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Incorporation of CF_3–Pseudoprolines into Peptides: A Methodological Study

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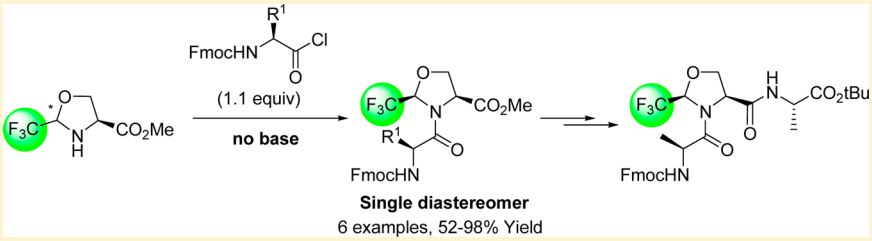
Supporting Information



ABSTRACT: The peptide coupling reactions allowing the incorporation of trifluoromethyl substituted oxazolidine-type pseudoprolines (CF_3-ΨPro) into peptide chains have been studied. While standard protocols can be used for the peptide coupling reaction at the *C*-terminal position of the CF_3-ΨPro, acid chloride activation has to be used for the peptide coupling reaction at the *N*-terminal position to overcome the decrease of nucleophilicity of the CF_3-ΨPro. We demonstrate that the *N*-amidification of a diastereomeric mixture of CF_3-ΨPro using Fmoc-protected amino acid chloride without base gave the corresponding dipeptides as a single diastereomer (6 examples). The ratio of the *cis* and *trans* amide bond conformers was determined by NMR study, highlighting the role of the Xaa side chains in the control of the peptide backbone conformation. Finally a tripeptide bearing a central CF_3-ΨPro has been successfully synthesized.

INTRODUCTION

Because of its cyclic structure, proline residue is recognized to play a unique and important role on the peptide backbone conformation. When included into a peptide, this feature prevents the proline from acting as a hydrogen bond donor and restrains the ϕ dihedral angle to about −60°.[1] The Xaa-Pro peptide bond is characterized by a small free energy difference (ΔG_{tc}) between the *trans* and the *cis* amide bond conformers, combined with a high activation energy ($\Delta G^{\ddagger}_{tc}$) for the *cis*–*trans* isomerization (Figure 1).[2−5] This isomerization is considered to play a key role for regulating many important biological processes in proline containing peptides and proteins, including the protein folding.[6−9] Numerous proline surrogates have been used to design peptides and peptidomimetics with defined conformation to improve their biological properties.[10−13] In this context, the use of pseudoprolines (ΨPro) appears as a very attractive approach.[14] In their pioneer works, Mutter et al. have shown that oxazolidine and thiazolidine derivatives could be obtained by the cyclocondensation of serine, threonine, and cysteine amino acids with aldehydes, ketones, and acetals.[15,16] C^{δ}-substituted pseudoprolines have gained in popularity because they exhibit a remarkable ability to induce *cis* amide bond conformations in peptide backbone.[17−20] While unsubstituted pseudoprolines are similar to proline and favor the *trans* amide bond conformation,[21,22] disubstitution at C^{δ} leads to a high *cis* content and a decrease of the $\Delta G^{\ddagger}_{tc}$.[17,18]

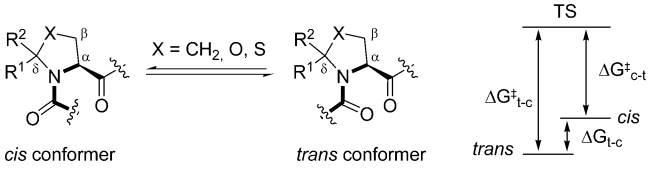


Figure 1. *cis*–*trans* conformer equilibrium in proline and pseudoprolines ($\Psi^{R1,R2}$Pro).

Therefore, pseudoprolines proved to be a valuable tool to tailor the *cis*–*trans* isomerization of the Xaa-ΨPro amide bonds for various applications in peptide engineering. The incorporation of pseudoprolines in small peptide chains is a powerful strategy to promote the head-to-tail cyclization.[23−27] Pseudoprolines are also broadly used in solid-phase peptide synthesis (SPPS) as temporary protecting groups to disrupt aggregate formation of the growing peptide chain and to significantly enhance the yield of the peptide synthesis.[28−34] Moreover, these proline surrogates are very useful tools for investigating the peptide bioactive conformations.[35−44] Despite these interesting features, pseudoprolines exhibit varied chemical

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stability under acidic conditions depending on the nature of the heteroatom and the degree of substitution at the C^{δ} carbon atom.[29] This drawback limits the use of oxazolidine-type pseudoprolines mostly as temporary protecting group in SPPS, while thiazolidine-type pseudoprolines can also be exploited for the synthesis of conformationally constrained peptides.

The introduction of fluorine atoms into biomolecules such as peptides is known to deeply influence their chemical and biological properties. It can provide a better affinity for lipid membranes, increased metabolic or thermal resistances, as well as improved autoassembly properties.[45−51] Fluorinated peptides can also be used as efficient probes for ^{19}F NMR studies.[52−56] However, regio- and stereoselective synthetic methods allowing the direct introduction of fluorine atoms or fluorinated groups into biomolecules are still challenging. Therefore, the development of syntheses of fluorinated biomolecules such as fluorinated amino acids has gained a considerable interest in peptide and protein chemistry.[57,58] Our group is focused on the stereoselective synthesis of trifluoromethylated amino acids (CF_3-AAs)[59−63] and their incorporation into peptides.[64] We have already reported the preparation of enantiomerically pure trifluoromethylated pseudoprolines (CF_3-ΨPro) and demonstrated that the stereoelectronic effects imparted by the trifluoromethyl group strongly increased the chemical stability of the oxazolidine core in acidic medium.[65] Accordingly, CF_3-substituted pseudoprolines behave as hydrolytically stable proline surrogates. We have also reported the electronic and geometric consequences due to the CF_3 group incorporation in CF_3-ΨPro containing tripeptide models **1** and **2** by theoretical calculations and NMR studies (Figure 2).[66,67] In particular, we have

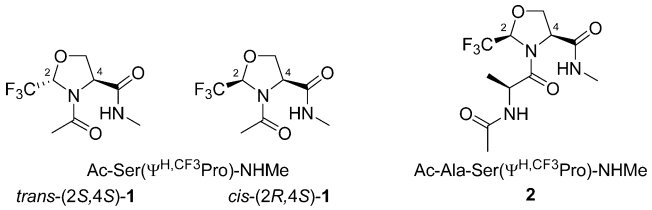


Figure 2. Structures of the CF_3-ΨPro containing tripeptide models.[66,67]

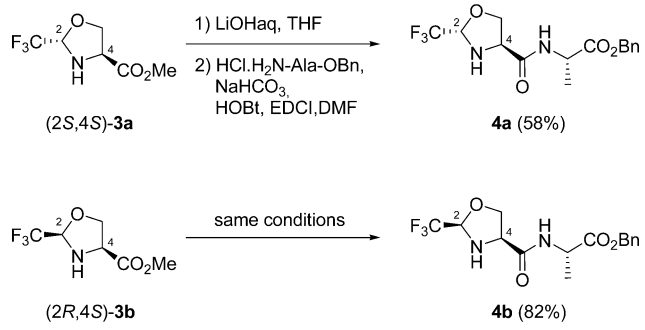
demonstrated that the incorporation of a Ser($\Psi^{CF3,H}$Pro) residue into a peptide chain can lower the rotational barrier of the *cis*−*trans* peptide bond isomerization, enhance the *cis* population ratio, freeze the puckering of the oxazolidine core and tune the peptide conformation depending of its absolute configuration and the solvent polarity.

Herein, we report a methodological study for the development of efficient coupling reactions allowing the incorporation of trifluoromethyl substituted oxazolidine-type pseudoprolines into peptide chains. We investigated the peptide coupling reaction at the *C*- and the *N*-termini of the CF_3-ΨPro residue. We also report the NMR study on five Fmoc-Xaa-Ser-($\Psi^{CF3,H}$Pro)-OMe derivatives highlighting the role of the Xaa side chains in the control of the peptide backbone conformation.

