Novel Design of Bicyclic β -Turn Dipeptides on Solid-Phase Supports and Synthesis of [3.3.0]-Bicyclo^[2,3]-Leu-enkephalin Analogues

Xuyuan Gu, Jinfa Ying, Richard S. Agnes, Edita Navratilova,[†] Peg Davis,[†] Gannon Stahl,[†] Frank Porreca,[†] Henry I. Yamamura,[†] and Victor J. Hruby^{*}

Department of Chemistry, University of Arizona, 1306 East University Boulevard, Tucson, Arizona 85721, and Department of Pharmacology, University of Arizona Health Science Center, Tucson, Arizona 85724

hruby@u.arizona.edu

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ABSTRACT



External bicyclic β -turn dipeptide mimetics provide an excellent design approach that can offer a rich chiral ensemble of structures with different backbone conformations. We report herein a novel design of a convergent combinatorial synthetic methodology, which is illustrated by the solid-phase synthesis of a series of [3.3.0]-bicyclo^[2,3]-Leu-enkephalin analogues. The reactions were optimized and the epimeric configurations were determined by 2D NMR spectroscopy. Biological assays show that these analogues have more potent δ binding affinity and bioactivity for δ vs μ opioid receptor, which may be related to the different conformations preferred by these analogues in our modeling studies.

Numerous efforts have been focused on building a variety of β -turn peptidomimetics.¹ Among them, bicyclic β -turn dipeptide (BTD) mimetics have been generally classified as internal or external β -turn mimetics^{2a} based on the covalent scaffold support located inside or outside the turn structure. Several types of internal β -turn-stabilizing structures have been developed,^{1c,d,2} a few of which have been used to generate combinatorial libraries by using solid-phase synthesis.^{2a,c,e} External bicyclic β -turn dipeptides^{2a} are especially interesting because they can control not only the ψ and φ dihedral angles of the backbone but also the χ_1 and χ_2 dihedral angles of side chain groups (Figure 1). Different kinds of external bicyclic scaffold synthetic methodologies have been developed by several groups,³ but a systematic application of these methods to solid-phase synthesis has not been reported. Due to the lack of efficient synthetic methodologies for preparing structurally diverse bicyclic

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Figure 1. Design of external BTD and its retrosynthetic analysis on the solid phase.⁴

dipeptides, most of this work has been limited to obtaining only a few of the possible isomers of bicyclic dipeptides in a particular scaffold.

Recently, we have been working on the asymmetric synthesis of different kinds of BTD mimetics⁵ to obtain a better understanding of the molecular basis of peptide ligand-protein receptor interactions that lead to high potency and receptor selectivity. The BTD design 2 (Figure 1) maintains the original peptide backbone. This design is general enough to be used for all peptides which may involve β -turn structures. The stereochemistry of these BTDs generally has been predetermined by the nature of the novel amino acid building blocks which are used for the synthesis. We envisioned that bicyclic dipeptides could be synthesized on a solid-phase support in conjunction with synthesis of larger peptides in a manner similar to solid-phase peptide chemistry. A retrosynthetic analysis of this concept indicates that these bicyclic dipeptides actually need only two nonstandard amino acids (Figure 1). They are β -substituted ω -unsaturated amino acids 4 and the β -substituted cysteines 5, respectively. Both of these enantiomerically pure nonproteinous amino acids can be synthesized using synthetic methodologies we recently have developed.6

