C^{α}-Methyl,C^{α}-allylglycine (Mag) Homooligomers

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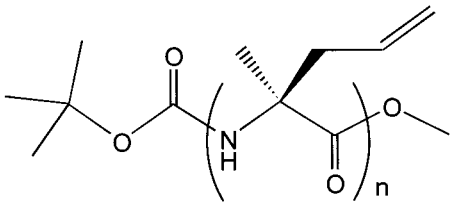
ABSTRACT: A complete series of N^{α}-protected, monodispersed homooligopeptide esters to the pentamer level from L-C^{α}-methyl,C^{α}-allylglycine (L-Mag) have been synthesized step-by-step in solution and fully characterized. The solution preferred conformation of these homooligomers has been assessed by FT-IR absorption and ^{1}H NMR techniques. Moreover, the molecular structures of the homodimer and trimer have been determined in the crystal state by X-ray diffraction, and the conformational energy map of the homotrimer has been computed. The results obtained point to the conclusion that right-handed, single, or multiple β-bends are preferentially adopted by the conformationally restricted L-Mag homooligomers. In particular, 3_{10}-helices are formed by the longest homooligomer (pleionomer). The implications for the use of the Mag residue in designing conformationally constrained peptide substrates for reactions involving the side-chain C═C functionality are briefly discussed.

Introduction

The stabilization of specific ordered secondary structures has recently become a major issue in peptide science, particularly in conjunction with the de novo design of protein and enzyme mimetics. In connection with this purpose one of the most effective strategies pursued for increasing the tendency to fold into β-bends and the 3_{10}-helical structure is C^{α}-methylation of the peptide chain.[1−7]

To gain a deeper understanding of the preferred conformation of this family of C^{α}-tetrasubstituted α-amino acids, we recently embarked on a program directed toward the structural analysis of the trifunctional C^{α}-methyl,C^{α}-allylglycine (Mag) peptides, characterized by a side chain C^{γ}═C^{δ} bond. By a chemoenzymatic approach, developed by DSM Research, we performed a large-scale synthesis of the optically pure L-Mag enantiomer.[8] We also prepared a set of model peptides containing L-Mag in combination with either Aib (α-aminoisobutyric acid) or L-Ala.[8] From our solution and crystal state conformational investigation it appeared that the Mag-based peptides have a marked tendency to fold into β-bends and 3_{10}-helices.

In the present extension of the above study we describe synthesis, characterization, and the results of experimental and theoretical conformational analyses (the former in solution by FT-IR absorption and ^{1}H NMR and in the crystal state by X-ray diffraction) of the homooligomeric, homochiral series Boc-(L-Mag)$_n$-OMe (n = 2−5; Boc, *tert*-butyloxycarbonyl; OMe, methoxy). A preliminary communication of part of this work has been reported.[9]



Boc-(L-Mag)$_n$-OMe

In recent years a number of methods on the synthesis of Mag and its derivatives have been described.[10−24] However, only a few papers have dealt with the incorporation of Mag into peptides.[8,25,26] Mag derivatives and peptides, along with those derived from other more common C^{α}-allyl substituted α-amino acid residues,[27−35] have been exploited in intramolecular C−C bond formation (ring-closing metathesis) and in the preparation of "buit-into" compounds by taking advantage of the readily available carbon−carbon double-bond functionality. Polypeptides have been prepared characterized by

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pendant carbon−carbon double-bond sites as handles for cross-linking reactions.[36,37]

Experimental Section

Synthesis of Peptides. The synthesis and characterization of the derivatives Boc-L-Mag-OH[8] and H-L-Mag-OMe[10] have been described. Newly synthesized homopeptides are as follows.

Boc-(L-Mag)$_2$-OMe. This compound was synthesized from Boc-L-Mag-OH and H-L-Mag-OMe [the latter obtained by reacting the corresponding Boc-protected α-amino ester[8] with a 30% solution of trifluoroacetic acid (TFA) in CH_2Cl_2 for 30 min, followed by treatment with *N*-methylmorpholine (NMM)] in anhydrous CH_2Cl_2 in the presence of *N*-ethyl,*N*′-(3-dimethylamino)propylcarbodiimide (EDC) hydrochloride and 1-hydroxy-7-azabenzotriazole (HOAt),[38] as the hydroxylamine-based additive, at room temperature for 1 day: yield 79%; mp 107−108 °C (from ethyl acetate−light petroleum). TLC (silica gel plates 60F-254 Merck): R_fI ($CHCl_3$−ethanol 9:1) 0.95, R_fII (1-butanol−acetic acid−water 3:1:1) 0.95, R_fIII (toluene−ethanol 7:1) 0.65; $[\alpha]^{20}_D$ −16.3° (*c* 0.5, methanol). IR absorption (KBr): ν_{max} 3397, 3296, 1728, 1708, 1660, 1516 cm^{-1}. ^{1}H NMR ($CDCl_3$, 10 mM): δ 7.09 (s, 1H, Mag NH), 5.70 (m, 2H, 2 Mag γCH), 5.12 (m, 4H, 2 Mag δCH_2), 5.06 (s, 1H, Mag NH), 3.74 (s, 3H, OMe CH_3), 2.90 and 2.59 (m, 4H, 2 Mag βCH_2), 1.57 (s, 3H, Mag βCH_3), 1.47 (s, 3H, Mag βCH_3), 1.44 (s, 9H, Boc CH_3).

