# Synthesis of a Potent and Selective $^{18}$F-Labeled δ-Opioid Receptor Antagonist Derived from the Dmt-Tic Pharmacophore for Positron Emission Tomography Imaging

Eun Kyoung Ryu,[‡,†] Zhanhong Wu,[‡] Kai Chen,[‡] Lawrence H. Lazarus,[§] Ewa, D. Marczak,[§] Yusuke Sasaki,[#] Akihiro Ambo,[#] Severo Salvadori,[||] Chuancheng Ren,[×] Heng Zhao,[×] Gianfranco Balboni,*[,⊥,||] and Xiaoyuan Chen*[,‡]

*Molecular Imaging Program at Stanford (MIPS), Department of Radiology and Bio-X Program, Stanford University School of Medicine, Stanford, California 94305-5484, Korea Basic Science Institute, Chuncheon Center, Korea, Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, Tohoku Pharmaceutical University, 4-1, Komatsushima 4-chome, Aoba-Ku, Sendai 981-8558, Japan, Department of Pharmaceutical Science and Biotechnology Center, University of Ferrara, I-44100, Ferrara, Italy, Department of Neurosurgery, Stanford University, Stanford, California 94305-5327, and Department of Toxicology, University of Cagliari, I-09124, Cagliari, Italy*

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Identification and pharmacological characterization of two new selective δ-opioid receptor antagonists, derived from the Dmt-Tic pharmacophore, of potential utility in positron emission tomography (PET) imaging are described. On the basis of its high δ selectivity, H-Dmt-Tic-ϵ-Lys(Z)-OH (reference compound **1**) is a useful starting point for the synthesis of $^{18}$F-labeled compounds prepared by the coupling of *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) with Boc-Dmt-Tic-ϵ-Lys(Z)-OH under slightly basic conditions at 37 °C for 15 min, deprotection with TFA, and HPLC purification. The total synthesis time was 120 min, and the decay-corrected radiochemical yield of [$^{18}$F]-**1** was about 25–30% ($n = 5$) starting from [$^{18}$F]SFB ($n = 5$) with an effective specific activity about 46 GBq/μmol. In vitro autoradiography studies showed prominent uptake of [$^{18}$F]-**1** in the striatum and cortex with significant blocking by **1** and UFP-501 (selective δ-opioid receptor antagonist), suggesting high specific binding of [$^{18}$F]-**1** to δ-opioid receptors. Noninvasive microPET imaging studies revealed the absence of [$^{18}$F]-**1** in rat brain, since it fails to cross the blood−brain barrier. This study demonstrates the suitability of [$^{18}$F]-**1** for imaging peripheral δ-opioid receptors.

## Introduction

Opioid receptors are classified into μ, δ, and κ subtypes and belong to the superfamily of G-protein-coupled receptors (GPCRs) that produce their effects by activation of intracellular Gi/Go proteins. The δ-opioid receptors play an important role in the modulation of nociceptive signaling encountered in animal models of pain.[1] Moreover, δ agonist administration or use of δ receptor-deficient mice confirmed that δ-opioid receptors are involved in emotional responses, such as depression-like behavior and anxiety.[2–4] These receptors are implicated in some aspects of morphine tolerance and dependence[5] with limited contribution compared to μ-opioid receptors.[1] Despite of the δ-opioid receptor involvement in several clinically relevant diseases and syndromes, such as analgesia, addiction, Parkinson's, Alzheimer's, and seizure disorders, their precise role in humans is incompletely understood.[6,7] Moreover, peripheral δ-opioid receptors seem to be involved in cancer,[8] cardiovascular disease,[9] gastrointestinal disorders,[10] and newer paradigms for pain relief that use peripherally restricted opioids.[11]

Radionuclide imaging techniques, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), are unique and complementary imaging techniques for in vivo assessment of drug distribution and interaction with biochemical targets systems.[12] Among opiate radioligands already available for PET imaging of δ-opioid receptors, only the antagonist [$^{11}$C]methylnaltrindole ([$^{11}$C]MeNTI) allows visualization of these receptors in the human brain.[13,14] Although it displayed high affinity for δ-opioid receptor ($K_i^{\delta} = 0.49$ nM) and moderate affinity for μ ($K_i^{\mu} = 39.2$ nM) and κ ($K_i^{\kappa} = 8.33$ nM) receptors, the selectivity was quite low ($K_i^{\mu}/K_i^{\delta} = 80$; $K_i^{\kappa}/K_i^{\delta} = 17$; $K_i^{\mu}/K_i^{\kappa} = 4.71$).[15] The development of tracers for PET or SPECT requires the presence of atoms (C, F, I) that can be substituted for their positron ($^{11}$C, $^{18}$F) or γ ($^{123}$I)-emitting isotope. Considering the usefulness of the reactant *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB)[16] in the synthesis of $^{18}$F labeled peptides and the versatility of the Dmt-Tic[a] as a δ-opioid pharmacophore,[3,4,17–21] we selected two δ-opioid reference compounds, H-Dmt-Tic-ϵ-Lys(Z)-OH[22] and H-Dmt-Tic-Phe-Lys(Z)-OH,[23] as potential tools for PET

* To whom correspondence should be addressed. For G.B.: phone, (+39) 532-455-275; fax, (+39) 532-455-953; e-mail, gbalboni@unica.it, bbg@unife.it. For X.C.: phone, (+1) 650-725-0950; fax, (+1) 650-736-7925; e-mail, shawchen@stanford.edu.

