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Design of peptides with α,β-dehydro-residues: Synthesis and crystal structure of a tetrapeptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3

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In order to develop general rules of peptide design with α,β-dehydro-residues, a peptide, Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3, was synthesized. The peptide was crystallized from its solution in an acetone:water mixture (70:30). The crystals belong to orthorhombic space group $P2_12_12_1$ with $a = 9.403(1)$ Å, $b = 16.871(1)$ Å, $c = 21.638(1)$ Å, and $Z = 4$. The peptide adopts a conformation with two overlapping types II′ and III β-turns having dihedral angles, $\phi_1 = 53.7(6)^\circ$, $\psi_1 = -135.9(4)^\circ$, $\phi_2 = -59.2(5)^\circ$, $\psi_2 = -17.9(5)^\circ$, $\phi_3 = -68.4(5)^\circ$, $\psi_3 = -18.8(6)^\circ$. The conformation was further characterized by two intramolecular $4 \rightarrow 1$ hydrogen bonds involving imino nitrogen atoms of ΔPhe^3 and Phe^4 as donors and carbonyl oxygen atoms of blocking group Boc and Ala^1 as acceptors. The packing of the molecules in the unit cell is stabilized by an intermolecular hydrogen bond, $N_2{-}H_2\cdots O'_3$ $[-x, y + 1/2, -z + 1/2] = 2.894$ Å and van der Waals forces involving aromatic side chains.

KEY WORDS: α,β-dehydro-residue; β-turn; hydrogen bond; conformation; crystal structure; consecutive dehydro-residues.

Introduction

The α,β-dehydro-residues are capable of producing predictable folded conformations in peptides.[1–11] The studies carried out so far have indicated that peptides containing two consecutive ΔPhe residues at $(i + 1)$ and $(i + 2)$ positions in peptides such as Ac-ΔPhe-ΔPhe-Gly[12] and Ac-ΔPhe-ΔPhe-Ala[13] generate a characteristic S-shaped structure with ϕ, ψ dihedral angles centred at $\pm 60^\circ, \pm 30^\circ$. On the other hand, the presence of two consecutive ΔPhe residues at $(i + 2)$ and $(i + 3)$ positions in sequences such as Boc-Ala-ΔPhe-ΔPhe-$NHCH_3$[14] induces a conformation with two overlapping type III β-turns resulting in an incipient 3_{10}-helix. However, the structure analysis of Boc-Val-ΔPhe-ΔPhe-Val-OCH_3[15] with two consecutive ΔPhe residues at $(i + 2)$ and $(i + 3)$ positions revealed the formation of a folded structure with two overlapping types II and I′ β-turns, respectively. These results indicate that the introduction of branched β-carbon residues on both sides of -ΔPhe-ΔPhe- segment induces a different conformation than the one obtained with non-branched β-carbon residues. Similarly, a peptide Boc-Val-ΔPhe-ΔPhe-Ala-OCH_3[16] with a branched β-carbon residue only on one side of -ΔPhe-ΔPhe- sequence prefers a conformation with two overlapping types II and III′ β-turns, respectively. These observations with fine variations

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in the conformations of peptides corresponding to different combinations of C^{β}-branched carbon residues with other non-C^{β}-branched carbons have contributed to new design rules. In order to further extend the scope of these studies, we have designed a tetrapeptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 and report here its synthesis, crystal structure and molecular conformation.

Experimental

The peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 was synthesized using azlactone procedure[17] having following steps:

Synthesis of Boc-Ala-(β-OH)-Phe-OH (1)

To a precooled solution (10°C) of Boc-Ala-OH (3 g, 15.8 mmol) in dry tetrahydrofuran (THF) (10 mL), *N*-methylmorpholine (NMM) (1.87 mL, 17.1 mmol) and isobutylchloroformate (IBCF) (2.21 mL, 13.8 mmol) were added. After 15 min of stirring a solution of DL-(β-OH)–Phe–OH (3.45 g, 19 mmol) in 1N NaOH (22 mL) was added to it and the mixture was stirred at 0°C. The resulting oily compound was obtained with a yield = 4.9 g (70%), $R_f = 0.40$ ($CHCl_3$:MeOH::9:1).

