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Preparation and conformational study of CF_3-containing enkephalin-derived oligopeptide

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Abstract

Incorporation of (2*S*,3*S*)-4,4,4-trifluorothreonine (F_3-Thr) instead of Thr in the enkephalin-derived hexapeptide led to the apparent conformational alteration due to the strong electron-withdrawing effect of the trifluoromethyl group by comparison with the original compound on the basis of their various NMR measurements.

Keywords: Fluorinated amino acid; Peptides; Conformations; NOESY spectra

1. Introduction

Completion of decoding of the human genomic base sequence in 2003 opened a new route to obtain the three-dimensional shapes and functions of the target active sites responsible for some specific diseases with the aid of computational technique.[1,2] Detailed analyses of such information will in the near future enable us to rationally design more effective drugs by installing appropriate elements so as to construct firm interaction with these reaction sites. When peptidic compounds are regarded as donors, introduction of fluorine-containing amino acids[3] would become one of the most useful methods for control of their conformations and physical properties. For example, a fluorine atom with three sets of lone pairs or a fluorinated substituent will endow ability to form hydrogen bonding (HB) and cause unfavorable electronic repulsion with other proximate electronegative atoms and/or groups, which would more or less bring about the conformational change of the original compounds. Although three-dimensional shapes of molecules are considered as one of the most important factors for determining whether a specific donor is accepted as a substrate or not, this issue seems not to have been paid significant attention thus far.[4] Based on such an idea, we have started our investigation to clarify how much a fluorinated substituent in amino acids affects the original peptidic conformation. For this purpose, we have selected the hexapeptide, Tyr-D-Ser-Gly-Phe-Leu-Thr **1** (DSLET[5]) known as the enkephalin-related peptide selectively bound to the δ opioid receptor. The fluorinated enkephalin-type peptides have been synthesized by utilization of, for instance, fluorine-containing phenylalanines[6] or leucines,[7] and their interesting biological activities were reported to be appeared when fluorine atoms were introduced at a judicious position. However, the relationship between substitution by fluorine and conformational change as well as the biological activity has not necessarily been clarified yet, and we therefore decided to start our research.

2. Results and discussion

2.1. Synthesis and conformational analysis of hexapeptide 2

In this study, most of peptide syntheses were performed by the solution-phase method using WSC·HCl[8] (water soluble

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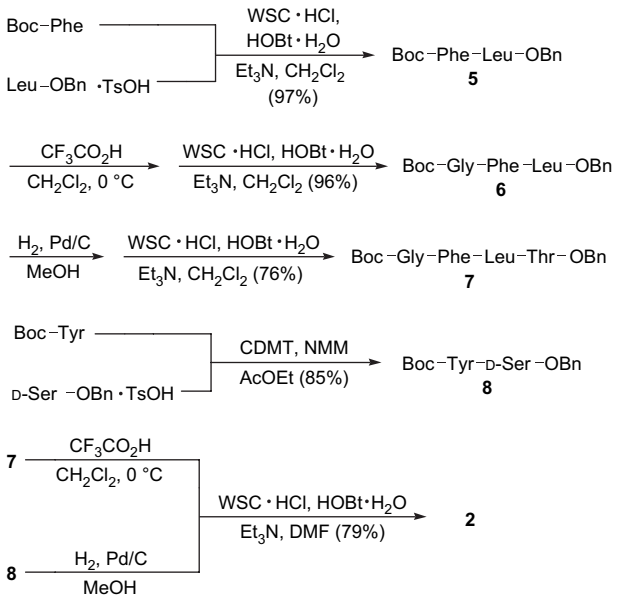
carbodiimide; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride)) in the presence of HOBt·H_2O[9] (1-hydroxybenzotriazole hydrate) as the representative condensation reagent partner, which usually attained good to excellent yields. However, preparation of Boc-Tyr-D-Ser–OBn **8** was the exception and only ca. 50% yield of **8** was furnished. Then, we have turned our attention to DMT-MM[10] (4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-ethylmorpholinium chloride) because other reagents like well-known DPPA (diphenylphosphoryl azide) resulted in almost no improvement. DMT-MM did not generally require any additional base for activation, but this was not the case here. Addition of 1.1 equiv of base was required to neutralize the trifluoroacetic acid salt of the N-terminal amine component in substrates, and a 1.1 equiv more amount was found to be necessary for effective transformation. After several experiments, conveniently prepared CDMT[11] (2-chloro-4,6-dimethoxy-1,3,5-triazine), the precursor of DMT-MM, was selected for this condensation in the presence of 2.2 equiv of NMM (*N*-methylmorpholine).[12] As a result, this step proceeded under the usual atmosphere in a conventional AcOEt solvent to attain 85% isolated yield of the product **8**. Combination of **7** and **8** in a usual manner furnished the desired hexapeptide **2** in 79% yield (Scheme 1).

The NOESY spectrum of **2** observed in DMSO-d_6 (Fig. 1) indicated that its conformation possessed two characteristic



Scheme 1. Preparation of Boc–Tyr-D-Ser-Gly-Phe-Leu-Thr–OBn **2**.

