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Novel Dmt-Tic Dipeptide Analogues as Selective Delta-Opioid Receptor Antagonists

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Abstract—A series of Dmt-Tic analogues with substitution on the Tic aromatic ring has been synthesized and evaluated for opioid receptor affinity and activation. Incorporation of large hydrophobic groups at position 7 of Tic did not greatly alter the δ opioid receptor binding affinities of the dipeptides whereas substitution at position 6 substantially diminished their affinity. These modified Dmt-Tic peptides showed binding affinities as low as 2.5 nM with up to 500-fold selectivity for the δ versus μ opioid receptor and proved to be δ receptor antagonists.

The development of highly selective and potent opioid agonists and antagonists has been slowed by the lack of knowledge about the geometry of the ligand-binding pocket. The direct evaluation of ligand receptor interactions using X-ray diffraction analysis or NMR spectroscopy is complicated by the fact that the opioid receptors, as members of the family of G-protein coupled receptors, are embedded in the cell membrane. Therefore, the efforts to characterize the ligand binding site have been focussed on extensive structure–activity studies of endogenous opioid peptides such as enkephalins, endorphins and dynorphins as well as the amphibian opioid peptides deltorphin and dermorphin.

It was shown recently that opioid peptide analogues containing a Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) residue in the 2-position are potent and highly selective δ antagonists.[1] The resulting N-terminal Tyr-Tic fragment has been introduced in a variety of opioid peptides with different receptor selectivity resulting not only in a change of their biological properties but also directing their affinity towards the δ receptor.[2,3] In fact, the smallest structure proven to be a δ antagonist is the Tyr-Tic dipeptide segment itself.[4] Methylation of the Tyr residue led to the Dmt-Tic dipeptide (Dmt = 2′,6′-dimethyl-tyrosine) which exhibited improved δ opioid affinity and selectivity and enhanced δ antagonist potency.[5] In addition, the methylation increased the hydrophobicity of the compound and added some conformational constraints on the aromatic ring. Further N-terminal modification added to the knowledge of the spatial requirements for δ-antagonism. *N*-mono and *N*,*N*-dimethylation,[6] reduction of the amide bond[7] or incorporation of β-methyl-2′,6′-Dmt[8] resulted in analogues with high δ receptor affinity and selectivity as well as δ antagonist potency, while alkylation with larger groups such as diethyl, piperidine, pyrrolidine or pyrrole decreased the potency as δ antagonists and led to a decrease in selectivity.[9] In order to gain further insight into the design of new opioid ligands we report herein a novel class of Dmt-Tic ligands with substitution on the Tic aromatic ring easily accessible by utilizing Suzuki cross-coupling chemistry.

The amino acid H-L-Tic(7–OH)–OH (**1**) was used as starting material for all examples, the hydroxyl group providing a handle for substitution at either positions 6, 7 or 8 (Scheme 1). Protection of the amine followed by esterification of the acid with trimethylsilyl diazomethane[10] provided Boc-L-Tic(7–OH)–OMe (**2**). Formation of the triflate (**4**) followed by Suzuki cross-coupling with a series of boronic acids afforded Boc-L-Tic(7–OR)–OMe derivatives **5a**–**e**. H-L-Tic(7–OH)–OH (**1**) could also be specifically mono- or bis-brominated (**6a**–**b**) which were in turn Boc-protected and esterified to give compounds **7a**–**b**. Formation of the methyl ether followed by Suzuki cross-coupling with boronic acids afforded compounds **9a**–**f**.

