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Enantiopure C^{α}-tetrasubstituted α-amino acids. Chemo-enzymatic synthesis and application to turn-forming peptides

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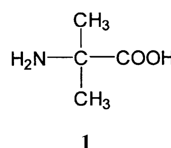
Abstract—By a chemo-enzymatic approach we carried out a large-scale synthesis of four enantiopure, sterically constrained, C^{α}-tetrasubstituted α-amino acids, all characterized by a sidechain $C^{\gamma}C^{\delta}$ double bond. By using one of them (L-Mag), we prepared an N^{α}-protected tetrapeptide benzylamide which was shown to adopt a β-turn conformation and to efficiently undergo ring-closing olefin metathesis. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Chiral C^{α}-tetrasubstituted α-amino acids are increasingly attracting attention as useful building blocks for the production of naturally occurring and tailor-made derivatives of pharmaceutical and agricultural interest.[1–4] In addition, it has been unambiguously demonstrated that these synthons, in analogy with their achiral prototype α-aminoisobutyric acid (Aib) (**1**),[5–10] are able to dramatically reduce the conformational freedom of the peptides in which they are incorporated. Indeed, detailed experimental investigations[11–13] have shown that peptides rich in chiral C^{α}-tetrasubstituted α-amino acids either fold in screw-sense biased β-turns[14–16] and 3_{10}-helical structures[17] or adopt planar, fully-extended (C_5) conformations.[15,18,19] The stabilization of specific ordered secondary structures has recently become a major issue in peptide science, with special emphasis to the design of rigidified catalysts, spacers, and templates (for leading review articles see Refs. 20–22).

In this context, allyl-type, chiral C^{α}-tetrasubstituted α-amino acids are of emerging relevance for the additional potential ability of their peptides to undergo a wide range of transformations at the $C^{\gamma}{=}C^{\delta}$ bond, including Grubbs' intramolecular, side-chain to side-chain, ring-closing olefin metathesis.[23,24] The preparation of 'built-into' compounds[25–35] and cyclic peptides via intramolecular carbon–carbon formation[36–43] has been already reported for allyl-based, chiral C^{α}-*tri*substituted α-amino acids and peptides. For the first time, Verdine and coworkers[44] have recently extended the exploding field of ring-closing metathesis to peptides characterized by C^{α}-methyl-L-allylglycine (L-Mag) (L-**2a**) and related residues.



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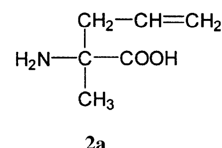

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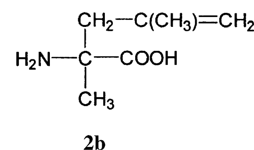

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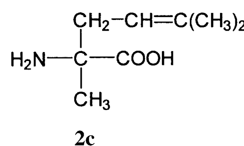

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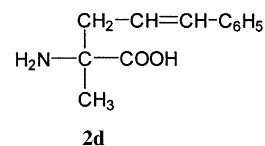

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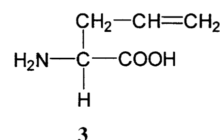

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Keywords: amino acids and derivatives; metathesis; NMR; peptides and polypeptides.

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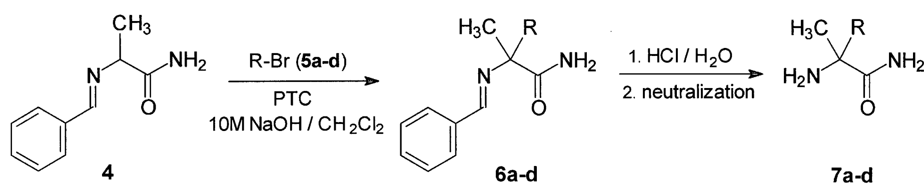
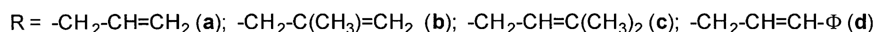




Scheme 1.

As part of a programme dealing with chemistry and structural characterization of allyl-type, C^{α}-tetrasubstituted α-amino acids, and their derivatives and peptides,[25,44–63] in this article we describe the large-scale synthesis of the four enantiopure α-amino acids L-**2a–d**. We also prepared and investigated the preferred conformation of: (i) a linear, N^{α}-protected tetrapeptide benzylamide containing one residue of L-Mag (L-**2a**) and one of its unmethylated counterpart L-allylglycine (L-Agl) (L-**3**), and (ii) its side-chain to side-chain, ring-closing olefin metathesis/hydrogenation derivative.

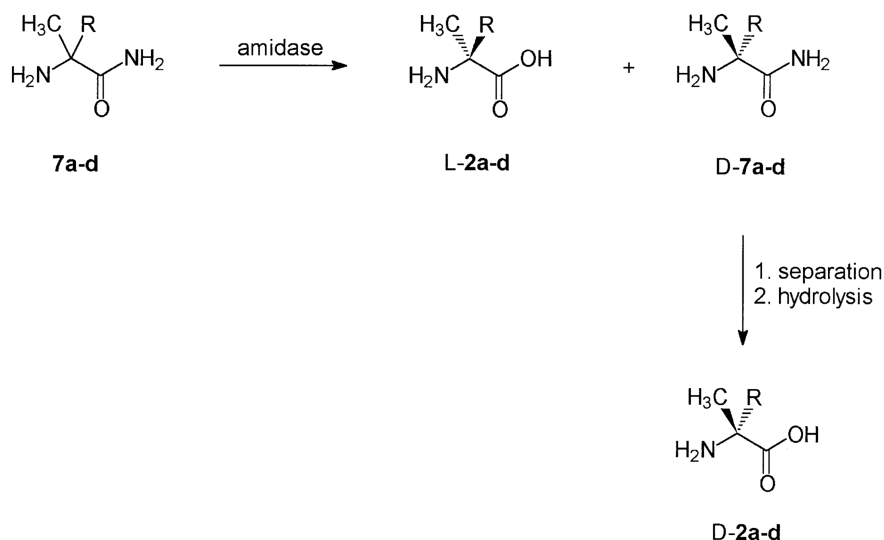
2. Results and discussion

2.1. Amino acid synthesis and enzymatic resolution

A variety of methods for the synthesis of enantiomerically pure C^{α}-tetrasubstituted α-amino acids (recently reviewed in Ref. 64) have been described. At DSM Research we concentrated on the preparation of this type of amino acids via enzymatic resolution of racemic α-amino amides. This route is especially attractive for large-scale preparation since the racemic α-amino amide substrates are readily accessible and the enzymatic resolution can be easily scaled-up and proceeds with high stereoselectivity. Currently, we have three amidases available for α-amino amide resolution.[65] The L-aminopeptidase from *Pseudomonas putida* ATCC 12633 is used for the enzymatic resolution of C^{α}-trisubstituted α-amino amides. This enzyme has recently been cloned and overexpressed in an *E. coli* K-12 host microorganism. For the resolution of C^{α}-tetrasubstituted α-amino amides two amidases (from *Mycobacterium neoaurum* ATCC 25795 and *Ochrobactrum anthropi* NCIMB 40321, respectively) are available, both exhibiting a high L-stereoselectivity. The amidase from the latter species is also able to stereoselectively hydrolyze C^{α}-trisubstituted α-amino amides.

