

Synthesis of Enantiomerically Pure *cis*- and *trans*-4-Amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic Acid: A Spin-Labelled, Cyclic, Chiral β -Amino Acid, and 3D-Structural Analysis of a Doubly Spin-Labelled β -Hexapeptide

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Amination of 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone (**1**) with either (*R*)- or (*S*)- α -methylbenzylamine gave corresponding enamines **2**. Whereas the reduction with NaBH₃CN/CH₃COOH afforded predominantly a mixture of two possible *cis* diastereomers of **3**, (1'*R*,3*S*,4*S*)/(1'*R*,3*R*,4*R*) or (1'*S*,3*R*,4*R*)/(1'*S*,3*S*,4*S*), which could be separated by crystallisation of their HCl salts, the use of NaBH₄/(CH₃)₂-CHCOOH as the reducing agent resulted in a mixture of one *trans*- and one *cis* diastereomer of **3** (1'*R*,3*S*,4*R*)/(1'*R*,3*R*,4*R*) or (1'*S*,3*R*,4*S*)/(1'*S*,3*S*,4*S*) in varying proportions depending upon the conditions used. The stereochemistry of the four diastereomers of **3** was clearly established by X-ray diffrac-

tion analysis of one of them, combined with ¹H NMR spectroscopic studies after nitroxide reduction. Removal of the chiral auxiliary from the separated diastereomers of **3** by hydrogenation and regeneration of the nitroxide radical gave expected amino esters **4**. A model β -hexapeptide containing (3*R*,4*S*)- β -TOAC combined with (1*S*,2*S*)-2-aminocyclohexane carboxylic acid was synthesised by solution methods and its preferred conformation (3₁₄-helix) was assessed by FTIR absorption, CD, and EPR spectroscopy.

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Introduction

Nitroxide free radicals derived from TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) have found applications in chemistry and medicine as spin labels,^[1] spin traps,^[2] oxidising agents,^[3] antioxidants^[4] and MRI contrast agents.^[5] To date, their most common use is in the study of conformation and structural mobility of peptides and proteins by EPR spectroscopy. Spin labels can be introduced either by functionalisation of the side chain of protein amino acids^[6] (for example, the thiol group of cysteine with methanethio-sulfonate spin labels) or by the insertion of synthetic nitroxide-bearing amino acids into the peptide chain. A variety of such novel amino acids have been prepared where the spin label has been placed either in the side chain of α -amino acids or incorporated into cyclic structures of α , α -disubstituted-, β - or γ -amino acids.^[7] These amino acids have been used to spin label biologically active peptides and

proteins, or introduced into model peptide systems for studies of physical effects such as intramolecular energy transfer (fluorescence quenching) and spin polarisation (CIDEP).^[6,8]

The TOAC (4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxylic acid) α -amino acid residue^[9–11] (Figure 1) has been widely used for these purposes. The tetrasubstituted α carbon of TOAC is responsible for its ability to induce β -turn or 3₁₀/ α -helical structures in peptides, but also for the reduced reactivity of its amino group. We wished to synthesise chiral, spin-labelled amino acids which retained the conformationally rigid character of TOAC, while allowing milder peptide coupling conditions. The racemic *trans*- β -amino acid POAC (3-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid) (Figure 1), first described by Rassat and Rey,^[11a] attracted our attention^[12]

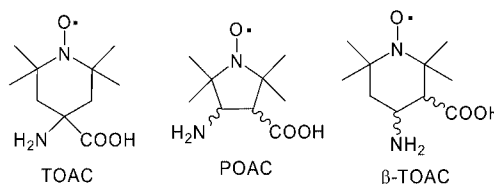


Figure 1. Chemical structures of the spin-labelled amino acids TOAC, POAC and β -TOAC.

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as its easy inclusion in a peptide synthesised by solid-phase methods had been demonstrated,^[13] whereas similar syntheses with the TOAC residue proved problematic.^[13,14] A β -amino acid form of TOAC (4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid, β -TOAC) was designed that could be obtained in enantiopure form.^[15,16]

The β -amino acids have been synthesised and studied in the last ten years since it was demonstrated that their oligomers may fold into helical conformations (stable in organic and aqueous solvents) and are resistant to enzymatic hydrolysis.^[17] Gellman and coworkers^[18,19] investigated the synthesis and conformation of peptides containing *trans*-ACHC (2-amino-cyclohexane-1-carboxylic acid) and *trans*-ApiC (4-aminopiperidine-3-carboxylic acid) and found that they form 3_{14} -helical structures. As the *trans*- β -TOAC residue has a similar structure to *trans*-ApiC, it was particularly interesting to assess if it could be used as a spin probe to study β -peptide secondary structures in the same way as TOAC had been exploited in the case of α -peptides. In this paper, full experimental details of the synthesis of *cis*- and *trans*- β -TOAC and the synthesis of peptides containing *trans*- β -TOAC and *trans*-ACHC up to the hexamer level with their conformational analyses by infrared, circular dichroism and EPR spectroscopy are reported.

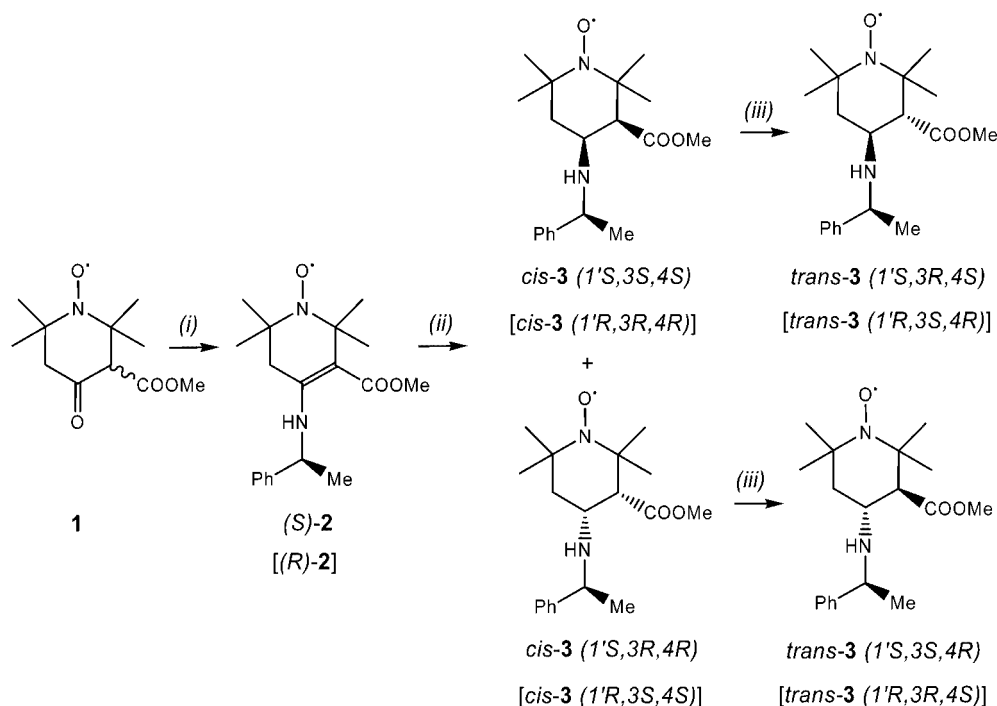
Results and Discussion

Synthesis and Characterisation

The syntheses of *cis*- and *trans*- β -TOAC were based on methods developed to prepare other cyclic β -amino acids.

Gellman and coworkers^[20] synthesised *trans*-3-amino-pyrrolidine-4-carboxylic acid and *trans*-2-amino-cyclopentanecarboxylic acid by reductive amination of a β -keto ester with either (*R*)- or (*S*)- α -methylbenzylamine in the presence of NaBH₃CN, and subsequent selective crystallisation of the hydrochloride salts of the obtained β -amino esters to provide either *trans* enantiomer. Cimarelli and Palmieri^[21] showed that cyclic β -enamino esters, derived from α -methylbenzylamine and a cyclic β -keto ester, could be selectively reduced by NaHB(OAc)₃ to give a predominant *cis* diastereomer. This method was developed further by Xu et al.^[22] for the preparation of *cis*-ACHC, who performed the reduction step using NaBH₄ and a bulky organic acid, and isolated the major diastereomer formed as its hydrobromide salt. This pathway was also used recently by Gellman and coworkers^[19] to obtain *trans*-2-amino-cyclohexanecarboxylic acid by epimerisation of the *cis* isomer. Similar methods applied to 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone (**1**) (Scheme 1) were attempted as a route to enantiopure β -TOAC.

