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Synthesis and Opioid Activity of *N,N*-Dimethyl-Dmt-Tic-NH-CH(R)-R′ Analogues: Acquisition of Potent δ Antagonism

Gianfranco Balboni,[a] Severo Salvadori,[b] Remo Guerrini,[b] Lucia Negri,[c] Elisa Giannini,[c] Sharon D. Bryant,[d] Yunden Jinsmaa[d] and Lawrence H. Lazarus[d,*]

[a]*Department of Toxicology, University of Cagliary, I-09126, Cagliary, Italy*
[b]*Department of Pharmaceutical Sciences and Biotechnology Center, University of Ferrara, I-44100 Ferrara, Italy*
[c]*Department of Human Physiology and Pharmacology 'Vittorio Erspamer,' University La Sapienza, I-00185 Rome, Italy*
[d]*Peptide Neurochemistry, LCBRA, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA*

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Abstract—*N,N*-Dimethylation of the H-Dmt-Tic-NH-CH(R)-R′ series of compounds produced no significant affect on the high δ-opioid receptor affinity ($K_i = 0.035$–0.454 nM), but dramatically decreased that for the μ-opioid receptor. The effect of *N*-methylation was independent of the length of the linker (R); however, the bioactivities were affected by the chemical composition of the third aromatic group (R′): phenyl (Ph) (**5′**–**8′**) elicited a greater reduction in μ-affinity (40–70-fold) compared to analogues containing 1*H*-benzimidazole-2-yl (Bid) (9-fold). The major consequences of *N,N*-dimethylation on in vitro bioactivity were: (i) a loss of δ-agonism coupled with the appearance of potent δ antagonism (**4′**–**7′**) ($pA_2 = 8.14$–9.47), while **1** exhibited only a 160-fold decreased δ agonism (**1′**) and the δ antagonism of **8** enhanced >10-fold ($pA_2 = 10.62$, **8′**); and (ii) a consistent loss of μ-affinity resulted in enhanced δ-opioid receptor selectivity. With the exception of compound **1′**, the change in the hydrophobic environment at the N-terminus and formation of a tertiary amine by *N,N*-dimethylation in analogues of the Dmt-Tic pharmacophore produced potent δ-selective antagonists.
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Introduction

N-Alkylation of opioid peptides containing the Dmt-Tic pharmacophore were initially designed to increase stability against cyclization between the C-terminal carboxylate and the N-terminal amine leading to diketopiperazine formation,[1,2] which drastically reduced both opioid receptor affinity and bioactivity.[3] While earlier studies demonstrated that *N,N*-methylation of enkephalin analogues yielded δ antagonists,[4,5] *N*-mono- or *N,N*-dimethylation of the Dmt-Tic pharmacophore also altered the pharmacologically defined profiles for δ- and μ-bioactivity in some analogues without substantially effecting δ-opioid receptor affinity.[6,7] The combined effect of *N,N*-dimethylation and C-terminal modification in the Dmt-Tic pharmacophore played a variable role on the acquisition of μ affinity and bioactivity profile. *N*-Alkylation with bulky, cyclic hydrophobic groups, such as piperidine, pyrrolidine or pyrrole, were detrimental to δ and μ bioactivity as well as affinity;[7] similar effects were observed when cyclopropylmethyl, dicyclopropylmethyl, benzyl or diallyl groups were incorporated into dynorphin A(1–11) analogues.[8]

It has been well documented that *N*-alkylation of a wide variety biologically relevant peptides[9–15] and model peptides produced physicochemical perturbations that affected their solution structure, conformation[16–20] and biological activities.[13,14,21] However, X-ray crystallography analyses of three Dmt-Tic analogues verified that *N*-methylation did not substantially effect the N-terminal conformations of these compounds.[22] In other compounds, the presence of *N*-alkylated amino acids influenced hydrogen bonding, *cis*/*trans* orientations, electrostatic interactions and stereochemistry,[18] as well as aggregation rates, conformational freedom and flexibility.[11,16] These alterations invariably affected their pharmacological properties,[13,14,21] as noted for other opioid peptides,[4,5,7,8,23–25] and the enhanced bioactivity in some *N*-alkylated peptide hormones resulted in resistance

