Comparative conformational analysis of peptides based on the two C^{α}-tetrasubstituted, C^{β}-branched, chiral α-amino acids (αMe)Dip and (αMe)Val †

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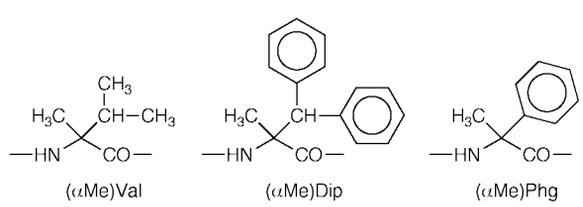
For the first time a number of terminally protected model peptides (to the pentamer level) of the sterically demanding α-amino acid C^{α}-methyl,C^{α}-diphenylmethylglycine, (αMe)Dip, in combination with either Ala or Gly residues, have been synthesized (by solution methods) and fully characterized. In a parallel synthesis the corresponding peptides based on the related α-amino acid C^{α}-methyl,C^{α}-isopropylglycine, (αMe)Val, have also been prepared. The results of a comparative conformational analysis, performed by using FTIR absorption, ^{1}H NMR, and X-ray diffraction techniques, favour the conclusion that, in contrast to the potent β-turn and 3_{10}-helix promoter (αMe)Val, (αMe)Dip may induce either a folded or a fully extended conformation. These findings indicate that, despite the common C^{α}-methylated and C^{β}-branched features, (αMe)Dip and (αMe)Val are characterized by partially divergent conformational bias.

Introduction

The stabilization of specific peptide structural motifs (*e.g.* turns, helices, sheets) has recently become a major issue in bioorganic chemistry. Short peptides with appropriate constraints on their conformational freedom can be used as: (i) precise molecular rulers or rigid scaffolding units in the *de novo* design of protein and enzyme mimetics and in the investigation of molecular/chiral recognition processes,[1–6] and (ii) building blocks for the synthesis of enzyme-resistant agonists and antagonists of bioactive peptides.[7,8] To this end one of the most effective strategies exploited for the stabilization of β-turns[9–11] and 3_{10}-/α-helical[12] conformations is C^{α}-methylation of the peptide main chain.[3,13,14] Within the class of C^{α}-methylated α-amino acids, the structural preferences of those with a linear side chain [Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine),[3,14] Iva (isovaline or C^{α}-methyl,C^{α}-ethylglycine),[15] (αMe)Aoc (C^{α}-methyl,C^{α}-*n*-hexylglycine),[16] and (αMe)Aun (C^{α}-methyl,C^{α}-*n*-nonylglycine)[17]], a γ-branched side chain [(αMe)Leu (C^{α}-methyl,C^{α}-isobutylglycine),[15] (αMe)-Phe (C^{α}-methyl,C^{α}-benzylglycine),[15] (αMe)Trp (C^{α}-methyl,C^{α}-indol-3-ylmethylglycine)[18]], and a δ-branched side chain [(αMe)Hph, C^{α}-methyl,C^{α}-phenylethylglycine][19,20] have already been described in detail.

Among the C^{α}-methylated α-amino acids with a *β-branched* side chain (αMe)Val (C^{α}-methyl,C^{α}-isopropylglycine)[21–24] and (αMe)Phg (C^{α}-methyl,C^{α}-phenylglycine)[25] have been extensively investigated. It was found that, while the C^{β}-*tri*substituted, *aliphatic* (αMe)Val is an extremely efficient β-turn and 3_{10}-helix former, the *aromatic* (αMe)Phg can induce either folded or fully-extended (C_5)[10,26] conformations.



To gain a better understanding of the preferred conformation of this sub-class of C^{α}-methylated α-amino acids we embarked on a program directed toward the first preparation and conformational characterization of peptides based on (αMe)Dip (C^{α}-methyl,C^{α}-diphenylmethylglycine)[27] characterized by a C^{β}-*tri*substituted, *aromatic* side chain. In particular, in this paper we describe the synthesis and a detailed conformational analysis in solution, using FTIR absorption and ^{1}H NMR techniques, of two terminally protected (αMe)Dip model peptide series (to the pentamer level) in combination with either an Ala or a Gly residue. These two protein amino acids are known to be easily accommodated in turns/helices (Ala), and either in turns or in extended conformations (Gly). For an optimal comparative analysis we have also prepared and

† Experimental procedures for the synthesis of the new derivatives and peptides are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p2/a9/a909856i. See Instructions for Authors available *via* the RSC web page (http://www.rsc.org/authors).

