Fluorine-Induced Helical Assembly of Dipeptides and its Remote Magnetic Alignment

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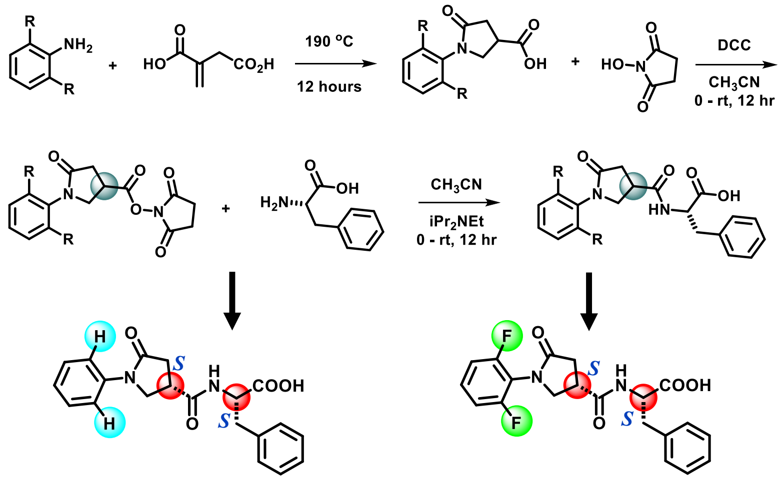
General Information:

NMR was obtained on a Bruker SampleCase Plus-500 MHz spectrometer. Bruker D8 QUEST diffractometer was used to obtain single-crystal X-ray diffraction data.

Synthesis of Compound 1 and 2:

γ-Lactam acid (Lactam, 0.965 g, 4 mmol) and N-hydroxysuccinimide (NHS, 460 mg, 4 mmol) were dissolved in 30 mL CH_3CN and N,N-dicylcohexylcarbodiimide (DCC, 867 mg, 4.2 mmol) was added at 0°C. The mixture was warmed up and continued to stir at room temperature for 8 h, then the resulting white solid was filtered off, and the filtrate was subjected to rotary evaporation. The crude product obtained (Lactam-NHS) was used without purification. L-Phenylalanine (660 mg, 4 mmol) and Hünig base (iPr_2NEt, 1.05 mL, 6 mmol) were dissolved in 15 mL of CH_3CN, the solution of the crude product (Lactam-NHS) (dissolved in 15 mL of CH_3CN) was added, and the resulting reaction mixture was stirred at room temperature overnight. The reaction mixture was subjected to rotary evaporation, and then 50 mL of water was added. The mixture was acidified pH 3 and extracted with 50 mL × 3 EtOAc. The organic phases were combined, dried over Na_2SO_4 and concentrated in vacuo. The residue was further purified using a flash column with EtOAc to MeOH/EtOAc (15%) as the eluent to afford the final compounds as illustrated in **Scheme S1**.



Scheme S1. Molecular synthesis strategy of dipeptide analogues.

Spectrum of Fourier transform infrared (FTIR)

Solid pellet (KBr pellet) method was employed.3 mg of the short peptide crystal powder was mixed with a small amount of potassium bromide (KBr) powder in an agate mortar and ground thoroughly until homogeneous. The mixture was then placed into a pellet die and pressed into a transparent or semi-transparent pellet using a hydraulic pellet press. Finally, the prepared sample pellet was scanned using a Fourier transform infrared (FTIR) spectrometer. The experimental parameters were set as follows: scan range from 4000 to 400 cm^{-1}, number of scans 128, resolution 4 cm^{-1}. The acquired data were smoothed using the OMNIC software.

Single-crystal X-ray diffraction

Single crystals of compound **1** and **2** were naturally grown in a glass vial at room temperature and low supersaturation with the solvent of EtOH:H_2O (3:1) to produce the crystal core. On a Bruker D8 QUEST diffractometer, single-crystal X-ray diffraction data were obtained using Cu (60 W, Diamond, $\mu K\alpha=12.894\ mm^{-1}$) micro-focus X-ray sources. Olex2[1] (v1.5) was used to solve the structure using the XT2[2] structure solution program with Intrinsic phasing. The XL[3] refinement software was then used to refine the structure using Least Squares minimization. The solvent mask[4] routine of OLEX2 was implemented to remove the contributions of the disorder water solvent to the observed structure factors.

Spectrum of Scanning Electron Microscope (SEM)

The model of scanning electron microscope used in this experiment is Hitachi SU-8600. The specific steps are as follows: (1) First, prepare the sample to be tested. Use a micropipette to aspirate approximately 5 μL of the sample (2 mg/mL, H_2O:DMSO = 95:5) assembled from different short peptides and drip it onto a silicon wafer. Allow it to dry at room temperature; alternatively, dry it using a freeze dryer, then mount the sample onto the sample stage. (2) Perform sputter coating with gold on the sample surface to enhance its conductivity. After loading the sample, adjust the appropriate voltage and current to obtain clear images.

