Electronic Supplementary Material (ESI) for New Journal of Chemistry

Supplementary information

Conformational properties of peptides incorporating a fluorinated pseudoproline residue

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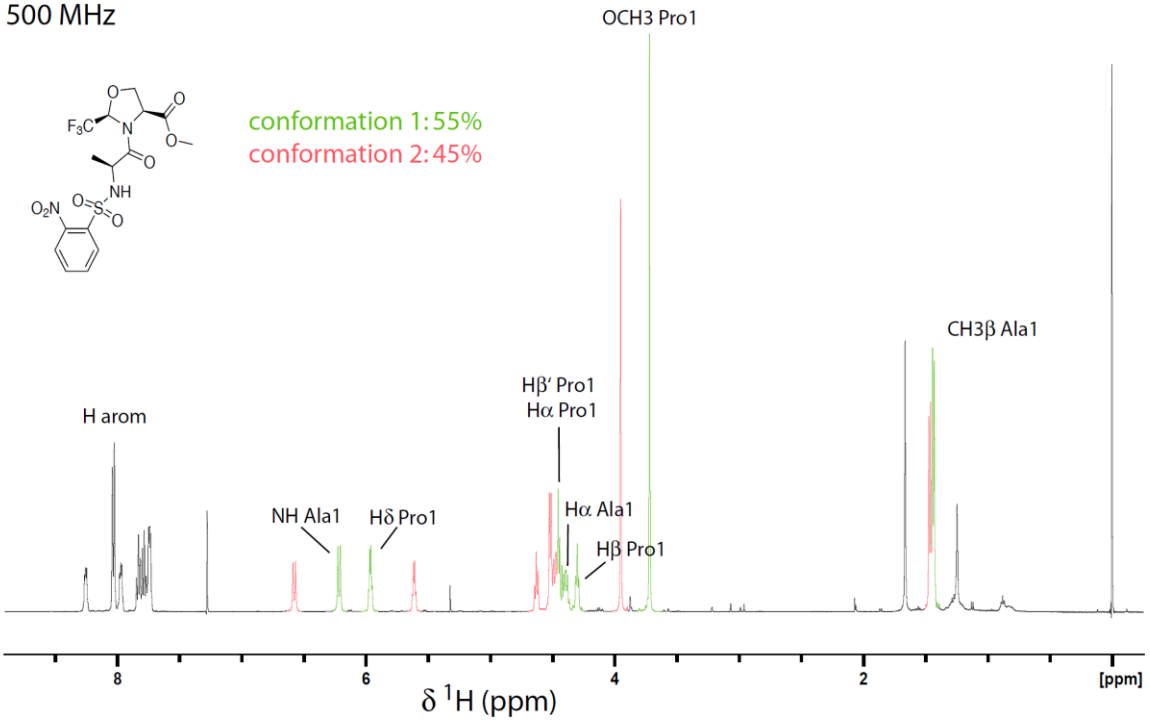
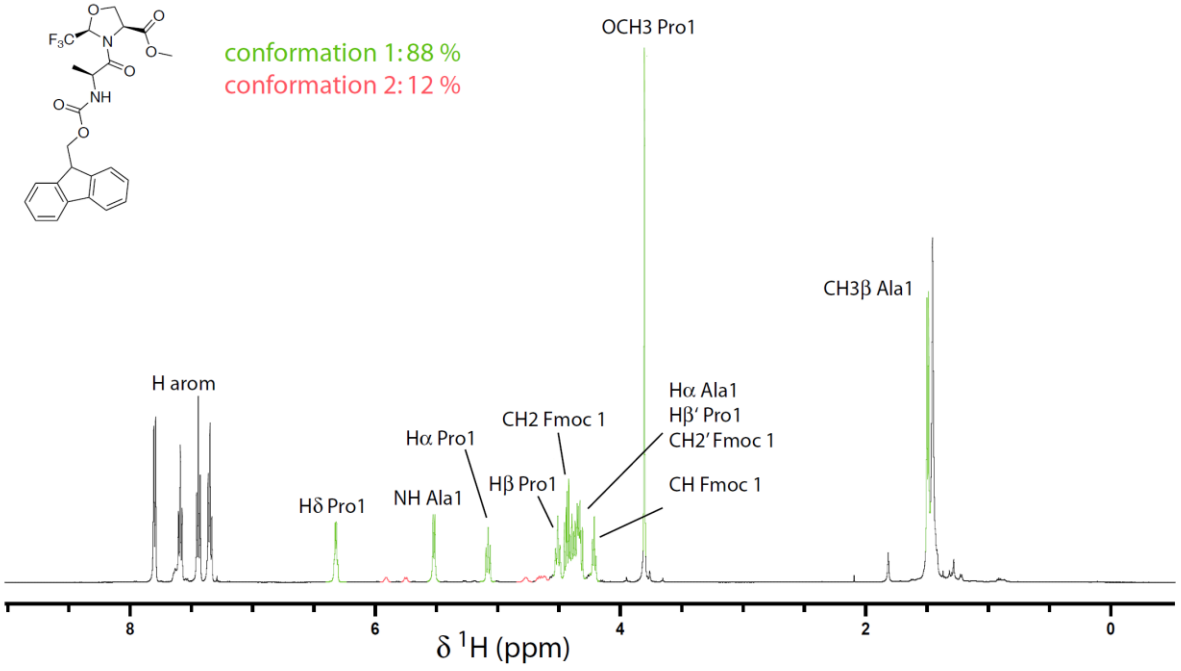
Proton NMR spectra of compounds 3-6

