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Supporting Information

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Supporting Information

Synthesis of Trifluoromethyl-Substituted Proline Analogues as ^{19}F-NMR Labels for Peptides in Polyproline II conformation

Pavel K. Mykhailiuk, Sergii Afonin, Gennady V. Palamarchuk, Oleg V. Shishkin, Anne S. Ulrich and Igor V. Komarov

Abbreviations

TMS: tetramethylsilane;

TIS: triisopropylsilane;

Fmoc: 9-fluorenylmethyloxycarbonyl;

Boc: *t*-butoxycarbonyl;

DIEA: *N,N*-diisopropylethylamine;

DMF: *N*,*N*-dimethylformamide;

DIC: diisopropylcarbodiimide;

6-ClHOBt: 6-chloro-*N*-hydroxybenzotriazole;

TFA: trifluoroacetic acid;

PyBop: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate;

TBTU: O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate;

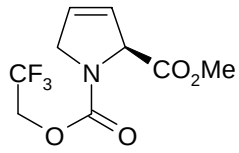
Experimental

General: Solvents were purified according to standard procedures. Alkenes **8**, **11** have been synthesised from 4-hydroxyproline following procedure described in the literature.[1] All other materials were purchased from Acros, Merck and Fluka. Melting points are uncorrected. Analytical TLC was perfomed using Polychrom SI F_{254} plates. Column chromatography was performed using Kieselgel Merck 60 (230-400 mesh) as the stationary phase. Peptides were analyzed by RP-HPLC instrument (Jasco, Japan) equipped with a diode-array detector and an analytical Vydac C18-column (4.6 mm × 250 mm). ^{1}H-, ^{13}C- and ^{19}F-NMR spectra were recorded either on a Varian Unity Plus 400 spectrometer (at 400, 101 and 377 MHz respectively) or on a Bruker Avance 500 spectrometer (at 500 MHz, 125 and 470 MHz). Chemical shifts are reported in ppm downfield from TMS (^{1}H, ^{13}C) or C_6F_6 (^{19}F) as internal standards. IR spectra were obtained on a Hewlett Packard UR 20 spectrometer. The ν_{max} (cm^{-1}) values of the IR spectra are given for the main absorbtion bands. Mass spectra were recorded either on an Agilent 1100 LCMSD SL instrument by chemical ionization (CI), or on a Bruker Biflex IV instrument (MALDI-TOF). MALDI samples were co-crystallized with a matrix of 3,5-dihydroxy-benzoic acid from acetonitrile/water solutions onto a stainless steel target. Elemental analysis: Mikroanalytisches Labor, Institute of Organic

Chemistry, University of Karlsruhe. Optical rotation values were measured on a PerkinElmer 341 polarimeter.

1) Synthesis of amino acids

2-methyl 1-(2,2,2-trifluoroethyl) 2,5-dihydro-1*H*-pyrrole-1,2-dicarboxylate (9)



An excess of trifluoromethyldiazomethane, obtained in a generator flask by reaction of 3,3,3-trifluoroethylamine hydrochloride with sodium nitrite, was gradually blown off by an inert gas through a drying tube ($MgSO_4$) into a vessel equipped with a condenser. The vessel contained a stirring solution of **8** (1.00 g, 4.41 mmol) and anhydrous CuCl (500 mg) in hexane. The mixture Ar/CF_3CHN_2 was blowing through an inlet in such a way, that it passed through a stirring solution. After 5 eq of $CF_3CH_2NH_2$*HCl had been used, the reaction was stopped; the conversion of **8** was 30% according to NMR of the reaction mixture. The mixture was diluted with CH_2Cl_2 and filtered. The filtrate was concentrated and the residue was purified by flash chromatography. Elution with hexane/EtOAc = 10/1 afforded starting material **8** first (R*f* = 0.40 in hexane/EtOAc = 4/1) and then **9** (290 mg, 1.15 mmol, 26%) as a colorless viscous oil (R*f* = 0.35 in hexane/EtOAc = 4/1).

^{1}H NMR (400 MHz, $CDCl_3$, rotamers), δ: 6.02 (m, 1H, 3-C*H*); 5.79 (m, 1H, 4-C*H*); 5.03 (m, 1H, 2-C*H*); 4.60 (m, 1H, OC*H*CF_3); 4.37 (m, 3H, OC*H*CF_3 + 5-CH_2); 3.77, 3.74 (two s, 3H, OCH_3).

^{19}F NMR (377 MHz, $CDCl_3$, rotamers), δ: 87.70, 87.52 (two t, ^3J(F, H) = 7.5 Hz, CF_3).

^{13}C NMR (125 MHz, $CDCl_3$, rotamers), δ: 170.02, 169.81 (two s, *C*$OOCH_3$); 152.52, 152.01 (two s, N*C*O), 129.00, 128.78 (two s, 3-*C*H); 125.22, 125.05 (two s, 4-*C*H); 130.8 (q, ^1J(C, F) = 267.1 Hz, *C*F_3); 66.74, 66.22 (two s, 2-*C*H); 61.49, 61.36 (two q, ^2J(C, F) = 37.8 and 35.2 Hz, O*C*H_2CF_3); 54.42, 53.53 (two s, 5-*C*H_2); 52.35 (s, O*C*H_3).

IR (neat): ν = 1757 (C=O in $COOCH_3$), 1735 (C=O in $COOCH_2CF_3$).

MS (CI): *m/z* (%): 254 (100) $[M+1]^+$.

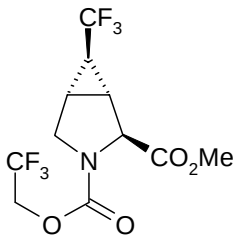
Anal. calcd. for $C_9H_{10}F_3NO_4$: C, 42.70; H, 3.98; N, 5.53. Found: C, 42.23; H, 3.60; N, 5.19.

2-methyl 3-(2,2,2-trifluoroethyl) (1*R*,2*S*,5*S,6R*)-6-(trifluoromethyl)-3-azabicyclo[3.1.0] hexane-2,3-dicarboxylate (10a),

2-methyl 3-(2,2,2-trifluoroethyl) (1*S*,2*S*,5*R,6S*)-6-(trifluoromethyl)-3-azabicyclo[3.1.0] hexane-2,3-dicarboxylate (10b)

An excess of CF_3CHN_2 (obtained as described above) was gradually blown off by an inert gas through a drying tube ($MgSO_4$) into a vessel containing a stirring mixture of **8** (4.00 g, 17.62 mmol) and CuOTf*0.5C_6H_6 (500 mg). The black tarry oil formed, which consisted of **10a**, **10b** and side products,

was dissolved in CH_2Cl_2 and triturated with acidic (pH ~ 5) 5% $KMnO_4$ solution (to remove compounds possessing C=C double bond). Water phase was separated and washed twice with CH_2Cl_2. Organic phases were combined, dried over $MgSO_4$, and evaporated. The residue was dissolved in CH_2Cl_2/TFA (1/4) mixture and stirred for 2 hours (to remove compounds possessing Boc-group). Finally, the solution was evaporated and the residue was purified by flash chromatography. Elution with hexane/EtOAc = 20/1 afforded **10a** first (590 mg, 1.76 mmol, 10%) as a yellowish oil. R*f* = 0.5 in hexane/EtOAc = 5/1.



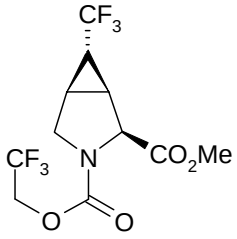
^{1}H NMR (400 MHz, $CDCl_3$, rotamers), δ: 4.46 (m, 1H, OC*H*CF_3); 4.43, 4.42 (two s, 1H, 2-C*H*); 4.30 (m, 1H, OC*H*CF_3); 3.76 (m, 1H, 4-C*H*); 3.74-3.72 (two s, 3H, OCH_3); 3.71, 3.68 (two d, *J* = 4.0 Hz, 1H, 4-C*H*); 2.13 (td, *J* = 8.0, 3.2 Hz, 1H, 1-C*H*); 2.02 (m, 1H, 5-C*H*); 1.52 (m, 1H, 6-C*H*).

^{19}F NMR (377 MHz, $CDCl_3$, rotamers), δ: 96.54, 96.50 (two d, ^3J(F, H) = 7.5 Hz, 3F, CHCF_3); 87.54, 87.35 (two t, ^3J(F, H) = 7.5 Hz, 3F, OCH_2CF_3).

IR (neat): ν = 1758 (C=O in $COOCH_3$), 1741 (C=O in $COOCH_2CF_3$).

MS (CI): *m/z* (%): 336 (100) $[M+1]^+$.

Further elution gave the isomer **10b** (240 mg, 0.88 mmol, 5%) as a yellowish oil. R*f* = 0.4 in hexane/EtOAc = 5/1.



^{1}H NMR (400 MHz, $CDCl_3$, rotamers), δ: 4.53 (m, 1H, OC*H*CF_3); 4.43, 4.40 (two d, *J* = 4.8 Hz, 1H, 2-C*H*); 4.30 (m, 1H, OC*H*CF_3); 3.81 (m, 1H, 4-C*H*); 3.77, 3.75 (two s, 3H, OCH_3); 3.69 (m, 1H, 4-C*H*); 2.26 (m, 1H, 1-C*H*); 2.07 (m, 1H, 5-C*H*); 2.20 (m, 1H, 6-C*H*).

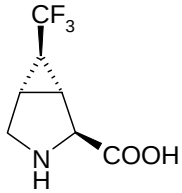
^{19}F NMR (377 MHz, $CDCl_3$, rotamers), δ: 96.02 (d, ^3J(F, H) = 7.5 Hz, 3F, CHCF_3), 87.67, 87.57 (two t, ^3J(F, H) = 7.5 Hz, 3F, OCH_2CF_3).

IR (neat): ν = 1760 (C=O in $COOCH_3$), 1735 (C=O in $COOCH_2CF_3$).

MS (CI): *m/z* (%): 336 (100) $[M+1]^+$.

(1*R*,2*S*,5S,6R)-6-(trifluoromethyl)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (6a)

A solution of **10a** (500 mg, 1.49 mmol) in aqueous HBr (48%, 10 ml) was refluxed for 6 h and evaporated. The black tarry residue was redissolved in H_2O (~1 ml), neutralized with aqueous NaOH (0.3M) to pH ~ 9 and submitted to an ion-exchange chromatography column (Dowex® 50 × 400). Elution with water and then with aqueous pyridine (10%) afforded **6a** (145 mg, 0.75 mmol, 50%) as a yellowish amorphous solid.



