Electronic Supplementary Information

Comparative effects of trifluoromethyl- and methyl-group substitutions in proline

by V. Kubyshkin,[a] S. Pridma[b] and N. Budisa[a]

a Biocatalysis group, Institute of Chemistry, Technical University of Berlin, Müller-Breslau-Str. 10, Berlin 10623, Germany

b Enamine Ltd., Chervonotkatska 78, Kyiv 02094, Ukraine

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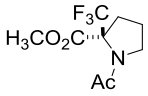
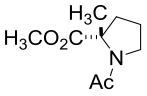
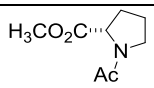
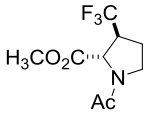
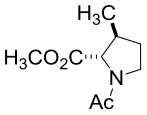
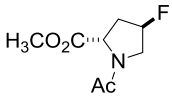
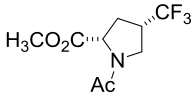
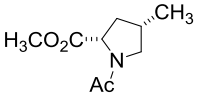
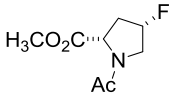
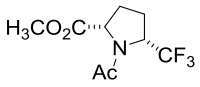
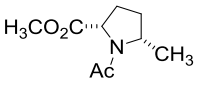
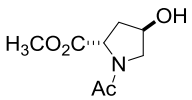
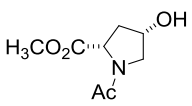
Supporting tables

Table S1 Summarized amide rotation properties as determined in salt samples of *N*-acetyl amino acids in aqueous medium by NMR.

AA	Ac-AA-O$^-$					
	$K_{trans/cis}$ (at 298 K)	kinetics				
		T, K	k, s^{-1}		$E^{\neq}$, kJ mol^{-1}	
			cis→trans	*trans→cis*	*cis→trans*	*trans→cis*
Pro	0.81±0.02	340	0.075±0.003	0.093±0.003	91.0±0.4	90.4±0.3
			trifluoromethylated			
2CF_3Pro	5.85±0.11	340	2.88±0.07	0.70±0.01	80.7±0.3	84.6±0.3
3CF_3Pro	1.27±0.02	340	0.229±0.022	0.220±0.007	87.8±0.6	87.9±0.4
4CF_3Pro	0.90±0.02	340	0.336±0.020	0.442±0.030	86.7±0.5	85.9±0.5
5CF_3Pro	0.42±0.01	310	0.308±0.005	0.713±0.013	79.1±0.1	76.9±0.1
			methylated			
2CH_3Pro	2.56±0.09	340	0.105±0.002	0.059±0.001	90.0±0.4	91.6±0.8
3CH_3Pro	0.90±0.01	340	0.056±0.001	0.067±0.002	91.8±0.3	91.3±0.3
4CH_3Pro	1.03±0.01	340	0.066±0.004	0.071±0.009	91.3±0.5	91.1±0.7
5CH_3Pro	0.65±0.01	340	0.645±0.028	1.083±0.072	84.9±0.4	83.4±0.5

Table S2 Summarized log$P_{octan\text{-}ol/water}$ values for methyl esters of *N*-acetyl amino acids.

compound	log*P*	compound	log*P*	compound	log*P*
trifluoromethylprolines		methylprolines		reference compounds	
	+0.41±0.04		−0.06±0.06		−0.44±0.05
	+0.35±0.05		−0.04±0.05		−0.66±0.03
	+0.24±0.06		−0.06±0.02		−0.84±0.05
	+0.28±0.06		−0.14±0.07		−1.24±0.08
					−1.43±0.06

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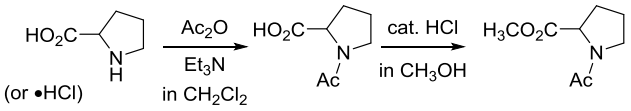
General protocols



***N*-acetylation:** An amino acid or an amino acid hydrochloride was mixed with acetic anhydride (2-5 equiv.) and triethylamine (2-5 equiv) in dichloromethane at the room temperature. The mixture was stirred for 30 min – 14 h until a clear solution was obtained. Dichloromethane was removed under reduced pressure. Residual anhydride was quenched by dissolving the residue in water, and this solution was freeze-dried. The residue was dissolved in some water and this solution was passed through a short cation exchange resin column. Acidic fractions were collected, and these were freeze-dried to give the product.

Esterification: An *N*-acetyl amino acid was dissolved in methanol (HLPC grade, about 10-20:1 v/w to the substance), and trimethylsilylchloride (2-5 equiv.) was added dropwise. The mixture was stirred at the room temperature for 14 hours. In the case of $2CF_3$Pro derivative the reaction time was prolonged to one week. The solvent was removed under reduced pressure and the product was purified by silica gel chromatography using ethyl acetate – methanol mixture (20:1) as an eluent (R_f in the range 0.4-0.7).

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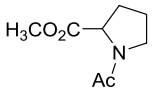
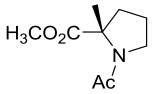
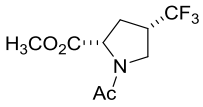
Resulting methyl esters of *N*-acetyl amino acids usually come out as oils. Exceptions were:



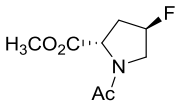
Ac-Pro-OMe, which was crystalized in the racemic form with s-*trans* amide, and the crystal structure reported with CCDC 1443104 [S1]. (*S*)-enantiomer crystalized with s-*cis* amide as reported in [S2].



Ac-2CH_3Pro-OMe, which crystalized as a single enantiomer with s-*trans* amide and the structure was reported with CCDC 1531999 [S5]



Ac-4CF_3Pro-OMe, which crystalized in the racemic form as s-*trans* rotamer and the crystal structure was reported with CCDC 1042476 [S3]



Ac-(*4R*)-Flp-OMe was obtained as a crystalline compound, the crustal structure in the form of s-*trans* rotamer was reported in [S2].

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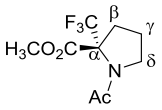
Compound characterization data

For all compounds ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were assigned using ^{1}H NOESY, $^{1}H\{^{13}C\}$ HSQC and $^{1}H^{13}C$ HMBC spectra. In few cases $^{19}F\{^{13}C\}$ HMQC and $^{19}F\{^{1}H\}$ HOESY were applied additionally.

Ac-2CF_3Pro-OMe

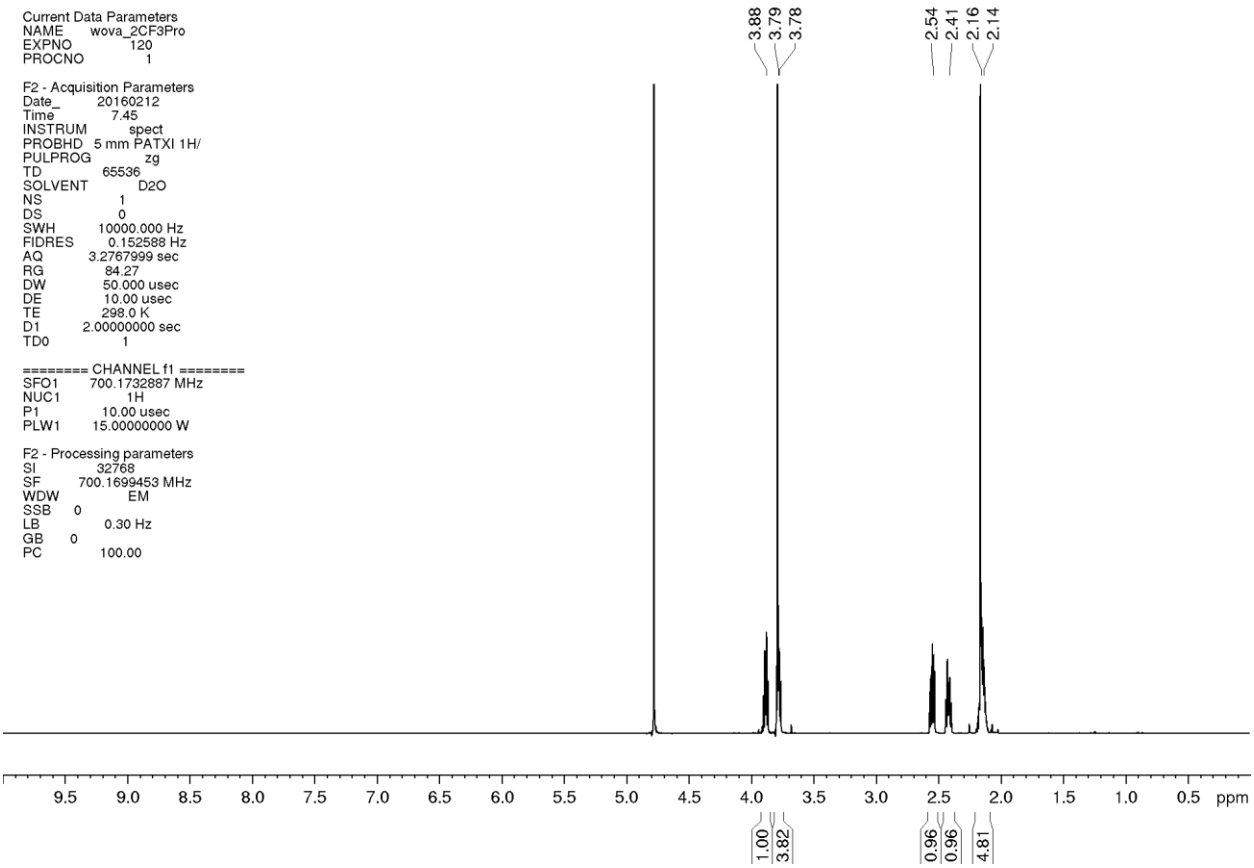
methyl (*R*)-1-acetyl-2-(trifluoromethyl)pyrrolidine-2-carboxylate



Original compound was purchased as a single enantiomer (*R*) in the zwitter-ionic form, and this was then processed according to the general procedures.

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{13}F_3NO_3^+$ 240.0842, found 240.0837.

^{1}H NMR (700 MHz, D_2O), δ, only s-*trans* rotamer ($K_{trans/cis}$ = 199±4): 3.88 and 3.78 (two m, 1H each, δ-CH_2), 3.79 (s, 3H, CH_3O), 2.54 and 2.41 (two m, 1H each, β-CH_2), 2.16 (s, 3H, Ac), 2.14 (m, 2H, γ-CH_2).



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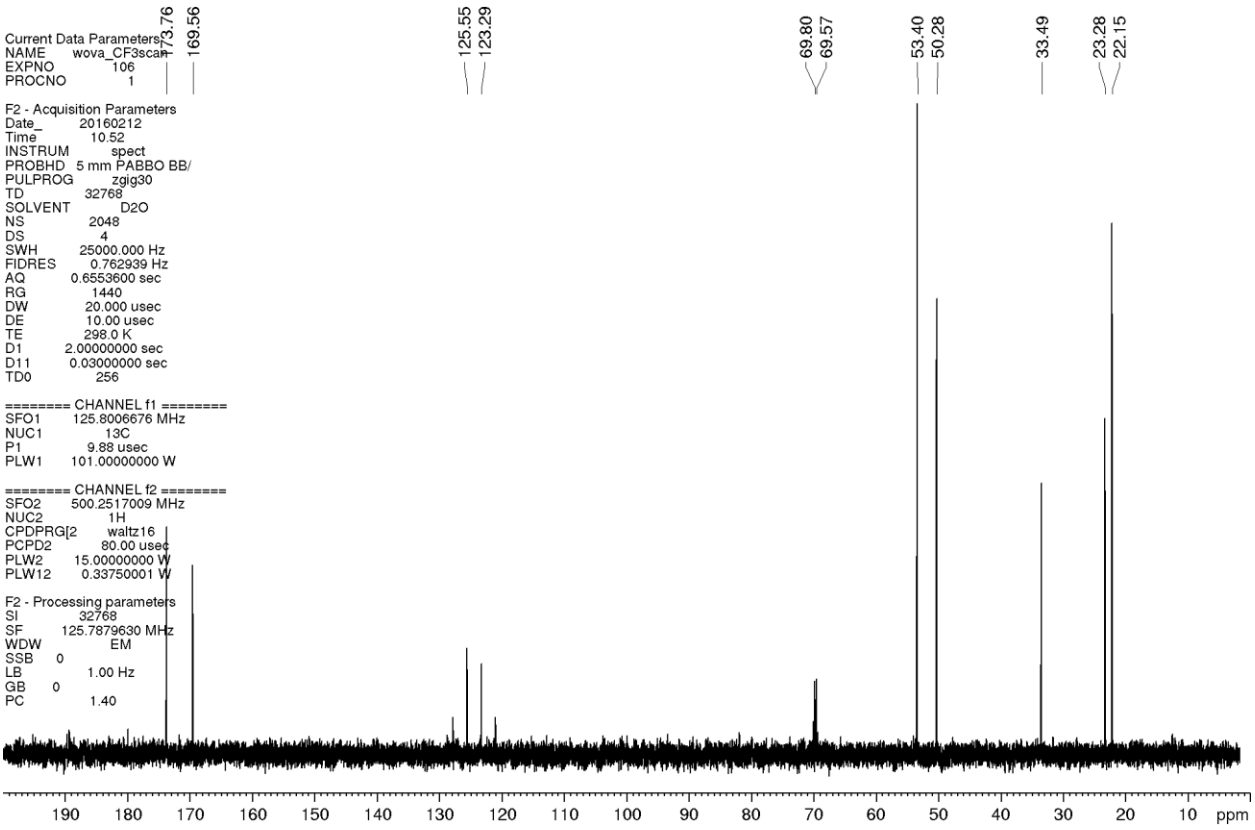
$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O), δ, only s-*trans* rotamer: 173.8 (s, C=O in Ac), 169.6 (s, C=O in CO_2Me), 124.4 (q, J_{CF} = 285 Hz, CF_3), 69.7 (q, J_{CF} = 29 Hz, α-C), 53.4 (s, CH_3O), 50.3 (s, δ-CH_2), 33.5 (s, β-CH_2), 23.3 (s, γ-CH_2), 22.2 (s, CH_3 in Ac).