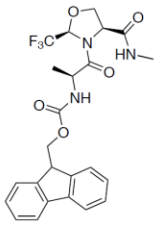
Compound **3**
$CDCl_3$, T = 274 K
500 MHz



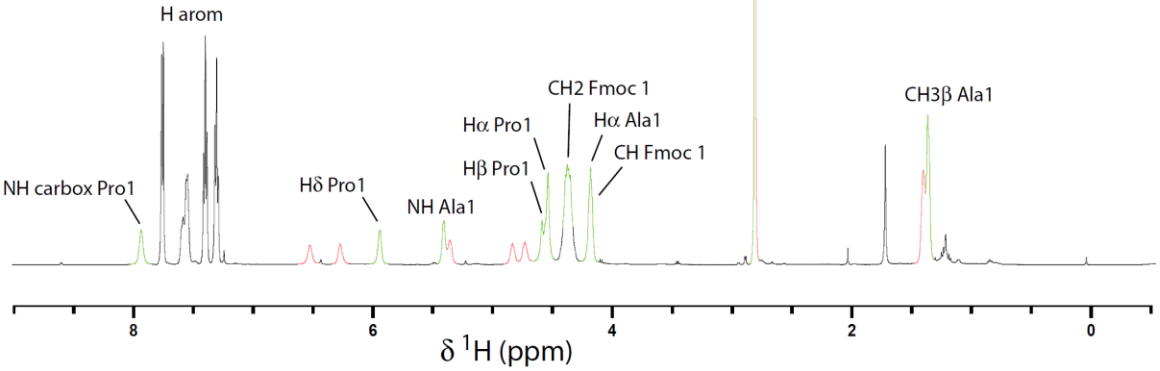
Compound **4**
$CDCl_3$, T = 274 K
500 MHz



Compound **5**
$CDCl_3$, T = 274 K
500 MHz



conformation 1: 58 %
conformation 2: 42 %



Compound **6**
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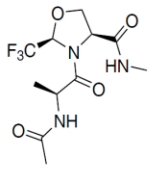
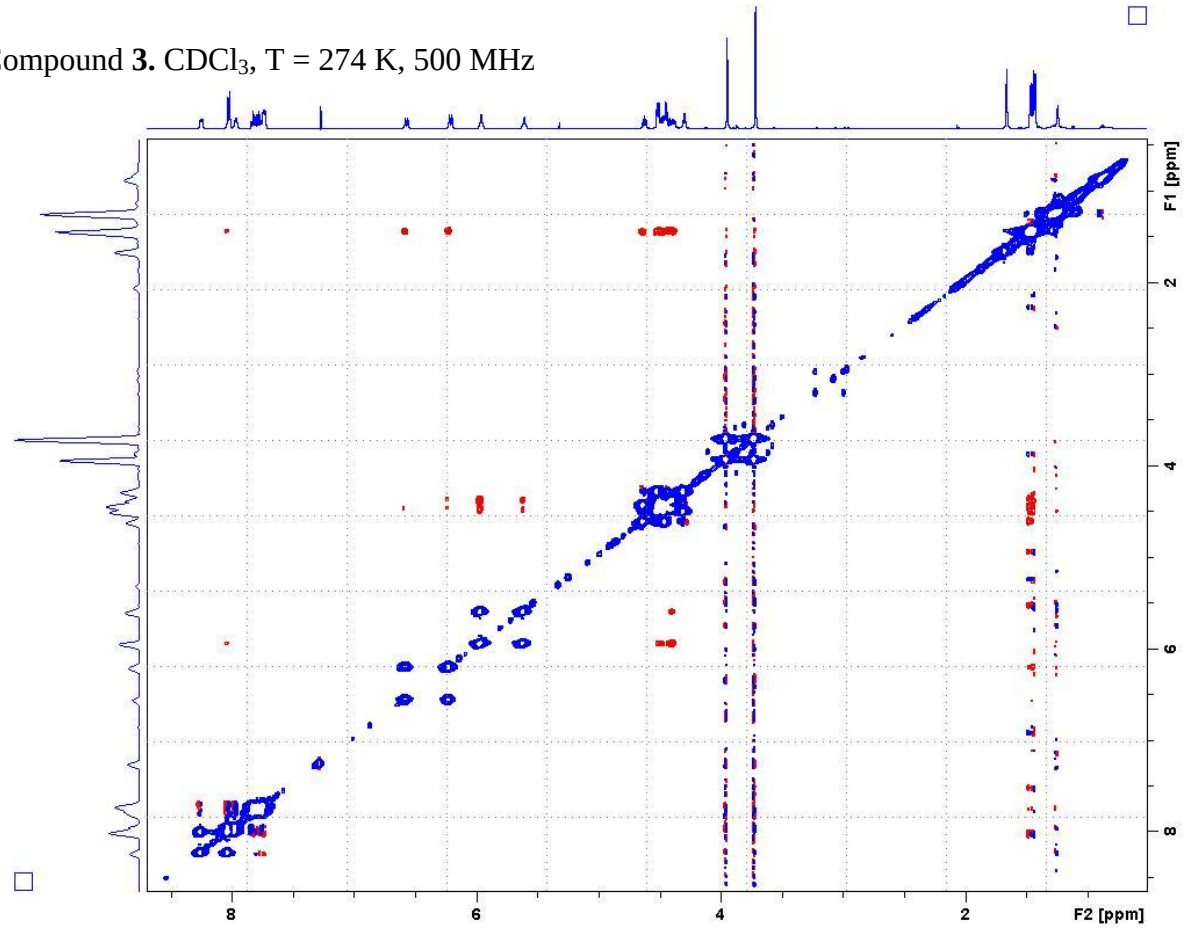
500 MHz



