Theoretical Study on Side-Chain Control of the 14-Helix and the 10/12-Helix of β-Peptides

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Abstract: The conformational features of a series of β-peptide models **1**−**11** have been studied by the molecular mechanics MM2* force-field and quantum mechanics methods. The geometries were optimized by the HF/6-31G** method. Energies were evaluated using the B3LYP/6-31G** method including solvent effect (SCIPCM). For the unsubstituted β-tripeptide model **1**, calculations indicate that a 12-membered-ring hydrogen-bonded structure and a 10-membered-ring hydrogen-bonded structure are low in energy. The coupling of these two structures forms the repeating unit for the 10/12-helix, indicating an intrinsic preference of the 10/12-helix for a β-polypeptide. Indeed, calculations predict that an unsubstituted β-heptapeptide model **2** favors the 10/12-helix over the 14-helix by 21.4 and 4.8 kcal/mol in the gas phase and methanol solution, respectively. The side-chain effect on the relative preferences of the 14- and the 10/12-helices is analyzed based on torsional and steric effects, and has been tested by the calculations on β-peptide models **3**−**11**. The methyl groups in (*S*)-β^2/β^3-polypeptide **9** and (*S*)-β^2-polypeptide **11** have little torsional and steric effects for right-handed 10/12-helix and left-handed 14-helix, and these β-peptides are predicted to adopt the intrinsically favored 10/12-helix. On the other hand, (*S*)-β^3-polypeptide **10** prefers to form a left-handed 14-helix in a polar solvent mainly because of torsional effects by three of the methyl groups in the 10/12-helix. The current study can be extended to evaluate the stabilities of the 10/12- and 14-helices for other sequences. For example, the 10/12-helix is predicted to be the accessible conformation for (*R*)-β^3/(*S*)-β^3-, (*S*)-β^2/(*R*)-β^2-, (*S*)-β^2/(*R*)-β^3-, and (*R*)-β^3/(*S*)-β^2-polypeptides.

Introduction

Unnatural oligomers that are able to form well-defined novel secondary structures have received intensive attention over the past few years.[1−2] The molecular designs around this topic often involve unnatural amino acids, such as β-amino acids,[3−6] γ-amino acids,[7] δ-amino acids,[8] α-aminoxy acids,[9] ω-amino acids,[10] α,α-disubstituted amino acids,[11] and *N*-alkylated amino acids.[12] Many of these are known to exhibit interesting secondary structures. In particular, β-peptides, consisting exclusively of β-amino acids, have recently emerged as a promising new class of compounds capable of forming stable helix, pleated-sheet, and turn secondary structures.[3−5]

Three different helical secondary structures (12-helix, 14-helix, and 10/12-helix) have been identified for β-peptides so far. Seebach et al. found that β^2- and β^3-peptides (**A** and **B**) favor a 3_1-helical structure (14-helix, **G**);[5a,f] Gellman's group reported that β-peptides with trans-substituted cyclohexane rings (**C**) strongly favor a 14-helix[4a,13] while β-peptides with trans-

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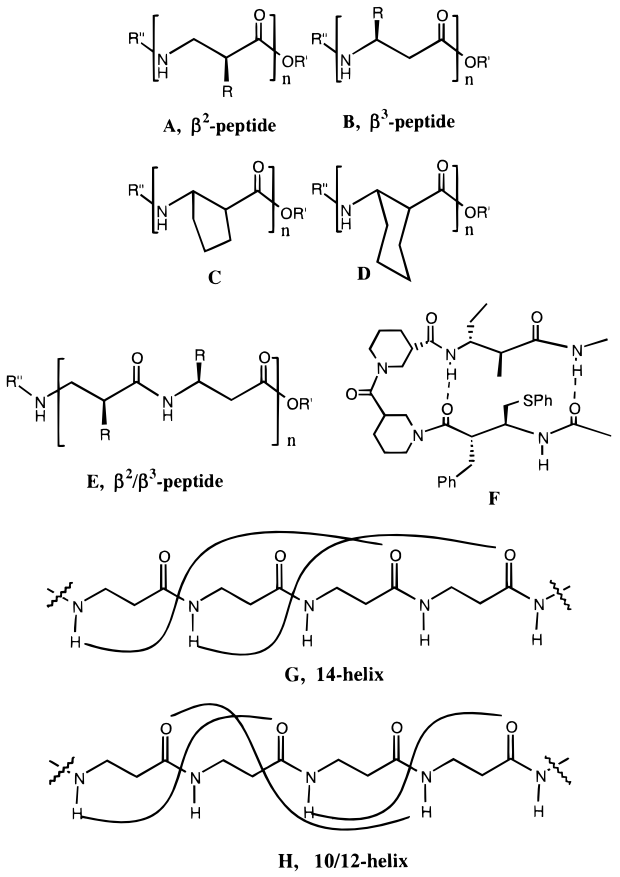
substituted cyclopentane rings (**D**) adopt a helical structure with 12-membered-ring hydrogen bonds (12-helix).[4b,14] More recently, Seebach et al. reported that several peptides with alternate α- and *β*-alkyl substitutions exist in a 12/10/12 sequence of hydrogen bonding pattern (10/12-helix, **H**).[5g,h] For example, an (*S*)-β^2/(*S*)-β^3-hexapeptide (**E**) appears to be in the 10/12-helix in both protected and nonprotected salt form. Seabach et al. have also found that for several β^2/β^3-peptides, deprotection of the two termini changes the secondary structure from the 10/12-helix to the 14-helix.[5h] Gellman et al. also found that a hairpin structure can be achieved by two consecutive disubstituted *β*-amino acids (**F**).[15]

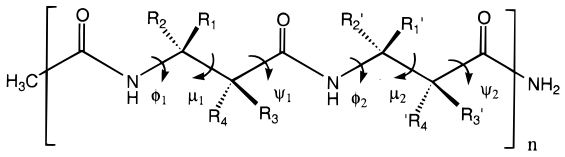
While the interesting chemistry of *β*-peptides is unfolding, a general understanding of the conformational features of *β*-peptides is still lacking.[16] We have reported a theoretical study on the conformational features of *β*-dipeptides.[17] We found that *β*-dipeptides have a tendency to form folded structures. That is, many conformational minima with gauche dihedral angles μ (see Scheme 2 for definition) can be located. In particular, solvent plays an important role in conformational preferences. Thus, conformations corresponding to the formation of the 12- and 14-helical structures are very unstable in the gas phase, but can be significantly stabilized by polar solvent effect. Two conformations corresponding to the formation of the 10/12-helix can also be located. However, such dipeptide models do not allow us to discuss the substituent effect on the formation of the 10/12-helix.

This paper reports an ab initio quantum mechanics study on peptide models **1**−**11**. First, we focus our attention on locating low-energy conformations, especially those corresponding to the secondary structure formation of *β*-tripeptide model **1**. We show that the global minimum of **1** in the gas phase is exactly the conformer that leads to the 10/12-helix and the dihedral angle constrained 12- and 14-helical conformers are still very high in energy. Second, we predict that unsubstituted *β*-heptapeptide model **2** intrinsically favors the 10/12-helix over the 14-helix both in the gas phase and in solution. Third, using a series of tri- and heptapeptide models **3**−**11**,[18] we show that the preferences for the 10/12-helix and the 14-helix are highly dependent upon the side-chain substitution pattern. Our calculations not only are in agreement with available experimental observations, but also allow the prediction for conformational preference of a wide range of *β*-peptide sequences. For example, we predict that several types of *β*-peptides such as (*R*)-β^3/(*S*)-β^3-, (*S*)-β^2/(*R*)-β^2-, (*S*)-β^2/(*R*)-β^3-, and (*R*)-β^3/(*S*)-β^2-polypeptides might also prefer the 10/12-helix. Thus, the current study makes suggestions for further experimental study. It should be useful for the design of peptides with special conformational features, which is of great importance in drug design and molecular design.[19]

Scheme 1



Scheme 2



1.	all Rs = H	n = 1	*β*-tripeptide
2.	all Rs = H	n = 3	*β*-heptapeptide
3.	$R_1 = CH_3$, $R_1' = CH_3$	n = 1	(*S*)-β^3/(*S*)-β^3-tripeptide
4.	$R_3 = CH_3$, $R_1' = CH_3$	n = 1	(*S*)-β^2/(*S*)-β^3-tripeptide
5.	$R_2 = CH_3$, $R_4' = CH_3$	n = 1	(*R*)-β^3/(*R*)-β^2-tripeptide
6.	$R_3 = CH_3$, $R_3' = CH_3$	n = 1	(*S*)-β^2/(*S*)-β^2-tripeptide
7.	$R_2 = CH_3$, $R_1' = CH_3$	n = 1	(*R*)-β^3/(*S*)-β^3-tripeptide
8.	$R_3 = CH_3$, $R_4' = CH_3$	n = 1	(*S*)-β^2/(*R*)-β^2-tripeptide
9.	$R_1 = CH_3$, $R_1' = CH_3$	n = 3	(*S*)-β^3/(*S*)-β^3-heptapeptide
10.	$R_3 = CH_3$, $R_1' = CH_3$	n = 3	(*S*)-β^2/(*S*)-β^3-heptapeptide
11.	$R_3 = CH_3$, $R_3' = CH_3$	n = 3	(*S*)-β^2/(*S*)-β^2-heptapeptide

Computational Methodology

In light of our previous calculation results on *β*-dipeptide models,[17] which suggest that the MM2*[20] force field can give relatively reasonable conformations and energetics compared to the ab initio results, we first

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carried out a Monte Carlo[21] conformational search for β-tripeptide model **1** using the MM2* force field with the Macromodel 6.0 program.[22] Totally, 5000 structures were optimized during the conformational search, and conformations within 50 kJ/mol with respect to the most stable conformation were accumulated. To ensure that all the conformers in the low-energy area were obtained, another conformational search starting with the lowest energy conformer that had been found in the first run was carried out and the same result was obtained. On the basis of the conformational search results, the 30 lowest energy conformers (within about 4.0 kcal/mol relative to the global minimum) and all other conformers with 10-m-r or 12-m-r hydrogen bonding were optimized with the HF/3-21G method of calculation using the GAUSSIAN94 program.[23] Finally, the 10 lowest energy conformers obtained by the HF/3-21G calculations were further optimized at the HF/6-31G** level. Conformations that correspond to the 12-helix and 14-helix were also calculated by the HF/6-31G** method. The dihedral angles ϕ, μ, and ψ of these two conformations were constrained at the values obtained previously for a β-hexapeptide,[17] to mimic the helical structure.

