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Design of peptides with α,β-dehydro-residues: syntheses, crystal structures and molecular conformations of two ΔPhe-Trp containing peptides

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Abstract

The ΔPhe-Trp is a newly designed moiety that was found inducing a unique conformation in peptides. The peptides Boc-L-Val-ΔPhe-L-Trp-OCH_3 (I) and Boc-L-Leu-ΔPhe-L-Trp-OCH_3 (II) were synthesized by azlactone method in solution phase. The peptide (I) was crystallized from its solution in ethanol–water mixture in orthorhombic space group $P2_12_12_1$ with $a = 10.663(3)$ Å, $b = 11.204(3)$ Å, $c = 26.516(10)$ Å and peptide (II) was crystallized from its solution in acetone in a monoclinic space group $P2_1$ with $a = 9.354(1)$Å, $b = 11.218(4)$Å, $c = 15.633(1)$Å and $\beta = 101.83(1)^\circ$. The structures were determined by direct methods. Peptide (I) was refined to an R value of 0.059 for 1554 observed reflections [$I \geq 2\sigma$ (I)] and peptide (II) was refined to an R value of 0.043 for 2920 observed reflections [$I \geq 2\sigma$ (I)]. The structures of peptides (I) and (II) were found to be identical. They formed an unusual type VIa β-turn conformation which is observed for the first time with a ΔPhe residue at $(i + 2)$ position indicating a unique influence of ΔPhe-Trp moiety in inducing a reproducible new structure in peptides.

Keywords: Peptide design; X-ray diffraction; ΔPhe-residue; ΔPhe-Trp moiety; Conformation; Crystal structure

1. Introduction

α,β-Dehydro-residues have been found to be strong inducers of specific conformations in peptides. The introduction of dehydro-residues in the peptide sequences has led to the design of a set of rules that can be applied to generate required structures of peptides [1]. It has been shown that the ΔPhe residue adopts a conformation corresponding to one of the three sets of torsion angles i.e. −60, 140°; 80, 0°; −60, −30° and their enantiomers [2]. Furthermore, these values are associated with a ΔPhe residue according to its site of substitution in a peptide i.e. a ΔPhe residue at $(i + 1)$ position adopts a conformation with ϕ, ψ value of −60, 140°, at $(i + 2)$ position 80, 0° and if it is repeated twice or more in a peptide with a sequence of atleast four amino acids, it adopted a conformation with ϕ, ψ values of −60, −30°. Thus a set of rules to design peptides with dehydro-residues has been reported [1]. In order to extend the scope of these rules, we had earlier reported the structure of Boc-Ile-ΔPhe-Trp-OCH_3

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[2]. This peptide with ϕ, ψ torsion angles: $\varphi_1 = 90.8(8)^\circ$, $\psi_1 = -151.6(6)^\circ$, $\varphi_2 = 89.0(8)^\circ$ and $\psi_2 = 15.9(9)^\circ$ was found in an uncommon conformation. A large number of structures with ΔPhe at $(i+2)$ position have indicated that a ΔPhe at $(i+2)$ position induces a common type II β-turn conformation with torsion angles centred around $\varphi_1 \approx -60^\circ$, $\psi_1 \approx 140^\circ$, $\varphi_{22} \approx 80^\circ$ and $\psi_2 \approx 0^\circ$. In order to establish the reproducibility of formation of the uncommon conformation in peptides with ΔPhe-Trp moiety as indicated by Boc-L-Ile-ΔPhe-L-Trp-OCH_3 [2], we have synthesized two peptides (I) Boc-L-Val-ΔPhe-L-Trp-OCH_3 and (II) Boc-L-Leu-ΔPhe-L-Trp-OCH_3 by varying the residue at $(i+1)$ position and keeping the ΔPhe-Trp-OCH_3 moiety unaltered. The detailed syntheses, crystal structures and molecular conformations of peptides (I) and (II) are reported here.

Table 1
The details of crystal data intensity data collection and refinement

	Peptide (I)	Peptide (II)
Molecular formula	$C_{31}H_{38}N_4O_6$	$C_{32}H_{40}N_4O_6$
Molecular weight	562.66	576.69
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
a (Å)	10.663(3)	9.354(1)
b/Å	11.204(3)	11.218(4)
c (Å)	26.516(10)	15.633(1)
β (°)	–	101.83(1)
Z (molecules/unit cell)	4	2
dc (gcm^{-3})	1.17	1.19
$F(000)$	1200	616
Radiation	Cu K_α ($\lambda = 1.5418$ Å)	Cu K_α ($\lambda = 1.5418$ Å)
Total no. of independent reflections	2302	3424
No of observed reflections ($I \geq 2\sigma(I)$)	1554	2920
Crystal dimensions (mm^3)	$1.5 \times 0.2 \times 0.1$	$1.6 \times 0.4 \times 0.3$
μr	0.66	0.67
Instrument used	Enraf-Nonius CAD4	Enraf-Nonius CAD4
Mode of data collection	$\omega - 2\theta$	$\omega - 2\theta$
Maximum 2θ (°)	110	140
R	0.059	0.043
Rw	0.059	0.049
W	Unit weight	$1.0963/(\sigma^2(F)+0.000768F^2)$
S (goodness of fit)	1.61	1.50
T (°K)	293	293

