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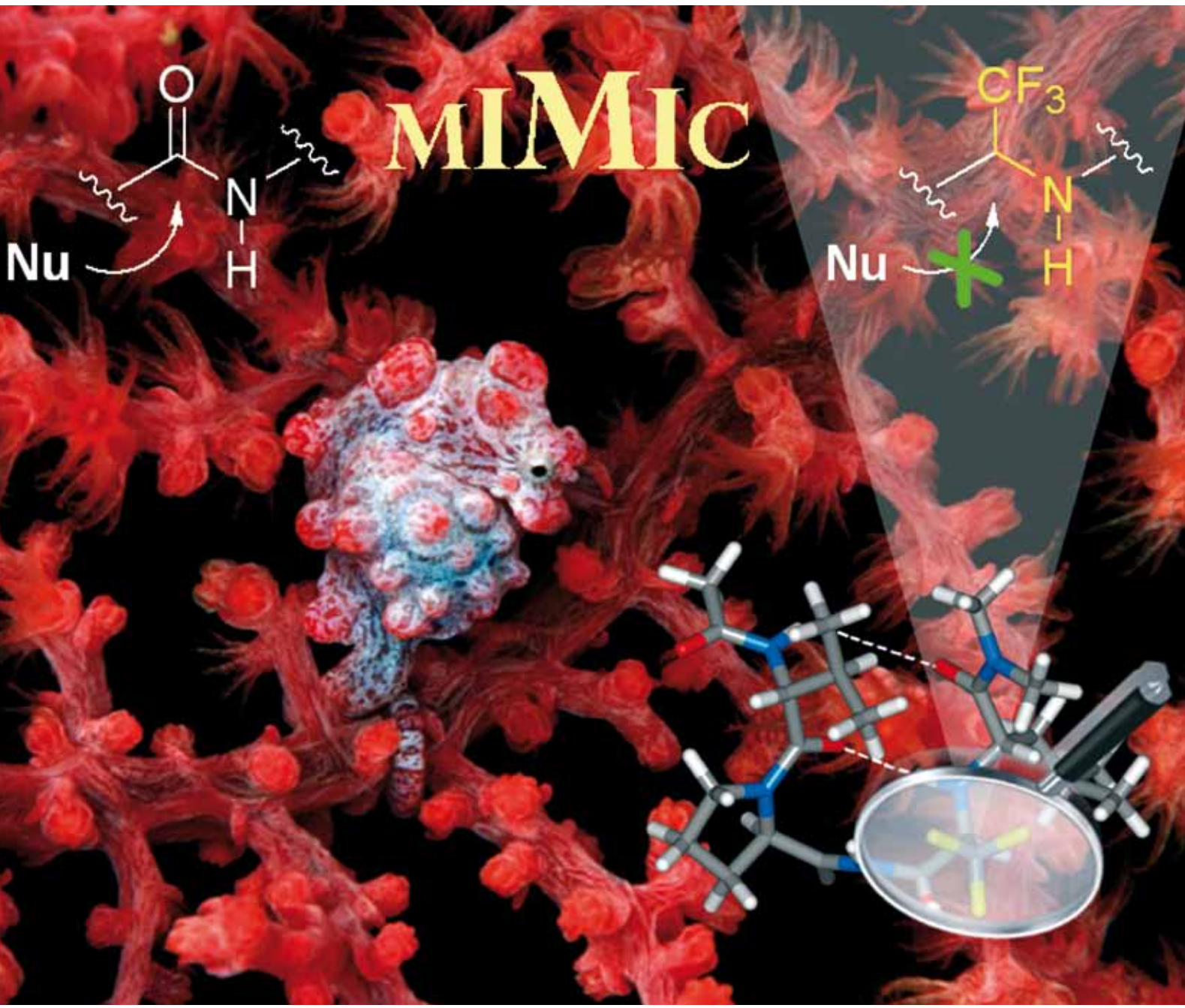

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Ψ[CH(CF_3)NH]Gly-peptides: synthesis and conformation analysis†

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Ψ[CH(CF_3)NH]Gly peptides, a conceptually new class of peptidomimetics having a stereogenic trifluoroethylamine group as a natural peptide-bond surrogate, have been synthesized by stereoselective addition of α-amino acid esters to trans-3,3,3-trifluoro-1-nitropropene. Long range nuclear Overhauser effects, detected *via* ROESY experiments, provided evidence that model Ψ[CH(CF_3)NH]Gly-tetrapeptides incorporating a trifluoroethylamine mimic of the dipeptide loop Pro-Gly can be represented by an ensemble of unfolded and folded conformations. The latter are driven by the formation of a hydrogen bond between a carbonyl group and the aminic proton of the trifluoroethylamine unit. MD calculations indicate a population of conformers which adopt folded β turn structures for all the L-peptides; on the other hand, a D-stereochemistry at the Pro residue induces a natural folding towards a β hairpin conformation driven by the formation of a second hydrogen bond, regardless of the stereochemistry of the stereogenic peptide bond surrogate.

Introduction

Peptides occur throughout nature in a wide range of roles essential to virtually every biochemical process. However, the pharmacological properties of most peptides preclude their use as drugs mainly because of their rapid in vivo degradation by action of proteolytic enzymes, and therefore by their low bioavailability.[1] The "druggability" of peptides could be achieved by the synthesis of suitable peptidomimetics able to retain both the activity and potency of the parent peptides, while at the same time be more metabolically stable, orally active and selective. In this context, the isosteric replacement of a scissile peptide bond represents a viable and popular approach in the rational design of peptidomimetics[2] because: 1) nearly all the peptide bond surrogates, with the notable exception of the ester and thioester functions, are more stable to enzymatic hydrolysis than the natural peptide bond;[3] 2) in the area of protease inhibitors, a nonscissile peptide bond replacement at the substrate cleavage site, particularly one that can mimic the transition state of amide bond hydrolysis, is key to achieving bioactivity;[2b,4] 3) most peptide bond surrogates influence the conformational preference of the residues in close proximity to them, and sometimes impart a conformational preference to a peptide region; 4) alterations of electronic properties introduced by peptide bond surrogates can significantly affect the transport properties of their parent peptides.[5]

The stereogenic trifluoroethylamine[6] function is a conceptually new peptide bond surrogate that has recently found the first validation in drug discovery thanks to the highly potent and metabolically stable Cathepsin K inhibitor Odanacatib, that is now in Phase III clinical trials for the therapy of postmenopausal osteoporosis.[7] This peptide-bond replacement has peculiar properties (Fig. 1). First of all, the sp^3 N atom of the trifluoroethylamine function has little Lewis basicity and is a bad hydrogen bond acceptor. In fact, the electron-withdrawing CF_3 group is instrumental in neutralizing the amine function generating a poorly-basic NH moiety. The latter is essentially non-protonated at physiological pH, in close similarity with the NH of an amide/peptide group. In fact, the pK_a of protonated trifluoroethylamine mimics was reported to be lower than 1.5.[7c] Moreover, the trifluoroethylamine NH moiety is a good hydrogen-bond donor, due to the increased acidity arising from the presence of the α-CF_3 group. On the contrary, the CF_3 group is a weak hydrogen-bond acceptor,[8] thus rendering the trifluoroethylamine function an effective peptide bond replacement only when the carbonyl group of the original ligand's amide/peptide-bond is not involved in essential hydrogen-bonding with the receptor. One should also notice that the trifluoroethylamine unit has an sp^3 tetrahedral configuration that can contribute to the optimization of the geometry and spatial orientations of the interactions (including

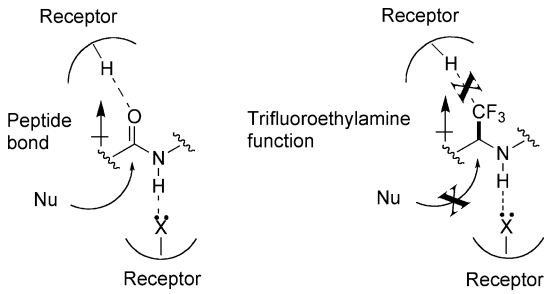

Fig. 1 Comparison between the peptide bond and the trifluoroethylamine function.

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† Electronic supplementary information (ESI) available: ^{1}H, ^{13}C, 2D ROESY and ^{1}H-^{13}C 2D HMBC spectra. See DOI: 10.1039/b901718f

hydrogen-bonds) between the original planar amide/peptide-moiety and the receptor. Last but not least, there is substantial evidence that the trifluoroethylamine unit has high metabolic stability.[7]

In our first accomplishment in this area,[9] we introduced the retro-trifluoroethylamine unit that was incorporated in partially modified retropeptides (PMR) **A** (Fig. 2) having a [CH(CF_3)NH] unit instead of the retro [CONH] peptide bond.

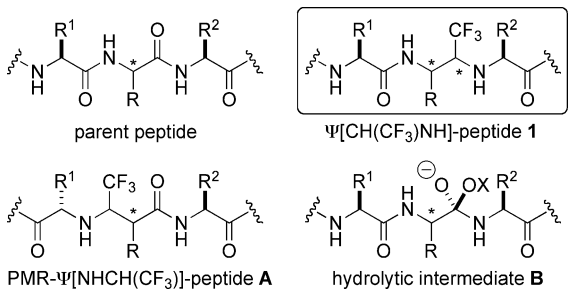


Fig. 2 Structure of natural peptides, PMR-Ψ[NHCH(CF_3)]Gly-peptides and Ψ[CH(CF_3)NH]Gly-peptides **1**.

