Intramolecular Hydrogen Bond-Controlled Prolyl Amide Isomerization in Glucosyl 3(*S*)-Hydroxy-5-hydroxymethylproline Hybrids: A Computational Study

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Peptide mimics containing spirocyclic glucosyl-(3(*S*)-hydroxy-5(*S*)-hydroxymethyl)proline (**1**) and glucosyl-(3(*S*)-hydroxy-5(*R*)-hydroxymethyl)proline (**2**) hybrids differing in the stereochemistry of the polar hydroxymethyl substitutent at the C-5- or (C_δ)-position have been investigated computationally. A computational "build and search" protocol of molecular mechanics systematic search/Monte Carlo search, followed by density functional theory (DFT), has been developed to ensure complete coverage of the large conformational space. Gas-phase DFT optimizations at the B3LYP level of theory lead to a strong preference for the *cis* conformation in the prolyl amide bond for both compounds **1** and **2**. However, inclusion of the solvent water by means of continuum solvation (PCM) results in a reduction of the prolyl amide *cis* population in both compounds, leading to good agreement with previous experimental observations. Intramolecular hydrogen bonding involving the C-5-hydroxymethyl substitutent is seen to play a crucial role to tune the thermodynamics of prolyl amide *cis*/*trans* isomerization and is responsible for the high *cis* prolyl amide population in compound **2**. Our results indicate that H-bond-forming substituents like the hydroxymethyl group at the C-5-position in proline can be used to control *cis*/*trans* prolyl amide isomerization. High *cis* prolyl amide conformer populations can be achieved by proper choice of the stereochemistry at the C-5-position.

Introduction

Proline (Pro) is unique among the 20 proteinogenic amino acids in that it incorporates the α-nitrogen in its side chain via a covalent bond, forming a five-membered pyrrolidine ring. This cyclization limits rotation of the ϕ dihedral angle, resulting in reduced conformational freedom of the amino acid. As a consequence, and contrary to all other proteinogenic amino acids which favor almost exclusively the *trans* isomer in polypeptides, proline can exist in both *cis* and *trans* configurations in peptides or proteins. The lack of an amide hydrogen atom on the α-amino group prevents the proline backbone from forming a hydrogen bond with the preceding part of the peptide structure. As a result, proline does not participate in forming intramolecular hydrogen bonds, a requirement for the formation of α-helices or β-sheets, and frequently acts as a structural disrupter of these secondary structures. Instead, the limited rotation of the ϕ dihedral angle in proline induces β-turns and extended helical structures (polyproline helix) in peptides that are crucial in protein/protein and protein/peptide interactions.[1,2] Moreover, the isomerization of the prolyl amide bond is extremely slow in H_2O and has been shown to be the rate-determining step in the folding pathways of many peptides and proteins.[3]

In nature, proline undergoes post-translational modifications such as hydroxylation to 4(*R*)-hydroxyproline (4-Hyp) and 3(*S*)-hydroxyproline (3-Hyp).[4−7] Hydroxylation controls the puckering of the pyrrolidine ring, affects the hydration sphere of proline, induces inductive effects, and introduces hydrogen-bond donor and acceptor properties in (poly)peptides.[8−11] These factors play a vital role in the stability of collagen.[10,12−14] In order to study structure−function and structure−activity relationships in proline-containing peptides, many proline analogues have been developed over the past years. These are relevant because the prolyl *N*-terminal *cis*/*trans* isomerization rate and equilibrium ratios of specific proline analogues are helpful in detecting and monitoring the local structure and environment of proline. Some examples include C-3-, C-4, and C^δ-substituted prolines,[15−18] silaproline,[19] azaprolines,[20,21] fused bicyclic proline,[22−26] pseudoprolines,[27−29] proline analogues with different ring sizes,[27,30,31] fused glucose-proline analogues,[32] and 4-hydroxyprolines and 4-fluoroprolines.[33,34] Recently, we have reported on the synthesis of spirocyclic glucose-3(*S*)-hydroxy-5(*S*)-hydroxymethyl)proline hybrid (Ac-Glc3(*S*)-5(*S*)-(CH_2OH)-HypH-OMe, **1**) and glucose-3(*S*)-hydroxy-5(*R*)-hydroxymethyl)proline hybrid (Ac-Glc3(*S*)-5(*R*)-(CH_2OH)HypH-OMe, **2**) (Scheme 1).[35,36] Peptide mimics **1** and **2** differ in the stereochemistry at the hydroxymethyl substitutent at the C-5-position of the pyrrolidine ring which is strategically located to form a hydrogen bond to the *cis* N-terminal carbonyl group in proline (Scheme 1). The thermodynamics and kinetics of the prolyl amide *cis*−*trans* isomerization in compounds **1** and **2** were investigated in detail with a large variety of different techniques.[36] Here, we report an extensive computational study on these compounds. Preliminary results thereof have been reported briefly in our previous experimental paper.[36]

Previous Computational Studies of Proline-Based Model Peptides. Given the importance of proline and its derivatives, computational studies of such compounds have a fairly long history. In 1970, Maigret and co-workers pioneered the com-

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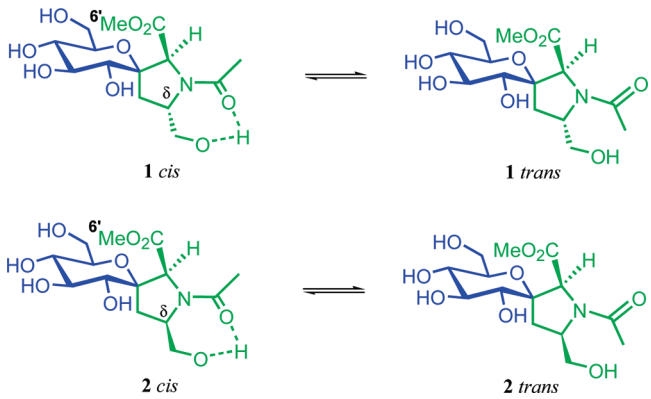
SCHEME 1: The *cis*−*trans* Isomerization of Peptide Mimics Ac-Glc3(*S*)-*δ*-(CH$_2$OH)Hyp-OMe 1 and 2 Differing in the Stereochemistry of the Hydroxymethyl Substitutent at C$_\delta$[a]