Boc-Ala-ΔPhe Azlactone (2)

Compound **1** (4.9 g, 13.9 mmol) was reacted with anhydrous sodium acetate (1.37 g, 16.7 mmol) and freshly distilled acetic anhydride (10 mL) for 72 h at room temperature. The yield was: 3.9 g (70%), $R_f = 0.92$ ($CHCl_3$:MeOH::9:1).

Boc-Ala-ΔPhe-(β-OH)-Phe-OH (3)

To a solution of compound **2** (3.0 g, 9.7 mmol) in acetone, (β-OH) Phe-OH (2.0 g, 11.6 mmol) in 1N NaOH (11.6 mL) was added with stirring. After 1 h of stirring at 45°C, the mixture was acidified at 0°C by the addition of 7 mL of 1N HCl. Acetone was removed under reduced pressure, the aqueous layer was extracted with ethyl acetate and washed with water, dried over anhydrous sodium sulphate and evaporated to yield compound **3**.

The yield of peptide was: 1.2 g (70%), $R_f = 0.3$ ($CHCl_3$:MeOH::9:1).

Boc-Ala-ΔPhe-ΔPhe Azlactone (4)

Compound **3** (2.7 g, 5 mmol) was reacted with anhydrous sodium acetate (0.5 g, 6 mmol) and freshly distilled acetic anhydride (10 mL) for 72 h at room temperature. The yield was: 1 g (40%), $R_f = 0.92$ ($CHCl_3$:MeOH::9:1).

Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 (5)

To a solution of compound **4** (0.5 g, 1.1 mmol) in DCM (10 mL), Phe-OCH_3. HCl (0.23 g, 1.6 mmol) was added followed by triethylamine (TEA) (0.23 mL, 1.6 mmol). This mixture was stirred at room temperature for 48 h. The solvent was evaporated, the residue was dissolved in ethyl acetate and washed with 10% sodium bicarbonate, 5% citric acid and water, respectively and dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure and the final product was obtained. The yield of peptide was: 0.45 g (80%), $R_f = 0.7$ ($CHCl_3$:MeOH::9:1).

^1H NMR of Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3

In order to confirm the correctness of the final product, ^1H NMR spectra were recorded in $CDCl_3$ with 400 MHz Bruker DRX 400 instrument and the following results were obtained: δ1.45 (s, 9 H, t-Bu, Boc); δ3.81 (s, 3 H, OCH_3); δ5.33, δ(bd, NH, Ala); δ7.25–7.28 (m, 12 H, ΔPhe, C^{β} ΔPhe); δ7.5 (s, 2 H, NH ΔPhe). The observed ^1H NMR spectra clearly indicated the presence of peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 in the solution.

Table 1. The Details of Intensity Data Collection and Refinement for Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3

CCDC no.	254434
Molecular formula	$C_{36}H_{40}N_4O_7$
Molecular weight	640.72
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	9.403(1)
b (Å)	16.871(1)
c (Å)	21.638(1)
Volume (Å^3)	2911.9(7)
Z (molecules/unit cell)	4
Crystal dimension (mm^3)	0.4 × 0.2 × 0.2
D_c (g cm^{-3})	1.240
μr (mm^{-1})	0.709
F(000)	1360
Total no. of independent reflections	3665
No. of observed reflections ($I \geq 2\sigma$ (*I*))	3142
Radiation (λ, Cu K_α/Å)	1.5418
Instrument used	Enraf–Nonius CAD4
Mode of data collection	ω-2θ
R	0.0667
R_W	0.1987
S (goodness-of-fit)	1.074
Temperature (K)	298