Boc-(L-Mag)$_3$-OMe. This compound was prepared from Boc-L-Mag-OH and H-(L-Mag)$_2$-OMe [the latter obtained by reacting the corresponding Boc-protected dipeptide ester (see above) with a 30% solution of TFA in CH_2Cl_2 for 30 min, followed by treatment with NMM] in anhydrous CH_2Cl_2 in the presence of EDC/HOAt at room temperature for 5 days: yield 43%; mp 130−131 °C (from diethyl ether−light petroleum). TLC R_fI 0.90, R_fII 0.95, R_fIII 0.40; $[\alpha]^{20}_D$ −60.1° (*c* 0.5, methanol). IR absorption (KBr): ν_{max} 3410, 3293, 1736, 1689, 1650, 1534 cm^{-1}. ^{1}H NMR ($CDCl_3$, 10 mM): δ 7.46 (s, 1H, Mag NH), 6.65 (s, 1H, Mag NH), 5.79 (m, 3H, 3 Mag γCH), 5.24−5.04 (m, 6H, 3 Mag δCH_2), 4.85 (s, 1H, Mag NH), 3.70 (s, 3H, OMe CH_3), 2.70−2.44 (m, 6H, 3 Mag βCH_2), 1.57 (s, 3H, Mag βCH_3), 1.55 (s, 3H, Mag βCH_3), 1.46 (s, 3H, Mag βCH_3), 1.44 (s, 9H, Boc CH_3).

Boc-(L-Mag)$_4$-OMe. This compound was prepared from Boc-L-Mag-OH and H-(L-Mag)$_3$-OMe [the latter obtained by reacting the corresponding Boc-protected tripeptide ester (see above) with a 30% solution of TFA in CH_2Cl_2 for 30 min, followed by treatment with NMM] in anhydrous CH_2Cl_2 in the presence of EDC/HOAt at room temperature for 6 days: yield 25%; mp 167−168 °C (from ethyl acetate−light petroleum); TLC R_fI 0.85, R_fII 0.95, R_fIII 0.35; $[\alpha]^{20}_D$ −79.6° (*c* 0.5, methanol). IR absorption (KBr): ν_{max} 3322, 1732, 1681, 1528 cm^{-1}. ^{1}H NMR ($CDCl_3$, 10 mM): δ 7.34 (s, 2H, 2 Mag NH), 6.53 (s, 1H, Mag NH), 5.71 (m, 4H, 4 Mag γCH), 5.20−5.02 (m, 8H, 4 Mag δCH_2), 4.82 (s, 1H, Mag NH), 3.69 (s, 3H, OMe CH_3), 2.55 (m, 8H, 4 Mag βCH_2), 1.57 (s, 3H, Mag βCH_3), 1.47 (s, 3H, Mag βCH_3), 1.45 (s, 9H, Boc CH_3), 1.40 (s, 3H, Mag βCH_3), 1.38 (s, 3H, Mag βCH_3).

Boc-(L-Mag)$_5$-OMe. This compound was synthesized from Boc-L-Mag-OH and H-(L-Mag)$_4$-OMe [the latter obtained by reacting the corresponding Boc-protected tetrapeptide ester (see above) with a 30% solution of TFA in CH_2Cl_2 for 30 min, followed by treatment with NMM] in anhydrous CH_2Cl_2 in the presence of EDC/HOAt at room temperature for 10 days: yield 30%; mp 175−177 °C (from ethyl acetate−light petroleum); TLC R_fI 0.85, R_fII 0.95, R_fIII 0.35; $[\alpha]^{20}_D$ −21.0° (*c* 0.2, methanol). IR absorption (KBr): ν_{max} 3298, 1733, 1680, 1659, 1532 cm^{-1}. ^{1}H NMR ($CDCl_3$, 10 mM): δ 7.66 (s, 1H, 1 Mag NH), 7.42 (s, 1H, 1 Mag NH), 7.16 (s, 1H, 1 Mag NH), 6.22 (s, 1H, Mag NH), 5.72 (m, 5H, 5 Mag γCH), 5.17−5.02 (m, 10H, 5 Mag δCH_2), 4.83 (s, 1H, Mag NH), 3.69 (s, 3H, OMe CH_3), 3.11−2.52 (m, 10H, 5 Mag βCH_2), 1.67−1.18 (m, 15H, 5 Mag βCH_3), 1.45 (s, 9H, Boc CH_3).

Infrared Absorption. The solid-state infrared absorption spectra (KBr disk technique) were recorded with a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer. The solution spectra were recorded using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. 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. Solvent (baseline) spectra were obtained under the same conditions.

Table 1. Crystal Data and Diffraction Parameters for the Boc-(L-Mag)$_n$-OMe (n = 2,3) Homooligomers

parameter	Boc-(L-Mag)$_2$-OMe	Boc-(L-Mag)$_3$-OMe
mol formula	$C_{18}H_{30}N_2O_5$	$C_{24}H_{39}N_3O_6$
mol wt, amu	354.4	465.6
crystal system	orthorhombic	tetragonal
space group	$P2_12_12_1$	$P4_1$
Z, mol/unit cell	4	4
a (Å)	23.296(1)	9.619(5)
b (Å)	8.604(2)	9.619(5)
c (Å)	10.418(1)	31.724(5)
V (Å^3)	2088.2(5)	2935(2)
d(calcd), g/cm^{-3}	1.127	1.054
radiation	Cu Kα (1.541 78 Å)	Cu Kα (1.541 78 Å)
data collect method	$\theta/2\theta$	$\theta/2\theta$
crystal size, mm	0.3 × 0.2 × 0.3	0.2 × 0.3 × 0.4
θ range, deg	1−70	1−70
indep refls	2290	2832
obs refls	1892 [$I > 2\sigma(I)$]	2509 [$I > 2\sigma(I)$]
goodness-of-fit	1.446	1.635
solved by	SIR 97[39]	SIR 97[39]
refined by	SHELXL 97[40]	SHELXL 97[40]
final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0780$, $wR_2 = 0.181$	$R_1 = 0.0669$, $wR_2 = 0.194$
R indices (all data)	$R_1 = 0.0904$, $wR_2 = 0.193$	$R_1 = 0.0726$, $wR_2 = 0.202$
$\Delta\rho$, e/Å^3	0.425/−0.499	0.594/−0.193
temp, K	293	293
crystallization solvent	EtOAc/PE[a]	EtOAc/PE[a]

