Potent δ-Opioid Receptor Agonists Containing the Dmt−Tic Pharmacophore

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Conversion of δ-opioid receptor antagonists containing the 2′,6′-dimethyl-L-tyrosine (Dmt)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) pharmacophore into potent δ-agonists required a third heteroaromatic nucleus, such as 1*H*-benzimidazole-2-yl (Bid) and a linker of specified length both located C-terminally to Tic in the general formula H−Dmt−Tic−NH−CH(R)−R′. The distance between Tic and Bid is a determining factor responsible for the acquisition of δ agonism (**2**, **2′**, **3**, **4**, **6**) or δ antagonism (**8**). Compounds containing a C-terminal Ala (**1**, **1′**), Asp (**5**), or Asn (**7**) with an amide (**1**, **1′**, **5**) or free acid group (**7**) served as δ-antagonist controls lacking the third heteroaromatic ring. A change in chirality of the spacer (**2**, **2′**) or inclusion of a negative charge via derivatives of Asp (**4**, **6**) resulted in potent δ agonism and moderate μ agonism, although δ-receptor affinity decreased about 10-fold for **4** while μ affinity fell by over 2 orders of magnitude. Repositioning of the negative charge in the linker altered activity: H−Dmt−Tic−NH−CH(CH$_2$−Bid)COOH (**6**) maintained high δ affinity (K_i = 0.042 nM) and δ agonism (IC$_{50}$ = 0.015 nM), but attachment of the free acid group to Bid [H−Dmt−Tic−NH−CH$_2$−Bid(CH$_2$−COOH) (**9**)] reconstituted δ antagonism (K_e = 0.27 nM). The data demonstrate that a linker separating the Dmt−Tic pharmacophore and Bid, regardless of the presence of a negative charge, is important in the acquisition of opioids exhibiting potent δ agonism and weak μ agonism from a parent δ antagonist.

Introduction

The simple pharmacophore Dmt−Tic[1] can acquire differential bioactivity through N- or C-terminal modifications; namely, N,N-methylation enhanced δ affinity, while C-terminal modifications by various hydrophobic groups or a linker and a Bid group increased binding to μ-opioid receptors by orders of magnitude, resulting in compounds exhibiting δ agonism in addition to substances with both δ antagonism and μ agonism.[2−5] Although the Dmt−Tic pharmacophore is the prototype for a family of highly selective δ-opioid receptor antagonists,[6] this remarkable pseudodipeptide can be transformed into a potent δ agonist by the C-terminal addition of a linker and a 1*H*-benzimidazole-2-yl (Bid) group.[4] Numerous data in the literature demonstrate the capacity of Dmt as the N-terminal residue to enhance and modify the biological activity of a vast array of opioid compounds, including transforming deltorphin B into a high dual affinity δ/μ agonist and enhancing the activity of DPDPE, DALDA, endomorphin-2 analogues, enkephalin, which also exhibited extraordinary stability, and [Dmt¹,Tic²]dynorphin A(1−11)−NH$_2$, which became a δ antagonist.[4−18] Moreover, the requirement of an unaltered Tic residue was estab-lished after modifications to the aromatic ring of Tic extensively weakened opioid activity.[14,17] Thus, the Dmt−Tic pharmacophore plays a significant role in activation (agonism) or inhibition (antagonism) of the biological response mechanism of opioid receptors that is mediated through a G-protein-coupled second messenger system.[19−24]

Rationale

The remarkable transformation of the Dmt−Tic pharmacophore from a δ antagonist into a potent δ agonist[4] initiated further investigations to examine our original hypothesis; namely, the length of the linker between Tic and Bid or another aromatic nucleus mediates the alteration of functional bioactivity.[4] It is well-known that the coupling of various hydrophobic substituents at the C terminus of Tic, a reaction that simultaneously removes the negative charge, promotes interaction with μ-opioid receptors[3,5,25] to produce bifunctional molecules.[3,4] Whereas studies on the TIPP family of opioid peptides indicated that hydrophobic C-terminal extensions resulted in analogues producing δ agonism or δ antagonism with μ agonism, it was only a Dmt derivative (DIPP−NH$_2$[ψ]) that exhibited the highest activity toward both δ- and μ-opioid receptors.[13] On the other hand, extensive studies by Salvadori et al.[3] and Pagé et al.[5] verified the appearance of dual bioactivity profiles (δ agonism/δ antagonism with μ agonism) using a series of Dmt−Tic analogues, which contained hydrophobic or aromatic substituents at the C terminus.

This study unveils a new series of H−Dmt−Tic−NH−CH(R)−R′ analogues (Figure 1) in which a third het-

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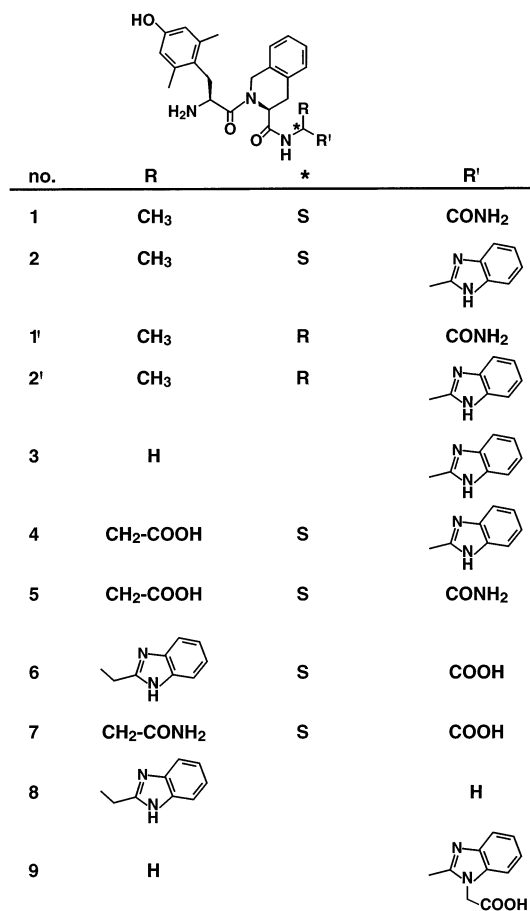
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Figure 1. General structures of H−Dmt−Tic−NH−CH(R)−R= analogues. The asterisk (∗) denotes chirality.

eroaromatic center (Bid) was used to evaluate the potential of converting the δ-opioid antagonist pharmacophore, H−Dmt−Tic−R (R = amino acid, −OH, −NH₂, −CHOH), into a δ-agonist pharmacophore. Analogues containing Ala and α-aminocarboxylic acids, such as Asp and Asn, were further tested as control compounds to explore the biological effect with δ- and μ-opioid receptors in the absence of Bid. In addition, the effect of a negative charge on bioactivity was investigated because previous studies with opioid peptides revealed that the presence of a negative charge repels ligands from the μ receptor with minimal effect on δ affinity.[26] Finally, four aspects regarding the linker between Tic[2] and Bid were examined: (i) the addition of linear and branched alkyl groups between the amide and Bid (**2**, **2′**, **3**, **8**); (ii) the stereochemistry of the carbon atom between the amide and Bid (**2**, **2′**); (iii) the position of Bid in the molecule (R or R′, Figure 1; **4**, **6**); and (iv) the addition of a carboxylic function to the linker or the nitrogen of Bid (**4**, **6**, **9**, Figure 1).