Magnetic alignment of dipeptides

The magnetic fields are composed of two parallel magnets, and their strength are detected by a magnetometer. Magnetic alignment of dipeptides was carried out under a self-established magnetic field of 60 mT in the laboratory. A volume of 3 μL dipeptide (2 mg/mL) aqueous solution (H_2O:DMSO = 95:5) was deposited on silicon substrates. By adjusting the magnetic field direction (horizontal and vertical) and regulating the drying process via sealed and humidified conditions, the samples were allowed to undergo self-assembly for six days. Subsequently, the sealed environment was removed to enable natural drying. The magnetic field direction was marked on the silicon substrates. After complete solvent evaporation, the samples were sputter-coated with gold and characterized by scanning electron microscopy (SEM).

Spectrum of Circular Dichroism Spectroscopy (CD)

(1) Prepare the corresponding short peptide solution (2mg/mL, H_2O:HFIP= 9:1). For the aging-dependent CD experiment, 2FPhlac-Phe was aged at 25 °C for 6 days before measurement. Turn on the circular dichroism spectrometer and allow it to pre-warm for 30 minutes. Continuously purge the instrument with nitrogen gas during use to protect its light source. (2) Add the sample into a quartz cuvette. Select the cuvette path length according to the sample concentration, adjust the parameters, and initiate the measurement. The specific parameters are as follows: scan wavelength range from 190 nm to 260 nm, scan speed of 50 nm/min, bandwidth of 1.0 nm, and a constant temperature of 25 °C (at a concentration capable of self-assembly). (3) To ensure the accuracy of the scan results, measure each sample at least three times. After automatically subtracting the background using the instrument's built-in software, calculate the average value and apply smoothing. Finally, obtain the sample's ellipticity value (in mdeg) and convert it to molar ellipticity [θ] using the corresponding formula:

$$[\theta] = \frac{100 * \theta}{C * l}$$

[θ]: Molar ellipticity ($deg \cdot cm^2 \cdot dmol^{-1}$), θ: Ellipticity measured directly by the instrument; C: Molar concentration (mol/L); l: Path length (cm). Typically, a quartz cuvette with a 0.1 cm or 1 cm path length is used; 100: A coefficient. This is a long-established constant arising from the conversion relationship between ellipticity and the difference in molar extinction coefficients.

Calculation of Diamagnetic Susceptibility Tensors

The crystal structure of 2FPhlac-Phe (S,S) dissected by experimental X-ray crystallography was initially refined using Olex2-1.5 with the Levenberg-Marquardt algorithm to minimize discrepancies between the structural model and experimental diffraction data (Dolomanov et al., 2009). A full unit cell was generated by applying crystal symmetry operations to the refined asymmetric unit.

Molecular magnetic susceptibility tensors (χ^{mol}) were computed in the principal axis system (Z-matrix coordinates) at the PBE/6-31G(d,p) level of theory (Guo et al., 2020). The crystal magnetic susceptibility tensor (χ^{cryst}) was then obtained by dividing χ^{mol} by Z = 6, corresponding to the number of molecules per unit cell.

Diagonalization of the χ^{cryst} tensor yielded three eigenvalues (χ_i^{cryst}) and the corresponding principal axes. The isotropic component (χ_{iso}^{cryst}) was calculated as the mean of the eigenvalues, while the anisotropy (χ_{aniso}^{cryst}) was defined as the difference between the maximum and minimum eigenvalues (Guo et al., 2020; Luyten et al., 1982).

To correlate magnetic anisotropy with crystal morphology, the diamagnetic susceptibility tensor per molecule was visualized relative to the crystal packing. Predicted crystal faces were generated using Bravais-Friedel-Donnay-Harker (BFDH) modeling in KrystalShaper v1.5 (Docherty et al., 1991), and the susceptibility tensor was overlaid to illustrate its orientation with respect to the dominant crystal habit.

((S)-5-oxo-1-phenylpyrrolidine-3-carbonyl)-L-phenylalanine (2HPhlac-Phe) compound 1

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 7.54 (d, *J* = 8.0 Hz, 2H), 7.36 (t, *J* = 7.7 Hz, 2H), 7.28 (t, *J* = 7.4 Hz, 2H), 7.26 – 7.19 (m, 3H), 7.18 (t, *J* = 7.4 Hz, 1H), 4.72 (dd, *J* = 9.8, 4.8 Hz, 1H), 4.06 – 3.94 (m, 2H), 3.34 (s, 0H), 3.27 (t, *J* = 4.5 Hz, 1H), 2.95 (dd, *J* = 14.0, 9.8 Hz, 1H), 2.68 (dd, *J* = 17.2, 9.5 Hz, 1H), 2.50 (dd, *J* = 17.2, 7.0 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.32, 173.22, 173.14, 138.66, 137.08, 128.88, 128.48, 128.09, 126.48, 124.99, 120.77, 53.66, 51.37, 37.02, 36.08, 35.60

((S)-1-(2,6-difluorophenyl)-5-oxopyrrolidine-3-carbonyl)-L-phenylalanine (2FPhlac-Phe) compound 2

