ORGANIC LETTERS

2011
Vol. 13, No. 13
3502–3505

A Highly Efficient Type I β-Turn Mimetic Simulating an Asx-Pro-Turn-Like Structure

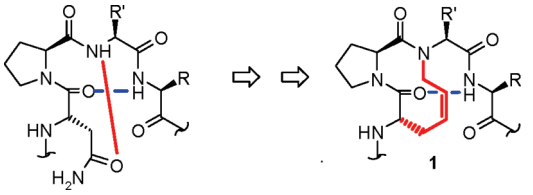
Andrea Pinsker, Jürgen Einsiedel, Steffen Härterich, Reiner Waibel, and Peter Gmeiner*

Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Schuhstrasse 19, 91052 Erlangen, Germany

peter.gmeiner@medchem.uni-erlangen.de

Received May 16, 2011

ABSTRACT



Asx-Pro-turns have been identified with high frequency in protein structures nucleating type I β-turns. By bridging the amino acid side chain in position *i* with a nitrogen substituent in position *i*+2 by ring-closing olefin metathesis (RCM), peptide mimetics of type 1 could be developed. NMR based conformational investigations indicated a stable intramolecular H-bond constraining a U-turn conformation that was predicted to simulate a type I β-turn.

Reverse-turn motifs are known as major recognition elements for receptor−ligand interactions.[1] As an example, more than 100 peptide-activated G-protein-coupled receptors bind biological effectors with U-turn-type structures.[2] Drug research has been directed to design conformationally restricted scaffolds nucleating reverse turns that allow a stable prearrangement of crucial pharmacophores.[3,4] Thus, pharmacological properties including binding affinity, target selectivity, and metabolic stability can be improved. Statistical analysis documented the preferential incorporation of amino acid residues in turn structures[5] when the tendency of asparagine, which is known to form a functionally relevant Asx-turn,[6] was found to predispose a type I β-turn.[7] Taking advantage of its frequent occurrence in the positions i+1 and i+2 of β-turns of type I/II and VI, respectively, powerful reverse-turn mimetics were derived from the functionally important amino acid proline.[5] Asx-Pro-turns exhibit a H-bonding network featuring both the i to i+3 backbone H-bond of the classical β-turn and a side chain to i+2 interaction forming the Asx-turn.[5] In fact, Asx-Pro-turns have been identified with high frequency in protein structures nucleating type I β-turns.[7] As a key recognition element for initiation of biological recognition events at the surface of proteins, the type I β-turn, which is by far the most common, has attracted considerable interest for the design of peptidomimetic moieties.[8] Bridging the amino acid side chains in positions i+1 and i+2 by ring-closing olefin metathesis (RCM) has been demonstrated to result in type I β-turn inducing scaffolds by J. A. Katzenellenbogen.[4a] As a complementary strategy that allows the incorporation of individual side chains into the positions i+1 and i+2, we aimed to replace the Asx-turn forming side-chain element in position i by a *cis*-olefin unit.

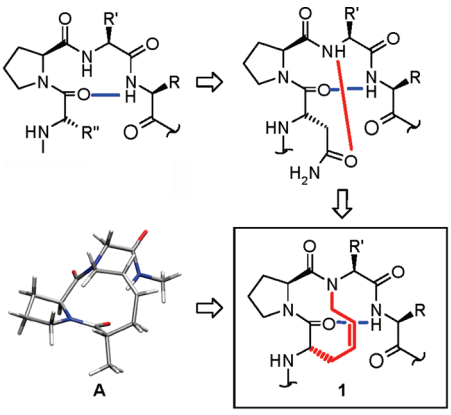
Employing the results of molecular dynamics simulations, we recently designed a highly efficient proline-based type VI β-turn mimetic.[9] Our design of length and geometry of the olefin linker for homologous and isomeric ring systems of type **A** was also based on molecular dynamics studies. Thus, a family of structures of type **A** including 9- to 12-membered ring systems were subjected to an energy minimization followed by molecular dynamics simulations

(1) Müller, G.; Hessler, G.; Decornez, H. Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 894–896.
(2) Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. *Chem. Rev.* **2005**, *105*, 793–826.
(3) (a) Blomberg, D.; Hedenström, M.; Kreye, P.; Sethson, I.; Brickmann, K.; Kihlberg, J. *J. Org. Chem.* **2004**, 3500–3508. (b) Bittermann, H.; Gmeiner, P. *J. Org. Chem.* **2006**, *71*, 97–102.

