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Conformational investigation of α,β-dehydropeptides. XIII. Conformational properties of *N*-acetyl-α,β-dehydrovaline *N*′,*N*′-dimethylamide★✪

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The crystal structure of Ac-ΔVal-NMe_2 (ΔVal = α,β-dehydrovaline) was determined by X-ray crystallography. The found angles ϕ = –60° and ψ = 125° correspond exactly to the respective values of the (*i* + 1)th residue in idealised β-turn II/VIa. *Ab initio*/DFT studies revealed that the molecule adopts the angle ψ restricted only to about |130°| and very readily attains the angle ϕ = about –50°. This is in line with its solid-state conformation. Taken together, these data suggest that the ΔVal residue combined with a C-terminal tertiary amide is a good candidate at the (*i* + 1)th position in a type II/VIa β-turn.

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Abbreviations: Δ, dehydro.

To achieve stereochemical control over the local folding of a peptide, both nature and researchers incorporate conformation-restricting amino acids into the peptide chain (Venkatraman *et al.*, 2001). Among them are α,β-dehydroamino acids with the $C^{\alpha}=C^{\beta}$ double bond. A set of design rules have been developed for (*Z*)-dehydrophenylalanine [(*Z*)-ΔPhe], (*Z*)-α,β-dehydroleucine [(*Z*)-ΔLeu] and dehydroalanine (ΔAla) (Singh & Kaur, 1996). However, much less is known about the conformational preferences of other α,β-dehydroamino acids. On the other hand, many modifications of peptides are based on the conversion of peptide bonds into tertiary amides, and the influence of the latter on the peptide conformation is also little recognised. *N*-Acetyl-α,β-dehydroamino acid *N*′,*N*′-dimethylamides, Ac-ΔXaa-NMe_2, are simple dipeptide models, which combine the two structural features: an α,β-dehydroamino acid residue and a tertiary amide bond. We have investigated the conformational preferences, in the solid state and as free entities, of the molecules with ΔXaa = ΔAla, (*Z*)-ΔPhe and (Z)-ΔAbu [(*Z*)-α,β-dehydrobutyrine] (Rzeszotarska *et al.*, 2002; Siodłak *et al.*, 2001; 2003; 2004a; 2004b). The crystal structure of Ac-(*Z*)-ΔLeu-NMe_2 is also known (El-Masdouri *et al.*, 1992). Herein, we report the X-ray structure analysis and *ab initio*/DFT calculations on Ac-ΔVal-NMe_2 (ΔVal = α,β-dehydrovaline, which has a branched β-carbon) as well as comparison of the obtained results with those on related molecules.

MATERIALS AND METHODS

Synthesis. Ac-ΔVal-NMe_2 was synthesised *via* *N*-carboxy anhydride of α,β-dehydrovaline, which was acetylated and opened with dimethylamine. The crude product was isolated by silica gel column chromatography and next crystallised from chloroform/*n*-hexane. The obtained crystals were of 99.6% purity, as determined by HPLC, and of m.p. 176–178°C. Details of this preparation are given by Smełka *et al.* (1997).

X-ray crystallography. Diffraction data were collected at 150 K using a Nonius Kappa CCD diffractometer and Mo K_{α} radiation (λ = 0.71073 Å). The structure was solved by direct methods and refined on F^2 by the full-matrix least-squares procedure using the program SHELXL-97 (Sheldrick, 1997a; 1997b). The H-atom positions were calculated from molecular geometry and 'riding model' was applied during the refinement, while the non-H atoms were refined with anisotopic displacement parameters. The crystallographic data for Ac-ΔVal-NMe_2 and structure refinement parameters are collected in Table 1.

