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Communication

Orientation of TOAC amino-acid spin labels in α-helices and β-strands

Derek Marsh *

Max-Planck-Institut für biophysikalische Chemie, Abt. Spektroskopie, 37070 Göttingen, Germany

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Abstract

The orientation of α-helices or β-strands, e.g., in membranes, can be determined from EPR order parameters of (2,2,6,6-tetramethyl-piperidine-1-oxy-4-amino-4-carboxylic acid) TOAC amino-acid spin labels incorporated in the polypeptide backbone. This requires knowledge of the inclination of the nitroxide axes, relative to the α-helix or β-strand axis. Crystal structures of TOAC-containing peptides are used to derive the spin-label orientation relative to refined α-poly-L-alanine and β-poly-L-alanine structures. The spin-label *z*-axes of the two mirror-image TOAC twist-boat conformers are inclined at $13 \pm 2°$ and $65 \pm 3°$, respectively, to the α-helix axis, or at $25 \pm 3°$ and $32 \pm 3°$ to the β-strand axis.

Keywords: TOAC; Nitroxide axes; Order parameter; α-Helix; β-Sheet; EPR

1. Introduction

The 2,2,6,6-tetramethyl-piperidine-1-oxy-4-amino-4-carboxylic acid (TOAC; see Fig. 1) nitroxyl amino acid was introduced by Nakaie et al. [1–3] as a means for spin-labelling the backbone of peptides. Because the nitroxide is rigidly coupled to the peptide backbone, measurement of angular order parameters, S_{zz}, from the spin label EPR spectrum provides direct information on the orientation of the secondary structural elements, e.g., in membranes [4,5]. The experimental order parameter, however, provides the orientation of the spin-label group, which must then be related to that of the secondary structural elements.

For uniaxial motional averaging, the EPR order parameter of TOAC in a regular secondary structure is given by:

$$S_{zz} = \langle P_2(\cos\gamma)\rangle \cdot P_2(\cos\theta_z), \tag{1}$$

where γ is the angle that the helix or β-strand axis makes with the director (e.g., membrane normal), and θ_z is the inclination of the nitroxide *z*-axis to the helix or β-strand axis. $P_2(x) = \frac{1}{2}(3x^2 - 1)$ is a second-order Legendre polynomial, and angular brackets indicate a time average over the molecular motion. The EPR order parameters are determined directly from the motionally averaged hyperfine splittings, in the case of fast motion (e.g., [6]), or by simulation in the case of slow motion [7]. To determine the orientational order parameter, $\langle P_2(\cos\gamma)\rangle$, of the helix or β-strand axis (or, in general, the molecular diffusion axis) from the EPR measurements, however, it is necessary to know the angle, θ_z, that the spin-label *z*-axis makes with the helix or strand axis. In the absence of uniaxial averaging, the static tilt of the α-helix or β-strand axis can only be determined from measurements on aligned samples. This then requires knowledge of the direction cosines ($\cos\theta_x$, $\cos\theta_y$, and $\cos\theta_z$) of all three nitroxide axes, relative to the helix or strand axis (see e.g., [8]).

Several crystal structures of the TOAC amino acid in peptides have revealed that the preferred conformation of the spin-label ring is the twist-boat form, of which there are two possible conformers [9,10]. The purpose of the present communication is to determine the orientation of the TOAC nitroxide to the α-helix axis, when the various TOAC crystal structures are built into the refined coordinates of α-poly-L-alanine [11]. It is found that one TOAC conformer is oriented with the spin-label *z*-axis close to the helix axis, whereas, in the other conformer, the *z*-axis

* Fax: +49 551 201 1501.
E-mail address: dmarsh@gwdg.de.

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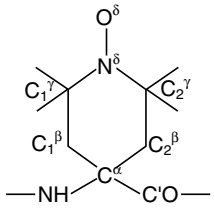

Fig. 1. TOAC amino acid spin label.

is tilted away from the axis of the helix. A similar determination is made also with the refined coordinates of β-poly-L-alanine [12] to determine the TOAC orientation in β-strands. In this case, it is found that the spin-label z-axis of both conformers is inclined at a considerable angle to the axis of the β-strands.

