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Agonist vs Antagonist Behavior of δ Opioid Peptides Containing Novel Phenylalanine Analogues in Place of Tyr[1]

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The novel phenylalanine analogues 4′-[*N*-((4′-phenyl)phenethyl)carboxamido]phenylalanine (Bcp) and 2′,6′-dimethyl-4′-[*N*-((4′-phenyl)phenethyl)carboxamido]phenylalanine (Dbcp) were substituted for Tyr[1] in the δ opioid antagonist TIPP (H-Tyr-Tic-Phe-Phe-OH; Tic = tetrahydroisoquinoline-3-carboxylic acid). Unexpectedly, [Bcp[1]]TIPP was a potent, selective δ opioid *agonist*, whereas [Dbcp[1]]TIPP retained high δ antagonist activity. Receptor docking studies indicated similar binding modes for the two peptides except for the biphenylethyl moiety which occupied distinct receptor subsites. The dipeptide H-Dbcp-Tic-OH was a highly selective δ antagonist with subnanomolar δ receptor affinity.

Introduction

On the basis of early structure−activity relationship (SAR[a]) studies performed with Met- and Leu-enkephalin (for a review, see ref 1), it was prematurely concluded that all structural modifications of the Tyr[1] phenolic ring in opioid peptides would be detrimental to opioid activity, a belief that was held for nearly 2 decades. In a key discovery, Mosberg and colleagues showed in 1992 that dimethylation at the 2′,6′-positions of the Tyr[1] residue of a cyclic enkephalin analogue, as achieved by substitution of 2′,6′-dimethyltyrosine (Dmt), increased opioid agonist potency by 1−2 orders of magnitude.[2] The most plausible explanation for this potency increase is that the two methyl groups may engage in additional binding interactions with residues in the receptor binding site. A more recent, interesting finding, independently reported by two groups,[3,4] was that substitution of a carboxamido ($-CONH_2$) group for the Tyr[1] phenolic hydroxyl group in opioid peptides, as achieved by replacement of the tyrosine with *p*-carboxamidophenylalanine, resulted in compounds that retained high opioid activity. Replacement of the phenolic hydroxyl group in non-peptide opiates with the $-CONH_2$ group had previously been shown to result in compounds retaining high μ receptor binding affinity,[5,6] and introduction of a (4′-phenyl)phenethyl substituent at the $-CONH_2$ group of 8-carboxamidocyclazocine did not diminish μ receptor binding affinity.[7] The latter finding prompted the design and synthesis of opioid peptide analogues in which the Tyr[1] hydroxyl function is replaced by a ((4′-phenyl)phenethyl)carboxamido group, which was achieved by substitution of 4′-[*N*-((4′-phenyl)phenethyl)carboxamido]phenylalanine (Bcp, Figure 1).[8] The cyclic enkephalin analogue H-Bcp-*c*[D-Cys-Gly-Phe(*p*NO_2)-D-Cys]NH_2 turned out to be a potent, μ-selective agonist, indicating that the large biphenylethyl substituent not only was tolerated at the μ opioid receptor but likely enhanced binding affinity by interacting with a receptor subsite.

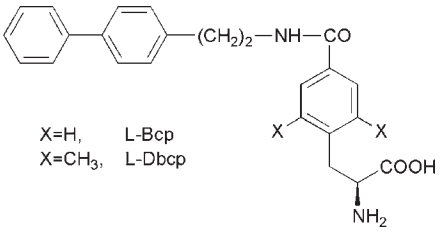

Figure 1. Structural formulas of L-Bcp and L-Dbcp.

