Structural Versatility of Peptides from $C^{\alpha,\alpha}$-Disubstituted Glycines. Synthesis, Characterization, and Solution Conformational Analysis of Homopeptides from C^{α}-Methyl-C^{α}-benzylglycine, [(α-Me)Phe]$_n$[1]

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ABSTRACT: A complete series of N- and C-blocked, monodispersed homooligopeptides from the sterically hindered (α-Me)Phe residue to the pentamer level was synthesized step-by-step by solution methods and fully characterized. The preferred conformation in chloroform solution was determined by FT-IR and ^{1}H NMR as a function of concentration and addition of a perturbing agent. In particular, the results obtained strongly support the view that a series of three consecutive β-turns (3_{10}-helix) is preferentially adopted by the pentamer, a clear indication that the (α-Me)Phe residue is an efficient β-turn and helix former, much stronger than its unmethylated parent compound (Phe). A comparison is also made with the conclusions extracted from a published work on homopeptides from Aib, the prototype of $C^{\alpha,\alpha}$-disubstituted glycines.

Introduction

The presence of a fully substituted α-carbon in the $C^{\alpha,\alpha}$-disubstituted glycines induces severe restrictions in the backbone conformation of the related homopolypeptides. This observation has motivated an intense investigation directed toward a comprehensive understanding of the conformational states and energetics of such polymers using both experimental and theoretical approaches (for recent review articles, see refs 2–5). This point is of special relevance to the exploitation of these compounds as precise molecular rulers or as scaffolding blocks in the *de novo* design of protein and enzyme mimetics.

A particularly interesting subfamily of $C^{\alpha,\alpha}$-disubstituted glycines is represented by C^{α}-*methylated* α-amino acids. For peptides rich in the Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine or C^{α}-methylalanine) residue an overall helical conformation for the peptide backbone has been unambiguously established. More specifically, monodispersed Aib homooligomers have been found to adopt the 3_{10}-helical structure.[2-5]

With the aim of expanding this line of research we have initiated a program that includes step-by-step synthesis and structural characterization of a complete, terminally-blocked, monodispersed homooligopeptide series from C^{α}-methyl-C^{α}-benzylglycine [or C^{α}-methylphenylalanine, (α-Me)Phe] to the pentamer level. This paper describes details of the synthetic work along with the solution conformational preferences, as assessed in chloroform by FT-IR absorption spectroscopy and ^{1}H NMR as a function of peptide concentration and addition of dimethyl sulfoxide (Me_2SO).

The selection of the (α-Me)Phe residue for this study was based on the following observations: (i) It has recently been incorporated in synthetic analogs of opioid peptides,[6-10] angiotensin II,[11-14] kinins,[15] atrial natriuretic factor,[16] luliberin,[17] angiotensin converting enzyme inhibitor,[18] and the formyl–methionyl tripeptide chemoattractant.[19] (ii) Its conformational preferences will be compared to the known tendency to form α-helices and β-sheets exhibited by Phe, its unmethylated protein analog.[20-24] (iii) The fact that there is limited number of papers incorporating synthetic details[25-33] and conformational analysis[19,32-41] of derivatives and peptides containing this $C^{\alpha,\alpha}$-disubstituted glycine.

Experimental Section

Synthesis of Peptides. The synthesis and characterization of the intermediates HCl·H-L-(α-Me)Phe-OMe (OMe, methoxy),[12,19,37] Z-D-(α-Me)Phe-NHMe (Z, benzyloxycarbonyl; NHMe, methylamino),[37] Z-L-(α-Me)Phe-OH,[37] and the 5(4*H*)-oxazolone from *p*BrBz-D-(α-Me)Phe-OH[37] (*p*BrBz, *para*-bromobenzoyl) have been described. Newly synthesized intermediates and homopeptides are as follows.

