α-Helix Mimetics

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A Single α-Helical Turn Stabilized by Replacement of an Internal Hydrogen Bond with a Covalent Ethylene Bridge**

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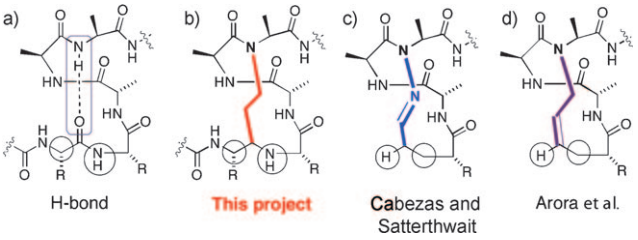
Well-defined peptide secondary structure is made possible by specific interactions and constraints, including hydrogen bonding (H-bonding), disulfide-bond bridges, lactam bridges, and ionic bonds. Peptidomimetics based on secondary structure defined by H-bonding include the γ turn,[1] β turn,[2] antiparallel β sheet,[3] α helix, and π helix.[4] The α helix is thought to account for approximately 30% of ordered protein structure and is implicated in binding interactions with a variety of biologically significant receptors.[5]

A minimum of 10–12 amino acids and the associated additive effect of the $(i,i+4)$ H-bonding network are generally required for a well-defined helical structure.[6] The construction of molecules aimed at reproducing the topology of an α helix in a more druglike (smaller, less peptidic) form (helix mimetics) has been extensively investigated.[7] Helix stabilization through side-chain constraints has been successfully achieved by approaches that employ two side-chain residues (for example, with lactams,[8,9] ring-closing metathesis (RCM),[10,11] disulfides). Other methods to enhance helicity include metal chelation,[12] unnatural amino acids,[13] peptoids,[14] and nucleating caps.[15] Nonpeptidic approaches include the use of terphenyl[16] and related heterocyclic scaffolds[17] that mimic the spatial side-chain arrangement.

Another strategy to stabilize an α helix is replacement of the weak $(i,i+4)$ H-bond with a covalent linkage. This concept was proposed in 1982 by Cabezas and Satterthwait, who developed a hydrazone linkage as an N-terminal H-bond replacement (Scheme 1).[18] More recently, Arora and coworkers have used an alkyl linkage formed by RCM,[19,20] but this approach also appears limited to the N-terminal position of a peptide. To date, no covalent H-bond replacement has been introduced at an internal helical turn. We report herein the first such covalent H-bond mimetic, which has significant helical character.

A H-bond mimetic is an alluring strategy for helix stabilization because it maintains side-chain functionality, has a small molecular weight, and is sequence independent. We set out to synthesize small, truncated peptides that have a well-defined helical structure by replacement of an $(i,i+4)$ H-bond with an ethylene bridge to give a 13-membered ring (Scheme 1b). The intent of the carbon bridge is to preorganize the peptide, which would otherwise have no well-defined secondary structure, into a helical conformation.[21] The presence of the ethylene bridge will result in a C(i) to N(i+4) distance of 3.8 Å, which is slightly shorter than the corresponding H-bond length (4.0 Å). Introduction of the ethylene bridge also changes the C(i) carbonyl group from sp^2 to sp^3 hybridized and the following peptide bond from an amide into a more flexible secondary amine linkage.



Scheme 1. H-bond mimetics. a) Peptide $(i,i+4)$ H-bond; b) internal ethylene bridge; c) N-terminal hydrazone;[18] d) N-terminal RCM.[19]

Galanin (Gal) was selected as the model for the H-bond replacement mimetics because Gal ligands have a range of possible roles in cancer, obesity, arthritis, and diabetes.[22] The Gal(1–16) N-terminal fragment (**10**) retains high biological activity compared to that of the full-length 29 amino acid Gal, and also maintains the partial helical structure that has been implicated in its biological activity.[23,24] The synthesis of helical Gal mimetics through a side-chain lactam approach has previously been attempted.[8] Our strategy was to construct a cyclic pentapeptide mimetic containing the H-bond replacement as a preorganized helical turn prior to its incorporation into Gal(1–16) by using solid-phase peptide synthesis (SPPS).