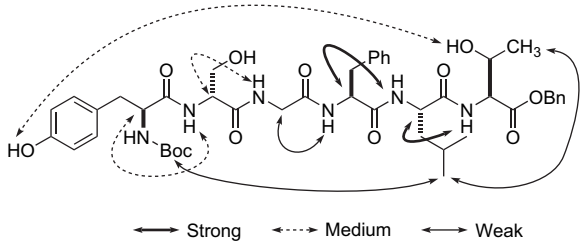


Figure 1. NOESY correlations of **2**.

long-range correlations between Tyr and Thr as well as Boc in Tyr and Leu–CH_3 groups apart from five and six residues, respectively, which assumed its partial helix- or spherical-type structure. The cross peak between Tyr phenol and Thr hydroxy protons led to speculation that Tyr–O*H* proton and Thr–*O*H oxygen worked as the HB donor and acceptor, respectively, when their acidity was considered.[13] If this was the case, it was strongly anticipated that substitution of Thr–CH_3 hydrogen atoms for fluorine should effectively lower the electron density of F_3-Thr–*O*H oxygen, which would apparently result in weakening the HB between Tyr and Thr. This idea was qualitatively supported by the acidity difference between CH_3CH_2OH and CF_3CH_2OH of ΔpK_a=3.1,[13,14] as well as HB stabilization energy difference of PhOH with Thr or F_3-Thr of 2.40 kcal/mol in favor of the former by ab initio calculation[15] using the B3LYP/6-311++G** level of theory with considering the DMSO solvent effect by the SCI-PCM model.

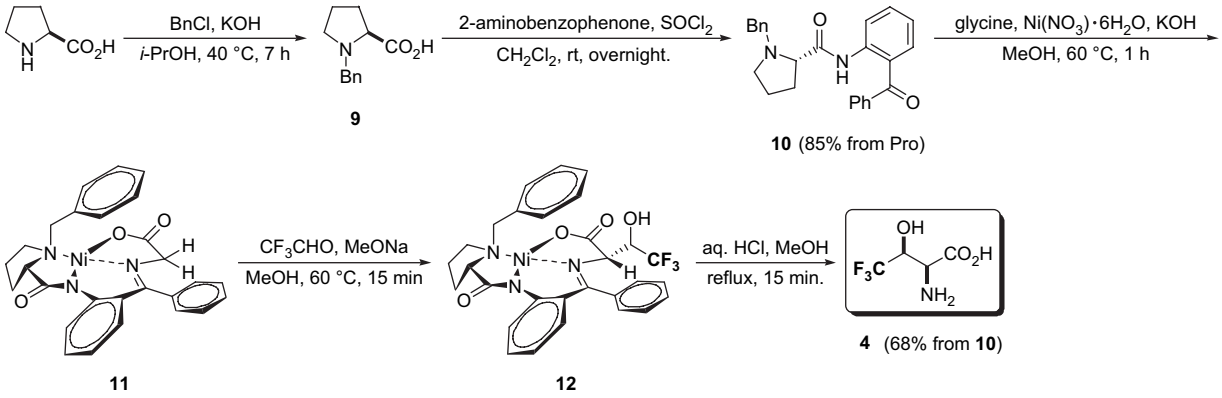
2.2. Synthesis and conformational analysis of fluorine-containing hexapeptide *3*

The synthesis of the compound **3** with a CF_3 group was initiated by construction of unnatural (2*S*,3*S*)-4,4,4-trifluorothreonine **4** (F_3-Thr) following the method reported by Soloshonok et al. (Scheme 2).[16] *N*-Benzylated L-proline was condensed with 2-aminobenzophenone to afford (*S*)-2-[(*N*-benzylprolyl)-amino]benzophenone (BPB) **10**. This chiral auxiliary **10** was then treated with glycine and $Ni(NO_3)\cdot 6H_2O$ and the resultant Gly–Ni–BPB **11** in turn was allowed to react with CF_3CHO to give F_3–Ni complex **12**. Hydrolysis of **12** led to recovery of **10** and isolation of F_3-Thr **4** whose stereoselectivity was determined to be at least 96.0% de[17] and 91.7% ee.[18]

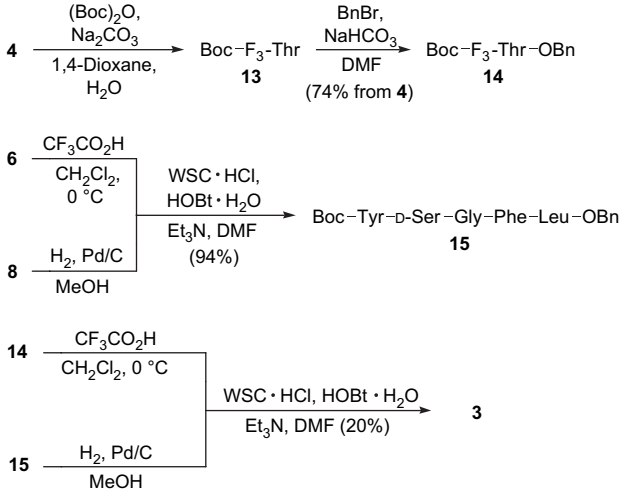
The obtained **4** was readily converted to the Boc–F_3-Thr–OBn **14** by protection of both at the N- and C-terminals in a usual manner.[19] After deprotection of N-terminal of **14** and C-terminal of **15**, their condensation reaction using WSC·HCl in the presence of HOBt·H_2O and Et_3N in DMF produced the requisite fluorine-containing hexapeptide **3** (Scheme 3). This condensation has been studied in detail under various conditions: more polar solvents and bulkier bases were found inappropriate, and moreover, CDMT and DMT-MM, which recorded an excellent yield for the preparation of **8**, could not improve the situation. The condensation reaction between non-fluorinated Thr and the pentapeptide **15** eventually led to the formation of the desired **3** in 20% isolated yield. This unsatisfactory result was in part as a result of epimerized byproduct with very close R_f value preventing ready purification.

