Solid-Phase Synthesis of Hydrogen-Bond Surrogate-Derived α-Helices

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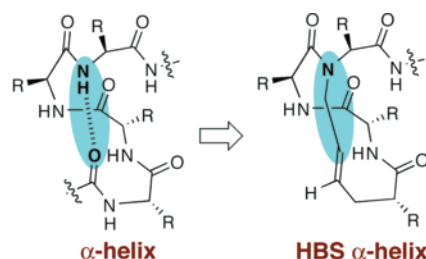# Solid-Phase Synthesis of Hydrogen-Bond Surrogate-Derived α-Helices

Gianluca Dimartino, Deyun Wang, Ross N. Chapman, and Paramjit S. Arora*

Department of Chemistry, New York University, 100 Washington Square East, New York, New York 10003

arora@nyu.edu

ABSTRACT

This report describes the solid-phase synthesis of hydrogen-bond surrogate-derived artificial α-helices by a ring-closing metathesis reaction. From a series of metathesis catalysts evaluated for the synthesis of these helices, the Hoveyda−Grubbs catalyst was found to afford high yields of the macrocycle irrespective of the peptide sequence.

The α-helix, a ubiquitous element of protein secondary structure, is intimately involved in biomolecular recognition.[1] Molecules that mimic the structure of α-helices have the potential to regulate these molecular processes.[2] We recently introduced a new approach for the development of short and highly stable artificial α-helices. These helices were obtained by replacement of an *N*-terminal main-chain hydrogen-bond with a carbon−carbon bond derived from a ring-closing metathesis (RCM) reaction.[3] An important feature of this hydrogen-bond surrogate (HBS) approach is that the internal placement of the cross-link allows synthesis of helices that allow full access to natural solvent-exposed surfaces. Our method, therefore, differs significantly from the commonly employed side-chain cross-linking methods for helix stabilization,[4] which sacrifice side-chain functionalities to nucleate

helical conformations. Modifying side chains makes them unavailable for molecular recognition; moreover, the resulting tether blocks at least one face of the target helix. Our approach uniquely allows synthesis of artificial helices in which all side chains are available for molecular recognition and no steric encumbrances are introduced on the helix surface. We believe that our artificial α-helices have the potential to target protein receptors and regulate protein−protein interactions more successfully than side-chain cross-linked helices.[5]

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The RCM reaction constitutes a key step for the synthesis of HBS α-helices.[6] Here we report detailed studies aimed at identifying the optimum reaction conditions for this crucial step on solid phases. Studies presented here were performed on two different bis-olefinic peptides **1a−b**, which upon macrocyclization afford artificial helices **2a−b** (Figure 1).
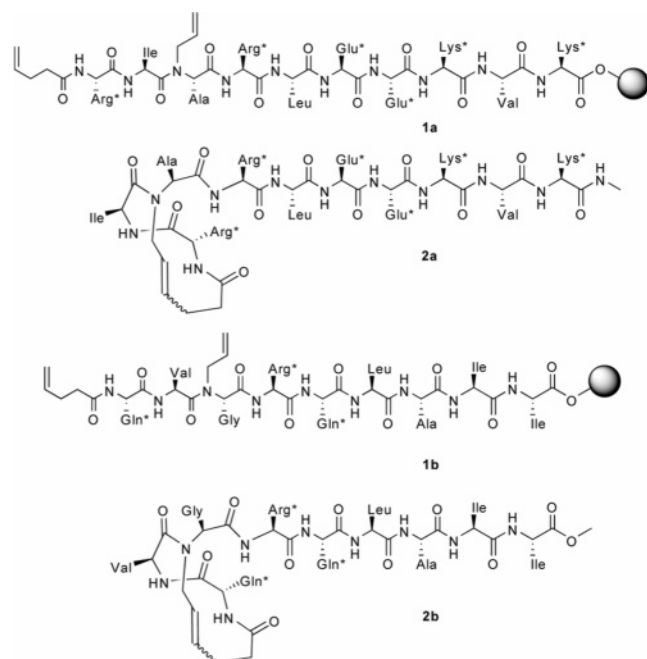

Figure 1. Structures of bis-olefinic peptides **1a−b** and HBS α-helices **2a−b**. Arg*, Pbf-protected Arg; Gln*, trityl-protected Gln; Glu*, *tert*-butyl-protected Glu; Lys*, Boc-protected Lys.

