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Article

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Incorporation of Trifluoromethylated Proline and Surrogates into Peptides: Application to the Synthesis of Fluorinated Analogues of the Neuroprotective Glycine-Proline-Glutamate (GPE) Tripeptide

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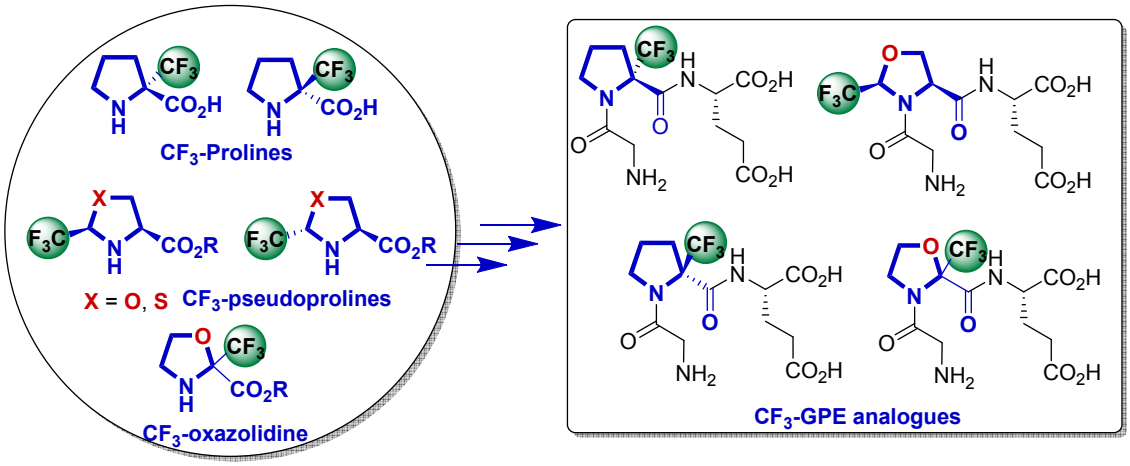


ABSTRACT: The incorporation into a peptide chain of highly hindered and weakly nucleophilic trifluoromethylated prolines, pseudoprolines and oxazolidines has been achieved. As an application, the synthesis of a new class of fluorinated analogues of the neuroprotective tripeptide glycine-proline-glutamate (GPE) is reported. These analogues have been elaborated from a panel of five-membered ring trifluoromethylated amino acids (Tfm-AAs) through the coupling reaction with a glutamate residue at the *C*-terminus and a glycine at the *N*-terminus. Although the peptide coupling reaction at the *C*-terminal position of the fluorinated amino acid was conveniently performed under standard

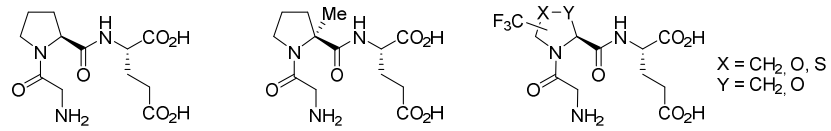
conditions, the very challenging coupling reaction at the highly deactivated *N*-terminal position proved to be much more problematic. A methodological study was needed to identify suitable reaction conditions for this difficult peptide coupling.

INTRODUCTION

The use of peptides as therapeutic agents has been quite limited due to several major drawbacks such as structural flexibility, rapid degradation by peptidases and low lipophilicity. However, because of recent advances in peptides chemistry and biology, peptide-based drug discovery constitutes now a serious alternative for addressing new therapeutic challenges.[1] The introduction of fluorine atoms into biomolecules such as peptides is known to deeply influence their chemical and biological properties[2-5] and consequently, represents an option to develop the therapeutic potential of peptides. The synthesis of fluorinated amino acids has gained a considerable interest in peptide and protein chemistry[6-8] as they are known to increase the chemical stability, the metabolic resistances[9,10] and to provide a better affinity of peptides for lipid membranes. Moreover, their incorporation into peptides can induce stabilization of particular conformations and better auto-assembly.[11-15] Fluorinated peptides can also be used as efficient probes for ^{19}F NMR studies.[16,17] Trifluoromethylated amino acids (Tfm-AAs) represent a special class of highly constrained non-proteogenic amino acids. While the coupling reactions of the Tfm-AAs at their *C*-termini can be efficiently achieved using standard protocols, their incorporation into peptides at the *N*-terminal position still remains challenging due to the stereo-electronic effects imparted by the CF_3 group which strongly decrease the nitrogen nucleophilicity.[18] Our group has developed the stereoselective synthesis of a panel of acyclic and cyclic Tfm-AAs in enantiopure form[19-26] and is now mainly focused on the development of efficient methodologies for their incorporation into peptides.[27-30] Although α-CF_3-alanine has been incorporated into peptides, the peptide coupling at the *N*-terminal position of α-CF_3-proline has not been reported so far. We report herein our investigations about the incorporation of α-CF_3-proline and various cyclic surrogates into short peptide sequences. The usefulness of this methodology for the synthesis of peptides of biological

interest is illustrated by the synthesis of new CF_3-tripeptide analogues of the neuroprotective glycine-proline-glutamate peptide (GPE) (Figure 1). It is assumed that the GPE is the result of the proteolytic cleavage of the insulin-like growth factor (IGF-1).[31] Even if the GPE neither binds to IGF-1 receptors nor has any neurotrophic effect, it displays remarkable CNS activities.[32-33] In different animal models, the GPE shows neuroprotective effects on neurodegenerative processes, such as Alzheimer's, Parkinson's and Huntington's diseases. There is also evidence that the GPE exhibits neuromodulatory activities.[34-36] Due to its structural simplicity, the GPE emerged as a lead for the development of potent neuroprotective agents for the treatment of various CNS injuries.[37] Numerous GPE analogues have been reported in the literature but most of them suffer of a lack of chemical stability and short half-time bioavailability.[38-45] It has been shown that proline residue is crucial due to its unique conformational constraint.[46] The tripeptide NNZ-2566, containing a 2-methylproline residue, shows higher activity compared to GPE and other analogues and is currently in phase II for several neurological indications.[47] Replacement of the proline residue by a CF_3-containing surrogate is expected to enhance the chemical stability of the GPE tripeptide together with its bioavailability profile.

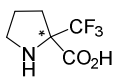
Figure 1. Chemical structure of GPE, NNZ-2566 and CF_3-GPE analogues



Three distinctive trifluoromethylated cyclic surrogates were considered for replacing the proline residue in the GPE sequence varying the position and the configuration of the CF_3 group along the 5-membered ring : α-CF_3-proline **1**, CF_3-pseudoprolines **2** and **3** derived from serine and cysteine respectively and CF_3-oxazolidines **4** and **5** derived from ethanolamine and (*R*)-phenylglycinol (Figure 2). Except for the oxazolidine **4**, the synthesis of enantiopure **1**,[20,22] **2**,[23] **3**[23] and **5**[20,21] have been previously described by our group.

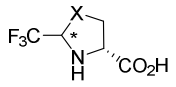
Figure 2. Chemical structure of Tfm-AAs as proline surrogates

CF_3-Proline template



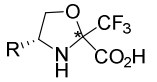
α-CF_3-Pro **1**

CF_3-Pseudoproline template



X = O Ser($\Psi^{CF3,H}$Pro) **2**
X = S Cys($\Psi^{CF3,H}$Pro) **3**

CF_3-Oxazolidine template

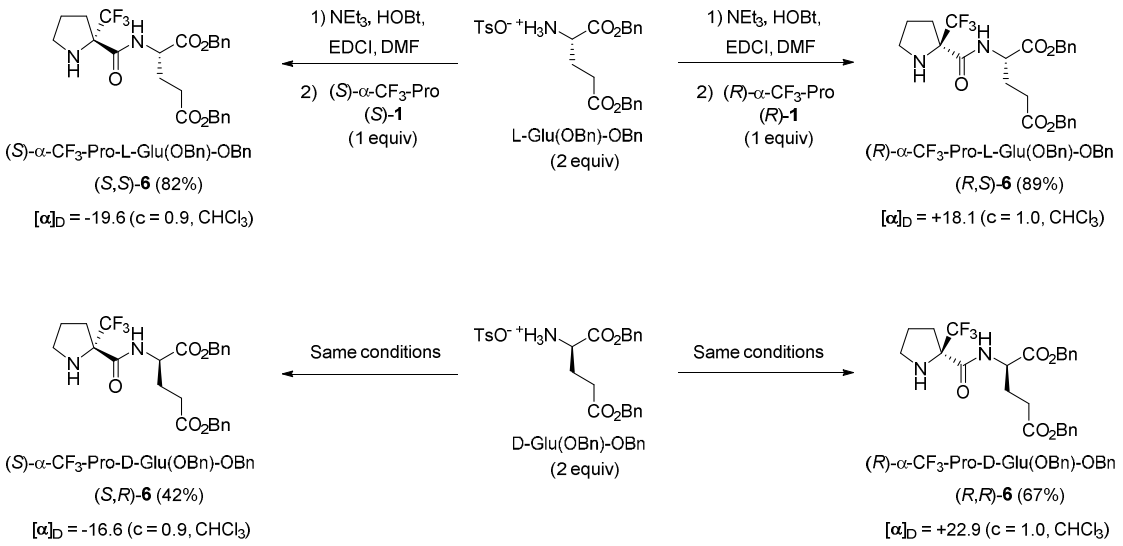


R = H CF_3-Oxa **4**
R = Ph CF_3-Oxa **5**

RESULTS AND DISCUSSION

CF_3-proline containing GPE analogues. The synthesis of CF_3-GPE analogues was first investigated using the (*S*)- and (*R*)-α-CF_3-proline **1**. Due to the very short GPE sequence, the synthesis was achieved in solution phase using the standard peptide elongation methodology. The coupling reaction between the glutamic acid residue (Glu) and the α-CF_3-proline **1** was performed following our reported procedure in order to avoid the diketopiperazine formation.[27] Because of the strong deactivation of the α-CF_3-proline **1** amino group induced by the electron-withdrawing effect of the neighboring CF_3 group,[17] its *N*-protection was not required to perform the coupling reaction in contrast with the non-fluorinated series. The four enantiopure dipeptides **6** [(*S*,*S*)-**6**, (*R*,*S*)-**6**, (*R*,*R*)-**6**, (*S*,*R*)-**6**] were obtained by coupling (*R*)- and (*S*)-α-CF_3-prolines **1** with L- or D-glutamic acids (Scheme 1). Therefore, the addition of 1 equivalent of α-CF_3-proline **1** to 2 equivalent amount of glutamic acid dibenzyl ester in the presence of coupling reagents afforded the corresponding dipeptides **6** without any trace of diketopiperazine. The expected dipeptides were obtained in 42 to 89% yield depending on the D- or L-series of the glutamic acid (Scheme 1).