Commercially available 2,2,6,6-tetramethyl-4-piperidone was oxidised by a described procedure to its 1-oxyl derivative.^[23] Carboxylation of this compound with carbon dioxide in the presence of potassium phenoxide following a known method^[24] did not give reproducible results in our hands, as the yield of keto ester **1** obtained after the reaction with diazomethane varied between 15–50% over different runs (35% yield in ref.^[24]). Amination of **1** with (*R*)- or (*S*)- α -methylbenzylamine in the presence of acetic acid proceeded smoothly to provide desired products (*R*)-**2** or (*S*)-**2** in 61 and 66% yields, respectively. The enamine struc-



Scheme 1. (i) (*S*)- [or (*R*)]- α -methylbenzylamine, AcOH, EtOH, 4-Å MS, room temp., 48 h. (ii) NaBH₃CN, AcOH, EtOH, 75 °C, 2 h; then HCl/EtOAc, 0 °C, filtration and recrystallisation from MeCN. (iii) NaOMe, MeOH, 70 °C, 18 h.

ture of **2** was determined from its ^1H NMR spectrum after nitroxide reduction.^[25,26]

Isolated enamine (*R*)-**2** was first reduced in the presence of NaBH_3CN and acetic acid to give a mixture of reduced products **3**. The ^1H NMR spectra of this mixture, after nitroxide reduction, showed that it contained the two *cis* diastereomers in a proportion of 1:1, with trace quantities of the two *trans* diastereomers. The hydrochloride salts were prepared by adding a solution of HCl in EtOAc to a cold solution of **3** in EtOAc . The precipitate formed was collected and recrystallised from MeCN to give (*1'R,3S,4S*)-**3** as a sole diastereomer as judged from its ^1H NMR spectrum. Diastereomer (*1'R,3R,4R*)-**3** was obtained from the EtOAc mother liquor. The same reaction sequence was followed starting from enamine (*S*)-**2** to give (*1'S,3R,4R*)-**3** as crystals and (*1'S,3S,4S*)-**3** from the EtOAc mother liquor [Scheme 1; products obtained from (*R*)-**2** are shown in brackets]. Derivative (*1'S,3R,4R*)-**3**· HCl was obtained after recrystallisation from MeOH/MeCN as large orange crystals suitable for X-ray diffraction analysis,^[15a,15c] which allowed the assignment of the configurations at C^3 and C^4 on the basis of the known configuration at $\text{C}^{1'}$ (Figure 2).

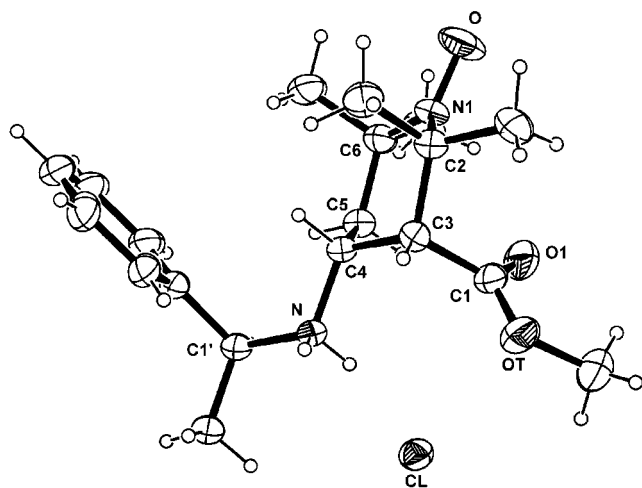


Figure 2. X-ray diffraction structure of *cis*-**3**(*1'S,3R,4R*) hydrochloride with selected atom numbering. Displacement ellipsoids for non-hydrogen atoms are drawn at the 30% probability level.

The X-ray diffraction structure shows that the piperidine ring adopts a slightly distorted chair conformation with puckering parameters $Q_T = 0.529(4)$ Å, $\theta_2 = 154.8(4)^\circ$ and $\phi_2 = 338.5(12)^\circ$. The amino and carboxyl substituents occupy the equatorial and axial positions, respectively. The values of the torsion angles θ [$\text{N}-\text{C}4-\text{C}3-\text{C}1$: $70.7(5)^\circ$] and ψ [$\text{C}4-\text{C}3-\text{C}1-\text{OT}$: $-117.1(4)^\circ$] are close to those reported for the β -peptide 3_{14} -helix (60° and -140° , respectively).^[17d,18] No significant comparison can be made between the value of the $\text{C}1'-\text{N}-\text{C}4-\text{C}3$ torsion angle, $172.2(3)^\circ$, with that of the ϕ torsion angle in β -peptides owing to the different nature (amino vs. amide) of the nitrogen atom.

Epimerisation of *cis* isomers (*1'S,3S,4S*)-**3**/(*1'S,3R,4R*)-**3** and (*1'R,3R,4R*)-**3**/(*1'R,3S,4S*)-**3** of known absolute configuration in the presence of NaOMe gave 1:1 *cis/trans* mix-

tures, which allowed the assignment of the absolute configuration of the resulting *trans* isomers (*1'S,3R,4S*)-**3**/(*1'S,3S,4R*)-**3** and (*1'R,3S,4R*)-**3**/(*1'R,3R,4S*)-**3**. Interestingly, the ^1H NMR spectra (Figure 3) of the four isomers of **3**· HCl after nitroxide reduction show different chemical shifts for H^3 , H^4 and H^5 – $\text{H}^{5'}$ in both pairs of isomers *cis*-(*1'R,3S,4S*)-**3**/*trans*-(*1'R,3R,4S*)-**3** and *cis*-(*1'R,3R,4R*)-**3**/*trans*-(*1'R,3S,4R*)-**3**, whereas the coupling constant of the H^3 proton is significantly higher for the *trans* isomers (11.4 and 11.8 Hz) than for the *cis* isomers (4.0 and 4.0 Hz) as expected. These differences allowed easy analysis of the crude *cis/trans* mixtures.

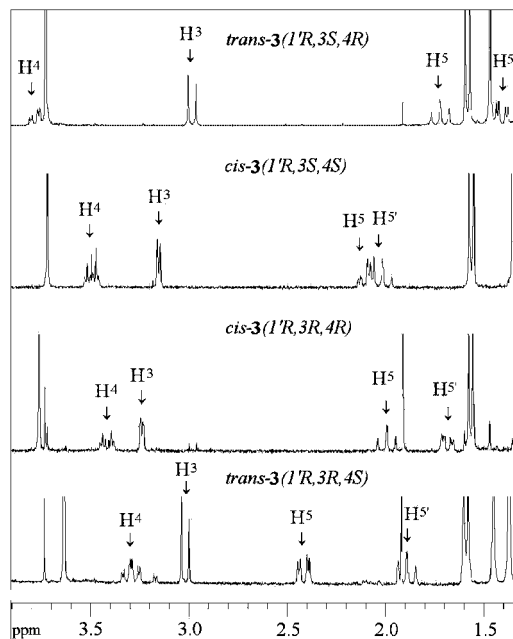
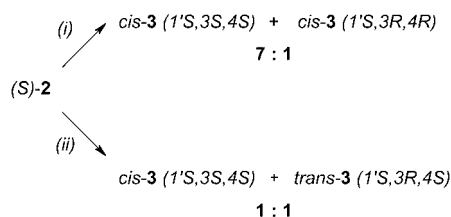


Figure 3. ^1H NMR spectra (D_2O) of the diastereomers **3**· HCl after nitroxide reduction.

Separation of the *cis/trans* isomers was troublesome: whereas 42% of (*1'S,3R,4S*)-**3** could be isolated from epimerisation of (*1'S,3S,4S*)-**3**, diastereomer (*1'S,3S,4R*)-**3** could not be separated by column chromatography from (*1'S,3R,4R*)-**3**. Repeated preparative thin-layer chromatography of the reaction mixture resulting from the epimerisation of (*1'R,3S,4S*)-**3** allowed the recovery of only a mixture of (*1'R,3R,4S*)-**3** and (*1'R,3S,4S*)-**3** in a ratio of 9:1.

To obtain greater quantities of a single diastereomer of *trans*-**3**, the reduction of enamine **2** was examined again. Xu et al.^[22] developed an asymmetric reductive amination method, first described by Cimarelli and Palmieri,^[21] to allow large-scale synthesis of ethyl 2-amino-1-cyclohexanecarboxylate from the corresponding α -methylbenzylamine-derived enamines. Whereas Cimarelli and Palmieri^[21] used $\text{NaHB}(\text{OAc})_3$ for the reduction of similar cyclic β -enamino esters, Xu et al.^[22] improved the stereoselectivity of the reduction step by using NaBH_4 in the presence of a bulky organic acid (e.g. isobutyric acid or pivalic acid). They achieved an optimal *cis/trans* selectivity of 60:1, with a *de* of 84% when using excess isobutyric acid and toluene as a cosolvent.

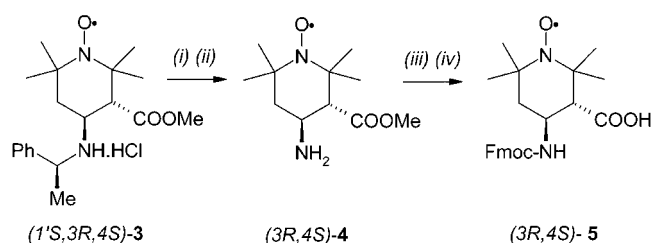
These optimised reaction conditions were applied to the reduction of enamine (*S*)-**2** (Scheme 2), with surprising results: NMR spectroscopic analysis of the crude reaction product revealed the presence of only the *cis* (1'*S*,3*S*,4*S*) and *trans* (1'*S*,3*R*,4*S*) diastereomers of **3** in a ratio of 1:1, with no trace of the other two diastereomers. After chromatography, 29% (1'*S*,3*S*,4*S*)-**3** and 30% (1'*S*,3*R*,4*S*)-**3** were obtained. The reduction was repeated with enamine (*R*)-**2** with similar results; 38% *cis*-(1'*R*,3*R*,4*R*)-**3** and 39% *trans*-(1'*R*,3*S*,4*R*)-**3** were isolated, whereas the other two diastereomers could not be detected in the NMR spectra of the crude product. Intrigued by this unexpected outcome, we returned to the reduction conditions proposed by Cimarelli and Palmieri;^[21] reduction of (*S*)-**2** with NaBH₄ in the presence of AcOH gave only the two *cis* diastereomers (1'*S*,3*S*,4*S*)-**3** and (1'*S*,3*R*,4*R*)-**3** in a ratio of 7:1, with no trace of the *trans* diastereomers in the NMR spectra of the crude product. Investigation of this reduction step was pursued by changing the acid or the ratio of acid to NaBH₄ used. The results are summarised in Table 1. Whereas trifluoroacetic acid and phenylacetic acid were unsuitable, pivalic acid gave a result similar to that of isobutyric acid with only (1'*S*,3*S*,4*S*)-**3** and (1'*S*,3*R*,4*S*)-**3** isolated from the reaction mixture, although in low yields. A lower yield was also observed with isobutyric acid when the excess of acid used was reduced, with the starting enamine recovered from the reaction mixture. A difference in *cis/trans* selectivity was noted when the relative proportion of NaBH₄:isobutyric acid was reduced; *cis* diastereomer (1'*S*,3*S*,4*S*)-**3** was favoured with a concomitant fall in the yield of the reduced product.