Racemic C^{α}-tetrasubstituted α-amino amides (**7b–d**) were prepared by PTC (phase-transfer catalyzed) alkylation of *N*-benzylidene-D,L-alaninamide **4** (Scheme 1), as previously described for **7a**.[46] With allyl-type bromides **5a**, **c** and **d** C^{α}-alkylation is fast and proceeds to high conversion. After acidic work-up of the racemic Schiff bases **6a**, **c** and **d**, the racemic C^{α}-tetrasubstituted α-amino amides **7a**, **c** and **d** were all obtained in 72–73% yield. In the alkylation reactions with prenyl (3,3-dimethallyl) bromide (**5c**) or cinnamyl bromide (**5d**) a complete S_N2 reaction occurred and no S_N2' product was observed. C^{α}-Alkylation of **4** with 3-bromo-2-methyl-1-propene **5b** (or 3-chloro-2-methyl-1-propene) proceeded only to low conversion. In addition, a substantial amount of byproducts occurred, which made the purification of the reaction mixture difficult. All attempts to improve the yield of this reaction failed.

The four racemic substrates **7a–d** were subjected to enzymatic hydrolysis using the amidases from *M. neoaurum*[48,66] and *O. anthropi*[67] (Scheme 2). Thus, the stereoselectivity of the two enzymes could be compared. In general, the



Scheme 2.

Table 1. Results of the enzymatic resolutions

Substrate	R	Amidase	Conversion (%)	e.e.$_{amide}$ (%)	e.e.$_{acid}$ (%)	*E*-ratio
7a	$-CH_2-CH=CH_2$	*M. neoaurum*	40	63	92	46
		O. anthropi	64	50	27	2.7
7b	$-CH_2-C(CH_3)=CH_2$	*M. neoaurum*	46	85	98	>250
		O. anthropi	46	72	85	27
7c	$-CH_2-CH=C(CH_3)_2$	*M. neoaurum*	45	80	98	>250
		O. anthropi	62	98	61	17
7d	$-CH_2-CH=CH-Ph$	*M. neoaurum*	51	≥98	95	≥180
		O. anthropi	31	45	96	79[a]

[a] At 40–50% conversion the *E*-ratio is 65–69.

enzymatic resolutions are performed on a 10% aqueous solution of the substrate. With the *M. neoaurum* amidase the initial pH was set at 8.0–8.5. With the amidase from *O. anthropi* a starting pH between 6 and 8 was applied as this enzyme has a much broader pH optimum. Since the enzyme reactions are not buffered, the pH during the reaction increased to 9.6 due to the formation of ammonia. The conversion of the resolution reactions was measured using an ammonia sensitive electrode. After the appropriate reaction time, in general between 6 and 24 h, the crude cell mass was removed by centrifugation and the amide and acid were separated by ion-exchange chromatography (strongly basic resin). The results of these enzymatic resolutions are listed in Table 1. If necessary, the e.e. values of the L-acids can be improved by recrystallization from isopropanol/water.

In all resolution experiments the L-enantiomer of the amide was preferably hydrolysed to the L-amino acid, L-**2a–d**, leaving the D-enantiomer of the amide, D-**7a–d**, behind. The observed stereoselectivity is clearly a result of steric interactions during the enzyme reaction. For the *M. neoaurum* amidase an active site model was suggested in which a small hydrophobic pocket is present and able to contain the C$^{\alpha}$-methyl substituent of the amides **7a–d**.[48,62,66] Larger substituents, like an ethyl or an allyl group, are accepted with great difficulty, which results in a slow hydrolysis of the D-enantiomer. In general, the enantiomeric ratio *E*[68] improves with increasing size of the large substituent. As already known from earlier results,[48,66] branching at the α-amino amide β-position results in a complete stereoselectivity (*E*>250) for the amidase from *M. neoaurum*, as well as for that from *O. anthropi*. From the current results it can be concluded that increasing the branching at the γ-(**7b**) and to a somewhat lesser extent also at the δ-position (**7c** and **7d**) has a similar effect.

Hydrogenation of the double bond of C$^{\alpha}$-methyl-D,L-allylglycinamide (**7a**) substantially improves the stereoselectivity of both enzymes. Indeed, C$^{\alpha}$-methyl-D,L-norvalinamide is hydrolysed by *M. neoaurum* amidase with a nearly complete stereoselectivity (*E*>232), while the enantiomeric ratio with the amidase from *O. anthropi* is enhanced from 27 to 97.[69] Similarly, C$^{\alpha}$-methyl-D,L-leucinamide, the hydrogenated analogue of **7b**, is hydrolysed with full stereoselectivity by the amidase from both species.[48,65,66,70]

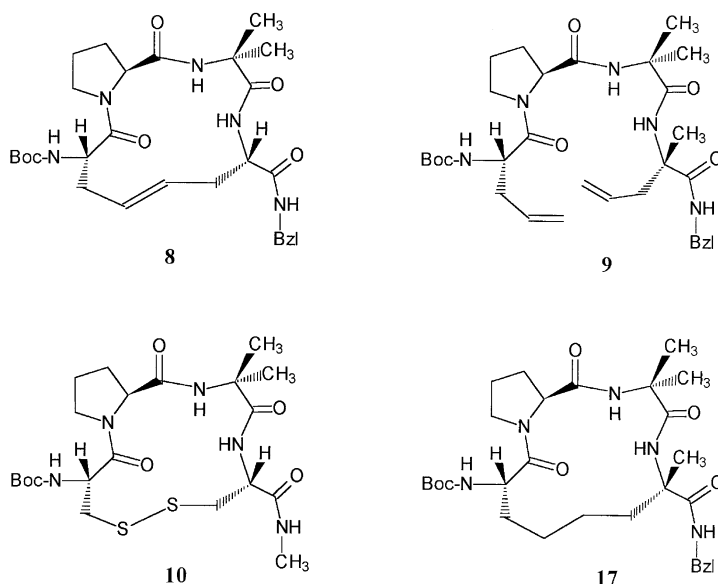
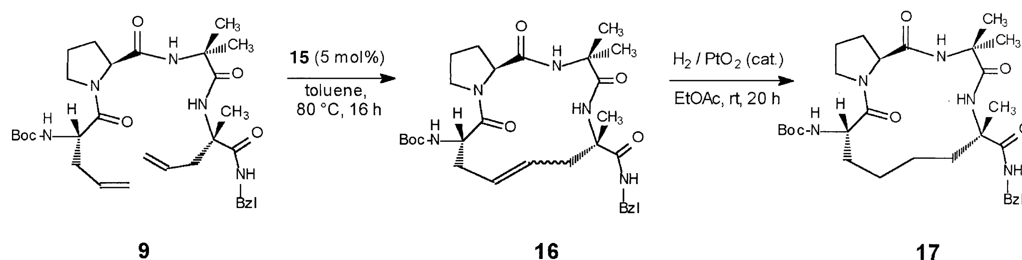


Figure 1. Representation of Grubbs' alkene-stabilized -L-Pro-Aib- β-turn peptide **8**, our linear β-turn peptide Boc-L-Agl-L-Pro-Aib-L-Mag-NHBzl **9**, Balaram's disulfide-stabilized -L-Pro-Aib- β-turn peptide **10**, and our alkane-stabilized -L-Pro-Aib- β-turn peptide **17**.



