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Proline Conformations

Conformational Properties of 4-Mercaptoproline and Related Derivatives**

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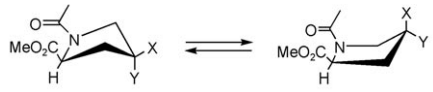
Proline residues have long been recognized to play a unique and important role in the structural properties of peptides and proteins. The *cis*/*trans* isomerization of the aminoacyl–proline bonds is critically involved in folding and stabilizing protein structures.[1] This general notion has inspired an intensive search for proline analogues that influence the equilibrium conformational populations of the *cis*/*trans* prolyl bonds and of the pyrrolidine ring-pucker isomers (*endo*/*exo* of C4) in order to possibly restrict at will the degree of conformational freedom of polypeptide chains and thus to modulate the thermodynamic stability of peptide and protein structures.[2] Of the various modified prolines found in nature, the most common are (2*S*,4*R*)-hydroxyproline (Hyp) and (2*S*,3*S*)-Hyp, which are generated in post-translational processes exclusively in Y and X positions of the collagen (Xaa-Yaa-Gly) repeats, respectively, with the enzymatic 4*R* hydroxylation being by far the dominant modification.[3] The stereoelectronic effects of this electronegative substituent at C4 or C3 of the pyrrolidine ring have been the subject of comparative analysis, particularly in synthetic model compounds with the fluorine substituent, in terms of (de)stabilization of the collagen triple helix.[2a,h,i,k,4] These studies on collagen model peptides have been extended to other proteins by exploiting the strong effects of 4-fluoroprolines (Flp).[2g,5]

Rather surprisingly, the non-natural synthetic 3- and 4-mercaptopyrrolidine-2-carboxylic acids (Mpc),[6] chalcogen analogues of hydroxyprolines, have been used only sporadically for side chain/side chain cyclization of peptides through thioether or disulfide bridges in attempts to restrict the conformational space of peptidic macrocycles.[7] Herein we report a structural comparison of the (2*S*,4*R*)- and (2*S*,4*S*)-Mpc epimers, in which replacement of the hydroxy group with the less electronegative thiol group results in altered conformational preferences: The 4*R* epimer of Mpc induces a C^{γ}-*endo* pucker while (4*R*)-Hyp and similar 4*R* substitutions generate the *exo* pucker (Scheme 1). This may have interesting structural implications for the design of peptides and proteins, particularly when the thiol group is exploited for thioether or disulfide intra- and interchain cross-bridging of polypeptide chains.



(4*R*)-Hyp (Y = OH; X = H): ***trans*, C^{γ}-*exo*** (4*S*)-Hyp (Y = H; X = OH): ***cis*, C^{γ}-*endo***
(4*S*)-Mpc (Y = H; X = SH): ***trans*, C^{γ}-*exo*** (4*R*)-Mpc (Y = SH; X = H): ***trans*, C^{γ}-*endo***

Scheme 1. Conformational equilibria of Ac-(4*R*/*S*)-Mpc-OMe (**1**) and Ac-(4*R*/*S*)-Hyp-OMe (**5**).

To evaluate the effect of 4-mercapto substitution on the pyrrolidine ring conformation the epimeric Ac-(2*S*,4*R*/*S*)-Mpc-OMe derivatives **1** were synthesized (Figure 1). In addition, to mimic the effect of side-chain bridging of such Mpc residues by thioethers or disulfides in peptides, the related alkyl and alkylthio epimer pairs Ac-(2*S*,4*R*/*S*)-Mpc(Me)-OMe (**2**) and Ac-(2*S*,4*R*/*S*)-Mpc(SMe)-OMe (**3**) were prepared, and their conformational preferences were compared with those of the known Ac-Pro-OMe (**4**), Ac-(2*S*,4*R*/*S*)-Hyp-OMe (**5**), and Ac-(2*S*,4*R*/*S*)-Flp-OMe (**6**) by NMR structural analysis in aqueous solution. In the model compounds the known effect of pH on the isomerization of Xaa–Pro bonds was prevented by the N and C derivatization.[8] Similarly, complications arising from hydrogen bonding in amide derivatives,[9] although weak in aqueous environments, are suppressed with C-terminal esters.