It would be reasonable to prepare the pentapeptide **15** for realization of the efficient construction of **2** and **3**, while the condensation of **15** after benzyl deprotection and Thr–OBn was unfortunately failed, which obliged us to select different routes to get access to the both targets.

In spite of an unacceptable isolated yield, its NMR study was carried out. As shown in Figure 2, as our expectation, entry of three fluorine atoms indeed altered the original three-dimensional shape and the unique long-range HB between Tyr–O*H* and Thr–*O*H found for the prototype **2** that



Scheme 2. Preparation of (2*S*,3*S*)-4,4,4-trifluorothreonine **4**.



Scheme 3. Preparation of Boc−Tyr-D-Ser-Gly-Phe-Leu-F3-Thr−OBn **3**.

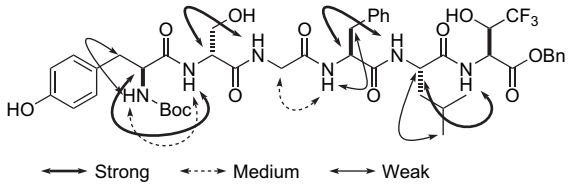


Figure 2. NOESY correlations of **3**.

was completely disappeared in this material **3**. Instead, the strong correlation between protons C*H*C(O)N*H* was noticed in all the amide linkage and very few cross-peaks between amino acids were observed, which would support the straight-type shape with the trans conformation at each amide bond.

Since there is a possibility that the conformation of **3** would possess a β-sheet type structure by construction of the intermolecular interaction with the other molecule. ^{1}H NMR spectrum was measured in a 0.1 mM solution by 200-fold dilution of the previous sample, whose chemical shifts of the amide and hydroxy protons with the ones in a 20 mM solution were shown in Table 1. In these instances, chemical shift differences Δ (ppm) were almost constant except for the Leu−N*H* proton. These results led us to conviction that the

Table 1
Concentration dependance of chemical shifts of **3**

	Chemical shift[a] (ppm)		Δ[b] (ppm)
	δ_{20}	$\delta_{0.1}$	
Tyr−N*H*	6.894	6.898	0.004
D-Ser−N*H*	7.940	7.941	0.001
Gly−N*H*	8.119	8.121	0.002
Phe−N*H*	8.066	8.066	0.000
Leu−N*H*	8.212	8.266	0.054
Thr−N*H*	8.445	8.450	0.005
Tyr−O*H*	9.152	9.147	−0.005

[a] Chemical shifts obtained by 20 mM and 0.1 mM solutions.
[b] Differences between δ_{20} and $\delta_{0.1}$.

structure **3** did not basically contain intermolecular interaction and their observed HB would be constructed at least mainly in an intramolecular manner.

To collect further conformational information, we tried to identify, which protons were responsible for HB by investigating chemical shift alteration under different solvent polarity (Fig. 3).[20] In our experiment, $CDCl_3$ and DMSO-d_6 were applied as the low and high polarity solvents, respectively, with the contents of the less polar former solvent of 0, 20, 40, 60, and 80%. A proton forming a stronger HB usually shows lower sensitivity toward the solvent polarity to be observed with smaller chemical shift difference even under larger polarity change. As a result, it was determined that HB forming protons were the amide protons of D-Ser, Gly, Phe, and the hydroxy proton of D-Ser in both **2** and **3**. Moreover, the more acidic hydroxy proton of F_3-Thr in **3** was also found to participate whose phenomenon was anticipated by incorporation of a strongly electron-withdrawing trifluoromethyl group, which should effectively decrease the electron density of F_3-Thr−*O*H, and resulted in increase of acidity of this O*H* group.

Examination of temperature dependance of these amide and hydroxy protons was also carried out on the basis of the proton NMR spectra obtained at 25, 35, and 45 °C (Table 2). Ishida and co-workers have reported on this issue and a proton involved in HB showed a high probability of the chemical shift difference Δ (ppb/K) less than 4.[21] With referring to this empirical threshold, both of Gly−N*H* and F_n-Thr−O*H* (n=0 and 3) were expected to form intramolecular HB and in

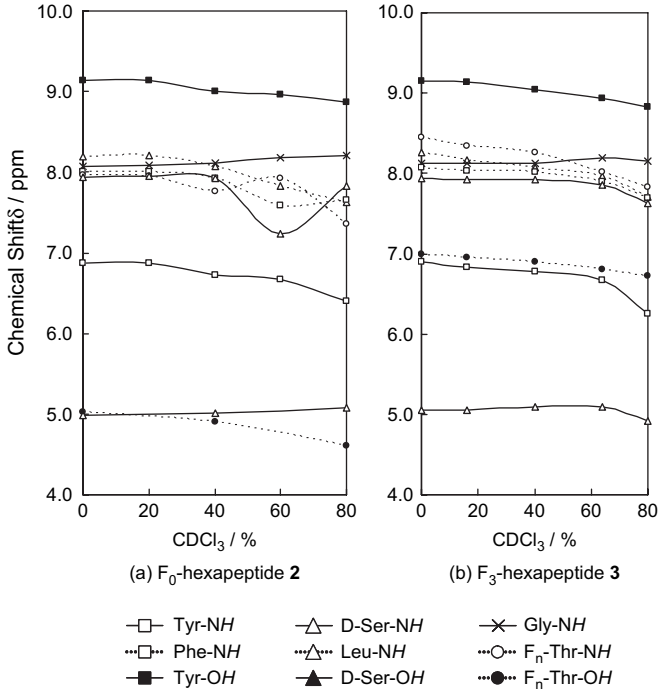


Figure 3. Chemical shift of the specific protons under different solvent ratios; (a) non-fluorinated hexapeptide **2**; (b) trifluorinated hexapeptide **3**.

