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Journal of Fluorine Chemistry 128 (2007) 1326–1334
JOURNAL OF FLUORINE CHEMISTRY
www.elsevier.com/locate/fluor

Syntheses and lipophilicity measurement of N^{α}/*N*-terminus-1, 1-dihydroperfluoroalkylated α-amino acids and small peptides

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Received 24 May 2007; received in revised form 2 July 2007; accepted 3 July 2007
Available online 21 August 2007

Abstract

(1,1-Dihydroperfluoroalkyl)phenyliodonium *N*,*N*-bis(trifluoromethylsulfonyl)imides (**4**, $n = 0$–2) were synthesized and used to transfer the corresponding 1,1-dihydroperfluoroalkyl groups to the α-amino group of (L)tyrosine. The obtained N^{α}-2,2,2-trifluoroethylated (L)tyrosine (**6**, $n = 0$) was further used as the *N*-terminus in the solid phase peptide synthesis of leucine enkephalin analogue. The lipophilicity of the N^{α}-1,1-dihydroperfluoroalkylated (L)tyrosines (**6**, $n = 0$–2) and *N*-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue (**7**), as well as the corresponding parent compounds, was measured.

Keywords: (1,1-Dihydroperfluoroalkyl)phenyliodonium *N*,*N*-bis(trifluoromethylsulfonyl)imides; 1,1-Dihydroperfluoroalkylation; (L)Tyrosine analogue; Leucine enkephalin analogue; Distribution coefficient; Lipophilicity

1. Introduction

Bioavailability is a major concern for peptide based drugs because of their poor biomembrane passage and rapid metabolism in the circulation systems. Examples are the enkephalins, of which the metabolic degradation at all absorptive mucosae and within the body presents a significant barrier to the use of these peptides as drugs [1–4]. Another drawback of enkephalins is that they are too hydrophilic to be absorbed by the transcellular route resulting in poor transport properties [5,6].

To overcome these difficulties, various prodrugs and peptidomimetics, such as 4-imidazolidinone enkephalin analogues [7,8], cyclic and branched enkephalin analogues [9,10], the leucine enkephalin analogues containing unnatural amino acid(s) [11], and glycosylated enkephalin analogues [12,13], have been synthesized. These modifications resulted in the stabilization of enkephalins against different enzymes as well as an improved delivery across bovine BMEC monolayers [6–8,14] or Caco-2 cell monolayers [13,15].

Fluoroalkylation can substantially affect both chemical and physical properties of substrates. For example, because of the inductive effect of CF_3CH_2- group, the lone pair electrons on oxygen in CF_3CH_2OH are less available for hydrogen bonding with the aqueous phase than that in CH_3CH_2OH. Therefore, CF_3CH_2OH ($\log P$ 0.41) is more lipophilic than CH_3CH_2OH ($\log P$ −0.32) [16], though trifluoroethanol (pK_a 12.4) [17] is considerably more acidic than ethanol (pK_a 15.9) [18]. Based on this rationale, it was expected that the attachment of CF_3CH_2- group to the α-amino group of amino acids could change both nucleophilicity [19–22] and lipophilicity of the parent compounds, and possibly, improve their resistance to degradation caused by aminopeptidases.

In this paper, we present the syntheses of (1,1-dihydroperfluoroalkyl)phenyliodonium *N*,*N*-bis(trifluoromethylsulfonyl)imides $CF_3(CF_2)_nCH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 0$–2) and the transfer of the corresponding fluoroalkyl moiety to the α-amino group of (L)tyrosine. The lipophilicity measurements of N^{α}-1,1-dihydroperfluoroalkylated (L)tyrosines (**6**, $n = 0$–2) and the *N*-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue (**7**) are given and compared to that of the corresponding parent compounds.

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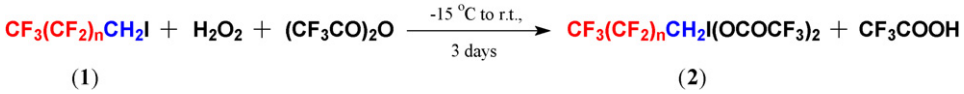
doi:10.1016/j.jfluchem.2007.07.003



Scheme 1.

2. Results and discussion

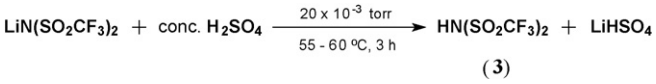
2.1. Preparation of (1,1-dihydroperfluoroalkyl)phenyliodonium N,N-bis(trifluoromethylsulfonyl)imides

Perfluoroalkyl aryliodonium salts were first synthesized by Yagupolskii et al. in the late 1970's [23]. In the early 1980's, Umemoto and Gotoh [24] synthesized perfluoroalkyl and 1,1-dihydroperfluoroalkyl aryliodonium triflates including (2,2,2-trifluoroethyl)phenyl iodonium triflate, which was later prepared using the modified method by Resnati [25] and used for *N*-trifluoroethylation of amino alcohols under dry conditions. In the late 1990's, DesMarteau and Montanari [19] introduced *N*,*N*-bis(trifluoromethylsulfonyl)imide into (2,2,2-trifluoroethyl)phenyliodonium salt to obtain a novel trifluoroethylating agent which has been used to transfer the 2,2,2-trifluoroethyl group to different nucleophiles in aqueous solutions [20–22]. Here, the higher homologues of this reagent are prepared and similarly utilized.

1,1-Dihydroperfluoroalkyl iodides (**1**, $n = 0$–2) were oxidized using hydrogen peroxide (50% aqueous solution) in the presence of excess trifluoroacetic anhydride to form the corresponding iodonium ditrifluoroacetates (**2**, $n = 0$–2) shown in Scheme 1.

N,*N*-Bis(trifluoromethylsulfonyl)imide (**3**) was obtained by sublimation from the corresponding lithium salt in concentrated H_2SO_4 under vacuum (Scheme 2).

The iodonium ditrifluoroacetates (**2**, $n = 0$–2) were reacted with sulfonamide (**3**) and benzene to form the desired 1,1-dihydroperfluoroalkylating agents (**4**, $n = 0$–2) by the elimination of trifluoroacetic acid and Friedel–Crafts like iodonium attachment to benzene ring (Scheme 3).



Scheme 2.