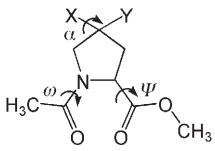


Figure 1. Chemical structure of *N*-acetylproline methyl ester (**4**, X = Y = H) and various derivatives, in which either X or Y is replaced by SH (**1**), SMe (**2**), SSMe (**3**), OH (**5**), or F (**6**).

trans/cis Equilibrium constants ($K_{t,c}$) and predominant ring puckerings were extracted from NMR spectral data to estimate the stereoelectronic effect of the thiol group on the conformational preference of the prolyl bond and on the ring puckering. These values are reported in Table 1 and compared to those known for the 4-hydroxy- and 4-fluoroproline epimer pairs. The equilibrium constants show that the 4*R*/*S* thiol group has a much weaker effect on the *trans*/*cis*

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Table 1: Thermodynamic parameters and conformational preferences of compounds **1**–**6**.

Cmpd	$K_{t/c}$[a]	Dominant conformation	$-\Delta H^{0}$[b] [kJ mol^{-1}]	ΔS^{0}[b] [J mol^{-1} K^{-1}]
1(4*R*)	5.4	*trans*, C$^{\gamma}$-*endo*	4.84 ± 0.8 %	−2.19 ± 5.9 %
1(4*S*)	4.7	*trans*, C$^{\gamma}$-*exo*	3.30 ± 1.0 %	1.89 ± 5.4 %
2(4*R*)	4.1	*trans*, C$^{\gamma}$-*endo*	1.93 ± 4.8 %	2.86 ± 2.8 %
2(4*S*)	3.1	*trans*, C$^{\gamma}$-*exo*	4.99 ± 2.5 %	−5.08 ± 5.0 %
3(4*R*)	4.3	*trans*, C$^{\gamma}$-*endo*	4.88 ± 2.6 %	−4.32 ± 4.3 %
3(4*S*)	3.6	*trans*, C$^{\gamma}$-*exo*	1.62 ± 4.6 %	5.25 ± 5.2 %
4[c]	4.8	*trans*, C$^{\gamma}$-*endo*	5.04 ± 1.0 %	−3.82 ± 4.3 %
5(4*R*)[d]	6.1	*trans*, C$^{\gamma}$-*exo*	7.84 ± 1.0 %	−10.7 ± 1.0 %
5(4*S*)[d]	2.4	*trans*, C$^{\gamma}$-*endo*	n.a.[e]	n.a.[e]
6(4*R*)[c]	7.3	*trans*, C$^{\gamma}$-*exo*	7.73 ± 3.3 %	−9.81 ± 8.3 %
6(4*S*)[c]	2.6	*trans*, C$^{\gamma}$-*endo*	3.04 ± 1.1 %	−2.47 ± 4.3 %

[a] Determined by integration of well-resolved signals in the ^{1}H NMR spectra in D_2O at 298 K. [b] The enthalpy (ΔH^{0}) and entropy (ΔS^{0}) contributions to the free energy difference between the *trans* and *cis* conformers were derived from van't Hoff plots (see Figure 1 in the Supporting Information); error limits were obtained from the residuals of the linear least-squares fitting. [c] Values from Ref. [2g]. [d] Values from Ref. [2k]. [e] Not available.