10.1021/ol201313q
Published on Web 06/09/2011

(100 ps) to probe the stability of the observed β-turn-like conformations. The length of the presumed H-bond during the resulting trajectory was plotted against the number of conformations observed with a specific distance when a clear prevalence for a H-bond (distance < 2.25 Å) was mainly observed for compounds bearing a *cis* configuration at the double bond. Especially, 9- and 10-membered ring systems showed a very narrow distance distribution indicating a stable H-bond. These molecules were selected for further *ab initio* geometry optimization with the Hartree–Fock algorithm at a 6-31G(d) level of theory. Dihedral angles of the calculated structures fit well to the ideal values for the βI-turn (Figure 1). Because the parent Asx-turn is formed by a pseudo-10-membered ring and structure **A** clearly fulfilled the stringent criteria for assigning a type I β-turn, we chose to approach the potential reverse turn mimetics of type **1** incorporating a diazecine based ring system. We herein present a highly practical synthetic approach to 10-membered unsaturated lactams as βI-turn mimetics, conformational investigations, and solid phase supported application toward artificial neuropeptide analogues.

Grubb's ring-closing olefin metathesis has been proven as an elegant technique for the synthesis of 10-membered rings when cyclization through RCM was explored as an alternative to the classic methods of lactam formation.[10] We intended to take advantage of ring-closing olefin metathesis to access the novel molecular scaffolds of type **1**.



	Φ i+1	Ψ i+1	Φ i+2	Ψ i+2	CO - HN
ideal βI-turn	-60	-30	-90	0	
A	-46.3	-26.7	-87.7	9.1	2.25 Å

Figure 1. Design and molecular dynamics investigations.

Our initial investigations were directed to the synthesis of the Asx-Pro-turn mimetic **6a** starting from the *N*-allylglycine derivative **2a**, which could be easily prepared by *N*-alkylation (Scheme 1). Coupling of Fmoc-protected proline acid chloride[11,12] with the secondary amine **2a** led to the synthetic intermediate **3a**. Subsequent *N*-deprotection and coupling with Fmoc-protected *C*-allylglycine acid chloride afforded the cyclization precursor **5a**. Ring closure metathesis was performed using a second generation Grubbs catalyst in refluxing dichloromethane to give access to the cyclic olefin **6a** in 41% yield. The control of *cis*/*trans*-geometry in generating double bonds of medium size, particularly 10-membered rings, is difficult and hard to predict[13] when the ratios of *E*/*Z*-isomers can be influenced by reaction temperature[10] or groups neighboring the olefins as described by Grubbs.[13] Some publications report the formation of only the *trans*-isomer,[3a,14] while others observe the generation of a *cis*-double bond exclusively.[15] For our 10-membered system, careful NMR analysis unambiguously proved the structure of **6a** with a diagnostic ^3J-coupling of 9.9 Hz for the *cis*-alkenyl partial structure (for *trans*-configuration, a coupling constant >15 Hz would be expected).[16] To demonstrate the versatility of the synthesis, the tyrosine analog **6b** was prepared

