Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Incorporation of CF_3-pseudoprolines into polyproline type II foldamers confers promising biophysical features

Chloé Cayrou, Astrid Walrant, Delphine Ravault, Karine Guitot, Sylvie Noinville, Sandrine Sagan, Thierry Brigaud, Simon Gonzalez,* Sandrine Ongeri, and Grégory Chaume*

TABLE OF CONTENTS

I. MATERIAL AND METHODS

Material. Unless specified otherwise, solvents were used as received. L-Ser-OBn was purchased from ABCR GmbH, PPTS and Pd/C from Alfa Aesar, $SOCl_2$ from Sigma Aldrich and trifluoroacetaldehyde methyl hemiacetal from Apollo Scientific. The reactions were monitored by ^{19}F NMR or by analytical thin-layer chromatography (TLC) using silica gel 60 pre-coated aluminum plates with fluorescent indicator. Visualization was accomplished by irradiation with a UV lamp and/or staining with phosphomolybdic acid or ninhydrin. For SPPS, DIC, Oxyma Pure, piperidine and TIPS were purchased from Iris Biotech, DIPEA and TFA from ABCR, HATU, TBTU from Fluorochem, Fmoc-Pro-OH from Iris Biotech, and finally Ac_2O from Sigma Aldrich. Fmoc-Rink Amide PEG AM resin (loading: 0.35 mmol/g), Fmoc-Rink Amide AM resin (loading: 0.71 mmol/g) were purchased from Iris Biotech and NovaPEG Rink Amide resin (loading: 0.49 mmol/g) from Novabiochem.

NMR. ^{1}H, ^{13}C, ^{19}F NMR spectra were measured on a BRUKER ADVANCE NEO 400 MHz spectrometer. NMR experiments were processed and analyzed with TOPSPIN 4.0.7 program. ^{1}H and ^{13}C chemicals shifts were referenced to the residual solvent signal ($CDCl_3$: 7.26 and 77.2 ppm, CD_3OD: 3.31 and 49.0 ppm, respectively). For water samples, 4,4-dimethyl-4-silapentane-1-sulfate (DSS) was used as an internal standard for ^{1}H chemical shift (0.00 ppm). Carbon assignments were obtained from heteronuclear 2D ^{1}H–^{13}C HSQC spectra. For ^{19}F NMR, C_6F_6 were used as internal standard (-161.64 ppm in $CDCl_3$, -165.37 ppm in CD_3OD and -163.01 ppm in D_2O). Data are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet and m = multiplet), coupling constant (Hz), integration. If nothing is specified for the multiplicity then the signal is a multiplet.

Infrared spectra. Infrared spectra were performed on a Perkin Elmer Spectrum Two FT-IR.

Specific rotations. Specific rotation was measured on a ANTON PAAR MCP 200 polarimeter.

High-Resolution Mass Spectra (HRMS). HRMS were recorded by electron spray (MS-ESI) techniques using QToF Impact HD Bruker spectrometer.

Melting points. Melting points (uncorrected) were measured in a capillary tube on a Buchi Melting Point M560.

UPLC-MS. Waters ACQUITY UPLC coupled to a single quadrupole ESI-MS (Waters 3100 Mass Detector) with a column Luna C18.

HPLC semi-preparative. HPLC semi-preparative purifications were performed with a Luna Omega 5 µm PS C18 100Å 250 X 10 column from Phenomenex on Agilent 1260 Infinity II machine.

Circular dichroism (CD) spectroscopy. Far-UV circular dichroism was performed using a Jasco 815 CD Spectrometer equipped with a Peltier. Spectra were recorded over the 185-270 nm wavelength range by using a 2 mm quartz cuvette. Spectra were obtained as an average of three scans at 4°C with a 20 nm/min scanning speed and a spectrometer bandwidth of 1 nm. The peptide concentration was 100 µM in different solvents, 20 mM sodium phosphate buffer (pH 7), *n*-propanol or a mixture of both.

Cytotoxicity assay. Wild type Chinese Hamster Ovary cells (CHO-K1, WT, ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM F12) supplemented with 10% fetal calf serum (FCS), penicillin (100,000 IU/L), streptomycin (100,000 IU/L), and amphotericin B (1 mg/L) in a humidified atmosphere containing 5% CO_2 at

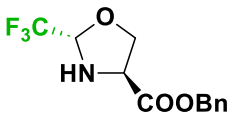
37 °C. CHO-K1 cells were seeded in a 96-well cell culture plate the day prior to the experiments (15,000 for 1 h assay and 7,000 for 24 h). The cells are incubated with 1, 5, 10, 25, 100 µM peptide for 1 h or 24 h (starting from a 1 mM peptide solution in 20 mM sodium phosphate buffer, pH 6.9 at 37°C. Three replicates were made for each condition. Cell viability was then evaluated using the CCK-8 kit (Dojindo), according to the supplyer's instructions. After peptide incubation and cell washing with HBSS, a 10% solution of CCK-8 reagent in DMEM was added. After 2 h incubation at 37°C, the absorbance at 450 nm was determined using a microplate reader (FLUOstar, BMG Labtech) DMEM F12 was used as 0% cytotoxicity control and 0.1% Triton X-100 in DMEM F12 as a 100% cytotoxicity control.

Stability test with Pronase. Pronase powder was purchased from Sigma-Aldrich. Hexamers **6** and **7** (final concentration: 200 µM) were diluted in a solution of Pronase (final concentration: 2.5 µg/mL) in 50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (w/v) Brij 35, pH 7.5 and placed under stirring at 37°C. The amount of intact peptide was quantified over time (5 min, 15 min, 30 min, 1 h, 1 h 30, 2 h, 3 h, 24 h and 48 h) by HPLC and by ^{19}F NMR in the case of hexamer **7**. Analyses were done in triplicate.

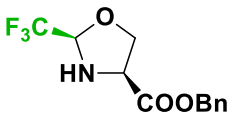
For HPLC monitoring, at each time point 20 µL of the enzymatic reaction was transferred in a HPLC vial containing 20 µL of 1% TFA solution in water. 10 µL of this quenched reaction was injected and the sample was analyzed with a gradient of 10 to 60% of acetonitrile in 10 min. The UV trace at 214 nm was used for peptide quantification. The area of peaks at 4.5 min (hexamer **6**) and 6.9 min (hexamer **7**) were integrated and used for quantification.

To monitor the degradation by ^{19}F NMR, analyses were conducted at 37°C in 5 mm tube with a final volume of 500 µL containing buffer and 10% of D_2O. ^{19}F NMR spectra were recorded at 37°C with a total of 64 scans for each spectrum. The data were multiplied with an exponential window function with 3 Hz line broadening prior to Fourier transformation. All fluorine signals were integrated and the residual TFA present in the sample was used as an internal standard. The ratio of fluorine signal integration from peptide over TFA was used to quantify the amount of peptide during time.

II. SYNTHESIS OF $CF_3\Psi$PRO



***trans*-$CF_3\Psi$Pro-OBn**



***cis*-$CF_3\Psi$Pro-OBn**

To a solution of L-serine benzyl ester hydrochloride (5.0 g, 21.58 mmol, 1.0 equiv.) in toluene (70 mL) were added trifluoroacetaldehyde methyl hemiacetal (2.17 mL, 22.67 mmol, 1.05 equiv.) and PPTS (543 mg, 2.16 mmol, 0.1 equiv.) at room temperature. The reaction mixture was stirred at 90°C for 1 h. Then a Dean Stark apparatus was added and the reaction mixture was heated at 140°C for 2 h 30. Trifluoroacetaldehyde methyl hemiacetal (1.0 mL, 10.79 mmol, 0.5 equiv.) was added and the reaction was left stirring for additional 1 h 30 under the same condition. The reaction mixture was filtered and evaporated under reduced pressure. The crude was purified using flash chromatography on silica gel using a gradient of cyclohexane/ethyl acetate as eluent (from (100/0) to (70/30) in 25 min then from (70/30) to (60/40) in 5 min and finally from (60/40) to (0/100) in 5 min) to give 0.48 g (8% yield) of the *cis*-$CF_3\Psi$Pro-OBn as white solid and 4.18 g (70% yield) of the *trans*-$CF_3\Psi$Pro-OBn as a colorless oil**.**

***cis*-$CF_3\Psi$Pro-OBn:** white solid; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.05 (m, 1 H, NH), 4.10-4.20 (m, 3 H, H_α, H_β-Ha and H_β-Hb), 4.95 (dq, J = 8.2, 5.0 Hz, 1 H, H_δ), 5.21 (s, 2 H, CH_2 OBn-Ha and CH_2 OBn-Hb), 7.34-7.37 (m, 5 H, OBn arom.); **^{13}C NMR** (100.5 MHz, $CDCl_3$): δ 59.0 (CH, C_α), 67.7 (CH_2, CH_2 OBn), 69.0 (CH_2, C_β), 87.9 (q, J = 34.4 Hz, CH, C_δ), 122.9 (q, J = 283.1 Hz, C, CF_3), 128.5 (CH, OBn arom.), 128.8 (CH, OBn arom.), 128.8 (CH, OBn arom.), 135.2 (C, OBn arom.), 170.6 (C, C=O); **^{19}F NMR** (376.2 MHz, $CDCl_3$): δ -80.7 (d, J = 5.6 Hz, CF_3).

***trans*-$CF_3\Psi$Pro-OBn:** Colorless oil; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.34 (m, 1 H, NH), 3.84 (dd, J = 7.8, 6.4 Hz, 1 H, H_β-Ha), 4.11 (ddd, J = 7.8, 7.3, 6.4 Hz, 1 H, H_α), 4.29 (t, J = 7.8 Hz, 1 H, H_β-Hb), 5.09 (dq, J = 8.1, 5.5 Hz, 1 H, H_δ), 5.22 (s, 2 H, CH_2 OBn-Ha and CH_2 OBn-Hb), 7.31-7.41 (m, 5H, OBn arom.); **^{13}C NMR** (100.5 MHz, $CDCl_3$): δ 58.8 (CH, C_α), 68.0 (CH_2, CH_2OBn), 69.8 (CH_2, C_β), 87.9 (q, J = 34.2 Hz, CH, C_δ), 123.3 (q, J = 283.3 Hz, C, CF_3), 128.5 (CH, OBn arom.), 128.9 (CH, OBn arom.), 128.9 (CH, OBn arom.), 134.9 (C, OBn arom.), 171.4 (C, C=O); **^{19}F NMR** (376.2 MHz, $CDCl_3$): δ -81.8 (d, J = 5.5 Hz, CF_3).

The obtained NMR data are in accordance with the literature.[1]

III. SYNTHESIS OF THE BUILDING BLOCK FOR SPPS

III.1 General procedure

III.1.1 General procedure A for acyl chloride synthesis

To a 0.2 M solution of Fmoc-Pro-OH (1.0 equiv.) in dried dichloromethane under argon, was added thionyl chloride (13.8 equiv.). The reaction mixture was sonicated at room temperature until the complete disappearance of the precipitate (from 30 min to 1 h) and then evaporated under vacuum. Co-evaporations with pentane were performed in order to remove the excess of thionyl chloride to give the Fmoc-Pro-Cl as a white solid directly used without further purification.

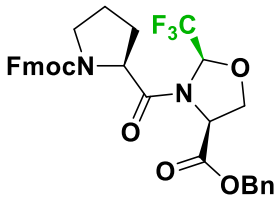
III.1.2 General procedure B for peptide coupling using acyl chloride activation

To a solution of fluorinated *cis*-CF_3ΨPro-OBn or *trans*-CF_3ΨPro-OBn pseudoproline (1 equiv.) in dried dichloromethane was added Fmoc-L-Pro-Cl or Fmoc-D-Pro-Cl (1.1 equiv. or 3.0 equiv.). The reaction mixture was stirred overnight at room temperature under argon atmosphere. After evaporation under vacuum, a purification by chromatography on silica gel gave pure products Fmoc-L-Pro-CF_3ΨPro-OBn and Fmoc-D-Pro-CF_3ΨPro-OBn in 76 and 97% yield, respectively.

III.1.3 General procedure C for the benzyl ester deprotection by hydrogenation

To a solution of protected Fmoc-L-Pro-CF_3ΨPro-OBn or Fmoc-D-Pro-CF_3ΨPro-OBn dipeptide (1 equiv.) in methanol was added 10% in weight palladium on carbon, 10% Pd. The mixture was stirred under 1 bar of hydrogen gas for 30 min to 50 min. The reaction was monitored by TLC (cyclohexane/ethyl acetate, 70/30) and was stopped when the starting material disappeared. Then, palladium was filtered over Celite® and methanol was removed under reduced pressure to give pure building blocks Fmoc-L-Pro-CF_3ΨPro-OH and Fmoc-D-Pro-CF_3ΨPro-OH as a white solid used directly without further purification in 97% and 100% yield, respectively.

III.2 Synthesis of the building block L-L, Fmoc-L-Pro-CF_3ΨPro-OH



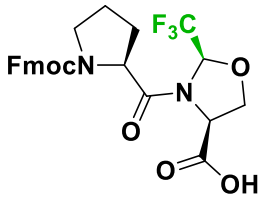
Fmoc-L-Pro-CF_3ΨPro-OBn

The reaction was performed following the general procedure A and B starting with Fmoc-L-Pro-OH (405 mg, 1.20 mmol, 1.1 equiv.), *cis*-CF_3ΨPro-OBn (300 mg, 1.09 mmol, 1.0 equiv.) in dried dichloromethane (7 mL). Purification by chromatography on silica gel (cyclohexane/ethyl acetate, 80/20 as eluent) give a pure dipeptide Fmoc-L-Pro-CF_3ΨPro-OBn (492 mg, 76%) as a 16/84 mixture of Fmoc *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

White solid; **mp** 56-59°C; **Rf** 0.17 (80/20 cyclohexane/ethyl acetate); **$[\alpha]^{20}_D$** -68.9° (*c* 1.0, $CHCl_3$); **IR** (neat) 3052, 2949, 2884, 1774, 1683, 1422, 1281, 1121, 760 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): (*84/16 mixture of rotational isomers*) *(major conformer) δ* 1.95 (m, 1 H, H_γ Pro-Ha), 2.03 (m, 1 H, H_β Pro-Ha), 2.14 (m, 1 H, H_β Pro-Hb), 2.28 (m, 1 H, H_γ Pro-Hb), 3.54 (m, 1 H, H_δ Pro-Ha), 3.69 (m, 1 H, H_δ Pro-Hb), 4.23 (t, *J* = 7.2 Hz, 1 H, CH Fmoc), 4.29-4.40 (m, 4 H, H_β CF_3ΨPro-Ha, CH_2 Fmoc-Ha, CH_2 Fmoc-Hb and H_α Pro), 4.51 (dd, *J* = 8.7, 1.2 Hz, 1 H, H^β ΨPro-Hb), 5.12 (m, 1 H, H_α CF_3ΨPro), 5.15 (d, *J* = 12.2 Hz, 1 H, CH_2 OBn-Ha), 5.21 (d, *J* = 12.2 Hz, 1 H, CH_2 OBn-Hb), 6.21 (q, *J* = 5.0 Hz, 1 H, H_δ CF_3ΨPro), 7.28–7.37 (m, 7 H, Fmoc arom. and OBn arom.), 7.40 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.58 (dd, *J* = 7.8, 7.3 Hz, 2 H, Fmoc arom.), 7.77 (d, *J* = 7.8 Hz, 2 H, Fmoc arom.); *(minor conformer) δ* 1.77 (m, 1 H, H_γ Pro-Ha), 1.85 (m, 1 H, H_β Pro-Ha), 2.02 (m, 1 H, H_β Pro-Hb), 2.04 (m, 1 H, H_γ Pro-Hb), 3.44 (m, 1 H, H_δ Pro-Ha), 3.64 (m, 1 H, H_δ Pro-Hb), 4.00 (m, 1 H, H_α Pro), 4.13-4.19 (m, 2 H, H_β CF_3ΨPro), 4.23 (t, *J* = 5.5 Hz, 1 H, CH Fmoc), 4.42 (dd, *J* = 10.9, 5.5 Hz, 1 H, CH_2 Fmoc-Ha), 4.66 (dd, *J* = 10.9, 5.5 Hz, 1 H, CH_2 Fmoc-Hb), 4.95 (t, *J* = 8.0 Hz, 1 H, CF_3ΨPro), 5.07 (m, 1 H, H_δ CF_3ΨPro), 5.09 (d, *J* = 12.2 Hz, 1 H, CH_2 OBn-Ha), 5.15 (d, *J* = 12.2 Hz, 1 H, CH_2 OBn-Hb), 7.28–7.43 (m, 9 H, Fmoc arom., OBn arom.), 7.49 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.77 (d, *J* = 7.8 Hz, 2 H, Fmoc arom.); **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K): (*84/16 mixture of rotational isomers*) (*major conformer*) *δ* 25.0 (CH_2, C_γ Pro), 30.4 (CH_2, C_β Pro), 47.1 (CH_2, C_δ Pro), 47.2 (CH, CH Fmoc), 57.0 (CH, C_α CF_3ΨPro), 58.5 (CH, C_α Pro), 67.7 (CH_2, CH_2 OBn), 67.9 (CH_2, CH_2 Fmoc), 69.1 (CH_2, C_β CF_3ΨPro), 85.2 (q, *J* = 34.9 Hz, CH, C_δ CF_3ΨPro), 120.1 (2 x CH, Fmoc arom.), 123.0 (q, *J* = 286.6 Hz, C, CF_3), 125.2 (CH, Fmoc arom), 125.3 (CH, Fmoc arom), 127.2 (2 × CH, Fmoc arom.), 127.9 (2 x CH, Fmoc arom.), 128.3 (2 x CH, OBn arom.), 128.6 (CH, OBn arom.), 128.7 (2 x CH, OBn arom.), 135.1 (C, OBn arom.), 141.4 (2 x C, Fmoc arom.), 143.8 (C, Fmoc arom.), 144.0 (C, Fmoc arom.), 155.4 (C, C=O), 168.3 (C, C=O), 172.9 (C, C=O); *(minor conformer) δ* 23.2 (CH_2, C_γ Pro), 31.7 (CH_2, C_β Pro), 47.5 (CH_2, C_δ Pro), 47.7 (CH, CH Fmoc), 56.7 (CH, Cα CF_3ΨPro), 57.8 (CH, Cα Pro), 66.9 (CH_2, CH_2 Fmoc), 67.7 (CH_2, CH_2 OBn), 68.5 (CH_2, Cβ CF_3ΨPro), 84.4* (q, *J* = 34.9 Hz, CH, Cδ CF_3ΨPro), 120.2 (2 x CH, Fmoc arom.), (C, CF_3)**, 124.7 (CH, Fmoc arom.), 124.9 (CH, Fmoc arom.), 127.2 (2 x CH, Fmoc arom.), 128.4 (2 x CH, OBn arom.), 128.6 (CH, OBn arom.), 128.7 (2 × CH, OBn arom.), 135.1 (C, OBn arom.), 141.3 (2 x C, Fmoc arom.), 143.8 (C, Fmoc arom.), 144.0 (C, Fmoc arom.), 155.4 (C, C=O), 168.0 (C, C=O), 172.2 (C, C=O); **^{19}F NMR** (376.2 MHz, $CDCl_3$, 298 K): (*84/16 mixture of rotational isomers*) (*major conformer*) *δ* -78.63 (d, *J* = 4.7 Hz, CF_3), (*minor conformer*) *δ* -78.35 ppm (d, *J* = 5.0 Hz, CF_3); **HRMS** (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{32}H_{29}F_3N_2O_6Na$: 617.1870, found: 617.1871.

* Some peaks are not visible in ^{13}C. Assignment was possible using 2D NMR (HSQC and HMQC).

** not observed



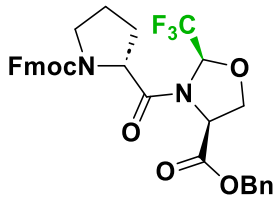
Fmoc-L-Pro-CF_3ΨPro-OH

The reaction was performed following the general procedure C starting with Fmoc-L-Pro-*cis*-CF_3ΨPro-OBn (3.87 g, 6.51 mmol, 1.0 equiv.) in methanol (190 mL) for 50 min to give pure building block Fmoc-L-Pro-CF_3ΨPro-OH (3.28 g, quantitative) as a 25/75 mixture of Fmoc *cis*/*trans* rotational isomers in CD_3OD at 298 K.

White solid; **mp** 141°C; **Rf** 0.13 (90/10 dichloromethane/methanol); **[α]$^{20}_D$** -52.45 (*c* 1.0, MeOH); **IR** (neat) 3450, 2982, 1751, 1687, 1425, 1287, 1153, 1121, 741 cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD, 298 K) (*75/25 mixture of rotational isomers*) *(major conformer) δ* 1.91 (m, 1 H, H_γ Pro-Ha), 2.04 (m, 1 H, H_β Pro-Ha), 2.13-2.26 (m, 2 H, H_β Pro-Hb and H_γ Pro-Hb), 3.39-3.63 (m, 2 H, H_δ Pro), 4.12-4.38 (m, 4 H, CH Fmoc, CH_2 Fmoc-Ha, H_β CF_3ΨPro), 4.39-4.60 (m, 2 H, H_α Pro and CH_2 Fmoc-Hb), 4.99 (t, *J* = 8.1 Hz, 1 H, H_α CF_3ΨPro), 6.28 (m, 1 H, H_δ CF_3ΨPro), 7.31 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.39 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.61 (t, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.80 (d, *J* = 7.5 Hz, 2 H, Fmoc arom.); *(minor conformer) δ* 1.89-2.11 (m, 4 H, H_γ Pro and H_β Pro), 3.39-3.63 (m, 2 H, H_δ Pro), 4.12-4.38 (m, 4 H, CH Fmoc, H_α Pro, CH_2 Fmoc), 4.39-4.60 (m, 2 H, H_β CF_3ΨPro), 4.89 (m, 1 H, H_α CF_3ΨPro), 5.51 (m, 1 H, H_δ CF_3ΨPro), 7.25-7.43 (m, 4 H, Fmoc arom.), 7.49-7.59 (m, 2 H, Fmoc arom.), 7.74-7.89 (m, 2 H, Fmoc arom.); **^{13}C NMR** (100.5 MHz, CD_3OD, 298 K): (*75/25 mixture of rotational isomers*) *(major conformer) δ* 25.7 (CH_2, C_γ Pro), 31.3 (CH_2, C_β Pro), 48.0 (CH_2, C_δ Pro), 48.3 (CH, CH Fmoc), 58.4 (CH, C_α CF_3ΨPro), 59.6 (CH, C_α Pro), 68.8 (CH_2, C_β CF_3ΨPro), 70.2 (CH_2, CH_2 Fmoc), 86.2 (q, *J* = 35.0 Hz, CH, C_δ CF_3ΨPro), 120.9 (CH, Fmoc arom.), 120.9 (CH, Fmoc arom.), 124.4 (q, *J* = 286.3 Hz, C, CF_3), 126.1 (2 x CH, Fmoc arom.), 128.2 (2 x CH, Fmoc arom.), 128.8 (2 x CH, Fmoc arom.), 142.5 (C, Fmoc arom.), 142.6 (C, Fmoc arom.), 145.1 (C, Fmoc arom.), 145.2 (C, Fmoc arom.), 156.7 (C, C=O), 171.4 (C, C=O), 174.8 (C, C=O); *(minor conformer) δ* 24.2 (CH_2, C_γ Pro), 32.5 (CH_2, C_β Pro), 48.3 (CH, CH Fmoc), 48.4 (CH_2, C_δ Pro), 58.3 (CH, C_α CF_3ΨPro), 59.0 (CH, C_α Pro), 68.3 (CH_2, C_β CF_3ΨPro), 69.8 (CH_2, CH_2 Fmoc), 85.7 (m, CH, C_δ CF_3ΨPro), 121.1 (2 x CH, Fmoc arom.), (C, CF_3)*****, 125.0 (CH, Fmoc arom.), 126.2 (CH, Fmoc arom.), 128.0 (2 x CH, Fmoc arom.), 128.7 (CH, Fmoc arom.), 128.9 (CH, Fmoc arom.), 142.4 (2 x C, Fmoc arom.), 145.2 (C, Fmoc arom.), 145.3 (C, Fmoc arom.), 155.6 (C, C=O), 171.2 (C, C=O), 174.3 (C, C=O); **^{19}F NMR** (CD_3OD, 376.2 MHz, 298 K): (*75/25 mixture of rotational isomers*) *(major conformer) δ* -79.98 (d, *J* = 5.2 Hz, CF_3); *(minor conformer) δ* -79.41 (d, *J* = 4.5 Hz, CF_3); **HRMS** (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{25}H_{23}F_3N_2O_6Na$: 527.1400, found: 527.1402.

***** *not observed*

III.3 Synthesis of the building block D-L, Fmoc-D-Pro-CF_3ΨPro-OH

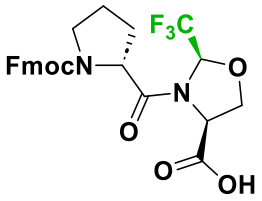


Fmoc-D-Pro-CF_3ΨPro-OBn

The reaction was performed following the general procedure A and B starting with Fmoc-D-Pro-OH (2.54 g, 7.56 mmol, 3.0 equiv.), *cis*-CF_3ΨPro-OBn (700 mg, 2.52 mmol, 1.0 equiv.) in dried dichloromethane (16 mL). Purification by chromatography on silica gel (70/30 cyclohexane/ethyl acetate as eluent) gave pure dipeptide Fmoc-D-Pro-CF_3ΨPro-OBn (1.46 g, 97%) as a 10/90 mixture of Fmoc *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

White solid; **mp** 56-59°C; **Rf** 0.14 (80/20 cyclohexane/ethyl acetate); **$[\alpha]^{20}_D$** -71.7° (*c* 0.59, $CHCl_3$); **IR** (neat) 3056, 2957, 1751, 1687, 1421, 1183, 1151, 741 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): (*90/10 mixture of rotational isomers*) *(major conformer) δ* 1.78-2.04 (m, 3 H, H_β Pro and H_γ Pro-Ha), 2.23 (m, 1 H, H_γ Pro-Hb), 3.51 (m, 1 H, H_δ Pro-Ha), 3.65 (m, 1 H, H_δ Pro-Hb), 4.18-4.39 (m, 4 H, CH Fmoc, H_α Pro, H_β CF_3ΨPro), 4.45-4.60 (m, 2 H, CH_2 Fmoc), 5.21 (dd, 2 H, CH_2 OBn), 5.54 (dd, *J* = 8.2, 4.7 Hz, 1 H, H_α CF_3ΨPro), 5.99 (q, *J* = 5.0 Hz, 1 H, H_δ CF_3ΨPro), 7.27–7.45 (m, 9 H, Fmoc arom. and OBn arom.), 7.56 (m, 2 H, Fmoc arom.), 7.76 (d, *J* = 7.6 Hz, 2 H, Fmoc arom.); *(minor conformer: too low to be observed)*; **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K): (*90/10 mixture of rotational isomers*) *(major conformer) δ* 25.0 (CH_2, C_γ Pro), 30.7 (CH_2, C_β Pro), 47.2 (CH_2, C_δ Pro and CH, CH Fmoc), 58.3 (2 x CH, C_α Pro and C_α CF_3ΨPro), 67.8 (CH_2, C_β CF_3ΨPro), 68.4 (CH_2, CH_2 OBn), 70.6 (CH_2, CH_2 Fmoc), 84.2 (q, *J* = 36.2 Hz, CH, C_δ CF_3ΨPro), 120.2 (2 x CH, Fmoc arom.), (C, CF_3)*, 125.2 (CH, Fmoc arom), 125.3 (CH, Fmoc arom), 127.2 (2 × CH, Fmoc arom.), 127.9 (2 x CH, Fmoc arom.), 128.8 (CH, OBn arom.), 128.9 (2 x CH, OBn arom.), 129.0 (2 x CH, OBn arom.), 134.7 (C, OBn arom.), 141.4 (2 x C, Fmoc arom.), 143.8 (C, Fmoc arom.), 144.1 (C, Fmoc arom.), 155.3 (C, C=O), 169.1 (C, C=O), 175.0 (C, C=O); *(minor conformer: too low to be observed)*; **^{19}F NMR** (376.2 MHz, $CDCl_3$, 298 K): (*90/10 mixture of rotational isomers*) (*major conformer*) *δ* -79.47 (d, *J* = 5.0 Hz, CF_3), (*minor conformer*) *δ* -79.41 ppm (d, *J* = 5.0 Hz, CF_3); **HRMS** (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{32}H_{29}F_3N_2O_6Na$: 617.1870, found: 617.1871.

not observed



Fmoc-D-Pro-CF_3ΨPro-OH

The reaction was performed following the general procedure C starting with Fmoc-D-Pro-*cis*-CF_3ΨPro-OBn (970 mg, 1.63 mmol, 1.0 equiv.) in methanol (30 mL) for 30 min to give pure building block Fmoc-D-Pro-CF_3ΨPro-OH (796 mg, 97%) as a 30/70 mixture of Fmoc *cis*/*trans* rotational isomers in CD_3OD at 298 K.

White solid; **mp** 202°C; **Rf** 0.13 (90/10 dichloromethane/methanol); **[α]$^{20}_D$** -50.24 (*c* 1.0, MeOH); **IR** (neat) 3333, 2972, 1745, 1683, 1423, 1151, 1119, 740 cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD, 298 K) (*70/30 mixture of rotational isomers*) *(major conformer) δ* 1.90 (m, 1 H, H_γ Pro-Ha), 2.03-2.20 (m, 2 H, H_β Pro-Ha and H_γ Pro-Hb), 2.28 (m, 1 H, H_β Pro-Hb), 3.43-3.58 (m, 2 H, H_δ Pro), 4.24 (m, 1 H, CH Fmoc), 4.30-4.41 (m, 2 H, H_β CF_3ΨPro), 4.47-4.56 (m, 3 H, CH_2 Fmoc and H_α Pro), 5.39 (t, *J* = 6.7 Hz, 1 H, H_α CF_3ΨPro), 5.92 (q, *J* = 5.1 Hz, 1 H, H_δ CF_3ΨPro), 7.31 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.40 (t, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.62 (t, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.80 (d, *J* = 7.8 Hz, 2 H, Fmoc arom.); *(minor conformer) δ* 1.79 (m, 1 H, H_γ Pro-Ha), 1.96-2.07 (m, 2 H, H_β Pro-Ha and H_γ Pro-Hb), 2.25 (m, 1 H, H_β Pro-Hb), 3.40-3.45 (m, 2 H, H_δ Pro), 3.98 (m, 1 H, CH_2 Fmoc-Ha), 4.17-4.38 (m, 4 H, CH Fmoc, CH_2 Fmoc-Hb, H_α Pro and H_α CF_3ΨPro), 4.44 (dd, *J* = 11.2, 5.1 Hz, 1 H, H_β CF_3ΨPro-Ha), 4.79 (dd, *J* = 11.2, 5.6 Hz, 1 H, H_β CF_3ΨPro-Hb), 5.78 (q, *J* = 5.1 Hz, 1 H, H_δ CF_3ΨPro), 7.27-7.44 (m, 4 H, Fmoc arom.), 7.57 (t, *J* = 7.2 Hz, 2 H, Fmoc arom.), 7.75-7.83 (m, 2 H, Fmoc arom.); **^{13}C NMR** (100.5 MHz, CD_3OD, 298 K): (*70/30 mixture of rotational isomers*) *(major conformer) δ* 25.7 (CH_2, C_γ Pro), 31.9 (CH_2, C_β Pro), 48.2 (CH_2, C_δ Pro), 48.4 (CH, CH Fmoc), 59.4 (CH, C_α CF_3ΨPro), 59.6 (CH, C_α Pro), 68.9 (CH_2, C_β CF_3ΨPro), 71.8 (CH_2, CH_2 Fmoc), 85.5 (q, *J* = 35.8 Hz, CH, C_δ CF_3ΨPro), 121.0 (2 x CH, Fmoc arom.), 124.3 (q, *J* = 286.3 Hz, C, CF_3), 126.1 (CH, Fmoc arom.), 126.2 (CH, Fmoc arom.), 128.2 (2 x CH, Fmoc arom.), 128.9 (2 x CH, Fmoc arom.), 142.6 (2 x C, Fmoc arom.), 145.1 (C, Fmoc arom.), 145.2 (C, Fmoc arom.), 156.8 (C, C=O), 171.9 (C, C=O), 175.8 (C, C=O); *(minor conformer) δ* 24.2 (CH_2, C_γ Pro), 32.9 (CH_2, C_β Pro), 48.4 (CH_2, CH Fmoc), 48.8 (CH, C_δ Pro), 58.8 (2 x CH, C_α Pro and C_α CF_3ΨPro), 67.9 (CH_2, C_β CF_3ΨPro), 71.8 (CH_2, CH_2 Fmoc), (CH, C_δ CF_3ΨPro)*, 121.0 (CH, Fmoc arom.), 121.2 (CH, Fmoc arom.), (C, CF_3)*, 125.7 (CH, Fmoc arom.), 126.2 (CH, Fmoc arom.), 128.4 (CH, Fmoc arom.), 128.9 (CH, Fmoc arom.), 129.1 (CH, Fmoc arom.), 128.9 (CH, Fmoc arom.), 142.6 (2 x C, Fmoc arom.), 144.7 (C, Fmoc arom.), 145.5 (C, Fmoc arom.), 155.7 (C, C=O), 171.3 (C, C=O), 175.2 (C, C=O); **^{19}F NMR** (CD_3OD, 376.2 MHz, 298 K): (*70/30 mixture of rotational isomers*) *(major conformer) δ* -80.63 (d, *J* = 4.7 Hz, CF_3); *(minor conformer) δ* -80.55 (d, *J* = 4.6 Hz, CF_3); **HRMS** (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{25}H_{23}F_3N_2O_6Na$: 527.1400, found: 527.1402.

not observed

IV. PEPTIDE SYNTHESIS OF OLIGOMERS 1-14 BY SPPS

IV.1 General procedure

All peptides were synthesized in a plastic syringe on 0.1 mmol scale using standard Fmoc-based solid phase peptide synthesis with Rink Amide resin. Three different loadings of resins were used during this work: Fmoc-Rink Amide PEG AM resin (loading: 0.35 mmol/g, 286 mg, 0.1 mmol scale), NovaPEG Rink amide resin (loading: 0.49 mmol/g, 204 mg, 0.1 mmol scale) and Fmoc-Rink Amide AM resin (loading: 0.71 mmol/g, 141 mg, 0.1 mmol scale).

After each Fmoc cleavage or coupling, the completion of the reaction was checked by performing a Kaiser Test (for primary amine) or a Chloranil Test (secondary amine). Peptides were cleaved from the resin thanks to a TFA/TIPS/H_2O (95/2.5/2.5, *v/v/v*) solution for 2 h. After filtration, the solvent was evaporated under air flow. Then, the crude peptides were dissolved in water, lyophilized and then purified by HPLC semi-preparative and all possess UPLC purities >95%.

IV.1.1 General procedure D for the manual synthesis of peptides

The loading of the resin was performed using Fmoc-L-Pro-OH (3 eq.) or Fmoc-D-Pro-OH (3 eq.) with TBTU (3 eq.) and DIPEA (5 eq.) in DMF (5 mL) for 1 h at r.t. The couplings of natural amino acids were achieved with Fmoc-L-Pro-OH (3 eq.) or Fmoc-D-Pro-OH (3 eq.) with HATU (3 eq.) and DIPEA (5 eq.) in DMF (5 mL) fo 1 h at r.t. Building blocks Fmoc-L-Pro-CF_3ΨPro-OH (2 eq.) or Fmoc-D-Pro-CF_3ΨPro-OH (2 eq.) were coupled using DIC (3 eq.) and Oxyma (3 eq.) in DMF (5 mL) at 75°C under microwave activation (100 W) for 10-20 min. All Fmoc deprotections were carried out by piperidine in DMF (20%, v/v) for 5 then 15 min at r.t. If needed, the acetylation was performed using Ac_2O (10 eq.) and DIPEA (10 eq.) in DMF (5 mL) for 2 x 30 min at r.t.

IV.1.2 General procedure E for the automated synthesis of peptides with LibertyBlue® (CEM)

Couplings of natural amino acid Fmoc-L-Pro-OH (5 eq., single coupling) were achieved with DIC (5 eq.), Oxyma (5 eq.) in DMF using standard coupling conditions under microwave activation (34 s at 75°C (170 W) then 210 s at 90°C (30 W)). Couplings of the building block Fmoc-L-Pro-CF_3ΨPro-OH was achieved using 2.5 eq. of the building block with DIC (5 eq.) and Oxyma (5 eq.) in DMF under microwave activation (10 min at 75°C (130 W) then 5 min at 90°C (100 W)). Fmoc deprotection were carried out by piperidine (20%) in DMF under microwave activation (30 s at 75°C (155 W) then 100 s at 90°C (30 W)). If needed, the acetylation was performed manually using Ac_2O (10 eq.) and DIPEA (10 eq.) in DMF (5 mL) for 2 x 30 min at r.t.

IV.2 Peptide synthesis

The synthesis conditions and yields of the synthesized peptides are listed in the following table.

Peptide		Resin loading	General procedure	Condition of purification*	Mass (yield)
Trimers	**1**	0.71 mmol/g	D	5-30% in 15 min	24.9 mg (71%)
	2	0.71 mmol/g	D	5-30% in 15 min	13.5 mg (30%)
	3	0.71 mmol/g	D	10-30% in 15 min	38.7 mg (93%)
	4	0.71 mmol/g	D	10-40% in 15 min	31.2 mg (74%)
	5	0.71 mmol/g	D	5-40% in 15 min	19.2 mg (46%)
Hexamers	**6**	0.49 mmol/g	D	10-40% in 15 min	17.0 mg (27%)
	7	0.49 mmol/g	E	10-90% in 20 min	24.4 mg (31%)
	8	0.49 mmol/g	E	10-50% in 15 min	33.7 mg (35%)**
	9	0.49 mmol/g	D	10-50% in 20 min	20.8 mg (16%)**
Nonamers	**10**	0.35 mmol/g	E	10-70% in 15 min	41.9 mg (25%)
	11	0.35 mmol/g	D	10-90% in 15 min	26.5 mg (26%)
	12	0.35 mmol/g	D	10-90% in 15 min	55.1 mg (55%)
	13	0.35 mmol/g	D	10-90% in 15 min	46.0 mg (46%)
	14	0.35 mmol/g	E	25-65% in 20 min	51.5 mg (45%)

*Gradient: percentage start-end of ACN + 0.1%. The co-solvent is H_2O + 0.1% TFA.