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 7.46 – 7.36 (m, 1H), 7.25 (tt, *J* = 15.6, 7.4 Hz, 5H), 7.08 (t, *J* = 9.0 Hz, 2H), 4.73 (dd, *J* = 9.8, 4.8 Hz, 1H), 3.92 – 3.82 (m, 2H), 3.42 (p, *J*

= 8.2 Hz, 1H), 3.29 – 3.23 (m, 1H), 2.95 (dd, *J* = 13.9, 9.8 Hz, 1H), 2.61 (dd, *J* = 17.3, 9.5 Hz, 1H), 2.51 (dd, *J* = 17.2, 7.8 Hz, 1H). ^{13}C NMR (126 MHz, MeOD) δ 173.98, 173.14, 172.65, 159.93, 157.92, 137.05, 129.87, 129.79, 129.71, 128.90, 128.09, 126.49, 114.39, 111.90, 111.74, 53.67, 51.43, 37.43, 37.04, 33.37. ^{19}F NMR (471 MHz, MeOD) δ -118.80, -119.96.

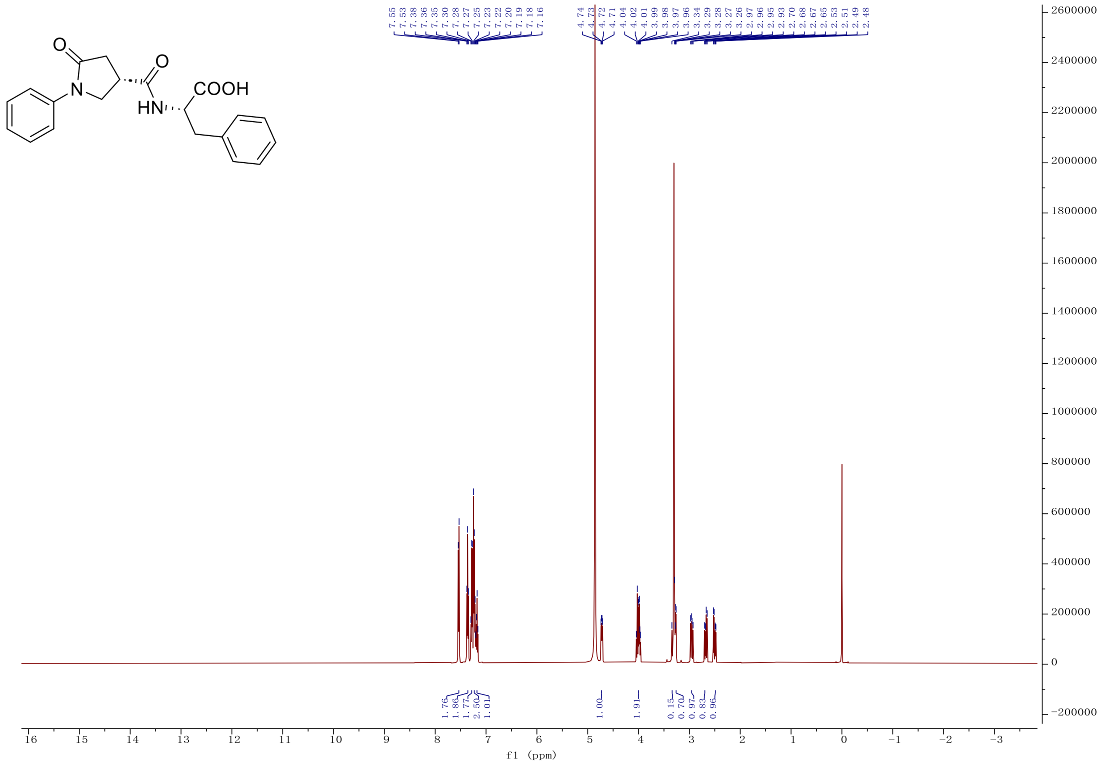


Fig. S1. ^{1}H-NMR spectrum of 2HPhlac-Phe.

