Helices with Additional H-bonds: Crystallographic Conformations of α,γ-Hybrid Peptides Helices Composed of β-Hydroxy γ-Amino acids (Statines)

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ABSTRACT: β-Hydroxy-γ-amino acids (Statines) are a class of naturally occurring non-ribosomal amino acids frequently found in many peptide natural products. Peptidomimetics constituted with statines have been used as inhibitors for various aspartic acid proteases. In contrast to the synthetic γ-amino acids, very little is known about the folding behavior of these naturally occurring β-hydroxy γ-amino acids. To understand the folding behavior of statines, three α,γ-hybrid peptides **P1** (Boc-Aib-γPhe-Aib-(*R, S*)Phesta-Aib-γPhe-Aib-$CONH_2$), **P2** (Boc-Aib-γPhe-Aib-(*S, S*)Phesta-Aib-γPhe-Aib-$CONH_2$) and **P3 (**Boc-Aib-γPhe-Aib-(*S, S*)Phesta-Aib-(*S, S*)Phesta-Aib-$CONH_2$) were synthesized on solid phase and their helical conformations in single crystals were studied. Results suggest that both *syn* and *anti* diastereoisomers of statines can be accommodated into the helix without deviating overall helical conformation of α,γ-hybrid peptides. In comparison with *syn* diastereoisomer, the *anti* diastereoisomer was found to be directly involved in the intramolecular H-bonding with the backbone carbonyl groups (*i to i+3*) similar to the backbone amide NHs in the helix.

Keywords: Amino acids; Peptides; Conformations: H-bonds; X-ray

INTRODUCTION

Hydrogen bonds play a significant role in the folding of proteins into well defined, three-dimensional structures. The hypothetical dissection of protein structures leads to helices, sheets and reverse turns along with loosely structured loops. On the basis of continuous H-bonding interactions between *i* and *i+4* residues in the polypeptide backbone amide groups Pauling proposed the α-helix.[1] The intramolecular hydrogen bonding is the key feature that holds together the α-helical fold, while the intermolecular H-bonds play crucial role in stabilizing the β-sheet structures.[2] Inspired by the nature's H-bond mediated α-peptide folded structures; researchers have explored amide H-bonding analogous to the α-peptide helices and sheets to create well defined, unnatural foldamers.[3-6] Incredible success has been achieved in this regard using non-natural β- and γ-peptide foldamers, initiated by Seebach[6-9], Gellman[10,11] and Hanessian.[12] The oligomers of β- and γ-amino acids have displayed well defined helical structures, and similar to the α-helix the major organizing force in folding of these non-natural peptides is the chains of intramolecular amide H-bonding. Based on the number of atoms involved in the H-bonding pseudocycle the β- and γ-peptide helices are classified as C_9-, C_{10}-, C_{12}-, C_{13}-, C_{14}-helices etc.[13-19] Further, the hybrid peptides composed of α-, β- and γ-amino acids displayed a variety of helices with different H-bonding patterns.[11,14,15,20-29] Both theoretical and experimental investigations revealed the stable 12-helical conformations from the α, γ-hybrid peptides composed of 1:1 alternating α- and γ-amino acids.[30-36]

Schultz and colleagues[37] systematically investigated the contribution of H-bonds to the stability of α-helix by replacing the amide bond (-CO-NH-) with an ester bond (-CO-O-). The substitution of amide by an ester significantly destabilizes the α-helix. Introducing additional H-bonds without deviating the overall helical fold may increase the stability of α-helix, however, it is rather difficult to introduce additional H-bond donors or acceptors on the α-peptide backbone. Recently Guichard and colleagues[38-40] and Clayden et. al[41] reported oligourea foldamers and related hybrids which can be described as γ-peptide analogues with additional H-bond donor groups. Seebach and colleagues examined the influence of backbone OH groups on the conformations of homooligomers of α-hydoxy β-amino acids[42] as well as β-hydroxy-γ-amino acids (statines).[43] In addition, Sharma and colleagues showed the intramolecular H-bonds

between the side-chain -OMe and the backbone amide NHs in α,β-hybrid peptides containing C-linked carbo-β-amino acid.[44]