RESULTS AND DISCUSSION

Coupling Reaction at the *C*-Terminal Position: Synthesis of *N*-Terminal CF_3−Pseudoproline Containing Dipeptides. To demonstrate the incorporation of the Ser($\Psi^{CF3,H}$Pro) residue into peptide chains, we first investigated the peptide coupling reaction at its *C*-terminal position. Because of the decrease of the nucleophilicity of the nitrogen atom due to the CF_3 group electron-withdrawing effect, no protection of the pseudoproline amino group is required.[64] The syntheses of the *N*-terminal Ser($\Psi^{CF3,H}$Pro) containing dipeptides **4** were achieved using a two-steps procedure involving the saponification of the ester function followed by the amidification reaction with alanine benzyl ester using a standard coupling protocol. Starting from oxazolidines *trans*-(2*S*,4*S*)-**3a** and *cis*-(2*R*,4*S*)-**3b**, the dipeptides **4a** and **4b** were obtained in, respectively, 58 and 82% yields without diketopiperazine side product formation and without epimerization at the C-2 or the C-4 center (Scheme 1). As we already

Scheme 1. Synthesis of *N*-Terminal CF_3−Pseudoproline Containing Dipeptides 4



observed for similar amidification reactions,[65] the yield difference between **4a** and **4b** could be due to steric factors. These dipeptides, bearing a Ser($\Psi^{CF3,H}$Pro) residue at the *N*-terminal position, constitute new building blocks with an anticipated increased lipophilicity since the presence of the vicinal CF_3 group dramatically decreases the protonation ability of the nitrogen atom.

Coupling at the *N*-Terminal Position: Synthesis of *C*-Terminal CF_3−Pseudoproline Containing Dipeptides. The synthesis of *C*-terminal Ser($\Psi^{CF3,H}$Pro) containing dipeptides was then investigated. Because of the lack of nucleophilicity of the amino group of the oxazolidines **3**, the amide bond formation using amino acids activated as an ester, a symmetric anhydride[68] or a mixed anhydride[69] failed to give the expected dipeptides. In contrast, we previously reported the peptide coupling reaction of the *cis*-(2*R*,4*S*)-**3b** oxazolidine using large amounts (7 equiv) of *o*-Nbs-alanine chloride **5** in the presence of collidine according to a reported procedure.[70,71] The expected dipeptide **6** was obtained in 91% yield as a 73:27 mixture of two diastereomers (Table 1, entry 1).[65] The major diastereomer of **6** was isolated by flash chromatography and fully characterized by 2D NMR spectroscopy. The assignment of its absolute configuration was confirmed by X-ray crystallographic analysis.[67,72] At this stage, we were not able to unambiguously assign the absolute configuration of the minor diastereomer of **6**. When the reaction was performed starting from the *trans*-(2*S*,4*S*)-**3a** oxazolidine, the same major dipeptide **6** was obtained with a similar diastereomeric ratio indicating that an epimerization reaction occurred at the C-2 center of the oxazolidine ring (Table 1, entry 2). As mentioned previously for the *N*-benzoylation reactions of the CF_3-substituted oxazolidines,[65] this result suggests an epimerization reaction of the *trans*-(2*S*,4*S*)-**3a** oxazolidine into the *cis*-

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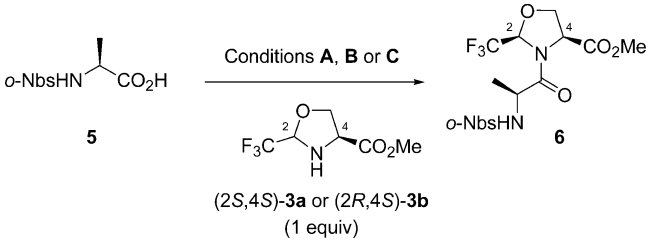
Table 1. Methodological Study of the Peptide Coupling Using *o*-Nbs-Alanine Chloride



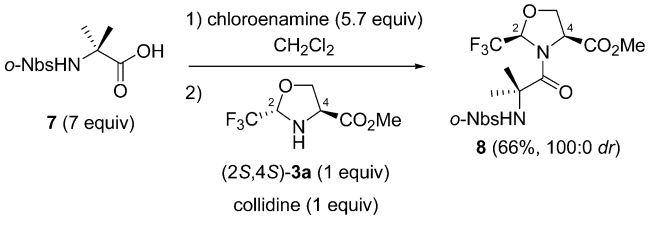
entry	conditions[a]	CF_3-ΨPro-OMe **3**	dipeptide **6**[b] (dr)[c]
1	**A**	(2*R*,4*S*)-**3b**	91% (73:27)[d]
2	**A**	(2*S*,4*S*)-**3a**	79% (85:15)
3	**B**	(2*S*,4*S*)-**3a**	86% (89:11)
4	**B**	(2*R*,4*S*)-**3b**	79% (82:18)
5	**C**	(2*S*,4*S*)-**3a**	78%, (87:13)
6	**C**	(2*R*,4*S*)-**3b**	87%, (90:10)

[a]Conditions **A**: **5** (7 equiv), 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (7 equiv), collidine (1 equiv), CH_2Cl_2. Conditions **B**: **5** (2 equiv), 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (2 equiv), CH_2Cl_2. Conditions **C**: **5** (2.2 equiv), $SOCl_2$ (3.3 equiv), TMU (0.44 equiv), collidine (1 equiv), CH_2Cl_2. [b]Isolated yield. [c]Measured by ^{19}F NMR of the crude reaction mixture. The dr represents the ratio of the major (2*R*,4*S*)-**6** diastereomer compared to a minor diastereomer which configuration was not assigned. [d]See ref 65.

(2*R*,4*S*)-**3b** oxazolidine under the reaction conditions. The *N*-coupling reactions of the *trans*-(2*S*,4*S*)-**3a** and the *cis*-(2*R*,4*S*)-**3b** oxazolidines occurred also using a smaller amount of *o*-Nbs-alanine chloride (2 equiv) without base to afford the dipeptide **6** in good yield (Table 1, entries 3 and 4). The presence or the absence of base for the coupling did not affect the epimerization level since the diastereomeric ratio remained almost identical in all cases. The coupling reactions of the *trans*-(2*S*,4*S*)-**3a** and the *cis*-(2*R*,4*S*)-**3b** oxazolidines with *o*-Nbs-alanine uronium in the presence of collidine gave the dipeptide **6** in similar ranges of yield and diastereomeric ratio (Table 1, entries 5 and 6).

The peptide coupling reaction of the *trans*-(2*S*,4*S*)-**3a** oxazolidine using the achiral protected amino isobutyric acid (Aib) chloride derived from **7** afforded the peptide **8** in moderate yield (66%) as a single diastereomer (Scheme 2). In

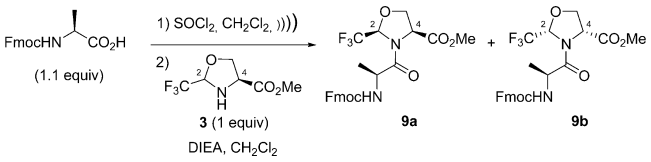
Scheme 2. Synthesis of *C*-Terminal CF_3−Pseudoproline Containing Dipeptide 8



accordance with the above results (see Table 1), the full NMR characterization of **8** revealed the relative (2,4)-*cis* configuration of the oxazolidine moiety indicating that the epimerization of the *trans*-(2*S*,4*S*)-**3a** oxazolidine into the *cis*-(2*R*,4*S*)-**3b** oxazolidine is total prior to the coupling reaction.

We then focused our attention on the N-amidification reactions of oxazolidines **3** with Fmoc-protected amino acid chloride prepared according to a reported procedure.[73] This approach allows the synthesis of *C*-terminal Ser(Ψ[CF3,H]Pro) containing dipeptides with a suitable protecting group for SPPS. We previously reported that the Fmoc-alanine chloride was electrophilic enough for the N-coupling of the (*R*)-(α-CF_3)Ala-L-Leu-OBn dipeptide to afford the corresponding Fmoc-Ala-(*R*)-(α-CF_3)Ala-L-Leu-OBn tripeptide in good yield.[64] We also recently reported that the amidification reaction of the *cis*-(2*R*,4*S*)-**3b** oxazolidine with the Fmoc-alanine chloride in the presence of diisopropylethylamine (DIEA) gave the corresponding dipeptides **9a** and **9b** in 90% yield as a 89:11 diastereomeric mixture (Table 2, entry 1).[67] As

Table 2. Methodological Study of the Peptide Coupling Using Fmoc-Alanine Chloride in Basic Conditions



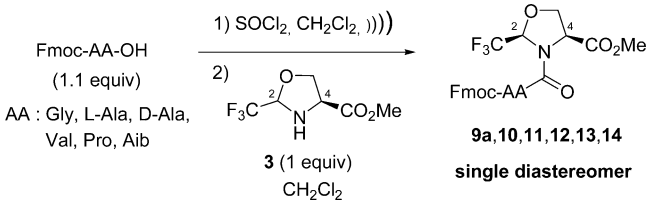
entry	CF_3-ΨPro-OMe **3**	DIEA (equiv)	dipeptides **9a**,**b**[a] (dr)[b]
1	(2*R*,4*S*)-**3b**	1	90% (89:11)[c]
2	(2*S*,4*S*)-**3a**	1	94% (80:20)
3	**3a**/**3b** (1:1)	1	92% (82:18)

[a]Isolated yield. [b]Measured by ^{19}F NMR of the crude reaction mixture. [c]Ref 67.

observed with *o*-Nbs protected amino acids, the reaction led to the same diastereomeric mixture of dipeptides **9a** and **9b** in high yield, whatever was the C-2 absolute configuration of the starting oxazolidines **3** (Table 2, entries 2 and 3).

