Linear oligopeptides. Part 329.[1] Synthesis, characterization and solution conformational analysis of C^{α}-ethyl, C^{α}-benzylglycine [(αEt)Phe] containing peptides

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For the first time a variety of derivatives and terminally blocked model peptides (to the pentapeptide level) of the sterically demanding (αEt)Phe residue have been synthesized (by solution methods) and fully characterized. The results of a solution conformational analysis, performed by using FTIR and ^{1}H NMR spectroscopy, favour the conclusion that (αEt)Phe is a β-turn and helix promoter as strong as (αMe)Phe (C^{α}-methyl, C^{α}-benzylglycine) but more efficient than the Phe parent amino acid. In addition, a CD study of N^{α}-*para*-bromobenzoylated peptides suggests that the relationship between (αEt)Phe chirality and the screw sense of the turn and helical structures that are formed is the same as that found for (αMe)Phe peptides, *i.e.* L-amino acids give left-handed helicities. Interestingly, this relationship is opposite to that exhibited by protein amino acids, including Phe.

Modifications of polypeptide backbones, that constrain and thereby limit the various conformations available to a particular peptide, have become valuable tools in the medicinal chemist's repertoire for the design of novel agonists and antagonists of peptide action.[2-4] Among the various possible forms of polypeptide backbone modification, methylation at the α-carbon atom of an amino acid residue proved to be most valuable for enhancing the tendency of β-turn[5-7] and $3_{10}/\alpha$-helix[8,9] formation.[10] In our continuing investigation of the conformational preferences of C^{α}-methylated amino acids we have recently been able to show that in the case of γ-branched, C^{α}-methylated L-amino acids, *e.g.* L-(αMe)Phe (C^{α}-methylphenylalanine), the relationship between α-carbon chirality and turn and helix handedness tends to be opposite to that exhibited by protein amino acids, including Phe [in other words, L-(αMe)Phe rich peptides preferentially fold in turns and helices of left-handed screw sense].[10-16]

With the aim of defining more precisely the role played by C^{α}-alkylation and position of side-chain branching on nature and handedness of the folded structure that is formed, we report here the synthesis, characterization, and a solution conformational study (by using FTIR absorption, ^{1}H NMR and CD techniques) of model oligopeptides (to the pentapeptide level) containing one or two L-(αEt)Phe residues. This is the first paper describing details of the synthetic methods and the conformational characterization of peptides rich in a C^{α}-ethylated amino acid. Only an X-ray diffraction analysis of the *N*-carboxy anhydride of L-(αEt)Phe has been reported to date.[17]

Experimental

Materials

For each type of preparation details of a representative example are reported below.

Z-L-(αEt)Phe–OH. To a solution of H–L-(αEt)Phe–OH (5 g, 26 mmol) in NaOH (2 mol dm^{-3}; 10 cm^3; 20 mmol) and acetone (100 cm^3) a solution of Z–Cl (4.40 cm^3, 31 mmol) in acetone (30 cm^3) was added dropwise at 0 °C. The pH was kept at about 10.9 with NaOH (2 mol dm^{-3}). After stirring the solution for 4 h at room temperature, the acetone was removed under reduced pressure. The aqueous layer was extracted twice with diethyl ether, acidified to about pH 3 with HCl (2 mol dm^{-3}), and extracted with AcOEt. The organic layer was washed with water, dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure.

Oxazol-5(4*H*)-one from *p*-BrC_6H_4CO–L-(αEt)Phe–OH. To a stirred suspension of H–L-(αEt)Phe–OH (0.386 g, 2 mmol) in anhydrous pyridine at 0 °C *p*-BrC_6H_4CO–Cl (1.756 g, 8 mmol) was added. After stirring for 2 h at room temperature, pyridine was removed under reduced pressure. The residue was dissolved in AcOEt and the insoluble material was filtered off. The organic solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, water, dried (Na_2SO_4), filtered, and evaporated to dryness. The product was purified by flash chromatography by eluting the column with an isocratic mixture of light petroleum–AcOEt, 4:1.