Theoretical analysis. The theoretical conformational properties were examined on a free Ac-ΔVal-NMe_2 molecule using the

Table 1. Crystallographic data and structure refinement parameters for Ac-ΔVal-NMe_2

Empirical formula	$C_9H_{16}N_2O_2$
Formula weight	184.24
Crystal system	Triclinic
Space group	$P\bar{1}$
Cell dimensions	
a (Å)	7.346(1)
b (Å)	8.613(1)
c (Å)	8.936(2)
α (°)	73.934(2)
β (°)	68.245(2)
γ (°)	85.772(2)
Volume (Å^3)	504.35(11)
Z	2
D_c (g·cm^{-3})	1.213
F(000)	200
θ range for data collection (°)	2.46 to 27.41
Reflections collected	4182
Independent refl. [R(int)]	2260 [0.0139]
Final *R* indices [$I > 2\sigma(I)$]	$R1$ = 0.0488 $wR2$ = 0.1383

Further details of the crystal structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (No. CCDC 231191). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: (44 1223) 336 033 or e-mail: deposit@chemcryst.cam.ac.uk).

GAUSSIAN 98 package (Frisch *et al.*, 1998). To generate the (ϕ,ψ) potential energy surface, 288 structures calculated at the *ab initio* HF/6–31G*//HF/3–21G level were used. In each structure, all geometrical parameters were fully relaxed, except for the constrained torsion angles ϕ and ψ. Values of these angles were chosen by using a step size of 15°, within the range from –180° to 180° for ϕ, and from 0° to 180° for ψ. Inversion through the achiral α-carbon [i.e. (ϕ,ψ) → (–ϕ, –ψ)] yields an equivalent structure; therefore a full (ϕ,ψ) potential energy surface map was obtained in this way (Head-Gordon *et al.*, 1991). The minima observed on the surface were then subjected to full geometry optimisation at the DFT/B3LYP/6-31+G** level, followed by a second derivative analysis (frequency), which proved all of them to be minima. The geometrical parameters of the corresponding energy-minimised conformers were then further discussed.

The accessible conformational space of the molecule studied was assumed on the basis of the close resemblance between the Ramachandran contact map and the energy contours map within the limit of 5.0 kcal · mol^{-1} (Ramachandran & Sasisekharan, 1968), as is also applied elsewhere (Zimmerman *et al.*, 1977; Herzberg & Moult, 1991). The space was calculated by way of the Surfer 8 programme using the radial basis function as a gridding method.

As the overall conformational profiles of modified peptide models can differ from those of common peptide models, we describe the energy-minimised conformers of the investigated molecule by the general short hand letter notation introduced by Zimmerman (1977).

RESULTS AND DISCUSSION

X-ray analysis

Figure 1 shows the molecular structure and the association mode of Ac-ΔVal-NMe$_2$ in the crystal state. Table 2 lists the values of selected geometrical parameters: bond lengths, bond angles, torsion angles and intermolecular contacts.

Bond lengths and angles of the ΔVal residue compare well with the values observed for other Ac-ΔXaa-NMe$_2$ as well as for known ΔVal peptides: Ac-Pro-ΔVal-NHMe (Ciszak *et al.*, 1992), Z-ΔVal-Trp-OMe (Z = benzyloxycarbonyl) (Vijayaraghavan *et al.*, 2001) and

Table 2. Geometry of the Ac-ΔVal-NMe$_2$ molecule in the crystal and intermolecular contacts

Bond lengths (Å)		
N(1)–C(1)		1.342(2)
N(1)–C(11)		1.457(2)
N(1)–C(12)		1.462(2)
C(1)–O(1)		1.241(2)
C(1)–C(2)		1.508(2)
C(2)–C(21)		1.335(2)
C(2)–N(2)		1.423(2)
C(21)–C(23)		1.501(2)
C(21)–C(22)		1.505(2)
N(2)–C(3)		1.348(2)
C(3)–O(2)		1.227(2)
C(3)–C(31)		1.510(2)
Bond angles (degrees)		
C(1)–N(1)–C(11)		124.4(2)
C(1)–N(1)–C(12)		119.0(2)
C(11)–N(1)–C(12)		116.5(2)
O(1)–C(1)–N(1)		121.5(2)
O(1)–C(1)–C(2)		119.3(2)
N(1)–C(1)–C(2)		119.2(2)
C(21)–C(2)–N(2)		122.0(2)
C(21)–C(2)–C(1)		123.8(2)
N(2)–C(2)–C(1)		114.5(2)
C(2)–C(21)–C(23)		123.0(2)
C(2)–C(21)–C(22)		121.6(2)
C(23)–C(21)–C(22)		115.3(2)
Torsion angles (degrees)		
ϕ	C(1)–C(2)–N(2)–C(3)	-60.2(2)
ψ	N(1)–C(1)–C(2)–N(2)	125.4(2)
ω_1	C(2)–N(2)–C(3)–C(31)	178.6(1)
ω_2^1	C(12)–N(1)–C(1)–C(2)	171.6(1)
ω_2^2	C(11)–N(1)–C(1)–O(1)	170.2(1)
ω_2^3	C(12)–N(1)–C(1)–O(1)	-6.1(2)
ω_2^4	C(11)–N(1)–C(1)–C(2)	-12.2(2)
χ^{11}	N(2)–C(2)–C(21)–C(22)	-8.9(2)
χ^{12}	N(2)–C(2)–C(21)–C(23)	170.1(1)