It was anticipated that basic reaction conditions would be responsible for the C-4 epimerization reaction. Indeed, when the reaction of a diastereomeric mixture of oxazolidines **3** was performed without base, the dipeptide **9a** was obtained as a single diastereomer in 66% yield (Table 3, entry 1). It should

Table 3. Synthesis of *C*-Terminal Tfm-Pseudoproline Containing Dipeptides 9a−14 without Base



entry	Fmoc-AA-OH	CF_3-ΨPro-OMe **3**	dipeptide	yield (%)[a]
1	L-Ala	**3a**/**3b** (84:16)	**9a**	66
2	Gly	**3a**/**3b** (84:16)	**10**	98
3	L-Val	**3a**/**3b** (84:16)	**11**	59
4	L-Pro	**3a**/**3b** (84:16)	**12**	52
5	Aib	**3a**/**3b** (84:16)	**13**	55[b]
6	D-Ala	**3a**/**3b** (100:0)	**14**	79

[a]Isolated yield. [b]3 equiv of Fmoc-Aib-OH were used.

be stressed that these conditions constitute a great improvement since only 1.1 equiv of Fmoc-protected amino acids is required and the only side product of the reaction is HCl.

These optimized conditions were successfully applied to coupling reaction of various Fmoc-protected amino acid chlorides with Tfm-pseudoprolines **3**. The dipeptides **10**, **11**,

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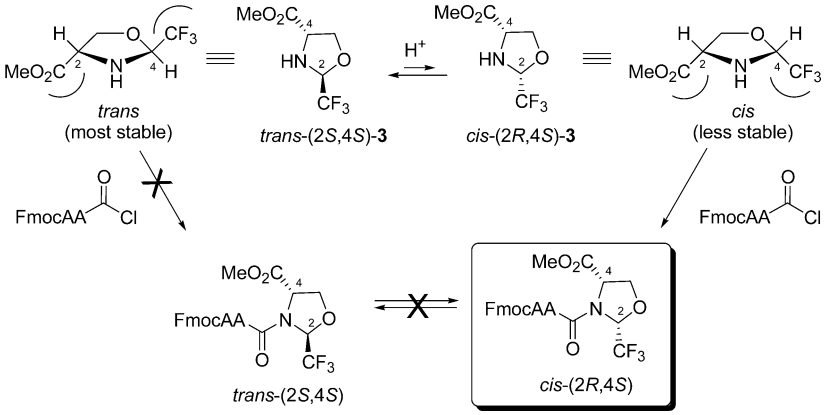
Scheme 3. Mechanism of the *N*-Acylation Reaction without Base

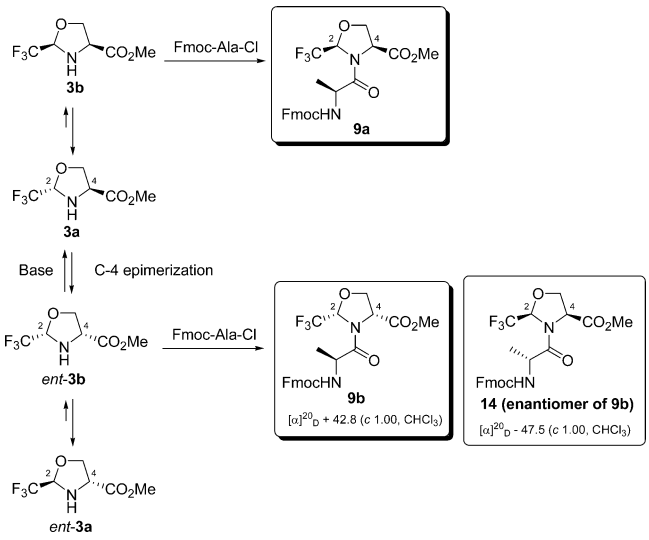


12, **13** and **14** derived respectively from glycine, valine, proline, aminoisobutyric acid (Aib) and D-alanine were obtained as a single diastereomer (Table 3, entries 2−6). The yield of the reaction decreased with the steric hindrance of the Fmoc-protected amino acid chloride. Larger amount (3 equiv) of the amino acid chloride was required in the case of Aib (Table 3, entry 4).

Configuration Assignments and Epimerization Explanation. The assignment of the relative configuration of the dipeptides **9a**−**14** was then investigated by NMR NOE analysis. When the coupling reactions were carried out without base, the exclusive formation of the *cis*-(2*R*,4*S*)-oxazolidine containing dipeptides was observed. This result is similar to those reported in the literature.[74,75] While in the *N*-unacetylated series the (2,4)-*trans* oxazolidine is found to be more stable than the (2,4)-*cis* isomer, the (2,4)-*cis*-*N*-acylated oxazolidines are favored compared to the corresponding (2,4)-*trans*-*N*-acylated isomers. This preferred (2,4)-*cis* relative configuration of *N*-acylated oxazolidine moieties is due to the all *trans* configuration minimizing the steric interactions (Scheme 3). The approach of the *cis*-oxazolidine amino group by the acylating reagent is easy from the opposite side of the sterically hindered face, while for the *trans*-oxazolidine, both sides are hindered. Because of the complete diastereoselectivity observed for the peptide coupling reactions without base, we can argue that the acid-catalyzed equilibrium between the (2,4)-*cis* and the (2,4)-*trans* oxazolidines **3** before the amide bond formation is critical for this outcome. The interconversion of the *trans*-(2*S*,4*S*)-**3** to the *cis*-(2*R*,4*S*)-**3** oxazolidine ring should proceed through an acidic promoted ring-opening/ring-closing process involving a protonated hydroxyimine intermediate. As known for *N*-acylated oxazolidine compounds, there is no equilibrium between the *cis*-(2*R*,4*S*)-oxazolidine containing dipeptides obtained and their *trans*-(2*S*,4*S*)- diastereomers.

When the coupling reaction of the *cis*-(2*R*,4*S*)-**3b** oxazolidine with the Fmoc-alanine chloride was carried out in the presence of base, the corresponding dipeptide was obtained as a mixture of two diastereomers **9a** and **9b** (see Table 2). The use of basic reaction conditions would be responsible for the epimerization reaction. The formation of the *trans*-oxazolidine containing dipeptide resulting from the C-2 epimerization of the oxazolidines ring was not considered because of the great steric hindrance of this position. However, it was anticipated that the epimerization reaction could occur either at the C-4 of the oxazolidine or at the C$^{\alpha}$ of the Ala residue. To discriminate these hypotheses, the coupling reaction of the oxazolidines **3** was performed using the Fmoc-D-alanine chloride applying our optimized conditions (Table 3, entry 6). The corresponding peptide **14** was obtained in 79% yield as a single diastereomer. Since the optical rotation value of **14** was opposite to that of the minor diastereomer **9b** and since their ^{1}H NMR spectra were indentical, **14** and **9b** should be enantiomers, and we assigned the (2*S*,4*R*)-configuration to the minor diastereomer **9b** (Scheme 4). We concluded that the amidification of Tfm-

Scheme 4. Configuration Assignments of the Minor Diastereomer of Dipeptide 9b



pseudoprolines in the presence of base led to the partial epimerization of the C-4 center. For the reasons exposed above (Scheme 3), the epimerization of the C-4 center prompted the epimerization of the C-2 center prior to the coupling and only the *cis*-(2*S*,4*R*)-oxazolidine **3b** or its *cis*-(2*R*,4*S*)-*ent*-**3b** enantiomer were *N*-acylated (Scheme 4).