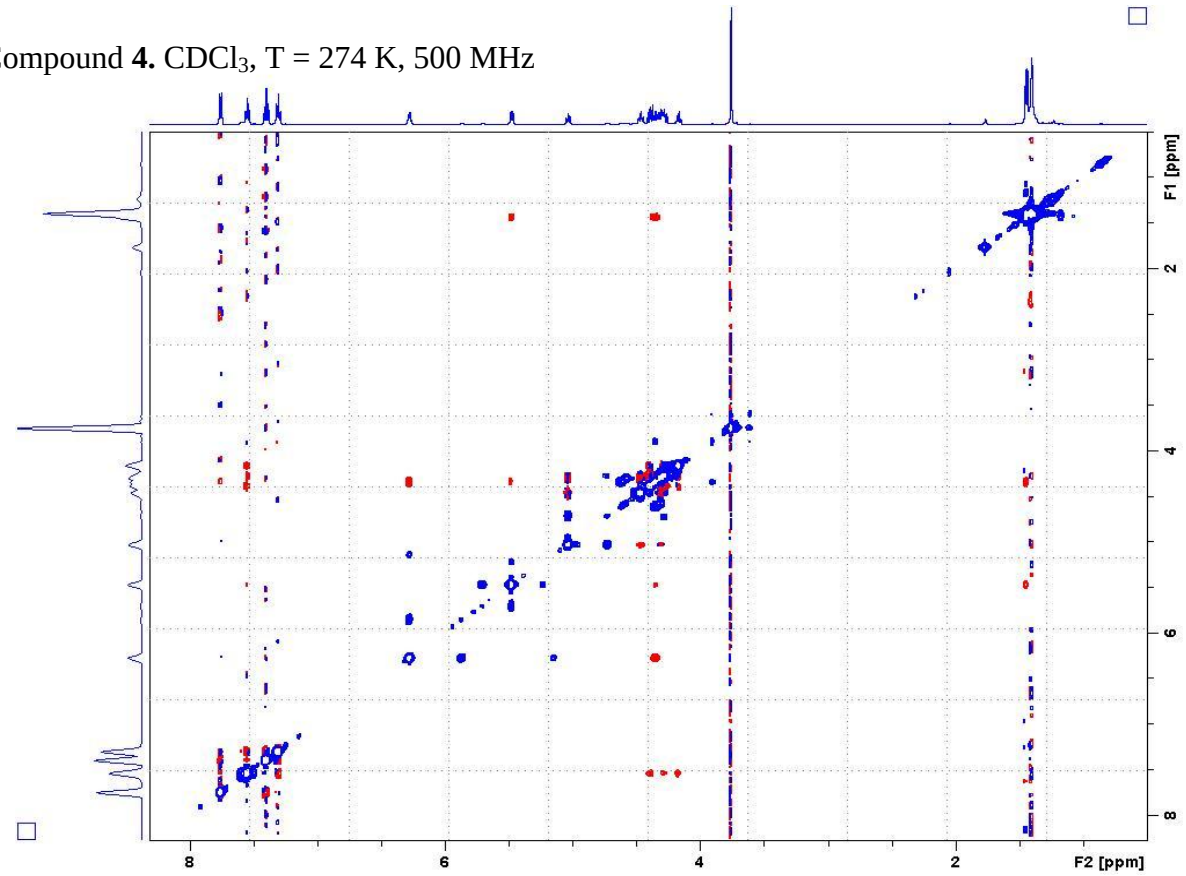
conformation 1: 70%
conformation 2: 30%

^{1}H-^{1}H NOESY spectra