Chemistry

All peptides and pseudopeptides were prepared in a stepwise procedure by standard solution peptide synthesis methods. Mixed carbonic anhydride coupling of *tert*-butyloxycarbonyl (Boc)−Ala−OH, Boc−D-Ala−OH, Boc−Asp−OBzl, or Boc−Asp(OBzl)−OH with *o*-phenyl-endiamine gave the corresponding crude intermediate monoamides, which were converted without purification to the desired 1*H*-benzimidazol-2-yl (Bid) derivatives by

Scheme 1. Synthesis of Compound **2**
[H−Dmt−Tic−NH−CH(CH₃)−Bid]
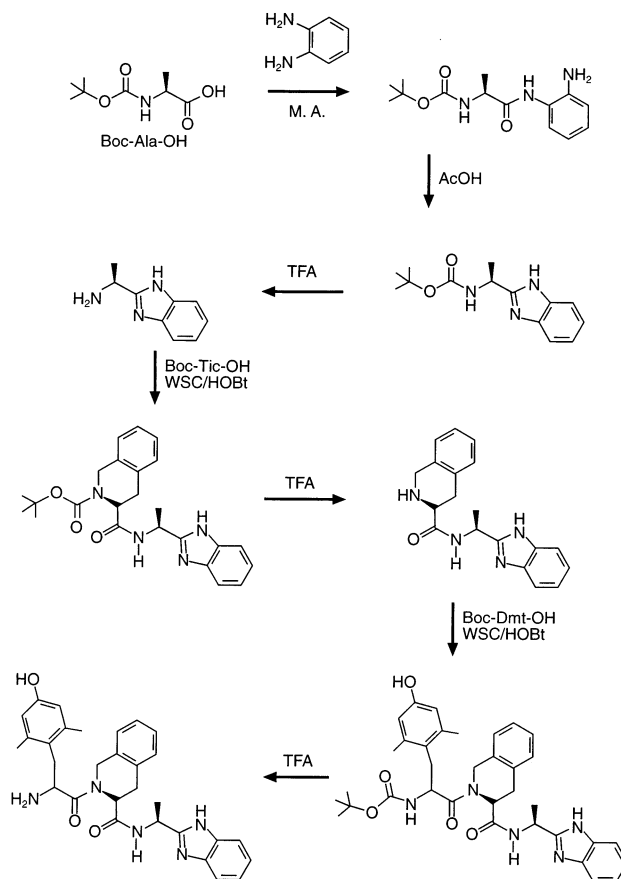
cyclization and dehydration in acetic acid (AcOH), as outlined in Schemes 1 and 2, respectively. N-Alkylation of Bid in position 1 (**9**) was obtained by treatment of Boc−NH−CH₂−Bid[4] with K₂CO₃ and Br−CH₂−COOEt (bromoacetic acid ethyl ester). After N-terminal deprotection with trifluoroacetic acid (TFA), each derivative was condensed with Boc−Tic−OH (Boc was deprotected with TFA) and then with Boc−Dmt−OH via WSC/HOBt (1-ethyl-3-[(3′-dimethyl)aminopropyl]carbodiimide/1-hydroxy-1,2,3-benzotriazole). In the final compounds, benzyl esters, ethyl ester, and Boc protecting groups were removed by catalytic hydrogenation (Pd/C, 5%), 1 N NaOH, and TFA treatment, respectively. H−Dmt−Tic−D-Ala−NH₂ was prepared as previously reported for H−Dmt−Tic−Ala−NH₂.[27] H−Dmt−Tic−Asn−OH was prepared in a stepwise procedure starting from H−Asn−OBzl[28] that was condensed with Boc−Tic−OH and then with Boc−Dmt−OH via WSC/HOBt.

Results

Receptor Interactions. While all the peptides listed in Table 1 exhibited high δ affinity, several analogues (**2**, **2′**, **3**, **6**, **8**, **9**) demonstrated exceptional K_i values (<0.1 nM). On the other hand, only three Bid-containing peptides had high μ affinities (K_i < 1 nM; **2**, **2′**, **3**); the μ affinities of the other compounds ranged from 5 to 80 nM. Alkylation of the carbon atom between the amide and Bid (**2**, Figure 1) did not substantially modify δ- and μ-receptor affinities and pharmacological activities (infra vide) compared to the reference δ agonist, Dmt−Tic−NH−CH₂−Bid (**3**). The corresponding diastereomer

Table 1. Receptor Affinity and Functional Bioactivity of Analogues Containing the Dmt−Tic Pharmacophore

| | receptor affinity[a] (nM) | | | functional bioactivity[b] | | |
| | | | | MVD | | GPI |
peptide	$K_i(\delta)$	$K_i(\mu)$	μ/δ	IC$_{50}$ (nM)	K_e (nM)	IC$_{50}$ (nM)
1, H−Dmt−Tic−Ala−NH$_2$	0.241 ± 0.02 (5)	47.1 ± 3.4 (4)	195		10.0 ± 1.3[c]	4740 ± 345[c]
1′, H−Dmt−Tic−D-Ala−NH$_2$	0.301 ± 0.021 (4)	47.5 ± 4.6 (3)	158		25.1 ± 1.1	>10000
2, H−Dmt−Tic−NH−CH(CH$_3$)−Bid	0.063 ± 0.004 (3)	0.411 ± 0.016 (3)	6.5	0.26 ± 0.03		71.7 ± 8.2
2′, H−Dmt−Tic−NH(R)CH(CH$_3$)−Bid	0.047 ± 001 (3)	0.233 ± 0.01 (3)	5	0.026 ± 0.002		6.36 ± 0.7
3, H−Dmt−Tic−NH−CH$_2$−Bid	0.035 ± 0.006 (3)	0.50 ± 0.054 (3)	14[d]	0.035 ± 0.003		40.7 ± 5
4, H−Dmt−Tic−NH−CH(CH$_2$−COOH)−Bid	0.443 ± 0.043 (4)	53.9 ± 4.4 (3)	122	0.12 ± 0.02		1724 ± 150
5, H−Dmt−Tic−Asp−NH$_2$	0.289 ± 0.018 (3)	2,565 ± 290 (3)	8875		7.58 ± 0.31	>10000
6, H−Dmt−Tic−NH−CH(CH$_2$−Bid)−COOH	0.042 ± 0.017 (4)	21.68 ± 5.5 (3)	516	0.015 ± 0.003		1558 ± 153
7, H−Dmt−Tic−Asn−OH	1.10 ± 0.26 (5)	80.3 ± 8.2 (4)	73		5.88 ± 0.21	>10000
8, H−Dmt−Tic−NH−CH$_2$−CH$_2$−Bid	0.067 ± 0.015 (4)	5.49 ± 0.03 (3)	82		4.78 ± 0.37[e]	107.5 ± 11.4[e]
9, H−Dmt−Tic−NH−CH$_2$−Bid(CH$_2$−COOH)	0.021 ± 0.0025 (4)	6.92 ± 0.25 (4)	330		0.27 ± 0.015	3193 ± 402
deltorphin C	0.24 ± 0.06 (6)	272 ± 50 (11)	1135[f]	0.30 ± 0.012		>1200
dermorphin	178.6 ± 18 (15)	1.22 ± 0.13 (22)	146[g]	16.5 ± 1.8		1.21 ± 0.18