Oxazol-5(4*H*)-one from Z–L-(αEt)Phe–OH. A solution of Z–L-(αEt)Phe–OH (0.35 g, 1.07 mmol) and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (0.21 g, 1.07 mmol) in CH_3CN (20 cm^3) was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue dissolved in AcOEt. The organic layer was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$ and water, dried (Na_2SO_4), filtered and evaporated to dryness.

Symmetrical anhydride of Z–L-(αEt)Phe–OH. A solution of Z–L-(αEt)Phe–OH (0.20 g, 0.6 mmol) and oxazol-5(4*H*)-one from Z–L-(αEt)Phe–OH (0.19 g, 0.6 mmol) in AcOEt (3 cm^3) was stirred for three days at 0 °C. The solvent was removed under reduced pressure and the product was purified by flash chromatography by eluting the column with an isocratic mixture of light petroleum–AcOEt, 9:2.

Z–D-Ala-L-(αEt)Phe-D-Ala–OMe (symmetrical anhydride method). A solution of Z–D-Ala–OH (0.18 g, 0.8 mmol) and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (0.08 g, 0.4 mmol) in CH_3CN (2 cm^3) was stirred for 40 min at room temperature. Then, H–L-(αEt)Phe-D-Ala–OMe [obtained by Pd-catalysed hydrogenolysis of 0.16 g (0.4 mmol) of the corresponding Z-protected dipeptide in methanol], dissolved in CH_3CN (2 cm^3), was added to the reaction mixture. The pH was kept at about 8.0 by dropwise addition of *N*-methylmorpholine (0.04 cm^3, 0.4 mmol). After stirring for five days

Table 1 Physical properties and analytical data for the (αEt)Phe derivatives and peptides

Compound	Yield (%)	Mp/°C [a]	Recryst. solvent [b]	$[\alpha]_D^{20}$ [c]	TLC [e] R_f(I)	R_f(II)	R_f(III)	ν/cm^{-1} [f]
(*a*) (αEt)Phe derivatives								
Z-L-(αEt)Phe-OH	81	oil	AcOEt–PE	46.4	0.50	0.95	0.30	3421, 1707 [g]
[Z-L-(αEt)Phe]$_2$O	37	oil	AcOEt–PE	−15.7 [d]	0.95	—	0.95	3421, 1818, 1717 [g]
Oxazol-5(4*H*)-one from Z-L-(αEt)Phe-OH	80	oil	AcOEt–PE	35.2 [d]	0.95	—	0.95	1829, 1682 [g]
Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-L-(αEt)Phe-OH	95	oil	AcOEt–PE	147.3 [d]	0.95	—	0.95	1814, 1654 [g]
mClAc-L-(αEt)Phe-OH	37	201–202	AcOEt–PE	50.3	0.50	0.95	0.20	3356, 1721, 1625
Oxazol-5(4*H*)-one from mClAc-L-(αEt)Phe-OH	38	oil	EE	−55.1 [d]	0.95	—	0.90	1822, 1676 [g]
Z-L-(αEt)Phe-NHPr^i	74	113–115	AcOEt–PE	0.0	0.95	0.95	0.55	3415, 3328, 1725, 1636
(*b*) (αEt)Phe/Ala peptides								
Z-L-(αEt)Phe-L-Ala-OMe	88	115–117	AcOEt–PE	−57.5	0.95	0.95	0.55	3435, 3381, 3355, 3280, 1751, 1736, 1720, 1669
Z-L-(αEt)Phe-D-Ala-OMe	30	oil	AcOEt–PE	29.2	0.95	0.95	0.55	3429, 3392, 1715, 1673
Z-L-Ala-L-(αEt)Phe-L-Ala-OMe	61	150–152	AcOEt–PE	−87.9	0.90	0.95	0.40	3326, 1745, 1702, 1674, 1648
Z-D-Ala-L-(αEt)Phe-D-Ala-OMe	64	86–88	AcOEt–PE	46.1	0.95	0.95	0.45	3350, 1737, 1719, 1704, 1672, 1646
Z-L-(αEt)Phe-(L-Ala)$_2$-OMe	50	59–61	EE–PE	−61.9	0.95	0.95	0.45	3327, 1742, 1725, 1644
Z-L-(αEt)Phe-(D-Ala)$_2$-OMe	37	63–65	EE	−28.6	0.95	0.95	0.45	3424, 1716, 1666
(*c*) (αEt)Phe/Aib peptides								
Z-L-(αEt)Phe-Aib-OBu^t	48	137–139	AcOEt–PE	16.6	0.95	0.95	0.70	3401, 3301, 1718, 1660
Z-Aib-L-(αEt)Phe-Aib-OBu^t	66	147–148	AcOEt–EE–PE	−32.6	0.95	0.95	0.45	3402, 3368, 3292, 1727, 1654
Z-Aib-L-(αEt)Phe-Aib-OH	93	124–125	AcOEt–PE	−28.6	0.55	0.95	0.30	3307, 1738, 1698, 1651
Z-L-(αEt)Phe-(Aib)$_2$-OBu^t	20	139–141	AcOEt–EE–PE	−82.3	0.90	0.95	0.40	3429, 3358, 3303, 1720, 1674
Z-(Aib)$_2$-L-(αEt)Phe-Aib-OBu^t	77	150–152	AcOEt–PE	−63.2	0.55	0.90	0.35	3428, 3340, 1729, 1703, 1674
Z-(Aib)$_2$-L-(αEt)Phe-Aib-OH	94	197–198	MeOH–EE	−60.4	0.50	0.95	0.25	3307, 1742, 1699, 1659
Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t	56	248–249	MeOH–EE	−72.7	0.60	0.90	0.15	3435, 3419, 3315, 1725, 1661
Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OH	91	277–278	MeOH–EE	−71.4	0.35	0.80	0.05	3352, 3302, 1742, 1657
p-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t	30	249–250	AcOEt–EP	−85.6	0.75	0.95	0.35	3429, 1727, 1668, 1643
p-BrC$_6$H$_4$CO-L-(αEt)Phe-(Aib)$_2$-L-(αEt)Phe-Aib-OBu^t	6	290–292	AcOEt–PE	−174.3	0.95	0.95	0.35	3431, 3324, 1728, 1663
(*d*) (αEt)Phe homo-peptides								
p-BrC$_6$H$_4$CO-[L-(αEt)Phe]$_2$-NHPr^i	18	87–89	AcOEt–PE	−120.1	0.95	0.95	0.50	3434, 3325, 1639