*Corresponding author. Tel.: +1-919-541-3238; fax: +1-919-541-0696; e-mail: lazarus@niehs.nih.gov

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to proteolytic degradation.[9] However, the loss of bioactivity in other peptides was attributed to a substantial conformational change,[13] or steric hindrance within the receptor binding site by the bulky N-terminal alkylated amine.[7,8] Nonetheless, the requirement for an N-terminal amine in opioid peptides was demonstrated by replacing it with a methyl group that obliterated δ-receptor affinity in analogues of the Dmt-Tic pharmacophore.[26] In contrast, the same modification in [Dmt[1]]dynorphin A(1–11)-NH_2, a κ-opioid agonist, produced a κ antagonist.[27]

As a general principle, the increase in overall hydrophobicity of the Dmt-Tic pharmacophore greatly enhanced receptivity to μ receptors with minimal effect on δ-receptor affinity although the spectrum of bioactivity in vitro changed dramatically. Recent data indicated the importance of a linker between the C-terminus of Tic and a third aromatic center [1*H*-benzimidazole-2-yl (Bid) or phenyl (Ph)] enhanced not only μ-receptor affinity, but also converted a potent δ antagonist into an exceptional δ agonist.[28,29] This communication further explores the effect of increased hydrophobicity through *N,N*-dimethylation in a series of H-Dmt-Tic-NH-CH(R)-R′ compounds on opioid receptor binding and functional bioactivity (Fig. 1).

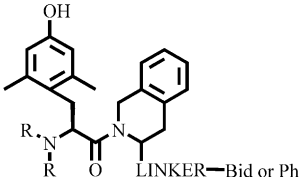


Figure 1. General structure of the reported compounds. R = H or CH_3; linker = none, CO–NH, CO–NH–CH_2, CO–NH–CH_2–CH_2, CO-Gly-NH, CO-Gly-NH–CH_2.

Results and Discussion

The receptor affinity constants (K_i values) for the eight pairs of peptides revealed that δ affinity essentially remained unaltered upon *N,N*-methylation of the Dmt-Tic pharmacophore, whereas μ affinity decreased substantially (Table 1) as seen with Dmt-Tic analogues containing C-terminal NH-*t*Bu, NHMe, 1-adamantyl[6] or a free acid functional group.[7] In our present series of compounds (Table 1), *N,N*-dimethylation decreased μ affinity by 40- (**5′**), 50- (**6′**), 60- (**8′**) and 70-fold (**7′**) relative to their non-methylated analogues (**5**–**8**), which was similar to data acquired in derivatives of the Dmt-Tic pharmacophore containing a variety of C-terminal hydrophobic groups.[7,30,31] This effect appeared to depend on the aromatic moiety (Bid or Ph) at the C-terminal of Dmt-Tic. Peptides with Ph (**5**–**8**, **5′**–**8′**) displayed a dramatic decrease in μ-affinity after *N*-alkylation compared to the derivatives containing Bid with similar alkyl linkers (**2**–**4**, **2′**–**4′**) (Table 1).

Studies reported that the length of the linker and composition of the C-terminal aromatic substituents caused a major shift in the spectrum of biological activity of the

Table 1. Opioid receptor affinity of H-Dmt-Tic-NH-CH(R)-R′ analogues with and without *N,N*-dimethylation

