Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 23, pp. 3049-3052, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10145-7

THE STEREOCHEMICAL REQUIREMENTS OF THE NOVEL δ-OPIOID SELECTIVE DIPEPTIDE ANTAGONIST TMT-TIC

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Abstract: Five conformationally constrained dipeptide TMT-*L*-Tic analogues have been synthesized and evaluated for their bioactivity using in vitro bioassays. The most potent and selective analogue (2*S*,3*R*)-TMT-*L*-Tic showed 9 nM binding affinity and 4000-fold selectivity for the δ vs μ opioid receptor. The lowest-energy conformation of (2*S*,3*R*)-TMT-*L*-Tic is suggested to be bioactive one in which the χ_1 torsional angle is *trans* for TMT and *gauche* (+) for Tic. © 1997 Elsevier Science Ltd.

The dipeptide segment Tyr-Tic has been demonstrated to be a universal δ-opioid receptor selective antagonist structure, and the minimum peptide unit that still maintains significant opioid receptor binding affinity.[1-3] Incorporation of this fragment into endogenous opioid peptides can dramatically change their biological properties. For example, both [Leu^5]enkephalin, a δ-selective agonist, and dermorphin, a μ-selective agonist, have been converted into δ-selective antagonists by incorporation of the Tyr-Tic segment into their N-terminal sequences.[3] Structure–activity studies of δ-opioid selective antagonists can provide new insight into the structural basis for the opioid ligand–receptor interaction and signal transduction, and crucial information for the rational design of a new generation of opioid ligands with potential clinical and therapeutic values. Recent studies have shown that the Tyr^1 residue is an important pharmacophore element in the dipeptide antagonist activity. Substitution of the Tyr^1 residue in this dipeptide fragment with the more hydrophobic 2′,6′-dimethyltyrosine (DMT) greatly improved the binding affinity and selectivity for the δ-opioid receptor over μ receptor.[4] Compared to DMT, β-methyl-2′,6′-dimethyltyrosine (TMT) has higher hydrophobicity and a more constrained side chain conformation, and has been demonstrated to be extremely useful probe to explore the stereochemical requirements of the side-chain pharmacophores in peptide-receptor interaction.[5] Therefore, in this letter, we report on the incorporation of the four TMT isomers into Tyr-Tic and the biological properties of the five resulting analogues.

The four optically pure diastereoisomers of Boc-β-methyl-2′,6′-dimethyltyrosine (N$^\alpha$-Boc-TMT) were synthesized by literature methods.[6] The synthesis of the TMT-Tic dipeptide started from commercially available N$^\alpha$-Boc-Tic (**1**) as shown in Scheme 1. The compound (**1**) was first converted to its benzyl ester (**2**) via reaction with benzyl bromide in the presence of phase transfer catalyst triethylbenzylammonium chloride.[7] Then the N$^\alpha$-Boc protecting group was removed with 1 N hydrochloric acid in acetic acid.

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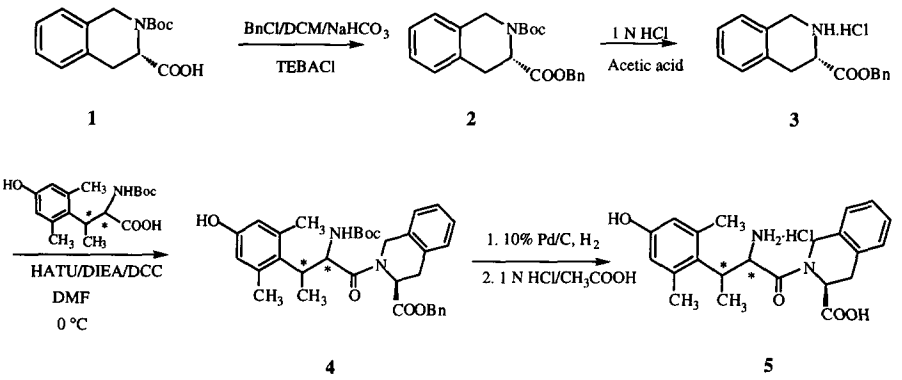
Scheme 1



The coupling of intermediate (**3**) with N^{α}-Boc-TMT was the most difficult step in the synthesis. It did not work using DIC/HOBt coupling reagents. However, it could be accomplished in 60–75% yields using HATU and DCC in the presence of diisopropylethylamine (DIEA) to give the protected dipeptide (**4**).[8] The protected dipeptide Boc-TMT-Tic-OBn (**4**) was hydrogenolyzed in the presence of 10% Pd/C to cleave the benzyl ester, and then treated with 1 N hydrochloric acid to remove the N^{α}-Boc protection. The final product (**5**)[15] was precipitated with ether, filtered, and then purified with RP-HPLC with 0.1% TFA/Acetonitrile as eluent.