Scheme 3.

On the other hand, replacing of the C$^{\alpha}$-methyl group of **7a** by a phenyl group (i.e. C$^{\alpha}$-phenyl-D,L-allylglycinamide) results in the complete loss of activity of the *M. neoaurum* amidase,[48] whereas the *S*-enantiomer is still hydrolysed by the *O. anthropi* amidase (E>200).[71]

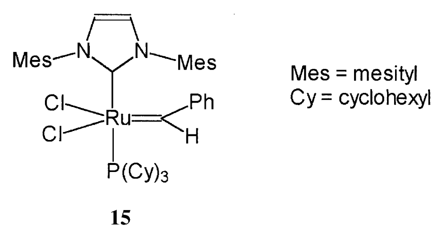
In summary, the active site of the *O. anthropi* amidase can bind larger substrates than those of the amidase from *M. neoaurum*. Therefore, the more open active site of *O. anthropi* results in a lower stereoselectivity for the substrates **7a–d** as compared to *M. neoaurum*.

2.2. Peptide synthesis, metathesis and conformational characterization

In this work we exploited the simplest of the four allyl-type, C$^{\alpha}$-tetrasubstituted α-amino acids discussed above, i.e. L-Mag (L-**2a**), in the C=C covalent stabilization of a tetrapeptide β-turn conformation involving a ring-closing metathesis/hydrogenation approach. To this aim we decided to partly modify the terminally blocked -L-Agl-L-Pro-Aib-L-Agl- sequence, shown to undergo olefin ring-closing metathesis to **8** (Fig. 1) in 60% yield by Grubbs and coworkers,[36] by replacing the L-Agl (L-**3**) residue in position 4 with its C$^{\alpha}$-methylated counterpart L-Mag (L-**2a**). The reasons for our choice of the -L-Agl-L-Pro-Aib-L-Mag- sequence **9** were based on the following observations: (i) The -L-Pro-Aib- dipeptide is expected to easily fold into a β-turn conformation.[36,72,73] (ii) L-Mag (L-2a) is known to overwhelmingly prefer ϕ,ψ backbone torsion angles in the right-handed helical region of the conformational space.[62,63] (iii) An X-ray diffraction analysis of **10**, the disulfide analogue of **8**, carried out by Balaram and coworkers,[72] clearly showed that, while Cys^1 is *semi*-extended, Cys^4 is helical.

The Boc (*tert*-butoxycarbonyl) N$^{\alpha}$-protected derivatives, including those of L-Agl (**11**)[74,75] and L-Mag,[62] were prepared in excellent yield by reacting the free amino acid with $(Boc)_2O$ (di-*tert*-butyldicarbonate) in an aqueous/dioxane mixture. The Boc-L-Mag-NHBzl (NHBzl, benzylamino) (**12**) C-terminal derivative was synthesized by using *N*-ethyl,*N′*-[3-(dimethylamino)propyl]-carbodiimide (EDC) as the dehydrating agent. Synthesis of the linear tetrapeptide **9** was performed step-by-step in solution via the intermediates Boc-Aib-L-Mag-NHBzl (**13**) and Boc-L-Pro-Aib-L-Mag-NHBzl (**14**), beginning from the C-terminal H-L-Mag-NHBzl. Peptide bond formation was achieved by the EDC/HOAt (1-hydroxy-7-aza-1,2,3-benzotriazole) method[76] in CH_2Cl_2 in the presence of the tertiary amine *N*-methylmorpholine (NMM). Using this approach, the three sterically hindered peptide bonds were obtained in satisfactory yields. Removal of the Boc N$^{\alpha}$-protecting group was performed by treatment with diluted trifluoroacetic acid (TFA).

While stirring of the linear Agl/Mag tetrapeptide diene **9** in the presence of 5 mol% of the conventional, Ru-based Grubbs' catalyst[77] resulted in complete recovery of the starting material (even at high temperatures), subjection of **9** to the newly developed, very active, thermally stable, dihydroimidazolylidene catalyst analogue (**15**)[78] led, after 16 h at 80°C, to the ring-closed, olefin product **16** as a mixture of two C=C bond diastereomers in 82% isolated yield (Scheme 3). Unfortunately, the isomers were not separable by regular column chromatography nor by HPLC. Subsequent hydrogenation of the isomeric mixture **16** with H_2/PtO_2 in ethyl acetate resulted in a clean reduction of the C=C bond to the saturated carbon–carbon cyclic tetrapeptide **17** in 85% isolated yield.



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To ascertain the type of conformation preferentially formed in solution by the cyclic compound **17** and its linear precursor **9** we performed a ^{1}H NMR investigation of the two tetrapeptides. The analysis was carried out in $CDCl_3$ at 1.0 mM concentration. 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 an increasing amount of the strong H-bonding acceptor solvent dimethylsulphoxide (DMSO)[79,80] 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).[81] Fig. 2 shows the behaviour of the NH resonances of the linear tetrapeptide **9** upon addition of DMSO and TEMPO. In this peptide only the N(3)H proton chemical shift is sensitive to the addition of DMSO and its resonance broadens 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

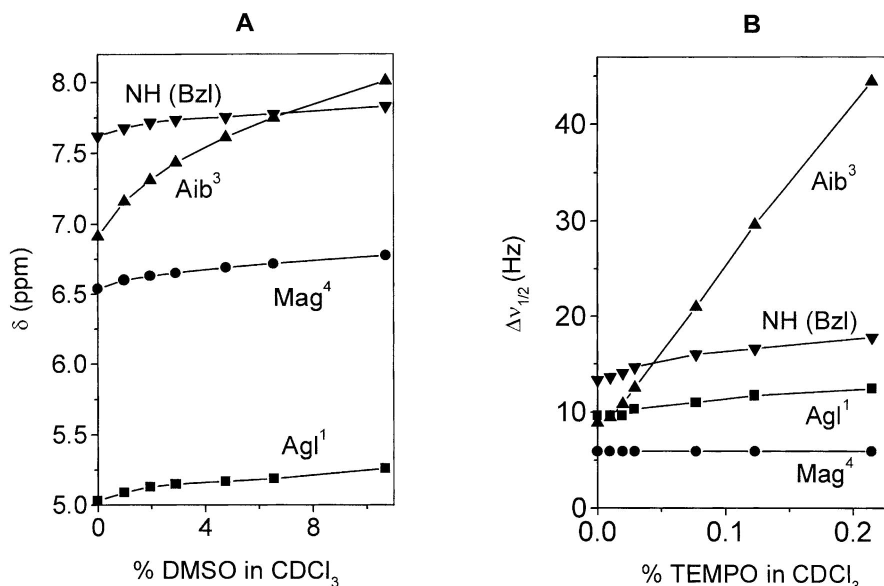

Figure 2. ^{1}H NMR titrations of the linear tetrapeptide Boc-L-Agl-L-Pro-Aib-L-Mag-NHBzl **9**. (A) Plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$ solution. Peptide concentration: 1.0 mM.