2. Conformation of TOAC in a peptide

Crystal structure studies on peptides have indicated a preference of the TOAC ring for the twist-boat conformation, although isolated occurrences of a near boat conformer and an approximate chair conformation are also found [9,10].

Of interest for the orientation of the TOAC spin label in peptides, is the orientation of the nitroxide p-π orbital (z-axis) relative to the NC$^{\alpha}$ C′ plane. The nitrogen p-π orbital is oriented perpendicular to the $C_1^{\gamma}(C_2^{\gamma})N^{\delta}O^{\delta}$ plane (cf. Fig. 1). The normal to the plane is defined by the vector

$$\mathbf{z} = \overline{C_1^{\gamma}N^{\delta}} \times \overline{N^{\delta}O^{\delta}}, \tag{2}$$

where C_1^{γ} is the (pro-L)C^{γ} atom. With appropriate permutation, the **z**-vector can alternatively be defined, in terms of the vector $C_2^{\gamma}N^{\delta}$, where C_2^{γ} is the (pro-D)C^{γ} atom. The nitroxide x-axis lies along the N-O bond and the y-axis lies closest to the C_1^{γ}-N^{δ} bond.

The normal to the NC$^{\alpha}$C′ plane is defined correspondingly by

$$\mathbf{n} = \overline{C^{\alpha}N^{\delta}} \times \overline{C^{\alpha}C'}, \tag{3}$$

where the direction of **n** lies closest to the C^{α}-C_1^{β} bond. The angle θ between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane is then given by

$$\cos\theta = \mathbf{z}\cdot\mathbf{n}/(|\mathbf{z}||\mathbf{n}|). \tag{4}$$

The inclination of the nitroxide z-axis to the NC$^{\alpha}$C′ plane is the complement of θ. The dihedral angle, ω, between the **z** and **n** vectors is given correspondingly by

$$\cos\omega = \frac{\left(\mathbf{z}\times\overline{C^{\alpha}N^{\delta}}\right)\cdot\left(\mathbf{n}\times\overline{C^{\alpha}N^{\delta}}\right)}{\left|\mathbf{z}\times\overline{C^{\alpha}N^{\delta}}\right|\left|\mathbf{n}\times\overline{C^{\alpha}N^{\delta}}\right|}, \tag{5}$$

where vertical bars indicate the lengths of the corresponding vectors.

Table 1 lists the values of the tilt angle θ and the dihedral angle ω between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane, for various TOAC peptide crystal structures. Differences between the values deduced taking the $C_1^{\gamma}N^{\delta}O^{\delta}$ normal or the $C_2^{\gamma}N^{\delta}O^{\delta}$ normal in the definition of the nitroxide z-axis indicate slight deviations from coplanarity, corresponding to a small pucker about the N^{δ} nitrogen. The near coincidence of the values for the inclination θ and the dihedral ω, arises because the **z** and **n** vectors are almost perpendicular to the $C^{\alpha}N^{\delta}$ vector. This somewhat simplifies visualisation of the orientation of the TOAC nitroxide in peptide helices.

Table 1
Angle, θ, between the nitroxide z-axis and the normal to the NC$^{\alpha}$C′ plane, and their dihedral angle, ω, for TOAC peptide derivatives

Peptide/residue[a]	θ(°)		ω(°)		Ref.
	$C_1^{\gamma}N^{\delta}O^{\delta}$[b]	$C_2^{\gamma}N^{\delta}O^{\delta}$[c]	$C_1^{\gamma}N^{\delta}O^{\delta}$[b]	$C_2^{\gamma}N^{\delta}O^{\delta}$[c]	
I/TOAC^{1}A	64.9	61.6	64.7	61.4	[8]
I/TOAC^{1}B	66.9	66.0	66.1	66.1	
II/TOAC^1	61.1	62.5	60.7	62.2	[8]
II/TOAC^4	62.8	67.0	62.9	67.1	
III/TOAC^1	64.8	66.2	64.6	66.0	[10]
IV/TOAC^{4}A	63.3	59.3	63.1	59.1	[9]
IV/TOAC^{4}B	62.1	64.0	62.2	64.1	
IV/TOAC^{8}A	63.9	66.3	63.8	66.3	
IV/TOAC^{8}B	63.8	68.0	62.8	67.2	
V/TOAC^1	−61.7	−57.1	−60.8	−56.2	[10]
VI/TOAC^1	−60.9	−62.2	−61.1	−62.3	[10]
VI/TOAC^2	−62.2	−67.2	−62.2	−67.2	