Table 2
Temperature dependance of **2** and **3**

	F_0-hexapeptide **2**		F_3-hexapeptide **3**	
	Δ^1	Δ^2	Δ^1	Δ^2
Leu−N*H*	4.9	4.9	4.9	4.7
Gly−N*H*	3.5	3.5	3.7	4.1
Phe−N*H*	3.9	3.5	4.2	4.4
F_n-Thr−N*H*	6.2	5.8	7.6	7.7
D-Ser−N*H*	5.0	4.8	4.6	4.6
Tyr−N*H*	5.4	5.6	5.0	5.5
Tyr−O*H*	4.6	4.5	4.3	4.7
F_n-Thr−O*H*	4.0	4.1	3.8	3.8
D-Ser−O*H*	4.0	3.9	4.0	4.7

Chemical shift difference Δ^1 (ppb/K)=−{δ (35 °C)−δ (25 °C)}/10; chemical shift difference Δ^2 (ppb/K)=−{δ (45 °C)−δ (35 °C)}/10.

addition, Phe−N*H* and D-Ser−O*H* in **2** were also suggested to be in a similar situation but with weaker interaction. The results from polarity and temperature dependance allowed us to convince the intramolecular HB formations of Gly−N*H*, Phe−N*H*, and D-Ser−O*H* in **2**, and Gly−N*H* and F_3-Thr−O*H* in **3**. Now, we are trying to analyze each of these conformations using a method such as X-ray crystallographic analysis in the solid state because the extensive computation by MM2 as well as MOPAC with considering solvent effects failed to obtain appropriate conformers, which could consistently explain a variety of NMR data described above. Since deprotection of **2** and **3** has not been succeeded yet, we are still concentrating our attention for attainment of the smooth cleavage of the protective groups, which will furnish an important information on the relationship between fluorine substitution and conformational change as well as their biological activity.

3. Conclusion

In summary, we have succeeded in preparation of trifluoromethyl-containing oligopeptide **3**, and clarified that CF_3-incorporation at a judicious position of **2** does lead to a significant conformational change by its strong electron-withdrawing effect at least in the present case. Accumulation of such types of information will eventually lead to control of the three-dimensional (3D) shapes. Therefore, we are attempting to analyze three-dimensional structures of these materials, and similar investigation about other peptidic targets has been in progress in this laboratory.

4. Experimental section

4.1. General

Most of reactions where an organic solvent was employed were performed under argon with magnetic stirring using flame-dried glassware. Anhydrous CH_2Cl_2 was purchased and used without further purification. DMF was freshly distilled from CaH_2. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. All protected amino acids (AAs) for peptide syntheses were prepared by *tert*-butyloxycarbonylation of an amino group or benzyl esterification of a carboxyl group of non-protected AAs. Analytical thin layer chromatography (TLC) was routinely used for monitoring reactions by generally using a mixture of *n*-hexane and ethyl acetate (v/v). Spherically-shaped neutral silica gel (63−210 μm or 40−50 μm) was employed for column chromatography or flush column chromatography, respectively. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded (^{1}H: 500, 400, or 300 MHz; ^{13}C: 125, 100, or 75 MHz; ^{19}F: 376 or 283 MHz) at room temperature whose data were reported as follows: chemical shift (δ scale) in parts per million (ppm) downfield from Me_4Si (δ=0.00) used as an internal standard, number of protons (integration), multiplicity (singlet, s; doublet, d; triplet, t; quartet, q; multiplet, m; broad peak, br; etc.), and coupling constant (*J*, Hz). Infrared (IR) spectra were reported in wave number (cm^{-1}). Optical rotation was measured as a MeOH solution with a cell of 50 mm length. FAB mass spectra were measured in a positive ion mode.

4.2. Experimental procedure

4.2.1. General procedure for the coupling of two amino acids

Preparation of Boc-Phe-Leu−OBn (**5**) is described as the representative example. Boc-Phe (13.3 g, 50.0 mmol) and Leu−OBn·TsOH (21.6 g, 55.0 mmol) in anhydrous CH_2Cl_2 (70 mL) were added to a 200 mL two-necked round-bottomed flask under argon. The mixture was cooled to 0 °C with an ice/water bath, and WSC·HCl (10.6 g, 55.1 mmol) and HOBt·H_2O (8.44 g, 55.1 mmol) were added in one portion, followed by the slow addition of Et_3N (7.7 mL, 55 mmol). After 0.5 h, the cold bath was removed, and the reaction mixture was stirred overnight at room temperature. The reaction