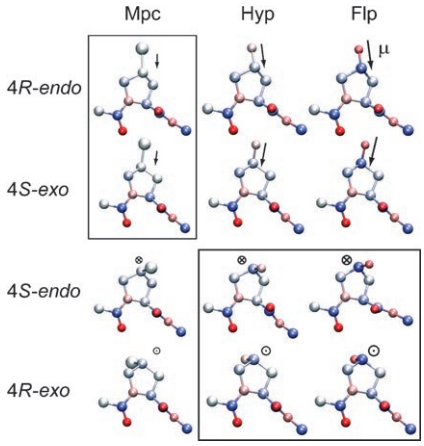

Figure 2. Optimized geometries of the L-proline derivatives as *trans* amide conformers: The strength and direction of the X–C$^{\gamma}$ bond dipoles μ are indicated by arrows. The atomic partial charges are indicated by a color code ranging from red (negative) through white (neutral) to blue (positive); adjacent red and blue atoms represent dipoles. The spheres represent "compound atoms"; the charges of hydrogen atoms are added to those of the neighboring heavy atoms. The experimentally observed conformers are marked by boxes.

conformational preference than the significantly more electronegative hydroxy and fluorine substituents. Indeed the $K_{t,c}$ values of **1**(4*R*) and **1**(4*S*) differ only slightly from that of unsubstituted proline. However, the ring-pucker preferences were reversed between the ring-substituted Ac-Pro-OMe species with similar *anti* (**1**(4*R*), **5**(4*R*), and **6**(4*R*)) and *syn* (**1**(4*S*), **5**(4*S*), and **6**(4*S*)) orientation of the electronegative substituent relative to the fixed L configuration of the C$^{\alpha}$ atom. Thus, an *anti* orientation of the 4-substituent resulted in a predominant C$^{\gamma}$-*endo* pucker for Mpc and in the known predominant C$^{\gamma}$-*exo* pucker for Hyp and Flp, whereas the opposite was true for the respective *syn*-oriented species.

The transformation of the thiol group into a methylthioether or methyldisulfide shifted the *trans*/*cis* equilibrium of the prolyl bond toward the *cis* conformation with the effect of the thioether being milder than that of the methyl disulfide. The ring-pucker preference was not affected by this type of derivatizations of the thiol group.

Quantum chemical calculations using density functional theory (DFT) were used to explain the experimental data obtained for compound **1** and **4**–**6**. In the case of Mpc (**1**) and Hyp (**5**) derivatives, potential curves were calculated for the dihedral angles ω, ψ, and α (Figure 1). The gas-phase energies and molecular geometries obtained by our DFT descriptions of **4**, **6**(4*R*), and **6**(4*S*) (see Tables II and III in the Supporting Information) agree very well with the results of previous DFT calculations,[2h] confirming our theoretical approach. The populations of the different conformers derived from NMR experimental data and those computed from DFT results are congruent with few exceptions (see Table IV in the Supporting Information). Most importantly, the calculations predict the initially unexpected but experimentally observed preference of **1**(4*R*) for the C$^{\gamma}$-*endo* pucker and of **1**(4*S*) for the C$^{\gamma}$-*exo* pucker.

The DFT calculations reveal that the pucker preference of a given substitution at the 4-position is mainly determined by the interaction between the bond dipole at the substitution site and the dipole of the preceding amide bond. Figure 2 shows how the combination of (4*R*)-Hyp or (4*R*)-Flp with a C$^{\gamma}$-*endo* pucker leads to an unfavorable antiparallel orientation of these dipoles, while the C$^{\gamma}$-*exo* pucker results in close to perpendicular dipole moments with an almost neutral contribution to the total energy. This results in the C$^{\gamma}$-*exo* pucker being more favorable for the (4*R*)-Hyp and the (4*R*)-Flp derivatives. In contrast, the dipole moment at the substitution site of Mpc is very weak and causes almost no energetic penalty for the 4*R*-*endo* combination. The experimentally observed preference of Mpc for the 4*R*-*endo* geometric variant implies that the net contributions of all interactions (electrostatic, van der Waals, bond geometries, etc.) except the decisive dipole–dipole interaction discussed before are slightly in favor of the C$^{\gamma}$-*endo* pucker for the (4*R*)-Mpc derivative **1**. Therefore, the preference of the (4*R*)-Hyp (**5**) and (4*R*)-Flp (**6**) derivatives for the C$^{\gamma}$-*exo* pucker is explained by a reduced unfavorable dipole–dipole orientation rather than a specific favorable interaction. One would expect that other 4*R* or 4*S* substitutions that introduce small dipole moments will also prefer the 4*R*-*endo* and 4*S*-*exo* combinations that had been hitherto considered unfavorable and unusual, unless steric effects prevail over electrostatic interaction as it is the case in the pair of 4-methylproline epimers.[2n,9]