2. Experimental

In order to synthesize the peptides Boc-L-Val-ΔPhe-L-Trp-OCH_3(I) and Boc-L-Leu-ΔPhe-L-Trp-OCH_3 (II) the following steps were used:

2.1. Synthesis of Boc-Val-(β-OH)-Phe-OH (1)

To a precooled solution (10 °C) of Boc-L-Val-OH (3 g, 13.8 mmol) in dry tetrahydrofuran (THF) (10 ml), N-methylmorpholine (NMM) (1.51 ml, 13.8 mmol) and isobutylchloroformate (IBCF) (1.79 ml, 13.8 mmol) were added. After 15 min of stirring a solution of DL-(β-OH)-Phe-OH (3.0 g, 16.5 mmol) in 1N NaOH (16.5 ml) was added to it and the mixture was stirred at 0 °C. The resulting oily compound had the yield = 4.6 g (68%), $R_f = 0.45$ ($CHCl_3$: MeOH: 9:1).

2.2. Boc-L-Val-ΔPhe azlactone (2)

Compound (1) (4.6 g, 12.1 mmol) was reacted with anhydrous sodium acetate (1.0 g, 12.1 mmol) and freshly distilled acetic anhydride (10 ml) for 96 h at room temperature. The yield was: 3.5 g (74%), $R_f = 0.97$ ($CHCl_3$: MeOH:: 9:1).

2.3. Boc-L-Val-ΔPhe-L-Trp-OCH_3 (I)

To a solution of compound (2) (1.0 g, 3.0 mmol) in dichloromethane (DCM), Trp-OCH_3.HCl (0.91 g. 3.6 mmol) neutralized by adding triethylamine (TEA) (0.5 ml, 3.6 mmol) and the solution was stirred for 200 h at room temperature. The yield of peptide (I) was: 0.98 g (72%), $R_f = 0.58$ ($CHCl_3$: MeOH:: 9:1).

Table 2a
Atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) of the peptide (I) (estimated standard deviations are given in parentheses)

Atoms	x	y	z	U_{eq} (Å^2)
C_0	−1677(9)	5316(8)	3682(3)	59(3)
C_{01}	−1129(10)	6041(9)	4102(4)	91(4)
C_{02}	−1591(5)	4016(8)	3788(4)	112(6)
C_{03}	−3012(8)	5663(11)	3581(4)	91(4)
O_0	−1059(6)	5594(5)	3201(2)	55(2)
C_0'	157(9)	5407(8)	3134(4)	57(3)
O_0'	879(7)	5102(8)	3454(3)	103(3)
N_1	480(6)	5609(6)	2657(3)	50(2)
C_1^{α}	1772(7)	5534(7)	2502(3)	47(3)
C_1^{β}	1918(1)	5043(8)	1962(4)	66(4)
$C_1^{\gamma 1}$	1452(11)	3741(8)	1938(4)	103(5)
$C_1^{\gamma 2}$	1253(10)	5783(9)	1569(3)	87(4)
C_1'	2444(8)	6726(7)	2554(3)	40(3)
O_1'	1897(6)	7653(5)	2528(2)	57(2)
N_2	3681(6)	6611(6)	2635(2)	45(2)
C_2^{α}	4425(8)	7650(7)	2721(3)	46(3)
C_2^{β}	4706(8)	8040(7)	3185(4)	50(3)
C_2^{γ}	4407(9)	7593(8)	3689(3)	55(3)
$C_2^{\delta 2}$	3724(9)	6554(8)	3793(4)	68(4)
$C_2^{\delta 1}$	4809(11)	8272(9)	4103(4)	79(4)
$C_2^{\epsilon 2}$	3505(11)	6223(10)	4276(4)	85(4)
$C_2^{\epsilon 1}$	4573(14)	7885(12)	4581(5)	99(5)
C_2^{η}	3905(13)	6865(12)	4671(4)	99(5)
C_2'	4954(8)	8255(8)	2282(4)	52(3)
O_2'	5423(6)	9260(5)	2298(2)	66(2)
N_3	4908(7)	7653(6)	1844(3)	53(2)
C_3^{α}	5558(10)	8001(8)	1398(4)	63(3)
C_3^{β}	6966(9)	7870(8)	1445(4)	65(4)
C_3^{γ}	7392(8)	6605(8)	1526(3)	49(3)
$C_3^{\delta 1}$	7402(8)	6006(9)	1974(4)	53(3)
$N_3^{\epsilon 1}$	7825(7)	4866(6)	1898(3)	60(3)
$C_3^{\delta 2}$	7829(9)	5799(7)	1150(4)	64(4)
$C_3^{\epsilon 2}$	8080(8)	4720(8)	1406(4)	55(3)
$C_3^{\epsilon 3}$	8050(9)	5860(11)	636(4)	78(4)
$C_3^{\xi 2}$	8538(10)	3745(9)	1145(5)	101(5)
C_3^{ω}	8749(8)	3830(6)	654(5)	96(6)
$C_3^{\xi 3}$	8508(11)	4890(8)	380(4)	81(4)
C_3'	5010(9)	7236(11)	969(4)	80(5)
O_3'	4386(8)	6383(7)	1032(3)	96(3)
O_4	5417(9)	7645(7)	530(3)	110(4)
C_4	5061(6)	6955(7)	96(4)	163(9)

