SUPPORTING INFORMATION

Incorporation of novel *cis*- and *trans*-4,5-Difluoromethanoprolines into Polypeptides

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Abbreviations

DIPEA	diisopropylethylamine
Np	p-nitrophenyl-
6-Cl-HOBt	6-chloro-N-hydroxybenzotriazole
TBTU	O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

Materials and methods

^1H - and ^{13}C -NMR spectra were recorded on a Bruker Avance 300, a Bruker Avance 400, and a Bruker Avance 500 spectrometers as specified below and referenced to TMS. ^{19}F -NMR spectra were recorded on a Bruker Avance 300 spectrometer and referenced to trifluoroacetic acid in water (set to -75 ppm) as an external standard, the experiments were run without proton decoupling. Mass spectra were recorded on a Finnigan MAT90 (70 eV) spectrometer using electron impact (EI) ionization. Infra-red spectra were recorded on a Bruker ALPHA FT-IR spectrometer with a diamond-ATR module, only values above 1500 cm^{-1} are denoted. CHN-analysis was done on an Elementar vario MICRO cube analyzer. Optical rotation at 20°C was analysed on a PerkinElmer 241 Polarimeter at 20°C . Melting points were measured on a Büchi 510 melting point apparatus ($3^\circ/\text{min}$ scan) and are uncorrected. Matrix-assisted laser desorption/ionization with time-of-flight detection (MALDI-TOF) mass spectra were recorded on a Bruker Autoflex III instrument. Reverse-phase high-performance liquid chromatography (RP-HPLC) was performed on a Jasco HPLC system with PU-2080 Plus pumps and a diode-array UV-detector. Vydac polymeric C18 columns were employed (4.6 mm x 250 mm as analytical (flow rate 1.5 mL/min), 10 mm x 250 mm as semi-preparative (6 mL/min) and 22 mm x 250 mm as preparative (20 mL/min)). Linear gradients, using as solvent A: 90% water, 10% acetonitrile, solvent B: 90% acetonitrile, 10% water; 5 mM hydrochloric acid was used as an ion-pairing agent in all casesⁱ. Tetrahydrofuran (THF), diglyme, DIPEA, and diethyl ether were freshly distilled under a nitrogen atmosphere from sodium/benzophenone. Toluene was distilled from sodium. Trifluoroacetic anhydride was distilled over dry potassium carbonate. All other chemicals were of appropriate commercial grades (purchased from Merck, Acros, ABCR, Fisher, Iris Biotech, Biosolve) and used without further purification unless otherwise stated.

Experimental procedures

Methyl (S)-5-Oxo-pyrrolidine-2-carboxylate (Pyr-OMe, S1)

The compound was prepared using the modified literature protocol.ⁱⁱ (S)-Pyroglutamic acid **5** (33 g, 0.26 mol) was dissolved in methanol (HPLC grade, 1 L); thionyl chloride (2.5 mL, 0.03 mol) was added dropwise upon stirring under argon within 1 min. The solution was kept

stirred at room temperature for 16 h. Saturated sodium carbonate solution was added until pH ~7. Methanol was removed under reduced pressure. The product was taken up in dichloromethane (250 mL), washed with brine (100 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure giving 29.75 g (81% yield) of the product as a pale yellow oil which was used in the next step without any further purification.

1-*tert*-Butyl 2-methyl (S)-5-Oxo-pyrrolidine-1,2-dicarboxylate (Boc-Pyr-OMe, S2)

The synthetic procedure published inⁱⁱⁱ was used, starting from Pyr-OMe (**S1**). The crude Boc-Pyr-OMe (**S2**) was purified by silica gel column chromatography, eluting with dichloromethane:diethyl ether (gradient from 5:0 to 3:2). 30.6 g of the product was obtained starting from 20 g of Pyr-OMe (90% yield). The measured $[\alpha]_D = -30.7^\circ$ (CHCl_3 , $c = 0.47$) was consistent with the value -30.4° (CHCl_3 , $c = 1.00$) reported in the literature.^{iv} $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ , ppm: 4.62 (dd, $J = 9, 3$ Hz, 1H), 3.72 (s, 3H), 2.45 (m, 2H), 2.31 (m, $J = 13, 3$ Hz, 1H), 1.90 (m, $J = 4$ Hz, 1H), 1.40 (s, 9H).

1-*tert*-Buthyl 2-methyl (S)-2-pyrrolin-1,5-dicarboxylate (Boc-DhPro-OMe, 4)

The compound was synthesized according to the published protocol.^v 7.80 g of the product was obtained from 10 g of starting **S2** (83% yield). $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) – (rotamers, 10:9, δ , ppm): 6.65 and 6.52 (two m, 1H, N-CH=CH), 4.96 and 4.91 (two m, 1H, N-CH=CH), 4.66 and 4.59 (two dd, $J = 12, 5$ Hz, 1H, $\text{CH-CO}_2\text{Me}$), 3.76 (s, 3H, CO_2CH_3), 3.06 (m, 1H, CHH), 2.67 (m, 1H, CHH), 1.49 and 1.44 (two s, 9H, $\text{C}(\text{CH}_3)_3$).

2-*tert*-butyl 3-methyl (1S,3S,5R)-6,6-difluoro-2-azabicyclo[3.1.0]hexane-2,3-dicarboxylate (Boc-*trans*-4,5- F_2 MePro-OMe, *trans*-6)

A 250 mL three-necked reactor equipped with a dropping funnel and a Liebig condenser was charged with Boc-DhPro-OMe (4.1 g, 4.4 mmol) and dry diglyme (60 mL) under argon. The mixture was heated to 177 °C in an oil bath, and slurry of sodium chlorodifluoroacetate (10.1 g, 66.4 mmol, 15 eq.) in diglyme (40 mL) was added under stirring through the dropping funnel within 15 min. After the addition, the stirred mixture was incubated for 5 min, keeping the temperature strictly within the range of 175-178 °C. Then the mixture was cooled to the room temperature and filtered through a paper filter. The retentate was washed with diethyl ether, the combined filtrate and washings were evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent – hexane:ethyl acetate 2:1, R_f = 0.5). The product, Boc-4,5-F₂MePro-OMe (0.860 g, 71% yield) was obtained as a pale yellow liquid.