δ opioid peptide antagonists containing 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic) in the 2-position of the peptide sequence, the so-called TIP(P) peptides, were first reported in 1992.[9] The two prototypes, TIPP (H-Tyr-Tic-Phe-Phe-OH) and TIP (H-Tyr-Tic-Phe-OH) displayed high δ antagonist activity and high δ receptor selectivity. Subsequent SAR studies led to a number of TIPP-derived δ antagonists with subnanomolar δ receptor binding affinity (for a review, see ref 10), including H-Dmt-Tic-Phe-Phe-OH (DIPP). The dipeptide H-Tyr-Tic-OH was shown to be a weak δ opioid antagonist, whereas H-Dmt-Tic-OH showed low nanomolar δ antagonist activity and high δ receptor selectivity.[11] Here, we describe the synthesis and opioid activity profiles of analogues of TIP(P) peptides, in which the Tyr[1] residue was replaced by Bcp or by the novel Tyr analogue 2′,6′-dimethyl-4′-[*N*-((4′-phenyl)phenethyl)carboxamido]phenylalanine (Dbcp) (Figure 1).

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[a] Abbreviations: Bcp, 4′-[*N*-((4′-phenyl)phenethyl)carboxamido]-phenylalanine; Cha, cyclohexylalanine; DAMGO, H-Tyr-D-Ala-Gly-Phe(NMe)-Gly-ol; Dbcp, 2′,6′-dimethyl-4′-[*N*-((4′-phenyl)phenethyl)-carboxamido]phenylalanine; DIC, 1,3-diisopropylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DIPP, H-Dmt-Tic-Phe-Phe-OH; Dmt, 2′,6′-dimethyltyrosine; DPDPE, H-Tyr-*c*[D-Pen-Gly-Phe-D-Pen]OH; DPPF, 1,1′-bis(diphenylphosphino)ferrocene; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; GPI, guinea pig ileum; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MVD, mouse vas deferens; SAR, structure−activity relationships; Tic, tetrahydroisoquinoline-3-carboxylic acid; TAPP, H-Tyr-D-Ala-Phe-Phe-NH_2; TIPP, H-Tyr-Tic-Phe-Phe-OH; TMH, transmembrane helix.

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Table 1. Opioid Receptor Binding Affinities of Bcp[1]- and Dbcp[1]-Analogues of TIPP Peptides[a]

compd	peptide	K_i^δ (nM)	K_i^μ (nM)	K_i^κ (nM)	K_i ratio $\delta/\mu/\kappa$
1	H-Bcp-Tic-Phe-Phe-OH	0.605 ± 0.058	87.7 ± 8.0	270 ± 74	1/145/446
2	H-Bcp-Tic-Phe-OH	0.536 ± 0.057	173 ± 10	1580 ± 720	1/323/2950
3	H-Bcp-Tic-OH	0.646 ± 0.088	2270 ± 210	4760 ± 200	1/3510/7370
4	H-Bcp-TicΨ[CH_2NH]Phe-Phe-OH	2.00 ± 0.19	323 ± 16	1240 ± 50	1/162/620
5	H-Bcp-TicΨ[CH_2NH]Cha-Phe-OH	3.31 ± 0.32	334 ± 64	1660 ± 60	1/101/502
6	H-Dbcp-Tic-Phe-Phe-OH	0.783 ± 0.046	142 ± 41	597 ± 73	1/181/762
7	H-Dbcp-Tic-OH	0.825 ± 0.107	615 ± 31	2750 ± 70	1/745/3330
8	H-Tyr-Tic-Phe-Phe-OH (TIPP)[b]	1.22 ± 0.07	1720 ± 50	> 1000	1/1410/ > 820
9	H-Tyr-Tic-OH	242 ± 28	> 1000	> 10000	
10	H-Dmt-Tic-Phe-Phe-OH[b]	0.248 ± 0.046	141 ± 24	> 1000	1/569/ > 4030
11	H-Dmt-Tic-OH[b]	1.84 ± 0.24	1360 ± 160	> 1000	1/739/ > 543
12	H-Cdp-Tic-Phe-Phe-OH	0.501 ± 0.060	2210 ± 490	> 1000	1/4410/ > 2000

[a] Values represent the mean of three to six determinations. [b] Data taken from Schiller et al.[10]

