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Convenient Synthesis of *N*-Terminal Tfm-Dipeptides from Unprotected Enantiopure α-Tfm-Proline and α-Tfm-Alanine

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A convenient procedure for the synthesis of highly lipophilic dipeptide building blocks from enantiopure α-trifluoromethyl α-amino acids is reported. Coupling reactions at the C termini of the trifluoromethyl α-amino acids were successfully performed with totally unprotected amino acids without formation of diketopiperazines. The synthesis of a tripeptide through a coupling reaction at the deactivated N-terminal position was achieved.

Introduction

The major disadvantages of peptides as therapeutic agents are their rapid degradation by peptidases and their low lipophilicities. One commonly adopted strategy to improve the stability of the peptide bond is the incorporation of α,α-disubstituted amino acids in the peptide chain. These constrained amino acids are also known to induce conformational restrictions, improving interaction with receptor sites.

In this context, α-trifluoromethyl α-amino acids (α-Tfm-AAs) are very attractive compounds for the design of biologically active molecules.[1] The incorporation of amino acids that contain trifluoromethyl groups into peptides can result in increased chemical and thermal stability, increased resistance to degradation by proteases,[2] and enhanced hydrophobicity, giving better affinities for lipid membranes. Moreover, their incorporation into peptides can induce stabilization of particular conformations and better auto-assembly.[3] Additionally, the trifluoromethyl group can serve as a label for ^{19}F NMR studies.[4]

Unfortunately, development of the introduction of α-trifluoromethyl α-amino acids (α-Tfm-AAs) into peptides is seriously limited by the fact that there are very few synthetic methods available for their synthesis in enantiopure form.[5] For this reason the chemical synthesis of α- peptides containing Tfm-AAs has mainly been performed with racemic mixture of the fluorinated amino acids.[6] To obtain enantiomerically pure peptides, this strategy requires tedious HPLC separations of diastereomeric mixtures.[6c–6f] As a complementary strategy, protease-catalyzed peptide synthesis has also proved to be efficient for the incorporation of sterically demanding racemic α-Tfm-AAs into peptides.[7] Recently, two methods involving original formation of the amide bond for the synthesis of dipeptides containing α-Tfm-AAs have been reported, but these methods were not well-suited for the production of enantiopure materials.[8]

Intriguingly, the synthesis of dipeptides containing α-Tfm-AAs by conventional peptide coupling of enantiopure fluorinated amino acids has not yet been extensively exploited, but in the course of our studies we have recently reported highly efficient and scalable methodologies for the synthesis of enantiopure α-Tfm-AAs.[9] Here we can now report coupling reactions of these enantiopure amino acids at their C termini, which should result in the design of new dipeptide building blocks with increased lipophilicity at their N-terminal positions; α-fluorination dramatically decreases the protonation capability of the nitrogen atom. Peptide coupling reactions generally require the use of *N*-protected amino acids in order to prevent the formation of diketopiperazines. Because of the decrease in the nucleophilicity of the nitrogen atom in an α-Tfm-amino acid, due to the strongly electron-withdrawing nature of fluorine atoms, however, we anticipated that coupling reactions of these compounds at their C termini might be achievable with unprotected amino acids. This would constitute a major improvement in the methodology of peptide coupling of fluorinated amino acids in relation to the case of nonfluorinated amino acids.

Results and Discussion

We recently reported a convenient gram-scale strategy for the synthesis of (*S*)- and (*R*)-α-Tfm-prolines [(*S*)-**1** and (*R*)-**1**].[9c] By this procedure we were able to prepare substantial amounts of enantiopure materials with which to undertake peptide coupling methodological studies (Scheme 1).

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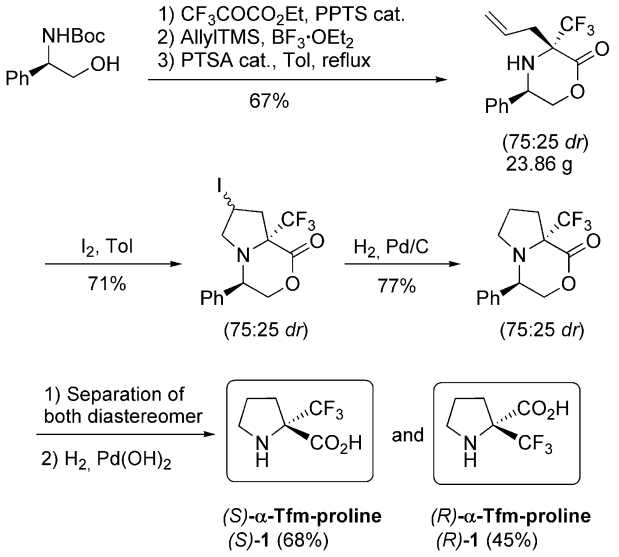
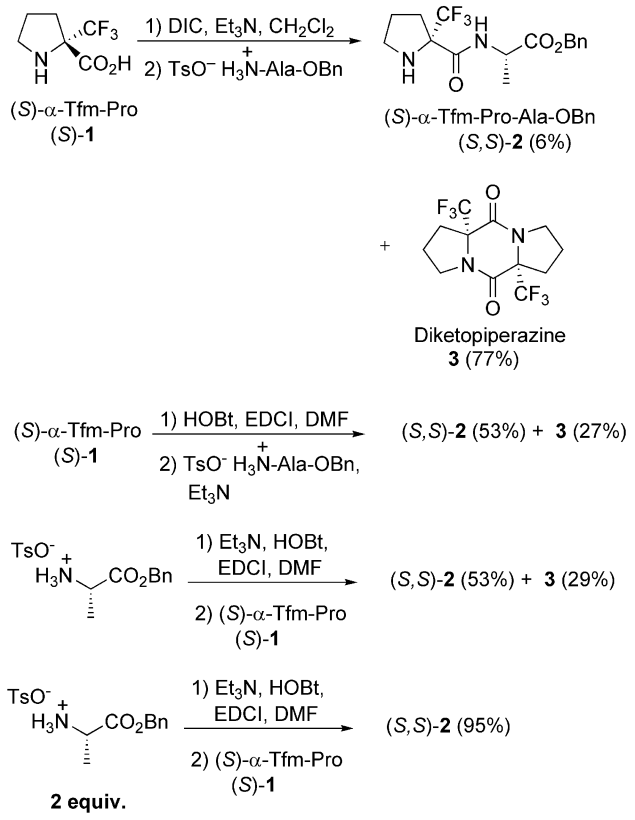


Scheme 1. Synthesis of (*S*)- and (*R*)-α-Tfm-prolines [(*S*)-**1** and (*R*)-**1**].[9c]

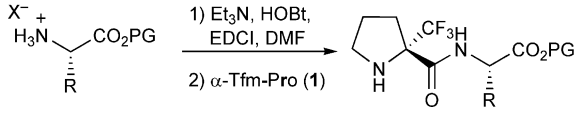
To the best of our knowledge the only example of an α-Tfm-pyrrolidine-type amino acid C-terminal coupling reaction reported in the literature involved α-Tfm-pyroglutamic acid derivatives in the racemic series.[10] As we anticipated that α-Tfm-amino acids should be deployable in peptide coupling reactions without protection of their nitrogen atoms, the enantiopure (*S*)-α-Tfm-proline [(*S*)-**1**] was subjected to DIC-mediated coupling with H_2N-Ala-OBn (Scheme 2). This procedure had previously proved to be efficient for the coupling of *N*-protected α-Tfm-alanine.[2] Unfortunately, though, under these conditions the expected dipeptide (*S*,*S*)-**2** was only obtained in 6% yield and the major product of the reaction was the diketopiperazine **3** (77%). The formation of the unexpected diketopiperazine was decreased to a 27–29% yield, however, when the less reactive HOBt/EDCI coupling system was used, whatever the order of introduction of the fluorinated and the non-fluorinated amino acid. The α-Tfm-Pro-Ala dipeptide (*S*,*S*)-**2** was in this case obtained in 53% yield. The isolated yield of (*S*,*S*)-**2** was increased to 95% when one equivalent of α-Tfm-proline [(*S*)-**1**] was added to two equivalent amounts of the non-fluorinated amino acid in the presence of the coupling reagent (Scheme 2). The formation of the diketopiperazine **3** was not observed under these conditions.