of compounds 3-6

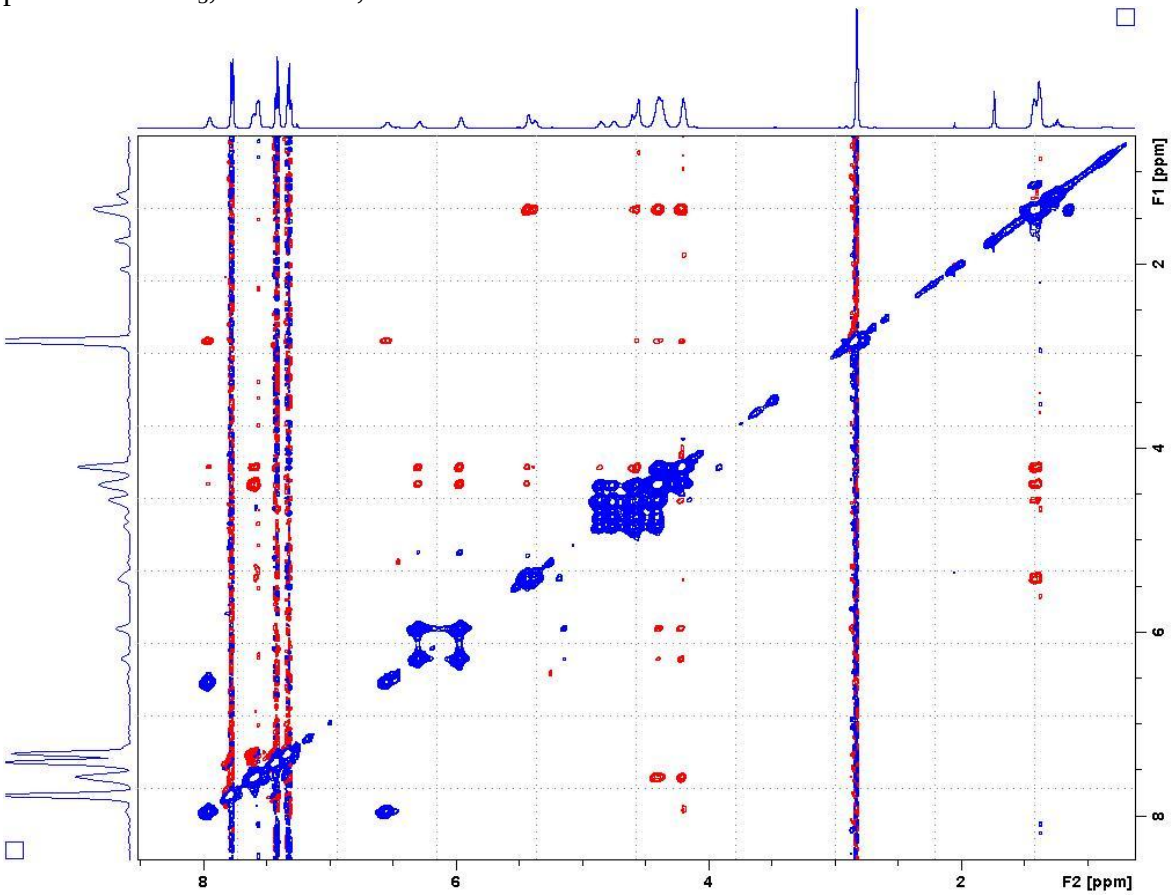
Compound **3.** $CDCl_3$, T = 274 K, 500 MHz



