Linear oligopeptides. Part 406.[1] Helical screw sense of peptide molecules: the pentapeptide system $(Aib)_4$/L-Val[L-(αMe)Val] in solution

PERKIN 2

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A variety of N^{α}-blocked pentapeptide esters, each containing four helicogenic, achiral α-aminoisobutyric acid residues and one chiral L-valine or C^{α}-methyl-L-valine residue in the N-terminal, internal or C-terminal position of the sequence, have been synthesized by solution methods and fully characterized. The results of a solution conformational analysis, performed by using FTIR absorption and ^{1}H NMR techniques, favour the conclusion that all of the pentapeptides examined fold into a 3_{10}-helical structure. In addition, a CD study of the N^{α}-*para*-bromobenzoylated peptides strongly supports the view that the prevailing screw sense of the 3_{10}-helical structure that is formed is strongly dependent upon the position in the sequence of the single chiral C^{α}-tri- or C^{α}-tetrasubstituted α-amino acid.

Introduction

A project is currently underway in our laboratories aimed at understanding the factors governing the helical screw sense of peptide molecules. In particular, the exploitation of peptides based on the highly helicogenic α-aminoisobutyric acid (Aib), the prototype of C^{α}-tetrasubstituted α-amino acids, is extremely advantageous as in this case a rather stable helical structure may be easily formed in solvents of relatively low polarity at very short main chain lengths, *e.g.* with about five amino acid residues.[2,3] This ordered peptide secondary structure is termed 3_{10}-helix,[4] the helical parameters of which are very close to those of the classical α-helix. However, the intramolecular C=O · · · H–N hydrogen bonding schemes are significantly different in the two types of helical structure, being of the 1←4 type or type-III (III′) β-bend[5] in the 3_{10}-helix, while of the 1←5 type or α-bend[6] in the α-helix.

Since Aib is an achiral residue, the helices adopted by its homo-oligomers do not exhibit a screw sense bias, the right- and left-handed forms being enantiomeric and hence isoenergetic and equally probable. However, if a chiral guest amino acid is incorporated in a host $(Aib)_n$ sequence, the resulting 3_{10}-helix is expected to exhibit a more or less markedly preferred screw sense, which will obviously be dependent on the absolute configuration and nature of the chiral residue. In the present work we have addressed the question of the effect induced in solution by an *additional* parameter, namely the *position* in the main chain of the chiral guest residue. As for the host sequence, we have designed an N^{α}-blocked, Aib-based, helical pentapeptide ester. As a guest residue, we have selected L-Val (a C^{α}-trisubstituted, protein α-amino acid) as well as its C^{α}-methylated counterpart, L-(αMe)Val (a strong helicogenic, C^{α}-tetrasubstituted α-amino acid).[7] These two amino acids have been inserted either at the N-terminus (residue 1), or in an internal position (residue 3), or at the C-terminus (residue 5) of the pentapeptide main chain.

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 [$^{2}H_6$]DMSO ([$^{2}H_6$]dimethyl sulfoxide) (99.96 $^{2}H_6$; 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}.

CD spectra

CD spectra were recorded using a Jasco model J-600 spectro-

Table 1 Physical properties and analytical data for the newly synthesized peptides described in this work