their linewidths are not influenced by addition of TEMPO. The cyclic tetrapeptide **17** behaves in a similar manner (Fig. 3)

From our ^{1}H NMR study and the known conformational tendencies of the Pro[82,83] and Aib[5–10] corner residues it is reasonable to conclude that the most populated conformation adopted in $CDCl_3$ solution by both terminally protected tetrapeptides **9** and **17** involves a β-turn centred at the -L-Pro^2-Aib^3- sequence with an intramolecular trans-annular H-bond connecting the N(4)H and C′1=O1 groups. The ROESY spectra of both peptides show a remarkably intense cross-peak between the $C^{\alpha}(2)$H and N(3)H protons (Fig. 4), the typical signature of a type-II β-turn conformation.[14–16] The overall preferred structure of both peptides must be quite compact, as the N(1)H and benzylamide NH protons as well appear to be highly solvent shielded.

3. Conclusions

By a chemo-enzymatic approach we have been able to synthesize in a large amount four enantiopure C^{α}-tetra-

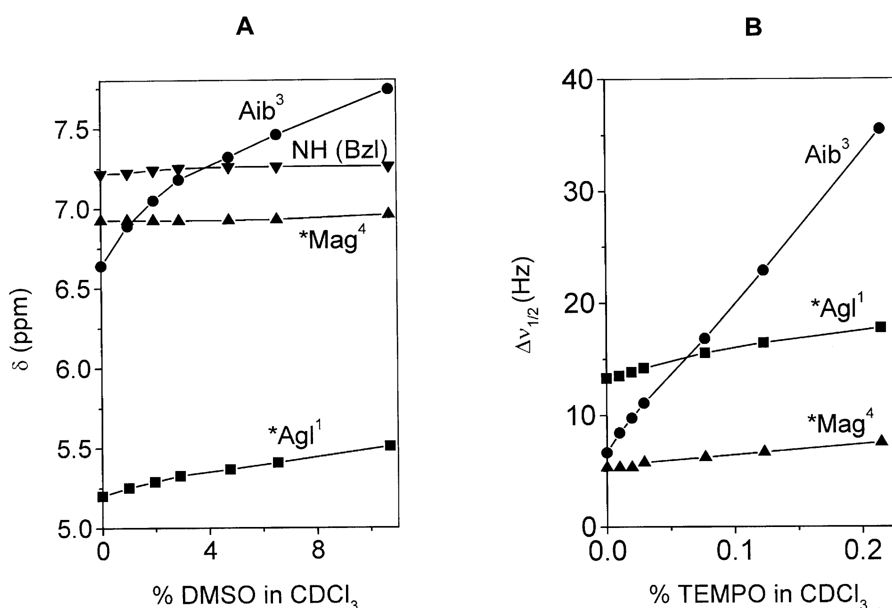

Figure 3. ^{1}H NMR titrations of the cyclized/reduced tetrapeptide Boc-L-Agl*-L-Pro-Aib-L-Mag*-NHBzl **17** (starred residues refer to those with the side chain modified by cyclization). (A) Plot of NH chemical shifts as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of bandwidth of the NH signals as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$ solution [the NH(Bzl) proton is under the benzyl aromatic protons]. Peptide concentration: 1.0 mM.

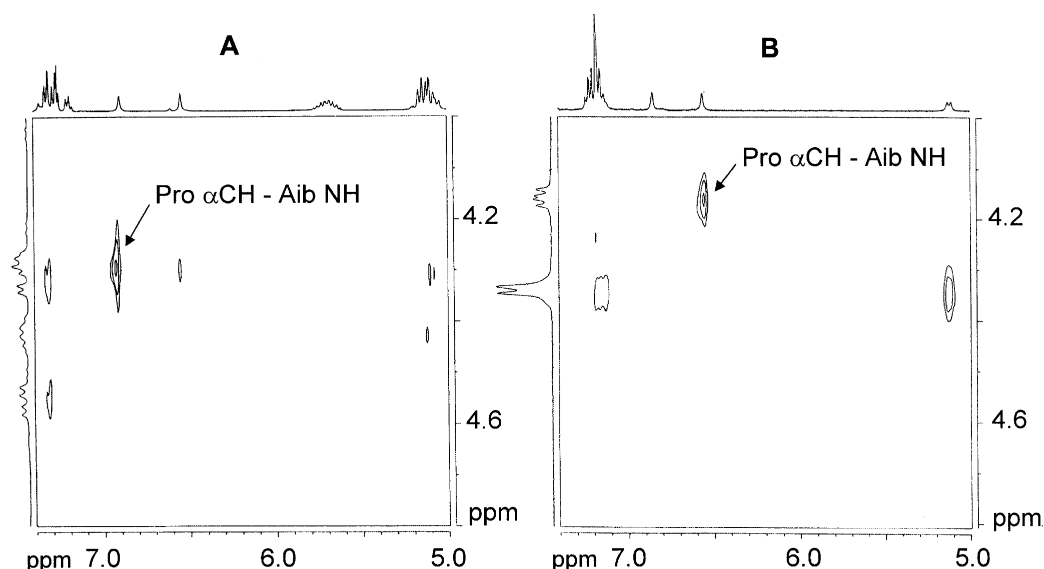

Figure 4. Partial ROESY spectra of (A) the linear tetrapeptide Boc-L-Agl-L-Pro-Aib-L-Mag-NHBzl **9** and (B) its cyclized/reduced derivative **17** in $CDCl_3$ solution.

substituted α-amino acids carrying a side chain of the allyl type. One of them, L-Mag (L-**2a**), was incorporated in a terminally blocked tetrapeptide (**9**), a model compound for ring-closing olefin metathesis. This reaction proceeds smoothly with a newly developed Grubbs' catalyst.[78] Reduction of the cyclized olefin diastereomeric mixture **16** afforded **17**, a peptide with a fully hydrogenated all-hydrocarbon cross-linking system.