Statines are naturally occurring γ-amino acids and peptides composed of statines displayed a variety of biological activities including aspartic acid protease inhibitors,[45,46] anticancer,[47] antimalarial properties[48] etc., In continuation of our efforts in understanding the structural properties of α,γ-peptide foldamers, we sought to investigate the influence of backbone –OH groups of statine diastereoisomers[49,50] on the conformations of hybrid helices. We anticipate that as the OH group is directly attached to the backbone of γ-amino acids, it may be possible to introduce additional intramolecular H-bonds along with the canonical CO---NH H-bonds in α,γ-hybrid peptide helices. Herein, we are reporting the synthesis and single crystal conformations of three α,γ-hybrid peptides (**P1-P3**) containing statine diastereoisomers. All three hybrid peptides adopted 12-helical conformations in single crystals. Further, we studied the structural analogy of these hybrid statine peptides with recently reported α,γ-hybrid peptide 12-helix **P4**.[51] Intriguingly, statine residues with *anti* stereochemistry (with respect to the amino acid side-chains) are involved in the intramolecular H-bonding with the backbone CO groups (C=O---H-O) along with the regular 12-membered C=O---HN H-bonding. In contrast, statine residue with *syn* stereochemistry (with respect to the amino acid side-chains) is not involved in the intramolecular H-bonding with the amide CO, however involved in the intermolecular H-bonding with the solvent molecule. The additional H-bonds of statines with *anti* stereochemistry can be utilized to stabilize the helical conformations.

MATERIALS AND METHODS

General Experimental Details: All amino acids, Weinreb amine hydrochloride salt, DCC, LAH, DIPEA, $SnCl_2$, Ethyl diazoacetate, $NaBH_4$, HOBt, HBTU, THF, DCM and DMF were used as commercially available. THF was dried over sodium and distilled prior to use. Column chromatography was performed on silica gel (100-200 mesh). ^{1}H NMR spectra were recorded on 400 MHz instrument (100 MHz for ^{13}C) using residual solvent as internal standard ($CDCl_3$ δ_H, 7.24 ppm, δ_c 77.0 ppm). The chemical shifts (δ) were reported in ppm and coupling constant (J) in Hz. High resolution mass spectra were obtained from ESI-TOF MS spectrometer and MALDI-

TOF/TOF spectrometer. The synthesis, CD and single crystal conformation (CCDC NO. 901428) of **P4** were reported earlier.[51]

General procedure for the synthesis of *N*-Boc-β-keto-γ-amino esters: The N-protected amino aldehyde (2.0 mmol) was dissolved in 15 mL of DCM at room temperature (20-25 ^{o}C) and then 0.0756 g (20 mol %) of tin (II) chloride was added followed by 0.239 g (2.1 mmol) of ethyl diazoacetate. Immediate gas evolution was observed. The reaction mixture was stirred and the progress of the reaction was monitored by TLC. After completion of the reaction, it was quenched with 10 mL of 0.5N HCl and the reaction mixture was extracted with DCM (30 mL x 3). The combined organic layer was washed with 20 mL of brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure to get greenish oily crude product which was purified on silica gel column chromatography.

(*S*)-ethyl 4-*(tert*-butoxycarbonylamino)-3- oxo-5-phenylpentanoate (1): White crystal (0.521 g, 78%); Melting Point = 61.4^{0} C; $[\alpha]_D^{25}$ = - 54.5 (c = 0.6, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.16 (s, 1H, enolic 17%), 7.24 –7.15 (m, 5H, C_6H_5), 5.03-5.01 (d, *J*= 7.3 Hz, 1H, NH), 4.57-4.52 (q, *J* =6.4 Hz, 1H, CH), 4.18-4.12(q, *J* = 7.2 Hz, 2H, -OCH2), 3.51-3.40 (dd, *J*=16 Hz, *J*=11.4 Hz, 2H, CH2, AB coupling), 3.15-2.95 (m, 2H, CH_2Ph), 1.38(s, 9H, $C(CH_3)_3$), 1.26-1.22 (t, *J*=7.2 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.96, 166.86, 155.18, 136.04, 129.24, 128.67, 127.00, 80.21, 61.45, 60.43, 46.86, 36.89, 28.20, 14.02; HR-MS m/z Calcd. for $C_{18}H_{25}NO_5$ $[M+Na]^+$ 358.1630, obsrvd 358.1633.

General procedure for the synthesis of *syn*- and *anti*-β-hydroxy γ-amino esters: The *N*-protected β-keto γ-amino ester (**1**) (1 mmol) was dissolved in 10 mL of dry THF under N_2 atmosphere, cooled to -78 ^{o}C, and then $NaBH_4$ (1.4 mmol, 0.142 g) was added in one portion. The reaction mixture was stirred for further 3 hrs to complete the reaction. The progress of the reaction was monitored by TLC. After completion of the reaction, it was quenched by pouring the mixture into ice-cold 1 *N* hydrochloric acid (10 mL). The aqueous phase was extracted with ethyl acetate (3 × 20 mL). Then the combined organic layers was washed with brine (60 mL) and dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The diastereoisomer mixtures (Table 4) were separated passing through normal silica gel column chromatography using pet- ether (60-80 ^{o}C)–ethyl acetate solvent system.