In our preceding synthetic efforts to control the folding/unfolding of a collagen triple helix by applying light we introduced two (4*S*)-Mpc residues, as the synthetically more readily accessible epimer, into the Ac-(Gly-Pro-Hyp)$_7$-Gly-Gly-NH$_2$ model collagen peptide for side chain/side chain cross-bridging of the two thiol groups with a suitable thiol-reactive azobenzene derivative (Figure 3).[11] Optimal locations for the Mpc residues according to modeling experiments are the i and $i+7$ positions corresponding to Xaa and Yaa residues of the classical (Xaa-Yaa-Gly) collagen triplets. High-resolution X-ray analysis of collagen model peptides[12]

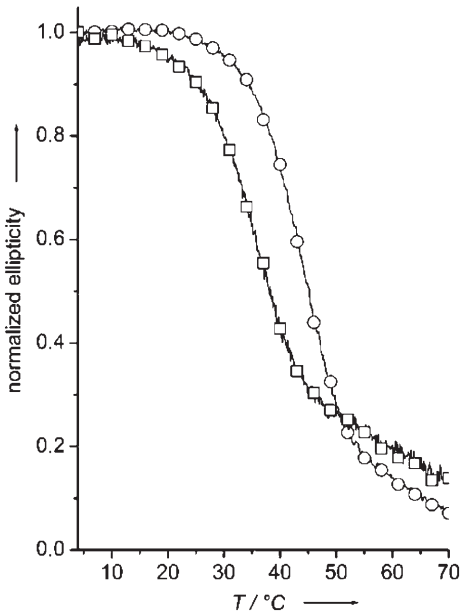

Figure 3. Thermal unfolding of the triple-helical Ac-(Gly-Pro-Hyp)$_7$-Gly-Gly-NH$_2$ (○) ($T_m = 43\,°C$) and of its analogue Ac-(GPO)$_2$-(G-(4*S*)-Mpc-O)-(GPO)-(GP-(4*S*)-MPC)-(GPO)$_2$-GG-NH$_2$ with (4*S*)-Mpc residues located in the X and Y positions (□) ($T_m = 34.5\,°C$). The unfolding was monitored by CD at 225 nm in aqueous solution at a peptide concentration of 1 mM; O = (4*R*)-hydroxyproline.

and computational analysis[13] revealed alternate C$^\gamma$-*endo* and C$^\gamma$-*exo* puckers of the Pro and Hyp residues in the X and Y positions of the triplets as a repetitive motif possibly involved in stabilizing the triple helix. Extensive comparative studies of synthetic collagen peptides containing (4*R*)-Hyp or (4*S*)-Hyp, and (4*R*)- or (4*S*)-Flp have shown that occupancy of the X and Y positions with proline analogues characterized by preferences for C$^\gamma$-*endo* and C$^\gamma$-*exo* puckers, respectively, leads to markedly increased thermal stabilities of the triple helix, while the opposite effect is induced with reversed ring puckerings.[2h,i,k,4a,b,12] However, more recently, in X-ray structures of [Gly-(4*R*)-Hyp-(4*R*)-Hyp]$_n$ peptides both Hyp residues assume *exo* conformations.[4g,j] In view of the still-evolving understanding of the mechanism of triple-helix stabilization and the weak electron-withdrawing property of the thiol group we assumed that replacement of a Pro and Hyp residue in Ac-(Gly-Pro-Hyp)$_7$-Gly-Gly-NH$_2$ with (4*S*)-Mpc should affect only marginally the triple-helix stability. In contrast, a rather substantial decrease in the thermal stability was observed as shown in Figure 3.

