Peptide Bond Isosteres: Ester or (*E*)-Alkene in the Backbone of the Collagen Triple Helix
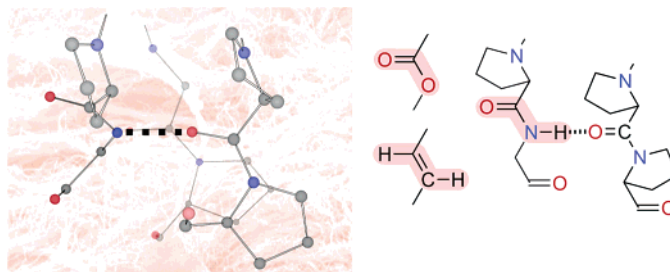
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ABSTRACT

Collagen is the most abundant protein in animals. Interstrand N–H⋯O=C hydrogen bonds between backbone amide groups form a ladder in the middle of the collagen triple helix. Isosteric replacement of the hydrogen-bond-donating amide with an ester or (*E*)-alkene markedly decreases the conformational stability of the triple helix. Thus, this recurring hydrogen bond is critical to the structural integrity of collagen. In this context, an ester isostere confers more stability than does an (*E*)-alkene.

Hydrogen bonds between main-chain amides are a dominant feature of folded proteins.[1] Perhaps the most recurrent main-chain—main-chain hydrogen bond is found in collagen, the most abundant protein in animals.[2] Collagen consists of a triple helix of (XaaYaaGly)$_n$ strands, in which Xaa is often (2*S*)-proline (Pro), Yaa is often (2*S*,4*R*)-4-hydroxyproline (Hyp), and *n* is ca. 300. The GlyN-H groups and XaaC=O groups form a ladder of hydrogen bonds that are buried in the middle of the triple helix and inaccessible to solvent (Figure 1).[3] The contribution of this prevalent hydrogen bond to the conformational stability of collagen is unknown.

One approach to estimating the strength of a main-chain—main-chain hydrogen bond is to replace the amide N—H with an ester O.[4] Substituting an ester for an amide is relatively conservative because the two functional groups are isosteric and have similar conformational preferences.[5] For example, replacing an alanine residue with a lactic acid in an α-helical model peptide has been shown to induce minimal structural perturbations.[6] On the other hand, there is more flexibility around an ester linkage than around an amide,[7] and the two

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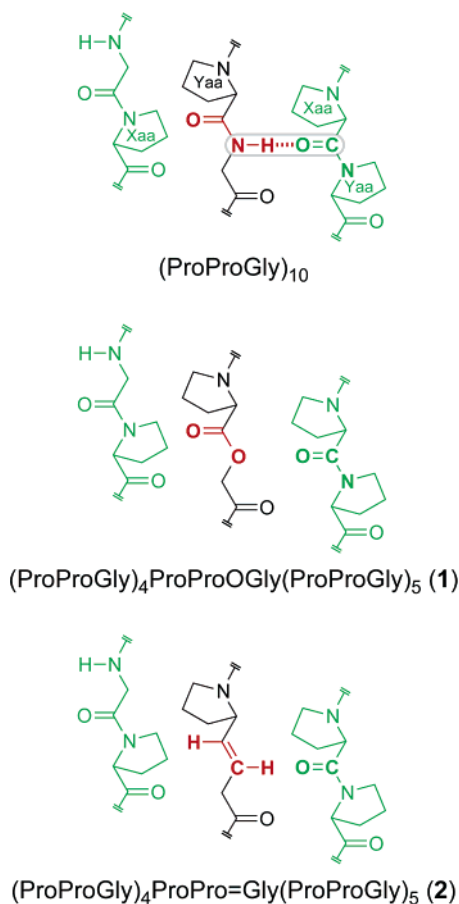
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(ProProGly)₁₀ represented as $(ProProGly)_{10}$

(ProProGly)₄ProProOGly(ProProGly)₅ (1) as $(ProProGly)_4ProProOGly(ProProGly)_5$ **(1)**

(ProProGly)₄ProPro=Gly(ProProGly)₅ (2) as $(ProProGly)_4ProPro{=}Gly(ProProGly)_5$ **(2)**