No.	Compound	δ (nM)	±	SE	*n*	μ (nM)	±	SE	*N*	μ/δ	Ref
1	H-Dmt-Tic-NH–CH_2-Bid	0.035	±	0.006	(3)	0.50	±	0.054	(3)	14	28
1′	*N,N*(Me)$_2$-Dmt-Tic-NH–CH_2-Bid	0.037	±	0.004	(3)	3.35	±	0.39	(3)	91	
2	H-Dmt-Tic-NH–CH_2–CH_2-Bid	0.067	±	0.015	(4)	5.49	±	0.093	(3)	82	28
2′	*N,N*(Me)$_2$-Dmt-Tic-NH–CH_2–CH_2-Bid	0.074	±	0.007	(3)	18.6	±	1.1	(3)	251	
3	H-Dmt-Tic-Gly-NH–CH_2-Bid	0.058	±	0.005	(3)	20.5	±	2.4	(3)	353	28
3′	*N,N*(Me)$_2$-Dmt-Tic-Gly-NH–CH_2-Bid	0.057	±	0.004	(4)	27.7	±	1.1	(3)	486	
4	H-Dmt-TIB	0.13	±	0.04	(4)	7.22	±	0.96	(4)	56	28, 35
4′	*N,N*(Me)$_2$-Dmt-TIB	0.454	±	0.018	(3)	66.2	±	4.7	(3)	146	
5	H-Dmt-Tic-NH–CH_2–Ph	0.069	±	0.01	(4)	0.95	±	0.23	(3)	14	
5′	*N,N*(Me)$_2$-Dmt-Tic-NH-CH_2-Ph	0.049	±	0.007	(4)	38.4	±	3.4	(3)	711	
6	H-Dmt-Tic-NH–Ph	0.093	±	0.015	(3)	1.45	±	0.23	(3)	16	
6′	*N,N*(Me)$_2$-Dmt-Tic-NH–Ph	0.099	±	0.21	(3)	72.1	±	6.8	(3)	728	
7	H-Dmt-Tic-Gly-NH–Ph	0.042	±	0.007	(3)	0.155	±	0.003	(3)	3.6	28
7′	*N,N*(Me)$_2$-Dmt-Tic-Gly-NH–Ph	0.051	±	0.008	(3)	10.7	±	0.33	(3)	210	
8	H-Dmt-Tic-Gly-NH–CH_2–Ph	0.031	±	0.002	(3)	0.163	±	0.018	(3)	5.3	28
8′	*N,N*(Me)$_2$-Dmt-Tic-Gly-NH–CH_2–Ph	0.034	±	0.003	(3)	9.44	±	0.11	(3)	278	

Me, methyl; Bid, 1*H*-benzimidazol-2-yl; TIB, 3-(1*H*-benzimidazol-2-yl)-1,2,3,4-tetrahydroisoquinolin-2-yl; Ph, phenyl. The number of independent repetitions *n* (in parentheses) used different synaptosomal preparations as summarized in the Experimental.

peptides, from being very strong δ antagonists to highly potent δ agonists.[28,29] As seen in Table 2, the functional bioactivity data indicated that *N,N*-dimethylation was detrimental to the observed δ agonist bioactivity of compounds **1**, **4**–**7**. Interestingly, **4**, **5** and **6** initially displayed weak multifunctional bioactivities, that is, weak δ agonism/δ antagonism and weak μ agonism; they became much more potent δ antagonists upon *N,N*-dimethylation while losing all bioactivity toward the μ-opioid receptor (GPI) and δ agonism as well (Table 2), except **1′** which had only a 160-fold decrease in δ agonism. In general, δ antagonism increased more than 10-fold for **2′**, **3′** and **8′**, and at the same time μ bioactivity decreased, whereas with **5′** and **6′** δ-receptor antagonism increased by more than two orders of magnitude and **8′** displayed a remarkably high δ-antagonism ($pA_2 = 10.62$), which accompanied a 1,300-fold decrease in μ-agonist activity (Table 2). Thus, the data indicated that μ agonism decreased substantially in all the analogues studied (**1′**–**8′**) (Table 2), which contrasted to the effects generated by non-*N,N*-dimethylated derivatives of the H-Dmt-Tic series of pseudopeptides.[7,28,29]

The effect of *N,N*-dimethylation on the biological activity of Dmt-Tic-containing peptides apparently involves several interrelated factors: (i) Stabilization against cyclization (diketopiperazine formation),[1,2] which is a spontaneous reaction that greatly reduced the interaction with δ receptors and eliminated biological activity.[3] (ii) Limitation in the rotation of the N-terminal amine due to steric hindrance by the bulky *N,N*-dimethyl groups[16,18] allowing the potential formation of more stable hydrogen bonds.[6] (iii) Increasing the hydrophobic interactions between the ligand and the receptor binding site could involve the spatial positioning of the pharmacophore[32] deduced from X-ray diffraction analyses of three Dmt-Tic pharmacophore analogues, two of which contained *N,N*-dimethylation.[22] (iv) Enhancement of the δ antagonist activity suggesting that *N,N*-dimethylation interfered with or inhibited the mechanism(s) of activating the δ-opioid receptor that gave rise to δ- or μ-receptor agonism.