Compound	Yield (%)	Mp/°C [a]	Recryst. solvent [b]	$[\alpha]_D^{20}$ [c]	TLC [d] R_f(I)	R_f(II)	R_f(III)	ν/cm^{-1} [e]
Z-(Aib)$_4$-L-Val-OBu^t	70	215–216	EtOAc	−42.5	0.75	0.95	0.25	3364, 3313, 1704, 1689, 1682, 1671, 1644, 1531
Bz-(Aib)$_4$-L-Val-OBu^t	77	233–234	EtOAc–LP	−38.1	0.70	0.95	0.20	3306, 1725, 1650, 1579, 1532
*p*BrBz-(Aib)$_4$-L-Val-OBu^t	86	228–229	MeCN	−38.1	0.80	0.95	0.25	3360, 3307, 1714, 1673, 1636, 1587, 1564, 1531
*p*IBz-(Aib)$_4$-L-Val-OBu^t	87	235–236	MeCN	−35.3	0.55	0.95	0.25	3437, 3315, 1713, 1671, 1587, 1530
*p*NO$_2$Bz-(Aib)$_4$-L-Val-OBu^t	90	225–226	MeCN	−37.1	0.50	0.95	0.20	3356, 3317, 1712, 1688, 1528
*p*MeOBz-(Aib)$_4$-L-Val-OBu^t	85	215–216	MeCN	−40.0	0.60	0.95	0.20	3437, 3370, 3329, 3302, 1714, 1666, 1636, 1574, 1533
*p*DMABz-(Aib)$_4$-L-Val-OBu^t	90	234–235	MeCN	−38.7	0.80	0.90	0.25	3362, 3330, 3301, 1711, 1667, 1650, 1622, 1525
Z-(Aib)$_4$-L-(αMe)Val-OBu^t	85	214–215	EtOAc–LP	−21.3	0.80	0.95	0.25	3431, 3329, 1703, 1670, 1530
Bz-(Aib)$_4$-L-(αMe)Val-OBu^t	88	245–246	CHCl$_3$–LP	−16.7	0.65	0.95	0.20	3423, 3298, 1732, 1722, 1658, 1644, 1579, 1534
*p*BrBz-(Aib)$_4$-L-(αMe)Val-OBu^t	78	227–228	MeCN	−16.9	0.75	0.95	0.20	3421, 3328, 1727, 1669, 1641, 1588, 1527
*p*IBz-(Aib)$_4$-L-(αMe)Val-OBu^t	89	224–225	EtOAc–LP	−36.9	0.50	0.95	0.25	3323, 1729, 1655, 1589, 1559, 1530
*p*NO$_2$Bz-(Aib)$_4$-L-(αMe)Val-OBu^t	86	250–251	MeCN	−23.2	0.45	0.95	0.20	3424, 3336, 1730, 1669, 1528
*p*MeOBz-(Aib)$_4$-L-(αMe)Val-OBu^t	84	222–223	EtOAc–LP	−38.2	0.60	0.95	0.20	3438, 3320, 1730, 1658, 1641, 1574, 1534
*p*DMABz-(Aib)$_4$-L-(αMe)Val-OBu^t	83	221–222	EtOAc–LP	−22.1	0.75	0.90	0.25	3434, 3376, 3351, 3330, 3302, 1710, 1688, 1625, 1522
Z-L-Val-(Aib)$_4$-OBu^t	74	181–182	EtOAc–LP	−25.1	0.65	0.95	0.25	3327, 1702, 1667, 1531
*p*BrBz-L-Val-(Aib)$_4$-OBu^t	88	235–236	EtOAc–LP	−32.7	0.65	0.95	0.30	3389, 3345, 1738, 1682, 1672, 1643, 1588, 1542, 1523
*p*IBz-L-Val-(Aib)$_4$-OBu^t	88	231–232	EtOAc–LP	−32.3	0.40	0.95	0.30	3345, 1735, 1645, 1586, 1538
Z-L-(αMe)Val-(Aib)$_4$-OBu^t	55	194–195	EtOAc–LP	31.8	0.70	0.95	0.30	3433, 3330, 1733, 1666, 1640, 1532
*p*BrBz-L-(αMe)Val-(Aib)$_4$-OBu^t	77	238–239	EtOAc–LP	28.6	0.60	0.95	0.25	3417, 3329, 1724, 1668, 1639, 1589, 1566, 1525
Z-L-Val-(Aib)$_2$-OBu^t	88	98–99	EtOAc–LP	−3.0	0.95	0.95	0.40	3325, 1729, 1703, 1657, 1529
*p*BrBz-(Aib)$_2$-L-Val-(Aib)$_2$-OBu^t	53	227–228	EtOAc–LP	31.4	0.50	0.95	0.20	3417, 3326, 1723, 1665, 1639, 1587, 1564, 1527

[a] Determined on a Leitz model Laborlux 12 apparatus (Wetzlar, Germany). [b] EtOAc, ethyl acetate, LP, light petroleum (bp 40–60 °C), MeCN, acetonitrile. [c] Determined on a Perkin-Elmer model 241 polarimeter (Norwalk, CT) equipped with a Haake model L thermostat (Karlsruhe, Germany); $c = 0.5$ (MeOH). [d] 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. [e] 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.

polarimeter (Tokyo, Japan) equipped with a Haake thermostat (Karlsruhe, Germany). Cylindrical, fused quartz cells of 0.2 mm path lengths were employed. The data are expressed in terms of $[\theta]_M$, the total molar ellipticity (deg cm^2 dmol^{-1}). Methanol (C. Erba, Rodano, Milan, Italy) was used as solvent.

Results and discussion

Synthesis and characterization

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 group a few years ago.[8,9] It involves a combination of organic synthesis for the preparation of the racemic α-amino acid amide followed by the use of a broadly specific amino acid amidase to achieve optical resolution.

The synthesis and characterization of eleven new L-Val and nine L-(αMe)Val pentapeptides were performed. Z-(Aib)$_4$-L-Val-OBu^t and Z-(Aib)$_4$-L-(αMe)Val-OBu^t were prepared using the oxazol-5(4*H*)-one from Z-(Aib)$_4$-OH.[10] Z-L-Val-(Aib)$_4$-OBu^t was synthesized from Z-L-Val-OH using the mixed anhydride method with isobutyl chloroformate, and Z-L-(αMe)Val-(Aib)$_4$-OBu^t *via* the symmetrical anhydride method with [Z-L-(αMe)Val]$_2$O.[7] Removal of the benzyloxycarbonyl N^α-protecting group was achieved by catalytic hydrogenation.

Incorporation of the benzoyl (Bz) group was obtained by treatment of the N^α-deprotected pentapeptide with benzoic anhydride. On the other hand, incorporation of the *para*-substituted Bz groups was achieved using the corresponding 1,2,3-benzotriazol-1-oxyl derivatives.[11–13] The synthesis of the pentapeptide with the L-Val residue in the central position was carried out from H-L-Val-(Aib)$_2$-OBu^t and the oxazol-5(4*H*)-one from *p*BrBz-(Aib)$_2$-OH.[14] The synthesis and characterization of the N^α-*para*-bromobenzoylated pentapeptide with a central L-(αMe)Val residue have already been reported.[7]

The various peptides and their synthetic intermediates were characterized (Table 1) by melting point determination, optical rotatory power, TLC (in three solvent systems), solid-state IR absorption spectroscopy and ^{1}H NMR spectrometry (the latter data are not reported).