Table 2. Opioid Agonist or Antagonist Activities of Bcp[1]- and Dbcp[1]-Analogues of TIPP Peptides[a]

		MVD		GPI	
compd	peptide	IC_{50} (nM)	K_e^δ (nM)	IC_{50} (nM)	K_e^μ (nM)
1	H-Bcp-Tic-Phe-Phe-OH	3.42 ± 0.36		223 ± 37 (IC_{40})[b]	
2	H-Bcp-Tic-Phe-OH		18.7 ± 1.2	458 ± 73 (IC_{35})[b]	
3	H-Bcp-Tic-OH		17.8 ± 0.7		5380 ± 380
4	H-Bcp-TicΨ[CH_2NH]Phe-Phe-OH	11.8 ± 1.6 (IC_{40})[b]		1460 ± 310 (IC_{40})[b]	
5	H-Bcp-TicΨ[CH_2NH]Cha-Phe-OH	32.0 ± 8.2 (IC_{40})[b]		721 ± 78 (IC_{35})[b]	
6	H-Dbcp-Tic-Phe-Phe-OH		3.07 ± 0.51		373 ± 71
7	H-Dbcp-Tic-OH		1.76 ± 0.30		2910 ± 570
8	H-Tyr-Tic-Phe-Phe-OH (TIPP)[c]		4.80 ± 0.20	> 10000	
9	H-Tyr-Tic−OH		263 ± 40	inactive	inactive
10	H-Dmt-Tic-Phe-Phe-OH[c]		0.196 ± 0.035	769 ± 142	
11	H-Dmt-Tic-OH[c]		6.55 ± 0.27	inactive	inactive
12	H-Cdp-Tic-Phe-Phe-OH		0.627 ± 0.058		3070 ± 260

[a] Values represent the mean of three to six determinations. [b] Partial agonist. [c] Data taken from Schiller et al.[10]

Boc-Bcp-OH was synthesized by activating the hydroxyl group of Boc-Tyr-OMe as the triflate, followed by carbonylation with carbon monoxide in the presence of potassium acetate, bis(diphenylphosphino)ferrocene (DPPF), and palladium acetate, as described by Wang et al.,[12] affording Boc-Phe(4′-COOH)-OMe in good yield. Subsequent reaction with 2-(4-biphenyl)ethylamine using *O*-(benzotriazol-1-yl)- *N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent followed by ester hydrolysis with 2 N NaOH yielded the target compound. Boc-Dbcp-OH was prepared in an analogous manner, starting with Boc-Dmt-OEt. Peptides were synthesized by standard solid-phase methods, were purified by preparative HPLC, and were obtained in >95% purity.

Results and Discussion

The Bcp[1]-tetrapeptide analogue H-Bcp-Tic-Phe-Phe-OH (**1**) showed about 2-fold higher δ receptor binding affinity (K_i^δ = 0.605 ± 0.058 nM) than the TIPP parent peptide (δ antagonist) (**8**) and retained high δ receptor binding selectivity (Table 1). Surprisingly, this compound turned out to be a potent full δ opioid agonist (IC_{50} = 3.42 ± 0.36 nM) in the MVD assay (Table 2). In the GPI assay, it displayed quite weak μ partial agonist properties. The C-terminally truncated tripeptide H-Bcp-Tic-Phe-OH (**2**) also showed subnanomolar δ receptor binding affinity, as well as high δ vs μ and δ vs κ binding selectivity. Unlike peptide **1**, however, the Bcp[1]-tripeptide was a δ antagonist (K_e^δ = 18.7 ± 1.2 nM) with low efficacy δ partial agonist activity (maximal inhibition of contractions = 15%) in the MVD assay. The low efficacy partial agonist component may explain the discrepancy between the K_e^δ and K_i^δ values determined for this compound. In the GPI assay, peptide **2** was characterized as a weak μ partial agonist. Further C-terminal truncation led to the dipeptide H-Bcp-Tic-OH (**3**) which also displayed subnanomolar δ receptor binding affinity and extraordinarily high δ receptor selectivity. In the MVD assay, compound **3** was a δ antagonist with a similar low efficacy δ partial agonist component as peptide **2**. The 375-fold higher δ receptor binding affinity of H-Bcp-Tic-OH (K_i^δ = 0.646 ± 0.088 nM) as compared to H-Tyr-Tic-OH (**9**, K_i^δ = 242 ± 28 nM) indicates that the biphenylethylcarboxamido group contributes in a major way to the binding interaction energy of **3**.