[a] Determined on a Leitz model Laborlux 12 apparatus (Wetzlar, Germany). [b] AcOEt, ethyl acetate; PE, light petroleum; EE, diethyl ether; MeOH, methanol. [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] c = 0.5 (AcOEt). [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 $CDCl_3$ solution ($c = 1 \times 10^{-2}$ mol dm^{-3}).

at room temperature the solvent was removed under reduced pressure and the residue dissolved in AcOEt. The organic layer was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$ and water, dried (Na_2SO_4), filtered and evaporated to dryness. The product was purified by flash chromatography by eluting the column with an isocratic mixture of $CHCl_3$–EtOH, 95:5.

Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t(oxazolone method). The oxazol-5(4*H*)-one from Ac-(Aib)$_2$-OH (0.20 g, 1 mmol) and H-L-(αEt)Phe-(Aib)$_2$-OBu^t (obtained by Pd-catalysed hydrogenolysis of 0.55 g, 1 mmol, of the corresponding Z-protected tripeptide in methanol), were refluxed in CH_3CN (2 cm^3) for 13 h. The solvent was removed under reduced pressure and the residue dissolved in AcOEt. The organic layer was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$ and water, dried (Na_2SO_4), filtered and evaporated to dryness.

Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OH. Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t (0.166 g, 0.26 mmol) was stirred at room temperature in trifluoroacetic acid (3 cm^3) for 3 h. The solvent was removed under reduced pressure and the residue evaporated a few times from diethyl ether.

