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ORIGINAL ARTICLE

Exploiting diverse stereochemistry of β-amino acids: toward a rational design of sheet-forming β-peptide systems

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Abstract Due to the two methylene groups in their backbone, β-amino acids can adopt numerous secondary structures, including helices, sheets and nanotubes. Chirality introduced by the additional side chains can significantly influence the folding preference of β-peptides composed of chiral β-amino acids. However, only conceptual suggestions are present in the literature about the effect of chirality on folding preferences. Summarizing both the experimental and computational results, Seebach (Chem Biodivers 1:1111–1240, 2004) has proposed the first selection rule on the effect of side chain chirality, on the folding preference of β-peptides. In order to extend and fine-tune the aforementioned predictions of Seebach, we have investigated its validity to the novel type of apolar sheet proposed recently (Pohl et al. in J Phys Chem B 114:9338–9348, 2010). In order to facilitate the rational design of sheet-like structures, a systematic study on the effect of chirality on "apolar" sheet stability is presented on disubstituted [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$ model peptides calculated at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) and B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) levels of theory both in vacuum and in polar and apolar solvents. In addition, both types of "apolar" sheets were investigated; the one with two strands of identical (AA) and enantiomeric (AB) backbone structure. Our results show that heterochirally disubstituted sheets have the greatest preference for sheet formation ($\Delta G \sim -11$ kcal mol^{-1}). However, in contrast to Seebach's predictions, "homochiral disubstitution" itself does not necessarily disrupt the sheet structure, rather it could result stable fold ($\Delta G \sim -5$ kcal mol^{-1}). Results indicate that both the methyl group orientation and the local conformational effect of substitution affects sheet stability, as point chirality was found to have influence only on the backbone torsional angles. These results enabled us to extend and generalize Seebach's predictions and to propose a more general and accurate "rule of thumb" describing the effect of chirality on sheet stability. This offers an easy-to-use summary on how to design β-peptide sheet structures. We conclude that heterochirally disubstituted models are the best candidates for sheet formation, if the two strands are substituted in a way to create identical torsional angle sets on the two backbones for ideal hydrogen-bonding pattern. With adequately selected side chains, homochirally disubtituted derivatives may also form sheet structures, and the position of methyl groups would prevent assembly of more than two strands making it ideal to create hairpins.

Keywords β-amino acid · β-peptide · Sheet structures · Computational chemistry · Chirality · Foldamer

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Abbreviations

β-Ala	3-Aminopropanoic-acid
$\beta^{2,3}$-hAla	3-Amino-2,3-dimethylpropanoic-acid

Introduction

Understanding the driving force of protein folding has long been a key issue in chemistry. Natural proteins are almost exclusively built of α-amino acids and details of their folded structures have been studied for more than a half century. The tremendous amount of data obtained enabled scientists to create rules in order to make predictions on the adopted structural motives and elements. More recently, although rarely found in nature, β-amino acid residues and β-peptides have also been extensively studied (Seebach 2004; Martinek and Fülöp 2003; Krauthauser et al. 1997) because of their unique properties. The two consecutive methylene groups could in principle result in a greater flexibility per subunits, which would allow for β-peptides to adopt numerous secondary structural elements including helices, sheets, "zigzag" structures etc. (Seebach 2004). Despite this flexibility, which allows the formation of many different secondary structures, most β-amino acid types are intrinsically more rigid than α-amino acids, thus the aforementioned ordered structures can form in shorter chains. However, due to the substantially lower amount of related experimental data and the higher amount of secondary structures available for folding, only a few general rules on the folding were derived. The importance of stereochemical patterning in the stability of secondary structures was already shown for α-peptides (Durani 2008) and mixed α/β-peptides (Sharma 2003, 2009). For β-peptides, an important rule was proposed by Seebach (2004) on the effect of point chirality of the side chains on folding preference (referred hereafter as Seebach's predictions). (i) Heterochirally (or "unlike") disubstituted β-amino acid residues form polar strands; (ii) mono- or homochirally (or "like") disubstituted derivatives tend to form helical structures; (iii) geminally disubstituted β-amino acids may form neither helices nor sheets because methyl groups would obstruct hydrogen bonds.

Recently, we proposed a new type of extended β-amino acid foldamer, a suitable candidate of forming extended β-pleated sheets (Pohl et al. 2010). The latter type of sheets were reported to be built of "apolar" strands (Fig. 1), which can be derived from the $H8_{PM}$ helical structure (Günther 2001; Beke et al. 2006a). The stability of this novel apolar sheet coupled with the enzymatic resistance of β-amino acids (Frackenpohl et al. 2001) makes them promising candidates for designing biocompatible polymers, which are of great importance in clinical uses for example. Furthermore, these apolar sheets present a negligible amount of backbone twisting and have a great stability compared to sheets made of polar strands. (Note that when Seebach's predictions were formulated only polar strands were yet discovered; Seebach 2004.) Even though NMR spectroscopic, X-ray crystallographic and MD calculations of shorter (4–6 residue long) hairpin structures of β-peptides were carried out (Krauthauser et al. 1997; Seebach et al. 1999; Daura et al. 2001; Karle et al. 2002; Chung et al. 2000), these experimental results are not sufficient to validate the existence of extended polar sheets. The introduction of hairpin-forming sequence alters their conformational preference, plus the sheet making sequences were too short to show the characteristic structural twisting of the latter type of sheets. Our previous calculations showed that in the absence of a closing turn-motif of the hairpin structure makes twisting to appear. In fact, in these longer polar sheet structures twisting (Lin et al. 2002; Beke et al. 2006b) is pronounced so much that self-forming nanotubes could emerge (Pohl et al. 2010; Beke et al. 2006b). Due to this strong twisting, and nanotube-forming characteristic of polar sheets, present work focuses on apolar sheets.

The relationship between the chirality of substitution and folding preference was studied by several groups (Günther 2001; Beke et al. 2006a; Seebach et al. 1999; Möhle et al. 1999; Wu and Wang 1998; Günther and Hofmann 2002; Martinek et al. 2002; Mandity et al. 2009). Oligomers composed of one to six residues of β-Ala,

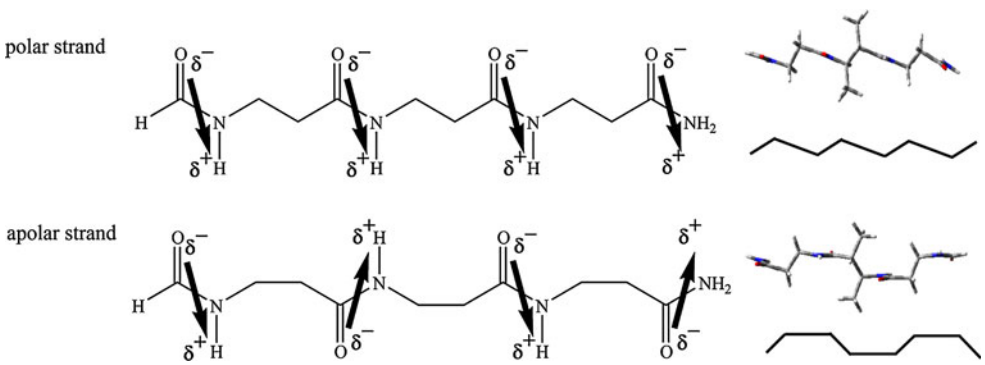


Fig. 1 Schematic structure of the two major types of β-peptide strands (*left*), their computed structure (*upper right*) and its representation (*lower right*): polar strand with carbonyl groups facing the same direction and apolar strand of alternating carbonyl groups. *Bolded arrows* indicate the dipole moment of the amide group

β^2-hAla, and β^3-hAla were investigated by MD and ab initio calculations in vacuum and in solvent at the HF/6-31G(d) and HF/3-21G levels of theory (Günther 2001; Beke et al. 2006a; Möhle et al. 1999; Wu and Wang 1998; Günther and Hofmann 2002). Parallel and antiparallel sheets of differently substituted β-amino acid derivatives were calculated at the B3LYP/6-31G(d,p)//RHF/6-31G(d) level of theory (Lin et al. 2002).