The position of the ethylene bridge in Gal(1–16) was chosen as G8–G12, which gave an appealing chemical access to the required GYLLG cyclic pentapeptide. The synthesis began with N-protected glycine **1**, which was converted into α,β-unsaturated ketone **2** (Scheme 2). This was followed by Michael addition of glycine *tert*-butyl ester to give **3**. Boc-leucine was coupled to amine **3** to yield secondary amide **4**, which afforded **5** after protecting-group manipulation and coupling to Alloc-leucine. Direct conversion of **3** into **5** through dipeptide segment coupling was attempted; however, racemization readily occurred. Reductive amination of **5** with a tyrosine derivative by using sodium triacetoxyborohydride afforded **6a** and **6b** as an inseparable mixture of diastereomers. Simultaneous deprotection of the Allyl/Alloc protecting groups, BOP-mediated cyclization, and separation of the

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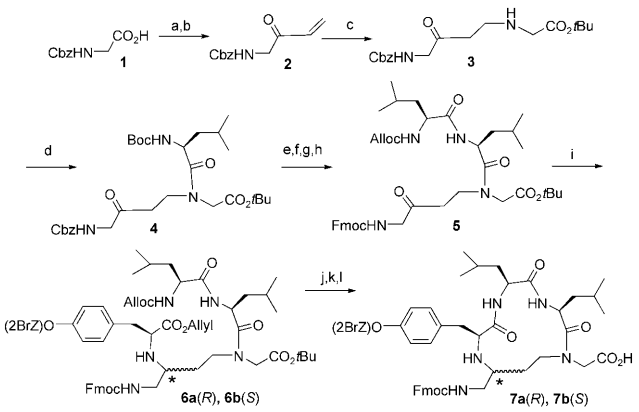


Scheme 2. Synthesis of cyclic pentapeptides. a) $CH_3ONHCH_3{\cdot}HCl$, HBTU, DIEA, DMF, 59%; b) $CH_2{=}CHMgBr$, THF, 0°C, 97%; c) Gly-O*t*Bu, DIEA, CH_2Cl_2; d) Boc-Leu, HATU, DIEA, DMF, 48% (2 steps); e) Pd/C, H_2, 0.1 M aq. HCl, EtOH, 95%; f) Fmoc-OSu, DIEA, dioxane, H_2O, 73%; g) 4 M HCl/dioxane, 0°C, 99%; h) Alloc-Leu, EDC, HOBt, DIEA, CH_2Cl_2, 87%; i) Tyr(2BrZ)-OAllyl, $(CH_3COO)_3BHNa$, $CH_3CH_2Cl_2$, 59% (isomer mix: *R*/*S* 1.7:1); j) barbituric acid, $[Pd\{P(C_6H_5)_3\}_4]$, CH_2Cl_2, vacuum, 98%; k) BOP, DIEA, DMF, syringe pump, 30%; l) TFA, CH_2Cl_2, 70–90%. HBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIEA = *N*,*N*-diisopropylethylamine; DMF = *N*,*N*-dimethylformamide; THF = tetrahydrofuran; Boc = *tert*-butoxycarbonyl; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazole[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate; Fmoc = 9-fluorenylmethoxycarbonyl; Su = succinimidyl; Alloc = allyloxycarbonyl; EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide; HOBt = 1-hydroxy-1*H*-benzotriazole; Z = benzyloxycarbonyl; BOP = 1-benzotriazolyloxytris(dimethylamino)phosphonium; TFA = trifluoroacetic acid.

diastereomers, followed by treatment with TFA gave SPPS building blocks **7a** and **7b**. The *R*/*S* stereochemistry at the reductive amination center was assigned by using ROESY 2D NMR correlations.

The SPPS building blocks **7a** and **7b** were deprotected and the *C*-terminus was methylated to give **8a** and **8b**, which were studied by using CD spectroscopy. The CD spectra of both **8a** and **8b** show double minima and positive value at 195 nm, whereas the linear analogue Ac-GYLLG-Me (**9**) showed random-coil behavior (Figure 1). Analysis of peptide

