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Incorporation of fluoroprolines to proctolin: Study on the effect of a fluorine atom toward peptidic conformation

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Abstract

In spite of quite small steric perturbation, substitution of proline (Pro) in the target pentapeptide proctolin (Arg-Tyr-Leu-Pro-Thr) for (4*R*)- as well as (4*S*)-4-fluoroproline led to apparent alteration of the pyrrolidine ring structure which eventually resulted in the significant conformational change of the whole molecules which was assumed on the basis of their NOESY spectra.

Keywords: Fluorinated amino acid; Peptidic conformations; NOESY spectra

1. Introduction

Proline (Pro) possesses an exceptional cyclic secondary amine structure among the 20 naturally occurring amino acids, which has been drawing a special interest in the field of medicinal chemistry. The peptide linkages composed of natural amino acids usually exist exclusively as the *s-trans* conformation mainly due to steric reasons, but the proline-related amide bond usually consists of a mixture of both *s-cis* and *s-trans* conformers by their structural similarity [1] (Fig. 1). These unique features sometimes affect their biological behavior like the case of protein foldings, and the examples where only one of the two isomers show bioactivity have been reported [2,3]. Therefore, incorporation of other elements or groups to the proline ring in target peptides has become one of the central methods for modification of the original biological activity.

Organofluorine compounds have played important roles in various areas as agrochemicals, pharmaceuticals, and polymers because fluorination can drastically affect the original chemical and physical properties of molecules [4]. Fluorinated amino acids [5] and peptides [6] are especially interesting as enzyme inhibitors in the development of medicines.

Our research group has previously reported the synthesis and conformational analysis of the fluorine-containing enkephalin hexapeptide derivative and successfully demonstrated that entry of three fluorine atoms to the terminal methyl group of the threonine residue clearly changed the original conformation, as our expectation, by elimination of the hydrogen bonding [7].

During our continuing study in this field, we selected biologically active proctolin **1** [8] (Arg-Tyr-Leu-Pro-Thr, Fig. 2) known as neurohormone of insects and shellfishes as the next target for investigation of the effect of a fluorine atom towards molecular conformation of **1** when this atom was substituted either for the pro-*R* or pro-*S* protons at the 4-position of Pro.

2. Results and discussion

2.1. Preparation of proctolin *1* and its monofluorinated analogues *2* and *3*

In Scheme 1 the synthetic sequence to assemble the target compound **1** is shown. For coupling reactions, the urea-type standard reagent WSC·HCl [9] was employed in the presence of an additive HOBt·H_2O [10] whose combination has advantages for realizing high yield with a lower chance of undesirable epimerization.

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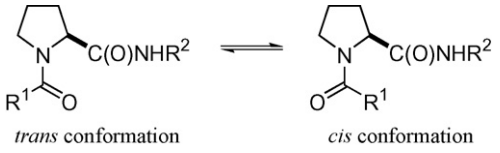


Fig. 1. Isomerism of the acylated Pro amide bond.

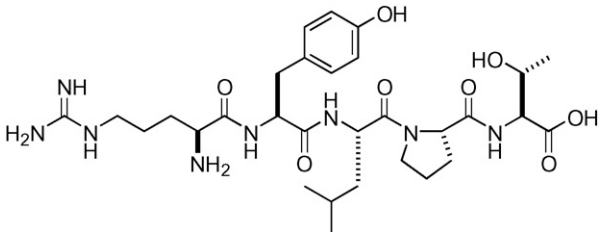
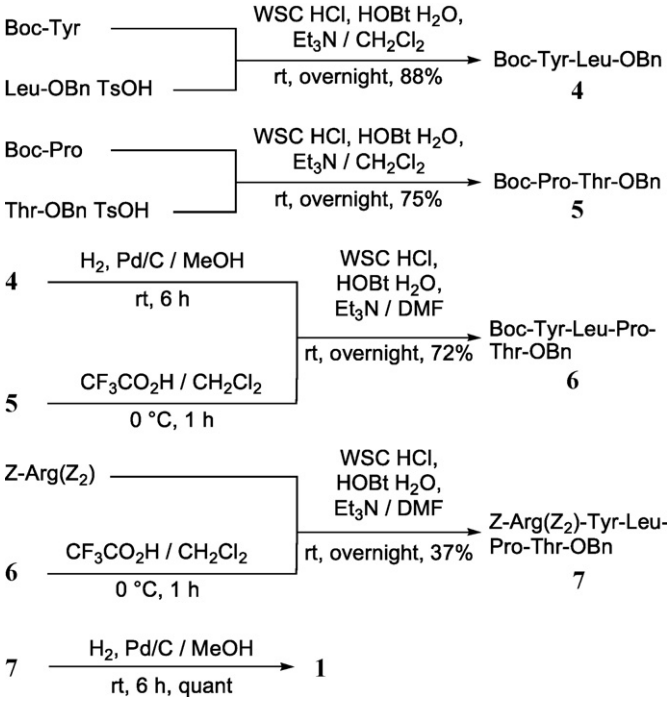


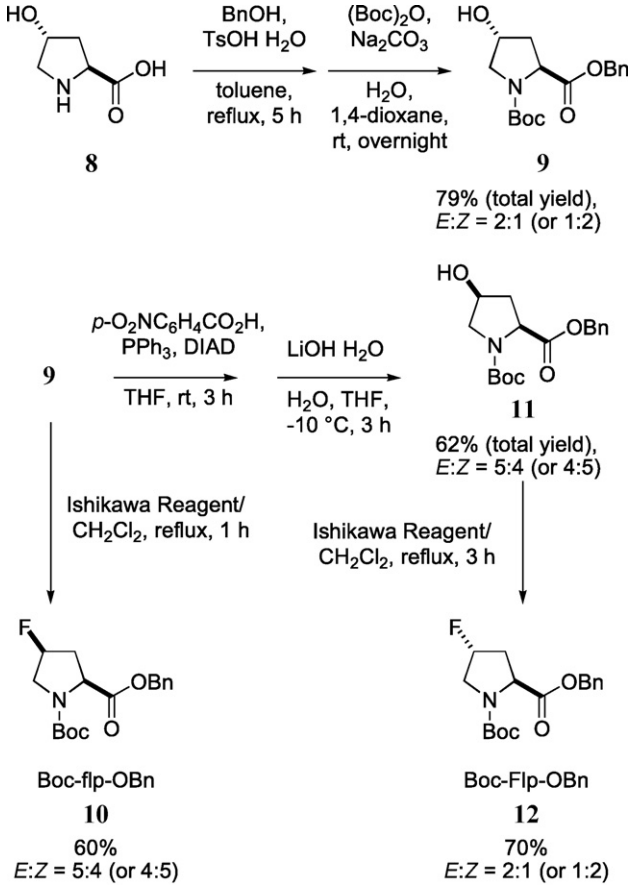
Fig. 2. Structure of Proctolin **1** (Arg-Tyr-Leu-Pro-Thr).

