Nitroxyl Peptides as Catalysts of Enantioselective Oxidations

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Abstract: The achiral, nitroxyl-containing α-amino acid TOAC (TOAC = 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), in combination with the chiral α-amino acid C^{α}-methyl valine [(αMe)Val], was used to prepare short peptides (from di- to hexa-) that induced the enantioselective oxidation of racemic 1-phenylethanol to acetophenone. The best catalyst was an N^{α}-acylated dipeptide alkylamide with the −TOAC-(αMe)Val− sequence folded in a stable, intramolecularly hydrogen-bonded β-turn conformation with large, lipophilic (hydrophobic) N- and C-terminal blocking groups. We rationalized our findings by proposing models for the diastereomeric intermediates between (*R*)-[and (*S*)]-1-phenylethanol and the catalyst Fmoc-TOAC-L-(αMe)Val-NH*i*Pr, based on the X-ray diffraction structure of the latter.

Keywords: alcohols · asymmetric catalysis · conformation analysis · oxidation · peptidomimetics

Introduction

TOAC (TOAC = 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid) is a member of the family of conformationally constrained C^{α}-tetrasubstituted α-amino acids, the prototype of which is α-aminoisobutyric acid (Aib). Aib and all the $C_i^{\alpha} \leftrightarrow C_i^{\alpha}$-cyclized α-amino acids of this family, like Ac_6c (Ac_6c = 1-amino-1-cyclohexanecarboxylic acid), the cycloaliphatic analogue of TOAC, strongly stabilize β-turn[1–3] and $3_{10}/\alpha$-helical[4] conformations in peptide molecules.[5, 6] The structures of Aib, Ac_6c and TOAC are given in Figure 1.

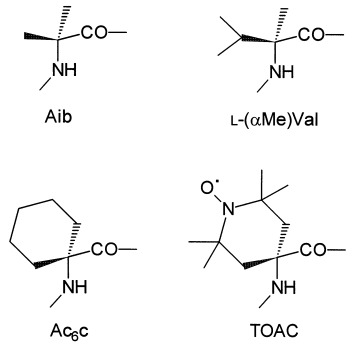

Figure 1. Chemical structures of the C^{α}-tetrasubstituted α-amino acids Aib, L-(αMe)Val, Ac_6c, and TOAC.

TOAC has a saturated heterocyclic structure containing a paramagnetic probe (a nitroxyl group) stabilized by the presence of two contiguous tetrasubstituted carbon atoms.[7] A favourable property of TOAC, over other spin-labelled amino acids connected to the peptide backbone through a flexible link, is the very restricted mobility owing to the C^{α}-tetrasubstitution and hampered rotation about side-chain bonds; this is a result of the incorporation of the nitroxyl nitrogen and the C^{α}, C^{β} and C^{γ} atoms into a cyclic moiety. Therefore, TOAC represents an extremely useful tool to

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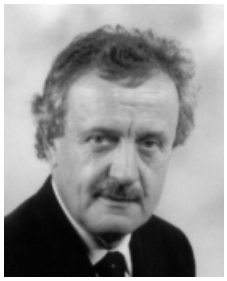
exploit sensitive electron spin resonance techniques for peptide and protein studies.[8, 9] In addition, as a free radical, TOAC is able to induce a dramatic intramolecular quenching of suitably tailored, fluorescence labelled peptides.[10, 11] We have also shown that TOAC undergoes a reversible, nitroxide-based redox process that can be monitored by cyclic voltammetry.[8]

In the present work we take advantage of the redox properties of TOAC-containing peptides by exploiting them as potential catalysts for the enantioselective oxidation of a secondary alcohol. As TOAC is an achiral amino acid residue, we incorporated it into short peptides based on the chiral C^{α}-tetrasubstituted (αMe)Val (C^{α}-methylvaline) (Figure 1). We chose this chiral residue because: 1) in analogy with Aib, Ac_6c and TOAC, it is a strong β-turn/3_{10}-helical inducer,[5–7, 12–17] 2) it contains an intrinsic source of chirality (the asymmetric α-carbon) and 3) among the chiral C^{α}-tetrasubstituted α-amino acids, it has the strongest known bias towards a specific turn/helix screw sense (right-handed for the L-enantiomer) (peptide overall molecular dissymmetry).[15–17]