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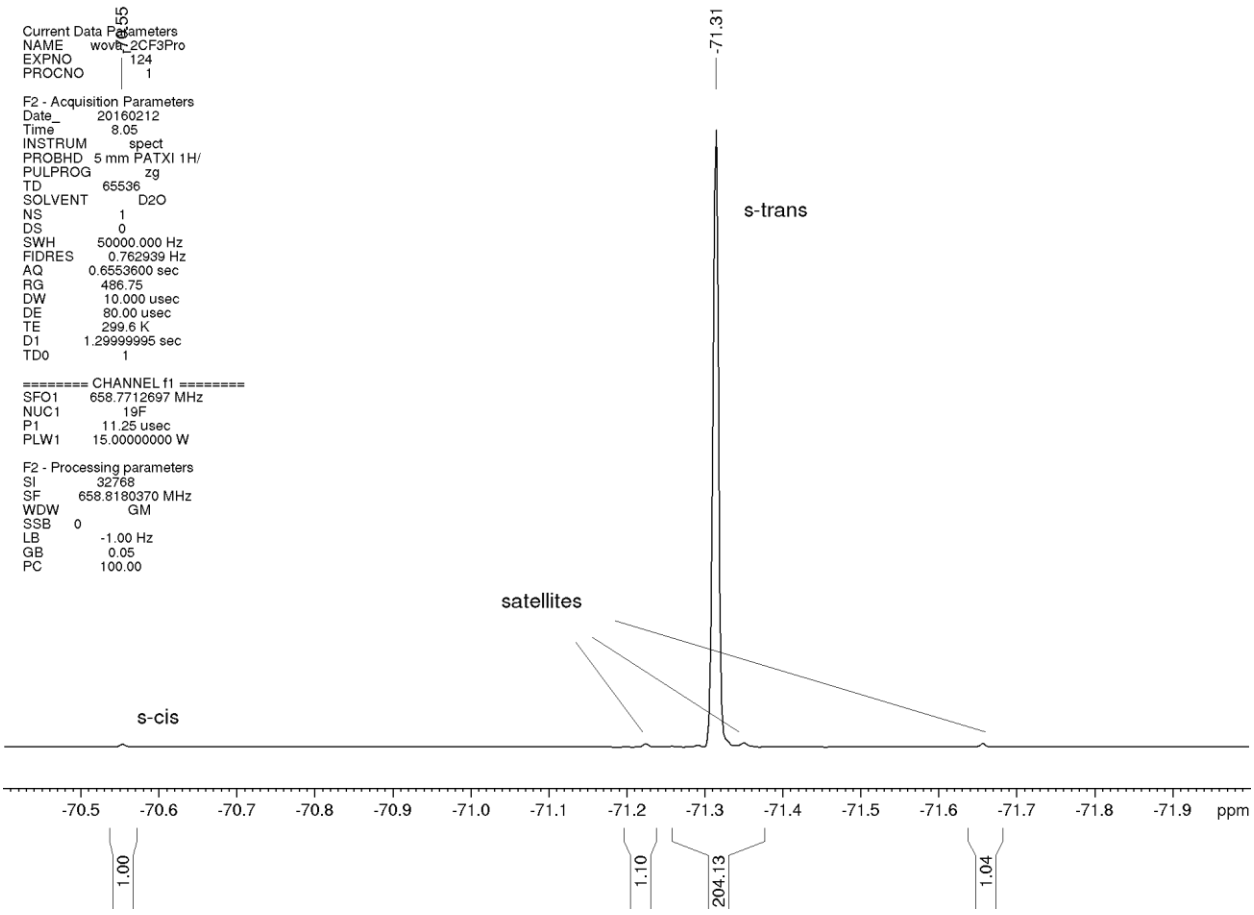
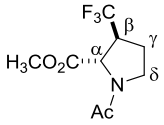
^{19}F NMR (659 MHz, D_2O), δ, two rotamers: −70.6 (s, s-*cis*), −71.3 (s, s-*trans*).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-3CF$_3$Pro-OMe

methyl (*2r,3r*)-1-acetyl-3-(trifluoromethyl)pyrrolidine-2-carboxylate



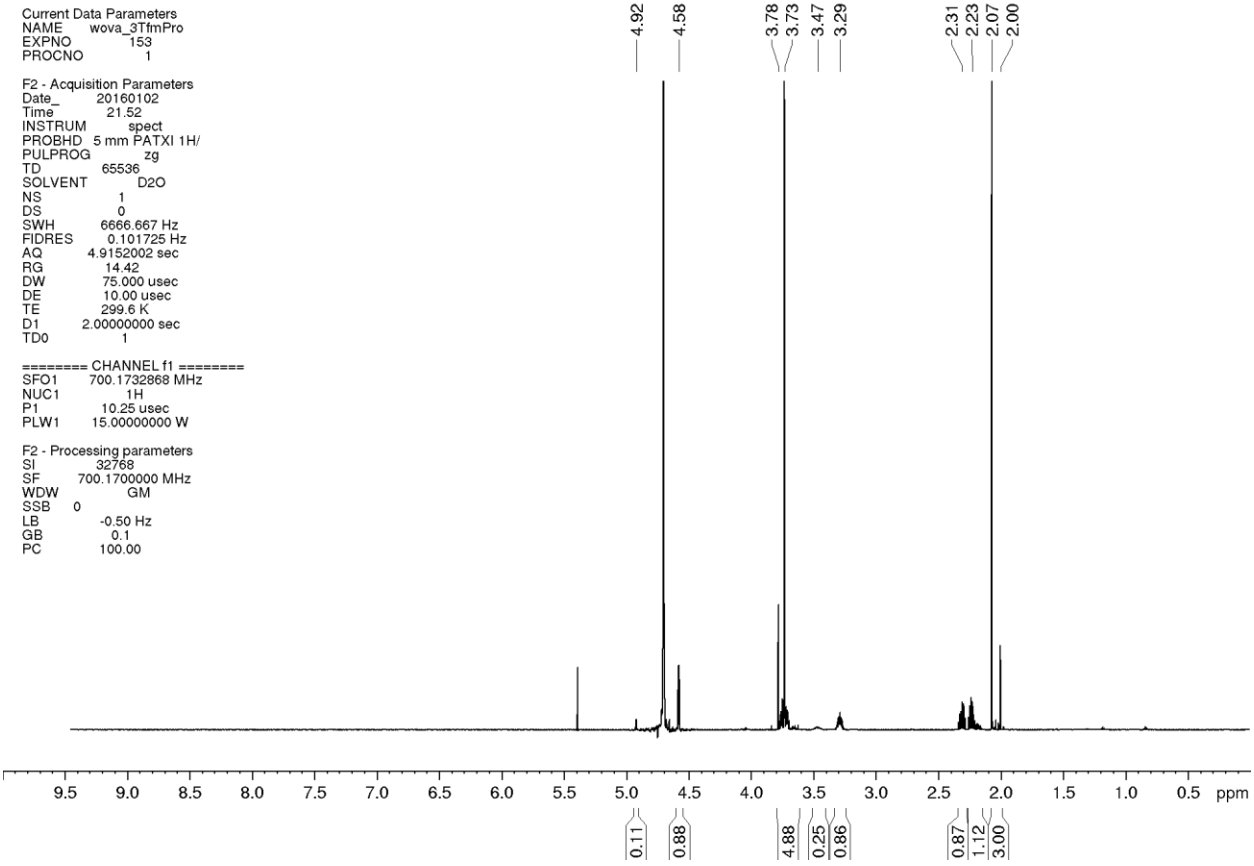
Original compound was supplied as a single diastereomer in the form of an amino acid hydrochloride, and this was then processed according to the general procedures.

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{13}F_3NO_3^+$ 240.0842, found 240.0838.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 6.60±0.15):

s-*trans*: 4.58 (d, *J* = 4.6 Hz, 1H, α-CH), 3.75 and 3.71 (two m, 2H, δ-CH_2), 3.73 (s, 3H, CH_3O), 3.29 (m, 1H, β-CH), 2.31 and 2.23 (two m, 2H, γ-CH_2), 2.07 (s, 3H, CH_3C);

s-*cis*: 4.92 (d, *J* = 2.5 Hz, 1H, α-CH), 3.78 (s, 3H, CH_3O), 3.66 and 3.48 (two m, 2H, δ-CH_2), 3.45 (m, 1H, β-CH), 2.19 (m, 2H, γ-CH_2), 2.00 (s, 3H, CH_3C).



(spectrum shows a resonance from dichloromethane additive)

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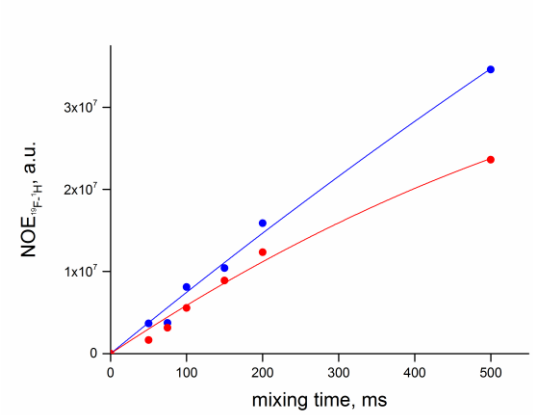
The diastereomer assignment was done using a series of $^{19}F\{^{1}H\}$ HOESY experiments with different mixing times, as shown below:

Ac-3CF_3Pro-OMe / D_2O sample

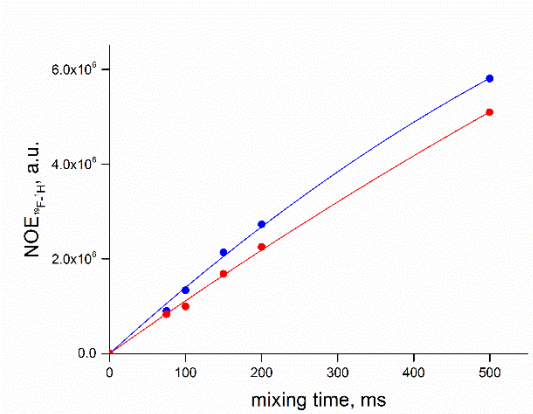
$^{19}F\{^{1}H\}$ HOESY at 471/500 MHz frequencies, 298 K

s-*trans* rotamer



quadratic fit y = a+bx+cx^2
b = 75969 (β-CH), 61518 (α-CH)
$\{r_\beta\}^6/\{r_\alpha\}^6$ = 75969/61518 = 1.24
$\{r_\beta\}/\{r_\alpha\}$ = 1.04

s-*cis* rotamer



quadratic fit y = a+bx+cx^2
b = 14556 (β-CH), 11396 (α-CH)
$\{r_\beta\}^6/\{r_\alpha\}^6$ = 14556/11396 = 1.28
$\{r_\beta\}/\{r_\alpha\}$ = 1.04

, where $\{r_x\}$ is the weighted distance of the corresponding hydrogen to the fluorine atoms of the CF_3-group:

$$\{r_x\} = \frac{1}{\sqrt[6]{\sum_{conformations} \frac{fraction}{r_{F-H_x}^6}}}$$

and $\sum_{conformations} fraction = 1$

Results show similar distance between CF_3-group and the α- and β-hydrogen atoms, which occurs when relative orientation of the CF_3- and carboxymethyl groups is *trans*-.