The two pseudopeptides containing a reduced peptide bond between the 2- and 3-position residues H-Bcp-TicΨ-[CH_2NH]Phe-Phe-OH (**4**) and H-Bcp-TicΨ[CH_2NH]Cha-Phe-OH (**5**, Cha = cyclohexylalanine) displayed 6- and 13-fold lower δ receptor binding affinity than their respective Tyr[1]-containing parent pseudopeptides, which are δ opioid antagonists,[10] and turned out to be nearly full δ agonists (e = 0.8) in the MVD assay with potencies 4- to 9-fold lower than those of tetrapeptide **1**. These results indicate that the altered rotational mobility around the reduced peptide bond in these two pseudopeptides may position the biphenylethylcarboxamido group in a location at the δ receptor binding site that is less favorable for optimal δ receptor binding and activation.

The tetrapeptide containing Dbcp (Figure 1) in place of Tyr[1], H-Dbcp-Tic-Phe-Phe-OH (**6**), showed subnanomolar δ receptor binding affinity comparable to that of the Bcp[1]-tetrapeptide **1** and similarly high δ vs μ and δ vs κ receptor binding selectivity. However, unlike agonist **1**, compound **6** retained high δ antagonist activity in the MVD assay and was a 2-fold more potent antagonist than the TIPP parent (**8**). H-Dbcp-Tic-Phe-Phe-OH (**6**) displayed 3-fold lower