[a] The *cis* prolyl amide bond in **1** and **2** may be stabilized by an intramolecular hydrogen bond between the hydroxymethyl substituent and the N-terminal carbonyl group of proline.

putational work on the *cis*−*trans* isomerization of the prolyl residue, using simple gas-phase calculations.[37,38] Farmer and Hopfinger[39] have done work on the conformational analysis to describe the molecular motion of the *cis*−*trans* isomerization in poly(L-proline), using force field methods. Force field approaches were applied by Delaney and Madison as well.[15] Karplus and co-workers[40] have studied the *cis*−*trans* isomerization reactions of proline dipeptides extensively, using the CHARMM force field[41,42] and (gas-phase) Hartree−Fock methods. Kang and Choi[43] investigated the conformational preference of *N*-acetyl-L-proline-*N*-methylamide (Ac-Pro-NHMe) and its prolyl *cis*−*trans* isomerization in the gas phase and solution using the HF/6-31+G(d) level of theory and the conductor-like polarizable continuum model (CPCM). They indicated that the free energy barriers in water for the *trans*−*cis* and *cis*−*trans* isomerizations are 19.0 and 18.8 kcal/mol, respectively, which is in accord with the experimental values of 20.4 and 19.8 kcal/mol, respectively. They also mentioned that the overall barrier for rotation is greatly affected by the polarity of the solvent—it is higher in polar solvents as compared to nonpolar ones. A more recent study by Kang[44] confirmed these results and demonstrated that the *cis*−*trans* isomerization proceeds in common through only the clockwise rotation. Moreover, it was found by analysis of the contributions to rotational barriers that the *cis*−*trans* isomerization for the nonprolyl and prolyl peptide bonds is entirely enthalpy driven in the gas phase and in solution.[44] *N*-Formyl-prolinamide is another model system that has been used to study the thermodynamics of prolyl amide *cis*/*trans* isomerization. This system has been studied by Enriz et al.[45] using gas-phase calculations. *N*-Formyl-prolinamide was chosen because the *cis*−*trans* isomerization of the *N*-formyl-prolinamide model peptide is considered to be an equilibrium between structures of comparable stabilities. The authors reported that the barrier heights for the *cis*−*trans* isomerization and ring puckering are in the ranges 19.6−24.6 and 1.8−7.5 kcal/mol, respectively. Che and Marshal[20] reported an extensive study of the amino acid analogue azaprolines (azPro) using NMR and computational studies for the exploration of the conformational preference (density functional theory DFT and *ab initio* methods up to MP2 with modest basis sets; PCM solvation model). This amino acid analogue contains a nitrogen atom in place of the C$_\alpha$ of proline. Sahai et al.[46] performed a computational study on the full conformational space of L-proline diamides, using high-level *ab initio* methods. Aliev and Courtier-Murias[47] performed a conformational analysis of L-proline in water using DFT and the IEFPCM solvation model, as well as molecular dynamics methods. Recently, they continued this work[48] in a combined NMR and computational study (DFT, Hartree−Fock, MP2, with continuum solvation included) of *N*-acetyl-L-proline (AcProOH) and *N*-acetyl-4-hydroxy-L-proline (AcHypOH). Moreover, recently a series of studies have reported the prolyl puckering transitions of the proline and pseudoproline residues.[49−52] Finally, we mention the recent work of Flores-Ortega et al.[53,54] who investigated different *N*-acetyl-*N*′-methylamide derivatives of L-proline using DFT and continuum solvation.

Computational Details

Carbohydrates such as the target molecules of this study and other flexible molecules provide a challenge to computational methods due to the enormous size of the conformation space, that grows exponentially with the overall size of the system. A reliable computational protocol needs to adequately sample that conformational space.[55] In the current study, we have addressed this problem by first performing molecular mechanics[56−58] (MM) searches for all possible conformers, followed by density functional theory[59] (DFT) based optimizations of these conformers.

MM searches were performed using the Monte Carlo[56,60] (MC) random searching algorithm and the MMFF94 force field[61−67] as implemented in the SPARTAN '02 program package.[68] The advantage of applying MC lies in its ability of sampling the entire potential energy surface (conformational space); however, due to the probabilistic nature of the method, one cannot necessarily be sure that all important low-energy conformers including the global energy minimum are found. Systematic search methods,[55,56] on the other hand, generate guess structures by systematically varying dihedral bond angles of all bonds in the system (for instance, in regular intervals of 30°). However, these methods are problematic for cyclic molecules and computationally expensive.

Here, we applied a combined method using both MC and systematic search in a "Build and Search" approach. In this approach, the search starts with a small molecule (that constitutes part of the target molecule) with few degrees of freedom. Its conformational space is mapped using systematic search and MC methods, depending on the system at hand (cyclic or not). Typically, this searching is repeated five times to ensure completeness, such that a redundant outcome (no more new conformers) is obtained. In this manner, a number of unique conformers are obtained for this small molecule. These conformers are used as starting structures for building the next bigger molecule. For each conformer of the bigger molecule, systematic and MC methods, respectively, are applied to obtain all possible conformers. This procedure is repeated until we reach the target molecule. Obviously, the procedure will sometimes lead to large numbers of identical, redundant conformers. From these, a set of unique conformers is obtained by superimposing all of the conformers and discarding any duplicates.

Applying this "Build and Search" approach specifically to compounds **1** and **2**, we started with the five-membered ring (CH$_2$−CH$_2$−CH$_2$−CH$_2$−NH). A systematic and MC search was employed that generated 12 unique structures. Once these unique structures were identified, the six-membered ring (i.e., CH$_2$−CH$_2$−CH$_2$−CH$_2$−CH$_2$−O) was attached to each conformer in a spirocyclic manner. These larger systems were again subjected to a MC conformational search, which generated 27

unique local minima (27 unique conformers for the spirocyclic system). Finally, the target compounds (compound **1** or **2**) were built on each of these 27 conformers and subjected to further conformational searches. This leads to approximately 2700 structures each, which were reduced by superposition to 443 unique structures at the MMFF94 level for compound **1** and 457 structures for compound **2**, respectively.