The NMR spectra of the new derivatives and peptides are available as supplementary data from BLDSC (SUPPL. NO. 57694, pp. 16) or the RSC Library. See Instructions for Authors available *via* the RSC web page (http://www.rsc.org/authors).

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investigated the conformational preferences of the two series of Ala and Gly peptides in which (αMe)Dip has been replaced by (αMe)Val.

A conformational study on the (αMe)Dip residue by means of computational methods at the molecular mechanics level has recently been published.[28] The X-ray diffraction analysis of an (αMe)Dip cyclic derivative (hydantoin) has been reported.[29]

Experimental

FTIR absorption spectra

FTIR absorption spectra were recorded using a Perkin-Elmer model 1720X FTIR spectrophotometer (Norwalk, CT) nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde [^{2}H]chloroform (99.8% ^{2}H) was purchased from Merck (Darmstadt, Germany).

^{1}H NMR spectra

^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer (Karlsruhe, Germany). Measurements were carried out in [^{2}H]chloroform (99.96 ^{2}H; Merck) and in [$^2H_6$]DMSO ([$^2H_6$]dimethyl sulfoxide) (99.96 $^2H_6$; Fluka, Buchs, Switzerland) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethylpiperidine-*N*-oxyl) was purchased from Sigma (Milwaukee, WI). The range of TEMPO concentration was 1.5–25 × 10^{-3} mol dm^{-3}.

Crystallographic data for the oxazol-5(4*H*)-one from Z-D,L-(αMe)Dip-OH §

$C_{24}H_{21}NO_3$, $M = 371.4$. Triclinic, $a = 10.316(2)$, $b = 10.864(2)$, $c = 11.577(2)$ Å, $\alpha = 116.2(1)$, $\beta = 113.5(1)$, $\gamma = 93.7(1)°$, $V = 1021(2)$ Å^3, space group $P\bar{1}$, $Z = 2$, $D_c = 1.208$ g cm^{-3}, $F(000) = 392$, $\mu = 0.074$ mm^{-1} (Mo-Kα), final R value 0.053.

Crystallographic data for Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe methanol solvate ¶

$C_{31}H_{51}N_5O_9$. $M = 637.8$. Monoclinic, $a = 10.080(2)$, $b = 17.853(3)$, $c = 10.185(2)$ Å, $\alpha = 90$, $\beta = 107.8(1)$, $\gamma = 90°$, $V = 1745.1(6)$ Å^3, space group $P2_1$, $Z = 2$, $D_c = 1.214$ g cm^{-3}, $F(000) = 688$, $\mu = 0.736$ mm^{-1} (Cu-Kα), final R value 0.035.

X-Ray crystal structure determinations

Colourless crystals (0.6 × 0.4 × 0.2 mm and 0.35 × 0.35 × 0.2 mm, respectively) of the oxazol-5(4*H*)-one and the pentapeptide were grown by slow evaporation of a methanol solution and a methanol–water solvent mixture, respectively. The two structures were solved by direct methods, using the SHELXS 86[30] program. Refinement for the oxazolone was performed using the SHELX 76[31] program, while that for the pentapeptide was performed using the SHELXL 93[32] program.

Fractional atomic coordinates, tables of hydrogen atom coordinates, thermal parameters, bond lengths, bond angles, and torsion angles for the oxazolone and the pentapeptide are available from the Cambridge Crystallographic Data Centre.

Results and discussion

Synthesis and characterization

A strategy of highly stereoselective enolate trapping of lithium (1*S*,2*R*,4*R*)-10-dicyclohexylsulfamoylisobornyl-2-cyano-3,3-diphenylpropanoate with methyl iodide, combined with the appropriate rearrangement process, has allowed the asymmetric synthesis of D-(αMe)Dip.[27] For the large scale production of the enantiomerically pure L-(αMe)Val we exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by the DSM Research group a few years ago.[33–35] It involves a combination of organic synthesis for the preparation of the racemic α-amino amide followed by the use of a broadly specific α-amino amidase to achieve optical resolution.