Table 2. Functional Bioactivity of H-Dmt-Tic-NH-CH(*R*)-R′ analogues with and without *N,N*-dimethylation

No.	MVD IC_{50} (nM)	±	SE	MVD pA_2	GPI IC_{50} (nM)	±	SE	Ref
1	0.035	±	0.003	—	40.7	±	5	28
1′	5.6	±	0.4	—	132.6	±	15	
2	—			8.32	107.5	±	11.4	28
2′	—			9.67	7152	±	350	
3	—			9.00	400			28
3′	—			9.90	>10 μM			
4	52.5			7.90	30	±	1.5	28, 35
4′	—			8.14	>10 μM			
5	111.0	±	13.4	6.01	850	±	110	
5′	—			9.51	>10 μM			
6	171.6	±	8.07	6.81	110	±	13	
6′	—			9.07	683	±	55	
7	3.0			—	2.57			28
7′				9.47	1720	±	112	
8	—			9.25	2.69			28
8′	—			10.62	3652	±	202	

Antagonist activity is defined by pA_2. Inactivity is denoted by a dash (—).

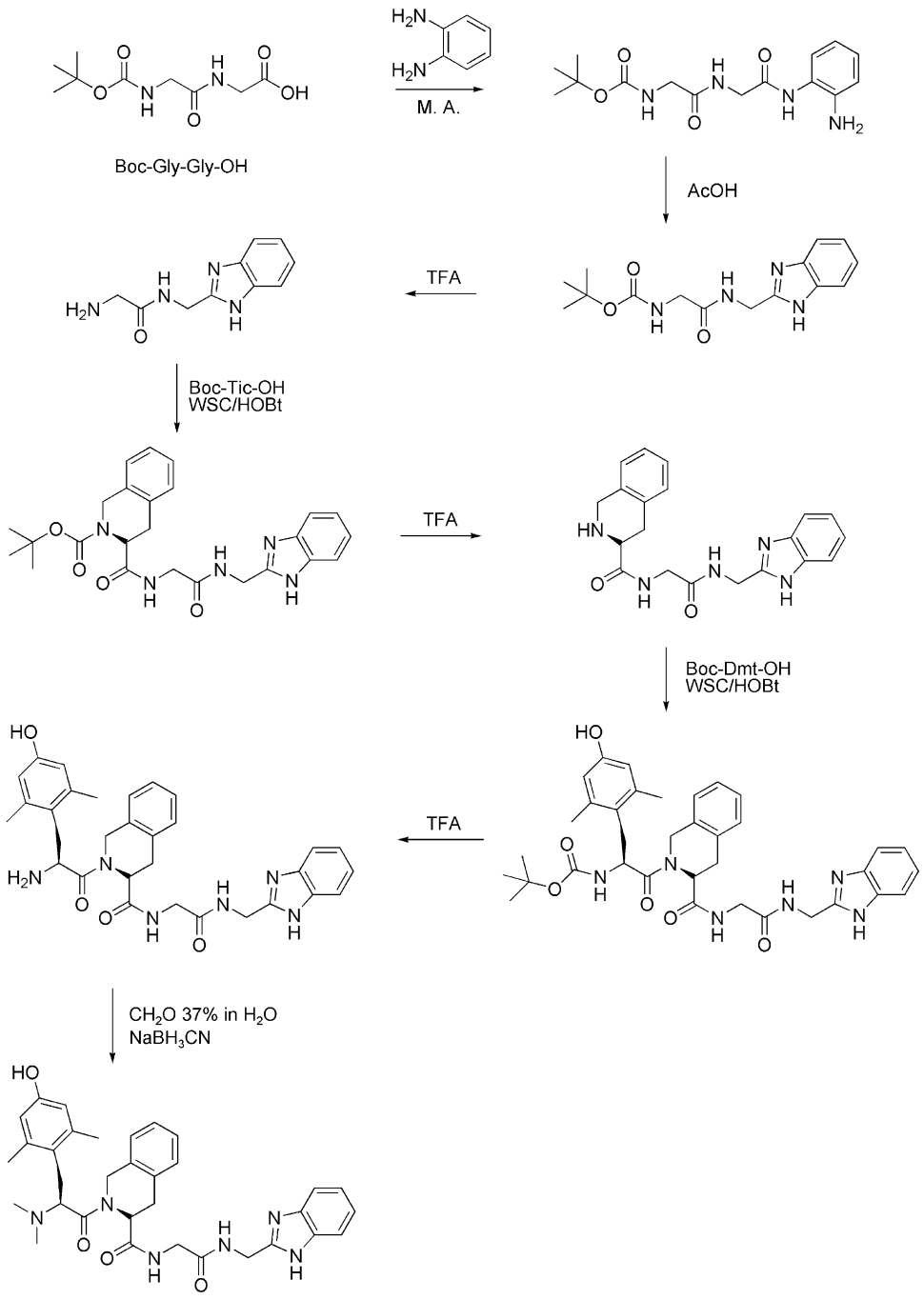
Conclusions

N,N-Dimethylation of C-terminally extended Dmt-Tic pharmacophore analogues containing an alkyl linker to a third aromatic center, either Ph or a Bid, had the following effects: (i) Minimal alteration of δ-opioid receptor affinity; (ii) reduction in the μ-receptor affinity relative to non-methylated analogues; (iii) drastically decreased δ-opioid receptor agonism while enhancing δ antagonism; and (iv) eliminated or greatly reduced the μ bioactivity for all compounds. The major consequence was the acquisition of potent δ-opioid receptor antagonism in several analogues, the degree to which this occurred involved the chemical composition of the third aromatic residue. These compounds may play a role determining the function of distinct opioid receptors in non-knockout animal models and might find potential clinical and therapeutic application as stable and highly selective δ-opioid receptor antagonists with enhanced hydrophobicity required for passage through membrane barriers.[26]

Experimental

Chemistry

Scheme 1 summarizes the basic synthesis of *N,N*-dimethyl-Dmt-Tic-NH-CH(R)-R′ analogues. H-L-Dmt-OH was prepared according to Dygos et al.,[33] and purity and chirality were compared to a sample generously donated by Dygos et al. Boc-Tic-OH was purchased from Bachem (Heidelberg, Germany). All pseudopeptides were prepared by standard solution step-by-step peptide synthesis. Mixed carbonic