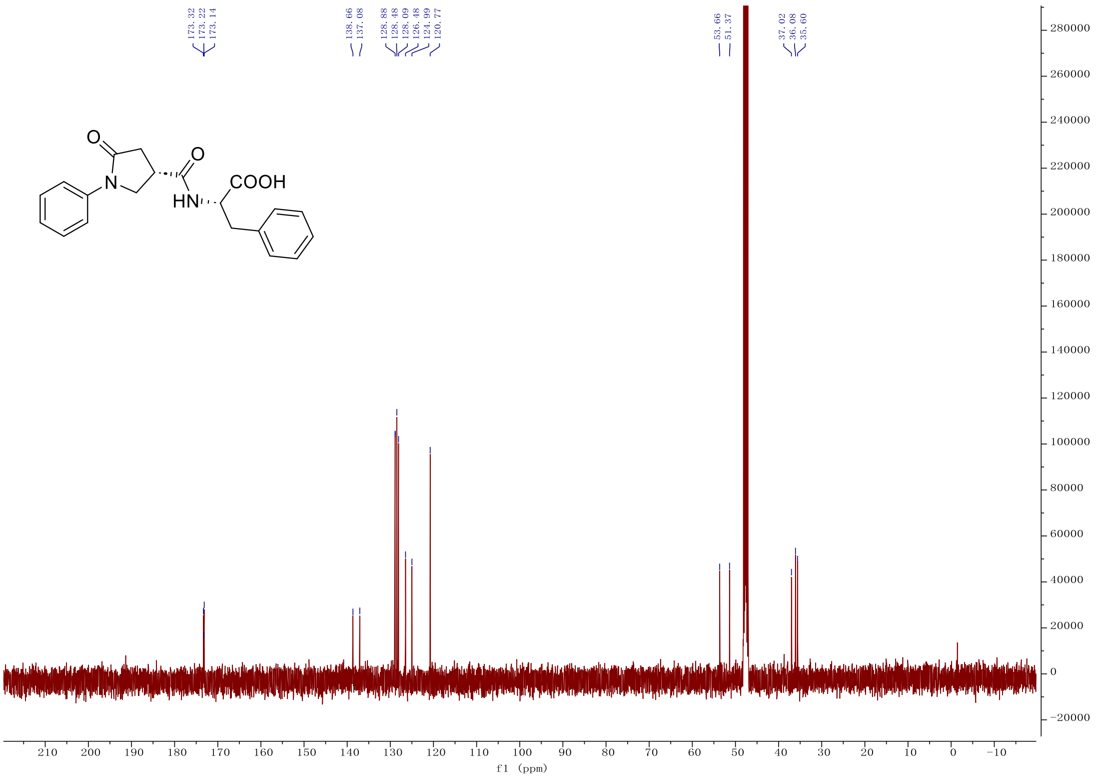


Fig. S2. ^{13}C-NMR spectrum of 2HPhlac-Phe.

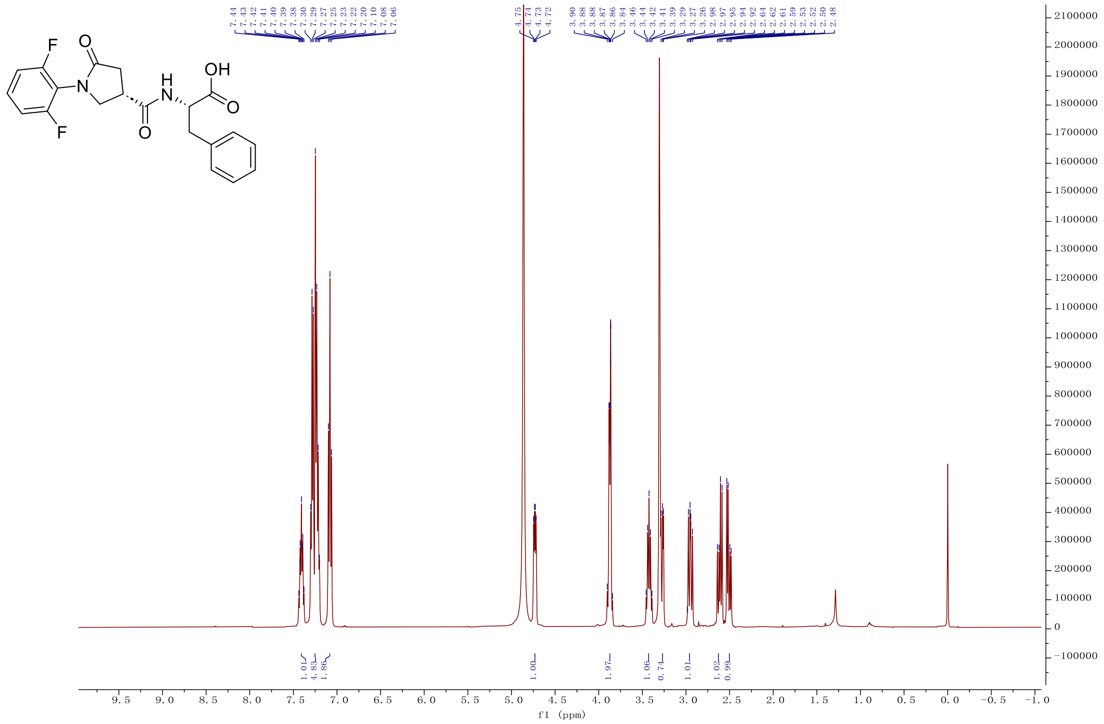


Fig. S3. ^{1}H-NMR spectrum of 2FPhlac-Phe.