Scheme 2. Reduction of enamine (*S*)-**2**. (i) NaBH₄, AcOH, EtOH, 0 °C to room temp., 7 h. (ii) NaBH₄, (CH₃)₂CHCOOH, toluene, 0 °C to room temp., 24 h.

Removal of the chiral auxiliary from **3** was achieved by hydrogenation over Pd/C for only a short time. Under these conditions, the nitroxide group was reduced to an intermediate N-hydroxy compound, from which the nitroxide function was regenerated by stirring the crude hydrogenated

product open to the air in the presence of Cu(OAc)₂ for 7 d.^[27] In this way, compounds (3*S*,4*S*)-**4**, (3*R*,4*R*)-**4**, (3*S*,4*R*)-**4** and (3*R*,4*S*)-**4** (Scheme 3) were obtained in 53,



Scheme 3. Removal of the chiral auxiliary from diastereomers **3**, followed by C-deprotection/N-protection of resulting β -amino ester enantiomer **4**. (i) H₂, Pd/C 10%, 95% EtOH, room temp., 30 min. (ii) Cu(OAc)₂, MeOH, air, room temp., 7 d. (iii) NaOH (aq), MeOH, reflux, 5 h. (iv) Fmoc-succinimidyl carbonate; NaHCO₃; acetone/water, 2:1; room temp.; 18 h.

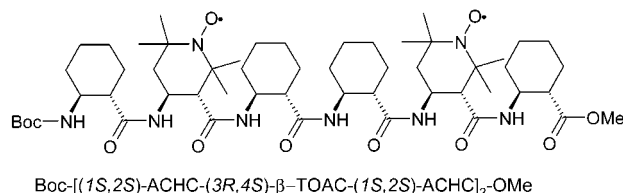
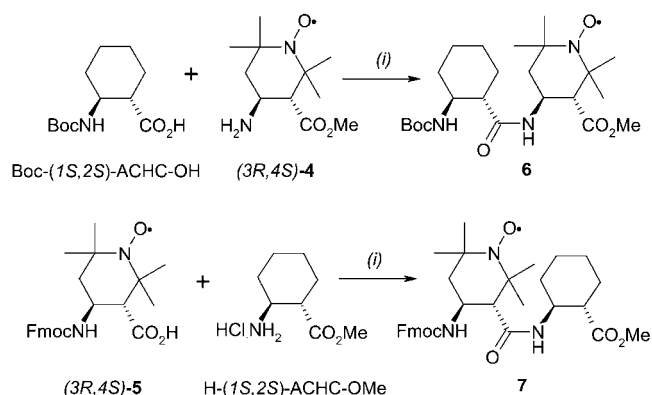


Figure 4. β -Hexapeptide **11** containing two (3*R*,4*S*)- β -TOAC residues at positions 2 and 5 (Boc is *tert*-butyloxycarbonyl).

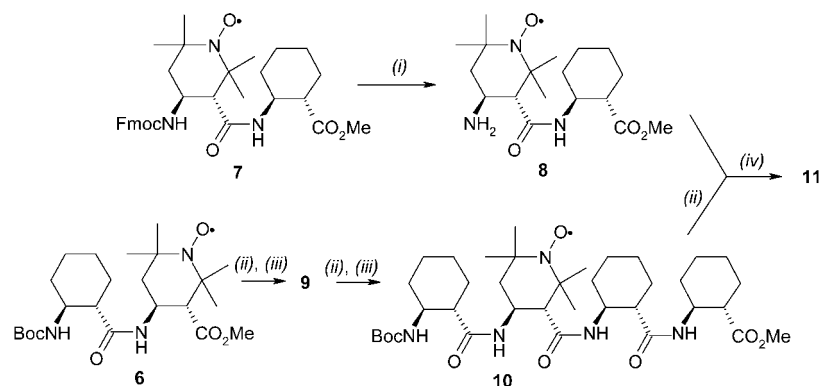


Scheme 4. Synthesis of dipeptides **6** and **7**. (i) EDC, HOAt, DIEA, CH₂Cl₂, 0 °C to room temp., 18 h {EDC, *N*-ethyl-*N'*-(3'-(dimethylamino)propyl)carbodiimide; HOAt, 7-aza-1-hydroxy-1,2,3-benzotriazole}.

Table 1. Reduction of (*S*)-**2** by NaBH₄ in the presence of organic acids.

Entry	Acid	Substrate/NaBH ₄ /acid	Cosolvent	Temp. [°C]	Yield [%]	Ratio <i>cis/trans</i> ^[a]
1	trifluoroacetic	1:5:20	toluene	20	decomposition	–
2	phenylacetic	1:10:20	toluene/acetonitrile	20	no reaction	–
3	pivalic	1:5:50	toluene	20	44	2.3:1
4	isobutyric	1:10:20	toluene	20	35	1.2:1
5	isobutyric	1:10:60	toluene	20	42	10:1
6	isobutyric	1:15:60	toluene	20	77	1:1

[a] (1'*S*,3*S*,4*S*)-**3** and (1'*S*,3*R*,4*S*)-**3**. Ratio determined from yields of isolated products after column chromatography.



Scheme 5. Synthesis of hexapeptide **11**. (i) $\text{CH}_3\text{CN}/\text{Et}_2\text{NH}$, 9:1; room temp.; 2 h. (ii) NaOH (aq), MeOH , reflux. (iii) $\text{H}-(1\text{S},2\text{S})\text{-ACHC-OMe}$, EDC, HOAt, DIEA, CH_2Cl_2 , 0 °C to room temp. (iv) EDC, HOAt, DIEA, CH_2Cl_2 , 0 °C to room temp., 18 h.

62, 57 and 41% yield, respectively. A derivative suitable for peptide synthesis was prepared from (3*R*,4*S*)-**4** by saponification of the methyl ester and Fmoc (9-fluorenylmethoxycarbonyl) protection of the amino group to provide building block (3*R*,4*S*)-**5** in 40% yield.

We wished to incorporate the (3*R*,4*S*)- β -TOAC residue into a model β -peptide to study in particular the resulting structure by EPR spectroscopy.^[28] We envisaged peptide **11**, shown in Figure 4, incorporating two (3*R*,4*S*)- β -TOAC residues combined with four (1*S*,2*S*)-ACHC residues, the homooligomers of which are known to fold into 3_{14} -helices.^[17] In this case, the β -TOAC residues at positions *i* and *i*+3 should be positioned on the same face of the ternary helix, which should allow an interaction between their two radical groups. With this aim in mind, we examined initially the coupling reaction between (3*R*,4*S*)-**4** and (3*R*,4*S*)-**5** with derivatives of (1*S*,2*S*)-ACHC (Scheme 4). Whereas the reaction of (3*R*,4*S*)-**4** with Boc-(1*S*,2*S*)-ACHC-OH in the presence of EDC/HOAt gave desired dipeptide **6** in 90% yield, the coupling of H-(1*S*,2*S*)-ACHC-OMe with (3*R*,4*S*)-**5** under the same conditions was less satisfactory, with product **7** recovered in only 40% yield.

Following these results, we decided to use a segment coupling approach to form the hexapeptide by reaction of a C-protected tetrapeptide with an N-protected dipeptide (Scheme 5). Dipeptide **7** was treated with diethylamine in MeCN to give N-deprotected dipeptide **8**. Elongation of dipeptide **6** by two cycles of saponification and reaction with H-(1*S*,2*S*)-ACHC-OMe in the presence of EDC/HOAt gave firstly tripeptide **9** in 49% yield, and secondly tetrapeptide **10** in 63% yield. Saponification of **10** and reaction with **8** in the presence of EDC/HOAt gave the desired hexapeptide Boc-[(1*S*,2*S*)-ACHC-(3*R*,4*S*)- β -TOAC-(1*S*,2*S*)-ACHC]₂-OMe (**11**) in 65% yield.

Conformational Analysis

We investigated the preferred conformations of the terminally protected, (1*S*, 2*S*)-ACHC/(3*R*, 4*S*)- β -TOAC β -peptides in structure-supporting solvents (CDCl_3 , MeOH) by using FTIR absorption, CD and EPR spectroscopic techniques.