Analysis of the *cis*−*trans* Amide Bond Conformer Ratio. The ratio of the *cis* and *trans* amide bond conformers was determined for the dipeptides **9a**, **10**, **11**, **12** and **13** at 274 K in $CDCl_3$ by integration of both ^{1}H and ^{19}F NMR isolated conformer resonances (Table 4). The Xaa-ΨPro amide bond conformation was assigned using inter-residue NOE correlations in the 2D NMR experiments. We observed a strong H^{α}_{Xaa}−$H^{\delta}_{\Psi Pro}$ cross peak for the unique spin system of peptide

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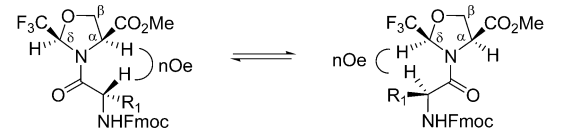
Table 4. *cis*–*trans* Ratio for Dipeptides 9a–13



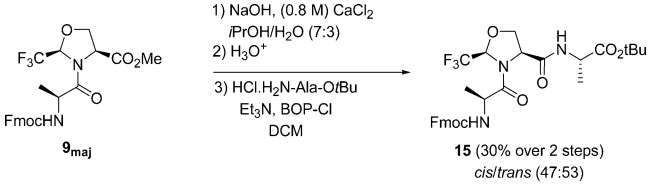
entry	AA	compound	*cis*/*trans*[a]
1	L-Pro	**12**	0/100
2	L-Val	**11**	7/93
3	L-Ala	**9a**	12/88[b]
4	Gly	**10**	61/39
5	Aib	**13**	85/15

[a]Measured by ^{1}H and ^{19}F NMR at 274 K in $CDCl_3$. [b]Ref 67.

12 (Xaa = Pro) in agreement with a *trans* amide bond conformation (Table 4, entry 1). The dipeptides **9a**, **10**, **11** and **13** showed an additional H^{α}_{Xaa}–$H^{\alpha}_{\Psi Pro}$ correlation, indicating the presence of a stabilized *cis* conformation. The dipeptides **9a** and **11**, bearing respectively the alanine and the valine residues, displayed a high proportion of the *trans* population, which seemed to be closely related with the steric hindrance of the amino acid side chain (Table 4, entries 2 and 3). The dipeptide **10**, bearing the unsubstitued glycine residue, exhibited a high proportion of *cis* conformer (61%, see Table 4, entry 4). This level was quite similar with the described disubstituted thiazolidine containing dipeptide Cbz-Gly-Thr($\Psi^{Me,Me}$Pro)-OMe (75% *cis*).[76] We suppose that the presence of the bulky Fmoc group could be responsible for the destabilization of the *cis* conformer because the *o*-Nbs *N*-protected peptide **6** showed a (55:45) isomeric ratio.[67] The incorporation of the Aib residue increased significantly the ratio of the *cis* conformer (85%, Table 4, entry 5) indicating that the *trans* isomer is destabilized by the presence of the methyl group substituting the H^{α} of the Ala residue.

Synthesis of a CF_3–Pseudoproline Containing Tripeptide. To demonstrate the potential incorporation of CF_3-pseudoprolines into peptide chains, a CF_3-pseudoproline containing tripeptide, bearing the Ser($\Psi^{H,CF3}$Pro) residue at the central position, was synthesized. To circumvent the risk of epimerization at both the C-2 and the C-4 centers of the Ser($\Psi^{H,CF3}$Pro) moiety, we adopted the unusual *N*-terminal to *C*-terminal peptide elongation starting from the diastereomerically pure Fmoc-Ala-Ser($\Psi^{H,CF3}$Pro)-OMe dipeptide **9a**. Treatment of **9a** under smooth conditions with a 0.8 M NaOH solution in the presence of $CaCl_2$ allowed the ester hydrolysis without traces of Fmoc deprotection.[77] Acidic treatment of the crude followed by the coupling of the L-alanine *tert*-butyl ester using BOP-Cl afforded the tripeptide **15** in moderate yield (Scheme 5). A 47:53 *cis*/*trans* population ratio was observed by ^{1}H NMR contrasting with the 12:88 ratio obtained for **9a**. The very low field resonance of the *C*-terminal alanine NH amide proton (δ_{NH} = 8.40 ppm) for the minor conformer suggested its involvement in a strong hydrogen bond, which could account for the stabilization of the *cis* Ala-Ser($\Psi^{H,CF3}$Pro) peptide bond in peptide **15**. As already observed with the pseudotetrapeptide Ac-Ala-Ser($\Psi^{CF3,H}$Pro)-NHMe, we anticipated the existence of a type VI β-turn conformation for the minor conformer of **15**.[67]

Scheme 5. Synthesis of the Tripeptide 15



CONCLUSION

Trifluoromethyl-group containing pseudoprolines Ser($\Psi^{H,CF3}$Pro) have been successfully incorporated into a peptide chain at both *N*- and *C*-termini. The coupling reactions at the *C*-terminus can be performed using standard protocols. The more challenging N-amidification reaction required specific coupling conditions to circumvent the lack of nucleophilicity of the Ser($\Psi^{CF3,H}$Pro) nitrogen atom and to prevent the epimerization of the Ser($\Psi^{H,CF3}$Pro) residue at the C-4 center. The use of Fmoc-protected amino acid chlorides without base allowed efficient coupling to afford several Fmoc-Xaa-Ser($\Psi^{CF3,H}$Pro)-OMe dipeptides as single diastereomers in good yield. Conformational studies by NMR spectroscopy revealed that the geometry of the amide bond was depending on both the nature of the side chain of the preceding amino acids. Finally, we synthesized a tripeptide bearing the Ser($\Psi^{H,CF3}$Pro) residue at the central position suitably protected and ready to be used in solid phase peptide synthesis.

EXPERIMENTAL SECTION

General 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. THF was distilled over sodium benzophenone ketyl 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 wave numbers are given in cm^{-1}. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were recorded in $CDCl_3$ (unless otherwise stated). ^{1}H NMR (400.00 MHz), ^{13}C NMR (100.50 MHz) and ^{19}F NMR (376.20 MHz) were measured on a spectrometer operating at a ^{1}H frequency of 400 MHz. ^{1}H NMR (500.00 MHz), ^{13}C NMR (125.75 MHz) were measured on a spectrometer operating at a ^{1}H frequency of 500 MHz and equipped with a triple resonance, *z*-axis pulsed-5 field-gradient cryogenic probehead, optimized for ^{1}H detection. Complete proton assignments were obtained from the analysis of 2D total correlation spectroscopy (TOCSY) experiments using 80 ms DIPSI-2 mixtime, and 2D nuclear Overhauser effect spectroscopy (NOESY) experiments (typically 500 ms mixing time). Homonuclear experiments were typically collected as 512 (t1) and 4096 (t2) time-domain matrices over a spectral width of 10 ppm, with 8 scans per t1 increment. Carbon assignment was deduced from heteronuclear 2D ^{1}H–^{13}C HSQC and 2D ^{1}H–^{13}C CH2-TROSY16 experiments, using 256 (t1) × 1024 (t2) timedomain matrices, with 32 scans per t1 increment. Chemical shifts of ^{1}H NMR are expressed in parts per million downfield from tetramethylsilane (δ = 0) in $CDCl_3$. Chemical shifts of ^{13}C NMR are expressed in parts per million downfield from $CDCl_3$ as internal standard (δ = 77.0). Chemical shifts of ^{19}F NMR are expressed in parts per million downfield from C_6F_6 as an internal standard (δ = −164.9). Coupling constants are reported in Hertz. Melting points were uncorrected. High-resolution mass spectra were obtained using electrospray ionization in positive ion mode and a TOF mass analyzer. Mass

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spectra were recorded using electrospray ionization in positive ion mode and a quadrupole mass analyzer.

Synthesis of *N*-Terminal CF_3−Pseudoproline Containing Dipeptides. *Representative Procedure for the Peptide Coupling Reaction at the C-Terminal Position.* To a solution of oxazolidine **3a** or **3b** (1 equiv) in THF at 0 °C was added a 1 M aqueous solution of LiOH (1.1 equiv). The reaction mixture was vigorously stirred for 4 h. Subsequently, Et_2O was added, and the reaction mixture was extracted with water (3×). The aqueous layers were combined, and water was removed under reduced pressure to give the corresponding lithium carboxylate, which was directly used without further purification.[65] The lithium carboxylate was diluted in DMF, and L-alanine benzylester hydrochloride (1.1 equiv), HOBt (1.0 equiv), $NaHCO_3$ (3.0 equiv) and EDCI (1.1 equiv) were successively added at room temperature. The reaction mixture was stirred overnight at room temperature and then diluted with AcOEt and water. The layers were separated, and the aqueous layer was extracted with AcOEt (3×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography gave dipeptides **4a** and **4b** in 58 and 82% yield, respectively.

