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Supporting Information

For

Peptide-Induced Chirality Transfer and Circularly Polarized Luminescence in Achiral BODIPY Emitters via Halogen Bonding

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Materials and Methods:

Chemicals and Reagents: All reagents and solvents were purchased from available commercial suppliers and further purified following standard procedures. All solvents were purified and dried before use following standard protocols. Spectroscopic-grade solvents were used for physical studies.

NMR spectroscopy: ^{1}H NMR, ^{13}C NMR, ^{11}B NMR and ^{19}F NMR spectra were obtained using Bruker 600 MHz and 400 MHz NMR spectrometers, with $CDCl_3$, DMSO-D_6 and D_2O as solvents. Chemical shifts (δ) are reported in parts per million (ppm) with tetramethylsilane (TMS) as the internal standard. Coupling constants (J) are given in hertz (Hz). For ^{11}B NMR spectroscopy, phenylboronic acid was used as an internal standard. Proton signal multiplicities are denoted as follows: singlet (s), doublet (d), triplet (t), quadruplet (q), and multiplet (m).

Mass spectrometry: For determination of mass of the synthesized compounds, an electron spray ionization (ESI) QTOF mass spectrometer was used.

UV-Vis spectroscopy: UV/Vis absorption spectra were measured using a JASCO V-750 spectrophotometer. The spectral bandwidth was maintained at 1.0 nm, and a scan rate of 500 nm per minute was used. The experiments were conducted in quartz cuvettes with optical path lengths of 10 mm or 2 mm. For variable-temperature UV/Vis studies, samples were taken in stoppered cuvettes and heated from 25 °C to 95 °C. The UV/Vis spectra were recorded at a 5 °C interval and every time the samples, allowing the samples to equilibrate for 2 mins after reaching the desired temperature each time. The melting curves were obtained by plotting the $\alpha_{aggregate}$ vs. temperature plots. Using the following equation, the $\alpha_{aggregate}$ was determined,

$$\alpha_{\mathrm{agg}}(T) \approx \frac{A(T) - A_{mono}}{A_{\mathrm{agg}} - A_{mono}}$$

where A_{agg}, A_{mono}, and $A(T)$ are the absorbance values at a particular wavelength in the UV/Vis spectra for the fully aggregated, monomeric, and in-between state at temperature T, respectively.

Fluorescence spectroscopy: Fluorescence spectra were recorded on a FluoroMax-3 spectrophotometer, from Horiba Jobin Yvon. The experiments were conducted in quartz cuvettes with optical path lengths of 10 mm.

Quantum Yield measurements: The samples for **A1**, **A2**, **D1-A1**, and **D1-A2** were prepared based on the previously mentioned method keeping the dye concentration fixed at 0.05 mM in 10% MeOH/water, and their relative quantum yields were determined using Rhodamine 6G as the reference in H_2O , whose quantum yield is reported.[1,2] The emission intensity measurements were performed using a 10 mm path length quartz cuvette. The excitation wavelength (λ_{ex}) was adjusted to 400 nm for the BODIPY homo-assemblies and co-assemblies, while for Rhodamine 6G, the excitation wavelength (λ_{ex}) was fixed at 526 nm. The excitation and emission bandwidths were maintained at 1 nm each in all the measurements. The measurements followed a literature protocol using the equation provided:[3]

$$Q_S = Q_R \times \frac{I_S}{I_R} \times \frac{A_R}{A_S} \times \left(\frac{\eta_S}{\eta_R}\right)^2$$

Q_S = quantum yield of the sample; Q_R = quantum yield of the reference; I_S = area under PL curve of the sample; I_R = area under PL curve of reference; A_R = absorbance of the reference; A_S = absorbance of the sample; η_S = refractive index of 10% MeOH/water =1.332; η_R = refractive index of water = 1.333.[4] The concentration of Rhodamine 6G was adjusted so that its absorbance was below 0.1 under the experimental condition.

CD spectroscopy: Circular dichroism experiments were carried out using a JASCO J-815 Circular Dichroism (CD) Spectropolarimeter. For the variable-temperature CD experiment, the samples were taken in a stoppered cuvette and heated from 25 ºC to 95 ºC. The CD spectra were recorded at 5 ºC intervals, allowing the samples to equilibrate for 2 mins after reaching the desired temperature each time. The melting curve was obtained by plotting the CD magnitude at a fixed wavelength vs. temperature.

CPL Spectroscopy: Circular polarized luminescence (CPL) measurements carried out in a JASCO CPL-300 Spectrometer. The instrument was equipped with a Peltier cell for temperature-dependent measurements. The data were collected in quartz cuvettes with optical path lengths of 2 mm, a DC voltage of 850 V, aperture of 40/40 nm, and a scanning rate of 200 nm/min.

FTIR spectroscopy: FTIR studies were carried out using a PerkinElmer Spectrum 100 FT-IR spectrometer. The samples were prepared by drop-casting the concentrated solutions of **D1** and its co-assemblies with **A1** and **A2**. The samples were slowly evaporated by air-drying overnight to obtain a thin film. The sample was subsequently scratched from the slide, and solid-state

FTIR measurements were performed using a KBr pellet in transmittance mode over a scan range of 4000-400 cm^{-1}.

TEM Imaging: Transmission Electron Microscopy (TEM) images were taken in a JEOL-2010EX machine with an accelerating voltage of 200 kV. The aggregated samples were drop-cast on TEM grids, typically made of copper, and air-dried overnight prior to the measurements.

Sample preparation

A stock solution of the peptides and the BODIPY dyes was prepared at a higher concentration of 1 mM in chloroform. 100 µL of $\mathbf{D_1}$ in chloroform was taken in a vial, and the solvent was evaporated by heating with a heat gun. The resulting film was re-dissolved in 100 µL of MeOH. To this, water (900 µL) was added to make a final concentration of 0.1 mM in 10% MeOH/H_2O mixture. In a similar way, the aggregated samples of the two BODIPY dyes ($\mathbf{A_1}$ and $\mathbf{A_2}$) and the control peptide, $\mathbf{D_2}$, were prepared. For the 1:1 co-assembly study, 100 µL of $\mathbf{D_1}$ and 100 µL of $\mathbf{A_1}$ in chloroform were mixed together in a small glass vial. The solvent was slowly evaporated by heating with a heat gun. The resulting film was redissolved in 100 µL of MeOH, followed by the addition of 900 µL of water to achieve a final concentration of 0.1 mM for both $\mathbf{D_1}$ and $\mathbf{A_1}$. A clear, transparent solution was obtained, which was allowed to stand at room temperature for at least 2 hours to reach equilibrium before conducting any physical measurements. This procedure was similarly followed for preparing $\mathbf{D_1}$-$\mathbf{A_2}$ co-assembly and also for studying the co-assembly of $\mathbf{A_1}$ and $\mathbf{A_2}$ with the control peptide donor, $\mathbf{D_2}$.

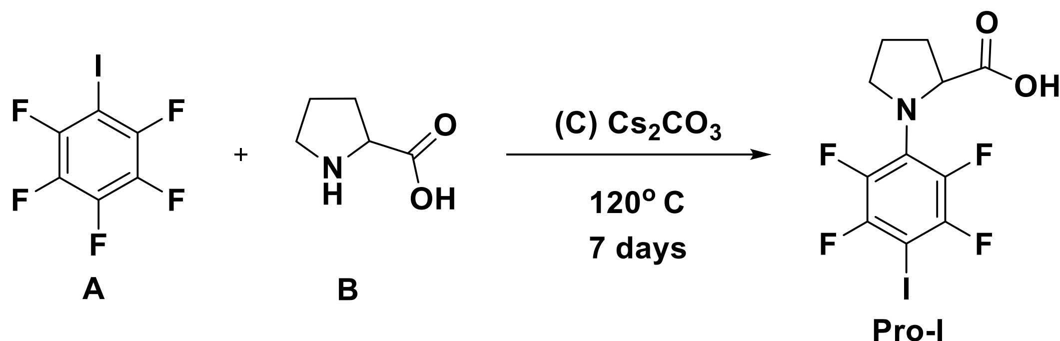
Synthesis and Characterization:

Synthesis of BODIPYs $\mathbf{A_1}$ and $\mathbf{A_2}$: BODIPYs $\mathbf{A_1}$ and $\mathbf{A_2}$ were synthesized as per the previously reported method.[5]

Synthesis of (2,3,5,6-tetrafluoro-4-iodophenyl)proline (Compound Pro-I)[6]

A measured quantity of proline (8.68 mmol) and Cs_2CO_3 (21.7 mmol) were taken in a sealed tube, to which 6 mL of pentafluoroiodobenzene (34.72 mmol) was added, and then the reaction mixture was stirred at 120 °C for 7 days. The resulting solution was cooled to RT. The mixture was diluted with 20 mL water, and then extracted with DCM (3 × 20 mL). The organic layer was dried over Na_2SO_4, and concentrated under vacuum in rotary evaporator. Purification of the crude on a silica gel column chromatography using hexane/ethyl acetate as eluent furnished **Pro-I** as a pure amorphous white product. (Yield = 282 mg; ~10 %). The compound was characterized by ^1H NMR, ^{13}C NMR, ^{19}F NMR and HR-MS mass spectrometry analyses. ^1H NMR (400 MHz, Chloroform-*d*): δ 4.67 (td, 1 H, J = 5.6, 2.8), 3.91-3.82 (m, 1H), 3.64- 3.55

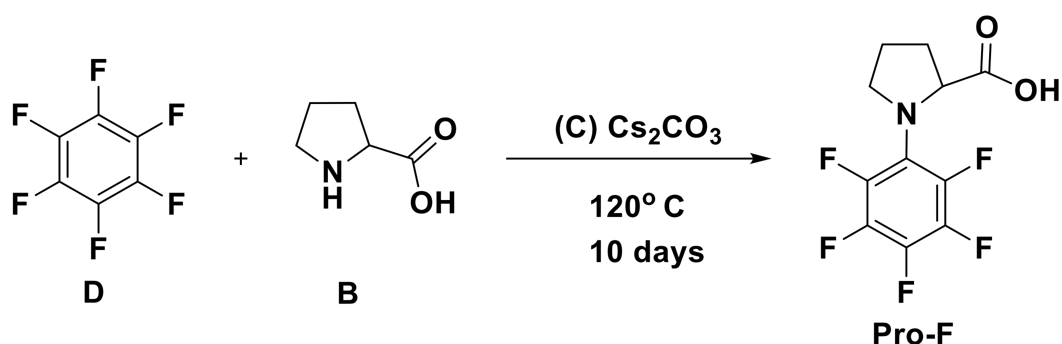
(m, 1H), 2.45-2.34 (m, 1H), 2.16 -2.05 (m, 1H), 2.05-1.87 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 178.47, 148.60-146.76 (m, 2C), 140.13-138.23 (m, 2C), 127.33, 62.23, 52.38, 52.19, 30.75, 24.27; ^{19}F NMR (565 MHz, $CDCl_3$, ppm): δ -123.14 (d, 2F, J = 17.4), -150.72 (d, 2F, J = 17.3); HRMS m/z calculated for [$C_{11}H_9F_4INO_2$] i.e. $[M+H]^+$: 389.9614; experimentally found = 389.9616.