Peptides **1a** and **1b** are derived from biologically important sequences and are related to projects currently underway in our lab. Our previous work involved an alanine-rich peptide.[3] The two peptides (**1a** and **1b**) contain different amino acid residues within the macrocycle, allowing us to observe the effect of peptide sequence on the efficiency of the metathesis reaction. On the basis of the presence of bulky amino acid residues within the putative α-turn, we expected bis-olefin **1b,** which contains valine (a β-branched amino acid disfavoring α-turns) and glutamine (featuring a large side-chain protecting group) residues, to be more difficult to cyclize than bis-olefin **1a**. We tested five metathesis catalysts including the Grubbs' Catalysts generations 1−3,[6−9] the Hoveyda−Grubbs Catalyst,[10] and the Ciba−Ruthenium

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Catalyst[11] to identify the best conditions for the preparation of HBS α-helices. Through the studies described herein, we find that only the Hoveyda−Grubbs catalyst affords acceptable yields of the RCM products **2a−b**. Remarkably, the peptide sequence appears to have a minimal effect on the efficiency of the RCM reaction, allowing the synthesis of any HBS α-helix of interest. As part of our overall program, we have now prepared approximately 10 different HBS α-helices and have found the optimized RCM conditions presented here to consistently afford high yields.

The artificial α-helices (**2a−b**) were generated from bis-olefin peptides (**1a−b**) following treatment of the resin with the RCM catalyst (Scheme 1). We typically isolate the HBS

Scheme 1. Solid-Phase Synthesis of HBS α-Helices.
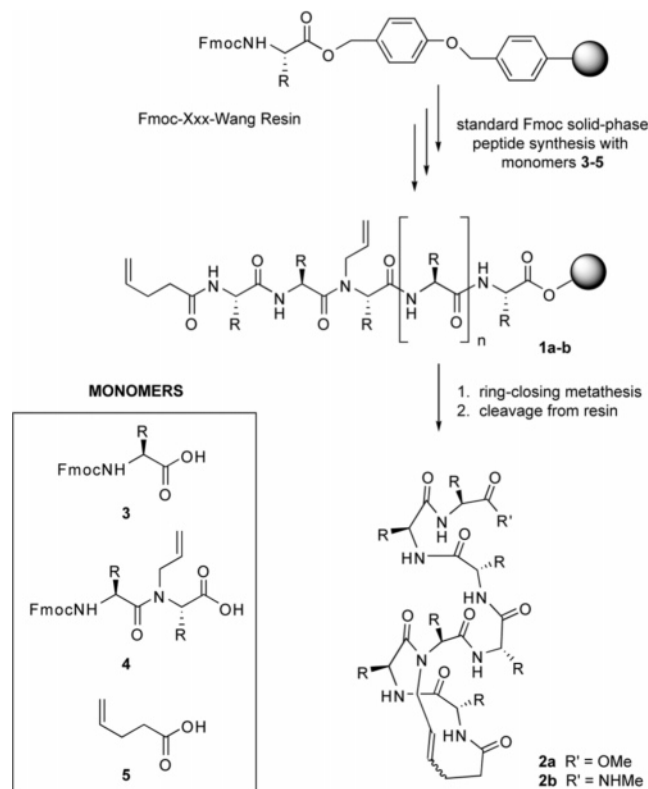
α-helices after deprotection of the amino acid side chains and cleavage from the resin. However, for the present study, which is aimed at identifying the optimum RCM reaction conditions and catalysts, we found that it was easier to quantify reaction progress with the protecting groups attached (especially the aromatic Pbf protecting group of arginine).

The RCM reaction is commonly performed in refluxing dichloromethane with Grubbs catalysts;[12] however, we found that these conditions did not yield significant amounts of the metathesized product from the bis-olefins shown in Figure 1. This preliminary result prompted us to systematically investigate several of the commonly used RCM