We synthesized, by solution methods, a set of linear TOAC/L-(or D-)(αMe)Val-containing di- to hexapeptides (**1–11** and **13–16**) with different N^{α}-protecting/blocking groups [Fmoc (Fmoc = fluoren-9-ylmethoxycarbonyl), Z (Z = benzyloxycarbonyl), Boc (Boc = *tert*-butoxycarbonyl), or Ac (Ac = acetyl)] and C-blocking/protecting groups (NH*t*Bu, NH*i*Pr, NHEt, NHMe, or OMe), in which the TOAC residue was inserted either at the N- or C-terminus, or in an internal position of the peptide sequence. The reference dipeptides Fmoc-TOAC-L-Val-NH*t*Bu (**12**) and *cyclo*[TOAC-L-(αMe)-Val] (**17**) were also prepared. The preferred conformations of these compounds in $CDCl_3$ were examined by IR spectroscopy and, for dipeptide **1** and pentapeptide **15**, also in the crystal state by X-ray diffraction. The capability of these peptides to act as mediators (catalysts) of chemical and electrochemical enantioselective oxidations was tested on the racemic secondary alcohol 1-phenylethanol. The results were interpreted by performing conformational energy computations on the mechanistically critical reaction intermediate.



Claudio Toniolo[*] received his Laurea in chemistry from the University of Padova, Italy, in 1965. Following post-doctoral study with Prof. M. Goodman at the Department of Chemistry, Section of Biopolymers, Polytechnic Institute of Brooklyn, New York, 1967-8, he joined the Faculty of Sciences, University of Padova, where he has been Professor of Organic Chemistry since 1980. He has been a Visiting Scholar or Professor at Portsmouth Polytechnic, Portsmouth, UK (Prof. E. M. Bradbury), the State University of New York at Binghamton (Prof. E. S. Stevens), the Indian Institute of Sciences, Bangalore (Prof. P. Balaram), University of California at San Diego (Prof. M. Goodman), Osaka University (Prof. Y. Kobayashi) and the National University of Somalia, Mogadishu. He is a member of the editorial board of several peptide-science and bioorganic-chemistry journals and co-author of over 500 publications on peptide synthesis, supramolecular chemistry and three-dimensional structure.

[*] Members of the Editorial Board will be introduced to readers with their first manuscript.

Results and Discussion

Synthesis and characterization: The synthesis of TOAC is described in references [7, 8]. For the large-scale production of enantiomerically pure L-(αMe)Val, an economically attractive and generally applicable chemo-enzymatic synthesis, developed by DSM Research, was exploited.[18, 19] This involved a combination of a partial Strecker synthesis, for the preparation of the racemic α-amino amide, followed by the use of the broadly specific α-amino amidase to achieve optical resolution. All peptides were prepared by solution methods. The homo-peptide series Z-[L-(αMe)Val]$_n$-NH*t*Bu ($n = 2-4$) was synthesized by using the acyl fluoride C-activation method,[20] which gives good yields for the coupling of two sterically demanding (αMe)Val residues. Z-L-(αMe)Val-F[17] was prepared from the N-protected amino acid[13, 16] and cyanuric fluoride in pyridine. The various Z-L-(or D-)(αMe)-Val-NHR (R = Me, Et, *i*Pr, *t*Bu) derivatives were synthesized by treating the N^{α}-protected amino acid with the relevant primary amine in the presence of EDC (EDC = 1-(3-dimethylamino)propyl-3-ethylcarbodiimide). Benzyloxycarbonyl N^{α}-deprotection was carried out by catalytic hydrogenolysis. The TOAC Fmoc,[21] Boc,[22] and Z[23] N^{α} derivatives were obtained according to published procedures. All coupling steps involving TOAC were performed by exploiting the highly efficient EDC/HOAt (HOAt = 1-hydroxy-7-aza-1,2,3-benzotriazole) C-activation method.[24] Fmoc N^{α}-deprotection was achieved by treatment with a diethylamine solution (25% v/v). The N^{α}-acetylated peptide was obtained by coupling the N^{α}-deprotected synthetic precursor with acetic anhydride. The N^{α}-free dipeptide ester H-TOAC-L-(αMe)-Val-OMe was cyclized to the 2,5-dioxopiperazine *cyclo*-[TOAC-L-(αMe)Val] (**17**) by heating at reflux in a toluene/acetic acid 20:1 (v/v) mixture. The synthesis and characterization of the penta- and hexapeptides Fmoc-[L-(αMe)Val]$_n$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**: $n = 2$; **16**: $n = 3$) are already published.[11]

All peptides were characterized by melting point and optical rotatory power determinations, thin-layer chromatography (TLC) in three solvent systems and solid-state IR absorption (Table 1).