These optimized conditions were then successfully applied to couplings of various amino acids with unprotected α-Tfm-prolines (Table 1). The enantiopure dipeptides derived from (*S*)-**1** and alanine, valine, phenylalanine, and leucine were conveniently obtained in good yields by this procedure (Table 1, Entries 1, 3–5). The coupling reaction starting from (*R*)-α-Tfm-proline [(*R*)-**1**] was also very efficient (Table 1, Entry 2). The formation of the diketopiperazine **3** was avoided in all cases.



Scheme 2. Methodological study of the coupling of unprotected α-Tfm-proline at its C terminus.

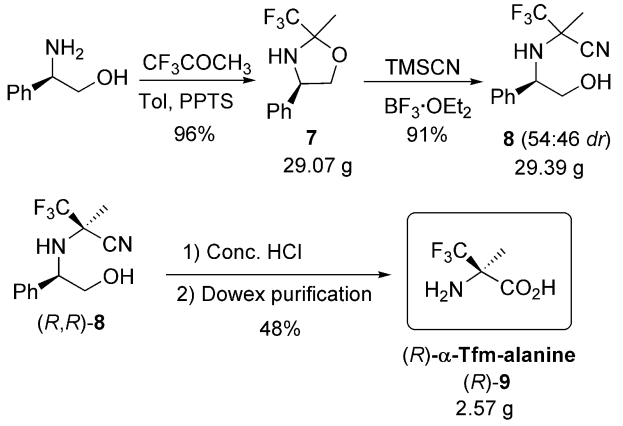
Table 1. Synthesis of α-Tfm-Pro dipeptides.[a]



Entry	X	R	PG	α-Tfm-Pro	Product (yield, %)[b]
1	TsO	Me	Bn	(*S*)-**1**	(*S*,*S*)-**2** (95)
2	TsO	Me	Bn	(*R*)-**1**	(*R*,*S*)-**2** (87)
3	Cl	*i*Pr	*t*Bu	(*S*)-**1**	(*S*,*S*)-**4** (87)
4	Cl	Bn	*t*Bu	(*S*)-**1**	(*S*,*S*)-**5** (76)
5	TsO	*i*Bu	Bn	(*S*)-**1**	(*S*,*S*)-**6** (80)

[a] Reaction conditions: HX·H_2N-AA-OGP (2 equiv.), Et_3N (4.1 equiv.), HOBt (1.5 equiv.), EDCI (1.5 equiv.), and α-Tfm-Pro (1 equiv.). [b] Isolated yield.

In order to extend the methodology to acyclic α-Tfm-amino acids, the coupling of α-Tfm-alanine was considered. Enantiopure (*R*)-Tfm-alanine [(*R*)-**9**, Scheme 3] was synthesized by an improved multigram scale procedure involving a Strecker-type reaction from the trifluoromethylated oxazolidines (Foxs) **7**.[9a] After separation of the two diastereomers, the hydrolysis of the amino nitrile (*R*,*R*)-**8** and the removal of the phenylglycinol side chain were performed in the same step by acidic treatment (Scheme 3).



Scheme 3. Multigram scale synthesis of enantiopure α-Tfm-alanine [(*R*)-**9**].

Coupling reactions between (*R*)-**9** and non-fluorinated α-amino acids were then achieved by the procedure designed for the α-Tfm-proline. The corresponding dipeptides were conveniently obtained in 77–82% isolated yields without formation of diketopiperazines (Table 2).

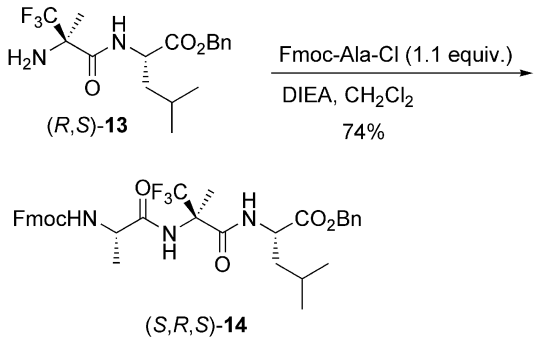
Table 2. Synthesis of α-Tfm-Ala dipeptides.[a]

Entry	X	R	PG	Product (yield, %)[b]
1	TsO	Me	Bn	(*R*,*S*)-**10** (82)
2	Cl	*i*Pr	*t*Bu	(*R*,*S*)-**11** (80)
3	Cl	Bn	*t*Bu	(*R*,*S*)-**12** (77)
4	TsO	*i*Bu	Bn	(*R*,*S*)-**13** (81)

[a] Reactions conditions: $HX{\cdot}H_2N$-AA-OGP (2 equiv.), Et_3N (4.1 equiv.), HOBt (1.5 equiv.), EDCI (1.5 equiv.), and (*R*)-α-Tfm-Ala (1 equiv.). [b] Isolated yield.

The dipeptides containing the fluorinated amino acids are highly stable both in the Tfm-proline and in the Tfm-alanine series. No cyclization into diketopiperazines had occurred after several months. This high stability is naturally related to the low nucleophilicity of the amino group.

As a proof-of-concept of the incorporation of enantiopure α-trifluoromethyl α-amino acids into peptide chains, coupling of the (*R*)-Tfm-Ala-L-Leu-OBn dipeptide (*R*,*S*)-**13** (Scheme 4) at its N terminus was investigated. Because of the strong deactivation of the amino group[11] and the steric bulkiness of the trifluoromethyl group, specific activation methods have to be used for N-terminal couplings of α-Tfm-amino acids. According to the pioneering works of Koksch et al.[5d] and Dal Pozzo et al.,[12,6e] highly electrophilic mixed anhydrides or amino acid bromides are required to achieve peptide coupling in good yields. In order to elaborate a tripeptide building block suitably protected for peptide synthesis we decided to perform the coupling reaction with a Fmoc-protected amino acid chloride; Fmoc-alanine chloride was efficiently prepared by a reported procedure.[13] Treatment of this amino acid chloride (1.1 equiv.) with the dipeptide (*R*,*S*)-**13**, containing the trifluoromethyl group, gave the corresponding tripeptide (*S*,*R*,*S*)-**14** in 74% yield without any epimerization (Scheme 4).