Figure 5 shows the FTIR absorption curves in the amide N–H stretching (amide A) region of the N-terminal di-, tri- and tetrapeptides of β -hexamer **11** in CDCl_3 solution. Our results clearly indicate that intense bands below 3400 cm^{-1} , typically exhibited by H-bonded -CONH- groups,^[29] are first seen at the level of tetramer. The curves of the dimer and trimer are dominated by the strong absorption above 3400 cm^{-1} , which is characteristic of free -CONH- groups. The spectrum of the hexapeptide was not recorded owing to its very poor solubility in this halohydrocarbon. Because the spectrum of the tetramer does not remarkably change upon dilution of the concentration from 1.0 to 0.1 mM, it is safe to conclude that the H-bonding observed in this concentration range is of the intramolecular type.

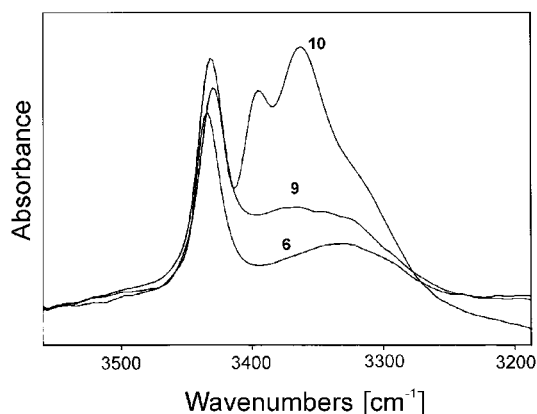


Figure 5. FTIR absorption spectra in the amide N–H stretching region of the Boc/OMe terminally protected, N-terminal, synthetic intermediates of hexapeptide **11**: dipeptide **6**, tripeptide **9** and tetrapeptide **10** in CDCl_3 solution (peptide concentration: 1 mM).

CD spectroscopy in the far-UV region (190–260 nm), where the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of chiral amide compounds are observed, is extensively used to analyse the secondary structure of polypeptides.^[30] Hexamer **11** displays an intense, negative Cotton effect centred at 218 nm in MeOH solution (Figure 6), which closely resembles that of 3_{14} -helical β -peptides.^[18b,31] A strictly related spectrum was published for the (1*S*, 2*S*)-ACHC homohexamer in the same solvent.^[18b] In addition, it is worth noting that in the

near UV and visible regions (between 350 and 600 nm) dichroic absorptions associated with the aminoxyl $n \rightarrow \pi^*$ transition^[32] (not shown) stand out clearly, which make this chromophoric group of β -TOAC potentially useful as a chiroptrospectroscopic probe for the assignment of the 3D-structure of β -polypeptides and detection of the environment polarity. Interestingly, an induced CD in the 350–550 nm region was already reported for TOAC-containing, chiral peptides.^[33]

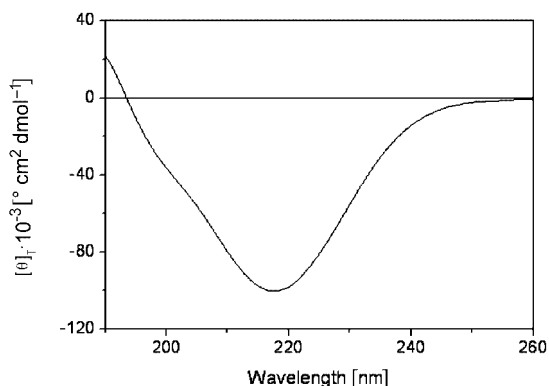


Figure 6. Far-UV CD spectrum of hexapeptide **11** in MeOH solution (peptide concentration: 1 mm).

The EPR spectrum of hexamer **11** in liquid MeOH solution at 258 K (Figure 7) consists of three sharp lines separated by 1.65 mT and centred at $g = 2.0058 \pm 0.0004$ and superimposed on a broad line spectrum. The broad and sharp components (Figure 7b and c, respectively) can be separated by fast Fourier transforming (FFT) the spectrum, filtering the short time components in the time domain and transforming them back into the frequency (magnetic field) domain (FFT procedure). The double integrated intensity of the sharp component corresponds to about 5% of the total intensity.

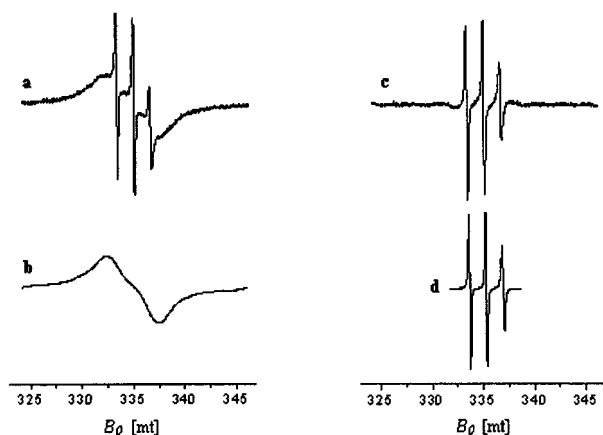


Figure 7. Analysis of the EPR spectrum (a) of dilabelled hexapeptide **11**. The sharp component (c) was obtained by subtracting the broad component (b) from spectrum (a). Trace (d) is the EPR spectrum of monolabelled tetrapeptide **10**. The two experimental spectra were obtained in liquid MeOH solution at 258 K (peptide concentration: 1 mm).

For comparison, Figure 7d shows the EPR spectrum of monolabelled tetrapeptide **10** in methanol solution at the same temperature ($a_{\text{iso}} = 1.63$ mT, $g = 2.0065 \pm 0.0004$). We have chosen this peptide as the reference in that it represents a nitroxide system with rotational diffusion characteristics as close as possible to those of **11**. The good agreement between the difference spectrum (c) and spectrum (d) suggests the attribution of the sharp lines to a monoradical impurity. The broad component (b) is that expected for peptide conformations where the two nitroxides are at a close distance and the electron–electron dipolar interaction is large and not fully averaged out by the molecular rotational diffusion motion. In frozen (120 K) MeOH solution, the spectrum shows indeed a well-resolved pattern (Figure 8b), typical of a two unpaired electron system in a triplet state. Here, again a narrow feature is present in the centre of the spectrum, consistent with a monoradical EPR spectrum (Figure 8c). The dipolar interaction parameter D , obtained by comparing the experimental spectrum with a computer simulated spectrum (Figure 8a), is 12.0 mT. The simulation was performed by assuming an isotropic hyperfine coupling of 0.8 mT between the two ^{14}N nuclei, which is one half the typical value for nitroxide radicals (1.65 mT in this solvent). This is expected for the case of a biradical with a large exchange interaction J compared with the hyperfine interaction.^[34,35] The same is true for the anisotropic tensor components. Moreover, the hyperfine tensor principal axes of the two ^{14}N nuclei were assumed to be coincident according to the expected peptide 3_{14} -helix conformation.^[18] The resulting, corresponding distance between the two radical centres is $R = 6.1$ Å, which was calculated with the assumption that the spin density was localised on the middle of the nitroxide N–O bond by the point dipole approximation $D = (3/2)g^2\beta^2/R^3$. According to a recent paper,^[36] the point dipole approximation applied to a pair of nitroxide groups at a distance $R < 9$ Å gives an overestimate of the R value (of the order of 5–10% depending on the relative nitroxide orientation). This result implies that the experimental 12.0 mT value should be associated to a lower nitroxide...nitroxide distance ($R = 5.5$ – 5.7 Å), which compares very well with the O...O distance (5.43 Å) extracted from our computer modelling (Figure 9)

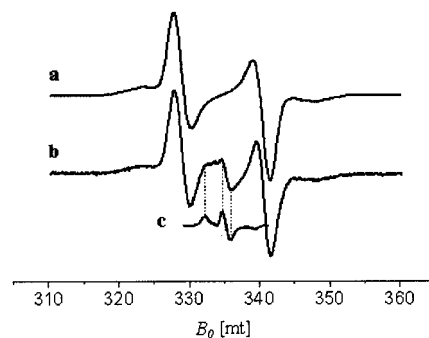


Figure 8. Simulated (a) and experimental (b) EPR spectra of dilabelled hexapeptide **11**. Trace (c) is the spectrum of monolabelled tetrapeptide **10**. The two experimental spectra were obtained in frozen MeOH solution at 120 K (peptide concentration: 1 mm).

for a regular 3_{14} -helix structure.^[18] The asymmetry parameter E is zero, as expected for interacting electron spins localised on N–O groups placed at a long distance.

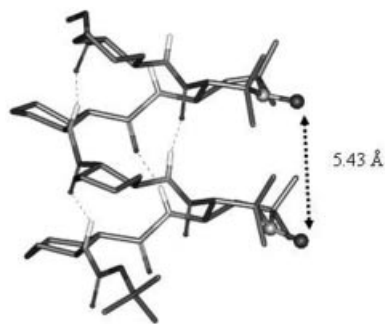


Figure 9. Model of hexapeptide **11** in the 3_{14} -helical structure, which highlights the distance between the oxygen atoms of the nitroxyl probes of the two (3*R*, 4*S*) β -TOAC residues.

The narrow triplet of lines in the solution spectrum (Figure 7c) and the central feature in the frozen spectrum (Figure 8b) can be attributed to a monoradical impurity in the sample, possibly a modified peptide with one of the two nitroxide groups reduced to a hydroxylamine functionality. However, it is not excluded that the observed spectral properties would be due, at least to a small extent, to peptide conformations where the distance between the nitroxides is large and both electron exchange and electron dipolar interactions are small relative to the nitrogen hyperfine coupling.