*(2S,4S)-H-Ser(Ψ^{CF3,H}Pro)-Ala-OBn (**4a**).* The dipeptide **4a** was prepared according to the representative procedure. The lithium carboxylate was prepared starting from oxazolidine (2*S*,*S*)-**3a** (796 mg, 4.0 mmol) in THF (22 mL), 1 M aqueous solution of LiOH (4.4 mL, 4.4 mmol, 1.1 equiv) as a yellow oil. The dipeptide **4a** was obtained starting from the crude lithium carboxylate in DMF (10.5 mL), L-alanine benzylester hydrochloride (951 mg, 1.1 equiv, 4.4 mmol), HOBt (541 mg, 1.0 equiv, 4.0 mmol), $NaHCO_3$ (1.00 g, 3.0 equiv, 12.0 mmol) and EDCI (844 mg, 1.1 equiv, 4.4 mmol). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the dipeptide **4a** (800 mg, 58%) as a white solid: mp 98−100 °C; R_f = 0.35 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{26}_D$ −37.1 (*c* 1.8, $CHCl_3$); IR (neat) 3308, 2977, 1730, 1654, 1549, 1455, 1143, 692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42 (d, *J* = 7.3 Hz, 3 H, H_β Ala-H), 3.28 (t, *J* = 8.2 Hz, 1 H, NH ΨPro), 3.83 (dd, *J* = 7.8, 6.9 Hz, 1 H, H_β ΨPro-Ha), 3.98 (ddd, *J* = 8.2, 7.8, 6.9 Hz, 1 H, H_α ΨPro-H), 4.19 (t, *J* = 7.8 Hz, 1 H, H_β ΨPro-Hb), 4.63 (quint, *J* = 7.3 Hz, 1 H, H_α Ala-H), 4.99 (dq, *J* = 8.2, 5.5 Hz, 1 H, H_δ ΨPro-H), 5.14 (d, *J* = 12.4 Hz, 1 H, Bn CH_2−Ha), 5.19 (d, *J* = 12.4 Hz, 1 H, Bn CH_2−Hb), 6.95 (d, *J* = 7.3 Hz, 1 H, NH Ala), 7.28−7.42 (m, 5 H, Bn arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 18.2 (CH_3, C_β Ala), 48.3 (CH, C_α Ala), 59.3 (CH, C_α ΨPro), 67.4 (CH_2, Bn CH_2), 70.2 (CH_2, C_β ΨPro), 88.0 (q, *J* = 33.6 Hz, CH, C_δ ΨPro), 123.0 (q, *J* = 282.7 Hz, CF_3), 128.2 (2 × CH, Bn arom.), 128.5 (CH, Bn arom.), 128.6 (2 × CH, Bn arom.), 135.0 (C, Bn arom.), 169.4 (C, C═O), 172.3 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ −84.6 (d, *J* = 5.5 Hz, CF_3); EIMS *m*/*z* M^+ 346, 277, 211, 164, 140, 112, 91 (100); HRMS (ESI-TOF) calcd for $C_{15}H_{17}F_3N_2O_4$ 346.1140, found 346.1134.

*(2R,4S)-H-Ser(Ψ^{CF3,H}Pro)-Ala-OBn (**4b**).* The dipeptide **4b** was prepared according to the representative procedure. The lithium carboxylate was prepared starting from oxazolidine (2*R*,4*S*)-**3b** (792 mg, 4.0 mmol) in THF (22 mL), 1 M aqueous solution of LiOH (4.4 mL, 4.4 mmol, 1.1 equiv) as a yellow oil. The dipeptide **4b** was obtained starting from the crude lithium carboxylate in DMF (10.5 mL), L-alanine benzylester hydrochloride (970 mg, 1.1 equiv, 4.4 mmol), HOBt (554 mg, 1.0 equiv, 4.0 mmol), $NaHCO_3$ (1.00 g, 3.0 equiv, 12.0 mmol) and EDCI (876 mg, 1.1 equiv, 4.4 mmol). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the dipeptide **4b** (1.18 g, 82%) as a colorless solid: mp 77−81 °C; R_f = 0.15 (60:40 cyclohexane/ethyl acetate); $[\alpha]^{27}_D$ −14.6 (*c* 2.25, $CHCl_3$); IR (neat) 3309, 2908, 1745, 1654, 1526, 1450, 1148, 1131, 804, 749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, *J* = 7.3 Hz, 3 H, H_β Ala-H), 3.25 (m, 1 H, NH ΨPro), 4.14 (dd, *J* = 8.0, 5.7 Hz, 1 H, H_β ΨPro-Ha), 4.22−4.31 (m, 2 H, H_α ΨPro-H, H_β ΨPro-Hb), 4.64 (dq, *J* = 8.0, 7.3 Hz, 1 H, H_α Ala-H), 5.04 (q, *J* = 5.5 Hz, 1 H, H_δ ΨPro-H), 5.17 (d, *J* = 12.4 Hz, 1 H, Bn CH_2−Ha), 5.20 (d, *J* = 12.4 Hz, 1 H, Bn CH_2−Hb), 7.32−7.41 (m, 5 H, Bn arom.), 7.63 (d, *J* = 8.0 Hz, 1 H, NH Ala); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 18.1 (CH_3, C_β Ala), 47.7 (CH, C_α Ala), 59.3 (CH, C_α ΨPro), 67.1 (CH_2, Bn CH_2), 70.3 (CH_2, C_β ΨPro), 87.6 (q, *J* = 34.5 Hz, CH, C_δ ΨPro), 123.2 (q, *J* = 283.7 Hz, CF_3), 128.1 (2 × CH, Bn arom.), 128.4 (CH, Bn arom.), 128.6 (2 × CH, Bn arom.), 135.3 (C, Bn arom.), 170.4 (C, C═O), 172.5 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ −83.8 (d, *J* = 5.5 Hz, CF_3); EIMS *m*/*z* M^+ 346, 277, 211, 164, 140, 112, 91 (100); HRMS (ESI-TOF) calcd for $C_{15}H_{17}F_3N_2O_4$ 346.1140, found 346.1154.

Synthesis of *C*-Terminal CF_3−Pseudoproline Containing Dipeptides. Synthesis of *o*-Nbs Protected *C*-Terminal Dipeptides (6 and 8). *Representative Procedure for the Peptide Coupling Reaction Using o-Nbs-Amino Acid Chloride. Method A.* To a solution of the *o*-Nbs-amino acid **5** or **7** (7 equiv) suspended in dichloromethane under argon was added 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (7 equiv) at 0 °C. The resulting solution was stirred at 0 °C until the disappearance of the precipitate (usually 20 min). The total conversion of the acid to chloride was checked by TLC after quenching with methanol. The resulting *o*-Nbs-amino acid chloride solution was added via cannula to a neat mixture of pseudoproline **3a** or **3b** (1.0 equiv) and collidine (1.0 equiv) at 0 °C. The temperature was allowed to warm to room temperature, and the solution was concentrated twice using a stream of argon. After 24 h, the resulting mixture was diluted with dichloromethane and quenched with a saturated aqueous solution of $NaHCO_3$. The layers were separated, and aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography gave dipeptides **6**[65] and **8** in 79 and 66% yield, respectively.

Method B. To a solution of the *o*-Nbs-alanine **5** (2 equiv) suspended in dichloromethane under argon was added 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (2 equiv) at 0 °C. The resulting solution was stirred at 0 °C until the disappearance of the precipitate (usually 20 min). The total conversion of the acid to chloride was checked by TLC after quenching with methanol. The resulting *o*-Nbs-alanine chloride solution was added via cannula to neat pseudoproline **3a** or **3b** (1.0 equiv) at 0 °C. The temperature was allowed to warm to room temperature, and the solution was concentrated twice using a stream of argon. After 24 h, the resulting mixture was diluted with dichloromethane and quenched with a saturated aqueous solution of $NaHCO_3$. The layers were separated, and aqueous layer was extracted with dichloromethane. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography gave dipeptide **6**[65] in 79−86% yield.