***p*BrBz-[D-(α-Me)Phe]$_2$-NHMe.** This compound was synthesized from the 5(4*H*)-oxazolone from *p*BrBz-D-(α-Me)Phe-OH and H-D-(α-Me)Phe-NHMe (obtained, in turn, by catalytic hydrogenation of the corresponding Z derivative) in anhydrous acetonitrile under reflux for 30 h: yield 55%; mp 204–205 °C (from ethyl acetate–petroleum ether); TLC (silica gel plates 60F-254 Merck) R_{f1} ($CHCl_3$–ethanol, 9:1) 0.80, R_{f3} (toluene–ethanol, 7:1) 0.10; $[\alpha]^{20}_D$ 77.0° (*c* 0.5, methanol); IR absorption (KBr) ν_{max} 3416, 3405, 3326, 3273, 1694, 1676, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56 and 7.37 (2m, 4 H, *p*BrBz phenyl), 7.36–6.78 [m, 10 H, (α-Me)Phe phenyls], 6.92 (q, 1 H, N*H*Me), 6.46 (s, 1 H, NH), 5.99 (s, 1 H, NH), 3.40 and 2.73 (2d, 2 H, β-CH_2), 3.24 (m, 2 H, β-CH_2), 2.80 (d, 3 H, NH*Me*), 1.68 (1, 3 H, β-CH_3), 1.58 (s, 3 H, β-CH_3). Anal. Calcd for $C_{28}H_{30}N_3O_3Br$: C, 62.7; H, 5.6; N, 7.8. Found: C, 62.1; H, 5.5; N, 7.7.

***p*BrBz-[L-(α-Me)Phe]$_2$-OMe.** This compound was prepared from the 5(4*H*)-oxazolone from *p*BrBz-L-(α-Me)Phe-OH and H-L-(α-Me)Phe-OMe in anhydrous acetonitrile under reflux for 30 h: yield 57%; mp 52–54 °C (from ethyl acetate–petroleum ether); TLC R_{f1} 0.90, R_{f2} (1-butanol–acetic acid–water, 3:1:1) 0.95, R_{f3} 0.70, R_{f6} (ethyl acetate–petroleum ether, 1:3) 0.25; $[\alpha]^{20}_D$ −96.2° (*c* 0.5, methanol); IR absorption ($CDCl_3$) ν_{max} 3404, 3378, 1733, 1673, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56 (m, 4 H, *p*BrBz phenyl), 7.29 (s, 1 H, NH), 7.22–6.95 [m, 10 H, (α-Me)Phe phenyls], 6.85 (s, 1 H, NH), 3.84 (s, 3 H, OMe), 3.67 and 3.14 (2d, 2 H, β-CH_2), 3.55 and 3.16 (2d, 2 H, β-CH_2), 1.63 (s, 3 H, β-CH_3), 1.57 (s, 3 H, β-CH_3). Anal. Calcd for $C_{28}H_{29}N_2O_4Br$: C, 62.6; H, 5.4; N, 5.2. Found: C, 62.4; H, 5.6; N, 5.0.

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Z-D-(α-Me)Phe-OtBu (OtBu, *tert*-Butoxy). This compound was prepared from Z-D-(α-Me)Phe-OH and isobutene in the presence of a catalytic amount of concentrated H_2SO_4 in anhydrous methylene chloride: yield 94%; oil; TLC R_{f1} 0.95, R_{f2} 0.90, R_{f3} 0.90; $[\alpha]^{20}_D$ 41.2° (*c* 0.5, methanol); IR absorption ($CDCl_3$) ν_{max} 3451, 3413, 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 5 H, Z phenyl), 7.18 and 7.03 [2m, 5 H, (α-Me)Phe phenyls], 5.55 (s, 1 H, NH), 5.12 (m, 2 H, Z CH_2), 3.43 and 3.12 (2d, 2 H, β-CH_2), 1.62 (s, 3 H, β-CH_3), 1.44 (s, 9 H, OtBu).

pBrBz-[D-(α-Me)Phe]$_2$-OtBu. This compound was synthesized from the 5(4*H*)-oxazolone from pBrBz-D-(α-Me)Phe-OH and H-D-(α-Me)Phe-OtBu (obtained, in turn, by catalytic hydrogenation of the corresponding Z derivative) in anhydrous acetonitrile under reflux for 64 h: yield 52%; oil; TLC R_{f1} 0.95, R_{f2} 0.95, R_{f3} 0.90, R_{f4} (ethyl acetate–petroleum ether, 1:4) 0.60; $[\alpha]^{20}_D$ 89.7° (*c* 0.5, methanol); IR absorption ($CDCl_3$) ν_{max} 3391, 1727, 1673, 1652, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56 (m, 4 H, pBrBz phenyl), 7.43 (s, 1 H, NH), 7.19 and 7.07 [2m, 10 H, (α-Me)Phe phenyls], 6.89 (s, 1 H, NH), 3.74 and 3.59 (2d, 2 H, β-CH_2), 3.13 and 3.10 (2d, 2 H, β-CH_2), 1.65 (s, 3 H, β-CH_3), 1.61 (s, 3 H, β-CH_3), 1.53 (s, 9 H, OtBu).

