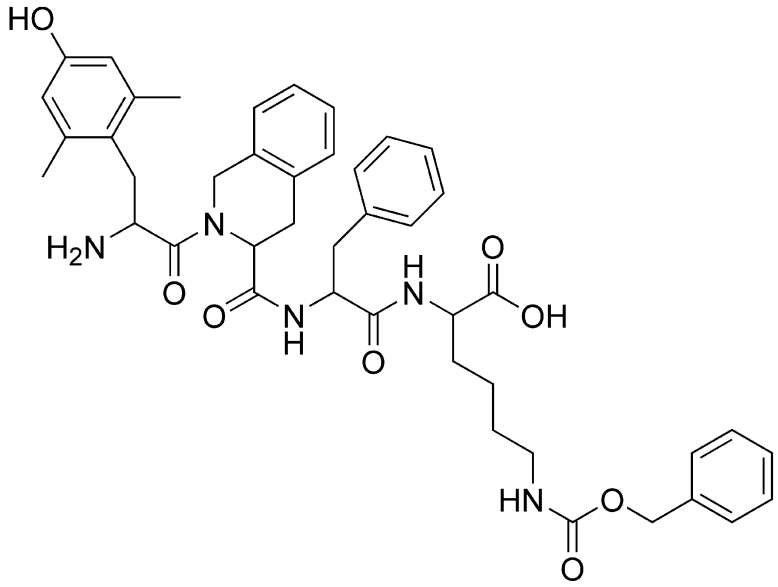
Journal of Medicinal Chemistry

Brief Article

From the Potent and Selective δ Opioid Receptor Agonist H-Dmt-d-Arg-Phe-Lys-NH to the Potent δ Antagonist H-Dmt-Tic-Phe-Lys(Z)-OH

Gianfranco Balboni, Maria Teresa Cocco, Severo Salvadori, Romeo Romagnoli, Yusuke Sasaki, Yoshio Okada, Sharon D. Bryant, Yunden Jinsmaa, and Lawrence H. Lazarus

J. Med. Chem., **2005**, 48 (17), 5608-5611• DOI: 10.1021/jm0504959 • Publication Date (Web): 30 July 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

From the Potent and Selective μ Opioid Receptor Agonist H-Dmt-D-Arg-Phe-Lys-NH_2 to the Potent δ Antagonist H-Dmt-Tic-Phe-Lys(Z)-OH

Gianfranco Balboni,*[,#,†] Maria Teresa Cocco,[#] Severo Salvadori,[†] Romeo Romagnoli,[†] Yusuke Sasaki,[‡] Yoshio Okada,[||] Sharon D. Bryant,[§] Yunden Jinsmaa,[§] and Lawrence H. Lazarus[§]

Department of Toxicology, University of Cagliari, I-09124, Cagliari, Italy, Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy, Tohoku Pharmaceutical University, 4-1, Komatsushima 4-chome, Aoba-Ku, Sendai 981-8558, Japan, Department of Medicinal Chemistry, Faculty of Pharmaceutical Science and High Tecnology Research Center, Kobe Gakuin University, Nishi-ku, Kobe 651-2180, Japan, and Medicinal Chemistry Group, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Received May 26, 2005

H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]DALDA) binds with high affinity and selectivity to the μ opioid receptor and is a potent and long-acting analgesic. Substitution of D-Arg in position 2 with Tic and masking of the lysine amine side chain by Z protection and of the C-terminal carboxylic function instead of the amide function transform a potent and selective μ agonist into a potent and selective δ antagonist H-Dmt-Tic-Phe-Lys(Z)-OH. Such a δ antagonist could be used as a pharmacological tool.