Structure determination

The peptide was crystallized from its solution in acetone–water (70:30) mixture at room temperature (298 K) by slow evaporation. The details of crystal data, intensity data collection and refinement are given in Table 1. The unit cell parameters were refined by the least-squares fit of 25 high angle ($25 \leq \theta \leq 40^\circ$) reflections. These reflections were centred individually on the diffractometer. The Lorentz and polarisation corrections were applied. The absorption correction was not applied due to small size of the crystals (0.4 mm × 0.2 mm × 0.2 mm). The structure was determined with direct methods using the program SHELXS 97.[18] The coordinates of non-hydrogen atoms were refined anisotropically using program SHELXL 97.[19] The positions of hydrogen atoms were obtained from difference Fourier maps and were included in the final cycles of refinement using isotropic temperature factors of non-hydrogen atoms to which they were attached. The final *R*-factor for 3142 observed reflections [$I \geq 2\sigma(I)$] was 0.066. The atomic scattering factors used in these calculations were those of Cromer and Mann[20] for non-hydrogen atoms and Stewart, Davidson and Simpson[21] for hydrogen atoms.

Results and discussion

The stereoview of the peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 is shown in Fig. 1. The conformation of the peptide molecule is characterized by two overlapping types II′ and III β-turns having dihedral angles, $\phi_1 = 53.7(6)^\circ$, $\psi_1 = -135.9(4)^\circ$, $\phi_2 = -59.2(5)^\circ$, $\psi_2 = -17.9(5)^\circ$, $\phi_3 = -68.4(5)^\circ$, $\psi_3 = -18.8(6)^\circ$.

Molecular dimensions

The introduction of a double bond between C^α and C^β atoms in the ΔPhe residue also affects the other bond lengths and bond angles in the dehydro-residue. The C=C double bonds of the two ΔPhe residues in the present structure have *trans* configurations of the phenyl groups with respect to the carbonyl groups. They have similar bond lengths of 1.336(6) and 1.331(6) Å, respectively which are typical values for styrenic double bond conjugated with an aromatic ring. The bond lengths $N_2-C_2^\alpha$ = 1.426(5) Å and $C_2^\alpha-C'_2$ = 1.505(5) Å in ΔPhe^2 and $N_3-C_3^\alpha$ = 1.416(5) Å and $C_3^\alpha-C'_3$ = 1.503(6) Å in ΔPhe^3 are significantly shorter than the corresponding bond distances in the saturated residues (1.45 Å for $N-C^\alpha$ and 1.53 Å for $C^\alpha-C'$).[22] The values of bond angles in ΔPhe^2, $N_2-C_2^\alpha-C'_2$, $N_2-C_2^\alpha-C_2^\beta$ and $C_2^\alpha-C_2^\beta-C_2^\gamma$ are 117.3(3)°, 124.4 (4)° and 129.4(4)°, respectively. The corresponding values in the ΔPhe^3 are 117.3(4)°, 124.4(4)° and 130.9(4)°. These values deviate considerably from the expected value of 120° for planar trigonal structures.[22] These deviations have been commonly observed in other dehydro-residues although minor differences occur due to the variations in the nature and size