The conformers obtained in this manner were used as input structures for subsequent DFT calculations using the Gaussian 03 (G03) program.[69] All calculations were performed at the B3LYP[70−72] level of theory using the standard 6-31+G(d,p) basis set. The use of diffuse and polarization functions should ensure reasonably converged geometries with respect to the basis set. We performed full geometry optimizations in the gas phase, followed by frequency calculations that were used to characterize the stationary points as true minima and to provide thermodynamic data. Except for four structures of compound **2** (that were discarded), no imaginary frequencies were observed. The gas-phase conformers were further reoptimized in solvation using the PCM continuum solvation model[73] and H_2O as the solvent (dielectric constant of 78). In solvation, only 355 and 258 conformers for compounds **1** and **2**, respectively, were located as true local minima. The few remaining structures had either large imaginary frequencies, which were very difficult to avoid, or some sort of difficulty with convergence. Those structures with convergence problems and/or high imaginary frequencies were checked for their SCF convergence profile. In particular, we examined the energy change for each iterative step, and it was found that there was no dramatic change in their energy profile from one step to the next. In addition, these structures were at very high energy levels relative to the conformers already collected. As a result, their impact on the conformational distribution was considered to be negligible, and these structures were discarded.

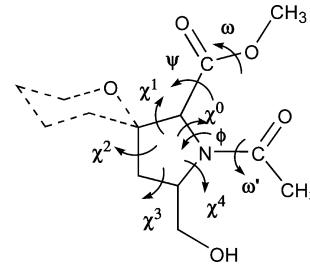
Boltzmann statistics (25.0 °C) were applied to the entire set of conformers in the gas phase and solution, respectively, in order to obtain the respective conformer distributions and the *cis*/*trans* ratios.

Geometrical Analysis. The most stable conformers have been investigated for internal hydrogen bonding. We used a bond length cutoff of ≤2.5 Å (for the distance between the hydrogen atom and an adjacent heavy atom) to state that a strong hydrogen bond exists between the hydrogen atom that is situated in between the donor and the acceptor atom.[74] The data has been investigated with bond distance cutoffs of ≤2.1 and ≤2.5 Å to check the sensitivity of the results to the choice of cutoff value. However, the results found from both cutoffs remain the same, proving that the approach taken is robust.

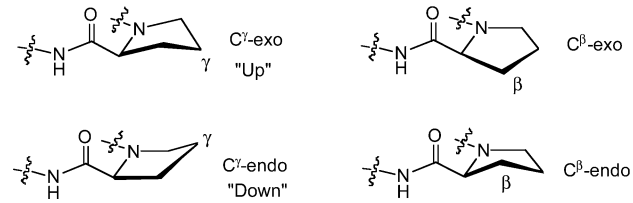
The backbone torsion and endocyclic angles were also characterized to determine the impact of C-5-hydroxymethylene on the peptide backbone chain and prolyl amide. The definitions of the backbone torsion and endocyclic angles are displayed in Scheme 2. These definitions are similar to those used in the work of Kang et al.[51,75,76]

Nomenclature. The nature of the different conformers was characterized using the systematic nomenclature of peptides that is based on the backbone torsion angles ϕ and ψ, Scheme 2.[37,77,78] In a general peptide, each of these two angles can have values corresponding to three general conformations, gauche$^-$ (g^-; angle around −60 or 300°), anti (a; angle around 180°) and gauche$^+$ (g^+, angle around 60°). This leads to nine possible conformations as far as these two angles are concerned. Due to the conformational restrictions imposed by its pyrrolidine ring, proline will only allow one ϕ value in the vicinity of 300° (−60°), i.e., g^-. Thus, only three conformations are possible for proline. These are termed α_L (ψ in the g^- conformation), ε_L (ψ in the a conformation), and γ_L (ψ in the g^+ conformation), respectively.

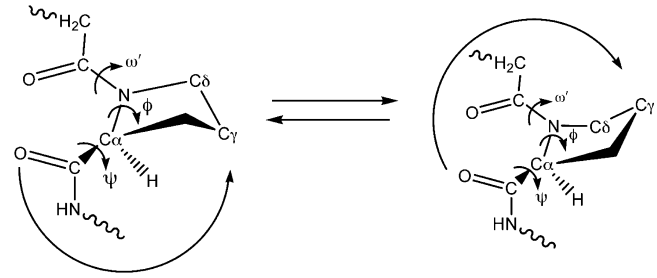
SCHEME 2: Definitions of the Backbone and Endocyclic Torsion Angles



SCHEME 3: Proline γ- and β-Exo and Endo Puckers; the γ- and β-Atoms Are Labeled



SCHEME 4: Conformational Equilibrium of the Two C_γ-Puckering States in Proline Containing Peptide Residues[a]



[a] Left, C_γ-exo or UP [u]; right, C_γ-endo or DOWN [d].

Proline contains a five-membered pyrrolidine ring (or side chain), which prefers to adopt distinct conformational states or puckers that are almost equal in energy.[79] A survey of proline residues in the crystal structures of 50 different proteins indicated that the proline side chain commonly adopts two distinct conformations (Scheme 4).[80] Proline is puckered in an envelope conformation, where mostly the γ- or C-4-carbon, but less frequently the β- or C-3-carbon, project out of the plane defined by the three atoms C_δ, N, and C_α in the pyrroldine ring (Scheme 3).[62] If the γ-carbon projects from the opposite face to the prolyl carboxyl group, then it is referred to as C$^\gamma$-exo or "UP". If the γ-carbon is projected onto the same face as the prolyl carboxyl group, then it is referred to C$^\gamma$-endo or "Down".[81] These two states are "DOWN", sometimes called *anti* [d], and "UP", sometimes called *syn* [u]. This definition is also illustrated in Scheme 4. The DOWN C_γ-puckering [d] is characterized by positive values of χ_1 and χ_3 and negative values of χ_2 and χ_4, and vice versa for the "UP" ring puckering [u], which is characterized by negative χ_1 and χ_3 and positive χ_2 and χ_4 (Schemes 2 and 4). In some cases, these definitions are not completely satisfied, although the conformers in question (mostly) resemble the DOWN-puckering type of conformer. These particular conformers are marked as [d]*. They are considered to be completely [d] unless otherwise indicated.

