Published in final edited form as:

Chem Commun (Camb). 2016 April 28; 52(33): 5738–5741. doi:10.1039/c6cc01104g.

Optimal Hydrogen-Bond Surrogate for α -Helices

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Abstract

Substitution of a main chain $i \rightarrow i+4$ hydrogen bond with a covalent bond can nucleate and stabilize the α -helical conformation in peptides. Herein we describe the potential of different alkene isosteres to mimic intramolecular hydrogen bonds and stabilize α -helices in diverse peptide sequences.

TOC image

Manuscript describes the impact of alkene-derived hydrogen bond isosteres on the stability of constrained helices.

Protein secondary and tertiary structures often serve as scaffolds for organization of critical functional groups at protein-protein interfaces.¹ Mimicry of these structured domains has emerged as an attractive strategy for the inhibition of aberrant protein-protein interactions (PPIs).² We have previously described a hydrogen bond surrogate (HBS) strategy for the stabilization of α -helices.³ In the HBS approach, an i to $i+4$ main-chain hydrogen bond is replaced with a covalent bond. Extensive biophysical characterization supports the hypothesis that appropriate placement of the surrogate nucleates helical conformation in the attached sequences, as expected from the classical helix-coil nucleation theories.⁴ Significantly, HBS helices have been shown by our group and others to modulate protein-protein interactions in cell free, cell culture and animal models.^{2h, 5}

In our prior studies, we have investigated hydrocarbon, thioether and disulfide linkages in place of the hydrogen bond to stabilize α -helices.^{3, 6} Facile access to the hydrocarbon bridge is possible due to the excellent functional group tolerance afforded by the olefin metathesis catalyst.⁷ The ring closing metathesis reaction predominantly yields an *E*-alkene in place of the putative C=O group in hydrogen bonded helices (Figure 1a). The *Z*-alkene HBS has not been extensively characterized due to the synthetic difficulty in accessing this geometry

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Electronic Supplementary Information (ESI) available: Synthetic procedures and characterization of peptides See DOI: 10.1039/x0xx00000x

from ring closing metathesis (RCM).⁸ Modeling suggests that the *Z*-alkene isostere might be a better mimic of the idealized helical hydrogen bond than the *E*-alkene. Although, neither alkene isostere captures the ideal main chain N-C=O—H(N) dihedral angle of 30–40°, the 0° torsion in *Z*-alkene is closer to the hydrogen bond than the 180° dihedral in the *E*-analog.⁹ The hydrogen bond differs from the possible alkene geometries in key aspects, for example, the “elevation angle” associated with the hydrogen is not adequately captured by alkenes.^{9–10} The C=O—H(N) angle in protein helices varies but has an average value of 155°,^{9–10} while the analogous C=C—C(N) angle in both alkene modified HBS helices remains fixed at ±120°. Figure 1b presents the two-dimensional models of geometries of N-C=O—H(N) hydrogen bonds in idealized α -helices and the analogous N-C=O—C(N) torsions in alkene isosteres.

We have previously analyzed the impacts of altering HBS macrocycle size and linker flexibility on helicity;^{4a, 11} however, the subtle effects of alkene geometry and other linker alterations have not been systematically investigated. Here, we tested the hypothesis that a *Z*-alkene isostere may lead to a more stable helical conformation.

A third variant of the alkene isosteres may also be designed since an alkene group in the HBS macrocycle can replace the resonance double bond forms shared between C—O or C—N atoms (Figures 1c and 2). Accordingly, we began by evaluating *Z*-alkene isosteres for both C—O and C—N to obtain *Z*^O-HBS and *Z*^N-HBS analogs, respectively. The *Z*-alkene isosteres were compared with the RCM reaction derived *E*-alkene in place of the C—O (*E*^O-HBS) bond. Preparation of the *Z*-analogs requires a non-RCM based synthetic method. We utilized a lactam ring closure approach to access *Z*-alkene HBS helices from hexenoate bridges with the desired alkene stereochemistry and position (Scheme 1 and ESI Figures S1–S2).

