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Conformational properties of peptides incorporating a fluorinated pseudoproline residue†

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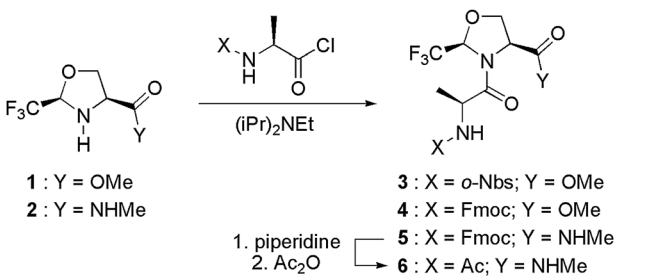
We have recently reported the synthesis of enantiomerically pure CF_3-oxazolidine pseudoprolines (CF_3-ΨPro). Complete NMR studies, together with DFT calculations, have highlighted the marked stereoelectronic effects of the CF_3 group on these new proline surrogates. In this paper, we describe for the first time the conformational features of dipeptides incorporating one CF_3-ΨPro residue. Extensive NMR analyses have been carried out in solution and revealed the presence of a stable type-VI β-turn in a pseudotetrapeptide sequence.

Introduction

The proline residue is of special significance in the structure of peptides and proteins. The pyrrolidine ring restricts the ϕ-dihedral angle to values around -60° and the presence of the tertiary amide leads to increased *cis* populations for the Xaa–Pro peptide bonds (lower free energy difference ΔG_{tc}).[1] These unique conformational properties of the peptide backbone are associated with a *cis–trans* isomerisation rate that is relatively high compared to the other amino acids (lower activation energy $\Delta G^{\ddagger}_{tc}$).[2] During the last two decades, it has been shown that proline analogues can be designed to further tailor the structural and thermodynamical features of the peptide backbone. C^{δ}-substituted prolines and pseudoprolines (ΨPro) have proved to be very useful as ΔG_{tc} and $\Delta G^{\ddagger}_{tc}$ can be finely tuned depending on the nature of the substituent and on the absolute configuration at C^{δ}.[3–6] Replacement of key proline residues by such analogues in biologically active peptides offers invaluable opportunities for the modulation of their activity.[7–9]

For a few years we have been interested in the stereoselective synthesis of trifluoromethyl group containing amino acids.[10] Recently, we reported the synthesis of CF_3-substituted pseudoprolines (CF_3-ΨPro)[11] and used both theoretical calculations and NMR studies to deliver a detailed analysis of the CF_3 stereoelectronic effects within these proline surrogates.[12] We demonstrated that the trifluoromethyl group (i) was responsible for freezing the puckering of the 5-membered oxazolidine ring, (ii) lowered the rotational barrier of the *cis–trans* peptide bond isomerization and (iii) locally promoted the stabilization of extended ψ-dihedral angles. We were then interested in the conformational studies of peptide oligomers containing such a CF_3-ΨPro amino acid. Herein, we report the structural properties of four peptides based on the X–Ala–Ser($\Psi^{CF_3,H}$Pro)–Y sequence, where X is a *o*-Nbs, Fmoc or Ac group and Y is a OMe or NHMe group (Scheme 1). For each peptidomimetic molecule, an extensive NMR study is provided, highlighting the influence of these terminal groups on the structure and stability of the backbone of the CF_3-ΨPro containing peptides **3–6**.



Scheme 1 Synthesis and chemical structures of compounds **3–6**.

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† Electronic supplementary information (ESI) available: NMR spectra of compounds **3–6**, method used for the determination of the stereochemistry of L-Ser($\Psi^{CF_3,H}$Pro) amino acids, tables with NMR restraints, thermodynamic and kinetic parameters of peptide **6**, and X-ray crystal data for peptide **3**. CCDC 910329. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3nj41084f

Results and discussion

Synthesis and characterization of peptides

(2*R*,4*S*)-CF_3-pseudoproline ester **1** is conveniently synthesized by condensation of serine esters with trifluoroacetaldehyde hemiacetal.[11] It can subsequently undergo a N-methylamidation in a good yield leading to compound **2**. Both compounds **1** and **2** were used as CF_3-ΨPro-building blocks for the synthesis of peptides **3–6** (Scheme 1). L-Ala with *o*-Nbs (**3**) or Fmoc (**4**, **5**) N-protecting groups was chosen as an amino acid model for the extension of the peptide backbone. Coupling reactions have been carried out using amino acid chlorides in the presence of base in order to overcome the low nucleophilicity of the CF_3-ΨPro amino group. Fmoc deprotection of peptide **5** followed by N-acetylation gave the pseudotetrapeptide **6**. Peptides **3**, **4** and **5** were obtained, respectively, from (2*R*,4*S*)-CF_3-pseudoproline ester **1** and (2*R*,4*S*)-CF_3-pseudoproline amide **2** in good yield as a diastereomeric mixture. Major compounds **3**, **4** and **5** (>70%) have been isolated using flash chromatography and were well characterized using 2D NMR spectroscopy as described in detail in the ESI.† In addition, we provide the X-ray structure for **3** (Fig. 1)† which nicely confirmed the stereochemistry determined by NMR. Although these major peptides were all found to incorporate a (2*S*)-alanine and a (2*R*,4*S*)-CF_3-pseudoproline, the presence of a minor diastereoisomer could be due to the epimerization of the alanine or the pseudoproline residues during the coupling reactions. Unfortunately, we are not able to assign the absolute configuration of the minor diastereomers at this stage. Further efforts will be made for the complete analysis of the epimerization process and for the optimization of the coupling conditions.