It has been found that side-chain configuration greatly influences the secondary structure of the peptide and that heterochirally disubstituted derivatives have the greatest preference for sheet forming.

β-peptides of cyclic side-chains are also good model systems to study the effect of chirality as the μ torsional angle is forced to adopt a *gauche* conformation (Martinek et al. 2002). More recently, a systematic study on oligomers of β-amino acids containing five membered rings was carried out (Mandity et al. 2009). For these structures, a general folding rule was proposed: homochiral models (φ and ψ torsional angles having the same sign) form helices, while heterochiral models (φ and ψ torsional angles having opposite sign) form strand structures.

Our goal here is to understand the effect of the chirality of substitution on the stability of apolar sheets formed of β-amino acid residues. The generalization and quantification of Seebach's predictions on substitution pattern would help synthetic chemists to design β-peptides of the desired secondary structural element, a key issue in future designs of extended sheet structures built from β-amino acids. In order to investigate the validity of Seebach's predictions to "apolar" sheets, a systematic theoretical study on the effect of chirality on "apolar" sheet stability was completed on disubstituted HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 model peptides in all the independent configuration (see Supplementary material for more details). The following questions were addressed:

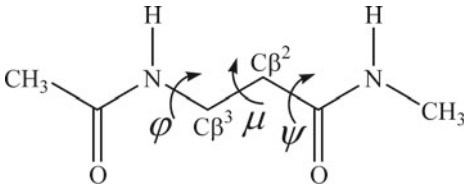
- Q1: What is the effect of methyl substitution and chirality on the stability of apolar sheets? Could methyl substitution pattern be used as a method to "fine-tune" backbone conformation?
- Q2: How accurate Seebach's predictions are in describing the stabilities of the differently disubstituted sheets? (e.g. If a homochirally disubstituted strand is expected to form helices, will it have low stability as sheet?)
- Q3: Is the formation of a stable sheet structure possible, when consisted of homochirally disubstituted ("like") amino acids?
- Q4: By extending Seebach's predictions, could there be a more general "rule of thumb" proposed for the relative stability of sheets, as function of strand orientation and side-chain chirality "pattern"?

Methods

Nomenclature

For the sake of simplicity sheets of β-peptides are referred as sheets throughout the paper. Strands with alternating carbonyl groups are referred as "apolar" strands, while strands in which carbonyl groups are facing the same spatial directions are referred as "polar" strands (Fig. 1). Regarding structural data backbone torsional angles are denoted as follows: C(O)-N–C–C torsional angle as φ, N–C–C–C(O) torsional angle as μ and C–C–C(O)-N torsional angle as ψ (Fig. 2). The previously described $H8_M$ (forming "A" type strand) and $H8_P$ (forming "B" type strand) helical structures were considered as sheet forming monomers for apolar sheets (Pohl et al. 2010).

The present work studies sheet-like structures built of two strands. Even though the two strands have the same



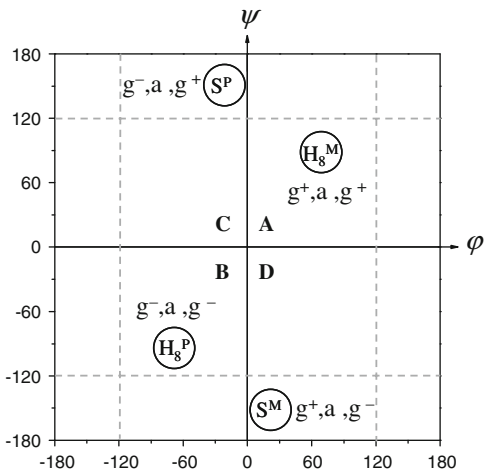


Fig. 2 Definition of torsional angles of β-amino acids forming peptides. The representation of the four basic extended-like strand structure on the *Ramachandran* surface at $\mu = 180°$. The *dashed lines* (120°) represent the border between *gauche* and *anti* regions. Conformational code (e.g. [g−, a, g+]), symbolic code introduced by Beke et al. (2006a) (e.g. S^P) and one-lettered code introduced by Pohl et al. (2010) (e.g. C) are given

constitution, in order to differentiate between them, one is referred to as "strand a" the other is referred to as "strand b". Peptides composed of β-amino acids can form a total of four different types of apolar sheets, two distinct types plus their conformational enantiomers, namely; AA, BB, AB and BA (Pohl et al. 2010). The first letter denotes the backbone conformation of "strand a" the second letter denotes the backbone conformation of "strand b". For symmetry reason, only the two distinct AA and AB types are reported hereafter (Fig. 3). Polar sheets (CC, DD, CD and DC) were found to adopt a twisted nanotube-like structure (Pohl et al. 2010).

As illustrated by Fig. 4, the present work studies dimers of tripeptides, in which the middle amino acid residue is substituted. All the other four terminal amino acid residues serve only as a "conformational lock", to hold the sheet like structure. A simplified nomenclature is introduced to differentiate the various possible disubstitution patterns of the middle amino acid residues of the peptide strands. Considering the absolute chirality of both β^2- and β^3- carbon atoms, a four letter code is introduced to denote the configuration and to identify the actual pair of strands. The first and second letters (R or S) denote the configuration of the middle residue of "strand a" (starting from the N-terminal, based on the convention in peptide chemistry), while the third and fourth letters denote the configuration of that of the middle residue of "strand b".

Computational details and considerations

All computations were carried out using the Gaussian 03 software package (Frisch 2004). Strands and both types of apolar β-pleated sheet assemblies (AA and AB) and both types of twisted polar sheet assemblies (CC and CD) of [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_{1-2}$ with all independent configurations (out of the 16 possible configuration, 10 can be ruled out based on symmetry reasons and thus, it is sufficient to study only the 6 basic configurations—see Supplementary information for details) as central amino acid residues (Figs. 3, 4), subjected to full geometry

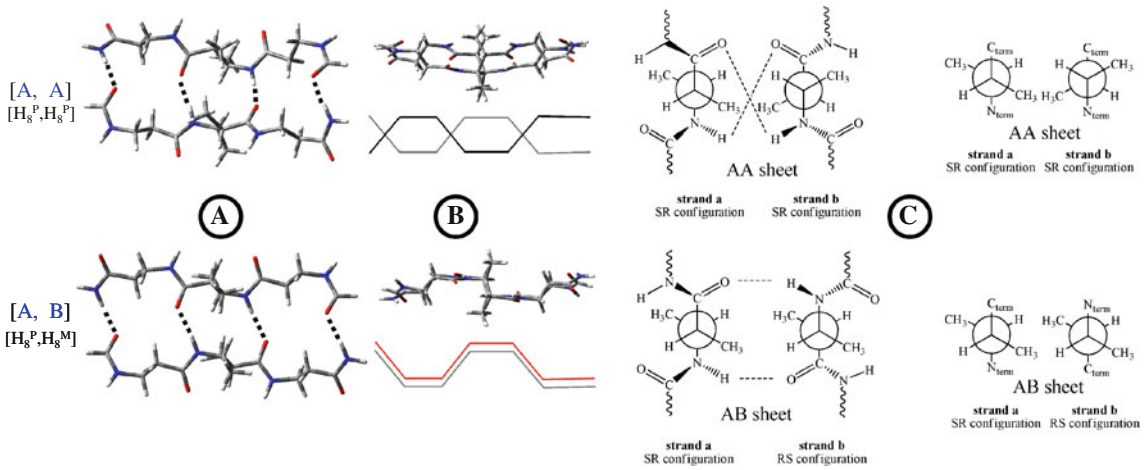


Fig. 3 Schematic representation of the two different types of apolar sheets of β-peptides. From *left to the right* conformational code, *top view* (**a**), *side view* and the schematic representation of it (**b**) is reported, plus the Newman projection of the μ torsional angle of the middle amino acid is depicted (**c**). The *dashed lines* indicate hydrogen bonds

