Published in final edited form as:

J Med Chem. 2006 September 7; 49(18): 5610–5617. doi:10.1021/jm060741w.

Effect of Lysine at C-Terminus of the Dmt-Tic Opioid Pharmacophore

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Abstract

Substitution of Gly with side-chain protected or unprotected Lys in lead compounds containing the opioid pharmacophore Dmt-Tic [H-Dmt-Tic-Gly-NH-CH₂-Ph, μ agonist / δ antagonist; H-Dmt-Tic-Gly-NH-Ph, μ agonist / δ agonist and H-Dmt-Tic-NH-CH₂-Bid, δ agonist (Bid = 1*H*-benzimidazole-2-yl)] yielded a new series of compounds endowed with distinct pharmacological activities. Compounds (**1–10**) included high δ - ($K_1^\delta = 0.068$ – 0.64 nM) and μ -opioid affinities ($K_1^\mu = 0.13$ – 5.50 nM) with a bioactivity that ranged from μ -opioid agonism [**10**, H-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid (IC₅₀ GPI = 39.7 nM)] to a selective μ -opioid antagonist [**3**, H-Dmt-Tic-Lys-NH-CH₂-Ph (pA₂ ^{μ} = 7.96)] and a selective δ -opioid antagonist [**5**, H-Dmt-Tic-Lys(Ac)-NH-Ph (pA₂ ^{δ} = 12.0)]. The presence of a Lys linker provides new lead compounds in the formation of opioid peptidomimetics containing the Dmt-Tic pharmacophore with distinct agonist and / or antagonist properties.

Introduction

The prototype δ -opioid receptor antagonist, H-Dmt-Tic-OH,^{1–3} which evolved from H-Tyr-Tic-OH,⁴ as a simplified form of TIP(P),⁵ represents the minimal sequence that selectively interacts with δ -opioid receptors as a potent δ -opioid antagonist. This dipeptide underwent extensive modifications by rationale drug design principles and structure-activity studies,^{6, 7} which included *N*-terminal modification with alkyl substitutions;^{3, 8} replacement of Tic by heteroaliphatic, heteroaromatic nuclei,⁹ or *p*-Phe;¹⁰ alteration of the C-terminus of Tic by substituents containing a hydrophobic groups;³ and addition of a third aromatic center with or without inserting interposing linkers.^{11–13} Many of the analogues had unique properties, including enhanced δ -opioid antagonism,^{3, 6–13} conversion from a δ -opioid antagonist to a δ -opioid agonist and vice versa,^{11, 14} mixed μ agonism / δ antagonism,^{3, 8, 11} or development of irreversible fluorescent δ -opioid antagonists.¹³ From these and other studies it was concluded that small, discrete and subtle modifications can drastically change the pharmacological profile in molecules related to the Dmt-Tic pharmacophore.^{15–17}

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Rationale

Our recent efforts focused attention on the presence of side chain protected Lys in the Dmt-Tic pharmacophore. The substitution of the third amino acid in H-Dmt-Tic-Xaa peptides with H-Lys(Ac)-OH, gave the most potent δ antagonist of the series [H-Dmt-Tic-Lys(Ac)-OH; $pA_2^{\delta} = 10.07$].¹⁸ Substitution of the C-Terminal Phe with H-Lys(Z)-OH in H-Dmt-Tic-Phe-Phe-OH [DIPP-OH; pA_2 (MVD) = 9.71] resulted an increase of about 50-fold in δ -opioid antagonist activity [H-Dmt-Tic-Phe-Lys(Z)-OH; $pA_2 = 11.43$].¹⁹ Starting from these considerations we introduced the side-chain protected or unprotected Lys into other peptides containing the Dmt-Tic pharmacophore: H-Dmt-Tic-Gly-NH-Ph (μ agonist / δ agonist), H-Dmt-Tic-Gly-NH-CH₂-Ph (μ agonist / δ antagonist) and H-Dmt-Tic-NH-CH₂-Bid (δ agonist) were selected as reference compounds. Compounds exhibiting pharmacological properties of μ agonism/ δ agonism could be interesting clinical analgesics which could have a low dependence for chronic use for the amelioration of pain.²⁰ Opioid ligands with a mixed μ agonist / δ antagonist activity profile may have diminished propensity to induce tolerance and therefore may have therapeutic advantages over μ agonist analgesics for long term treatment of pain.^{21, 22} δ opioid receptor agonists, such as H-Dmt-Tic-NH-CH₂-Bid and H-Dmt-Tic-NH-CH(CH₂-COOH)-Bid, are attractive as potential analgesics, since δ opioid agonists exhibit strong antinociceptive activity with relatively few side effects.²³ Furthermore, δ opioid receptor agonists produce antidepressant-like and anxiolytic-like effects and regulate BDNF mRNA expression in rodents,^{24, 25} such that the regulation of BDNF mRNA expression could be useful in the treatment of multiple sclerosis and related diseases.²⁶ Moreover, δ -opioid receptor activation protects cortical neurons, producing hibernation and neuroprotection.²⁷⁻²⁹ Activation of δ and κ opioid receptors affords cardioprotection.³⁰