Taking into account that Pro and/or Hyp replacements in single triplets of (Gly-Pro-Hyp)$_n$ peptides, that is, in host–guest peptides, lead to results that are significantly different than those from repetitive replacements,[4k] a rational interpretation of the drop of the T_m value by about 8 °C can be put forward. The stereoelectronic effects of substituted prolines in the (Gly-Pro-Hyp) collagen repeats are not additive in terms of triple-helix (de)stabilization,[4i] but the steric effects seem to be so.[2n] By the single substitution of a Hyp residue in Y with (4*S*)-Mpc the *trans* peptide bond is less favored and similar to that of a Pro residue (Table 1). This negative effect, which leads to a 2 °C lower T_m in a (Gly-Pro-Hyp)$_8$ host peptide containing one Gly-Pro-Pro repeat,[4k] should be fully compensated by the favored C$^\gamma$-*exo* pucker of the (4*S*)-Mpc. Similarly, like a Pro residue in X position combined with Hyp in Y, (4*S*)-Mpc should marginally affect the triple-helical fold despite its favored C$^\gamma$-*exo* pucker. The rather strong experimentally observed destabilization must therefore be assigned mainly to steric effects, fully supporting a strong interplay between stereoelectronic and steric effects in the assembly of collagen triple helices.

Despite the limitations of the predictive power of the simple Ac-Pro-OMe system for values in protein environments, the results of this study offer a more general view of the relation between 4-substitutions of proline and resulting conformational properties of the amide bond and, especially, of the proline ring pucker. The increased understanding of the determinants of proline geometry together with the decisive role of proline residues and related analogues in peptide and protein structures can provide a powerful tool in the design and folding studies of polypeptides. In contrast, their application in proteins must await improved methodologies for an efficient incorporation of such non-natural amino acids into expressed proteins unless synthetic and semisynthetic ligation strategies suffice for the purpose.

Experimental Section

Details of the synthesis of Ac-(4*R*/*S*)-Mpc-OMe (**1**), Ac-(4*R*/*S*)-Mpc(Me)-OMe (**2**), and Ac-(4*R*/*S*)-Mpc(SMe)-OMe (**3**) are reported in Supporting Information. Solutions in D_2O were used for NMR measurements, and in the case of Ac-Mpc-OMe tris-(2-carboxyethyl)-phosphine hydrochloride (1 equiv) was added to prevent oxidation. NMR experiments were performed with a Bruker DRX500 spectrometer using a triple-resonance ($^{15}N/^{13}C/^1H$) inverse probe. Assignment of ^1H and ^{13}C NMR resonances was based on homonuclear 2D ^1H-^1H NOESY and TOCSY experiments and heteronuclear 2D ^{13}C-^1H COSY experiments.[15]

Thermodynamics and kinetics of amide-bond isomerization: Equilibrium constants ($K_{t/c}$) for the *trans*/*cis* conformer ratios at various temperatures were determined by integration of the signals of the α and γ protons in 1D ^1H NMR spectra. The enthalpic and entropic contributions to the free energy difference between the *cis* and *trans* conformers were obtained from van't Hoff plots according to $\ln(K_{ZE}) = (-\Delta H^0/R)(1/T) + \Delta S^0/R$.

NMR conformation analysis: The pucker of the proline ring was identified by the method of Gerig and McLeod[16] by means of the distinct pattern of the ^1H–^1H coupling constants observed in 1D ^1H NMR spectra; for example, the C$^\gamma$-*exo* pucker results in large and similar coupling constants $J(H_\alpha,H_{\beta1})$ and $J(H_\alpha,H_{\beta2})$, whereas for the C$^\gamma$-*endo* pucker one of the coupling constants is large and the other small. The *cis*-to-*trans* isomerization of the amide bond is a slow process on the NMR time scale, and therefore two distinct signals are observed for the two conformers. On the other hand, the pucker inversion is a fast process, and therefore the signal obtained for the *trans* conformer is an averaging of the *trans*, C$^\gamma$-*endo* and *trans*, C$^\gamma$-*exo* pucker; the same is true for the *cis* conformer. An estimation of the ratio of the two puckerings for the *trans* and *cis* conformers was obtained by the equation $\Delta J_{exp} = x\Delta J_{endo} + y\Delta J_{exo}$ where ΔJ refers to the difference between $J(H_\alpha,H_{\beta1})$ and $J(H_\alpha,H_{\beta2})$, while ΔJ_{endo} and ΔJ_{exo} were calculated with the program MestRe-J[17] using the dihedral angles obtained from the lowest energy conformers produced by quantum chemical calculations (see Table I in the Supporting Information).

