Self-assembly of diphenylalanine with preclick components as capping groups

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METHODS

Peptide synthesis and characterization

Amino acids were supplied by PolyPeptide. $N$-[3-(dimethylamino)-propyl]-$N'$-ethylcarbodiimide hydrochloride was a product from Bachem and all other reagents for peptide synthesis were purchased from Sigma-Aldrich. Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were registered on a Thermo Nicolet Avatar 360 FTIR spectrophotometer; $\nu_{\text{max}}$ is given for the main absorption bands. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AV-400 or ARX-300 instrument at room temperature unless otherwise indicated, using the residual solvent signal as the internal standard, chemical shifts ($\delta$) are expressed in ppm and coupling constants ($J$) in Hertz. Optical rotations were measured on a JASCO P-1020 polarimeter. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer.

General procedure for peptide-bond formation: To a solution of the appropriately $N^\alpha$-protected $\alpha$-amino acid (4.00 mmol) in dichloromethane (DCM), 1-hydroxybenzotriazole (4.40 mmol) was added, and the solution was cooled to 0 °C in an ice bath. $N$-[3-(dimethylamino)-propyl]-$N'$-ethylcarbodiimide hydrochloride (4.40 mmol) was added, followed by the solution of the amino component (4.40 mmol) in DCM, obtained after acidolytic removal of the protecting group and $N$-methylmorpholine (4.40 mmol) or $N$-ethyldiisopropylamine (4.40 mmol). The reaction mixture was stirred 1 h at 0 °C, then at room temperature for 24 h, by keeping the pH (moistened pH paper) to 8. The reaction mixture was repeatedly washed with 5% KHSO$_4$, 5% NaHCO$_3$ and water. The organic phase was dried over MgSO$_4$ and evaporated to dryness. The peptide product was purified by flash chromatography.
Characterization of the final peptides (Poc-FF-N$_3$, N$_3$-FF-OPrp; pictures of the NMR and IR spectra are shown in Figures S8-S13) and all intermediates is as follows:

**Poc-FF-N$_3$.** White solid, mp 125°C. [$\alpha$$_D$$^20$] = −16.3 (c = 0.50, MeOH). IR (KBr) ν 3318, 3281, 2109, 1735, 1700, 1656 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 400 MHz) δ 2.50 (t, 1H, J = 2.4 Hz), 2.99–3.16 (m, 4H), 3.34–3.50 (m, 2H), 4.15–4.31 (m, 2H), 4.41 (dd, 1H, J = 14.0 Hz, J = 6.8 Hz), 4.66 (d, 2H, J = 2.3 Hz), 4.82 (dd, 1H, J = 13.4 Hz, J = 6.4 Hz), 5.36 (d, 1H, J = 7.1 Hz), 6.24 (d, 1H, J = 6.4 Hz), 6.99–7.06 (m, 2H), 7.17–7.35 (m, 8H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ 37.94, 38.29, 49.59, 53.05, 53.49, 56.14, 75.10, 77.95, 127.28, 127.39, 128.80, 128.89, 129.30, 129.49, 135.45, 136.17, 155.10, 170.30, 170.70. HRMS (ESI) $^{[M+Na]}$+: calcd. 486.1768, found 486.1765.

**N$_3$-FF-OPrp.** Oil which solidifies on standing, mp 86°C. [$\alpha$$_D$$^20$] = −20.1 (c = 0.45, DMSO). IR (KBr) ν 3302, 2115, 1746, 1687 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 300 MHz) δ 2.45 (t, 1H, J = 2.5 Hz), 2.82–3.06 (m, 3H), 3.20 (dd, 1H, J = 14.1 Hz, J = 4.1 Hz), 4.13 (dd, 1H, J = 8.0 Hz, J = 4.1 Hz), 4.64 (qd, 2H, J = 15.5 Hz, J = 2.5 Hz), 4.84 (dt, 1H, J = 8.3 Hz, J = 5.8 Hz), 6.60 (d, 1H, J = 8.1 Hz), 6.81–6.88 (m, 2H), 7.13–7.30 (m, 8H). $^{13}$C NMR (CDCl$_3$, 75 MHz) δ 37.86, 38.46, 52.83, 52.97, 65.35, 75.78, 76.93, 127.41, 127.46, 128.73, 128.79, 129.44, 129.73, 135.14, 136.04, 168.15, 170.28. HRMS (ESI) $^{[M+Na]}$+: calcd. 399.1428, found 399.1438.