For substituted β-tripeptide models **3**−**8**, the focus was on the preference for the formation of different helical structures. Only two structures were optimized for each β-tripeptide model. One is the fully optimized 10/12-helical conformer and the other is the 14-helical conformer with torsional angle constraint. The geometry optimization was at the HF/6-31G** level.

Electron correlation energy is important to hydrogen bonding. To evaluate the energetics, single-point calculations were performed on the HF/6-31G** geometries of all the conformers of β-tripeptide models **1** and **3**−**8** with the density functional theory B3LYP/6-31G** method.[24] To account for the solvent effect on the conformational preferences, the energy of each structure was further calculated by the self-consistent isosurface polarization continuum model (SCIPCM)[25] at the B3LYP/6-31G** level. There has been ample evidence to suggest that this method is superior to the Orsager-based moethd,[26] but the calculation result could be sensitive to isodensity value. A dielectric constant of 33.0 was used to model methanol solvent in which most experimental observations were obtained. An isodensity value of 0.0004 au was used for all the calculations. To test the validity of the solvent model, we have carried out a Monte Carlo QM/MM real solvent (CH_3OH) simulation[27] for a simple β-dipeptide model $CH_3C(O)NHCH_2$-$CH_2C(O)NH_2$. The solvent effects for the conformational preferences agree very well with those calculated by the SCIPCM method.[17,28]

For β-heptapeptide models **2** and **9**−**11**, the 14-helix and 10/12-helix were fully optimized with the HF/6-31G* method. The energies and solvent effect were also evaluated with the B3LYP/6-31G* method.

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Results and Discussion

Unsubstituted β-Tripeptide Model 1. The Monte Carlo conformational search for model **1** resulted in 159 unique conformers with the MM2* force field. Among these conformers, 21 were in 10-m-r or 12-m-r hydrogen-bonded structures, and 10 were among the 30 lowest energy conformers. In the second process, the 30 lowest energy conformers along with the other conformers with 10-m-r or 12-m-r hydrogen bond were further studied with the quantum mechanics method. In total, 41 conformers were optimized with the HF/3-21G method. The dihedral angles (ϕ, μ, and ψ, for definition, see Scheme 2) and energies of the 41 MM2* conformations were collected in Table 1 of the Supporting Information. The HF/3-21G geometry optimizations resulted in 36 unique conformers, and their dihedral angles (ϕ, μ, and ψ) and total and relative energies are given in Table 2 of the Supporting Information; the stereoviews of the 36 conformers are given in Figure 1 of the Supporting Information.

The third step involved the optimization with the HF/6-31G** method of the 10 most stable conformers obtained by the HF/3-21G method. With two of the structures converted to one, a total of nine conformers (**1a**−**i**) were obtained, as shown in Figure 1. The relative energies and dihedral angles of these structures are presented in Table 1.

Conformers **1a** and **1b** have two consecutive C6 (formal 6-m-r hydrogen bond) local structures. While **1a** is in an extended helical form, **1b** forms a turn structure. As expected, the two conformers have very similar stabilities. Interestingly, these are not the global minima in the gas phase, but the SCIPCM calculation predicts that they are global minima in CH_3OH solution.

Conformers **1c** and **1d** have 12-m-r and 10-m-r hydrogen bonds, respectively. While **1c** is the global minimum in the gas phase, **1c** and **1d** have similar stabilities in CH_3OH solution, both about 1 kcal/mol less stable than **1b**. They have several similar structural features: (1) Both have a strong hydrogen bond, which is reflected by short O- - -H bond lengths (2.188 Å for **1c** and 2.153 Å, for **1d**) and large N−H- - -O bond angles (146.6° for **1c** and 152.8° for **1d**). (2) Apart from the hydrogen bond, there are several additional short carbonyl oxygen/amide hydrogen distances. The electrostatic interactions are partially responsible for the stability of the two conformers. (3) Both of them have alternate up and down carbonyl groups. (4) As indicated in Tabel 1, The six dihedral angles from ϕ_1 to ψ_2 are almost symmetrical, especially in **1d** (77.9°, 61.9°, −110.5°, −90.8°, 59.2°, and 81.4°). Most importantly, ϕ_1, μ_1, and ψ_1 of **1c** are almost the same as ϕ_2, μ_2, and ψ_2 of **1d**, and vice versa. All these indicate that if an additional β-peptide residue extends at either end of conformer **1c** or **1d**, a new 10-m-r or 12-m-r stable hydrogen bond will form. Continuation of this process leads to alternate 10-m-r and 12-m-r hydrogen bonded structures, or a 10/12-helix. Thus, the repeating unit in this kind of helix involves two residues and two sets of dihedral angles ϕ, μ, and ψ.

Conformer **1e** is also in a 12-m-r hydrogen-bonded structure with a short hydrogen-bond length of 2.194 Å and a good bond angle of 157.9°. It is a perfect turn structure. It is predicted that in CH_3OH this conformer is only slightly higher in energy than **1a** and **1b**. This suggests that a β-alanine-β-alanine unit might be a potential β-turn promoter in designing a hairpin structure. Indeed, this unit is found in many cyclic peptides, and has been suggested to form a turn structure.[29] This turn structure has been further recognized by Seebach et al. with β-peptides consisting of geminally disubstituted $\beta^{2,2}$- and $\beta^{3,3}$-amino acids.[30]