First, Boc-Tyr was condensed with Leu-OBn·TsOH under the above condition to yield the dipeptide **4**. Removal of the Boc group of the dipeptide **5**, prepared in a same manner as **4** from Boc-Pro and Thr-OBn·TsOH, followed by the coupling with **4** after hydrogenolytic cleavage of the *C*-terminal benzyl group furnished the tetrapeptide **6**. Finally, the pentapeptide **7** was constructed by the WSC-mediated condensation of Boc-deprotected **6** and Z-Arg(Z_2) prepared following to the synthetic method reported by Zervas et al. [11]. Catalytic hydrogenation affected the spontaneous removal of the benzyl ester and the Z groups at the same instance to provide the desired target pentapeptide **1** as a single stereoisomer.

At the next stage, (2*S*,4*S*)- and (2*S*,4*R*)-4-fluoroproline derivatives **10** [12] (Boc-flp-OBn) and **12** (Boc-Flp-OBn) were prepared following to the procedure shown in Scheme 2. The commercially available (4*R*)-4-hydroxyproline **8** (Hyp)



Scheme 1. Preparation of Arg-Tyr-Leu-Pro-Thr **1**.



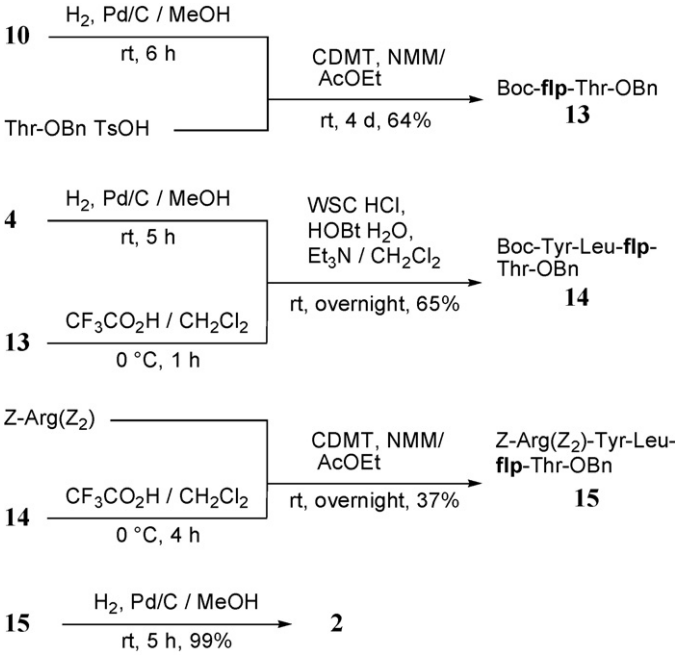
Scheme 2. Syntheses of fluoroprolines **10** and **12**.

was protected as a benzyl ester by BnOH and a catalytic amount of acid, and subsequently by $(Boc)_2O$ with a base to yield **9** which was further converted to Boc-flp-OBn **10** by the fluorination using perfluoropropene diethylamine adduct [13] (PPDA; Ishikawa reagent). Inversion of stereochemistry of the hydroxy group in **9** by the Mitsunobu procedure [12a,14] prior to the PDDA fluorination afforded the different diastereomer Boc-Flp-OBn **12** in a stereospecific manner.

Fluorinated prolines **10** and **12** thus obtained were incorporated to proctolin **1** instead of Pro. The same synthetic protocol for **1** was used for the construction of both **2** and **3** (Schemes 3 and 4). Most of coupling reactions proceeded in good to high yields, while CDMT [15] was found especially effective in some specific steps such as preparation of the dipeptide **13** and the pentapeptide **15**. The final hydrogenolysis was smoothly completed for **15** under an ambient pressure to furnish **2** in a quantitative yield with a 2:1 isomeric ratio [16], while the same process to **3** proceeded with difficulty under the same condition. However, deprotection from **18** was eventually realized under a 0.46 MPa pressure of H_2 and **3** was afforded in an acceptable yield in a 2:1 isomeric ratio [16].

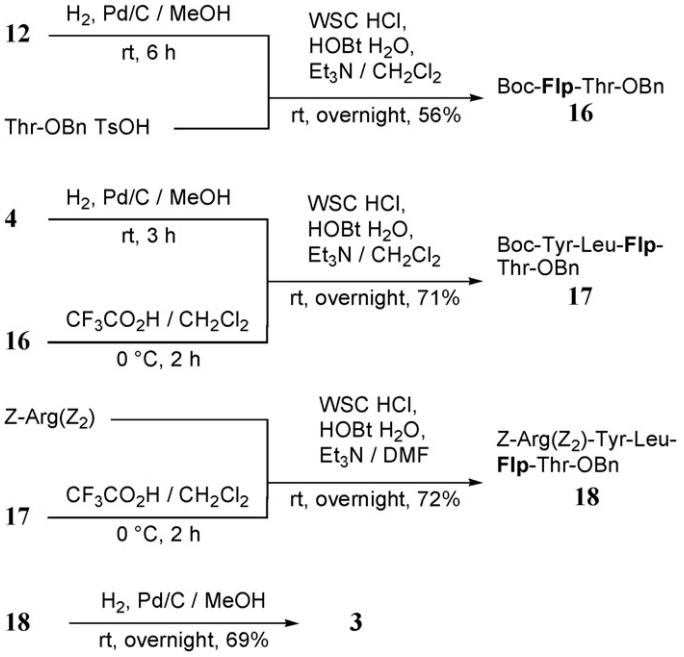
2.2. Conformational analysis of proctolin *1* and its monofluorinated analogues *2* and *3*

Extensive studies have been carried out by the Raines' research group [17] on disclosing the pyrrolidine ring structure



Scheme 3. Preparation of Arg-Tyr-Leu-flp-Thr **2**.

in Pro derivatives. For example, Ac-Pro-OMe **19** was found to exist as a conformational mixture of C$^{\gamma}$-*endo*:C$^{\gamma}$-*exo* = 66:34, while introduction of only one fluorine to the 4-position of the pyrrolidine ring of Pro affected this ratio quite significantly. Thus, in spite of imposing the least steric perturbation, Ac-flp-OMe **20** and Ac-Flp-OMe **21** [18] gave the isomeric preference of *endo*:*exo* = 95:5 and 14:86, respectively, which was explained as a result of the *gauche* effect. The amide *cis*:*trans* ratios of **19**–**21** were 3.0, 4.0, and 1.2, respectively, in favor of the *trans* form and the crystallographic analysis of **21** with the C$^{\gamma}$-*exo*-*trans* conformation led to the speculation that this



Scheme 4. Preparation of Arg-Tyr-Leu-flp-Thr **3**.