Conclusions

The *cis* and *trans* enantiomers of the β -TOAC residue bearing a nitroxide function were obtained in an enantiopure form by selective reduction of the corresponding enamines. Derivatives suitable for peptide synthesis were also prepared. The *trans*-(3*R*,4*S*)- β -TOAC enantiomer was incorporated into a β -hexapeptide in combination with (1*S*,2*S*)-ACHC. Conformational analysis of this peptide by a combination of optical spectroscopies showed that it largely populates the classical 3_{14} -helical structure of β -polypeptides.

We believe that these spin-labelled, rigid and chiral, β -amino acids will find interesting applications in peptidomimetic conformational and biological studies.

Experimental Section

General: Melting points were measured by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with a Bruker WM300 spectrometer operating at 300 MHz and 77 MHz, respectively, the solvent being used as the internal standard. Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. The optical rotations were measured in a 1-dm thermostatted cell with a Perkin–Elmer 241 polarimeter, with an accuracy of 0.3%. Elemental analyses were performed by the C.N.R.S. Service of Microanalyses in Gif-sur-Yvette (France). Mass spectra (electrospray mode) were recorded with a

Hewlett–Packard HP5989MS spectrometer by Vincent Steinmetz (ILV). High-resolution mass spectra were performed by the CNRS Central Analytical Service in Vernaison (France). Analytical TLC and preparative column chromatography were performed on Kieselgel F254 and Kieselgel 60 (0.040–0.063 mm) (Merck), respectively. UV light ($\lambda = 254$ nm) allowed visualisation of the spots after TLC runs for all compounds. Except when noted, all starting materials and solvents were obtained from commercial suppliers and were used as received. Boc-(1*S*,2*S*)-ACHC-OH was obtained from NeoMPS SA, Strasbourg, France.

Preparation of NMR Samples: Well-resolved ^1H and ^{13}C NMR spectra of the nitroxide compounds described in this study could only be obtained by preliminary use of the sodium dithionite reduction procedure described in ref.^[26] The nitroxide sample was dissolved in $[\text{D}_6]$ acetone or D_2O and sodium dithionite (2 equiv.) dissolved in D_2O was added.

Enamines (R)-2 and (S)-2: Keto ester **1**^[23,24] (5.24 g, 23 mmol) was dissolved in absolute EtOH (85 mL) and 4-Å MS were added. The mixture was placed under an Ar atmosphere and (*R*)- α -methylbenzylamine (20.7 mL, 160.9 mmol) and glacial acetic acid (9.2 mL, 160.9 mmol) were added. The mixture was stirred at room temp. for 48 h. The mixture was concentrated, and the residue taken up in CH_2Cl_2 . The mixture was filtered, and the filtrate was washed successively with saturated aqueous NaHCO_3 and brine. The organic phase was dried with MgSO_4 , filtered and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 9:1) to give enamine (*R*)-2 (6.63 mg, 87%) as an orange oil. $[a]_D^{25} = -345$ (589), -367 (578), -441 (546), -914 (436), abs.(365), ($c = 0.25$, MeOH). ^1H NMR (300 MHz, $\text{D}_2\text{O}/[\text{D}_6]$ acetone): $\delta = 7.48$ –7.63 (m, 5 H, ArH), 4.98 (m, 1 H, CH), 3.96 (s, 3 H, OCH_3), 2.75, 2.39 (2 d, $J = 16.9$ Hz, 2 H, CH_2), 1.71 (d, $J = 6.6$ Hz, 3 H, CHCH_3), 1.68, 1.64, 1.34, 1.10 (4s, 12 H, 4CH_3) ppm. ^{13}C NMR (77 MHz, $\text{D}_2\text{O}/[\text{D}_6]$ acetone): $\delta = 172.1$ (C=O), 158.1 (C^4), 147.0, 130.3, 126.9, 116.7 (ArC), 98.9 (C^3), 61.8 (C^2), 56.8 (C^6), 53.3 (NCH), 51.4 (OCH_3), 41.4 (C^5), 28.4, 27.6, 26.0, 25.3 (CH_3) ppm. ES-MS: m/z (%) = 685.4 (55) $[\text{2M} + \text{Na}]^+$, 354.2 (72) $[\text{M} + \text{Na}]^+$, 332.2 (64) $[\text{M} + \text{H}]^+$. $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_3$ (331.4): calcd. C 68.85, H 8.21, N 8.45; found C 68.61, H 8.13, N 8.24. Enamine (*S*)-2 was prepared in the same way from keto ester **1** and (*S*)- α -methylbenzylamine in 82% yield. $[a]_D^{25} = +357$ (589), $+380$ (578), $+456$ (546), $+954$ (436), abs.(365), ($c = 0.26$, MeOH). ^1H and ^{13}C NMR ($\text{D}_2\text{O}/[\text{D}_6]$ acetone): identical to (*R*)-2. $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_3$ (331.4): calcd. C 68.85, H 8.21, N 8.45; found C 68.58, H 8.41, N 8.25.

(3*S*,4*S*)-4-[(1*R*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl Hydrochloride [(1*R*,3*S*,4*S*)-3-HCl] and (3*R*,4*R*)-4-[(1*R*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl Hydrochloride [(1*R*,3*R*,4*R*)-3-HCl]: Enamine (*R*)-2 (543 mg, 1.64 mmol) was dissolved in absolute EtOH (10 mL) and glacial acetic acid (0.19 mL, 3.28 mmol) was added. The mixture was placed under an Ar atmosphere and sodium cyanoborohydride (310 mg, 4.92 mmol) was added. The mixture was heated at 70 °C for 1 h. The mixture was cooled and then concentrated. The residue was taken up in ether and washed with saturated aqueous NaHCO_3 . The aqueous phase was extracted with diethyl ether. The combined ether phases were washed with brine, then dried with MgSO_4 , filtered and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 2:1) to give **3 (371 mg, 68%) as an orange oil. The residue was dissolved in EtOAc (5 mL) and cooled to 0 °C. A solution of HCl in EtOAc (approx. 3.4 M, 0.35 mL) was added. The mixture was kept at 4 °C overnight. The resulting precipitate was filtered off. The collected**

solid was recrystallised from MeCN to give (1'*R*,3*S*,4*S*)-**3**·HCl [157 mg, 26% from (*R*)-**2**] as light orange crystals. M.p. 202–204 °C. $[a]_D^{25} = +29$ (589), +32 (578), +48 (546), –39 (436), –115 (365), ($c = 0.25$, MeOH). ^1H NMR (300 MHz, D_2O): $\delta = 7.55$ (s, 5 H, ArH), 4.67 (m, 1 H, CH), 3.87 (s, 3 H, OCH_3), 3.65 (m, 1 H, H^4), 3.31 (d, $J = 4.0$ Hz, 1 H, H^3), 2.23 (m, 2 H, H^5), 1.73 (d, $J = 6.6$ Hz, 3 H, CHCH_3), 1.51, 1.44, 1.36, 1.32 (4 s, 12 H, 4 CH_3) ppm. ^{13}C NMR (77 MHz, D_2O): $\delta = 173.1$ (C=O), 137.6, 133.1, 132.7, 130.5 (ArC), 60.7, 60.0 (C^2 , C^6), 59.4 (CHPh), 56.3 (OCH_3), 50.5 (C^3 , C^4), 34.7 (C^5), 32.8, 29.3, 27.1, 27.0, (CH_3), 20.9 (CH_3) ppm. ES-MS: m/z (%) = 335.2 (100) $[\text{M} + 2\text{H}]^+$, 334.2 (98) $[\text{M} + \text{H}]^+$. $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\cdot\text{HCl}$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 61.75, H 8.22, N 7.41. The EtOAc filtrate was evaporated to give (1'*R*,3*R*,4*R*)-**3**·HCl [127 mg, 21% from (*R*)-**2**] as an orange foam. $[a]_D^{25} = +41$ (589), +41 (578), +41 (546), +105 (436) ($c = 0.25$, MeOH). ^1H NMR (300 MHz, D_2O): $\delta = 7.54$ – 7.60 (m, 5 H, ArH), 4.14 (m, 1 H, CH), 3.92 (s, 3 H, OCH_3), 3.58 (m, 1 H, H^4), 3.40 (d, $J = 4.0$ Hz, 1 H, H^3), 2.15, 1.82 (2 m, 2 H, H^5), 1.73 (d, $J = 6.9$ Hz, 3 H, CHCH_3), 1.45, 1.44, 1.31, 1.26 (4 s, 12 H, 4 CH_3) ppm. ^{13}C NMR (77 MHz, D_2O): $\delta = 173.1$ (C=O), 137.4, 133.1, 132.7, 130.6 (ArC), 60.6, 59.9 (C^2 , C^6), 59.7 (CHPh), 56.6 (OCH_3), 50.8, 49.6 (C^3 , C^4), 36.0 (C^5), 32.8, 29.4, 27.1, 26.9, (CH_3), 21.9 (CH_3) ppm. ES-MS: m/z (%) = 335.2 (100) $[\text{M} + 2\text{H}]^+$, 334.2 (61) $[\text{M} + \text{H}]^+$. $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\cdot\text{HCl}$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 61.11, H 8.41, N 7.05.