Intermolecular contacts of the carbonyl O atoms			
Acceptor...H–Donor	A...D (Å)	A...H (Å)	∠ (deg)
O(1)···H(2n)–N(2) (*a*)	2.847(2)	2.03	175
O(1)···H(12c)–C(12) (*b*)	3.229(2)	2.67	118
O(2)···H(11b)–C(11) (*c*)	3.484(2)	2.65	145
O(2)···H(12b)–C(12) (c)	3.293(2)	2.44	148

Symmetry codes: (*a*) -1-*x*, -1-*y*, -1-*z*; (*b*) -1-*x*, -1-*y*, -2-*z*; (*c*) -1-*x*, -*y*, -2-*z*

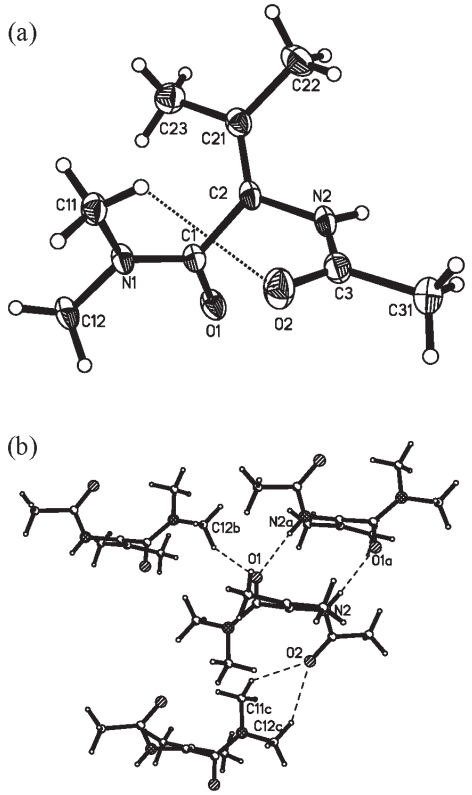

Figure 1. The Ac-ΔVal-NMe_2 molecule (a) and its environment in the crystal structure (b).

Parameters for intramolecular C(11)–H(11c)···O(2) contact are: C···O 3.353(2) Å, H···O 2.75 Å, ∠C–H···O 122°.

Z-ΔVal-Val-ΔPhe-Ile-OMe (Makker *et al.*, 2003). Analysis of the crystal packing of Ac-ΔXaa-NMe_2 reveals also similarities in the mode of intermolecular contacts: except Ac-ΔAla-NMe_2, centrosymmetric dimers are formed through a pair of N–H···O(1) hydrogen bonds. Those interactions are accompanied by C–H···O contacts. In the crystal of Ac-ΔVal-NMe_2, however, solely the N′-methyl groups are involved in the C–H···O contacts: both intermolecular as well as one intramolecular (Fig. 1). The latter is exceptionally favourable for Ac-ΔVal-NMe_2, with the shortest C(11)···O(2) distance observed of the studied Ac-ΔXaa-NMe_2 (Rzeszotarska *et al.*, 2002; Siodłak *et al.*, 2003; 2004b). This contact is also seen in theoretical analysis of Ac-ΔXaa-NMe_2 (Siodłak *et al.*, 2004a) and seems to be common for this group of compounds.