[a] Peptides and coordinates: I, Z-TOAC-(L-Ala)$_2$-NHtBu, CCDC 123753; II, pBrBz-TOAC-(L-Ala)$_2$-TOAC-L-Ala-NHtBu, CCDC 123754; III, Boc-TOAC-[L-(αMe)Val]$_4$-NHtBu, CCDC 257672; IV, trichogin GA IV nOct-[TOAC^{4,8},Leu-OMe^{11}], CCDC 120048; V, Ac-TOAC-(Aib)$_3$-L-Trp-Aib-OtBu, CCDC 257673; VI, Fmoc-(TOAC)$_2$-(Aib)$_4$-OtBu, CCDC 257674. A and B indicate two inequivalent molecules in the asymmetric unit.

[b] Nitroxide z-axis defined as normal to (pro-L)$C_1^{\gamma}N^{\delta}O^{\delta}$ plane.

[c] Nitroxide z-axis defined as normal to (pro-D)$C_2^{\gamma}N^{\delta}O^{\delta}$ plane.

The peptides divide themselves into two groups of twist-boat conformers. The larger group, with $\theta = +(64.1 \pm 2.3)°$ and $\omega = +(63.9 \pm 2.3)°$, corresponds to the conformer with $^6T_2$ ring puckering [10], where 6 refers to the (pro-L)C_1^{γ} and 2 to the (pro-D)C_2^{γ} atom. The smaller group, with $\theta = -(61.9 \pm 3.2)°$ and $\omega = -(61.5 \pm 3.5)°$, corresponds to the mirror-image conformer with $^2T_6$ ring puckering, except that peptide V is closer to the $^3T_1$ twist-boat disposition [10].

3. Orientation of TOAC in an α-helix

To determine the orientation of the TOAC nitroxide axes in an α-helix it is necessary to transform the TOAC local axis system (x, y, and z) to that of the α-helix (X, Y, Z)

$$\mathbf{X} = \mathbf{R}_z(\gamma)\mathbf{R}_x(\beta)\mathbf{R}_z(\alpha)(\mathbf{x} - \mathbf{x}_o), \tag{6}$$

where $\mathbf{x}_o$ is the position of the origin of the TOAC axes in the helix axis system. $\mathbf{R}_i$ are rotation matrices and α, β, and γ are the Euler angles relating the two systems of axes.

The refined coordinates of Arnott and Dover [11] for α-poly-L-alanine are taken for the α-helix system. The helix axis is directed from N- to C-terminal along Z, and the radial vector of the C^{α} atom is directed along X. The parameters of the transformation, (α, β, γ) and $\mathbf{x}_o$, are determined by constraining the transformed coordinates of the TOAC N, C^{α}, and C' atoms to coincide with those in the α-helix. This is done by non-linear least squares optimisation. Representative coordinates for TOAC residues in the two mirror-image twist-boat configurations are given in Table 2. The structures of these two TOAC conformers in a poly-alanine helix are shown in Fig. 2.