Chemistry

Peptides (**1-6**) and pseudopeptides (**7-10**) were prepared stepwise by solution peptide synthetic methods as outlined in Schemes 1 and 2, respectively. Boc-Lys(Z)-OH or Boc-Lys(Ac)-OH was condensed with benzylamine or aniline via WSC/HOBt. After N-terminal Boc deprotection with TFA, Lys side-chain protected amides were condensed with Boc-Tic-OH via WSC/HOBt. N-terminal Boc protected dipeptide amides were treated with TFA and condensed with Boc-Dmt-OH via WSC/HOBt. Final N-terminal Boc deprotection with TFA gave compounds (**1, 2, 4** and **5**) (Scheme 1). Catalytic hydrogenation (5% Pd/C) and TFA treatment of Boc-Dmt-Tic-Lys(Z)-amides gave the final products **3** and **6** (Scheme 1). Pseudopeptides (**7-10**) containing C-terminal 1*H*-benzimidazol-2-yl were synthesized in a similar manner. Mixed carbonic anhydride coupling of Boc-Lys(Z)-OH or Boc-D-Lys(Z)-OH or Boc-Lys(Ac)-OH with *o*-phenyldiamine gave the corresponding crude intermediate monoamides, which were converted without purification to the desired heteroaromatic derivatives by cyclization and dehydration in acetic acid as outlined in Scheme 2. As detailed in Scheme 1, after N $^{\alpha}$ -deprotection with TFA, each derivative was condensed with Boc-Tic-OH and then with Boc-Dmt-OH via WSC/HOBt. Final N-terminal Boc deprotection with TFA gave compounds (**7-9**) (Table 1). Catalytic hydrogenation (5% Pd/C) and TFA treatment of Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid gave the final product **10** (Table 1). Final compounds (**1-10**) were purified by preparative HPLC.

Results and Discussion

Receptor Affinity Analysis

Receptor binding and functional bioactivities are reported in Table 1. All new compounds (**1-10**) had subnanomolar affinity for δ -opioid receptors ($K_i^{\delta} = 0.068$ - 0.64 nM). As expected, the lack of a free carboxylic function induces an increase in the μ -receptor affinity ($K_i^{\mu} = 0.13$ - 5.50 nM).^{18, 31} In general, none of these compounds (**1-10**) are highly receptor selective;

however, the benzyl amides (**1-3**) and phenyl amides (**4-6**) were slightly more selective for δ receptors, and **7-10** containing Bid at the C-terminus exhibited a modest selectivity for μ -opioid receptors. Selectivity (K_i^μ / K_i^δ) of pseudopeptides containing Bid at the C-terminus depends on the side-chain of the amino acid transformed in Bid. In fact, selectivity decreased from 122 in H-Dmt-Tic-NH-CH(CH₂-COOH)-Bid (carboxylic function) to 14 in H-Dmt-Tic-NH-CH₂-Bid (no side chain) and further declined to 0.33 ($K_i^\delta / K_i^\mu = 3.1$) in **10** (H-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid, with an amine function). The functionalization of the C-terminal carboxylic acid in H-Dmt-Tic-Lys(Ac)-OH with benzylamine (**2**), aniline (**5**) and Bid (**9**) caused a highly significant drop in selectivity by several orders of magnitude (276-, 4472- and 31057-fold, respectively).

Functional Bioactivity

Compounds (**1-10**) were tested in the electrically stimulated MVD and GPI assays for intrinsic functional bioactivity (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 definitive nor comprehensive answers for these observations.¹⁸ Our data reveal that all of the analogues were inactive as δ opioid agonists in the MVD assay. Substitution of side chain protected or unprotected Lys in the δ agonists [H-Dmt-Tic-NH-CH₂-Bid and H-Dmt-Tic-NH-CH(CH₂-COOH)-Bid] and δ agonist / μ agonist (H-Dmt-Tic-Gly-NH-Ph) reference compounds caused a complete loss of δ agonist activity. The new compounds containing Bid at the C-terminus (**7-10**) show μ opioid agonism in the same order of magnitude as the endogenous μ agonist endomorphin-2.³² The Lys side-chain (unprotected or protected as an acetyl) in place of the Asp side-chain is able to transform a selective δ agonist into selective μ agonists (**7-10**). Unexpectedly, and contrary to our preceding results,³¹ the stereochemistry of Lys seems to be quite important; in fact, H-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid is 6-fold less active than the corresponding diastereoisomer containing *D*-Lys (**8**). Among the C-terminal phenyl amide compounds (**4-6**) having μ agonist activity in the μ M range, only H-Dmt-Tic-Lys(Ac)-NH-Ph (**5**) is an very potent and selective δ antagonist (GPI, IC₅₀ = 1248 nM; MVD, pA₂ = 12.0). Among C-terminal benzyl amide derivatives, **1** is almost inactive as an agonist or an antagonist; H-Dmt-Tic-Lys(Ac)-NH-CH₂-Ph is a nonselective δ and μ antagonist (MVD pA₂ = 10.4; GPI pA₂ = 8.16). Finally, **3** (H-Dmt-Tic-Lys-NH-CH₂-Ph) shows an interesting selective μ antagonist bioactivity (GPI, pA₂ = 7.96).

