COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Stereoselective synthesis of individual isomers of Leu-enkephalin analogues containing substituted β-turn bicyclic dipeptide mimetics†

Chiyi Xiong,[a] Junyi Zhang,[a] Peg Davis,[b] Wei Wang,[ac] Jinfa Ying,[a] Frank Porreca[b] and Victor J. Hruby*[a]

[a] *Department of Chemistry, University of Arizona, 1306 E. University Blvd., Tucson, AZ 85721, USA*
[b] *Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA*
[c] *Genomics Institute of the Novartis Research Foundation, 10675 John Jay Hopkins Drive, San Diego, CA 92121, USA*

Received (in Corvallis, OR, USA) 21st February 2003, Accepted 1st May 2003
First published as an Advance Article on the web 4th June 2003

Novel constrained β-turn dipeptide mimetics, 8-phenyl thiaindolizidinone amino acids 3, have been synthesized stereoselectively and incorporated into Leu-enkephalin peptides as a replacement of dipeptide Gly³–Phe⁴ to afford four individual isomers of Leu-enkephalin analogues 6.

In recent years, a great deal of effort has focused on the design of β-turn peptidomimetics, which are conformationally constrained to provide a better understanding of the stereostructural basis of peptide and protein interactions.[1] One of the pharmaceutically interesting biological targets for which the peptidomimetic approach has been applied recently is Leu-enkephalin.[2]

Previous studies on Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) and related cyclic analogues have suggested that it adopts, predominantly, a β-turn centered at Gly³-Phe⁴.[2a,3] These studies also suggested that for opioid peptides, the turn position residues not only play a role of conformation restriction, but also are involved in direct interaction with receptor(s).[2b,3] Furthermore, our studies have indicated that the conformational requirements for optimal interaction with the μ and δ receptors differ in a subtle manner.[4] With these criteria in mind, we propose that conformationally restricted, substituted bicyclic β-turn dipeptide (BTD) mimetics with different stereochemical configurations (Figure 1) would be a desirable peptidomimetic moiety. The compounds were designed to constrain two backbone angles (ψ_2 and φ_3), of the four torsion angles that define a β-turn, and most importantly, to place the critical phenyl side chain of the Phe⁴ residue of Leu-enkephalin correctly in 3D space. Incorporations of unsubstituted BTD into biologically active peptides have been used to investigate the structure–activity relationship between peptides and receptors.[5,2b] For example, Nagai and co-workers have incorporated unsubstituted thiaindolizidinone amino acid as a Gly-Gly replacement in Leu-enkephalin to produce an analogue, which exhibited 1/500 of the activity of the parent peptide.[2b] Lubell *et al.*employed the indolizidin-9-one amino acids as a constrained Gly-Gly surrogate in a Leucine-enkephalin mimic, and their compound showed affinities for opioid receptors that were three orders of magnitude lower than that of Leu-enkephalin.[5f] However due to the lack of a robust methodology, to the best of our knowledge, there is no report of the incorporation of 8-substituted BTDs into a biologically active peptide to make individual isomers which could truly mimic the peptide functions. In this communication we wish to report a flexible synthesis which allows for the preparation of diastereoisomers of Leu-enkephalin analogues containing the substituted chiral β-turn bicyclic dipeptide mimetics.