anhydride coupling of Boc-Gly-OH, Boc-βAla-OH or Boc-Gly-Gly-OH with *o*-phenylendiamine gave the corresponding crude intermediate amides, which were converted without purification to the desired heteroaromatic derivatives by cyclization and dehydration in acetic acid. After deprotection with TFA, each derivative was condensed with Boc-Tic-OH and then with Boc-Dmt-OH via WSC/HOBt. Di- and tripeptides containing C-terminal benzyl amide or phenyl amide were obtained in a similar manner starting from the condensation of the appropriate Boc-amino acid with benzyl amine or aniline via WSC/HOBt, respectively. *N,N*-dimethylation was accomplished with 37% aqueous formaldehyde and $NaBH_3CN$ according to the procedure of Borch and Hassid as outlined in Scheme 1.[34] Crude pseudopeptides were purified by preparative reversed-phase high performance liquid chromatography (HPLC) using a Waters Delta Prep 4000 system with Waters Prep LC 40 mm Assembly C_{18} column (30×4 cm; 15 μm particle size). 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% solvent B (60% acetonitrile in 0.1% TFA, v/v) in 25 min was adopted for the elution of the products. Analytical



Scheme 1. Synthesis of *N,N*-(Me)$_2$-Dmt-Tic-Gly-NH–CH$_2$-Bid (**3′**).

HPLC analyses were performed with a Beckman System Gold with a Beckman ultrasphere ODS column (250×4.6 mm, 5 μm). Analytical determinations and capacity factor (K') of the products were determined using the HPLC in the above solvent systems (solvents A and B) programmed at flow rates 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 nm.

TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany) using the following solvent systems: (A) 1-butanol/AcOH/H_2O (3:1:1, v/v/v); and (B) CH_2Cl_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 chromatography (2×70 cm, 0.7–1 g 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 ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer. MALDI-TOF analyses (matrix assisted laser desorption ionization time-of-flight mass spectrometry) of peptides were 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.