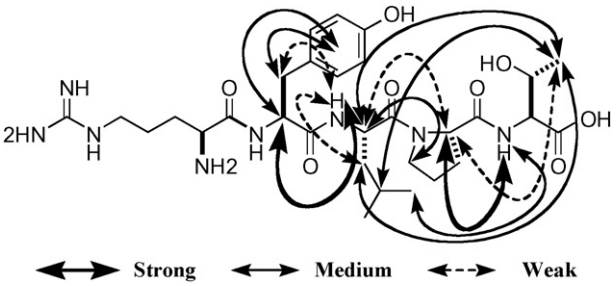


Fig. 3. NOESY correlation of **1**.

material was further stabilized by the $n-\pi^*$ interaction of amide oxygen with the methoxycarbonyl carbon atom [19].

With such basic information on the three-dimensional shapes of Pro derivatives, NOESY spectrum of **1** obtained in DMSO-d_6 (Fig. 3) was analyzed. The amide bonds of Tyr-Leu and Pro-Thr were determined to possess *trans* conformation on the basis of the correlations between C*H*–C(O)N*H*–, including the one between Leu and Pro which was strongly suggested by the definite cross peak between Leu-α-C*H* and Pro-δ-CH_2. Moreover, a number of cross peaks between Leu and Thr were observed which led to anticipation that Leu-C*O* and Thr-N*H* formed a hydrogen bond to construct γ-turn conformation. In addition, the zwitterionic interaction between the both terminal amino acids Arg and Thr would also contribute to the close proximity of the side chains of Leu and Thr. Absence of the cross peak between Pro-α-C*H* and -γ-CH_2 expected the pyrrolidine ring conformation of Pro as C$^{\gamma}$-*endo*.

In case of compound **2**, it was interesting to note that no long range correlations were obtained from the NOESY spectrum except for three cross peaks between adjacent amino acids (Fig. 4). The pentapeptide **2** was proved to possess *trans* amide conformation between Tyr-Leu and Pro-Thr similar to the corresponding non-fluorinated counterpart **1**. The cross peak between Leu-α-C*H* and Pro-δ-CH_2 clearly demonstrated the *trans* relationship of the amide linkage which allowed us to assume the major isomer being *trans*. In addition, the C$^{\gamma}$-*exo* ring pucker of the pyrrolidine ring was deduced to be formed at the flp residue on the basis of the obvious correlation between α-C*H* and γ-C*H* of flp, which was not found for **1**. This C$^{\gamma}$-*exo* preference was totally contradicted to the issue already discussed above. This reversal would be occurred to avoid the undesirable electronic repulsive interaction of the fluorine atom with the carbonyl oxygen at the same residue when C$^{\gamma}$-*endo* conformation was occupied. This conformational

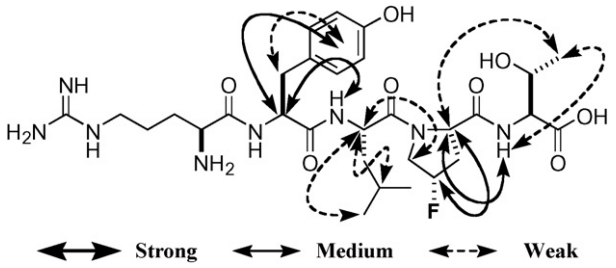


Fig. 4. NOESY correlation of **2**.

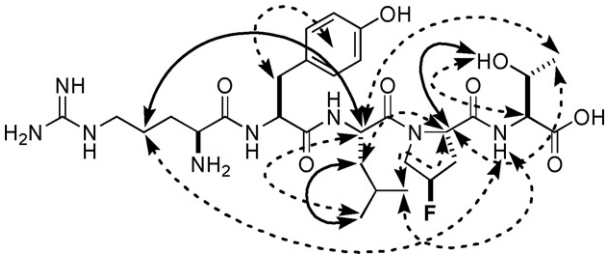


Fig. 5. NOESY correlation of **3**.

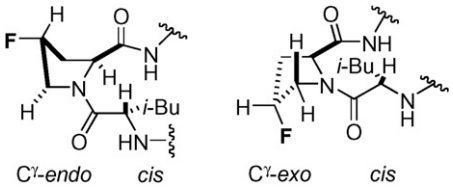


Fig. 6. Plausible pyrrolidine ring structure of **3**.

modification would be energetically favorable so that it could keep γ-turn conformation by forming the hydrogen bonding between Leu-C*O* and Thr-N*H* by sacrificing the ring conformational stability.

One of the most interesting points for the NOESY results of **3** would be the fact that, different from both **1** and **2**, this compound **3** possessed *cis* conformation at the Leu-Pro amide bond which was assumed from the correlation between Pro-α-C*H* and Leu-β-CH_2 (Fig. 5). Furthermore, the pyrrolidine ring in **3** indicated a similar tendency to the compound **2** with occupying the opposite conformation to the expected C$^{\gamma}$-*exo* structure, and C$^{\gamma}$-*endo* was the actual three-dimensional shape. This phenomena would be understood from the fact that the C$^{\gamma}$-*exo* form would be encumbered by the steric hindrance of the *i*-Bu group as supported by the cross peaks between protons in this group and Pro-α-*H* (Fig. 6). The C$^{\gamma}$-*endo* structure, therefore, was presumed to be formed as the most stable pyrrolidine ring conformation.

Finally, insecticidal activities were investigated briefly for the pentapeptides **1**–**3**. At the 500 ppm concentration, the Flp-containing **3** showed a biological activity towards *tortrix* while **1** and **2** being inactive. On the other hand, only the non-fluorinated **1** was found to be potent against *spider mite*.

3. Conclusion

In summary, we could clarify obvious conformational change including alteration of the following proline-related characteristic structures of the C$^{\gamma}$-*endo* or C$^{\gamma}$-*exo* pyrrolidine ring conformations and *cis* or *trans* stereochemistry for the amide linkage just by entry of only one fluorine atom to Pro. Moreover, such conformational change would be one of the important factors for appearance of biological activities in spite of imposing the least steric perturbation. Our research group is going to clarify the solid state conformations of the pentapeptides **1**–**3** synthesized here by X-ray crystallographic structure analysis for obtaining their three-dimensional feature.

4. Experimental

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 and THF were 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 *t*-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). Spherical neutral silica gel (63–210 μm or 40–50 μm) was employed for column chromatography and flush 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 ($\delta = 0.00$) 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 by a cell with a 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-Tyr-Leu-OBn (**4**) is described as the representative example. Boc-Tyr (8.44 g, 30.0 mmol) and Leu-OBn·TsOH (11.8 g, 30.0 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (100 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 (6.33 g, 33.0 mmol, 1.1 equiv) and HOBt·H_2O (5.05 g, 33.0 mmol, 1.1 equiv) were added in one portion, followed by the slow addition of Et_3N (4.6 mL, 33 mmol, 1.1 equiv). 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 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 work-up procedure, the extracts were dried over anhydrous $MgSO_4$, filtered, and evaporated. After purification by column chromatography, the dipeptide compound, **4** (12.8 g, 26.5 mmol) was obtained as white foam.