The development of tolerance and physical dependence induced by chronic morphine administration limits its prolonged use in the treatment of pain.[1] Analgesia and tolerance to morphine are abolished in μ opioid receptor knock-out mice, implicating the μ opioid receptor as the primary receptor type mediating both of these effects.[2−4] However, several lines of evidence suggest the additional involvement of the δ opioid receptor in morphine tolerance. Initial studies using δ opioid receptor antagonists[5] and more recent studies using δ opioid receptor knock-out mice[6] were shown to disrupt the development of tolerance. In pharmacological studies, the selective δ receptor antagonist naltridole has been shown to interact with alternative receptors because naltridole binding was still detected in the $\mu/\delta/\kappa$ triple knock-out mice.[7] Furthermore, at high concentrations, NTI has been shown to lose its δ selectivity and act as an agonist in some cell types.[8]

The N-terminal dimethylation of opioid peptides containing the Dmt-Tic pharmacophore drastically decreases δ opioid receptor agonism while enhancing δ antagonism.[9,10] Fortunately, during an attempt to prepare N-terminal dimethylated analogues of selective μ agonists dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), endomorphin-1 (H-Tyr-Pro-Trp-Phe-NH_2), endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2), and H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]DALDA) (reference compound, Table 1),[11] we found a side chain protected [Dmt[1]]-DALDA (H-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-NH_2) (compound **1**, Table 1) analogue with potent activity on GPI and MVD assays (IC_{50} = 0.509 and 1.69 nM, respectively). Considering the correlation between side chain positive charge masking of D-Arg[2] and Lys[4] and the potency increase for δ receptors (binding and agonist functional bioactivity), we decided to examine thoroughly other modifications with the aim of converting the potent μ agonist [Dmt[1]]DALDA into a potent δ antagonist. In this work, starting from side chain positive charge masking, we also considered the presence of a C-terminal carboxylic group (**2** and **4**, Table 1) and the substitution of D-Arg[2] with Tic (**3** and **4**, Table 1) to enhance the δ opioid agonist activity and reduce μ opioid agonist activity. The substitution of D-Arg[2] with Tic gives peptides containing the Dmt-Tic pharmacophore identified by us as a potent δ opioid antagonist.[12] The same modification (substitution of the second amino acid with Tic) was reported by some of us for Leu-enkephalin and dermorphin[13] to yield quite potent δ antagonists (pA_2 = 6.5−7.5).

All peptides were prepared in a stepwise procedure by standard solution peptide synthesis as outlined in Scheme 1. As an example, in this scheme is reported the synthesis of H-Dmt-Tic-Phe-Lys(Z)-OH (**4**). H-Lys-(Z) OMe or H-Lys(Z)-NH_2 was condensed with Boc-Phe-OH via WSC/HOBt. After Boc N-terminal deprotection with TFA, each derivative was condensed with Boc-D-Arg(NO_2)-OH or Boc-Tic-OH and finally, after Boc deprotection, with Boc-Dmt-OH (WSC/HOBt). Final C-terminal amide compounds were obtained by Boc deprotection with TFA, while C-terminal free carboxylic acid was obtained by hydrolysis of the methyl ester with 1 N NaOH and then TFA treatment. Crude peptides were purified by preparative reversed-phase HPLC.

Receptor Affinity Analysis

Receptor binding and functional bioactivity are reported in Table 1. [Dmt[1]]DALDA, the reference compound, shows high affinity and selectivity for μ receptors. D-Arg[2] and Lys[4] side chain protection with NO_2 and Z, respectively, (**1**) induces an 87-fold increase in δ receptor affinity, while μ receptor affinity remains

* To whom correspondence should be addressed. Phone: +39-532-291-275. Fax: +39-532-291-296. E-mail: gbalboni@unica.it; bbg@unife.it.
[#] University of Cagliari.
[†] University of Ferrara.
[‡] Tohoku Pharmaceutical University.
[||] Kobe Gakuin University.
[§] National Institute of Environmental Health Sciences.

