Supporting Information

CF_2H as hydrogen bond donor group for the fine tuning of peptide bond geometry with difluoromethylated pseudoprolines

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Table of contents

1. General methods

Unless otherwise mentioned, all the reagents were purchased from commercial source. All glassware was dried in an oven at 150 °C prior to use. All solvents were purified and dried by standard techniques and distilled prior to use. Dichloromethane was distilled over calcium hydride under argon. All organic extracts were dried over $MgSO_4$, unless otherwise noted. Silica gel (230−400 mesh) was used for flash column chromatography, eluting (unless otherwise stated) with cyclohexane/ethyl acetate. Silica TLC plates were visualized under UV light, by a 10% solution of phosphomolybdic acid in ethanol followed by heating. Infrared spectra (IR) were obtained by Fourier transformation and wavenumbers are given in cm^{-1}. ^1H NMR (400.00 MHz), ^{13}C NMR (100.50 MHz), and ^{19}F NMR (376.20 MHz) spectra were measured on a spectrometer operating at a ^1H frequency of 400 MHz. ^1H NMR (500.00 MHz) and ^{13}C NMR (125.75 MHz) were measured on a spectrometer operating at a ^1H frequency of 500 MHz and equipped with a triple resonance, z-axis pulsed-5 field-gradient cryogenic probehead, optimized for 1H detection. Complete proton assignments were obtained from the analysis of 2D total correlation spectroscopy (TOCSY) experiments using 80 ms DIPSI-2 mixtime, and 2D nuclear Overhauser effect

Spectroscopy (NOESY) experiments (typically 500 ms mixing time). Homonuclear experiments were typically collected as 512 (t1) and 4096 (t2) time-domain matrices over a spectral width of 10 ppm, with 8 scans per t1 increment. Carbon assignment was deduced from heteronuclear 2D ^1H–^{13}C HSQC and 2D ^1H–^{13}C CH_2-TROSY16 experiments, using S3 256 (t1) × 1024 (t2) timedomain matrices, with 32 scans per t1 increment. Chemical shifts of ^1H NMR are expressed in parts per million downfield from tetramethylsilane (δ= 0) in $CDCl_3$ or in D_2O. Chemical shifts of ^{13}C NMR are expressed in parts per million downfield from $CDCl_3$ as internal standard (δ= 77.16). Chemical shifts of ^{19}F NMR are expressed in parts per million downfield from C_6F_6 as an internal standard (δ= –164.9) in $CDCl_3$ or from TFA as an internal standard (δ= –75.6) in D_2O. Coupling constants are reported in Hertz. Melting points were uncorrected. High-Resolution Mass Spectra (HRMS) were obtained using ElectroSpray Ionization (ESI) in positive ion mode and a TOF mass analyzer orusing Electronic Impact (EI) in positive ion mode by direct insertion probe and double focusing magnetic sector mass analyzer.

2. Experimental section

(4*S*)-2-Difluoromethyloxazolidine-4-carboxylic Acid Benzyl Esters (*S*,*S*)-1 and (*R*,*S*)-1: To a solution of L-serine benzyl ester hydrochloride (5.00 g, 21.58 mmol, 1.0 equiv) in toluene (100 mL), were added PPTS (0.542 g, 2.16 mmol, 0.1 equiv) and difluoroacetaldehyde ethyl hemiacetal (2.86 g, 22.66 mmol, 1.05 equiv). The resulting mixture was warmed to reflux using a Dean-Stark apparatus for 1h30. The reaction mixture was then cooled to 0°C with an ice bath and filtered. Toluene was evaporated. Purification by flash chromatography (90:10 to 70:30 cyclohexane/ethyl acetate) gave pseudoprolines (*S*,*S*)-**1** (2.46 g, 44%) as a colorless oil and (*R*,*S*)-**1** (1.04 g, 19%) as a white solid.

(*S*,*S*)-1: Colorless oil; R_f = 0.38 (80:20 Cyclohexane/Ethyl acetate); $[\alpha]^{20}_D$: -55.5 (*c* 1.01, $CHCl_3$); IR (neat, cm^{-1}): 3331, 2972, 1738, 1498, 1456, 1380, 1316, 1199, 1076; ^1H NMR (400 MHz, $CDCl_3$, 293 K) δ 3.21 (s, 1 H, NH), 3.78 (dd, *J* = 7.8, 6.7 Hz, 1 H, H_β ΨPro-Ha), 4.06 (dd, *J* = 7.8, 6.7 Hz, 1 H, H_α ΨPro-H), 4.22 (t, *J* = 7.8 Hz, 1 H, H_β ΨPro-Hb), 4.95 (ddd, *J* = 10.1, 8.7, 2.8 Hz, 1 H, H_δ ΨPro-H), 5.19 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Ha), 5.23 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Hb), 5.55 (ddd, *J* = 55.5, 54.6, 2.8 Hz, 1 H, CF_2H-H), 7.31-7.44 (m, 5 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) δ 59.0 (CH, C_α ΨPro), 67.7 (CH_2, CH_2 Bn), 69.5 (CH_2, C_β ΨPro), 89.2 (t, *J* = 25.4 Hz, CH, C_δ ΨPro), 114.0 (t, *J* = 245.9 Hz, CH, CF_2H), 128.5 (2 CH, C-arom), 128.8 (3 CH, C-arom), 135.0 (C, C-arom), 171.5 (C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 293 K) δ -133.7 (ddd, *J* = 289.6, 54.6, 8.7 Hz, 1 F), -135.6 (ddd, *J* = 289.6, 55.5, 10.1 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{12}H_{13}F_2NO_3$ 258.0936, found 258.0940.

(*R*,*S*)-1: White solid; m.p. 70-72 °C; R_f = 0.18 (80:20 Cyclohexane/Ethyl acetate); $[\alpha]^{20}_D$: -35.2 (*c* 1.15, $CHCl_3$); IR (neat, cm^{-1}): 3362, 2895, 1739, 1498, 1456, 1382, 1340, 1195, 1116, 1073; ^1H NMR (400 MHz, $CDCl_3$, 293 K) δ 3.03 (s, 1 H, NH), 4.04 (m, 2 H, H_β), 4.12 (m, 1 H, H_α), 4.73 (ddd, *J* = 6.9, 6.1, 4.6 Hz, 1 H, H_δ ΨPro-H), 5.17 (d, *J* = 12.0 Hz, 1 H, Bn CH_2-Ha), 5.21 (d, *J* = 12.0 Hz, 1 H, Bn CH_2-Hb), 5.68 (ddd, *J* = 55.5, 54.6, 4.6 Hz, 1 H, CF_2H-H), 7.29-7.42 (m, 5 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) δ 58.9 (CH, C_α ΨPro), 67.5 (CH_2, Bn CH_2), 69.3 (CH_2, C_β ΨPro), 89.7 (t, *J* = 26.8 Hz, CH, C_δ ΨPro), 113.7 (t, *J* = 244.4 Hz, CH, CF_2H), 128.5 (2 CH, C-arom), 128.7 (CH, C-arom), 128.8 (2 CH, C-arom), 135.1 (C, C-arom), 171.7 (C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 293 K) -131.9 (ddd, *J* = 293.9, 55.5, 6.1 Hz, 1 F), -134.0 (ddd, *J* = 293.9, 54.6, 6.9 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{12}H_{13}F_2NO_3$ 258.0936, found 258.0937.

Acylation Reactions.

Representative Procedure for the Acylation using Acid Anhydride with Iodine Catalysis: A solution of oxazolidine **1** (1.0 equiv) and iodine (0.1 equiv) in acetic anhydride under argon was stirred at room temperature until the reaction was completed as monitored by TLC and ^{19}F NMR analysis. DCM was added and the mixture was washed with a 1 M aqueous solution of $NaHSO_3$ (3 x). The aqueous layer was extracted with DCM (3 x) and the combined organic extracts are dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography gave the corresponding acylated oxazolidine **2**.

Representative Procedure for the Acylation using Acyl Chloride: To a solution of oxazolidine **1** (1.0 equiv) in DCM at 0°C was added under argon pyridine (3.0 equiv) and acyl chloride (3 equiv). The mixture was stirred at room temperature until the reaction was completed as monitored by TLC and ^{19}F NMR. DCM was added and the mixture was washed with brine (3 x). The aqueous layer was extracted with DCM (3 x) and the combined organic layer were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography gave the corresponding acylated oxazolidine **2**.

(2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Benzyl Ester (*S*,*S*)-2: The product was prepared by the acid anhydride procedure with iodine catalysis using 1.37 g of (*S*,*S*)-**1** (5.32 mmol, 1 equiv) in acetic anhydride (5 mL, 52.8 mmol, 10 equiv), iodine (135 mg, 0.53 mmol, 0.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave 625 mg of (*R*,*S*)-**2** (2.09 mmol, 39%) as a 50/50 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K and 338 mg of (*S*,*S*)-**2** (1.13 mmol, 21%) as an 81/19 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K.

The product was prepared by the acyl chloride procedure using 500 mg of (*S*,*S*)-**1** (1.94 mmol, 1.0 equiv) in DCM (3 mL), 471 µL of pyridine (5.83 mmol, 3 equiv) and 415 µL of acetyl chloride (5.83 mmol, 3.0 equiv). Purification by flash chromatography (70:30 to 50:50 cyclohexane/ethyl acetate) gave 469 mg of (*S*,*S*)-**2** (0.469 g, 1.57 mmol, 81%) as an 81/19 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K: Yellow oil; R_f = 0.55 (60:40 Cyclohexane/Ethyl acetate); $[\alpha]^{20}_D$: -77.2° (*c* 1.10, $CHCl_3$); IR (neat, cm^{-1}): 2917, 1746, 1667, 1499, 1456, 1398, 1353, 1279, 1192, 1156, 1108, 1067; ^1H NMR (400 MHz, $CDCl_3$, 293 K) δ (81:19 mixture of rotational isomers) (major conformer) δ 1.98 (s, 3 H, Ac CH_3-H), 4.35 (m, 1 H, H_β-Ha), 4.50-4.57 (m, 2 H, H_β-Hb and H_α), 5.22 (d, *J* = 12.0 Hz, 1 H, CH_2 Bn), 5.27 (d, *J* = 12.0 Hz, 1 H, CH_2 Bn), 5.53 (dd, *J* = 20.0, 2.6 Hz, 1 H, H_δ), 6.21 (dd, *J* = 55.5, 53.8 Hz, 1 H, CF_2H), 7.29-7.42 (m, 5 H, H-arom); (minor conformer) δ 2.17 (s, 3 H, Ac CH_3-H), 4.16 (dd, *J* = 9.6, 1.4 Hz, 1 H, H_β ΨPro-Ha), 4.35 (m, 1 H, H_β ΨPro-Hb), 4.63 (dd, *J* = 7.3, 1.4 Hz, 1 H, H_α ΨPro-H), 5.15 (d, *J* = 12.0 Hz, 1 H, Bn CH_2-Ha), 5.22 (d, *J* = 12.0 Hz, 1 H, Bn CH_2-Hb), 5.47 (ddd, *J* = 12.4, 5.1, 2.3 Hz, 1 H, H_δ ΨPro), 5.78 (ddd, *J* = 54.6, 53.8, 2.3 Hz, 1 H, CF_2H-H), 7.29-7.42 (m, 5 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) δ (81:19 mixture of rotational isomers) (major conformer) δ 22.7 (CH_3, Ac), 58.9 (CH, C_α ΨPro), 68.3 (CH_2, CH_2 Bn), 72.6 (CH_2, C_β ΨPro), 87.0 (dd, *J* = 28.8, 19.2 Hz, CH, C_δ ΨPro), 112.2 (t, *J* = 246.3 Hz, CH, CF_2H), 128.6 (2 CH, C-arom), 128.9 (2 CH, C-arom), 129.0 (CH, C-arom), 134.6 (C, C-arom), 169.7 (C, C=O), 169.9 (C, C=O); (minor conformer) δ 22.7 (CH_3, Ac), 58.5 (CH, C_α ΨPro), 67.6 (CH_2, CH_2 Bn), 70.1 (CH_2, C_β ΨPro), 86.3 (t, *J* = 25.4 Hz, CH, C_δ ΨPro), 113.3 (t, *J* = 250.2 Hz, CH, CF_2H), 128.4 (2 CH, C-arom), 128.8 (2 CH, C-arom), 135.2 (C, C-arom), 169.0 (C, C=O), 169.7 (C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 293 K) δ (81:19 mixture of rotational isomers) (major conformer) δ -133.6 (ddd, *J* = 287.5, 53.8, 2.6 Hz, 1 F), -144.3 (ddd, *J* = 287.5, 55.5, 20.0 Hz, 1 F); (minor conformer) δ -128.5 (ddd, *J* = 293.5, 53.8, 5.1 Hz, 1 F), -135.0 (ddd, *J* = 293.5, 54.6, 12.4 Hz, 1 F).; HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{14}H_{15}F_2NO_4$ 300.1042, found 300.1051.

(2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Benzyl Ester (*R*,*S*)-2: The product was prepared by the acyl chloride procedure using 1.04 g of (*R*,*S*)-**1** (4.03 mmol, 1 equiv) in DCM (8 mL), 977 µL of pyridine (12.08 mmol, 3 equiv) and 862 µL of acetyl chloride (12.08 mmol, 3 equiv). Purification by flash chromatography (60:40 cyclohexane/ethyl acetate) gave 906 mg of (*R*,*S*)-**2** (3.03 mmol, 75%) as a 50/50 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K.

The product was prepared by the acid anhydride procedure with iodine catalysis using 300 mg of (*R*,*S*)-**1** (1.16 mmol, 1 equiv) in acetic anhydride (2 mL, 11.66 mmol, 10 equiv), iodine (30 mg, 0.12 mmol, 0.1 equiv). Purification by flash chromatography (60:40 cyclohexane/ethyl acetate) gave 262 mg of (*R*,*S*)-**2** (0.88 mmol, 76%) as a 50/50 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K: Yellow oil; R_f = 0.36 (60:40 Cyclohexane/ethyl Acetate); $[\alpha]^{20}_D$: -36.9 (*c* 1.31, $CHCl_3$); IR (neat, cm^{-1}): 2917, 1747, 1670, 1500, 1456, 1397, 1352, 1275, 1194, 1112, 1070; ^1H NMR (400 MHz, $CDCl_3$, 293 K) δ (50:50 mixture of rotational isomers) δ 2.09 (s, 3 H, Ac CH_3-H), 2.20 (s, 3 H, Ac CH_3-H), 4.17 (m, 1 H, H_β ΨPro-Ha), 4.31 (t, *J* = 8.2 Hz, 1 H, H_β ΨPro-Hb), 4.40 (t, *J* = 8.2 Hz, 1 H, H_β

ΨPro-Ha), 4.51 (m, 1 H, H_β ΨPro-Hb), 4.61 (m, 1 H, H_α ΨPro-H), 5.01 (m, 1 H, H_α ΨPro-H), 5.17-5.25 (m, 4 H, 2 Bn CH_2-H), 5.32 (m, 1 H, H_δ ΨPro-H), 5.60 (m, 1 H, H_δ ΨPro-H), 5.76 (ddd, *J* = 55.5, 55.0, 4.1 Hz, 1 H, CF_2H-H), 5.97 (dd, *J* = 54.6, 54.2 Hz, 1 H, CF_2H-H), 7.32-7.40 (m, 10 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) δ (50:50 mixture of rotational isomers) δ 22.5 (2 CH_3, Ac), 57.1 (CH, C_α ΨPro), 58.4 (CH, C_α ΨPro), 67.8 (CH_2, CH_2 Bn), 68.2 (CH_2, CH_2 Bn), 69.2 (CH_2, C_β ΨPro), 70.9 (CH_2, C_β ΨPro), 86.3-87.6 (m, 2 CH, C_δ ΨPro), 112.2 (t, *J* = 247.3, CH, CF_2H), 113.0 (t, *J* = 246.3, CH, CF_2H), 128.4 (4 CH, CH-arom), 128.6 (4 CH, CH-arom), 128.9 (2 CH, CH-arom), 134.6 (C, C-arom), 134.9 (C, C-arom), 168.7 (C, C=O), 168.9 (C, C=O), 169.9 (2 C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 293 K) δ (50:50 mixture of rotational isomers) (conformer 1) δ -129.7 (dd, *J* = 297.4, 55.5 Hz, 1 F), -132.3 (dd, *J* = 297.4, 55.0, Hz, 1 F); (conformer 2) δ -133.3 (dd, *J* = 289.6, 54.2, Hz, 1 F), -140.2 (ddd, *J* = 289.6, 54.6, 16.5 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{14}H_{15}F_2NO_4$ 300.1042, found 300.1045.

(2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Acid (*S*,*S*)-3: To a solution of (*S*,*S*)-**2** (0.405 g, 1.35 mmol, 1.0 equiv) in methanol (3 mL) was added 10% Pd/C (0.144 g, 0.14 mmol, 0.1 equiv). The mixture was placed under hydrogen atmosphere (4 bars) and stirred vigourously until the starting material diseappared. The crude mixture was filtered under a microporous filter and evaporated under reduced pressure. The acidic compound (*S*,*S*)-**3** was obtained quantitatively (0.304g, 1.45 mmol) as an 83/17 inseparable mixture of *cis*/*trans* rotational conformers in CD_3OD at 293 K and used in the next step without further purification: White solid; R_f = 0.41 (90:10 DCM/MeOH); $[\alpha]^{20}_D$: -89.4 (*c* 0.83, MeOH); IR (neat, cm^{-1}): 3399, 2923, 2508, 2075, 1728, 1645, 1404, 1360, 1231, 1157, 1109, 1065; ^{1}H NMR (400 MHz, CD_3OD, 293 K) δ (83:17 mixture of rotational isomers) (major conformer) δ 2.05 (s, 3 H, CH_3 Ac), 4.36 (m, 1 H, H_β-Ha), 4.45 (m, 1 H, H_β-Hb), 4.73 (m, 1 H, H_α), 5.40 (dd, *J* = 19.1, 3.0 Hz, 1 H, H_δ), 6.12 (dd, *J* = 55.5, 54.2 Hz, 1 H, CF_2H); (minor conformer) δ 2.15 (s, 3 H, CH_3 Ac), 4.19 (m, 1 H, H_β-Ha), 4.45 (m, 1 H, H_β-Hb), 4.50 (m, 1 H, H_α), 5.63 (ddd, *J* = 8.7, 6.9, 3.2 Hz, 1 H, H_δ), 5.99 (td, *J* = 54.4, 3.2 Hz, 1 H, CF_2H); ^{13}C NMR (100.5 MHz, CD_3OD, 293 K) (83:17 mixture of rotational isomers) (major conformer) δ 23.5 (CH_3, Ac), 61.1 (CH, C_α ΨPro), 74.8 (CH_2, C_β ΨPro), 88.9 (dd, *J* = 28.8, 20.1 Hz, CH, C_δ ΨPro), 114.6 (t, *J* = 244.9 Hz, CF_2H), 173.9 (C, C=O), 174.3 (C, C=O); (minor conformer) δ 23.5 (CH_3, Ac), 60.4 (CH, C_α ΨPro), 72.3 (CH_2, C_β ΨPro), 88.5 (t, *J* = 27.8 Hz, CH, C_δ ΨPro), 115.8 (t, *J* = 247.8 Hz, CF_2H), 172.4 (C, C=O), 174.1 (C, C=O); ^{19}F (376.2 MHz, CD_3OD, 293 K) (83:17 mixture of rotational isomers) (major conformer) δ -134.9 (ddd, *J* = 288.7, 54.2, 3.0 Hz, 1 F), -144.7 (ddd, *J* = 288.7, 55.5, 19.1 Hz, 1 F); (minor conformer) δ -131.9 (ddd, *J* = 293.9, 54.2, 6.9 Hz, 1 F), -136.2 (ddd, *J* = 293.9, 54.6, 8.7 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_7H_{10}F_2NO_4$ 210.0572, found 210.0578.

(2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Acid (*R*,*S*)-3: To a solution of (*R*,*S*)-**2** (0.605 g, 2.02 mmol, 1 equiv) in methanol (10 mL) was added 10% Pd/C (0.215 g, 0.20 mmol, 0.1 equiv). The mixture was placed under hydrogen atmosphere (4 bars) and stirred vigourously until the diseapparence of the starting material. The crude mixture was filtered under a microporous filter and evaporated under reduced pressure. The acidic compound (*R*,*S*)-**3** was obtained quantitatively (0.422g, 2.02 mmol) as a 60/40 inseparable mixture of *cis*/*trans* rotational conformers in CD_3OD at 293 K and used in the next step without further purification: White solid; R_f = 0.40 (90:10 DCM/MeOH); $[\alpha]^{20}_D$: -52.9 (*c* 0.81, MeOH); IR (neat, cm^{-1}) 3349, 2945, 1738, 1652, 1407, 1213, 1158, 1113, 1071, 1022; ^{1}H NMR (400 MHz, CD_3OD, 293 K) (60:40 mixture of rotational isomers) (major conformer) δ 2.13 (s, 3 H, Ac CH_3-H), 4.33-4.43 (m, 2 H, 2 H_β ΨPro-H), 4.81 (m, 1 H, H_α ΨPro-H), 5.51 (m, 1 H, H_δ ΨPro-H), 5.91 (dd, *J* = 55.0, 54.6 Hz, 1 H, CF_2H-H); (minor conformer) δ 2.15 (s, 3 H, Ac CH_3-H), 4.17 (m, 1 H, H_β ΨPro-Ha), 4.38 (m, 1 H, H_β ΨPro-Hb), 4.81 (m, 1 H, H_α ΨPro-H), 5.47 (m, 1 H, H_δ ΨPro-H), 5.89 (t, *J* = 54.6 Hz, 1 H, CF_2H-H); ^{13}C NMR (100.5 MHz, CD_3OD, 293 K) (60:40 mixture of rotational isomers) (major conformer) δ 23.3 (CH_3, Ac), 60.6 (CH, C_α ΨPro), 72.7 (CH_2, C_β ΨPro), 88.5 (dd, *J* = 28.8, 24.0 Hz, CH, C_δ ΨPro), 114.7 (t, *J* = 246.3 Hz, CH, CF_2H), 173.5 (C, C=O), 173.7 (C, C=O); (minor conformer) δ 23.3 (CH_3, Ac), 59.3 (CH, C_α ΨPro), 71.2 (CH_2, C_β ΨPro), 89.1 (t, *J* = 30.2 Hz, CH, C_δ ΨPro), 115.5 (t, *J* = 245.9 Hz, CH, CF_2H), 172.6 (C, C=O), 173.9 (C, C=O); ^{19}F (376.2 MHz, CD_3OD, 293 K) : (60:40 mixture of rotational isomers) (major conformer) δ -134.6 (dd, *J* = 290.9, 54.6 Hz, 1 F), -139.6 (ddd, *J* = 290.9, 55.0, 14.3 Hz, 1 F); (minor conformer) δ -132.0 (ddd, *J* = 295.8, 54.6, 5.2 Hz, 1 F), -134.4 (dd, J = 295.8, 54.6 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_7H_{10}F_2NO_4$ 210.0572, found 210.0575.

(2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*S*,*S*)-4: To a solution of (*S*,*S*)-**3** (0.205 g, 0.98 mmol, 1.0 equiv) in DMF (5 mL) were successively added under argon at 0 °C HOBt (150 mg, 0.98 mmol, 1.0 equiv) and EDCI (207 mg, 1.08 mmol, 1.1 equiv). After stirring at 0 °C for 30 min, methylamine hydrochloride (132 mg, 1.96 mmol, 2.0 equiv) and $NaHCO_3$ (247 mg, 2.94 mmol, 3

equiv) were added. The reaction mixture was stirred at room temperature until the reaction was completed as monitored by TLC and ^{19}F NMR analysis. The reaction was quenched with water (3 mL) and AcOEt (5 mL), the aqueous layer was extracted with AcOEt (3 x) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (96:4 AcOEt/MeOH) gave 88 mg of (*S*,*S*)-**4** (0.40 mmol, 40%) as a 70/30 inseparable mixture of *cis*/*trans* rotational conformers in CD_3OD at 293 K: White solid, mp 167°C; R_f = 0.52 (96:4 AcOEt/MeOH); $[\alpha]^{20}_D$: -86.3 (*c* 1.35, MeOH); IR (neat, cm^{-1}): 3307, 2965, 2452, 1652, 1578, 1397, 1353, 1259, 1226, 1160, 1114, 1064; ^1H NMR (400 MHz, CD_3OD, 293 K) δ (70:30 mixture of rotational isomers) (*cis* conformer) δ 1.99 (s, 3 H, Ac CH_3-H), 2.76 (s, 3 H, NMe), 4.19 (d, *J* = 8.7 Hz, 1 H, H_β ΨPro-Ha), 4.46 (ddd, *J* = 8.7, 6.9, 1.8 Hz, 1 H, H_β ΨPro-Hb), 4.58 (d, *J* = 6.0 Hz, 1 H, H_α ΨPro-H), 5.50 (dd, *J* = 19.9, 3.0 Hz, 1 H, H_δ ΨPro-H), 6.13 (dd, *J* = 55.5, 54.2 Hz, 1 H, CF_2H-H); (*trans* conformer) δ 2.13 (s, 3 H, Ac CH_3-H), 2.71 (s, 3 H, NMe), 4.05 (d, *J* = 7.8 Hz, 1 H, H_β ΨPro-Ha), 4.34-4.42 (m, 2 H, H_β ΨPro-Hb and H_α ΨPro-H), 5.64 (ddd, *J* = 10.0, 6.9, 2.8 Hz, 1 H, H_δ ΨPro-H), 6.04 (td, *J* = 54.6, 2.8 Hz, 1 H, CF_2H-H); ^1H NMR (400 MHz, H_2O/D_2O 90/10, 278 K) δ (60:40 mixture of rotational isomers) (*cis* conformer) δ 2.06 (s, 3 H, Ac CH_3-H), 2.81 (d, *J* = 4.3 Hz, 3 H, NMe), 4.34 (dt, *J* = 9.4, 1.4 Hz, 1 H, H_β ΨPro-Ha), 4.58 (ddd, *J* = 9.3, 7.6, 1.7 Hz, 1 H, H_β ΨPro-Hb), 4.79 (m, 1 H, H_α ΨPro-H), 5.67 (ddd, *J* = 18.2, 3.2, 1.0 Hz, 1 H, H_δ ΨPro-H), 6.22 (td, *J* = 54.6, 53.5, 1.0 Hz, 1 H, CF_2H-H), 8.48 (d, *J* = 4.3 Hz, 1 H, NH); (*trans* conformer) δ 2.22 (s, 3 H, Ac CH_3-H), 2.76 (d, *J* = 4.8 Hz, 3 H, NMe), 4.21 (ddd, *J* = 9.3, 3.2, 1.7 Hz, 1 H, H_β ΨPro-Ha), 4.49 (ddt, *J* = 9.3, 8.0, 1.4 Hz, 1 H, H_β ΨPro-Hb), 4.56 (dd, *J* = 7.6, 2.1 Hz, 1 H, H_α ΨPro-H), 5.81 (ddd, *J* = 9.3, 8.1, 2.8 Hz, 1 H, H_δ ΨPro-H), 6.10 (td, *J* = 53.8, 2.8 Hz, 1 H, CF_2H-H), 8.32 (d, *J* = 4.8 Hz, 1 H, NH); ^{13}C NMR (100.5 MHz, CD_3OD, 293 K) δ (70:30 mixture of rotational isomers) (*cis* conformer) δ 23.5 (CH_3, Ac), 27.5 (CH_3, NMe), 61.6 (CH, C_α ΨPro), 75.5 (CH_2, C_β ΨPro), 89.3 (dd, *J* = 28.8, 20.1 Hz, CH, C_δ ΨPro), 114.7 (t, *J* = 242.4 Hz, CH, CF_2H), 172.0 (C, C=O), 173.4 (C, C=O); (*trans* conformer) δ 23.7 (CH_3, Ac), 27.3 (CH_3, NMe), 61.2 (CH, C_α ΨPro), 72.9 (CH_2, C_β ΨPro), 88.6 (dd, *J* = 27.1, 24.3 Hz, CH, C_δ ΨPro), 116.0 (t, *J* = 248.2 Hz, CH, CF_2H), 173.4 (C, C=O), 173.7 (C, C=O); ^{13}C NMR (125 MHz, D_2O, 298 K) δ (60:40 mixture of rotational isomers) (*cis* conformer) δ 21.7 (CH_3, Ac), 26.1 (CH_3, NMe), 59.1 (CH, C_α ΨPro), 73.1 (CH_2, C_β ΨPro), 86.6 (dd, *J* = 28.0, 22.1 Hz, CH, C_δ ΨPro), 112.3 (t, *J* = 245.4 Hz, CH, CF_2H), 172.1 (C, C=O), 172.9 (C, C=O); (*trans* conformer) δ 21.9 (CH_3, Ac), 25.9 (CH_3, NMe), 58.6 (CH, C_α ΨPro), 70.8 (CH_2, C_β ΨPro), 86.1 (t, *J* = 26.0 Hz, CH, C_δ ΨPro), 113.1 (t, *J* = 247.5 Hz, CH, CF_2H), 171.9 (C, C=O), 172.2 (C, C=O); ^{19}F (376.2 MHz, CD_3OD, 293 K) (70:30 mixture of rotational isomers) (*cis* conformer) δ -135.0 (ddd, *J* = 288.7, 54.2, 3.0 Hz, 1 F), -144.3 (ddd, *J* = 288.7, 55.5, 19.9 Hz, 1 F); (*trans* conformer) δ -131.7 (ddd, *J* = 293.0, 54.6, 6.9 Hz, 1 F), -136.1 (ddd, *J* = 293.0, 54.6, 10.0 Hz, 1 F); ^{19}F (376.2 MHz, D_2O, 298 K) (60:40 mixture of rotational isomers) (*cis* conformer) δ -131.3 (ddd, *J* = 287.8, 53.5, 3.2 Hz, 1 F), -138.4 (dddt, *J* = 287.8, 54.6, 18.2, 1.7 Hz, 1 F); (*trans* conformer) δ -129.2 (dddq, *J* = 293.1, 53.8, 8.1, 1.4 Hz, 1 F), -132.5 (dddq, *J* = 293.1, 53.8, 9.3, 1.4 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_8H_{12}F_2NO_3$ 223.0889, found 223.0890.

(2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*R*,*S*)-4: To a solution of (*R*,*S*)-**3** (0.225 g, 1.08 mmol, 1.0 equiv) in DMF (5 mL) were successively added under argon at 0 °C HOBt (165 mg, 1.08 mmol, 1.0 equiv) and EDCI (227 mg, 1.18 mmol, 1.1 equiv). After stirring at 0 °C for 30 min, methylamine hydrochloride (145 mg, 2.15 mmol, 2.0 equiv) and DIPEA (576 µL, 3.33 mmol, 3.1 equiv) were added. The reaction mixture was stirred at room temperature until the reaction was completed as monitored by TLC and ^{19}F NMR analysis. The reaction was quenched with water (3 mL) and AcOEt (5 mL), the aqueous layer was extracted with AcOEt (3 x) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (99:1 DCM/MeOH) gave 79 mg of (*R*,*S*)-**4** (0.36 mmol, 33%) as a 56/44 inseparable mixture of *cis*/*trans* rotational conformer in CD_3OD at 293 K: Yellow oil; R_f = 0.39 (97:3 DCM/MeOH); $[\alpha]^{20}_D$: -46.9 (*c* 0.93, MeOH); IR (neat, cm^{-1}): 3311, 2947, 2834, 1657, 1553, 1411, 1354, 1205, 1164, 1115, 1073, 1023; ^1H NMR (400 MHz, CD_3OD, 293 K) (56:44 mixture of rotational isomers) (*cis* conformer) δ 2.15 (s, 3 H, Ac CH_3-H), 2.71 (s, 3 H, NMe), 4.11 (m, 1 H, H_β ΨPro-Ha), 4.34 (t, *J* = 8.5 Hz, 1 H, H_β ΨPro-Hb), 4.69 (m, 1 H, H_α), 5.50 (m, 1 H, H_δ ΨPro-H), 6.00 (dd, *J* = 55.5, 54.6 Hz, 1 H, CF_2H-H); (*trans* conformer) δ 2.07 (s, 3 H, Ac CH_3-H), 2.75 (s, 3 H, NMe), 4.25 (m, 1 H, H_β ΨPro-Ha), 4.34 (t, *J* = 8.5 Hz, 1 H, H_β ΨPro-Hb), 4.64 (m, 1 H, H_α ΨPro-H), 5.45 (m, 1 H, H_δ ΨPro-H), 5.99 (dd, *J* = 54.6, 53.8 Hz, 1 H, CF_2H-H); ^1H NMR (600 MHz, D_2O, 283 K) (53:47 mixture of rotational isomers) (*cis* conformer) δ 2.14 (s, 3 H, Ac CH_3-H), 2.80 (s, 3 H, NMe), 4.38 (ddd, *J* = 9.1, 4.2, 1.4 Hz, 1 H, H_β ΨPro-Ha), 4.44 (ddd, *J* = 9.1, 7.5, 1.8 Hz, 1 H, H_β ΨPro-Hb), 4.83 (dd, *J* = 7.6, 4.4 Hz, 1 H, H_α ΨPro-H), 5.61 (ddd, *J* = 14.1, 4.6, 1.8 Hz, 1 H, H_δ ΨPro-H), 6.15 (ddd, *J* = 54.8, 53.1, 1.8 Hz, 1 H, CF_2H-H);

(*trans* conformer) δ 2.24 (s, 3 H, Ac CH_3-H), 2.75 (s, 3 H, NMe), 4.20 (ddd, *J* = 8.9, 7.1, 1.5 Hz, 1 H, H_β ΨPro-Ha), 4.50 (td, *J* = 8.9, 1.4 Hz, 1 H, H_β ΨPro-Hb), 4.79 (dd, *J* = 8.9, 7.1 Hz, 1 H, H_α ΨPro-H), 5.73 (ddd, *J* = 8.1, 6.8, 4.0 Hz, 1 H, H_δ ΨPro-H), 6.07 (td, *J* = 54.2, 4.0 Hz, 1 H, CF_2H–H); ^{13}C NMR (100.5 MHz, CD_3OD, 293 K) δ (56:44 mixture of rotational isomers) (*cis* conformer) δ 23.5 (CH_3, Ac), 27.5 (CH_3, NMe), 60.4 (CH, C_α ΨPro), 71.4 (CH_2, C_β ΨPro), 89.3 (t, *J* = 27.8 Hz, CH, C_δ ΨPro), 115.3 (t, *J* = 246.0 Hz, CH, CF_2H), 171.9 (C, C=O), 173.2 (C, C=O); (*trans* conformer) δ 23.7 (CH_3, Ac), 27.3 (CH_3, NMe), 62.0 (CH, C_α ΨPro), 73.4 (CH_2, C_β ΨPro), 89.0 (t, *J* = 20.1 Hz, CH, C_δ ΨPro), 114.7 (t, *J* = 246.3 Hz, CH, CF_2H), 172.5 (C, C=O), 174.2 (C, C=O); ^{13}C NMR (125 MHz, D_2O, 298 K) δ (53:47 mixture of rotational isomers) δ 21.8 (2 CH_3, Ac), 25.9 (CH_3, NMe), 26.0 (CH_3, NMe), 58.4 (CH, C_α ΨPro), 59.8 (CH, C_α ΨPro), 69.3 (CH_2, C_β ΨPro), 71.1 (CH_2, C_β ΨPro), 86.6 (q, *J* = 26.6 Hz, 2 CH, C_δ ΨPro), 112.1 (t, *J* = 245.3 Hz, CH, CF_2H), 112.8 (t, *J* = 245.0 Hz, CH, CF_2H), 171.2 (2 C, C=O), 173.7 (C, C=O), 174.3 (C, C=O); ^{19}F (376.2 MHz, CD_3OD, 293 K) (56:44 mixture of rotational isomers) (*cis* conformer) δ -132.6 (ddd, *J* = 295.0, 55.5, 5.2 Hz, 1 F), -135.3 (ddd, *J* = 295.0, 54.6, 3.5 Hz, 1 F); (*trans* conformer) δ -134.8 (dd, *J* = 290.0, 53.8 Hz, 1 F), -140.0 (ddd, *J* = 290.0, 54.6, 14.7 Hz, 1 F); ^{19}F (564.6 MHz, D_2O, 283 K) (53:47 mixture of rotational isomers) (*cis* conformer) δ -131.1 (ddd, *J* = 289.9, 53.1, 4.6 Hz, 1 F), -135.5 (ddd, *J* = 289.9, 54.8, 14.1 Hz, 1 F); (*trans* conformer) δ -128.8 (ddd, *J* = 295.2, 54.2, 6.8 Hz, 1 F), -131.9 (ddd, *J* = 295.2, 54.2, 8.1 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_8H_{12}F_2NO_3$ 223.0889, found 223.0893.

Peptide Coupling Reactions.

Representative Procedure for the Preparation of Fmoc-Aminoacid Chloride Assisted by Ultrasonication: To a 0.2 M solution of the Fmoc-aminoacid (1.0 equiv) suspended in DCM under argon, was added freshly distilled $SOCl_2$ (13.0 equiv). The mixture was sonicated at room temperature until the complete disappearence of the precipitate (from 30 min to 1 h), and then solvent and excess of $SOCl_2$ were removed in vacuo. The crude was washed with pentane to give the Fmoc-aminoacid chloride as a white solid directly used without further purification.

Base-Free Representative Procedure for the Peptide Coupling Reaction: To a solution of pseudoprolines **1** in DCM was added Fmoc-amino acid chloride solution in DCM. The reaction mixture was stirred at room temperature under inert atmosphere until the reaction was completed as monitored by TLC and ^{19}F NMR (usually 18 h), and then quenched with a solution of saturated $NaHCO_3$. The aqueous phase was extracted with DCM (2 x) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography gave the corresponding peptide (*R*,*S*)-**6** in 82% yield.

Pyridine Representative Procedure for Peptide Coupling Reaction: To a solution of pseudoprolines **1** in DCM was successively added pyridine and Fmoc-amino acid chloride solution in DCM. The mixture was stirred at room temperature until the reaction was completed as monitored by TLC and ^{19}F NMR, and then quenched with a solution of saturated $NaHCO_3$. The aqueous phase was extracted with DCM (2 x) and the combined organic extracts were washed with a 1 M aqueous HCl solution (1 x), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography gave the corresponding peptides (*S*,*S*)-**5**, (*R*,*S*)-**5** and (*S*,*S*)-**6** in 57−86% yield.

