Pergamon

Tetrahedron 56 (2000) 3589–3601

TETRAHEDRON

Mag: a C^{α}-Methylated, Side-chain Unsaturated α-Amino Acid. Introduction into Model Peptides and Conformational Preference

Cristina Peggion,[a] Roberto Flammengo,[a] Eric Mossel,[a] Quirinus B. Broxterman,[a] Bernard Kaptein,[a] Johan Kamphuis,[b] Fernando Formaggio,[c] Marco Crisma[c] and Claudio Toniolo[c,*]

[a]*DSM Research, Organic Chemistry and Biotechnology Section, 6160 MD Geleen, The Netherlands*
[b]*DSM Food Speciality, Nutritional Ingredients, P.O. Box 1, 2600 MA Delft, The Netherlands*
[c]*Biopolymer Research Centre, C.N.R., Department of Organic Chemistry, University of Padova, 35131 Padova, Italy*

Received 22 October 1999; revised 14 March 2000; accepted 30 March 2000

Abstract—By a chemo-enzymatic approach we synthesized the chiral, C^{α}-methylated α-amino acid Mag, characterized by a side-chain $C^{\gamma}=C^{\delta}$ bond. We also prepared a series of model peptides containing Mag in combination with Aib and Ala. All of the peptides were fully characterized and their conformational preference was determined in solution by FT-IR absorption and ^{1}H NMR investigations. X-Ray diffraction analyses of L-Mag, a derivative and three peptides are also presented. We find that this C^{α}-methylated α-amino acid is an excellent β-turn and 3_{10}-helix former. A peptide with two Mag residues one on top of the other after one complete turn of the 3_{10}-helix has been synthesized and characterized. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

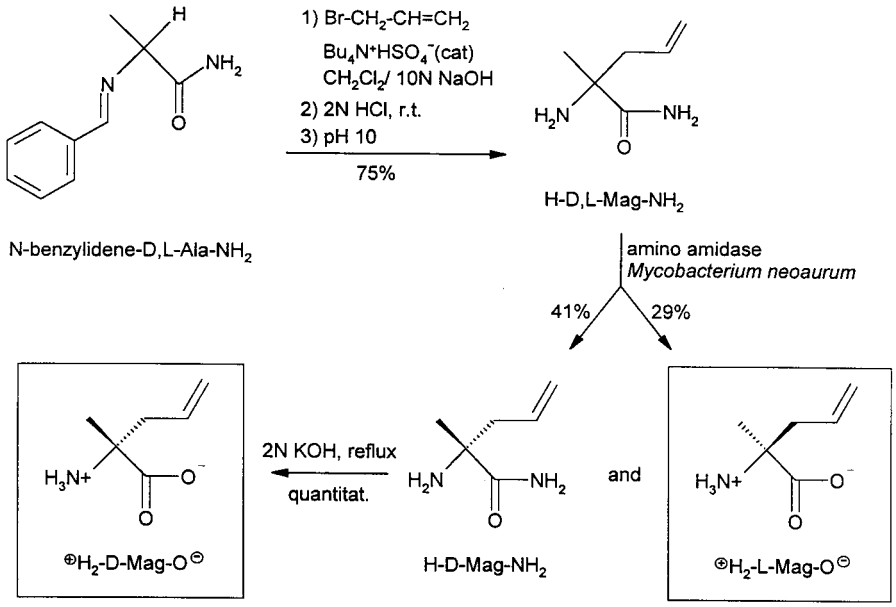
The relevance of stable secondary structure elements in peptides and proteins has inspired considerable effort toward the design of rigidified artificial molecules. In particular, significant progress has been made in engineering and synthesizing turn and helix inducers and mimetics. Indeed, turn and helical motifs are fundamental constituents of many biologically active peptides and are often critical for their biological activity. Among the many approaches to the synthesis of rigidified peptides a widely used strategy has been represented by the exploitation of structurally restricted C^{α}-substituted α-amino acids able to drastically reduce the conformational freedom of the peptide in which they are inserted.[1–5] More specifically, the conformational preferences of C^{α}-methylated α-amino acids have been extensively studied and their tendency to fold into a β-turn and the 3_{10}-helical structure unambiguously demonstrated.[4–6]

Interest in allyl C^{α}-substituted α-amino acids arises from the wide versatility of their $C^{\gamma}=C^{\delta}$ bond towards a number of chemical reactions. Polypeptides have been prepared containing a pendant unsaturation site as a handle for cross-linking reactions.[7,8] Recently, syntheses of derivatives and short peptides containing different types of allyl C^{α}-substituted α-amino acids have been published.[9–15] C^{α}-Allyl amino acid derivatives have proven to be useful intermediates in the synthesis of conformationally restricted peptidomimetics.[9,10] More interestingly, derivatives and peptides of C^{α}-allyl amino acids may step up to metathesis reactions that lately have been improved by the development of efficacious ruthenium catalysts.[11–15] In particular, ringclosing metathesis reactions form cycles of various sizes. For example, Blackwell and Grubbs[11] efficiently stabilized a 3_{10}-helical peptide by ringclosing metathesis between two homoserine *O*-allyl esters. Another useful application of this family of amino acids dealt with their incorporation into peptides followed by addition of an array of thiol-based radicals to the alkene function ('built-into' peptides).[16]

Therefore, we decided to take advantage of C^{α}-methyl-C^{α}-allylglycine (Mag), which combines the structural features of a C^{α}-methylated α-amino acid with those of a C^{α}-allyl, functionalizable side chain. In the last few years a variety of methods aiming at synthesizing Mag and its derivatives has been published.[16–22] Mag has also been inserted into a dipeptide.[23] In the present work we describe a chemo-enzymatic synthesis of Mag and the preparation of a series of Mag model peptides in combination with Aib (α-aminoisobutyric acid) and Ala. Peptide preferred conformations have been assessed in solution by FT-IR absorption and

Keywords: amino acids and derivatives; NMR; peptides; X-ray crystallography.
* Corresponding author. Tel.: +390-49-827-5247; fax: +390-49-827-5239; e-mail: biop02@mail.chor.unipd.it

0040–4020/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4020(00)00274-X



Scheme 1.

^{1}H NMR techniques. In addition, the 3D-structures of the free amino acid $^{\oplus}H_2$-L-Mag-$O^{\ominus}$, the derivative Piv-L-Mag-NH*t*Bu (Piv, pivaloyl; NH*t*Bu, *tert*-butylamino), and the peptides Boc-L-Mag-D-Ala-OMe (Boc, *tert*-butyloxycarbonyl; OMe, methoxy), Boc-Aib-L-Mag-Aib-OMe, and Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe have been solved by X-ray diffraction analysis.

Results and Discussion

Amino acid and peptide synthesis

For the large-scale production of the enantiomerically pure L-Mag we exploited an economically attractive and generally applicable chemo-enzymatic synthesis developed by DSM Research[19–21,24,25] a few years ago. At first, we performed a phase-transfer catalyzed allylation of N^{α}-benzylidene-D,L-alanine amide for the preparation of the racemic α-amino amide (Scheme 1) 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,[16,19–21] 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 *iso*-propanol/water 3:1. Thus, the L- and D-Mag were obtained in enantiomeric excess >99%.

The Boc N^{α}-protected L-derivative was prepared by reacting the free amino acid with $(Boc)_2O$ (di-*tert*-butyldicarbonate) in an aqueous/dioxane mixture. For the synthesis of

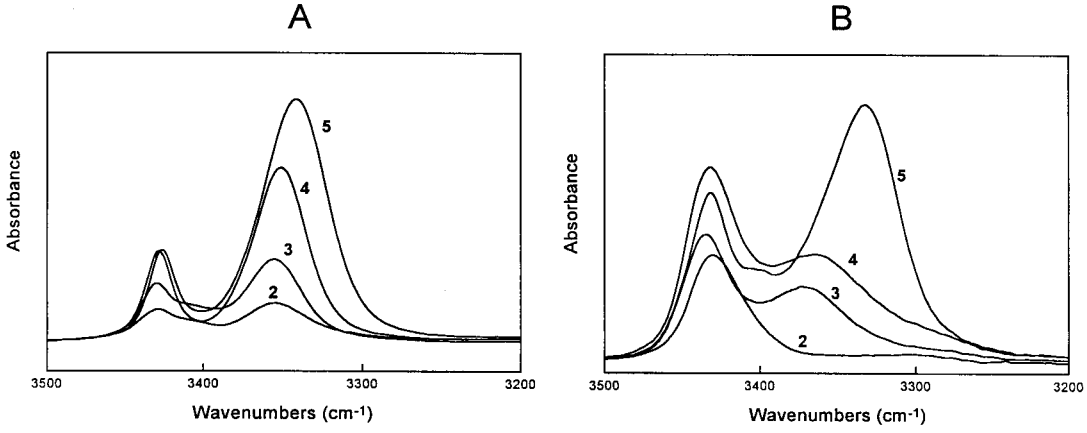


Figure 1. FT-IR absorption spectra (3500–3200 cm^{-1} region) in $CDCl_3$ solution of (A) Boc-L-Mag-Aib-OMe (**2**), Boc-Aib-L-Mag-Aib-OMe (**3**), Boc-Aib-Aib-L-Mag-Aib-OMe (**4**), and Boc-L-Mag-Aib-Aib-L-Mag-Aib-OMe (**5**); (B) Boc-L-Mag-L-Ala-OMe (**2**), Boc-L-Ala-L-Mag-L-Ala-OMe (**3**), Boc-L-Ala-L-Ala-L-Mag-L-Ala-OMe (**4**), and Boc-L-Mag-L-Ala-L-Ala-L-Mag-L-Ala-OMe (**5**). Peptide concentration: 1.0 mM.