To demonstrate this novel concept, we chose Leuenkephalin, an endogenous opioid peptide as a target peptide.⁷ Enkephalin analogues with δ and μ opioid agonist activities have been extensively studied.⁸ Previous work indicated that although enkephalin itself has a random structure in water, a β -turn conformation at the Glv²-Glv³ positions is important for its biological activity.⁹ A (3S,6S,9R)-[4.3.0]-azobicyclo^[2,3]-Leu-enkephalin^{10a} and a (2R,5S,8S)-[3.4.0]-thiazobicyclo^[2,3]-Leu-enkephalin^{10b} have been prepared previously by conventional methods.¹⁰ However, one single isomer inserted in the enkephalin structure gives a very limited picture. Here we report a novel synthesis of several [3.3.0]-bicyclo^[2,3]-Leu-enkephalin analogues with different geometries by nonconventional solid-phase synthesis.¹¹ The synthesis of S^{β} -Fm-cysteine was accomplished by a modified method.¹² S^{β}-Fm protection was chosen so that both the N^{α} -Fmoc and S^{β} -Fm protecting groups can be simultaneously deprotected by piperidine. Allylglycine was protected by an N^{α} -Fmoc, and the terminal alkene was oxidized to the aldehyde by OsO₄/NaIO₄.13

 N^{α} -Fmoc-Leu-Wang resin was used to begin our synthesis of the [3.3.0]-bicyclo^[2,3]-Leu-enkephalin analogues (Scheme 1). N^{α} -Fmoc deprotection and the subsequent coupling steps





^{*a*} Key: (i) 25% piperidine in DMF; (ii) Fmoc-Phe-OH, HBTU, HOBT, DIEA; (iii) identical to i; (iv) Fmoc-L-Cys-S(Fm)-OH, DCM/DMF, HBTU, HOBT, DIEA; (v) 50% piperidine, 5% TIPS, DMF; (vi) *S*-2-(9*H*-fluorenyl-9-methoxycarbonylamino)-4-oxobutyric acid, DMF, DIEA; (vii) HBTU, HOBT, DIEA; (viii) identical to i; (ix) Fmoc-Tyr-(O-'Bu)-OH, HBTU, HOBT, DIEA; (x) identical to i; (xi) 90%TFA, 5% H₂O, 5% TIPS.

were accomplished by conventional methods.¹³ After cysteine coupling, the N^{α} -Fmoc and S^{β} -Fm were deprotected using 50% piperidine in DMF and 5% TIPS to guarantee the completeness of the reaction in 30 min. The Kaiser test¹⁴ for Cys residues does not show a deep blue color in the

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presence of the unprotected thiol because the sulfur reacts in an intramolecular reaction¹⁵ to give a thiazolidine. As a matter of fact, a positive color indicated an incomplete deprotection of the S^{β} -Fm on sulfur. The *N*,*S*-thiazolidine formation with R- or S-2-(9H-fluorenyl-9-methoxycarbonylamino)-4-oxobutyric acid followed and was optimized in DMF with 2 equiv of DIEA within 2 h. A similar strategy for thiazolidine synthesis on a solid-phase support has been reported previously.¹⁶ Diastereomeric peptides with the epimeric bridge head H were formed at this stage. The lactamization of the secondary amine using HBTU and HOBT in the presence of DIEA progressed quickly. Finally, N^{α} -Fmoc-Tyr-(O-^{*t*}Bu)-OH was introduced by conventional peptide synthesis.¹³ After N^{α} -Fmoc deprotection, the peptide was cleaved from resin. The peptide solution was evaporated and neutralized, and the diastereomeric analogues were isolated and purified on a reversed-phase HPLC column.¹³

This 11-step synthesis of bicyclo^[2,3]-Leu-enkephalin thus was completed, and two bridgehead diastereomeric peptides were generated in about 12 h time. The combined yield of the final products was 48% with clean HPLC spectra.¹³ This optimized procedure was used to synthesize two other diastereomers **13a** and **13b** from D-allylglycine in a combined yield of 43%. The diastereomeric ratio of **12a** (2R,5R,7S) to **12b** (2R,5S,7S) was 47:53 while the ratio of **13a** (2R,5R,7R) to **13b** (2R,5S,7R) was 40:60.¹³ The bridgehead configuration were assigned on the basis of ROE connectivity patterns (Figure 2). We were fortunate that the bridgehead hydrogens (4.8–5.0 ppm) are well separated from other protons in the D₂O solution NMR spectra, allowing the relationship of these isomers to be assigned unambiguously.

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Figure 2. Stereochemistry of bicyclic dipeptides and the bridgehead ROEs.

