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Note

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Homochiral versus Heterochiral Trifluoromethylated Pseudoproline Containing Dipeptides: A Powerful Tool to Switch the Prolyl-Amide Bond Conformation

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Supporting Information Placeholder

ABSTRACT: The design of constrained peptides is of prime importance in the development of bioactive compounds and for applications in supramolecular chemistry. Due to its nature, the peptide bond undergoes a spontaneous *cis-trans* isomerism and the *cis* isomers are much more difficult to stabilize than the *trans* forms. By using oxazolidine-based pseudoprolines (ΨPro) substituted by a trifluoromethyl group, we show that the *cis* peptide bond can be readily switched from 0% to 100% in Xaa-ΨPro dipeptides. Our results prove that changing the configuration of the C^{α} in Xaa or in ΨPro is sufficient to invert the *cis*:*trans* populations while changing the nature of the Xaa side chain finely tuned the conformers ratio. Moreover, a strong correlation is found between the puckering of the oxazolidine ring and the peptide bond conformation. This finding highlights the role of the trifluoromethyl group in the stabilization of the peptide bond geometry. We anticipate that such templates will be very useful to constrain the backbone geometry of longer peptides.

Heterochiral templates have proved to be potent tools to control the peptide backbone conformation.[1] For instance, D-amino acids have been largely employed in the design of *β*-turn like structures.[2] The common type I and II are readily obtained from homochiral (LL) and heterochiral (LD) Xaa-Yaa moieties, respectively. Their mirror images (types I' and II'), widely used as strong β-hairpin nucleators, are preferred with residue pairs (DD) and (DL), which display the opposite chirality.[3] However, these homo- and heterochiral templates usually share a common *trans* peptide bond geometry.[4] To constrain the amide bond conformation, numerous alkyl-substituted pyrrolidine rings have been introduced at the Yaa position as proline surrogates (Figure 1). While they have almost no effect at the C^{β} and C^{γ} positions, C^{α} and C^{δ} alkylations lead to a strong preference for the *trans* and *cis* isomers, respectively.[5] Furthermore, comprehensive studies on pseudoproline (ΨPro) surrogates have shown that changing the substituent nature at the C^{δ} position tuned the *cis*/*trans* isomer ratio.[6] The *cis* content increasing with the degree of substitution at C^{δ}, a dimethyl substitution gave almost 100% of *cis* isomer. The configuration at the C^{δ} position plays also an important role. When the C^{δ} substituent and the C^{α} carbonyl group of a proline ring surrogates

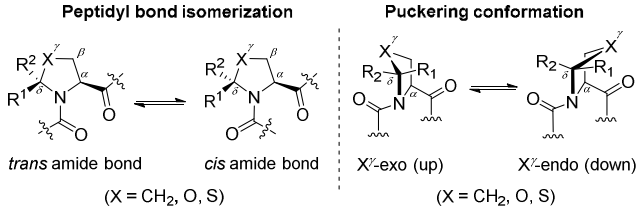


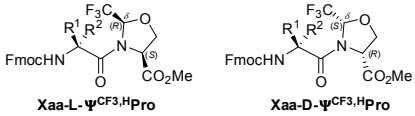
Figure 1. *Cis*–*trans* conformers and puckering equilibrium in L-proline (X = CH_2) and L-pseudoprolines ($\Psi^{R1,R2}$Pro, X = O, S).

are on the same side (R^1 position, Figure 1), the *trans* Xaa-Pro amide bond is favored. Conversely, when they are located on opposite sides (R^2 position, Figure 1), the *cis* Xaa-Pro amide bond content is enhanced.[6,7] In *N*-acetyl-thiazolidine tripeptide models, Dumy et al. have shown that the replacement of the *C*-terminal methyl ester with a methyl amide enhanced this behavior.[6d] In contrast, Lubell et al. have reported that the replacement of the proline with the (2*S*,5*R*)-5-*tert*-butylproline (R^1 substituent) into Ac-Xaa-Pro-NHMe dipeptides strongly displaced the *cis-trans* equilibrium toward the *cis* conformer, whatever was the nature of the Xaa residue (alkyl, aromatic, hydrogen-bond donors or acceptors).[8] Overall, these results prove that C^{δ}-substituted pseudoprolines represent outstanding tools to constrain the peptide bond geometry.

Our group is strongly involved in the synthesis of trifluoromethyl (CF_3) containing prolines and their pseudoproline surrogates[9] as well as their incorporation in peptide chains.[10] In particular, we have reported several studies based on CF_3-pseudoprolines ($\Psi^{CF3,H}$Pro) model peptides which established the stereoelectronic effects imparted by the CF_3 group at the C^{δ} position.[11] As observed in other C^{δ}-substituted pseudoprolines, we found that the CF_3 group led to greater *cis* peptide bond content when placed at the R^2 position (Figure 1). Moreover, the electronic effects of the CF_3 group were responsible for a low rotational barrier of the *cis-trans* peptide bond isomerization as observed in model peptides exhibiting intramolecular hydrogen bond[12] and for freezing the puckering of the oxazolidine core (Figure 1).[11a] We then focused on a methodological study for the synthesis of Fmoc-Xaa-Ser($\Psi^{CF3,H}$Pro)-OMe dipeptides (Xaa = Gly, L-Ala, L-Val, L-Pro and Aib).[10b] NMR conformational studies of these peptides re-

vealed that the geometry of the amide bond closely depends on the nature of the preceding amino acid Xaa.

In the present paper, we show for the first time that the configuration of the Xaa residue tightly control the *cis*-*trans* ratio in ($\Psi^{CF3,H}$Pro)-containing dipeptides. For this purpose, a systematic study of twelve homo- and heterochiral Fmoc-Xaa-Ser($\Psi^{CF3,H}$Pro)-OMe dipeptides has been conducted (Figure 2). In addition four dipeptides incorporating an achiral Xaa residue have been synthesized and compared to the previous series. Each enantiomer has been analyzed by NMR spectroscopy. Structural features (*cis*-*trans* amide bond contents and vicinal coupling constants) are reported allowing us to depict the local role of the CF_3-pseudoprolines in the peptide conformation.