Solution conformational analysis

The conformational preferences adopted by the terminally blocked (Aib)$_4$/L-Val and (Aib)$_4$/L-(αMe)Val peptides were ascertained in the structure supporting solvents CDCl$_3$ (by FTIR absorption and ^{1}H NMR techniques) and MeOH (by CD spectroscopy). Fig. 1 illustrates the FTIR absorption spectra (N–H stretching region) and Figs. 2 and 3 the ^{1}H NMR data of six selected pentapeptides. The C=O···H–N intramolecular hydrogen-bonding pattern characterizing a 3_{10}-helical, N^α-acylated pentapeptide sequence is reported in Fig. 4. Fig. 5 shows CD spectra of the same six N^α-*para*-bromobenzoylated pentapeptide sequences. The relevant parameters for the CD and UV absorption spectra of the N^α-benzoyl and N^α-*para*-substituted benzoyl-peptides are listed in Table 2.

The FTIR absorption curves are characterized by bands at 3454–3431 cm^{-1} (free, solvated NH groups) and at 3357–3347 cm^{-1} (strongly hydrogen-bonded NH groups)[15–17] (Fig. 1). No appreciable differences are seen in the spectra between 1×10^{-3}

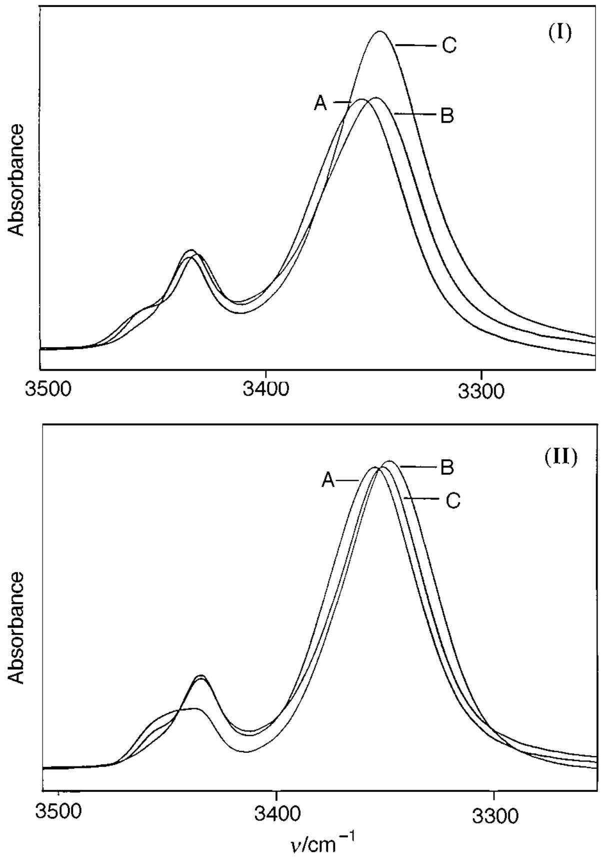

Fig. 1 FTIR absorption spectra (3500–3250 cm^{-1} region) of *p*BrBz-L-Xxx-(Aib)$_4$-OBu^t (A), *p*BrBz-(Aib)$_2$-L-Xxx-(Aib)$_2$-OBu^t (B), and *p*BrBz-(Aib)$_4$-L-Xxx-OBu^t (C) in $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}. Part (I): Xxx = L-Val; part (II): Xxx = L-(αMe)Val.

and 1×10^{-4} mol dm^{-3} peptide concentration (results not shown). Therefore, the observed hydrogen bonding should be interpreted as arising almost exclusively from intramolecular C=O···H–N interactions. The intensity of the low-frequency band relative to the high-frequency band (A_H/A_F ratio) is remarkable in all cases, suggesting the occurrence of a large population of intramolecularly hydrogen-bonded folded (helical) species. A comparison of the six curves shown in Fig. 1 indicates that the A_H/A_F ratio is lower in the pentapeptides -L-Val-(Aib)$_4$- and -(Aib)$_2$-L-Val-(Aib)$_2$-, the only two sequences where L-Val residue is internal to the helical structure [in the pentapeptide -(Aib)$_4$-L-Val- the C-terminal L-Val residue is mostly external to the helical structure].

In summary, the FTIR absorption results are consistent with the hypothesis that in $CDCl_3$ solution all of the pentapeptides investigated are almost completely folded in an intramolecularly hydrogen-bonded helical conformation. Not surprisingly, the two C^α-tetrasubstituted residues Aib and (αMe)Val are found to be more efficient helix formers than the C^α-trisubstituted residue L-Val.[3,7,18,19] To get more detailed information on the preferred conformations of these peptides in $CDCl_3$ solution we carried out a 400 MHz ^{1}H NMR investigation. The delineation of inaccessible (presumably intramolecularly hydrogen-bonded) NH groups by ^{1}H NMR was performed by using (i) solvent dependence of NH chemical shifts by adding increasing amounts of the hydrogen bonding acceptor DMSO[20,21] to the $CDCl_3$ solution and (ii) free radical TEMPO-induced line broadening of NH resonances.[22]

Unambiguous assignments have been performed only for the L-Val NH protons of the three (Aib)$_4$/L-Val pentapeptides *via* an inspection of their multiplicities and for the pentapeptide -(Aib)$_2$-L-(αMe)Val-(Aib)$_2$- by means of a ROESY experiment,[7] which allowed a sequential assignment of all its NH protons to be made.

