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Exploring β-Hydroxy γ-Amino Acids (Statines) in the Design of Hybrid Peptide Foldamers

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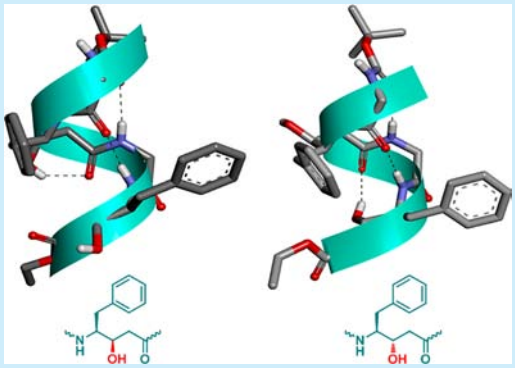
Supporting Information

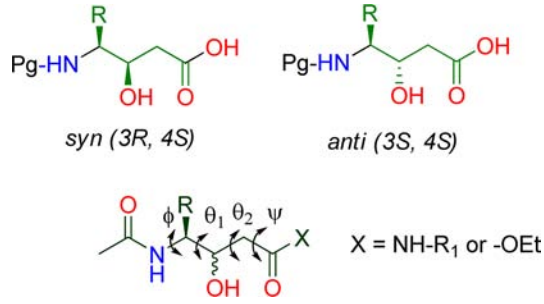
ABSTRACT: The synthesis and characterization of *syn* and *anti* β-hydroxy γ-amino acid (statine) diastereoisomers, their utilization in the design of hybrid peptide foldamers, and their single crystal conformations are studied.



γ-Amino-β-hydroxy acids (statines) are naturally occurring nonribosomal amino acids widely present in many peptide natural products. Peptides constituted with statines have been used as protease inhibitors. For example, pepstatin,[1] a naturally occurring peptide, has shown broad inhibitory activities against various aspartic acid proteases,[2] such as pepsin, cathespin D and E, rennin, HIV-1 protease, β-secretase, plasmepsin I and II of the malarial parasite *Plasmodium falciparum*, etc. In addition, several natural peptides containing statines or modified statines, such as didemnins,[3] dolastatins,[4] hapalosin,[5] tamandarins,[6] etc., displayed promising anticancer properties. Inspired by the remarkable biological applications of these peptide natural products, various synthetic strategies have also been developed for the synthesis of statines.[7] Although the peptides containing statines have been extensively investigated for their biological properties, very little is known regarding their conformational properties. The natural occurrence and their excellent biological activities inspired us to investigate the conformational behavior of these amino acids in hybrid peptides.

The conformational properties of γ- and hybrid γ-peptides with various types of γ-amino acids such as γ^4-amino acids,[8] 3,3-dialkyl γ-amino acids, cyclic γ-amino acids,[10] and 2,3,4-alkyl substituted γ-amino acids have been thoroughly investigated. Results suggest that most of these γ- and hybrid γ-peptides prefer to adopt helical conformations. However, peptides with α,β-unsaturated γ-amino acids[12] and 4,4-dimethyl substituted γ-amino acids[13] prefer to adopt an extended sheet type of structure. We have been interested in the conformational analysis of various γ- and α,γ-hybrid peptides and sought to investigate the influence of β-hydroxyl groups of statine residues in the folding of hybrid γ-peptides. As we are interested in understanding the conformational behavior of both the *syn* and *anti* (with respect to side chains of the amino acids) diastereoisomers of statines (Scheme 1), β-keto-γ-amino esters were chosen as starting materials for the synthesis.

Scheme 1. *Syn* and *Anti* β-Hydroxy-γ-amino Acids (Statines)[a]



[a]Local torsional variables of statines are also shown.

Recently, we reported the mild protocol for the synthesis of β-keto-γ-amino esters[14] starting from the *N*-protected amino aldehydes by adopting the Roskamp's procedure.[15] Rich et al. reported the synthesis of *syn* and *anti* diastereoisomers of statines through the mild reduction of β-keto γ-amino esters using $NaBH_4$ in various sovlent combinations.[16] We chose to utilize THF as a sovlent in the reduction of β-keto esters to reduce the diastereoselectivity in the reaction. The schematic representation of the synthesis of statines is shown in Scheme 2. By utilizing the reported protocol,[14] various *N*-Boc-β-keto-γ-amino esters (Table 1, **1a**–**e**) were synthesized starting from the corresponding amino aldehyde and ethyl diazoacetate in the

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Scheme 2. Synthesis of Statines Starting from Boc-Amino Aldehydes