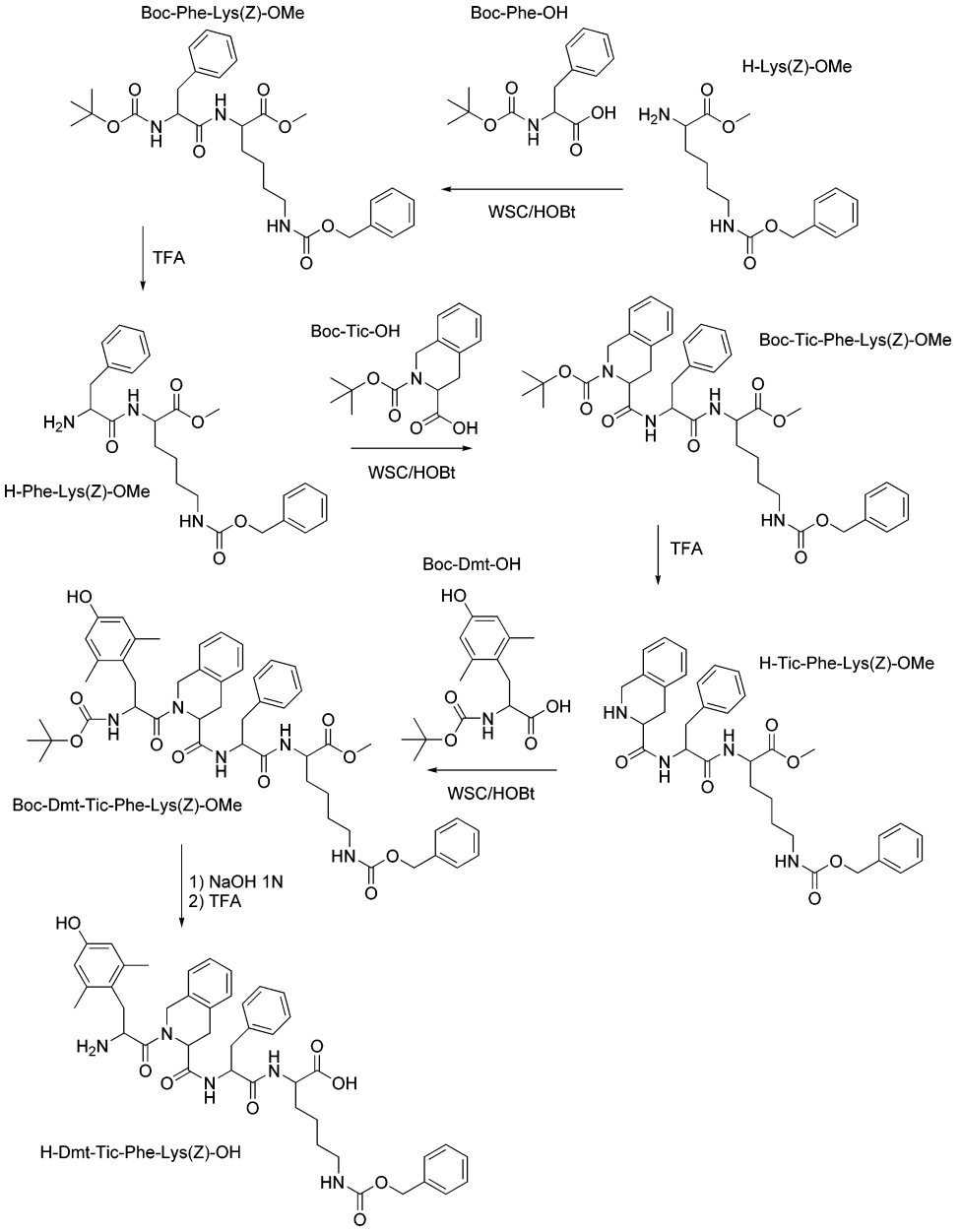
10.1021/jm0504959 CCC: $30.25
Published on Web 07/30/2005

Table 1. Receptor Binding and Functional Bioactivity

		receptor affinity[a]		selectivity		functional bioactivity		
compd	structure	K_i^δ (nM)	K_i^μ (nM)	δ/μ	μ/δ	GPI IC_{50} (nM)[c]	MVD IC_{50} (nM)[c]	MVD pA_2 [b]
	H-Dmt-D-Arg-Phe-Lys-NH_2[d]	2100 ± 310	0.143 ± 0.015	14700		1.41 ± 0.29	23.1 ± 2.0	
1	H-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-NH_2	24.2 ± 1.2 (3)	0.15 ± 0.055 (6)	161		0.509 ± 0.151	1.69 ± 0.26	
2	H-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-OH	47.6 ± 18 (3)	1.45 ± 0.12 (4)	33		63.0 ± 15.5	682 ± 147	
3	H-Dmt-Tic-Phe-Lys(Z)-NH_2	0.23 ± 0.04 (4)	1.14 ± 0.18 (4)		5	1887 ± 887	>10000	8.42
4	H-Dmt-Tic-Phe-Lys(Z)-OH	0.019 ± 0.009 (4)	2.75 ± 0.34 (4)		145	>10000	>10000	11.43

