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Use of Disulfide "Staples" to Stabilize β-Sheet Quaternary Structure

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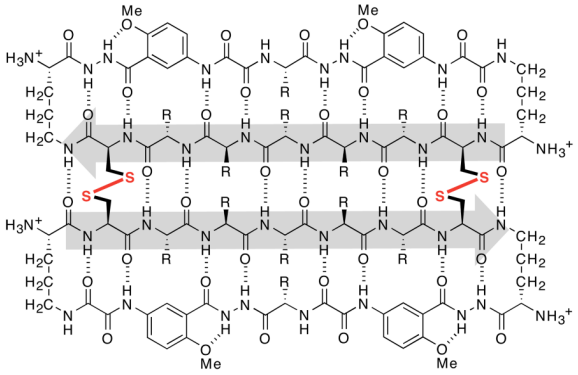
Abstract



This letter reports the use of disulfide linkages to stabilize a β-sheet dimer with a well-defined structure in aqueous and dimethyl sulfoxide solutions. In this dimer, two cyclic β-sheet peptides are connected by disulfide linkages at the non-hydrogen-bonded rings. The cyclic β-sheet "domains" interact through hydrogen bonding to form a four-stranded β-sheet structure. This interaction results in enhanced folding of the cyclic β-sheet peptides.

Disulfide bonds are important in stabilizing protein quaternary structures.[1] Our laboratory has previously developed chemical model systems that use hydrogen bonding and hydrophobic interactions to mimic β-sheet quaternary structures.[2] Here, we report the use of disulfide linkages to stabilize a β-sheet dimer with a well-defined structure in competitive solvents.

Disulfide bonds in antiparallel β-sheets generally occur at the non-hydrogen-bonded amino acid pairs, rather than the hydrogen-bonded amino acid pairs. This pairing preference has been demonstrated both statistically in proteins[3] and empirically in a β-hairpin model system.[4] A recent study of a tripeptide cystine dimer has demonstrated that disulfide linkages can induce β-sheet hydrogen-bonding interactions in noncompetitive organic solvents when the cysteine residues form non-hydrogen-bonded pairs of antiparallel β-sheets.[5]

We previously introduced cyclic β-sheet peptide **1a** (Figure 1) that self-associates in water through edge-to-edge and face-to-face intermolecular β-sheet interactions to form a tetrameric β-sheet sandwich.[6] Cyclic β-sheet peptide **1a** consists of a heptapeptide β-strand, a β-strand mimic comprising two unnatural amino acid Hao units,[7] and two δ-linked ornithine ($^{\delta}$Orn) turn units[8] connecting the two strands. Significantly, the antiparallel β-sheet dimer of cyclic peptide

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Supporting Information Available Experimental procedures and spectroscopic and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

1a is only stable within the tetramer (dimer of dimers) and is not observed as a discrete species. Studies of analogues of this peptide showed that the formation of this tetrameric β-sandwich requires hydrophobic interactions.

To test whether disulfide linkages can stabilize a hydrogen-bonded β-sheet dimer in competitive solvents, we have now introduced cysteine residues at positions R_1 and R_7 of peptide **1a**, thus creating peptide **1b**. Upon oxidation, peptide **1b** forms β-sheet dimer **2**, with two disulfide linkages (Cys1—Cys7) at these non-hydrogen-bonded positions (Figure 2). These disulfide linkages not only covalently connect the monomeric "domains" in an antiparallel fashion but also substantially stabilize β-sheet folding.

Dimer **2** was synthesized efficiently by oxidation of peptide **1b** with a 20% aqueous DMSO solution.[9] Oxidation of a 2.5 mM solution of peptide **1b** was completed in 24 h and provided dimer **2** as the major product (35–75% isolated yield after HPLC purification). Figure 3 illustrates the progress of the oxidation reaction through analytical RP-HPLC traces of the crude oxidation mixture after 0.25, 1, 6, and 12 h. Although the oxidation could have resulted in two different isomeric bisdisulfides, one parallel and one antiparallel, only the antiparallel isomer was observed upon completion of the reaction.[10,11]

