

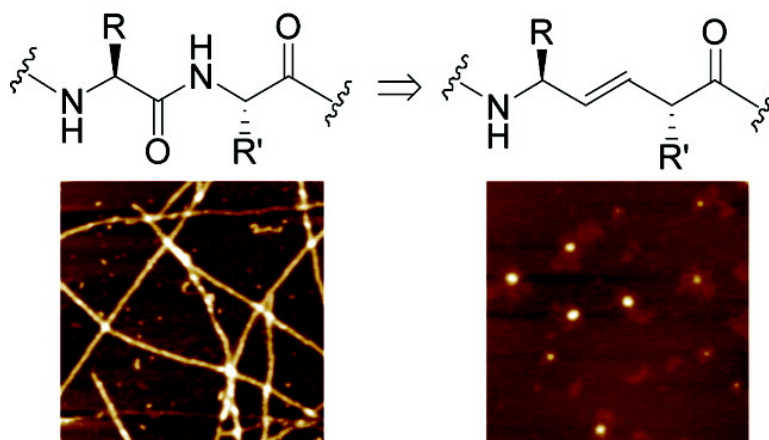
Communication

***E*-Olefin Dipeptide Isostere Incorporation into a Polypeptide Backbone Enables Hydrogen Bond Perturbation: Probing the Requirements for Alzheimer's Amyloidogenesis**

Yanwen Fu, Jan Bieschke, and Jeffery W. Kelly

J. Am. Chem. Soc., **2005**, 127 (44), 15366-15367 • DOI: 10.1021/ja0551382 • Publication Date (Web): 14 October 2005

Downloaded from <http://pubs.acs.org> on March 25, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 6 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)



ACS Publications
 High quality. High impact.

E-Olefin Dipeptide Isostere Incorporation into a Polypeptide Backbone Enables Hydrogen Bond Perturbation: Probing the Requirements for Alzheimer's Amyloidogenesis

Yanwen Fu, Jan Bieschke, and Jeffery W. Kelly*

Department of Chemistry and The Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC 506, La Jolla, California 92037

Received July 28, 2005; E-mail: jkelly@scripps.edu

Intermolecular backbone–backbone hydrogen bonding is observed in quaternary structures and is believed to be an important stabilizing force in protein–protein interactions. It is possible to eliminate both a backbone H-bond acceptor (C=O) and a donor (N–H) by replacing the amide functionality in a single dipeptide substructure within a protein with an *E*-alkene moiety (Figure 1). The idea of incorporating *E*-olefin dipeptide isosteres into polypeptides to perturb backbone H-bonding is not new. However, this strategy has been rarely realized in large part because of the difficulties involved in the chemical synthesis of *E*-alkene dipeptide isosteres and the challenge of incorporating these dipeptide isosteres into proteins.¹ Protein backbone H-bond perturbation has been realized using amide-to-ester backbone mutations.² Like ester perturbations, amide-to-*E*-alkene substitutions are ideal in that the conformational and geometrical preferences of the backbone are maintained (Figure 1). Moreover, unlike the situation in esters, where the non-carbonyl oxygen experiences an unfavorable electrostatic interaction with the amide carbonyl the NH once H-bonded to, *E*-olefin perturbations do not introduce unfavorable electrostatic interactions, making it easier to extract H-bond energies from $\Delta\Delta G$ measurements.

Herein, we describe a stereoselective *E*-olefin dipeptide isostere synthesis (Scheme 1) that can be used to make gram quantities of the amino acid required for eliminating a specific H-bond donor and acceptor in a protein while maintaining side-chain structures. The Phe–Phe *E*-olefin dipeptide isostere (5) was incorporated into the 40 residue Alzheimer's amyloid peptide, referred to as A β (1–40), in place of phenylalanines 19 and 20 utilizing a Boc/benzyl solid-phase synthesis strategy. The aim is to examine the role of intermolecular H-bonding in the process of amyloidogenesis thought to cause Alzheimer's disease. This amide-to-*E*-alkene backbone mutation precludes amyloid formation, but not spherical aggregate formation, providing insight into the structural requirements of amyloidogenesis.