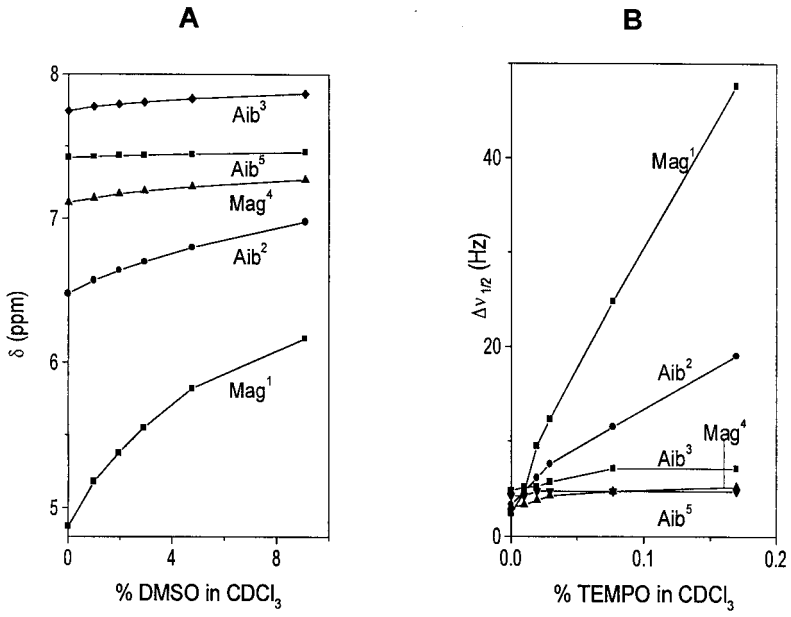


Figure 2. ^{1}H NMR titrations of Boc-L-Mag-Aib-Aib-L-Mag-Aib-OMe. (A) Plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (*v/v*). (B) Plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (*w/v*) in $CDCl_3$. Peptide concentration: 1.0 mM.

Piv-L-Mag-NH*t*Bu L-Mag was first treated with trimethylsilylchloride. Then, the *O,N-bis*-silyl Mag derivative was reacted with Piv-Cl. Acidic hydrolysis gave Piv-L-Mag-OH which was dehydrated to the corresponding 5(4*H*)-oxazolone with *N*-ethyl, *N′*-[3-(dimethylamino)propyl]-carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt). Finally, the oxazolone was reacted with *tert*-butylamine in *N,N*-dimethylformamide (DMF). Syntheses of the three peptide series (to the pentamer level) were performed step-by-step in solution, beginning from the C-terminal amino acid methyl ester. Peptide bond formation was achieved by the EDC/HOAt (1-hydroxy-7-azabenzotriazole method)[26] in CH_2Cl_2 in the presence of *N*-methylmorpholine (NMM). Using this approach, the sterically hindered L-Mag-Aib and Aib-L-Mag peptide bonds were obtained in satisfactory yields (70–90%). Removal of the Boc N^{α}-protecting group was performed by treatment with diluted TFA (trifluoroacetic acid).

Solution conformational analysis

A solution conformational analysis of the three series was performed by using FT-IR absorption and ^{1}H NMR in a solvent of low polarity ($CDCl_3$) at different peptide concentrations. Fig. 1A shows the FT-IR absorption spectra of the Boc/OMe-protected L-Mag/Aib peptide series in the 3500–3200 cm^{-1} (N–H stretching) region. The curves are characterized by two bands at about 3430 cm^{-1}, assigned to free (solvated) NH groups, and at 3360–3340 cm^{-1}, assigned to H-bonded NH groups.[27] The intensity of the low-frequency band, relative to the high-frequency band, increases linearly as the main-chain length increases. Concomitantly, the absorption maximum markedly shifts to lower wavenumbers. A similar trend is also observed for the two Mag/Ala series (Fig. 1B shows the spectra of the all-L-series). When in combination with L-Ala, the L-Mag peptides still exhibit a tendency to fold into a turn or a helical conformation, but a higher flexibility is observed. Indeed, the di-, tri-, and tetrapeptides show a significant amount of unordered conformation, while in the pentapeptide (containing two L-Mag residues in positions 1 and 4) the helical character becomes predominant. No significant difference is observed in the spectra of the pentapeptides with either L-Ala or D-Ala in position 5. All of the peptides investigated tend to self-associate only marginally in the concentration range examined (10–0.1 mM) (not shown).

To better understand the type of helix that is formed, we performed a ^{1}H NMR investigation of the three pentapeptides. The analysis was carried out in $CDCl_3$ at 1.0 mM concentration where self-association is absent. All NH proton resonances were assigned by means of 2D ROESY experiments. The participation of specific NH groups in H-bonding was established by examining the behaviour of the NH resonances upon addition of perturbing agents. In particular, we investigated the solvent dependence of NH chemical shifts, by adding increasing amounts of the strong H-bonding acceptor solvent dimethylsulphoxide (DMSO)[28] to the $CDCl_3$ solution, and the line broadening of NH resonances induced by adding the free radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxo).[29] Fig. 2 illustrates the behaviour of the NH resonances of the L-Mag/Aib pentapeptide upon addition of DMSO and TEMPO. In this peptide only the N(1)H and N(2)H proton chemical shifts, in particular that of the N(1)H proton, are sensitive to the addition of DMSO and their resonances broaden upon addition of the paramagnetic TEMPO. All the other protons display a behaviour characteristic of shielded protons, as their chemical shifts appear relatively insensitive to solvent composition and their linewidths are not influenced by addition of TEMPO.

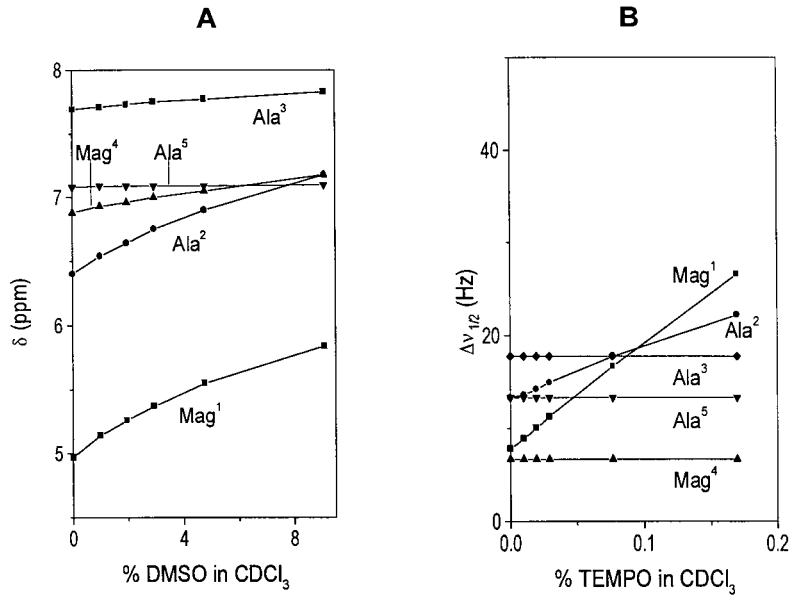


Figure 3. ^{1}H NMR titrations of Boc-L-Mag-L-Ala-L-Ala-L-Mag-L-Ala-OMe. (**A**) Plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (*v/v*). (**B**) Plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (*w/v*) in $CDCl_3$. Peptide concentration: 1.0 mM.

The two Mag/Ala pentapeptides behave in a similar manner, more specifically as shown in Fig. 3 for the all-L diastereomer. From our ^{1}H NMR study it is reasonable to conclude that the most populated conformation adopted in $CDCl_3$ solution by the terminally protected Mag containing pentapeptides is the 3_{10}-helix, where only the two N-terminal NH protons do not participate in the intramolecular H-bonding scheme.

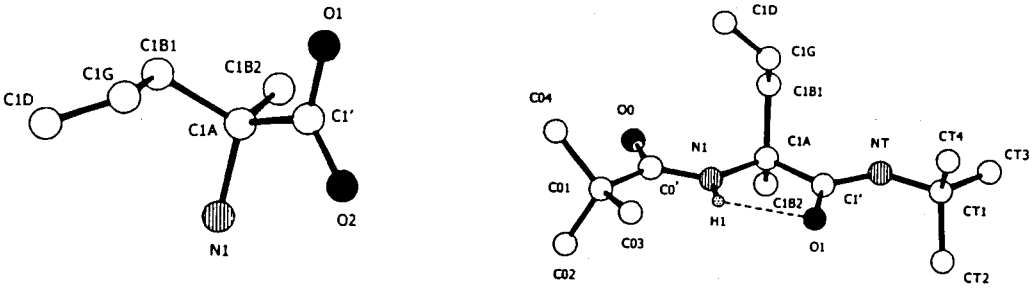


Figure 4. Left: X-ray diffraction structure of $^{\oplus}H_2$-L-Mag-$O^{\ominus}$ monohydrate with numbering of the atoms. Only one of the two side-chain conformer is shown. Right: X-ray diffraction structure of Piv-L-Mag-NH*t*Bu with numbering of the atoms. The intramolecular H-bond is represented by a dashed line.

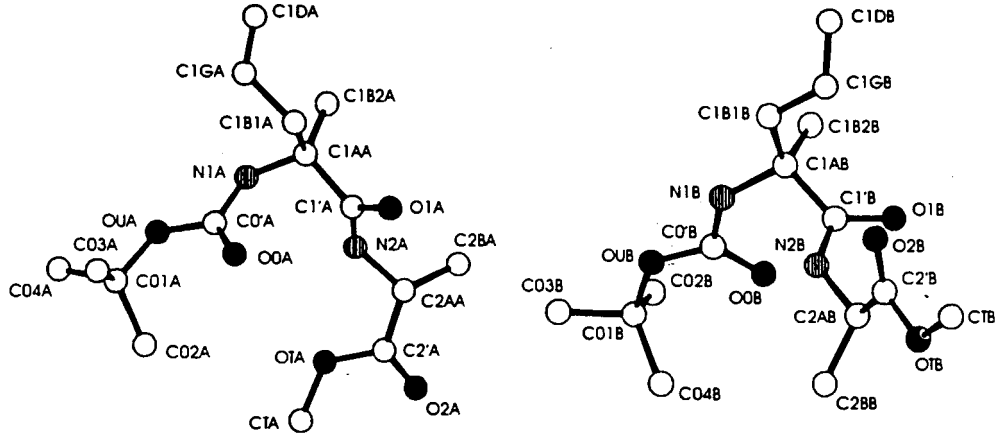


Figure 5. X-Ray diffraction structures of the two independent molecules **A** and **B** of Boc-L-Mag-D-Ala-OMe with numbering of the atoms.