(*3R*, 4*S*)-ethyl 4-((*tert*-butoxycarbonyl)amino)-3-hydroxy-5-phenylpentanoate (2): White powder (0. 196 g, 60%); **$[\alpha]_D^{20}$:** –14.3 (c = 1, MeOH); **^{1}H NMR** (400 MHz, $CDCl_3$ **:** δ_H 7.32-7.22 (m, 5H, -Ph), 4.56-4.54 (d, *J* = 9.8, 1H, NH), 4.21-4.16 (q, *J* = 7.2, 2H, -OC*H*$_2$), 4.00-3.99 (d, *J* = 6.5, 1H,-C*H*-OH), 3.90-3.84 (m, 1H, -C*H*-), 3.61 (b, 1H, -OH), 3.01-2.82 (m, 2H, -C*H*$_2$-Ph), 2.61-2.47 (m, 2H, -C*H*$_2$CO), 1.36 (s, 9H, Boc -(C*H*$_3$)$_3$) ,1.30-1.26 (t, *J* = 7.2, 3H, -C*H*$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$)**:** δ_C 173.01, 155.71, 137.60, 129.47, 128.45, 126.43, 79.60, 70.06, 60.89, 55.11, 38.12, 35.79, 28.24, 14.13; **ESI** *m/z* for molecular formula $C_{18}H_{27}NO_5$ Calcd. $[M+Na]^+$ 360.1786, observed 360.1789.

(*3S*, 4*S*)-ethyl 4-((*tert*-butoxycarbonyl)amino)-3-hydroxy-5-phenylpentanoate (3): White powder (0.134 g, 40%); **$[\alpha]_D^{20}$:** – 36.2 (*c* = 1, MeOH); **^{1}H NMR** (400 MHz, $CDCl_3$ **:** δ_H 7.31-7.22 (m, 5H, -Ph), 4.97-4.95 (d, *J* = 9.8, 1H, NH), 4.16-4.11 (q, *J* = 7.2, 2H, -OC*H*$_2$), 4.00-3.97 (d, *J* = 8, 1H,-C*H*-OH), 3.76-3.70 (m, 1H, -C*H*-), 3.52 (b, 1H, -OH), 2.93-2.91 (m, 2H, -C*H*$_2$-Ph), 2.63-2.35 (m, 2H, -C*H*$_2$CO), 1.42 (s, 9H, Boc -(C*H*$_3$)$_3$) ,1.27-1.25 (t, *J* = 7.0, 3H, -C*H*$_3$); **^{13}C NMR** (100 MHz, $CDCl_3$) **:** δ_C 173.61, 155.81, 138.14, 129.41, 128.45, 126.36, 79.40, 66.94, 60.85, 55.34, 38.53, 29.68, 28.33, 14.09; **ESI** *m/z* for molecular formula $C_{18}H_{27}NO_5$ Calcd. $[M+Na]^+$ 360.1786, observed 360.1792.

General procedure for the synthesis of Fmoc-β-hydroxy γ-amino acid: The pure diastereomers of Boc-NH-γPhe(*β*-OH)OEt (**3**, 1.0 g, 3 mmol) was dissolved in MeOH (5 mL) and add 1 *N* NaOH (2 eq.) and stir the reaction for 4 hr. After the completion of reaction evaporate the solvent methanol and acidified with 10% HCl and extract the compound using EtOAc (3 × 30 mL). The combined organic layer was washed with brine solution (1 × 30 mL). Then Boc-NH-γPhe(*β*-OH)OH was dissolved in DCM (3 mL). The solution was cooled to 0 °C and to this added TFA (3 mL). The reaction mixture was stirred for another 1 hr at RT. After completion of the reaction, solvent DCM and TFA were evaporated under reduced pressure and residue was co-evaporated with DCM (three times). Then the residue was dissolved in 10 % aqueous Na_2CO_3 (12 mL) and added THF (3 mL). To this reaction mixture Fmoc-OSu (0.91 g, 2.7 mmol) in THF (8 mL) was added. The reaction mixture was stirred for another 8 hr. After the completion of reaction, solvent THF was evaporated and residue was treated with 5 % aqueous HCl to make pH ~2. Then the aqueous layer was extracted with ethyl acetate (50 mL × 3) and the

combined organic layer was washed with brine solution (50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get Fmoc-NH-*γ*Phe(*β*-OH)-OH (**4**, 1.04 g) in 90 % yield, which was used for solid phase peptide synthesis without further purification.