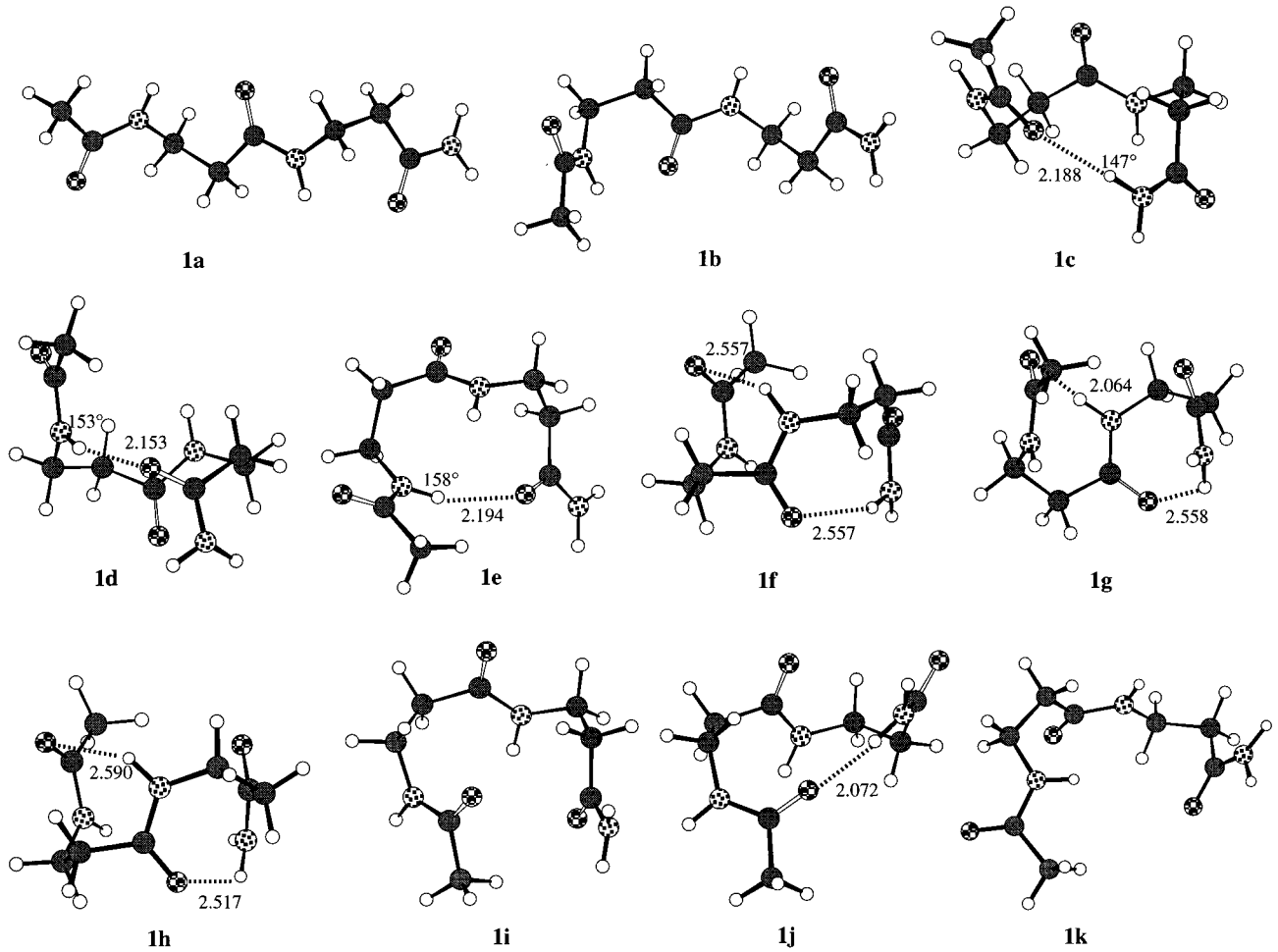


Figure 1. HF/6-31G** optimized structures of tripeptide model **1**. (a) 12-m-r conformer for mixed helix; (b) 10-m-r conformer for mixed helix; (c and d) 6-m-r conformers; (e) 10-m-r reverse turn conformer; (f−h) 8-m-r conformers; (i) 6-m-r and weak 12-m-r conformer; (j) torsion constrained 12-helix conformer; and (k) torsion constrained 14-helix conformer.

Table 1. Calculated Dipole Moments, Relative Energies, and Dihedaral Angles of Low-Energy Conformers, 12-Helix, and 14-Helix Conformers of β-Tripeptide Model **1**[a]

		rel energy(kcal/mol)			dihedral angles					
sructure	dipole(D)	HF	B3LYP	B3LYP (SCIPCM)	ϕ_1	μ_1	ψ_1	ϕ_2	μ_2	ψ_2
1a	6.6	0.6	1.4	−0.9	−99.2	−63.0	−176.7	−93.5	−62.6	−178.8
1b	5.8	0.7	1.3	−1.1	98.8	63.2	173.0	−99.6	−62.6	−177.4
1c	4.0	0.0	0.0	0.0	−97.2	57.2	100.4	81.8	60.0	−142.5
1d	4.8	1.6	1.8	0.1	77.9	61.9	−110.5	−90.8	59.2	81.4
1e	7.0	1.8	1.7	−0.6	92.0	71.2	−78.6	−69.7	−64.2	−160.0
1f	1.1	2.1	1.2	0.7	−60.0	−51.8	100.6	102.8	−61.3	−37.5
1g	3.9	3.8	2.6	0.6	111.6	−60.9	−14.2	−53.5	−45.0	113.9
1h	2.4	4.0	4.3	3.1	−57.2	−51.9	98.9	48.0	51.0	−120.8
1i	3.6	2.4	2.7	0.2	103.4	−79.9	98.0	82.0	66.0	173.9
1j	9.6	8.2	7.2	1.9	90.0	−89.0	110.0			
1k	10.0	11.0	11.6	4.6	−154.7	64.3	−135.9			

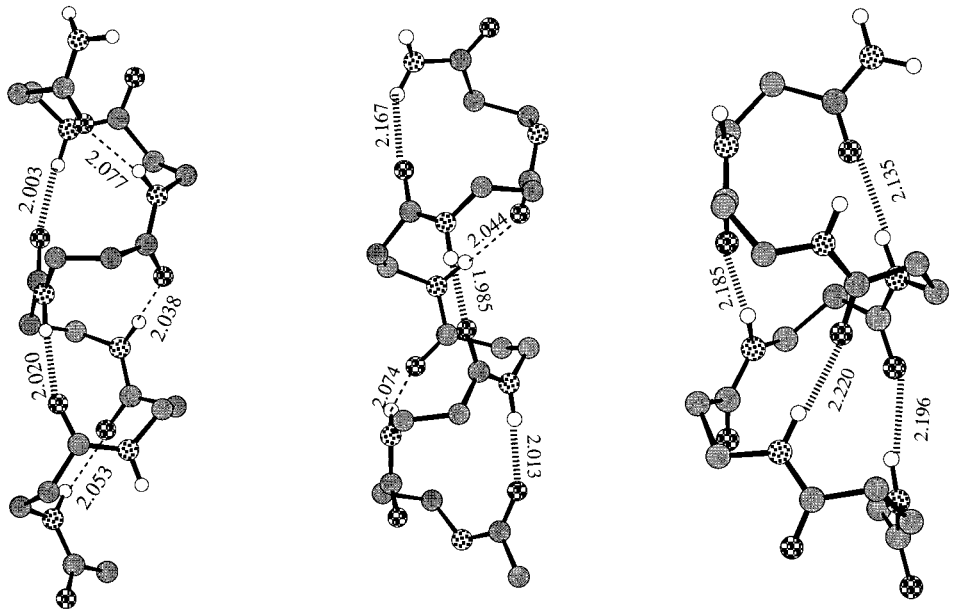
[a] All calculations are with the 6-31G** basis set on the HF/6-31G** geometries.

Conformers **1f** and **1g** can be considered as having two consecutive C8 local structures. A check of dihedral angles of the two conformers indicates that they have the same pattern as those in conformers **1c** and **1d**. Therefore, the combination of the two conformers also leads to a helical structure. However, this helical structure is higher in energy than the helix formed by **1c** and **1d** by about 1.5 kcal/mol for each two residues, and therefore, can be ruled out.

Conformer **1h** also has two consecutive C8 (8-m-r hydrogen bond) structures, which are in a reverse turn. This structure is quite high in energy, and can be ruled out. Conformer **1i** has one C6 unit and another in a conformation for the 12-helix. It is also a turn structure, but is about 1.3 kcal/mol less stable than **1b**.

Conformers **1j** and **1k** are dihedral angle-constrained 12-helical and 14-helical conformers, respectively. The 12-helical conformer (**1j**) is 1.9 kcal/mol higher in energy than conformer **1a** despite the already formed 12-m-r hydrogen bond; the 14-helical conformer is 4.6 kcal/mol higher in energy than **1a** in

(29) Pavone, V.; Lombardi, A.; Yang, X.; Pedone, C.; Di Blasio, B. *Biopolymers* **1990**, *30*, 189.
(30) Seebach, D.; Abele, S.; Sifferien, T.; Hänggi, M.; Gruner, S.; Seiler, P. *Helv. Chim. Acta* **1998**, *81*, 2218.



	2a, 10/12/10/12/10 helix	**2b**, 12/10/12/10/12 helix	**2c**, 14-helix
ΔE(Gas):	0.0 (0.0)	-1.4	24.0 (21.4)
ΔE(Sol.)	0.0 (0.0)	0.3	7.4 (4.8)
Dipole:	4.4	3.8	29.5

Figure 2. The HF/6-31G* optimized helical structures of heptapeptide model **2**. The relative energies (kcal/mol) are with the B3LYP/6-31G* method. The values in parentheses are relative Gibbs free energies.

solution. These indicate that there is an intrinsic preference for the 10/12-helix in β-polypeptides.

Unsubstituted β-Heptapeptide Model 2. The above calculations for the tripeptide model **1** suggest that several helical structures are possible for β-peptides. However, the 10/12-helix formed by the combination of **1c** and **1d** is predicted to be the most stable. This seems surprising because the first reported helices are 14- and 12-helices,[3−5] and the 10/12-helices that have been reported so far all contain a β^2- and β^3-mixed sequence. To verify the prediction and to investigate the structural features of the 10/12-helix, calculations on the β-heptapeptide model **2** were then studied. This molecule allows five consecutive hydrogen bonds for the 10/12-helix.[31] There are two possible helices depending upon the first hydrogen bonding: 12/10/12/10/12-helix and 10/12/10/12/10-helix. Both helices were studied. The 14-helical structure was also fully optimized for comparison. The 12-helical conformation was not calculated in this study because the 12-helix is less stable than the 14-helix in most cases when the solvation effect is taken into account as indicated by our previous calculations.[17]

The optimized structures and relative energies are given in Figure 2, and the results can be summarized as follows. (1) The 12/10/12/10/12-helix is more stable than the 10/12/10/12/10-helix by about 1.4 kcal/mol in the gas phase. But when the solvent effect is taken into account, they have comparable stabilities. This is almost the same as the energy difference between conformers **1c** and **1d** of the tripeptide model **1**, where the methanol solvent stabilizes **1d** (10-m-r) more than **1c** (12-m-r) by about 1.7 kcal/mol. (2) Compared to the 14-helix, the 10/12-helix can form more stable hydrogen bonds as indicated by the shorter hydrogen bond lengths in **2a** and **2b** (see Figure 2). (3) Due to the alternating up and down carbonyl groups, the 10/12-helices have small dipole moments of 3.8 and 4.4 D, respectively. The 14-helix, however, has a large dipole moment of 29.5 D. (4) The 14-helix structure is less stable than the 12/10-helix by about 25 kcal/mol in the gas phase. This destabilization is reduced to 7.1 kcal/mol in methanol solvent.