(4) (a) Kahn, M.; Chen, B. *Tetrahedron Lett.* **1987**, *28*, 1623–1626. (b) Kahn, M.; Wilke, S.; Chen, B.; Fujita, K. *J. Am. Chem. Soc.* **1988**, *110*, 1638–1639. (c) Ball, J. B.; Alewood, P. F. *J. Mol. Recog.* **1990**, *2*, 55–64. (d) Hinds, M. G.; Welsh, J. H.; Brennand, D. M.; Fisher, J.; Glennie, M. J.; Richards, N. G. J.; Turner, D. L.; Robinson, J. A. *J. Med. Chem.* **1991**, *34*, 1777–1789. (e) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. *Tetrahedron* **1997**, *38*, 12789–12854. (f) Fink, B. E.; Kym, P. R.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 4334–4344. (g) Souers, A. J.; Virgilio, A. A.; Rosenquist, A.; Fenuik, W.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 1817–1825. (h) Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. *J. Am. Chem. Soc.* **1999**, *121*, 12204–12205. (i) Haskell-Luevano, C.; Rosenquist, A.; Souers, A.; Khong, K. C.; Ellman, J. A.; Cone, R. D. *J. Med. Chem.* **1999**, 4380–4387. (j) Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, 7431–7448. (k) Qiu, W.; Gu, X.; Soloshonok, V. A.; Carducci, M. D.; Hruby, V. J. *Tetrahedron Lett.* **2001**, *42*, 145–148. (l) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. *Tetrahedron Lett.* **2001**, 1237–1239. (m) Wang, W.; Xiong, C.; Hruby, V. J. *Tetrahedron Lett.* **2001**, *18*, 3159–3161. (n) Freidinger, R. M. *J. Med. Chem.* **2003**, *46* (26), 5553–5566. (o) Banfi, L.; Basso, A.; Guanti, G.; Riva, R. *Tetrahedron Lett.* **2003**, *44*, 7655–7658. (p) Jeanotte, G.; Lubell, W. D. *J. Am. Chem. Soc.* **2004**, *126*, 14334–14335. (q) Kaul, R.; Surprenant, S.; Lubell, W. D. *J. Org. Chem.* **2005**, *10*, 3838–3844. (r) Stepién, A.; Loska, R.; Cmoch, P.; Stalinsky, K. *Synlett* **2005**, *1*, 83–86. (s) Pawar, V. G.; De Borggraeve, W. M.; Maes, V.; Tourwé, D. A.; Compernolle, F.; Hoornaert, G. J. *Tetrahedron Lett.* **2005**, 1707–1710. (t) Surprenant, S.; Lubell, W. D. *Org. Lett.* **2006**, *13*, 2851–2854. (u) Albericio, F.; Arvidson, P. I.; Bisetty, K.; Giralt, E.; Govender, T.; Jali, S.; Kongsaeree, P.; Kruger, H. G.; Prabpai, S. *Chem. Biol. Drug Des.* **2008**, *71*, 125–130. (v) Ko, E.; Burgess, K. *Org. Lett.* **2011**, *5*, 980–983.

(5) (a) Hutchinson, E. G.; Thornton, J. M. *Protein Sci.* **1994**, *3*, 2207–2216. (b) Guruprasad, K.; Rajkumar, S *J. Biosci.* **2000**, *25*, 143–156.

(6) (a) Mcharfi, M.; Aubry, A.; Boussard, G; Marraud, M. *Eur. Biophys. J.* **1986**, *14*, 43–51. (b) Imperiali, B.; Shannon, K. L. *Biochemistry* **1991**, *30*, 4374–4380.

(7) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167–339.

(8) (a) Bieräugel, H.; Jansen, P. T.; Schoemaker, H. E.; Hiemstra, H.; van Maarseveen, J. H. *Org. Lett.* **2002**, *16*, 2673–2674. (b) David, O.; Meester, W. J. N.; Bieräugel, H.; Schoemaker, H. E.; Hiemstra, H.; van Maarseveen, J. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 4373–4375.

(9) Hoffmann, T.; Lanig, H.; Waibel, R.; Gmeiner, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 3361–3364.

(10) Fürstner, A.; Müller, T. *Synlett* **1997**, *8*, 1010–1012.

(11) Beyermann, M.; Bienert, M.; Niedrich, H.; Carpino, L. A.; Sadat-Aalaee, D. *J. Org. Chem.* **1990**, *55*, 721–728.

(12) Oku, A.; Yamaura, Y.; Harada, T. *J. Org. Chem.* **1986**, *52*, 3732–3734.