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

The *C*-terminal BnO deprotection of **4** is described as the representative example. At 0 °C, **4** (4.02 g, 8.30 mmol) in MeOH (8.3 mL) was added to 10% Pd/C (0.087 g, 0.98 mol%) under argon in a 50 mL two-necked round-bottomed flask. After argon was replaced with hydrogen at the same temperature, the reaction mixture was stirred for 6 h at room temperature. The catalyst was then removed by filtration, and the filtrate was concentrated on a rotary evaporator. Boc-Tyr-Leu-OH was used for the next condensation reaction without further purification.

The *N*-terminal Boc deprotection of **5** is described as the representative example. **5** (3.32 g, 8.17 mmol) in CH_2Cl_2 (5.0 mL) was added to a 100 mL round-bottomed flask at room temperature. The solution was cooled to 0 °C with an ice/water bath, and CF_3CO_2H (13 mL, 160 mmol) was slowly added, and the mixture was stirred at 0 °C for 1 h. Excess CF_3CO_2H was then removed as an azeotropic mixture with CH_2Cl_2. Boc-deprotected H-Pro-Thr-OBn·CF_3CO_2H thus obtained was used for the next condensation step without further purification.

4.2.3. Preparation of Boc-Hyp-OBn (9)

(2*S*,4*R*)-4-Hydroxyproline (6.57 g, 50.1 mmol), toluene (50 mL), BnOH (24 mL, 230 mmol, 4.6 equiv), and *p*-TsOH·H_2O (11.5 g, 60.4 mmol, 1.2 equiv) were added to a 200 mL round-bottomed flask. The mixture was refluxed for 2 h, and the residue was then washed with Et_2O (100 mL) and *n*-hexane (100 mL) until it was crystallized. The resulting precipitate was filtered off with suction and was washed with *n*-hexane. Hyp-OBn·TsOH obtained as a white foam was used for the next step.

Hyp-OBn·TsOH (19.7 g, 50.0 mmol), 1,4-dioxane (100 mL), H_2O (50 mL), and Na_2CO_3 (5.83 g, 55.0 mmol, 1.1 equiv) were added to a 300 mL round-bottomed flask. The mixture was cooled to 0 °C with an ice/water bath, and $(Boc)_2O$ (12.2 g, 55.8 mmol, 1.1 equiv) was added in one portion. After 0.5 h, the cold bath was removed, and the reaction mixture was stirred overnight at room temperature. It was concentrated on a rotary evaporator and the residue was diluted with water. To this solution was added 4 N HCl aq. until pH 1 checked by pH-test paper. The usual workup procedure and purification by column chromatography afforded **9** (13.0 g, 40.4 mmol) as an oil.

4.2.4. Preparation of Boc-flp-OBn (10)

9 (6.43 g, 2.20 mmol) in anhydrous CH_2Cl_2 (10 mL) was added to a 50 mL two-necked round-bottomed flask under argon. The mixture was cooled to 0 °C with an ice/water bath, and PPDA (4.7 mL, 26 mmol, 1.3 equiv) was slowly added. After 0.5 h, the cold bath was removed, and the reaction mixture was refluxed for 1 h. It was concentrated on a rotary evaporator and the residue was diluted with water. The usual workup procedure and purification by column chromatography afforded **10** (3.89 g, 12.0 mmol) as an oil.

4.2.5. Preparation of Boc-hyp-OBn (11)

9 (3.22 g, 10.0 mmol) was dissolved in anhydrous THF (50 mL), and it was added with Ph_3P (3.40 g, 13.0 mmol, 1.3 equiv) and PNBOH (2.19 g, 13.1 mmol, 1.3 equiv) to a 100 mL two-necked round-bottomed flask under argon. After cooling the solution in a 0 °C bath, DIAD (2.6 mL, 13 mmol, 1.3 equiv) was slowly added. The resulting solution was stirred overnight at rt and then concentrated to a yellow oil under reduced pressure. Adding *n*-hexane-AcOEt solution to the oil resulted in the formation of a colorless solid which was separated by vacuum filtration. This solid was not the desired product, but most likely triphenylphosphine oxide and diisopropylhydrazine. The crude Boc-hyp(PNB)-OBn obtained as an oil from the filtrate was used for the next step.

To a solution of attained *p*-nitrobenzoic acid ester in THF (60 mL) and H_2O (20 mL) was added LiOH·H_2O (0.44 g, 10 mmol, 1.0 equiv) at −10 °C. After stirring for 3 h, 4 N HCl aq. was added to this solution until pH 1 with checking by pH-test paper. The usual workup procedure and purification by column chromatography afforded **11** (1.98 g, 6.18 mmol) as a white foam.

4.3. Experimental data

4.3.1. Boc-Tyr-Leu-OBn (4) [20]

Yield 88.4%. R_f 0.79 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.86 (d, J = 6.5 Hz, 3H), 0.87 (d, J = 7.0 Hz, 3H), 1.41 (s, 9H), 1.42–1.61 (m, 3H), 2.93–3.02 (m, 2H), 4.29 (br s, 1H), 4.60 (br s, 1H), 5.10 (d, J = 12.5 Hz, 1H), 5.14 (d, J = 12.5 Hz, 1H), 5.14 (br s, 1H), 6.42 (d, J = 8.0 Hz, 1H), 6.69 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 7.31-7.38 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 21.8, 22.7, 24.6, 28.2, 37.3, 41.4, 50.9, 55.8, 67.1, 80.4, 115.5, 127.8, 128.2, 128.4, 128.6, 130.4, 135.2, 155.1, 155.6, 171.3, 172.3.

4.3.2. Boc-Pro-Thr-OBn (5) [21]

Yield 74.9%. R_f 0.61 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.18 (d, J = 6.5 Hz, 3H), 1.45 (s, 9H), 1.76–2.11 (m, 2H), 2.11–2.28 (m, 2H), 3.29–3.57 (m, 2H), 4.18–4.44 (br, 2H), 4.61 (dd, J = 2.2, 8.8 Hz, 1H), 5.18 (d, J = 12.0 Hz, 1H), 5.21 (d, J = 12.5 Hz, 1H), 7.08 (br s, 1H), 7.26–7.46 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 20.0, 28.3, 47.1, 57.6, 60.0, 60.4, 61.0, 67.2, 68.7, 128.2, 128.4, 128.6, 135.2, 155.5, 170.6, 172.5, 175.0.