Fmoc-Gly-Ser(Ψ^{CF2H,H}Pro)-OBn (*S*,*S*)-5: The product was prepared following the pyridine representative procedure using (*S*,*S*)-**1** (0.98 g, 3.8 mmol, 1.0 equiv) in DCM (7 mL), pyridine (617 µL, 6.1 mmol, 1.6 equiv) and Fmoc-Gly-Cl (1.926 g, 6.1 mmol, 1.6 equiv) in DCM (15 mL). Purification by flash chromatography (80:20 Cyclohexane/Ethyl acetate) gave 1.755 g of the Fmoc-Gly-(2*S*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*S*,*S*)-**5** (86%, 3.27 mmol) as an 83/17 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K along with 122 mg of Fmoc-Gly-(2*R*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-**5** (6%, 0.23 mmol) as an 58/42 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K: White solid; mp 134 °C; R_f = 0.29 (70:30 Cyclohexane/AcOEt); $[\alpha]^{20}_D$: -67.6 (*c* 1.03, $CHCl_3$); IR (neat, cm^{-1}): 3335, 2925, 1723, 1673, 1519, 1451, 1429, 1247, 1214, 1193, 1062; ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) (83:17 mixture of rotational isomers) (major conformer) δ 3.79 (dd, *J* = 16.9, 4.0 Hz, 1 H, H_α Gly-Ha), 4.06 (dd, *J* = 16.9, 5.8 Hz, 1 H, H_α Gly-Hb), 4.23 (t, *J* = 7.1 Hz, 1 H, Fmoc CH-H), 4.40 (d, *J* = 7.1 Hz, 2 H, Fmoc CH_2-H), 4.40 (m, 1 H, H_β ΨPro-Ha), 4.52 (dd, *J* = 8.2, 6.9 Hz, 1 H, H_β ΨPro-Hb), 4.59 (m, 1 H, H_α ΨPro), 5.19 (d, *J* = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.29 (d, *J* = 12.1 Hz, 1 H, Bn CH_2-Hb), 5.51 (m, 1 H, NH Gly), 5.57 (d, *J* = 19.9 Hz, 1 H, H_δ ΨPro-H), 6.22 (dd, *J* = 55.5, 53.8 Hz, 1 H, CF_2H-H), 7.30-7.44 (m, 9 H, H-arom), 7.60 (d, *J* = 7.3 Hz, 2 H, H-arom), 7.77 (d, *J* = 7.3 Hz, 2 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) (83:17 mixture of rotational isomers) (major conformer) δ 43.7 (CH_2, C_α Gly), 47.2 (CH, CH Fmoc), 57.5 (CH, C_α ΨPro), 67.4 (CH_2, CH_2 Fmoc), 68.7 (CH_2, CH_2 Bn), 72.8 (CH_2, C_β ΨPro), 87.3 (dd, *J* = 28.8,

18.2 Hz, CH, C_δ ΨPro), 112.0 (t, *J* = 245.4 Hz, CH, CF_2H), 120.2 (CH, C-arom), 125.2 (CH, C-arom), 127.2 (CH, C-arom), 127.9 (CH, C-arom), 128.8 (CH, C-arom), 129.0 (CH, C-arom), 129.7 (CH, C-arom), 134.4 (C, C-arom), 141.4 (C, C-arom), 143.9 (C, C-arom), 156.2 (C, C=O), 168.2 (C, C=O), 169.1 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 293 K) (83:17 mixture of rotational isomers) (major conformer) δ -133.6 (dd, *J* = 289.2, 53.8 Hz, 1 F), -143.6 (ddd, *J* = 289.2, 55.5, 19.9 Hz, 1 F); (minor conformer) δ -127.9 (dd, *J* = 299.8, 57.2 Hz, 1 F), -135.4 (ddd, *J* = 299.8, 54.2, 12.1 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{29}H_{27}F_2N_2O_6$ 537.1832, found 537.1823.

Fmoc-Gly-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-5: The product was prepared following the pyridine representative procedure using (*R*,*S*)-**1** (100 mg, 0.39 mmol, 1.0 equiv) in DCM (2 mL), pyridine (62 µL, 0.77 mmol, 2.0 equiv) and Fmoc-Gly-Cl (220 mg, 0.70 mmol, 1.8 equiv) in DCM (2 mL). Purification by flash chromatography (70:30 Cyclohexane/Ethyl acetate) gave 159 mg of the Fmoc-Gly-(2*R*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-**5** (76%, 0.30 mmol) as an 58/42 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K: White solid, mp 120 °C; R_f = 0.18 (70:30 Cyclohexane/AcOEt); $[\alpha]^{20}_D$: -46.0 (*c* 1.03, $CHCl_3$); IR (neat, cm^{-1}): 3333, 2956, 1723, 1674, 1519, 1450, 1428, 1213, 1193, 1104, 1070; ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) (58:42 mixture of rotational isomers) (major conformer) δ 3.95 (m, 1 H, H_α Gly-Ha), 4.17-4.25 (m, 2 H, H_α Gly-Hb and Fmoc CH-H), 4.35 (m, 1 H, H_β ΨPro-Ha), 4.39 (d, *J* = 7.3 Hz, 2 H, Fmoc CH_2-H), 4.57 (m, 1 H, H_β ΨPro-Hb) 4.77 (m, 1 H, H_α ΨPro-H), 5.15-5.30 (m, 2 H, Bn CH_2-H), 5.67 (m, 1 H, H_δ ΨPro-H), 5.80 (m, 1 H, NH Gly), 5.97 (t, *J* = 55.1, 1 H, CF_2H-H), 7.30-7.46 (m, 9 H, H-arom), 7.61 (d, *J* = 7.3 Hz, 2 H, H-arom), 7.78 (d, *J* = 7.3 Hz, 2 H, H-arom); (minor conformer) δ 3.95 (m, 1 H, H_α Gly-Ha), 4.16 (m, 1 H, H_β ΨPro-Ha), 4.22 (m, 1 H, H_α Gly-Hb), 4.30 (m, 1 H, Fmoc CH-H), 4.38 (m, 1 H, H_β ΨPro-Hb), 4.39 (d, *J* = 7.3 Hz, 2 H, Fmoc CH_2-H), 5.06 (m, 1 H, H_α ΨPro-H), 5.15-5.30 (m, 2 H, Bn CH_2-H), 5.49 (m, 1 H, H_δ ΨPro-H), 5.80 (m, 1 H, NH Gly), 5.97 (t, *J* = 55.9 Hz, 1 H, CF_2H-H), 7.30-7.46 (m, 9 H, H-arom), 7.61 (d, *J* = 7.3 Hz, 2 H, H-arom), 7.78 (d, *J* = 7.3 Hz, 2 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) δ (58:42 mixture of rotational isomers) (major conformer) δ 43.4 (CH_2, C_α Gly), 47.1 (CH, Fmoc CH), 57.1 (CH, C_α ΨPro), 67.3 (CH_2, Fmoc CH_2), 68.4 (CH_2, C_β ΨPro), 68.6 (CH_2, CH_2 Bn), 85.6-87.4 (m, C_δ ΨPro), 112.1 (t, *J* = 247.3 Hz, CF_2H), 120.1 (2 CH, C-arom), 125.2 (2 CH, C-arom), 127.2 (2 CH, C-arom), 127.8 (2 CH, C-arom), 128.6 (CH, C-arom), 128.8 (2 CH, C-arom), 134.5 (C, C-arom), 141.3 (2 C, C-arom), 143.8 (2 C, C-arom), 156.5 (C, C=O), 168.0 (C, C=O), 168.4 (C, C=O); (minor conformer) δ 43.4 (CH_2, C_α Gly), 47.1 (CH, Fmoc CH), 57.1 (CH, C_α ΨPro), 67.3 (CH_2, Fmoc CH_2), 68.6 (CH_2, CH_2 Bn), 70.8 (CH_2, C_β ΨPro), 85.6-87.4 (m, C_δ ΨPro), 112.7 (t, *J* = 249.2 Hz, CF_2H), 120.1 (2 CH, C-arom), 125.2 (2 CH, C-arom), 127.2 (2 CH, C-arom), 127.8 (2 CH, C-arom), 128.6 (CH, C-arom), 128.8 (2 CH, C-arom), 134.8 (C, C-arom), 141.3 (2 C, C-arom), 143.8 (2 C, C-arom), 156.5 (C, C=O), 168.3 (C, C=O), 169.3 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 293 K) (58:42 mixture of rotational isomers) (major conformer) δ -133.6 (dd, *J* = 290.5, 55.1 Hz, 1 F), -138.9 (ddd, *J* = 290.5, 55.1, 10.8 Hz, 1 F); (minor conformer) δ -129.4 (dd, *J* = 296.9, 55.9 Hz, 1 F), -132.7 (dd, *J* = 296.9, 55.9 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{29}H_{27}F_2N_2O_6$ 537.1832, found 537.1823.

Fmoc-L-Ala-Ser(Ψ^{CF2H,H}Pro)-OBn (*S*,*S*)-6: The product was prepared following the pyridine representative procedure using (*S*,*S*)-**1** (100 mg, 0.39 mmol, 1.0 equiv) in DCM (3 mL), pyridine (56 µL, 0.69 mmol, 1.8 equiv) and Fmoc-Ala-Cl (206 mg, 0.62 mmol, 1.6 equiv) in DCM (3 mL). Purification by flash chromatography (80:20 Cyclohexane/Ethyl acetate) gave 121 mg of the Fmoc-L-Ala-(2*S*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*S*,*S*)-**6** (57%, 0.22 mmol) as an 76/24 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K along with 60 mg of Fmoc-L-Ala-(2*R*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-**6** (28%, 0.11 mmol) as an 78/22 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K: White solid; mp 132°C; R_f = 0.42 (50:50 Pentane/Et_2O); $[\alpha]^{20}_D$: -40.2 (*c* 1.30, $CHCl_3$); IR (neat, cm^{-1}): 2927, 1721, 1668, 1451, 1419, 1215, 1155, 1072; ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) (76:24 mixture of rotational isomers) (major conformer) δ 1.39 (d, *J* = 6.9 Hz, 3 H, C_β Ala), 4.20 (m, 1 H, Fmoc CH-H), 4.30 (m, 1 H, Fmoc CH_2-Ha), 4.38 (m, 1 H, Fmoc CH_2-Hb), 4.39 (m, 1 H, H_α Ala-H), 4.41 (m, 1 H, H_β ΨPro-Ha), 4.52 (m, 1 H, H_β ΨPro-Hb), 4.60 (m, 1 H, H_α ΨPro-H), 5.18 (d, *J* = 12.4 Hz, 1 H, CH_2 Bn-Ha), 5.22 (d, *J* = 12.4 Hz, 1 H, CH_2 Bn-Hb), 5.62 (dd, *J* = 19.1, 2.6 Hz, 1 H, H_δ ΨPro-H), 5.61 (m, 1 H, NH Ala), 6.13 (dd, *J* = 55.5, 53.3 Hz, 1 H, CF_2H-H), 7.27-7.44 (m, 9 H, H-arom), 7.56-7.62 (m, 2 H, H-arom), 7.77 (d, *J* = 7.3 Hz, 2 H, H-arom); (minor conformer) δ 1.34 (d, *J* = 6.9 Hz, 3 H, C_β Ala-H), 4.20 (m, 2 H, Fmoc CH-H and H_β ΨPro-Ha), 4.30 (m, 1 H, Fmoc CH_2-Ha), 4.38 (m, 1 H, Fmoc CH_2-Hb), 4.42 (m, 1 H, H_α Ala-H), 4.45 (m, 1 H, H_β ΨPro-Hb), 4.73 (m, 1 H, H_α ΨPro-H), 5.19 (d, *J* = 12.4 Hz, 1 H, CH_2 Bn-Ha), 5.23 (d, *J* = 12.4 Hz, 1 H, CH_2 Bn-Hb), 5.50 (dd, *J* = 11.7, 7.8 Hz, 1 H, H_δ ΨPro-H), 5.61 (m, 1 H, NH Ala), 6.15 (t, *J* = 53.8 Hz, 1 H, CF_2H-H), 7.27-7.44 (m, 9 H, H-arom), 7.56-7.62 (m, 2 H, H-arom), 7.77 (d, *J* = 7.3 Hz, 2 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) (76:24 mixture of rotational isomers) (major conformer) δ 19.6 (CH_3, C_β Ala), 47.0 (CH, CH Fmoc), 49.2 (CH, C_α Ala), 58.0 (CH, C_α

ΨPro), 67.0 (CH_2, CH_2 Fmoc), 68.2 (CH_2, CH_2 Bn), 72.5 (CH_2, C_β ΨPro), 86.7 (dd, *J* = 27.8, 21.1 Hz, CH, C_δ ΨPro), 111.7 (t, *J* = 246.3 Hz, CH, CF_2H), 120.0 (2 CH, C-arom), 125.1 (2 CH, C-arom), 127.0 (2 CH, C-arom), 127.7 (2 CH, C-arom), 128.2 (CH, C-arom), 128.4 (CH, C-arom), 128.7 (3 CH, C-arom), 134.4 (C, C-arom), 141.2 (2 C, C-arom), 143.6 (C, C-arom), 143.8 (C, C-arom), 155.2 (C, C=O), 169.0 (C, C=O), 172.7 (C, C=O); (minor conformer) δ 18.1 (CH_3, C_β Ala), 47.0 (CH, Fmoc CH), 48.9 (CH, C_α Ala), 57.7 (CH, C_α ΨPro), 67.2 (CH_2, Fmoc CH_2), 67.7 (CH_2, Bn CH_2), 70.2 (CH_2, C_β ΨPro), 85.3 (m, CH, C_δ ΨPro), 113.2 (t, *J* = 250.2 Hz, CH, CF_2H), 120.0 (2 CH, C-arom), 125.1 (2 CH, C-arom), 127.0 (2 CH, C-arom), 127.7 (2 CH, C-arom), 128.2 (CH, C-arom), 128.4 (CH, C-arom), 128.7 (3 CH, C-arom), 134.8 (C, C-arom), 141.4 (2 C, C-arom), 143.6 (C, C-arom), 143.8 (C, C-arom), 155.7 (C, C=O), 168.9 (C, C=O), 170.8 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 293 K) (76:24 mixture of rotational isomers) (major conformer) δ -134.0 (ddd, *J* = 290.5, 53.3, 2.6 Hz, 1 F), -143.2 (ddd, *J* = 290.5, 55.5, 19.1 Hz, 1 F); (minor conformer) δ -133.0 (ddd, *J* = 292.2, 53.8, 7.8 Hz, 1 F), -135.7 (ddd, *J* = 292.2, 53.8, 11.7 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{30}H_{29}F_2N_2O_6$ 551.1988, found 551.1987.

Fmoc-L-Ala-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-6: The product was prepared following the base free representative procedure using (*S*,*S*)-**1** (100 mg, 0.39 mmol, 1.0 equiv) in DCM (2 mL), Fmoc-Ala-Cl (192 mg, 0.58 mmol, 1.5 equiv) in DCM (2 mL). Purification by flash chromatography (80:20 Cyclohexane/Ethyl acetate) gave 176 mg of the Fmoc-L-Ala-(2*R*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-**6** (82%, 0.32 mmol) as an 78/22 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K.

The product was prepared following the base free representative procedure using (*R*,*S*)-**1** (100 mg, 0.39 mmol, 1.0 equiv) in DCM (2 mL), Fmoc-L-Ala-Cl (206 mg, 0.62 mmol, 1.6 equiv) in DCM (2 mL). Purification by flash chromatography (90:10 Cyclohexane/Ethyl acetate) gave 190 mg of the Fmoc-L-Ala-(2*R*,4*S*)-Ser(Ψ^{CF2H,H}Pro)-OBn (*R*,*S*)-**6** (88%, 0.34 mmol) as an 78/22 inseparable mixture of *cis*/*trans* rotational conformers in $CDCl_3$ at 293 K : White solid, mp 82°C; R_f = 0.49 (70:30 Cyclohexane/AcOEt); $[\alpha]^{20}_D$: -61.9 (*c* 1.03, $CHCl_3$); IR (neat, cm^{-1}): 3318, 2948, 1743, 1709, 1668, 1520, 1478, 1377, 1296, 1189, 1071; ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) (78:22 mixture of rotational isomers) (major conformer) δ 1.39 (d, *J* = 6.9 Hz, 3 H, H_β Ala), 4.14-4.21 (m, 2 H, Fmoc CH-H + H_β ΨPro-Ha), 4.27-4.36 (m, 2 H, H_β ΨPro-Hb + Fmoc CH_2-Ha), 4.36-4.47 (m, 2 H, Fmoc CH_2-Hb and H_α Ala), 5.09 (m, 1 H, H_α ΨPro-H), 5.16 (d, *J* = 12.2 Hz, 1 H, CH_2 Bn-Ha), 5.20 (d, *J* = 12.2 Hz, 1 H, CH_2 Bn-Hb), 5.37 (d, *J* = 6.4 Hz, 1 H, NH Ala), 5.76 (ddd, *J* = 55.5, 55.1, 3.7 Hz, 1 H, CF_2H), 6.00 (dd, *J* = 6.9, 3.7 Hz, 1 H, C_δ ΨPro-H), 7.25-7.45 (m, 9 H, H-arom), 7.52-7.61 (m, 2 H, H-arom), 7.77 (d, *J* = 7.3 Hz, 2 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 293 K) (78:22 mixture of rotational isomers) (major conformer) δ 17.9 (CH_3, C_β Ala), 47.0 (CH, Fmoc CH), 48.6 (CH, C_α Ala), 57.0 (CH, C_α ΨPro), 67.2 (CH_2, CH_2 Fmoc), 67.7 (CH_2, CH_2 Bn), 68.6 (CH_2, C_β ΨPro), 86.4 (dd, *J* = 28.8, 25.9 Hz, CH, C_δ ΨPro), 112.9 (t, *J* = 248.2 Hz, CH, CF_2H ΨPro), 120.0 (2 CH, C-arom), 125.0 (2 CH, C-arom), 127.0 (2 CH, C-arom), 127.7 (2 CH, C-arom), 128.2 (CH, C-arom), 128.6 (3 CH, C-arom), 134.8 (C, C-arom), 141.2 (2 C, C-arom), 143.6 (2 C, C-arom), 156.1 (C, C=O), 169.2 (C, C=O), 172.8 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 293 K) (78:22 mixture of rotational isomers) (major conformer) δ -129.5 (dd, *J* = 297.4, 55.5 Hz, 1 F), -134.1 (ddd, *J* = 297.4, 55.1, 6.9 Hz, 1 F); (minor conformer) δ -132.8 (dd, *J* = 289.0, 53.8 Hz, 1 F), -138.8 (dd, *J* = 289.0, 51.2 Hz, 1 F); HRMS (ESI-TOF) : $[M+H]^+$ calcd for $C_{30}H_{29}F_2N_2O_6$ 551.1988, found 551.1990.

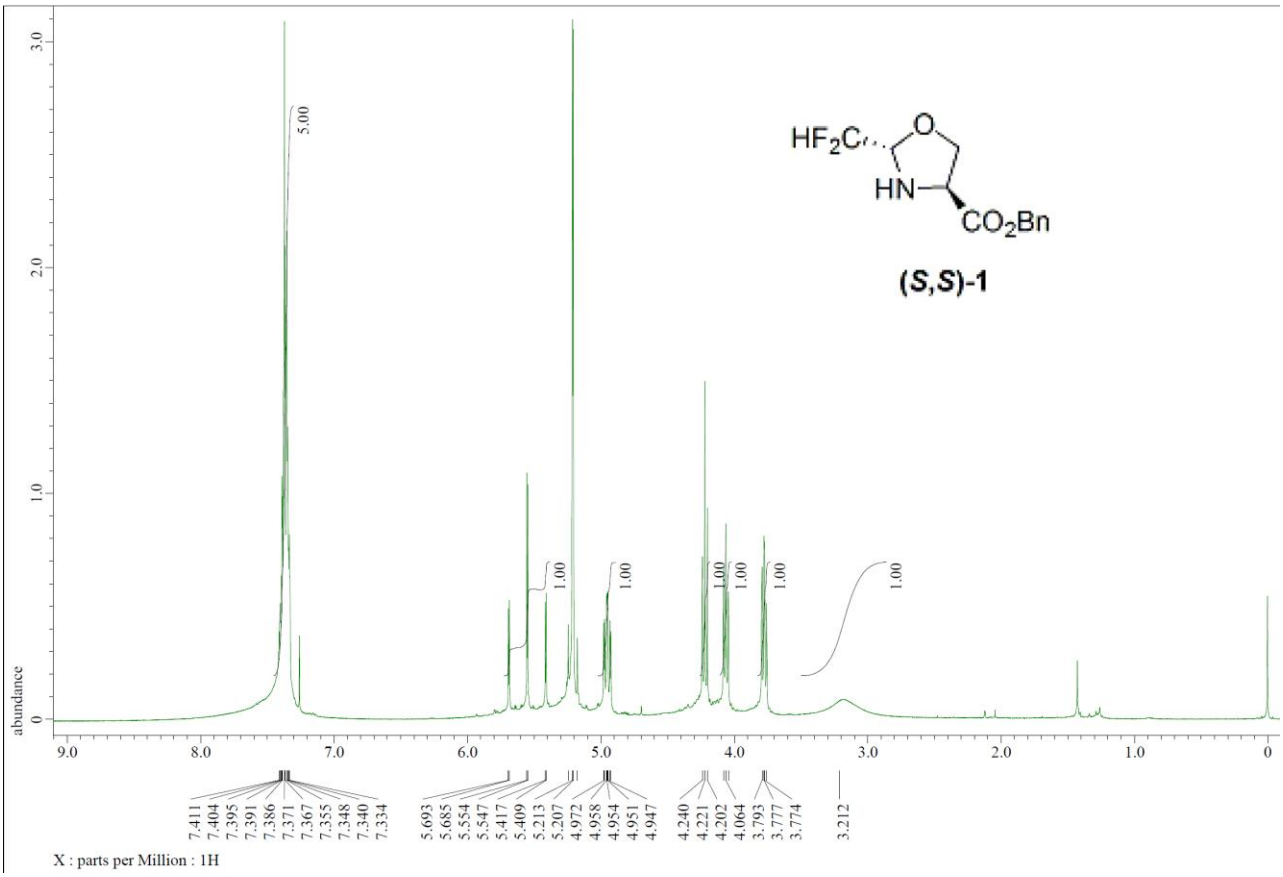
Fmoc-L-Ala-Ser(Ψ^{CF2H,H}Pro)-L-Ala-O*t*Bu (*S*,*S*)-7: To a solution of the dipeptide (*S*,*S*)-**6** (0.270 g, 0.49 mmol, 1 equiv) in AcOEt (5 mL) and MeOH (5 mL) was added 52 mg of 10% Pd/C (0.049 mmol, 0.1 equiv). The mixture was placed under hydrogen atmosphere (1 bar) and stirred vigourously until the starting material's disappearance. The crude mixture was filtered under a microporous filter and evaporated under reduced pressure to afford quantitatively the crude acidic compound (0.225 g, 0.49 mmol) as a 95/5 inseparable mixture of *cis*/*trans* rotational conformers (in $CDCl_3$ at 293 K) which was used in the next step without further purification. To a solution of the corresponding acid (0.225 g, 0.49 mmol, 1 equiv) in dichloromethane (20 mL) were successively added L-alanine *tert*-butyl ester hydrochloride (0.178 g, 0.98 mmol, 2 equiv), Et_3N (205µL, 1.47 mmol, 3 equiv) and after 15 minutes stirring at room temperature BOP-Cl (0.25 g, 0.98 mmol, 2 equiv). The reaction mixture was stirred 12 h at room temperature, then quenched with HCl 1 M (20 mL). The layers were separated and the aqueous phase was extracted with DCM (3 x 20mL). The combined organic layers were washed with a saturated $NaHCO_3$ aqueous solution (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (90:10 cyclohexane/ethyl acetate) gave 154 mg of the pure tripeptide (*S*,*S*)-**7** (56%, 0.27 mmol) as a 85/15 inseparable mixture of rotational conformers in $CDCl_3$ at 298 K : White solid; mp 166 °C; R_f = 0.60 (3:2 Cyclohexane/Ethyl acetate); $[\alpha]^{20}_D$: -52.4 (*c* 1.1, $CHCl_3$); IR (neat, cm^{-1}): 1721, 1696, 1660, 1539, 150, 1419, 1362; ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) (major rotational isomer) δ 1.36 (s, 9 H, 3 CH_3, *t*Bu), 1.38-1.48 (m, 6 H, 2 CH_3, H_β Ala-1 & Ala-2), 4.19 (t, *J* = 6.9 Hz, 1 H, Fmoc CH-H), 4.31 (d, *J* = 6.9 Hz, 2 H,

Fmoc CH_2-H), 4.34-4.42 (m, 2 H, H_α Ala_1-H and Ala_2-H), 4.34-4.57 (3 H, H_β ΨPro-H and H_α ΨPro-H), 5.43 (d, *J* = 6.0 Hz, 1 H, NH Ala_1), 5.65 (d, *J* = 20.8 Hz, 1 H, C_δ ΨPro-H), 6.35 (t, *J* = 54.5 Hz, 1 H, CF_2H-H), 7.32 (t, *J* = 7.3 Hz, 2 H, H-arom), 7.40 (t, *J* = 7.3 Hz, 2 H, H-arom), 7.44 (d, *J* = 7.8 Hz, 1 H, NH Ala_2), 7.56 (d, *J* = 7.3 Hz, 1 H, H-arom), 7.58 (d, *J* = 7.3 Hz, 1 H, H-arom), 7.76 (d, *J* = 7.3 Hz, 2 H, H-arom); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (major rotational isomer) δ 17.0 (CH_3, C_β Ala), 17.5 (CH_3, C_β Ala), 27.8 (3 CH_3, *t*Bu), 47.0 (CH, Fmoc CH), 49.5 (2 CH, C_α Ala), 58.7 (CH, C_α ΨPro), 67.5 (CH_2, Fmoc CH_2), 73.6 (CH_2, C_β ΨPro), 81.8 (C, Cq, *t*Bu), 87.0-87.7 (m, CH, C_δ ΨPro), 111.8 (t, *J* = 245.4 Hz, CH, CF_2H), 120.0 (2 CH, C-arom), 125.1 (2 CH, C-arom), 127.1 (2 CH, C-arom), 127.8 (2 CH, C-arom), 141.2 (2 C, C-arom), 143.5 (C, C-arom), 143.7 (C, C-arom), 156.2 (C, C=O) 168.5 (C, C=O), 171.3 (C, C=O), 171.9 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 298 K) (85:15 mixture of rotational isomers) (major conformer) δ -134.8 (dd, *J* = 290.4, 53.8 Hz, 1 F), -142.7 (ddd, *J* = 290.4, 55.4, 20.8 Hz, 1 F); (minor conformer) δ -132.9 (ddd, *J* = 291.3, 54.7, 8.3 Hz, 1 F), -134.9 (ddd, *J* = 291.3, 53.8, 11.3 Hz, 1 F); HRMS (ESI-TOF) : $[M+Na]^+$ calcd for $C_{30}H_{35}F_2N_3O_7Na$ 610.2335, found 610.2335.

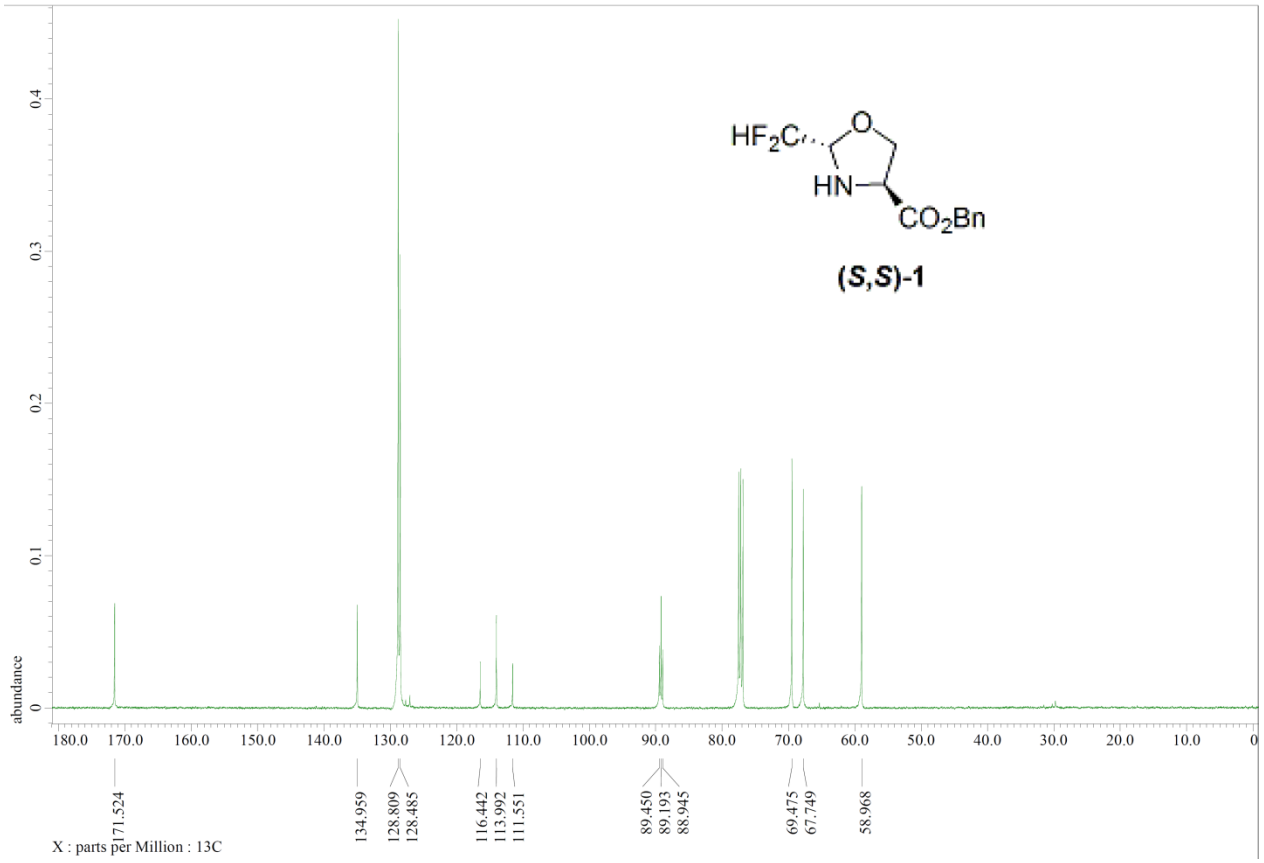
Fmoc-L-Ala-Ser(Ψ^{CF2H,H}Pro)-L-Ala-O*t*Bu (*R,S*)-7: To a solution of the dipeptide (*R,S*)-**6** (0.357 g, 0.65 mmol, 1 equiv) in AcOEt (5 mL) and MeOH (5 mL) was added 69 mg of 10% Pd/C (0.065 mmol, 0.1 equiv). The mixture was placed under hydrogen atmosphere (1 bar) and stirred vigourously until the starting material's disappearance. The crude mixture was filtered under a microporous filter and evaporated under reduced pressure to afford quantitatively the crude acidic compound (0.299 g, 0.65 mmol) as a 65/35 inseparable mixture of *cis*/*trans* rotational conformers (in $CDCl_3$ at 293 K) which was used in the next step without further purification. To a solution of the corresponding acid (0.240 g, 0.52 mmol, 1 equiv) in dichloromethane (20 mL) were successively added L-alanine *tert*-butyl ester hydrochloride (0.189 g, 1.04 mmol, 2 equiv), Et_3N (217 µL, 1.56 mmol, 3 equiv) and after 15 minutes stirring at room temperature BOP-Cl (0.265 g, 1.04 mmol, 2 equiv). The reaction mixture was stirred 12 h at room temperature, then quenched with HCl 1 M (20 mL). The layers were separated and the aqueous phase was extracted with DCM (3 x 20mL). The combined organic layers were washed with a saturated $NaHCO_3$ aqueous solution (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave 183 mg of the pure tripeptide (*R,S*)-**7** (63%, 0.33 mmol) as a 59/41 inseparable mixture of rotational conformers in $CDCl_3$ at 293 K: White solid; mp 101°C; R_f = 0.27 (3:2 Cyclohexane/Ethyl acetate); $[\alpha]^{20}_D$: -40.9 (*c* 1.2, $CHCl_3$); IR (neat, cm^{-1}): 1735, 1685, 1660, 1560, 1542, 1520, 1248, 1148, 1074; ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) (59:41mixture of rotational isomers) δ 1.30-1.46 (m, 12 H, 4 CH_3, H_β Ala-1 & Ala-2), 1.33 (s, 9 H, 3 CH_3, *t*Bu), 1.46 (s, 9 H, 3 CH_3, *t*Bu), 4.16 (t, *J* = 6.9 Hz, 2 H, Fmoc CH-H), 4.13-4.25 (4 H, H_β ΨPro-H and Fmoc CH_2-Ha), 4.28 (2 H, *J* = 6.9 Hz, Fmoc CH_2-H), 4.30-4.50 (m, 5 H, Fmoc CH_2-Hb, H_α Ala_1-H and Ala_2-H), 4.50-4.55 (1 H, H_α ΨPro-H), 4.62-4.75 (1 H, H_β ΨPro-Hb), 4.81-4.89 (1 H, H_α ΨPro-H), 5.53-5.61 (m, 1 H, C_δ ΨPro-H), 5.64 (d, *J* = 6.0 Hz, 2 H, NH Ala_1), 5.83-5.95 (m, 2 H, CF_2H-H), 5.92-6.08 (m, 1 H, C_δ ΨPro-H), 7.29 (t, *J* = 7.3 Hz, 2 H, H-arom), 7.30 (t, *J* = 7.3 Hz, 2 H, H-arom), 7.39 (t, *J* = 7.3 Hz, 4 H, H-arom), 7.55 (d, *J* = 7.3 Hz, 2 H, H-arom), 7.56 (d, *J* = 7.3 Hz, 2 H, H-arom), 7.75 (d, *J* = 7.3 Hz, 4 H, H-arom), 8.08 (bs, 2 H, NH Ala_2); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (59:41mixture of rotational isomers) δ 16.2 (CH_3, C_β Ala), 16.6 (CH_3, C_β Ala), 17.9 (CH_3, C_β Ala), 18.1 (CH_3, C_β Ala), 27.8 (6 CH_3, *t*Bu), 46.9 (CH, Fmoc CH), 48.6 (CH, C_α Ala), 48.9 (2 CH, C_α Ala), 49.5 (CH, C_α Ala), 57.6 (CH, C_α ΨPro), 58.7 (CH, C_α ΨPro), 67.3 (CH_2, Fmoc CH_2), 68.1 (CH_2, Fmoc CH_2), 71.5 (2 CH_2, C_β ΨPro), 81.3 (C, Cq, *t*Bu), 82.1 (C, Cq, *t*Bu), 86.2-87.6 (m, 2 CH, C_δ ΨPro), 111.5 (t, *J* = 247.3 Hz, CH, CF_2H), 112.5 (t, *J* = 246.3 Hz, CH, CF_2H), 120.0 (4 CH, C-arom), 124.9 (2 CH, C-arom), 125.0 (2 CH, C-arom), 127.0 (4 CH, C-arom), 127.7 (4 CH, C-arom), 141.2 (4 C, C-arom), 143.5 (2 C, C-arom), 143.6 (2 C, C-arom), 156.2 (C, C=O), 156.5 (C, C=O), 167.8 (2 C, C=O), 171.6 (2 C, C=O), 172.9 (C, C=O), 173.9 (C, C=O); ^{19}F (376.2 MHz, $CDCl_3$, 298 K) (59:41mixture of rotational isomers) (major conformer) δ -129.4 (dd, *J* = 295.6, 53.8 Hz, 1 F), -135.3 (dd, *J* = 295.6, 55.5 Hz, 1 F); (minor conformer) δ -134.4 (dd, *J* = 288.7, 52.9 Hz, 1 F), -139.8 (dd, *J* = 288.7, 55.5 Hz, 1 F); HRMS (ESI-TOF) : $[M+Na]^+$ calcd for $C_{30}H_{35}F_2N_3O_7Na$ 610.2335, found 610.2336.