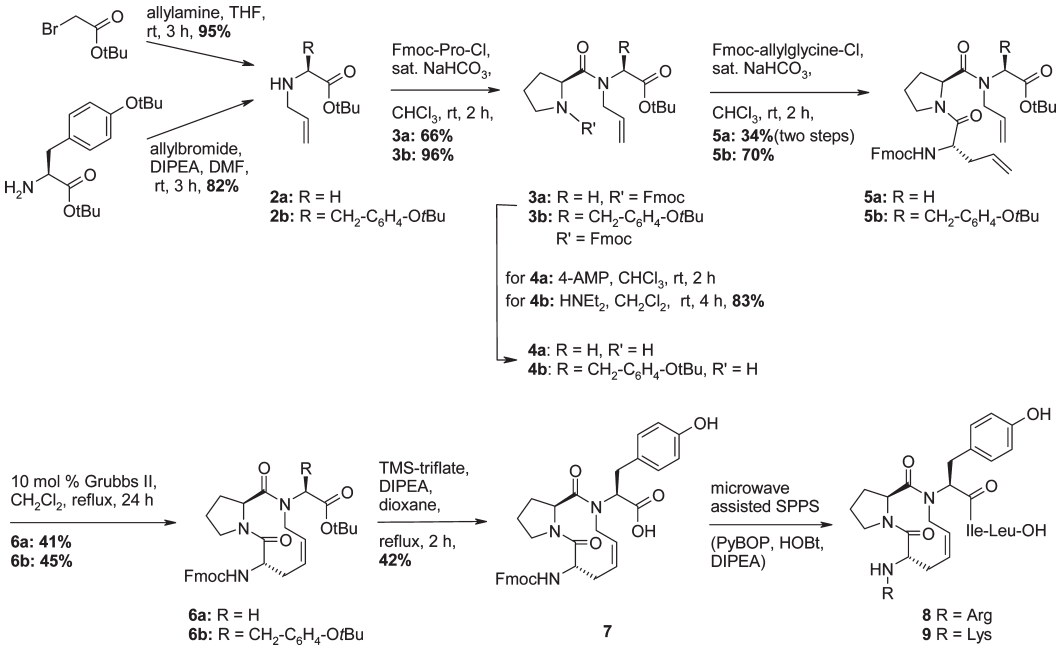
(13) Mohapatra, D. K.; Ramesh, D. K.; Giardello, M. A.; Chorghade, M. S.; Gurjar, M. K.; Grubbs, R. H. *Tetrahedron Lett.* **2007**, *48*, 2621–2625.

(14) Srihari, P.; Kumaraswamy, B.; Somaiah, R.; Yadav, J. S. *Synthesis* **2010**, 1039–1045.

(15) (a) Hoffmann, T.; Waibel, R.; Gmeiner, P. *J. Org. Chem.* **2003**, *68*, 62–69. (b) Szostak, M.; Aubé, J. *Org. Lett.* **2009**, *11*, 3878–3881.

(16) (a) Smith, G. V.; Kriloff, H. *J. Am. Chem. Soc.* **1963**, *85*, 2016–2017. (b) Pawar, M. D. *J. Am. Chem. Soc.* **1996**, *118*, 12821–12825.

Scheme 1. Synthesis of the Molecular Scaffold **6a,b** and the Neurotensin Analogs **8** and **9**



following an analogous reaction protocol. Thus, we started from bis-*tert*-butyl protected tyrosine which was *N*-alkylated to give the secondary amine **2b**. Subsequent ligation with proline, *N*-deprotection, coupling with allylglycine, and RCM gave rise to the conformationally restricted peptide mimetic **6b** via intermediates **3b**, **4b**, and **5b**.

We have recently shown that peptide backbone modifications of neurotensin analogues substantially control their receptor affinity and subtype selectivity.[17] To take advantage of our novel peptide mimetic for the investigation of receptor ligand interactions and to demonstrate the suitability of our molecular scaffold for solid phase supported peptide synthesis (SPPS), the conformationally constrained neurotensin mimetics **8** and **9** were prepared starting from Wang resin preloaded with the sequence Ile-Leu. Deprotection of our scaffold **6b** was done by trimethylsilyl trifluoromethansulfonate to give the carboxylic acid **7**. Attachment of the building block to the functionalized Wang resin and subsequent ligation with Fmoc-Arg and Fmoc-Lys followed by deprotection and cleavage led to the neurotensin mimetics **8** and **9**, respectively. Standard solid phase protocols for microwave assisted SPPS were employed using PyBOP/HOBt/DIPEA for the coupling steps. Epimerization could be observed to a minor extent when the impurities of the final products **8** and **9** could be separated by preparative HPLC.