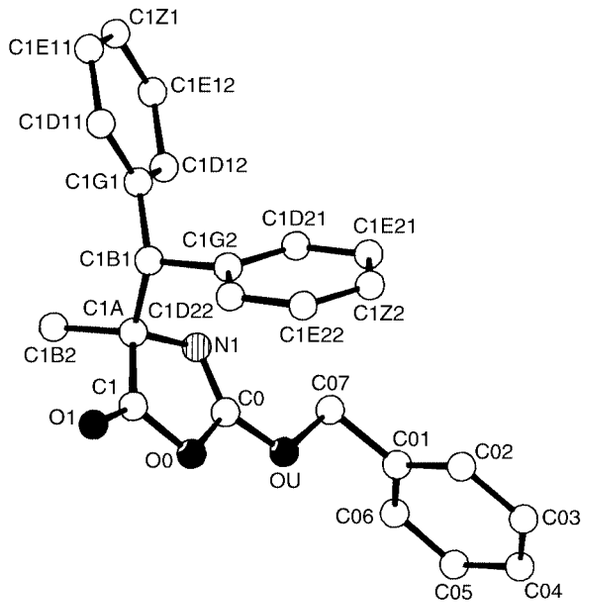

Fig. 1 X-Ray diffraction structure of the oxazol-5(4*H*)-one from Z-D,L-(αMe)Dip-OH. Only the D-enantiomer is shown.

The synthesis and characterization of ten (αMe)Dip and four (αMe)Val new peptides (to the pentamer level) were performed (Table 1). The synthesis and characterization of Z-L-(αMe)Val-OH, and the L-(αMe)Val/L-Ala and L-(αMe)Val/Gly di- and tripeptides have already been reported.[21] The benzyloxycarbonyl (Z) N$^{\alpha}$-protected (αMe)Dip was obtained by reacting the free amino acid with Z-OSu (OSu, 1-oxysuccinimido)[36] in a 1,4-dioxane–alkaline aqueous solvent mixture. The oxazol-5(4*H*)-one from Z-D,L-(αMe)Dip-OH was prepared by treating the N$^{\alpha}$-protected amino acid with EDC [*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide]. During peptide bond formation involving the sterically hindered (αMe)Dip and (αMe)Val residues the carboxy group of the N$^{\alpha}$-protected amino acid or dipeptide was activated using the highly efficient EDC–HOAt (1-hydroxy-7-aza-1,2,3-benzotriazole) method.[37] Optimization of the reaction yields was not attempted. The tripeptide Z-D,L-(αMe)Dip-(D-Ala)$_2$-OMe was prepared as a diastereomeric mixture using the racemic Z-D,L-(αMe)Dip-OH. Table 1 lists the physical properties and analytical data for the major (more retained) diastereomer purified by HPLC, the (αMe)Dip C$^{\alpha}$-configuration of which is undetermined. Removal of the Z N$^{\alpha}$-protecting group was performed by catalytic hydrogenation. *tert*-Butyl (Bu^t) ester formation was achieved by H_2SO_4-catalysed reaction of the corresponding Z-protected amino acid with 2-methylpropene, while methyl (Me) ester formation was achieved by treatment of the free amino acid with a $SOCl_2$–MeOH mixture.[38]

The newly synthesized derivatives and peptides were characterized by melting point determination, optical rotatory power, TLC (in three solvent systems), solid-state IR absorption spectroscopy and ^{1}H NMR (the latter data are not reported †). The crystalline oxazol-5(4*H*)-one from Z-D,L-(αMe)Dip-OH was also characterized by X-ray diffraction (Fig. 1). The bond lengths and bond angles for the oxazol-5(4*H*)-one ring agree well with the corresponding mean values published in a survey

§ CCDC reference number 188/226.
¶ CCDC reference number 188/226. See http://www.rsc.org/suppdata/p2/a9/a909856i for crystallographic files in .cif format.

Table 1 Physical properties and analytical data for the compounds synthesized in this work