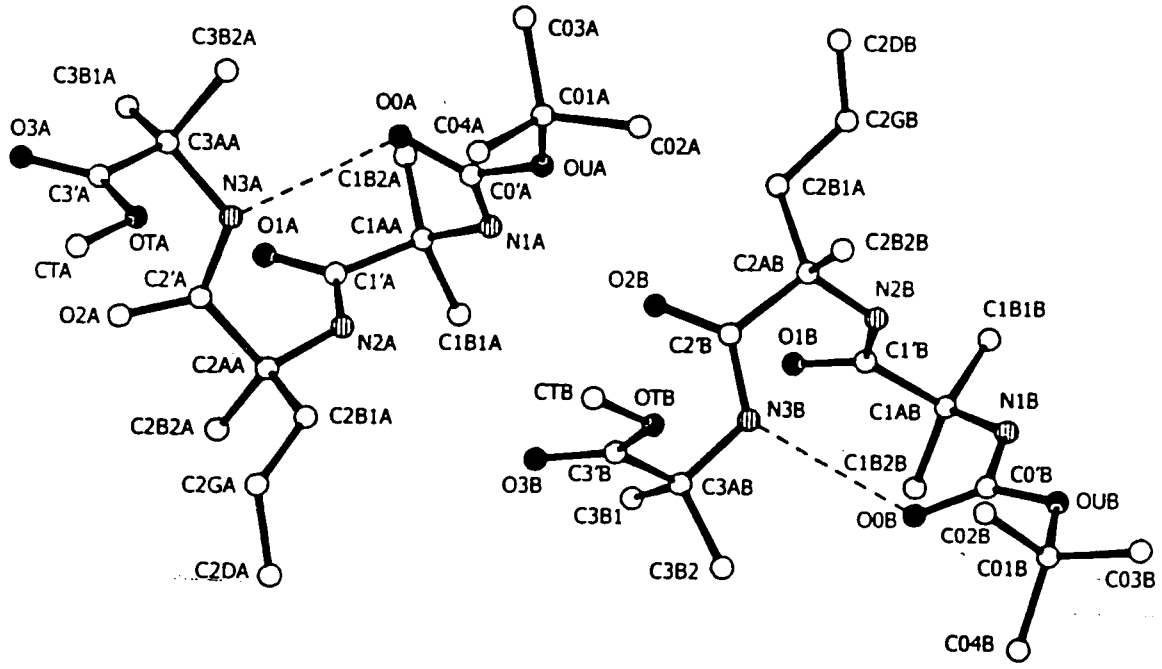


Figure 6. X-Ray diffraction structures of the two independent molecules **A** and **B** of Boc-Aib-L-Mag-Aib-OMe with numbering of the atoms. The intramolecular H-bonds are represented by a dashed line.

Crystal-state conformational analysis

The molecular and crystal structures of the free amino acid $^{\oplus}H_2$-L-Mag-$O^{\ominus}$ monohydrate, the derivative Piv-L-Mag-NH*t*Bu, the dipeptide Boc-L-Mag-D-Ala-OMe, the tripeptide Boc-Aib-L-Mag-Aib-OMe, and the pentapeptide Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe were determined by X-ray diffraction. The di- and tripeptides crystallize with two independent molecules, **A** and **B**, in the asymmetric unit. The molecular structures of the five compounds with the atomic numbering schemes are illustrated in Figs. 4–7, respectively. Relevant N^{α}-protecting group, main-chain, and side-chain torsion angles[30] are given in Table 1. In Table 2, the intra- and intermolecular H-bond parameters are listed.

Bond lengths and bond angles are in general agreement with previously reported values for the geometry of the *tert*-butyloxycarbonylamino moiety,[31] the methylester group, the Aib and Ala residues, and the peptide unit. The average bond distances and bond angles for the Mag side-chain allyl moiety (as obtained from the experimental values with estimated standard deviations lower than 0.02 Å and 2°, respectively) are: CD═CG 1.31 Å, CG–CB 1.47 Å, and

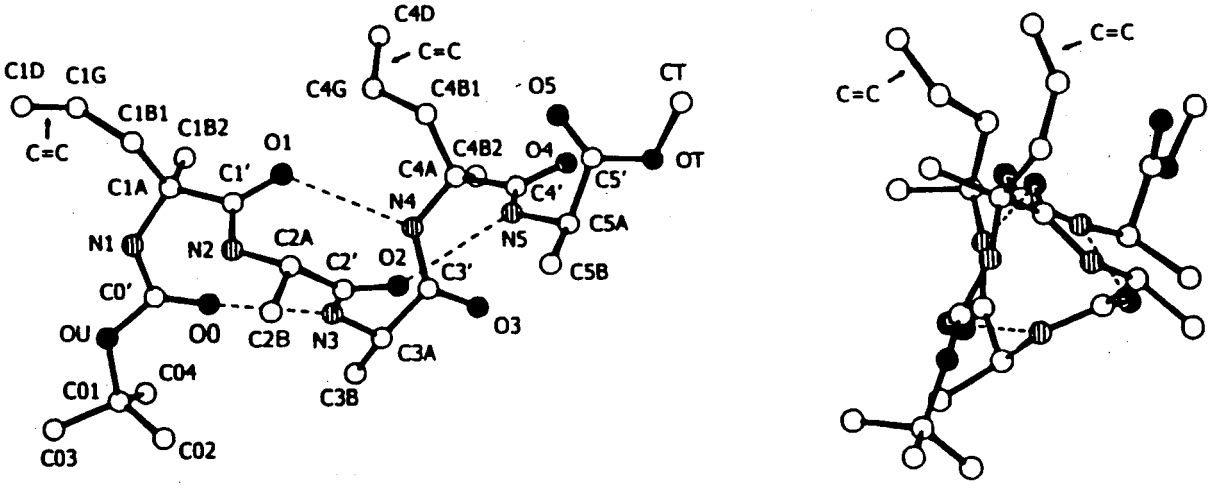


Figure 7. Left: X-ray diffraction structure of Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe with numbering of the atoms (a view parallel to the helix axis). Right: A view of the same molecule along the helix axis. In both views of the molecule the intramolecular H-bonds are represented by a dashed line, the two side-chain C═C bonds are marked, and only one of the side-chain conformers is shown.

Table 1. Selected N^{α}-protecting group, backbone and side-chain torsion angles (the torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, ω) and side chains (χ) are described in Ref. 30. For the torsion angle for rotation about bonds of the Boc-protecting group (θ) see Ref. 31 (deg) for Mag, its derivative and peptides

Torsion angle	Amino acid monohydrate	Amino acid derivative	Dipeptide mol. **A**/mol. **B**	Tripeptide mol. **A**/mol. **B**	Pentapeptide
θ^1			177.6(6)/−179.9(6)	−177.6(5)/174.2(4)	−173.7(6)
ω_0		−175.0(4)	172.9(5)/−172.8(5)	−174.6(5)/177.2(4)	−170.1(6)
ϕ_1		180.0(4)	58.4(7)/−56.0(7)	−55.9(7)/58.5(7)	−57.4(9)
ψ_1		−179.5(4)	42.8(7)/−46.0(6)	−33.5(7)/27.9(7)	−33.5(9)
ω_1		177.5(4)	171.5(5)/−175.6(5)	−174.9(5)/−177.7(5)	−177.4(6)
ϕ_2			145.8(6)/95.1(6)	−59.5(6)/50.2(6)	−63.4(10)
ψ_2			−24.4(9)[a]/−177.9(5)[a]	−29.2(7)/37.9(7)	−20.5(10)
ω_2			179.7(7)[b]/176.5(7)[b]	172.8(5)/−179.4(5)	−179.9(7)
ϕ_3				50.4(8)/−49.5(8)	−63.9(9)
ψ_3				49.6(8)[a]/−49.4(8)[a]	−18.8(10)
ω_3				175.9(5)[b]/−176.9(5)[b]	175.0(6)
ϕ_4					−53.9(9)
ψ_4					−31.6(10)
ω_4					179.0(8)
ϕ_5					69.2(12)
ψ_5					−150.8(8)[a]
ω_5					−174.8(10)[b]
χ_1^1	54(2)[c]/72(2)[c]	56.9(5)	−54.5(9)/−171.7(8)		−179.9(16)[c]/−76.2(16)[c]
χ_1^2	−123(3)[c]/106(5)[c]	−115.8(8)	−122.7(9)/123.2(10)		126(3)[c]/114(4)[c]
χ_2^1				−158.2(8)/−59.6(6)	
χ_2^2				110.5(13)/−129.6(8)	
χ_4^1					−71.7(10)
χ_4^2					−109.5(14)

[a] N-CA-C′-OT torsion angle.
[b] CA-C′-OT-CT torsion angle.
[c] The C1G atom is disordered over two sites.

CB–CG=CD 123.3°. The corresponding literature mean values for the H_2C=CH– and =CH–CH_2– distances are 1.30 and 1.50 Å, respectively.[32]

The conformationally informative τ (N–CA–C′) bond angle for the L-Mag residue of the amino acid derivative is very narrow, 105.2(3°), a preliminary indication of the onset of the fully extended (C_5) conformation.[33]

All six L-Mag residues included in the peptides examined populate the helical region (A or A^*) of the conformational space.[34] However, the L-Mag residue of the amino acid derivative is fully extended (region E or E^*). The average value for the Φ, Ψ backbone torsion angles of the Mag residues forming helical turns are ±57.1, ±36.7°, in the range of those expected for a regular 3_{10}-helix (±57, ±30°).[35]

The (amide) N–H···O=C′ (amide) intramolecularly H-bonded C_5 conformation, exhibited by the Mag amino

Table 2. Intra- and intermolecular H-bond parameters for Mag, its derivative and peptides

Peptide	Donor D–H	Acceptor A	Symmetry operation	Distance (Å) D…A	Distance (Å) H…A	Angle (deg) D–H…A
$^{\oplus}H_2$-L-Mag-$O^{\ominus}$ H_2O	N1–HA	O2	x, y, z	2.631(13)	2.47	89.3
	OW–HA	O1	x, y, z	2.702(13)	1.94(6)	155(15)
	N1–HA	O2	$x-1, y+1, -z$	2.802(13)	1.86	171.6
	N1–HB	O1	$-x+1/2, y+1/2, z-1/4$	2.785(11)	1.84	175.8
	N1–HC	OW	$x-1, y, -z$	2.882(15)	1.99	155.9
	OW–HB	OW	$x, y, -z$	2.82(2)	2.06(2)	153(16)
Piv-L-Mag-NH*t*Bu	N1–H	O1	x, y, z	2.561(5)	2.10	113.1
	NT–H	O0	$x+1/2, -y+1/2, -z+1$	3.093(5)	2.30	154.2
Boc-L-Mag-D-Ala-OMe	N1A–H	O0B	x, y, z	2.880(6)	2.02	173.7
	N2A–H	O1B	x, y, z	2.910(5)	2.27	131.8
	N1B–H	O0A	$x-1, y, z$	3.038(5)	2.19	168.3
	N2B–H	O1A	$x-1, y, z$	2.843(6)	2.10	143.7
Boc-Aib-L-Mag-Aib-OMe	N3A–H	O0A	x, y, z	2.975(6)	2.22	145.9
	N3B–H	O0B	x, y, z	2.961(6)	2.18	151.4
	N1A–H	O2B	x, y, z	2.833(6)	1.98	174.0
	N1B–H	O2A	$x+1, y, z$	2.811(6)	1.96	171.0
Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe	N3–H	O0	x, y, z	3.017(8)	2.19	162.1
	N4–H	O1	x, y, z	3.229(9)	2.39	165.9
	N5–H	O2	x, y, z	3.103(10)	2.28	160.6
	N1–H	O4	$x, y-1, z$	2.938(9)	2.11	161.0

acid derivative, although uncommon,[5] has been previously reported for other chiral C^{α}-tetrasubstituted α-amino acids.[36] In addition to the narrow τ bond angle mentioned above, two other typical features of this conformation are: (i) the very short intramolecular N1···O1 separation, and (ii) the narrow N1–H···O1 angle. Interestingly, an intramolecularly H-bonded C_5 conformation, but of the ammonium ··· carboxylate type is also shown by the free amino acid $^{\oplus}$H$_2$-L-Mag-O$^{\ominus}$.