** Yield taking into account the TFA salt.

*Table S1: Protocols for the synthesis of oligomers **1**-**14** and their corresponding yields*

IV.3 HRMS values of the synthesized peptides

Peptide		Sequence	HRMS		
			Ion species	Calculated	Found
Trimers	**1**	Ac-[L-Pro]$_3$-NH$_2$	$[M+Na]^+$	373.1846	373.1850
	2	Ac-L-Pro-CF_3ΨPro-L-Pro-NH$_2$	$[M+Na]^+$	443.1513	443.1515
	3	Ac-D-Pro-CF_3ΨPro-L-Pro-NH$_2$	$[M+Na]^+$	443.1513	443.1515
	4	Ac-L-Pro-CF_3ΨPro-D-Pro-NH$_2$	$[M+Na]^+$	443.1513	443.1518
	5	Ac-D-Pro-CF_3ΨPro-D-Pro-NH$_2$	$[M+Na]^+$	443.1513	443.1516
Hexamers	**6**	Ac-[L-Pro]$_6$-NH$_2$	$[M+Na]^+$	664.3429	664.3436
	7	Ac-[L-Pro-CF_3ΨPro-L-Pro]$_2$-NH$_2$	$[M+Na]^+$	804.2762	804.2762
	8	H-[L-Pro-CF_3ΨPro-L-Pro]$_2$-NH$_2$	$[M+Na]^+$	762.2659	762.2657
	9	H-[L-Pro-CF_3ΨPro-L-Pro]$_2$-OH	$[M+H]^+$	741.2677	741.2665
Nonamers	**10**	Ac-[L-Pro]$_9$-NH$_2$	$[M+Na]^+$	955.5012	955.5023
	11	Ac-L-Pro-CF_3ΨPro-L-Pro-[Pro]$_6$-NH$_2$	$[M+H]^+$	1003.4859	1003.4879
	12	Ac-[Pro]$_3$-L-Pro-CF_3ΨPro-L-Pro-[Pro]$_3$-NH$_2$	$[M+H]^+$	1003.4859	1003.4880
	13	Ac-[Pro]$_6$-L-Pro-CF_3ΨPro-L-Pro-NH$_2$	$[M+H]^+$	1003.4859	1003.4879
	14	Ac-[L-Pro-CF_3ΨPro-L-Pro]$_3$-NH$_2$	$[M+Na]^+$	1165.4014	1165.4011

*Table S2: HRMS values of oligomers **1**-**14***

IV.4 UV-purity of peptides 1-14 by UPLC-MS

The UV-purity of the synthesized peptides was checked by UPLC-MS and are listed in the following table.

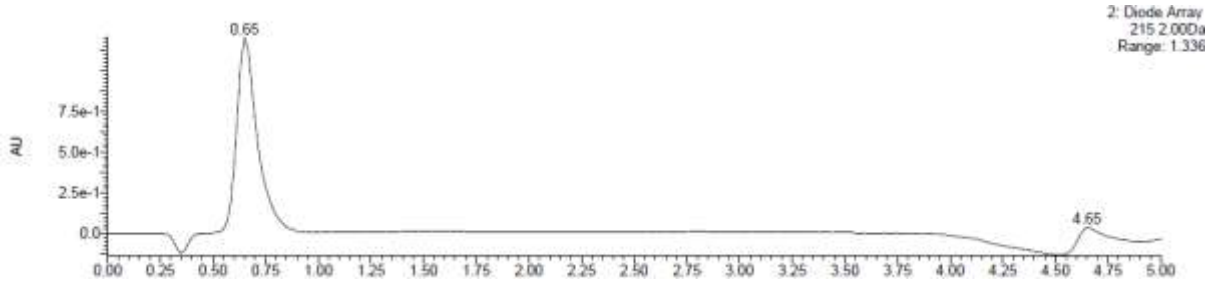
Peptide		Sequence	Purity	UPLC/MS	
				Gradient*	Rt (min)
Trimers	**1**	Ac-[L-Pro]$_3$-NH$_2$	> 95%	10-50	0.65
	2	Ac-L-Pro-*cis*-CF$_3$ΨPro-L-Pro-NH$_2$	> 95%	10-70	1.03
	3	Ac-D-Pro-*cis*-CF$_3$ΨPro-L-Pro-NH$_2$	> 95%	10-50	0.98
	4	Ac-L-Pro-*cis*-CF$_3$ΨPro-D-Pro-NH$_2$	> 95%	10-50	1.25
	5	Ac-D-Pro-*cis*-CF$_3$ΨPro-D-Pro-NH$_2$	> 95%	10-50	1.08
Hexamers	**6**	Ac-[L-Pro]$_6$-NH$_2$	> 95%	10-90	1.34
	7	Ac-[L-Pro-*cis*-CF$_3$ΨPro-L-Pro]$_2$-NH$_2$	> 95%	10-90	1.71
	8	H-[L-Pro-*cis*-CF$_3$ΨPro-L-Pro]$_2$-NH$_2$	> 95%	10-90	1.27
	9	H-[L-Pro-*cis*-CF$_3$ΨPro-L-Pro]$_2$-OH	> 95%	10-50	1.60
Nonamers	**10**	Ac-[L-Pro]$_9$-NH$_2$	> 95%	20-60	1.22
	11	Ac-L-Pro-*cis*-CF$_3$ΨPro-L-Pro-[Pro]$_6$-NH$_2$	>95%	10-90	1.85
	12	Ac-[Pro]$_3$-L-Pro-*cis*-CF$_3$ΨPro-L-Pro-[Pro]$_3$-NH$_2$	>95%	10-90	1.80
	13	Ac-[Pro]$_6$-L-Pro-*cis*-CF$_3$ΨPro-L-Pro-NH$_2$	>95%	10-90	1.80
	14	Ac-[L-Pro-*cis*-CF$_3$ΨPro-L-Pro]$_3$-NH$_2$	> 95%	10-90	2.03

*Gradient: percentage start-end of ACN + 0.1%. The co-solvent is H_2O + 0.1% TFA.

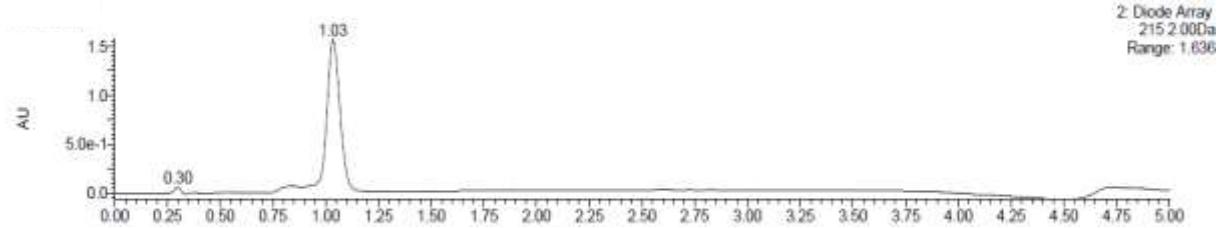
*Table S3: UV-purity at 215 nm of peptides **1**-**14***

<u>UPLC Chromatograms at 215 nm:</u>

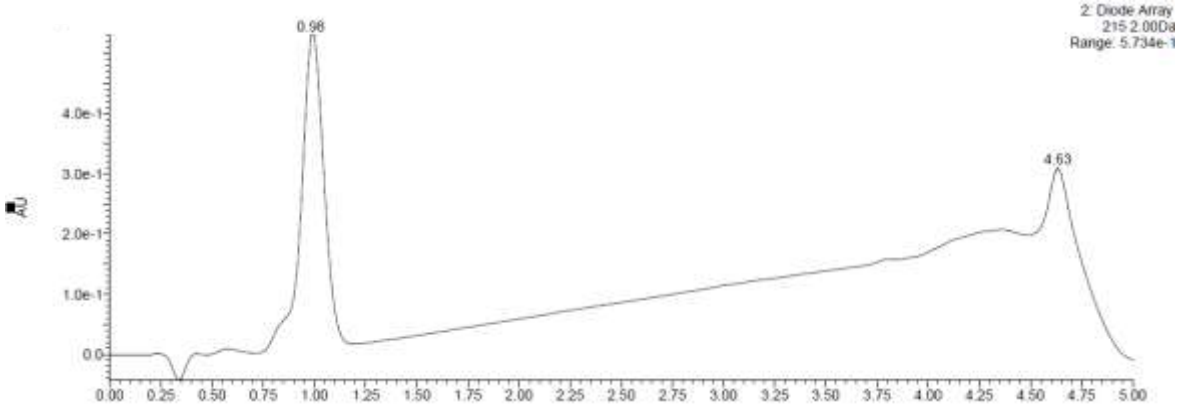
Trimer 1



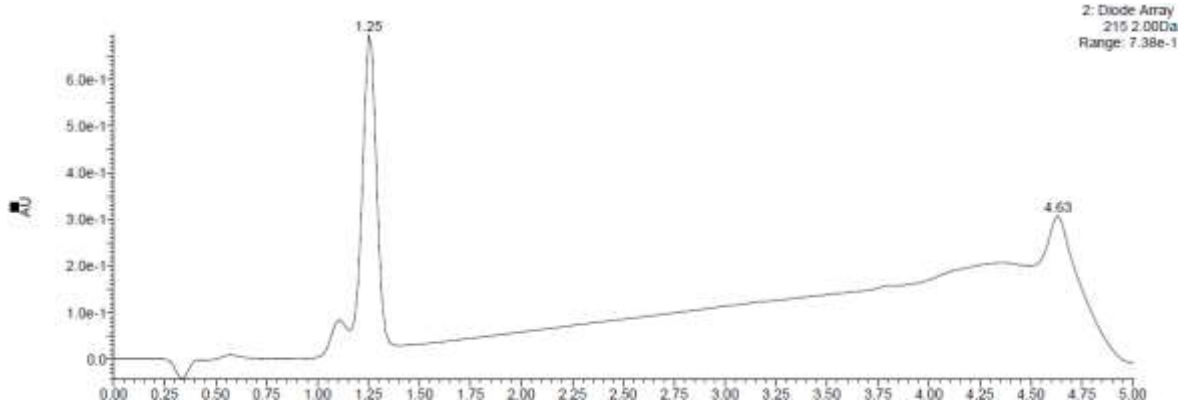
Trimer 2



Trimer 3

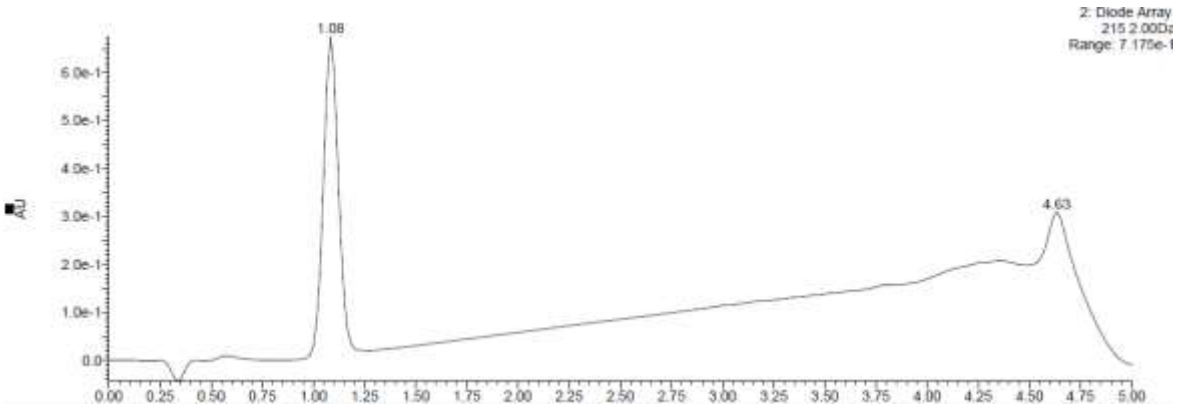


Trimer 4

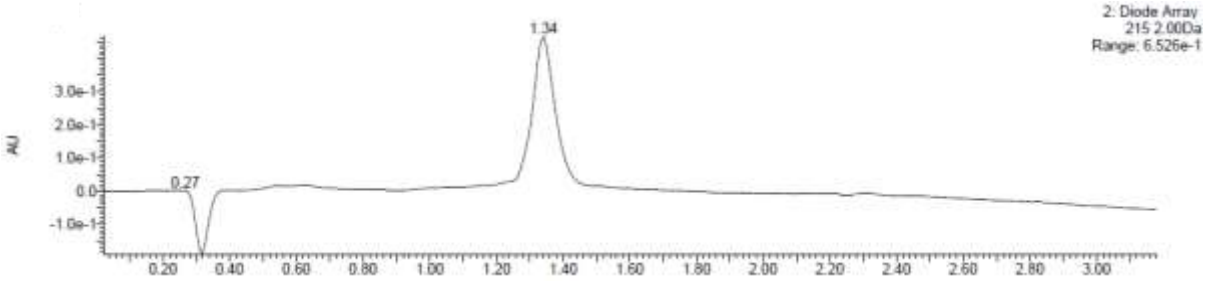


Shoulder and main peak of the same mass.

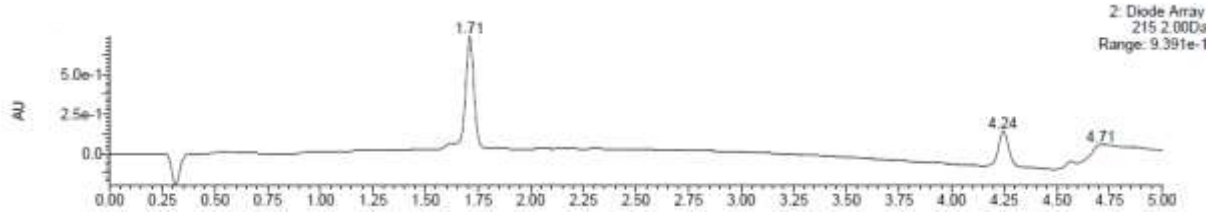
Trimer 5



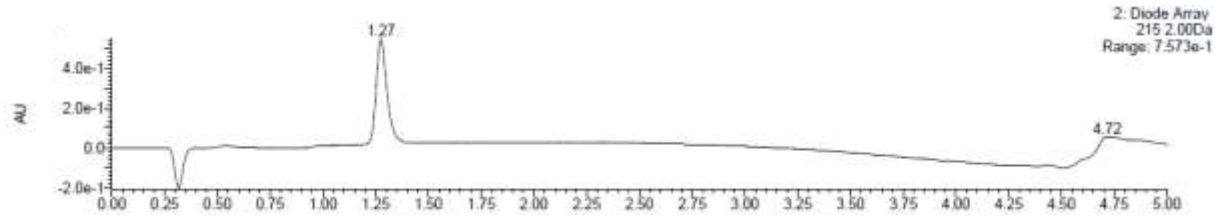
Hexamer 6



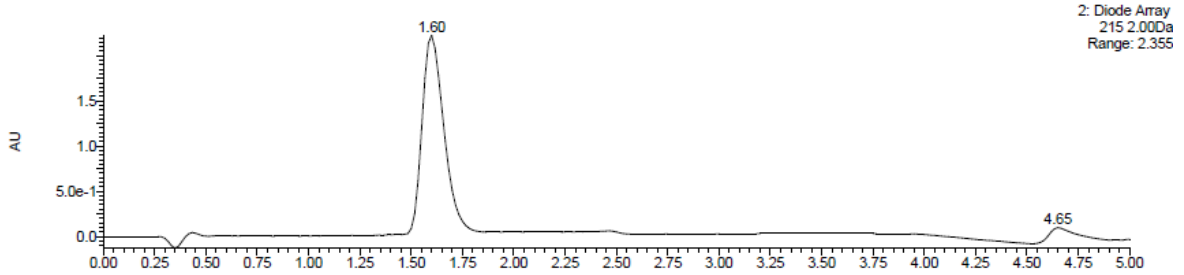
Hexamer 7



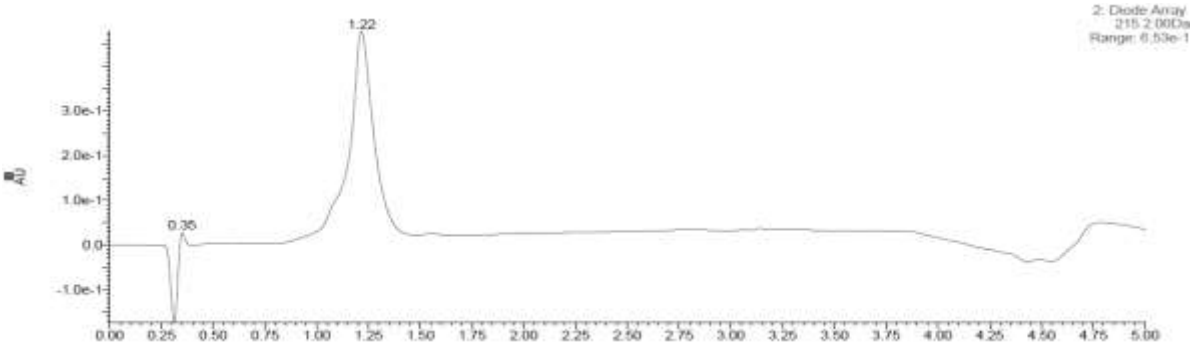
Hexamer 8



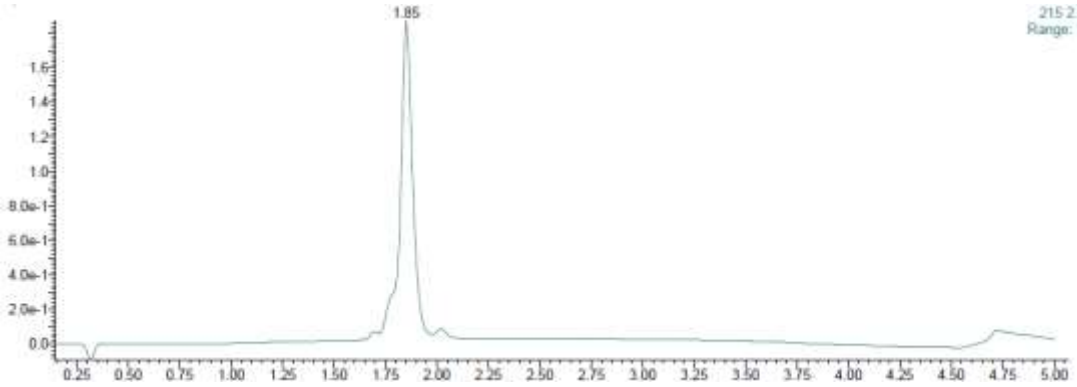
Hexamer 9



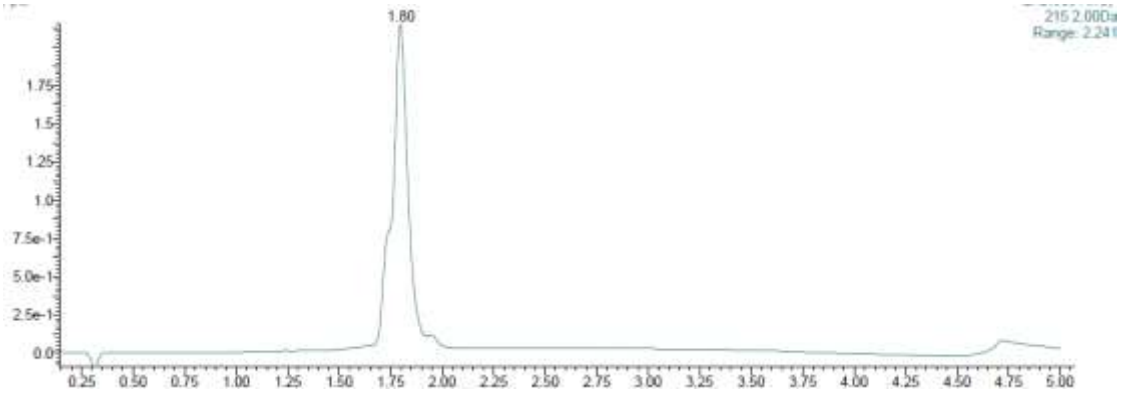
Nonamer 10



Nonamer 11

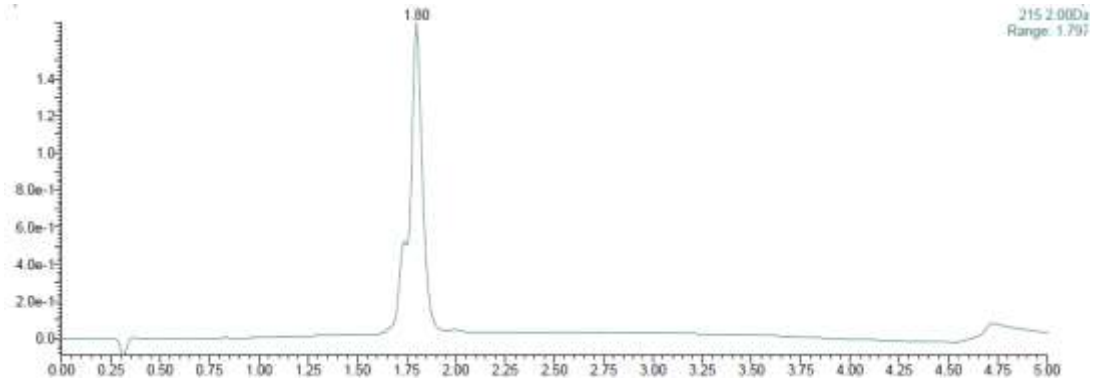


Nonamer 12



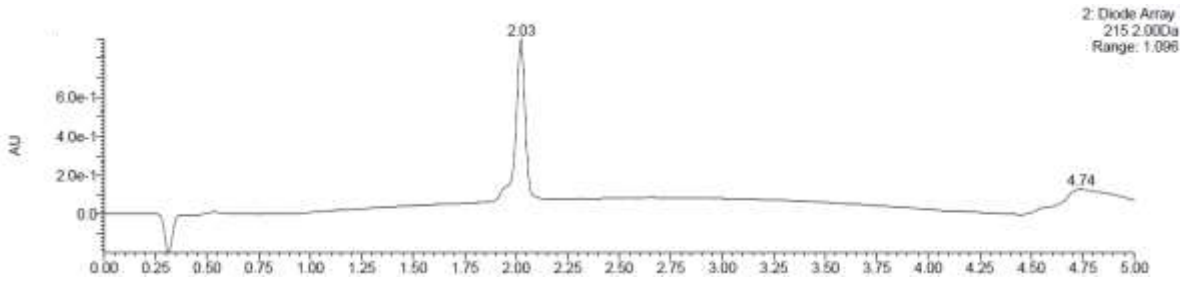
Shoulder and main peak of the same mass.

Nonamer 13



Shoulder and main peak of the same mass.

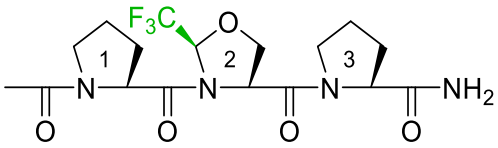
Nonamer 14



V. NMR CHARACTERIZATION OF TRIPEPTIDES 2-5, HEXAMER 7 AND NONAMER 14

V.1 Trimers

V.1.1 Trimer 2, one rotamer (>95%)



V.1.1.1 Characterization of trimer 2 by NMR

^{1}H NMR (400 MHz, D_2O, 298 K): Major rotamer (>95%) all-*trans*

	H_α	H_β		H_γ		H_δ	
Ac	2.08 (s)	-		-		-	
Pro^1	4.59 (t, *J* = 6.7 Hz)	1.98	2.30	2.02	2.14	3.66 (t app., *J* = 6.2 Hz)	
CF_3ΨPro^2	5.11 (t, *J* = 8.8 Hz)	4.25 (t, *J* = 9.0 Hz)	4.79 (t, *J* = 8.7 Hz)	-		6.33 (q, *J* = 5.2 Hz)	
Pro^3	4.40 (dd, *J* = 8.5, 5.8 Hz)	1.94	2.32	2.04		3.58 (tt, *J* = 9.9, 6.8 Hz)	3.85 (tt, *J* = 9.9, 6.7 Hz)

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (>95%) all-*trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	20.9	-	-	-	-	[c]
Pro^1	58.5	29.4-29.6[a]	24.7	48.5	-	
CF_3ΨPro^2	57.7	69.2	-	84.6 (q, *J* = 35.2 Hz)	[b]	
Pro^3	60.6	29.4-29.6[a]	24.6	47.6	-	

[a]: The two C_β chemical shifts (29.4 and 29.6 ppm) cannot be assigned to a specific Pro residue.

[b]: Not observed.

[c]: The following carbonyl chemical shifts (167.6, 172.6, 174.6 and 176.6 ppm) were not assigned.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (> 95%) all-trans)* δ -77.54 (d, *J* = 4.90 Hz, CF_3ΨPro^2 CF_3).

V.1.1.2 Amide bonds assignment of trimer 2

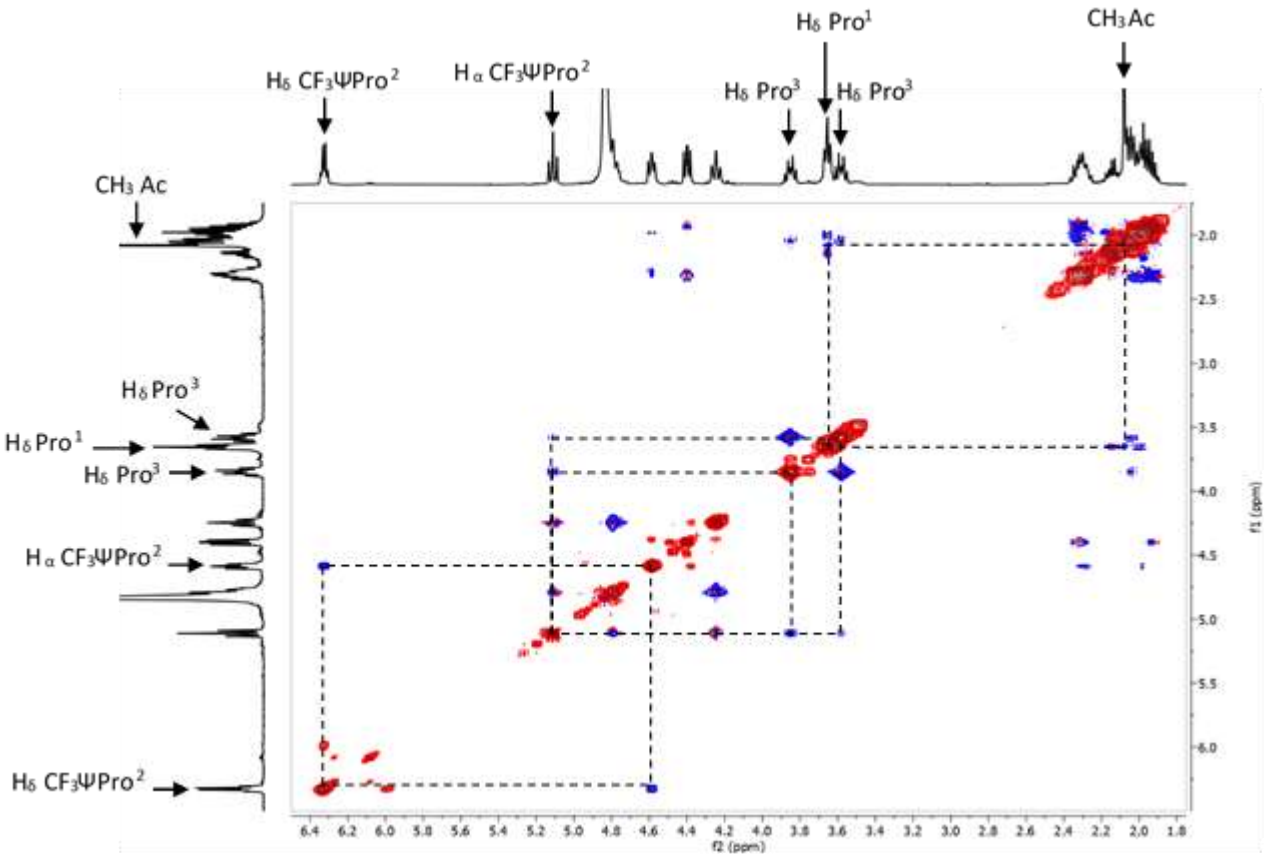
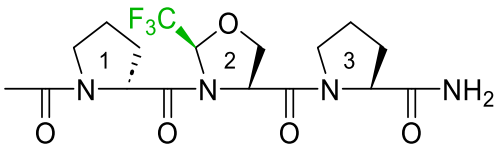


*Figure S1: 2D ROESY spectra of trimer **2**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

V.1.2 Trimer 3, four rotamers (65/15/13/7)



V.1.2.1 Characterization of trimer 3 by NMR

^{1}H NMR (400 MHz, D_2O, 298 K): Major rotamer (65%) *trans-cis-trans*

	H_α	H_β	H_γ	H_δ
Ac	2.09 (s)	-	-	-
Pro^1	4.25	1.96 2.19	1.97 2.13	3.65
$CF_3\Psi Pro^2$	5.53 (t, *J* = 8.2 Hz)	4.41 (t, *J* = 8.7 Hz) 4.97 (t, *J* = 8.3 Hz)	-	6.08 (q, *J* = 5.4 Hz)
Pro^3	4.45	1.91 2.35	2.03	3.50 3.79

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (65%) *trans-cis-trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	20.9	-	-	-	-	[c]
Pro^1	58.3	29.8	24.6-24.9[a]	48.6	-	
$CF_3\Psi Pro^2$	58.3	71.0	-	84.5 (q, *J* = 35.7 Hz)	[b]	
Pro^3	60.7	29.3	24.6-24.9[a]	47.3	-	

[a]: The two C_γ chemical shifts (24.6 and 24.9 ppm) cannot be assigned to a specific Pro residue.

[b]: Not observed.

[c]: The following carbonyl chemical shifts (167.4, 172.7, 175.1 and 176.4 ppm) were not assigned.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (65%) trans-cis-trans) δ* -77.81 (d, *J* = 5.5 Hz, $CF_3\Psi Pro^2 CF_3$).

V.1.2.2 Amide bonds assignment of trimer 3

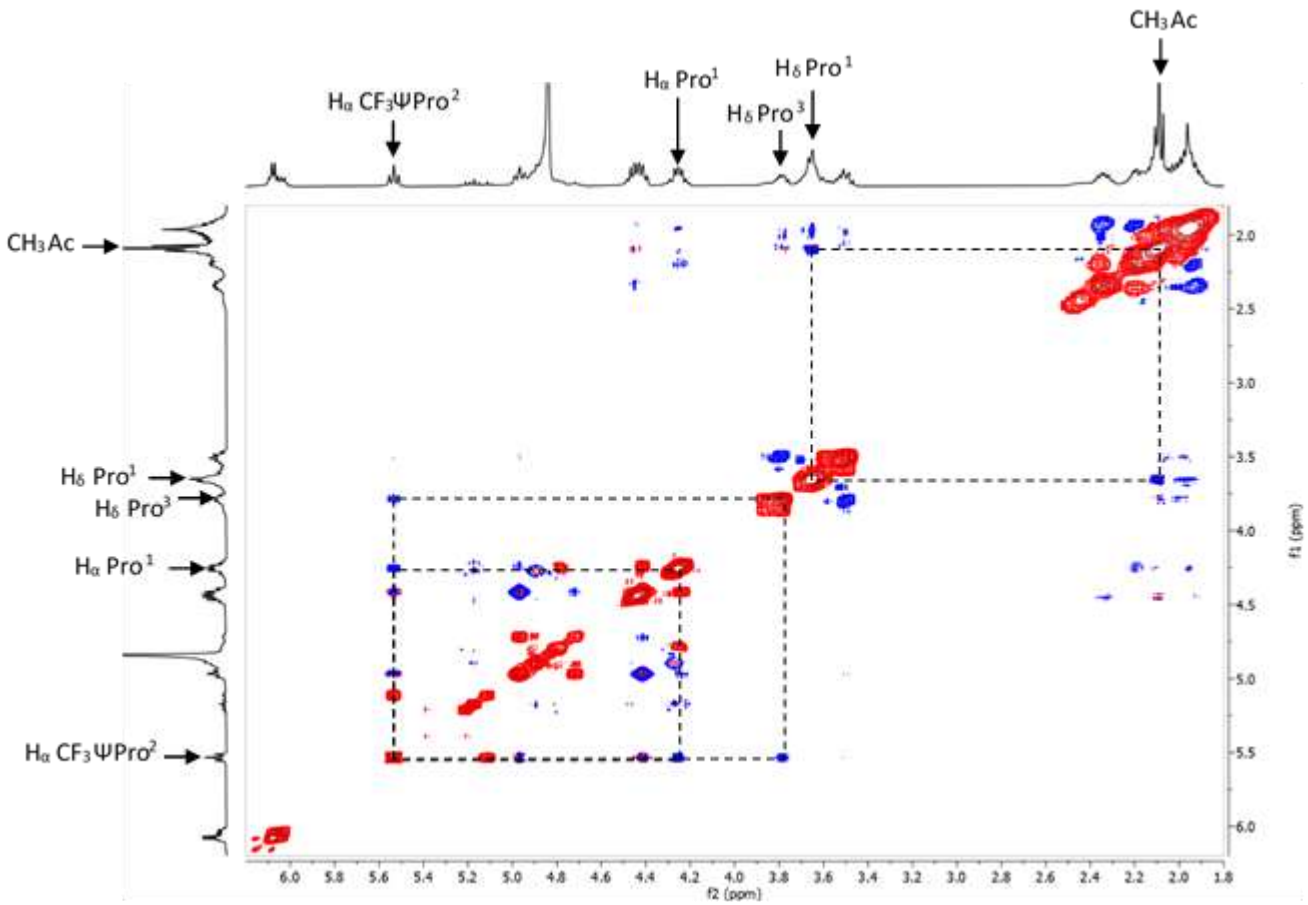
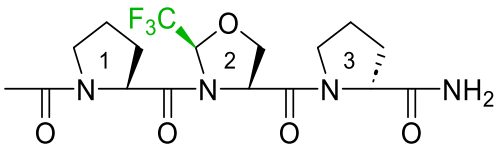


*Figure S2: 2D ROESY spectra of trimer **3**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

V.1.3 Trimer 4, three rotamers (70/22/8)



V.1.3.1 Characterization of trimer 4 by NMR

^{1}H NMR (400 MHz, D_2O, 298 K): Major rotamer (70%) all-*trans*

	H_α	H_β		H_γ		H_δ	
Ac	2.08 (s)	-		-		-	
Pro^1	4.59 (t, *J* = 6.6 Hz)	1.98	2.29	2.00	2.13	3.65	
CF_3ΨPro^2	5.11 (t, *J* = 8.9 Hz)	4.23 (t, *J* = 8.9 Hz)	4.78 (t, *J* = 8.9 Hz)	-		6.33 (q, *J* = 5.1 Hz)	
Pro^3	4.37	2.03	2.26	2.03		3.55	3.93

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (70%) all-*trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	21.8	-	-	-	-	[b]
Pro^1	58.5	29.6	24.7	48.5	-	
CF_3ΨPro^2	51.1	68.9	-	84.6 (q, *J* = 34.8 Hz)	[a]	
Pro^3	60.7	29.3	24.0	47.4	-	

[a]: Not observed.

[b]: The following carbonyl chemical shifts (168.0, 172.6, 174.8 and 176.8 ppm) were not assigned.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (70%) all-trans)* δ -77.50 (d, *J* = 5.1 Hz, CF_3ΨPro^2 CF_3).