**Boc-L-Phe-OCH$_2$C≡CH.** Oil. [$\alpha$$_D$$^20$] = −14.3 (c = 0.50, MeOH). IR (neat) ν 3363, 2099, 1743, 1715 cm$^{-1}$. $^1$H NMR (CDCl$_3$, 400 MHz) δ 1.43 (s, 9H), 2.53 (t, 1H, J = 2.5 Hz), 3.13 (qd, 2H, J = 13.9 Hz, J = 5.9 Hz), 4.60–4.83 (m, 3H), 4.97 (d, 1H, J = 7.5 Hz), 7.14–7.35 (m, 5H). $^{13}$C NMR (100 MHz, CDCl$_3$) δ 28.40, 38.21, 52.76, 54.42,
75.57, 80.17, 127.24, 128.72, 129.53, 135.81, 155.18, 171.27. HRMS (ESI) 
C_{17}H_{21}NO_4Na [M+Na]^+: calcd. 326.1363, found 326.1352.

**Boc-L-Phe-OCH<sub>2</sub>CH<sub>2</sub>Br**. White solid, mp 63°C. [α]<sub>D</sub><sup>20</sup> = –8.2 (c = 0.50, MeOH). IR (KBr) ν 3366, 1745, 1691 cm<sup>–1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 1.44 (s, 9H), 3.13 (dd, 2H, J = 11.2 Hz, J = 6.2 Hz), 3.46 (t, 2H, J = 6.2 Hz), 4.42 (td, 2H, J = 6.2 Hz, J = 4.2 Hz), 4.63 (q, 1H, J = 6.3 Hz), 4.99 (d, 1H, J = 7.6 Hz), 7.14–7.36 (m, 5H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 28.19, 28.39, 38.36, 54.50, 64.57, 80.15, 127.23, 128.72, 129.42, 135.92, 155.17, 171.63. HRMS (ESI) C_{16}H_{22}BrNO<sub>4</sub>Na [M+Na]^+: calcd. 394.0624, found 394.0627.

**Boc-L-Phe-OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>**. Oil. [α]<sub>D</sub><sup>20</sup> = –4.3 (c = 0.50, MeOH). IR (neat) ν 3365, 2106, 1748, 1716 cm<sup>–1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 1.43 (s, 9H), 3.02–3.19 (m, 2H), 3.33–3.51 (m, 2H), 4.25 (t, 2H, J = 5.2 Hz), 4.62 (q, 1H, J = 6.3 Hz), 5.02 (d, 1H, J = 7.5 Hz), 7.12–7.37 (m, 5H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 28.31, 38.27, 49.57, 54.53, 63.74, 80.05, 127.15, 128.66, 129.32, 135.90, 155.11, 171.72. HRMS (ESI) C_{16}H_{22}N<sub>4</sub>O<sub>4</sub>Na [M+Na]^+: calcd. 357.1533, found 357.1540.

