Brief Articles

Endomorphin-2 with a β-Turn Backbone Constraint Retains the Potent μ-Opioid Receptor Agonist Properties

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The constitutional similarity with different secondary structure preference between the Aba-Gly and the spiro-Aba-Gly scaffolds were exploited to design the novel endomorphin-2 analogs Tyr-spiro-(*R*/*S*)-Aba-Gly-Phe-NH_2 (**1** and **2**) and Tyr-(*R*/*S*)-Aba-Gly-Phe-NH_2 (**3** and **4**). The (*R*)-spiro analog **1** was found to be a potent and selective μ-opioid agonist/partial agonist ($K_{i\mu}$ = 29.3 nM, IC_{50} = 50 nM, K_e = 0.57). NMR experiments and molecular modeling indicated that its backbone adopts mainly a β-turn in aqueous solution.

Introduction

Endomorphin-2 (H-Tyr-Pro-Phe-Phe-NH_2) is a highly potent and selective agonist acting at μ-opioid receptors.[1] Because the μ-opioid receptor agonists display the most potent antinociceptive activity, endomorphins are important model peptides in the search toward new analgesics. However, the three-dimensional structure of the μ-opioid receptor and great details of its binding site are unknown. A possible way to explore the structural features of the μ-opioid receptor—μ-agonist interaction is the introduction of conformational constraints into the ligands. Recently, the conformational freedom of the Phe side chains of endomorphin-2 has been reduced, resulting in [β-MePhe]endomorphin-2 derivatives with enhanced affinity and selectivity.[2] Not only the side chains, but also the peptide backbone of the endomorphin-2 are highly flexible as the formation of a number of secondary structural elements (different β- and γ-turns) was revealed by conformational analysis.[3] In one type of the β-turns, an intramolecular hydrogen bond was also present. Doi et al. reported the substitution of the Pro^2 residue of endomorphin-2 with 1-aminocyclohexane-1-carboxylic acid and it was found that the [Ac_6c^2]endomorphin-2 derivative adopted a β-turn-like structure with an intramolecular hydrogen bond.[4] The relevance of β-turns in the μ-opioid receptor—ligand interaction has been further emphasized when the β-turn mimetic 4,7-dioxo-hexahydro-pyrazino[1,2-*a*]pyrimidine scaffold was used to orient the opioid pharmacophore elements. This bicyclic scaffold mimics a type III β-turn, and the corresponding peptidomimetic retained the bioactivity of endomorphin-2.[5] Another type of turn, the N−O turn, was also introduced into the endomorphin-2 sequence, which resulted in compounds with high μ-opioid receptor affinity but low δ- over μ-opioid receptor selectivity.[6]

Recently, we have found that in a series of 4-amino-1,2,4,5-tetrahydro-2-benzazepine-3-one containing tetrapeptide models (Ac-Aba-Xaa-NHMe), the [5,7]spirocyclic derivative, Ac-spiro-Aba-Gly-NHMe exclusively formed a β-turn, while other lactams, such as Ac-Aba-Gly-NHMe adopted mainly an extended conformation.[7] These pseudodipeptides allowed us to design unique endomorphin-2 mimetics that are constitutionally similar to endomorphin-2 but are different in secondary structure preferences. With this novel approach, we could directly explore the effect of the backbone conformation on the μ-opioid receptor—agonist interaction. The target compounds were obtained by the substitution of the middle Pro^2-Phe^3 sequence of endomorphin-2 with the spiro-Aba-Gly and Aba-Gly building blocks. The structure of the resulting peptide amides, Tyr-(*R*/*S*)-spiro-Aba-Gly-Phe-NH_2 and Tyr-(*R*/*S*)-Aba-Gly-Phe-NH_2 were then examined by NMR spectroscopy and molecular modeling, and their function was examined by different in vitro assays.