Structures **2a** and **2c** have also been optimized by the HF/3-21G method, and harmonic vibrational frequency calculations for the two structures were carried out at the same level to estimate the thermal properties.[32] Structure **2a** is destabilized by entropy and thermal energy correction by 2.6 kcal/mol at room temperature with respect to **2c**. Thus, the free energy of **2a** is still 4.8 kcal/mol smaller than that of **2c** in methanol solution, confirming an intrinsic preference for the 10/12-helix over the 14-helix.

Analysis of Substituent Effect on Helix Formation. Just as in α-peptides, the substituents play important roles in determining the secondary structure of β-peptides. To understand the substituent effect, it is beneficial to analyze the local conformational features of β-peptides. The conformational potential energy surfaces of 2-methylpropanamide and *N*-isopropylformamide were studied previously by Maxwell and Jorgensen using the HF/6-31G* method.[33] To make a more systematic comparison, we studied the two model systems with the B3LYP/6-31G** method, that is, constrained geometry optimization followed by single point energy evaluation with the SCIPMC calculation.

Our calculation results are shown in Figure 3. Solvent (methanol) has not much effect on the calculated potential energy surface (solid vs dash curves). The best conformation for 2-methylpropanamide has both methyl groups gauche to the carbonyl group. The conformational maximum has both methyl

(31) Daura, X.; Gademann, K.; Jaun, B.; Seebach, D.; van Gunsteren, W. F.; Mark, A. E. *Angew. Chem., Int. Ed. Engl*. **1999**, *38*, 236.

(32) The calculated energy, entropy, and thermal energy with the HF/3-21G method are −1673.78993 au, 213.7 cal/(mol·K), and 419.0 kcal/mol for **2a**, and −1673.74691 au, 217.6 cal/(mol·K), and 417.6 kcal/mol for **2c**.

(33) Maxwell, D. S.; Tirado-Rives, J.; Jorgensen, W. L. *J. Comput. Chem.* **1995**, *16*, 984.

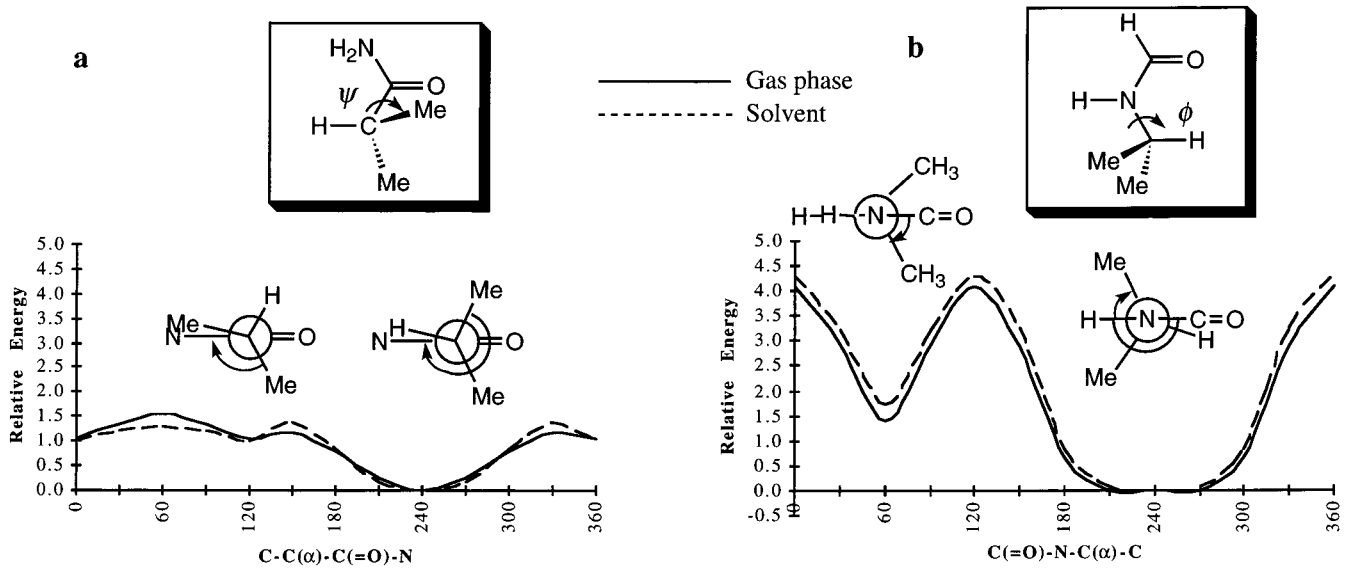


Figure 3. The B3LYP/6-31G** energy profiles of 2-methylpropanamide (a) and *N*-isoproylformamide (b), energy in kcal/mol.

groups gauche to the amino group. There is a shallow minimum when one of the methyl groups is eclipsed with the amino group. Overall, the potential energy surface for the Cα−C(=O) bond rotation is relatively flat, with the highest barrier of about 1.6 kcal/mol in the gas phase and 1.5 kcal/mol in methanol. For *N*-isopropylformamide, the situation is quite different. The conformational minimum has both methyl groups gauche to the amide N−H bond. The potential energy surface is very flat in the dihedral angle ϕ region of 200−280°. There is a local minimum in the vicinity of $\phi = 60°$, which is higher in energy by about 1.4 kcal/mol. The barrier of rotation is high, over 4 kcal/mol, and appears at the dihedral angle ϕ of 0° and 120°. This high barrier is apparently caused by the severe steric interaction between the methyl group and the carbonyl oxygen, which are eclipsing. These conformational features indicate that the β-substitution is more efficient than the α-substitution in reducing the flexibility of the β-peptide backbone.

Figure 4 shows the repeating units for the 10/12-helix and the 14-helix. Since the 10/12-helix has two alternate sets of dihedral angles ϕ, μ, and ψ and two alternate units (10-m-r and 12-m-r), there are eight unique substitution sites. Substitution at $\mathbf{R_1}$, $\mathbf{R_3}$, $\mathbf{R_1}'$, or $\mathbf{R_3}'$ by an alkyl group leads to an *S* configuration, and substitution at $\mathbf{R_2}$, $\mathbf{R_4}$, $\mathbf{R_2}'$, or $\mathbf{R_4}'$ by an alkyl group leads to an *R* configuration. The effect of alkyl substitution can be divided into two parts. One is the effect on local conformational stability, which can be analyzed qualitatively based on the potential energy profiles shown in Figure 3. This is referred to as "torsional effect". The other is the steric effect of the alkyl substituent with the other amino acid residues of the helix backbone, which is referred to as "steric effect". This can be analyzed qualitatively based on the orientation of the substituent group with respect to the axis of the helix. The result of the analysis is given in Table 2.

For the 10-m-r unit of the 10/12-helix (Figure 4a), ϕ_1, ψ_1, ϕ_2, and ψ_2 are about 90°, −100°, −100°, and 90°, respectively. Torsionally, $\mathbf{R_3}$, $\mathbf{R_1}'$, and $\mathbf{R_4}'$ substitutions are in the most favorable conformational region in comparison with the potential energy profiles shown in Figure 3. $\mathbf{R_2}$ is also a favorable position in terms of ϕ_1 dihedral angle, but is gauche in terms of μ_1. As will be shown later, a methyl group at $\mathbf{R_2}$ causes a destabilization of about 1.5 kcal/mol. $\mathbf{R_1}$ or $\mathbf{R_2}'$ substitution would cause a large destabilization of about 3 kcal/mol, and these positions are designated as "bad". $\mathbf{R_4}$ and $\mathbf{R_3}'$ substitutions also cause

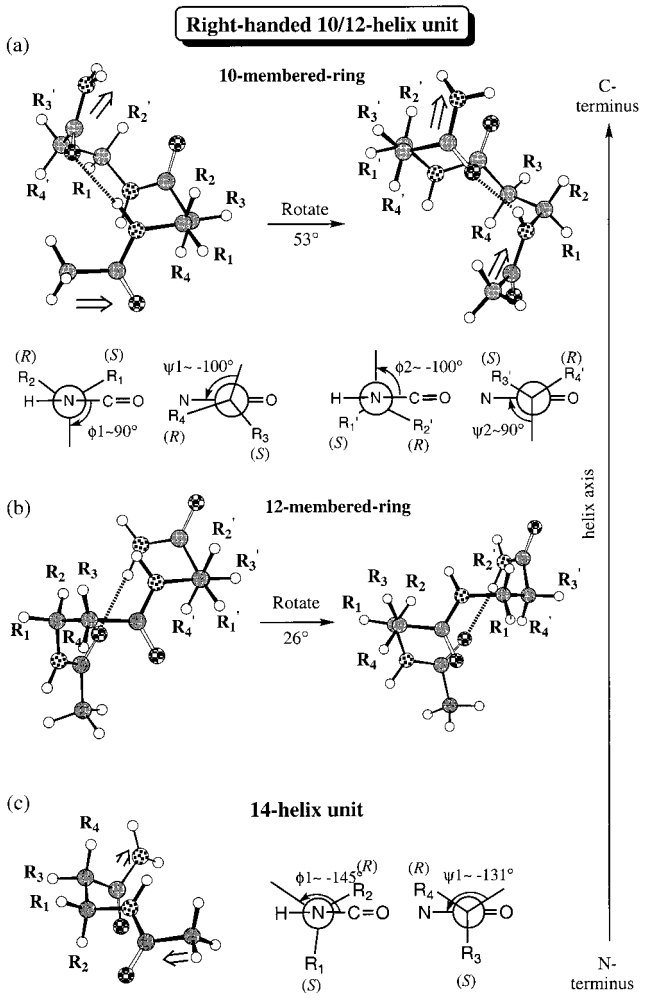


Figure 4. Eight and four substitution patterns in the repeating units of right-handed 10/12-helix and left-handed 14-helix, respectively.