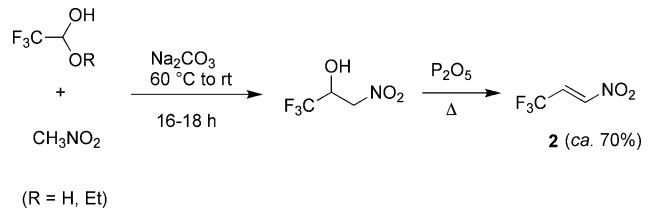
We showed[9e] that this unit is a sort of hybrid between a peptide bond mimic and a proteolytic transition state analogue, as it combines some of the properties of a peptidyl–CONH-group (low NH basicity, a CH(CF_3)-NH-CH backbone angle close to 120°, a C-CF_3 bond substantially isopolar with the C=O) with properties of the tetrahedral intermediate **B** involved in the protease-mediated hydrolysis reaction of a peptide bond (high electron density on the trifluoromethyl group, tetrahedral backbone carbon). Moreover, the presence of the bulky CF_3 group, rather than the presence of an intramolecular hydrogen bond, is probably the driving force for the high stability of the turn-like conformations displayed by appropriately configured retropeptides **A** both in low polarity organic solvent solutions and in the solid state.

Next, we communicated the stereocontrolled synthesis of a related class of peptidomimetics, the Ψ[CH(CF_3)NH]Gly-peptides **1** (Fig. 2, R = H).[10] These peptidomimetics are much closer to the natural peptides, as they feature a trifluoroethylamine unit replacing the native peptidic amide bond. More recently we also described the synthesis of differently fluorinated Ψ[CH(R_F)NH]Gly-peptides (R_F = CF_2H, CF_2Cl, CF_2CH_3, *etc.*).[11] In this paper we report a full account on the stereoselective synthesis of these peptidomimetics with a special emphasis on the synthesis of rationally designed Ψ[CH(CF_3)NH]Gly-tetrapeptides and the studies on their propensity to assume turn-like conformations in organic solvents, as demonstrated by means of NMR and molecular modeling analysis. Thus, for the first time, the trifluoroethylamine NH moiety is shown to be a good hydrogen bond donor. Attempts to synthesize more complex Ψ[CH(CF_3)NH]-peptides **1** (Fig. 2, R = alkyl) through the same chemistry will be also discussed.

Results and discussion

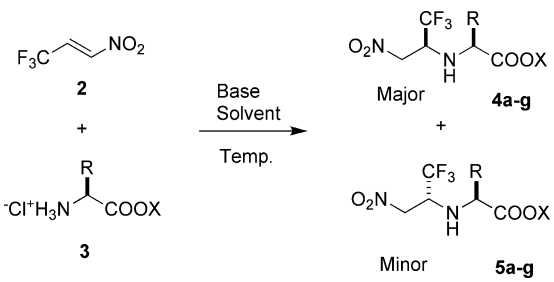
Chemistry

Electron poor carbon–carbon double bonds having a fluoroalkyl group in β position constitute interesting and versatile building blocks for further functionalization because they are much more reactive than the unfluorinated counterparts.[12] While for the stereoselective synthesis of PMR-Ψ[NHCH(CF_3)]Gly-peptides we exploited the reactivity of enantiomerically and geometrically pure fluorinated enoyl oxazolidin-2-ones, that reacted even with poorly nucleophilic α-amino acid esters, in this case the key step for the assembling of the amide bond surrogate was achieved using highly reactive *trans*-2-trifluoromethyl-1-nitroethene **2** that was prepared by Henry reaction[13] of aqueous fluoral with an excess of nitrometane, followed by dehydration of the intermediate nitroaldol on P_2O_5 and distillation (Scheme 1).[14]



Scheme 1 Synthesis of 2-trifluoromethyl-1-nitroethene **2**.

Nucleophilic addition of α-amino acid esters,[15] generated in situ from the corresponding hydrochloride salts **3** in the presence of a base (Scheme 2), to **2** gave rise to the formation of α′-Tfm-β′-nitro α-amino esters diastereoisomers **4** (major) and **5** (minor). The reaction is operatively very simple, took place almost instantaneously at room temperature and the final diastereoisomers were in all cases easily separable by flash chromatography.



Scheme 2 The key aza-Michael step.

The diastereoselection of the reaction was dependent on different factors such as the solvent, the structure and the amount of the base, the side chain R of the α-amino acid esters **3** and the temperature.[16] To fine tune such parameters in order to optimize diastereoselectivities we carried out a series of experiments at rt using L-Val benzyl and *tert*-butyl ester hydrochlorides **3a**,**b** as model nucleophiles (Table 1). First we investigated the influence of the base (1.1 equiv) using DCM as a model solvent (entries 1–4). The best results were achieved with organic bases and in particular with the more sterically hindered DIPEA (entry 4) as compared to TMP (entry 2) and DABCO (entry 3). Unsatisfactory results were obtained using an inorganic base such as $NaHCO_3$ (entry 1). Next, using DIPEA as a model base we focussed on the role of the solvent (entries 4–8). As expected,[16] the best results were achieved using low-polarity solvents such as CCl_4 (entry 6) and in particular toluene (entries 7, 8) while with more polar DCM (entry 4) and THF (entry 5) less stereoselective reactions were observed. To our surprise, carrying out the reaction with the free α-amino acid ester derived from **3b**, therefore without using any base,[17] a remarkable

Table 1 Aza-Michael reaction with α-amino acid esters nucleophiles

Entry[a]	α-amino acid ester	Major product	R	X	Base (equiv)	Solvent	dr[c]	Yield (%)
1	L-**3a**	**4a**	*i*Pr	Bn	$NaHCO_3$ (1.1)	DCM	1.7:1.0	>98[c]
2	L-**3a**	**4a**	*i*Pr	Bn	TMP (1.1)	DCM	2.5:1.0	>98[c]
3	L-**3a**	**4a**	*i*Pr	Bn	DABCO (1.1)	DCM	3.0:1.0	79[d]
4	L-**3a**	**4a**	*i*Pr	Bn	DIPEA (1.1)	DCM	4.4:1.0	70[d]
5	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.1)	THF	3.7:1.0	>98[c]
6	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.1)	CCl_4	7.9:1.0	>98[c]
7	L-**3a**	**4a**	*i*Pr	Bn	DIPEA (1.1)	Toluene	11.2:1.0	79[d]
8	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.1)	Toluene	11.7:1.0	65[c]
9	L-**3b**	**4b**	*i*Pr	*t*-Bu	none[b]	Toluene	3.8:1.0	>98[c]
10	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.0)	Toluene	4.0:1.0	93[c]
11	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.3)	Toluene	8.5:1.0	88[c]
12	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.5)	Toluene	5.1:1.0	77[c]
13	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (1.7)	Toluene	3.6:1.0	70[c]
14	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (2.0)	Toluene	3.7:1.0	75[c]
15	L-**3b**	**4b**	*i*Pr	*t*-Bu	DIPEA (5.0)	Toluene	3.2:1.0	74[c]
16	L-**3c**	**4c**	Me	*t*-Bu	DIPEA (1.1)	Toluene	5.8:1.0	60[d]
17	L-**3c**	**4c**	Me	*t*-Bu	none[b]	Toluene	1.6:1.0	87[d]
18	L-**3d**	**4d**	*s*Bu	Me	DIPEA (1.1)	Toluene	7.5:1.0	72[d]
19	L-**3d**	**4d**	*s*Bu	Me	none[b]	Toluene	4.4:1.0	88[d]
20	L-**3e**	**4e**	Bn	*t*-Bu	DIPEA (1.1)	Toluene	8.5:1.0	60[d]
21	L-**3f**	**4f**	*i*Bu	*t*-Bu	DIPEA (1.1)	Toluene	6.0:1.0	75[d]
21	D-**3g**	**4g**	Bn	Me	none[b]	Toluene	1.8:1.0	98[d]

[a] All the reactions were carried out at rt. [b] α-Amino acid ester was preliminarily treated with $NaHCO_3$ [c] Determined by ^{19}F and ^{1}H NMR. [d] Overall isolated yield.

drop of stereoselectivity was observed (from a dr of about 12:1 to less than 4:1, entries 8 and 9 respectively). We next investigated the influence of the base stoichiometry on the stereoselection of the reaction (entries 10–15), using the optimized conditions (DIPEA as base and toluene as solvent). The best result was obtained with a catalytic amount of the base (1.1 equiv., entries 7 and 8) while an increasing amount of the base resulted in a progressive decrease of diastereoselectivity (entries 11–15). Very low diasteroselection was achieved also when the added base was completely neutralized by the hydrochloride **3** (entry 10). As expected, the X group on the ester function of the nucleophile has a negligible effect (compare entries 7 and 8). Finally, the effect of the bulkiness of the R side chains of **3** was investigated performing the reaction with different α-amino acid esters (entries 16–21). Accordingly, the bulkier is the R group the higher resulted to be the diastereoisomeric ratio of the process, as exemplified by R = *iso*-propyl (entries 7 and 8) as compared to benzyl (entry 20), *iso*-butyl (entry 21) and methyl (entry 16). An important effect of the stereogenic *sec*-butyl side-chain is more than likely in the case of L-Ile-OMe (entries 18, 19), so these results should be weighed in a different manner. In an attempt to improve further the diastereoselectivities, we undertook a series of experiments lowering the temperature of the reaction. Rather surprisingly, room temperature seems to be essential in order to obtain the desired products because carrying out the reaction at –40 °C and –70 °C in different solvents invariably resulted in the formation of complex mixtures of unidentified compounds.

The stereochemistry of the minor diastereoisomer **5a** was assessed by X-ray diffraction (see ESI†),[18] whereas the configurations of the other adducts **4** and **5** were confidentially assigned on the basis of their spectroscopic features in comparison with those of **4a** and **5a**. In fact, the ^{19}F NMR signals of the fluorinated group of the major diasteroisomers **4** were always observed at higher fields than those of the minor diastereoisomer **5** (see Experimental Section).