(3*R*,4*R*)-4-[(1'*S*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl Hydrochloride [(1'*S*,3*R*,4*R*)-3**·HCl] and (3*S*,4*S*)-4-[(1'*S*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl Hydrochloride [(1'*S*,3*S*,4*S*)-**3**·HCl]:** *Method (i)*: Enamine (*S*)-**2** (1533 mg, 4.63 mmol) was dissolved in absolute EtOH (30 mL) and glacial acetic acid (0.53 mL, 9.26 mmol) was added. The mixture was placed under an Ar atmosphere and sodium cyanoborohydride (872 mg, 13.89 mmol) was added. The mixture was heated at 70 °C for 2 h. The mixture was cooled and then concentrated. The residue was taken up in CH_2Cl_2 and washed with saturated aqueous NaHCO_3 . The aqueous phase was extracted with CH_2Cl_2 . The combined CH_2Cl_2 phases were washed with brine, then dried with MgSO_4 , filtered and concentrated. The residue was purified by chromatography (cyclohexane/EtOAc, 2:1) to give **3** (1078 mg, 70%) as an orange oil. The residue was dissolved in EtOAc (15 mL) and cooled to 0 °C. A solution of HCl in EtOAc (approx. 3.4 mL, 0.95 mL) was added. The mixture was kept at 0 °C for 30 min. The resulting precipitate was filtered off. The collected solid was recrystallised from MeCN to give (1'*S*,3*R*,4*R*)-**3**·HCl [157 mg, 26% from (*S*)-**2**] as light orange crystals. M.p. 209–211 °C (dec.). $[a]_D^{25} = -29$ (589), –36 (578), –52 (546), +40 (436) ($c = 0.26$, MeOH). ^1H and ^{13}C NMR (300 MHz, D_2O): identical to (1'*R*,3*S*,4*S*)-**3**. $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\cdot\text{HCl}$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 61.49, H 8.34, N 7.41. Recrystallisation from MeOH/MeCN (1:10) at 0 °C gave large orange crystals suitable for X-ray diffraction analysis. The EtOAc filtrate was evaporated to give (1'*S*,3*S*,4*S*)-**3**·HCl [533 mg, 31% from (*S*)-**2**] as an orange oil. $[a]_D^{25} = -45$ (589), –45 (578), –47 (546), –130 (436), –248 (365) ($c = 0.25$, MeOH). ^1H and ^{13}C NMR (D_2O): identical to (1'*R*,3*R*,4*R*)-**3**. $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\cdot\text{HCl}$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 61.11, H 8.41, N 7.05. *Method (ii)*: A solution of glacial acetic acid (4.3 mL, 75.5 mmol) in MeCN (3 mL) was cooled to 0 °C. Sodium borohydride (283 mg, 7.5 mmol) was added slowly in portions over 40 min. A solution of the enamine (*S*)-**2** (503 mg, 1.5 mmol) in MeCN (3 mL) was added dropwise. The mixture was stirred at 0 °C for 3 h, then at room temp. for 4 h. The mixture was diluted with CH_2Cl_2 , and then washed twice with a

saturated NaHCO_3 solution and once with brine. The organic phase was dried with MgSO_4 , filtered and concentrated. Column chromatography (cyclohexane/EtOAc, 2:1) of the residue successively gave enamine (*S*)-**2** (165 mg, 33%) and a mixture of (1'*S*,3*R*,4*R*)-**3** and (1'*S*,3*S*,4*S*)-**3** (298 mg, 59%). The *cis* diastereomers **3** were converted into their HCl salts by the addition of a solution of HCl in EtOAc for ^1H NMR characterisation, which showed the ratio of (1'*S*,3*R*,4*R*)-**3**·HCl/(1'*S*,3*S*,4*S*)-**3**·HCl to be 1:7.

(3*R*,4*S*)-4-[(1'*S*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl [(1'*S*,3*R*,4*S*)-3**·HCl]:** *Method (i)*: Sodium (55 mg, 2.4 mmol) was added slowly in portions to MeOH (3 mL), and a solution of (1'*S*,3*S*,4*S*)-**3** (125 mg, 0.37 mmol) dissolved in MeOH (1 mL) and added. The mixture was heated at reflux for 24 h. The resulting solution was cooled to 0 °C, and the pH of the mixture was adjusted to 6 by the addition of 1 N HCl. The mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 , and washed with a saturated NaHCO_3 solution. The organic phase was dried with MgSO_4 , filtered and concentrated. Column chromatography (cyclohexane/EtOAc, 2:1) of the residue gave (1'*S*,3*R*,4*S*)-**3** (51 mg, 42%) and (1'*S*,3*S*,4*S*)-**3** (70 mg, 57%). *Method (ii)*: Isobutyric acid (3.5 mL, 30 mmol) was cooled to 0 °C and NaBH_4 (288 mg, 7.6 mmol) was added slowly in portions over 40 min. The mixture was then stirred at room temp. for 30 min. Enamine (*S*)-**2** (497 mg, 1.5 mmol) was dissolved in toluene (4 mL) and added dropwise to the mixture. Further portions of NaBH_4 (57 mg, 1.5 mmol) were added to the mixture after 2, 4 and 12 h. After 20 h, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with a saturated NaHCO_3 solution (2×30 mL) and with brine (30 mL). The organic phase was dried with MgSO_4 , filtered and concentrated. Column chromatography (cyclohexane/EtOAc, 2:1) of the residue successively gave enamine (*S*)-**2** (67 mg, 13%), (1'*S*,3*R*,4*S*)-**3** (151 mg, 30%) and (1'*S*,3*S*,4*S*)-**3** (149 mg, 29%). *trans* Diastereomer (1'*S*,3*R*,4*S*)-**3** was converted into its HCl salt by the addition of a solution of HCl in EtOAc for characterisation. $[a]_D^{25} = -39$ (589), –44 (578), –52 (546) ($c = 0.25$, MeOH). ^1H NMR (300 MHz, D_2O): $\delta = 7.42$ (m, 5 H, ArH), 4.52 (q, $J = 11.7$ Hz, 1 H, PhCHCH_3), 3.78 (m, 1 H, H^4), 3.72 (s, 3 H, OCH_3), 2.97 (d, $J_{3,4} = 11.8$ Hz, 1 H, H^3), 1.70, 1.40 (2 m, 2 H, H^5), 1.58 (d, $J = 6.7$ Hz, 3 H, PhCHCH_3), 1.46, 1.25, 1.16, 1.04 (4 s, 12 H, 4 CH_3) ppm. ^{13}C NMR (77 MHz, D_2O): $\delta = 173.1$ (C=O), 151.9, 137.9, 133.1, 132.5, 130.9 (ArC), 62.7, 61.2 (C^2 , C^6), 58.8 (CHPh), 56.4 (OCH_3), 55.1 (C^4), 53.3 (C^3), 39.8 (C^5), 31.5, 31.3, 25.6, 24.5, (CH_3), 21.0 (PhCH_3) ppm. ES-MS: m/z (%) = 335.2 (100) $[\text{M} + 2\text{H}]^+$, 334.2 (61) $[\text{M} + \text{H}]^+$. $\text{C}_{19}\text{H}_{29}\text{N}_2\text{O}_3\cdot\text{HCl}$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 61.55, H 8.29, N 6.95.

(3*S*,4*R*)-4-[(1'*R*)-Phenylethyl]amino-3-methoxycarbonyl-2,2,6,6-tetramethyl-piperidine-1-oxyl Hydrochloride [(1'*R*,3*S*,4*R*)-3**·HCl]:** Isobutyric acid (3.7 mL, 40 mmol) was cooled to 0 °C and NaBH_4 (380 mg, 10 mmol) was added slowly in portions over 40 min. The mixture was then stirred at room temp. for 30 min. Enamine (*R*)-**2** (497 mg, 1.5 mmol) was dissolved in toluene (4 mL) and added dropwise to the mixture. Further portions of NaBH_4 (76 mg, 2 mmol) were added to the mixture after 2 and 12 h. After 20 h, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with a saturated NaHCO_3 solution (2×30 mL) and with brine (30 mL). The organic phase was dried with MgSO_4 , filtered and concentrated. Column chromatography (cyclohexane/EtOAc, 2:1) of the residue successively gave enamine (*R*)-**2** (193 mg, 29%), (1'*R*,3*S*,4*R*)-**3** (92 mg, 14%) and (1'*R*,3*R*,4*R*)-**3** (186 mg, 28%). *trans* Diastereomer (1'*R*,3*S*,4*R*)-**3** was converted into its HCl salt by the addition of a solution of HCl in EtOAc for characterisation. Orange foam. $[a]_D^{25} = +37$ (589), +40 (578), +47 (546) ($c = 0.26$, MeOH); ^1H and ^{13}C NMR (D_2O): identical to (1'*S*,3*R*,4*S*)-**3**.

$C_{19}H_{29}N_2O_3 \cdot HCl$ (369.9): calcd. C 61.69, H 8.17, N 7.57; found C 62.01, H 8.34, N 7.12.

General Method for the Reduction of (S)-2 by $NaBH_4$ in the Presence of Organic Acids: A solution of the organic acid in toluene (and additionally MeCN in the case of phenylacetic acid) was cooled to 0 °C and $NaBH_4$ was added slowly in portions over 40 min. Enamine (S)-2 was dissolved in toluene and added dropwise to the mixture. The temperature of the mixture was allowed to rise to room temp., and then stirred for 3 h. The mixture was diluted with CH_2Cl_2 and washed with a saturated $NaHCO_3$ solution and with brine. The organic phase was dried with $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (cyclohexane/EtOAc, 2:1) to successively give enamine (S)-2, (1'S,3R,4S)-3 and (1'S,3S,4S)-3.

