

Supporting Information

Crystallization and Lamellar Nanosheet Formation of an Aromatic Dipeptoid

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Materials and Methods

Peptoid Synthesis and purification. Peptoid design was synthesized manually on rink amide MBHA resin using well established submonomer peptoid synthesis procedure reported previously.¹⁻³ Briefly, resin (Cat no. 855003, Merck, UK) was treated with de-protecting reagent (20% piperidine in NMP) for 20 min under shaking and repeated twice. Further, bromoacetylated intermediate was formed on terminal secondary amine groups present on deprotected resin by activating BAA (20x excess of resin) with DIC (18.5x excess) for 15 min. Later, bromine was substituted by Benzylamine (F) (20x excess, 2M) through S_N2 displacement and this substitution reaction was carried out for 30 min under continuous shaking. Bromoacetylation and S_N2 displacement steps were repeated one more time to the growing peptoid chain from C to N terminal direction to make a dipeptoid (i.e. N(FF)). Briefly, synthesized N(FF) on resin was suspended in a reaction mixture of 20x excess of acetic anhydride (ca. 2M) prepared in DCM. The reaction mixture was initially stirred for 2 h at 37°C and later recoupled for 4 h to ensure complete coupling/capping. Post synthesis, the peptoid chains were cleaved by treating resin with a cleavage recipe (TFA:TIPS:H₂O=95:2.5:2.5 v/v/v) for 10 min. Cleaved peptoid product was purified using preparative gradient RP-HPLC (Dionex Ultimate 3000) using a 250x10 mm Phenomenex Jupiter C18 column. LC-MS data are shown in SI Fig.1.

ESI-MS and HPLC. HPLC fractions containing the pure product were identified by ESI-LC-MS analysis (Agilent) and the ACN/water solutions were lyophilized. A 5-95% ACN gradient over 6 min with 0.1% formic acid mobile phase was used. A 100x4.6 mm “Porosil” C18 column was used. The purity of each final product was confirmed by analytical RP-HPLC using a 250x4.6 mm

diameter “Nucleosil” C18 column (Macherey-Nagel) and a gradient of 5-95% ACN run over 30 min with 0.1% TFA (SI Fig.2). The mass was determined by ESI-MS (SI Fig.3) to be 353.7 [M+H]⁺ (exact mass is 353.17 g mol⁻¹).

Polarized Optical Microscopy. Crystals or spherulites grown on a microscope coverslip were observed using an Olympus BX41 polarized microscope in a crossed polarizer configuration. Images were captured with a Canon G2 digital camera.

Crystal Preparation. An aliquot of 20 μl of CapNFF dissolved in 40 μl of a mixture 70 wt% acetonitrile and 30 wt% water was evaporated and subsequently resuspended in 20 μl of DMSO. The DMSO solution of peptoid was then injected into 15 ml of water and left to evaporate for 2 weeks at room temperature inside a closed plastic box.

X-ray Diffraction. X-ray diffraction measurements were performed in Experiments Hutch 1 (EH1) of Beamline I19, at Diamond Light Source.⁴ The data were collected at a wavelength of 0.6889 Å on a Fluid Film Devices 3-circle fixed-chi diffractometer using a Dectris Pilatus 2M detector. The crystal was mounted on a MiTeGen micromount using a perfluoropolyether oil, and cooled for data collection by a Cryostream nitrogen-gas stream.⁵ The collected frames were integrated using XIA2⁶⁻⁹ software and the data were corrected for absorption effects using AIMLESS,¹⁰ an empirical method. The structure was solved by dual-space methods¹¹ and refined by least-squares refinement on all unique measured F₂ values.¹¹

Transmission Electron Microscopy (TEM). TEM imaging was performed using a JEOL 2100Plus TEM microscope operated at 200 kV. Droplets of 2 wt% capFNN dissolved in 70%Acetonitrile:30% water were placed on Cu grids coated with a carbon film (Agar Scientific, UK), stained with uranyl acetate acid (1 wt %) (Sigma-Aldrich, UK), washed with water and dried.

