF (10 x 20 mL). The DMF was removed and the resin was treated twice with 20 % piperidine in DMF (20 mL) for 10 min. The resin was then washed with DMF (10 x 20 mL).
Fmoc-Pro-OH (4.68 g, 13.86 mmol), HATU (5.08 g, 13.4 mmol) and DIEA (4.82 mL, 27.7 mmol) were dissolved in DMF (10 mL) and added to the resin. The suspension was agitated for 1 h and the resin washed with DMF (5 x 20 mL). The coupling was repeated twice, each time with fresh reagents. The resin product was washed with DMF (10 x 20 mL), CH_2Cl_2 (10 x 20 mL) and hexane (10 x 20 mL). The resin was collected and dried *in vacuo*.
The dried resin material was treated with 20 % 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in CH_2Cl_2 (100 mL) for 1 h, filtered and the filtrate was evaporated to dryness *in vacuo* to afford the title compound as a white solid (4.10 g, 79 %).
R_T (system 1b) = 7.22 min;
m/z (ES^+) calcd for $C_{31}H_{38}N_3O_7^+$ (MH^+) 564.27, found 564.26; calcd for $C_{62}H_{74}N_6NaO_{14}^+$ ($[2M+Na]^+$) 1149.52, found 1149.44.

1. E. D. Goddard-Borger and R. V. Stick, *Org. Lett.* (2007), **9**, 3797-3800.
2. E. Sabido, T. Tarrago and E. Giralt. *Bioorg. Med. Chem. Lett.* (2009), **19**, 3752-3755.
3. L. Garcia, A. Pla-Quintana and A. Roglans, *Org. Biomol. Chem.* (2009), **7**, 5020-5027.
4. B. Sacca and L. Moroder, *J. Pept. Sci.* (2002), **8**, 192-204.

Solid-phase peptide synthesis

Peptides were synthesized in a glass column on a continuous flow peptide synthesizer using NovaGel^{TM} Rink amide resin (0.67 mmol g^{-1}; Merck Chemicals Ltd. # 01-64-0286). Acylation with activated Fmoc-amino acids were typically accomplished with a four-fold excess of Fmoc-amino acid, 3.95 equivalents of HATU and eight equivalents of DIEA. Coupling cycles were carried out for a minimum of one hour and all proline residues were triple coupled. The column volume was ~2 mL. Washing and Fmoc-deprotection cycles were carried out at a flow rate of 2.8 mL min^{-1} with DMF and 20% piperidine in DMF, respectively. The Fmoc-deprotection was monitored using an in-line UV detector and monitoring at 290 nm.

Acidolysis was achieved using the cocktail TFA – *i*Pr_3SiH (TIPS) – H_2O (95:2.5:2.5), approximately 10 mL per gram of peptide resin, typically for 2 h. The suspension was filtered, the filtrate was evaporated to dryness *in vacuo*, and the residual material was triturated with ice-cold ether (x 3) to furnish a white or off-white solid. The peptide product was dissolved in Milli-Q water and lyophilized before analysis and purification by semi-preparative RP-HPLC.

Synthesis of DS1

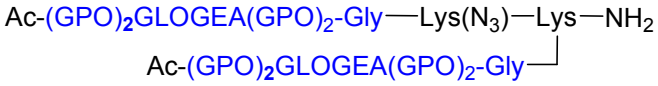
NovaGel^{TM} Rink amide resin (150 mg, 0.1 mmol) was swollen in DMF (~ 2 mL) for 30 min.
Fmoc-Lys(ivDde)-OH (230 mg, 0.4 mmol) was pre-activated using HATU (148 mg, 0.39 mmol) and DIEA (139 μL, 0.8 mmol) in DMF (~1.5 mL), added to the resin and gently stirred for 1 h. The resin was washed with DMF (10 min) to afford the resin product **1**, which was then treated with 20 % piperidine in DMF (10 min) and washed with DMF (10 min). Fmoc-Lys(N_3)-OH **2** (158 mg, 0.4 mmol), HATU (148 mg, 0.39 mmol) and DIEA (139 μL, 0.8 mmol) were dissolved in DMF (1.5 mL) and added to the resin. Coupling was carried out for 1 h, and the resin was washed with DMF (10 min). The ivDde and Fmoc groups were removed by treatment with 2–3 % hydrazine hydrate in DMF (1 h) followed by 20 % piperdine in DMF (10 min). The resin product **3** was then washed with DMF (10 min).
A mixture of Fmoc-Gly-OH (240 mg, 0.8 mmol – effectively 4 eq), HATU (296 mg, 0.78 mmol) and DIEA (278 μL, 1.6 mmol) was added to the resin and left for 1h. The resin product was then washed with DMF (10 min), treated with 20% piperidine in DMF (10 min) and then washed with DMF (10 min). Fmoc-Hyp(*t*Bu)-OH (0.8 mmol), HATU (296 mg, 0.78 mmol) and DIEA (278 μL, 1.6 mmol) were dissolved in DMF (1.5 mL) and added to the resin, which was then left with gentle agitation for 1 h. After the cycles of washing, Fmoc deprotection and washing, activated Fmoc-Pro-OH (270 mg, 0.8 mmol) was introduced using the standard coupling methodology. After washing, the proline coupling was repeated twice with fresh reagents.
At this point, Fmoc-Pro-Hyp(*t*Bu)-Gly-OH **4** (225 mg, 0.4 mmol – effectively 2 eq) was activated using HATU (148 mg, 0.39 mmol) and DIEA (139 μL, 0.8 mmol) in DMF (~1.5 mL) and added to the resin. The suspension was gently stirred for 2 h. This coupling was repeated once. Couplings were continued in the usual manner using appropriately protected Fmoc amino acids or Fmoc-Pro-Hyp(*t*Bu)-Gly-OH units. After incorporation of the final Fmoc-tripeptide unit **4**, half the resin was taken away for synthesis of **DP2**.
For the remaining resin material, a mixture of Fmoc-Gly-OH (240 mg, 0.8 mmol – effectively 8 eq), HATU (296 mg, 0.78 mmol) and DIEA (278 μL, 1.6 mmol) was added to the resin and left for 1h. After washing and Fmoc deprotection, a solution of $(Ac)_2O$ (142 μL, 1.5 mmol) and DIEA (35 μL, 0.2 mmol) in DMF (1 mL) was added to the resin and left for 30 min. The

resin was transferred to a Buchner funnel and washed with DMF (10 x 2 mL), CH_2Cl_2 (10 x 2 mL) and hexane (10 x 2 mL). The resin was dried *in vacuo*.
The peptide resin was suspended in TFA – TIPS – H_2O (95:2.5:2.5; ~5 mL) for 2 h. The suspension was filtered, the filtrate was evaporated to dryness *in vacuo*, and triturated with ice-cold ether (3 x 3 mL) to afford a white solid. The title peptide product was dissolved in water (10 mL) and lyophilized to yield a white fluffy solid (147 mg).
Semi-preparative RP-HPLC (10 mg peptide in 1 mL H_2O – CH_3CN (80:20); 5 injections of 200 µL) yielded 3.0 mg of purified **DS1**.