Compound	Yield (%)	Mp/°C[b]	Recryst. solvent[c]	$[\alpha]_D^{20}$[d]	TLC[e] R_f(I)	R_f(II)	R_f(III)	IR (KBr) ν/cm^{-1}[f]
(*a*) *Derivatives*								
Z-D-(αMe)Dip-OH	60	82–83	EtOAc–LP	14.1	0.80	0.95	0.45	3412, 1704
Oxazol-5(4*H*)-one from Z-D,L-(αMe)-Dip-OH	72	99–101	EtOAc–LP	—	0.90	—	0.85	1826, 1694
(*b*) *(αMe)Dip/Ala and (αMe)Val/Ala peptides*								
Z-D-(αMe)Dip-D-Ala-OBu^t	95	Oil	EtOAc–LP	41.4	0.95	0.95	0.80	3401, 1730, 1666[g]
Z-D-Ala-D-(αMe)Dip-D-Ala-OBu^t	62	142–143	EtOAc–LP	117.8	0.90	0.95	0.50	3359, 1733, 1700, 1677, 1622, 1525
Z-(αMe)Dip[a]-(D-Ala)$_2$-OMe	45	72–73	EtOAc–LP	25.4	0.90	0.95	0.50	3414, 3362, 1732, 1675, 1647
Z-(D-Ala)$_2$-D-(αMe)Dip-D-Ala-OBu^t	80	148–149	DE–LP	74.6	0.85	0.95	0.50	3417, 3364, 1719, 1672, 1654
Z-D-(αMe)Dip-(D-Ala)$_2$-D-(αMe)Dip-D-Ala-OBu^t	40	127–128	$CHCl_3$–LP	42.0	0.85	0.95	0.50	3348, 1724, 1701, 1669, 1653
Z-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe	81	Oil	$CHCl_3$–LP	−26.3	0.70	0.90	0.35	3321, 1742, 1703, 1660, 1535[g]
Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe	86	168–169	EtOAc–LP	−21.0	0.50	0.90	0.20	3431, 3413, 3318, 1737, 1694, 1675, 1662, 1533
(*c*) *(αMe)Dip/Gly and (αMe)Val/Gly peptides*								
Z-D-(αMe)Dip-Gly-OBu^t	80	Oil	EtOAc–LP	12.9	0.95	0.95	0.80	3399, 1732, 1668[g]
Z-Gly-D-(αMe)Dip-Gly-OBu^t	68	78–80	EtOAc–LP	54.8	0.80	0.95	0.40	3369, 1730, 1680
Z-D-(αMe)Dip-(Gly)$_2$-OBu^t	30	60–62	EtOAc–LP	−7.5	0.80	0.95	0.40	3407, 3342, 1707, 1658, 1650
Z-(Gly)$_2$-D-(αMe)Dip-Gly-OBu^t	62	82–84	EtOAc–LP	24.6	0.55	0.95	0.35	3339, 1726, 1668
Z-D-(αMe)Dip-(Gly)$_2$-D-(αMe)Dip-Gly-OBu^t	65	128–130	EtOAc–LP	8.1	0.75	0.95	0.40	3334, 1736, 1662
Z-(Gly)$_2$-L-(αMe)Val-Gly-OBu^t	85	131–132	EtOAc–LP	9.5	0.60	0.90	0.30	3329, 1713, 1662, 1529
Z-L-(αMe)Val-(Gly)$_2$-L-(αMe)Val-Gly-OBu^t	70	92–94	EtOAc–LP	15.0	0.50	0.85	0.25	3331, 1744, 1664, 1528

[a] Undetermined configuration at (αMe)Dip. [b] Determined on a Leitz model Laborlux apparatus (Wetzlar, Germany). [c] EtOAc, ethyl acetate; LP, light petroleum (bp 40–60 °C); DE, diethyl ether. [d] Determined on a Perkin-Elmer model 241 polarimeter (Norwalk, CT) equipped with a Haake model L thermostat (Karlsruhe, Germany); $c = 0.5$ (MeOH). [e] Silica gel plates (60F-254 Merck, Darmstadt, Germany) using the following solvent systems: (I) chloroform–ethanol 9:1; (II) butan-1-ol–acetic acid–water 6:2:2; (III) toluene–ethanol 7:1. The compounds were revealed either with the aid of a UV lamp or with the hypochlorite–starch–iodide chromatic reaction. A single spot was observed in each case. [f] Determined in KBr pellets on a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer. [g] Determined in a film.

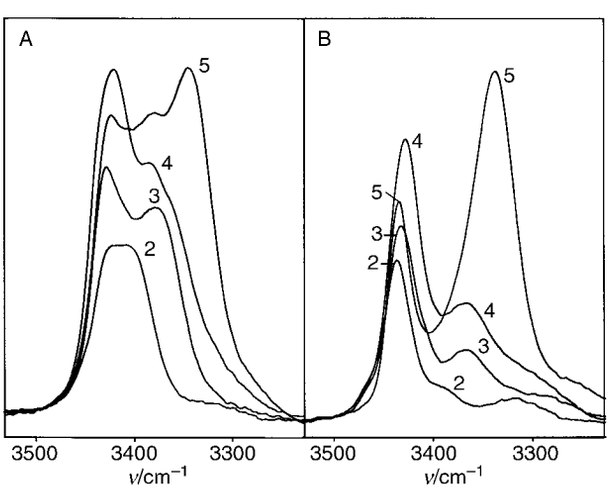

Fig. 2 FTIR absorption spectra (3500–3200 cm^{-1} region) of the terminally protected (αMe)Dip/Ala (A) and (αMe)Val/Ala (B) series from dipeptide through pentapeptide in $CDCl_3$ solution. The numbers on the curves refer to the peptide length. Peptide concentration: 1×10^{-3} mol dm^{-3}.