mixture was then concentrated on a rotary evaporator and the residue was diluted with water. The aqueous layer was extracted with AcOEt (three times) and the combined organic layer was successively washed with 5% $NaHCO_3$ aq (three times), 5% citric acid aq (three times), and brine (once). After this typical workup procedure, the extracts were dried over anhydrous $MgSO_4$, filtered, and evaporated. After purification by column chromatography, the dipeptide compound **5** (22.8 g, 48.7 mmol) was obtained as white foam. Data for **5**:[22] yield 97.4%; R_f 0.51 (*n*-hexane−AcOEt=2:1); ^{1}H NMR δ ($CDCl_3$) 0.86 (3H, d, J=6.4 Hz), 0.88 (3H, d, J=6.1 Hz), 1.40 (9H, s), 1.45−1.62 (3H, m), 3.05 (2H, d, J=6.7 Hz), 4.35 (1H, q, J=5.8 Hz), 4.60 (1H, dt, J=5.2, 8.4 Hz), 5.01 (1H, br s), 5.11 (1H, d, J=12.2 Hz), 5.15 (1H, d, J=12.2 Hz), 6.30 (1H, d, J=8.2 Hz), 7.18−7.28 (5H, m), 7.32−7.38 (5H, m); ^{13}C NMR δ ($CDCl_3$) 21.8, 22.6, 24.5, 28.1, 38.0, 41.3, 50.8, 55.4, 66.8, 80.0, 126.7, 128.1, 128.3, 128.4, 128.5, 129.3, 135.3, 136.5, 155.4, 171.1, 172.2.

4.2.2. General procedure for the deprotection of the N-terminal Boc group

The N-terminal Boc deprotection of **5** is described as the representative example. Compound **5** (4.69 g, 10.0 mmol) in CH_2Cl_2 (4.0 mL) was added to a 50 mL round-bottomed flask at room temperature. The solution was cooled to 0 °C with an ice/water bath, and CF_3CO_2H (7.7 mL, 100 mmol) was slowly added, and the mixture was stirred at 0 °C for 2.5 h. Excess CF_3CO_2H was then removed as an azeotropic mixture with CH_2Cl_2. Boc-deprotected H−Phe-Leu−OBn·CF_3CO_2H thus obtained was used for the next condensation step without further purification.

4.2.3. General procedure for the deprotection of the C-terminal BnO group

The C-terminal BnO deprotection of Boc−Gly-Phe-Leu−OBn (**6**) is described as the representative example. At 0 °C, **6** (0.549 g, 1.04 mmol) in MeOH (6.0 mL) was added to 10% Pd/C (0.020 g, 1.8 mol %) under argon in a 10 mL two-necked round-bottomed flask. After argon was replaced with hydrogen at the same temperature, the reaction mixture was stirred overnight at room temperature. The catalyst was then removed by filtration, and the filtrate was concentrated on a rotary evaporator. Boc−Gly-Phe-Leu−OH was used for the next condensation reaction without further purification.

4.2.4. Preparation of Boc−F_3-Thr−OBn (*14*)[19]

F_3-threonine **4** (2.40 g, 13.9 mmol), 1,4-dioxane (28 mL), H_2O (28 mL), and Na_2CO_3 (1.63 g, 15.3 mmol) were added to a 300 mL round-bottomed flask. The mixture was cooled to 0 °C with an ice/water bath, and $(Boc)_2O$ (3.39 g, 15.5 mmol) was added in one portion. After 1 h, the cold bath was removed, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then concentrated on a rotary evaporator and the residue was diluted with water. The usual workup procedure and evaporation of the solvent after drying with anhydrous Na_2SO_4 furnished Boc−F_3-Thr (**13**) (3.34 g, 12.2 mmol, 88.1% yield) as oily residue, which was used for the next step.

To a mixture of **13** (1.63 g, 5.98 mmol) and $NaHCO_3$ (1.53 g, 18.2 mmol) in DMF (24 mL) was added BnBr (0.81 mL, 6.8 mmol), and the reaction mixture was stirred overnight at room temperature. The usual workup procedure and purification by column chromatography afforded **14** (1.84 g, 5.05 mmol) as an oil. Data for **14**: yield 84.5% from **4**; R_f 0.65 (*n*-hexane−AcOEt=2:1); ^{1}H NMR δ (DMSO-d_6) 1.44 (9H, s), 4.05 (1H, br s), 4.56 (1H, br s), 4.67 (1H, dd, J=2.5, 9.0 Hz), 5.23 (2H, s), 5.343 (1H, d, J=9.0 Hz), 7.26−7.39 (5H, m); ^{13}C NMR δ 28.0, 53.6, 67.8, 69.3 (q, J=31.7 Hz), 80.7, 123.9 (q, J=283.1 Hz), 128.0, 128.4, 128.5, 134.7, 156.0, 169.4; ^{19}F NMR δ ($CDCl_3$) −78.0 (d, J=6.8 Hz); IR (neat) ν 678, 694, 735, 775, 850, 908, 941, 1028, 1055, 1108, 1140, 1151, 1248, 1335, 1365, 1452, 1500, 1513, 1675, 1702, 1720, 1738, 2950, 3300 cm^{-1}; $[\alpha]_D^{29}$ +17.2 (*c* 1.00, MeOH). Anal. Calcd for $C_{16}H_{20}F_3NO_5$: C, 52.89; H, 5.55; N, 3.86. Found: C, 53.30; H, 5.91; N, 3.99.