TABLE 1: Total Population Distribution (%) of Compounds 1 and 2

	gas phase	in H_2O	exp., in H_2O[a]
	Compound **1**		
cis isomers	74.92	29.15	23
trans isomers	25.08	70.85	77
	Compound **2**		
cis isomers	68.63	57.75	53
trans isomers	31.37	42.25	47

[a] References 35 and 36.

The *trans* ($\omega = 180°$) and *cis* ($\omega = 0°$) conformations of proline-containing peptides are defined by the orientation of the methyl carbon of the terminal proline *N*-acetyl group and the C_α-carbon of the proline residue, Schemes 1 and 2. They are denoted by "t" and "c", respectively. On the basis of the three parameters (backbone conformation angles ϕ and ψ, ring puckering, and peptide bond isomer), a total of 12 different types of conformations is theoretically possible.

Results

The total conformational distribtutions for compounds **1** and **2** in the gas phase and water are presented in Table 1. Detailed information on the energies and geometries of the most stable conformers (those that contribute at least 0.5% to the total distribution, according to Boltzmann statistics at 25.0 °C) is provided in Tables S1−S4 of the Supporting Information. Selected additional geometry parameters for the most stable conformers of compounds **1** and **2** in water are provided in Table 2 and Tables S5 and S6 of the Supporting Information; see also below.

Prolyl Amide *cis/trans* Distributions. The calculated population distributions in the gas phase do not match the experimental observations that predict a strong preference for the *trans* conformers of compound **1**, while the preference for the *cis* prolyl amide population of compound **2** in solution is correctly predicted, Table 1.[35,36] Indeed, gas phase calculations predict about 75 and 69% *cis*-populations for peptide mimics **1** and **2**, respectively. This is significantly changed when the effects of the bulk solvent are included. The distribution is reversed to 29% *cis* (71% *trans*) for compound **1**, and it is reduced to 58% *cis* (42% trans) for compound **2**. With the inclusion of the solvent, the calculated distributions of prolyl amide *cis*/*trans* isomers in peptide mimics **1** and **2** are in good agreement with the experimental data determined by ^{1}H NMR, Table 1.[35,36] To understand this effect, we note that polar solvent such as water will stabilize different isomers to differing degrees, depending on the details of the electronic distributions of the conformers (dipole and higher-order moments). To first order, the free energy of solvation for such neutral species is proportional to the dipole moment squared.[55] For example, the most stable conformers in the gas phase for compound **1**, which are all *cis*, are not the most stable structures in water anymore. The higher energy gas-phase *trans* conformers are the most stable conformers in water, while the most stable *cis* conformers in the gas phase became higher energy conformers in water. This could be due to stabilizing interactions between the electronic distribution of the conformers that tend to stabilize trans conformers relative to the cis conformers.

Conformers and Relative Energies in the Gas Phase and Aqueous Solution. For compound **1**, nine types of local minima, t-ε_L[d], t-α_L[d], t-ε_L[u], t-ε_L[d]*, c-ε_L[u], c-α_L[d], c-ε_L[d], c-α_L[d]*, and c-ε_L[d]*, were found for the gas phase, whereas only four types of local minima, t-ε_L[d], t-α_L[d], c-ε_L[d], and c-α_L[d], were identified in water. Representative examples for these conformers are shown in Figures 1 and S1 (Supporting Information), respectively.

In the gas phase, the relative energies of the conformations of the peptide mimic **1** follow the order c-ε_L[u] < c-α_L[d] < c-ε_L[d] < t-ε_L[d] < t-α_L[d] < t-ε_L[u] (Figure S1, Supporting Information). The most stable conformer, c-ε_L[u], is characterized by a *cis* prolyl amide bond, the ε_L backbone conformation, and "UP" puckering (C_γ-exo) in which the reference plane is defined by C_β, C_α, N, and C_δ. It should be noted that the energy difference between c-ε_L[u], c-α_L[d], and c-ε_L[d] is very low (less than 0.1 kcal/mol) relative to the energy difference between t-ε_L[d], t-α_L[d], and t-ε_L[u], which is approximately 0.33 kcal/mol. This could be because the hydrogen-bonding interactions stabilize the *cis* structures more than the *trans* structures; see below. The relative energy difference between the most stable *cis* and *trans* conformers of compound **1** is approximately 0.36 kcal/mol.

For compound **1** in water, t-ε_L[d] is the most stable conformer, and the relative energies of the conformations are calculated to be t-ε_L[d] < t-α_L[d] < c-ε_L[d] < c-α_L[d] (Figure 1). The lowest energy conformer, t-ε_L[d], in water is characterized by a *trans* prolyl amide bond, the ε_L backbone conformation, and the "DOWN" puckering (C_γ-endo). However, the low absolute value for the dihedral angle χ^4 when compared to χ^0 for the most

TABLE 2: Backbone Torsion Angles, Endocyclic Torsion Angles, Relative Energies (ΔE, kcal/mol), and Conformational Distribution of the Selected *cis*−*trans* Isomers of Compounds 1 and 2, Optimized at the B3LYP/6-31+G(d, p) Level of Theory in Water (See Tables S2 and S4 of the Supporting Information for More Complete Data)