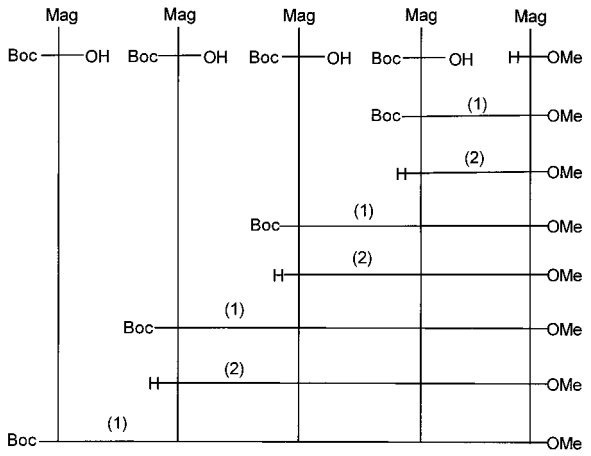
[a] EtOAc, ethyl acetate; PE, petroleum ether. [b] Vapor diffusion.

^{1}H Nuclear Magnetic Resonance. The ^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer. Measurements were carried out in deuteriochloroform (99.96% *d*; Aldrich) and dimethyl-d_6 sulfoxide (Me_2SO) (99.96% d_6; Acros Organics) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) was purchased from Sigma.

X-ray Diffraction. Colorless single crystals of Mag homodimer and trimer were grown at room temperature from the solvents reported in Table 1. The X-ray data were collected on an Enraf-Nonius CAD4 diffractometer of the Biocrystallography Research Center, CNR, at the University of Naples "Federico II". During data collections three standard reflections were periodically measured in order to check the stability of crystals and the electronics. The observed intensity variations were within 3%. The intensities were corrected for Lorentz and polarization factors, but no absorption correction was applied. Unit cell determinations were carried out for all crystals by least-squares refinement of the setting angles of 25 high angle reflections accurately centered.

Both structures were solved by direct methods using the programs mentioned in Table 1. The solution with the best figure of merit revealed the coordinates of all non-H atoms. Refinement of the two structures was performed by full-matrix least-squares procedures with the programs listed in Table 1. All non-H atoms were refined anisotropically. H atoms of the two oligomers were calculated, and during the refinement they were allowed to ride on their carrying atoms with U_{iso} set equal to 1.2 times the U_{eq} of the attached atom. The scattering factors for all atomic species were calculated from Cromer and Waber.[41] Details of the crystallographic data and diffraction parameters for the two structures are given in Table 1. Further details of the crystal structures, including final atomic parameters for the non-H atoms, have been deposited with and

Chart 1



Conditions: (1) EDC/HOAt/NMM in CH_2Cl_2. (2) dil. TFA in CH_2Cl_2, followed by NMM.

are available on request from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (England), on quoting the full journal citation.

Conformational Energy Computations. The theoretical conformational analysis of Boc-(L-Mag)$_3$-OMe was performed using the parameters extracted from the X-ray diffraction structure of the homotrimer. Conformational energy computations were performed using the INSIGHT/DISCOVER package with the consistent valence force field CVFF.[42−44] A dielectric constant of 1 was assumed in all calculations. The conformational space was mapped by calculating the conformational energy at 20° intervals for the ϕ, ψ torsion angles. In all conformational search, the ω torsion angles were fixed at 180°, and the ϕ and ψ angles of the Mag^2 residue were fixed at −60° and −30°, respectively. Minimum-energy conformations were obtained in the low-energy regions located in the above search, minimizing the energy using the Conjugate Gradients algorithm.[45] Conformational energy is expressed as $\Delta E = E - E_0$, where E_0 is the energy of the most stable conformation. All computations were performed on O2 Silicon Graphics workstations of the Biocrystallography Research Center (CNR), University of Naples.

Results and Discussion

Peptide Synthesis. An economically attractive and generally applicable chemoenzymatic synthesis developed by DSM Research[11−14,46,47] was used for the large-scale production of the optically pure L-Mag enantiomer.[8] At first, we performed a phase-transfer catalyzed allylation of N$^\alpha$-benzylidene-DL-alanine amide for the preparation of the racemic α-amino amide which was purified by distillation. Then, we used a broadly specific amino amidase from *Mycobacterium neoaurum* ATCC 25795 to achieve optical resolution, affording the free L-amino acid and the D-amino amide,[11−14] which were separated by ion-exchange chromatography. Alkaline hydrolysis of the D-amino amide gave the corresponding free D-amino acid. Since the enzymatic resolution is not fully enantioselective (E-ratio: 40), a final optical purification was performed by crystallization from 2-propanol/water 3:1. Thus, the L- and D-Mag were obtained in enantiomeric excess >99%. Then, the preparation and full characterization of the terminally protected homooligomeric, homochiral Boc-(L-Mag)$_n$-OMe peptides (to the pentamer level) were performed. The synthesis was carried out step-by-step in solution, beginning from the C-terminal α-amino methyl ester (Chart 1). Peptide bond formation was achieved by the EDC/HOAt C-activation procedure[38] in methylene chloride in the presence of a tertiary amine (NMM). Using this methodology, the sterically hindered L-Mag-L-Mag peptide bonds were formed in variable yields, generally decreasing with increasing peptide size. Removal of the Boc N$^\alpha$-protection was carried out by mild acidolysis (diluted TFA) without disturbing the side-chain C=C double bond(s).