4.3.3. Boc-Tyr-Leu-Pro-Thr-OBn (6)

Yield 72.0%. R_f 0.57 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.85 (d, J = 6.5 Hz, 3H), 0.89 (d, J = 7.0 Hz, 3H), 1.18 (d, J = 6.0 Hz, 3H), 1.37–1.62 (m, 3H), 1.41 (s, 9H), 1.86–2.18 (m, 4H), 2.83–2.95 (m, 2H), 3.54–3.75 (m, 2H), 4.27–4.33 (m, 2H), 4.44–4.48 (m, 1H), 4.60 (dd, J = 3.2, 9.2 Hz, 1H), 4.70–4.76 (m, 1H), 5.15 (d, J = 12.5 Hz, 1H), 5.20 (d, J = 12.5 Hz, 1H), 5.23 (d, J = 8.5 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.5 Hz, 2H), 7.25–7.37 (m, 5H), 7.41 (d, J = 8.5 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 20.1, 21.7, 23.1, 28.2, 38.0, 41.2, 47.6, 48.9, 55.7, 57.9, 60.0, 67.3, 68.5, 80.1, 115.5, 127.6, 128.1, 128.2 128.4, 128.5, 128.6, 130.2, 135.1, 155.2, 155.4, 171.0, 171.3, 171.5, 172.0. IR (KBr) ν: 661, 740, 917, 1017, 1043, 1100, 1160, 1208, 1362, 1438, 1493, 1619, 1651, 2300, 2950, 3270 cm^{-1}. $[\alpha]_D^{18}$ −59.6 (*c* 1.03, MeOH). mp 98–103 °C.

HRMS-FAB (*m/z*): $[M + H]^+$ calcd for $C_{36}H_{51}N_4O_9$, 683.3656; found, 683.3698.

4.3.4. Z-Arg(Z$_2$)-Tyr-Leu-Pro-Thr-OBn (7)

Yield 37.4%. R_f 0.73 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.84 (d, J = 6.0 Hz, 3H), 0.87 (d, J = 6.5 Hz, 3H), 1.07 (d, J = 6.5 Hz, 3H), 1.41-2.32 (m, 11H), 2.61–2.93 (m, 2H), 3.54–3.65 (m, 2H), 3.74–3.98 (m, 2H), 4.16–4.25 (m, 1H), 4.25–4.37 (m, 1H), 4.53 (dd, J = 2.5, 9.0 Hz, 1H), 4.59–4.67 (m, 1H), 4.73–4.85 (m, 1H), 4.85–5.01 (m, 1H), 5.01–5.24 (m, 8H), 6.52 (d, J = 8.0 Hz, 2H), 6.65 (d, J = 7.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.15–7.27 (m, 20H), 7.26 (br s, 1H), 7.44 (d, J = 9.0 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 20.0, 21.8, 23.2, 24.3, 24.7, 28.9, 41.7, 44.1, 47.4, 57.8, 59.8, 66.9, 67.1, 69.0, 115.3, 115.6, 127.3, 127.6, 127.8, 128.0, 128.2, 128.2, 128.3, 128.3, 128.4, 128.5, 128.8, 128.9, 130.2, 134.5, 135.3, 136.3, 155.7, 160.7, 163.3, 170.9, 172.1. IR (KBr) ν: 660, 695, 738, 898, 1000, 1092, 1204, 1368, 1435, 1502, 1603, 1700, 2300, 2930, 3000, 3250, 3350 cm^{-1}. $[\alpha]_D^{19}$ −48.5 (*c* 1.03, MeOH). mp 93–95 °C. HRMS-FAB (*m/z*): $[M + H]^+$ calcd for $C_{61}H_{73}N_8O_{14}$, 1141.5246; found, 1141.5208.

4.3.5. Arg-Tyr-Leu-Pro-Thr (1) [21]

Yield 99.5%. ^{1}H NMR (500 MHz, DMSO-d_6, δ): 0.71–1.01 (m, 9H), 1.07–2.27 (m, 11H), 2.62–2.95 (m, 2H), 3.02 (t, J = 7.0 Hz, 2H), 3.14 (t, J = 6.2 Hz, 1H), 3.43-3.69 (m, 2H), 3.72–4.08 (m, 2H), 4.38–4.42 (m, 1H), 4.43–4.50 (m, 1H), 4.52–4.63 (m, 1H), 6.62 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.5 Hz, 1H), 7.97 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 6.5 Hz, 1H). ^{13}C NMR (500 MHz, DMSO-d_6, δ): 19.2, 21.5, 23.2, 24.1, 24.5, 24.9, 28.5, 31.8, 36.5, 36.6, 40.6, 40.9, 46.7, 48.5, 53.4, 54.0, 57.5, 59.6, 66.2, 114.8, 127.5, 130.2, 155.8, 157.1, 170.2, 170.7, 170.8, 173.8.

4.3.6. Boc-Hyp-OBn (9) [22]

Yield 80.8%, 2:1 (*E*,*Z* isomeric ratio). R_f 0.40 (Et_2O). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.34 (s, 9H), 2.01–2.10 (m, 1H), 2.22–2.34 (m, 1H), 2.40 (br s, 1H), 3.42–3.65 (m, 2H), 4.41–4.53 (m, 2H), 5.13 (d, J = 12.3 Hz, 1H), 5.18 (d, J = 12.3 Hz, 1H), 7.32–7.37 (m, 5H). (minor) 1.45 (s, 9H), 2.01–2.10 (m, 1H), 2.22–2.34 (m, 1H), 2.40 (br s, 1H), 3.42–3.65 (m, 2H), 4.41–4.53 (m, 2H), 5.09 (d, J = 12.9 Hz, 1H), 5.26 (d, J = 12.6 Hz, 1H), 7.32–7.37 (m, 5H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 27.6, 38.5, 54.0, 57.7, 66.2, 68.4, 79.9, 127.5, 127.9, 128.1, 135.0, 153.7, 172.6. (minor) 27.9, 37.8, 54.2, 57.4, 66.2, 69.1, 79.7, 127.7, 127.9, 128.0, 135.2, 154.2, 172.4.

4.3.7. Boc-flp-OBn (10) [12]

Yield 60.1%, 5:4 (*E*,*Z* isomeric ratio). R_f 0.64 (*n*-Hexane:AcOEt = 1:1). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.34 (s, 9H), 2.31–2.42 (m, 1H), 2.50 (dd, J = 15.0, 17.5 Hz, 1H), 3.58–3.73 (m, 2H), 4.46 (d, J = 9.5 Hz, 1H), 5.12 (s, 2H), 5.20 (dm, J = 52.0 Hz, 1H), 7.29–7.36 (m, 5H). (minor) 1.46 (s, 9H), 2.28 (ddd, J = 4.5, 9.5, 14.5 Hz, 1H), 2.44 (dd, J = 4.5, 9.5 Hz, 1H), 3.76–3.89 (m, 2H), 4.60 (d, J = 9.5 Hz, 1H), 5.09 (d, J = 12.5 Hz, 1H), 5.20 (dm, J = 52.0 Hz, 1H), 5.27 (d, J = 12.5 Hz, 1H), 7.29–7.36 (m, 5H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 28.3, 37.5 (d, J = 20.6 Hz), 53.2 (d, J = 24.6 Hz), 66.9, 80.4, 91.2 (d, J = 174.8 Hz), 128.0, 128.3, 128.4, 135.5, 153.6, 171.5. (minor) 28.1, 36.6 (d, J = 20.6 Hz), 52.9 (d, J = 22.6 Hz), 67.0, 80.4, 91.7 (d, J = 176.9 Hz), 128.1, 128.3, 128.5, 135.6, 154.0, 171.2. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −174.28 to −173.50 (m).