The linear tetrapeptide diene precursor **9** and the final cyclic product **17** share a common conformational preference, in particular an intramolecularly H-bonded type-II β-turn motif. We conclude that preorganization in the linear precursor **9**, characterized by two allyl-based residues separated by two turn-forming residues in the amino acid sequence, favours ring closure and generates peptide **17**, in which the β-turn conformation is frozen by a side-chain to side-chain, 4-carbon cross-link. Compared to Grubbs' olefin cyclopeptide **8** and Balaram's disulfide analogue **10**, our linear and cyclic peptides (**9** and **17**, respectively) appear to be more markedly intramolecularly H-bonded, and hence, presumably, more extensively folded. We attribute this structural propensity to the presence of the severely conformationally constrained, turn/helix forming, C^{α}-tetrasubstituted α-amino acid Mag in **9** and its metathesized side-chain derivative in **17**.

4. Experimental

4.1. General

4.1.1. Amino acid and peptide synthesis. L-Allylglycine (2-amino-L-4-pentenoic acid) (L-**3**) is an ACROS product. 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); (IV) ethyl acetate (EtOAc). 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.[84] [eluent: 50 mM sodium acetate solution (titrated to pH 6.0 with acetic acid)/methanol 7:3]. Grubbs' catalyst **15** was prepared according to the literature procedure.[78] The synthesis and characterization of H-D,L-Mag-NH_2 (**7a**), H-L-Mag-OH (L-**2a**), and H-D-Mag-NH_2 (D-**7a**) have already been described.[46]

4.1.2. C^{α}-Methyl-D,L-(γ-methyl)allylglycinamide (7b). This compound was prepared as described for H-D,L-Mag-NH_2 (**7a**),[46] starting from *N*-benzylidene-D,L-alaninamide (**4**) (470 g, 2.7 mol) and 3-chloro-2-methyl-1-propene (285 mL, 2.9 mol). The *title compound*, obtained as a colourless oil after vacuum distillation (39 g, 10%), crystallized on standing, mp 70–72°C, bp 115–130°C (0.2 mmHg); δ_H (200 MHz, $CDCl_3$) 7.48 and 6.00 (2H, 2 br s, amide NH_2), 4.82 and 4.66 (2H, 2m, allyl δCH_2), 2.80 and 1.99 (2H, 2 d, allyl βCH_2), 1.64 (3H, s, allyl δCH_3), 1.45 (2H, s, amine NH_2), 1.25 (3H, s, βCH_3); δ_C (50 MHz, $CDCl_3$) 178.78 (s, *C*O), 141.71 (γ*C*), 114.63 (t, δCH_2), 55.84 (s, α*C*), 48.28 (t, βCH_2), 28.41 (q, δCH_3), 22.79 (q, βCH_3); HRMS (FAB): MH^+, found 143.1169. $C_7H_{15}N_2O$ requires 143.1184.

4.1.3. C^{α}-Methyl-D,L-prenylglycinamide (7c). This compound was prepared as described for H-D,L-Mag-NH_2 (**7a**),[46] starting from *N*-benzylidene-D,L-alaninamide (**4**) (17.2 g, 0.09 mol) and prenyl bromide (1-bromo-3-methyl-2-butene)[85] (16.0 g, 0.11 mol). The *title compound* (**7c**) (7.0 g, 50%) was obtained as a colourless oil after vacuum distillation, bp 131–134°C (1.8 mmHg); δ_H (50 MHz,

$CDCl_3$) 7.40 and 5.82 (2H, 2 br s, amide NH_2), 5.13 (1H, t, allyl γC*H*), 2.56 and 2.21 (2H, 2 dd, allyl βCH_2), 1.83 and 1.74 (6H, 2 s, εCH_3), 1.44 (2H, br s, amine NH_2), 1.34 (3H, s, βCH_3); δ_C (50 MHz, $CDCl_3$) 179.49 (s, *C*O), 135.15 (s, δ*C*), 117.71 (d, γ*C*H), 56.74 (s, α*C*), 38.42 (t, βCH_2), 26.16 and 25.01 (2 q, 2 εCH_3), 17.00 (q, βCH_3); HRMS (FAB): MH^+, found 157.1328. $C_8H_{17}N_2O$ requires 157.1341.

4.1.4. C$^{\alpha}$-Methyl-D,L-cinnamylglycinamide (7d). This compound was prepared as described for H-D,L-Mag-NH_2 (**7a**),[46] except for the toluene extraction that was omitted, and the final extraction performed with neat $CHCl_3$ (2×250 mL). Starting from *N*-benzylidene-D,L-alaninamide (**4**) (27.6 g, 0.16 mol) and cinnamyl bromide (24 mL, 0.16 mol), the *title compound* (22.7 g, 71%) was obtained as a white crystalline solid after crystallization from EtOAc/pentane, mp 112–114°C; δ_H (200 MHz, $CDCl_3$) 7.49 and 5.78 (2H, 2 s, amide NH_2), 7.35 (m, 5H, phenyl C*H*), 6.52 (1H, dd, allyl δC*H*), 6.17 (1H, m, allyl γC*H*), 2.94 and 2.30 (2H, 2 dd, allyl βCH_2), 1.51 (2H, s, amine NH_2), 1.43 (3H, s, βCH_3); δ_C (50 MHz, $CDCl_3$) 177.87 (s, *C*O), 135.07 (s, phenyl *C*), 132.54 (d, γ*C*H), 126.64, 125.54 and 124.30 (3d, phenyl *C*H), 122.98 (d, δ*C*H), 55.67 (s, α*C*), 43.07 (t, βCH_2), 25.68 (q, βCH_3); HRMS (FAB): MH^+, found 205.1330. $C_{12}H_{17}N_2O$ requires 205.1341.

4.2. General procedure for the enzymatic resolution of 7a–d with *Mycobacterium neoaurum*

To a solution of racemic α-amino amides (**7a–d**) (100 g) in water (1 L), adjusted to pH 8.0–8.5 with acetic acid, the freeze-dried *M. neoaurum* ATCC 25795[48,66] (8–10 g) was added. The mixture was shaken (150 rpm) at 40°C until a 40–50% conversion was reached (15–24 h, depending on the substrate) as determined by ammonia development or HPLC analysis. After removing the cell mass by centrifugation, the reaction mixture was filtered over *Hyflo*, concentrated under reduced pressure (to remove NH_3) and, finally, diluted to 500 mL with water. The L-amino acids (L-**2a–c**) were separated from the D-amino amides (D-**7a–c**) by ion-exchange column chromatography (Amberlyst A26, strongly basic ion-exchange resin). The D-amino amides were eluted first and recovered by evaporating the aqueous solution. The L-amino acids were obtained by eluting the ion-exchange column with a 2N acetic acid solution and evaporating the eluent to dryness. The L-amino acid L-**2d** was separated from its D-amino amide (D-**7d**) by extracting with $CHCl_3$ (5×100 mL) the aqueous solution which was basified to pH 9 with 5N NaOH. The D-amino amide precipitated from the organic phase on concentrating and cooling, while the L-amino acid crystallized from the aqueous layer after neutralization to pH 7 with 5N HCl and concentration under reduced pressure.