Table 2
TOAC Cartesian coordinates from Z-TOAC-(L-Ala)$_2$-NHtBu molecule B[9][a], in the α-poly-L-alanine axis system [11][b]

Atom	X (Å)	Y (Å)	Z (Å)
N	1.386 (1.372)	−0.706 (−0.698)	−0.900 (−0.889)
C^{α}	2.282 (2.301)	−0.014 (−0.004)	0.000 (0.000)
C'	1.476 (1.471)	0.770 (0.752)	1.067 (1.043)
C_1^{β}	3.026 (3.205)	1.024 (0.942)	−0.898 (−0.768)
C_1^{γ}	4.501 (4.518)	1.291 (0.370)	−0.535 (−1.247)
N^{δ}	5.201 (5.228)	0.041 (−0.157)	−0.274 (−0.050)
O^{δ}	6.470 (6.508)	0.097 (−0.107)	−0.332 (−0.003)
C_2^{γ}	4.563 (4.539)	−1.247 (−0.722)	0.043 (1.126)
C_2^{β}	3.246 (3.117)	−0.950 (−1.082)	0.727 (0.746)

[a] Values in parentheses are for the mirror-image conformation: TOAC[1] of Fmoc-(TOAC)$_2$-(Aib)$_4$-OtBu [10].

[b] Z-axis is the helix axis (directed from N- to C-terminus); X-axis is directed to the C^{α}-atom; the Y-axis lies closest to the (pro-L)C^{β} direction.

The angle, θ_z, that the nitroxide z-axis makes with the helix axis is then given simply by the transformed z-coordinate

$$\cos\theta_z = (\mathbf{z})_Z/|\mathbf{z}|, \tag{7}$$

where the nitroxide **z**-vector is defined in Eq. (2). Similarly, the orientation, θ_x, of the nitroxide x-axis is given by

$$\cos\theta_x = \left(\overline{N^{\delta}O^{\delta}}\right)_Z \Big/ \left|\overline{N^{\delta}O^{\delta}}\right| \tag{8}$$

The orientation, θ_y, of the nitroxide y-axis is further given by the orthonormality condition

$$\cos^2\theta_x + \cos^2\theta_y + \cos^2\theta_z = 1. \tag{9}$$

The orientations of the nitroxide axes to the α-helix axis are given for the various TOAC structures in Table 3. It is evident that the orientation of the nitroxide to the helix axis is very different for the two groups of TOAC conformers.