[a] The K_i values were determined according to Chang and Prusoff,[27] as detailed in the Supporting Information. The mean ± SE with n repetitions in parentheses is based on independent duplicate binding assays with five to eight peptide doses using several different synaptosomal preparations. [b] pA_2 is the negative logarithm to the base 10 of the molar concentration of an antagonist that is necessary to double the concentration of agonist needed to elicit the original submaximal response. The antagonist properties of these compounds were tested using deltorphin C (δ opioid receptor agonist) or dermorphin (μ opioid receptor agonist). [c] Agonist activity was expressed as IC_{50} obtained from dose−response curves. These values represent the mean ± SE for at least five fresh tissue samples. Deltorphin C and dermorphin were the internal standards for MVD (δ opioid receptor bioactivity) and GPI (μ opioid receptor bioactivity) tissue preparations, respectively. [d] Data taken from Schiller et al.[11]

Scheme 1. Synthesis of H-Dmt-Tic-Phe-Lys(Z)-OH



unchanged, with a loss of 90-fold selectivity. The substitution of the C-terminal primary amide (**1**) with a carboxylic acid (**2**) causes a small drop in δ affinity (2-fold), μ affinity (10-fold), and selectivity (5-fold). The replacement of D-Arg(NO_2)[2] with Tic (**3**) produces a 106-fold increase of δ binding and an 8-fold decrease of μ binding compared to **1**, giving a compound that is essentially nonselective (μ/δ = 5). The change of C-

terminal amide in **3** with a carboxylic acid gives **4**, endowed with high δ affinity (0.019 nM) with an increase of 12 and more than 100000-fold compared to **3** and [Dmt[1]]DALDA, respectively. The affinity for μ receptors remains in the same order of magnitude for **2** and **3**. Compound **4** is quite selective for δ receptor (μ/δ = 145). Despite the κ receptor affinity reported for [Dmt[1]]DALDA (K_i^κ = 22.3 nM),[11] c**1**−**4** did not show affinity at concentrations up to 10 μM for this receptor.

Functional Bioactivity

Compounds **1**−**4** were tested in the electrically stimulated MVD and GPI assays for intrinsic activity (Table 1). We and other investigators have previously discussed the discrepancy of the correlation between receptor binding affinities and functional bioactivity. Unfortunately, we have neither a definitive nor comprehensive answer for this observation.[12] Our data reveal that all analogues were inactive as antagonists in the GPI assay. Compound **1** shows agonist activities for GPI and MVD comparable to that of the reference compound. The change of the C-terminal amide with the carboxylic function (**2**) reduces the agonist activity for GPI and MVD (120- and 400-fold, respectively) compared to **1**. The substitution of D-Arg(NO_2)[2] with Tic drastically transforms the compound's behavior. In fact, **3** and **4** show interesting δ antagonist activity with pA_2 of 8.42 and 11.43, respectively. At the same time, they demonstrate negligible or no agonist activity for the μ receptor. As expected, the C-terminal amide (**3**) maintains a little μ agonist activity (IC_{50} = 1887 nM) compared to the C-terminal carboxylic acid (**4**).

Starting from the well-known μ opioid agonist tetrapeptide [Dmt[1]]DALDA, it is possible to change its activity from μ agonist to a very potent δ antagonist by applying modifications inducing δ antagonism, such as the following: (1) Masking side chain positive charges decreases μ and κ affinity. (2) Introduction of a carboxylic function, especially at the C-terminal, increases δ selectivity, and most important, (3) introduction of a Tic residue in position 2 gives Tyr/Dmt-Tic pharmacophore derivatives (Dmt induces opioid affinity and potency increase without modification of receptor selectivity).[14] Schiller P. W. et al. reported the synthesis of [Tic[2]]-deltorphin I endowed with δ selective partial agonist activity.[15] The same introduction of Tic in position 2 (combined with the other modifications reported above) transforms [Dmt[1]]DALDA into more potent δ antagonists such as naltridole (K_e = 0.21 nM, pA_2 = 9.68),[16] TIPP[Ψ] (K_e = 2.89 nM, pA_2 = 8.54),[17] N(Me)$_2$Dmt-Tic-OH (K_e = 0.28 nM, pA_2 = 9.55),[10] and H-Dmt-Tic-NH-CH_2-Bid(CH_2COOH) (K_e = 0.27 nM, pA_2 = 9.57).[18]

