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ORIGINAL ARTICLE

Chiral, fully extended helical peptides

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Abstract The synthesis of the N-protected (blocked) homo-peptide esters from the chiral C^{α}-ethyl, C^{α}-*n*-pentylglycine was performed in solution to the hexapeptide level. The conformational propensity exhibited by these oligomers in chloroform solution and in the crystal state was assessed by use of FTIR absorption, NMR, and X-ray diffraction. The results indicated that fully extended helical structures (2.0_5-helices) are overwhelmingly adopted irrespective of the peptide main-chain length. This oligomeric series is of great interest as it is characterized by the longest C^{α}_{i},…, C^{α}_{i+1} (per residue) separation achievable in the class of chiral, rigid, helical peptide spacers based on α-amino acids.

Keywords Fully extended helix · Homo-oligopeptides · Infrared absorption · Nuclear magnetic resonance · C^{α}-Tetrasubstituted α-amino acids · X-ray diffraction

Abbreviations

Ac	Acetyl
Ac_nc	1-Aminocycloalkane-1-carboxylic acid
Aib	α-Aminoisobutyric acid or C^{α}-methylalanine or $C^{\alpha,\alpha}$-dimethylglycine
(αMe)AA	C^{α}-Methylated α-amino acid
Beg	C^{α}-*n*-Butyl, C^{α}- ethylglycine
Deg	$C^{\alpha,\alpha}$-Diethylglycine
DMAP	4-(dimethylamino)Pyridine
DMSO	Dimethylsulphoxide
Dpg	$C^{\alpha,\alpha}$-di-*n*-Propylglycine
EDC	*N*-Ethyl,*N*′-[3-(dimethylamino) propyl]carbodiimide
Epg	C^{α}-Ethyl, C^{α}-*n*-pentylglycine
Etn	C^{α}-Ethylnorvaline or C^{α}-ethyl, C^{α}-*n*-propylglycine
EtOH	Ethanol
Iva	Isovaline or C^{α}-methyl, C^{α}-ethylglycine
MeOH	Methanol
MTBE	Methyl, *tert*-butyl ether
OMe	Methoxy
OSu	Succinimido
O*t*Bu	*tert*-Butoxy
TEMPO	2,2,6,6-Tetramethylpiperidinyl-1-oxy
Tfa	Trifluoroacetyl
Z	Benzyloxycarbonyl

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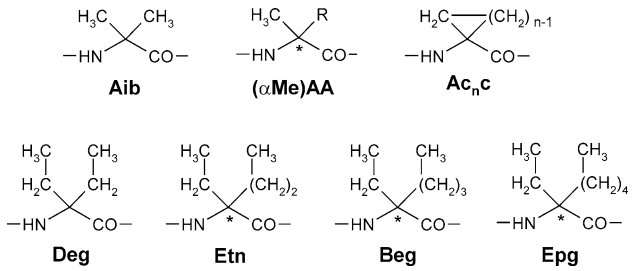
Introduction

It is well recognized that C^{α}-methylated α-amino acids, such as the achiral Aib and its chiral higher homologues (αMe)AAs (Scheme 1) almost exclusively favor the onset of a (3_{10}- or α-) helical structure (Toniolo and Benedetti 1991a) in peptides, even at a very short main-chain length (Karle and Balaram 1990; Toniolo and Benedetti 1991b; Toniolo 1993; Toniolo et al. 2001, 2004; Benedetti et al. 2002). An extensive occurrence of Aib and Iva, the latter being the chiral (αMe)AA with R = CH_2CH_3, has been demonstrated in a variety of natural peptaibiotics (Valle et al. 1989b; Toniolo and Brückner 2009; De Zotti et al. 2010).



Scheme 1 Chemical structures of the C^{α}-tetrasubstituted α-amino acids discussed in this paper

The same conformational propensity is exhibited by the large number of $C_i^{\alpha} \rightarrow C_i^{\alpha}$ cyclized residues of the Ac$_n$c family (Scheme 1) investigated so far, including the smallest member ($n = 3$), although in this latter case the helix is somewhat distorted as a significant ring strain effect is operative (Valle et al. 1989a). It is worth pointing out that the Ac$_3$c residue has been recently shown to occur in two groups of peptaibiotics, namely neoefrapeptins (Fredenhagen et al. 2006) and acretocins (Brückner and Kirschbaum 2010). Natural peptides containing other members of the Ac$_n$c family have not been discovered so far.

The 3D-structural tendencies of α-amino acids are quite different when *both* side chains of a $C^{\alpha,\alpha}$-disubstituted Gly residue are the same, longer than a methyl group and not interconnected in a cyclic system (Toniolo and Benedetti 1991b, c; Toniolo 1993; Imawaka et al. 2000; Toniolo et al. 2001, 2004; Tanaka 2007), as in the achiral Deg shown in Scheme 1. Other examples of this class of residues include $C^{\alpha,\alpha}$-di-*n*-propyl, diphenyl and dibenzyl α-amino acids. All of them overwhelmingly induce the formation of the multiple fully extended C_5-conformation (2.0_5-helix).

The fully extended peptide conformation was proposed at an early stage in structural studies of proteins. In this form H-bonding takes place between the N–H groups of one chain and the C=O groups of the chains on either side, thus making a planar sheet held together by intermolecular H-bonds directed approximately perpendicular to the main-chain axis. Pauling and Corey (1951) investigated the possibility of small contractions of the peptide chains and proposed precise conformations for parallel and antiparallel, *pleated*, *β*-sheet forms which better satisfy stereochemical and H-bonding requirements and have main-chain repeat lengths nearer those found experimentally. These authors were also able to show that steric hindrance between adjacent main chains prevents the onset of the planar sheet in case the side chain is anything but a hydrogen, that is, it could be formed only by -(Gly)$_n$- homopeptides.

The repeating motif of the fully extended polypeptide conformation is the $2 \rightarrow 2$ intramolecularly H-bonded form (Toniolo 1980). The relative disposition of the two dipoles, N_i–H_i and C'_i=O_i, is such that there is obviously some interaction between them. Since these four atoms, together with the $C^{\alpha}{}_i$ atom, are involved in a pentagonal cyclic structure, this conformation is also called the C_5 structure. The C_5 structure has been taken into consideration (and often overemphasized) in conformational energy calculations. Its occurrence in apolar non-interacting solvents has been proposed using mainly IR absorption and ^{1}H NMR measurements of model peptides (Cung et al. 1972; Toniolo et al. 1988). Gly derivatives have the highest population of C_5 structure when compared to the derivatives of residues carrying a side chain. The influence of the bulkiness of the lateral substituent can easily be explained by considering the intramolecular non-bonded interactions between the side group R and the atoms H_{i+1} and O_{i-1}, which induce a warping of these nonsymmetric molecules. Unequivocal verification of the occurrence of the C_5 form has been obtained in the crystal state by the X-ray diffraction analyses of a few favorable compounds, i.e. Gly-rich short peptides (Toniolo 1980; Birkedal et al. 2002).

In globular proteins, a repeating C_5 motif (2.0_5-helix) has so far been authenticated only in the X-ray diffraction analysis of the -(Gly)$_4$- sequence of His-*t*RNA-synthetase (Åberg et al. 1977). In addition, a recent statistical analysis of proteins (Hovmöller et al. 2002) has shown that more than 99% of the residues populating the C_5 region of the ϕ,ψ map are Gly.