‡ Stanford University School of Medicine.

† Korea Basic Science Institute.

§ National Institute of Environmental Health Sciences.

# Tohoku Pharmaceutical University.

|| University of Ferrara.

× Stanford University.

⊥ University of Cagliari.

[a] Abbreviations. In addition to the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14–42), this paper uses the following additional symbols and abbreviations: AcOEt, ethyl acetate; AcOH, acetic acid; Bid, 1*H*-benzimidazole-2-yl; Boc, *tert*-butyloxycarbonyl; DAMGO, [D-Ala$^2$,*N*-Me-Phe$^4$,Gly-ol$^5$]enkephalin; DEL C, deltorphin II (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-$NH_2$); DMF, *N*,*N*-dimethylformamide; DMSO-$d_6$, hexadeuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; DPDPE, (D-Pen$^2$,D-Pen$^5$)-enkephalin; $Et_2O$, diethyl ether; GPI, guinea pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; MALDI-TOF, matrix assisted laser desorption ionization time-of-flight; MVD, mouse vas deferens; NMM, 4-methylmorpholine; $pA_2$, negative log of the molar concentration required to double the agonist concentration to achieve the original response; Pe, petroleum ether; TEA triethylamine; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[3′-dimethyl)aminopropyl]carbodiimide hydrochloride; Z, benzyloxycarbonyl.

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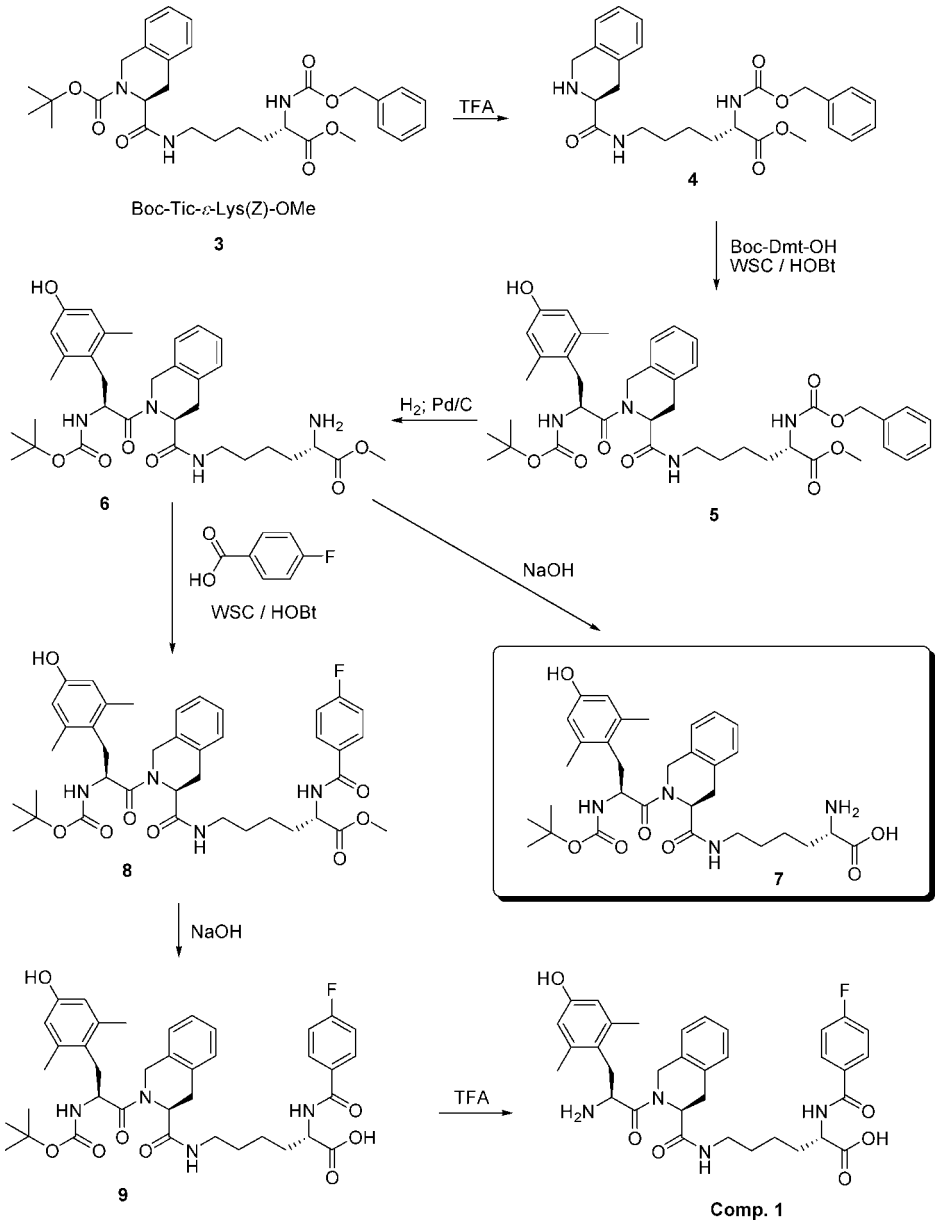
**Scheme 1.** Synthesis of Compound **1**




imaging on the basis of the similarity between the Z protecting group and the 4-fluorobenzoyl substituent.