^{1}H NMR studies of dimer **2** were performed in both DMSO-d6 and D_2O solutions. The NMR resonances of **2** are sharp and dispersed in DMSO-d6 at room temperature. The NMR resonances of **2** in D_2O are broad at room temperature (298 K) and submillimolar concentrations but sharpen at higher temperature (318 K). This broadening may arise from conformational exchange between disulfide bond rotamers occurring at an intermediate rate on the NMR time scale. The NMR resonances of dimer **2** in D_2O also sharpen with increasing concentration. This sharpening suggests that covalent dimer **2** forms non-covalent oligomers at millimolar concentrations and that these oligomers likely stabilize a particular conformer.[12]

Downfield shifting of the α-proton resonances of dimer **2** suggests that the dimer strongly folds into a β-sheet structure. Figure 4 illustrates the average deviation of the α-proton chemical shifts from published random coil values[13] ($\Delta\delta H_\alpha$) of the residues at positions R_2 to R_6 for covalent dimer **2**, the monomer of cyclic peptide **1a**, and the tetramer of cyclic peptide **1a** in D_2O and DMSO-d6 solutions. The average $\Delta\delta H_\alpha$ values for dimer **2** in both solvents (0.85 and 0.98 ppm, respectively) are significantly larger than those for the monomer of cyclic peptide **1a** (0.01 and −0.05 ppm, respectively) and are comparable to that of the tetramer of cyclic peptide **1a** in D_2O (0.70 ppm).

We previously showed that the β-sheet folding is only partial in the monomer of cyclic peptide **1a** but strongly increases upon formation of the tetramer.[6] The large average $\Delta\delta H_\alpha$ value for the tetramer arises form its strong β-sheet folding. The large average $\Delta\delta H_\alpha$ values for dimer **2** in DMSO and water also indicate strong β-sheet folding of the dimer in these solvents.

The difference in the chemical shifts of the diastereotopic δ-protons of a $^{\delta}$Orn turn unit ($\Delta\delta^{\delta}$Orn) reflects the degree of folding of the turn unit, and hence the peptide.[8] We have previously observed an average $\Delta\delta^{\delta}$Orn value of 0.15 ppm for the partially folded monomer of cyclic peptide **1a** and an average Δ^{δ}Orn value of 0.69 ppm for the highly folded tetramer of cyclic peptide **1a** in D_2O.[6] Dimer **2** exhibits large average $\Delta\delta^{\delta}$Orn values of 0.68 and 0.60 ppm respectively in DMSO-d6 and in D_2O. As in the noncovalent tetramer of cyclic peptide **1a**, the large average Δ^{δ}Orn values for dimer **2** indicate predominant or complete folding.

800 MHz NOESY studies of dimer **2** in both D_2O and DMSO-d6 reveal a network of NOEs associated with β-sheet folding and antiparallel edge-to-edge interactions. Figure 5 illustrates the key NOEs involving the backbone CH_α and Hao_{H6} protons in these solvents. Figure 6

illustrates these NOE cross-peaks that are observed in DMSO-d6. The intradomain NOEs between the α-protons of Thr4 and Lys8 ($T4_{\alpha}/K8_{\alpha}$), between the α-proton of Ser2 and H6 of Hao2 ($S2_{\alpha}/Hao2_{H6}$), and between the α-proton of Thr6 and H6 of Hao1 ($T6_{\alpha}/Hao1_{H6}$) indicate β-sheet folding within each domain. The interdomain NOEs between the α-protons of Cys1 and Cys7 ($C1_{\alpha}/C7_{\alpha}$) and between the α-protons of Phe3 and Tyr5 ($F3_{\alpha}/Y5_{\alpha}$) indicate edge-to-edge dimerization. Collectively, these NOEs establish that dimer **2** folds into a β-sheet structure in which the domains participate in antiparallel edge-to-edge β-sheet interactions.

The large downfield shifting of the α-proton resonances, the large difference in the chemical shifts of the diastereotopic δ-protons of the $^{\delta}$Orn turn units, and the NOEs arising from *intra*domain and *inter*domain contacts collectively establish that dimer **2** is strongly folded into β-sheet structure in both water and DMSO.