Substitution of an alkene in place of the C—N bond results in a β,γ -unsaturated amide, which can potentially undergo alkene isomerization to yield a conjugated amide.¹² We placed a dimethyl group at the α -position (position 2 in Figure 2) to remove the acidic proton and avoid alkene isomerization. The α -dimethyl group is reminiscent of α -aminoisobutyric (Aib) residue – a known helix inducer.¹³ We conjectured that the rigidification afforded by α -dimethyl substitution may also enhance helical stability in HBS peptides derived from carbonyl alkene isosteres. Overall, we evaluated five different constraints as part of these studies: *E* and *Z* carbonyl–alkene isosteres, with and without the dimethyl substitutions, and the dimethyl *Z*-alkene C—N isostere. (Figure 2).

We began analysis of the conformational stability endowed by each HBS constraint on an alanine-rich sequence. Although alanine-rich peptides have high helical stability, our earlier studies indicated that polyalanine HBS macrocycles are not optimal nucleators as opposed to macrocycles that contain bulkier residues. Alanine-rich macrocycles, thus, provide a rigorous test for preliminary evaluation of a constraint’s potential. The five constraints were incorporated into the sequence X*AKG*AAAAAKAY-NH₂ (**1**), where X* and G* denote the bridging positions. The sequence contains two lysine groups to enhance water solubility and a tyrosine residue as a spectroscopic probe.

The lactam approach requires access to free carboxylic acid and amine partners. We found cyclization in solution to be more efficient although this approach requires selective removal of protecting groups and cleavage of the peptide from resin. Full synthetic details are included in the ESI. We utilized Fukuyama nitrobenzenesulfonamide chemistry to install constraints into resin bound peptides (Scheme 1 and ESI Figures S6–S7).¹⁴ The lactam formation was accomplished using standard coupling agents to afford the desired HBS peptide. The helicity of HPLC purified peptides was determined using circular dichroism (CD). Canonical α -helices display double minima at 208 and 222 nm and maxima near 190 nm.^{4f} The relative percent helicity of peptides can be estimated by the mean residue ellipticity, θ , at 222 nm, although these estimates are often not accurate for short helices.^{4b, 15} Trifluoroethanol (TFE) is known to induce helical structure in a concentration dependent manner.¹⁶ The CD ellipticity for peptides typically plateaus near 30–40% TFE in aqueous buffers, and the value attained at these high TFE concentrations may be used to benchmark “100%” helicity for a given peptide.¹⁷ This individual benchmarking is necessary because short peptides typically do not obey the %helicity equations developed for long peptides and proteins.^{4f, 15a}

Figure 3a compares the relative helicity of the unconstrained peptide (Ac-**1**: AKGAAAAAKAY-NH₂) and the five HBS constructs used to stabilize polyalanine sequence **1**. The mean residue ellipticity value at 222 nm for each peptide in phosphate buffered saline (PBS) is normalized to that of the unconstrained peptide in 30% TFE in PBS as the “maximal helicity” for the unconstrained peptide. The raw CD plots are shown in the ESI, Figures S9–S10. The results suggest that both carbonyl alkene surrogates (*E*^O-HBS and *Z*^O-HBS) stabilize the peptide conformation to similar extent, with the *Z*-alkene derivative slightly more helical. Importantly, both analogs provide roughly six-fold enhancement in helicity as compared to the unconstrained peptide in aqueous solution. The constrained peptides in pure aqueous buffers also display higher helical stability than the unconstrained peptide in buffer with a high TFE concentration.

α -Dimethylation of carbonyl alkene isosteres *E*^O-HBS and *Z*^O-HBS to obtain *E*^O-HBS^{DM} and *Z*^O-HBS^{DM} leads to divergent results. The dimethylated linker with an *E*-alkene in place of the C–O bond has higher conformational stability, whereas the analog with the *Z*-alkene substitution is largely unstructured. Similarly, *Z*^N-HBS^{DM} peptide that contains a *Z*-alkene in place of the C–N bond and an α -dimethyl substitution is non-helical (Figures 4 and S9). We obtained similar trends with two biological sequences derived from HIF-1 α and p53 activation domain (ESI, Figures 3b, S11 and S13). The RCM derived *E*^O-HBS helix derivatives of both of these sequences have been previously described from our group.^{2h, 18} In each case, α -dimethylation of RCM-derived *E*-alkene boosts helicity. Temperature denaturation scans performed on the p53 derivatives are also consistent with previous results showing a broad melting transition indicative of a helix that can only denature in one direction.^{4a, 11} The overall results from the three sequences are summarized in Figure 4.