4.2.1. Resolution of C$^{\alpha}$-methyl-D,L-(γ-methyl)allylglycinamide (7b). The enzymatic resolution of C$^{\alpha}$-methyl-D,L-(γ-methyl)allylglycinamide (32 g, 0.22 mol) was stopped after 46% conversion.

*C$^{\alpha}$-Methyl-L-(γ-methyl)allylglycine (L-**2b**)* (15.0 g, 46%, e.e. 98%) was obtained as a pale yellow solid; δ_H (200 MHz, D_2O+DCl) 4.06 and 3.90 (2H, 2 br s, allyl δCH_2), 1.77 and 1.58 (2H, 2 d, allyl βCH_2), 0.71 (3H, s, allyl δCH_3), 0.62 (3H, s, allyl βCH_3); δ_C (50 MHz, D_2O+DCl) 173.36 (s, *C*O), 137.96 (s, γ*C*), 118.16 (t, δCH_2), 58.89 (s, α*C*), 44.18 (t, βCH_2), 23.33 and 22.21 (2 q, δCH_3 and βCH_3); HRMS (FAB): MH^+, found 144.1003. $C_8H_{16}NO_2$ requires 144.1025.

*C$^{\alpha}$-Methyl-D-(γ-methyl)allylglycinamide (D-**7b**)* (20 g, content 86 wt%, e.e. 85%) was recovered as a pale yellow oil.

4.2.2. Resolution of C$^{\alpha}$-methyl-D,L-prenylglycinamide (7c). The enzymatic resolution of C$^{\alpha}$-methyl-D,L-prenylglycinamide (61 g, 0.39 mol) was stopped after 45% conversion.

*C$^{\alpha}$-Methyl-L-prenylglycine (L-**2c**)* (21 g, 34%, e.e. 98%) was recovered as a pale yellow solid; δ_H (200 MHz, D_2O+DCl) 4.77 (br s, allyl γCH and solvent signal), 2.31 (2H, 2dd, allyl βCH_2), 1.41 (3H, s, allyl ε-CH_3), 1.34 (3H, s, allyl ε-CH_3), 1.27 (3H, s, βCH_3); δ_C (50 MHz, D_2O+DCl) 174.00 (s, *C*O), 140.91 (s, δ*C*), 114.41 (d, γ*C*H), 60.61 (s, α*C*), 35.53 (t, βCH_2), 25.47 (q, βCH_3), 21.45 and 17.58 (2 q, 2 εCH_3); HRMS (FAB): MH^+, found 158.1186. $C_8H_{16}NO_2$ requires 158.1181.

*C$^{\alpha}$-Methyl-D-prenylglycinamide (D-**7c**)* [47 g, approximately 50% (still containing some water), e.e. 80%] was obtained as a pale yellow oil.

4.2.3. Resolution of C$^{\alpha}$-methyl-D,L-cinnamylglycinamide (7d). Due to the poor solubility of C$^{\alpha}$-methyl-D,L-cinnamylglycinamide (15 g, 0.074 mol), the enzymatic resolution was performed on a 5% (w/w) suspension. The reaction was stopped after 51% conversion.

*C$^{\alpha}$-Methyl-L-cinnamylglycine (L-**2d**)* (7.04 g, 47%, e.e. 95%) was obtained as a white solid; δ_H (200 MHz, D_2O) 7.39 (5H, m, phenyl C*H*), 6.55 (1H, d, allyl δC*H*), 6.21 (1H, m, allyl γ-C*H*), 2.65 and 2.40 (2H, 2 dd, allyl βCH_2), 1.36 (3H, s, βCH_3); δ_C (50 MHz, D_2O+DCl) 173.65 (s, *C*O), 136.57 (d, γ*C*H), 136.50 (s, phenyl *C*), 129.08, 128.41 and 126.66 (3d, phenyl *C*H), 120.85 (d, δ*C*H), 60.18 (s, α*C*), 40.20 (t, βCH_2), 21.43 (q, βCH_3); HRMS (FAB): MH^+, found 206.1176. $C_{12}H_{16}NO_2$ requires 206.1181.

*C$^{\alpha}$-Methyl-D-cinnamylglycinamide (D-**7d**).* (6.98 g, 46%, e.e.≥98%) was obtained as a pale yellow solid.

4.3. General procedure for the enzymatic resolution of 7a–d with *Ochrobactrum anthropi*

To a solution of racemic α-amino amides (**7a–d**) (10 g) and $MnSO_4{\cdot}H_2O$ (15 mg) in H_2O (100 mL), adjusted to pH 6.0–8.0 with acetic acid or H_2SO_4, a crude cell suspension (10 wt%) of *O. anthropi* NCIMB 40321[67] (1 g) was added. The mixture was shaken (150 rpm) at 50°C until a conversion of 40–50% was reached (ammonia determination). The work-up of the enzymatic resolutions was performed as described above for the enzymatic resolutions with *M. neoaurum*.

4.3.1. Resolution of C$^{\alpha}$-methyl-D,L-(γ-methyl)allylglycinamide (7b). After a conversion of 46% was reached,

chiral HPLC analysis revealed 85% and 72% e.e. values for C$^{\alpha}$-methyl-L-(γ-methyl)allylglycine (L-**2b**) and C$^{\alpha}$-methyl-D-(γ-methyl)allylglycinamide (D-**7b**), respectively. Further work-up of the reaction was omitted.

4.3.2. Resolution of C$^{\alpha}$-methyl-D,L-prenylglycinamide (7c). After a conversion of 62% was reached, chiral HPLC analysis revealed 61% and 98% e.e. values for C$^{\alpha}$-methyl-L-prenylglycine (L-**2c**) and C$^{\alpha}$-methyl-D-prenylglycinamide (D-**7c**), respectively. Further work-up of the reaction was omitted.

4.3.3. Resolution of C$^{\alpha}$-methyl-D,L-cinnamylglycinamide (7d). After a conversion of 31% was reached, the work-up of the reaction yielded C$^{\alpha}$-methyl-L-cinnamylglycine (L-**2d**) (e.e. 96%) and C$^{\alpha}$-methyl-D-cinnamylglycinamide (D-**7b**) (e.e.$\geq$98%) as white solids. Further work-up of the reaction was omitted. The e.e. of L-**2d** improves by re-crystallization from an *i*PrOH/H_2O mixture.