V.1.3.2 Amide bonds assignment for trimer 4

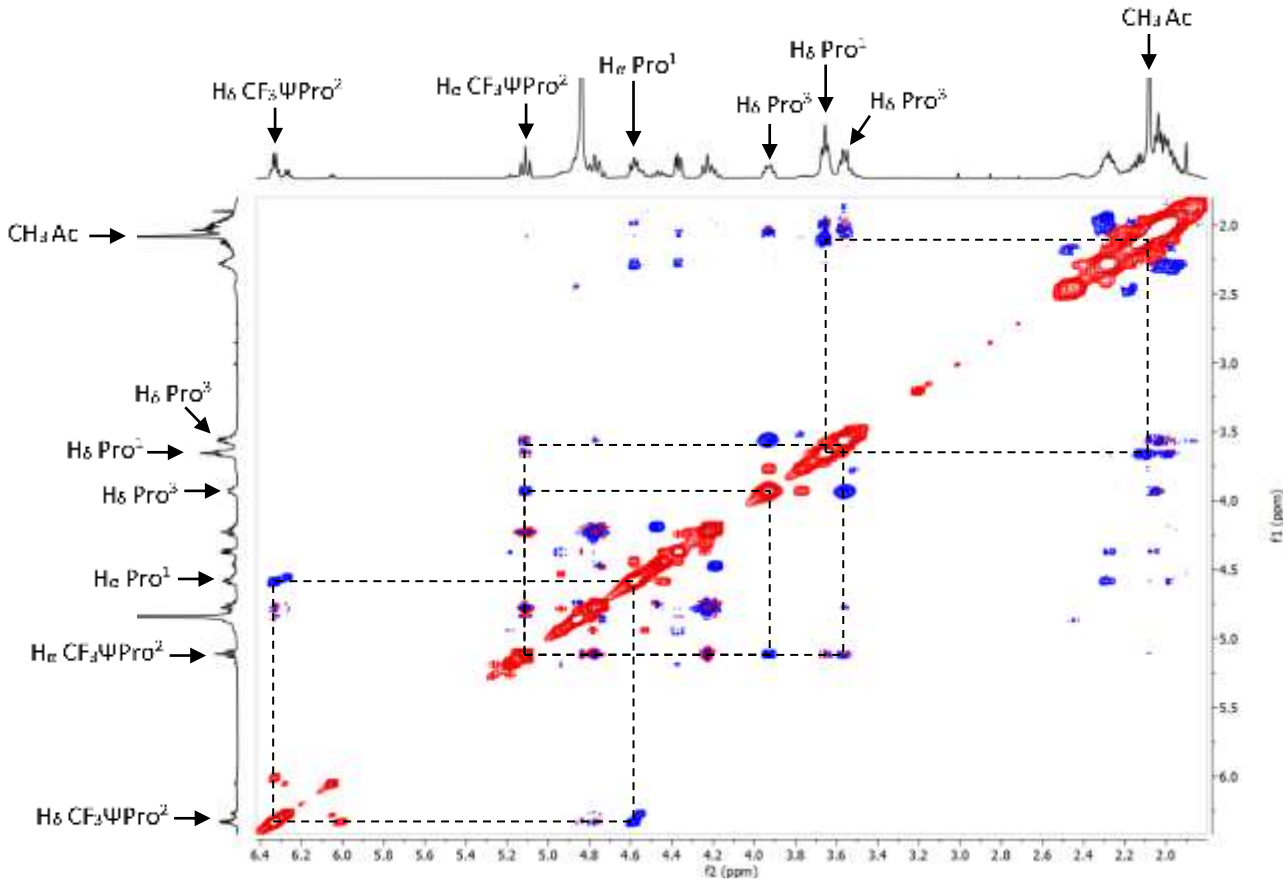
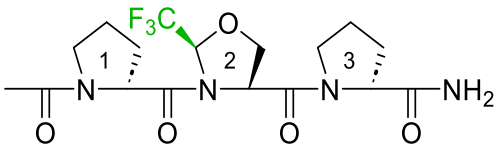


*Figure S3: 2D ROESY spectra of trimer **3**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

V.1.4 Trimer 5, one rotamer (>95%)



V.1.4.1 Protons assignment

^{1}H NMR (400 MHz, D_2O, 298 K): Major rotamer (>95%) *trans-cis-trans*

	H_α	H_β		H_γ		H_δ	
Ac	2.10 (s)	-		-		-	
Pro^1	4.22 (t, *J* = 7.1 Hz)	1.88	2.15	1.95	2.10	3.65	
CF_3ΨPro^2	5.49 (t, *J* = 8.4 Hz)	4.31 (t, *J* = 8.5 Hz)	4.95 (t, *J* = 8.6 Hz)	-		6.06 (q, *J* = 5.4 Hz)	
Pro^3	4.41 (dd, *J* = 8.7, 4.8 Hz)	1.97	2.31	2.05		3.51	3.80

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (>95%) *trans-cis-trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	20.9	-	-	-	-	[b]
Pro^1	58.7	29.9	24.7	48.6	-	
CF_3ΨPro^2	58.5	70.8	-	84.6 (q, *J* = 35.4 Hz)	[a]	
Pro^3	60.6	29.3	24.4	47.2	-	

[a]: Not observed.

[b]: The following carbonyl chemical shifts (167.2, 172.6, 175.1 and 176.6 ppm) were not assigned.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (>95%) trans-cis-trans) δ* -77.65 (d, *J* = 5.5 Hz, CF_3ΨPro^2 CF_3).

V.1.4.2 Amide bonds assignment

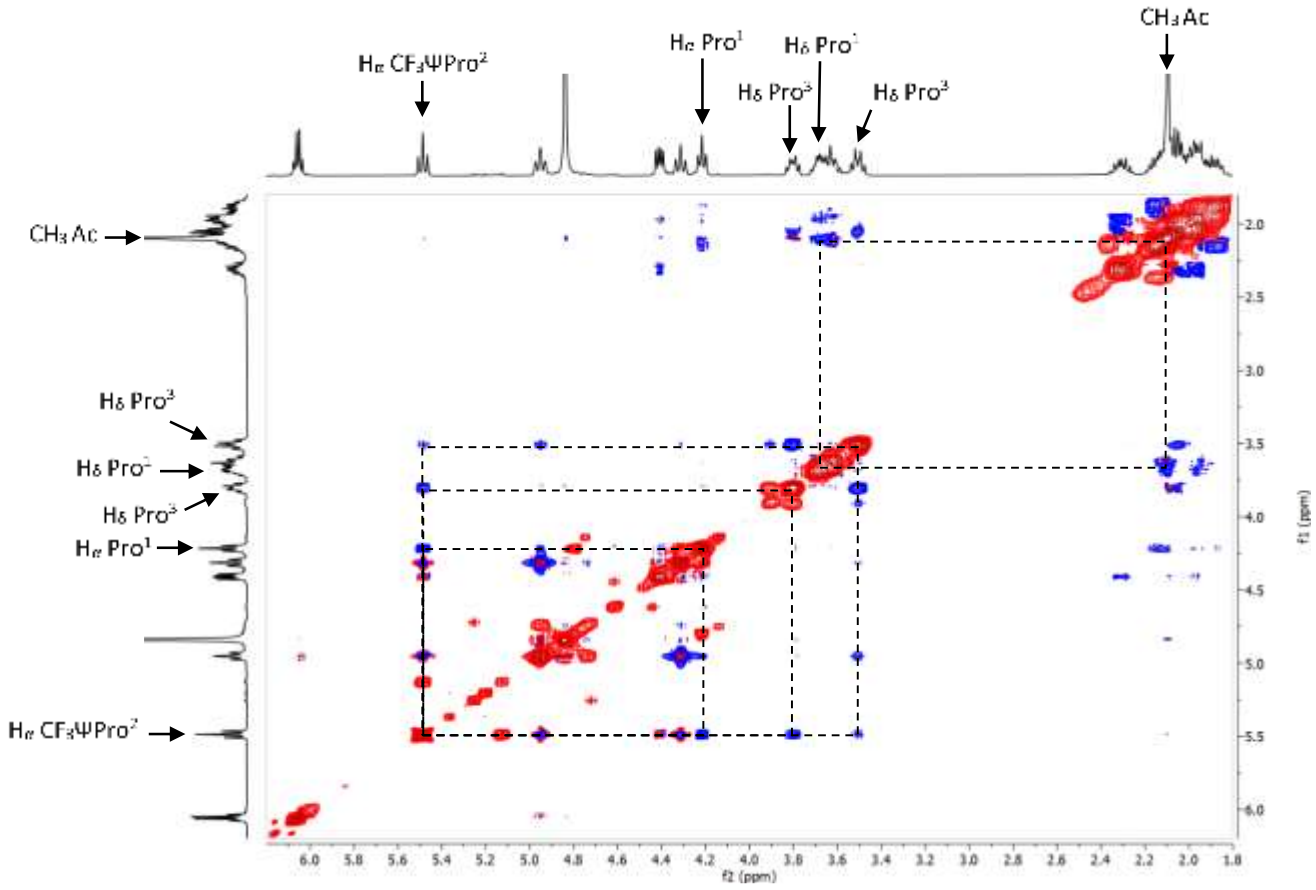
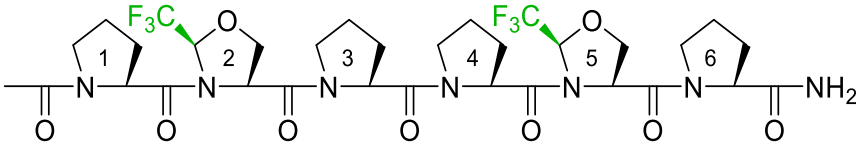


*Figure S4: 2D ROESY spectra of trimer **5**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

V.2 Hexamers

V.2.1 Hexamer 7, one rotamer (>95%)



V.2.1.1 Protons assignment

^{1}H NMR (400 MHz, D_2O, 298K): Major rotamer (> 95%) all-*trans*

	H_α	H_β		H_γ		H_δ	
Ac	2.08 (s)	-		-		-	
Pro^1	4.58 (t, *J* =6.7 Hz)	1.98	2.29	2.03	2.15	3.65	
CF_3ΨPro^2	5.09 (dt, *J* = 8.8, 3.9 Hz)	4.21	4.78	-		6.32	
Pro^3	4.72	1.79	2.34	2.03		3.53	3.87
Pro^4	4.64 (t, *J* = 7.1 Hz)	1.96	2.30	2.03	2.15	3.65	3.82
CF_3ΨPro^5	5.09 (dt, *J* = 8.8, 3.9 Hz)	4.21	4.78	-		6.34	
Pro^6	4.39 (dd, *J* = 8.5, 5.7 Hz)	1.94	2.32	2.02		3.58	3.84

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (> 95%) all-*trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	20.9	-	-	-	-	
Pro^1	58.5	29.6	24.6-25.0[b]	48.3	-	
CF_3ΨPro^2 [a]	57.7	69.1	-	84.6	122.5 (q, *J* = 286.5 Hz)	
Pro^3	58.7	27.6	24.6-25.0[b]	47.5-47.6[c]	-	[d]
Pro^4	58.9	29.1	24.6-25.0[b]	47.5-47.6[c]	-	
CF_3ΨPro^5 [a]	57.7	69.1	-	84.6	122.5 (q, *J* = 286.5 Hz)	
Pro^6	60.6	29.4	24.6-25.0[b]	47.5-47.6[c]	-	

[a]: The two CF_3ΨPro could not be distinguished.

[b]: The four C_γ chemical shifts (2 x 24.6, 24.7 and 25.0 ppm) cannot be assigned to a specific Pro residue.

[c]: The three C_δ chemical shifts (2 x 47.5 and 47.6 ppm) cannot be assigned to a specific Pro residue.

[d]: The following carbonyl chemical shifts (167.1, 167.6, 171.3, 172.6, 174.1, 174.7, 176.6) were not assigned.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (> 95%) all-trans)* δ -77.53 (d, J = 4.2 Hz, $CF_3\Psi Pro^{[a]}$ CF_3), -77.62 (d, J = 4.2 Hz, $CF_3\Psi Pro^{[a]}$ CF_3).

[a]: The two CF_3 chemical shift cannot be assigned to a specific $CF_3\Psi Pro$ residue as the $CF_3\Psi Pro$ cannot be differentiated.

V.2.1.2 Amide bonds assignment

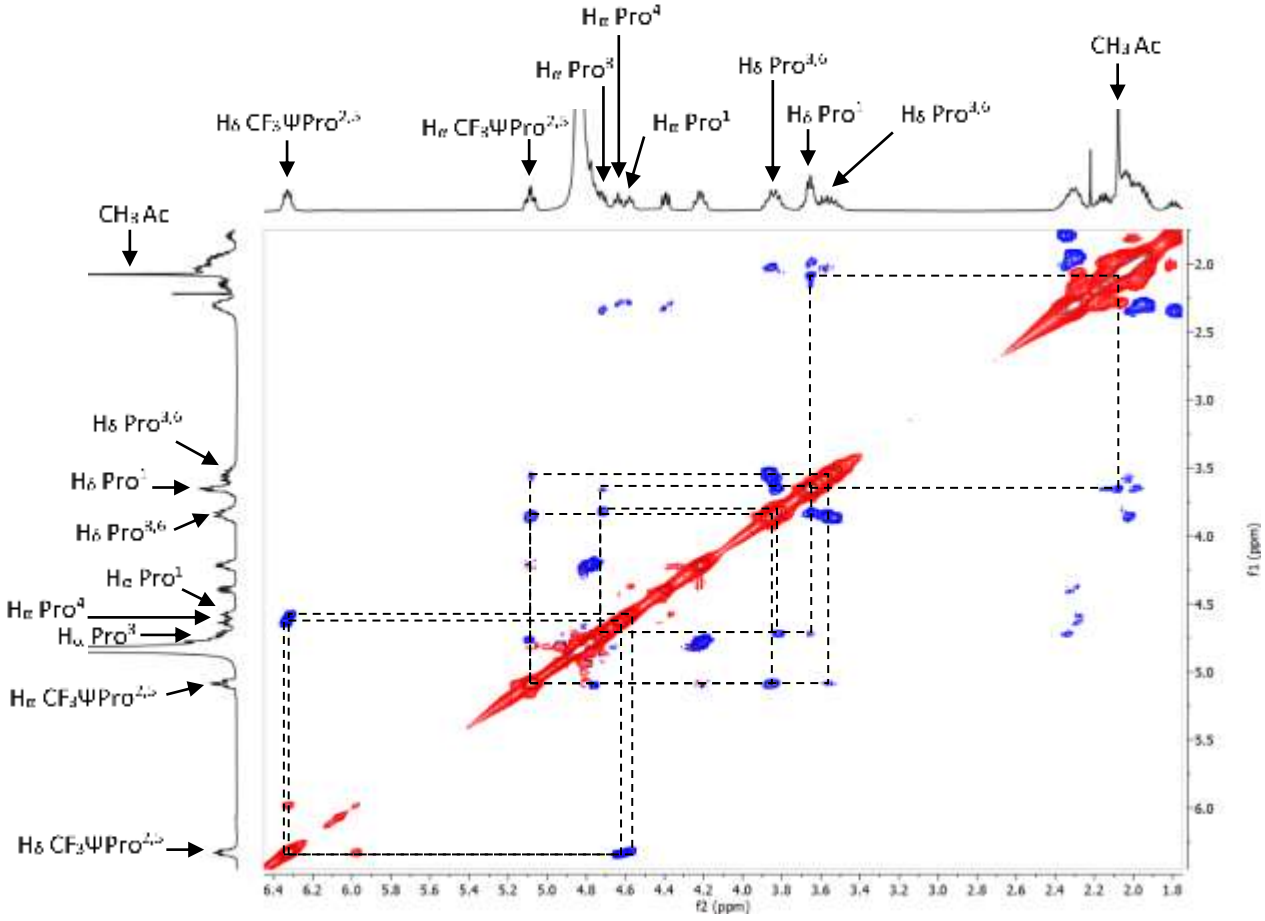
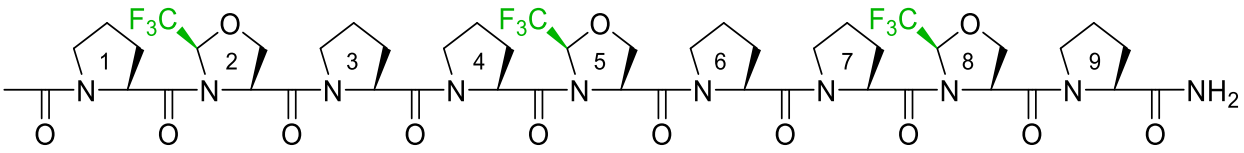


*Figure S5: 2D ROESY spectra of hexamer **7**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

V.3 Nonamers

V.3.1 Nonamer 14, one rotamer (>95%)



V.3.1.1 Protons assignment

^{1}H NMR (400 MHz, D_2O, 298K): Major rotamer (> 95%) all-*trans*

	H_α	H_β		H_γ		H_δ	
Ac	2.08 (s)	-		-		-	
Pro^1	4.58 (t, *J* = 6.8 Hz)	1.98	2.30	2.14		3.65	
$CF_3\Psi Pro^2$ [a]	5.08	4.21	4.78	-		6.33	
Pro^3 [b]	4.71	1.80	2.34	2.02		3.53	3.86
Pro^4 [c]	4.63 (t app., *J* = 6.9 Hz)	1.96	2.30	2.07	2.17	3.65	3.82
$CF_3\Psi Pro^5$ [a]	5.08	4.21	4.78	-		6.33	
Pro^6 [b]	4.71	1.80	2.34	2.02		3.53	3.86
Pro^7 [c]	4.63 (t app., *J* = 6.9 Hz)	1.96	2.30	2.07	2.17	3.65	3.82
$CF_3\Psi Pro^8$ [a]	5.08	4.21	4.78	-		6.33	
Pro^9	4.39 (dd, *J* = 8.5, 5.6 Hz)	1.95	2.32	2.05		3.58	3.84

[a]: The three $CF_3\Psi Pro$ could not be distinguished.

[b]: The two prolines, Pro^3 and Pro^6 could not be distinguished.

[c] : The two proline, Pro^4 and Pro^7 could not be distinguished.

^{13}C NMR (100.5 MHz, D_2O, 298 K): Major rotamer (> 95%) all-*trans*

	C_α	C_β	C_γ	C_δ	CF_3	C=O
Ac	20.9	-	-	-	-	[e]
Pro[1]	58.5	29.6	24.6-25.0[d]	48.5	-	
CF_3ΨPro[2] [a]	57.7	69.1	-	84.6 (q, *J* = 34.8 Hz)	123.9 (q, *J* = 286.8 Hz)	
Pro[3] [b]	58.7	27.6	24.6-25.0[d]	47.5	-	
Pro[4] [c]	58.9	29.1	24.6-25.0[d]	47.6	-	
CF_3ΨPro[5] [a]	57.7	69.1	-	84.6 (q, *J* = 34.8 Hz)	123.9 (q, *J* = 286.8 Hz)	
Pro[6] [b]	58.7	27.6	24.6-25.0[d]	47.5	-	
Pro[7] [c]	58.9	29.1	24.6-25.0[d]	47.6	-	
CF_3ΨPro[8] [a]	57.7	69.1	-	84.6 (q, *J* = 34.8 Hz)	123.9 (q, *J* = 286.8 Hz)	
Pro[9]	60.6	29.4	24.6-25.0[d]	47.5	-	

[a]: The three CF_3ΨPro could not be distinguished.

[b]: The two prolines, Pro^3 and Pro^6 could not be distinguished.

[c] : The two proline, Pro^4 and Pro^7 could not be distinguished

[d]: The C_γ chemical shifts (24.6, 24.7 and 25.0 ppm) cannot be assigned to a specific Pro residue. Moreover, due to resonance overlap only 3 peaks were found.

[e]: The following carbonyl chemical shifts (2 x 167.1, 167.6, 2 x 171.3, 172.6, 2 x 174.1, 174.7, 176.6) were not assigned. Due to resonance overlap, only 6 carbonyl peaks were found.

^{19}F NMR (376.2 MHz, D_2O, 298 K): *(Major rotamer (> 95%) all-trans) δ* -76.36 (d, *J* = 4.7 Hz, CF_3ΨPro[a] CF_3), -76.44 (m, 2 x CF_3ΨPro[a] CF_3).

[a]: The three CF_3 chemical shift cannot be assigned to a specific CF_3ΨPro residue as the CF_3ΨPro cannot be differentiated.

V.3.1.2 Amide bonds assignment

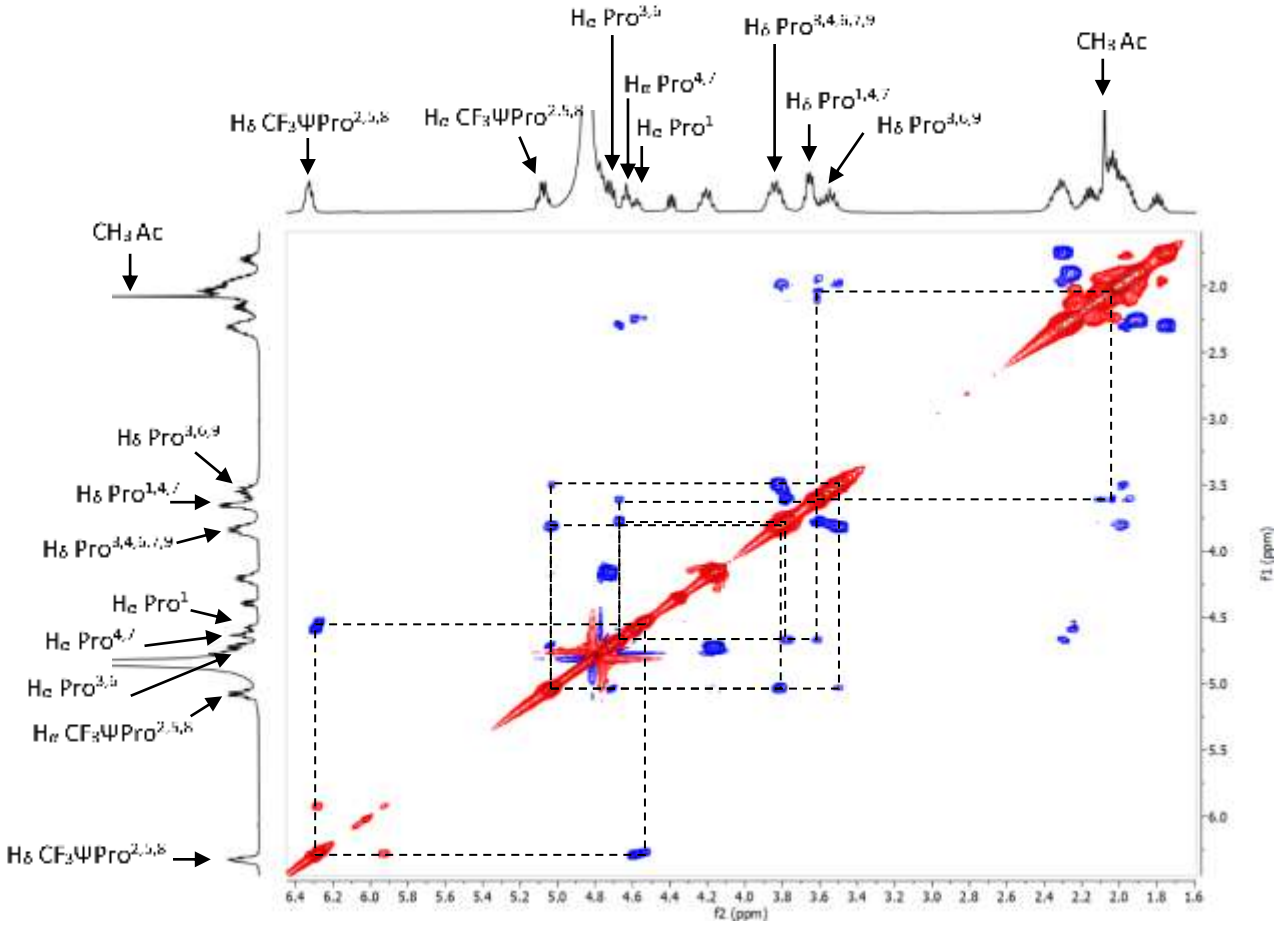
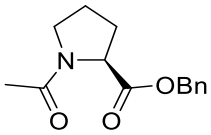


*Figure S6: 2D ROESY spectra of nonamer **14**, 400 MHz, 20 mM in D_2O, 298 K, 16 scans, 200 ms as mixing time*

VI. SYNTHESIS AND CD STUDIES OF RESIDUE CHROMOPHORES

VI.1 Synthesis of Ac-L-Pro-NHMe

VI.1.1 Ac-L-Pro-OBn

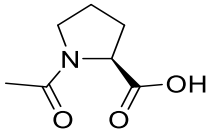


H-L-Pro-OBn.HCl (500 mg, 2.07 mmol, 1 eq.) was dissolved in dry DCM (7 mL) under argon atmosphere. Pyridine (500 µL, 6.20 mmol, 3 eq.) was added and then AcCl (440 µL, 6.20 mmol, 3 eq.) was added dropwise. The reaction mixture was left stirred at r.t overnight. The reaction mixture was then diluted with DCM (10 mL) and washed with HCl (1M, 4 mL). The resulting aqueous phase was extracted with DCM (2 x 10 mL). The organic phases were gathered, washed with brine (15 mL), dried over Na_2SO_4, filtered and evaporated. The crude was purified by chromatography on silica gel with cyclohexane/ethyl acetate (80/20) to give the pure compound Ac-L-Pro-OBn (453 mg, 88%) as a 22/78 mixture of Ac *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

Oil; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) *(22/78 mixture of rotational isomers*) *(trans rotational isomer) δ* 1.48-2.31 (m, 4 H, H_β-Pro and H_γ-Pro), 2.10 (s, 3 H, CH_3 Ac), 3.35-3.69 (m, 2 H, H_δ-Pro), 4.54 (dd, *J* = 8.6, 3.4 Hz, 1 H, H_α Pro), 5.12-5.20 (m, 2 H, CH_2 OBn), 7.28-7.40 (m, 5 H, OBn arom.); *(cis rotational isomer) δ* 1.48-2.31 (m, 4 H, H_β-Pro and H_γ-Pro), 1.92 (s, 3 H, CH_3 Ac), 3.35-3.69 (m, 2 H, H_δ-Pro), 4.39 (dd, *J* = 8.6, 2.9 Hz, 1 H, H_α Pro), 5.12-5.20 (m, 2 H, CH_2 OBn), 7.28-7.40 (m, 5 H, OBn arom.); **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K) *(22/78 mixture of rotational isomers*) *(trans rotational isomer) δ* 22.4 (CH_3, CH_3 Ac), 24.9 (CH_2, C_γ), 29.5 (CH_2, C_β), 47.9 (CH_2, C_δ), 58.7(CH, C_α), 66.9 (CH_2, CH_2 OBn), 128.1 (2 x CH, OBn arom.), 128.2 (CH, OBn arom.), 128.6 (2 x CH, OBn arom.), 135.9 (C, OBn arom.), 169.6 (C, C=O), 172.3 (C, C=O); *(cis rotational isomer) δ* 22.3 (CH_3, CH_3 Ac), 22.9 (CH_2, C_γ), 31.6 (CH_2, C_β), 46.4 (CH_2, C_δ), 60.4 (CH, C_α), 67.4 (CH_2, CH_2 OBn), 128.4 (2 x CH, OBn arom.), 128.7 (CH, OBn arom.), 128.8 (2 x CH, OBn arom.), 135.3 (C, OBn arom.), 168.9 (C, C=O), 172.1 (C, C=O).

In accordance with the literature.[2]

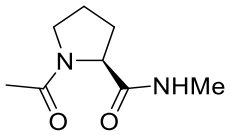
VI.1.2 Ac-L-Pro-OH



Ac-L-Pro-OBn (450 mg, 1.82 mmol, 1 eq.) was dissolved in MeOH (12 mL) and Pd/C, 10% (45 mg, 10% in weight) was added. The reaction medium was stirred under H_2 atmosphere overnight at r.t. The reaction mixture was filtered through celite and then evaporated. The crude was directly engaged in the next step.

Colorless oil. Product commercially available (CAS: 68-95-1).

VI.1.3 Ac-L-Pro-NHMe



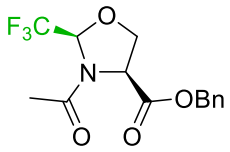
Ac-L-Pro-OH (330 mg, 2.10mmol, 1 eq.) was dissolved in DMF (12 mL). $MeNH_2$.HCl (241 mg, 3.57 mmol, 1.7 eq.), HOBt (321 mg, 2.38 mmol, 1.1 eq.), $NaHCO_3$ (600 mg, 7.14 mmol, 3.4 eq.), EDCI (483 mg, 2.52 mmol, 1.2 eq.) were successively added. The reaction medium was stirred overnight at r.t. Water (25 mL) and EtOAc (25 mL) were added and the layers were separated. The aqueous layer was extracted with EtOAc (7 x 25 mL) and the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude was purified by chromatography on silica gel with dichloromethane/methanol from (100/0) to (90/10) to give the desire compound (120 mg, 39% over two steps) as a 10/90 mixture of Ac *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

White solid; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) (*10/90 mixture of rotational isomers*) *(trans rotational isomer) δ* 1.82 (m, 1 H, H_β-Pro-Ha), 2.02 (m, 1 H, H_γ-Pro-Ha), 2.11 (s, 3 H, CH_3 Ac), 2.14 (m, 1 H, H_γ-Pro-Hb), 2.49 (m, 1 H, H_β-Pro-Hb), 2.76 (d, *J* = 4.9 Hz, 3 H, CH_3 NHMe), 3.41 (dt, *J* = 9.8, 7.0 Hz, 1 H, H_δ-Pro-Ha), 3.54 (dt, *J* = 10.1, 2.3 Hz, 1 H, H_δ-Pro-Hb), 4.56 (d, *J* = 8.0 Hz, 1 H, H_α-Pro), 7.14 (m, 1 H, NH); *(cis rotational isomer) δ* 1.73-2.27 (m, 4 H, H_β-Pro and H_γ-Pro), 2.04 (s, 3 H, CH_3 Ac), 2.79 (d, *J* = 4.9 Hz, 3 H, CH_3 NHMe), 3.60 (m, 2 H, H_δ-Pro), 4.30 (dd, *J* = 7.7, 3.7 Hz, 1 H, H_α-Pro), 6.1 (m, 1 H, NH); **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K) (*10/90 mixture of rotational isomers*) *(trans rotational isomer) δ* 22.7 (CH_3, CH_3 Ac), 25.2 (CH_2, C_γ), 26.3 (CH_3, CH_3 NHMe), 27.2 (CH_2, C_β), 48.5 (CH_2, C_δ), 59.6 (CH, C_α), 171.3 (C, C=O), 171.8 (C, C=O); *(minor conformer: too low to be observed)*.

Product commercially available (CAS: 19701-85-0).

VI.2 Synthesis of Ac-CF_3ΨPro-OBn

VI.2.1 Ac-CF_3ΨPro-OBn

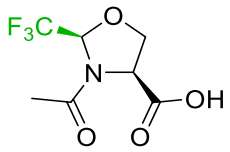


H-CF_3ΨPro-OBn (416 mg, 1.51 mmol, 1 eq.) was dissolved in acetic anhydride (1.4 mL, 15.1 mmol, 10 eq.) and iodine (38 mg, 0.15 mmol, 0.1 eq.) was added. The reaction mixture was stirred overnight at r.t. Then, DCM (15 mL) was added and the organic layer was washed with $NaHSO_3$ (1 M). The aqueous layer was extracted with DCM (3 x 10 mL). The resulting organic layers were gathered, washed with brine, dried with Na_2SO_4, filtered and evaporated. The crude was purified by chromatography on silica gel with cyclohexane/ethyl acetate (60/40) to give the pure compound Ac-CF_3ΨPro-OBn (413 mg, 86%) as a 50/50 mixture of Ac *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

Oil; **Rf** 0.48 (65/35 cyclohexane/ethyl acetate); **[α]$^{20}_D$** -70.0° (*c* 1.0, $CHCl_3$); **IR** (neat) 2961, 1753, 1682, 1388, 1286, 1176, 1148, 1120, 953, 847, 754, 695 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K) *(50/50 mixture of rotational isomers impossible to distinguish) δ* 2.14 (s, 3 H, CH_3 Ac), 2.22 (s, 3 H, CH_3 Ac), 4-30-4.56 (m, 4 H, 2 x H_β-CF_3ΨPro), 4.68 (s,

1 H, Hα-CF_3ΨPro), 5.03 (s, 1 H, Hα-CF_3ΨPro), 5.21 (s, 4 H, 2 x CH_2 OBn), 5.50 (s, 1 H, H_δ-CF_3ΨPro), 5.92 (s, 1 H, H_δ-CF_3ΨPro), 7.31-7.40 (m, 10 H, 2 x OBn arom.); **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K) *(50/50 mixture of rotational isomers impossible to distinguish)* δ 22.4 (CH_3, CH_3 Ac), 31.1 (CH_3, CH_3 Ac), 57.0 (CH, C_α), 58.5 (CH, C_α), 67.7 (CH_2, CH_2 OBn), 68.3 (CH_2, CH_2 OBn), 69.6 (CH_2, C_β), 70.6 (CH_2, C_β), 84.3 (m, CH, C_δ), 85.4 (m, CH, C_δ), 122.8 (q, J = 286.6 Hz, 2 C, 2 x CF_3), 128.1-129.8 (10 x CH, OBn arom.), 134.6 (2 x C, OBn arom.), 135.1 (2 x C, OBn arom.), 168.4 (2 x C, C=O), 169.7 (2 x C, C=O); **^{19}F NMR** (376.2 MHz, $CDCl_3$, 298 K) *(50/50 mixture of rotational isomers impossible to distinguish)* δ -78.57 (s, CF_3), -78.81 (s, CF_3); **HRMS** (ESI-TOF) *m/z* [M + Na]$^+$ calculated for $C_{14}H_{14}F_3NO_4Na$: 340.0773, found: 340.0761.

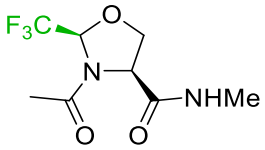
VI.2.2 Ac-CF_3ΨPro-OH



Ac-CF_3ΨPro-OBn (400 mg, 1.26 mmol, 1 eq.) was dissolved in MeOH (6 mL) and Pd/C, 10% (40 mg, 10% in weight) was added. The reaction mixture was stirred under H_2 (1 bar) at r.t. overnight. The reaction mixture was filtered through Celite and the solvent was evaporated. The crude was directly engaged in the next step.

Colorless oil.

VI.2.3 Ac-CF_3ΨPro-NHMe



Ac-CF_3ΨPro-OH (250 mg, 1.11 mmol, 1 eq.) was dissolved in DMF (6 mL) and $MeNH_2$.HCl (128 mg, 1.89 mmol, 1.7 eq.), HOBt (170 mg, 1.25 mmol,1.1 eq.), EDCI (255 mg, 1.33 mmol, 1.2 eq.), $NaHCO_3$ (317 mg, 3.77 mmol, 3.4 eq.) were successively added. The reaction mixture was stirred 22 hours at r.t. Water (20 mL) and EtOAc (20 mL) were added and the layers were separated. The aqueous phase was extracted with EtOAc (6 x 20 mL) and the organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated. The crude was purified by chromatography on silica gel from Cyclohexane/AcOEt (50/50) to (0/100) to give the desired compound (185 mg, 62% over two steps) as a 24/76 mixture of Ac *cis*/*trans* rotational isomers in $CDCl_3$ at 298 K.

Colorless oil; **^{1}H NMR** (400 MHz, $CDCl_3$, 298 K): *(24/76 mixture of rotational isomers*) *(trans rotational isomer)* δ 2.25 (m, 3 H, CH_3 Ac), 2.82 (m, 3 H, CH_3 NHMe), 4.21-4.95 (m, 3 H, H_α-CF_3ΨPro, H_β-CF_3ΨPro), 5.52 (m, 1 H, H_δ-CF_3ΨPro), 6.70 (m, 1 H, NH); *(cis rotational isomer: too low to be observed)*; **^{13}C NMR** (100.5 MHz, $CDCl_3$, 298 K): *(24/76 mixture of rotational isomers) (trans rotational isomer)* δ 22.6 (CH_3, CH_3 Ac), 26.5 (CH_3, CH_3 NHMe), 57.7 (CH_2, C_α), 69.0 (CH_2, C_β), 85.6 (q, J = 35.1 Hz, CH, C_δ), 122.5 (q, J = 287.8 Hz, C, CF_3), 168.5 (C, C=O), 171.4 (C, C=O); *(cis rotational isomer: too low to be observed)*; **^{19}F NMR** (376.2 MHz, $CDCl_3$, 298 K) *(24/76 mixture of rotational isomers) (trans rotational isomer)* δ -79.23 (s, CF_3); *(cis rotational isomer)* δ -78.08 (s, CF_3).

In accordance with the literature.[1]

VI.3 Circular dichroism characterization

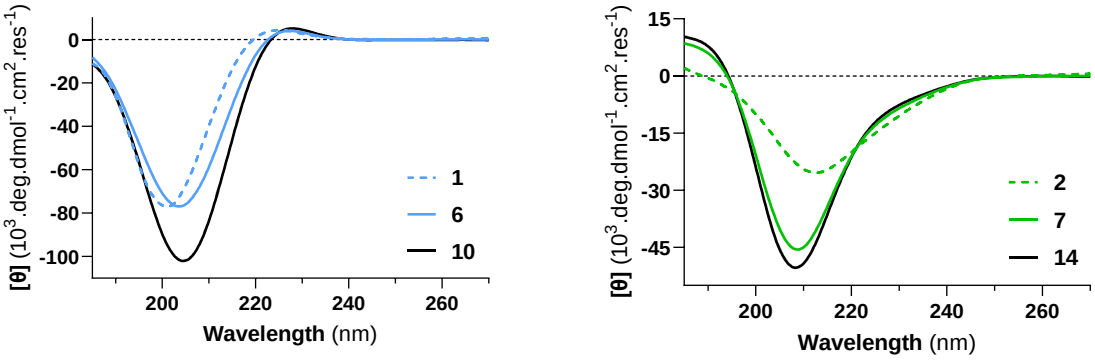


*Figure S7: Comparison of CD spectra of non-fluorinated peptides **1**, **6** and **10** and fluorinated peptides **2**, **7** and **14** chromophores Ac-L-Pro-NHMe and Ac-CF₃ΨPro-NHMe, 100 µM in PBS 20 mM, 4°C*

Trimer **1** shows a CD spectrum similar to that of hexamer **6**, with a slight blue shift of the negative band around 207 nm. The fluorinated trimer **2** exhibits a red shifted and less intense negative band compared to the hexamer **8** and nonamer **14**. The absence of the hallmark positive band around 228 nm for the trimer **2** is also noted, as observed with the fluorinated hexamer **7** and nonamer 14.

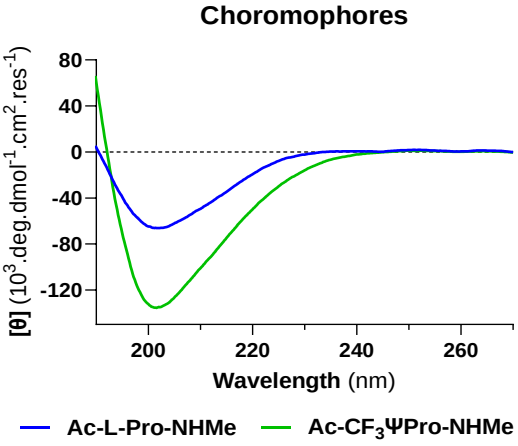


Figure S8: CD spectra of chromophores Ac-L-Pro-NHMe and Ac-CF₃ΨPro-NHMe, 100 µM in PBS 20 mM, 4°C

The direct proximity of the chiral environment (C_δ-CF_3) on Ac-CF₃ΨPro-NHMe CD chromophore (amide bond) significantly impacts the magnitude of the CD signal which appears more negative compared to that of the Ac-L-Pro-NHMe.