To evaluate whether the cysteine residues had to be at the non-hydrogen-bonded positions of the β-sheet, we prepared and studied cyclic peptide **1c**, which is a sequence isomer of peptide **1b** with cysteine at positions R_2 and R_6, rather than R_1 and R_7. We attempted to form the dimer of peptide **1c** by oxidation and found dimer formation to be strikingly challenging. Oxidation with aqueous DMSO solution provided a bicyclic peptide with an *intra*molecular Cys2—Cy6 disulfide linkage as the major product. Only prolonged oxidation of a concentrated aqueous solution of the cyclic peptide with air afforded a small quantity of dimeric product. The dimer of **1c** proved difficult to characterize by NMR spectroscopy, because it showed broad resonances and multiple sets of peaks. The positions of the α-proton resonances and small $\Delta\delta^{\delta}$Orn values of this compound suggest that this dimer is poorly folded. The difficult synthesis, complex NMR spectra, and poor folding suggest that disulfide linkages at hydrogen-bonded positions do not induce and stabilize β-sheet structure.[3,4]

In conclusion, the Cys—Cys disulfide linkages at non-hydrogen-bonded positions stabilize β-sheet dimers through antiparallel edge-to-edge β-sheet interactions. Covalent dimer **2** in which 54-membered-ring cyclic peptides (domains) are linked by two Cys1—Cys7 disulfide linkages exhibits strong *intra*domain β-sheet folding and *inter*domain antiparallel edge-to-edge β-sheet interactions in water and in DMSO. Such disulfide linkages serve as "staples"[16] and constitute a new tool for stabilizing β-sheet quaternary structure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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10. A minor monomeric oxidation side product was detected by mass spectrometric analysis of the crude oxidation mixture but was not isolated or further characterized. This product likely contains an *intra*molecular Cys1—Cys7 disulfide linkage.
11. Hydrogen-bonding complementarity between domains may explain the exclusive formation of the antiparallel isomer; a parallel isomer could not form a full complement of interdomain hydrogen bonds while maintaining intradomain hydrogen bonds.
12. NMR studies of cyclic peptide **1b** (the domain building block) were not performed, because the peptide oxidizes rapidly in aqueous solutions.
13. Wishart DS, Sykes BD. Methods Enzymol 1994;239:363–392. [PubMed: 7830591]
14. The interdomain $F3_{\alpha}/Y5_{\alpha}$ NOE could not be established with certainty in D_2O, because the $F3_{\alpha}$ and $Y5_{\alpha}$ resonances are close in this solvent.
15. The sample was lyophilized with D_2O twice before NMR studies to attenuate resonances associated with water and exchangeable protons.
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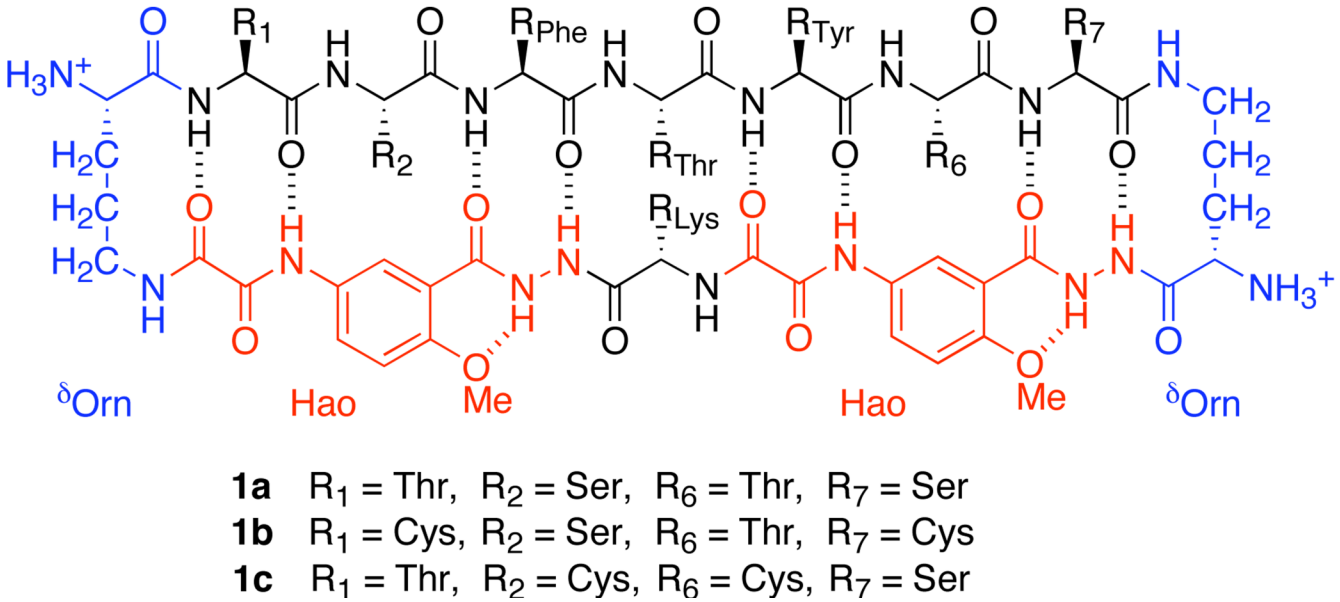