Scheme S1: Synthetic scheme for **Pro-I**.

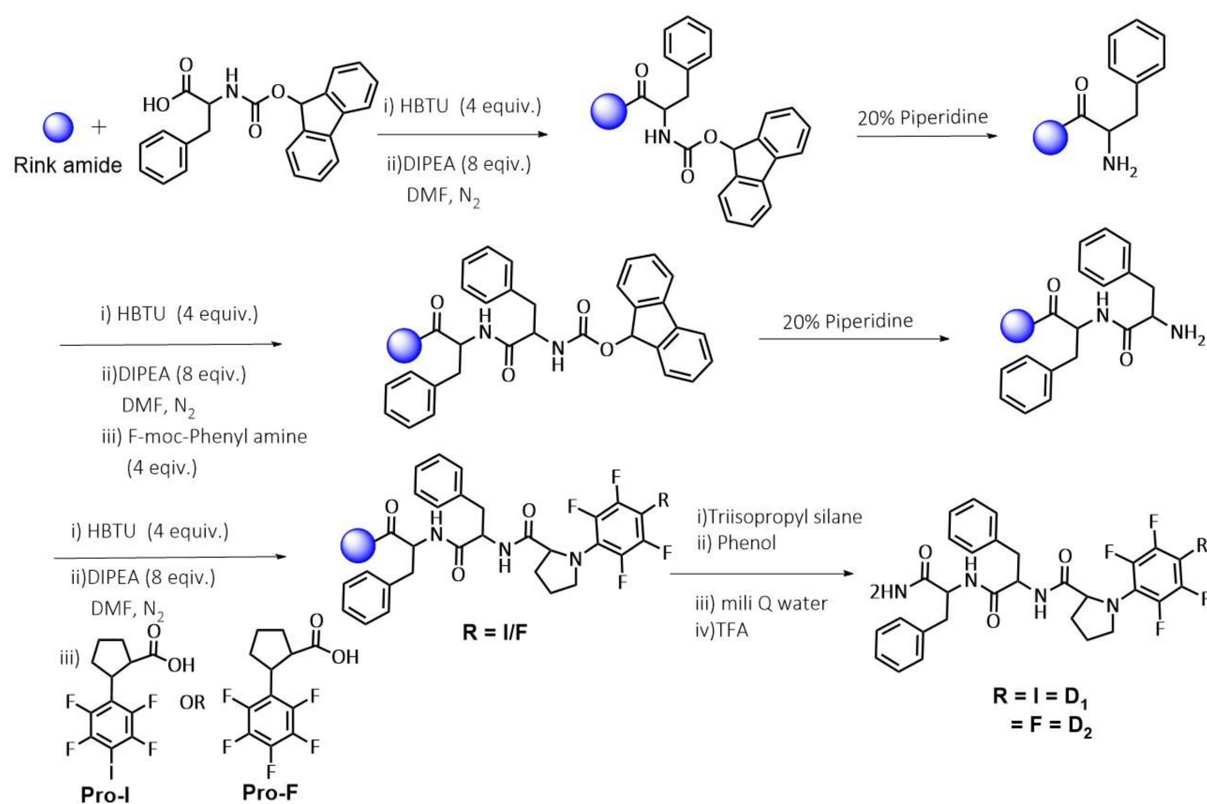
Synthesis of (perfluorophenyl)proline (Compound Pro-F)[6]

A measured quantity of proline (8.68 mmol) and $CsCO_3$ (21.7 mmol) were taken in a sealed tube to which 6 mL pentafluoroiodobenzene (34.72 mmol) was added, and then the reaction mixture was stirred at 120 °C for 10 days. The resulting solution was cooled to RT. The mixture was diluted with 20 mL water, then extracted with DCM (3 × 20 mL). The organic layer was dried over Na_2SO_4, and concentrated under vacuum in a rotary evaporator. Purification of the crude on a silica gel column chromatography using hexane/ethyl acetate as eluent furnished **Pro-F** as a brown sticky liquid (Yield = 212 mg; ~ 10 %). The compound was characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and HR-MS mass spectrometry analyses. ^{1}H NMR (400 MHz, Chloroform-*d*): δ 4.69 – 4.60 (m, 1H), 3.92 – 3.82 (m, 1H), 3.63-3.53 (m, 1H), 2.47- 2.35 (m, 1H), 2.18- 2.06 (m, 1H), 2.06-1.88 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 177.45, 149.64-146.20 (m, 2C), 141.28-137.56 (m, 2C), 127.61, 62.48, 58.31, 52.78, 31.14, 24.71; ^{19}F NMR (565 MHz, Chloroform-*d*): δ -148.35 to -148.52 (m, 2F), -158.90 (t, 1F, J = 21.7), -161.69 (td, 2F, J = 22.3, 5.6); HRMS m/z calculated for [$C_{11}H_9F_5NO_2$] i.e. $[M+H]^+$: 282.0853; experimentally found: 282.0805.



Scheme S2: Synthetic scheme for **Pro-F**.

Solid-phase peptide synthesis:[7]



Scheme S3: Synthetic scheme for peptides **D1** and **D2**.

Peptides **D1** and **D2** were prepared following a solid-phase peptide synthesis technique that involved the sequential addition of amino acids from the C-terminus to the N-terminus. Fmoc-protected Rink amide resin was taken in a peptide synthesizer tube, and it served as the solid support. The following key steps were maintained for the complete synthesis of the two peptides, **D1** and **D2**:

1. Resin Swelling: The Rink amide resin was first swollen in DMF to increase its surface area and reactivity.

2. Fmoc Deprotection: The Fmoc (9-fluorenylmethyloxycarbonyl) group was cleaved from the resin-bound amino acid using piperidine as a base.

3. Amino Acid Coupling: In the next step, the second Fmoc-protected amino acid was activated using O-Benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU) and N,N-Diisopropylethylamine (DIPEA), and coupled to the deprotected amine of the amino acid bound to the resin.

4. Repetition: Steps 2 and 3 were repeated for the subsequent attachment of the proline derivative (**Pro-I** or **Pro-F**).

5. Final Deprotection and Cleavage: Once the tripeptide chain was prepared in the resin, the peptide was cleaved from the resin using a cleavage cocktail solution.

6. Purification: The peptide was finally purified using column chromatography in neutral alumina.

Resin swelling:

Protected Rink amide resin (0.3 g, 4.77 mmol) was allowed to swell in 10 mL of DMF overnight in a refrigerator. After swelling, the resin was transferred into a specialized apparatus equipped with a G-5 sintered bed. The solvent was then removed by suction using an oil-free piston pump.

Deprotection of the Fmoc group:

To the pre-swollen resin, 15 mL of 20% piperidine in DMF was added and stirred for 15 minutes under a nitrogen atmosphere. The solution was then drained, and the resin was washed twice with 10 mL of DMF under nitrogen. The deprotection process was repeated, followed by a thorough wash with DMF to ensure complete deprotection of the Fmoc group.

General coupling procedure:

Fmoc-amino acid (19 mmol, 4 eqv.) and HBTU (2 mmol, 4 eqv.) were dissolved in 10 mL of DMF, followed by the addition of DIPEA (38 mmol, 8 eqv.). This well-mixed solution was then added to the resin, and the mixture was stirred for two hours under a nitrogen atmosphere. Once the reaction was complete, the solution was drained, and the resin was washed alternately with DMF and DCM (4-6 times for 30 seconds each) under nitrogen. The Kaiser Test was performed following standard procedure, and the pale-yellow coloration indicated successful coupling. This cycle of deprotection, coupling, and washing was repeated until the desired

peptide was synthesized. After the final reaction with **Pro-I** / **Pro-F**, the solution was drained, and the resin was washed first with DMF and then with DCM. The peptidyl resin was thoroughly dried in preparation for the next process.

Cleavage:

The purpose of cleavage is to separate the peptide from the solid support. For that, the peptidyl resin was treated with a cleavage cocktail solution that leads to a series of complex reactions. The cleavage cocktail used had the following combination: TFA/phenol/water/TIPS (88/5/5/2). This cocktail was added to the dried resin and stirred for 2 hours. After stirring, the solution was drained, and the resin was washed with the cocktail. The filtrate was collected in a conical flask and placed in a vacuum desiccator containing powdered KOH. After the solvent evaporated completely, the peptide was washed several times with cold ether. The crude peptide was then dissolved in distilled water-acetonitrile mixture (80/20) and lyophilized. The lyophilized peptide was purified by column chromatography using silica as the stationary phase and 20% DCM/MeOH as eluent to obtain the pure products, **D_1** and **D_2** as a white powder (yield = 90 mg, 13 % and 64 mg, 11 %, respectively). All the compounds were characterized by ^{1}H NMR, ^{19}F NMR, and HR-MS mass spectral analyses.