Figure 1. Interstrand hydrogen bond in the middle of a collagen triple helix, and ester and (*E*)-alkene isosteres of the hydrogen bond donor.
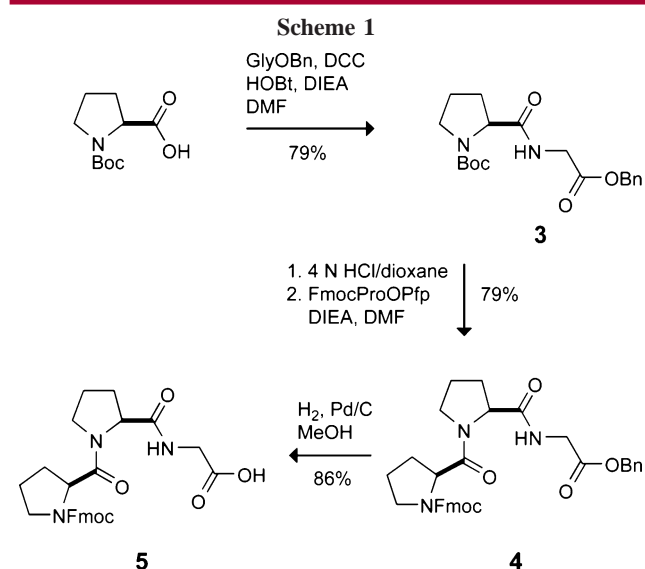
functional groups have distinct electronic properties.[8] Accordingly, hydrogen bond strengths obtained by ester substitutions are estimates that, nonetheless, have been informative.[4]

An alternative approach is to replace the amide NH with an alkene CH.[9] This approach is beyond the realm of biosynthesis.[10] Although alkene isosteres have been incorporated into peptides by chemical synthesis,[11] we are not aware of a comparison between an ester and alkene as a surrogate for an amide that forms a solvent-inaccessible main-chain–main-chain hydrogen bond.

Here, we synthesize collagen strands in which a single amide bond is replaced with an ester (depsipeptide **1**) or (*E*)-alkene (alkenyl peptide **2**). We compare the conformational stability of the resulting triple helices with that of a collagen

triple helix containing a native backbone. The results provide new information on the contribution of the prevalent interstrand hydrogen bonds to triple helix stability and differentiate between an ester and (*E*)-alkene as an amide bond isostere in this context.

In previous work, we determined that the H/D fractionation factors of the GlyN–H···O=CXaa hydrogen bonds in (ProProGly)₁₀ and (ProHypGly)₁₀ triple helices were not distinguishable, suggesting that the GlyN–H···O=CXaa hydrogen bonds have a similar strength in these two contexts.[12] Accordingly, we limit our analysis herein to isosteres of (ProProGly)₁₀. Also in previous work, we reported a five-step synthesis for FmocProFlpGly (where Flp refers to (2*S*,4*R*)-4-fluoroproline) based on standard Boc chemistry with an average overall yield of 17%.[13] We have now made this route more efficient by eliminating the need to switch from a Boc to Fmoc protecting group. Following the route in Scheme 1 and monitoring the hydrogenolysis

Scheme 1

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reaction with care, we achieved an overall yield of 54% over three steps for the synthesis of FmocProProGly from BocPro. One particular advantage of the route in Scheme 1 is the facile purification of the product in the final step.

To minimize diketopiperazine formation, we synthesized depsipeptide **1** by condensing two dipeptide segments, FmocProOGly (**6**, Scheme 2) and FmocGlyPro, to install the
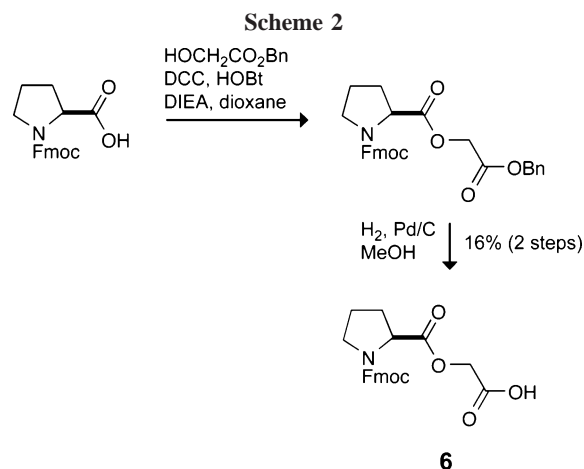
Scheme 2

residues near the labile ester linkage. This strategy (Scheme 3) avoided the presence of an unprotected (and thus nucleo-

