Supramolecular Design by Covalent Capture. Design of a Peptide Cylinder via Hydrogen-Bond-Promoted Intermolecular Olefin Metathesis

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Noncovalent chemical approaches to supramolecular design often exploit self-assembling and self-organizing molecular processes to establish equilibrium conditions for the production of thermodynamically stable aggregates of predetermined form and function.[1] Such strategies are inherently efficient and have several distinguishing attributes, among which the most noteworthy are minimization of molecular information and synthetic effort through the use of modular subunits, high cooperativity in the assembly process, and built-in error-checking. However, the "equilibrium aggregates" produced in this way often possess low kinetic stabilities. Therefore, the utility of such processes could be greatly enhanced if desired noncovalent ensembles could be kinetically captured and stabilized by covalent-bond-forming processes.[2] Here we report one such approach in the context of the design and synthesis of a novel peptide-based cylindrical structure possessing a hydrophilic cavity and hydrophobic outside surface characteristics. Directed hydrogen-bonding interactions in nonpolar organic solvents are used to effect structural self-assembly and self-organization of a flat ring-shaped peptide subunit into two thermodynamically favored interconverting antiparallel β-sheet-like hydrogen-bonded cylindrical ensembles.[3] Hydrogen-bond-promoted 38-member ring forming olefin metathesis[4] in the presence of $(Cy_3P)_2Cl_2Ru{=}CHCH{=}CPh_2$ is shown to be an effective means for the selective capture of the noncovalent peptide ensemble in the desired covalently stabilized form.

The synthetic strategy employed in this study is based on the previously described design principles employed in the construction of peptide tubular ensembles[3,5] as well as recent advances in the design of remarkably chemoselective olefin metathesis catalysts[4] (Figure 1). Cyclic peptides made up of an even number of alternating D- and L-amino acids bearing selective N-methylated peptide backbone amide functionalities have been shown to self-assemble in nonpolar organic solvents to form hydrogen-bonded cylindrical ensembles.[3] It was hypothesized that such ensembles, when endowed with intersubunit juxtapositioning of appropriate functionalities, should be able to readily participate in intermolecular covalent-bond-forming processes to form stabilized supramolecular cylindrical structures. The eight-residue cyclic peptide *cyclo*[-(L-Phe-D-^{Me}NAla-L-Hag-D-^{Me}NAla)$_2$-] was designed for the task at hand.[6] The peptide ring structure was equipped with two L-homoallylglycine (Hag) residues for use in olefin metathesis reactions. The C_2 symmetry of the peptide subunit (perpendicular to the plane of the ring structure) allows for the formation of two interconverting hydrogen-bonded diastereomeric ensembles **2a** and **2b**, which are distinguished by the identity of their cross-strand near-neighbor residues.[3b] It was hypothesized that the hydrogen-bonded ensemble **2a**, which favors a productive orientation of the olefinic side chain moieties, would lower the activation entropy for the intermolecular carbon−carbon bond forming olefin metathesis process. In this way, the equilibrium would siphon through the kinetically labile ensemble **2a** and convert into the covalently stabilized tricyclic structure **3**.

In nonpolar organic solvents, the peptide subunit self-assembles to form two slow-exchanging antiparallel β-sheet-like hydrogen-bonded cylinders with an association constant of $K_a(CDCl_3) = 99 \pm 1\ M^{-1}$ (Figures 1 and 2a). Remarkably, in the presence of Grubbs ruthenium carbene catalyst,[4b] the desired covalent supramolecular structure **3** is formed in 65% yield.[8] The metathesis reaction gave three inseparable diastereomeric products, *cic,cis*, *trans,trans*, and *cis,trans*, as evidenced by ^{1}H NMR spectroscopy and ion-spray mass spectrometry[8] (Figure

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(6) Homoallylglycine (Hag) was prepared from ethyl acetamidocyanoacetate and 4-bromo-1-butene following the procedure of Albertson[7a] and resolved (porcine kidney acylase I) following the procedures of Chenault et al.[7b] Standard Boc protection and purification by flash chromatography gave the requisite amino acids having >99% ee according to Marfey's test.[7c] The linear peptide (D-^{Me}NAla-L-Hag-D-^{Me}NAla-L-Phe)$_2$ was synthesized on commercially available *N*-Boc-L-Phe-PAM resin using standard Boc chemistry, cleaved from the solid support using tetrabutylammonium fluoride in DMF,[7d] and purified by RP-HPLC (44% yield). Linear peptide (1 mM) was cyclized at 0 °C in the presence of 3.0 equiv of 1-hydroxy-7-azabenzotriazole[7e] (HOAt), 3.0 equiv of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate[7f] (HATU) in 99.5% DMF, 0.5% v/v DIEA to give a 70% yield of the cyclic peptide following purification by RP-HPLC (IS-MS: MW_{found} = 857.4).