**Preparation of peptide solutions.** Organic solvents were purchased from Sigma-Aldrich, Fisher Scientific and Scharlab. The peptide concentration in the prepared solutions ranged from 0.05 to 5 mg/mL. Solutions or dispersions (25 or 100 μL) of the peptides were prepared from 5 mg/mL stocks. The solvents used to dissolve the synthesized peptides, Poc-FF-N<sub>3</sub> and N<sub>3</sub>-FF-OPrp were hexafluoropropanol (HFIP), dichloromethane (DCM) and tetrahydrofuran (THF). Milli-Q water, acetonitrile (ACN), methanol (MeOH) and isopropanol (iPrOH) was added as co-solvents to reduce the peptide concentration and alters the polarity of the environment. The solvent:co-solvent ratio was systematically varied from 4:1 to 1:99 (i.e. 4:1, 2:3, 1:4, 1:9, 1:19;
1:24, 1:49 and 1:99). Finally, 10 or 20 μL aliquots were placed on microscope coverslips or glass slides (glass sample holders) and kept at room temperature (25 °C). The humidity was kept constant at 50%.

**Surface-mediated assemblies.** Peptide solutions using HFIP and MeOH as solvent and co-solvent, respectively, were prepared considering 1:0 to 1:99 ratios (*i.e.* the peptide concentrations ranged from 5.0 to 0.05 mg/mL). 20 μL aliquots of HFIP:MeOH peptide solution were deposited onto glass coverslips, silanized glass coverslips, plasma-functionalized polystyrene pieces or stainless steel AISI 316 sheets.

Glass coverslips were purchased from Agar Scientific. Silanized coverslips were produced by shaking 30 glass coverslips in 4 mL of 5% dimethyldichlorosilane in heptane for 2 hours, repeatedly washed in MeOH and allowed to cure overnight at 100 °C. Steel AISI 316L sheets were acquired in commercial sources. Polystyrene pieces were cut from Nunc™ 24-well cell-culture treated multidishes that received a Nunclon™ Delta surface treatment.

**Optical microscopy.** Morphological observations were performed using a Zeiss Axioskop 40 microscope. Micrographs were taken with a Zeiss AxiosCam MRC5 digital camera.

**Scanning electron microscopy (SEM).** SEM studies were performed in a Focussed Ion Beam Zeiss Neon 40 scanning electron microscope operating at 5 kV and equipped with an EDX spectroscopy system. Samples were mounted on a double-side adhesive carbon disc and sputter-coated with a thin layer of carbon to prevent sample charging problems.
**Fractal characterization of dendritic microstructures.** The dendritic assembly behavior of Poc-FF-N3 was observed by optical microscopy. The fractal dimension of the dendritic morphology was determined by the fractal box-counting method\(^1\) using ImageJ software version 1.50e. (version: 2.0.0-rc-43/1.50e, Fiji package).\(^2\) For this analysis, images of dendritic microstructures were converted into 8-bit binary format images, which were covered by square box arrays. The number of boxes occupied by the underlying dendritic morphology \((N)\) and the side length of boxes \((L)\) were plotted in logarithmic scale to determine the fractal dimension \((FD)\) of the dendrites.

**Circular Dichroism (CD).** CD spectra at the far-UV region were recorded with a Chirascan-plus qCD spectrometer (Applied Photophysics, APL; UK) equipped with a temperature-controlled cell, using a call path length 10 mm. Dilute peptides solutions (0.01 mg/mL) were studied at temperatures comprised between -50 °C and 60 °C using different solvent:co-solvent mixtures.

**Theoretical calculations.** All Density Functional Theory (DFT) calculations were carried out using the Gaussian 09 computer program.\(^3\) The geometries of the different systems investigated were fully optimized by using the M06L, M06L-D3 and BLYP-D3 functionals, which were combined with the 6-31G(d) basis set. The former was developed by Zhao and Truhlar\(^4\) whilst the latter is the combination of the Becke exchange functional and the LYP correlation functional.\(^5\)\(^6\) Dispersion was considered by incorporating the original D3 damping function of Grimme in both functionals.\(^7\)

Binding energies (BEs) were corrected with the basis set superposition error (BSSE) by means of the standard counterpoise.
REFERENCES