	B3LYP/6-31+G(d, p)		backbone torsion angles and endocyclic torsion angles[a]								
conformers	H_2O (in kcal/mol) ΔE	conformer distribution (%)	ω'	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	χ^0
			Compound **1**								
1, t-ε_L[d]	0.0[b]	4.7002	175.253	−64.198	153.736	177.502	28.915	−34.773	26.879	−8.378	−13.164
3, t-α_L[d]	0.12174	3.8271	175.55	−61.41	−28.681	−179.498	27.616	−33.469	26.027	−8.356	−12.356
11, c-ε_L[d]	0.54656	1.8683	−10.903	−63.708	154.37	177.909	31.884	−34.728	23.936	−3.193	−18.36
17, c-α_L[d]	0.68963	1.4674	−10.68	−62.642	−27.379	−177.946	30.963	−34.117	23.815	−3.727	−17.428
			Compound **2**								
1, c-ε_L[d]	0.0[b]	4.1504	4.031	−81.347	154.516	176.927	32.683	−37.378	27.191	−6.035	−16.964
3, t-ε_L[d]*	0.01757	4.0291	−170.077	−85.486	154.199	177.889	32.851	−33.196	20.473	1.061	−21.596
13, c-α_L[d]	0.42733	2.0175	3.512	−81.193	−28.105	−177.812	32.361	−36.72	26.421	−5.464	−17.112
20, t-α_L[d]*	0.64006	1.4089	−170.052	−84.046	−27.779	−179.098	32.501	−32.811	20.21	1.031	−21.333

[a] Defined in Scheme 2; angles in degrees. [b] Total energy $E = -1279.13604$ a.u. The population distributions were calculated using Boltzmann statistical weights at 25 °C.

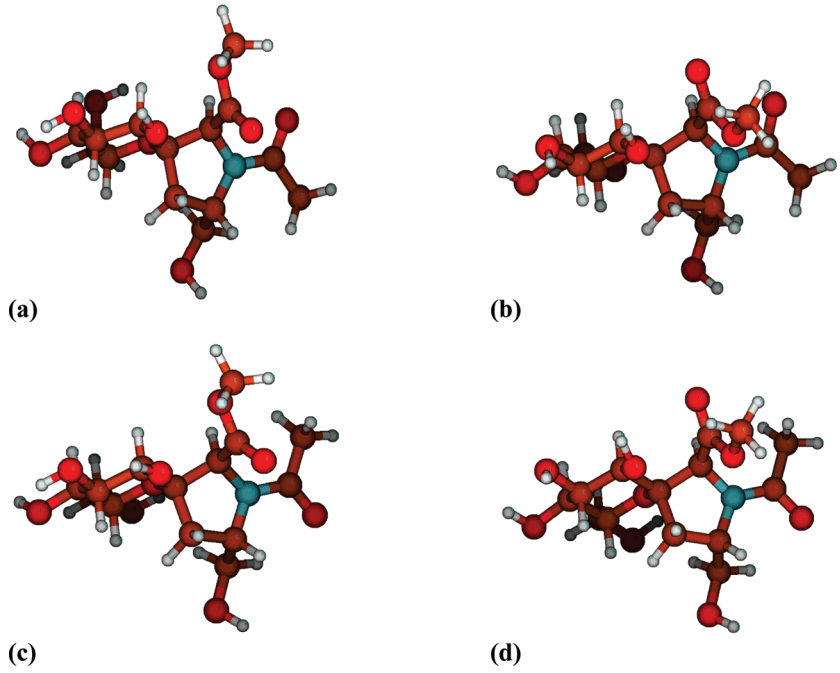


Figure 1. Representative solution-phase (water) optimized structures of compound **1**: (a) t-ε_L[d]; (b) t-α_L[d]; (c) c-ε_L[d]; (d) c-α_L[d].

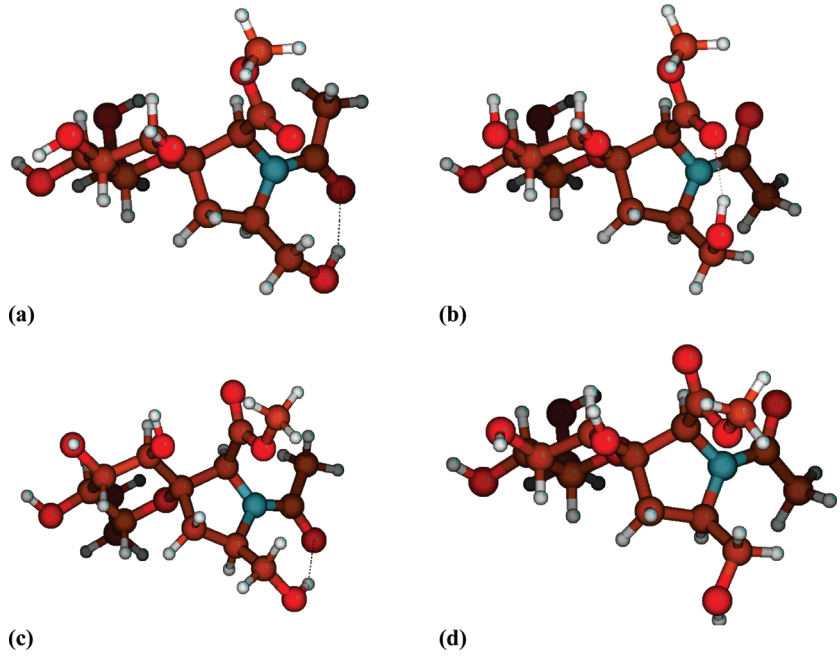


Figure 2. Representative solution-phase (water) optimized structures of compound **2**: (a) c-ε_L[d]; (b) t-ε_L[d]*; (c) c-α_L[d]; (d) t-α_L[d]*.

stable *trans* conformers of **1** in water indicates that the preferred pucker of *cis or trans* prolyl amide **1** is better described as a C_β-exopucker than C_γ-endopucker, with the basal plane defined by C_α, N, C_δ, and C_γ. Depending on the extent of the puckering degrees, these two terms will be used interchangeably. The relative energy difference between t-ε_L[d] and t-α_L[d] is about 0.12 kcal/mol, while there is a gap of 0.14 kcal/mol between c-ε_L[d] and c-α_L[d]. Thus, unlike in the gas phase, the relative energy differences among *cis* and among *trans* conformers are almost identical in water. The relative energy difference between the most stable *cis* and *trans* conformers in water is about 0.54 kcal/mol.