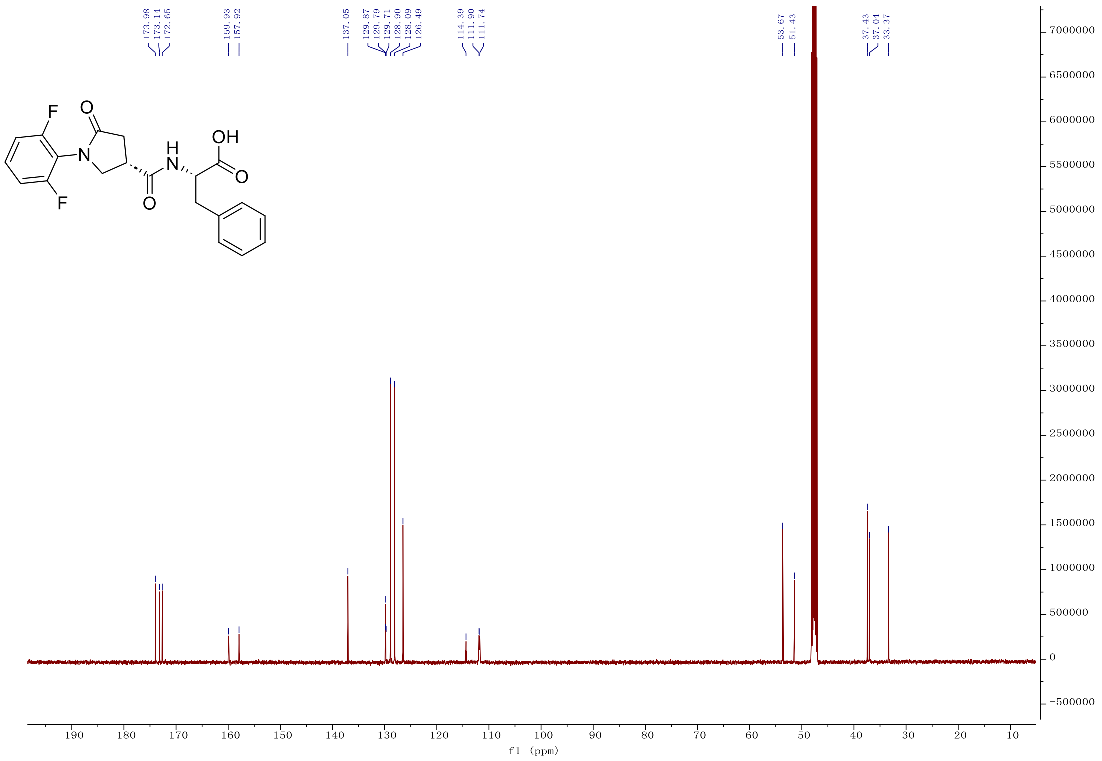


Fig. S4. ^{13}C-NMR spectrum of 2FPhlac-Phe.

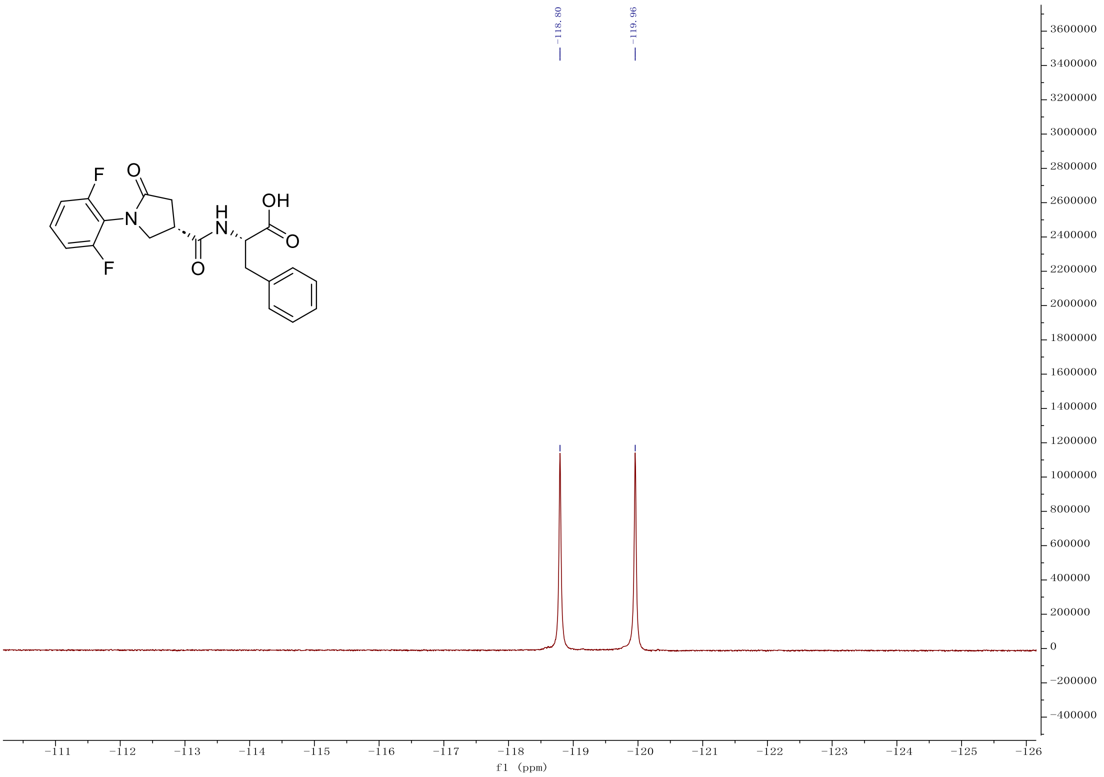


Fig. S5. ^{19}F-NMR spectrum of 2FPhlac-Phe.