Xaa-L-$\Psi^{CF3,H}$Pro **Xaa-D-$\Psi^{CF3,H}$Pro**

Achiral Xaa:	Gly (**2**) Aib (**6**)	Gly (***ent*-2**) Aib (***ent*-6**)
Homochiral series:	L-Ala (**3**) L-Val (**4**) L-Pro (**5**)	D-Ala (***ent*-3**) D-Val (***ent*-4**) D-Pro (***ent*-5**)
Heterochiral series:	D-Ala (**7**) D-Val (**8**) D-Pro (**9**)	L-Ala (***ent*-7**) L-Val (***ent*-8**) L-Pro (***ent*-9**)

Figure 2. Chemical structure of the Fmoc-Xaa-Ser($\Psi^{CF3,H}$Pro)-OMe dipeptides.

The dipeptides have been synthesized starting from Fmoc-protected L- or D-Xaa amino acid chlorides and L- or D-$\Psi^{CF3,H}$Pro (**1** or ***ent*-1**), readily obtained from the condensation between the fluoral hemiacetal and the (*S*)- or (*R*)-serine methyl ester, respectively.[9b] The coupling reaction in base-free conditions gave the corresponding dipeptides **2-9** and ***ent*-(2-9)** in moderate to good yields as >95% enantiopure diastereomers (Table 1).[13] As previously reported, a dynamic kinetic resolution process (DKR) occurred during the *N*-amidification leading exclusively to pseudoproline moieties bearing the CF_3 group in a *cis* configuration relative to the methyl ester termination (Figure 2).[10b] The conformation of the Xaa-ΨPro peptide bonds has been determined by NMR spectroscopy at 274 K in $CDCl_3$. *Cis* and *trans* conformers were in the slow exchange regime at this temperature. Typical *cis*/*trans* exchange cross peaks were observed in the Roesy spectra, whereas dipolar interactions between neighboring protons were associated with negative cross peaks (Figure 3). *Cis* conformers were characterized by strong H^{α}_{Xaa}-$H^{\alpha}_{\Psi pro}$ correlations, while *trans* forms were assigned from H^{α}_{Xaa}- $H^{\delta}_{\Psi pro}$ cross peaks.[14] Populations were quantified by the integration of ^1H and ^{19}F NMR of isolated resonances. When a glycine precedes the L- or D-$\Psi^{CF3,H}$Pro residue the corresponding dipeptide **2** (or ***ent*-2**) exhibits a *cis* conformer content of 61% (Table 1). Thus, in the absence of any side chain at the Xaa position, the peptide backbone already displays a slight preference for the *cis* conformation. This tendency is significantly enhanced with the Aib residue (85%) which bears two methyl groups (Table 1, dipeptides **6** and ***ent*-6**). To assess the influence of each methyl group on the peptide bond geometry, we compared the *cis* content in Xaa-L-ΨPro and Xaa-D-ΨPro dipeptides with Xaa = L-Ala or D-Ala. In all cases, we observed a marked unbalance between the *cis* and *trans* populations. While the *cis* amide bond was strongly stabilized in heterochiral peptides **7** and ***ent*-7** (92%), the opposite trend was found in homochiral **3** and ***ent*-3** peptides (*cis* content: 12%). Note that these population ratio were only slightly displaced in a polar solvent (see supporting information). It is well known that the nature of the amino acid preceding the proline residue is of crucial significance for both the *cis*-*trans* ratio[4b,c] and

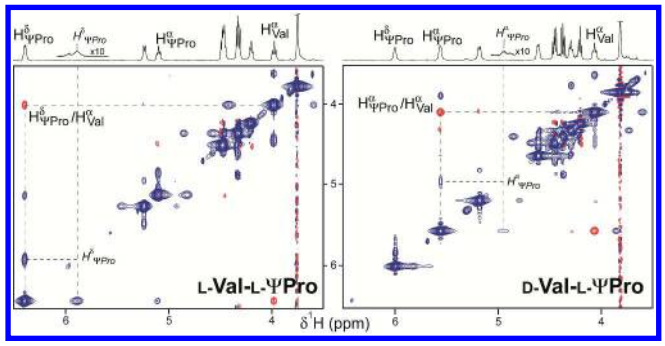


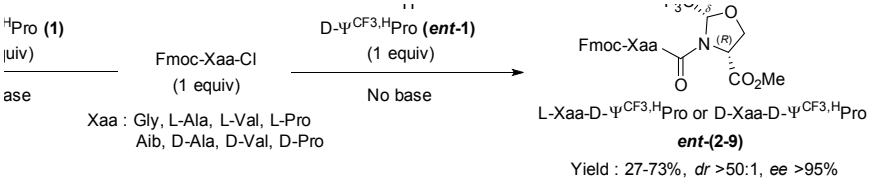
Figure 3. H^{δ}-H^{α} region of the Roesy spectra of Fmoc-L/D-Val-L-Ser($\Psi^{CF3,H}$Pro)-OMe. Spectra have been recorded in $CDCl_3$, at 500 MHz and 274 K. In each spectrum, assignments of the H^{α} are reported for the two residues together with the ΨPro H^{δ}. Assignments of the minor ΨPro H^{δ} or H^{α} correspond to the italicized labels (~5% in these two examples).

the rate constants of the spontaneous isomerization.[15] Aromatic residues (Ar) at the Xaa position gives the highest fraction of *cis* conformer,[16] probably as a result of Ar-Pro interactions.[8c,17] However, very few examples are available on hetero- and homochiral dipeptides series. Lubell et *al.* reported that the incorporation of a L-Xaa or a D-Xaa residue at the *N*-terminal position of L-Pro poorly affects the *cis*-*trans* ratio in Ac-Xaa-Pro-NHMe dipeptides, the *trans* conformer always being the major one.[8c] Replacing the proline with a (2*S*,5*R*)-5-*tert*-butylproline switches the isomeric equilibrium, both the homochiral and heterochiral dipeptides preferentially adopting a *cis* peptide conformation. Importantly, we examined a methyl ester dipeptides series so that no hydrogen bond can modulate the *cis*/*trans* ratio. Our analyses have shown that homochiral and heterochiral dipeptides have inverted isomer populations but share the same major/minor ratio. These observations highlight the unique behavior of the CF_3-pseudoproline in the Xaa-ΨPro series. As mentioned before, the configuration at the C^{δ} is entirely controlled by the L/D stereochemistry of the CF_3-pseudoproline (Figure 2). Therefore, this striking observation suggests that the stereospecific interaction between the CF_3 group of the ΨPro and the methyl substituent of the alanine controls the prolyl amide bond geometry. So, when the C^{δ} of the ΨPro and the C^{α} of the Ala residue display the same absolute configuration, the *cis* amide bond is stabilized.