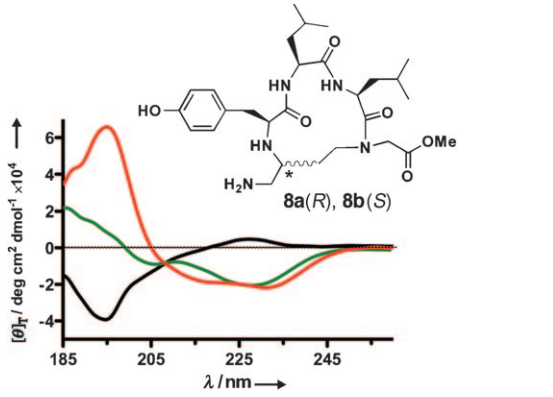


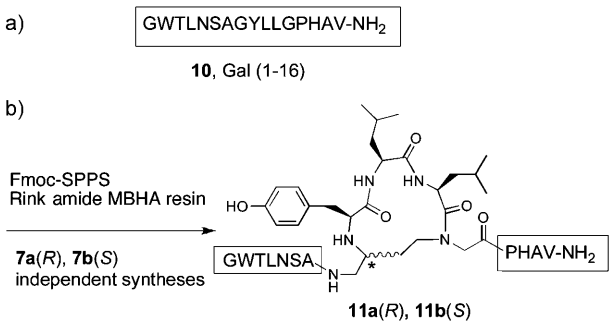
Figure 1. CD spectra of **8a** (*R* isomer; green), **8b** (*S* isomer; red), and linear analogue Ac-GYLLG-Me (**9**; black) in 10 mM sodium phosphate buffer (pH 7.4).

secondary structure by CD spectroscopy assumes that the backbone amide transitions dominate the far-UV spectrum. However, it must be noted that aromatic groups can also make a small contribution to far-UV electron transitions, although a more dominant contribution occurs in the near-UV (250–300 nm) region (see the Supporting Information, Figure S2).

In particular, the CD spectrum of **8b** shows a negative value at 208 nm and a large positive value at 195 nm, which is typical for an α-helical conformation.[25] We were encouraged by this result, which indicated that the *S* diastereomer of the ethylene-bridge H-bond mimic displayed α-helical properties.

The NMR spectra of **8a** and **8b** were examined for evidence of a helical structure. Traditional indicators of peptide helicity include certain $d_{\alpha N}(i,i+3)$ and $d_{\alpha N}(i,i+4)$ NOE correlations, $^3J_{NHC\alpha}$ values, NH temperature dependence, and H/D exchange experiment results. However, these are not applicable to **8a** and **8b** because of the short peptide sequence and absence of possible $(i,i+3)$ or $(i,i+4)$ hydrogen bonds. Instead of the $\delta(H\alpha)$ value found in a random coil,[26] an upfield $\delta(H\alpha)$ shift is considered characteristic of a helical structure. The $\delta(H\alpha)$ value for each leucine residue in both **8a** and **8b** was shifted upfield, most prominently by $\delta = -0.8$ ppm for Leu3 of **8b**; however, possible ring current effects from the tyrosine aromatic ring may also contribute to this upfield shift (see the Supporting Information for further discussion).

The building blocks **7a** and **7b** were then independently incorporated into the Gal(1–16) sequence by using standard Fmoc-SPPS to give **11a** and **11b** (Scheme 3). The structures of



Scheme 3. a) Gal(1–16) (**10**); b) synthesis of peptides **11a** and **11b** containing the ethylene-bridged H-bond mimetic.

peptides **11a** and **11b** were investigated by using CD spectroscopy in 10 mM phosphate buffer (pH 7.4) and with addition of 30% trifluoroethanol (TFE)[27] (Figure 2), with comparison to the results for linear analogue **10.**

The CD spectra of **11a** and **11b** show a more helical structure than linear **10** in buffer and with TFE. Comparison of the diastereomers reveals subtle differences, with the *S* isomer **11b** displaying more typical α-helical behavior (negative at 205 and 222 nm) with a larger positive 190 nm transition, whereas the *R* isomer **11a** reveals a structure that is barely altered by the addition of TFE. The CD spectra in buffer/sodium dodecylsulfate (SDS) solutions[28] were comparable to those of the 30% TFE solutions.

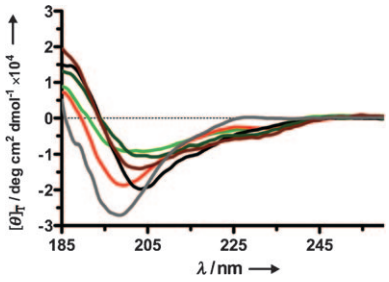


Figure 2. CD spectra of linear Gal(1–16) (**10**) in buffer (gray) and with 30% TFE added (black); *R* isomer **11a** in buffer (light green) and with 30% TFE added (dark green); *S* isomer **11b** in buffer (red) and with 30% TFE added (dark red).