Table 1. Binding Affinities of dipeptide TMT-Tic analogues

Compound	[^{3}H]p-ClDPDPE (δ) (IC_{50}, nM)	[^{3}H]DAMGO (μ)(IC_{50}, nM)	δ/μ
L-Tyr-*L*-Tic[a]	191 ± 48.8	28411 ± 2941	148
(2*S*,3*S*)-TMT-*L*-Tic-OH (**5a**)	124 ± 26.5	>80000	> 500
(2*S*,3*R*)-TMT-*L*-Tic-OH (**5b**)	9.3 ± 0.53	35000 ± 18000	3800
(2*S*,3*R*)-TMT-*L*-Tic-OBn (**4b**)	19 ± 4.7	8400 ± 62	450
(2*R*,3*S*)-TMT-*L*-Tic-OH (**5c**)	>10000	>80000	N/A
(2*R*,3*R*)-TMT-*L*-Tic-OH (**5d**)	>10000	>80000	N/A
Bioassay	MVD (δ)	GPI (μ)	
(2*S*,3*R*)-TMT-*L*-Tic-OH (**5b**)	1 μM shifts DPDPE 547-fold	30% at 30 μM; does not antagonize PL-017	

[a]The binding affinities are Ki (nM) values, cited from ref 4.

The binding afffinities of all four TMT-Tic analogues were examined using the radiolabeled opioid ligand [^{3}H]p-ClDPDPE for δ-opioid receptor,[9] and [^{3}H]DAMGO ([D-Ala^2, MePhe^4, Glyol^5]enkephalin) for μ-opioid receptor. As shown in Table 1, both *D*-TMT-*L*-Tic dipeptide analogues (**5c** and **5d**) did not show significant binding affinity for either μ or δ-opioid receptors. Of the *L*-TMT-*L*-Tic analogues (**5a** and **5b**), analogue (**5b**) had a 9.3 nM binding affinity and 3800-fold selectivity for the δ-opioid receptor over the μ-receptor, whereas (**5a**) had substantially lower binding affinity and selectivity. Therefore, we conclude that the preferred stereo configuration of the TMT residue for interaction of the TMT-Tic dipeptide with the δ-opioid receptor is S at the α carbon and R

at the β carbon, which is the same preferred stereochemical requirements of δ-opioid selective peptide agonists.[5] Benzylation of the free carboxylic acid group at the C-terminal of (**5b**) did not significantly effect the binding affinity of the dipeptide for the δ-opioid receptor; (2*S*,3*R*)-TMT-*L*-Tic-OBn (**4b**) had 19 nM binding affinity, and a 450 - fold selectivity for the δ-opioid receptor. Apparantly, the free carboxylic acid group at the C-terminal of the antagonist dipeptide inhibits ligand interact with μ-opioid receptor in a manner similar to the Asp[4] residue in the δ-opioid selective DELT I agonist.[10] Further bioassay studies of the potent (2*S*,3*R*)-TMT-*L*-Tic-OH (**5b**, Table 1) was performed on the MVD and the GPI,[11] and showed that this dipeptide is a very potent and highly selective δ-opioid receptor antagonist.

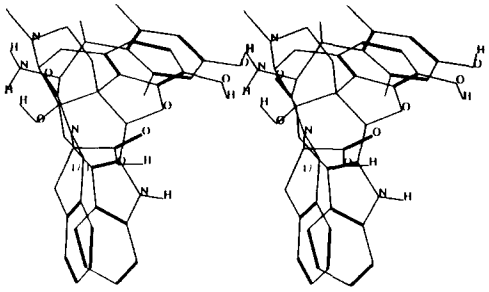


Figure 1. The Stereoview of the superpositioned low-energy conformations of (2*S*,3*R*)-TMT-*L*-Tic (**5b**) and OMI

In order to examine the possible bioactive conformation of the δ-opioid selective TMT-Tic dipeptide antagonist, a computer-assisted conformational search of the most potent and selective dipeptide (2*S*,3*R*)-NH_2-TMT-*L*-Tic-OH (**5b**) was performed. The initial results indicated that this conformationally constrained dipeptide analogue does not have much freedom to access variable conformational spaces, only one possible low energy conformation is available for this molecule within a 5 kcal/mol energy. In this low-energy conformation, the Tic residue has a *gauche*(+) side-chain conformation, and the (2*S*,3*R*)-TMT residue has a *trans* side chain conformation around the χ_1 dihedral angle; these values are consistent with the results reported from this laboratory for other bioactive peptides.[12,13] Overall inspection of this conformation revealed that the two aromatic rings in the (2*S*,3*R*)-NH_2-TMT-*L*-Tic-OH are oriented approximate 90° relative to each other. In this conformation **5b** overlaps very well with the two aromatic rings of the highly conformationally constrained non-peptide δ-opioid selective antagonist OMI[14] which only has one principal low-energy conformation available (Figure 1). Detailed conformational and structure–activity relationship studies of this dipeptide are currently under investigation, and will be reported elsewhere.

