Synthesis of Stapled β^3 -Peptides through Ring-Closing Metathesis

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ABSTRACT



The first synthesis of carbon-stapled β^3 -peptides is reported. The precursor β^3 -peptides, with *O*-allyl β -serines located in an *ili*+3 relationship, were prepared on solid phase. We show that efficient ring-closing metathesis (RCM) of these new β^3 -peptides proceeds smoothly either in solution or on an appropriate solid support. All products were generated with high selectivity for the *E*-isomer.

The design and application of foldamers¹ is expanding and maturing rapidly. In particular, the propensity of β -peptides (i.e., peptides consisting entirely of cyclic and/or acyclic β -amino acids) to form stable, helical structures²⁻⁴ in solution has led to applications in peptidomimetics^{3,5-12} as well as new materials.^{13,14} The 14-helix⁴ is particularly important because of its almost perfect pitch (i.e., almost exactly three residues per turn).

Strategies adopted for further stabilizing 14-helical β^3 -peptides in solution mostly rely on favorable interactions

(3) Wu, Y.-D.; Gellman, S. Acc. Chem. Res. 2008, 41, 1231–1232.



Figure 1. Summary of NOE correlations observed for the stapled 2a peptide consistent with the formation of a 14-helix. Gray dots or diamonds represent NOEs ambiguously assigned due to spectral overlap.

between side chain residues located in an *i/i*+3 relationship which are in relatively close proximity to each other (\sim 4.8 Å).⁴ These include a disulfide clamp,¹⁵ one or more salt bridges,^{7,16–18} and a lactam bridge.^{15,19} Other important

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⁽¹⁾ Foldamers: Structure, Properties, and Applications; Hecht, S., Huc, I., Eds.; Wiley-VCH: New York, 2007.

⁽²⁾ Seebach, D.; Becak, A. K.; Bierbaum, D. J. Chem. Biodiversity 2004, 1, 1111–1239.

⁽⁴⁾ Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. Chem. Rev. 2001, 101, 3219–3232.

strategies include the control of helix macrodipole orientation²⁰ and the incorporation of varying numbers of cyclic β -amino acids.^{21,22}

We describe here a new, ring-closing metathesis (RCM)derived staple for β^3 -peptide 14-helices. Such staples have found extensive use in α -peptides since Grubbs reported the first carbon-stapled α -peptides in 1998.^{23–25} We were interested in evaluating RCM-derived staples as a means of introducing synthetically flexible functionality on or near the *surface* of such helices. This should provide the opportunity to assemble new structures, via functional group manipulation of, in this case, alkenes, through supramolecular and/or covalent processes. We chose to study hexa- β -peptides **1a–1d** as our RCM precursor candidates (Scheme 1). In each case, two β^3 -serines bearing an *O*-allyl side chain²⁶ are located in an i/(i+3) relationship.

Scheme 1. Sequences for All New Hexa- β -peptides (2a-d) and Their Precursors (1a-d) as well as Conditions for Ring Closure



All RCM precursor β^3 -peptides were prepared on resin using standard SPPS methods (Scheme 1). Peptides **1a**-c

- (5) Bautista, A. D.; Craig, C. J.; Harker, E. A.; Schepartz, A. Curr. Opin. Chem. Biol. 2007, 11, 685–692.
- (6) Harker, E. A.; Daniels, D. S.; Guarracino, D. A.; Schepartz, A. Bioorg. Med. Chem. 2009, 17, 2038–2046.
- (7) Petersson, E. J.; Schepartz, A. J. Am. Chem. Soc. 2008, 130, 821-823.
- (8) Müller, M. M.; Windsor, M. A.; Pomerantz, W. C.; Gellman, S. H.; Hilvert, D. Angew. Chem., Int. Ed. **2009**, 48, 922–925.
- (9) Murray, J. K.; Sadowsky, J. D.; Scalf, M.; Smith, L. M.; Tomita, Y.; Gellman, S. H. J. Comb. Chem. 2008, 10, 204–15.
- (10) Akkarawongsa, R.; Potocky, T. B.; English, E. P.; Gellman, S. H.; Brandt, C. R. Antimicrob. Agents Chemother. **2008**, *52*, 2120–2129.
- (11) Potocky, T. B.; Silvius, J.; Menon, A. K.; Gellman, S. H. ChemBioChem 2007, 8, 917–926.
- (12) Murray, J. K.; Gellman, S. H. Biopolymers 2007, 88, 657-686.
- (13) Pomerantz, W. C.; Yuwono, V. M.; Pizzey, C. L.; Hartgerink, J. D.; Abbott, N. L.; Gellman, S. H. Angew. Chem., Int. Ed. 2008, 47, 1241–
- (14) Pizzey, C. L.; Pomerantz, W. C.; Sung, B.-J.; Yuwono, V. M.;