The ΔVal residue adopts in the Ac-ΔVal-NMe_2 crystal the torsion angles ϕ, ψ = –60°, 125°. They depart somewhat from those about (–45°, 130°) found in the crystal structures of other Ac-ΔXaa-NMe_2, however, correspond well to the ϕ, ψ values (–60°, 120°) of the (i + 1)th residue in the idealised β-turn II (Rose *et al.*, 1985). Similar values (–44°, 136°) were found in the crystal structure of Z-ΔVal-Trp-OMe and the ΔVal residue at the (i + 1)th position was recognised as a β-turn II inducer (Vijayaraghavan *et al.*, 2001). However, in Ac-Pro-ΔVal-NHMe (Ciszak *et al.*, 1992) and Z-ΔVal-Val-ΔPhe- Ile-OMe (Makker *et al.*, 2003), which are other ΔVal peptides of known crystal structure, ΔVal adopts the ϕ, ψ angles of (–74°, –14°) and (–391°, –411°), respectively.

Theoretical analysis

Figure 2 presents the results of calculations on free Ac-ΔVal-NMe_2 molecule, and Table 3 shows its selected conformational parameters in the solid state and in all energy-minimised conformers.

The Ramachandran diagram of the (ϕ, ψ) surface for Ac-ΔVal-NMe_2 shows three minima, E, H/F and E* as well as their mirror image with respect to the (0°, 0°) origin. The global minimum is positioned in the region of extended conformations. The corresponding conformer E (ϕ, ψ = –120°, 123°) is stabilised by the intramolecular conventional N–H···O hydrogen bond closing a five-membered ring, with a geometry far away from the optimal hydrogen-bond parameters (Steiner, 2002), and by two weaker C–H···O hydrogen bonds. The remaining conformers contain no conventional N–H···O hydrogen bond. There are only unconventional interactions such as the weaker C–H···O hydrogen bonds as well as non-covalent attractions between two $C^{\delta+} = O^{\delta-}$ dipoles not mediated by a hydrogen atom. Recent reports indicate such interactions, however, as important factors in stabilising energy-minimised structures (Kim &

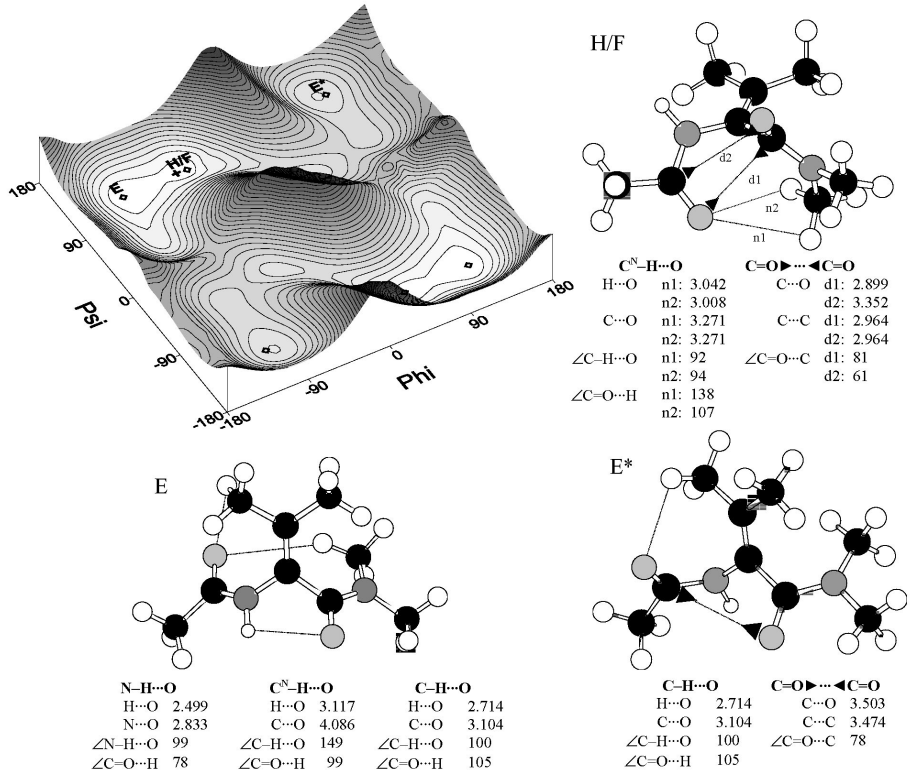

Figure 2. Landscape representation of the ϕ, ψ potential energy surface of the free Ac-ΔVal-NMe$_2$ molecule[a] along with the minima found on this surface and the corresponding three conformers (E, H/F and E*)[b] stabilized by the enumerated hydrogen bonds (·····) and dipole interactions (▶···◀). + – Solid state conformer.