Previously reported synthetic approaches to *E*-olefin isosteres often relied on S_N2' substitutions or rearrangements of allylic substrates³ or generation of the double bond via 1,2 elimination.⁴

In the *E*-olefin dipeptide isostere synthesis described herein, the amino terminal C α stereocenter comes from an amino aldehyde (derived from an amino acid), whereas the C-terminal stereocenter is produced via the diastereoselective (11:1) Evans alkylation of an oxazolidinone enolate (Scheme 1). The synthesis commenced with a Wittig reaction, followed by a hydroboration–oxidation, affording the *N*-Boc-Phe-Gly dipeptide *E*-olefin isostere using established procedures.⁵ The acid within **2** was then condensed with Evans' chiral oxazolidinone⁶ utilizing a mixed anhydride approach. Diastereoselective alkylation of the C-terminal C α carbon in **3** is the key step in this approach. To avoid formation of N-alkylated or C γ -alkylated side products and decomposition of the enolate,

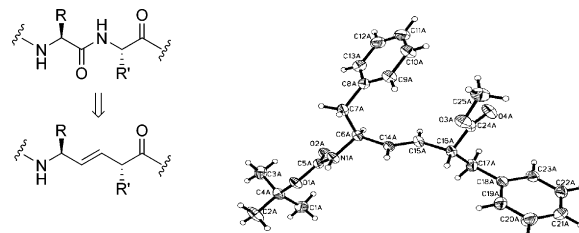
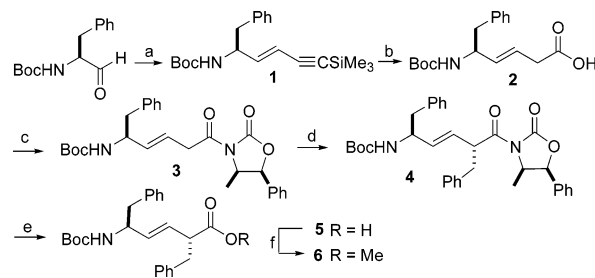


Figure 1. The backbone amide-to-*E*-olefin mutation eliminates both a H-bond acceptor (C=O) and a donor (N–H) (left). The X-ray structure of the Phe–Phe *E*-olefin dipeptide isostere synthesized herein (right).

Scheme 1. Synthesis of *E*-Olefin Isostere^a



^a Conditions: (a) Ph₃PCH₂C≡CSiMe₃Br, *n*-BuLi, *E/Z* 7:1, 99% ee, 70%. (b) (i) cyclohexene, BH₃; (ii) H₂O₂, 82% for two steps. (c) (i) *t*-BuCOCl, Et₃N; (ii) oxazolidinone, *n*-BuLi, 85% for two steps. (d) LDA, PhCH₂Br, 70%, dr 11:1. (e) LiOH, H₂O₂, 88%. (f) TMS–CHN₂, 92%.

the reaction was conducted under kinetically controlled conditions, where **3** was added to **2** equiv of freshly prepared LDA at –78 °C. Addition of excess benzyl bromide afforded the desired product with a high diastereoselectivity ratio (11:1) and in good chemical yield (70%). Precursor **4** was treated with basic hydrogen peroxide to remove the oxazolidinone chiral auxiliary affording **5**, ready for incorporation into a polypeptide chain by solid-phase peptide synthesis. The methyl ester **6**, afforded by TMSCHN₂ treatment, was employed to confirm the structure of the Phe–Phe dipeptide *E*-olefin isostere by X-ray crystallography (Figure 1).

The misassembly of A β , ultimately affording insoluble amyloid fibrils, is genetically linked to the pathogenesis of Alzheimer's disease.⁷ A β (1–40) contains two hydrophobic subsequences which are underlined in one letter code below: DAEFRHDSGYEVH-HQKL**LVFFA**EDVGSNKGAIIGLMVGGVV. The central hydrophobic core LVFFA (17–21) is essential for amyloidogenesis.⁸ Since it has been argued that it is primarily the backbone that enables so many peptides to form cross- β -sheet-based amyloid fibrils,⁹ it follows that intermolecular H-bonding should be essential. An amide-to-*E*-olefin substitution was therefore introduced into the key Phe19–Phe20 subsequence, removing both a hydrogen-bond donor and acceptor thought to make H-bonds with different neighboring A β molecules.¹⁰