In the six pentapeptides examined in the $CDCl_3$–DMSO mixtures and in the presence of the paramagnetic perturbing agent TEMPO two classes of NH protons were observed (Figs. 2 and 3). Class (i) includes protons whose chemical shifts are remarkably sensitive to the addition of DMSO and whose resonances broaden significantly upon addition of TEMPO. The fully (NH) assigned pentapeptide -(Aib)$_2$-L-(αMe)Val-(Aib)$_2$- exhibits two protons of this type, N(1)H and N(2)H. All other five pentapeptides show an analogous behaviour (two protons of this type). In particular, also the N(1)H proton of -L-Val-(Aib)$_4$- is a member of this class. Class (ii) includes those protons displaying a behaviour characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition and of linewidths to the presence of TEMPO). The pentapeptide -(Aib)$_2$-L-(αMe)Val-(Aib)$_2$- exhibits three protons of this type, N(3)H to N(5)H. All other five pentapeptides behave similarly (three protons of this type). In particular, also the N(3)H and N(5)H protons of -(Aib)$_2$-L-Val-(Aib)$_2$- and -(Aib)$_4$-L-Val-, respectively, are members of this class.

In conclusion, these ^{1}H NMR results allow us to define the N(3)H to N(5)H protons of the pentapeptides as almost inaccessible to perturbing agents, and therefore, most probably, intramolecularly hydrogen bonded. This situation is indeed that expected for a pentapeptide in a regular 3_{10}-helix structure, characterized by three consecutive intramolecularly hydrogen-bonded β-turns with the N(3)H to N(5)H protons acting as hydrogen bonding donors (Fig. 4). This conformational conclusion is supported by the observation of the L-Val $^3J_{HN\alpha}$ coupling constants. In $CDCl_3$ solution (peptide concentration: 1×10^{-3} mol dm^{-3}) and in the pentapeptides where the L-Val residue is either in the N-terminal or in an internal position of the sequence (*i.e.* inside the 3_{10}-helix), the $^3J_{HN\alpha}$ value is in the range 5.9–6.2 Hz, implying a φ torsion angle[23] of about $-70°$[24] (close to that expected for a 3_{10}-helical structure[4]). In contrast, in the pentapeptides where the L-Val residue is C-terminal (*i.e.* mostly external to the 3_{10}-helix) the $^3J_{HN\alpha}$ value is about 8.0 Hz, corresponding to consistently wider φ torsion angles, $-90°$ or $-145°$, typical of more extended peptide conformations.

The screw sense of the 3_{10}-helical structure adopted by the pentapeptides was assessed by CD spectroscopy, by taking advantage of the *para*-bromobenzamido chromophore. In this connection we have recently reported that the *para*-bromobenzoyl group linked at the N-terminus of a peptide chain is an excellent CD probe for the assignment of the screw sense of 3_{10}-helical peptides, irrespective of the C^α configuration of the constituent α-amino acids.[25] Two oppositely signed bands are visible in the CD spectra of the six pentapeptides in MeOH solution illustrated in Fig. 4. The cross-over point between the two components of this exciton splitting is seen at 238 ± 1 nm, close to the region (241–242 nm) where the absorption maximum of the *para*-bromobenzamide chromophore is found.[26] A CD pattern with the positive component at higher wavelengths is indicative of the predominant population of a right-handed 3_{10}-helical structure.[25] This same pattern is shown by the pentapeptides with the guest L-Val or L-(αMe)Val residue either at the N-terminus or in an internal position of the main chain. Conversely, the CD spectra of the -(Aib)$_4$-L-Val- and -(Aib)$_4$-L-(αMe)Val- pentapeptides (negative component at higher wavelengths) reflect a higher population of the left-handed 3_{10}-helical structure. However, the lower dichroic intensities of the bands of these latter two pentapeptides compared to that of the former four pentapeptides might be, at least in part, interpreted as arising from a relatively modest predominance of the left-handed helix in the two compounds with the C-terminal, chiral guest residue [an additional factor responsible for this phenomenon might be that in the -(Aib)$_4$-L-Val- and