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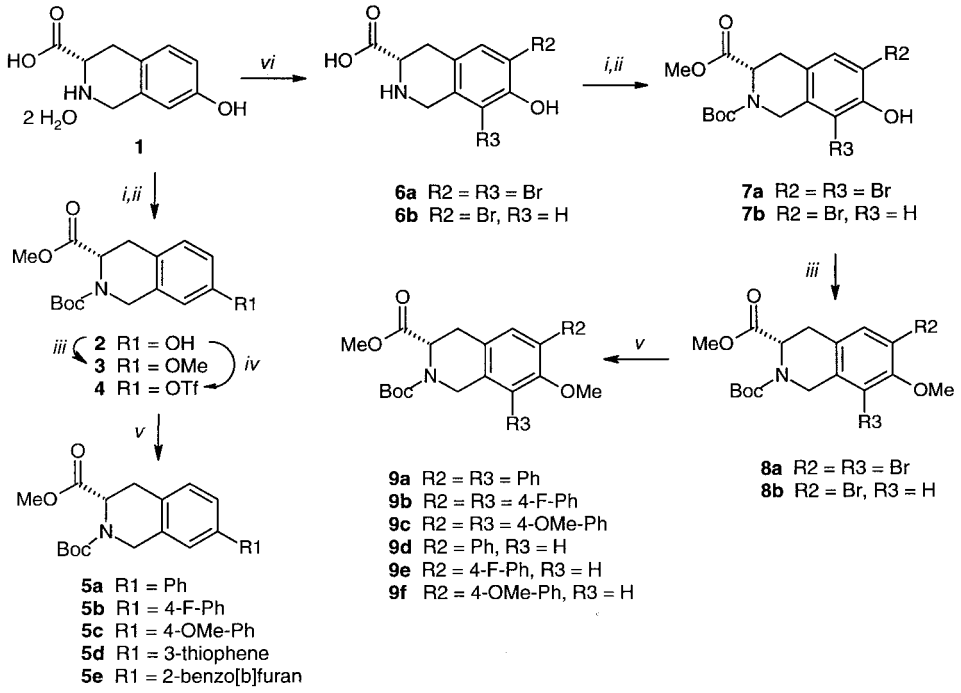
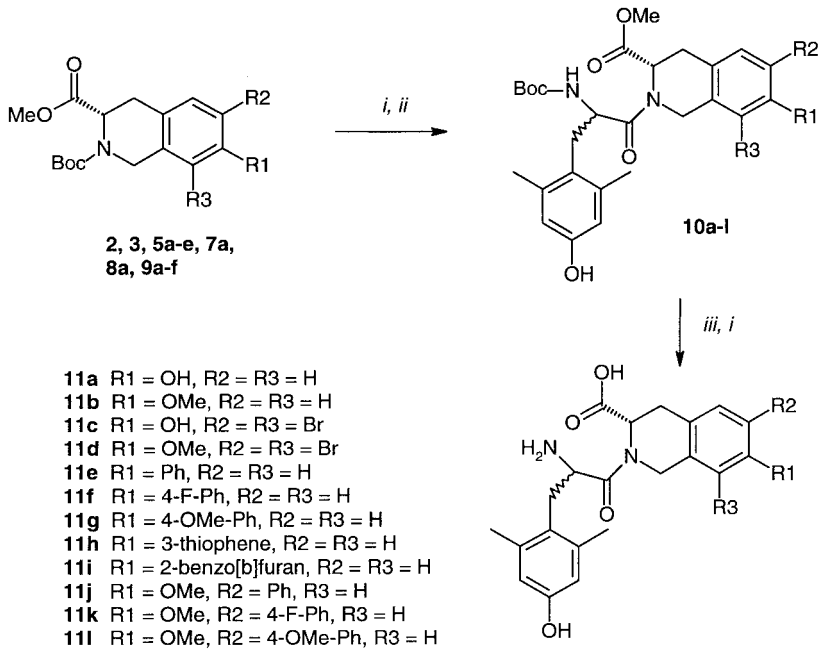


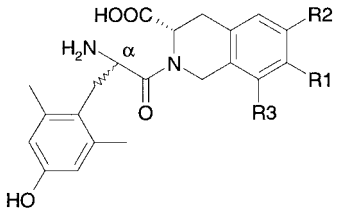
Scheme 1. (i) di-*tert*-butyldicarbonate, Na_2CO_3, H_2O/dioxane, 16 h, quant.; (ii) $TMSCHN_2$, MeOH/C_6H_6, 30 min, 99%; (iii) NaH, MeI, DMF, 30 min, 93%; (iv) triflic anhydride, Et_3N, CH_2Cl_2, 20 min, 85%; (v) Ar–$B(OH)_2$, $(Ph_3P)_4Pd$, Na_2CO_3, toluene/EtOH, 70 °C, 12 h, 75–95%; (vi) Br_2, AcOH, 1–12 h, 95%.