Computational methods: Density functional theory (DFT) calculations were carried out with the program TURBOMOLE 5.6.[18] Here, the B3LYP functional[19] and the TZVP basis set[20] (a Gaussian basis set of triple-zeta valence quality augmented by polarization functions) were employed. Various isomeric structures of compound **4** as well as of its mercapto- (**1**), hydroxy- (**5**), and fluoro dervatives (**6**) were calculated both for the gas phase and with the continuum solvent model COSMO[21] (dielectric constant $\varepsilon = 80$) to account for the dielectric shielding by the D_2O solvent used in the NMR experiments. Mulliken population analyses[22] were performed to determine partial atomic charges. For further details see the Supporting Information.

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[1] a) J. F. Brandts, H. R. Halvorson, M. Brennan, *Biochemistry* **1975**, *14*, 4953 – 4963; b) W. J. Wedemeyer, E. Welker, H. A. Scheraga, *Biochemistry* **2002**, *41*, 14637 – 14644; c) G. Fischer, *Chem. Soc. Rev.* **2000**, *29*, 119 – 127; d) F. X. Schmid in *Protein Folding Handbook*, *Vol. 2* (Eds: J. Buchner, T. Kiefhaber), Wiley-VCH, Weinheim, **2005**, pp. 916 – 945.

[2] a) E. S. Eberhardt, N. Panasik, R. T. Raines, *J. Am. Chem. Soc.* **1996**, *118*, 12261 – 12266; b) N. Panasik, E. S. Eberhardt, A. S. Edison, D. R. Powell, R. T. Raines, *Int. J. Pept. Protein Res.* **1994**, *44*, 262 – 269; c) D. Kern, M. Schutkowski, T. Drakenberg, *J. Am. Chem. Soc.* **1997**, *119*, 8403 – 8408; d) M. Keller, C. Sager, P. Dumy, M. Schutkowski, G. S. Fischer, M. Mutter, *J. Am. Chem. Soc.* **1998**, *120*, 2714 – 2720; e) E. Beausoleil, R. Sharma, S. W. Michnick, W. D. Lubell, *J. Org. Chem.* **1998**, *63*, 6572 – 6578; f) S. S. A. An, C. C. Lester, J. L. Peng, Y. J. Li, D. M. Rothwarf, E. Welker, T. W. Thannhauser, L. S. Zhang, J. P. Tam, H. A. Scheraga, *J. Am. Chem. Soc.* **1999**, *121*, 11558 – 11566; g) C. Renner, S. Alefelder, J. H. Bae, N. Budisa, R. Huber, L. Moroder, *Angew. Chem.* **2001**, *113*, 949 – 951; *Angew. Chem. Int. Ed.* **2001**, *40*, 923 – 925; h) M. L. DeRider, S. J. Wilkens, M. J. Waddell, L. E. Bretscher, F. Weinhold, R. T. Raines, J. L. Markley, *J. Am. Chem. Soc.* **2002**, *124*, 2497 – 2505; i) J. A. Hodges, R. T. Raines, *J. Am. Chem. Soc.* **2003**, *125*, 9262 – 9263; j) Y. Che, G. R. Marshall, *J. Org. Chem.* **2004**, *69*, 9030 – 9042; k) L. E. Bretscher, C. L. Jenkins, K. M. Taylor, M. L. DeRider, R. T. Raines, *J. Am. Chem. Soc.* **2001**, *123*, 777 – 778; l) C. M. Taylor, R. Hardre, P. J. B. Edwards, *J. Org. Chem.* **2005**, *70*, 1306 – 1315; m) W. Kim, K. I. Hardcastle, V. P. Conticello, *Angew. Chem.* **2006**, *118*, 8321 – 8325; *Angew. Chem. Int. Ed.* **2006**, *45*, 8141 – 8145; n) M. D. Shoulders, J. A. Hodges, R. T. Raines, *J. Am. Chem. Soc.* **2006**, *128*, 8112 – 8113; o) S. K. M. Umashankara, I. R. Babu, K. N. Ganesh, *Chem. Commun.* **2003**, 2606 – 2607; p) I. R. Babu, K. N. Ganesh, *J. Am. Chem. Soc.* **2001**, *123*, 2079 – 2080.