On the contrary, opposite configurations of these two stereocenters favor the *trans* amide bond. Moreover, a 85% *cis* content is observed for the Aib-ΨPro dipeptides which revealed a significant difference of the CF_3/CH_3 interactions between the two homotopic methyl groups. In dipeptide **6**, the (*R*)-C^{δ}-CF_3/*pro*-*R* CH_3 interaction prevails and is responsible for the *cis* peptide bond geometry preference. In dipeptide ***ent*-6**, it is the (*S*)-C^{δ}-CF_3/*pro*-*S* CH_3 interaction that dominates. Increasing the steric hindrance of the Xaa residue enhanced the *cis*/*trans* unbalance. A *cis* content of 95% was found in the heterochiral Val-ΨPro dipeptides **8** and ***ent*-8** while only 7% of the related homochiral compounds (**4** and ***ent*-4**) displayed the *cis* peptide bond geometry (Table 1 and Figure 3). Finally, when Xaa was a Pro residue, a single conformer was detected in solution, which was assigned to the *cis* for the heterochiral and to the *trans* for the homochiral Pro-ΨPro dipeptides, respectively (Table 1).

The stereo-electronic effects of the trifluoromethyl group seem crucial in the modulation of the conformational preferences. We have previously reported the ability of the CF_3 substituent to efficiently constrain the puckering in 5-membered rings, the (2*R*)-$\Psi^{CF3,H}$Pro being up-puckered and the (2*S*)-$\Psi^{H,CF3}$Pro being

Table 1. Synthesis, *cis/trans* populations and vicinal couplings of dipeptides 2-9 and *ent*-(2-9).

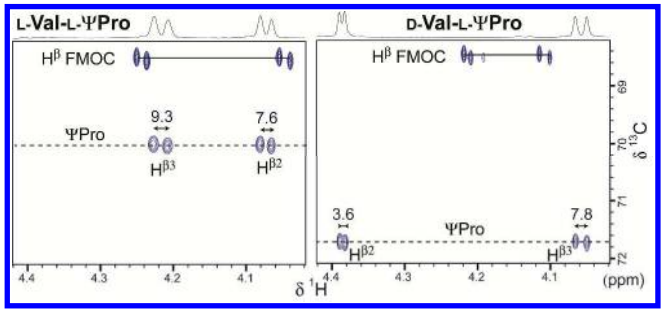


Compound (% yield)[a]				Conformer populations		Vicinal couplings[b] (Hz)	
	Xaa-L-$\Psi^{CF3,H}$Pro		Xaa-D-$\Psi^{CF3,H}$Pro	% cis[c]	% trans[c]	$^3J_{H\alpha-H\beta2}$	$^3J_{H\alpha-H\beta3}$
2	Gly-L-$\Psi^{CF3,H}$Pro (96)	**ent-2**	Gly-D-$\Psi^{CF3,H}$Pro (63)[d]	**61**	39	6.9	6.9
3	L-Ala-L-$\Psi^{CF3,H}$Pro (66)	**ent-3**	D-Ala-D-$\Psi^{CF3,H}$Pro (73)	12	**88**	7.8	8.6
4	L-Val-L-$\Psi^{CF3,H}$Pro (59)	**ent-4**	D-Val-D-$\Psi^{CF3,H}$Pro (53)	7	**93**	7.6	9.4
5	L-Pro-L-$\Psi^{CF3,H}$Pro (86)	**ent-5**	D-Pro-D-$\Psi^{CF3,H}$Pro (53)	0	**100**[f]	7.5	9.2
6	Aib-L-$\Psi^{CF3,H}$Pro (55)[g]	**ent-6**	Aib-D-$\Psi^{CF3,H}$Pro (63)[h]	**85**	15	4.2	7.9
7	D-Ala-L-$\Psi^{CF3,H}$Pro (79)	**ent-7**	L-Ala-D-$\Psi^{CF3,H}$Pro (56)[i]	**92**	8	4.8	8.4
8	D-Val-L-$\Psi^{CF3,H}$Pro (79)	**ent-8**	L-Val-D-$\Psi^{CF3,H}$Pro (67)	**95**	5	3.6	7.9
9	D-Pro-L-$\Psi^{CF3,H}$Pro (92)	**ent-9**	L-Pro-D-$\Psi^{CF3,H}$Pro (27)[e]	**100**[f]	0	4.9	8.2

[a]Yield of the isolated compound. [b]Measured on H^{α} resonances and/or on the CH_2-TROSY spectra of the major conformer. [c]Measured by ^1H and ^{19}F NMR at 274 K in $CDCl_3$. [d]69% conversion. [e]Not optimized reaction. [f]Only one conformer is observed for the Xaa-Pro amide bond. [g]Three equivalents of Fmoc-Aib-Cl were used. [h]Two equivalents of Fmoc-Aib-Cl were used. [i]65% conversion.

down-puckered in oxazolidine rings.[11a] Statistics performed on X-ray structures databases have revealed a significant prevalence of the C^{γ}-exo conformation for the *trans* Xaa-Pro bond, while a C^{γ}-endo puckering preference was associated with the *cis* peptide bond.[4b,18] We thus investigated the correlations between the peptide bond geometry and the ring puckering of the ΨPro moieties.[19] $^3J_{H\alpha-H\beta2}$ and $^3J_{H\alpha-H\beta3}$ coupling constants were used to determine the ring puckering preferences. These values are extracted from 1D spectra when H^{α} resonances were well-resolved and/or in a straightforward manner from the CH_2-TROSY experiments (Figure 4).[20] Both vicinal couplings constants should be comparable for O^{γ}-exo puckered rings (O^{γ} and C' on the same side) with $^3J_{H\alpha-H\beta2} \sim {}^3J_{H\alpha-H\beta3} \sim 8$ Hz, whereas in O^{γ}-endo puckering (O^{γ} and C' on the opposite side), typical values are $^3J_{H\alpha-H\beta2} \sim 3$ Hz and $^3J_{H\alpha-H\beta3} \sim 8$ Hz.[21] Our measurements unambiguously showed that the homochiral dipeptides, which strongly prefer the *trans* peptide bond, were prone to adopt the O^{γ}-exo puckering (Table 1). Conversely, heterochiral dipeptides displayed significantly reduced $^3J_{H\alpha-H\beta2}$ values. This corresponds to high O^{γ}-endo contents that are associated with the *cis* peptide bonds. The conformational correlations observed in proteins *trans*/C^{γ}-exo and *cis*/C^{γ}-endo) were then confirmed in the Xaa-ΨPro series. Interestingly, when the Xaa was a Gly residue, the absence of any side chain allowed the combination of an O^{γ}-exo puckering and a *cis* peptide bond. The minor *trans* form of the Gly-L/D-$\Psi^{CF3,H}$Pro dipeptides was also associated with the O^{γ}-exo puckering ($^3J_{H\alpha-H\beta2}$ = 7.5 Hz and $^3J_{H\alpha-H\beta3} \sim 8.5$ Hz, see experimental section), which recalls the freezing of the puckering previously observed in the isolated $\Psi^{CF3,H}$Pro residues.[11a] However, the interactions with the side chain of Xaa govern both the puckering of the oxazolidine and the peptide bond. Changing the Xaa configuration results in the inversion of both the peptide bond and the oxazolidine puckering. In a similar manner, inverting the configuration of the oxazolidine C^{δ} which bears the CF_3 group restores the puckering and the peptide bond geometry.