The stereochemical outcome may be rationalized by using the predictive model depicted in Fig. 3, which resembles those of similar aza-Michael reactions involving fluorinated acceptors.[19] The α-amino ester nucleophile reacts from the less hindered diastereoface of the five-membered ring resulting from an intramolecular H-bond involving a carboxyl oxygen and an amine hydrogen H_a. The *trans*-nitroalkene acceptor places the sterically demanding CF_3 group in the less crowded position, namely pseudo-equatorially. One of the nitro O-atoms could be involved in H-bonding with the amino-ester's H_b.[20] A further stabilizing polar interaction between one of the F-atoms and the amino-ester's H_a is not unlikely.[21] In this picture, the Hunig's base would play a key role by promoting the transfer of the H_b proton to the carbon in α-position to the nitro group.

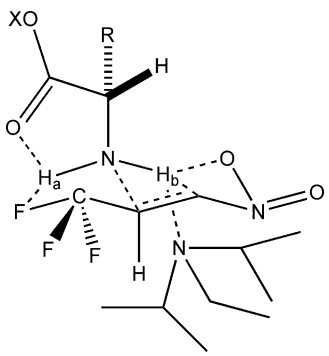
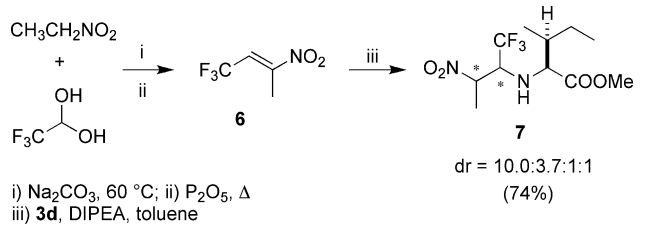


Fig. 3 Predictive model for the formation of the major diastereomers **4a–g**.

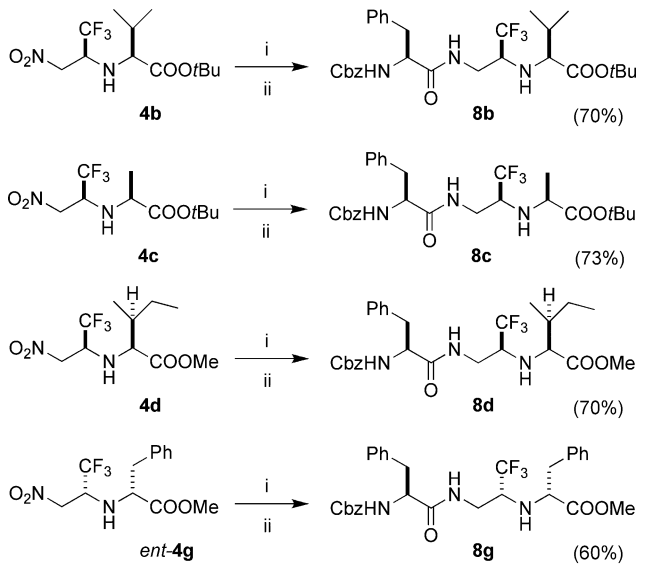
The synthesis of more complex Ψ[CH(CF_3)NH]Ala-peptides **1** (R = Me, Fig. 2) was attempted by means of an aza-Michael addition of L-Ile-OMe **3d** to 3,3,3-trifluoro-1-methyl-1-nitropropene **6** under optimized conditions (Scheme 3). The



Scheme 3 Synthesis of nitro-adducts **7**.

latter was obtained in geometrically pure form following the same strategy outlined for compound **2** (see Scheme 1).[22] The reaction, although moderately stereoselective, gave rise to the formation of all of the four diastereoisomers **7** which could not be isolated in analytically pure form. Owing to the difficulties connected with the isolation and purification of compounds like **7**, the synthesis of Ψ[CH(CF_3)NH]Ala-peptides was not investigated further.[23]

With a number of α′-fluoroalkyl-β′-nitro α-amino esters **4**,**5** in hand we addressed their elaboration into the target Ψ[CH(CF_3)NH]Gly peptides **8** (Scheme 4).



Scheme 4 Elaboration of aza-Michael adducts **4** into tripeptide mimetics **8**.

Reduction of the nitro group was accomplished by using $Pd(OH)_2$ catalyst in the presence of a 1 N solution of HCl to trap the free amino function as hydrochloride salt. Coupling with Cbz-^{L}Phe-OH using HOBt/EDC or HATU/HOAt led to the formation of tripeptide mimics **8b-d**,**g** in good overall yields.

Secondary structure of PMR-Ψ[CH(CF_3)NH]Gly-peptides

Three types of regular secondary structures are recognized in proteins: helix, sheet and turn. While many model studies of α-helices and β-turn stability have been reported, sheets have received less attention probably because β-sheets in proteins often form from discontinuous polypeptide segments.[24] For this reason it is important to identify the most effective strategies for assembling peptide strands into β-sheets. The simplest way to bring two antiparallel strands together is to connect them by a short dipeptide segment (loop) between the C-terminus of one strand and the N-terminus of the other able to generate a ten-membered intramolecularly hydrogen-bonded (C=O···H-N) ring involving four amino-acid residues, namely a β-turn motif.[25] This strand-loop-strand arrangement is referred to as "β-hairpin" (Fig. 4).

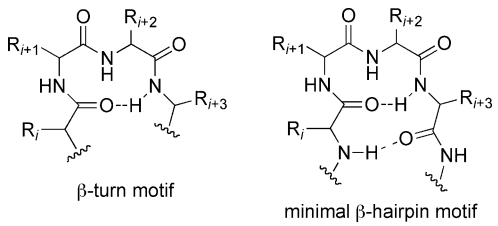


Fig. 4 The β-turn and β-hairpin motif.

A number of recent studies have reported the incorporation of peptidomimetics into β-sheet or β-hairpin structures such as 1,6-dehydro-3(2H) pyridinone, referred to as @-tide residue,[26] templated β-sheet,[27] alkene isosteres[28] and cis-azobenzene turn mimic.[29] It is known that in small tetrapeptides, in which intramolecular hydrogen bonding provides a major driving force for secondary structure formation, the presence of proline at the second of the four residues promotes formation of turns.[30] Specifically, we focussed our attention on two tetrapeptides, previously studied by Gellman *et al.*, namely ^{L}Val-^{L}Pro-Gly-^{L}Leu **9** and ^{L}Val-^{D}Pro-Gly-^{L}Leu **10** that differ from each other only for the configuration of the proline moiety on the dipeptide loop Pro-Gly.[31] By means of NMR studies in a nonpolar solvent (*i.e.* DCM) Gellman *et al.* demonstrated that the presence of L-proline in tetrapeptide **9** promotes formation of type I and type II turns while the "mirror image" β-turns (D-Pro-Gly in tetrapeptide **10**) promotes β-hairpin formation (Fig. 5).

Recently we have demonstrated that the trifluoroethylamine peptide-bond replacement, when incorporated into PMR-peptides, is responsible for an increased tendency of these peptides to assume turn-like conformations in organic solvents even if they are not directly involved in the formation of intramolecular hydrogen bonds.[9e] In order to improve our knowledge on the influence of this stereogenic surrogate on the conformation of peptides we decided to assess whether some analogies exist between the Gellman's tetrapeptides **9** and **10** and their fluorinated parent peptides. For this purpose we synthesized four diastereoisomeric Ψ[CH(CF_3)NH]Gly tetrapeptides **11–14** that differ from each other for the configurations of (1) the key proline amino acid and (2) the CF_3-substitued stereogenic center (Fig. 6). It should be stressed that the key amide bond between the Pro-Gly loop and H-^{L}Leu-NMe_2, in which the NH proton is involved in an intramolecular hydrogen bond stabilizing the secondary structures, is replaced by the trifluoroethylamine function in which the above mentioned hydrogen is an α-CF_3-amine proton.

The key diastereomeric intermediates **17a**,**b** were obtained in good overall yields by performing an aza-Michael addition to **2** with H-^{L}Leu-NMe_2 hydrochloride **16**, using non-stereoselective conditions (excess of $NaHCO_3$, DCM, 1:1 dr) (Scheme 5). The amide **16** was obtained by condensation of Cbz-^{L}Leu-OH **15** with Me_2NH followed by *N*-deprotection.[32]

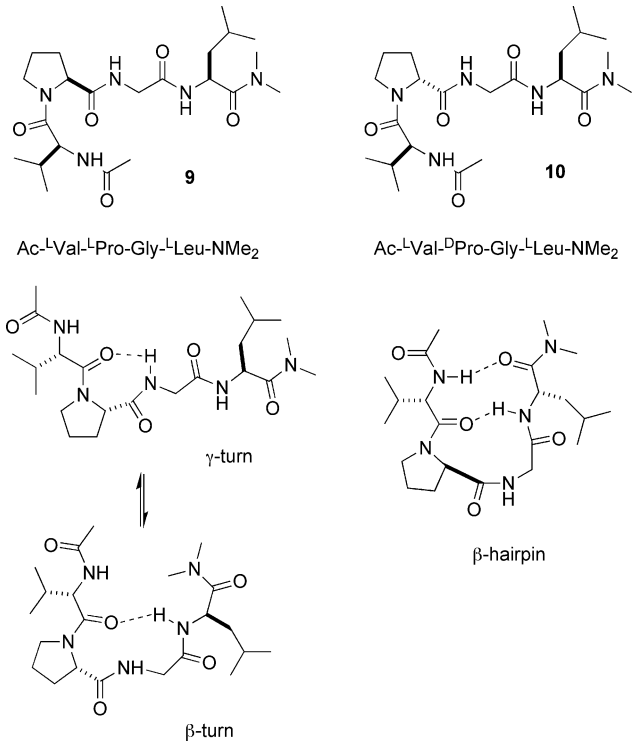
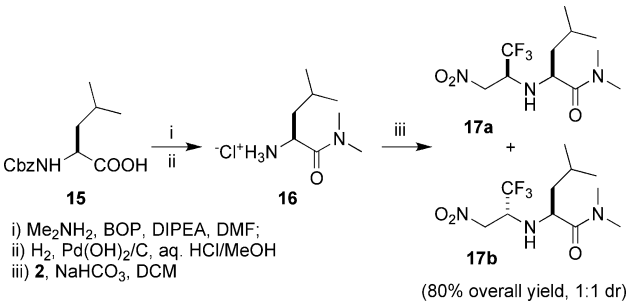
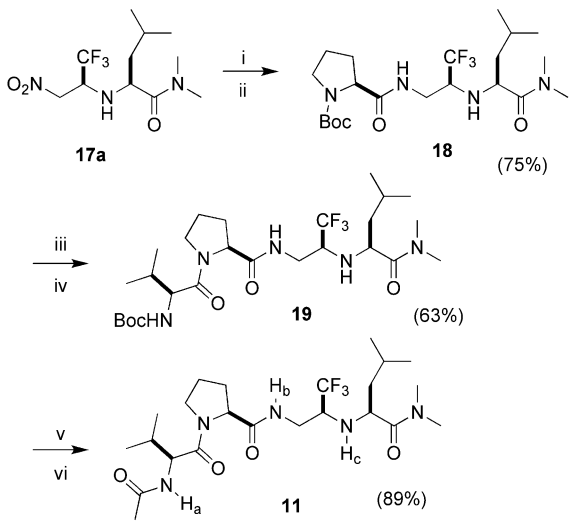


Fig. 5 Major folding patterns for tetrapeptides **9** and **10** in DCM solution.