pBrBz-[D-(α-Me)Phe]$_2$-OH. This compound was prepared by treatment of pBrBz-[D-(α-Me)Phe]$_2$-OtBu with a 1:1 mixture of trifluoroacetic acid–methylene chloride: yield 80%; mp 171–172 °C (from ethyl acetate–petroleum ether); R_{f1} 0.35, R_{f2} 0.95, R_{f3} 0.30; $[\alpha]^{20}_D$ 79.0° (*c* 0.5, methanol); IR absorption (KBr) ν_{max} 3393, 3320, 1730, 1661, 1630, 1589, 1564 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.53 (m 4 H, pBrBz phenyl), 7.26–7.07 [m, 10 H, (α-Me)Phe phenyls], 6.94 (s, 1 H, NH), 6.77 (s, 1 H, NH), 3.50–3.26 (m, 4 H, β-CH_2), 1.67 (s, 3 H, β-CH_3), 1.58 (s, 3 H, β-CH_3).

5(4*H*)-Oxazolone from pBrBz-[D-(α-Me)Phe]$_2$-OH. This compound was synthesized from pBrBz-[D-(α-Me)Phe]$_2$-OH in anhydrous acetonitrile in the presence of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride: yield 95%; oil; TLC R_{f1} 0.95, R_{f3} 0.95, R_{f4} 0.70; $[\alpha]^{20}_D$ −50.3° (*c* 0.5, ethyl acetate); IR absorption (KBr) ν_{max} 3398, 1824, 1658, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.59 (m, 4 H, pBrBz phenyl), 7.26–6.95 [m, 11 H, (α-Me)Phe phenyls and NH], 3.77 and 2.88 (2d, 2 H, β-CH_2), 3.05 (m, 2 H, β-CH_2), 1.40 (s, 3 H, β-CH_3), 1.32 (s, 3 H, β-CH_3).

pBrBz-[D-(α-Me)Phe]$_3$-OtBu. This compound was prepared from the 5(4*H*)-oxazolone from pBrBz-[D-(α-Me)Phe]$_2$-OH and H-D-(α-Me)Phe-OtBu in anhydrous acetonitrile under reflux for 15 h: yield 71%; mp 152–154 °C (from ethyl acetate–petroleum ether); TLC R_{f1} 0.95, R_{f2} 0.95, R_{f3} 0.70; $[\alpha]^{20}_D$ 124.7° (*c* 0.5, methanol). IR absorption (KBr) ν_{max} 3330, 1716, 1692, 1650, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.51 (m, 4 H, pBrBz phenyl), 7.29–6.99 [m, 18 H, (α-Me)Phe phenyls and NH], 3.68 and 3.17 (2d, 2 H, β-CH_2), 3.56 and 3.23 (2d, 2 H, β-CH_2), 3.41 and 3.13 (2d, 2 H, β-CH_2), 1.65 (s, 3 H, β-CH_3), 1.60 (s, 3 H, β-CH_3), 1.53 (s, 9 H, OtBu), 1.43 (s, 3 H, β-CH_3).

pBrBz-[D-(α-Me)Phe]$_3$-OH. This compound was synthesized by treatment of pBrBz-[D-(α-Me)Phe]$_3$-OtBu with a 1:1 mixture of trifluoroacetic acid–methylene chloride: yield 92%; mp 210–211 °C (from diethyl ether); TLC R_{f1} 0.45, R_{f2} 0.95, R_{f3} 0.15; $[\alpha]^{20}_D$ 137.3° (*c* 0.5, methanol); IR absorption (KBr) ν_{max} 3396, 3302, 1739, 1654, 1589, cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 8.18 (s, 1 H, NH), 7.65 (s, 1 H, NH), 7.62 (m, 4 H, pBrBz phenyl), 7.20–7.02 [m, 16 H, (α-Me)Phe phenyls and NH], 3.61–2.92 (m, 6 H, β-CH_2), 1.34 (s, 3 H, β-CH_3), 1.27 (s, 3 H, β-CH_3), 1.11 (s, 3 H, β-CH_3).