3. NMR spectra of synthesized compounds

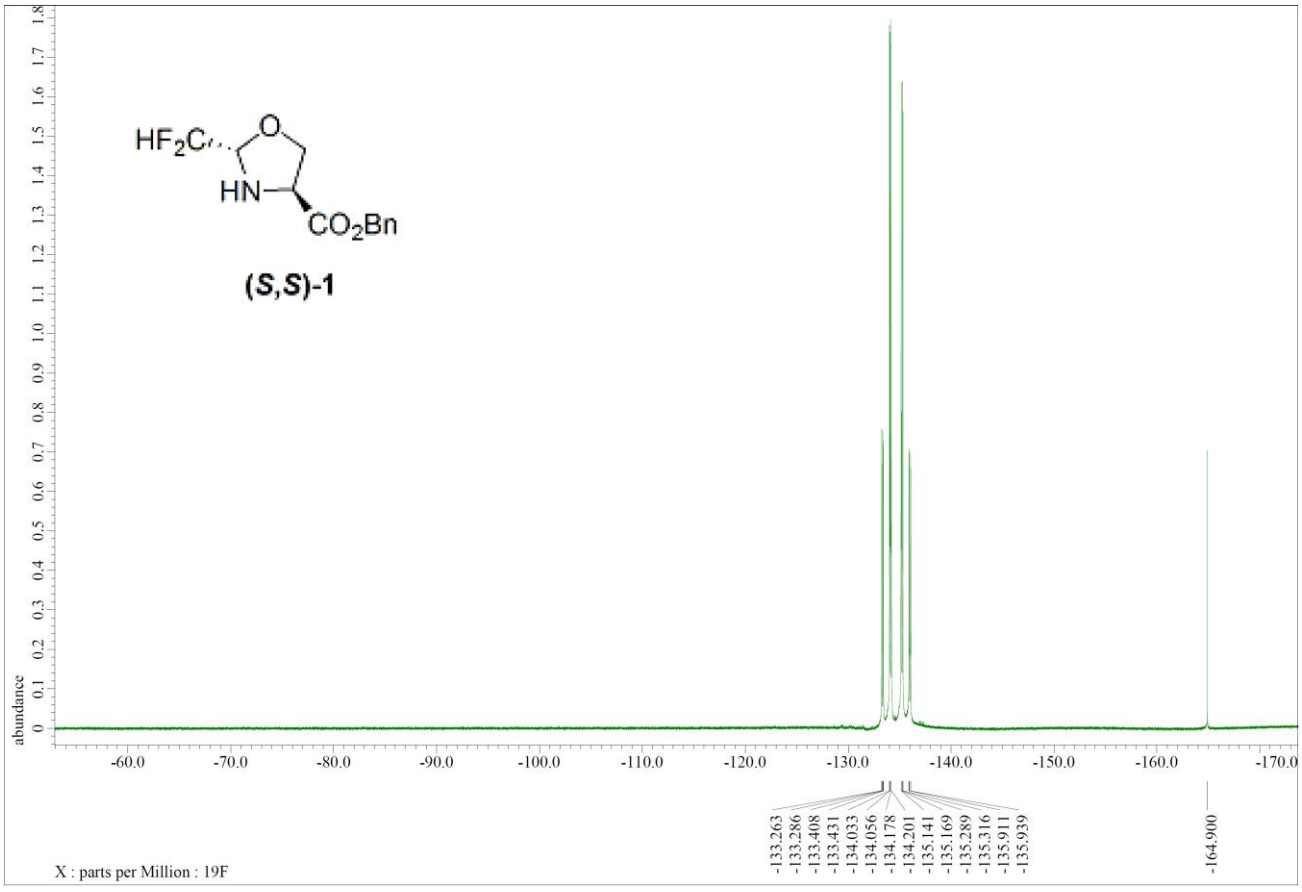
3.1. (2S,4S)-2-Difluoromethyloxazolidine-4-carboxylic Acid Benzyl Esters (S,S)-1



^{1}H NMR spectrum at 293 K in $CDCl_3$

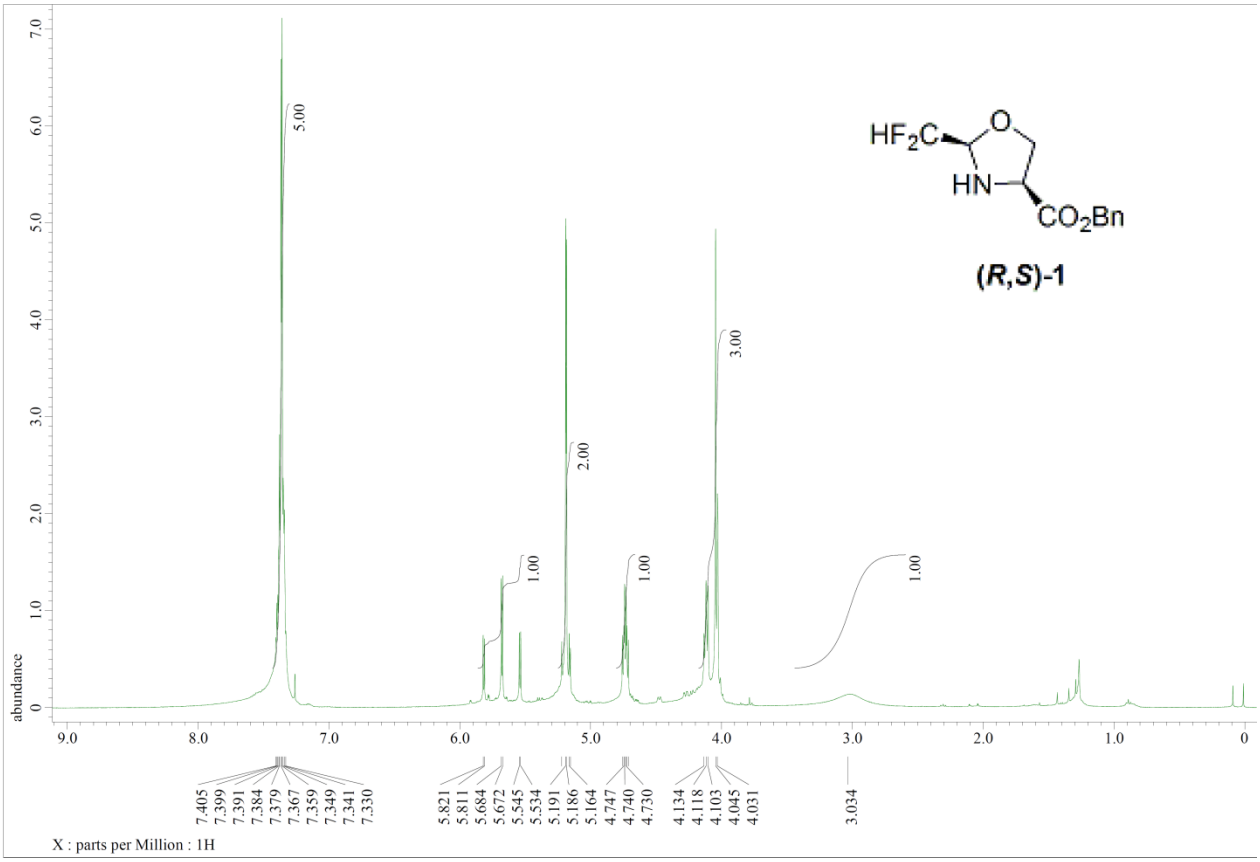


^{13}C NMR spectrum at 293 K in $CDCl_3$

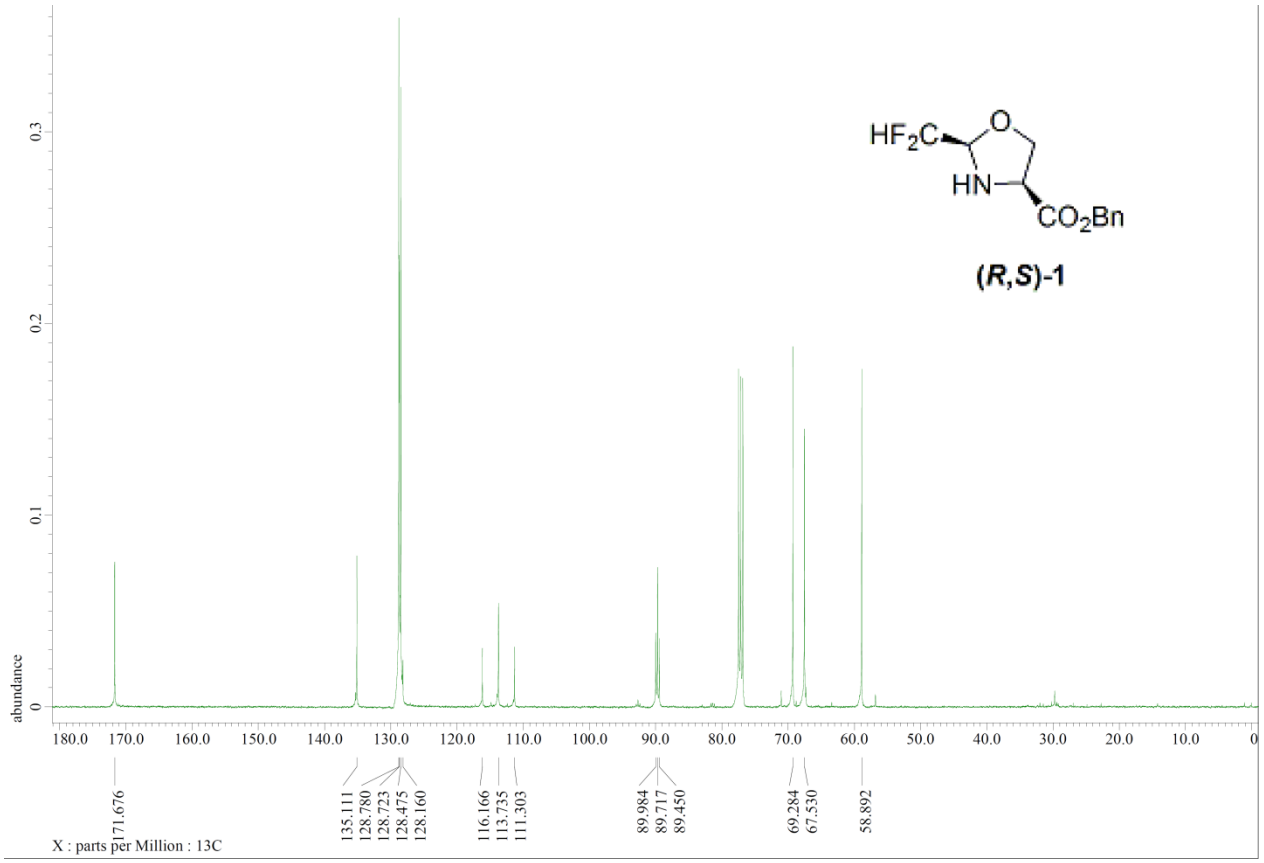


^{19}F NMR spectrum at 293 K in $CDCl_3$

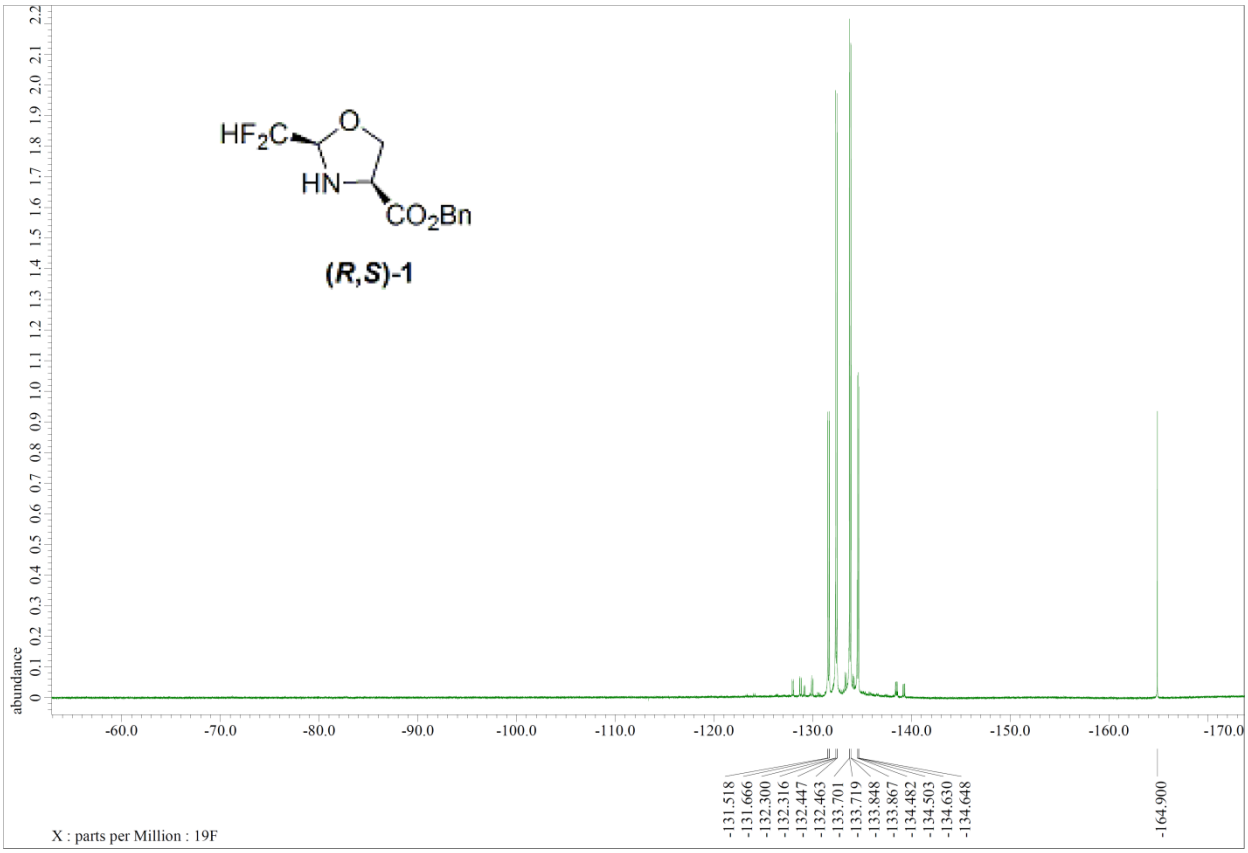
3.2. (2*R*,4*S*)-2-Difluoromethyloxazolidine-4-carboxylic Acid Benzyl Esters (*R*,*S*)-1



^{1}H NMR spectrum at 293 K in $CDCl_3$

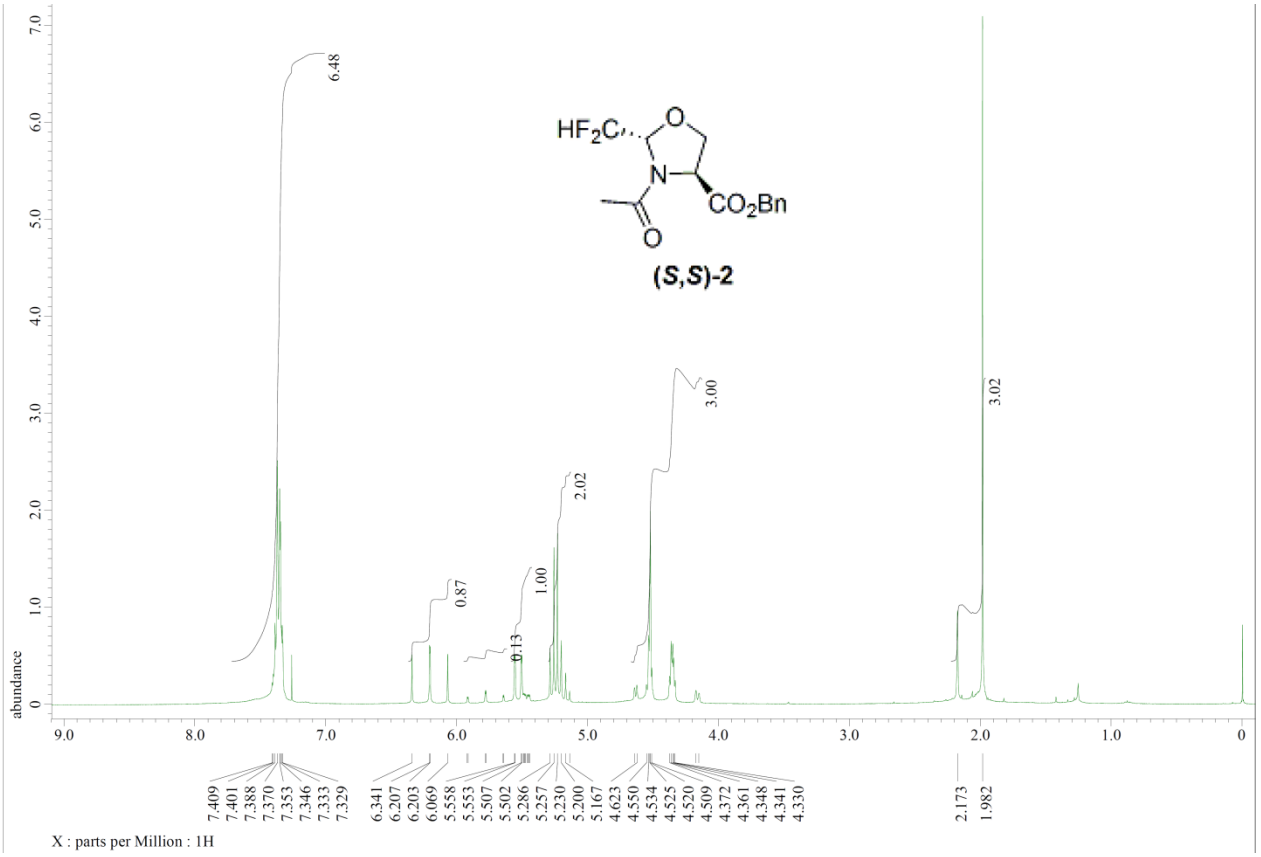


^{13}C NMR spectrum at 293 K in $CDCl_3$

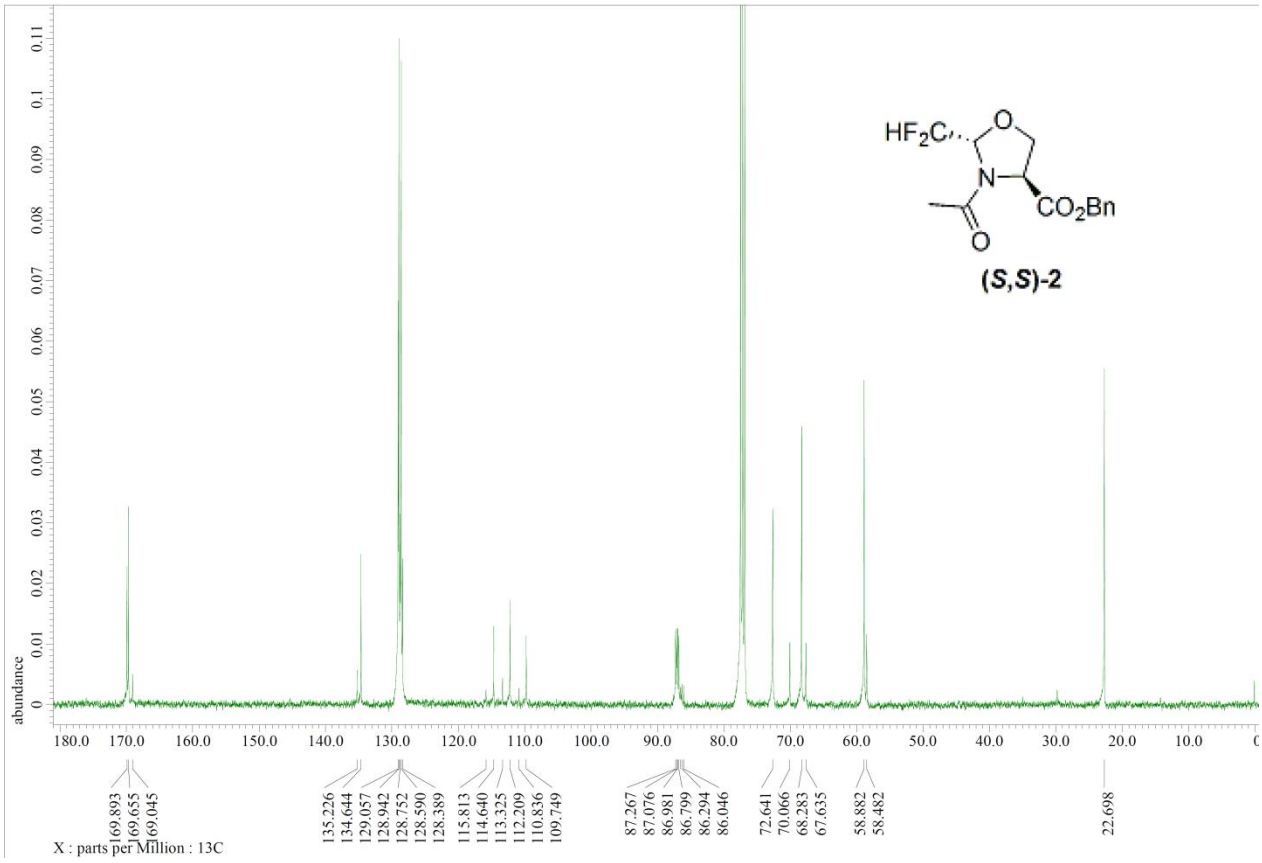


^{19}F NMR spectrum at 293 K in $CDCl_3$

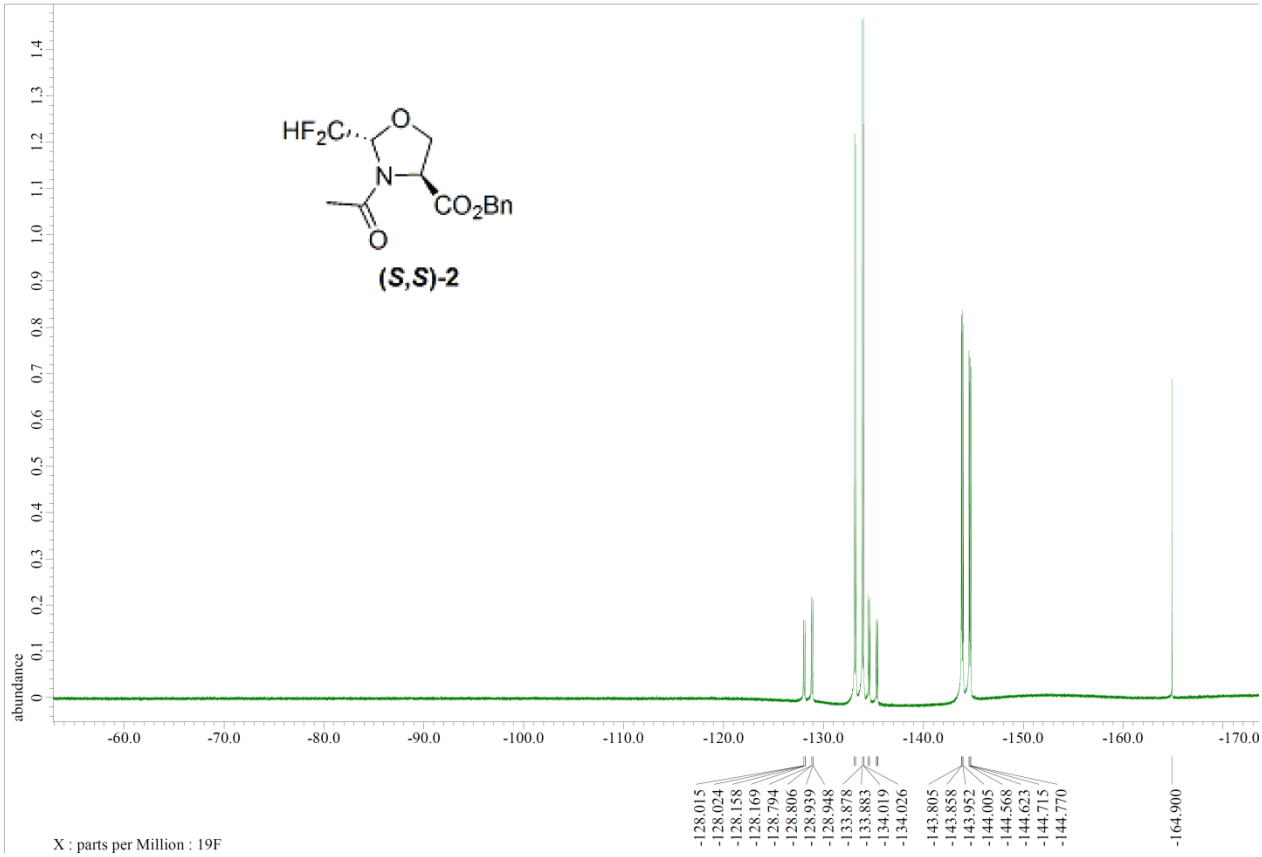
3.3. (2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Benzyl Ester (*S*,*S*)-2



^{1}H NMR spectrum at 293 K in $CDCl_3$

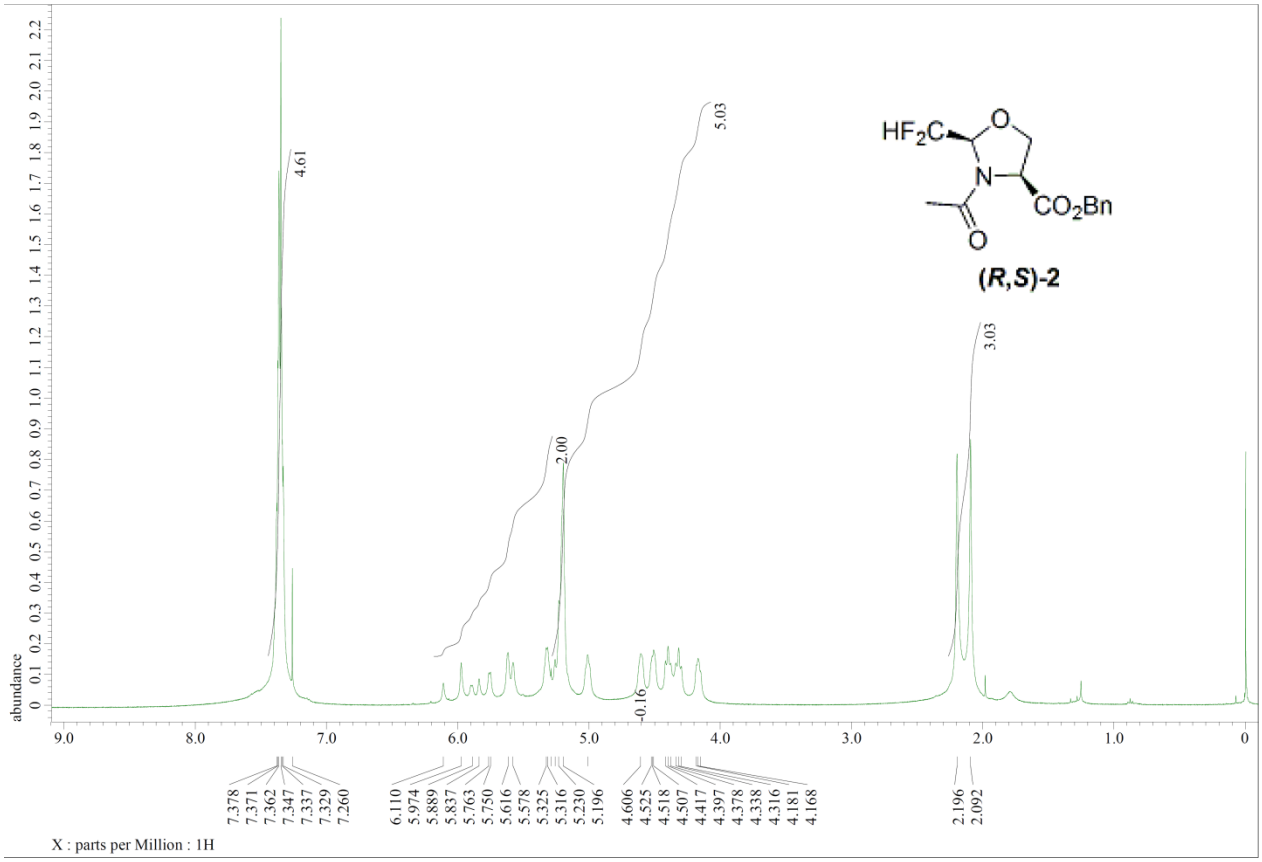


^{13}C NMR spectrum at 293 K in $CDCl_3$

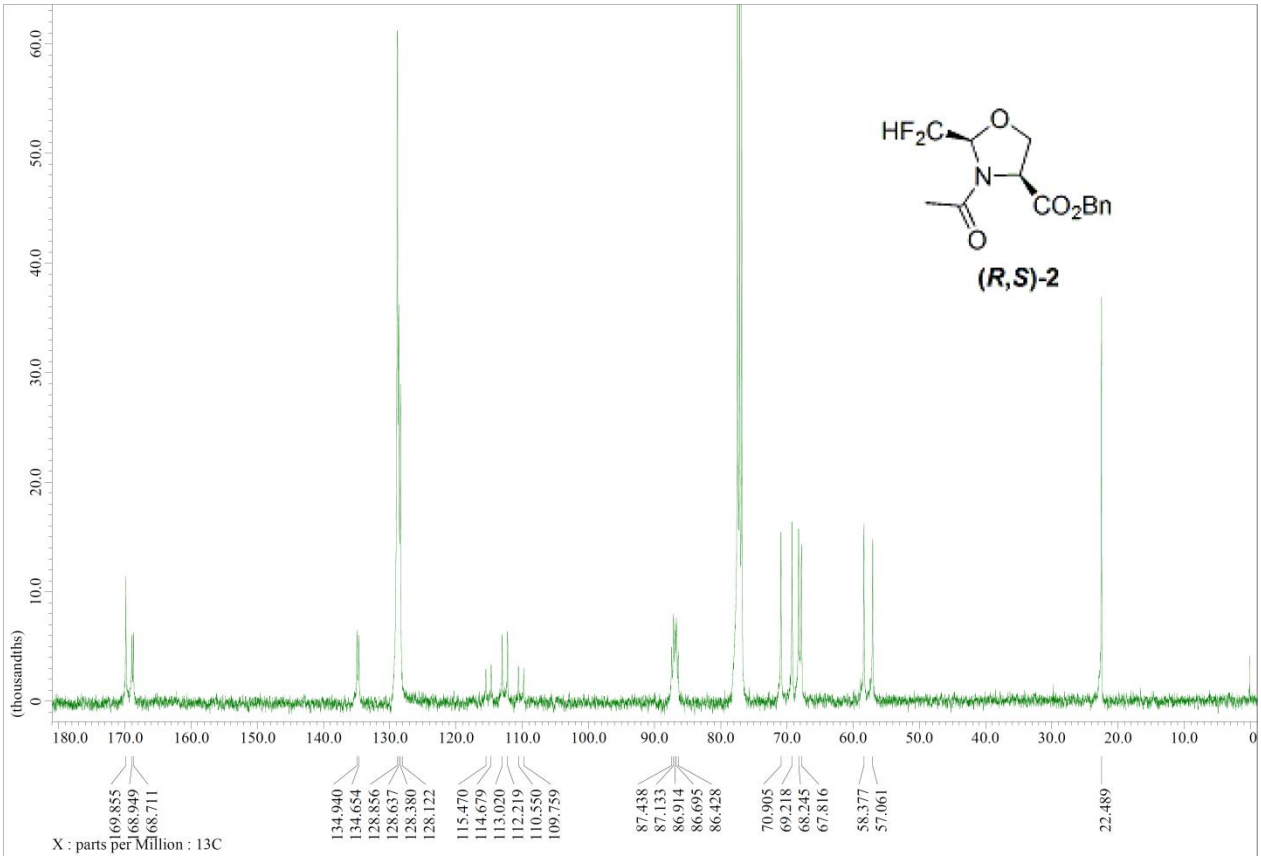


^{19}F NMR spectrum at 293 K in $CDCl_3$

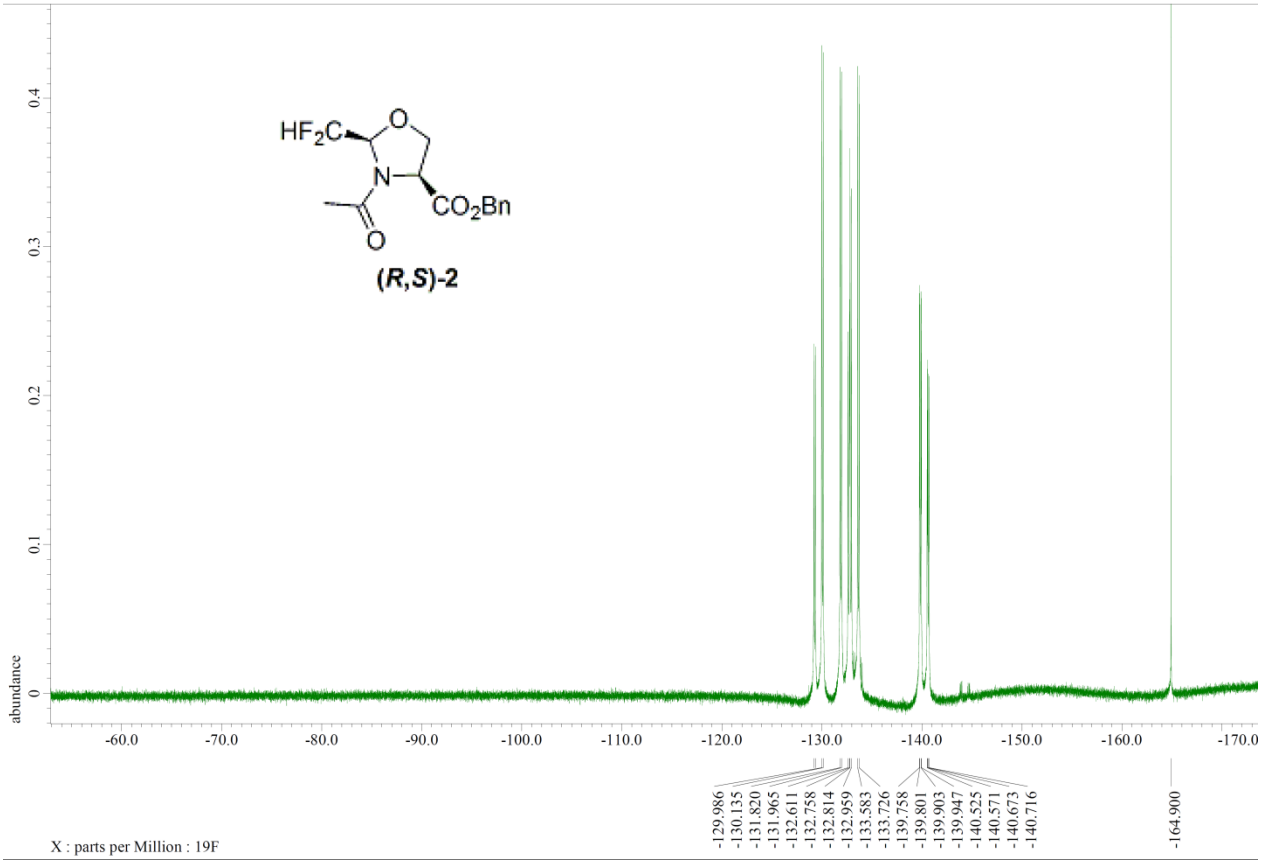
3.4. (2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Benzyl Ester (*R*,*S*)-2



^{1}H NMR spectrum at 293 K in $CDCl_3$

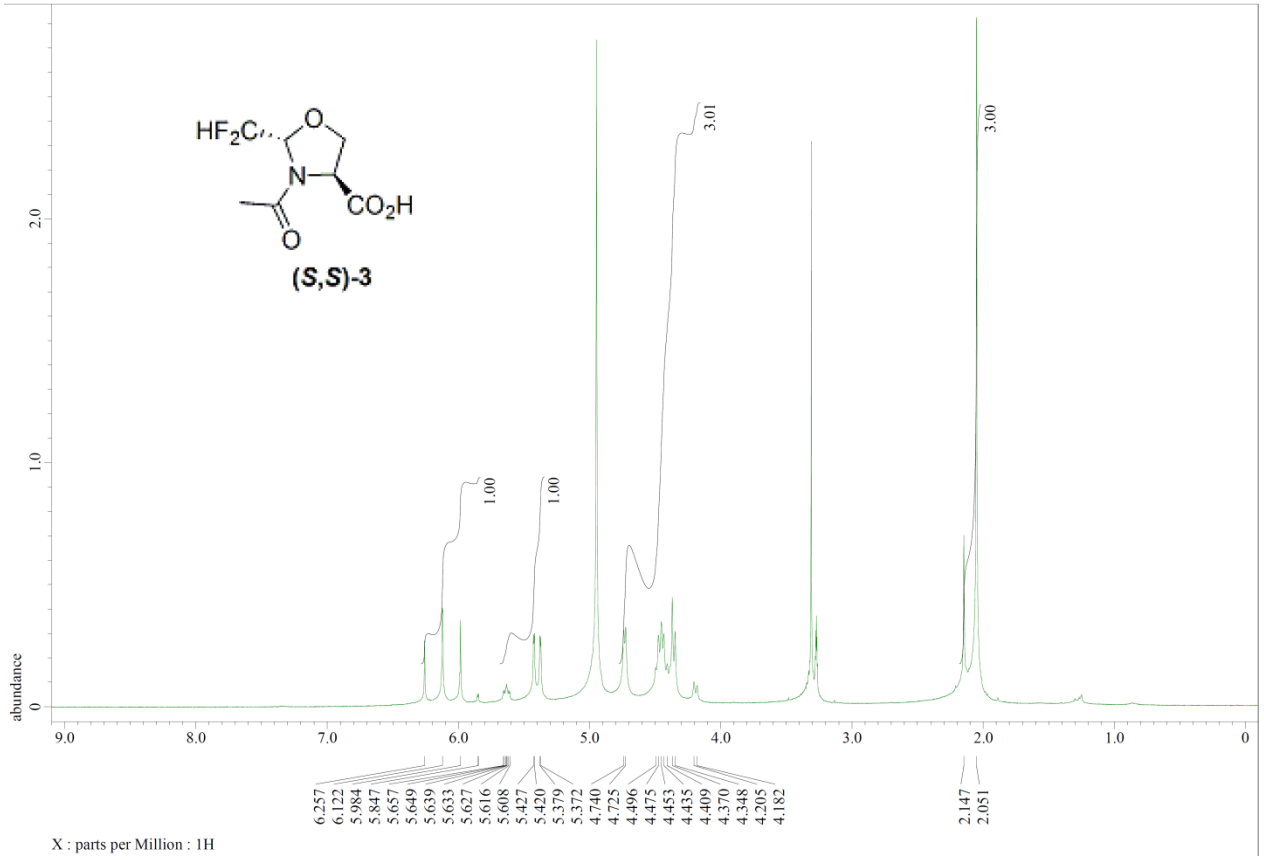


^{13}C NMR spectrum at 293 K in $CDCl_3$

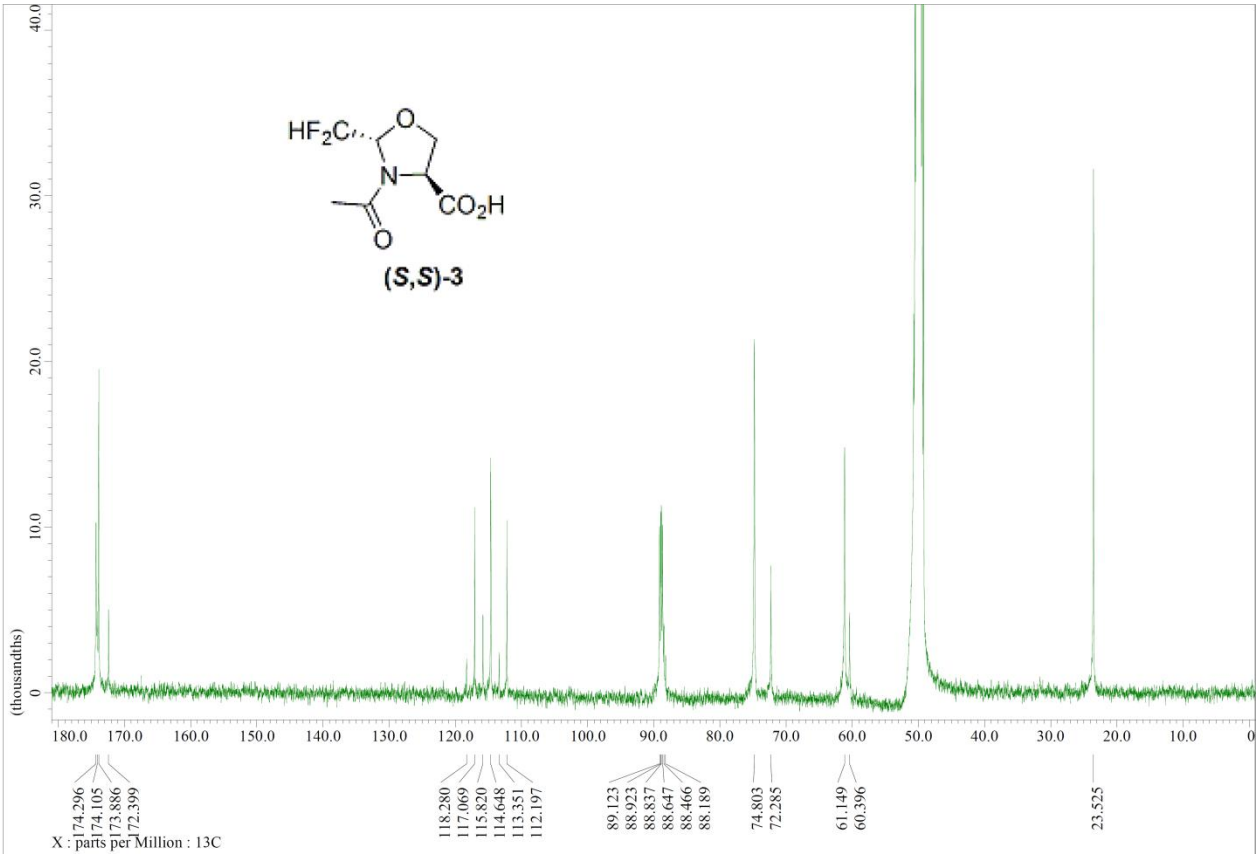


^{19}F NMR spectrum at 293 K in $CDCl_3$

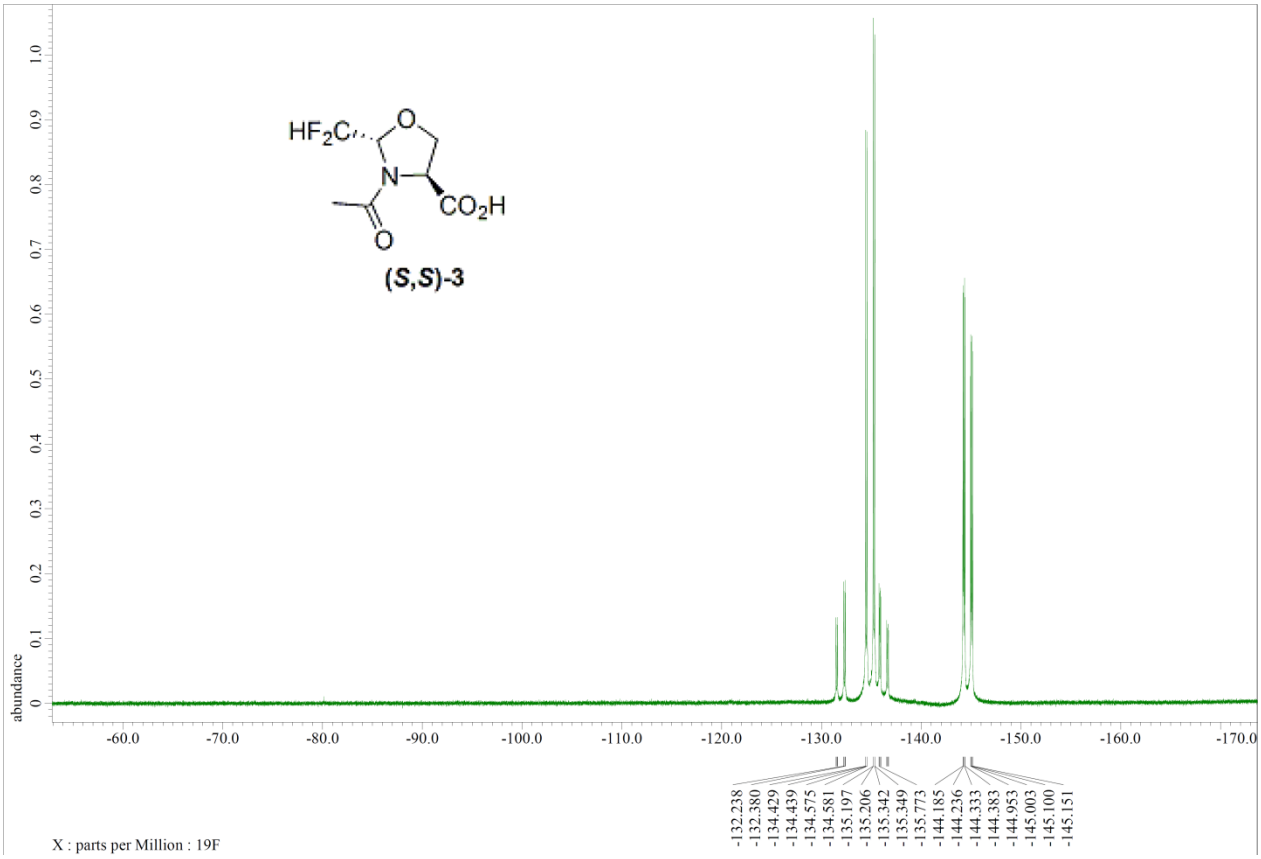
3.5. (2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Acid (*S*,*S*)-3