The nitro group of diastereomerically pure **17a** was hydrogenated to an amino group and the latter was coupled with Boc-^{L}Pro-OH using HOBt/EDC, affording the tripeptide **18** (Scheme 6). Standard Boc deprotection using TFA/DCM followed by neutralization of the resulting trifluoroacetate salt and coupling with Boc-^{L}Val-OH afforded the tetrapeptide **19**. The target Ac-^{L}Val-^{L}Pro-(*S*)Ψ[CH(CF$_3$)NH]Gly-^{L}Leu-NMe$_2$ tetrapeptide **11** was obtained by deprotection of the amino moiety of **19** and subsequent acetylation with Ac$_2$O in DCM. Using the same synthetic pathway and Boc-^{D}Pro-OH in the second step of the synthesis we prepared also the epimeric tetrapeptide **12**. Analogously, starting from diastereomerically pure **17b** we obtained the tetrapeptides **13** and **14** containing ^{L}Pro and ^{D}Pro, respectively, at the second of the four residues.



Scheme 5 Synthesis of intermediates **17a**,**b**.



Scheme 6 Synthesis of Ac-^{L}Val-^{L}Pro-(*S*)Ψ[NHCH(CF$_3$)]Gly-^{L}Leu-NMe$_2$ **11**.

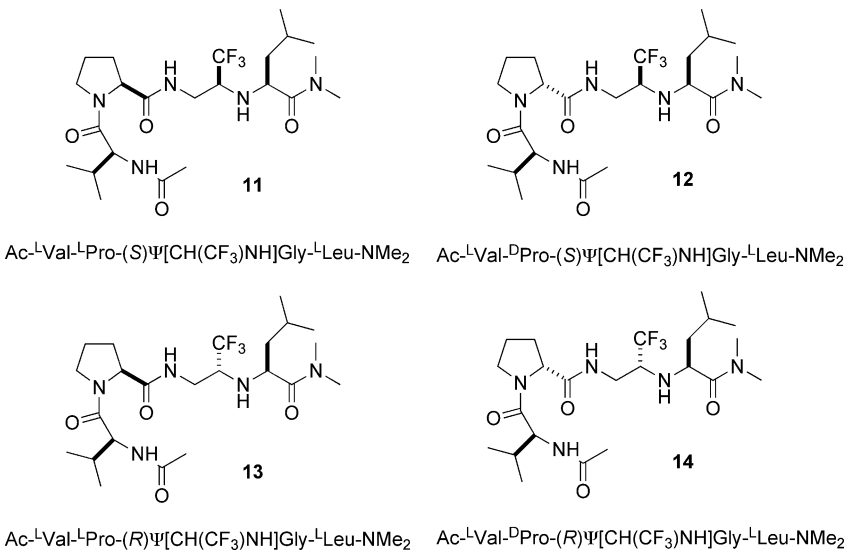


Fig. 6 Ψ[CH(CF$_3$)NH]Gly tetrapeptides.

NOE experiments

The $CDCl_3$ solution structure of the peptides **11**, **12** and **13** were investigated using 2D ROESY experiments (Rotating frame Overhauser Enhancement SpectroscopY) at 298 K and 273 K. The peptides' sequential assignment was performed starting from the NH_a and NH_b amidic signals by means of an integrated series of ^1H-^1H (COSY, TOCSY and ROESY)[33–35] and ^1H-^{13}C (one-bond and long range)[36] 2D experiments.

The main problem is the high flexibility of small peptides, which assume different conformations in solution. Although ROEs cannot be interpreted in terms of a unique structure due to the conformational averaging, we obtained a model structure using MD simulations with a minimum number of distance constraints converting the intensities of the cross peaks in the ROESY spectra in distances.

Information on the conformations adopted by these peptides can be obtained examining the results reported in Table 2, which lists the intermolecular NOE interactions and interproton distance values (Å) obtained from MD calculations. It should be pointed out that, in comparison with the Gellman's tetrapeptides, our peptides have two amidic protons, NH_a and NH_b, and an aminic proton NH_c vicinal to the trifluoromethyl group. The experimental problem associated to the aminic nature of the NH_c was mainly that this proton resonates in the same region of the methylene group of Pro (see ESI†). In order to detect the important ROEs contacts defining a folded conformation, we had therefore to assign, case by case, the NH_c. The COSY experiments showed a cross peak connecting by scalar coupling the Hα Leu with the NH_c. For compounds **11** and **13** we detected ROEs contacts between NH_b and the aminic proton NH_c, and NH_a and NH_c with Hα of the L-Pro that give a clear indication of the presence of a folded structure (Fig. 7).

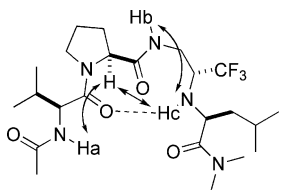


Fig. 7 Schematic representation of the structure of **13** in $CDCl_3$. The arrows indicate the diagnostic NOE interactions. No NOEs were found between terminal CH_3 and NHa with the N-methyl terminal groups.

The NH_b and NH_c as well as NH_a and Hα of the L-Pro protons belong to adjacent residues, so that the cross-peaks can be consistent with a folded conformation but can also derive from other conformations available to flexible peptides. On the other hand, the cross-peak between aminic NH_c and Hα of the Pro (*i, i*+2) is more diagnostic for a folded structure because a comparison of the value of this distance in an "all-extended" conformation results in a distance longer than 5 Å. Consequently, compounds **11** and **13** can be realistically represented by an ensemble of unfolded and folded conformations. These findings are in accordance with the MD calculations which indicate a population of conformers which adopt a folded β turn structure forming a 10 membered ring featuring a hydrogen bond between the aminic proton NH_c of L-Leu and the CO of L-Val (2.3 Å, Fig. 8). This observation validates further the concept that the trifluoroethylamino function is an effective mimic for the peptide bond.[37] We also considered the distances between C-terminal and N-terminal methyl groups. The lack of cross peaks between the terminal CH_3 groups of the diethylamide function and the CH_3 of the acetyl group and/or NH_a allows us to exclude the presence of a hairpin conformation. It is noteworthy that the different configuration of the stereogenic trifluoromethylamino group for peptides **11** and **13** doesn't affect their conformation.

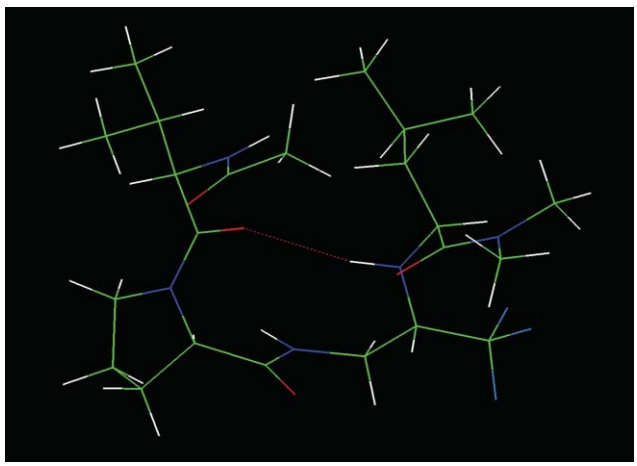


Fig. 8 Conformation of **13** obtained from molecular modeling.

We performed also experiments to determine the temperature coefficients for the exchangeable amidic protons of the peptides, in $CDCl_3$ solution, over a temperature range of 233 to 298K. Literature data[38] report that values of $-\Delta\delta/\Delta T \times 10^3$ ppb/K below 4 for amidic NH are consistent with intra-molecular hydrogen-bonding, while values ranging between 4 and 6 or greater, are typical of an exposition to the solvent. The coefficient values obtained for the NH amidic protons (NH_a showed values of 10 and 5 ppb/K and NH_b showed values of 5 and 6 ppb/K for **13** and **11** respectively) suggest the lack of hydrogen bonds.

Basically for the compound **12** we detected the same ROEs interactions: NH_b with the aminic proton NH_c, NH_a and NH_c with the Hα of D-Pro where the last interaction (*i, i*+2) is diagnostic for a folded structure. The temperature coefficient obtained for the amidic NH_b showed a value of 7 ppb/K suggesting a solvent exposition of this proton, while NH_a showed a "borderline" value of 4.0 ppb/K. In addition to these interactions we found two other weak long range cross peaks between NH_a and NH_c, and between NH_a and the N-terminal methyl groups, which were not observed for the L-peptides (Fig. 9).

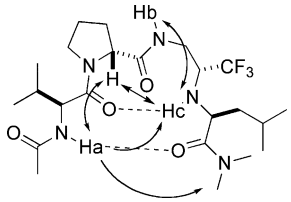


Fig. 9 Schematic representation of the structure of **12** in $CDCl_3$. The arrows indicate the diagnostic NOE interactions. NOEs were found between NH_a an NH_c and between NH_a and the N-methyl terminal group.