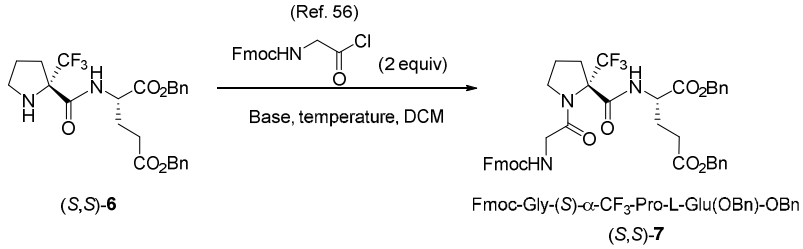
Scheme 1. Synthesis of dipeptides 6



The next step involved the coupling reaction of the glycine residue (Gly) at the *N*-terminal position of the dipeptides **6**. Despite the small steric hindrance of the Gly residue, this reaction remained highly challenging because of the very low nucleophilicity of the dipeptide amino group and the steric bulkiness of the α-CF_3-group. Only very few examples are found in the literature for the *N*-terminal coupling of acyclic α-CF_3-amino acids.[9,48-50] All these examples required specific activation methods, such as mixed anhydrides or acyl halides, to promote the peptide coupling in good yield. To our knowledge, the *N*-terminal coupling reaction has never been reported so far for cyclic α-CF_3-amino acids such as α-CF_3-prolines. Firstly, we checked the use of a mixed anhydride obtained from isobutylchloroformate and Cbz-glycine. However no coupling product was obtained from the dipeptide (*S*,*S*)-**6** whatever the solvent used (ethyl acetate or DMF) and only starting material was recovered. The use of acyl halide activation was then investigated. Amino acid fluorides are less moisture sensitive than amino acid chlorides and are recognized as excellent coupling reagents for both solution and solid-phase peptide syntheses.[51] They are particularly efficient for the coupling of sterically hindered amino acids but require the treatment with a silylating agent such as *N*,*O*-bis(trimethylsilyl)acetamide (BSA).[52] A coupling test between the pre-activated dipeptide (*S*,*S*)-**6** and the Fmoc-glycine fluoride, prepared according to Olah's method,[53] was attempted without success and only starting material was recovered. We postulated that the reactivity of the acyl fluoride was not

enough to overcome the stereoelectronic deactivation of the fluorinated dipeptide **6** and we decided to try the more reactive amino acid chlorides. Because of the hydrolysis risk, epimerization and other side reactions, the acyl chlorides have been considered for a long time as overactivated species.[54] Thanks to the significant contribution of Carpino and his group, the use of the Fmoc-amino acid chlorides have turned out to be one of the most efficient coupling strategies.[55] However, particular attention is required to ensure the coupling. The Fmoc-glycyl chloride was prepared by treatment of the Fmoc-glycine with thionyl chloride in DCM under ultrasonication and obtained in pure form by crystallization from pentane.[56] Because of the release of HCl during the amide bond formation, the coupling reaction of the Fmoc-glycyl chloride with the dipeptide (*S*,*S*)-**6** was first performed in the presence of DIEA. As summarized in Table 1, no reaction occurred at room temperature and only the starting material was recovered even after 48 h (Table 1, entry 1). The increase of the temperature at 60°C in a sealed tube led to the formation of the tripeptide (*S*,*S*)-**7** in a low yield (Table 1, entry 2). We postulated that the neutralization of the HCl released from the reaction would prevent the acidic activation of the amino acid chloride. As the amino group of the fluorinated dipeptide **6** is strongly deactivated, we anticipated that the HCl release would not be a problem and the reaction was performed without base. Indeed, the coupling reaction of the Fmoc-glycyl chloride with the dipeptide (*S*,*S*)-**6** at 60°C in the absence of DIEA gave the corresponding tripeptide (*S*,*S*)-**7** in a very good yield (88%) without epimerization (Table 1, entry 3). The substitution of the conventional heating method by microwave irradiation, reported to accelerate the peptide syntheses, allowed the coupling in about 30 min but the yield decreased to 54% (Table 1, entry 4).

Table 1. Optimization of the synthesis of the tripeptide (*S*,*S*)-7 using amino acid chloride activation

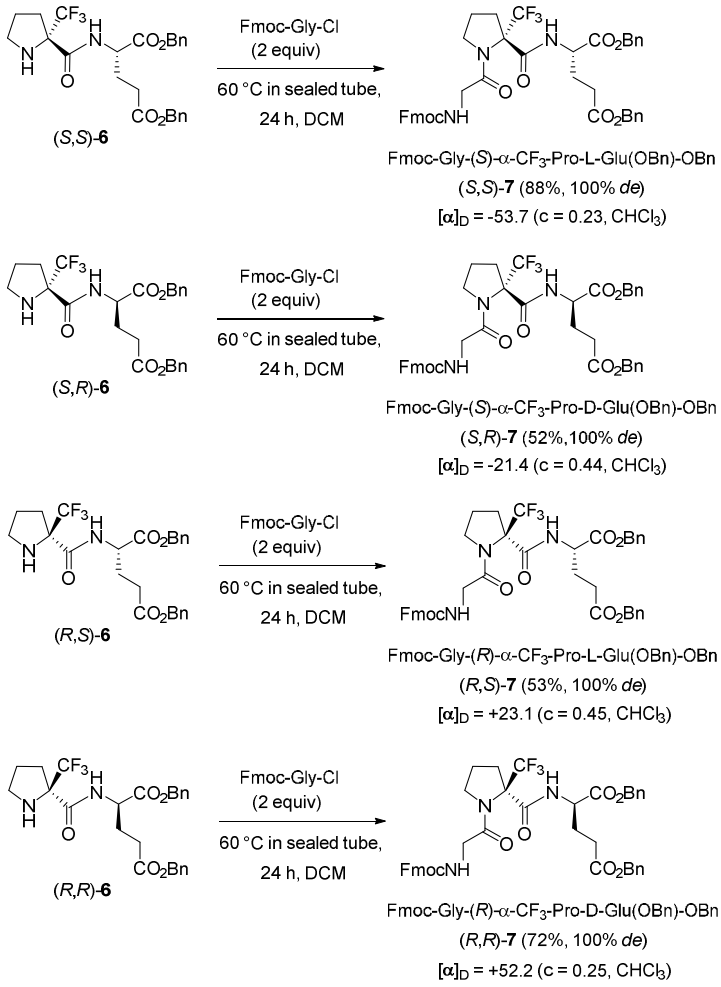


Entry	Base (equiv)	Temperature (°C)	Time (h)	Tripeptide (*S*,*S*)-**7** Yield (%)
1	DIEA (1)	r.t.	48	0[a]
2	DIEA (2)	60[b]	24	15[c]
3	-	60[b]	24	88[c]
4	-	Microwave	0.5	54[c]

[a]Starting material was recovered. [b]Reaction performed in a sealed tube. [c]Obtained as a single diastereomer.

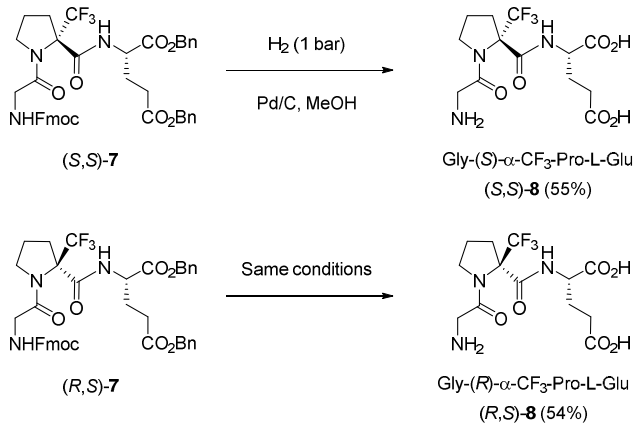
The optimized coupling conditions (Table 1, entry 3) were then successfully applied to the dipeptides (*R*,*S*)-**6**, (*S*,*R*)-**6** and (*R*,*R*)-**6** to afford the corresponding tripeptides **7** in 52-67% yield without epimerization (Scheme 2).

Scheme 2. Synthesis of tripeptides 7



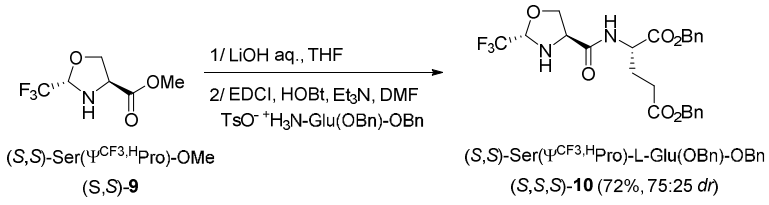
The access to the α-CF_3-proline containing GPE analogues required the full removal of the protecting groups. The sequential cleavages were first considered in classical manner as usually reported in peptide chemistry when using orthogonal protecting groups. Unexpectedly the standard Fmoc deprotection by treatment with piperidine in DMF led to degradation. The use of DBU in DCM gave the same result. Since both Fmoc and Bn protecting groups are sensitive to hydrogenolysis, their removal in a single step was envisioned. The hydrogenolysis of (*S*,*S*)-**7** and (*R*,*S*)-**7** tripeptides was carried out under hydrogen atmosphere (1 bar) in methanol in the presence of an excess amount of Pd/C catalyst (140 %mol of Pd) to afford the CF_3-GPE analogues (*S*,*S*)-**8** and (*R*,*S*)-**8** in 55% and 54% yield respectively (Scheme 3). Surprisingly the use of higher hydrogen pressure (3-5 bars) led only to degradation.

Scheme 3. Synthesis of CF_3-proline containing GPE analogues (*S*,*S*)-8 and (*R*,*S*)-8



CF_3-pseudoproline containing GPE analogues. We then focused our attention on the CF_3-pseudoproline template. It is anticipated that this 5-CF_3-proline surrogate should constitute a valuable tool to control the *cis-trans* isomerisation of the glycyl-pseudoprolyl bond.[23,28,30] The synthesis was first attempted following the same peptide elongation sequence as used for the α-CF_3-proline. The pseudoproline (*S*,*S*)-**9** was prepared according to our reported procedure by condensation of serine methyl ester with fluoral.[23] After saponification of the ester function of the pseudoproline **9**, its coupling reaction with L-glutamic acid dibenzyl ester was carried out at the *C*-terminal position according to the standard procedure using EDCI and HOBt (Scheme 4). The corresponding dipeptide (*S*,*S*,*S*)-**10** was obtained in 72% yield as a diastereomeric mixture.