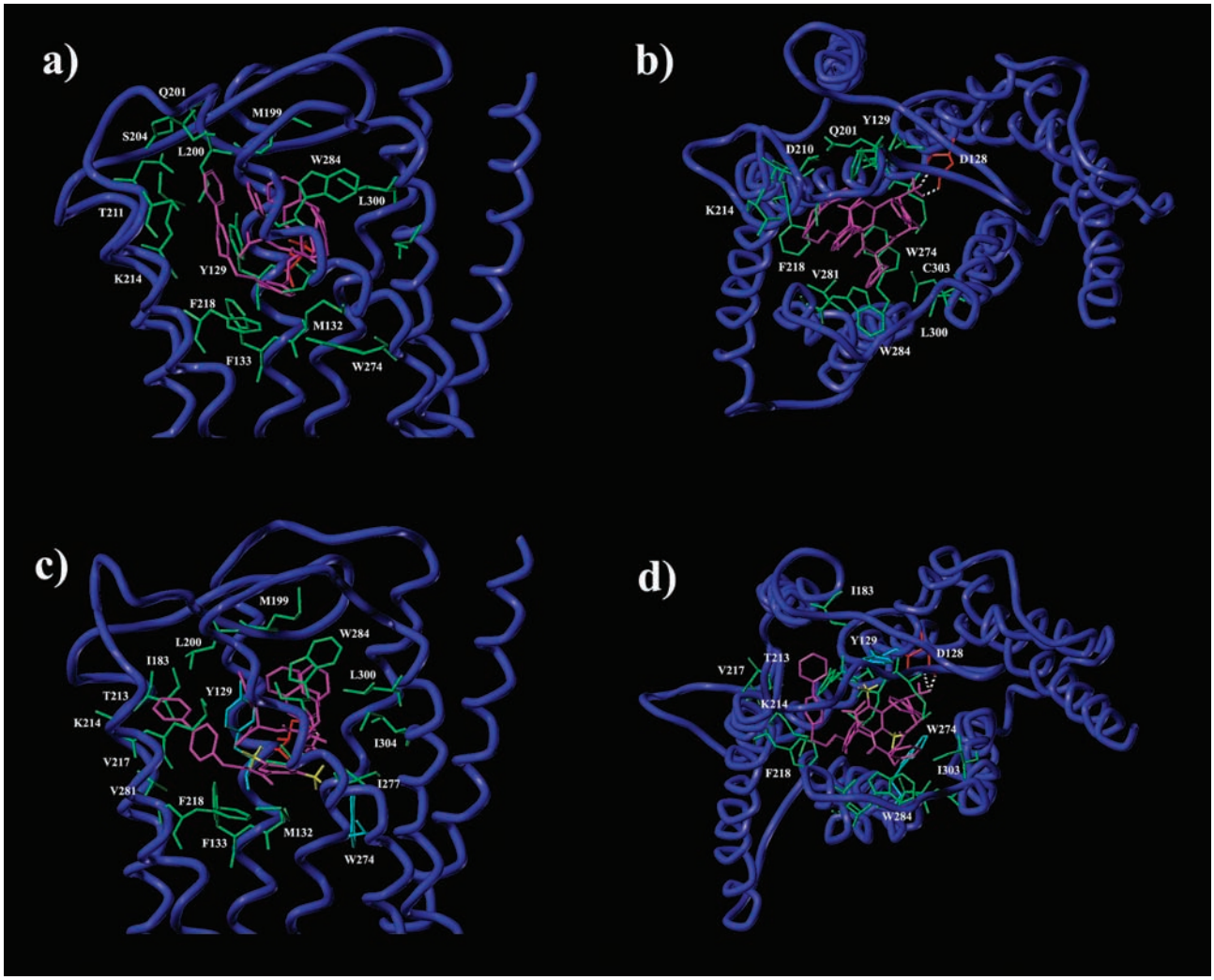

Figure 2. Receptor docking studies. [Bcp[1]]TIPP bound to the δ receptor in the active state: (a) front view; (b) top view. [Dbcp[1]]TIPP bound to the δ receptor in the inactive state: (c) front view; (d) top view. The peptide ligands are in magenta, and the two methyl groups of Dbcp are in yellow. Selected residues of the receptor are in green, red (D128), or cyan (Y129 and W274). The hydrogen bonds between D128 and the amino group of Bcp or Dbcp are indicated as white dashed lines.

δ receptor binding affinity than H-Dmt-Tic-Phe-Phe-OH (**10**) and 1.5-fold lower δ affinity than H-Cdp-Tic-Phe-Phe-OH (**12**; Cdp = 4′-carboxamido-2′,6′-dimethylphenylalanine). Similarly, in the MVD assay, **6** also showed 15- and 5-fold decreases in δ antagonist activity (K_e) compared to **10** and **12**, respectively. These comparisons indicate that the presence of the biphenylethyl substituent in H-Dbcp-Tic-Phe-Phe-OH does not cause an increase in δ receptor binding affinity. It is also possible that the interaction of the biphenylethyl substituent with the receptor may slightly alter the positioning of the 2′,6′-dimethylphenylalanine moiety at the receptor, resulting in the observed decrease in δ receptor binding affinity and δ antagonist activity. H-Dbcp-Tic-Phe-Phe-OH (**6**) has the same low μ receptor binding affinity as H-Dmt-Tic-Phe-Phe-OH (**10**) but interestingly 15-fold higher μ affinity than H-Cdp-Tic-Phe-Phe-OH (**12**). In agreement with the result of the μ receptor binding assay, **6** also showed about 8-fold higher μ antagonist activity than **12** in the GPI assay. This result indicates that the biphenylethyl substituent of **6** does strengthen binding to the μ receptor in the inactive state to some extent. On the other hand, **10** behaves as a weak agonist at the μ receptor.