Table 2 FTIR absorption maxima for the terminally blocked (αEt)Phe peptides in $CDCl_3$ solution[a,b]

Peptide	$\lambda_{3500-3250}/cm^{-1}$	$\lambda_{1800-1600}/cm^{-1}$
Z-L-(αEt)Phe-L-Ala-OMe	3427, 3390	1739. 1715w, 1675
Z-L-(αEt)Phe-D-Ala-OMe	3453w, 3426, 3392	1739, 1715w, 1676
Z-L-Ala-L-(αEt)Phe-L-Ala-OMe	3429, 3367	1738, 1718, 1699w, 1684w, 1664
Z-D-Ala-L-(αEt)Phe-D-Ala-OMe	3424, 3365	1737, 1719, 1686w, 1665
Z-L-(αEt)Phe-(L-Ala)$_2$-OMe	3440w, 3423, 3390w, 3352w	1742, 1716, 1687, 1666
Z-L-(αEt)Phe-(D-Ala)$_2$-OMe	3439, 3424, 3390w, 3352w	1741, 1717, 1685w, 1665
Z-L-(αEt)Phe-Aib-OBu^t	3451w, 3429w, 3396	1714, 1674
Z-Aib-L-(αEt)Phe-Aib-OBu^t	3429, 3395w, 3366	1720, 1686, 1659
Z-L-(αEt)Phe-(Aib)$_2$-OBu^t	3454w, 3429w, 3373	1719, 1684w, 1663
Z-(Aib)$_2$-L-(αEt)Phe-Aib-OBu^t	3427, 3357	1720, 1682, 1657w
Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t	3428, 3345	1726, 1681w, 1673, 1657w
p-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t	3434, 3342	1726, 1681w, 1669, 1656w
p-BrC$_6$H$_4$CO-L-(αEt)Phe-(Aib)$_2$-L-(αEt)Phe-Aib-OBu^t	3432, 3340	1727, 1678w, 1665
p-BrC$_6$H$_4$CO-[L-(αEt)Phe]$_2$-NHPr^i	3436, 3356	1687w, 1675w, 1649

[a] Peptide concentration = 2×10^{-3} mol dm^{-3}. [b] w = weak bands.

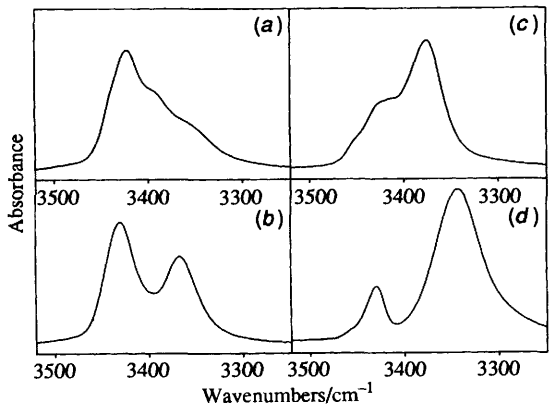

Fig. 1 FTIR absorption spectra (3500–3250 cm^{-1} region) of (*a*) Z-L-(αEt)Phe-(L-Ala)$_2$-OMe, (*b*) Z-L-Ala-L-(αEt)Phe-L-Ala-OMe, (*c*) Z-L-(αEt)Phe-(Aib)$_2$-OBu^t, and (*d*) Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t in $CDCl_3$ solution. Peptide concentration = 2×10^{-3} mol dm^{-3}.

The physical properties and analytical data for the (αEt)Phe peptides discussed in this work and their synthetic intermediates are listed in Table 1.

FTIR absorption spectra

FTIR absorption spectra were recorded with a Perkin-Elmer model 1720X spectrophotometer (Norwalk, CT), nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (base-line) 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 [$^2H_2$]chloroform (99.8% ^2H) was purchased from Merck (Darmstadt, Germany).

^1H NMR spectra

^1H NMR spectra were recorded with a Bruker model AM 400 spectrometer (Karlsruhe, Germany). Measurements were carried out in [$^2H_2$]chloroform (99.96% ^2H; Merck) and in [$^2H_6$]DMSO ([$^2H_6$]dimethyl sulfoxide) (99.96% $^2H_6$; Fluka, Büchs, Switzerland) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethyl-1-piperidyloxyl) was purchased from Sigma (Milwaukee, WI).