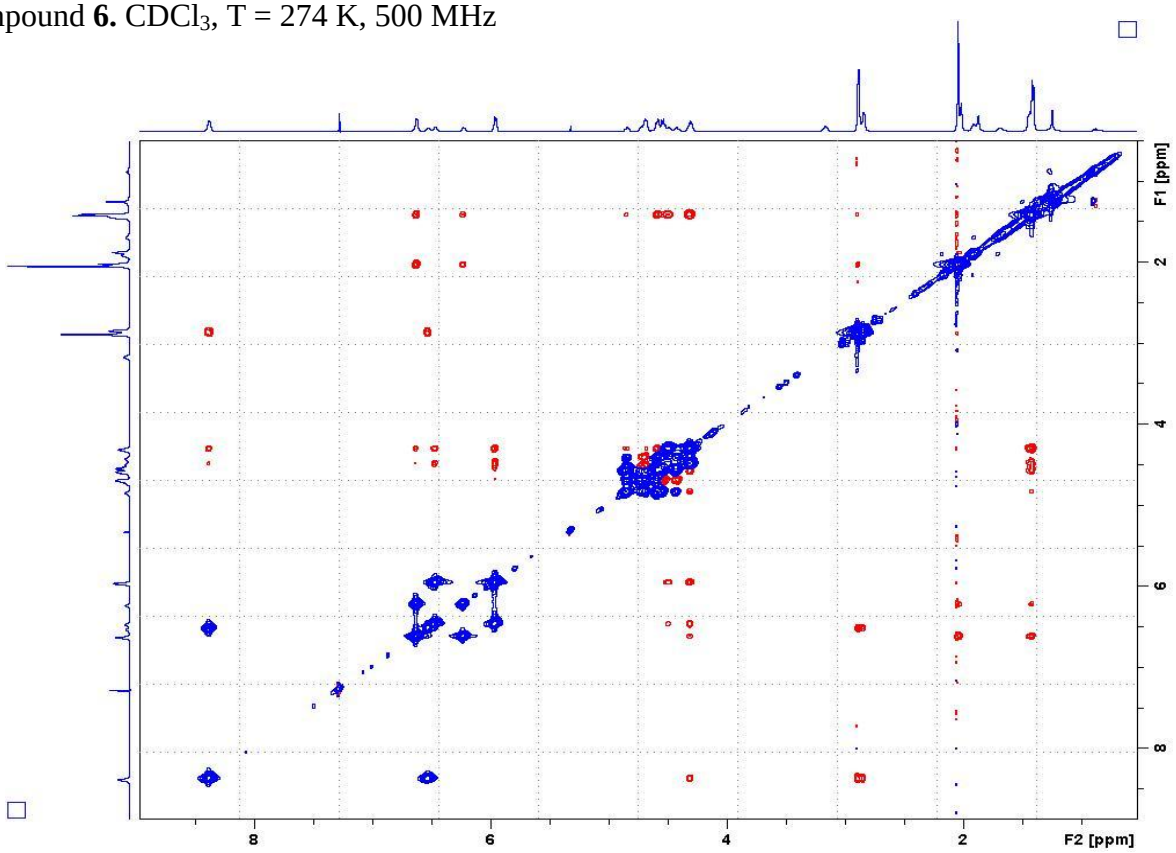
Compound **4.** $CDCl_3$, T = 274 K, 500 MHz



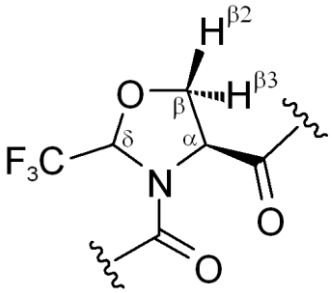
Compound **5.** $CDCl_3$, T = 274 K, 500 MHz



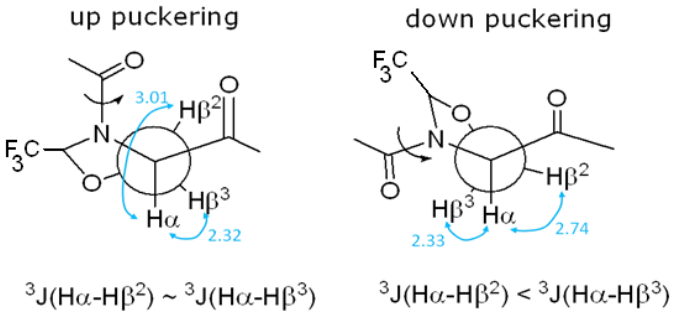
Compound **6.** $CDCl_3$, T = 274 K, 500 MHz



Determination of CF_3-ΨPro stereochemistry using 2D NMR

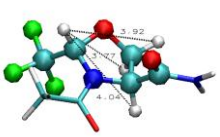
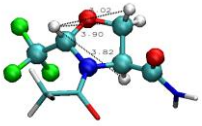
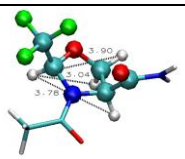
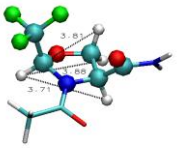