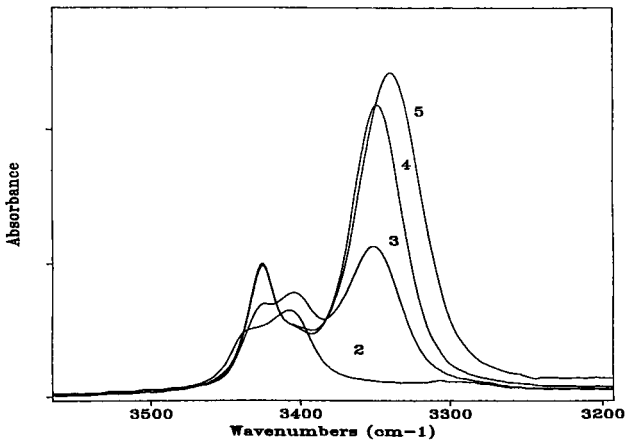


Figure 1. FT-IR absorption spectra in the N−H stretching region of the Boc-(L-Mag)$_n$-OMe (n = 2−5) homooligomers in $CDCl_3$ solution. Peptide concentration: 1 mM.

Solution Conformation. The conformational preferences of the Boc/OMe protected L-Mag homooligomers were investigated in a structure-supporting solvent ($CDCl_3$) by FT-IR absorption and ^{1}H NMR over the peptide concentration range 10−0.1 mM.

The FT-IR absorption spectra in the N−H stretching (amide A) region (peptide concentration: 1 mM) are illustrated in Figure 1. The curves are characterized by bands (shoulders) at 3439−3426 cm^{-1} (free, solvated NH groups),[48] 3404−3400 cm^{-1} (weakly H-bonded NH groups of fully extended conformations),[49] and 3351−3339 cm^{-1} (strongly H-bonded NH groups of folded conformations).[48] The intensity of the low-frequency band relative to those of the high-frequency bands increases significantly as main-chain length increases. We have also been able to demonstrate that even at 10 mM concentration self-association via intermolecular N−H···O=C 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.

The present FT-IR absorption analysis has provided evidence that intramolecular H bonding typical of folded conformations is the predominant feature of the terminally protected, longer L-Mag homooligomers (pleionomers)[50] in $CDCl_3$, a solvent of low polarity.

To get more detailed information on the preferred conformation in $CDCl_3$ solution of Boc-(L-Mag)$_5$−OMe, the longest and most significant homopeptide of this series, we carried out a 400 MHz ^{1}H NMR investigation. All five NH proton resonances were assigned by means of a 2D ROESY experiment beginning from the urethane N(1)H proton at higher field. An analysis of the spectra as a function of peptide concentration (not shown) indicates that a 10-fold dilution (from 10 to 1 mM) produces a variation, albeit small (Δppm = 0.02, to higher fields), only of the chemical shift of the N(1)H proton. For the N(2)H−N(5)H protons the concentration effect is negligible. In agreement with the FT-IR absorption results discussed above, we conclude that at 10 mM concentration in the modest self-association

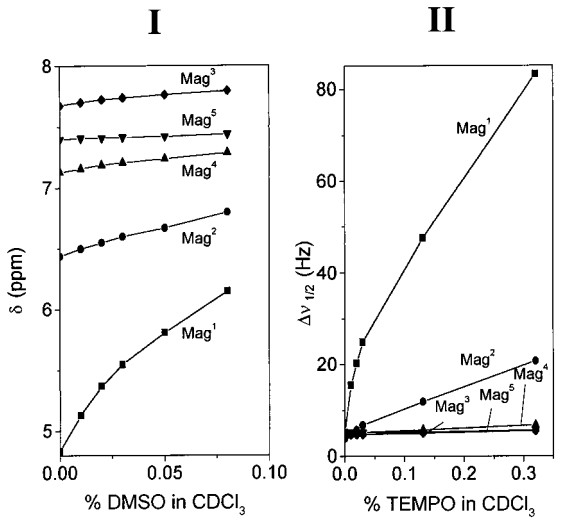


Figure 2. (I) Plot of NH chemical shifts in the ^{1}H NMR spectra of the Boc-(L-Mag)$_5$-OMe homooligomer as a function of increasing percentages of Me_2SO to the $CDCl_3$ solution (v/v). (II) Plot of the bandwidth of the NH protons of the same homooligomer as a function of increasing percentages of TEMPO to the $CDCl_3$ solution (w/v). Peptide concentration: 1.0 mM.

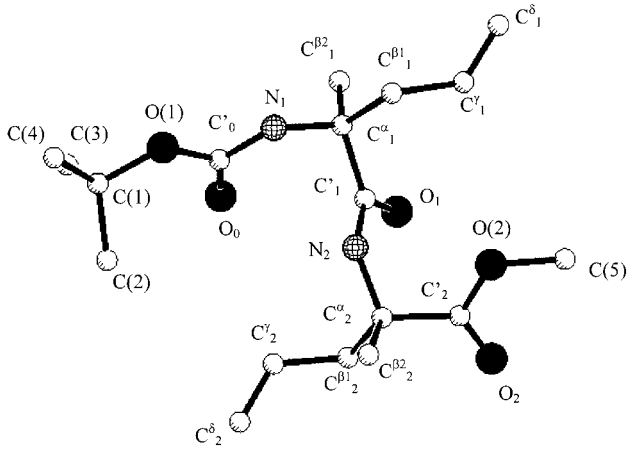


Figure 3. X-ray diffraction structure of the Boc-(L-Mag)$_2$-OMe homooligomer with numbering of the atoms.

phenomenon the urethane N(1)H group plays the role of the H bonding donor.[48,51]

In the absence of self-association (peptide concentration: 1 mM) the delineation of inaccessible (or intramolecular H bonded) NH protons was carried out with use of solvent (Me_2SO)[52] dependence of NH proton chemical shifts and free-radical (TEMPO)[53] induced line broadening of NH proton resonances. Figure 3 (parts I and II) graphically describes the results obtained. Two classes of NH protons were clearly observed: (1) The first class [N(1)H and N(2)H protons] includes protons whose chemical shifts are sensitive to the addition of the strong H bonding acceptor solvent Me_2SO[54] and whose resonances significantly broaden upon addition of TEMPO. Interestingly, the sensitivity of the N(1)H proton is significantly higher than that of the N(2)H proton. (2) The second class [N(3)H−N(5)H protons] include those displaying a behavior characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition and of line widths to the presence of the paramagnetic agent TEMPO).