¹H-NMR (CDCl₃, 400 MHz) – (rotamers, 5:4, δ , ppm): 4.39 (major) and 4.17 (minor) (m, 1H, N-CH-CO₂Me), 3.99 (minor) and 3.80 (major) (m, 1H, N-CH-CF₂), 3.76 (s, 3H, CO₂CH₃), 2.62 (m, J = 10-11 Hz, 1H, CHH), 2.34 and 2.28 (two m, 2H, CH-CH-CHH), 1.48 (major) and 1.43 (minor) (s, 9H, (CH₃)₃C). ¹³C-NMR (CDCl₃, 101 MHz) – (rotamers, δ , ppm): 172.1 (s, CO₂Me), 155.0 and 154.3 (s, N-CO₂Bu^t), 111.9 (t, J = 293 Hz, CF₂), 81.8 and 81.7 (s, O-CMe₃), 62.3 and 61.6 (s, CH-CO₂Me), 52.6 and 52.4 (s, CO₂CH₃), 42.9 (two t, J = 12 Hz, N-CH-CF₂), 29.3 (s, CH₂), 28.4 and 28.2 (s, C(CH₃)₃), 26.4 and 24.7 (t, J = 12 Hz, CH-CH-CH₂). ¹⁹F-NMR (CDCl₃, 282 MHz) – (rotamers, δ , ppm): -129.7 (major) and -130.0 (minor) (dd, J_{F-F} = 160 Hz, J_{F-H} = 10 Hz, exo-F), -155.7 (minor) and -156.2 (major) (d, J_{F-F} = 160 Hz, endo-F). Mass spectrum, m/z : 277 [M]⁺, 221 [M-Bu^t]⁺, 177 [M-Boc]⁺ (100%), 163 [M-Boc-Me]⁺, 118 [M-Boc-CO₂Me]⁺. IR (cm⁻¹): 2978, 1750, 1704. CHN-analysis (found/calculated), %: C 52.02/51.98, H 6.17/6.18, N 4.82/5.05. $[\alpha]_D$ = 105° (c = 1.01, CHCl₃).

(1S,3S,5R)-2-(tert-butoxycarbonyl)-6,6-difluoro-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (Boc-*trans*-4,5-F₂MePro-OH, *trans*-7)

Boc-*trans*-4,5-F₂MePro-OMe (*trans*-6, 0.5 g, 1.8 mmol) was dissolved in methanol (15 mL). A sodium hydroxide solution (1 M in water, 7.3 mL, 4 eq.) was added to the solution within two minutes upon stirring. After stirring for 6 h at room temperature, methanol was evaporated under reduced pressure (keeping the bath temperature below 40 °C). The residue was taken up in water (15 mL) and washed with diethyl ether (2 x 15 mL). The water layer

was then acidified by 1 M hydrogen chloride solution until acidic (pH 1-2) and the product was extracted with dichloromethane (4 x 15 mL). The combined extracts were dried over magnesium sulfate, filtered and evaporated giving 0.433 g of the product (91% yield) as pale fluffy crystals.

^1H -NMR (CDCl_3 , 500 MHz) – (rotamers, 5:2, δ , ppm): 8.80 (broad s, 1H, CO_2H), 4.39 (major) and 4.24 (minor) (m, 1H, $\text{CH-CO}_2\text{Me}$), 4.02 and 3.76 (m, 1H, N-CH-CF_2), 2.73 (m, 1H), 2.61 (major) and 2.47 (minor) (m, 1H, both CH_2), 2.36 (m, 1H, CH-CH-CH_2); the assignment was accomplished using HMQC experiment (see below). ^{13}C -NMR (CDCl_3 , 126 MHz) – (rotamers, δ , ppm): 176.7 and 173.6 (s, CO_2H), 157.3 and 154.1 (s, $\text{N-CO}_2\text{Bu}^t$), 111.5 (t, $J = 293$ Hz, CF_2), 83.0 and 82.0 (s, O-CMe_3), 62.6 and 61.5 (s, $\text{CH-CO}_2\text{Me}$), 43.1 and 42.6 (t, $J = 12$ Hz, N-CH-CF_2), 29.3 and 28.2 (s, $\text{O-C(CH}_3)_3$), 27.3 (s, CH_2), 26.3 and 24.6 (t, $J = 12$ Hz, CH-CH-CH_2). ^{19}F -NMR (CDCl_3 , 282 MHz) – (rotamers, δ , ppm): -129.9 (minor) and -130.8 (major) (dd, $J_{\text{F-F}} = 162$ Hz, $J_{\text{F-H}} = 12$ Hz, exo-F), -155.9 (minor) and -156.3 (major) (d, $J_{\text{F-F}} = 161$ Hz, endo-F). The two ^{19}F -NMR signals (N-Boc rotamers) merged on heating the sample to 60 °C (see below). The assignment in the ^{19}F -NMR spectrum was done based on the results from the ^1H - ^{19}F NOE saturation experiment. The $J_{\text{F-H}}$ coupling were assumed to be characteristic and used for the ^{19}F -NMR signal assignments for all the other difluorocyclopropane derivatives described below. Mass spectrum, m/z : 263 $[\text{M}]^+$ (traces), 163 $[\text{M-Boc}]^+$ (100%), 118 $[\text{M-Boc-CO}_2\text{H}]^+$. IR (cm^{-1}): broad 3300-2500, 2981, 2935, 1747, 1632. CHN-analysis (found/calculated), %: C 49.94/50.19, H 5.57/5.74, N 5.18/5.32. M.p. = 125 °C. $[\alpha]_{\text{D}} = -126^\circ$ ($c = 1.00$, CHCl_3). The crystals for the X-ray diffraction (see below) were obtained by crystallization from CDCl_3 .

***tert*-Butyl (S)-5-Oxo-pyrrolidine-2-carboxylate (Pyr-OBu^t, 10)**

The compound was prepared according to the literature procedure.^{vi} 17.8 g of the product (white crystals) was obtained from 12.9 g of the starting pyroglutamic acid **5** (yield 96%). ^1H -NMR (400 MHz, CDCl_3) δ , ppm: 6.34 (broad s, 1H, NH), 4.16 (m, 1H, $\text{HN-CH-CO}_2\text{Bu}^t$), 2.50-2.31 (m, 3H), 2.24-2.25 (m, 1H), 1.49 (s, 9H, $\text{C(CH}_3)_3$). ^{13}C -NMR (CDCl_3 , 101 MHz), δ , ppm: 177.9 (s, HN-C=O), 171.0 (s, CO_2Bu^t), 82.5 (s, CMe_3), 56.1 ($\text{HN-CH-CO}_2\text{Bu}^t$), 29.4 (s, CH_2), 28.0 (s, $\text{C(CH}_3)_3$), 24.8 (s, CH_2).