Solid Phase Peptide Synthesis: All peptides were synthesized at 0.2 mmol scales on Rink amide resin using standard Fmoc-chemistry. All peptide coupling reactions were carried out using HBTU/HOBt as coupling agents. Fmoc deprotections were facilitated using 20% piperidine in DMF. The coupling reactions were monitored by Kaiser Test. Double couplings were used in the case statine and Aib residues. Final coupling was performed using Ac-Aib to avoid acetylation of free OH groups in statines. After completion of the synthesis, peptides were cleaved from resin using TFA. After cleavage, the resin was filtered and washed with TFA. The filtrate was evaporated under reduced pressure to give gummy product. The gummy product was triturated with cold diethyl ether to give crude peptides. These crude peptides were further purified using reverse phase HPLC on C_{18} column using methanol/water gradient system. The mass of the pure peptides was confirmed by MALDI-TOF/TOF.

Crystal Structure Analysis

Crystal data analysis of Ac-Aib-*γ*Phe-Aib-(*3R, 4S*)-*β*-hydroxy-*γ*Phe-Aib-*γ*Phe-Aib-CONH$_2$ (P1): Crystals were grown by slow evaporation from a solution of trifluoroethanol. A single crystal (0.1 × 0.08 × 0.02 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100 K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 50.48), for a total of 24569 independent reflections. Space group P 21, a = 12.60 (2), b = 17.38 (3), c = 14.02 (2), β = 107.54 (2), V = 2928 (8) Å^3, Monoclinic, Z = 2 for chemical formula C_{51} H_{72} N_8 O_9 2 (C_2 H_3 F_3 O), with one molecule in asymmetric unit; ρ calcd. = 1.295 gcm^{-3}, μ = 0.103 mm^{-1}, F (000) = 1212, R_{int} = 0.2381. The final R value was 0.0998 (wR2 = 0.2834) 7413 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 733 variables, S = 1.016. The structure was obtained by direct methods using SHELXS-97.[52] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. The largest difference peak and hole were 0.331 and -0.309 eÅ^3, respectively.

Crystal data analysis of Ac-Aib-*γ*Phe-Aib-(*3S, 4S*)-*β*-hydroxy-*γ*Phe-Aib-*γ*Phe-Aib-CONH$_2$ (P2): Crystals were grown by slow evaporation from a solution of trifluoroethanol. A single crystal (0.18 × 0.09 × 0.01 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100 K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 50.996), for a total of 40794 independent reflections. Space group P 21, a = 10.722 (11), b = 17.315 (18), c = 15.790 (17), β = 108.633 (17), V = 2791(5) Å^3, Monoclinic, Z = 2 for chemical formula C_{51} H_{72} N_8 O_9 1(C_2 H_3 F_3 O), with one molecule in asymmetric unit; ρ calcd. = 1.239 gcm^{-3}, μ = 0.093 mm^{-1}, F (000) = 1112, R_{int} = 0.406. The final R value was 0.0885 (wR2 = 0.2549) 10129 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 678 variables, S = 0.911. The structure was obtained by direct methods using SHELXS-97.[52] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. The largest difference peak and hole were 0.297 and -0.330 eÅ^3, respectively. After thorough crystallization process, we are able to get small size crystals in trifluoroethanol solvent, which results in moderate quality data leads to R_{int} value above 0.25.

Crystal data analysis of Ac-Aib-*γ*Phe-Aib-(*3S, 4S*)-*β*-hydroxy-*γ*Phe-Aib-(*3S, 4S*)-*β*-hydroxy-*γ*Phe-Aib-CONH$_2$ (P3): Crystals were grown by slow evaporation from a solution of trifluoroethanol. A single crystal (0.1 × 0.08 × 0.1 mm) was mounted on loop with a small amount of the paraffin oil. The X-ray data were collected at 100 K temperature on a Bruker APEX DUO CCD diffractometer using Mo K_α radiation (λ = 0.71073 Å), ω-scans (2θ = 50.996), for a total of 40794 independent reflections. Space group P 21, a = 10.612 (2), b = 17.651 (4), c = 15.805 (3), β = 108.640 (5), V = 2805.3 (10) Å^3, Monoclinic, Z = 4 for chemical formula C_{51} H_{72} N_8 O_{10} 1(C_3 H_8 O), with one molecule in asymmetric unit; ρ calcd. = 1.16 gcm^{-3}, μ = 0.084 mm^{-1}, F (000) = 1042, R_{int} = 0.1933. The final R value was 0.0778 (wR2 = 0.2170) 13646 observed reflections ($F_0 \geq 4\sigma$ ($|F_0|$)) and 672 variables, S = 0.893. The structure was obtained by direct methods using SHELXS-97.[52] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed geometrically in the idealized position and refined in the final cycle of refinement as riding over the atoms to which they are bonded. The largest difference peak and hole were 0.582 and -0.591 eÅ^3, respectively.