destabilization. Since the potential energy surface is flatter for dihedral angle ψ, the destabilization is only about 1 kcal/mol, and these positions are designated as "ok". For the part of steric effect, $\mathbf{R_3}$ and $\mathbf{R_1}'$ are nearly perpendicular to the helix axis, and they have minimum steric effect on the formation of the helix. $\mathbf{R_4}$ and $\mathbf{R_2}'$ point toward N- and C-terminus, respectively. They would be buried in the helix structure, and would disrupt

Table 2. Conformational Preferences of Alkyl Substituents in the Right-Handed 10/12-Helix and Left-Handed 14-Helix[a]

	10/12-mixed helix									
	10-m-r				12-m-r				14-helix	
	torsional		steric		torsional		steric		torsional	steric
$\mathbf{R_1}$	bad	(3.0)	ok	(0.5)	good	(0.0)	good	(0.0)	good	good
$\mathbf{R_2}$	ok	(1.5)	ok	(0.5)	bad	(3.0)	bad	(>3.0)	bad	bad
$\mathbf{R_3}$	good	(0.0)	good	(0.0)	ok	(0.5)	ok	(0.0)	good	good
$\mathbf{R_4}$	ok	(1.0)	bad	(>3.0)	good	(0.0)	ok	(1.5)	bad	bad
$\mathbf{R_1}'$	good	(0.0)	good	(0.0)	bad	(3.0)	ok	(0.5)		
$\mathbf{R_2}'$	bad	(3.0)	bad	(>3.0)	ok	(1.5)	ok	(0.5)		
$\mathbf{R_3}'$	ok	(0.5)	ok	(0.0)	good	(0.0)	good	(0.0)		
$\mathbf{R_4}'$	good	(0.0)	ok	(1.5)	ok	(1.0)	bad	(>3.0)		

[a] The values in parenthesis are roughly estimated destabilization energies (kcal/mol) by methyl groups.

Table 3. Calculated Relative Energies (kcal/mol) and Dihedral Angles of the 10-m-r Conformer of the 10/12-Helix and the 14-helix Conformer of Tripeptide Models **1** and **3**−**8**[a]

	HF	B3LYP	B3LYP (SCIPCM)	dihedral angles					
structure	E_{rel}	E_{rel}	E_{rel}	ϕ_1	μ_1	ψ_1	ϕ_2	μ_2	ψ_2
1									
1c, 10-m-r	0.0	0.0	0.0	77.9	61.9	−110.5	−90.8	59.2	81.4
1k, 14-helix	9.4	9.8	4.5	−154.7	64.3	−135.9			
3, $\mathbf{R_1} = \mathbf{R_1}' =$ Me									
3a, 10-m-r	0.0	0.0	0.0	56.8	54.8	−101.8	−89.2	58.0	77.2
3b, 14-helix	7.2	7.9	1.2	−154.7	64.3	−135.9			
4, $\mathbf{R_3} = \mathbf{R_1}' =$ Me									
4a, 10-m−r	0.0	0.0	0.0	79.3	60.9	−108.3	−91.4	57.8	80.3
4b, 14-helix	9.2	9.7	5.0	−154.7	64.3	−135.9			
5, $\mathbf{R_2} = \mathbf{R_4}' =$ Me									
5a, 10-m-r	0.0	0.0	0.0	−80.2	−59.0	104.5	94.8	−57.8	−83.8
5b, 14-helix	7.6	8.6	3.4	−154.7	64.3	−135.9			
6, $\mathbf{R_3} = \mathbf{R_3}' =$ Me									
6a, 10-m−r	0.0	0.0	0.0	78.8	60.7	−105.9	−93.0	57.7	75.0
6b, 14-helix	9.1	10.0	4.4	−154.7	64.3	−135.9			
7, $\mathbf{R_2} = \mathbf{R_1}' =$ Me									
7a, 10-m-r	0.0	0.0	0.0	69.0	53.8	−99.8	−91.0	57.6	80.0
7b, 14-helix	13.8	13.6	8.5	154.7	−64.3	135.9			
8, $\mathbf{R_3} = \mathbf{R_4}' =$ Me									
8a, 10-m-r	0.0	0.0	0.0	82.4	61.7	−113.3	−93.0	59.9	82.7
8b, 14-helix	12.1	12.2	7.6	−154.7	64.3	−135.9			

[a] All energies are calculated with the 6-31G** basis set on HF/6-31G** geometries.

the formation of the helix. Therefore, these positions are forbidden for alkyl substitution, and are designated as "bad". $\mathbf{R_1}$, $\mathbf{R_2}$, $\mathbf{R_3}'$, and $\mathbf{R_4}'$ point outward, but have a dihedral angle of about 20−40° with the helix axis. These positions allow small alkyl substituents but do not allow bulky ones. For the 12-m-r unit, the situation is the same as the 10-m-r unit, except that the set of groups $\mathbf{R_1}$, $\mathbf{R_2}$, $\mathbf{R_3}$, and $\mathbf{R_4}$ and the set of groups $\mathbf{R_1}'$, $\mathbf{R_2}'$, $\mathbf{R_3}'$, and $\mathbf{R_4}'$ should be exchanged. Thus, $\mathbf{R_1}$ and $\mathbf{R_3}'$ are perfect for the 12-m-r.

The situation for the 14-helix is much simpler. The repeating unit is a dipeptide model. There are only four unique substitution positions. $\mathbf{R_1}$ and $\mathbf{R_3}$ are both tortionally and sterically favorable, while $\mathbf{R_2}$ and $\mathbf{R_4}$ are highly unfavorable. Therefore, any substituent at the $\mathbf{R_2}$ or $\mathbf{R_4}$ position would disrupt the 14-helix.

Substituted Tripeptide Models 3−8. To substantiate the above analysis, several substituted tripeptide models were calculated. For the 10/12-helix, only the 10-m-r structure was calculated. For the 14-helix, dihedral angles ϕ, μ, and ψ were constrained during geometry optimization. The calculated energies and dihedral angles of **1** and **3**−**8** are given in Table 3. A comparison of energy difference between the conformer for the 10/12-helix (**3a**−**8a**) and the conformer for the 14-helix (**3b**−**8b**) with that between **1c** and **1k** should tell the torsional effect of the substituents discussed in the last section.

For the unsubstituted model **1**, the conformer for the 10/12-helix **1c** is more stable than the conformer for the 14-helix **1k** by 4.5 kcal/mol in methanol solution. Upon methyl substitution at the $\mathbf{R_1}$ and $\mathbf{R_1}'$ positions (refer to Figure 4a), **3a** is found to be only 1.2 kcal/mol more stable than **3b**. This 3.3 kcal/mol reduction in the preference for the 10/12-helix is mainly caused by the steric interaction between the methyl at the $\mathbf{R_1}$ position and the carbonyl group (torsional effect). It is also reflected by the change of ϕ_1 dihedral angle. The decrease in the dihedral angle from 78° in **1** to 57° in **3a** is to reduce the steric interaction.

Model **4** has the $\mathbf{R_3}$ and $\mathbf{R_1}'$ replaced by methyl groups. These positions are ideal for both the 10/12-helix and the 14-helix. The calculated energy difference between **4a** and **4b** is 5 kcal/mol in methanol, very similar to the situation of **1**. On the other hand, when $\mathbf{R_2}$ and $\mathbf{R_1}'$ are replaced by methyl groups, the preference for **5a** over **5b** is decreased by over 1 kcal/mol. This is due to the torsional effect of the methyl group at $\mathbf{R_2}$. Calculations for model **6** indicate that methyl groups at the $\mathbf{R_3}$ and $\mathbf{R_3}'$ positions have little effect on the preference between the 10/12-helix and the 14-helix.

Model **7** has methyl groups at the $\mathbf{R_2}$ and $\mathbf{R_1}'$ positions. The methyl groups slightly destabilize the 10-m-r conformer **7a**, but cause a large destabilization to the 14-helix conformer **7b**. As a result, the 10/12-helix is favored over the 14-helix. Model **8** has methyl groups at the $\mathbf{R_3}$ and $\mathbf{R_4}'$ positions. There is no

Table 4. Predicted Preferences for the Right-Handed 10/12-Helix and Left-Handed 14-Helix for β-Peptides of the Type $(\beta/\beta)_n$

−(β/β)− unit		10/12-helix		14-helix
		10/12/10∼	12/10/12∼	
(*S*)-β^3/(*S*)-β^3	R_1, R_1'	unfavorable	unfavorable	favorable
(*S*)-β^2/(*S*)-β^3	R_3, R_1'	favorable[a]	unfavorable	favorable
(*S*)-β^3/(S)-β^2	R_1, R_3'	unfavorable	favorable[a]	favorable
(*S*)-β^2/(S)-β^2	R_3, R_3'	favorable[a]	favorable[a]	favorable
(*R*)-β^3/(*S*)-β^3	R_2, R_1'	favorable[b]	unfavorable	unfavorable
(*S*)-β^2/(*R*)-β^2	R_3, R_4'	favorable[b]	unfavorable	unfavorable
(*S*)-β^2/(*R*)-β^3	R_3, R_2'	unfavorable	favorable	unfavorable
(*R*)-β^3/(*S*)-β^2	R_2, R_3'	favorable	unfavorable	unfavorable

[a] Predicted to be favored over the 14-helix. [b] Left-handed 12/10/12-helix is equally favorable.

torsional problem for the 10-m-r conformer, but a torsional destabilization in the 14-helix conformer **8b**. The 10/12-helix is favored over the 14-helix.