2.2. Preparation of N^{α}-1,1-dihydroperfluoroalkylated (L)tyrosines

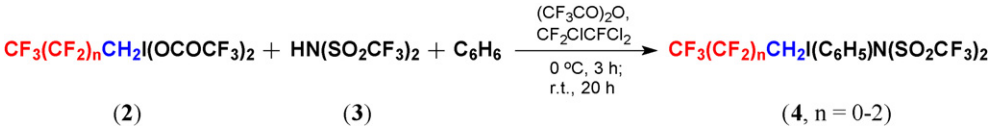
The reaction of (L)tyrosine methyl/ethyl ester with $CF_3(CF_2)_nCH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 0$–2) in two phase solvents CH_2Cl_2/H_2O resulted in ester intermediates (**5**, $n = 0$–2). The esters were then cleaved by basic hydrolysis followed by acidification with conc. HCl to pH 4.5 to give N^{α}-1,1-dihydroperfluoroalkylated (L)tyrosines (**6**, $n = 0$–2) shown in Scheme 4.

2.3. Preparation of N-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue

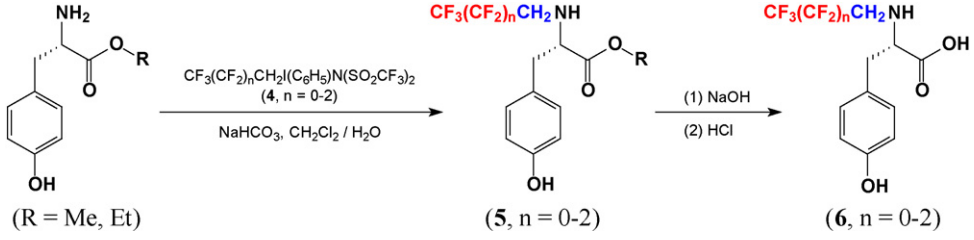
As shown in Scheme 5, Wang resin was used in the solid phase peptide synthesis. The 9-fluorenylmethyloxy carbonyl (Fmoc-) group was used as the N^{α}-protective group. (L)Leucine, (L)phenylalanine, glycine, and the second glycine were attached sequentially onto the Wang resin. N^{α}-2,2,2-trifluoroethylated (L)tyrosine (**6**, $n = 0$) was finally coupled to give the desired *N*-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue (**7**) after the cleavage of pentapeptide from the Wang resin.

2.4. Preparation of N^{α}-ethylated (L)tyrosine

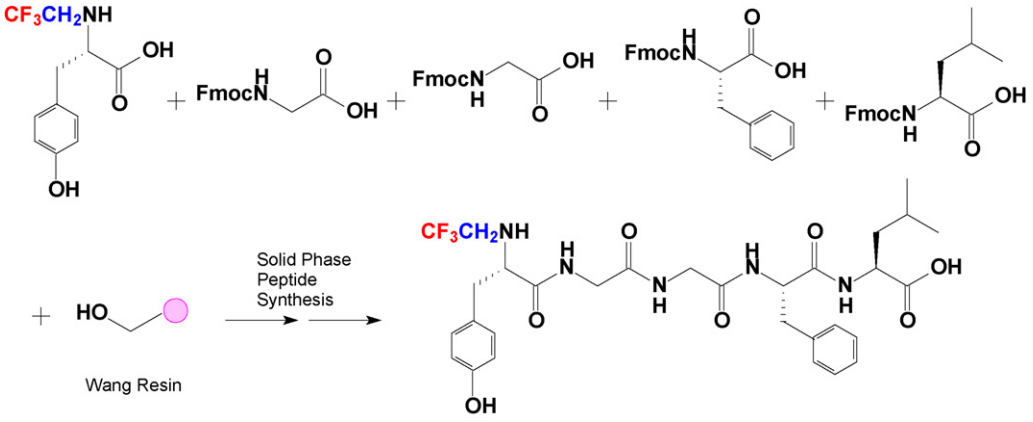
The reaction of (L)tyrosine methyl ester with $(CH_3CH_2)_3OBF_4$ resulted in the N^{α}-ethylated intermediate (**8**). The methyl ester was then cleaved by basic hydrolysis



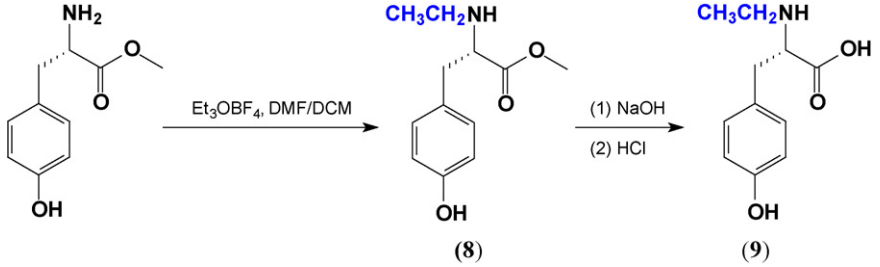
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

followed by acidification to give N^{α}-ethylated (L)tyrosine (**9**) shown in Scheme 6.

2.5. Lipophilicity measurement

1-Octanol and aqueous NaH_2PO_4/NaOH buffer solution (pH 7.07) were used as partition solvents. (L)Tyrosine, N^{α}-CH_3CH_2-(L)TyrOH (**9**), N^{α}-$CF_3(CF_2)_nCH_2$-(L)TyrOH (**6**, $n = 0$–2), leucine enkephalin, and *N*-CF_3CH_2-Leu-enkephalin (**7**) were used as substrates. The distribution coefficient $D_{7.07}$ was calculated as follows [26,27].

$$D_{7.07} = \frac{[\text{Substrate}]_{\text{1-octanol}}}{[\text{Substrate}]_{\text{aqueous}}} \quad (1)$$

2.5.1. (L)Tyrosine

The aqueous buffer solutions containing different concentrations of (L)tyrosine were prepared and subjected to UV measurement as shown in Fig. 1.

The absorbance (at 275 nm) of (L)tyrosine in aqueous buffer solution was plotted versus the corresponding concentration to give the calibration curve shown in Fig. 2.

(L)Tyrosine was partitioned between 1-octanol and aqueous buffer solution. The concentration of (L)TyrOH in aqueous buffer solution was obtained by UV measurement. The concentration of (L)TyrOH in 1-octanol layer and the distribution coefficient $D_{7.07}$ of (L)TyrOH between 1-octanol and aqueous buffer solution were calculated.

$$D_{7.07} = \frac{[(\text{L})\text{TyrOH}]_{\text{1-octanol}}}{[(\text{L})\text{TyrOH}]_{\text{aqueous}}} = \frac{2.80 \times 10^{-3}}{0.922} = 3.04 \times 10^{-3}$$

$$\log D_{7.07} = \log 3.04 \times 10^{-3} = -2.52$$

The value obtained is comparable to the literature value log $P = -2.66$ for (L)TyrOH [28].