Conformational energy computations indicated that the ϕ,ψ space explorable by the "monopeptides" from C^{α}-tetrasubstituted, achiral (but not $C^{\alpha,\alpha}$-dimethylated) α-amino acids is severely restricted and the minimum energy conformation corresponds to the C_5 structure (Benedetti et al. 1988; Toniolo and Benedetti 1991c).

On the basis of the aforementioned results, for many years the general view held by structural peptide chemists was that most of the achiral α-amino acids could represent useful building blocks for the production of the 2.0_5-helix structure. However, more recently, in a paper published by Imawaka et al. (2000), it was clearly shown that the essential prerequisite for the peptide C_5-structure formation is not side-chain symmetry (i.e. amino acid achirality), but rather any kind of substitution at *both* C^{β} atoms (i.e. even chiral C^{α}-tetrasubstituted α-amino acids can be involved). In particular, their most exciting result was the X-ray diffraction structure of the 2.0_5-helical, terminally protected, homotetramer from (*S*)-Beg (Scheme 1). This finding changed the picture of the conformational preferences of C^{α}-tetrasubstituted α-amino acids to a relevant extent, in the sense that it paved the way to the current belief that a sequence of α-amino acid residues with *both* side chains longer than a methyl group into a linear peptide might result in a marked stabilization of the uncommon 2.0_5-helix.

The principal aim of the present work is to corroborate the pioneering data of the Japanese authors (Imawaka et al. 2000) by extending the conformational investigation to homo-peptides based on other, chiral, C^{α}-ethylated α-amino acids. To this end, we have synthesized and studied a set of terminally protected (blocked) $[(S)\text{-Epg}]_n$ (Scheme 1) homo-oligomers to the hexamer level. A preliminary report of part of this work has been recently published (Toniolo et al. 2009). Interestingly, the presence of a chiral, C^{α}-ethylated α-amino acid (Etn), the lower homologue of Beg, has been reported in a peptaibiotic (Tsantrizos et al. 1996; Rainaldi et al. 2003).

Materials and methods

α-Amino acid synthesis

(R,S)-2-Amino-2-ethylheptanenitrile

NaCN (49.5 g, 1.01 mol) was dissolved in aqueous ammonia (25%, 700 ml, 5 mol) at room temperature, followed by a slow addition of AcOH (60.9 g, 1.01 mol) while the temperature increased to 38°C. After cooling to room temperature, 3-octanone (128.2 g, 1 mol) was slowly added to the solution, giving rise to a biphasic reaction mixture. MeOH (50 ml) was added to the mixture to increase the solubility of the ketone and, therefore, the rate of the reaction (in fact, after the MeOH addition the temperature rose from 28 to 32°C). The mixture was stirred overnight, after which time the conversion was 56% (according to an ^{1}H-NMR measurement). The conversion increased to 72% by stirring for further 24 h. Additional NaCN (24.5 g, 0.5 mol) and NH_4Cl (26.5 g, 0.5 mol) were added and the reaction was stirred for another 24 h. At approximately 90% conversion, the reaction was worked up. The aqueous solution was extracted with $CHCl_3$ (3 × 400 ml), the organic solution was dried (Na_2SO_4), and the solvent completely removed under reduced pressure. This procedure afforded the title compound as an oil (144.6 g, 0.94 mol; yield: 93.1%).

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 2.08–1.20 [m, 12H, $(C\underline{H}_2)_5$ and $N\underline{H}_2$], 1.20–1.03 (t, 3H, $CH_2C\underline{H}_3$), and 0.98–0.82 ppm [t, 3H, $(CH_2)_4C\underline{H}_3$].

(R,S)-2-Amino-2-ethylheptanoic amide

The nitrile prepared as above discussed (0.93 mol, 143.5 g) was added dropwise to H_2SO_4 (96%, 500 ml) at 6°C (ice/water bath). During the addition, the temperature increased to 39°C. The solution was stirred for 18 h, after which water (16.8 g, 1 eq.) was added and stirring was continued for an additional hour. The resulting yellow solution was then filtered in order to remove $(NH_4)_2SO_4$ and the pH was adjusted to 8.7 by slow addition of concentrated ammonia. The precipitated salts were filtered off and the aqueous layer was extracted with $CHCl_3$ (3 × 400 ml) giving the title compound (138.3 g, 0.80 mol; yield, 86.0%). As, according to ^{1}H-NMR data, a small amount of ketone was still present from the first step, an HCl solution (3 N, 1 eq.) was added and the ketone was extracted with MTBE (300 ml). The pH was then again adjusted to 8.7 with concentrated ammonia and the product extracted from the aqueous layer with $CHCl_3$ to afford the pure amide as an oil (132.3 g, 0.77 mol; yield 83%).

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.38 (bs, 1H, $CON\underline{H}_2$), 5.71 (bs, 1H, $CON\underline{H}_2$), 2.00–1.65 (m, 2H, $C\underline{H}_2$), and 1.65–1.10 ppm [m, 11H, $(C\underline{H}_2)_4$ and $N\underline{H}_2$]. ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 179.47, 61.11, 40.54, 33.41, 32.17, 23.50, 22.58, 14.02, and 8.06 ppm.

Enzymatic resolution of (R,S)-2-amino-2-ethylheptanoic amide

The racemic amide (101 g, 0.59 mol) was mixed with H_2O (915 g, 10% w/w) to a final concentration of 0.56 mol/kg. After adjusting the pH to 7.1 with H_2SO_4 (96%), a cell suspension containing the amidase from *Ochrobactrum anthropi* [expressed in *E. coli* (Sonke et al. 2005)] (10 g, 2,000 units/g) was added and the mixture was shaken at 55°C for 48 h to reach a conversion of 40% according to ammonia determination. The pH was adjusted from 7.5 to 2.0 with 6 M H_2SO_4 to dissolve the precipitated amino acid. The mixture was centrifuged (1,300 × *g*, 20°C, 20 min) to remove the cell mass. The supernatant was partially concentrated to isolate the (*R*)-amide and the (*S*)-acid by ion-exchange chromatography on the strongly basic Amberlyst A-26 resin. The (*R*)-amide was recovered by eluting with water, followed by extraction with $CHCl_3$ (3 × 400 ml). After drying (Na_2SO_4) and concentration under reduced pressure, 50.6 g (50%) of (*R*)-2-amino-2-ethylheptanoic amide was isolated as an oil. e.e. 74% (*R*). ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.38 (bs, 1H, $CON\underline{H}_2$), 5.71 (bs, 1H, $CON\underline{H}_2$), 2.00–1.65 (m, 2H, $C\underline{H}_2$), and 1.65–1.10 ppm [m, 11H, $(C\underline{H}_2)_4$ and $N\underline{H}_2$]. ^{13}C-NMR ($CDCl_3$, 50 MHz): δ 179.47, 61.11, 40.54, 33.41, 32.17, 23.50, 22.58, 14.02, and 8.06 ppm.

The (*S*)-amino acid was obtained by eluting the ion-exchange column with 1 N AcOH and concentrating to dryness. This procedure gave 31.3 g (31%) of (*S*)-2-amino-2-ethylheptanoic acid as a solid. e.e. > 99%. ^{1}H-NMR (D_2O, 200 MHz): δ 2.10–1.55 (m, 4H, $\alpha C\underline{H}_2$), 1.55–1.04 (m, 6H, $\beta\gamma\delta C\underline{H}_2$), and 1.04–0.40 ppm (m, 6H, $C\underline{H}_3$). ^{13}C-NMR (D_2O, 50 MHz): δ 175.00. 64.55, 35.07, 30.84, 28.90, 22.40, 21.58, 13.27, and 7.22 ppm.