Chemistry

The novel endomorphin-2 mimetics were prepared by standard solid-phase methodology using 4-methylbenzhydrylamine (MBHA[a]) resin. Boc-(*S*)- and Boc-(*R*)-Aba-Gly-OH as well as racemic Moc-spiro-Aba-Gly-OBn were prepared as described earlier.[8,7] The latter was converted into the SPPS-compatible racemic Boc-spiro-Aba-Gly-OH (Scheme 1). The Moc-protected benzyl ester was hydrolyzed with 33% hydrobromic acid in

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[a] Abbreviations: H-Aba-Gly-OH, 2-(4-amino-4,5-dihydro-3-oxo-1*H*-benzo[*c*]azepin-2(3*H*)-yl)acetic acid; Boc, *tert*-butyloxycarbonyl; CHO, Chinese hamster ovary; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]enkephalin; DIEA, *N*,*N*-diisopropylethylamine; DMSO-d_6, hexadeuterodimethylsulfoxide; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; MBHA, 4-methylbenzhydrylamine; Moc, methyloxycarbonyl; MVD, mouse vas deferens; NOE, nuclear Overhauser effect; ROESY, rotating-frame Overhauser effect spectroscopy; RP-HPLC, reversed-phase high-performance liquid chromatography; TBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium tetrafluoroborate; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; TOCSY, totally correlated spectroscopy.

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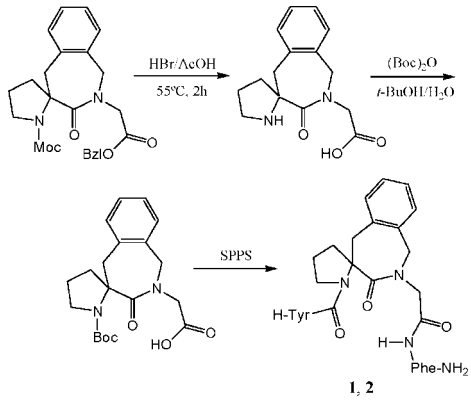
Scheme 1. Preparation of the Spirocyclic Endomorphin-2 Mimetics



1, **2**

acetic acid[9] at 55 °C for 2 h, and the resulting secondary amine, which was quantitatively formed, was acylated with Boc_2O. Cleavage of the Moc-protected benzyl ester failed with other reagents, such as trimethylsilyl iodide[10] or 6 M HCl, as the amide bond in the benzazepinone ring was also hydrolyzed, resulting in α-(*o*-((carboxymethyl-amino)-methyl)-benzyl)-proline. The racemic Boc-spiro-Aba-Gly-OH was coupled to Phe-MBHA resin in the presence of TBTU and DIEA. After deprotection, Boc-Tyr(2,6-Cl_2-Bzl)-OH was coupled under the same conditions. Finally, the diastereomeric mixture of the endomorphin-2 mimetic **1** and **2** was cleaved from the resin with liquid HF in the presence of dimethyl sulfide and anisole. The peptide diastereomers were separated by RP-HPLC on a Vydac 218TP1010 column. In a water−acetonitrile gradient system, their resolution was sufficiently high as the capacity factors of **1** and **2** were 2.47 and 3.72, respectively. The derivatives **3** and **4** were prepared similarly from Boc-(*R*)- and (*S*)-Aba-Gly-OH.

To determine the absolute configuration of the quaternary carbon atom in **1** and **2**, the Boc-(*S*)-spiro-Aba-Gly-OH was prepared by asymmetric synthesis, using the method described for the racemic compound,[7] but starting from (*S*)-*o*-cyanobenzyl-Pro. Then the Tyr-(*S*)-spiro-Aba-Gly-Phe-NH_2 diastereomer was synthesized, which was used to assign the absolute configuration of the HPLC-separated peptide diastereomers. It turned out that the first eluting endomorphin-2 derivative (**1**) has the (*R*)-configuration, and the isomer with higher retention time (**2**) has the (*S*)-configuration at the spiro-fused ring carbon atom (see Supporting Information).

Biological Data

The effect of the structural modification on the bioactivity of endomorphin-2 was examined by different bioassays. First, the in vitro enzymatic stability of the peptides **1**–**4** was measured in a rat brain homogenate lacking the cerebellum, as described previously.[11] The kinetics of their metabolism was characterized by analyzing the digestion mixtures with RP-HPLC. The velocity constants and half-lives were then calculated on the basis of pseudo-first-order kinetics. The spirocyclic derivatives **1** and **2** were found to be resistant to proteolytic hydrolysis ($t_{1/2} > 24$ h), compound **3** was stable for hours, while compound **4** was rapidly degraded ($t_{1/2} < 1$ min; see Supporting Information).