***tert*-Butyl (2S)-2-{[(2S)-2-(*tert*-butoxycarbonyl)-5-oxopyrrolidin-1-yl]carbonyl}pyrrolidine-1-carboxylate (Boc-Pro-Pyr-OBu^t, 11)**

The compound was prepared analogously to the published procedure.⁵ Sodium hydride (60% emulsion in paraffin, 0.59 g, 14.8 mmol) was washed with dry toluene under an argon atmosphere (3 x 5 mL), and then suspended in toluene (70 mL). Solid Pyr-OBu^t (**10**, 2.60 g, 14 mmol) was added to the suspension in small portions upon stirring at room temperature, and the resulting mixture was kept stirred for 30 min. Commercially available Boc-Pro-ONp (4.5 g, 13.4 mmol) was suspended in dry toluene (45 mL) and the suspension was combined with the reaction mixture under stirring. The stirring continued for 21 h. The mixture became yellow first, then color gradually changed into orange towards the end of this period. The solid material was filtered-off, the filtrate was washed with 10% sodium hydroxide aqueous solution (2 x 50 mL) and brine (2 x 75 mL), dried over magnesium sulfate, filtered and evaporated. The product was purified on a silica gel column using toluene:ethyl acetate mixture (9:2) as an eluent ($R_f = 0.4$) giving 4.25 g (83% yield) as white crystals.

¹H-NMR (CDCl₃, 400 MHz) – (two rotamers, 10:7, δ , ppm): 5.37 (m, 1H, N-CH-C=O), 4.70 (m, 1H, N-CH-C=O), 3.58 (m, 1H, N-CHH), 3.44 (m, 1H, N-CHH), 2.76-2.49 (m, 2H), 2.43-2.29 (m, 2H), 2.13-1.96 (m, 2H), 1.89 (m, 2H), 1.47, 1.46, 1.45 and 1.38 (four s, 18H, C(CH₃)₃). ¹³C-NMR (CDCl₃, 101MHz) – (two rotamers δ , ppm): 174.5, 173.8 and 173.2 (three s, two C-C(=O)-N), 170.2 and 169.9 (two s, C-CO₂Bu^t), 154.3 and 153.6 (two s, tBuO-C(=O)-N), 82.6, 82.3, 79.6 and 79.4 (four s, two O-CMe₃), 59.9, 59.8, 58.5 and 58.4 (four s, two N-CH-C=O), 47.0 and 46.7 (two s, N-CH₂), 31.9 and 31.8 (two s, CH₂), 30.2 and 29.3 (two s, CH₂), 28.5, 28.3 and 27.9 (three s, two C(CH₃)₃), 23.7 and 23.0 (two s, CH₂), 21.8 and 21.6 (two s, CH₂). Mass spectrum, m/z : 382 [M]⁺, 326 [M-Bu^t]⁺, 270 [M-2Bu^t]⁺, 253, 255 [M-(Bu^t,Boc)]⁺. IR (cm⁻¹): 2976, 2940, 2891, 1740, 1691. CHN-analysis (found/calculated), %: C 59.62/59.67, H 7.75/7.91, N 7.19/7.32. M.p. = 106 °C. $[\alpha]_D = -116^\circ$ ($c = 1.01$, CHCl₃).

***tert*-Butyl (5S)-1-{[(2S)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]carbonyl}-2-pyrrolin-5-carboxylate (Boc-Pro-DhPro-OBu^t, 12)**

Boc-Pro-Pyr-OBu^t (**11**, 3 g, 7.9 mmol) was dissolved in dry THF (50 mL) under an argon atmosphere. The solution was cooled in a dry ice-acetone bath. LiBHEt₃ (1M solution in THF, 8.6 mL, 8.6 mmol) was added within 2 min to the cooled solution upon stirring and the resulting mixture was kept stirred for 1 h. 4-Dimethylaminopyridine (50 mg, 0.4 mmol), DIPEA (8.2 mL, 47.4 mmol) and trifluoroacetic acid anhydride (1.6 mL, 11.9 mmol) were added sequentially. The mixture was stirred for the next 20 h, during which the bath was allowed to warm to the room temperature. Water (10 mL) was added, organic solvents were removed under reduced pressure and water layer was extracted by diethyl ether (3 x 75 mL). The combined extracts were dried over magnesium sulfate, filtered and evaporated. The crude product was purified by silica gel column chromatography (eluent - toluene:ethyl acetate (9:2), R_f = 0.6) obtaining 2 g of the Boc-Pro-DhPro-OBu^t (70% yield) as fluffy crystals.

¹H-NMR (CDCl₃, 400 MHz, two rotamers, 10:9, δ, ppm): 6.55 (m, 1H, N-CH=CH), 5.07 (m, 1H), 4.70 (m, 1H), 4.52 and 4.39 (two m, 1H), 3.53 (m, 1H), 3.42-3.28 (m, 1H), 2.97-2.86 (m, 1H), 2.55-2.43 (three m, 1H), 2.18-2.05 (m, 1H), 2.05-1.91 (m, 2H), 1.86-1.74 (m, 1H), 1.39, 1.38, 1.38 and 1.31 (four s, 18H). ¹³C-NMR (CDCl₃, 101 MHz, two rotamers, δ, ppm): 170.2, 170.0, 168.7 and 168.1 (two C-C=O groups), 154.5 and 153.7 (Bu^tO-C(=O)-N), 128.2 and 128.1 (N-CH=CH), 109.2 and 109.1 (N-CH=CH), 81.6, 81.4 and 79.6 (two O-CMe₃ groups), 58.6, 58.5 and 57.5 (two N-CH-C=O groups), 46.8 and 46.6 (N-CH₂), 33.3 and 33.2 (CH₂), 30.4 and 29.3 (CH₂), 28.5, 28.4 and 27.1 (two C(CH₃)₃ groups), 24.2 and 23.6 (CH₂). Mass spectrum, m/z: 366 [M]⁺, 293 [M-OBu^t]⁺, 237 [M-(OBu^t, Bu^t)]⁺, 209 [M-(Bu^t, Boc)]⁺. IR (cm⁻¹): 2971, 2926, 2870, 1746, 1698, 1660, 1625. CHN-analysis (found/calculated), %: C 62.21/62.27, H 8.13/8.25, N 7.40/7.64. M.p. = 130°C. [α]_D = -160° (c = 1.01, CHCl₃).

***tert*-Butyl (1*S*,3*S*,5*R*)-6,6-difluoro-2-{[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]carbonyl}-2-azabicyclo[3.1.0]hexane-3-carboxylate (Boc-Pro-*trans*-4,5-F₂MePro-OBu^t, *trans*-13)**

Boc-Pro-DhPro-OBu^t (**12**, 1 g, 2.7 mmol) was dissolved in dry diglyme (25 mL), and the solution was immersed in an oil bath which was then heated to 175 °C. Suspension of sodium difluorochloroacetate (11.33 g, 74.6 mmol) in diglyme (55 mL) was added within 20 min to the stirred solution, keeping the bath temperature in the range of 174-179 °C. After additional 10 min of incubation upon stirring the bath was removed and the mixture was allowed to cool to the room temperature. The solution was filtered, evaporated, and the residue was subjected to the two successive purifications with silica gel column chromatography (eluent -, hexane:ethyl acetate (2:1), R_f = 0.3). The mixed fractions of Boc-Pro-*cis*-4,5-F₂MePro-OBu^t and Boc-Pro-*trans*-4,5-F₂MePro-OBu^t eluted first, followed by the fractions containing pure Boc-Pro-*trans*-4,5-F₂MePro-OBu^t. The overall yield of the collected isomers was 657 mg (58%), the ratio of the isomers in the initial mixture (1:5) was estimated from ¹⁹F-NMR spectra and from analytical RP-HPLC. Pure Boc-Pro-*trans*-4,5-F₂MePro-OBu^t (**trans-13**) was obtained as a white solid.