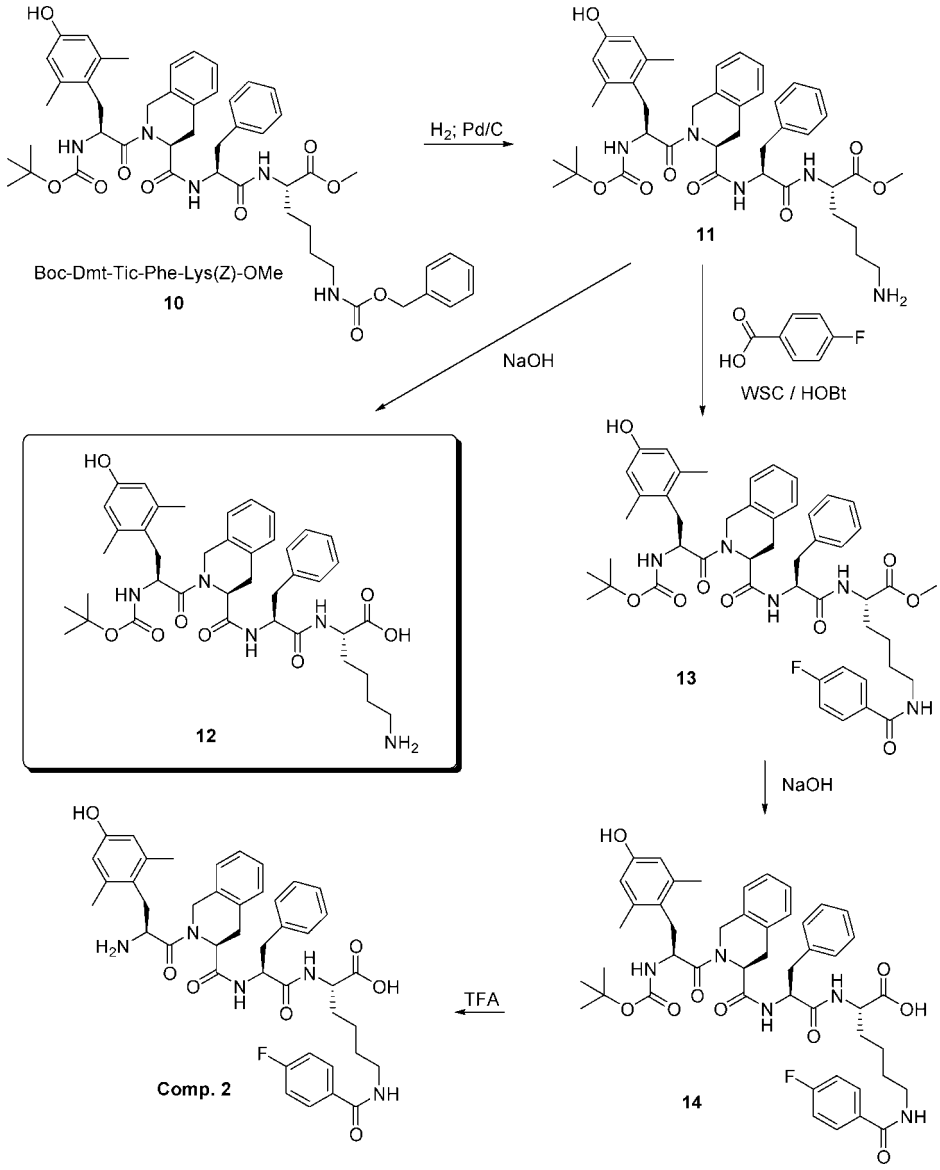
## Results and Discussion

**Chemistry and Radiochemistry.** Compounds **1** [H-Dmt-Tic-ϵ-Lys(Z)-OH] and **2** [H-Dmt-Tic-Phe-Lys(Z)-OH] were prepared stepwise by solution peptide synthetic methods, outlined in Schemes 1 and 2, respectively. Boc-Tic-ϵ-Lys(Z)-OMe (**3**)[22] was deprotected at its N-terminus by TFA treatment and condensed with Boc-Dmt-OH via WSC/HOBt. The resulting fully protected intermediate Boc-Dmt-Tic-ϵ-Lys(Z)-OMe (**5**) was Z deprotected by catalytic hydrogenation ($H_2$, 10% Pd/C) and condensed with 4-fluorobenzoic acid via WSC/HOBt. Hydrolysis of C-terminal methyl ester (NaOH) and Boc removal (TFA) at N-terminus gave the final compound **1**. Compound **2** was synthesized starting from the fully protected intermediate Boc-Dmt-Tic-Phe-Lys(Z)-OMe (**10**)[23] (Scheme 2). After Z removal ($H_2$, 10% Pd/C), it was coupled with 4-fluorobenzoic acid via WSC/HOBt. Hydrolysis of C-terminal methyl ester (NaOH) and N-terminal Boc deprotection (TFA) gave the final compound **2** (Scheme 2).