[a] The K_i values (nM) were determined according to the Chang and Prusoff[43] as detailed in Experimental Section. The mean ± SE with n repetitions in parentheses is based on independent assays with five to eight peptide doses using multiple synaptosome preparations. [b] Agonist activity was expressed as IC$_{50}$ (K_e) obtained from concentration−response curves. These values represent the mean ± SE of at least five fresh tissue samples. [D-Ala^2]Deltorphin I (deltorphin C)[11] and dermorphin were the internal standards for MVD (δ bioactivity) and GPI (μ bioactivity) tissue preparations, respectively. [c] Data from Salvadori et al.[6] [d] Receptor binding data from Balboni et al.[4] These bioassay data were not reported previously. [e] Data taken from Balboni et al.[4] [f] Binding assay data from Salvadori et al.[44] [g] Receptor data from Lazarus et al.[41]

Scheme 2. Synthesis of Compound **6** [H−Dmt−Tic−NH−CH(CH$_2$−Bid)−COOH]
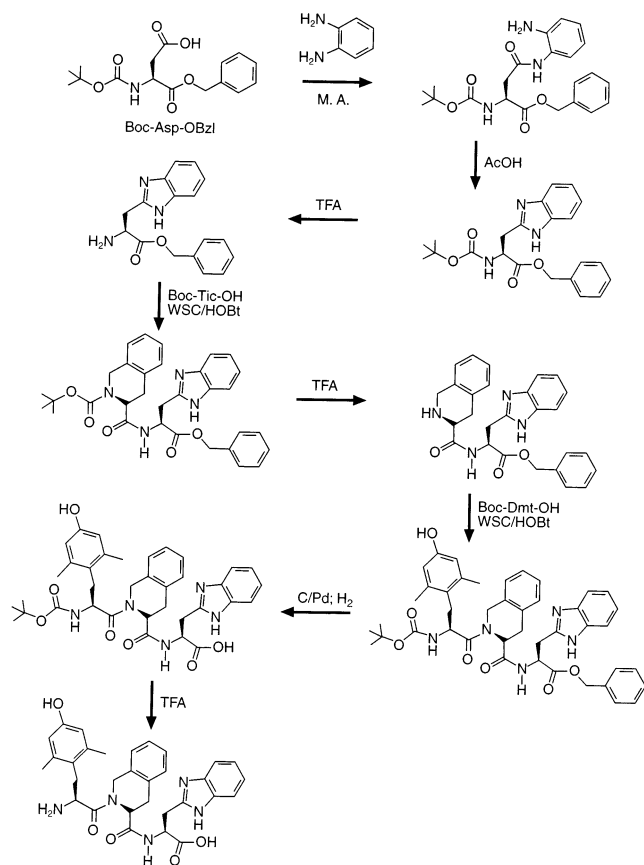
of **2** (**2′**, Figure 1) maintained comparable δ selectivity and potent agonist activity even though μ affinity and agonist potency increased. The control compounds, containing Ala−NH$_2$ instead of Bid (**1**, **1′**, Figure 1), exhibited equivalent δ affinities and selectivities. In addition, the tripeptide analogue controls with C-terminal Asp and Asn (**5** and **7**, respectively) had relatively good δ affinities (0.289 and 1.10 nM, respectively) and δ-antagonist bioactivities [K_e(mouse vas deferens) = 7.58 and 5.88 nM, respectively]. The alkylation of the side chain of compound **2** with a carboxylic

function (**4**, Figure 1) improved δ selectivity relative to **2**, **2′**, and **3** even though δ affinity fell by about 10-fold (Table 1). Compound **5** with a very weak $K_i(\mu)$ (2565 nM) had the highest δ selectivity, comparable to many Dmt−Tic analogues with a free C-terminal carboxyl group.[2,3,5,6]

In the Bid analogues containing carboxylate groups (**4**, **6**, **9**), μ affinities diminished and δ selectivities increased, which verified the hypothesis that a negative charge in an opioid ligand retards binding to μ receptors without detrimental effects on δ affinity.[11,26] However, the position of the carboxyl group relative to the Bid moiety (compare **6** and **9**) influenced μ binding to a greater extent than that for the δ receptor. Bioactivity data for analogues containing a different chirality at the C-terminal of Dmt−Tic indicated that the change in chirality had essentially no influence on the bioactivity profiles (compare pairs of compounds **1** + **2** and **1′** + **2′**).

Pharmacological Activity in Vitro. Several peptides (**2**, **2′**, **3**, **4**, **6**) acted as potent δ-opioid agonists. The IC$_{50}$ values ranged nearly 20-fold, from 0.015 to 0.26 nM in isolated organ preparations (Table 1), and they were many times more effective in inhibiting electrically evoked contractions in the mouse vas deferens (MVD) than in the guinea-pig ileum (GPI). Interestingly, the analogues containing carboxylic acids (**4**, **6**, **9**) exhibited very weak μ agonism relative to **2**, **2′**, and **3**. In addition, compound **9**, which displayed the highest δ affinity, was inactive as an agonist in the MVD preparation at a concentration as high as 137 μM. In fact, compound **9** behaved as a potent antagonist. displacing the [D-Ala^2]-deltorphin I dose response curve to the right (K_e = 0.27 nM) with a weak bioactive response on GPI (IC$_{50}$ = 3193 nM).

Analogues **1**, **1′**, **5**, and **7** were pure δ antagonists that lacked the third aromatic center, Bid. These control compounds exhibited micromolar activity on GPI, while in contrast, compound **8**, a potent δ antagonist containing Bid, displayed weak μ agonism (Table 1). None of the peptide analogues (Table 1) exhibited μ antagonism.