The dipeptide H-Dbcp-Tic-OH (**7**) also displayed subnanomolar δ opioid receptor binding affinity ($K_i^{\delta} = 0.825 \pm 0.107$ nM), very high δ receptor binding selectivity, and subnanomolar δ antagonist activity ($K_e^{\delta} = 1.76 \pm 0.30$ nM) in the MVD assay. In comparison with H-Dmt-Tic-OH (**11**),[10,11] it showed 2-fold higher δ receptor binding affinity and 4-fold higher δ antagonist activity. Thus, unlike in the case of tetrapeptide **6**, the biphenylethyl moiety of **7** strengthens binding to the δ receptor to some extent. This result can be explained with the different binding mode of the biphenylethyl moiety in the dipeptide compared to the tetrapeptide (see below). Compound **7** also displayed about 2-fold higher μ receptor binding affinity than **11**, and, consequently, these two dipeptides have about the same high δ vs μ receptor selectivity (Table 1). With the exception of a slightly elevated molecular weight (MW = 576), the physicochemical properties of H-Dbcp-Tic-OH are in agreement with Lipinski's rule of five, and this compound may turn out to be of interest as a pharmacological tool. Unlike peptides **2** and **3**, which have a weak δ partial agonist component, **6** and **7** showed no δ opioid agonist activity in the MVD assay at concentrations up to 10 μM.

Studies of flexible docking of ligands to the δ opioid receptor were performed using Mosberg's models of the receptor in the inactive and the activated state. The tetrapeptides H-Bcp-Tic-Phe-Phe-OH (**1**, δ agonist) and H-Dbcp-Tic-Phe-Phe-OH (**6**, δ antagonist) were docked to the activated and the inactive forms of the δ receptor, respectively (Figure 2). In general, a comparison of the ligand−receptor interactions of agonist **1** bound to the active form of the receptor with those of antagonist **6** bound to the inactive receptor form revealed that many of the interactions involved the same receptor residues, with notable exceptions. In both cases, the N-terminal amino group of the ligand formed a salt bridge with Asp^{128} of the receptor. The Phe^4 residue of the two peptides bound to their respective receptor forms occupied only slightly different receptor regions, 2−3 Å apart. While in both cases interactions with Leu^{200} and Trp^{284} are seen, the Phe^4 side chain of **1** also interacts with Gln^{201} and Ser^{204} in the active form of the receptor. The Phe^3 residue in both peptides interacts with Val^{281}, Trp^{284}, and Leu^{300} and, additionally, with Cys^{303} in the case of agonist **1** and with Ile^{304} in the case of antagonist **6**. The Tic^2 residue in the two ligands also occupies a very similar receptor site, interacting with Met^{199} and Leu^{300}, and in addition, this residue in the agonist interacts with Cys^{303}, while in the antagonist it is close to Ile^{304}. In both ligands the phenyl ring of the phenylalanine portion of the 1-position residue interacts with Tyr^{129}, Met^{132}, Phe^{133}, and Trp^{274} and additionally with Ile^{277} in the case of the antagonist.

Interestingly, the biphenylethyl substituent in agonist **1** and antagonist **6** interacts with different receptor regions and are seen to be about 4−5 Å apart from each other when the two receptor complexes are compared. While the biphenyl moiety of both ligands interacts with Lys^{214} and Phe^{218} of the receptor, it differentially interacts with Glu^{201}, Ser^{204}, Asp^{210}, and Thr^{211} in the case of the agonist and with Ile^{183}, Thr^{213}, and Val^{217} in the case of the antagonist. The different positioning of the biphenyl moiety in the agonist− vs antagonist−receptor complexes appears to be due to additional hydrophobic interactions of the 2′,6′-dimethyl groups of $Dbcp^1$ in the antagonist with Tyr^{127} and Trp^{274} of the receptor in the inactive state and is reflected in different dihedral angles for receptor-bound peptide **1** ($\Psi_1 = 89°, \chi_1 = 177°, \chi_2 = 69°$) vs receptor-bound peptide **6** ($\Psi_1 = 132°, \chi_1 = 161°, \chi_2 = 89°$).