The receptor binding properties of the novel analogs were examined by radioligand binding assays on a crude membrane fraction of Wistar rat forebrains (Table 1). The incorporation of a spirocyclic benzazepinone β-turn mimetic resulted in the derivative **1** with 3-fold lower μ-opioid receptor affinity than endomorphin-2 but with no affinity to the δ-opioid receptor. As a result, its μ- over δ-selectivity was found to be very high. The configuration of the spiro carbon atom in this derivative is (*R*), which gives the same spatial arrangement as in L-Pro. This finding is in agreement with the fact that the L-configuration of Pro was considered vital for μ-opioid activity and selectivity.[12,13] Furthermore, the endomorphin-2 derivative **4** containing the extended conformation preferring pseudo dipeptide and having also L-stereochemistry at residue 2, displayed low affinity to the μ- and the δ-opioid receptors. The other spirocyclic diastereomer **2** and the nonspiro derivative **3** had practically no affinity to the opioid receptors.

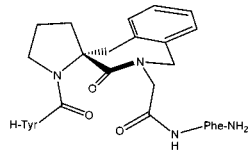
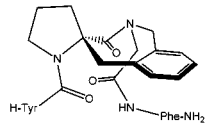
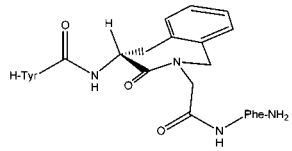
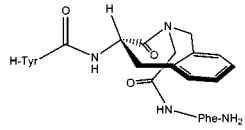
Analog **1** efficiently stimulated regulatory GTP binding proteins both in rat brain membrane fractions and in homogenates of CHO cells, stably expressing μ-opioid receptors, as determined in [^{35}S]GTPγS binding assays (Supporting Information). Addition of the opioid antagonist naloxone (1 μM) reversed this effect, clearly indicating the involvement of opioid receptors in the activation of the signal transduction pathways. In the recombinant receptor system E_{max} value of receptor-mediated G-protein activation stimulated by compound **1** was found to be fairly less than by endomorphin-2 or by the prototype synthetic μ-opioid agonist peptide DAMGO, but it was quite similar to those of morphine in rat brain membranes. The intermediate efficacy (E_{max}) values for **1** relative to the full agonist effect of DAMGO are consistent with the agonist/partial agonist property of **1**.

The opioid properties of **1** were further examined in the MVD bioassay, which contains all three opioid receptor types.[14] The potencies of **1**, endomorphin-2, and DAMGO to inhibit the electrically evoked contractions of MVD were compared (Table 1). The peptide **1** was found to be approximately three times less potent than endomorphin-2 and almost equipotent with DAMGO, as the IC_{50} values were calculated to be 50 nM for **1**, 17 nM for endomorphin-2, and 76 nM for DAMGO. The K_e value of naltrexone against **1** was similar to that of DAMGO (0.57 and 0.24, respectively), but it was approximately six times higher than that of endomorphin-2. These results indicate that **1** exerts its agonist/partial agonist action through μ-opioid receptors because the naltrexone K_e value was falling into the 0.2–0.6 nM range.[15]

Conformational Analysis

The structure of **1** was examined by NMR spectroscopy, and compounds **1**, **4**, and the parent endomorphin-2 were further studied by molecular modeling. The NMR studies of **1** were done in 90% H_2O/10% D_2O solution. To enhance the solubility of **1**, two drops of DMSO-d_6 were added to the solution. The ^{1}H chemical shifts were assigned by 2D NMR experiments, including 2D TOCSY and ROESY measurements augmented with gradient-enhanced water suppression schemes. The resulting ^{1}H NMR parameters are summarized in Supporting Information. In contrast to endomorphin-2, for which two sets of signals were observed in the NMR spectra corresponding to the *cis*- and *trans*-rotamers around the Tyr-Pro amide bond,[16] only one set of signals was present for **1**. Based on the observed NOEs between the Tyr^1 $H_{\beta\beta'}$ and spiro-Aba^2 H_δ and between the Tyr^1 H_α and spiro-Aba^2 $H_{\delta\delta'}$ protons, these signals were assigned to the *trans*-rotamer. A large difference in chemical shifts was found between the two nonequivalent Gly^3 H_α protons of **1** ($\Delta\delta = 1.24$ ppm), which is the consequence of the anisotropic shielding effect of the neighboring carbonyl groups originated from the constrained φ and ψ torsion angles. The