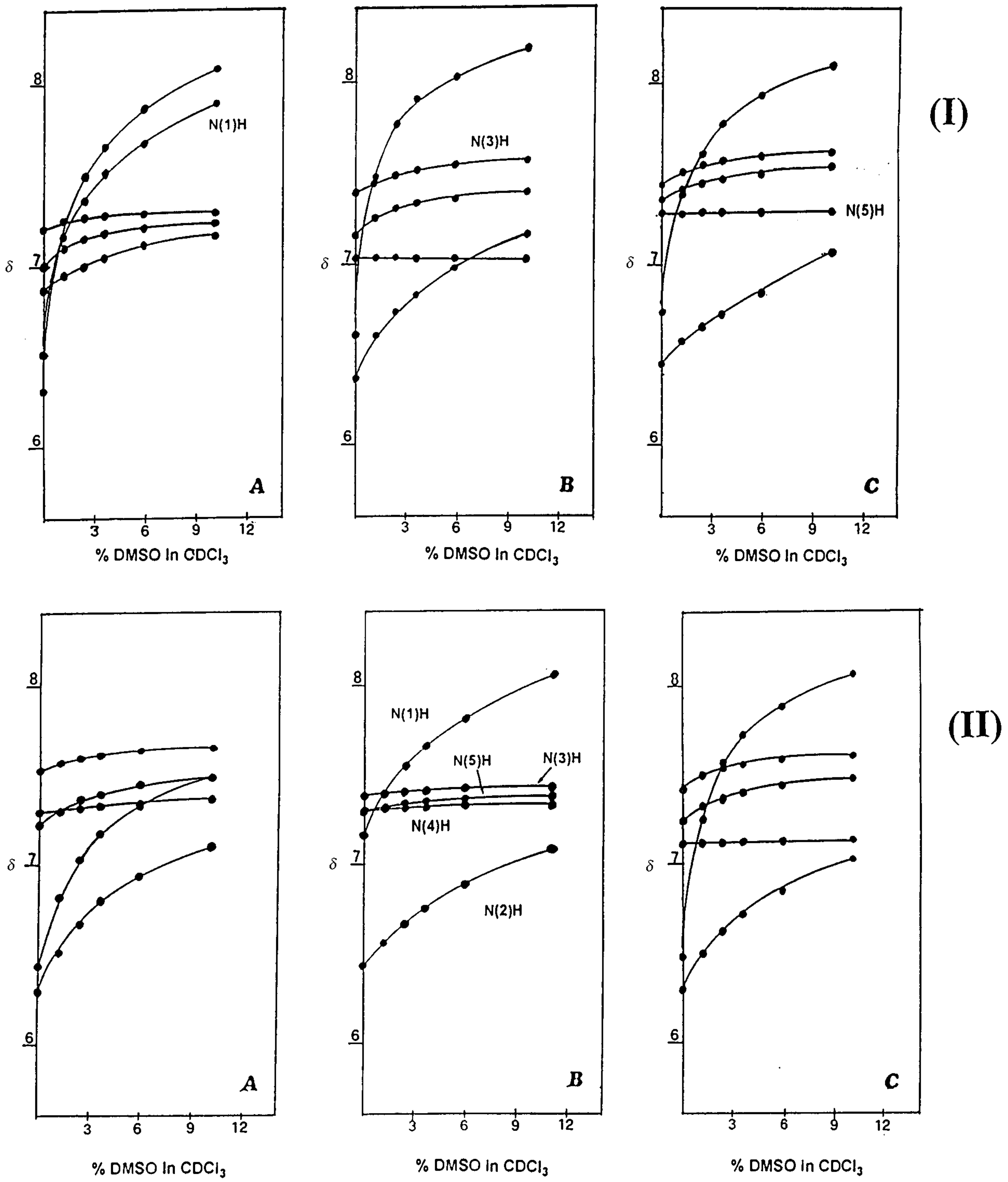

Fig. 2 Plots of NH chemical shifts in the ^{1}H NMR spectra of *p*BrBz-L-Xxx-(Aib)$_4$-OBu^t (A), *p*BrBz-(Aib)$_2$-L-Xxx-(Aib)$_2$-OBu^t (B), and *p*BrBz-(Aib)$_4$-L-Xxx-OBu^t (C) as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}. Part (I): Xxx = L-Val; part (II): Xxx = L-(αMe)Val. Unambiguously assigned NH proton resonances are explicitly indicated. For the assignment of the NH proton resonances of *p*BrBz-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-OBu^t see ref. 7.

-(Aib)$_4$-L-(αMe)Val- pentapeptides the distance between the N-terminal chromophore and the chiral centre is much longer].

Table 2 shows that the unsubstituted benzamido and all types of *para*-substituted benzamido chromophores examined give rise to an exciton splitting with signs of the Cotton effects paralleling those of the *para*-bromobenzamido chromophore discussed above. However, only in the Bz- and *p*BrBz-peptides, where the absorption maximum of the N-terminal chromophore (at 226 nm and 241–242 nm, respectively)[26] is closer to those of the peptide chromophore (below 230 nm),[27] the ellipticities of the CD bands are remarkably high and the exciton split is regular, *i.e.* the CD cross-over point is close to the UV absorption maximum and the dichroic intensities of the positive and negative maxima are comparable. In any event, these additional CD data confirm the screw sense of the -(Aib)$_4$-L-Val- and -(Aib)$_4$-L-(αMe)Val- pentapeptides.

Taken together, our CD data favour the conclusion that the L-Val and L-(αMe)Val residues, when incorporated at the N-terminus or in an internal position of the peptide sequence preferentially induce a normal helix screw sense (*i.e.* an L-amino