Analysis of the *cis–trans* populations

For each compound **3–6**, two slowly exchanging conformers were observed at 274 K in $CDCl_3$, as attested by the presence of exchange cross peaks in the ^1H–^1H EXSY spectra.[5,13] Integration of isolated resonances has been used to determine the corresponding populations. For **3** and **5** the two conformers were almost equally populated whereas an imbalance of 9 : 1 and 7 : 3 was observed in **4** and **6**, respectively (Table 1). The Ala–CF_3-ΨPro peptide bond conformations were then established using inter-residue NOE correlations. We observed

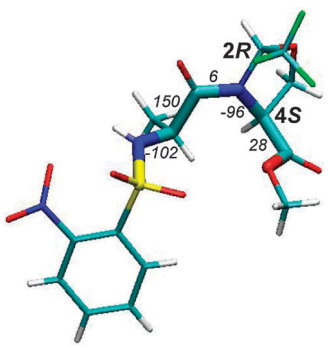

Fig. 1 Overview of the X-ray structure obtained for **3**. The stereochemistry of the CF_3-ΨPro residue and backbone dihedral angles (in degree) have been reported.

Table 1 *cis* : *trans* ratio calculated for compounds **3–6** at 274 K.[a] The geometry of the peptide bond was not clearly established for peptide **3** in $CDCl_3$

Comp.	Solvent	*Cis* : *trans*
3	$CDCl_3$	45 : 55 or 55 : 45
4	$CDCl_3$	12 : 88
5	$CDCl_3$	58 : 42
6	$CDCl_3$	70 : 30
6	DMSO-d_6[a]	66 : 34
6	H_2O : D_2O 90 : 10	58 : 42

[a] 294 K for **6** in DMSO.

a strong H^{α}_{Ala}–$H^{\delta}_{\Psi Pro}$ cross peak for the major spin system of **4** in agreement with a *trans* peptide bond, whereas compounds **5** and **6** displayed an additional H^{α}_{Ala}–$H^{\alpha}_{\Psi Pro}$ correlation, indicating a stabilized *cis* conformation. We conclude that replacing the *o*-Nbs (**3**) by the bulky Fmoc group (**4**) strongly destabilized the Ala CF_3-ΨPro *cis* peptide bond. This effect was alleviated by the incorporation of the NHMe C-terminal group (**5**) leading to almost equal populations. A further stabilization of the *cis* conformation was finally obtained in the pseudotetrapeptide **6**, which lacks the Fmoc protecting group.

Interestingly, the population levels of **6** were strongly influenced by the solvent. The highest stabilization of the *cis* state was obtained in $CDCl_3$ followed by DMSO-d_6 and H_2O. The presence of an intramolecular hydrogen bond involving the NH carboxamide could be responsible for the relatively high *cis* population observed in peptides **5** and **6** in apolar solvent. This has been assessed by comparing the solvent dependence of the NH protons chemical shifts ($\Delta\delta$) as well as their temperature coefficients ($\Delta\delta/\Delta T$).[14] When changing the solvent from $CDCl_3$ to DMSO or from $CDCl_3$ to H_2O, typical $\Delta\delta$ values were 2 ppm for the NH protons of pseudotetrapeptides **6**, except for the terminal NH methylamide in the *cis* conformer which displayed very low values of 0.31 ppm and 0.37 ppm, respectively (Table 2). The highest temperature coefficient was also obtained for this proton (−5.8 ppb K^{-1}), which was in agreement with a weak hydrogen bond in water.

To complete the analysis of the *cis–trans* isomerization on the pseudotetrapeptide **6**, we evaluated the energy barriers $\Delta G^{\ddagger}_{ct}$ by performing quantitative analyses of the EXSY experiments[5] and by measuring the coalescence temperatures.[15] The relatively low values obtained in $CDCl_3$, DMSO or in water (~15 kcal mol^{-1}, see ESI†) were ascribed to the presence of the (2*R*)-CF_3 substituent, acting as a stabilizing polar group of the transition state.[12] The $\Delta G^{\ddagger}_{ct}$ values corresponding to the *cis* to *trans* isomerization were not greatly influenced by the solvent. This could be due to the fact that increasing the solvent polarity

Table 2 Solvent dependence and temperature coefficients of the NH protons in peptide **6**

	NH		NH carbox	
	Ala *cis*	Ala *trans*	*cis*	*trans*
$\Delta\delta_{CDCl_3 \rightarrow DMSO}$ (ppm)	1.30	2.33	0.31	2.04
$\Delta\delta_{CDCl_3 \rightarrow H_2O}$ (ppm)	2.17	1.96	0.37	2.09
$\Delta\delta/\Delta T$ (H_2O) (ppb K^{-1})	−8.5	−7.8	−5.8	−8.8

destabilized the transition state, but also the *cis* conformation by disrupting the H-bond.

Structural analysis of the pseudotetrapeptide 6

We have previously shown that the presence of the CF_3 group in the CF_3-ΨPro residue was also responsible for stereoelectronic effects that efficiently constrain the ring puckering.[12] In Ac–(2*R*,4*S*)-CF_3-ΨPro–NHMe, the ring geometry always adopted an up puckering, independently of the solvent or the peptide bond conformation. We have then performed CH_2-TROSY experiments to precisely measure the vicinal $^3J_{H\alpha-H\beta}$ couplings in the longer peptide **6** (Fig. 2).[16] These constants enable the precise determination of the 5-membered ring conformation.

Whereas the *trans* conformer displayed the characteristic vicinal coupling values for an up puckering ($^3J_{H\alpha H\beta 2}$ = 7.2 Hz and $^3J_{H\alpha H\beta 3}$ = 8.8 Hz), no canonical conformation was assigned to the *cis* form ($^3J_{H\alpha H\beta 2}$ = 4.6 Hz and $^3J_{H\alpha H\beta 3}$ = 8.2 Hz). An envelope conformation of the 5-membered ring ($\chi_1 \sim 0^\circ$) or fast exchanging and equally populated up/down puckerings could account for these coupling values. This is in strong contrast to the features observed on the pseudopeptide lacking the Ala residue (Ac–CF_3-ΨPro–NHMe). First, it was found to adopt the up puckering, whatever the peptide bond geometry or the solvent polarity. Second, the C-terminal carboxamide proton was also involved in an H-bond but for the *trans* conformer only, which was consequently the major conformer in $CDCl_3$. The peculiar features of the peptide **6** are then clearly related to the presence of the preceding Ala residue. The unusual geometry observed for the oxazolidine ring may be of importance for the establishment of the H-bond in the *cis* conformer.