Small-Angle X-ray Scattering (SAXS). SAXS experiments were performed on the bioSAXS beamline B21 at Diamond light source, U.K. A solution containing 2 wt% peptide in 70% acetonitrile: 30 % water were loaded in a PCR tube in an automated sample changer. Data was collected using a Dectris PILATUS 2 M detector at a fixed camera length of 3.9 m with a wavelength $\lambda = 1 \text{ \AA}$. Data are presented as a function of $q = 4\pi \sin \theta / \lambda$, where 2θ is the scattering angle.

Fluorescence Spectroscopy. Fluorescence spectra were recorded with a Varian Cary Eclipse fluorescence spectrometer with samples in 4 mm inner width quartz cuvettes. The excitation wavelength was $\lambda_{\text{ex}} = 265 \text{ nm}$.

Table S1. Crystallization experiments

Sample	Step 1	Step 2	Step 3
A	S₂ : Leave S₁ to evaporate and re-suspend in 20 μ l of DMSO	Inject 10 μ l of S₂ into 15 μ l of water placed on a microscope coverslip	Seal preparation in step 2 in a flask and leave to evaporate for 14 days at RT. Complete evaporation by opening the flask inside a fume cupboard, for a further 2 weeks, at RT
B	---	Place 15 μ l of S₁ on a microscope coverslip	As above
C	S₄ : Leave S₃ to evaporate and re-suspend in 50 μ l of DMSO	Inject 10 μ l of S₄ into 30 μ l of water placed on a microscope coverslip	As above
D	As above	Place 15 μ l of S₄ on a microscope coverslip	As above
E	---	Place 15 μ l of S₅ on a microscope coverslip	As above

Key: **S₁** and **S₃** are 20 and 40 μ l respectively of 10 wt% capFNN dissolved in 30%:70% water: acetonitrile. **S₅** is 2 wt% capFNN dissolved in 30%:70% water: acetonitrile.

Table S2. SAXS fit parameters for the data in Fig.2f. Obtained using the Bilayer Gauss model in SASfit.¹²

Parameter	Value
Scale factor	0.416
Bilayer thickness, t (nm)	6.95
Gaussian dispersity in t (nm)	1.45
Sigma(outer) (nm)	0.68
Relative contrast inner (a.u.)	4.09×10^{-7}
Sigma(inner) (nm)	0.58
Relative contrast outer (a.u.)	-1.11×10^{-6}
Diameter (nm) (fixed)	50
Background ^a c_0	0.021
Background c_1	2.4×10^{-9}
Background α	3.77

^a Background represented as $I_{\text{back}}(q) = c_0 + c_1 q^{-\alpha}$

Table S3. Hydrogen Bond geometry (Å, °).

D – H...A	D – H	H...A	D...A	D – H...A
N(22) – H(22A)...O(21) ⁱ	0.89(2)	1.92 (2)	2.8040(14)	169.3(18)
N(22) – H(22B)...O(25) ⁱⁱ	0.92(2)	1.99(2)	2.8743(13)	161.5(17)

Symmetry Codes: (i) $x, y, z - 1$; (ii) $\frac{1}{2} - x, \frac{1}{2} + y, -\frac{1}{2} + z$