$[\alpha]_D^{20}$ = -23.3 (*c* = 0.167 g cm^{-3} in MeOH).

^{1}H NMR (400 MHz, D_2O), δ: 4.32 (s, 1H, 2-C*H*); 3.69 (dd, J = 12.0, 4.0 Hz, 1H, 4-C*H*); 3.53 (d, J = 12.0 Hz, 1H, 4-C*H*); 2.51 (dd, 1H, J = 7.8, 3.6 Hz, 1-C*H*); 2.35 (ddd, 1H, J = 7.8, 3.6, 4.0 Hz, 5-C*H*); 1.81 (m, 1H, 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD), δ: 95.48 (d, ^3J(H, F) = 3.8 Hz, CF_3).

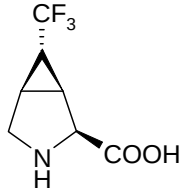
IR (KBr): ν = 1617 (ν_{as} COO^-), 1386 (ν_s COO^-).

MS (CI): *m/z* (%): 254 (100) $[M+1]^+$.

Anal. calcd. for $C_7H_8F_3NO_2$: C, 43.09; H, 4.13; N, 7.18. Found: C, 42.75; H, 3.75; N, 6.80.

(1*S*,2*S*,5*R*,6*S*)-6-(trifluoromethyl)-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (6b)

6b was synthesized from **10b** in the same way as it was described for **6a** (60% yield).



^{1}H NMR (400 MHz, D_2O), δ: 4.28 (d, J = 4.0 Hz, 1H, 2-C*H*); 3.58 (d, J = 12.0 Hz, 1H, 4-C*H*); 3.53 (dd, J = 12.0, 4.0 Hz, 1H, 4-C*H*); 2.50 (dt, 1H, J = 7.8, 4.0, Hz, 1-C*H*); 2.25 (dt, 1H, J = 7.8, 4.0 Hz, 5-C*H*); 1.93 (m, 1H, 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD), δ: 95.49 (d, ^3J(H, F) = 3.8 Hz, CF_3).

^{13}C NMR (125 MHz, CD_3OD), δ: 169.60 (s, *C*OOH); 124.94 (q, ^1J(C, F) = 270.4 Hz, *C*F_3); 61.94 (s, 2-*C*H); 46.50 (s, 4-*C*H_2); 22.79 (s, 1-*C*H); 19.57 (s, 5-*C*H); 19.03 (q, ^2J(C, F) = 36.5 Hz, 6-*C*H).

IR (KBr): ν = 1620 (ν_{as} COO^-), 1391 (ν_s COO^-).

MS (CI): *m/z* (%): 196 (100) $[M+1]^+$.

Anal. calcd. for $C_7H_8F_3NO_2$: C, 43.09; H, 4.13; N, 7.18. Found: C, 42.77; H, 3.82; N, 6.97.

2-*tert*-butyl 3-methyl (1*R*,3*S*,5*R*,6*R*)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-2,3-dicarboxylate (12a),

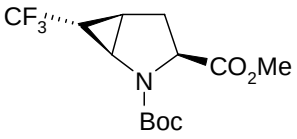
2-*tert*-butyl 3-methyl (1*S*,3*S*,5*S*,6*S*)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-2,3-dicarboxylate (12b),

2-*tert*-butyl 3-methyl (1*S*,3*S*,5*S*,6*R*)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-2,3-dicarboxylate (12c),

3-methyl 2-(2,2,2-trifluoroethyl) (1*S*,3S,5*S*,6*S*)-6-(trifluoromethyl)-2-azabicyclo[3.1.0] hexane-2,3-dicarboxylate (13)

CF_3CHN_2 (obtained as described above) was gradually blown off by an inert gas from the generator flask and passed through a drying tube ($MgSO_4$) into a vessel containing a stirring mixture of **11** (4.00 g, 17.62 mmol) and anhydrous CuCl (500 mg). The reaction was monitored by means of ^{1}H-, ^{19}F-NMR and HPLC. After the starting material had been disappeared, the trifluoromethyldiazomethane bubbling was immediately stopped. The reaction mixture was diluted with CH_2Cl_2, filtered, and evaporated. The oily residue was submitted to flash chromatography. Elution with hexane/EtOAc = 20/1 produced **12a** (1.46 g,

4.75 mmol, 27%) first as a colorless viscous oil. R*f* = 0.50 in hexane/EtOAc = 5/1. $[\alpha]_D^{20}$ = +11.2 (*c* = 0.67 g cm^{-3} in MeOH).



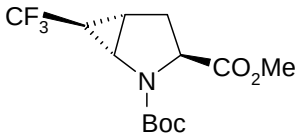
^{1}H NMR (400 MHz, $CDCl_3$, rotamers), *δ*: 4.42 (two dd, *J* = 11.6, 3.2 Hz, 1H, 3-C*H*); 3.78, 3.62 (two dd, *J* = 7.2, 1.6 Hz, 1H, 1-C*H*); 3.72, 3.71 (two s, OCH_3); 2.64 (m, 1H, 4-C*H*); 2.23-2.08 (m, 2H, 4-C*H,* 6-C*H*); 1.90 (m, 1H, 5-C*H*); 1.47, 1.38 (two s, 9H, C(CH_3)$_3$).

^{19}F NMR (377 MHz, $CDCl_3$, rotamers), *δ*: 97.04, 96.73 (two d, ^3J(H, F) = 7.5 Hz, CF_3).

^{13}C NMR (125 MHz, $CDCl_3$, rotamers), *δ*: 173.65, 173.51 (two s, *C*OOCH$_3$); 153.99, 153.38 (two s, N*C*O); 124.22 (two q, ^1J(C, F) = 270.4 Hz, *C*F$_3$); 81.14, 81.03 (two s, O*C*(CH$_3$)$_3$); 59.53, 59.18 (two s, 3-*C*H); 52.50, 52.36 (two s, O*C*H$_3$); 39.72 (two overlapped q, ^3J(C, F) = 3.8 Hz, 1-*C*H); 30.82, 29.84 (two s, *C*H$_2$); 28.29, 28.20 (two s, C(*C*H$_3$)$_3$); 25.95 (two overlapped q, ^2J(C, F) = 36.5 Hz, 6-*C*H); 18.94, 18.83 (two overlapped q, ^3J(C, F) = 3.8 Hz, 5-*C*H).

IR (neat): *ν* = 1744 (C=O in COOCH$_3$), 1711 (*ν* C=O in COOC(CH$_3$)$_3$).

MS (CI): *m/z* (%): 210 (100) [M-Boc+2]$^+$. Anal. calcd. for $C_{13}H_{18}F_3NO_4$: C, 50.49; H, 5.87; N, 4.53. Found: C, 50.33; H, 5.49; N, 4.23.



Further elution afforded **12b** (1.31 g, 4.23 mmol, 22 %) as a white solid. Crystallization from cyclohexane gave crystals suitable for an X-ray analysis.

R*f* = 0.40 in hexane/EtOAc = 5/1. M.p. 86-87ºC. $[\alpha]_D^{20}$ = -135.2 (*c* = 0.55 g cm^{-3} in MeOH).

^{1}H NMR (400 MHz, $CDCl_3$, rotamers), *δ*: 4.03 (broad s, 1H, 3-C*H*); 3.72 (broad s, 4H, 1-C*H* + OCH_3); 2.42 (m, 1H, 4-C*H*); 2.32 (m, 1H, 4-C*H*); 2.03 (broad s, 1H, 5-C*H*); 1.47 (broad s, 10H, 6-C*H*+C(CH_3)$_3$).

^{19}F NMR (377 MHz, $CDCl_3$, rotamers), *δ*: 96.96, 96.54 (two broad s, CF_3).

^{13}C NMR (125 MHz, $CDCl_3$, rotamers), *δ*: 171.78 (s, *C*OOCH$_3$); 154.91 (broad s, N*C*O); 124.01 (q, ^1J(C, F) = 271.6 Hz, *C*F$_3$); 81.27 (s, O*C*(CH$_3$)$_3$); 60.30 (s, 3-*C*H); 52.43 (s, O*C*H$_3$); 39.72 (q, ^3J(C, F) = 2.5 Hz, 1-*C*H); 31.34 (broad s, *C*H$_2$); 29.27 (broad s, 6-*C*H); 28.21 (s, C(*C*H$_3$)$_3$); 19.92 (s, 5-*C*H).

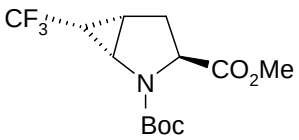
IR (KBr): *ν* = 1758 (C=O in COOCH$_3$), 1711 (*ν* C=O in COOC(CH$_3$)$_3$).

MS (CI): *m/z* (%): 210 (100) [M-Boc+2]$^+$.

Anal. calcd for $C_{13}H_{18}F_3NO_4$: C, 50.49; H, 5.87; N, 4.53. Found: C, 50.21; H, 5.53; N, 4.18.

The isomer **12c** (0.93 g, 3.00 mmol, 17%) was eluted from the column immediately after **12b** as a colourless viscous oil. R*f* = 0.35 in hexane/EtOAc = 5/1.

^{1}H NMR (400 MHz, $CDCl_3$, rotamers), *δ*: 4.38, 4.22 (two dd, *J* = 10.0, 4.4 Hz, 1H, 3-C*H*); 3.82 (m, 1H, 1-C*H*); 3.75, 3.74 (two s, 3H, OCH_3); 2.65 (m, 1H, 4-C*H*); 2.30 (m, 1H, 4-C*H*); 2.07 (m, 1H, 5-C*H*); 1.58 (m, 1H, 6-C*H*); 1.47, 1.41 (two s, 9H, C(CH_3)$_3$).



^{19}F NMR (377 MHz, $CDCl_3$), *δ*: 103.65 (d, ^3J(H, F) = 7.5 Hz, CF_3).