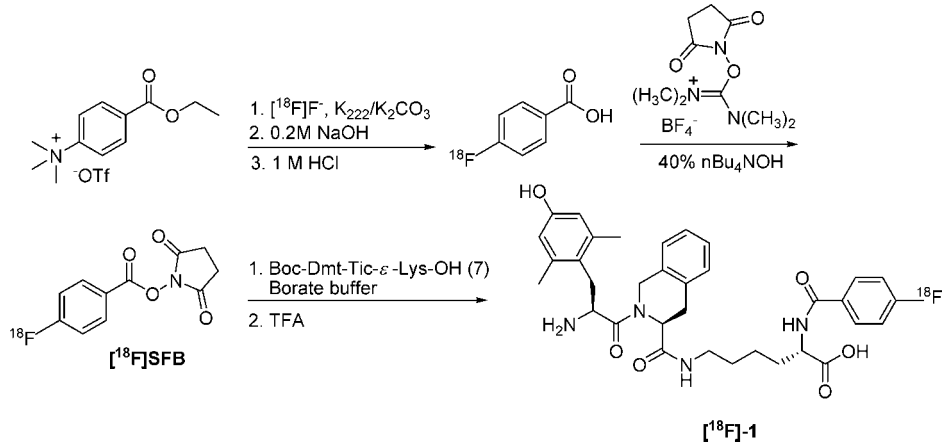
[$^{18}$F]-**1** was prepared by coupling *N*-succinimidyl 4-[$^{18}$F]fluorobenzoate ([$^{18}$F]SFB) with Boc-Dmt-Tic-ϵ-Lys-OH (7) under slightly basic conditions at 37 °C for 15 min and deprotected with TFA, followed by HPLC purification (Scheme 3). The radiochemical yield was 25–30% ($n = 5$) from [$^{18}$F]SFB with high radiochemical purity (>99%). The effective specific activity was about 46 GBq/$\mu$mol.

**Receptor Affinity Analysis.** Receptor binding and functional bioactivities are reported in Table 1. Like the majority of the compounds of general formula H-Dmt-Tic-ϵ-Lys(R)-R′ (R = −$NH_2$, −NH−Ac, −NH−Z; R′ = −CONH−Ph, CONH−$CH_2$−Ph, −Bid [Bid, $^1$H-benzimidazole-2-yl]),[22] **1** exhibited subnanomolar affinity for δ-opioid receptors ($K_i^\delta = 0.17$ nM). As expected, the presence of a free carboxylic function in compounds containing the Dmt-Tic pharmacophore increases δ selectivity ($K_i^\mu / K_i^\delta = 240$) by suppressing μ-opioid receptor affinity ($K_i^\mu = 41.2$ nM). Compound **2** arose from the second reference compound [H-Dmt-Tic-Phe-Lys(Z)-OH] by substitution of the Z protecting group in the Lys$^4$ side chain with a 4-fluorobenzoyl function, causing a slight decrease in δ affinity

**Scheme 2.** Synthesis of Compound **2**



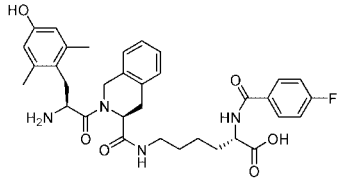
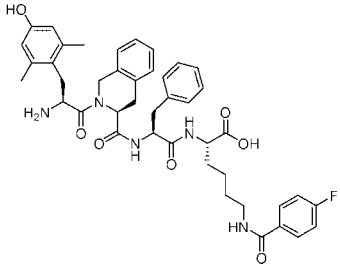

**Scheme 3.** Synthesis of [$^{18}$F]-**1**




($K_i^{\delta}$ = 0.25 nM) and $\mu$ affinity 3-fold ($K_i^{\mu}$ = 13.2 nM) and resulting in a 4.5-fold loss in $\delta$ selectivity ($K_i^{\mu}/K_i^{\delta}$ = 53).

**Functional Bioactivity.** Analogues **1** and **2** were tested in the electrically stimulated MVD and GPI assays for intrinsic functional bioactivity (Table 1). Similar to the reference compound containing Lys linked to the Dmt-Tic pharmacophore through its $\epsilon$-amine group, **1** maintained the same $\delta$ antagonism (MVD, p$A_2$ = 8.25) and weak $\mu$ agonism (GPI, $IC_{50}$ = 1916 nM). For the second reference [H-Dmt-Tic-Phe-Lys(Z)-OH], in which the replacement of the Z protecting group with the

**Table 1.** Receptor Binding Affinities and Functional Bioactivities of Compounds **1** and **2** and $\delta$ Selectivity $K_i^{\mu}/K_i^{\delta a}$

| Comp. | Structure | Receptor affinity[a] (nM) | | Selectivity | Functional bioactivity | | |
|---|---|---|---|---|---|---|---|
| | | $K_i^{\delta}$ | $K_i^{\mu}$ | $K_i^{\mu}/K_i^{\delta}$ | MVD $pA_2$[c] | MVD $IC_{50}$[b] (nM) | GPI $IC_{50}$[b] (nM) |
| *Ref. 1* | *H-Dmt-Tic-ε-Lys(R)-R'*[d] *R = -NH2, -NHCO-Ac, -NH-Z R' = -CONH-Ph, -CONH-CH2-Ph, -Bid* | *0.21-2.64* | *0.60-3.43* | *1.7-16.3* | *7.81-8.27* | | *434-1990* |
| **1** |  | 0.172±0.003 (3) | 41.2±1.76 (3) | 240 | 8.25 | | 1916±554 |
| *Ref. 2* | *H-Dmt-Tic-Phe-Lys(Z)-OH*[e] | *0.019* | *2.75* | *145* | *11.43* | | *>10000* |
| **2** |  | 0.248±0.019 (3) | 13.2±1.13 (3) | 53 | 9.45 | 5176±1400 | 50.6±6.9 |