4.3.8. Boc-hyp-OBn (11) [12a]

Yield 61.6%, 5:4 (*E*,*Z* isomeric ratio). R_f 0.43 (*n*-Hexane:AcOEt = 3:2). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.34 (s, 9H), 2.11 (br d, J = 12.3 Hz, 1H), 2.25–2.40 (m, 1H), 3.24 (d, J = 9.3 Hz, 1H), 3.50–3.72 (m, 2H), 4.31–4.44 (m, 2H), 5.20 (s, 2H), 7.32–7.39 (m, 5H). (minor) 1.47 (s, 9H), 2.11 (br d, J = 12.3 Hz, 1H), 2.25–2.40 (m, 1H), 3.34 (d, J = 9.9 Hz, 1H), 3.50–3.72 (m, 2H), 4.31–4.44 (m, 2H), 5.14 (d, J = 12.3 Hz, 1H), 5.31 (d, J = 12.3 Hz, 1H), 7.34–7.37 (m, 5H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 28.0, 38.5, 55.2, 57.9, 67.2, 70.0, 80.3, 128.0, 128.4, 128.5, 128.6, 135.0, 153.6, 174.4. (minor) 28.3, 37.7, 55.6, 57.7, 67.2, 71.0, 80.2, 128.2, 128.4, 128.5, 128.6, 135.2, 154.3, 174.5.

4.3.9. Boc-Flp-OBn (12) [12a]

Yield 70.0%, 2:1 (*E*,*Z* isomeric ratio). R_f 0.63 (*n*-Hexane:AcOEt = 1:1). ^{1}H NMR (300 MHz, $CDCl_3$, δ): (Major) 1.36 (s, 9H), 1.96–2.08 (m, 1H), 2.51–2.67 (m, 1H), 3.63 (ddd, J = 3.3, 6.6, 36.6 Hz, 2H), 3.92 (ddd, J = 2.1, 12.9, 22.2 Hz, 1H), 4.45 (dd, J = 7.8, 9.0 Hz, 1H), 5.08–5.31 (m, 3H), 7.36 (br s, 5H). (minor) 1.47 (s, 9H), 2.10–2.21 (m, 1H), 2.51–2.67 (m, 1H), 3.59 (ddd, J = 3.3, 6.6, 36.6 Hz, 2H), 4.54 (ddd, J = 8.4, 8.4, 8.4 Hz, 1H), 4.54 (t, J = 8.4 Hz, 1H), 5.08-5.31 (m, 3H), 7.36 (br s, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 27.9, 37.3 (d, J = 22.9 Hz), 52.7 (d, J = 22.3 Hz), 57.5, 66.7, 80.3, 90.8 (d, J = 178.6 Hz), 127.9, 128.0, 128.2, 128.3, 128.4, 135.1, 153.4, 172.2. (minor) 28.1, 36.3 (d, J = 22.9 Hz), 53.0 (d, J = 23.5 Hz), 57.3, 66.7, 80.2, 91.6 (d, J = 178.6 Hz), 127.9, 128.0, 128.2, 128.3, 128.4, 135.4, 153.9, 171.9. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −178.77 (ddddd, J = 22.6, 22.9, 38.7, 38.7, 52.6 Hz). (minor) −178.09 (ddddd, J = 22.6, 22.9, 36.5, 36.5, 52.6 Hz).

4.3.10. Boc-flp-Thr-OBn (13)

Yield 63.8%. R_f 0.60 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.17 (d, J = 6.5 Hz, 3H), 1.46 (s, 9H), 2.01–2.84 (m, 2H), 3.55–3.88 (m, 2H), 4.30 (br, 1H), 4.48 (br s, 1H), 4.64 (dd, J = 2.2, 8.8 Hz, 1H), 5.22 (??, 2H), 5.23 (td, J = 3.5, 52.0 Hz, 1H), 7.26 (br s, 1H), 7.30–7.38 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 19.7, 28.1, 37.7, 53.9 (d, J = 20.6 Hz), 57.0, 59.9, 67.2, 68.5, 81.7, 92.0 (d, J = 172.7 Hz), 128.2, 128.4, 128.6, 135.3, 154.7, 170.2, 172.1. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −174.06 to −173.32 (m). (minor) −175.38 to −174.59 (m). $[\alpha]_D^{19}$ −53.9 (*c* 1.04, MeOH). mp 118–119 °C. Anal. Calcd for $C_{21}H_{29}FN_2O_6$: C, 59.42; H, 6.89; N, 6.60. Found: C, 59.59; H, 6.81; N, 6.57.

4.3.11. Boc-Tyr-Leu-flp-Thr-OBn (14)

Yield 64.7%. R_f 0.39 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.86–0.94 (m, 6H), 1.18 (d, J = 6.5 Hz, 3H), 1.40 (s, 9H),

1.28–1.72 (m, 3H), 2.11–2.82 (m, 2H), 2.81–3.12 (m, 2H), 3.58–4.12 (m, 2H), 4.18–4.46 (m, 2H), 4.50–4.65 (m, 1H), 4.59 (dd, J = 2.5, 8.5 Hz, 1H), 4.66–4.91 (d, J = 9.8, 1H), 5.12 (d, J = 12.5, 1H), 5.14 (d, J = 13.0, 1H), 5.06–5.23 (m, 1H), 5.21–5.29 (br, 1H), 6.64 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.0 Hz, 1H), 7.22 (d, J = 8.5, 1H), 7.26–7.38 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 20.0, 22.8, 23.0, 24.4, 28.3, 33.8, 37.1, 40.9, 52.0, 55.8, 58.1, 58.5, 67.4, 75.8, 115.6, 121.4, 127.6, 128.2, 128.3, 128.4, 128.6, 128.6, 130.3, 135.1, 155.2, 170.5, 170.5, 170.8, 171.5, 172.4.

^{19}F NMR (283 MHz, $CDCl_3$, δ): −172.34 to −171.73 (m). IR (KBr) ν: 658, 731, 920, 1037, 1157, 1202, 1358, 1419, 1500, 1639, 2340, 2980, 3290, 4170 cm^{-1}. $[\alpha]_D^{19}$ −53.9 (c 1.04, MeOH). mp 115–117 °C. HRMS-FAB (m/z): $[M + H]^+$ calcd for $C_{36}H_{50}FN_4O_9$, 701.3562; found, 701.3535.

