Linear oligopeptides. Part 352.[1] Synthesis, characterization and solution conformational analysis of C^{α}-methyl-homo-phenylalanine [(αMe)Hph] containing peptides

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For the first time a number of derivatives and terminally blocked model peptides (to the pentapeptide level) of the sterically demanding C^{α}-methyl-homo-phenylalanine, (αMe)Hph, 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 spectroscopies, favour the conclusion that (αMe)Hph is as potent a β-turn and helix promoter as (αMe)Phe (C^{α}-methylphenylalanine) and (αEt)Phe (C^{α}-ethylphenylalanine), and more potent than the Phe parent amino acid. In addition, a CD study of N^{α}-*para*-bromobenzoylated peptides suggests that the relationship between (αMe)Hph α-carbon chirality and the prevailing screw sense of the turn and helical structures that are formed is opposite to that found for (αMe)Phe and (αEt)Phe peptides, *i.e.* L-amino acids give right-handed helicities. This relationship is the same as that exhibited by protein amino acids, including Phe.

Introduction

In the last decade medicinal chemists have become interested in analogues of bioactive peptides containing chiral C^{α}-alkylated α-amino acids, as these sterically demanding, backbone modified, non-coded residues tend to freeze specific conformations and dramatically slow down enzymatic degradation.[2-4] In our ongoing study of the preferred conformations of C^{α}-alkylated amino acids we have recently demonstrated that γ-branched C^{α}-alkylated L-amino acids, *e.g.* L-(αMe)Phe (C^{α}-methylphenylalanine) and L-(αEt)Phe (C^{α}-ethylphenylalanine), (*i*) are extremely effective β-turn[5-7] and 3_{10}/α-helix[8,9] promoters and (*ii*) the handedness of the turns and helices that are formed is opposite to that exhibited by protein amino acids, including Phe [in other words, L-(αMe)Phe-[10-17] and L-(α-Et)Phe[1,18,19]-rich peptides preferentially fold in turns and helices of left-handed screw sense]. Similar results for (αMe)Phe peptides have been published by other groups.[20-22]

With the aim of defining more precisely the role played by the position of side-chain branching on nature and handedness of the folded structure that is formed, we report here the synthesis, the characterization and a solution conformational study (by using FTIR absorption, ^{1}H NMR and CD techniques) of model peptides (including homo-peptides) of (αMe)Hph (C^{α}-methyl-homo-phenylalanine) to the pentamer level. This is the first paper describing details of synthetic methods and conformational analyses of peptides rich in this C^{α}-methylated, δ-branched α-amino acid. Only an X-ray diffraction investigation of an (αMe)Hph derivative has been reported to date.[23] X-Ray diffraction results of a variety of pseudopeptides of the unmethylated counterpart Hph have been published,[24-31] as some of these compounds are orally active angiotensin-converting enzyme inhibitors and have been successfully introduced as antihypertensive drugs.[32-34]

Experimental

Materials

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

Z-D-(αMe)Hph-OH. H-D-(αMe)Hph-OH (1.04 g, 5.4 mmol) was suspended in dioxane (5 cm^3) and kept at 0 °C. Then NaOH (5.3 mmol), $NaHCO_3$ (0.44 g, 5.2 mmol) and Z–OSu (Z, benzyloxycarbonyl and OSu, 1-hydroxysuccinimido) (1.32 g, 5.3 mmol) were added. The reaction mixture was stirred at room temperature for 40 h. Dioxane was evaporated under reduced pressure. The oily product was dissolved in 5% $NaHCO_3$ and the unreacted Z–OSu was extracted with diethyl ether. The aqueous layer was acidified to pH 3 with $KHSO_4$ and the product was extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4) and evaporated to dryness under reduced pressure.