Method C. To a solution of *o*-Nbs-alanine **5** (2.2 equiv) in THF at 0 °C under argon was added dropwise freshly distilled $SOCl_2$ (3.3 equiv) and TMU (0.44 equiv). The resulting mixture was stirred for 2 h at 0 °C, and then temperature was allowed to warm to room temperature, and solvent was removed under a vacuum. The corresponding *o*-Nbs-alanine chloride was directly used in the peptidic coupling reaction without further purification. A solution of pseudoproline **3a** or **3b** (1.0 equiv) and collidine (1.0 equiv) in dichloromethane was added at 0 °C via cannula to the freshly prepared *o*-Nbs-alanine chloride (neat). The resulting mixture was stirred overnight at room temperature, diluted with dichloromethane, and quenched with a saturated aqueous solution of $NaHCO_3$. The layers were separated, and aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography gave dipeptide **6**[65] in 78−87% yield.

*o-Nbs-Aib-Ser(Ψ^{CF3,H}Pro)-OMe (**8**).* The dipeptide **8** was prepared according to the method A starting from *o*-Nbs-2-methylalanine **7** (2.02 g, 7.0 mmol, 7.0 equiv) in dichloromethane (7 mL), 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine (750 μL, 5.67 mmol, 5.7 equiv), pseudoproline (2*S*,4*S*)-**3a** (260 mg, 1.0 mmol, 1.0 equiv) and collidine (133 μL, 1.0 mmol, 1.0 equiv). The crude was purified by flash chromatography (70:30 cyclohexane/ethyl acetate) to give the dipeptide **8** (310 mg, 66%) as 100% *cis* rotational isomer in $CDCl_3$ at 273 K: pale yellow solid; mp 62−63 °C; R_f = 0.68 (60:40 petroleum ether/ethyl acetate); $[\alpha]^{22}_D$ +77.4 (*c* 1.02, $CHCl_3$); IR (neat) 3338, 2957, 1743, 1677, 1539, 1175, 1145 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 298 K) (single rotational isomer) δ 1.45 (s, 3 H, H_β Aib-H),

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1.60 (s, 3 H, H_β Aib-H), 3.81(s, 3 H, OMe), 4.41 (t, J = 7.8 Hz, 1 H, H_β ΨPro-Ha), 4.63 (dd, J = 7.8, 2.8 Hz, 1 H, H_β ΨPro-Hb), 5.84 (dd, 1 H, J = 7.8, 2.8 Hz, H_α ΨPro-H), 6.00 (s, 1 H, NH Aib), 6.05 (q, J = 4.6 Hz, 1 H, H_δ ΨPro-H), 7.79−7.84 (m, 2 H, *o*-Nbs arom.), 7.96 (m, 1 H, *o*-Nbs arom.), 8.18 (m, 1 H, *o*-Nbs arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (single rotational isomer) δ 26.6 (CH_3, C_β Aib), 27.4 (CH_3, C_β Aib), 52.9 (CH_3, OMe), 58.8 (CH, C_α ΨPro), 61.4 (C, C_α Aib), 70.3 (CH_2, C_β ΨPro), 86.1 (q, J = 36.4 Hz, CH, C_δ ΨPro), 122.4 (q, J = 285.6 Hz, CF_3), 125.8 (CH, *o*-Nbs arom.), 131.1 (CH, *o*-Nbs arom.), 133.6 (CH, *o*-Nbs arom.), 134.2 (CH, *o*-Nbs arom.), 135.4 (C, *o*-Nbs arom.), 147.6 (C, *o*-Nbs arom.), 169.6 (C, C═O), 171.6 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) (single rotational isomer) δ −80.8 (d, J = 4.6 Hz, CF_3); ESIMS m/z 492.17 $[M + Na]^+$, 470.19 $[M + H]^+$; HRMS (ESI-TOF) calcd for $C_{16}H_{18}F_3N_3O_8SNa$ 492.0664, found 492.0659.

Synthesis of Fmoc-Protected *C*-Terminal Dipeptides (9−14). *Representative Procedure for the Preparation of Fmoc-Aminoacid Chloride Assisted by Ultrasonication.*[73] 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 room temperature until the complete disappearence of the precipitate (from 30 min to 1 h), and 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.

DIEA Representative Procedure for Peptide Coupling Reaction. To a solution of pseudoprolines **3** (1.0 equiv) in dichloromethane was added DIEA (1.0 equiv). The resulting mixture was added via cannula to the freshly prepared Fmoc-amino acid chloride solid (1.1 equiv). The reaction mixture was stirred for 24 h at room temperature, diluted with dichloromethane, and washed with 1 M aqueous solution of HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography gave a diastereomeric mixture of dipeptides **9a** and **9b**[67] in 90−94% yield.

Base-Free Representative Procedure for the Peptide Coupling Reaction. To a solution of pseudoprolines **3** (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, and then the solvent was evaporated under reduced pressure. Purification by flash chromatography gave pure dipeptides **9a**−**14** in 52−98% yield.

Fmoc-Gly-L-Ser(Ψ^{CF3,H}Pro)-OMe (**10**). The reaction was performed following the base-free representative procedure starting from pseudoproline (2*S*,4*S*)-**3a** (256 mg, 1.29 mmol, 1.0 equiv) in dichloromethane (4 mL) and Fmoc-Gly-Cl (446 mg, 1.42 mmol, 1.1 equiv). Purification by flash chromatography (60:40 cyclohexane/ethyl acetate) gave the pure dipeptide **10** (604 mg, 98%) as a 61/39 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K: white solid; mp 88−92 °C; R_f = 0.24 (60:40 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ −36.0 (*c* 1.06, $CHCl_3$); IR (neat) 3338, 2955, 1686, 1518, 1150, 727 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 274 K) (*trans* rotamer) δ 3.79 (s, 3 H, OMe), 4.00 (m, 1 H, H_α Gly-Ha), 4.20 (m, 1 H, H_α Gly-Hb), 4.23 (t, J = 7.2 Hz, 1 H, Fmoc CH), 4.39 (d, J = 7.2 Hz, 2 H, Fmoc CH_2), 4.48−4.54 (m, 2 H, H_β ΨPro-H), 5.05 (t, J = 7.9 Hz, 1 H, H_α ΨPro-H), 5.67 (q, J = 4.7 Hz, 1 H, H_δ ΨPro-H), 5.70−5.75 (m, 1 H, NH Gly), 7.32 (t, J = 7.6 Hz, 2 H, Fmoc arom.), 7.42 (t, J = 7.4 Hz, 2 H, Fmoc arom.), 7.60 (d, J = 7.4 Hz, 2 H, Fmoc arom.), 7.78 (d, J = 7.6 Hz, 2 H, Fmoc arom.); (*cis* rotamer) δ 3.82 (s, 3 H, OMe), 4.05 (m, 1 H, H_α Gly-Ha), 4.22 (m, 1 H, H_α Gly-Hb), 4.23 (t, J = 7.2 Hz, 1 H, Fmoc CH), 4.39 (d, J = 7.2 Hz, 2 H, Fmoc CH_2), 4.54 (d, J = 6.8 Hz, 2 H, H_β ΨPro-H), 4.80 (t, J = 6.8 Hz, 1 H, H_α ΨPro-H), 5.70−5.75 (m, 1 H, NH Gly), 5.97 (q, J = 5.0 Hz, 1 H, H_δ ΨPro-H), 7.32 (t, J = 7.6 Hz, 2 H, Fmoc arom.), 7.42 (t, J = 7.4 Hz, 2 H, Fmoc arom.), 7.60 (d, J = 7.4 Hz, 2 H, Fmoc arom.), 7.78 (d, J = 7.6 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ 42.8 (CH_2, C_α Gly), 46.2 (CH, Fmoc CH), 52.4 (CH_3, OMe), 56.3 (CH, C_α ΨPro), 66.8 (CH_2, Fmoc CH_2), 68.2 (CH_2, C_β ΨPro), 83.6 (q, J = 36.4 Hz, CH, C_δ ΨPro), 119.4 (2 × CH, Fmoc arom.), 121.8 (q, J = 286.6 Hz, CF_3), 124.9 (2 × CH, Fmoc arom.), 126.9 (2 × CH, Fmoc arom.), 127.6 (2 × CH, Fmoc arom.), 140.6 (2 × C, Fmoc arom.), 143.0 (2 × C, Fmoc arom.), 155.7 (C, C═O), 167.2 (C, C═O), 167.9 (C, C═O); (*cis* rotamer) δ 42.5 (CH_2, C_α Gly), 46.3 (CH, Fmoc CH), 53.0 (CH_3, OMe), 56.1 (CH, C_α ΨPro), 66.7 (CH_2, Fmoc CH_2), 69.9 (CH_2, C_β ΨPro), 83.7 (q, J = 36.4 Hz, CH, C_δ ΨPro), 119.4 (2 × CH, Fmoc arom.), 121.8 (q, J = 286.6 Hz, CF_3), 124.5 (2 × CH, Fmoc arom.), 126.4 (2 × CH, Fmoc arom.), 127.1 (2 × CH, Fmoc arom.), 140.6 (2 × C, Fmoc arom.), 143.0 (2× CH, Fmoc arom.), 155.6 (C, C═O), 167.8 (C, C═O), 167.9 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ −81.8 (s, CF_3); (*cis* rotamer) δ −82.2 (s, CF_3); ESIMS m/z 501.18 $[M + Na]^+$, 479.20 $[M + H]^+$; HRMS (ESI-TOF) calcd for $C_{23}H_{21}F_3N_2O_6$ 478.1352, found 478.1408.