Gellman, S. H.; Hartgerink, J. D.; Yethiraj, A.; Abbott, N. L J. Chem. Phys. **2008**, *129*, 095103/1–095103/8.

- (15) Rueping, M.; Jaun, B.; Seebach, D. Chem. Commun. 2000, 2267-2268.
- (16) Arvidsson, P. I.; Rueping, M.; Seebach, D. Chem. Commun. 2001, 649–650.
- (17) Seebach, D.; Schreiber, J. V.; Arvidsson, P. I.; Frackenpohl, J. *Helv. Chim. Acta* **2001**, *84*, 271–279.

(18) Cheng, R. P.; DeGrado, W. F. J. Am. Chem. Soc. 2001, 123, 5162–5163.

were prepared on polyethylene-glycol based NovaPEG Wang resin with a view to evaluating peptide RCMs on a rigid solid support. Such resins have been shown to have good swelling properties in both polar and apolar solvents.^{27,28} **1d** was prepared on standard Wang resin, and RCM was also performed on resin. The product was then N-acetylated and cleaved from resin.

Extensive screening of catalyst/solvent combinations typically employed in RCM²⁹ yielded little success. However, it was ultimately determined that a 4:1 mixture of TFE and CH₂Cl₂ worked well in combination with Hoveyda-Grubbs generation II catalyst (Scheme 1)³⁰ both in solution and on solid support. Conversion in all cases was >90% as evidenced by LCMS. To obtain such high conversion, it was found that RCMs on Novapeg resin required only 20 mol % catalyst, whereas reaction on Wang resin required approximately twice the amount of catalyst. Lower catalyst loadings led to incomplete conversion due, possibly, to catalyst decomposition and/or adsorption onto the resin. LCMS analysis of crude reaction material indicated minimal byproduct formation had occurred.³¹ Optimum yields (typically 20-30% overall for on-resin peptide synthesis, RCM, capping and cleavage) were obtained by washing the resin prior to cleavage with DMSO:DMF (1:1) overnight (to remove catalyst and catalyst-derived impurities) and by performing HPLC purification at an elevated temperature of 60 °C. All the peptide RCMs gave the E-alkene product with high selectivity. This outcome is similar to a recent report from Grubbs.²⁴

We were also interested to establish whether or not this chemistry would apply to longer peptides. Thus nona- β^3 peptide **1e** (Scheme 2) was synthesized on Wang resin,

Scheme 2. Synthesis of the New Stapled Nona- β^3 -peptide (**2e**) via Solution Phase RCM of the Nona- β^3 -peptide (**1e**)



N-acetylated, and then cleaved prior to RCM to evaluate the efficiency of solution phase peptide RCM. Solution phase RCM under our standard conditions, with no side chain protection of the three aspartic acid residues, proceeded smoothly providing stapled **2e**.

NMR spectroscopy was used to determine the solution structure of our peptides. The peptide RCM pair **1a** and **2a**

⁽¹⁹⁾ Vaz, E.; Brunsveld, L. Org. Lett. 2006, 8, 4199-4202.

⁽²⁰⁾ Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. J. Am. Chem. Soc. 2003, 125, 4022–4023.

⁽²¹⁾ Vaz, E.; Pomerantz, W. C.; Geyer, M.; Gellman, S. H.; Brunsveld, L. *ChemBioChem* **2008**, *9*, 2254–2259.