Z-D-(αMe)Hph-OBu^t. 2-Methylpropene (20 cm^3) was bubbled into a solution of Z-D-(αMe)Hph-OH (3.78 g, 11.6 mmol) in anhydrous CH_2Cl_2 (50 cm^3) and cooled to −60 °C. Then, concentrated H_2SO_4 (0.1 cm^3) was added. The reaction mixture was kept at room temperature for 7 days and then poured into 25 cm^3 of a 5% aqueous solution of $NaHCO_3$. CH_2Cl_2 was removed under reduced pressure and the aqueous phase was extracted with AcOEt. The organic layer was washed with 5% aqueous $NaHCO_3$ and H_2O, dried (Na_2SO_4), filtered and evaporated to dryness.

Oxazol-5(4*H*)-one from *p*-BrC_6H_4CO-D-(αMe)Hph-OH. To a stirred suspension of H-D-(αMe)Hph-OH (1.35 g, 7.0 mmol) in anhydrous pyridine at 0 °C, *p*-BrC_6H_4CO-Cl (6.18 g, 28.2 mmol) was added. After stirring the reaction for 22 h at room temperature, the pyridine was removed *in vacuo*. The residue was dissolved in diethyl ether and the insoluble salts were filtered off. The product was then purified by flash-chromatography by eluting the column with a 1:5 isocratic mixture of AcOEt:light petroleum.

[Z-L-(αMe)Hph)]$_2$O. Z-L-(αMe)Hph-OH (1.24 g, 3.80 mmol) was added to a solution of the oxazol-5(4*H*)-one from Z-L-(αMe)Hph-OH (1.18 g, 3.83 mmol) [obtained *in situ* by reaction of Z-L-(αMe)Hph-OH (1.39 g, 4.27 mmol) with 1.2 equivalents of EDC·HCl] in AcOEt [EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide]. After stirring the solution for 4 days at room temperature, the organic phase was washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, dried ($NaSO_4$) and evaporated to dryness.

Z-L-Ala-L-(αMe)Hph-L-Ala-OMe (symmetrical anhydride method). H-L-(αMe)Hph-L-Ala-OMe (0.094 g, 0.34 mmol) [obtained from Pd-catalysed hydrogenolysis of 0.145 g (0.35 mmol) of the corresponding Z-protected dipeptide ester in MeOH] was dissolved in anhydrous CH_2Cl_2 (3 cm^3). (Z-L-Ala)$_2$O (0.17 g, 0.4 mmol) was added, followed by *N*-methyl morpholine (0.4 mmol). After stirring the reaction at room temperature for 24 h, CH_2Cl_2 was evaporated under reduced pressure. The oily residue was dissolved in AcOEt and washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, dried (Na_2SO_4) and evaporated to dryness under reduced pressure.

***p*-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αMe)Hph-(Aib)$_2$-OBu^t (oxazolone method).** The oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-(Aib)$_2$-OH [35,36] (0.082 g, 0.23 mmol) and H-L-(αMe)Hph-(Aib)$_2$-OBu^t (0.105 g, 0.21 mmol) [obtained from Pd-catalysed hydrogenolysis of the corresponding Z-protected tripeptide ester] were refluxed in CH_3CN for 16 h. The solvent was removed under reduced pressure, the residue dissolved in AcOEt, washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated under reduced pressure.

***p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OH.** *p*-BrC$_6$H$_4$CO-[D-(αMe)-Hph]$_2$-OBu^t (1.75 g, 2.9 mmol) was stirred for 2 h at room temperature in 20 cm^3 of a 1 : 1 mixture of CF_3CO_2H : CH_2Cl_2. The solvent was removed *in vacuo* and the residue was washed several times from diethyl ether and dried under reduced pressure.

Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OH. A solution of *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OH (1.33 g, 2.4 mmol) and EDC·HCl (0.57 g, 2.8 mmol) in CH_3CN (15 cm^3) was stirred at room temperature for 2 h. The solvent was removed under reduced pressure, the residue dissolved in AcOEt and the organic phase washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, dried (Na_2SO_4), filtered and evaporated to dryness.