Figure 4. C^{β} region of the CH2 TROSY spectra of Fmoc-L/D-Val-D-Ser($\Psi^{CF3,H}$Pro)-OMe. Spectra have been recorded in $CDCl_3$, at 500 M Hz and 274 K. A cross-section through each H^{β} resonance is displayed to show the pure doublets obtained.



In summary, we have shown that the *cis-trans* ratio of the Xaa-CF_3-ΨPro amide bond conformation is strongly influenced by the ΨPro C^{δ} and the Xaa C^{α} configurations. A strong correlation is observed between the oxazolidine ring puckering and the peptide bond geometry which corresponds to the trends observed in proteins for Xaa-Pro moieties. Since the ΨPro C^{δ} configuration is governed by the L/D stereochemistry of the CF_3-pseudoproline, the backbone geometry is readily tuned by using homochiral or heterochiral Xaa-CF_3-ΨPro dipeptides. *Cis*/*trans* or *trans*/*cis* ratio of 20:1 were obtained in the Val-ΨPro series, whereas a single conformer was observed for Pro-ΨPro dipeptides. We have described a complete set of 16 dipeptide building blocks that are ready to use for solid-phase peptide synthesis (SPPS). We anticipate that such templates will be very useful to constrain the backbone geometry in longer peptides.

EXPERIMENTAL SECTION

General Experimental Methods. Unless otherwise mentioned, all the reagents were purchased from commercial source. All glassware was dried in an oven at 150 °C prior to use. All solvents were purified and dried by standard techniques and distilled prior to use. Dichloromethane was distilled over calcium hydride under argon. All organic extracts were dried over $MgSO_4$, unless otherwise noted. Silica gel (230−400 mesh) was used for

flash column chromatography, eluting (unless otherwise stated) with cyclohexane/ethyl acetate. Silica TLC plates were visualized under UV light, by a 10% solution of phosphomolybdic acid in ethanol followed by heating. Infrared spectra (IR) were obtained by Fourier transformation and wavenumbers are given in cm^{-1}. ^1H NMR (400.00 MHz), ^{13}C NMR (100.50 MHz), and ^{19}F NMR (376.20 MHz) spectra were measured on a JEOL 400 spectrometer. 2D NMR spectra were acquired on a spectrometer operating at a ^1H frequency of 500 MHz and equipped with a triple resonance, z-axis pulsed-5 field-gradient cryogenic probehead, optimized for 1H detection. Complete proton assignments were obtained from the analysis of 2D total correlation spectroscopy (TOCSY)[22] experiments using 80 ms DIPSI-2 mixtime,[23] and 2D rotating frame Overhauser effect spectroscopy (ROESY)[24] experiments (typically 250 ms mixing time). Homonuclear experiments were typically collected as 256 (t1) and 2048 (t2) time-domain matrices over a ^1H spectral width of 12 ppm, with 8 scans per t1 increment. Carbon assignment was deduced from heteronuclear 2D $^1H–^{13}C$ HSQC experiments,[25] using 512 (t1) × 1024 (t2) time domain matrices, with 16 scans per t1 increment. 2D $^1H–^{13}C$ CH_2-TROSY experiments were recorded to extract proton-proton vicinal couplings.[20b] Data were typically collected as 512 (t1) and 8192 (t2) time-domain matrices over a ^1H spectral width of 9 ppm, with 16 scans per t1 increment. 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. Spectra were analyzed using the TOPSPIN® software. Chemical shifts of ^1H NMR are expressed in parts per million downfield from $CHCl_3$ (δ = 7.26) 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). Melting points were uncorrected. High-Resolution Mass Spectra (HRMS) were obtained using ElectroSpray Ionization (ESI) in positive ion mode and a TOF mass analyzer or using Electronic Impact (EI) in positive ion mode by direct insertion probe and double focusing magnetic sector mass analyzer. High Performance Liquid Chromatography (HPLC) was performed on a Daicel Chiralpak IA column (250×4.6 mm, 5 μm) or Phenomenex Lux Amylose-2 column (250×4.6 mm, 5 μm). An isocratic program was applied with n-Hexane (A) and isopropanol (B) (v/v) at a flow rate of 1.0 mL/min. HPLC analyses were performed on an UV detector (254 nm) or ELSD detector (temperature of the nebulization was set at 30 °C or 40 °C). The injection volume was 10 μL for quantitative analysis.

Representative procedure for the preparation of pseudoproline methyl ester.[9b] To a solution of Boc-serine methyl ester (1.0 equiv) in toluene at room temperature were added pyridinium p-toluenesulfonate (PPTS) (0.2 equiv) and trifluoroacetaldehyde hydrate (1.2 equiv). The resulting mixture was stirred at room temperature for 1 h, then toluene was added and the reaction mixture was warmed to reflux using a Dean-Stark apparatus. The progress of the reaction was checked by ^1H NMR (usually each 5 h). After cooling, trifluoroacetaldehyde hydrate (0.2 equiv) was added and the reaction mixture rewarming to reflux until the total conversion. The reaction mixture was then cooled to 0 °C with an ice bath and filtered, and toluene was evaporated. Purification by flash chromatography gave the corresponding pseudoprolines **1** or ***ent*-1** as a mixture of two separable diastereomers.