Table 2 Intramolecular NOE interactions and interproton distances values (Å) for **11**, **12** and **13**

		Model distances		
Experimental NOE	Intensities[a]	**11**	**12**	**13**
NHb···NHa	*ww*	4.47	5.28	4.31
NHb···Hα Val	*w*	4.83	4.56	4.87
NHb···Hα Pro	*s*	2.49	3.50	2.44
NHb···Hα Leu	*w*	5.47	5.00	5.62
NHb···Hα Gly (pro S)	*s*	3.07	3.09	2.44
NHb···Hα Gly (pro R)		2.51	2.79	2.85
NHb···$CH(CF_3)$	*s*	4.04	3.93	3.86
NHb···Hβ Pro (pro S)	*m*	4.67	2.84	4.57
NHb···Hβ Pro(pro R)	*m*	4.44	4.01	4.42
NHb···NHc	*m*	3.63	2.73	2.99
NHb···Hδ (pro S)	*w*	4.50	4.61	4.65
NHb···Hδ (pro R)		5.01	3.63	5.01
NHa···Hα Val	*m*	3.06	3.06	3.08
NHa···Hα Pro	*w*[b]	5.67	5.37 (*zero NOE*)	5.53
NHa···Hδ (pro S)	*w*[b]	4.40	4.76	4.33
NHa···Hδ (pro R)		5.50	5.33 (*no NOE*)	5.05
NHa···terminal CH_3	*s*	2.43	2.44	2.43
Hα Val···. Hα Pro	*w*	4.55	4.63	4.58
Hα Val ···Hδ Pro(pro S)	*s*	2.50	2.61	2.52
Hα Val···Hδ Pro (pro R)		2.29	2.24	2.33
Hα Val ···Hβ Val	*s*	2.47	3.06	3.06
Hα Val ···γCH_3 Val(pro S)	*s*	2.64	2.79	2.81
Hα Val···γCH_3 Val (pro R)		3.52	2.79	2.79
Hα Pro ···Hδ Pro (pro S)	*m*	4.19	3.43	4.19
Hα Pro ···Hδ Pro (pro R)		3.78	3.92	3.76
Hα Pro ···Hα Gly (pro S)	*w*	4.52	4.53	4.61
Hα Pro ···Hα Gly (pro R)		4.64	5.26	4.71
Hα Pro ···Hβ Pro (pro S)	*s*	2.34	3.07	2.38
Hα Pro ···Hβ Pro (pro R)		2.73	2.42	2.73
Hα Pro ···Hγ (pro S)	*s*	3.89	2.78	3.89
Hα Pro ···Hγ (pro R)		4.20	3.86	4.16
Hα Pro ···NHc	*w*	4.76	4.23	4.11
Hδ,δ'···Hβ, β' Pro	*s*	2.94–4.20	2.90–4.20	2.97–4.19
Hδ,δ'···Hγ, γ' Pro	*s*	2.37–3.88	2.40–3.90	2.40–3.00
Hα Leu ···$CH(CF_3)$	*s*	2.94	2.43	2.85
Hα Leu ···NCH_3 terminals	*m*	2.56, 4.20	2.54, 4.18	2.70, 4.22
Hα Leu ···NHc	*s*	3.07	3.08	3.02
Hα Leu ···Hγ Leu	*s*	2.47	3.12	3.11
Hα Leu ···Hβ Leu *(pro S)*	*s*	3.07	2.52	3.05
Hα Leu ···Hβ Leu(*pro R*)		2.52	3.07	2.50
$CH(CF_3)$···Hα Gly (*pro S*)	*s*	2.45	2.47	3.06
$CH(CF_3)$···Hα Gly (*pro R*)		2.32	2.37	2.43
$CH(CF_3)$···Hβ Leu (*pro S*)	*m*	4.27	4.27	4.49
$CH(CF_3)$···Hβ Leu (*pro R*)	*w*	4.22	3.77	4.50
$CH(CF_3)$···NHc	*s*	2.74	2.88	2.78
NCH_3 terminals···NHc	*m*	4.70, 5.10	4.65, 5.00	5.26, 4.82
NHc···Hγ Leu	*s*	4.00	3.24	4.50
NHc···Hβ Leu (*pro S*)	*s*	2.75	3.00	2.44
NHc···Hβ Leu (*pro R*)	*m*	3.03	2.64	2.43
NHc···δCH_3 Leu (*pro S*)	*m*	5.04	4.70	4.98
NHc···δCH_3 Leu (*pro R*)		5.33	4.84	5.02
NHc···Hα Gly (*pro S*)	*s*	2.29	2.70	2.42
NHc···Hα Gly (*pro R*)	*m*	3.39	3.54	3.42
Hβ Val···γ, γ' NCH_3	*s*	2.13, 2.40	2.16, 2.16	2.16, 2.16
NHa···NCH_3 terminal	*w*[c]	5.88	4.28	5.58
NHa···NHc	*w*[c]	6.50	4.06	7.05

[a] Cross peaks intensities referred to the compounds **11**, **12** and **13**. [b] Cross peaks intensities referred to **11** and **13**. [c] Cross peaks intensities referred to **12**.

On the basis of these interactions the MD calculations showed structures forming a 10 membered-ring with a hydrogen bond between the aminic proton NH_c of L-Leu and the CO of L-Val (1.94 Å) and a second hydrogen bond between NH_a and the CO of L-Leu (2.55 Å, Fig. 10). This evidence confirmed that the change of stereochemistry at the Pro stereocenter induces a natural folding towards a β hairpin conformation.

Conclusions

The design and synthesis of metabolically stable peptide analogs that can either mimic or block the bioactivity of natural peptides or enzymes is an important constituent of bioorganic and medicinal chemistry. Isosteric replacement of a scissile peptide bond with surrogates able to maintain the "favorable" features of the latter

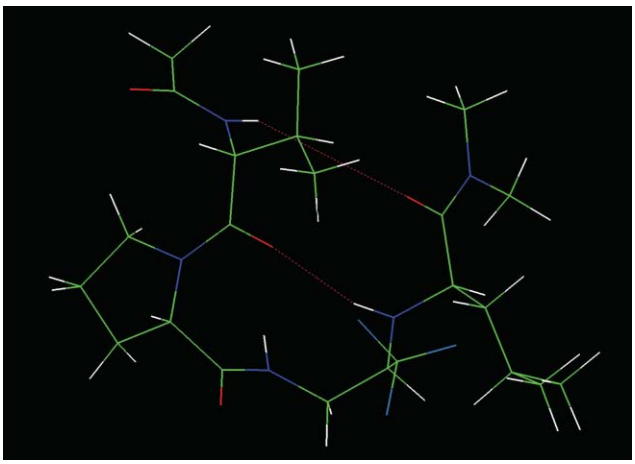


Fig. 10 Conformation of **12** obtained from molecular modeling.

represents a viable and popular approach in the rational design of peptidomimetics. In this sense, we introduced recently the trifluoroethylamino function that proved to be an excellent peptide bond surrogate because: 1) it has a better metabolic stability; 2) it can behave as a sort of hybrid between a peptide bond mimic and a proteolytic transition state analogue; 3) the stereo-electronic features of the trifluoromethyl group are responsible for an increased tendency of PMR-Ψ[NHCH(CF_3)]Gly-peptides to assume turn-like conformations in organic solvents. To investigate further the latter issue, several Ψ[CH(CF_3)NH]Gly tetrapeptides containing the trifluoroethylamino function in a Pro-Gly loop have been synthesized and their conformation has been investigated by 1D and 2D NMR experiments and MD calculations. Independently from the stereochemistry of the sterogenic trifluoroethylamino function these tetrapeptides were found to exist in well defined secondary structures stabilized by an intramolecular hydrogen bond involving the aminic proton of the surrogate. These findings are important because one of the most significant differences between the peptide bond and the trifluoroethylamine surrogate is the nature of the hydrogen atom which is amidic in the former, and aminic in the latter. However, we have shown herein that due to the presence of the strongly electron-withdrawing α-CF_3 group, the NH moiety of the trifluoroethylamine function is functionally very close to the amidic NH. Indeed, it can act intramolecularly as a hydrogen bond donor like an amidic NH, thus inducing the formation of stable secondary structures in small peptidomimetics. All the experimental evidence described here and in previous reports confirm that the trifluoroathylamino function is a promising peptide- and amide-bond replacement that will likely find further application in medicinal chemistry and drug discovery.

Experimental

General details

Commercially available reagent-grade solvents were employed without purification. All reactions where an organic solvent was employed were performed under a nitrogen atmosphere, after flame-drying of the glass apparatus. Melting points (m.p.) are uncorrected and were obtained on a capillary apparatus. TLC was run on silica gel 60 F_{254} Merck. Flash chromatography (FC) was performed with silica gel 60 (60–200 μm, Merck). ^{1}H-, ^{13}C-, and ^{19}F-NMR spectra were run at 250, 400 or 500 MHz. Chemical shifts are expressed in ppm (δ), using tetramethylsilane (TMS) as internal standard for ^{1}H and ^{13}C nuclei (δ_H and $\delta_C = 0.00$), while C_6F_6 was used as external standard (δ_F −162.90) for ^{19}F.

Experimental for the synthesis of nitropropene 2

See ref. 14.

Experimental for the aza-Michael reaction

Synthesis of **4**,**5d** is described as an example. To a stirred solution of **2** (0.76 mmol, 107 mg) and **3d** (0.51 mmol, 92 mg) in toluene (7 ml) at rt, DIPEA was added (0.56 mmol, 73 μl). After half an hour at rt, the solvent was removed in vacuo, the crude material was dissolved in EtOAc and washed once with 1 N HCl. The organic layer was dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by FC (hexane/diisopropyl ether 9:1) affording 106 mg (72%) of the two pure diastereoisomers **4d** and **5d** in a 7.5:1 ratio.