Repeating the enzymatic resolution on a 5 g substrate scale under identical conditions, already resulted in 50%

conversion after 20 h, most likely as a result of better mixing in the presence of the solid acid formed during the hydrolysis. This experiment resulted in (*S*)-acid and (*R*)-amide with 95 and 98% e.e., respectively.

Chemical hydrolysis of (R,S)-2-amino-2-ethylheptanoic amide

The racemic amide (1.0 g, 5.8 mmol) was dissolved in H_2O (10 ml), 12 N HCl solution (10 ml) was added and the solution was stirred for 3 h. After completion of the reaction according to TLC, the solvent was completely removed under reduced pressure, affording the racemic 2-amino-2-ethylheptanoic acid hydrochloride, together with 1 eq. of NH_4Cl. This material was used as such as reference compound for the e.e. determination by HPLC.

Determination of e.e. by HPLC of (R)-2-amino-2-ethylheptanoic amide and (S)-2-amino-2-ethylheptanoic acid

The following method was used for the e.e. determination of the amide and the acid.

Column: Nucleosil, 250/4 mm, dd 5 µm; column temperature: 40°C; eluant: aqueous NaOAc buffer (pH = 5.3) containing either 48% MeOH (for the amide) or 40% MeOH (for the acid); flow: 1.0 ml/min; detection: UV, 254 nm; sample derivatization: (2*S*,3*S*)-dibenzoyltartaric acid anhydride; injection volume: 100 µl.

Alternatively, the e.e.'s of the acid and amide were determined by the following conditions using a chiral HPLC column.

Column: Chiralpak AD, 250/46 mm; column temperature: room temperature; eluant: hexane/EtOH/MeOH 93.5/3.8/2.7% v/v; flow: 1.0 ml/min; pre-column reaction: *o*-phthaldialdehyde/3-mercapto-D-isobutyric acid (for the α-amino acid) (Duchateau et al. 1992), while *o*-phthaldialdehyde/*N*-acetyl-L-cysteine (for the α-amino amide); detection: UV, 210 nm; injection volume: 20 µl.

Peptide synthesis

General procedure

For the in situ pre-formation of the 5(4*H*)-oxazolone, Tfa-(*S*)-Epg-OH was dissolved in anhydrous CH_3CN (15 ml) and 1 eq of EDC × HCl was added. After 10 min, 1 eq of H-[(*S*)-Epg]$_n$-O*t*Bu (n = 1–5) was added and the reaction mixture was refluxed for 36 h under stirring. The product was isolated by flash chromatography.

For the Tfa N^{α}-deprotection step, a weighted amount of N^{α}-trifluoroacetylated amino acid derivative or peptide was dissolved in EtOH and treated with $NaBH_4$ under reflux. The reaction was periodically monitored by TLC until disappearance of the starting material. Then, the solvent was evaporated to dryness and the resulting product (without any purification) was subject to the coupling step.

Characterization

The various peptides and their synthetic intermediates were characterized by melting point determination, TLC (in three solvent systems), solid-state IR absorption spectroscopy (Table 1) and ^{1}H NMR spectrometry (the latter data not reported).

IR absorption

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

NMR

The NMR spectra were recorded with a Bruker (Karlsruhe, Germany) model AM 400 spectrometer. Measurements were carried out in deuterochloroform (99.96% *d*; Aldrich) and deuterated DMSO (99.96% *d*; Acros Organics, Geel, Belgium) with tetramethylsilane as the internal standard. The free-radical TEMPO was purchased from Sigma-Aldrich (St. Louis, MO).

X-ray diffraction

Colorless crystals of Tfa-[(*S*)-Epg]$_2$-O*t*Bu and Tfa-[(*S*)-Epg]$_3$-O*t*Bu were grown from methanol by slow evaporation at room temperature and from hot acetonitrile, respectively. Data collections were performed on a Philips PW1100 four-circle diffractometer in the θ–2θ scan mode using graphite-monochromated CuKα radiation. Cell parameters were obtained by least-squares refinement of the angular settings of 48 carefully centred reflections in the 12 ÷ 20° θ range. Three standard reflections, periodically monitored, did not show any significant intensity variation. This finding implies stability of the crystals and the electronics. Intensities were corrected for Lorentz and polarization effects, not for absorption. Both structures were solved by direct methods of the SHELXS 97 program (Sheldrick 2008) and refined by full-matrix block least-squares procedures on F^2, using all data, by application of the SHELXL 97

Table 1 Physical properties and analytical data for the Epg derivatives and peptides synthesized in this work

Compound	Mp/°C[a]	Recryst. solvent[b]	TLC[c]			V/cm^{-1} [d]
			Rf(I)	Rf(II)	Rf(III)	
Z-(S)-Epg-OH	Oil	–	0.70	0.95	0.50	3,408, 1,708
Tfa-(S)-Epg-OH	Oil	–	0.45	0.90	0.25	3,356, 1,709
Z-(S)-Epg-O*t*Bu	Oil	–	0.90	0.95	0.90	3,419, 1,732, 1,717
Ac-(S)-Epg-O*t*Bu	Oil	–	0.95	0.95	0.70	3,410, 3,308, 1,731, 1,681, 1,658
Tfa-(S)-Epg-OMe	Oil	–	0.95	0.85	0.85	3,386, 1,724
Z-[(S)-Epg]$_2$-O*t*Bu	Oil	–	0.95	0.95	0.90	3,400, 1,725, 1,673
Z-[(S)-Epg]$_3$-O*t*Bu	Oil	–	0.95	0.95	0.90	3,385, 3,353, 1,729, 1,674
Ac-[(S)-Epg]$_2$-O*t*Bu	104–106	EtOAc/LP	0.95	0.95	0.60	3,392, 3,306, 1,724, 1,676, 1,660
Ac-[(S)-Epg]$_3$-O*t*Bu	135–136	EtOAc/LP	0.95	0.95	0.60	3,403, 3,335, 3,316, 1,722, 1,674, 1,653
Ac-[(S)-Epg]$_4$-O*t*Bu	141–142	EtOAc/LP	0.95	0.95	0.60	3,397, 3,360, 3,332, 1,721, 1,675, 1,652
Ac-[(S)-Epg]$_5$-O*t*Bu	148–149	EtOAc/LP	0.95	0.95	0.55	3,419, 3,360, 3,309, 1,726, 1,667, 1,643
Ac-[(S)-Epg]$_6$-O*t*Bu	189–191	EtOAc/LP	0.85	0.80	0.40	3,408, 3,293, 1,724, 1,667, 1,661
Tfa-[(S)-Epg]$_2$-O*t*Bu	72–74	MeCN	0.95	0.95	0.85	3,386, 3,319, 1,728, 1,671
Tfa-[(S)-Epg]$_3$-O*t*Bu	136–138	MeCN	0.95	0.95	0.90	3,391, 3,348, 1,731, 1,663
Tfa-[(S)-Epg]$_4$-O*t*Bu	184–186	MeCN	0.95	0.90	0.90	3,393, 3,345, 1,730, 1,678, 1,659
Tfa-[(S)-Epg]$_5$-O*t*Bu	215–217	MeCN	0.95	0.90	0.90	3,394, 3,346, 1,730, 1,678, 1,656
Tfa-[(S)-Epg]$_6$-O*t*Bu	223–225	MeCN	0.95	0.90	0.90	3,436, 3,394, 3,346, 1,723, 1,678, 1,655