Scheme 2. (i) 1 M HCl/AcOH, 1 h, quant; (ii) Boc-D/L-Dmt-OH, EDC, HOBt, DIPEA, DMAP, DMF, 2–4 days, 15–55%; (iii) 1 M LiOH, EtOH/H_2O, Δ, 3 h, quant.

After *N*-deprotection these amino acid derivatives were used to prepare the different Dmt-Tic dipeptides (Scheme 2) by coupling with Boc-D/L-Dmt–OH[11] yielding compounds **10a**–**l**. Peptide couplings were found to be slow and gave low yields likely due to the bulky nature of the amino acids. The best results were obtained using a combination of EDC, HOBt and DMAP as coupling reagents. Bis-substituted Tic derivatives **9a**–**c** failed to give any of the desired dipeptide products using different coupling strategies (HATU, EDC, HOBt, DPPA) probably due to steric hindrance. Saponification of the methyl ester followed by removal of the *N*-protecting group afforded the different dipeptides **11a**–**l**.[12] The formation of small amounts of the respective diketopiperazines was observed but these were easily removed by reverse-phase HPLC. The binding affinities of dipeptides **11a**–**l** were determined at the three opioid receptors (δ, μ, κ) (Table 1). The diastereomeric dipeptides were

Table 1. Binding affinities of Dmt-Tic dipeptide analogues **11a–l** on opioid receptors



	R1	R2	R3	α[a]	δ receptor ^{125}I-Deltorphin II IC_{50} (nM)	μ receptor ^{125}I-FK-33824 IC_{50} (nM)	κ receptor ^{125}I-DPDYN IC_{50} (nM)	μ/δ Ratio
Dmt-Tic	H	H	H	L	1.6	894	37503	558
11a	OH	H	H	D/L	236	2160	9290	9.2
11b	OMe	H	H	D/L	10.9	2280	9000	211
11b	OMe	H	H	L	12.1	2870	9440	237
11c	OH	Br	Br	D	>10000	8130	n/d[b]	—
11c	OH	Br	Br	L	1080	1240	>10000	1.1
11d	OMe	Br	Br	D	571	8240	n/d	14.4
11d	OMe	Br	Br	L	2.5	1670	>10000	505
11e	Ph	H	H	D	122	1940	>10000	15.9
11e	Ph	H	H	L	13.2	635	5740	48.1
11f	4-F-Ph	H	H	D	81.7	n/d	>10000	—
11f	4-F-Ph	H	H	L	3.8	587	7560	155
11g	4-OMe-Ph	H	H	D/L	4.9	2650	>10000	546
11g	4-OMe-Ph	H	H	L	5.8	1140	>10000	211
11h	3-thiophene	H	H	D/L	13.5	142	2730	10.5
11i	2-benzo[b]furan	H	H	D/L	52.7	2440	>10000	546
11j	OMe	Ph	H	D/L	2540	>10000	>10000	—
11k	OMe	4-F-Ph	H	D/L	1160	7220	>10000	6.2
11l	OMe	4-OMe-Ph	H	D/L	1520	4640	>10000	3.1

[a]Based on chromatography retention time.
[b]n/d: Not tested.

separated by reverse phase HPLC[13] and the compounds with higher δ receptor affinities were assumed to be the L-Dmt containing isomers based on the SAR of published Dmt-Tic analogues.[9]

The presence of the hydroxyl group at R_1 (compounds **11a** and **11c**) greatly reduces the binding affinities of the dipeptides towards the δ opioid receptor, however, when the hydroxyl group was methylated (**11b** and **11d**) the binding affinities for the L isomer were restored. This result indicates that an H-bond donating group is detrimental to an efficient ligand-receptor interaction whereas a sterically more demanding H-bond acceptor is well tolerated. Furthermore, the fact that the 6,8-dibromo compound **11d** (L isomer) displayed an IC_{50} of 2.5 nM and a 500-fold selectivity for δ over μ receptor, similar to the values observed for Dmt-Tic, indicates that the hydrophobic binding pocket is 'spacious' enough to accommodate bulkier ligands.