Table 1. Opioid Receptor Binding Potencies and Agonist Action on Isolated Tissue

no.	cmpd	receptor affinity			MVD[a]	
		$K_{i\mu}$	$K_{i\delta}$	$K_{i\delta}$ / $K_{i\mu}$	IC_{50} (nM)	K_e (nM)[b]
	endomorphin-2	9.5 ± 2.0	n.d.	–	16.79 ± 3.75	0.09 ± 0.03
		0.69 ± 0.16[1]	9233 ± 201[1]	13381[1]		
	DAMGO	2.4 ± 0.2	n.d.	–	75.55 ± 6.98	0.24 ± 0.01
		0.34 ± 0.07[1]	190 ± 16[1]	559[1]		
1		29.3 ± 2.9	>10000	>350	49.81 ± 3.99	0.57 ± 0.24
	Tyr-(*R*)-spiro-Aba-Gly-Phe-NH_2					
2		8732 ± 819	2312 ± 820	0.26	n.d.	n.d.
	Tyr-(*S*)-spiro-Aba-Gly-Phe-NH_2					
3		2245 ± 104	2965 ± 129	1.32	n.d.	n.d.
	Tyr-(*R*)-Aba-Gly-Phe-NH_2					
4		373 ± 66	778 ± 101	2.08	n.d.	n.d.
	Tyr-(*S*)-Aba-Gly-Phe-NH_2					

[a] Agonist potencies of opioid peptides in mouse vas deferens (MVD) preparation. Data are presented as means ± S.E.M of 3–7 independent duplicate experiments. [b] The equilibrium dissociation constant of naltrexone.

formation of a stable secondary structure was further confirmed by the temperature coefficient of the Gly NH proton. It was found to be −4.2 ppb/K for Ac-spiro-Aba-Gly-NHMe,[7] and −5.2 ppb/K for **1** in DMSO-d_6, that is, the turn structure of the pseudo dipeptide could be transposed into the tetrapeptide **1**. In contrast, a significantly higher temperature dependence was found in water for **1** (−7.6 ppb/K), indicating that this amide proton is exposed to the aqueous solvent and does not participate in an intramolecular hydrogen bond. In such turn structures without intramolecular H-bonds, the aromatic–aromatic and proline−aromatic interactions stabilize the conformers.[17] The backbone conformation and the mean orientation of the side chain groups are related to the vicinal coupling constants $^3J_{NH}$ and $^3J_{H\alpha\beta}$ (see Supporting Information). The population of χ_1 rotamers (*gauche* (−), *trans*, and *gauche* (+)) calculated from the measured $^3J_{H\alpha\text{-}H\beta}$ coupling constants varied between 20 and 40% in both Tyr^1 and Phe^4 residues, suggesting their side chain flexibility due to rotation around the $C_\alpha-C_\beta$ bond.

Structures of **1** and **4** were compared with endomorphin-2 by molecular modeling to investigate possible structural determinants of the opioid activity. Although **1** and **4** contain unnatural amino acid residues, each molecule has a native peptide backbone. Therefore, these peptides can adopt analogous conformations and their secondary structure is directly comparable. A 2000-membered conformational ensemble was generated for the peptides using distance geometry, and then the unique geometries were optimized using the AM1 method and analyzed after the removal of the duplicate structures and the structures with relative potential energy higher than 15 kcal/mol.[18] This procedure was repeated for **1**, with the inclusion of constrained H−H distances derived from ROESY experiments (for a detailed description, see Supporting Information). The volumes of ROESY cross peaks were converted to distance restraints using the intensities of Gly^3 H_α,$H_{\alpha'}$ peaks for calibration. To avoid biasing the structure calculations, only unambiguously assigned ROESY cross peaks were used. A total of 12 different conformational families of endomorphin-2 were identified, including extended and bent structures, classic and inverse γ-turns around Phe^3, inverse γ-turn around Pro^2, and type I, II, and V β-turns (Table 2). These findings are in agreement with the results of a previous simulated annealing study on endomorphin-2.[3] In contrast, type I and V β-turns and inverse γ-turn around Pro^2 were not found in the conformational ensemble of **4**, however, an additional type II′ β-turn was found. This difference is the result of the constraint introduced by the benzazepinone ring. Incorporation of the more constrained spirocyclic benzazepinone moiety resulted in an even more rigid