[a] Determined on a Leitz model Laborlux 12 apparatus (Wetzlar, Germany)

[b] *EtOAc* ethyl acetate, *LP* light petroleum (bp 40–60°C), *MeCN* acetonitrile

[c] Silica gel plates (60F-254 Merck, Darmstadt, Germany) using the following solvent systems: (I) chloroform–ethanol 9:1; (II) butan-1-ol–acetic acid–water 6:2:2; (III) toluene–ethanol 7:1. The compounds were revealed either with the aid of a UV lamp or with the hypochlorite–starch–iodide chromatic reaction. A single spot was observed in each case

[d] Determined in KBr pellets (or as a film between KBr disks in the case of oils) on a Perkin Elmer model 1720X FTIR spectrophotometer

program (Sheldrick 2008) with all non-H atom anisotropic, and allowing the positional parameters and the anisotropic displacement parameters of the non-H atoms to refine at alternate cycles.

In both structures the N-terminal Tfa group shows rotational disorder. It was refined on two sets of positions [atoms F1, F2, and F3 in one conformer, while F1′, F2′, and F3′ in the other (the latter is rotated by about 60° with respect to the former)], with occupancies 0.70:0.30 in the dipeptide and 0.50:0.50 in the tripeptide. In the structure of the tripeptide the C^{ζ} atom of Epg(2) is also disordered. It was refined over two positions (atoms C2Z and C2Z′) with occupancy of 0.65 and 0.35, respectively. Restraints were applied to the bond distances and bond angles involving the disordered groups and side-chain atoms. H-atoms were calculated at idealized positions and refined using a riding model. Relevant crystal data and structure refinement parameters are listed in Table 2.

CCDC 795268 and 795269 contain the supplementary crystallographic data for this paper. These data can be obtained from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Results and discussion

α-Amino acid synthesis and optical resolution

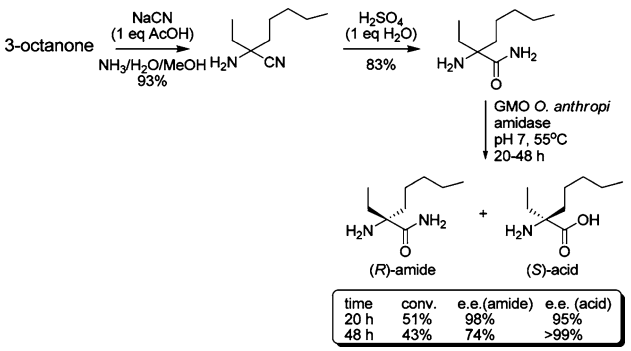
Enantiomerically pure H-(*R*)-Epg-NH_2 and H-(*S*)-Epg-OH were prepared by the chemo-enzymatic process described in Scheme 2 (Sonke et al. 2000). Strecker reaction of 3-octanone in MeOH/water resulted in a 93% yield of crude α-aminonitrile after 3 days. This latter compound was hydrolyzed in concentrated sulfuric acid to the racemic 2-amino-2-ethylheptanoic amide. Enzymatic resolution using the amidase from *Ochrobactrum anthropi* NCIMB 40321, overexpressed in *E. coli* (Sonke et al. 2005), gave the (*S*)-acid and (*R*)-amide in high enantiomeric excess (e.e.) of >99% and >98%, respectively (corresponding to an E-ratio of 180–220 for the enzymatic reaction) (Chen et al. 1982).

Peptide synthesis

The Z-protected H-(*S*)-Epg-OH was prepared using Z-OSu (Paquet 1982) in CH_3CN/H_2O in the presence of triethylamine. The Tfa-protected amino acid was synthesized using

Table 2 Crystal and refinement data for Tfa-[(*S*)-Epg]$_2$-O*t*Bu and Tfa-[(*S*)-Epg]$_3$-O*t*Bu

Parameter	Tfa-[(*S*)-Epg]$_2$-O*t*Bu	Tfa-[(*S*)-Epg]$_3$-O*t*Bu
Empirical formula	C_{24} H_{43} F_3 N_2 O_4	C_{33} H_{60} F_3 N_3 O_5
M_r	480.60	635.84
T (K)	293(2)	293(2)
λ (Å)	1.54178	1.54178
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1$
a (Å)	8.428(2)	6.612(2)
b (Å)	16.525(3)	30.544(5)
c (Å)	10.325(2)	9.847(3)
β (°)	95.08(8)	97.86(8)
Volume (Å^3)	1,432.3(5)	1,970.0(9)
Z	2	2
ρ_{calc} (Mg m^{-3})	1.114	1.072
μ (mm^{-1})	0.732	0.664
F(000)	520	692
Crystal size (mm^3)	0.40 × 0.20 × 0.07	0.40 × 0.35 × 0.30
θ range for data collection (°)	5.07–60.10	4.76–60.01
Index ranges	$-9 \le h \le 9$	$-7 \le h \le 7$
	$0 \le k \le 18$	$0 \le k \le 34$
	$0 \le l \le 11$	$0 \le l \le 11$
Reflections collected/ unique	2355/2,212 (R_{int} = 0.0782)	3,169/2,991 (R_{int} = 0.0297)
Data/restraints/ parameters	2,212/67/325	2,991/108/434
Goodness-of-fit on F^2	0.891	0.908
Final R indices [$I \ge 2\sigma(I)$]	R_1 = 0.0912, wR_2 = 0.2207	R_1 = 0.0827, wR_2 = 0.2163
R indices (all data)	R_1 = 0.1487, wR_2 = 0.2492	R_1 = 0.1056, wR_2 = 0.2370
Residuals (e· Å^{-3})	0.354/−0.290	0.334/−0.203



Scheme 2 Chemo-enzymatic synthesis of H-(*R*)-Epg-NH$_2$ and H-(*S*)-Epg-OH

trifluoroacetic acid anhydride. Tfa-(*S*)-Epg-OMe was obtained from Tfa-(*S*)-Epg-OH and MeOH in anhydrous CH_3CN in the presence of EDC.HCl and DMAP. Preparation

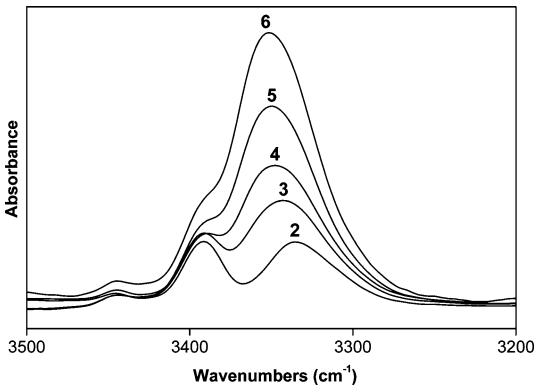


Fig. 1 FTIR absorption spectra (N–H stretching region) of the Tfa-[(*S*)-Epg]$_n$-O*t*Bu (n = 2–6) homo-peptides in $CDCl_3$ solution. Peptide concentration: 1 mM

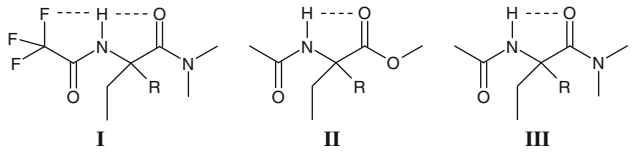
of *Z*-(*S*)-Epg-O*t*Bu was achieved by H_2SO_4-catalyzed treatment of the Z-protected amino acid with isobutylene in anhydrous CH_2Cl_2. Ac-(*S*)-Epg-O*t*Bu was synthesized from H-(*S*)-Epg-O*t*Bu (prepared in turn by catalytic hydrogenation of the corresponding Z-derivative) by reaction with a 50% solution of acetic anhydride in CH_2Cl_2.