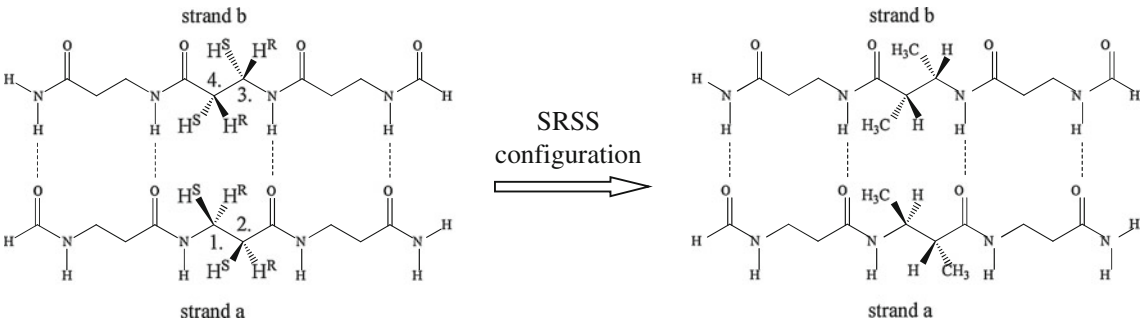


Fig. 4 Nomenclature used in the present work for sheets built of strands containing differently disubstituted β-amino acids: (*left*), an example of its application in the case of a sheet structure with SRSS configuration (*right*): e.g.: 1S2R3S4S configuration is denoted by SRSS

optimization at the RHF/3-21G level of theory. Subsequently, selected models were subjected to full geometry optimization at the B3LYP/6-31G(d) and M05-2X/6-31G(d) levels of theory. The optimized M05-2X structures were then submitted to frequency calculations at the M05-2X/6-31G(d) level of theory. The results of frequency calculations were used to compute thermodynamic parameters, entalphy (H), Gibbs Free Energy (G), and Entropy (S) using statistical thermodynamics. Moreover, single point energy calculations in vacuum, water and heptane, using the IEF-PCM model (Tomasi and Persico 1994) were completed on the optimized structures at the M05-2X/6-311++G(d,p) level of theory. It has been shown that at this level of theory basis set superposition error (BSSE) is not too significant (less than 0.5 kcal mol^{-1}/residue, Beke et al. 2006b). Heptane herein is used to mimic the interior conditions of a membrane (Stenberg et al. 1999). In order to confirm the validity of the single point energy calculations, full optimization and frequency calculation were also completed on two selected structures at the M05-2X/6-311++G(d,p) level of theory, by using the IEF-PCM solvent model.

Precision and accuracy

The effect of basis set and electron correlation on structure and stability of non-covalently attached subunits has already been investigated on parallel sheets of β-amino acids (Beke et al. 2006b). Optimizations at B3LYP/6-31G(d) or M05-2X/6-31G(d) levels of theory, followed by single point energy calculations on a higher basis set, 6-311++G(d,p), to minimize the BSSE, were found to be adequate to characterize the structures of peptide systems of similar magnitude (Beke et al. 2006b). Based on these results, calculations of the present work were carried out at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory unless mentioned otherwise.

Results and discussion

The effect of chirality on the stability of the different sheet foldamers of [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH$_2$]$_2$

In order to validate the applicability of Seebach's predictions on apolar sheets, stability of dimers of the differently disubstituted tripeptide models were investigated. Since we found that the effect of substitution on stability varies for the different types of sheet structures, the disubstituted tripeptide models were used to study both AA and AB types of sheets.

AA type sheets

As an extension to Seebach's predictions, not only pure heterochiral and homochiral, but also mixed dimers were studied. The overall conformation of the strand plays a major role in sheet stability. In differently disubstituted derivatives strands adopt slightly different backbone conformations, based on their local configurations (Table 1). This can be attributed to the effect of point chirality (Möhle et al. 1999): Cβ (β^3) substitution affects the φ torsional angle, whereas Cα (β^2) substitution has a greater effect on torsional angle ψ.

R or S configuration has been found to have the opposite effect on torsional angle values. In the case of Cβ (β^3) substitution of S configuration value of φ decreases, while increases in R substituted derivatives. (The magnitude of the above shift is $-20°$ or $+20°$, respectively, compared to the value in unsubstituted sheet like structure.) Likewise, in the case of Cα (β^2) substitution, ψ torsional angle is shifted to lower values in S substituted derivatives, while it is shifted to higher values in R substituted derivatives. Once again the magnitude of changes is about the same as spelled out above compared to the value of the unsubstituted sheet.

Table 1 The effect of point chirality on the backbone torsional angles of strands of AA type: backbone torsional angles of the central units of HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH$_2$

Chirality of disubstitution		Torsional angles strand a			Torsional angles strand b			ΔE^{a}_{rel}/kcal mol^{-1}
Strand a	Strand b	φ	M	ψ	φ	μ	ψ	
SR	SR	60 (63)	160 (160)	118 (126)	59 (63)	160 (160)	155 (126)	0.00 (0.00)
SR	RS	60 (64)	160 (161)	141 (124)	104 (114)	165 (168)	82 (78)	0.45 (0.67)
SR	SS	60 (64)	163 (166)	133 (137)	84 (86)	151 (154)	137 (116)	1.62 (0.91)
RS	SS	103 (111)	173 (166)	84 (83)	81 (82)	151 (149)	123 (120)	2.41 (1.06)
SS	SS	88 (88)	159 (155)	131 (130)	88 (88)	159 (155)	131 (130)	3.69 (1.73)
SS	RR	82 (82)	146 (146)	135 (118)	73 (79)	154 (164)	101 (90)	5.71 (6.83)

All sensible configurations (Fig. 4) were calculated at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory and at the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) (values in parentheses) levels of theory

[a] Relative to the most stable conformer of AA type sheets: SRSR (M05-2X: $E = -1{,}981.385062$ Hartree; B3LYP: $E = -1{,}981.63758274$ Hartree)

Table 2 The effect of the chirality of disubstitution on the overall sheet stability of β-peptides forming AA type sheets

Chirality of disubstitution		Relative values of thermodynamic functions			
Strand a	Strand b	ΔE^{a}_{rel}/kcal mol^{-1}	ΔH^{a}_{rel}/kcal mol^{-1}	ΔG^{a}_{rel}/kcal mol^{-1}	$T\Delta S^{a}_{rel}$/kcal mol^{-1}
SR	SR	0.00 (0.00)	0.00 (0.00)	0.00 (0.80)	0.00 (−0.80)
SR	RS	0.45 (0.67)	0.35 (0.51)	0.60 (0.00)	−0.25 (0.51)
SR	SS	1.62 (0.91)	1.86 (0.89)	2.10 (1.81)	−0.24 (0.92)
RS	SS	2.41 (1.06)	2.65 (1.01)	3.28 (2.29)	−0.63 (−1.28)
SS	SS	3.69 (1.73)	3.95 (1.91)	5.17 (3.38)	−1.22 (−1.47)
SS	RR	5.71 (6.83)	6.10 (6.93)	6.88 (8.16)	−0.78 (−1.23)

For HCO-β-Ala-β2,3-hAla-β-Ala-NH2 all possible configurations (Fig. 4) were calculated at the M05-2X/6-311 ++G(d,p)//M05-2X/6-31G(d) and at the B3LYP/6-311 ++G(d,p)//B3LYP/6-31G(d) (values in parentheses) levels of theory

[a] Relative to the most stable conformer of AA type sheets: SRSR (M05-2X: $E = -1{,}981.385062$ Hartree, $H = -1{,}980.605368$ Hartree, $G = -1{,}980.74093$ Hartree; B3LYP: $E = -1{,}981.63758274$ Hartree, $H = -1{,}980.870425$ Hartree, $G = -1{,}981.008856$ Hartree)

Interestingly enough, even in sheet structures, the opposite strand has little or no effect on the torsional angles of a strand; its structure is only affected by local chirality. Because of this, four distinct [φ, ψ] pairs can be attributed to the four possible substitution patterns of a strand (e.g. for all structures containing a strand with *SR* configuration the torsional angle values are the same for "strand a" or "strand b", Table 1).