[a] The $K_i$ values (nM) were determined according to Cheng and Prusoff.[33] The mean ± SE (*n* repetitions in parentheses) is based on independent duplicate binding assays with five to eight peptide doses using several different synaptosomal preparations. [b] Agonist activity was expressed as $IC_{50}$ obtained from dose–response curves representing the mean ± SE for at least five to six fresh GPI tissue samples. Deltorphin II and endomorphin-2 were the internal standards for MVD ($\delta$-opioid receptor bioactivity) and GPI ($\mu$-opioid receptor bioactivity) in the tissue preparations, respectively. [c] The $pA_2$ values of opioid antagonists were determined against the agonists deltorphin II and endomorphin-2 according to the method of Kosterlitz and Watt.[36] [d] Data taken from Balboni et al.[22] [e] Data taken from Balboni et al.[23]

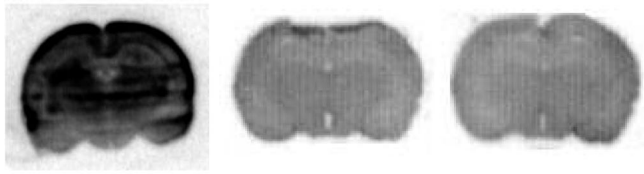



**Figure 1.** Autoradiography of rat brain slices incubated with [$^{18}$F]-**1**. In vitro autoradiograms of coronal sections from rat brain in the presence of (A) [$^{18}$F]-**1**, (B) [$^{18}$F]-**1** and **1** (10 $\mu$M), and (C) [$^{18}$F]-**1** and UPF-501 (10 $\mu$M).

4-fluorobenzoyl substituent was detrimental for functional bioactivity (**2**), $\delta$ antagonism fell 95-fold from $pA_2 = 11.43$ to $pA_2 = 9.45$ accompanied by increased $\mu$ agonism (GPI, $IC_{50} =$ 50.6 nM), which was absent in the corresponding reference compound. Compound **2** also displayed weak $\delta$ agonism at concentrations greater than 5 $\mu$M, which was not observed by the reference compound. Thus, on the basis of the better selectivity for the $\delta$-opioid receptor and a high level of $\delta$ antagonism, **1** was exploited for labeling with $^{18}$F.

**Autoradiography Studies.** In vitro autoradiography revealed that [$^{18}$F]-**1** exhibited high uptake in the caudate putamen (striatum) and cortex of rat brain slices, regions known to have a high expression of opioid receptors. The radiotracer uptake into both the striatum and the cortex was significantly blocked by **1** or UPF-501 [*N*,*N*(Me)$_2$-Dmt-Tic-OH, a potent and selective $\delta$-pioid antagonist],[17] indicating that [$^{18}$F]-**1** binds specifically and selectively to $\delta$-opioid receptors (Figure 1).

**MicroPET Imaging.** Static microPET scans were performed on a Sprague–Dawley rat and selected coronal, sagittal, and transaxial images at 15 min after femoral vein injection of [$^{18}$F]-**1** (Figure 2). Despite the fact that [$^{18}$F]-**1** exhibited prominent accumulation in $\delta$-opioid receptor positive regions of the brain by in vitro autoradiography, noninvasive PET imaging clearly demonstrated the absence of uptake into intact rat brain in vivo, indicating that this compound does not cross the blood−brain barrier (BBB).

## Conclusions

This study showed that **1** is a potent and selective $\delta$-opioid receptor antagonist, and although [$^{18}$F]-**1** specifically binds to $\delta$-opioid receptor in brain slices in vitro, there was an absence of uptake in brain because of limited penetration of the BBB. At present, there is an increasing demand of radioligands for in vivo imaging studies of peripheral opioid receptors to help assess the roles they may play in cancer, cardiovascular disease, gastrointestinal disorders, and pain relief.[24] The feasibility of imaging $\delta$-opioid receptors in normal human heart[25] and $\delta$ sites overexpressed in primary tumors of lung[26] and breast cancer patients[27] was initially demonstrated in limited studies using *N*1′-([$^{11}$C]methyl)naltrindole and PET; however, because of the higher affinity and selectivity, [$^{18}$F]-**1** could represent a new useful tracer for PET imaging of peripheral $\delta$-opioid receptors as a marker in various disease states.