4d. $R_f = 0.31$, (*n*-Hex/*i*Pr_2O 7:3); $[\alpha]^{23}_D = -16.2°$ ($c = 1.1$, $CHCl_3$); FT IR (film): $\nu_{max} = 3365, 2967, 2930, 1732, 1567, 1379$ cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.67$ (dd, $J = 13.1$, 4.7 Hz, 1H), 4.52 (dd, $J = 13.1$, 7.8 Hz, 1H), 3.86 (m, 1H), 3.68 (s, 3H), 3.32 (d, $J = 5.13$ Hz, 1H), 1.91 (br s, 1H), 1.71 (m, 1H), 1.41(m, 1H), 1.14 (m, 1H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.86 (t, $J = 7.5$, 3H); ^{19}F NMR (470.6 MHz, $CDCl_3$): $\delta = -75.9$ (d, $J = 7.0$ Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 174.5, 124.5$ (q, $J = 282.9$ Hz), 74.4, 65.8, 58.2 (q, $J = 29.0$ Hz), 52.0, 38.6, 24.6, 15.7, 11.4; MS (70 eV): *e*/*z* (%): 287 [M^+ + 1] (3), 227 (100).

5d. $R_f = 0.41$ (*n*-Hex/*i*Pr_2O 7:3); ^{19}F NMR (470.6 MHz, $CDCl_3$): $\delta = -75.9$ (d, $J = 7.0$ Hz); $[\alpha]^{23}_D = +34.1°$ ($c = 1.8$, $CHCl_3$); FT IR (film): $\nu_{max} = 3349, 2967, 1735, 1561, 1382, 1156$ cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.60$ (dd, $J = 12.8$, 3.7 Hz, 1H), 4.40 (dd, $J = 12.8$, 10.3 Hz, 1H), 3.96 (m, 1H), 3.72 (s, 3H), 3.22 (d, 5.1 Hz, 1H), 2.19 (br s, 1H), 1.60 (m, 1H), 1.39 (m, 1H), 1.11 (m, 1H), 0.86 (d, $J = 6.9$ Hz, 3H), 0.83 (t, $J = 7.5$, 3H); ^{19}F NMR (470.6 MHz, $CDCl_3$): $\delta = -75.1$ (d, $J = 7.0$ Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 174.2, 124.7$ (q, $J = 283.2$ Hz), 74.8, 65.3, 59.2 (q, $J = 29.7$ Hz), 52.0, 39.5, 24.5, 15.4, 11.4.

Typical procedure for the synthesis of Ψ[CH(CF_3)NH]Gly-tetrapetides

Synthesis of **8c** is described as an example. A solution of **4c** (0.69 mmol, 197 mg) and 1 N HCl (0.69 mmol, 690 μl), in MeOH (7 ml), in the presence of a catalytic amount of palladium hydroxide on carbon, was stirred at rt for five hours, under a hydrogen atmosphere. Then, the mixture was filtered on a Celite pad, and the solvent removed in vacuo. The crude material was dissolved in dry DMF (5 ml) and Cbz-L-Phe-OH, (0.90 mmol, 258 mg), *sym* collidine (2.07 mmol, 274 μl), followed by solid HOAt (0.69 mmol, 94 mg) and solid HATU (0.69 mmol, 262 mg) were added at rt. The mixture was stirred overnight, quenched with 1 N HCl, and extracted with Et_2O. The organic layer was washed once with water and then dried on Na_2SO_4. The solvent was removed in vacuo, and the crude purified by FC (hexane/AcOEt 70:30) affording 270 mg of **8c** (73%).

8c. $[\alpha]^{23}_{D} = -12.4°$ (c = 1.9, CH_3COCH_3); FT IR (microscope): $\nu_{max} = 3319, 2980, 2930, 1727, 1691, 1661, 1543, 1370\ cm^{-1}$; ^{1}H NMR: (250 MHz, $CDCl_3$): δ = 7.40–7.14 (m, 11H), 5.56 (br d, J = 7.6 Hz, 1H), 5.13 (d, J = 12.25 Hz, 1H), 5.00 (d, J = 12.25 Hz, 1H), 4.60 (m, 1H), 3.39 (m, 3H), 3.14 (m, 2H), 2.90 (m, 1H), 1.50 (br s, 1H) 1.40 (s, 9H), 1.22 (d, J = 7.2 Hz, 3H); ^{19}F NMR: (235.4 MHz, $CDCl_3$): $\delta = -77.3$ (d, J = 7.2 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 176.0, 171.6, 156.0, 136.5, 136.3, 129.5, 128.6, 128.5, 128.1, 127.0, 125,5 (q, J = 282.7 Hz), 82.3, 67.0, 60.4, 58.0 (q, J = 29.6 Hz), 56.1, 38.6, 28.0, 19.4, 14.2; MS (70 eV): e/z (%): 538 [M^+ + 1] (1), 436 (20), 91 (100).

Procedure for the synthesis of 17a,b

To a solution of Cbz-^{L}Leu-OH **15** (5.76 mmol, 1.53 g) in dry DMF (15 ml), BOP (5.76 mmol, 2.55 g), dimethylamine hydrochloride (5.76 mmol, 0.47 g) followed by DIPEA (17.28 mmol, 2.9 ml) were added at rt and the solution stirred for two hours. 1 N HCl was added, the mixture extracted with AcOEt and the organic phase washed with saturated $NaHCO_3$ and brine. The organic phase was dried on Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude was purified by FC (hexane:AcOEt 1:1) affording 1.43 g (85%) of **16**. A solution of **16** (4.90 mmol, 1.43 g) and 1 N HCl (4.90 mmol, 4.9 ml), in MeOH (30 ml), in the presence of a catalytic amount of palladium hydroxide on carbon, was stirred at rt for three hours, under a hydrogen atmosphere. Then, the mixture was filtered on a Celite pad, and the solvent removed in vacuo. The crude material obtained was dissolved in DCM (29 ml) and a solution of **2** (4.90 mmol, 691 mg) in DCM (2 ml) followed by an excess of $NaHCO_3$ were added and the mixture left to stir at rt for two hours. Water was added and the mixture extracted with DCM, the collected organic layers dried on Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude product was purified by FC (toluene:AcOEt 5:1) affording 1.2 g (3.9 mmol, 80% yield) of a 1:1 mixture of **17a** and **17b**.

17a. R_f: 0.46 Toluene/AcOEt 4:1; $[\alpha]^{23}_{D} = -29.3°$ (c = 0.9, $CHCl_3$); FT IR (film): $\nu_{max} = 1650, 1582\ cm^{-1}$; ^{1}HNMR (400 MHz, $CDCl_3$): δ= 4.61 (m, 2H), 3.78 (m, 1H), 3.67 (m, 1H), 3.00 (s, 3H), 2.93 (s, 3H), 2.19 (m, 1H), 1.98 (m, 1H), 1.34 (m, 1H), 1.20 (m, 1H), 0.91 (m, 6H),; ^{19}F NMR (235.4 MHz, $CDCl_3$): $\delta = -76.5$ (d, J = 7.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 174.4, 124.7 (q, J = 281.4 Hz), 74.5, 58.5 (q, J = 30.5 Hz), 57.3, 43.1, 36.4, 36.0, 24.4, 23.6, 21.2; MS (70 eV): e/z (%): 322 [M^+ + Na] (100), 300 [M + 1] (31), 159 (97).

17b. R_f: 0.40 Toluene/AcOEt 4:1; $[\alpha]^{23}_{D} = -12.8°$ (c = 2.9, $CHCl_3$); FT IR (film): $\nu_{max} = 1643, 1563\ cm^{-1}$; ^{1}HNMR (250 MHz, $CDCl_3$): δ= 4.60 (dd, J = 13.1, 3.3 Hz, 1H), 4.42 (dd, J = 13.1, 9.7 Hz, 1H), 3.94 (m, 1H), 3.58 (dd, J = 10.9, 2.7 Hz, 1H), 2.99 (s, 3H), 2.96 (s, 3H), 2.80 (br s, 1H), 1.78 (m, 1H), 1.36 (ddd, J = 13.6, 10.9, 3.6 Hz, 1H), 1.11 (ddd, J = 13.6, 9.7, 3.1 Hz, 1H), 0.89 (m, 6H); ^{19}F NMR (235.4 MHz, $CDCl_3$): $\delta = -75.1$ (d, J = 7.5 Hz); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 174.1, 124.7 (q, J = 283.5 Hz), 74.9, 59.3 (q, J = 26.1 Hz), 56.0, 44.0, 36.5, 35.8, 24.0, 23.6, 21.1; MS (70 eV): e/z (%): 300 [M + 1] (15), 227 (80), 86 (55), 43 (100).

Typical procedure for the coupling with Boc-^{L,D}Pro-OH and Boc-Val-OH

Synthesis of **18** is described as an example. The nitro group of pure diasteroisomer **17a** was hydrogenated as described above. The crude material obtained after the hydrogenation (2.02 mmol, 616 mg) was dissolved in dry DMF (7 ml) and Boc-^{L}Pro-OH (2.22 mmol, 477 mg), *sym* collidine (1.3 ml), followed by solid HOBT (2.22 mmol, 299 mg) and solid EDC (2.22 mmol, 425 mg) were added at 0 °C. The mixture was stirred overnight at room temperature, quenched with HCl 1 N and extracted with ethyl acetate. The organic layer was washed once with water, once with HCl 1 N, once with sodium hydrogencarbonate and finally once with brine. The organic layer was dried on Na_2SO_4 and filtered; the solvent was removed in vacuo, and the crude product purified by FC (AcOEt:-hexane 6:4), affording 692 mg of **18** (75%).