[a]The potential energy surface calculated at the *ab initio* HF/6–31G*//HF/3–21G level of theory (energy contours are drawn every 1 kcal · mol^{-1}). [b]The conformers were optimized at the DFT/B3LYP/6–31+G** level of theory. Geometric parameters of (·····) hydrogen bond and (▶···◀) dipole attraction are presented below each conformer. The structural parameters for the internal X–H···A interactions presented only for the contacts (X = N, C; A = O, C) with H···A ≤ 3.2 Å and ∠X–H···A > 90° acc. to Steiner (2002). The structural parameters for the internal C=O▶···◀C=O contacts presented only for the distances C···O ≤ 3.6 Å acc. to Allen *et al.* (1998). Distances are in Å, angles are in degrees.

Friesner, 1997; Vargas *et al.*, 2000; 2001; Maccallum *et al.*, 1995; Allen *et al.*, 1998). The conformer H/F (ϕ, ψ = –49°, 124°), second in the energy order, is stabilised by two chelating intramolecular C–H···O hydrogen bonds and a pair of slightly sheared antiparallel dipole C=O▶···◀C=O attractions (Allen *et al.*, 1998). All these contacts are relatively strong and this is also proved by the solid state conformation (Fig. 1a). On the other hand, the relatively weaker N–H···O hydrogen bond in conformer E, makes the gap in energy $\Delta E_{H/F-E}$ very small, 0.68 kcal · mol^{-1}. The H/F conformer is advantageous at the (i + 1)th position for the β-turn II promotion. The highest-energy conformer E* (ϕ, ψ = 112°, 136°) is stabilised by one C–H···O hydrogen bond and one sheared parallel dipole C=O▶···◀C=O interaction.

All conformers show some departure from planarity of the C-terminal tertiary amide bond as compared to the standard peptide bond (MacArthur & Thornton, 1996). This deviation can be fully described by the twisting parameter τ and the out-of-plane parameters χ_C and χ_N (Winkler & Dunitz, 1971). The twisting parameter τ is influenced by the steric repulsion between the CH_3 groups on the C^β and N atoms, and the higher the value of the torsion angle ψ, the higher the value of

τ. The out-of-plane parameters χ_C and χ_N reflect not only steric repulsion, but also internal attractions. The highest values of these parameters are observed in conformer H/F, in which the $CON(CH_3)_2$ group is involved in relatively strong contacts.

Comparing the results of calculations on Ac-ΔVal-NMe_2 with those on other Ac-ΔXaa-NMe_2 (Siodłak *et al.*, 2004a) we see the same stabilising interactions and a great impact of the unconventional ones. The lowest-energy conformers are conformers E and the second lowest ones are H/F (for Ac-(*Z*)-ΔPhe-NMe_2 with its specific N–H···π interaction, the order is reversed). However, one should notice that the energetic gap $\Delta E_{H/F-E}$ for Ac-ΔVal-NMe_2 is extremely small and the deviation from planarity – i.e. from values of 0 or 180° – of both ϕ, ψ torsion angles in these conformers is the greatest of all Ac-ΔXaa-NMe_2 molecules studied so far. In consequence, the possible π-cross conjugation, which is for ΔXaa (Thormann & Hofmann, 1998) cannot stabilise considerably the Ac-ΔVal-NMe_2 conformers, to any significant extent. Further prominent feature of the Ac-ΔVal-NMe_2 molecule is a lack of conformers with the ψ torsion angle close to 0°, which are typical of other Ac-ΔXaa-NMe_2 molecules. Only the structures with the ψ torsion angle fixed at about |130°| are found. Based on a 5.0 kcal · mol^{-1} cut-off (Zimmerman *et al.,* 1977; Herzberg & Moult, 1991) we can assume that the Ac-ΔVal-NMe_2 molecule has access to 26% of the total structural space. Despite the presence of two CH_3 groups on the C^β atom – one in the *Z* and the other in the *E* position – and the above restricted ψ torsion angle, this is more than in the case of the (*Z*)-ΔAbu (21%) and even the ΔAla (24%) analogue that has no β-substituent. In contrast to ΔAla and (*Z*)-ΔPhe, for which the conformational space is focused mainly around the global minimum (E and H/F, respectively), the space of ΔVal is not focused on any distinctive minimum. The conformer E of ΔAla is stabilised by a short N–H···O hydrogen bond and π-cross conjugation. The conformer H/F of (*Z*)-ΔPhe is additionally stabilised by an N–H···π interaction. The flatness of the available conformational space of ΔVal shows that the forces stabilising its conformers E and H/F are of similar magnitude and only moderately influenced by the change of the ϕ, ψ torsion angles.