CD spectra

CD spectra were recorded with a JASCO model J-600 spectropolarimeter (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-(αEt)Phe we exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by the DSM group a few years ago.[18,19] 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 seven derivatives and 17 peptides (to the pentapeptide level), the latter incorporating one or two L-(αEt)Phe residues and including a terminally blocked homodipeptide, were performed. The benzyloxycarbonyl (Z) and monochloroacetyl (mClAc) N-blocked derivatives were obtained by reacting the free amino acid with a slight excess of Z–Cl and mClAc–Cl, respectively. Conversely, a large excess of mClAc–Cl and *p*-BrC$_6$H$_4$CO–Cl gave the corresponding oxazol-5(4*H*)-ones to a large extent. The oxazol-5(4*H*)-one from Z-L-(αEt)Phe–OH was prepared by treating the N$^\alpha$-protected amino acid with *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide. Reaction of Z-L-(αEt)Phe–OH with the oxazol-5(4*H*)-one from Z-L-(αEt)Phe–OH gave the symmetrical anhydride [Z-L-(αEt)Phe]$_2$O. During amide or peptide bond formation involving this sterically hindered residue the carboxy group of the N$^\alpha$-blocked amino acid or peptide was activated using the symmetrical anhydride or the oxazol-5(4*H*)-one method. Optimization of the reaction yields was not attempted. The N$^\alpha$-blocked peptide free acids were obtained by treatment of the corresponding *tert*-butyl esters with trifluoroacetic acid. Removal of the benzyloxycarbonyl N$^\alpha$-protecting group was performed by catalytic hydrogenation.

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 ^1H NMR spectroscopy (the latter data are not reported).

Solution conformational analysis

The preferred conformations adopted by the terminally blocked (αEt)Phe containing peptides were determined in the structure supporting solvents $CDCl_3$ and MeOH by FTIR absorption and ^1H NMR spectroscopy, and by CD spectroscopy, respectively. The FTIR absorption maxima in $CDCl_3$ solution at the 2×10^{-3} mol dm^{-3} concentration are listed in Table 2. Figs. 1 and 2 illustrate FTIR absorption spectra (N–H stretching region) and ^1H NMR data, respectively, of selected (αEt)-Phe/Ala and (αEt)Phe/Aib peptides. Fig. 3 shows the CD

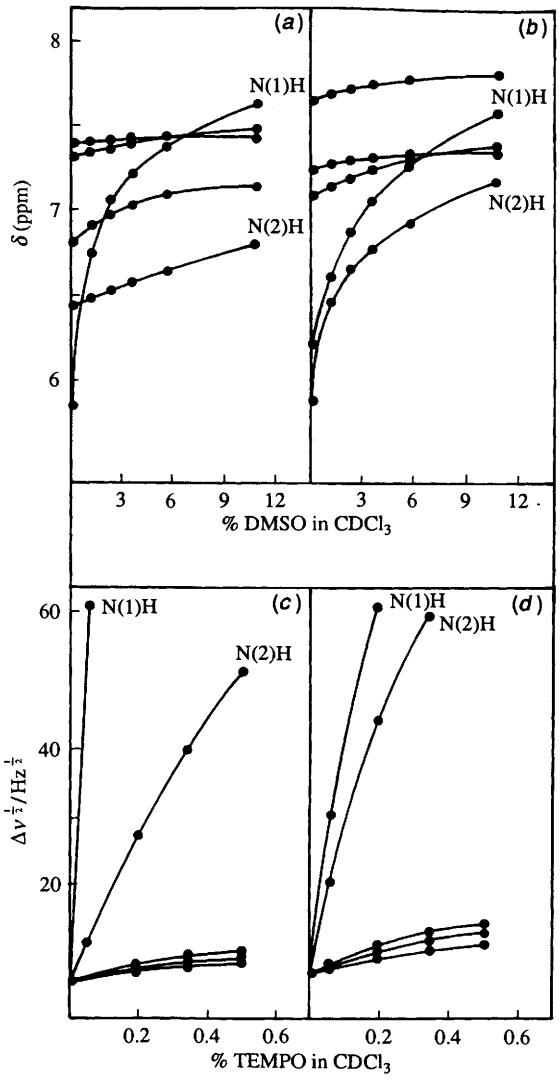

Fig. 2 Plot of NH chemical shifts in the ^{1}H NMR spectra of (*a*) Ac-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t and (*b*) *p*-BrC$_6$H$_4$CO-L-(αEt)Phe-(Aib)$_2$-L-(αEt)Phe-Aib-OBu^t as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. Plot of the bandwidth of the NH protons of the same peptides [(*c*) and (*d*), respectively] as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration = 2 × 10^{-3} mol dm^{-3}.

spectra in the region of absorption of the *para*-bromobenzamido chromophoric probe of a (αEt)Phe/Aib pentapeptide and the homo-dipeptide.