Compound **D_1**

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.04 (d, 1H, *J*=8.4), 7.93 (d, 1H, *J*=8.1), 7.26 – 7.08 (10 H, m), 4.45 (tt, 3 H, *J* 8.3, 4.0), 3.76 – 3.63 (m,2 H), 3.47- 3.35 (m,1 H,), 3.01-2.91 (m, 2 H), 2.84-2.77 (m, 1H), 2.75-2.66 (m, 1H), 1.76-1.68 (m, 1H), 1.65-1.57 (m, 1H); ^{19}F NMR (565 MHz, DMSO-d_6): δ -125.06 (d, *J* = 19.6), -150.43 (d, *J* =18.4); HRMS m/z calculated for [$C_{29}H_{27}F_4NINaO_3$] i.e. $[M+Na]^+$: 705.0692; experimentally found: 705.0632.

Compound **D_2**

^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.44 (d, 1H, *J=6.6*), 7.33-7.29 (m, 2H), 7.27-7.23 (m, 1H), 7.22-7.17 (m, 4H), 7.16-7.12 (m, 2 H), 4.63 (q,1H, *J*= 7.2), 4.46 (m,1H), 3.92 (1 H, dd, *J*= 9.3, 2.6), 3.27-3.20 (m, 2H), 3.06-3.96 (m, 3 H), 3.90-3.84 (m, 1H), 3.28-3.18 (m, 1H), 1.97-1.92 (m, 1H), 1.85-1.71 (m, 1H); ^{19}F NMR (565 MHz, Chloroform-*d*): δ -148.16 to -148.72 (m), -158.90 (t, *J* = 21.7), -161.69 (td, *J* = 22.3, 5.6); HRMS m/z calculated for [$C_{29}H_{27}F_5N_4NaO_3$] i.e. $[M+Na]^+$: 597.1901; experimentally found: 597.1734.

Additional Figures:

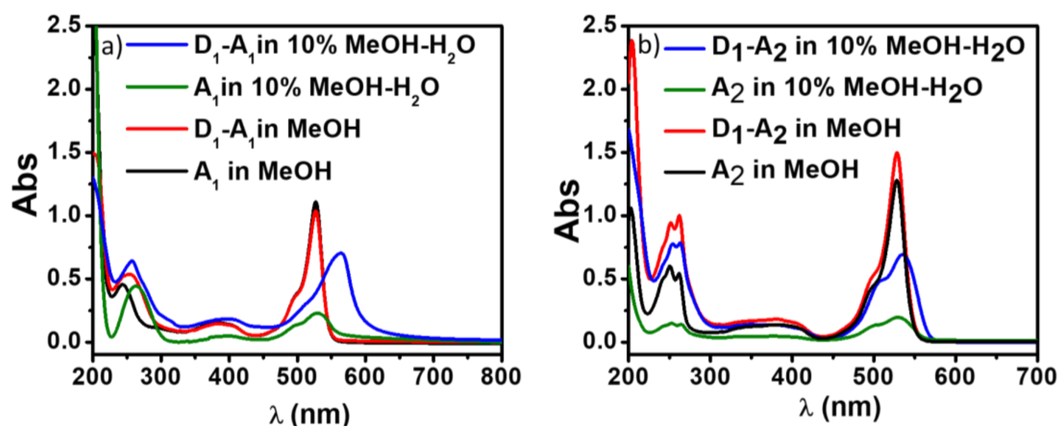


Figure S1: Compared UV-Vis absorption spectra of (a) $\mathbf{A_1}$ and $\mathbf{D_1}$-$\mathbf{A_1}$, and (b) $\mathbf{A_2}$ and $\mathbf{D_1}$-$\mathbf{A_2}$ in MeOH and 10% MeOH-H_2O mixture at 298 K. Individual Conc. = 0.1 mM.

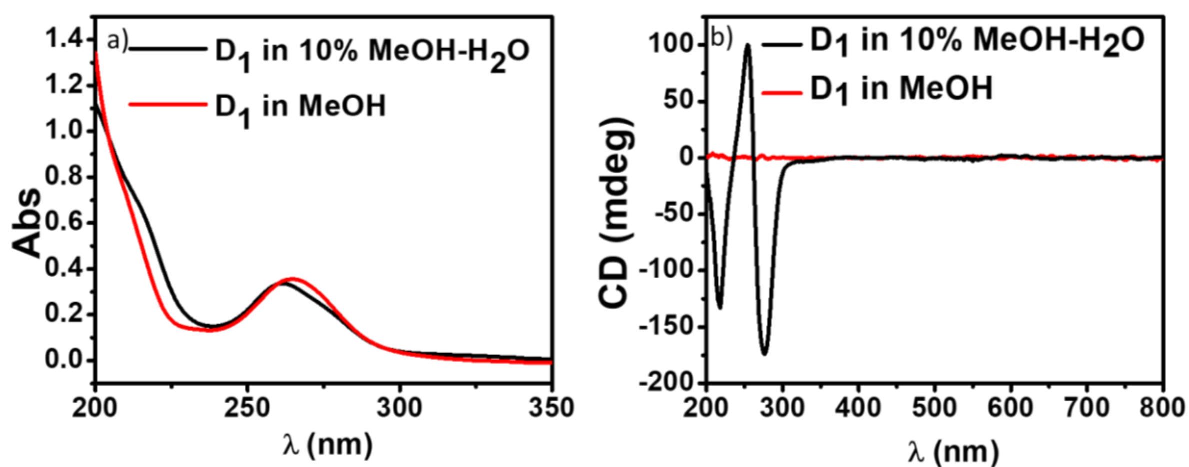


Figure S2: (a) UV-Vis absorption spectra and (b) CD spectra of $\mathbf{D_1}$ in MeOH and 10% MeOH-H_2O mixture at 298 K. Conc. = 0.1 mM.

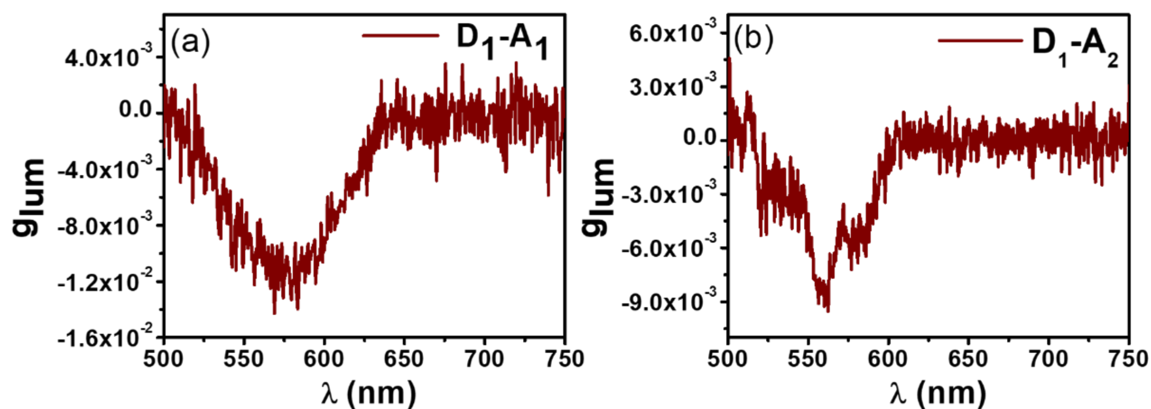


Figure S3: Luminescence dissymmetry factor (g_{lum}) versus wavelength plot from CPL analysis for (a) **D_1-A_1** co-assembly, and (b) **D_1-A_2** co-assembly at 25 °C.

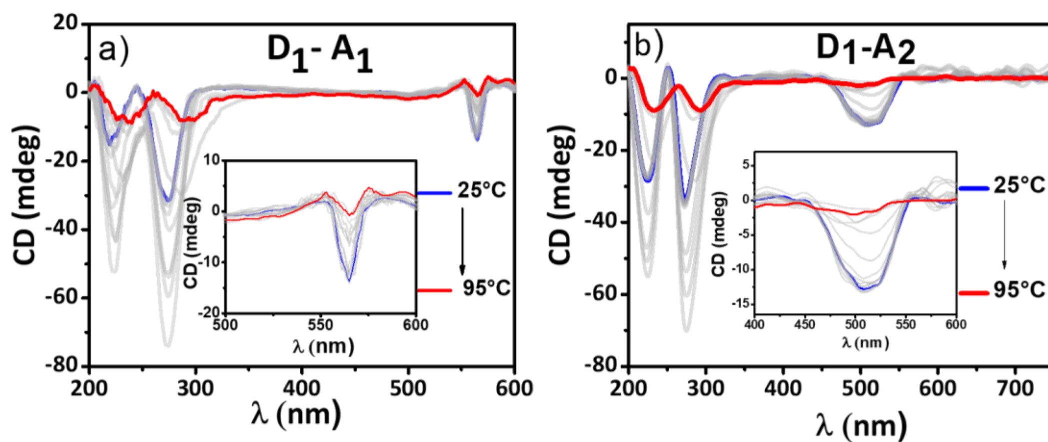


Figure S4: Variable-temperature CD spectra of (a) **D_1-A_1** co-assembly, and (b) **D_1-A_2** co-assembly. With increasing temperature, induced CD signals for **A_1** and **A_2** disappeared.