Note: During the latter stages of the peptide assembly, longer Fmoc deprotection times (40–60 min) were necessary.

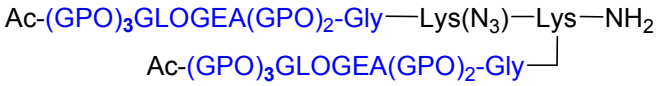
n = 2, **DS1**; n = 3, **DS2**
Red indicates segment insertion using the trimer **4**.

DS1



Semi-preparative (20–30% B in 10 min) R_T = 6.22 min
R_T (system 1a) = 9.56 min
m/z (ES^+): ($[M+3H]^{3+}$) calcd 1261.24, found 1261.23.

DS2



Semi-preparative (20–30% B in 10 min) R_T = 5.99 min
R_T (system 1a) = 9.52 min
m/z (ES^+): ($[M+3H]^{3+}$) calcd 1417.67, found 1417.66
m/z (MALDI-TOF): $[M+H]^+$ calcd 4249.0, found 4248.2.

The linear peptides **SS1**–**SS6** were assembled stepwise using standard methods and appropriately protected amino acids.

Ac-GPOGPOGLOGEAGPOGPO-NH_2 (SS1)

Semi-preparative (10–19% B in 10 min) R_T = 9.82 min
R_T (system 1a) = 7.05 min
m/z (ES^+): ($[M+H]^+$) calcd 1668.78, found 1668.71.

Ac-GPOGPOGPOGLOGEAGPOGPO-NH_2 (SS2)

Semi-preparative (10–19% B in 10 min) R_T = 9.96 min
R_T (system 1a) = 7.20 min
m/z (ES^+): ($[M+H]^+$) calcd 1935.91, found 1935.77.

Ac-GPOGPOGLOGEAGPOGPOG-Pra-NH_2 (SS3)

Semi-preparative (10–22% B in 10 min) R_T = 7.05 min
R_T (system 1a) = 5.82 min
m/z (ES^+): ($[M+Na]^+$) calcd 1842.83, found 1843.35.

Ac-GPOGPOGPOGLOGEAGPOGPOG-Pra-NH_2 (SS4)

Semi-preparative (10–22% B in 10 min) R_T = 7.41 min
R_T (system 1a) = 6.18 min
m/z (MALDI-TOF): $[M+Na]^+$ calcd 2109.9, found 2109.9

Ac-GPOGPOGLOGENGPOGPOG-Pra-NH_2 (SS5)

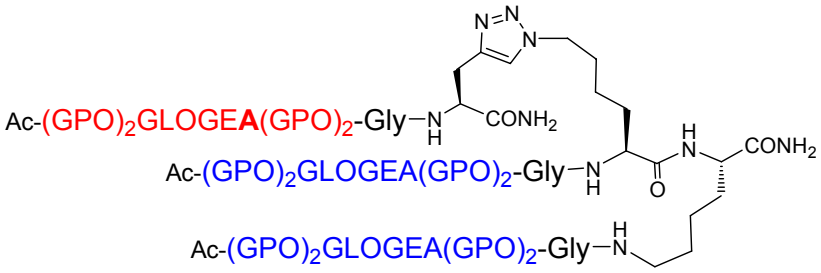
Semi-preparative (15–23% B in 8 min) R_T = 6.51 min
R_T (system 1a) = 7.40 min
m/z (ES^+): $[M+H]^+$ calcd 1863.85, found 1863.90.

AcGPOGPOGPOGLOGENGPOGPOG-Pra-NH_2 (SS6)

Semi-preparative (15–26% B in 10 min) R_T = 6.56 min
R_T (system 1a) = 7.55 min
m/z (ES^+): $[M+2Na]^{2+}$ calcd 1087.97, found 1087.94
m/z (MALDI-TOF): $[M+Na]^+$ calcd 2152.96, found: 2152.1.

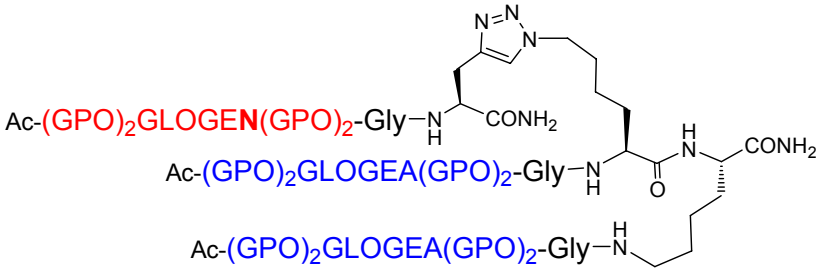
CuAAC reactions

CP1



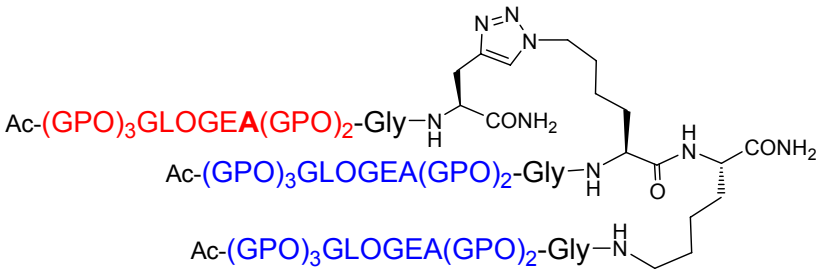
To a solution of **SS3** (1.96 mg, 1.08 µmol) and **DS1** (2.0 mg, 0.54 µmol) in water (400 µL) was added a solution of $CuSO_4$•5HO (0.67 mg, 2.7 µmol) in 5 % DMF in H_2O (80 µL). Sodium ascorbate (2.1 mg, 10.8 µmol) was added and the solution mixed for 1–2 min. The mixture was immediately injected onto a RP-HPLC column and purified (system 1a). The collected fraction was lyophilized to afford a flocculent white solid (2.05 mg, 69 %).
R_T (system 1a) = 8.96 min
m/z (ES^+): ($[M+3H+Na]^{4+}$,100 %) calcd 1390.89, found 1390.89; ($[M+2H+Na]^{3+}$, 60 %) calcd 1853.53, found: 1854.22.