Molecular Modeling. H−Dmt−Tic−NH−CH$_2$−Bid, a δ-opioid receptor agonist (**3**), was modeled using coordinates for Dmt and Tic from the X-ray crystal

Figure 2. Backbone superimpositions of four low-energy conformers of the δ-opioid receptor agonist H−Dmt−Tic−NH−CH$_2$−Bid (**3**).

structure of *N,N*-(Me)$_2$−Dmt−Tic−OH (δ antagonist)[29b] (Figure 2). Features of the Dmt−Tic pharmacophore include cis orientation around the peptide bond, approximately 5 Å between the aromatic rings, and near-parallel orientation of the aromatic ring structures.[29] Conformations describing the C-terminal modifications were generated by extensive conformational searching and energy minimization using InsightII. One-hundred forty-seven low-energy conformers were generated by the search, and four of the lowest energy conformations with unique C-terminal orientations were superimposed for comparison and evaluation as potential bioactive forms (Figure 2). The relative energies of the four unique low-energy structures ranged from 67 to 97 kcal/mol, and centroid distances between the aromatic rings of Tic and Bid ranged from 6.7 to 9.9 Å. The most notable structural variations were observed around bonds between the amide and methylene linker, as well as between the methylene linker and Bid (Figure 2).

Discussion

Opioid peptide agonists for the δ-opioid receptor include a vast array of enkephalin derivatives,[30] as well as JOM-13 cyclic analogues,[31−33] the potent deltorphin family of amphibian skin peptides,[11] and the unique peptide H−Dmt−Tic−NH−CH$_2$−Bid[4] (Figure 1 and Table 1). These new pseudopeptides containing Dmt and Tic, well-known for their δ antagonism[25,29] and potent δ-inverse agonism,[34] provided templates for the formation of δ opioids endowed with potent δ-agonist activity (Table 1).

The improvement in δ selectivity of **4**, **6**, and **9** relative to other Bid-containing peptides and the elevated selectivity of **5** confirm earlier observations that a negative charge in an opioid peptide is detrimental for μ-receptor interaction.[26] Furthermore, the absence of the Bid pharmacophore (**1**, **1′**, **5**, **7**) (Figure 1 and Table 1) resulted in δ-antagonist activity as observed previously.[6,35] On the other hand, Bid is not exclusively associated with δ agonism because compounds **8** and **9** were potent δ antagonists (Table 1). Quite interestingly, deletion of the carboxylic acid function of **6** to yield **8** (Figure 1, Table 1) not only improved μ affinity as expected but also shifted the pharmacological activity from a δ agonist to a δ antagonist. Similarly, the same

chemical modification applied to **4** to obtain analogue **2** elevated μ affinity about 130-fold, since it is known that hydrophobic peptides enhance μ-receptor interaction.[3,5] However, a change in pharmacological activity was not observed; in other words, both **4** and **2** retained strong δ agonism. In a similar chemical approach, deletion of the COOH function of TIP gave weak δ agonists,[36] while in analogues containing Trp, removal of the COOH function failed to modify δ antagonism.[37]

Finally, alkylation with CH$_2$−COOH on the nitrogen of Bid (**9**) was carried out in order to determine if a negative charge on the heteroaromatic function could modify δ selectivity as well as the biological properties. Comparison of the biological data of **9** with data of **6** and **4** (Figure 1 and Table 1) indicated a δ affinity relatively similar to that of **6** but 20-fold greater than that of **4**; compound **9** quite unexpectedly revealed potent δ-antagonist activity with $K_e = 0.27$ nM. These data suggest that the alkylation of the nitrogen of Bid can affect the acquisition of agonism or antagonism. A previous study exploring the chemical composition and length of the linker between Tic and Bid indicated that the distance between the aromatic groups may be responsible for the manifestation of δ agonism (compare **3** and **8**), but the present study demonstrated that compound **6** with the same distance as that of **8** was a potent δ agonist.[4]

Molecular modeling of **3** revealed that a number of low-energy forms were accessible with unique orientations of the Bid pharmacophore (Figure 2) and the addition of an acetate group to Bid may limit the conformational freedom, thereby resulting in δ antagonism. The adaptability of the ligand to the δ-receptor binding pocket may be an important factor for receptor activation as seen in the X-ray diffraction analysis of three Dmt−Tic analogues,[29b] which were, along with other opioid molecules, docked into a model of the δ-opioid receptor based on the crystalline structure of bovine rhodopsin.[29c] Those docking studies[29c] further demonstrated that binding sites for δ agonists and δ antagonists were contained in distinct but overlapping regions of the δ-opioid receptor. The molecular modeling demonstrated the accessible conformational space of the C-terminal portion of these peptides based on the Dmt−Tic pharmacophore generated from the X-ray crystallography data.[29b] These conformation studies provide valuable information for docking to opioid receptors[29c] in comparison with other bioactive opioids in order to develop a working hypothesis for the mechanism of activating their respective receptors. Most importantly, however, the models provide a molecular template upon which to design new opioid compounds that interact with specific opioid receptors.

Conclusions

Several analogues in the H−Dmt−Tic−NH−CH(R)−R′ series of peptides containing Bid have exceptionally high δ agonism in vitro, while slight modifications reverted the peptide back to its original state, namely, to a potent δ antagonist. Peptides **2**, **2′**, **3**, and **4** contained a relatively short linker, −NH−CH$_2$− (Figure 1), between the Dmt−Tic pharmacophore and the heteroaromatic ring Bid that yielded δ agonism regardless of the side chain (**2**, **2′**, **4**) or chirality (**2**, **2′**). As seen

Table 2. Analysis Results of Dmt−Tic Pharmacophore Compounds[a]

compound	MH$^+$, m/z	analytical
New Compounds		
TFA·H−Dmt−Tic−D-Ala−NH$_2$ (**1'**)	439	(C$_{24}$H$_{30}$N$_4$O$_4$·TFA) C, H, N
2TFA·H−Dmt−Tic−NH−(*S*)CH(CH$_3$)−Bid (**2**)	512	(C$_{30}$H$_{33}$N$_5$O$_3$·2TFA) C, H, N
2TFA·H−Dmt−Tic−NH−(*R*)CH(CH$_3$)−Bid (**2'**)	512	(C$_{30}$H$_{33}$N$_5$O$_3$·2TFA) C, H, N
2TFA·H−Dmt−Tic−NH−(*S*)CH(CH$_2$−COOH)−Bid (**4**)	556	(C$_{31}$H$_{33}$N$_5$O$_5$·2TFA) C, H, N
TFA·H−Dmt−Tic−Asp−NH$_2$ (**5**)	483	(C$_{25}$H$_{30}$N$_4$O$_6$·TFA) C, H, N
2TFA·H−Dmt−Tic−NH−(*S*)CH(CH$_2$−Bid)−COOH (**6**)	556	(C$_{31}$H$_{33}$N$_5$O$_5$·2TFA) C, H, N
TFA·H−Dmt−Tic−Asn−OH (**7**)	483	(C$_{25}$H$_{30}$N$_4$O$_6$·TFA) C, H, N
2TFA·H−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOH) (**9**)556		(C$_{31}$H$_{33}$N$_5$O$_5$·2TFA) C, H, N
Published Compounds		
TFA·H−Dmt−Tic−Ala−NH$_2$ (**1**)[6]		
2TFA·H−Dmt−Tic−NH−CH$_2$−Bid (**3**)[4]		
TFA·H−Dmt−Tic−NH−CH$_2$−CH$_2$−Bid (**8**)[4]		