Peptide synthesis

2TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH-CH$_2$-Bid (1′). To a stirred solution of 2TFA·H-Dmt-Tic-NH–CH$_2$-Bid[28] (0.29 g, 0.40 mmol) in acetonitrile/H$_2$O (1:1, v/v) (10 mL) were added NMM (0.09 mL, 0.80 mmol), 37% aqueous formaldehyde (0.32 mL, 4 mmol) and sodium cyanoborohydride (0.08 g, 1.2 mmol). Glacial acetic acid (0.04 mL) was added over 10 min and the reaction was stirred at room temperature for 15 min. The reaction mixture was acidified with TFA (0.1 mL) and directly purified by preparative HPLC: yield 0.26 g. (88%); R_f (A) 0.35; HPLC $K'=5.70$; mp 158–160 °C; $[\alpha]_D^{20}$ −31.8; MH$^+$ 527.

2TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–CH$_2$–CH$_2$-Bid (2′). This was obtained by exhaustive methylation of 2TFA·H-Dmt-Tic-NH–CH$_2$–CH$_2$-Bid[28] as reported for 2TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–CH$_2$-Bid: yield 0.048 g. (81%); R_f (A) 0.38; HPLC $K'=5.29$; mp 165–167 °C; $[\alpha]_D^{20}$ −27.2; MH$^+$ 541.

2TFA·*N,N*-(Me)$_2$-Dmt-Tic-Gly-NH–CH$_2$-Bid (3′). This was obtained by exhaustive methylation of 2TFA·H-Dmt-Tic-Gly-NH–CH$_2$-Bid[28] as reported for 2TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–CH$_2$-Bid: yield 0.048 g. (89%); R_f (A) 0.39; HPLC $K'=4.76$; mp 159–161 °C; $[\alpha]_D^{20}$ −27.2; MH$^+$ 584.

2TFA·*N,N*-(Me)$_2$-Dmt-3-(1*H*-benzimidazol-2-yl)-1,2,3,4-tetrahydroisoquinoline-2-yl (TIB) (4′). This was obtained by exhaustive methylation of 2TFA·H-Dmt-TIB[35,28] as reported for 2TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–CH$_2$-Bid: yield 0.058 g. (90%); R_f (A) 0.48; HPLC $K'=3.92$; mp 168–170 °C; $[\alpha]_D^{20}$ −35.1; MH$^+$ 470.

TFA·*N,N*-(Me)$_2$-Dmt-Tic-Gly-NH–Ph (7′). To a stirred solution of TFA·H-Dmt-Tic-Gly-NH–Ph[28] (0.1 g, 0.17 mmol) in acetonitrile/H$_2$O (1:1, v/v) (10 mL) were added NMM (0.02 mL, 0.17 mmol), 37% aqueous formaldehyde (0.14 mL, 1.7 mmol) and sodium cyanoborohydride (0.032 g, 0.51 mmol). Glacial acetic acid (0.04 mL) was added over 10 min and the reaction was stirred at room temperature for 15 min. The reaction mixture was acidified with TFA (0.1 mL) and directly purified by preparative HPLC: yield 0.08 g. (84%); R_f (A) 0.48; HPLC $K'=9.36$; mp 155–157 °C; $[\alpha]_D^{20}$ −18.3; MH$^+$ 487.

Boc-Tic-NH–CH$_2$–Ph. This compound was obtained by condensation of Boc-Tic-OH with benzylamine as reported for Boc-Gly-NH–CH$_2$–Ph:[28] yield 0.81 g. (87%); R_f (B) 0.81; HPLC $K'=12.1$; mp 132–134 °C; $[\alpha]_D^{20}$ −40.8; MH$^+$ 367; ^{1}H NMR (DMSO) δ = 1.39, 1.46 (2×s, 9H), 3.06 (m, 2H), 4.22–4.96 (m, 5H), 6.95–7.15 (m, 9H), 8.26 (m, 1H).