18. R_f: 0.41 AcOEt/*n*-Hexane 7:3 $[\alpha]^{23}_{D} = -44.5$ (c = 1.1, $CHCl_3$); FT IR (film): ν_{max} = 3380, 1632, 1529, 1471, 1114 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ = 7.99 (br s, 1H), 4.15 (br s, 1H), 3.86 (m, 1H), 3.44 (m, 4H), 3.23 (m, 1H), 3.10 (s, 3H), 2.94 (s, 3H), 2.21 (m, 1H), 1.89 (m, 4H), 1.41 (s, 9H), 1.33 (m, 2H), 0.95 (m, 6H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 176.7, 176.2, 156.1, 127.4 (q, J = 283.5 Hz), 81.5, 62.1, 59.9 (q, J = 26.1 Hz), 56.1, 44.3, 39.7, 37.3, 36.2, 32.4, 31.4, 28.7, 25.8, 25.4, 24.7, 23.8, 22.2; ^{19}F NMR (235.4 MHz, $CDCl_3$): $\delta = -75.0$, J = 7.5 Hz; MS (70 eV): e/z (%): 467[M^+ + 1] (20), 367 (35), 294 (55), 70 (100), 57 (50), 43 (20).

18. (1.03 mmol, 480 mg) was dissolved in a 20% solution of TFA in DCM, stirred for an hour at room temperature, and the solvent was removed in vacuo. The residue was dissolved in a minimal amount of AcOEt, washed with a 5% aqueous solution of $NaHCO_3$ 5%, the organic layer dried on Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The crude material obtained was coupled with Boc-LVal-OH following the HOBt/EDC protocol described above affording 367 mg of tetrapeptide **19** (63%).

19. R_f: 0.48 (AcOEt/*n*-Hexane 4:1); $[\alpha]^{23}_{D} = -39.0°$ (c = 2.3, $CHCl_3$); FT IR (film): ν_{max} = 3440, 1637, 1501, 1441, 1394, 1164 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): δ = 7.02 (br s, 1H), 5.18 (br d, J = 8.6 Hz,1H), 4.46 (m, 1H), 4.26 (br t, J = 7.3 Hz), 3.72 (m, 2H), 3.56 (m, 2H), 3.17 (m, 2H), 3.02 (s, 3H), 2.94 (s, 3H), 2.21 (m, 2H), 2.07 (m, 1H), 1.96 (m, 3H), 1.86 (m, 1H), 1.41 (s, 9H), 1.36 (m, 1H), 1.24 (m, 1H), 0.97 (d, J = 7.3 Hz), 0.91 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.8, 172.3, 171.6, 155.9, 125.9 (q, J = 284.4 Hz), 79.5, 60.2, 58.4 (q, J = 26.1 Hz), 56.9, 53.9, 47.6, 43.5, 37.8, 36.6, 35.9, 31.4, 28.3, 27.9, 25.1, 24.6, 23.5, 21.8, 19.4, 17.3; ^{19}F NMR (470.6 MHz, $CDCl_3$): δ = 74.7 (d, J = 7.7 Hz); MS (70 eV): e/z (%): 566 [M^+ + 1] (20), 466 (10), 294 (25), 70 (100), 57 (70), 43 (20).

Typical procedure for the synthesis of final Ac-^{L}Val-^{L,D}Pro-Ψ[CH(CF_3)NH]Gly-^{L}Leu-NMe_2 tetrapetides

Synthesis of **11** is described as example. **19** was Boc-deprotected and the resulting trifluoroacetate salt neutralized following the procedure described above. The crude (0.12 mmol, 55 mg) was dissolved in dry DCM and TEA (36 µl) was added at 0 °C. After two minutes Ac_2O (12 µl) was added and the mixture was

stirred for one hour at 0 °C. The solution was washed once with HCl 1 N, twice with $NaHCO_3$ 5% and then dried on Na_2SO_4. The solvent was removed in *vacuo* and the crude purified by FC ($CHCl_3$/MeOH 98:2), affording 54 mg (89%) of **11**.

11. $[\alpha]^{23}_D = -62.2$ ($c = 2.1$, $CHCl_3$); FT IR (film): $\nu_{max} = 3325$, 2961, 2244, 1634, 1539, 1446, 1390 cm^{-1}; ^{1}HNMR (400 MHz, $CDCl_3$): $\delta = 7.92$ (br s, 1H), 6.15 (br d, $J = 5.0$ Hz, 1H), 4.65 (dd, $J = 8.9$,6.3 Hz, 1H), 4.50 (m, 1H), 3.83 (dd, $J = 10.9$, 2.7 Hz, 1H), 3.78 (m, 1H), 3.70 (m, 1H), 3.51 (m, 1H), 3.34 (m, 1H), 3.01 (s, 3H) 2.98 (s, 3H), 2.10 (m, 10H), 1.45 (ddd, $J = 13.7$, 10.6, 3.9 Hz, 1H), 1.24 (ddd, $J = 13.7$, 10.0, 3.0 Hz, 1H), 0.93 (m, 13H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 175.6$, 172.3, 171.2, 170.0, 125.7 (Q, $J = 281.8$ Hz), 60.5, 58.5 (Q, $J = 27.4$ Hz), 56.3, 55.6, 47.7, 42.2, 36.7, 36.0, 31.4, 29.1, 25.1, 24.5, 23.6, 21.0, 19.3, 17.8; ^{19}F NMR (235.4 MHz, $CDCl_3$): $\delta = -76.8$ (d, $J = 5.0$ Hz); MS (70 eV): *e*/*z* (%): 508 [$M^+ + 1$] (10), 367(30), 294(98), 70(100).

NMR experiments

The NMR spectra were recorded on a Bruker AMX 600 spectrometer operating at a frequency of 600.13 MHz for ^{1}H nucleus. The chemical shifts (δ) were measured in ppm and referenced to external DSS signal for $CDCl_3$ solution. Estimated accuracy ±0.01 ppm. The experiments were performed at 25 °C. The concentration of peptides was 4.5 mM. ROESY and COSYmqf spectra were acquired in the phase sensitive TPPI mode, with 1K × 512 complex FIDs, spectral width of 6329 Hz, recycling delay of 1.2 s, 48 scans. The mixing times for ROESY experiments ranged between 150 ms and 800 ms. TOCSY spectra were recorded with the use of a MLEV-17 spin-lock pulse (field strength of 7550 Hz, 60 ms total duration, 2.5 ms of TRIM pulse). All spectra were transformed and weighted with a 90° shifted sine-bell squared function to 1K × 1K real data points.^{13}C NMR spectra were obtained by means of one-bond and long range correlation spectroscopy using HMQC and HMBC in the TPPI mode. The NH temperature coefficients were obtained in $CDCl_3$, by monitoring the amide and amine NH chemical shifts over a temperature range of 233 to 298 K. All changes in NH chemical shifts ($-\Delta\delta/\Delta T$) were linear over the above temperature range.

Molecular modeling

Molecular models were built using a Silicon Graphics 4D35GT workstation running the Insight II & Discover software. Molecular mechanics (MM) and molecular dynamics (MD) were carried out using *cvff* force-fields. The starting geometry of the peptides was generated using standard bond lengths and angles. The simulations were performed *in vacuo* with relative permittivity $\varepsilon = 1.0$. At the first step we performed a minimization by Discover with steepest-descendent algorithm followed by conjugate gradient minimization for a maximum of 2000 iterations each or RMS deviation of 0.001 Kcal/mol. Then we performed MD 100 ps simulations at a constant temperature of 300 K with distance constraints derived from the ROE cross peaks from the ROESY spectra in $CDCl_3$. Distance constraints with a force constant of 15 Kcal/Å were applied with a range of lower and upper bound of 2.0–3.0, 3.0–4.0, 4.0–5.0 Å in accordance with the NOE intensities of strong, medium and weak, respectively. No H-bonding constraints was used. Every structure obtained from MD was further minimized. An average structure was created for each MD simulation.