2.4. H-NMR Spectra of Boc-L-Val-ΔPhe-L-Trp-OCH_3 (I)

In order to confirm the synthesis of the peptide (I) ^{1}H-NMR spectra of the peptide was obtained in $CDCl_3$ with 400 MHz Bruker DRX 400 instrument. The following characteristics peaks were observed: δ 8.4 (s, 1H, NH ΔPhe); δ 7.5 (d, 1H, NH Trp); δ 7.0–7.4 (m, 12H, ΔPhe Ar, Olefinic and indole); δ 6.7 (d, 1H, NH Urethane); δ 5.0 (q, 1H, C^{α}Val); δ 4.04 (q, 1H, C^{α}Trp); δ 3.7 (s, 3H, –OCH_3); δ 3.4 (m, 2H, C^{β}Trp); δ 2.1 (m, 1H, C^{β}Val); δ

Table 2b
Atomic coordinates ($\times 10^4$) and equivalent isotropic thermal parameters ($\times 10^3$) for peptide (II) (estimated standard deviations are given in parentheses)

Atoms	x	y	z	U_{eq} (Å^2)
C_0	0891(5)	6449(0)	0460(3)	456(1)
C_{01}	1696(9)	7627(8)	0490(4)	728(2)
C_{02}	−0509(6)	6438(11)	−0207(3)	823(2)
C_{03}	1839(7)	5413(8)	0292(4)	641(1)
O_0	0411(3)	6242(6)	1282(2)	477(8)
C_0'	1389(4)	6176(7)	2038(3)	361(1)
O_0'	2696(3)	6280(6)	2127(2)	484(8)
N_1	0719(4)	5991(6)	2710(2)	374(9)
C_1^{α}	1587(4)	5858(6)	3587(2)	314(1)
C_1^{β}	0587(5)	5679(7)	4240(3)	360(1)
C_1^{γ}	−0453(5)	6700(8)	4314(3)	436(1)
$C_1^{\delta 1}$	0347(7)	7854(8)	4558(4)	758(2)
$C_1^{\delta 2}$	−1375(6)	6346(9)	4985(4)	715(2)
C_1'	2568(4)	4764(6)	3658(3)	312(9)
O_1'	2087(3)	3793(6)	3391(2)	533(9)
N_2	3954(3)	4928(6)	4099(2)	316(8)
C_2^{α}	4896(4)	3926(6)	4335(3)	335(1)
C_2^{β}	5893(4)	3530(7)	3899(3)	393(1)
C_2^{γ}	6365(4)	3978(7)	3124(3)	428(1)
$C_2^{\delta 1}$	5879(5)	5044(7)	2696(3)	492(1)
$C_2^{\epsilon 1}$	6395(6)	5387(8)	1966(3)	563(1)
$C_2^{\epsilon 2}$	7882(6)	3669(9)	2070(4)	717(2)
$C_2^{\delta 2}$	7388(5)	3303(8)	2801(3)	563(1)
C_2^{η}	7391(7)	4719(9)	1646(4)	645(2)
C_2'	4763(4)	3304(6)	5154(3)	377(1)
O_2'	5273(3)	2296(6)	5340(2)	506(1)
N_3	4083(4)	3912(6)	5691(2)	367(9)
C_3^{α}	3966(5)	3498(7)	6556(3)	402(1)
C_3^{β}	5424(5)	3597(7)	7221(3)	455(1)
C_3^{γ}	5935(4)	4862(7)	7395(3)	408(1)
$C_3^{\delta 1}$	5741(4)	5595(7)	8100(3)	438(1)
$C_3^{\epsilon 3}$	5162(6)	5428(9)	8850(4)	603(1)
$C_3^{\xi 3}$	5169(7)	6418(12)	9401(5)	817(3)
C_3^{η}	5721(8)	7528(11)	9226(6)	863(3)
$C_3^{\xi 2}$	6287(7)	7691(8)	8501(6)	741(2)
$C_3^{\epsilon 2}$	6316(5)	6732(7)	7950(4)	557(1)
$N_3^{\epsilon 1}$	6830(5)	6659(7)	7207(3)	408(1)
$C_3^{\delta 2}$	6600(5)	5549(7)	6883(4)	501(1)
C_3'	2764(6)	4216(8)	6827(3)	456(1)
O_3'	2273(4)	5118(7)	6486(2)	582(1)
O_4	2357(5)	3689(7)	7507(2)	654(1)
C_4	1228(11)	4314(15)	7841(6)	453(3)