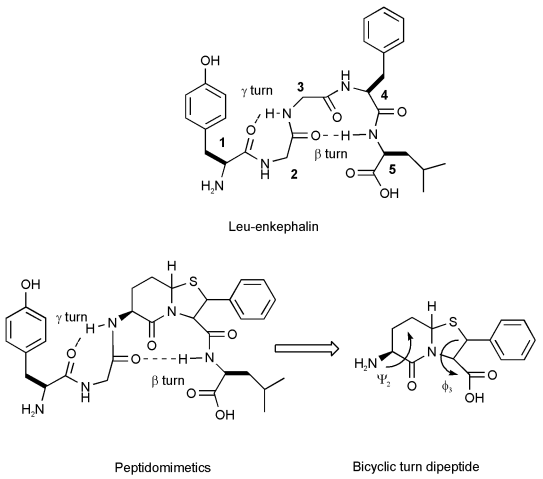
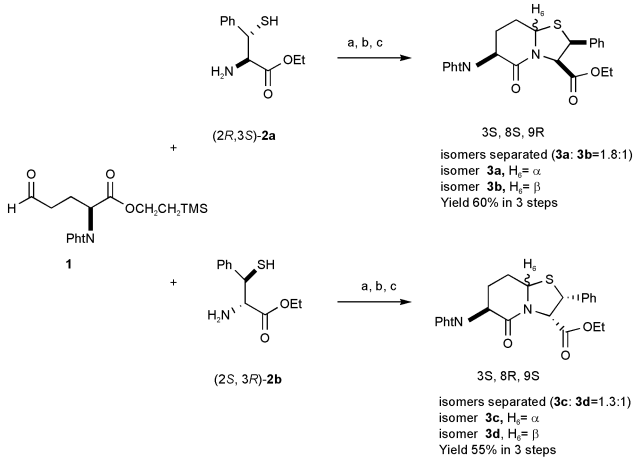


Fig. 1

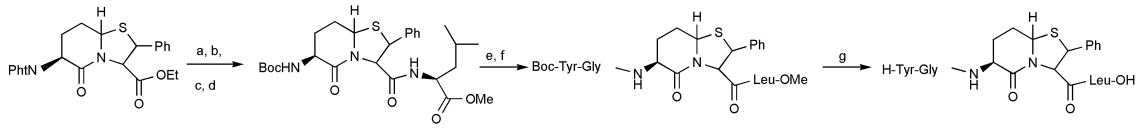
The synthesis of 8-phenyl-substituted thiaindolizidinone amino acids was accomplished using the convergent synthetic strategy in Scheme 1. The β-phenylcysteine derivatives (2*R*, 3*S*)-**2a** and (2*S*, 3*R*)-**2b**, were prepared according to our new protocol.[6] The doubly protected glutamic acid γ-aldehyde **1** was prepared according to a procedure previously described.[5a] Aldehyde **1** was converted to a mixture of thiazolidines by condensation with the β-phenylcysteine derivative (2*R*, 3*S*)-**2a**, or (2*S*, 3*R*)-**2b** in buffered aqueous ethanol. Sequential fluoride-mediated deprotection and cyclization of the resulting amino acids with the aid of carbodiimide coupling reagent resulted in clean formation of the bicyclic lactams **3a/3b–3c/3d** as epimers at the bridgehead. These diastereoisomers can be readily separated. The ratio of **3a/3b**, **3c/3d** ranged from 1.8–1.3 :1. The reaction behavior of the β-phenylcysteines is quite different than the unsubstituted counterpart, in which only one epimer was formed during the cyclization step.[5a] In addition, in the case of β-phenyl-substituted cysteines, the addition of the carbodiimide coupling reagent was necessary for efficient cyclization,



Scheme 1 Reagents: a, HOAc, EtOH, H_2O; b, $PhCH_2N(CH_3)_3F$; c, DCC/HOBt

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b3/b302235h/

DOI: 10.1039/b302235h



(3*S*, 6R, 8S, 9*R*)-**3a**
(3*S*, 6S, 8S, 9*R*)-**3b**
(3*S*, 6R, 8R, 9S)-**3c**
(3*S*, 6S, 8R, 9S)-**3d**

(3*S*, 6R, 8S, 9*R*)-**4a**
(3*S*, 6S, 8S, 9*R*)-**4b**
(3*S*, 6R, 8R, 9S)-**4c**
(3*S*, 6S, 8R, 9S)-**4d**

(3*S*, 6R, 8S, 9*R*)-**5a**
(3*S*, 6S, 8S, 9*R*)-**5b**
(3*S*, 6R, 8R, 9S)-**5c**
(3*S*, 6S, 8R, 9S)-**5d**

(3*S*, 6R, 8S, 9*R*)-**6a**
(3*S*, 6S, 8S, 9*R*)-**6b**
(3*S*, 6R, 8R, 9S)-**6c**
(3*S*, 6S, 8R, 9S)-**6d**