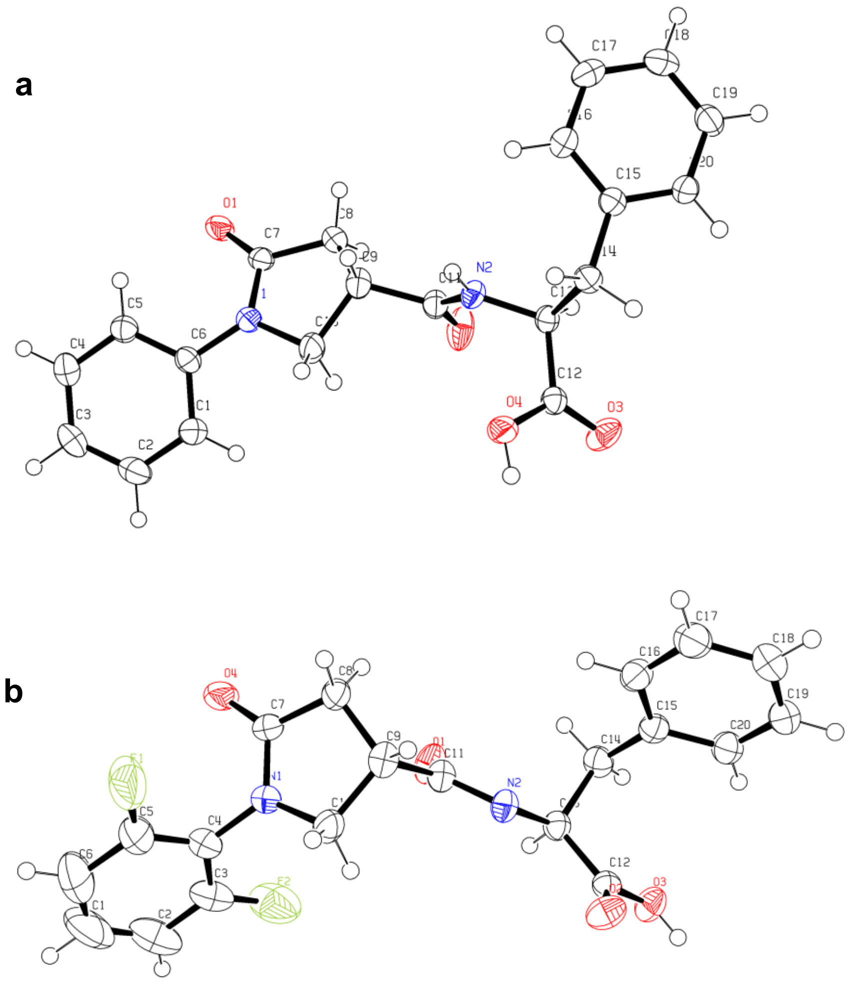


Fig. S6. X-ray diffraction (XRD) of ORTEP style for a) compound **1**; and b) compound **2**. The different colored atoms represent different elements, such as carbon (gray), fluorine (yellowgreen), nitrogen (blue), oxygen (red), and hydrogen (white).