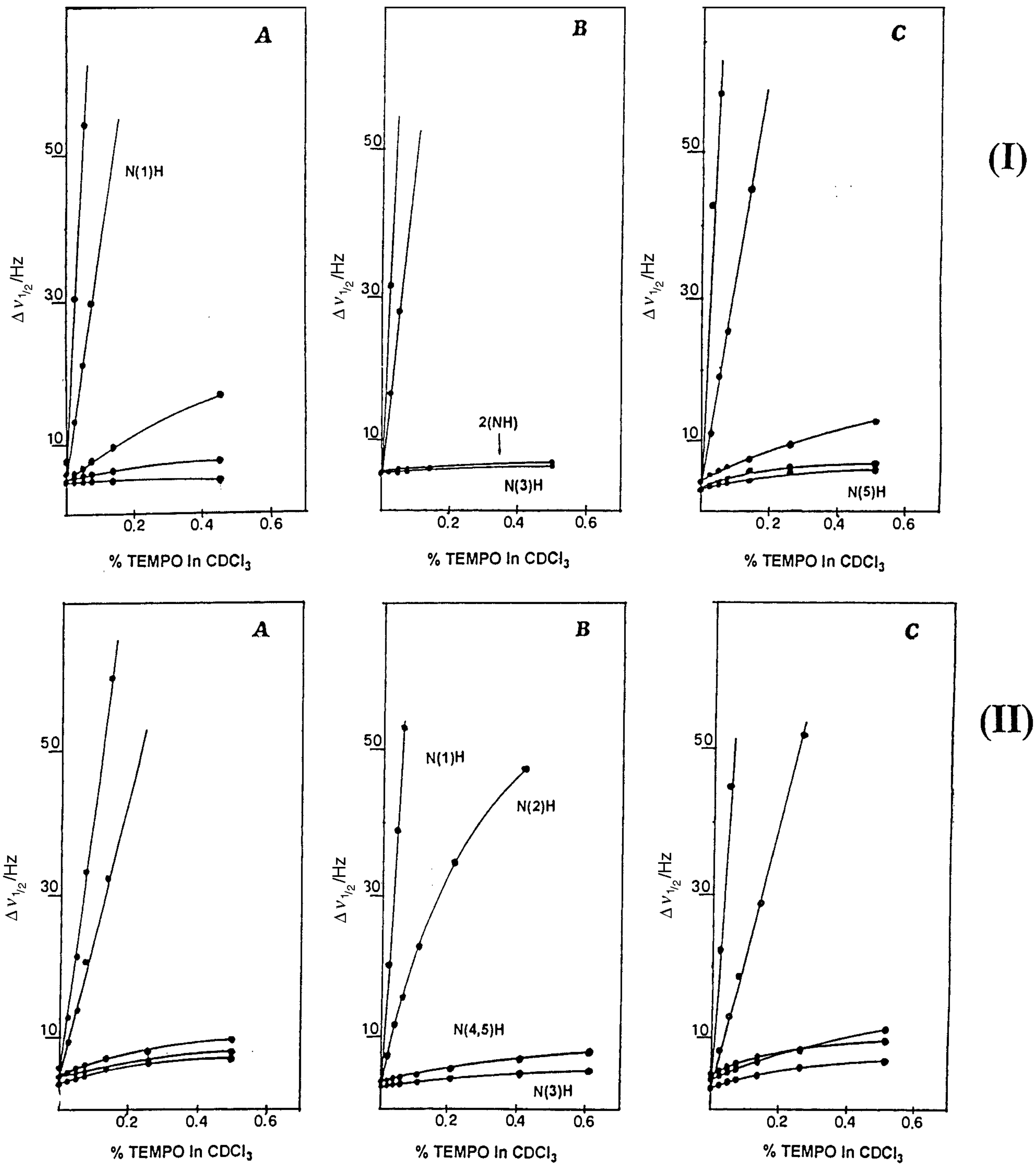

Fig. 3 Plots of the bandwidths of the NH proton in the ^{1}H NMR spectra of *p*BrBz-L- Xxx-(Aib)$_4$-OBu^t (A), *p*BrBz-(Aib)$_2$-L-Xxx-(Aib)$_2$-OBu^t (B), and *p*BrBz-(Aib)$_4$-L-Xxx-OBu^t (C) as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}. Part (I): Xxx = L-Val; part (II): Xxx = L-(αMe)Val. Unambiguously assigned NH proton resonances are explicitly indicated. For the assignment of the NH proton resonances of *p*BrBz-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-OBu^t see ref. 7.

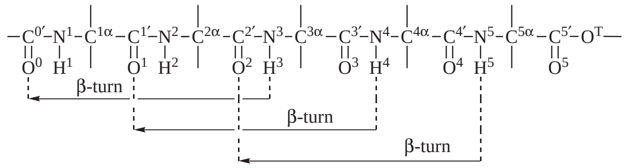

Fig. 4 The C=O···H–N intramolecular hydrogen-bonding pattern characterizing the 3_{10}-helical, N^{α}-acylated pentapeptide system studied in this work

acid gives a right-handed helical structure), while such a relationship is opposite when the chiral, guest residue is inserted at the C-terminus.

Conclusions

In this first systematic investigation[28,29] of the preferred screw sense of the 3_{10}-helical peptides in solution we have designed a pentapeptide system based on four helicogenic, achiral Aib residues, the prototype of C^{α}-tetrasubstituted α-amino acids, and a single chiral α-amino acid, either C^{α}-trisubstituted (L-Val) or C^{α}-tetrasubstituted [L-(αMe)Val]. The conformational results obtained confirmed our working hypothesis, in the sense that in all of the compounds examined the 3_{10}-helical structure is indeed formed to a significant extent.

