Novel C-Terminus Modifications of the Dmt-Tic Motif: A New Class of Dipeptide Analogues Showing Altered Pharmacological Profiles Toward the Opioid Receptors

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Received May 8, 2001

Abstract: The design, synthesis and pharmacological evaluation of a novel class of Dmt-Tic dipeptide analogues are described. These resulting analogues bearing different C-terminal functionalities were found to bind to the human δ receptor with high affinity. One specific class of dipeptides bearing urea/thiourea functionalities showed partial to full activation of the δ receptor. Several dipeptides also showed good binding affinities with full activation of the human κ receptor, a novel property for those ligands.

Introduction. The discovery of the first class of opioid peptide analogues (H-Tyr-Tic-Phe-Phe; TIPP) incorporating a Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) residue in the position 2 of enkephalin derivatives was a major breakthrough in the development of selective δ ligands.[1] Extensive work on the TIPP opioid family analogues gave birth to a diversity of peptide derivatives demonstrating a wide range of agonist/antagonist effects[2] toward the different opioid receptors. In fact, the H-Tyr-Tic-OH dipeptide was shown to be the smallest fragment that still retained potent δ antagonist properties. But substitution of the Tyr by Dmt (2′,6′-dimethyltyrosine)[3] produced a dipeptide which exhibited improved δ opioid affinity having even enhanced δ antagonist properties. Such synthetic peptides not only represent relevant pharmacological tools for the study of opioid receptors but also show potential therapeutic uses since δ opioid antagonists are known to possess immunosuppressive activity[4] and to alleviate effects of drug addictions.[5]

Within the past decade, several structural and chemical modifications on the Dmt-Tic pharmacophore have been reported, modifications affecting the overall binding properties of these dipeptides.[6] Many N-terminal modifications, such as mono- or dimethylation,[6b,7] peptide bond reductions,[8] or incorporation of a β-methyl-2′,6′-Dmt[9] proved to greatly increase the δ affinity, selectivity, and antagonist potency over the other opioid receptors. However, N-alkylation with larger groups such as piperidine, pyrrole, or pyrrolidine[6a,10] decreased both the δ selectivity and antagonist potency. The importance of the Tic motif was also demonstrated by replacement with various heteroaromatic residues producing peptide analogues with decreased δ binding affinites.[11] Further substitutions at positions 5, 6, and 7 of the Tic moiety resulted in analogues showing altered opioid receptor binding affinities.[12]

Modifications on the C-terminus of Dmt-Tic have also been reported and shown to represent crucial features that influence the pharmacological profile of these peptides. Elimination of the negative charge from the carbonyl function of Tic by amidation or reduction,[3] or simple deletion of the carbonyl functionality,[12b] proved to greatly reduce the μ/δ selectivity ratio. Furthermore, Lazarus and co-workers[10] reported that incorporation of lipophilic substituents at the C-terminus of Dmt-Tic yielded δ antagonists also having enhanced μ affinity, therefore providing ligands with bifunctional activity. Analogous observations were also previously reported when lipophilic substituents were inserted at the C-terminus of the H-Tyr-Tic dipeptide motif.[13] Because of the potential clinical effectiveness of such ligands, opioids having dual activities[14] could represent targets of choice not only in pain management but also in transplantation studies.[15] However, most classes of peptides with C-terminal modifications reported so far were restricted to amide bonds resulting in analogues showing, with a few exceptions,[16] mainly δ antagonist effects. To enlarge the scope of these structural modifications, we report herein the synthesis and pharmacological characterizations of a larger series of Dmt-Tic analogues bearing modified methylene-extended C-terminal functionalities.

Chemistry. The aim of this study was to prepare a series of Dmt-Tic analogues having a diversity of modified C-terminal functionalities such as size, orientation, charge, and H-bonding character that could affect the opioid receptors interactions.