^{1}H NMR spectrum at 293 K in CD_3OD

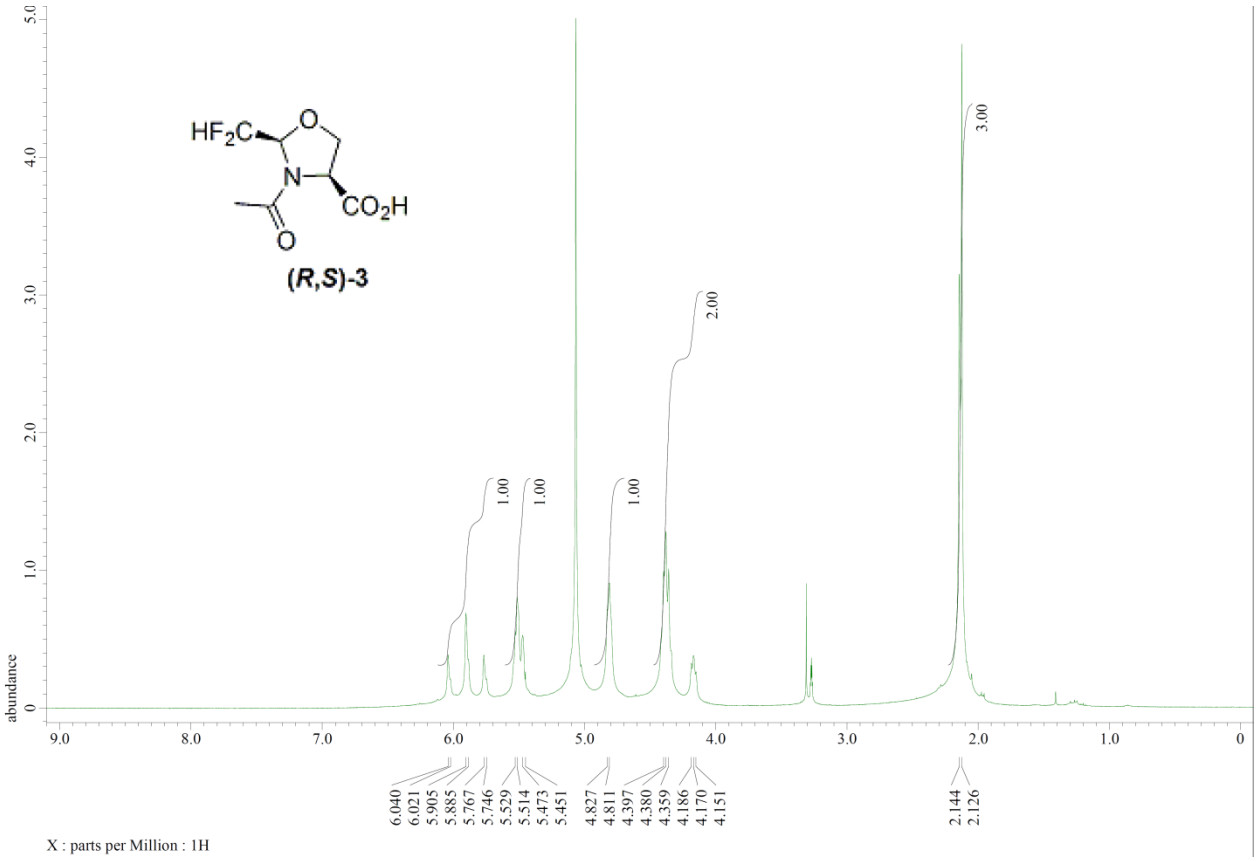


^{13}C NMR spectrum at 293 K in CD_3OD

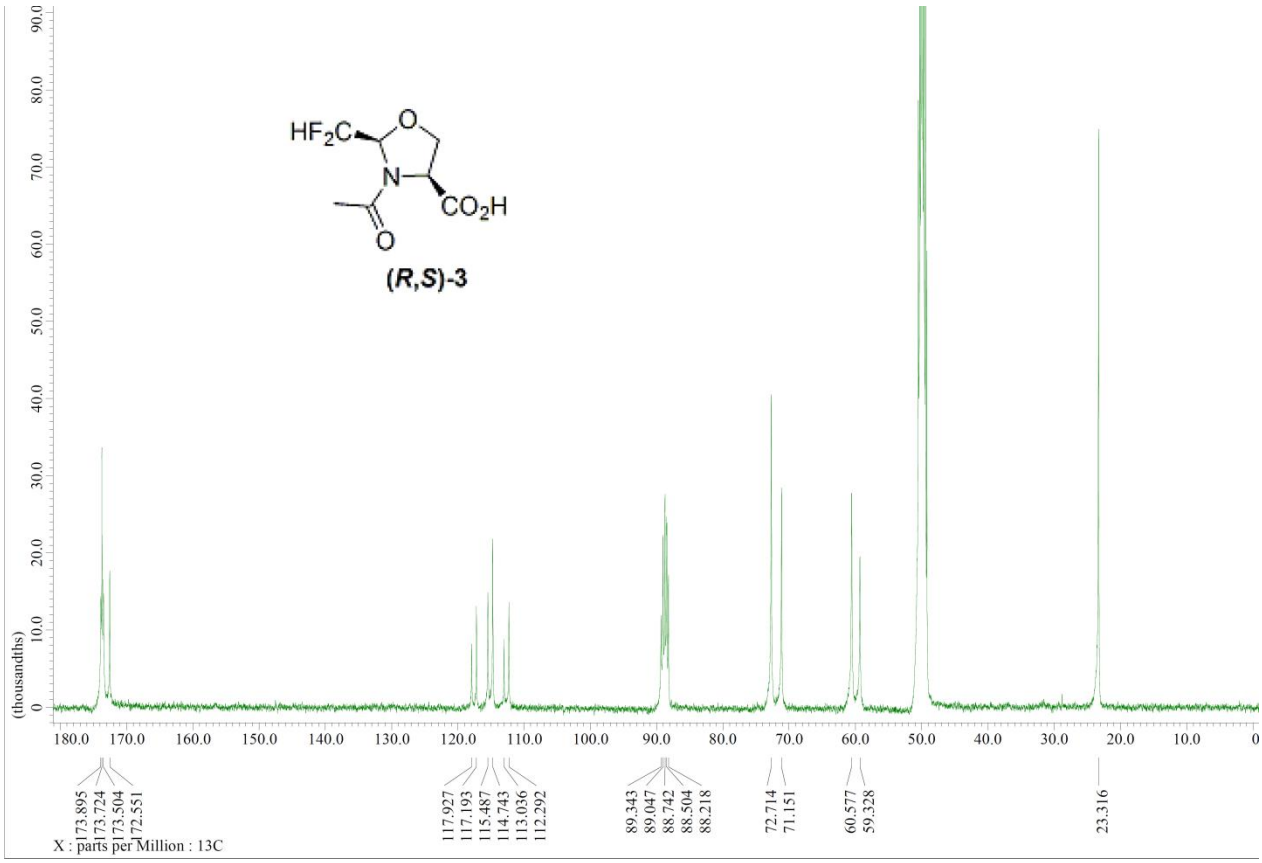


^{19}F NMR spectrum at 293 K in CD_3OD

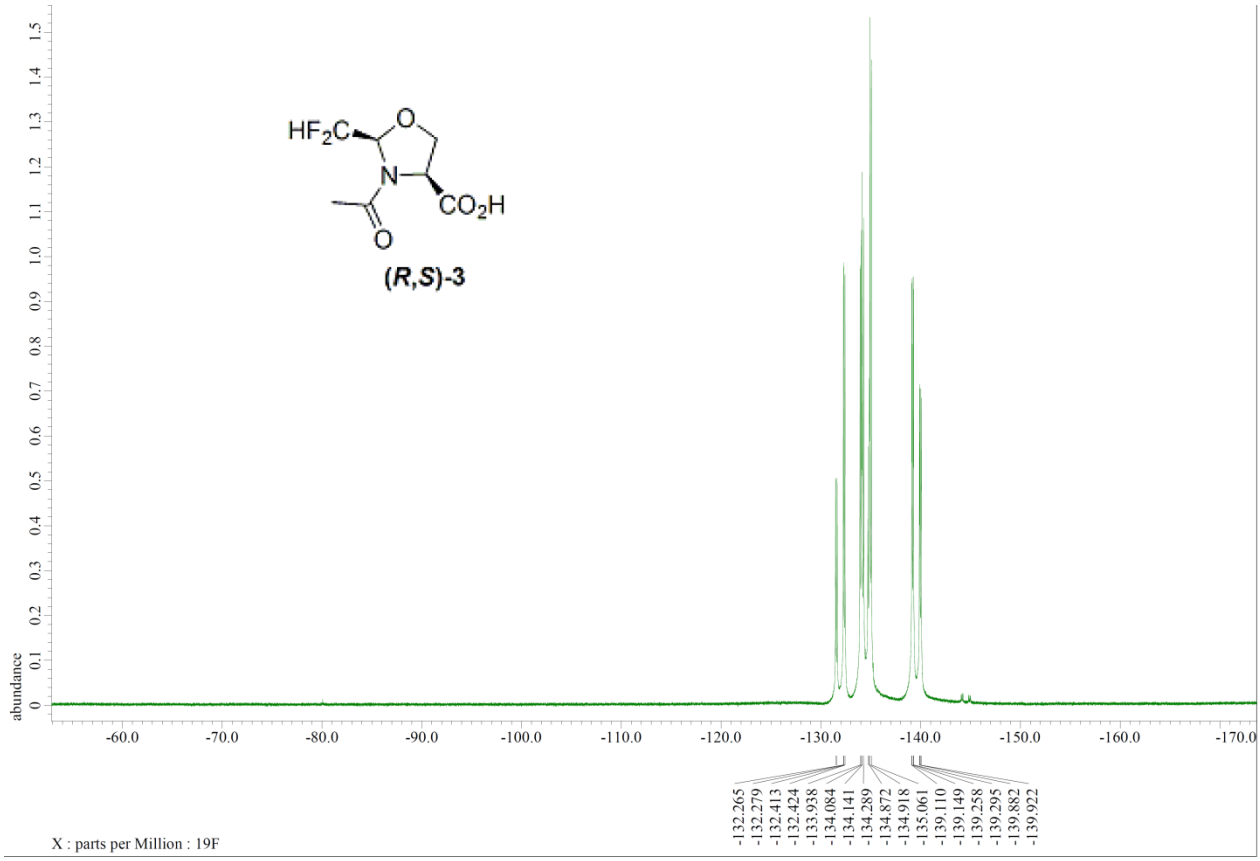
3.6. (2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-carboxylic Acid (*R*,*S*)-3



^{1}H NMR spectrum at 293 K in CD_3OD

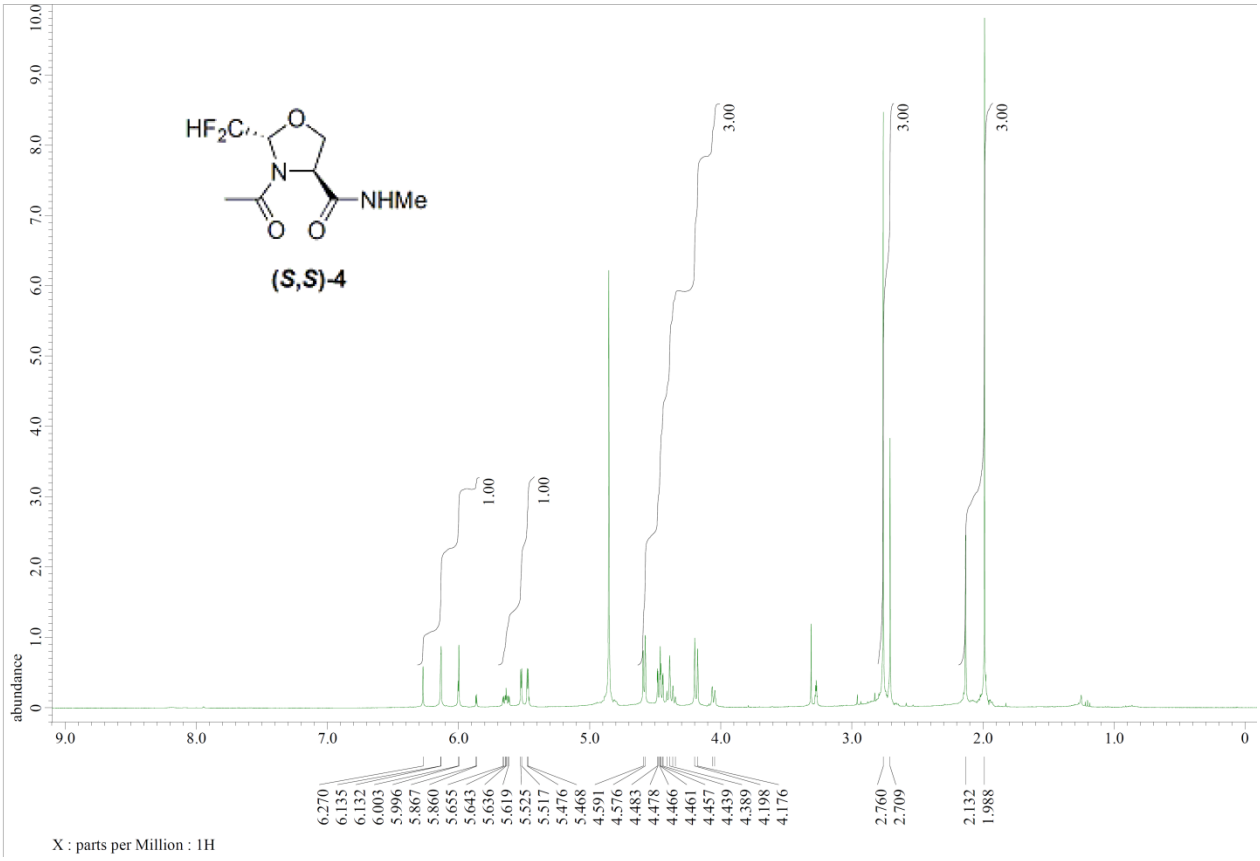


^{13}C NMR spectrum at 293 K in CD_3OD

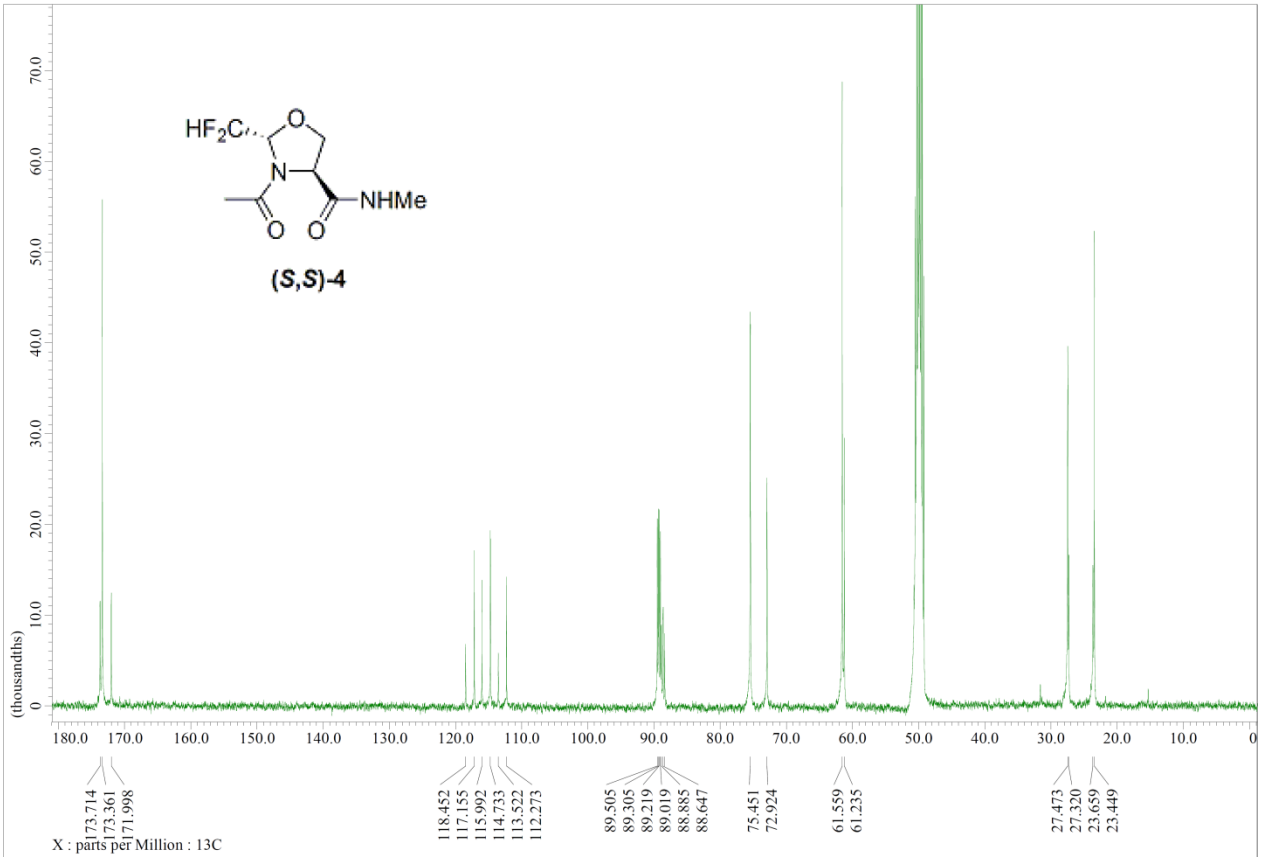


^{19}F NMR spectrum at 293 K in CD_3OD

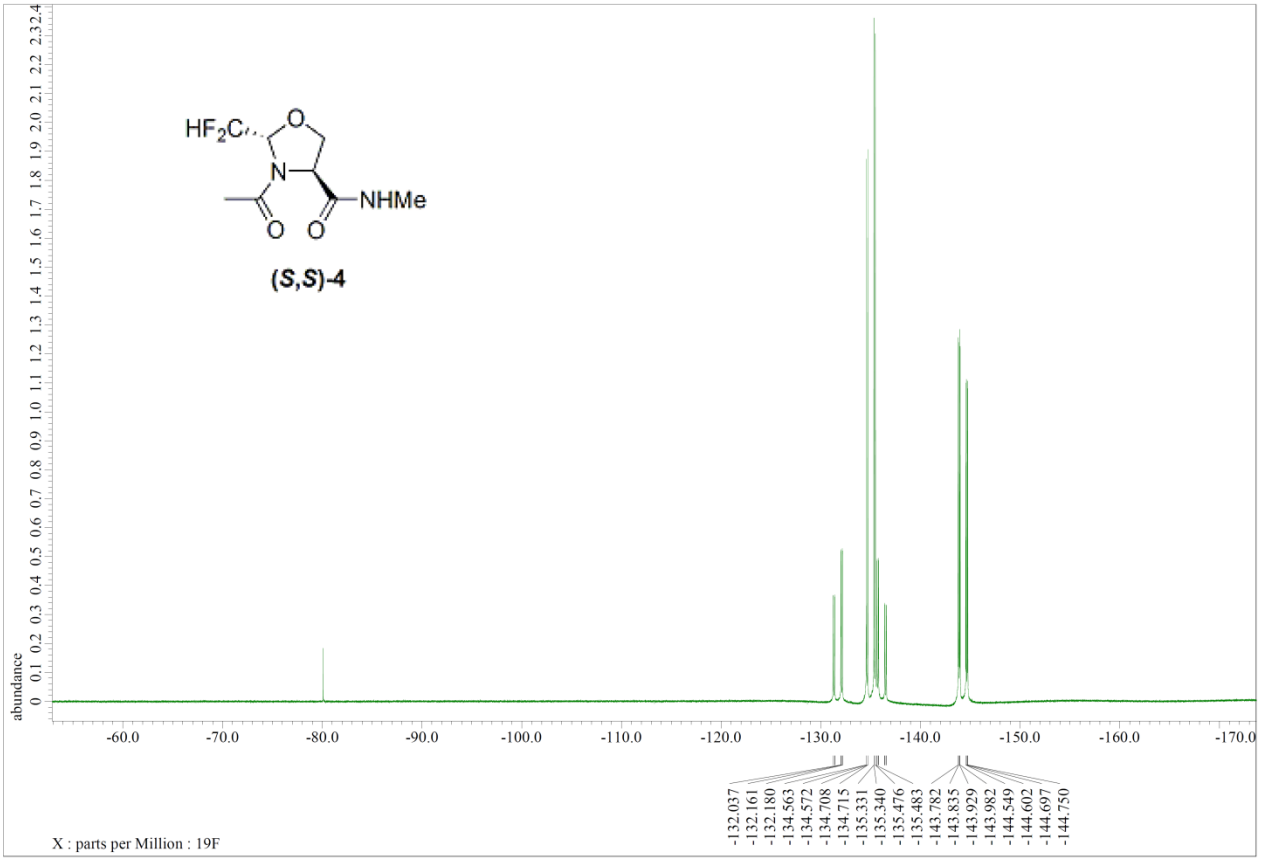
3.7. (2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*S*,*S*)-4



^{1}H NMR spectrum at 293 K in CD_3OD

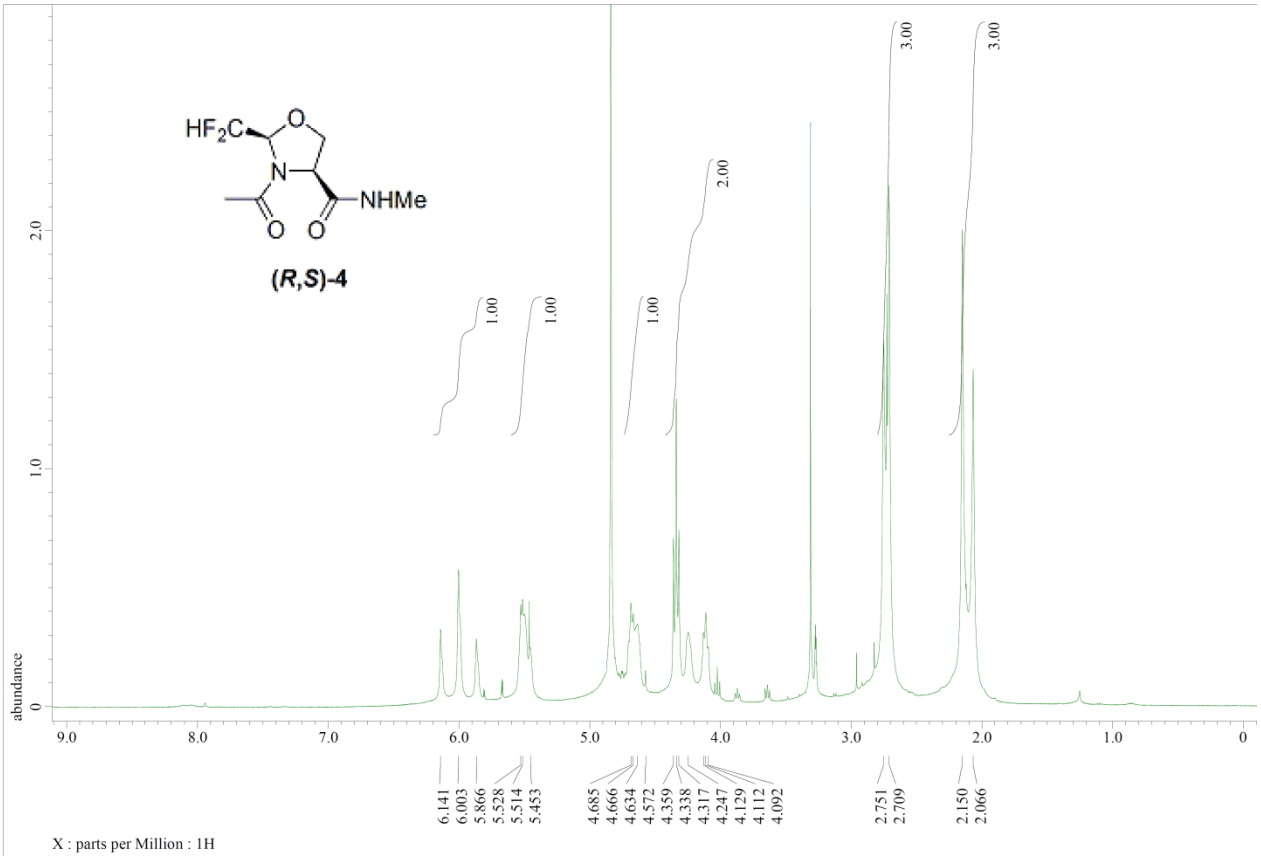


^{13}C NMR spectrum at 293 K in CD_3OD

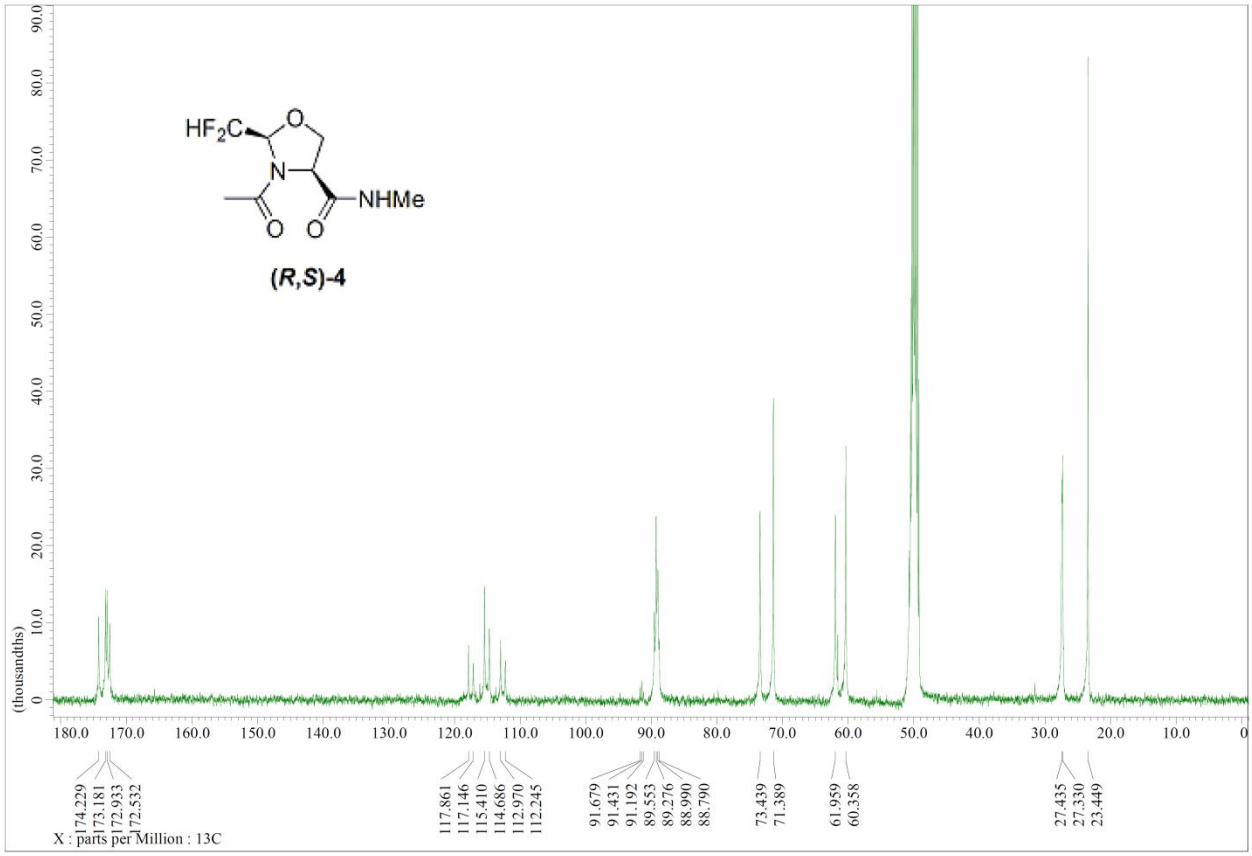


^{19}F NMR spectrum at 293 K in CD_3OD

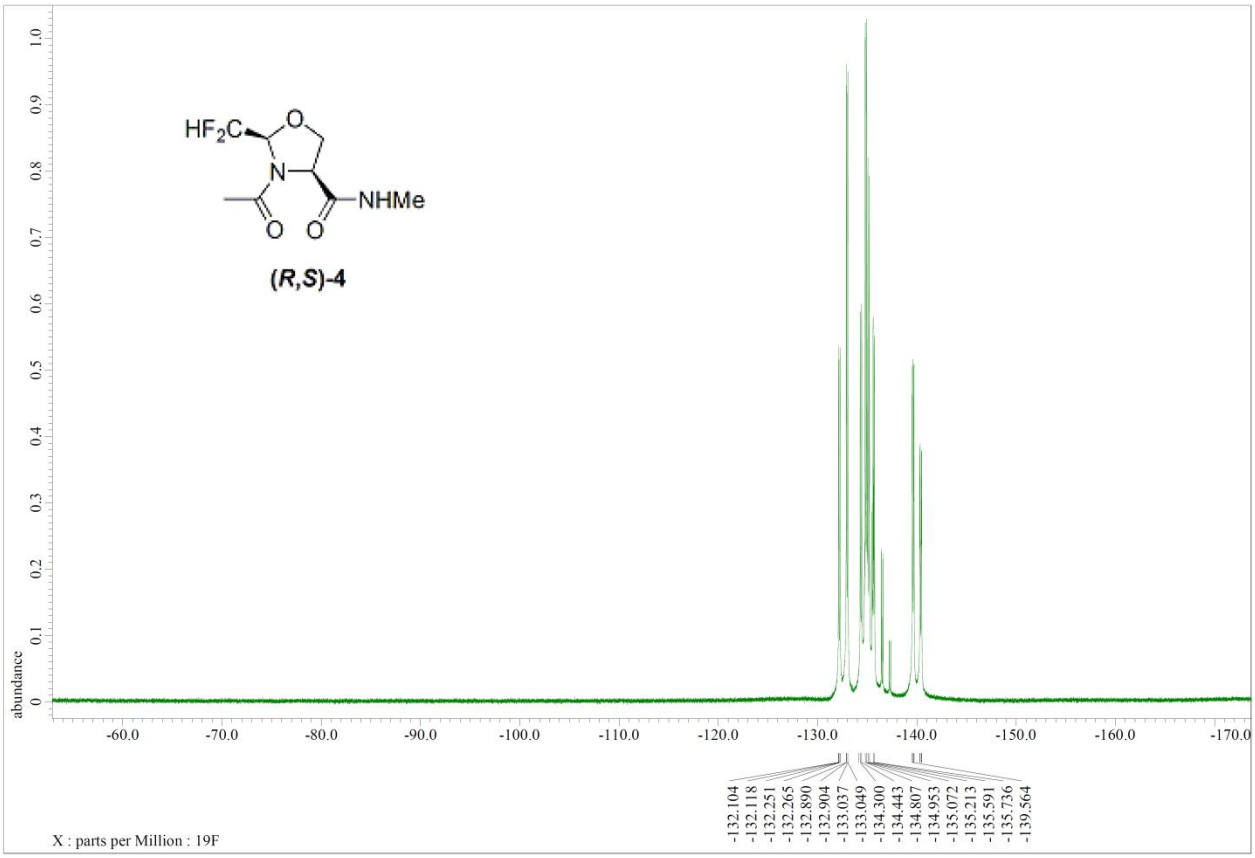
3.8. (2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*R*,*S*)-4



^{1}H NMR spectrum at 293 K in CD_3OD

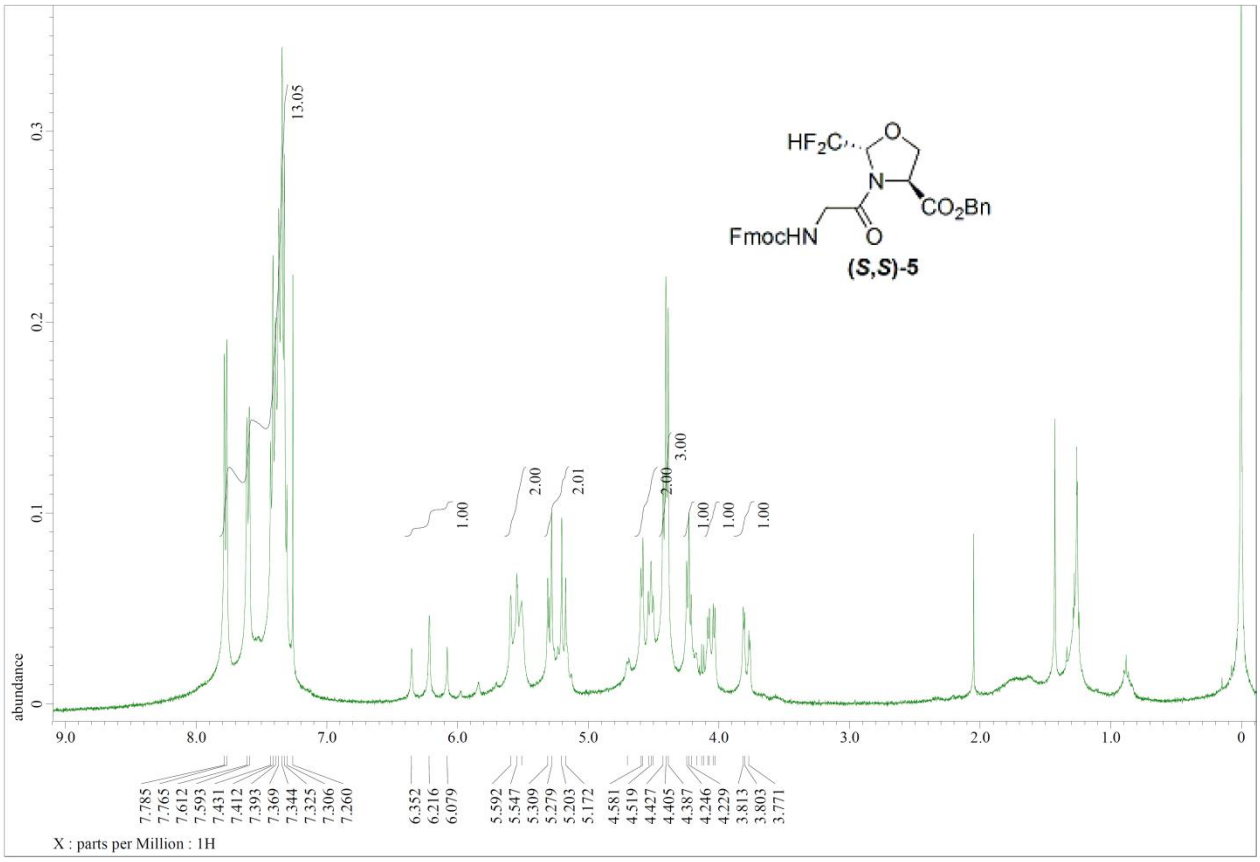


^{13}C NMR spectrum at 293 K in CD_3OD

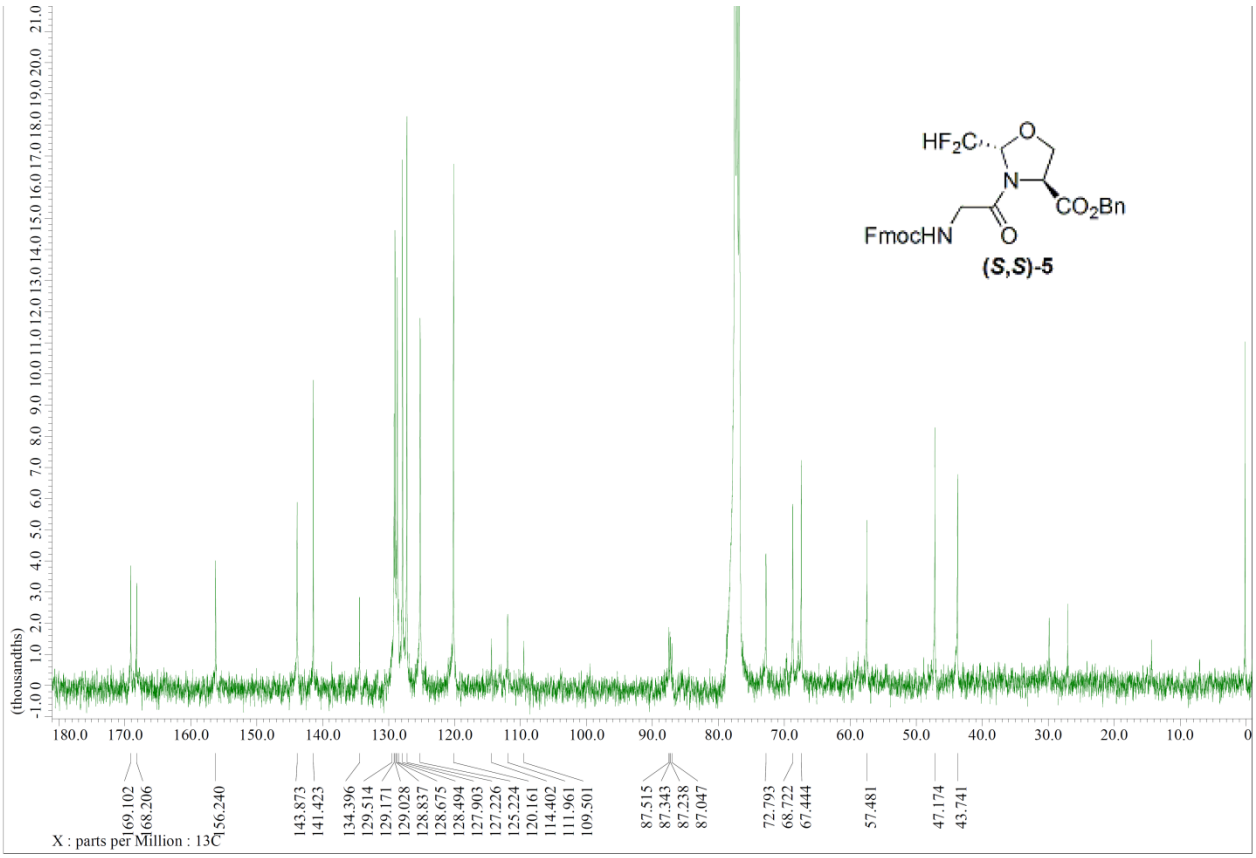


^{19}F NMR spectrum at 293 K in CD_3OD

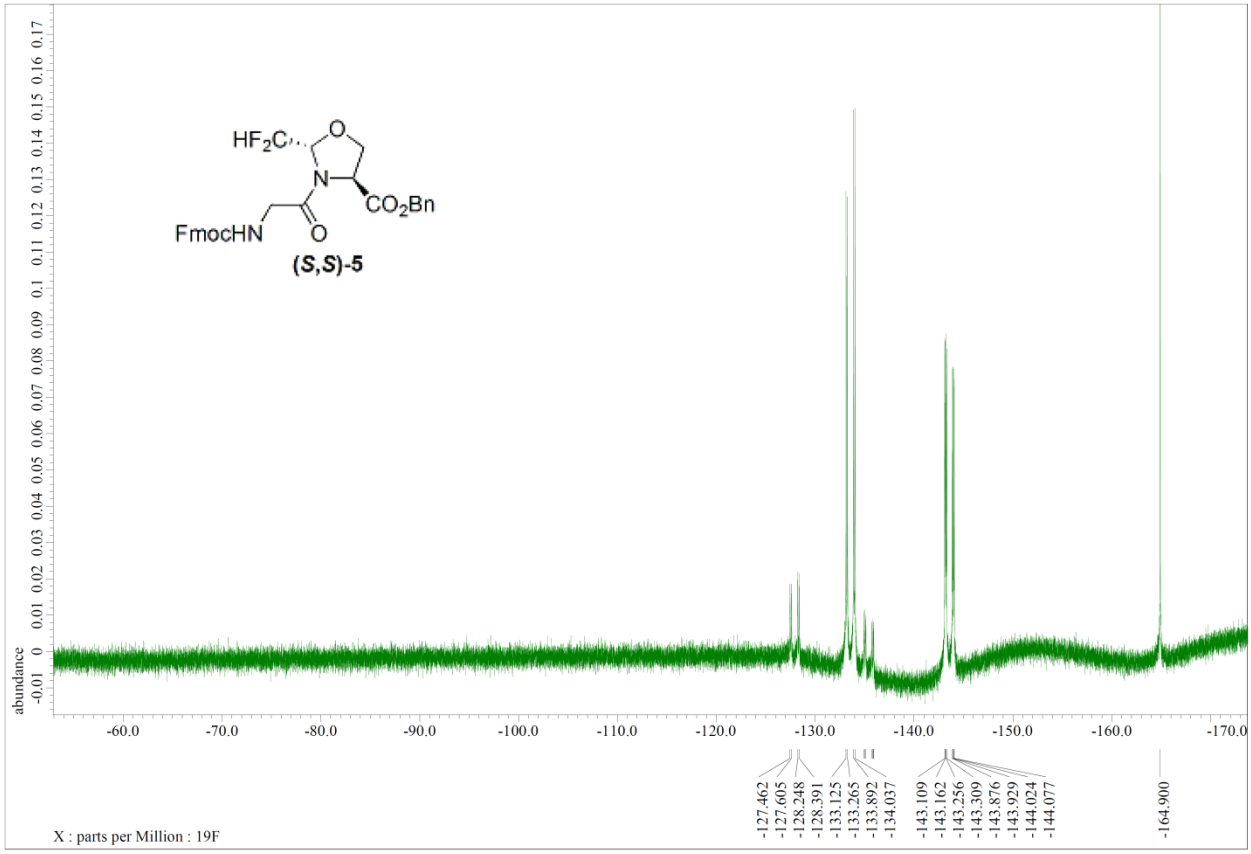
3.9. Fmoc-Gly-(2*S*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-OBn 5a



