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Synthesis of a potent and selective ^{18}F -labeled δ -opioid receptor antagonist derived from the Dmt-Tic pharmacophore for PET imaging

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Abstract

H-Dmt-Tic- ϵ -Lys(Z)-OH (**1**) was used in the synthesis of ^{18}F -labeled opioids for positron emission tomography (PET) imaging by coupling *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, deprotected with TFA and HPLC purification in 120 min with a decay-corrected radiochemical 25–30% yield of ^{18}F -**1** (*n* = 5) and specific activity ca. 46 GBq/ μmol . Autoradiography uptake of ^{18}F -**1** in striatum and cortex was blocked by **1** and UFP-501 demonstrating specific binding to δ -opioid receptors. MicroPET imaging revealed the absence of ^{18}F -**1** in rat brain, suggesting its suitability 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 signalling 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 dependence⁵ 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

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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 [¹¹C]-methylnaltrindole ([¹¹C]-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 (¹¹C, ¹⁸F) or γ (¹²³I)-emitting isotope. Considering the usefulness of the reactant *N*-succinimidyl-4-[¹⁸F]fluorobenzoate ([¹⁸F]SFB)¹⁶ in the synthesis of ¹⁸F labelled peptides and the versatility of the Dmt-Tic as a δ -opioid pharmacophore,^{3, 4, 17–21} we selected two δ -opioid reference compounds, H-Dmt-Tic- ϵ -Lys(Z)-OH²² and H-Dmt-Tic-Phe-Lys(Z)-OH,²³ as potential tools for PET imaging on the basis of the similarity between the Z protecting group and the 4-fluoro-benzoyl 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 Scheme 1 and Scheme 2, respectively. Boc-Tic- ϵ -Lys(Z)-OMe²² 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 was Z deprotected by catalytic hydrogenation (H₂; 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)-OMe²³ (Scheme 2). After Z removal (H₂; 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**.

[¹⁸F]-**1** was prepared by coupling *N*-succinimidyl-4-[¹⁸F]fluorobenzoate ([¹⁸F]SFB) with Boc-Dmt-Tic- ϵ -Lys-OH under slightly basic condition 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 [¹⁸F]-SFB with high radiochemical purity (> 99%). The effective specific activity was ca. 46 GBq/ μ mol.

Receptor affinity analysis

Receptor binding and functional bioactivities are reported in Table 1. Of the majority of the compounds of general formula H-Dmt-Tic- ϵ -Lys(R)-R' (R = -NH₂, -NH-Ac, -NH-Z; R' = -CONH-Ph, CONH-CH₂-Ph, -Bid [Bid, 1H-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 Z protecting group in the Lys⁴ side chain with a 4-fluorobenzoyl function, caused a slight decrease in δ affinity ($K_i^\delta = 0.25$ nM) and increased μ affinity 3-fold ($K_i^\mu = 13.2$ nM) resulting in a 4.5-fold loss in δ 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 ϵ -amine group, **1** maintained the same δ antagonism (MVD, $pA_2 = 8.25$) and weak μ agonism (GPI, $IC_{50} = 1916$ nM). The second reference [H-Dmt-Tic-Phe-Lys(Z)-OH], in which the replacement of Z protecting group with the 4-fluorobenzoyl substituent was detrimental for functional bioactivity (**2**); δ antagonism fell 95-fold, from a $pA_2 = 11.43$ to $pA_2 = 9.45$ accompanied by increased μ agonism (GPI, $IC_{50} = 50.6$ nM) which was absent in the corresponding reference compound. Compound **2** also displayed weak δ agonism at concentrations > 5 μ M, which was not observed by the reference compound. Thus, on the basis of the better selectivity for the δ -opioid receptor and a high level of δ antagonism, **1** was exploited for labelling 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 δ -opioid antagonist],¹⁷ indicating that [^{18}F]-**1** binds specifically and selectively to δ -opioid receptors (Fig. 1).