Boc-L-Tic-OH **1** (Scheme 1) was first reduced to the corresponding alcohol **2** using a previously reported mixed anhydride procedure.[17] The resulting alcohol was either reduced to the corresponding alkane (**3**), esterified (**4**), or alkylated to form the corresponding methyl (**5**), allyl (**6**), and benzyl (**7**) ethers (61−63% yields). The alcohol derivative **2** was also protected under its *tert*-butyldimethylsilyl form **8** following previous nitrogen deprotection. Furthermore, the alcohol **2** was also transformed into the azide **9** in 86% yield using standard Mitsunobu conditions. After N-deprotection under acidic conditions (1 M HCl/AcOH), compounds **2**−**7**, along with silyl **8** and azide **9**, were coupled to Boc-L-Dmt-OH using standard peptide coupling strategy. The L-isomer of Dmt was used in this study since it is well established that the affinity of L-Dmt containing peptides for opioid receptors is greater than those containing the D-isomer. Carbodiimide coupling reagents (DCC or EDC) were found to give better and more consistent results for this coupling step compared to other coupling reagents such as HATU, TBTU, or DPPA. Final removal of the *tert*-butoxy N-protecting groups afforded the dipeptide analogues **10**−**15** and **17** with overall yields of 25−80%.

The other series of Dmt-Tic analogues based on a nitrogen scaffold were prepared via a similar route.

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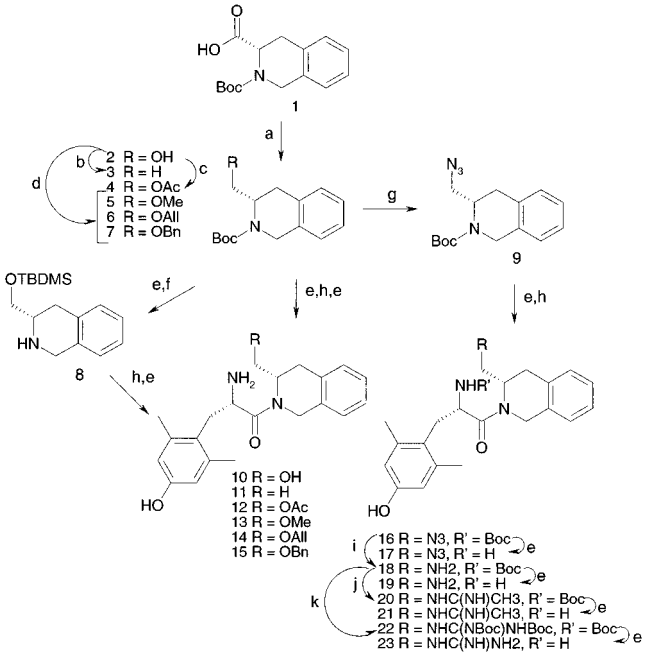
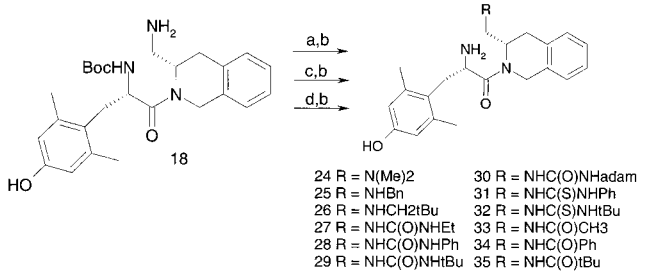
10.1021/jm015532k CCC: $20.00
Published on Web 06/16/2001

Scheme 1[a]



[a] Reagents and conditions: (a) (i) isobutyl chloroformate, NMM, DME, 0 °C, 5 min; (ii) $NaBH_4$, H_2O, 0 °C → rt, 20 min; (b) (i) MsCl, TEA, CH_2Cl_2, 0 °C → rt, 1 h; (ii) $LiAlH_4$, THF, −78 °C → rt, 24 h; (c) AcCl, DIPEA, CH_2Cl_2, 0 °C, 45 min; (d) R-I/R-Br, NaH, DMF, 0 °C → rt, 2 h; (e) 1 M HCl/AcOH, rt, 1−24 h; (f) TBDMS-Cl, TEA, DMF, 0 °C → rt, 5 h; (g) PPh_3, DEAD, DPPA, THF, 0 °C → rt, 4 h; (h) Boc-L-Dmt-OH, DCC, HOBt, DIPEA, DMF, 0 °C → rt, 24 h; (i) H_2, 10% Pd/C, MeOH, rt, 18 h; (j) methyl acetimidate hydrochloride, DIPEA, DMF, rt, 24 h; (k) 1-*H*-pyrazole-1-[*N*,*N*′-bis(*tert*-butoxycarbonyl)]carboxamidine, DIPEA, DMF, 35 °C, 18 h.