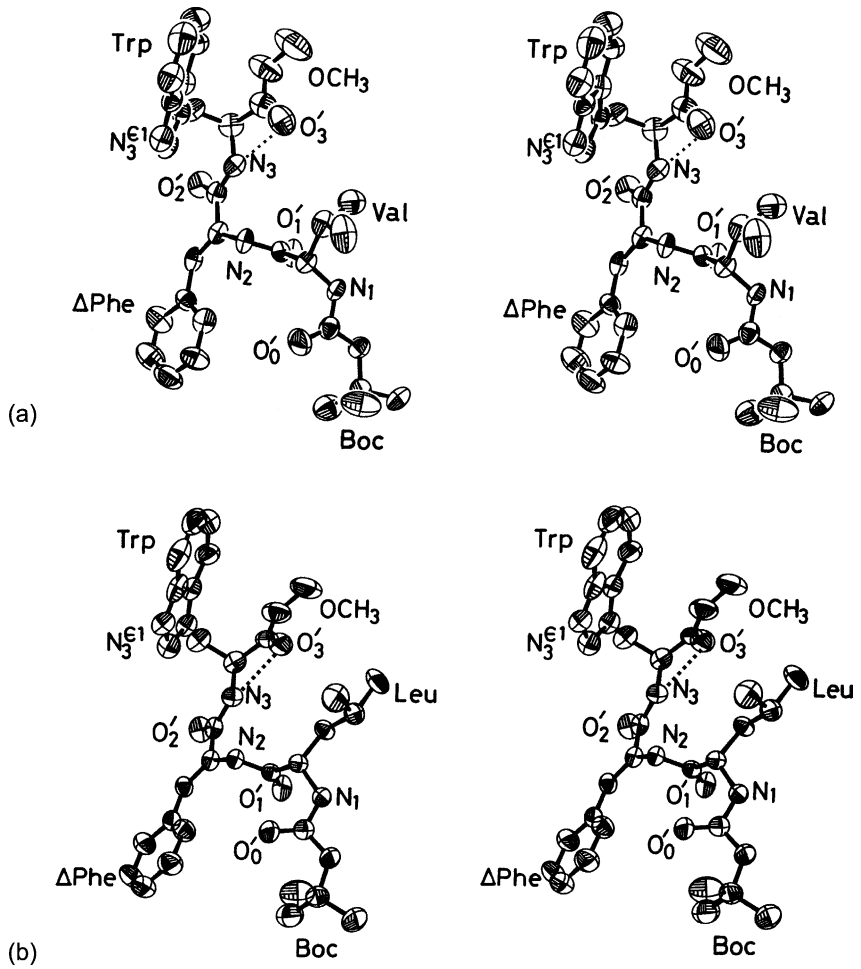

Fig. 1. Boc-Val-ΔPhe-Trp-OCH_3 (a) and Boc-Ile-ΔPhe-Trp-OCH_3 (b)

1.5 (s, 9H, Boc); δ 0.95 (d, 3H, $C^{\gamma 1}$Val); δ 0.82 (d, 3H, $C^{\gamma 2}$Val); δ 0.82 (d, 3H, $C^{\gamma 2}$Val). The assignments of peaks as described above clearly indicated the correct synthesis of the required peptide.

2.5. Synthesis of Boc-L-Leu-(β-OH)-Phe-OH (3)

To a precooled solution (10 °C) of Boc-L-Leu-OH (2 g, 8.6 mmol) in dry tetrahydrofuran (THF) (10 ml), *N*-methylmorpholine (NMM) (1.51 ml, 13.8 mmol) and isobutylchloroformate (IBCF) (0.95 ml, 0.6 mmol) were added. After 15 min of stirring a solution of DL-(β-OH)-Phe-OH (1.87 g, 10.3 mmol) in 1N NaOH (10.3 ml) was added and the mixture was stirred at 0 °C. The yield was 2.5 g (63%), $R_f = 0.42$ ($CHCl_3$: MeOH:: 9:1).