1a R_1 = Thr, R_2 = Ser, R_6 = Thr, R_7 = Ser
1b R_1 = Cys, R_2 = Ser, R_6 = Thr, R_7 = Cys
1c R_1 = Thr, R_2 = Cys, R_6 = Cys, R_7 = Ser

Figure 1.
Cyclic β-sheet peptides **1a–1c**.

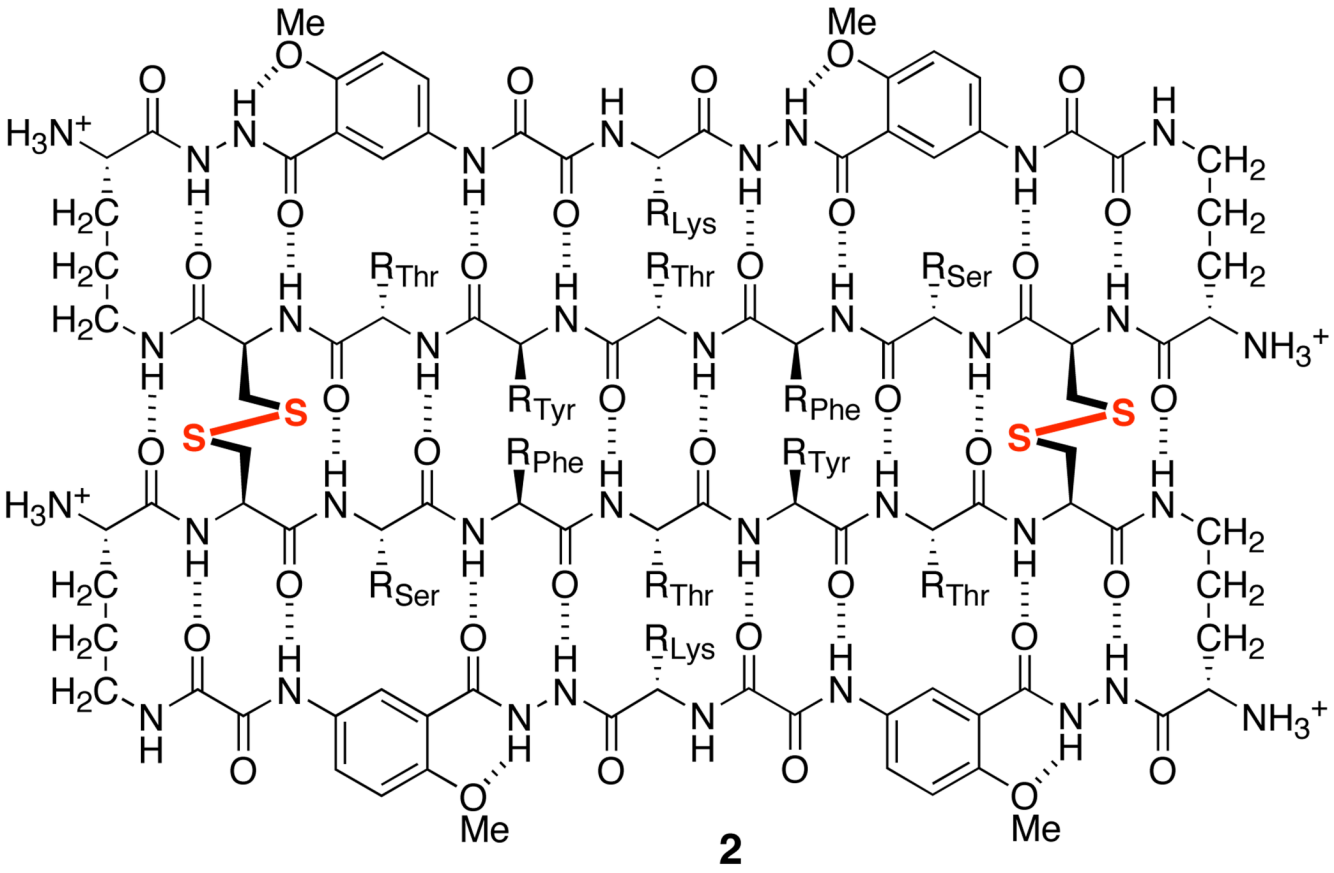

Figure 2.
β-Sheet dimer **2**.