*4.3.12. Z-Arg(Z$_2$)-Tyr-Leu-flp-Thr-OBn (**15**)*

Yield 37.1%. R_f 0.49 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.85–0.93 (m, 6H), 1.09 (d, J = 6.5 Hz, 3H), 1.18–1.35 (m, 2H), 1.38–1.73 (m, 5H), 1.95–3.13 (m, 4H), 3.72–4.08 (m, 4H), 4.20–4.33 (m, 1H), 4.39–4.52 (m, 1H), 4.54 (dd, J = 2.0, 9.0 Hz, 1H), 4.55–4.66 (m, 1H), 4.66–4.82 (m, 1H), 4.84–4.97 (m, 1H), 4.98–5.20 (m, 8H), 5.15–5.30 (m, 1H), 6.54 (d, J = 7.5 Hz, 2H), 6.79 (d, J = 8.0 Hz, 2H), 7.10 (br s, 1H), 7.22 (d, J = 6.5 Hz, 1H), 7.24–7.37 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 19.8, 19.9, 21.8, 23.0, 23.2, 24.4, 24.8, 24.8, 29.6, 29.6, 29.6, 29.7, 29.8, 53.8, 57.6, 59.0, 66.8, 66.8, 66.9, 66.9, 67.0, 67.0, 67.0, 67.1, 67.1, 67.2, 67.2, 68.1, 69.0, 115.2, 127.4, 127.8, 127.9, 128.0, 128.1, 128.1, 128.3, 128.4, 128.4, 128.6, 128.7, 128.8, 128.9, 130.2, 134.5, 155.2, 155.6, 170.6, 170.7, 172.1. ^{19}F NMR (283 MHz, Acetone-d_6, δ): −172.80 to −171.90 (m). IR (KBr) ν: 705, 778, 1000, 1078, 1204, 1370, 1425, 1500, 1602, 1630, 1700, 3020, 3300 cm^{-1}. $[\alpha]_D^{19}$ −46.4 (c 0.70, MeOH). mp 97–101 °C. HRMS-FAB (m/z): $[M]^+$ calcd for $C_{61}H_{71}FN_8O_{14}$, 1158.5074; found, 1158.5077.

*4.3.13. Arg-Tyr-Leu-flp-Thr (**2**)*

Yield 99.1%, 2:1 (*E*,*Z* isomeric ratio). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 0.73–1.02 (m, 9H), 1.16–1.77 (m, 7H), 2.14–2.49 (m, 2H), 2.62–2.90 (m, 2H), 2.85–3.13 (m, 2H), 3.55–4.24 (m, 5H), 4.24–4.78 (m, 3H), 5.24 (d, J = 54.0 Hz, 1H), 6.64 (dd, J = 8.2, 13.8 Hz, 2H), 7.00 (dd, J = 8.0, 30.0 Hz, 2H), 7.16 (d, J = 5.5 Hz, 1H), 7.88 (br s, 1H), 8.39 (br s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 18.4, 21.4, 23.2, 24.1, 30.5, 31.9, 34.7, 36.0, 36.4, 36.8, 48.6, 53.4 (d, J = 20.5 Hz), 53.8, 57.2, 58.7, 65.6, 92.3, 114.8, 127.6, 130.1, 155.8, 157.2, 169.0, 170.8, 171.1, 171.7, 173.5, 174.4. ^{19}F NMR (283 MHz, DMSO-d_6, δ): −172.29 to −171.21 (m). IR (KBr) ν: 594, 808, 832, 880, 914, 964, 1081, 1174, 1244, 1388, 1444, 1517, 1636, 2960, 3310 cm^{-1}. $[\alpha]_D^{19}$ −53.4 (c 0.82, MeOH). mp 181–186 °C. HRMS-FAB (m/z): $[M]^+$ calcd for $C_{30}H_{47}FN_8O_8$, 666.3501; found, 666.3465.

*4.3.14. Boc-Flp-Thr-OBn (**16**)*

Yield 56.0%. R_f 0.69 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.21 (d, J = 6.3 Hz, 3H), 1.45 (s, 9H), 2.39–2.60 (br m, 2H), 3.20 (d, J = 6.9 Hz, 1H), 3.56 (dd, J = 3.0, 36.3 Hz, 1H), 3.83 (d, J = 22.2 Hz, 1H), 4.25–4.30 (m, 1H), 4.44 (t, J = 7.8 Hz, 1H), 4.65 (dd, J = 3.0, 9.6 Hz, 1H), 5.14–5.32 (m, 3H), 7.17 (d, J = 9.3 Hz, 1H), 7.36 (m, 5H). (minor) 1.18 (d, J = 8.1 Hz, 3H), 1.45 (s, 9H), 2.39–2.60 (br m, 2H), 2.80 (br s, 1H), 3.51 (dd, J = 3.3, 36.0 Hz, 1H), 3.88 (d, J = 22.2 Hz, 1H), 4.25–4.30 (m, 1H), 4.44 (t, J = 7.8 Hz, 1H), 4.62 (dd, J = 2.7, 9.6 Hz, 1H), 5.14–5.32 (m, 3H), 6.78 (d, J = 8.4 Hz, 1H), 7.36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 19.7, 28.0, 35.7 (d, J = 21.1 Hz), 53.4 (d, J = 22.3 Hz), 57.7, 58.3, 67.0, 68.5, 80.8, 91.8 (d, J = 176.8 Hz), 127.9, 128.2, 128.4, 135.0, 154.6, 170.5, 171.7. (minor) 20.0, 28.0, 37.8 (d, J = 21.7 Hz), 53.2 (d, J = 21.1 Hz), 57.3, 58.9, 67.0, 67.4, 80.8, 91.1 (d, J = 178.0 Hz), 127.9, 128.2, 128.4, 135.0, 154.2, 170.5, 172.8. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −177.63 (ddddd, J = 20.6, 20.6, 36.5, 36.6, 57.1 Hz). (minor) −178.80 (ddddd, J = 19.8, 19.8, 36.5, 36.5, 57.1 Hz). IR (KBr) ν: 765, 972, 1034, 1074, 1116, 1166, 1207, 1249, 1318, 1378, 1409, 1456, 1540, 1674, 1698, 1744, 2337, 2354, 2364, 2929, 2978, 3410 cm^{-1}. $[\alpha]_D^{26}$ −65.3 (c 0.11, MeOH). mp 134–136 °C. HRMS-FAB (m/z): $[M + H]^+$ calcd for $C_{21}H_{30}FN_2O_6$, 425.2088; found, 425.2085.