¹H-NMR (CDCl₃, 400 MHz, two rotamers, 5:2, δ , ppm): 4.53 (m, both N-CH-C=O of the minor isomer, both amino acid residues), 4.47 (m, N-CH-C=O major isomer, the F₂MePro residue), 4.40 (m, N-CH-C=O, major isomer, the Pro residue) – 2H in total, 3.72 (m, 1H, N-CH-CF₂), 3.53-3.30 (m, 2H, N-CH₂), 2.44 (m, 2H, CF₂-CH-CHH), 2.24 (m, 2H, CF₂-CH-CHH and N-C(=O)-CH-CHH), 2.01 (m, 1H, N-C(=O)-CH-CHH), 1.96-1.77 (m, 2H, N-CH₂-CH₂), 1.39 and 1.33 (two s, C(CH₃)₃). ¹³C-NMR (CDCl₃, 101 MHz, two rotamers δ , ppm): 171.6 and 171.5, 170.2 and 170.0 (two C-C=O), 154.3 and 154.0 (Bu^tO-C(=O)-N), 111.6 (t, J = 295 Hz, CF₂), 82.1 and 81.9, 79.9 and 79.4 (two O-CMe₃), 64.6 and 64.4 (N-CH-C=O the F₂MePro residue), 58.3 and 58.0 (N-CH-C=O, the Pro residue), 46.9 and 46.7 (N-CH₂), 41.9 (m, N-CH-CF₂), 30.4 and 29.4 (CH₂), 28.5, 28.3 and 27.9 (two C(CH₃)₃), 27.7 (t, J = 12 Hz, CF₂-CH-CH₂), 27.0 (CH₂), 23.9 and 23.1 (CH₂). ¹⁹F-NMR (CDCl₃, 282 MHz, two rotamers, δ , ppm): -126.7 (minor), -127.0 (major) (ddd, J_{F-F} = 158 Hz, J_{F-H} = 14, 3 Hz, *exo*-F), -154.2 (major), -154.5 (minor) (d, J_{F-F} = 158 Hz, *endo*-F). Mass spectrum, m/z: 417 [M]⁺, 360 [M-Bu^t]⁺, 343 [M-OBu^t]⁺, 287 [M-(OBu^t, Bu^t)]⁺, 259 [M-(Bu^t, Boc)]⁺. IR (cm⁻¹): 2973, 2931, 2877, 1718, 1688, 1662. CHN-analysis (found/calculated), %: C 57.44/57.68, H 7.01/7.26, N 6.48/6.73. M.p. = 152 °C. [α]_D = -138° (c = 1.01, CHCl₃).

***tert*-Butyl (1R,3S,5S)-6,6-difluoro-2-[[*(2S)*-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]carbonyl]-2-azabicyclo[3.1.0]hexane-3-carboxylate (Boc-Pro-*cis*-4,5-F₂MePro-OBu^t, *cis*-13)**

The mixed fraction from the previous synthesis (see the procedure above, 120 mg) was dissolved in a THF-dichloromethane mixture (0.5 mL) and subjected to an RP-HPLC purification on the preparative scale. Water-methanol gradient (48 to 66%) was used for elution, the flow rate was 20 mL/min. The *trans*-isomer (Boc-Pro-*trans*-4,5-F₂MePro-OBu^t) eluted first (49 mg), followed by the *cis*-isomer, Boc-Pro-*cis*-4,5-F₂MePro-OBu^t (54 mg). The solvent was removed under reduced pressure and the residue was lyophilized yielding the purified compound as white sugar-like crystals.

¹H-NMR (CDCl₃, 300 MHz, two rotamers, 5:2, δ, ppm): 4.78 (m, 1H, N-CH-CO₂Bu^t), 4.55 and 4.45 (two m, 1H, N-CH-C(=O)-N), 4.19 and 3.91 (two m, 1H, N-CH-CF₂), 3.60-3.29 (m, 2H, N-CH₂), 2.57 and 2.21 (two m, 2H, CH₂, the F₂MePro residue), 2.32 (m, 1H, CF₂-CH-CH₂), 2.05 (m, 3H, CH-CH₂-CHH-CH₂), 1.81 (m, 1H, CH-CH₂-CHH-CH₂), 1.38, 1.35 and 1.34 (three s, 18H, two C(CH₃)₃). ¹³C-NMR (CDCl₃, 101 MHz, two rotamers δ, ppm): 171.6 and 171.5 (CO₂Bu^t), 168.6 and 168.4 (C(=O)-N), 154.7 and 153.5 (N-C(=O)-OBu^t), 111.5 (t, J = 293 Hz, CF₂), 81.7 and 81.5, 79.8 and 79.7 (CMe₃), 64.0 and 62.8 (N-CH-C=O, the F₂MePro residue), 57.9 and 57.9 (N-CH-C=O, the Pro residue), 46.9 and 46.7 (N-CH₂), 43.6 (m, J = 17Hz, N-CH-CF₂), 30.6 and 29.6 (CH₂), 28.5, 28.4 and 27.8 (C(CH₃)₃), 27.5 (m, CF₂-CH-CH₂), 26.3 (CH₂), 24.4 and 23.6 (CH₂). ¹⁹F-NMR (CDCl₃, 282 MHz, two rotamers, δ, ppm): -126.0 (minor) and -126.3 (major) (dd, J_{F-F} = 162 Hz, J_{F-H} = 14 Hz, *exo*-F), -151.7 (major) and -151.9 (minor) (d, J_{F-F} = 162 Hz, *endo*-F). IR (cm⁻¹): 2973, 2939, 2882, 1746, 1681. CHN-analysis (found/calculated), %: C 57.55/57.68, H 7.16/7.26, N 6.31/6.73. M.p. = 119 °C. [α]_D = -48° (c = 0.98, CHCl₃).

The configuration of the isomers was first established using ¹⁹F-¹H NOE NMR experiment. The major isomer showed stronger NOE correlation between α-CH <-> *endo*-F resonances (comparable to that for Boc-*trans*-F₂MePro-OH) than in the case of the minor isomer (see below). Crystals of Boc-Pro-*cis*-4,5-F₂MePro-OBu^t suitable for the X-ray analysis were grown from methanol. The X-ray diffraction data later confirmed the configuration.