Table 2. Available Conformations of Endomorphin-2, **1**, and **4** Obtained from Molecular Modeling

		Peptide			
	conformation	endomorphin-2	**4**	**1**	**1**[b]
trans[a]	random/extended	+	+	−	−
	bend	+	+	+	+
	classic γ-turn around Phe^3/Gly^3	+	+	+	+
	inverse γ-turn around Phe^3/Gly^3	+	+	+	−
	inverse γ-turn around Pro^2	+	−	−	−
	type I β-turn	+	−	−	−
	type II β-turn	+	+	+	+
	type II′ β-turn	−	+	−	−
	type V β-turn	+	−	−	−
cis[a]	random/extended	+	+	+	−
	bend	+	+	+	−
	classic γ-turn around Phe^3/Gly^3	+	+	+	−
	inverse γ-turn around Phe^3/Gly^3	+	+	+	−

[a] Rotamers around the Tyr^1−Pro^2 amide bond. [b] Conformations for **1** obtained by using ROE distance restraints.

structure, as confirmed by the NMR studies: only eight conformational families were identified for **1** by the modeling method. Compared to **4**, the possible conformations for **1** were further reduced. It suggests that the only turn geometries facilitated by the spirocyclic benzazepinone moiety in **1** are the γ-turns around Gly^3 and the type II β-turn. This is further supported by the results obtained with the inclusion of ROESY-derived distance restraints in the calculations. Only three conformational families were found for **1**, including bent structures, classic γ-turns formed around Gly^3, and type II β-turns (Figure 1), that is, in aqueous environment, a turn structure is more readily adopted than extended or random conformations by **1**. Although the relative population of the different conformational families can not be given with our modeling data, it is hypothesized that endomorphin-2 and **1** fold into a turn conformation more readily than **4** does, and such turns are responsible for advantageous orientation of the pharmacophore groups of endomorphin-2.

Conclusions

Without the high resolution structure of the μ-opioid receptor protein, it remains difficult to study the interaction of an opioid ligand with the receptor and to optimize this interaction. In this work, we performed a comparative study on constitutionally very similar endomorphin-2 mimetics, which remarkably tend to adopt different secondary structures. Recently, a stable β-turn inducing mimetic, Ac-spiro-Aba-Gly-NHMe has been developed and the related compound, Ac-Aba-Gly-NHMe, has been found to prefer an extended conformation.[7] The secondary structure of the μ-opioid receptor agonist endomorphin-2 was altered by these Aba-Gly pseudo dipeptides, resulting in the native backbone sequence. NMR spectroscopic and molecular modeling methods revealed that the spirocyclic benzazepinone derivative retained its turn structure in the endomorphin-2 tetrapeptide sequence, that is, this core structure constrained the endomorphin-2 backbone into a bent structure. It is important to note that the introduction of the spirocyclic lactam resulted in a modified distance between the first and second aromatic side chains (Tyr^1 and Phe^3 in endomorphin-2 vs Tyr^1 and Aba in **1**–**4**). However, **1** retained the bioactivity of endomorphin-2, which means that the obtained structure–activity conclusions must be relevant to the endomorphin-2–μ-opioid receptor interacting system.