Conclusions

In the light of the objectives of this study, we evaluated the possibility to improve the potency of some reference opioid compounds (δ agonists, μ agonist / δ agonist and μ agonist / δ antagonist) through the substitution of Gly with a side-chain unprotected or protected (Z or Ac) Lys. Quite surprisingly, as seen in Table 1, while none of the new compounds confirmed our hypothesis (substitution of Gly with Lys is could be able to improve potency), considerably more interesting results were obtained. Starting from the prototypic δ selective antagonist pharmacophore (Dmt-Tic) in our previous studies, we were able to transform this pharmacophore into selective δ agonists and vice versa, non-selective δ agonists/ μ agonists and non-selective μ agonists / δ antagonists. The introduction of a C-terminal Lys residue further increased the versatility of this pharmacophore; in fact, we now obtained μ -selective agonists H-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid (GPI; IC₅₀ = 39.7 nM), and a μ -selective antagonist H-Dmt-Tic-Lys-NH-CH₂-Ph (GPI; pA₂ = 7.96) endowed with a potency comparable to the reference μ antagonists CTOP [*D*-Phe-c(Cys-Tyr-*D*-Trp-Orn-Thr-Pen)-Thr-NH₂] and CTAP [*D*-Phe-c(Cys-Tyr-*D*-Trp-Arg-Thr-Pen)-Thr-NH₂].³³ The μ -opioid antagonist H-Dmt-Tic-Lys-NH-CH₂-Ph could find wide use as a pharmacological tool in opioid research and may also have potential as a therapeutic agent as in the regulation of food intake³⁴ and in the treatment of alcoholism.³⁵

Experimental Section

Peptide Synthesis

Boc-Lys(Z)-NH-CH₂-Ph.⁴²—To a solution of Boc-Lys(Z)-OH (0.23 g, 0.62 mmol) and benzylamine (0.07 mL, 0.62 mmol) in DMF (10 mL) at 0 °C, HOBt (0.10 g, 0.68 mmol), and WSC (0.13 g, 0.68 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 0.26 g (89%); *R_f*(B) 0.94; HPLC *K'* 5.55; mp 93-95 °C; [α]_D²⁰ -12.1; *m/z* 471 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.96 (t, 2H), 4.46-4.53 (m, 3H), 5.34 (s, 2H), 7.06-7.19 (m, 10H).

TFA-H-Lys(Z)-NH-CH₂-Ph—Boc-Lys(Z)-NH-CH₂-Ph (0.20 g, 0.43 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et₂O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.16 g (98%); *R_f*(A) 0.77; HPLC *K'* 3.55; mp 112-114 °C; [α]_D²⁰ -13.4; *m/z* 371 (M+H)⁺.

Boc-Tic-Lys(Z)-NH-CH₂-Ph—To a solution of Boc-Tic-OH (0.13 g, 0.46 mmol) and TFA-H-Lys(Z)-NH-CH₂-Ph (0.22 g, 0.46 mmol) in DMF (10 mL) at 0 °C, NMM (0.05 mL, 0.46 mmol), HOBt (0.07 g, 0.51 mmol), and WSC (0.09 g, 0.51 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 0.23 g (80%); *R_f*(B) 0.82; HPLC *K'* 5.61; mp 105-107 °C; [α]_D²⁰ -18.2; *m/z* 630 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.92-3.17 (m, 4H), 4.17-4.53 (m, 5H), 4.92-5.34 (m, 3H), 6.96-7.19 (m, 14H).

TFA-H-Tic-Lys(Z)-NH-CH₂-Ph—Boc-Tic-Lys(Z)-NH-CH₂-Ph (0.17 g, 0.27 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et₂O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.16 g (96%); *R_f*(A) 0.49; HPLC *K'* 4.28; mp 118-120 °C; [α]_D²⁰ -20.3; *m/z* 530 (M+H)⁺.

Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph—To a solution of Boc-Dmt-OH (0.10 g, 0.32 mmol) and TFA-H-Tic-Lys(Z)-NH-CH₂-Ph (0.21 g, 0.32 mmol) in DMF (10 mL) at 0 °C, NMM (0.03 mL, 0.32 mmol), HOBt (0.05 g, 0.35 mmol), and WSC (0.07 g, 0.35 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 0.25 g (95%); *R_f*(B) 0.78; HPLC *K'* 5.41; mp 132-134 °C; [α]_D²⁰ -17.1; *m/z* 821 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.35 (s, 6H), 2.92-3.17 (m, 6H), 4.41-4.53 (m, 5H), 4.92-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.19 (m, 14H).