Fmoc-L-Val-L-Ser(Ψ^{CF3,H}Pro)-OMe (**11**). The reaction was performed starting from a 84:16 diastereomeric mixture of (2*S*,4*S*)-**3a** and (2*R*,4*S*)-**3b** 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 **11** (3.09 g, 59%) as a 7/93 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 300 K: white solid; mp 112 °C; R_f = 0.42 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{25}_D$ −74.5 (*c* 1.0, $CHCl_3$); IR (neat) 3312, 2928, 1764, 1716 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 300 K) (*trans* rotamer) δ 1.03 (d, J = 7.0 Hz, 3 H, H_γ Val-Ha), 1.05 (d, J = 7.3 Hz, 3 H, H_γ Val-Hb), 2.06−2.15 (m, 1 H, H_β Val-H), 3.75 (s, 3 H, OMe), 3.97 (t, J = 9.0 Hz, 1 H, H_α Val-H), 4.19 (t, J = 6.9 Hz, 1 H, Fmoc CH), 4.31 (dd, J = 9.8, 6.9 Hz, 1 H, H_β ΨPro-Ha), 4.33 (d, J = 7.8 Hz, 1 H, Fmoc CH_2−Ha), 4.46 (d, J = 7.8 Hz, 1 H, Fmoc CH_2−Hb), 4.47 (dd, J = 9.8, 6.4 Hz, 1 H, H_β ΨPro-Hb), 5.10 (t, J = 8.2 Hz, 1 H, H_α ΨPro-H), 5.26 (d, J = 8.7 Hz, 1 H, NH Val), 6.40 (q, J = 4.6 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, 2 H, Fmoc arom.), 7.76 (d, J = 7.3 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ 18.5 (CH_3, C_γ Val), 18.6 (CH_3, C_γ Val), 31. Six (CH, C_β Val), 47.0 (CH, Fmoc CH), 52.7 (CH_3, OMe), 56.5(CH, C_α ΨPro), 58.6 (CH, C_α Val), 67.3 (CH_2, Fmoc CH_2), 68.7 (CH_2, C_β ΨPro), 85.0 (q, J = 37.4 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 122.7 (q, J = 287.5 Hz, CF_3), 124.9 (CH, Fmoc arom.), 125.0 (CH, Fmoc arom.), 127.0 (2 × CH, Fmoc arom.), 127.8 (2 × CH, Fmoc arom.), 141.3 (2 × C, Fmoc arom.), 143.5 (C, Fmoc arom.), 143.6 (C, Fmoc arom.), 156.5 (C, C═O), 168.6 (C, C═O), 172.5 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ −81.0 (d, J = 4.6 Hz); HRMS (ESI-TOF) calcd for $C_{26}H_{27}F_3N_2O_6$ 520.1821, found 520.1906.

Fmoc-L-Pro-L-Ser(Ψ^{CF3,H}Pro)-OMe (**12**). The reaction was performed following the base-free representative procedure starting from a 84:16 diastereomeric mixture of (2*S*,4*S*)-**3a** and (2*R*,4*S*)-**3b** pseudoprolines (1.02 g, 5.13 mmol, 1.0 equiv) in dichloromethane (7.5 mL) and Fmoc-L-Pro-Cl (2.00 g, 5.62 mmol, 1.1 equiv). Purification by flash chromatography (80:20 cyclohexane/ethyl acetate) gave the pure dipeptide **12** (1.39 g, 52%) as a *trans* rotational isomer in $CDCl_3$ at 300 K: white solid; mp 115 °C; R_f = 0.26 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{21}_D$ −56.1 (*c* 1.0, $CHCl_3$); IR (neat) 2956, 1763, 1689 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, 300 K) (*trans* rotamer) δ 1.94−2.05 (m, 1 H, H_γ Pro-Ha), 2.10−2.26 (m, 2 H, H_β Pro-H), 2.27−2.39 (m, 1 H, H_γ Pro-Hb), 3.52−3.60 (m, 1 H, H_δ Pro-Ha), 3.67−3.74 (m, 1 H, H_δ Pro-Hb), 3.77 (s, 3 H, OMe), 4.23 (t, J = 7.1 Hz, 1 H, Fmoc CH), 4.32 (dd, J = 8.5, 6.9 Hz, 1 H, H_β ΨPro-Ha), 4.34 (d, J = 7.1 Hz, 2 H, Fmoc CH_2−H), 4.36−4.40 (m, 1 H, 1 H, H_α Pro-H), 4.50 (t, J = 8.5 Hz, 1 H, H_β ΨPro-Hb), 5.06 (t, J = 7.8 Hz, 1 H, H_α ΨPro-H), 6.23 (q, J = 4.8 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 (dd, J = 11.0, 7.6 Hz, 2 H, Fmoc arom.), 7.77 (d, J = 7.3 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ 24.8 (CH_2, C_γ Pro), 30.9 (CH_2, C_β Pro), 47.1 (CH_2, C_δ Pro), 47.2 (CH, Fmoc CH), 52.8 (CH_3, OMe), 56.9 (CH, C_α ΨPro), 58.3 (CH, C_α Pro), 67.7 (CH_2, Fmoc CH_2), 68.9 (CH_2, C_β ΨPro), 85.1 (q, J = 30.7 Hz, CH, C_δ ΨPro), 120.1 (2 × CH, Fmoc arom.), 122.9 (q, J = 286.6 Hz, CF_3), 125.2 (2 × CH, Fmoc arom.), 127.1 (2 × CH, Fmoc arom.), 127.8 (2 × CH, Fmoc arom.), 141.4 (2 × C, Fmoc arom.), 143.8 (C, Fmoc arom.), 143.9 (C, Fmoc arom.), 155.4 (C, C═O), 168.8 (C, C═O), 173.0 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) (*trans* rotamer) δ −82.0 (d, J = 4.8 Hz); ESIMS m/z 519.28

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$[M + H]^+$; Anal. Calcd for $C_{26}H_{25}F_3N_2O_6$ (518.17) C, 60.23; H, 4.86; N, 5.40, found C, 60.22; H, 4.84; N, 5.51.

*Fmoc-Aib-Ser($\Psi^{CF3,H}$Pro)-OMe (**13**).* The reaction was performed following the base-free representative procedure starting from a 84:16 diastereomeric mixture of (2*S*,4*S*)-**3a** and (2*R*,4*S*)-**3b** 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 **13** (700 mg, 55%) as a 85/15 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 274 K: white solid; mp 107−112 °C; R_f = 0.24 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{25.0}_D$ −28.4 (*c* 0.95, $CHCl_3$); IR (neat) 3321, 2952, 1709, 1677, 1513, 1144, 739 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 274 K) δ 1.54 (br s, 6 H, H_β Aib-H), 3.69 (s, 3 H, OMe), 4.16 (m, 1 H, H_β ΨPro-Ha), 4.18 (m, 1 H, Fmoc CH), 4.39 (m, 1 H, H_β ΨPro-Hb), 4.50−4.52 (m, 2 H, Fmoc CH_2−H), 5.10 (s, 1 H, NH Aib), 5.14 (dd, *J* = 7.5, 4.1 Hz, 1 H, H_α ΨPro-H), 6.04 (q, *J* = 4.9 Hz, 1 H, H_δ ΨPro-H), 7.34 (t, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.43 (t, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.57 (d, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.76 (d, *J* = 7.4 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$, 298 K) δ 25.7 (2 × CH_3, C_β Aib), 46.5 (CH, Fmoc CH), 52.4 (CH_3, OMe), 57.6 (CH, C_α ΨPro), 57.9 (C, C_α Aib), 65.9 (CH_2, Fmoc CH_2), 69.5 (CH, C_β ΨPro), 84.9 (q, *J* = 35.5 Hz, CH, C_δ ΨPro), 119.4 (2 × CH, Fmoc arom.), 121.9 (q, *J* = 285.6 Hz, CF_3), 124.1 (2 × CH, Fmoc arom.), 126.5 (2 × CH, Fmoc arom.), 127.3 (2 × CH, Fmoc arom.), 140.7 (2 × C, Fmoc arom.), 142.7 (2 × C, Fmoc arom.), 154.7 (C, C═O), 169.0 (C, C═O), 172.6 (C, C═O); ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) δ −80.9 (d, *J* = 5.2 Hz); ESIMS *m*/*z* 507.29 $[M + H]^+$; HRMS (ESI-TOF) calcd for $C_{25}H_{25}F_3N_2O_6Na$ 529.1562, found 529.1554.