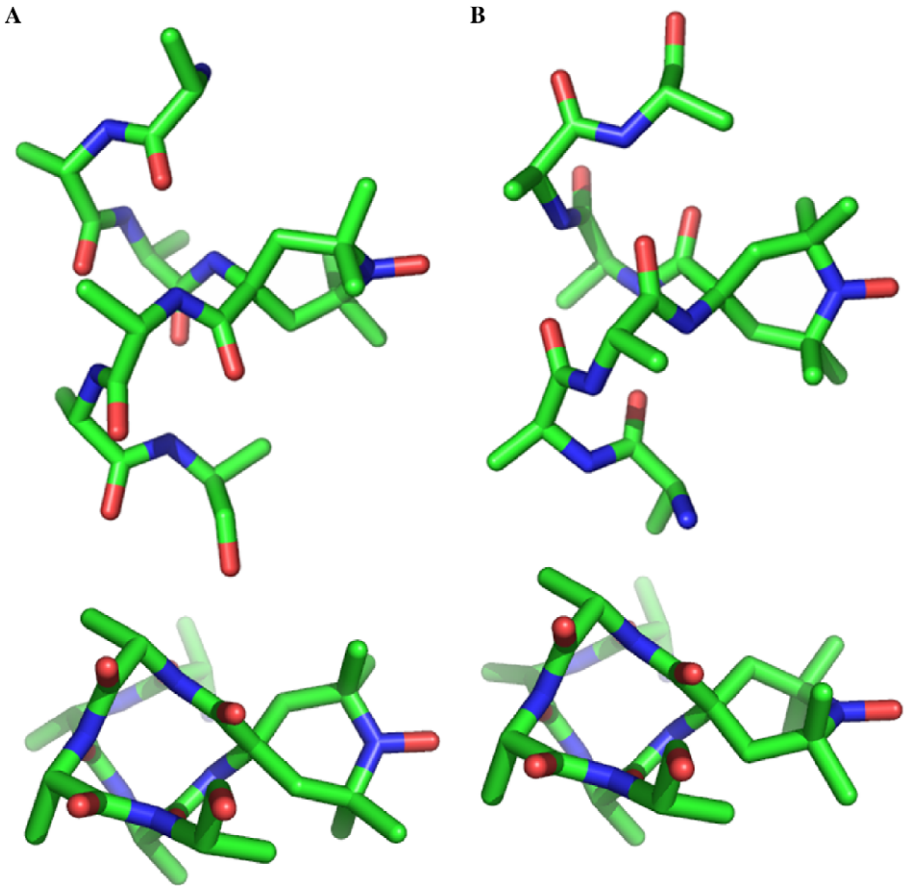

Fig. 2. TOAC[1] ring of: A. Z-TOAC-(L-Ala)$_2$-NHtBu molecule B [9], and B. Fmoc-(TOAC)$_2$-(Aib)$_4$-OtBu [10], incorporated in α-poly-L-alanine [11] (for details see text). Two orthogonal views are given for each peptide.

Table 3
Angles θ_x, θ_y, and θ_z that the TOAC nitroxide x, y, and z axes make with the α-helix axis (α-poly-L-alanine), for different TOAC-containing peptide crystal structures[a]

Peptide/ residue[b]	θ_x(°)	θ_y(°)		θ_z(°)		Pucker
		$C_1^\gamma N^\delta O^\delta$	$C_2^\gamma N^\delta O^\delta$	$C_1^\gamma N^\delta O^\delta$	$C_2^\gamma N^\delta O^\delta$	
I/TOAC[1]A	80.0	78.5	81.7	15.3	13.0	$^6T_2$
I/TOAC[1]B	87.4	77.3	77.3	13.0	13.0	
II/TOAC[1]	75.9	82.0	80.6	16.3	17.0	$^6T_2$
II/TOAC[4]	87.8	80.4	76.2	9.8	13.9	
III/TOAC[1]	81.4	78.7	77.3	14.2	15.4	$^6T_2$
IV/TOAC[4]A	81.2	80.0	84.0	13.4	10.7	$^6T_2$
IV/TOAC[4]B	86.5	81.1	79.2	9.6	11.4	
IV/TOAC[8]A	83.8	79.5	77.1	12.2	14.4	
IV/TOAC[8]B	65.7	78.7	74.8	27.1	29.2	
V/TOAC[1]	80.8	26.7	22.5	65.1	69.7	$^3T_1$
VI/TOAC[1]	92.1	25.4	24.2	64.7	65.9	$^2T_6$
VI/TOAC[2]	92.6	25.5	30.5	64.6	59.6	

[a] N–C^α–C′ coordinates of TOAC peptides (see Table 1 for references) were transformed to those of α-poly-L-alanine [11].
[b] see Table 1 for peptides and references.

For the larger $^6T_2$ group, the mean values are: $\theta_x = 83.0 \pm 4.2°$ and $\theta_z = 13.3 \pm 2.2°$ ($\theta_y = 79°$); and for the mirror image conformer are: $\theta_x = 88.5 \pm 6.7°$ and $\theta_z = 64.9 \pm 3.2°$ ($\theta_y = 25°$). In the first group, TOAC[8] in molecule B of trichogin GA IV is anomalous, because it is intermediate between the twist-boat and boat conformations [13]; it has been omitted from evaluation of the mean.

4. Trichogin GA IV helix

In the crystal structures of the *n*Oct-[TOAC[4,8],Leu-OMe[11]] derivative, the central and C-terminal regions of the 11-residue peptaibol trichogin GA IV are mainly α-helical [13]. This therefore offers the opportunity for comparison with the TOAC orientation when incorporated into the α-poly-L-alanine structure. The helix axis of the TOAC[4,8] trichogin analogue can be defined as the line equidistant from the C^α atoms of residues 1–9, for which the mean radial distance is 2.28 Å, similar to that in α-poly-L-alanine [11]. The resulting mean orientations for TOAC[4]A and B, and TOAC[8]A are: $\theta_x = 84.1 \pm 9.9°$ and $\theta_z = 14.4 \pm 1.9°$ ($\theta_y = 77°$), as compared with $\theta_x = 83.8 \pm 2.7°$ and $\theta_z = 12.0 \pm 1.8°$ ($\theta_y = 80°$) from Table 3 for the poly-alanine coordinates. The anomalous TOAC[8]B yields values of $\theta_x = 59.9°$ and $\theta_z = 33.7°$ ($\theta_y = 76°$), compared with $\theta_x = 65.7°$ and $\theta_z = 28.2°$ ($\theta_y = 77°$) for poly-alanine. For pure twist-boat conformations, at least, the orientations relative to the helix axis, in the two cases, are in reasonable agreement.