*4.3.15. Boc-Tyr-Leu-Flp-Thr-OBn (**17**)*

Yield 71.4%. R_f 0.54 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.84 (d, J = 7.0 Hz, 3H), 0.87 (d, J = 6.0 Hz, 3H), 1.22 (d, J = 6.5 Hz, 3H), 1.41 (s, 9H), 1.44 (m, 2H), 1.54 (m, 1H), 2.18–2.30 (m, 1H), 2.40–2.53 (m, 1H), 2.76–2.96 (m, 2H), 3.73 (dd, J = 3.0, 35.5 Hz, 1H), 3.97 (br m, 1H), 4.25–4.33 (m, 4H), 4.61–4.65 (m, 2H), 4.72 (dd, J = 8.5, 14.0 Hz, 1H), 5.19 (br m, 2H), 5.27 (d, J = 9.0 Hz, 1H), 5.33 (br m, 1H), 6.62 (br m, 1H), 6.67 (d, J = 8.5, 2H), 6.92 (d, J = 8.5 Hz, 2H), 7.34–7.38 (m, 5H), 7.49 (d, J = 8.5 Hz, 1H), 7.61 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 19.8, 21.8, 22.7, 24.1, 28.1, 35.6 (d, J = 22.7 Hz), 38.0, 41.2, 50.2, 54.0 (d, J = 22.7 Hz), 55.3, 58.0, 58.6, 67.2, 68.2, 79.8, 91.8 (d, J = 179.8 Hz), 115.4, 128.0, 128.3, 128.4, 130.1, 134.9, 155.2, 155.4, 170.8, 171.6, 171.8. IR (KBr) ν: 699, 745, 846, 1013, 1066, 1175, 1252, 1370, 1452, 1521, 1662, 1882, 1936, 2235, 2311, 2395, 2494, 2606, 2756, 2815, 2888, 2973, 3083, 3294, 3487, 3538, 3623, 3742, 3822, 3862, 3942 cm^{-1}. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −177.74 (ddddd, J = 20.4, 20.6, 38.7, 38.7, 54.8 Hz). $[\alpha]_D^{26}$ −51.2 (c 0.11, MeOH). mp 97–102 °C. HRMS-FAB (m/z): $[M + H]^+$ calcd for $C_{36}H_{50}FN_4O_9$, 701.3562; found, 701.3569.

*4.3.16. Z-Arg(Z$_2$)-Tyr-Leu-Flp-Thr-OBn (**18**)*

Yield 71.6%. R_f 0.65 (AcOEt). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.85 (d, J = 5.5 Hz 3H), 0.87 (d, J = 6.0 Hz, 3H), 1.06 (d, J = 6.0 Hz, 3H), 1.39–1.64 (br m, 7H), 2.14–2.22 (m, 1H), 2.40–2.51 (m, 1H), 2.59–2.83 (m, 2H), 3.72–3.81 (m, 1H), 3.88 (br s, 1H), 4.13 (br m, 1H), 4.25–4.31 (m, 2H), 4.49 (br m, 1H), 4.55 (dd, J = 1.5, 9.5 Hz, 1H), 4.63 (m, 1H), 4.81–4.95 (m, 3H), 5.04–5.21 (m, 8H), 5.27–5.31 (m, 1H), 6.00 (br m, 1H), 6.52 (d, J = 7.5 Hz, 2H), 6.65 (d, J = 7.5 Hz, 2H), 7.19 (d, J = 6.5 Hz, 1H), 7.25 (m, 20H), 7.31 (br s, 1H), 7.62–7.79 (m, 1H), 9.27 (br s, 1H), 9.41–9.44 (br m, 1H). ^{13}C NMR (125 MHz, CD_3OD, δ): 11.0, 17.0, 19.0, 20.1, 22.0, 26.4, 33.6 (d, J = 20.6 Hz), 34.8, 38.2, 42.0, 47.1, 51.8 (d, J = 22.7 Hz), 52.8, 56.1, 56.4, 58.0, 64.3, 64.6, 64.7, 65.1, 66.4, 90.0 (d, J = 177.8 Hz), 112.8,

125.4, 125.6, 125.8, 125.8, 125.9, 126.0, 126.1, 126.3, 128.0, 133.0, 133.6, 134.5, 134.8, 153.3, 153.6, 158.3, 161.4, 168.2, 169.5, 169.6, 170.6. ^{19}F NMR (283 MHz, $CDCl_3$, δ): −177.92 to −177.23 (m). (minor) −177.20 to −177.60 (m). IR (KBr) ν: 705, 745, 848, 915, 1007, 1094, 1248, 1374, 1448, 1516, 1645, 1710, 2316, 2386, 2473, 2602, 2688, 2773, 2885, 2967, 3037, 3086, 3293, 3372, 3486, 3547, 3627, 3704, 3747, 3817, 3863, 3941 cm^{-1}. $[\alpha]_D^{26}$ −36.4 (*c* 0.11, MeOH). mp 87–91 °C. HRMS-FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{61}H_{72}FN_8O_{14}$, 1159.5152; found, 1159.5161.

*4.3.17. Arg-Tyr-Leu-Flp-Thr (**3**)*

Yield 68.9%, 2:1 (*E*,*Z* isomeric ratio). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 0.88 (d, J = 7.0 Hz, 6H,), 0.95 (d, J = 4.5 Hz, 3H), 1.24–1.64 (br m, 7H), 2.09–2.44 (m, 2H), 2.68 (dd, J = 9.0, 13.5 Hz, 1H), 2.84–2.89 (m, 1H), 3.02 (br m, 2H), 3.11 (t, J = 6.0 Hz, 1H), 3.71 (dd, 1H, J = 10.0, 37.5 Hz), 3.98 (br m, 3H), 4.47 (br m, 1H), 4.54–4.60 (m, 2H), 5.35 (d, 1H, J = 54.0 Hz), 6.62 (d, 2H, J = 7.5 Hz), 6.95 (d, 2H, J = 8.5 Hz), 7.46 (br s, 1H), 7.53 (br s, 1H), 8.01 (br s, 1H), 8.08 (d, 1H, J = 7.5 Hz), 9.27 (br s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6, δ): 19.9, 22.0, 23.4, 24.4, 25.1, 29.3, 32.0, 35.9 (d, J = 20.6 Hz), 37.4, 40.5, 41.0, 49.0, 49.2, 53.8, 54.4, 58.8 (d, J = 18.6 Hz), 67.4, 93.0 (d, J = 175.8 Hz), 115.3, 130.6, 137.2, 156.2, 157.4, 170.6, 171.2, 171.6, 174.6. ^{19}F NMR (283 MHz, CD_3OD, δ): −176.01 to −175.42 (m). (minor) −176.92 to −176.34 (m). IR (KBr) ν: 625, 731, 772, 793, 802, 822, 833, 977, 1108, 1175, 1204, 1393, 1444, 1522, 1656, 2330, 2360, 2689, 2828, 2863, 2934, 2976, 3199, 3391, 3788, 3840 cm^{-1}. $[\alpha]_D^{24}$ −39.6 (*c* 0.11, MeOH). mp 166–169 °C. HRMS-FAB (*m*/*z*): $[M]^+$ calcd for $C_{30}H_{47}FN_8O_8$, 666.3501; found, 666.3465.

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