⁽²²⁾ However, multiple salt bridges on a single face of the helix, correctly oriented with respect to the helix macrodipole, are effective. See: ref 20.

was chosen because these peptides displayed the best dispersion in their 1D NMR spectra at 500 MHz in d_3 -MeOH. NOESY experiments of **1a** and stapled **2a** revealed all the expected²¹ i/i+2 and i/i+3 connectivities for a 14-helix (Figure 1).

Thus, the excellent results for the peptide RCMs described here are, very likely, due to the relatively close proximity of the two alkenes as a result of the helical nature of each RCM precursor in solution. In addition to the NMR evidence cited above, CD analysis showed that all new β^3 -peptides, closed as well as unclosed, exhibited spectra in TFE at ambient temperature, consistent with a 14-helix,⁴ with a minimum between 211 and 214 nm and a maximum between 195 and 198 nm.³² Indeed, **1a** and **2a** were consistently helical in a range of solvents, including TFE, MeOH, and 75% acetonitrile/25% 5 mM phosphate buffer pH 7 (Figure

(25) For reviews on the use of RCM-derived staples in helical α -peptides, see: (a) Kim, Y.-W.; Verdine, G. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2533–2536. (b) Brik, A. *Adv. Synth. Catal.* **2008**, 1661–1675. (c) Davis, B. G. *Chembiochem* **2009**, 959–969.

(26) Bergman, Y.; Ciampini, M.; Jalal, S.; Lagiakos, H.; Aguilar, M.-I.; Perlmutter, P. *Tetrahedron: Asymmetry* **2008**, *19*, 2861–2863.

(27) Camperi, S. A.; Marani, M. M.; Iannucci, N. B.; Cote, S.; Albericio, F.; Cascone, O. *Tetrahedron Lett.* **2005**, *46*, 1561–1564.

(28) Garcia-Martin, F.; Quintanar-Audelo, M.; Garcia-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Cote, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, 8, 213–220.

(29) Grubbs, R. H. Handbook of Metathesis; Wiley-VCH: Weinheim, Germany, 2003.

(30) Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H. J. Am. Chem. Soc. 2000, 122, 8168–8179.

(31) Byproduct formation can be an issue with Grubbs catalysis, see: Hong, S. H.; Day, M. W.; Grubbs, R. H J. Am. Chem. Soc. 2004, 126, 7414–7415.

(32) See Supporting Information.

2(a)). The two water-soluble pairs, 1c/2c and 1e/2e, were also helical in 5 mM phosphate buffer pH 7 (Figure 2(b)).



Figure 2. CD spectra of (a) β^3 -peptides **1a** and **2a** (50 μ g mL⁻¹) in TFE, MeOH, and 75% acetonitrile/25% 5 mM phosphate buffer pH 7 and (b) β^3 -peptides **1c/2c** and **1e/2e** in 5 mM phosphate buffer pH 7.

In summary, we have developed the first synthesis of RCM-stapled β^3 -peptides. We have also demonstrated that efficient RCM of suitably substituted β^3 -peptides is possible either in solution or on an appropriate solid support. All RCMs generated the *E*-isomer with high selectivity. Furthermore we have shown that RCM-stapled β -peptides form 14-helices in both organic solvents and water. This study provides new avenues for the design of β^3 -peptides where defined three-dimensional structure is a prerequisite for further functionalization and assembly.

Supporting Information Available: Experimental procedures, HPLC, MS data, CD, and 1D NMR spectra of all peptides. TOCSY and NOESY data for **1a** and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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⁽²³⁾ Blackwell, H. E.; Grubbs, R. H. Angew. Chem., Int. Ed. 1998, 37, 3281–3284.

⁽²⁴⁾ Boal, A. K.; Guryanov, I.; Moretto, A.; Crisma, M.; Lanni, E. L.; Toniolo, C.; Grubbs, R. H.; O'Leary, D. J. J. Am. Chem. Soc. **2007**, *129*, 6986–6987.