In the case of heterochiral disubstitution, methyl groups can adopt the ideal *anti* conformation. Beke et al. (2004) showed that *gauche* configuration is preferred for φ torsional angle and a more extended conformation is preferred for ψ. Although being heterochiral in nature, there is a structural difference between SRSR and SRRS configurations. In the first case, both strands have (*R*)-Cα and (*S*)-Cβ configuration, both φ and ψ torsional angles are shifted towards their "ideal" orientations (*gauche* and *anti*, respectively). Thus, both strands adopt a favorable structure, resulting in greater stabilization. In the latter case, **strand a** has (*R*)-Cα and (*S*)-Cβ, **strand b** has (*S*)-Cα and (*R*)-Cβ configuration. Thus, based on the changes in torsional angle values upon chiral substitution as discussed above, for **strand b** both φ and ψ are shifted from the ideal values, which results a less optimal strand structure.

In the case of homochiral disubstitution, methyl groups cannot adopt an *anti* conformation as they would be positioned in a *gauche* orientation. In contrast to Seebach's predictions, strands with homochiral configuration were also found to be able to form apolar sheet structures. In this case, φ and ψ torsional angles are similar to those of the unsubtituted sheets. For the (*S*)-Cα and (*S*)-Cβ derivatives, φ torsional angle is slightly shifted towards its favored conformation and also the methyl groups are facing away from the interstrand H-bonds (Fig. 5). As a result, no methyl groups are found in the "forbidden" positions contradicting the prediction of Seebach. However, even if the H-bonding pattern as well as the sheet structure is undisturbed, weak repulsion arises between the facing methyl groups in *gauche* position ($\sim$3 kcal mol^{-1} less stability, Table 1).

Properties of mixed sheets built of a heterochiral ("unlike") and a homochiral ("like") strand are similar to that of sheets built of heterochiral strands. Both strands adopt the φ, ψ values characteristic to their substitutional pattern. In the case of homochirally disubtituted derivatives with methyl groups facing away from the H-bond network, backbone torsional angles remain similar and thus the sheet structure remains undisturbed.

These structural considerations can be used to explain stability differences between the differently disubstituted AA type sheets (relative stabilities summarized in (Table 2).

Clearly heterochirally substituted species seem to be the best candidates for sheet formation as having the lowest relative ΔG values (0.00 kcal mol^{-1} for SRSR and

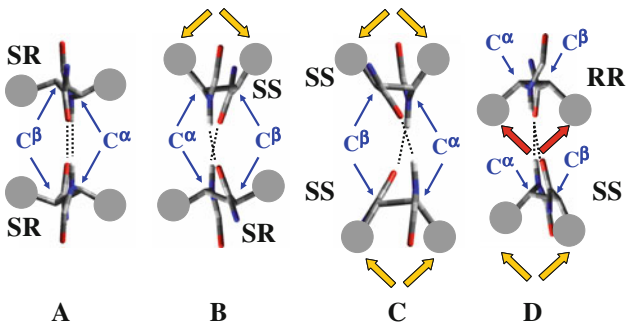


Fig. 5 Possible dihedral conformations of the methyl groups (*grey circles*) and their repulsive interactions (*indicated by arrows*). Heterochiral strands (**a**) are optimal for sheet formation (with minimized methyl–methyl repulsion). Methyl groups adopt *gauche* conformation on homochirally substituted strands (**b–d**): based on chirality they can face away (external (ext.)) from the H-bonds (**b**, **c**) or face towards (internal (int.)) the H-bonds (D). Note: hydrogen atoms not taking part in H-bonding are not shown. Strand a and strand b are the lower and upper strands, respectively

0.60 kcal.mol^{-1} for SRRS, respectively), in accordance with Seebach's predictions. As methyl groups are in the *anti* orientation, intrastrand methyl–methyl repulsions are most likely the smallest possible (Fig. 5). The SRSR structure has greater stability than that of SRRS. This difference cannot be explained by Seebach's predictions as both derivative has heterochiral (or "unlike") configuration. Consequently, both should have a roughly equal stability. The reason for the difference lies in the aforementioned local effect of point chirality. The more favorable hydrogen bonding scheme and the more favorable φ and ψ values for "strand b" (vide infra) result in greater stability for the SRSR derivative when compared to that of the SRRS (Table 2).

Sheets built only of homochirally disubstituted strands have lower stability because of the destabilizing interactions operative between the methyl groups all in *gauche* conformation. This effect is more pronounced when the methyl groups are facing the hydrogen bond system ($\Delta G_{SSSS} = 5.17$ and $\Delta G_{SSRR} = 6.88$ kcal mol^{-1}, respectively). This is in agreement with the notion of "forbidden" position in previous publications (Seebach 2004). However, structures containing homochiral strands [or "like" configuration based on the nomenclature proposed by Seebach (2004)] still retain their sheet like structure. Thus homochirality (or "like" configuration) itself (because of "forbidden" side-chain positions, Seebach 2004) is not the only reason for the reduced sheet stability (Fig. 5; Table 2). The repulsion between methyl groups and the conformation of the strands also have to be taken into the account.

Mixed sheets built of a heterochiral ("unlike") and a homochiral ("like") strand are similar to that of sheets composed of heterochiral strands. If the methyl groups of the homochirally disubstituted strand are facing away from the interstrand H-bonds, the stability loss of sheet structure is negligible. The lower stability compared to pure heterochiral sheets (Table 3) can be attributed to methyl–methyl repulsions of the homochirally disubstituted strand. However, as only one strand possess homochiral configuration, this effect is lower compared to the purely homochiral sheets. Thus the relative stabilities of mixed sheets are in between the pure heterochiral and pure homochiral nanosystems (Table 3).

The stability difference between SRSS and RSSS sheets ($\Delta G_{SRSS} = 2.10$ and $\Delta G_{RSSS} = 3.28$ kcal mol^{-1}) can again be attributed to the unfavourable φ values in "strand a" for the latter RSSS derivative.

AB type sheets

In this type of apolar sheet, the two strands form mirror image structures (Table 3). Thus, (*R*)-Cα and (*S*)-Cβ substitution in one strand is the same as (*S*)-Cα and (*R*)-Cβ substitution on the other one. Because of this, unlike AA sheet, SRRS conformer is the most stable foldamer (containing ideal φ, ψ torsional angle values) and SRSR conformer has a lower stability because of the unfavorable φ value in "strand b".

The relative stabilities of differently disubstituted AB type sheets are summarized in Table 4. Trends and conclusions observed in the case of AA sheets are also valid for the AB sheets.

Heterochiral configurations possess the greatest stability in agreement with Seebach's predictions ($\Delta G_{SRRS} = 0.00$ and $\Delta G_{SRSR} = 1.55$ kcal mol^{-1}). Homochiral foldamers retain the sheet structure, but possess lower stability ($\Delta G_{SSSS} = 7.85$ and $\Delta G_{SSRR} = 9.12$ kcal mol^{-1}), and mixed AB sheets are more stable than pure homochiral but less stable than pure heterochiral AB sheets ($\Delta G_{SRRR} = 3.01$ and $\Delta G_{RSRR} = 5.10$ kcal mol^{-1}). The stability differences for heterochiral and mixed sheets can again be attributed to the unfavorable φ values in the case of (*S*)-Cα and (*R*)-Cβ substitution. It is important to note that even though SSSS configuration with a pair of methyl groups facing the hydrogen bond system, have found to have greater stability for AB sheets, it is clearly the least stable configuration with longer side-chains.