We will now consider compound **2**. In the gas phase, eight types of local minima, c-ε_L[u]*, t-ε_L[u]*, c-α_L[d], c-ε_L[u], c-ε_L[d], c-ε_L[d]*, t-ε_L[d]* and t-γ_L[d], were identified on the basis of the backbone and endocyclic torsion angles. Likewise, seven types of local minima, c-ε_L[d], t-ε_L[d]*, c-α_L[d], c-ε_L[d]*, t-α_L[d]*, t-α_L[d], and t-ε_L[d], were found in water. Representative examples for these conformers are shown in Figures 2 and S2 (Supporting Information), respectively. The corresponding backbone torsion angles are provided in Table 2.

The relative energies of the conformations in the gas phase are determined in the order c-ε_L[u]* < t-ε_L[u]* < c-α_L[d] < c-ε_L[u] < c-ε_L[d] < c-ε_L[d]* < t-ε_L[d]* < t-γ_L[d]. The most stable conformer c-ε_L [u]* is characterized by a *cis* prolyl amide bond, an ε_L backbone conformation, and distorted "UP" puckering (distorted C_γ-exo). The small values for the dihedral angles χ^1 and χ^2 and the large values for χ^4 or χ^3 indicate the presence of distorted puckering. The relative energy difference between the c-ε_L[u]* and c-ε_L[u] conformers of the *cis*-compound **2** of

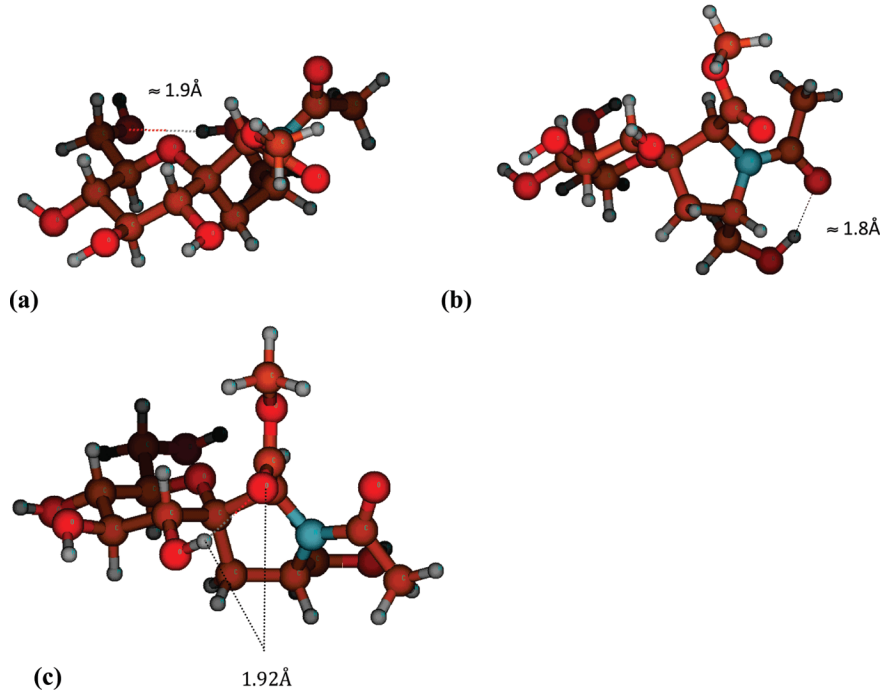


Figure 3. The three types of hydrogen bonds that exist in water for compound **1**: (a) C_δ—CH_2—OH···HO—C_6; (b) C_δ—CH_2—OH···O═C—N; (c) C_2—OH···O═C—C_α.

approximately 1.1 kcal/mol indicates that the distortion of the "UP" ring puckering stabilizes the structure by this amount if all other torsional angles remain the same for both structures. The relative energy difference between the most stable *cis* and *trans* conformers of compound **2** is approximately 0.11 kcal/mol. We note the appearance of a γ_L backbone conformation among the important *trans* conformers, which corresponds to a reverse γ turn.

The most stable conformer of compound **2** in water is characterized by a *cis* prolyl amide bond, an ε_L backbone conformation, and "DOWN" puckering (C_γ-endo). However, the smaller absolute value of the dihedral angle χ^4 when compared to χ^1 indicates that the pucker of the eight most stable conformers may be better described as C_β-exo. The relative energies of the conformations in water were obtained in the order of c-ε_L[d] < t-ε_L[d]* < c-α_L[d] < c-ε_L[d]* < t-α_L[d]* < t-α_L[d] < t-ε_L[d]. The relative energy difference between c-ε_L[d] and c-α_L[d], the two most stable *cis* conformations of compound **2** in water, is approximately 0.42 kcal/mol. This indicates that the backbone ε_L stabilizes the structure much better than α_L. It could be hypothesized that this energy difference between the two conformers might be relevant for the induction of secondary structures such as β-turn motifs and polyproline helices. The relative energy difference between the most stable *cis* (c-ε_L[d]) and the most stable *trans* (t-ε_L[d]*) conformers is only 0.018 kcal/mol, meaning that the molecules are effectively thermoneutral. Additionally, in water, the "UP" puckering conformation is not found, unlike in the gas phase. Instead, the "DOWN" puckering conformers are much more stable in water for compound **2**.

In the following, we discuss qualitative geometry features of the most important conformers of compounds **1** and **2** in water, i.e., those with >0.5% contribution. (We do not discuss the gas-phase conformers any further, since their distribution is very different from the experimental observations.) For these conformers, the six-membered sugar residue is always in a chair conformation, which is consistent with the NMR structure of the compound in solution.[82]

An inspection of the geometric parameters of compound **1** in water (Tables S5 and S6, Supporting Information) indicates that the bond angles of the five-membered prolyl residue in the *cis* and *trans* conformations deviate by less than 0.5° from one another. This suggests that the *cis*−*trans* isomerization of the prolyl amide does not have a great impact on the five-membered pyrrolidine ring. Furthermore, the $\angle C^\delta$−N−C^α bond angle around the N-terminal shows a larger deviation of 8−11° than that for the other bond angles.