2.6. Boc-L-Leu-ΔPhe Azlactone (4)

Compound (3) (2.5 g, 6.3 mmol) was reacted with anhydrous sodium acetate (0.52 g, 6.3 mmol) and freshly distilled acetic anhydride (10 ml) for 96 h at room temperature. The yield was 1.90 g (80%), $R_f = 0$. ($CHCl_3$: MeOH:: 9:1).

2.7. Boc-L-Leu-ΔPhe-L-Trp-OCH_3 (II)

To a solution of compound (4) (1.15 g, 3.1 mmol) in dichloromethane (DCM), Trp-OCH_3.HCl (0.94 g.

Table 3
Torsion angles (°) with estimated standard deviations in parentheses)

		Peptide (I)	Peptide (II)
θ_0	$C_0-O_0-C'_0-N_1$	173.7(7)	179.6(4)
ω_0	$O_0-C'_0-N_1-C_1^{\alpha}$	176.1(7)	178.3(4)
ϕ_1	$C'_0-N_1-C_1^{\alpha}-C'_1$	−89.9(9)	−62.9(6)
Ψ_1	$N_1-C_1^{\alpha}-C'_1-N_2$	152.0(6)	135.9(5)
χ_1	$N_1-C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma}$	–	−61.8(6)
$\chi_1^{1,1}$	$N_1-C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma 1}$	−64.7(9)	–
$\chi_1^{1,2}$	$N_1-C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma 2}$	59.2(9)	–
$\chi_1^{2,1}$	$C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma}-C_1^{\delta 1}$	–	−57.5(7)
$\chi_1^{2,2}$	$C_1^{\alpha}-C_1^{\beta}-C_1^{\gamma}-C_1^{\delta 2}$	–	179.0(5)
ω_1	$C_1^{\alpha}-C'_1-N_2-C_2^{\alpha}$	−176.4(7)	169.2(5)
ϕ_2	$C'_1-N_2-C_2^{\alpha}-C'_2$	−88.3(9)	−83.8(6)
Ψ_2	$N_2-C_2^{\alpha}-C'_2-N_3$	−13.0(10)	−16.9(7)
χ_2^1	$N_2-C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}$	1.0(11)	3.0(10)
$\chi_2^{2,2}$	$C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}-C_2^{\delta 2}$	2.0(11)	−176.2(5)
$\chi_2^{2,1}$	$C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}-C_2^{\delta 1}$	−177.0(11)	5.3(1)
ω_2	$C_2^{\alpha}-C'_2-N_3-C_3^{\alpha}$	−168.9(8)	−174.2(5)
ϕ_3	$C'_2-N_3-C_3^{\alpha}-C'_3$	−167.6(9)	−163.0(5)
Ψ_3	$N_3-C_3^{\alpha}-C'_3-O_4$	169.7(9)	164.8(5)
χ_3	$N_3-C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}$	63.0(11)	64.6(6)
χ_3^1	$C'_3-C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}$	−57.0(10)	−56.0(7)
$\chi_3^{2,1}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 1}$	97.0(10)	97.2(7)
$\chi_3^{2,2}$	$C_3^{\alpha}-C_3^{\beta}-C_3^{\gamma}-C_3^{\delta 2}$	−81.0(10)	−78.8(8)

3.7 mmol) neutralized by adding triethylamine (TEA) (0.5 ml, 3.7 mmol) and the solution was stirred for 200 h at room temperature. The yield of peptide (II) was 0.95 g (72%) $R_f = 0.58$ ($CHCl_3$: MeOH:: 9:1).

2.8. H-NMR Spectra of Boc-L-Leu-ΔPhe-L-Trp-OCH_3

In order to ascertain the correctness of the sequence of the peptide a ^{1}H-NMR spectra of the peptide was observed in $CDCl_3$ with 400 MHz Bruker DRX 400 instrument. The observed proton peaks were assigned as follows: δ 7.0–7.6 (m, 11H, Ar ΔPhe, indole); δ 7.0 (s, 1H, ΔPhe olefinic); δ 8.4 (bs, 1H, NH, ΔPhe); δ 6.9 (bd, 1H, NH Trp); δ 5.0 (q, 1H, C^{α}Trp); δ 4.9 (bd, NH Urethane); δ 4.2 (q, C^{α} Leu); δ 3.6 (s, 3H, −OMe); δ 3.4 (d, 2H, C^{β}Trp); δ 1.4 (s, 9H, t-Bu); δ 1.2 (m, 4H, C^{β} and C^{γ} Leu); δ 0.9 (d, 6H, C^{δ}Leu). The unambiguous assignments of peaks confirmed the right sequence of the peptide (II).