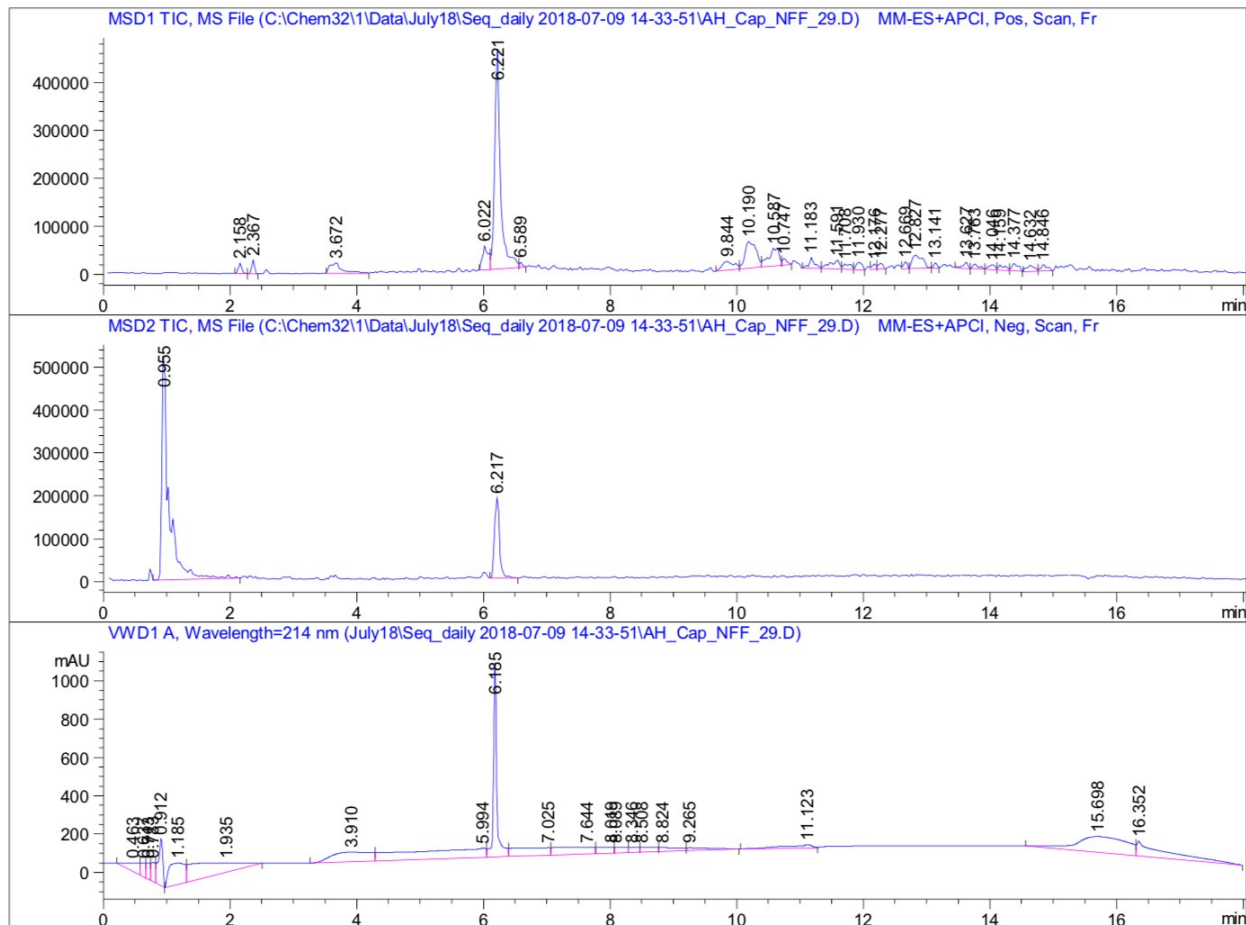


Figure S1. LC-MS (ESI) spectra for purified CapN(FF). The top, middle and bottom traces show the positive ion, negative ion, and 214 nm LC chromatograms, respectively. CapN(FF) is seen to elute at 6.2 min. No other peptoid peaks are observed in the LC trace at 214 nm, corresponding to the amide bonds of along the peptoid backbone, indicating purity.

