Substitution of the Side-Chain-Constrained Amino Acids β-Methyl-2′,6′-Dimethyl-4′-Methoxytyrosine in Position 2 of a Bicyclic Oxytocin Analogue Provides Unique Insights into the Bioactive Topography of Oxytocin Antagonists

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Stereochemical properties of the side-chain functional groups of amino acid residues in endogenous peptide hormones and transmitters play crucial roles in the regulation of their physiological properties.[1] To provide insight into the specificity of peptide ligand−receptor recognition, a systematic topographical approach has been developed in which novel side-chain-constrained amino acids are incorporated into peptide templates to reveal specific stereochemical requirements of the binding pharmacophores for recognizing a particular receptor or for differentiation between receptor subtypes.[1c,2] In this paper, we demonstrate, for the first time to our knowledge, that the bioactive topography of a peptide ligand can be achieved with opposite stereoconfigurations of a pair of enantiomeric amino acids containing a crucial binding pharmacophore.

Oxytocin, H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2 (OT), is a neurohypophyseal hormone physiologically important for milk-ejecting and uterine-contracting activity in mammals.[3] Oxytocin antagonists have been shown to have therapeutic potential for the treatment of pre-term labor by inhibiting uterine contractions.[4] Detailed structure−activity relationship studies of oxytocin analogues have revealed that oxytocin agonists[5,6a] and antagonists[5c,6] have different receptor-binding conformations[5,6] and that the chirality[5c,7] and hydrophobicity[8] of the aromatic amino acid residue at position 2 of OT are extremely important for determining the inhibitory properties and high affinity of antagonists for the uterine oxytocin receptor. Recent NMR and molecular dynamics studies[6] on the constrained bicyclic oxytocin antagonist, Xxx-Tyr^2-Ile^3-Glu^4-Asn^5-Cys^6-Pro^7-Lys^8-Gly^9-NH_2 (BC-OT), where Xxx = deamino-Cys^{9a} or deamino-Pen (dPen, Figure 1)[9b] have resulted in a new model of the bioactive conformation for antagonists of the OT receptor.[6a] However, due to high side-chain flexibility, this model was not able to specify the bioactive topography of the critical Tyr^2 side chain in the bicyclic antagonists. By reason of their highly constrained side-chain conformations (Figure 2), diastereoisomers of β-methyl-2′,6′-dimethyltyrosine (TMT) have been used for a systematic side-chain "rotamer scan" that allows one to examine the topographical requirements for an aromatic pharmacophore of peptide ligands.[1c,10] In the present study, we have used four optically pure diastereoisomers of β-methyl-2′,6′-dimethyl-4′-methoxytyrosine (*p*-MeOTMT),[11] incorporated into the 2-position of the bicyclic antagonist [dPen^1]BC-OT to examine stereochemical requirements of the crucial Tyr^2 pharmacophore. The newly designed bicyclic oxytocin analogues were synthesized on solid-phase supports using previously reported methodology[12] and tested in the classical oxytocin assays for their *in vitro* and *in vivo* uterotonic antago-

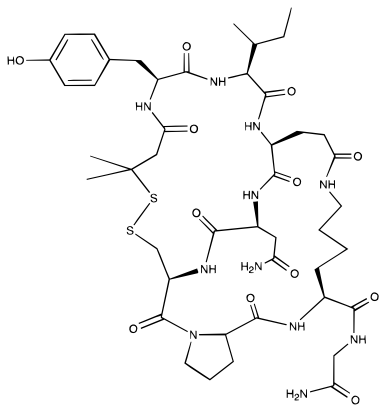

Figure 1. The chemical structure of [dPen^1,cyclo(Glu^4,Lys^8)]oxytocin (**BC-OT**).

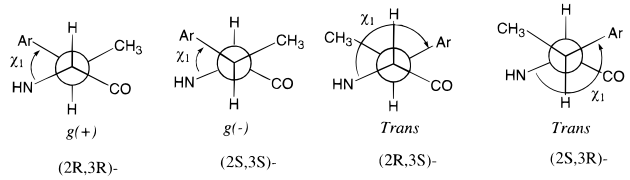

Figure 2. The favorable rotamers of diastereoisomeric β-methyl-2′,6′-dimethyl-4′-methoxytyrosine (***p*-MeOTMT**), (Ar = 2′,6′-dimethyl-4′-methoxyphenyl; the (2*S*,3*S*)- and (2*S*,3*R*)-isomers are viewed from the β-carbon to α-carbon in order to pinpoint the topographical similarity and difference among these four diastereoisomers).

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Table 1. Biological Activities of Bicyclic Oxytocin Antagonist Analogues[a]

peptides	uterotonic activities (pA_2) in vitro (no Mg^{2+})	uterotonic activities (pA_2) in vitro (1 mM Mg^{2+})	*in vivo*	pressor (pA_2)	binding affinity IC_{50} (nM)
[dPen^1,Glu^4,Lys^8]OT	8.10	7.30	6.03	6.27	128
[dPen^1,(2*S*,3*S*)-*p*-MeOTMT^2,Glu^4,Lys^8]OT	8.26	7.85	6.88	7.10	8
[dPen^1,(2*S*,3*R*)-*p*-MeOTMT^2,Glu^4,Lys^8]OT	~5.60	0	0	0	3.6×10^4
[dPen^1,(2*R*,3*R*)-*p*-MeOTMT^2,Glu^4,Lys^8]OT	7.80	7.60	6.08	6.00	160
[dPen^1,(2*R*,3*S*)-*p*-MeOTMT^2,Glu^4,Lys^8]OT	~5.50	0	0	0	1.9×10^4