Scheme 2[a]

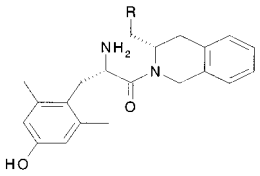


[a] Reagents and conditions: (a) RCHO, $NaCNBH_3$, MeOH/AcOH, 0 °C → rt, 1 h; (b) 1 M HCl/AcOH, rt, 1 h; (c) R−N═C═O/R−N═C═S, DIPEA, CH_2Cl_2, rt, 18 h; (d) RC(O)Cl, DIPEA, CH_2Cl_2, 0 °C → rt, 1 h.

Dipeptide **16** having the azido side chain could also be reduced to its corresponding amine derivative by catalytic hydrogenation to yield intermediate **18**. This resulting dipeptide was either deprotected under acidic conditions to give the diamino-peptide **19** in 52% yield or transformed to its corresponding amidine **20** or guanidine[18] **22** derivative. These two dipeptides were then fully deprotected to afford Dmt-Tic analogues **21** and **23** in 21−27% yield (three steps).

Intermediate **18** was also used to prepare three other classes of dipeptide derivatives (amino, urea/thiourea, and amido) (Scheme 2). The amine **18** reacted with formaldehyde, benzaldehyde, or trimethylacetaldehyde under reductive amination conditions, followed by removal of the nitrogen Boc-protecting group to afford the resulting amino-dipeptide analogues **24**−**26** in 15−64% yield. Ureas **27**−**30** and thioureas **31**−**32** were obtained similarly from dipeptide **18** after reaction with different isocyanates/isothiocyanates in dichloromethane. Finally, the amido-dipeptides **33**−**35** were obtained in 20−50% yields by reacting different acid chlorides to amine **18** prior to N-deprotection.

Table 1. Binding Affinities of Dmt-Tic Analogues for Opioid Receptors



	R[a]	δ IC_{50} (nM)	μ IC_{50} (nM)	κ IC_{50} (nM)	μ/δ ratio	κ/δ ratio
Deltorphin II		1.3	225	7330	173	5640
Dmt-Tic		1.6	894	37500	558	23400
10	OH	2.0	421	>10000	211	>5000
11	H	1.7	3.0	764	1.8	450
12	OAc	1.1	4.7	3100	4.3	2820
13	OMe	1.3	458	2510	352	1930
14	OAll	0.98	108	1450	110	1480
15	OBn	0.33	12.5	64.6	37.9	196
17	N_3	1.1	94.6	1070	86.0	973
19	NH_2	4.9	113	521	23.1	106
21	$NHC(NH)CH_3$	9.9	33.2	996	3.4	101
23	$NHC(NH)NH_2$	3.2	12.8	345	4.0	108
24	$N(Me)_2$	0.97	83.2	793	85.8	818
25	NHBn	0.41	0.65	1.5	1.6	3.7
26	$NHCH_2t$-Bu	0.99	1.3	1.5	1.3	1.5
27	NHC(O)NHEt	0.99	169	321	171	324
28	NHC(O)NHPh	0.13	1.0	4.8	7.7	36.9
29	NHC(O)NH*t*-Bu	0.17	61.8	1.3	364	7.6
30	NHC(O)NHadam	0.29	1.1	4.1	3.8	14.1
31	NHC(S)NHPh	0.27	5.4	51.4	20.0	190
32	NHC(S)NH*t*-Bu	0.22	36.0	2.8	164	12.7
33	NHAc	0.79	16.1	9380	20.3	11900
34	NHC(O)Ph	0.24	0.98	307	4.1	1280
35	NHC(O)*t*-Bu	0.79	25.3	394	32.0	499

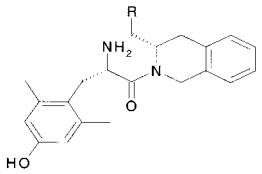
[a] R groups: Ac, acetate; Me, methyl; All, allyl; Bn, benzyl; *t*-Bu, *tert*-butyl; Et, ethyl; adam, 1-adamantyl; Ph, phenyl.

The final compounds were purified by reversed-phase chromatography and isolated and tested as their corresponding TFA salts. ^1H NMR of the final dipeptides clearly demonstrated in some cases the presence of cis/trans isomers[19] arising from the Dmt-Tic peptide bond. This cis/trans ratio seemed to vary according to the size of the C-terminal group and/or to the presence of a carbonyl-type functionality and the C-terminus. No tentative assignment was addressed to identify which of the two isomers was predominantly present for the different dipeptides. The Dmt-Tic analogues were also found to be much more stable in aqueous conditions since the well-known diketopiperizine formation[20] is excluded for these classes of dipeptide analogues.