Details of data collection, coordinates, bond lengths and angles, anisotropic thermal parameters, and hydrogen coordinates all three peptides (**P1-P3**) are deposited in the Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, UK, ref CCDC No. 1036533-35.

RESULTS AND DISCUSSION

The sequences of the peptides **P1**-**P3** are shown in Scheme 1. The stereochemically constrained helix favoring α-amino isobutyric acid (Aib) was used as an α-amino acid component in all hybrid peptide sequences.[53,54] To have a direct comparison to the new statine peptides (**P1-P3**) we selected recently reported, well characterized α, γ-hybrid peptide **P4** as a model peptide.[51] The γ-Phe was synthesized using Witting reaction followed by the catalytic hydrogenation as reported earlier.[55] The phenylalanine statines were synthesized from the mild $NaBH_4$ reduction of the ethyl ester of *N*-Boc-β-keto γ-Phe (1) and the *syn* (2) and *anti* (3) diastereoisomers were separated using column chromatography.[49,50] Ethyl ester of *N*-Boc-β-keto-γ-Phe was synthesized from Roskamp's procedure[56] using Boc-phenylalaninal and ethyl diazoacetate as reported earlier.[57] The solid phase compatible *N*-Fmoc-protected statine diastereoisomers were synthesized after the saponification, *N*-Boc deprotection followed by the Fmoc- protection of the free amine. The schematic representation of the synthesis of statines is shown in Scheme 2. The *syn* diastereoisomer was used to synthesize **P1** and the *anti* diastereoisomer was used to construct **P2** and **P3**. Heptapeptide **P3** was synthesized by replacing the two C-terminal γ-Phe residues of **P4** and γ-Phe was retained at the N-terminus, because similar to the *N*-terminal amide NHs in the helix there is no H-bond acceptor for the *N*-terminal β-hydroxyl group.

All three peptides, **P1-P3** were synthesized using solid phase method on Rink Amide resin using standard Fmoc- chemistry. All peptide couplings were performed using standard HBTU/HOBt coupling conditions. After the synthesis, peptides were cleaved from the resin and purified using reverse phase HPLC on C_{18} column.

As crystal structures provide unambiguous structural information, we subjected all three peptides to the crystallization in various solvent combinations. The X-ray quality crystals of all three peptides were obtained from isopropanol/trifluoroethanol solvent combination. The crystal structures of the peptides, **P1-P3**, are shown in Figure 1. All three peptides adopted 12-helical conformations similar to the **P4**[51] (Figure 2). The 12-helix adopted by the **P1** is stabilized by six

consecutive intramolecular H-bonds. Similar to the other two γ^4-Phe residues, the (*R, S*) Phesta also adopted g^+, g^+ conformation along the C^{γ}-C^{β} and C^{β}-C^{α} bonds to accommodate into the 12-helix. The β-hydroxyl group is not involved in the intramolecular H-bonding with the backbone amide carbonyl groups, however is involved in the intermolecular H-bonding with the solvent trifluoroethanol. The insertion of *syn* hydroxyl group did not affect the overall 12-helical conformation of α, γ-hybrid peptide, suggesting that small functional groups can be accommodated at the β-position of γ-amino acids without deviating the overall helical fold. The ϕ and ψ dihedral angles of γ-residues are defined by comparison with α-residues, and two additional torsion angles along C_{γ}–C_{β} and C_{β}–C_{α} bonds are designated as θ_1 and θ_2, respectively (Figure 1B). The torsion angles of P1 are shown in Table 1. The H-bond distances and angles of **P1** are tabulated and given in the supporting information.

Crystal structure analysis of **P2** reveals that the peptide adopted a 12-helical conformation. In contrast to **P1**, the helical structure of **P2** is stabilized by seven intramolecular H-bonds. Both (*S, S*) Phesta and the γ-Phe adopted g^+, g^+ conformation. Detailed structure analysis reveals that both *anti* β-OH and the amide NH of Phesta4 are involved in the intramolecular bifurcated H-bonding with the Aib1 carbonyl group ($i \rightarrow i+3$). In addition to the regular 12-membered H-bonding existed between the amide groups of *i* and *i+3* residues, an additional 15-membered H-bond existed between the C=O and β-OH group. The C=**O**---**H**-O and **O**---**O** distances were found to be 2.13 Å and 2.94 Å, respectively and the C=O---H bond angle was found to be 145°. It has been observed that the hydroxyl and carboxylate amino acid side-chains are often part of capping motifs in stabilizing α-helices in proteins.[58] In addition, the frequent occurrence of asparagine at the *N*-terminus of the helices has also been believed that it may supply the H-bond partners for the unpaired backbone amide NHs to induce the stability of the helix through backbone side-chain interactions.[59-61]