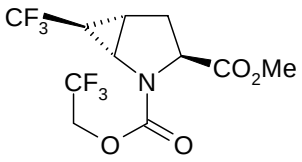
^{13}C NMR (125 MHz, $CDCl_3$, rotamers), *δ*: 172.60 (s, *C*OOCH$_3$); 154.57, 154.05 (two s, N*C*O); 126.16 (two q, ^1J(C, F) = 275.4 Hz, *C*F$_3$); 80.96, 80.91 (two s, O*C*(CH$_3$)$_3$); 63.48, 62.94

(two q, ^{5}J(H, F) = 1.3 Hz, 3-*C*H); 52.39, 52.21 (two s, O*C*H_3); 41.02, 40.62 (two q, ^{3}J(C, F) = 1.3 Hz, 1-*C*H); 28.98, 27.88 (two s, *C*H_2); 28.17, 28.11 (two s, C(*C*H_3)$_3$); 27.41 (two overlapped q, ^{2}J(C, F) = 35.2 Hz, 6-*C*H); 21.26, 19.92 (two q, ^{3}J(C, F) = 1.3 Hz, 5-*C*H).

IR (neat): ν = 1752 (C=O in $COOCH_3$), 1705 (ν C=O in $COOC(CH_3)_3$).

MS (CI): *m/z* (%): 210 (100) $[M\text{-Boc}+2]^+$.

Anal. calcd. for $C_{13}H_{18}F_3NO_4$: C, 50.49; H, 5.87; N, 4.53. Found: C, 50.37; H, 5.56; N, 4.35.



In a case the reaction was not stopped after the starting material **11** had been disappeared, the $COOC(CH_3)_3$-group transformed into the $COOCH_2CF_3$-group. Compound **13** was isolated from the complex reaction mixture by column chromatography with the purity of ~ 80% (containing ~ 20% of **12b**). Crystals of **13** suitable for an X-ray structure analysis were obtained by crystallization from cyclohexane. R*f* = 0.38 in hexane/EtOAc = 5/1. M.p. 56-57ºC.

^{1}H NMR (400 MHz, $CDCl_3$, rotamers), δ: 4.48 (broad s, 1H, OC*H*CF_3); 4.27 (broad s, 1H, OC*H*CF_3); 4.08 (broad s, 1H, 3-C*H*); 3.81, 3.68 (two broad s, 1H, 1-C*H*); 3.62 (s, 3H, O*CH*$_3$); 2.36 (broad s, 1H, 4-C*H*); 2.30 (broad s, 1H, 4-C*H*); 1.99 (broad s, 1H, 5-C*H*); 1.46 (broad s, 1H, 6-C*H*).

^{19}F NMR (377 MHz, $CDCl_3$, rotamers), δ: 96.53, 96.15 (two broad s, 3F, CHC*F*$_3$), 87.36, 87.28 (two broad s, 3F, OCH_2CF_3).

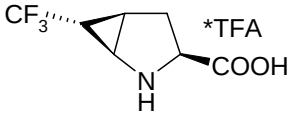
^{13}C NMR (125 MHz, $CDCl_3$, rotamers), δ: 170.91 (s, *C*$OOCH_3$); 153.44, 152.97 (two s, N*C*O); 123.62 (q, ^{1}J(C, F) = 271.6 Hz, *C*F_3); 122.80 (q, ^{1}J(C, F) = 276.8 Hz, *C*F_3), 61.89, 61.57 (two s, O*C*H_3); 61.73 (q, ^{2}J(C, F) = 37.8 Hz, O*C*H_2CF_3); 61.58, 60.83 (two broad s, 3-*C*H); 52.74 (s, O*C*H_3); 40.80, 39.75 (two broad s, 1-*C*H); 32.30, 31.19 (two s, *C*H_2); 30.25 (q, ^{2}J(C, F) = 34.0 Hz, 6-*C*H); 20.05, 18.64 (two s, 5-*C*H).

IR (KBr): ν = 1752 (C=O in $COOCH_3$), 1731 (C=O in $COOCH_2CF_3$).

MS (CI): *m/z* (%): 336 (100) $[M\text{-Boc}+2]^+$.

Anal. calcd. for $C_{11}H_{11}F_6NO_4$: C, 39.42; H, 3.31; N, 4.18. Found: C, 39.20; H, 3.00; N, 3.98.

(1*R*,3*S*,5*R*,6*R*)-3-carboxy-6-(trifluoromethyl)-2-azoniabicyclo[3.1.0]hexane trifluoroacetate (7a*TFA)



An aqueous solution of NaOH (13.8 ml, 0.93M, 12.8 mmol) was slowly added to a stirring solution of **12a** (1.00 g, 3.23 mmol) in MeOH (10 ml) and the resulting suspension was stirred for 2 hours. The transparent solution formed was evaporated (to remove MeOH), redissolved in H_2O (10 ml) and extracted with CH_2Cl_2 (2 × 3 ml). The organic layer was discarded; water phase was acidified to pH ~ 2 with aqueous HCl and extracted again with CH_2Cl_2 (3 × 5 ml). After being dried ($MgSO_4$), the organic layer was evaporated to produce Boc-**7a** as a white solid. Boc-**7a** was dissolved in 5 ml of TFA/CH_2Cl_2 (1/4), and the mixture was stirred

for 2 hours. Evaporation of the solvent afforded **7a***TFA (1.13 g, 3.22 mmol, quant.) as a white solid. M.p. 145-146ºC.

^{1}H NMR (400 MHz, CD_3OD), δ: 4.68 (dd, J = 10.8, 3.2 Hz, 1H, 3-C*H*), 3.82 (dd, J = 6.4, 2.0 Hz, 1H, 1-C*H*), 2.82 (m, 1H, 4-C*H*), 2.53 (dd, J = 14.0, 2.8 Hz, 1H, 4-C*H*), 2.38 (dd, J = 10.8, 6.4 Hz, 1H, 5-C*H*), 2.10 (m, 1H, 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD), δ: 95.31 (d, ^3J(H, F) = 3.8 Hz, CF_3); 85.43 (s, CF_3COO).

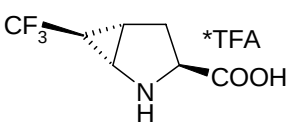
^{13}C NMR (125 MHz, CD_3OD), δ: 171.12 (s, *C*OOH); 161.15 (q, ^2J(C, F) = 36.5 Hz, *C*OO$^-$ of CF_3COO^-); 123.72 (q, ^1J(C, F) = 270.4 Hz, *C*F_3); 116.48 (q, ^1J(C, F) = 292.3 Hz, *C*F_3 of CF_3COO^-); 59.85 (s, 3-*C*H); 38.17 (q, ^3J(C, F) = 3.8 Hz, 1-*C*H); 29.58 (s, *C*H_2); 23.08 (q, ^2J(C, F) = 36.5 Hz, 6-*C*H); 20.81 (q, ^3J(C, F) = 3.8 Hz, 5-*C*H).

IR (KBr): ν = 1730 (C=O in COOH), 1683 (ν_{as} COO^-), 1632 (ν_s COO^-).

MS (CI): *m/z* (%): 196 (100) $[M\text{-}CF_3COO]^+$.

Anal. calcd. for $C_9H_9F_6NO_4$: C, 34.97; H, 2.93; N, 4.53. Found: C, 34.60; H, 2.63; N, 4.40.

(1*S*,3*S*,5*S*,6*S*)-3-carboxy-6-(trifluoromethyl)-2-azoniabicyclo[3.1.0]hexane trifluoroacetate (7b*TFA)



7b*TFA was synthesized from **12b** analogously to **7a***TFA (quant. yield). White solid. M.p. 143-144ºC. $[\alpha]_D^{20}$ = -25.0 (c = 0.187 g cm^{-3} in MeOH).

^{1}H NMR (400 MHz, CD_3OD), δ: 4.21 (t, J = 8.8 Hz, 1H, 3-C*H*); 3.75 (dd, J = 6.0, 2.0 Hz, 1H, 1-C*H*); 2.66 (dd, J = 12.8, 8.0 Hz, 1H, 4-C*H*); 2.56 (m, 1H, 4-C*H*); 2.38 (m, 2H, 5-C*H* and 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD), δ: 104.13 (d, ^3J(H, F) = 7.5 Hz, CF_3CH); 85.37 (s, CF_3COO$^-$).

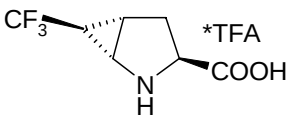
^{13}C NMR (101 MHz, CD_3OD), δ: 169.71 (s, *C*OOH); 161.15 (q, ^2J(C, F) = 35.2 Hz, *C*OO$^-$ of CF_3COO^-); 125.52 (q, ^1J(C, F) = 270.9 Hz, CH*C*F_3); 116.49 (q, ^1J(C, F) = 289.2 Hz, *C*F_3 of CF_3COO^-); 58.06 (s, 3-*C*H); 37.67 (q, ^3J(C, F) = 4.0 Hz, 1-*C*H); 29.76 (s, 4-*C*H_2); 21.22 (q, ^2J(C, F) = 37.3 Hz, 6-*C*H); 20.53 (q, ^3J(C, F) = 2.0 Hz, 5-*C*H).

IR (KBr): ν = 1733 (C=O in COOH), 1679 (ν_{as} COO^-), 1636 (ν_s COO^-).

MS (CI): *m/z* (%): 196 (100) $[M\text{-}CF_3COO]^+$.

Anal. calcd. for $C_9H_9F_6NO_4$: C, 34.97; H, 2.93; N, 4.53. Found: C, 34.74; H, 2.73; N, 4.33.

(1*S*,3*S*,5*S*,6*R*)-3-carboxy-6-(trifluoromethyl)-2-azoniabicyclo[3.1.0]hexane trifluoroacetate (7c*TFA)



7c*TFA was synthesised from **12c** analogiously to **7a***TFA (quant. yield). White solid. M.p. 143-144ºC.

^{1}H NMR (400 MHz, CD_3OD), δ: 4.39 (t, J = 8.8 Hz, 1H, 3-C*H*); 3.76 (t, J = 6.4

Hz, 1H, 1-C*H*); 2.69 (m, 2H, 4-CH_2); 2.44 (m, 1H, 5-C*H*); 2.21 (m, 1H, 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD), δ: 104.13 (d, ^3J(H, F) = 7.5 Hz, CF_3CH); 85.37 (s, CF_3COO^-).