CP2



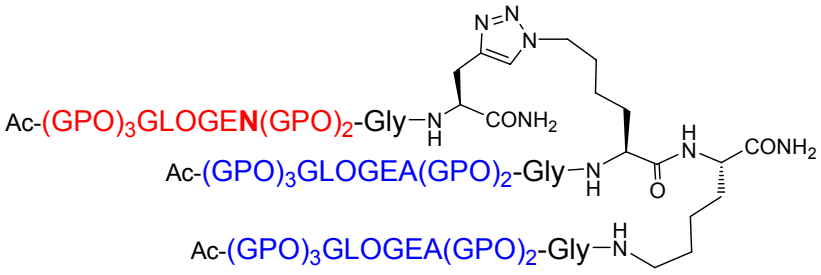
To a solution of **SS5** (1.68 mg, 0.90 µmol) and **DS1** (1.67 mg, 0.45 µmol) in water (400 µL) was added a solution of $CuSO_4$•5HO (0.56 mg, 2.25 µmol) in 5 % DMF in H_2O (80 µL). Sodium ascorbate (1.8 mg, 9.1 µmol) was added and the solution mixed for 1–2 min. The solution was immediately injected onto a RP-HPLC column and purified (system 1a), the collected fraction was lyophilized, to yield a flocculent white solid (1.45 mg, 58 %).
R_T (system 1a) = 9.41 min
m/z (ES+): ($[M+4H]^{4+}$, 100 %) calcd 1394.66, found: 1395.68; ($[M+3H]^{3+}$, 55 %) calcd 1860.88, found: 1860.92.

CP3



To a solution of **SS4** (1.96 mg, 0.94 µmol) and **DS2** (2.0 mg, 0.47 µmol) in water (400 µL) was added a solution of $CuSO_4$•5HO (0.59 mg, 2.4 µmol) in 5 % DMF in H_2O (80 µL). Sodium ascorbate (1.9 mg, 9.6 µmol was added and the solution mixed for 1–2 min. The solution was immediately injected onto a RP-HPLC column and purified (system 1a), the collected fraction was lyophilized, to afford a flocculent white solid (1.26 mg, 42 %).
R_T (system 1a) = 8.88 min
m/z (MALDI-TOF): ($[M+H]^+$) calcd 6358.9, found: 6362.9.

CP4



To a solution of **SS6** (1.93 mg, 0.91 µmol) and **DS2** (1.93 mg, 0.45 µmol) in water (400 µL) was added a solution of $CuSO_4$•5HO (0.56 mg, 2.3 µmol) in 5 % DMF in H_2O (80 µL). Sodium ascorbate (1.8 mg, 9.1 µmol was added and the solution mixed for 1–2 min. The solution was immediately injected onto a HPLC column and purified (system 1a), the collected fraction was lyophilized, to yield a flocculent white solid (1.24 mg, 43 %).
R_T (system 1a) = 9.45 min
m/z (ES+): ($[M+3H+Na]^{4+}$) calcd 1591.24, found: 1591.96; ($[M+4H+Na]^{5+}$) calcd 1273.59, found: 1273.34.

Circular dichroism

Circular dichroism spectroscopy was carried out on a Jasco 810 Circular Dichroism Spectropolarimeter with a Peltier CDF-426S to control the temperature. The peptide solutions (200 μM in 10 mM phosphate buffered saline (PBS), pH 7.2) were incubated at 2–4 °C for 24 h. Immediately prior to acquiring data, the peptide solutions were incubated at 2 °C for 20 min in the cell compartment. Spectra were then recorded in 0.5 nm increments with a 3 s averaging time, 1 nm bandpass and 0.2 cm pathlength. Melt temperature curves were recorded using a temperature change rate of 10 °C h^{-1} and the ellipticity was monitored at 223 nm. Binomial smoothing was applied to melt transition curves using the Jasco analysis software.

Differential scanning calorimetry

DSC was carried out using using a VP-DSC MicroCalorimeter.
A baseline scan was carried out with 10 mM PBS in the reference and sample cells. Heating/cooling cycles were performed from 5 to 60 °C at 6 °C h^{-1} until two scans overlapped fully and the second scan was used as a baseline. The **CP3** peptide solution (1 mg mL^{-1}; PBS, pH 7.2) were incubated at 4 °C for at least 16 h in degassed buffer and melt transitions were then recorded at 6 °C h^{-1}. The peptide sample was subjected to repeated 5–60–5 °C cycles Repeated scans overlapped identically, indicating complete re-formation of triple helices, most likely as a result of the very slow cooling process. Melt temperatures were deconvoluted using the instrument software and fitted to a two-state system.