[a] Binding affinity is with uterine membrane receptor; 0 means no activity up to concentrations of 10^{-5} M or dose of 4×10^{-2} mg per rat.

nistic activities and in the vasopressin pressor assay for their antipressor activity *in vivo*.[13] Further, we determined binding affinities to uterine membrane receptors.[14]

As shown in the Table 1, substitution of the Tyr^2 residue in the BC-OT with *p*-MeOTMT (4 isomers) led to dramatically different biological properties of the resulting analogues.[15] Incorporation of (2*S*,3*S*)-*p*-MeOTMT^2 and (2*R*,3*R*)-*p*-MeOTMT^2 into the bicyclic template resulted in two very active peptide analogues. [(2*S*,3*S*)-MeOTMT^2]BC-OT is one of the most potent peptide antagonists of OT reported so far ($pA_2 = 8.26$) and also shows potent antagonistic activity in the vasopressin assay, whereas [(2*R*,3*R*)-MeOTMT^2]BC-OT has slightly lower potency ($pA_2 = 7.80$). Incorporation of the other pair of enantiomers, (2*S*,3*R*)- and (2*R*,3*S*)-*p*-MeOTMT, produced two bicyclic OT analogues which were almost inactive *in vitro* and *in vivo*. The biological activity data are highly consistent with the binding affinities to the uterine OT receptor. Both L- and D-aromatic amino acids in position 2 of oxytocin are known to produce potent OT antagonists,[5] and methylation of the 4′-hydroxyl group of Tyr^2 increases the antagonistic potency.[7] Therefore, the high antagonistic potency of two analogues with opposite chiralities at C^{α} in position 2 is not surprising. However, to our knowledge, this is the first example where inversion of the chirality at the C^{β} position changes the biological activity of a peptide ligand by as much as 3 orders of magnitude. Furthermore, the chirality inversion changes the antagonist activities of the bicyclic analogues in a highly correlated way. Apparently, these unusual structure−activity relationships are a result of the strong conformational preferences of the *p*-MeOTMT side chains.[1c,10]

As illustrated in Figure 2, both the (2*S*,3*R*)- and (2*R*,3*S*)-*p*-MeOTMT residues prefer the trans χ^1 rotamer, while (2*S*,3*S*)- and (2*R*,3*R*)-*p*-MeOTMT residues prefer the gauche (−) or the gauche (+) χ^1 rotamer, respectively. To compare the overall topographical features of all four [*p*-MeOTMT^2]BC-OT analogues, energy minimization using the OPLS force field[16] implemented in Macromodel 4.5 software[17] has been performed starting with the proposed bioactive conformation of BC-OT,[6] and with the favored conformations of the *p*-MeOTMT^2 side chains.[10] As shown in Figure 3, incorporation of four *p*-MeOTMT diastereoisomers did not affect significantly the overall backbone conformation of the BC-OT analogues. However, the orientation of the aromatic side chain in *p*-MeOTMT^2 residues differs dramatically between the active and inactive analogues. It seems that the preferred gauche rotamers of (2*R*,3*R*)- and (2*S*,3*S*)-*p*-MeOTMT^2 can result in a similar orientation of the critical aromatic binding pharmacophores to favorably interact with a hydrophobic binding pocket in the uterine receptor, whereas the favorable trans rotamers of both (2*S*,3*R*)- and (2*R*,3*S*)-*p*-MeOTMT residues misplace the aromatic pharmacophore. Thus, the differential topography of the aromatic pharmacophore in position 2 leads to a 1000-fold or greater difference in receptor affinity and antagonist potency of the two pairs of enantiomeric BC-OT analogues. Therefore, it may be concluded that the bioactive rotamer of the aromatic side chain in position 2 of bicyclic oxytocin antagonist is gauche (−) for L-amino acids and gauche (+) for D-amino acids. In conjuction with the previously proposed backbone structure of BC-OT,[6] our present study supplies a well-defined model of the biologically active conformation for oxytocin antagonists. This series of oxytocin analogues also provides an excellent example of the importance of topographical considerations in the design of novel bioactive peptides to explore in detail information about peptide−receptor recognition.

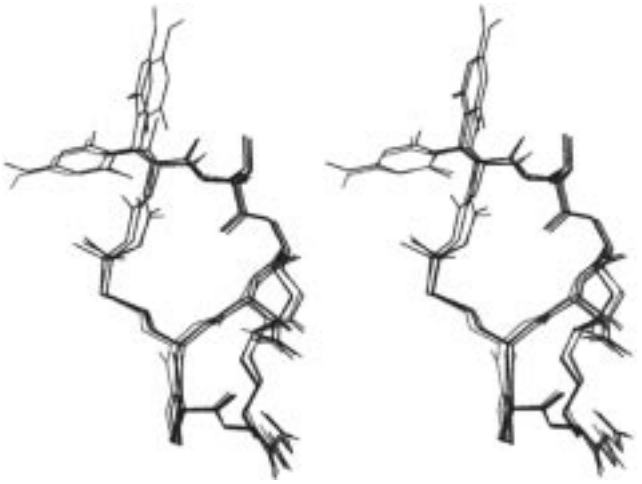

Figure 3. Overlapped stereoview of the miminum energy conformations of all four [*p*-MeOTMT^2]BC-OT analogues: (2*S*,3*S*)-*p*-MeO-TMT^2-BC-OT (dark green); (2*R*,3*R*)-*p*-MeO-TMT^2-BC-OT (red); (2*S*,3*R*)-*p*-MeO-TMT^2-BC-OT (green); (2*R*,3*S*)-*p*-MeOTMT^2-BC-OT (blue).

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Supporting Information Available: Synthetic methodology, analytical data on new compounds, and bioassay methods (7 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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