TFA·H-Tic-NH–CH$_2$–Ph. Boc-Tic-NH–CH$_2$–Ph was treated with TFA as reported for TFA·H-Gly-NH–Bzl:[28] yield 0.31 g. (98%); R_f (A) 0.52; HPLC $K'=6.51$; mp 155–157 °C; $[\alpha]_D^{20}$ −31.8; MH$^+$ 267.

Boc - Dmt - Tic - NH–CH$_2$–Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-NH–CH$_2$–Ph as reported for Boc-Dmt-Tic-Gly-NH–CH$_2$–Ph:[28] yield 0.15 g. (83%); R_f (B) 0.73; HPLC $K'=10.9$; mp 145–147 °C; $[\alpha]_D^{20}$ −22.6; MH$^+$ 558; ^{1}H NMR (DMSO) δ = 1.40, 1.42 (2×s, 9H), 2.17 (s, 6H), 3.08–3.15 (m, 4H), 4.22–4.92 (m, 5H), 6.37 (s, 2H), 6.91–7.31 (m, 10H), 8.25 (m, 1H).

TFA·H - Dmt - Tic - NH–CH$_2$–Ph. Boc-Dmt-Tic-NH–CH$_2$–Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Gly-NH–CH$_2$–Ph:[28] yield 0.07 g. (96%); R_f (A) 0.49; HPLC $K'=9.03$; mp 158–160 °C; $[\alpha]_D^{20}$ −23.4; MH$^+$ 458.

TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–CH$_2$–Ph (5′). This was obtained by exhaustive methylation of TFA·H-Dmt-Tic-NH-CH$_2$-Ph as reported for TFA·*N,N*-(Me)$_2$-Dmt-Tic-Gly-NH-Ph: yield 0.66 g. (91%); R_f (A) 0.52; HPLC $K'=9.36$; mp 164–166 °C; $[\alpha]_D^{20}$ −21.9; MH$^+$ 487.

Boc-Tic-NH–Ph. This compound was obtained by condensation of Boc-Tic-OH with aniline as reported for Boc-Gly-NH–CH$_2$–Ph:[28] yield 0.78 g. (85%); R_f (B) 0.78; HPLC $K'=11.5$; mp 139–141 °C; $[\alpha]_D^{20}$ −31.5; MH$^+$ 353; ^{1}H NMR (DMSO) δ = 1.40, 1.42 (2×s, 9H), 3.06–3.15 (m, 2H), 4.22–4.92 (m, 3H), 6.95–7.15 (m, 9H), 8.96 (bs, 1H).

TFA·H-Tic-NH–Ph. Boc-Tic-NH–Ph was treated with TFA as reported for TFA·H-Gly-NH–CH$_2$–Ph:[28] yield 0.27 g. (96%); R_f (A) 0.48; HPLC $K'=6.27$; mp 150–152 °C; $[\alpha]_D^{20}$ −33.1; MH$^+$ 253.

Boc-Dmt-Tic-NH–Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-NH–Ph as reported for Boc-Dmt-Tic-Gly-NH–CH$_2$–Ph;[28] yield 0.16 g. (82%); R_f (B) 0.69; HPLC $K'=10.1$; mp 140–142 °C; $[\alpha]_D^{20}$ −24.1; MH$^+$ 544; ^{1}H NMR (DMSO) δ = 1.40, 1.42 (2×s, 9H), 2.17 (s, 6H), 3.08–3.15 (m, 4H), 4.22–4.92 (m, 4H), 6.37 (s, 2H), 6.91–7.31 (m, 10H), 8.95 (bs, 1H).

TFA·H - Dmt - Tic - NH–Ph. Boc-Dmt-Tic-NH–Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Gly-NH–CH$_2$–Ph:[28] yield 0.065 g. (97%); R_f (A) 0.46; HPLC $K'=8.25$; mp 146–148 °C; $[\alpha]_D^{20}$ −25.8; MH$^+$ 445.

TFA·*N,N*-(Me)$_2$-Dmt-Tic-NH–Ph (6′). This product was obtained by exhaustive methylation of TFA·H-Dmt-Tic-NH–Ph as reported for TFA·*N,N*-(Me)$_2$-Dmt-Tic-Gly-NH–Ph: yield 0.58 g (89%); R_f (A) 0.51; HPLC $K'=8.39$; mp 149–151 °C; $[\alpha]_D^{20}$ −27.5; MH$^+$ 472.