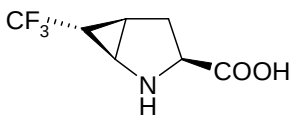
^{13}C NMR (101 MHz, CD_3OD), δ: 169.19 (s, *C*OOH); 160.18 (q, ^2J(C, F) = 36.2 Hz, *C*OO^- of CF_3COO^-); 125.54 (q, ^1J(C, F) = 273.6 Hz, CH*C*F_3); 116.00 (q, ^1J(C, F) = 292.6 Hz, *C*F_3 of CF_3COO^-); 61.11 (q, ^5J(C, F) = 4.0 Hz, 3-*C*H); 39.81 (q, ^3J(C, F) = 2.0 Hz, 1-*C*H); 27.24 (s, 4-*C*H_2); 21.46 (q, ^2J(C, F) = 39.2 Hz, 6-*C*H); 22.02 (q, ^3J(C, F) = 2.0 Hz, 5-*C*H).

IR (KBr): ν = 1731 (C=O in COOH), 1680 (ν_{as} COO^-), 1635 (ν_s COO^-).

MS (CI): *m/z* (%): 196 (100) $[M\text{-}CF_3COO]^+$.

Anal. calcd for $C_9H_9F_6NO_4$: C, 34.97; H, 2.93; N, 4.53. Found: C, 34.68; H, 2.76; N, 4.25.

(1*R*,3*S*,5*R*,6*R*)-6-(trifluoromethyl)-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (7a)



7a*TFA was neutralized with aqueous NaOH (0.3M) and submitted to an ion exchange chromatography column (Dowex® 50 × 400). Elution with water followed by aqueous pyridine (10%) afforded **7a** in quant. yield.

M.p. > 200ºC. $[\alpha]_D^{20}$ = -37.0 (*c* = 0.544 g cm^{-3} in H_2O).

^{1}H NMR (400 MHz, D_2O), δ: 4.39 (dd, *J* = 11.2, 2.8 Hz, 1H, 3-C*H*); 3.80 (dd, *J* = 6.8, 2.4 Hz, 1H, 1-C*H*); 2.72 (m, 1H, 4-C*H*); 2.48 (dd, *J* = 14.0, 2.8 Hz, 1H, 4-C*H*); 2.38 (dd, *J* = 10.8, 6.4 Hz, 1H, 5-C*H*); 2.05 (m, 1H, 6-C*H*).

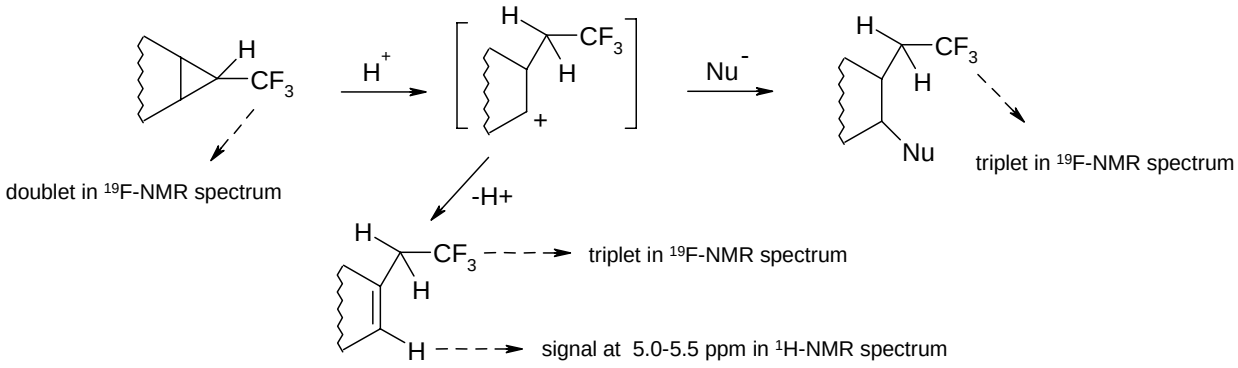
^{19}F NMR (377 MHz, D_2O), δ: 95.81 (d, ^3J(H, F) = 3.8 Hz, CF_3).

MS (CI): *m/z* (%): 196 (100) $[M+1]^+$.

IR (KBr): ν = 1636 (ν_{as} COO^-), 1388 (ν_s COO^-).

Anal. calcd. for $C_7H_8F_3NO_2$: C, 43.09; H, 4.13; N, 7.18. Found: C, 42.82; H, 3.85; N, 6.80.

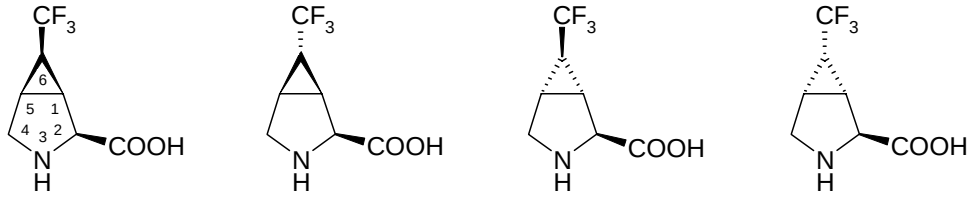
Under the same purification conditions the isomers **7**(**b**,**c**)*TFA decomposed partially (**7b***TFA) or completely (**7c***TFA). Unfortunately, the products of decomposition were not isolated due to the formation of rather complex reaction mixtures (in a case of **7c***TFA there were at least six different α-C*H* signals present in ^{1}H-NMR spectrum). However, typical triplets in ^{19}F-NMR spectra and signals at 5.0-5.5 ppm in ^{1}H-NMR spectra indicated that cleavage of the cyclopropane ring may have happened as shown below schematically.



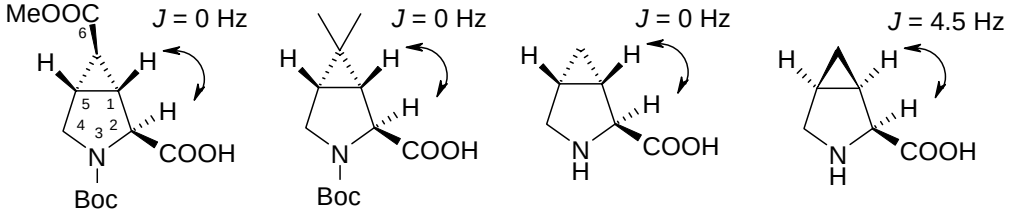
2) Assignment of stereochemistry of 6(a,b), 7(a,b,c) and 13

6(**a**,**b**)

Formation of four stereoisomers of **6**, possessing (*S*)-configuration at the aminocarboxylate moiety, is theoretically possible:



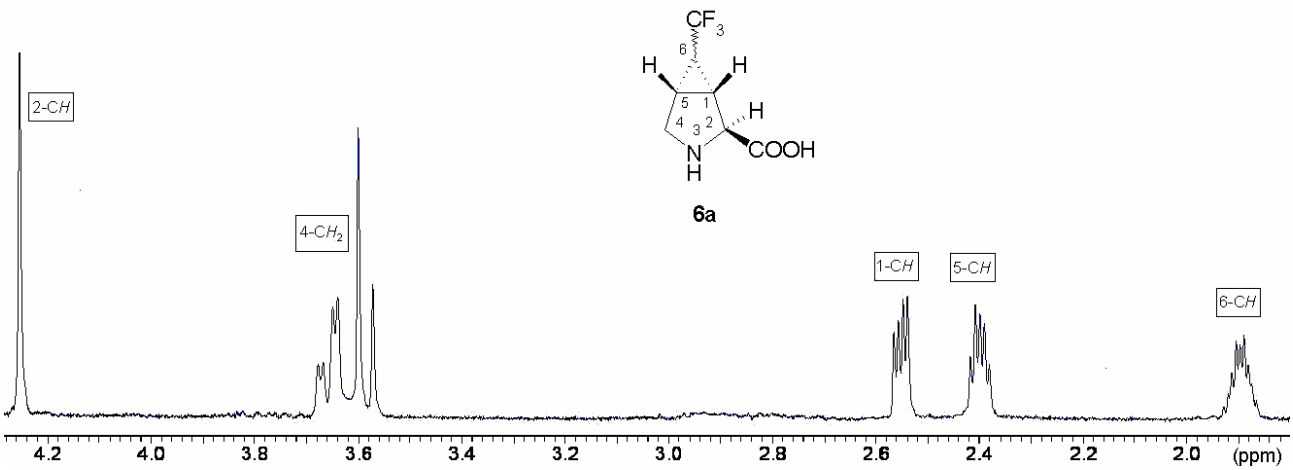
First, we established the configuration at the C(1) and C(5) atoms in the cyclopropane rings of **6**(**a**,**b**). We compared ^{1}H-NMR spectra of **6**(**a**,**b**) and the known structurally related amino acids **X1**, **X2** (which were obtained by stereoselective synthesis)[2] and **X3**, **X4** (the absolute configurations of which were determined by X-ray analysis).[3]



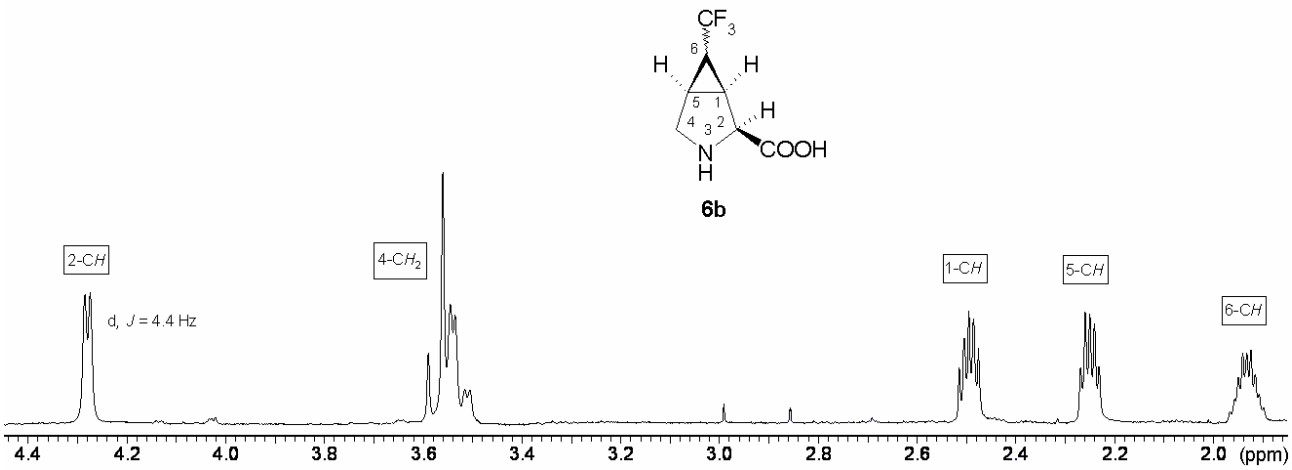
X1 **X2** **X3** **X4**

In the ^{1}H-NMR spectra of three *trans*-methanoprolines **X1**, **X2**, **X3** the signal of the proton at C(2) is either singlet (**X3**) or pair of singlets (two rotamers are observed for **X1**, **X2**). Contrastingly, in the ^{1}H-NMR spectrum of *cis*-isomer **X4** the same proton is doublet (^{3}J(H, H) = 4.5 Hz). In the ^{1}H-NMR spectrum of **6a** proton at the C(2) is a singlet, but in **6b** – a doublet with ^{3}J(H, H) = 4.4 Hz. This confirms

that **6a** possesses the cyclopropane fragment in *trans*-configuration with respect to the COOH-group, **6b** - in *cis*-configurations, as shown below.