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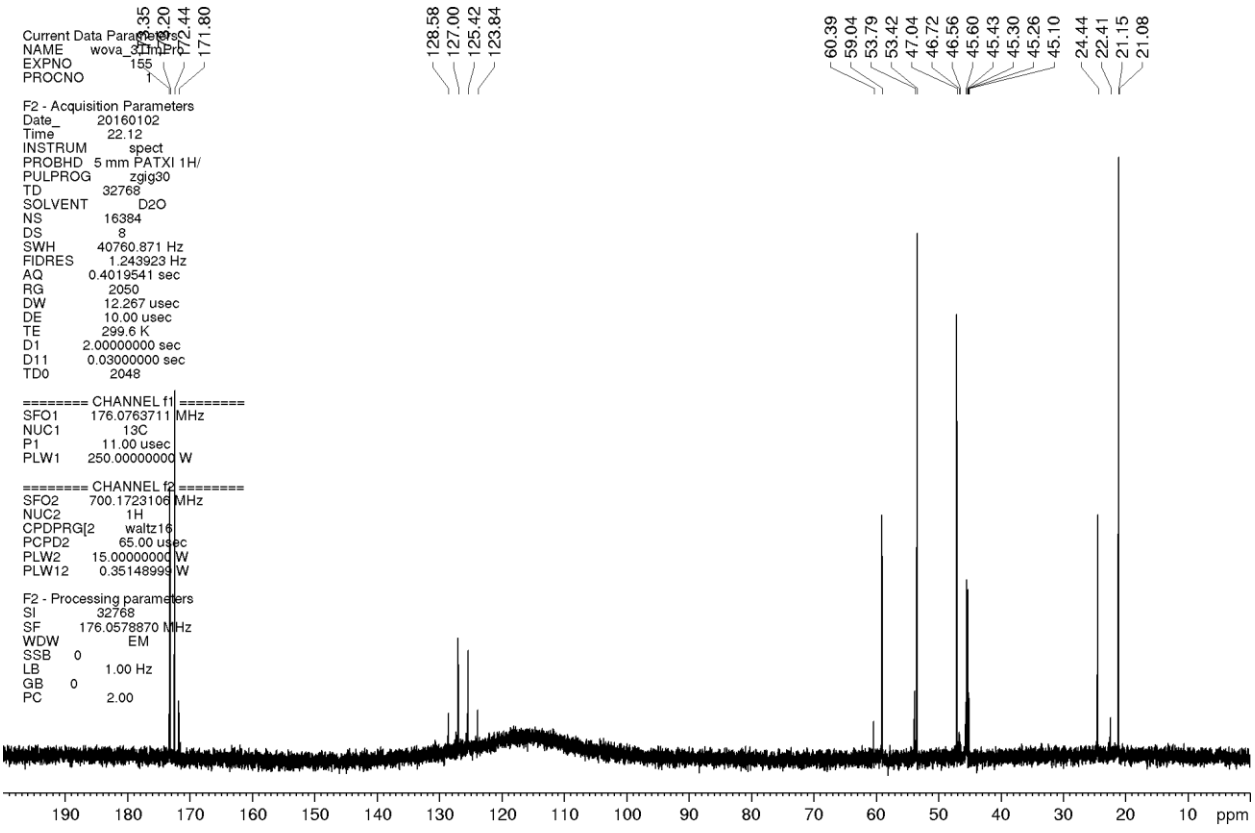
$^{13}C\{^{1}H\}$ NMR (176 MHz, D_2O), δ, only s-*trans* rotamer: 173.2 (s, C=O in Ac), 172.4 (s, CO_2Me), 126.2 (q, J_{CF} = 279 Hz, CF_3), 59.0 (s, α-CH), 53.4 (s, CH_3O), 47.1 (s, δ-CH_2), 45.3 (q, J_{CF} = 29 Hz, β-CH), 24.4 (s, γ-CH_2), 21.1 (s, CH_3).



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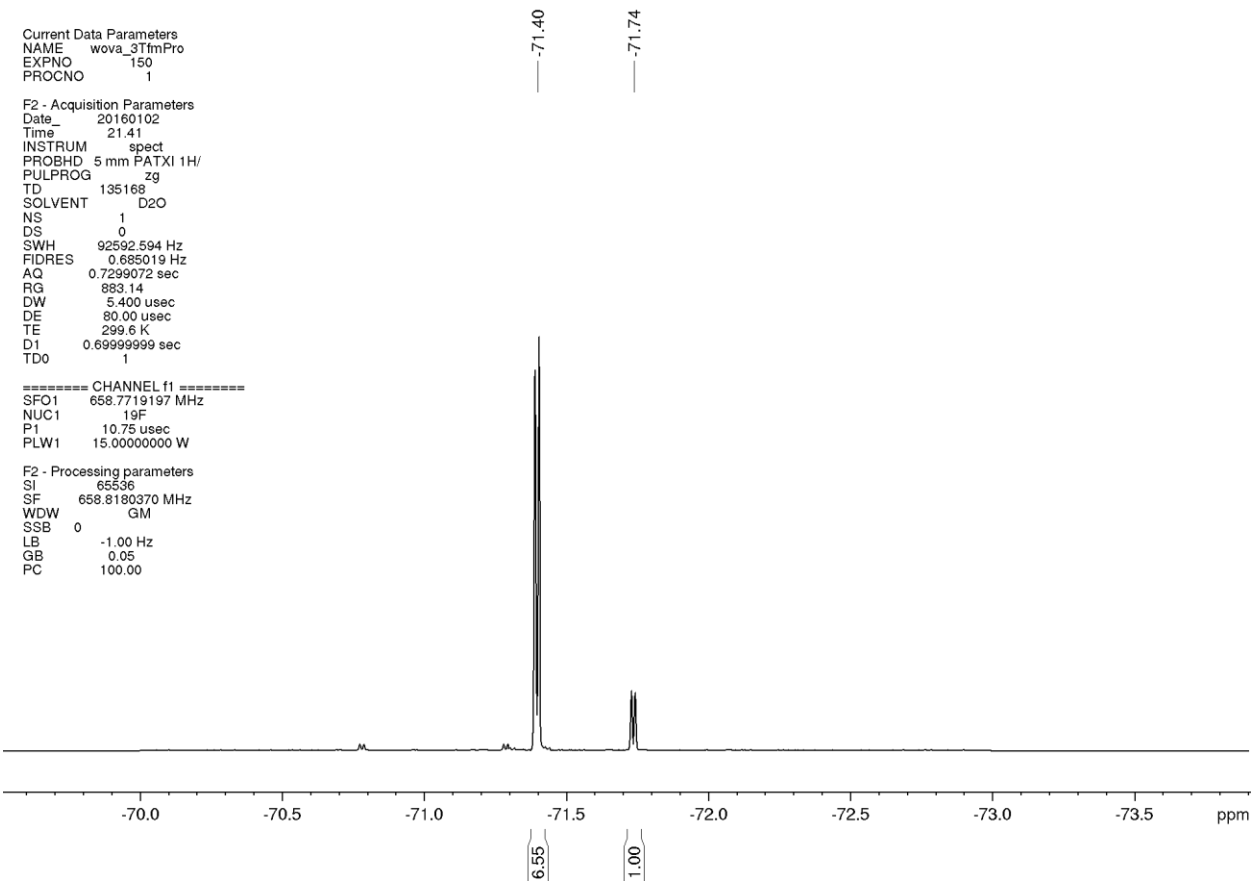
^{19}F NMR (659 MHz, D_2O), δ, two rotamers: −71.4 (d, J_{FH} = 9 Hz, s-*trans*), −71.7 (d, J_{FH} = 9 Hz, s-*cis*).



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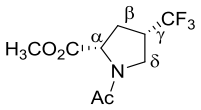
Ac-4CF_3Pro-OMe

methyl (*2S,4S*)-1-acetyl-4-(trifluoromethyl)pyrrolidine-2-carboxylate



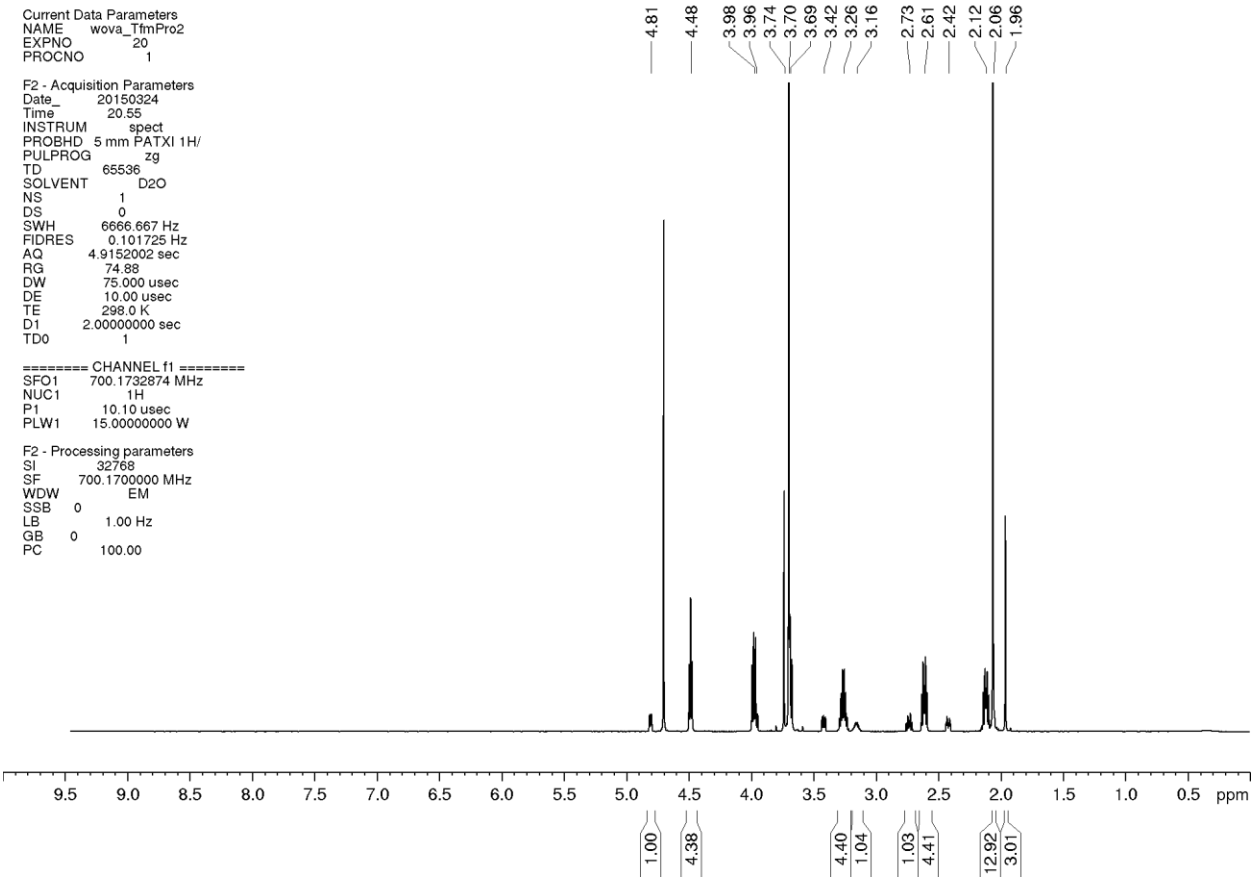
The synthesis of the enantiomeric amino acid and the model compound is as reported previously in [S3].

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{13}F_3NO_3^+$ 240.0842, found 240.0836.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 4.36±0.18):

s-*trans*: 4.48 (t, *J* = 8.3 Hz, 1H, α-CH), 3.98 (dd, *J* = 10.7, 8.7 Hz, 1H, δ-CH), 3.70 (s, 3H, CH_3O), 3.69 (dd, *J* = 10.8, 9.2 Hz, 1H, δ-CH), 3.26 (m, 1H, γ-CH), 2.61 (dt, *J* = 13.5, 8.3 Hz, 1H, β-CH), 2.11 (dt, *J* = 13.3, 8.5 Hz, 1H, β-CH), 2.06 (s, 3H, CH_3 in Ac);

s-*cis*: 4.81 (dd, *J* = 9.7, 4.0 Hz, 1H, α-CH), 3.96 (m, 1H, δ-CH), 3.74 (s, 3H, CH_3O), 3.42 (dd, *J* = 12.8, 6.3 Hz, 1H, δ-CH), 3.16 (m, 1H, γ-CH), 2.73 (dt, *J* = 14.4, 9.5 Hz, 1H, β-CH), 2.42 (dt, J = 14.4, 4.7 Hz, 1H, β-CH), 1.96 (s, 3H, CH_3 in Ac).