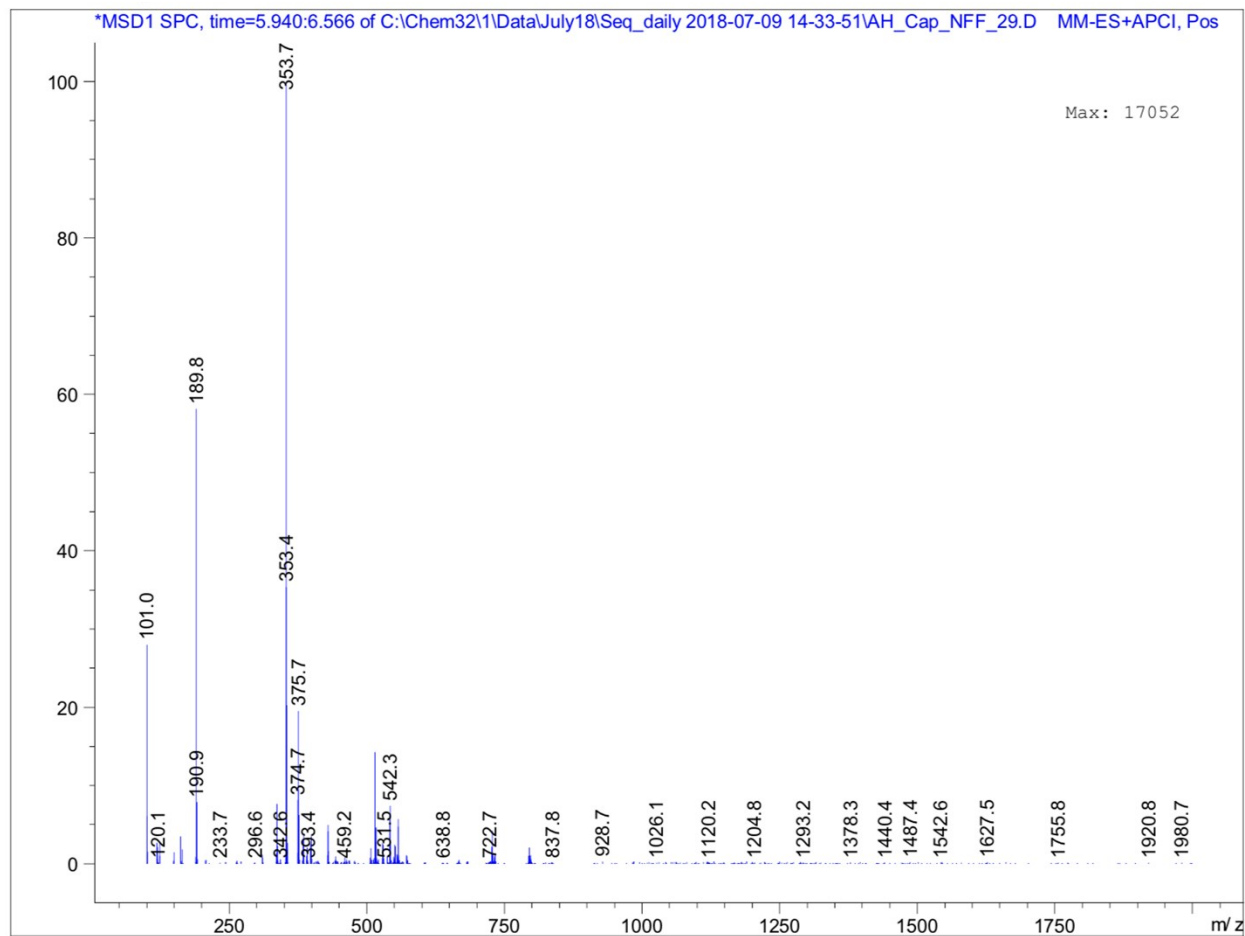


Figure S2. The mass spectrum of positive ions identified at the 6.2 min peak of LC-MS. The peak at 353.7 m/z is assigned to the target $[M+H]^+$. The 0.5 m/z discrepancy is assigned to a calibration issue. Other peaks are assigned to fragments belonging to the solvent and impurities in the column.