The present ^{1}H NMR data support the view that at low concentration (1 mM) in $CDCl_3$ solution the N(3)H−N(5)H protons of the pentamer are inaccessible to solvent and perturbing agents and therefore, most probably, intramolecularly H bonded. The intramolecular H bonding scheme of the pentamer does not appear to change upon self-association [involving the N(1)H proton as the donor of the intermolecular H-bond]. Since all NH protons, beginning from the N(3)H proton of Boc-(L-Mag)$_5$-OMe form intramolecular H bonds, we are inclined to conclude that the structure predominantly adopted in $CDCl_3$ by these peptides is the 3_{10}-helix[55] (a series of consecutive, type III β-bends)[56−58] 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.[55] These more detailed conclusions are in full agreement with the preliminary indications extracted from the FT-IR absorption study discussed above.

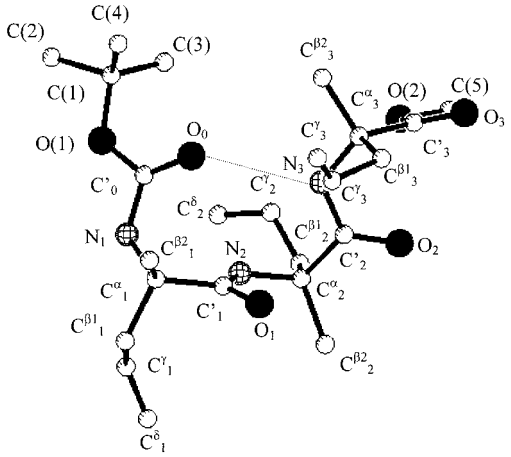


Figure 4. X-ray diffraction structure of the Boc-(L-Mag)$_3$-OMe homooligomer with numbering of the atoms. The intramolecular H bond is represented by a dotted line.

Table 2. Torsion Angles (deg) for the Boc-(L-Mag)$_n$-OMe (n = 2, 3) Homooligomers

torsion angle	Boc-(L-Mag)$_2$-OMe	Boc-(L-Mag)$_3$-OMe
θ^1	170.0 (4)	−177.4 (4)
ω_0	−173.1 (3)	−163.4 (4)
ϕ_1	−55.0 (4)	−63.0 (5)
ψ_1	−42.8 (3)	−26.8 (5)
ω_1	−172.7 (3)	−178.6 (3)
ϕ_2	56.1 (4)	−63.7 (5)
ψ_2	34.7 (4)[a]	−28.1 (5)
ω_2	179.9 (4)[b]	−176.9 (3)
ϕ_3		47.7 (5)
ψ_3		42.4 (5)[c]
ω_3		−176.2 (4)[d]
χ_1^1	−176.9 (3)	58.6 (5)
χ_1^2	130.4 (6)	123.2 (8)
χ_2^1	−58.7 (5)	49.0 (7)
χ_2^2	−114.1 (6)	−113.3 (8)
χ_3^1		−51.5 (6)
χ_3^2		−116.4 (10)

[a] $N_2-C^\alpha_2-C'_2-O(2)$. [b] $C^\alpha_2-C'_2-O(2)-C(5)$. [c] $N_3-C^\alpha_3-C'_3-O(2)$. [d] $C^\alpha_3-C'_3-O(2)-C(5)$.

Crystal-State Conformation. We determined by X-ray diffraction the molecular and crystal structures of two Boc/OMe protected L-Mag homooligomers, namely the dimer and the trimer. The molecular structures with the atomic numbering schemes are shown in Figures 3 and 4, respectively. Protecting groups, backbone, and side-chain torsion angles[59] are given in Table 2. In Table 3 the intra- and intermolecular H-bond parameters are listed.

Table 3. Intra- and Intermolecular H-Bond Parameters for the Boc-(L-Mag)$_n$-OMe (n = 2, 3) Homooligomers

peptide	donor	acceptor	symmetry operation	N ··· O distance, Å	C′═O···N angle, deg
Boc-(L-Mag)$_2$-OMe	N1H	O_1	$-x + 3/2, -y, z + 1/2$	2.940 (4)	142.0 (2)
	N2H	O_2	$-x + 3/2, -y - 1, z + 1/2$	3.055 (4)	163.5 (3)
Boc-(L-Mag)$_3$-OMe	N3H	O_0	x, y, z	2.964 (4)	133.8 (3)
	N1H	O_2	$-y, -x + 2, z + 1/4$	2.933 (4)	164.8 (3)

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the *tert*-butyloxycarbonyl-amino urethane[60] and methyl ester[61] moieties, the Mag residue,[8] and the peptide unit.[62,63] In particular, the bond angles indicate an asymmetric geometry for the C^{α} atom of all Mag residues. More specifically, the bond angles involving the $C^{\beta 1}$ atom are narrower than those involving the $C^{\beta 2}$ atom. This observation is common to Aib and other C^{α}-tetrasubstituted α-amino acids.[6,64] The average value for the conformationally sensitive N−C^{α}−C′ (τ) bond angle is 110.4°, comparable to that exhibited by the C^{α}-tetrasubstituted α-amino acids forming regular bends and helices.[6,65]