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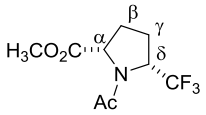
Ac-5CF_3Pro-OMe

methyl (*2S,5R*)-1-acetyl-5-(trifluoromethyl)pyrrolidine-2-carboxylate



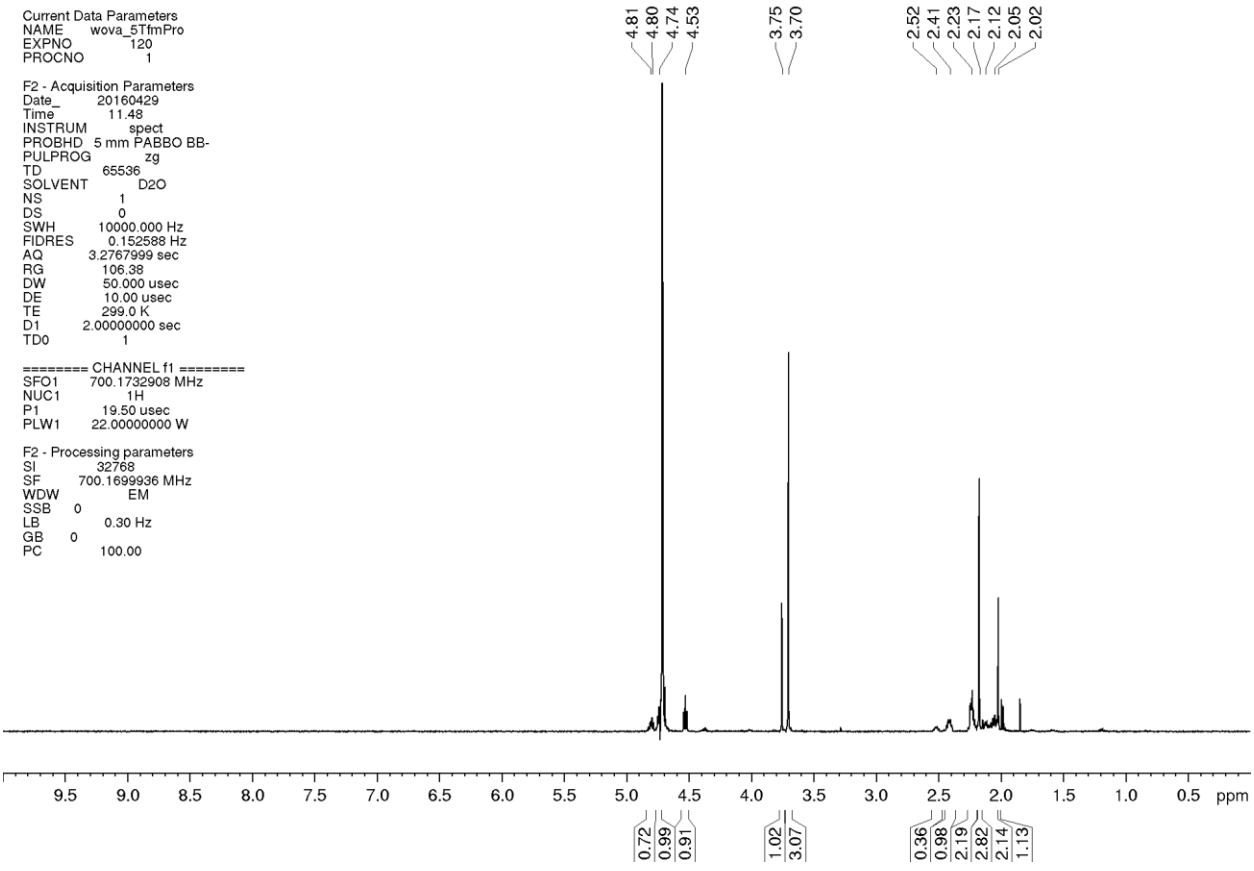
Original compound was purchased as a single diastereomer in the form of an amino acid hydrochloride, and this was then processed according to the general procedures. The assignment of the diastereomer was done following the detection of the amide barriers. The latter would increase in case of a *trans*-diastereomer and decrease when the disatereomer is *cis* [S4]. Significant decrease of the barriers indicated the *cis*-diastereomer.

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{13}F_3NO_3^+$ 240.0842, found 240.0838.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 2.78±0.18):

s-*trans*: 4.74 (m, 1H, δ-CH), 4.53 (t, *J* = 9.2 Hz, 1H, α-CH), 3.70 (s, 3H, CH_3O), 2.41 (m, 1H, β-CH), 2.23 (m, 2H, γ-CH_2), 2.17 (s, 3H, CH_3 in Ac), 2.05 (m, 1H, β-CH);

s-*cis*: 4.81 (m, 1H, δ-CH), 4.80 (t, *J* = 8.5 Hz, 1H, α-CH), 3.75 (s, 3H, CH_3O), 2.51 (m, 1H, β-CH), 2.12 (m, 2H, γ-CH_2), 2.09 (m, 1H, β-CH), 2.02 (s, 3H, CH_3 in Ac).

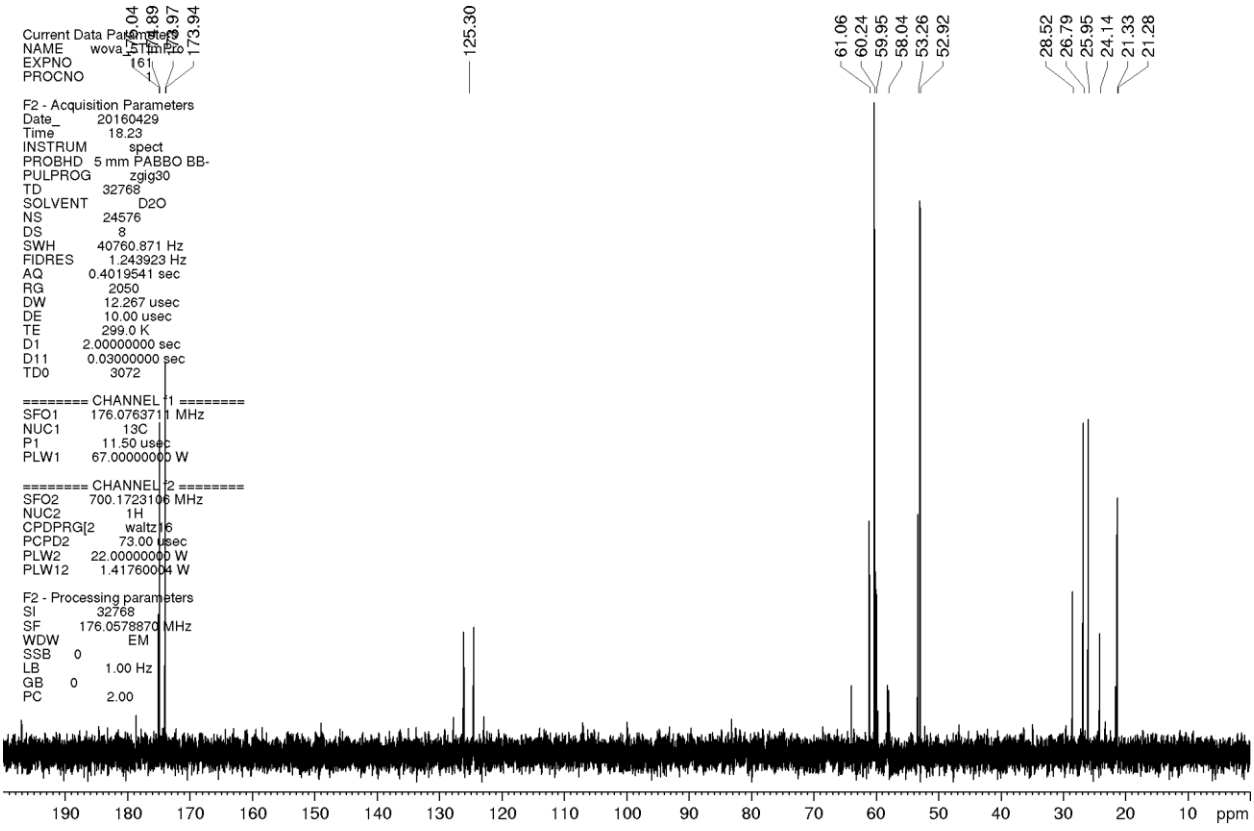


New J. Chem., **2018**, doi: 10.1039/c8nj02631a

$^{13}C\{^{1}H\}$ NMR (176 MHz, D_2O), δ, two rotamers:

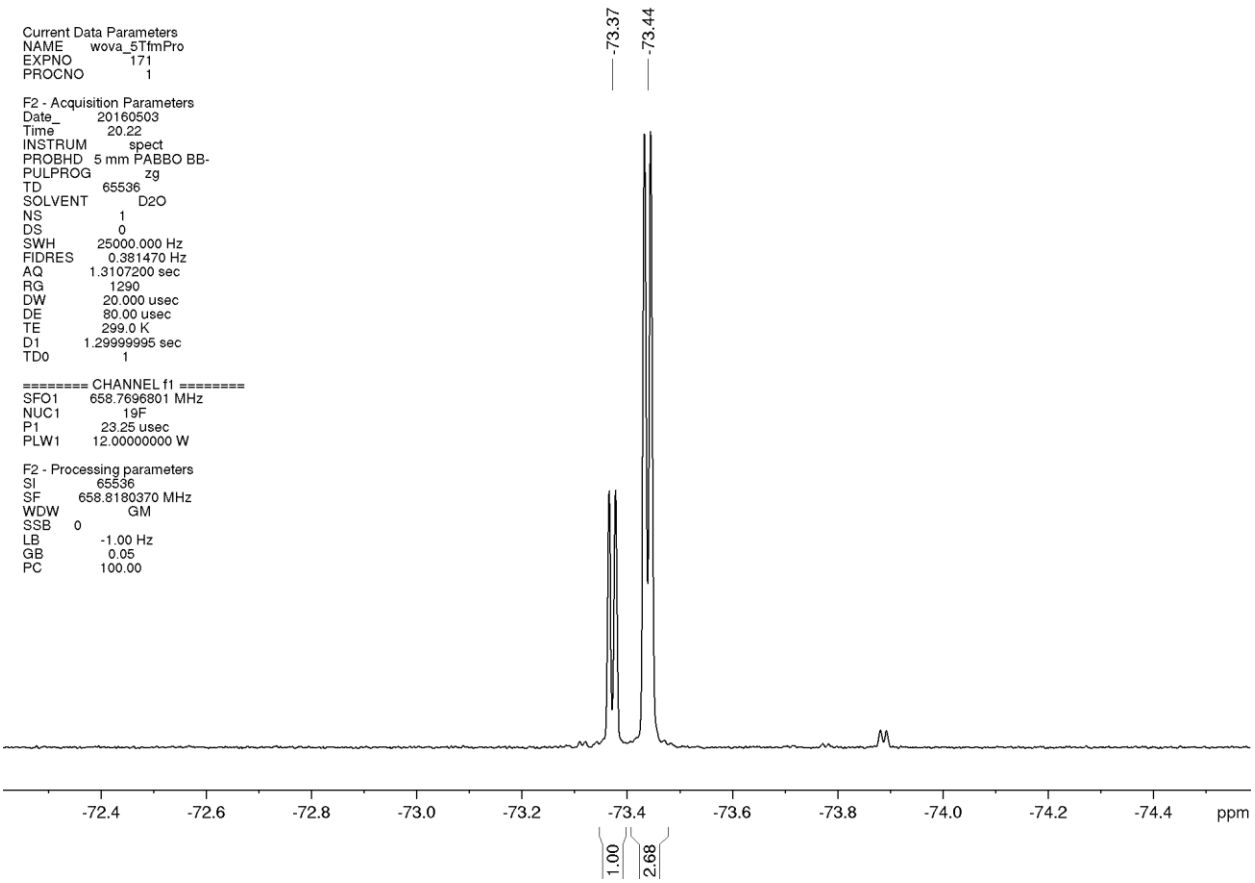
s-*trans*: 174.9 (s, C=O in Ac), 173.9 (s, CO_2Me), 125.3 (q, J_{CF} = 282 Hz, CF_3), 60.2 (s, α-CH), 59.9 (q, J_{CF} = 31 Hz, δ-CH), 52.9 (s, CH_3O), 26.8 (s, β-CH_2), 26.0 (s, γ-CH_2), 21.3 (s, CH_3 in Ac);

s-cis: 175.0 (s, C=O in Ac), 174.0 (s, CO_2Me), 125.4 (q, J_{CF} = 282 Hz, CF_3), 61.1 (s, α-CH), 58.4 (q, J_{CF} = 30 Hz, δ-CH), 53.3 (s, CH_3O), 28.5 (s, β-CH_2), 24.1 (s, γ-CH_2), 21.3 (s, CH_3 in Ac).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

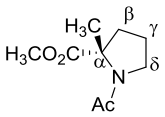
^{19}F NMR (659 MHz, D_2O), δ, two rotamers: −73.37 (d, J_{FH} = 8 Hz, s-*cis*), −73.44 (d, J_{FH} = 8 Hz, s-*trans*).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-2CH_3Pro-OMe