[a] The analysis of the new compounds detailed in Experimental Section are included. The data on those compounds previously published are referenced.

previously[4] and with compound **8** (Table 1), increasing the length of the linker reconstituted δ antagonism; however, the addition of a carboxyl group restored δ agonism (**6**). Furthermore, the alkylation of the Bid nitrogen (**9**) caused an unanticipated reversion from δ agonism to δ antagonism (Table 1). These data signify that the addition of Bid and the carboxylic group to improve δ-opioid receptor selectivity of the Dmt−Tic pharmacophore does not guarantee δ agonism. Instead, the precise location and special orientation of these chemical groups is critical for exhibiting agonist or antagonist behaviors. These stimulating data serve as a springboard to launch the design of new opioid analogues containing other types of linkers, functional groups, and heteroaromatic nuclei that affect bioactivity in a similar manner that may lead to answers regarding opioid receptor activation and the development of compounds suitable for clinical and therapeutic applications.

Experimental Section

Materials. H−Dmt−OH was prepared according to Dygos et al.,[38] and the purity and chirality were compared to a sample generously donated by J. H. Dygos. Boc−Tic−OH was purchased from Bachem (Heidelberg, Germany). The radioactive opioid ligands were from commercial sources: [³H]DPDPE (32.0 Ci/mmol; NEN-DuPont, Billerica, MA) and [³H]DAGO (58.0 Ci/mmol, Amersham, Arlington Heights, IL).

General Methods. Crude peptides were purified by preparative reversed-phase high-performance liquid chromatography (HPLC) using a Waters Delta Prep 4000 system with a Waters PrepLC 40 mm assembly column C$_{18}$ (30 cm × 4 cm, 300 Å, 15 μm particle size column). The column was perfused at a flow rate of 40 mL/min with mobile-phase solvent A (10% acetonitrile in 0.1% TFA, v/v), and a linear gradient from 0% to 50% of solvent B (60%, acetonitrile in 0.1% TFA, v/v) in 25 min was adopted for the elution of the products. Analytical HPLC analyses were performed with a Beckman System Gold with a Beckman ultrasphere ODS column (5 μm; 4.6 mm × 250 mm). Analytical determinations and capacity factor (K') of the products were determined using HPLC conditions in the above solvent systems (solvents A and B) programmed at a flow rate of 1 mL/min using the following linear gradients: (a) from 0% to 100% B in 25 min and (b) from 10% to 70% B in 25 min. All analogues showed less than 1% impurities when monitored at 220 and 254 nm.

Thin-layer chromatography (TLC) was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany) using the following solvent systems: (A) 1-butanol/AcOH/H$_2$O (3:1:1, v/v/v); (B) CH$_2$Cl$_2$/toluene/methanol (17:1:2, v/v/v). Ninhydrin (1%, Merck), fluorescamine (Hoffman-La Roche), and chlorine reagents were used as sprays. Open column chroma-

tography (2 cm × 70 cm, 0.7−1 g of material) was run on silica gel 60 (70−230 mesh, Merck) using the same eluent systems.

Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were determined at 10 mg/mL in methanol with a Perkin-Elmer 241 polarimeter with a 10 cm water-jacketed cell. All ¹H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 200 MHz spectrometer. MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry of peptides was conducted using a Hewlett-Packard G 2025 A LD-TOF system. The samples were analyzed in the linear mode with 28 kV accelerating voltage, mixing them with a saturated solution of α-cyano-4-hydroxycinnamic acid matrix. The analysis results for the new compounds are in Table 2.

Boc−Tic−NH−(*S*)CH(CH$_3$)−Bid. To a solution of Boc−Tic−OH (0.55 g, 2 mmol) and 2 TFA·H$_2$N−(*S*)CH(CH$_3$)−Bid [1-(1*H*-benzimidazol-2-yl)ethylamine][39] (0.8 g, 2 mmol) in DMF (10 mL) at 0 °C were added NMM (0.44 mL, 4 mmol), HOBt (0.34 g, 2.2 mmol), and WSC (0.42 g, 2.2 mmol). The reaction mixture was stirred for 3 h at 0 °C and for 24 h at room temperature. After DMF was evaporated, the residue was solubilized in EtOAc and washed with NaHCO$_3$ (5%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et$_2$O/Pe (1:9, v/v): yield 0.7 g (83%); R_f(B) = 0.81; HPLC K' = 7.42; mp 215−217 °C; [α]^{20}$_D$ −18.5°; MH$^+$ 421; ¹H NMR (DMSO) δ 1.38−1.42 (d, 9H), 1.56 (d, 3H), 3.08−3.15 (m, 2H), 4.22−4.53 (m, 4H), 7.05−7.71 (m, 9H), 7.97 (s, 1H).

2TFA·H−Tic−NH−(*S*)CH(CH$_3$)−Bid. Boc−Tic−NH−(*S*)-CH(CH$_3$)−Bid (0.4 g, 0.95 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et$_2$O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.49 g (95%); R_f(A) = 0.36; HPLC K' = 5.32; mp 180−182 °C; [α]^{20}$_D$ −20.9°; MH$^+$ 321.

Boc−Dmt−Tic−NH−(*S*)CH(CH$_3$)−Bid. To a solution of Boc−Dmt−OH (0.11 g, 0.37 mmol) and 2TFA·H−Tic−NH−(*S*)CH(CH$_3$)−Bid (0.2 g, 0.37 mmol) in DMF (10 mL) at 0 °C were added NMM (0.08 mL, 0.74 mmol), HOBt (0.063 g, 0.41 mmol), and WSC (0.079 g, 0.41 mmol). The reaction mixture was stirred for 3 h at 0 °C and for 24 h at room temperature. After DMF was evaporated, the residue was solubilized in EtOAc and washed with NaHCO$_3$ (5%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et$_2$O/Pe (1:9, v/v): yield 0.18 g (81%); R_f(B) = 0.77; HPLC K' = 7.8; mp 143−144 °C; [α]^{20}$_D$ −24.6°; MH$^+$ 611; ¹H NMR (DMSO) δ 1.32−1.39 (d, 9H), 1.55 (d, 3H), 2.17 (s, 6H), 2.85−3.10 (m, 4H), 4.22−4.65 (m, 5H), 6.37 (s, 2H), 7.05−7.71 (m, 10H), 7.97 (s, 1H).

2TFA·H−Dmt−Tic−NH−(*S*)CH(CH$_3$)−Bid (2). Boc−Dmt−Tic−NH−(*S*)CH(CH$_3$)−Bid (0.061 g, 0.1 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et$_2$O/Pe (1:1, v/v) was added to the solution until the product precipitated: yield 0.09 g (93%); R_f(A) = 0.46; HPLC K' = 4.34; mp 158−160 °C; [α]^{20}$_D$ −26.1°; MH$^+$ 512. Anal. (C$_{30}$H$_{33}$N$_5$O$_3$·2TFA) C, H, N.