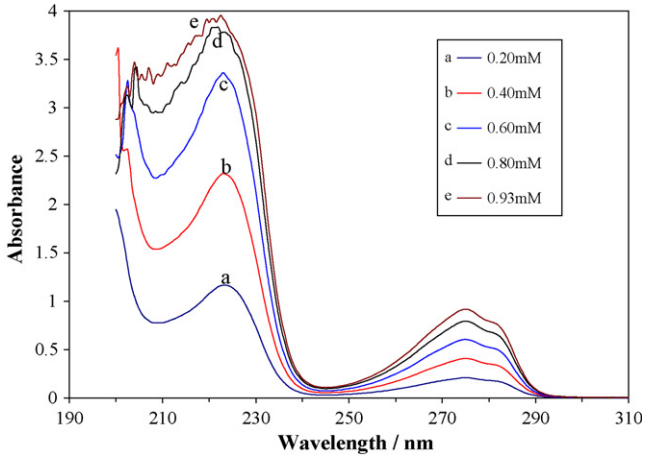


Fig. 1. UV absorbance of (L)TyrOH in aqueous buffer solution (22 °C, with subtraction of absorbance of blank aqueous buffer solution).

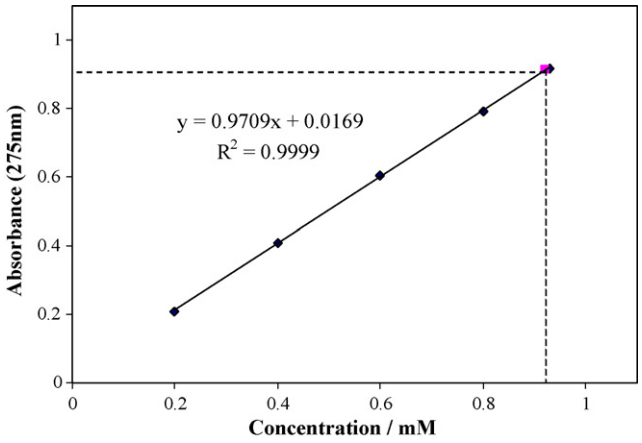


Fig. 2. Calibration curve (at 275 nm) of (L)TyrOH in aqueous buffer solution at 22 °C.

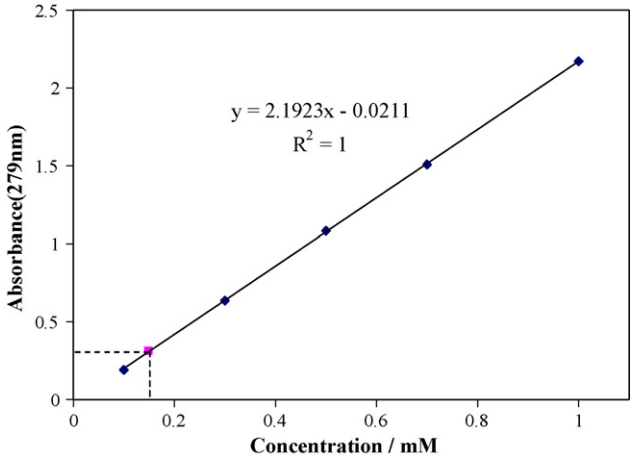


Fig. 4. Calibration curve (at 279 nm) of N^{α}-CF_3CH_2-(L)TyrOH (**6**, $n = 0$) in 1-octanol at 22 °C.

2.5.2. N^{α}-ethylated (L)tyrosine (**9**)

The aqueous buffer solutions containing different concentrations of N^{α}-ethylated (L)tyrosine (**9**) were prepared and subjected to UV measurement. The absorbance (at 274 nm) of N^{α}-CH_3CH_2-(L)TyrOH in aqueous buffer solution was plotted versus the corresponding concentration to give the calibration curve shown in Fig. 3.

N^{α}-CH_3CH_2-(L)TyrOH was partitioned between 1-octanol and aqueous buffer solution. The concentration of N^{α}-CH_3CH_2-(L)TyrOH in aqueous buffer solution was obtained by UV measurement. The concentration of N^{α}-CH_3CH_2-(L)TyrOH in 1-octanol layer and the distribution coefficient $D_{7.07}$ of N^{α}-CH_3CH_2-(L)TyrOH between 1-octanol and aqueous buffer solution were calculated.

$$D_{7.07} = \frac{[N^{\alpha}\text{-}CH_3CH_2\text{-(L)TyrOH}]_{1-\text{octanol}}}{[N^{\alpha}\text{-}CH_3CH_2\text{-(L)TyrOH}]_{\text{aqueous}}} = \frac{6.14 \times 10^{-3}}{0.263}$$

$$= 2.33 \times 10^{-2}$$

$$\log D_{7.07} = \log 2.33 \times 10^{-2} = -1.63$$

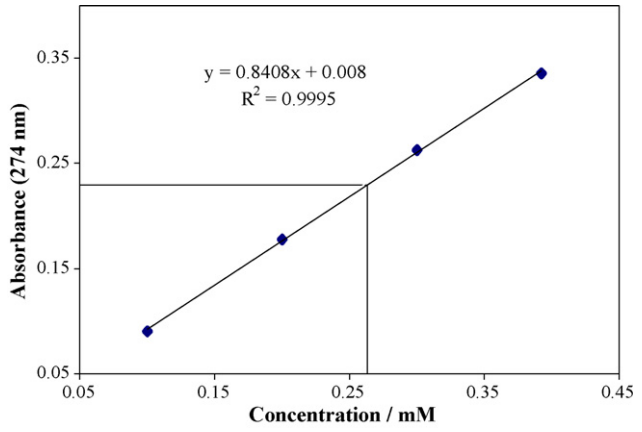


Fig. 3. Calibration curve (at 274 nm) of N^{α}-CH_3CH_2-(L)TyrOH (**9**) in aqueous buffer solution at 22 °C.

The lipophilicity substituent constant π for CH_3CH_2- group attached to α-amino group of (L)tyrosine was calculated as follows [27].

$$\begin{aligned}\pi(CH_3CH_2-) &= \log D(N^{\alpha}\text{-}CH_3CH_2\text{-(L)TyrOH}) \\ &\quad - \log D(\text{(L)TyrOH}) \\ &= -1.63 - (-2.52) = 0.89\end{aligned}$$

2.5.3. N^{α}-2,2,2-trifluoroethylated (L)tyrosine (**6**, $n = 0$)

The 1-octanol solutions containing different concentrations of N^{α}-CF_3CH_2-(L)TyrOH (**6**, $n = 0$) were prepared and subjected to UV measurement. The absorbance (at 279 nm) of N^{α}-CF_3CH_2-(L)TyrOH in 1-octanol was plotted versus the corresponding concentration to give the calibration curve shown in Fig. 4.