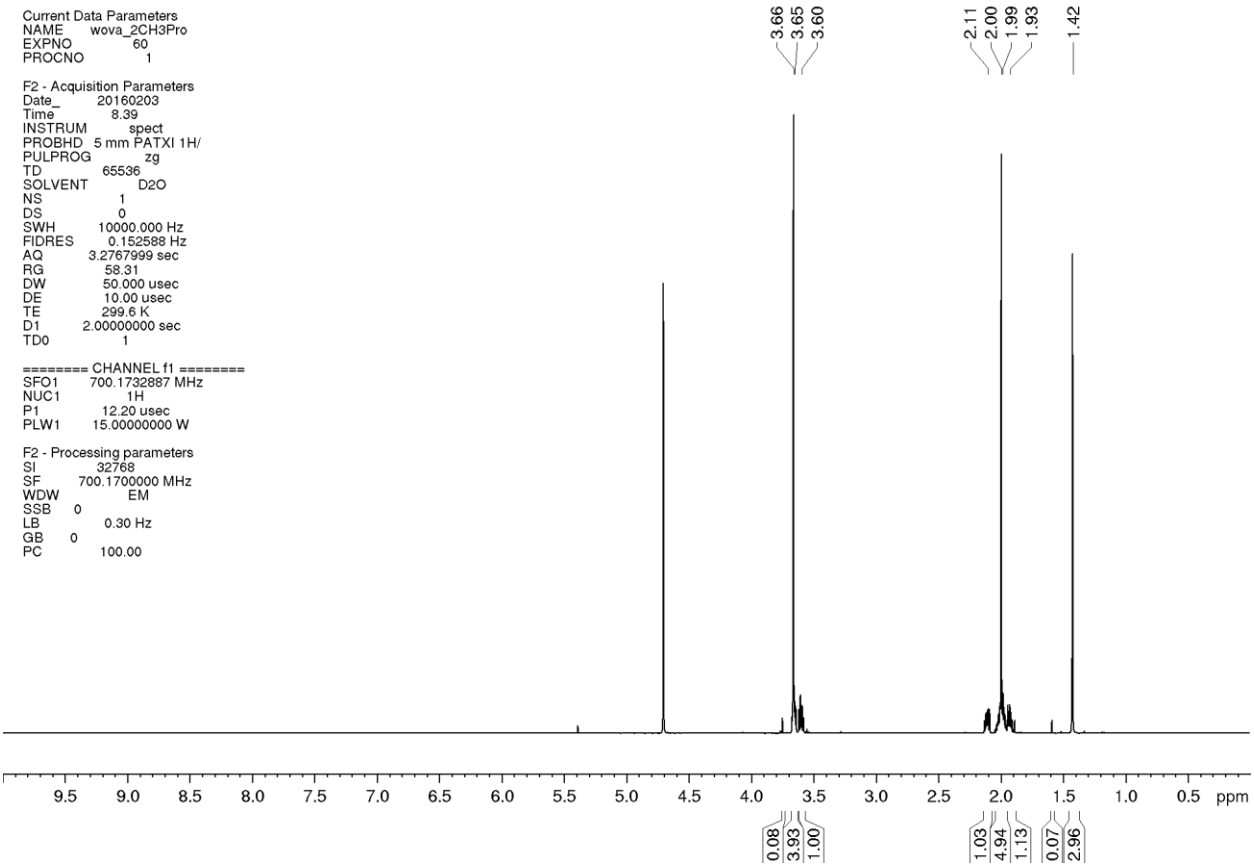
methyl (*S*)-1-acetyl-2-methylpyrrolidine-2-carboxylate



This compound was prepared from commercially available enantiomeric (*S*)-methylproline using general procedures. Detailed experimental characterization was reported in [S5].

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{16}NO_3^+$ 186.1125, found 186.1123.

^{1}H NMR (700 MHz, D_2O), δ, only s-*trans* rotamer ($K_{trans/cis}$ = 39±1): 3.66 (s, 3H, CH_3O), 3.65 (m, 1H, δ-CH), 3.60 (m, 1H, δ-CH), 2.11 (m, 1H, β-CH), 2.00 (s, 3H, CH_3 in Ac), 1.99 (m, 2H, γ-CH_2), 1.93 (m, 1H, β-CH), 1.42 (s, α-CH_3).

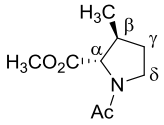


(there is a little signal from a dichloromethane additive in the spectrum)

New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-3CH_3Pro-OMe

methyl (*2S,3S*)-1-acetyl-3-methylpyrrolidine-2-carboxylate



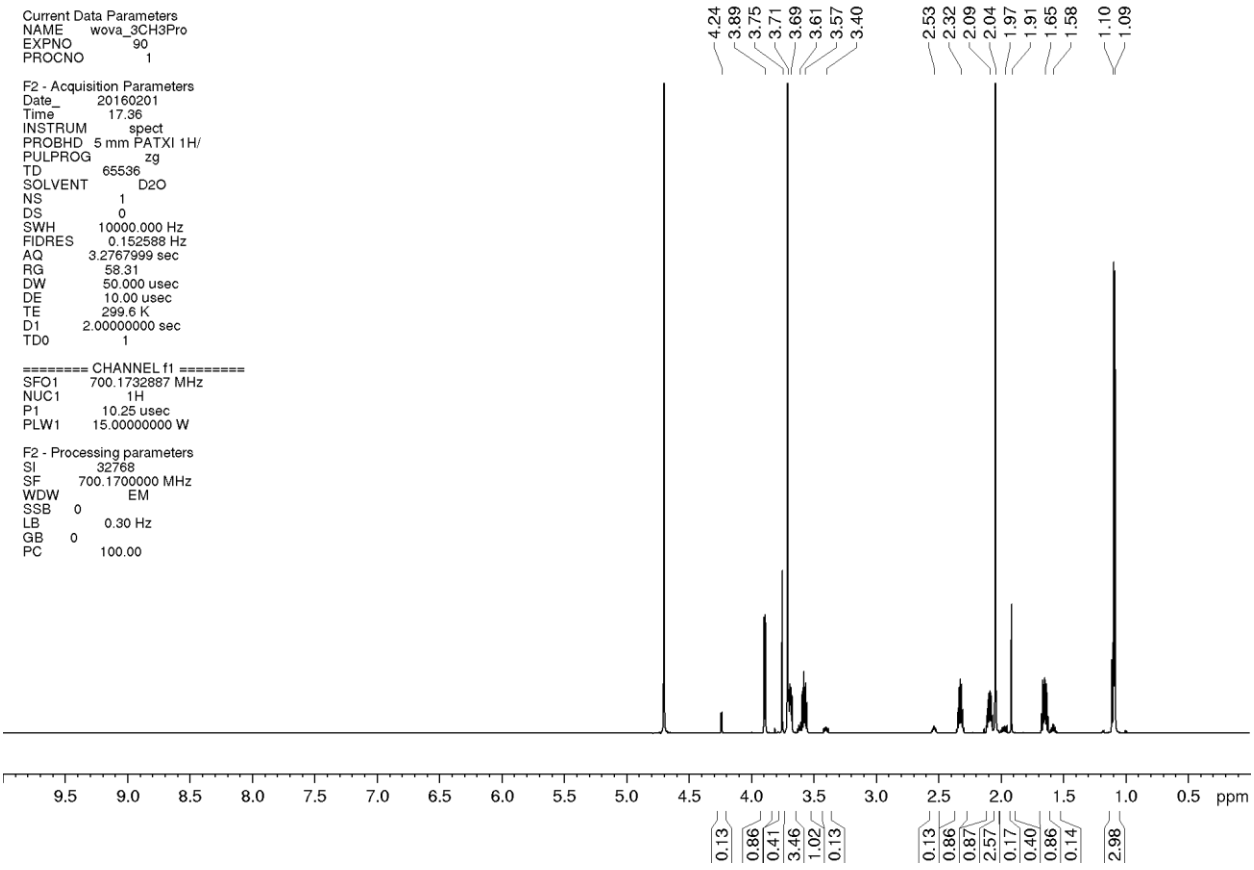
Starting amino acid was purchased in enantiomericaly pure form as a zweitter-ion. This was processed according to the general procedures.

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{16}NO_3^+$ 186.1125, found 186.1121.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 6.51±0.10):

s-*trans*: 3.89 (d, *J* = 6.9 Hz, 1H, α-CH), 3.71 (s, 3H, CH_3O), 3.69 and 3.57 (two m, 2H, δ-CH_2), 2.32 (m, 1H, β-CH), 2.09 and 1.65 (two m, 2H, γ-CH_2), 2.04 (s, 3H, CH_3 in Ac), 1.09 (d, *J* = 6.8 Hz, β-CH_3);

s-*cis*: 4.24 (d, *J* = 4.0 Hz, 1H, α-CH), 3.75 (s, 3H, CH_3O), 3.61 and .40 (two m, 2H, δ-CH_2), 2.53 (m, 1H, β-CH), 1.97 and 1.58 (two m, 2H, γ-CH_2), 1.91 (s, 3H, CH_3 in Ac), 1.10 (d, *J* = 6.9 Hz, 3H, β-CH_3).

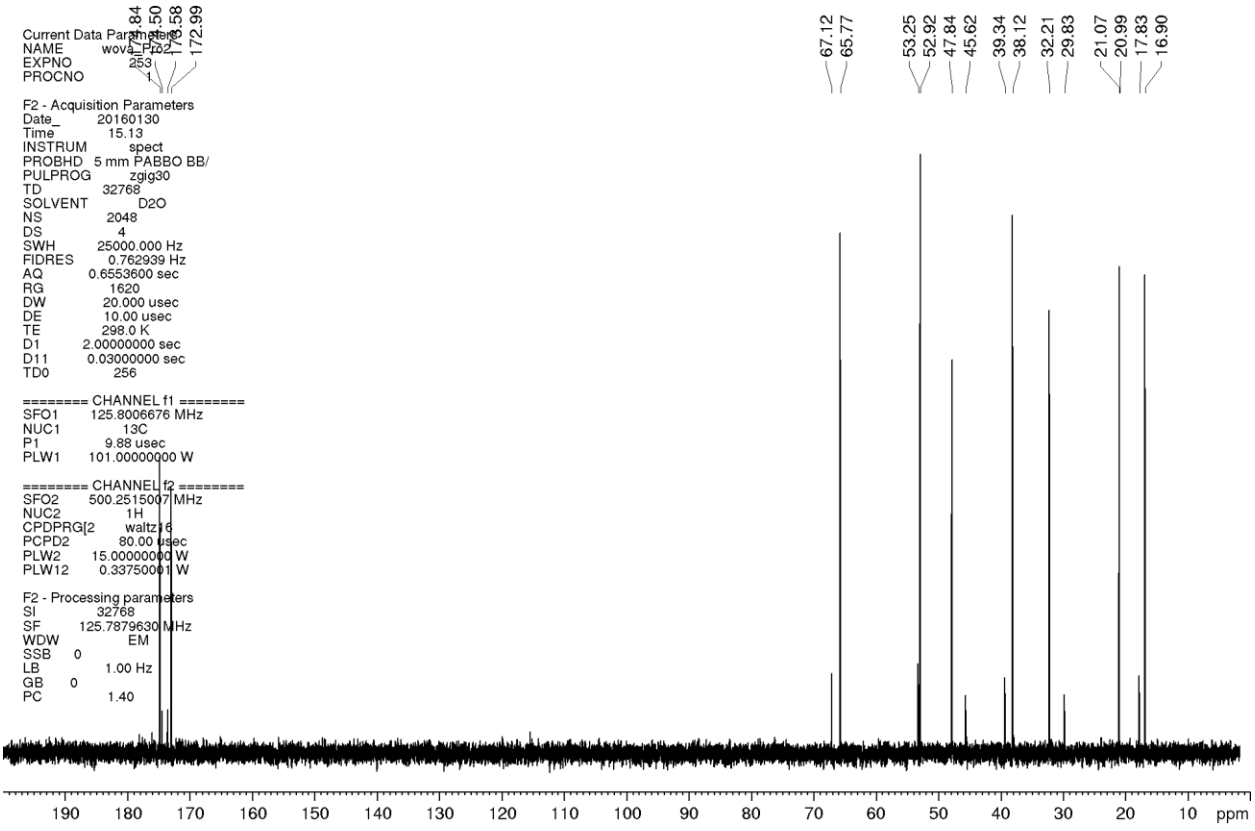


New J. Chem., **2018**, doi: 10.1039/c8nj02631a

$^{13}C\{^{1}H\}$ NMR (126 MHz, D_2O), δ, two rotamers:

s-*trans*: 174.9 (s, CO_2Me), 173.0 (s, C=O in Ac), 65.8 (s, α-CH), 52.9 (s, CH_3O), 47.8 (s, δ-CH_2), 38.1 (s, β-CH), 32.2 (s, γ-CH_2), 21.0 (s, CH_3 in Ac), 16.9 (s, β-CH_3);

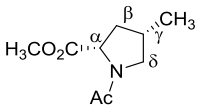
s-*cis*: 174.5 (s, CO_2Me), 173.6 (s, C=O in Ac), 67.1 (s, α-CH), 53.3 (s, CH_3O), 45.6 (s, δ-CH_2), 39.3 (s, β-CH), 29.8 (s, γ-CH_2), 21.1 (s, CH_3 in Ac), 17.8 (s, β-CH_3).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-4CH_3Pro-OMe