2.9. Structure determination

The peptide Boc-Val-ΔPhe-Trp-OCH_3 (I) was crystallized from its saturated solution in ethanol–water mixture while the peptide Boc-Leu-ΔPhe-Trp-OCH_3 (II) gave diffractable crystals from its saturated solution in acetone. The detailed crystal data are given in Table 1. The structures were determined by direct methods using the program SHELX 86 [3]. The coordinates of non-hydrogen atoms were refined anisotropically using the program SHELX 76 [4]. The positions of hydrogen atoms were obtained from difference Fourier maps and were included in the final cycles of refinements using isotropic thermal parameters of the non-hydrogen atoms to which they were attached. During the refinement the function minimized was $\sum w(|F\text{obs}| - |F\text{cal}|)$ where the weights were $w = 1.0$ for peptide (I) and $w = 1.0963/(\sigma^2(F) + 0.000768|F|^2)$ for peptide (II). The final R factors for observed reflections 1554 in (I) and 2920 in (II) were 0.059 and 0.043, respectively. The atomic scattering factors used in these calculations were those of Cromer and Mann [5] for non-hydrogen atoms and of Stewart, Davidson and Simpson [6] for hydrogen atoms. The atomic coordinates for peptides (I) and (II) are listed in Tables 2a and 2b, respectively. The solvent molecules were not observed in these

Table 4
The ϕ, ψ torsion angles (°) in sequences containing ΔPhe-Trp element: (I) Boc-L-Val-ΔPhe-L-Trp-OCH_3 (II) Boc-L-Leu-ΔPhe-L-Trp-OCH_3 and (III) Boc-L-Ile-ΔPhe-L-Trp-OCH_3

Peptides	ϕ_1	ψ_1	ϕ_2	ψ_2	ϕ_3	ψ_3	Ref
(I)	−89.9(9)	152.0(6)	−88.3(9)	−13.0(10)	−167.6(9)	169.7(9)	Present study
(II)	−62.9(6)	135.9(5)	−83.8(6)	−16.9(7)	−163.0(5)	164.8(5)	Present study
(III)	−90.8	151.6(6)	−89.0(8)	−15.5(5)	−167.7(7)	166.0(7)	2