The spatial arrangement corresponding to the L-Pro in endomorphin-2 is found in **1** and **4**, while that equivalent with the D-Pro is found in **2** and **3**. Derivative **1** exhibited high affinity and selectivity toward the μ-opioid receptor, while **4** displayed very low μ- and δ-opioid receptor affinity, and further, **2** and **3** lost their bioactivity. In different in vitro bioassays, **1** turned out to be a potent opioid agonist that action was naloxone or naltrexone reversible. As a consequence, both the spirocyclic ring and the proper chirality are essential for retaining the bioactivity of endomorphin-2. Furthermore, because the core spiro-Aba-Gly structure bends the tetrapeptide backbone and this structure establishes proper interactions with the receptor, we can hypothesize that for proper orientation of the pharmacophore groups it is necessary to bend the peptide backbone and the μ-opioid receptor prefers such conformers from the whole endomorphin-2 conformer population.

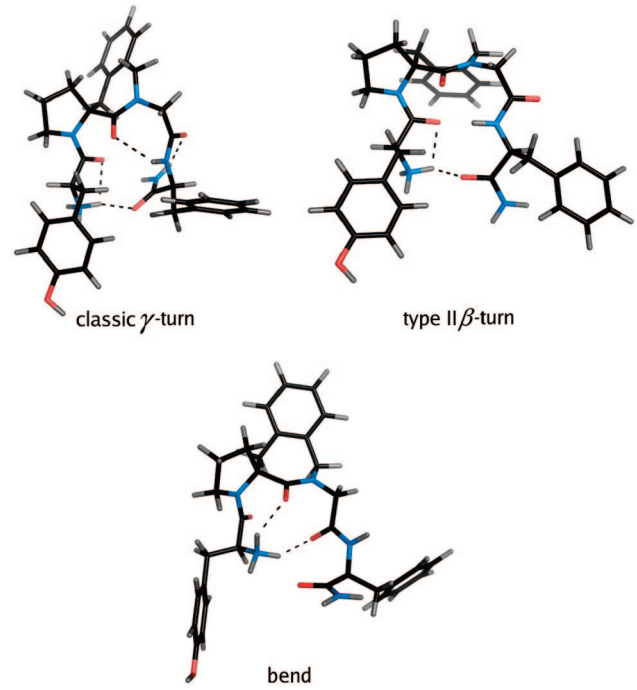


Figure 1. Representative conformers of **1** obtained with ROE distance restraints.

Beyond the influence on the binding properties, a structural consequence of our designed turn in endomorphin-2 is that the Tyr^1−Pro^2 amide bond must be in the *trans*-conformation, otherwise, there is no way to form a turn structure. The importance of the Tyr^1−Pro^2 amide bond conformation of endomorphins in their bioactive form is a controversial and maybe overstressed problem. Earlier, the *trans*-conformer, which is present in higher portion in the *cis*/*trans*-equilibrium, was concluded to be the bioactive conformer,[19] and later, the *cis*-conformer was also directly evidenced to be the bioactive conformer.[20] This is not an ambiguity when the opioid receptor protein selects its ligand by conformational selection in a dynamic environment, and thus, the conformation of a certain amide bond alone cannot be a strict determinant for a stable ligand–receptor interaction. It works in the case of DAMGO, which is highly flexible and very potent, or it can be the situation when the turn conformers of endomorphin-2 are selected.

Experimental Section

Boc-spiro-Aba-Gly-OH. A total of 900 mg of Moc-spiro-Aba-Gly-OBn[7] (2.13 mmol) was dissolved in 40 mL of 33% HBr in AcOH and it was stirred under an Ar atmosphere at 55 °C for 2.5 h. The solution was evaporated then redissolved in glacial acetic acid and evaporated again. The crude product was dried under vacuum: H-spiro-Aba-Gly-OH; yield 990 mg; ESI-MS 275 $[M + H]^+$;