All five L-Mag residues populate the helical region (A or A*)[66] of the conformational (ϕ, ψ) space.[8] The average value for the ϕ, ψ backbone torsion angles of the Mag residue are ±57.1° and ±35.0°, close to those expected for a 3_{10}-helix.[55] In the dimer the two sets of ϕ, ψ torsion angles have opposite signs. The same behavior holds true for the ϕ, ψ values of the third residue of the trimer with respect to those of the preceding ones. This observation is quite common in the X-ray diffraction structures of peptides heavily based on C^{α}-methylated α-amino acids.[6] The 1−2 sequence of the tripeptide is folded in a 1←4 C′═O···H−N intramolecularly H-bonded β-turn conformation of the helical (III) type.[56−58] The C'_0═O_0···H−N_3 intramolecular separation is within the limits expected for such H-bonds.[67−69]

In the two molecules only one significant deviation of the ω torsion angles ($|\Delta\omega| > 8°$) from the ideal value of the trans-planar urethane, peptide, and ester units (180°) is observed: the urethane ω_0 value of the tripeptide, which differs by 16.6°. The trans arrangement of the θ^1 torsion angle of the Boc−NH− moiety found for both homooligomeric molecules is that commonly reported for Boc-protected peptides (type *b* conformation).[60] The methyl ester conformation of both dimer and trimer with respect to the preceding C^{α}−N bond is intermediate between the *anticlinal* and *antiperiplanar* conformations.[70] In the five L-Mag residues of the two compounds examined the N−C^{α}−C^{β}−C^{γ} (χ^1) torsion angle is either in the g^+ conformation (twice) or in the g^- (twice) and t (once) conformations; i.e. no clear side-chain conformation bias is observed for this parameter.[8] Conversely, the values for the χ^2 (C^{α}−C^{β}−C^{γ}−C^{δ}) torsion angle are in the rather restricted range ±110−130° (skew conformations).

The Boc-(L-Mag)$_2$-OMe molecules pack together along the *b* direction, generating rows of molecules stabilized by N−H···O═C intermolecular H bonds (N_1−H···O_1═C'_1 and N_2−H···O_2═C'_2). Then, hydrophobic interactions link together rows of peptide molecules running in the *a* and *c* directions.

The packing mode of the Boc-(L-Mag)$_3$-OMe molecules is characterized by one intermolecular H bond between the (urethane) N−H group of the first residue and the (peptide) C′═O group of the second residue (N_1−H···O_2═C'_2). This H bond is established along the *c* direction, giving rise to rows of molecules aligned in a head-to-tail fashion. The crystal structure is further stabilized by van der Waals interactions between the hydrophobic groups along the other crystallographic directions.

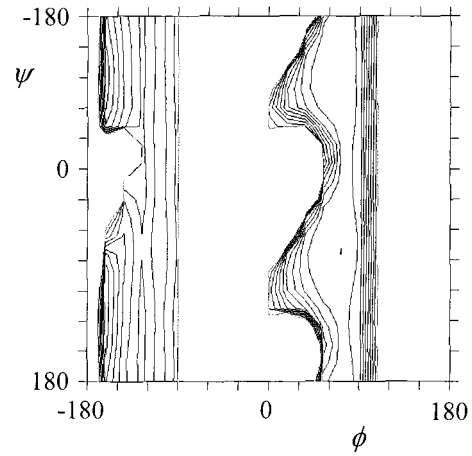


Figure 5. Rigid-rotor (ϕ, ψ) map of Boc-(L-Mag)$_3$-OMe with the Mag^1 and Mag^2 conformations set to −60°, −30°.

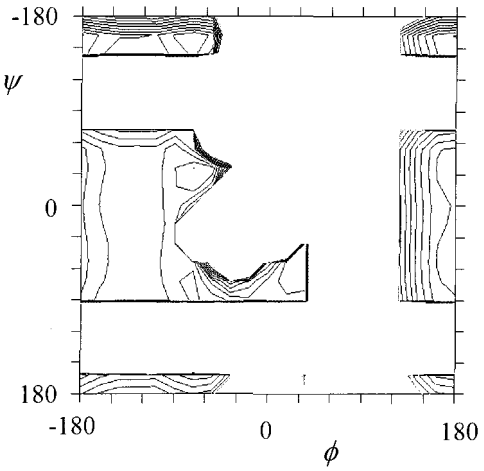


Figure 6. Rigid-rotor (ϕ, ψ) map of Boc-(L-Mag)$_3$-OMe with the Mag^2 conformation set to −60°, −30° and the Mag^3 conformation to 60°, 30°.

Conformational Energy Computations. Conformational energy maps have been computed for the tripeptide Boc-(L-Mag)$_3$-OMe using the crystallographic parameters reported here. Two rigid-rotor (ϕ, ψ) maps of this peptide were computed: the first keeping fixed the Mag^1 and Mag^2 residues in a folded conformation ($\phi = -60°$, $\psi = -30°$) and the second keeping fixed the Mag^2 residue at $\phi = -60°$, $\psi = -30°$ and the Mag^3 residue in a helical conformation of opposite handedness ($\phi = 60°$, $\psi = 30°$). The two rigid-rotor maps of the Mag^3 and Mag^1 residues are shown in Figures 5 and 6, respectively. The minimum-energy conformations from the rigid-rotor maps after the full minimization procedure are given in Table 4.