The FTIR curves are characterized by bands at 3454–3423 cm^{-1} (free, solvated NH groups) and at 3373–3340 cm^{-1} (strongly hydrogen-bonded NH groups) (Fig. 1 and Table 2).[20,21] The intensity of the low-frequency band relative to the high-frequency band significantly increases: (*i*) as main-chain length increases, (*ii*) from (αEt)Phe/Ala to (αEt)Phe/Aib peptides, and (*iii*) from (αEt)Phe at position 1 to (αEt)Phe at position 2 in the (αEt)Phe/Ala peptides. No appreciable differences are seen in the spectra of the diastereoisomeric (αEt)Phe/Ala peptides. In addition, a weak band (shoulder) in the 3396–3390 cm^{-1} region (weakly hydrogen-bonded NH groups) is visible in the spectra of the shortest peptides (di- and tri-peptides).

We have also been able to demonstrate that even at 1 × 10^{-2} mol dm^{-3} concentration, self-association *via* N–H···O=C intermolecular hydrogen bonding is negligible (less than 5%) for all peptides (results not shown). Therefore, the observed

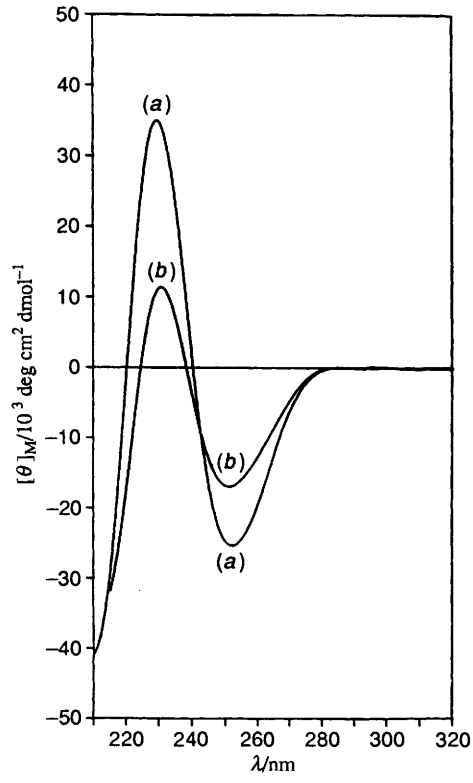

Fig. 3 CD spectra in the 210–320 nm region of (*a*) *p*-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αEt)Phe-(Aib)$_2$-OBu^t and (*b*) *p*-BrC$_6$H$_4$CO-[L-(αEt)Phe]$_2$-NHPr^i in MeOH solution. Peptide concentration = 1 × 10^{-3} mol dm^{-3}.

hydrogen bonding should be interpreted as arising almost exclusively from intramolecular N–H···O=C interactions. In any event, even at the highest dilution examined (1 × 10^{-4} mol dm^{-3}), the intensity of the band of the longest peptides related to strongly hydrogen-bonded NH groups is remarkable, suggesting the occurrence of large populations of intramolecularly hydrogen-bonded folded species. The observation of the 3373–3340 cm^{-1} band in the tri-, tetra- and penta-peptides, which is absent in the dipeptides, seems to indicate that the (αEt)Phe peptides do not tend to adopt a γ-turn (C_7) conformation[6,22,23] even in a solvent of low polarity ($CDCl_3$), and highlights the tendency of the (αEt)Phe tripeptides to fold into a β-turn conformation which may evolve in a series of consecutive β-turns (3_{10}-helices) in longer peptides. Interestingly, by increasing the number of residues in the peptide chain it appears that the population of the weakly hydrogen-bonded, fully extended (C_5) conformers[23-25] tends to decrease relative to the strongly hydrogen-bonded folded conformers.