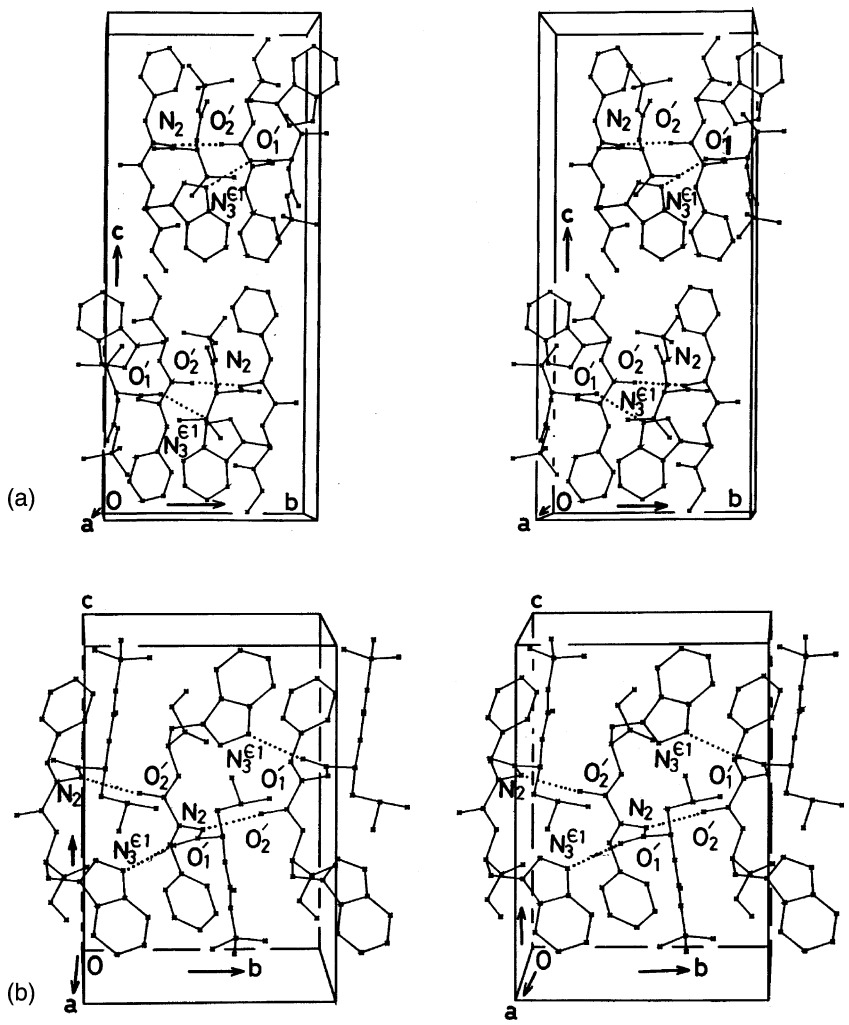

Fig. 2.

Table 5a
Hydrogen bonds for peptide (I) and peptide (II) with estimated standard deviations in parentheses

Type	H bond	Lengths (Å)	Angle (Å) ∠N–H···O	Symmetry
Peptide (I)				
Intermolecular	$N_2 - H_2 \cdots O'_2$	2.830(9)	170.9(7)	$-x+1, y-1/2\text{-}z+1/2$
Intermolecular	$N_3^{\epsilon 1} - H_3^{\epsilon 1} \cdots O'_1$	2.944(9)	159.8(8)	$-x+1, y-1/2, -z+1/2$
Intramolecular (intra-residue)	$N_3 - H_3 \cdots O'_3$	2.648(10)	107.4(7)	x, y, z
Peptide (II)				
Intermolecular	$N_3 - H_2 \cdots O'_2$	2.844(9)	169.7(4)	$-x+1, y-1/2, -z+1/2$
Intermolecular	$N_3^{\epsilon 1} - H_3^{\epsilon 1} \cdots O'_1$	2.832(9)	166.1(6)	$-x+1, y-1/2, -z+1/2$
Intramolecular (intra-residue)	$N_3 - H_3 \cdots O'_3$	2.665(7)	106.3(4)	x, y, z

Table 5b
The van der Waals interactions with distances ≤3.5 (Å) between pairs of symmetry related atoms for peptides (I) and (II)

van der Waals pairs in peptide I	Distances (Å)	van der Waals pairs in peptide II	Distances (Å)
$C_{03}\cdots C_2^{\gamma}$ (3)	3.544(16)	$C_{02}\cdots C_3^{\zeta 2}$ (1)	3.547(8)
$O_0'\cdots C_3^{\beta}$ (2)	3.415(12)	$O_0\cdots C_2^{\eta}$ (3)	3.446(7)
$C_1^{\alpha}\cdots O_2'$ (2)	3.375(9)	$O_0'\cdots C_2^{\beta}$ (4)	3.186(7)
$C_2^{\beta}\cdots N_3^{\epsilon 1}$ (1)	3.419(11)	$N_1\cdots C_2^{\eta}$ (3)	3.512(6)
$C_2^{\gamma}\cdots C_{03}$ (3)	3.544(17)	$C_1^{\alpha}\cdots O_2'$ (4)	3.468(5)
$C_2^{\gamma}\cdots C_3^{\zeta 2}$ (1)	3.439 (14)	$C_2^{\beta}\cdots N_3^{\epsilon 1}$ (2)	3.481(6)
$C_2^{\delta 2}\cdots C_3^{\zeta 2}$ (1)	3.458(14)	$C_2^{\gamma}\cdots C_3^{\zeta 2}$ (2)	3.482(6)
$O_2'\cdots C_1^{\alpha}$ (1)	3.375(9)	$C_2^{\eta}\cdots O_0$ (5)	3.446(7)
$C_1^{\beta}\cdots O_0'$ (1)	3.415(12)	$C_2^{\eta}\cdots N_1$ (5)	3.512(6)
$N_3^{\epsilon 1}\cdots C_2^{\beta}$ (2)	3.419(11)	$O_2'\cdots C_1^{\alpha}$ (2)	3.468(5)
$C_3^{\zeta 2}\cdots C_2^{\gamma}$ (2)	3.438(14)	$C_3^{\beta}\cdots O_0'$ (2)	3.186(7)
$C_3^{\zeta 2}\cdots C_2^{\delta 2}$ (2)	3.458(14)	$C_3^{\zeta 2}\cdots C_2'$ (6)	3.472(8)
		$C_3^{\zeta 2}\cdots C_2^{\gamma}$ (4)	3.482(6)
		$N_3^{\epsilon 1}\cdots C_2^{\beta}$ (4)	3.481(6)

Peptide I: (1) $-x+1, y+1/2, -z+1/2$; (2) $-x+1, y-1/2, -z+1/2$; (3) $x+1, y, z$. Peptide II: (1) $x-1, y, z-1$; (2) $-x+1, y-1/2, -z+1$; (3) $x-1, y, z$; (4) $-x+1, y+1/2, -z+1$ (5) $x+1, y, z$; (6) $x+1, y, z+11$.

structures and hence the interactions of peptides with solvent molecules were not determined.