4.3.4. *N-tert*-Butyloxycarbonyl-L-allylglycine, Boc-L-Agl-OH (11).[74,75] H-L-Agl-OH (0.50 g, 4.34 mmol) and NaOH (0.21 g, 5.21 mmol) in 30 mL of a 1:1 mixture of dioxane/H_2O was cooled to 0°C. Di-*tert*-butyl-dicarbonate (Boc_2O) (1.14 g, 5.21 mmol) was added in three portions and the reaction was stirred at room temperature for 24 h. Then, dioxane was evaporated under reduced pressure. Excess of Boc_2O was extracted with diethyl ether and the aqueous layer was acidified to pH 3 with 10% $KHSO_4$. The aqueous solution was extracted with EtOAc. The organic solution was washed with 10% $KHSO_4$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness to give the *title compound* (0.90 g, 96%) as a colourless oil; R_fI 0.35, R_fII 0.95, R_fIII 0.15; $[\alpha]_D^{20}=+9.5$ (*c* 0.5, MeOH); ν_{max} (KBr) 3363, 1715, 1698 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 5.71 (1H, m, γC*H*), 5.21 (2H, m, δC*H*$_2$), 5.14 (1H, d, *J*=8.3 Hz, N*H*), 4.39 (1H, m, αC*H*), 2.58 (2H, m, βC*H*$_2$), 1.45 (9H, s, Boc C*H*$_3$); HRMS (FAB): MH$^+$, found 215.2496. $C_{10}H_{17}NO_4$ requires 215.2493.

4.3.5. *N-tert*-Butyloxycarbonyl-C$^{\alpha}$-methyl-L-allylglycine benzylamide, Boc-L-Mag-NHBzl (12). To a solution of Boc-L-Mag-OH[62] (900 mg, 3.9 mmol) in CH_2Cl_2 (10 mL) cooled to 0°C, EDC·HCl (752 mg, 3.9 mmol) was added. After 20 min, benzylamine (4.5 mL, 39 mmol) was added and the reaction was stirred at room temperature for 2 days. Then, the solvent was removed and the residue dissolved in EtOAc. The solution was washed with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$ and H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (559 mg, 48%) was crystallized as a white solid from EtOAc/petroleum ether, mp 79–80°C; R_fI 0.95, R_fII 0.95, R_fIII 0.55; $[\alpha]_D^{20}=-15.3$ (*c* 0.5, MeOH); ν_{max} (KBr) 3332, 1732, 1682, 1650, 1540 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.30 (5H, m, Bzl C*H*), 6.73 (1H, br s, N*H*Bzl), 5.74 (1H, m, Mag γC*H*), 5.16 (2H, m, Mag δC*H*$_2$), 4.88 (1H, s, Mag N*H*), 4.45 (2H, d, *J*=5.5 Hz, Bzl C*H*$_2$), 2.73 (1H, dd, *J*=7.3, 14.1 Hz, Mag βC*H*$_2$), 2.55 (1H, dd, *J*=7.6, 14.1 Hz, Mag βC*H*$_2$), 1.51 (3H, s, Mag βC*H*$_3$), 1.40 (9H, s, Boc C*H*$_3$); HRMS (FAB): MH$^+$, found 319.2023. $C_{18}H_{35}N_3O_6$ requires 319.2022.

4.3.6. *N-tert*-Butyloxycarbonyl-α-aminoisobutyryl-C$^{\alpha}$-methyl-L-allylglycine benzylamide, Boc-Aib-L-Mag-NHBzl (13). To a solution of Boc-Aib-OH[86] (565 mg, 2.78 mmol) in CH_2Cl_2 (10 mL) cooled to 0°C, EDC·HCl (533 mg, 2.78 mmol) and HOAt (379 mg, 2.78 mmol) were added. After 30 min $CF_3CO_2^{\ominus}$ $^{\oplus}H_2$-L-Mag-NHBzl [obtained by treatment of the corresponding Boc-protected amino acid benzylamide (**12**) (590 mg, 1.85 mmol) with a 30% solution of TFA in CH_2Cl_2 for 30 min], neutralized with NMM (1.23 mL, 11.13 mmol), was added. The reaction mixture was stirred for 2 days. Then, EtOAc was added and the solution was extracted with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (373 mg, 50%) was crystallized as a white solid from EtOAc/petroleum ether, mp 178–179°C; R_fI 0.85, R_fII 0.95, R_fIII 0.35; $[\alpha]_D^{20}=-19.9$ (*c* 0.5, MeOH); ν_{max} (KBr) 3411, 3319, 3294, 1693, 1677, 1646, 1547 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.76 (1H, br s, N*H*Bzl), 7.30 (5H, m, Bzl C*H*), 6.47 (1H, s, Mag N*H*), 5.66 (1H, m, Mag γC*H*), 5.15 (2H, m, Mag δC*H*$_2$), 4.61 (1H, s, Aib N*H*), 4.52 (1H, dd, *J*=7.5, 18.2 Hz, Bzl C*H*$_2$), 4.38 (1H, dd, *J*=7.0, 18.2 Hz, Bzl C*H*$_2$), 2.86 (1H, dd, *J*=8.3, 16.2 Hz, Mag βC*H*$_2$), 2.42 (1H, dd, *J*=10.6, 16.2 Hz, Mag βC*H*$_2$), 1.59 (3H, s, Aib βC*H*$_3$), 1.57 (3H, s, Aib βC*H*$_3$), 1.41 (3H, s, Mag βC*H*$_3$), 1.27 (9H, s, Boc C*H*$_3$); HRMS (FAB): MH$^+$, found 404.2555. $C_{22}H_{33}N_3O_4$ requires 404.2549.

4.3.7. *N-tert*-Butyloxycarbonyl-L-prolyl-α-aminoisobutyryl-C$^{\alpha}$-methyl-L-allylglycine benzylamide, Boc-L-Pro-Aib-L-Mag-NHBzl (14). To a solution of Boc-Pro-OH (267 mg, 1.24 mmol) in CH_2Cl_2 (5 mL) cooled to 0°C, EDC·HCl (237 mg, 1.24 mmol) and HOAt (168 mg, 1.24 mmol) were added. After 30 min $CF_3CO_2^{\ominus}$ $^{\oplus}H_2$-Aib-L-Mag-NHBzl [obtained by treatment of the corresponding Boc-protected dipeptide benzylamide (**13**) (250 mg, 0.619 mmol) with a 30% solution of TFA in CH_2Cl_2 for 1 h], neutralized with NMM (409 μL, 3.71 mmol), was added. The reaction mixture was stirred for 2 days. Then, EtOAc was added and the solution was extracted with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (294 mg, 95%) was crystallized as a white solid from EtOAc/petroleum ether; mp 58–62°C; R_fI 0.80, R_fII 0.90, R_fIII 0.30; $[\alpha]_D^{20}=-57.9$ (*c* 0.5, MeOH); ν_{max} (KBr) 3307, 1672, 1541 cm^{-1}; δ_H (250 MHz, $CDCl_3$) 7.73 (1H, br s, N*H*Bzl), 7.31 (5H, m, Bzl C*H*), 7.09 (1H, s, N*H*), 6.50 (1H, s, N*H*), 5.67 (1H, m, Mag γC*H*), 5.13 (2H, m, Mag δC*H*$_2$), 4.46 (2H, m, Bzl C*H*$_2$), 4.09 (1H, m, Pro αC*H*), 3.36 (2H, m, Pro δC*H*$_2$), 2.94 (1H, dd, *J*=8.1, 17.6 Hz, Mag βC*H*$_2$), 2.46 (1H, dd, *J*=11.0, 17.6 Hz, Mag βC*H*$_2$), 2.06–1.84 (4H, m, Pro βC*H*$_2$ and γC*H*$_2$), 1.61 (3H, s, βC*H*$_3$), 1.54 (3H, s, βC*H*$_3$), 1.44 (9H, s, Boc C*H*$_3$), 1.43 (3H, s, βC*H*$_3$); HRMS (FAB): MH$^+$, found 501.3060. $C_{27}H_{40}N_4O_5$ requires 501.3077.