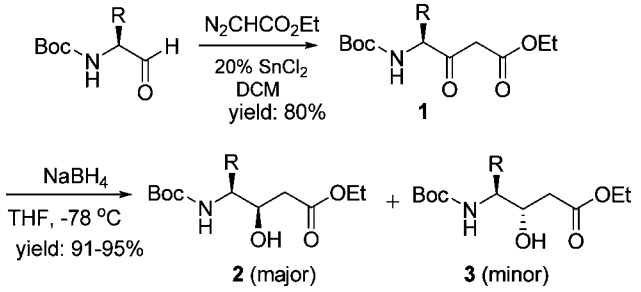
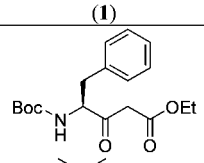
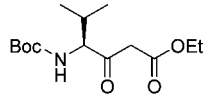
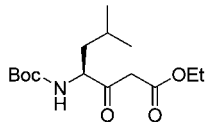
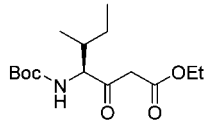
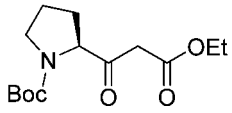


Table 1. Diastereomeric Ratio of Statines Obtained after Column Purification

no.	beta-keto-esters (**1**)	yield(%) (*2*+**3**)	statine (dr) **2**	**3**
a		95	65	35
b		93	68	32
c		92	64	36
d		90	68	32
e		91	58	42

presence of anhydrous tin chloride. Out of all the β-keto-γ-amino esters in Table 1, we were able to obtain single crystals for **1a** and its X-ray structure is shown in Figure 1. To understand the compatibility and the stereochemical output, we initially subjected **1a** to the $NaBH_4$ reduction in dry THF at −78 °C. The two diastereoisomers **2a** (major) and **3a** (minor) obtained after the mild $NaBH_4$ reduction were separated using column chromatography. The major isomer (**2a**) gave single crystals after slow evaporation of a methanol solution, and its X-ray structure is shown in Figure 1. Similar to the other γ-amino acids, the torsional values of β-keto and β-hydroxy γ-amino acids were measured by introducing two additional variables θ_1 (N−C^{γ}−C^{β}−C^{α}) and θ_2 (C^{γ}−C^{β}−C^{α}−C′) along with ϕ (C′−N−C^{γ}−C^{β}) and ψ (C^{β}−C^{α}−C′−N/O) (Scheme 1). Instructively, the crystal structure of **1a** reveals that the carbonyls (C═O) of keto and ester functional groups oriented ∼90° perpendicular to one another. In contrast to the vinylogous amino acids[8d] where the side chain is almost perpendicular to the double bond, the amino acid side chain adopted a skew conformation (−18°) with the keto functional group. The analysis of the statine **2a** suggested that the molecule adopted *gauche*$^+$ (g^+) and *anti* (t) conformations along C^{γ}−C^{β} and C^{β}−C^{α} bonds, respectively. Further, the β-hydroxyl group was involved in the network of bifurcated intermolecular H-bonding with the other hydroxyl groups (see Supporting Information (SI)). Encouraged by these results, we subjected other β-keto-γ-amino esters (**1**) in Table 1 to the mild $NaBH_4$ reduction in THF. Both major (**2**, *syn*) and minor (**3**, *anti*) diastereoisomers were separated using column chromatography. The yield (**2** + **3**) and the diastereomeric ratio of the statines are given in the Table 1. Interestingly, all *syn* diastereoisomers (**2a**−**d**) displayed a more downfield amide NH shift over their *anti* diastereoisomers (**3a**−**d**).

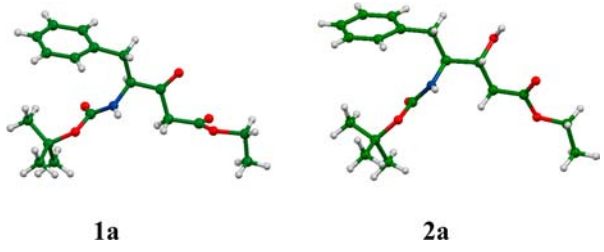
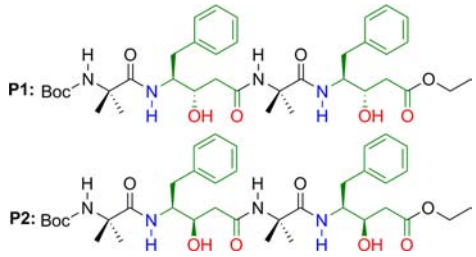


Figure 1. X-ray structures of β-keto-γ-phenylalanine and *syn*-(3*R*,4*S*)-β-hydroxy γ-phenylalanine (major product).