TFA-H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph (1)—Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph (0.19 g, 0.23 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et₂O/Pe (1:1, v/v) were added to the solution until the product precipitated: yield 0.16 g (96%); *R_f*(A) 0.45; HPLC *K'* 4.81; mp 128-130 °C; [α]_D²⁰ -15.1; *m/z* 721 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 6H), 2.35 (s, 6H), 2.92-3.17 (m, 6H), 3.95-4.53 (m, 4H), 4.92-5.34 (m, 3H), 6.29 (s, 2H), 6.96-7.19 (m, 14H).

Boc-Lys(Ac)-NH-CH₂-Ph—This compound was obtained by condensation of Boc-Lys(Ac)-OH with benzylamine via WSC/HOBt as reported for Boc-Lys(Z)-NH-CH₂-Ph: yield 0.32 g (82%); *R_f*(B) 0.88; HPLC *K'* 3.76; mp 101-103 °C; $[\alpha]^{20}_{\text{D}}$ -13.0; *m/z* 379 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.02 (s, 3H), 3.20-4.53 (m, 5H), 7.06-7.14 (m, 5H).

TFA·H-Lys(Ac)-NH-CH₂-Ph—Boc-Lys(Ac)-NH-CH₂-Ph was treated with TFA as reported for TFA·H-Lys(Z)-NH-CH₂-Ph: yield 0.19 g (98%); *R_f*(A) 0.74; HPLC *K'* 2.14; mp 120-122 °C; $[\alpha]^{20}_{\text{D}}$ -14.3; *m/z* 279 (M+H)⁺.

Boc-Tic-Lys(Ac)-NH-CH₂-Ph—This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Ac)-NH-CH₂-Ph via WSC/HOBt as reported for Boc-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.46 g (83%); *R_f*(B) 0.76; HPLC *K'* 5.02; mp 111-113 °C; $[\alpha]^{20}_{\text{D}}$ -19.1; *m/z* 537 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.02 (s, 3H), 3.05-3.20 (m, 4H), 4.22-4.92 (m, 7H), 4.92-5.34 (m, 3H), 6.96-7.14 (m, 9H).

TFA·H-Tic-Lys(Ac)-NH-CH₂-Ph—Boc-Tic-Lys(Ac)-NH-CH₂-Ph was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.32 g (98%); *R_f*(A) 0.46; HPLC *K'* 3.02; mp 124-126 °C; $[\alpha]^{20}_{\text{D}}$ -21.2; *m/z* 437 (M+H)⁺.

Boc-Dmt-Tic-Lys(Ac)-NH-CH₂-Ph—This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Ac)-NH-CH₂-Ph via WSC/HOBt as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.15 g (85%); *R_f*(B) 0.72; HPLC *K'* 4.94; mp 138-140 °C; $[\alpha]^{20}_{\text{D}}$ -18.0; *m/z* 729 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05-3.20 (m, 6H), 4.46-4.53 (m, 5H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96-7.14 (m, 9H).

TFA·H-Dmt-Tic-Lys(Ac)-NH-CH₂-Ph (2)—Boc-Dmt-Tic-Lys(Ac)-NH-CH₂-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.07 g (95%); *R_f*(A) 0.42; HPLC *K'* 3.62; mp 134-136 °C; $[\alpha]^{20}_{\text{D}}$ -16.0; *m/z* 629 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05-3.20 (m, 6H), 3.95-4.53 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.14 (m, 9H).

Boc-Dmt-Tic-Lys-NH-CH₂-Ph—To a solution of Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph (0.10 g, 0.12 mmol) in methanol (30 mL) was added Pd/C (10%, 0.07 g), and H₂ was bubbled for 1 h at room temperature. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et₂O/Pe (1:9, v/v): yield 0.08 g (96%); *R_f*(B) 0.65; HPLC *K'* 4.99; mp 141-143 °C; $[\alpha]^{20}_{\text{D}}$ -18.3; *m/z* 687 (M+H)⁺.

2TFA·H-Dmt-Tic-Lys-NH-CH₂-Ph (3)—Boc-Dmt-Tic-Lys-NH-CH₂-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.07 g (95%); *R_f*(A) 0.39; HPLC *K'* 3.32; mp 147-149 °C; $[\alpha]^{20}_{\text{D}}$ -16.2; *m/z* 587 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 6H), 2.35 (s, 6H), 2.65-3.17 (m, 6H), 3.95-4.53 (m, 6H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.14 (m, 9H).

Boc-Lys(Z)-NH-Ph—This compound was obtained by condensation of Boc-Lys(Z)-OH with aniline via WSC/HOBt as reported for Boc-Lys(Z)-NH-CH₂-Ph: yield 0.23 g (82%); *R_f*(B) 0.89; HPLC *K'* 5.15; mp 90-92 °C; $[\alpha]^{20}_{\text{D}}$ -15.2; *m/z* 456 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 15H), 2.96 (t, 2H), 4.53-5.34 (m, 3H), 7.00-7.64 (m, 10H).