The single crystal analysis of **P3** reveals a helical conformation similar to the **P2** structure. The *anti* Phesta residues and γPhe adopted g^+, g^+ conformations to accommodate nicely into the helix. Careful structure analysis suggests that in contrast to the six H-bonds between *i* and *i+3* residues in **P1,** or a regular α,γ-hybrid heptapeptide helix (**P4**), the helical structure of **P3** is stabilized by eight intramolecular H-bonds (*i* and *i+3* residues). The *anti* β-OH groups of both

Phesta4 and Phesta6 are involved in the 15-membered intramolecular H-bonding with the carbonyl groups of Aib1 and Aib3, respectively along with the six regular 12-membered H-bonds. The carbonyl groups of Aib1 and Aib3 are involved in the bifurcated H-bonding with the β-OH groups as well as amide NHs of statines. The bond angle and bond distances of C=O---O-H H-bonds are found to be very similar to that of CO---H-N H-bonds (Figure 2). The torsional angels of γ-residues are given in the Table 1. The H-bond parameters are tabulated in the supporting information. The overlay of α,γ-hybrid peptide 12-helix **P4** and **P3** (Figure 2) confirms the very close similarity between the two backbones, which only differ by the presence of additional H-bonds in **P3**. Overall, the crystallographic analyses of all three peptides reveal that statines with *anti* stereochemistry with respect to the amino acid side-chains may stabilize the hybrid helix with additional H-bonds along with the regular backbone amide H-bonds. We further subjected all these peptides for the CD analysis to understand whether the insertion of additional hydroxyl groups may influence CD signature of the 12-helix. All peptides showed CD negative minima at 220 nm and positive maxima at 205 nm similar to the β-peptide[62-64] as well as α,γ-hybrid peptide 12-helices.[51,65] The CD spectra of hybrid peptides are shown in Figure 3.

CONCLUSION

In conclusion, we are reporting the conformational properties of α,γ-hybrid peptides composed of naturally occurring β-hydroxy γ-amino acids. Single crystal analysis suggests that both *syn* and *anti* diastereoisomers of statines can be accommodated into the 12-helix without deviating the overall helical conformation of α,γ-hybrid peptides. In contrast to the *syn* diastereoisomer, the *anti* diastereoisomers were found to be directly involved in the intramolecular H-bonding with the backbone carbonyl group ($i \rightarrow i+3$) similar to the amide NHs. As helices are generally held together by the intramolecular H-bonds, we speculated that the utilization of *anti* diastereoisomers of statines in the design of helices may increase the stability through additional H-bonds. As statines have been recognized as protease inhibitors for various bacterial and viral infections as well as other diseases, the remarkable structural properties of statines reported here can be utilized further in the design of functional foldamers and peptidomimetics.

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SCHEMES AND FIGURE LEGENDS

SCHEME 1: Sequences of α,γ-hybrid peptides composed of γ- and β-hydroxy γ-amino acids. Chemical structures of γ-Phe and β-hydroxy γ-Phe diastereoisomers are shown below the peptide sequences.

SCHEME 2: Synthesis of statines starting from *N*-Boc-amino aldehydes

FIGURE 1: a) X-ray structures of **P1**, **P2** and **P3**. b) Local torsion angles of γ-residues. c) Two different types of intramolecular H-bonds observed in **P2** and **P3** crystal structures are highlighted in red [regular CO(i)---NH (i+3)12-membered H-bonds] and green [15-membered H-bonds exists between CO(i)----H-O (i+3) residues].

FIGURE 2: a) X-ray structure of α,γ^4-hybrid peptide **P4**.[52] B) Superposition of **P4** and **P3**. C) Parameters of bifurcated H-bonds between *i* and *i+3* residues in **P3** are highlighted.

FIGURE 3: Circular dichroism spectra of statine contained peptides **P1**, **P2** and **P3** peptides (0.2 mM) in methanol at 20 °C.