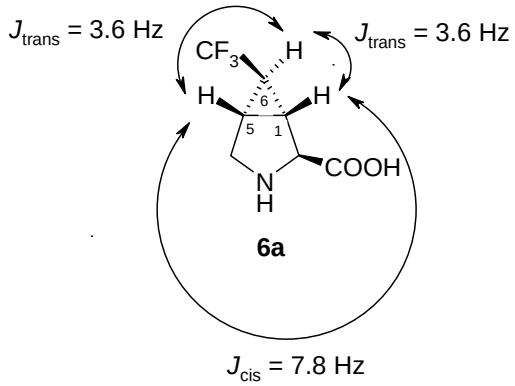


6a

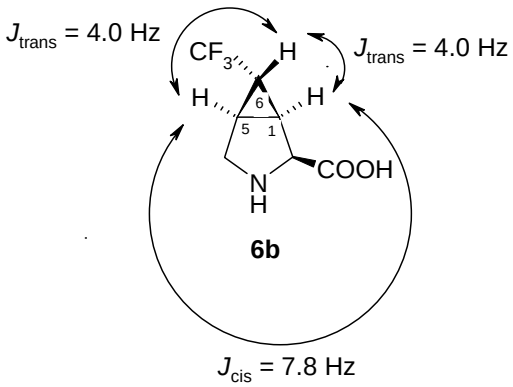


6b

Next question to be answered is the configuration at C(6) in **6**(**a,b**). It is generally known for cyclopropane derivatives that *cis*-^3J(H, H) coupling constant is larger than the corresponding *trans*-^3J(H, H) constant. [4] In each isomers **6**(**a**,**b**) two types of coupling constants are seen:



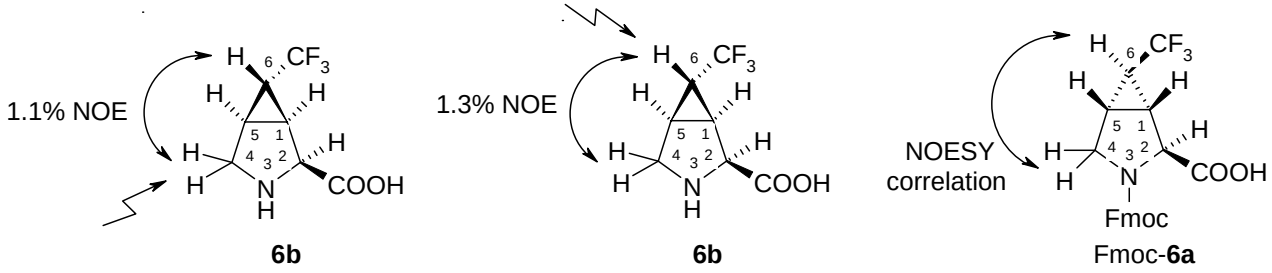
6a



6b

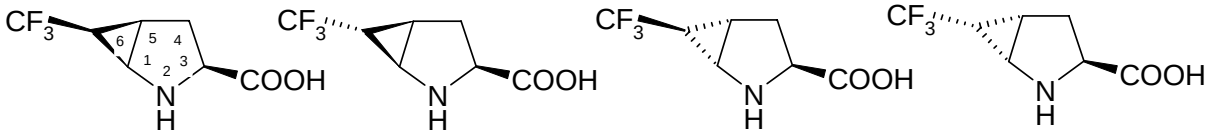
This observation suggests that in both **6a** and **6b** the proton at C(6) has an *anti*-orientation relatively to the protons at C(1) and C(5). For isomer **6b** this assumption was readily confirmed by NOE between

protons at C(4) and C(6). Although the analogous correlations were not detected for **6a** itself, they were found in the NOESY spectrum of Fmoc-**6a**.[1]

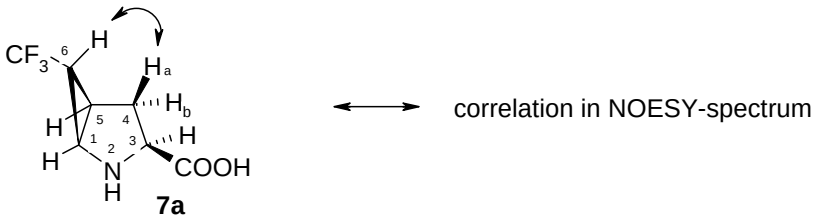


7(**a**,**b**,**c**)

As in the case of **6**, a formation of four stereoisomers of **7**, possessing (*S*)-configuration at the aminocarboxylate moiety, is theoretically possible:



The major experimental evidence proving the configuration of the stereocenters in **7a** is the presence of correlation peak between 6-H and 4-H_a in the NOESY spectrum. These protons could be in spatial proximity in only one case, shown below:

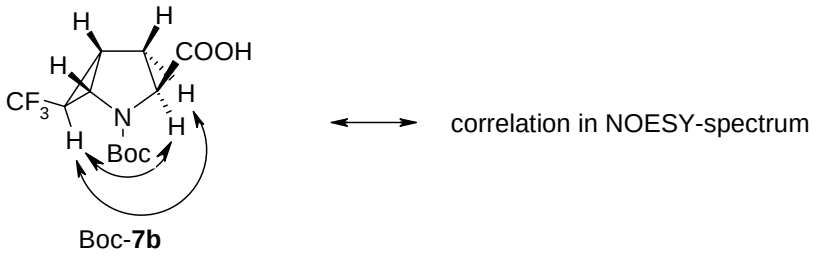


Structure assignment of **7b** was carried out on its precursor - Boc-**7b**.[2] The NOESY correlations which undoubtedly prove the relative configuration of the stereocenters in Boc-**7b** (and hence, in **7b**) are shown below:

---

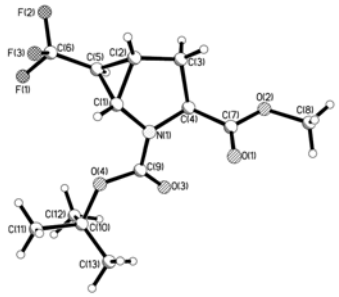
[1] Fmoc-**6a** was synthesised from **6a** using a standard FmocCl/Na_2CO_3 protocol.
M.p. 193-194 ºC. White solid. ^{1}H NMR (400 MHz, CD_3OD, rotamers), δ: 7.80 (t, J = 6.8, 5.6 Hz, 2H); 7.63 (t, J = 10.8, 7.2 Hz, 2H); 7.40 (t, J = 7.2 Hz, 2H); 7.32 (t, J = 7.2 Hz, 2H); 4.55-4.10 (m, 4H, 2-C*H*, OCH_2CH and OCH$_2$C*H*); 3.71, 3.62 (two broad s, 2H, 4-CH_2); 2.46, 2.38 (two m, 1H, 1C*H*); 2.19 (m, 1H, 5-C*H*); 1.93 (m, 1H, 6-C*H*). ^{19}F NMR (377 MHz, $CDCl_3$, rotamers), δ: 95.71, 95.66 (two broad s, CF_3). MS (CI): *m/z* (%): 418 (100) $[M+1]^+$.

[2] Boc-**7b** was obtained from **12b** analogously to Boc-**7a**, which is described in details in the protocol of obtaining of **7a**. White solid. M.p. 121-122ºC. ^{1}H NMR (400 MHz, CD_3OD, rotamers), δ: 4.10 (broad s, 1H, 3-C*H*); 3.76, 3.66 (two broad s, 1H, 1-C*H*); 2.55 (two d, J = 9.6 Hz, 1H, 4-C*H*); 2.34 (m, 1H, 4-C*H*); 2.11 (broad s, 1H, 5-C*H*); 1.89 (m, 1H, 6-C*H*); 1.46, 1.44 (two s, 9H, C(CH_3)$_3$). ^{19}F NMR (377 MHz, CD_3OD, rotamers), δ: 95.87, 95.62 (two broad s, CF_3).
^{13}C NMR (101 MHz, CD_3OD, rotamers), δ: 173.56 (broad s, *C*OOH); 155.18, 154.78 (two broad s, N*C*O); 124.30 (q, ^1J(C, F) = 270.6 Hz, *C*F$_3$); 81.23, 80.65 (broad s, O*C*(CH$_3$)$_3$); 60.22 (m, 3-C*H*); 39.69 (s, 1-*C*H); 31.35, 30.77 (two q, ^3J(C, F) = 2.8

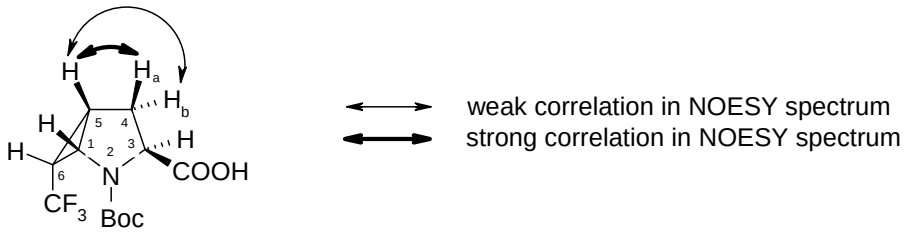


Boc-**7b**

The structural assignment of **7b** was latter confirmed by an X-ray study of its other precursor **12b** (the molecular structure is shown below):



Determination of the relative configuration of the stereogenic centers in **7c** was also performed on its precursor – Boc-**7c**.[3] In the NOESY spectrum of Boc-**7c** the area of corelation peak between 4-H_a and 5-H is much larger than that of the correlation between 4-H_a and 5-H. This means, that the protons 5-H and 4-H_a are in a *syn*-orientation. Thus, Boc-**7c** possesses the same configuration of the cyclopropane ring with regard to the COOH group as its isomer, Boc-**7b**. Apparently, Boc-**7c** differs from Boc-**7b** by only the configuration at C(6).