VII. SUPPLEMENTARY CD EXPERIMENT

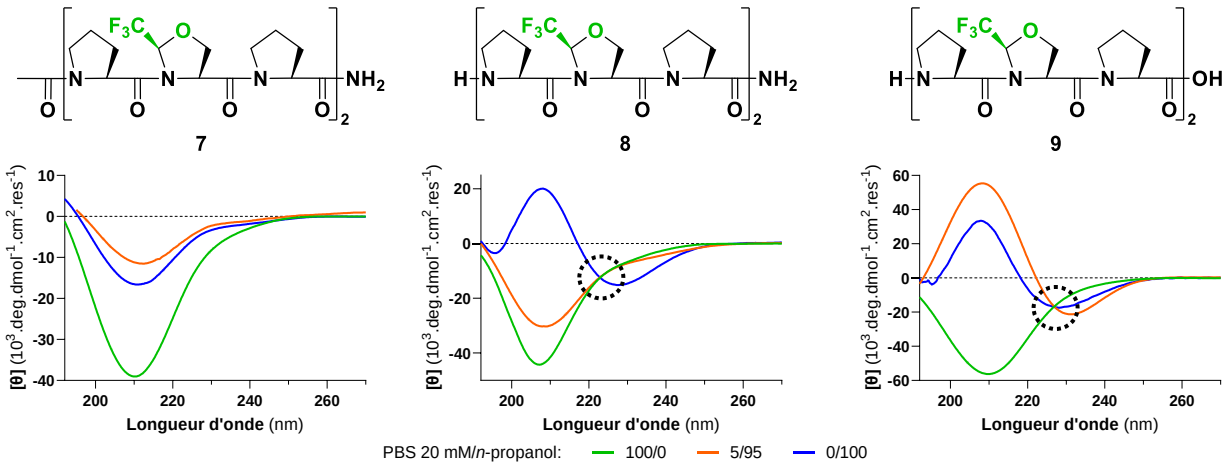


*Figure S9: CD spectra of hexamers **7**-**9**, 100 µM, 4°C with different ratio of the solvent PBS 20 mM/n-propanol: green line (100/0), orange line (5/95) and blue line (0/100). Circle highlight the isosbestic point*

Figure S9 repeats Figure 1.C presented in the article, with the addition of the CD spectrum in the solvent PBS 20 mM/*n*-propanol (5/95), revealing the presence of an isosbestic point for oligomers **8** and **9**. These two isosbestic points indicate that only two conformations are engaged in the transition process and then strongly support the PPII folding of our fluorinated oligomers in aqueous media.

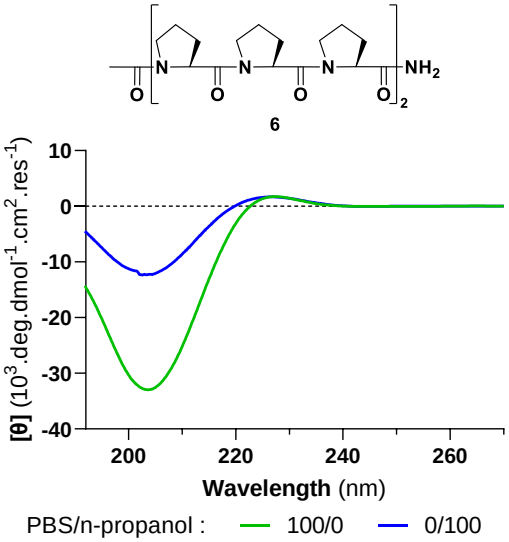


*Figure S10: CD spectra of hexamer **6**, 100 µM, 4°C with different ratio of the solvent PBS 20 mM/n-propanol: green line (100/0) and blue line (0/100).*

The non-fluorinated hexamer **6** does not promote a conversion of PPII to PPI folding in PBS 20 mM/*n*-propanol (0/100), but only a decrease of the signal ellipticity is observed.

VIII. X-RAY STRUCTURE OF NONAMER 14

VIII.1 Crystallographic data collection, structure determination and refinement

Suitable crystals for single crystal X-ray diffraction (SCXRD) analysis were obtained for the compound **14** by slow evaporation of methanol solvent. Methanol was not dried but contained residual water and was exposed to air during vapor diffusion. X-ray diffraction data were measured at low temperature (123 K) using a RIGAKU *XtaLabPro* diffractometer consisting of a Mo microfocus sealed-tube *MM003* generator coupled to a Max-Flux® double-bounce confocal multilayer optic and a HPAD *PILATUS3R 200K* detector. The data were processed using *CrysAlisPro*[3] with *SCALE3 ABSPACK* scaling algorithm implemented for the empirical absorption correction using spherical harmonics, combined with a numerical absorption correction based on Gaussian integration over a multifaceted rectangular platelet model. The weak diffraction exhibited little signal beyond atomic resolution, yet sufficient to obtain a structure solution by intrinsic phasing methods with complete connectivity using *SHELX-T*. The working data set was truncated at a resolution limit of sinq/l=0.515 (d_{min}= 0.97Å) in order to attempt to anisotropic refinement of the model with complete connectivity using full-matrix least-squares methods on F^2 using *SHELX-L.*[4,5] In the absence of any anomalous signal, the model was refined as a two-component inversion twin, with the non-significant BASF fraction value being disregarded, since the absolute configuration is determined by the *L*-proline constituents. Three badly-fitting reflections, which were shaded by the beamstop, were omitted, and the displacement factors were severely restrained to compensate for the low ratio of observables-to-parameters. To keep them in check, the RIGU (strict sd 0.001) and ISOR (sd 0.03 except for three non-hydrogen atoms sd 0.001) commands were applied throughout the entire model. All carbon-bound and nitrogen-bound hydrogen atoms were geometrically positioned and refined using riding models with constrained distances set 0.98 Å (RCH_3), 0.99 Å (R_2CH_2), 1.00 Å (R_3CH), 0.88 Å (N-H), and U_{iso}(H) parameters with assigned values of either 1.2U_{eq} or 1.5U_{eq} (RCH_3 only) of the attached atom. Crystal data, data collection and structure refinement details are summarized in Table S4.

Despite the poor refinement statistics, the model was able to evidence two different puckering features for Pro^1 and Pro^3, whose occupancy parameter ratio were adjusted to 0.60/0.40 and 0.65/0.35, respectively (Fig. S11 and S12). The mean of helical pitch (Table S5) and dihedral angles values (Table S6) were calculated. Additionally, three vicinal water molecules were observed in the model together with hydrogen bonding patterns (Table S7, Figure S13 and S14). The O-H distances were restrained to a value of 0.84 Å. Finally, electrostatic potentials mapped on Hirshfeld surface were studied (Figure S15).

CCDC 2352908 for **14** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Identification code	**14**
Empirical formula	$C_{47}H_{53}F_3N_5O_5$, *3* H_2O
Formula weight	1191.04
Crystal form	
Crystal system, Space group	Triclinic, P 1
Unit cell dimensions a (Å) b (Å) c (Å) α (°) β (°) γ (°)	 6.7234(16) 10.105(2) 19.638(5) 91.057(19) 90.07(2) 93.418(18)
Volume ($Å^3$)	1331.6(5)
Z, Calculated density (Mg/m^3)	1, 1.493
Absorption coefficient (mm^{-1})	0.132
F(000)	626
Crystal size	0.13 x 0.06 x 0.02
Data collection	
Diffractometer	Rigaku XtaLABPro mm003 Pilatus 200K
Temperature (K)	123.0 (2)
Wavelength (Å)	0.71073
θ range for data collection	3.035 to 21.485
Limiting indices	$-6 \le h \le 6$, $-10 \le k \le 10$, $-20 \le l \le 20$
Reflections collected / unique	10538 / 5817 [R(int) = 0.1186]
Completeness to θ_{full} = 21.5°	99.2 %

Absorption correction	Gaussian
Max. and min. transmission	1.000 and 0.808
Structure Refinement	
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5814 / 1240 / 779
Goodness-of-fit on F^2	1.078
Final R indices [I>2σ(I)]	R1 = 0.1101, wR2 = 0.2404
R indices (*all data*)	R1 = 0.1488, wR2 = 0.2600
Largest diff. peak and hole (e.Å^{-3})	0.516 and -0.404
CCDC deposit number	2352908

Table S4: Crystal data, data collection and structure refinement details for ***14***

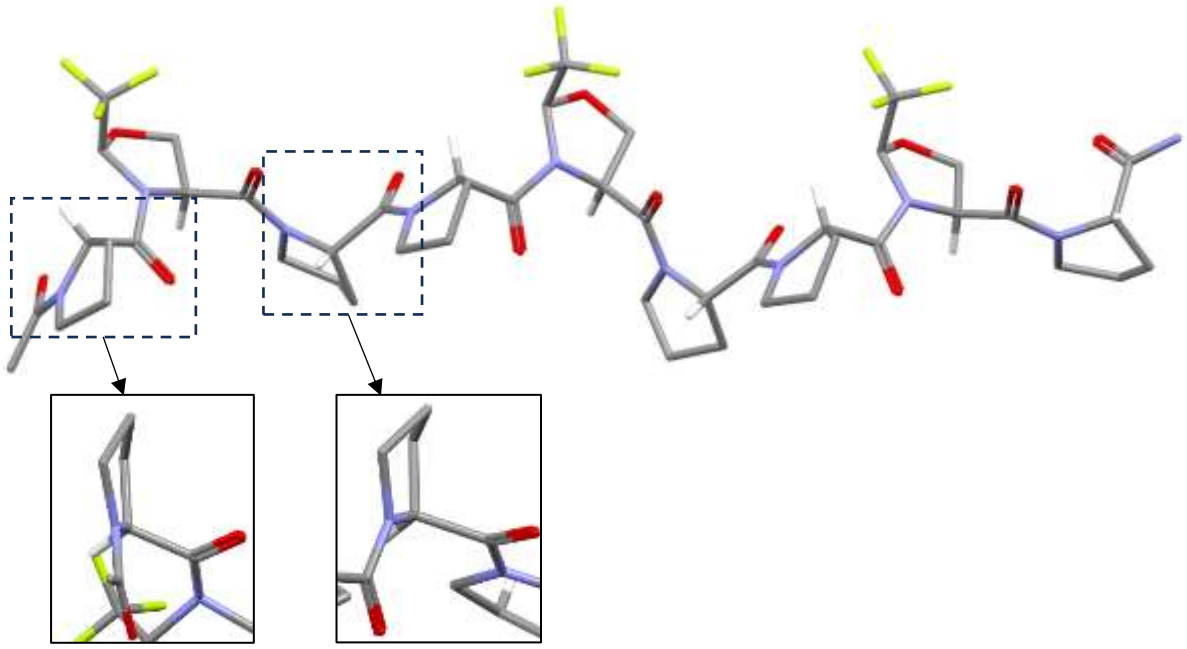


Figure S11: Major X-ray structure of nonamer ***14*** *with Pro[1] (60%) and Pro[3] (65%) in C_γ-endo puckering*

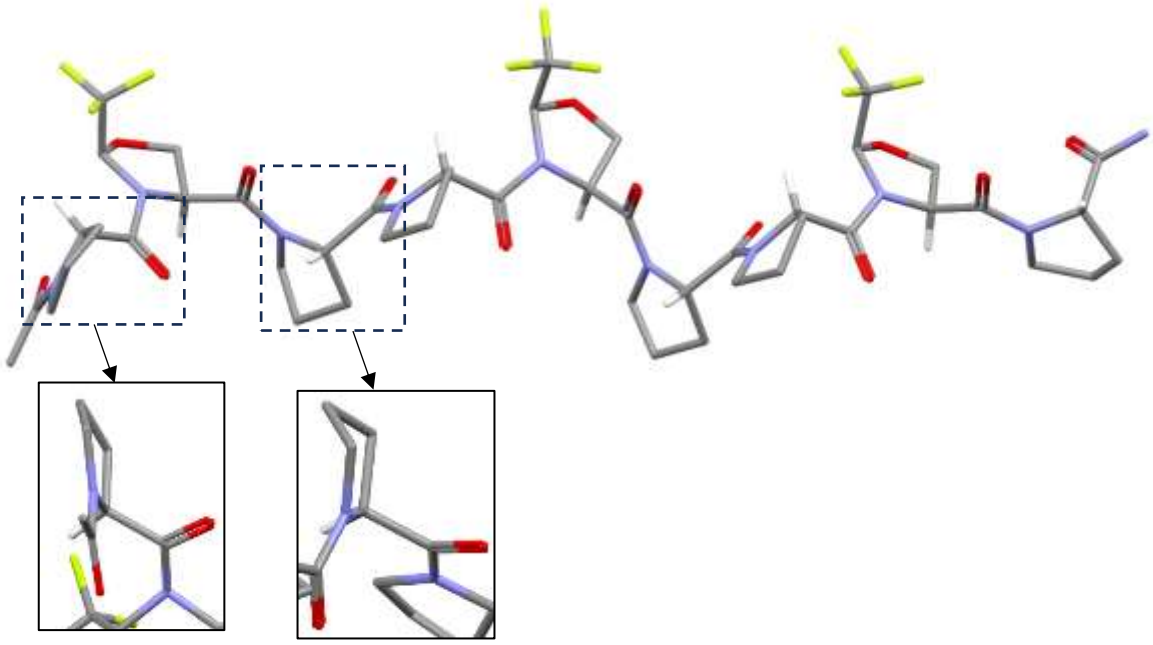


*Figure S12: Minor X-ray structure of nonamer **14** with Pro[1] (40%) and Pro[3] (35%) in C_γ-exo puckering*

VIII.2 Helical pitch

A. Major X-ray structure

Distance every three residues (Å)	Pro^1-Pro^4	$CF_3\Psi Pro^2$-$CF_3\Psi Pro^5$	Pro^3-Pro^6	Pro^4-Pro^7	$CF_3\Psi Pro^5$-$CF_3\Psi Pro^8$	Pro^6-Pro^9
C_α	8.913	9.894	8.99	8.863	8.473	9.84
C_β	8.616	10.809	8.869	8.902	7.835	10.289
C_γ or O	8.227	11.19	8.154	8.823	7.641	10.844
C_δ	8.283	10.578	8.743	8.826	8.052	9.837
C (carbonyl)	8.553	9.728	9.117	8.927	8.466	9.356
O (carbonyl)	8.962	9.484	9.018	8.841	8.724	9.15
Mean (Å)	8.55 ± 0.24	10.18 ± 0.58	8.87 ± 0.24	8.90 ± 0.07	8.25 ± 0.34	9.60 ± 0.69
Global mean (Å)	**9.06 ± 0.62**					

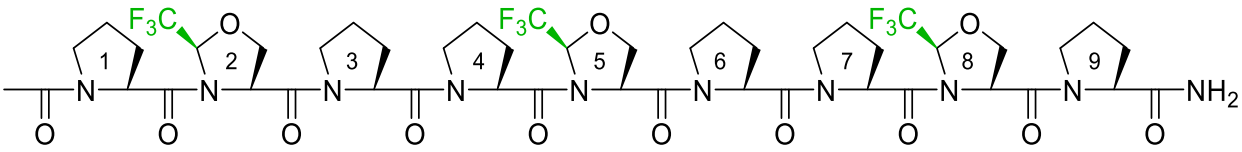
B. Minor X-ray structure

Distance every three residues (Å)	Pro^1-Pro^4	$CF_3\Psi Pro^2$-$CF_3\Psi Pro^5$	Pro^3-Pro^6	Pro^4-Pro^7	$CF_3\Psi Pro^5$-$CF_3\Psi Pro^8$	Pro^6-Pro^9
C_α	8.913	9.894	8.99	8.863	8.473	9.84
C_β	8.616	10.809	8.869	8.902	7.835	10.289
C_γ or O	8.874	11.19	8.868	8.823	7.641	10.844
C_δ	8.283	10.578	8.743	8.826	8.052	9.837
C (carbonyl)	8.553	9.728	9.117	8.927	8.466	9.356
O (carbonyl)	8.962	9.484	9.018	8.841	8.724	9.15
Mean (Å)	8.64 ± 0.23	10.18 ± 0.58	8.97 ± 0.12	8.90 ± 0.07	8.25 ± 0.34	9.60 ± 0.69
Global mean (Å)	**9.09 ± 0.60**					

*Table S5: Distance every three residues between the C_α, C_β, C_γ or O, C_δ, C carbonyl and O carbonyl atoms for the major (**A**) and minor (**B**) X-ray structure. The average of all these distances gives the value of the helix pitch. The calculated error corresponds to the standard deviations. Red values correspond to differences between the two X-ray structure*

The major and minor X-ray structures give access to very close helix pitch values since only two distances differ between C_γ(Pro^1) - C_γ(Pro^4) and C_γ(Pro^3) - C_γ(Pro^6) due to the different C_γ-*exo* puckering of Pro^1 and Pro^3 in these two structures.

VIII.3 Dihedral angles values



	Ac	**Pro^1**	**CF$_3$ΨPro^2**	**Pro^3**	**Pro^4**	**CF$_3$ΨPro^5**	**Pro^6**	**Pro^7**	**CF$_3$ΨPro^8**	**Pro^9**
ϕ (°)	-	-56	-75	-81	-74	-79	-47	-69	-85	-82
ψ (°)	-	+142	+140	+145	+154	+137	+136	+132	+168	+174
ω (°)	+176	-173	-156	+176	+178	+175	-178	+179	+172	-

*Table S6: Dihedral angles table of nonamer **14** X-ray structure. The two X-ray structures obtained have the same ϕ, ψ and ω dihedral angles, since they differ only in the puckering on prolines 1 and 3. φ angles: (C_{i-1}-N_i-$C_{\alpha i}$-C_i); ψ angles: (N_i-$C_{\alpha i}$-C_i-N_{i+1}); ω angles: ($C_{\alpha i}$-C_i-N_{i+1}-$C_{\alpha i+1}$). Ideal values for PPII helix: ϕ = -75°, ψ = 150 ° and ω = 180° or -180°*

The introduction of trifluoromethyl-pseudoproline residues caused distortions of certain dihedral angle values which deviate from those reported for an ideal PPII-helix.

VIII.4 Hydrogen-bonding patterns in nonamer 14

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O1W-H1W1...O6	0.84(3)	2.24(5)	3.02(2)	158(13)
O1W-H1W2...O2W$^{\#1}$	0.84(3)	1.99(5)	2.75(2)	150(12)
O2W-H2W1...O9	0.84(3)	1.93(5)	2.75(2)	167(20)
O2W-H2W2...O3W$^{\#5}$	0.84(3)	1.96(5)	2.78(2)	164(19)
O3W-H3W1...O8	0.84(3)	2.14(4)	2.969(19)	169(19)
O3W-H3W2...O1W$^{\#2}$	0.84(3)	2.02(6)	2.81(2)	156(15)
N10-H10A...O10$^{\#2}$	0.88	2.55	3.42(3)	169.9
N10-H10B...O10$^{\#3}$	0.88	2.11	2.92(3)	152.0

Table S7: Water hydrogen bonding pattern for 14 (Å and °). Symmetry transformations used to generate equivalent atoms: #1 x,y+1,z ; #2 x,y-1,z ; #3 x,y-2,z+1 ; #5 x-1,y,z

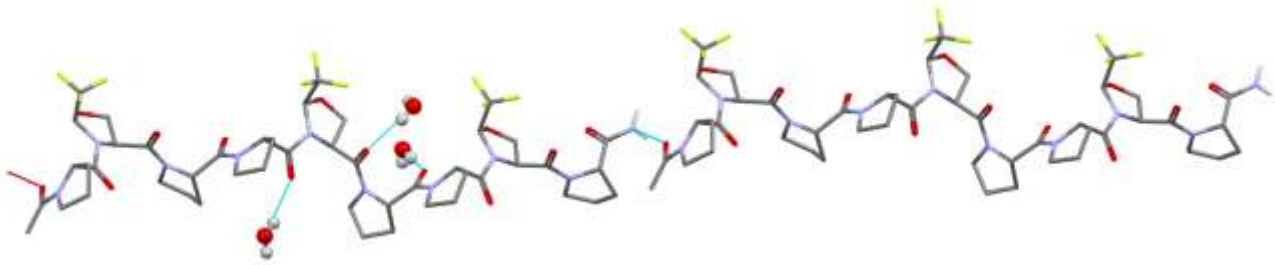


*Figure S13: H-bonding patterns (cyan dashed lines) between nonamers **14** (capped sticks style) and water molecules (ball and stick style)*

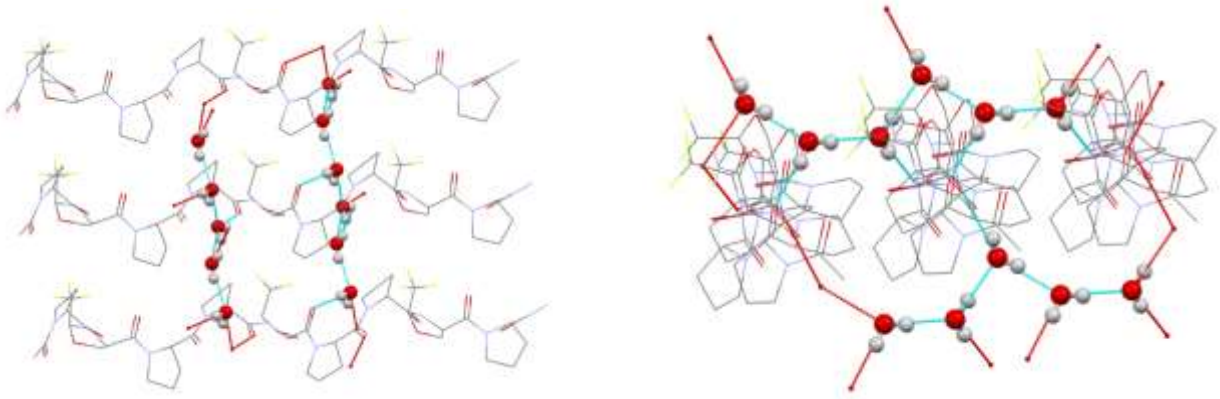
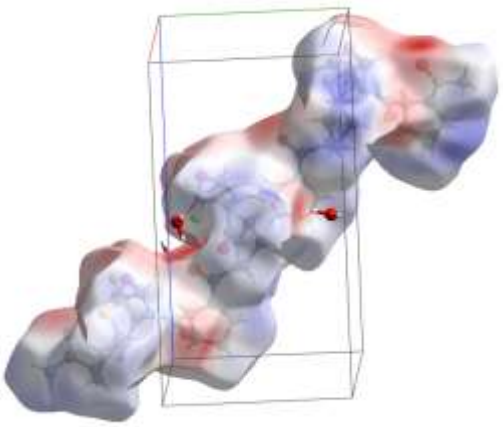


*Figure S14: H-bonding patterns between water molecules (ball and stick style) and three molecules of nonamer **14** (wireframe style); A. from a side view B. from a top view*

VIII.5 Electrostatic potentials mapped on Hirshfeld surface of nonamer 14

A.



B.

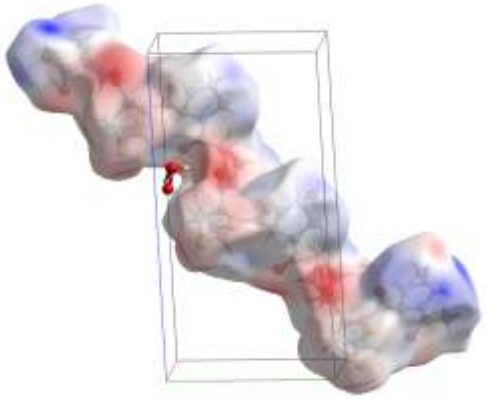


*Figure S15: Electrostatic potentials mapped on Hirshfeld surface of nonamer **14** X-ray structure. **A**. from prolines side (positive potential), **B**. from trifluoromethyl-pseudoprolines side (negative potential)*

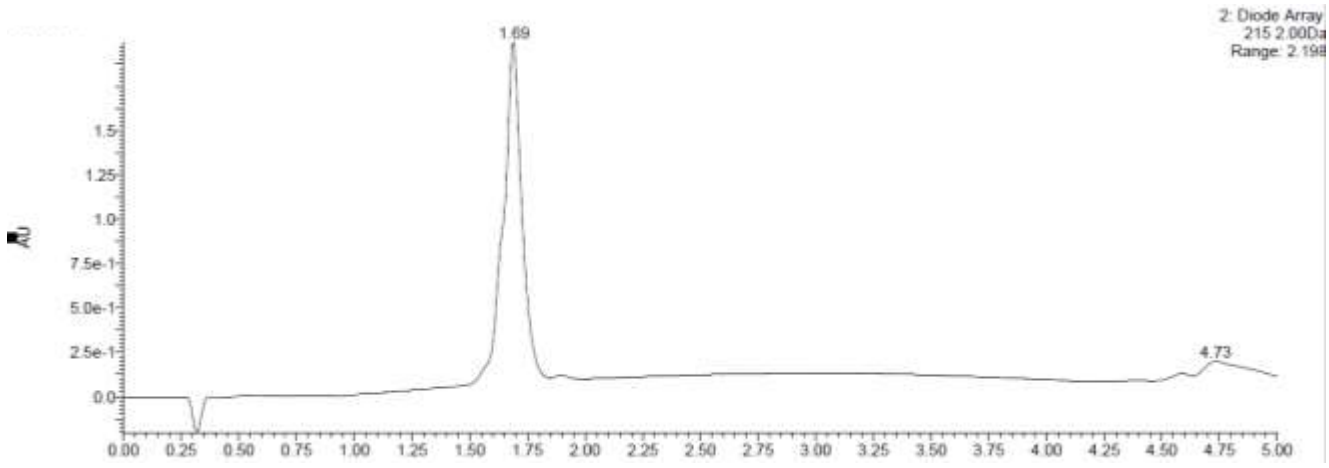
IX. COMPARISON OF HYDROPHOBICITY BY UPLC-MS RETENTION TIME

Oligomers	Retention time (min)
Ac-[Pro]$_6$-NH$_2$ **6**	1.34
Ac-[Pro-CF$_3$ΨPro-Pro]$_2$-NH$_2$ **7**	1.71
Ac-[Pro]$_9$-NH$_2$ **10**	1.69
Ac-[Pro-CF$_3$ΨPro-Pro]$_3$-NH$_2$ **14**	2.03

Table S8: Peptide retention time for hydrophobicity comparison (measured with a 10-90% ACN + 0.1% TFA gradient over 3 min)

The hydrophobicity of the oligomers has been assessed thanks to the retention time values obtained by RP-UPLC (Table S8). Retention time values of hexamers **6** and **7** and nonamer **14** were reported in Table S3 (see section IV.4). For comparison propose, the UPLC-MS of nonamer **10** was performed using the same gradient, *i.e* 10-90% ACN + 0.1% TFA using H_2O + 0.1% TFA as a co-solvent (UV chromatogram below). The retention time values of fluorinated oligomers were found all superior to that of their non-fluorinated analogues, in accordance with an increase of hydrophobicity.

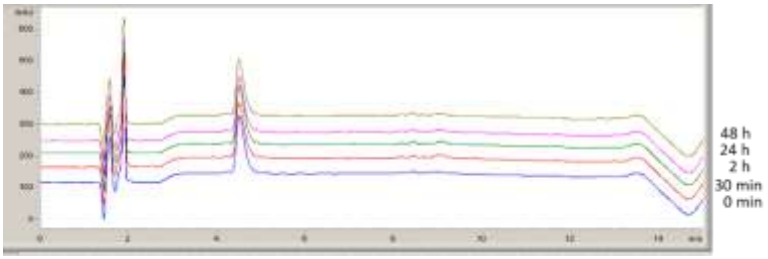
Nonamer **10**



X. RESULTS OF STABILITY TEST WITH PRONASE OF HEXAMERS 6-7

X.1 Test followed by UPLC-MS

A.



B.

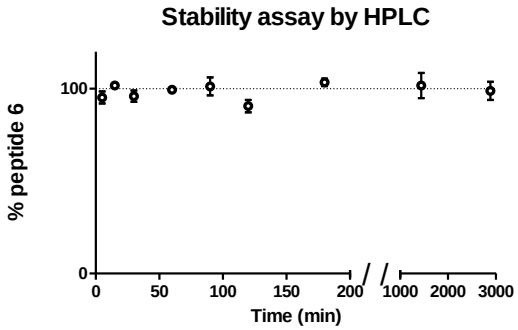
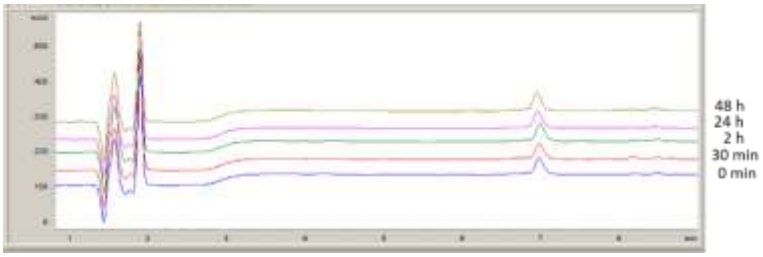


*Figure S16: **A**. HPLC chromatogram at 214 nm of hexamer **6**; **B**. Amount of intact hexamer **6** (%) quantified over time by HPLC*

A.



B.

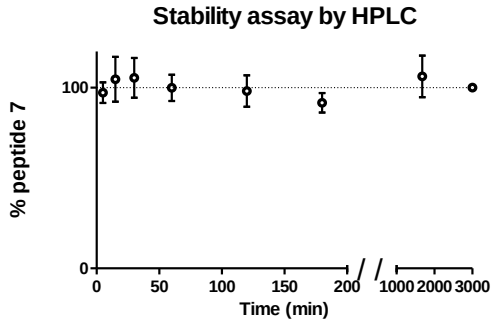


*Figure S17: **A**. HPLC chromatogram at 214 nm of hexamer **7**; **B**. Amount of intact hexamer **7** (%) quantified over time by HPLC*

Time	Ac-[Pro]$_6$-NH$_2$ 6		Ac-[Pro-CF$_3$ΨPro-Pro]$_2$-NH$_2$ 7	
	Peak area (UV trace at 214 nm)	% peptide	Peak area (UV trace at 214 nm)	% peptide
5 min	409	108	2319	99
	357	94	2254	97
	337	89	2068	89
15 min	409	108	2319	99
	357	94	2254	97
	337	89	2068	89
30 min	321	84	2115	91
	463	122	2213	95
	412	108	2360	101
60 min	345	91	2263	97
	432	114	2338	100
	356	94	2330	100
90 min	332	87	2298	98
	436	115	2190	94
	345	91	2571	110
120 min	332	87	2261	97
	436	115	2039	87
	345	91	2017	86
180 min	326	97	2340	100
	288	*76*	2376	102
	367	86	2495	107
1440 min	358	94	2129	91
	388	102	2664	114
	432	114	2295	98
2880 min	358	94	2216	95
	445	117	2152	92
	n.o	n.o	2519	108

n.o. : not observed

Table S9: Peptide stability (%) monitoring by HPLC

X.2 Followed by ^{19}F NMR

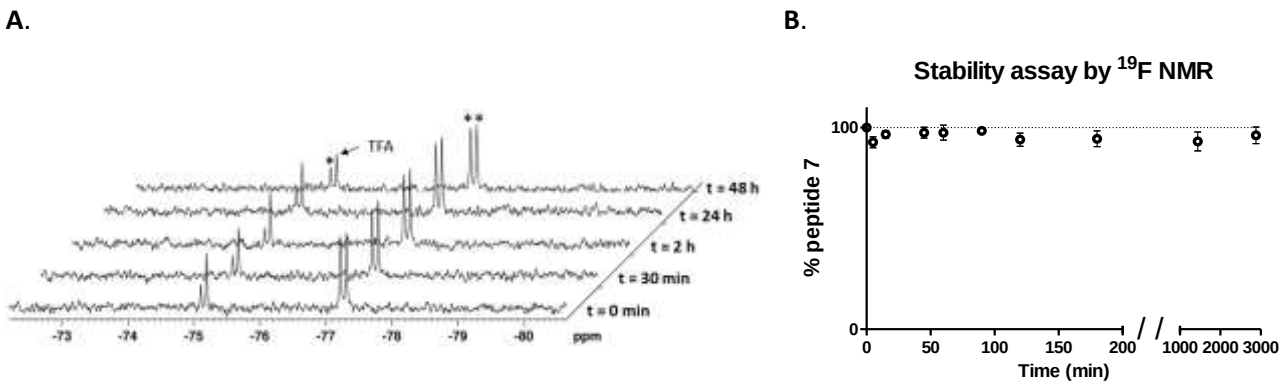


*Figure S18: **A**. ^{19}F NMR spectra of hexamer **7**; **B**. Amount of intact hexamer **7** (%) quantified over time by ^{19}F NMR*

A second peak close to the signal of TFA is observed on ^{19}F NMR spectra. This signal only appears when using Tris buffer solution. Despite several attempts to control whether it could correspond to a TFA salt of Tris compound, we were unable to confirm such hypothesis.

Time	Ac-[Pro-CF_3ΨPro-Pro]$_2$-NH_2 7	
	Peptide integration vs TFA peak = 1	**% peptide**
0 min	3,41 (mean value from triplicate)	100
5 min	3,12	92
	3,11	91
	3,26	96
15 min	3,33	98
	3,29	97
	3,22	94
45 min	3,41	100
	3,33	98
	3,22	94
60 min	3,41	100
	3,15	93
	3,41	100
90 min	3,33	98
	3,11	97
	3,41	100
120 min	3,29	97
	3,11	91
	3,22	94
180 min	3,33	98
	3,10	91
	3,22	94
1440 min	2,9	88
	3,29	97
	3,22	94
2880 min	3,43	101
	3,22	94
	3,15	93

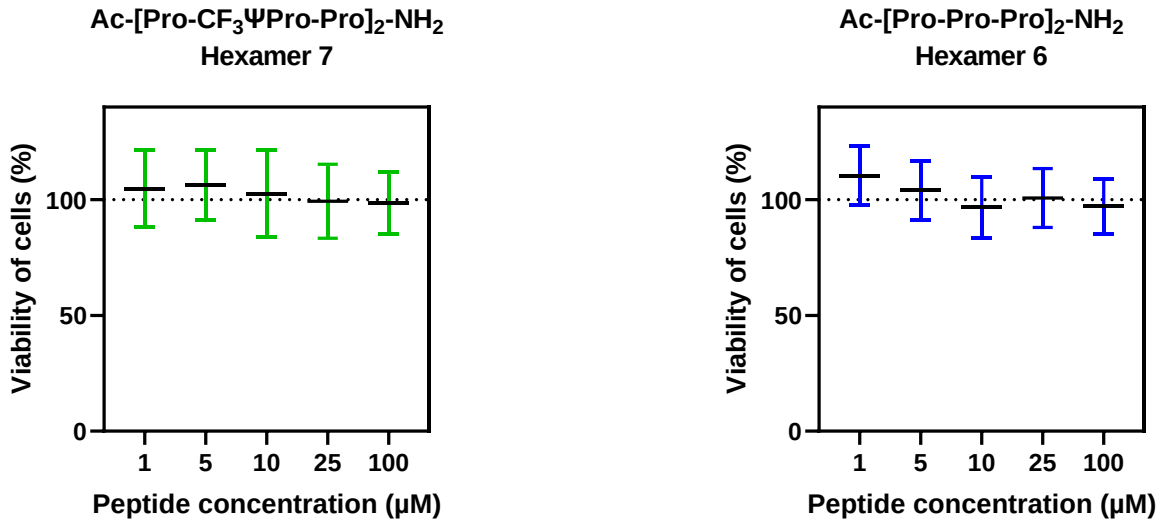
Table S9: Peptide stability (%) monitoring by ^{19}F NMR

X.3 Positive control assay

In order to control the Pronase activity, we performed a positive control assay. This test was performed on the peptide Mca-GKPILFFRLK(DNP)R-NH_2 substrate of cathepsin D. Complete degradation was observed after 15 min.