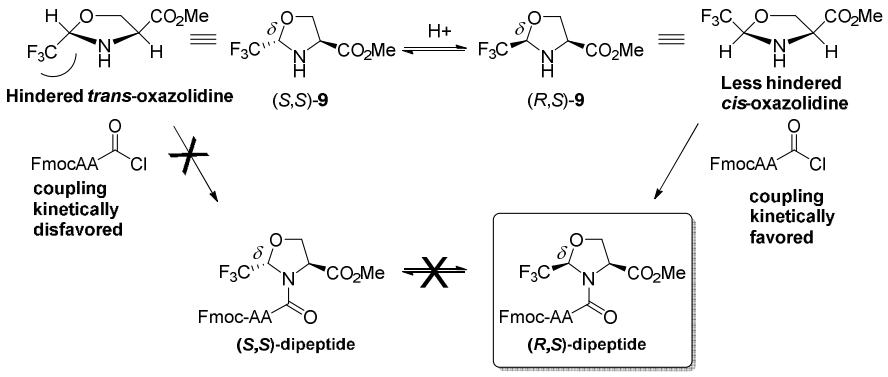
Scheme 4. *C*-terminal coupling reaction of the pseudoproline (*S*,*S*)-9



At this stage, the absolute configuration of the minor diastereomer could not be unambiguously assigned. This synthetic pathway being stereochemically irrelevant, we decided to adopt a reverse strategy for the peptide elongation and to start with the *N*-terminal coupling of pseudoprolines **9**. We

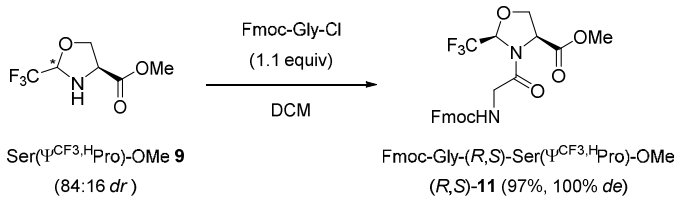
have recently reported the coupling reactions of a mixture of *cis* and *trans* pseudoprolines **9** with various Fmoc protected amino acid chlorides in base free conditions.[30] We demonstrated that the reaction involved a dynamic kinetic resolution (DKR) process. Indeed only the corresponding dipeptides bearing a *cis* oxazolidine were obtained regardless the configuration at the C^{δ} position of the starting pseudoproline **9**. We postulated that the *trans* (*S*,*S*)-**9** and the *cis* (*R*,*S*)-**9** pseudoprolines interconvert rapidly throughout a ring opening equilibrium promoted by the acidic conditions (Scheme 5). Due to steric effects, the *N*-acylation of the *trans* pseudoproline (*S*,*S*)-**9** is strongly disfavored and only the *cis* (*R*,*S*)-**9** pseudoproline reacts to afford exclusively the dipeptide bearing the *cis* (*R*,*S*)-oxazolidine.[57,58] It is important to note that no epimerization of the C^{δ} occurs once the pseudoprolines are *N*-acylated.

Scheme 5. Dynamic kinetic resolution process during *N*-coupling of pseudoprolines 9



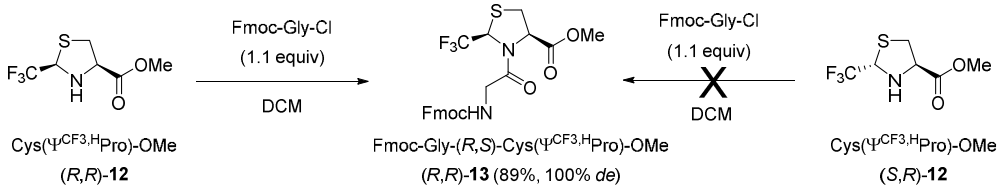
Applied to Fmoc-glycine chloride, the reaction of a diastereomeric mixture of pseudoprolines (*S*,*S*)-**9** and (*R*,*S*)-**9** gave the dipeptide (*R*,*S*)-**11** in 97% yield as a single diastereomer (Scheme 6). Compared to the conditions used with the CF_3-proline template (Table 1), the reaction was carried out in milder conditions at room temperature using only a slight excess amount (1.1 equiv) of acyl chloride.

Scheme 6. *N*-terminal coupling reaction of pseudoprolines 9



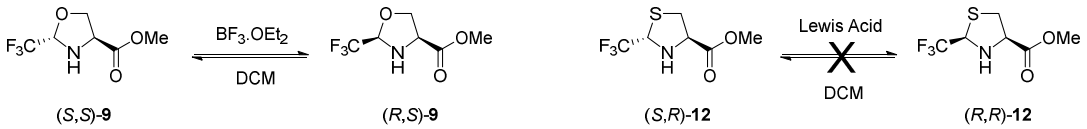
These conditions were applied to the thiazolidines **12** prepared by condensation of cysteine methyl ester with fluoral.[23] Starting from a diastereomeric mixture of the *trans* (*S*,*R*)-**12** and *cis* (*R*,*R*)-**12** thiazolidines, the coupling reaction led to the expected dipeptide (*R*,*R*)-**13** but with a low conversion. The monitoring of the reaction progress by TLC and ^{19}F NMR analyses shown a complete disappearance of the *cis* thiazolidine (*R*,*R*)-**12** without any reaction of the *trans* (*S*,*R*)-**12** diastereomer. The coupling reactions with Fmoc-Gly-Cl were then performed separately on the *trans* (*S*,*R*)-**12** and *cis* (*R*,*R*)-**12** thiazolidines (Scheme 7). The thiazolidine (*R*,*R*)-**12** afforded the expected dipeptide (*R*,*R*)-**13** in 89% yield as a single diastereomer while no reaction occurred with the (*S*,*R*)-**12** thiazolidine (Scheme 7).

Scheme 7. *N*-terminal coupling reaction of pseudoprolines 12



As observed with the oxazolidines **9**, the *N*-coupling reaction is strongly dependent of steric effects and only the *cis* thiazolidine (*R*,*S*)-**12** reacts to afford the corresponding (*R*,*S*)-**13** dipeptide. In agreement with the literature in the non-fluorinated series,[59,60] we assumed that, unlike the oxazolidines **9**, the trifluoromethylated thiazolidines (*R*,*R*)-**12** and (*S*,*R*)-**12** are stable towards acidic media and cannot interconvert throughout a ring opening equilibrium. In a previous work,[23] we reported that a Lewis acid treatment ($BF_3 \cdot OEt_2$) can mediate the ring opening and promote the epimerization of the *trans* (*S*,*S*)-**9** oxazolidine into the *cis* (*R*,*S*)-**9** (Scheme 8). The higher thermodynamic stability of the *trans* (*S*,*S*)-**9** oxazolidine compared to the *cis* (*R*,*S*)-**9** one induces a slow equilibrium shift toward the *trans* (*S*,*S*)-**9**. In contrast, no epimerization occurred when the *trans* (*S*,*R*)-**12** thiazolidine was treated with $BF_3 \cdot OEt_2$, $Ti(OiPr)_4$ or $Ti(OiPr)_3Cl$ as Lewis acids. The reaction with $TiCl_4$ or $SnCl_4$ gave only degradation products.

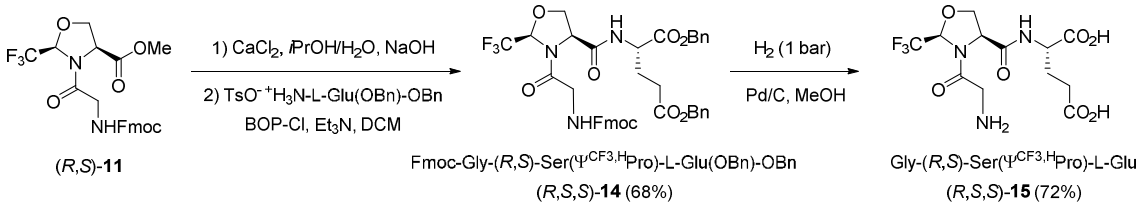
Scheme 8. Lewis acid-mediated epimerization of pseudoprolines 9 and 12



t (h)	(*S*,*S*)-**9** / (*R*,*S*)-**9**
0	100:0
18	22:78
48	66:34

In order to synthesize oxazolidine-type CF_3-pseudoproline containing GPE analogues, the saponification of the methyl ester dipeptide (*R*,*S*)-**11** was carried out under mild conditions to avoid early Fmoc deprotection.[61] The treatment using NaOH in *i*PrOH/H_2O (7:3) solution of $CaCl_2$ (0.84 M) gave the corresponding acid which was directly engaged in the coupling reaction with respectively L- and D-glutamic acid dibenzyl ester (Scheme 9). Surprisingly, the reaction under the standard conditions (EDCI/HOBt) which were effective for the CF_3-proline template (Scheme 1) failed and the starting material was recovered. The use of BOP-Cl allowed the synthesis of the expected tripeptide (*R*,*S*,*S*)-**14** in 68% yield without epimerization (Scheme 9). The removal of all the protecting groups was performed following the same procedure used for the CF_3-proline template. In a single step, the hydrogenolysis of the tripeptide (*R*,*S*,*S*)-**14** afforded the corresponding CF_3-GPE analogues (*R*,*S*,*S*)-**15** in good yield (72%).

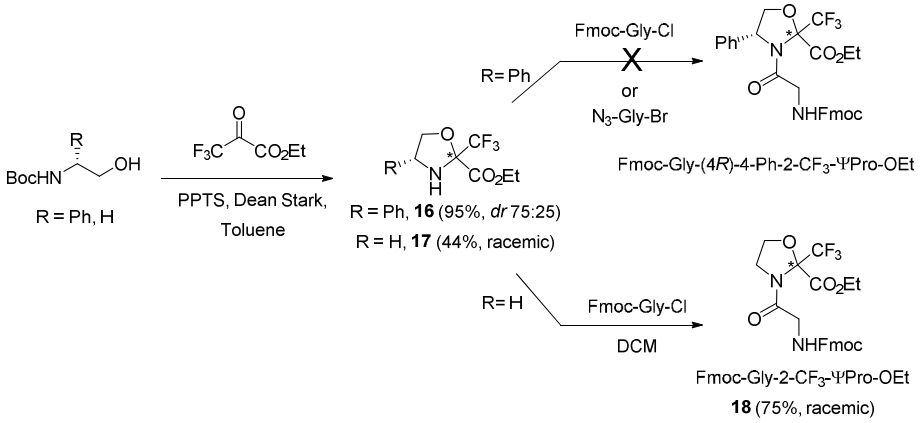
Scheme 9. Synthesis of CF_3-pseudoproline containing GPE analogues (*R*,*S*,*S*)-14 and (*R*,*S*,*S*)-15



These optimized conditions were applied for the coupling of the thiazolidine containing dipeptide (*R*,*R*)-**13** with L-glutamic acid dibenzyl ester. Unfortunately, the reaction led only to the recovery of the starting material. Other attempts using EDCI/HOBt coupling reagents or the mixed anhydride activation by treatment of (*R*,*R*)-**13** with isobutylchloroformate in the presence of NMM failed.