Table 3 The effect of chirality on the backbone torsional angles of strands of AB type: backbone torsional angle values of the central unit of HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 were calculated for all independent configurations (Fig. 4) at the M05-2X/6-311 ++G(d,p)// M05-2X/6-31G(d) level of theory

Chirality of disubstitution		Torsional angles strand a			Torsional angles strand b			ΔE^{a}_{rel}/kcal mol^{-1}
Strand a	Strand b	φ	*M*	ψ	φ	μ	ψ	
SR	RS	58 (60)	161 (159)	117 (119)	−58 (−60)	−161 (−159)	−117 (−119)	0.00 (0.00)
SR	SR	56 (56)	158 (156)	123 (125)	− 99 (−103)	−160 (−157)	−78 (−78)	3.26 (2.31)
SR	RR	57 (60)	161 (161)	117 (120)	−83 (−81)	−163 (−154)	−110 (−111)	3.98 (2.56)
RS	RR	98 (104)	163 (163)	77 (76)	−79 (−79)	−154 (−150)	−114 (−110)	5.99 (3.63)
SS	RR	83 (82)	159 (154)	112 (112)	−83 (−82)	−160 (−154)	−112 (−112)	7.98 (5.18)
SS	SS	80 (79)	156 (148)	116 (114)	−70 (−77)	−149 (−152)	−91 (−94)	8.70 (6.84)

[a] Relative to the most stable conformer of AB type sheets: SRRS (M05-2X: E = –1,980.7836989 Hartree; B3LYP: E = –1,981.649983 Hartree)

Table 4 The effect of the chirality of disubstitution on the overall sheet stability of β-peptides forming AB type sheets

Chirality of disubstitution		Relative values of thermodynamic functions			
Strand a	Strand b	ΔE^{a}_{rel}/kcal mol^{-1}	ΔH^{a}_{rel}/kcal mol^{-1}	ΔG^{a}_{rel}/kcal mol^{-1}	$T\Delta S^{a}_{rel}$/kcal mol^{-1}
SR	RS	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SR	SR	3.26 (2.19)	3.10 (2.10)	1.55 (1.96)	1.55 (0.15)
SR	RR	3.98 (2.79)	3.88 (2.75)	3.02 (2.34)	0.86 (0.41)
RS	RR	5.99 (3.63)	5.88 (3.48)	5.10 (2.61)	0.79 (0.88)
SS	RR	7.98 (5.71)	8.15 (5.79)	7.85 (5.68)	0.30 (0.11)
SS	SS	8.70 (7.03)	8.61 (7.02)	9.12 (6.49)	−0.51 (0.24)

For HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 all possible configurations (Fig. 4) were calculated at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) and at the B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) (values in parentheses) levels of theory

[a] Relative to the most stable conformer of AB sheets: SRRS (M05-2X: $E = -1{,}980.7836989$ Hartree, $H = -1{,}980.003438$ Hartree, $G = -1{,}980.135712$ Hartree; B3LYP: $E = -1{,}981.649983$ Hartree, $H = -1{,}980.882350$ Hartree, $G = -1{,}981.016132$ Hartree)

Relative stability of apolar dimeric sheets in polar and apolar solvents

To better support the practical design of various sheet structures of β-peptides, the effect of the environment has to be taken into account on the relative stability distribution. Here both polar or apolar solvents were tested for the AA and AB type sheets, which were the most stable sheets based on gas phase calculations (Table 5). These structures were submitted for solvent calculations using the IEFPCM model. For a polar solvent environment water, while for apolar heptane was chosen, which could qualitatively reproduce a hydrophobic environment such as interior of a lipid bilayer (Stenberg et al. 1999).

Interestingly, applying implicit solvent models does not significantly alter the stability order of the lower energy stereoisomers of apolar AA type strands. SRSR remains the most stable one however the difference between the two most stable configurations (SRSR and SRRS) is almost negligible in water. Note that both polar and apolar matrices enhance the destabilizing effect of methyl–methyl repulsion, lowering the stability of both homochiral and mixed apolar AA type of strands (i.e. for SRSS $\Delta G = 2.10$ kcal mol^{-1} in vacuum, 5.24 kcal mol^{-1} in water and 3.52 kcal mol^{-1} in heptane). This is a rather important observation as according to the gas phase calculations five out of six configurations could have similar affinity to form AA type sheets, whereas the notable distinction in relative stability for water and heptane gives a much clearer suggestion for 'sheet-favoring' chiral residues (Table 5). A change of order for SSSS (all methyl groups facing outward to the environment) and SSRR (only one pair of the methyl groups faces the environment) derivatives was also observed.

The same distinction between the most stable and less stable configurations is observed for stability of AB type apolar sheets in water and also in heptane (Table 6). While the first two most stable configurations are within 1.6 kcal mol^{-1} for all three environments, the ΔG between the second and third most stable structures is $\sim$1.5 kcal mol^{-1} for vacuum, $\sim$4 kcal mol^{-1} for water and $\sim$2.5 kcal mol^{-1} in heptane. This again clearly suggests that preference

Table 5 The effect of polar and apolar solvents on the stability of chiraly disubstituted AA type sheets of β-peptides, as calculated for HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 (Fig. 4) at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory

Chirality of disubstitution		ΔE/kcal mol^{-1a}			ΔH / kcal mol^{-1a}			ΔG/kcal mol^{-1a}		
Strand a	Strand b	Vacuum	Water	Heptane	Vacuum	Water	Heptane	Vacuum	Water	Heptane
SR	SR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SR	RS	0.45	0.29	0.54	0.35	0.19	0.44	0.60	0.45	0.70
SR	SS	1.62	4.76	3.04	1.86	5.00	3.29	2.10	5.24	3.52
RS	SS	2.41	6.38	4.18	2.65	6.62	4.42	3.28	7.25	5.05
SS	SS	3.69	8.52	6.36	3.95	8.78	6.63	5.17	10.01	7.85
SS	RR	5.71	6.51	5.75	6.10	6.91	6.15	6.88	7.69	6.93

Optimization was carried out in vacuum, single point energy calculation was carried out in solvent

[a] Relative to the most stable conformer of AA sheets: SRSR (vacuum: $E = -1{,}981.385062$ Hartree, $H = -1{,}980.605368$ Hartree, $G = -1{,}980.74093$; water: $E = -1{,}981.41099$ Hartree, $H = -1{,}980.631298$ Hartree, $G = -1{,}980.76686$ Hartree; heptane: $E = -1{,}981.391012$ Hartree, $H = -1{,}980.611318$ Hartree, $G = -1{,}980.74688$ Hartree). Absolute energy values can be found in supplementary Tables 5, 7, 9

Table 6 The effect of polar and apolar solvents on the stability of chiraly disubstituted AB type sheets of β-peptides, as calculated for HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 (Fig. 4) at the M05-2X/6-311++G(d,p) level of theory

Chirality of disubstitution		ΔE/kcal mol^{-1}[a]			ΔH/kcal mol^{-1}[a]			ΔG/kcal mol^{-1}[a]		
Strand a	Strand b	Vacuum	Water	Heptane	Vacuum	Water	Heptane	Vacuum	Water	Heptane
SR	RS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SR	SR	3.26	2.49	2.92	3.10	2.32	2.75	1.55	0.77	1.21
SR	RR	3.98	5.65	4.71	3.88	5.55	4.61	3.02	4.70	3.75
RS	RR	5.99	8.02	6.82	5.88	7.91	6.71	5.10	7.12	5.92
SS	RR	8.70	11.33	9.87	8.61	11.24	9.78	9.12	11.75	10.29
SS	SS	7.98	6.90	7.01	8.15	7.06	7.17	7.85	6.77	6.87

Using gas phase geometries obtained at the M05-2X/6-31G(d) level of theory

[a] Relative to the most stable conformer of AB sheets: SRRS (vacuum: $E = -1{,}981.397979$ Hartree, $H = -1{,}980.617718$ Hartree, $G = -1{,}980.749992$ Hartree; water: $E = -1{,}981.420424$ Hartree, $H = -1{,}980.640163$ Hartree, $G = -1{,}980.772437$ Hartree; heptane: $E = -1{,}981.401774$ Hartree, $H = -1{,}980.621513$ Hartree, $G = -1{,}980.753787$ Hartree). Absolute energy values can be found in supplementary Tables 6, 8, 10

of a certain sheet type is seriously affected by the chirality of the disubstituted β-amino acid residues. Unlike AA type apolar sheet, in this case the least stable structure in vacuum is the same as it is in solvents. A change in stability order can also be seen in this case, namely in polar solvent the stability of the less favored mixed sheet seems to be lowered, making the homochirally disubstituted SSSS derivative to be more stable. As mentioned before, this should not be the case with longer side-chains. Nevertheless, it is important to observe that for both AA and AB type sheets the qualitative stability order of the most stable configurations is correctly predicted by gas phase calculations.

Stability changes of apolar sheets of β-peptides in polar and apolar solvents

Since in the present study, our aim is to give a systematic proposal on which β-amino acids should be used to materialize certain sheet structures, we mostly focused on the relative stability of the different configurations in a given subset of sheets. However, it is useful to consider whether sheet formation out of two single-stranded peptide segments would be preferred thermodynamically at all. Thus, both for AA- and for AB-type sheet structures energetic properties of dimerization were considered.