RP-HPLC analyses of trimer building block and purified peptides

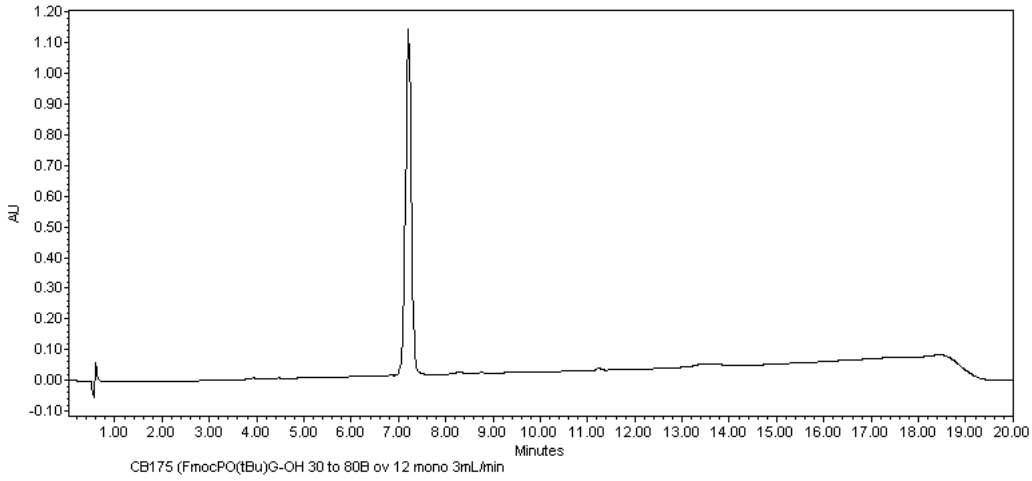


Figure S1 RP-HPLC analysis (system 1b) of purified **4**

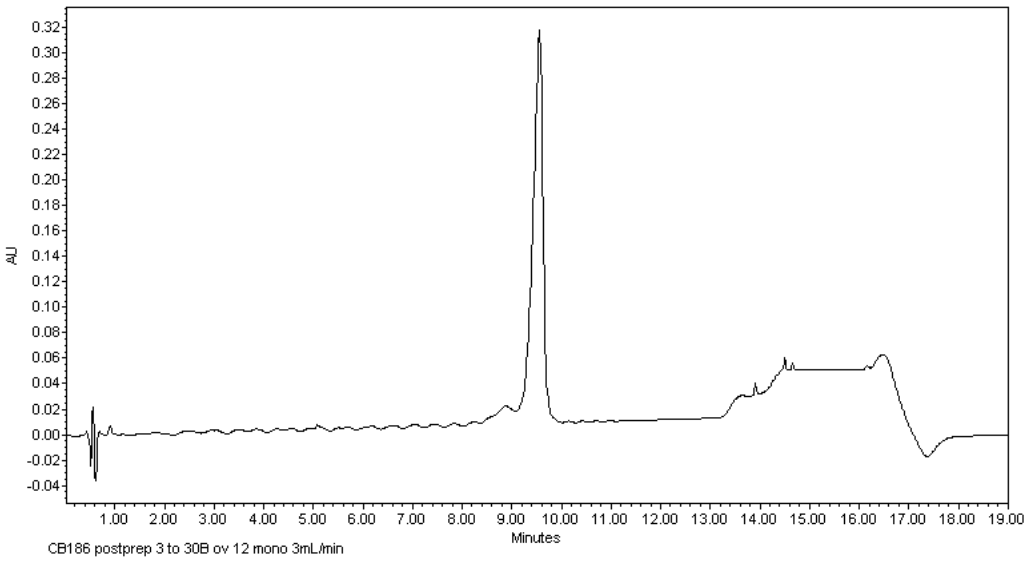


Figure S2 RP-HPLC analysis (system 1a) of purified **DS1**

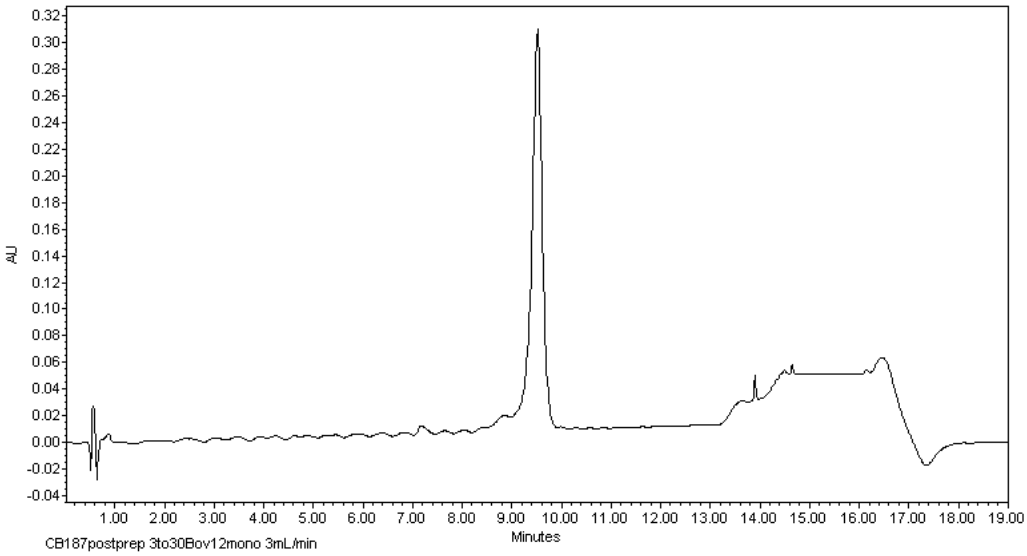


Figure S3 RP-HPLC analysis (system 1a) of purified **DS2**

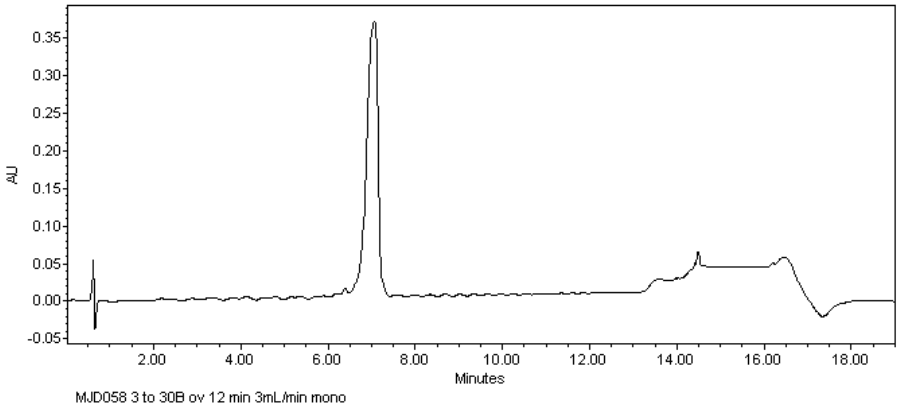


Figure S4 RP-HPLC analysis (system 1a) of purified **SS1**

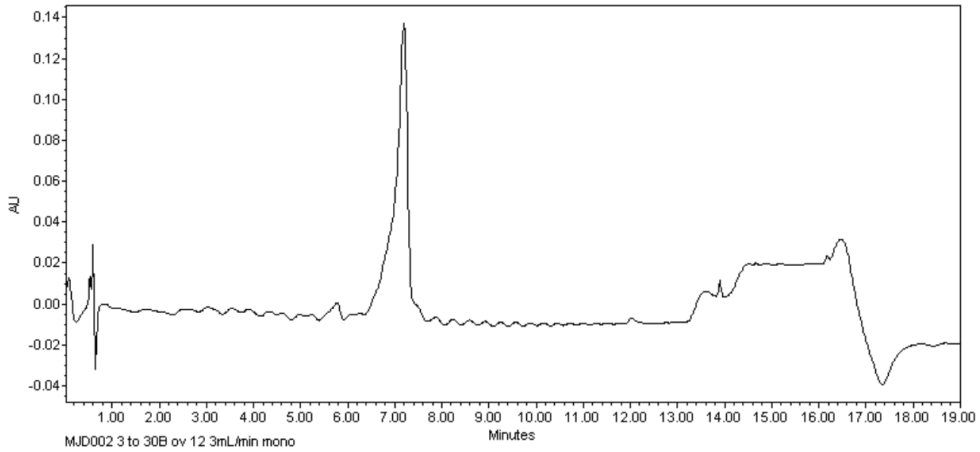


Figure S5 RP-HPLC analysis (system 1a) of purified **SS2**

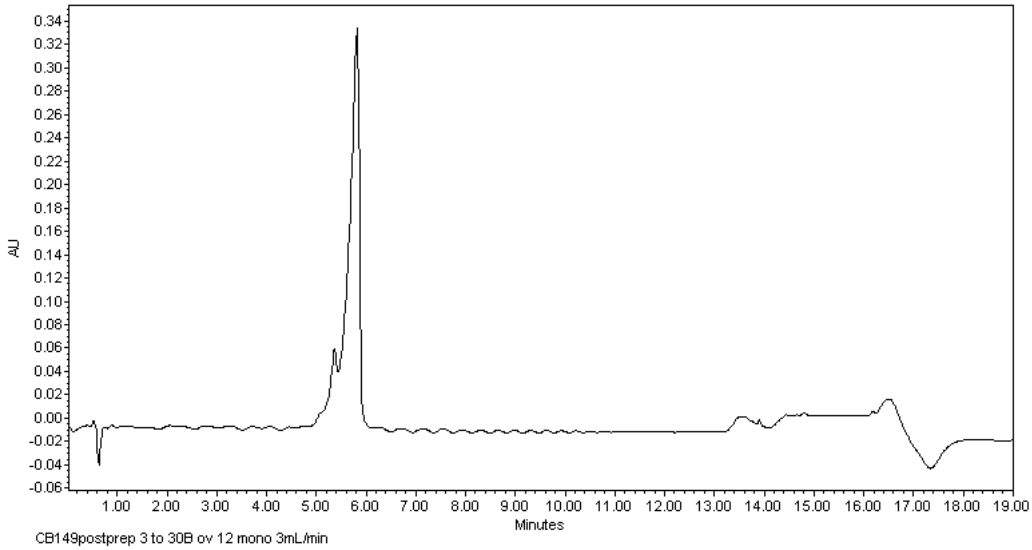


Figure S6 RP-HPLC analysis (system 1a) of purified **SS3**

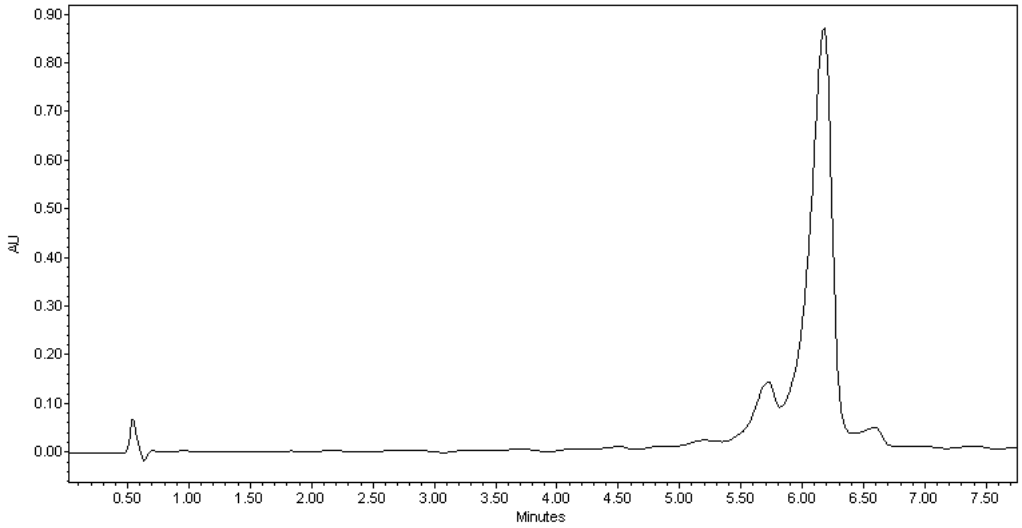