Scheme 4. Synthesis of the Fmoc-L-Ala-(*R*)-α-Tfm-Ala-L-Leu-OBn tripeptide (*S*,*R*,*S*)-**14**.

Conclusions

In conclusion, we report a convenient procedure for the synthesis of highly lipophilic dipeptide building blocks from unprotected enantiopure α-trifluoromethyl α-amino acids. To allow access to a tripeptide, the coupling reaction at the N-terminal position deactivated by the trifluoromethyl group requires the use of an amino acid chloride. We are currently investigating the scope of the incorporation of enantiopure α-trifluoromethyl α-amino acids into peptides and our results will be reported in due course.

Experimental Section

General: Unless otherwise mentioned, all the reagents were purchased from commercial sources. All glassware was dried in an oven prior to use. Ether and THF were distilled under nitrogen from sodium/benzophenone prior to use. CH_2Cl_2 was distilled under nitrogen from CaH_2 prior to use. ^1H NMR (400.00 MHz), ^{13}C NMR (100.50 MHz), and ^{19}F NMR (376.20 MHz) were measured with a JEOL 400 spectrometer. Chemical shifts of ^1H NMR were expressed in parts per million downfield from tetramethylsilane (δ = 0 ppm) in $CDCl_3$. ^{13}C NMR chemical shifts are expressed in parts per million downfield from $CDCl_3$ as internal standard (δ = 77.0 ppm). ^{19}F NMR chemical shifts are expressed in parts per million downfield from C_6F_6 as internal standard (δ = –164.9 ppm). Coupling constants are reported in Hertz. Column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm), with the specified mixtures of solvents as eluents. Thin-layer chromatography (TLC) was performed on Merck silica gel (Merck 60 PF_{254}) plates. Silica TLC plates were visualized under UV light and by use of a solution of phosphomolybdic acid in ethanol (10%) followed by heating. Gas chromatography (CPV) was performed on an Agilent 6890 N instrument (flame ionization detector) with

a polydimethylsiloxane column (HP ultra I, 25 m × 3.2 mm × 0.52 μm layer thickness). Mass spectra (MS) were obtained with a GC/MS apparatus (HP 5973 MSD) with an HP 6890 Series GC. Ionization was obtained by electronic impact (EI 70 eV). Infrared spectra (IR) were obtained by Fourier transformation with a Bruker Tensor 27 instrument; wavenumbers are given in cm^{-1}. Elemental analyses were performed by the CNRS analysis central service. Optical rotations are reported as their specific rotations at 25 °C in g/100 mL, determined with a JASCO P1010 polarimeter. Melting points were obtained on a Büchi apparatus and are uncorrected.

Synthesis of α-Tfm-amino Acids: (*R*)- and (*S*)-α-Tfm-prolines [(*R*)-**1** and (*S*)-**1**] were prepared by our previously reported procedure.[9c]

Multigram Synthesis of Enantiopure (*R*)-α-Tfm-alanine. (2*S*,4*R*)-2-Methyl-4-phenyl-2-trifluoromethyl-1,3-oxazolidine (7): (*R*)-Phenylglycinol (17.98 g, 131 mmol) was added at 0 °C to a solution of trifluoroacetone (14.4 mL, 161 mmol, 1.23 equiv.) in toluene (680 mL), followed by the addition of PPTS (3.34 g, 0.1 equiv.). The reaction mixture was stirred for 1 h at room temperature and then warmed to reflux under a Dean–Stark apparatus. The reaction progress was monitored by ^1H NMR after each 24 h, and trifluoroacetone (7 mL, 78 mmol, 0.6 equiv.) was added to the resulting mixture at 0 °C until the disappearance of the (*R*)-phenylglycinol (usually 57 h). The reaction mixture was cooled to 0 °C with an ice-bath and filtered, and toluene was evaporated. The crude oxazolidine **7** (29.07 g, 96%) was obtained as a single diastereomer and was used in the next step without further purification. A pure analytical sample of **7** was isolated by flash chromatography (petroleum ether/AcOEt, 90:10); yellow oil, $[\alpha]_D = -23.2$ ($c = 1.8$, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.62$ (s, 3 H, Me), 2.25 (d, $J = 8.7$ Hz, 1 H, NH), 3.82 (t, $J = 7.8$ Hz, 1 H, 5-Ha), 4.40 (t, $J = 7.8$ Hz, 1 H, 5-Hb), 4.58 (dt, $J = 8.7$, 7.8 Hz, 1 H, 4-H), 7.27–7.42 (m, 5 H, Ph) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 20.6$ (CH_3, Me), 62.0 (CH, C4), 73.6 (CH_2, C5), 94.2 (q, $J = 30.8$ Hz, C2, C), 124.8 (q, $J = 287.2$ Hz, CF_3, C), 126.7 (2 × CH, Ph), 128.2 (CH, Ph), 128.9 (2 × CH, Ph), 138.8 (C, Ph) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): $\delta = -85.7$ (s, CF_3) ppm. IR (neat): $\tilde{\nu} = 3356$, 3033, 2999, 1458, 1338, 1156 cm^{-1}. MS (EI): *m*/*z* (%) = 232 $[M + H]^+$, 200, 162 (100), 132, 120, 77. $C_{11}H_{12}F_3NO$ (231.09): calcd. C 57.14, H 5.23, N 6.06; found C 56.80, H 5.05, N 5.85.

3,3,3-Trifluoro-2-[(1*R*)-2-hydroxy-1-phenylethylamino]-2-methylpropionitrile (8): Cyanotrimethylsilane (25 mL, 186.8 mmol, 1.5 equiv.) and $BF_3{\cdot}OEt_2$ (23.7 mL, 186.8 mmol) were added under argon at 0 °C to a solution of the crude oxazolidine **7** (28.8 g, 124.5 mmol) in dichloromethane (400 mL). The reaction mixture was stirred at room temperature until disappearance of the starting material (20 h, GC monitoring). The reaction mixture was then poured into saturated aqueous $NaHCO_3$ (400 mL) and vigorously stirred for 1 h. The aqueous layer was extracted with dichloromethane (3 × 100 mL), and the combined organic extracts were washed with water (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash chromatography (petroleum ether/AcOEt, 85:15) gave pure isolated fractions of (*R*,*R*)-**8** (17.44 g, 54%) and (*S*,*R*)-**8** (11.95 g, 37%).

Diastereoisomer (*R*,*R*)-8: Pale yellow oil, $R_f = 0.46$ (petroleum ether/AcOEt,, 85:15). $[\alpha]_D = -139.0$ ($c = 1.0$, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.32$ (s, 3 H, Me), 1.91–1.98 (m, 1 H, OH), 2.84 (s, 1 H, NH), 3.49 (dd, $J = 11.2$, 9.6 Hz, 1 H, 2′-Ha), 3.80 (dd, $J = 11.2$, 4.1 Hz, 1 H, 2′-Hb), 4.10 (dd, $J = 9.6$, 4.1 Hz, 1 H, 1′-H), 7.28–7.41 (m, 5 H, Ph) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 20.2$ (CH_3, Me), 59.9 (q, $J = 30.3$ Hz, C2, C), 61.2 (CH, C1′), 66.8 (CH_2, C2′), 116.5 (C, CN), 123.1 (q, $J = 283.6$ Hz, CF_3, C), 127.0 (2 × CH, Ph), 128.0 (CH, Ph), 128.7 (2 × CH, Ph), 140.3 (C, Ph) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): $\delta = -82.6$ (s, CF_3) ppm. IR (neat): $\tilde{\nu} = 3329$, 3032, 2937, 1603, 1455, 1174 cm^{-1}. MS (EI): *m*/*z* (%) = 258 $[M]^+$, 227, 200 (100), 162, 120, 77. $C_{12}H_{13}F_3N_2O$ (258.10): calcd. C 55.81, H 5.07, N 10.85; found C 55.45, H 5.15, N 10.51.