For the synthesis of the *Z*-[(*S*)-Epg]$_n$-O*t*Bu homo-peptide series, we first used the acylfluoride C-activation method (Carpino et al. 1990). Since the coupling yields in the formation of dimer and trimer were poor (10–30%), we decided to follow the procedure described by Imawaka et al. (2000) for the synthesis of the (*S*)-Beg homo-oligomers. It involves Tfa N$^{\alpha}$-protection (see above) and subsequent, selective, reductive Tfa removal by $NaBH_4$ in EtOH. Peptide bond formation was achieved in anhydrous CH_3CN using the in situ pre-formed 5(4*H*)-oxazolone from the Tfa-protected amino acid. The reaction yields were acceptable (35–65%), although decreasing with peptide main-chain length elongation. The 5(4*H*)-oxazolone was synthesized by treating Tfa-(*S*)-Epg-OH with EDC. The electronegative trifluoromethyl group is probably the main responsible for the good electrophilicity of the carbonyl moiety of the otherwise modestly reactive 5(4*H*)-oxazolone. Peptide N$^{\alpha}$-acetylation was obtained in excellent yields by treating the N$^{\alpha}$-deprotected homo-peptides with an excess of acetic anhydride.

Solution conformational analysis

The solution conformational preferences of the Tfa (or Ac) N$^{\alpha}$-protected (*S*)-Epg homo-peptide *tert*-butylester series from dimer through hexamer were examined in a solvent of low polarity ($CDCl_3$) at varying concentrations in the range 10–0.1 mM by using FTIR absorption and ^{1}H NMR.

The FTIR absorption spectra in the N–H stretching region (3,500–3,200 cm^{-1}) of the Tfa-[(*S*)-Epg]$_n$-O*t*Bu (n = 2–6) homo-peptide series are reported in Fig. 1. In



Scheme 3 Intraresidue, intramolecularly H-bonded conformers occurring in N^{α}-trifluoroacetylated peptides based on C^{α}-ethylated α-amino acids

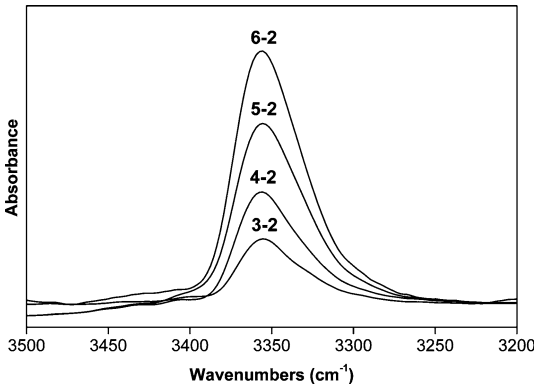


Fig. 2 FTIR absorption difference spectra (N–H stretching region) between each of the Tfa-[(*S*)-Epg]$_n$-O*t*Bu (n = 3–6) homo-peptides and that of the corresponding homo-dipeptide in $CDCl_3$ solution. Peptide concentration: 1 mM

the dipeptide the two bands at about 3,335 and 3,385 cm^{-1} are assigned to N–H vibrators involved in intramolecular H-bonds of the types F···H(N)···O=C (peptide) (conformer I) and (peptide) N–H···O=C (ester) (conformer II) (Scheme 3), at the N- and C-termini of the main chain (Cung et al. 1972; Toniolo et al. 1988). Notably, in both conformers the N–H···O=C H-bonding is of the intraresidue (i, i or C_5) type. The amount of free (non-H-bonded) N–H stretching vibrators (very weak band near 3,440 cm^{-1}) (Pysh and Toniolo 1977; Toniolo et al. 1988) is low. Upon backbone elongation to tri-, tetra-, penta- and hexapeptides, the absorption maximum of the band at lower frequency (3,335 cm^{-1}) significantly shifts to higher wavenumbers and its relative intensity increases linearly and markedly. The "internal" C_5 conformer III (Scheme 3), (peptide) N_i-H_i···O_i = C_i (peptide), appears to provide an ever increasing contribution to the changes observed in the spectra of the four latter peptides. This effect is particularly evident from the FTIR absorption difference spectra shown in Fig. 2 (see also Toniolo et al. 2009), where the contributions of conformers I and II (both present in the dipeptide) are subtracted from the spectra of the higher homo-oligomers. The resulting maximum, perfectly aligned in all oligomers, of ever increasing intensity along the peptide series and attributable to conformer III, is seen at 3,356 cm^{-1}. Very similar data (not shown) were also found for the N^{α}-acetylated (*S*)-Epg homo-peptide series, where the absorption maximum in the difference spectra is only slightly shifted (3,359 cm^{-1}). In the concentration range examined (10–0.1 mM) the FTIR absorption spectra of the (*S*)-Epg homo-peptides changes only slightly in the 3,500–3,200 cm^{-1} region. This finding indicates that the observed N–H···O = C H-bonds are almost exclusively of the intramolecular type.

In the C=O stretching region, a quite intense band is visible for all oligomers at 1,720 cm^{-1}, due to overlapping of the carbonyl vibrators of the trifluoroacetamido and *tert*-butylester moieties (Toniolo et al. 1988). As for the peptide C=O groups, a single amide I band at 1,666 cm^{-1} is found for the tripeptide, whereas in the spectra of the higher oligomers two bands are visible near 1,675 cm^{-1} and at 1,654–1,648 cm^{-1} (not shown). The stronger absorption ($\approx$1,650 cm^{-1}) can be associated with H-bonded carbonyls, while the position of the weaker absorption is in the range usually attributed to free carbonyls (Toniolo et al. 1988; Kennedy et al. 1991). However, the very low amount of free N–H groups (see above) makes the occurrence of a significant amount of free carbonyls unlikely. It is worth recalling that peptides in the antiparallel β-sheet conformation give rise to a strong C=O absorption at about 1,630 cm^{-1} accompanied by a weak band at 1,680–1,690 cm^{-1} (Miyazawa 1967). In that case, the splitting of the amide I band has been ascribed to coupling of neighboring vibrators. Interestingly, the split character of the IR amide I band is observed also in spectra computed for single-stranded peptides in the β-sheet conformation (Kubelka and Keiderling 2001; Kubelka et al. 2006). In the experimental spectra of our Epg homo-oligomers, the splitting of the amide I band arises at the level of the tripeptide, in which two peptide carbonyls are present (beside the trifluoroacetyl and ester C=O groups). In the higher homologues, the intensity ratio between the two bands does not change dramatically if compared to that of the tripeptide. On the basis of these observations, we are inclined to hypothesize that the occurrence of two amide I bands at about 1,675 cm^{-1} and 1,650 cm^{-1} might be the result of the coupling of consecutive peptide carbonyls each involved in an intraresidue (i, i or C_5) H-bond, as computationally assessed for the Deg homo-peptides (Maekawa et al. 2010, in press).