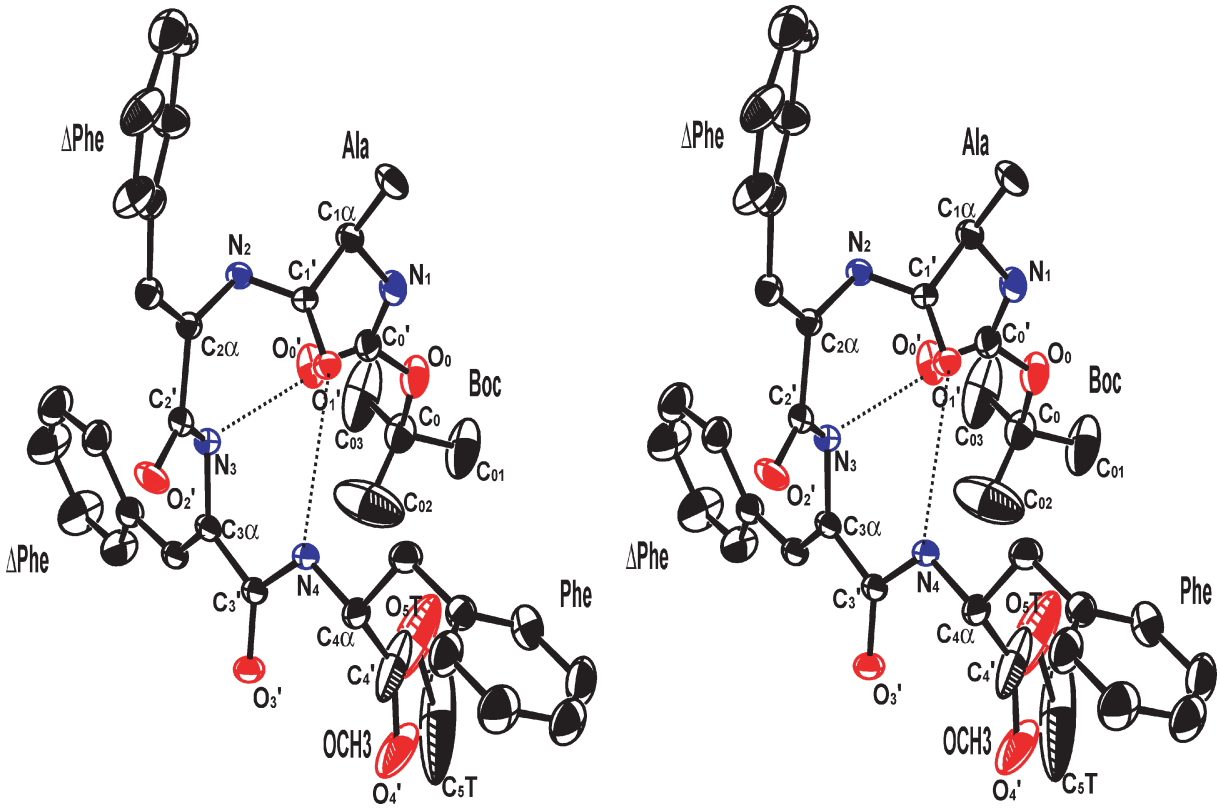

Fig. 1. Stereoview of the peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 at 30% ellipsoidal probability. The main chain atoms are labeled.

of the side chains of neighbouring residues. There are other atoms in the structure such as C_{01}, C_{02}, C_{03}, $C_3^{\varepsilon 2}$, C_3^{ζ}, C'_4, O'_4, O_5^t and C_5^t that are thermally disordered. Therefore, bond lengths and angles including dihedral show significant distortions due to uncertainty in their positions.

Conformation of the peptide

The dihedral angles (shown in Fig. 2) that define the conformation of the peptide are given in Table 2. The values of $\theta_0 = -175.8(4)^\circ$ and $\omega_0 = 176.2(4)^\circ$ show that the conformation of the Boc group corresponds to *trans–trans*.[23] The atoms N_1, C_0, O'_0 and O_0 are coplanar and the dihedral angle O^1 ($C_0{-}O_0{-}C'{-}O'_0$) is 6.9(8)°. This shows that the $C'_0{-}O'_0$ bond is *syn* planar with the $C_0{-}O_0$ bond as seen for esters in general.[24,25]

The peptide backbone adopts a conformation with two overlapping types II′ and III β-turns having dihedral angles $\phi_1 = 53.7(6)^\circ$, $\psi_1 = -135.9(4)^\circ$, $\phi_2 = -59.2(5)^\circ$, $\psi_2 = -17.9(5)^\circ$, $\phi_3 = -68.4(5)^\circ$, $\psi_3 = -18.8(6)^\circ$. It is stabilized by two 4 → 1 intramolecular hydrogen bonds involving imino nitrogen atoms of ΔPhe[3] and Phe[4] and the carbonyl oxygen atoms of Boc group and Ala[1] residue. All peptide units are *trans* with $\omega \cong 180^\circ$. It may be stated that the constraints generated by dehydro-residue are steric in nature and are not significantly affected by the behaviour of the solvent or crystal packing forces. Thus, the conformations induced by the dehydro-residues are similar in both solution and solid states.[26–29]