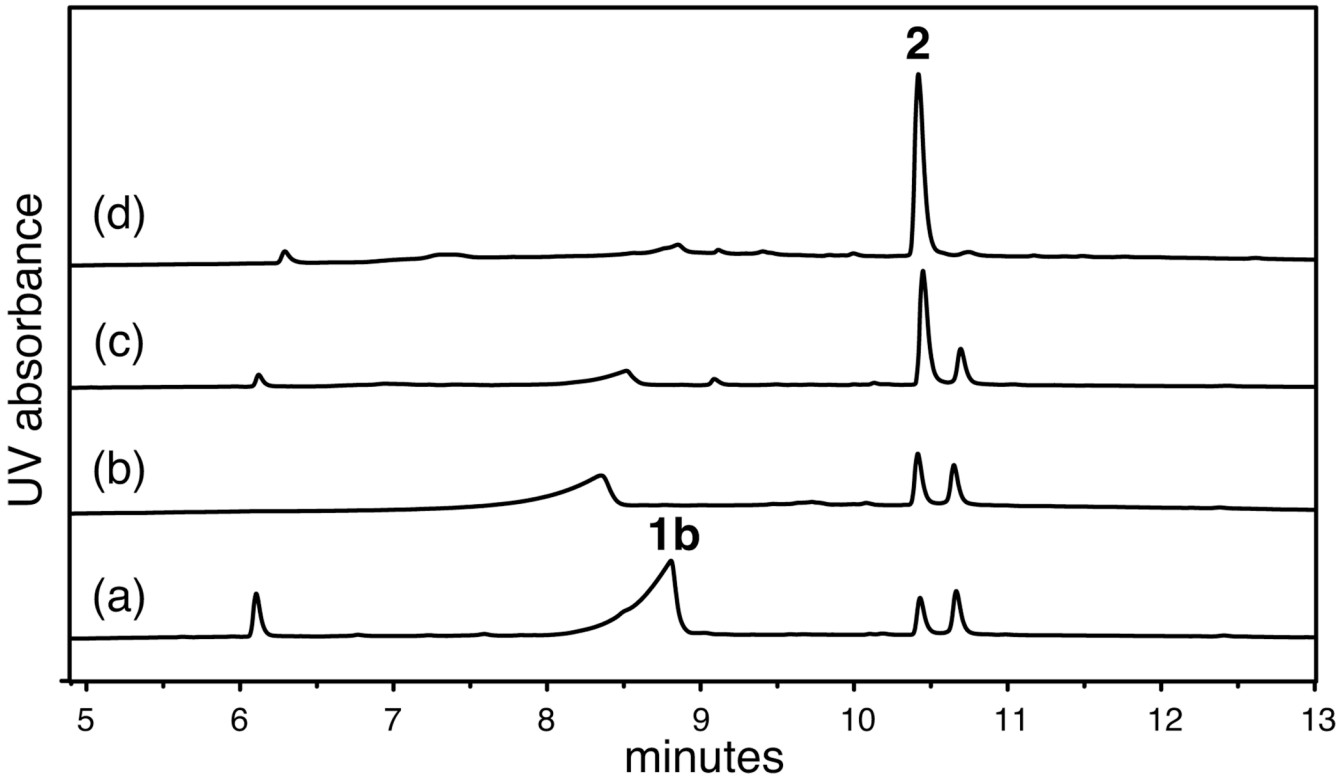

Figure 3.
Analytical RP-HPLC traces of the oxidation reaction of peptide **1b** by a 