Figure S7 RP-HPLC analysis (system 1a) of purified **SS4**

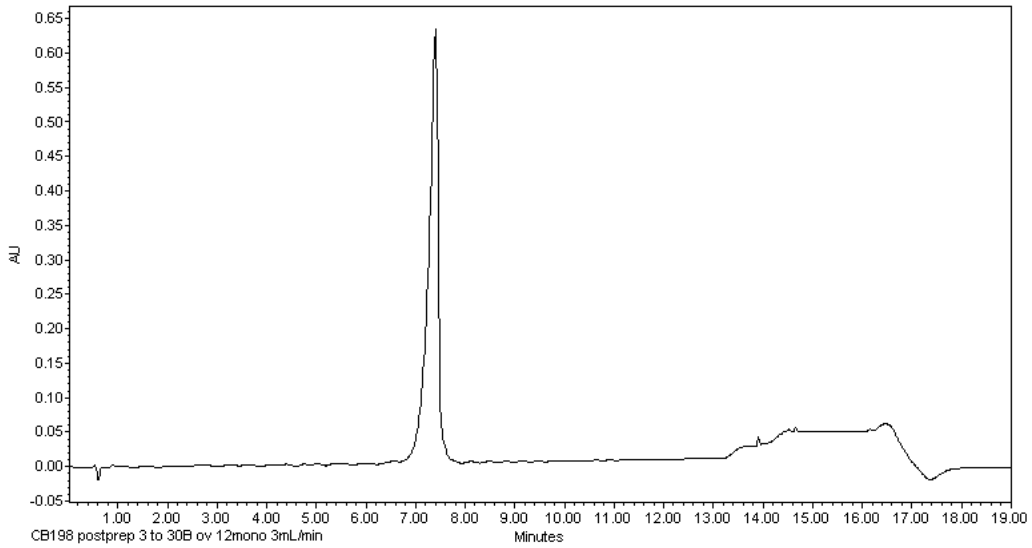


Figure S8 RP-HPLC analysis (system 1a) of purified **SS5**

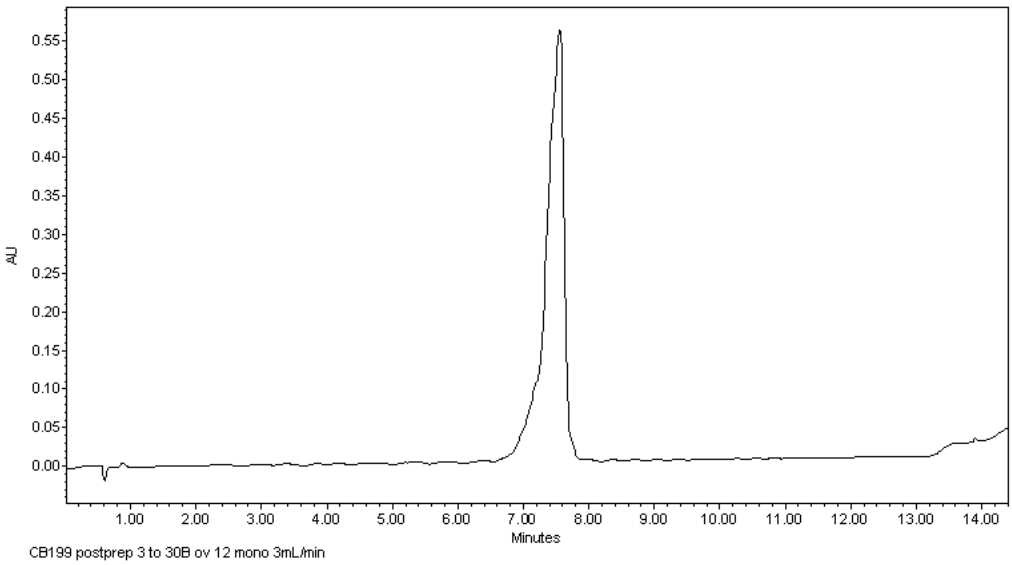


Figure S9 RP-HPLC analysis (system 1a) of purified **SS6**

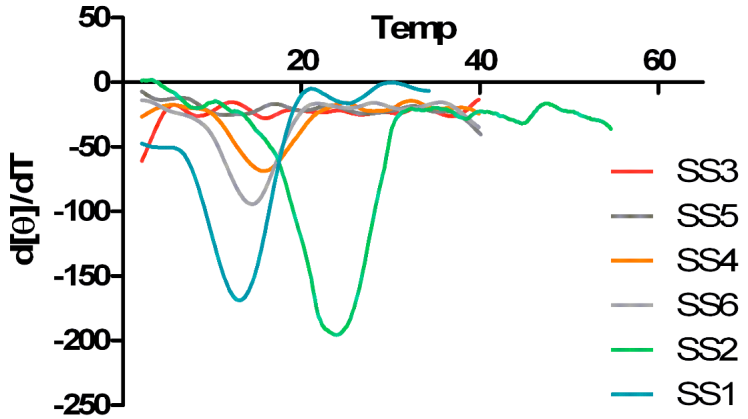


Figure S10 First derivative of melt transition curves of self-assemblies derived from **SS1**– **SS6**. The peptides **SS3** and **SS5** appeared not to form triple helical assemblies at temperatures above 2 °C.

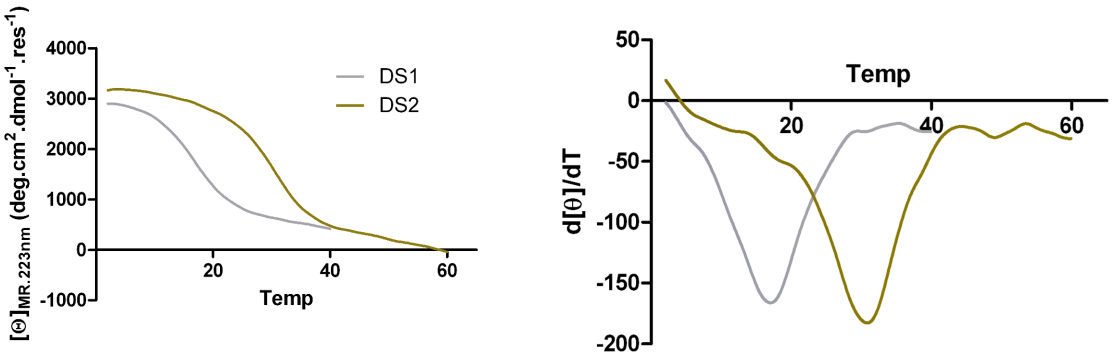


Figure S11. Molar residual ellipticity (223 nm) melt transition and first derivative of melt transition curves of "dual-stranded" peptides **DS1** and **DS2**. These peptides apparently have the capacity to form triple helices; **DS1** and **DS2** gave T_M = 17 and 30 °C, respectively.

Azide: Alkyne (eq.)	$CuSO_4 \cdot 5H_2O$/ NaAsc /TBTA	Time	Solvent	Conversion (%)[a]
1:1	1:5 ± TBTA	24–72 h	H_2O/ CH_3CN (1:1)	<1
1:1	1:5 ± TBTA	24–72 h	H_2O/ DMF (1:1)	<1
1:1	1:5 ± TBTA	24–72 h	H_2O/ CH_2Cl_2 (1:1)	<1
1:1	1:5 ± TBTA	24–72 h	H_2O/ *t*BuOH (1:1)	<1
1:1	1:5 ± TBTA	90 min	H_2O/DMF (1:1) (microwave 25 W)	~ 2
1:1	5:25	24 h	H_2O/CH_3CN (1:1)	<1
1:1	5:25	90 min	H_2O/DMF (1:1)	~ 50
1:1	5:25	5 min	5% DMF in H_2O	>90
1:2	5:25	2 min	5% DMF in H_2O	>95

Table S1 A selection of investigated CuAAC reactions.
[a] conversion was measured by disappearance of the azido-bearing peptide, determined by RP-HPLC.