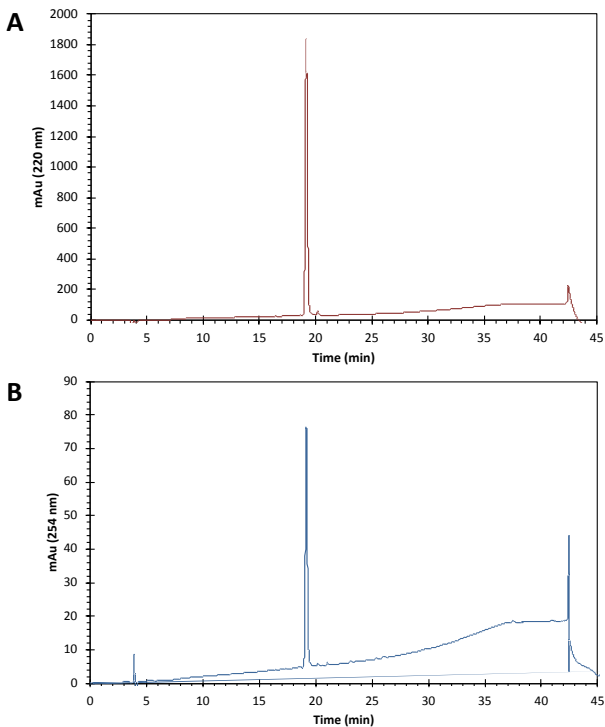


Figure S3. HPLC chromatograms of purified CapN(FF). A 5-95% ACN gradient elution over 30 min was used. A) the absorbance signal at 220 nm. B) the absorbance signal at 254 nm. The single peak appearing at ca. 19 min at both 220 nm (side of amide peak) and 254 nm (benzyl sidechain of Nphe) shows the purity of the peptoid (98.2% according to the 220 nm trace). The small peak at ca. 4 min is the injection peak, followed by a short isocratic segment until the start of the gradient at ca. 7 min. The disturbance at ca. 42 min corresponds to the end of a 5 min rinse at 95% ACN and the return of the solvent to 5% ACN equilibration. A C18 column was used (see Experimental).

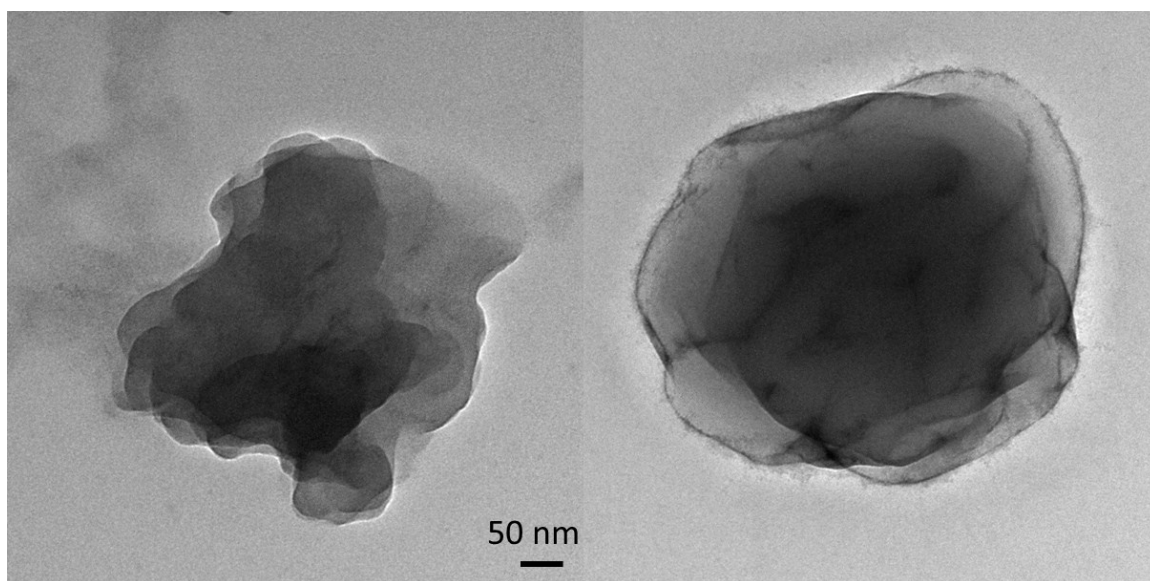


Figure S4. Additional TEM images of nanosheets.