L-Ψ^{CF3,H}Pro-OMe (1). The reaction was performed following the representative procedure starting from Boc-L-serine methyl ester (21.64 g, 98.79 mmol, 1.0 equiv) in toluene (50 mL), PPTS (4.97 g, 19.76 mmol, 0.2 equiv) and trifluoroacetaldehyde hydrate (9.8 mL, 118.23 mmol, 1.2 equiv). After 1 h at room temperature, 800 mL of toluene were added before warming. Purification by flash chromatography (90:10 cyclohexane/ethyl acetate) gave pseudoprolines (2*S*)-**1a** (10.61 g, 54%) as a colorless oil and (2*R*)-**1b** (1.87 g, 10%) as a white solid.

(2*S*)-L-Ψ^{CF3,H}Pro-OMe (1a): colorless oil; *Rf* = 0.31 (80:20 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ –50.4 (*c* 4.95, $CHCl_3$); HRMS (EI) m/z: M^+ Calcd for $C_6H_8F_3NO_3$ 199.0456; Found 199.0457; HPLC-ELSD (Chiralpak IA, 30 °C) *rt* = 4.30 min (60:40, *n*-hexane/isopropanol); see ref. 9b for spectral data of **1a**.

(2*R*)-L-Ψ^{CF3,H}Pro-OMe (1b): white solid; mp 65-76 °C; *Rf* = 0.10 (80:20 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ –17.6 (*c* 4.9, $CHCl_3$); HRMS (EI) m/z: M^+ Calcd for $C_6H_8F_3NO_3$ 199.0456; Found 199.0458; HPLC-ELSD (Chiralpak IA, 30 °C) *rt* = 4.49 min (60:40, *n*-hexane/isopropanol); see ref. 9b for spectral data of **1b**.

D-Ψ^{CF3,H}Pro-OMe (*ent*-1). The reaction was performed following the representative procedure starting from Boc-D-serine methyl ester (16.67 g, 76.10 mmol, 1.0 equiv) in toluene (34 mL), PPTS (3.82 g, 15.20 mmol, 0.2 equiv) and trifluoroacetaldehyde hydrate (7.6 mL, 91.69 mmol, 1.2 equiv). After 1 h at room temperature, 680 mL of toluene were added before warming. Purification by flash chromatography (90:10 cyclohexane/ethyl acetate) gave pseudoprolines (2*R*)-***ent*-1a** (7.80 g, 52%) as a colorless oil and (2*S*)-***ent*-1b** (1.31 g, 9%) as a white solid.

(2*R*)-D-Ψ^{CF3,H}Pro-OMe (*ent*-1a): colorless oil; *Rf* = 0.31 (80:20 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ +46.4 (*c* 4.95, $CHCl_3$); IR (neat) 3338, 2962, 1740, 1439, 1287, 1223, 1158, 1131, 665 cm^{-1}; HPLC-ELSD (Chiralpak IA, 30 °C) *rt* = 4.05 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-1a** are similar to those of **1a**.[9b]

(2*S*)-D-Ψ^{CF3H}Pro-OMe (*ent*-1b): white solid; mp 65-76 °C; *Rf* = 0.10 (80:20 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ +19.2 (*c* 4.9, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_6H_8F_3NO_3Na$ 222.0349; Found 222.0349; HPLC-ELSD (Chiralpak IA, 30 °C) *rt* = 4.78 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-1b** are similar to those of **1b**.[9b]

Representative procedure for the preparation of Fmoc-aminoacid chloride assisted by ultrasonication.[26] To a 0.2 M solution of the Fmoc-aminoacid (1.0 equiv) suspended in dichloromethane under argon, was added freshly distilled $SOCl_2$ (13.8 equiv). The mixture was sonicated at romm temperature until the complete disappearence of the precipitate (from 30 min to 1 h), then solvent and excess of $SOCl_2$ were removed *in vacuo* to give the Fmoc-aminoacid chloride as a white solid directly used without further purification.

Representative procedure for the peptide coupling reaction.[10b] To a solution of pseudoprolines **1** or ***ent*-1** (1.0 equiv) in dichloromethane was added Fmoc-amino acid chloride (1.1 equiv). The reaction mixture was stirred for 18 h at room temperature under inert atmosphere, then the solvent was evaporated under reduced pressure. Purification by flash chromatography gave pure dipeptides **2-9** and ***ent*-(2-9)** in 27-96% yield.

Fmoc-Gly-L-Ψ^{CF3,H}Pro-OMe (2). The reaction was performed following the representative procedure starting from a 84:16 diastereomeric mixture of (2*S*)-**1a** and (2*R*)-**1b** pseudoprolines (7.94 g, 39.90 mmol, 1.0 equiv) in dichloromethane (120 mL) and Fmoc-Gly-Cl (13.83 g, 43.83 mmol, 1.1 equiv). Purification by flash chromatography (60:40 cyclohexane/ethyl acetate) gave the pure dipeptide **2** (18.4 g, 96%) as a 61/39 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 63–83 °C; R_f = 0.24 (60:40 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ – 36.0 (*c* 1.06, $CHCl_3$); HRMS (EI) m/z: $[M + Na]^+$ Calcd for $C_{23}H_{21}F_3N_2O_6Na$ 501.1244; Found 501.1248; HPLC-ELSD (Chi-

ralpak IA, 40 °C) *rt* = 6.54 min (60:40, *n*-hexane/isopropanol); see ref. 10b for spectral data of **2**.

Fmoc-Gly-D-Ψ^{CF3,H}Pro-OMe (*ent*-2). The reaction was performed following the representative procedure starting from pseudoproline (2*R*)-***ent*-1a** (307 mg, 1.54 mmol, 1.0 equiv) in dichloromethane (6 mL) and Fmoc-Gly-Cl (535 mg, 1.70 mmol, 1.1 equiv). Purification by flash chromatography (75:25 cyclohexane/ethyl acetate) gave recovered pseudoproline (2*R*)-***ent*-1a** (66 mg, 21%) and the pure dipeptide ***ent*-2** (462 mg, 63%) as a 61/39 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 64–76 °C; $[\alpha]^{23}_D$ +44.2 (*c* 1.05, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{23}H_{21}F_3N_2O_6Na$ 501.1244; Found 501.1242; HPLC-ELSD (Chiralpak IA, 40 °C) *rt* = 6.22 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-2** are similar to those of **2**.[10b]

Fmoc-L-Ala-L-Ψ^{CF3,H}Pro-OMe (3). The reaction was performed following the representative procedure starting from a 84:16 diastereomeric mixture of (2*S*)-**1a** and (2*R*)-**1b** pseudoprolines (1 g, 5.03 mmol, 1.0 equiv) in dichloromethane (20 mL) and Fmoc-L-Ala-Cl (1.82 g, 5.50 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave 1.65 g (66%) of pure dipeptide **3** as a 12/88 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 65-69 °C; R_f = 0.29 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{24}_D$ –81.2 (*c* 0.95, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{23}F_3N_2O_6Na$ 515.1400; Found 515.1386; HPLC-UV (Lux Amylose-2) *rt* = 5.31 min (60:40, *n*-hexane/isopropanol); see Ref. 11b for spectral data of **3**.