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Notes and references

1 A. Loffet, *J. Pept. Sci.*, 2002, **8**, 1–7.
2 (*a*) G. L. Olson, D. R. Bolin, M. P. Bonner, M. Bös, C. M. Cook, D. C. Fry, B. J. Graves, M. Hatada, D. E. Hill, M. Kahn, V. S. Madison, V. K. Rusiecki, R. Sarabu, J. Sepinwall, G. P. Vincent and M. E. Voss, *J. Med. Chem*, 1993, **36**, 3039–3049; (*b*) J. Gante, *Angew. Chem.*, 1994, **106**, 1780–1802; J. Gante, *Angew. Chem. Int. Ed. Engl.*, 1994, **33**, 1699–1720; (*c*) D. Leung, G. Abbenante and D. P. Fairlie, *J. Med. Chem.*, 2000, **43**, 305–341.
3 J.-L. Fauchère and C. Thurieau, *Adv. Drug Res.*, 1992, **23**, 127–159.
4 A. F. Spatola, In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Vol 7 (Ed. B. Weinstein) Marcel Dekker, New York, 1983, 267-357.
5 (*a*) J. S. Morley, T. D. Hennessey and J. W. Payne, *Biochem. Soc. Trans.*, 1983, **11**, 798–800; (*b*) A. B. Smith, III, R. Hirschmann, A. Pasternak, M. C. Guzman, A. Yokoyama, P. A. Sprengeler, P. L. Darke, E. A. Emini and W. A. Schleif, *J. Am. Chem. Soc.*, 1995, **117**, 11113–11123.
6 For a review on the trifluoethylamine unit, see: M. Sani, A. Volonterio and M. Zanda, *ChemMedChem.*, 2007, **2**, 1693–1700.
7 (*a*) W. C. Black, C. I. Bayly, D. E. Davies, S. Desmarais, J.-P. Falgueyret, S. Léger, C. S. Li, F. Massé, D. J. McKay, J. T. Palmer, M. D. Percival, J. Robichaud, N. Tsou and R. Zamboni, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4741–4744; (*b*) C. S. Li, D. Deschenes, S. Desmarais, J.-P. Falgueyret, J. Y. Gauthier, D. B. Kimmel, S. Léger, F. Massé, M. E. McGrath, D. J. McKay, M. D. Percival, D. Riendeau, S. B. Rodan, M. Thérien, V.-L. Truong, G. Wesolowski, R. Zamboni and W. C. Black, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1985–1989; (*c*) W. C. Black and M. D. Percival, *ChemBioChem*, 2006, **7**, 1525–1535; (*d*) J. Y. Gauthier, N. Chauret, W. Cromlish, S. Desmarais, L. T. Duong, J.-P. Falgueyret, D. B. Kimmel, S. Lamontagne, S. Léger, T. LeRiche, C. Sing Li, F. Massé, D. J. McKay, D. A. Nicoll-Griffith, R. M. Oballa, J. T. Palmer, M. D. Percival, D. Riendeau, J. Robichaud, G. A. Rodan, S. B. Rodan, C. Seto, M. Thérien, V.-L. Truong, M. C. Venuti, G. Wesolowski, R. N. Young, R. Zamboni and W. C. Black, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 923–928; (*e*) P. D. O'Shea, C. Chen, D. Gauvreau, F. Gosselin, G. Hughes, C. Nadeau and R. P. Volante, *J. Org. Chem.*, 2009, in press, http://pubs.acs.org/doi/pdfplus/10.1021/jo8020314.
8 J. D. Dunitz and R. Taylor, *Chem. Eur. J.*, 1997, **3**, 89–98.
9 (*a*) A. Volonterio, P. Bravo and M. Zanda, M., *Org. Chem.*, 2000, **2**, 1827–1830; (*b*) A. Volonterio, P. Bravo and M. Zanda, *Tetrahedron Lett.*, 2001, **42**, 3141–3144; (*c*) A. Volonterio, S. Bellosta, P. Bravo, M. Canavesi, E. Corradi, S. V. Meille, M. Monetti, N. Moussier and M. Zanda, *Eur. J. Org. Chem.*, 2002, 428–438; (*d*) M. Sani, P. Bravo, A. Volonterio and M. Zanda, *Colelct. Czech. Chem. Commun.*, 2002, **67**, 1305–1319; (*e*) A. Volonterio, S. Bellosta, F. Bravin, M. C. Bellucci, L. Bruché, G. Colombo, L. Malpezzi, S. Mazzini, S. V. Meille, M. Meli, C. Ramirez de Arellano and M. Zanda, M., *Chem. Eur. J.*, 2003, **9**, 4510–4522.
10 M. Molteni, A. Volonterio and M. Zanda, *Org. Lett.*, 2003, **5**, 3887–3890.
11 S. Bigotti, A. Volonterio and M. Zanda, *Synlett*, 2008, 958–962.
12 T. Billard, *Chem. Eur. J.*, 2006, **12**, 974–979.
13 For a review, see: F. A. Luzzio, *Tetrahedron*, 2001, **57**, 915–945.
14 Nitro-alkene **2** was prepared starting from a commercial available aqueous solution of fluoral hydrate: O. Klenz, R. Evers, R. Miethchen and M. Michalik, *J. Fluorine Chem.*, 1997, **81**, 205–210See also:; M. Molteni, R. Consonni, T. Giovenzana, L. Malpezzi and M. Zanda, *J. Fluorine Chem.*, 2006, **127**, 901–908.
15 For a review of asymmetric conjugate additions to nitroalkenes, see: T. A. Johnson, D. O. Jang, B. W. Slafer, M. D. Curtis and P. Beak, *J. Am. Chem. Soc.*, 2002, **124**, 11689–11698 and references therein.
16 In a recent work concerning a tandem process involving an aza-Michael reaction with fluorinated acceptors, we found that the polarity of the

solvent as well as the structural features of the base govern remarkably the diastereoselection of the process: M. Sani, L. Bruché, G. Chiva, S. Fustero, J. Piera, A. Volonterio and M. Zanda, *Angew. Chem. Int. Ed.*, 2003, **42**, 2060–2063. However, it should be stressed that the two processes are conceptually and mechanistically unrelated, since the stereocontrol of the reaction described in the reference above arose from an enolate protonation following the aza-Michael reaction, that per se was not stereogenic.

17 α-Amino acid ester hydrochloride **3b** was preliminarly neutralized by treating with an aqueous solution of $NaHCO_3$.

18 S. Bigotti, S. V. Meille, A. Volonterio and M. Zanda, *J. Fluorine Chem.*, 2008, **129**, 767–774.

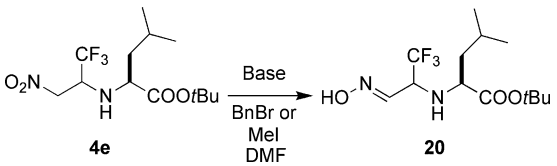
19 S. Fustero, G. Chiva, J. Piera, A. Volonterio, M. Zanda, J. Gonzalez and A. Moran Ramallal, *Chem. Eur. J.*, 2007, **13**, 8530–8542.

20 For examples of conjugate additions to nitroolefins where a hydrogen bond involving a nitro group is proposed in the transition state, see: (*a*) D. Enders and A. Seki, *Synlett*, 2002, 26–28; (*b*) W. Wang, J. Wang and H. Li, *Angew. Chem. Int. Ed.*, 2005, **44**, 1369–1371; (*c*) M. Wiesner, J. D. Revell and H. Wennemers, *Angew. Chem. Int. Ed.*, 2008, **47**, 1871–1874.

21 For an overview on the capacity of F atoms to undertake polar interactions, rather than hydrogen bonds,see: K. Müller, C. Faeh and F. Dietrich, *Science*, **317**, 1881–1886and references therein.

22 Spectroscopic data revealed the presence of only one out of the two possible isomers. Thermodynamically more stable *trans* geometry was confidently assigned to **6** in analogy with the nitro-alkene **2**.

23 In order to obtain α-substituted nitro-compounds like **7**, we also tried to alkylate compound **4e** in the presence of different bases such as NaH, *t*BuOK, Cs_2CO_3, NH_4OAc. In all cases, we obtained the corresponding oxime **20** derived from deoxygenation of the starting material. For a related reaction, see: C. Czekelius and E. M. Carreira, *Angew. Chem. Int. Ed.*, 2005, **44**, 612–615..



24 For a recent review concerning model systems for β-hairpins and β-sheets, see: R. M. Huges and M. L. Waters, *Curr. Opin. Chem. Biol.*, 2006, **16**, 514–524.

25 S. H. Gellman, *Curr. Opin. Chem. Biol.*, 1998, **2**, 717–725.

26 (*a*) S. T. Phillips, L. K. Blasdel and P. A. Bartlett, *J. Org. Chem.*, 2005, **70**, 1865–1871; (*b*) S. T. Phillips, L. K. Blasdel and P. A. Bartlett, *J. Am. Chem. Soc.*, 2005, **127**, 4193–4198; (*c*) S. T. Phillips, G. Piersanti and P. A. Bartlett, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 13737–13742.

27 C. Schmuck, M. Heil, J. Scheiber and K. Baumann, *Angew. Chem. Int. Ed. Engl.*, 2005, **44**, 7208–7212.

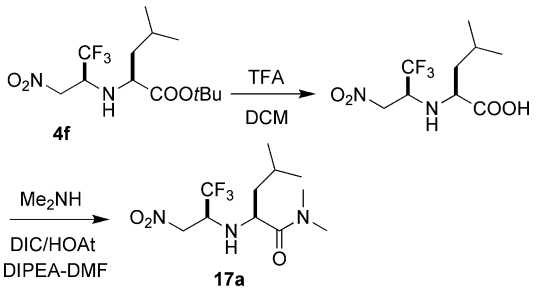
28 Y. Fu, J. Bieschke and J. W. Kelly, *J. Am. Chem. Soc.*, 2005, **127**, 15366–15367.

29 (*a*) A. Aemissegger, V. Krautler, W. F. van Gunsteren and D. Hilvert, *J. Am. Chem. Soc.*, 2005, **127**, 2929–2936; (*b*) V. Krautler, A. Aemissegger, P. H. Hunenberger, D. Hilvert, T. Hansson and W. F. van Gunsteren, *J. Am. Chem. Soc.*, 2005, **127**, 4935–4942; (*c*) S. L. Dong, M. Lowenec, T. E. Schrader, W. J. Schreier, W. Zinth, L. Moroder and C. Renner, C., *Chem. Eur. J.*, 2006, **12**, 1114–1120.

30 G. D. Rose, L. M. Gierasch and J. A. Smith, *Adv. Protein Chem.*, 1985, **37**, 1.

31 (*a*) T. S. Haque, J. C. Little and S. H. Gellman, *J. Am. Chem. Soc.*, 1996, **118**, 6975–6985; (*b*) J. D. Fisk, D. R. Powell and S. H. Gellman, *J. Am. Chem. Soc.*, 2000, **122**, 5443–5447.

32 The stereochemistry of adducts **17a**,**b** was determined by chemical correlation with major diastereoisomer **4f** as depicted below.



33 M. Rance, O. W. Sørensen, G. Bodenhausen, G. Wagner, R. R. Ernst and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 1983, **117**, 479–485.

34 L. Braunschweiler and R. R. Ernst, *J. Magn. Reson*, 1983, **53**, 521.

35 A. A. Bothner-By, R. L. Stephensen and J. Lee, *J.Am.Chem. Soc*, 1984, **106**, 811.

36 A. Bax, R. H. Griffey and B. L. Hawkins, *J. Magn. Reson.*, 1983, **55**, 301.

37 It is noteworthy that for PMR-Ψ[NHCH(CF_3)]Gly peptides, secondary structures were stabilized by the presence of the bulky CF_3 group rather than the presence of an intramolecular hydrogen bond involving the aminic proton of our surrogate which was never observed (see ref. 9e).

38 A. Torganis, I. P. Gerothanassis, Z. Athanassiou, T. Mavromoustakos, G. E. Hawkes and C. Sakarellos, *Biopolymers*, 2000, **53**, 72–83.