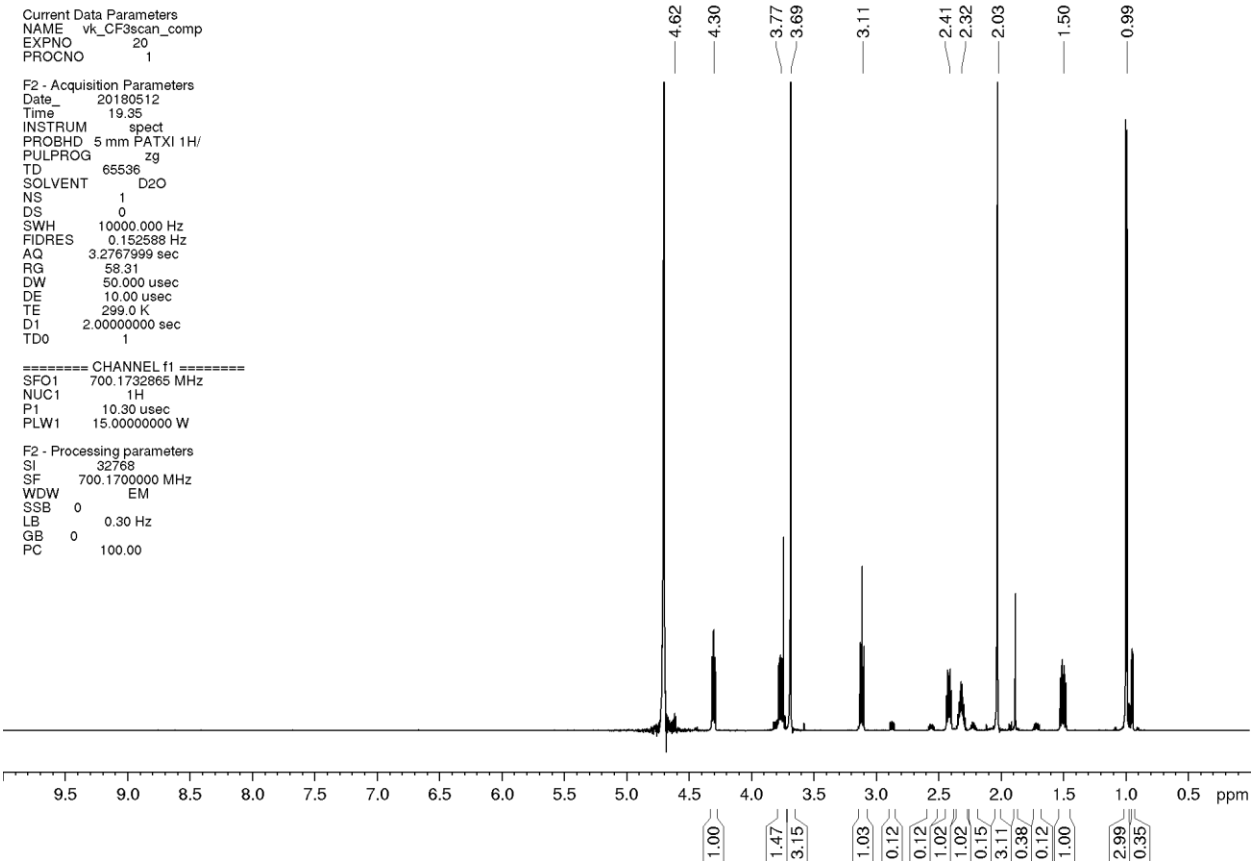
methyl (*2S,4S*)-1-acetyl-4-methylpyrrolidine-2-carboxylate



We prepared the derivation according to the general procedure starting from enantiomerically pure amino acid hydrochloride. The latter was prepared according to the protocol [S6] as described previously in [S7]. The final substance was previously described in [S8,S9].

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{16}NO_3^+$ 186.1125, found 186.1123.

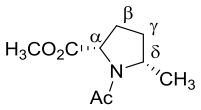
^{1}H NMR (700 MHz, D_2O), δ, s-*trans* rotamer ($K_{trans/cis}$ = 8.05±0.13): 4.30 (t, *J* = 8.5 Hz, 1H, α-CH), 3.77 (dd, *J* = 10.4, 7.3 Hz, 1H, δ-CH), 3.69 (s, 3H, CH_3O), 3.11 (t, *J* = 10.2 Hz, 1H, δ-CH), 2.41, dt, *J* = 12.4, 7.0 Hz, 1H, β-CH), 2.32 (m, 1H, γ-CH), 2.03 (s, 3H, CH_3 in Ac), 1.50 (m, 1H, β-CH), 0.99 (d, *J* = 6.7 Hz, 3H, γ-CH_3).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-5CH_3Pro-OMe

methyl (*2S,5S*)-1-acetyl-5-methylpyrrolidine-2-carboxylate



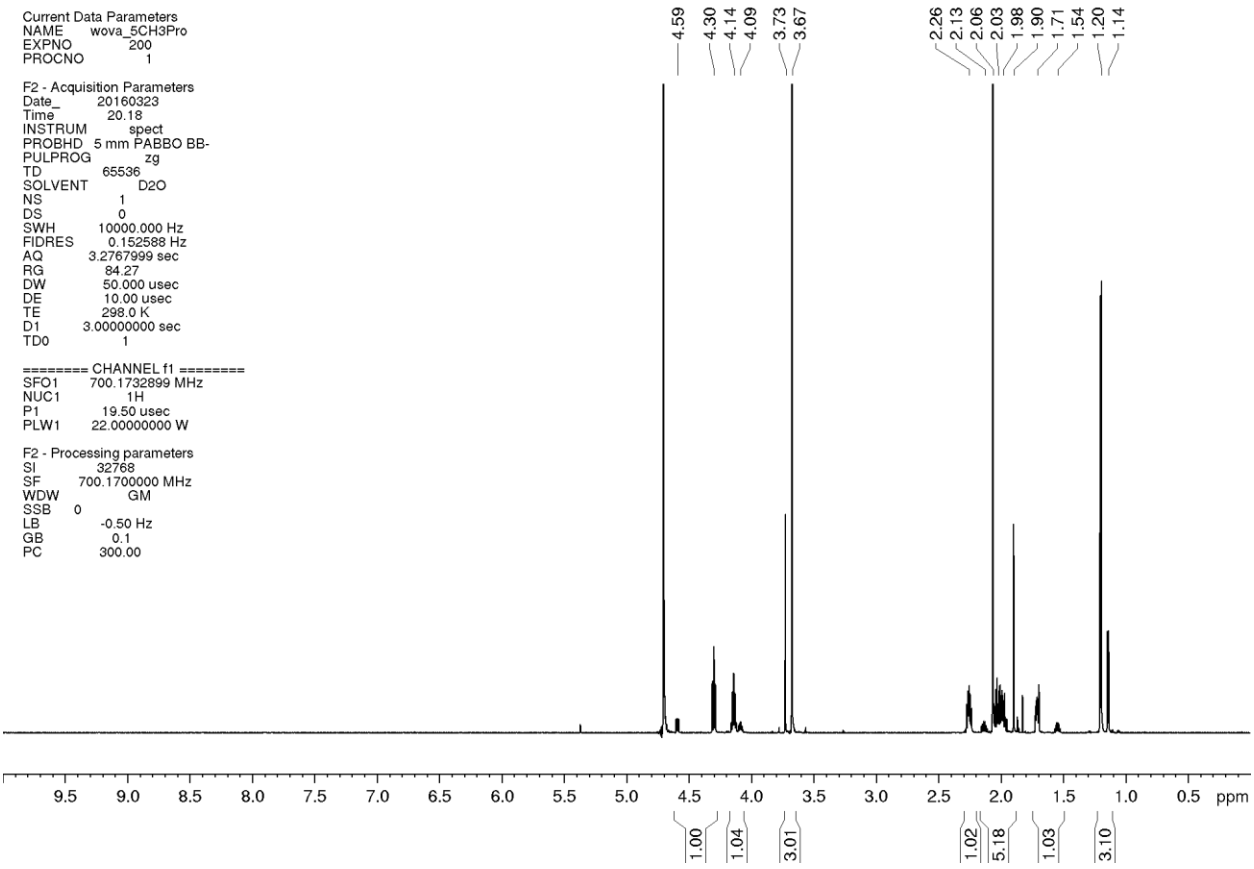
Starting amino acid was synthesized in enantiomerically pure form starting from a glutamic acid derivative according to [S10]. This was functionalized according to the general procedures.

Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_9H_{16}NO_3^+$ 186.1125, found 186.1122.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 4.64±0.02):

s-*trans*: 4.30 (t, *J* = 8.6 Hz, 1H, α-CH), 4.14 (m, 1H, δ-CH), 3.67 (s, 3H, CH_3O), 2.26 (m, 1H, β-CH), 2.06 (s, 3H, CH_3 in Ac), 2.03 (m, 1H, γ-CH), 1.98 (m, 1H, β-CH), 1.71 (m, 1H, γ-CH), 1.20 (d, *J* = 6.6 Hz, 1H, δ-CH_3);

s-*cis*: 4.59 (dd, *J* = 8.6, 5.5 Hz, 1H, α-CH), 4.09 (m, 1H, δ-CH), 3.73 (s, 3H, CH_3O), 2.26 and 2.13 (two m, 1H each, β-CH_2), 2.00 (m, 1H, γ-CH), 1.90 (s, 3H, CH_3 in Ac), 1.54 (m, 1H, γ-CH), 1.14 (d, *J* = 6.4 Hz, 1H, δ-CH_3).



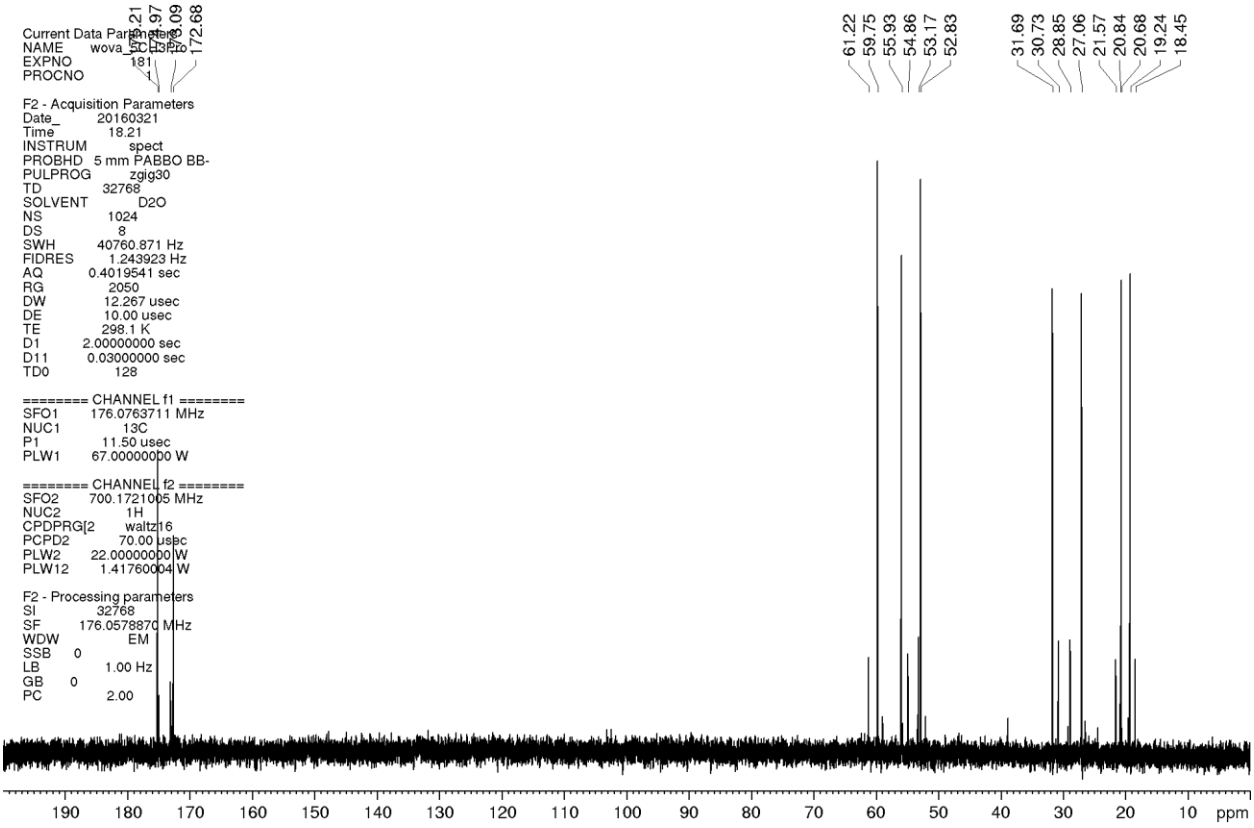
New J. Chem., **2018**, doi: 10.1039/c8nj02631a

(there is a little signal from a dichloromethane additive in the spectrum)

$^{13}C\{^{1}H\}$ NMR (176 MHz, D_2O), δ, two rotamers:

s-*trans*:175.2 (s, CO_2Me), 172.7 (s, C=O in Ac), 59.8 (s, α-CH), 55.9 (s, δ-CH), 52.8 (s, CH_3O), 31.7 (s, β-CH_2), 27.1 (s, γ-CH_2), 20.7 (s, CH_3 in Ac), 19.2 (s, δ-CH_3);