Two main conformational features distinguish molecule **A** from molecule **B** of the dipeptide. First, the N-terminal L-Mag residue is left-handed helical in molecule **A**, but right-handed helical in molecule **B**. Secondly, the C-terminal D-Ala residue, although in a partially extended conformation in both molecules, has two sets of largely divergent Φ, Ψ torsion angles.

In both molecules **A** and **B** 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/III′) type.[37] The C0′–O0···H–N3 intramolecular H-bond is of normal strength. The β-turn conformation has opposite handedness in the two molecules. In both molecules also the C-terminal Aib residue is helical, but its handedness is opposite to that of the preceding residues, a common observation for Aib-based helical peptides.

The backbone of the pentapeptide adopts a regular right-handed 3_{10}-helical structure. Peptide groups N3–H to N5–H and O0=C0′ to O2=C2′ participate in three consecutive 1←4 (type III β-turn) C′=O···H–N intramolecular H-bonds, appropriate for a 3_{10}-helix. The range of observed N···O distances is 3.017(8)–3.229(9) Å, while that of N–H···O angles is 160.6–165.9°. The C-terminal D-Ala residue is *semi*-extended.

In the amino acid derivative and peptides investigated no significant deviation of the ω torsion angles ($|\Delta\omega|>10°$) from the ideal values of the *trans* planar amide, urethane, peptide, and ester units (180°) is observed. The *trans* arrangement of the θ^1 torsion angle of the Boc-NH– moiety, found for all of the molecules of di-, tri-, and pentapeptides, is that commonly reported for Boc-protected peptides (type *b* conformation).[31]

In the ten L-Mag residues of the five compounds examined (the di- and tripeptides are each characterized by two independent molecules, and in the free amino acid and in L-Mag[1] of the pentapeptide the side-chain CG atom is disordered over two sites) the N–CA–CB–CG (χ^1) torsion angle is either in the g^+ conformation (three times) or in the g^- (four times) and t (three times) conformations, i.e. no side-chain conformational bias is observed for this parameter. Conversely, the values for the χ^2 torsion angles are in the range ±106–130° (*skew* conformations). The two Mag allyl side chains in positions 1 and 4 of the pentapeptide seat one on top of the other after one complete turn of the 3_{10}-helix (Fig. 7, right view). The intramolecular distances C1A···C4A, C1B1···C4B1, C1G···C4G (or C1G′···C4G), and C1D···C4D (or C1D′···C4D) are 6.13, 6.41, 6.49 (or 5.24 Å), and 8.29 (or 5.97 Å). It is worth remembering that the reported pitch (CA···CA) for the 3_{10}-helix is 6.29 Å.[35]

In the crystal the five H-atoms of the ammonium and water donors and the three oxygen atoms of the carboxylate and water acceptors of $^{\oplus}$H$_2$-L-Mag-O$^-$ monohydrate are all involved in a complex network of intermolecular H-bonds. The N···O and O···O H-bonds have normal lengths and angles. The crystal structure of the fully extended amino acid derivative is characterized by a single intermolecular N–H···O=C′ H-bond between the C-terminal amide NT-H group and the N-terminal amide O0=C0′ group of a symmetry related molecule, generating rows along the *x*-direction.

In the crystal packing mode of the dipeptide four intermolecular N–H···O=C′ H-bonds are observed, each connecting a molecule **A** to a molecule **B** (and vice versa). The urethane N1–H group is linked to the urethane O0=C0′ group, while the peptide N2–H group is linked to the peptide O1=C1′ group. In the unit cell of the turn-forming tripeptide each of the two intermolecular N–H···O=C′ H-bonds connects a molecule **A** to a molecule **B** (and vice versa). The urethane N1 group forms a H-bond with the peptide O2=C2′ group. The molecules of the helical pentapeptide are held together in a head-to-tail fashion along the *y*-direction in rows stabilized by (urethane) N1H···O4=C4′ (peptide) intermolecular H-bonds. Neither in the tripeptides, nor in the pentapeptide does the N2–H group of the L-Mag[2] residue seem to be involved in the intermolecular H-bonding scheme.

Conclusions

By a chemo-enzymatic approach we have been able to prepare both Mag enantiomers in a large amount and by solution methods to incorporate L-Mag into a variety of model peptides to the pentamer level. The results of the solution conformational analysis described in this work, combined with those extracted from an X-ray diffraction study, also reported here, strongly favor the conclusion that N^{α}-acylated Mag-based tripeptide esters have a great tendency to fold in a β-turn conformation, while the most populated structures adopted by tetra and pentapeptides are two consecutive β-turns (incipient 3_{10}-helix) and the 3_{10}-helix, respectively. These conclusions are in excellent agreement with those already reported for other C^{α}-methylated α-amino acids.[5] As for the relationship between Mag chirality and peptide helix handedness, the available X-ray diffraction data indicate that L-Mag can easily be accommodated into a right-handed helix, but unfortunately do not allow us to conclude that there is a univocal relationship between these two configurational and conformational properties. Hopefully, this issue will be solved by a conformational analysis of the L-Mag homo–oligomer series, the synthesis of which is currently in progress in our laboratories.

In any case, we expect that Mag, with its remarkable conformational bias towards β-turns and the 3_{10}-helix and the concomitant chemical reactivity of its allyl side chain, would become an important component in the arsenal of peptide chemists.

Experimental

General

Amino acid and peptide synthesis. Melting points were determined using a Leitz model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin–Elmer model 241 polarimeter equipped with a Haake model D thermostat. Thin-layer chromatography was performed on Merck Kieselgel 60/F_{254} precoated plates. The solvent systems used are: I, chloroform/ethanol (9:1); II, 1-butanol/acetic acid/water (3:1:1); III, toluene/ethanol (7:1). The chromatograms were developed by quenching of UV fluorescence, chlorine–starch–potassium iodide or ninhydrin chromatic reaction, as appropriate. Enantiomeric excesses (e.e.) were determined by HPLC after precolumn derivatization with *o*phthaldehyde/(R)-3-mercaptoisobutyric acid according to Duchateau et al.[38] [eluant: 50 mM sodium acetate solution (titrated to pH 6.0 with acetic acid)/methanol 7:3].

D,L-2-Amino-2-methyl-4-pentenoic acid amide or C^{α}-methyl-D,L-allylglycine amide (H-D,L-Mag-NH_2). To a solution of 176 g of *N*-benzylidene-D,L-alanineamide[19] (1.0 mol) and 35 g of $Bu_4N^+ \cdot HSO_4^-$ (10 mol%) in 1 L of CH_2Cl_2 in a 2 L Erlenmeyer flask were added 700 mL of a 10 N NaOH solution and 96 mL of allyl bromide (1.1 mol). The reaction mixture was stirred vigorously overnight at room temperature. TLC (EtOAc/*n*hexane 7:3) revealed that the reaction was at completion. The organic phase was separated from the aqueous phase and washed with water (3×500 mL). To the combined organic layers were added 700 mL of a 2 N HCl solution and the mixture was stirred at room temperature for 2 h. After separation of the organic and the aqueous phases, the organic phase was extracted with a 0.1 N HCl solution (3×350 mL). The combined aqueous phases were washed with CH_2Cl_2 (3×600 mL) and added with a 10 N NaOH solution to pH 10 under stirring. After washing of the aqueous solution with toluene and re-extraction of the toluene layer with water, the combined aqueous phases were saturated with NaCl and extracted with $CHCl_3$/EtOH 9:1 (5×700 mL). The combined $CHCl_3$ phases were dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the *title compound* (96 g, 75%) as a slowly crystallizing oil, mp 46–47°C (after distillation, bp 115–130°C/0.2 Torr); R_fI 0.55; δ_H (200 MHz, DMSO d_6) 7.31 and 6.95 (2H, 2 br s, amide NH_2), 5.70 (1H, m, γC*H*), 5.00 (2H, m, δCH_2), 2.35 and 2.12 (2H, 2m, βCH_2), 1.75 (2H, s, NH_2), 1.12 (3H, s, βCH_3).

Enzymatic resolution of H-D,L-Mag-NH_2. To a solution of 180 g of H-D,L-Mag-NH_2 (1.41 mol) in 1.45 L of water (approximate pH 9.5), adjusted to pH 8.4 with acetic acid, were added 20 g of freeze-dried *Mycobacterium neoaurum* ATCC 25795.[20] The mixture was shaken (150 rpm) at 40°C for 18 h. At a conversion of 40% (HPLC) the cell mass was removed by centrifugation, followed by filtration over *Hyflo*. The filtrate was first concentrated under reduced pressure to remove NH_3 and then diluted to 2.5 L with water. The acid and the amide were separated by an ion-exchange column chromatography (Amberlyst A26, a strongly basic ion-exchange resin). After evaporation of the first eluant 120 g of H-D-Mag-NH_2 (content 80 wt%, yield 53%, e.e. 63%) were recovered as an oil. After elution of the ion-exchange column with a 2 N acetic acid solution and evaporation of the eluant 89 g of H-L-Mag-OH (content 77 wt%, yield 38%, e.e. 92%) were isolated as a solid.