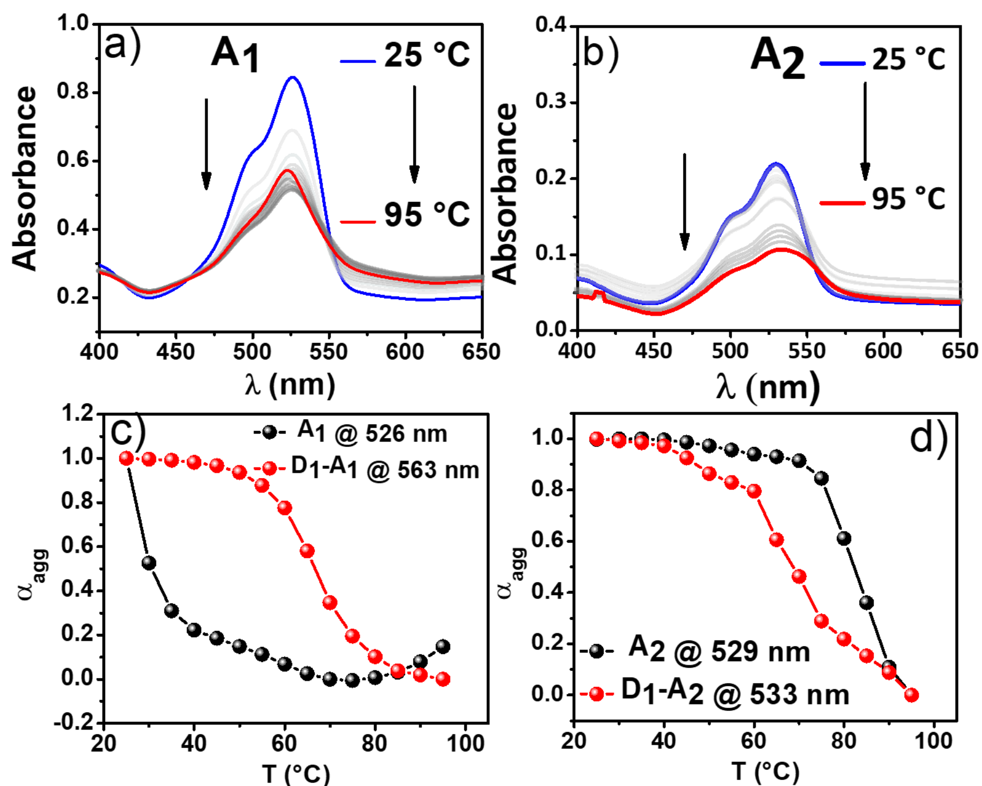


Figure S5: (a) Temperature-dependent UV-Vis absorption spectra of (a) $\mathbf{A_1}$ in 10 mm path length cuvette and (b) $\mathbf{A_2}$ in 2 mm path length cuvette; (c) Compared α_{agg} vs. Temperature plot for (c) $\mathbf{A_1}$ and $\mathbf{D_1}$-$\mathbf{A_1}$ co-assembly, and (d) $\mathbf{A_2}$ and $\mathbf{D_1}$-$\mathbf{A_2}$ co-assembly.

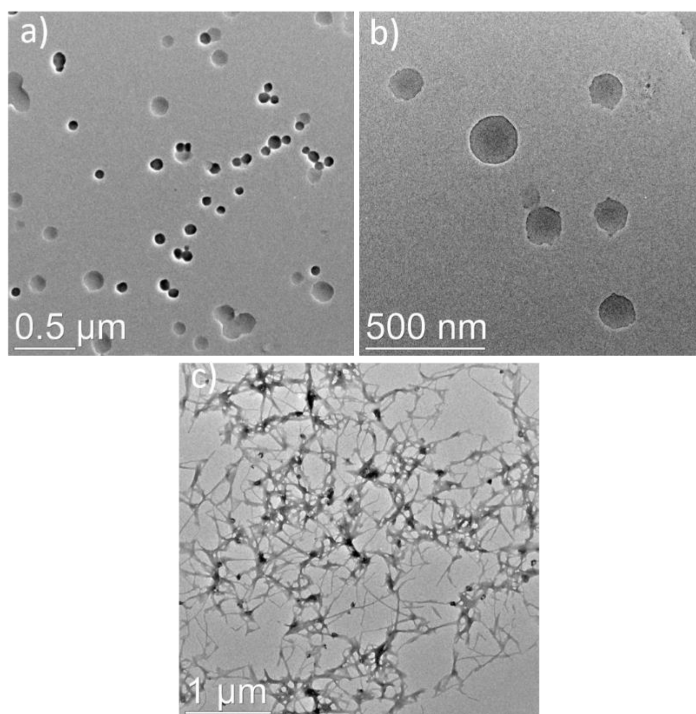


Figure S6: TEM images of (a) $\mathbf{A_1}$, (b) $\mathbf{A_2}$ and (c) $\mathbf{D_1}$-$\mathbf{A_2}$ in 10% MeOH-water.

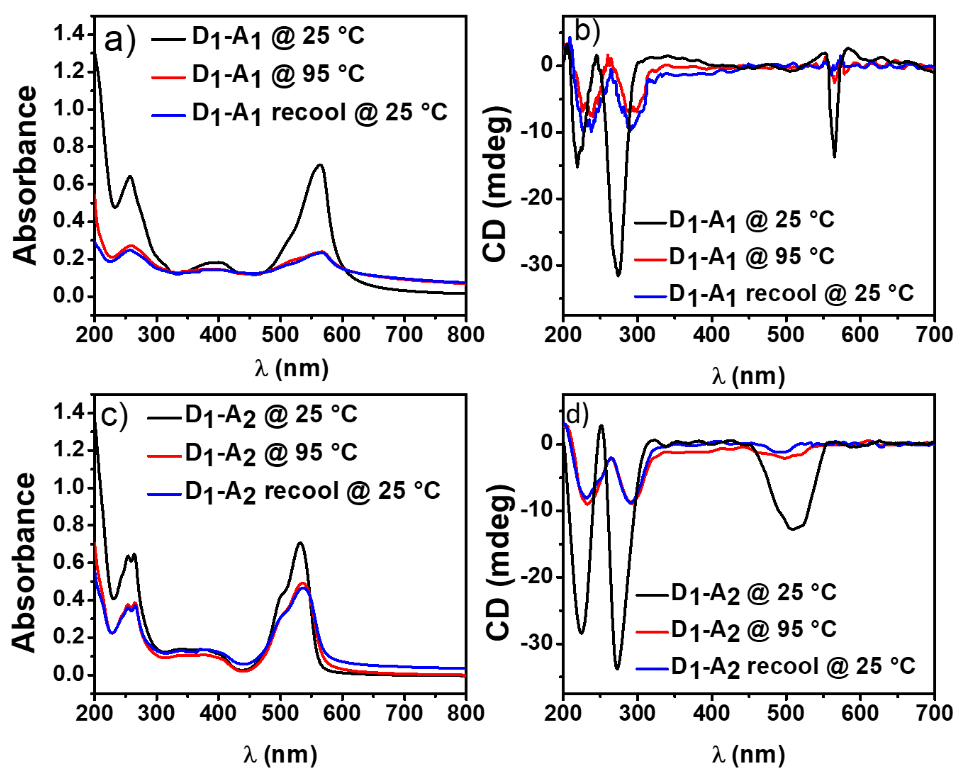


Figure S7: UV-Vis absorption spectra of co-assembled (a) **D_1-A_1** and (c) **D_1-A_2** under different conditions; CD spectra of co-assembled (b) **D_1-A_1** and (d) **D_1-A_2** under different conditions. Individual conc. = 0.1 mM in 10% MeOH-H_2O. At 95 ºC, the induced CD signal of the co-assembly disappeared, and that didn't regenerate after cooling back the sample to 25 ºC.

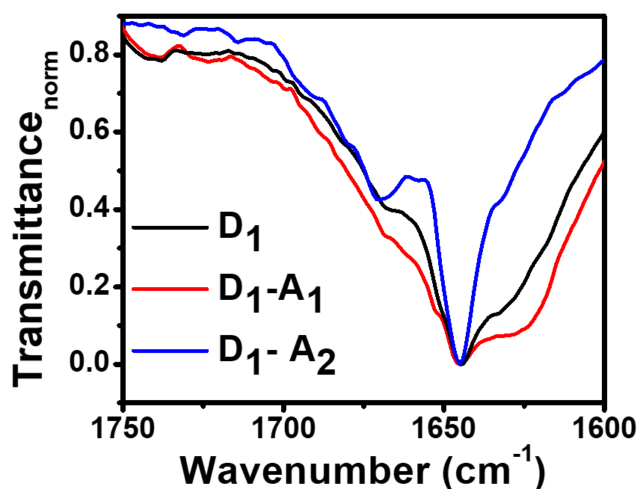


Figure S8: Compared FTIR spectra (showing the amide C=O stretching frequency region) of co-assembled **D_1-A_1** and **D_1-A_2** with **D_1** in 10% MeOH/H_2O.

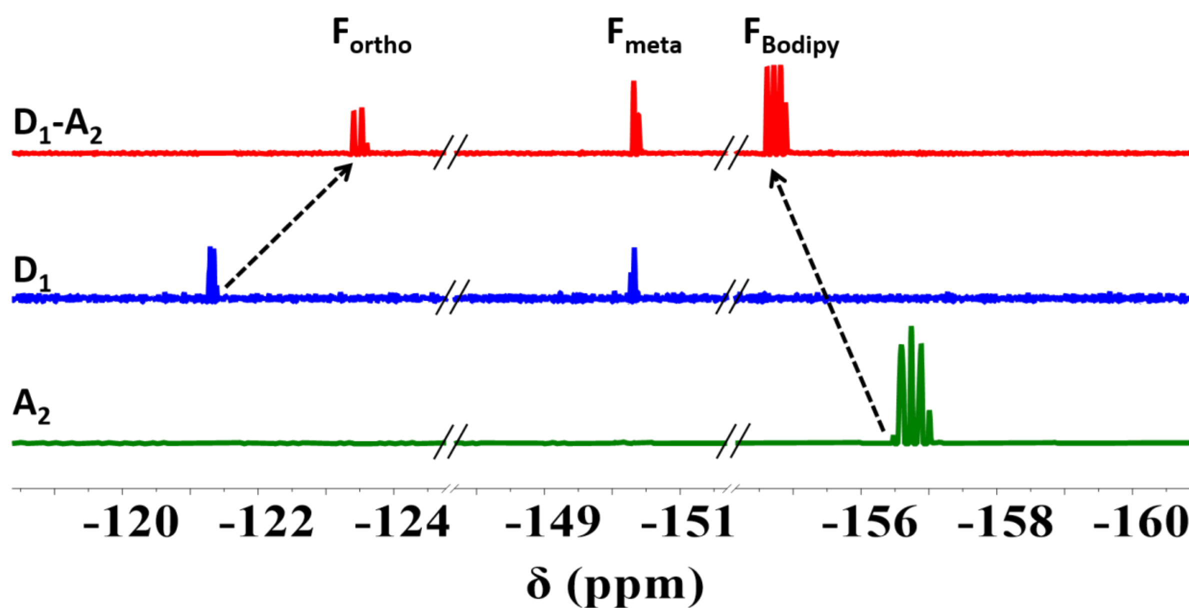


Figure S9: ^{19}F NMR spectra of $\mathbf{A_2}$, $\mathbf{D_1}$, and $\mathbf{D_1}$-$\mathbf{A_2}$ in 10% MeOH-water. D_2O was used as a locking solvent. Ortho- and meta-fluorine atoms (with respect to iodine) of peptide $\mathbf{D_1}$ showed an upfield shift, and BODIPY fluorine atoms showed a downfield shift upon halogen bonding with $\mathbf{A_2}$ in the co-assembled state.