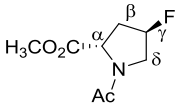
s-cis: 175.0 (s, CO_2Me), 173.1 (s, C=O in Ac), 61.2 (s, α-CH), 54.9 (s, δ-CH), 53.2 (s, CH_3O), 30.7 (s, β-CH_2), 28.8 (s, γ-CH_2), 21.6 (s, CH_3 in Ac), 18.5 (s, δ-CH_3).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-(*4R*)-Flp-OMe

methyl (*2S*,*4R*)-1-acetyl-4-fluoropyrrolidine-2-carboxylate

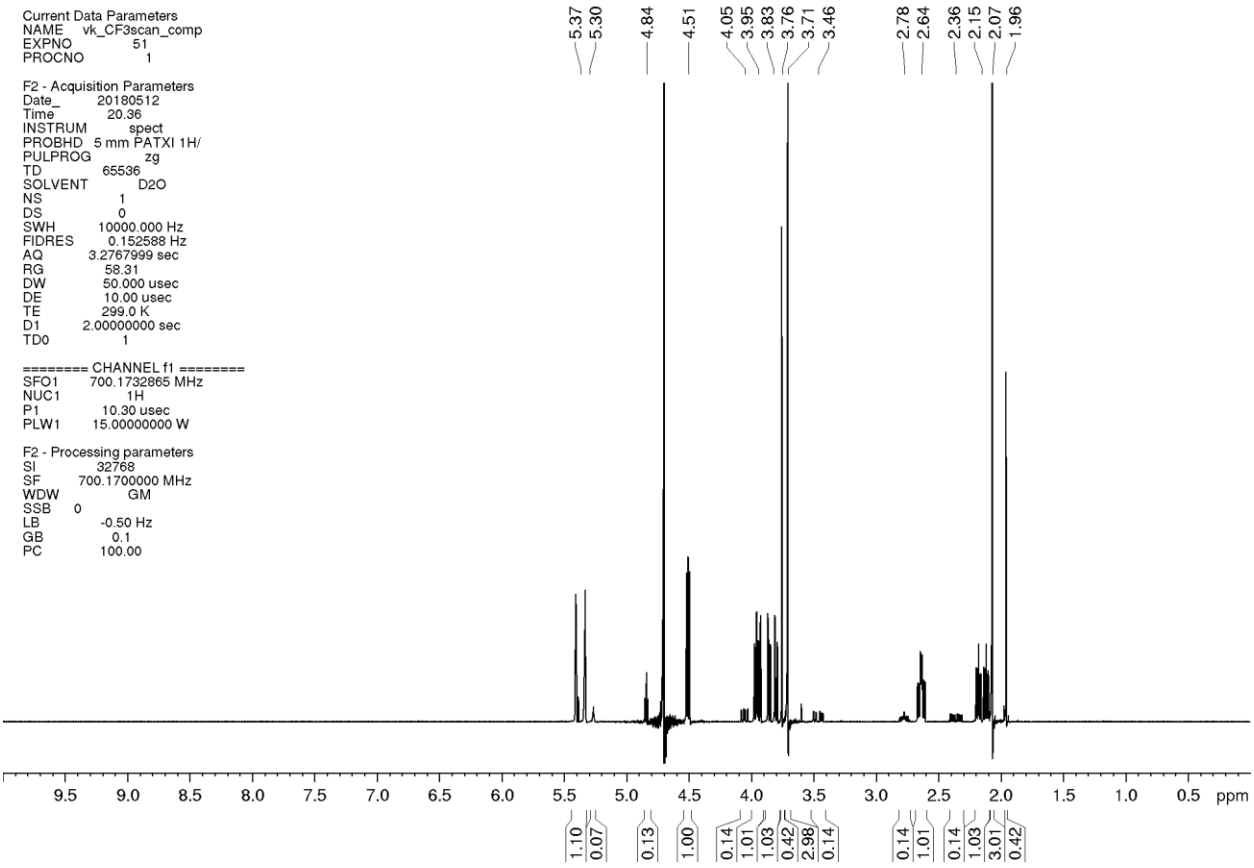


Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_8H_{13}FNO_3^+$ 190.0874, found 190.0870.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 7.16±0.31):

s-*trans*: 5.37 (dt, J_{HF} = 52 Hz, J_{HH} = 3.3 Hz, 1H, γ-CHF), 4.51 (dd, J = 9.9, 7.8 Hz, 1H, α-CH), 3.97 (ddd, J_{HF} = 22 Hz, J_{HH} = 13.0, 2.3 Hz, 1H, δ-CH), 3.83 (ddd, J_{HF} = 38 Hz, J_{HH} = 13.0, 3.1 Hz, 1H, δ-CH), 3.71 (s, 3H, CH_3O), 2.64 (ddddd, J_{HF} = 19 Hz, J_{HH} = 14.8, 7.7, 2.2, 1.2 Hz, 1H, β-CH), 2.15 (dddd, J_{HF} = 42 Hz, J_{HH} = 14.9, 10.1, 4.0 Hz, 1H, β-CH), 2.07 (s, 3H, CH_3 in Ac);

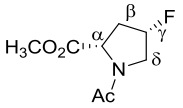
s-*cis*: 5.30 (dt, J_{HF} = 52 Hz, J_{HH} = 3.6 Hz, 1H, γ-CHF), 4.84 (t, J = 8.4 Hz, 1H, α-CH), 4.05 (ddd, J_{HF} = 21 Hz, J_{HH} = 14.0, 2.7 Hz, 1H, δ-CH), 3.75 (s, 3H, CH_3O), 3.46 (ddd, J_{HF} = 37 Hz, J_{HH} = 14.1, 3.4 Hz, 1H, δ-CH), 2.77 (ddddd, J_{HF} = 21 Hz, J_{HH} = 15.0, 8.6, 2.6, 1.4 Hz, 1H, β-CH), 2.36 (dddd, J_{HF} = 39 Hz, J_{HH} = 15.1, 8.2, 4.4 Hz, 1H, β-CH), 1.96 (s, 3H, CH_3 in Ac).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-(*4S*)-Flp-OMe

methyl (*2S*,*4S*)-1-acetyl-4-fluoropyrrolidine-2-carboxylate

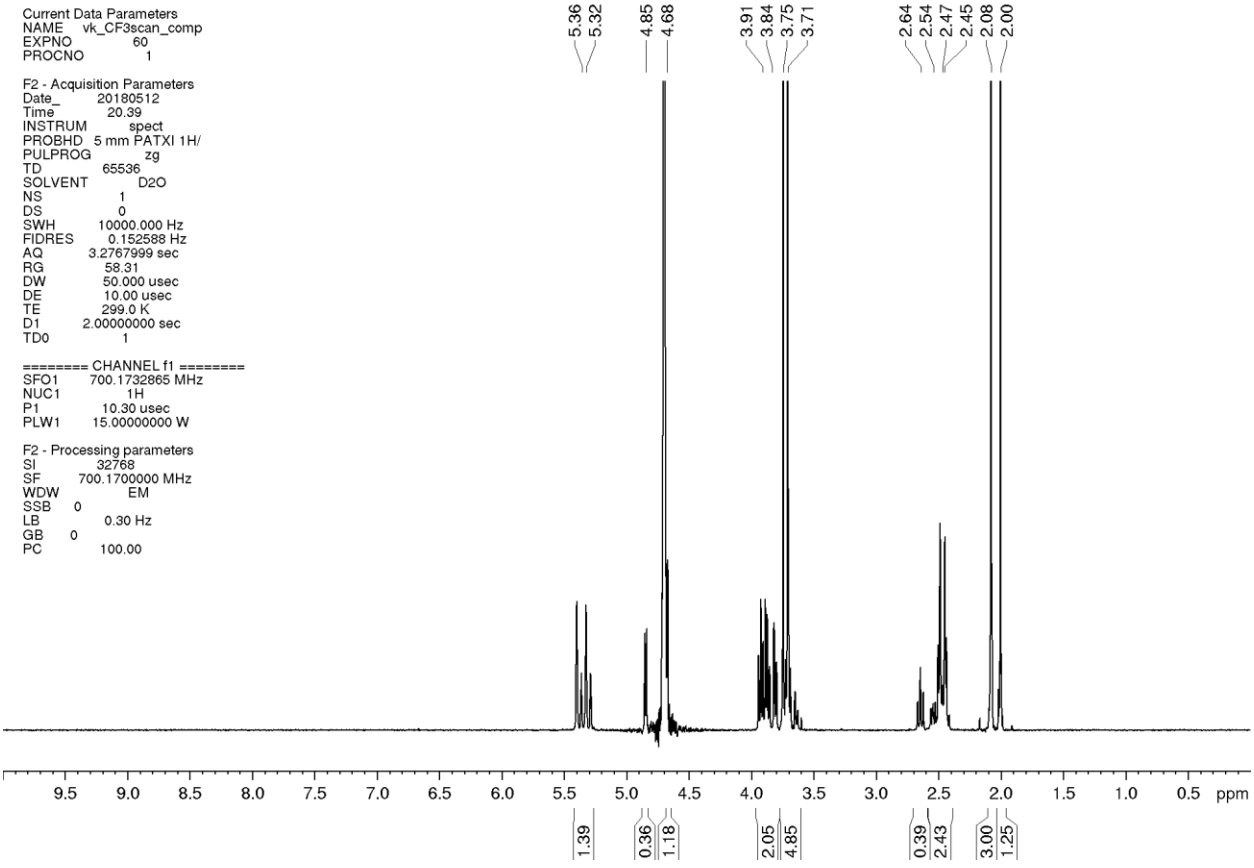


Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_8H_{13}FNO_3^+$ 190.0874, found 190.0872.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 2.62±0.07):

s-*trans*: 5.36 (dt, J_{HF} = 52 Hz, J_{HH} = 3.2 Hz, 1H, γ-CHF), 4.68 (m, 1H, α-CH), 3.91 (dd, J_{HF} = 25 Hz, J_{HH} = 13.2 Hz, 1H, δ-CH), 3.84 (ddd, J_{HF} = 39 Hz, J_{HH} = 13.3, 3.6 Hz, 1H, δ-CH), 3.71 (s, 3H, CH_3O), 2.49 (m, 1H, β-CH), 2.48 (m, 1H, β-CH), 2.08 (s, 3H, CH_3 in Ac);

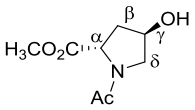
s-*cis*: 5.32 (dt, J_{HF} = 52 Hz, J_{HH} = 3.5 Hz, 1H, γ-CHF), 4.85 (d, J = 9.8 Hz, 1H, α-CH), 3.75 (s, 3H, CH_3O), 3.71 (ddd, J_{HF} = 28 Hz, J_{HH} = 14.4, 1.6 Hz, 1H, δ-CH), 3.70 (ddd, J_{HF} = 38 Hz, J_{HH} = 14.4, 3.8 Hz, 1H, δ-CH), 2.66 (ddd, J_{HF} = 16 Hz, J_{HH} = 14.6, 1.6 Hz, 1H, β-CH), 2.52 (m, 1H, β-CH), 2.00 (s, 3H, CH_3 in Ac).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-(*4R*)-Hyp-OMe

methyl (*2S,4R*)-1-acetyl-4-hydroxypyrrolidine-2-carboxylate

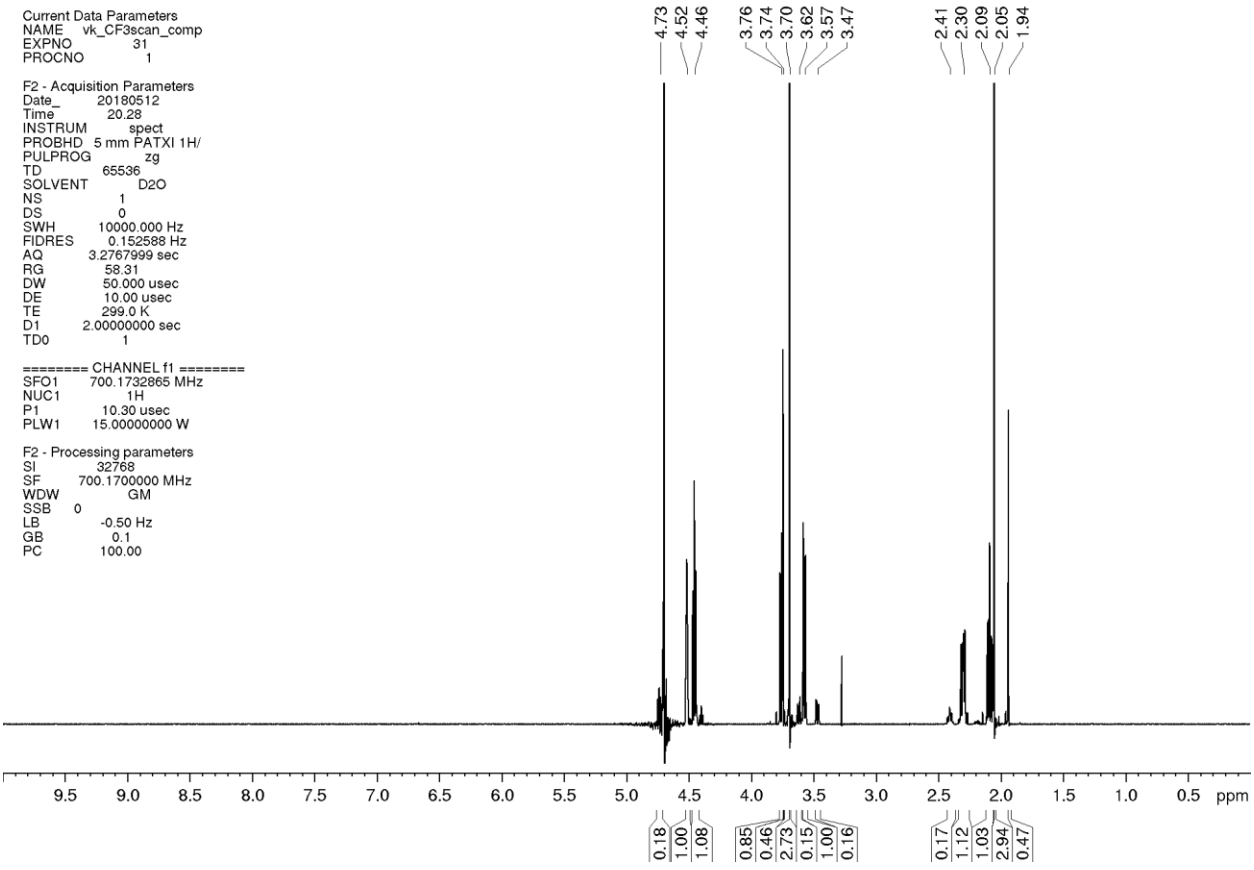


Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_8H_{14}NO_4^+$ 188.0917, found 188.0915.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 6.22±0.40):

s-*trans*: 4.52 (m, 1H, γ-CH), 4.46 (t, *J* = 8.6 Hz, 1H, α-CH), 3.76 (dd, *J* = 11.5, 4.0 Hz, 1H, δ-CH), 3.70 (s, 3H, CH_3O), 3.57 (dt, *J* = 11.8, 1.6 Hz, 1H, δ-CH), 2.30 (ddt, *J* = 13.7, 7.8, 2.0 Hz, 1H, β-CH), 2.09 (ddd, *J* = 13.8, 9.2, 4.6 Hz, 1H, β-CH), 2.05 (s, 3H, CH_3 in Ac);