C^{α}-Methyl-L-allylglycine, H-L-Mag-OH. H-L-Mag-OH (89 g, content 77 wt%, e.e. 92%), obtained from the enzymatic resolution, was flushed with toluene to remove the remaining water and acetic acid. The residue was recrystallized from isopropanol/water 3:1 (950 mL). After 4 d the chemically pure *title compound* was isolated by filtration and dried on air (48 g, 77%; content >99 wt%, e.e. 99.3%); $[\alpha]_D^{20}=-17.8$ (*c* 1.3, 1 N HCl); δ_H (200 MHz, DMSO d_6) 7.40 (3H, br s, NH_3^+), 5.78 (1H, m, γC*H*), 5.07 (2H, m, δCH_2), 2.37 (2H, m, βCH_2), 1.20 (3H, s, βCH_3); HRMS (EI): MH^+, found 130.0873. $C_6H_{12}NO_2$ requires 130.0868.

C^{α}-Methyl-D-allylglycine, H-D-Mag-NH_2. A second enzymatic resolution was performed on the partially enriched H-D-Mag-NH_2 (105 g, content 80 wt%, e.e. 63%). The amide was dissolved in 0.95 L of water (approximate pH 9.5), the pH was adjusted to 8.4 with acetic acid, and *Mycobacterium neoaurum* (22 g) was added. The mixture was shaken (170 rpm) at 40°C for 3 d, after which the conversion had reached 23% (HPLC). The enzyme was removed by centrifugation, and the acid and amide were separated by ion-exchange column chromatography and isolated as described above. H-D-Mag-NH_2 (62 g, 78%) was obtained as an oil, e.e. >99.5%, $[\alpha]_D^{20}=4.7$ (*c* 3.7, 1 N HCl); the NMR data were identical to those of H-D,L-Mag-NH_2. HRMS (EI): MH^+, found 129.1021. $C_6H_{13}N_2O$ requires 129.1028.

C^{α}-Methyl-D-allylglycine, H-D-Mag-OH. H-D-L-Mag-OH with e.e.>99% was obtained in quantitative yield by alkaline hydrolysis of H-D-Mag-NH_2 with a 2 N KOH solution at reflux for 2 h. The NMR data were identical to those of H-L-Mag-OH.

***N*-Pivaloyl-C^{α}-methyl-L-allylglycyl *tert*-butylamide, Piv-L-Mag-NH*t*Bu.** To a suspension of H-L-Mag-OH (0.516 g, 4.0 mmol) in anhydrous pyridine (10 mL) at room temperature were added under stirring Piv-Cl (1.446 g, 12.0 mmol) and after 30 min NMM (0.44 mL, 4.0 mmol). The mixture was stirred at room temperature overnight. Then, the solvent was evaporated to dryness. The residue was dissolved in EtOAc and the organic solution was quickly washed with water, 5% $NaHCO_3$, water, 0.5 M citric acid, and water, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The resulting 5(4*H*)oxazolone from Piv-L-Mag-OH was treated without further purification with *tert*-butylamine (2.10 mL, 20.0 mmol) in DMF (10 mL) at 65°C for 24 h. After evaporation of the solvent in vacuo, purification of the crude product by flash chromatography gave the *title compound* (0.68 g, 64%) as a solid, mp 120–121°C (toluene/pentane); R_fI 0.90, R_fII 0.90, R_fIII 0.60; $[\alpha]_D^{20}=+3.6$ (*c* 0.5, MeOH); ν_{max} (KBr) 3373, 3350, 1667, 1634, 1539 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 6.64 (1H, s, N*H*), 6.19 (1H, s, N*H*), 5.69 (1H, m, γC*H*), 5.14 (2H, m, δCH_2), 2.84 (2H, m, βCH_2), 1.56 (3H, s, βCH_3), 1.34 (9H, s, NH*t*Bu

CH_3), 1.18 (9H, s, Piv CH_3). HRMS (EI): M^+, found 268.2143. $C_{15}H_{28}N_2O_2$ requires 268.2151.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycine, Boc-L-Mag-OH.** A solution of H-L-Mag-OH (1.00 g, 7.74 mmol) and NaOH (0.31 g, 7.74 mmol) in a 1:1 dioxane/water solution (20 mL) was cooled to 0°C. Boc_2O (1.85 g, 8.51 mmol) was added in three portions and the reaction was stirred at room temperature for 10 h and at 35°C for 16 h. The pH was kept at 10 by addition of 2 N NaOH. Then, dioxane was evaporated under reduced pressure. Excess of Boc_2O was extracted with diethyl ether (Et_2O) and the aqueous layer was acidified to pH 3 with 10% $KHSO_4$. The product was extracted with EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and filtered. Evaporation to dryness under reduced pressure gave the *title compound* (1.59 g, 90%) as an oil; R_fI 0.40, R_fII 0.90, R_fIII 0.20; $[\alpha]_D^{20}=-19.0$ (*c* 0.5, MeOH); ν_{max} (film) 3434, 1712, 1635 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 10.62 (1H, s, COO*H*), 5.72 (1H, m, γC*H*), 5.19 (1H, s, N*H*), 5.13 (2H, m, δCH_2), 2.66 (2H, m, βCH_2), 1.54 (3H, s, βCH_3), 1.44 (9H, s, Boc 3 CH_3); HRMS (EI): MH^+, found 230.1383. $C_{11}H_{20}NO_4$ requires 230.1392.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycine methylester, Boc-L-Mag-OMe.** To a solution of Boc-L-Mag-OH (0.52 g, 2.26 mmol) and TEA (0.94 mL, 6.78 mmol) in CH_2Cl_2, EDC·HCl (0.48 g, 2.48 mmol) and HOAt (0.34 g, 2.48 mmol) were added at 0°C. After 1 h MeOH (about 20 equiv.) was added and the solution was stirred at room temperature for 24 h. Then, the solvent was evaporated to dryness. The residue was dissolved in EtOAc, the organic solution washed with 10% $KHSO_4$, water, 5% $NaHCO_3$, water, dried over anhydrous Na_2SO_4, and filtered. Evaporation to dryness gave the *title compound* (0.58 g, 75%) as an oil; R_fI 0.90, R_fII 0.90, R_fIII 0.85; $[\alpha]_D^{20}=-14.8$ (*c* 0.5, MeOH); ν_{max} (film) 3428, 3376, 1742, 1718 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 5.66 (1H, m, γC*H*), 5.15 (1H, s, N*H*), 5.13 (2H, m, δCH_2), 3.74 (3H, s, OMe CH_3), 2.69 and 2.56 (2H, 2m, βCH_2), 1.53 (3H, s, βCH_3), 1.43 (9H, s, Boc CH_3); HRMS (EI): M^+, found 243.1465. $C_{12}H_{21}NO_4$ requires 243.1471.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycine-α-aminoisobutyric acid methylester, Boc-L-Mag-Aib-OMe.** To a solution of BocLMag-OH (2.11 g, 9.18 mmol) in CH_2Cl_2 (10 mL) and TEA (3.83 mL, 27.54 mmol) cooled to 0°C, HOAt (1.78 g, 12.85 mmol) and EDC·HCl (2.50 g, 12.85 mmol) were added. After 2 min HCl·H-Aib-OMe[39] (2.30 g, 14.90 mmol) was added and the reaction was stirred at room temperature for 2 d. Then, the solvent was removed and the residue redissolved in EtOAc. The organic solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and filtered. Evaporation to dryness gave the *title compound* (2.71 g, 90%) as an oil; R_fI 0.80, R_fII 0.90, R_fIII 0.40; $[\alpha]_D^{20}=60.0$ (*c* 0.5, MeOH); ν_{max} (KBr) 3359, 3330, 1743, 1731, 1684, 1524 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.04 (1H, s, Aib N*H*), 5.73 (1H, m, Mag γC*H*), 5.16 (2H, m, Mag δCH_2), 4.97 (1H, s, Mag N*H*), 3.73 (3H, s, OMe CH_3), 2.722.48 (m, 2H, Mag βCH_2), 1.541.48 (9H, 3s, Mag βCH_3 and Aib 2 βCH_3), 1.43 (9H, s, Boc 3 CH_3); HRMS (EI): M^+, found 328.2004. $C_{16}H_{28}N_2O_5$ requires 328.1998.

***N-tert*-Butyloxycarbonyl-α-aminoisobutyryl-C$^\alpha$-methyl-L-allylglycyl-α-aminoisobutyric acid methylester, Boc-AibL-Mag-Aib-OMe.** TFA·H-L-Mag-Aib-OMe [obtained by treatment with a 30% solution of TFA in CH_2Cl_2 of the corresponding Boc-protected dipeptide methylester (1.36 g, 4.15 mmol)] was dissolved in CH_2Cl_2 (20 mL) and NMM (1.37 mL, 12.44 mmol) and the solution cooled to 0°C. To a solution of Boc-Aib-OH[40] (1.35 g, 6.63 mmol) in CH_2Cl_2 (20 mL) and NMM (1.37 mL, 12.44 mmol), EDC·HCl (1.27 g, 6.64 mmol) and HOAt (0.90 g, 6.64 mmol) were added at 0°C. After 15 min the two solutions were combined and the resulting mixture was stirred at room temperature for 4 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. The *title compound* (1.32 g, 77%) was obtained by precipitation with light petroleum ether as a solid, mp 155–156°C; R_fI 0.85, R_fII 0.95, R_fIII 0.40; $[\alpha]_D^{20}=-30.8$ (*c* 0.5, MeOH); ν_{max} (KBr) 3392, 3296, 1728, 1691, 1657, 1531 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.50 (1H, s, N*H*), 6.53 (1H, s, N*H*), 5.84 (1H, m, Mag γC*H*), 5.185.12 (2H, m, Mag δCH_2), 4.84 (1H, s, N*H*), 3.69 (3H, s, OMe CH_3), 2.822.74 and 2.392.33 (2H, 2m, Mag βCH_2), 1.591.40 (m, 15H, Mag βCH_3 and 2 Aib 4 βCH_3), 1.45 (9H, s, Boc 3 CH_3); HRMS (EI): M^+, found 413.2527. $C_{20}H_{35}N_3O_6$ requires 413.2526.