[3] a) I. Wagner, H. Musso, *Angew. Chem.* **1983**, *95*, 827 – 839; *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 816 – 828; b) A. B. Mauger, *J. Nat. Prod.* **1996**, *59*, 1205 – 1211; c) L. Moroder, C. Renner, J. J. Lopez, M. T. Mutter, G. Tuchscherer in *Cis-trans Isomerization in Biochemistry* (Ed.: C. Dugave), Wiley-VCH, Weinheim, **2006**, pp. 225 – 259.

[4] a) S. K. Holmgren, K. M. Taylor, L. E. Bretscher, R. T. Raines, *Nature* **1998**, *392*, 666 – 667; b) S. K. Holmgren, L. E. Bretscher, K. M. Taylor, R. T. Raines, *Chem. Biol.* **1999**, *6*, 63 – 70; c) C. L. Jenkins, L. E. Bretscher, I. A. Guzei, R. T. Raines, *J. Am. Chem. Soc.* **2003**, *125*, 6422 – 6427; d) J. A. Hodges, R. T. Raines, *J. Am. Chem. Soc.* **2005**, *127*, 15923 – 15932; e) M. Doi, Y. Nishi, S. Uchiyama, Y. Nishiuchi, H. Nishio, T. Nakazawa, T. Ohkubo, Y. Kobayashi, *J. Pept. Sci.* **2005**, *11*, 609 – 616; f) M. Doi, Y. Nishi, S. Uchiyama, Y. Nishiuchi, T. Nakazawa, T. Ohkubo, Y. Kobayashi, *J. Am. Chem. Soc.* **2003**, *125*, 9922 – 9923; g) K. Kawahara, Y. Nishi, S. Nakamura, S. Uchiyama, Y. Nishiuchi, T. Nakazawa, T. Ohkubo, Y. Kobayashi, *Biochemistry* **2005**, *44*, 15812 – 15822; h) Y. Nishi, S. Uchiyama, M. Doi, Y. Nishiuchi, T. Nakazawa, Z. Ohkubo, Y. Kobayashi, *Biochemistry* **2005**, *44*, 6034 – 6042; i) D. Barth, A. G. Milbradt, C. Renner, L. Moroder, *ChemBioChem* **2004**, *5*, 79 – 86; j) M. Schumacher, K. Mizuno, H. P. Bächinger, *J. Biol. Chem.* **2005**, *280*, 20397 – 20403; k) A. V. Persikov, J. A. M. Ramshaw, A. Kirkpatrick, B. Brodsky, *J. Am. Chem. Soc.* **2003**, *125*, 11500 – 11501.

[5] a) W. Kim, R. A. McMillan, J. P. Snyder, V. P. Conticello, *J. Am. Chem. Soc.* **2005**, *127*, 18121 – 18132; b) R. Golbik, C. Yu, E. Weyher-Stingl, R. Huber, L. Moroder, N. Budisa, C. Schiene-Fischer, *Biochemistry* **2005**, *44*, 16026 – 16034; c) S. C. R. Lummis, D. L. Beene, L. W. Lee, H. A. Lester, R. W. Broadhurst, D. A. Dougherty, *Nature* **2005**, *438*, 248 – 252.

[6] a) V. Eswarakrishnan, L. Field, *J. Org. Chem.* **1981**, *46*, 4182 – 4187; b) A. J. Verbiscar, B. Witkop, *J. Org. Chem.* **1970**, *35*, 1924 – 1927.