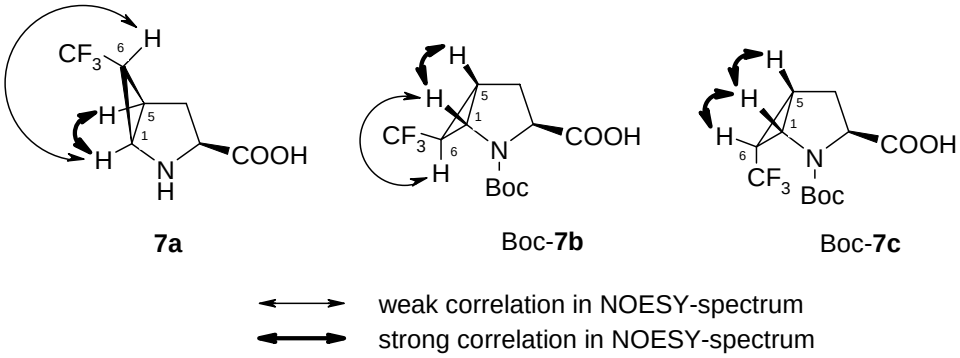


Boc-**7c**

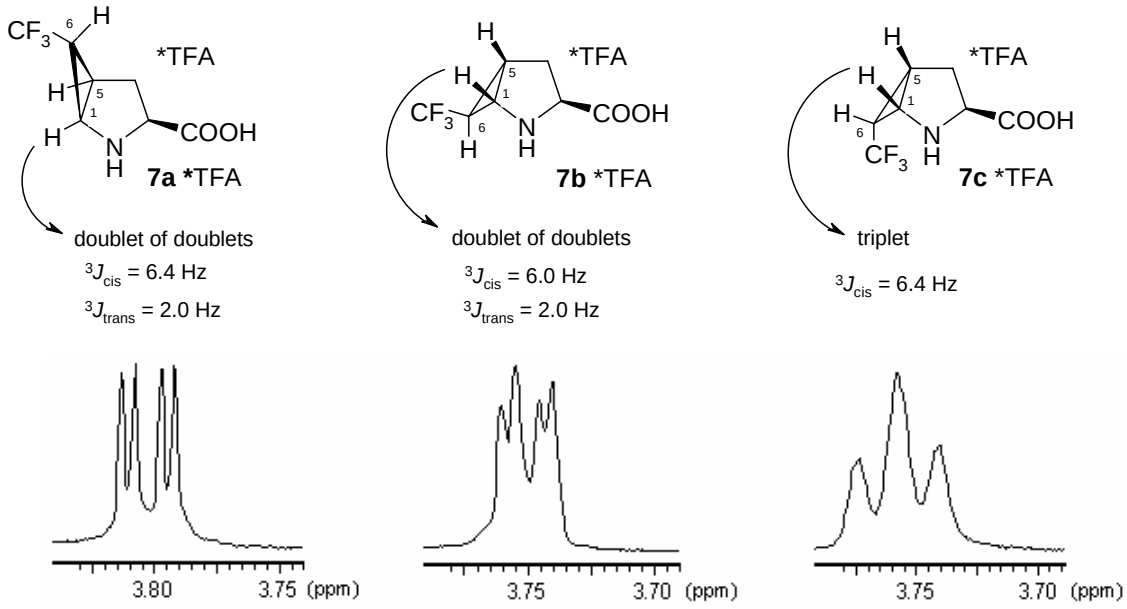
Hz, *C*H_2); 27.95 (broad s, 6-*C*H); 26.97 (s, C(*C*H_3)$_3$); 19.49, 18.02 (two broad s, 5-*C*H). IR (KBr): ν = 1756 (C=O in COOH), 1654 (C=O in COOC(CH$_3$)$_3$). MS (CI): *m/z* (%): 296 (100) $[M+1]^+$. Anal. calcd for $C_{12}H_{16}F_3NO_4$: C, 48.82; H, 5.46; N, 4.74. Found: C, 48.53; H, 5.22; N, 4.33.

[3] Boc-**7c** was synthesised from **12c** using the same procedure applied for the Boc-**7a**. White solid. M.p. = 120-121ºC. H NMR (400 MHz, CD_3OD, rotamers), δ: 4.20 (two dd, J = 10.0; 3.6 Hz, 1H, 3-C*H*); 3.73 (m, 1H, 1-C*H*); 2.64 (q, J = 10.0 Hz, 1H, 4-C*H*); 2.35 (m, 1H, 4-C*H*); 2.18 (m, 1H, 5-C*H*); 1.77 (m, 1H, 6-C*H*); 1.47, 1.42 (two s, 9H, C(CH_3)$_3$). ^{19}F NMR (377 MHz, CD_3OD, rotamers), δ: 103.17, 103.09 (two d, ^3J(H, F) = 7.5 Hz, CF_3). ^{13}C NMR (101 MHz, CD_3OD, rotamers), δ: 173.73, 173.58 (two s, *C*OOCH$_3$); 156.73, 156.39 (two s, N*C*O); 128.21 (q, ^1J(C, F) = 272.9 Hz, *C*F_3); 82.57, 82.27 (two s, O*C*(CH$_3$)$_3$); 65.27, 65.05 (two q, ^5J(C, F) = 2.0 Hz, 3-C*H*); 42.24, 42.01 (two q, ^3J(C, F) = 2.0 Hz, 1-*C*H); 30.03, 29.14 (two s, *C*H_2); 28.71, 28.62 (two s, C(*C*H$_3$)$_3$); 22.31 (two overlapped q, ^2J(C, F) = 34.2 Hz, 6-*C*H); 22.46, 21.17 (two q, ^3J(C, F) = 2.0 Hz, 5-*C*H). IR (KBr): ν = 1720 (C=O in COOH), 1694 (C=O in COOC(CH$_3$)$_3$). MS (CI): *m/z* (%): 296 (100) $[M+1]^+$. Anal. calcd for $C_{12}H_{16}F_3NO_4$: C, 48.82; H, 5.46; N, 4.74. Found: C, 48.46; H, 5.11; N, 4.25.

All abovementioned assumptions on the structure of **7a** and Boc-**7**(**b**,**c**) are in accordance with the observed ^{1}H-^{1}H NOESY-correlations in the cyclopropane rings of these compounds. In both **7a** and Boc-**7b**, the correlation peak between 1-H and 5-H (*syn*-protons) is stronger than that between 1-H та 6-H (*anti*-protons). Contrastingly, in Boc-**7c** both correlations are of the same value due to the *syn*-arrangement of all the three protons:



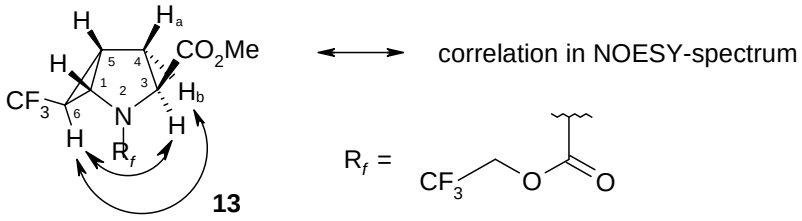
The determined structures can be additionally confirmed by the multiplicity of the proton at respective C(1) in ^{1}H-NMR spectra of **7**(**a**,**b**,**c**)*TFA:



In **7a***TFA the 1-H proton signal is a doublet of doublets ($^{3}J_{cis}$ = 6.4 Hz, $^{3}J_{trans}$ = 2.0 Hz), which is in an accordance with *anti*-orientation of 6-H with regard to 1-H and 5-H. In **7b***TFA the same situation is observed, as the 1-H proton signal is a doublet of doublets ($^{3}J_{cis}$ = 6.0 Hz, $^{3}J_{trans}$ = 2.0 Hz). On the contrary to **7**(**a**,**b**)*TFA, in **7c***TFA the 1-H proton peak is a triplet with a characteristic *cis*-constant $^{3}J_{cis}$ = 6.4 Hz, which again confirms that all the three cyclopropane protons are in a *syn*-orientation.

13

The structure of **13** was determined based on the correlations between protons 6-H and 3-H; 6-H and 4-H_b in the NOESY spectrum.

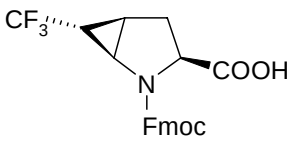


Later, the established structure was confirmed by an X-ray analysis of **13**.

3) Peptide synthesis

For peptide synthesis the Fmoc-protected derivatives of **7a** and **2** were obtained first.

Fmoc-**7a**



A solution of Fmoc-Cl (670 mg, 2.69 mmol) in dioxane (10 ml) was added dropwise over 15 min to a solution of **7a** (500 mg, 2.56 mmol) and Na_2CO_3 (1 g) in dioxane-water (25 ml, 2/3, v/v) cooled to 0ºC (ice bath). After being stirred at this temperature for 30 min, the reaction was left overnight at room temperature. Water (500 ml) was added and the formed mixture was extracted with Et_2O (2 × 25 ml). The organic layer was discarded; water phase was acidified with aqueous HCl to pH ~ 1 and extracted with EtOAc (3 × 50 ml). After drying over $MgSO_4$ the solution was evaporated to obtain Fmoc-**7a** (855 mg, 2.05 mmol, 80%) which was used for peptide synthesis without additional purification. An analytically pure sample was obtained by crystallization from hexane-EtOAc mixture. White solid. M.p. 193-194ºC.

^{1}H NMR (500 MHz, CD_3OD, rotamers), δ: 7.82 (t, J = 8.0 Hz, 2H); 7.69 (t, J = 7.0 Hz, 1.2H); 7.61 (dd, J = 7.0, 3.5 Hz, 0.8H); 7.42 (pseudo q, J = 7.0 Hz, 2H); 7.33 (t, J = 7.0 Hz, 2H); 4.55, 4.49 (two overlapped dd, J = 8.5, 3.0 Hz, 1H, 3-C*H*); 4.51 (dd, J = 10.0, 6.5 Hz, 0.6H); 4.38 (m, 2H); 4.19 (t, J = 6.5 Hz, 0.4H); 3.92, 3.82 (two dd, J = 7.0, 1.5 Hz, 1H, 1-C*H*); 2.82 (m, 1H, 4-C*H*); 2.27-2.07 (m, 3H, 4-C*H*, 5-C*H* and 6-C*H*).