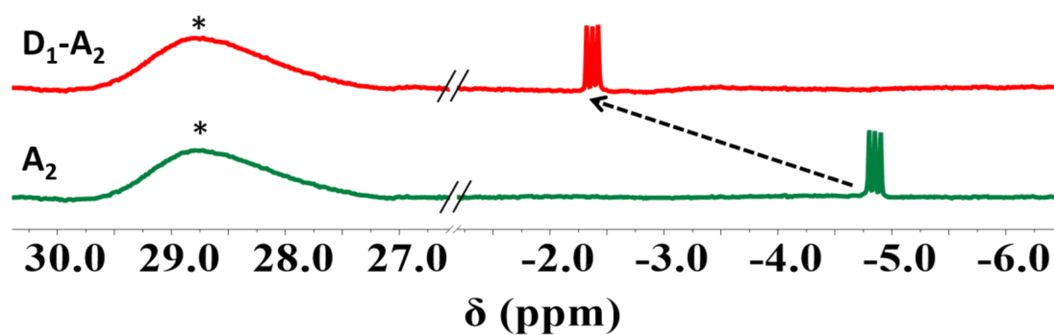


Figure S10: ^{11}B NMR spectra of $\mathbf{A_2}$ and co-assembled $\mathbf{D_1}$-$\mathbf{A_2}$ in 10 % MeOH/water. D_2O was used as a locking solvent and phenylboronic acid as an internal standard. $\mathbf{A_2}$ boron atom experienced a significant downfield shift upon halogen bonding with $\mathbf{D_1}$ in the co-assembly.

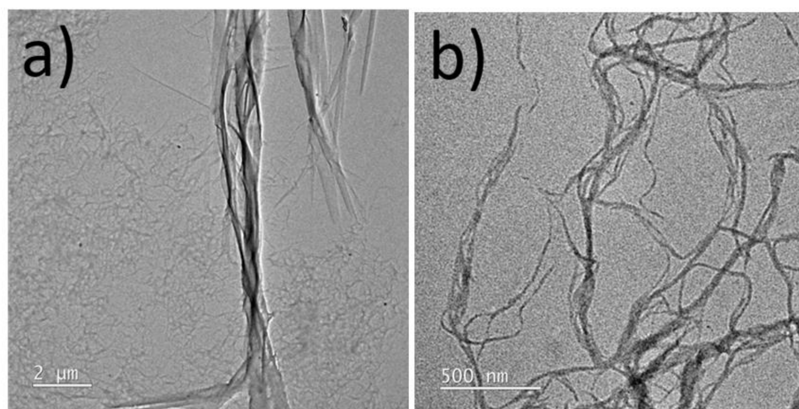


Figure S11: TEM images of self-assembled **D_2** in 10% MeOH/water mixture.

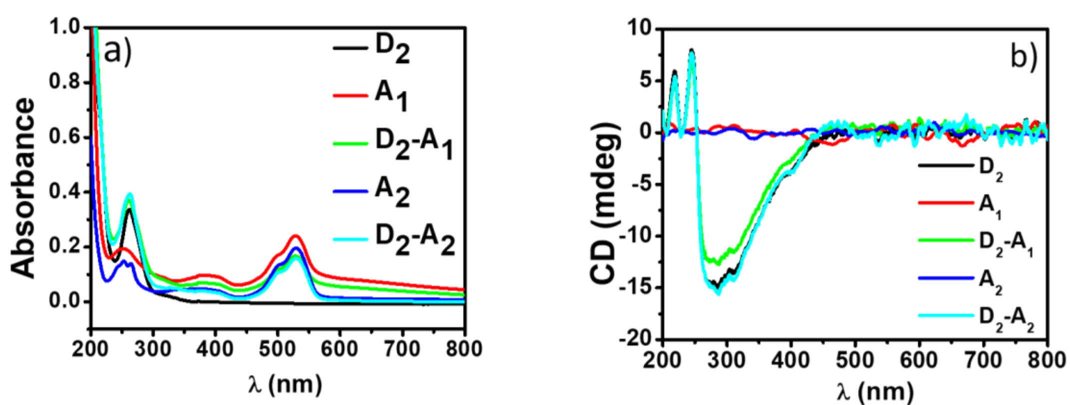
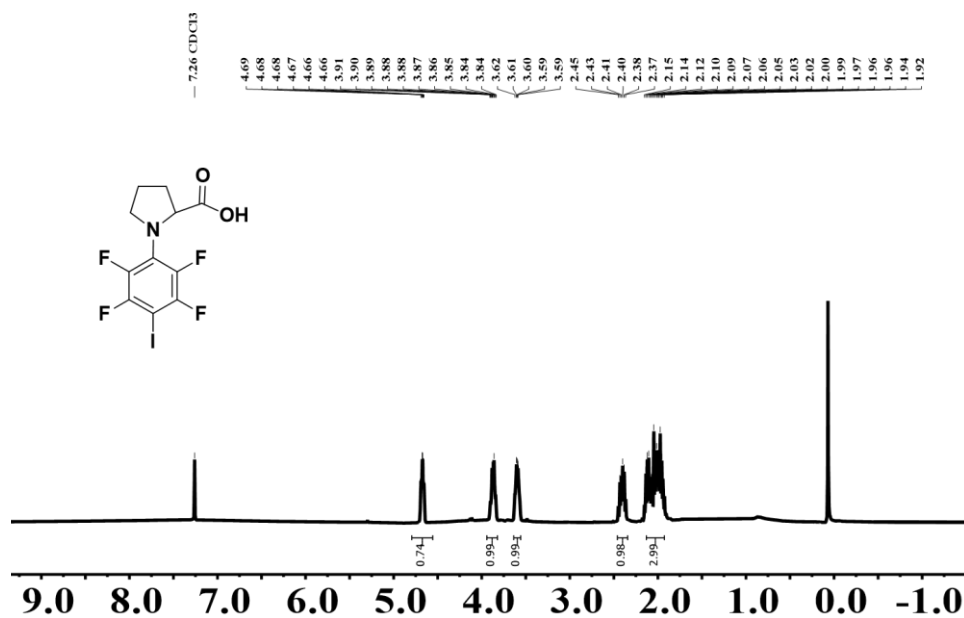
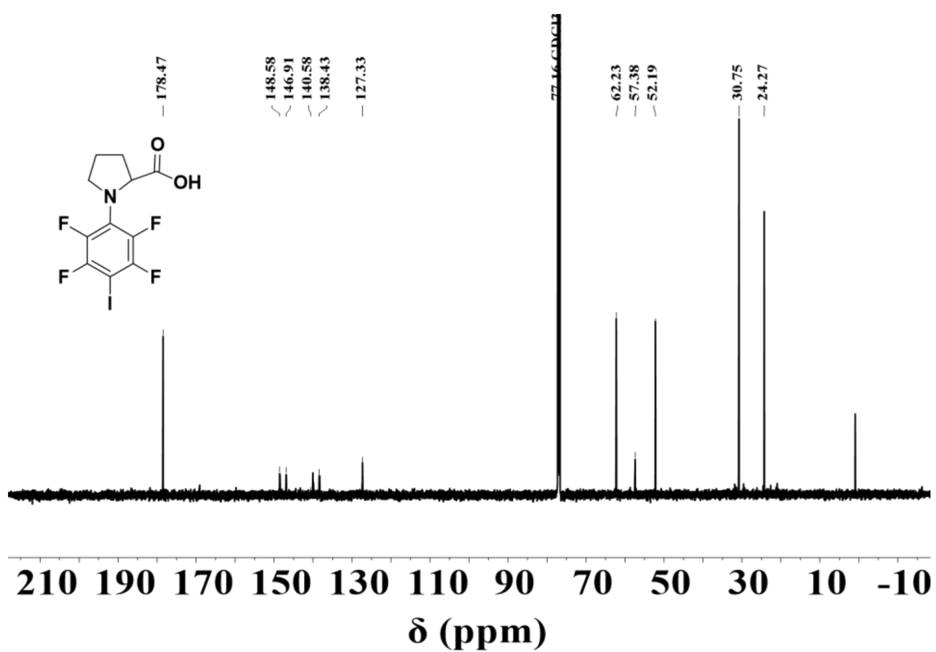


Figure S12: (a) UV-Vis spectra and (b) CD spectra of **A_1**, **A_2**, **D_2** and their 1:1 mixtures (**D_2-A_1** and **D_2-A_2**) in a 10% MeOH/H_2O. No induced CD signals for **A_1** or **A_2** appeared in the presence of the control peptide **D_2** lacking a halogen bond-donating iodine atom.