(1S,3S,5R)-6,6-difluoro-2-[(2S)-1-(9H-fluoren-9-ylmethoxy)carbonylpyrrolidin-2-yl]carbonyl}-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (Fmoc-Pro-*trans*-4,5-F₂MePro-OH, S3)

Boc-Pro-*trans*-4,5-F₂MePro-OBu^t (*trans*-**13**, 130 mg, 0.31 mmol) was dissolved in dichloromethane (6 mL). Trifluoroacetic acid (3 mL) was added to the solution upon stirring at room temperature and the stirring continued for the next 1 h. The volatiles were removed under reduced pressure. The residue was dissolved in dichloromethane (5 mL) and evaporated under reduced pressure, this operation was performed twice. 1 M sodium carbonate aqueous solution (1.5 mL) was added, followed by acetone (1 mL) and water (3 mL). The mixture was cooled in an ice bath. Fmoc-OSu (116 mg, 0.34 mmol) in acetone (1 mL) was added within 1 min, water (1 mL) and acetone (5 mL) were additionally added (the composition was adjusted such that the solution was eventually transparent), the bath was removed and the mixture was stirred the next 23 h at ambient temperature. Acetone was removed under reduced pressure, the residue was poured into water (40 mL), the resulting mixture was washed with diethyl ether (2 x 15 mL), and the organic layers were discarded. The remaining water layer was acidified with 1M aqueous hydrogen chloride until pH 1-2 and extracted with ethyl acetate (4 x 25 mL). The combined ethyl acetate extracts were dried over sodium sulfate, filtered, and evaporated. The residue was taken-up in acetonitrile-water and lyophilized. Fmoc-Pro-*trans*-4,5-F₂MePro-OH (141 mg, 94% yield) was obtained as white powder.

¹H-NMR (CDCl₃, 400 MHz, two rotamers, 5:2, δ, ppm): 7.67 (m, 2H), 7.56-7.44 (m, 2H), 7.31 (m, 2H), 7.23 (m, 2H), 7.07 (broad s, 1H), 4.70-4.10 (m, 5H), 3.73-3.44 (m, 3H), 2.57-1.83 (m, 7H). ¹³C-NMR (CDCl₃, 101 MHz, two rotamers, δ, ppm): 174.0, 173.7, 172.3, 171.9, 155.0, 154.6, 144.2, 144.1, 144.1, 143.8, 141.3, 141.3, 141.2, 127.8, 127.7, 127.6, 127.1, 126.8, 125.4, 125.3, 125.1, 125.1, 120.0, 119.9, 68.3, 67.7, 64.6, 64.4, 58.7, 58.3, 47.4, 47.2, 47.1, 46.8, 41.8 (m), 30.6, 29.3, 28.3 (t, J = 11 Hz), 28.0, 26.4, 26.2, 25.4, 24.3, 23.3. ¹⁹F-NMR (CDCl₃, 282Hz, two rotamers, δ, ppm): -128.0 (dm, J = 161 Hz, *exo*-F), -153.9 (minor) and -154.1 (major) (two d, J = 160 Hz, *endo*-F). Mass spectrum, m/z: 178 [fluorenylmethyl]⁺. IR (cm⁻¹): 3600-2400 (broad), 2958, 2889, 1706, 1674, 1522. M.p.=55-65 °C. [α]_D = -109° (c = 1.00, CHCl₃).

(1R,3S,5S)-6,6-difluoro-2-[(2S)-1-(9H-fluoren-9-ylmethoxy)carbonylpyrrolidin-2-yl]carbonyl}-2-azabicyclo[3.1.0]hexane-3-carboxylic acid (Fmoc-Pro-*cis*-4,5-F₂MePro-OH, S4)

The compound was prepared analogously to Fmoc-Pro-*trans*-4,5-F₂MePro-OH (**S3**) above, starting from the Boc-Pro-*cis*-4,5-F₂MePro-OBu^t (*cis*-**13**, 42 mg, 0.1 mmol). As the amount of the compound synthesized (33 mg, 67% yield) was too small for its full characterization, only ¹⁹F-NMR spectrum was measured and the compound was immediately used for the peptide synthesis. The peptide synthesized from the product was then fully characterized (see below).

¹⁹F-NMR (CDCl₃, 282 MHz, two rotamers, 2:1, δ, ppm): -126.9 (minor) and -127.4 (major) (dm, J_{F-F} = 162 Hz, *exo*-F), -151.4 (major) and -151.8 (minor) (d, J_{F-F} = 163 Hz, *endo*-F).

¹H-NMR spectrum of Boc-*trans*-4,5-F₂MePro-OMe (*trans*-6, CDCl₃, 400 MHz).

¹³C-NMR spectrum of Boc-*trans*-4,5-F₂MePro-OMe (*trans*-6, CDCl₃, 101 MHz).

¹H-NMR spectrum of Boc-*trans*-4,5-F₂MePro-OH (*trans*-7, CDCl₃, 500 MHz).

in CDCl₃

¹³C-NMR spectrum of Boc-*trans*-4,5-F₂MePro-OH (*trans*-7, CDCl₃, 126 MHz).

^1H - ^{13}C HMQC spectrum of Boc-*trans*-4,5-F₂MePro-OH (*trans*-7, CDCl₃, 500 and 126 MHz).

```

NAME      kubmz20
EXPNO     6
PROCNO    1
Date_     20090321
Time      3.43
INSTRUM   spect
PROBHD    5 mm Multinucl
PULPROG   hmqcqh
TD         2048
SOLVENT   CDCl3
NS         32
DS         16
SWH        5144.033 Hz
FIDRES     2.511735 Hz
AQ         0.1991156 sec
RG         256
DW         97.200 usec
DE         6.00 usec
TE         298.1 K
CNST2     145.0000000
d0         0.00000300 sec
D1         1.50000000 sec
d2         0.00344828 sec
d13        0.00000400 sec
IN0        0.00002368 sec
MCREST     0.00000000 sec
MCWRK     1.50000000 sec
    
```

```

===== CHANNEL f1 =====
NUC1      1H
P1        16.70 usec
p2        33.40 usec
PL1       0.00 dB
SFO1      499.9323997 MHz
    
```

```

===== CHANNEL f2 =====
CPDPRG2   garp
NUC2      13C
P3         9.60 usec
PCPD2     53.98 usec
PL2       3.00 dB
PL12      18.00 dB
SFO2      125.7180574 MHz
ND0        2
TD         512
SFO1      125.7181 MHz
FIDRES     41.248680 Hz
SW         167.990 ppm
FnMODE     QF
SI         1024
SF         499.9300000 MHz
WDW        QSINE
SSB        2
LB         0.00 Hz
GB         0
PC         1.40
SI         1024
MC2        QF
SF         125.7074980 MHz
WDW        QSINE
SSB        2
LB         0.00 Hz
GB         0
    
```