Two-dimensional ^{1}H NMR spectroscopy in H_2O/D_2O (90:10) was conducted for **11a** and **11b**. The NOESY spectrum of **11a** revealed short-range $d_{\alpha N}(i,i+1)$ and $d_{\beta N}(i,i+1)$ NOEs along the peptide sequence, but non-overlapping medium- or long-range NOEs (for example, $d_{\alpha N}(i,i+3)$ or $d_{\alpha N}(i,i+4)$) were not observed. Two sequential $d_{NN}(i,i+1)$ NOEs were observed between Ser6, Ala7, and Gly8, a result indicating that the peptide region immediately prior to the ethylene bridge may have a helical secondary structure. Analysis of the two-dimensional ^{1}H NMR spectrum of **11b** proved troublesome because of overlapping spin systems.

The stability of Gal(1–16) (**10**) and **11b** was investigated by thermal denaturation experiments (Figure 3). The variable-temperature CD spectra of Gal(1–16) (**10**) show decreased peptide secondary structure with increased temperature (minimum deepens and moves to the left). Remarkably different are the variable-temperature CD spectra of the H-bond mimetic *S* isomer, **11b**, which show little difference within the 5–75 °C range.

The biological activity of **10**, **11a**, and **11b** was tested by using a competitive radioligand binding assay against full-length ^{125}I-labeled Gal (porcine) at the GAL1 and GAL2 receptors. Both **11a** and **11b** showed potency but were more than 250-fold less active than Gal(1–16) (**10**). This may indicate that ligand flexibility is required to enable correct ligand–receptor interactions. More positively, both **11a** and **11b** were more stable than Gal (1–16) (**10**) in rat plasma (refer to the Supporting Information, Table 2).

The position of the ethylene-bridge mimetic in **11a**/**11b** (G8–G12) was selected for ease of chemical access to the required cyclic pentapeptide, but it results in a proline being C terminal to the mimetic. We are currently evaluating H-bond replacements in other model peptides, in which helix propagation C terminal to the mimetic can be better evaluated (see the Supporting Information) and the NMR solution structure can be determined.

In summary, we have demonstrated that the *S* diastereomer of cyclic pentapeptide **8b** shows potential as a single-turn helical mimetic. Incorporation of the ethylene-bridged H-bond mimetic scaffolds **7a** and **7b** into Gal(1–16) to give **11a** and **11b** resulted in increased helicity and increased stability towards thermal denaturation and proteolytic degradation but incurred a decrease in biological activity compared to that of unmodified **10**. This work represents the first example of a covalent H-bond replacement contained internally within a peptide sequence, with potential for wide use in understanding the molecular basis of ligand–receptor interactions.

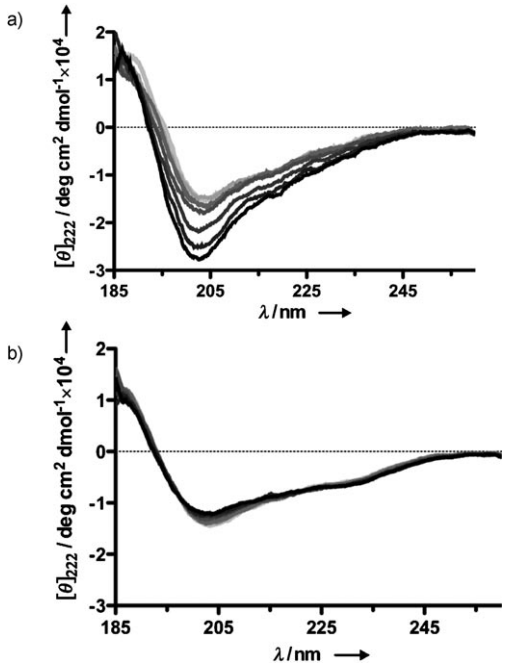


Figure 3. Variable-temperature CD spectra of a) **10** and b) **11b** in 10 mM sodium phosphate buffer (pH 7.4) with 30% TFE added. Temperatures from 75 °C (black) to 5 °C (light gray) are represented by increasing color lightness.

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