MicroPET Imaging

Static microPET scans were performed on a Sprague-Dawley rat and selected coronal, sagittal, and transaxial images at 15 min after tail-vein injection of [^{18}F]-**1** (Fig. 2). Despite the fact that [^{18}F]-**1** exhibited prominent accumulation in δ -opioid receptor positive regions of the brain by in vitro autoradiography, non-invasive 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 δ -opioid receptor antagonist, and although [^{18}F]-**1** specifically binds to δ -opioid receptor in brain slices in vitro, there was an absence of uptake in brain due to 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 δ -opioid receptors in normal human heart,²⁵ and δ sites overexpressed in primary tumors of lung²⁶ and breast cancer patients²⁷, was initially demonstrated in limited studies using *N*1'-([^{11}C]methyl)naltrindole and PET; however, due to the higher affinity and selectivity, [^{18}F]-**1** could represent a new useful tracer for PET imaging of peripheral δ -opioid receptors as markers in various disease states.

Experimental Section

Chemistry

General Methods—Crude compounds were purified by preparative reversed-phase HPLC [Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly column C18 (30 $\times$ 4 cm, 15 μ m particle size)] and eluted at a flow rate of 20 mL/min with mobile phase solvent A (10% acetonitrile + 0.1% TFA in H₂O, v/v), and a linear gradient from 10 to 60% solvent B (60%, acetonitrile + 0.1% TFA in H₂O, v/v) in 30 min. Analytical HPLC analyses were performed with a Beckman System Gold (Beckman ultrasphere ODS column, 250 $\times$ 4.6 mm, 5 μ m particle size). Analytical determinations and capacity factor (K') of the products used HPLC in solvents A and B programmed at flow rate of 1 mL/min with linear gradient from 0

to 100% B in 25 min. Analogues had less than 1% impurities when monitored at 220 and 254 nm. TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany): (A) 1-butanol/AcOH/H₂O (3:1:1, v/v/v); (B) CH₂Cl₂/toluene/methanol (17:1:2). Ninhydrin (1% ethanol, Merck), fluorescamine (Hoffman-La Roche) and chlorine spray reagents. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were assessed at 10 mg/mL in methanol with a Perkin-Elmer 241 polarimeter in a 10 cm water-jacketed cell. Molecular weights of the compounds were determined by a MALDI-TOF analysis (Hewlett Packard G2025A LDTOF system mass spectrometer) and α -cyano-4-hydroxycinnamic acid as a matrix. ¹H NMR (δ) spectra were measured, when not specified, in DMSO-*d*₆ solution using a Bruker AC-200 spectrometer, and peak positions are given in parts per million downfield from tetramethylsilane as internal standard.

Peptide Synthesis

TFA.H-Tic- ϵ -Lys(Z)-OMe—Boc-Tic- ϵ -Lys(Z)-OMe²² (1.67 g, 3.02 mmol) was treated with TFA (2 mL) for 0.5 h at room temperature. Et₂O/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; [α]_D²⁰ –19.6; *m/z* 454 (M+H)⁺.

Boc-Dmt-Tic- ϵ -Lys(Z)-OMe—To a solution of Boc-Dmt-OH (0.22 g, 0.71 mmol) and TFA.H-Tic- ϵ -Lys(Z)-OMe (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 H₂O), NaHCO₃ (5% in H₂O), and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.46 g (87%); *R*_f(B) 0.91; HPLC *K'* 7.30; mp 133–135 °C; [α]_D²⁰ –17.5; *m/z* 746 (M+H)⁺; ¹H-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—To a solution of Boc-Dmt-Tic- ϵ -Lys(Z)-OMe (0.37 g, 0.5 mmol) in methanol (30 mL) was added Pd/C (10%, 0.1 g), and H₂ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.27 g (90%); *R*_f(B) 0.74; HPLC *K'* 5.06; mp 139–141 °C; [α]_D²⁰ –18.1; *m/z* 612 (M+H)⁺.