Temperature-induced changes in the appearance of the ^{19}F -NMR spectrum of Boc-*trans*-4,5- $\text{F}_2\text{MePro-OH}$ (*trans*-7, $\text{H}_2\text{O-CH}_3\text{CN}$, 282 MHz)

```

NAME   Boc-F2MePro-OH
EXPNO   22
PROCNO   1
Date_    20110323
Time     20.54
INSTRUM   spect
PROBHD   10 mm Multinuc
PULPROG   hpdec
TD       16384
SOLVENT   H2O- acetonitrile
NS       16
DS       0
SWH      100000.000 Hz
FIDRES   6.103516 Hz
AQ       0.0819750 sec
RG       8192
DW       5.000 usec
DE       10.00 usec
TE       variable
D1       1.50000000 sec
TD0      1
    
```

```

===== CHANNEL f1 =====
NUC1     19F
P1       10.00 usec
PL1      12.00 dB
SFO1     282.3899245 MHz
    
```

```

===== CHANNEL f2 =====
NUC2     1H
PL2      12.00 dB
PL12     120.00 dB
SFO2     300.1587580 MHz
SI       16384
SF       282.4304534 MHz
WDW      no
SSB      0
LB       0.00 Hz
GB       0
PC       1.00
    
```

¹H-NMR spectrum of Boc-Pro-Pyr-OBu^t (11, CDCl₃, 400 MHz).

^{13}C -NMR spectrum of Boc-Pro-Pyr-OBu^t (11, CDCl₃, 101 MHz).

in CDCl₃

^1H -NMR spectrum of Boc-Pro-DhPro-OBu^t (12, CDCl₃, 400 MHz).

in CDCl₃

^{13}C -NMR spectrum of Boc-Pro-DhPro-OBu^t (12, CDCl₃, 101 MHz).

in CDCl₃

^1H -NMR spectrum of Boc-Pro-*trans*-4,5- F_2MePro -OBu^t (*trans*-13, CDCl_3 , 400 MHz).

in CDCl_3

^{13}C -NMR spectrum of Boc-Pro-*trans*-4,5-F₂MePro-OBu^t (*trans*-13, CDCl₃, 101 MHz).

¹H-NMR spectrum of Boc-Pro-*cis*-4,5-F₂MePro-OBu^t (*cis*-13, CDCl₃, 300 MHz).

in CDCl₃

^{13}C -NMR spectrum of Boc-Pro-*cis*-4,5- F_2MePro -OBu^t (*cis*-13, CDCl_3 , 101 MHz).

¹H-NMR spectrum of Fmoc-Pro-*trans*-4,5-F₂MePro-OH (S3, CDCl₃, 400 MHz).

^{13}C -NMR spectrum of Fmoc-Pro-*trans*-4,5-F₂MePro-OH (S4, CDCl₃, 101 MHz).

Heteronuclear ^{19}F - ^1H NOE experiment for Boc-Pro-*trans*-4,5- $\text{F}_2\text{MePro-OBu}^t$ (*trans*-13). Lower trace: ^1H -NMR spectrum; middle trace: ^1H NOE difference spectrum recorded under saturation of the ^{19}F -NMR resonance at -127.57 ppm (exo-F); upper trace: ^1H NOE difference spectrum recorded under saturation of the ^{19}F -NMR resonance at -154.21 ppm (endo-F).

^{19}F saturation at -154.21ppm (endo-F)

^{19}F saturation at -127.57ppm (exo-F)

Boc-Pro-F2MePro-OBu
major isomer

```

NAME 01Apr2012-Boc-Pro-F2MePro-2major
EXPNO 122
PROCNO 1
Date_ 20120402
Time 5.38
INSTRUM spect
PROBHD 10 mm Multinuc
PULPROG noediff
TD 65536
SOLVENT CDCl3
NS 2048
DS 0
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2769001 sec
RG 256
DW 50.000 usec
DE 10.00 usec
TE 301.3 K
D1 2.00000000 sec
d11 0.03000000 sec
D20 0.50000000 sec
DELTA1 1.50000000 sec
L4 1
NBL 3

===== CHANNEL f1 =====
NUC1 1H
P1 20.00 usec
PL1 5.75 dB
SFO1 300.1734217 MHz

===== CHANNEL f2 =====
NUC2 19F
PL2 12.00 dB
PL14 40.00 dB
SFO2 282.4325830 MHz
SI 131072
SF 300.1735708 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
    
```


Heteronuclear ^{19}F - ^1H NOE experiment for Boc-Pro-*cis*-4,5- $\text{F}_2\text{MePro-OBu}^t$ (*cis*-13). Lower trace: ^1H -NMR spectrum; middle trace: ^1H NOE difference spectrum recorded under saturation of the ^{19}F -NMR resonance at -127.57 ppm (exo-F); upper trace: ^1H NOE difference spectrum recorded under saturation of the ^{19}F -NMR resonance at -154.21 ppm (endo-F).

Peptide synthesis

Val-Arg-Leu-Pro-Pro-Pro-Val-Arg-Leu-Pro-*trans*-4,5-F₂MePro-Pro-Val-Arg-Leu-Pro-Pro-Pro (SAP-11(Pro)-*trans*-4,5-F₂MePro, 14)

The peptide was synthesized manually by the solid phase method using standard Fmoc strategy^{vii,viii}. Proline pre-loaded 2-chlorotrytyl resin (85 mg, 100-200 mesh, 0.59 mmol/g loading) was used. Corresponding Fmoc amino acids (6 eq. calculating on the resin loading, 0.3 mmol each: Fmoc-Pro, 101.5 mg; Fmoc-Leu, 106.3 mg; Fmoc-(Pbf)Arg, 195.2 mg; and Fmoc-Val, 102.1 mg) were pre-mixed with 6-Cl-HOBt (51.1 mg, 0.3 mmol, 6 eq.), TBTU (93.4 mg, 0.24 mmol, 4.8 eq.), and DIPEA (105 μ L, 0.6 mmol, 12 eq.) in N-methylpyrrolidone (2 mL) for 2 min immediately before coupling and then added to the resin. For the coupling of the Fmoc-Pro-*trans*-4,5-F₂MePro-OH, two equivalents of the dipeptide were used (calculating on the resin loading, 48.4 mg). The dipeptide was pre-mixed with 6-Cl-HOBt (17 mg, 0.1 mmol, 2 eq.), TBTU (31.4 mg, 1.6 eq.), and DIPEA (35 μ L, 4 eq.) in N-methylpyrrolidone (2 mL) for 2 min before coupling. Standard coupling time for the amino acids was 2 h, but extended to 4 h for the dipeptide and to 3 h for the amino acid (Pro) next to the dipeptide. Fmoc-protection was cleaved by mixing the resin with 22% piperidine in dimethylformamide (1.5 mL) for 30 min. After the synthesis, the resin was washed sequentially with methanol, THF, acetonitrile, and dichloromethane and dried in vacuum for 24 h. The peptide was cleaved from the resin by the treatment with 95% trifluoroacetic acid (2 x 2 mL). The trifluoroacetic acid was blown-off by the argon flow, the residue was lyophilized from acetonitrile-water. Purification was done by RP-HPLC on a semi-preparative scale.