Strands with $H8^P$ (A) and $H8^M$ (B) conformation were calculated, in the same way as in the case of sheet structures, with all possible chirality, as they have significant stability on their own and possess a conformation that is most similar to the strand conformation in apolar sheets. In these structures μ torsional angle is shifted from 160 to 120, thus intrastrand 8-membered hydrogen bonds are formed. Also, because of the helical nature of the strand, homochiral disubstitutions are more preferred than heterochiral ones.

For the calculations thermodynamic parameters (ΔX, $X = E$, H or G) of dimerization of a sheet with a given configuration of "strand a" and "strand b", the sum of the thermodynamic parameters (X, $X = E$, H or G) of the two strands (A and A for AA sheets; A and B for AB sheets) with the same configurations as "strand a" and "strand b", respectively, was substracted from the thermodynamic parameters of the sheet (Eq. 1):

$$\Delta X_{\text{formation}} = X_{\text{sheet}}(\text{ab}) - [X_{\text{H8}}(\text{a}) + X_{\text{H8}}(\text{b})] \quad (1)$$

Thus, for example, in the case of AA sheet with SRSS configuration the dimerization energy was calculated as follows (Eq. 2.):

$$\Delta X_{\text{formation}} = X_{\text{AA}}(\text{SRSS}) - [X_{\text{A}}(\text{SR}) + X_{\text{A}}(\text{SS})]. \quad (2)$$

According to gas phase calculations, the dimerization is favored for every configuration for both AA and AB sheets (Tables 7, 8). However, when effects of the environment are considered, the magnitude of enthalpy and Gibbs free energy of dimerization was found to decrease in the range 3–5 kcal mol^{-1}. Because of this decrease, for AA sheets (Table 7), only the formation of the two heterochiral structures, SRSR and SRRS, was thermodynamically preferred in water. The stability decrease in heptane was somewhat smaller (with 2–4 kcal mol^{-1}), thus the formation of one of the mixed type sheets was also found to be preferred ($\Delta G < 0$ for SRSR, SRRS and SRSS). These stability values are in line with the relative sheet preferences found in the previous section (Table 5).

For AB type of sheets, the two heterochirally disubtituted derivative, one mixed and one homochirally disubtituted derivative was found to prefer dimerization in water (i.e. SRRS, SRSR, SRRR and SSSS had $\Delta G < 0$) (Table 8). Again, heptane had a lesser destabilizing effect on the formation thus only the least stable homochiral

Table 7 The effect of polar and apolar solvents on the formation (ΔE, ΔH and ΔG) of chiral disubstituted AA type sheets of β-peptides HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 (Fig. 4) obtained at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory

Chirality of disubstitution		ΔE/kcal mol^{-1a}			ΔH/kcal mol^{-1a}			ΔG/kcal mol^{-1a}		
Strand a	Strand b	Vacuum	Water	Heptane	Vacuum	Water	Heptane	Vacuum	Water	Heptane
SR	SR	−16.99	−13.73	−12.87	−16.38	−13.13	−12.26	−7.38	−4.12	−3.26
SR	RS	−15.68	−12.31	−11.45	−15.01	−11.65	−10.79	−5.45	−2.09	−1.23
SR	SS	−16.82	−8.86	−10.40	−15.85	−7.89	−9.43	−6.73	1.23	−0.31
RS	SS	−15.16	−6.11	−8.39	−14.04	−4.98	−7.26	−4.22	4.83	2.55
SS	SS	−16.20	−4.98	−7.65	−15.09	−3.87	−6.55	−5.10	6.12	3.45
SS	RR	−10.93	−6.17	−6.63	−8.91	−4.15	−4.61	−0.29	4.48	4.01

Optimization was carried out in vacuum, single point energy calculation was carried out in solvent

[a] Relative to the sum of the two strands with identical chiralities (STables 11, 12, 13)

Table 8 The effect of polar and apolar solvents on the formation (ΔE, ΔH and ΔG) of AB type sheets of β-peptides HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2 (Fig. 4) obtained at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory

Chirality of disubstitution		ΔE/kcal mol^{-1a}			ΔH/kcal mol^{-1a}			ΔG/kcal mol^{-1a}		
Strand a	Strand b	Vacuum	Water	Heptane	Vacuum	Water	Heptane	Vacuum	Water	Heptane
SR	RS	−25.09	−19.65	−19.62	−24.13	−18.69	−18.66	−13.06	−7.62	−7.59
SR	SR	−20.97	−16.04	−15.83	−20.01	−15.08	−14.87	−10.19	−5.26	−5.05
SR	RR	−22.57	−13.88	−15.49	−21.59	−12.90	−14.51	−11.49	−2.81	−4.41
RS	RR	−19.69	−10.39	−12.50	−18.56	−9.25	−11.37	−8.09	1.21	−0.91
SS	RR	−19.29	−8.09	−10.89	−17.40	−6.20	−10.76	−6.99	4.21	1.41
SS	SS	−16.76	−11.70	−12.12	−15.40	−10.34	−9.01	−4.86	0.20	−0.23

Optimization was carried out in vacuum, single point energy calculation was carried out in solvent

[a] Relative to the sum of the two strands with identical chiralities (STables 11, 12, 13)

configuration had higher ΔG values than two separate single strands. Note that these values also help to identify that which sheets would be most likely formed in a certain environment: For instance, comparing values from Tables 7 and 8, it can be concluded that in water a short sequence with SRSR or RSRS chirality pattern on the opposing strands would readily form a AA and AB sheet motifs, most likely preferring AB type for both SRSR ($\Delta\Delta G = -1.12$ kcal mol^{-1} favoring AB over AA) and SRRS ($\Delta\Delta G = -5.03$ kcal mol^{-1} favoring AB). Along the same lines, a relative sheet preference can be proposed. The authors hope that such a quick guide can efficiently help the future design of new β-peptide secondary structures.

Structural variations

In order to further clarify the effect of substitution, single point energy calculations on dimers containing only the disubstituted amino acids (Fig. 6) were also investigated. The conclusions and trends observed in the case of tripeptide dimers were also valid for dimers of [HCO-$\beta^{2,3}$-hAla-NH_2]$_2$, thus the differences observed are indeed the results of substitution (see supplementary Table 2). Thus without the unsubstituted ends the structures are still stable. In order to further confirm these results, full optimization was carried out on the most stable AB sheet: SRRS, and on the homochirally disubstituted AB sheet: SSRR at the M05-2X/6-31G(d) level of theory. The stability difference obtained is the same as those of the single point energy calculations (Table 9).

Along the same line, in order to validate the results of our single point energy calculations in water and in heptane at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory, full geometry optimization and frequency calculation at the M05-2X/6-311++G(d,p) level of theory were carried out in water on the most stable AB sheet: SRRS and the homochirally disubstituted AB sheet: SSRR of [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$. It can be seen, that although the destabilization effect obtained is higher in this case (Table 10), the homochirally disubstituted derivative retained its sheet structure, further confirming the existence of such secondary structural element.