5(4*H*)-Oxazolone from pBrBz-[D-(α-Me)Phe]$_3$-OH. This compound was prepared from pBrBz-[D-(α-Me)Phe]$_3$-OH in anhydrous acetonitrile in the presence of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride: yield 95%; oil; TLC R_{f1} 0.95, R_{f3} 0.90, R_{f4} 0.40; $[\alpha]^{20}_D$ 2.5° (*c* 0.5, ethyl acetate); IR absorption (KBr) ν_{max} 3384, 1826, 1657, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.55 (m, 4 H, pBrBz phenyl), 7.32 (s, 1 H, NH), 7.20, 7.07, and 6.90 [3m, 15 H, (α-Me)Phe phenyls], 7.00 (s, 1 H, NH), 3.87 and 3.02 (2d, 2 H, β-CH_2), 3.57 and 2.82 (2d, 2 H, β-CH_2), 3.10 (m, 2 H, β-CH_2), 1.72 (s, 3 H, β-CH_3), 1.37 (s, 3 H, β-CH_3), 1.30 (s, 3 H, β-CH_3).

pBrBz-[D-(α-Me)Phe]$_4$-OtBu. This compound was synthesized from the 5(4*H*)-oxazolone from pBrBz-[D-(α-Me)Phe]$_3$-OH and H-D-(α-Me)Phe-OtBu in anhydrous acetonitrile under reflux for 60 h: yield 56%; mp 224–226 °C (from ethyl acetate–petroleum ether); TLC R_{f1} 0.95, R_{f2} 0.90, R_{f3} 0.60; $[\alpha]^{20}_D$ 172.6° (*c* 0.5, methanol); IR absorption (KBr) ν_{max} 3433, 3330, 1723, 1670, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50 (m, 4 H, pBrBz phenyl), 7.44 (s, 1 H, NH), 7.29 (s, 1 H, NH), 7.26–6.97 [m, 20 H, (α-Me)Phe phenyls], 6.15 (s, 1 H, NH), 6.12 (s, 1 H, NH), 3.67 and 3.33 (2d, 2 H, β-CH_2), 3.49 and 3.39 (2d, 2 H, β-CH_2), 3.38 (m, 2 H, β-CH_2), 3.27 and 3.16 (2d, 2 H, β-CH_2), 1.60 (s, 3 H, β-CH_3), 1.55 (s, 3 H, β-CH_3), 1.48 (s, 9 H, OtBu), 1.37 (s, 3 H, β-CH_3), 1.28 (s, 3 H, β-CH_3).

pBrBz-[D-(α-Me)Phe]$_4$-OH. This compound was prepared by treatment of pBrBz-[D-(α-Me)Phe]$_4$OtBu with a 1:1 mixture of trifluoroacetic acid–methylene chloride: yield 90%; mp 294–296 °C (from acetone–ethyl acetate); TLC R_{f1} 0.50, R_{f2} 0.90, R_{f3} 0.15; $[\alpha]^{20}_D$ 198.2° (*c* 0.5, acetone); IR absorption (KBr) ν_{max} 3297, 1743, 1658, 1603, 1589, 1566 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 8.06 (s, 1 H, NH), 7.97 (s, 1 H, NH), 7.71 (s, 1 H, NH), 7.60 (s, 1 H, NH), 7.57–7.41 (m, 4 H, pBrBz), 7.23–6.90 [m, 20 H, (α-Me)Phe phenyls], 3.63–2.82 (m, 8 H, β-CH_2) 1.42 (s, 3 H, β-CH_3), 1.35 (s, 3 H, β-CH_3), 1.23 (s, 3 H, β-CH_3), 1.05 (s, 3 H, β-CH_3).