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

FTIR absorption spectra

FTIR absorption spectra were recorded using 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 [^{2}H]chloroform (99.8% ^{2}H) was purchased from Merck (Darmstadt, Germany).

^{1}H NMR spectra

^{1}H NMR spectra were recorded using 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, 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 using 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 term 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- and D-(αMe)Hph we exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by the DSM group a few years ago.[37,38] 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.

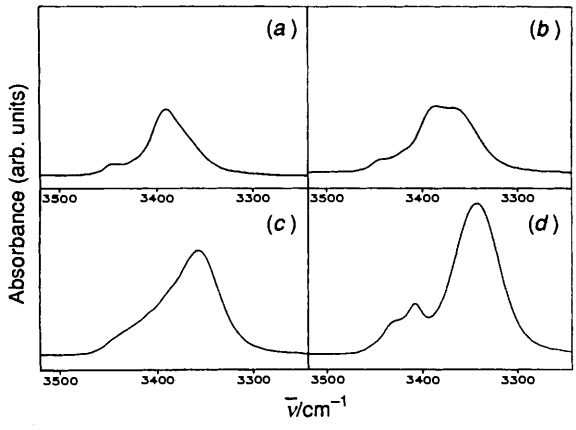

Fig. 1 FTIR absorption spectra (3500–3250 cm^{-1} region) of *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_n$-OBu^t homo-peptides (*a*) $n = 2$; (*b*) $n = 3$; (*c*) $n = 4$; (*d*) $n = 5$ in $CDCl_3$ solution. Peptide concentration: 1×10^{-3} mol dm^{-3}.

The synthesis and characterization of four (αMe)Hph derivatives and twenty two peptides (to the pentapeptide level), the latter including a series of homo-peptides, were performed. The benzyloxycarbonyl (Z) derivative was obtained by reacting the free amino acid with Z–OSu (OSu, 1-hydroxysuccinimido). A large excess of *p*-BrC$_6$H$_4$CO-Cl over the free amino acid gave the corresponding oxazol-5(4*H*)-one to a large extent. The oxazol-5(4*H*)-one from Z-L-(αMe)Hph-OH was prepared by treating the N$^\alpha$-protected amino acid with EDC. Reaction of Z-L-(αMe)Hph-OH with the oxazol-5(4*H*)-one from Z-L-(αMe)Hph-OH gave the symmetrical anhydride [Z-L-(αMe)-Hph]$_2$O. During 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. *tert*-Butyl ester formation was achieved by H_2SO_4-catalysed reaction of the corresponding free acid with 2-methylpropene.

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 spectroscopy (the latter data are not reported).

Solution conformational analysis

The preferred conformations adopted by the terminally blocked (αMe)Hph containing peptides were determined in the structure supporting solvents $CDCl_3$ (by FTIR absorption and ^{1}H NMR spectroscopies) and MeOH (by CD spectroscopy). The FTIR absorption maxima in $CDCl_3$ solution at the 1×10^{-3} mol dm^{-3} concentration are listed in Table 2. Figs. 1–3 illustrate FTIR absorption spectra (N–H stretching region) and ^{1}H NMR data of selected peptides. Fig. 4 shows the CD spectra in the region of absorption of the *para*-bromobenzamido chromophoric probe of the (α-Me)Hph homo-tripeptide and, for comparison, an (αMe)Phe [16] and an (αEt)Phe [19] homo-peptide.