In 1992, Schiller et al. reported a very similar study without knowing the starting point endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2), a potent and selective μ agonist identified in 1997.[19] In fact, the introduction of Tic in position 2 gave H-Tyr-Tic-Phe-Phe-NH_2 (TIPP-NH_2) (GPI, IC_{50} = 1700 nM; MVD, K_e = 18.0 nM, pA_2 = 7.74). The substitution of the C-terminal amide with the carboxylic acid gave the first δ antagonist containing Tic, H-Tyr-Tic-Phe-Phe-OH (TIPP-OH) (K_e = 4.80 nM, pA_2 = 8.32).[20] The introduction of Dmt[1] in place of Tyr transforms TIPP-NH_2 and TIPP-OH into the more potent H-Dmt-Tic-Phe-Phe-NH_2 (DIPP-NH_2) (GPI, IC_{50} = 18.2 nM; MVD, K_e = 0.209 nM, pA_2 = 9.68) and H-Dmt-Tic-Phe-Phe-OH (DIPP-OH) (K_e = 0.196 nM, pA_2 = 9.71).[21] Finally, considering the structures of H-Dmt-Tic-Phe-Phe-OH and H-Dmt-Tic-Phe-Lys(Z)-OH and their δ antagonist potency (K_e = 0.196 nM and K_e = 0.0037 nM), the increase of about 50-fold activity must to be assigned to the protected Lys at the C-terminal position. Further studies are in progress about this position. H-Dmt-Tic-Phe-Lys(Z)-OH is likely to find wide use as a pharmacological tool in opioid research and may also have potential as a therapeutic agent.

Experimental Section

Chemistry. Boc-D-Arg(NO_2)-Phe-Lys(Z)-NH_2. To a solution of Boc-D-Arg(NO_2)-OH (0.13 g, 0.40 mmol) and TFA·H-Phe-Lys(Z)-NH_2[22] (0.22 g, 0.40 mmol) in DMF (10 mL) at 0 °C were added NMM (0.04 mL, 0.40 mmol), HOBt (0.07 g, 0.44 mmol), and WSC (0.08 g, 0.44 mmol). The mixture was stirred for 3 h at 0 °C and for 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 by Na_2SO_4 and then by evaporation. The residue was precipitated from Et_2O/Pe (1:9, v/v): yield 0.27 g (93%); R_f(B) = 0.84; HPLC K' = 6.55; mp 141−143 °C; $[\alpha]^{20}_D$ +28.2; MH^+ 729.

Boc-D-Arg(NO_2)-Phe-Lys(Z)-OMe. Yield 0.32 g (90%); R_f(B) = 0.92; HPLC K′ 7.49; mp 150−152 °C; $[\alpha]^{20}_D$ +25.4°; MH^+ 744.

Boc-Tic-Phe-Lys(Z)-NH_2. Yield 0.48 g (91%); R_f(B) = 0.88; HPLC K' = 7.98; mp 157−159 °C; $[\alpha]^{20}_D$ +29.3°; MH^+ 687.

Boc-Tic-Phe-Lys(Z)-OMe. Yield 0.55 g (94%); R_f(B) = 0.94; HPLC K' = 8.64; mp 141−143 °C; $[\alpha]^{20}_D$ +28.5; MH^+ 702.

TFA·H-D-Arg(NO_2)-Phe-Lys(Z)-NH_2. Boc-D-Arg(NO_2)-Phe-Lys(Z)-NH_2 (0.27 g, 0.37 mmol) was treated with TFA (2 mL) for 30 min at room temperature. Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.27 g (96%); R_f(A) = 0.80; HPLC K' = 5.68; mp 155−157 °C; $[\alpha]^{20}_D$ +30.4; MH^+ 629.