To fully characterize the structural properties of the *cis* conformation of the pseudotetrapeptide **6**, we have conducted structure calculations using a restrained dynamic simulated annealing. A total of 11 proton–proton distance restraints have been extracted from the NOESY spectrum in $CDCl_3$ and 3 coupling constants have been measured (see ESI†). Despite the limited number of NMR constraints, the 5-lowest energy structures were well superimposed as shown in Fig. 3. We observed an H-bond between the NH carboxamide proton and the oxygen of the N-terminal acetyl carbonyl, which was in perfect agreement

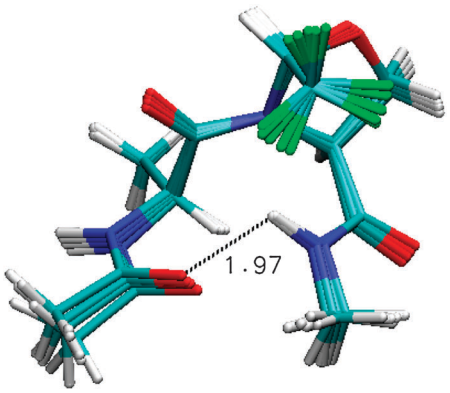

Fig. 3 Overlay of the 5-lowest energy structures obtained by molecular dynamics under NMR restraints. The NH···OC distance is displayed in Å.

with our previous observations. The *cis* Ala–ΨPro peptide bond conformation together with the backbone dihedral angles of the Ala and the CF_3-ΨPro residues (ϕ_{Ala} = −66°; ψ_{Ala} = 140°; ω = −1°; $\phi_{\Psi Pro}$ = −76° and $\psi_{\Psi Pro}$ = 2°) defined a nice type-VI a1 β-turn (canonical dihedral angles are ϕ_{i+1} = −60°; ψ_{i+1} = 120°; ω = 0°, ϕ_{i+2} = −90° and ψ_{i+2} = 0°). Similar dihedral angles were also measured in the crystal structure of **3** for the Ala–CF_3-ΨPro pair (Fig. 1), suggesting that the X-ray peptide scaffold was maintained in solution. Similarly, the χ_1 angle of the ΨPro residue measured on the calculated structures of **6** was *ca.* (5 ± 3)°, which corresponds to a slightly down-puckered envelope and is in fair agreement with the X-ray structure of **3** (χ_1 = +15°).

It should be stressed that both the relatively high population of the *cis* conformer in water (58%) and the temperature coefficient for the NH carboxamide (−5.8 ppb K^{-1}) indicate the persistence of an H-bond in this solvent, although partly destabilized. Therefore, the Ala–CF_3-ΨPro unit appears to be very valuable for inducing type-VI β-turn in solution. This original secondary structure has attracted considerable interest because it is implicated in the function of important protein and is associated with the high potency of numerous peptide hormone analogues.[9,17–22] However, the design of new molecules mimicking the type-VI β-turn remains a challenge since it requires the stabilization of the *cis* peptide bond. Several attempts have been made in the past fifteen years. Direct cyclization of the peptide backbone has been considered and led to several templates such as 8-membered ring lactam,[18] functionalized piperidinone,[19] aminopyroglutamate,[20] or bicyclic systems.[21] Alternatively, proline surrogates that favor the *cis* peptide bond conformation have been assessed for their propensity to induce type-VI β-turns. This included C^δ-substituted prolines,[14,22] pseudoprolines[9,23] or azaprolines.[24] It has been predicted that the combination of these different modifications would enhance the *cis* preference for the peptide bond, by taking advantage of the steric and the electronic effects.[25] Herein, we used the (2*R*,4*S*)-CF_3-pseudoproline that bears the highly electronegative and bulky CF_3 group in addition to the oxazolidine ring oxygen. We demonstrated that the (2*R*,4*S*)-CF_3-ΨPro induces a 58% *cis* peptide bond conformation in water for a minimal

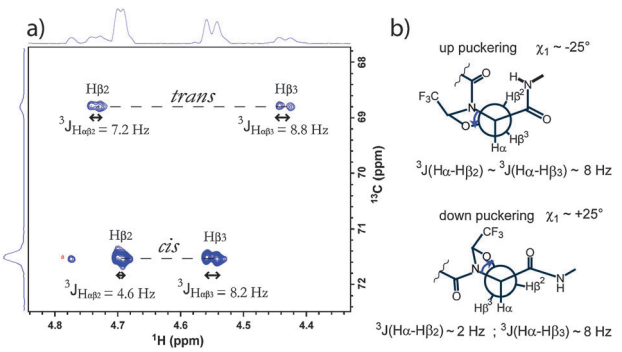

Fig. 2 (a) $C^\beta H_2$ region of the CH_2-TROSY experiment recorded on compound **6** in $CDCl_3$, 274 K, 500 MHz. (b) Schematic representation of the up and down puckerings with the corresponding $^3J_{H\alpha H\beta}$ vicinal couplings.

pseudotetrapeptide sequence. The MD calculations matched the X-ray structure and revealed a preference for a type VI β-turn conformation in solution. Further structural studies on longer peptides incorporating the CF_3-ΨPro residue are currently under investigation in our laboratory.