Flexible docking of the dipeptide antagonists H-Bcp-Tic-OH (**3**) and Dbcp-Tic-OH (**7**) to the δ receptor in the inactive state was also performed. A superposition of the receptor-bound conformations of the dipeptide antagonists **3** and **7** and the tetrapeptide antagonist **6** is shown in Figure 3. As in the case of the tetrapeptide, the N-terminal amino group of the two dipeptides forms a salt bridge with Asp^{128} of the receptor. The Tic^2 residue and the phenyl ring of the phenylalanine portion of the 1-position residue occupy the same receptor binding subsites as the corresponding moieties in the tetrapeptide. However, in comparison with the 2′,6′-dimethylphenyl moiety in docked tetrapeptide **6**, that same moiety in the docked dipeptides **3** and **7** has a twisted orientation. As a consequence of this twist, the interaction of one of the 2′,6′-dimethyl groups in dipeptide **7** with Trp^{274}, as seen in the case of the tetrapeptide, is lost, whereas one of the methyl groups still interacts with Tyr^{129} as in the case of the tetrapeptide. Most interestingly, the biphenyl moiety of the dipeptides assumes a completely different orientation compared to the one in the tetrapeptide and interacts with the same receptor region as the phenyl rings of Phe^3 and Phe^4 in the tetrapeptide, particularly with residues Leu^{200} and Trp^{284}. These interactions may explain the increased δ receptor binding affinity of H-Dbcp-Tic-OH compared to H-Dmt-Tic-OH.

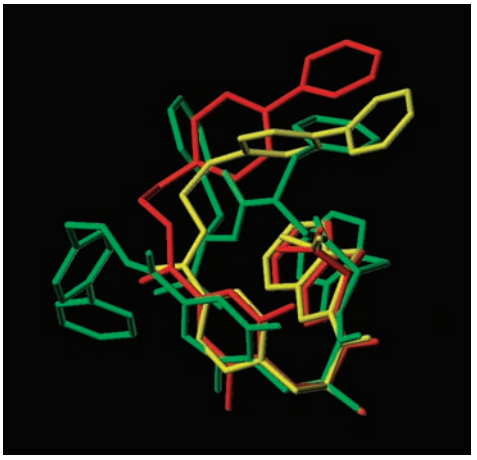

Figure 3. Conformations of H-Bcp-Tic-OH (**3**, yellow), H-Dbcp-Tic-Phe-Phe-OH (**6**, green), and H-Dbcp-Tic-OH (**7**, red) bound to the δ receptor in the inactive state.

Conclusions

Replacement of the Tyr^1 residue in TIPP peptides with a Bcp or Dbcp residue resulted in a general increase in δ opioid receptor binding affinity. All Bcp^1- or $Dbcp^1$- tetra-, tri-, and dipeptide analogues displayed similar subnanomolar δ receptor binding affinity and high δ receptor binding selectivity. The dipeptide H-Dbcp-Tic-OH (**7**) is a low molecular weight δ opioid antagonist with subnanomolar δ receptor binding affinity and extraordinarily high δ receptor selectivity and represents a novel pharmacological tool. The observation that [Bcp^1]TIPP (**1**) is a δ opioid agonist, whereas [$Dbcp^1$]TIPP (**6**) is a δ antagonist, is of great significance. The flexible docking studies performed with these two tetrapeptides indicated that the large biphenylethyl group contained in the Bcp residue of the δ agonist interacts with an accessory binding site of the activated receptor distinct from the binding site of the biphenylethyl group of the Dbcp residue of the δ antagonist bound to the receptor in the inactive state. This finding demonstrates that the differential orientation of a large hydrophobic substituent introduced at an appropriate site of a ligand may result in the induction of or interaction with a distinct active or inactive receptor conformation. In the two docked dipeptide antagonists, the biphenylethyl moiety assumes yet another very different orientation, permitting it to interact with a receptor region similar to that with which the Phe^3 and Phe^4 residues of the tetrapeptide antagonist (**6**) interact. These results indicate that the δ opioid receptor can accommodate the biphenylethyl moiety contained in the 1-position residue of TIPP peptides in a number of different binding modes that promote the interaction of the ligand with the receptor in an activated or an inactive state. In future studies we will replace the biphenylethyl group in the Bcp^1- and $Dbcp^1$-TIPP analogues described here with a variety of substituents containing large aromatic moieties that might interact with different hydrophobic subsites at the δ receptor, in an effort to further explore the agonist vs antagonist behavior of this class of δ opioid receptor ligands.