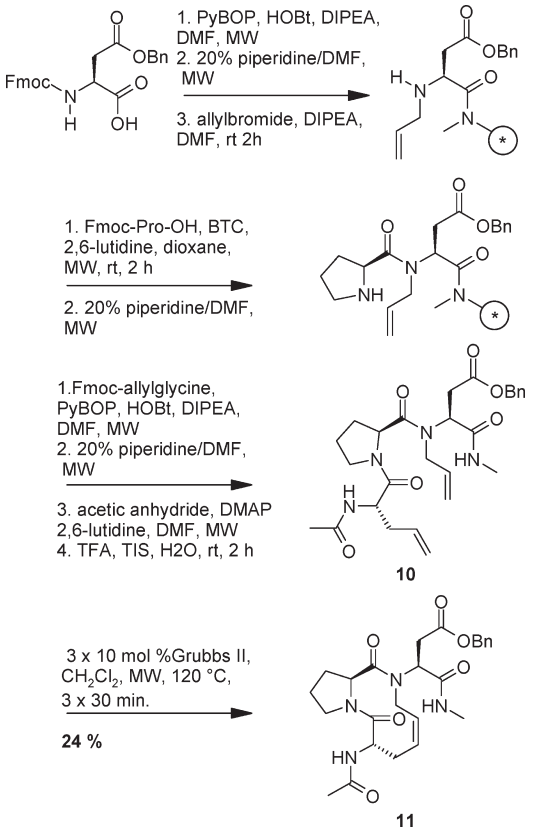
Aspartate is the most prevalent amino acid in position $i+2$ of type I β-turns in naturally occurring proteins.[5] Thus, we envisioned investigating a further model system that incorporates an aspartate derivative adjacent to the proline residue. Because we planned to investigate this model peptide surrogate for its conformational properties emphasizing its capability to form a stable type I β-turn, *N*-acetylation and *N*-methyl carboxamide functionalization should be realized. Moreover, we were intrigued by the question if our RCM precursor sequence can be also constructed by solid phase supported peptide synthesis. Thus, we chose *N*-methyl aminomethylindole resin as a commercially available backbone amide linker (BAL) that was *N*-acylated with Fmoc-protected γ-benzyl aspartate by microwave assisted coupling when we employed PyBOB/HOBt as an activating mixture (Scheme 2).[17a] After *N*-allylation and coupling with triphosgene-activated Fmoc-proline, Fmoc-allylglycine was ligated. After *N*-acetylation, TFA promoted cleavage in the presence of triisopropylsilane gave access to the fully functionalized cyclization precursor **10**. Employing microwave irradiation at 120 °C, the target compound **11** could be synthesized upon treatment of the *bis*-olefine **10** with a Grubbs II catalyst.[18] The stereochemistry of the proline-allylglycine peptide bond was assigned *trans* by NOE experiments.

To investigate the ability of our molecular scaffold to nucleate a stable type I β-turn, NMR spectroscopic studies were performed comparing the model peptide surrogate **11** with the *N*-acetylated tripeptidyl carboxamides **12**, **13**, and **14** that were expected to show the differential prevalence of

(17) (a) Bittermann, H.; Einsiedel, J.; Hübner, H.; Gmeiner, P. *J. Med. Chem.* **2004**, *47*, 5587–5590. (b) Bittermann, H.; Boeckler, F.; Einsiedel, J.; Gmeiner, P. *Chem.—Eur. J.* **2006**, *12*, 6315–6322. (c) Einsiedel, J.; Lanig, H.; Waibel, R.; Gmeiner, P. *J. Org. Chem.* **2007**, *72*, 9102–9113. (d) Einsiedel, J.; Huebner, H.; Hervet, M.; Haerterich, S.; Koschatzky, S.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2013–2018. (e) Härterich, S.; Koschatzky, S.; Einsiedel, J.; Gmeiner, P. *Bioorg. Med. Chem.* **2008**, *16*, 9359–9368. (f) Lomlim, L.; Einsiedel, J.; Heinemann, F. W.; Meyer, K.; Gmeiner, P. *J. Org. Chem.* **2008**, *73*, 3608–3611. (g) Einsiedel, J.; Held, C.; Hervet, M.; Plomer, M.; Tschammer, N.; Huebner, H.; Gmeiner, P. *J. Med. Chem.* **2011**, *54*, 2915–2923.