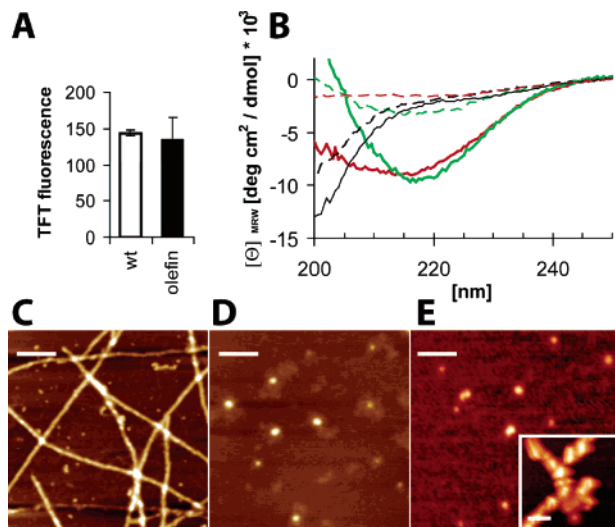


Figure 2. (A) Tft fluorescence of wt A β and the EOA β analogue after agitated incubation at 37 °C for 25 h. (B) Mean residue ellipticity of A β (1–40) (solid lines) and EOA β (dotted lines) before aggregation (black), after 25 h (red), and 6 weeks (green). AFM images (scale bars: 200 nm) of aggregated A β (C) and the EOA β after 25 h (D) and 6 weeks (E).

The Phe19–Phe20 *E*-olefin dipeptide isostere was incorporated into A β by manual solid-phase peptide synthesis using HATU activation. The reverse phase HPLC purified Phe19–Phe20 *E*-olefin analogue of A β (1–40), hereafter referred to as EOA β , was subjected to high pH treatment and membrane filtration to monomerize the sample.¹¹ Tft is an environmentally sensitive fluor that binds and fluoresces when bound to spherical and cross- β -sheet aggregates of A β .¹¹ Incubation of EOA β (50 μ M) with agitation on a rocker (30/min) in the buffer (150 mM NaCl, 50 mM phosphate, pH 7.4, 37 °C) that typically leads to the aggregation of A β (1–40) resulted in a strong thioflavin T signal after 25 h, comparable to that exhibited by wt A β (Figure 2A), indicating that EOA β was able to self-assemble. Tft fluorescence remained essentially constant for both peptides over the course of 6 weeks.

To examine whether aggregated EOA β adopts a β -sheet rich structure analogous to that exhibited by wt A β , far-UV circular dichroism (CD) was employed. The CD spectra of unaggregated A β peptides display strong minima around 200 nm, whereas aggregated wt A β exhibits a strong minimum at 217 nm, characteristic of cross- β -sheet structure. The amplitude at 217 nm was very weak if present at all in EOA β (25 h), but increased very slowly over 6 weeks, long after the Tft signal had plateaued (Figure 2B). Absorbance at 280 nm revealed that the EOA β concentrations remained very similar to those of wt A β .

Since the differences in secondary structure suggested differences in aggregate morphology, atomic force microscopy (AFM) was employed to examine the wt A β and EOA β assembled structures. Fibrillar aggregates were observed with wt A β (Figure 2C), but not with EOA β , which aggregated predominantly, if not exclusively, into spherical aggregates (Figure 2D) that partially assembled into larger amorphous aggregates after 6 weeks (Figure 2E, inset). In each case, \sim 50% of the sample could be pelleted at 100 000g after 4 days of aggregation. No fibril formation was observed with EOA β even after 6 weeks of incubation. Electron micrographs of these samples revealed analogous results, suggesting that it is unlikely that these results are biased by the mica- or carbon-coated Cu surfaces used for AFM and EM, respectively. AFM measurements determined the height of the spherical aggregates to be \sim 5 nm,

EM measurements reveal a \sim 10 nm diameter, similar to the dimensions seen in diffusible oligomer aggregation intermediates (“ADDLS”)¹² and in cholesterol metabolite induced spherical aggregates.¹¹ There is substantial evidence that A β aggregation proceeds first by forming spherical aggregates which transform into protofilaments and then into fibrils having a cross- β -sheet structure.^{7b} It is striking that removal of 1 of the 39 amide bonds in A β , and thus one H-bond donor and one acceptor, could prevent the typical progression of spherical aggregates into protofilaments and fibrils. The results herein indicate that the 19–20 amide bond is critical for protofilament and fibril formation, but not for spherical aggregate formation. Ongoing studies to discern whether replacement of other amides will influence amyloidogenesis will be reported in due course. The discovery of an A β analogue that can form spherical aggregate morphologies but cannot progress to protofilaments or fibrils is very valuable for delineating the structure, toxicity, and antigenicity of spherical A β aggregates.