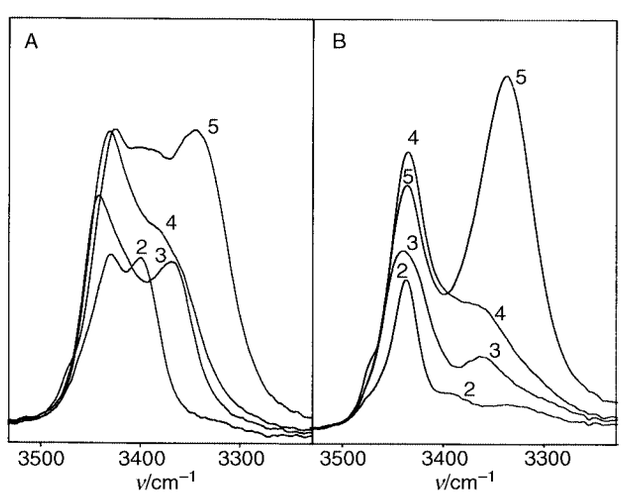

Fig. 3 FTIR absorption spectra (3500–3200 cm^{-1} region) of the terminally protected (αMe)Dip/Gly (A) and (αMe)Val/Gly (B) series from dipeptide through pentapeptide in $CDCl_3$ solution. The numbers on the curves refer to the peptide length. Peptide concentration: 1×10^{-3} mol dm^{-3}.

of crystal structures of this heterocyclic moiety.[39] The values for the $\chi^{1,1}$ (N–C1A–C1B1–C1G1) and $\chi^{1,2}$ (N–C1A–C1B1–C1G2) side-chain torsion angles[40] are −76.2(7)° and 53.7(7)°, respectively. The dihedral angle between the planes of the two phenyl rings is 110.1(2)°.

Solution conformational analysis

The preferred conformations adopted by the terminally protected (αMe)Dip/Ala (or Gly) and (αMe)Val/Ala (or Gly) peptide series were determined in the structure supporting solvent $CDCl_3$ by FTIR absorption and ^{1}H NMR techniques. Figs. 2 and 3 illustrate the FTIR absorption spectra in the informative N–H stretching region, while Figs. 4 and 5 show the ^{1}H NMR titrations of the four pentapeptides.

The FTIR absorption curves of the (αMe)Val peptides are characterized by two bands in the regions 3445–3430 cm^{-1} (free, solvated NH groups) and 3370–3330 cm^{-1} (strongly H-bonded NH groups) respectively.[41–43] The intensity of the low-frequency

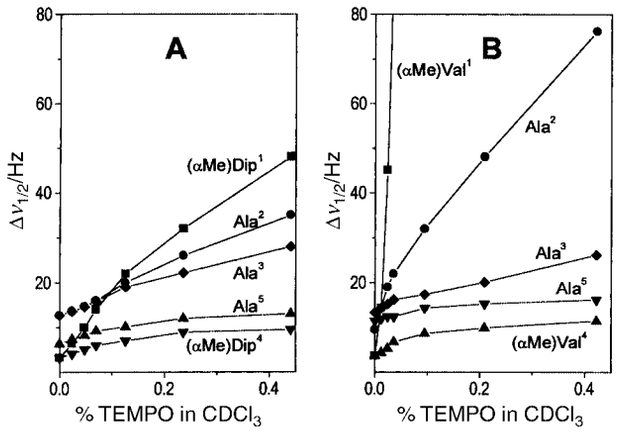

Fig. 4 Plots of the bandwidths of the NH protons in the ^{1}H NMR spectra of the terminally protected (αMe)Dip/Ala (A) and (αMe)Val/Ala (B) pentapeptides as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}.