All of the results of our FTIR absorption investigation fit beautifully with those already published for the Deg (Toniolo et al. 1988) and Beg (Imawaka et al. 2000) homo-peptides found to adopt consecutive C_5 conformations (2.0_5-helices) (Toniolo and Benedetti 1991c).

To obtain further information on the 3D-structural tendency of the N^{α}-trifluoacetylated (*S*)-Epg homo-peptide esters in $CDCl_3$ solution, the NH chemical shifts in their ^1H NMR spectra were investigated as a function of addition of DMSO and TEMPO. The polar solvent DMSO is expected

to interact strongly with the exposed amide NH protons via N–H···O = S H-bonds, thus inducing a downfield shift in their resonances (Kopple et al. 1969; Martin and Hauthal 1975). The paramagnetic free-radical TEMPO, on the other hand, is known to perturb the ^{1}H NMR spectra of compounds containing exposed –CONH– groups by broadening the resonances of their NH groups by virtue of nitroxide radical–amide association of the N–H···O–N= type (Kopple and Schamper 1972).

The trifluoroacetamido NH signal of the (*S*)-Epg peptides was identified by virtue of its low-field position (≈8.0 ppm) (Toniolo et al. 1988). The C-terminal NH signal of the dimer was found near 6.85 ppm. By analogy, we related the 6.8–6.9 ppm signal in the spectra of the higher homologues to their C-terminal NH group. The internal NH signal of the tripeptide (at 7.4 ppm) was assigned by elimination. These assignments for the tripeptide were confirmed by a combination of 2D-NMR HMBC and ROESY spectral experiments. It is clear that the NH proton at ≈8.0 ppm, which gives a cross-peak with the quartet signal of the Tfa carbonyl carbon at about 195 ppm (Fig. 3a) is that of the N-terminal residue. By using the ROESY spectra (Fig. 3b), it was then possible to assign the N(2)H proton (at approximately 7.4 ppm) and the N(3)H proton (near 6.85 ppm) from the known N(1)H proton. Interestingly, the observation of the occurrence of the internal NH signals of all homo-oligomers in the narrow range 7.40–7.45 ppm (see, for example, Fig. 4a) highlights their closely related 3D-structural environment.

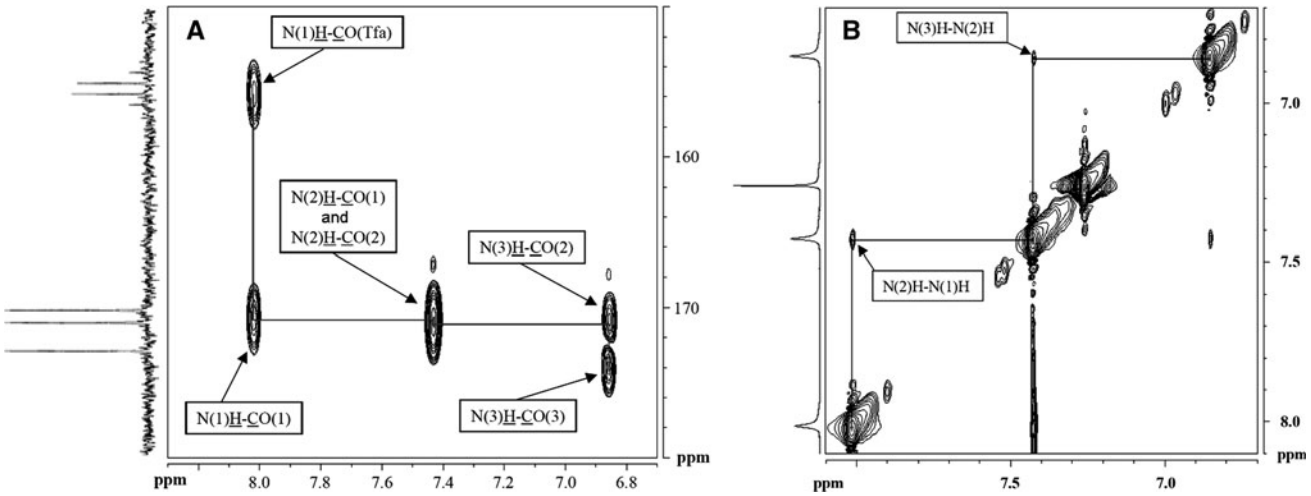


Fig. 3 Section of the HMBC spectrum (**a**) and the ROESY spectrum (**b**) of Tfa-[(*S*)-Epg]$_3$-O*t*Bu in $CDCl_3$ solution. Peptide concentration: 10 mM

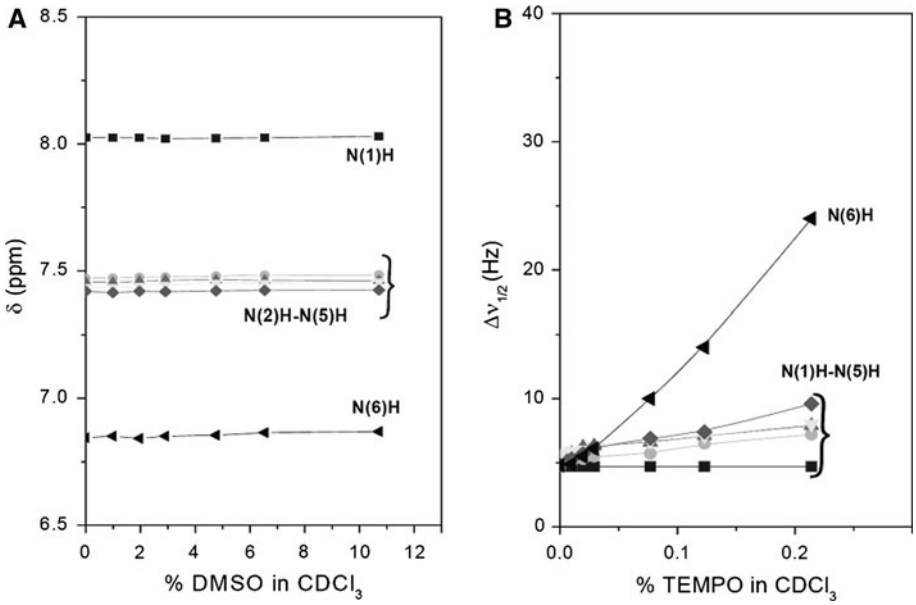


Fig. 4 **a** Plot of the NH proton chemical shifts in the ^{1}H NMR spectra of Tfa-[(*S*)-Epg]$_6$-O*t*Bu as a function of increasing percentages of DMSO (v/v) added to the $CDCl_3$ solution. **b** Plot of bandwidths of the NH proton signals in the ^{1}H NMR spectra of the same homo-hexapeptide as a function of increasing percentages of TEMPO (w/v) added to the $CDCl_3$ solution. Peptide concentration: 1 mM