N^{α}-CF_3CH_2-(L)TyrOH was partitioned between 1-octanol and aqueous buffer solution. The concentration of N^{α}-CF_3CH_2-(L)TyrOH in 1-octanol was obtained by UV measurement. The concentration of N^{α}-CF_3CH_2-(L)TyrOH in aqueous buffer solution and the distribution coefficient $D_{7.07}$ of N^{α}-CF_3CH_2-(L)TyrOH between 1-octanol and aqueous buffer solution were calculated.

$$D_{7.07} = \frac{[N^{\alpha}\text{-}CF_3CH_2\text{-(L)TyrOH}]_{1-\text{octanol}}}{[N^{\alpha}\text{-}CF_3CH_2\text{-(L)TyrOH}]_{\text{aqueous}}} = \frac{0.151}{4.62}$$

$$= 3.27 \times 10^{-2}$$

$$\log D_{7.07} = \log 3.27 \times 10^{-2} = -1.49$$

The lipophilicity substituent constant π for CF_3CH_2- group attached to the α-amino group of (L)tyrosine was calculated as follows.

$$\pi(CF_3CH_2-)$$

$$= \log D(N^{\alpha}\text{-}CF_3CH_2\text{-(L)TyrOH}) - \log D(\text{(L)TyrOH})$$

$$= -1.49 - (-2.52) = 1.03$$

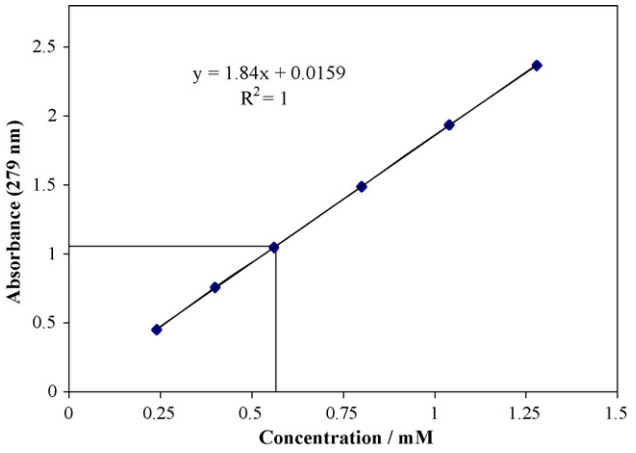


Fig. 5. Calibration curve (at 279 nm) of N^{α}-$CF_3CF_2CH_2$-(L)TyrOH (**6**, $n = 1$) in 1-octanol at 22 °C.

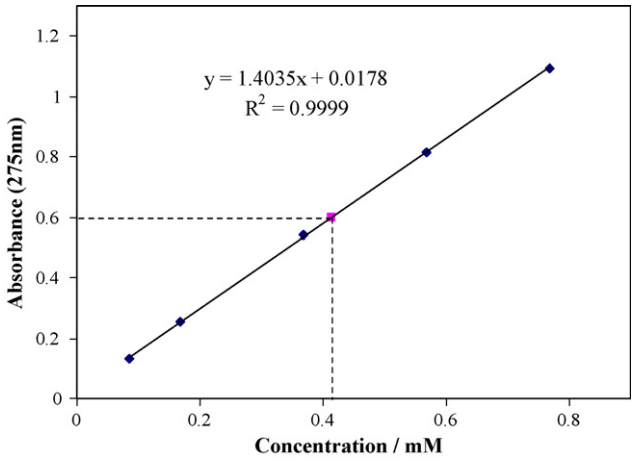


Fig. 7. Calibration curve (at 275 nm) of leucine enkephalin in aqueous buffer solution at 22 °C.

2.5.4. N^{α}-1,1-dihydropentafluoropropylated (L)tyrosine (**6**, $n = 1$) and N^{α}-1,1-dihydroheptafluorobutylated (L)tyrosine (**6**, $n = 2$)

Similarly, the 1-octanol solutions of **6** ($n = 1$, 2) were examined at 279 nm to give the calibration curves as shown in Figs. 5 and 6. The distribution coefficients $D_{7.07}$ and the lipophilicity substituent constants π were then determined.

For N^{α}-$CF_3CF_2CH_2$-(L)TyrOH (**6**, $n = 1$):

$$D_{7.07} = \frac{[N^{\alpha}\text{-}CF_3CF_2CH_2\text{-}(L)\text{TyrOH}]_{1-\text{octanol}}}{[N^{\alpha}\text{-}CF_3CF_2CH_2\text{-}(L)\text{TyrOH}]_{\text{aqueous}}} = \frac{0.566}{3.33} = 0.170$$

$$\log D_{7.07} = \log 0.170 = -0.769$$

$$\pi(CF_3CF_2CH_2-) = \log D(N^{\alpha}\text{-}CF_3CF_2CH_2\text{-}(L)\text{TyrOH}) - \log D((L)\text{TyrOH}) = -0.769 - (-2.52) = 1.75$$

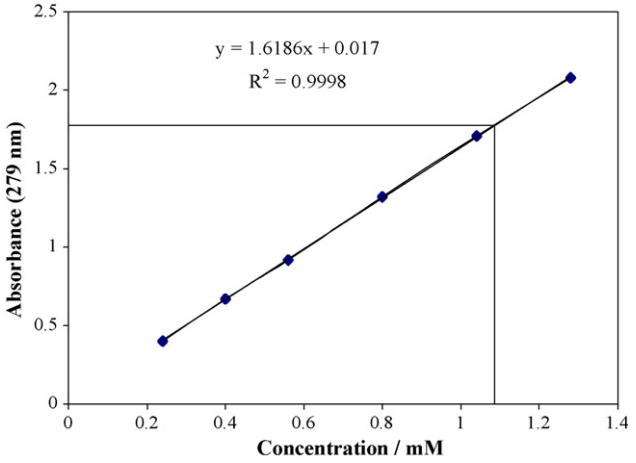


Fig. 6. Calibration curve (at 279 nm) of N^{α}-$CF_3CF_2CF_2CH_2$-(L)TyrOH (**6**, $n = 2$) in 1-octanol at 22 °C.

For N^{α}-$CF_3CF_2CF_2CH_2$-(L)TyrOH (**6**, $n = 2$):

$$D_{7.07} = \frac{[N^{\alpha}\text{-}CF_3CF_2CF_2CH_2\text{-}(L)\text{TyrOH}]_{1-\text{octanol}}}{[N^{\alpha}\text{-}CF_3CF_2CF_2CH_2\text{-}(L)\text{TyrOH}]_{\text{aqueous}}} = \frac{1.09}{2.03} = 0.537$$

$$\log D_{7.07} = \log 0.537 = -0.270$$

$$\pi(CF_3CF_2CF_2CH_2-) = \log D(N^{\alpha}\text{-}CF_3CF_2CF_2CH_2\text{-}(L)\text{TyrOH}) - \log D((L)\text{TyrOH}) = -0.270 - (-2.52) = 2.25$$

2.5.5. Leucine enkephalin

The aqueous buffer solutions containing different concentrations of leucine enkephalin (Sigma–Aldrich Co.) were prepared and subjected to UV measurement. The absorbance (at 275 nm) of leucine enkephalin in aqueous buffer solution was plotted versus the corresponding concentration to give the calibration curve shown in Fig. 7.