## Experimental Section

**Chemistry. TFA·H-Tic-ε-Lys(Z)-OMe (4).** Boc-Tic-ε-Lys(Z)-OMe (**3**)[22] (1.67 g, 3.02 mmol) was treated with TFA (2 mL) for

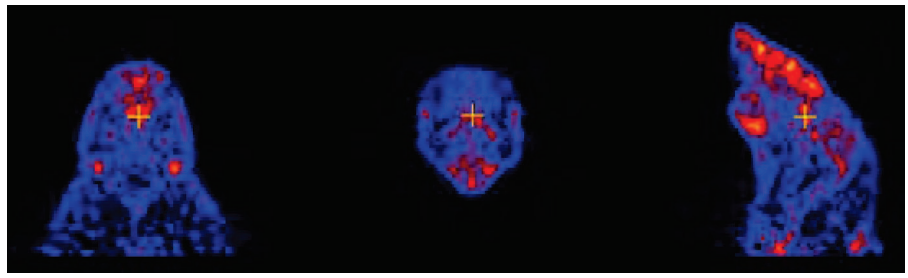


**Figure 2.** In vivo PET analysis of the distribution of [$^{18}$F]-**1** in rats. Representative coronal, transaxial, and sagittal microPET images of [$^{18}$F]-**1** in the normal Sprague–Dawley rat brain 15 min after femoral vein injection as detailed in the Experimental Section.

0.5 h at room temperature. $Et_2O$/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 1.58 g (92%); $R_f$(A) = 0.49; HPLC $K'$ = 4.96; mp 118–120 °C; $[\alpha]^{20}_D$ −19.6; *m/z* 454 $(M + H)^+$.

**Boc-Dmt-Tic-ϵ-Lys(Z)-OMe (5).** To a solution of Boc-Dmt-OH (0.22 g, 0.71 mmol) and TFA·H-Tic-ϵ-Lys(Z)-OMe (**4**) (0.4 g, 0.71 mmol) in DMF (10 mL) at 0 °C, NMM (0.08 mL, 0.71 mmol), HOBt (0.12 g, 0.78 mmol), and WSC (0.15 g, 0.78 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in $H_2O$), $NaHCO_3$ (5% in $H_2O$), and brine. The organic phase was dried ($Na_2SO_4$) and evaporated to dryness. The residue was precipitated from $Et_2O$/Pe (1:9, v/v): yield 0.46 g (87%); $R_f$(B) = 0.91; HPLC $K'$ = 7.30; mp 133–135 °C; $[\alpha]^{20}_D$ −17.5; *m/z* 746 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.90 (m, 15H), 2.35 (s, 6H), 2.92–3.20 (m, 6H), 3.67 (s, 3H), 4.41–5.34 (m, 7H), 6.29 (s, 2H), 6.96–7.19 (m, 9H).

**Boc-Dmt-Tic-ϵ-Lys-OMe (6).** To a solution of Boc-Dmt-Tic-ϵ-Lys(Z)-OMe (**5**) (0.37 g, 0.5 mmol) in methanol (30 mL) was added Pd/C (10%, 0.1 g), and $H_2$ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was precipitated from $Et_2O$/Pe (1:9, v/v): yield 0.27 g (90%); $R_f$(B) = 0.74; HPLC $K'$ = 5.06; mp 139–141 °C; $[\alpha]^{20}_D$ −18.1; *m/z* 612 $(M + H)^+$.

**Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OMe (8).** To a solution of Boc-Dmt-Tic-ϵ-Lys-OMe (**6**) (0.43 g, 0.7 mmol) and 4-fluorobenzoic acid (0.1 g, 0.7 mmol) in DMF (10 mL) at 0 °C, HOBt (0.12 g, 0.77 mmol) and WSC (0.15 g, 0.77 mmol) were added. The reaction mixture was stirred for 3 h at 0 °C and 24 h at room temperature. After DMF was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in $H_2O$), $NaHCO_3$ (5% in $H_2O$), and brine. The organic phase was dried ($Na_2SO_4$) and evaporated to dryness. The residue was precipitated from $Et_2O$/Pe (1:9, v/v): yield 0.45 g (88%); $R_f$(B) = 0.79; HPLC $K'$ = 5.25; mp 134–136 °C; $[\alpha]^{20}_D$ −17.3; *m/z* 734 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.94 (m, 15H), 2.35 (s, 6H), 2.92–3.20 (m, 6H), 3.67 (s, 3H), 4.41–4.92 (m, 5H), 6.29 (s, 2H), 6.96–7.93 (m, 8H).

**Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OH (9).** To a solution of Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OMe (**8**) (0.51 g, 0.7 mmol) in ethanol (10 mL) at room temperature, 1 N NaOH (1.1 mL, 1.1 mmol) was added. The reaction mixture was stirred for 4 h at room temperature. After ethanol was evaporated, the residue was dissolved in EtOAc and washed with citric acid (10% in $H_2O$) and brine. The organic phase was dried ($Na_2SO_4$) and evaporated to dryness. The residue was precipitated from $Et_2O$/Pe (1:9, v/v): yield 0.45 g (90%); $R_f$(B) = 0.75; HPLC $K'$ = 5.14; mp 141–143 °C; $[\alpha]^{20}_D$ −17.9; *m/z* 720 $(M + H)^+$.