We determined if the binding affinity of different p53 analogs for the biological receptor MDM2 correlated with their conformational stability. We utilized a previously described competition fluorescence polarization assay with fluorescein-labeled derivative of p53 for these studies.¹⁸ Competition experiments revealed that α -dimethylation of the linker

improves affinity of p53- E^O -HBS^{DM} ($K_D = 63 \pm 30$ nM) by three-folds over the previously reported E^O -HBS analog ($K_D = 216 \pm 57$ nM) (Fig. 5).¹⁸ A somewhat surprising result is the slight decrease in binding affinity for Z^O -HBS ($K_D = 330 \pm 72$ nM) relative to the E-analog in spite of the slight increase in helicity.

We utilized computational modeling with MacroModel to probe the effect of dimethyl group substitution on the C1–C2 and C2–C3 dihedrals as compared to the ϕ and ψ angles in an amino acid residue (Figure 4).¹⁹ A full discussion of the conformational analysis studies is included in the ESI. The analysis suggests that while E and Z alkene torsion angles differ around the four atoms in the surrogate, the rest of the macrocycle adjusts to accommodate the fluctuations (ESI, Table S2, Figures S16–17). However, placement of the dimethyl group strains the macrocycle through A-1,3 strain involving the dimethyl groups and the alkene or the amide groups in Z^N -HBS^{DM} and Z^O -HBS^{DM}, respectively. Overall, we find that placement of the α -dimethyl group in E^O -HBS boosts helicity by 10–20% over the hydrocarbon HBS motif we have extensively utilized. Importantly, this motif is easily accessible via an RCM reaction with the commercially available pentenoic acid analog (2,2-dimethyl-4-pentenoic acid).

In summary, we have comprehensively analyzed various hydrocarbon linkers for stabilization of helical peptides using the hydrogen bond surrogate. These studies, along with those described earlier,^{4a, 11} indicate that small changes in the HBS macrocycle can substantially alter its nucleation potential. The present studies reveal an optimized constraint for sequence-independent stabilization of peptide helices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the U.S. National Institutes of Health (R01GM073943) for financial support of this work. S.T.J. is grateful for the Margaret and Herman Sokol Predoctoral Fellowship and the Margaret Strauss Kramer Graduate Fellowship from the NYU Chemistry Department.

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Fig. 1.

(a) Hydrogen bond surrogate (HBS) helices feature a covalent bond in place of an intramolecular hydrogen bond. HBS helices with an *E*-alkene surrogate can be accessed from a bis-olefin peptide through the ring closing metathesis (RCM) reaction. (b) Relative geometries of alkene isostere as compared to idealized hydrogen bonds in peptide helices. (c) Various alkene isosteres are designed to study their effect on helix nucleation. The alternative alkene isosteres were accessed via lactam ring closure.

Fig. 2.

Design of HBS macrocycles with amide bond isosteres. The C–O or the C–N bonds of an amide may be substituted with an alkene.

Fig. 3.

(a) The bar graph compares the relative helicity (θ_{222}) of the unconstrained peptide, Ac-AKG*AAAAAKAY-NH₂, to those of five HBS derivatives stabilizing the alanine-rich sequence. The mean residue ellipticity (θ) value at 222 nm for each peptide in phosphate buffered saline (PBS) is normalized to that of the unconstrained peptide in 30% TFE in PBS as the “maximal helicity” for the polyalanine sequence. Similar results were obtained for biological sequences derived from p53 and HIF-1 α (ESI). (b) Circular dichroism spectra and temperature scans (inset) for the p53-derived E^O -HBS and E^O -HBS^{DM} in 0.1X PBS, pH = 7.6 with 10% TFE. Thermal denaturation of the p53 analogs was assessed by monitoring changes in ellipticity at 222 nm.

Fig. 4.

Summary of the results from CD studies on three different sequences.

Fig. 5. Determination of binding affinity of p53-derived sequences to His6-tagged MDM2 by a competition fluorescence polarization assay. The effect of alkene isosteres on binding of p53 HBS sequences parallels the relative helical stability endowed by each constraint.

Scheme 1.

Synthesis of HBS helices via lactam ring closure. $R_0 = \text{H or Me}$