**Figure S1.** Representative Poc-FF-N$_3$ assemblies observed onto silanized glass. Optical micrographs of the supramolecular doughnut-like structures derived from (a) 4 (4:1), (b) 0.5 (1:9) and (c) 0.1 mg/mL (1:49) solutions HFIP:MeOH (solvent:co-solvent ratio indicated in parenthesis).
Figure S2. Representative Poc-FF-N$_3$ assemblies observed onto plasma-treated polystyrene. Optical micrographs of (a) disordered agglomerate of fibres derived from 4 mg/mL peptide solution in HFIP:MeOH; and (b) dendritic-like microstructures derived from 0.5 (left) and 0.1 mg/mL (right) peptide solutions in HFIP:MeOH.
**Figure S3.** CD spectra of Poc-FF-N$_3$ recorded at different temperatures for 0.01 mg/mL peptide solutions in (a) 1:9 DCM:MeOH; (b) 1:9 HFIP:water; (c) HFIP:water; and (d) 1:9 HFIP:PrOH.
Figure S4. Lateral and top views of the (a,c) antiparallel and the (b,d) parallel β-sheet assemblies obtained for a complex with three Poc-FF-N$_3$ strands. Geometries were optimized at the (a,b) M06L-D3/6-31G(d) and (c,d) M06L/6-31G(d) levels. Intermolecular hydrogen bonds are represented by black dashed lines, the (N–)H···O distances (in Å) being displayed. Intermolecular azide···alkyne, azide···azide, and alkyne···alkyne interactions are represented by pink dashed lines. These three π-stacking interactions have been considered to occur when the distance between the two motifs is lower than 4.5 Å (values are provided in the graphic). Azide···alkyne distances in the antiparallel disposition, have been determined considering the central nitrogen atom of the azide group and each of the two carbons of the alkyne group. Azide···azide and alkyne···alkyne distances in the parallel disposition have been determined considering the central nitrogen atom of each azide group and the geometric center of each C≡C bond, respectively.
Figure S5. Representative optical micrographs of the irregular assemblies formed by N$_3$-FF-OPrp in: (a) glass using a peptide concentration of 2 mg/mL in 2:3 HFIP:water; (b) glass using a peptide concentration of 1 mg/mL in 1:4 HFIP:MeOH; (c) silanized glass using a peptide concentration of 4 mg/mL in 4:1 HFIP:MeOH; (d) plasma-treated polystyrene a using peptide concentration of 0.5 mg/mL in 1:9 HFIP:MeOH.
Figure S6. CD spectra of N$_3$-FF-OPrp recorded at different temperatures for 0.01 mg/mL peptide solutions in (a) 1:9 DCM:MeOH; (b) 1:9 HFIP:water; (c) HFIP:water; and (d) 1:9 HFIP:iPrOH.
Figure S7. Lateral and top views of the geometries obtained after optimization of the (a) antiparallel and the (b) parallel β-sheet assemblies constructed using three N$_3$-FF-OPrp strands. Geometries were optimized at the B3LYP-D3/6-31G(d) level. Intermolecular hydrogen bonds are represented by black dashed lines, the (N–)H⋯O distances (in Å) being displayed. Intermolecular alkyne⋯alkyne interactions are represented by pink dashed lines. These interactions have been considered to occur when the distance between the geometric centre of two adjacent alkyne motifs is lower than 4.5 Å (values are provided in the graphic). Azide⋯azide distances were larger than 5 Å.
Figure S8. $^1$H NMR (CDCl$_3$, 400 MHz) of dipeptide Poc-FF-N$_3$.

Figure S9. $^{13}$C NMR (CDCl$_3$, 100 MHz) of dipeptide Poc-FF-N$_3$. 
Figure S10. $^1$H NMR (CDCl$_3$, 300 MHz) of dipeptide N$_3$-FF-OPrp.

Figure S11. $^{13}$C NMR (CDCl$_3$, 75 MHz) of dipeptide N$_3$-FF-OPrp.
Figure S12. IR spectrum (KBr) of dipeptide Poc-FF-N$_3$.

Figure S13. IR spectrum (KBr) of dipeptide N$_3$-FF-OPrp.