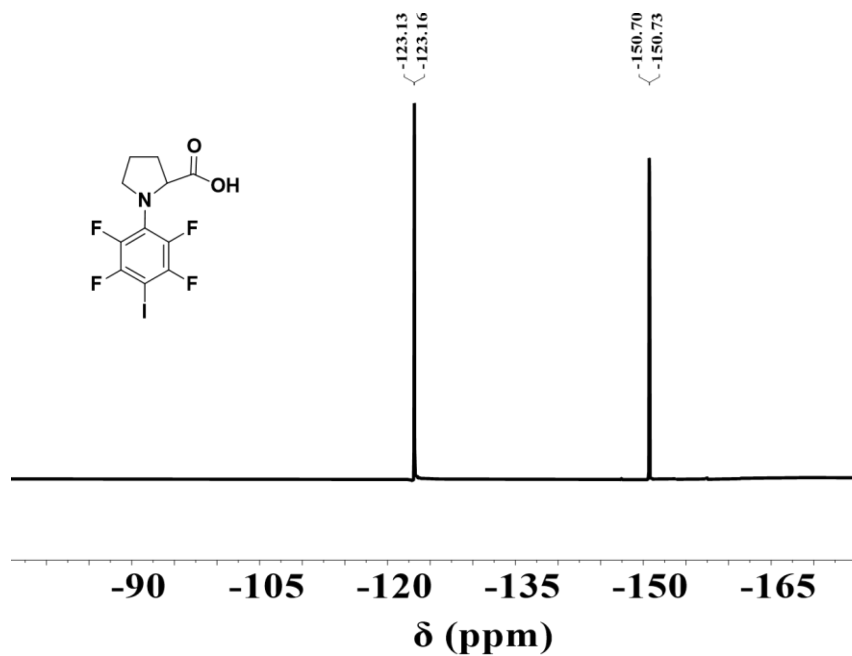
NMR and Mass Spectra



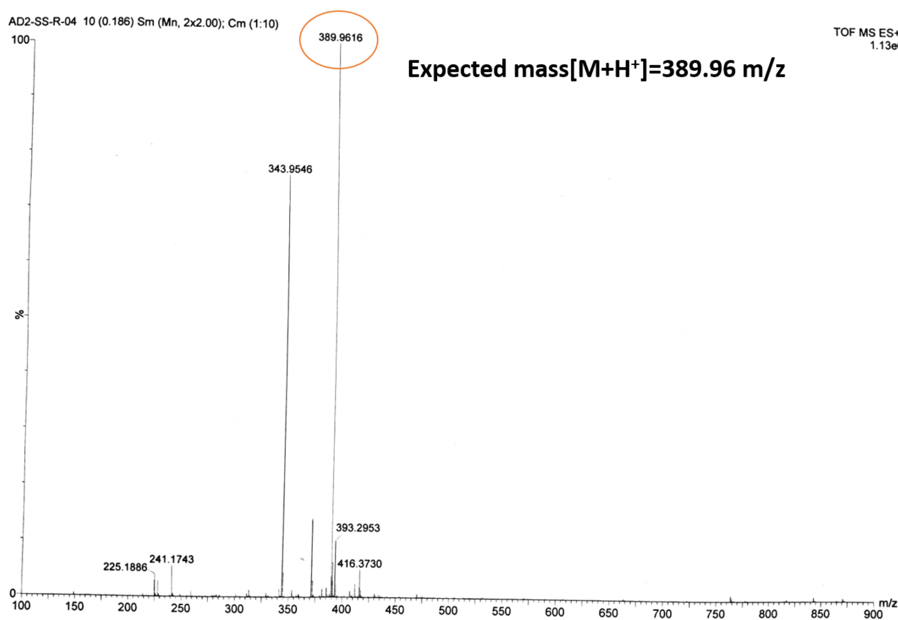
^{1}H NMR spectrum of compound **Pro-I** in $CDCl_3$.



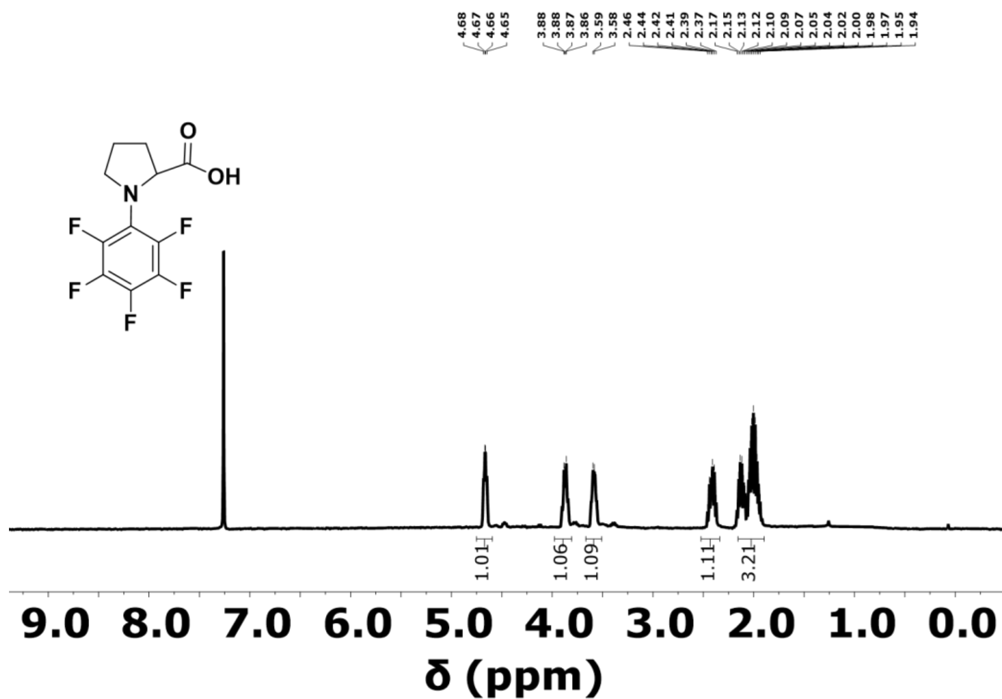
^{13}C NMR spectrum of the compound **Pro-I** in $CDCl_3$.



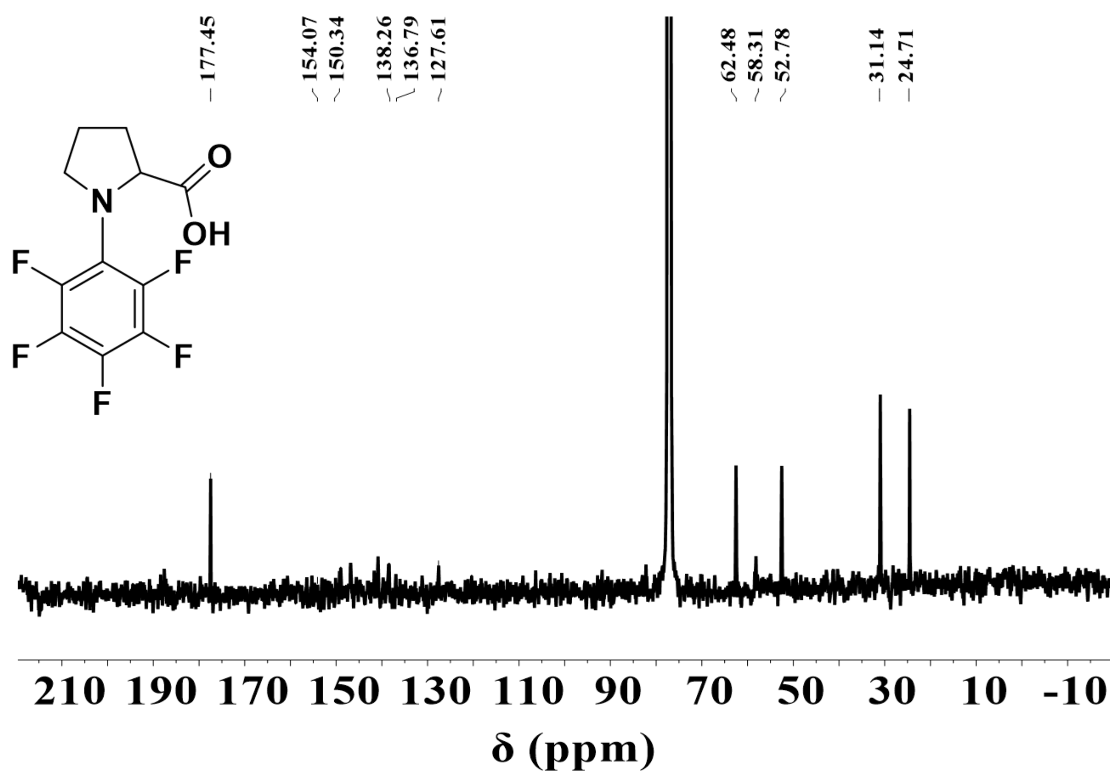
^{19}F NMR spectrum of compound **Pro-I** in $CDCl_3$.



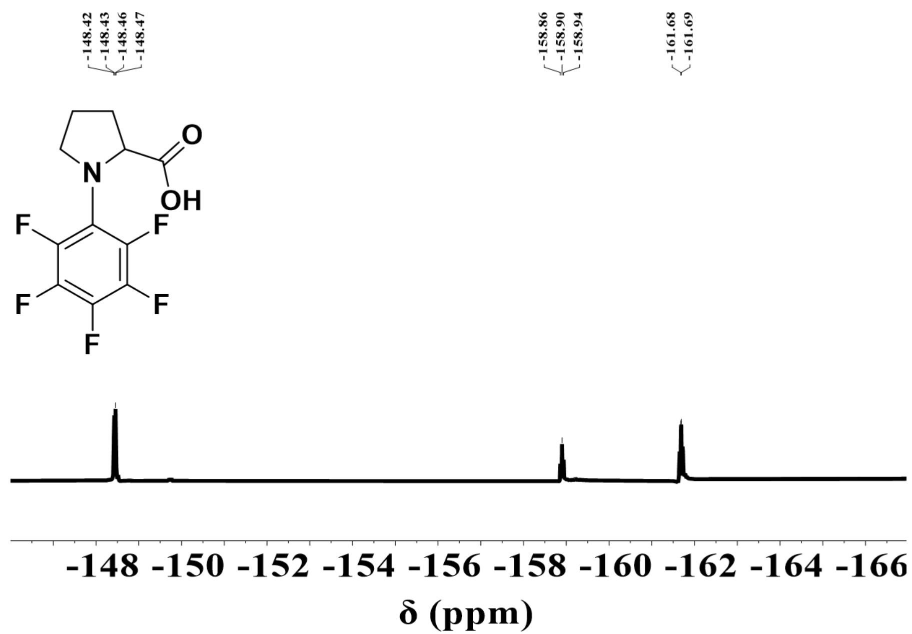
HRMS spectrum of the compound **Pro-I.**