(3S,4S)-4-Amino-3-methoxycarbonyl-2,2,6,6-tetramethylpiperidine-1-oxyl [(3S,4S)-4]: The hydrochloride salt of (1'R,3S,4S)-3 (400 mg, 1.08 mmol) was dissolved in 95% EtOH (40 mL). The solution was placed under an Ar atmosphere and 10% Pd/C (400 mg) was added. The mixture was stirred under H_2 for 30 min. The mixture was filtered and the filtrate concentrated. The residue was dissolved in MeOH (30 mL) and copper (II) acetate (20 mg) was added. The mixture was stirred rapidly open to air for 24 h. The mixture was concentrated, and the residue was purified by chromatography (CH_2Cl_2 /MeOH, 9:1) to give amine (3S,4S)-4 (131 mg, 53%) as an amorphous orange solid. M.p. 94–96 °C. $[a]^{25}_D = +10$ (589), +11 (578), +23 (546), –56 (436), –197 (365) ($c = 0.105$, MeOH). 1H NMR (300 MHz, D_2O) [HCl salt]: $\delta = 4.28$ (m, 1 H, H^4), 3.87 (s, 3 H, OCH_3), 3.28 (d, $J_{3,4} = 4.8$ Hz, 1 H, H^3), 2.15, 2.07 (2 m, 2 H, H^5), 1.62, 1.57, 1.53, 1.48 (4 s, 12 H, 4 CH_3) ppm. ^{13}C NMR (77 MHz, D_2O) [HCl salt]: $\delta = 173.5$ (C=O), 60.6, 60.0 (C^2 , C^6), 56.3 (OCH_3), 51.5 (C^4), 46.1 (C^3), 36.7 (C^5), 32.9, 29.5, 27.5, 27.2, (CH_3) ppm. ES-MS: m/z (%) = 231.1 (70) $[M + 2H]^+$, 230.1 (100) $[M + H]^+$, 215.1 (100), 213.1 (34). $C_{11}H_{21}N_2O_3$ (229.3): calcd. C 57.61, H 9.23, N 12.22; found C 57.85, H 9.28, N 12.08.

(3R,4R)-4-Amino-3-methoxycarbonyl-2,2,6,6-tetramethylpiperidine-1-oxyl [(3R,4R)-4]: The hydrochloride salt of (1'R,3R,4R)-3 (452 mg, 1.08 mmol) was dissolved in 95% EtOH (45 mL). The solution was placed under an Ar atmosphere and 10% Pd/C (290 mg) was added. The mixture was stirred under H_2 for 30 min. The mixture was filtered and the filtrate concentrated. The residue was dissolved in MeOH (20 mL) and copper (II) acetate (10 mg) was added. The mixture was stirred rapidly open to air for 7 d. The mixture was concentrated, and the residue was purified by chromatography (CH_2Cl_2 /MeOH, 9:1) to give amine (3R,4R)-4 (131 mg, 62%) as an amorphous orange solid. M.p. 88–91 °C. $[a]^{25}_D = -10$ (589), –11 (578), –22 (546), +38 (436), +140 (365) ($c = 0.104$, MeOH). 1H and ^{13}C NMR (D_2O): identical to (3S,4S)-4. $C_{11}H_{21}N_2O_3$ (229.3): calcd. C 57.61, H 9.23, N 12.22; found C 57.39, H 9.23, N 11.64.

(3S,4R)-4-Amino-3-methoxycarbonyl-2,2,6,6-tetramethylpiperidine-1-oxyl [(3S,4R)-4]: The hydrochloride salt of (1'R,3S,4R)-3 (1035 mg, 2.80 mmol) was dissolved in 95% EtOH (100 mL). The solution was placed under an Ar atmosphere and 10% Pd/C (489 mg) was added. The mixture was stirred under H_2 for 30 min. The mixture was filtered and the filtrate concentrated. The residue was dissolved in MeOH (40 mL) and copper (II) acetate (5 mg) was added. The mixture was stirred rapidly open to air for 5 d. The mixture was concentrated, and the residue was purified by chromatography (CH_2Cl_2 /MeOH, 95:5) to give amine (3S,4R)-4 (365 mg, 57%) as an orange oil. $[a]^{25}_D = +23$ (589), +24 (578), +32 (546), –89 (436) ($c = 0.1$, MeOH). 1H NMR (300 MHz, D_2O) [HCl

salt]: $\delta = 4.19$ (m, 1 H, H^4), 3.84 (s, 3 H, OCH_3), 3.04 (d, $J_{3,4} = 11.4$ Hz, 1 H, H^3), 2.31, 1.93 (2 m, 2 H, H^5), 1.61, 1.53, 1.48 (3 s, 12 H, 4 CH_3) ppm. ^{13}C NMR (77 MHz, D_2O) [HCl salt]: $\delta = 170.1$ (C=O), 58.7, 56.8 (C^2 , C^6), 54.3 (C^4), 52.9 (OCH_3), 44.5 (C^3), 37.9 (C^5), 29.5, 28.8, 24.1, 22.2 (CH_3) ppm. ES-MS: m/z (%) = 252.5 (41) $[M + Na]^+$, 230.6 (100) $[M + H]^+$. $C_{11}H_{21}N_2O_3 \cdot 2H_2O$ (265.326): calcd. C 49.79, H 9.49, N 10.56; found C 49.64, H 9.67, N 10.00.

(3R,4S)-4-Amino-3-methoxycarbonyl-2,2,6,6-tetramethylpiperidine-1-oxyl [(3R,4S)-4]: The hydrochloride salt of (1'S,3R,4S)-3 (1025 mg, 2.77 mmol) was dissolved in 95% EtOH (100 mL). The solution was placed under an Ar atmosphere and 10% Pd/C (660 mg) was added. The mixture was then stirred under H_2 for 20 min. The mixture was filtered and the filtrate concentrated. The residue was dissolved in MeOH (100 mL) and copper (II) acetate (10 mg) was added. The mixture was stirred rapidly open to air for 7 d. The mixture was concentrated, and the residue was purified by chromatography (CH_2Cl_2 /MeOH, 9:1) to give amine (3R,4S)-4 (258 mg, 41%) as an orange oil. $[a]^{25}_D = -21$ (589), –22 (578), –24 (546) +99 (436) ($c = 0.1$, MeOH). 1H and ^{13}C NMR (D_2O): identical to (3S,4R)-4. ES-MS: m/z (%) = 230.5 (100) $[M + H]^+$. FAB-HRMS: calcd. for $C_{11}H_{21}N_2O_3 + 2H$ 231.1709; found 231.1705.

(3R,4S)-4-(9H-Fluoren-9-ylmethoxycarbonylamino)-2,2,6,6-tetramethylpiperidine-1-oxyl-3-carboxylic Acid [(3R,4S)-5]: Amino ester (3R,4S)-4 (320 mg, 1.39 mmol) was dissolved in MeOH (15 mL) and water (6 mL). A 1 N solution of NaOH (6 mL) was added, and the mixture was heated at 75 °C for 2 h. The mixture was cooled in an ice bath and diluted with water (10 mL). The pH was adjusted to approximately 6 by the addition of a 2 N HCl solution. The solution was cooled to 0 °C and acetone (40 mL), $NaHCO_3$ (1170 mg, 13.9 mmol) and Fmoc-OSu (612 mg, 1.82 mmol) were added. The mixture was stirred at room temp. for 18 h. The acetone was removed under reduced pressure. The solution was diluted with water and washed twice with diethyl ether. The aqueous phase was acidified by the addition of a 0.5 N HCl solution and extracted twice with CH_2Cl_2 . The combined organic extracts were washed with water, dried with $MgSO_4$, filtered and concentrated. The residue was purified by chromatography (CH_2Cl_2 /MeOH, 9:1) to give acid (3R,4S)-5 (246 mg, 40%) as a light orange solid. $[a]^{25}_D = -2$ (589), –3 (578), –2 (546) ($c = 0.2$, MeOH). FAB-HRMS: calcd. for $C_{25}H_{29}N_2O_5 + 2H$ 439.2233; found 439.2231.

Boc-(1S,2S)-ACHC-(3R,4S)- β -TOAC-OMe (6): Boc-(1S,2S)-ACHC-OH (294 mg, 1.21 mmol) and (3R,4S)-4 (213 mg, 0.93 mmol) were dissolved in CH_2Cl_2 (7 mL). HOAt (253 mg, 1.86 mmol) was added, and the mixture was cooled in an ice bath. DIEA (0.16 mL, 0.93 mmol) was added, followed by EDC (268 mg, 1.40 mmol). The mixture was stirred at room temperature for 15 h. The mixture was diluted with CH_2Cl_2 and washed successively with 0.5 N HCl, a saturated $NaHCO_3$ solution and water. The organic phase was dried with $MgSO_4$, filtered and concentrated. The residue obtained was purified by preparative thin-layer chromatography (CH_2Cl_2 /MeOH, 9:1) to give dipeptide 6 (379 mg, 90%). $[a]^{25}_D = -4$ (589), –4 (578), –4 (546), +53 (436), +53 (365) ($c = 0.1$, MeOH). ES-MS: m/z (%) = 477.7 (100) $[M + H + Na]^+$. $C_{23}H_{40}N_3O_6$ (454.574): calcd. C 60.77, H 8.87, N 9.24; found C 60.81, H 8.79, N 9.05.