4.3. Experimental data

4.3.1. Boc−Gly-Phe-Leu−OBn (*6*)[23]

Yield 95.6%; R_f 0.54 (*n*-hexane−AcOEt=1:2); ^{1}H NMR δ ($CDCl_3$) 0.85 (3H, d, J=6.4 Hz), 0.86 (3H, d, J=6.4 Hz), 1.39−1.62 (3H, m), 1.42 (9H, s), 3.02 (1H, dd, J=6.7, 13.7 Hz), 3.09 (1H, dd, J=6.4, 14.0 Hz), 3.72 (1H, dd, J=5.3, 16.9 Hz), 3.77 (1H, dd, J=5.6, 16.9 Hz), 4.56 (1H, dt, J=5.4, 8.5 Hz), 4.72 (1H, q, J=7.1 Hz), 5.11 (1H, d, J=12.2 Hz), 5.14 (1H, d, J=12.5 Hz), 5.19 (1H, t, J=5.6 Hz), 6.53 (1H, br s), 6.82 (1H, d, J=7.9 Hz), 7.16−7.25 (5H, m), 7.32−7.38 (5H, m); ^{13}C NMR δ ($CDCl_3$) 21.8, 22.5, 24.6, 28.2, 38.2, 40.9, 43.9, 50.9, 54.0, 66.8, 79.8, 126.7, 128.1, 128.2, 128.3, 128.4, 129.2, 135.3, 136.3, 155.9, 169.5, 170.7, 172.1.

4.3.2. Boc−Gly-Phe-Leu-Thr−OBn (*7*)[24]

Yield 75.7%; R_f 0.81 (AcOEt); ^{1}H NMR δ ($CDCl_3$) 0.84 (3H, d, J=6.4 Hz), 0.87 (3H, d, J=5.8 Hz), 1.17 (3H, d, J=6.4 Hz), 1.35 (9H, s), 1.45−1.52 (3H, m), 3.03 (1H, dd, J=5.8, 14.0 Hz), 3.15 (1H, dd, J=6.1, 14.0 Hz), 3.68 (1H, dd, J=5.3, 16.9 Hz), 3.73 (1H, dd, J=4.1, 16.9 Hz), 4.30−4.34 (1H, m), 4.62 (1H, dd, J=3.4, 8.8 Hz), 4.66 (1H, q, J=4.9 Hz), 4.73 (1H, q, J=6.4 Hz), 5.17 (1H, d, J=12.5 Hz), 5.20 (1H, d, J=12.2 Hz), 5.42 (1H, br s), 7.14 (1H, d, J=6.7 Hz), 7.12−7.36 (10H, m), 7.25 (1H, d, J=7.3 Hz), 7.45 (1H, d, J=8.8 Hz); ^{13}C NMR δ ($CDCl_3$) 19.6, 22.1, 22.4, 24.4, 28.2, 38.0, 40.8, 44.0, 51.9, 54.2, 58.1, 67.2, 68.0, 80.2, 126.9, 128.1, 128.2, 128.5, 128.5, 129.2, 135.2, 135.9, 156.3, 170.1, 170.5, 171.2, 172.7.

4.3.3. Boc−Tyr-D-Ser−OBn (*8*)

Yield 84.6%; R_f 0.71 (AcOEt); ^{1}H NMR δ ($CDCl_3$) 1.40 (9H, s), 2.94 (2H, d, J=6.7 Hz), 3.74 (1H, dd, J=3.2, 11.4 Hz), 3.85 (1H, d, J=11.3 Hz), 4.33 (1H, q, J=7.3 Hz), 4.59 (1H, br), 5.14 (1H, d, J=12.2 Hz), 5.17 (1H, d, J=12.2 Hz), 5.26 (1H, d, J=7.6 Hz), 6.73 (2H, d, J=8.5 Hz), 6.88 (1H, d, J=7.3 Hz), 7.02 (2H, d, J=8.2 Hz), 7.30−7.37 (5H, m); ^{13}C NMR

δ ($CDCl_3$) 28.2, 37.8, 54.7, 56.2, 62.3, 67.5, 80.7, 115.6, 127.5, 128.0, 128.4, 128.4, 128.5, 130.3, 135.0, 155.8, 170.1, 171.9; IR (KBr) ν 694, 732, 851, 1020, 1163, 1235, 1364, 1453, 1508, 1634, 2300, 2910, 3250 cm^{-1}; $[\alpha]_D^{21}$ −7.46 (*c* 1.05, MeOH); mp 118–123 °C. Anal. Calcd for $C_{24}H_{30}N_2O_7$: C, 62.87; H, 6.59; N, 6.11. Found: C, 62.68; H, 6.80; N, 6.34.