***N-tert*-Butyloxycarbonyl-α-aminoisobutyryl-α-aminoisobutyryl-C$^\alpha$-methyl-L-allylglycyl-α-aminoisobutyric acid methylester, Boc-Aib-Aib-L-Mag-Aib-OMe.** TFA·H-AibLMag-Aib-OMe [obtained by treatment with TFA of the corresponding Boc-protected tripeptide methylester (0.70 g, 1.69 mmol)] was dissolved in CH_2Cl_2 (15 mL) and NMM (0.55 mL, 5.07 mmol) and the solution was cooled to 0°C. Boc-Aib-OH (0.55 g, 2.70 mmol) was dissolved in CH_2Cl_2 (10 mL) and NMM (0.55 mL, 5.07 mmol), then EDC·HCl (518 mg, 2.70 mmol) and HOAt (368 mg, 2.70 mmol) were added at 0°C. The two solutions were combined and the reaction mixture was stirred at room temperature for 4 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. The *title compound* (0.78 g, 93%) was obtained by precipitation with light petroleum as a solid, mp 186–187°C; R_fI 0.60, R_fII 0.85, R_fIII 0.20; $[\alpha]_D^{20}=-2.5$ (*c* 0.5, MeOH); ν_{max} (KBr) 3335, 3309, 1726, 1679, 1652, 1522 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.34 (1H, s, N*H*), 7.27 (1H, s, N*H*), 6.53 (1H, s, N*H*), 5.68 (1H, m, Mag γC*H*), 5.05 (2H, m, Mag δCH_2), 4.89 (1H, s, Aib N*H*), 3.69 (3H, s, OMe CH_3), 3.042.52 (2H, m, Mag βCH_2), 1.571.38 (21H, m, Mag βCH_3 and 3 Aib 6 βCH_3), 1.46 (s, 9H, Boc 3 CH_3); HRMS (EI): MH^+, found 499.3120. $C_{24}H_{43}N_4O_7$ requires 499.3132.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycyl-α-aminoisobutyryl-α-aminoisobutyryl-C$^\alpha$-methyl-L-allylglycyl-α-aminoisobutyric acid methylester, Boc-L-Mag-Aib-Aib-L-Mag-Aib-OMe.** TFA·H-Aib-Aib-L-Mag-Aib-OMe [obtained by treatment with TFA of the corresponding Boc-protected tetrapeptide methylester (0.44 g, 0.89 mmol)] was dissolved in CH_2Cl_2 (15 mL) and NMM (0.29 mL, 2.68 mmol) and the solution cooled to 0°C. Boc-L-Mag-OH (348 mg, 1.52 mmol) was dissolved in

CH_2Cl_2 (15 mL) and NMM (0.29 mL, 2.68 mmol), EDC·HCl (291 mg, 1.52 mmol) and HOAt (207 mg, 1.52 mmol) were added at 0°C. The two solutions were combined and the reaction mixture was stirred at room temperature for 3 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound* (379 mg, 70%) as a solid, mp 240–241°C; R_fI 0.65, R_fII 0.85, R_fIII 0.20; $[\alpha]_D^{20}=-6.3$ (*c* 0.5, MeOH); ν_{max} (KBr) 3310, 1727, 1661, 1530 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.72(1H, s, N*H*), 7.41(1H, s, N*H*), 7.10 (1H, s, N*H*), 6.47 (1H, s, N*H*), 5.74 (2H, m, 2 Mag 2 γC*H*), 5.06 (4H, m, 2 Mag 2 δCH_2), 4.87 (1H, s, Mag N*H*), 3.69 (3H, s, OMe CH_3), 3.052.51 (4H, m, 2Mag 2 βCH_2), 1.551.39 (24H, m, 2 Mag 2 βCH_3 and 3 Aib 6 βCH_3), 1.46 (9H, s, Boc 3 CH_3); HRMS (EI): M^+, found 609.3739. $C_{30}H_{51}N_5O_8$ requires 609.3738.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycyl-L-alanine methylester, Boc-L-Mag-L-Ala-OMe.** To a solution of Boc-L-Mag-OH (750 mg, 3.27 mmol) in CH_2Cl_2 (10 mL) and NMM (1.1 mL, 9.6 mmol) cooled to 0°C, HOAt (899 mg, 6.60 mmol) and EDC·HCl (1.26 g, 6.60 mmol) were added. After 15 min HCl·H-L-Ala-OMe (1.0 g, 7.0 mmol) was added and the reaction was stirred at room temperature for 20 h. Then, the solvent was removed and the residue redissolved in EtOAc. The solution was extracted with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound* (0.98 g, 96%) as a solid, mp 90–91°C; R_fI 0.95, R_fII 0.95, R_fIII 0.50; $[\alpha]_D^{20}=-40.8$ (*c* 1, MeOH); ν_{max} (KBr) 3314, 1751, 1712, 1684, 1653, 1524 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 6.95 (1H, d, *J*=7.0 Hz, Ala N*H*), 5.72 (1H, m, Mag γC*H*), 5.15 (2H, m, Mag δCH_2), 4.91 (1H, s, Mag N*H*), 4.57 (1H, dq, *J*=7.0, 7.3 Hz, Ala αC*H*), 3.74 (3H, s, OMe CH_3), 2.762.53 (2H, m, Mag βCH_2), 1.48 (3H, s, Mag βCH_3), 1.44 (9H, s, Boc 3 CH_3), 1.40 (3H, d, *J*=7.3 Hz, Ala βCH_3); HRMS (EI): M^+, found 314.1857. $C_{15}H_{26}N_2O_5$ requires 314.1842.

***N-tert*-Butyloxycarbonyl-L-alanyl-C$^\alpha$-methyl-L-allylglycyl-L-alanine methylester, Boc-L-Ala-L-Mag-L-Ala-OMe.** To a solution of Boc-L-Ala-OH (1.07 g, 5.4 mmol) in CH_2Cl_2 (10 mL) and NMM (0.89 mL, 8.1 mmol) cooled to 0°C, HOAt (734 mg, 5.4 mmol) and EDC·HCl (1.05 g, 5.4 mmol) were added. After 15 min TFA·H-L-Mag-L-Ala-OMe [obtained by treatment of the corresponding Boc-protected dipeptide methylester (850 mg, 2.70 mmol) with a 30% solution of TFA in CH_2Cl_2)] in CH_2Cl_2 (10 mL) and NMM (0.89 mL, 8.1 mmol) were added and the reaction was stirred at room temperature for 24 h. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound* (1.00 g, 97%) as a solid, mp 154–156°C; R_fI 0.95, R_fII 0.95, R_fIII 0.35; $[\alpha]_D^{20}=-64.8$ (*c* 0.5, MeOH); ν_{max} (KBr) 3390, 3294, 1746, 1683, 1644, 1529 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.11 (1H, d, *J*=6.3 Hz, Ala N*H*), 6.60 (1H, s, Mag N*H*), 5.69 (1H, m, Mag γC*H*), 5.17 (2H, m, Mag δCH_2), 4.90 (1H, br d, *J*=6.0 Hz, Ala N*H*), 4.52 (1H, dq, *J*=6.3, 7.3 Hz, Ala αC*H*), 4.05 (1H, dq, *J*=6.0, 7.0 Hz, Ala αC*H*), 3.73 (3H, s, OMe CH_3), 2.822.62 (2H, m, Mag βCH_2), 1.57 (3H, s, Mag βCH_3), 1.45 (9H, s, Boc 3 CH_3), 1.41 (3H, d, *J*=7.3 Hz, Ala βCH_3), 1.36 (3H, d, *J*=7.0 Hz, Ala βCH_3); HRMS (EI): M^+, found 385.2227. $C_{18}H_{31}N_3O_6$ requires 385.2213.

N-*tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-C$^\alpha$-methyl-L-allylglycyl-L-alanine methylester, Boc-L-Ala-L-Ala-L-Mag-L-Ala-OMe. To a solution of Boc-L-Ala-OH (666 mg, 3.52 mmol) in CH_2Cl_2 (10 mL) and NMM (0.64 mL, 6.21 mmol) cooled to 0°C, HOAt (479 mg, 3.52 mmol) and EDC·HCl (675 mg, 3.52 mmol) were added. After 15 min TFA·H-L-Ala-L-Mag-L-Ala-OMe [obtained by treatment of the corresponding Boc-protected tripeptide methylester (800 mg, 2.07 mmol) with a 30% solution of TFA in CH_2Cl_2)] in CH_2Cl_2 (10 mL) and NMM (0.64 mL, 6.21 mmol) were added and the reaction was stirred at room temperature for 2 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (680 mg, 72%) was recrystallized from Et_2O/light petroleum, mp 164–165°C; R_fI 0.90, R_fII 0.90, R_fIII 0.30; $[\alpha]_D^{20}=-69.6$ (*c* 0.5, MeOH); ν_{max} (KBr)3369, 3284, 1745, 1705, 1657, 1539 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.00 (1H, d, *J*=7.0 Hz, Ala N*H*), 6.61 (1H, d, *J*=6.2 Hz, Ala N*H*), 6.60 (1H, s, Mag N*H*), 5.64 (1H, m, Mag γC*H*), 5.14 (2H, m, Mag δCH_2), 4.91 (1H, br d, *J*=6.5 Hz, Ala N*H*), 4.52 (1H, dq, *J*=7.0, 7.3 Hz, Ala αC*H*), 4.28 (1H, dq, *J*=6.2, 7.3 Hz, Ala αC*H*), 4.13 (1H, dq, *J*=6.5, 6.6 Hz, Ala αC*H*), 3.74 (3H, s, OMe CH_3), 2.772.66 (2H, m, Mag βCH_2), 1.54 (3H, s, Mag βCH_3), 1.44 (9H, s, Boc 3 CH_3), 1.40 (3H, d, *J*=7.3 Hz, Ala 3 βCH_3), 1.39 (3H, d, *J*=7.3 Hz, Ala 3 βCH_3), 1.36 (3H, d, *J*=6.6 Hz, Ala 3 βCH_3); HRMS (EI): MH^+, found 457.2665. $C_{21}H_{37}N_4O_7$ requires 457.2662.