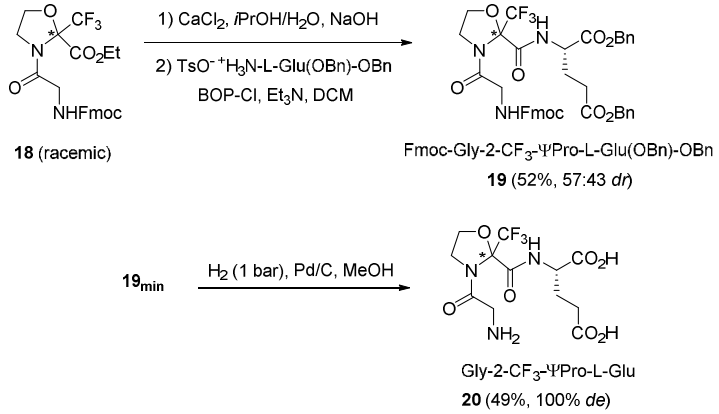
2-CF_3-oxazolidine containing GPE analogues. The last investigated GPE analogue series ambitioned to incorporate the trifluoropyruvate oxazolidine template in place of the proline. According to our previously reported procedure,[21] the CF_3-oxazolidine **16** was obtained in a high yield (95%) as a 75:25 diastereomeric mixture by the condensation of the ethyl trifluoropyruvate with *N*-Boc-(*R*)-phenylglycinol in the presence of PPTS (Scheme 10). We already reported that this oxazolidine proved to be a convenient building block for the synthesis of various enantiopure α-CF_3-amino acids[20-22,24] and amino alcohols.[62] In other respect, we consider herein this kind of oxazolidines as original stable proline surrogates. Indeed, the conjugated electron withdrawing effect of both the trifluoromethyl and the carboxylic group prevents the oxazolidine ring opening. Likewise, the unsubstituted racemic oxazolidine **17** was obtained in 44% yield through the condensation reaction of ethyl trifluoropyruvate with *N*-Boc-ethanolamine (Scheme 10). As both diastereomers of **16** and the racemic oxazolidine **17** can be considered as 2-CF_3-proline surrogates (2-CF_3-ΨPro), we decided to investigate their incorporation into the GPE peptide sequence. Following the synthetic strategy successfully employed in the CF_3-pseudoproline series based on the *N*- to *C*-termini peptide elongation (*vide supra*), the coupling reaction between a diastereomeric mixture of oxazolidine **16** and the glycine residue was attempted (Scheme 10). However, the expected dipeptide was not obtained whatever was the glycine activation (acyl chloride or acyl bromide) or the protocol used (room temperature, conventional heating or microwave activation, zinc activation, stoichiometry and solvent nature). In order to decrease the steric impact of the oxazolidine, we decided to perform the coupling reaction starting from the unsubstituted racemic oxazolidine **17** (Scheme 10). Therefore **17** was coupled with Fmoc-glycine chloride to afford the racemic dipeptide **18** in a good yield (75%).

Scheme 10. *N*-terminal coupling reaction of oxazolidines 16 and 17



The rest of the synthesis to get the GPE analogues was achieved following the protocol described for the CF_3-pseudoproline template. A saponification reaction followed by the coupling reaction of the dipeptide **18** with the dibenzylated glutamic acid gave the tripeptide **19** as a 57:43 diastereomeric mixture in 52% yield (Scheme 11). A pure fraction of each diastereomer was obtained by flash chromatography separation. However, we were unable to assign the absolute configuration of the oxazolidine core of each diastereomer. Finally, the isolated diastereomer of the tripeptide $\mathbf{19_{min}}$ was submitted to hydrogenolysis to give the CF_3-GPE analogue **20** in 49% yield.

Scheme 11. Synthesis of the 2-CF_3-oxazolidine containing GPE analogue 20



CONCLUSIONS

We have developed convenient methods for the incorporation of various CF_3-proline surrogates (α-CF_3-prolines, CF_3-pseudoprolines and 2-CF_3-oxazolidines) within peptide sequences. As a result of this study, we demonstrated that the choice of the peptide elongation route (*C*- to *N*-terminus or *N*- to

C-terminus) was crucial for the success of the peptide synthesis and also to guarantee its configuration integrity. For the CF_3-proline template, the standard peptide elongation route (*C*- to *N*-terminus) was applied while the reverse strategy was required for the CF_3-pseudoproline and CF_3-oxazolidine templates. Noteworthy, the Fmoc-benzyl strategy allowed the final full removal of the protecting groups in single step by hydrogenolysis. The methods described in this paper have been successfully applied to the solution phase synthesis of several novel trifluoromethylated analogues of the neuroprotective GPE peptide. Evaluation of their neuroprotective effects on different types of neurons from diverse induced injuries activity are in progress and will be reported in due time. Moreover, for future developments, the fluorinated Fmoc-protected peptide intermediates elaborated in this work will constitute suitable peptides bocks for the solid phase synthesis of longer peptides incorporating CF_3-proline or its surrogates.

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}. ^1H NMR, ^{13}C NMR, and ^{19}F NMR spectra were recorded in $CDCl_3$ (unless otherwise stated). ^1H 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 ^1H frequency of 400 MHz. Chemical shifts of ^1H NMR are expressed in parts per million downfield from tetramethylsilane ($\delta = 0$) in $CDCl_3$. Chemical shifts of ^{13}C NMR are expressed in parts per million downfield from $CDCl_3$ as internal standard ($\delta = 77.0$). Chemical shifts of ^{19}F NMR are expressed in parts per million downfield from C_6F_6 as an internal standard ($\delta =$

−164.9). Coupling constants are reported in Hertz. Assignments were obtained from the analysis of 2D $^{1}H–^{13}C$ heteronuclear correlation (HETCOR) spectroscopy. Correlation spectroscopy (COSY) was used to correlate chemical shifts of protons coupled to one another. Melting points were uncorrected. High-resolution mass spectra were obtained using electrospray ionization (ESI) in positive ion mode and a TOF mass analyzer or using direct inlet probe (DI-HRMS).

CF_3-proline containing GPE analogues.

General procedure for the synthesis of dipeptides (*S*,*S*)-6, (*S*,*R*)-6, (*R*,*S*)-6, (*R*,*R*)-6. Triethylamine (4.1 equiv), HOBt (1.5 equiv), EDCI (1.5 equiv), and finally α-Tfm-proline **1** (1 equiv) were successively added at 0 °C to a stirred solution of glutamic dibenzylester tosylate salt (2 equiv) in DMF (0.25 M/α-Tfm amino acid). DMF was added and the resulting mixture was stirred at 0 °C for 20 min and then at room temperature for 24 h. The mixture was diluted with DCM and water. The aqueous layer was extracted with DCM (3 ×) and the combined organic layers were washed with water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography to afford the corresponding dipeptides in 42–89% yields.

H-(*S*)-α-Tfm-Pro-L-Glu(OBn)-OBn (*S*,*S*)-6. The dipeptide (*S*,*S*)-**6** was prepared by the General Procedure, with L-glutamic dibenzylester tosylate salt (961 mg, 1.92 mmol, 2 equiv), triethylamine (540 µL, 3.94 mmol, 4.1 equiv), HOBt (195 mg, 1.44 mmol, 1.5 equiv), EDCI (275 mg, 1.44 mmol, 1.5 equiv), and (*S*)-α-Tfm proline **1** (176 mg, 0.96 mmol) in DMF (4 mL). Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*S*,*S*)-**6** (388 mg, 82%) as a colorless oil; $[\alpha]^{25}_{D}$ −19.6 (*c* 0.9, $CHCl_3$); IR (neat) : 3021, 1740, 1685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.70 (m, 1 H, H_γ Pro-Ha), 1.85 (m, 1 H, H_γ Pro-Hb), 2.07 (m, 1 H, H_β Glu-Ha), 2.15–2.46 (m, 6 H, NH Pro, H_β Glu-Hb, H_γ Glu and H_β Pro), 3.01–3.11 (m, 2 H, H_δ Pro-Ha), 4.62 (m, 1 H, H_α Glu), 5.09 (s, 2 H, Bzl CH_2), 5.14 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Ha), 5.18 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Hb), 7.30–7.38 (m, 10 H, Bn arom.), 8.11 (d, *J* = 6.9 Hz, 1 H, NH Glu); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 25.4 (CH_2, C_γ Pro), 26.9 (CH_2, C_β Glu), 30.1 (CH_2, C_γ Glu), 32.3 (CH_2, C_β Pro), 47.5 (CH_2, C_δ Pro), 51.9 (CH, C_α Glu), 66.6 (CH_2, Bn

CH_2), 67.4 (CH_2, Bn CH_2), 70.7 (q, J = 26.8 Hz, CH, C_α Pro), 125.9 (q, J = 284.7 Hz, CF_3), 128.2, 128.3, 128.5, 128.6, 128.6, 135.0, 135.6, 169.5, 171.1, 172.3; ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –77.8 (s, CF_3); DI-HRMS calcd. for $C_{25}H_{27}F_3N_2O_5$ $[M]^+$: 492.1872; found 492.1873.

H-(*R*)-α-Tfm-Pro-D-Glu(OBn)-OBn (*R,R*)-6. The dipeptide (*R*,*R*)-**6** was prepared by the General Procedure, from D-glutamic dibenzylester tosylate salt (332 mg, 0.67 mmol, 2 equiv), triethylamine (181 µL, 1.36 mmol, 4.1 equiv), HOBt (68 mg, 0.50 mmol, 1.5 equiv), EDCI (96 mg, 0.50 mmol, 1.5 equiv), and (*R*)-α-Tfm proline **1** (61 mg, 0.33 mmol) in DMF (1.5 mL). Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*R*)-**6** (110 mg, 67%) as a colorless oil; $[\alpha]^{25}_D$ +22.9 (*c* 1.0, $CHCl_3$); The spectral data of (*R,R*)-**6** were identical to those of (*S,S*)-**6**; DI-HRMS calcd. for $C_{25}H_{27}F_3N_2O_5$ $[M]^+$: 492.1872; found 492.1873.