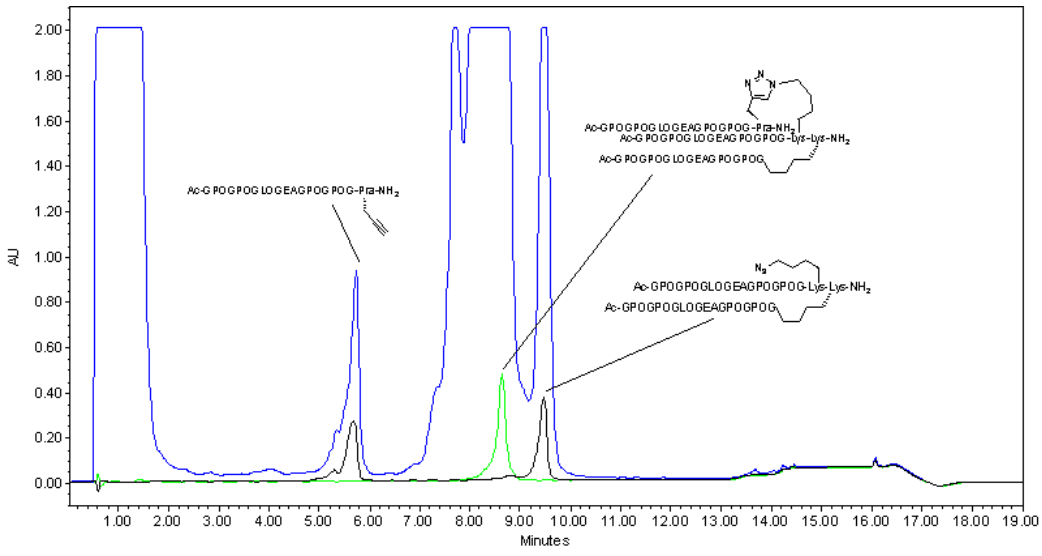


Figure S12a RP-HPLC analysis (system 1a) of **CP1** synthesis. **Black**: the peptide precursors; **Blue**: after 30–60 s of addition of all reagents; **Green**: RP-HPLC analysis (system 1a) of purified **CP1**.

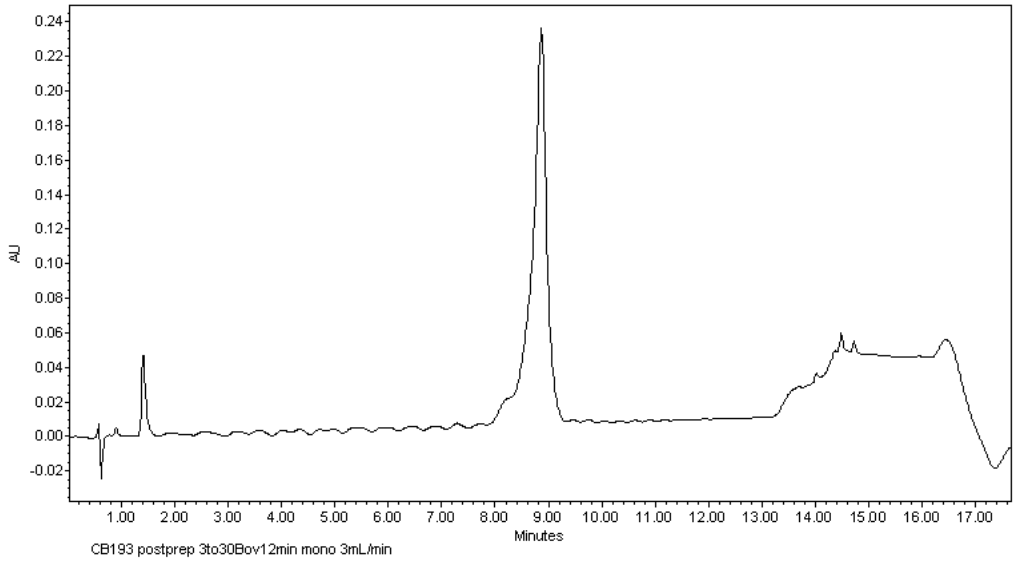


Figure S12b RP-HPLC analysis (system 1a) of purified **CP1**.