^{1}H NMR spectrum at 293 K in $CDCl_3$

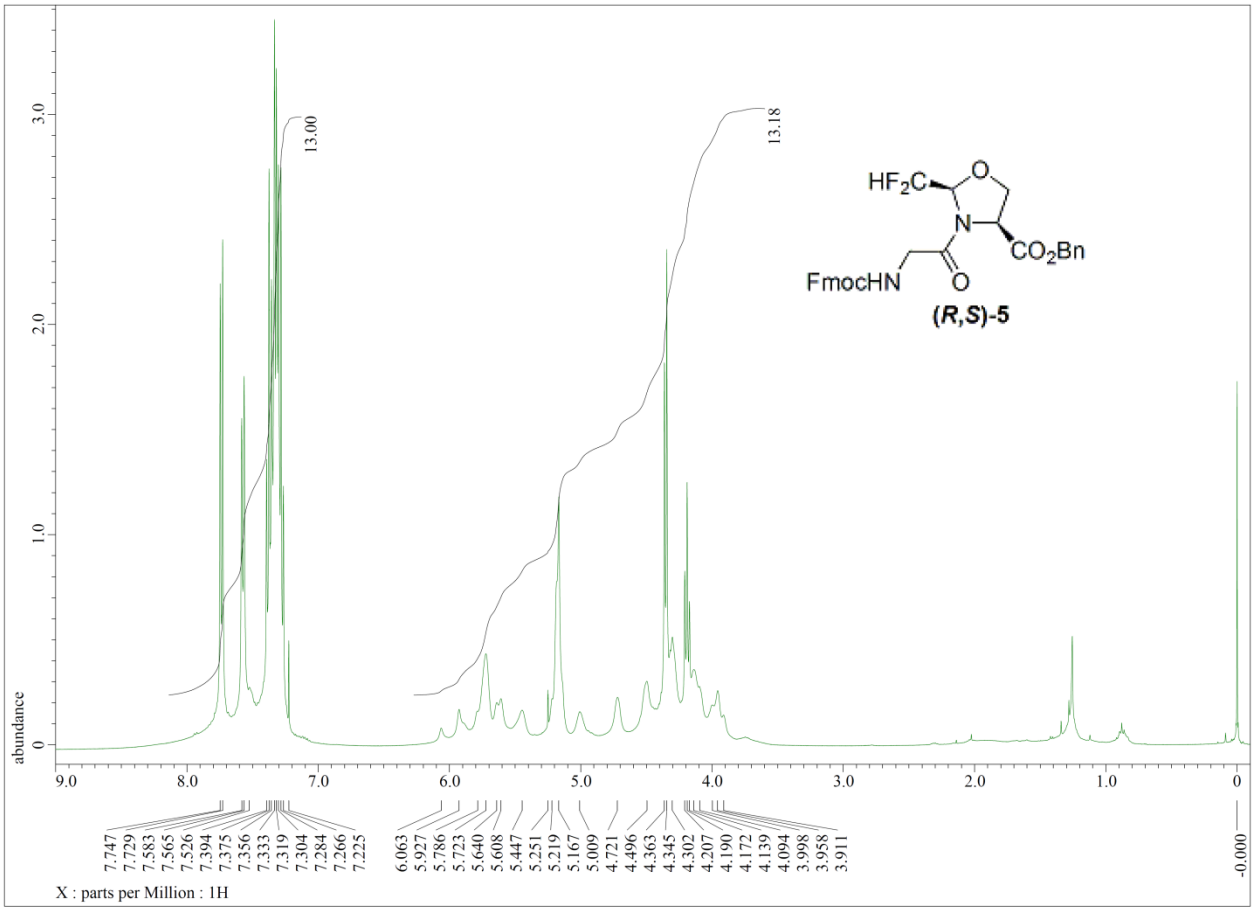


^{13}C NMR spectrum at 293 K in $CDCl_3$

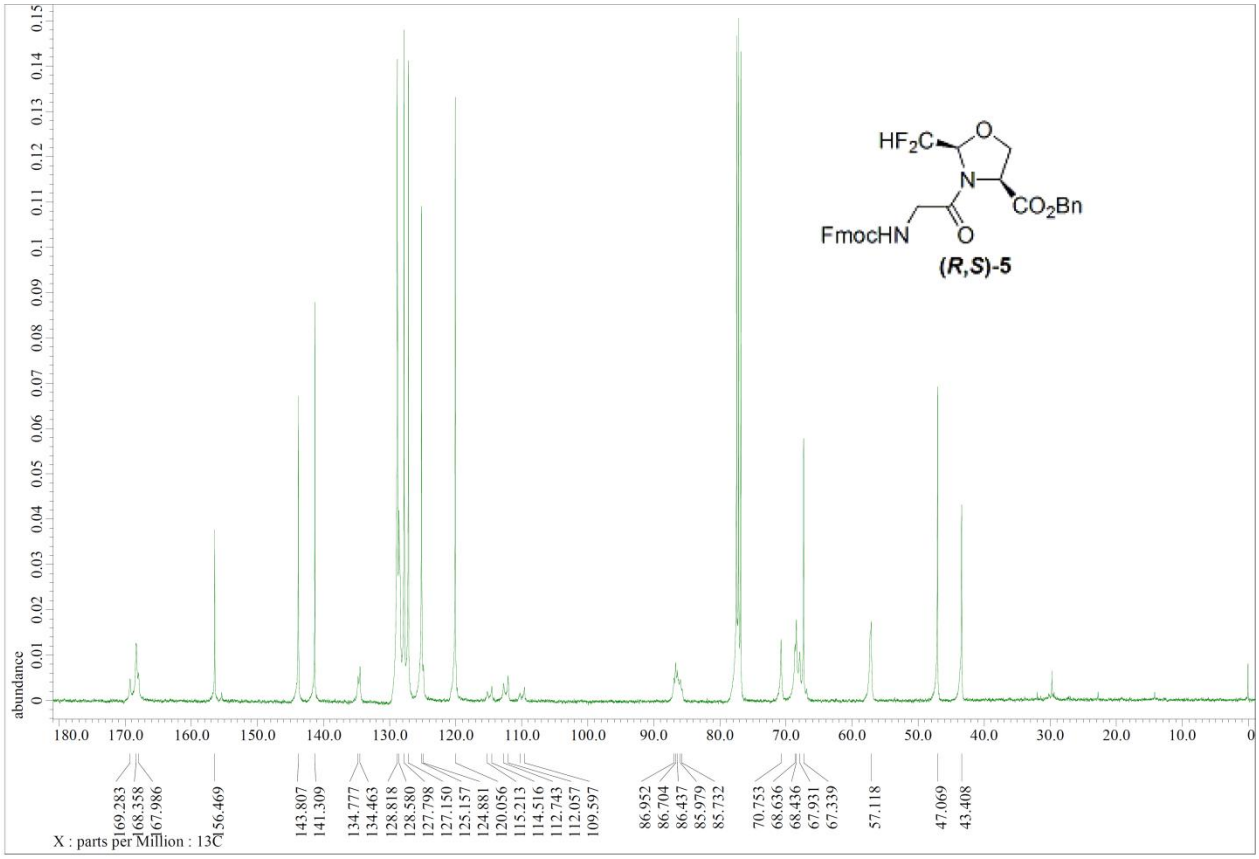


^{19}F NMR spectrum at 293 K in $CDCl_3$

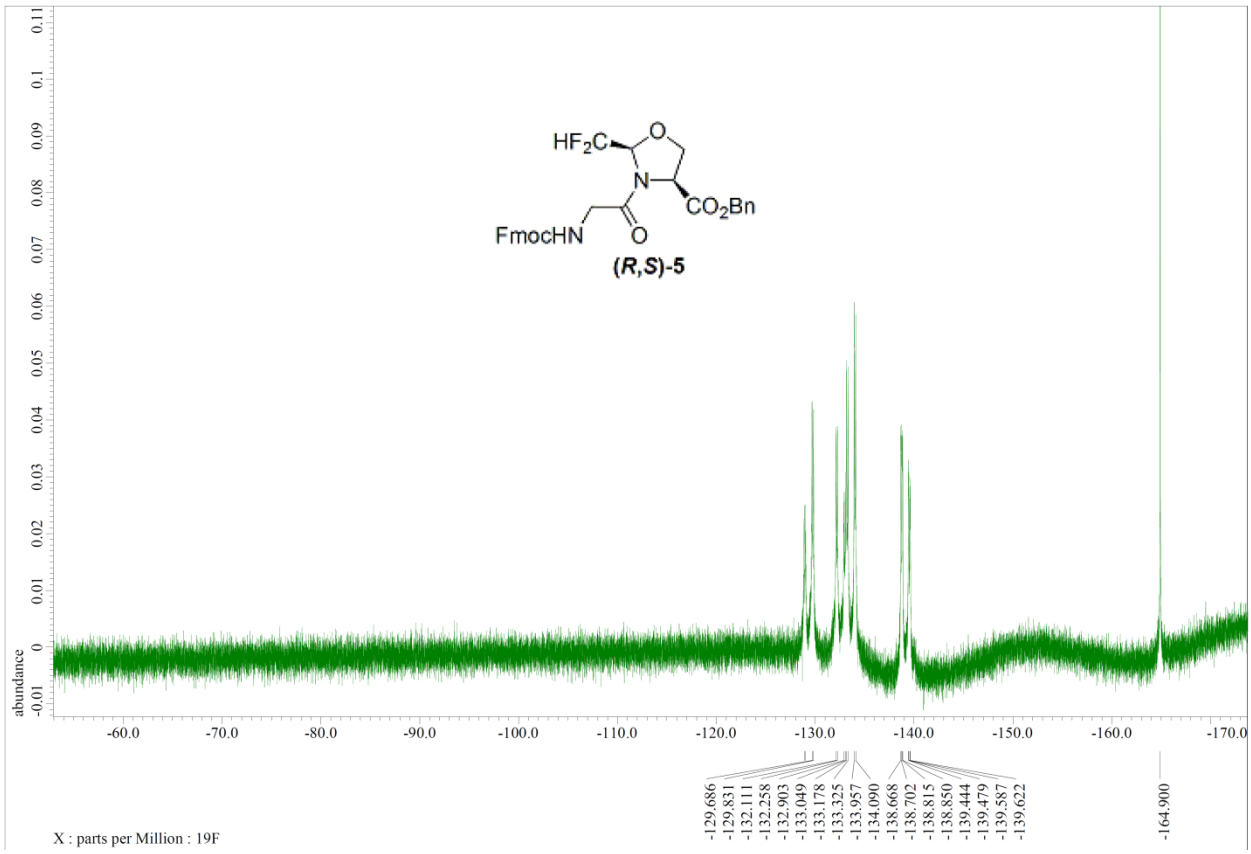
3.10. Fmoc-Gly-(2*R*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-OBn 5b



^{1}H NMR spectrum at 293 K in $CDCl_3$

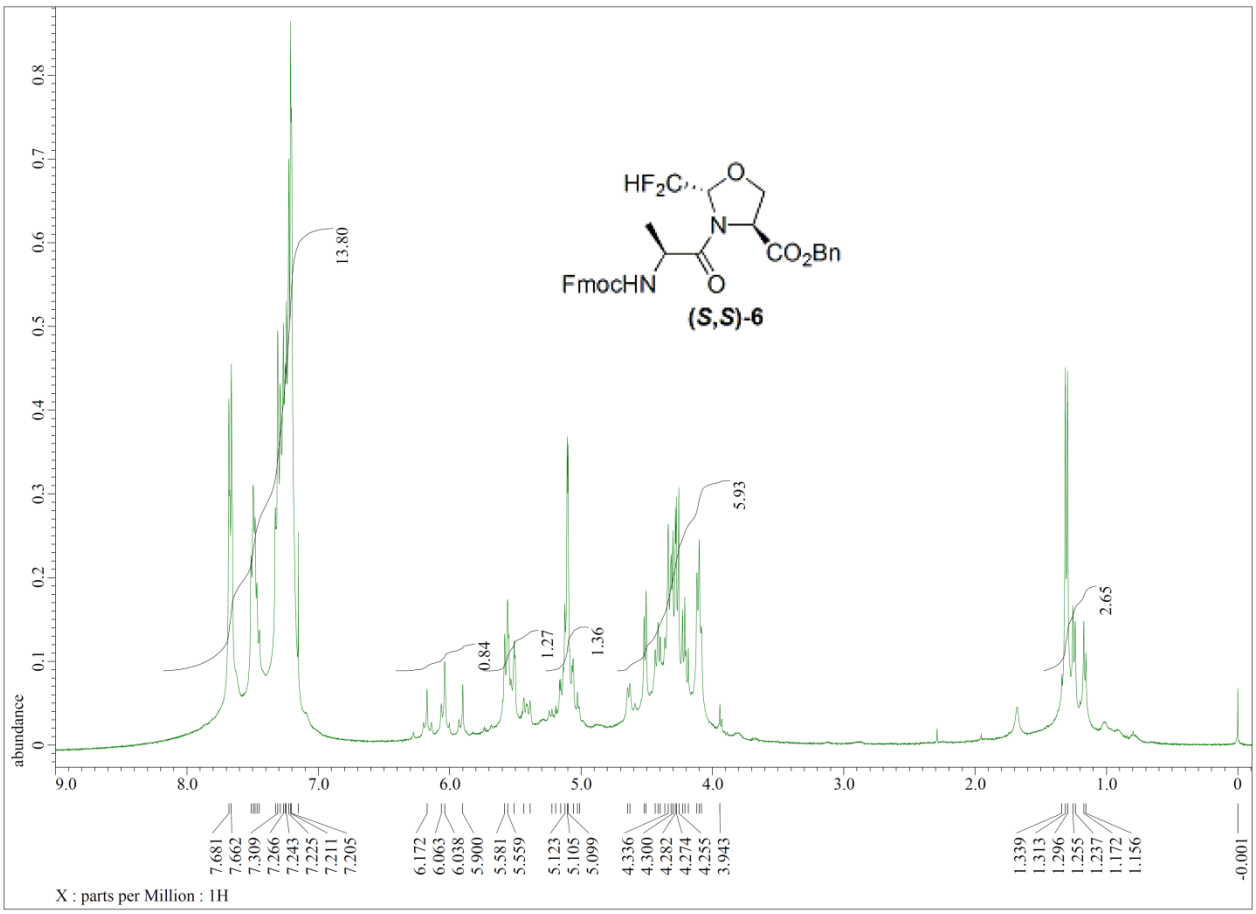


^{13}C NMR spectrum at 293 K in $CDCl_3$

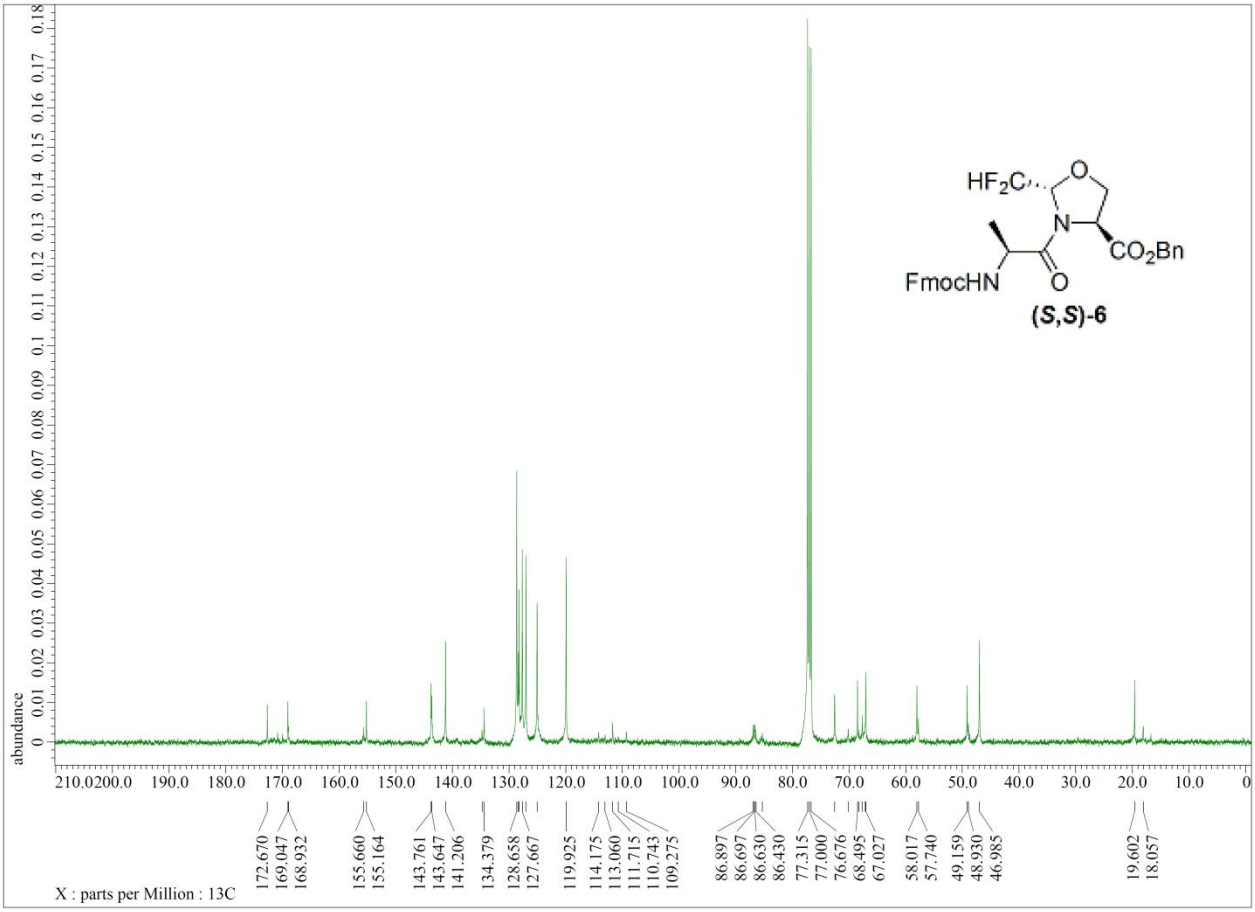


^{19}F NMR spectrum at 293 K in $CDCl_3$

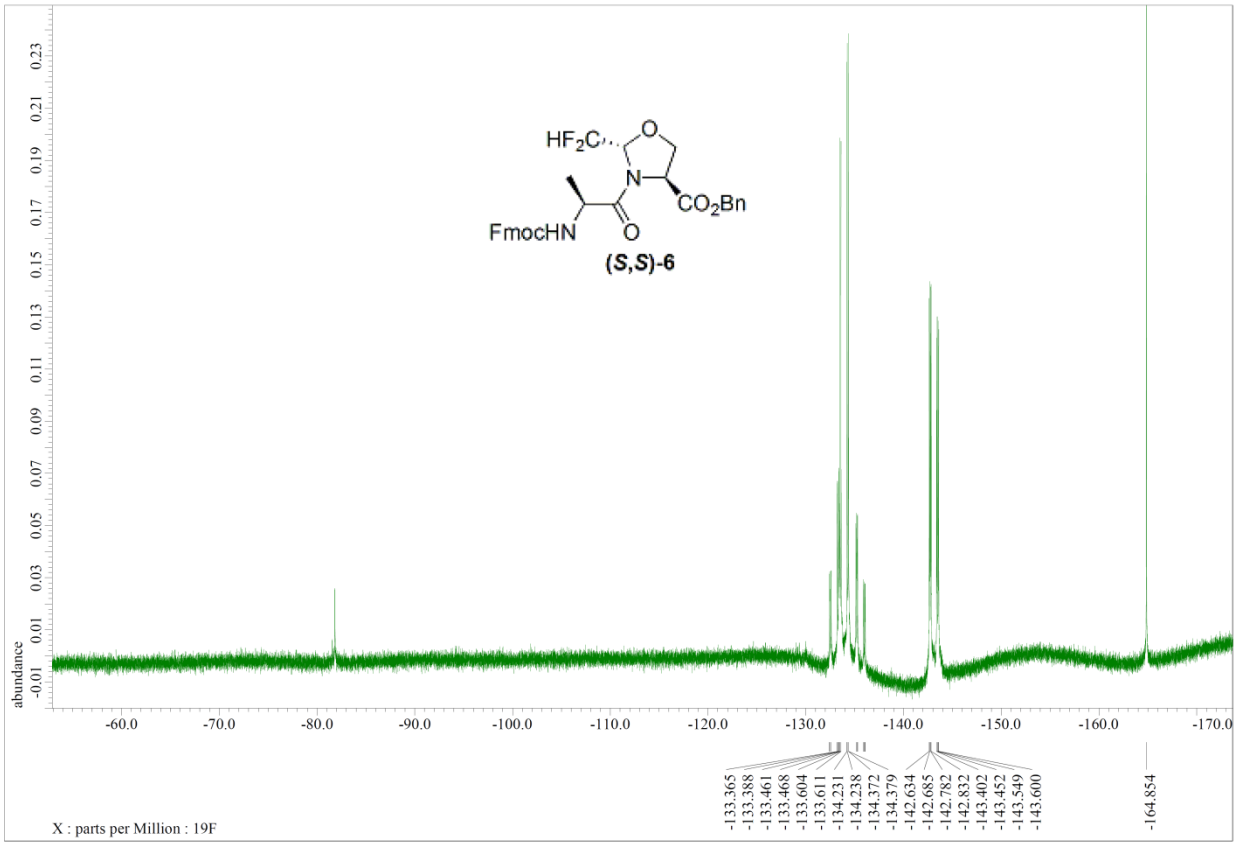
3.11. Fmoc-L-Ala-(2*S*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-OBn 6a



^{1}H NMR spectrum at 293 K in $CDCl_3$

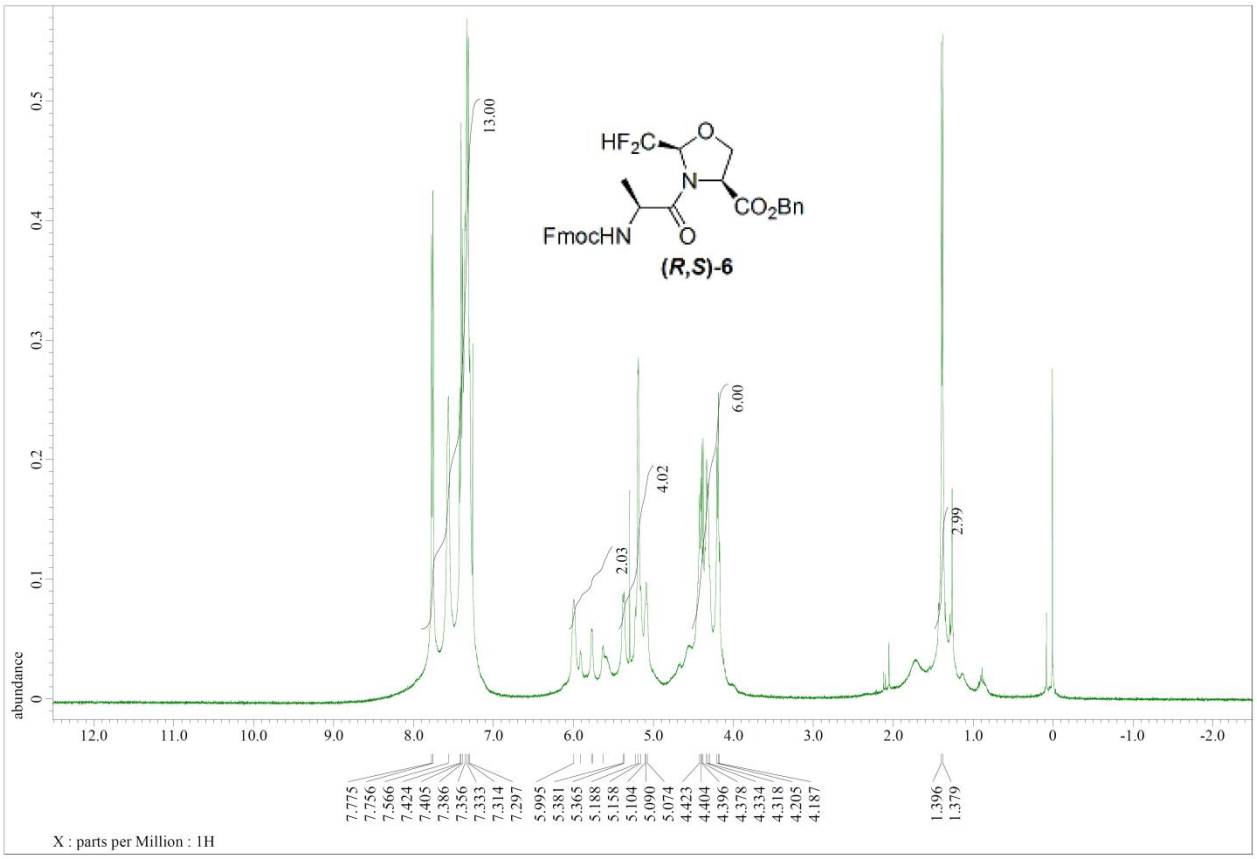


^{13}C NMR spectrum at 293 K in $CDCl_3$

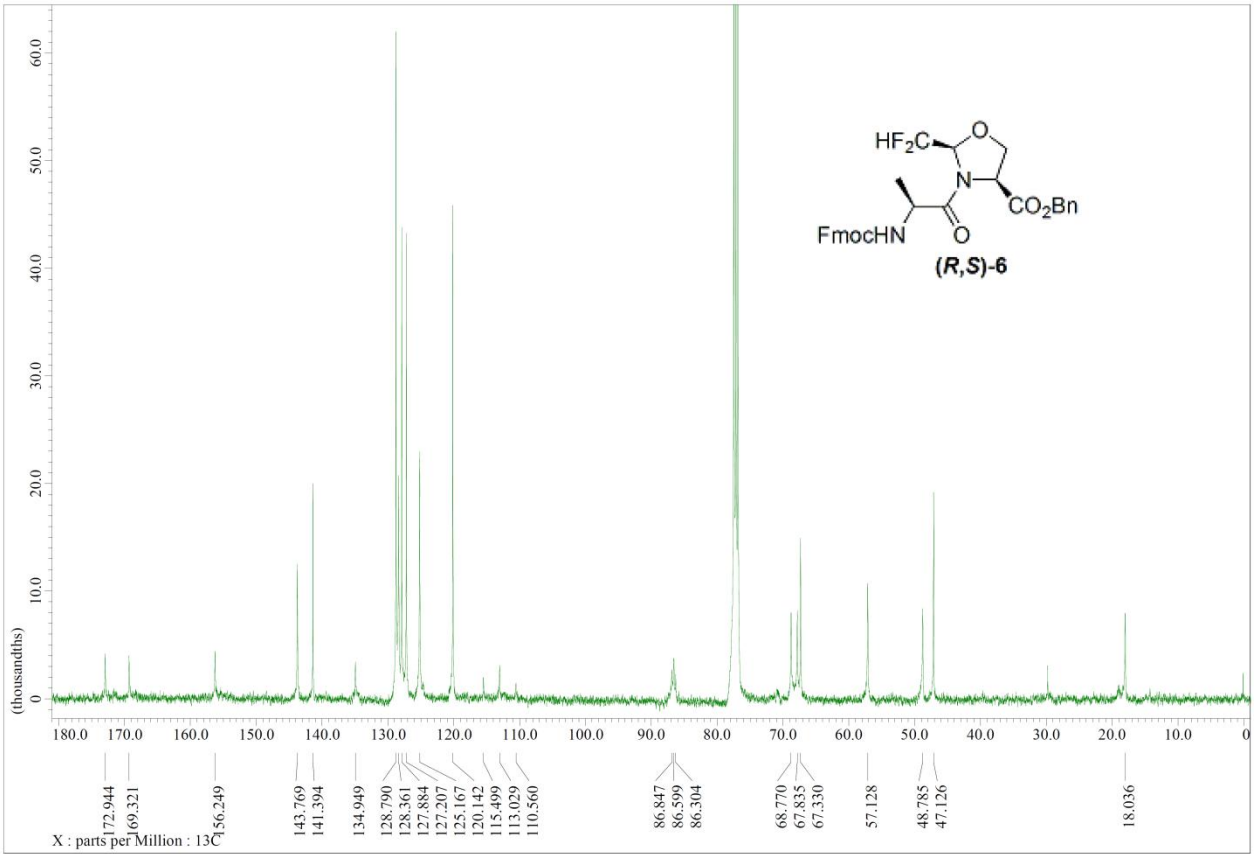


^{19}F NMR spectrum at 293 K in $CDCl_3$

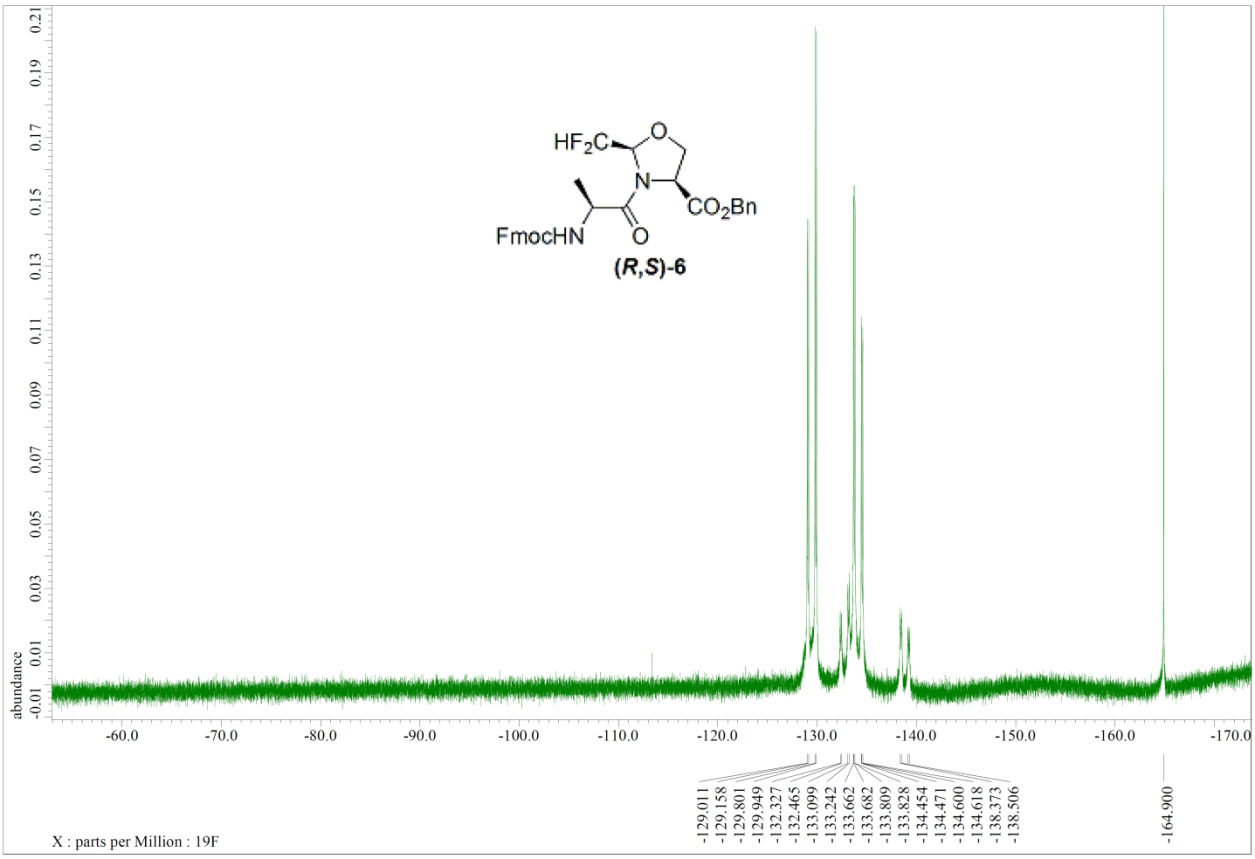
3.12. Fmoc-L-Ala-(2*R*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-OBn 6b