5(4*H*)-Oxazolone from pBrBz-[D-(α-Me)Phe]$_4$-OH. This compound was synthesized from pBrBz-[D-(α-Me)Phe]$_4$-OH in anhydrous acetonitrile in the presence of *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide hydrochloride: yield 84%; mp 95–97 °C (from ethyl acetate–petroleum ether); TLC R_{f1} 0.95, R_{f3} 0.75, R_{f4} 0.25; $[\alpha]^{20}_D$ 99.5° (*c* 0.5, ethyl acetate); IR absorption (KBr) ν_{max} 3379, 1820, 1659, 1603, 1589, 1566 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.55 (m, 4 H, pBrBz phenyl), 7.26–6.94 [m, 23 H, (α-Me)Phe phenyls and NH], 3.60 and 3.05 (2m, 8 H, β-CH_2), 1.57 (s, 3 H, β-CH_3), 1.52 (s, 3 H, β-CH_3), 1.36 (s, 3 H, β-CH_3), 1.34 (s, 3 H, β-CH_3).

pBrBz-[D-(α-Me)Phe]$_5$-OtBu. This compound was prepared from the 5(4*H*)-oxazolone from pBrBz-[D-(α-Me)Phe]$_4$-OH and H-D-(α-Me)Phe-OtBu in anhydrous acetonitrile under reflux for 24 h: yield 56%; mp 326–327 °C (from diethyl ether–petroleum ether); TLC R_{f1} 0.95, R_{f2} 0.90, R_{f3} 0.55; $[\alpha]^{20}_D$ 390.9° (*c* 0.5, methanol); IR absorption (KBr) ν_{max} 3431, 3325, 1728, 1667, 1532 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1 H, NH), 7.55 (s, 1 H, NH), 7.46 and 6.99 (2m, 4 H, pBrBz phenyl), 7.25–6.72 [m, 27 H, (α-Me)Phe phenyls and NH], 5.65 (s, 1 H, NH), 5.59 (s, 1 H, NH), 3.85 and 3.22 (2d, 2 H, β-CH_2), 3.76 and 3.36 (2d, 2 H, β-CH_2), 3.42 and 3.21 (2d, 2 H, β-CH_2), 3.33 and 2.94 (2d, 2 H, β-CH_2), 2.98 and 2.66 (2d, 2 H, β-CH_2), 1.57 (s, 3 H, β-CH_3), 1.55 (s, 3 H, β-CH_3), 1.51 (s, 3 H, β-CH_3), 1.49 (s, 9 H, OtBu), 1.22 (s, 3 H, β-CH_3), 1.10 (s, 3 H, β-CH_3).

Infrared Absorption. Infrared absorption spectra were recorded with a Perkin-Elmer Model 1720X FT-IR spectrometer, nitrogen flushed, at 2-cm^{-1} nominal resolution, averaging 16 scans for 10 and 1.0 mM sample concentrations or 64 scans for 0.1 mM sample concentration. 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 deuteriochloroform (99.8% *d*) was purchased from Merck. For the solid-state measurements the KBr disk technique was used.

^{1}H Nuclear Magnetic Resonance. The ^{1}H NMR nuclear magnetic resonance spectra were recorded either with a Bruker Model WP 200 SY or with a Bruker Model AM 400 spectrometer (processed on a Bruker X-32 computer). Measurements were carried out in deuteriochloroform (99.96% *d*; Merck) and dimethyl-d_6 sulfoxide (99.96% d_6; Stohler) with tetramethylsilane as the internal standard. Two-dimensional ROESY (Rotating-frame Overhauser Effect SpectroscopY)[42] spectra were obtained with a continuous-wave spin-locking pulse of 200 and 250 ms delivered with a power of $\gamma B_2/2\pi$ = 10 kHz. Pure absorption spectra were obtained according to the TPPI (Time Proportional Phase Incrementation) method[43,44] with 400 experiments. Final spectra of 2K × 2K real points were obtained from data matrices acquired as 400 experiments of 2K complex points. Shifted sine bell functions were applied in both dimensions prior to Fourier transformation.