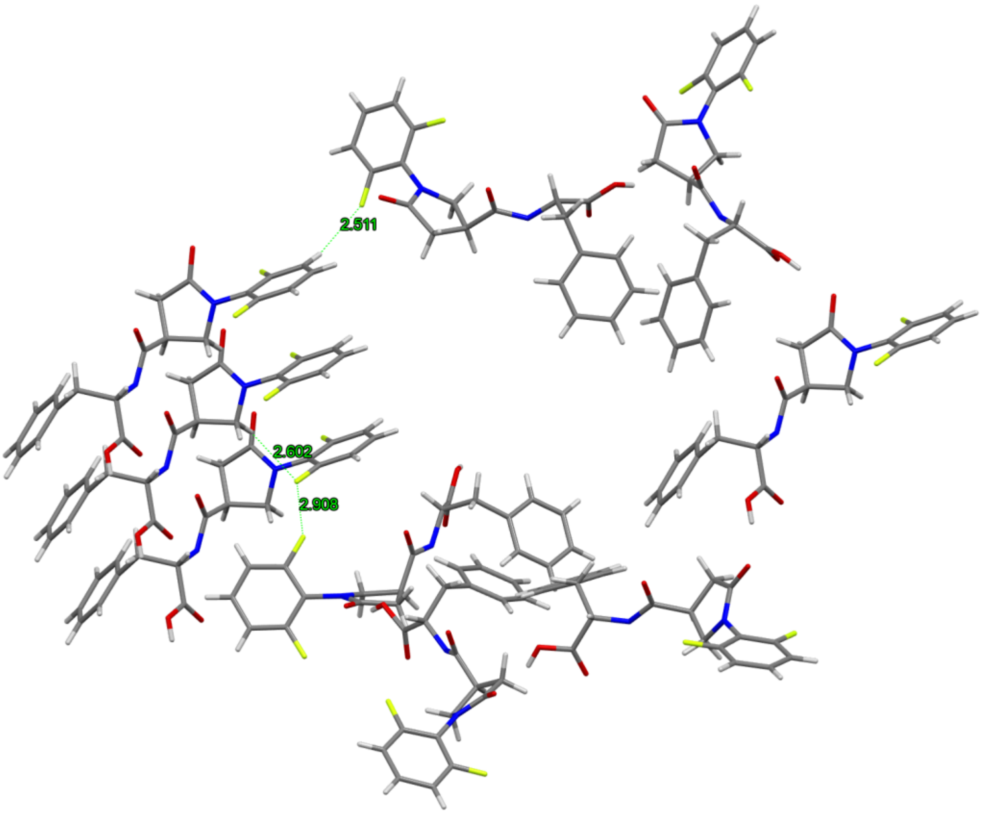


Fig. S7. X-ray diffraction (XRD) of the influence of 2,6-difluoro substituents.

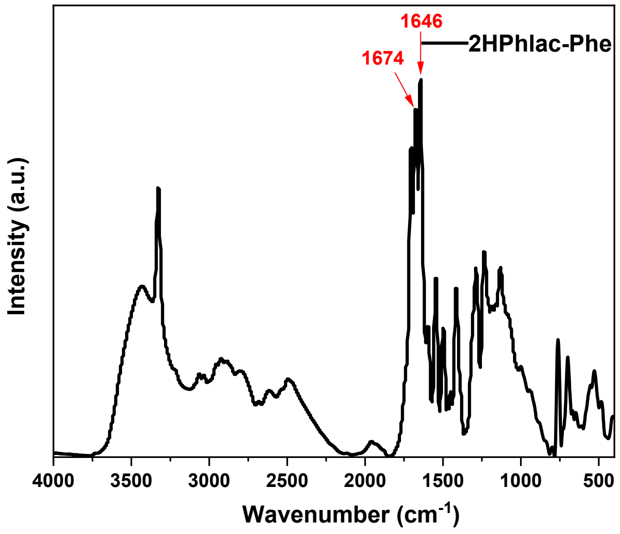


Fig. S8. FTIR spectroscopy spectrum of compound **1**.

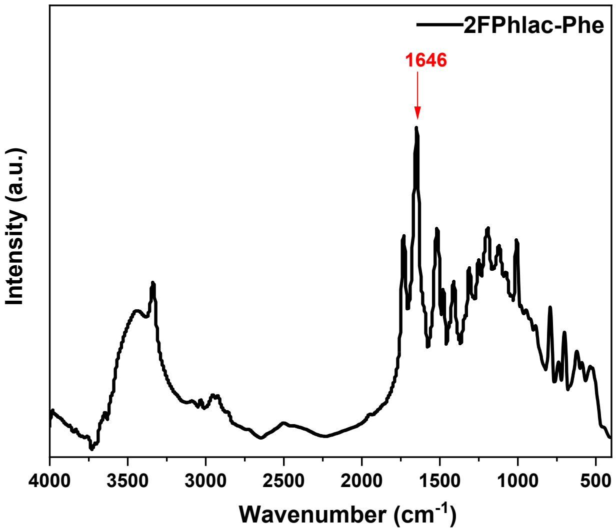


Fig. S9. FTIR spectroscopy spectrum of compound **2**.