Leucine enkephalin was partitioned between 1-octanol and aqueous buffer solution. The concentration of leucine enkephalin in aqueous buffer solution was obtained by UV measurement. The concentration of leucine enkephalin in 1-octanol layer and the distribution coefficient $D_{7.07}$ of leucine enkephalin between 1-octanol and aqueous buffer solution were calculated.

$$D_{7.07} = \frac{[\text{Leu-enkephalin}]_{1-\text{octanol}}}{[\text{Leu-enkephalin}]_{\text{aqueous}}} = \frac{0.059}{0.414} = 0.143$$

$$\log D_{7.07} = \log 0.143 = -0.845$$

2.5.6. N-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue (7)

The 1-octanol solutions containing different concentrations of N-terminus-CF_3CH_2-leucine enkephalin analogue (**7**) were

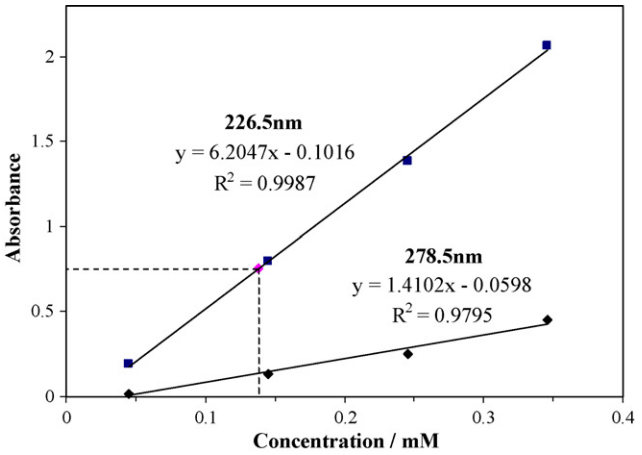


Fig. 8. Calibration curves (at 226.5 nm and 278.5 nm) of *N*-CF_3CH_2-Leu-enkephalin analogue (**7**) in 1-octanol at 22 °C.

prepared and subjected to UV measurement. The absorbance (at 226.5 and 278.5 nm, respectively) of *N*-CF_3CH_2-Leu-enkephalin analogue in 1-octanol was plotted versus the corresponding concentration to give the calibration curves shown in Fig. 8. The calibration curve at 226.5 nm was used to determine the concentration of the substrate in 1-octanol layer after partition.

N-CF_3CH_2-Leu-enkephalin analogue (**7**) was partitioned between 1-octanol and aqueous buffer solution. The concentration of *N*-CF_3CH_2-Leu-enkephalin in 1-octanol was obtained by UV measurement. The concentration of *N*-CF_3CH_2-Leu-enkephalin in aqueous buffer solution and the distribution coefficient $D_{7.07}$ of *N*-CF_3CH_2-Leu-enkephalin between 1-octanol and aqueous buffer solution were calculated.

$$D_{7.07} = \frac{[N\text{-}CF_3CH_2\text{-Leu-enkephalin}]_{1-\text{octanol}}}{[N\text{-}CF_3CH_2\text{-Leu-enkephalin}]_{\text{aqueous}}} = \frac{0.138}{0.415}$$

$$= 0.332$$

$$\log D_{7.07} = \log 0.332 = -0.479$$

Therefore, the lipophilicity substituent constant π for the CF_3CH_2- group attached to *N*-terminus of leucine enkephalin was calculated as follows.

$$\pi(CF_3CH_2-)$$
$$= \log D(N\text{-}CF_3CH_2\text{-Leu-enkephalin})$$
$$- \log D(\text{Leu-enkephalin})$$
$$= -0.479 - (-0.845) = 0.366$$

The distribution coefficient $D_{7.07}$, $\log D_{7.07}$, and lipophilicity substituent constant π of fluoroalkylated (L)tyrosines and leucine enkephalin are compared in Table 1.

In summary, 1,1-dihydroperfluoroalkylating agents $CF_3(CF_2)_nCH_2I(C_6H_5)N(SO_2CF_3)_2$ (n = 0–2) have been synthesized and used to transfer the corresponding 1,1-dihydroperfluoroalkyl groups to the α-amino group of (L)tyrosine and *N*-terminus of leucine enkephalin. Fluoroalkylation of the above α-amino acid and peptide resulted in the increased lipophilicity. The lipophilicity substituent constant depends on both the fluoroalkyl substituent and the substrate. As expected, extended fluorine content leads to increases in π values.

Table 1
Lipophilicity of fluoroalkylated (L)TyrOH and Leu-enkephalin (22 °C)

	$D_{7.07}$	log $D_{7.07}$	π $CF_3(CF_2)_nCH_2$–
(L)Tyrosine	3.04×10^{-3}	−2.52	
N^{α}-CH_3CH_2-(L)tyrosine	2.33×10^{-2}	−1.63	0.89
N^{α}-CF_3CH_2-(L)tyrosine	3.27×10^{-2}	−1.49	1.03
N^{α}-$CF_3CF_2CH_2$-(L)tyrosine	0.170	−0.769	1.75
N^{α}-$CF_3CF_2CF_2CH_2$-(L)tyrosine	0.537	−0.270	2.25
Leu-enkephalin	0.143	−0.845	
N-terminus-CF_3CH_2-Leu-enkephalin	0.332	−0.479	0.366

3. Experimental

The reagents and solvents were obtained from commercial suppliers and used without further purification. NMR spectra were obtained on Bruker AC-200, Bruker Avance 300, JEOL ECX-300, and JEOL Eclipse+ 500 spectrometers. Chemical shifts are given in ppm relative to $CFCl_3$ for ^{19}F, and relative to deuterated solvents used for ^{1}H and ^{13}C NMR. UV measurement was carried out on UV-2101PC Scanning Spectrophotometer. Melting points are uncorrected.

*3.1. Preparation of $CF_3(CF_2)_nCH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, n = 0) [19] and NMR data (**4**, n = 0–2)*

A three necked round bottom flask (300 mL) was equipped with a thermometer and a septum. Trifluoroacetic anhydride (130 mL, 925 mmol) was added into the flask. The flask was protected with flowing dry nitrogen and cooled at −15 °C. H_2O_2 aqueous solution (50%, 13.5 mL, 238 mmol) was added slowly with stirring. Then CF_3CH_2I (**1**, n = 0) (19.9 mL, 200 mmol) was added. The reaction was allowed to continue under nitrogen protected from light with aluminium foil for 3 days. After evaporation of the volatiles, the resulting thick oil was left in the closed flask standing in a freezer until it solidified to a crystalline product $CF_3CH_2I(OCOCF_3)_2$ (**2**, n = 0) (85.7 g, 196 mmol, 98.0%).