Boc−Tic−NH−(*R*)CH(CH₃)−Bid. This intermediate was obtained by condensation of Boc−Tic−OH with 2TFA·H₂N−(*R*)CH(CH₃)−Bid via WSC/HOBt as reported for Boc−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.68 g (82%); R_f(B) = 0.79; HPLC K' = 7.38; mp 215−217 °C; $[\alpha]^{20}_D$ +3.5°; MH⁺ 421; ¹H NMR (DMSO) δ 1.38−1.40 (d, 9H), 1.56 (d, 3H), 3.08−3.17 (m, 2H), 4.20−4.54 (m, 4H), 7.05−7.71 (m, 9H), 7.97 (s, 1H).

2TFA·H−Tic−NH−(*R*)CH(CH₃)−Bid. Boc−Tic−NH−(*R*)-CH(CH₃)−Bid was treated with TFA as reported for 2TFA·H−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.36 g (94%); R_f(A) = 0.41; HPLC K' = 5.75; mp 180−182 °C; $[\alpha]^{20}_D$ +7.9°; MH⁺ 321.

Boc−Dmt−Tic−NH−(*R*)CH(CH₃)−Bid. This compound was obtained by condensation of Boc−Dmt−OH with 2TFA·H−Tic−NH−(*R*)CH(CH₃)−Bid via WSC/HOBt as reported for Boc−Dmt−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.17 g (79%); R_f(B) = 0.85; HPLC K' = 7.2; mp 142−144 °C; $[\alpha]^{20}_D$ +12.6°; MH⁺ 611; ¹H NMR (DMSO) δ 1.32−1.39 (d, 9H), 1.55 (d, 3H), 2.17 (s, 6H), 2.85−3.15 (m, 4H), 4.20−4.65 (m, 5H), 6.34 (s, 2H), 7.05−7.72 (m, 10H), 7.97 (s, 1H).

2TFA·H−Dmt−Tic−NH−(*R*)CH(CH₃)−Bid (2′). Boc−Dmt−Tic−NH−(*R*)CH(CH₃)−Bid was treated with TFA as reported for 2TFA·H−Dmt−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.088 g (96%); R_f(A) = 0.40; HPLC K' = 3.78; mp 152−154 °C; $[\delta]^{20}_D$ +16.1°; MH⁺ 512. Anal. (C₃₀H₃₃N₅O₃·2TFA) C, H, N.

3-(1*H*-Benzimidazol-2-yl)-2-*tert*-butoxycarbonylamino-propionic Acid Benzyl Ester [Boc−NH−(*S*)CH(CH₂−Bid)−COOBzl]. A solution of Boc−Asp−OBzl (1 g, 3.1 mmol) and 4-methylmorpholine (NMM, 0.34 mL, 3.1 mmol) in *N,N*-dimethylformamide (DMF, 10 mL) was treated at −20 °C with isobutyl chloroformate (IBCF, 0.4 mL, 3.1 mmol). After 10 min at −20 °C, *o*-phenylendiamine (0.33 g, 3.1 mmol) was added. The reaction mixture was allowed to stir while slowly warming to room temperature (1 h) and was then stirred for 3 h. The solvent was evaporated, and the residue was partitioned between ethyl acetate (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 crystallized from diethyl ether (Et₂O)/petroleum ether (Pe) (1:9, v/v): yield 0.98 g (80%); R_f(B) = 0.79; HPLC K' = 6.6; mp 141−143 °C; $[\alpha]^{20}_D$ +12.1°; MH⁺ 396; ¹H NMR (DMSO) δ 1.40 (s, 9H), 3.09 (d, 2H), 4.86−5.30 (m, 3H), 7.19−7.70 (m, 10H), 7.98 (s, 1H).

2TFA·H₂N−(*S*)CH(CH₂−Bid)−COOBzl. Boc−NH−(*S*)-CH(CH₂−Bid)−COOBzl (0.9 g, 2.28 mmol) was treated with TFA (1 mL) for 0.5 h at room temperature. Et₂O/Pe (1:1, v/v) was added to the solution until the product precipitated; yield 1.11 g (93%); R_f(A) = 0.83; HPLC K' = 4.85; mp 152−154 °C; $[\alpha]^{20}_D$ +15.3°; MH⁺ 296.

Boc−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl. This substance was obtained by condensation of Boc−Tic−OH with 2TFA·H₂N−(*S*)CH(CH₂−Bid)−COOBzl via WSC/HOBt as reported for Boc−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.74 g (79%); R_f(B) = 0.74; HPLC K' = 4.5; mp 166−168 °C; $[\alpha]^{20}_D$ +8.2°; MH⁺ 555; ¹H NMR (DMSO) δ 1.40 (s, 9H), 3.04−3.17 (m, 4H), 4.22−5.32 (m, 6H), 7.01−7.70 (m, 14H), 7.98 (s, 1H).

2TFA·H−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl. Boc−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl was treated with TFA as reported for 2TFA·H−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.73 g (92%); R_f(A) = 0.71; HPLC K' = 5.31; mp 158−160 °C; $[\alpha]^{20}_D$ +10.4°; MH⁺ 455.

Boc−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl. This compound was obtained by condensation of Boc−Dmt−OH with 2TFA·H−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl via WSC/HOBt as reported for Boc−Dmt−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.44 g (81%); R_f(B) = 0.65; HPLC K' = 9.7; mp 165−167 °C; $[\alpha]^{20}_D$ +3.5°; MH⁺ 746; ¹H NMR (DMSO) δ 1.40 (s, 9H), 2.35−3.14 (m, 8H), 4.48−5.33 (m, 7H), 6.32−7.70 (m, 16H), 7.98 (s, 1H).

Boc−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOH. To a solution of Boc−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOBzl (0.3 g, 0.4 mmol) in MeOH (30 mL) was added C/Pd (10%, 0.05 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 0.24 g (94%); R_f(B) = 0.76; HPLC K' = 7.1; mp 175−177 °C; $[\alpha]^{20}_D$ +4.8°; MH⁺ 656.

2TFA·H−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOH (6). Boc−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOH was treated with TFA as reported for 2TFA·H−Dmt−Tic−NH−(*S*)CH-(CH₃)−Bid: yield 0.17 g (96%); R_f(A) = 0.87; HPLC K' = 2.93; mp 165−167 °C; $[\alpha]^{20}_D$ +6.2°; MH⁺ 556. Anal. (C₃₁H₃₃N₅O₅·2TFA) C, H, N.