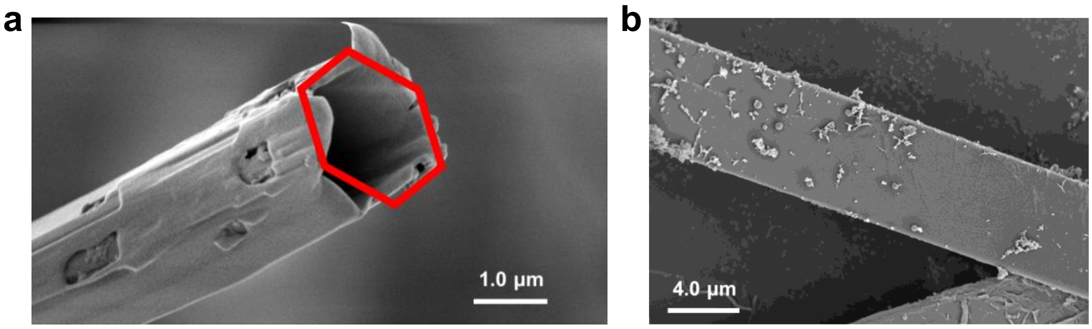
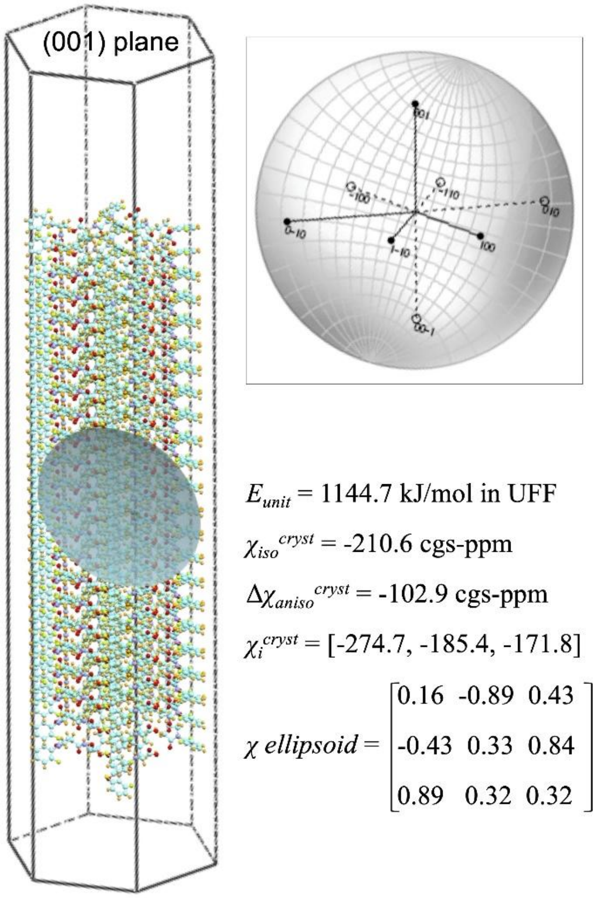


Fig. S10. Final SEM of a) compound **1** and b) compound **2**.



E_{unit} = 1144.7 kJ/mol in UFF

$\chi_{iso}{}^{cryst}$ = -210.6 cgs-ppm

$\Delta\chi_{aniso}{}^{cryst}$ = -102.9 cgs-ppm

χ_i^{cryst} = [-274.7, -185.4, -171.8]

$$\chi\ ellipsoid = \begin{bmatrix} 0.16 & -0.89 & 0.43 \\ -0.43 & 0.33 & 0.84 \\ 0.89 & 0.32 & 0.32 \end{bmatrix}$$

Fig. S11. Calculated crystal morphology and diamagnetic susceptibility of compound **2** (S,S). BFDH-predicted crystal morphology with overlaid diamagnetic susceptibility tensor ellipsoids per molecule, illustrating stereographic projection on Wulff-net of crystal planes, annotated with Miller indices.

Table S1. Crystallographic data and structure refinement of compound **1**.

Empirical formula	$C_{20}H_{20}N_2O_4$
Formula weight	352.38
Temperature/K	200.0
Crystal system	monoclinic
Space group	$P2_1$
a/Å	7.8132(4)
b/Å	5.6230(3)
c/Å	19.9273(9)
α/°	90
β/°	97.0615(18)
γ/°	90
Volume/Å^3	868.84(8)
Z	2
ρ_{calc}g/cm^3	1.347
μ/mm^{-1}	0.776
F(000)	372.0
Crystal size/mm^3	0.02 × 0.008 × 0.006
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	4.468 to 136.542
Index ranges	$-9 \leq h \leq 9$, $-5 \leq k \leq 6$, $-24 \leq l \leq 24$
Reflections collected	15292
Independent reflections	3097 [R_{int} = 0.0462, R_{sigma} = 0.0313]
Data/restraints/parameters	3097/1/236
Goodness-of-fit on F^2	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0274, wR_2 = 0.0680
Final R indexes [all data]	R_1 = 0.0282, wR_2 = 0.0685
Largest diff. peak/hole / e Å^{-3}	0.11/-0.15
Flack parameter	-0.01(8)

Table S2. Crystallographic data and structure refinement of compound **2**.

Empirical formula	$C_{20}H_{18}F_2N_2O_4$
Formula weight	388.37
Temperature/K	168.00
Crystal system	hexagonal
Space group	$P6_4$
a/Å	26.4437(8)
b/Å	26.4437(8)
c/Å	4.9534(3)
α/°	90
β/°	90
γ/°	120
Volume/Å^3	2999.7(3)
Z	6
ρ_{calc}g/cm^3	1.322
μ/mm^{-1}	0.910
F(000)	1242.0
Crystal size/mm^3	0.05 × 0.0026 × 0.0026
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	6.684 to 133.054
Index ranges	$-31 \leq h \leq 31$, $-31 \leq k \leq 31$, $-5 \leq l \leq 5$
Reflections collected	33287
Independent reflections	3509 [R_{int} = 0.1260, R_{sigma} = 0.0607]
Data/restraints/parameters	3509/1/254
Goodness-of-fit on F^2	1.010
Final R indexes [I>=2σ (I)]	R_1 = 0.0477, wR_2 = 0.1220
Final R indexes [all data]	R_1 = 0.0590, wR_2 = 0.1281
Largest diff. peak/hole / e Å^{-3}	0.26/-0.17
Flack parameter	0.1(2)

Reference:

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