***N-tert*-Butyloxycarbonyl-C$^\alpha$-methyl-L-allylglycyl-L-alanyl-L-alanyl-C$^\alpha$-methyl-L-allylglycyl-L-alanine methylester, Boc-L-Mag-L-Ala-L-Ala-L-Mag-L-Ala-OMe.** To a solution of Boc-L-Mag-OH (392 mg, 1.71 mmol) in CH_2Cl_2 (5 mL) and NMM (0.33 mL, 3.0 mmol) cooled to 0°C, HOAt (233 mg, 1.71 mmol) and EDC·HCl (328 mg, 1.71 mmol) were added. After 15 min TFA·H-L-Ala-L-Ala-L-Mag-L-Ala-OMe [obtained by treatment of the corresponding Boc-protected tetrapeptide methylester (520 mg, 1.14 mmol) with a 30% solution of TFA in CH_2Cl_2)] in CH_2Cl_2 (5 mL) and NMM (0.33 mL, 3.0 mmol) were added and the reaction was stirred at room temperature for 24 h. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound*(582 mg, 90%) as a solid, mp 132–134°C; R_fI 0.90, R_fII 0.90, R_fIII 0.25; $[\alpha]_D^{20}=-46.6$ (*c*0.5, MeOH); ν_{max} (KBr) 3314, 1742, 1665, 1526 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.71 (1H, d, *J*=7.0 Hz, Ala N*H*), 7.10 (1H, d, *J*=7.3 Hz, Ala N*H*), 6.90 (1H, s, Mag N*H*), 6.42 (1H, d, *J*=5.5 Hz, Ala N*H*), 5.78 (2H, m, 2Mag 2 γC*H*), 5.315.11 (4H, m, 2 Mag 2 δCH_2), 5.03 (s, 1H, Mag N*H*), 4.51 (1H,dq, *J*=7.3, 6.3 Hz, Ala αC*H*), 4.32 (1H, dq, *J*=7.0, 7.0 Hz, Ala αC*H*), 4.24 (1H, dq, *J*=5.5, 7.0 Hz, Ala αC*H*), 3.70 (s, 3H, OMe CH_3), 2.812.42 (4H, m, 2 Mag 2 βCH_2), 1.52 (6H, s, 2 Mag 2 βCH_3), 1.45 (9H, s, Boc 3 CH_3), 1.44 (6H, d, *J*=7.0 Hz, 2 Ala 2 βCH_3), 1.39 (3H, d, *J*=6.3 Hz, Ala βCH_3); HRMS (EI): M^+, found 567.3260. $C_{27}H_{45}N_5O_8$ requires 567.3268.

***N-tert*-Butyloxycarbonyl-C$^{\alpha}$-methyl-L-allylglycyl-D-alanine methylester, Boc-L-Mag-D-Ala-OMe.** To a solution of Boc-L-Mag-OH (1.12 g, 4.88 mmol) in CH_2Cl_2 (10 mL) and NMM (1.60 mL, 14.6 mmol) cooled to 0°C, HOAt (1.19 g, 8.78 mmol) and EDC·HCl (1.68 g, 8.78 mmol) were added. After 15 min HCl·H-D-Ala-OMe (1.23 g, 8.78 mmol) was added and the reaction was stirred at room temperature for 3 d. Then, the solvent was removed and the residue redissolved in EtOAc. The organic solution was washed with 10% $KHSO_4$, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound* (1.39 g, 91%) as a solid, mp 102–103°C; R_fI 0.90, R_fII 0.85, R_fIII 0.50; $[\alpha]_D^{20}$=1.2 (*c* 0.5, MeOH); ν_{max} (KBr) 3318, 1752, 1713, 1685, 1652, 1522 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 6.89 (1H, d, *J*=7.1 Hz, Ala N*H*), 5.75 (1H, m, Mag γC*H*), 5.17 (2H, m, Mag δCH_2), 4.96 (1H, s, Mag N*H*), 4.57 (1H, dq, *J*=7.1, 7.3 Hz, Ala αC*H*), 3.74 (3H, s, OMe CH_3), 2.662.54 (2H, m, Mag βCH_2), 1.51 (3H, s, Mag βCH_3), 1.44 (9H, s, Boc 3 CH_3), 1.40 (3H, d, *J*=7.3 Hz, Ala βCH_3); HRMS (EI): M$^+$, found 314.1856. $C_{15}H_{26}N_2O_5$ requires 314.1842.

***N-tert*-Butyloxycarbonyl-L-alanyl-C$^{\alpha}$-methyl-L-allylglycyl-D-alanine methylester, Boc-L-Ala-L-Mag-D-Ala-OMe.** To a solution of Boc-L-Ala-OH (1.24 g, 6.56 mmol) in CH_2Cl_2 (10 mL) and NMM (1.27 mL, 11.57 mmol) cooled to 0°C, HOAt (0.89 g, 6.56 mmol) and EDC·HCl (1.25 g, 6.56 mmol) were added. After 15 min TFA·H-L-Mag-D-Ala-OMe [obtained by treatment of the corresponding Boc-protected dipeptide methylester (1.21 g, 3.86 mmol) with a 30% solution of TFA in CH_2Cl_2] in CH_2Cl_2 (10 mL) and NMM (1.27 mL, 11.57 mmol) were added and the reaction was stirred at room temperature for 3 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the *title compound* (1.30 g, 88%) as a solid, mp 129–131°C; R_fI 0.70, R_fII 0.80, R_fIII 0.30; $[\alpha]_D^{20}$=−2.5 (*c* 0.5, MeOH); ν_{max} (KBr) 3355, 3310, 1735, 1711, 1667, 1648, 1541 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.20 (1H, br d, *J*=6.5 Hz, Ala N*H*), 6.65 (1H, s, Mag N*H*), 5.87 (1H, m, Mag γC*H*), 5.18 (2H, m, Mag δCH_2), 4.86 (1H, br d, *J*=6.2 Hz, Ala N*H*), 4.49 (1H, dq, *J*=6.5, 7.3 Hz, Ala αC*H*), 3.95 (1H, dq, *J*=6.2, 7.2 Hz, Ala αC*H*), 3.71 (3H, s, OMe CH_3), 2.702.42 (2H, m, Mag βCH_2), 1.62 (3H, s, Mag βCH_3), 1.46 (9H, s, Boc 3 CH_3), 1.43 (3H, d, *J*=7.3 Hz, Ala βCH_3), 1.36 (3H, d, *J*=7.2 Hz, Ala βCH_3); HRMS (EI): M$^+$, found 385.2214. $C_{18}H_{31}N_3O_6$ requires 385.2213.

***N-tert*-Butyloxycarbonyl-L-alanyl-L-alanyl-C$^{\alpha}$-methyl-L-allylglycyl-D-alanine methylester, Boc-L-Ala-L-Ala-L-Mag-D-Ala-OMe.** To a solution of Boc-L-Ala-OH (973 mg, 4.90 mmol) in CH_2Cl_2 (10 mL) and NMM (0.95 mL, 8.64 mmol) cooled to 0°C, HOAt (666 mg, 4.90 mmol) and EDC·HCl (938 mg, 4.90 mmol) were added. After 15 min TFA·H-L-Ala-L-Mag-D-Ala-OMe [obtained by treatment of the corresponding Boc-protected tripeptide methylester (1.11 g, 2.88 mmol) with a 30% solution of TFA in CH_2Cl_2] in CH_2Cl_2 (10 mL) and NMM (0.95 mL, 8.64 mmol) were added and the reaction was stirred at room temperature for 4 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (1.02 g, 78%) was recrystallized from Et_2O/lightpetroleum, mp 72–74°C; R_fI 0.55, R_fII 0.80, R_fIII 0.25; $[\alpha]_D^{20}$=−3.3 (*c* 0.5, MeOH); ν_{max} (KBr) 3322, 1742, 1656, 1527 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 6.96 (1H, d, *J*=5.8 Hz, Ala N*H*), 6.73 (1H, br d, *J*=6.0 Hz, Ala N*H*), 6.66 (1H, s, Mag N*H*), 5.81 (1H, m, Mag γC*H*), 5.13 (2H, m, Mag δCH_2), 4.90 (1H, d, *J*=6.2 Hz, Ala N*H*), 4.52 (1H, dq, *J*=5.8, 7.3 Hz, Ala αC*H*), 4.23 (1H, dq, *J*=6.0, 7.1 Hz, Ala αC*H*), 4.14 (1H, dq, *J*=6.2, 7.0 Hz, Ala αC*H*), 3.73 (3H, s, OMe CH_3), 2.71–2.56 (2H, m, Mag βCH_2), 1.57 (3H, s, Mag βCH_3), 1.46 (9H, s, Boc 3 CH_3), 1.42 (3H, d, *J*=7.3 Hz, Ala βCH_3), 1.39 (3H, d, *J*=7.1 Hz, Ala βCH_3), 1.37 (3H, d, *J*=7.0 Hz, Ala βCH_3); HRMS (EI): M$^+$, found 456.2595. $C_{21}H_{36}N_4O_7$ requires 456.2584.

***N-tert*-Butyloxycarbonyl-C$^{\alpha}$-methyl-L-allylglycyl-L-alanyl-L-alanyl-C$^{\alpha}$-methyl-L-allylglycyl-D-alanine methylester, Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe.** To a solution of Boc-L-Mag-OH (624 mg, 2.72 mmol) in CH_2Cl_2 (5 mL) and NMM (0.56 mL, 5.10 mmol) cooled to 0°C, HOAt (370 mg, 2.72 mmol) and EDC·HCl (521 mg, 2.72 mmol) were added. After 15 min TFA·H-L-Ala-L-Ala-L-Mag-D-Ala-OMe [obtained by treatment of the corresponding Boc-protected tetrapeptide methylester (700 mg, 1.70 mmol) with a 30% solution of TFA in CH_2Cl_2)] in CH_2Cl_2 (5 mL)and NMM (0.56 mL, 5.10 mmol) were added and the reaction was stirred at room temperature for 4 d. Then, EtOAc was added and the mixture was washed with 0.5 M citric acid, water, 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and concentrated. Addition of light petroleum gave the title compound (801 mg, 83%) as a solid, mp 83–85°C; R_fI 0.90, R_fII 0.90, R_fIII 0.25; $[\alpha]_D^{20}$=7.2 (*c* 0.5, MeOH); ν_{max} (KBr) 3313, 1742, 1658, 1530 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.74 (1H, d, *J*=7.0 Hz, Ala N*H*), 7.10 (1H, d, *J*=6.7 Hz, Ala N*H*), 6.91 (1H, s, Mag N*H*), 6.40 (1H, d, *J*=5.1 Hz, Ala N*H*), 5.84 (2H, m, 2 Mag 2 γC*H*), 5.325.11 (4H, m, 2 Mag 2 δCH_2), 4.97 (1H, s, Mag N*H*), 4.51 (1H, dq, *J*=6.7, 6.3 Hz, Ala αC*H*), 4.20 (2H, m, 2Ala 2 αC*H*), 3.70 (3H, s, OMe CH_3), 2.722.42 (4H, m, 2 Mag 2 βCH_2), 1.52 (6H, s, 2 Mag 2 βCH_3), 1.46 (9H, s, Boc 3 CH_3), 1.44 (3H, d, *J*=7.0 Hz, Ala βCH_3), 1.43 (3H, d, *J*=7.1 Hz, Ala βCH_3), 1.42 (3H, d, *J*=6.3 Hz, Ala βCH_3); HRMS (EI): M$^+$, found 567.3292. $C_{27}H_{45}N_5O_8$ requires 567.3268.