The synthesized [3.3.0]-bicyclo^[2,3]-Leu-enkephalin analogues were examined for their binding affinities to opioid receptors in competition with [³H]-deltorphin II (δ) and [³H]-DAMGO (μ) (Table 1). All the analogues show binding affinities with IC₅₀ values in the micromolar range. In comparing the δ and μ binding affinities, we observed they

Table 1. Binding Affinity in Competition with [³H]-DAMGO and [³H]-Deltorphin II in Mouse Brain Membranes and Potency in MVD and GPI/LMMP Bioassays

peptide	Del II (δ) (%)	DAMGO	MVD	GPI/LMMP
analogues	IC ₅₀ (μ M)	(µ) (%)	(δ) (%)	(µ) (%)
12a (2 <i>R</i> ,5 <i>R</i> ,7 <i>S</i>)	$egin{array}{c} 22.5^a \ 47\pm9 \ 2.4\pm0.9 \ 33.1^a \end{array}$	0.6 ^a	14.4^b	3.8^b
12b (2 <i>R</i> ,5 <i>S</i> ,7 <i>S</i>)		0 ^a	10.3^b	6.1^b
13a (2 <i>R</i> ,5 <i>R</i> ,7 <i>R</i>)		20.6 ^a	15.5^b	5.9^b
13b (2 <i>R</i> ,5 <i>S</i> ,7 <i>R</i>)		2.6 ^a	11.5^b	4.6^b

 a Percent decrease of maximum binding at 10 μM peptide. b Percent decrease of maximum effect at 1 μM peptide.

all are more potent δ opioid ligands. The binding affinity of **13a** (2.4 μ M) with a D-amino acid in position-2 is about 20-fold better than that of **12b** (47 μ M) with an L-amino acid at this position. The effect of D-amino acids in Leuenkephalin analogues DPDPE has been previously discussed.¹⁷ The functional assays (MVD and GPI/LMMP) also show more potent δ activity than μ activity (Table 1).

Modeling studies¹³ have shown that the backbones conformations of [3.3.0]-bicyclo^[2,3]-Leu-enkephalin are controlled by different bicyclic chiralities. Although [3.3.0]-BTDs have been proposed as β -turn mimetics,¹⁸ their conformations may be modulated not only by the configurations of the BTDs but also by their specific peptide sequence. It is not surprising that no typical β -turn structure was

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observed for the bicyclo^[2,3]-analogues. However, a type I β -turn conformation in **13a** occurs at positions 3-4 ($\phi_3 = -46.0^\circ$, $\psi_3 = -43.9^\circ$, $\phi_4 = -94.9^\circ$, $\psi_4 = +7.3^\circ$), consistent with the X-ray structure of Leu-enkephalin previously reported.¹⁹ In addition, the distance (8.78 Å) between the two aromatic ring centroids fits well with expected distances for δ receptor ligands.²⁰ In light of the type I turn structure and the pharmacophore distance requirements, no other analogues show a better fit, which may provide the structural basis for the higher δ binding affinity and δ bioactivity of **13a** relative to the other analogues.

In summary, we have developed a novel methodology for the synthetic assembly of external bicyclic dipeptide containing peptides from chiral precursors on solid-phase supports. The synthesis of four isomers of [3.3.0]-bicyclo^[2,3]-Leuenkephalin represents the first example in this area. This fast and efficient synthetic strategy makes it possible to obtain diastereomeric libraries of these novel compounds. It should provide a unique tool for studying structure-biological activity relationships in combination with NMR and modeling techniques. The method is general enough for parallel synthesis of all chiral side-chain containing bicyclic dipeptides using the appropriate amino acid precursors. The syntheses of [3.3.0]-bicyclic dipeptides with side-chain groups and [3.4.0]- and [3.5.0]-bicyclic dipeptides on solidphase supports are currently under investigation.

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Supporting Information Available: Experimental procedures, purification, and characterization of all compounds; 2D NMR and computational study. This material is available free of charge via the Internet at http://pubs.acs.org.

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