^{1}H NMR spectrum at 293 K in $CDCl_3$

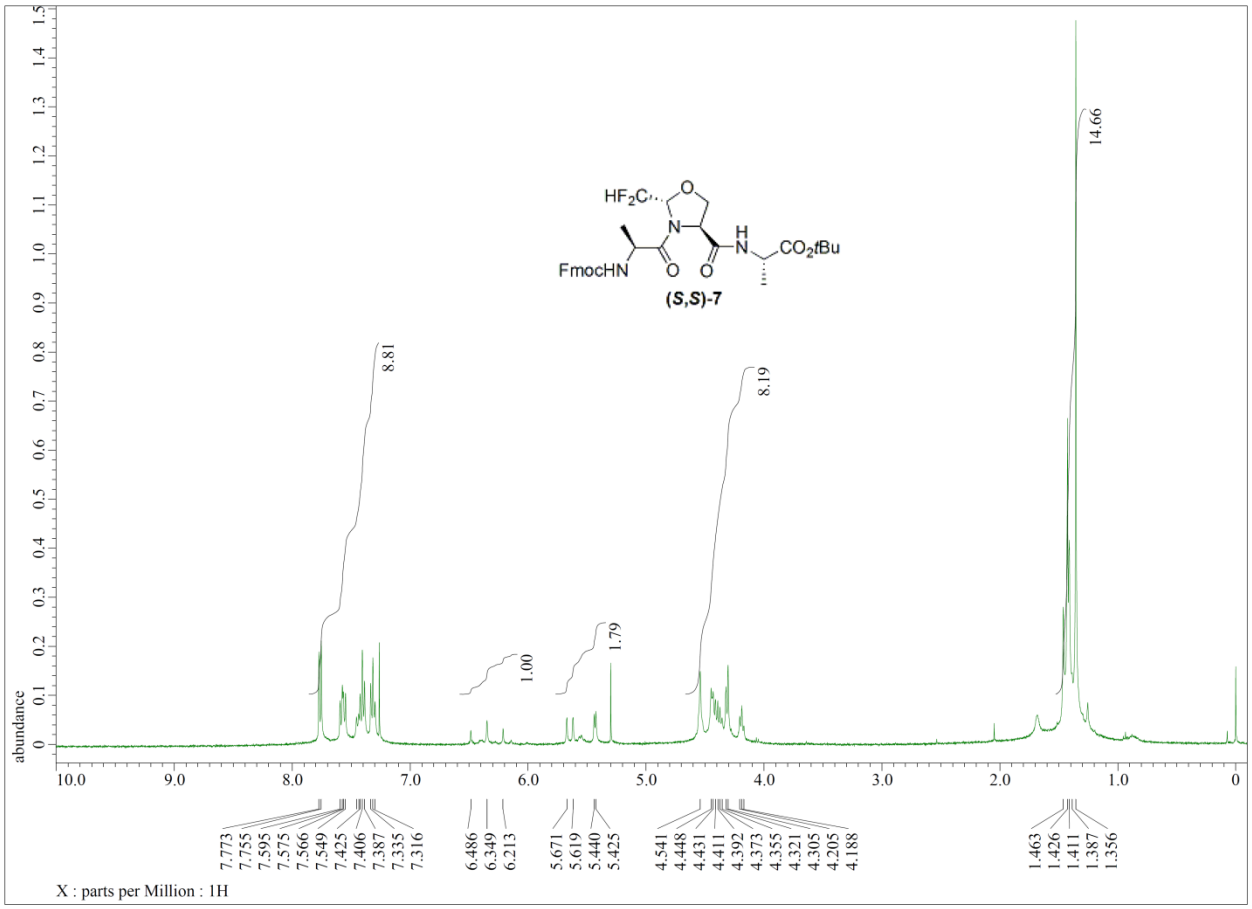


^{13}C NMR spectrum at 293 K in $CDCl_3$

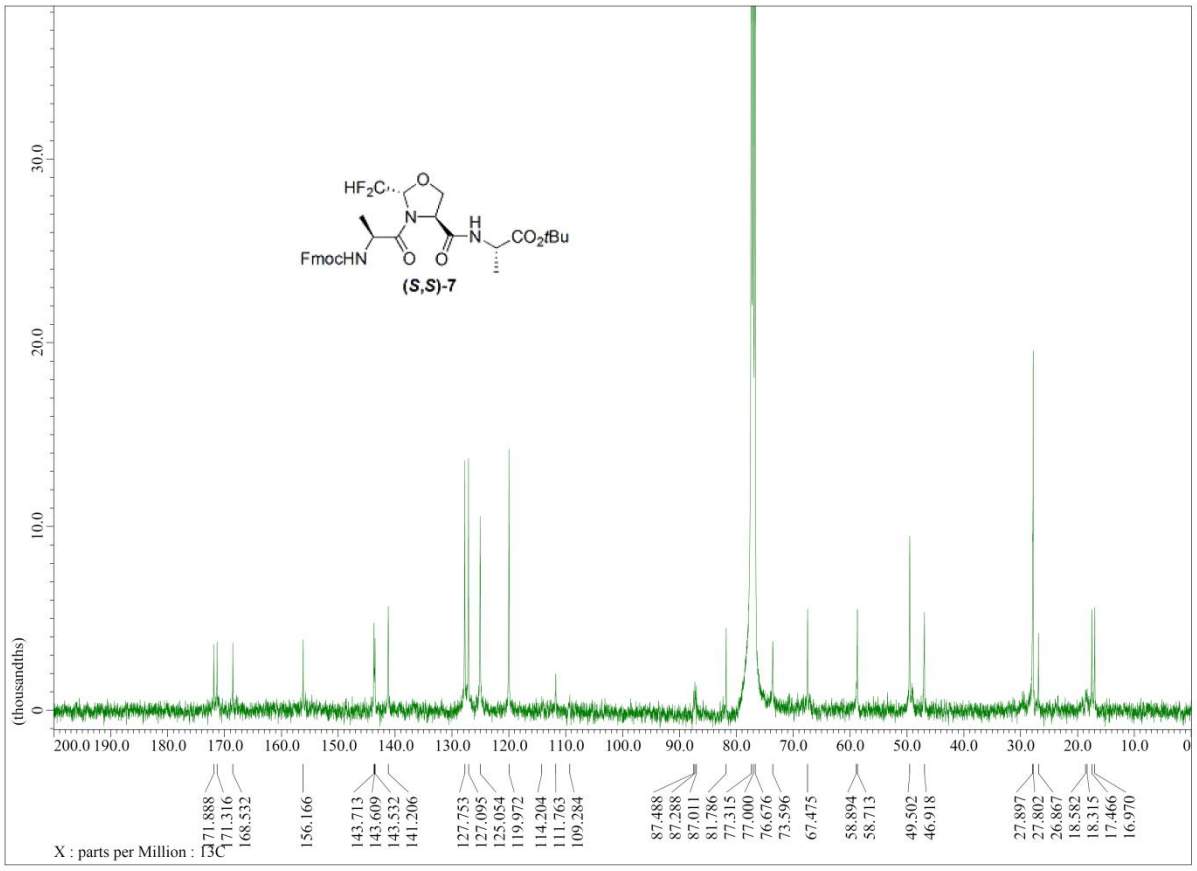


^{19}F NMR spectrum at 293 K in $CDCl_3$

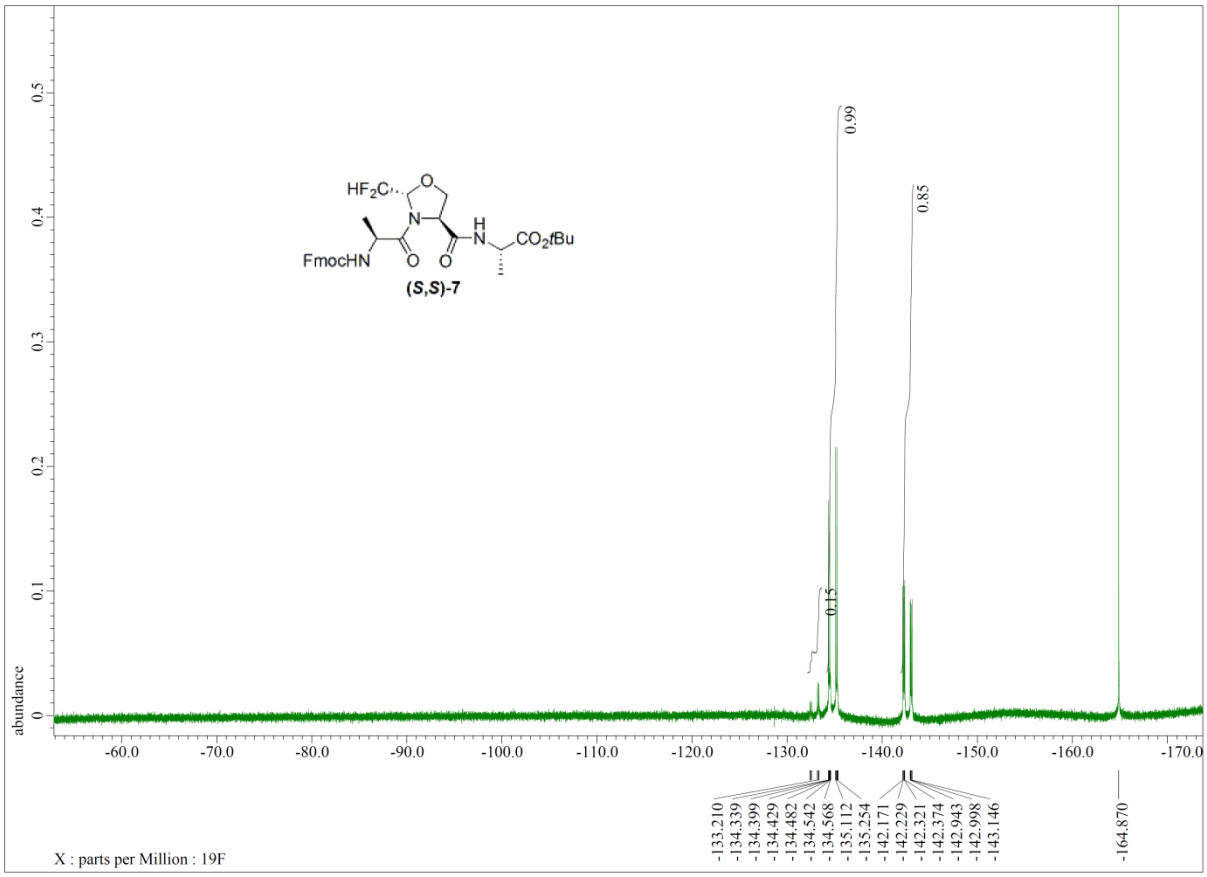
3.13. Fmoc-L-Ala-(2*S*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-L-Ala-O*t*Bu 7a



^{1}H NMR spectrum at 293 K in $CDCl_3$

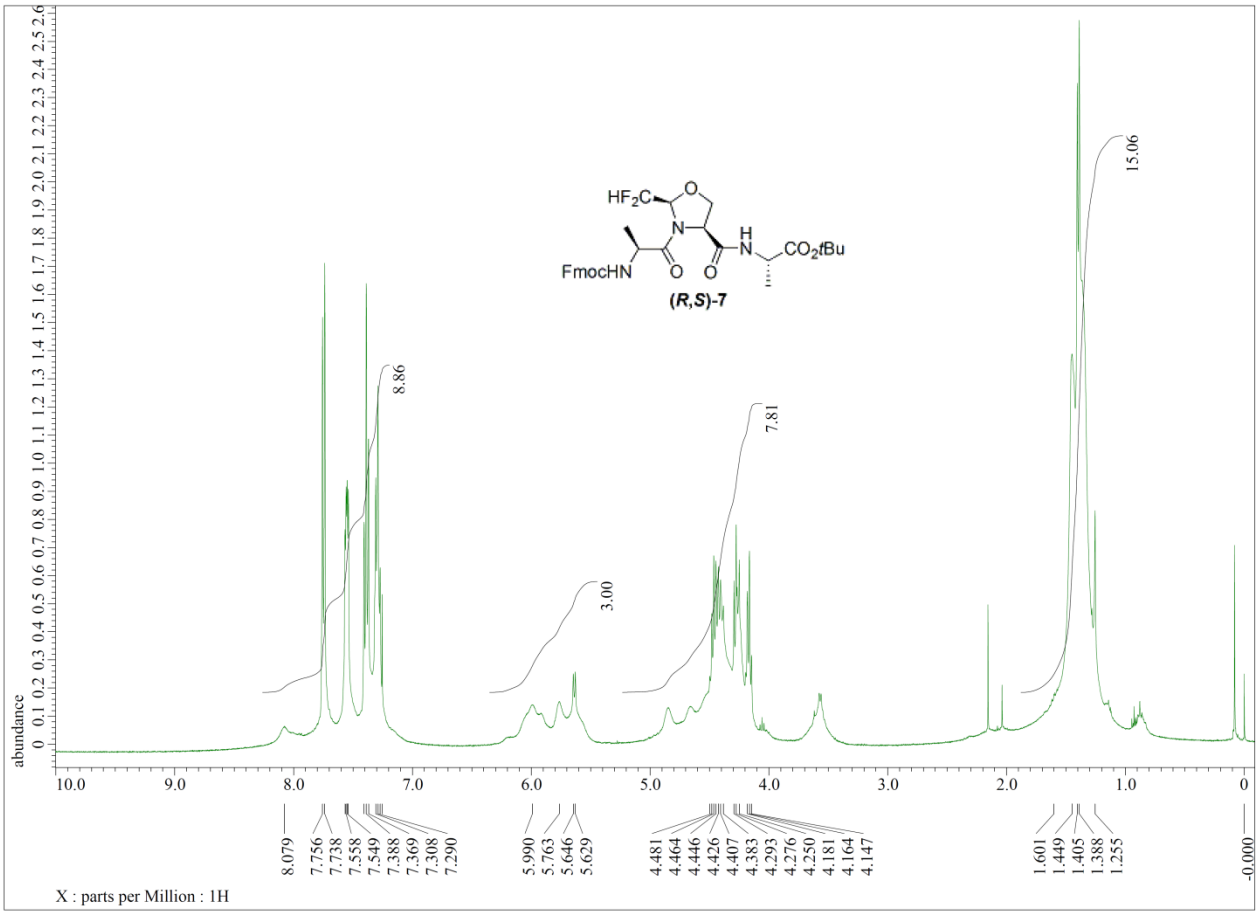


^{13}C NMR spectrum at 293 K in $CDCl_3$

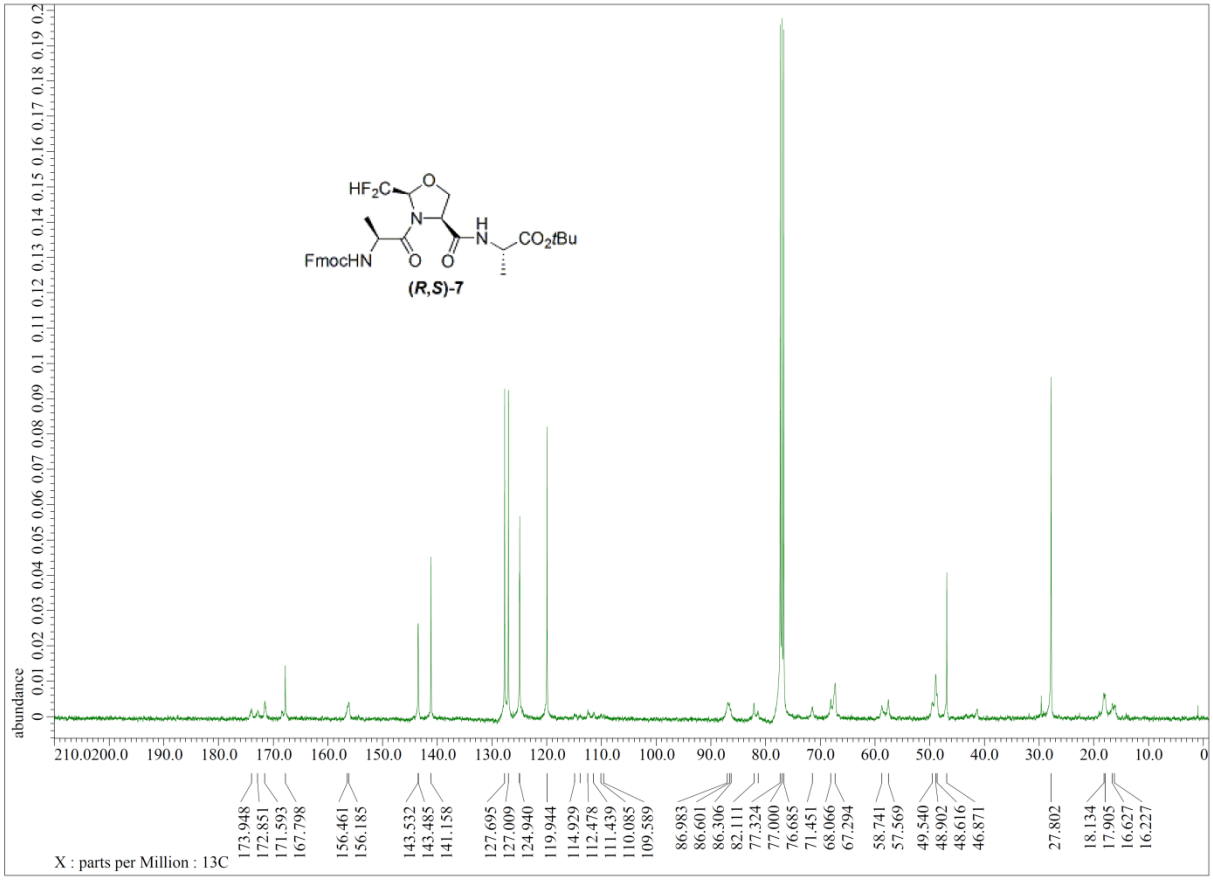


^{19}F NMR spectrum at 293 K in $CDCl_3$

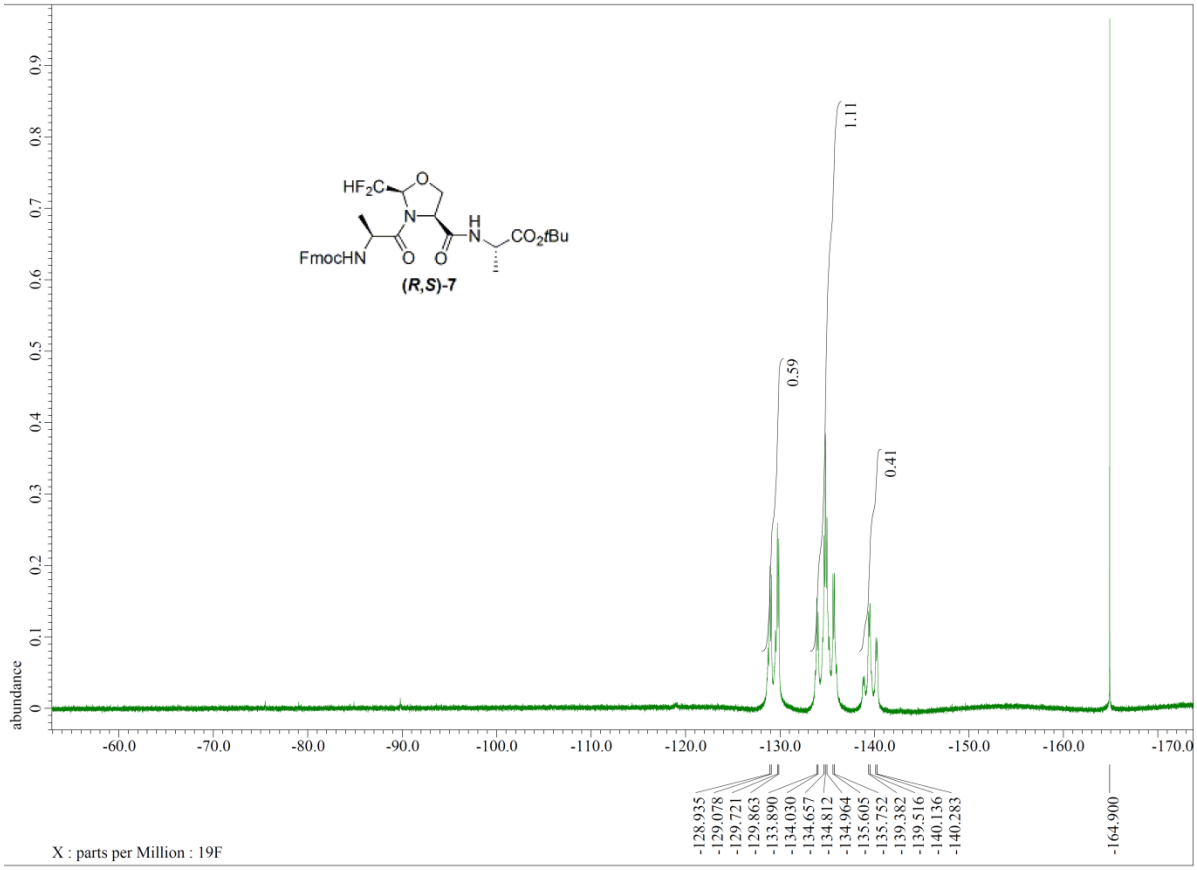
3.14. Fmoc-L-Ala-(2*R*,4*S*)-Ser($\Psi^{CF2H,H}$Pro)-L-Ala-O*t*Bu 7b



^{1}H NMR spectrum at 293 K in $CDCl_3$



^{13}C NMR spectrum at 293 K in $CDCl_3$



^{19}F NMR spectrum at 293 K in $CDCl_3$

4. Calculated Karplus-type curve of $^3J_{(FH)}(\phi)$

The Karplus-type curve representing the angular ϕ dependence upon the NMR vicinal fluorine-proton couplings $^3J_{(FH)}$ was calculated from the reported equation:[1]

$$
\begin{aligned}
{}^3J_{\mathrm{HF}}^{X_1X_2/X_3X_4} = {} & \left[25.1 - 3.5\left(\Delta x_{X_1} + \Delta x_{X_2}\right) - 5.1\left(\Delta x_{X_3} + \Delta x_{X_4}\right)\right] \\
& + \left[-7.0 - 0.8\left(\Delta x_{X_1} + \Delta x_{X_2}\right) + 1.2\left(\Delta x_{X_3} + \Delta x_{X_4}\right)\right] \times \cos\left(\Phi_{\mathrm{FH}}\right) + \left[20.2 - 1.9\left(\Delta x_{X_1} + \Delta x_{X_2}\right) - 10.7\left(\Delta x_{X_3} + \Delta x_{X_4}\right)\right] \\
& \times \cos\left(2\Phi_{\mathrm{FH}}\right) + \left[-1.7\left(\Delta x_{X_1} - \Delta x_{X_2}\right) - 2.2\left(\Delta x_{X_3} - \Delta x_{X_4}\right)\right] \times \sin\left(\Phi_{\mathrm{FH}}\right) + \left[6.1\left(\Delta x_{X_1} - \Delta x_{X_2}\right) + 6.5\left(\Delta x_{X_3} - \Delta x_{X_4}\right)\right] \\
& \times \sin\left(2\Phi_{\mathrm{FH}}\right) + 3.4\left(\Delta x_{X_3}^2 + \Delta x_{X_4}^2\right) \times \cos\left(2\Phi_{\mathrm{FH}}\right) - \left[2.0(\delta_1 - \delta_2 + \delta_3 - \delta_4)\right] \times \sin\left(2\Phi_{\mathrm{FH}}\right)
\end{aligned}
$$

were:

- X_1, X_2, X_3 and X_4 represent the substituents of the fluoroethane (CFX_1X_2-CHX_3X_4)

- $\Delta\chi_{Xi}$ represents the Huggins relative electronegativies and corresponds to $\Delta\chi_{Xi} = \chi_{Xi} - \chi_{H}$

- Φ represents the torsion angle (F-C-C-H)

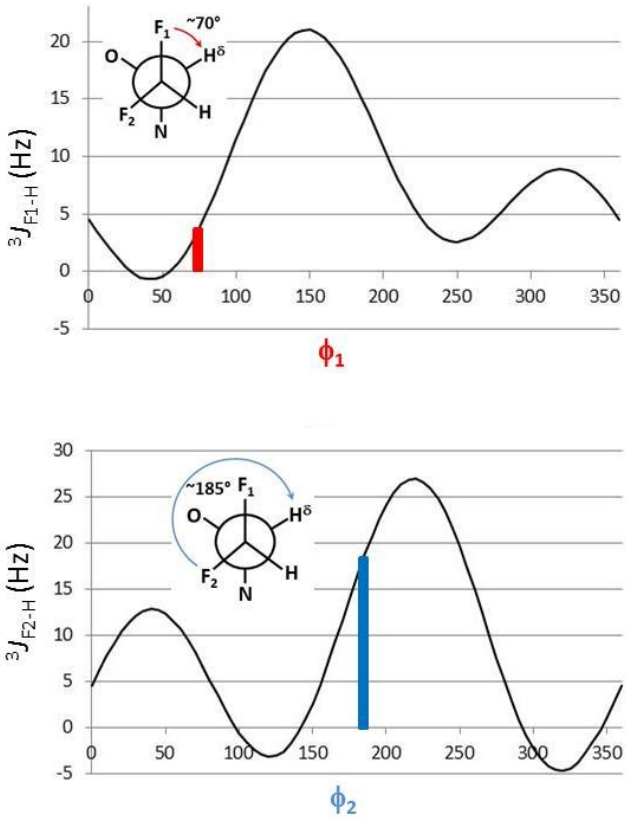
- δ_i is set equal to 0 when the position i is an hydrogen and equal to 1 when the position i is substituted

here, we used:

- X_1 = F or H, X_2 = H or F, X_3 = O and X_4 = N

- $\Delta\chi_{X1}$ = 1.78, $\Delta\chi_{X2}$ = 0, $\Delta\chi_{X3}$ = 1.24 and $\Delta\chi_{X4}$ = 0.84 with χ_F = 3.98, χ_H = 2.20, χ_O = 3.44, χ_N = 3.04

- δ_1 = 1, δ_2 = 0, δ_3 = 1 and δ_4 = 1



Calculated Karplus-type curve of $^3J_{(FH)}(\Phi)$

[1] J. San Fabian, J. Guilleme and E. Diez, *J. Magn. Reson.*, 1998, **133**, 255.

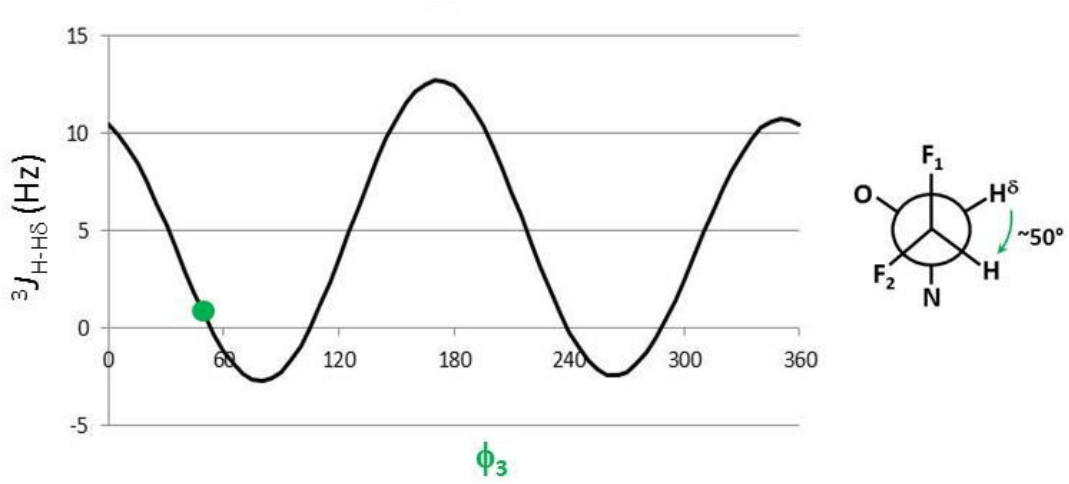
5. Calculated Karplus-type curve of $^{3}J_{(HH)}$ (ϕ)

The Karplus-type curve representing the angular ϕ dependence upon the NMR vicinal proton =-proton couplings $^{3}J_{(HH)}$ was calculated from the reported equations:[2]

$$^{3}J_{HH} = P_1 \cos^2\phi + P_2 \cos\phi + P_3 + \sum [\Delta\chi_i \ (P_4 + P_5 \cos^2(\xi_i \ \phi + P6 \ |\Delta\chi_i|))]$$

were:

- $\Delta\chi_i$ represents the Huggins relative electronegativies of the substituents S_i
- Φ represents the torsion angle (H-C-C-H)
- ξ_i is set equal to -1 or +1 depending on the position *i*
- $P_1 = 13.22$; $P_2 = -0.99$; $P_3 = 0$; $P_4 = 0.53$; $P_5 = -2.46$; $P_6 = 19.9$



Calculated Karplus-type curve of $^{3}J_{(HH)}$ (Φ)

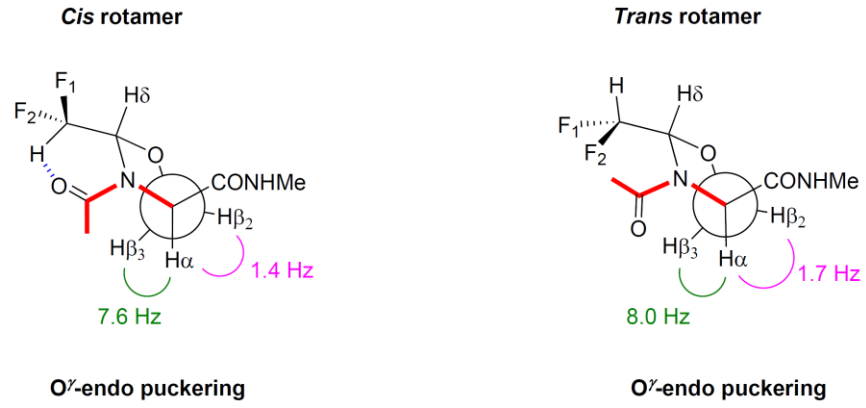
[2] C. A. G.Haasnoot, F. A. A. M. De Leeuw and C. Altona, *Tetrahedron*, 1979, **36**, 2783.

6. NMR conformational analysis

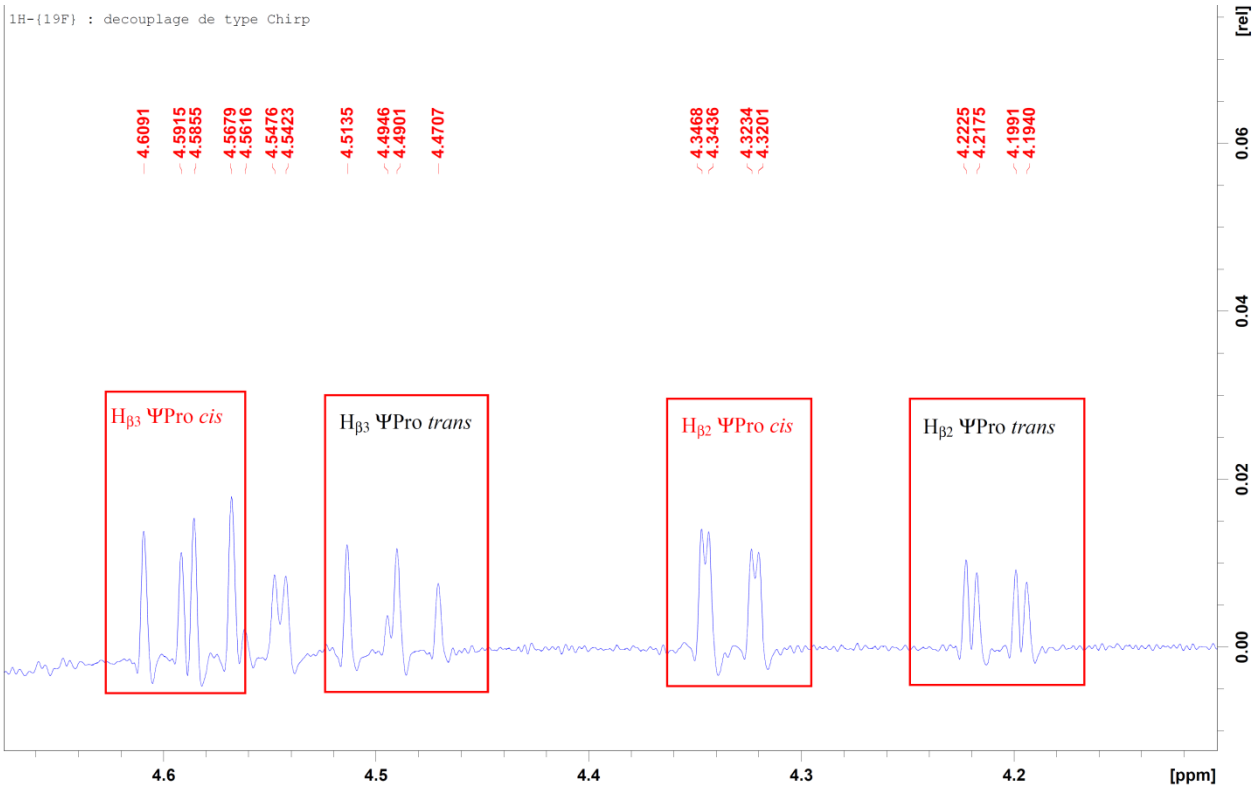
6.1. NMR conformational assignment of (2*S*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*S*,*S*)-4

A. Puckering

The puckering preferences have been determined from well-resolved ^{1}D spectra or from CH2-TROSY experiments[3] using $^{3}J_{H\alpha\text{-}H\beta2}$ and $^{3}J_{H\alpha\text{-}H\beta3}$ coupling constants. A signature of the puckering can be easily obtained by looking at the multiplicity of the H_α protons. H_α resonance appears as a triplet (t) in the O^γ-exo-puckered form, and as a doublet of doublets (d × d) in the O^γ-endo-puckered form. $^{3}J_{H\alpha\text{-}H\beta}$ vicinal coupling constants can also be accurately measured on the H_β resonances using extensive apodization as shown in the following spectra.



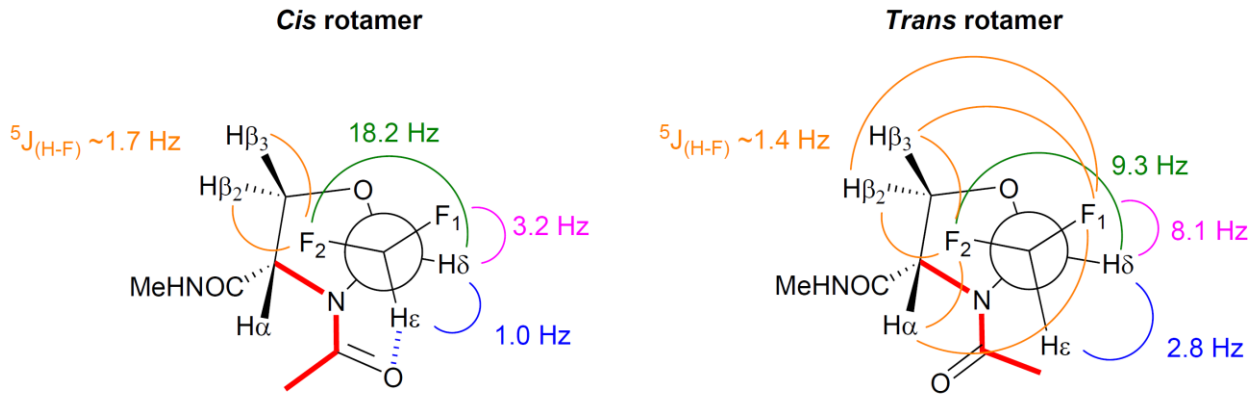
Coupling constants from ^{1}H NMR 500 MHz in H_2O/D_2O (90:10)



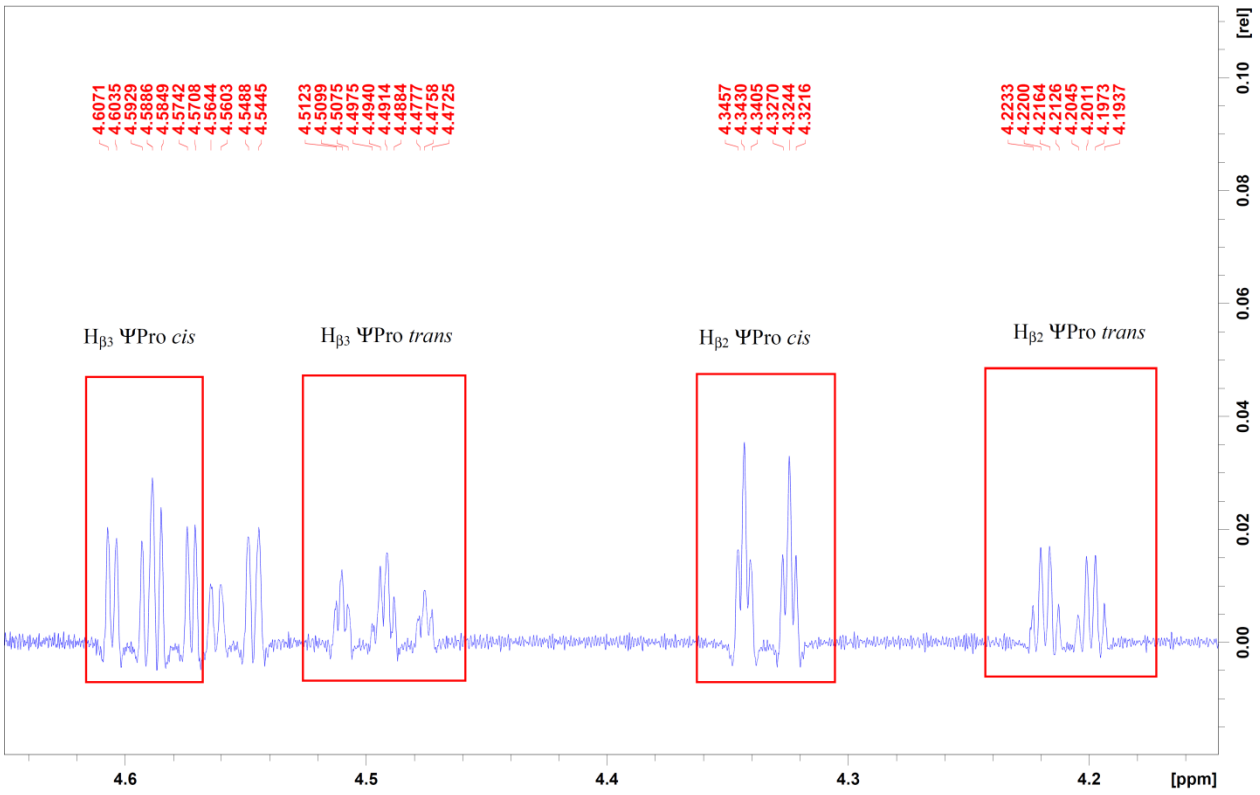
^{1}H-{^{19}F} NMR spectrum at 278 K in H_2O/D_2O (90:10) with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -3.0 Hz, GB = 0.4 (TD = 24590, SI = 65536)

[3] (*a*) G. Guichard, A. Violette, G. Chassaing and E. Miclet, *Magn. Reson. Chem.* 2008, **46**, 918; (*b*) D. Feytens, G. Chaume, G. Chassaing, S. Lavielle, T. Brigaud, B. J. Byun, Y. K. Kang and E. Miclet, *J. Phys. Chem. B*, 2012, **116**, 4069; (c) G. Chaume, J. Simon, N. Lensen, J. Pytkowicz, E. Miclet and T. Brigaud, *J. Org. Chem.*, 2017, **82**, 13602..