The FTIR curves are characterized by bands at 3460–3408 cm^{-1} (free, solvated NH groups) and at 3372–3341 cm^{-1}

Table 1 Physical properties and analytical data for the (αMe)Hph derivatives and peptides

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]
(a) (αMe)Hph derivatives								
Z-D-(αMe)Hph-OH	75	oil	AcOEt–LP	−6.8	0.70	0.95	0.45	3414, 3327, 1709[f]
Z-D-(α-Me)Hph-OBu^t	79	oil	AcOEt–LP	5.1	0.95	0.95	0.85	3419, 1716[f]
[Z-L-(αMe)Hph]$_2$O	76	100–101	AcOEt–LP	−2.7[g]	0.95	0.95	0.35	3373, 1815, 1744, 1707
Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-D-(αMe)Hph-OH	74	oil	AcOEt–LP	−20.3[g]	0.95	0.90	0.90	1820, 1650[f]
(b) (αMe)Hph-Ala peptides								
Z-L-(αMe)Hph-L-Ala-OMe	67	oil	AcOEt–LP	−7.8	0.80	0.95	0.50	3348, 1724, 1659[f]
Z-L-(αMe)Hph-D-Ala-OMe	73	93–94	AcOEt–LP	15.6	0.95	0.95	0.50	3447, 1744, 1725, 1653, 1540
Z-L-Ala-L-(αMe)Hph-L-Ala-OMe	79	145–146	AcOEt–LP	−32.7	0.80	0.95	0.40	3373, 1745, 1705, 1648, 1538
Z-D-Ala-L-(αMe)Hph-D-Ala-OMe	69	189–190	AcOEt–LP	23.8	0.90	0.95	0.40	3370, 3308, 1744, 1679, 1638, 1541
Z-L-(αMe)Hph-(L-Ala)$_2$-OMe	30	83–85	AcOEt–LP	−25.1	0.70	0.95	0.45	3327, 1706, 1654
Z-L-(αMe)Hph-(D-Ala)$_2$-OMe	35	123–124	AcOEt–LP	44.6	0.80	0.95	0.40	3345, 3311, 1757, 1746, 1706, 1650, 1549
(c) (αMe)Hph-Aib peptides								
Z-L-(αMe)Hph-Aib-OBu^t	57	137–138	AcOEt–LP	−1.8[g]	0.60	0.95	0.60	3401, 3307, 1651, 1539
Z-Aib-L-(αMe)Hph-Aib-OBu^t	62	oil	AcOEt–LP	2.2	0.75	0.95	0.45	3356, 1731, 1706, 1671
Z-L-(αMe)Hph-(Aib)$_2$-OBu^t	67	oil	AcOEt–LP	−10.8	0.70	0.95	0.40	3362, 1730, 1706, 1666, 1526
Z-(Aib)$_2$-L-(αMe)Hph-Aib-OBu^t	66	oil	AcOEt–LP	−16.4	0.65	0.95	0.40	3341, 1731, 1704, 1671, 1527
Ac-(Aib)$_2$-L-(αMe)Hph-(Aib)$_2$-OBu^t	46	209–210	MeOH–EE	−4.6	0.50	0.90	0.20	3398, 1720, 1662, 1649
p-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αMe)Hph-(Aib)$_2$-OBu^t	63	237–238	AcOEt–LP	16.1	0.65	0.95	0.35	3326, 1727, 1668, 1642
(d) (αMe)Hph homo-peptides								
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OBu^t	70	oil	AcOEt–LP	−6.5	0.95	0.95	0.70	3352, 1718, 1654[f]
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OH	70	174–175	AcOEt–LP	−8.3	0.95	0.95	0.30	3398, 3323, 1726, 1672, 1627
Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OH	96	oil	AcOEt–LP	21.4[g]	0.95	0.95	0.75	3395, 1817, 1671[f]
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_3$-OBu^t	54	168–169	AcOEt–LP	−5.6	0.95	0.95	0.60	3310, 1700, 1650, 1529
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_3$-OH	98	150–152	AcOEt–LP	−12.5	0.50	0.95	0.30	3408, 3341, 1727, 1698, 1661
Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_3$-OH	92	oil	AcOEt–LP	7.3[g]	0.95	0.95	0.70	3359, 1820, 1652
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_4$-OBu^t	41	98–100	AcOEt–LP	−17.9	0.95	0.95	0.55	3375, 1725, 1670
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_4$-OH	50	103–104	AcOEt–LP	−20.9	0.55	0.95	0.40	3421, 3334, 1732, 1657
Oxazol-5(4*H*)-one from *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_4$-OH	83	105–106	EE–LP	43.1[g]	0.95	0.90	0.65	3376, 3336, 1814, 1673
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_5$-OBu^t	42	124–125	AcOEt–LP	−16.1	0.95	0.90	0.60	3328, 1729, 1666, 1528