TFA·H-Lys(Z)-NH-Ph—Boc-Lys(Z)-NH-Ph was treated with TFA as reported for TFA·H-Lys(Z)-NH-CH₂-Ph: yield 0.13 g (97%); *R_f*(A) 0.65; HPLC *K'* 3.63; mp 110-120 °C; $[\alpha]^{20}_{\text{D}}$ -15.9; *m/z* 356 (M+H)⁺.

Boc-Tic-Lys(Z)-NH-Ph—This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Z)-NH-Ph via WSC/HOBt as reported for Boc-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.26 g (88%); *R_f*(B) 0.77; HPLC *K'* 5.47; mp 97-99 °C; [α]²⁰_D -19.5; *m/z* 616 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.79 (m, 15H), 2.92-3.17 (m, 4H), 4.17-4.53 (m, 2H), 4.92-5.34 (m, 3H), 6.96-7.19 (m, 14H).

TFA·H-Tic-Lys(Z)-NH-Ph—Boc-Tic-Lys(Z)-NH-Ph was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.17 g (98%); *R_f*(A) 0.44; HPLC *K'* 4.37; mp 114-116 °C; [α]²⁰_D -20.4; *m/z* 516 (M+H)⁺.

Boc-Dmt-Tic-Lys(Z)-NH-Ph—This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Z)-NH-Ph via WSC/HOBt as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.22 g (84%); *R_f*(B) 0.73; HPLC *K'* 5.44; mp 127-129 °C; [α]²⁰_D -16.4; *m/z* 807 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 15H), 2.35 (s, 6H), 2.92-3.17 (m, 6H), 4.41-4.53 (m, 3H), 4.92-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.64 (m, 14H).

TFA·H-Dmt-Tic-Lys(Z)-NH-Ph (4)—Boc-Dmt-Tic-Lys(Z)-NH-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.13 g (93%); *R_f*(A) 0.38; HPLC *K'* 4.70; mp 124-126 °C; [α]²⁰_D -14.4; *m/z* 707 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 6H), 2.35 (s, 6H), 2.96-3.05 (m, 4H), 3.95-4.53 (m, 4H), 4.92-5.34 (m, 3H), 6.29 (s, 2H), 6.96-7.64 (m, 14H).

Boc-Lys(Ac)-NH-Ph—This compound was obtained by condensation of Boc-Lys(Ac)-OH with aniline via WSC/HOBt as reported for Boc-Lys(Z)-NH-CH₂-Ph: yield 0.35 g (92%); *R_f*(B) 0.83; HPLC *K'* 3.85; mp 96-98 °C; [α]²⁰_D -16.1; *m/z* 365 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 15H), 2.02 (s, 3H), 3.20-4.53 (m, 3H), 7.00-7.64 (m, 5H).

TFA·H-Lys(Ac)-NH-Ph—Boc-Lys(Ac)-NH-Ph was treated with TFA as reported for TFA·H-Lys(Z)-NH-CH₂-Ph: yield 0.21 g (98%); *R_f*(A) 0.62; HPLC *K'* 2.31; mp 116-118 °C; [α]²⁰_D -16.8; *m/z* 265 (M+H)⁺.

Boc-Tic-Lys(Ac)-NH-Ph—This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Lys(Ac)-NH-Ph via WSC/HOBt as reported for Boc-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.45 g (83%); *R_f*(B) 0.71; HPLC *K'* 4.86; mp 103-105 °C; [α]²⁰_D -20.4; *m/z* 523 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 15H), 2.02 (s, 3H), 3.05-3.20 (m, 4H), 4.22-4.92 (m, 5H), 6.96-7.64 (m, 9H).

TFA·H-Tic-Lys(Ac)-NH-Ph—Boc-Tic-Lys(Ac)-NH-Ph was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.30 g (96%); *R_f*(A) 0.41; HPLC *K'* 3.28; mp 120-122 °C; [α]²⁰_D -21.3; *m/z* 423 (M+H)⁺.

Boc-Dmt-Tic-Lys(Ac)-NH-Ph—This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Lys(Ac)-NH-Ph via WSC/HOBt as reported for Boc-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.14 g (80%); *R_f*(B) 0.67; HPLC *K'* 4.25; mp 133-135 °C; [α]²⁰_D -17.3; *m/z* 715 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05-3.20 (m, 6H), 4.46-4.53 (m, 3H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96-7.64 (m, 9H).

TFA·H-Dmt-Tic-Lys(Ac)-NH-Ph (5)—Boc-Dmt-Tic-Lys(Ac)-NH-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.07 g (98%); *R_f*(A) 0.35; HPLC *K'* 3.73; mp 130-132 °C; [α]²⁰_D -145.3; *m/z* 615 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05-3.20 (m, 6H), 3.95-4.53 (m, 4H), 4.92 (m, 3H), 6.29 (s, 2H), 6.96-7.64 (m, 9H).

Boc-Dmt-Tic-Lys-NH-Ph—Boc-Dmt-Tic-Lys(Z)-NH-Ph was treated with H₂ in presence of Pd/C 10% as reported for Boc-Dmt-Tic-Lys-NH-CH₂-Ph: yield 0.18 g (94%); *R*_f(B) 0.64; HPLC *K'* 4.71; mp 138-140 °C; [α]_D²⁰ -17.8; *m/z* 673 (M+H)⁺.