Substituent Effects on the 14-Helix and the 10/12-Helix. The above analysis of the individual substituent effect, calculation results for the substituted tripeptide models, along with the fact that there is an intrinsic preference for the 10/12-helix without any substituent (model **2**) allow us to qualitatively predict the relative preferences for the 10/12-helix and the 14-helix.

Consider β-peptides of the type $(\beta/\beta)_n$, with each β-amino acid monosubstituted; there are 16 possible combinations for the alkyl substituents for the 10/12-helix. Only eight molecules need to be analyzed because these 16 molecules are eight pairs of enantiomers. Since the first hydrogen bond from the N-terminus can be either 10-m-r or 12-m-r, two types of 10/12-helices should be analyzed. A helix starting with 10-m-r from the N-terminus is termed 10/12/10-helix, and a helix starting with 12-m-r from the N-terminus is termed 12/10/12-helix. The analysis for the right-handed 10/12-helix and left-handed 14-helix is summarized in Table 4.

For [(*S*)-β^3/(*S*)-β^3]$_n$- or (*S*)-β^3-peptides, both 10-m-r and 12-m-r have $\mathbf{R_1}$ and $\mathbf{R_1'}$ substitutions. While $\mathbf{R_1'}$ is allowed, each methyl group at $\mathbf{R_1}$ causes over 3 kcal/mol destabilization (see Table 2). Thus, both the 10/12/10- and 12/10/12-helices are unfavorable. On the other hand, the substitution pattern is ideal for the left-handed 14-helix. Therefore, the 14-helix is favored.

For [(*S*)-β^2/(*S*)-β^3]$_n$-peptides, the 10/12/10-helix would have $\mathbf{R_3}$ and $\mathbf{R_1'}$ substitutions in the 10-m-r and $\mathbf{R_1}$ and $\mathbf{R_3'}$ substitutions in the 12-m-r—both are favorable. On the other hand, the 12/10/12-helix would have $\mathbf{R_3}$ and $\mathbf{R_1'}$ substitutions in the 12-m-r and $\mathbf{R_1}$ and $\mathbf{R_3'}$ substitutions in the 10-m-r—both are unfavorable. The substitution pattern is also favorable for the 14-helix. While both the 10/12/10-helix and the 14-helix are favorable, we predict that the former is more stable because of an intrinsic preference for the helix as demonstrated by the heptapeptide model **2**. Following a similar argument, the 12/10/12-helix is predicted to be most favorable for [(*S*)-β^3/(*S*)-β^2]$_n$-peptides.

[(*S*)-β^2/(*S*)-β^2]$_n$- or (*S*)-β^2-peptides have all the substituents in favorable positions for all the three types of helices, that is, $\mathbf{R_3}$ and $\mathbf{R_3'}$ substitutions for both the 10/12/10- and 12/10/12-helices and $\mathbf{R_3}$ substitutions for the 14-helix. Since the calculations on tripeptide model **6** suggest that $\mathbf{R_3'}$ substitution causes little destabilization to the 10/12-helix, we predict that the 10/12/10- and 12/10/12-helices are more favorable than the 14-helix because of their intrinsic preference.

A common feature for peptides [(*R*)-β^3/(*S*)-β^3]$_n$, [(*S*)-β^2/(*R*)-β^2]$_n$, [(*S*)-β^2/(*R*)-β^3]$_n$, and [(*R*)-β^3/(*S*)-β^2]$_n$ is that they have different chiralities at β- and α-carbon centers, respectively. As clearly stated by Seebach et al.,[5h] the alternating chiral β^3- or β^2-polypeptide is sterically not allowed for the 14-helix because half of the side chains interfere with the backbone. However, they are sterically allowable for the 10/12-helices if the substituents are not too bulky. For both [(*R*)-β^3/(*S*)-β^3]$_n$ and [(*S*)-β^2/(*R*)-β^2]$_n$-peptides, we predict that the right-handed 10/12/10- and left-handed 12/10/12-helices are equally favorable but the right-handed 12/10/12- and left-handed 10/12/10-helices are unfavorable. Peptides [(*S*)-β^2/(*R*)-β^3]$_n$ should favor the right-handed 12/10/12-helix while [(*R*)-β^3/(*S*)-β^2]$_n$ should favor the right-handed 10/12/10-helix. It should be noted that all these favored helices are still somewhat destabilized compared to the unsubstituted 10/12-helix. We expect that each dipeptide unit causes about 1−2 kcal/mol destabilization. Whether these peptides can exist in β-sheet or other structures needs to be further studied. It has been shown that poly-β-alanine exists in a β-sheet form.[34,35] However, side-chain substituents reduce the tendency for β-sheet formation.[17]

14-Helix vs 10/12-Helix for Heptapeptide Models 9−11. To verify the above predictions and compare our results with existing experimental results, we carried out calculations on heptapeptide models **9**−**11**. The results are summarized in Table 5 and Figures 5−7.

The right-handed 10/12/10/12/10- and right-handed 12/10/12/10/12-helices and the 14-helix of heptapeptide model **9** were fully optimized. These structures are given in Figure 5. At each calculation level, the 12/10/12/10/12-helix is slightly more stable than the 10/12/10/12/10-helix. However, the two 10/12-helices are about 3 kcal/mol less stable than the 14-helix. If the entropy and enthalpy corrections for **9a** and **9c** are assumed to be about the same as that for **2a** and **2c**, that is, a destabilization of 2.6 kcal/mol for **9c** with respect to **9a**,[36] the free energy of **9c** would be about 5 kcal/mol smaller than those of **9a** and **9b**, suggesting that the 14-helix is more stable than the 10/12-helix for β^3-peptide. This is in agreement with Seebach's experimental observation.[5a]

A comparison between **9** and **2** indicates that, as expected, the methyl groups cause about 10 kcal/mol destabilization to the 10/12-helix with respect to the 14-helix. The heptapeptide model **9** can be constructed from three units of tripeptide model **3**. As discussed earlier, each unit of tripeptide causes about 3.3 kcal/mol destabilization to the 10/12-helix by one unfavorable methyl group (indicated by an arrow), amounting to about 10 kcal/mol by three units.

Seebach et al. have proposed that the 14-helix might be stabilized by a favorable hydrophobic interaction because there are three pairs of alkyl groups which are nearly staggered in the 14-helix.[5h] Because the pair of methyl groups in **9c** are separated by about 5.5 Å, the hydrophobic interaction cannot be important in our calculations, as indicated by the above energetic analysis. It could be important for larger alkyl side chains which were used in their experiment.

The right-handed 10/12/10/12/10-helix, left-handed 12/10/12/10/12-helix, and left-handed 14-helix of heptapeptide model **10** are shown in Figure 6. **10a** and **10b** can be considered as constructed from three 10/12-helical conformers of tripeptide models **4** and **5**, respectively. A comparison of energies of **4** and **5** indicates that the 10/12-helix conformer **5a** is destabilized

(34) Narita, M.; Doi, M.; Kudo, K.; Terauchi, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3553.

(35) Yuki, H.; Okamoto, Y.; Taketani, Y.; Tsubota, T.; Marubayashi, Y. *J. Polym. Sci. Polym. Chem. Ed.* **1978**, *16*, 2237.

(36) Vibration frequency calculation was not carried out for substituted heptapeptides **9**−**11**. The assumption that the entropy and enthalpy correction between the 10/12-helix and the 14-helix of **9**−**11** is the same as that of unsubstituted heptapeptide **2** only gives a rough estimate.