$CF_3CH_2I(OCOCF_3)_2$ (**2**, n = 0): crystal; ^{1}H NMR (200.1 MHz, $CDCl_3$): δ 4.84–4.97 (2H, q, J = 8.79 Hz); ^{19}F NMR (188.3 MHz, $CDCl_3$): δ −74.0 (6F, s), −63.9 (3F, t, J = 8.79 Hz).

In a dry box, $(CF_3SO_2)_2NLi$ (65.1 g, 227 mmol) was added into a 500 mL round bottom flask. H_2SO_4 (95–98%, 200 mL) was then added. The mixture was heated in an oil bath at 55–60 °C. The white product $(CF_3SO_2)_2NH$ (**3**) (62.4 g, 222 mmol, 98.1%) was sublimed under vacuum (20×10^{-3} torr) and collected in a 500 mL two necked round bottom flask (one neck leading to sublimation connector, the other leading to vacuum) cooled in an ice bath during 3 h.

$(CF_3SO_2)_2NH$ (**3**): white solid; ^1H NMR (200.1 MHz, $CDCl_3$): δ 7.84 (1H, s) (moisture dependent); ^{19}F NMR (188.3 MHz, $CDCl_3$): δ −75.6 (6F, s).

Under a slow flow of nitrogen, trifluoroacetic anhydride (35 mL) and $CF_2ClCFCl_2$ (100 mL) were added into the flask containing $CF_3CH_2I(OCOCF_3)_2$ (**2**, $n = 0$) (196 mmol). The solution of $CF_3CH_2I(OCOCF_3)_2$ was transferred to the flask containing $(CF_3SO_2)_2NH$ (**3**) (222 mmol). The resulting suspension was cooled in an ice bath, stirred under nitrogen, and protected from light with Al-foil. After 15 min, benzene (20.0 mL, 223 mmol) was added. The reaction mixture was stirred with ice bath cooling for 3 h before it was allowed to warm up to room temperature. After stirring 15 h at room temperature, the second portion of benzene (7.0 mL, 78 mmol) was added and the stirring was continued for another 5 h. The reaction mixture was then transferred into a 500 mL round bottom flask, and the volatiles were evaporated at 22 °C using rotary evaporator. The obtained oily dark brown residue was pumped under vacuum (5×10^{-3} torr) for 2 h. Crushed ice (*ca.* 150 g) was added into the flask with vigorous shaking to cause the rapid formation of light gray solid which was separated from water and crystallized from CH_2Cl_2. The white crystalline product $CF_3CH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 0$) was obtained (87.8 g, 155 mmol, 79.1%).

$CF_3CH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 0$): crystal; mp 74–77 °C; ^1H NMR (200.1 MHz, CD_3CN): δ 4.69–4.83 (2H, q, $J = 9.72$ Hz), 7.56–7.64 (2H, m), 7.77–7.85 (1H, m), 8.10–8.14 (2H, m); ^{19}F NMR (188.3 MHz, CD_3CN): δ −78.9 (6F, s), −61.8 (3F, t, $J = 9.72$ Hz).

$CF_3CF_2CH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 1$): semisolid; ^1H NMR (300.5 MHz, $(CD_3)_2CO$): δ 5.29 (2H, t, $J = 17.2$ Hz), 7.62–7.68 (2H, m), 7.82–7.87 (1H, m), 8.37–8.41 (2H, d, $J = 7.90$ Hz); ^{19}F NMR (282.8 MHz, $(CD_3)_2CO$): δ −78.8 (6F, s), −83.2 (3F, s), −107.8 (2F, t, $J = 16.9$ Hz).

$CF_3CF_2CF_2CH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 2$): thick liquid; ^1H NMR (300.5 MHz, CD_3CN): δ 4.86 (2H, t, $J = 17.6$ Hz), 7.60–7.66 (2H, m), 7.82–7.87 (1H, m), 8.17–8.20 (2H, d, $J = 8.10$ Hz); ^{19}F NMR (282.8 MHz, CD_3CN): δ −78.2 (6F, s), −79.9 (3F, t, $J = 9.61$ Hz), −104.2 (2F, m), −125.0 (2F, s).

3.2. Preparation of N^{α}-$CF_3(CF_2)_nCH_2$-(L)tyrosine (**6**, $n = 0$) and NMR data (**6**, $n = 0$–2)

(L)Tyrosine ethyl ester (3.299 g, 15.77 mmol) was dissolved in 50 mL of CH_2Cl_2. Water (50 mL) and $NaHCO_3$ (1.596 g, 19.00 mmol) were added and the mixture was stirred. $CF_3CH_2I(C_6H_5)N(SO_2CF_3)_2$ (**4**, $n = 0$) (9.836 g, 17.35 mmol) was added with stirring at room temperature. After 4 h, the CH_2Cl_2 layer was separated and washed with 3×50 mL of water. After evaporation of organic solvent, the obtained residue was subjected to column chromatography using 10–40% acetone in *n*-hexane. The obtained ester intermediate (**5**, $n = 0$) was stirred in 50 mL of 1 M NaOH aqueous solution at room temperature for 10 h. The aqueous solution was then acidified with conc. HCl to pH around 4.5 in an ice bath cooling to form white precipitate, which was filtered, washed with H_2O, and dried under vacuum to yield white product N^{α}-CF_3CH_2-(L)tyrosine (**6**, $n = 0$) (3.939 g, 14.97 mmol, 94.9%).

N^{α}-CF_3CH_2-(L)tyrosine (**6**, $n = 0$): white solid; mp 210–213 °C; ^1H NMR (300.5 MHz, $(CD_3)_2CO$): δ 2.91 (2H, qd, $J = 9.73$ Hz, $J = 5.83$ Hz), 3.10–3.47 (2H, m), 3.59 (1H, dd, $J = 6.36$ Hz, $J = 6.36$ Hz), 6.74 (2H, d, $J = 8.60$ Hz), 7.09 (2H, d, $J = 8.60$ Hz); ^{13}C NMR (75.6 MHz, $(CD_3)_2CO$): δ 38.9, 49.3 (q, $J = 31.1$ Hz), 63.2, 115.8, 126.8 (q, $J = 277.4$ Hz), 129.0, 131.2, 156.9, 174.8; ^{19}F NMR (282.8 MHz, $(CD_3)_2CO$): δ −71.9 (3F, t, $J = 9.73$ Hz); ESMS (M + H, $C_{11}H_{12}NO_3F_3$): *m/z* 264.0737.