Stereochemistry of the C^{δ} center in CF_3-ΨPro can be determined from the precise analysis of ^1H-^1H NOESY experiments. First, the puckering of the 5-membered ring and the stereospecific assignment of the H^{β} methylene protons have to be determined. This can be readily obtained from the measurement of the $^3J_{H\alpha\text{-}H\beta3}$ and $^3J_{H\alpha\text{-}H\beta2}$ couplings and the comparison of the volume of the $H^{\alpha}/H^{\beta2}$ and $H^{\alpha}/H^{\beta3}$ cross peaks in the NOESY experiment. H^{α}-C^{α}-C^{β}-$H^{\beta3}$ and H^{α}-C^{α}-C^{β}-$H^{\beta2}$ dihedral angles obtained in CF_3-ΨPro minimized structures are associated with $^3J_{H\alpha\text{-}H\beta2} \approx {}^3J_{H\alpha\text{-}H\beta3}$ for the up puckering, and $^3J_{H\alpha\text{-}H\beta2} < {}^3J_{H\alpha\text{-}H\beta2}$ for the down puckering. Moreover, H^{α}-$H^{\beta3}$ distance is significantly lower than the H^{α}-$H^{\beta2}$ distance for the puckering up puckering whereas this difference is attenuated for the down puckering.



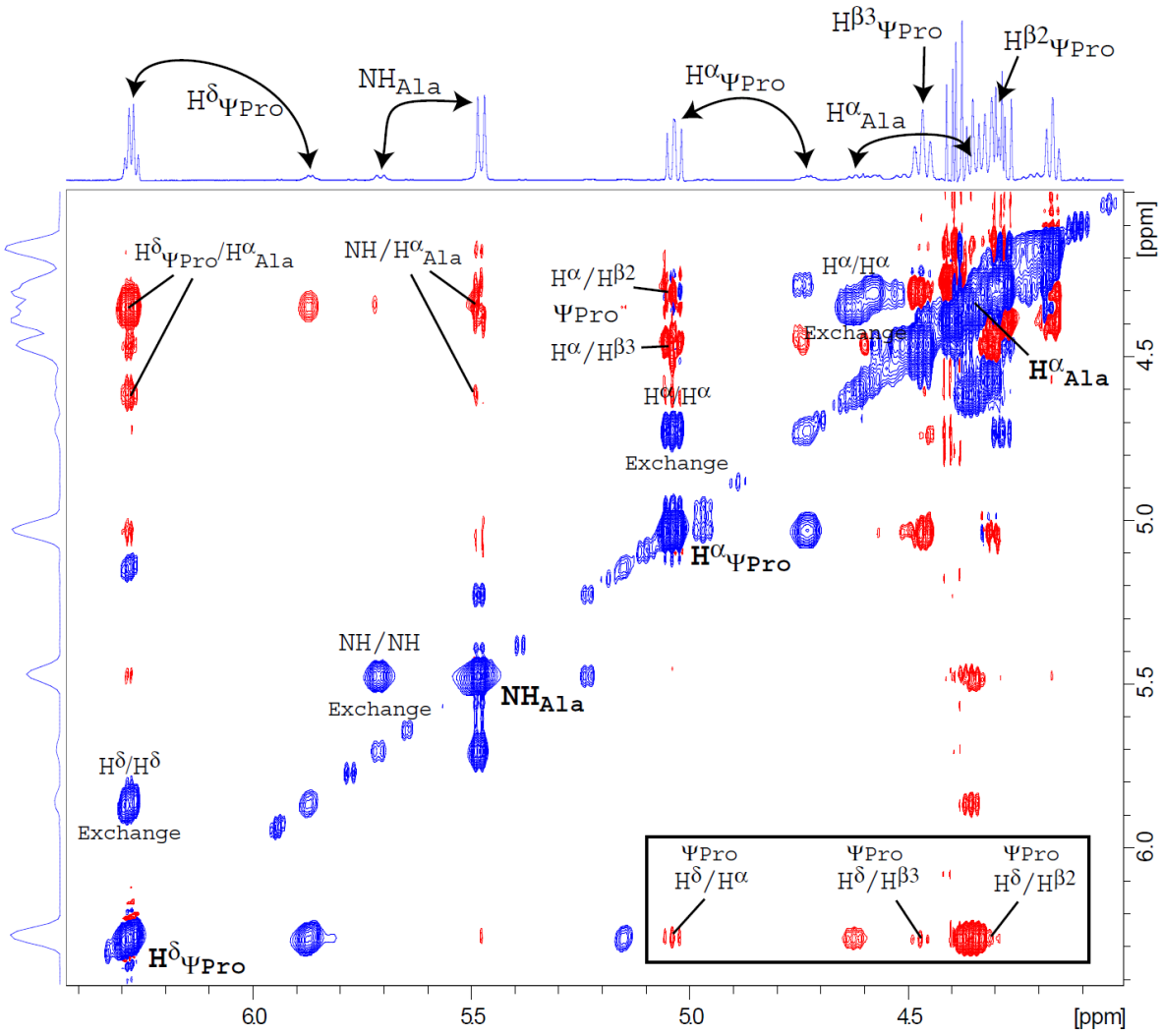
Once the stereospecific assignment and the puckering are known, observation of H^{δ}-$H^{\beta2}$; H^{δ}-$H^{\beta3}$ and H^{δ}-H^{α} nOe correlations unambiguously associate a configuration at the C^{δ} center (position 2). For a (2*S*) configuration and a down puckering or a (2*R*) configuration and a up puckering, d(H^{δ}-$H^{\beta2}$), d(H^{δ}-$H^{\beta3}$) and d(H^{δ}-H^{α}) are all close to 4 Å and the corresponding correlations are weak. For a (2*S*) configuration and a up puckering, d(H^{δ}-$H^{\beta2}$) is relatively short (~ 3 Å) and the corresponding nOe is much higher than those observed for the H^{δ}- $H^{\beta3}$ and H^{δ}-H^{α} correlations. On the contrary, for a (2*R*) configuration and a down puckering,