5. Application to membranes

From the above analysis of TOAC peptide crystal structures, the orientation of the nitroxide z-axis relative to the axis of an α-helix is characterised by $P_2(\cos\theta_z) = 0.92 \pm 0.03$ and $P_2(\cos\theta_z) = -0.20 \pm 0.06$, for the two different ring conformers, respectively. The EPR experiment is unable to determine the sign of the order parameter. From Eq. 1, it is hence clear that experimental order parameters $|S_{zz}| > 0.26$ for uniaxial ordering are consistent only with the $^6T_2$ conformer, for which $P_2(\cos\theta_z) = 0.92$. For membrane-bound α-helical TOAC peptides, this appears to be the case [4,5].

TOAC spin-labelled *n*Oct-[Leu-OMe[11]] trichogin GA IV displays axially averaged EPR spectra when bound to egg phosphatidylcholine bilayers at 27 °C [4]. Using the spin Hamiltonian parameters of 2,2,6,6-tetramethylpiperidine-1-oxyl [14], the effective order parameters are: $S_{zz} = 0.56$, 0.63, and 0.74 for the TOAC[1], TOAC[4], and TOAC[8] derivatives. For the TOAC[4] and TOAC[8] derivatives, the spin-label orientation in TOAC[4,8] trichogin is given by $P_2(\cos\theta_z) = 0.92$ and 0.89, respectively (neglecting the anomalous TOAC[8]B). For TOAC[4] positioned at the residue 1 position $P_2(\cos\theta_z) = 0.96$. Evidently, the differences in experimental order parameter at the different positions do not represent kinking in the helix, but rather must reflect a dynamic loosening of the helix towards the N-terminal. The TOAC[8] derivative thus best reflects the order parameter of the helix axis $\langle P_2(\cos\gamma)\rangle = 0.83$. The angular motion of the whole trichogin molecule is therefore considerably restricted at the membrane surface.

TOAC-labelled [Ala[36],Phe[41], and Ala[46]] phospholamban has been reconstituted in bilayers of dioleoyl phosphatidylcholine/dioleoyl phosphatidylethanolamine (4:1 mol/mol) by Karim et al. [5]. The order parameters for TOAC substituted at residue positions 11, 24, or 46 in this α-helical protein are found to be: $S_{zz} = 0.71 \pm 0.09$, 0.83 ± 0.09, and 0.94 ± 0.03, respectively, at 4 °C. These high values are consistent with the $^6T_2$ TOAC conformer. The value for the 46-position, which is thought to be located in the hydrophobic interior of the membrane, indicates that the transmembrane section of the protein is oriented close to the membrane normal. The somewhat lower values at the 11- and 24-positions correspond to effective order parameters for the helix of $\langle P_2(\cos\gamma)\rangle = 0.77$ and 0.79, respectively. As for the trichogin example, this implies a loosening of the helix structure in these regions of the protein that can be characterised by wobble in a cone of half-angles 33° and 31° at the 11- and 24-positions, respectively. Note that the very high effective order parameter, $\langle P_2(\cos\gamma)\rangle \approx 1$, that is found for the 46-position would also be obtained if axial averaging were absent. Therefore, a static tilt of the phospholamban helix cannot be excluded. Additional measurements at higher temperatures and on aligned samples could be useful in such cases.