Scheme 2 Reagents: a, BBr_3, DCM; b, Leu-OMe·HCl, BOP, HOBt, DIPEA, DMF; c, NH_2NH_2, EtOH,$CHCl_3$, H_2O; d, $(BOC)_2O$, THF, TEA; e, TFA, 0 °C, 20 min; f, Boc-Tyr-Gly-OH, HOBt, BOP, DIPEA, DMF; g, BBr_3, DCM, −10 °C, 4 h, 9–15% overall yields.

while the unsubstituted bicyclic lactam can be formed through catalysis of dilute HCl in the case of unsubstituted cysteine.[5a] The stereochemistries at the bridgehead carbons (C-6) of the 8-substituted bicyclic thiazolidine lactams were assigned on the basis of selective 1D transient NOE experiments performed on **3a–d** (Figure 2). For example, in the experiment performed on **3a**, relatively strong NOEs were observed between H_6 and H_8, H_9, H_3 respectively, whereas weaker NOEs were observed between H_6 and H_8, H_9, H_3 respectively in the case of **3b**. Similar results were seen with bicyclic lactams **3c** and **3d**. These data support the assignment of a *R*-configuration at C-6 in **3a**/**3c** and a *S*-configuration at C-6 in **3b**/**3d**.

Incorporation of the 8-phenyl BTD into Leu-enkephalin using similar methodology as described before[5a] suffered some problems. For example, after converting the phthalyl protecting group to the N^{α}-Boc protecting group (Scheme 2), we tried to hydrolysize the ethyl ester group using standard base saponification conditions, However this failed because of the steric hindrance provided by the neighboring phenyl group, which is on the same face as the carboxylate function, and when longer reaction times were employed, racemization occurred. The problem was solved by using boron tribromide to selectively cleave the ethyl ester group,[7] while keeping the phthalyl protecting group and thiazolidine ring intact. Subsequently, the leucine methyl ester was treated with Pht-8-phenyl-BTD-OH in DMF using BOP and HOBt as the coupling reagent to yield the tripeptide Pht-8-phenyl-BTD-Leu-OMe. Then the phthalyl protecting group was removed by hydrazinolysis and the α-amino group reprotected with di-*tert*-butyl dicarbonate to afford tripeptide **4** (Scheme 2). Cleavage of the N^{α}-Boc group of **4** and coupling with N^{α}-Boc-Tyr-Gly-OH gave the desired Leu-enkephalin analogues **5** in a protected form which were deprotected by treatment with BBr_3 to give peptides **6** (Scheme 2). After purification by RP-HPLC, the Leu-enkephalin analogues were isolated in 9–15% overall yield. There was no evidence of racemization at any stage of the synthesis by ^1H NMR or TLC analysis. The purity of peptides **6** was examined by analytical HPLC and their compositions were verified by high resolution fast atom bombardment mass spectrometry.

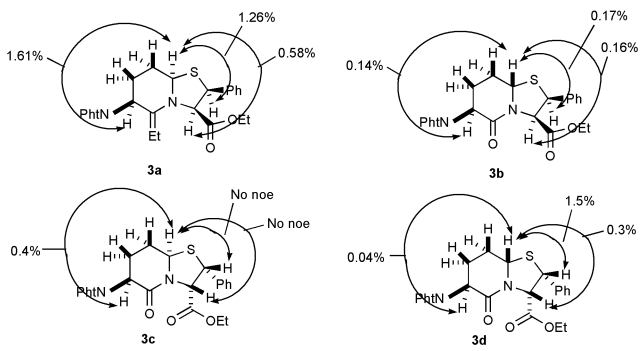


Fig. 2 NOEs observed for the bicyclic thiazolidine lactam intermediates **3a-d**.

Compounds **6a–d** were evaluated in the isolated mouse vas deferens (MVD, for δ-receptor) and guinea pig ileum (GPI, for μ receptor) bioassays. At 1μM concentration, compounds **6a–c** showed 3.3, 8.9, 0 and 1% agonist activity for MVD respectively, and 2.9, 2.6, 4 and 4.6% agonist activity for GPI respectively. These correspond to a loss of potency of approximately three orders of magnitude compared with Leu-enkephalin, and suggest that the correct spatial distances and orientations of the two aromatic pharmacophores (Tyr[1] phenol group and Phe[4] phenyl group) in Leu-enkephalin are critical for high potency.