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(8) In a typical experiment, a dried solution of cyclic peptide (3−18 mg, 5.0 mM in $CDCl_3$) and the ruthenium carbene catalyst (20−25 mol %) was allowed to react at room temperature under an Ar atmosphere for 48 h to give a 65% yield of the metathesis product **3** as a mixture of three olefin isomers, which were collectively isolated by RP-HPLC (MH^+_{Found} = 1657.5). Hydrogenation in the presence of 1 wt equiv of 10% Pd/C in MeOH can be performed either on the purified products or directly on the crude reaction mixture to give quantitatively the reduced product **4** (MH^{++}_{Found} = 1661.7): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, J = 7.6 Hz, 4 H, Hag NH), 7.56 (br, 4 H, Phe NH), 7.29−7.16 (m, 20 H, Phe), 5.16 (q, J = 7.0 Hz, 4 H, D-^{Me}NAla(a) α-C*H*), 5.12 (m, 4 H, Phe α-CH), 4.76 (m, 4 H, Hag α-CH), 4.59 (br, 4 H, D-MeNAla(b) α-C*H*), 3.06 (s, 12 H, D-^{Me}NAla(b) NCH_3), 3.04−2.92 (m, 8 H, Phe β-CH_2), 2.48 (s, 12 H, D-^{Me}NAla(a) NCH_3), 1.54 (m, 8 H, H, Hag β-CH_2), 1.40 (d, J = 7.3 Hz, D-^{Me}NAla(b) β-CH_3), 1.26−1.18 (m, 16 H, Hag, $\gamma + \delta$ CH_2), 1.09 (d, J = 7.0 Hz, 12 H, D-^{Me}NAla(a) β-CH_3).

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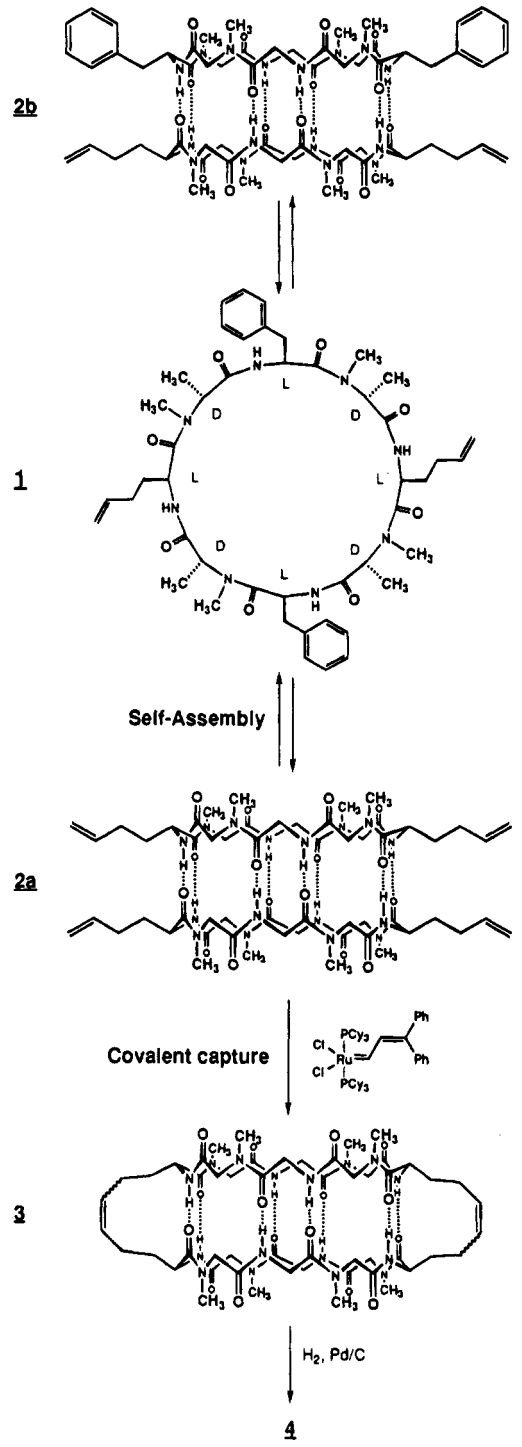

Figure 1. Schematic representation of the synthetic strategy employed in the design of the tricyclic cylindrical peptide structure. The flat ring-shaped peptide subunit **1** self-assembles in nonpolar organic solvents to give an equal mixture of the hydrogen-bonded peptide ensembles **2a** and **2b**. Only ensemble **2a**, which has the proper juxtaposition of olefinic side chains, can participate in the olefin metathesis which siphons the equilibrium toward the kinetically stable tricyclic structure **3**. For clarity most side chains are omitted. D and L refer to the amino acid chirality.