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catalysts and reaction conditions. The results of these experiments are summarized in Figures 2−5. Full experimental details and a list of all metathesis experiments performed on bis-olefins **1a−b** are included in the Supporting
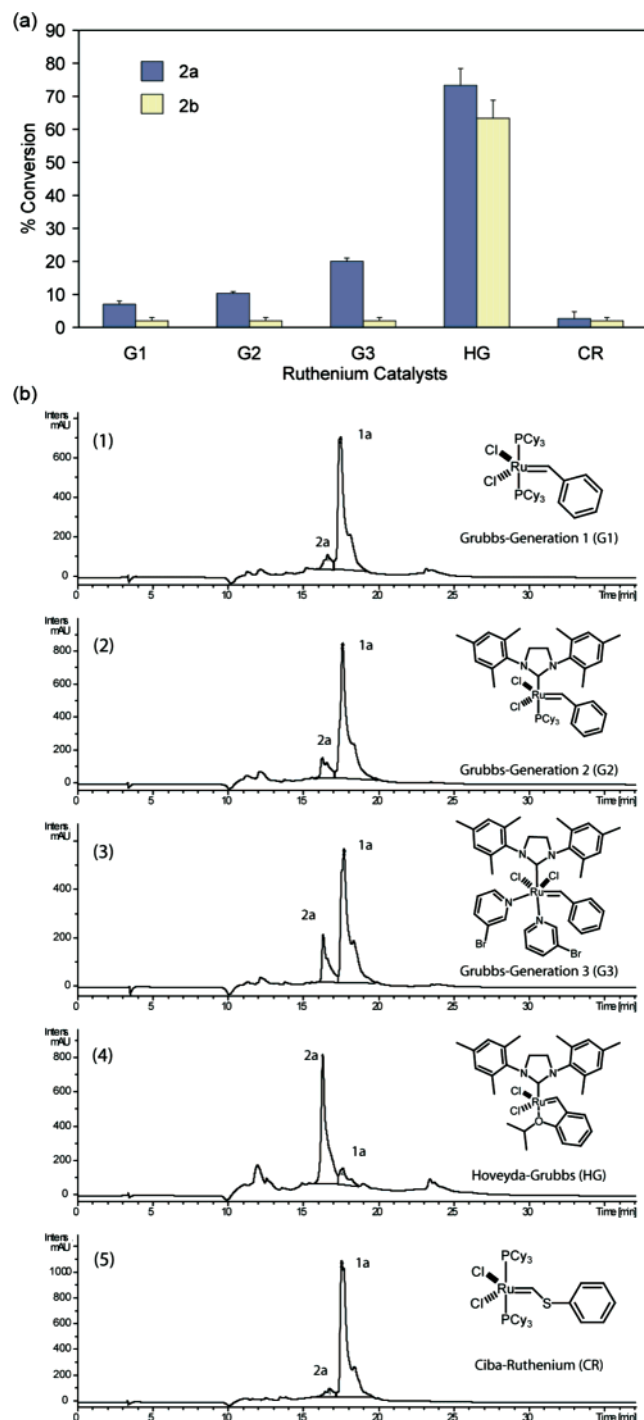

Figure 2. (a) Graph summarizes efficacy of five different metathesis catalysts: Grubbs' catalysts generations 1−3 (G1−3), the Hoveyda−Grubbs catalyst (HG), and the Ciba−Ruthenium Catalyst (CR) for the conversion of bis-olefins **1a−b** to α-helices **2a−b**. (b) Representative HPLC plots for the conversion of **1a** to **2a** with the five catalysts. Conditions: 15 mol% of each catalyst at 60 °C for 24 h in dichloroethane.
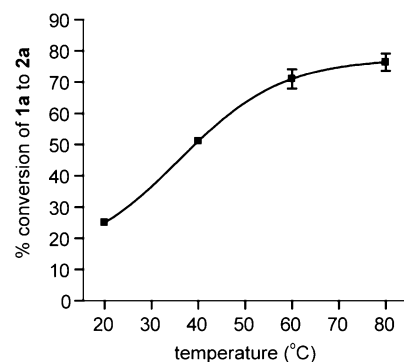

Figure 3. Effect of temperature on the conversion of **1a** to **2a**. The reactions were performed with 15 mol% Hoveyda−Grubbs catalyst in dichloroethane for 24 h.
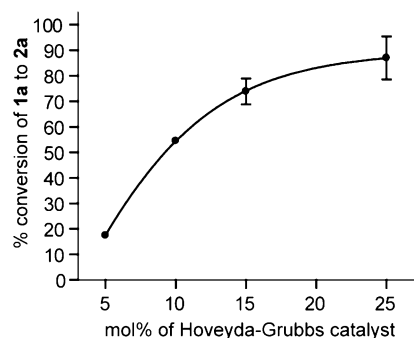