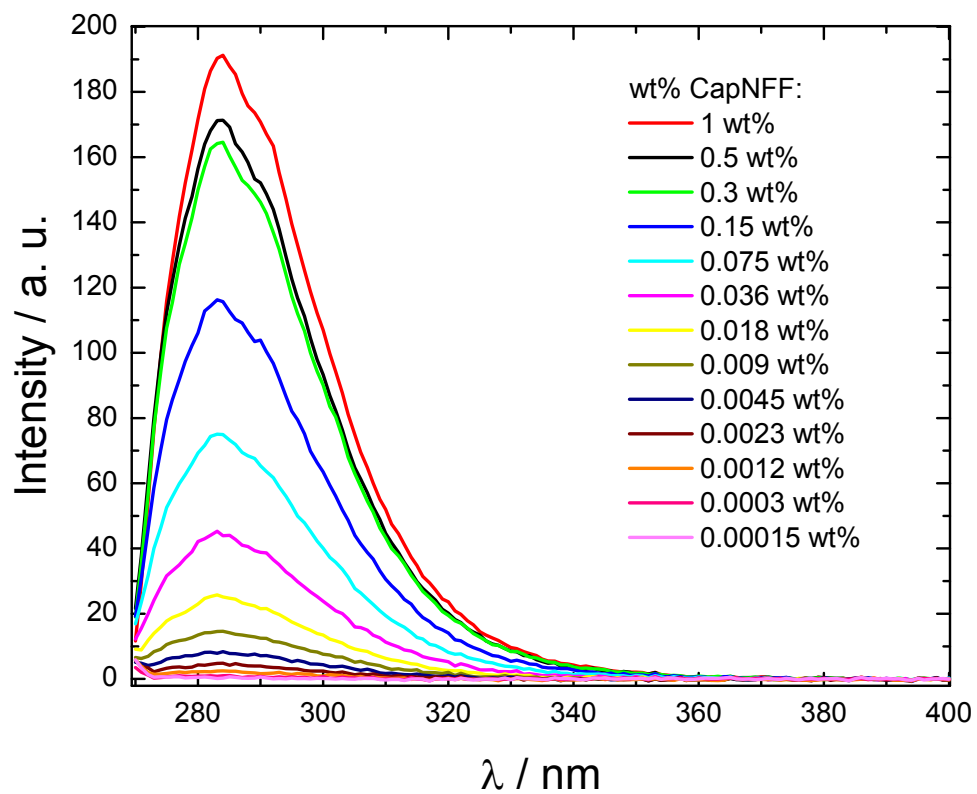


Figure S5. Fluorescence spectra of CapNFF in 70% acetonitrile: 30% water ($\lambda_{\text{ex}} = 265$ nm).