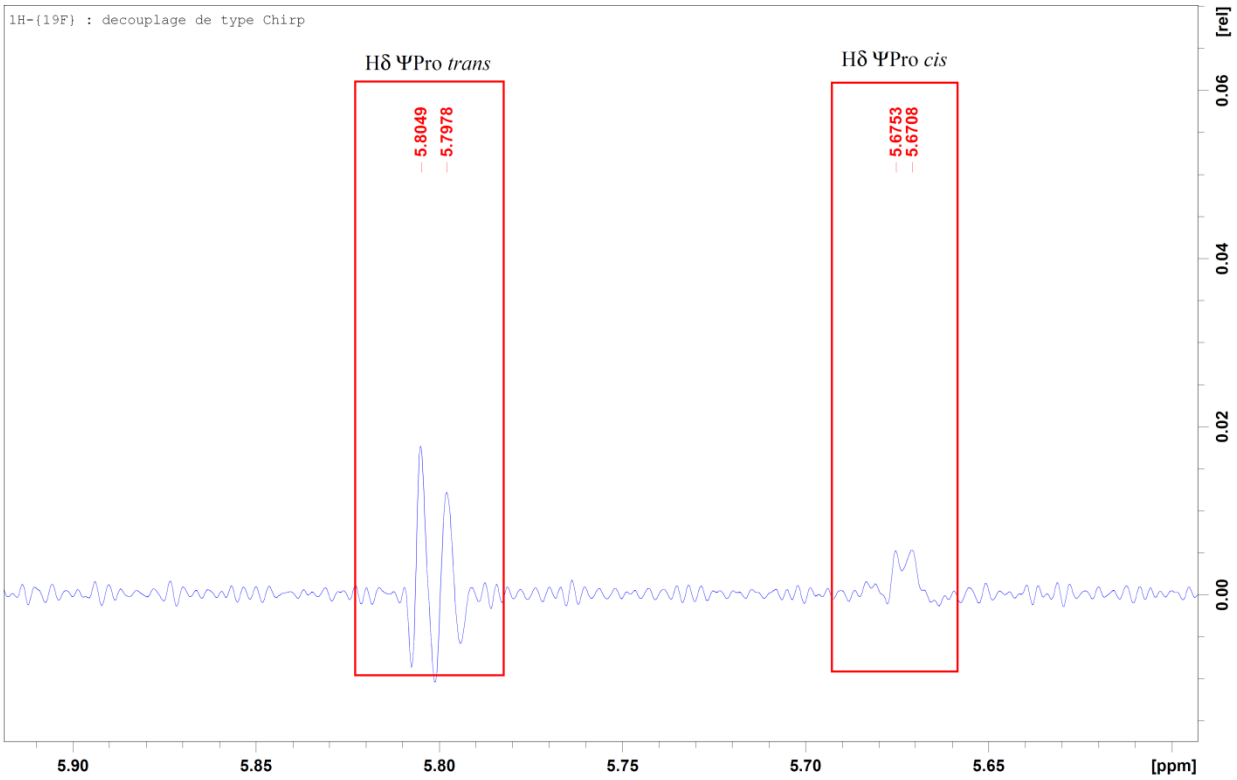
B. Coupling constants



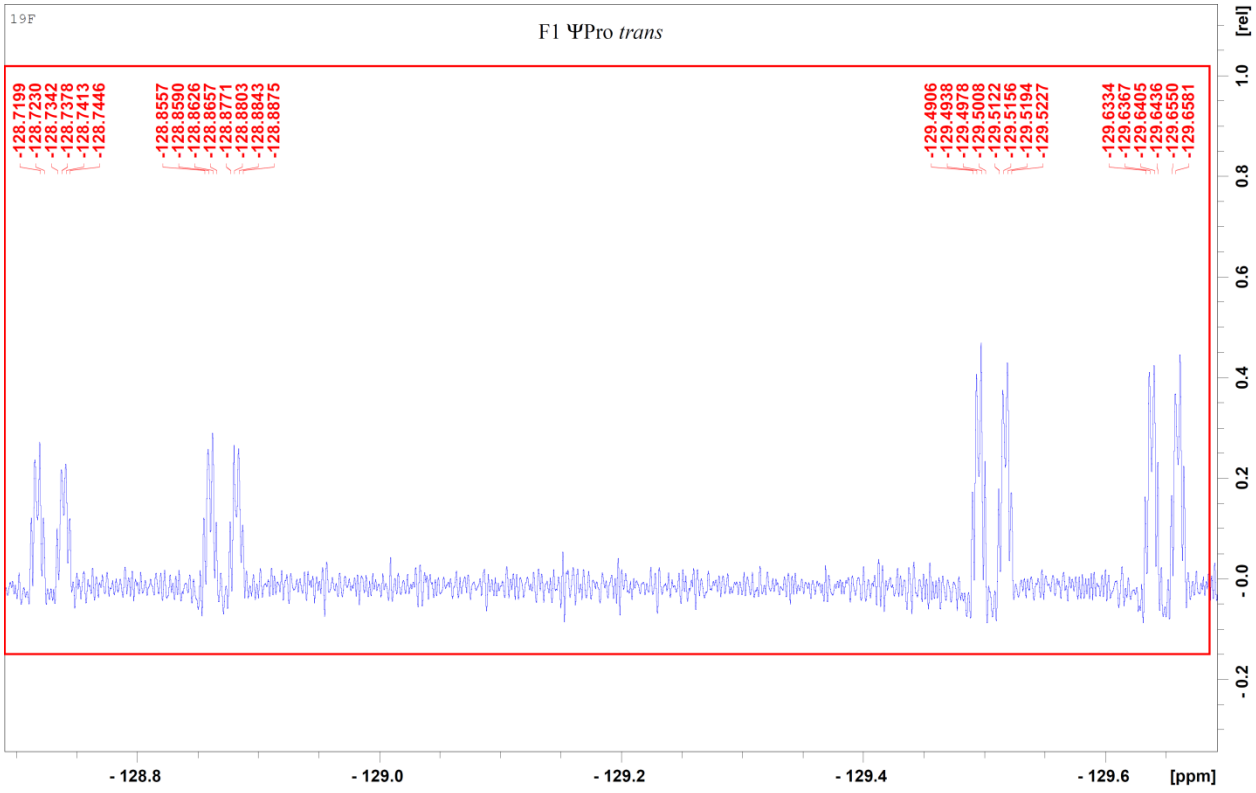
Coupling constants from ^{1}H NMR 400 MHz in H_2O/D_2O = 90/10

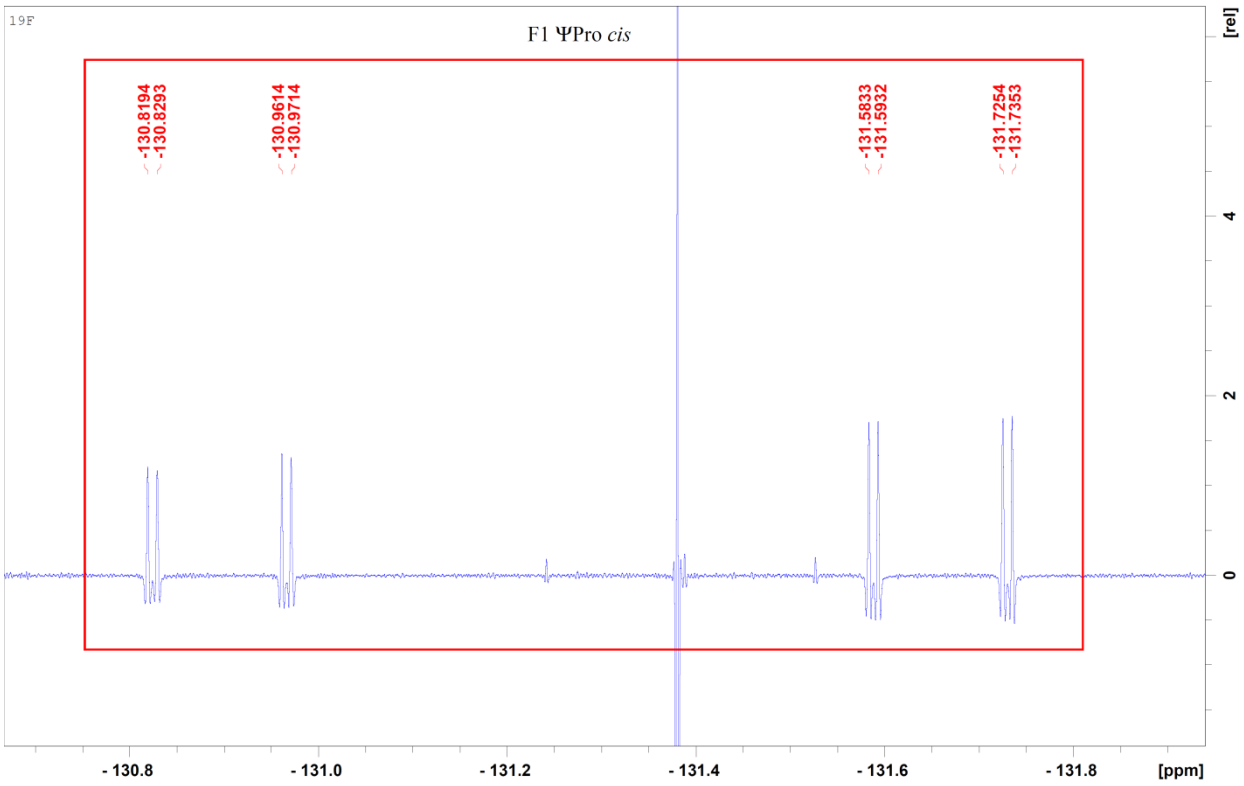


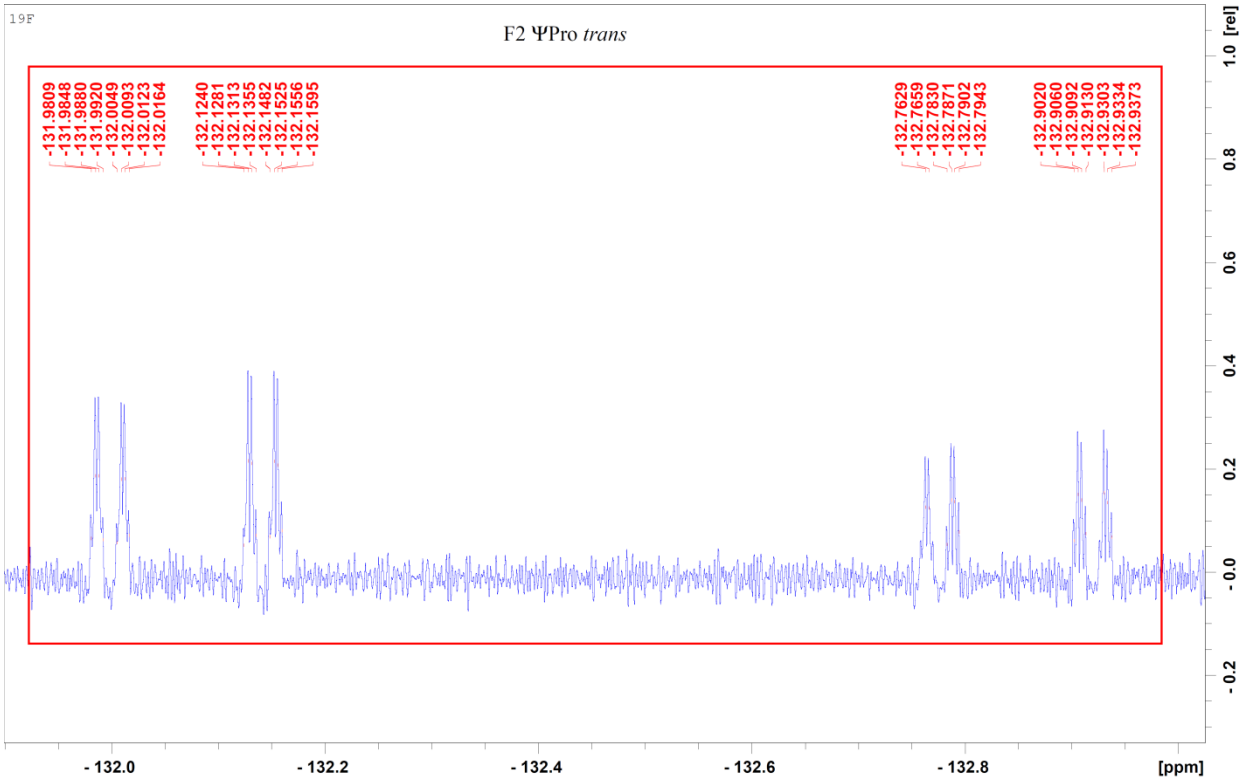
^{1}H NMR spectrum at 298 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -0.9 Hz, GB = 0.3 (TD = 32768, SI = 65536)

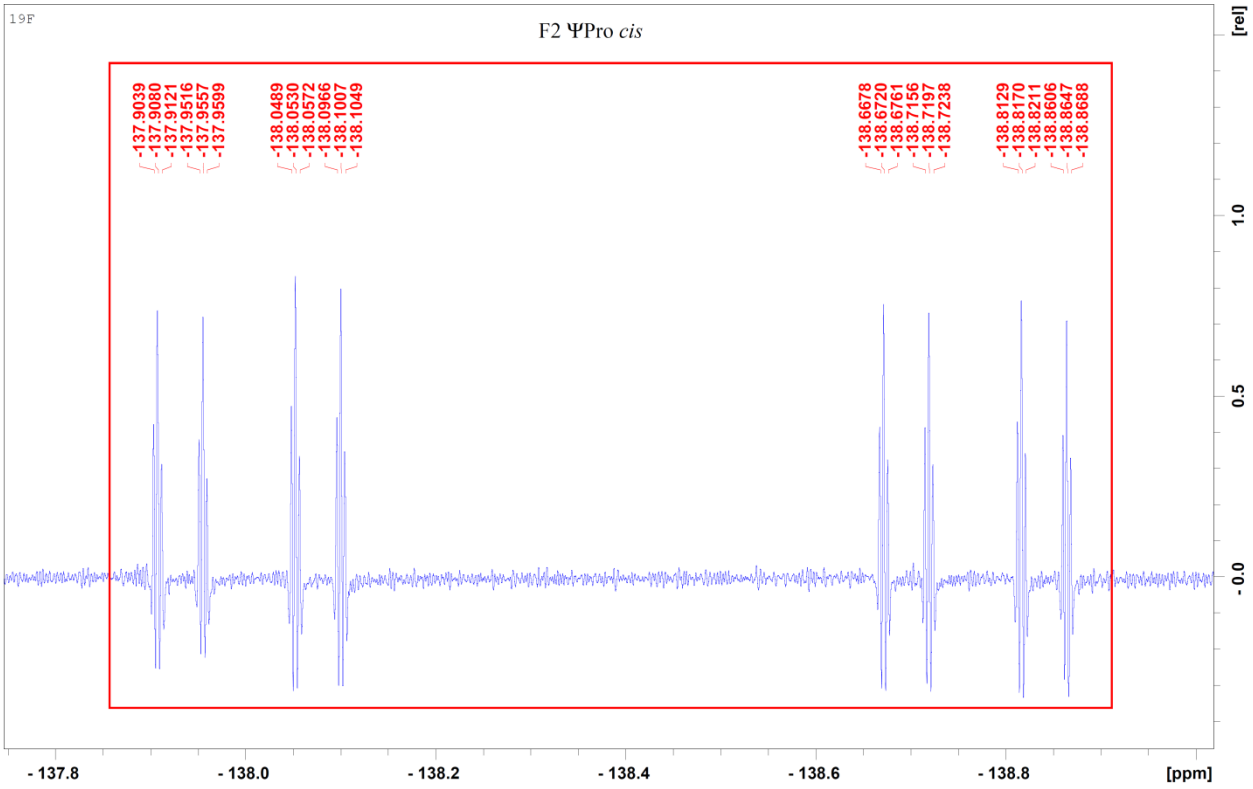


^{1}H-{^{19}F} NMR spectrum at 278 K in H_2O/D_2O 90/10 Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -5.0 Hz, GB = 0.45 (TD = 24590, SI = 65536)




19F
F1 ΨPro *cis*
-130.8194
-130.8293
-130.9614
-130.9714
-131.5833
-131.5932
-131.7254
-131.7353
[rel]
4
2
0
- 130.8
- 131.0
- 131.2
- 131.4
- 131.6
- 131.8
[ppm]

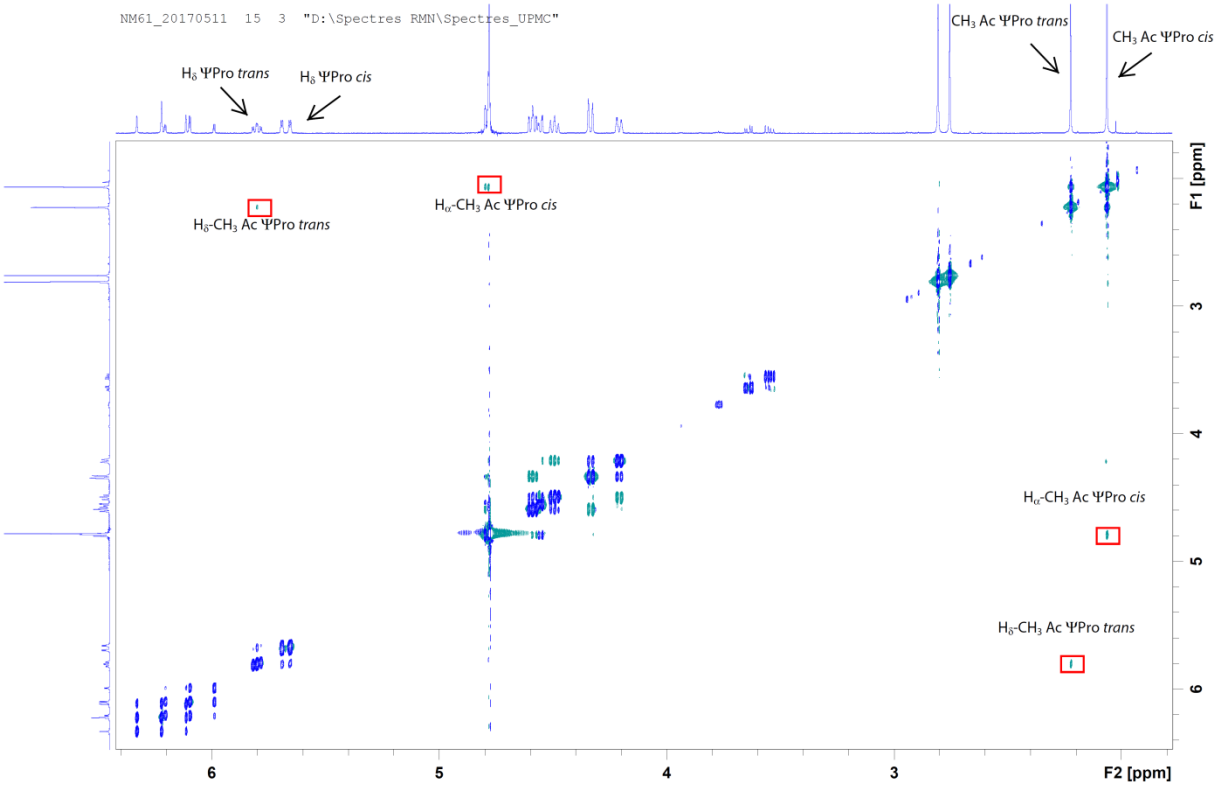

19F
F2 ΨPro *trans*
-131.9809
-131.9848
-131.9880
-131.9920
-132.0049
-132.0093
-132.0123
-132.0164
-132.1240
-132.1281
-132.1313
-132.1355
-132.1482
-132.1525
-132.1556
-132.1595
-132.7629
-132.7659
-132.7830
-132.7871
-132.7902
-132.7943
-132.9020
-132.9060
-132.9092
-132.9130
-132.9303
-132.9334
-132.9373
1.0 [rel]
0.8
0.6
0.4
0.2
- 0.0
- 0.2
- 132.0
- 132.2
- 132.4
- 132.6
- 132.8
[ppm]



^{19}F NMR spectra at 278 K in H_2O/D_2O 90/10 with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -2.3 Hz, GB = 0.5 (TD = 187500, SI = 524288)

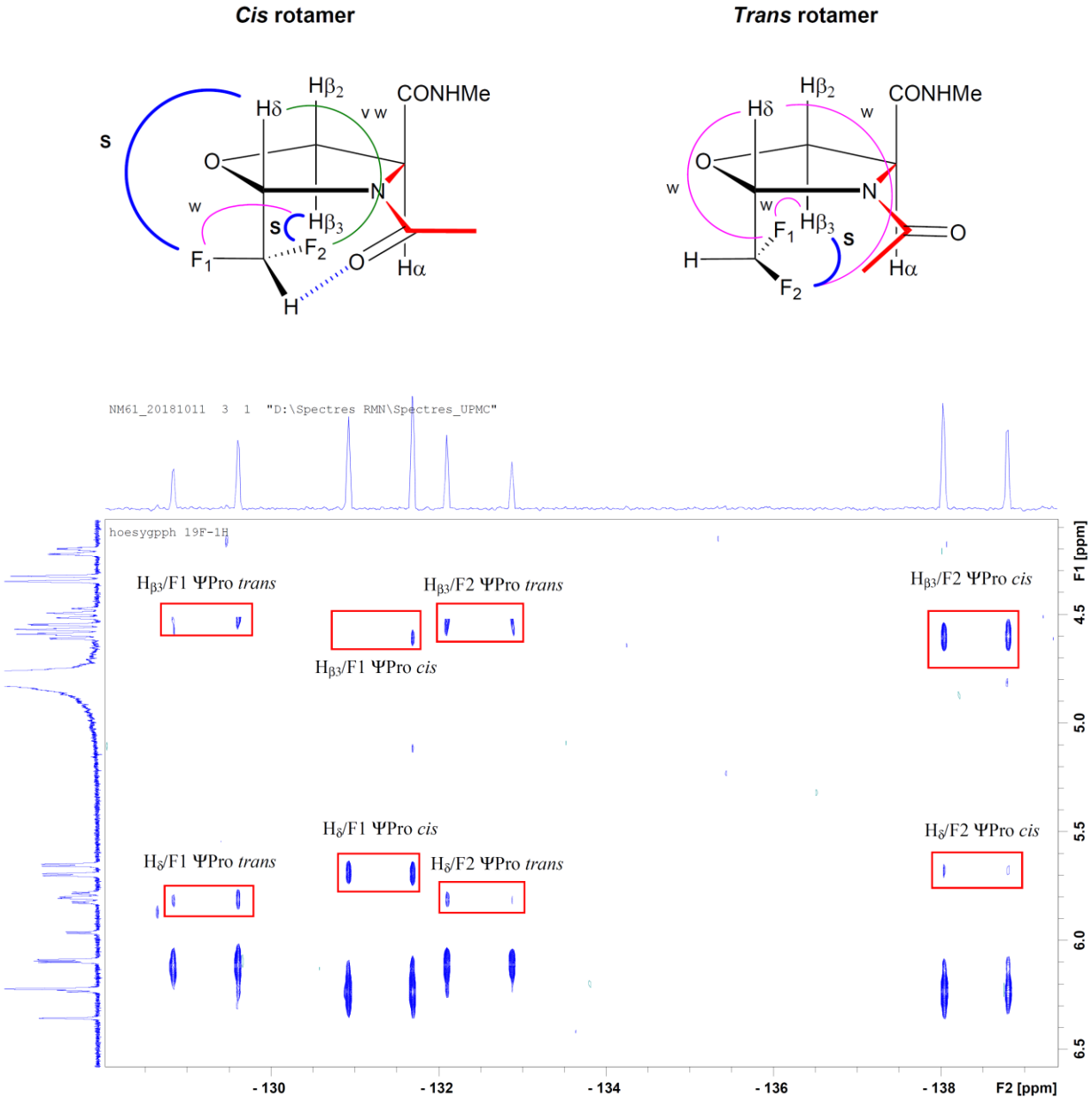
C. NOESY spectrum

The conformation of the ΨPro amide bonds has been unambiguously assigned using 2D NMR spectroscopy at 278 K in D_2O. *Cis* and *trans* conformers were in the slow exchange regime at this temperature. Dipolar interactions between neighboring protons were associated with negative cross peaks in the NOESY spectra. *Cis* conformers were characterized by strong CH_3(Acetyl)−H_α Ψpro correlations, while *trans* forms were assigned from CH_3(Acetyl)−H_δ Ψpro cross peaks.



NOESY NMR spectrum of (*S*,*S*)-**4** (20 mM) in phosphate buffer (50 mM, pH 7) at 278 K in D_2O (mixing time 800 ms)

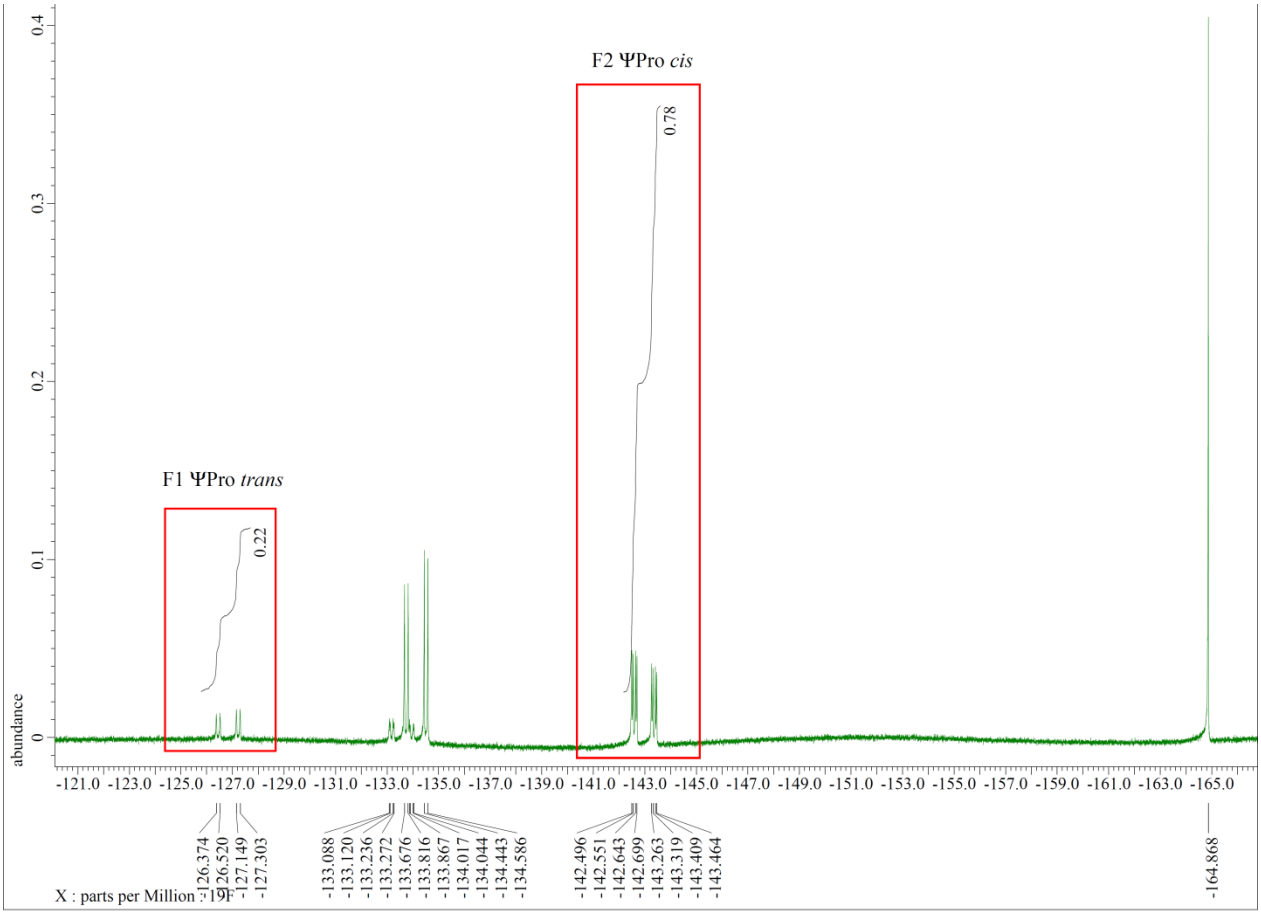
D. HOESY spectrum



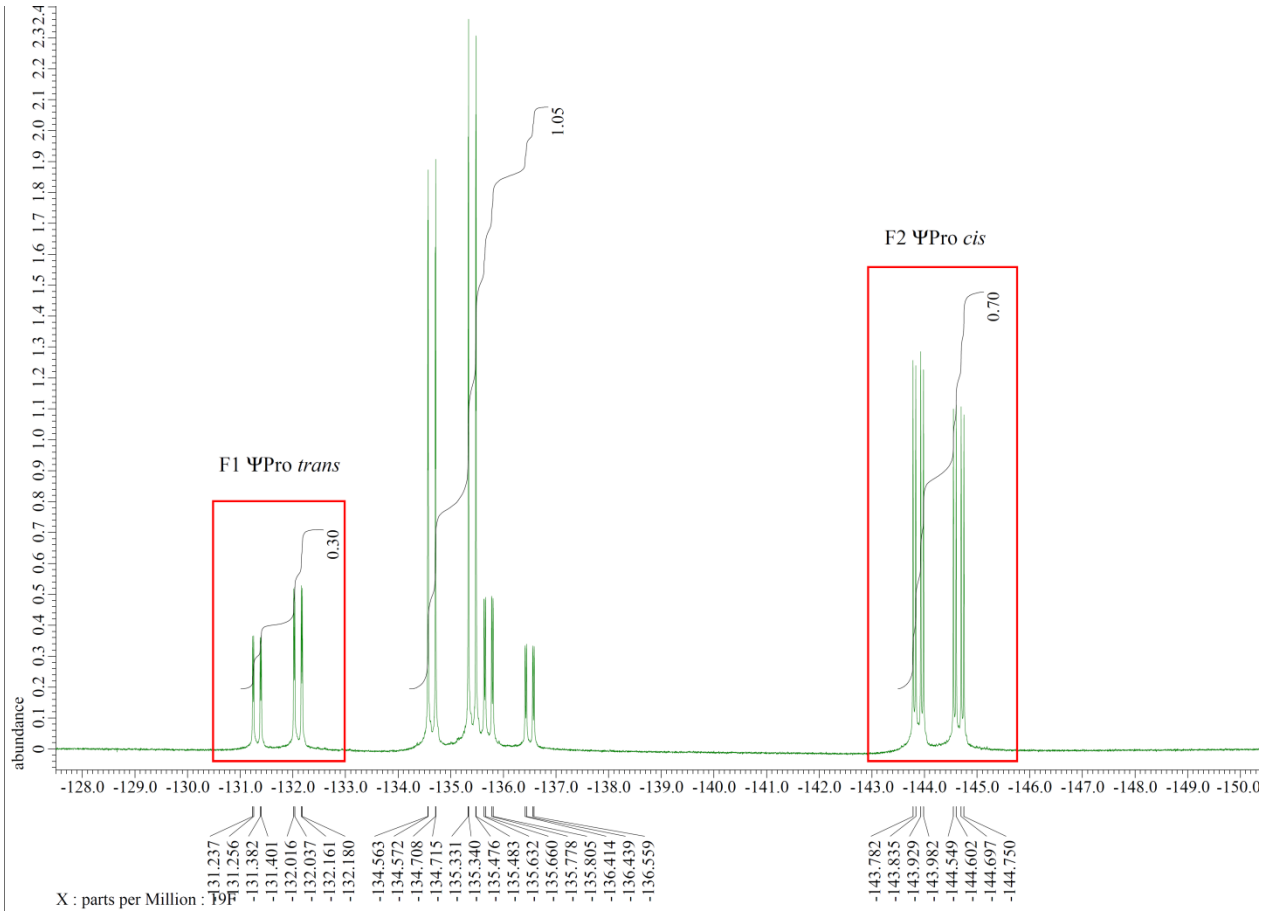
^{1}H-^{19}F HOESY NMR spectrum at 278 K in H_2O/D_2O 90/10

E. Cis/trans population

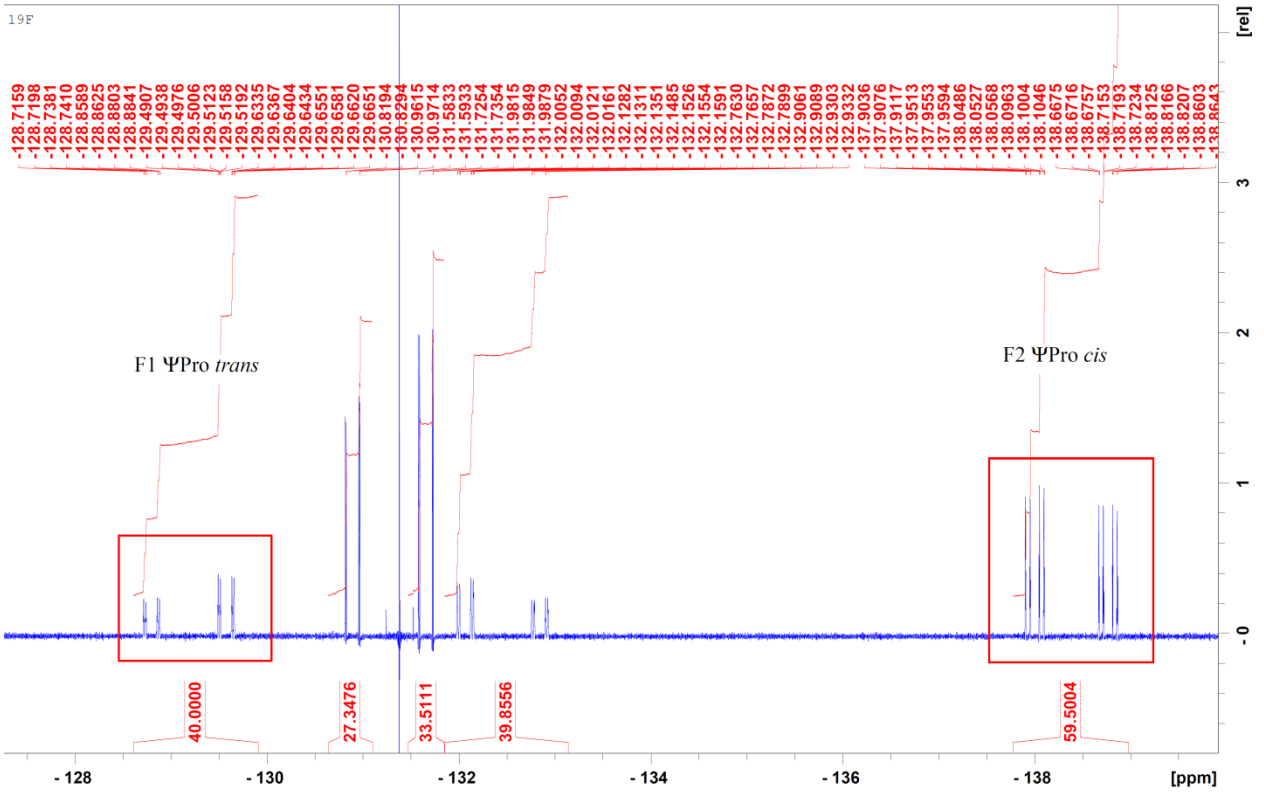
The *cis*/*trans* populations for a given solvent were quantified by the integration of ^{1}H and ^{19}F isolated resonances.



^{19}F NMR spectrum at 298 K in $CDCl_3$



^{19}F NMR spectrum at 293 K in CD_3OD

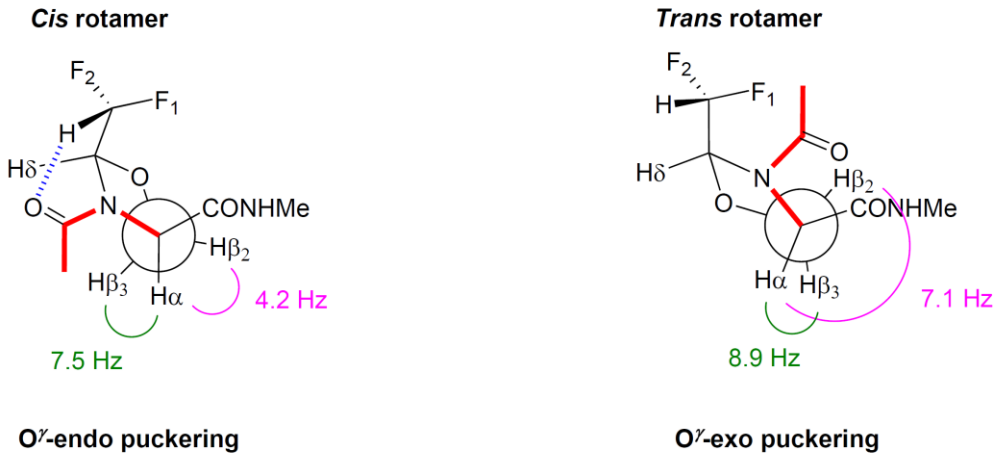


^{19}F NMR spectrum at 298 K in D_2O

6.2. NMR conformational assignment of (2*R*,4*S*)-*N*-Acetyl-2-difluoromethyloxazolidine-4-*N*-methylamide (*R*,*S*)-4

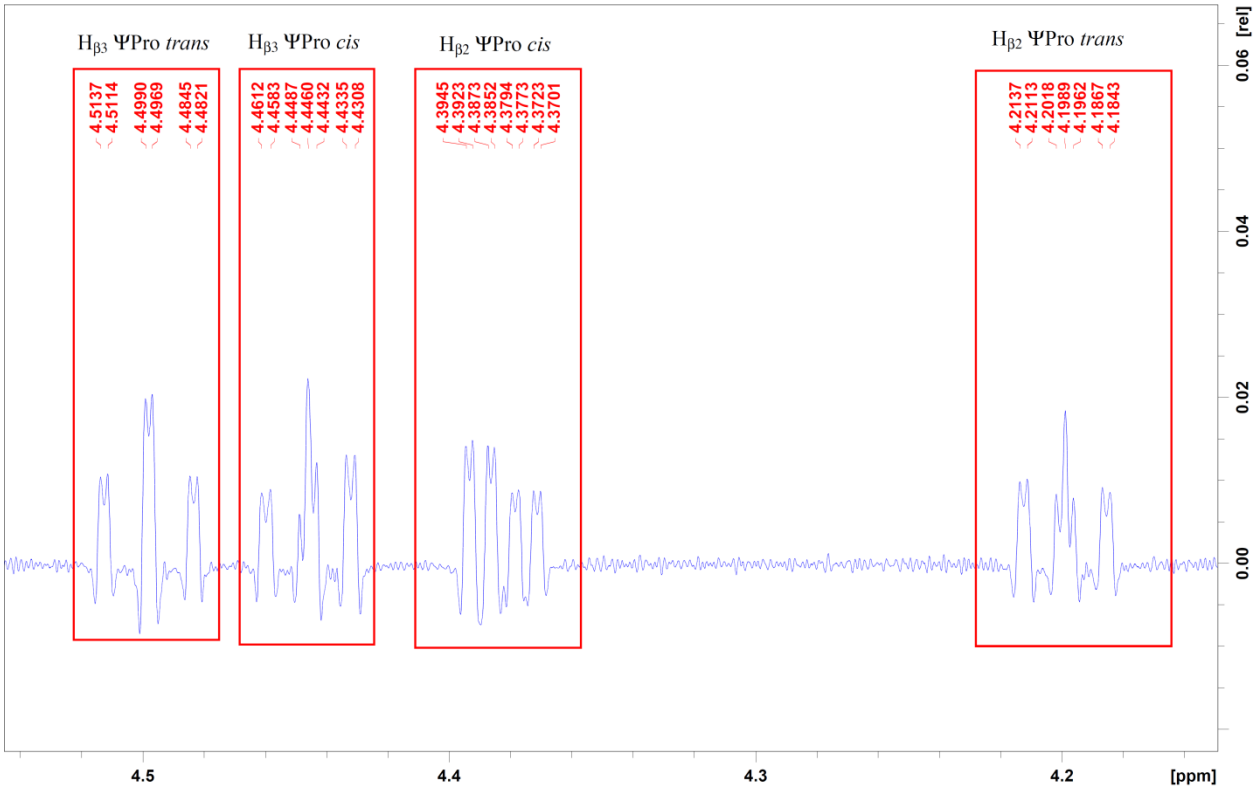
A. Puckering

The ring puckering was obtained following the methodology described above (see Part 6.2.A, p. S40).