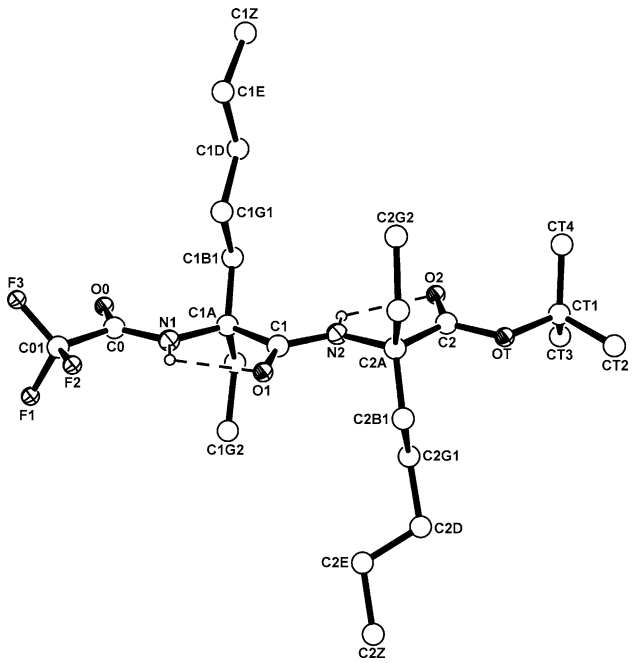


Fig. 5 X-ray diffraction structure of Tfa-[(*S*)-Epg]$_2$-O*t*Bu with atom numbering. Only the major-occupancy conformer of the disordered Tfa group is shown. The two intramolecular N–H···O=C H-bonds are represented by dashed lines (the intramolecular N–H···F H-bond is not shown)

The study of the solvent accessibilities of the NH protons of the five homo-peptides, indicative of their participation as donors to peptide—additive or intramolecular peptide—peptide H-bonds and tested by using the DMSO and TEMPO acceptors, strongly support the view that almost all of the NH protons are totally insensitive to the presence of the two perturbing agents. The results for the longest homo-peptide (the hexamer) are reported in Fig. 4. Indeed, according to the TEMPO experiments, the NH proton of the C-terminal residue of each of the tri-, tetra-, penta- and hexapeptides (for the latter, see Fig. 4b) exhibits a (modest) sensitivity, that increases slightly upon main-chain elongation. This finding suggests a limited fraying of the peptide C-termini. In this connection, it is worth recalling that the C-terminal carbonyl ester is a poorer H-bond acceptor if compared to the internal peptide carbonyl groups, as indicated by the results of our FTIR absorption analysis (see above). Again, the data of these conformational investigations in solution agree well with those of the N$^{\alpha}$-trifluoacetylated Deg (Toniolo et al. 1988) and Beg (Imawaka et al. 2000) homo-oligomers. Interestingly, our corresponding TEMPO titrations with the N$^{\alpha}$-acetylated (*S*)-Epg homo-peptides (not shown) are strongly in favor of the conclusion that in this series the N-terminal proton is significantly more exposed to the perturbing agent than its corresponding C-terminal proton. These data underscore the important role played by the N$^{\alpha}$-trifluoroacetyl group in the stabilization of the N-terminal C_5 conformer.

Crystal-state conformational analysis

Numerous attempts to grow single crystals appropriate for an X-ray diffraction analysis allowed us to solve the 3D-structures of the homo-dipeptide Tfa-[(*S*)-Epg]$_2$-O*t*Bu (Toniolo et al. 2009) and the homo-tripeptide Tfa-[(*S*)-Epg]$_3$-O*t*Bu (Figs. 5, 6, respectively). Lists of the most relevant conformational and intramolecular H-bond parameters for the two oligomers are reported in Tables 3 and 4, respectively.

The τ (N–C$^{\alpha}$–C′) bond angle (Touw and Vriend 2010) ranges from 103.9(3)° to 104.6(5)°. These values, characteristic of the internal angles of a pentagonal structure (Toniolo and Benedetti 1991c), are markedly narrower than those of a regular tetrahedral sp^3 atom (109.5°). This finding represents a clear evidence for the presence of the strain introduced by an intramolecularly H-bonded structure of the C_5-pseudoring type.

In all five (*S*)-Epg residues investigated (in particular in the three residues of the trimer) the sets of ϕ,ψ backbone torsion angles show values close to planarity (180°, 180°), the largest deviation observed being 3.1°. This is the strongest indication for the occurrence of consecutive, fully extended (C_5) conformers (Toniolo and Benedetti 1991c). An additional, although indirect, proof to this assumption is based on the observation that all N–H and C=O groups, which generate the C_5 conformers, are not involved in any intermolecular H-bond interaction. On the average, the intramolecular N···O distance in each residue is 2.578 Å. Also, the ω (peptide and ester) torsion angles show values in the proximity of 180°, the largest deviation being noted for the (ester) ω_2 angle of the dipeptide, −174.6(6)°. The trifluoroacetamido moieties of the two peptides are close to planarity, the related ω torsion angles being −176.7(6)° and −173.9(5)°, respectively. In both structures, the N-terminal NH group is also within H-bonding distance from one of the fluorine atoms of one of the conformers of the rotationally disordered Tfa group. This finding supports the occurrence of an intramolecular, bifurcated H-bond of the type F···H(N)···O=C (peptide) (conformer I in Scheme 3). All these crystal-state results agree very well with those already published for the Deg (Toniolo et al. 1988) and Beg (Imawaka et al. 2000) homo-peptides. The ethyl and *n*-pentyl side chains of the five (*S*)-Epg residues adopt a fully extended conformation and point outwards orthogonally from the peptide backbone. Such a side-chain disposition allows the residues to release unfavorable intramolecular interactions.

The packing mode of both structures (for that of the tripeptide, see Fig. 7) is characterized by the lack of any

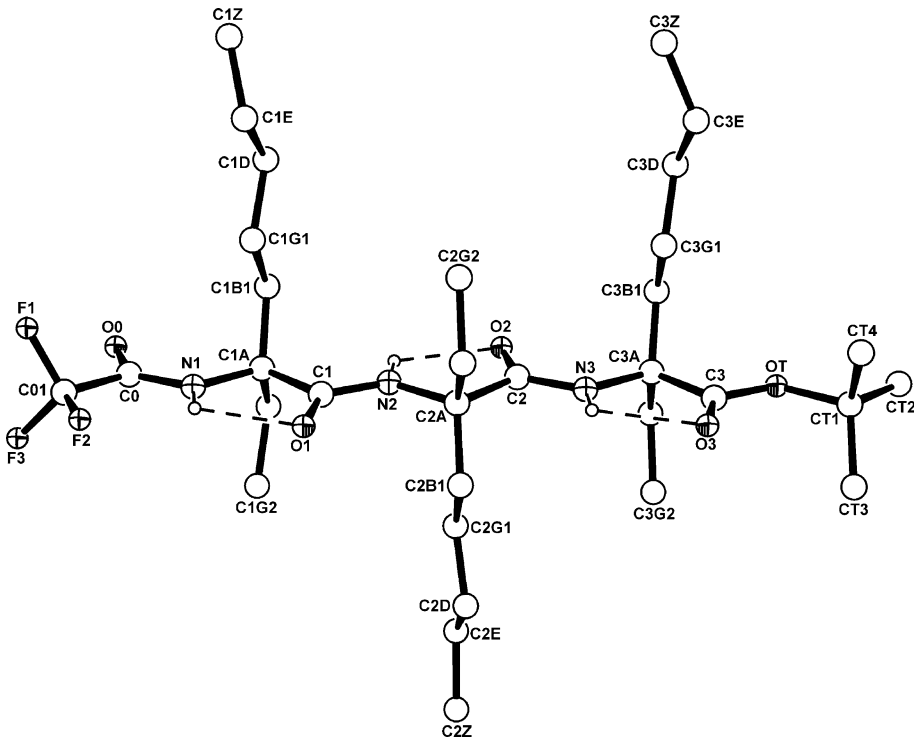


Fig. 6 X-ray diffraction structure of Tfa-[(*S*)-Epg]$_3$-O*t*Bu with atom numbering. Only the major-occupancy conformers of the disordered Tfa group and of Epg(2) *n*-pentyl side chain are shown. The three intramolecular N–H···O=C H-bonds are represented by dashed lines (the intramolecular N–H … F H-bond is not shown)