¹⁹F-NMR (H₂O, 282 MHz, δ , ppm): -129.3 (dd, J_{F-F} = 161 Hz, J_{F-H} = 12 Hz), -152.9 (d, J_{F-F} = 161 Hz). MALDI-TOF mass spectrum, m/z : 2046 [M+H]⁺ (expected: 2046).

Analytical RP-HPLC chromatogram, ^{19}F -NMR (H_2O , 282 MHz) and MALDI-TOF mass spectra of SAP-11(Pro)-*trans*-4,5- F_2MePro (14).

Val-Arg-Leu-Pro-Pro-Pro-Val-Arg-Leu-Pro-*cis*-4,5-F₂MePro-Pro-Val-Arg-Leu-Pro-Pro-Pro (SAP-11(Pro)-*cis*-4,5-F₂MePro, 15) was synthesized and purified analogously to SAP-11(Pro)-*trans*-4,5-F₂MePro (above), using 3 times less resin (28 mg). The peptide was cleaved from the resin by the treatment with hexafluoroisopropanol-dichloromethane cleavage cocktail (3:7 vol; 2 x 15 min). After blowing-off the solvent (argon flow) and lyophilisation (from acetonitrile-water), the crude mixture was purified on semi-preparative RP-HPLC column (isocratic elution with A:B 1:1 for 10 minutes). After lyophilisation, the peptide was treated with trifluoroacetic acid-water (95:5 vol., with few drops of triisopropylsilane) cocktail (5 mL) in order to deprotect the arginine side chains. After one hour the deprotection was complete (RP-HPLC monitoring), the cocktail was blown-off (argon current), the peptide was lyophilized (from acetonitrile-water). The crude peptide was subjected to a semi-preparative scale RP-HPLC. 2.6 mg of peptide was obtained after lyophilisation.

¹⁹F-NMR (H₂O, 282 MHz, δ , ppm): -125.9 (dm, J_{F-F} = 160 Hz, J_{F-H} = 14 Hz), -151.7 (d, J_{F-F} = 159 Hz). MALDI-TOF mass spectrum, m/z : 2046 [M+H]⁺ (expected: 2046).

Analytical RP-HPLC chromatogram, ^{19}F -NMR (H_2O , 282 MHz) and MALDI-TOF mass spectra of SAP-11(Pro)-*cis*-4,5- F_2MePro (15).

Kinetics of the F₂MePro·TFA decomposition

Boc-F₂MePro-OH (12 mg) dissolved in CDCl₃ (0.4 mL) was mixed with TFA (200 μL) at room temperature. After 15 min, the volatilities were blown-off by the argon flow within 2 min. The solid residue was washed with CDCl₃. The ¹H- and ¹⁹F-NMR spectra of the washings showed that they contained only the starting material (Boc-F₂MePro-OH). The crystalline residue was taken up into D₂O and a series of ¹H-, ¹⁹F-NMR spectra were run at 23 °C. The relaxation delays (acquisition time + recycling delay) were set as follows: 5.28 (3.28 + 2.00) sec for ¹H-NMR measurements, 3.02 (1.02 + 2.00) sec for ¹⁹F-NMR measurements. The first set of spectra (starting point) was measured ca. 40 min after the addition of TFA. According to the NMR spectral data, the solution contained F₂MePro-OH·TFA and the starting Boc-F₂MePro-OH in a ca. 10:1 ratio. The signals from the amino acid salt disappeared within the next 200 min (see plots below).

The amount of the main decomposition product (assigned to the α-unsaturated aldehyde **9**, here named as additive) increased during the degradation, reaching its maximum around 200th min, and then decreased. The structure of the additive was suggested based on the ¹⁹F-NMR resonances (dd, 32 and 19 Hz at -131.1 ppm) and ¹H-NMR spectrum (d, 19 Hz at 9.21 ppm; dt, 32, 7 Hz at 6.31 ppm; t, 7 Hz at 4.26 ppm; m, at 3.02 ppm). Another intermediate gave a broad signal at -160.0 in the ¹⁹F-NMR spectrum, which showed complicated evolution, and significantly decreased in the intensity towards at the end of the measurements.

a) ^{19}F -NMR peak intensities (I) of the: CF_2 group in $\text{F}_2\text{MePro-OH-TFA}$ (dm at -129.8 ppm, exo-F and d at -148.3 ppm, endo-F; $J_{\text{F-F}} = 178 \text{ Hz}$) and the signal from the additive (dd at -131.1 ppm), plotted against time (t). b) Linearization of the time-dependence in the coordinates: $\ln I_{\text{endo-F}} - \text{time}$. c) Evolution of the ormalized peak intensities (I_n) with time, as observed in the ^{19}F - and ^1H -NMR spectra of the compound 9.

Representative ^1H -NMR spectra of $\text{F}_2\text{MePro-OH-TFA}$, showing its time-dependent decomposition in D_2O at 23°C .

NAME 08Mar2012-Boc-F2MePro-OH
 EXPNO 11
 PROCNO 1
 Date_ 20120308
 Time_ 14.45
 INSTRUM spect
 PROBHD 5 mm Multinuc
 PULPROG zg
 TD 65536
 SOLVENT D2O
 NS 16
 DS 0
 SWH 10000.000 Hz
 FIDRES 0.152588 Hz
 AQ 3.2769001 sec
 RG 256
 DW 50.000 usec
 DE 10.00 usec
 TE 296.4 K
 D1 2.00000000 sec
 TD0 1

===== CHANNEL f1 =====
 NUC1 1H
 P1 30.00 usec
 PL1 9.00 dB
 SFO1 300.1581668 MHz
 SI 65536
 SF 300.1577425 MHz
 WDW no
 SSB 0
 LB 0.00 Hz
 GB 0
 PC 1.00

Representative ^{19}F -NMR spectra of $\text{F}_2\text{MePro-OH}\cdot\text{TFA}$, showing its time-dependent decomposition in D_2O at 23°C .

Molecular and crystal structure of compound *trans*-7.

Figure S1. Structure of compound *trans*-7, according to X-ray diffraction data. Thermal ellipsoids are shown at the 50% probability level.