TFA·H-D-Arg(NO_2)-Phe-Lys(Z)-OMe. Yield 0.29 g (95%); R_f(A) = 0.89; HPLC K' = 6.29; mp 162−164 °C; $[\alpha]^{20}_D$ +27.4°; MH^+ 644.

TFA·H-Tic-Phe-Lys(Z)-NH_2. Yield 0.45 g (95%); R_f(A) = 0.84; HPLC K' = 6.11; mp 165−167 °C; $[\alpha]^{20}_D$ +32.7°; MH^+ 587.

TFA·H-Tic-Phe-Lys(Z)-OMe. Yield 0.54 g (96%); R_f(A) = 0.77; HPLC K' = 7.00; mp 153−155 °C; $[\alpha]^{20}_D$ +30.4; MH^+ 602.

Boc-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-NH_2. To a solution of Boc-Dmt-OH (0.10 g, 0.32 mmol) and TFA·H-D-Arg(NO_2)-Phe-Lys(Z)-NH_2 (0.24 g, 0.32 mmol) in DMF (10 mL) at 0 °C were added NMM (0.03 mL, 0.32 mmol), HOBt (0.05 g, 0.35 mmol), and WSC (0.07 g, 0.35 mmol). The mixture was stirred for 3 h at 0 °C and for 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 0.27 g (91%); R_f(B) = 0.75; HPLC K' = 6.57; mp 144−146 °C; $[\alpha]^{20}_D$ +23.1; MH^+ 920.

Boc-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-OMe. Yield 0.32 g (90%); R_f(B) = 0.83; HPLC K' = 7.55; mp 141−143 °C; $[\alpha]^{20}_D$ +20.4; MH^+ 935.

Boc-Dmt-Tic-Phe-Lys(Z)-NH_2. Yield 0.18 g (85%); R_f(B) = 0.84; HPLC K' = 7.74; mp 161−163 °C; $[\alpha]^{20}_D$ +35.6°; MH^+ 878.

Boc-Dmt-Tic-Phe-Lys(Z)-OMe. Yield 0.38 g (83%); R_f(B) = 0.89; HPLC K' = 8.53; mp 154−156 °C; $[\alpha]^{20}_D$ +33.6; MH^+ 893.

Boc-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-OH. To a solution of Boc-Dmt-Tic-Phe-Lys(Z)-OMe (0.17 g, 0.22 mmol) in EtOH (10 mL) at room temperature, 1 N NaOH (0.27 mL, 0.27 mmol) was added. The mixture was stirred for 3 h at room temperature. After EtOH 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 crystallized from Et_2O/Pe (1:9, v/v): yield 0.17 g (87%); R_f(B) = 0.64; HPLC K' = 7.25; mp 154−156 °C; $[\alpha]^{20}_D$ +22.8; MH^+ 921.

Boc-Dmt-Tic-Phe-Lys(Z)-OH. Yield 0.18 g (89%); R_f(B) = 0.68; HPLC K' = 8.03; mp 165−167 °C; $[\alpha]^{20}_D$ +24.1; MH^+ 879.

TFA·H-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-NH_2 (1). Boc-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-NH_2 (0.27 g, 0.29 mmol) was treated with TFA (2 mL) for 30 min at room temperature. Et_2O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.26 g (96%); R_f(A) = 0.64; HPLC K' = 5.17; mp 165−167 °C; $[\alpha]^{20}_D$ +26.7; MH^+ 820; ^{1}H NMR (DMSO) δ 1.29−1.79 (m, 10H), 2.35 (s, 6H), 2.65−3.95 (m, 9H), 4.53−5.34 (m, 5H), 6.29 (s, 2H), 7.08−7.21 (m, 10H).

TFA·H-Dmt-D-Arg(NO_2)-Phe-Lys(Z)-OH (2). Yield 0.15 g (93%); R_f(A) = 0.52; HPLC K' = 6.15; mp 170−172 °C; $[\alpha]^{20}_D$ +27.9°; MH^+ 821; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 10H), 2.35 (s, 6H), 2.65−3.95 (m, 9H), 4.46−5.34 (m, 5H), 6.29 (s, 2H), 7.08−7.21 (m, 10H).