**TFA·H-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OH (1).** Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OH (**9**) was treated with TFA as reported for TFA·H-Tic-ϵ-Lys(Z)-OMe: yield 0.09 g (94%); $R_f$(A) = 0.42; HPLC $K'$ = 3.34; mp 149–151 °C; $[\alpha]^{20}_D$ −18.5; *m/z* 620 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.82 (m, 6H), 2.35 (s, 6H), 2.92–3.20 (m, 6H), 3.95–4.92 (m, 5H), 6.29 (s, 2H), 6.96–7.93 (m, 8H). Anal. ($C_{36}H_{40}F_4N_4O_8$) C, H, N.

**Boc-Dmt-Tic-ϵ-Lys-OH (7).** To a solution of Boc-Dmt-Tic-ϵ-Lys-OMe (**6**) (0.09 g, 0.15 mmol) in ethanol (10 mL) at room temperature, 1 N NaOH (0.23 mL, 0.23 mmol) was added. The reaction mixture was stirred for 4 h at room temperature. After ethanol was evaporated, the residue was dissolved in solvent B and directly purified by preparative HPLC as reported above in general methods: yield 0.1 g (92%); $R_f$(B) = 0.67; HPLC $K'$ = 4.73; mp 146–148 °C; $[\alpha]^{20}_D$ −18.4; *m/z* 598 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.78 (m, 15H), 2.35 (s, 6H), 2.92–3.49 (m, 7H), 4.41–4.92 (m, 4H), 6.29 (s, 2H), 6.96–7.02 (m, 4H).

**Boc-Dmt-Tic-Phe-Lys-OMe (11).** To a solution of Boc-Dmt-Tic-Phe-Lys(Z)-OMe (**10**)[23] (0.71 g, 0.8 mmol) in methanol (30 mL) was added Pd/C (10%, 0.2 g), and $H_2$ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was precipitated from $Et_2O$/Pe (1:9, v/v): yield 0.58 g (88%); $R_f$(B) = 0.81; HPLC $K'$ = 5.24; mp 144–146 °C; $[\alpha]^{20}_D$ +35.2; *m/z* 759 $(M + H)^+$.

**Boc-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OMe (13).** This compound was obtained by condensation of Boc-Dmt-Tic-Phe-Lys-OMe (**11**) with 4-fluorobenzoic acid via WSC/HOBt as reported for Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OMe: yield 0.23 g (85%); $R_f$(B) = 0.78; HPLC $K'$ = 5.10; mp 135–137 °C; $[\alpha]^{20}_D$ +30.6; *m/z* 881 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.90 (m, 15H), 2.35 (s, 6H), 2.92–3.20 (m, 8H), 3.67 (s, 3H), 4.41–4.92 (m, 6H), 6.29 (s, 2H), 6.96–7.93 (m, 13H).

**Boc-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OH (14).** This compound was obtained by hydrolysis of Boc-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OMe (**13**) with 1 N NaOH as reported for Boc-Dmt-Tic-ϵ-Lys(4-fluorobenzoyl)-OH: yield 0.15 g (91%); $R_f$(B) = 0.75; HPLC $K'$ = 5.0; mp 139–141 °C; $[\alpha]^{20}_D$ +31.4; *m/z* 867 $(M + H)^+$.

**TFA·H-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OH (2).** Boc-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OH (14) was treated with TFA as reported for TFA·H-Tic-ϵ-Lys(Z)-OMe: yield 0.08 g (96%); $R_f$(A) = 0.40; HPLC $K'$ = 3.86; mp 146–149 °C; $[\alpha]^{20}_D$ +31.1; *m/z* 767 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.78 (m, 6H), 2.35 (s, 6H), 2.92–3.20 (m, 8H), 3.95–4.92 (m, 5H), 6.29 (s, 2H), 6.96–7.93 (m, 13H). Anal. ($C_{45}H_{49}F_4N_5O_9$) C, H, N.

**Boc-Dmt-Tic-Phe-Lys-OH (12).** This compound was obtained by hydrolysis of Boc-Dmt-Tic-Phe-Lys-OMe (11) with 1N NaOH as reported for Boc-Dmt-Tic-ϵ-Lys-OH: yield 0.06 g (86%); *Rf*(B) 0.65; HPLC $K'$ 4.75; mp 140–142 °C; $[\alpha]^{20}_D$ +36.1; *m/z* 745 $(M + H)^+$; $^1$H NMR (DMSO-$d_6$) δ 1.29–1.78 (m, 15H), 2.35 (s, 6H), 2.92–3.17 (m, 8H), 4.41–4.92 (m, 6H), 6.29 (s, 2H), 6.96–7.21 (m, 9H).

**Radiochemistry.** For radiochemistry, analytical and semi-preparative reversed-phase HPLC were performed on a Dionex 680 chromatography system with a UVD 170U absorbance detector and model 105S single-channel radiation detector (Carroll & Ramsey Associates). The recorded data were processed using Chromeleon software (version 6.50). Isolation of peptides and $^{18}$F-labeled peptides was performed using a Vydac protein and peptide column (218TP510, 5 μm, 250 mm × 10 mm). The flow was set to 5 mL/min using a gradient system starting from 95% solvent A (0.1% trifluoroacetic acid [TFA] in water) and 5% solvent B (0.1% TFA in acetonitrile [ACN]) (0–2 min) and increased to 35% A and 65% B at 32 min. Analytical HPLC used the same gradient system but with another Vydac column (218TP54, 5 μm, 250 mm × 4.6 mm) and with a flow of 1 mL/min. The ultraviolet (UV) absorbance

was monitored at 218 nm, and the identification of the peptides was confirmed on the basis of the UV spectrum acquired using a PDA detector.