(18) Chapman, R. N.; Arora, P. S. *Org. Lett.* **2006**, *8*, 5825–5828.

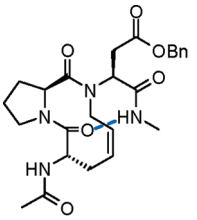
Scheme 2. Solid Phase Supported Synthesis of the Peptide Mimetic **11**



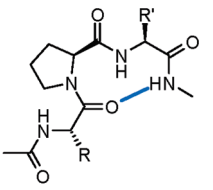
type I β-turns. These peptides were obtained by microwave assisted Fmoc-solid phase peptide synthesis (see Supporting Information). The experiments were done in 2 mM solution to exclude intermolecular interactions.[19] Interestingly, NMR-derived $\delta(NH)$ values demonstrated the existence of an intramolecular hydrogen bond when the NH(i+3) signals in the conformationally restricted peptide mimetic **11** and the reference agents **12** and **13** resonated significantly more downfield (6.8−6.9 ppm) than the Ala-Pro-Ala derivative (6.2 ppm).[15] To further investigate the stability of the intramolecular hydrogen bond, increasing amounts of DMSO-d_6, which acts as a competitive hydrogen bond acceptor, were added to a solution of **11** and **12**−**14** in $CDCl_3$ (Table 1). Interestingly, the peptide mimetic **11** and the Asx-Pro-turn inducing sequences **12** and **13** did not reveal a substantial alteration of the NH shift ($\Delta\delta$ 0.2−0.4), thus indicating a stable intramolecular

(19) (a) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173. (b) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228–4245.

Table 1. Amide Proton Chemical Shift (δ) of NH(i+3) As a Function of the Percentage of DMSO-d_6 in $CDCl_3$ for the Peptide Mimetic **11** and the Reference Peptides **12**, **13**, **14**



11



12: R=Asn, R'=Asp(OBn)
13: R=Asn, R'=Ala
14: R=Ala, R'=Ala

% DMSO	**11**	**12**	**13**	**14**
0	6.7	6.9	6.9	6.3
5	6.8	6.9	7.0	6.8
10	6.9	7.0	7.1	7.1
20	7.0	7.1	7.1	7.3
30	7.1	7.1	7.2	7.4
$\Delta\delta$	0.4	0.2	0.3	1.1

H-bond in the presence of even 30% DMSO. Temperature dependent chemical shift changes of ^{1}H NMR signals as a measure for the stability of secondary structures corroborated the assumption that the peptide mimetic **11** might form a stable intramolecular H-bond when a comparatively low $\Delta\delta/\Delta T$ value of −4.7 ppb/K was observed.[3b]

In summary, novel reverse-turn mimetics could be developed by bridging the amino acid side chain and a nitrogen substituent in positions i and i+2, respectively, by ring-closing olefin metathesis. NMR based conformational investigations indicated a stable intramolecular H-bond constraining a U-turn conformation that was predicted to simulate a type I β-turn.

Acknowledgment. The Volkswagen Foundation is acknowledged for finacial support (Project I/81 615). We thank Prof. Ivana Ivanović-Burmazović and Leanne Nye (Department of Chemistry and Pharmacy, Chair of Bioinorganic Chemistry, Friedrich Alexander University Erlangen-Nürnberg) for high resolution ESI-TOF mass spectra.

Supporting Information Available. Additional information regarding the synthesis and characterization of peptide analogs by HPLC, MS, and NMR. This material is available free of charge via the Internet at http://pubs.acs.org.