However, the most relevant information extracted from our chirospectroscopic data strongly supports the view that the pos-

Table 2 Relevant parameters for the CD and UV absorption spectra[a] of the peptides discussed in this work

Peptide	$[\theta]_{max}/10^3$ (λ)[b]	λ_0[c]	$[\theta]_{max}/10^3$ (λ)[a]	$\varepsilon/10^4$ (λ_{max})[d]
Bz-(Aib)$_4$-L-Val-OBu^t	−10.6 (239)	231	+16.8 (221)	1.3 (226)
Bz-(Aib)$_4$-L-(αMe)Val-OBu^t	−8.2 (239)	230	+8.6 (222)	1.3 (226)
*p*BrBz-L-Val-(Aib)$_4$-OBu^t	+22.5 (247)	237	−26.6 (226)	1.7 (241)
*p*BrBz-(Aib)$_2$-L-Val-(Aib)$_2$-OBu^t	+18.5 (249)	239	−25.7 (229)	1.7 (242)
*p*BrBz-(Aib)$_4$-L-Val-OBu^t	−7.8 (248)	238	+12.6 (227)	1.6 (241)
*p*BrBz-L-(αMe)Val-(Aib)$_4$-OBu^t	+21.9 (249)	238	−19.9 (229)	1.5 (241)
*p*BrBz-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-OBu^t	+15.6 (247)	237	−17.0 (228)	1.7 (241)
*p*BrBz-(Aib)$_4$-L-(αMe)Val-OBu^t	−7.2 (249)	239	+9.9 (229)	1.7 (241)
*p*IBz-L-Val-(Aib)$_4$-OBu^t	+13.6 (252)	240	−13.6 (216)	1.3 (253)
*p*IBz-(Aib)$_4$-L-Val-OBu^t	−4.9 (258)	242	+7.0 (222)	1.5 (253)
*p*IBz-(Aib)$_4$-L-(αMe)Val-OBu^t	−4.5 (257)	243	+4.2 (232)	1.5 (253)
*p*MeOBz-(Aib)$_4$-L-Val-OBu^t	−6.7 (255)	241	+11.0 (227)	1.4 (254)
*p*MeOBz-(Aib)$_4$-L-(αMe)Val-OBu^t	−5.8 (255)	241	+8.4 (230)	1.2 (255)
*p*NO$_2$Bz-(Aib)$_4$-L-Val-OBu^t	−2.7 (280)	260	+2.1 (245)	0.9 (263)
*p*NO$_2$Bz-(Aib)$_4$-L-(αMe)Val-OBu^t	−2.7 (281)	260	+2.9 (242)	1.0 (264)
*p*DMABz-(Aib)$_4$-L-Val-OBu^t	−2.6 (306)	264	+7.8 (225)	2.0 (307)
*p*DMABz-(Aib)$_4$-L-(αMe)Val-OBu^t	−3.4 (300)	263	+6.1 (227)	2.4 (307)

[a] In methanol solution (peptide concentration: 1×10^{-4} mol dm^{-3}). [b] $[\theta]_{max}$ is the total molar ellipticity (deg cm^2 dmol^{-1}) at λ_{max} (the wavelength in nm corresponding to the dichroic maximum). [c] Wavelength in nm corresponding to the cross-over point. [d] ε is the molar extinction coefficient at λ_{max} (the wavelength in nm corresponding to the UV absorption maximum).