d(H^{δ}-$H^{\beta3}$) is relatively short (~ 3 Å) and the H^{δ}-$H^{\beta3}$ nOe is much higher than those observed for the H^{δ}-$H^{\beta2}$ and H^{δ}-H^{α} correlations. This is summarized in the following table.

				
CF_3 configuration	**2*S*, 4*S***	**2*S*, 4*S***	**2*R*, 4*S***	**2*R*, 4*S***
puckering	down (χ_1 = +25.3°)	up (χ_1 = -31.3°)	down (χ_1 = +31.2°)	up (χ_1 = -12.3°)
^3J	$^3J_{H\alpha\text{-}H\beta3} > {}^3J_{H\alpha\text{-}H\beta2}$	$^3J_{H\alpha\text{-}H\beta3} \approx {}^3J_{H\alpha\text{-}H\beta2}$	$^3J_{H\alpha\text{-}H\beta3} > {}^3J_{H\alpha\text{-}H\beta2}$	$^3J_{H\alpha\text{-}H\beta3} \approx {}^3J_{H\alpha\text{-}H\beta2}$
Noe	$I_{H\alpha\text{-}H\beta3} \approx I_{H\alpha\text{-}H\beta2}$	$I_{H\alpha\text{-}H\beta3} > I_{H\alpha\text{-}H\beta2}$	$I_{H\alpha\text{-}H\beta3} \approx I_{H\alpha\text{-}H\beta2}$	$I_{H\alpha\text{-}H\beta3} > I_{H\alpha\text{-}H\beta2}$
d (H^{δ}-H^{α})	4.04	3.82	3.78	3.71
d (H^{δ}-$H^{\beta3}$)	3.77	3.90	**3.04**	3.88
d (H^{δ}-$H^{\beta2}$)	3.92	**3.02**	3.90	3.81

As an example, the NOESY spectrum is provided for compound **4**. Here, the CF_3-ΨPro residue is found with $^3J_{H\alpha\text{-}H\beta2} \approx {}^3J_{H\alpha\text{-}H\beta3}$ (H^{α} resonance appears as a triplet, $^3J_{H\alpha\text{-}H\beta2}$ and $^3J_{H\alpha\text{-}H\beta3}$ are also precisely measured in the CH_2-TROSY experiment)[1]. These features characterize the up puckering and allow the $H^{\beta2}$/$H^{\beta3}$ stereospecific assignment. Then, the volumes of the H^{δ}-$H^{\beta2}$, H^{δ}-$H^{\beta3}$ and H^{δ}-H^{α} correlations are compared for allowing the determination of the configuration at C^{δ}. Despite a partial overlap of the $H^{\delta}{}_{\Psi Pro}$-$H^{\beta2}{}_{\Psi Pro}$ and the $H^{\delta}{}_{\Psi Pro}$-$H^{\alpha}{}_{Ala}$, it is found in this example that volumes of the H^{δ}-$H^{\beta2}$, H^{δ}-$H^{\beta3}$ and H^{δ}-H^{α} correlations are of the same order of magnitude which corresponds to the *R* configuration at C^{δ} (2*R*).

[1] Guichard, G.; Violette, A.; Chassaing, G.; Miclet, E. *Magn. Reson Chem*. **2008**, 46, 918-924.



Compound **4**
NOESY 500 MHz, T=274 K

Coupling constants and NOE correlations of peptide 6.