Completely analogous, an inspection of the geometric parameters of compound **2** in water shows that the bond angles of the pyrrolidine five-membered prolyl residue in the *cis* and *trans* conformations are similar. This suggests again that the *cis*−*trans* isomerization of the prolyl amide does not have a great impact on the five-membered pyrrolidine ring.

The various angles characterizing the structures of compounds **1** and **2** (Scheme 2) fall into distinct ranges, as would be expected, Table 2. A detailed summary thereof is provided in the Supporting Information (Tables S1−S4). We note that there are marked differences in the values of the backbone torsion angles and endocyclic angles between the gas phase and aqueous solution, illustrating the importance of full optimizations in the presence of the solvent.

Intramolecular Hydrogen Bonding in Solution. The most stable conformers in water have been investigated for internal hydrogen bonding using a bond length cutoff of 2.5 Å, as discussed in the Computational Details section. We have focused exclusively on the aqueous solution results, since the gas phase calculations do not reproduce the observed *cis*−*trans* ratios (see above). On the basis of this criteria, we found three types of hydrogen bonds in compound **1** in water for the conformations that contribute a population distribution of >0.5%, Figure 3. Likewise, two major types of hydrogen bonds were found for compound **2**, Figure 4.

In compound **1**, the first major type of hydrogen bond exists between the prolyl C_δ-hydroxymethyl group and the primary sugar hydroxyl group at C6 (C_δ—CH_2—OH···HO—C_6, see Figure 3a). The bond distance is approximately 1.9 Å. This

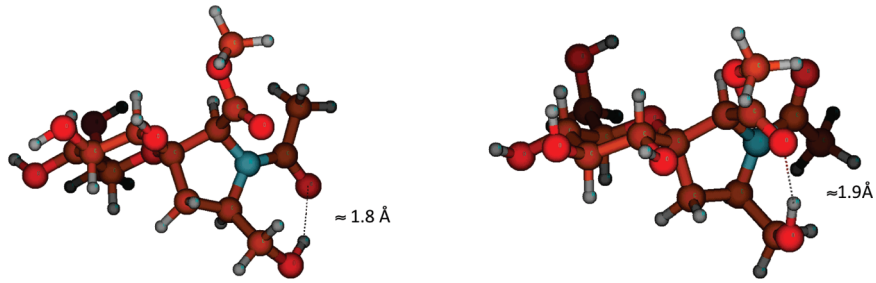


Figure 4. The two types of hydrogen bonds that exist in water for compound **2**: (a) C_δ—CH_2—OH···O=C—N; (b) C_δ—CH_2—OH···O=C—C_α.

hydrogen bond was found in prolyl amide *trans* conformers (10, 16, 23, 30, 37, 56; Table S2, Supporting Information), which contribute about 7% of the total distribution. The second type of hydrogen bond exists between the prolyl C_δ-hydroxymethyl group and the prolyl N-terminal carbonyl oxygen (C_δ—CH_2—OH···O=C—N, see Figure 3b) and is found in *cis* conformers only. The conformers possessing this hydrogen bond (22, 25, 32, 46, 48, 53, 84; Table S2, Supporting Information) contribute 5.7% of the total distribution. The bond distance of this hydrogen bond (∼1.8 Å) is shorter than the previous hydrogen bond. The third type of intramolecular hydrogen bond exists between the sugar C-2-hydroxyl group and the C-terminal carbonyl (C_2—OH···O=C—C_α, see Figure 3c). It is found in only one of the 43 *trans* conformers (conformer 55; population 0.6%, Table S2, Supporting Information). We note that these three types of hydrogen bonds do not exist at all in the most stable *cis* or *trans* conformers.

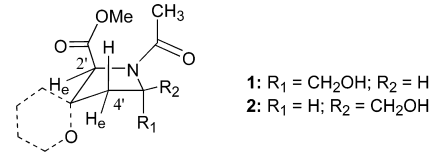
For compound **2**, two major types of hydrogen bonds were found (Figure 4). The first type occurs between the prolyl C_δ-hydroxymethyl group and the prolyl N-terminal carbonyl oxygen (i.e., C_δ—CH_2—OH···O=C—N, see Figure 4a) in several *cis* conformers. The bond distance is approximately 1.8 Å. A total of 21 *cis* conformers, including the most stable structure, possess this type of hydrogen bond (conformers 1, 2, 4, 6, 7, 8, 12, 13, 17, 18, 19, 21, 22, 25, 33, 37, 44, 47, 48, 51, 55; Table S4, Supporting Information). These conformers contribute 37.3% of the total population distribution. The second type of intramolecular hydrogen bond exists between the prolyl C_δ-hydroxymethyl group and C-terminal carbonyl (C_δ—CH_2—OH···O=C—C_α, see Figure 4b). It is found in both *cis* and *trans* conformations and has a longer bond length (∼1.9 Å). The *cis* conformers possessing this type of hydrogen bond contribute approximately 7% to the total distribution (conformers 15, 16, 23, 30, 39, 52; Table S4, Supporting Information), whereas the respective *trans* conformers contribute approximately 13.9% (conformers 3, 5, 10, 32, 36, 40, 49, 50, 53; Table S4, Supporting Information).

At least one of the above-mentioned hydrogen bonds exists in the most stable conformers of compound **2**. This indicates that intramolecular hydrogen bond formation in compound **2** has a more pronounced effect on prolyl amide *cis*/*trans* isomerization when compared to compound **1**.