20% aqueous DMSO solution after: (a) 0.25 h, (b) 1 h, (c) 6 h, and (d) 12 h.

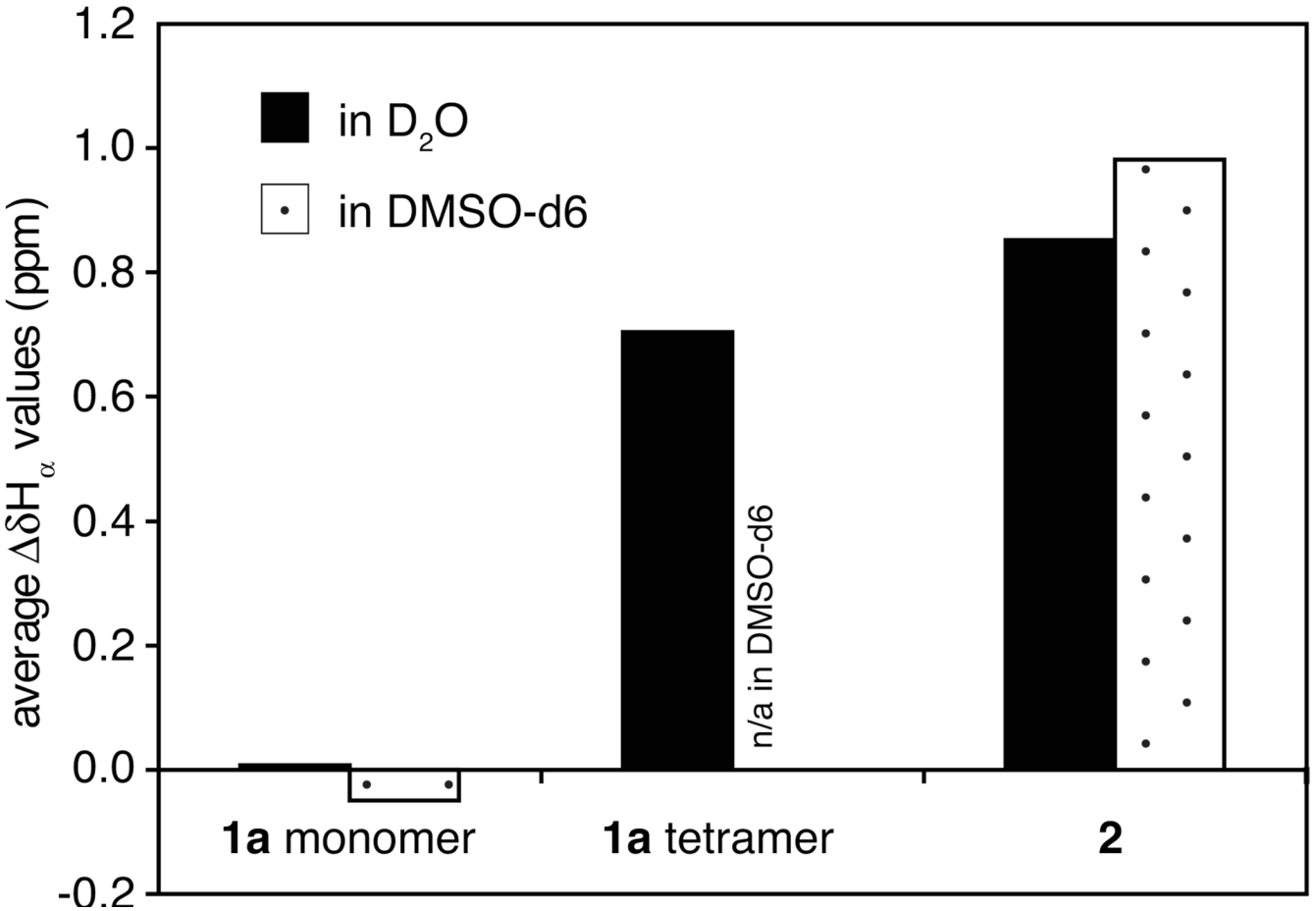

Figure 4.