4.3.4. Boc–Tyr-D-Ser-Gly-Phe-Leu-Thr–OBn (**2**)

Yield 78.7%; R_f 0.83 (AcOEt–MeOH=2:1); ^1H NMR δ (DMSO-d_6) 0.82 (3H, d, *J*=6.5 Hz), 0.86 (3H, d, *J*=6.0 Hz), 1.09 (3H, d, *J*=6.5 Hz), 1.29 (9H, s), 1.44–1.52 (2H, m), 1.56–1.63 (1H, m), 2.63 (1H, dd, *J*=11.0, 12.5 Hz), 2.74 (1H, dd, *J*=10.5, 12.5 Hz), 2.86 (1H, dd, *J*=3.0, 13.0 Hz), 3.02 (1H, dd, *J*=2.8, 13.8 Hz), 3.58 (1H, dd, *J*=5.5, 11.5 Hz), 3.60 (1H, dd, *J*=5.5, 16.8 Hz), 3.72 (1H, dd, *J*=5.3, 16.8 Hz), 4.13 (1H, dt, *J*=4.0, 9.0 Hz), 4.19 (1H, dd, *J*=6.2, 8.8 Hz), 4.26 (1H, td, *J*=5.0, 6.0 Hz), 4.25 (1H, dd, *J*=3.0, 8.5 Hz), 4.44 (1H, dt, *J*=6.0, 8.5 Hz), 4.55 (1H, br t, *J*=8.5 Hz), 4.99 (1H, t, *J*=5.8 Hz), 5.02 (1H, d, *J*=5.50 Hz), 5.10 (1H, d, *J*=12.5 Hz), 5.14 (1H, d, *J*=12.5 Hz), 6.63 (2H, d, *J*=7.0 Hz), 6.88 (1H, d, *J*=8.2 Hz), 7.03 (2H, d, *J*=7.5 Hz), 7.16–7.23 (5H, m), 7.31–7.37 (5H, m), 7.94 (1H, d, *J*=9.5 Hz), 7.96 (1H, d, *J*=7.5 Hz), 8.00 (1H, d, *J*=8.5 Hz), 8.07 (1H, t, *J*=5.5 Hz), 8.20 (1H, d, *J*=8.0 Hz), 9.14 (1H, s); ^{13}C NMR δ (DMSO-d_6) 20.1, 21.6, 23.0, 24.1, 28.1, 36.6, 37.6, 40.7, 42.0, 51.0, 53.8, 54.9, 56.1, 57.8, 61.7, 65.9, 66.3, 78.2, 114.8, 126.2, 127.7, 127.9, 128.0, 128.3, 129.2, 130.1, 135.9, 137.7, 155.3, 155.7, 168.4, 170.2, 170.4, 170.8, 171.9, 172.4; IR (KBr) ν 411, 453, 488, 580, 698, 746, 831, 1022, 1080, 1105, 1163, 1252, 1367, 1388, 1454, 1516, 1649, 2335, 2362, 2871, 2931, 2958, 3032, 3066, 3292, 3811, 3847, 3857 cm^{-1}; $[\alpha]_D^{21}$ −8.32 (*c* 0.23, MeOH); mp 160–163 °C; HRMS-FAB (*m/z*): $[M]^+$ calcd for $C_{45}H_{60}N_6O_{12}$, 876.4269; found, 876.4233.

4.3.5. (S)-2-[(N-Benzylprolyl)amino]benzophenone (BPB) (**10**)[16c]

Yield 85.0%; R_f 0.70 (*n*-hexane–AcOEt=2:1); ^1H NMR δ ($CDCl_3$) 1.73–1.86 (2H, m), 1.96 (1H, ddd, *J*=4.4, 8.4, 16.8 Hz), 2.25 (1H, ddd, *J*=9.8, 13.0, 18.1 Hz), 2.41 (1H, dt, *J*=6.8, 9.6 Hz), 3.21 (1H, ddd, *J*=2.4, 6.8, 9.2 Hz), 3.32 (1H, dd, *J*=4.7, 10.2 Hz), 3.59 (1H, d, *J*=12.8 Hz), 3.92 (1H, d, *J*=12.8 Hz), 7.07–7.62 (12H, m), 7.78 (1H, d, *J*=7.9 Hz), 8.56 (1H, d, *J*=8.5 Hz), 11.52 (1H, s); ^{13}C NMR δ ($CDCl_3$) 24.1, 30.9, 53.8, 59.8, 68.2, 121.4, 122.1, 125.2, 127.0, 128.1, 128.2, 129.0, 130.1, 132.5, 132.5, 133.3, 138.1, 138.5, 139.1, 174.6, 198.0.

4.3.6. F_3-Threonine (**4**)[16e]

Yield 65.2%; ^1H NMR (DMSO-d_6) δ 2.86 (1H, dd, *J*=10.5, 12.5 Hz), 3.10 (1H, d, *J*=13.0 Hz), 4.37 (1H, br s); ^{13}C NMR (DMSO-d_6) δ 52.9, 67.7 (q, *J*=30.17 Hz), 125.4 (q, *J*=281.04 Hz), 169.4; ^{19}F NMR δ (D_2O) −77.4 (d, *J*=6.8 Hz).