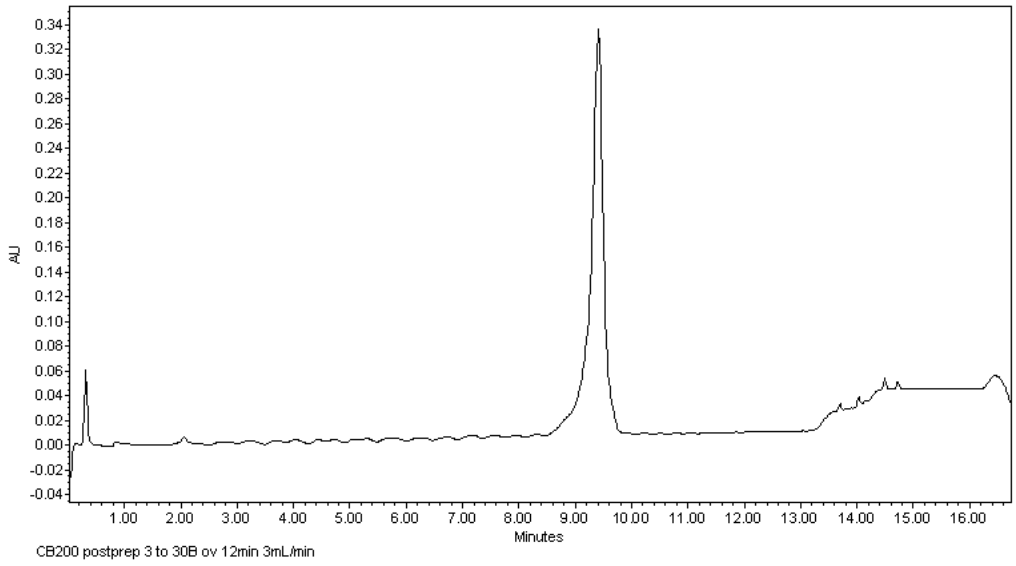


Figure S13 RP-HPLC analysis (system 1a) of purified **CP2**

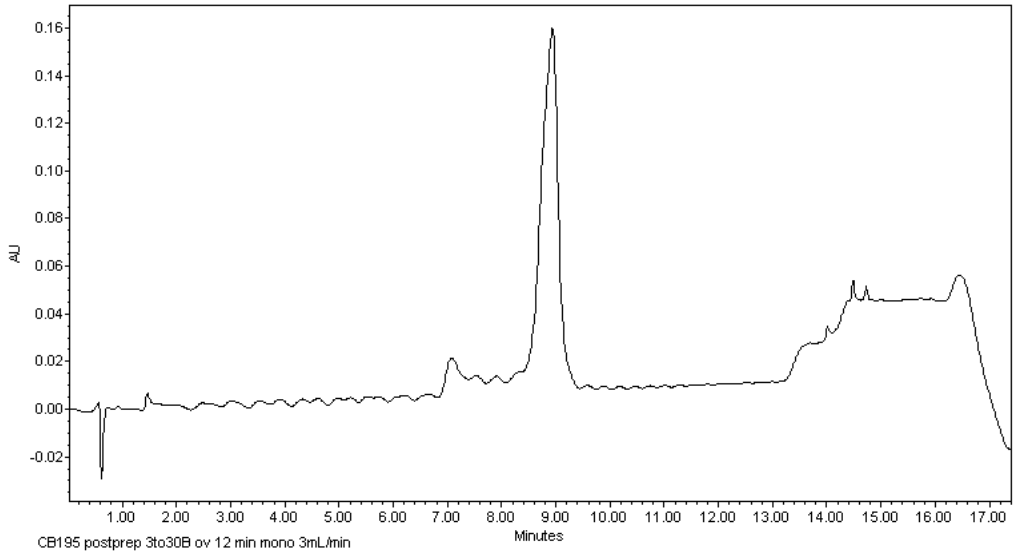


Figure S14 RP-HPLC analysis (system 1a) of purified **CP3**

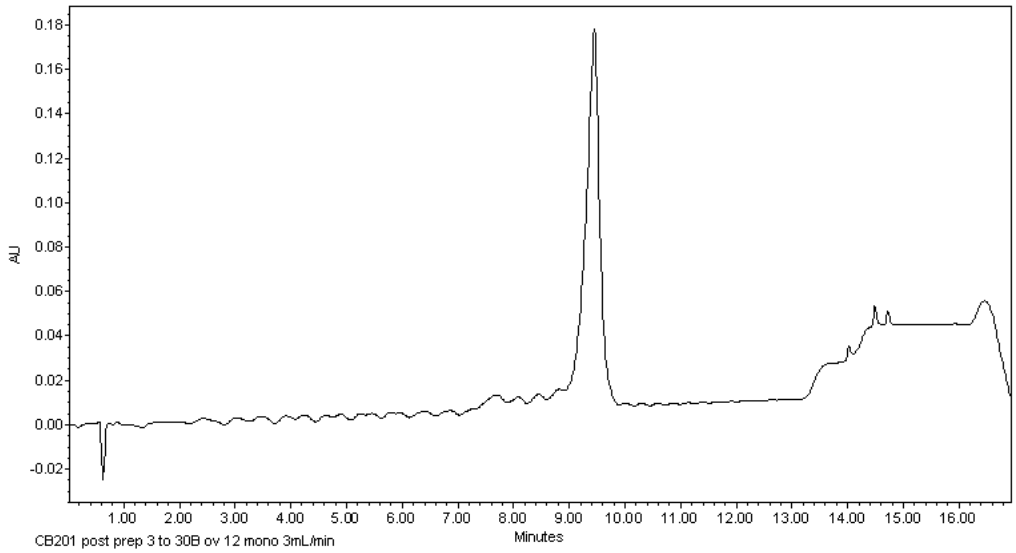


Figure S15 RP-HPLC analysis (system 1a) of purified **CP4**

X-ray crystal data for SS1

The structure was deposited in the Protein Data Bank under PDB ID 3P46.

DATA COLLECTION		
Spacegroup	C2	
CELL		
A, B, C (Å)	84.02, 17.29, 26.05	
A, β, γ (°)	90.00, 91.35, 90.00	
Resolution (Å)	22.4-1.70 (1.76-1.7)	
Rmerge	0.052	(0.251)
I/σI	14.1	(3.5)
Completeness (%)	98.0	(83.0)
Redundancy	3.45	(3.02)
REFINEMENT		
Resolution	22.4-1.7	
No. Of Reflections	4110	
Rwork/Rfree	20.0/24.4	
Atom count/ B factor		
Peptide	350/19.0	
Solvent	80/30.5	
R.M.S. Deviations		
Bond Lengths (Å)	0.023	
Bond Angles (°)	2.57	

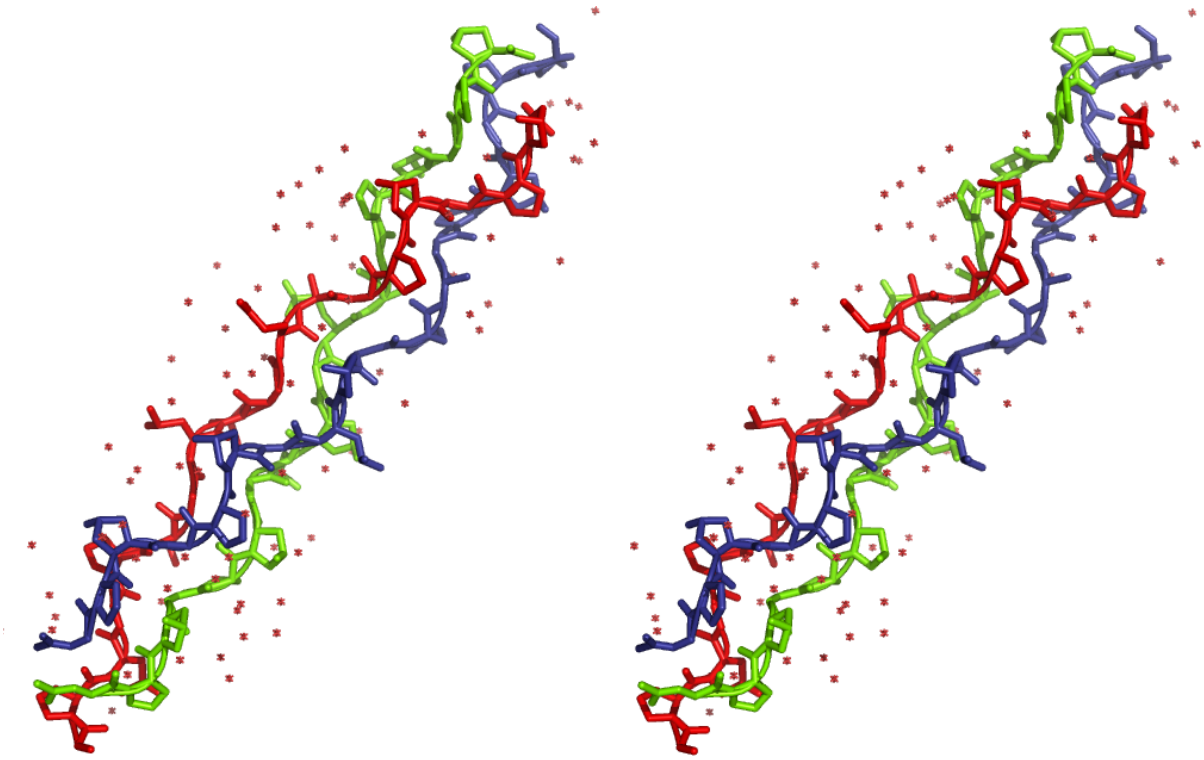


Figure S16 Stereoview of the structure of the self-assembled triple helix derived from **SS1**, presented using PyMOL™.