^{19}F NMR (377 MHz, CD_3OD, rotamers), δ: 96.61, 96.41 (two broad s, CF_3).

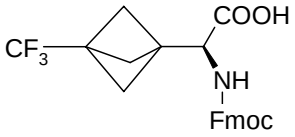
^{13}C NMR (125 MHz, CD_3OD, rotamers), δ: 174.65, 174.33 (two s, *C*OOH); 143.84, 143.65 (two s, N*C*O); 143.84, 143.77, 143.65, 143.56 (four s, Fmoc-residue); 141.26, 141.20, 141.10 (three s, Fmoc-residue); 127.49 (s, *C*H); 126.87, 126.81 (two s, *C*H); 124.82, 124.74 (two s, *C*H); 124.42 (two q, ^1J(C, F) = 270.4 Hz, *C*F_3); 119.58 (s, *C*H); 67.98, 67.85 (two s, O*C*H_2CH); 59.36, 58.71 (two s, 3-*C*H); 46.97,

46.86 (two s, OCH_2CH); 39.79, 39.22 (two q, ^3J(C, F) = 3.8 Hz, 1-*C*H); 30.48, 29.36 (two s, 4-*C*H_2); 28.28, 24.87 (two overlapped q, ^2J(C, F) = 36.3 Hz, 6-*C*H); 19.36, 18.22 (two s, 5-*C*H).

MS (CI): *m/z* (%): 418 (100) $[M+1]^+$.

Anal. calcd for $C_{18}H_{22}F_3NO_4$: C, 63.31; H, 4.35; N, 3.36. Found: C, 62.95; H, 4.19; N, 3.54.

Fmoc-**2** was synthesised as described previously.[5]



7a was incorporated into the 18-mer peptide **14** - SAP[6] $(VRLPPP)_3$ at the position of Pro^{11} to produce **15**. **2** was incorporated into SAP at the position of Leu^9 to obtain **16**.

All peptides (**14-16**) have been synthesized manually using standard solid phase Fmoc-protocols. 2-Chlorotrityl resin. While incorporating Arg into the peptide sequence, side chain protected Fmoc-(Pbf)Arg was used. Incorporation of natural amino acids: Fmoc-amino acids (4 eq) / TBTU (4 eq) / 6-Cl-HOBT (4 eq) and DIEA (8 eq) for 2 h. **7a** was incorporated by utilizing Fmoc-**7a** (2 eq) / DIC (6 eq) / 6-Cl-HOBt (2 eq) for 2 h. The incorporation of **2** was achieved by using Fmoc-**2** (2 eq) / TBTU (2 eq) / 6-Cl-HOBt / DIEA (4 eq) for 2 h. While incorporating Pro^{10} (next to **7a**), Val and Arg double couplings were used. Second couplings were done by using Fmoc-amino acid (4 eq) / PyBop (4 eq) / DIEA (8 eq). The coupling efficiency was monitored by Kaiser test (in all cases except Pro couplings). Deprotection was carried out with 20% piperidine in DMF for 20 min. The peptides were cleaved from the resin at room temperature by treatment with a cocktail of TFA (95%), water (2.5%) and TIS (2.5%) for 12 h with occasional shaking. The resin was filtered off and washed with pure TFA twice. The combined filtrates were evaporated under a gentle stream of nitrogen and the products were precipitated with cold diethyl ether. After centrifugation the supernatant (diethyl ether) was decanted. The solid precipitate was resuspended in H_2O and lyophilized. Peptides were purified by preparative RP-HPLC (Jasco, Japan; 25 × 250 mm Vydac C18-column). The H_2O/MeOH gradients were individually optimized for each peptide at 40°C. The crude peptides were loaded on the column as 50 mg/ml solution in MeOH. 5 mM HCl was used as ion-pairing agent instead of conventional TFA.

After the purification, all peptides were of > 95% purity according to analytical HPLC and they were stored as lyophilized powders at -40°C until use.

SAP $(VRLPPP)_3$, **14**

MALDI-TOF: *m/z*: 1998.67 $[M+1]^+$, calculated mass 1997.52.

VRLPPPVRLP-**7a**-PVRLPPP, **15**

MALDI-TOF: *m/z*: 2077.7 $[M]^+$, calculated mass 2077.53.

VRLPPPVR-**2**-PPPVRLPPP), **16**

MALDI-TOF: *m/z*: 2075.9 $[M]^+$, calculated mass 2075.52.

Circular dichroism spectroscopy.

Circular dichroism spectra were recorded on a Jasco 800 instrument with a 1 mm cuvette in aqueous buffer of 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.0. The peptides (0.25 mg) were dissolved in MeOH and the corresponding aliquot was taken away into 2-ml tube. The solvent was removed with a gentle N_2 stream followed by vacuum overnight. To the dried peptide the corresponding volume of buffer was added in order to obtain solution of 5-150 μM concentration. The solutions were transferred into the cuvettes and equilibrated for 10 min at 20°C prior to measurements. In the temperature series, the same conditions were used. The temperature was changed from cold (5° C) up to warm (50° C) with a step of 5° C. Each measurement was preceded by equilibration (50 min) at the corresponding temperature.

4) X-ray diffraction study of compounds 12b and 13

X-ray diffraction study of compounds **12b** and **13** revealed that asymmetric part of crystal unit cell contains one molecule of **12b** and two molecules (A and B) of **13**. Molecules A and B have almost the same geometrical parameters and they differ only in orientation of substituents at the N(1) and C(4) atoms.

Experimental part

Intensities of reflections were measured on an automatic «Xcalibur 3» diffractometer (graphite monochromatic MoKα radiation, CCD-detector ω scanning). The crystal data and parameters of experiments are given in Table S1. All structures were solved by direct method using SHELX97 package.[7] Positions of the hydrogen atoms were located from electron density difference maps and refined by "riding" model with $U_{iso} = nU_{eq}$ of carrier non-hydrogen atom (n = 1.5 for methyl group and n = 1.2 for other hydrogen atoms). Full-matrix least-squares refinement against F^2 was performed in anisotropic approximation for non-hydrogen atoms. Final atomic coordinates, geometrical parameters and crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk). Values of bond lengths and angles are listed in Tables S2 and S3.

Table S1. Crystal data and structure refinement for $C_{13}H_{18}F_3NO_4$ (**12b**), $C_{11}H_{11}F_6NO_4$ (**13**).

Structure	**12b**	**13**
Mr	309.28	335.21
Unit cell parameters, Å, deg.	*a*= 8.760(4), *b*= 11.256(6), *c*= 16.466(5),	*a*= 8.474(3), *b*= 21.767(2), *c*= 8.8358(13), β = 114.51(2)
Temperature, K	293	293
V, $Å^3$	1623.6(12)	1482.9(6)
Crystal System	orthorhombic	monoclinic
Space group	$P2_12_12_1$	$P2_1$
Z	4	4
F(000)	648	680
D_{calc}(g/sm^3)	1.265	1.502
μ(MoK_α), mm^{-1}	0.115	0.160
$2\theta_{max}$, deg.	51	55
Measured refl.	8876	34972
Independent refl.	1733	3480
R_{int}	0.040	0.022
Refl. with F>4σ(F)	811	2509
Number of parameters	194	397
wR_2	0.149	0.129
R_1(F>4σ(F))	0.045	0.044
S	0.854	1.026
CCDC dep. Number	676507	676508

Table S2. Bond lengths (Å) in **12b** and **13**.

Bond, Å	Compound **12b**	Compound **13**	
		A	B
F(1)-C(6)	1.288(7)	1.350(7)	1.324(6)
F(2)-C(6)	1.275(7)	1.308(6)	1.329(6)
F(3)-C(6)	1.323(9)	1.318(7)	1.334(6)
F(4)-C(11)		1.336(7)	1.339(6)
F(5)-C(11)		1.284(7)	1.308(5)
F(6)-C(11)		1.328(6)	1.312(6)
N(1)-C(9)	1.356(5)	1.348(4)	1.349(4)
N(1)-C(1)	1.413(5)	1.445(4)	1.430(4)
N(1)-C(4)	1.450(5)	1.457(4)	1.453(4)
O(1)-C(7)	1.184(5)	1.182(4)	1.194(4)
O(2)-C(7)	1.313(5)	1.314(4)	1.314(4)
O(2)-C(8)	1.440(6)	1.457(5)	1.446(6)
O(3)-C(9)	1.190(5)	1.201(4)	1.200(4)
O(4)-C(9)	1.333(5)	1.362(4)	1.360(4)
O(4)-C(10)	1.449(5)	1.425(5)	1.426(4)
C(1)-C(2)	1.458(6)	1.487(5)	1.483(5)
C(1)-C(5)	1.497(6)	1.499(5)	1.503(4)
C(2)-C(3)	1.486(6)	1.505(5)	1.502(6)
C(2)-C(5)	1.484(6)	1.509(5)	1.508(5)
C(3)-C(4)	1.549(6)	1.544(4)	1.553(5)
C(4)-C(7)	1.496(6)	1.517(4)	1.497(4)
C(5)-C(6)	1.466(7)	1.477(6)	1.469(6)
C(10)-C(11)	1.534(8)	1.465(8)	1.484(6)
C(10)-C(13)	1.461(8)		
C(10)-C(12)	1.496(9)		