Dihedral restraints:

$^{3}J_{HN\text{-}H\alpha}$ of the Ala residue has been extracted and averaged from 1D proton and well-resolved NOESY cross peaks. The $^{3}J_{H\alpha\text{-}H\beta}$ couplings have been meassured using of the $^{1}H\text{-}^{13}C$ CH_2 TROSY experiment. Dihedral restraints have been directly derived from the coupling values, using the Karplus equation (Karplus parameters: A=8.77, B=-1.18, C=1.62 for $^{3}J_{H\alpha H\beta}$ and A=6.51, B=-1.76, C=1.60).[2]

$^{3}J_{HNH\alpha}$	4.9
$^{3}J_{H\alpha H\beta 2}$	4.6
$^{3}J_{H\alpha H\beta 3}$	8.2

Noe distances restraints :

The volumes of the NOESY cross peaks (I_{ab}) were converted into target distances d_{ab} using $d_{ab} = k/(I_{ab})^{6}$. The k value has been calibrated using the well resolved $HN_{Ala}\text{-}H^{\alpha}_{Ala}$ correlation and the $^{3}J_{HNH\alpha}$ coupling. Distance ranges ($d_{low} < d < d_{up}$) were then defined, with d_{low} = (d_{ab} - 0.5) Å and d_{up} = (d_{ab} + 0.5) Å.

NOE	proton i		proton j		d_{low} (Å)	d_{up} (Å)
1	ALA	Hα	ALA	CH3	2.13	3.13
2	ALA	Hα	ΨPRO	Hα	2.23	3.23
3	ALA	Hα	ALA	HN	2.60	3.60
4	ALA	Hα	Carbox	HN	2.36	3.36
5	ΨPRO	Hα	ALA	CH3	2.78	3.78
6	ALA	HN	ALA	CH3	2.55	3.55
7	Carbox	HN	Carbox	CH3	2.40	3.40
8	ΨPRO	Hδ	ALA	CH3	3.97	4.97
9	ΨPRO	Hδ	ΨPRO	Hβ2	3.46	4.46
10	ΨPRO	Hα	Carbox	HN	3.40	4.40
11	ΨPRO	Hβ2	Carbox	HN	3.85	4.85

[2] (a) Schmidt J.M. *J Biomol NMR*. 2007, **37**: 287-301. (b) Hansen E, Prog. NMR Spect, 1981, 14, 175-296.

Thermodynamic and kinetic parameters of peptide 6

Cis-trans isomerization rate constants were determined from the coalescence temperatures observed in 1D spectra (temperature studies between 274-323 K in $CDCl_3$, 278-353 K in D_2O:H_2O 1:9, or 294-353 K in DMSO-d_6).[3] For each sample, typically three coalescence temperatures were determined on three couple of resonances, which were used for the calculation of independent rate constants. An additional method based on 2D EXSY experiments was used for the measurements of rate constants (mixing times from 20 ms to 100 ms).[4] A very good agreement with the coalescence temperature method was obtained. Rotational barriers were then calculated using the Eyring equation.

6	$CDCl_3$	DMSO-d_6	D_2O
cis:trans	70:30	66:34	58:42
ΔG_{ct} (kCal/mol)	0.49	0.23	0.20
$\Delta G^{\ddagger}_{ct}$ (kCal/mol)	15.0 ± 0.3	15.0 ± 0.2	15.6 ± 0.2

[3] D. H. Williams, I. Fleming. Spectroscopic Methods in Organic Chemistry, 4th ed.; McGraw-Hill: London, 1987. H. Friebolin. Basic One- and Two-dimensional NMR Spectroscopy; VCH Verlagsgesellschaft: Weinheim, 1991.

[4] M. Keller, C. Sager, P. Dumy, M. Schutkowski, G. S. Fischer, M. Mutter. *J. Am. Chem. Soc.* **1998**, *120*, 2714–2720.

X-ray crystal data for peptide 3

Table 1. Crystal data for peptide **3** (chaume_C15H16F3N3O8S)

Compound	chaume_C15H16F3N3O8S
Molecular formula	$C_{15}H_{16}F_3N_3O_8S$
Molecular weight	455.37
Crystal habit	Colorless Block
Crystal dimensions(mm)	0.34x0.16x0.10
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a(Å)	8.177(1)
b(Å)	11.319(1)
c(Å)	20.788(1)
α(°)	90.00
β(°)	90.00
γ(°)	90.00
V(Å^3)	1924.0(3)
Z	4
d(g-cm^{-3})	1.572
F(000)	936
μ(cm^{-1})	0.247
Absorption corrections	multi-scan ; 0.9207 min, 0.9757 max
Diffractometer	KappaCCD
X-ray source	MoKα
λ(Å)	0.71069
Monochromator	graphite
T (K)	150.0(1)
Scan mode	phi and omega scans
Maximum θ	30.01
HKL ranges	-11 11 ; -15 15 ; -21 29
Reflections measured	18849
Unique data	5601
Rint	0.0285
Reflections used	4878
Criterion	I > 2σI)
Refinement type	Fsqd
Hydrogen atoms	mixed
Parameters refined	277
Reflections / parameter	17
wR2	0.0829
R1	0.0345
Flack's parameter	0.04(6)
Weights a, b	0.0342 ; 0.3983
GoF	1.056
difference peak / hole (e Å^{-3})	0.166(0.043) / -0.311(0.043)