4.3.7. Boc–Tyr-D-Ser-Gly-Phe-Leu–OBn (**15**)

Yield 94.2%; R_f 0.68 (*n*-hexane–AcOEt=1:5); ^1H NMR δ (DMSO-d_6) 0.83 (3H, d, *J*=6.4 Hz), 0.89 (3H, d, *J*=6.4 Hz), 1.29 (9H, s), 1.51–1.66 (3H, m), 2.63 (1H, dd, *J*=9.9, 13.6 Hz), 2.70 (1H, dd, *J*=10.1, 13.7 Hz), 2.86 (1H, dd, *J*=4.4, 13.7 Hz), 2.96 (1H, dd, *J*=3.7, 14.0 Hz), 3.45–3.49 (1H, m), 3.56–3.62 (1H, m), 3.71 (1H, dd, *J*=6.0, 16.6 Hz), 4.13 (1H, dt, *J*=4.6, 8.8 Hz), 4.25 (1H, q, *J*=6.0 Hz), 4.33 (1H, dt, *J*=5.2, 8.2 Hz), 4.55 (1H, dt, *J*=3.4, 9.1 Hz), 5.00 (1H, t, *J*=5.3 Hz), 5.10 (1H, d, *J*=12.0 Hz), 5.13 (1H, d, *J*=12.5 Hz), 6.63 (2H, d, *J*=7.9 Hz), 6.86 (1H, d, *J*=8.2 Hz), 7.02 (2H, d, *J*=8.2 Hz), 7.16–7.38 (10H, m), 7.93 (1H, d, *J*=7.3 Hz), 8.02 (1H, d, *J*=8.5 Hz), 8.07 (1H, t, *J*=5.5 Hz), 8.43 (1H, d, *J*=7.6 Hz), 9.13 (1H, s); ^{13}C NMR δ (DMSO-d_6) 21.4, 22.8, 24.3, 28.2, 36.7, 37.7, 39.7, 42.1, 50.6, 53.8, 55.1, 56.2, 61.7, 66.0, 78.3, 114.9, 126.4, 127.9, 127.9, 128.1, 128.1, 128.5, 128.5, 129.2, 130.2, 136.0, 137.8, 155.4, 155.8, 168.5, 170.5, 171.4, 172.2; IR (KBr) ν 409, 592, 698, 748, 820, 1053, 1169, 1250, 1367, 1454, 1516, 1658, 2349, 2958, 3064, 3307, 3662, 3789, 3811 cm^{-1}; $[\alpha]_D^{21}$ −8.61 (*c* 1.02, MeOH); mp 149–152 °C; HRMS-FAB (*m/z*): $[M]^+$ calcd for $C_{41}H_{53}N_5O_{10}$, 775.3792; found, 775.3788.

4.3.8. Boc–Tyr-D-Ser-Gly-Phe-Leu-F_3-Thr–OBn (**3**)

Yield 20.0%; R_f 0.62 (AcOEt–MeOH=9:1); ^1H NMR δ (DMSO-d_6) 0.74 (3H, d, *J*=6.0 Hz), 0.77 (3H, d, *J*=6.0 Hz), 1.29 (9H, s), 1.18–1.37 (3H, m), 2.62 (1H, dd, *J*=10.0, 14.0 Hz), 2.77 (1H, dd, *J*=9.0, 13.5 Hz), 2.87 (1H, dd, *J*=4.0, 13.5 Hz), 2.94 (1H, dd, *J*=5.8, 13.2 Hz), 3.47 (1H, ddd, *J*=6.3, 12.2, 23.0 Hz), 3.60 (1H, ddd, *J*=5.2, 10.0, 20.0 Hz), 3.64 (1H, dd, *J*=5.5, 17.2 Hz), 3.70 (1H, dd, *J*=5.8, 17.2 Hz), 4.13 (1H, dt, *J*=4.5, 9.0 Hz), 4.27 (1H, dt, *J*=5.5, 7.0 Hz), 4.48 (1H, dt, *J*=8.7, 9.5 Hz), 4.19 (1H, dd, *J*=2.1, 7.1 Hz), 4.62 (1H, dt, *J*=6.5, 8.0 Hz), 4.91 (1H, dd, *J*=2.2, 9.2 Hz), 5.06 (1H, t, *J*=5.2 Hz), 5.15 (1H, d, *J*=12.5 Hz), 5.20 (1H, d, *J*=12.5 Hz), 6.63 (2H, d, *J*=8.5 Hz), 6.90 (1H, d, *J*=8.5 Hz), 7.01 (1H, d, *J*=7.5 Hz), 7.03 (2H, d, *J*=8.0 Hz), 7.16–7.26 (5H, m), 7.32–7.38 (5H, m), 7.94 (1H, d, *J*=8.0 Hz), 8.06 (1H, d, *J*=8.5 Hz), 8.12 (1H, t, *J*=5.8 Hz), 8.20 (1H, d, *J*=9.0 Hz), 8.44 (1H, d, *J*=9.0 Hz), 9.17 (1H, s); ^{13}C NMR δ (DMSO-d_6) 21.2, 23.3, 23.9, 28.1, 36.6, 38.2, 40.7, 42.0, 50.6, 51.7, 54.0, 54.8, 56.1, 61.7, 66.8, 68.3 (q, *J*=30.2 Hz), 78.2, 114.8, 124.4 (q, *J*=281.8 Hz), 126.3, 127.8, 127.9, 128.0, 128.1, 128.4, 128.4, 129.2, 130.2, 135.5, 137.4, 155.3, 155.7, 168.4, 168.7, 170.4, 171.9, 172.6; ^{19}F NMR δ (DMSO-d_6) −76.0 (d, *J*=7.9 Hz); IR (KBr) ν 833, 908, 949, 1032, 1049, 1101, 1165, 1252, 1338, 1367, 1390, 1454, 1512, 1624, 1670, 1745, 1890, 2343, 2388, 2872, 2935, 2964, 3034, 3068, 3284 cm^{-1}; $[\alpha]_D^{21}$ +15.7 (*c* 0.09, MeOH); mp 101–105 °C. Anal. Calcd for $C_{45}H_{57}F_3N_6O_{12}$: C, 58.06; H, 6.17; N, 9.03. Found: C, 57.64; H, 6.31; N, 8.79.

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18. Enantiomeric excess (ee) was analyzed after the reaction of Boc–F_3-Thr and (*S*)-phenethylamine leading to the diastereomer mixture of the amides. Five doublet peaks were found by ^{19}F NMR, and a similar calculation as in Ref. 17 afforded 91.7% ee as the minimum level of enantioselectivity.
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