Table S3. Bond angles (deg.) in **12b** and **13**

Bond angle	Compound **12b**	Compound **13**	
		A	B
C(9)-N(1)-C(1)	124.0(3)	125.5(2)	125.7(2)
C(9)-N(1)-C(4)	119.4(3)	118.7(2)	119.6(2)
C(1)-N(1)-C(4)	112.8(3)	113.0(2)	113.4(2)
C(7)-O(2)-C(8)	116.9(4)	116.9(3)	118.0(3)
C(9)-O(4)-C(10)	123.4(4)	116.0(3)	116.2(3)
N(1)-C(1)-C(2)	108.1(4)	106.3(3)	106.6(3)
N(1)-C(1)-C(5)	116.2(4)	115.0(2)	115.8(2)
C(2)-C(1)-C(5)	60.3(3)	60.7(2)	60.7(2)
C(1)-C(2)-C(5)	61.1(3)	60.0(2)	60.3(2)
C(1)-C(2)-C(3)	108.4(4)	108.9(3)	108.9(3)
C(5)-C(2)-C(3)	118.2(4)	117.6(3)	117.1(3)
C(2)-C(3)-C(4)	106.3(4)	106.1(3)	105.8(3)
N(1)-C(4)-C(7)	111.6(3)	110.9(2)	112.0(2)
N(1)-C(4)-C(3)	104.2(3)	105.0(2)	104.6(3)
C(7)-C(4)-C(3)	111.3(4)	110.2(3)	112.2(3)
C(6)-C(5)-C(2)	121.7(5)	118.5(3)	119.6(3)
C(6)-C(5)-C(1)	120.1(5)	118.7(3)	119.5(3)
C(2)-C(5)-C(1)	58.6(3)	59.3(2)	59.0(2)
F(2)-C(6)-F(1)	107.5(8)	106.7(5)	105.0(5)
F(2)-C(6)-F(3)	104.6(6)	108.0(5)	106.3(4)
F(1)-C(6)-F(3)	104.3(7)	104.0(5)	105.9(3)
F(2)-C(6)-C(5)	114.7(6)	112.5(4)	113.0(3)
F(1)-C(6)-C(5)	112.8(5)	111.3(4)	112.9(4)
F(3)-C(6)-C(5)	112.0(7)	113.7(4)	113.1(4)
O(1)-C(7)-O(2)	123.7(4)	125.2(3)	124.4(3)
O(1)-C(7)-C(4)	126.2(4)	124.6(3)	125.0(3)
O(2)-C(7)-C(4)	110.1(4)	110.2(2)	110.6(3)
O(3)-C(9)-O(4)	127.0(4)	124.9(3)	124.8(3)
O(3)-C(9)-N(1)	124.4(4)	124.9(3)	125.7(3)
O(4)-C(9)-N(1)	108.6(4)	110.2(3)	109.5(2)
O(4)-C(10)-C(13)	110.8(5)	109.4(4)	106.9(3)

O(4)-C(10)-C(12)	107.7(5)		
C(13)-C(10)-C(12)	116.4(7)		
O(4)-C(10)-C(11)	102.9(4)		
C(13)-C(10)-C(11)	109.0(6)		
C(12)-C(10)-C(11)	109.2(6)		
F(5)-C(11)-F(6)		108.6(5)	108.4(4)
F(5)-C(11)-F(4)		107.1(6)	107.5(4)
F(6)-C(11)-F(4)		105.6(5)	107.2(4)
F(5)-C(11)-C(10)		114.7(5)	112.1(4)
F(6)-C(11)-C(10)		111.1(5)	112.3(4)
F(4)-C(11)-C(10)		109.2(5)	109.2(4)

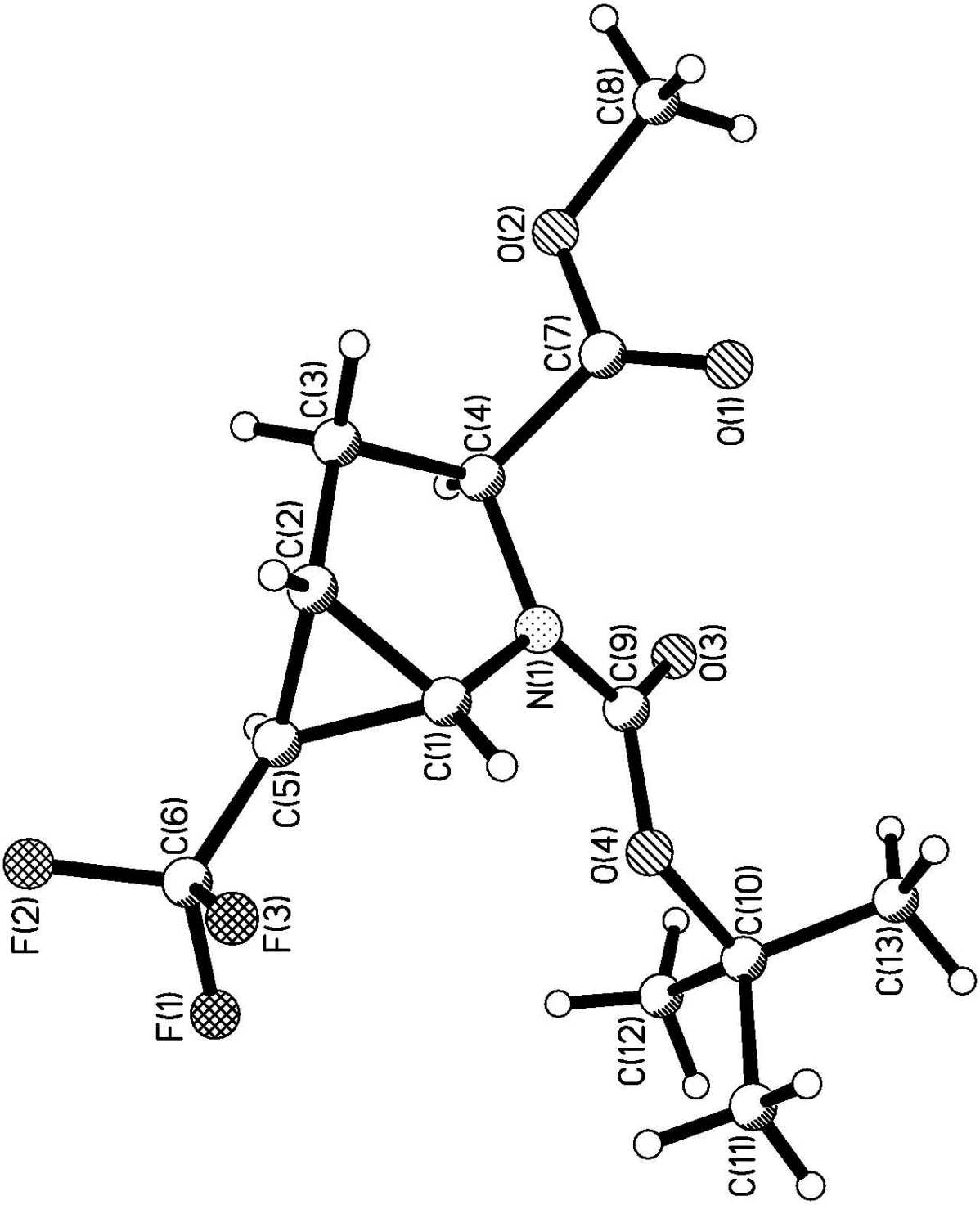


Figure 1. Molecular structure of **12b**.

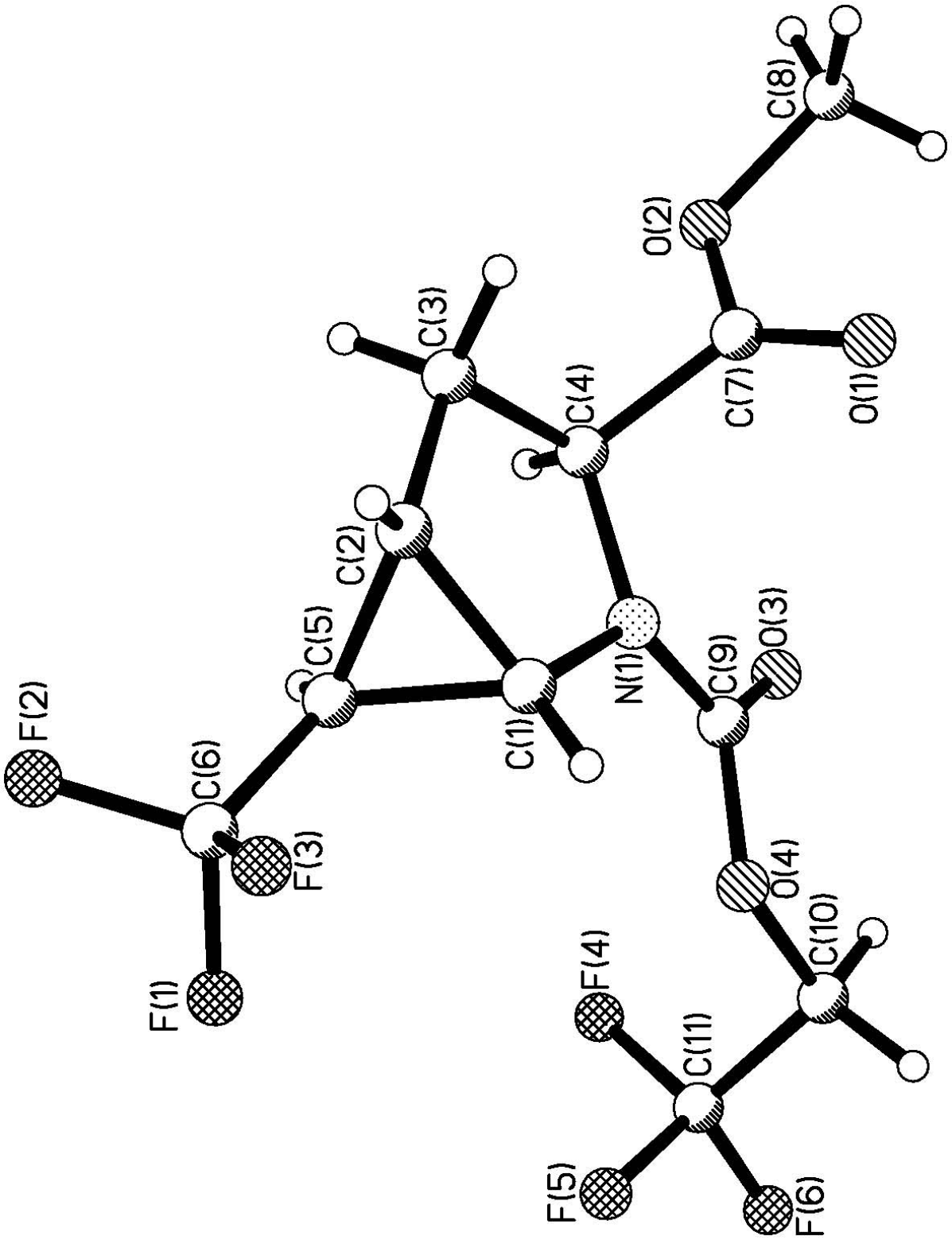


Figure 2. Molecular structure of **13**.

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Full citation for Reference 4c within main text:

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