HPLC $k' = 2.43$. The crude H-spiro-Aba-Gly-OH (990 mg) was dissolved in 20 mL of *t*-BuOH and 8.5 mL of water, and pH 9 was adjusted with 5 M aqueous NaOH solution. Then 890 mg of $(Boc)_2O$ (4.09 mmol) was added to the solution in small portions, followed by addition of 20 mL of *t*-BuOH. The turbid solution was stirred at room temperature overnight and after dilution with 50 mL of water it was extracted three times with 80 mL of pentane. The water phase was cooled in ice, acidified with 1 M H_2SO_4 and extracted with EtOAc. The EtOAc extract was washed with brine, dried over $MgSO_4$, and evaporated. Yield 790 mg (98%); ESI-MS 375 $[M + H]^+$, 319, 275 $[(M\text{-}Boc) + H]^+$; TLC R_f 0.66 ($CHCl_3$−MeOH−AcOH 90:8:2); HPLC $k' = 5.58$; ^{1}H NMR (DMSO-d_6) δ 7.28 (m, 4H, Ar-H), 5.00 and 4.92 (2 × d, 1H, J = 14.6 Hz, 1-H), 4.31 and 4.22 (2 × d, 1H, J = 17.1 Hz, Gly-α-H), 4.21 and 4.09 (2 × d, 1H, J = 14.6 Hz, 1-H′), 4.05 and 3.94 (2 × d, 1H, J = 17.1 Hz, Gly-α-H′), 4.06 and 3.99 (2 × d, 1H, J = 14.0 Hz, 5-H), 3.44 (m, 2H, δ-H_2), 2.69 and 2.60 (2 × d, 1H, J = 14.0 Hz, 5-H′), 1.69 (m, 4H, β-H_2, γ-H_2), 1.42 and 1.40 (2 × s, 9H, $C(C\underline{H}_3)_3$). *cis*−*trans* ratio 1:3 (calculated from the proton signal intensities). ^{13}C NMR (DMSO-d_6) δ 174.8 and 174.4 (C-3), 171.0 and 170.9 (Gly CO), 153.7 and 153.3 (carbamate CO), (138.5 and 138.2, 138.0) (C-6, C-7), 130.2 and 130.0 (C-11), (128.5, 128.4, 127.2 and 127.0) (C-8, C-9, C-10), 79.5 and 79.0 ($\underline{C}(CH_3)_3$), 69.0 and 68.6 (C-4), 52.7 and 52.5 (Gly C-α), 52.2 and 52.1 (C-1), 48.8 and 48.6 (C-δ), 40.5 (C-5), 39.5 (C-β), 28.6 and 28.3 (3C, $C(\underline{C}H_3)_3$), 23.0 and 22.1 (C-γ). For numbering see ref 21.

Boc-(*S*)-spiro-Aba-Gly-OH. This compound was prepared as described for the racemic compound,[7] but starting from commercially available (*S*)-α-(*o*-cyanobenzyl)proline; $[\alpha]^{20}_D$ +36 (*c* 1, MeOH).

H-Tyr-spiro-Aba-Gly-Phe-NH_2 (1 and 2). Boc-spiro-Aba-Gly-OH (188 mg, 0.5 mmol) was activated with TBTU in the presence of DIEA, and it was coupled to the Phe-MBHA resin (0.3 mmol) for 4 h. After deprotection, Boc-Tyr(2′,6′-Cl_2-Bzl)-OH (0.9 mmol) was coupled two times in the presence of TBTU and DIEA. The peptide was cleaved from the resin with HF in the presence of dimethyl sulfide and anisole. The mixture of diastereomers **1** and **2** was obtained in 67% yield. ESI-MS 584 $[M + H]^+$; HPLC $k' =$ 3.03 (**1**) and 4.50 (**2**).

H-Tyr-Aba-Gly-Phe-NH_2 (3 and 4). The peptides were prepared as above, but Boc-(*R*)-Aba-Gly-OH and Boc-(*S*)-Aba-Gly-OH[8] were used for **3** and **4**, respectively. Compound **3** was obtained in 68% yield; ESI-MS 544 $[M + H]^+$; HPLC $k' = 2.37$. Compound **4** was obtained in 66% yield; ESI-MS 544 $[M + H]^+$; HPLC $k' = 2.14$.

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Supporting Information Available: Additional experimental details, HPLC and NMR data, and radioligand binding curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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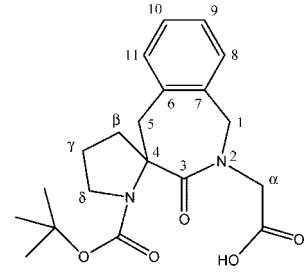

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