TFA·H-Dmt-Tic-Phe-Lys(Z)-NH_2 (3). Yield 0.18 g (93%); R_f(A) = 0.70; HPLC K' = 6.80; mp 157−159 °C; $[\alpha]^{20}_D$ +35.1°; MH^+ 778; ^{1}H NMR (DMSO-d_6) δ 1.29−1.79 (m, 6H), 2.35 (s, 6H), 2.92−3.95 (m, 9H), 4.41−5.34 (m, 7H), 6.29 (s, 2H), 6.96−7.21 (m, 14H).

TFA·H-Dmt-Tic-Phe-Lys(Z)-OH (4). Boc, yield 0.13 g (95%); R_f(A) = 0.62; HPLC K' = 6.83; mp 164−166 °C; $[\alpha]^{20}_D$ +36.7°; MH^+ 779; ^{1}H NMR (DMSO-d_6) δ 1.29−1.78 (m, 6H), 2.35 (s, 6H), 2.92−3.95 (m, 9H), 4.41−5.34 (m, 7H), 6.29 (s, 2H), 6.96−7.21 (m, 14H).

Supporting Information Available: Experimental details, NMR data, elemental analysis results, and refs 23−29. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) In addition to the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1985**, *260*, 14−42), this paper uses the following symbols and abbreviations: DAMGO, [D-Ala^2,*N*-Me-Phe^4,Glyol^5]enkephalin; Boc, *tert*-butyloxycarbonyl; DELT or deltorphin C, [D-Ala^2]deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2); dermorphin, H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2; DMF, *N*,*N*-dimethylformamide; DMSO-d_6, hexadeuteriodimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; [Dmt^1]DALDA, H-Dmt-D-Arg-Phe-Lys-NH_2; DPDPE, *cyclo*-[D-Pen^{2,5}]enkephalin; endomorphin-1, H-Tyr-Pro-Trp-Phe-NH_2; endomorphin-2, H-Tyr-Pro-Phe-Phe-NH_2; Et_2O, ethyl ether; EtOAc, ethyl acetate; EtOH, ethyl alcohol; 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; pA_2, negative log of the molar concentration required to double the agonist concentration to achieve the original response; Pe, petroleum ether; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TLC, thin-layer chromatography; WSC, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide·HCl; Z, benzyloxycarbonyl.

(2) Fang, F. G.; Fields, H. L.; Lee, N. M. Action at the μ receptor is sufficient to explain the supraspinal analgesic effects of opiates. *J. Pharmacol. Exp. Ther.* **1986**, *238*, 1039−1044.

(3) Matthes, H. W.; Maldonado, R.; Simonin, F.; Valverde, O.; Slowe, S.; Kitchen, I.; Befort, K.; Dierich, A.; Le Meur, M.; Dolle, P.; Tzavara, E.; Hanoune, J.; Roques, B. P.; Kieffer, B. L. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. *Nature* **1996**, *383*, 819−823.

(4) Sora, I.; Funada, M.; Uhl, G. R. The μ-opioid receptor is necessary for [D-Pen^2,D-Pen^5]enkephalin-induced analgesia. *Eur. J. Pharmacol.* **1997**, *324*, R1−R2.

(5) Abdelhamid, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. Selective blockage of δ opioid receptors prevents the development of morphine tolerance and dependence in mice. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 299−303.

(6) Nitsche, F.; Schuller, A. G.; King, M. A.; Zengh, M.; Pasternak, G. W.; Pintar, J. E. Genetic dissociation of opiate tolerance and physical dependence in δ-opioid receptor-1 and preproenkephalin knock-out mice. *J. Neurosci.* **2002**, *22*, 10906−10916.

(7) Gaveriaux-Ruff, C.; Filliol, D.; Simonin, F.; Matthes, H. W.; Kieffer, B. L. Immunosuppression by δ-opioid antagonist NTI: δ- and triple $\mu/\delta/\kappa$ opioid receptor knockout mice reveal a nonopioid activity. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 1193−1198.

(8) Chen, Y. L.; Law, P. Y.; Loh, H. H. Inhibition of akt/protein kinase B signalling by naltrindole in small cell lung cancer cells. *Cancer Res.* **2004**, *64*, 8723−8730.