XI. RESULTS OF VIABILITY ASSAYS OF HEXAMERS 6-7

Incubation time with CHO-K1 cells: 1h



Incubation time with CHO-K1 cells: 24h

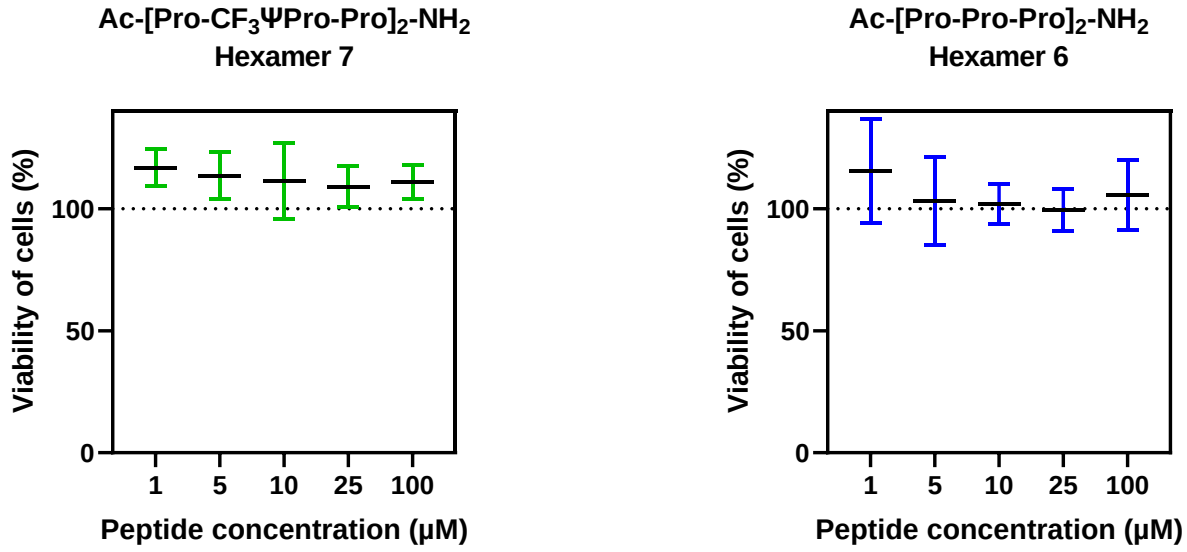


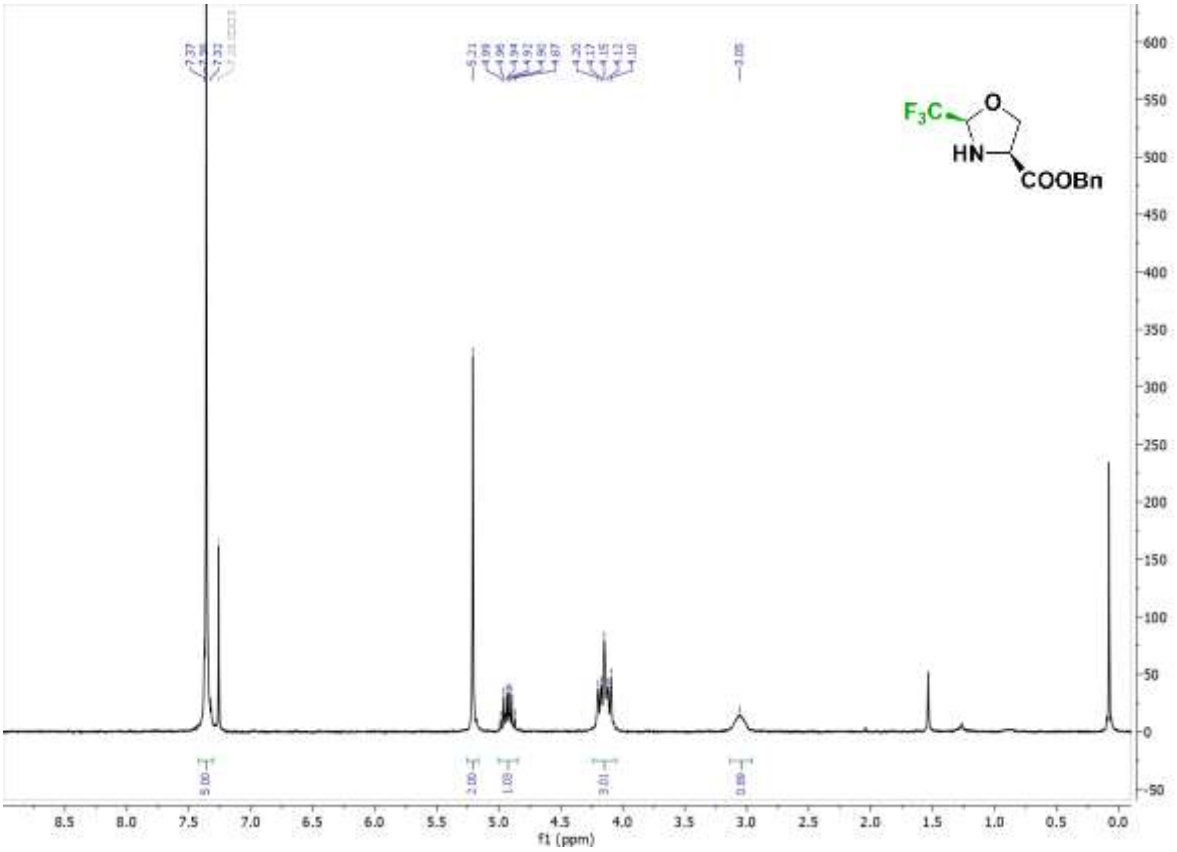
Figure S19: Results of viability assay on CHO-K1 with hexamers 6-7 with 1 hour incubation (top) and 24h incubation (bottom)

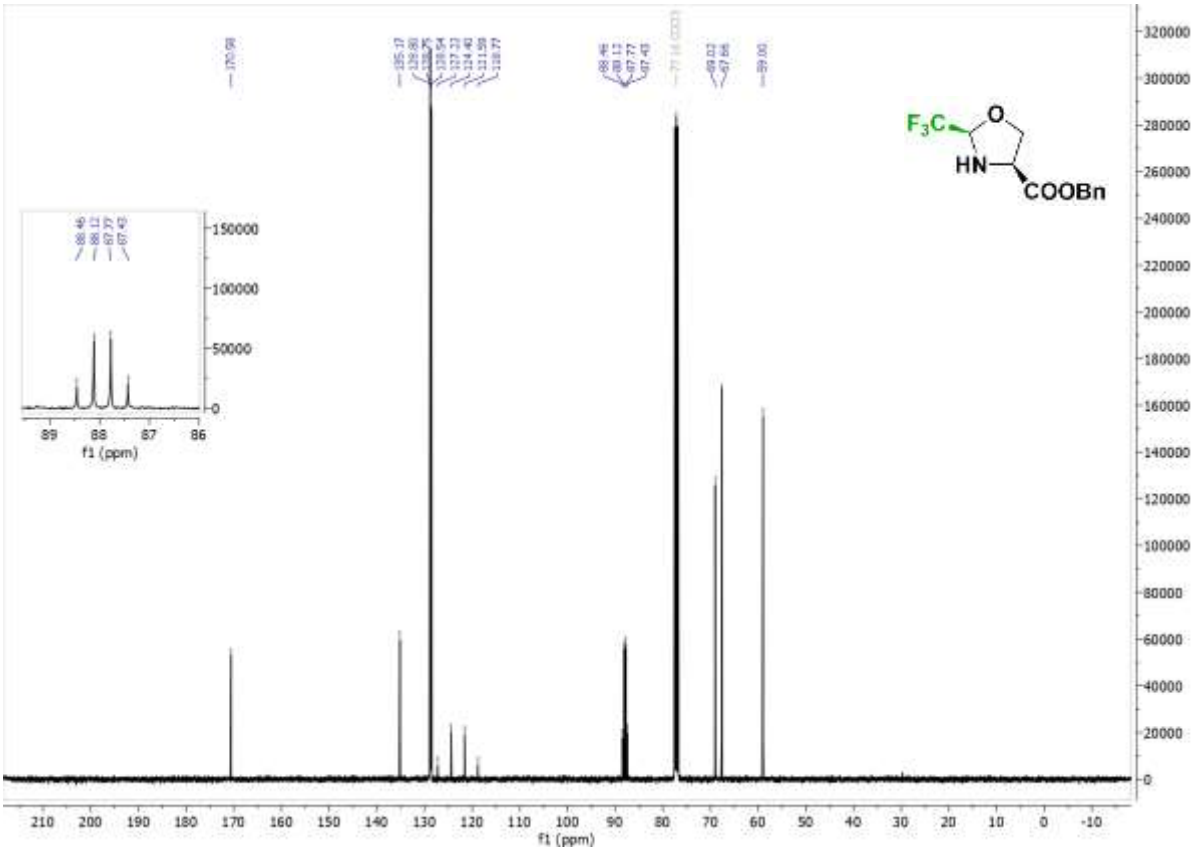
After 1h or 24h incubation of healthy CHO-K1 mammalian cells with our foldamers **6** and **7**, no toxicity was revealed. Thus, the fluorinated foldamer **7** is as non-cytotoxic as its non-fluorinated analogue **6** counterpart.

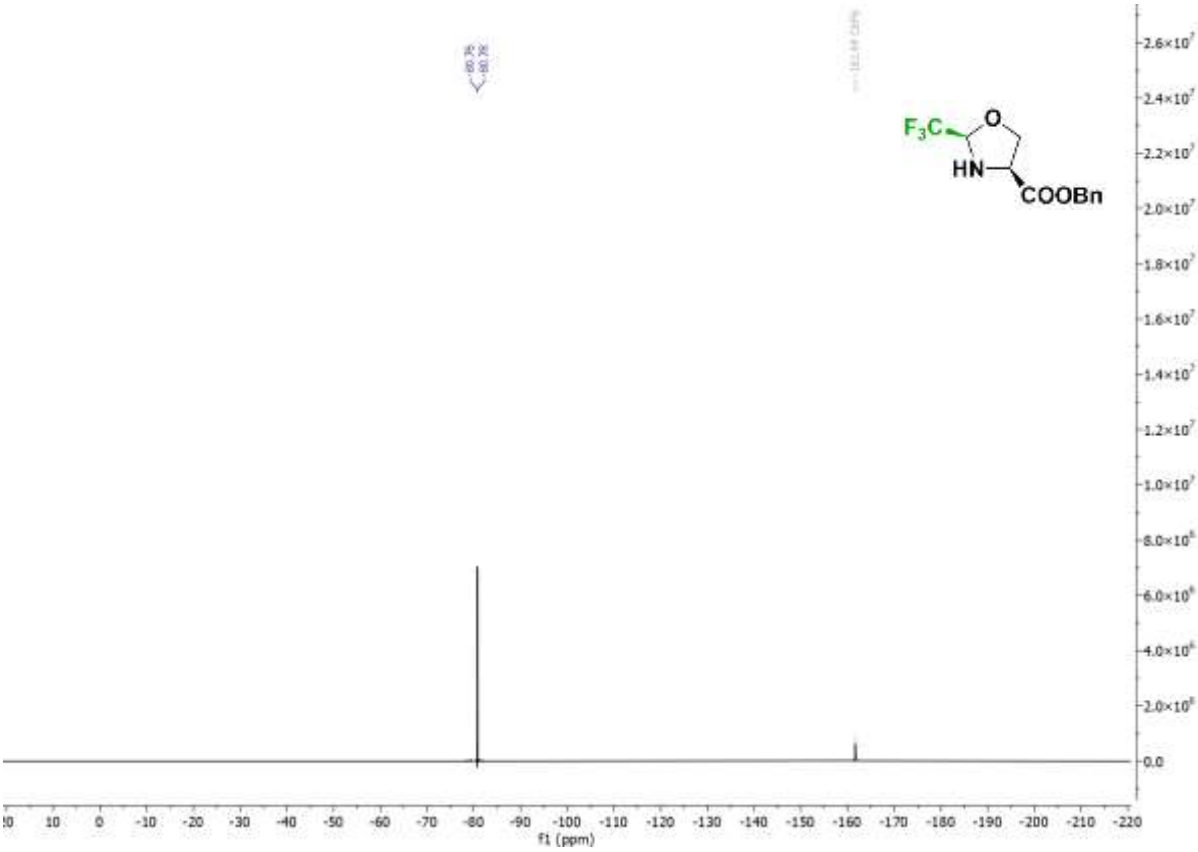
XII. NMR SPECTRA

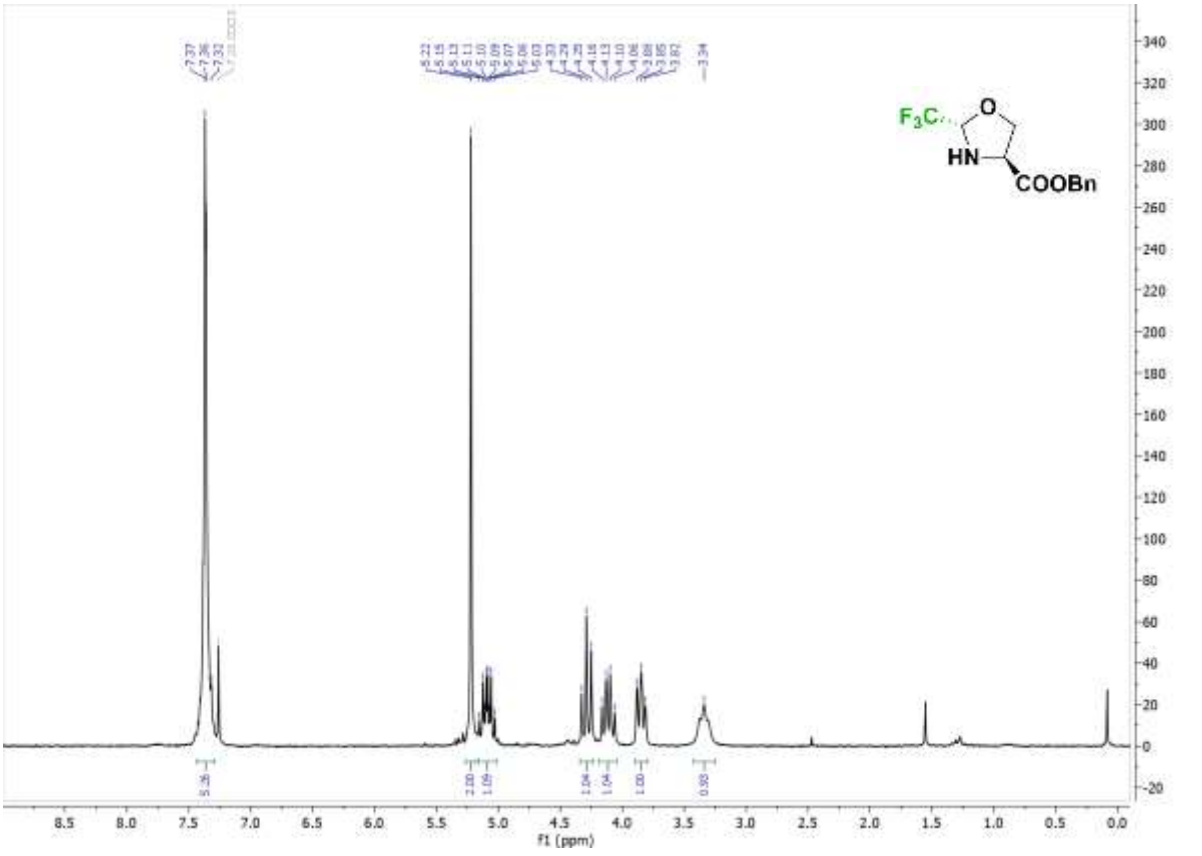
XII.1 Building blocks Fmoc-Pro-CF_3ΨPro

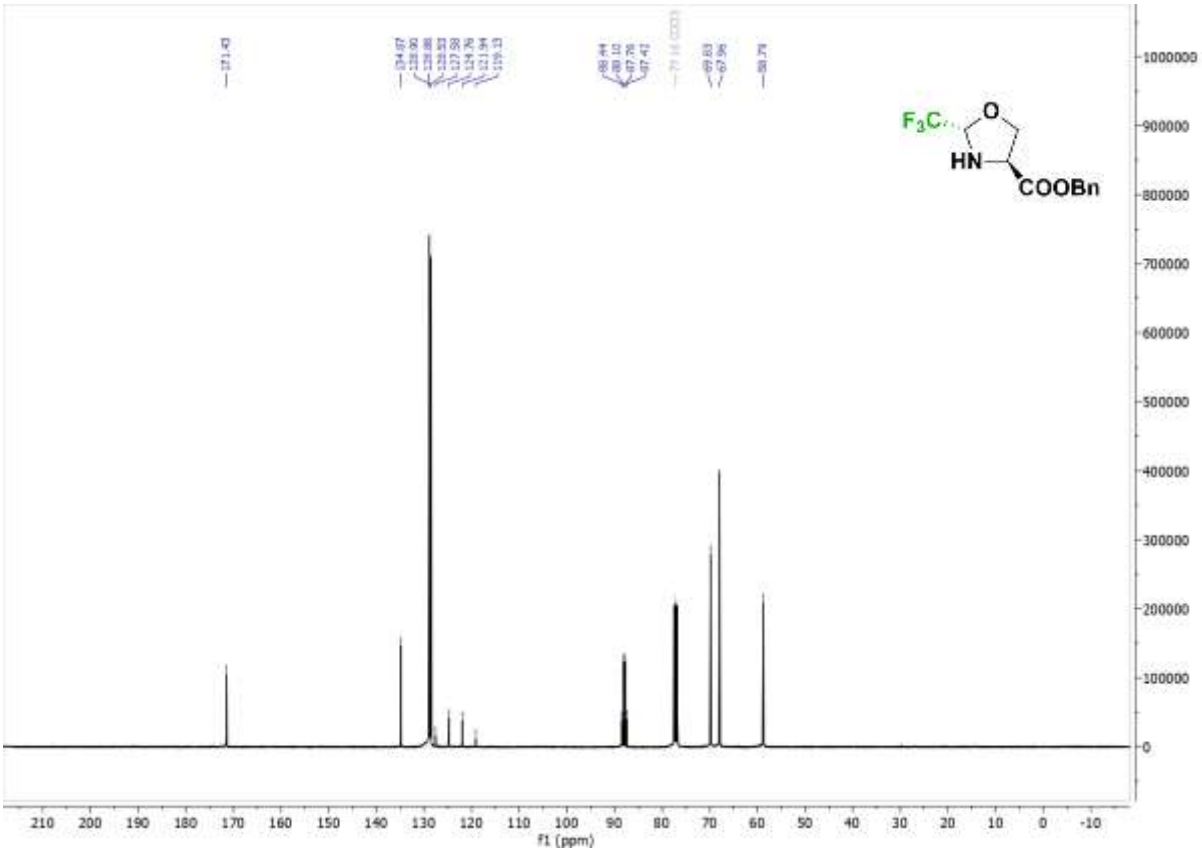
XII.1.1 CF_3ΨPro

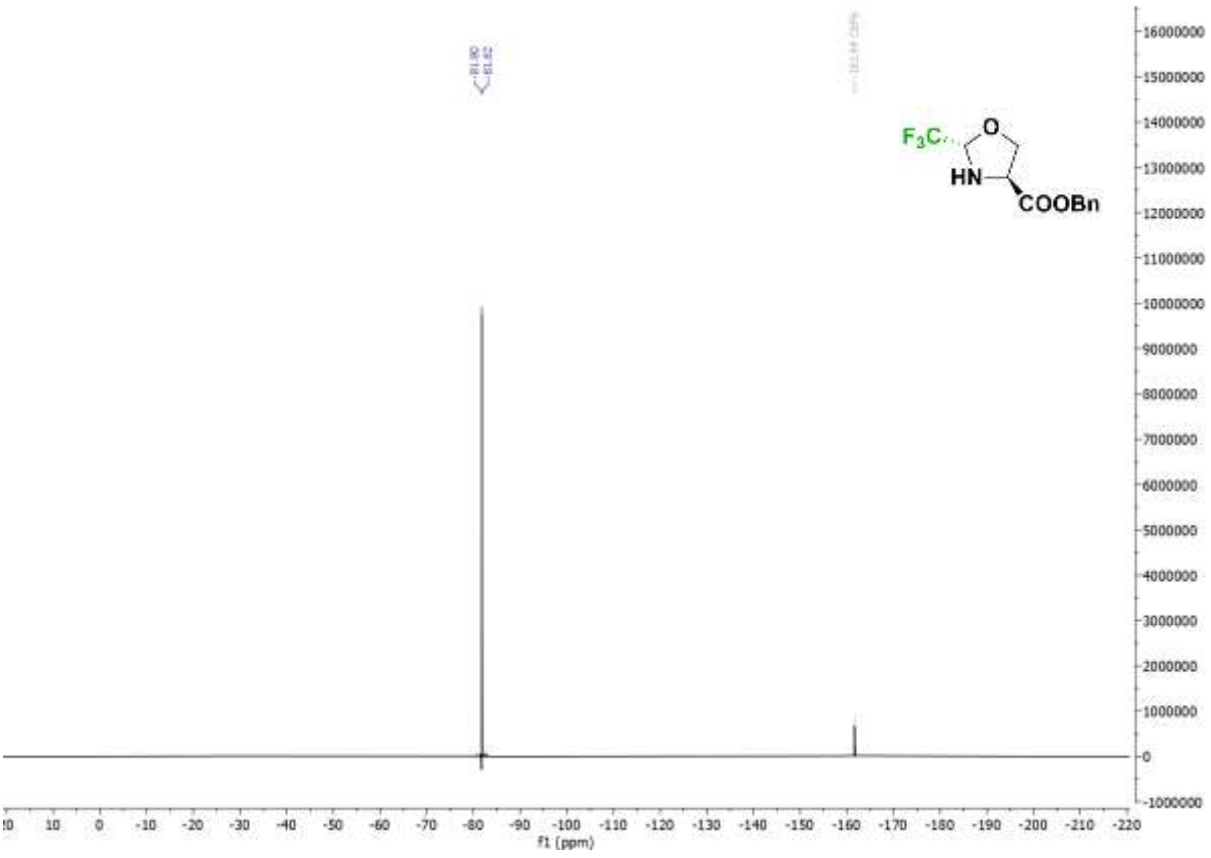



F3C
O
HN
COOBn
f1 (ppm)

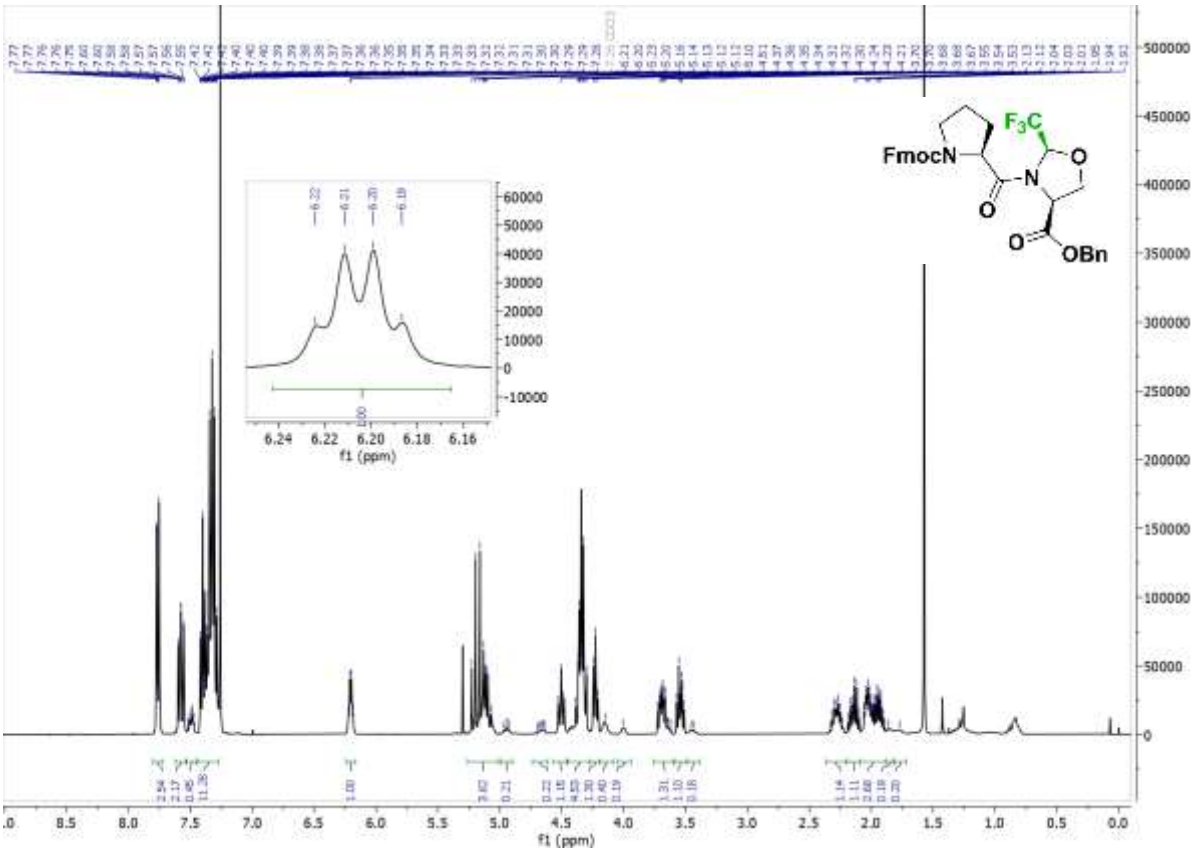

F3C
O
HN
COOBn
f1 (ppm)

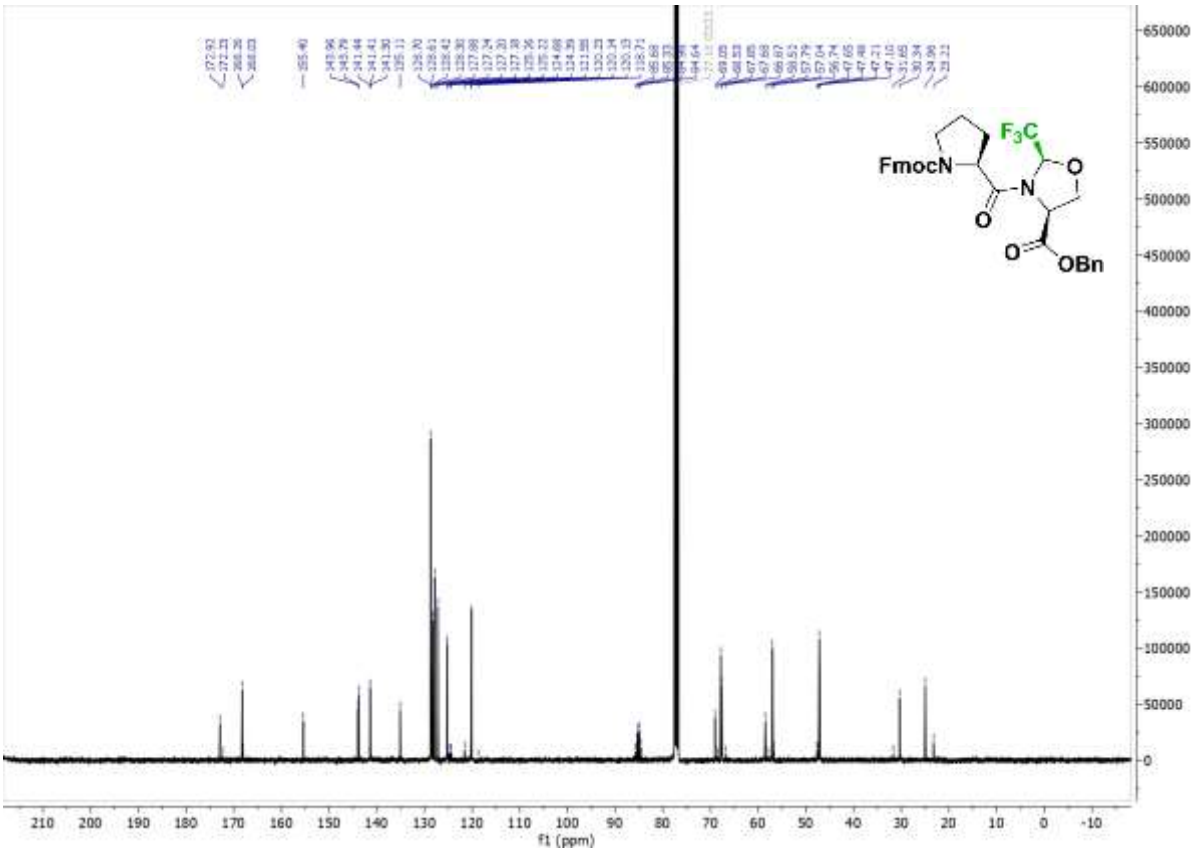

F3C
O
HN
COOBn
f1 (ppm)

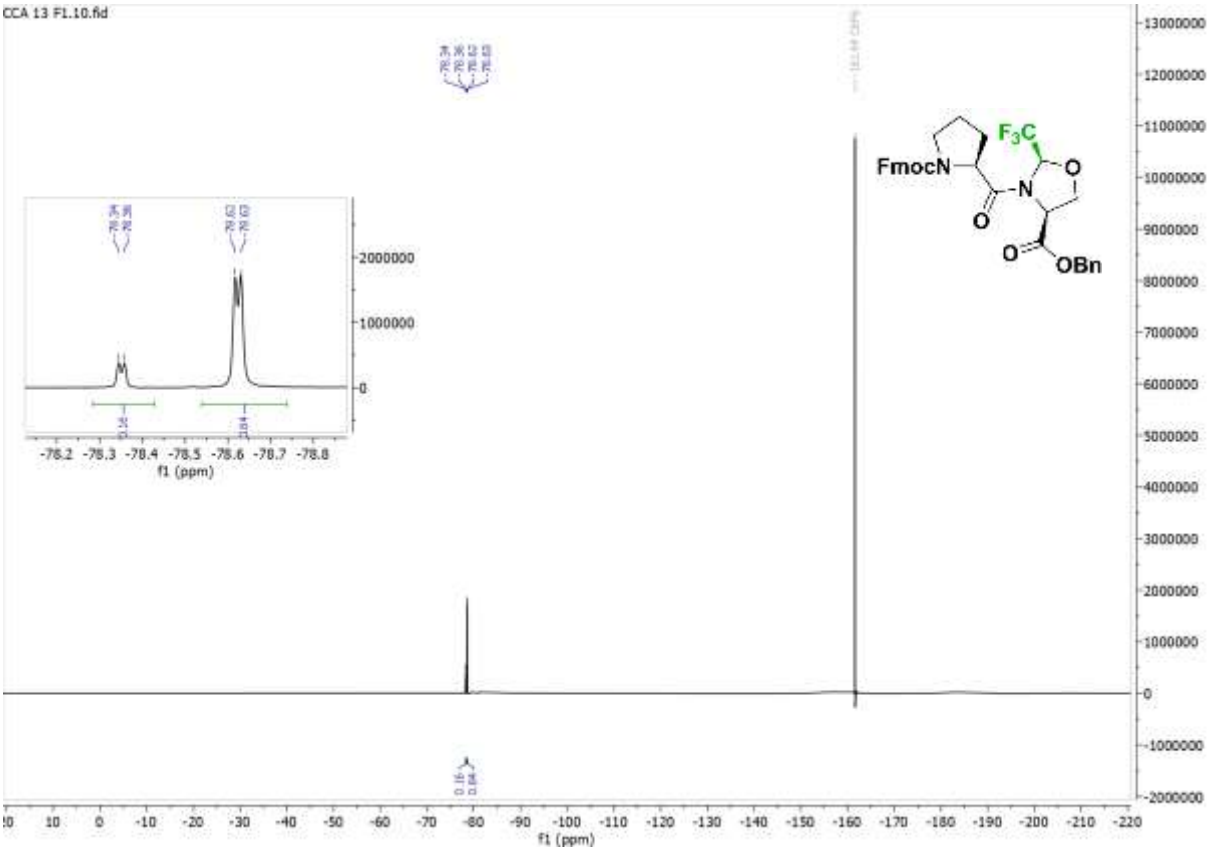

F3C
O
HN
COOBn
f1 (ppm)


F3C
O
HN
COOBn
f1 (ppm)