Results and Discussion

Peptide Synthesis. For the large-scale production of the optically pure (α-Me)Phe enantiomers we have exploited an economically attractive, chemoenzymatic synthesis recently described by some of us.[27] The preparation and characterization of six terminally blocked homopep-

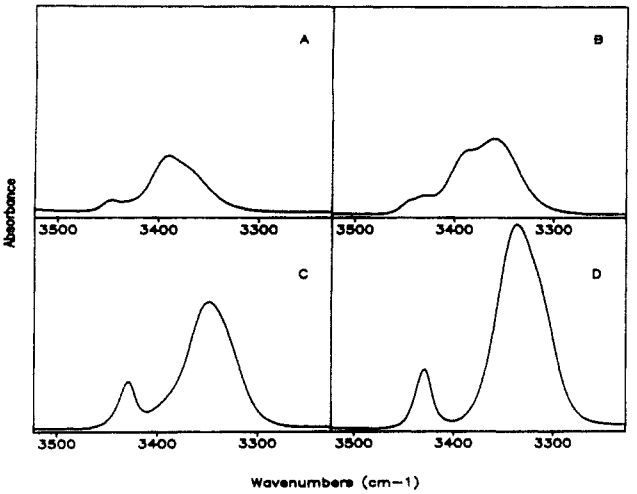

Figure 1. FT-IR absorption spectra in the N–H stretching region of the *p*BrBz-[D-(α-Me)Phe]$_n$-O*t*Bu (A, n = 2; B, n = 3; C, n = 4; D, n = 5) homopeptides in $CDCl_3$ solution (concentration 1 mM).

tides (to the pentamer level) from this sterically hindered residue have been performed. During the coupling reactions (in anhydrous acetonitrile under reflux for 15–60 h) the carboxyl group of the N$^{\alpha}$-blocked amino acid or peptide has been activated using the 5(4*H*)-oxazolone method. After purification the terminally blocked homooligomers were obtained in reasonably good yields (50–70%). The various peptide 5(4*H*)-oxazolones have been prepared by treating the corresponding N$^{\alpha}$-blocked peptide free acids with *N*-ethyl-N′-[3-(dimethylamino)propyl]-carbodiimide. The N$^{\alpha}$-blocked peptide free acids have been obtained by treatment of the corresponding *tert*-butyl esters with diluted trifluoroacetic acid. Removal of the benzyloxycarbonyl N$^{\alpha}$-protecting group was carried out by catalytic hydrogenation. The synthesis of the fully protected amino acid Z-D-(α-Me)Phe-O*t*Bu was achieved by treating Z-D-(α-Me)Phe-OH with isobutene in the presence of a catalytic amount of concentrated H_2SO_4 in anhydrous methylene chloride.

Solution Conformation. The conformational preferences of the terminally blocked (α-Me)Phe homooligomers were investigated in a structure-supporting solvent ($CDCl_3$) by FT-IR absorption and ^{1}H NMR at various concentrations (over the range 10–0.1 mM).

The FT-IR absorption spectra in the N–H stretching region (amide A) of the complete *p*BrBz-[D-(α-Me)Phe]$_n$-O*t*Bu (n = 2–5) series (concentration 1 mM) are illustrated in Figure 1. The curves are characterized by bands at 3449–3430 cm^{-1} (free, solvated NH groups), 3393–3386 cm^{-1} (weakly H-bonded NH groups), and 3358–3337 cm^{-1} (strongly H-bonded NH groups).[45] The intensity of the low-frequency band relative to the high-frequency band(s) increases significantly with increasing main-chain length; in parallel, the absorption maximum shifts markedly to lower wavenumbers (to 3337 cm^{-1} in the pentamer).

Using the Mizushima's dilution method,[46] we have been able to show that even at 10 mM concentration self-association *via* N–H···O=C intermolecular H-bonding is negligible (less than 5%) for all oligomers (results not shown). Therefore, the observed H-bonding should be interpreted as arising almost exclusively from intramolecular N–H···O=C interactions.

Additional structural information has been extracted by analyzing the amide I and II vibrational modes (second derivative technique). The frequencies of the main bands for the two longest oligomers fall at 1669–1664 and 1533–1532 cm^{-1}, respectively (results not shown). In a recent

Table I
Resonance Assignments of the Protons of *p*BrBz-[D-(α-Me)Phe]$_5$-O*t*Bu[a]

groups/residues	NH	CH_3	CH_2	aromatics
*p*BrBz				7.00 (*o*), 7.46 (*m*)
1	5.60	1.22	2.67, 2.98	6.89 (2 H), 7.24 (3 H)
2	5.59	1.10	2.95, 3.33	6.98 (2 H), 7.22 (3 H)
3	7.62	1.52	3.37, 3.77	7.20, 7.26 (5 H)
4	*b*	1.56	3.24, 3.86	6.72 (*p*), 7.03 (*m*), 7.19 (*o*)
5	7.55	1.58	3.23, 3.43	6.90 (*p*), 7.12 (*m*), 7.26 (*o*)
O*t*Bu		1.47		

[a] $CDCl_3$, 1 mM concentration. [b] Under the envelope of aromatic protons around 7.2 ppm.