Fmoc-D-Ala-L-Ψ^{CF3,H}Pro-OMe (7). The reaction was performed following the base free representative procedure starting from (2*S*)-**1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Ala-Cl (547 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide **7** (545 mg, 79%) as a 92/8 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 300 K : white solid; mp 87-90 °C; R_f = 0.40 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ –47.5 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{23}F_3N_2O_6Na$ 515.1400; Found 515.1400; HPLC-UV (Chiralpak IA) *rt* = 4.76 min (60:40, *n*-hexane/isopropanol); see Ref. 11b for spectral data of **7**.

Fmoc-L-Ala-D-Ψ^{CF3,H}Pro-OMe (*ent*-7). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (294 mg, 1.48 mmol, 1.0 equiv) in dichloromethane (6 mL) and Fmoc-L-Ala-Cl (536 mg, 1.63 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave recovered pseudoproline (2*R*)-***ent*-1a** (102 mg, 35%) and the pure dipeptide ***ent*-7** (406 mg, 56%) as a 92/8 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 305 K : white solid; mp 87-90 °C; $[\alpha]^{23}_D$ +43.2 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{23}F_3N_2O_6Na$ 515.1400; Found 515.1397; HPLC-UV (Chiralpak IA) *rt* = 4.32 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-7** are similar to those of **7**.[11b]

Fmoc-D-Ala-D-Ψ^{CF3,H}Pro-OMe (*ent*-3). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Ala-Cl (547 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide ***ent*-3** (541 mg, 73%) as a 12/88 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 54–67 °C; $[\alpha]^{23}_D$ +80.3 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{23}F_3N_2O_6Na$ 515.1400; Found 515.1387; HPLC-UV (Lux Amylose-2) *rt* = 7.48 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-3** are similar to those of **3**.[11b]

Fmoc-L-Val-L-Ψ^{CF3,H}Pro-OMe (4). The reaction was performed starting from a 84:16 diastereomeric mixture of (2*S*)-**1a** and (2*R*)-**1b** pseudoprolines (2.00 g, 10.0 mmol, 1.0 equiv) in dichloromethane (30 mL) and Fmoc-L-Val-Cl (3.95 g, 11.0 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave the pure dipeptide **4** (3.09 g, 59%) as a 7/93 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 300 K : white solid; mp 115-117 °C; R_f = 0.42 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{25}_D$ –74.5 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{27}F_3N_2O_6Na$ 543.1713; Found 543.1698; HPLC-UV (Chiralpak IA) *rt* = 4.71 min (60:40, *n*-hexane/isopropanol); see Ref. 10b for spectral data of **4**.

Fmoc-D-Val-L-Ψ^{CF3,H}Pro-OMe (8). The reaction was performed starting from (2*S*)-**1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Val-Cl (594 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave the pure dipeptide **8** (620 mg, 79%) as a 95/5 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 305 K : white solid; mp 58-62 °C; R_f = 0.61 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{25}_D$ –29.9 (*c* 1.0, $CHCl_3$); IR (neat) 3321, 2968, 1681 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 305 K) : (*cis* rotamer) δ 1.03 (d, *J* = 6.9 Hz, 6 H, H_γ Val-H), 2.12 (m, 1 H, H_β Val-H), 3.82 (s, 3 H, OMe), 4.05 (t, *J* = 8.7 Hz, 1 H, H_α Val-H), 4.20 (t, *J* = 6.9 Hz, 1 H, Fmoc CH), 4.29 (t, *J* = 8.2 Hz, 1 H, $H_{\beta3}$ Ψpro-H), 4.35 (dd, *J* = 10.5, 7.3 Hz, 1 H, Fmoc CH_2-Ha), 4.45 (dd, *J* = 10.5, 6.1 Hz, 1 H, Fmoc CH_2-Hb), 4.60 (dd, *J* = 8.5, 3.4 Hz, 1 H, $H_{\beta2}$ Ψpro-H), 5.22 (d, *J* = 8.2 Hz, 1 H, NH Val), 5.57 (dd, *J* = 7.3, 3.2 Hz, 1 H, H_α Ψpro-H), 6.00 (q, *J* = 5.0 Hz, 1 H, H_δ Ψpro-H), 7.31 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.41 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.55 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.56 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.78 (d, *J* = 7.8 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100,5 MHz, $CDCl_3$, 298 K) : (*cis* rotamer) δ 18.4 (CH_3, C_γ Val), 19.0 (CH_3, C_γ Val), 31.4 (CH, C_β Val), 47.0 (CH, Fmoc CH), 52.9 (CH_3, OMe), 58.0 (CH, C_α Ψpro), 58.4 (CH, C_α Val), 67.2 (CH_2, Fmoc CH_2), 70.4 (CH_2, C_β Ψpro), 83.9 (q, *J* = 35.5 Hz, CH, C_δ Ψpro), 120.1 (2 × CH, Fmoc arom.), 122.4 (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.), 143.4 (C, Fmoc arom.), 143.5 (C, Fmoc arom.), 156.6 (C, C=O), 169.1 (C, C=O), 173.2 (C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) : (*cis* rotamer) δ –82.8 (d, *J* = 4.3 Hz); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{27}F_3N_2O_6Na$ 543.1713; Found 543.1710; HPLC-UV (Chiralpak IA) *rt* = 5.63 min (60:40, *n*-hexane/isopropanol).