FTIR 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. The solution IR absorption spectra were recorded using a Perkin–Elmer model 1720X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were obtained under the same conditions. Cell with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% D) was purchased from Fluka.

Nuclear magnetic resonance. The ^{1}H NMR spectra were recorded with a Bruker model AM 400 spectrometer.

Measurements were carried out in deuterochloroform (99.96% D; Aldrich) and deuterated DMSO (99.96% d6; Acros Organics) with tetramethylsilane as the internal standard. The free radical TEMPO was purchased from Sigma.

Mass spectrometry. HRMS was carried out on a Micromass AutoSpecE mass spectrometer tuned to resolution 6000 (at 5% peak height). Spectra were scanned at a speed of 2 s/dec for the full mass range (*m/z* 20 900). Acquisitions were performed by means of a water-cooled high temperature solid probe with a constant bleed of perfluorokerosene (reference) via the septum inlet. The 'secondary reference correction' method was used to compensate for the scan-to-scan errors which occur when acquiring data by scanning the magnet (e.g. drift and hysteresis). This result was achieved by looking for the peaks of the secondary reference file, which have to be present during the acquisition. When the data file was mass measured using 'accurate correction', we located all reference peaks within the secondary reference file. The located secondary reference peaks were then flagged as reference peaks and their masses reassigned to their real mass. With a reference peak on either side of an unknown mass peak, and knowing the exponential scan law between the two reference peaks, the mass of the unknown peak could be calculated.

X-Ray diffraction. Single crystals of $^{\oplus}H_2$-L-Mag-$O^{\ominus}$ monohydrate, Boc-Aib-L-Mag-Aib-OMe and Boc-L-Mag-L-Ala-L-Ala-L-Mag-D-Ala-OMe were obtained by slow evaporation at room temperature from 2-propanol/H_2O, EtOAc/light petroleum and MeOH, respectively. Single crystals of Piv-L-Mag-NH*t*Bu and Boc-L-Mag-D-Ala-OMe were grown by vapour diffusion at room temperature from $CHCl_3$/*n*pentane and EtOAc/light petroleum, respectively. Intensity data collection was performed using a Philips PW 1100 four-circle diffractometer. Graphite-monochromated CuKα radiation (λ=1.54178 Å) and $\theta/2\theta$ scan mode were used. Cell parameters were obtained by least-squares refinements of the angular setting of 48 carefully centred high angle reflections. The structures were solved by direct methods (SHELXS 86[41] program for Piv-L-Mag-NH*t*Bu, while SHELXS 97[42] program for the other compounds). Refinement was carried out on F^2, with all non-H atoms anisotropic, by application of the SHELXL 97[43] program.

In $^{\oplus}H_2$-L-Mag-O^{-} monohydrate (**1**) the CG and CD atoms of the allyl side chain were refined over two sets of positions (C1G, C1D and C1G′, C1D′ atoms, respectively) with population parameters of 0.70 and 0.30, respectively. A similar side-chain disorder is shown by the N-terminal Mag residue in the structure of the pentapeptide, in which the major (C1G, C1D atoms) and the minor (C1G′ C1D′ atoms) conformers were refined with population parameters of 0.58 and 0.42, respectively. Restraints were applied to the bond distances and bond angles for the refinement of the disordered moieties. It has to be mentioned that displacement parameters significantly higher than the average characterize the Mag side-chain CG and CD atoms in all structures. However, the data did not support a viable model for disorder apart from the two cases discussed above. In the free amino acid the positions of the H-atoms of the $-NH_3^+$ moiety and the co-crystallized water molecule were recovered from a difference Fourier map. All other H-atoms in this structure and the remaining structures were calculated at idealized positions. During the refinement these H-atoms were allowed to ride on their carrying atom, with U_{iso} set equal to 1.2 (or 1.5 for methyl groups) times the U_{eq} of the parent atom. The crystal of the pentapeptide did not significantly diffract above θ=50° (1.0 Å resolution). The relatively high values of R_{int}, as well as of the R_1 and wR_2 factors might be ascribed to the far from optimal crystal quality. However, we are confident that the conformational features of the molecule, as discussed in this work, are firmly established.

Acknowledgements

The authors thank Dr Gerard T. C. Kwakkenbos of DSM Research for the HRMS determinations.

References

1. Spatola, A. F. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, Weinstein, B., Ed.; Marcel Dekker: New York, 1983; vol. 7, pp 267–357.
2. Aubry, A.; Boussard, G.; Cung, M. T.; Marraud, M.; Vitoux, B. *J. Chim. Phys.* **1988**, *85*, 345–359.
3. Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. *Biochem. J.* **1990**, *268*, 249–262.
4. Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747–6756.
5. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061–1072.
6. Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. *Chem. Eur. J.* **1996**, *2*, 1104–1111.
7. Poché, D. S.; Thibodeaux, S. J.; Rucker, V. C.; Warner, I. M.; Daly, W. H. *Macromolecules* **1997**, *30*, 8081–8084.
8. Guinn, R. M.; Margot, A. O.; Taylor, J. R.; Schumacher, M.; Douglas, S. C.; Harvey, W. B. *Biopolymers* **1995**, *35*, 503–512.
9. Semple, J. E.; Minami, N. K.; Tamura, S. Y.; Brunck, T. K.; Nutt, R. F.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2421–2426.
10. Badorrey, R.; Cativiela, C.; Diaz-de-Villegas, M. D.; Galvez, J. A.; Lapeña, Y. *Tetrahedron: Asymmetry* **1997**, *8*, 311–317.
11. Blackwell, H. E.; Grubbs, R. H. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 3281–3284.
12. Clark, T. D.; Kobayashi, K.; Ghadiri, M. R. *Chem. Eur. J.* **1999**, *5*, 782–792.
13. 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**, 2583–2589.
14. Biagini, S. C. G.; Davies, R. G.; Gibson, V. C.; Giles, M. R.; Marshall, E. L.; North, M.; Robson, D. A. *J. Chem. Soc., Chem. Commun.* **1999**, 235–236.
15. Hammer, K.; Romming, C.; Undheim, K. *Tetrahedron* **1998**, *54*, 10837–10850.
16. Broxterman, Q. B.; Kaptein, B.; Kamphuis, J.; Schoemaker, H. E. *J. Org. Chem.* **1992**, *57*, 6286–6294.
17. van der Werf, A.; Kellogg, R. M. *Tetrahedron Lett.* **1988**, *29*, 4981–4984.

18. Williams, R. M. In *Methods in Molecular Medicine: Peptidomimetics Protocols*, Kazmierski, W. M., Ed.; Humana Press: Totowa, NJ, 1999; vol. 23, pp 339–356.
19. Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron Lett.* **1992**, *33*, 6007–6010.
20. Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Peters, P. J. H.; Schoemaker, H. E.; Kamphuis, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1113–1116.
21. 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*, 1171–1175.
22. Rutjes, F. P. J. T.; Veerman, J. J. N.; Meester, W. J. N.; Hiemstra, H.; Schoemaker, H. E.; Eur *J. Org. Chem.* **1999**, 1127–1135.
23. Ojima, I.; Delaloge, F. *Chem. Soc. Rev.* **1997**, *26*, 377–386.
24. 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*, 1826–1827.
25. 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*, 63–74.
26. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
27. Bonora, G. M.; Mapelli, C.; Toniolo, C.; Wilkening, R. R.; Stevens, E. S.; Int *J. Biol. Macromol.* **1984**, *6*, 179–188.
28. Kopple, K. D.; Ohnishi, M. *Biochemistry* **1969**, *8*, 4087–4095.
29. Kopple, K. D.; Schamper, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 3644–3646.
30. IUPAC-IUB Commission on Biochemical Nomenclature *J. Mol. Biol.* **1970**, *52*, 1–17.
31. Benedetti, E.; Pedone, C.; Toniolo, C.; Némethy, G.; Pottle, M. S.; Scheraga, H. A.; Int *J. Pept. Protein Res.* **1980**, *16*, 156–172.
32. Allen, F. H.; Kennard, O.; Watson, D. G.; Brammer, L.; Orpen, A. G.; Taylor, R. *J. Chem. Soc., Perkin Trans. II* **1987**, S1–S19.
33. Toniolo, C.; Benedetti, E. *Molecular Conformation and Biological Interactions*; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, India, 1991, pp 511–521.
34. Zimmerman, S. S.; Pottle, M. S.; Némethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1–9.
35. Toniolo, C.; Benedetti, E. *Trends Biochem. Sci.* **1991**, *16*, 350–353.
36. Toniolo, C.; Pantano, M.; Formaggio, F.; Crisma, M.; Bonora, G. M.; Aubry, A.; Bayeul, D.; Dautant, A.; Boesten, W. H.; Schoemaker, H. E.; Kamphuis, J. *Int. J. Biol. Macromol.* **1994**, *16*, 7–14.
37. Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425–1436.
38. Duchateau, A. L. L.; Knuts, H.; Boesten, J. M. M.; Guns, J. J. *J. Chromatogr.* **1992**, *623*, 237–245.
39. Paterson, Y.; Stimson, E. R.; Evans, D. J.; Leach, S. J.; Scheraga, H. A.; Int *J. Pept. Protein Res.* **1982**, *20*, 468–480.
40. Mayr, W.; Oekonomopulos, R.; Jung, G. *Biopolymers* **1979**, *18*, 425–450.
41. Sheldrick, G. M. SHELXS 86. Program for the Solution of Crystal Structures; University of Göttingen: Göttingen, Germany, 1986.
42. Sheldrick, G. M. SHELXS 97. Program for the Solution of Crystal Structures; University of Göttingen: Göttingen, Germany, 1997.
43. Sheldrick, G. M. SHELXS 97. Program for Crystal Structure Refinement; University of Göttingen: Göttingen, Germany, 1997.