Scheme 3
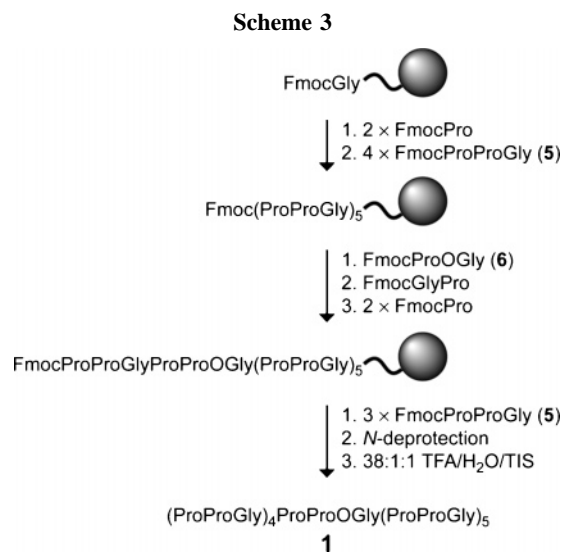
philic) amino group two residues from the labile ester group during the synthesis. Cleavage from the resin and purification by HPLC yielded depsipeptide **1**.

A solution of depsipeptide **1** (0.2 mM) in 50 mM AcOH was incubated at 4 °C for 24 h and then analyzed by circular dichroism (CD) spectroscopy. Wavelength scans from 200 to 260 nm indicated that the peptide had not assembled into a triple helix. The absence of the triple helix was confirmed by the linear decrease in ellipticity at 225 nm, which is
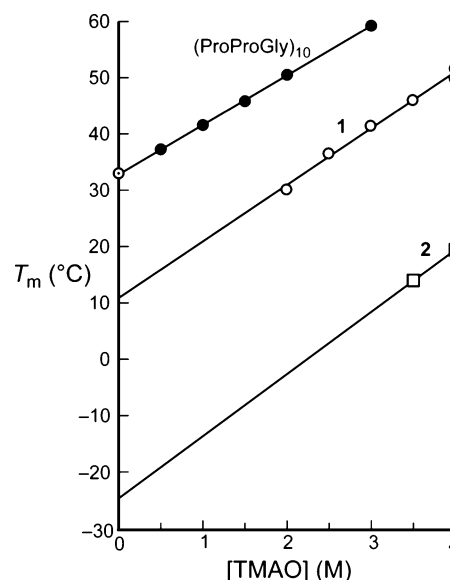
Figure 2. Effect of trimethylamine *N*-oxide concentration on the value of T_m for triple helical (ProProGly)$_{10}$, depsipeptide **1**, and alkenyl peptide **2** in PBS. Lines were obtained by linear least-squares analysis of the data in PBS containing TMAO (0.5−4.0 M).

characteristic of triple helix content, with increasing temperature. In contrast, (ProProGly)$_{10}$ had a T_m value (which is the temperature at the midpoint of the thermal transition between native and unfolded states) of 41 °C in 50 mM AcOH.[13] The failure of depsipeptide **1** to assemble into triple helices indicates that each interstrand hydrogen bond contributes substantially to triple helix stability.

The natural osmolyte trimethylamine *N*-oxide (TMAO) enhances the conformational stability of collagen[14] and other proteins,[15] presumably by enhancing water structure and thereby discouraging backbone−water interactions.[16] Incubating depsipeptide **1** with various concentrations of TMAO in phosphate-buffered saline (PBS) at 4 °C for 24 h led to an increase in ellipticity at 225 nm with increasing TMAO concentration. Triple helices of depsipeptide **1** began to show a thermal transition in solutions containing 2 M TMAO or higher. MALDI-TOF mass spectrometric analysis showed negligible decomposition of depsipeptide **1** after thermal denaturation experiments.