H-(*R*)-α-Tfm-Pro-L-Glu(OBn)-OBn (*R,S*)-6. The dipeptide (*R*,*S*)-**6** was prepared by the General Procedure, with L-glutamic dibenzylester tosylate salt (764 mg, 1.53 mmol, 2 equiv), triethylamine (420 µL, 3.14 mmol, 4.1 equiv), HOBt (155 mg, 1.15 mmol, 1.5 equiv), EDCI (219 mg, 1.15 mmol, 1.5 equiv), and (*R*)-α-Tfm proline **1** (140 mg, 0.76 mmol) in DMF (3 mL). Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*S*)-**6** (334 mg, 89%) as a colorless oil; $[\alpha]^{25}_D$ +18.1 (*c* 0.9, $CHCl_3$); IR (neat): 3332, 3019, 2925, 1736, 1685 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.69 (m, 1 H, H_γ Pro-Ha), 1.79 (m, 1 H, H_γ Pro-Hb), 2.04 (m, 1 H, H_β Glu-Ha), 2.11–2.48 (m, 6 H, NH Pro, H_β Glu-Hb, H_γ Glu and H_β Pro), 3.00–3.12 (m, 2 H, H_δ Pro), 4.60 (m, 1 H, H_α Glu), 5.09 (s, 2 H, Bn CH_2), 5.12 (d, J = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.18 (d, J = 12.1 Hz, 1 H, Bn CH_2-Hb), 7.28–7.37 (m, 10 H, Bn arom.), 8.29 (d, J = 8.5 Hz, 1 H, NH Glu); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 25.1 (CH_2, C_γ Pro), 26.9 (CH_2, C_β Glu), 30.2 (CH_2, C_γ Glu), 32.0 (CH_2, C_β Pro), 47.5 (CH_2, C_δ Pro), 51.8 (CH, C_α Glu), 66.5 (CH_2, Bn CH_2), 67.4 (CH_2, Bn CH_2), 71.0 (q, J = 26.8 Hz, CH, C_α Pro), 125.8 (q, J = 283.7 Hz, CF_3), 127.9, 127.9, 128.0, 128.2, 128.3, 135.1, 135.7, 169.4, 171.1, 172.4; ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –77.8 (s, CF_3); DI-HRMS calcd. for $C_{25}H_{27}F_3N_2O_5$ $[M]^+$: 492.1872; found 492.1874.

H-(*S*)-α-Tfm-Pro-D-Glu(OBn)-OBn (*S,R*)-6. The dipeptide (*S,R*)-**6** was prepared by the General Procedure, with D-glutamic dibenzylester tosylate salt (605 mg, 1.12 mmol, 2 equiv), triethylamine (326 µL, 2.46 mmol, 4.1 equiv), HOBt (122 mg, 0.90 mmol, 1.5 equiv), EDCI (173 mg, 0.90 mmol, 1.5 equiv), and (*S*)-α-Tfm proline **1** (111 mg, 0.60 mmol) in DMF (3 mL). Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*S,R*)-**6** (124 mg, 42%) as a colorless oil; $[\alpha]^{25}_{D}$ –16.6 (*c* 1.0, $CHCl_3$); The spectral data of (*S,R*)-**6** were identical to those of (*R,S*)-**6**; DI-HRMS calcd. for $C_{25}H_{27}F_3N_2O_5$ $[M]^+$: 492.1872; found 492.1874.

General procedure for the synthesis of dipeptides (*S,S*)-7, (*S,R*)-7, (*R,S*)-7, (*R,R*)-7. To a solution of dipeptide **6** (1 equiv) in DCM under argon was added Fmoc-Gly-Cl (2 equiv) prepared according to our reported procedure.[30] The mixture was refluxed for 24 h and then quenched by saturated $NaHCO_3$ solution. The aqueous phase was extracted by DCM (3 ×) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by flash chromatography to afford the corresponding tripeptides in 52–88% yield.

Fmoc-Gly-(*S*)-α-Tfm-Pro-L-Glu(OBn)-OBn (*S,S*)-7. The tripeptide (*S,S*)-**7** was prepared following the General Procedure, using dipeptide (*S,S*)-**6** (552 mg, 1.12 mmol, 1 equiv) and Fmoc-Gly-Cl (708 mg, 2.24 mmol, 2 equiv) in DCM (4 mL). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave pure (*S,S*)-**7** (757 mg, 88 %) as a white solid. Mp 110–112°C; $[\alpha]^{25}_{D}$ – 53.7 (*c* 0.23, $CHCl_3$); IR (neat): 3300, 1728, 1681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.95–2.15 (m, 3 H, H_γ Pro and H_β Glu-Ha), 2.29 (m, 1 H, H_β Glu-Hb), 2.30–2.59 (m, 4 H, H_γ Glu and H_β Pro), 3.60 (q, *J* = 8.0 Hz 1 H, H_δ Pro-Ha), 3.71 (q, *J* = 8.0 Hz, 1 H, H_δ Pro-Hb), 4.03–4.09 (m, 2 H, H_α Gly), 4.20 (t, *J* = 7.2 Hz, 1 H, Fmoc CH), 4.30–4.40 (m, 2 H, Fmoc CH_2), 4.66 (m, 1 H, H_α Glu), 5.09 (s, 2 H, Bn CH_2), 5.14 (d, *J* = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.16 (d, *J* = 12.1 Hz, 1 H, Bn CH_2-Hb), 5.73 (s, 1 H, NH Gly), 7.21 (d, *J* = 6.9 Hz, 1 H, NH Glu), 7.27–7.35 (m, 12 H, Fmoc arom. and Bn arom.) 7.41 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.60 (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, $CDCl_3$) δ 23.3 (CH_2, C_γ Pro), 26.4 (CH_2, C_β Glu), 29.9 (CH_2, C_γ Glu), 34.1 (CH_2, C_β Pro), 44.1 (CH_2, C_α Gly), 47.0 (CH, Fmoc CH), 48.5 (CH_2, C_δ Pro), 52.7 (CH, C_α Glu),

66.5 (CH_2, Bn CH_2), 67.1 (CH_2, Fmoc CH_2), 67.4 (CH_2, Bn CH_2), 72.0 (q, J = 28.8 Hz, CH, C_α Pro), 119.9, 124.9 (q, J = 286.6 Hz, CF_3), 125.1, 127.0, 127.6, 128.1, 128.2, 128.3, 128.5, 128.6, 135.0, 135.6, 141.2, 143.8, 156.1, 165.9, 167.9, 170.9, 172.9; ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –71.3 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{42}H_{41}F_3N_3O_8$ $[M+H]^+$: 772.2846; found 772.2881.

Fmoc-Gly-(*S*)-α-Tfm-Pro-D-Glu(OBn)-OBn (*S,R*)-7. To a solution of dipeptide (*S,R*)-**6** (67 mg, 1.12 mmol, 1 equiv) in DCM (1 mL) under argon was added Fmoc-Gly-Cl (86 mg, 2.24 mmol, 2 equiv). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave pure (*S,R*)-**7** (55 mg, 52%) as a white solid. Mp 110–112°C; $[\alpha]^{25}_D$ –21.4 (*c* 0.44, $CHCl_3$); IR (neat): 1728, 1681, 1519, 1450 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92–2.15 (m, 3 H, H_γ Pro and H_β Glu-Ha), 2.20 (m, 1 H, H_β Glu-Hb), 2.34–2.52 (m, 4 H, H_γ Glu and H_β Pro), 3.55 (q, J = 8.0 Hz 1 H, H_δ Pro-Ha), 3.67 (q, J = 8.0 Hz, 1 H, H_δ Pro-Hb), 3.97 (dd, J = 17.4, 4.0 Hz, 1 H, H_α Gly-Ha), 4.11–4.21 (m, 2 H, H_α Gly-Hb and Fmoc CH), 4.28 (dd, J = 10.3, 6.0 Hz, 1 H, Fmoc CH_2-Ha), 4.37 (dd, J = 10.3, 7.3 Hz, 1 H, Fmoc CH_2-Hb), 4.63 (m, 1 H, H_α Glu), 5.01 (d, J = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.09 (d, J = 12.1 Hz, 1 H, Bn CH_2-Hb), 5.10 (d, J = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.19 (d, J = 12.1 Hz, 1 H, Bn CH_2-Hb), 5.71 (m, 1 H, NH-Gly), 7.27–7.35 (m, 13 H, NH Glu, Fmoc arom. and Bn arom.), 7.40 (t, J = 7.6 Hz, 2 H, Fmoc arom.), 7.57 (d, J = 7.6 Hz, 2 H, Fmoc arom.), 7.76 (d, J = 7.6 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 22.9 (CH_2, C_γ Pro), 25.7 (CH_2, C_β Glu), 30.0 (CH_2, C_γ Glu), 34.4 (CH_2, C_β Pro), 44.2 (CH_2, C_α Gly), 46.9 (CH, Fmoc CH), 48.5 (CH_2, C_δ Pro), 52.3 (CH, C_α Glu), 66.8 (CH_2, Bn CH_2), 67.1 (CH_2, Fmoc CH_2), 67.3 (CH_2, Bn CH_2), 72.0 (q, J = 27.8 Hz, CH, C_α Pro), 119.9, 124.5 (q, J = 286.6 Hz, CF_3), 125.1, 127.0, 127.7, 127.9, 128.3, 128.4, 128.5, 128.6, 135.0, 135.3, 141.2, 143.8, 156.1, 166.2, 167.9, 171.0, 174.2; ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –71.1 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{42}H_{41}F_3N_3O_8$ $[M+H]^+$: 772.2846; found 772.2864.

Fmoc-Gly-(*R*)-α-Tfm-Pro-L-Glu(OBn)-OBn (*R,S*)-7. The tripeptide (*R,S*)-**7** was prepared following the General Procedure, using dipeptide (*R,S*)-**6** (190 mg, 0.39 mmol, 1 equiv) and Fmoc-Gly-Cl (243 mg, 0.77 mmol, 2 equiv) in DCM (2 mL). Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave pure (*R,S*)-**7** (159 mg, 53%) as a white solid; Mp 110-112°C; $[\alpha]^{25}_D$

+23.1 (*c* 0.45, $CHCl_3$); The spectral data of (*R*,*S*)-**7** were identical to those of (*S*,*R*)-**7**; HRMS (ESI-TOF) calcd. for $C_{42}H_{41}F_3N_3O_8$ $[M+H]^+$: 772.2846; found 772.2864.