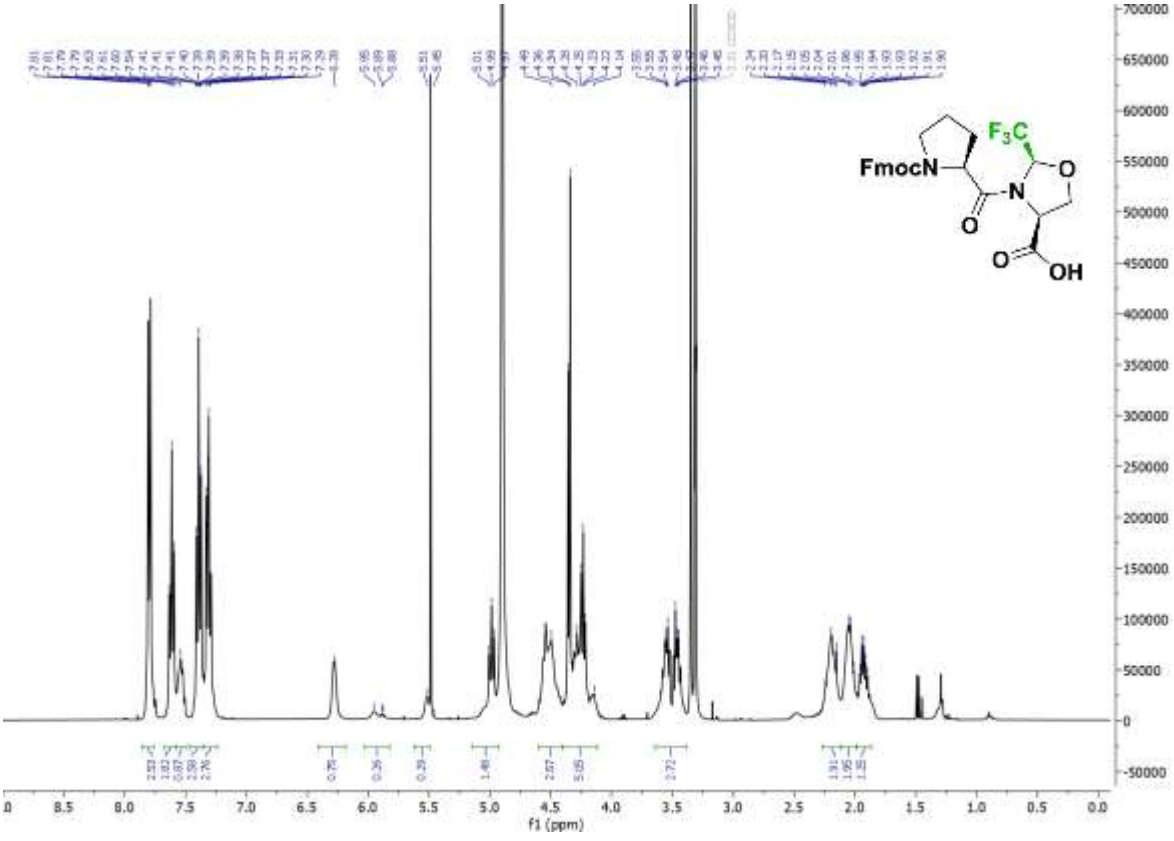
XII.1.2 Fmoc-L-Pro-$CF_3\Psi$Pro-OBn

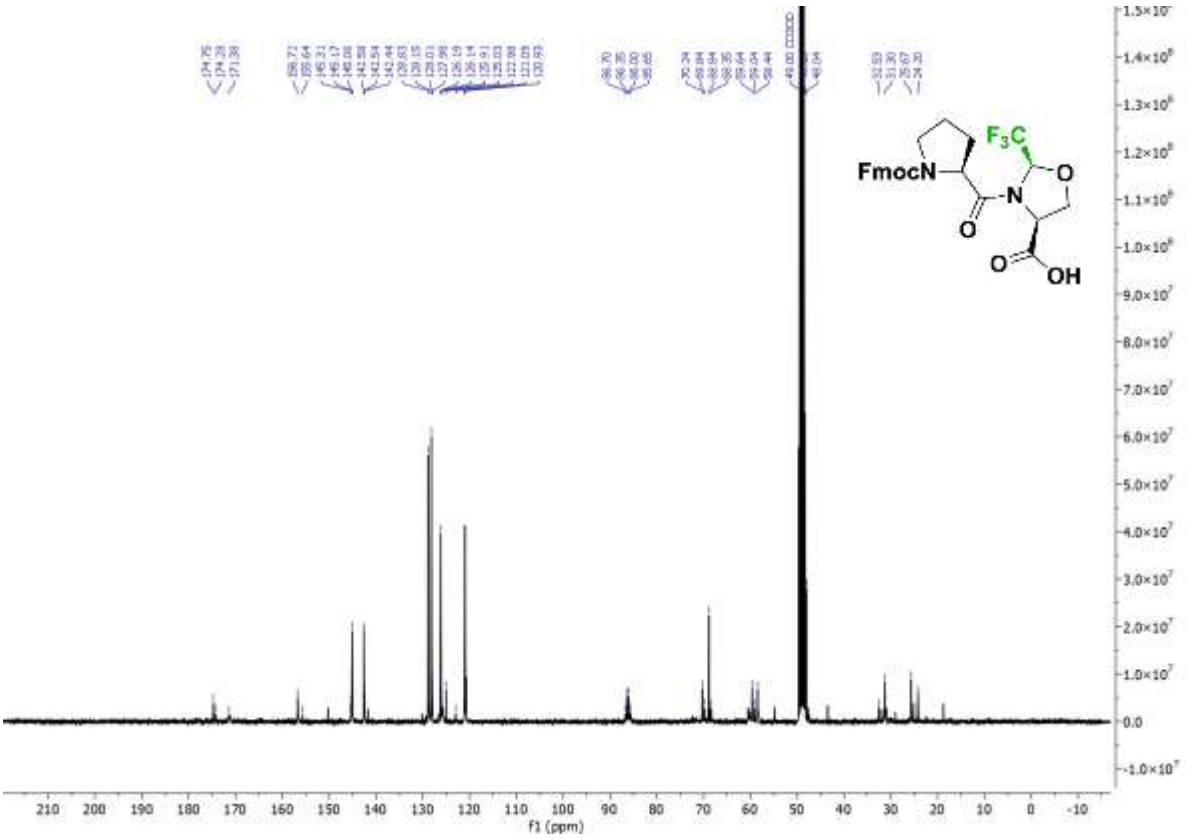


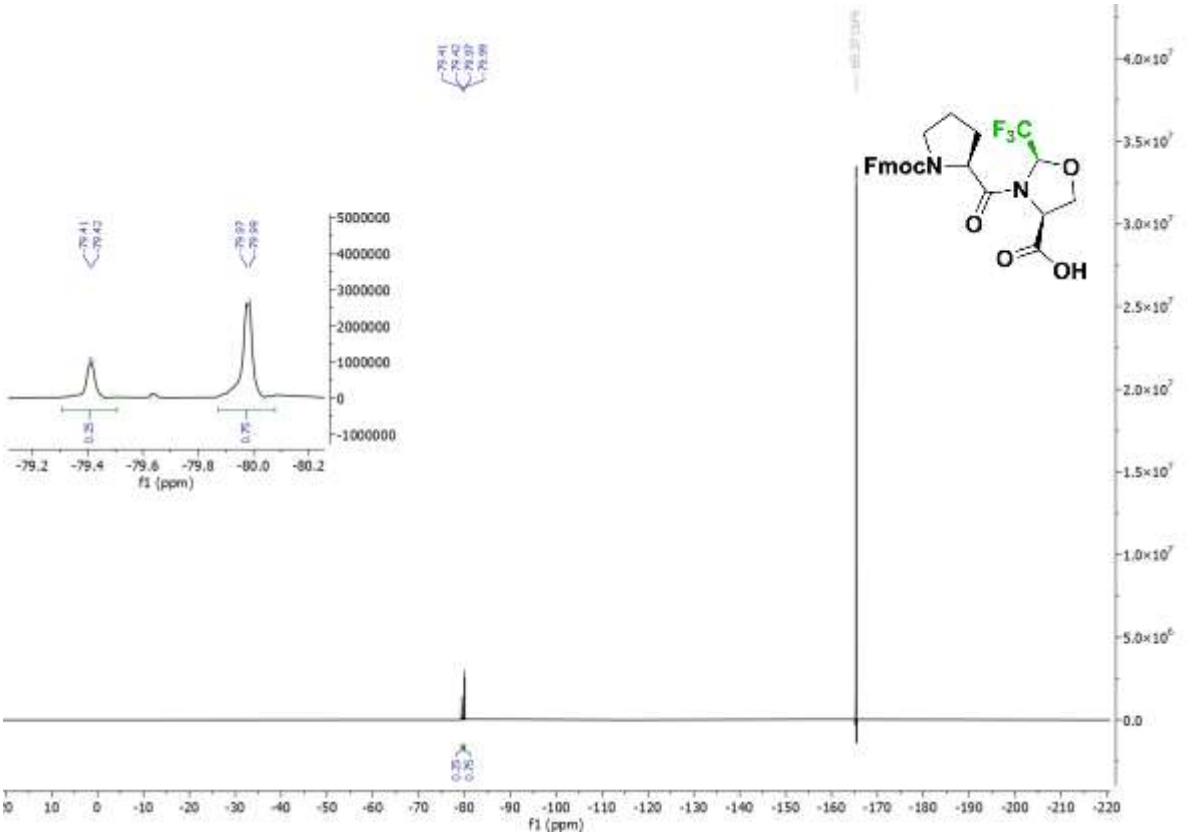




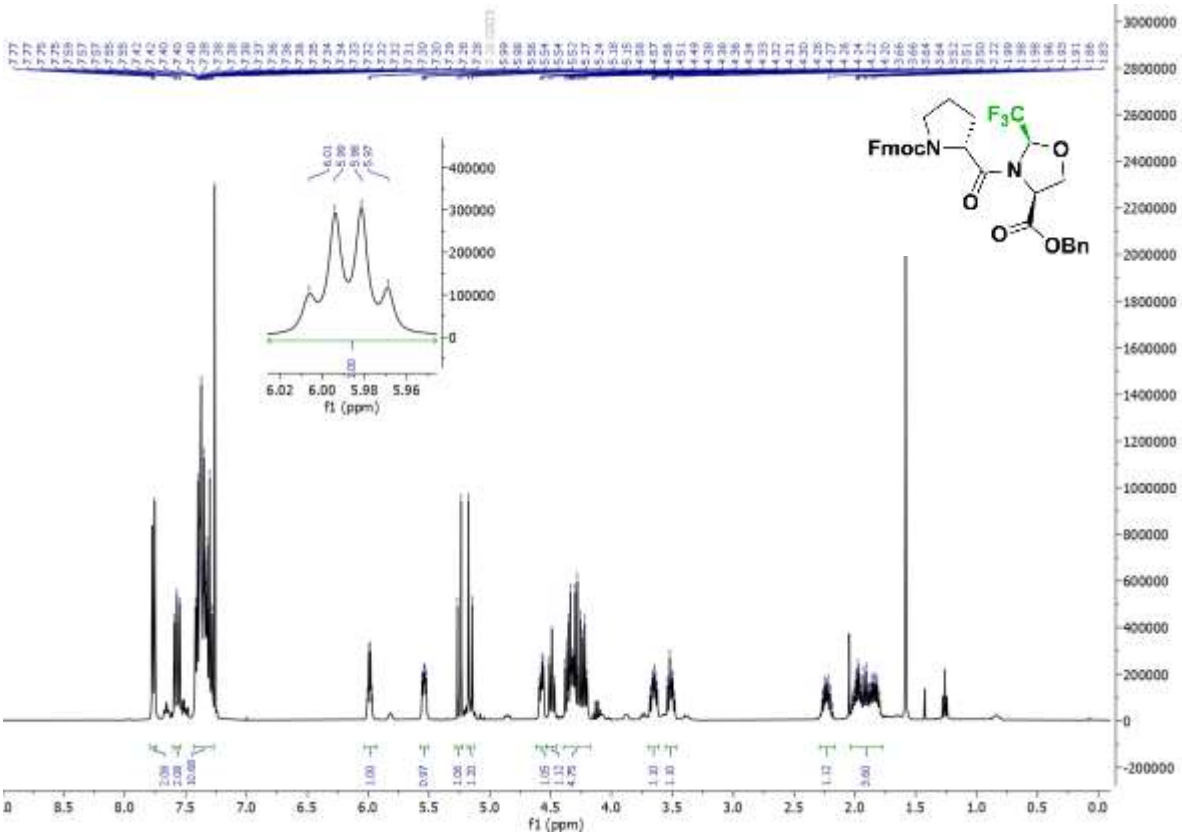
XII.1.3 Fmoc-L-Pro-CF_3ΨPro-OH

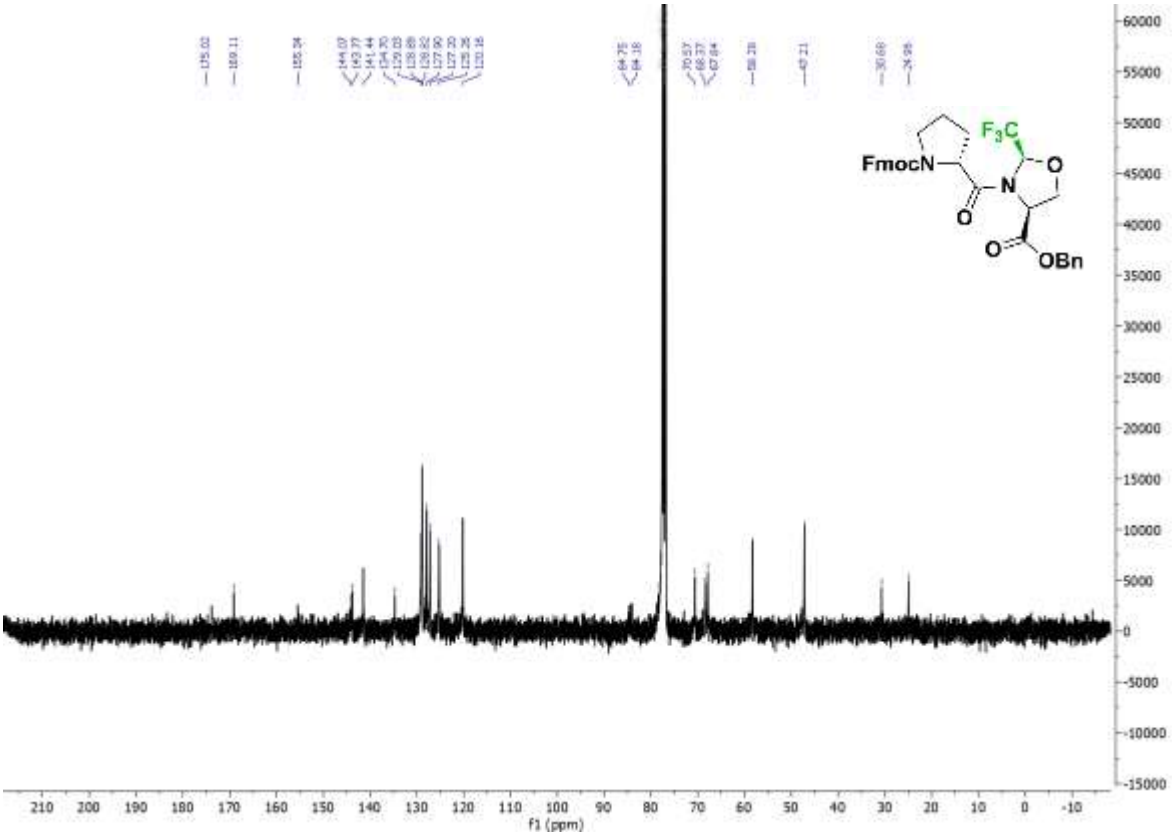


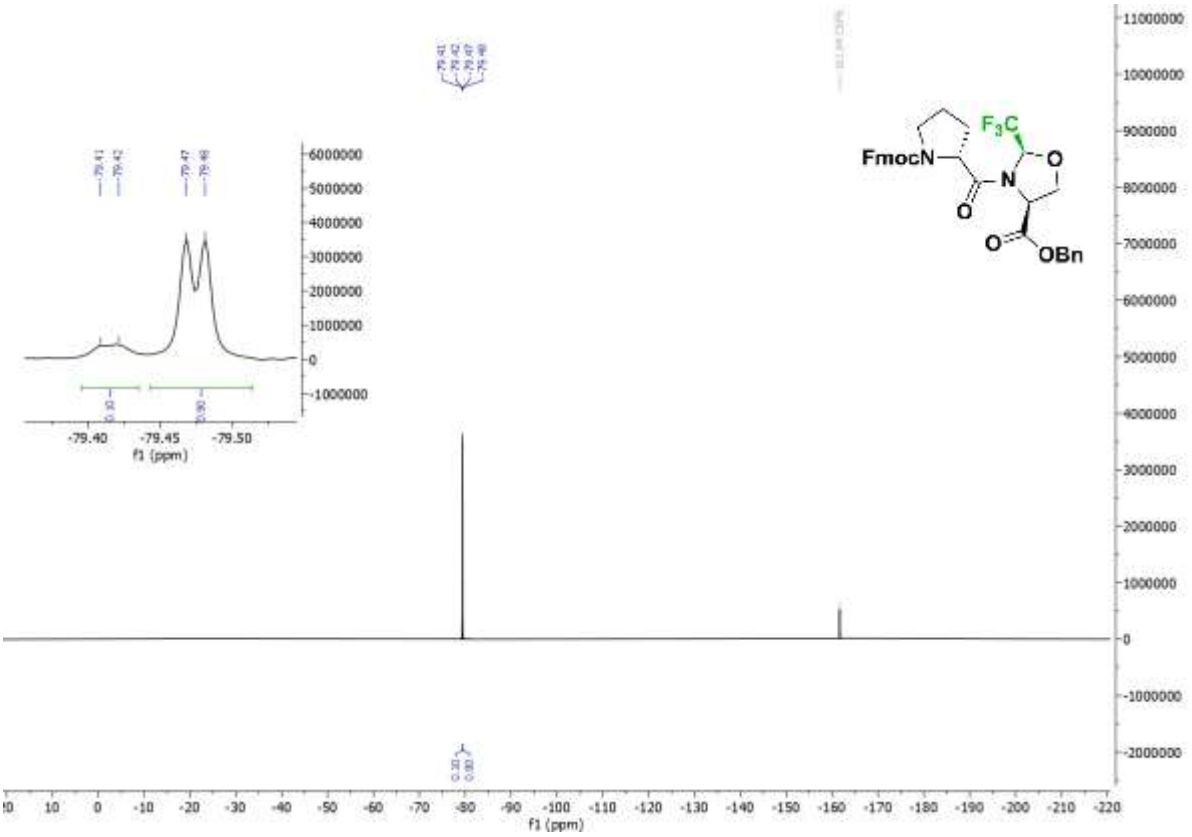

FmocN
F3C
O
N
O
O
OH
f1 (ppm)


FmocN
F3C
O
N
O
O
OH
f1 (ppm)

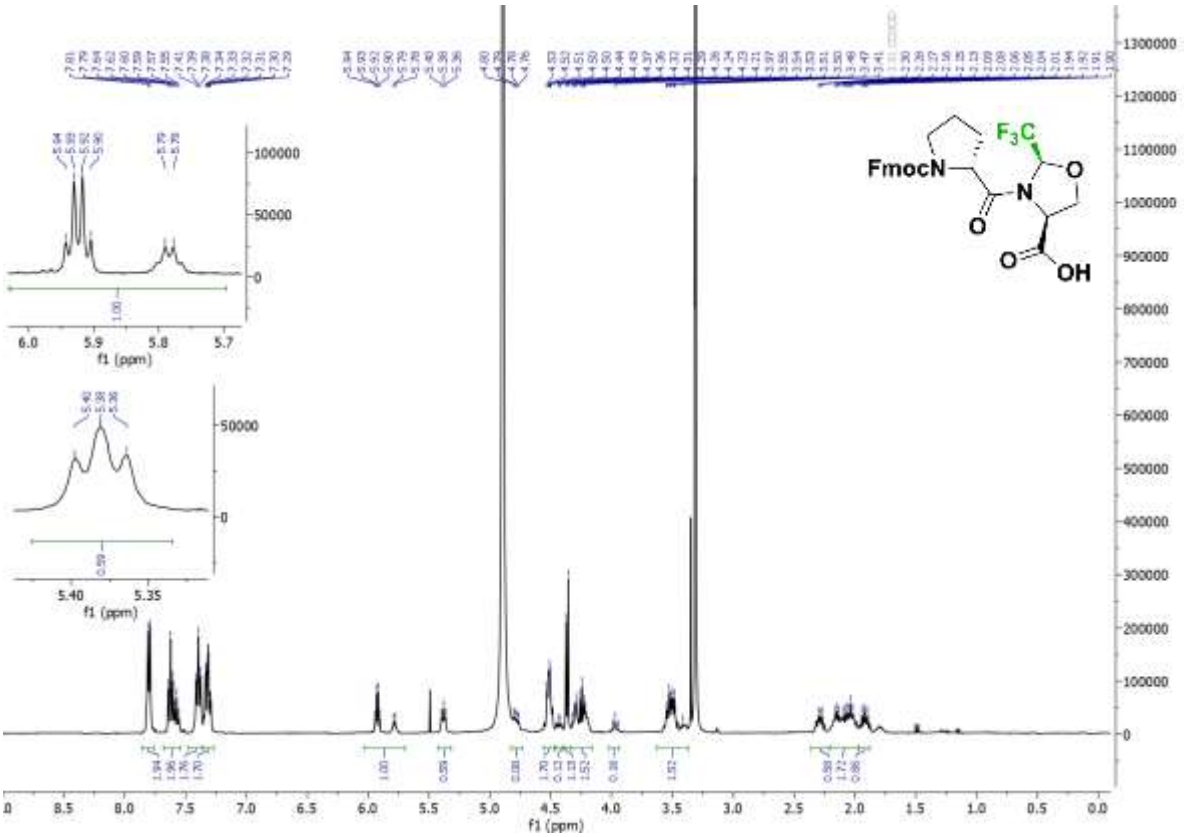
XII.1.4 Fmoc-D-Pro-$CF_3\Psi$Pro-OBn

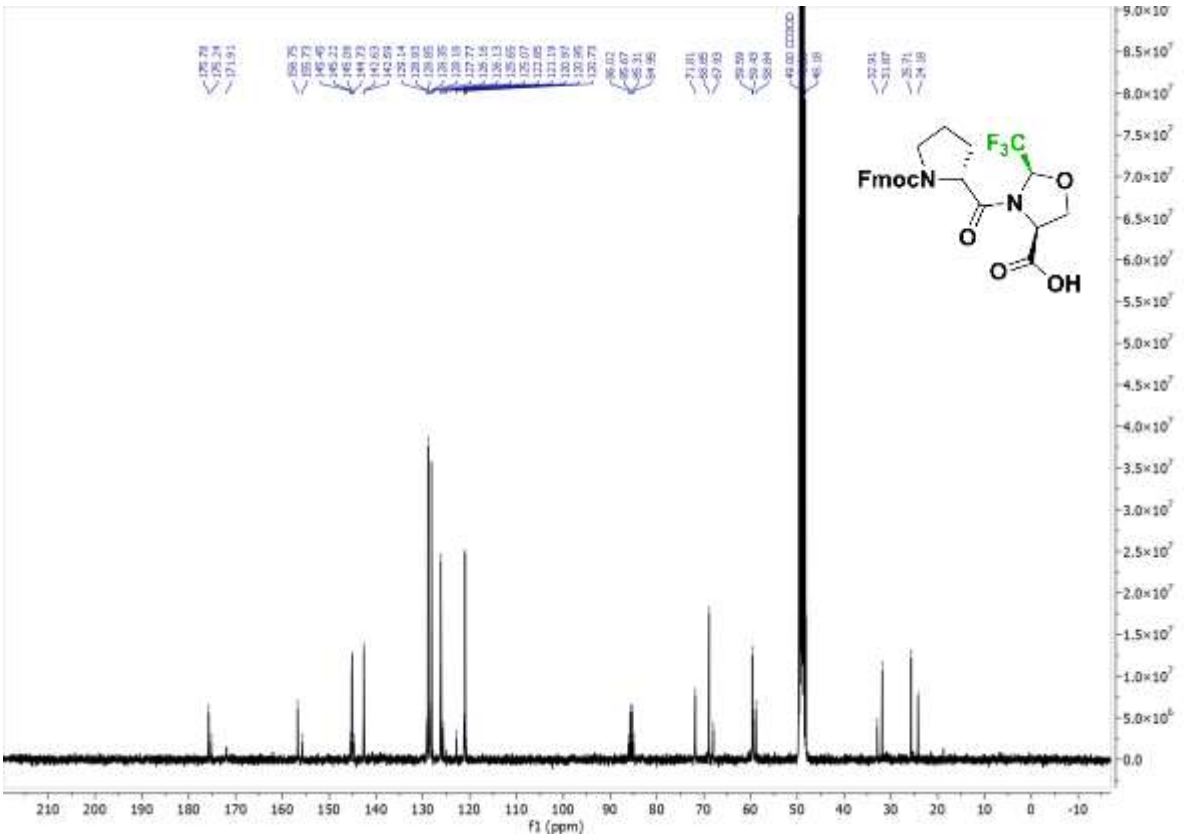


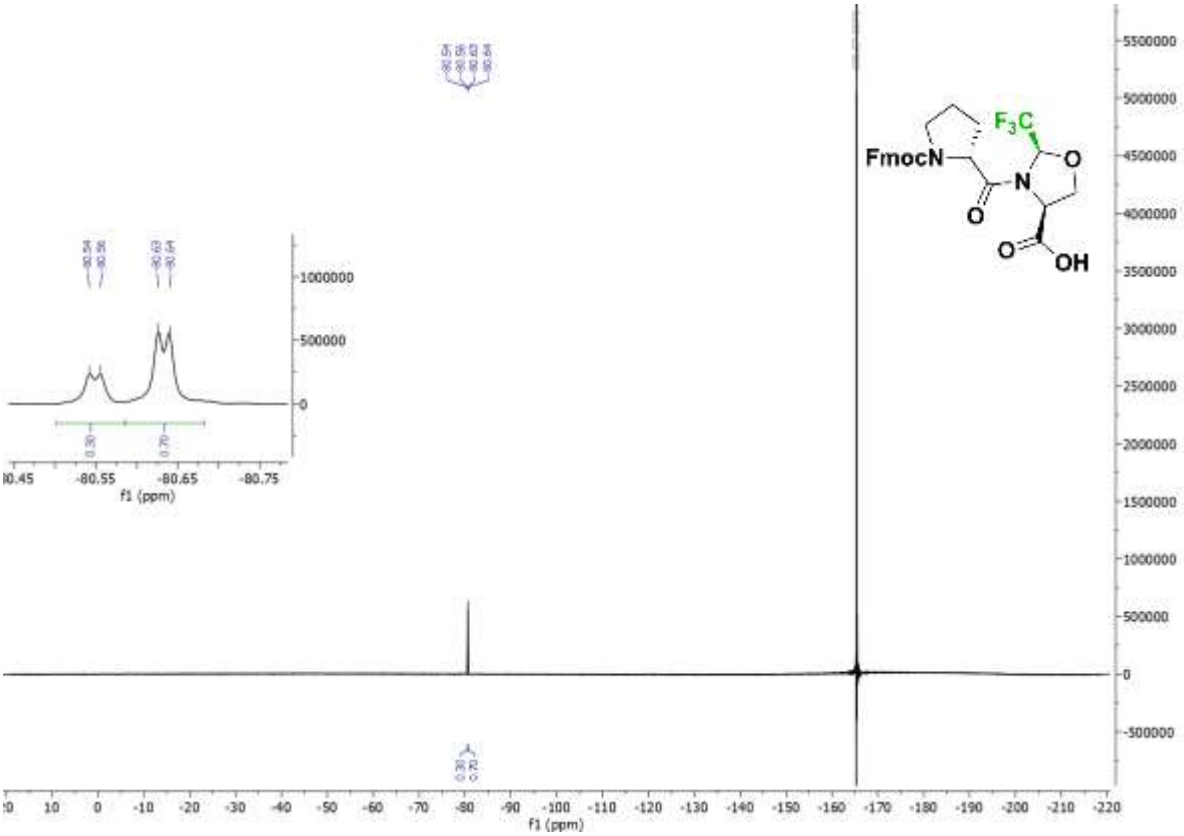




XII.1.5 Fmoc-D-Pro-CF_3ΨPro-OH

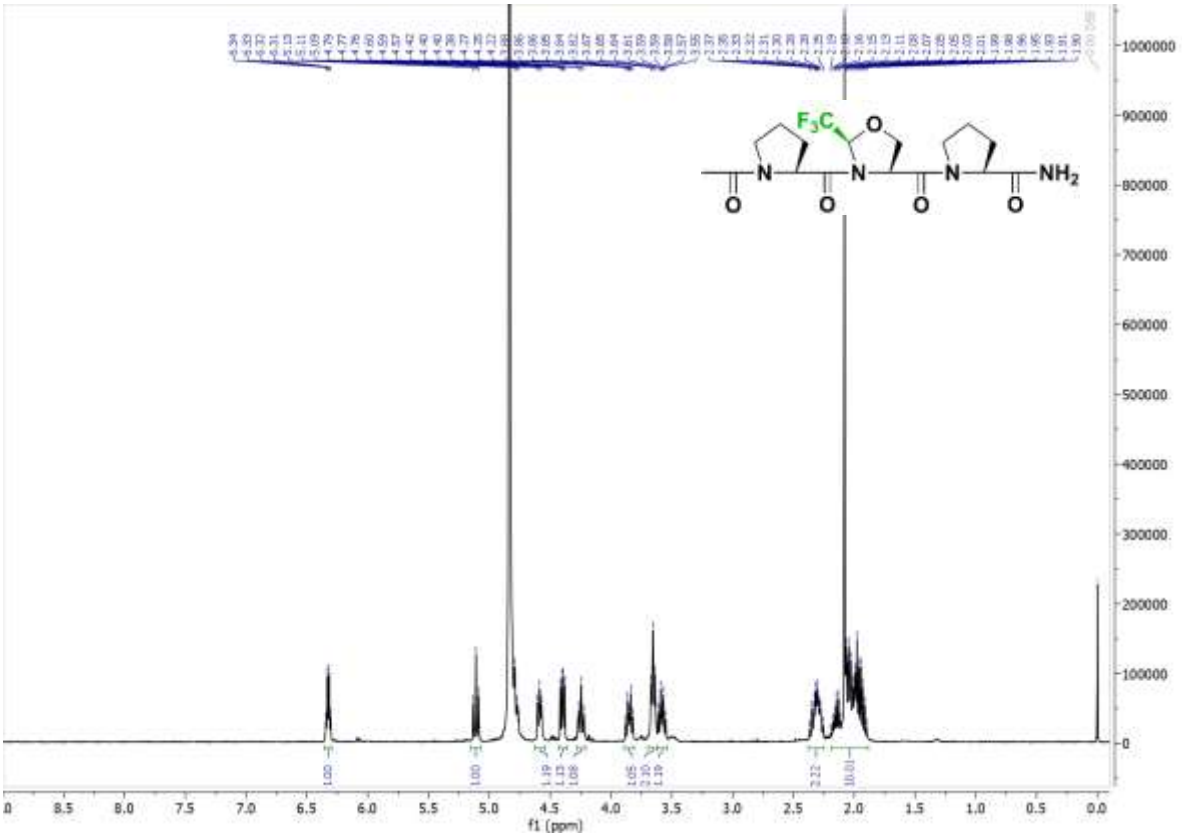


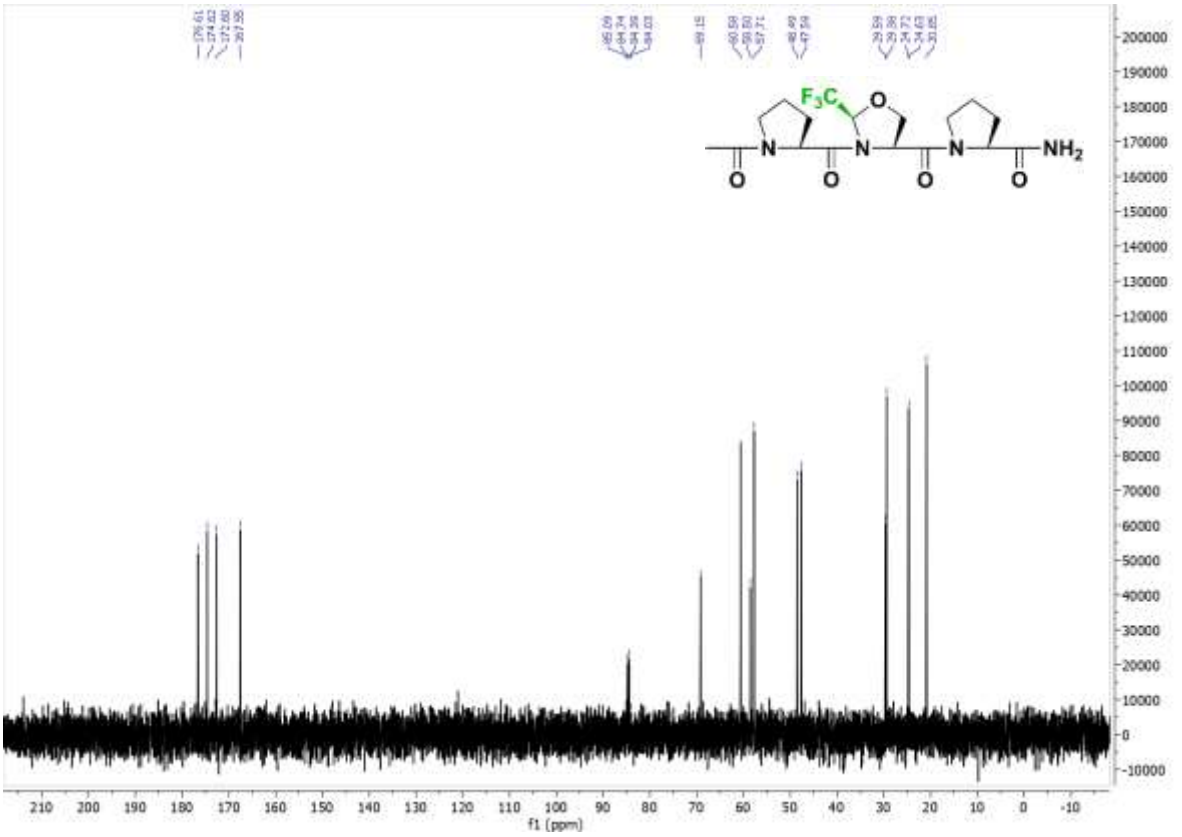

FmocN
F3C
O
N
O
O
OH
f1 (ppm)

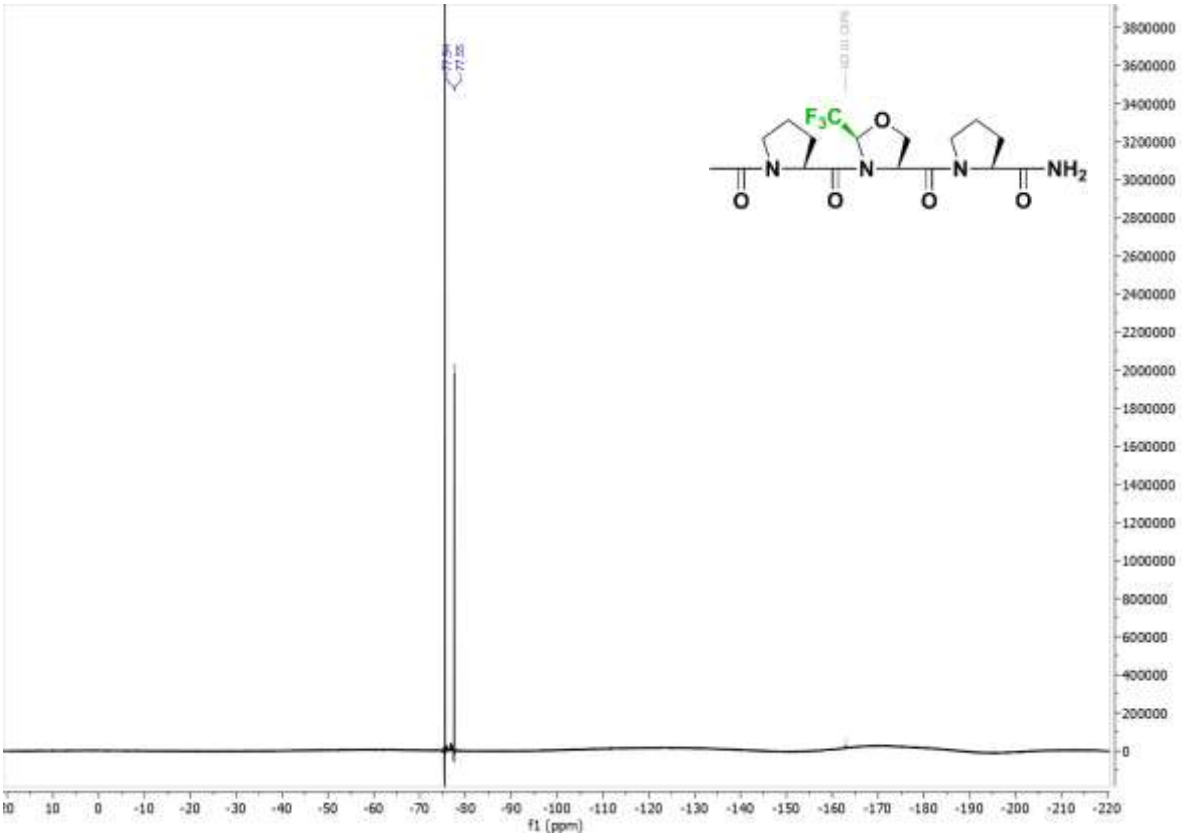

FmocN
F3C
O
N
O
O
OH
f1 (ppm)

XII.2 Oligomers

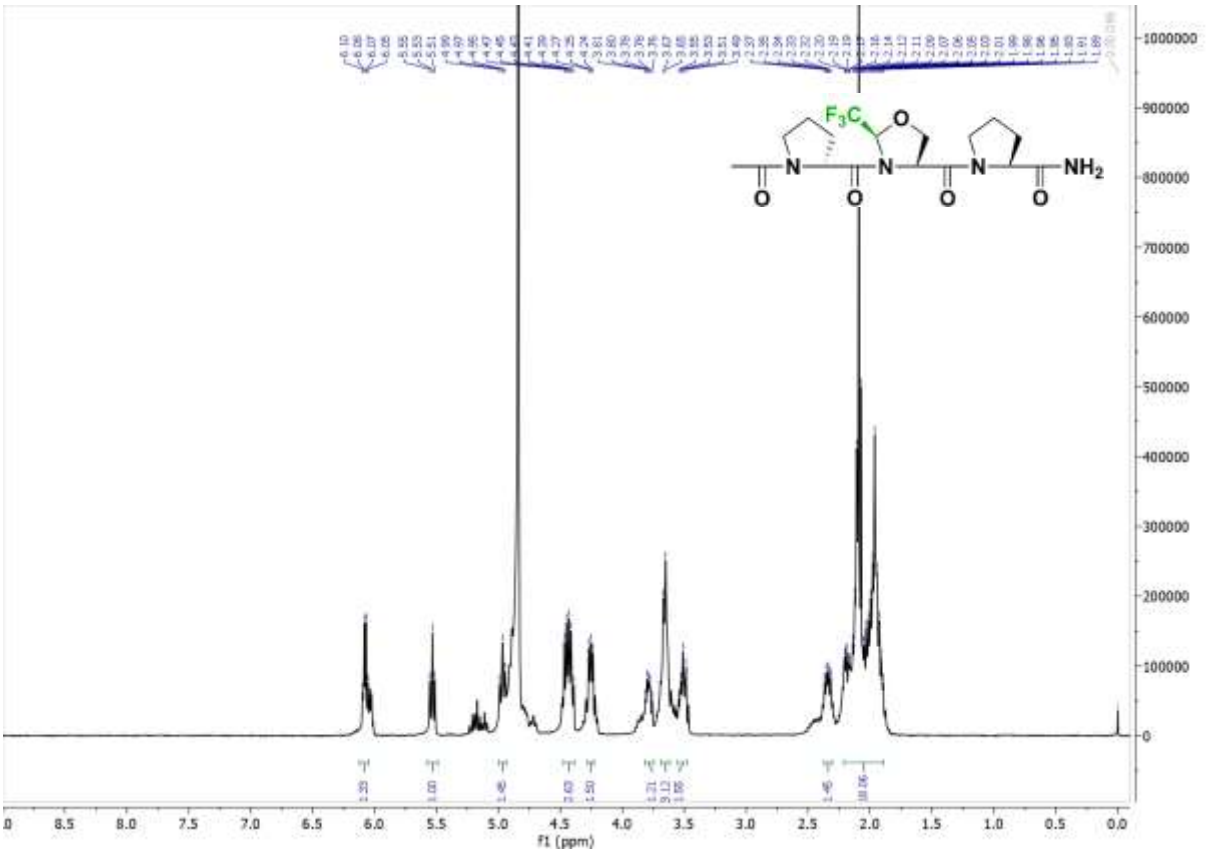
XII.2.1 Trimer 2

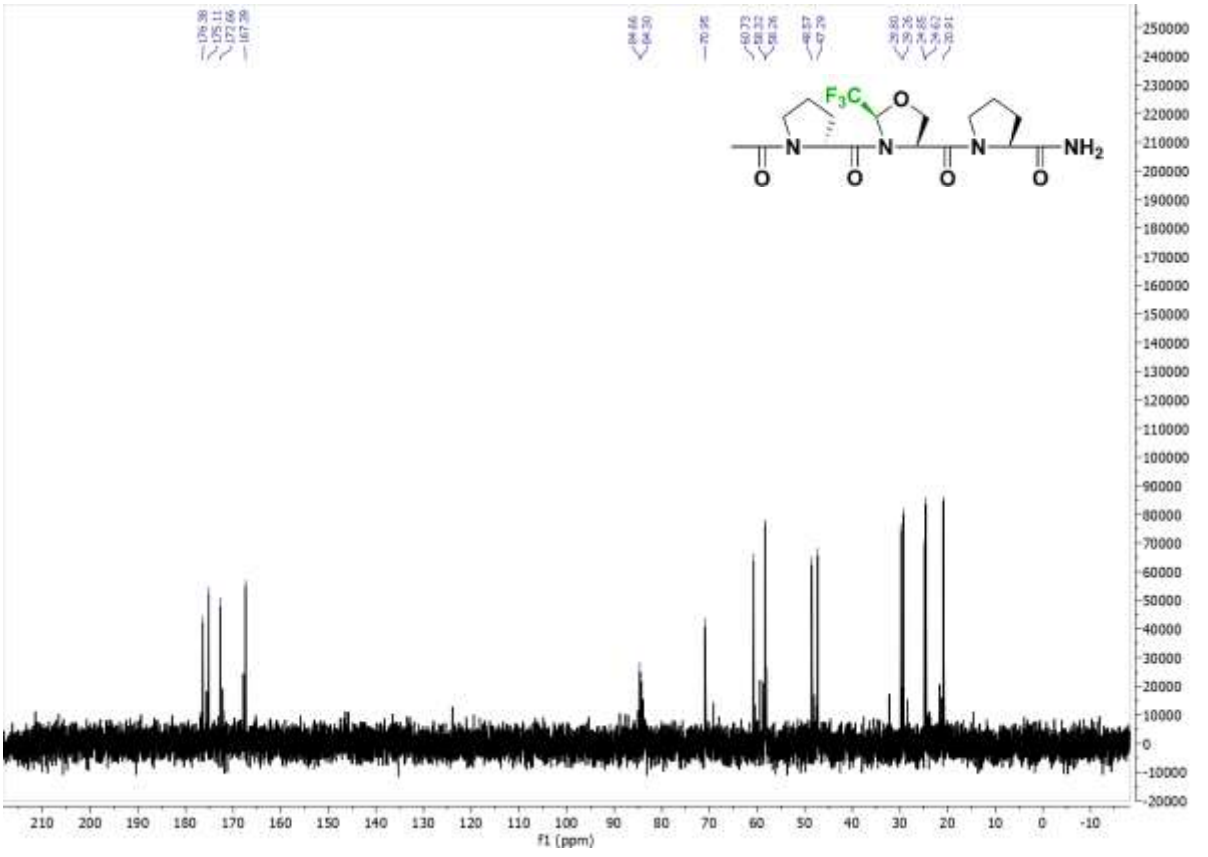


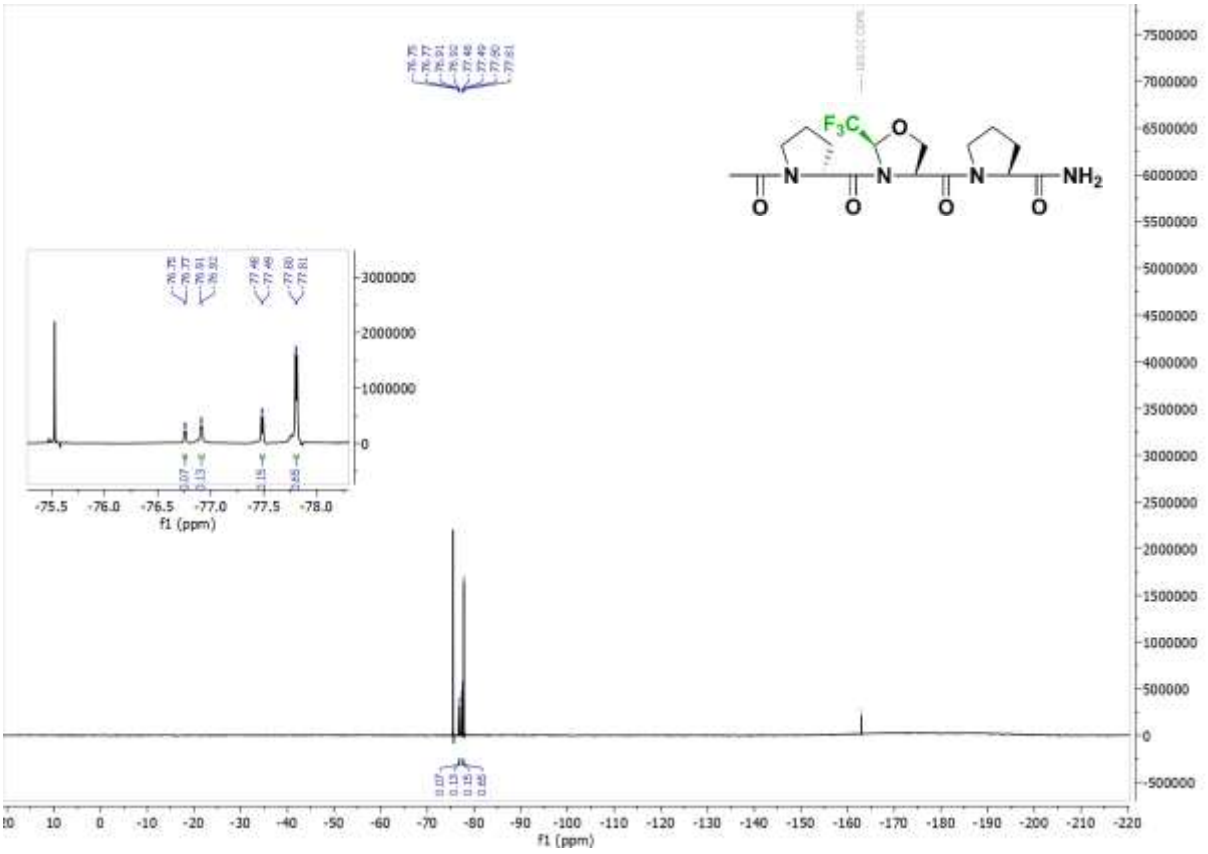

F3C
O
N
N
N
NH2
O
O
O
O
f1 (ppm)