2b). The reaction appears to proceed in a stepwise fashion as judged by the transient buildup of small quantities of an intermediate dimeric species where only the first intermolecular side chain−side chain olefin bond has formed. Purified samples of this intermediate, when subjected to the methathesis reaction conditions, convert to the tricyclic products **3**. Reaction products **3** were hydrogenated in the presence of Pd/C in order to eliminate ^{1}H NMR spectrum complexity due to the presence of olefin isomers. Reduction afforded, quantitatively, saturated

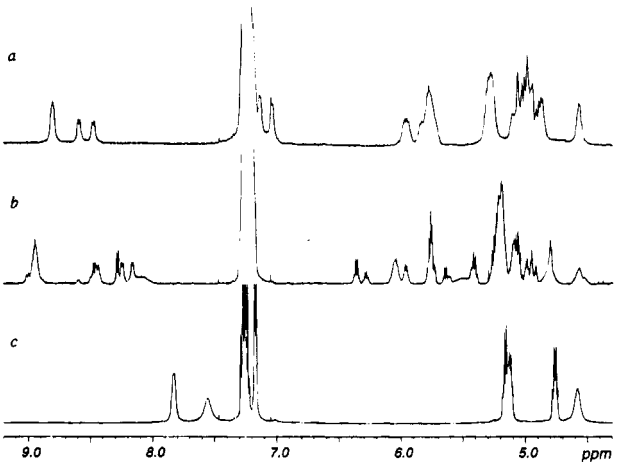

Figure 2. ^{1}H NMR (500 MHz, $CDCl_3$) spectra displaying NH and C_α resonances of (a) peptide **1** (5.0 mM), (b) metathesis products **3** (7.1 mM at 293 K, mixture of three olefin isomers), and (c) product **4** (4.8 mM, at 283 K). Spectrum *a* displays non-hydrogen-bonded NH resonances for the peptide monomer **1** at δ_{NH} = 7.04 and 7.14 ppm and hydrogen-bonded NH resonances for the two equally populated dimeric cylindrical ensembles **2a** and **2b** at δ_{NH} = 8.48, 8.59, and 8.81 (2H). Spectrum *b* reflects the presence of the three diasteromeric (*cic,cis*, *trans,cis*, and *trans,trans*) metathesis products. Reduction of the olefins produces a single symmetrical product with δ_{NH} = 7.56 and 7.83 ppm (spectrum *c*).[8]

product **4**, which was characterized by ^{1}H NMR spectroscopy (ROESY, COSEY), mass spectrometry, and FT-IR spectroscopy.[8] The ^{1}H NMR ($CDCl_3$) spectrum is indicative of the expected symmetrical structure, having hydrogen-bonded NH resonances appearing at 7.83 and 7.56 ppm (Figure 2c). The FT-IR spectrum displays amide-I resonances at 1632 and 1674 cm^{-1}, closely resembling that of previously characterized peptide nanotube structures,[3a,b,5] and an NH stretching frequency at 3310 cm^{-1}, which is also in agreement with the expected 4.7−4.8 Å hydrogen-bonded backbone-backbone distance.[9] A requirement for intersubunit hydrogen-bonding interaction for the promotion of olefin metathesis is supported by the following observations. When the reaction was conducted in polar organic solvents (1:1 DMF/$CHCl_3$ or EtOH), which are known from ^{1}H NMR studies to disrupt intersubunit hydrogen-bonding interactions, no metathesis products were observed even after prolonged reaction times.[10] Moreover, the directive effects of H-bonding interactions may have also contributed in preventing formation of undesired side products such as the intramolecularly metathesized peptide structure and oligomeric species as judged by ion-spray mass spectrometry performed on the crude reaction mixture.

In summary, we have presented a novel approach for the design and synthesis of a covalently stabilized β-sheet-like peptide cylindrical ensemble. The covalent capture strategy presented here is expected to be useful in stabilizing kinetically labile α-helical and β-sheet peptide secondary structures and may also find utility in *de novo* design of artificial proteins.

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