Experimental Section

Peptide Synthesis. Peptides were prepared by the manual solid-phase technique using Boc protection for the α-amino group and DIC/HOBt or HBTU/DIEA as coupling agents. Peptides **1**, **2**, **4**, **5**, **6**, and **12** were assembled on a polystyrene−divinylbenzene (1%) resin (100−200 mesh) (Boc-Phe-resin, 0.65 equiv/g, Bachem Bioscience, King of Prussia, PA). For the synthesis of dipeptides **3** and **7** a Boc-Tic-resin (0.35 equiv./g) was prepared according to the Gisin method,[13] using a Merrifield resin (D-2120, 100−200 mesh, 1.05 mM/g Bachem Bioscience). Peptide assembly was carried out according to a published protocol.[14] To introduce the reduced peptide bond between the Tic^2 and Phe^3 (or Cha^3) residues, a reductive alkylation reaction[15] between 2-Boc-1,2,3,4-tetrahydroisoquinoline-3-aldehyde[14] and the amino group of the resin-bound H-Phe-Phe- or H-Cha-Phe- dipeptide was performed as follows. 2-Boc-1,2,3,4-tetrahydroisoquinoline-3-aldehyde (2 equiv) in DMF containing 1% AcOH was added to the resin. Sodium cyanoborohydride (5.0 equiv) was then added portionwise over a period of 40 min, and the reaction was allowed to continue for 3 h. After the resin was washed, deprotection and coupling of the Boc-Bcp-OH residue were performed according to the standard protocol. Peptides were cleaved from the resin and deprotected by HF/anisole treatment in the usual manner. After evaporation of the HF, the resin was extracted three times with Et_2O and subsequently three times with glacial AcOH. The peptides were obtained in solid form through lyophilization of the acetic acid extracts. Peptides were purified by preparative reversed phase HPLC. Analytical parameters are listed in Supporting Information.

Theoretical Conformational Analyses. All calculations were performed using the molecular modeling software SYBYL, version 7.0 (Tripos Associates). The standard Tripos force field was used for energy calculations and a dielectric constant of 1 was used. The TIPP analogues were constructed as previously described,[16,17] using the standard fragment library in SYBYL, and were subjected to 300 ps of molecular dynamics simulation. Conformations were sampled every 10 ps and were minimized, and the lowest-energy conformation from the dynamics simulation was used as the starting structure for flexible docking to the receptor. The resulting overall conformations of the Bcp^1- and $Dbcp^1$-peptide analogues were similar to the previously proposed δ receptor-bound conformations of TIPP peptides.[16,17] Models of the δ opioid receptor in the inactive and in the activated state, constructed by Mosberg et al. by homology modeling based on the crystal structure of rhodopsin (http://mosberglab.phar.umich.edu/resources/), were used in the docking studies. Flexible docking was performed using the software program GLIDE (Schrödinger LLC). Each of the resulting ligand−receptor complexes was minimized using the conjugate gradient approach.[18] Molecular dynamics simulations of 100 ps at 300 K were performed in order to assess the stability of each complex. In each case no significant change in the complex structure was observed during the simulation.

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Supporting Information Available: Experimental details and refs 19−28. This material is available free of charge via the Internet at http://pubs.acs.org.

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