Diastereoisomer (*S*,*R*)-8: White solid; m.p. 81–83 °C, $R_f = 0.19$ (petroleum ether/AcOEt, 85:15). $[\alpha]_D = -89.0$ ($c = 1.0$, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.74$ (s, 3 H, Me), 1.98 (dd, $J = 6.0$, 5.6 Hz, 1 H, OH), 2.46 (d, $J = 5.5$ Hz, 1 H, NH), 3.57 (ddd, $J = 11.0$, 7.6, 5.6 Hz, 1 H, 2′-Ha), 3.80 (ddd, $J = 11.0$, 6.0, 4.6 Hz, 1 H, 2′-Hb), 4.13 (ddd, $J = 7.6$, 5.5, 4.6 Hz, 1 H, 1′-H), 7.27–7.40 (m, 5 H, Ph) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 20.5$ (CH_3, Me), 58.3 (q, $J = 31.6$ Hz, C2, C), 60.9 (CH, C1′), 67.0 (CH_2, C2′), 116.2 (C, CN), 123.0 (q, $J = 284.7$ Hz, CF_3, C), 126.9 (2 × CH, Ph), 128.1 (CH, Ph), 128.7 (2 × CH, Ph), 139.1 (C, Ph) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): $\delta = -81.5$ (s, CF_3) ppm.

(*R*)-α-Tfm-Alanine (9): A solution of the amino nitrile (*R*,*R*)-**8** (8.8 g, 34 mmol, 1 equiv.) in concentrated aqueous HCl (100 mL) was heated to reflux. After 16 h, the solution was allowed to cool to room temperature and diluted with Et_2O (100 mL). The layers were separated, the aqueous phase was extracted, and the combined organic fraction was concentrated under reduced pressure. The crude residue was loaded onto a DOWEX 50W8-400 column to afford the pure (*R*)-α-Tfm-alanine [(*R*)-**9**, 2.57 g, 48%] as a white solid without m.p. (sublimation). $[\alpha]_D = +11.2$ ($c = 0.9$, HCl). ^1H NMR (400 MHz, D_2O): $\delta = 1.51$ (s, 3 H, Me) ppm. ^{13}C NMR (100.5 MHz, D_2O): $\delta = 16.6$ (C, Me), 62.1 (q, $J = 28.8$ Hz, CNH_2, C), 123.6 (q, $J = 282.8$ Hz, CF_3, C), 168.2 (C, CO_2H) ppm. ^{19}F NMR (376.2 MHz, D_2O): $\delta = -79.2$ (s, CF_3) ppm. IR (neat): $\tilde{\nu} = 3506$, 3257, 2959, 2592, 1652, 1605, 1538, 1156 cm^{-1}. MS (EI): *m*/*z* (%) = 112 $[M - CO_2H]^+$ (100). HRMS (EI): $C_4H_6F_3NO_2 - CO_2H$: 112.0374; found 112.0371.

Methodological Study of the Coupling Reaction between (*S*)-α-Tfm-proline (1) and L-Alanine: DIC (46 μL, 0.30 mmol, 1.1 equiv.), triethylamine (25 μL, 0.27 mmol, 1.0 equiv.), and L-alanine benzyl ester hydrogen *p*-toluenesulfonate (96 mg, 0.27 mmol, 1 equiv.) were successively added at room temperature to a stirred solution of (*S*)-α-Tfm-proline [(*S*)-**1**, 50 mg, 0.27 mmol, 1 equiv.] in CH_2Cl_2 (6 mL). The reaction mixture was stirred for 3 d and then diluted with CH_2Cl_2 (20 mL) and HCl (0.1N, 10 mL). The layers were separated, the aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), and the combined chlorinated extracts were washed with water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (cyclohexane/AcOEt, 80:20) to afford dipeptide (*S*,*S*)-**2** (6 mg, 6%) and diketopiperazine **3** (35 mg, 77%).

HOBt (55 mg, 0.41 mmol, 1.5 equiv.) and EDCI (79 mg, 0.41 mmol, 1.5 equiv.) were successively added at room temperature to a stirred solution of (*S*)-α-Tfm-proline [(*S*)-**1**, 50 mg, 0.27 mmol, 1 equiv.] in DMF (760 μL). The reaction mixture was stirred for 10 min and cooled to 0 °C, and then L-alanine benzyl ester hydrogen *p*-toluenesulfonate (96 mg, 0.27 mmol, 1 equiv.) and triethylamine (156 μL, 1.12 mmol, 4.1 equiv.) were successively added. After 1 h at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred overnight. The resulting solution was diluted with CH_2Cl_2 (20 mL), washed successively with aqueous HCl (1 N, 20 mL), saturated aqueous $NaHCO_3$ (20 mL), and water (20 mL), dried with $MgSO_4$, filtered, and concentrated under re-

duced pressure. The crude residue was purified by flash chromatography (cyclohexane/AcOEt, 80:20) to afford (*S*,*S*)-**2** (50 mg, 53%) and diketopiperazine **3** (12 mg, 27%).

Triethylamine (156 μL, 1.12 mmol, 4.1 equiv.), HOBt (55 mg, 0.41 mmol, 1.5 equiv.), and EDCI (79 mg, 0.41 mmol, 1.5 equiv.) were successively added at room temperature to a stirred solution of L-alanine benzyl ester hydrogen *p*-toluenesulfonate (96 mg, 0.27 mmol, 1 equiv.) in DMF (360 μL). The reaction mixture was cooled to 0 °C and a suspension of (*S*)-α-Tfm-proline [(*S*)-**1**, 0.27 mmol, 1 equiv.] in DMF (400 μL) was added to the reaction mixture, followed by DMF (200 μL). After 1 h at 0 °C, the reaction mixture was allowed to warm to room temperature and stirred for 3 d. The resulting solution was diluted with CH_2Cl_2 (20 mL), washed successively with HCl (1 N, 20 mL), saturated aqueous $NaHCO_3$ (20 mL), and water (20 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (cyclohexane/AcOEt, 80:20) to afford (*S*,*S*)-**2** (50 mg, 53%) and diketopiperazine **3** (13 mg, 29%).