2TFA·H-Dmt-Tic-Lys-NH-Ph (6)—Boc-Dmt-Tic-Lys-NH-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.05 g (92%); *R*_f(A) 0.34; HPLC *K'* 3.15; mp 149-151 °C; [α]_D²⁰ -15.8; *m/z* 573 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.89 (m, 6H), 2.35 (s, 6H), 2.65-3.05 (m, 4H), 3.95-4.53 (m, 4H), 4.92 (m, 1H), 6.29 (s, 2H), 6.96-7.64 (m, 9H).

Benzyl 5-(tert-butyl 3-carbamoyl-3,4-dihydroisoquinoline-2(1*H*)-carboxyloyl)-5-(1*H*-benzo[*d*]imidazol-2-yl)pentylcarbamate {Boc-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid}—To a solution of Boc-Tic-OH (0.33 g, 1.20 mmol) and 2TFA·H₂N-CH[(CH₂)₄-NH-Z]-Bid [benzyl 5-amino-5-(1*H*-benzo[*d*]imidazol-2-yl)pentylcarbamate]⁴³ (0.70 g, 1.20 mmol) in DMF (10 mL) at 0 °C, NMM (0.26 mL, 2.40 mmol), HOBt (0.20 g, 1.32 mmol), and WSC (0.25 g, 1.32 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 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 0.59 g (80%); *R*_f(B) 0.63; HPLC *K'* 4.94; mp 137-139 °C; [α]_D²⁰ -13.3; *m/z* 613 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.92-3.17 (m, 4H), 4.17-4.87 (m, 3H), 4.92-5.34 (m, 3H), 6.96-7.70 (m, 13H).

2TFA·H-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid—Boc-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.43 g (97%); *R*_f(A) 0.45; HPLC *K'* 3.72; mp 140-142 °C; [α]_D²⁰ -14.6; *m/z* 513 (M+H)⁺.

Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid—To a solution of Boc-Dmt-OH (0.10 g, 0.32 mmol) and 2TFA·H-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid (0.24 g, 0.32 mmol) in DMF (10 mL) at 0 °C, NMM (0.07 mL, 0.64 mmol), HOBt (0.05 g, 0.35 mmol), and WSC (0.07 g, 0.35 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 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 0.23 g (91%); *R*_f(B) 0.63; HPLC *K'* 4.88; mp 140-142 °C; [α]_D²⁰ -14.8; *m/z* 804 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.35 (s, 6H), 2.92-3.17 (m, 6H), 4.41-4.87 (m, 3H), 4.92-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

2TFA·H-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid (7)—Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.04 g (96%); *R*_f(A) 0.31; HPLC *K'* 3.90; mp 146-148 °C; [α]_D²⁰ -18.3; *m/z* 704 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 6H), 2.35 (s, 6H), 2.96-3.05 (m, 6H), 3.95-4.46 (m, 3H), 4.87-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

Boc-Tic-NH-(*D*)-CH[(CH₂)₄-NH-Z]-Bid—This compound was obtained by condensation of Boc-Tic-OH with 2TFA·H₂N-(*D*)-CH[(CH₂)₄-NH-Z]-Bid via WSC/HOBt as reported for Boc-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid: yield 0.56 g (83%); *R*_f(B) 0.64; HPLC *K'* 4.87; mp 139-141 °C; [α]_D²⁰ +6.8; *m/z* 613 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.92-3.17 (m, 4H), 4.17-4.87 (m, 3H), 4.92-5.34 (m, 3H), 6.96-7.70 (m, 13H).

2TFA·H-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid—Boc-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.39 g (92%); *R*_f (A) 0.47; HPLC *K'* 3.71; mp 143-145 °C; [α]²⁰_D +7.5; *m/z* 513 (M+H)⁺.

Boc-Dmt-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid—This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid via WSC/HOBt as reported for Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid: yield 0.17 g (87%); *R*_f(B) 0.65; HPLC *K'* 5.17; mp 142-144 °C; [α]²⁰_D +4.9; *m/z* 804 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.35 (s, 6H), 2.92-3.17 (m, 6H), 4.41-4.87 (m, 3H), 4.92-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

2TFA·H-Dmt-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid (8)—Boc-Dmt-Tic-NH-(D)-CH[(CH₂)₄-NH-Z]-Bid was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.06 g (91%); *R*_f(A) 0.33; HPLC *K'* 3.90; mp 149-151 °C; [α]²⁰_D +5.6; *m/z* 704 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 6H), 2.35 (s, 6H), 2.96-3.05 (m, 6H), 3.95-4.46 (m, 3H), 4.87-5.34 (m, 4H), 6.29 (s, 2H), 6.96-7.70 (m, 13H).