Experimental

Synthesis and characterization of compounds 3–6

Unless otherwise mentioned, all the reagents were purchased from a commercial source. All glassware was dried in an oven at 150 °C prior to use. CH_2Cl_2 was distilled under nitrogen from CaH_2 prior to use. ^{1}H NMR (400.00 MHz), ^{13}C NMR (100.50 MHz) and ^{19}F NMR (376.20 MHz) were measured on a JEOL ECX400 spectrometer, ^{1}H NMR (500.00 MHz) and ^{13}C NMR (125.75 MHz) were measured on a Bruker Avance III spectrometer operating at a 1H frequency of 500 MHz and equipped with a triple resonance, *z*-axis pulsed-field-gradient cryogenic probehead, optimized for ^{1}H detection. Complete proton assignments were obtained from the analysis of 2D total correlation spectroscopy (TOCSY) experiments using 80 ms DIPSI-2 mixtime and 2D nuclear Overhauser effect spectroscopy (NOESY) experiments (typically 500 ms mixing time). Homonuclear experiments were typically performed using 512 (t_1) and 4096 (t_2) time-domain matrices over a spectral width of 10 ppm, with 8 scans per t_1 increment. Carbon assignment was deduced from heteronuclear 2D ^{1}H–^{13}C HSQC and 2D ^{1}H–^{13}C CH_2-TROSY[16] experiments, using 256 (t_1) × 1024 (t_2) time-domain matrices, with 32 scans per t_1 increment. Data were processed using the TOPSPIN 2.0 software (Bruker). Shifted sine-bell window functions were applied in both indirect and direct detected dimensions and extensive zero-filling prior to Fourier transformation was used to yield high digital resolution. Chemical shifts of ^{1}H NMR are expressed in parts per million downfield from tetramethylsilane (δ = 0) in $CDCl_3$. Chemical shifts of ^{13}C NMR are expressed in parts per million downfield from $CDCl_3$ as internal standard (δ = 77.0). Chemical shifts of ^{19}F NMR are expressed in parts per million downfield from C_6F_6 as an internal standard (δ = −164.9). Coupling constants are reported in Hertz. Column chromatography was performed on SDS 60 Å (40–63 μm) silica gel, employing a mixture of the specified solvent as an eluent. Thin-layer chromatography (TLC) was performed on Merck silica gel (Merck 60 PF254) plates. Silica TLC plates were visualized under UV light, by a 10% solution of phosphomolybdic acid in ethanol followed by heating. Mass spectra (MS) were obtained on a GC/MS apparatus HP 5973 MSD with an HP 6890 Series GC. Ionization was obtained by electronic impact (EI 70 eV). Infrared spectra (IR) were obtained by Fourier-transformation on Bruker Tensor 27, wavenumbers are given in cm^{-1}. Liquid Chromatography-Mass Spectrometry (LC-MS) analyses were done on a Waters (LC) ESI/TOF spectrometer and MS spectrum was recorded using an ESI ionization and positive ion mode. Elemental analyses were performed on a Perkin–Elmer CHN 2400. Optical rotations were determined using a JASCO P1010 polarimeter. HRMS analyses were performed on a Jeol JMS-GC Mate II or using a Waters (LC) ESI/TOF spectrometer. Melting points were obtained on a Büchi apparatus and are uncorrected.

***o*-Nbs-Ala-Ser($\Psi^{CF_3,H}$Pro)-OMe (3)[11].** To a solution of the *o*-Nbs-alanine[26] (1.30 g, 4.74 mmol, 7 equiv.) suspended in dichloromethane (4.8 mL), under argon, was added 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (628 μL, 4.74 mmol, 7 equiv.) at 0 °C. The resulting solution was stirred at 0 °C until the disappearance of the precipitate (usually 20 min). The total conversion of the acid to chloride was checked by TLC after quenching with methanol. The *o*-Nbs-alanine chloride solution was added *via* cannula to a neat mixture of pseudoprolines (*R*,*S*)-**1** (135 mg, 0.68 mmol, 1.0 equiv.) and collidine (90 μL, 0.68 mmol, 1.0 equiv.) at 0 °C. The temperature was allowed to warm to room temperature and the solution was concentrated twice using a stream of argon. After 24 h, the resulting mixture was diluted with dichloromethane, and quenched with a saturated aqueous solution of $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude 73 : 27 mixture of diastereomers was purified by flash chromatography (70 : 30 cyclohexane–ethyl acetate) to give 70 mg (23%) of minor diastereomer $\mathbf{3_{min}}$ and 210 mg (68%) of major diastereomer (*R*,*S*)-**3** as a 45 : 55 inseparable mixture of *cis–trans* rotational isomers in $CDCl_3$ at 274 K. (*R*,*S*)-**3** *major diastereomer*: white solid; mp 182–183 °C; R_f = 0.30 (60 : 40 cyclohexane–ethyl acetate); $[\alpha]_D^{24}$ −122.5 (*c* 1.8, $CHCl_3$); IR (neat) 3300, 3107, 1730, 1677, 1535, 1440, 1154 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 274 K) (major rotamer) δ 1.44 (d, *J* = 5.5 Hz, 3H, H_β Ala-H), 3.71 (s, 3H, OMe), 4.30 (t, *J* = 6.4 Hz, 1H, H_β ΨPro-Ha), 4.39 (m, 1H, H_α Ala-H), 4.45 (m, 2H, H_α and H_β ΨPro-Hb), 5.97 (q, *J* = 5.2 Hz, 1H, H_δ ΨPro-H), 6.22 (d, *J* = 9.5 Hz, 1H, NH Ala); 7.73–7.85 (m, 2H, *o*-Nbs arom.), 7.95–8.05 (m, 2H, *o*-Nbs arom.); (minor rotamer) δ 1.46 (d, *J* = 5.5 Hz, 3H, H_β Ala-H), 3.95 (s, 3H, OMe), 4.49 (m, 1H, H_α Ala-H), 4.52 (d, *J* = 7.1 Hz, 2H, H_β ΨPro-H), 4.63 (t, *J* = 6.9 Hz, 1H, H_α ΨPro-H), 5.61 (q, *J* = 4.9 Hz, 1H, H_δ ΨPro-H), 6.57 (d, *J* = 10.1 Hz, 1H, NH Ala), 7.73–7.85 (m, 2H, *o*-Nbs arom.), 8.03 (m, 1H, *o*-Nbs arom.), 8.25 (m, 1H, *o*-Nbs arom.); ^{13}C NMR (125.75 MHz, DMSO-d_6, 298 K) (major rotamer) δ 18.8 (CH_3, C_β Ala), 51.5 (CH, C_α Ala), 53.1 (CH_3, OMe), 56.3 (CH, C_α ΨPro), 68.9 (CH_2, C_β ΨPro), 84.5 (q, *J* = 36.4 Hz, CH, C_δ ΨPro), 122.3 (q, *J* = 279.9 Hz, CF_3), 124.3 (CH, *o*-Nbs arom.), 129.5 (CH, *o*-Nbs arom.), 132.6 (CH, *o*-Nbs arom.), 133.1 (CH, *o*-Nbs arom.), 134.2 (C, *o*-Nbs arom.), 147.3 (C, *o*-Nbs arom.), 168.8 (C═O), 170.8 (C═O); (minor rotamer) δ 19.3 (CH_3, C_β Ala), 51.9 (CH, C_α Ala), 53.8 (CH_3, OMe), 56.6 (CH, C_α ΨPro), 70.4 (CH_2, C_β ΨPro), 84.4 (q, *J* = 36.4 Hz, CH, C_δ ΨPro), 122.3 (q, *J* = 279.9 Hz, CF_3), 124.3 (CH, *o*-Nbs arom.), 129.5 (CH, *o*-Nbs arom.), 132.6 (CH, *o*-Nbs arom.), 133.5 (CH, *o*-Nbs arom.), 133.8 (C, *o*-Nbs arom.), 147.3 (C, *o*-Nbs arom.), 168.7 (C═O), 170.8 (C═O); ^{19}F NMR (376.2 MHz, DMSO-d_6, 298 K) (major rotamer) δ −80.9 (s, CF_3); (minor rotamer) δ −81.7 (s, CF_3); LCMS (ES^+) *m*/*z* = 456.46 $[M + H]^+$; anal. calcd for $C_{15}H_{16}F_3N_3O_8S$ (455.06): C, 39.56; H, 3.54; N, 9.23. Found: C, 39.45; H, 3.47; N, 8.99%. *Minor diastereomer* $\mathbf{3_{min}}$: colorless oil; R_f = 0.41 (50 : 50 cyclohexane–ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$, 323 K) (single rotamer):