*Fmoc-D-Ala-L-Ser($\Psi^{CF3,H}$Pro)-OMe (**14**).* The reaction was performed following the base-free representative procedure starting from pseudoproline (2*S*,4*S*)-**3a** (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 **14** (545 mg, 79%) as a 92/8 inseparable mixture of *cis*/*trans* rotational isomers in $CDCl_3$ at 300 K: white solid; mp 64−66 °C; R_f = 0.40 (70:30 cyclohexane/ethyl acetate); $[\alpha]^{23}_D$ −47.5 (*c* 1.0, $CHCl_3$); HRMS (ESI-TOF) calcd for $C_{24}H_{23}F_3N_2O_6Na$ 515.1406, found 515.1400; spectral data of **14** are similar to those of **9b**.[67]

Synthesis of the Tripeptide. Fmoc-L-Ala-L-Ser($\Psi^{CF3,H}$Pro)-L-Ala-OtBu (15). Saponification of the dipeptide methyl ester **9a** (1.41 g, 2.86 mmol) was performed following a described method by addition of NaOH (137 mg, 2.44 mmol, 1.2 equiv) to a 0.8 M $CaCl_2$ solution in *i*PrOH-$H_2$0 7:3 (68 mL).[75] The reaction mixture was stirred for 4 h at room temperature, quenched with 1 M HCl, concentrated under reduced pressure and diluted with H_2O (20 mL). The aqueous solution was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with H_2O (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (95:5 dichloromethane/methanol) gave the corresponding acid (1.14 g, 83%). To a solution of the Fmoc-Ala-Ser($\Psi^{H,CF3}$Pro)-OH dipeptide (337 mg, 0.705 mmol) in dichloromethane (40 mL) were successively added L-alanine *tert*-butyl ester hydrochloride (192 mg, 1.06 mmol, 1.5 equiv), NEt_3 (406 μL, 2.90 mmol, 4.1 equiv), and after stirring for 20 min at room temperature, BOP-Cl (270 mg, 1.06 mmol, 1.5 equiv). The reaction mixture was stirred overnight at room temperature and then quenched with 1 M HCl (15 mL). The layers were separated, and the aqueous phase was extracted with dichloromethane (3 × 30 mL). The combined organic layers were washed with saturated $NaHCO_3$ aqueous solution (20 mL) and H_2O (20 mL) and then dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the pure tripeptide **15** (180 mg, 42%) as a 53/47 inseparable mixture of rotational isomers in $CDCl_3$ at 274 K: white solid; mp 93−95 °C; R_f = 0.36 (50:50 cyclohexane/ethyl acetate); $[\alpha]^{26}_D$ −52.3 (*c* 1.2, $CHCl_3$); IR (neat) 3448, 3315, 3006, 2970, 2944, 1739, 1369, 1216 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$, 274 K) (Major rotamer) δ 1.32 (br s, 9 H, *t*Bu), 1.33 (d, *J* = 6.6 Hz, 3 H, H_β Ala3-H), 1.48 (d, *J* = 7.6 Hz, 3 H, H_β Ala1-H), 4.19 (br s, 1 H, Fmoc CH), 4.27 (m, 1 H, Fmoc CH_2−Ha), 4.33 (m, 1 H, Fmoc CH_2−Hb), 4.39 (m, 1 H, H_α Ala3-H), 4.40 (m, 1 H, H_β ΨPro-Ha), 4.54 (m, 1 H, H_α Ala1-H), 4.62 (m, 1 H, H_β ΨPro-Hb), 4.95 (t, *J* = 8.1 Hz, 1 H, H_α ΨPro-H), 5.35 (m, 1 H, NH Ala1), 6.27 (m, 1 H, H_δ ΨPro-H), 7.11 (d, *J* = 6.4 Hz, 1 H, NH Ala3), 7.32 (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, 2 H, Fmoc arom.), 7.77 (d, *J* = 7.3 Hz, 2 H, Fmoc arom.); (minor rotamer) δ 1.39 (d, *J* = 7.9 Hz, 3 H, H_β Ala3-H), 1.44 (m, 3 H, H_β Ala1-H), 1.46 (s, 9 H, *t*Bu), 4.19 (br s, 1 H, Fmoc CH), 4.35 (m, 1 H, Fmoc CH_2−Ha), 4.39 (m, 1 H, H_α Ala3-H), 4.40 (m, 1 H, H_α Ala1-H), 4.42 (m, 1 H, Fmoc CH_2−Hb), 4.56 (m, 1 H, H_β ΨPro-Ha), 4.61 (m, 1 H, H_β ΨPro-Hb), 4.62 (m, 1 H, H_α ΨPro-H), 5.34 (m, 1 H, NH Ala1), 5.99 (m, 1 H, H_δ ΨPro-H), 7.32 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.41 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.57 (d, *J* = 7.2 Hz, 2 H, Fmoc arom.), 7.77 (d, *J* = 7.3 Hz, 2 H, Fmoc arom.), 8.39 (d, *J* = 7.5 Hz, 1 H, NH Ala3); ^{13}C NMR (100,5 MHz, $CDCl_3$, 298 K) (rotamer 1) δ 17.9 (CH_3, C_β Ala1), 18.3 (CH_3, C_β Ala3), 27.7 (3 × CH_3, O*t*Bu), 46.6 (CH, Fmoc CH), 48.7 (CH, C_α Ala3), 49.3 (CH, C_α Ala1), 58.1 (CH, C_α ΨPro), 67.2 (CH_2, Fmoc CH_2), 68.7 (CH_2, C_β ΨPro), 81.8 (C, O*t*Bu), 84.5 (q, *J* = 34.5 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 122.8 (q, *J* = 286.6 Hz, CF_3), 125.0 (2 × CH, Fmoc arom.), 127.1 (2 × CH, Fmoc arom.), 127.8 (2 × CH, Fmoc arom.), 141.2 (2 × C, Fmoc arom.), 143.4 (2 × C, Fmoc arom.), 156.5 (C, C═O), 167.2 (C, C═O), 171.4 (C, C═O), 174.3 (C, C═O); (rotamer 2) δ 16.0 (CH_3, C_β Ala1), 16.4 (CH_3, C_β Ala3), 27.8 (3 × CH_3, O*t*Bu), 46.6 (CH, Fmoc CH), 48.6 (CH, C_α Ala1), 49.0 (CH, C_α Ala3), 57.3 (CH, C_α ΨPro), 67.5 (CH_2, Fmoc CH_2), 71.4 (CH_2, C_β ΨPro), 81.8 (C, O*t*Bu), 84.8 (q, *J* = 34.5 Hz, CH, C_δ ΨPro), 120.0 (2 × CH, Fmoc arom.), 122.8 (q, *J* = 286.6 Hz, CF_3), 125.0 (2 × CH, Fmoc arom.), 127.1 (2 × CH, Fmoc arom.), 127.8 (2 × CH, Fmoc arom.), 141.2 (2 × C, Fmoc arom.), 143.4 (2 × C, Fmoc arom.), 156.5 (C, C═O), 167.2 (C, C═O), 171.4 (C, C═O), 174.3 (C, C═O); ^{19}F NMR (376,2 MHz, $CDCl_3$, 298 K) δ −81.6 (s, CF_3) and −81.9 (s, CF_3); ESIMS *m*/*z* 606.32 $[M + H]^+$; HRMS (ESI-TOF) calcd for $C_{30}H_{34}F_3N_3O_7Na$ 628.2247, found 628.2244.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental information and proton, fluorine, and carbon NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).
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