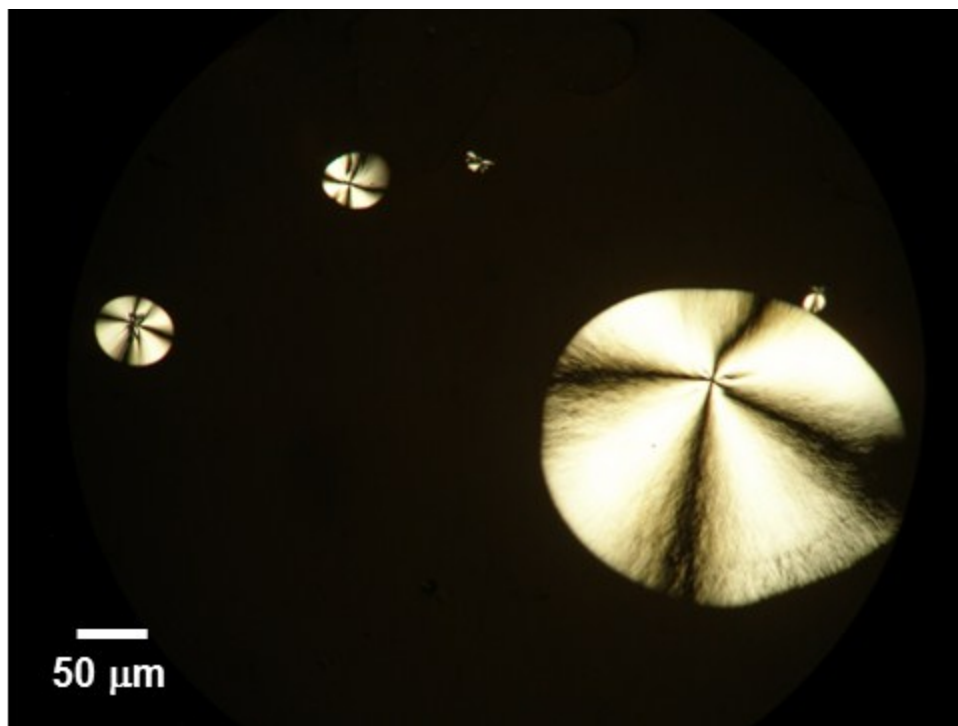


Figure S6. POM images from sample B, showing Maltese cross patterns from spherulites.

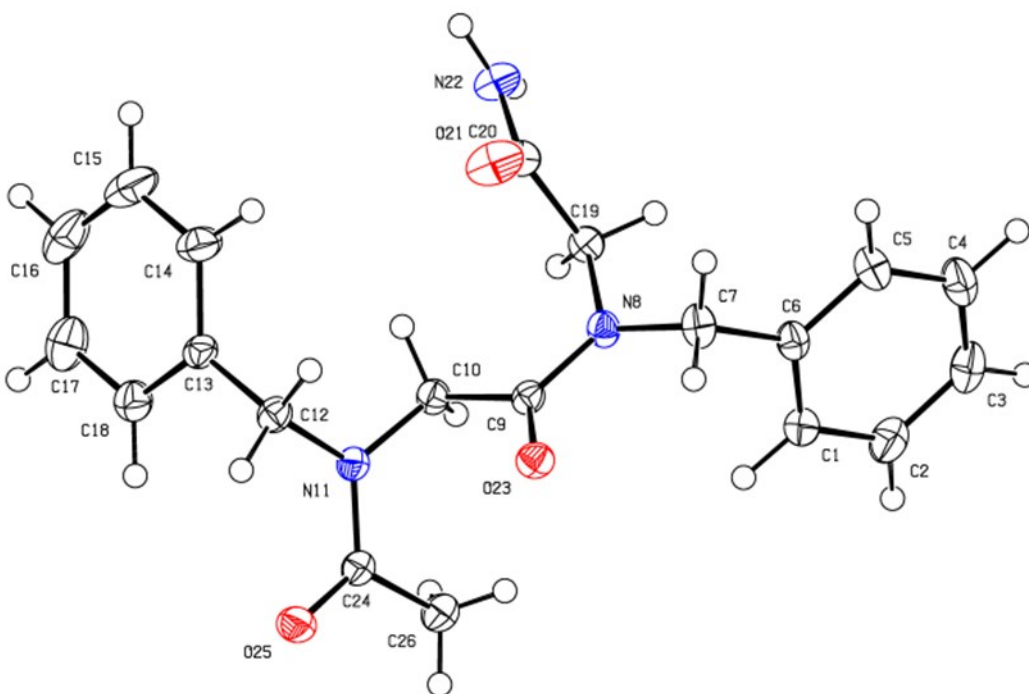


Figure S7. Numbering scheme of atoms from single crystal XRD.

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