N^{α}-$CF_3CF_2CH_2$-(L)tyrosine (**6**, $n = 1$): white solid; mp 218–221 °C; ^1H NMR (300.5 MHz, $(CD_3)_2SO$): δ 2.74 (2H, m), 3.13 (1H, q, $J = 15.7$ Hz), 3.34 (1H, q, $J = 15.7$ Hz), 3.38 (1H, dd, $J = 6.55$ Hz, $J = 6.55$ Hz), 6.63 (2H, d, $J = 8.43$ Hz), 6.96 (2H, d, $J = 8.43$ Hz), 9.18 (1H, s, br); ^{13}C NMR (75.6 MHz, $(CD_3)_2SO$): δ 38.2, 46.7 (t, $J = 22.4$ Hz), 62.9, 115.1 (m), 115.4, 119.3 (m), 128.1, 130.6, 156.4, 175.3; ^{19}F NMR (282.8 MHz, $(CD_3)_2SO$): δ −82.5 (3F, s), −120.2 (2F, t, $J = 15.8$ Hz). ESMS (M + H, $C_{12}H_{12}NO_3F_5$): *m/z* 314.0687.

N^{α}-$CF_3CF_2CF_2CH_2$-(L)tyrosine (**6**, $n = 2$): white solid; mp 206–209 °C; ^1H NMR (300.5 MHz, $(CD_3)_2SO$): δ 2.73 (2H, m), 3.16 (1H, q, $J = 16.0$ Hz), 3.38 (1H, q, $J = 16.0$ Hz), 3.39 (1H, dd, $J = 6.55$ Hz, $J = 6.55$ Hz), 6.63 (2H, d, $J = 8.41$ Hz), 6.95 (2H, d, $J = 8.41$ Hz), 9.23 (1H, s, br); ^{13}C NMR (75.6 MHz, $(CD_3)_2SO$): δ 38.2, 46.8 (t, $J = 22.4$ Hz), 63.0, 115.1 (m), 115.4, 119.3 (m), 120.2 (m), 128.1, 130.6, 156.4, 175.2; ^{19}F NMR (282.8 MHz, $(CD_3)_2SO$): δ −79.9 (3F, t, $J = 9.47$ Hz), −117.5 (2F, m), −126.6 (2F, s); ESMS (M + H, $C_{13}H_{12}NO_3F_7$): *m/z* 365.0538.

3.3. Solid phase synthesis of N-terminus-2,2,2-trifluoroethylated leucine enkephalin (**7**)

Wang resin (1.000 g, 0.980 meq) was pre-swelled in CH_2Cl_2 and used in solid phase syntheses. Coupling reactions were carried out in the presence of N^{α}-Fmoc protected amino acids (6.000 mmol), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (1.927 g, 6.000 mmol), and diisopropylethylamine (1.8 mL, 10 mmol) in *N*,*N*-dimethylformamide (25 mL) at room temperature (60 °C for the coupling reaction of N^{α}-CF_3CH_2-(L)tyrosine) for 20 h with nitrogen gas sparging. Fmoc-removal was carried out in the presence of 25 mL of 20% piperidine in DMF for 15 min twice. Trifluoroacetic acid (95%, 25 mL) was used to cleave the peptide from resin at room temperature for 1 h twice. *N*-terminus-2,2,2-trifluoroethylated leucine enkephalin analogue (**7**) (0.336 g, 0.527 mmol, 53.8% in yield based on resin substitution level 0.980 meq/g resin) was obtained after recrystallization of the product from CH_3CN twice and lyophilization.

N-CF_3CH_2-Leu-enkephalin analogue (**7**): white solid; mp 85–88 °C; ^1H NMR (500.1 MHz, $(CD_3)_2SO$): δ 0.85 (3H, d, $J = 6.42$ Hz), 0.90 (3H, d, $J = 6.42$ Hz), 1.53 (2H, m), 1.63 (1H, m), 2.61 (1H, m), 2.76 (2H, m), 3.06 (2H, m), 3.20 (1H, m), 3.62 (2H, m), 3.73 (2H, m), 4.22 (1H, m), 4.56 (1H, m), 6.63 (2H, d, $J = 8.71$ Hz), 6.99 (2H, d, $J = 8.71$ Hz), 7.18 (1H, m), 7.25 (4H, m), 7.98 (1H, m), 8.08 (1H, d, $J = 8.25$ Hz), 8.16 (1H,

m), 8.29 (1H, d, $J = 7.79$ Hz), 9.17 (1H, br, s); ^{13}C NMR (125.7 MHz, $(CD_3)_2SO$): δ 21.3, 22.9, 24.3, 37.6, 38.1, 41.7, 41.9, 47.7 (q, $J = 30.7$ Hz), 50.3, 53.6, 63.1, 114.9, 125.9 (q, $J = 278$ Hz), 126.3, 128.1, 128.2, 129.3, 130.1, 137.8, 155.7, 168.4, 168.9, 171.2, 173.3, 173.9; ^{19}F NMR (470.6 MHz, $(CD_3)_2SO$): δ −71.7 (3F, t, $J = 10.1$ Hz). MALDI ($C_{30}H_{38}N_5O_7F_3$): *m/z* 637.064.

3.4. Preparation of N^{α}-CH_3CH_2-(L)tyrosine (**9**)

(L)Tyrosine methyl ester (0.882 g, 4.518 mmol) was dissolved in 6 mL of DMF and cooled in an ice bath. Triethyloxonium tetrafluoroborate $(C_2H_5)_3OBF_4$ (1.0 M solution in dichloromethane) (15.0 mL, 15.0 mmol) was injected under nitrogen protection. The reaction mixture was then allowed to warm up to room temperature and stirred for 24 h. After washing with 0.5 M of Na_2CO_3 aqueous solution, the organic solvent was evaporated. The obtained residue was subjected to column chromatography using 10–40% acetone in n-hexane to yield white crystalline N^{α}-CH_3CH_2-(L)tyrosine methyl ester (**8**) (0.538 g, 2.410 mmol, 53.3%).

The intermediate (**8**) was stirred with 20 mL of 1 M NaOH aqueous solution at room temperature overnight. The aqueous solution was then cooled in an ice bath and acidified using conc. HCl to pH 4.5. The solvent H_2O was evaporated, and the residue was dried under vacuum. Extraction of the dried residue using dry CH_3CN yielded the white product N^{α}-CH_3CH_2-(L)tyrosine (**9**) (0.435 g, 2.078 mmol, 86.2%).