Fmoc-L-Val-D-Ψ^{CF3,H}Pro-OMe (*ent*-8). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (108 mg, 0.54 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-L-Val-Cl (213 mg, 0.60 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide ***ent*-8** (188 mg, 67%) as a 95/5 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 300 K : white solid; mp 58-62 °C; $[\alpha]^{23}_D$ +30.2 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{27}F_3N_2O_6Na$ 543.1713; Found 515.1710; HPLC-UV (Chiralpak IA) *rt* = 4.67 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-8** are similar to those of **8**.

Fmoc-D-Val-D-Ψ^{CF3,H}Pro-OMe (*ent*-4). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Val-Cl (594 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide ***ent*-4** (414 mg, 53%) as a 7/93 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 278 K : white solid; mp 114-117 °C; $[\alpha]^{23}_D$ +83.4 (*c* 1.0, $CHCl_3$); IR (neat) 3318, 2968, 1678, 1519, 1151, 735 cm^{-1};

HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{27}F_3N_2O_6Na$ 543.1713; Found 543.1709; HPLC-UV (Chiralpak IA) *rt* = 5.54 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-4** are similar to those of **4**.[10b]

Fmoc-L-Pro-L-Ψ^{CF3,H}Pro-OMe (5). The reaction was performed following the base free representative procedure starting from (2*R*)-**1a** pseudoprolines (544 mg, 2.73 mmol, 1.0 equiv) in dichloromethane (9 mL) and Fmoc-L-Pro-Cl (1.05 g, 2.96 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave the pure dipeptide **5** (1.22 g, 86%) as a *trans* rotational isomer in $CDCl_3$ at 300 K : white solid; mp 113-116 °C; R_f = 0.26 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{21}_D$ –56.1 (*c* 1.0, $CHCl_3$); Anal. Calcd for $C_{26}H_{25}F_3N_2O_6$: C, 60.23; H, 4.86; N, 5.40; Found: C, 60.22; H, 4.84; N, 5.51. HPLC-UV (Chiralpak IA) *rt* = 8.72 min (60:40, *n*-hexane/isopropanol); see Ref. 10b for spectral data of **5**.

Fmoc-D-Pro-L-Ψ^{CF3,H}Pro-OMe (9). The reaction was performed following the base free representative procedure starting from (2*S*)-**1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Pro-Cl (591 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave the pure dipeptide **9** (722 mg, 92%) as a 13/87 inseparable mixture of Fmoc *cis*/*trans* rotational isomer in $CDCl_3$ at 274 K : white solid; mp 62-65° C; R_f = 0.33 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{22}_D$ –78.0 (*c* 1.0, $CHCl_3$); IR (neat) 2958, 1754, 1688, 1421 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 274 K) : (*cis* Fmoc rotamer) δ 1.81 (m, 1 H, H_β Pro-Ha), 2.08 (m, 2 H, H_γ Pro-H), 2.10 (m, 1 H, H_β Pro-Hb), 3.45 (m, 1 H, H_δ Pro-Ha), 3.65 (m, 1 H, H_δ Pro-Hb), 3.78 (m, 1 H, $H_{\beta3}$ Ψpro-H), 3.80 (s, 3 H, OMe), 4.00 (m, 1 H, H_α Pro-H), 4.23 (m, 1 H, Fmoc CH), 4.33 (m, 1 H, $H_{\beta2}$ Ψpro-H), 4.40 (m, 1 H, Fmoc CH_2-Ha), 4.81 (m, 1 H, Fmoc CH_2-Hb), 5.50 (dd, *J* = 7.6, 5.3 Hz, 1 H, H_α Ψpro-H), 5.87 (q, *J* = 5.0 Hz, 1 H, H_δ Ψpro-H), 7.31 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.40 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.57 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.60 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.78 (d, *J* = 7.3 Hz, 2 H, Fmoc arom.); (*trans* Fmoc rotamer) δ 1.99 (m, 1 H, H_β Pro-Ha), 2.23 (m, 2 H, H_γ Pro-H), 2.33 (m, 1 H, H_β Pro-Hb), 3.60 (m, 1 H, H_δ Pro-Ha), 3.73 (m, 1 H, H_δ Pro-Hb), 3.82 (s, 3 H, OMe), 4.25 (t, *J* = 6.9 Hz, 1 H, Fmoc CH), 4.27 (dd, *J* = 10.0, 7.3 Hz, 1 H, Fmoc CH_2-Ha), 4.36 (dd, *J* = 10.0, 7.3 Hz, 1 H, Fmoc CH_2-Hb), 4.38 (m, 1 H, H_α Pro-H), 4.56 (m, 2 H, H_β Ψpro-H), 5.50 (dd, *J* = 7.6, 5.3 Hz, 1 H, H_α Ψpro-H), 6.03 (q, *J* = 5.0 Hz, 1 H, H_δ Ψpro-H), 7.31 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.40 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.56 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.60 (d, *J* = 7.3 Hz, 1 H, Fmoc arom.), 7.78 (d, *J* = 7.3 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 300 K) : (*cis* Fmoc rotamer) δ 23.3 (CH_2, C_β Pro), 31.8 (CH_2, C_γ Pro), 47.6 (CH_2, C_δ Pro), 47.6 (CH, Fmoc CH), 53.1 (CH_3, OMe), 57.3 (CH, C_α Pro), 57.9 (CH, C_α Ψpro), 66.4 (CH_2, Fmoc CH_2), 70.4 (CH_2, C_β Ψpro), 84.0 (q, *J* = 35.5 Hz, CH, C_δ Ψpro), 120.0 (2 × CH, Fmoc arom.), 122.5 (q, *J* = 284.7 Hz, CF_3), 125.0 (CH, Fmoc arom.), 125.1 (CH, Fmoc arom.), 127.2 (2 × CH, Fmoc arom.), 127.9 (2 × CH, Fmoc arom.), 141.2 (2 × C, Fmoc arom.), 143.6 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 153.6 (C, C=O), 168.8 (C, C=O), 173.1 (C, C=O); (*trans* Fmoc rotamer) δ 24.8 (CH_2, C_β Pro), 30.6 (CH_2, C_γ Pro), 46.9 (CH, Fmoc CH), 47.1 (CH_2, C_δ Pro), 53.0 (CH_3, OMe), 57.9 (CH, C_α Ψpro), 58.1 (CH, C_α Pro), 67.7 (CH_2, Fmoc CH_2), 70.4 (CH_2, C_β Ψpro), 84.0 (q, *J* = 35.5 Hz, CH, C_δ Ψpro), 120.0 (2 × CH, Fmoc arom.), 122.8 (q, *J* = 286.2 Hz, CF_3), 125.0 (CH, Fmoc arom.), 125.1 (CH, Fmoc arom.), 127.0 (2 × CH, Fmoc arom.), 127.7 (2 × CH, Fmoc arom.), 141.2 (2 × C, Fmoc arom.), 143.6 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 155.2 (C, C=O), 169.5 (C, C=O), 173.7 (C, C=O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) : (*cis* Fmoc and *trans* Fmoc rotamers) δ –82.8 (d, *J* = 5.5 Hz); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{25}F_3N_2O_6Na$ 541.1557; Found 541.1553; HPLC-UV (Lux Amylose-2) *rt* = 8.19 min (60:40, *n*-hexane/isopropanol).