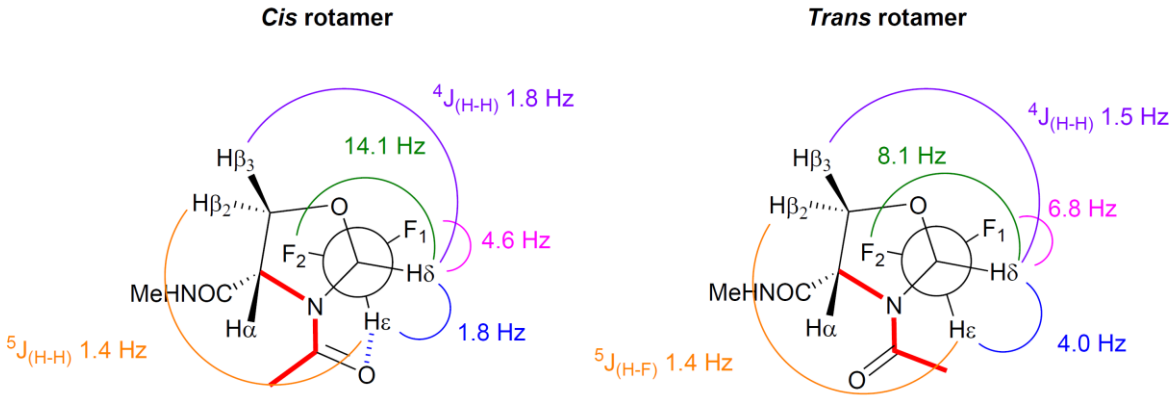
O$^{\gamma}$-endo puckering **O$^{\gamma}$-exo puckering**

Coupling constants from ^{1}H NMR 600 MHz in D_2O

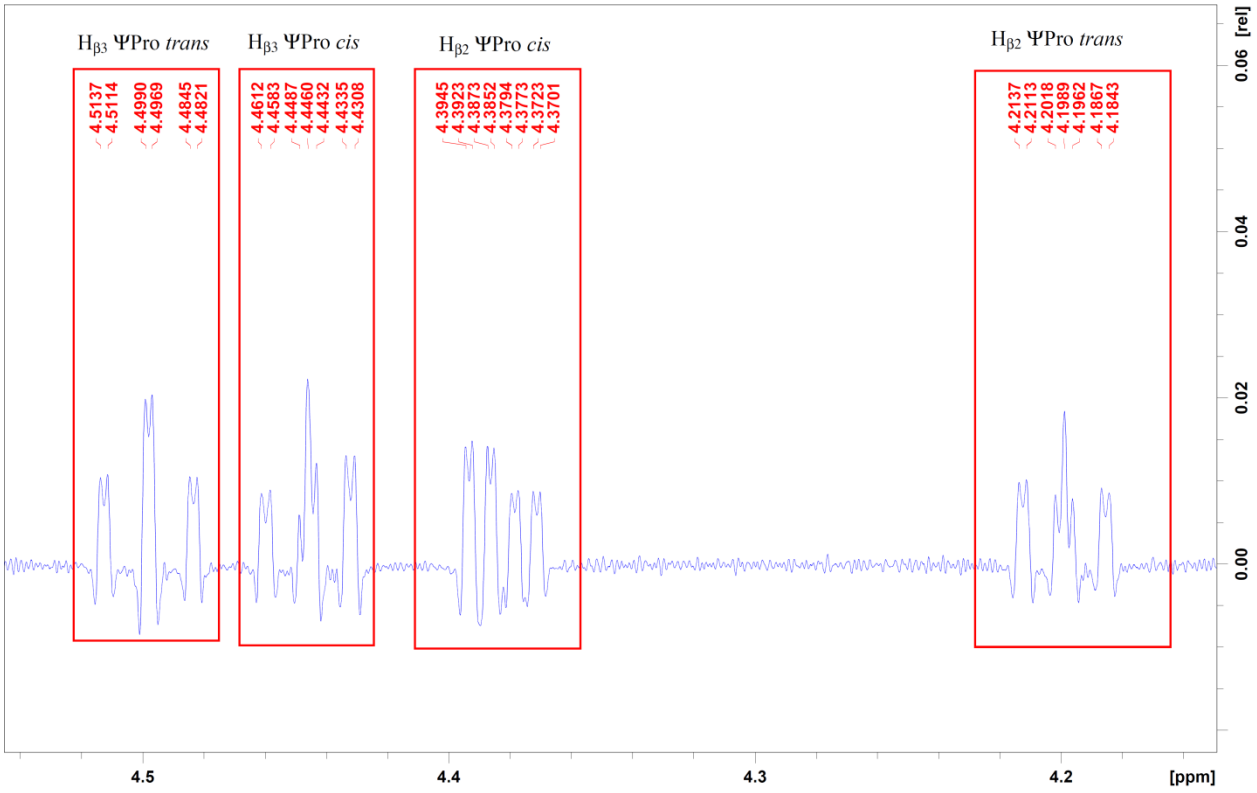


^{1}H-{^{19}F} NMR spectrum at 283 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -3.0 Hz, GB = 0.4 (TD = 32768, SI = 65536)

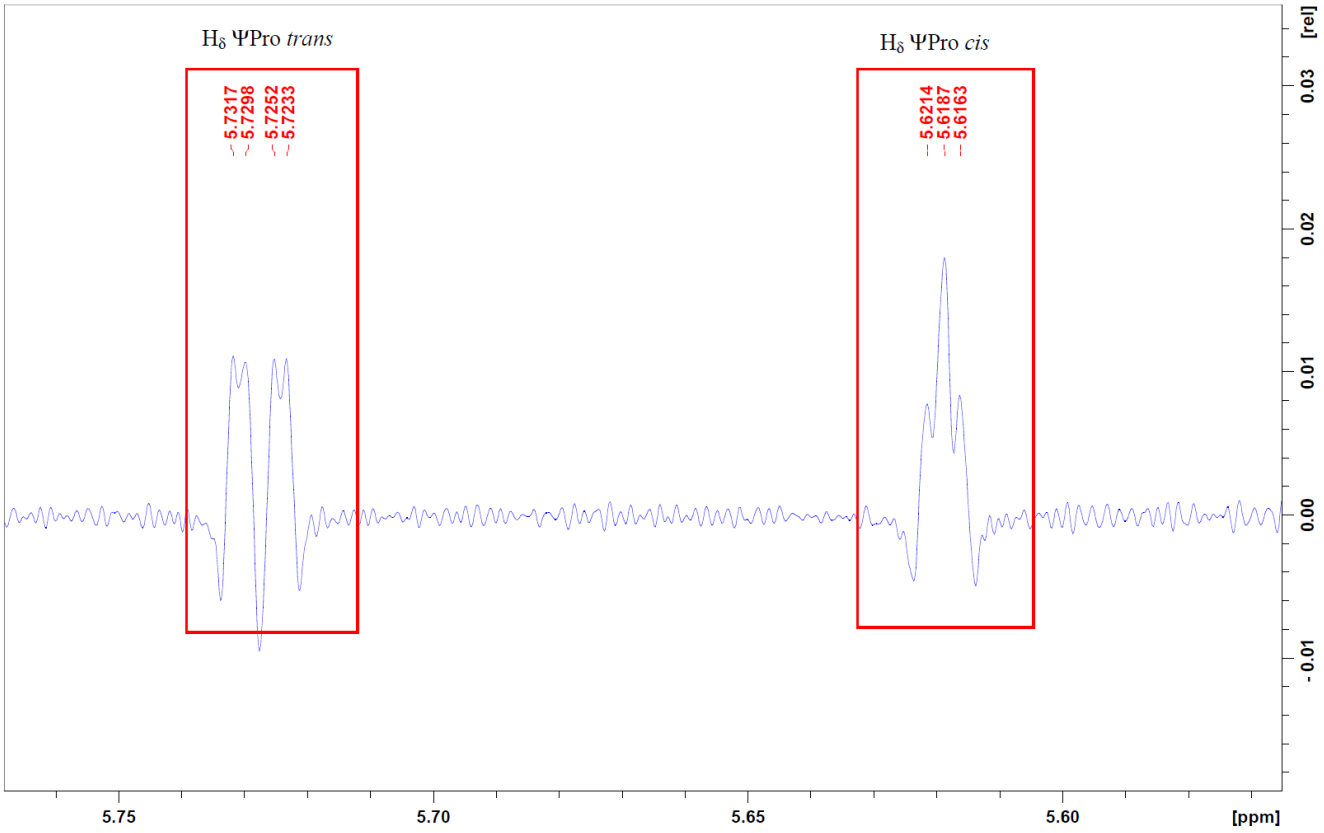
B. Coupling constants



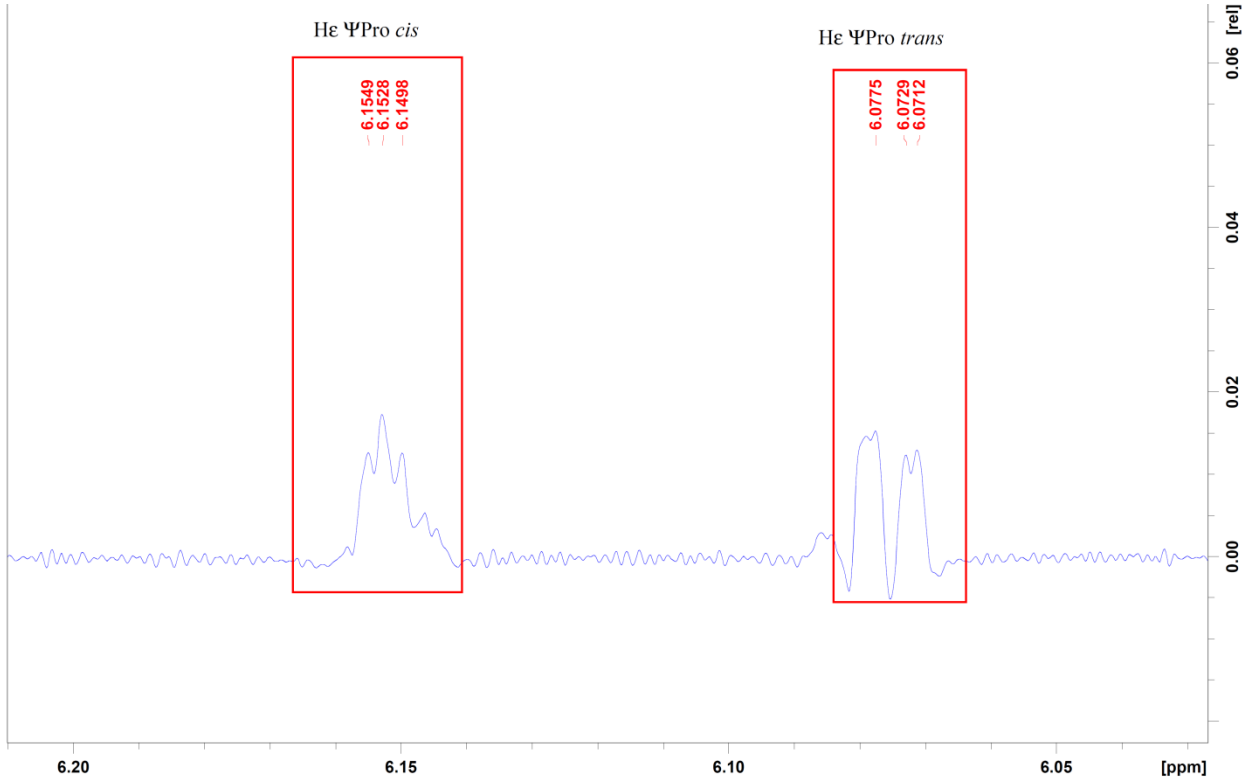
Coupling constants from ^{1}H NMR 600 MHz in D_2O



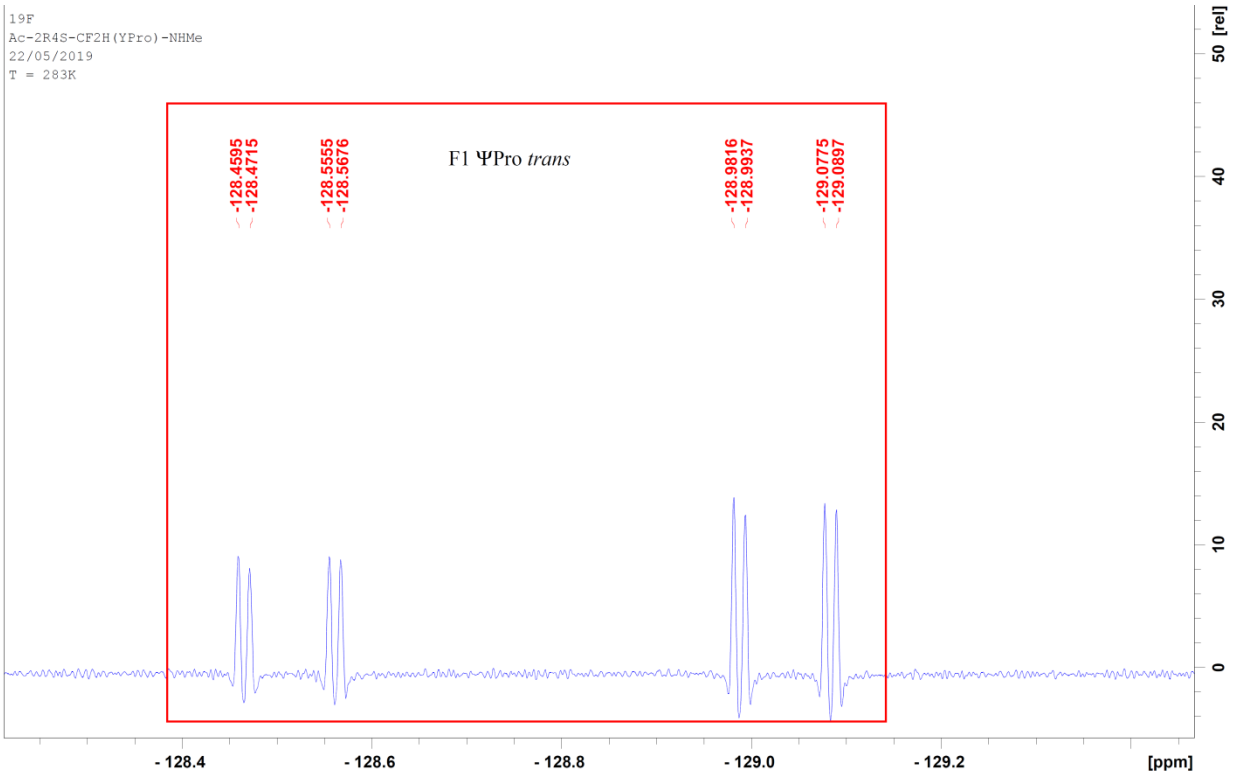
^{1}H-{^{19}F} NMR spectrum at 283 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -3.0 Hz, GB = 0.4 (TD = 32768, SI = 65536)

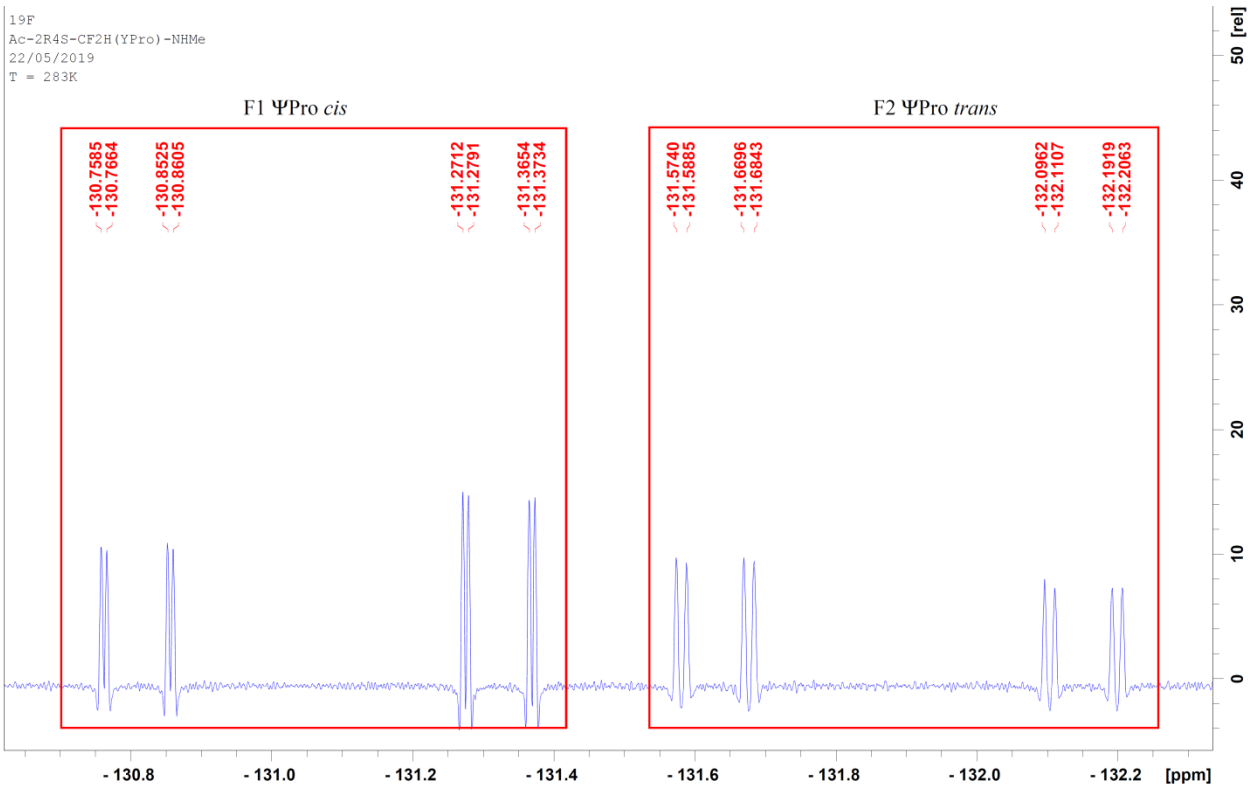


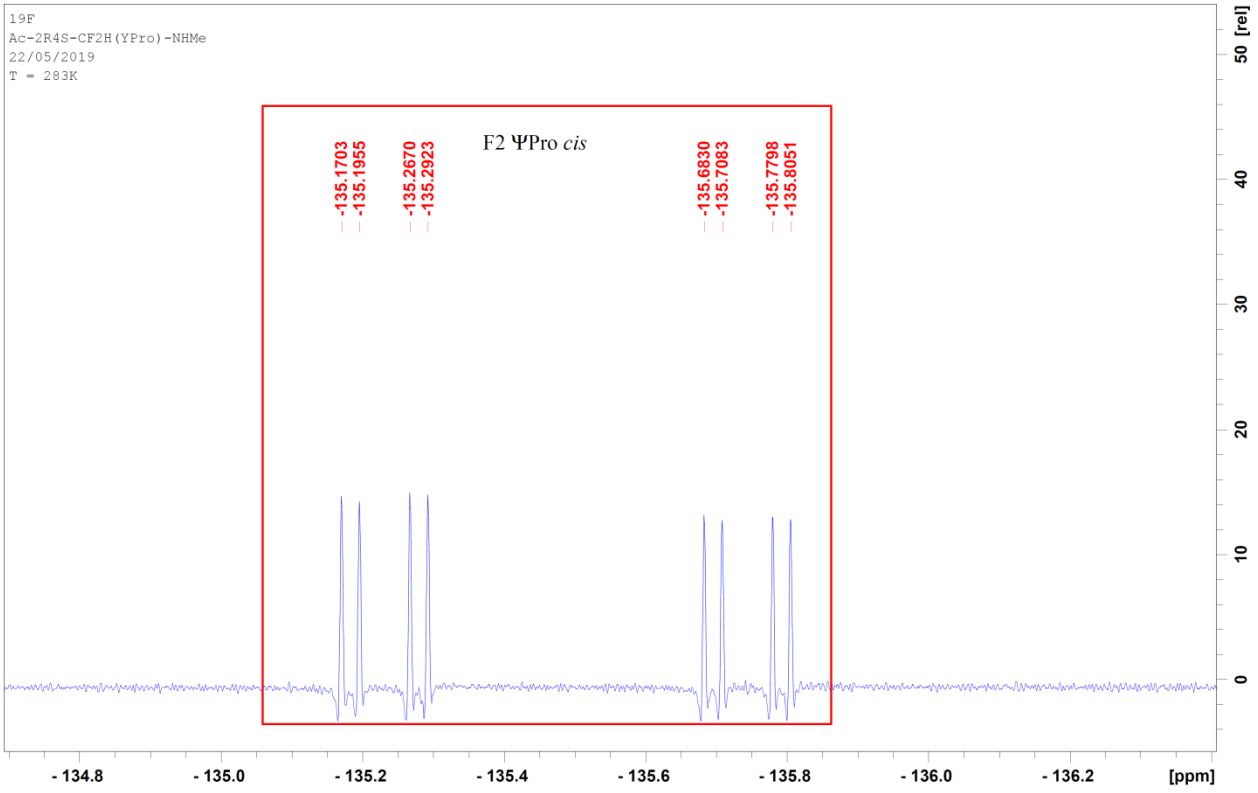
^{1}H-{^{19}F} NMR spectrum at 283 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -3.5 Hz, GB = 0.4 (TD = 32768, SI = 65536)



^{1}H-{^{19}F} NMR spectrum at 283 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -3.0 Hz, GB = 0.4 (TD = 32768, SI = 65536)


19F
Ac-2R4S-CF2H(YPro)-NHMe
22/05/2019
T = 283K
F1 ΨPro *trans*
-128.4595
-128.4715
-128.5555
-128.5676
-128.9816
-128.9937
-129.0775
-129.0897
50 [rel]
40
30
20
10
0
- 128.4
- 128.6
- 128.8
- 129.0
- 129.2
[ppm]

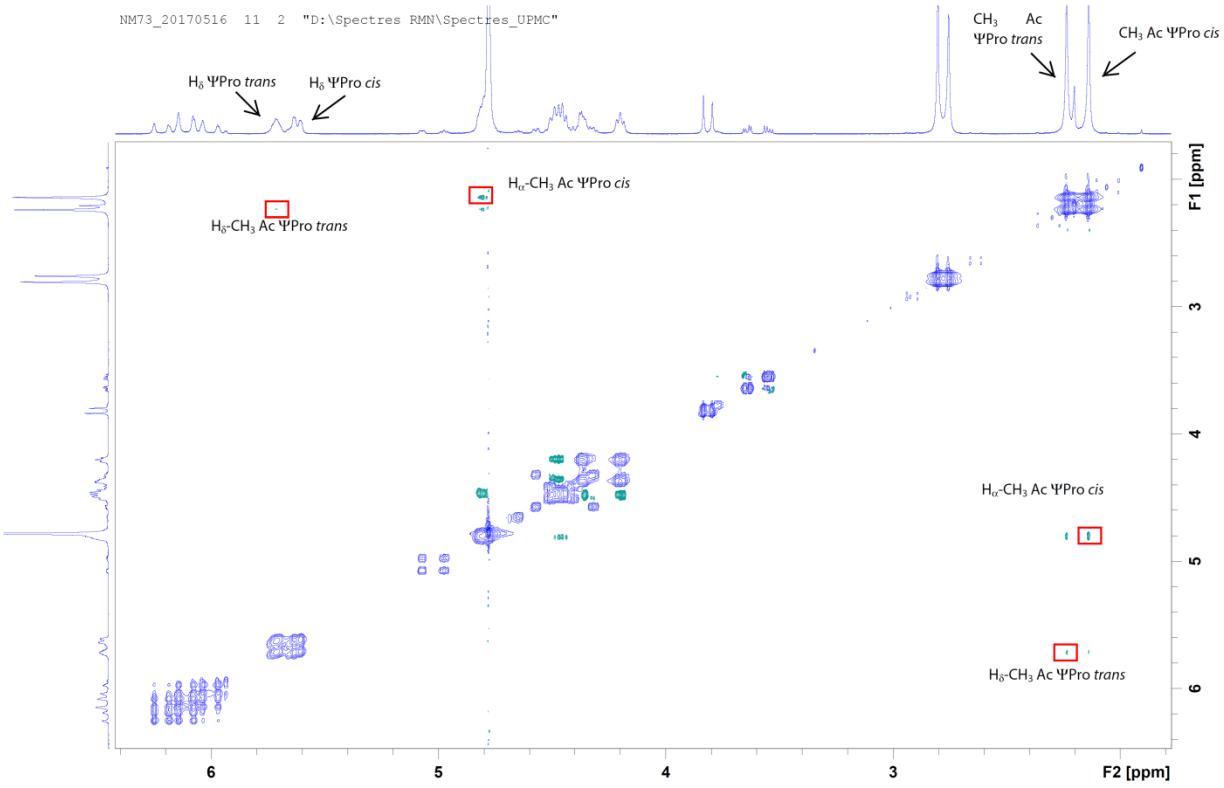

19F
Ac-2R4S-CF2H(YPro)-NHMe
22/05/2019
T = 283K
F1 ΨPro *cis*
F2 ΨPro *trans*
-130.7585
-130.7664
-130.8525
-130.8605
-131.2712
-131.2791
-131.3654
-131.3734
-131.5740
-131.5885
-131.6696
-131.6843
-132.0962
-132.1107
-132.1919
-132.2063
50 [rel]
40
30
20
10
0
- 130.8
- 131.0
- 131.2
- 131.4
- 131.6
- 131.8
- 132.0
- 132.2
[ppm]



^{19}F NMR spectra at 283 K in D_2O with Lorentz-Gauss apodization for resolution enhancement with corresponding parameters: LB = -5.0 Hz, GB = 0.5 (TD = 32768, SI = 65536)

C. NOESY spectrum

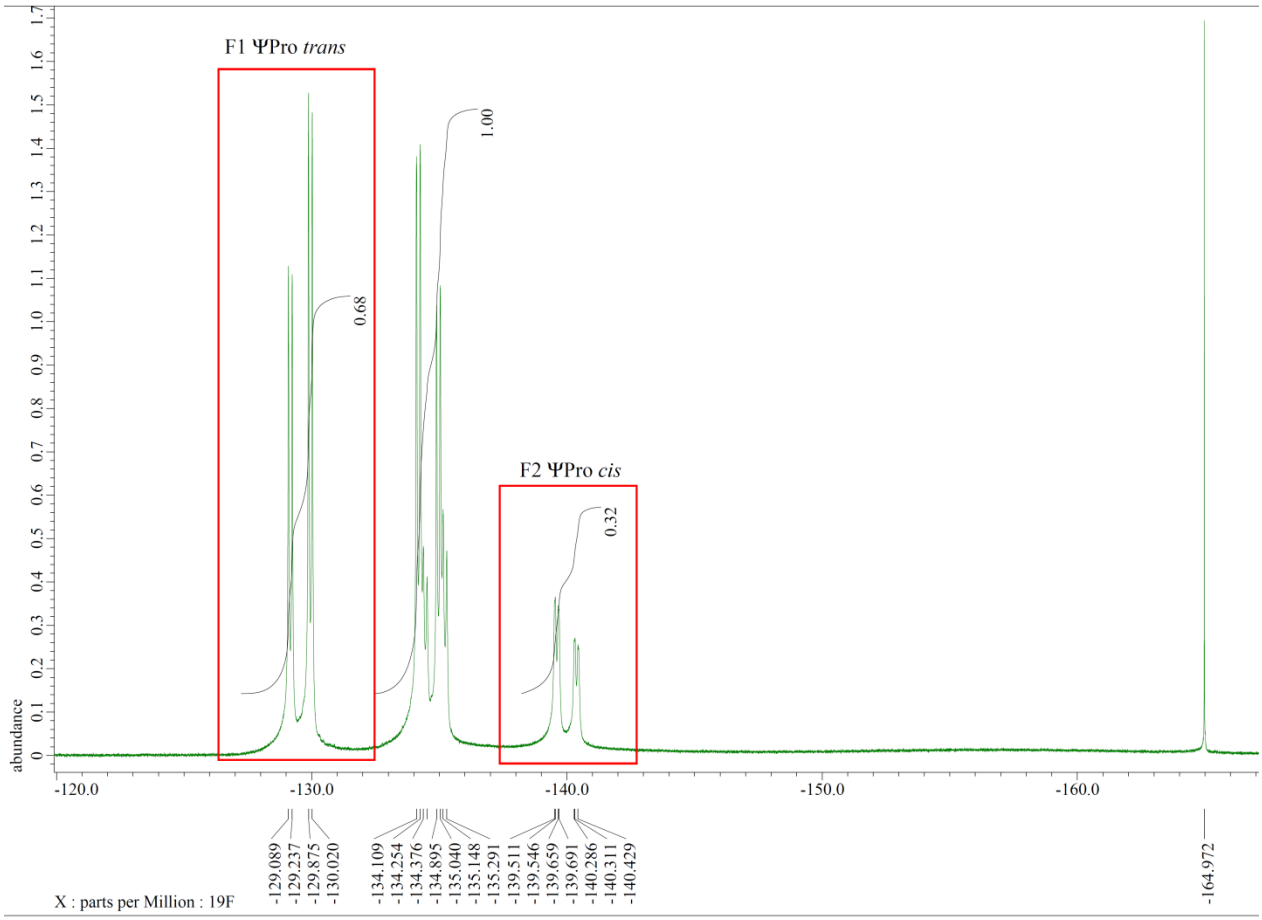
The conformation of the ΨPro amide bonds has been unambiguously assigned using 2D NMR spectroscopy at 278 K in D_2O. *Cis* and *trans* conformers were in the slow exchange regime at this temperature. Dipolar interactions between neighboring protons were associated with negative cross peaks. *Cis* conformers were characterized by strong CH_3(Acetyl)−Hα Ψpro correlations, while *trans* forms were assigned from CH_3(Acetyl)−H_δ Ψpro cross peaks.



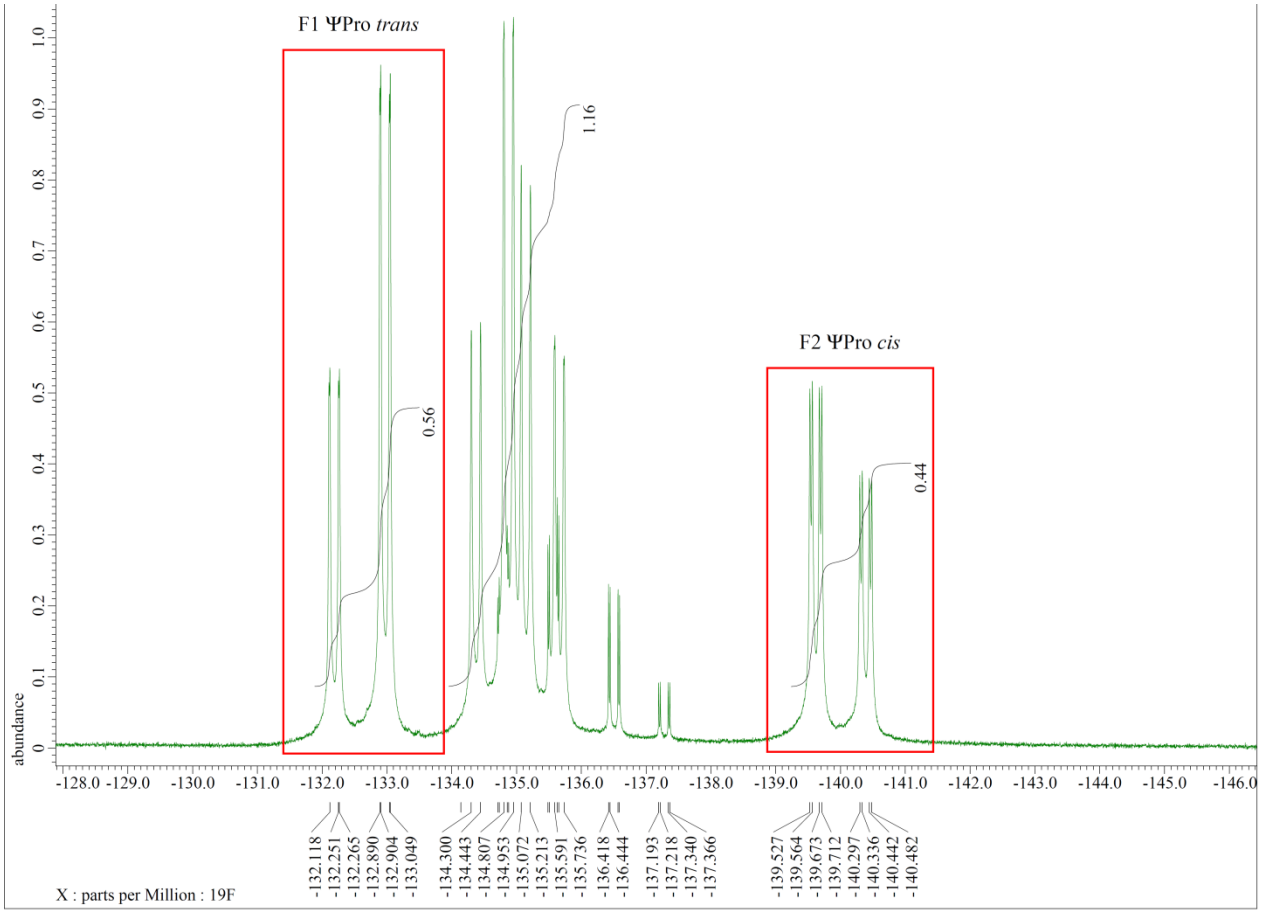
NOESY NMR spectrum of (*R*,*S*)-**4** (20 mM) in phosphate buffer (50 mM, pH 7) at 278 K in D_2O (mixing time 800 ms)

D. Cis/trans population

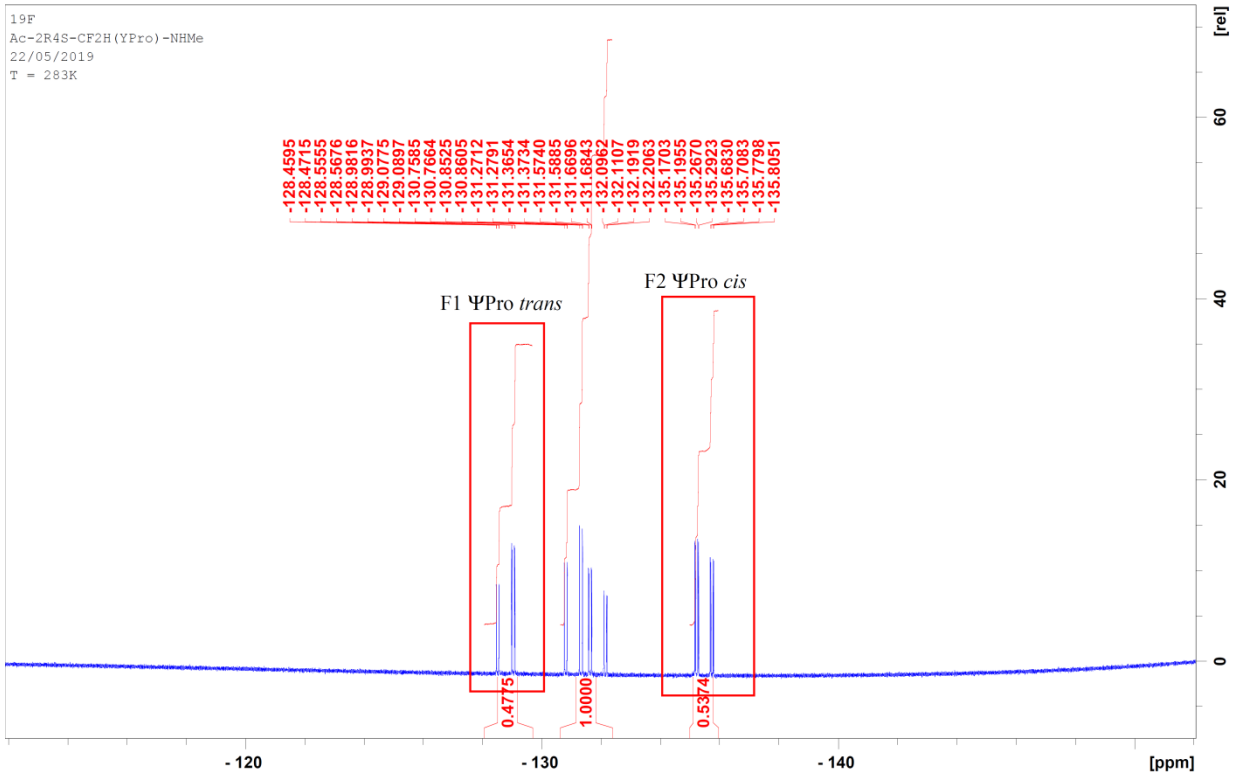
The *cis*/*trans* populations for a given solvent were quantified by the integration of ^{1}H and ^{19}F NMR of isolated resonances.



^{19}F NMR spectrum at 298 K in $CDCl_3$



^{19}F NMR spectrum at 293 K in CD_3OD



^{19}F NMR spectrum at 283 K in D_2O

7. Determination of the rotational barriers for *cis-trans* isomerisation

The coalescence temperature method was used to determine the rotational barriers for *cis*−*trans* isomerisation ($\Delta G^{\ddagger}_{c\text{-}t}$) by application of the Eyring equation (1).[4]

$$k_r = \frac{k_b T}{h} e^{\frac{-\Delta G^{\ddagger}}{RT}} \text{ (1)}$$

were :
- k_r is the exchange rate constant
- k_b is the Boltzman's constant
- h is the Planck's constant
- T is the temperature (°K)
- R is the gaz constant (8.3144 J/deg/mol)

$\Delta G^{\ddagger}_{c\text{-}t}$ and $\Delta G^{\ddagger}_{t\text{-}c}$ values were calculated from the exchange rate constant (k_r) and the coalescence temperature (Tc) using equation (2) and (3) respectively.

$$\Delta G^{\ddagger}_{c-t} = RT\left[23.760 + \ln\left(\frac{Tc}{k_{c-t}}\right)\right] \text{ (1)}$$

$$\Delta G^{\ddagger}_{t-c} = RT\left[23.760 + \ln\left(\frac{Tc}{k_{t-c}}\right)\right] \text{ (2)}$$

were :

- $k_{c\text{-}t}$ and $k_{t\text{-}c}$ represent the rate constant for the isomerisation process, from the *cis* to the *trans* isomer and the *trans* to *cis* isomer respectively. Values are calculated from equation (3) and (4)

$$k_{c-t} = (1 - \Delta p) \times k_c \text{ (3)}$$

$$k_{c-t} = (1 + \Delta p) \times k_c \text{ (4)}$$

were :
- Δp represents the absolute difference between *cis* and *trans* population

- k_c represents the rate exchange at the coalescence temperature and is given by equation (5)

$$k_c = \pi \Delta \nu / \sqrt{2} \text{ (5)}$$

- $\Delta \nu$ represents the frequency difference (in Hz) of a given nucleus between the *cis* and the *trans* isomers in the slow-exchange regime.

(*S*,*S*)-**4** (Δp = 0.20)

nucleus	δ *trans* (ppm)	δ *cis* (ppm)	$\Delta \nu$ (Hz)	*Tc* (K)	$\Delta G^{\ddagger}_{c\text{-}t}$ (kcal.mol^{-1})	$\Delta G^{\ddagger}_{t\text{-}c}$ (kcal.mol^{-1})
N**H**	8.32	8.48	80	318	15.45	15.19
H$^{\delta}$	5.81	5.67	70	>318	nd	nd
C**H**$_3$-NMe	2.76	2.81	25	>318	nd	nd

nd : not determined

(*R*,*S*)-**4** (Δp = 0.06)

nucleus	δ *trans* (ppm)	δ *cis* (ppm)	$\Delta \nu$ (Hz)	*Tc* (K)	$\Delta G^{\ddagger}_{c\text{-}t}$ (kcal.mol^{-1})	$\Delta G^{\ddagger}_{t\text{-}c}$ (kcal.mol^{-1})
N**H**	8.42	8.23	95	323	15.55	15.47
H$^{\delta}$	5.73	5.61	60	318	15.73	15.65
C**H**$_3$-NMe	2.76	2.80	70	318	16.42	16.35
				Mean	15.90	15.82

[4] K. D. Zimmer, R. Shoemaker and R. R. Ruminski, *Inorg. Chim. Acta,* 2006, **359**, 1478.