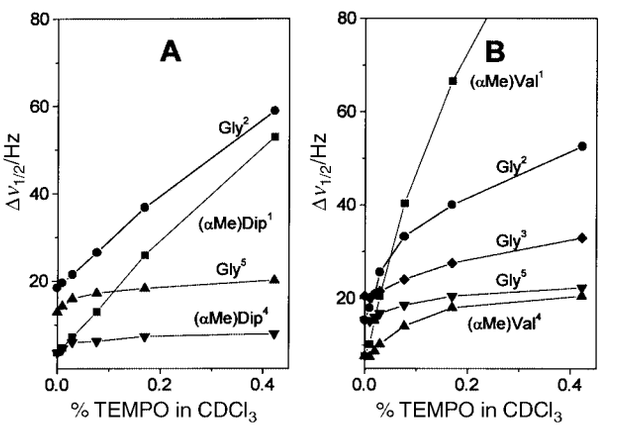

Fig. 5 Plots of the bandwidths of the NH protons in the ^{1}H NMR spectra of the terminally protected (αMe)Dip/Gly (A) and (αMe)Val/Gly (B) pentapeptides as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}. In the (αMe)Dip/Gly pentapeptide the Gly N(3)H resonance is hidden under the peaks of the aromatic protons.

band relative to the high-frequency band significantly increases as the main-chain length increases; concomitantly, the absorption maximum shifts markedly to lower wavenumbers. The most significant difference for both the Ala and the Gly series is observed between the tetra- and pentapeptides, induced by the incorporation of an additional (αMe)Val residue. In general, no appreciable differences are seen in the spectra between the Ala and the Gly series. The band related to H-bonded NH groups is almost negligible in the two dipeptides. Using Mizushima's dilution method,[44] we have been able to demonstrate that even at 1×10^{-2} mol dm^{-3} peptide concentration self-association *via* N–H···O=C intermolecular H-bonding is of minor significance for all of the (αMe)Val peptides investigated (not shown). Therefore, the observed H-bonding should be interpreted as arising almost exclusively from intramolecular N–H···O=C interactions. The remarkable intensity of the low-frequency band relative to the high-frequency band (high A_H/A_F ratio) and the position of the former in the spectrum (3340–3335 cm^{-1}) suggest the occurrence of a large population of intramolecularly H-bonded folded (helical) species for the two pentapeptides. More specifically, the observation of the 3370–3330 cm^{-1} band in the tri-, tetra-, and pentapeptides, which is almost absent in the dipeptides, confirms that the (αMe)Val peptides[21–24] do not tend to adopt a γ-turn (C_7) conformation[10,45,46] even in a solvent of low polarity and highlights their propensity to fold into a β-turn conformation (tripeptides) which may evolve in a series of consecutive β-turns (3_{10}-helices) in the longer peptides.

Conversely, the FTIR absorption spectra of both (αMe)Dip series are more complex. In addition to the two bands typical of the (αMe)Val peptides, one (or more) absorption(s) in the 3415–3380 cm^{-1} region characterize the spectra of the (αMe)Dip peptides. The relative band intensities change only slightly with dilution (in the 1×10^{-2}–1×10^{-4} mol dm^{-3} range). These findings strongly support the view that, at variance with (αMe)Val peptides, weakly H-bonded, fully-extended (C_5) species[10,26] are also highly populated in the conformational equilibria of all (αMe)Dip peptides in $CDCl_3$ solution.

To get more detailed information on the preferred conformation of the (αMe)Val and (αMe)Dip peptides in $CDCl_3$ solution we carried out a 400 MHz ^{1}H NMR investigation. The delineation of inaccessible (or intramolecularly H-bonded) NH groups by ^{1}H NMR was performed by using: (i) free-radical TEMPO-induced line broadening of NH resonances[47] and (ii) solvent dependences of NH chemical shifts by adding increasing amounts of the H-bonding acceptor DMSO to the $CDCl_3$ solution.[48,49]

With regard to the conformationally significant pentapeptides, unambiguous assignments for the NH protons have been performed *via* inspection of chemical structure [the most upfield Z-urethane N(1)H proton], analysis of their multiplicities, and ROESY experiments. From an analysis of the various spectra as a function of peptide concentration (in the 1×10^{-2}–1×10^{-3} mol dm^{-3} range) we have been able to conclude that dilution induces a modest shift (to higher fields) of all NH resonances. Interestingly, however, the most sensitive protons are the N(1)H and N(2)H protons for the (αMe)Val-based pentapeptides, compared with the N(2)H and N(3)H protons for the (αMe)Dip-based pentapeptides.