[a] Determined on a Leitz model Laborlux 12 apparatus (Wetzlar, Germany). [b] AcOEt, ethyl acetate; LP, 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] 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. [f] Determined in a film. [g] c = 0.5 (AcOEt).

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

Peptide	$\lambda_{3500-3300}$/cm^{-1}	$\lambda_{1800-1620}$/cm^{-1}
Z-L-(αMe)Hph-L-Ala-OMe	3431, 3395	1740, 1719$_w$, 1674
Z-L-(αMe)Hph-D-Ala-OMe	3431, 3394	1740, 1719$_w$, 1673
Z-L-Ala-L-(αMe)Hph-L-Ala-OMe	3429, 3372	1741, 1717, 1703$_w$, 1685$_w$, 1665
Z-D-Ala-L-(αMe)Hph-D-Ala-OMe	3430, 3371	1742, 1718$_w$, 1702, 1685$_w$, 1665
Z-L-(αMe)Hph-(L-Ala)$_2$-OMe	3429, 3390$_w$, 3352	1741$_w$, 1719, 1686$_w$, 1664
Z-L-(αMe)Hph-(D-Ala)$_2$-OMe	3429, 3397$_w$, 3356	1741, 1718, 1686$_w$, 1664
Z-L-(αMe)Hph-Aib-OBu^t	3452$_w$, 3434, 3395	1722, 1672
Z-Aib-L-(αMe)Hph-Aib-OBu^t	3432, 3393$_w$, 3372	1720, 1688$_w$, 1662
Z-L-(αMe)Hph-(Aib)$_2$-OBu^t	3455$_w$, 3430, 3395$_w$, 3374	1721, 1687$_w$, 1663
Z-(Aib)$_2$-L-(αMe)Hph-Aib-OBu^t	3428, 3361	1718, 1684, 1661$_w$
Ac-(Aib)$_2$-L-(αMe)Hph-(Aib)$_2$-OBu^t	3460$_w$, 3442$_w$, 3427, 3349	1727, 1679, 1659$_w$
p-BrC$_6$H$_4$CO-(Aib)$_2$-L-(αMe)Hph-(Aib)$_2$-OBu^t	3457$_w$, 3434, 3349	1727, 1677, 1660$_w$
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_2$-OBu^t	3448, 3391	1722, 1714$_w$, 1676, 1654
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_3$-OBu^t	3446, 3421$_w$, 3393, 3358	1722, 1714$_w$, 1680, 1652
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_4$-OBu^t	3448$_w$, 3410$_w$, 3392$_w$, 3356	1722, 1682, 1655
p-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_5$-OBu^t	3433$_w$, 3408, 3341	1724, 1673, 1655

[a] Peptide concentration = 1 × 10^{-3} mol dm^{-3}. [b] w = Weak band.