Other structural variation included the full methyl substitution of the tripeptide dimers (Fig. 6). Full geometry

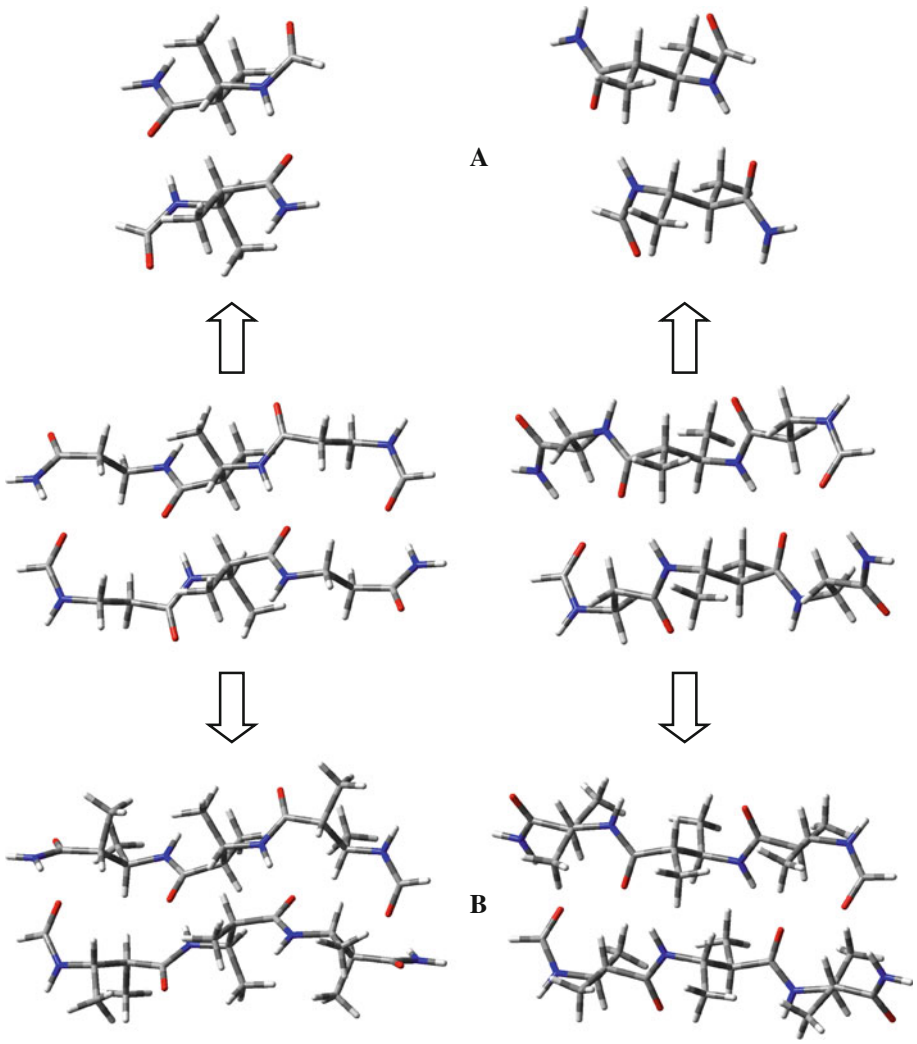


Fig. 6 Summary of other studied model systems: structure of the dimers of heterochirally and homochirally disubstituted mono-β-peptides: (For-$\beta^{2,3}$-hAla-NH_2)$_2$ (**a**) and the dimers of heterochirally and homochirally disubstituted tri-β-peptides: (For-($\beta^{2,3}$-hAla)$_3$-NH_2)$_2$ (**b**) as obtained at the M05-2X/6-611++G(d,p)//M05-2X/6-31G(d) level of theory

Table 9 The effect of chirality on the backbone torsional angles of strands in case of AB type sheets of beta peptides: backbone torsional angle values and relative stabilities of fully optimized HCO-$\beta^{2,3}$-hAla-NH_2 in selected configurations at the M05-2X/6-31G(d) level of theory

Chirality of disubstitution		Torsional angles strand a			Torsional angles strand b			ΔE_{rel}^{a}/kcal mol^{-1}
Strand a	Strand b	φ	μ	ψ	φ	μ	ψ	
SR	RS	58	164	123	−58	−164	−123	0.00 (0.00)
SS	RR	85	166	117	−85	−166	−117	5.99 (5.99)

The relative stabilities obtained from single point energy calculations are given in parentheses

[a] Relative to the fully optimized most stable conformer of AB type sheets: SRRS

optimization on the pure heterochiral: RS-SR-RS SR-RS-SR, and pure homochiral: RR-SS-RR SS-RR-SS derivative of For-[HCO-($\beta^{2,3}$-hAla)$_3$-NH_2]$_2$ were carried out (Table 11). The optimized structure parameters revealed that both sheet structures are stable. Even though experimentalists documented so far only the existence of the helical structures(s) nevertheless here we predict that the potential energy hypersurface exhibits multiple minima. Even if the helix would be the global minimum the optimized sheet structure cleanly is at least a higher energy minimum on the potential energy hypersurface.

Summary

The effect of chirality on apolar sheet stability has been investigated on [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$ peptide model. In accordance with Seebach's predictions,

Table 10 The effect of chirality on the backbone torsional angles of strands in case of AB type sheets of beta peptides: backbone torsional angle values and relative stabilities of fully optimized [HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$ in selected configurations in water at the M05-2X/6-311++G(d,p) level of theory

Chirality of disubstitution		Torsional angles strand a			Torsional angles strand b			ΔE^{a}_{rel}	ΔH^{a}_{rel}	ΔG^{a}_{rel}	$T\Delta S^{a}_{rel}$
Strand a	Strand b	φ	μ	ψ	φ	μ	ψ				
SR	RS	59 (58)	165 (161)	122 (114)	−59 (−58)	−165 (−161)	−122 (−114)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SS	RR	88 (83)	169 (159)	114 (112)	88 (−83)	169 (−160)	114 (−112)	6.96 (6.90)	8.06 (7.06)	8.27 (6.77)	−0.21 (0.30)

The values in parentheses are the corresponding torsional angles and relative stabilities of the structures used in single point calculations

[a] In kcal mol^{-1}; relative to the fully optimized most stable conformer of AB type sheets: SRRS

Table 11 The effect of chirality on the backbone torsional angles of strands in case of AB type sheets of beta peptides: averages of backbone torsional angle values and relative stabilities of fully optimized For-[HCO-($\beta^{2,3}$-hAla)$_3$-NH_2]$_2$ in selected configurations at the M05-2X/6-31G(d) level of theory

Chirality of disubstitution		Torsional angles strand a			Torsional angles strand b			ΔE^{a}_{rel}	ΔH^{a}_{rel}	ΔG^{a}_{rel}	$T\Delta S^{a}_{rel}$
Strand a	Strand b	φ	μ	ψ	φ	μ	ψ				
SRSRSR	RSRSRS	59	161	143	−59	−161	−142	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
RRSSRR	SSRRSS	96	176	102	−80	−178	−123	10.81 (7.98)	11.14 (8.15)	10.92 (7.85)	0.22 (0.30)

As a comparison, the values in parentheses show the stability difference obtained for the original For-[HCO-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$ model system

[a] In kcal mol^{-1}; relative to the fully optimized most stable conformer of AB type sheets: SRSRSR-RSRSRS

heterochirally disubstituted sheets have the greatest preference for sheet formation ($\Delta G_{formation} \sim -11$ kcal mol^{-1} for AB and ~ -7 kcal mol^{-1} for AA sheets).

In contrast to Seebach's predictions, homochiral substitution itself does not hinder sheet formation. Even though these structures haven't yet been observed experimentally, there is no theoretical reason for their non-existence: it depends on the orientation of the *gauche* methyl groups. In the case of AA sheets with (S)-Cα and (S)-Cβ substitution methyl groups are facing away from H-bonding system (Figs. 5, 7). The sheet has $\sim$2–3 kcal mol^{-1} less stability, compared to heterochiral disubstitution, because of methyl–methyl repulsion, but the structure is retained. However, in the case of AA sheets with (R)-Cα and (R)-Cβ substitution, methyl groups facing the H-bonding system (Figs. 5, 7) cause great ($\sim$6 kcal mol^{-1}) destabilization resulting in sheets of very little stability.

The stability of mixed sheets is between the stabilities of homo- and heterochirally substituted sheets as only one strand contains methyl groups in *gauche* orientation.

In order to simplify the rational design of such sheet structures, the aforementioned properties are summarized in a visual representation of formation Gibbs free energies for all 16 configurations in Fig. 8. As AA and BB sheets, as well as AB and BA sheets can form equally, enantiomeric structures are included in the figure (e.g. SRSR is the most stable configuration for AA sheet, while RSRS is the most stable configuration for BB sheet and they have the same stability, being enantiomeric pairs). As both sheet type has equal stability, depending on configuration either AA or BB sheet is formed. The general tendencies mentioned above holds true for structures not only computed in vacuum but also in polar (water) and apolar (heptane) molecular environments. Heterochiral structures possess great stability in all solvents. The destabilizing effect of *gauche* methyl groups in homochiral and mixed sheets are further enhanced by solvent effect. However, in the case of AB type of sheets even fully homochirally disubstituted sheets have a tendency to form ($-2 < \Delta G < 0$ kcal mol^{-1}) to some extent in all solvents tried. This tendency could possibly be further enhanced by adding longer sidechains resulting in favorable sidechain–sidechain interactions.