Further substitutions at this 7-position (R_1) of Tic with bulkier hydrophobic groups seemed to confirm the hydrophobic pocket hypothesis. Introduction of a phenyl group did not greatly alter the δ receptor affinity since dipeptide **11e** still showed low nM binding affinity. Addition of *p*-methoxybenzene (**11g**) produced one of the most selective compounds for the δ-receptor. The affinity of the *p*-fluorobenzene analogue (**11f**) was comparable to that of **11e** but a loss of selectivity was due to a 2-fold increased μ-receptor affinity. The 3-thiophene derivative (**11h**) showed good binding affinity at the δ receptor albeit with poor selectivity. The benzo[*b*]furan derivative (**11i**) displayed only moderate affinity for the δ-receptor probably due to its larger size creating some kind of steric hindrance. Introduction of bulky substituents R_2 (**11j–l**) at position 6 of Tic was

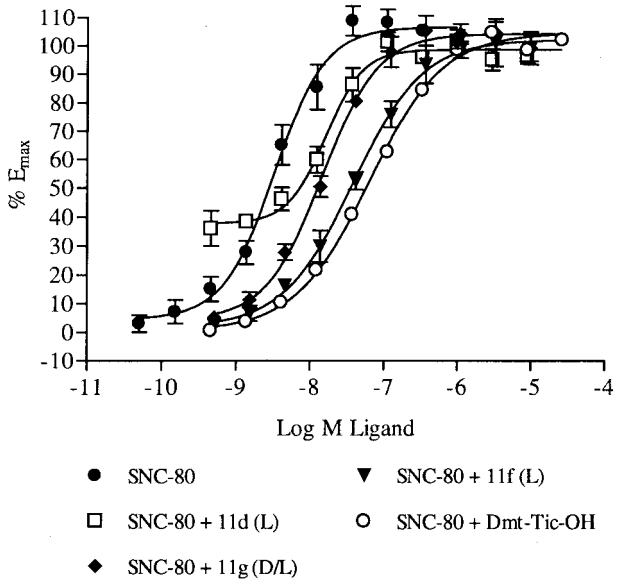


Figure 1. Effect of 100 nM Dmt-Tic–OH analogues, on SNC80 GTP[γ][^{35}S] binding dose response curve on human δ receptor.

detrimental to δ opioid receptor binding. Except for compound **11d** (L), which turned out to be a partial agonist at 100 nM (EC_{50} 28 nM), none of the dipeptides showed δ agonist activity up to 30 μM in the GTP[γ]^{35}S functional assay. Analogues **11f** (L) and **11g** (D/L) were shown to block the effect of the δ selective opioid agonist SNC-80 with K_e values of 11.5 and 38.6 nM, respectively, compared to 5.3 nM for Dmt-Tic (Fig. 1).