(5a*S*,10a*S*)-5a,10a-Bis(trifluoromethyl)octahydrodipyrrolo[1,2-*a*:1′,2′-*d*]pyrazine-5,10-dione (3): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.91–2.20 (m, 6 H, 2 × H_γ Pro-H, 2 × H_β Pro-Ha), 2.75–2.84 (m, 2 H, 2 × H_β Pro-Hb), 3.53–3.62 (m, 2 H, 2 × H_δ Pro-Ha), 4.05–4.15 (m, 2 H, 2 × H_δ Pro-Hb) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 20.0 (2 × CH_2, C_γ Pro), 33.5 (2 × CH_2, C_β Pro), 47.3 (2 × CH_2, C_δ Pro), 71.3 (2 × C, C_α Pro), 123.7 (q, J = 287.5 Hz, CF_3, 2 × C), 161.1 (2 × C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –74.6 (s, 2 × CF_3) ppm. IR (neat): $\tilde{\nu}$ = 2922, 1670, 1418, 1178, 1158 cm^{-1}. MS (EI): *m*/*z* (%) = 330 $[M]^+$, 261 (100), 233, 96. HRMS (EI): calcd. for $C_{12}H_{12}F_6N_2O_2$: 330.0803; found 330.0807.

Optimized General Procedure for Coupling of α-Tfm Amino Acids: Triethylamine (4.1 equiv.), HOBt (1.5 equiv.), EDCI (1.5 equiv.), and finally α-Tfm amino acid (1 equiv.) were successively added at 0 °C to a stirred solution of an amino ester salt (2 equiv.) in DMF (0.273 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 until the reaction was complete as monitored by ^{19}F NMR spectroscopy. The mixture was diluted with CH_2Cl_2 and water. The aqueous layer was extracted with CH_2Cl_2 (3×) and the combined chlorinated extracts 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 76–95% yields.

(*S*)-α-Tfm-Pro-L-Ala-OBn [(*S*,*S*)-2]: The dipeptide (*S*,*S*)-**2** was prepared by the General Procedure, with L-alanine benzyl ester hydrogen *p*-toluenesulfonate (192 mg, 0.55 mmol, 2 equiv.), triethylamine (156 μL, 1.12 mmol, 4.1 equiv.), HOBt (74 mg, 0.55 mmol, 2 equiv.), EDCI (105 mg, 0.55 mmol, 2 equiv.), and (*S*)-α-Tfm proline (50 mg, 0.27 mmol) in DMF (1 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 48 h. Purification on silica gel (cyclohexane/AcOEt, 80:20) gave pure (*S*,*S*)-**2** (89 mg, 95%) as a colorless oil, R_f = 0.43 (cyclohexane/AcOEt, 70:30). $[\alpha]_D$ = –46.0 (c = 1.9, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43 (d, J = 7.4 Hz, 3 H, H_β Ala-H), 1.66–1.78 (m, 1 H, H_γ Pro-Ha), 1.84–1.93 (m, 1 H, H_γ Pro-Hb), 2.18–2.28 (m, 2 H, H_β Pro-H), 2.50 (br. s, 1 H, NH Pro), 3.09 (dd, J = 7.3, 5.9 Hz, 2 H, H_δ Pro-H), 4.60 (quint, J = 7.4 Hz, 1 H, H_α Ala-H), 5.15 (d, J = 12.4 Hz, 1 H, Bn CH_2-Ha), 5.21 (d, J = 12.4 Hz, 1 H, Bn CH_2-Hb), 7.31–7.39 (m, 5 H, Bn arom.), 7.91 (d, J = 7.4 Hz, 1 H, NH Ala) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 18.1 (CH_3, C_β Ala), 25.4 (CH_2, C_γ Pro), 32.3 (CH_2, C_β Pro), 47.6 (CH_2, C_δ Pro), 48.3 (CH, C_α Ala), 67.2 (CH_2, Bn CH_2), 70.7 (q, J = 26.8 Hz, C_α Pro, C), 126.0 (q, J = 284.7 Hz, CF_3, C), 128.1 (2 × CH, Bn arom.), 128.4 (CH, Bn arom.), 128.6 (2 × CH, Bn arom.), 135.2 (C, Bn arom.), 169.0 (C, C=O), 172.3 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –77.8 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3336, 1740, 1676, 1508, 1453, 1148 cm^{-1}. MS (EI): *m*/*z* (%) = 344 $[M]^+$ (100). HRMS (EI): $C_{16}H_{19}F_3N_2O_3$: 344.1348; found 344.1352.

(*R*)-α-Tfm-Pro-L-Ala-OBn [(*R*,*S*)-2]: The dipeptide (*R*,*S*)-**2** was prepared by the General Procedure, with L-alanine benzyl ester hydrogen *p*-toluenesulfonate (192 mg, 0.55 mmol, 2 equiv.), triethylamine (156 μL, 1.12 mmol, 4.1 equiv.), HOBt (74 mg, 0.55 mmol, 2 equiv.), EDCI (105 mg, 0.55 mmol, 2 equiv.), and (*R*)-α-Tfm proline (50 mg, 0.27 mmol) in DMF (1 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 24 h. Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*S*)-**2** (82 mg, 87%) as a colorless oil, R_f = 0.23 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = +16.4 (c = 4.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (d, J = 7.3 Hz, 3 H, H_β Ala-H), 1.64–1.74 (m, 1 H, H_γ Pro-Ha), 1.74–1.85 (m, 1 H, H_γ Pro-Hb), 2.12–2.27 (m, 2 H, H_β Pro-H), 2.34 (br. s, 1 H, NH Pro), 3.03–3.10 (m, 2 H, H_δ Pro-H), 4.59 (quint, J = 7.3 Hz, 1 H, H_α Ala-H), 5.11 (d, J = 12.2 Hz, 1 H, Bn CH_2-Ha), 5.19 (d, J = 12.2 Hz, 1 H, Bn CH_2-Hb), 7.31–7.39 (m, 5 H, Bn arom.), 8.18 (d, J = 7.3 Hz, 1 H, NH Ala) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 17.9 (CH_3, C_β Ala), 25.1 (CH_2, C_γ Pro), 32.1 (CH_2, C_β Pro), 47.5 (CH_2, C_δ Pro), 48.0 (CH, C_α Ala), 67.1 (CH_2, Bn CH_2), 70.7 (q, J = 25.9 Hz, C_α Pro, C), 125.9 (q, J = 283.7 Hz, CF_3, C), 128.1 (2 × CH, Bn arom.), 128.4 (CH, Bn arom.), 128.6 (2 × CH, Bn arom.), 135.2 (C, Bn arom.), 169.0 (C, C=O), 172.3 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –77.8 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3329, 1740, 1677, 1508, 1151 cm^{-1}. MS (EI): *m*/*z* (%) = 344 $[M]^+$, 138 (100), 91. HRMS (EI): calcd. for $C_{16}H_{19}F_3N_2O_3$: 344.1348; found 344.1348.