Fmoc-Gly-(*R*)-α-Tfm-Pro-D-Glu(OBn)-OBn (*R*,*R*)-7. The tripeptide (*R*,*R*)-**7** was prepared following the General Procedure, using dipeptide (*R*,*R*)-**6** (62 mg, 0.13 mmol, 1 equiv) in DCM (1 mL) under argon was added Fmoc-Gly-Cl (80 mg, 0.26 mmol, 2 equiv. Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave pure (*R*,*R*)-**7** (70 mg, 72%) as a white solid; Mp 110–112°C; $[\alpha]^{25}_D$ +52.2 (*c* 0.25, $CHCl_3$); The spectral data of (*R*,*R*)-**7** were identical to those of (*S*,*S*)-**7**; HRMS (ESI-TOF) calcd. for $C_{42}H_{41}F_3N_3O_8$ $[M+H]^+$: 772.2846; found 772.2864.

H-Gly-(*S*)-α-Tfm-Pro-L-Glu(OH)-OH (*S*,*S*)-8. A solution of tripeptide (*S*,*S*)-**7** (440 mg, 0.57 mmol, 1 equiv) in MeOH (2.5 mL) was hydrogenated over 20% Pd/C (440 mg) at room temperature for 24 h under hydrogen atmosphere (1 bar). The reaction mixture was filtered and evaporated. The crude was precipitated in pentane and filtrated to afford pure (*S*,*S*)-**8** (116 mg, 55%) as a white solid; $[\alpha]^{25}_D$ –55.5 (*c* 1.3, H_2O); IR (neat): 3600-2750, 1667, 1538 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 1.90 (m, 1 H, H_β Glu-Ha), 1.98–2.18 (m, 3 H, H_γ Pro-H and H_β Glu-Hb), 2.28–2.38 (m, 3 H, H_β Pro-Ha and H_γ Glu-H), 2.55 (m, 1 H, H_β Pro-Hb), 3.65 (q, *J* = 8.0 Hz 1 H, H_δ Pro-Ha), 3.79–3.88 (m, 1 H, H_δ Pro-Hb), 3.98 (d, *J* = 16.7 Hz,1 H, H_α Gly-Ha), 4.09 (d, *J* = 16.7 Hz,1 H, H_α Gly-Hb), 4.21 (m, 1 H, H_α Glu-H); ^{13}C NMR (100.5 MHz, D_2O) δ 22.7 (CH_2, C_γ Pro), 27.0 (CH_2, C_β Glu), 30.9 (CH_2, C_γ Glu), 34.5 (CH_2, C_β Pro), 41.3 (CH_2, C_α Gly), 48.7 (CH_2, C_δ Pro), 54.4 (CH, C_α Glu), 71.8 (q, *J* = 28.8 Hz, CH, C_α Pro), 124.5 (q, *J* = 285.6 Hz, CF_3), 166.0, 167.8, 176.8, 178.6; ^{19}F NMR (376.2 MHz, D_2O) δ –71.7 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{13}H_{19}F_3N_3O_6$ $[M+H]^+$: 370.1226; found 370.1221.

H-Gly-(*R*)-α-Tfm-Pro-L-Glu(OH)-OH (*R*,*S*)-8. A solution of tripeptide (*R*,*S*)-**7** (159 mg, 0.21 mmol, 1 equiv) in MeOH (1.0 mL) was hydrogenated over 20% Pd/C (159 mg) at room temperature for 24 h under hydrogen atmosphere (1 bar). The reaction mixture was filtered and evaporated. The crude was precipitated in pentane and filtrated to afford pure (*R*,*S*)-**8** (41 mg, 54%) as a white solid; $[\alpha]^{25}_D$ –49.3 (*c* 0.85, H_2O); IR (neat): 3600-2750, 1667, 1538 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 1.90 (m, 1 H, H_β

Glu-Ha), 2.00–2.18 (m, 3 H, H_γ Pro-H and H_β Glu-Hb), 2.28–2.48 (m, 3 H, H_β Pro-Ha and H_γ Glu-H), 2.58 (m, 1 H, H_β Pro-Hb), 3.69 (m, 1 H, H_δ Pro-Ha), 3.87 (m, 1 H, H_δ Pro-Hb), 4.01 (d, J = 16.5 Hz, 1 H, H_α Gly-Ha), 4.15 (d, J = 16.5 Hz,1 H, H_α Gly-Hb), 4.20 (m, 1 H, H_α Glu-H); ^{13}C NMR (100.5 MHz, D_2O) δ 22.7 (CH_2, C_γ Pro), 26.1 (CH_2, C_β Glu), 31.7 (CH_2, C_γ Glu), 34.5 (CH_2, C_β Pro), 41.5 (CH_2, C_α Gly), 48.9 (CH_2, C_δ Pro), 54.9 (CH, C_α Glu), 71.8 (q, J = 27.8 Hz, CH, C_α Pro), 124.5 (q, J = 285.6 Hz, CF_3), 166.3, 168.0, 177.0, 179.6; ^{19}F NMR (376.2 MHz, D_2O) δ –71.5 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{13}H_{19}F_3N_3O_6$ $[M+H]^+$: 370.1226; found 370.1222.

CF_3-pseudoproline containing GPE analogues.

H-(*S*)-Ser(Ψ^{CF3,H}Pro)-L-Glu(OBn)-OBn (*S,S,S*)-10. To a solution of oxazolidine (*S,S*)-**9** (2 g, 10.0 mmol) in THF (40 mL) was slowly added at 0°C 1 M aqueous solution of LiOH (11 mL, 11 mmol, 1.1 equiv). The solution mixture was vigorously stirred at 0°C until the disappearance of the starting material (usually 1 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. A fraction of the crude lithium carboxylate (210 mg, 1.1 mmol) was diluted in DMF (6 mL), and L-glutamic acid dibenzylester tosylate salt (1.1 g, 2.2 mmol, 2.0 equiv), NEt_3 (630 μL, 4.5 mmol, 4.1 equiv), HOBt (223 mg, 1.65 mmol, 1.5 equiv),and EDCI (316 mg, 1.65 mmol, 1.5 equiv) were successively added at room temperature. The reaction mixture was stirred overnight at room temperature and then diluted with DCM and water. The layers were separated, and the aqueous layer was extracted with DCM (3×). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (70:30 cyclohexane/ethyl acetate) gave the dipeptide (*S,S,S*)-**10** (392 mg, 72%) as an inseparable 75:25 diastereomeric mixture. ^{1}H NMR (400 MHz, $CDCl_3$) (*major*) δ 2.08–2.12 (m, 1 H, H_β Glu-Ha), 2.20-2.31 (m, 1 H, H_β Glu-Hb), 2.32–2.50 (m, 2 H, H_γ Glu), 2.85 (m, 1 H, NH ΨPro), 3.81 (t, J = 7.0 Hz, 1 H, H_β ΨPro-Ha), 3.93 (t, J = 7.0 Hz, 1 H, H_α ΨPro), 4.17 (t, J = 7.8 Hz, 1 H, H_β ΨPro-Hb), 4.62–4.69 (m, 1 H, H_α Glu), 5.01 (q, J = 5.5 Hz, 1 H, H_δ ΨPro), 5.10 (s, 2 H, Bn CH_2-H), 5.14 (d, J = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.18

(d, J = 12.1 Hz, 1 H, Bn CH_2-Hb), 7.10 (d, J = 7.1 Hz, 1 H, NH-Glu), 7.26–7.40 (m, 10 H, Bn arom.); (*minor*) δ 2.08–2.12 (m, 1 H, H_β Glu-Ha), 2.20-2.31 (m, 1 H, H_β Glu-Hb), 2.32–2.50 (m, 2 H, H_γ Glu), 2.85 (m, 1 H, NH ΨPro), 3.86-3.93 (m, 2 H, H_β ΨPro-Ha and H_α ΨPro), 4.11 (t, J = 7.1 Hz, 1 H, H_β ΨPro-Hb), 4.62–4.69 (m, 1 H, H_α Glu), 4.90 (q, J = 5.1 Hz, 1 H, H_δ ΨPro), 5.10 (s, 2 H, Bn CH_2-H), 5.14 (d, J = 12.1 Hz, 1 H, Bn CH_2-Ha), 5.18 (d, J = 12.1 Hz, 1 H, Bn CH_2-Hb), 7.10 (d, J = 7.1 Hz, 1 H, NH-Glu), 7.26–7.40 (m, 10 H, Bn arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$) (*major*) δ 26.7 (CH_2, C_β Glu), 30.2 (CH_2, C_γ Glu), 52.0 (CH, C_α Glu), 59.3 (CH, C_α ΨPro), 66.7 (CH_2, Bn CH_2), 67.6 (CH_2, Bn CH_2), 70.2 (CH_2, C_β ΨPro), 87.9 (q, J = 33.6 Hz, CH, C_δ ΨPro), 123.1 (q, J = 283.7 Hz, CF_3), 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 134.9, 135.5, 170.0, 171.1, 172.7; (*minor*) δ 26.8 (CH_2, C_β Glu), 30.2 (CH_2, C_γ Glu), 51.8 (CH, C_α Glu), 59.3 (CH, C_α ΨPro), 66.6 (CH_2, Bn CH_2), 67.6 (CH_2, Bn CH_2), 70.0 (CH_2, C_β ΨPro), 87.9 (q, J = 33.6 Hz, CH, C_δ ΨPro), 123.0 (q, J = 282.8 Hz, CF_3), 128.3, 128.3, 128.4, 128.5, 128.6, 128.7, 134.9, 135.6, 170.2, 171.0, 172.5; ^{19}F NMR (376.2 MHz, $CDCl_3$) (*major*) δ –84.6 (d, J = 5.5 Hz, CF_3); (*minor*) δ –84.4 (d, J = 5.1 Hz, CF_3); HRMS (ESI-TOF) calcd. for $C_{24}H_{26}F_3N_2O_6$ $[M+H]^+$: 495.1743; found 495.1732.