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12. Selected analytical characterizations of the dipeptide analogues: (**11b**, D/L isomers) ^{1}H NMR (CD_3OD) δ 6.94 (d, 1H), 6.70 (m, 2H), 6.55, 6.44, 6.33, 6.28 (4s, 2H), 5.25 (m, 1H), 4.63 (m, 2H), 4.41 (m, 3H), 3.79 (m, 1H), 3.75, 3.72 (2s, 3H), 3.38 (d, 1H), 3.22 (m, 3H), 3.11 (m, 2H), 2.74 (m, 2H), 2.26, 2.18 (2s, 6H), 1.86 (dd, 1H); MS for $C_{22}H_{26}N_2O_5$ 399.18 (MH+); $C_{22}H_{26}N_2O_5$; calc: C 53.49%, H 5.26%, N 5.11%; found: C 53.39%, H 5.19%, N 5.21%; (**11d**, L isomer) ^{1}H NMR (CD_3 OD) δ 7.34, 7.27, 7.18 (3s, 1H), 6.47, 6.35, 6.31 (3s, 2H), 4.74 (d, 1H), 4.39 (m, 1H), 4.20 (m, 1H), 3.77 (m, 4H), 3.03 (m, 1H), 2.89 (m, 1H), 2.72 (dd, 1H), 2.27, 2.17 (2s, 6H), 1.71 (dd, 1H); MS for $C_{22}H_{24}Br_2N_2O_5$ 557.25 (MH+); $C_{22}H_{24}Br_2$ N_2O_5; calc: C 41.79%, H 4.08%, N 4.09%; found: C 41.80%, H 4.13%, N 4.10%; (**11f**, D/L isomers) ^{1}H NMR (CD_3OD) δ 7.57 (m, 2H), 7.36 (m, 2H), 7.30–6.99 (m, 3H), 6.57, 6.44, 6.26 (3s, 2H), 5.36 (m, 1H), 4.81 (d, 1H), 4.63 (m, 1H), 4.51 (d, 2H), 4.39 (m, 1H), 3.82 (m, 1H), 3.50 (d, 1H), 3.23 (m, 3H), 3.11 (m, 2H), 2.84 (m, 2H), 2.27 (m, 6H), 1.98 (dd, 1H); MS for $C_{27}H_{28}N_2O_4$ 463.01 (MH+); $C_{27}H_{28}N_2O_4$; calc: C 58.55%, H 5.13%, N 4.61%; found: C 58.48%, H 5.10%, N 4.55%; (**11g**, D/L isomers) ^{1}H NMR (CD_3OD) δ 7.48 (m, 2H), 7.36 (m, 2H), 7.05 (dd, 1H), 6.97 (m, 2H), 6.57, 6.44, 6.29 (3s, 2H), 5.32 (m, 1H), 4.78 (d, 1H), 4.63 (m, 1H), 4.50 (d, 2H), 4.38 (m, 1H), 3.82 (m, 1H), 3.79 (m, 3H), 3.46 (d, 1H), 3.24 (m, 3H), 3.10 (m, 2H), 2.81 (m, 2H), 2.30 (m, 6H), 1.96 (dd, 1H); MS for $C_{28}H_{30}N_2O_5$ 475.02 (MH+); $C_{28}H_{30}N_2O_5$; calc: C 58.69%, H 5.25%, N 4.50%; found: C 58.78%, H 5.29%, N 4.43%; (**11j**, D/L isomers) ^{1}H NMR (CD_3OD) δ 7.48 (m, 1H), 7.39 (m, 2H), 7.15 (t, 1H), 7.06 (d, 1H), 6.92 (m, 2H), 6.43, 6.30 (2s, 2H), 5.15 (m, 1H), 4.37 (d, 1H), 4.25 (m, 1H), 4.13 (d, 1H), 3.96 (d, 1H), 3.90 (m, 1H), 3.61 (m, 2H), 3.15 (m, 3H), 3.00 (m, 2H), 2.75 (m, 2H), 2.10 (m, 6H), 2.04 (dd, 1H); MS for $C_{28}H_{30}N_2O_5$ 475.15 (MH+); $C_{28}H_{30}N_2O_5$; calc: C 52.09%, H 4.67%, N 3.75%; found: C 52.14%, H 4.66%, N 3.84%.
13. Preparative HPLC purification was performed on a Gilson system using a Luna C18 (250×20.2 mm) column and one of the two following gradients: Gradient I—20–50% B; Gradient II—30–80% B in 25 min (A 0.1% TFA in H_2O, B 0.1% TFA in CH_3CN), flow rate 40 mL/min at room temperature.