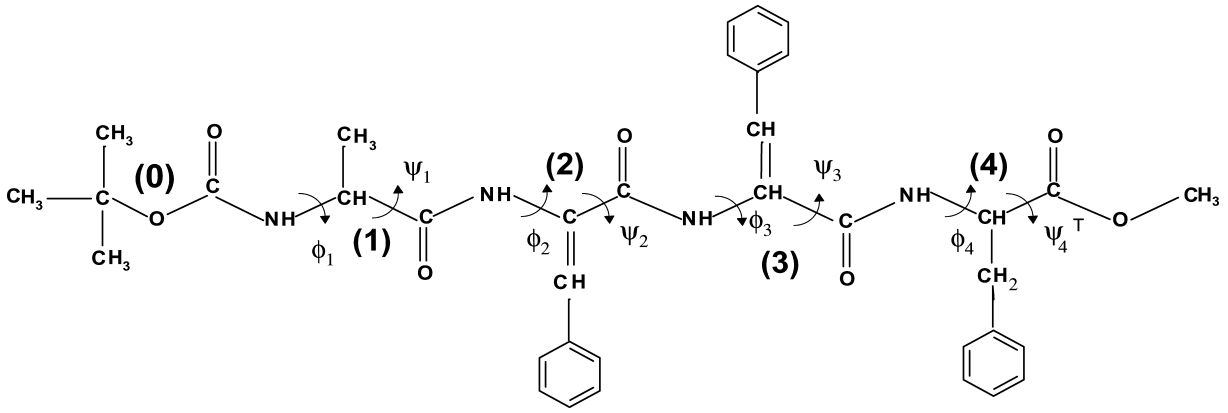

Fig. 2. Schematic representation of dihedral angles in the peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3.

As seen from Table 3, various tetrapeptides with two consecutive ΔPhe residues at $(i+2)$ and $(i+3)$ positions tend to form either overlapping types II and III β-turn conformations[15,16,30] or two overlapping type III β-turns resulting in the formation of an incipient 3_{10}-helix.[14]

Table 2. Selected Dihedral Angles (°) Involving Non-Hydrogen Atoms in Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 (Estimated Standard Deviations are Given in Parentheses)

θ_0	$C_0-O_0-C'_0-N_1$	−175.8(4)
ω_0	$O_0-C'_0-N_1-C_1^{\alpha}$	176.2(4)
ϕ_1	$C'_0-N_1-C_1^{\alpha}-C'_1$	53.7(6)
ψ_1	$N_1-C_1^{\alpha}-C'_1-N_2$	−135.9(4)
ω_1	$C_1^{\alpha}-C'_1-N_2-C_2^{\alpha}$	−176.8(4)
ϕ_2	$C'_1-N_2-C_2^{\alpha}-C'_2$	−59.2(7)
χ_2^1	$N_2-C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}$	−5.2(7)
$\chi_2^{2,1}$	$C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}-C_2^{\delta 1}$	32.0(7)
$\chi_2^{2,2}$	$C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}-C_2^{\delta 2}$	−145.2(5)
ψ_2	$N_2-C_2^{\alpha}-C'_2-N_3$	−17.9(5)
ω_2	$C_2^{\alpha}-C'_2-N_3-C_3^{\alpha}$	173.6(4)
ϕ_3	$C'_2-N_3-C_3^{\alpha}-C'_3$	−68.4(5)
χ_3^1	$N_3-C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}$	−3.7(8)
$\chi_3^{2,1}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 1}$	−19.0(8)
$\chi_3^{2,2}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 2}$	162.5(6)
ψ_3	$N_3-C_3^{\alpha}-C'_3-N_4$	−18.8(6)
ω_3	$C_3^{\alpha}-C'_3-N_4-C_4^{\alpha}$	−179.7(4)
ϕ_4	$C'_3-N_4-C_4^{\alpha}-C'_4$	63.6(8)
χ_4^1	$N_4-C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma}$	176.0(5)
$\chi_4^{2,1}$	$C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma}-C_4^{\delta 1}$	−83.5(7)
$\chi_4^{2,2}$	$C_4^{\alpha}-C_4^{\beta}-C_4^{\gamma}-C_4^{\delta 2}$	96.1(6)
ψ_4^T	$N_4-C_4^{\alpha}-C'_4-O_5$	48.0(12)