In both (αMe)Val pentapeptides examined in the presence of the paramagnetic perturbing agent TEMPO and in $CDCl_3$–DMSO solvent mixtures (the latter results are not shown) at 1×10^{-3} mol dm^{-3} peptide concentration, two classes of NH protons were observed. Class (i) [N(1)H and N(2)H protons] includes protons whose resonances broaden significantly upon addition of TEMPO and whose chemical shifts are sensitive to the addition of DMSO. In both compounds the sensitivity of the N(1)H proton is markedly higher than that of the N(2)H proton. Class (ii) [N(3)H to N(5)H protons] includes those displaying a behaviour characteristic of shielded protons (relative insensitivity of linewidths to the presence of TEMPO and of chemical shifts to solvent composition). Interestingly, the difference in the extent of perturbation induced by TEMPO and DMSO between proton classes (i) and (ii) is less significant in the two (αMe)Dip pentapeptides compared to the corresponding (αMe)Val pentapeptides.

In summary, these ^{1}H NMR results agree well with the FTIR absorption data discussed above, allowing us to conclude that in $CDCl_3$ solution in the absence of self-association the terminally protected (αMe)Val-based pentapeptides are largely folded in a 3_{10}-helical structure, while this ordered conformation is much less populated in the (αMe)Dip-based pentapeptides. In addition, these conformational biases do not seem to be dictated by the Ala and Gly residues, despite their known divergent tendencies, but rather by the different structural propensities of the C^α-tetrasubstituted (αMe)Val *versus* (αMe)Dip residues.

Crystal-state conformational analysis

We determined by X-ray diffraction the molecular and crystal structure of the pentapeptide Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)-Val-L-Ala-OMe methanol solvate. The molecular structure with the atomic numbering scheme is illustrated in Fig. 6. Relevant N-protecting group, backbone and side-chain torsion angles[40] are given in Table 2. In Table 3 the intra- and intermolecular H-bond parameters are listed. Despite a number of attempts,

we were unable to grow single crystals suitable for an X-ray diffraction analysis from any of the (αMe)Dip-based peptides.

Bond lengths and bond angles (deposited) of the (αMe)Val pentapeptide are in general agreement with previously reported values for the geometry of the benzyloxycarbonylamino moiety,[50] the methyl ester group,[51] the peptide unit[52,53] and the (αMe)Val[22–24] residue.

Both L-(αMe)Val residues are found in the helical region *A* of the conformational map.[54] Globally, the pentapeptide is folded in a right-handed 3_{10}-helical structure characterized by two 1←4 C=O···H–N intramolecular H-bonds. The N4···O1 intramolecular distance, 3.416(4) Å, is too long for an H-bond.[55–57] Interestingly, this slight distortion from a regular 3_{10}-helix involves the only dipeptide sequence of the pentamer, -Ala[2]-Ala[3]-, lacking any C^{α}-tetrasubstituted amino acid. The usual inversion of the handedness of the C-terminal helical residue with respect to that of the preceding ones[58] is also found in this 3_{10}-helical peptide ester.

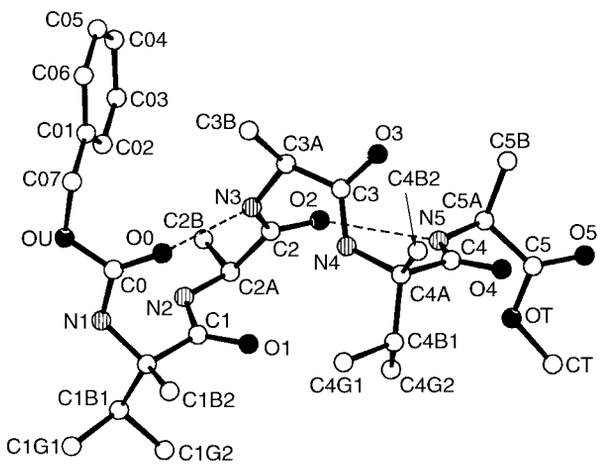

Fig. 6 X-Ray diffraction structure of Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe. The intramolecular H-bonds are indicated by dashed lines.

Table 2 Selected torsion angles (degrees)[40] for Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe methanol solvate

N-Protecting group		Backbone		Side chains	
θ^3	−13.6(4)	φ_1	−52.9(4)	$\chi_1^{1,1}$	−68.2(3)
θ^2	86.5(4)	ψ_1	−44.4(3)	$\chi_1^{1,2}$	166.7(3)
θ^1	−177.4(3)	ω_1	−173.6(3)	$\chi_4^{1,1}$	−58.9(5)
ω_0	−177.3(3)	φ_2	−65.9(4)	$\chi_4^{1,2}$	70.7(4)
		ψ_2	−21.5(4)		
		ω_2	−174.6(3)		
		φ_3	−69.8(4)		
		ψ_3	−13.4(4)		
		ω_3	172.2(3)		
		φ_4	−62.6(4)		
		ψ_4	−17.4(4)		
		ω_4	173.1(3)		
		φ_5	52.3(4)		
		ψ_5[a]	53.4(4)		
		ω_5[b]	174.3(3)		

[a] N5–C5A–C5–OT. [b] C5A–C5–OT–CT.