In summary, this communication described the synthesis of novel, conformationally constrained 8-phenyl substituted β-turn dipeptide mimetics (3*S*, 6*R*, 8*S*, 9*R*)-**3a**, (3*S*, 6*S*, 8*S*, 9*R*)-**3b**, (3*S*, 6*R*, 8*R*, 9*S*)-**3c** and (3*S*, 6*S*, 8*R*, 9*S*)-**3d**. Incorporation of these substituted dipeptide mimetics into Leu-enkephalin was accomplished by using modified solution phase peptide synthesis methods. Extensive structure–activity relationship studies are under the investigation.

The authors thank the USPHS grants DA 06284 and DA 13449 for support.

Notes and references

1 (*a*) L. Halab, F. Gosselin and W. D. Lubell, *Biopolymers (Peptide Sci.)*, 2000, **55**, 101; (*b*) S. Hanessian, G. McNaughton-Smith, H.-G. Lombart and W. D. Lubell, *Tetrahedron*, 1997, **53**, 12789.

2 (*a*) T. D. W. Claridge, H. Christopher, J. K. Richard, L. Victor, A. N. Ian and J. S. Christopher, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 485; (*b*) U. Nagai, S. Kazuki, N. Rika and K. Rika, *Tetrahedron*, 1993, **49**, 3577; (*c*) M. Eguchi, R. Y. W. Shen, J. P. Shea, M. S. Lee and M. Kahn, *J. Med. Chem.*, 2002, **45**, 1395; (*d*) Y. Rew and M. Goodman, *J. Org. Chem.*, 2002, **67**, 8820.

3 (*a*) B. Vesterman, J. Saulitis, J. Betins, E. Liepins and G. V. Nikiforovich, *Biochim. Biophys. Acta*, 1989, **998**, 204; (*b*) V. J. Hruby and C. A. Gehrig, *Med. Res. Rev.*, 1989, **9**, 343; (*c*) R. Schwyzer, P. Moutevelis-Minakakis, S. Kimura and H.-U. Gremlich, *J. Peptide Sci.*, 1997, **3**, 65; (*d*) E. R. Stimson, Y. C. Meinwald and H. A. Scheraga, *Biochemistry*, 1979, **18**, 1661.

4 (*a*) H. I. Mosberg, R. Hurst, V. J. Hruby, K. Gee, H. I. Yamamura, J. J. Galligan and T. F. Burks, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 5871; (*b*) V. J. Hruby, L.-F. Kao, B. M. Pettitt and M. Karplus, *J. Am. Chem. Soc.*, 1988, **110**, 3351.

5 (*a*) A. Bach, II, J. A. Markwalder and W. C. Ripka, *Int. J. Peptide Protein Res.*, 1991, **38**, 314; (*b*) S. Roy, H.-G. Lombart, W. D. Lubell, R. E. W. Hancock and S. W. Farmer, *J. Peptide Res.*, 2002, **60**, 198; (*c*) W. Li and K. D. Moeller, *J. Am. Chem. Soc.*, 1996, **118**, 10106; (*d*) S. Hanessian and G. McNaughton-Smith, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1567; (*e*) S. Y. Tamura, E. A. Goldman, T. K. Brunck, W. C. Ripka and J. E. Semple, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 331; (*f*) F. Gosselin, D. Tourwe, M. Ceusters, T. Meert, L. Heylen, M. Jurzak and W. D. Lubell, *J. Peptide Res.*, 2001, **57**, 337.

6 (*a*) C. Xiong, W. Wang, C. Cai and V. J. Hruby, *J. Org. Chem.*, 2002, **67**, 1399; (*b*) C. Xiong, W. Wang and V. J. Hruby, *J. Org. Chem.*, 2002, **67**, 3514.

7 (*a*) A. M. Felix, *J. Org. Chem.*, 1974, **39**, 1427; (*b*) P. G. Mattingly and M. J. Miller, *J. Org. Chem.*, 1981, **46**, 1557; (*c*) M. Wakselman and M. Zrihen, *Chem. Lett.*, 1982, **3**, 333.