[7] a) D. S. Kemp, J. H. Rothman, T. C. Curran, D. E. Blanchard, *Tetrahedron Lett.* **1995**, *36*, 3809 – 3812; b) G. V. Nikiforovich, J. L. F. Kao, K. Plucinska, W. J. Zhang, G. R. Marshall, *Biochemistry* **1994**, *33*, 3591 – 3598; c) K. Plucinska, T. Kataoka, M. Yodo, W. L. Cody, J. X. He, C. Humblet, G. H. Lu, E. Lunney, T. C. Major, R. L. Panek, P. Schelkun, R. Skeean, G. R. Marshall, *J. Med. Chem.* **1993**, *36*, 1902 – 1913; d) T. Kataoka, D. D. Beusen, J. D. Clark, M. Yodo, G. R. Marshall, *Biopolymers* **1992**, *32*, 1519 – 1533.

[8] C. Grathwohl, K. Wüthrich, *Biopolymers* **1976**, *15*, 2025 – 2041.

[9] G. B. Liang, C. J. Rito, S. H. Gellman, *J. Am. Chem. Soc.* **1992**, *114*, 4440 – 4442.

[10] J. L. Flippen-Anderson, R. Gilardi, I. L. Karle, M. H. Frey, S. J. Opella, L. M. Gierasch, M. Goodman, V. Madison, N. G. Delaney, *J. Am. Chem. Soc.* **1983**, *105*, 6609 – 6614.

[11] U. Kusebauch, S. A. Cadamuro, H. J. Musiol, M. O. Lenz, J. Wachtveitl, L. Moroder, C. Renner, *Angew. Chem.* **2006**, *118*, 7170 – 7173; *Angew. Chem. Int. Ed.* **2006**, *45*, 7015 – 7018.

[12] a) V. Nagarajan, S. Kamitori, K. Okuyama, *J. Biochem.* **1999**, *125*, 310 – 318; b) R. Z. Kramer, L. Vitagliano, J. Bella, R. Berisio, L. Mazzarella, B. Brodsky, A. Zagari, H. M. Berman, *J. Mol. Biol.* **1998**, *280*, 623 – 638; c) R. Berisio, L. Vitagliano, L. Mazzarella, A. Zagari, *Protein Sci.* **2002**, *11*, 262 – 270; d) L. Vitagliano, R. Berisio, A. Mastrangelo, L. Mazzarella, A. Zagari, *Protein Sci.* **2001**, *10*, 2627 – 2632; e) L. Vitagliano, R. Berisio, L. Mazzarella, A. Zagari, *Biopolymers* **2001**, *58*, 459 – 464.

[13] R. Improta, C. Benzi, V. Barone, *J. Am. Chem. Soc.* **2001**, *123*, 12568 – 12577.

[14] R. T. Raines, *Protein Sci.* **2006**, *15*, 1219 – 1225.

[15] K. Wütrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**.

[16] J. T. Gerig, R. S. McLeod, *J. Am. Chem. Soc.* **1973**, *95*, 5725 – 5729.

[17] A. Navarro-Vazquez, J. C. Cobas, F. J. Sardina, J. Casanueva, E. Diez, *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 1680 – 1685.

[18] a) R. Ahlrichs, M. Bär, M. Häser, H. Horn, C. Kölmel, *Chem. Phys. Lett.* **1989**, *162*, 165 – 169; b) O. Treutler, R. Ahlrichs, *J. Chem. Phys.* **1995**, *102*, 346 – 354.

[19] a) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648 – 5652; b) C. T. Lee, W. T. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785 – 789.

[20] A. Schäfer, C. Huber, R. Ahlrichs, *J. Chem. Phys.* **1994**, *100*, 5829 – 5835.

[21] A. Klamt, G. Schüürmann, *J. Chem. Soc. Perkin Trans. 2* **1993**, 799 – 805.

[22] R. S. Mulliken, *J. Chem. Phys.* **1955**, *23*, 1833 – 1840.