F3C
O
N
N
N
NH2
O
O
O
O
f1 (ppm)

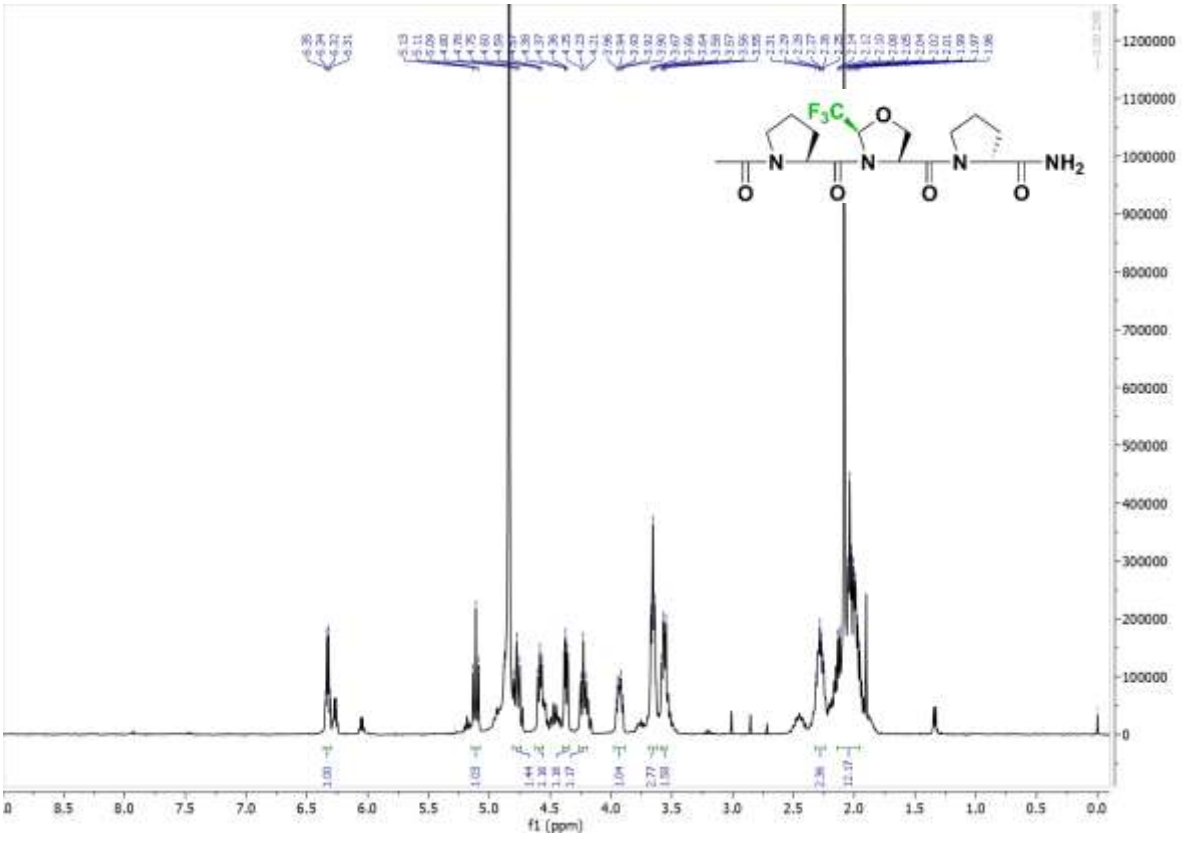
XII.2.2 Trimer 3

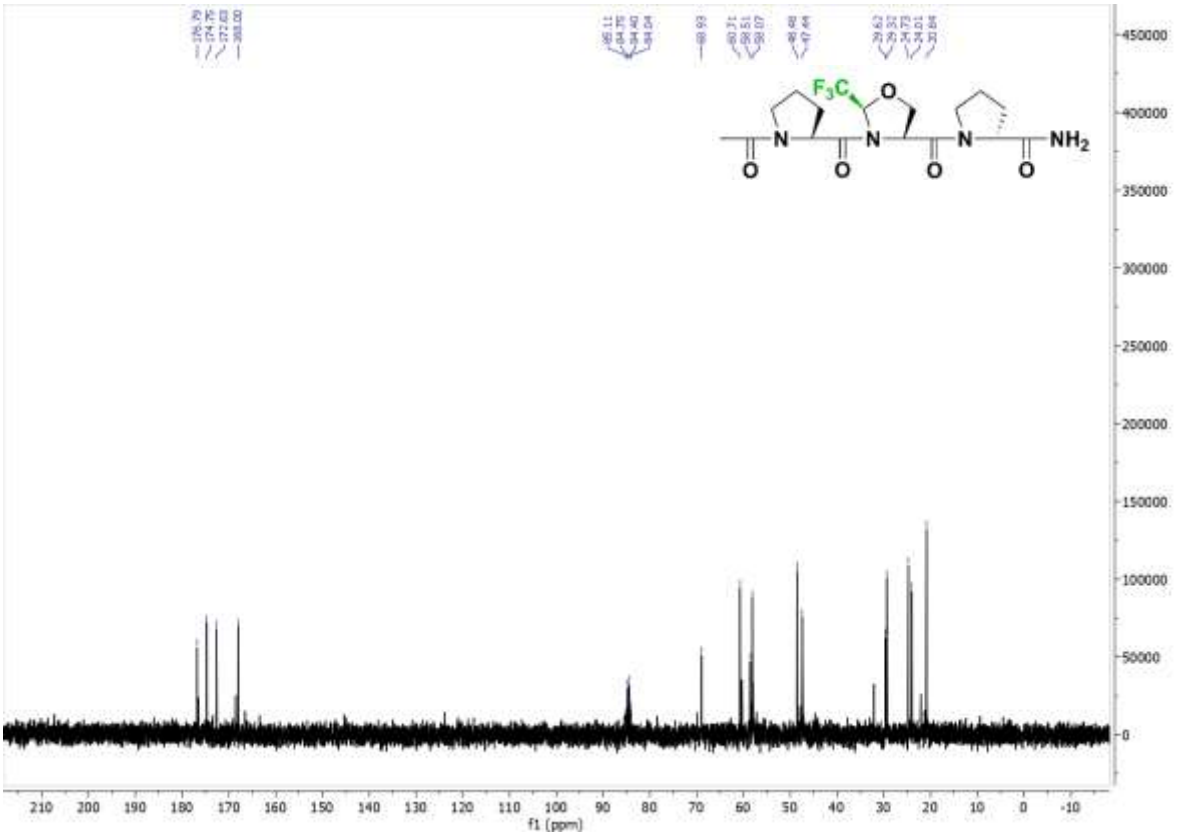


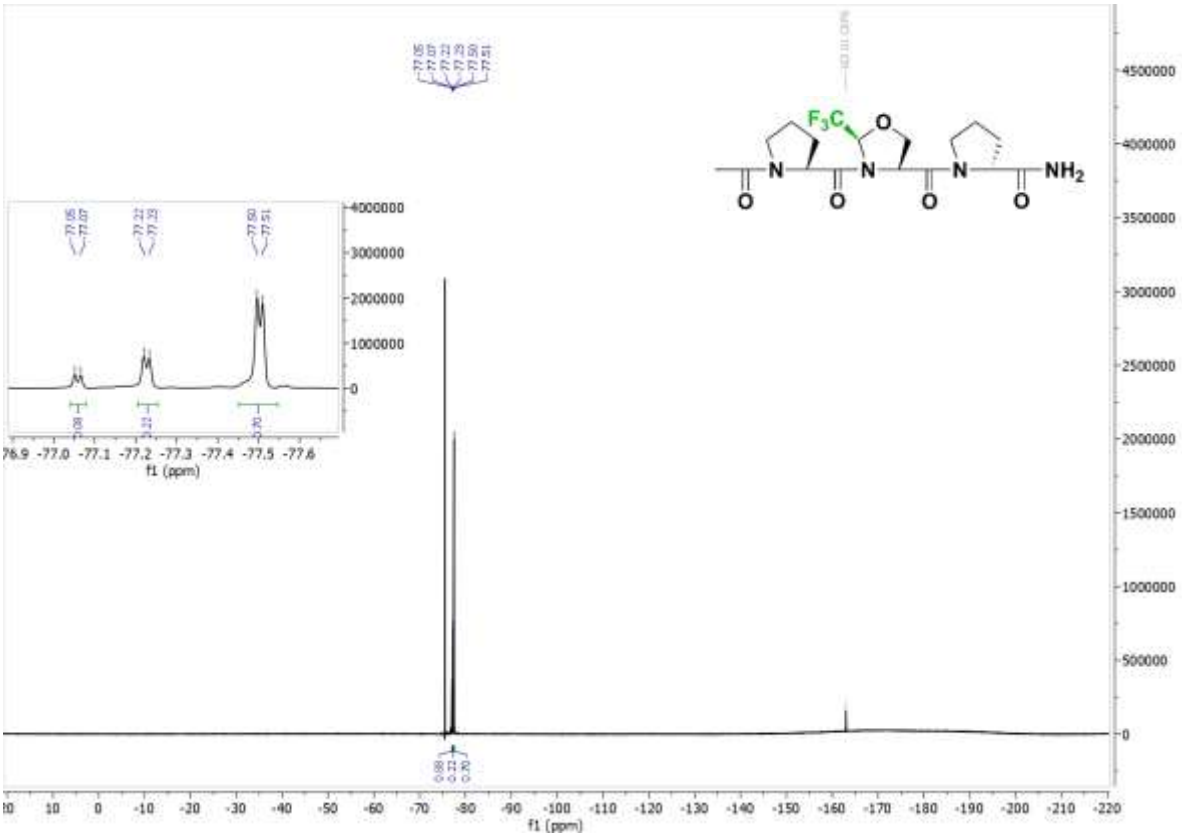




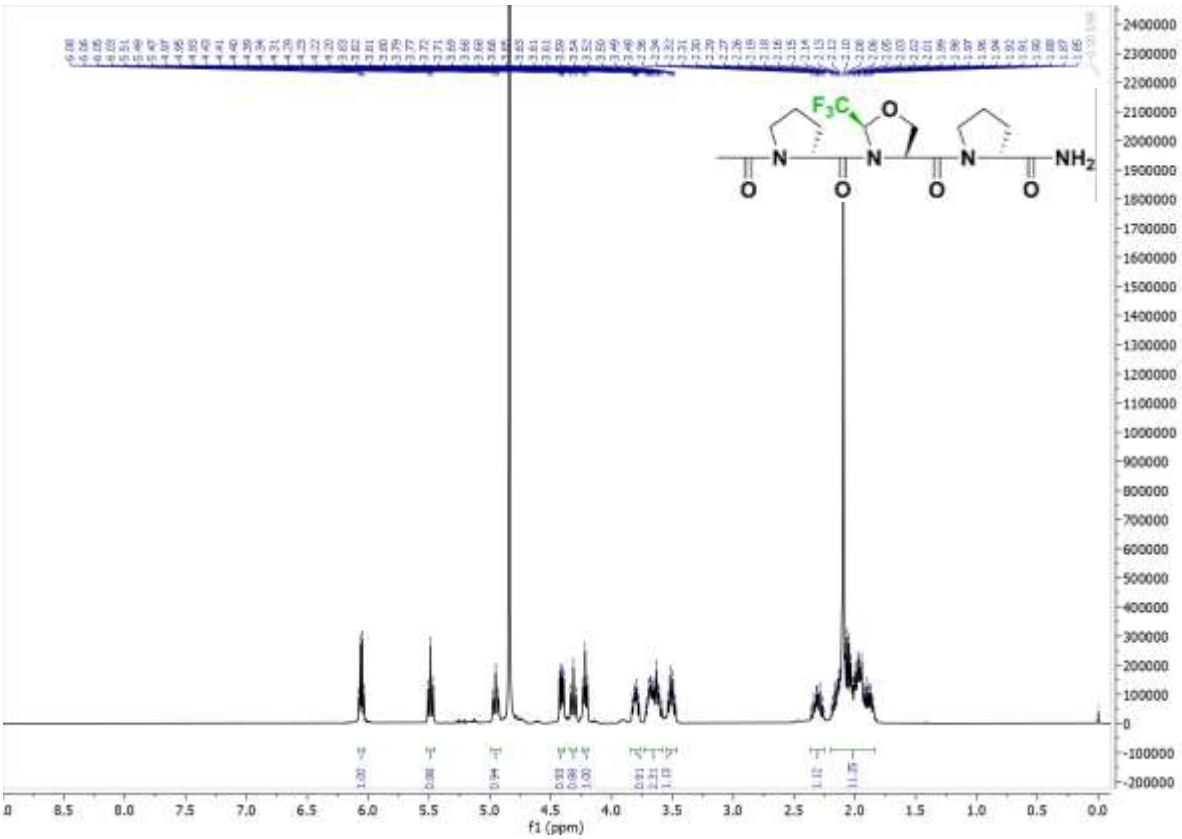
XII.2.3 Trimer 4

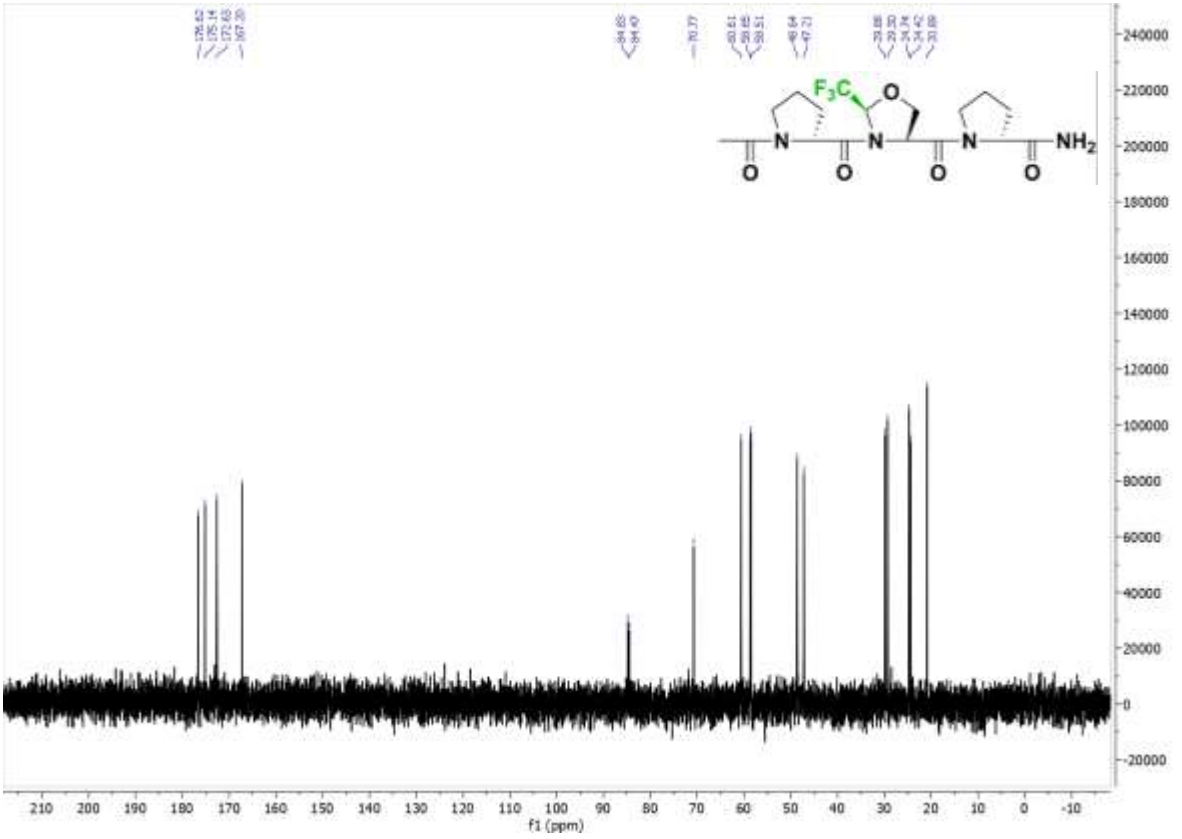


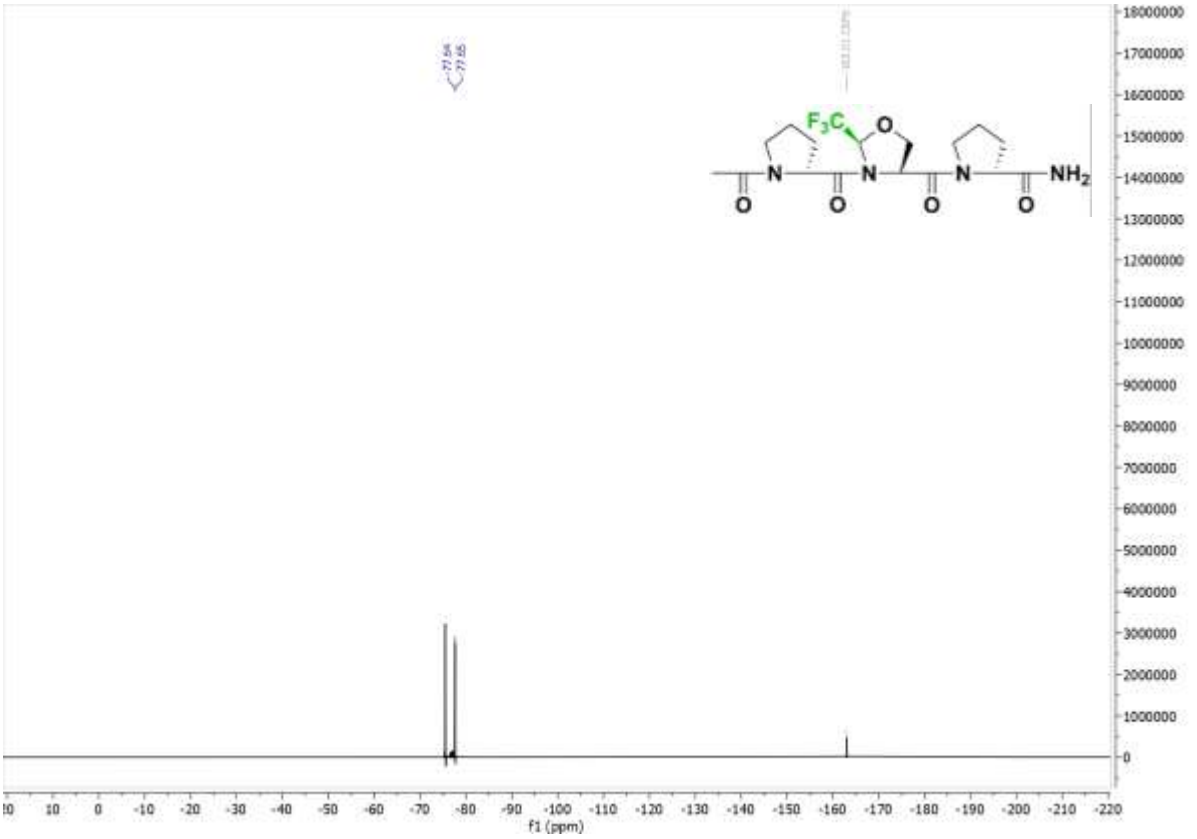

F3C
O
N
N
N
NH2
O
f1 (ppm)


F3C
O
N
N
N
NH2
O
f1 (ppm)

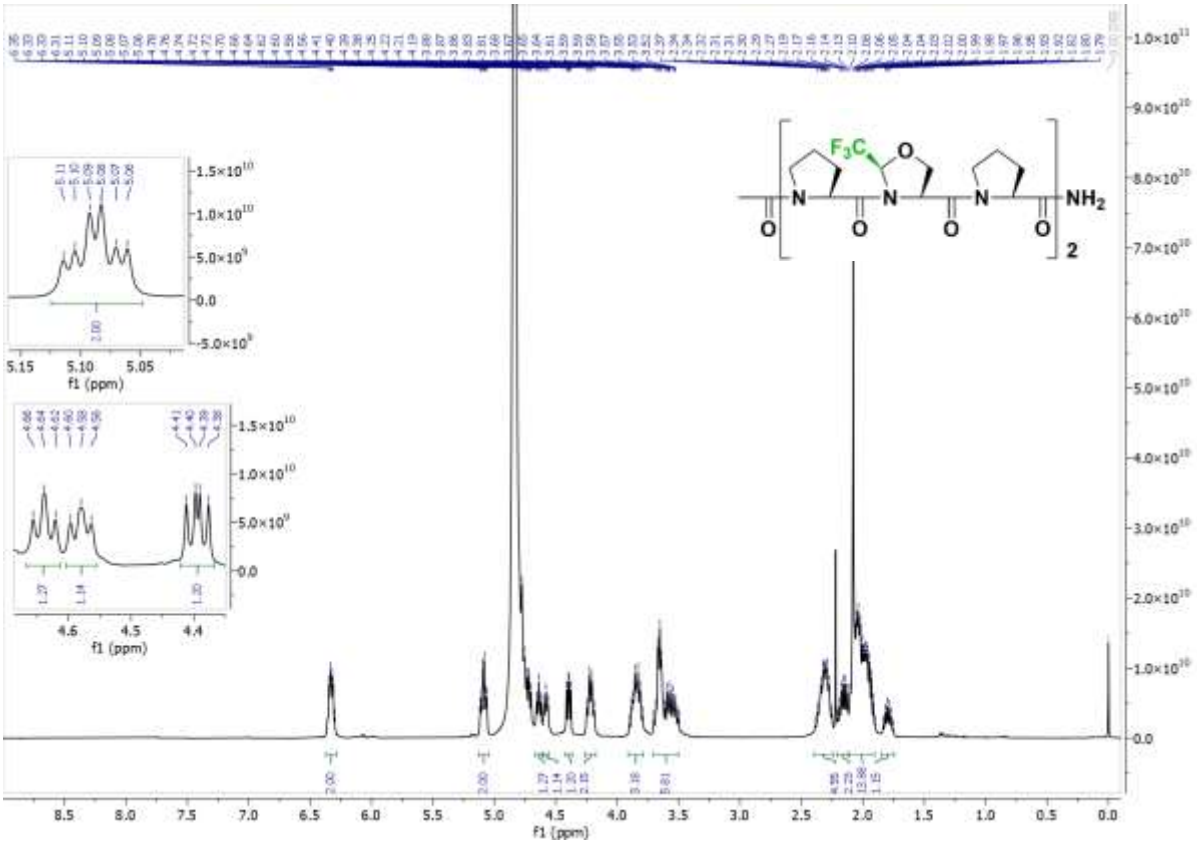
XII.2.4 Trimer 5

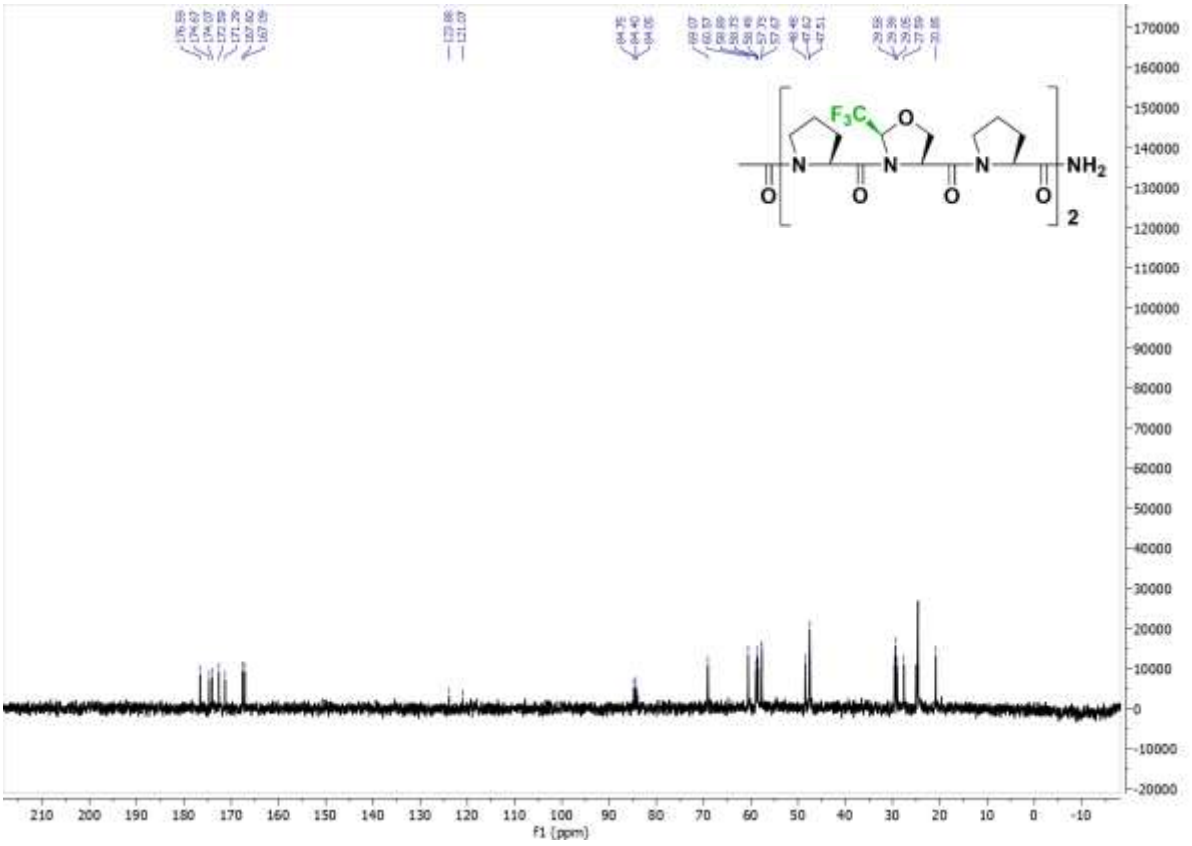


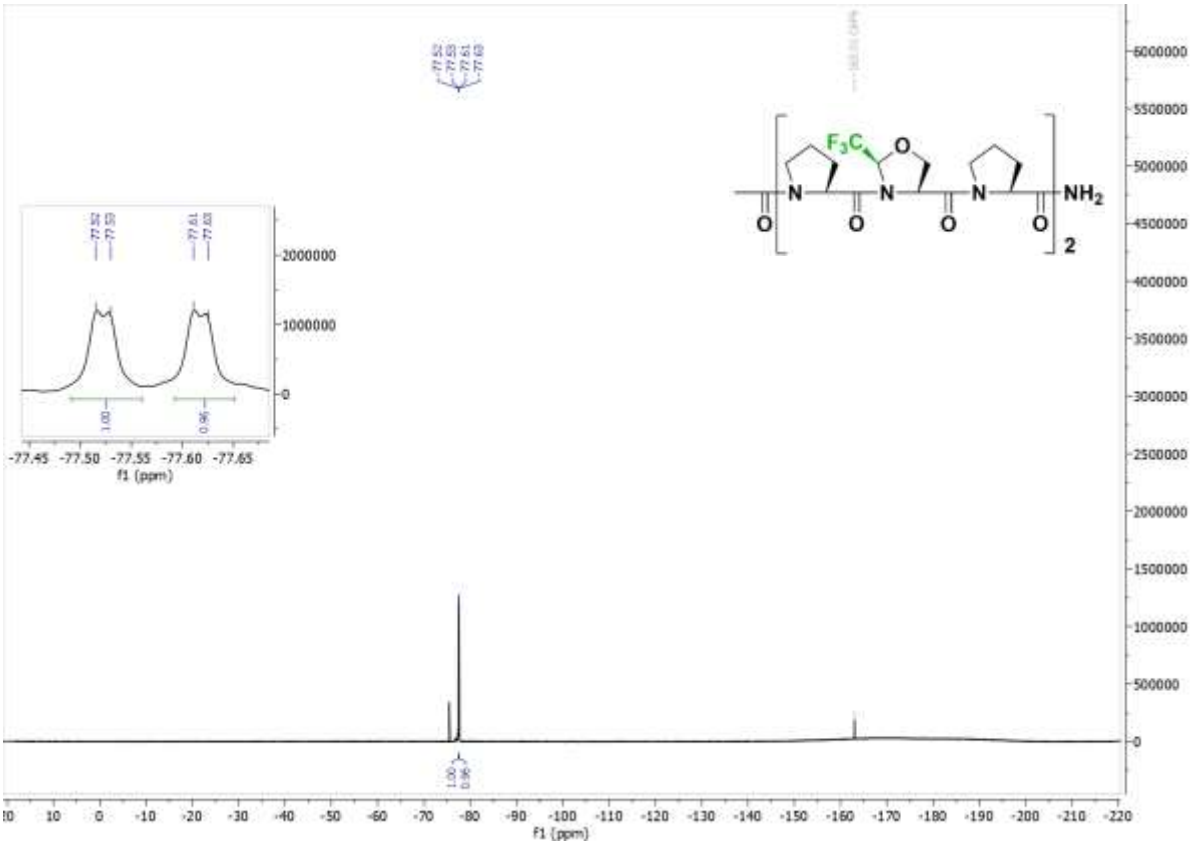




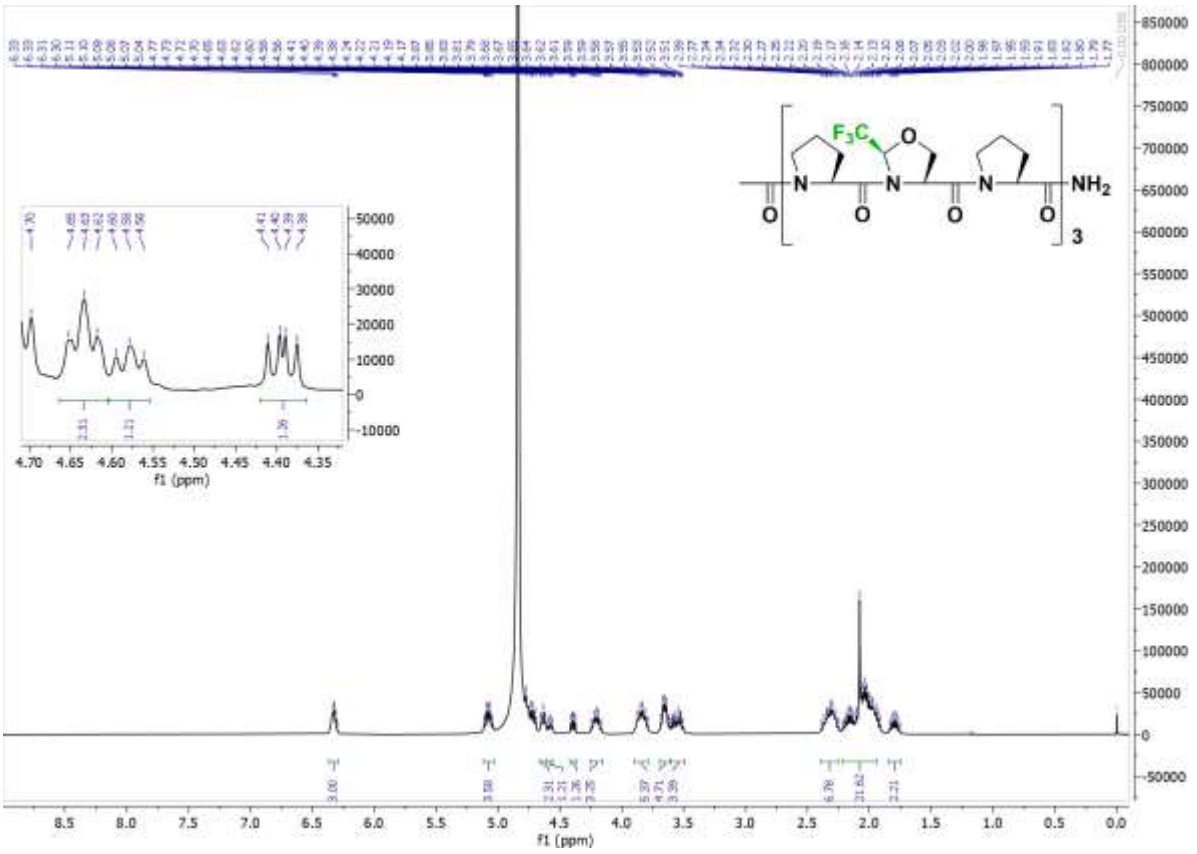
XII.2.5 Hexamer 7

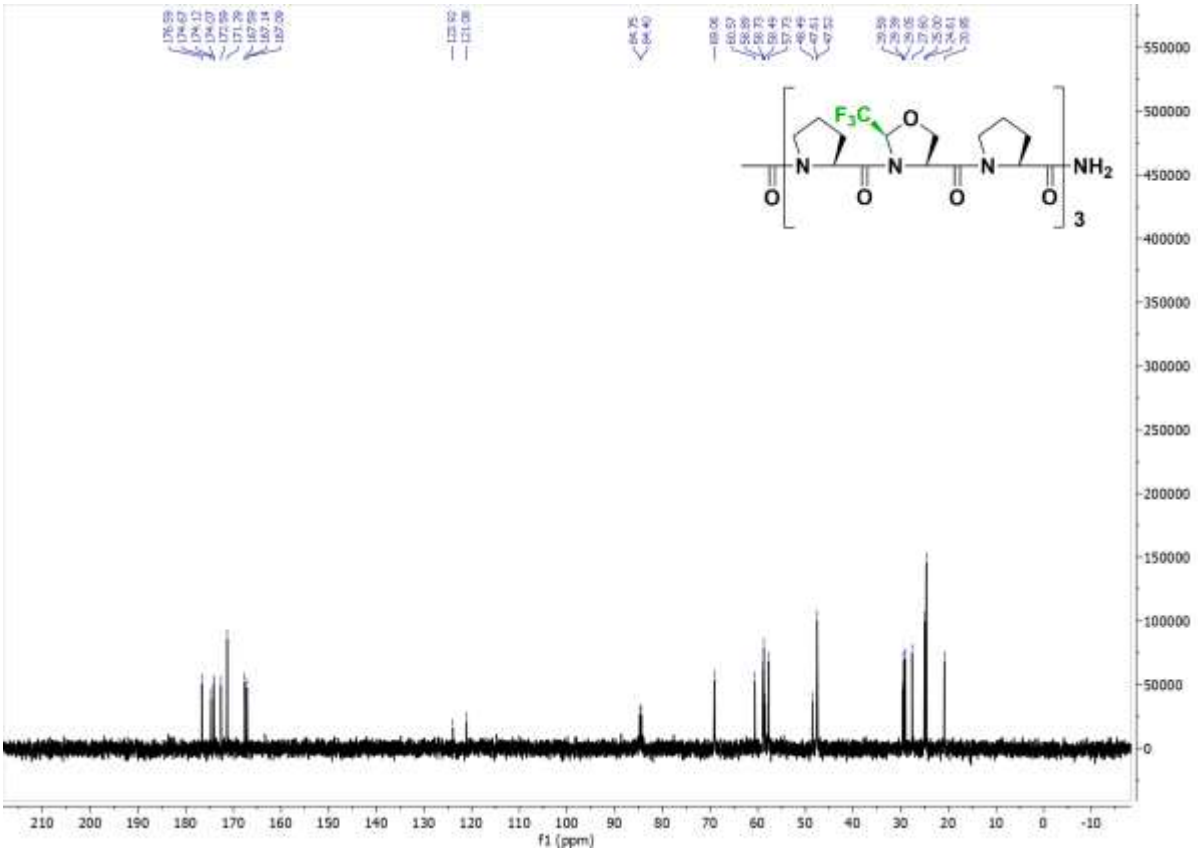


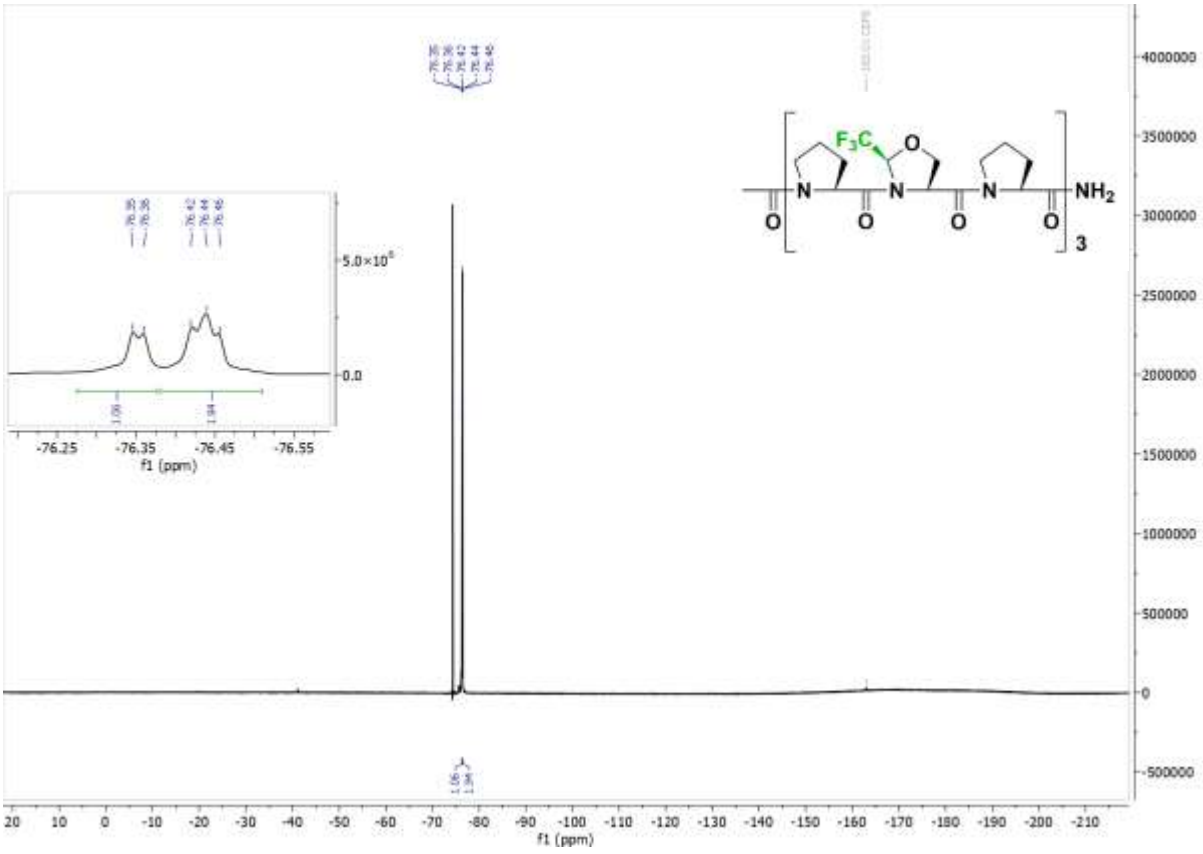

F3C
O
N
NH2
2
F1 (ppm)