Conformational analysis: The preferred conformation adopted by the linear, N^{α}-acylated TOAC/(αMe)Val dipeptide amides, in a structure supporting solvent ($CDCl_3$), was investigated by FTIR spectroscopy. ^{1}H NMR spectroscopy can not routinely be used with TOAC peptides because the nitroxyl group broadens the resonances of nearby protons beyond detection (radical-induced relaxation effect). Figure 2 illustrates the FTIR absorption spectra in the N–H stretching region of three relevant N^{α}-acylated dipeptide amides. The

Table 1. Physical properties and analytical data for new TOAC and (αMe)Val derivatives and peptides.

Compound	M.p.[°C][a]	Recryst. solvent[b]	$[\alpha]_D^{20}$[c]	TLC[e] R_f(I)	R_f(II)	R_f(III)	IR ν [cm^{-1}][f]
Fmoc-TOAC-NH*t*Bu	138 – 140	EtOAc/PE	-	0.95	0.95	0.45	3334, 1727, 1702, 1678, 1523
Z-L-(αMe)Val-NH*t*Bu	116 – 117	EtOAc/PE	8.1	0.95	0.95	0.50	3417, 3298, 1722, 1663, 1538
Z-[L-(αMe)Val]$_2$-NH*t*Bu	126 – 127	EtOAc/PE	12.2	0.95	0.95	0.40	3359, 3296, 1713, 1685, 1657, 1522
Z-[L-(αMe)Val]$_3$-NH*t*Bu	162 – 163	EtOAc/PE	18.4	0.95	0.95	0.35	3343, 3309, 1702, 1673, 1654, 1515
Z-[L-(αMe)Val]$_4$-NH*t*Bu	245 – 246	DCM/PE	17.8	0.95	0.90	0.30	3340, 1707, 1668, 1528
Z-D-(αMe)Val-NH*t*Bu	115 – 117	EtOAc/PE	– 8.0	0.95	0.95	0.50	3418, 3299, 1718, 1659, 1535
Z-L-(αMe)Val-NH*i*Pr	92 – 94	EtOAc/PE	– 0.4[d]	0.95	0.95	0.50	3383, 3327, 3297, 1727, 1708, 1693, 1660, 1645, 1539
Z-L-(αMe)Val-NHEt	oil	EtOAc/PE	0.4[d]	0.90	0.90	0.40	3344, 3316, 1708, 1635, 1535
Z-L-(αMe)Val-NHMe	oil	EtOAc/PE	– 0.6[d]	0.80	0.90	0.40	3334, 1708, 1653, 1526
Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**)	165 – 167	EtOAc/PE	14.8	0.90	0.95	0.40	3341, 1725, 1652, 1522
Fmoc-TOAC-D-(αMe)Val-NH*t*Bu (**2**)	165 – 167	EtOAc/PE	– 14.9	0.90	0.95	0.40	3340, 1722, 1652, 1520
Fmoc-TOAC-L-(αMe)Val-NH*i*Pr (**3**)	155 – 157	EtOAc/PE	18.8	0.75	0.95	0.40	3371, 1702, 1631, 1527
Fmoc-TOAC-L-(αMe)Val-NHEt (**4**)	162 – 164	EtOAc/PE	19.7	0.75	0.95	0.35	3378, 1701, 1636, 1539
Fmoc-TOAC-L-(αMe)Val-NHMe (**5**)	158 – 160	EtOAc/PE	23.5	0.70	0.90	0.40	3427, 3319, 1718, 1664, 1523
Z-TOAC-L-(αMe)Val-NH*t*Bu (**6**)	156 – 157	EtOAc/PE	41.2	0.75	0.95	0.35	3434, 3378, 3289, 1710, 1684, 1658, 1521
Boc-TOAC-L-(αMe)Val-NH*t*Bu (**7**)	208 – 209	EtOAc/PE	40.8	0.80	0.95	0.35	3394, 1707, 1681, 1637, 1523
Boc-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**8**)	213 – 214	DCM/PE	58.6	0.90	0.95	0.25	3338, 1694, 1669, 1658, 1510
Boc-TOAC-[L-(αMe)Val]$_3$-NH*t*Bu (**9**)	251 – 252	DCM/PE	55.2	0.95	0.90	0.20	3351, 3337, 1691, 1671, 1659, 1646, 1532
Boc-TOAC-[L-(αMe)Val]$_4$-NH*t*Bu (**10**)	244 – 245	DCM/PE	53.2	0.95	0.85	0.20	3343, 1664, 1523
Ac-TOAC-L-(αMe)Val-NH*t*Bu (**11**)	181 – 182	EtOAc/PE	46.4	0.65	0.85	0.30	3441, 3383, 1677, 1660, 1533
Fmoc-TOAC-L-Val-NH*t*Bu (**12**)	165 – 167	EtOAc/PE	14.6	0.90	0.95	0.40	3341, 1725, 1652, 1522
Fmoc-TOAC-L-(αMe)Val-OMe (**13**)	165 – 167	EtOAc/PE	1.3[d]	0.95	0.95	0.50	3323, 1743, 1721, 1660
Fmoc-L-(αMe)Val-TOAC-NH*t*Bu (**14**)	131 – 133	EtOAc/PE	4.3[d]	0.95	0.95	0.40	3365, 1720, 1670, 1525
cyclo[TOAC-L-(αMe)Val] (**17**)	240 – 242	hot toluene	– 8.9[d]	0.80	0.85	0.40	3426, 3315, 1662, 1557