Acknowledgment: The authors acknowledge the financial support of grants from NIDA DA 06284 and the U.S. Public Health Service DK 17420, the Dean's Fellowship from the Grad. College of the University of Arizona for S.L., and the financial support from Yunnan Provincial Education Committee, P.R.C. for J.L. as a visiting scholar in the U.S.A. The contents are the responsibility of the authors and do not necessarily represent the official views of the USPHS.

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15. Selected analytical characterizations of the dipeptide analogues: (**5a**) White solid (TFA salt), $[\alpha]_D^{22}$ + 23.06° (*c* 1.0); ^{1}H NMR (CD_3OD) δ 7.18 - 7.03 (m, 4H, aromatic protons), 6.44 (s, 2H, aromatic protons), 5.28 (t, *J* = 4.2 Hz, 1H, -CH), 4.89 (m, 2H, $-CH_2$), 3.58 (m, 2H, $-CH_2$), 3.17 (m, 1H, -CH), 2.45 (s, 3H, $-CH_3$), 2.23 (s, 3H, $-CH_3$), 1.39 (d, *J* = 7.4 Hz, 3H, $-CH_3$); MS for $C_{22}H_{26}N_2O_4$: 383.1979 (M + H$^+$); (**5b**) White solid (TFA salt); $[\alpha]_D^{22}$ + 28.17° (*c* 0.2); ^{1}H NMR (CD_3OD) δ 8.94 (s, 1H, -OH), 7.73–7.32 (m, 4H, aromatic protons), 6.28 (s, 2H, aromatic protons), 4.88 (d, *J* = 11.42 Hz, 1H, -CH), 4.78–4.80 (s, 2H, CH_2, buried in solvent peak), 3.44 (m, 2H, $-CH_2$), 3.32–3.17 (m, 2H, -CH and CH), 2.31 (s, 3H, $-CH_3$), 2.15 (s, 3H, $-CH_3$), 1,21 (d, *J* = 7.2 Hz, 3H, $-CH_3$). MS for $C_{22}H_{26}N_2O_4$: 383.1972 (M + H$^+$); (**5c**) White solid (TFA salt); $[\alpha]_D^{22}$ −23.75° (*c* 1.0); ^{1}H NMR (CD_3OD) δ 7.14–6.94 (m, 4H, aromatic protons), 6.30 (s, 2H, aromatic protons), 5.11 (m, 1H, -CH), 4.83 (d, *J* = 11.4 Hz, 1H, -CH), 4.56 (d, *J* = 15.2 Hz, 1H, -CH), 3.90 (d, *J* = 15.3 Hz, 1H, -CH), 3.10 (dd, *J* = 15.7, 2.0 Hz, 1H, -CH), 2.49(s, 3H, $-CH_3$), 2.43 (s, 3H, $-CH_3$), 1.51(d, *J* = 7.3 Hz, 3H, $-CH_3$). MS for $C_{22}H_{26}N_2O_4$: 383.1961 (M + H$^+$); (**5d**) White solid (TFA salt); $[\alpha]_D^{22}$ −28.0° (*c* 0.2); ^{1}H NMR (CD_3OD) δ 7.12-7.02 (m, 4H, aromatic protons), 6.41 (d, *J* = 2.45 Hz, 1H, aromatic protons), 6.31 (d, *J* = 2.60 Hz, aromatic proton), 5.18 (dd, *J* = 6.1, 3.7 Hz, 1H, -CH), 4.73 (m, 1H, -CH), 4.39 (m, 1H, -CH), 3.58 (m, 1H, -CH), 3.24–3.16 (m, 2H, -CH, and -CH), 2.31 (s, 3H, $-CH_3$), 2.25 (s, 3H, $-CH_3$), 1.22 (d, *J* = 7.4 Hz, 3H, $-CH_3$). MS for $C_{22}H_{26}N_2O_4$: 383.1974 (M + H$^+$).

(*Received in USA* 28 *August* 1997; *accepted* 10 *October* 1997)