Figure 4. Effect of the amount of the Hoveyda−Grubbs catalyst on the conversion of **1a** to **2a**. The reactions were performed in dichloroethane at 60 °C for 24 h.
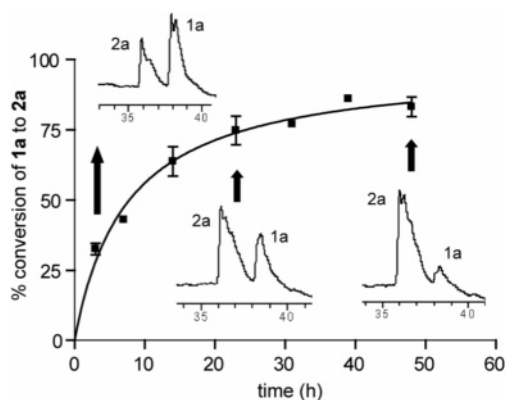

Figure 5. Effect of reaction time on the conversion of **1a** to **2a**. Figure also shows LC-MS traces with peaks corresponding to bis-olefin **1a** and product **2a** at the indicated period of reaction time. The reactions were performed with 15 mol% Hoveyda−Grubbs catalyst in dichloroethane at 60 °C.

Information (Table S1). Briefly, the resin-bound bis-olefin peptides were placed in a solid-phase synthesis vessel and treated with the RCM catalyst in dichloroethane. The reaction

mixture was shaken in a temperature-controlled environment. After the indicated reaction times, the peptides were cleaved from the resin and analyzed by LC-MS. All percent conversion values reported are averages of two to five independent experiments.

Figure 2 depicts percentages of products **2a**−**b** obtained from RCM reactions performed with 15 mol% of the given catalyst at 60 °C for 24 h. This figure represents optimum conditions established after significant efforts to determine suitability of each catalyst under various conditions (Supporting Information, Table S1). We find that the Hoveyda− Grubbs catalyst affords the highest amounts (60−75% conversion) of the macrocyclized products **2a**−**b** under these conditions. The Grubbs third generation catalyst (G3) also affords the desired products but in lower yields than the Hoveyda−Grubbs catalyst for the bis-olefins **1a**−**b**. The other metathesis catalysts (Grubbs' generations 1 and 2 and the Ciba catalyst) did not provide appreciable amounts of the desired metathesized products.

Figures 3−5 report a subset of the data we obtained for macrocyclization of bis-olefin **1a** with respect to temperature, time, and amount of the Hoveyda−Grubbs catalyst to arrive at the optimum conditions (Supporting Information, Table S1). Figure 3 shows that the yield of the macrocycle **1b** improves at elevated temperatures reaching an optimum between 60 and 80 °C; we typically carry out the solid-phase reactions at 60 °C. Figure 4 describes the effect of increasing the amount of the catalyst on efficiency of the reaction. We find that 15−25 mol% catalyst is needed for effective conversion; however, due to the cost of catalyst, we typically use 15 mol% for routine syntheses. Figure 5 shows that longer (24−48 h) reaction times lead to higher conversions. From these studies, we find that the optimized reaction times and conditions for the synthesis of HBS α-helices (24−48 h at 60 °C with 15−25 mol% Hoveyda−Grubbs catalyst in dichloroethane) lead to respectable yields (50−90%) for the RCM step for a variety of bis-olefin peptides of the type shown in Figure 1.

Importantly, we find that the optimized conditions work effectively for the synthesis of artificial helices on preparative scale (Supporting Information). As expected, the RCM step affords a mixture of cis and trans isomers with the trans isomer forming in higher amounts. The ratios of the cis and trans alkene isomers obtained from the RCM reactions on **1a**−**b** were determined by HPLC and ^{1}H NMR spectroscopy (Supporting Information). We observed a cis/trans ratio of 1:3 for **2a** and 1:2.5 for **2b**. The α-helical conformations of the constrained peptides **2a**−**b** were confirmed by circular dichroism spectroscopy (Supporting Information).

In conclusion, we find that hydrogen-bond surrogate-derived artificial α-helices can be efficiently synthesized on solid phases with the Hoveyda−Grubbs catalyst. Importantly, the synthesis of our α-helices does not require the preparation of new enantiomerically pure amino acids and is achieved by standard peptide synthesis protocols with easily available monomers. Our studies highlight the capability of the metathesis reaction, as these products would be significantly more difficult to obtain by alternative methods. We anticipate that the results described herein will be of significant interest because of the wide-ranging potential of this powerful reaction.

Supporting Information Available: Synthesis, ^{1}H NMR, ^{13}C NMR, and HRMS of modified amino acids; synthesis, RCM, and characterization of peptides; circular dichroism spectra for **2a**-**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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