Tuneable Fmoc-Phe(4-X)-Phe-NH₂ nanostructures by variable electronic substitution

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Materials and Methods

All Fmoc-para-substituted derivatives were purchased from Novabiochem, UK except Fmoc-Phenylalanine which was purchased from Sigma Aldrich. Phenylalanine amide was purchased from Bachem and thermolysin form Sigma Aldrich. Purity of all amino acid derivatives was >97%. All reagents were used without further purification.

Samples Preparation:

For the amide condensation we used 20 mM of the electrophile and 80 mM of the nucleophile in the presence of 1 mg of thermolysin in 1 ml of pH 8 (100 mM) sodium phosphate buffer. All samples were vortexed and sonicated in order to obtain a homogenous mixture.

Transition electron microscopy (TEM): Carbon-coated copper grids (200 mesh) were glow discharged in air for 30 s. The support film was touched onto the gel surface for 3 s and blotted down using filter paper. Negative stain (20 ml, 1% aqueous methylamine vanadate obtained from Nanovan; Nanoprobes) was applied and the mixture blotted again using filter paper to remove excess. The dried specimens were then imaged using a LEO 912 energy filtering transmission electron microscope operating at 120kV fitted with 14 bit/2 K Prosan CCD camera.

Fluorescence spectroscopy: 1 ml samples were prepared in a 1 cm$^2$ quartz cuvette. Fluorescence emission spectra were measured on a Jasco FP-6500 spectrofluorometer at a scanning speed of 200 nm.min$^{-1}$. The emission spectra were recorded between 300 and 600 nm resulting from excitation at 280 nm, using a bandwidth of 3 nm with a medium response and a 1 nm data pitch.

FTIR spectroscopy: Spectra were acquired using a Bruker Vertex 70 spectrometer with a spectral resolution of 1 cm$^{-1}$. The spectra were obtained by averaging 25 scans per sample. Measurements were performed in a standard IR cuvette (Harrick Scientific), in which the sample was contained between two CaF$_2$ windows (thickness, 2 mm) separated by a 25 µm PTFE spacer. All sample manipulations were performed in a glove box to minimize interference from atmospheric water vapour. D$_2$O (Sigma-Aldrich) was used as solvent for all the infrared spectral measurements.

High-performance liquid chromatography (HPLC): A Dionex P680 high-performance liquid chromatography pump was used to quantify conversions of the enzymatic reaction. A 20 µl sample was injected onto a Macherey-Nagel C18 column with a length of 250 mm and an internal diameter of 4.6 mm and 5-mm fused silica particles at a flow rate of 1 ml.min$^{-1}$. The eluting solvent system had a linear gradient of 20% (v/v) acetonitrile in water for 4 min, gradually rising to 80% (v/v) acetonitrile in water at 35 min. This concentration was kept constant until 40 min when the gradient was decreased to 20% (v/v) acetonitrile in water at 42 min. Sample preparation involved mixing 50 ml of gel with acetonitrile–water (1000 ml, 70:30
mixture) containing 0.1% trifluoroacetic acid. The purity of each identified peak was determined by UV detection at 280 nm.

**Circular Dichroism (CD):** Spectra were measured on a JascoJ600 spectropolarimeter with 1 s integrations with a step size of 1 nm and a single acquisition with a slit width of 1 nm. A circular CD cell(Hellma) was used with a path length of 0.1 mm. All the measured CD spectra had values of HT lower than saturation at all wavelengths in all the samples.
Supporting Data

Table S1. Chosen substituents and their corresponding Hammet-σp values.1

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substitution (X)</th>
<th>Hammett-σp value</th>
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<tbody>
<tr>
<td>1</td>
<td>OH</td>
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<tr>
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<td>6</td>
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*Bulky group with stereoelectronic properties.

Figure S1. Fluorescence spectra of Fmoc-diphenylalanine derivatives before and after thermolysin addition. Normalised spectra before (a), after (b) non-normalised spectra before (c) and after (d).
Figure S2. High Tension (HT) voltage.

Figure S3. CAC values determination by monitoring fluorescence intensity with different concentrations.

References