δ 1.43 (d, J = 6.9 Hz, 3H, H_β Ala-H), 3.82 (s, 3H, OMe), 4.43 (dq, J = 8.7, 6.9 Hz, 1H, H_α Ala-H), 4.57 (d, J = 6.4 Hz, 2H, H_β ΨPro-H), 5.16 (t, J = 6.4 Hz, 1H, H_α ΨPro-H), 5.56 (q, J = 5.0 Hz, 1H, H_δ ΨPro-H), 6.15 (d, J = 8.7 Hz, 1H, NH Ala), 7.70–7.75 (m, 2H, *o*-Nbs arom.), 7.88–7.92 (m, 2H, *o*-Nbs arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 323 K) (single rotamer): δ 18.7 (CH_3, C_β Ala), 51.3 (CH, C_α Ala), 53.4 (CH_3, OMe), 57.2 (CH, C_α ΨPro), 70.4 (CH_2, C_β ΨPro), 84.0 (q, J = 36.4 Hz, CH, C_δ ΨPro), 122.4 (q, J = 286.6 Hz, CF_3), 125.8 (CH, *o*-Nbs arom.), 129.6 (CH, *o*-Nbs arom.), 133.0 (CH, *o*-Nbs arom.), 134.1 (CH, *o*-Nbs arom.), 134.3 (C, *o*-Nbs arom.), 147.4 (C, *o*-Nbs arom.), 168.7 (C, C═O), 171.2 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 323 K) (single rotamer): δ −82.3 (d, J = 5.0 Hz, CF_3); MSMS (ES^+) m/z = 456.15 $[M + H]^+$, 478.14 $[M + Na]^+$.

Representative procedure for the preparation of Fmoc-alanine chloride assisted by ultrasonication[27]. To a 0.2 M solution of the Fmoc-alanine (1.0 equiv.) suspended in dichloromethane under argon was added freshly distilled $SOCl_2$ (13.8 equiv.). The mixture was sonicated until the complete disappearance of the precipitate (usually 30 min), then solvent and excess of $SOCl_2$ were removed *in vacuo* to give the Fmoc-alanine chloride as a white solid directly used in the next step without further purification.