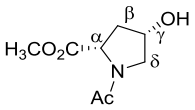
s-*cis*: 4.74 (m, 1H, α-CH), 4.45 (m, 1H, γ-CH), 3.74 (s, 3H, CH_3O), 3.62 (dt, *J* = 12.6, 1.8 Hz, 1H, δ-CH), 3.47 (dd, *J* = 12.7, 4.7 Hz, 1H, δ-CH), 2.41 (dddd, *J* = 14.2, 8.7, 3.3, 1.6 Hz, 1H, β-CH), 2.28 (m, 1H, β-CH), 1.94 (s, 3H, CH_3 in Ac).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Ac-(*4S*)-Hyp-OMe

methyl (*2S,4S*)-1-acetyl-4-hydroxypyrrolidine-2-carboxylate

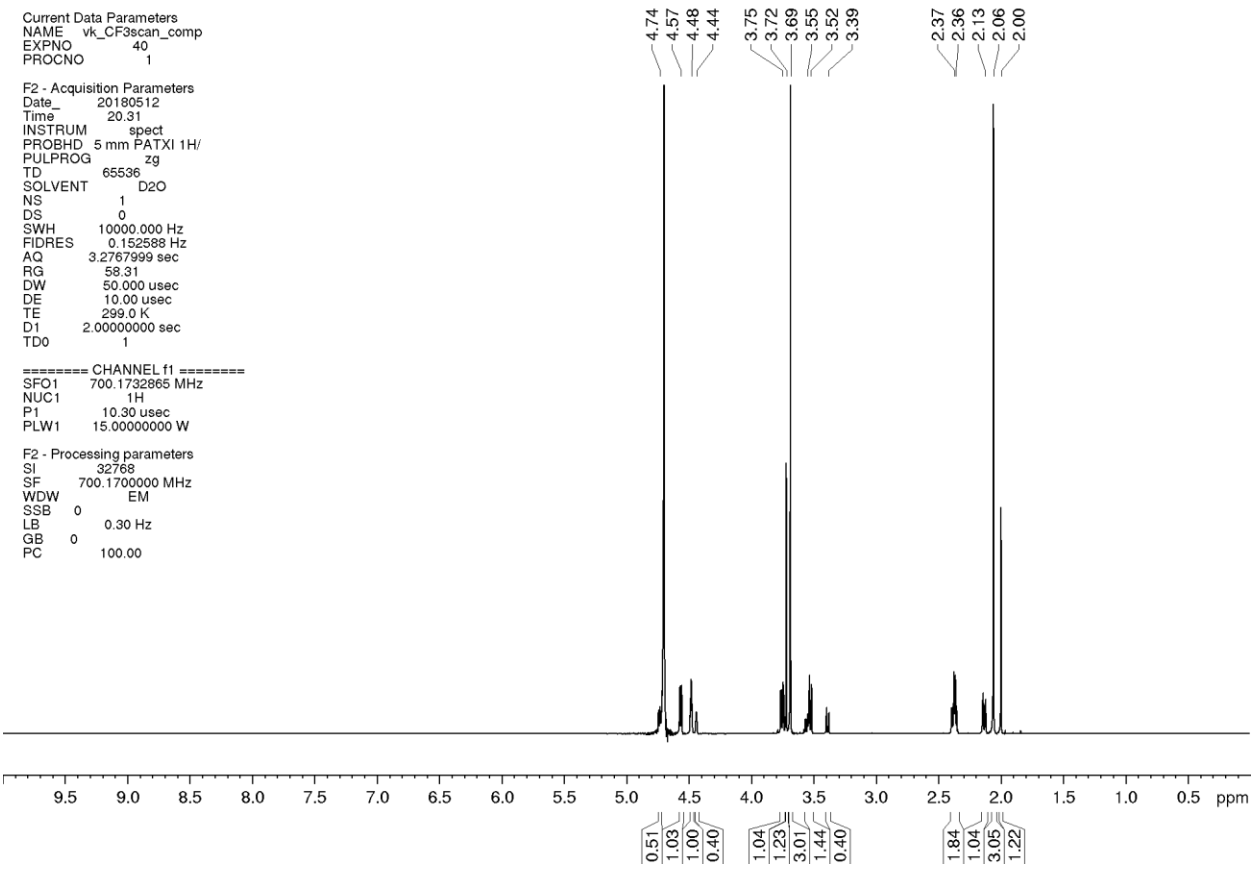


Mass-spectrum (ESI-Orbitrap): calcd. for $[M+H]^+$ $C_8H_{14}NO_4^+$ 188.0917, found 188.0914.

^{1}H NMR (700 MHz, D_2O), δ, two rotamers ($K_{trans/cis}$ = 2.49±0.05):

s-*trans*: 4.57 (dd, *J* = 9.6, 2.5 Hz, 1H, α-CH), 4.48 (m, 1H, γ-CH), 3.75 (dd, *J* =11.6, 4.6 Hz, 1H, δ-CH), 3.69 (s, 3H, CH_3O), 3.52 (dt, *J* = 11.6, 1.5 Hz, 1H, δ-CH), 2.36 and 2.13 (two m, 1H each, β-CH_2), 2.06 (s, 3H, CH_3 in Ac);

s-*cis*: 4.74 (dd, *J* = 7.6, 3.1 Hz, 1H, α-CH), 4.44 (m, 1H, γ-CH), 3.72 (s, 3H, CH_3O), 3.55, (dd, *J* = 13.1, 4.5 Hz, 1H, δ-CH), 3.39 (d, *J* = 13.1 Hz, 1H, δ-CH), 2.37 (m, 2H, β-CH_2), 2.00 (s, 3H, CH_3 in Ac).



New J. Chem., **2018**, doi: 10.1039/c8nj02631a

Physical chemistry

More detailed descriptions of the pK_a measurements, amide equilibrium constant determination and kinetic measurements can be found in [S11,S12]. Here are shortened descriptions:

Amino acid pK_a: aqueous solution of an amino acid (about 10-50 mM) and potassium phosphate (75-100 mM) and in some cases glycine (50 mM) was titrated by KOH or HCl solutions to different pH values at 294±2 K. 0.5 ml aliquots were taken, and to this 0.05 ml deuterium oxide solution was added for locking purposes. The samples also contained about 1 mM sodium 3-(trimethylsilyl)propane-1-sulfonate (TPS) standard for referencing. ^{1}H, ^{19}F and ^{19}F{^{1}H} NMR spectra were acquired at 298 K, for proton detection W5 water suppression pulse tray was applied. Chemical shifts were plotted against pH, these were fitted according to a Boltzmann fit, and the bending point was considered as the pK_a value.

Amino acid methyl ester pK_a: aqueous buffers containing potassium phosphate (75-100 mM) and one of the glycine (50 mM) or potassium citrate (50 mM) were prepared by titration with KOH and HCl solutions at 294±2 K. 0.5 ml aliquots were taken, to this 0.05 ml deuterium oxide was added, and the samples also contained about 1 mM TPS. ^{1}H NMR spectra were acquired using W5 water suppression scheme at 298 K.

Amino acid methyl esters were prepared beforehand by shaking an amino acid in acidic methanol solution for 14 hours. These were prepared by adding either 0.1 ml trimethylsilyl chloride or 0.2 ml thionyl chloride to about 1.5 ml methanolic solution of about 25-50 mg of an amino acid. After reaction completion the solvent was removed under reduced pressure, the residue was dissolved in deuterium oxide, and 1 μl of this solution was added to the NMR tubes containing buffers with different pH to give about 1 mM final concentration of the analyte. The samples were then measured within about 2 min after addition of the analyte stock. ^{1}H W5 NMR spectra were acquired at 298 K.

Chemical shift of the buffer (glycine or citric acid) recorded in the reference series were plotted agains pH, and these were used for correction pH pf the samples after addition of the amino acid methyl ester hydrochlorides.

Chemical shift of the analytes were then plotted against corrected pH, Boltzmann fit was analyzed to deliver the pK_a value.

***N*-acetyl amino acid pK_a:** aquous solution containing *N*-acetyl amino acid (about 2-5 mM) and potassium phosphate (about 7 mM) were titrated to different pH values at 294±2 K using KOH and HCl solutions. 0.5 ml aliquots were taken, 0.05 ml deuterium oxide was added for locking, the samples also contained about 1 mM TPS standard for referencing (methanol referencing below pH 2). ^{1}H NMR spectra were acquired with W5 water suppression at 298 K. Chemical shifts were plotted against pH, Boltzmann fits were analysed to deliver the pK_a values.

Equilibrium populations:

Salt samples: an *N*-acetyl amino acid (10 mg) and potassium hydrogen phosphate (10 mg) were titrated in 1 ml aquous solution to neutral pH about 7 according to pH paper. 0.55 ml aliquote was

taken, this was freeze-dried, then freeze-dried from some amount of deuterium oxide (0.3-0.5 ml), and then dissolved in 0.55 ml deuterium oxide for measurements.

Acid samples: an *N*-acetyl amino acid (5 mg) and potassium hydrogen sulphate (10 mg) were dissolved in deuterium oxide (~0.5 ml), this solution was freeze-dried, then freeze-dried from another portion of deuterium oxide (~0.3-0.5 ml), and then dissolved in 0.55 ml of deuterium oxide for measurements.

Methyl ester samples: an *N*-acetyl amino acid methyl ester (~ 5 mg) was dissolved in 0.55 ml deuterium oxide for measurements.

Measurements: ^{1}H and ^{19}F NMR spectra were acquired at 700 and 659 MHz respectively at 298 K according to conventional methanol standard calibration. ^{1}H{^{19}F} NMR spectra were acquired at 500/471 MHz with inverse-gated decoupling (during acquisition only). The spectra were acquired using pre-calibrated 90-degree pulses in one scan in order to ensure complete pre-relaxation. The time-domain spectra were processed with an appropriate window function, background was corrected and resulting frequency domain spectra were integrated. Integral ratios for the rotameric forms were considered as equilibrium constants. Integration of different resonances, repetition of the spectra acquisition and application of different window functions delivered values with some discrepancies. These were averaged, and the root-mean square deviation of the values was considered as the error.

Amide rotation kinetics: Exchange was measured in 2D z-cross relaxation experiments (NOESY/EXSY) with z-gradients using ^{1}H or ^{19}F NMR detection. Frequency domain spectra were analyzed with EXSYCalc freeware (Mestrec). The rotation barrier were calculated by using Eyring equation assuming single transition state. The ^{1}H-detected spectra were also analysed as NOESY to confirm the rotameric assignment.

Partitioning: a compound (~ 5 mg) was shaken with octan-1-ol (1.00 ml) and water (1.00 ml) for 17-24 hours at 294±2 K. 0.30 ml aliquots of each phase were taken, 0.30 ml of acetonitrile-d_3 was added to each sample. ^{1}H, ^{19}F and ^{19}F{^{1}H} NMR spectra were acquired at 298 K. The samples were well tuned and calibrated 90-degree pulses were used, spectra were typically acquired in one scan to ensure complete pre-relaxation. For reprocessing between the spectra acquisition of different phase only zero-order phase was readjusted. Absolute integral ratio between equivalent resonances observed in water and octan-1-ol phase sample spectra was considered as partitioning constant. The whole procedure was performed in triplicate. The error takes into account discrepancies between different samples as well as the error of the NMR integration and acquisition.

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The data for the amino acid derivatives summarized in the paper has been reported in:

Pro: amide properties, pK_a [S11,S13], pK_a of the methyl ester and log$P$ [S12];

4CF_3Pro: amide properties and pK_a in [S1,S3]. Note, that the amide equilibrium constant for Ac-4CF_3Pro-OMe reported in [S3] is somewhat smaller than reported here. The contradiction is most likely a concertation effect due to a very high concentration of the analyte used in [S3] (~ 50 mM);

2CH_3Pro: pK_a data and amide properties in [S5];

4CH_3Pro: pK_a data and amide properties in [S11,S13];

5CH_3Pro: pK_a data and amide properties in [S13].

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