The colourless crystals of *trans*-7 ($C_{11}H_{15}NO_4F_2$) are orthorhombic. At 293 K $a = 6.3209(3)$, $b = 10.0193(6)$, $c = 19.857(1)$ Å, $V = 1257.6(1)$ Å³, $M_r = 263.24$, $Z = 4$, space group $P2_12_12_1$, $d_{\text{calc}} = 1.390$ g/cm³, $\mu(\text{MoK}\alpha) = 0.124$ mm⁻¹, $F(000) = 552$. Compound *trans*-7 is crystallized in non-centrosymmetric space group which means that one enantiomer only presents in the crystal phase. The configurations of the chiral centres at the C1 and C2 atoms (S- and R-, respectively) were determined relatively to the known configuration of the chiral centre at the C4 atom (S-configuration).

Tetrahydropyrrole and cyclopropane rings (Figure S1) are *cis*-type jointed (the H1-C1-C2-H2 torsion angle is 0°). The saturated heterocyclic ring adopts a flattened envelope conformation. The deviation of the C4 atom from the mean plane of the remaining atoms of the ring is -0.15 Å. The carboxylic substituent is in axial position and is almost orthogonal to the plane of the heterocycle (the C2-C3-C4-C6 and C3-C4-C6-O1 torsion angles are -111.7(2)° and -97.0(2)°, respectively). The carboxylic fragment of the substituent at the N1 atom is coplanar to the C1-N1 endocyclic bond (the C1-N1-C7-O3 torsion angle is 4.0(2)°). The *tert*-butyl group has *ap*-conformation relatively the N1-C7 bond and is turned in such a way that the C8-C9 bond is

antiperiplanar to the C7-O4 bond (the C8-O4-C7-N1 and C7-O4-C8-C9 torsion angles are $169.1(1)^\circ$ and $179.1(1)^\circ$, respectively).

In the crystal phase the molecules of *trans*-7 form the infinite chains (Figure S2) along the [1 0 0] crystallographic direction bonded by the O2-H...O3' ($x-1, y, z$) intermolecular hydrogen bond (H...O 1.87 Å O-H...O 175°). The formation of the hydrogen bond results also in the elongation of the O3-C7 bond ($1.228(1)$ Å) as compared with its mean value^{ix} of 1.210 Å and the shortening of the O2-C6 bond ($1.325(2)$ Å as compared with 1.333 Å).

Figure S2. Packing of the molecules *trans*-7 in the crystal phase.

Molecular and crystal structure of the compound *cis*-13.

Figure S3. Structure of compound *cis*-13, according to X-ray diffraction data. Thermal ellipsoids are shown at the 50% probability level.

The colourless crystals of *cis*-13 ($C_{20}H_{30}N_2O_5F_2$) are orthorhombic. At 293 K $a = 9.8867(4)$, $b = 10.9713(4)$, $c = 20.0679(8)$ Å, $V = 2176.8(2)$ Å³, $M_r = 416.46$, $Z = 4$, space group $P2_12_12_1$, $d_{\text{calc}} = 1.271$ g/cm³, $\mu(\text{MoK}\alpha) = 0.102$ mm⁻¹, $F(000) = 888$. The compound *cis*-13 is crystallized in non-centrosymmetric space group which means that one enantiomer presents only in the crystal phase. The configurations of the chiral centres at the C1 and C3 atoms (R- and S-, respectively) were determined relatively to the known configurations of the chiral centres at the C5 and C12 atoms (S- configurations for both).

Tetrahydropyrrole and propane cycles (Figure S3) are *cis*-type joined (the H1-C1-C3-H3 torsion angle is 1°). At that the saturated heterocycle is planar within 0.01 Å. The carboxylic fragment of the substituent at the C5 atom is turned relatively the N1-C5 endocyclic bond (the N1-C5-C6-O1 torsion angle is -29.0(2)°). The *tert*-butyl group is located in *ap*-conformation relatively the C5-C5 bond and is turned in such way that the C7-C9 bond is antiperiplanar to the C6-O2 bond (the C7-O2-C6-C5 and C6-O2-C7-C9 torsion angles are 174.8(1)° and -176.0(2)°, respectively). The carbonyl group of the substituent at the nitrogen atom is almost coplanar to the heterocycle plane (the C5-N1-C11-O3 torsion angle is -6.5(2)°). Tetrahydropyrrole ring adopts a twist conformation where deviations of the C13 and C14

atoms are -0.27 Å and 0.29 Å, respectively. This cycle is turned in such a way that the O3-C11-C12-N2 torsion angle is -25.9(2)°. The angle between planar heterocycle and the flat fragment of the other heterocycle is 86°. The steric repulsion is observed between two heterocycles (the shortened intramolecular contacts H1...H12 2.24 Å (the sum of the van der Waals radii^x is 2.34 Å), H12...C1 2.56 Å (2.87 Å)). The carboxylic fragment of the substituent at the N2 atom is almost coplanar to the C15-N2 bond (the C15-N2-C16-O4 torsion angle is 7.3(3)°). The *tert*-butyl group of this substituent is located in *ap*-conformation relatively the C16-N2 bond and is turned in such a way that the C17-C18 bond is antiperiplanar to the C16-O5 bond (the C17-O5-C16-N2 and C16-O5-C17-C18 torsion angles are -163.9(2)° and -178.2(2)°, respectively).

Experimental details

For compound ***trans*-7**, intensities of 7979 reflections (3665 independent, $R_{\text{int}}=0.017$) were measured on the «Xcalibur-3» diffractometer (graphite monochromated MoK_α radiation, CCD detector, ω -scanning, $2\Theta_{\text{max}} = 60^\circ$). For compound ***cis*-13**, intensities of 12550 reflections (6302 independent, $R_{\text{int}}=0.026$) were measured on the same instrument.

The structures were solved by a direct method using SHELXTL package.^{xi} Positions of the hydrogen atoms were identified from the electron density difference maps and refined using the “rider” model with $U_{\text{iso}} = nU_{\text{eq}}$ of the carrier atom, connected to the given hydrogen ($n = 1.5$ for methyl groups and $n = 1.2$ for other hydrogens). The hydrogen atom of the carboxyl group was refined using isotropic approximation.

The final anisotropic full-matrix least-squares refinement of the structures against F^2 (for the non-hydrogen atoms using 3608 (***trans*-7**), and 6236 (***cis*-13**) reflections) converged to: $wR_2 = 0.088$ ($R_1 = 0.036$ for 2832 reflections with $F > 4\sigma(F)$, $S = 1.024$) for the structure ***trans*-7**; and $wR_2 = 0.106$ ($R_1 = 0.044$ for 4214 reflections with $F > 4\sigma(F)$, $S = 0.967$) for the structure ***cis*-13**. The final atomic coordinates, and crystallographic data for molecules ***trans*-7** and ***cis*-13** have been deposited to the Cambridge Crystallographic Data Centre, 12 Union Road, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk) and are available on request quoting the deposition numbers CCDC 871117 for ***trans*-7** and CCDC 888092 for ***cis*-13**).

Supplementary references:

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