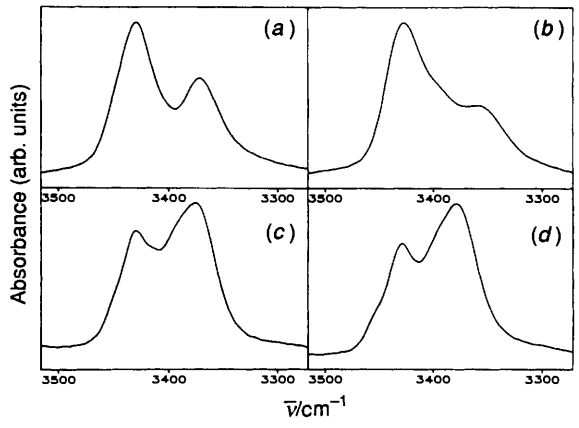

Fig. 2 FTIR absorption spectra (3500–3250 cm^{-1} region) of (*a*) Z-L-Ala-L-(αMe)Hph-L-Ala-OMe, (*b*) Z-L-(αMe)Hph-(L-Ala)$_2$-OMe, (*c*) Z-Aib-L-(αMe)Hph-Aib-OBu^t and (*d*) Z-L-(αMe)Hph-(Aib)$_2$-OBu^t in $CDCl_3$ solution. Peptide concentration: 1 × 10^{-3} mol dm^{-3}.

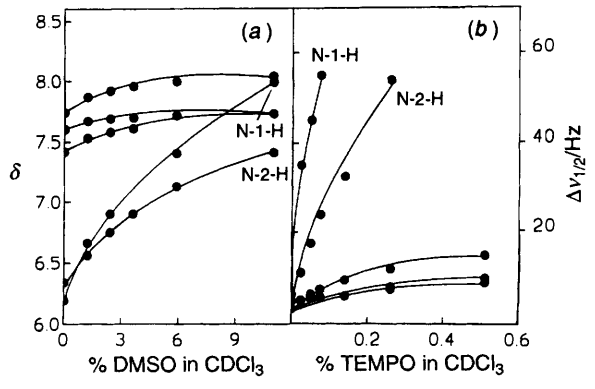

Fig. 3 (*a*) Plot of NH chemical shifts in the ^{1}H NMR spectrum of *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_5$-OBu^t as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. (*b*) Plot of the bandwidth of the NH protons of the same peptide as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration: 1 × 10^{-3} mol dm^{-3}.

(strongly hydrogen-bonded NH groups) (Figs. 1 and 2 and Table 2).[39,40] The intensity of the low-frequency band relative to the high-frequency band significantly increases: (*i*) as main-chain length increases, (*ii*) from (αMe)Hph-Ala to (αMe)Hph-Aib peptides and (*iii*) from (αMe)Hph at position 1 to (αMe)Hph at position 2 in the (αMe)Hph-Ala peptides. No appreciable differences are seen in the spectra of the diastereoisomeric (αMe)Hph-Ala peptides. In addition, a weak band (shoulder) in the 3397–3390 cm^{-1} region (weakly

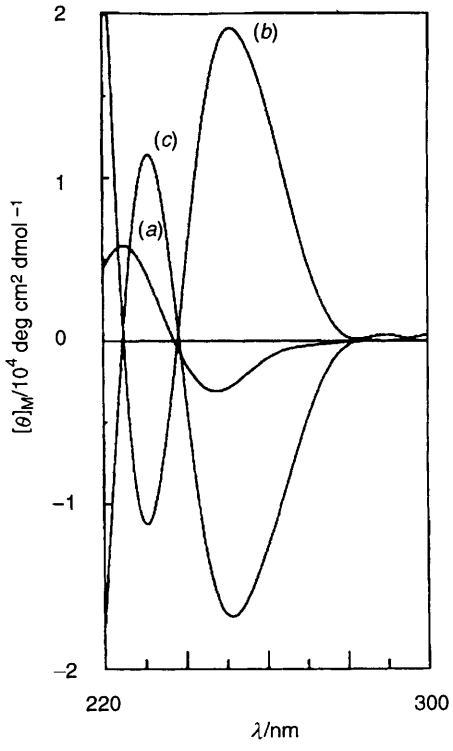

Fig. 4 CD spectra in the 220–300 nm region of (*a*) *p*-BrC$_6$H$_4$CO-[D-(αMe)Hph]$_3$-OBu^t, (*b*) *p*-BrC$_6$H$_4$CO-[D-(αMe)Phe]$_3$-OBu^t and (*c*) *p*-BrC$_6$H$_4$CO-[L-(αEt)Phe]$_2$-NHPr^i in MeOH solution. Peptide concentration: 1 × 10^{-3} mol dm^{-3}.