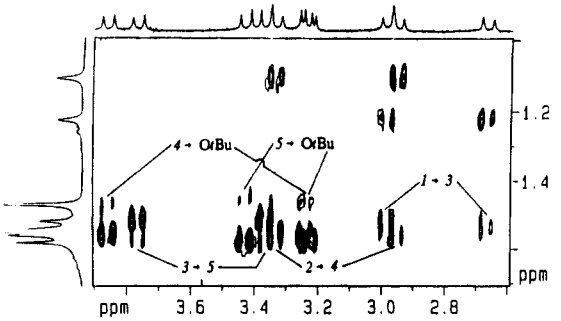

Figure 2. Portion of a ROESY spectrum of *p*BrBz-[D-(α-Me)-Phe]$_5$-O*t*Bu showing the connectivities between the β-methylene and the β-methyl regions. Only negative peaks are plotted. Interresidue connectivities are indicated.

FT-IR study of peptides forming helices in organic solution amide I and II bands in the above regions have been assigned to intramolecularly H-bonded peptide groups of 3_{10}-helical stuctures.[47]

The present FT-IR absorption analysis has provided evidence that main-chain dependent intramolecular H-bonding is the predominant factor for the terminally-blocked (α-Me)Phe homopeptides in $CDCl_3$ solution. To get additional information on the preferred conformation in a $CDCl_3$ solution of *p*BrBz-[D-(α-Me)Phe]$_5$-O*t*Bu, the longest and most significant homopeptide, we carried out a detailed 400-MHz ^{1}H NMR investigation.

Table I lists the resonance assignments. Intraresidue cross-peaks between the β-methyl group and both the amide NH and the β-methylene protons were detected for all residues. On the other hand, only one intraresidue connectivity between the NH and the methylene protons was found and later assigned to the C-terminal residue. The resonance position of two NH protons is upfield shifted and so is that of the corresponding β-methyl group. Residue 1 was assigned based on a connectivity between its NH and the easily distinguished protons of the *p*BrBz moiety. The other set of resonances was assigned to residue 2 by analogy with other similar compounds.[19,37] An interresidue NH → NH cross-peak involving N(2)H was interpreted as sequential [N(2)H → N(3)H], which led to the assignment of residue 3. This assignment was confirmed by a sequential CH_3(2)H → N(3)H connectivity. One of the two remaining NH protons showed an interaction with both remaining β-methyl groups. The most logical assignment is an intraresidue [CH_3(4) → N(4)H] and a sequential interaction [CH_3(4) → N(5)H]. The interresidue connectivities found between β-methylene and β-methyl protons (Figure 2) are consequently assigned to $CH_2(i)$ → $CH_3(i+2)$. This completes the sequential assignment.

In the aromatic region scalar connectivities indicate that the ortho, meta, and para protons of two out of five phenyl rings are not equivalent. For a third ring, a possible connectivity was detected near the diagonal, indicating

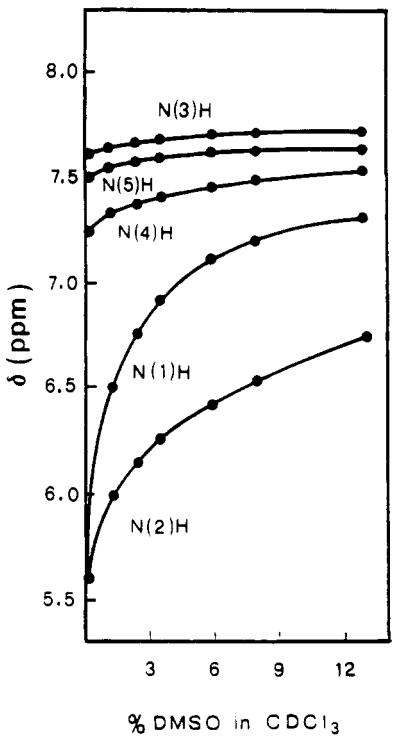

Figure 3. Plot of NH chemical shifts in the ^{1}H NMR spectra of *p*BrBz-[D-(α-Me)Phe]$_5$-O*t*Bu as a function of increasing percentages of Me_2SO added to the $CDCl_3$ solution (v/v). Peptide concentration 1 mM.