Fmoc-Ala-Ser($\Psi^{CF_3,H}$Pro)-OMe (4). To a solution of pseudoproline (*R*,*S*)-**1** (60 mg, 0.3 mmol, 1.0 equiv.) in dichloromethane (300 μL) was added DIEA (50 μL, 0.3 mmol, 1.0 equiv.). The resulting mixture was added *via* cannula to the freshly prepared Fmoc-alanine acid chloride solid (109 mg, 0.33 mmol, 1.1 equiv.). The reaction mixture was stirred for 24 h, diluted with dichloromethane, and washed with 1 M aqueous solution of HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude 89 : 11 mixture of diastereomers was purified by flash chromatography (80 : 20 cyclohexane–ethyl acetate) to give 26 mg (18%) of pure minor diastereomer $\mathbf{4_{min}}$, 44 mg (30%) of a mixture of both diastereomers and 62 mg (42%) of pure major diastereomer (*R*,*S*)-**4** as a 12/88 inseparable mixture of *cis*–*trans* rotational isomers in $CDCl_3$ at 274 K. (*R*,*S*)-**4** *major diastereomer*: white solid; mp 56–60 °C; R_f = 0.29 (70 : 30 cyclohexane–ethyl acetate); $[\alpha]_D^{24}$ −81.2 (*c* 0.95, $CHCl_3$); IR (neat) 3324, 3015, 2955, 1743, 1684, 1523, 1154, 757, 743 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 274 K): (*trans* rotamer) δ 1.45 (d, J = 6.7 Hz, 3H, H_β Ala-H), 3.76 (s, 3H, OMe), 4.17 (t, J = 7.3 Hz, 1H, Fmoc CH); 4.30 (dd, J = 10.5, 7.3 Hz, 1H, Fmoc CH_2-Ha), 4.31–4.35 (m, 1H, H_β ΨPro-Ha), 4.31–4.37 (m, 1H, H_α Ala-H), 4.42 (dd, J = 10.5, 7.1 Hz, 1H, Fmoc CH_2-Hb), 4.47 (t, J = 8.9 Hz, 1H, H_β ΨPro-Hb), 5.04 (t, J = 8.2 Hz, 1H, H_α ΨPro-H), 5.48 (d, J = 7.6 Hz, 1H, NH Ala), 6.28 (q, J = 4.8 Hz, 1H, H_δ ΨPro-H), 7.31 (t, J = 7.3 Hz, 2H, Fmoc arom.), 7.40 (t, J = 7.5 Hz, 2H, Fmoc arom.), 7.55 (t, J = 7.4 Hz, 2H, Fmoc arom.), 7.76 (d, J = 7.5 Hz, 2H, Fmoc arom.); (*cis* rotamer) δ 1.38 (d, J = 6.4 Hz, 3H, H_β Ala-H), 3.76 (s, 3H, OMe), 4.20 (t, J = 7.1 Hz, 1H, Fmoc CH); 4.30–4.36 (m, 1H, Fmoc CH_2-Ha), 4.38–4.44 (m, 1H, Fmoc CH_2-Hb), 4.52–4.56 (m, 1H, H_β ΨPro-Ha), 4.58–4.61 (m, 1H, H_β ΨPro-Hb), 4.58–4.66 (m, 1H, H_α Ala-H), 4.71–4.78 (m, 1H, H_α ΨPro-H), 5.69 (d, J = 7.8 Hz, 1H, NH Ala), 5.88 (q, J = 4.3 Hz, 1H, H_δ ΨPro-H), 7.33 (t, J = 7.3 Hz, 2H, Fmoc arom.), 7.43 (t, J = 7.5 Hz, 2H, Fmoc arom.), 7.58 (t, J = 7.4 Hz, 2H, Fmoc arom.), 7.79 (d, J = 7.5 Hz, 2H, Fmoc arom.); ^{13}C NMR (125.75 MHz, $CDCl_3$, 274 K): (*trans* rotamer) δ 17.8 (CH_3, C_β Ala), 46.8 (CH, Fmoc CH), 48.7 (CH, C_α Ala), 53.1 (CH_3, OMe), 56.6 (CH, C_α ΨPro), 67.2 (CH_2, Fmoc CH_2), 69.0 (CH_2, C_β ΨPro), 84.9 (q, J = 36.0 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 121.8 (q, J = 286.6 Hz, CF_3), 125.0 (2 × CH, Fmoc arom.), 127.6 (2 × CH, Fmoc arom.), 127.7 (2 × CH, Fmoc arom.), 140.6 (2 × C, Fmoc arom.), 143.0 (2 × C, Fmoc arom.), 155.6 (C, C═O), 167.8 (C, C═O), 167.9 (C, C═O); (*cis* rotamer) δ 19.1 (CH_3, C_β Ala), 46.8 (CH, Fmoc CH), 48.5 (CH, C_α Ala), 53.7 (CH_3, OMe), 56.8 (CH, C_α ΨPro), 67.2 (CH_2, Fmoc CH_2), 70.4 (CH_2, C_β ΨPro), 84.5 (q, J = 34.0 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 121.8 (q, J = 286.6 Hz, CF_3), 125.0 (2 × CH, Fmoc arom.), 127.6 (2 × CH, Fmoc arom.), 127.7 (2 × CH, Fmoc arom.), 140.6 (2 × C, Fmoc arom.), 143.0 (2 × C, Fmoc arom.), 155.7 (C, C═O), 167.2 (C, C═O), 167.9 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K): (*trans* rotamer) δ −82.0 (s, CF_3); (*cis* rotamer) δ −81.8 (s, CF_3); MSMS (ES^+) m/z = 493.27 $[M + H]^+$, 515.23 $[M + Na]^+$, 531.23 $[M + K]^+$; HRMS (EI) calcd for $C_{24}H_{23}F_3N_2O_6$ 492.1508, found: 492.1590. *Minor diastereomer* $\mathbf{4_{min}}$: white solid; mp 64–66 °C; R_f = 0.40 (70 : 30 cyclohexane–ethyl acetate); $[\alpha]_D^{23}$ +42.8 (*c* 1.0, $CHCl_3$); IR (neat): 3326, 2956, 1752, 1686, 1524, 1153, 742 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 302 K): δ 1.47 (d, J = 6.5 Hz, 3H, H_β Ala-H), 3.83 (s, 3H, OMe), 4.19 (t, J = 6.9 Hz, 1H, Fmoc CH); 4.32–4.42 (m, 3H, H_α Ala-H, Fmoc CH_2), 4.46 (t, J = 8.2 Hz, 1H, H_β ΨPro-Ha), 4.52–4.60 (m, 1H, H_β ΨPro-Hb), 5.30 (d, J = 6.4 Hz, 1H, NH Ala), 5.47 (dd, J = 7.6, 4.4 Hz, 1H, H_α ΨPro-H), 5.98 (q, J = 5.1 Hz, 1H, H_δ ΨPro-H), 7.31 (t, J = 7.5 Hz, 2H, Fmoc arom.), 7.41 (t, J = 7.5 Hz, 2H, Fmoc arom.), 7.56 (t, J = 7.5 Hz, 2H, Fmoc arom.), 7.79 (d, J = 7.5 Hz, 2H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 323 K) δ 18.0 (CH_3, C_β Ala), 47.2 (CH, Fmoc CH), 48.6 (CH, C_α Ala), 53.0 (CH_3, OMe), 57.8 (CH, C_α ΨPro), 67.3 (CH_2, Fmoc CH_2), 70.3 (CH_2, C_β ΨPro), 84.2 (q, J = 35.5 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 122.6 (q, J = 285.6 Hz, CF_3), 124.9 (2 × CH, Fmoc arom.), 127.0 (2 × CH, Fmoc arom.), 127.8 (2 × CH, Fmoc arom.), 141.3 (2 × C, Fmoc arom.), 143.6 (2 × C, Fmoc arom.), 156.3 (C, C═O), 169.2 (C, C═O), 173.8 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K): δ −82.6 (s, CF_3); MSMS (ES^+) m/z = 493.26 $[M + H]^+$, 515.22 $[M + Na]^+$, 531.22 $[M + K]^+$.