The Fmoc-Gly-(*R*)-Ser($\Psi^{CF3,H}$Pro)-OMe (*R,S*)-**11** was prepared according to our previously reported procedure.[30]

Fmoc-Gly-(*R*)-Cys($\Psi^{CF3,H}$Pro)-OMe (*R,R*)-13. To a solution of (*R,R*)-**12** (3.0 g, 13.9 mmol, 1 equiv) in DCM (150 mL) was added Fmoc-Gly-Cl (4.820 g, 15.3 mmol, 1.1 equiv).The mixture is stirred at room temperature for 24 h then quenched with saturated $NaHCO_3$ solution (150 mL). The aqueous solution was extracted with DCM (3 x 100 mL). The combined organic extracts were washed with brine (150 mL) and were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture is purified by flash chromatography (80:20 cyclohexane/ethyl acetate) to afford pure dipeptide (*R,R*)-**13** (6.08 g, 89%) as a 57/43 inseparable mixture of rotational isomers in $CDCl_3$ at 298 K: yellow solid. Mp 72–74°C; $[\alpha]^{25}_D$ –95.9 (*c* 1.07, $CHCl_3$); IR (neat): 3342, 1716, 1682, 1519, 1118 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 323 K) δ 3.33-3.65 (m, 2 H, H_β ΨPro), 3.79 (s, 3 H, OMe), 4.12

(m, 1 H, H_α Gly-Ha), 4.22 (t, J = 6.9 Hz, 1 H, Fmoc CH), 4.35-4.47 (m, 3 H, H_α Gly-Hb and Fmoc CH_2), 5.03 (m, 1 H, H_α ΨPro), 5.69 (m, 1 H, NH, Gly), 6.02 (m, 1 H, H_δ ΨPro), 7.31 (t, J = 7.3 Hz, 2 H, Fmoc arom.), 7.40 (t, J = 7.3 Hz, 2 H, Fmoc arom.), 7.59 (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, $CDCl_3$, 323 K) δ 31.9 (CH_2, C_β ΨPro), 43.2 (CH_2, C_α Gly), 47.2 (CH, Fmoc CH), 53.0 (CH_3, OMe), 62.2 (q, J = 30.7 Hz, C_δ ΨPro), 62.8 (CH, C_α ΨPro), 67.4 (CH_2, Fmoc CH_2), 119.9, 124.2 (q, J = 281.8 Hz, CF_3), 125.0, 127.1, 127.7, 141.3, 143.8, 156.3, 168.4, 168.9; ^{19}F NMR (376.2 MHz, $CDCl_3$, 298 K) δ (*majo* rotamer) δ –77.1 (s, CF_3), (*mino* rotamer) δ –77.3 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{23}H_{21}F_3N_2O_5SNa$ $[M+Na]^+$: 517.1021; found: 517.1028.

Fmoc-Gly-(*R*)-Ser(Ψ^{CF3,H}Pro)-L-Glu(OBn)-OBn (*R,S,S*)-14. The saponification of the dipeptide methyl ester (*R*,*S*)-**11** (15.0 g, 31.4 mmol) was performed following a described method by addition of NaOH (1.5 g, 37.6 mmol, 1.2 equiv) to a 0.8 M $CaC1_2$ solution in iPrOH-H_2O 7:3 (715 mL).[61] The reaction mixture was stirred for 12 h at room temperature, quenched with 1 M HCl, concentrated under reduced pressure and diluted with H_2O (200 mL). The aqueous solution was extracted with ethyl acetate (3 ×). The combined organic layers were washed with H_2O, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude acid was used in the next step without further purification. To a solution of the crude acid (6.0 g, 12.9 mmol, 1 equiv) in DCM (690 mL) were added L-glutamic dibenzylester tosylate salt (8.06 g, 16.1 mmol, 1.25 equiv) and Et_3N (5.5 mL, 39.7 mmol, 3.2 equiv). After 20 min at room temperature BOP-Cl (4.26 g, 16.8 mmol, 1.3 equiv) was added and the mixture was stirred at room temperature for 24 h then quenched with water. The aqueous solution was extracted with DCM (3 x 200 mL). The organic layer was washed with brine (100 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture is purified by flash chromatography (80:20 cyclohexane/ethyl acetate) to give pure (*R*,*S*,*S*)-**14** (6.82 g, 68%) as a 57/43 inseparable mixture of rotational isomers in $CDCl_3$ at 298 K: yellow oil; $[\alpha]^{25}_D$ –27.2 (*c* 0.9, $CHCl_3$); IR (neat): 3310, 2928, 1731, 1677, 1529, 1150, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ (mixture of rotational isomers) 2.00-2.12 (m, 1 H, H_β Glu-Ha), 2.21-2.32

(m, 1 H, H_β Glu-Hb), 2.34-2.54 (m, 2 H, H_γ Glu), 3.82-4.02 (m, 2 H, H_α Gly), 4.17-4.26 (m, 2 H, Fmoc CH and H_β ΨPro-Ha), 4.34-4.44 (m, 3 H, Fmoc CH_2 and H_β ΨPro-Hb), 4.67-4.77 (m, 1 H, H_α Glu), 5.02-5.20 (m, 5 H, H_α ΨPro and Bn CH_2), 5.69-6.09 (m, 2 H, H_δ Pro and NH-Gly), 7.12-7.21 (m, 1 H, NH Glu), 7.27-7.37 (m, 12 H, Bn arom. and Fmoc arom.), 7.41 (t, J = 7.3 Hz, 2 H, Fmoc arom.), 7.62 (d, J = 7.3 Hz, 2 H, Fmoc arom.), 7.77 (d, J = 7.3 Hz, 2 H, Fmoc arom.); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ (mixture of rotational isomers) 26.8 (CH_2, C_β Glu), 30.0 (CH_2, C_γ Glu), 43.3 and 44.0 (CH_2, C_α Gly), 46.8 (CH, Fmoc CH), 51.6 and 51.9 (CH, C_α Glu), 58.4 (CH, C_α ΨPro), 66.4 (CH_2, Bn CH_2), 67.0 (CH_2, Fmoc CH_2), 67.2 (CH_2, Bn CH_2), 67.3 (Fmoc CH_2), 67.4 (CH_2, C_β ΨPro), 84.7 (CH, C_δ Pro), 119.8, 122.5 (q, J = 287.5 Hz, CF_3), 124.9, 126.9, 127.6, 128.0, 128.1, 128.3, 128.4, 134.9, 135.4, 141.0, 143.5, 143.6, 156.5, 168.0, 169.2, 170.9, 171.3, 172.4, 172.5; ^{19}F NMR (376.2 MHz, $CDCl_3$) δ (mixture of rotational isomers) –81.8 (s, CF_3) and -81.3 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{41}H_{39}F_3N_3O_9$ $[M+H]^+$: 774.2638; found 774.2653.

H-Gly-(*R*)-Ser(Ψ^{CF3,H}Pro)-L-Glu(OH)-OH-(*R,S,S*)-15. A solution of tripeptide (*R*,*S*,*S*)-**14** (4.0 g, 5.16 mmoles, 1 equiv.) in MeOH (40 mL) was hydrogenated over 20% Pd/C (4.0 g) at room temperature for 24 h under 1 bar pressure of hydrogen. The reaction mixture was filtered and concentrated under reduced pressure. Water and Et_2O were added, aqueous phase was washed with Et_2O (3 x 50 mL). The aqueous phase was concentrated under reduced pressure to give pure deprotected peptide (*R*,*S*,*S*)-**15** (1.75 g, 72 %) as a single rotational isomer in D_2O at 353 K: yellow solid; $[\alpha]^{25}_D$ –31.1 (*c* 1.2, H_2O); IR (neat): 3600-2750, 1667, 1538 cm^{-1}; ^{1}H NMR (400 MHz, D_2O, 353K) δ 2.38-2.52 (m, 1 H, H_β Glu-Ha), 2.56-2.70 (m, 1 H, H_β Glu-Hb), 2.82-2.91 (m, 2 H, H_γ Glu), 4.57-4.62 (m, 2 H, H_α Gly), 4.80-4.92 (m, 2 H, H_α Glu, H_β ΨPro-Ha), 5.10-5.20 (m, 1 H, H_β ΨPro-Hb), 5.40-5.50 (m, 1 H, H_α ΨPro), 6.45-6.52 (m, 1 H, H_δ ΨPro); ^{13}C NMR (100.5 MHz, D_2O, 353K) δ 27.7 (CH_2, C_β Glu), 31.7 (CH_2, C_γ Glu), 41.6 (CH_2, C_α Gly), 55.3 (CH, C_α Glu), 59.4 (CH, C_α ΨPro), 71.1 (CH_2, C_β ΨPro), 85.5 (q, J = 34.5 Hz, C_δ ΨPro), 123.0 (q, J = 285.6 Hz, CF_3), 168.3, 169.1, 177.2, 178.8; ^{19}F NMR (376.2 MHz, D_2O, 353K) δ –80.4 (s, CF_3); HRMS (ESI-TOF) calcd. for $C_{12}H_{17}F_3N_3O_7$ $[M+H]^+$: 372.1019; found 372.1019.

2-CF_3-oxazolidine containing GPE analogues

The compound **16** was prepared as a mixture of diastereomer (75:25) according to our previously reported procedure.[20]

2-CF_3-ΨPro-OEt-17. To a solution of *N*-Boc-ethanolamine (5.200 g, 33 mmol, 1 equiv) in toluene (100 mL) was added PPTS (829 mg, 3.3 mmol, 0.1 equiv) and ethyl trifluoropyruvate (5.610 g, 102 mmol, 1.2 equiv). After stirring the mixture for 1 h at room temperature, the reaction was heated to reflux with a Dean Stark apparatus for 24 h then cooled to 0°C, filtrated and concentrated under reduced pressure. Purification by flash chromatography (90:10 cyclohexane/ethyl acetate) gave a racemic mixture of **17** (5.320 g, 44 %) as a yellow oil. IR (neat): 3342, 2987, 2904, 1742, 1448, 1372, 1322, 1279, 1232, 1177, 1016, 987, 943, 858, 817, 747, 681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 7.1 Hz, H_{Et}), 3.16 (q, 1H, J = 9.9 Hz, H_δ-Ha), 3.32 (bs, 1H, NH), 3.38-3.45 (m, 1H, H_δ-Hb), 3.77 (q, 1H, J = 7.9 Hz, H_γ–Ha), 4.15 (ddd, 1H, J = 7.9 Hz, 6.9 Hz, 2.9 Hz, H_γ-Hb), 4.27-4.42 (m, 2H, H_{Et}); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 13.6 (C_{Et}), 46.6 (C_δ), 63.6 (C_{Et}), 68.5 (C_γ), 93.3 (q, J= 31.6 Hz, C_α), 122.7 (q, J = 286.6 Hz, CF_3), 166.2 (CO); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –83.1 (s, CF_3). HRMS (ESI-TOF) calcd. $C_7H_{11}F_3NO_3$ $[M+H]^+$: 214.0691, found 214.0695.