(9) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Bryant, S. D.; Jinsmaa, Y.; Lazarus, L. H. Synthesis and opioid activity of *N*,*N*-dimethyl-Dmt-Tic-NH-CH(R)-R′ analogues: acquisition of potent δ antagonism. *Bioorg. Med. Chem.* **2003**, *11*, 5435−5441.

(10) Salvadori, S.; Balboni, G.; Gerrini, R.; Tomatis, R.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. Evolution of the Dmt-Tic pharmacophore: N-terminal methylated derivatives with extraordinary δ opioid antagonist activity. *J. Med. Chem.* **1997**, *40*, 3100−3108.

(11) Schiller, P. W.; Nguyen, T. M.-D.; Berezowska, I.; Dupuis, S.; Weltrowska, G.; Chung, Nga N.; Lemieux, C. Synthesis and in vitro opioid activity profiles of DALDA analogues. *Eur. J. Med. Chem.* **2000**, *35*, 895−901.

(12) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Bryant, S. D.; Jinsmaa, Y.; Lazarus, L. H. Direct influence of C-terminally substituted amino acids in the Dmt-Tic pharmacophore on δ-opioid receptor selectivity and antagonism. *J. Med. Chem.* **2004**, *47*, 4066−4071 and references cited herein.

(13) Tancredi, T.; Salvadori, S.; Amodeo, P.; Picone, D.; Lazarus, L. H.; Bryant, S. D.; Guerrini, R.; Marzola, G.; Temussi, P. A. Conversion of enkephalin and dermorphin into δ-selective opioid antagonists by single-residue substitution. *Eur. J. Biochem.* **1994**, *224*, 241−247.

(14) Bryant, S. D.; Jinsmaa, Y.; Salvadori, S.; Okada, Y.; Lazarus, L. H. Dmt and opioid peptides: a potent alliance. *Biopolymers* **2003**, *71*, 86−102.

(15) Schiller, P. W.; Weltrowska, G.; Nguyen, T. M.-D.; Wilkes, B. C.; Chung, N. N.; Lemieux, C. Conformationally restricted deltorphin analogues. *J. Med. Chem.* **1992**, *35*, 3956−3961.

(16) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. Application of the message−address concept in the design of highly potent and selective non-peptide δ opioid receptor antagonists. *J. Med. Chem.* **1988**, *31*, 281−282.

(17) Schiller, P. W.; Weltrowska, G.; Nguyen, T. M.-D.; Wilkes, B. C.; Chung, N. N.; Lemieux, C. TIPP[Ψ]: a highly potent and stable pseudopeptide δ opioid receptor antagonist with extraordinary δ selectivity. *J. Med. Chem.* **1992**, *36*, 3182−3187.

(18) Balboni, G.; Salvadori, S.; Guerrini, R.; Negri, L.; Giannini, E.; Jinsmaa, Y.; Bryant, S. D.; Lazarus, L. H. Potent δ-opioid receptor agonists containing the Dmt-Tic pharmacophore. *J. Med. Chem.* **2002**, *45*, 5556−5563.

(19) Zadina, J. E.; Hackler, L.; Ge, L.-I.; Kastin, A. J. A potent and selective endogenous agonist for the μ-opiate receptor. *Nature* **1997**, *386*, 499−502.

(20) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Wilkes, B. C.; Marsden, B. J.; Lemieux, C.; Chung, N. N. Differential stereochemical requirements of μ vs δ opioid receptors for ligand binding and signal transduction: development of a class of potent and highly δ-selective peptide antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11871−11875.

(21) Schiller, P. W.; Weltrowska, G.; Berezowska, I.; Nguyen, T. M.-D.; Wilkes, B. C.; Lemieux, C.; Chung, N. N. The TIPP opioid peptide family: development of δ antagonists, δ agonists, and mixed μ agonist/δ antagonists. *Biopolymers* **1999**, *51*, 411−425.

(22) Salvadori, S.; Marastoni, M.; Balboni, G.; Tomatis, R.; Borea, P. A. Opioid peptides. Synthesis and binding assays of desamino-Tyr^1 dermorphin analogues. *Farmaco, Ed. Sci.* **1987**, *42*, 931−940.

JM0504959