hydrogen-bonded NH groups) is visible in the spectra of the shortest 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 10%) for all peptides (results not shown). Therefore, the observed 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 a large population of intramolecularly hydrogen-bonded folded species. The observation of the 3372–3341 cm^{-1} band in the tri-, tetra- and penta-peptides, which is absent in the dipeptides, seems to indicate that the (αMe)Hph peptides do not tend to adopt a γ-turn (C_7) conformation[6,41,42] even in a solvent of low polarity ($CDCl_3$)

and highlights the tendency of the (αMe)Hph 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[42-44] tends to decrease relative to the strongly hydrogen-bonded folded conformers.

To get more detailed information on the preferred conformation of these peptides in $CDCl_3$ solution we carried out a 400 MHz ^1H NMR investigation. The delineation of inaccessible (or intramolecularly hydrogen-bonded) NH groups by ^1H NMR spectroscopy was performed by using (*i*) solvent dependence of NH chemical shifts by adding increasing amounts of the hydrogen bonding acceptor DMSO[45,46] to the $CDCl_3$ solution and (*ii*) free-radical TEMPO-induced line broadening of NH resonances.[47]

With regard to the conformationally significant homo-pentapeptide, 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^{α}-*para*-bromobenzoylated peptides from different types of $C^{\alpha,\alpha}$-disubstituted glycines.[12,19] From an analysis of the spectrum as a function of concentration (1×10^{-2}–1×10^{-3} mol dm ^{3}) in $CDCl_3$ solution (results not shown), we have been able to conclude that dilution induces a very modest shift to higher fields of the NH resonances. In particular, the most sensitive N(1)H proton shifts only by 0.05 ppm.

In the homo-pentapeptide examined in the $CDCl_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO at 1×10^{-3} mol dm^{-3} peptide concentration, two classes of NH protons were observed (Fig. 3). 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 ^1H NMR results allow us to conclude that, in $CDCl_3$ solution at 1×10^{-2} mol dm^{-3} concentration, the homo-pentapeptide has 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 ^1H 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 recently 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 oppositely signed bands, negative at higher wavelengths, are visible in the CD spectrum of the D-(αMe)Hph homo-tripeptide in MeOH solution (Fig. 4). The cross-over point between the two components of this exciton splitting is seen in the vicinity of 240 nm, the region where the absorption maximum of the *para*-bromobenzamido chromophore is found.[48] This CD pattern is indicative of the onset of a predominant population of left-handed helical structures for the D-(αMe)Hph peptide in MeOH solution. Interestingly, the screw sense preference is opposite to that shown by (αMe)Phe[16] and (αEt)Phe[19] peptides with the same α-carbon absolute configuration.

Conclusions

In the first detailed investigation of the preferred conformation of peptides containing a C^{α}-methylated, δ-branched amino acid [(αMe)Hph] we have been able to show that this sterically demanding $C^{\alpha,\alpha}$-disubstituted glycine tends to induce either 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 the γ-branched (αMe)Phe[10-17], (αEt)Phe[1,18,19] and Phe[11,39,49,50] containing peptides allows us to conclude that the (αMe)Hph residue is as potent a β-turn and helix inducer as (αMe)Phe and (αEt)Phe, and more potent than the unalkylated parent compound Phe.

As for the relationship between α-carbon chirality and turn and helix handedness of the (αMe)Hph peptides, the CD data point to a behaviour similar to that characteristic of protein amino acids, including Phe, namely L-(αMe)Hph peptides give right-handed turns and helical structures. This property makes the (αMe)Hph peptides conformationally dissimilar from their (αMe)Phe and (αEt)Phe analogues. In summary, these findings confirm that the major factor responsible for the screw sense preference adopted by peptides from C^{α}-alkylated phenyl-containing α-amino acids is the position of their side-chain branching.

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