TABLES

Table 1: Torsion angles(°) of peptides P1, P2 and P3

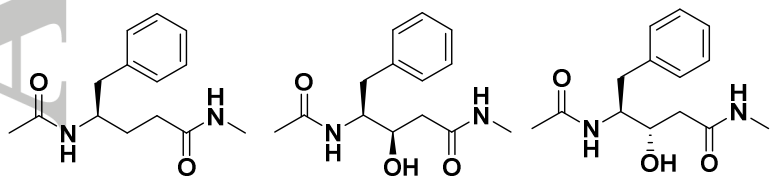
Pept.	Resd.	ϕ	θ_1	θ_2	ψ	ω
P1	Aib1	-59	-	-	-50	-175
	γPhe2	-117	52	64	-135	-168
	Aib3	-60	-	-	-33	-175
	(*R, S*)γPhesta4	-126	48	60	-120	-172
	Aib5	-53	-	-	-43	-170
	γPhe6	-130	55	58	-118	-173
	Aib7	-54	-	-	-40	-
P2	Aib1	-58	-	-	-41	-178
	γPhe2	-116	49	66	-137	-169
	Aib3	-62	-	-	-36	-175
	(*S, S*)γPhesta4	-122	46	64	-124	-165
	Aib5	-64	-	-	-35	-176
	γPhe6	-127	51	60	-116	-175
	Aib7	-54	-	-	-43	-
P3	Aib1	-59	-	-	-41	-178
	γPhe2	-116	49	66	-135	-169
	Aib3	-63	-	-	-34	-174
	(*S, S*)γPhesta4	-123	44	63	-127	-165
	Aib5	-61	-	-	-38	-175
	(*S, S*)γPhesta6	-124	47	65	-117	-176
	Aib7	-54	-	-	-44	-

P1: Ac-Aib- Phe-Aib-(*R, S*)Phesta-Aib- Phe-Aib-$CONH_2$

P2: Ac-Aib- Phe-Aib-(*S, S*)Phesta-Aib- Phe-Aib-$CONH_2$

P3: Ac-Aib- Phe-Aib-(*S, S*)Phesta-Aib-(*S, S*)Phesta-Aib-$CONH_2$

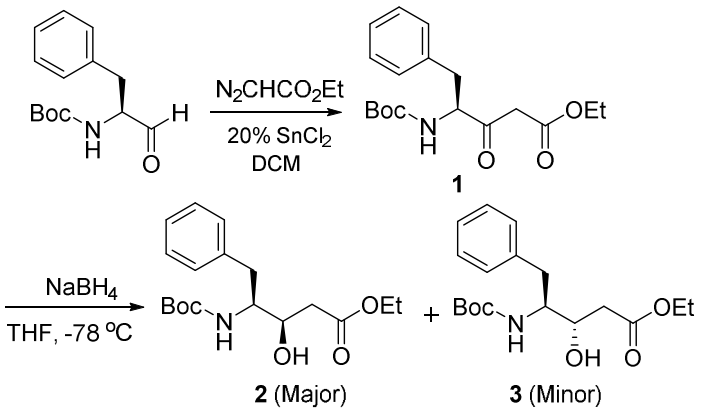
P4: Ac-Aib- Phe-Aib- Phe-Aib- Phe-Aib-$CONH_2$ (control)