The [$^{18}$F]SFB was synthesized following a reported procedure.[28,29] The [$^{18}$F]SFB was dissolved in DMSO (100 $\mu$L) and added to the Boc-protected-compound (Boc-Dmt-Tic-$\epsilon$-Lys-OH, 200 $\mu$g, 0.95 $\mu$mol) in borate buffer (300 $\mu$L, 0.05 M, pH 8.5) at 37 °C for 15 min. The Boc group was removed with anhydrous TFA (500 $\mu$L) for 5 min at room temperature (Scheme 3). The mixture was purified by HPLC, and the collected fractions (retention time: 17.9–18.1 min) were evaporated. The radioactivity was then reconstituted in phosphate-buffered saline (PBS) and passed through a 0.22-$\mu$m Millipore filter into a sterile multidose vial for in vivo applications.

**Pharmacology. Competitive Binding Assays.** Opioid receptor affinities were determined under equilibrium conditions [2.5 h at room temperature (23 °C)] in competition assays using brain $P_2$ synaptosomal membranes prepared from Sprague–Dawley rats.[30,31] Synaptosomes were preincubated to remove endogenous opioid peptides and stored at −80 °C in buffered 20% glycerol.[30,32] Each analogue was analyzed in duplicate assays using five to eight dosages and three to five independent repetitions with different synaptosomal preparations ($n$ values are listed in Table 1 in parentheses and results are the mean ± SE). Unlabeled peptide (2 $\mu$M) was used to determine nonspecific binding in the presence of 1.9 nM [$^3$H]deltorphin II (45.0 Ci/mmol, Perkin-Elmer, Boston, MA; $K_D$ = 1.4 nM) for $\delta$-opioid receptors and 3.5 nM [$^3$H]DAMGO (50.0 Ci/mmol, Amersham Bioscience, Buckinghamshire, U.K.; $K_D$ = 1.5 nM) for $\mu$-opioid receptors. Glass fiber filters (Whatman GFC) were soaked in 0.1% polyethylenimine in order to enhance the signal-to-noise ratio of the bound radiolabeled synaptosome complex, and the filters were washed thrice in ice-cold buffered BSA.[30] The affinity constants ($K_i$) were calculated according to Cheng and Prusoff.[33]

**Biological Activity in Isolated Tissue Preparation.** The myenteric plexus longitudinal muscle preparations (2–3 cm segments) from the small intestine of male Hartley strain of guinea pigs (GPI) measured $\mu$-opioid receptor agonism, and a single mouse vas deferens (MVD) was used to determine $\delta$-opioid receptor agonism as described previously.[34] The isolated tissues were suspended in organ baths containing balanced salt solutions in a physiological buffer, pH 7.5. Agonists were tested for the inhibition of electrically evoked contraction and expressed as $IC_{50}$ (nM) obtained from the dose–response curves. The $IC_{50}$ values represent the mean ± SE of five or six separate assays, and the $\delta$-antagonist potencies in the MVD assay were determined against the $\delta$-agonist deltorphin-II, while $\mu$-antagonism (GPI assay) used the $\mu$-agonist endomorphin-2. Antagonism is expressed as p$A_2$ determined using the Schild plot.[35]

**Animals for in Vitro and in Vivo Studies.** Laboratory animals were used under protocols approved and governed by the Animal Care and Use Committees of Stanford University, Tohoku Pharmaceutical University, and the National Institute of Environmental Health Sciences.

**In Vitro Autoradiography.** Male Sprague–Dawley rats (260–300 g) were sacrificed by $CO_2$ inhalation and then 20 $\mu$m coronal sections of the brain were cut with a cryostat (Microm HM505N, Carl Zeiss, Waldorf, Germany) and stored at −78 °C until used. Tissue was preincubated for 3 min in cold acetone before the sections were incubated in PBS containing [$^{18}$F]-**1** (0.37 MBq) for 1 h. After incubation the slices were rinsed three times in PBS and air-dried and the slides were taped to a autoradiography cassette containing a Super Resolution screen (Packard, Meriden, CT) for overnight exposure. The films were analyzed using a Typhoon Trio scanner (Amersham Biosciences, U.K.). For a receptor blocking experiments, brain slices were incubated with [$^{18}$F]-**1** in the presence of 10 $\mu$M reference compound or UFP-501 [$N$,$N$-(Me)$_2$-Dmt-Tic-OH].[17]

**MicroPET Imaging.** PET scans and image analysis are performed using a rodent scanner (microPET R4, Siemens Medical Solutions). About 27.6–32.8 MBq of [$^{18}$F]-**1** was injected into a Sprague–Dawley rat through the femoral vein under isoflurane anesthesia. Five minute static scans were taken 15 min after injection, and the images were reconstructed using a two-dimensional ordered-subsets expectation maximum (OSEM) algorithm. No correction was necessary for attenuation and scattering.

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**Supporting Information Available:** Chemistry general methods, elemental analysis results, MS and HPLC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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