To get more detailed information on the preferred conformation of these peptides in this halocarbon, we carried out a 400 MHz ^{1}H NMR investigation. The delineation of inaccessible (or 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[26,27] to the $CDCl_3$ solution and (*ii*) free-radical (TEMPO)-induced line broadening of NH resonances.[28]

With regard to the conformationally significant pentapeptides, a partial tentative assignment has been performed for the two upfield resonances to the N(1)H and N(2)H protons, by analogy with the chemical shifts in chloroform of other N$^{\alpha}$-acetylated and N$^{\alpha}$-*para*-bromobenzoylated peptides from different types of C$^{\alpha,\alpha}$-disubstituted glycines.[11,12,24] From an analysis of the spectra as a function of concentration (1 × 10^{-2}

to 1×10^{-3} mol dm^{-3}) in $CDCl_3$ solution (results not shown), we have been able to conclude that dilution induces a modest shift to higher fields of the NH resonances. In particular, the sensitive N(1)H and N(2)H protons shift only by 0.10–0.35 and by 0.03–0.05 ppm, respectively. The N^{α}-acetylated pentapeptide has a more pronounced tendency to self-associate than the N^{α}-*para*-bromobenzoylated pentapeptides.

In the pentapeptides examined in the $CDCl_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO at 2×10^{-3} mol dm^{-3} peptide concentration, two classes of NH protons were observed (Fig. 2). Class (*i*) [N(1)H and N(2)H protons] includes protons whose chemical shifts are sensitive to the addition of DMSO and whose resonances broaden significantly upon addition of TEMPO. Interestingly, the sensitivity of the N(1)H proton is 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 chemical shifts to solvent composition, and of linewidths to the presence of TEMPO).

In summary, these ^{1}H NMR results allow us to conclude that, in $CDCl_3$ solution at 1×10^{-2} mol dm^{-3} concentration, the pentapeptides have a tendency (although modest) to self-associate and that in this process, the amide N(1)H proton plays a major role as hydrogen bonding donor. At lower concentrations, the N(3)H to N(5)H protons are almost inaccessible to perturbing agents and are, therefore, most probably intramolecularly hydrogen-bonded. In view of these FTIR and ^{1}H NMR observations, it is reasonable to conclude that the most populated structures adopted in $CDCl_3$ solution by the terminally blocked tri-, tetra- and penta-peptides are the β-turn, two consecutive β-turns and the 3_{10}-helix, respectively.

We have previously shown 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.[16] Two intense, oppositely signed bands, negative at higher wavelengths, are visible in the CD spectra of an L-(αEt)Phe/Aib pentapeptide and the L-(αEt)Phe homo-dipeptide in MeOH solution (Fig. 3). The cross-over points between the two components of this exciton splitting are seen in the vicinity of 240 nm, the region where the absorption maximum of the *para*-bromobenzamido chromophore is found.[29] These CD patterns are indicative of the onset of a significant amount of left-handed helical structure for the L-(αEt)Phe peptides in MeOH solution.

Conclusions

In the first detailed investigation of the preferred conformation of peptides containing a sterically hindered C^{α}-ethylated amino acid, (αEt)Phe, we have been able to show that these compounds tend to fold either into β-turns or (incipient) 3_{10}-helical structures depending upon main-chain length. A comparison of the results described here with the corresponding findings already reported for (αMe)Phe[10–16] and Phe[11,20,30,31] containing peptides allows us to conclude that the (αEt)Phe residue is a β-turn and helix inducer as efficient as (αMe)Phe, but stronger than the unalkylated parent compound Phe.

As for the relationship between α-carbon chirality and turn and helix handedness of the (αEt)Phe peptides, the CD data available so far point to a behaviour opposite to that characteristic of protein amino acids, including Phe, namely L-(αEt)Phe peptides give left-handed turns and helical structures. Again, this property makes (αEt)Phe peptides conformationally similar to their (αMe)Phe analogues. In summary, these findings confirm that the major factor responsible for the abnormal behaviour of C^{α}-alkylated Phe residues is their side-chain γ-branching.

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