# Introduction of $F_3$-Threonine to the Hexapeptide, DSLET: Investigation of the Effect of Fluorine Atoms toward Peptidic Conformations

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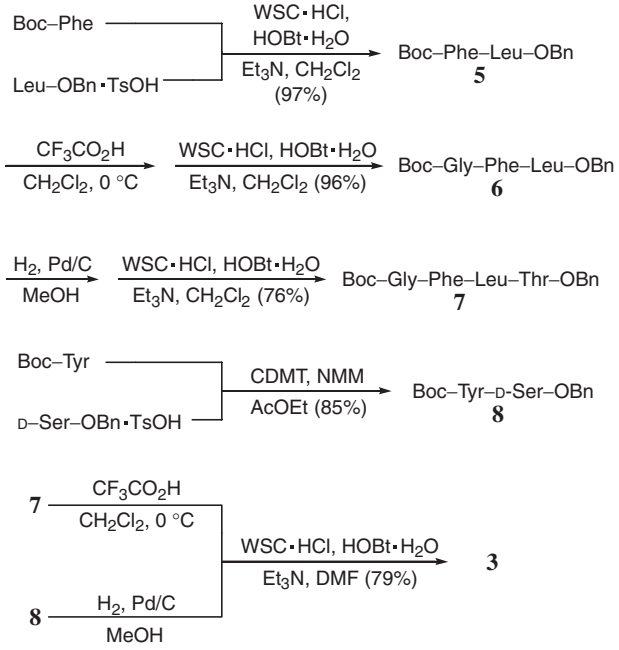
Introduction of trifluoromethyl-containing threonine to the target hexapeptide, Tyr–D-Ser–Gly–Phe–Leu–Thr (DSLET), led to the apparent conformational alteration due to the electron-withdrawing effect of the $CF_3$ group when compared with the original DSLET on the basis of their NOESY spectra.

Accomplishment of the human genomic base sequence decoding in 2003 allowed us to obtain information about proteins responsible for some specific diseases,[1] which will lead to clarification of the three-dimensional shapes of their active sites with the aid of computational technique.[2] This complete analysis enables us to rationally design more effective drugs by installing appropriate elements so as to construct firm interaction with their target active sites.

Incorporation of a few fluorine atoms to an organic molecule possibly brings about enhancement of the original biological activity and improvement of the functional selectivity because of the similar atomic size of fluorine to hydrogen,[3] but such substitution would affect formation of additional hydrogen bonds and cause unfavorable electronic repulsion with other electronegative groups. Conformational change is then expected based on these phenomena, which is considered as one of the most important determinants whether donor molecules fit target active sites, but such issue seems not to have been drawing significant attention thus far.[4] These facts prompted us to start our investigation to clarify how much a fluorine atom or a fluorinated substituent in amino acids[5] affects original peptidic conformations. For this purpose, we have selected the hexapeptide, Tyr–D-Ser–Gly–Phe–Leu–Thr **1** (DSLET[6]) known as the enkephalin-related peptide selectively bound to the $\delta$ opioid receptor.

In this study, most of the peptide syntheses were performed by the solution-phase method using WSC·HCl[7] in the presence of HOBt·$H_2O$[8] as the representative condensing reagent partner which usually attained good to excellent yields. However, Boc–Tyr–D-Ser–OBn **8** was the exception furnished only in ca. 50% yield and this problem was solved by CDMT[9] (2-chloro-4,6-dimethoxy-1,3,5-triazine) in the presence of 2.2 equiv. of NMM (*N*-methylmorpholine).[10] As a result, this step effectively proceeded under the usual atmosphere in an AcOEt solvent to attain 85% isolated yield of the product **8**. Combination of **7** and **8** in a usual manner produced the desired compound **3** in 79% yield (Scheme 1).

The NOESY spectrum of **3** observed in DMSO-$d_6$ (Figure 1) indicated that its conformation possessed two characteristic long-range interactions between two amino acids apart from five and six residues, which led to estimation of its helix- or spherical-type structure where *N*-terminal Tyr should be observed around the region of *C*-terminal Thr. The cross peak between Tyr phenol and Thr hydroxy protons led to speculation that




**Scheme 1.** Synthesis of protected $F_0$-hexapeptide **3**.

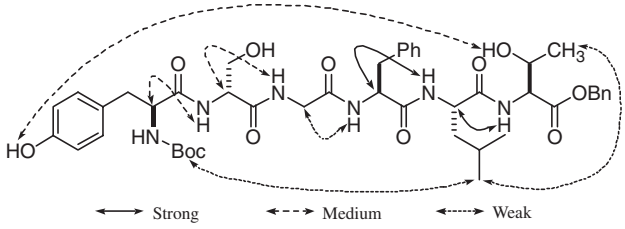
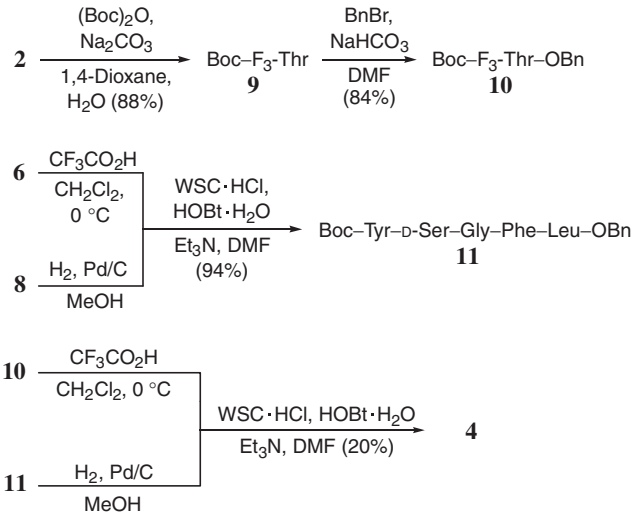