that all the protons are nearly equivalent. As for two remaining rings, two protons have different chemical shifts from the other three. The aromatic protons of residue **5** were easily identified as the only ones displaying a proximity to the C-terminal *tert*-butyl group. The aromatic spin systems of residues **2** and **3** were assigned through intraresidue connectivities to the methylene protons. Parts of the spin system of residue **3** and the ortho protons of residue **4** are overlapped. The assignment of the latter was possible because of a cross-peak connecting them to their methylene group and was confirmed by a weaker cross-peak between the same methylene group and the meta protons of the same aromatic system.

An analysis of the spectra of the homopentamer as a function of peptide concentration (20–1 mM) in $CDCl_3$ solution (results not shown) indicates that dilution produces an extremely modest ($0.01 < \text{ppm} < 0.06$) shift of all NH resonances. The delineation of inaccessible (or intramolecularly H-bonded) NH groups was performed with use of solvent (Me_2SO)[48] dependencies of NH chemical shifts. Figure 3 clearly shows two classes of NH protons: (1) The first class [N(1)H and N(2)H protons] includes protons whose chemical shifts are dramatically sensitive to the addition of the strong H-bonding acceptor solvent Me_2SO.[49] (2) The second class (all other NH protons) includes those displaying a behavior characteristic of shielded protons (very modest sensitivity of chemical shifts to solvent composition).

The present ^{1}H NMR results support the view that in $CDCl_3$ solution the N(3)H and the following protons in the peptide chain of the (α-Me)Phe homopentamer are not freely accessible to the perturbing solvent Me_2SO and therefore, most probably, intramolecularly H-bonded. In view of these observations it seems reasonable to conclude that the ordered secondary structure predominantly assumed in this solvent by the (α-Me)Phe homopentamer is the 3_{10}-helix[50] (a series of three consecutive type-III β-bends[51]) rather than the α-helix, which would require the NH protons involved in the intramolecular H-bonding to begin from the N(4)H proton. As additional proof of this structural assignment, the presence of the $CH_2(i) \rightarrow CH_3(i+2)$ interresidue connectivities and the lack of sequential $NH \rightarrow CH_2$ connectivities strongly suggest that the conformation of the peptide is a right-handed 3_{10}-helix. In fact, model building shows that this conformation can account for all the interresidue cross-peaks found. Interestingly, in this context it is worth noting that the X-ray diffraction structure of *p*BrBz-[D-(α-Me)-Phe]$_4$-O*t*Bu, recently solved in the Padova laboratory, is indicative of the occurrence of a regular, right-handed 3_{10}-helix in the crystal state for this homotetramer.[52] These more detailed ^{1}H NMR conclusions are in full agreement with the indications extracted from the FT-IR absorption study discussed above.

Conclusions

In this work we have described the successful step-by-step, solution synthesis of the sterically hindered [(α-Me)-Phe]$_n$ homopeptides to the pentamer using the 5(4*H*)-oxazolone method for the activation of the carboxyl component in the coupling reactions. In addition, we have examined the preferred conformation of the aforementioned oligomers in chloroform solution by using FT-IR and ^{1}H NMR. From the results obtained we are inclined to conclude that the homopentamer preferentially forms a 3_{10}-helical structure.

A comparison of the findings obtained for [(α-Me)Phe]$_n$ homopeptides in solution with the corresponding results already reported for (Phe)$_n$ homooligopeptides[21,23,45,53–56] and the homopolymer[20,24,57] allows us to conclude that the (α-Me)Phe residue is an efficient β-turn and helix former, much stronger than its unmethylated parent compound (Phe). In addition, in contrast to the Phe oligomers, there is no tendency for the (α-Me)Phe oligomers to adopt the self-associated β-sheet conformation.

The solution conformational preferences of the (α-Me)-Phe homopeptides, together with the corresponding tendencies of the homopeptides derived from Aib,[2–5,58] the prototype of C^α-methylated α-amino acids, reinforce the conclusion that C^α-methylation induces a significant propensity for β-turn and 3_{10}-helix formation in the resulting homooligomers.

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References and Notes

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