Discussion

Conformational analysis of the pyrrolidine ring in the highly populated conformers in peptide mimics **1** and **2** indicates that the pucker resembles "DOWN" (C_γ-endo) or C_β-exo pucker. The C_β-exo conformation has been previously observed in the crystal structure of 3(*S*)-hydroxyproline containing model peptides.[83] This conformation places the endocyclic oxygen

SCHEME 5: C_β-Exo Conformations of Compounds 1 and 2[a]



[a] The substitutents on the glucose ring are omitted for clarity.

substituent in an axial position as observed for *trans*-3(*S*)-hydroxyproline-containing dipeptides.[84] In this conformation, the pyrrolidine ring is stabilized by a gauche interaction between the endocyclic nitrogen and endocyclic oxygen and a stabilizing $\sigma(C^\gamma—H) \rightarrow \sigma^*(C^\beta—O)$ interaction. A similar C^β-exo conformation was also observed in the more lipophilic silaproline (Sip) analogue, Scheme 5.[82]

Previous studies have shown that the hydroxyl group at the β- or γ-position in proline affects the puckering of the pyrrolidine ring in the model peptides without influencing the kinetics and thermodynamics of prolyl amide *cis*/*trans* isomerization.[8,10,36] These findings are in contrast to our results obtained with Glc3(*S*)-5-(CH_2OH)HypHs-containing peptide mimics **1** and **2**. Compounds **1** and **2** demonstrate that the kinetics and thermodynamics of *cis*/*trans* isomerization are greatly affected by the presence of polar groups either by hybridization with D-glucose or incorporation of a hydroxymethyl substituent into the C_δ-position of proline.[28] The stereochemistry of the hydroxymethyl substituent at the δ-position influences both the rate of *cis*/*trans* isomerization and the stability of the *cis*/*trans* isomers.[28] Compared to proline and 3(*S*)Hyp-containing peptide mimics, both Glc3(*S*)-δ-(CH_2OH)HypHs-modified peptide mimics **1** and **2** display a higher *cis* isomer population.[28] This is most likely the result of intramolecular hydrogen bonding between the C_δ-hydroxymethyl group and the N-terminal carbonyl of proline. Experimental evidence for the presence of this intramolecular hydrogen bond was previously obtained via measurements of the temperature coefficients for all hydroxyl groups in DMSO-d_6.[28] These measurements indicate that the hydroxymethyl group located at the C_δ-position of proline is involved in intramolecular hydrogen bond formation to an acceptor atom.[28] Analysis of the most stable prolyl amide *cis* conformers in **1** and **2** indicates the presence of a hydrogen bond between the C_δ-hydroxymethyl group and the carbonyl group of NAc (C_δ—CH_2OH···O=C—N). The higher *cis* isomer ratio in **2** is due to the formation of a second hydrogen bond involving the C_δ-hydroxymethyl group and the C-terminal carboxymethyl group. Alternatively, the *trans* prolyl amide bond in **1** may be stabilized by the formation of a hydrogen bond between C_δ—CH_2OH and the primary sugar hydroxyl group 6′-OH. In addition, the calculations support the notion that

the H-bond between C_δ—$CH_2OH\cdots O{=}C$—N is stronger in compound **2** when compared to **1**. For instance, for compound **2**, 10 out of the 20 most stable conformers possess this type of H-bond, while the same H-bond was not present in the 20 most stable conformers of compound **1**.

Conclusion

We have studied the thermodynamic properties of two polyhydroxylated hydroxyproline-containing peptide mimics **1** and **2** using DFT calculations. A reliable computational protocol has been developed in the form of the "Build and Search" approach to ensure complete coverage of the large conformational space of these highly flexible molecules. Particular care in this respect has been necessitated by the existence of the five- and six-membered rings that render the sole application of systematic search approaches impractical. In addition, systematic search methods are entirely impractical for larger molecules due to the enormous computational cost. On the basis of the "Build and Search" protocol, we have found 355 and 258 gas-phase conformers for compounds **1** and **2**, respectively, at the B3LYP/6-31+G(d,p) level of theory.

Gas-phase calculations result in a strong preference for prolyl amide *cis* conformers, which is at odds with experiment.[35,36] However, after inclusion of the polarizable solvent (water), we achieve excellent agreement with experiment for the *cis/trans* distribution. Clearly, this is a differential effect of the electronic distribution in the molecule, which can be represented by the dipole and higher-order multipole moments. Solvation is also seen to have a strong influence on various geometrical parameters. This emphasizes the need for full optimizations and frequency calculations in the presence of the solvent. On the other hand, the relatively simple PCM continuum solvation model appears to be sufficient to capture the essential effects.

Computational modeling of the pyrroldine ring indicates that the pyrrolidine ring prefers a "Down" pucker in **1** and **2**, which is closely related to the C^β-exo pucker. Our study indicates that the insertion of polar substitutents capable of forming hydrogen bonds in the δ-position of proline greatly impacts the kinetics and thermodynamics of prolyl amide *cis*/*trans* isomerization. Our calculations demonstrate that the observed differences in prolyl amide isomerization are not due to conformational changes in the pucker of the pyrrolidine ring but rather are the result of intramolecular hydrogen bonding in water. The preferable adoption of the prolyl amide *cis* conformation in model peptide **2** permits its use as a selective *cis*-XAA-GlcProH bond inducer to chemically introduce constraint into peptides and proteins and to test the *cis*-imide bond as a structural requirement for the bioactive conformation. Moreover, the presence of the unprotected glucose moiety in GlcProH provides opportunities to explore the effect of glycosylation in unusual glycopeptides, while decoration of the gluco-based polyol scaffold provides rich opportunities to tailor the physical, chemical, hydrophobic, lipophilic, nucleophilic, and pharmacodynamic properties of proline mimetics and proline-containing peptidomimetics.

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Supporting Information Available: Full citation for refs 42 and 69, Figures S1 and S2 showing selected gas-phase geometries, geometrical parameters and conformational distributions of the most important isomers of compounds **1** and **2** in the gas phase and aqueous solution (Tables S1−S4), selected bond angles and bond lengths (Tables S5 and S6), and further discussion of these geometrical parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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