(*S*)-α-Tfm-Pro-L-Val-O*t*Bu [(*S*,*S*)-4]: The dipeptide (*S*,*S*)-**4** was prepared by the General Procedure, with L-valine *tert*-butyl ester hydrochloride (115 mg, 0.55 mmol, 2 equiv.), triethylamine (156 μL, 1.12 mmol, 4.1 equiv.), HOBt (55 mg, 0.41 mmol, 1.5 equiv.), EDCI (79 mg, 0.41 mmol, 1.5 equiv.), and (*S*)-α-Tfm proline (50 mg, 0.27 mmol) in DMF (1 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 48 h. Purification on silica gel (cyclohexane/AcOEt, 80:20) gave pure (*S*,*S*)-**4** (80 mg, 87%) as a white solid; m.p. 108 °C, R_f = 0.45 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = –23.8 (c = 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, J = 6.9 Hz, 3 H, H_γ Val-H), 0.88 (d, J = 6.9 Hz, 3 H, H_γ Val-H), 1.42 (s, 9 H, *t*Bu 3 × CH_3-H), 1.66–1.77 (m, 1 H, H_γ Pro-Ha), 1.83–1.92 (m, 1 H, H_γ Pro-Hb), 2.13–2.26 (m, 3 H, H_β Pro-H, H_β Val-H), 2.56 (br. s, 1 H, NH Pro), 3.03–3.13 (m, 2 H, H_δ Pro-H), 4.38 (dd, J = 9.2, 4.6 Hz, 1 H, H_α Val-H), 7.75 (d, J = 9.2 Hz, 1 H, NH Val) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 17.2 (CH_3, C_γ Val), 18.9 (CH_3, C_γ Val), 25.5 (CH_2, C_γ Pro), 27.9 (3 × CH_3, *t*Bu CH_3), 31.3 (CH, C_β Val), 32.5 (CH_2, C_β Pro), 47.6 (CH_2, C_δ Pro), 57.4 (CH, C_α Val), 70.7 (q, J = 26.9 Hz, C_α Pro, C), 82.0 (C, *t*Bu C), 126.0 (q, J = 283.7 Hz, CF_3, C), 169.0 (C, C=O), 170.5 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –77.7 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3338, 3300, 2967, 2934, 2873, 1737, 1676, 1515, 1145 cm^{-1}. MS (EI): *m*/*z* (%) = 338 $[M]^+$, 138 (100). $C_{15}H_{25}F_3N_2O_3$ (338.18): calcd. C 53.24, H 7.45, N 8.28; found C 53.57, H 7.72, N 8.35.

(*S*)-α-Tfm-Pro-L-Phe-O*t*Bu [(*S*,*S*)-5]: The dipeptide (*S*,*S*)-**5** was prepared by the General Procedure, with L-phenylalanine *tert*-butyl ester hydrochloride (141 mg, 0.55 mmol, 2 equiv.), triethylamine (156 μL, 1.12 mmol, 4.1 equiv.), HOBt (55 mg, 0.41 mmol, 1.5 equiv.), EDCI (79 mg, 0.41 mmol, 1.5 equiv.), and (*S*)-α-Tfm proline (50 mg, 0.27 mmol) in DMF (1 mL). After 20 min at 0 °C,

the resulting mixture was stirred at room temperature for 48 h. Purification on silica gel (cyclohexane/AcOEt, 80:20) gave pure (*S*,*S*)-**5** (80 mg, 76%) as a white solid; m.p. 88 °C, R_f = 0.38 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = +0.7 (*c* = 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 9 H, *t*Bu 3 × CH_3-H), 1.36–1.52 (m, 1 H, H_γ Pro-Ha), 1.66–1.76 (m, 1 H, H_γ Pro-Hb), 1.96 (ddd, *J* = 13.8, 7.8, 4.4 Hz, 1 H, H_β Pro-Ha), 2.07 (ddd, *J* = 13.8, 9.2, 7.8 Hz, 1 H, H_β Pro-Hb), 2.33 (br. s, 1 H, NH Pro), 2.84–2.94 (m, 2 H, H_δ Pro-H), 2.97 (dd, *J* = 14.0, 6.9 Hz, 1 H, H_β Phe-Ha), 3.11 (dd, *J* = 14.0, 6.0 Hz, 1 H, H_β Phe-Hb), 4.63 (ddd, *J* = 7.8, 6.9, 6.0 Hz, 1 H, H_α Phe-H), 7.04–7.09 (m, 2 H, arom. Phe-H), 7.12–7.22 (m, 3 H, arom. Phe-H); 7.81 (d, *J* = 7.8 Hz, 1 H, NH Phe) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 25.5 (CH_2, C_γ Pro), 27.8 (3 × CH_3, *t*Bu CH_3), 32.2 (CH_2, C_β Pro), 37.7 (CH_2, C_β Phe), 47.4 (CH_2, C_δ Pro), 53.4 (CH, C_α Phe), 70.5 (q, *J* = 26.8 Hz, CH_2, C_α Pro, C), 82.3 (C, *t*Bu C), 125.9 (q, *J* = 283.7 Hz, CF_3, C), 126.9 (CH, Phe arom.), 128.2 (2 × CH, Phe arom.), 129.3 (2 × CH, Phe arom.), 136.0 (C, Phe arom.), 168.7 (C, C=O), 170.1 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –77.6 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3343, 1712, 1672, 1604, 1164 cm^{-1}. MS (EI): *m*/*z* (%) = 386 $[M]^+$, 138 (100). $C_{19}H_{25}F_3N_2O_3$ (386.18): C 59.06, H 6.52, N 7.25; found C 59.08, H 6.52, N 7.15.

(*S*)-α-Tfm-Pro-L-Leu-OBn [(*S*,*S*)-6]: The dipeptide (*S*,*S*)-**6** was prepared by the General Procedure with L-leucine *tert*-butyl ester hydrochloride (215 mg, 0.55 mmol, 2 equiv.), triethylamine (156 µL, 1.12 mmol, 4.1 equiv.), HOBt (55 mg, 0.41 mmol, 1.5 equiv.), EDCI (79 mg, 0.41 mmol, 1.5 equiv.), and (*S*)-α-Tfm proline (50 mg, 0.27 mmol) in DMF (1 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 30 h. Purification on silica gel (cyclohexane/AcOEt, 80:20) gave pure (*S*,*S*)-**6** (87 mg, 80%) as a colorless oil, R_f = 0.34 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = –46.6 (*c* = 2.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (d, *J* = 6.4 Hz, 3 H, H_δ Leu-H), 0.92 (d, *J* = 6.4 Hz, 3 H, H_δ Leu-H), 1.52–1.62 (m, 2 H, H_β Leu-Ha, H_γ Leu-H), 1.65–1.76 (m, 2 H, H_γ Pro-Ha, H_β Leu-Hb), 1.84–1.93 (m, 1 H, H_γ Pro-Hb), 2.13–2.28 (m, 2 H, H_β Pro-H), 2.53 (br. s, 1 H, NH Pro), 3.09 (dd, *J* = 7.0, 5.7 Hz, 2 H, H_δ Pro-H), 4.64 (td, *J* = 8.7, 5.2 Hz, 1 H, H_α Leu-H), 5.13 (d, *J* = 12.2 Hz, 1 H, Bn CH_2-Ha), 5.19 (d, *J* = 12.2 Hz, 1 H, Bn CH_2-Hb), 7.30–7.40 (m, 5 H, Bn arom.), 7.74 (d, *J* = 8.7 Hz, 1 H, NH Leu) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 21.7 (CH_3, C_δ Leu), 22.8 (CH_3, C_δ Leu), 25.0 (CH, C_γ Leu), 25.4 (CH_2, C_γ Pro), 32.4 (CH_2, C_β Pro), 41.1 (CH_2, C_β Leu), 47.6 (CH_2, C_δ Pro), 50.9 (CH, C_α Leu), 67.1 (CH_2, Bn CH_2), 70.7 (C, q, *J* = 25.9 Hz), 126.0 (C, q, *J* = 284.7 Hz, CF_3), 128.2 (2 × CH, Bn arom.), 128.4 (CH, Bn arom.), 128.5 (2 × CH, Bn arom.), 135.2 (C, Bn arom.), 169.1 (C, C=O), 172.2 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –77.7 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3338, 1740, 1677, 1509, 1147 cm^{-1}. MS (EI): *m*/*z* (%) = 386 $[M]^+$, 138 (100). $C_{19}H_{25}F_3N_2O_3$ (386.18): C 59.06, H 6.52, N 7.25; found C 59.31, H 6.52, N 7.01.