Fmoc-(3R,4S)- β -TOAC-(1S,2S)-ACHC-OMe (7): Boc-(1S,2S)-ACHC-OH (100 mg, 0.41 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled in an ice bath. Trifluoroacetic acid (1 mL) was added, and the mixture was stirred at 0 °C for 15 min and then at room temperature for 1 h. The mixture was concentrated under reduced pressure. The resulting residue was dissolved in MeOH (3 mL) and

cooled in an ice bath. Thionyl chloride (0.7 mL) was added dropwise. The mixture was stirred from 0 °C to room temperature for 18 h. The mixture was concentrated under reduced pressure. Diethyl ether was added to the residue, and the mixture was again concentrated under reduced pressure. This procedure was repeated three times to give the crude amino ester hydrochloride, which was used as such without further purification. Thus, the obtained hydrochloride HCl·H-(1*S*,2*S*)-ACHC-OMe (72 mg, 0.37 mmol) and (3*R*,4*S*)-**5** (123 mg, 0.28 mmol) were dissolved in CH₂Cl₂ (5 mL) and THF (5 mL). HOAt (77 mg, 0.56 mmol) was added, and the mixture was cooled in an ice bath. DIEA (0.14 mL, 0.84 mmol) was added, followed by EDC (83 mg, 0.42 mmol). The mixture was stirred at room temperature for 16 h. The mixture was diluted with CH₂Cl₂ and washed successively with 0.5 N HCl, a saturated NaHCO₃ solution and water. The organic phase was dried with MgSO₄, filtered and concentrated. The residue obtained was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 92:8) to give dipeptide **7** (63 mg, 40%). [α]_D²⁵ = −2 (589), −3 (578), −3 (546), +31 (436), +13 (365) (*c* = 0.09, MeOH). ES-MS: *m/z* (%) = 599.3 (100) [M + Na]⁺, 577.9 (7) [M + 2H]⁺. C₃₃H₄₂N₃O₆·0.5H₂O (585.698): calcd. C 67.67, H 7.40, N 7.17; found C 67.35, H 7.65, N 6.69.

H-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-OMe (8**):** Dipeptide **7** (54 mg, 0.094 mmol) was dissolved in MeCN (9 mL) and diethylamine (1 mL) was added. The mixture was stirred at room temperature for 2 h. The mixture was concentrated, and the residue obtained was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 90:10) to give dipeptide **8** (31 mg, 91%). [α]_D²⁵ = +12 (589), +12 (578), +17 (546), +45 (436), +27 (365) (*c* = 0.08, MeOH). ES-MS: *m/z* (%) = 377.2 (11) [M + Na]⁺, 355.3 (100) [M + H]⁺. ES-HRMS: calcd. for C₁₈H₃₂N₃O₄ + H 355.2471; found 355.2452.

Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-OMe (9**):** Dipeptide **6** (338 mg, 0.744 mmol) was dissolved in MeOH (15 mL) and a 1 M aqueous NaOH solution (6 mL) was added. The mixture was heated at 75 °C for 24 h. The mixture was cooled, and the pH was adjusted to 5–6 by the addition of a 0.5 M aqueous HCl solution. The mixture was concentrated under reduced pressure to remove MeOH. The remaining solution was diluted with water, and extracted with CH₂Cl₂. The combined organic extracts were washed with water, then dried with MgSO₄, filtered and concentrated. The crude Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-OH (208 mg, 0.472 mmol, 63%) obtained was used as such in the next step without further purification. This residue and the hydrochloride HCl·H-(1*S*,2*S*)-ACHC-OMe (119 mg, 0.614 mmol) were dissolved in CH₂Cl₂ (5 mL), and the mixture was cooled in an ice bath. HOAt (128 mg, 0.944 mmol) and DIEA (0.24 mL, 1.42 mmol) were added, followed by EDC (136 mg, 0.71 mmol). The mixture was stirred at room temperature for 48 h. The mixture was diluted with CH₂Cl₂ and washed successively with 0.5 N HCl, water and a saturated NaHCO₃ solution. The organic phase was dried with MgSO₄, filtered and concentrated. The residue obtained was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 9:1) to give tripeptide **9** (133 mg, 49%). [α]_D²⁵ = −50 (589), −51 (578), −48 (546), −24 (436), −52 (365) (*c* = 0.1, MeOH). ES-MS: *m/z* (%) = 601.9 (100) [M + Na]⁺. ES-HRMS: calcd. for C₃₀H₅₁N₄O₇ + H 580.3836; found 580.3867.

Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-(1*S*,2*S*)-ACHC-OMe (10**):** Tripeptide **9** (128 mg, 0.221 mmol) was dissolved in MeOH (5 mL) and a 1 M aqueous NaOH solution (1 mL) was added. The mixture was heated at 75 °C for 15 h. The mixture was cooled, and the pH was adjusted to 5–6 by the addition of a 0.5 M

aqueous HCl solution. The mixture was concentrated under reduced pressure to remove MeOH. The remaining solution was diluted with water, and extracted with CH₂Cl₂. The combined organic extracts were washed with water, then dried with MgSO₄, filtered and concentrated. The crude Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-OH (101 mg, 0.179 mmol, 81%) obtained was used as such in the next step without further purification. This residue and the hydrochloride HCl·H-(1*S*,2*S*)-ACHC-OMe (69 mg, 0.359 mmol) were dissolved in CH₂Cl₂ (5 mL), and the mixture was cooled in an ice bath. HOAt (49 mg, 0.359 mmol) and DIEA (0.12 mL, 0.72 mmol) were added, followed by EDC (52 mg, 0.269 mmol). The mixture was stirred at room temperature for 18 h. The mixture was diluted with CH₂Cl₂ and washed successively with 0.5 N HCl, water and a saturated NaHCO₃ solution. The organic phase was dried with MgSO₄, filtered and concentrated. The residue obtained was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 9:1) to give tetrapeptide **10** (80 mg, 63%). [α]_D²⁵ = −2 (589), −2 (578), 0 (546), −26 (436), −108 (365) (*c* = 0.1, MeOH). ES-MS: *m/z* (%) = 727.4 (100) [M + Na]⁺, 705.5 (42) [M + H]⁺. C₃₇H₆₂N₅O₈·2H₂O (740.938): calcd. C 59.97, H 8.97, N 9.45; found C 60.15, H 8.87, N 9.09.

Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-OMe (11**):** Tetrapeptide **10** (70 mg, 0.221 mmol) was dissolved in MeOH (3 mL) and a 1 M aqueous NaOH solution (1 mL) was added. The mixture was heated at 75 °C for 15 h. The mixture was cooled, and the pH was adjusted to 5–6 by the addition of a 0.5 M aqueous HCl solution. The mixture was concentrated under reduced pressure to remove MeOH. The remaining solution was diluted with water, and extracted with CH₂Cl₂. The combined organic extracts were washed with water, then dried with MgSO₄, filtered and concentrated. The crude Boc-(1*S*,2*S*)-ACHC-(3*R*,4*S*)-β-TOAC-(1*S*,2*S*)-ACHC-(1*S*,2*S*)-ACHC-OH (67 mg, 0.097 mmol, 97%) obtained was used as such in the next step without further purification. This residue and dipeptide **8** (37 mg, 0.105 mmol) were dissolved in CH₂Cl₂ (5 mL), and the mixture was cooled in an ice bath. HOAt (26 mg, 0.194 mmol) and DIEA (0.03 mL, 0.194 mmol) were added, followed by EDC (28 mg, 0.146 mmol). The mixture was stirred at room temperature for 18 h. The mixture was diluted with CH₂Cl₂ and washed successively with 0.5 N HCl, water and a saturated NaHCO₃ solution. The organic phase was dried with MgSO₄, filtered and concentrated. The residue obtained was purified by preparative thin layer chromatography (CH₂Cl₂/MeOH, 9:1) to give hexapeptide **11** (64 mg, 65%). [α]_D²⁵ = −37 (589), −41 (578), −46 (546), −38 (436), −137 (365) (*c* 0.09, MeOH); ES-MS: *m/z* (%) = 1051.2 (100) [M + Na + 2H]⁺, 1029.3 (27) [M + 3H]⁺. FAB-HRMS: calcd. for C₅₄H₉₀N₈O₁₁ + Na + 2H 1051.6783; found 1051.6779; calcd. for C₅₄H₉₀N₈O₁₁ + Na + H 1050.6705; found 1050.6709.

FTIR Absorption: The FTIR absorption spectra were recorded with a Perkin–Elmer model 1720X spectrophotometer, nitrogen-flushed, equipped with a sample shuttle device, at 2 cm^{−1} nominal resolution, averaging 100 scans. Cells with path lengths of 0.1, 1.0, and 10 mm (with CaF₂ windows) were used. Spectrograde deuteriochloroform (99.8% D) was purchased from Aldrich. Solvent (baseline) spectra were recorded under the same conditions.

Circular Dichroism: The CD spectra were obtained with a Jasco J-710 dichrograph. Cylindrical fused quartz cells of 10, 1.0, 0.2 and 0.1-mm path length (Hellma) were used. The values are expressed in terms of [θ]_T, the total molar ellipticity (deg × cm² × dmol^{−1}). Spectrograde MeOH (Aldrich) was used as solvent.

Electron Paramagnetic Resonance: The EPR spectra were recorded with an X-band (9.5 GHz) Bruker ER2000 spectrometer. Tempera-

ture control was achieved with the aid of a Bruker BVT2000 nitrogen-flow system. A 1 mM solution of hexapeptide **11** (or tetrapeptide **10**) in MeOH solution was prepared and put into an EPR quartz tube of 1 mm inner diameter. The solution in the tube was carefully degassed in a vacuum line by several pump-freeze-thaw cycles and finally sealed off.

Modelling: The molecular model of β -hexapeptide **11** was created by means of the WebLab ViewerPro software (Molecular Simulations Inc.), version 3.7. Dihedral angles of an ideal 3_1 -helix of β -peptides^[18] were used to draw the structure.

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