Boc-Dmt-Tic- ϵ -Lys(4-fluorobenzoyl)-OMe—To a solution of Boc-Dmt-Tic- ϵ -Lys-OMe (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 H₂O), NaHCO₃ (5% in H₂O), and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.45 g (88%); *R*_f(B) 0.79; HPLC *K'* 5.25; mp 134–136 °C; [α]_D²⁰ –17.3; *m/z* 734 (M+H)⁺; ¹H-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—To a solution of Boc-Dmt-Tic- ϵ -Lys(4-fluorobenzoyl)-OMe (0.51 g, 0.7 mmol) in ethanol (10 mL) at room temperature, 1N 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 H₂O) and brine. The organic phase was dried (Na₂SO₄) and evaporated to dryness. The residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.45 g (90%); *R*_f(B) 0.75; HPLC *K'* 5.14; mp 141–143 °C; [α]_D²⁰ –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 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' 4.86; mp 149–151 °C; $[\alpha]^{20}_D$ –18.5; m/z 620 (M+H)⁺; ¹H-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. Calcd. for C₃₆H₄₀F₄N₄O₈: C, 59.01; H, 5.50; N, 7.65. Found: C, 58.85; H, 5.43; N, 7.48.

Boc-Dmt-Tic- ϵ -Lys-OH—To a solution of Boc-Dmt-Tic- ϵ -Lys-OMe (0.09 g, 0.15 mmol) in ethanol (10 mL) at room temperature, 1N 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)⁺; ¹H-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—To a solution of Boc-Dmt-Tic-Phe-Lys(Z)-OMe²³ (0.71 g, 0.8 mmol) in methanol (30 mL) was added Pd/C (10%, 0.2 g), and H₂ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was precipitated from Et₂O/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—This compound was obtained by condensation of Boc-Dmt-Tic-Phe-Lys-OMe 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)⁺; ¹H-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—This compound was obtained by hydrolysis of Boc-Dmt-Tic-Phe-Lys(4-fluorobenzoyl)-OMe with 1N 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 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' 2.87; mp 146–149 °C; $[\alpha]^{20}_D$ +31.1; m/z 767 (M+H)⁺; ¹H-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. Calcd. for C₄₅H₄₉F₄N₅O₉: C, 61.43; H, 5.61; N, 7.96. Found: C, 61.68; H, 5.77; N, 7.78.

Boc-Dmt-Tic-Phe-Lys-OH—This compound was obtained by hydrolysis of Boc-Dmt-Tic-Phe-Lys-OMe with 1N NaOH as reported for Boc-Dmt-Tic- ϵ -Lys-OH: yield 0.06 g (86%); R_f (B) 0.65; HPLC K' 4.75; mp 140–142 °C; $[\alpha]^{20}_D$ +36.1; m/z 745 (M+H)⁺; ¹H-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 separations 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 (v. 6.50). Isolation of peptides and ¹⁸F-labeled peptides was performed using a Vydac protein and peptide column (218TP510,