To understand the conformational properties of statines in peptide sequences, we designed two hybrid peptides **P1**and **P2**. The sequences of these peptides are shown in Scheme 3. In the case of **P1**, we incorporated the minor diastereoisomer (3*S*,4*S*) **3a**, and in the case of **P2**, the major diastereoisomer (3*R*,4*S*) **2a** was used along with an alternating α-amino acid, Aib. Both **P1** and **P2** were synthesized in solution phase through a 2 + 2 convergent strategy using Boc chemistry (see SI). Statines were used in the coupling reaction without protection of the β-hydroxyl group. All coupling reactions were performed using HBTU/HOBt coupling conditions, and peptides were isolated in moderate to good yields (see SI) after silica gel column chromatography. Instructively, all coupling reactions were found to be clean and efficient, suggesting that unprotected hydroxyl groups experienced no interference during the peptide synthesis.

Scheme 3. Sequences of Hybrid Peptides P1 and P2 Containing *Anti* (3*S*,4*S*) and *Syn* (3*R*,4*S*) Diastereoisomers of β-Hydroxy-γ-phenylalanine, Respectively



Single crystals of peptides **P1** and **P2** were obtained from the solution of aqueous methanol after slow evaporation, and their X-ray structures are shown in Figure 2. The analysis of the crystal structure of **P1** reveals that the presence of two molecules in the asymmetric unit which are interconnected by two water molecules. Both molecules in the asymmetric unit adopted a helical conformation with a slight variation in their torsional variables. The helical conformation of **P1** is stabilized by two intramolecular 12-membered H-bonds between the amide NH and C═O of *i* and *i*+3 residues similar to the α,γ^4-

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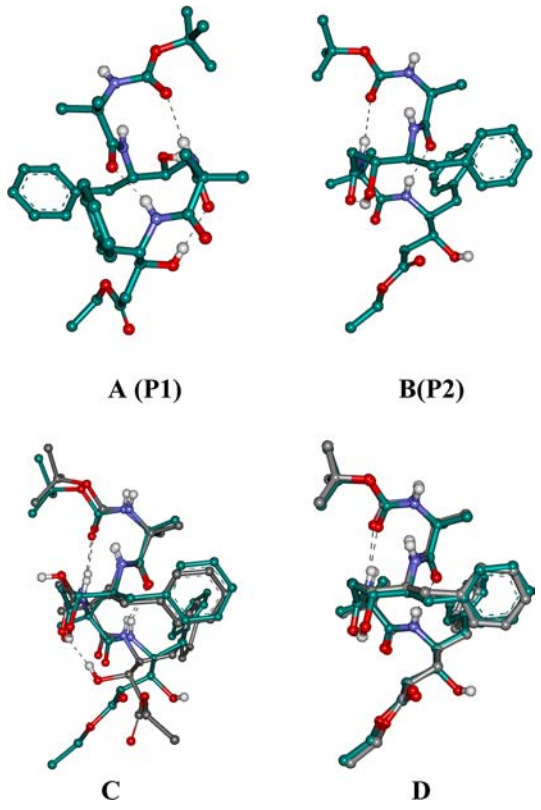

Figure 2. X-ray structures of (A) **P1**, (B) **P2**, (C) Superposition of **P1** and **P2**, and (D) Overlay of **P2** and α,γ^4-hybrid tetrapeptide 12-helix. Side-chain hydrogens are not shown for clarity.