The ϕ, ψ torsion angle values in the minimized conformations are in good agreement with the experimental values found in the crystal state. These theoretical results show that the folded backbone conformation is the preferred conformation for the Mag residue in

Table 4. Minimum-Energy Conformations for the Boc-(L-Mag)$_3$-OMe Homooligomer

residue	ϕ (deg)	ψ (deg)	ΔE (kcal/mol)
L-Mag^3	72	54	0.0
	72	−126	1.4
	−162	90	33.8
	−162	−126	35.2
L-Mag^1	−72	−36	0.0
	180	54	7.7
	180	−36	8.0
	−72	72	9.4

isolated homopeptides. It is also interesting to note that the folded conformation with the C-terminal L-Mag residue in the opposite handedness with respect to the preceding L-Mag residues is the lowest energy structure. Taken together, these data indicate that bulky, chiral residues (such as Mag) show a conformational behavior similar to that observed for smaller, achiral C$^\alpha$-tetrasubstituted α-amino acids (such as Aib).[6] In addition, this structural propensity is not attributable to molecular self-assembling as that operative in the presence of crystal packing forces.

Conclusions

In this work we have described the successful step-by-step solution synthesis of the sterically hindered L-Mag homooligomers to the pentamer level using the EDC/HOAt method for activation of the carboxyl component in the coupling reactions. Furthermore, the results of the solution conformational analysis, combined with those extracted from the crystal-state X-ray diffraction study and conformational energy computations, also reported here, definitely confirm our earlier findings[8] that Mag is a conformationally constrained amino acid and has a remarkable propensity for β-bend and 3_{10}-helix formation. This conclusion strictly parallels those already reported for other C$^\alpha$-methylated α-amino acids.[7] As for the relationship between Mag α-carbon chirality and the screw sense of the bend/helix that is adopted by its peptides, the X-ray diffraction data available from this investigation strongly support the view that this structural property is analogous to that exhibited by protein amino acids, i.e., L-amino acids fold into right-handed bends/helices.

It is reasonable to foresee that the strong conformational bias of peptides rich in the C$^\alpha$-methylated L-Mag residue for *right*-handed β-bends and 3_{10}- (or the closely related α-) helices would have an influence on rate, yield, and stereochemistry of complexation and chemical reactions of the side-chain carbon−carbon double-bond function.[26,71] An investigation along this line is currently in progress in our laboratories.

Supporting Information Available: Tables of positional parameters, bond distances, bond angles, and torsion angles for the X-ray diffraction structures of Boc-(L-Mag)$_2$-OMe and Boc-(L-Mag)$_3$-OMe. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Marshall, G. In *Intra-Science Chemistry Reports*; Kharasch, N., Ed.; Gordon and Breach: New York, 1971; pp 305−316.
(2) Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1983; Vol. 7, pp 267−357.
(3) Aubry, A.; Boussard, G.; Cung, M. T.; Marraud, M.; Vitoux, B. *J. Chim. Phys.* **1988**, *85*, 345.
(4) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249.
(5) Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747.
(6) Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004.
(7) Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061.
(8) Peggion, C.; Flammengo, R.; Mossel, E.; Broxterman, Q. B.; Kaptein, B.; Kamphuis, J.; Formaggio, F.; Crisma, M.; Toniolo, C. *Tetrahedron* **2000**, *56*, 3589.
(9) Crisma, M.; Peggion, C.; Formaggio, F.; Toniolo, C.; Vitale, R. M.; Iacovino, R.; Saviano, M.; Benedetti, E.; Broxterman, Q. B.; Kaptein, B.; Kamphuis, J. In *Peptides 2000*; Martinez, J., Epton, R., Eds.; Mayflower Scientific: Kingswinford, U.K., in press.
(10) van der Werf, A.; Kellogg, R. M. *Tetrahedron Lett.* **1988**, *29*, 4981.
(11) Broxterman, Q. B.; Kaptein, B.; Kamphuis, J.; Schoemaker, H. E. *J. Org. Chem.* **1992**, *57*, 6286.
(12) Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron Lett.* **1992**, *33*, 6007.
(13) Schoemaker, H. E.; Boesten, W. H. J.; Kaptein, B.; Hermes, H. F. M.; Sonke, T.; Broxterman, Q. B.; van den Tweel, W. J. J.; Kamphuis, J. *Pure Appl. Chem.* **1992**, *64*, 1171.
(14) Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Peters, P. J. H.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1113.
(15) Rutjes, F. P. J. T.; Schoemaker, H. E. *Tetrahedron Lett.* **1997**, *38*, 677.
(16) Veerman, J. J. N.; van Maarseveen, J. H.; Visser, G. M.; Kruse, C. G.; Schoemaker, H. E.; Hiemstra, H.; Rutjes, F. P. J. T. *Eur. J. Org. Chem.* **1998**, 2583.
(17) Rutjes, F. P. J. T.; Veerman, J. J. N.; Meester, W. J. N.; Hiemstra, H.; Schoemaker, H. E. *Eur. J. Org. Chem.* **1999**, 1127.
(18) Genet, J. P.; Juge, S.; Besnier, I.; Uziel, J.; Ferroud, D.; Kardos, N.; Achi, S.; Ruiz-Montes, J.; Thorimbert, S. *Bull. Soc. Chim. Fr.* **1990**, *127*, 781.
(19) Williams, R. M.; Im, M. N. *J. Am. Chem. Soc.* **1991**, *113*, 9276.
(20) Berkowitz, D. B.; Pedersen, M. L.; Jahng, W. J. *Tetrahedron Lett.* **1996**, *37*, 4309.
(21) Badorrey, R.; Cativiela, C.; Diaz-de-Villegas, M. D.; Galvez, J. A.; Lapeña, Y. *Tetrahedron: Asymmetry* **1997**, *8*, 311.
(22) Loreto, M. A.; Pompei, F.; Tardella, P. A.; Tofani, D. *Tetrahedron* **1997**, *53*, 15853.
(23) Chinchilla, R.; Galindo, N.; Najera, C. *Synthesis* **1999**, *4*, 704.
(24) Trost, B. M.; Ariza, X. *J. Am. Chem. Soc.* **1999**, *121*, 10727.
(25) Ojima, I.; Delaloge, F. *Chem. Soc. Rev.* **1997**, *26*, 377.
(26) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 5891.
(27) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606.
(28) Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413.
(29) Blackwell, H. E.; Grubbs, R. H. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 3281.
(30) Armstrong, S. K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 371.
(31) Ripka, A. S.; Bohacek, R. S.; Rich, D. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 357.
(32) Williams, R. M.; Liu, J. *J. Org. Chem.* **1998**, *63*, 2130.
(33) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J., Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 11638.
(34) Blackwell, H. E.; O'Leary, D. J.; Chatterjee, A. K.; Washenfelder, R. A.; Bussmann, D. A.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 58.
(35) Undheim, K.; Efskind, J. *Tetrahedron* **2000**, *56*, 4847.
(36) Guinn, R. M.; Margot, A. O.; Taylor, J. R.; Schumacher, M.; Douglas, S. C.; Harvey, W. B. *Biopolymers* **1995**, *35*, 503.
(37) Poché, D. S.; Thibodeaux, S. J.; Rucker, V. C.; Warner, I. M.; Daly, W. H. *Macromolecules* **1997**, *30*, 8081.
(38) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(39) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115.
(40) Sheldrick, G. M. *SHELXL 97. Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.
(41) Cromer, D. T.; Waber, J. T. In *International Tables for X-ray Crystallography*, 2nd ed.; Kynoch Press: Birmingham, U.K., 1974; Vol. IV, Table 2.2B (now distributed by D. Reidel Publ. Co., Dordrecht, The Netherlands).
(42) Lifson, S.; Hagler, A. T.; Dauber, P. J. *J. Am. Chem. Soc.* **1979**, *101*, 5111.
(43) Hagler, A. T.; Lifson, S.; Dauber, P. J. *J. Am. Chem. Soc.* **1979**, *101*, 5122.