3-(1*H*-Benzimidazol-2-yl)-3-*tert*-butoxycarbonylamino-propionic Acid Benzyl Ester [Boc−NH−(*S*)CH(CH₂−COOBzl)−Bid]. This compound was obtained by condensation of Boc−Asp(OBzl)−OH with *o*-phenylendiamine via IBCF as reported for Boc−NH−(*S*)CH(CH₂−Bid)−COOBzl: yield 1.62 g (82%); R_f(B) = 0.52; HPLC K' = 6.5; mp 136−138 °C; $[\alpha]^{20}_D$ +15.8°; MH⁺ 396; ¹H NMR (DMSO) δ 1.40 (s, 9H), 2.80 (d, 4H), 5.34−5.51 (m, 3H), 7.19−7.70 (m, 10H), 7.99 (s, 1H).

2TFA·H₂N−(*S*)CH(CH₂−COOBzl)−Bid. Boc−NH−(*S*)-CH(CH₂−COOBzl)−Bid was treated with TFA as reported for 2TFA·H₂N−(*S*)CH(CH₂−Bid)−COOBzl: yield 1.26 g (95%); R_f(A) = 0.78; HPLC K' = 4.2; mp 142−144 °C; $[\alpha]^{20}_D$ +17.3°; MH⁺ 296.

Boc−Tic−NH−(*S*)CH(CH₂−COOBzl)−Bid. This compound was obtained by condensation of Boc−Tic−OH with 2TFA·H₂N−(*S*)CH(CH₂−COOBzl)−Bid via WSC/HOBt as reported for Boc−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.76 g (84%); R_f(B) = 0.76; HPLC K' = 4.05; mp 159−161 °C; $[\alpha]^{20}_D$ +10.3°; MH⁺ 555; ¹H NMR (DMSO) δ 1.40 (s, 9H), 2.80−3.05 (m, 4H), 4.22−5.51 (m, 6H), 6.96−7.70 (m, 14H), 7.98 (s, 1H).

2TFA·H−Tic−NH−(*S*)CH(CH₂−COOBzl)−Bid. Boc−Tic−NH−(*S*)CH(CH₂−COOBzl)−Bid was treated with TFA as reported for 2TFA·H−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.62 g (91%); R_f(A) = 0.67; HPLC K' = 3.49; mp 152−154 °C; $[\alpha]^{20}_D$ +11.8°; MH⁺ 455.

Boc−Dmt−Tic−NH−(*S*)CH(CH₂−COOBzl)−Bid. This compound was obtained by condensation of Boc−Dmt−OH with 2TFA·H₂N−(*S*)CH(CH₂−COOBzl)−Bid via WSC/HOBt as reported for Boc−Dmt−Tic−NH−(*S*)CH(CH₃)−Bid: yield 0.47 g (83%); R_f(B) = 0.63; HPLC K' = 9.11; mp 171−173 °C; $[\alpha]^{20}_D$ +4.3°; MH⁺ 746; ¹H NMR (DMSO) δ 1.40 (s, 9H), 2.35−3.05 (m, 12H), 4.46−5.51 (m, 7H), 6.29 (s, 2H), 6.96−7.70 (m, 13H), 7.99 (s, 1H).

Boc−Dmt−Tic−NH−(*S*)CH(CH₂−COOH)−Bid. Boc−Dmt−Tic−NH−(*S*)CH(CH₂−COOBzl)−Bid was treated with C/Pd and H₂ as reported for Boc−Dmt−Tic−NH−(*S*)CH(CH₂−Bid)−COOH: yield 0.28 g (92%); R_f(B) = 0.79; HPLC K' = 6.9; mp 173−175 °C; $[\alpha]^{20}_D$ +5.2°; MH⁺ 656.

2TFA·H−Dmt−Tic−NH−(*S*)CH(CH₂−COOH)−Bid (4). Boc−Dmt−Tic−NH−(*S*)CH(CH₂−COOH)−Bid was treated with TFA as reported for 2TFA·H−Dmt−Tic−NH−(*S*)CH-(CH₃)−Bid: yield 0.17 g (90%); R_f(A) = 0.67; HPLC K' = 2.90; mp 164−166 °C; $[\alpha]^{20}_D$ +7.9°; MH⁺ 556. Anal. (C₃₁H₃₃N₅O₅·2TFA) C, H, N.

[2-(*tert*-Butoxycarbonylaminomethyl)benzimidazol-1-yl]acetic Acid Ethyl Ester [Boc−NH−CH₂−Bid(N¹−CH₂−COOEt)]. To a solution of Boc−NH−CH₂−Bid⁴ (0.5 g, 1.63 mmol) in DMF (10 mL) at room temperature, K₂CO₃ (0.79 g, 5.72 mmol) and, after 1 h, bromoacetic acid ethyl ester (0.19 mL, 1.73 mmol) were added. The reaction mixture was stirred for 86 h at room temperature. After DMF was evaporated, the residue was solubilized in EtOAc and washed with NaHCO₃ (5%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et₂O/Pe (1:9, v/v): yield 0.46 g (84%); R_f(B) = 0.93; HPLC K' = 4.95; mp 139−141 °C; MH⁺ 334; ¹H NMR (DMSO) δ 1.30−1.40 (m, 12H), 4.20−4.69 (m, 6H), 7.26−7.70 (m, 4H), 8.00 (bs, 1H).

2TFA·H₂N−CH₂−Bid(N¹−CH₂−COOEt). Boc−NH−CH₂−Bid(N¹−CH₂−COOEt) was treated with TFA as reported for 2TFA·H₂N−(*S*)CH(CH₂−Bid)−COOBzl: yield 0.51 g (98%); R_f(A) = 0.88; HPLC K' = 2.50; mp 222−224 °C; MH⁺ 234.

Boc−Tic−NH−CH₂−Bid(N¹−CH₂−COOEt). This compound was obtained by condensation of Boc−Tic−OH with 2TFA·H₂N−CH₂−Bid(N¹−CH₂−COOEt) via WSC/HOBt as

reported for Boc−Tic−NH−(*S*)CH(CH$_3$)−Bid: yield 0.42 g (86%); R_f(B) = 0.92; HPLC *K'* = 9.12; mp 148−150 °C; [α]$^{20}_D$ −11.6°; MH$^+$ 493; ^{1}H NMR (DMSO) δ 1.30−1.40 (m, 12H), 3.05 (m, 2H), 4.12−4.92 (m, 9H), 6.96−7.70 (m, 8H), 8.00 (bs, 1H).

2TFA·H−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOEt). Boc− Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOEt) was treated with TFA as reported for 2TFA·H−Tic−NH−(*S*)CH(CH$_3$)−Bid: yield 0.36 g (96%); R_f(A) = 0.93; HPLC *K'* = 6.11; mp 187−189 °C; [α]$^{20}_D$ −14.3°; MH$^+$ 393.