Fmoc-Gly-2-CF_3-ΨPro-OEt (18). To a solution of **17** (220 mg, 1.03 mmol, 1 equiv) in DCM (3 mL) was added Fmoc-Gly-Cl (490 mg, 1.55 mmol, 1.5 equiv). The mixture is stirred at room temperature for 24 h then quenched with saturated $NaHCO_3$ solution (5 mL). The aqueous solution was extracted with DCM (3 x 10 mL). The organic layer was washed with a saturated NaCl solution (10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture is purified by flash chromatography (80:20 cyclohexane/ethyl acetate) to give the racemic dipeptide **18** (378 mg, 75 %) as a white solid. Mp 54-56°C; IR (neat): 3330, 2922, 1758, 1715, 1682, 1203, 1170, 1033, 759, 738 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (t, J = 7.3 Hz, 3H, Et), 3.7 (dd, J = 8.2, 7.8 Hz,1H, H_δ ΨPro-Ha), 3.92 (m, 1H, H_δ ΨPro-Hb), 4.02 (dd, J = 17.4, 4.6 Hz, 1H, H_α Gly-Ha), 4.11 (dd, J = 17.4, 6.9 Hz, 1H, H_α Gly-Hb), 4.18 (dd, J = 7.3, 7.3 Hz, 1H, CHFmoc),

4.29 (q, J = 7.3 Hz, 2H, H_{Et}), 4.35-4.39 (m, J = 7.3 Hz, 4H, H_γ ΨPro and CH_2Fmoc), 5.72 (bs, 1H, NH), 7.28 (t, J = 7.3, 2H, H_{Ar}Fmoc), 7.38 (t, J = 7.3 Hz, 2H, H_{Ar}Fmoc), 7.57 (d, J = 7.3 Hz, 2H, H_{Ar}Fmoc), 7.74 (d, J = 7.3 Hz, 2H, H_{Ar}Fmoc); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 13.9 (C_{Et}), 44.1 (C_α Gly), 45.2 (C_δ ΨPro), 47.1 (CHFmoc), 63.1 (C_{Et}), 67.4(C_γ ΨPro), 69.0 (CH_2Fmoc), 89.5 (q, J = 32.6 Hz, C_α ΨPro), 120.1 (CFmoc), 122.4 (q, J = 288.5 Hz, CF_3), 125.2, 127.2, 127.9, 141.4, 143.8 (CFmoc), 156.3, 163.5, 167.1 (CO); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ −79.7 (s, CF_3); DI-HRMS calcd. for $C_{24}H_{23}F_3N_2O_6$ $[M]^+$: 492.1508, Found 492.1491.

Fmoc-Gly-2-CF_3-ΨPro-L-Glu(OBn)-OBn (19). To a solution of calcium chloride (0.84 M) in isopropanol/water (7:3) (11 mL) were added the compound **18** (237 mg, 0.48 mmol, 1 equiv) and NaOH (23 mg, 0.58 mmol, 1.2 equiv).[61] The mixture was stirred at room temperature for 24 h then quenched by HCl 1 M. The aqueous solution was extracted with ethyl acetate (3 x 10 mL). The organic layer was washed with a saturated NaCl solution (10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the intermediate acid which was used directly in the next step without further purification. To a solution of the crude carboxylic acid intermediate (173 mg, 0.37 mmol, 1 equiv) in DCM (20 mL) were added L-Glu(OBn)-OBn (240 mg, 0.48 mmol, 1.30 equiv) and Et_3N (120 mg, 1.19 mmol, 3.2 equiv). After 20 min at room temperature BOP-Cl (122 mg, 254.57 mmol, 1.3 equiv) was added and the mixture was stirred at room temperature for 24 h then quenched with water. The aqueous solution was extracted with DCM (3 x 10 mL). The organic layer was washed with a saturated NaCl solution (10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (50:50 cyclohexane/ethyl acetate) to give access to the separate tripeptide diastereomers $\mathbf{19_{maj}}$ (85 mg, 30 %) and $\mathbf{19_{min}}$ (64 mg, 22 %).

The diastereomer $\mathbf{19_{maj}}$ was obtained as colorless foam. $[\alpha]^{20}_D$ +15.0 (c 0.2, $CHCl_3$); IR (neat): 3375, 2924, 1694, 1524, 1451, 1164, 1125, 1003, 740, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01-2.10 (m, 1H, H_β Glu-Ha), 2.19-2.29 (m, 1H, H_β Glu-Hb), 2.36-2.43 (m, 2H, H_γ Glu), 3.71 (dd, J = 8.2, 7.3 Hz, 1H, H_δ ΨPro-Ha), 3.86 (dd, J = 8.0, 6.2 Hz, 1H, H_δ ΨPro-Hb), 4.02-4.07 (m, 2H, H_α Gly), 4.18

(dd, J = 7.3, 6.9 Hz, 1H, CHFmoc), 4.29-4.40 (m, 4H, CH_2Fmoc and H_γ ΨPro), 4.63-470 (m, 1H, H_α Glu), 5.03 (d, J = 14.6 Hz,1H, HBn), 5.06 (d, J = 14.6 Hz, 1H, HBn), 5.12 (d, J = 12.4 Hz, 1H, HBn), 5.14 (s, 1H, NH), 5.19 (d, J = 12.4 Hz, 1H, HBn), 5.65 (s, 1H, NH), 7.28-7.33 (m, 12 H, H_{Ar}Fmoc and H_{Ar}), 7.40 (t, J = 7.3 Hz, 2 H, H_{Ar}Fmoc), 7.51 (d, J = 7.3 Hz, 1H, NH), 7.59 (d, J = 7.8 Hz, 2 H, H_{Ar}Fmoc), 7.75 (d, J = 7.8 Hz, 2 H, H_{Ar}Fmoc); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 26.6 (C_β Glu), 30.0 (C_γ Glu), 44.3 (C_α Gly), 45.5 (C_δ ΨPro), 47.1 (CHFmoc), 52.4 (C_α Glu), 66.8, 67.4, 67.7, 67.9 (CBn CH_2Fmoc and C_γ ΨPro), 90.9 (q, J = 30.7 Hz, C_α ΨPro), 120.1 (CFmoc), 122.6 (q, J = 289.5 Hz, CF_3), 125.2, 127.2, 127.8, 128.2, 128.4, 128.7, 128.7 (CFmoc), 135.5, 141.2, 143.7 (CFmoc), 156.2, 162.6, 167.0, 170.7, 173.2 (CO); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –77.5 (s, CF_3). HRMS (ESI-TOF) calcd. for $C_{41}H_{39}F_3N_3O_9$ $[M+H]^+$: 774.2638, found 774.2643.

The diastereomer **19_{min}** is obtained as colorless foam. $[\alpha]^{20}_D$ −15.5 (*c* 0.5, $CHCl_3$); IR (neat): 3333, 2916, 1694, 1246, 1207, 1164, 1125, 1103, 1081, 1002, 758, 740, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00-2.17 (m, 1H, H_β Glu-Ha), 2.20-2.36 (m, 1H, H_β Glu-Hb), 2.39-2.53 (m, 2H, H_γ Glu), 3.73 (dd, J = 15.1, 7.3 Hz, 1H, H_δ ΨPro-Ha), 3.94 (dd, J = 15.1, 6.9 Hz, 1H, H_δ ΨPro-Hb), 4.05-4.09 (m, 2H, H_α Gly), 4.21 (dd, J = 7.8, 6.9 Hz, 1H, CHFmoc), 4.30-4.40 (m, 4H, CH_2Fmoc and H_γ ΨPro), 4.60-4.70 (m, 1H, H_α Glu), 5.08 (AB, 2H, HBn), 5.14 (AB, 2H, HBn), 5.75 (s, 1H, NH), 7.25-7.43 (m, 15 H, H_{Ar}Fmoc, $H_{Ar,}$ and NH), 7.60 (d, J = 7.3 Hz, 2 H, H_{Ar}Fmoc), 7.76 (d, J = 7.3 Hz, 2H, H_{Ar}Fmoc); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 26.4 (C_β Glu), 29.9 (C_γ Glu), 44.1 (C_α Gly), 45.5 (C_δ ΨPro), 47.0 (CHFmoc), 52.3 (C_α Glu), 66.5, 67.2, 67.4, 67.8 (CBzl CH_2Fmoc and C_γ ΨPro), 90.7 (q, J = 32.6 Hz, C_α ΨPro), 119.9 (CFmoc), 122.4 (q, J = 291.7 Hz, CF_3), 125.0, 127.0, 127.6, 128.1, 128.2, 128.4, 128.5 (CFmoc), 134.9, 135.6, 141.1, 143.7 (CFmoc), 156.2, 162.7, 167.2, 170.8, 172.8 (CO); ^{19}F NMR (376.2 MHz, $CDCl_3$) δ –77.5 (s, CF_3). HRMS (ESI-TOF) calcd. for $C_{41}H_{39}F_3N_3O_9$ $[M+H]^+$: 774.2638, Found 774.2660.

H-Gly-2CF_3-ΨPro-L-Glu(OH)-OH (20). The diastereomer **19_{min}** (86 mg, 0.11 mmole, 1 equiv) in MeOH (2 mL) was hydrogenated over 10% Pd/C (86 mg) at room temperature for 24 h under 1 bar

pressure of hydrogen. The reaction mixture was filtered, concentrated under reduced pressure and purified by reverse phase semi-preparative HPLC to give pure deprotected tripeptide **23** as a mixture of two conformers (59/41 determined by ^{19}F NMR) (20 mg, 49 %). White solid. ^{1}H NMR (400 MHz, D_2O, 353K) δ 1.99 (ddd, J = 14.2, 7.1, 6.9 Hz, 1H, H_β Glu-Ha), 2.22 (ddd, J = 14.2, 8.2, 6.9 Hz, 1H, H_β Glu-Hb), 2.42 (t, J = 6.9 Hz, 2H, H_γ Glu), 3.91 (dt, J = 8.2, 7.1 Hz, 1H, H_α Gly-Ha), 4.02-4.10 (m, 3 H, H_α Gly-Hb, H_δ ΨPro), 4.42-4.52 (m, 3 H, Ha Glu, H_γ ΨPro); ^{13}C NMR (100.5 MHz, D_2O, 353K) δ 27.3 (CH_2, C_β Glu minor), 27.8 (CH_2, C_β Glu major), 31.1 (CH_2, C_γ Glu minor), 31.5 (CH_2, C_γ Glu major), 42.4 (CH_2, C_α Gly major), 45.1 (CH_2, C_α Gly minor), 46.6 (CH_2, C_δ ΨPro major), 46.9 (CH_2, C_δ ΨPro minor), 54.4 (CH, C_α Glu), 67.8 (CH_2, C_γ ΨPro minor), 69.5 (CH_2, C_γ ΨPro major), 91.8 (C_α ΨPro), 127.3 (q, J = 302.9 Hz, CF_3), 162.5 (CO Gly minor), 164.9 (CO Gly major), 165.9 (CO ΨPro), 175.9 (C_δ Glu), 177.0 (CO Glu); ^{19}F NMR (376.2 MHz, D_2O, 353K) δ –79.9 (s, CF_3 major), –78.8 (s, CF_3, minor); HRMS (ESI-TOF) calcd. for $C_{12}H_{17}F_3N_3O_7$ $[M+H]^+$: 372.1019, Found 372.1023.

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Notes

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ASSOCIATED CONTENT

Supporting Information

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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