γ-Phe (*R, S*)-γ-Phestatine (*S, S*)-γ-Phestatine

(*R, S*)-γ-Phesta (*S, S*)-γ-Phesta

Scheme 1



Scheme 2

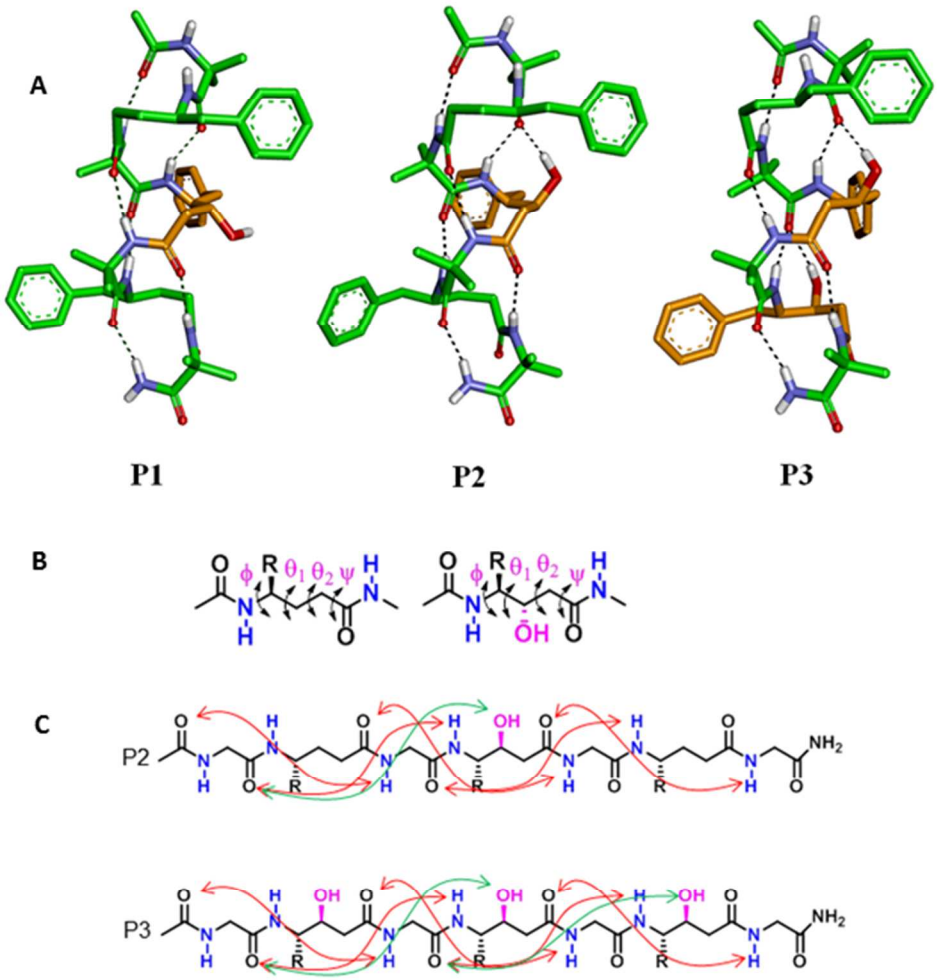


Figure 1

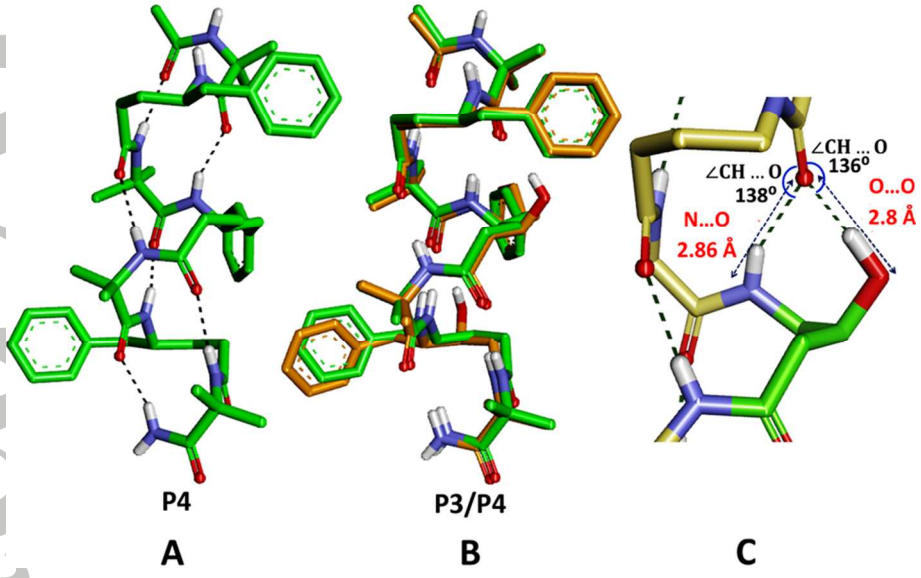


Figure 2

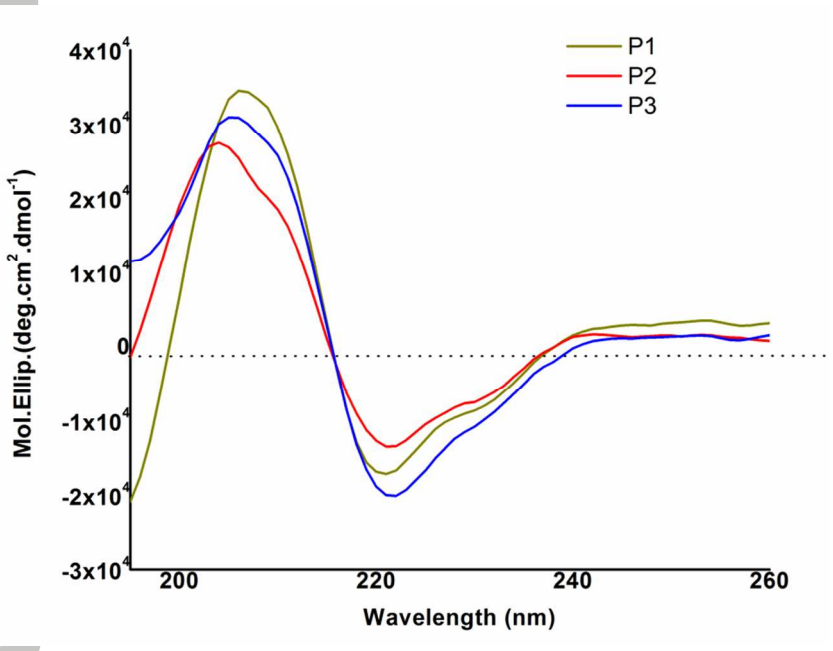


Figure 3

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