Fmoc-Ala-Ser($\Psi^{CF_3,H}$Pro)-NHMe (5). To a solution of pseudoproline (*R*,*S*)-**2** (110 mg, 0.55 mmol, 1.0 equiv.) in dichloromethane (1 mL) was added DIEA (91 μL, 0.55 mmol, 1.0 equiv.). The resulting mixture was added *via* cannula to the freshly prepared Fmoc-alanine chloride solid (0.61 mmol, 1.1 equiv.). The reaction mixture was stirred for 24 h, diluted with dichloromethane, and washed successively with 1 M aqueous solution of HCl and brine. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography (40 : 60 cyclohexane–ethyl acetate) gave 102 mg (37%) of a mixture of both diastereomers and 77 mg (28%) of pure major diastereomer (*R*,*S*)-**5** as a 58/42 inseparable mixture of *cis*–*trans* rotational isomers in $CDCl_3$ at 298 K. (*R*,*S*)-**5** *major diastereomer*: white solid; R_f = 0.34

(40 : 60 cyclohexane–ethyl acetate); $[\alpha]_D^{24}$ −60.5 (*c* 1.1, $CHCl_3$); IR (neat) 3322, 3066, 2947, 1667, 1530, 1252, 1152, 731 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 274 K): (*trans* rotamer) δ 1.43 (d, J = 6.3 Hz, 3H, H_β Ala-H), 2.83 (d, J = 4.7 Hz, 3H, NHMe), 4.20 (t, J = 6.6 Hz, 1H, Fmoc CH), 4.39 (m, 1H, H_α Ala-H), 4.40 (m, 3H, Fmoc CH_2 and H_β ΨPro-Ha), 4.75 (t, J = 7.6 Hz, 1H, H_β ΨPro-Hb), 4.85 (t, J = 7.6 Hz, 1H, H_α ΨPro-H), 5.37 (d, J = 6.9 Hz, 1H, NH Ala), 6.27–6.30 (m, 1H, H_δ ΨPro-H), 6.52–6.56 (m, 1H, NHMe), 7.32 (t, J = 7.3 Hz, 2H, Fmoc arom.), 7.42 (t, J = 7.0 Hz, 2H, Fmoc arom.), 7.61 (d, J = 7.8 Hz, 2H, Fmoc arom.), 7.77 (d, J = 7.3 Hz, 2H, Fmoc arom.); (*cis* rotamer) δ 1.39 (d, J = 6.9 Hz, 3H, H_β Ala-H), 2.83 (d, J = 4.7 Hz, 3H, NHMe), 4.20 (t, J = 6.6 Hz, 2H, Fmoc CH and H_α Ala-H), 4.40 (m, 2H, Fmoc CH_2), 4.54–4.63 (m, 3H, H_β ΨPro-H, H_α ΨPro-H), 5.43 (d, J = 6.1 Hz, 1H, NH Ala), 5.96 (q, J = 4.9 Hz, 1H, H_δ ΨPro-H), 7.32 (t, J = 7.3 Hz, 2H, Fmoc arom.), 7.42 (t, J = 7.0 Hz, 2H, Fmoc arom.), 7.57 (d, J = 7.5 Hz, 2H, Fmoc arom.), 7.77 (d, J = 7.3 Hz, 2H, Fmoc arom.), 7.93–7.97 (m, 1H, NHMe); ^{13}C NMR (125.75 MHz, $CDCl_3$): (*trans* rotamer) δ 18.0 (CH_3, C_β Ala), 26.6 (CH_3, NHMe), 46.8 (CH, Fmoc CH), 48.5 (CH, C_α Ala), 57.7 (CH, C_α ΨPro), 67.4 (CH_2, Fmoc CH_2), 68.7 (CH_2, C_β ΨPro), 85.0 (q, J = 32.6 Hz, CH, C_δ ΨPro), 120.2 (2 × CH, Fmoc arom.), 122.4 (q, J = 285.9 Hz, CF_3), 125.1 (2 × CH, Fmoc arom.), 127.2 (2 × CH, Fmoc arom.), 127.9 (2 × CH, Fmoc arom.), 141.3 (2 × C, Fmoc arom.), 143.0 (2 × C, Fmoc arom.), 156.3 (C, C═O), 168.2 (C, C═O), 175.1 (C, C═O); (*cis* rotamer) δ 16.1 (CH_3, C_β Ala), 26.6 (CH_3, NHMe), 46.8 (CH, Fmoc CH), 49.4 (CH, C_α Ala), 58.5 (CH, C_α ΨPro), 67.4 (CH_2, Fmoc CH_2), 71.6 (CH, C_β ΨPro), 84.8 (q, J = 36.7 Hz, CH, C_δ ΨPro), 120.2 (2 × CH, Fmoc arom.), 122.4 (q, J = 285.9 Hz, CF_3), 125.1 (2 × CH, Fmoc arom.), 127.2 (2 × CH, Fmoc arom.), 127.9 (2 × CH, Fmoc arom.), 141.3 (2 × C, Fmoc arom.), 143.0 (2 × C, Fmoc arom.), 156.8 (C, C═O), 168.2 (C, C═O), 173.2 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K): (*trans* rotamer) δ −82.0 (s, CF_3); (*cis* rotamer) δ −81.8 (s, CF_3); MSMS (ES^+) m/z = 492.2 $[M + H]^+$, 514.2 $[M + Na]^+$; HRMS (ES^+) calcd for $C_{24}H_{25}F_3N_3O_5$: 492.1746, found 492.1755.