Results and Discussion. The binding affinities (IC_{50}) of all compounds were determined using cloned human δ, μ, and κ opioid receptors, along with their corresponding agonist potencies (EC_{50}) using GTPγ[^{35}S] binding assays for the δ receptor. Only selected compounds having IC_{50} values in the 1−5 nM range were tested for GTPγ[^{35}S] binding assays on μ and κ opioid receptors. The opioid receptor binding affinities and selectivity of the dipeptides are listed in Table 1 and compared to the values for Deltorphin II and Dmt-Tic. The GTPγ[^{35}S] binding values for δ, μ, and κ are reported in Table 2.

Reduction of the carboxyl group to the alcohol provided dipeptide **10** which showed binding affinities and

Table 2. GTPγ[^{35}S] Binding Assays of Dmt-Tic Dipeptide Analogues on Opioid Receptors



		δ		μ		κ	
	R	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
Deltorphin II		4.71	78.7	1130	21.8	ns	
10	OH	ns		ns		nd	
11	H	ns		nd		nd	
12	OAc	ns		ns		nd	
13	OMe	ns		nd		nd	
14	OAll	ns		nd		nd	
15	OBn	0.97[a]	26.9	ns		nd	
17	N_3	49.4[a]	10.4	nd		nd	
19	NH_2	ns		nd		nd	
24	$N(Me)_2$	ns		nd		nd	
25	NHBn	0.92[a]	31.5	37.1[a]	50.2	6.8	100
26	$NHCH_2t$-Bu	2.8[a]	24.3	ns		1.4	102
27	NHC(O)NHEt	5.7[a]	67.0	nd		nd	
28	NHC(O)NHPh	0.75	92.9	18.8[a]	60.1	161	81.7
29	NHC(O)NH*t*-Bu	0.65	95.0	ns		6.9	96.9
30	NHC(O)NHadam	ns		ns		11.5	81.7
31	NHC(S)NHPh	1.7	128	nd		nd	
32	NHC(S)NH*t*-Bu	0.32[a]	74.7	nd		24.3	99.6
33	NHAc	1.50[a]	16.9	nd		nd	
34	NHC(O)Ph	ns		109[a]	42.9	nd	
35	NHC(O)*t*-Bu	ns		nd		nd	

[a] EC_{50} values are reported as $EC_{max/2}$ for partial agonists. ns: inactive; no stimulative effect up to 1 μM. nd: not determined.

selectivity (μ/δ: 211) within the same range as previously reported values.[6b] Reincorporation of a carbonyl functionality by acetylation of the alcohol gave dipeptide **12** which kept good δ binding affinity (1.1 nM) but suffered from a 25-fold loss in μ/δ selectivity. Ethers **13**−**15** showed great binding affinities toward the δ receptor, proving once again that a carboxyl/carbonyl functional group is not required for δ binding.[21] Furthermore, the δ binding affinity seemed to increase with the bulkiness of the side chain, going from 1.3 nM for methoxy to 0.33 nM for benzyloxy. However, the selectivity over the μ and κ opioid receptors was also shown to decrease with bulkier side chains. The increased binding affinity for the μ receptor could also be related to the hydrophobicity of the C-terminal substituents, an observation previously reported for similar analogues.[10] For comparison, the D-Tic isomer of dipeptide **15** was also prepared via a similar synthesis route (not shown) but showed much lower δ binding affinity,[22] proving again the importance of the C-terminus chirality. Dipeptide **11**, in which the C-terminus lacks any dipole (R=H), proved that hydrogen bonding is not required in receptor binding (IC_{50} 1.7 nM). Incorporation of a C-terminal azido group resulted in dipeptide **17** which still retained good binding affinity (IC_{50} 1.1 nM) while maintaining respectable selectivity over the other receptors.

The insertion of basic residues was shown to be somehow detrimental as dipeptides **19**, **21**, and **23**, bearing primary amino-, amidino-, and guanidino-moieties, respectively, demonstrated the lowest δ binding affinities (3.2 to 9.9 nM) of all the dipeptides reported herein. However, amine alkylations produced analogues with restored binding affinities below 1 nM. As observed for the ether analogues, incorporation of bulkier and more hydrophobic groups such as benzyl **25** and *tert*-butyl **26** yielded nonselective compounds. These two latter dipeptides demonstrated particularly good binding toward the κ receptor with affinities of 1.5 nM as well as full receptor activation with EC_{50} values of 6.8 and 1.4 nM, respectively, which represents, to our knowledge, a property that has not been reported for Dmt-Tic analogues.