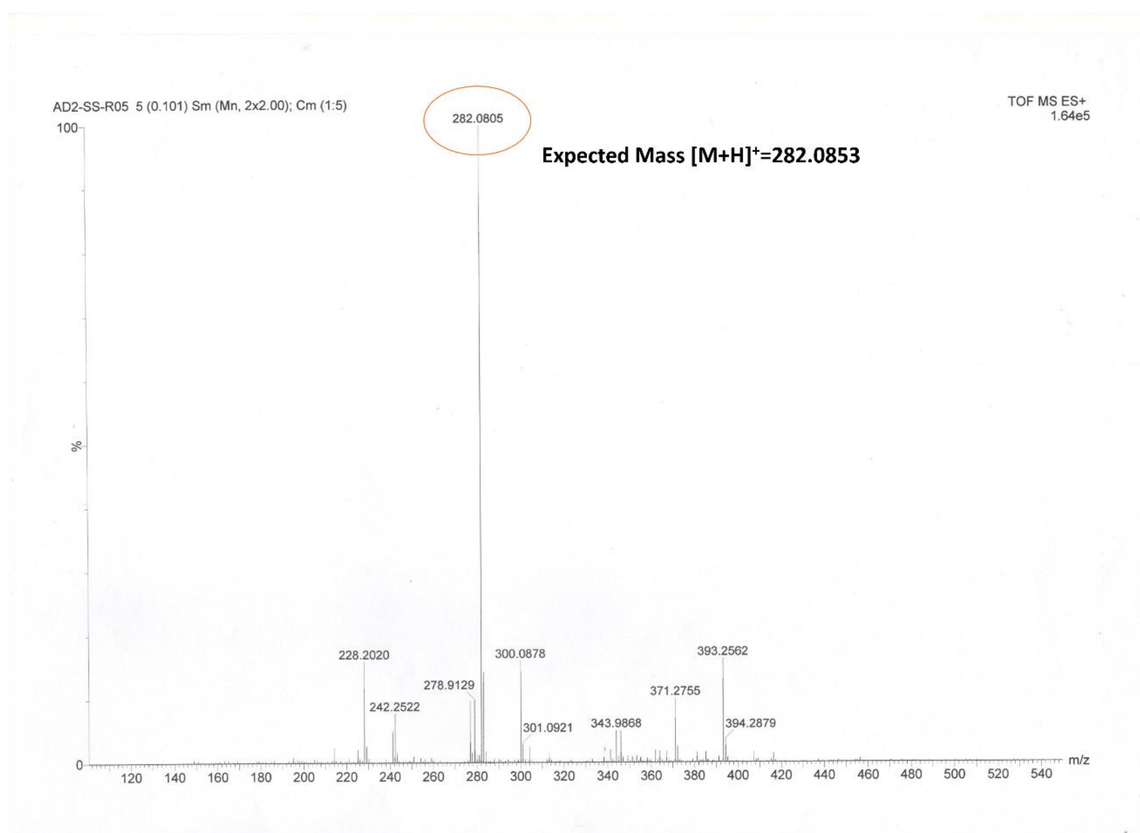
^{1}H NMR spectrum of the compound **Pro-F** in $CDCl_3$.



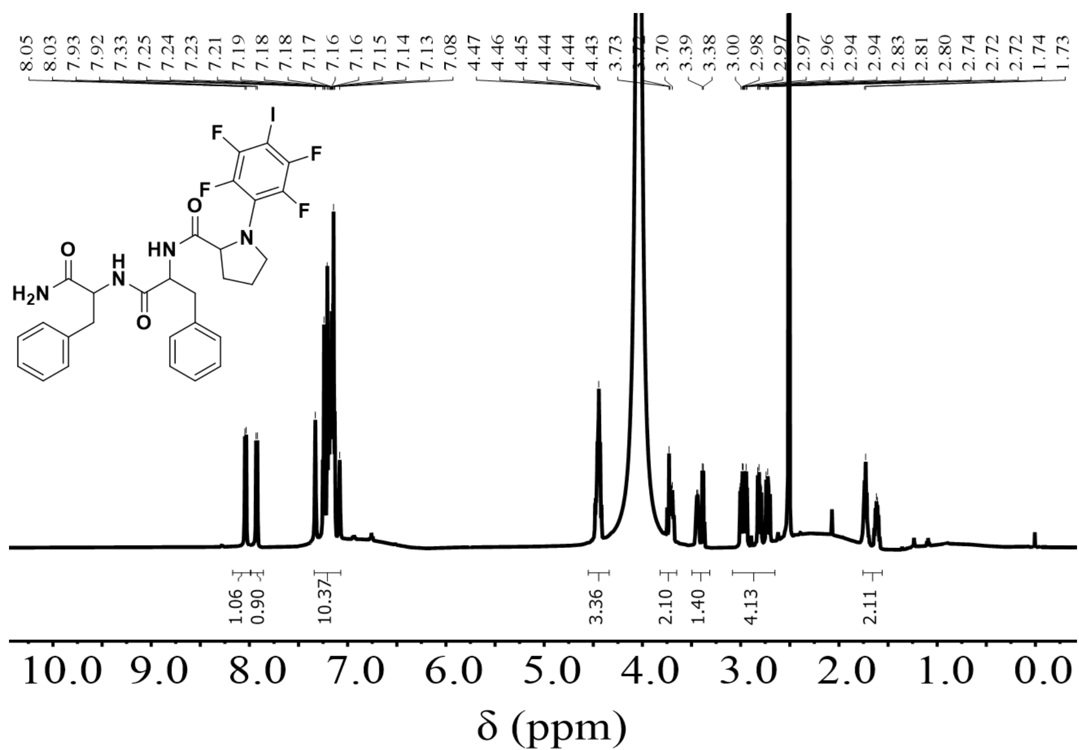
^{13}C NMR spectrum of the compound **Pro-F** in $CDCl_3$.



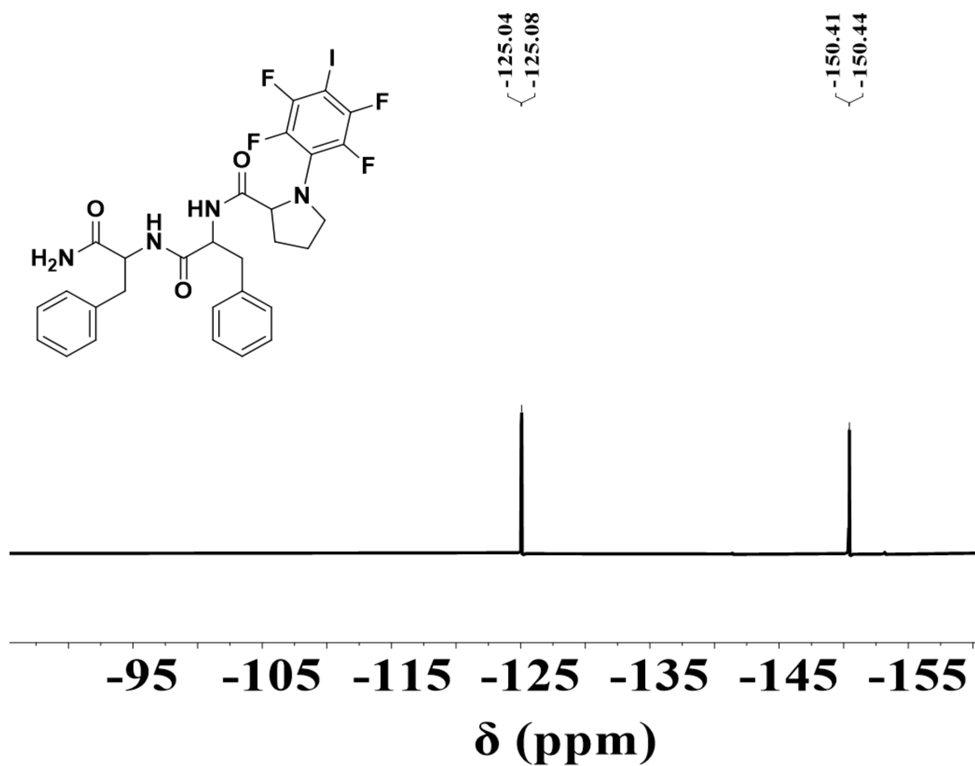
^{19}F NMR spectrum of compound **Pro-F** in $CDCl_3$.