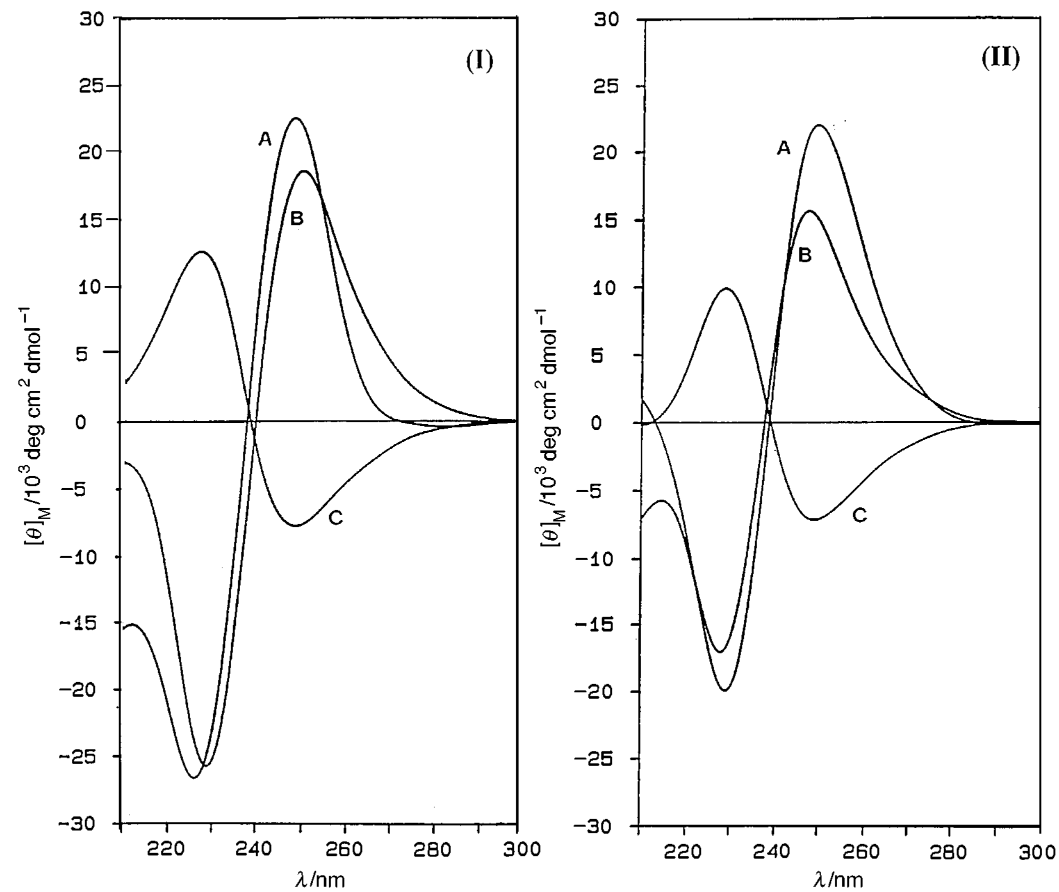

Fig. 5 CD spectra in the 210–300 nm region of *p*BrBz-L-Xxx-(Aib)$_4$-OBu^t (A), *p*BrBz-(Aib)$_2$-L-Xxx-(Aib)$_2$-OBu^t (B), and *p*BrBz-(Aib)$_4$-L-Xxx-OBu^t (C) in MeOH solution. Peptide concentration: 1×10^{-3} mol dm^{-3}. Part (I): Xxx = L-Val; part (II): Xxx = L-(αMe)Val.

ition where the single chiral residue is inserted in the main-chain is critical in directing 3_{10}-helical handedness. More specifically, if the chiral, helical residue is incorporated either at the N-terminal or in an internal position the relationship between α-carbon chirality and helical screw sense is that found in proteins (*i.e.* an L-amino acid gives a *right*-handed helix), whereas if the chiral residue is located at the C-terminus that relationship is 'inverse' (*i.e.* an L-amino acid gives a *left*-handed helix). Since this study has been conducted in solution, obviously crystal packing forces cannot be invoked[30] as responsible for the experimentally observed data. Rather, we believe that the 'inverse' relationship described in this work might be explained on the basis of an unfavourable O···O interaction taking place between the carbonyl oxygen atom of the *i*-2 amino acid from the C-terminus and either oxygen atom of the ester functionality if the sign of the φ torsion angle of the C-terminal (*i*) residue is the same as that of the preceding 3_{10}-helical residues, and that this interaction can be removed by changing the sign of φ, *i.e.* by rotating it by 180° as illustrated in Fig. 6. Since in general L-amino acids have a strong tendency to adopt negative φ values,[31] then it is not surprising that they would tend to induce a left-handed screw sense (positive φ values) in the

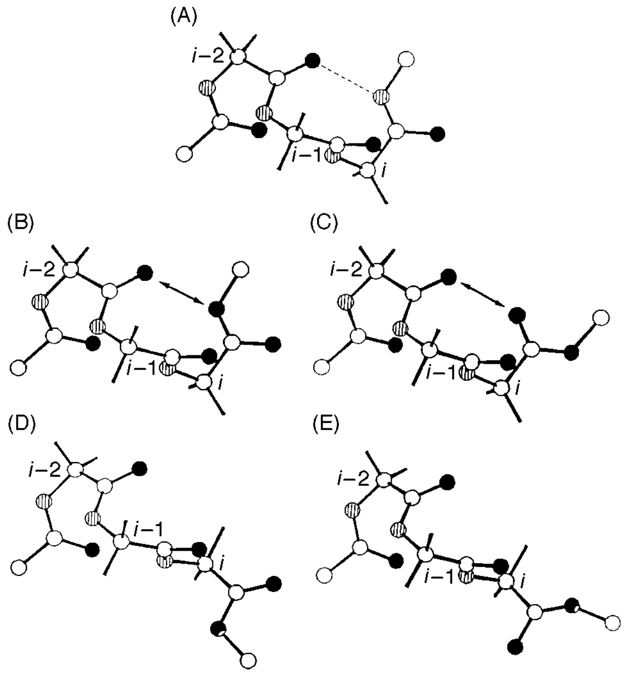

Fig. 6 (A) Model of a right-handed, 3_{10}-helical peptide *N-alkyl amide*, showing the C=O···H–N intramolecular hydrogen bond typical of the type-III β-turn conformation at the C-terminus (in this model the torsion angles φ_i, ψ_i are −60°, −60°, where *i* is the C-terminal residue). (B) Model of a right-handed 3_{10}-helical peptide *ester*, showing the unfavourable C-terminal interaction between the C=O oxygen of the *i*-2 residue and the C–OR oxygen of the *i* residue taking place if φ_i, ψ_i are −60°, −60°. (C) Model of a right-handed 3_{10}-helical peptide *ester*, showing the unfavourable C-terminal interaction between the C=O oxygen of the *i*-2 residue and the C=O oxygen of the *i* residue taking place if φ_i, ψ_i are −60°, +120°. (D) Model of a right-handed 3_{10}-helical peptide *ester*, where φ_i, ψ_i are +60°, +60°. (E) Model of a right-handed 3_{10}-helical peptide *ester*, where φ_i, ψ_i are +60°, −120°. In models (D) and (E) the unfavourable O···O interaction is removed by rotation of the φ_i torsion angle by 180°.

preceding residues if located at the C-terminus of an otherwise achiral 3_{10}-helix.

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