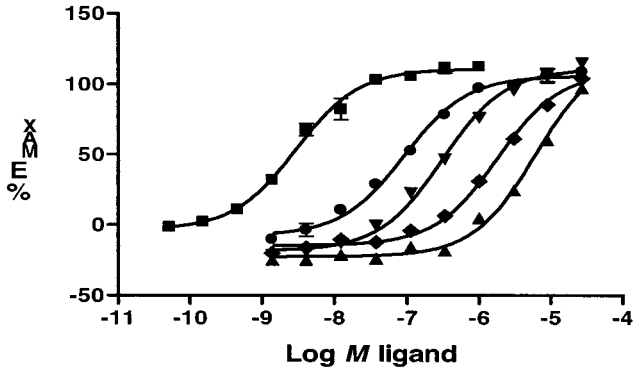


Figure 1. Effect of 100 nM dipeptides on SNC-80 in the binding of GTPγ[^{35}S] to δ opioid receptor: (■) SNC-80 alone (1 μM); (▲) SNC-80 + dipeptide **30**; (◆) SNC-80 + dipeptide **34**; (▼) SNC-80 + dipeptide **35**; (●) SNC-80 + Dmt-Tic.

The urea/thiourea functionalities greatly influenced the nature of the dipeptides. Impressive δ binding affinities were obtained in this series showing IC_{50} values down to 0.13 nM (compound **28**). The presence of the *tert*-butyl side chain seemed to greatly improve the μ/δ selectivity compared to analogues containing a phenyl group. Dipeptide **30**, bearing an adamantyl side chain, also bound well to all three opioid receptors. Furthermore, these compounds were able to partially or even fully activate the δ and κ receptors (Table 2). The binding and activation of the κ receptor represent novel properties for such types of analogues. The only exception of this class was compound **30**, bearing the adamantyl side chain, which acted as a powerful δ antagonist, as shown in the GTPγ[^{35}S] functional assay (Figure 1), by blocking the effect of the δ selective opioid agonist SNC-80[23] with a K_e value of 0.102 nM compared to 4.60 nM for Dmt-Tic. This antagonist potency is comparable to those of the widely used nonpeptidic δ antagonist naltrindole. The bulkier nature of the adamantyl group could be the explanation for aversing the agonistic effects. Only partial activation of the μ receptor was observed with analogues bearing benzylic side chains, as in dipeptides **25**, **28**, and **34**, invoking therefore not only the need of bulky substituents but also somekind of $\pi-\pi$ interactions for activation.

Compounds **33**−**35** bearing C-terminal amido groups showed similar binding profiles to the previous described analogues without noticeable activation of the δ receptor. Dipeptides **34** and **35** rather acted as δ antagonists (Figure 1) with K_e values of 0.37 and 1.26 nM, respectively. It seems therefore that both the size and chemical nature of the C-terminal substitution are important factors for the degree of activation of the different opioid receptors.

In conclusion, novel classes of potent δ opioid Dmt-Tic dipeptide analogues were identified as a result of C-terminal modifications using either O or N substituents. These dipeptides demonstrated various selectivities while showing sometimes dual agonist/antagonist

activities over the μ and κ receptors. Dipeptides bearing urea/thiourea C-terminal functionalities proved to represent a new class of powerful δ agonists. The results of this study place in evidence that the activation of the opioid receptors is not only determined by the size of the C-terminus group but also by its chemical nature. It is nonetheless surprising to observe that small modifications, as subtle as going from an amide to a urea bond, can drastically change the pharmacological profile of the compounds. In the specific case of the δ receptor, the order of activation was shown to occur as follows: urea/thiourea > amino ≫ amido for bulky subtituents (R = phenyl or *tert*-butyl) at the C-terminus. Other types of C-terminal substituents are currently under investigation in our labs to further explore their effects on the activation of the opioid receptors.

Acknowledgment. The authors are thankful to Dr. K. Carpenter for NMR analysis of the dipeptides and Dr. C. Walpole for helpful discussions. A sample of Dmt-Tic was kindly provided by Dr. L. H. Lazarus (NIEHS).

Supporting Information Available: Experimental procedures, ^{1}H NMR, MS, elemental analyses, HPLC *K*'s, and biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM015532K