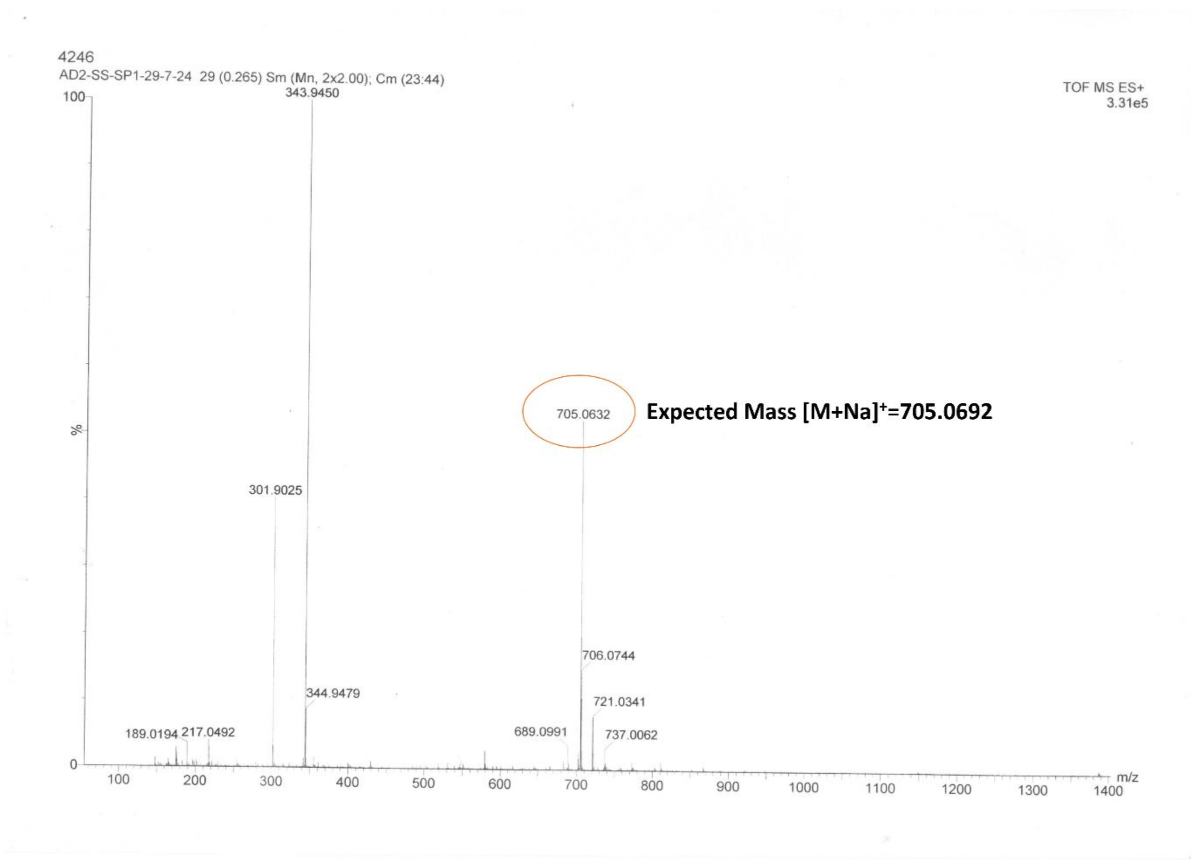
HRMS spectrum of the compound **Pro-F**.



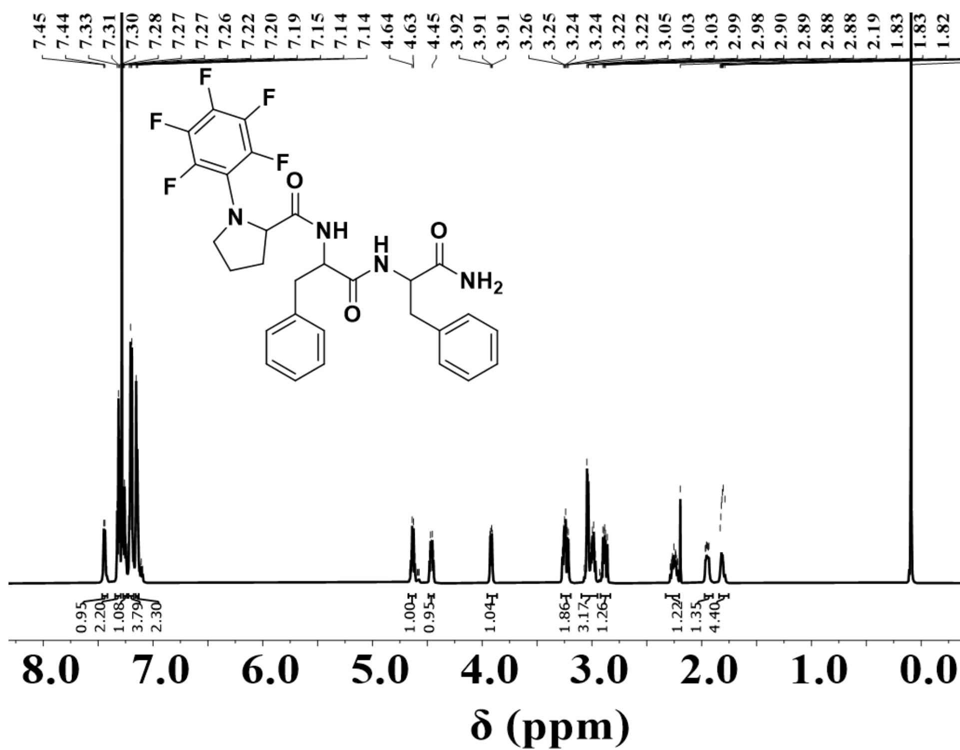
^{1}H NMR spectrum of peptide **D1** in DMSO-d6.



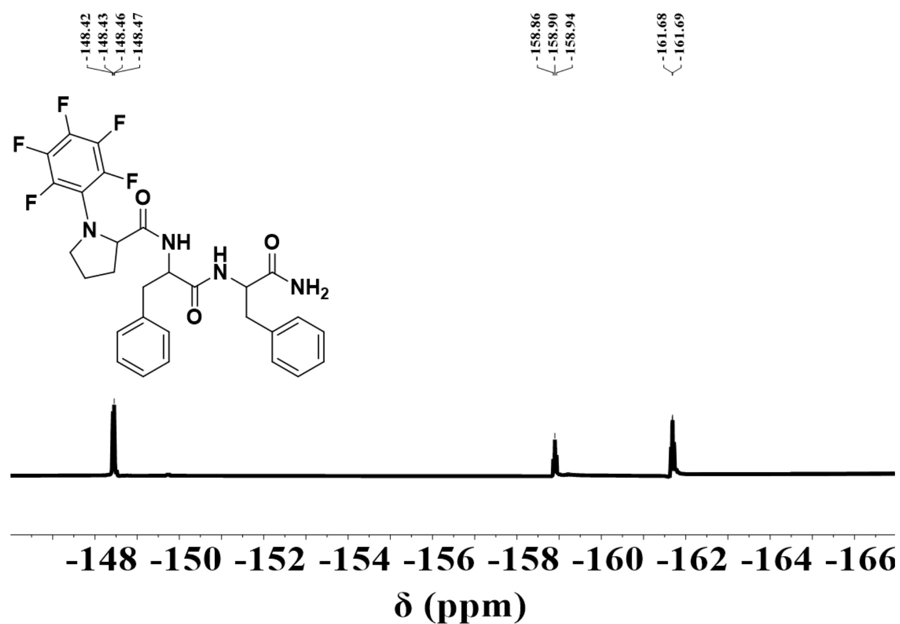
^{19}F NMR spectrum of peptide **D1** in DMSO-d6.



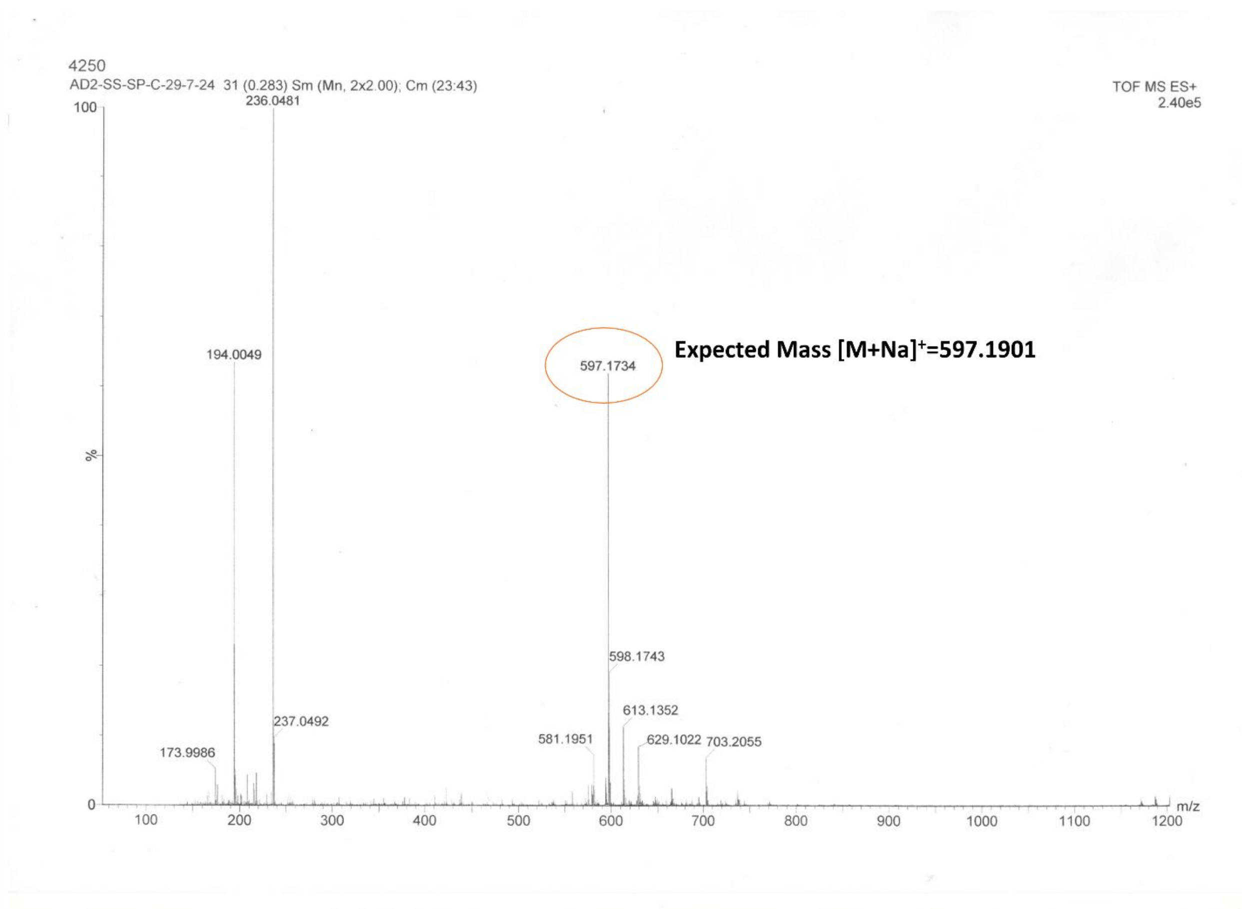
HRMS spectrum of peptide **D1**.



^{1}H NMR spectrum of peptide **D2** in $CDCl_3$



^{19}F NMR spectrum of peptide **D2** in $CDCl_3$.



HRMS spectrum of peptide **D2**.

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