As indicated by the dihedral angles of the side chains of ΔPhe^2 and ΔPhe^3, ($\chi_2^1 = -5.2(7)$ Å, $\chi_2^{2,1} = 32.0(7)$ Å, $\chi_2^{2,2} = -145.2(5)$ Å and $\chi_3^1 = -3.7(8)$ Å, $\chi_3^{2,1} = -19.0(8)$ Å, $\chi_3^{2,2} = 162.5(6)$ Å, the phenyl ring and peptide unit in ΔPhe^2 is significantly distorted from planarity while it is only slightly distorted in ΔPhe^3. It may be noted that ΔPhe^2 is located at $(i+2)$ position of the type II′ β-turn and at $(i+1)$ position of type III β-turn while ΔPhe^3 occupies $(i+2)$ position of type III β-turn conformation. In order to adjust to two different requirements of ϕ, ψ dihedral angles in ΔPhe^2, its side chain is accommodated with respect to the backbone with significant distortions from planarity. Similar deviations have been reported in other structures having two different types of overlapping β-turn conformations.[15,16,30,31]

Molecular packing and hydrogen bonding

The packing of molecules in the unit cell is illustrated in Fig. 3. The structure is stabilized by an intermolecular hydrogen bond involving NH of ΔPhe^2 and carbonyl oxygen atom of a symmetry related $[-x,\ y+1/2,\ -z+1/2]$ ΔPhe^3 residue. The parameters of hydrogen

Table 3. Dihedral Angles for Peptides with Two Consecutive ΔPhe Residues at $(i + 2)$ and $(i + 3)$ Positions

Peptides	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	ψ_2
Boc-Val-ΔPhe-ΔPhe-Val-OCH_3	−54.8	130.5	65.8	12.8	79.4	3.9	−106.4	−54.6
Boc-Val-ΔPhe-ΔPhe-Ala-OCH_3	−54.0	129.0	57.0	15.0	80.0	7.0	−108.0	−34.0
Boc-Ala-ΔPhe-ΔPhe-Ala-OCH_3	−61.7	132.2	58.1	17.0	72.1	12.1	−103.1	−45.6
Boc-Leu-ΔPhe-ΔPhe-Ile-OCH_3	−51.1	−43.5	−56.4	−22.2	−99.3	2.3	−122.7	−159.2
Boc-Ala-ΔPhe-ΔPhe-$NHCH_3$	−71.0	−25.0	−63.1	−11.5	−62.4	−24.2	—	—

bonds are given in Table 4. The packing of the molecules gives rise to clustering of aromatic rings between the parallel columns. The columns are stacked vertically and the interior is stabilized by van der Waals forces involving aromatic rings and blocking groups. The unit cell appears to be uniformly packed in all the three directions.

Conclusions

The following conclusions can be drawn for the structures of peptides containing two consecutive ΔPhe residues:

(i) The three peptide unit sequences with two consecutive ΔPhe residues at $(i + 1)$ and