Boc-Tic-Gly-NH–Ph. This compound was obtained by condensation of Boc-Tic-OH with TFA·H-Gly-NH–Ph as reported for Boc-Tic-Gly-NH–Bzl: yield 0.24 g. (85%); R_f (B) 0.67; HPLC $K'=10.73$; mp 131–133 °C; $[\alpha]_D^{20}$ −36.8; MH$^+$ 410; ^{1}H NMR (DMSO) δ = 1.39, 1.41 (2×s, 9H), 3.08–3.15 (m, 2H), 3.61 (d, 2H), 4.22–4.92 (m, 3H), 6.91–7.31 (m, 10H), 8.96 (bs, 1H).

TFA·H-Tic-Gly-NH–Ph. Boc-Tic-Gly-NH–Ph was treated with TFA as reported for TFA·H-Tic-Gly-NH–Bzl: yield 0.18 g. (96%); R_f (A) 0.35; HPLC $K' = 6.07$; mp 165–167 °C; $[\alpha]_D^{20}$ −32.5; MH^+ 310.

Boc-Dmt-Tic-Gly-NH–Ph. This compound was obtained by condensation of Boc-Dmt-OH with TFA·H-Tic-Gly-NH–Ph as reported for Boc-Dmt-Tic-Gly-NH–Bzl: yield 0.14 g. (84%); R_f (B) 0.64; HPLC $K' = 9.9$; mp 144—146 °C; $[\alpha]_D^{20}$ −19.7; MH^+ 601; ^1H NMR (DMSO) δ = 1.40, 1.42 (2×s, 9H), 2.17 (s, 6H), 3.08–3.15 (m, 4H), 3.61 (d, 2H), 4.22–4.92 (m, 3H), 6.37 (s, 2H), 6.91–7.31 (m, 11H), 8.94 (bs, 1H).

TFA·H-Dmt-Tic-Gly-NH–Ph. Boc-Dmt-Tic-Gly-NH-Ph was treated with TFA as reported for TFA·H-Dmt-Tic-Gly-NH-Bzl: yield 0.07 g. (97%); R_f (A) 0.41; HPLC $K' = 7.18$; mp 155–157 °C; $[\alpha]_D^{20}$ −21.8; MH^+ 444.

TFA·*N,N*-$(Me)_2$-Dmt-Tic-Gly-NH–CH_2–Ph (8′). This was obtained by exhaustive methylation of TFA·H-Dmt-Tic-Gly-NH–CH_2–Ph[28] as reported for TFA·*N,N*-$(Me)_2$-Dmt-Tic-Gly-NH–Ph: yield 0.07 g. (86%); R_f (A) 0.45; HPLC $K' = 9.19$; mp 158–160 °C; $[\alpha]_D^{20}$ −20.4; MH^+ 473.

Biological assays

Receptor binding. Receptor affinities of the peptides were obtained using rat brain synaptosome preparations under equilibrium binding conditions as described previously[6,7] (Table 1). The δ-opioid receptors were labeled with [^{3}H]DPDPE (32.0 Ci/mmol; NEN-DuPont, Billerica, MA, USA) and μ-opioid receptors with [^{3}H]DAGO (58.0 Ci/mmol: Amersham, Arlington Heights, IL, USA) in which 2 μM unlabeled peptide established the level of nonspecific binding. The K_i values were determined according to Cheng and Prusoff[36] and given as the mean ± standard error (SE) with *n* (in parentheses) to indicate between three and five independent repetitions with different synaptosomal preparations for each analogue.

Functional bioactivity

Functional bioassays in vitro utilized guinea-pig ileum (myenteric plexus longitudinal muscle from the small intestine; GPI) for μ receptors and mouse vas deferens (MVD) for δ receptors. Agonism was determined by inhibition of electrically stimulated relaxation of muscle twitch as published previously.[29] The IC_{50} values represent the mean ± SE of five tissue samples based on deltorphin B and dermorphin as the internal standards for MVD and GPI assays, respectively. The pA_2 is the negative log of the molar concentration necessary to double the agonist concentration (deltorphin B) to achieve the original response.

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