Fmoc-L-Pro-D-Ψ^{CF3,H}Pro-OMe (*ent*-9). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (123 mg, 0.62 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-L-Pro-Cl (242 mg, 0.68 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide ***ent*-9** (87 mg, 27%) as a 13/87 inseparable mixture of Fmoc *cis*/*trans* rotational isomer in $CDCl_3$ at 274 K : white solid; mp 62-65° C; R_f = 0.33 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{22}_D$ +79.2 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{25}F_3N_2O_6Na$ 541.1557; Found 541.1553; HPLC-UV (Lux Amylose-2) *rt* = 7.40 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-9** are similar to those of **9**.

Fmoc-D-Pro-D- Ψ^{CF3,H}Pro-OMe (*ent*-5). The reaction was performed following the representative procedure starting from (2*R*)-***ent*-1a** pseudoproline (300 mg, 1.51 mmol, 1.0 equiv) in dichloromethane (5 mL) and Fmoc-D-Pro-Cl (591 mg, 1.66 mmol, 1.1 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide ***ent*-5** (414 mg, 53%) as a *trans* rotational isomers in $CDCl_3$ at 300 K : white solid; mp 113-116 °C; $[\alpha]^{21}_D$ +53.0 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{25}F_3N_2O_6Na$ 541.1557; Found 541.1554; HPLC-UV (Chiralpak IA) *rt* = 6.03 min (60:40, *n*-hexane/isopropanol); spectral data of ***ent*-5** are similar to those of **5**.[10b]

Fmoc-Aib-L-Ψ^{CF3,H}Pro-OMe (6). The reaction was performed following the base free representative procedure starting from a 84:16 diastereomeric mixture of (2*S*)-**1a** and (2*R*)-**1b** pseudoprolines (500 mg, 2.51 mmol, 1.0 equiv) in dichloromethane (7.5 mL) and Fmoc-Aib-Cl (2.59 g, 7.74 mmol, 3.0 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure dipeptide **6** (700 mg, 55%) as a 85/15 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 64-79 °C; R_f = 0.24 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{22}_D$ –35.5 (*c* 1.05, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{25}F_3N_2O_6Na$ 529.1557; Found 529.1554; HPLC-UV (Chiralpak IA) *rt* = 6.41 min (80:20, *n*-hexane/isopropanol); see Ref. 10b for spectral data of **6**.

Fmoc-Aib-D-Ψ^{CF3,H}Pro-OMe (*ent*-6). The reaction was performed following the representative procedure starting from pseudoproline (2*R*)-***ent*-1a** (508 mg, 2.55 mmol, 1.0 equiv) in dichloromethane (10 mL) and Fmoc-Aib-Cl (1.75 g, 5.1 mmol, 2.0 equiv). Purification by flash chromatography (70:60 cyclohexane/ethyl acetate) gave pure dipeptide ***ent*-6** (462 mg, 63%) as a 85/15 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K : white solid; mp 64-79 °C; $[\alpha]^{22}_D$ +36.5 (*c* 1.00, $CHCl_3$); HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{25}H_{25}F_3N_2O_6Na$ 529.1557; Found 529.1554; HPLC-UV (Chiralpak IA) *rt* = 6.75 min (80:20, *n*-hexane/isopropanol); spectral data of ***ent*-6** are similar to those of **6**.

ASSOCIATED CONTENT

Supporting Information

1D proton, 1D fluorine, and 1D carbon NMR spectra for the described compounds; 2D Roesy and CH_2-Trosy spectra of L-Val-L-Ψ^{CF3,H}Pro, D-Val-D-Ψ^{CF3,H}Pro, L-Val-D-Ψ^{CF3,H}Pro and D-Val-L-Ψ^{CF3,H}Pro; complete set of ^3J measurements, chiral HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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(13) Each enantiomer has been synthetized independently in a stereoselective way. Diastereomeric and enantiomeric purities have been confirmed by analytical chiral HPLC of the enantiomers and their co-mixture (see Experimental Section and Supporting Information). The chromatogram of **2** showed a slightly weaker 91% enantiomeric excess.

(14) For the dipeptides incorporating the Aib residue, CH_3 resonances have been used to discriminate *cis*/*trans* isomers.

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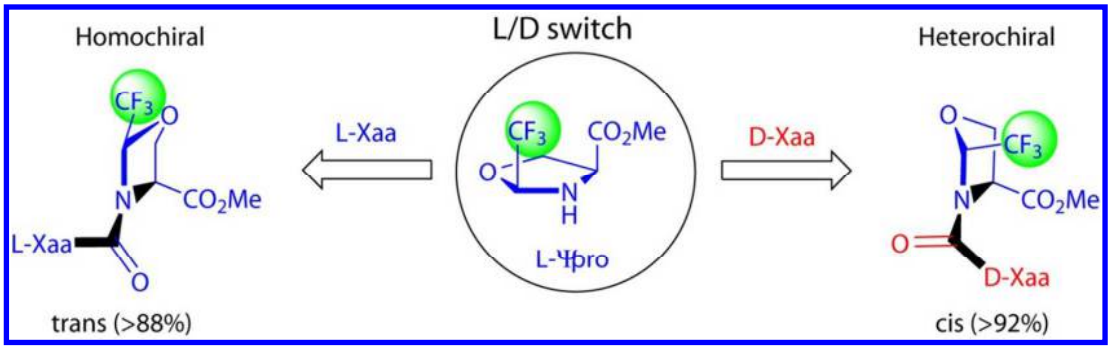
Homochiral
L/D switch
Heterochiral
L-Xaa
D-Xaa
CF3
CO2Me
L-Ψpro
D-Xaa
trans (>88%)
cis (>92%)