In the case of polar type, CC sheets no clear trends were observable for the effect of chirality on sheet stability. It is hypothesized that the energy differences between the backbone conformers are comparable with the energy differences between differently disubstituted conformers (See Supplementary Material).

It should be noted that methyl group orientation alone is not enough to predict sheet stability, the local conformational effect of substitution also have to be considered as point chirality was found to have a local effect on torsional angles. *R*-substitution shifts torsional angles towards *anti* position whereas *S*-substitution shifts torsional angles towards *gauche* position on both Cα and Cβ. The torsional angle values of the two strands in the sheets are independent

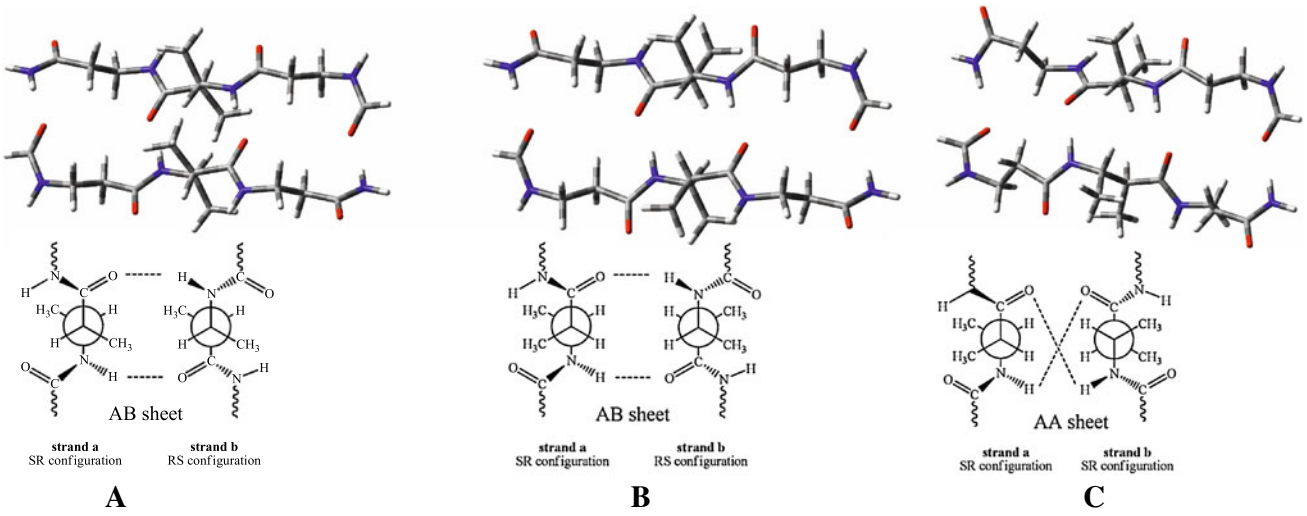


Fig. 7 Schematic structures and the corresponding Newman projections of **a** The most stable heterochiral conformer (AB type of sheet); **b** the homochirally disubstituted AB type of sheet; **c** the homochirally disubstituted AA type of sheet

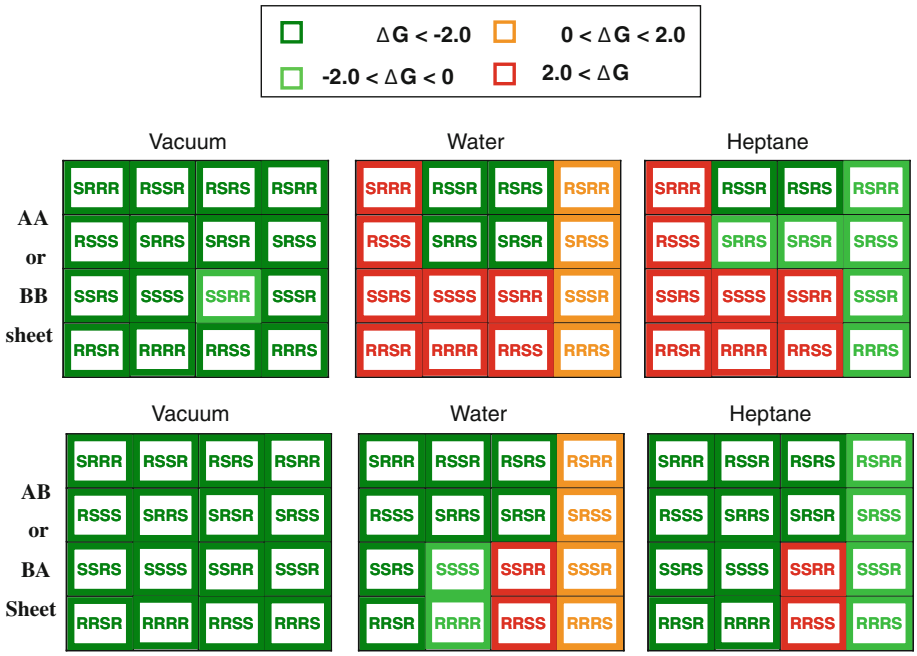


Fig. 8 Graphic summary of the thermodynamic parameters of formation of differently disubstituted AA (or BB) and AB (or BA) apolar sheets as obtained at the M05-2X/6-311++G(d,p)//M05-2X/6-31G(d) level of theory

of each other. Sheet stability is mostly affected by the torsional angles of the two backbones: strands with identical substitution patterns have similar backbone torsional angles thus can form stable sheets because of the favorable hydrogen bonding pattern.

Sheets containing (*S*)-Cβ carbon were found to possess greater stability than sheets containing (*R*)-Cβ substitution, because of the more favorable φ torsional angle values in the former. The effect of Cβ substitution was found to have greater effect on sheet stability compared to Cα substitution. This is in agreement with the results of previous publications (Möhle et al. 1999; Wu and Wang 1998; Günther and Hofmann 2002; Beke et al. 2004): the value of φ torsional angle (affected by Cβ substitution) is more "rigid" and has greater effect on sheet stability, while ψ torsional angle (affected by Cα substitution) has greater conformational freedom.

Based on these observations, the four questions asked at the introduction can be answered:

A1: We have found that heterochirally disubstituted derivatives have the greatest why homochirally disubstituted derivatives have the least preference for sheet forming. Mixed (one heterochirally and one homochirally

substituted) derivatives had a stability between the aforementioned two. A local effect of chirality on backbone torsion angles is observed.

A2: We can conclude that Seebach's predictions provided a good qualitative description of folding preference. However it lacked the consideration of some key issues. The trends observed in sheet stability correlates well with the statements of Seebach's predictions: homochiral disubstitution is the least, while heterochiral disubstitution is the most preferred setup for sheet formation.

A3: In contrast to the some statement of Seebach's predictions, sheet structures can also be formed from homochirally disubstituted derivatives. In the case of methyl groups facing away from the hydrogen bonding system, sheet formation is not hindered and the clashing of sidechains doesn't occur either. However this derivative can only form two stranded sheets as subsequent self-association is blocked by methyl groups.

A4: Our results enabled us to extend and generalize this rule to propose a general "rule of thumb" to describe the effect of chirality on sheet stability: Heterochirally disubstituted models are the best candidates for sheet forming if the two strands are substituted in a way to create matching torsional angle sets on the two backbone for ideal hydrogen-bonding pattern (*e.g.*: SRSR for AA sheets and SRRS for AB sheets). With adequately selected side chains, homochirally disubtituted derivatives may also form sheet structures, and the position of methyl groups would prevent further aggregation which would make it an ideal motif to create hairpins (the addition of a 3rd strand would be hindered by the methyl groups).

Finally, the results of our calculations in vacuum are further confirmed by single point calculations in water and heptane (IEF-PCM) and by calculations completed at different levels of theory (See supplementary material).

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