(*R*)-α-Tfm-Ala-L-Ala-OBn [(*R*,*S*)-10]: The dipeptide (*R*,*S*)-**10** was prepared by the General Procedure, with L-alanine benzyl ester hydrogen tosylate (447 mg, 1.27 mmol, 2 equiv.), triethylamine (363 µL, 2.54 mmol, 4.1 equiv.), HOBt (129 mg, 0.95 mmol, 1.5 equiv.), EDCI (183 mg, 0.95 mmol, 1.5 equiv.), and (*R*)-α-Tfm alanine (100 mg, 0.64 mmol) in DMF (3 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 24 h. Purification on silica gel (dichloromethane/AcOEt, 90:10) gave pure (*R*,*S*)-**10** (166 mg, 82%) as a colorless oil, R_f = 0.15 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = –12.7 (*c* = 0.86, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42 (d, *J* = 7.3 Hz, 3 H, H_β Ala-H), 1.46 (s, 3 H, H_β Tfm-Ala-H), 1.85 (br. s, 2 H, NH_2 Tfm-Ala), 4.57 (qd, *J* = 7.3, 6.9 Hz, 1 H, H_α Ala-H), 5.12 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Ha), 5.19 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Hb), 7.22–7.42 (m, 5 H, Bn arom.), 7.87 (d, *J* = 6.9 Hz, 1 H, NH Ala) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 17.8 (CH_3, C_β Ala), 20.7 (CH_3, C_β Tfm-Ala), 47.9 (CH, C_α Ala), 60.5 (C, q, *J* = 27.8 Hz, C_α Tfm-Ala), 67.0 (CH_2, Bn CH_2), 125.5 (C, q, *J* = 284.7 Hz, CF_3), 128.0 (2 × CH, Bn arom.), 128.3 (CH, Bn arom.), 128.5 (2 × CH, Bn arom.), 135.1 (C, Bn arom.), 168.9 (C, C=O), 172.2 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –81.2 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3373, 1738, 1678, 1148 cm^{-1}. MS (EI): *m*/*z* (%) = 318 $[M]^+$, 298, 275 (100), 206. HRMS (EI): calcd. for $C_{14}H_{17}F_3N_2O_3$: 318.1191; found 386.1181.

(*R*)-α-Tfm-Ala-L-Val-O*t*Bu [(*R*,*S*)-11]: The dipeptide (*R*,*S*)-**11** was prepared by the General Procedure, with L-valine *tert*-butyl ester hydrochloride (266 mg, 1.27 mmol, 2 equiv.), triethylamine (363 µL, 2.54 mmol, 4.1 equiv.), HOBt (129 mg, 0.95 mmol, 1.5 equiv.), EDCI (183 mg, 0.95 mmol, 1.5 equiv.), and (*R*)-α-Tfm alanine (100 mg, 0.64 mmol) in DMF (3 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 24 h. Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*S*)-**11** (159 mg, 80%) as a white solid; m.p. 68 °C, R_f = 0.39 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = +12.5 (*c* = 1.37, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87 (d, *J* = 6.9 Hz, 3 H, H_γ Val-H), 0.91 (d, *J* = 6.9 Hz, 3 H, H_γ Val-H), 1.45 (s, 9 H, *t*Bu 3 × CH_3-H), 1.50 (s, 3 H, H_β Ala-H), 1.81 (br. s, 2 H, NH_2 Ala), 2.20 (septd, *J* = 6.9, 4.1 Hz, 1 H, H_β Val-H), 4.37 (dd, *J* = 9.2, 4.1 Hz, 1 H, H_α Val-H), 7.81 (d, *J* = 9.2 Hz, 1 H, NH Val) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 17.3 (CH_3, C_γ Val), 18.9 (CH_3, C_γ Val), 21.0 (CH_3, C_β Ala), 27.9 (3 × CH_3, *t*Bu CH_3), 31.3 (CH, C_β Val), 57.3 (CH, C_α Val), 60.8 (C, q, *J* = 27.8 Hz, C_α Ala), 82.0 (C, *t*Bu C), 125.7 (C, q, *J* = 283.7 Hz, CF_3), 168.8 (C, C=O), 170.6 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –81.4 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3368, 3354, 3308, 1723, 1686, 1158 cm^{-1}. MS (EI): *m*/*z* (%) = 312 $[M]^+$, 211 (100), 112. $C_{13}H_{23}F_3N_2O_3$ (312.17): C 49.99, H 7.42, N 8.97; found C 49.70, H 7.40, N 8.76.

(*R*)-α-Tfm-Ala-L-Phe-O*t*Bu [(*R*,*S*)-12]: The dipeptide (*R*,*S*)-**12** was prepared by the General Procedure, with L-phenylalanine *tert*-butyl ester hydrochloride (326 mg, 1.27 mmol, 2 equiv.), triethylamine (363 µL, 2.54 mmol, 4.1 equiv.), HOBt (129 mg, 0.95 mmol, 1.5 equiv.), EDCI (183 mg, 0.95 mmol, 1.5 equiv.), and (*R*)-α-Tfm alanine (100 mg, 0.64 mmol) in DMF (3 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 24 h. Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*S*)-**12** (177 mg, 77%) as a white solid; m.p. 68 °C, R_f = 0.36 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = +53 (*c* = 3.31, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.36 (s, 3 H, H_β Ala-H), 1.40 (s, 9 H, *t*Bu 3 × CH_3-H), 1.60 (br. s, 2 H, NH_2 Ala), 3.04 (dd, *J* = 14.2, 6.0 Hz, 1 H, H_β Phe-Ha), 3.09 (dd, *J* = 14.2, 6.0 Hz, 1 H, H_β Phe-Hb), 4.62 (dt, *J* = 7.8, 6.0 Hz, 1 H, H_α Phe-H), 7.04–7.09 (m, 2 H, arom. Phe-H), 7.12–7.25 (m, 3 H, arom. Phe-H); 7.63 (d, *J* = 7.8 Hz, 1 H, NH Phe) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 20.9 (CH_3, C_β Ala), 27.9 (3 × CH_3, *t*Bu CH_3), 37.6 (CH_2, C_β Phe), 53.4 (CH, C_α Phe), 60.6 (C, q, *J* = 26.8 Hz, C_α Ala), 82.5 (C, *t*Bu C), 125.7 (C, q, *J* = 284.7 Hz, CF_3), 127.0 (CH, Phe arom.), 128.3 (2 × CH, Phe arom.), 129.4 (2 × CH, Phe arom.), 135.9 (C, Phe arom.), 168.5 (C, C=O), 170.1 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –81.3 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3407, 3370, 3338, 1708, 1673, 1147 cm^{-1}. MS (EI): *m*/*z* (%) = 360 $[M]^+$, 304 (100), 259, 193, 148, 120. $C_{17}H_{23}F_3N_2O_3$ (360.17): calcd. C 56.66, H 6.43, N 7.77; found C 56.68, H 6.63.