hybrid peptides.[8c,d] Interestingly, the β-hydroxyl groups are pointed toward the N-terminus of the helix similar to the amide NHs. Instructively, as a result of this unique projection of the β-OH toward the N-terminus, the Phe-sta4 β-OH is involved in the 10-membered intramolecular H-bonding with the CO group of the Phe-sta2. Further, β-OH of the Phe-sta2 is involved in the strong intermolecular H-bonding with the water molecule which connects the other helix through intermolecular H-bonding with the penultimate Aib3 CO group. Further, analysis of the torsional variables reveals that Phe-sta2 adopted g^+, g^+ conformations along $C^\gamma-C^\beta$ and $C^\beta-C^\alpha$ bonds and anticlinal conformations along $N-C^\gamma$ and $C^\alpha-C(=O)$ bonds. In contrast, the Phe-sta4 adopted a fully extended conformation due to the lack of terminal amide NH to participate in the intramolecular H-bonding. The torsional values of Phe-sta residues are given in Table 2. The H-bond parameters are tabulated in the Supporting Information. Analysis of the crystal structure of **P2** with (3*R*,4*S*) β-hydroxy-γ-phenylalanine (*syn*) suggests the presence of a single molecule in the asymmetric unit. Similar to **P1**, **P2** also adopted a 12-helical conformation and the structure is stabilized by two intramolecular H-bonds between the residues *i* and *i*+3. The Phe-sta2 is accommodated nicely into the 12-helical conformation by adopting the g^+, g^+ conformation along θ_1 and θ_2. Further, Phe-sta4 in **P2** adopted an extended conformation by having the torsional values θ_2 and ψ −170° and 160°, respectively, similar to **P1**. Due to the stereochemical requirement, the β-OH group of (3*R*,4*S*) β-hydroxy-γ-phenylalanine in **P2** pointed toward the C-terminus of the helix similar to the amide CO groups. In contrast to the β-hydroxyl group of *anti* (3*S*,4*S*)Phe-sta2 in **P1**, the β-OH group of the Phe-sta2 in **P2** is involved in the six-membered intramolecular H-bonding with CO of the same residue with a C═O···H distance of 2.09 Å and C═O···H−O angle of 142°. This intraresidue H-bonding is not observed in the terminal Phe-sta4, as it does not participate in the helix due to the lack of C-terminal amide NH; however, it is involved in the strong intermolecular H-bonding with the solvent methanol. The structural analysis of **P1** and **P2** reveal that, irrespective of the stereochemistry at the β-position and the projection of the hydroxyl groups, both peptides adopted the right-handed helical conformation. Further, the superposition of the hybrid statine peptide **P2** with the known α,γ^4-hybrid tetrapeptide 12-helix[8d] (Boc-Aib-γ^4Phe-Aib-γ^4Phe-OEt) reveals a very nice backbone correlation (Figure 2D) suggesting that statines can be utilized as surrogates for other γ-amino acids in the design of functional foldamers. Overall, these results suggest that *syn* and *anti* diastereoisomers of β-hydroxy γ-amino acids can be accommodated nicely into the 12-helical conformation without much deviation from the α,γ^4-hybrid peptide 12-helix. Although both *syn* and *anti* diastereoisomers adopted the required g^+, g^+ to accommodate into the helix similar to the γ^4-residues, *anti* diastereoisomers may stabilize the helical folds through additional inter-residue H-bonding compared to the *syn* diastereoisomers and γ^4-residues.

Table 2. Torsional Variables (deg) of β-Keto and β-Hydroxy γ-Amino Acid Residues

no.	residue	ϕ	θ_1	θ_2	ψ
AAs	1a	−135	35	162	94
	2a	−131	59	−169	−172
P1	Phe-sta2	−123 ± 3	53 ± 3	60 ± 3	−122 ± 1
	Phe-sta4	−105 ± 3	173 ± 3	168 ± 9	154 (−57)
P2	Phe-sta2	−126	51	63	−121
	Phe-sta4	−105	56	−170	−158

In conclusion, we demonstrated the facile synthesis of various β-hydroxy γ-amino acids starting from β-keto-γ-amino esters and studied their single crystal conformations. The stereochemically pure *anti* (3*S*,4*S*) and *syn* (3*R*,4*S*) β-hydroxy γ-phenylalanine were utilized in the synthesis of hybrid peptides with alternating α-amino acids. Results suggest that irrespective of the stereochemistry at the β-carbon center, both the α,γ-hybrid peptides adopted 12-helical conformations. However, the β-hydroxyl groups in *anti* diasteroisomers projected toward the N-terminus of the helix similar to the amide NHs, while in *syn* diastereoisomers the β-hydroxyl group projected toward the C-terminus of the helix similar to the amide carbonyl. These stereochemical requirement enabled the β-hydroxyl groups to be involved in the 6-membered intraresidue H-bonds in the *syn* diasteroisomers and 10-membered interresidue H-bonds in the *anti* diastereoisomers, respectively. The influence of these additional −OH group interactions with the backbone carbonyl groups in the higher ordered helical peptides is yet to be investigated. Overall, the facile synthesis of statines and stereochemical analysis of monomers as well as hybrid peptides of statines reported here may provide realistic information regarding the structure-based design of peptides containing β-hydroxy γ-amino acids.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, compound characterization, and crystallographic information for **1a**, **2a**, **P1**, and **P2**. This

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material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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