6. Orientation of TOAC in a β-sheet

Exactly the same procedure as for the α-helix can be used to determine the orientation of TOAC in a β-strand. The refined coordinates of Arnott et al. [12] for β-poly-L-alanine

Table 4
TOAC Cartesian coordinates from *Z*-TOAC-(L-Ala)$_2$-NH*t*Bu molecule B [9][a], in the β-poly-L-alanine axis system [12][b]

Atom	*X* (Å)	*Y* (Å)	*Z* (Å)
N	0.322 (0.335)	0.141 (0.126)	2.548 (2.541)
C^{α}	−0.140 (−0.151)	0.787 (0.802)	1.340 (1.340)
C′	0.498 (0.496)	0.122 (0.122)	0.094 (0.128)
$C_1{}^{\beta}$	0.405 (0.169)	2.248 (2.285)	1.436 (1.368)
$C_1{}^{\gamma}$	−0.511 (−0.884)	3.346 (3.185)	0.857 (1.970)
N^{δ}	−1.890 (−2.144)	3.139 (2.948)	1.277 (1.215)
O^{δ}	−2.653 (−2.965)	4.153 (3.916)	1.182 (1.309)
$C_2{}^{\gamma}$	−2.449 (−2.508)	1.878 (1.648)	1.791 (0.619)
$C_2{}^{\beta}$	−1.658 (−1.672)	0.743 (0.555)	1.176 (1.255)

[a] Values in parentheses are for the mirror-image conformation: TOAC[1] of Fmoc-(TOAC)$_2$-(Aib)$_4$-O*t*Bu [10].
[b] *Z*-axis is the β-strand axis (directed from N- to C-terminus); *X*-axis is directed along the intersection of adjacent peptide planes.

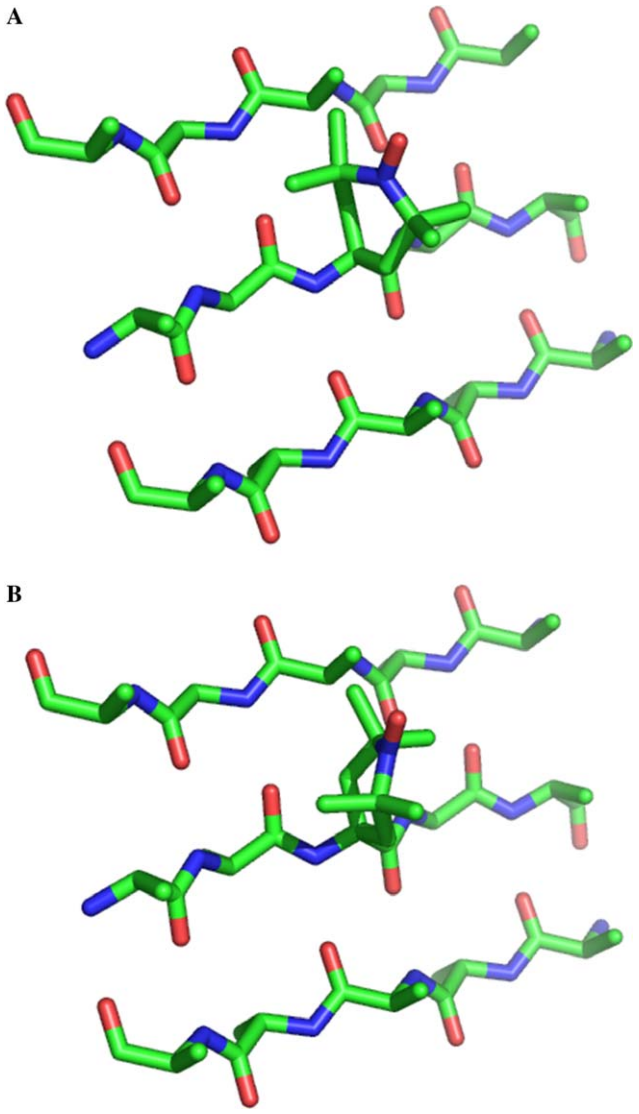

Fig. 3. TOAC[1] ring of: A. *Z*-TOAC-(L-Ala)$_2$-NH*t*Bu molecule B [9], and B. Fmoc-(TOAC)$_2$-(Aib)$_4$-O*t*Bu [10], incorporated in β-poly-L-alanine [12], for details see text.