All urethane,[50] peptide[52,53] and ester[51] groups are *trans* (ω torsion angles) with no deviation >8° from planarity. The conformation of the Z-urethane group is the usual *trans*,*trans* (θ^1 and ω_0 torsion angles) or type-*b* conformation.[50] Also the values of the θ^2 and θ^3 torsion angles are typical of the Z-urethane group. The methyl ester group adopts a conformation with respect to the C5A–N5 bond between the *antiplanar* and *anticlinal* conformations,[59] the N5–C5A–C5–O5 torsion angle being −132.2(4)°. The conformation of the L-(αMe)Val[1] isopropyl side chain ($\chi_1^{1,1}$ and $\chi_1^{1,2}$ torsion angles) is the common (*t*, g^-) conformation;[22–24,60] however, this disposition is (g^+, g^-) in L-(αMe)Val[4].

In the crystals of the terminally protected pentapeptide the molecules are held together in the *x*,*z* plane through head-to-tail intermolecular H-bonds involving the (urethane) N1–H and (peptide) N2–H groups as donors and the (peptide) O4=C4 and (ester) O5=C5 groups as acceptors, respectively, of symmetry related molecules. The methanol molecule plays the role of the donor of the H-bond to the (peptide) C3=O3 group within the same asymmetric unit. The O–H···O H-bond is of normal strength.[61,62]

Conclusions

In this work we have been able to synthesize step-by-step by solution methods model peptides based on the sterically demanding (αMe)Dip residue. For a comparative conformational analysis the analogous peptides with (αMe)Dip→(αMe)Val replacement(s) have also been prepared. The present detailed study not only confirms[21–24] that the C^{β}-*tris*substituted, *aliphatic* (αMe)Val residue is a strong β-turn and 3_{10}-helix former, but it additionally strongly supports the view that the C^{β}-*tris*substituted, *aromatic* (αMe)Dip residue, investigated for the first time, can either fold in turns/helices or adopt a fully-extended conformation. The conclusions obtained in this experimental study for (αMe)Dip agree well with those recently extracted from a theoretical conformational investigation,[28] in the sense that (α/3_{10})helical conformations are the most stable structures for this residue *in vacuo*. However, from these computations it was also concluded that extended structures are significantly destabilized for (αMe)Dip.

It is also noteworthy that the experimental conformational preferences of (αMe)Dip described here closely match those previously found for (αMe)Phg, also an *aromatic* residue.[25] Taken together, all these results favour the conclusion that it is the aromatic character or its related steric hindrance, not the degree of the common C^{β}-substitution, that governs the struc-

Table 3 Intra- and intermolecular H-bond parameters for Z-L-(αMe)Val-(L-Ala)$_2$-L-(αMe)Val-L-Ala-OMe methanol solvate

Donor D–H	Acceptor A	Symmetry operations of A	Distance/Å D···A	Distance/Å H···A	Angle/degrees D···A=C
Intramolecular H-bonds					
N3–H	O0	*x*, *y*, *z*	3.015(4)	2.228(4)	151.9(3)
[a]N4–H	O1	*x*, *y*, *z*	3.416(4)	2.574(4)	166.4(3)
N5–H	O2	*x*, *y*, *z*	3.101(4)	2.262(4)	164.3(3)
Intermolecular H-bonds					
N1–H	O4	1 + *x*, *y*, 1 + *z*	2.858(4)	2.029(4)	161.6(3)
N2–H	O5	1 + *x*, *y*, 1 + *z*	3.116(4)	2.413(4)	139.3(3)
[b]O_M–H	O3	*x*, *y*, *z*	2.776(5)	2.016(6)	153.8(5)

[a] Too long to be considered a (peptide) N–H···O=C (peptide) H-bond. [b] The donor is the solvent (methanol).

tural bias of this sub-class of C^{α}-methylated α-amino acids. A future study from our laboratories will try to dissect the relative roles of aromaticity *versus* steric requirements by investigating the structural preferences of peptides characterized by the fully hydrogenated side-chain derivatives from (αMe)Dip and (αMe)Phg residues.

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