**Figure 1.** NOESY spectrum of **3**.

Tyr–O*H* proton and Thr–*O*H oxygen worked as the hydrogen-bond donor and acceptor, respectively, when their acidity was considered.[11] Thus, it was strongly anticipated that substitution of a Thr–$CH_3$ group for a $CF_3$ moiety should effectively lower the electron density of $F_3$-Thr–*O*H which would apparently result in weakening the hydrogen bond between Tyr and Thr. This is qualitatively supported by the acidity difference between $CH_3CH_2OH$ and $CF_3CH_2OH$ of $\Delta pK_a = 3.5$.

For this purpose, we have prepared (2*S*,3*S*)-4,4,4-trifluorothreonine **2** ($F_3$-Thr) following to the synthetic method reported by Soloshonok et al.[12] which was readily converted to the desired protected hexapeptide **4** (Scheme 2). As shown in Figure 2, as our expectation, introduction of three fluorine atoms indeed altered the original three-dimensional shape and the long-range hydrogen bonding between Tyr and Thr was




**Scheme 2.** Synthesis of protected $F_3$-hexapeptide **4**.

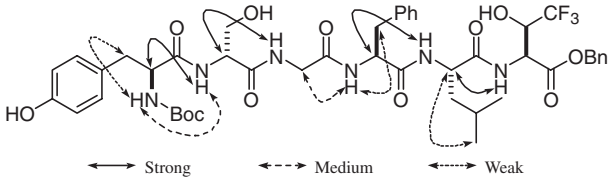



**Figure 2.** NOESY spectrum of **4**.

totally disappeared. Instead, the strong correlation between protons C*H*C(O)N*H* was noticed in the all amide linkage, which would support its straight shape.

To collect further conformational information, we tried to confirm which protons are responsible for hydrogen bonding by investigating chemical shift alteration under different solvent polarity (Table 1).[13] In our experiment, $CDCl_3$ and DMSO-$d_6$ as the lower and higher polarity solvents, respectively, were applied with the contents of the former solvent of 0, 20, 40, 60, and 80%. A proton forming a stronger hydrogen bonding usually shows lower sensitivity toward the solvent polarity to be observed with smaller chemical shift difference even under bigger polarity charge. It was determined on the basis of Table 1 that hydrogen bond forming protons were the amide protons of D-Ser, Gly, Phe, and the hydroxy proton of D-Ser in both **3** and **4**. Moreover, the hydroxy proton of $F_3$-Thr in **4** was found to participate, too. The latter phenomenon was anticipated by incorporation of a strong electron-withdrawing trifluoromethyl group which should effectively decrease the electron density of $F_3$-Thr–*O*H as our expectation, and resulted in increase of acidity of the O*H* group. Because chemical shifts of these protons were almost constant in both 20 and 0.1 mM solutions, we believe that these protons constructed intramolecular hydrogen bonding.

**Table 1.** Chemical shift of the specific protons under different solvent ratio

| | $F_0$-hexapeptide ($n = 0$) **3** ($\delta$/ppm) | | | $F_3$-hexapeptide ($n = 3$) **4** ($\delta$/ppm) | | |
|---|---|---|---|---|---|---|
| $CDCl_3$/% | 0 | 40 | $\Delta\delta^a$ | 0 | 40 | $\Delta\delta^a$ |
| Tyr–N*H* | 6.876 | 6.729 | −0.147 | 6.828 | 6.781 | −0.117 |
| D-Ser–N*H* | 7.940 | 7.920 | −0.020 | 7.941 | 7.924 | −0.017 |
| Gly–N*H* | 8.069 | 8.109 | 0.040 | 8.121 | 8.130 | 0.009 |
| Phe–N*H* | 7.998 | 7.920 | −0.078 | 8.066 | 8.014 | −0.052 |
| Leu–N*H* | 8.197 | 8.074 | −0.123 | 8.266 | 8.076 | −0.190 |
| $F_n$-Thr–N*H* | 7.962 | 7.762 | −0.200 | 8.450 | 8.260 | −0.190 |
| Tyr–O*H* | 9.136 | 9.008 | −0.128 | 9.147 | 9.043 | −0.104 |
| D-Ser–O*H* | 4.989 | 5.007 | 0.018 | 5.047 | 5.090 | 0.043 |
| $F_n$-Thr–O*H* | 5.018 | 4.898 | −0.120 | 6.996 | 6.904 | −0.092 |

[a] $\Delta\delta = \delta(CDCl_3\ 40\%) - \delta(CDCl_3\ 0\%)$.

As described above, we have successfully demonstrated that entry of fluorine atoms at a judicious position of organic molecules possibly affected the neighboring functional groups, leading to significant change of the original conformation. Accumulation of this type of information will eventually allow us to control three-dimensional shapes of molecules at our own will. Further study for clarification of the relationship between fluorine-substitution and conformational alteration is in progress in this laboratory.

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