Ac-Ala-Ser(Ψ^{CF_3,H}Pro)-NHMe (6). To a solution of the Fmoc-dipeptide (*R*,*S*)-**5** (300 mg, 0.61 mmol, 1.0 equiv.) in dichloromethane (12 mL) under argon was added dropwise piperidine (600 μL, 6.10 mmol, 10.0 equiv.) at room temperature. The reaction mixture was stirred until it was completed as monitored by TLC (approximately 80 min), then solvent was removed *in vacuo*. Purification by flash chromatography (90 : 10 ethyl acetate–methanol) gave 164 mg (98%) of pure deprotected dipeptide which was then diluted in dichloromethane (18 mL). After the addition of acetic anhydride (62 μL, 0.66 mmol, 1.1 equiv.), the reaction mixture was stirred for 1 h then solvent was removed under reduced pressure. The solid residue was washed with diethyl ether, diluted in dichloromethane, filtered and evaporated under reduced pressure to afford 184 mg (97%) of pure tetrapeptide mimic **6** as a 70/30 inseparable mixture of *cis*–*trans* rotational isomers in $CDCl_3$ at 274 K. (*R*,*S*)-**6**: white solid; mp 145–155 °C; R_f = 0.51 (90 : 10 ethyl acetate–methanol); $[\alpha]_D^{26}$ −113.6 (*c* 1.0, $CHCl_3$); IR (neat) 3390, 2989, 1652, 1544, 1151 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 274 K): (*trans* rotamer) δ 1.44 (d, J = 7.1 Hz, 3H, H_β Ala-H), 2.02 (s, 3H, CH_3 Ac), 2.84 (d, J = 4.6 Hz, 3H, CH_3 NHMe), 4.43 (t, J = 8.5 Hz, 1H, H_β ΨPro-Ha), 4.47–4.51 (m, 1H, H_α Ala-H), 4.72 (t, J = 8.5 Hz, 1H, H_β ΨPro-Hb), 4.85 (t, J = 8.5 Hz, 1H, H_α ΨPro-H), 6.23 (d, J = 6.0 Hz, 1H, NH Ala), 6.47 (q, J = 5.3 Hz, 1H, H_δ ΨPro-H), 6.51–6.55 (m, 1H, NHMe); (*cis* rotamer) δ 1.41 (d, J = 7.0 Hz, 3H, H_β Ala-H), 2.04 (s, 3H, CH_3 Ac), 2.89 (d, J = 4.6 Hz, 3H, CH_3 NHMe), 4.31 (quint, J = 6.3 Hz, 1H, H_α Ala-H), 4.54 (t, J = 8.3 Hz, 1H, H_β ΨPro-Ha), 4.59 (dd, J = 8.3, 4.3 Hz, 1H, H_α ΨPro-H), 4.69 (dd, J = 8.3, 4.3 Hz, 1H, H_β ΨPro-Hb), 5.96 (q, J = 5.1 Hz, 1H, H_δ ΨPro-H), 6.63 (d, J = 4.9 Hz, 1H, NH Ala), 8.38 (q, J = 4.6 Hz, 1H, NHMe); ^{13}C NMR (125.75 MHz, $CDCl_3$, 274 K): (*trans* rotamer) δ 17.5 (CH_3, C_β Ala), 22.5 (CH_3, Ac), 26.6 (CH_3, NHMe), 47.5 (CH, C_α Ala), 57.8 (CH, C_α ΨPro), 68.8 (CH_2, C_β ΨPro), 84.8 (q, J = 38.5 Hz, CH, C_δ ΨPro), 122.5 (q, J = 284.5 Hz, CF_3), 168.3 (C, C═O), 172.1 (C, C═O), 173.2 (C, C═O); (*cis* rotamer) δ 15.7 (CH_3, C_β Ala), 22.3 (CH_3, Ac), 26.7 (CH_3, NHMe), 48.7 (CH, C_α Ala), 58.6 (CH, C_α ΨPro), 71.5 (CH_2, C_β ΨPro), 84.9 (q, J = 38.5 Hz, CH, C_δ ΨPro), 122.6 (q, J = 284.5 Hz, CF_3), 168.3 (C, C═O), 171.0 (C, C═O), 175.1 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K): (single rotamer) δ −81.9 (s, CF_3); MSMS (ES^+) m/z = 312.1 $[M + H]^+$, 334.1 $[M + Na]^+$; HRMS (ES^+) calcd for $C_{11}H_{17}F_3N_3O_4$: 312.1171, found 312.1172.

Structure calculations

Simulated annealing (SA) calculations were carried out with the program DYNAMO 2.1[28] and consisted of three stages. The first stage comprised an initialization period of 1000 steps (3 fs long) of molecular dynamics at 4000 K and very tight temperature control. In the second stage, the coordinates were allowed to vary at 4000 K with loose temperature control for 4000 steps. In the final stage, the structure was annealed by slowly reducing the temperature from 4000 K to 0 K over the space of 20 000 molecular dynamic steps (3 fs long). In the first two stages, force constants were set as follows: 1000 kcal mol^{-1} $Å^{-2}$, 250 kcal mol^{-1} rad^{-2}, 50 kcal mol^{-1} rad^{-2}, 2 kcal mol^{-1} $Å^{-2}$ for bonds, angles, impropers and NOE constraints, respectively. No van der Waals interactions were operative. During the final stage, bonds force constant was maintained at 1000 kcal mol^{-1} $Å^{-2}$, both angles and impropers at 500 kcal mol^{-1} rad^{-2}, the NOE term was increased exponentially from 2 to 30 kcal mol^{-1} $Å^{-2}$. The van der Waals interactions and *J*-coupling restraints were slowly introduced during cooling to reach the final values of 4 kcal mol^{-1} $Å^{-2}$ and 1 kcal mol^{-1} rad^{-2}, respectively. NMR constraints consisted of 3 ^1H–^1H vicinal *J*-coupling constants and 11 NOE (see ESI†). For each oligomer 100 structures have been calculated starting from an extended fold. For the 5 lowest energy structures, typical final energies (kcal mol^{-1}) were 0.39 ± 0.04; 1.13 ± 0.07; 0.12 ± 0.01; 2.77 ± 0.02; 6.79 ± 0.10; and 1.31 ± 0.05 for *J*-coupling, bond, impropers, angle, NOE and van der Waals force constants, respectively.

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