5 μ m, 250 $\times$ 10 mm). The flow was set at 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 μ m, 250 $\times$ 4.6 mm) and flow of 1 mL/min. The ultraviolet (UV) absorbance was monitored at 218 nm and the identification of the peptides was confirmed based on 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 μ L) and added to the Boc-protected-compound (Boc-Dmt-Tic- ϵ -Lys-OH, 200 μ g, 0.95 μ mol) in borate buffer (300 μ L, 0.05 M, pH 8.5) at 37 $^{\circ}$ C for 15 min. The Boc group was removed with anhydrous TFA (500 μ 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- μ 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 $^{\circ}$ C)] in competition assays using brain P₂ 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 parenthesis and results are mean $\pm$ SE). Unlabeled peptide (2 μ M) was used to determine non-specific binding in the presence of 1.9 nM [3 H]deltorphan II (45.0 Ci/mmol, Perkin Elmer, Boston, MA; K_D = 1.4 nM) for δ -opioid receptors and 3.5 nM [3 H] DAMGO (50.0 Ci/mmol), Amersham Bioscience, Buckinghamshire, U. K.; K_D = 1.5 nM) for μ -opioid receptors. Glass fibre 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 and 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 μ -opioid receptor agonism, and a single mouse vas deferens (MVD) was used to determine δ -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 IC₅₀ (nM) obtained from the dose-response curves. The IC₅₀ values represent the mean $\pm$ SE of five or six separate assays, and the δ -antagonist potencies in the MVD assay were determined against the δ -agonist deltorphan-II, while μ -antagonism (GPI assay) used the μ -agonist endomorphin-2. Antagonism is expressed as pA₂ 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 CO₂ inhalation and then 20- μ 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 [¹⁸F]-**1** (0.37 MBq) for 1 h. After incubation the slices were rinsed three times in PBS, 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 typhoon Trio scanner (Amersham Biosciences, United Kingdom). For a receptor blocking experiments, brain slices were incubated with [¹⁸F]-**1** in the presence of 10 μM 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 [¹⁸F]-**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 2-dimensional ordered-subsets expectation maximum (OSEM) algorithm. No correction was necessary for attenuation and scattering.

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(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*-butoxycarbonyl
DAMGO, [*D*-Ala², *N*-Me-Phe⁴, Gly-ol⁵]enkephalin
DEL C, deltorphin II (H-Tyr-*D*-Ala-Phe-Asp-Val-Val-Gly-NH₂)
DMF, *N,N*-dimethylformamide
DMSO-*d*₆, hexadeuteriodimethyl sulfoxide
Dmt, 2',6'-dimethyl-*L*-tyrosine
DPDPE, (D-Pen², D-Pen⁵)-enkephalin
Et₂O, 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
pA₂, 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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Figure 1.

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

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.

Scheme 1.
Synthesis of compound 1.

Scheme 2.
Synthesis of compound 2.

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

Table 1
Receptor binding affinities and functional bioactivities of compounds **1–2**.

Comp.	Structure	Receptor affinity ^a (nM)		Selectivity	Functional bioactivity	
		K _i ^δ	K _i ^μ	K _i ^μ /K _i ^δ	MVD IC ₅₀ ^b (nM)	GPI IC ₅₀ ^b (nM)
Ref. 1	<i>H</i> -Dmt-Tic- <i>e</i> -Lys(R)-R', ^d					
1	<p><i>R</i> = -NH₂, -NHCO-Ac, -NH-Z</p> <p><i>R</i>' = -CONH-Ph, -CONH-CH₂-Ph, -Bid</p>	0.21–2.64 0.172±0.003 (3)	0.60–3.43 41.2±1.76 (3)	1.7–16.3 240	7.81–8.27 8.25	434–1990 1916±554
Ref. 2	<i>H</i> -Dmt-Tic-Phe-Lys(Z)-OH ^e					
2		0.019 0.248±0.019 (3)	2.75 13.2±1.13 (3)	145 53	11.43 9.45	>10000 50.6±6.9

 μ selectivity K_1^δ/K_1^μ .^{*}

^aThe K_1 values (nM) were determined according to Cheng and Prusoff.³³ The mean $\pm$ SE (n repetitions in parenthesis) is based on independent duplicate binding assays with five to eight peptide doses using several different synaptosomal preparations.

^bAgonist activity was expressed as IC₅₀ obtained from dose-response curves representing the mean $\pm$ SE for at least five to six fresh GPI tissue samples. Deltorphin II and endomorphin-2 were the internal standards for MVD (δ -opioid receptor bioactivity) and GPI (μ -opioid receptor bioactivity) in the tissue preparations, respectively.

^cThe pA₂ values of opioid antagonists were determined against the agonists deltorphin II and endomorphin-2 according to the method of Kosterlitz and Watt.³⁶

^dData taken from Balboni et al.²²

^eData taken from Balboni et al.²³