In summary, we report a synthesis of *E*-olefin isosteres that can be prepared on a scale that enables their incorporation into polypeptide sequences to examine the role of individual hydrogen bonds in protein structure acquisition.

Acknowledgment. We gratefully acknowledge financial support from the NIH (GM 51105), the Lita Annenberg Hazen Foundation, and the Bundy Foundation, and thank I. Yonemoto for EM images.

Supporting Information Available: Experimental details and characterization data for the synthesis of **6**; crystal structure of **6**; procedures for peptide assembly. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- Jenkins, C. L.; Vasbinder, M. M.; Miller, S. J.; Raines, R. T. *Org. Lett.* **2005**, *7*, 2619–2622.
- For examples, see: (a) Lu, W.; Qasim, M. A.; Laskowski, M., Jr.; Kent, S. B. H. *Biochemistry* **1997**, *36*, 673–679. (b) Koh, J. T.; Cornish, V. W.; Schultz, P. G. *Biochemistry* **1997**, *36*, 11314–11322. (c) Beligere, G. S.; Dawson, P. E. *J. Am. Chem. Soc.* **2000**, *122*, 12079–12082. (d) Nakhle, B. M.; Silinski, P.; Fitzgerald, M. C. *J. Am. Chem. Soc.* **2000**, *122*, 8105–8111. (e) Deechongkit, S.; Nguyen, H.; Powers, E. T.; Dawson, P. E.; Gruebele, M.; Kelly, J. W. *Nature* **2004**, *430*, 101–105.
- (a) Ibuka, T.; Habashita, H.; Funakoshi, S.; Fujii, N.; Oguchi, Y.; Ueyehara, T.; Yamamoto, Y. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 801–803. (b) Wipf, P.; Fritch, P. *J. Org. Chem.* **1994**, *59*, 4875–4886. (c) Bohnstedt, A. C.; Vara Prasad, J. N. V.; Rich, D. H. *Tetrahedron Lett.* **1993**, *34*, 5217–5220. (d) Wang, X. J.; Hart, S. A.; Bailing, X.; Mason, M. D.; Goodell, J. R.; Etkorn, F. A. *J. Org. Chem.* **2003**, *68*, 2343–2349.
- Miles, N. J.; Sammes, P. G.; Kennewell, P. D.; Westwood, R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2299–2305.
- Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc., Chem. Commun.* **1980**, 234–235.
- For an example, see: Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
- (a) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353–356. (b) Lashuel, H. A.; Hartley, D.; Petre, B. M.; Walz, T.; Lansbury, P. T. *Nature* **2002**, *418*, 291.
- (a) Wood, S. J.; Wetzel, R.; Martin, J. D.; Hurler, M. R. *Biochemistry* **1995**, *34*, 724–730. (b) Esler, W. P.; Stimson, E. R.; Ghilardi, J. R.; Lu, Y.-A.; Felix, A. M.; Vinters, H. V.; Mantyh, P. W.; Lee, J. P.; Maggio, J. E. *Biochemistry* **1996**, *35*, 13914–13921.
- Fandrich, M.; Dobson, C. M. *EMBO J.* **2002**, *21*, 5682–5690.
- Petkova, A. T.; Ishii, Y.; Balbach J. J.; Antzutkin O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 383–385.
- (a) Zhang, Q.-H.; Powers, E. T.; Nieva, J.; Huff, M. E.; Dendle, M. A.; Bieschke, J.; Glabe, C. G.; Eschenmoser, A.; Wentworth, P., Jr.; Lerner, R. A.; Kelly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4752–4757. (b) Bieschke, J.; Zhang, Q.-H.; Powers, E. T.; Lerner, R. A.; Kelly, J. W. *Biochemistry* **2005**, *44*, 4977–4983.
- Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.; Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6448–6453.

JA0551382