4.3.8. *N-tert*-Butyloxycarbonyl-L-allylglycyl-L-prolyl-α-aminoisobutyryl-C$^{\alpha}$-methyl-L-allylglycine benzylamide, Boc-L-Agl-L-Pro-Aib-L-MagNHBzl (9). To a solution of Boc-L-Agl-OH (**11**) (223 mg, 1.04 mmol) in CH_2Cl_2 (5 mL) cooled to 0°C, EDC·HCl (199 mg, 1.04 mmol) and HOAt (141 mg, 1.04 mmol) were added. After 30 min $CF_3CO_2^{\ominus}$ $^{\oplus}H_2$-L-Pro-Aib-L-Mag-NHBzl [obtained by treatment of the corresponding Boc-protected tripeptide

benzylamide (**14**) (260 mg, 0.519 mmol) with a 30% solution of TFA in CH_2Cl_2 for 1 h], neutralized with NMM (343 μL, 3.11 mmol), was added. The reaction mixture was stirred for 2 days. Then, EtOAc was added and the solution was extracted with 10% $KHSO_4$, H_2O, 5% $NaHCO_3$, H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness. The *title compound* (239 mg, 77%) was crystallized as a white solid from EtOAc/petroleum ether, mp 87–88°C; R_fI 0.75, R_fII 0.90, R_fIII 0.30; $[\alpha]_D^{20}=-63.2$ (*c* 0.5, MeOH); ν_{max} (KBr) 3310, 1710, 1674, 1640, 1539 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 7.63 (1H, br t, *J*= 5.6 Hz, N*H*Bzl), 7.31 (5H, m, Bzl C*H*), 6.90 (1H, s, Aib N*H*), 6.54 (1H, s, Mag N*H*), 5.71 (1H, m, Agl γC*H*), 5.66 (1H, m, Mag γC*H*), 5.12 (4H, m, Agl and Mag δCH_2), 5.05 (1H, d, *J*=8.8 Hz, Agl N*H*), 4.55 (1H, dd, *J*=6.5, 14.9 Hz, Bzl CH_2), 4.42 (1H, m, Agl αC*H*), 4.31 (1H, dd, *J*=5.3, 14.9 Hz, Bzl CH_2), 4.28 (1H, m, Pro αC*H*), 3.67 and 3.47 (2H, 2m, Pro δCH_2), 3.01 (1H, dd, *J*=6.8, 14.0 Hz, Mag βCH_2), 2.48 (1H, dd, *J*=8.2, 14.0 Hz, Mag βCH_2), 2.30 (2H, m, Agl βCH_2), 1.91 (4H, m, Pro βCH_2 and γCH_2), 1.53 (3H, s, Mag βCH_3), 1.44 (3H, s, Aib βCH_3), 1.43 (9H, s, Boc CH_3), 1.40 (3H, s, Aib βCH_3); HRMS (FAB): MH^+, found 598.3598. $C_{32}H_{47}N_5O_6$ requires 598.3605.

4.3.9. Cyclic peptide 17. Boc-L-Agl-L-Pro-Aib-L-Mag-NHBzl (**9**) (80 mg, 0.13 mmol) and Grubbs' catalyst **15** (5.7 mg, 6.7 μmol) were combined in dry and deoxygenated toluene (8 mL). After stirring for 16 h at 80°C under N_2, the solution was concentrated and purified by flash chromatography (EtOAc) to give the cyclic peptide **16** (63 mg, 82%) as a pale yellow oil (mixture of two C=C bond diastereomers). Part of this compound (14 mg, 0.024 mmol) and PtO_2 (cat.) were dissolved in EtOAc (2 mL) under an H_2-atmosphere. After stirring for 20 h at room temperature, the solution was filtrated through celite, concentrated, and purified by flash chromatography (EtOAc) to give the *title compound* (12 mg, 85%) as a waxy solid; R_fIV 0.25; ν_{max} (film) 3295, 1679, 1642 cm^{-1} δ_H (400 MHz, $CDCl_3$) 7.25 (6H, m, Bzl N*H* and aromatic C*H*), 6.93 (1H, s, Mag N*H*), 6.64 (1H, s, Aib N*H*), 5.19 (1H, d, *J*=8.4 Hz, Agl N*H*), 4.42 (3H, m, Bzl CH_2 and Agl αC*H*), 4.23 (1H, m, Pro αC*H*), 3.83 and 3.65 (2H, 2m, Pro δCH_2), 2.35–2.20 (2H, m, Pro βCH_2), 2.05–1.90 (4H, m, Agl 1 βCH_2, Mag 1 βCH_2 and Pro γCH_2), 1.75–1.25 (13H, m, Agl 1 βCH_2, Mag 1 βCH_2, Agl γCH_2 and Aib and Mag βCH_3), 1.44 (9H, s, Boc CH_3), 0.88 (2H, m, Agl δCH_2); HRMS (FAB): MH^+, found 572.3433. $C_{30}H_{46}N_5O_6$ requires 572.3448.

4.4. Mass spectrometry

Fast Atom Bombardment (FAB) mass spectrometry was carried out using a JEOL SX/SX model 102A four-sector tandem mass spectrometer ($B_1E_1B_2E_2$ geometry), coupled to a JEOL MS/MP9021D/UPD data system. The samples were loaded in a nitrobenzyl alcohol solution onto a stainless steel probe and bombarded with xenon atoms with an energy of 8 KeV. During the high-resolution FABMS measurements a resolving power of 5000–10,000 (10% valley definition) was used. Polyethyleneglycol (PEG) 300 and 600 was used to calibrate the mass spectrometer.

4.5. Nuclear magnetic resonance

The ^1H NMR spectra were recorded by means of Bruker model AC 200, AC250 or AM 400 spectrometers. Deuterated chloroform (99.96% D) and water (99.98% D) were purchased from Aldrich, while deuterated DMSO (99.96% D_6) was obtained from Acros Organics. The free-radical TEMPO was a Sigma product.

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