Table 3 Relevant conformational parameters (°) observed for the (*S*)-Epg residues in the crystal state in Tfa-[(*S*)-Epg]$_2$-O*t*Bu and Tfa-[(*S*)-Epg]$_3$-O*t*Bu

Peptide	Residue	τ	ϕ	ψ	ω
Tfa-[(*S*)-Epg]$_2$-O*t*Bu	1	104.6(5)	176.4(7)	−176.9(5)	179.1(6)
	2	104.4(5)	−178.8(6)	−176.9(5)[a]	−174.6(6)[b]
Tfa-[(*S*)-Epg]$_3$-O*t*Bu	1	104.4(3)	179.9(5)	−178.9(4)	−179.7(4)
	2	104.6(3)	179.7(4)	179.7(4)	178.5(4)
	3	103.9(3)	−179.9(4)	−179.9(4)[c]	177.9(5)[d]

[a] N2-C2A-C2-OT

[b] C2A-C2-OT-CT1

[c] N3-C3A-C3-OT

[d] C3A-C3-OT-CT1

Table 4 Intramolecular H-bond parameters for Tfa-[(*S*)-Epg]$_2$-O*t*Bu and Tfa-[(*S*)-Epg]$_3$-O*t*Bu

Peptide	Donor D–H	Acceptor A	Symmetry equiv. of A	Distance (Å) D···A	Distance (Å) H···A	Angle (°) D–H···A
Tfa-[(*S*)-Epg]$_2$-O*t*Bu	N1–H1	F2	*x*, *y*, *z*	2.643(8)	2.21	111
	N1–H1	O1	*x*, *y*, *z*	2.536(7)	2.08	112
	N2–H2	O2	*x*, *y*, *z*	2.585(7)	2.14	111
Tfa-[(*S*)-Epg]$_3$-O*t*Bu	N1–H1	F2	*x*, *y*, *z*	2.630(7)	2.20	111
	N1–H1	O1	*x*, *y*, *z*	2.529(5)	2.07	112
	N2–H2	O2	*x*, *y*, *z*	2.558(4)	2.11	112
	N3–H3	O3	*x*, *y*, *z*	2.573(4)	2.13	112

intermolecular N–H···O=C H-bond. In the structure of the dipeptide a relatively short C–H···O contact is observed between the C2Z methyl group and the O1 carbonyl oxygen atom of a $(-1 + x, y, z)$ symmetry related molecule [C···O distance 3.580(14) Å, H···O distance 2.69 Å, C–H···O angle 155°]. In addition, a C–H···F contact is found between the C-terminal CT4 methyl group and a $(x, y, 1 + z)$ equivalent of the F2′ atom belonging to the minor

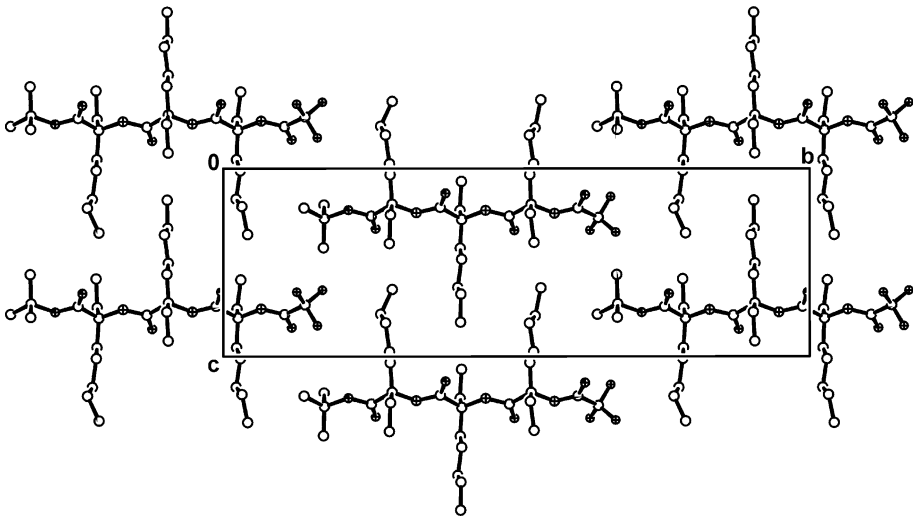


Fig. 7 Packing mode of the Tfa-[(*S*)-Epg]$_3$-O*t*Bu molecules in the crystal as viewed along the *a* direction

conformer of the disordered N-terminal Tfa group [C···F distance 3.42(3) Å, H···F distance 2.58 Å, C–H···O angle 146°]. These two interactions connect molecules along the *a* and *c* directions, respectively.

Weak C–H···O and C–H···F interactions characterize also the packing mode of the tripeptide. Specifically, the shortest intermolecular C–H···O contacts [with C···O distances ranging from 3.228(18) to 3.310(6) Å, H···O distances within 2.62–2.70 Å and C–H···O angles between 124 and 121°] are observed between C2Z and O2 (symmetry equivalence: $1 + x$, y, $-1 + z$), C3Z and O3 (x, y, $1 + z$), and between C2G2 and O2 ($-1 + x$, y, z). A C–H···F contact occurs between the C-terminal CT3 methyl group and a ($-x$, $-1/2 + y$, $1 - z$) symmetry equivalent of the F2 atom of the disordered N-terminal Tfa group [C···F distance 3.379(15) Å, H···F distance 2.55 Å, C–H···O angle 144°]. This latter interaction connects molecules related by the twofold screw axis parallel to the *b* direction.

Conclusions

In this work we unambiguously demonstrated that (*S*)-Epg is the second chiral C^{α}-ethylated α-amino acid, the N^{α}-protected (blocked) homo-oligomer *tert*-butyl esters of which possess a largely prevailing tendency to adopt the fully extended, 2.0_5-helix structure. In our view this conformation will find extensive applications as a molecular spacer or bridge in future energy/electron transfer studies and spectroscopic investigations in that its per residue separation (3.85 Å) is by far the longest among those of all peptide structures (Toniolo and Benedetti 1991c). These homo-oligomers, along with those based on its lower homologue (*S*)-Beg (Imawaka et al. 2000), are characterized by the additional, spectroscopically useful property of being potential substrates for electronic and vibrational CD chiroptical analyses. The Tfa/O*t*Bu protected (*S*)-Epg homo-oligopeptides synthesized in this work dissolve readily in $CDCl_3$, an appropriate solvent to record vibrational CD spectra. However, $CDCl_3$ is not transparent for use in the far-UV region where peptide electronic transitions are known to absorb (Beychock 1967). On the other hand, solvents suitable for electronic CD studies of peptides (e.g., alcohols) tend to destabilize, at least partially, the 2.0_5-helix (unpublished results). To this end, we are planning to synthesize a longer (*S*)-Epg homo-oligomer series carrying N- and/or C-terminal blocking groups suitable for increasing peptide solubility in far-UV transparent solvents of low polarity.

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