Table 5. Calculated Relative Energies (kcal/mol) and Average Dihedral Angles of the 14-Helix and the 10/12-Helix of Heptapeptide Models **2** and **9**−**11**[a]

	HF	B3LYP	B3LYP (SCIPCM)[b]	av dihedral angles					
structure	E_{rel}	E_{rel}	E_{rel}	ϕ_1	μ_1	ψ_1	ϕ_2	μ_2	ψ_2
2									
2a, 10/12/10/12/10	0.0	0.0	0.0 (0.0)	88.1	65.1	−109.4	−97.7	60.3	87.5
2b, 12/10/12/10/12	−1.3	−1.4	0.3	86.7	63.7	−122.1	−99.7	60.3	92.7
2c, 14-helix	22.0	24.0	7.4 (4.8)	−141.9	61.4	−137.4			
9									
9a, 10/12/10/12/10	0.0	0.0	0.0 (0.0)	69.9	66.0	−108.9	−97.5	60.8	91.9
9b, 12/10/12/10/12	−0.3	−0.4	−0.5	73.5	68.3	−121.0	−100.6	61.3	99.5
9c, 14-helix	11.4	14.8	−3.4 (−5.0)	−144.4	59.9	−135.3			
10									
10a, 10/12/10/12/10	0.0	0.0	0.0 (0.0)	87.4	64.1	−107.6	−99.3	59.9	86.4
10b, 12/10/12/10/12	9.9	7.5	8.5	−97.6	−58.3	116.3	100.9	−54.8	−95.2
10c, 14-helix	21.4	24.2	7.6 (5.0)	−144.1	59.2	−134.0			
11									
11a, 10/12/10/12/10	0.0	0.0	0.0 (0.0)	87.6	66.1	−106.5	−98.6	61.5	82.4
11b, 14-helix	18.9	22.6	6.0 (3.4)	−144.3	58.0	−130.6			

[a] All energies are calculated with the 6-31G* basis set on HF/6-31G* geometries. [b] The values in parentheses are estimated relative Gibbs free energies. **2c** is 2.6 kcal/mol less stable than **2a** by enthalpy and entropy corrections based on the HF/3-21G frequency calculations.

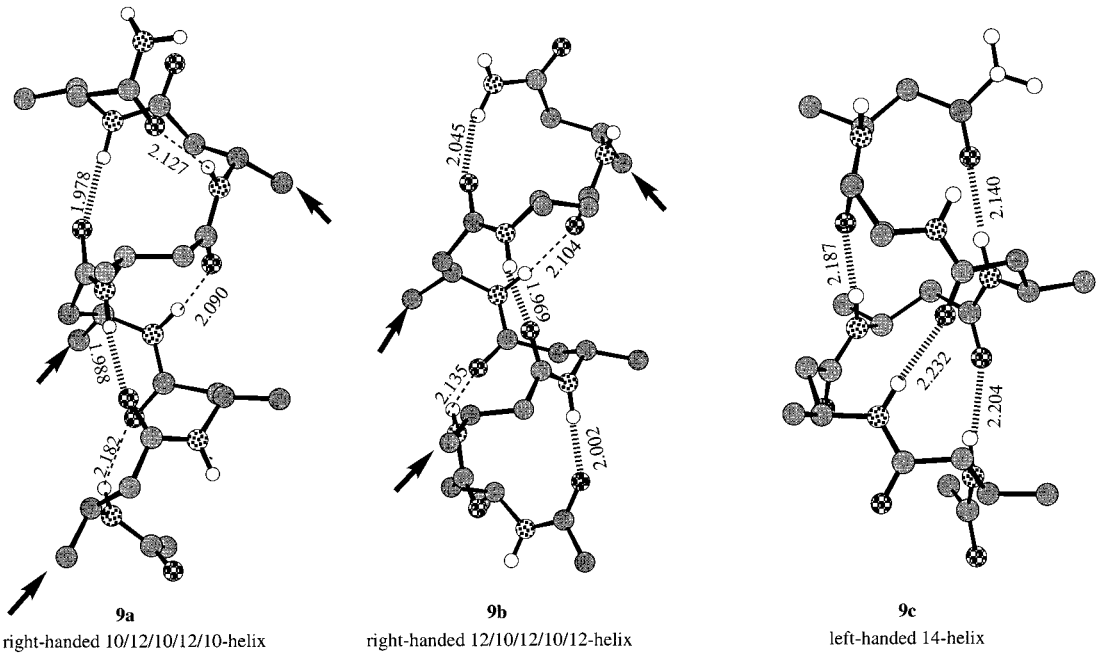
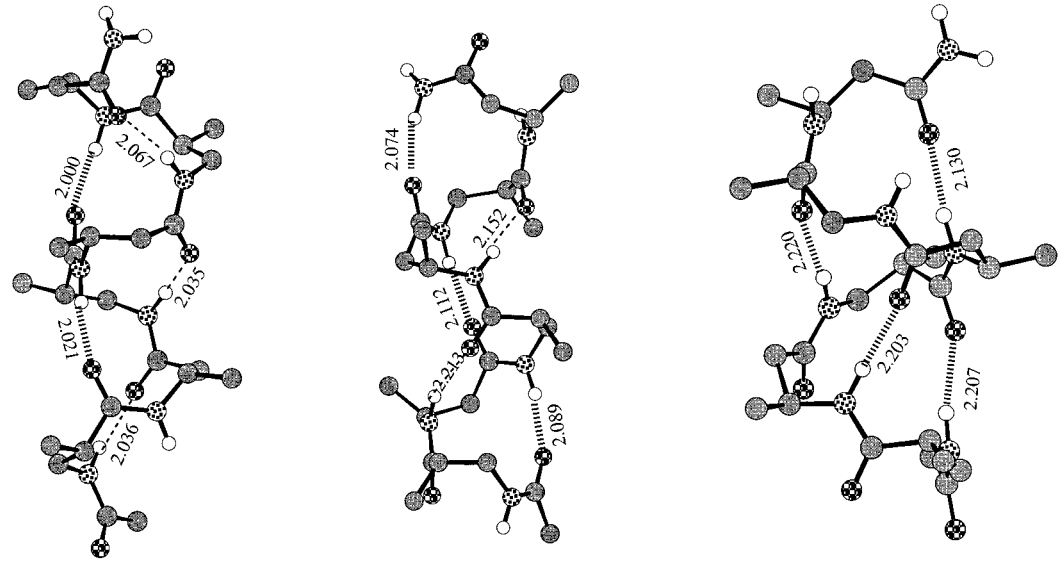


Figure 5. HF/6-31G* optimized 10/12/10/12/10-, 12/10/12/10/12-, and 14-helices of heptapeptide model **9**.

by torsional interaction by about 1.6 kcal/mol. Thus, **10b** should be destabilized by the torsional effect of the methyl groups by about 5 kcal/mol while **10a** has no torsional effect. The energy difference of 8.5 kcal/mol between **10a** and **10b** suggests that **10b** is also destabilized by the steric effect of the methyl groups. Indeed, in **10b**, there are two close H/H distances of 2.17 Å involving two of the α-methyl groups. The calculated preference for the 10/12/10/12/10-helix over the 14-helix is 7.6 kcal/mol, very similar to that calculated for the unsubstituted model **2** (7.1 kcal/mol). This clearly indicates that substituents cause little steric interaction in these two helices. Assuming the entropy and enthalpy corrections for **10a** and **10c** are the same as those for **2a** and **2c**,[36] which is 2.6 kcal/mol, the free energy of **10a** is lower than that of **10c** by about 5 kcal/mol. Thus, the right-handed 10/12-helix is predicted to be more stable than the 14-helix for (*S*)-β^2/(*S*)-β^3-peptides. This is once again in agreement with Seebach's experiment.[5h] Since the methyl groups have little effect on the stabilities of **10a** and **10c**, we conclude that the preference for the 10/12-helix is due to the intrinsic preference for the 10/12-helix backbone.

The right-handed 10/12/10/12/10-helix (**11a**) and the left-handed 14-helix (**11b**) of heptapeptide model **11** are given in Figure 7. The right-handed 12/10/12/10/12-helix was not calculated because it is expected that it should have similar stability as **11a**. **11** can be constructed from three units of tripeptide model **6**. As shown earlier, the methyl groups in **6** cause only a small destabilization to the 10/12-helix. The calculated energy difference between **11a** and **11b** is 6 kcal/mol, only about 1 kcal/mol smaller than that between **2a** and **2c**. With entropy and enthalpy corrections based on **2a** and **2c**, we still predict that the 10/12-helix is more stable than the 14-helix by over 3 kcal/mol.

It should be pointed out that our models are different from most experimental β-peptides in two important aspects: (1) We replace the normal carboxylic acid C-termimus with an amide group. This allows an additional 12-m-r hydrogen bond at the C-terminus for the 12/10/12-helix in our model. The allowed hydrogen bonds are not affected for the 10/12/10-helix and the 14-helix. (2) Our models have an acetyl group at the N-terminus, which resembles only the N-protected β-peptides studied

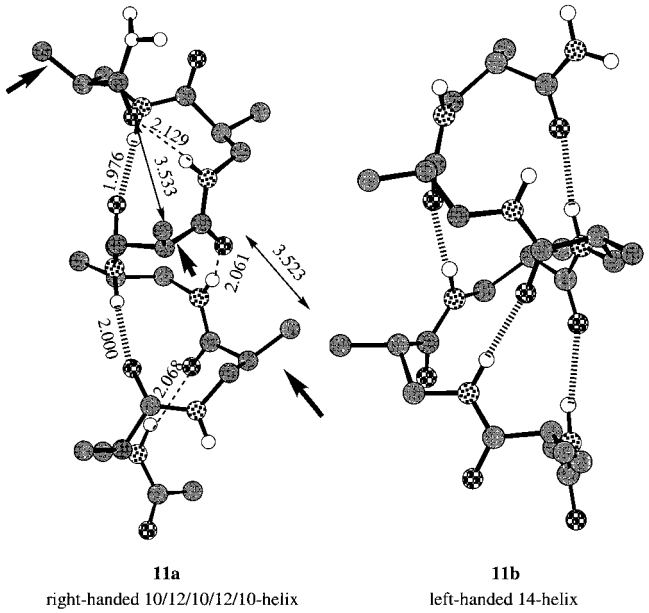


10a right-handed 10/12/10/12/10-helix

10b left-handed 12/10/12/10/12-helix

10c left-handed 14-helix

Figure 6. HF/6-31G* optimized 10/12/10/12/10-, 12/10/12/10/12-, and 14-helices of heptapeptide model **10**.