3. Results and discussion

The stereoviews of peptides (I) and (II) are shown in Fig. 1a and b, respectively. The conformations of both peptides are similar. These structures are characterized by an unusual type VIa β-turn conformation which is observed for the first time with a ΔPhe residue at $(i+2)$ position indicating the significance of a new element ΔPhe-Trp in these peptides.

3.1. Molecular dimensions

The C=C double bond of ΔPhe in the two peptides has the trans configuration of the phenyl group with respect to the carbonyl group. The C=C bond lengths of 1.34(1) Å in peptide (I) and 1.33(7) Å in peptide (II) correspond to the typical value for a styrenic double bond conjugated with an aromatic ring. The values of bond angles $C_2^{\alpha}-C_2^{\beta}-C_2^{\gamma}$ in ΔPhe of the two peptides are 132(2) and 131(5)°, respectively. The large deviations of these angles from 120° of a trigonal arrangement are notable features caused by steric constraints in a flattened ΔPhe residue and have been observed invariably [1].

3.2. Conformation of the peptides

The torsion angles that define the conformations of the two peptides are listed in Table 3. The backbone torsion angles: $\varphi_1 = -89.9(9)°$, $\psi_1 = 152.0(6)°$, $\varphi_2 = -88.3(9)°$, $\psi_2 = -13.0(10)°$, $\varphi_3 = -167.6(9)°$, $\psi_2^T = 169.7(9)°$ in peptide (I) and $\varphi_1 = -62.9(6)°$, $\psi_1 = 135.9(5)°$, $\varphi_2 = -83.8(6)°$, $\psi_2 = -16.9(7)°$, $\varphi_3 = -163.0(5)°$, $\psi_2^T = 164.8(5)°$ in peptide (II) are identical and clearly indicate the formation of an unusual type VIa β-turn conformation [7]. This has been observed for the first time with a ΔPhe residue. The structure of a peptide Boc-L-Ile-ΔPhe-L-Trp-OCH_3 reported earlier also showed the formation of a similar conformation (Table 4). Although, three peptides listed in Table 4 have different residues at $(i+1)$ position yet they adopt identical conformations. The common moiety in all of them is ΔPhe-Trp. Thus it can be termed as the inducer of the observed similar conformations in these peptides. Therefore, an unusual type VIa β-turn conformation can be generated by substituting a ΔPhe at $(i+2)$ and Trp at $(i+3)$ positions. This is a new rule for peptide design with ΔPhe residue.

3.3. Molecular packing and hydrogen bonding

The molecular packing in the crystals of peptides (I) and (II) are illustrated in Fig. 2a and b,

respectively. As seen from Table 5a, the molecules in the two structures are linked by intermolecular hydrogen bonds involving NH of ΔPhe and its carbonyl oxygen of a symmetry related molecule. The other intermolecular hydrogen bond involves $N^{\epsilon 1}$ of Trp residue as a donor and carbonyl oxygen of a symmetry related Val residue in peptide (I) and symmetry related Leu residue in peptide (II) as acceptors. The packing of the molecules in these peptides are further stabilized by van der Waals forces (Table 5b) involving hydrophobic groups (Fig. 2a and b).

4. Conclusions

The peptide design rules with a ΔPhe residue at $(i+2)$ position are summarized below:

1. The ΔPhe residue has been found to adopt one out of the three conformations belonging to the following three sets of ϕ, ψ torsion angles, -60, 140°; 80, 0° and 60, -30° or their enantiomers [1].
2. A peptide with a ΔPhe residue at $(i+2)$ position prefers a type II β-turn conformation [1].
3. A peptide with a ΔPhe residue at $(i+2)$ position and a branched β-carbon residue (Val) at $(i+3)$ position adopts a distorted β-turn II conformation [8].
4. A peptide having a ΔPhe residue at $(i+2)$ position with branched β-carbon residues on its both sides, adopts a characteristic unfolded S-shaped conformation with the values of ϕ, ψ torsion angles in which their signs change alternately [9,10].
5. As observed in the present case, a peptide with a ΔPhe residue at $(i+2)$ position and a Trp residue at $(i+3)$ position induces an unusual type VIa β-turn conformation. This is a new design rule with a ΔPhe at $(i+2)$ position and is a valuable addition to the already existing design rules with dehydro-residues that can be exploited to provide specific structures for useful applications.

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