Boc-NH-CH[(CH₂)₄-NH-Ac]-Bid—A solution of Boc-Lys(Ac)-OH (0.3 g, 1.04 mmol) and NMM (0.11 mL, 1.04 mmol) in DMF (10 mL) was treated at -20 °C with IBCF, 0.14 mL, 1.04 mmol). After 10 min at -20 °C, *o*-phenyldiamine (0.11 g, 1.04 mmol) was added. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h) and was then stirred for an additional 3 h. The solvent was evaporated, and the residue was partitioned between EtOAc and H₂O. The EtOAc layer was washed with 5% NaHCO₃ and brine and dried over Na₂SO₄. The solution was filtered, the solvent was evaporated, and the residual solid was dissolved in glacial AcOH (10 mL). The solution was heated at 65 °C for 1 h. After the solvent was evaporated, the residue was precipitated from Et₂O/Pe (1:9, v/v): yield 0.3 g (80%); *R*_f (B) 0.71; HPLC *K'* 3.03; mp 141-143 °C; [α]²⁰_D -7.6; *m/z* 362 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.02 (s, 3H), 3.20 (m, 2H), 4.87 (m, 1H), 7.26-7.70 (m, 4H).

2TFA·H₂N-CH[(CH₂)₄-NH-Ac]-Bid—Boc-NH-CH[(CH₂)₄-NH-Ac]-Bid was treated with TFA as reported for TFA·H-Lys(Z)-NH-CH₂-Ph: yield 0.16 g (90%); *R*_f(A) 0.48; HPLC *K'* 2.08; mp 149-151 °C; [α]²⁰_D -9.8; *m/z* 262 (M+H)⁺.

Boc-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid—This compound was obtained by condensation of Boc-Tic-OH with 2TFA·H₂N-CH[(CH₂)₄-NH-Ac]-Bid via WSC/HOBt as reported for Boc-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid: yield 0.45 g (85%); *R*_f(B) 0.57; HPLC *K'* 4.09; mp 143-145 °C; [α]²⁰_D -14.2; *m/z* 521 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.02 (s, 3H), 3.05-3.20 (m, 4H), 4.22-4.92 (m, 5H), 6.96-7.70 (m, 8H).

2TFA·H-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid—Boc-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid was treated with TFA as reported for TFA·H-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.31 g (97%); *R*_f(A) 0.42; HPLC *K'* 3.38; mp 146-148 °C; [α]²⁰_D -15.5; *m/z* 421 (M+H)⁺.

Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid—This compound was obtained by condensation of Boc-Dmt-OH with 2TFA·H-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid via WSC/HOBt as reported for Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid: yield 0.13 g (78%); *R*_f(B) 0.57; HPLC *K'* 4.31; mp 150-152 °C; [α]²⁰_D -15.7; *m/z* 712 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29-1.84 (m, 15H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05-3.20 (m, 6H), 4.46 (s, 2H), 4.87-4.92 (m, 3H), 6.29 (s, 2H), 6.96-7.70 (m, 8H).

2TFA·H-Dmt-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid (9)—Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Ac]-Bid was treated with TFA as reported for TFA·H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.06

g (93%); *Rf*(A) 0.28; HPLC *K'* 3.16; mp 153–155 °C; $[\alpha]^{20}_{\text{D}}$ -19.2; *m/z* 612 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29–1.84 (m, 6H), 2.02 (s, 3H), 2.35 (s, 6H), 3.05–3.20 (m, 6H), 3.95–4.46 (m, 3H), 4.87–4.92 (m, 3H), 6.29 (s, 2H), 6.96–7.70 (m, 8H).

Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid—Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH-Z]-Bid was treated with H₂ in presence of Pd/C 10% as reported for Boc-Dmt-Tic-Lys-NH-CH₂-Ph: yield 0.17 g (92%); *Rf*(B) 0.52; HPLC *K'* 4.02; mp 147–149 °C; $[\alpha]^{20}_{\text{D}}$ -16.3; *m/z* 670 (M+H)⁺.

3TFA-H-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid (10)—Boc-Dmt-Tic-NH-CH[(CH₂)₄-NH₂]-Bid was treated with TFA as reported for TFA-H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph: yield 0.03 g (93%); *Rf*(A) 0.29; HPLC *K'* 2.98; mp 156–158 °C; $[\alpha]^{20}_{\text{D}}$ -20.4; *m/z* 570 (M+H)⁺; ¹H-NMR (DMSO-*d*₆) δ 1.29–1.84 (m, 6H), 2.35 (s, 6H), 2.65–3.05 (m, 6H), 3.95–4.46 (m, 3H), 4.87–4.92 (m, 2H), 6.29 (s, 2H), 6.96–7.70 (m, 8H).

Supplementary Material

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Acknowledgements

This research was supported in part by the University of Cagliari (PRIN 2004), University of Ferrara (Italy), and the Intramural Research Program of NIH, and NIEHS. The authors appreciate the professional expertise and assistance of the library staff and the Comparative Medicine Branch at NIEHS.

References

- 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: Ac, acetyl; 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; 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; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP(P), H-Tyr-Tic-Phe-(Phe)-OH.; TLC, thin-layer chromatography; WSC, 1-ethyl-3-[3'-(dimethyl)aminopropyl]-carbodiimide hydrochloride; Z, benzyloxycarbonyl;
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Scheme 1.
 Synthesis of compounds **1-6**.