The T_m value of triple helical (ProProGly)$_{10}$ and depsipeptide **1** showed a similar linear dependence on TMAO concentration (Figure 2). For triple helical (ProProGly)$_{10}$, the extrapolated T_m value at 0 M TMAO is 32.8 °C, which is in gratifying agreement to the T_m value of 33 °C measured in PBS. For triple helical depsipeptide **1**, the extrapolated T_m value is 10.7 °C. With a ΔS_m value of 0.21 kcal/mol,[13b] this $\Delta T_m = 22.1$ °C corresponds to $\Delta\Delta G_m = \Delta T_m \Delta S_m = 4.2$

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kcal/mol.[17] This value does not take into account the solvation of amides being greater than that of esters.

Differences in solvation between amides and esters can be estimated by the $\Delta\Delta G°$ of octanol → water partitioning of analogous small-molecule amides and esters. For example, $\Delta\Delta G°_{octanol\rightarrow water} = -1.7$ kcal/mol for methyl acetate versus N-methylacetamide.[18] Then according to the analysis of Schultz and co-workers,[4b] each GlyN−H···O=CXaa hydrogen bond contributes approximately $\Delta\Delta G°_{GlyN-H\cdots O=CXaa} = (\Delta\Delta G°_{octanol\rightarrow water} - \Delta\Delta G_m)/3 = -2.0$ kcal/mol to the conformational stability of the collagen triple helix. This value is typical for a solvent-inaccessible main-chain−main-chain hydrogen bond.[1,4]

The central ProGly unit in (ProProGly)$_{10}$ was also replaced with alkene **7**, which was synthesized by olefin cross-metathesis as described previously,[11h] to yield alkenyl peptide **2**. As with depsipeptide **1**, incubation of alkenyl peptide **2** for 24 h in 50 mM AcOH yielded no evidence for triple helix formation, as probed by CD spectroscopy. The CD spectrum from 200 to 260 nm exhibited only a small maximum near 225 nm, similar to the signature spectra of a polyproline type II helix.[19] The molar ellipticity at 227 nm decreased linearly with temperature, consistent with the absence of a triple helix.

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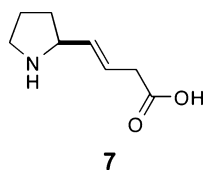
Repeating the thermal denaturation experiments on alkenyl peptide **2** in the presence of TMAO revealed that a transition could be observed at ≥3.5 M TMAO. The T_m value of triple helical **2** in 3.5 M TMAO was 14.0 °C and that in 4.0 M TMAO was 19.5 °C. Extrapolation of these data gives a T_m value of −24.7 °C at 0 M TMAO. Thus, in the context of a collagen triple helix, replacing a ProGly segment with alkene isostere **7** results in a triple helix that is much less stable than one in which the ProGly segment is replaced with an isosteric ester.

The conformational stability conferred by the two peptide bond isosteres examined herein decreases in the order: ester > (*E*)-alkene (Figure 2). This order could arise from the (*E*)-alkene isostere not adopting ϕ or ψ torsion angles amenable to triple helix formation[20] or having a low dipole moment.[21] A disruption in the solvation of the triple helix[22] by the alkenyl group could also play a role. It is noteworthy that formation of a C−H···O=C hydrogen bond[23] in a triple helix of alkenyl peptide **2** (Figure 1) is apparently not able to compensate for the loss of an N−H···O=C hydrogen bond and other deleterious effects.

Our analysis of collagen triple helices containing backbone isosteres has demonstrated that the prevalent interstrand hydrogen bonds make a substantial contribution to the conformational stability of the collagen triple helix. From our data with an ester isostere, we estimate the strength of each hydrogen bond to be 2.0 kcal/mol. Interestingly, an (*E*)-alkene isostere confers much less stability than does an ester isostere and is thus a less well-suited surrogate for a peptide bond, at least in the context of the collagen triple helix.

Supporting Information Available: Procedures for the preparation of compounds **1** and **2**, and related analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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