Table 5
Angles θ_x, θ_y and θ_z that the TOAC nitroxide *x*, *y* and *z* axes make with the β-strand axis (β-poly-L-alanine), for different TOAC-containing peptide crystal structures[a]

Peptide/ residue[b]	θ_x(°)	θ_y(°)		θ_z(°)		Pucker
		$C_1^{\gamma}N^{\delta}O^{\delta}$	$C_2^{\gamma}N^{\delta}O^{\delta}$	$C_1^{\gamma}N^{\delta}O^{\delta}$	$C_2^{\gamma}N^{\delta}O^{\delta}$	
I/TOAC[1]A	94.2	64.3	67.5	26.1	22.9	$^6T_2$
I/TOAC[1]B	85.7	69.1	69.1	21.4	21.4	
II/TOAC[1]	99.8	63.8	65.2	28.3	27.0	$^6T_2$
II/TOAC[4]	86.3	65.7	69.9	24.6	20.4	
III/TOAC[1]	92.2	67.4	68.9	22.7	21.2	$^6T_2$
IV/TOAC[4]A	92.9	66.1	62.1	24.1	28.1	$^6T_2$
IV/TOAC[4]B	87.3	65.1	67.0	25.1	23.2	
IV/TOAC[8]A	89.8	66.7	69.2	23.3	20.8	
IV/TOAC[8]B	111.5	67.0	70.9	32.4	29.5	
V/TOAC[1]	88.9	58.2	53.5	31.9	36.5	$^3T_1$
VI/TOAC[1]	82.1	58.5	59.7	32.7	31.5	$^2T_6$
VI/TOAC[2]	83.2	59.9	64.8	31.0	26.2	

[a] N–C^{α}–C′ coordinates of TOAC peptides (see Table 1 for references) were transformed to those of β-poly-L-alanine [12].
[b] See Table 1 for peptides and references.

are taken for the β-sheet axis system. The β-strand axis is directed from N- to C-terminal along *Z*, and the *X*-axis lies along the intersection of the peptide planes in the antiparallel β-pleated sheet. Representative coordinates for TOAC residues in the two twist-boat conformations are given in Table 4. The structures of these two TOAC conformers in a poly-alanine antiparallel β-sheet are shown in Fig. 3. The orientations of the nitroxide axes to the β-strand axis are given for the various TOAC twist-boat structures in Table 5. For β-strands, the difference in orientation of the nitroxide *z*-axis between the two mirror-image conformers is not so great as for α-helices. For the $^6T_2$ conformers, the mean values are $\theta_x = 91.0 \pm 4.8°$ and $\theta_z = 24.9 \pm 3.4°$ ($\theta_y = 65°$), whereas for the mirror-image TOAC conformers $\theta_x = 84.7 \pm 3.7°$ and $\theta_z = 31.6 \pm 3.3°$ ($\theta_y = 59°$). As expected, the orientations in a β-strand differ considerably from those in an α-helix.

Currently, there are no experimental examples of TOAC in a β-sheet peptide. Although the TOAC residue is intrinsically helicogenic, incorporation in β-strands should be possible, because there are several instances of TOAC in α-helices [4,5] in spite of the fact that α,α-substituted amino acids favour the 3_{10}-helix. Incorporation in extended β-sheets is likely to be more restrictive than in α-helices, however. As seen from Fig. 3, strong steric clashes may occur with the side chain from one of the adjacent strands, unless this residue is glycine, or the sheet is strongly twisted. Otherwise, TOAC label positions would be limited to β-sheet edges, or, e.g., in two-stranded anti-parallel β-ribbons. Knowledge of the orientation of the TOAC axes will be essential to the interpretation of future experiments on these types of β-strands. It should be noted that when the molecular diffusion axis does not coincide with the β-strand direction, it is necessary also to know the orientation of the β-strand axis to the molecular diffusion axis (cf., [15]).

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