Average $\Delta\delta H_\alpha$ values of the residues at positions R_2 to R_6 for the monomer and tetramer of peptide **1a** and for dimer **2**. The NMR data were obtained from 0.5 and 8.5 mM D_2O solutions of peptide **1a** at 280 K, a 0.7 mM DMSO-d6 solution of peptide **1a** at 298 K, a 0.2 mM D_2O solution of dimer **2** at 318 K, and a 0.7 mM DMSO-d6 solution of dimer **2** at 298 K.

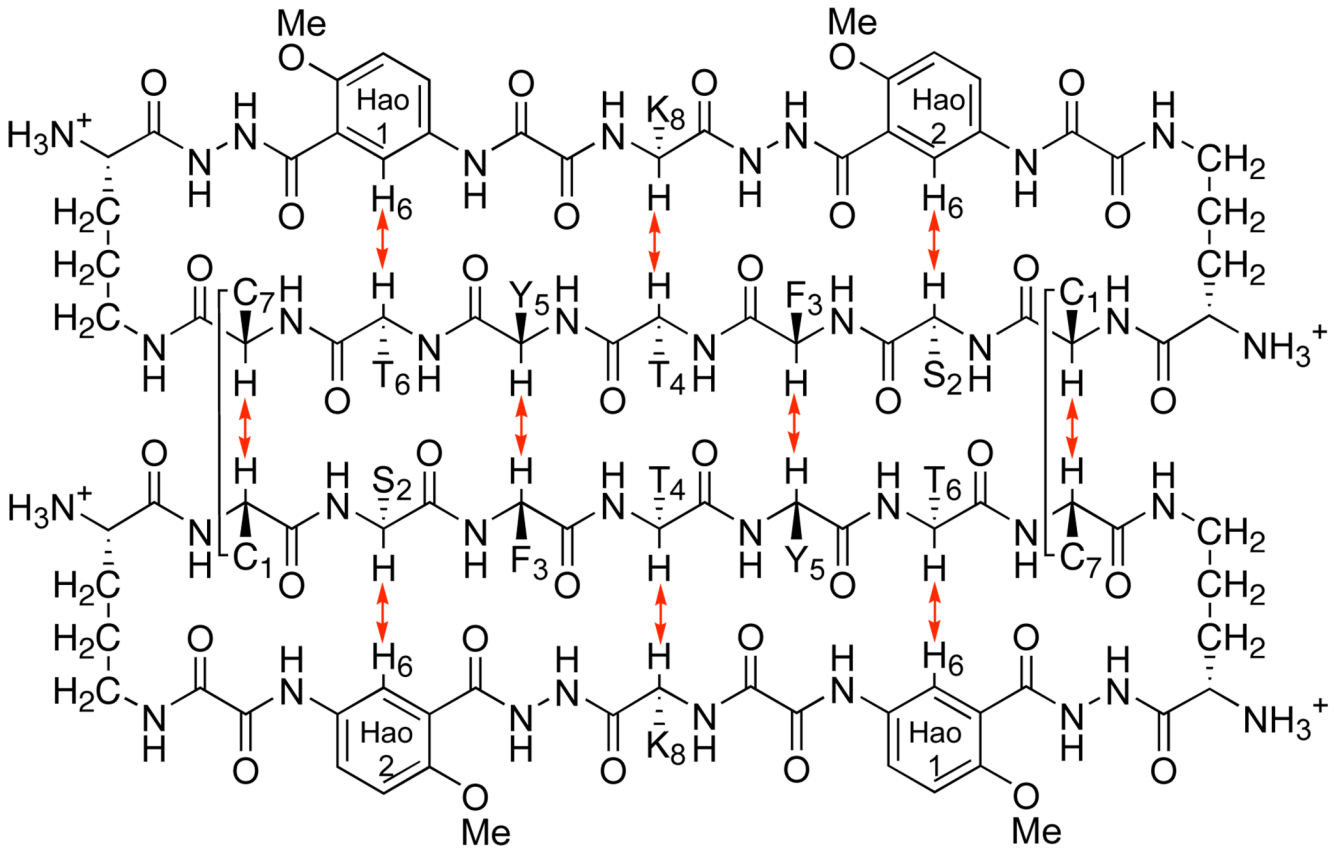

Figure 5.
Key NOEs associated with the *intra*domain and *inter*domain main-chain contacts of dimer **2** observed in D_2O and in DMSO-d6.[14]

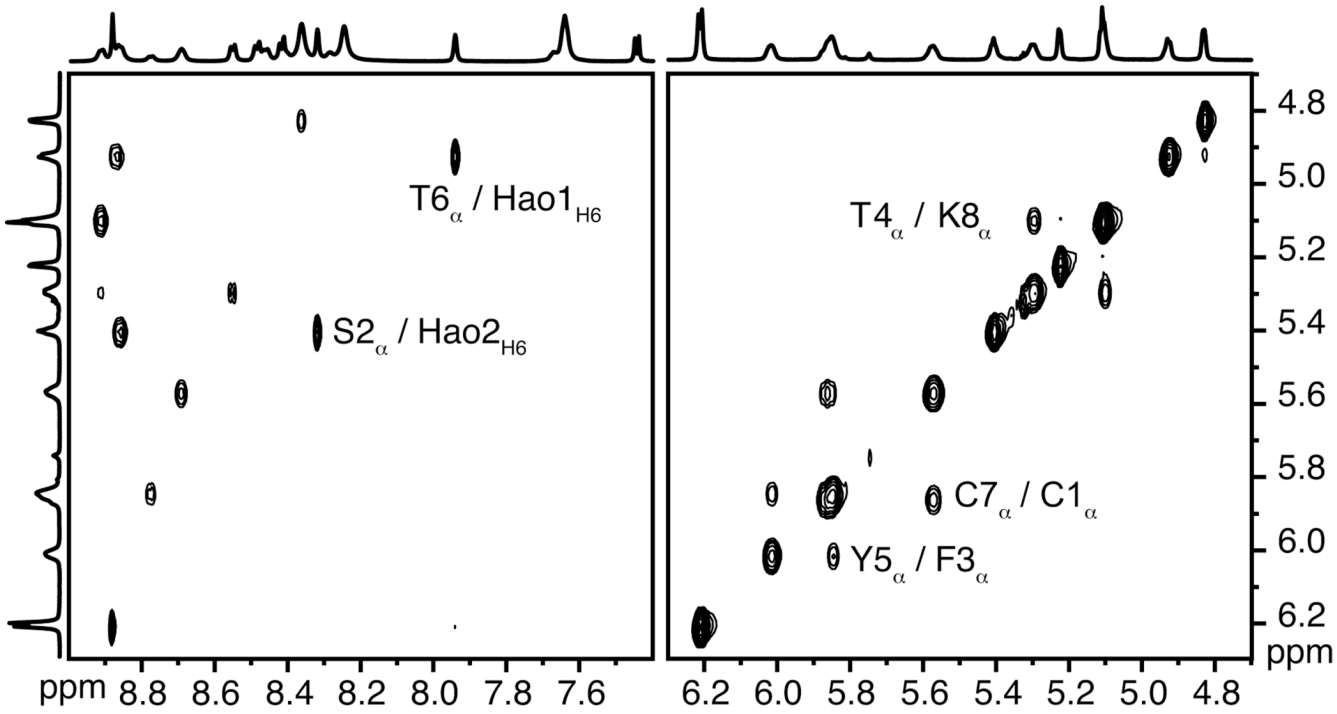

Figure 6.
Selected expansions of the 800 MHz NOESY spectrum of 0.7 mM dimer **2** in DMSO-d6 at 298 K.[15]