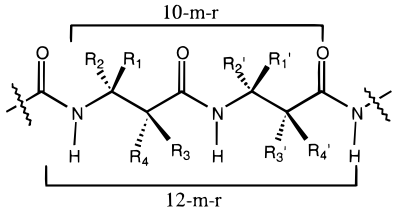


11a right-handed 10/12/10/12/10-helix

11b left-handed 14-helix

Figure 7. HF/6-31G* optimized 10/12/10/12/10-mixed and 14-helices of heptapeptide model **11**.

Table 6. Substituent Effect on the Stability of the 10/12-Helix



	right-handed helix		left-handed helix		
entry	10-m-r	12-m-r	10-m-r	12-m-4	energy
1	R_3, R_1'	R_1, R_3'	R_4, R_2'	R_2, R_4'	0.0
2	R_3, R_3'	R_3, R_3'	R_4, R_4'	R_4, R_4'	0.5
3	R_3, R_4'	R_4, R_3'	R_4, R_3'	R_3, R_4'	1.5
4	R_2, R_1'	R_1, R_2'	R_1, R_2'	R_2, R_1'	2.0
5	R_2, R_3'	R_3, R_2'	R_1, R_4'	R_4, R_1'	2.5
6	R_2, R_4'	R_4, R_2'	R_1, R_3'	R_3, R_1'	3.5[a]
7	R_1, R_1'	R_1, R_1'	R_2, R_2'	R_2, R_2'	3.5

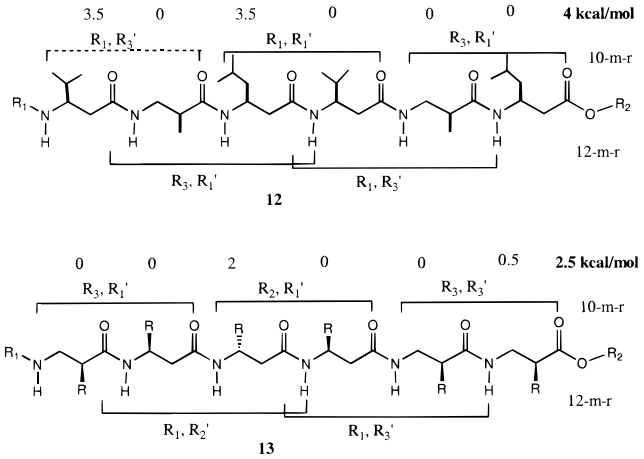
[a] Reduce to about 2 kcal/mol if the ring is the first ring from N-terminus.

experimentally.[3−5] Once again, this allows one more 12-m-r hydrogen bond at the N-terminus for the 12/10/12-helix but has no effect on the 10/12/10-helix and the 14-helix. Therefore, for many of the β-peptides studied by Seebach et al., the 10/12-helix is actually a 10/12/10-helix.[5h]

The prediction that the 10/12-helix is preferred over the 14-helix for β^2-peptides is less certain. First of all, the preference for the former is reduced with respect to the unsubstituted model **2**. This is because in **11a**, three of the methyl groups (with arrows) are not in perfect steric-free positions. When these methyl groups are replaced by bulkier groups, even larger destabilization is expected, reducing the preference for the 10/12-helix. Experimentally, there has been no reports of structural determination for protected β^2-peptides. Seebach et al. have reported the CD spectrum of a deprotected β^2-hexapeptide.[5f,h] The spectrum is similar to that of a typical 14-helix, but the cotton effect of CD is weak. It has been found that deprotection in certain cases converts a 10/12-helix back to a 14-helix, which is reasonable based on dipole-charge interactions.[5h]

So far, the β-peptides that have been discussed are in the form $(\beta/\beta)_n$. The above calculations and analysis allow us to estimate roughly the destabilizations caused by methyl substitutions at different positions, as shown in parentheses in Table 2. The combinations of the individual substitutions can provide a guideline for the derivation and design of 10/12-helix with a random sequence. As shown in Table 6, the ($\mathbf{R_3}$, $\mathbf{R_1'}$) and ($\mathbf{R_1}$, $\mathbf{R_3'}$) substitutions are the best for the 10-m-r and 12-m-r structures, respectively. ($\mathbf{R_3}$, $\mathbf{R_3'}$) substitution is the next best for both 10-m-r and 12-m-r structures, which only causes about 0.5 kal/mol destabilization. Others can be derived in the same way. It should be noted that Table 6 only gives normal situations, that is, both torsional and steric effects operate. When a substituent is involved in a terminal ring, its steric effect may disappear. For example, $\mathbf{R_4}$ in a right-handed 12-m-r or $\mathbf{R_4'}$ in a right-handed 10-m-r has a steric effect of about 1.5 kcal/mol (Table 2). If the ring is the first ring from the N-terminus, this steric effect disappears (check with Figure 4). Similarly, $\mathbf{R_4}$ in

Scheme 3



a 10-m-r or $\mathbf{R_4'}$ in a 12-m-r has a large steric effect. This steric effect also disappears if the ring is at the N-terminus.

For the substituent effect on the stability of the left-handed 10/12-helix, everything can be derived by the image of the right-handed helix. That is, $\mathbf{R_4}$ and $\mathbf{R_2'}$ are most favored for a 10-m-r, and $\mathbf{R_2}$ and $\mathbf{R_4'}$ are most favored for a 12-m-r (Table 6). To illustrate the possible applications of the above qualitative analysis of substituent effect, Scheme 3 shows two examples.

Seebach et al. have reported the CD spectrum of hexapeptide **12**. It has a single absorption maximum at about $\lambda = 200$ nm.[5h] Since the CD absorption is quite different from that of a typical 14-helix, it would be tempting to assign the structure as a 10/12-helix. A closer examination using the conditions in Table 6 indicates that the first two rings from the C-terminus are favorable; the middle 10-m-r would cause about 3.5 kcal/mol destabilization; the 10-m-r at the N-terminus would be highly unlikely to form because it would also cause about 3.5 kcal/mol destabilization. Thus, a total of about 4 kcal/mol destabilization to the right-handed 10/12-helix is predicted. On the other hand, the side-chain substituents do not cause destabilization to the left-handed 14-helix. Therefore, the intrinsic preference for the 10/12-helix over the 14-helix is canceled out by the side-chain substituents, and it is quite possible that these two helices are in equilibrium.

Peptide **13** has all the chiral carbons in the (*S*) configuration except one (the third from the left). This prevents the formation of the 14-helix. However, it should still be possible to form a 10/12/10/12/10-helix because substituents only cause about 2.5 kcal/mol destabilization.

Summary

We have theoretically studied the conformational features of a series of β-tripeptide and β-heptapeptide models. The results provide novel explanations for the experimental observations of the significant influence of substitution patterns on the formation of various helical structures. It also allows prediction for the stability of the 10/12- and 14-helices for a variety of sequences of β-peptides. These are as follows:

(1) While several helical structures are possible for unsubstituted β-peptides, the 10/12-helix with alternate 10-m-r and 12-m-r hydrogen bonds is intrinsically favored. In particular, the 10/12-helix is favored over the 14-helix by about 21 and 5 kcal/mol in the gas phase and CH_3OH solution, respectively, if the peptide is protected (not zwitterionic).

(2) Conformational analysis indicates that while substitutions at both the α- and β-carbons of the β-amino residue reduce the flexibility of the β-peptide backbone, the β-substitution is more efficient.

(3) For (*S*)-β^2/(S)-β^3-peptides and (*S*)-β^2-peptides, all substituents are allowable for the right-handed 10/12-helix and the left-handed 14-helix; the 10/12-helix is predicted to be more favored because it is intrinsically favorable.

(4) β^3-Peptides are predicted to adopt the 14-helix instead of the 10/12-helix because in the 10/12-helix half of the substituents are in unfavorable positions.

(5) Several patterns of alkyl substitutions would disrupt the formation of the 14-helix, but only cause mild destabilization to the 10/12-helix. These include (*R*)-β^3/(*S*)-β^3-, (*S*)-β^2/(*R*)-β^2-, (*S*)-β^2/(*R*)-β^3- ·and (*R*)-β^3/(*S*)-β^2-peptides. Therefore, these β-peptides might also form the 10/12-helix.

It should be pointed out that in deriving the above predictions, the possible formation of β-sheet or other secondary structures is not considered explicitly. The effect of the protection for C- and N-termini also needs to be studied. When short β-peptide units such as dipeptide or tripeptide units are incorporated into normal α-peptide sequences, special and interesting secondary structures are also possible. In addition, it is necessary to answer the question why β-peptides[3−5] (and likewise γ-peptides[7]) have a higher tendency to form secondary structures than α-peptides do. Research attempting to address these issues is currently underway in this group.

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Supporting Information Available: Tables of dihedral angles, energies, and structures of MM2* and HF/3-21G optimized conformations of tripeptide model **1**, and HF/6-31G** and B3LYP/6-31G** calculated total energies of conformations of **1**−**11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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