Boc−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOEt). This compound was obtained by condensation of Boc−Dmt−OH with 2TFA·H−Tic−NH−CH$_2$−Bid(N1−CH$_2$−COOEt) via WSC/ HOBt as reported for Boc−Dmt−Tic−NH−(*S*)CH(CH$_3$)−Bid: yield 0.33 g (92%); R_f(B) = 0.96; HPLC *K'* = 9.8; mp 154−156 °C; [α]$^{20}_D$ −22.0°; MH$^+$ 684; ^{1}H NMR (DMSO) δ 1.30−1.40 (m, 12H), 2.35 (s, 6H), 3.05 (m, 4H), 4.12−4.92 (m, 10H), 6.29 (s, 2H), 6.96−7.70 (m, 8H), 8.00 (m, 2H).

2TFA·H−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOEt). Boc−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOEt) was treated with TFA as reported for 2TFA·H−Dmt−Tic−NH−(*S*)CH- (CH$_3$)−Bid: yield 0.16 g (92%); R_f(A) = 0.81; HPLC *K'* = 6.40; mp 162−164 °C; [α]$^{20}_D$ −34.7°; MH$^+$ 584.

2TFA·H−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$−COOH) (9). To a solution of 2TFA·H−Dmt−Tic−NH−CH$_2$−Bid(N^1−CH$_2$− COOEt) (0.15 g, 0.22 mmol) in EtOH (10 mL) at room temperature, 1 N NaOH (1 mL, 1 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. After EtOH was evaporated, the residue was purified by preparative HPLC without any treatment: yield 0.116 g (89%); R_f(A) = 0.93; HPLC *K'* = 7.8; mp 179−181 °C; [α]$^{20}_D$ −36.2°; MH$^+$ 556. Anal. (C$_{31}$H$_{33}$N$_5$O$_5$·2TFA) C, H, N.

Biological Activity in Isolated Tissue Preparations. Preparations of the myenteric plexus longitudinal muscle, obtained from the small intestine of guinea pigs (rich in μ-opioid receptors) and preparations of mouse vas deferens (containing δ-opioid receptors), were used for field stimulation with bipolar rectangular pulses of supramaximal voltage. Agonists were evaluated for their inhibition of the electrically evoked twitch. The results were expressed as the IC$_{50}$ values obtained from concentration−response curves (Tallarida and Murray, 1986) and converted to pEC$_{50}$. The IC$_{50}$ values represent the mean ± SE of not less than five tissue samples. [D-Ala^2]Deltorphin I[11] and dermorphin[40] were used as internal standards with MVD and GPI tissue preparations, respectively.

Receptor Binding Assays. The binding affinity of all opioid analogues was determined under equilibrium conditions (2 h at room temperature) in a competition assay system using rat brain P$_2$ synaptosomes previously preincubated in 0.1 M NaCl, 0.4 mM GDP, 50 mM HEPES, pH 7.5, and 50 μg/mL soybean trypsin inhibitor for 60 min at room temperature to remove endogenous opioids.[41] After extensive washing of synaptosomes in ice-cold buffer containing protease inhibitor, the material was resuspended in buffer, pH 7.5, containing 50 μg/mL soybean trypsin inhibitor and 20% glycerol, aliquoted, and stored at −80 °C. The δ- and μ-opioid receptors were radiolabeled with [^{3}H]DPDPE (32.0 Ci/mmol; NEN-DuPont, Billerica, MA) and [^{3}H]DAGO (58.0 Ci/mmol; Amersham, Arlington Heights, IL), respectively, as described in detail elsewhere.[26] Excess unlabeled peptide (2 μM) established nonspecific binding. Labeled membranes were rapidly filtered on Whatman GF/C glass fiber filters, thoroughly washed, and dried, and the radioactivity was determined using CytoScint (ICN). All analogues were analyzed in duplicate using five to eight dosages and three to five independent repetitions using different synaptosomal preparations for each analogue (actual *n* values are listed in Table 1 in parentheses) with results given as mean ± SE. The affinity constants (*K*$_i$) were calculated according to Cheng and Prusoff.[42]

Molecular Modeling. H−Dmt−Tic−NH−CH$_2$−Bid, a δ-opioid receptor agonist (**3**), was modeled using InsightII Discover software (version 98.0) manufactured by Accelrys. Coordinates for Dmt and Tic were derived from the X-ray crystal structure of *N,N*-(Me)$_2$−Dmt−Tic−OH (δ antagonist).[29b]

Conformations describing the C-terminal modifications were generated by extensive conformational searching and energy minimization. The Dmt−Tic portion of the molecule was constrained by tethering with a force constant of 1000 kcal mol^{-1} Å^{-1} to preserve the X-ray coordinates and minimized for 1000 steps or until the root-mean-square (rms) gradient was 0.0002 kcal mol^{-1} Å^{-2} using the cff91 force field and the conjugate gradient algorithm. Minimization parameters included a distant-dependent dielectric [ε = 1.0(*r*)], and all scale terms except nonbonded and Coulombic terms were set to 1.0; the latter terms were 2.0. Conformational searches were performed via a torsion force with a force constant of 100 kcal mol^{-1} Å^{-1} that was applied to three dihedrals labeled C1 (peptide bond between Tic and NH$_2$), C2 (bond between −NH− and −CH$_2$−), and C3 (bond between −CH$_2$− and −Bid). The region evaluated ranged from 0° and 360°: C1 for two intervals (180°); C2 and C3 for six intervals (60°). All final conformers were minimized for an additional 200 iterations. The C-terminal region was evaluated using conformational searching techniques, which forces angles of rotation and does not require solvation.

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: Bid, 1*H*-benzimidazole-2-yl; Boc, *tert*-butyloxycarbonyl; Boc−Gly−Bid, Boc−βAla−Bid, Boc−Gly−Gly−Bid, benzimidazole derivatives obtained from the carboxylic functions of Boc−Gly−OH, Boc−βAla−OH, and Boc−Gly−Gly−OH, respectively; Bzl, benzyl (CH$_2$−Ph); DAGO, [D-Ala^2,*N*-Me−Phe^4,Gly-ol^5]enkephalin; DALDA, Tyr−D-Arg−Phe−Lys−NH$_2$; DCC, *N,N*-dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; Dmt, 2′,6′-dimethyl-L-tyrosine; DPDPE, *cyclic*[D-Pen^{2,5}]enkephalin; DTLET, Tyr−D-Thr−Gly−Phe−Leu; Et$_2$O, diethyl ether; EtPt, petroleum ether; GPI, guinea-pig ileum; HOBt, 1-hydroxybenzotriazole; HPLC, high-performance liquid chromatography; *K*$_e$, the antilog of pA$_2$, which is determined as the negative log of the molar concentration of the antagonist necessary to double the agonist (deltorphin B) concentration; LiAlH$_4$, lithium aluminum hydride; Me, methyl; MeOH, methanol; MVD, mouse vas deferens; NaBH$_3$CN, sodium cyanoborohydride; NMM, 4-methylmorpholine; NTI, naltrindole; NH−tBut, *tert*-butylamine; OMe, methyl ester; Pe, petroleum ether; Ph, phenyl; TEA, triethylamine; 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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