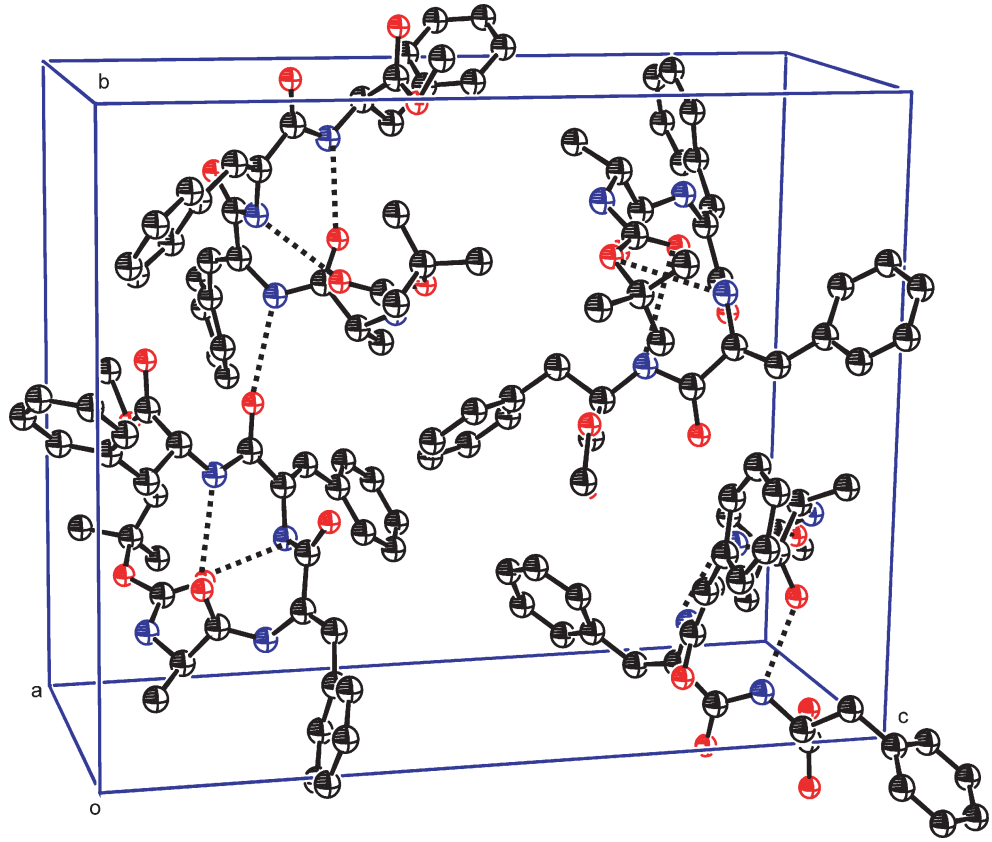

Fig. 3. Crystal packing of the peptide Boc-Ala-ΔPhe-ΔPhe-Phe-OCH_3 as viewed down the *a*-axis.

Table 4. List of Hydrogen Bonds for Peptide Boc–Ala–ΔPhe–ΔPhe–Phe–OCH_3 (Estimated Standard Deviations are Given in Parentheses)

Type	D−H···A	D···A (Å)	D−H···A (°)	Symmetry
Intramolecular	$N_3-H_3\cdots O'_0$	2.943(6)	140.0(2)	x, y, z
Intramolecular	$N_4-H_4\cdots O'_1$	2.964(7)	166.5(6)	x, y, z
Intermolecular	$N_2-H_2\cdots O'_3$	2.894(7)	174.4(1)	$-x, y + 1/2, -z + 1/2$

($i + 2$) positions adopt an unfolded S-shaped structure with dihedral angles ϕ, ψ centred at $\pm 60^\circ$, $\pm 30^\circ$.[12,13]

(ii) The peptides containing two consecutive ΔPhe residues at ($i + 2$) and ($i + 3$) positions with residues other than the branched β-carbons at ($i + 1$) and ($i + 4$) positions form two overlapping type III β-turns (incipient 3_{10}-helix).[14,30]

(iii) The sequences containing two consecutive ΔPhe residues at ($i + 2$) and ($i + 3$) positions with branched β-carbon residue only at ($i + 1$) position adopt a conformation with two overlapping types II and III′ β-turns.[16]

(iv) The peptides containing two consecutive ΔPhe residues at ($i + 2$) and ($i + 3$) positions with branched β-carbon residues such as Val and Ile at both ($i + 1$) and ($i + 4$) positions form two overlapping types II and I′ β-turns.[15]

These results indicate that specific conformations of peptides can be generated with α,β-dehydro-residues. The predictable nature of these conformations makes the design of peptides with α,β-dehydro-residues a useful method for developing specific ligands for various biological applications including drug design. The rational structure-based drug design requires the knowledge of the three-dimensional structures of the target molecules and the tools to design ligands with complementary structures. A number of design rules with α,β-dehydro-residues provide a useful method for developing peptide molecules with desired structures.

Supplementary material The crystallographic data was submitted in the Cambridge Crystallographic Data Centre (CCDC) with the deposition number CCDC 254434. The data is accessible from the website www.ccdc.cam.ac.uk.

Acknowledgments

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