Scheme 2.
 Synthesis of compounds **7-10**.

Table 1

Receptor Binding and Functional Bioactivity. ^aThe K_i values (nM) were determined according to Chang and Prusoff.³⁹ The mean $\pm$ SE with n repetitions in parenthesis 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. These values represent the mean $\pm$ SE for at least five to six fresh tissue samples. Deltorphan II and endomorphin-2 were the internal standards for MVD (δ -opioid receptor bioactivity) and GPI (μ -opioid receptor bioactivity) tissue preparation, respectively. ^cThe pA_2 values of opioid antagonists against the agonists (deltorphan II and endomorphin-2) were determined by the method of Kosterlitz and Watt.⁴⁰ Data taken from Balboni *et al.*^{11e} Data taken from Balboni *et al.*³¹ Data taken from Balboni *et al.*^{18g} Data taken from Balboni *et al.*¹⁹ * μ Selectivity $K_i\delta/K_{i\mu}$. n.a. = no antagonism.

Comp.	Structure	receptor affinity ^a		selectivity	functional bioactivity			
		$K_i\delta$ (nM)	$K_i\mu$ (nM)		MVD IC_{50} (nM) ^b	GPI IC_{50} (nM) ^b	MVD pA_2 ^c	GPI pA_2 ^c
	<i>H-Dmt-Tic-Gly-OH</i>	1.38 $\pm$ 0.09 (7)	529.0 $\pm$ 50 (6)	383			8.85	
	<i>H-Dmt-Tic-Gly-NH-CH₂-Ph^d</i>	0.031	0.16	5.3		2.69	9.25	
	<i>H-Dmt-Tic-Gly-NH-Ph^d</i>	0.042	0.16	3.6	3.02	2.57		
	<i>H-Dmt-Tic-NH-CH₂-Bid^d</i>	0.035	0.50	14	0.13	26.92		
	<i>H-Dmt-Tic-NH-CH(CH₂-COOH)-Bid^e</i>	0.443	53.9	122	0.12	1724		
	<i>H-Dmt-Tic-Lys(Ac)-OH^f</i>	0.047	1051	22361			10.07	
	<i>H-Dmt-Tic-Phe-Lys(Z)-OH^g</i>	0.019	2.75	145		13.7	11.43	
	<i>Endomorphin-2</i>							
	<i>Deltorphan-II</i>				0.371			
1	<i>H-Dmt-Tic-Lys(Z)-NH-CH₂-Ph</i>	0.31 $\pm$ 0.02 (3)	4.41 $\pm$ 0.52 (5)	14	>10000	>10000	6.21	5.82
2	<i>H-Dmt-Tic-Lys(Ac)-NH-CH₂-Ph</i>	0.068 $\pm$ 0.009 (3)	5.50 $\pm$ 0.18 (3)	81	>10000	>10000	10.4	8.16
3	<i>H-Dmt-Tic-Lys-NH-CH₂-Ph</i>	0.50 $\pm$ 0.07 (4)	4.05 $\pm$ 0.54 (5)	8	>10000	>10000	6.01	7.96
4	<i>H-Dmt-Tic-Lys(Z)-NH-Ph</i>	0.57 $\pm$ 0.06 (3)	1.47 $\pm$ 0.20 (4)	3	>10000	438 $\pm$ 65	5.77	-
5	<i>H-Dmt-Tic-Lys(Ac)-NH-Ph</i>	0.13 $\pm$ 0.003 (4)	0.63 $\pm$ 0.065 (3)	5	>10000	1248 $\pm$ 194	12.0	-
6	<i>H-Dmt-Tic-Lys-NH-Ph</i>	0.42 $\pm$ 0.02 (4)	0.93 $\pm$ 0.05 (4)	2	>10000	1254 $\pm$ 205	5.38	-
7	<i>H-Dmt-Tic-NH-CH[(CH₂)₄NH-Z]-Bid</i>	0.64 $\pm$ 0.02 (3)	0.37 $\pm$ 0.040 (3)	1.7*	9049 $\pm$ 1128	375 $\pm$ 33	<5	-
8	<i>H-Dmt-Tic-NH-(D)-CH[(CH₂)₄NH-Z]-Bid</i>	0.40 $\pm$ 0.08 (4)	0.15 $\pm$ 0.018 (3)	2.7*	>10000	62.5 $\pm$ 13.5	<5	-
9	<i>H-Dmt-Tic-NH-CH[(CH₂)₄NH-Ac]-Bid</i>	0.18 $\pm$ 0.03 (4)	0.13 $\pm$ 0.02 (4)	1.4*	>10000	53.9 $\pm$ 7.3	n.a.	-
10	<i>H-Dmt-Tic-NH-CH[(CH₂)₄NH₂]-Bid</i>	0.49 $\pm$ 0.04 (3)	0.16 $\pm$ 0.015 (3)	3.1*	>10000	39.7 $\pm$ 6.9	n.a.	-