N^{α}-CH_3CH_2-(L)tyrosine (**9**): white solid; mp 249–252 °C; ^{1}H NMR (300.5 MHz, D_2O): δ 1.13 (3H, t, $J = 7.21$ Hz), 2.98 (2H, m), 3.06 (2H, m), 3.89 (1H, dd, $J = 6.55$ Hz, $J = 6.55$ Hz), 6.76 (2H, d, $J = 8.78$ Hz), 7.06 (2H, d, $J = 8.78$ Hz); ^{13}C NMR (75.6 MHz, D_2O): δ 10.5, 34.6, 42.3, 62.0, 115.8, 125.9, 130.8, 155.0, 172.1; ESMS (M + H, $C_{11}H_{15}NO_3$): *m/z* 210.0987.

3.5. Lipophilicity measurement [27]

NaH_2PO_4 (15.17 g) and NaOH (2.943 g) were dissolved in 2.0 L of deionized water to give aqueous buffer solution with pH 7.07.

Above aqueous buffer solution (10.00 mL) and 1-octanol (25.00 mL) were vigorously stirred together for 96 h and then kept contacted with each other in a separatory funnel. The aqueous layer and 1-octanol layer were used as the blank solution for UV measurement in the partition experiments respectively.

All partition experiments were carried out at 22 °C.

3.5.1. (L)Tyrosine

(L)Tyrosine in aqueous buffer solution (0.929 mM, 10.00 mL) was vigorously stirred with 1-octanol (25.00 mL) for 96 h. The aqueous layer was separated and subjected to UV measurement (at 275 nm) to give an absorbance of 0.912 after subtraction of the absorbance of blank aqueous buffer solution. From the calibration curve in Fig. 2, the corresponding concentration of 0.922 mM of (L)TyrOH in aqueous buffer solution was obtained. The concentration of (L)TyrOH in 1-octanol layer was calculated as 2.80×10^{-3} mM.

3.5.2. N^{α}-CH_3CH_2-(L)tyrosine (**9**)

N^{α}-CH_3CH_2-(L)tyrosine (**9**) in aqueous buffer solution (0.300 mM, 5.00 mL) was vigorously stirred with 1-octanol (30.00 mL) for 96 h. The aqueous layer was separated and subjected to UV measurement (at 274 nm) to give an absorbance of 0.229 after subtraction of the absorbance of blank aqueous buffer solution. From the calibration curve in Fig. 3, the corresponding concentration of 0.263 mM of N^{α}-CH_3CH_2-(L)TyrOH in aqueous buffer solution was obtained. The concentration of N^{α}-CH_3CH_2-(L)TyrOH in 1-octanol layer was calculated as 6.14×10^{-3} mM.

3.5.3. N^{α}-CF_3CH_2-(L)tyrosine (**6**, $n = 0$)

N^{α}-CF_3CH_2-(L)tyrosine (**6**, $n = 0$) in 1-octanol (2.000 mM, 25.00 mL) was stirred vigorously with aqueous buffer solution (10.00 mL) for 96 h. The 1-octanol layer was separated and subjected to UV measurement (at 279 nm) to give an absorbance of 0.309 after subtraction of the absorbance of blank 1-octanol. From the calibration curve in Fig. 4, the corresponding concentration of 0.151 mM of N^{α}-CF_3CH_2-(L)TyrOH in 1-octanol was obtained. The concentration of N^{α}-CF_3CH_2-(L)TyrOH in aqueous layer was calculated as 4.62 mM.

3.5.4. N^{α}-$CF_3CF_2CH_2$-(L)tyrosine (**6**, $n = 1$)

N^{α}-$CF_3CF_2CH_2$-(L)tyrosine (**6**, $n = 1$) in 1-octanol (1.900 mM, 25.00 mL) was stirred vigorously with aqueous buffer solution (10.00 mL) for 96 h. The 1-octanol layer was separated and subjected to UV measurement (at 279 nm) to give an absorbance of 1.056 after subtraction of the absorbance of blank 1-octanol. From the calibration curve in Fig. 5, the corresponding concentration of 0.566 mM of N^{α}-$CF_3CF_2CH_2$-(L)TyrOH in 1-octanol was obtained. The concentration of N^{α}-$CF_3CF_2CH_2$-(L)TyrOH in aqueous layer was calculated as 3.33 mM.

3.5.5. N^{α}-$CF_3CF_2CF_2CH_2$-(L)tyrosine (**6**, $n = 2$)

N^{α}-$CF_3CF_2CF_2CH_2$-(L)tyrosine (**6**, $n = 2$) in 1-octanol (1.900 mM, 25.00 mL) was stirred vigorously with aqueous buffer solution (10.00 mL) for 96 h. The 1-octanol layer was separated and subjected to UV measurement (at 279 nm) to give an absorbance of 1.776 after subtraction of the absorbance of blank 1-octanol. From the calibration curve in Fig. 6, the corresponding concentration of 1.087 mM of N^{α}-$CF_3CF_2CF_2CH_2$-(L)TyrOH in 1-octanol was obtained. The concentration of N^{α}-$CF_3CF_2CF_2CH_2$-(L)TyrOH in aqueous layer was calculated as 2.033 mM.

3.5.6. Leucine enkephalin

Leucine enkephalin (Sigma–Aldrich Co.) in aqueous buffer solution (0.769 mM, 5.00 mL) was stirred vigorously with 1-octanol (30.00 mL) for 96 h. The aqueous layer was separated and subjected to UV measurement (at 275 nm) to give an absorbance of 0.599 after subtraction of the absorbance of

blank aqueous buffer solution. From the calibration curve in Fig. 7, the corresponding concentration of 0.414 mM of leucine enkephalin in aqueous buffer solution was obtained. The concentration of leucine enkephalin in 1-octanol layer was calculated as 0.059 mM.

3.5.7. *N*-CF_3CH_2-Leu-enkephalin (**7**)

N-CF_3CH_2-Leu-enkephalin analogue (**7**) in 1-octanol (0.345 mM, 20.00 mL) was stirred vigorously with aqueous buffer solution (10.00 mL) for 96 h. The 1-octanol layer was separated and subjected to UV measurement (at 226.5 nm) to give an absorbance of 0.753 after subtraction of the absorbance of blank 1-octanol. From the calibration curve (at 226.5 nm) in Fig. 8, the corresponding concentration of 0.138 mM of *N*-CF_3CH_2-Leu-enkephalin in 1-octanol was obtained. The concentration of *N*-CF_3CH_2-Leu-enkephalin in aqueous layer was calculated as 0.415 mM.

Acknowledgements

Financial support of this research by the National Science Foundation is gratefully acknowledged. The authors thank Dr. Dev P. Arya and Prof. Joel F. Liebman for their suggestions.

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