(44) Hagler, A. T.; Dauber, P. J.; Lifson, S. *J. Am. Chem. Soc.* **1979**, *101*, 5131.
(45) Brooks, C. L., III.; Pettitt, M. B.; Karplus, M. In *Proteins: a Theoretical Perspective of Dynamics, Structure and Thermodynamics*; Wiley: New York, 1988.
(46) Kruizinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. *J. Org. Chem.* **1988**, *53*, 1826.
(47) Elferink, V. H. M.; Breitgoff, D.; Kloosterman, M.; Kamphuis, J.; Van den Tweel, W. J. J.; Meijer, E. M. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 63.
(48) Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S. *Int. J. Biol. Macromol.* **1984**, *6*, 179.
(49) Toniolo, C.; Benedetti, E. In *Molecular Conformation and Biological Interactions*; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, 1991; pp 511−521.
(50) Toniolo, C.; Bonora, G. M.; Barone, V.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C. *Macromolecules* **1985**, *18*, 895.
(51) Iqbal, M.; Balaram, P. *Biopolymers* **1982**, *21*, 1427.
(52) Kopple, K. D.; Ohnishi, M. *Biochemistry* **1969**, *8*, 4087.
(53) Kopple, K. D.; Schamper, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 3644.
(54) Martin, D.; Hauthal, H. G. In *Dimethyl Sulphoxide*; van Nostrand-Reinhold: Wokingham, UK, 1975.
(55) Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350.
(56) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425.
(57) Toniolo, C. *C.R.C. Crit. Rev. Biochem* **1980**, *9*, 1.
(58) Rose, G. D.; Gierasch, L. M.; Smith, J. P. *Adv. Protein Chem.* **1985**, *37*, 1.
(59) IUPAC-IUB Commission on Biochemical Nomenclature. *J. Mol. Biol.* **1970**, *52*, 1.
(60) Benedetti, E.; Pedone, C.; Toniolo, C.; Némethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1980**, *16*, 156.
(61) Schweizer, W. B.; Dunitz, J. D. *Helv. Chim. Acta* **1982**, *65*, 1547.
(62) Benedetti, E. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1982; Vol. 6, pp 105−184.
(63) Ashida, T.; Tsunogae, Y.; Tanaka, I.; Yamane, T. *Acta Crystallogr.* **1987**, *B43*, 212.
(64) Moretto, V.; Formaggio, F.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Benedetti, E.; Santini, A.; Saviano, M.; Di Blasio, B.; Pedone, C. *J. Pept. Sci.* **1996**, *2*, 14.
(65) Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Némethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947.
(66) Zimmerman, S. S.; Pottle, M. S.; Némethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1.
(67) Ramakrishnan, C.; Prasad, N. *Int. J. Protein Res.* **1971**, *3*, 209.
(68) Taylor, R.; Kennard, O.; Versichel, W. *Acta Crystallogr.* **1984**, *B40*, 280.
(69) Görbitz, C. H. *Acta Crystallogr.* **1989**, *B45*, 390.
(70) Dunitz, J. D.; Strickler, P. In *Structural Chemistry and Molecular Biology*; Rich, A., Davidson, N., Eds.; Freeman: San Francisco, 1968; pp 595−602.
(71) Moretto, A.; Peggion, C.; Formaggio, F.; Crisma, M.; Toniolo, C.; Piazza, C.; Kaptein, B.; Broxterman, Q. B.; Ruiz, I.; Diaz-de-Villegas, M. D.; Galvez, I. A.; Cativiela, C. *J. Pept. Res.* **2000**, *56*, 283.

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