F3C
O
N
NH2
2
1.00
0.96
f1 (ppm)

XII.2.6 Nonamer 14

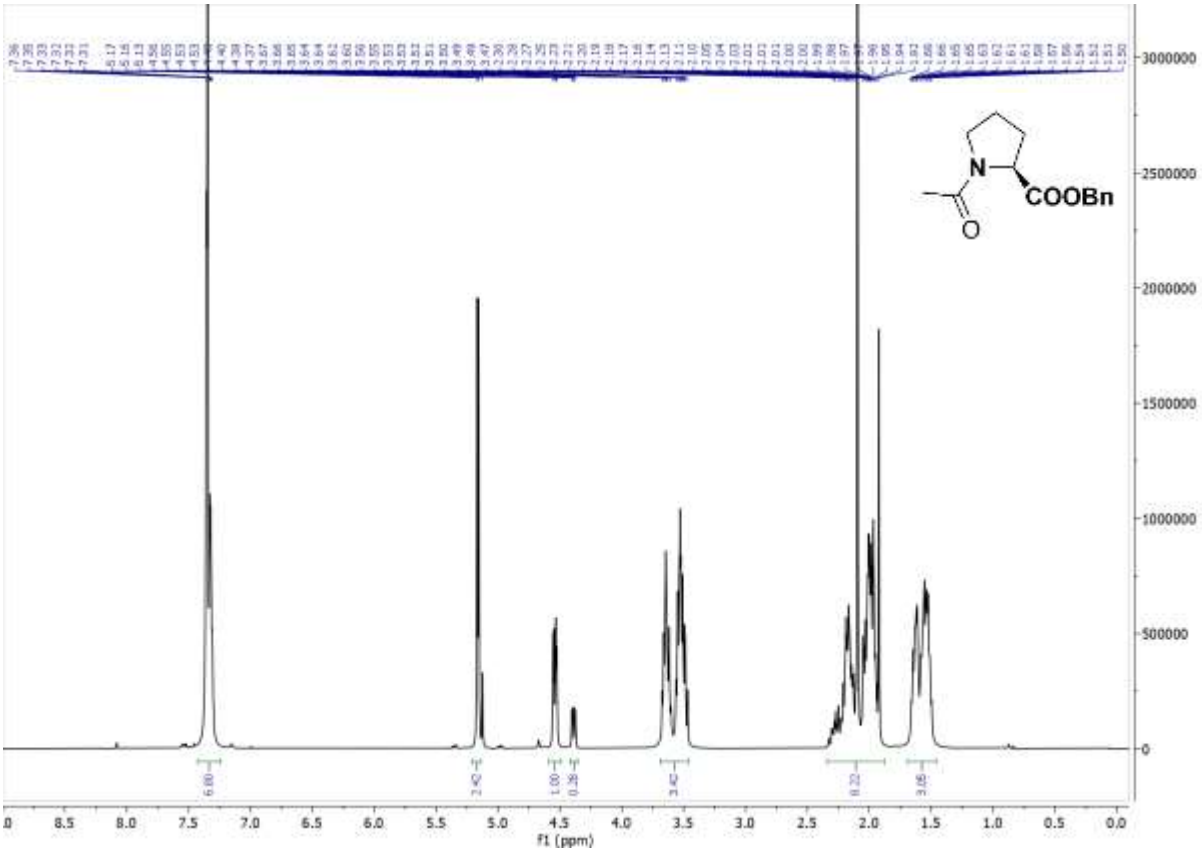


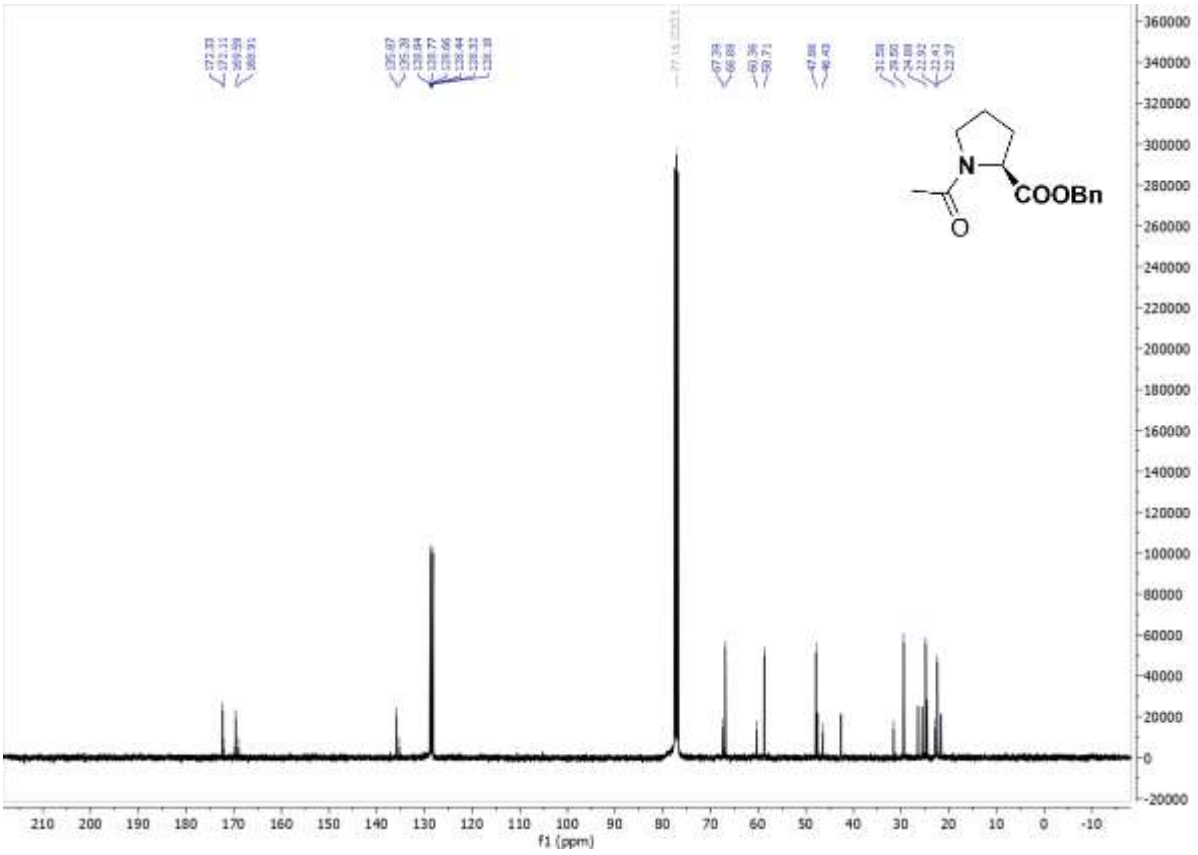

F3C
O
N
N
N
NH2
O
O
O
O
3
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

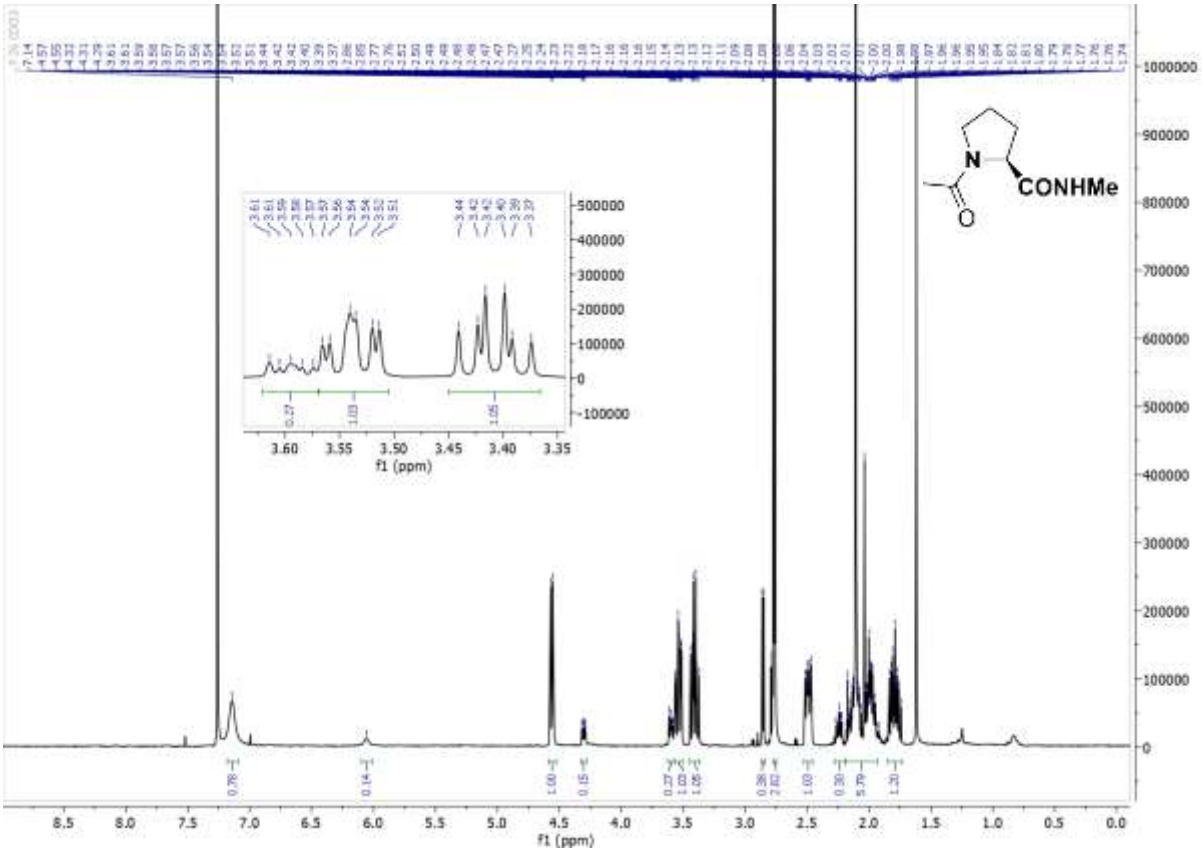

F3C
O
N
N
N
NH2
O
O
O
O
3
-76.25
-76.35
-76.45
-76.55
f1 (ppm)
5.0×10^6
0.0
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
-500000
20 10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

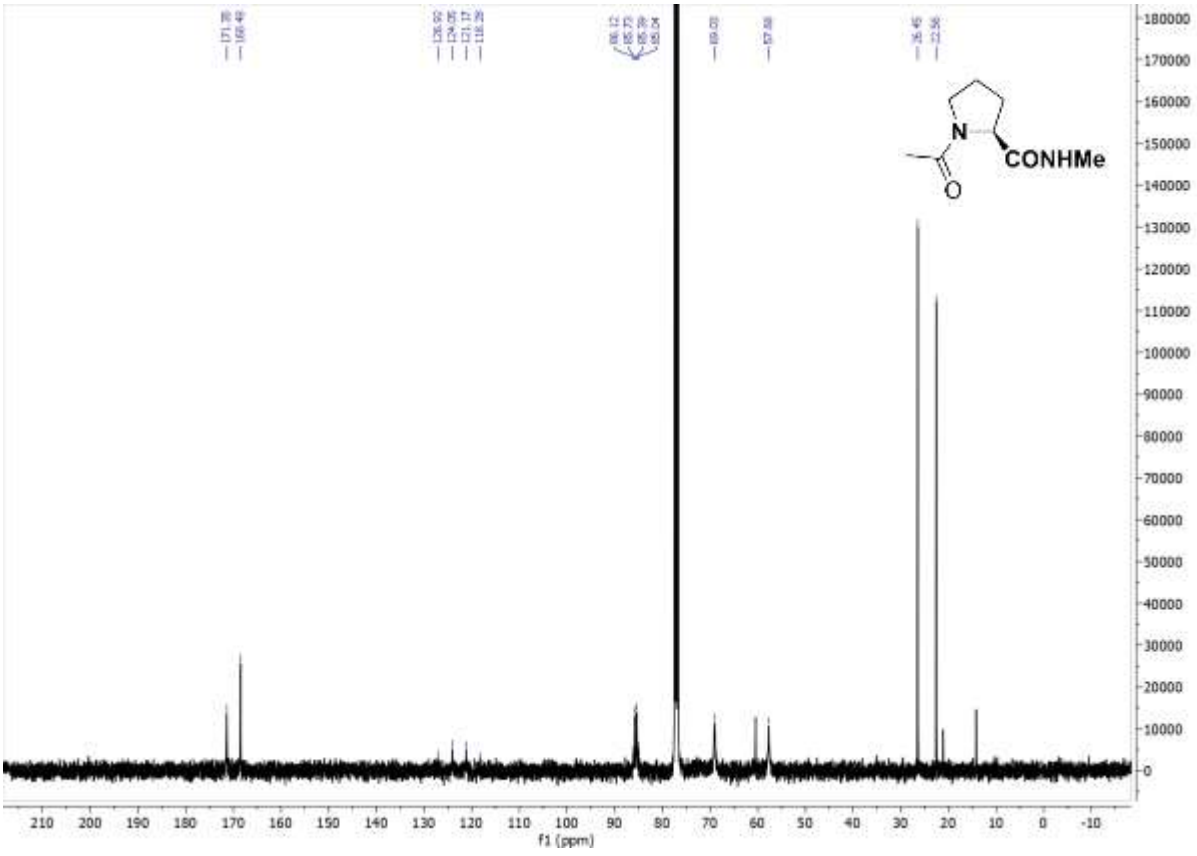
XII.3 Chromophores

XII.3.1 Ac-Pro-NHMe

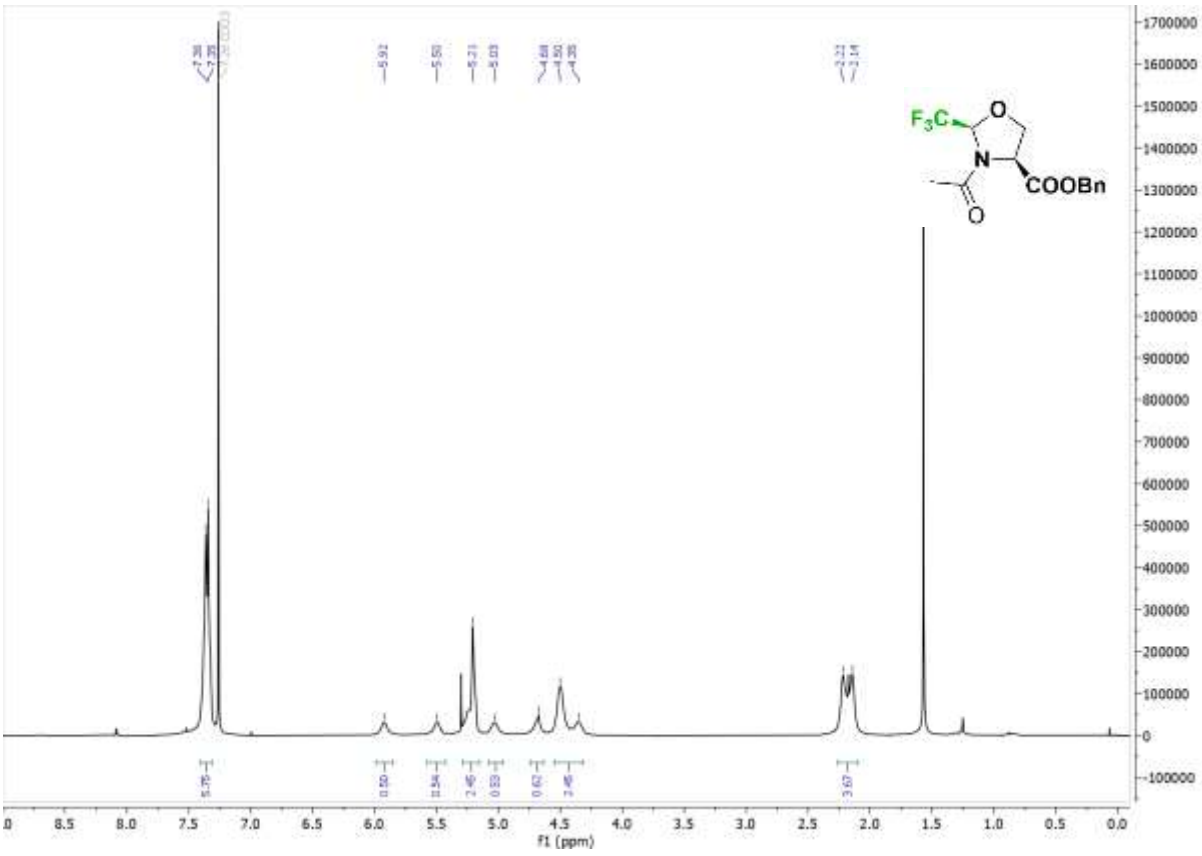


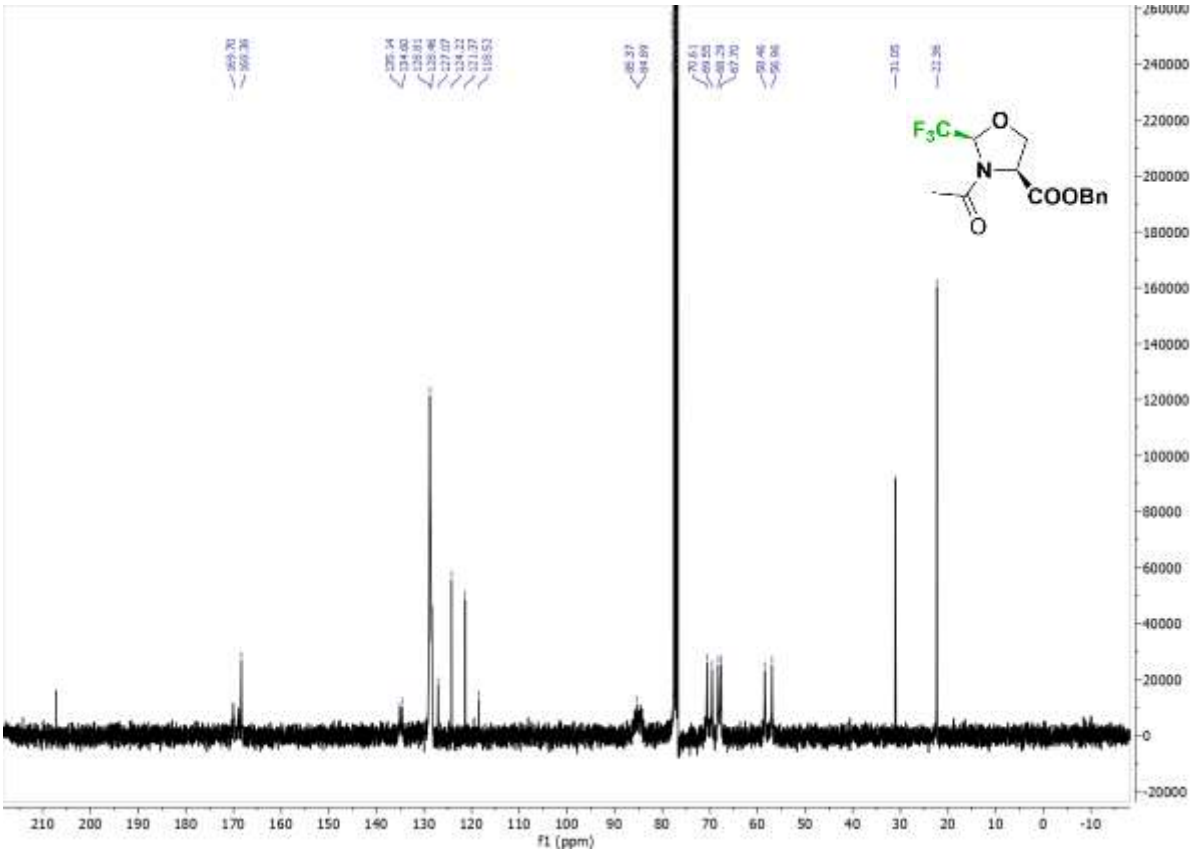

N
COOBn
O
f1 (ppm)

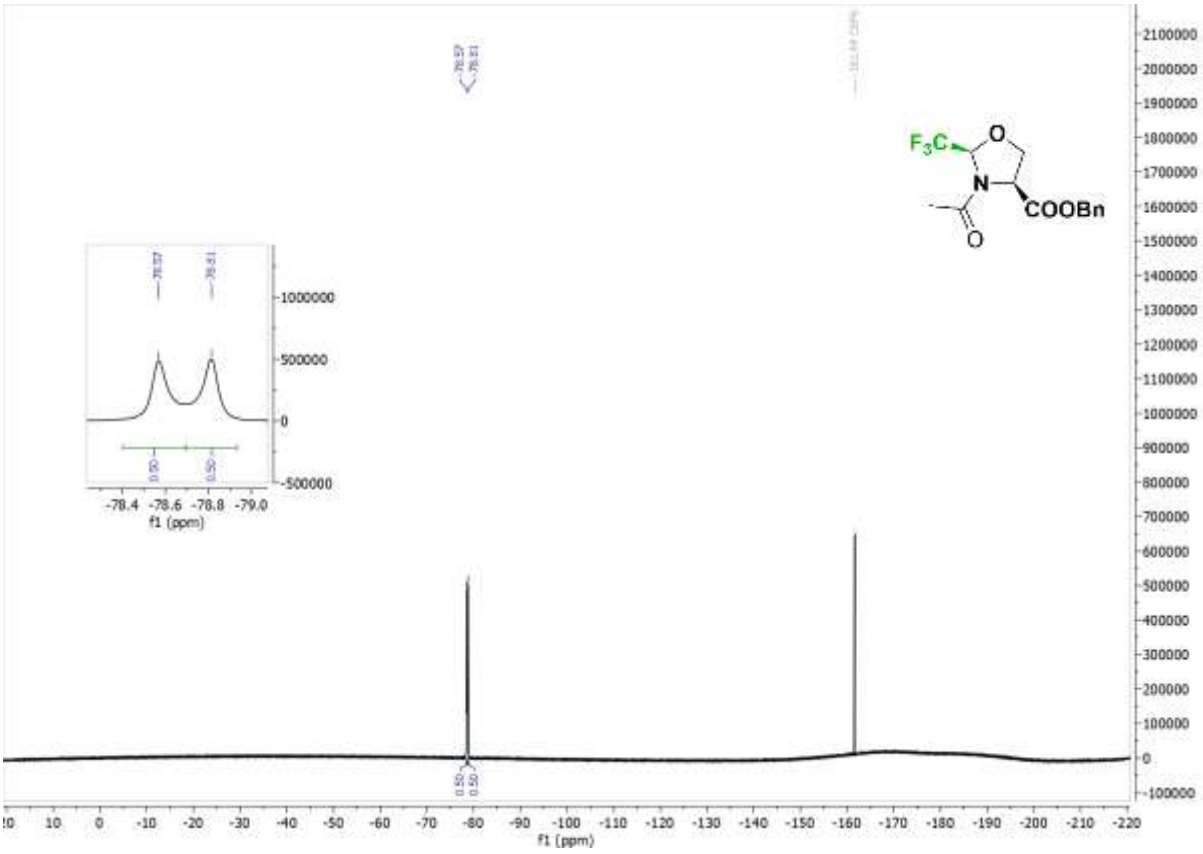

CONHMe
f1 (ppm)

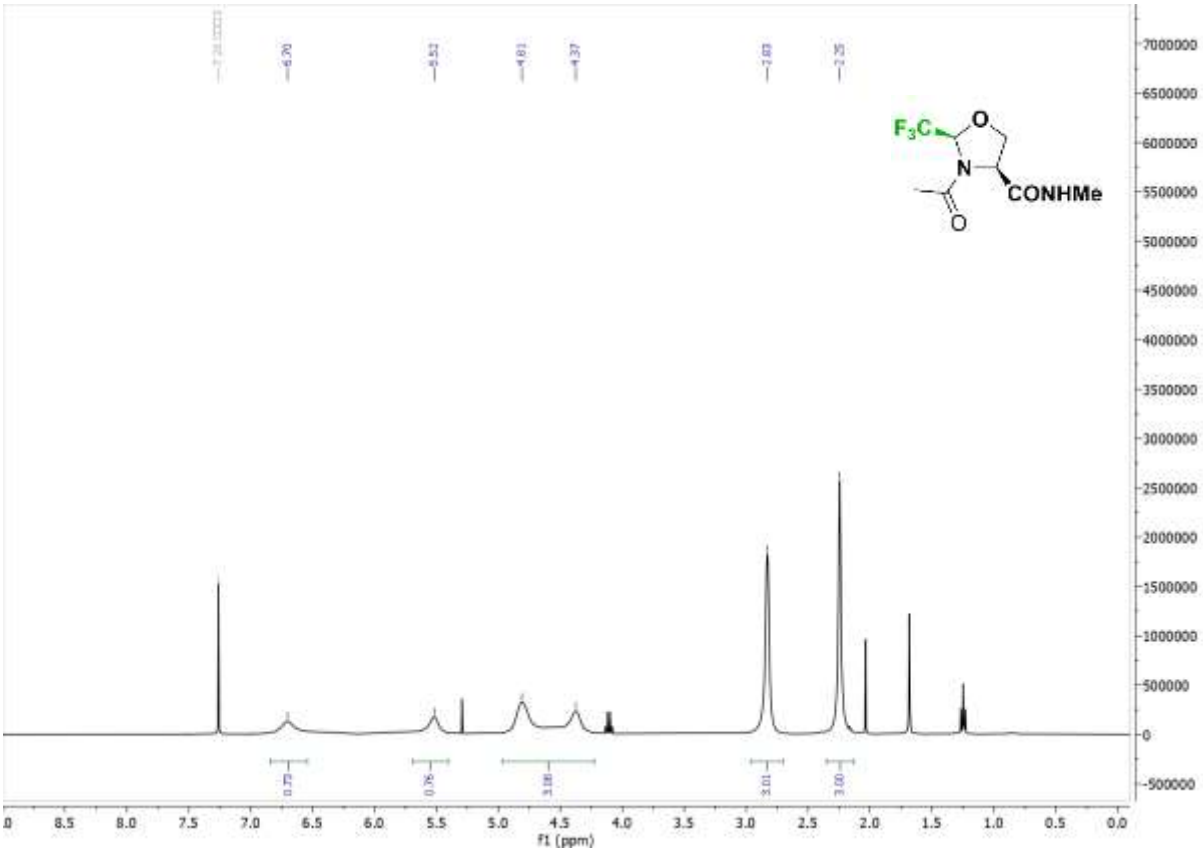

171.38
169.49
126.92
124.35
121.17
118.28
66.12
65.73
65.39
65.04
59.03
57.88
35.45
22.56
N
CONHMe
O
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

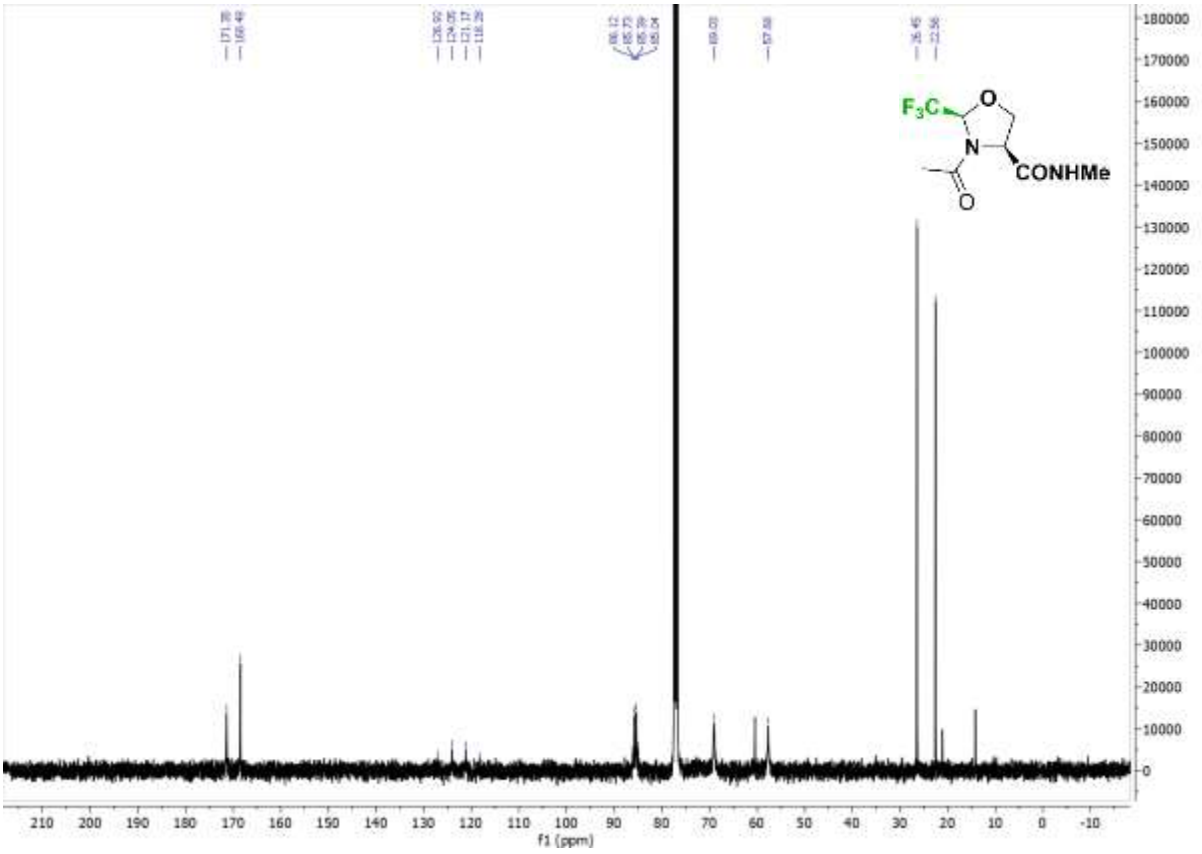
XII.3.2 Ac-CF_3ΨPro-NHMe

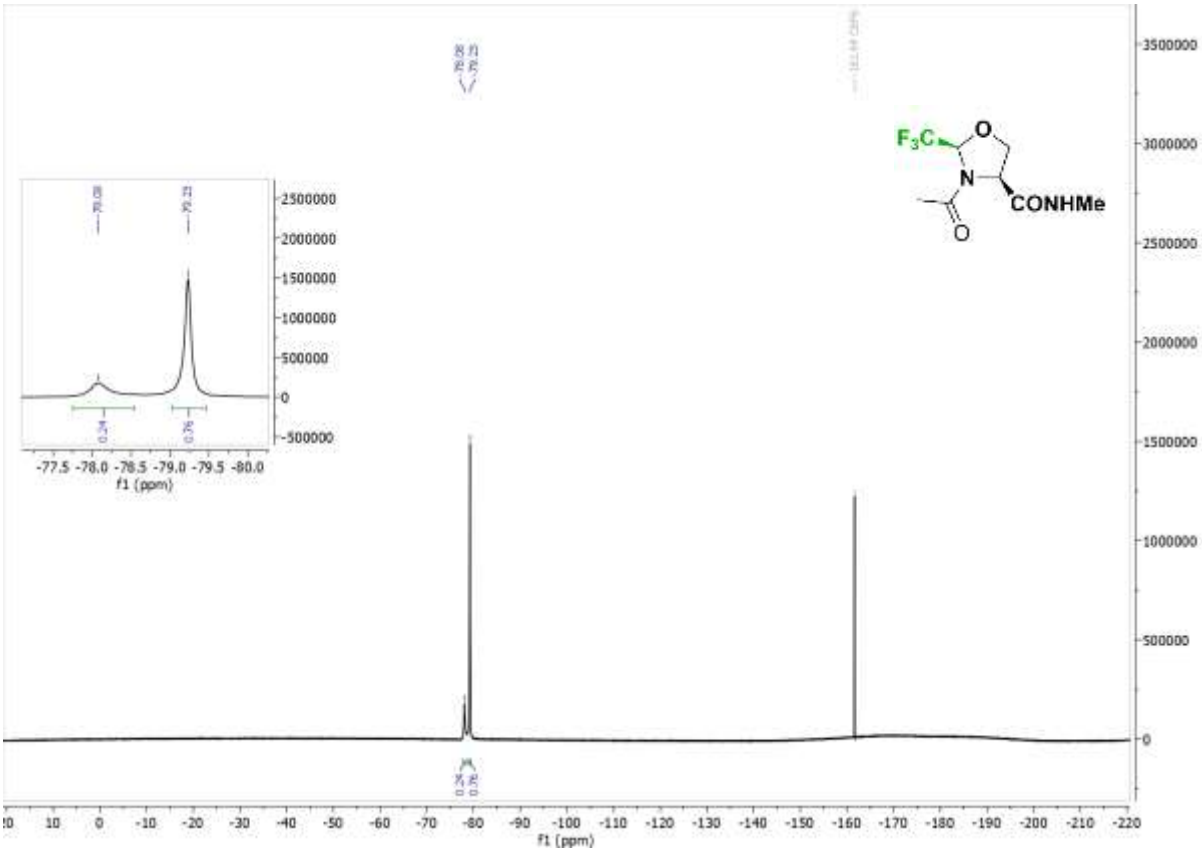





F3C
O
N
COOBn
O
f1 (ppm)


F3C
O
N
CONHMe
O
f1 (ppm)


F3C
O
N
CONHMe
O
f1 (ppm)


F3C
O
N
CONHMe
O
f1 (ppm)

XIII. BIBLIOGRAPHY

(1) Chaume, G.; Barbeau, O.; Lesot, P.; Brigaud, T. Synthesis of 2-Trifluoromethyl-1,3-Oxazolidines as Hydrolytically Stable Pseudoprolines. *J. Org. Chem.* **2010**, *75* (12), 4135–4145. https://doi.org/10.1021/jo100518t.

(2) Das, S.; Li, Y.; Bornschein, C.; Pisiewicz, S.; Kiersch, K.; Michalik, D.; Gallou, F.; Junge, K.; Beller, M. Selective Rhodium-Catalyzed Reduction of Tertiary Amides in Amino Acid Esters and Peptides. *Angewandte Chemie International Edition* **2015**, *54* (42), 12389–12393. https://doi.org/10.1002/anie.201503584.

(3) Rigaku, O. D. *CrysAlis PRO*. 2021, Rigaku Oxford Diffraction, Yarnton, England.

(4) Sheldrick, G. M. A Short History of SHELX. *Acta Cryst A* **2008**, *64* (1), 112–122. https://doi.org/10.1107/S0108767307043930.

(5) Sheldrick, G. M. SHELXT – Integrated Space-Group and Crystal-Structure Determination. *Acta Cryst A* **2015**, *71* (1), 3–8. https://doi.org/10.1107/S2053273314026370.