(*R*)-α-Tfm-Ala-L-Leu-OBn [(*R*,*S*)-13]: The dipeptide (*R*,*S*)-**13** was prepared by the General Procedure, with L-leucine benzyl ester hy-

drogen *p*-toluenesulfonate (500 mg, 1.27 mmol, 2 equiv.), triethylamine (363 μL, 2.54 mmol, 4.1 equiv.), HOBt (129 mg, 0.95 mmol, 1.5 equiv.), EDCI (183 mg, 0.95 mmol, 1.5 equiv.), and (*R*)-α-Tfm alanine (100 mg, 0.64 mmol) in DMF (3 mL). After 20 min at 0 °C, the resulting mixture was stirred at room temperature for 24 h. Purification on silica gel (cyclohexane/AcOEt, 90:10) gave pure (*R*,*S*)-**13** (187 mg, 81%) as a colorless oil, R_f = 0.33 (cyclohexane/AcOEt, 80:20). $[\alpha]_D$ = –9.2 (*c* = 3.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.91 (d, *J* = 5.1 Hz, 6 H, H_δ Leu-H), 1.48 (s, 3 H, H_β Ala-H), 1.55–1.71 (m, 3 H, H_β Leu-H, H_γ Leu-H), 1.79 (br. s, 2 H, NH_2 Ala), 4.59–4.64 (m, 1 H, H_α Leu-H), 5.12 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Ha), 5.19 (d, *J* = 12.4 Hz, 1 H, Bn CH_2-Hb), 7.26–7.38 (m, 5 H, Bn arom.), 7.72 (d, *J* = 8.3 Hz, 1 H, NH Leu) ppm. ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 20.9 (CH_3, C_β Ala), 21.7 (CH_3, C_δ Leu), 22.7 (CH_3, C_δ Leu), 24.9 (CH, C_γ Leu), 41.0 (CH_2, C_β Leu), 50.8 (CH, C_α Leu), 60.7 (C, q, *J* = 26.8 Hz, C_α Ala), 67.1 (CH_2, Bn CH_2), 125.6 (C, q, *J* = 284.7 Hz, CF_3), 128.2 (2×CH, Bn arom.), 128.4 (CH, Bn arom.), 128.6 (2×CH, Bn arom.), 135.2 (C, Bn arom.), 167.0 (C, C=O), 172.3 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $CDCl_3$): δ = –81.4 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3371, 1738, 1682, 1149 cm^{-1}. MS (EI): *m*/*z* (%) = 360 $[M]^+$, 340, 317, 248, 225, 206. HRMS (EI): calcd. for $C_{17}H_{23}F_3N_2O_3$: 360.1661; found 360.1660.

Procedure for the Preparation of Fmoc-alanine Chloride by Ultrasonication: Freshly distilled $SOCl_2$ (956 μL, 13.1 mmol, 14 equiv.) was added under argon to a suspension of the Fmoc-alanine acid (299 mg, 0.960 mmol, 1.0 equiv.) in DCM (5.6 mL). The mixture was sonicated at room temperature for 30 min, and solvent and excess $SOCl_2$ were then removed in vacuo. The white solid residue was dissolved in DCM (1 mL).

Fmoc-L-Ala-(*R*)-α-Tfm-Ala-L-Leu-OBn [(*S*,*R*,*S*)-14]: A solution of dipeptide **13** (315 mg, 0.874 mmol, 1.0 equiv.) and DIEA (145 μL, 0.874 mmol, 1.0 equiv.) in DCM (3 mL) was added by cannula at 0 °C to a stirred freshly prepared solution of Fmoc-alanine chloride (0.960 mmol, 1.1 equiv.) in DCM (1 mL). The reaction mixture was stirred for 18 h at room temperature, and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography on silica gel (cyclohexane/AcOEt, 80:20) to afford the tripeptide **14** (421 mg, 74%) as a white powder; m.p. 135–137 °C, R_f = 0.21 (cyclohexane/AcOEt, 70:30). $[\alpha]_D$ = –29.2 (*c* = 0.98, MeOH). ^{1}H NMR (400 MHz, $[D_6]$DMSO): δ = 0.74 (d, *J* = 6.2 Hz, 3 H, H_δ Leu-H), 0.77 (d, *J* = 6.0 Hz, 3 H, H_δ Leu-H), 1.24 (d, *J* = 6.9 Hz, 3 H, H_β Ala-H), 1.35–1.65 (m, 3 H, H_γ Leu-H, H_β Leu-H), 1.57 (s, 3 H, H_β Tfm-Ala-H), 4.10–4.40 (m, 5 H, H_α Ala-H, H_α Leu-H, Fmoc CH_2, Fmoc CH), 5.10 (d, *J* = 12.7 Hz, 1 H, Bn CH_2-Ha), 5.15 (d, *J* = 12.7 Hz, 1 H, Bn CH_2-Hb), 7.33–7.41 (m, 7 H, Fmoc arom., Bn arom.), 7.46 (t, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.62 (d, *J* = 6.6 Hz, 1 H, NH), 7.73 (d, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.90 (d, *J* = 7.3 Hz, 1 H, NH), 7.93 (d, *J* = 7.6 Hz, 2 H, Fmoc arom.), 8.58 (s, 1 H, NH Tfm-Ala) ppm. ^{13}C NMR (100.5 MHz, $[D_6]$DMSO): δ = 18.5 (CH_3, C_β Ala), 20.3 (CH_3, C_β Tfm-Ala), 21.9 (CH_3, C_δ Leu), 23.7 (CH_3, C_δ Leu), 24.6 (CH, C_γ Leu), 41.2 (CH_2, C_β Leu), 47.6 (CH, Fmoc CH), 50.8 (CH), 51.5 (CH), 61.9 (C, q, *J* = 27.8 Hz, C_α Tfm-Ala), 66.7 (CH_2, Bn CH_2), 66.9 (CH_2, Fmoc CH_2), 121.1 (2×CH, Fmoc arom.), 125.7 (C, q, *J* = 286.6 Hz, CF_3), 126.2 (2×CH, Fmoc arom.), 128.0 (2×CH, Fmoc arom.), 128.6 (2×CH, Fmoc arom.), 128.8 (2×CH, Bn arom.), 129.0 (CH, Bn arom.), 129.3 (2×CH, Bn arom.), 136.9 (C, Bn arom.), 141.7 (2×C, Fmoc arom.), 144.7 (2×C, Fmoc arom.), 156.7 (C, C=O), 167.4 (C, C=O), 172.9 (C, C=O) ppm. ^{19}F NMR (376.2 MHz, $[D_6]$DMSO) δ = –75.7 (s, CF_3) ppm. IR (neat): $\tilde{\nu}$ = 3365, 3313, 1732, 1693, 1659, 1531, 1149 cm^{-1}.

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