Theoretical conformational analysis of Ac-ΔVal-NHMe (Thormann & Hofmann, 1998) indicates B (ϕ, ψ = –81°, 54°) and H/F (ϕ, ψ = –58°, 126°) to be two lowest-energy conformers with a small energetic gap, $\Delta E_{H/F-B}$ = 0.83 kcal · mol^{-1}. The distortion from planarity of their ϕ, ψ torsion angles is the highest among the Ac-ΔXaa-NHMe molecules, thus a π-cross conjugation cannot be expected either. However, unlike the dimethyl analogue, energy-minimised conformers with the ψ torsion angle different from about |130°| exist as well.

Table 3. Selected conformational parameters (degrees) of the Ac-ΔVal-NMe_2 molecule in the solid state and all its energy-minimised conformers

Ac-ΔVal-NMe_2	Conformer[a]	Energy (Hartrees)	Δ Energy (kcal · mol^{-1})	ϕ	ψ	χ_C[b]	χ_N[b]	τ[b]
Crystal structure	H/F		–	-60.2	125.4	-2.3	-3.7	170.9
B3LYP/6-31+G**	E	-612.605720700	0.00	-120.1	122.6	1.7	-11.9	171.4
	H/F	-612.604630400	0.68	-48.6	124.2	-2.8	-19.7	171.0
	E*	-612.600351364	3.37	111.8	136.1	1.2	-15.8	167.9

[a]Energy regions of the (ϕ,ψ) conformational map are denoted in terms of the short-hand letter notation introduced by Zimmerman *et al.* (1977). [b]Amide bond deformation parameters introduced by Winkler & Dunitz (1971).

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CONCLUSION

Only three examples of peptide crystal structures containing ΔVal: Ac-Pro-ΔVal-NHMe (Ciszak *et al.*, 1992), Z-ΔVal-Trp-OMe (Vijayaraghavan *et al.*, 2001) and Z-ΔVal-Val-ΔPhe-Ile-OMe (Makker *et al.*, 2003) are known and only one paper deals with energy-minimised conformers of For/Ac-ΔVal-NHMe/NH_2 (Thormann & Hofmann, 1998). This work presents both X-ray analysis of the Ac-ΔVal-NMe_2 crystal and theoretical conformational analysis on the free molecule of Ac-ΔVal-NMe_2 as compared to Ac-ΔVal-NHMe and other related molecules.

The calculations on both dehydrovaline models, i.e. mono- and dimethylamide, make it possible to infer that the conformer H/F, which, when located at the (i + 1)th position of a β-turn promotes its βII-type, can be very readily attained by the ΔVal residue in both molecules. However, the ΔVal residue combined with a C-terminal tertiary amide seems to be a better β-turn II inducer, as this kind of residues should have no other choice for the angle ψ than about $|130°|$, whereas the ΔVal residue with the typical C-terminal peptide bond experiences less restriction on the ϕ, ψ angles. This conclusion is supported by the known crystal structures of ΔVal peptides. Moreover, because for this kind of residues the *cis*-form peptide bond is easily accessible, such a residue at the (i + 1)th position should be also suitable for promotion of β-turn VIa (Richardson, 1981). Clearly, however, many more conformational studies, in both solid state and in solution, need to be carried out to elaborate the rules of peptide design with the ΔVal residue contribution.

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