[a] Determined on a Leitz model Laborlux 12 apparatus (Wetzlar, Germany). [b] EtOAc = ethyl acetate, PE = petroleum ether, DCM = dichloromethane. [c] Determined on a Perkin – Elmer model 241 polarimeter (Norwalk, CT) equipped with a Haake model L thermostat (Karlsruhe, Germany): $c = 0.5$ (methanol). [d] $[\alpha]_{546}^{20}$. [e] Silica gel plates (60F-254 Merck), solvent systems: I) chloroform/ethanol 9:1, II) butan-1-ol/water/acetic acid 3:1:1, III) toluene/ethanol 7:1; the plates were developed with a UV lamp or with the hypochlorite/starch/iodide chromatic reaction, as appropriate; a single spot was observed in each case. [f] Determined in KBr pellets on a Perkin – Elmer model 580 B spectrophotometer equipped with a Perkin – Elmer model 3600 IR data station.

curves are characterized by two bands, at around 3435 cm^{-1} (free, solvated NH groups) and 3380 cm^{-1} (H-bonded NH groups).[25, 26] The spectra do not vary appreciably with changing peptide concentration (in the range 10 – 0.1 mM) (not shown). Therefore, the observed hydrogen bonding arises almost exclusively from intramolecular C=O ··· H–N interactions. The remarkable intensity of the low-frequency band shown by all the N$^{\alpha}$-acylated TOAC/(αMe)Val dipeptide amides (for peptide **1** see Figure 2, curve A) suggests a significant population of intramolecularly H-bonded species in these compounds. Interestingly, this band is: 1) much less strong in the TOAC/Val analogue **12** (see curve B), highlighting the positive effect of C$^{\alpha}$-tetrasubstitution [cf.(αMe)-Val vs Val] on intramolecular H-bond formation [6, 26] and 2) of appreciable intensity in the (αMe)Val/TOAC dipeptide **14** (see curve C) indicating that a set of two consecutive C$^{\alpha}$-tetrasubstituted α-amino acids, but not necessarily their precise sequence, is important for a high content of intramolecular H-bonding. The known conformational preferences of TOAC[7] and (αMe)Val,[12–17] combined with the position of the low-frequency band, strongly support the view that the intramolecularly H-bonded species formed by the N$^{\alpha}$-

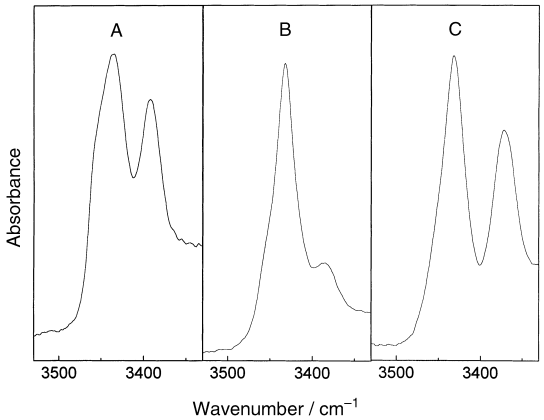


Figure 2. FTIR absorption spectra in the N–H stretching region (3500 – 3350 cm^{-1} region) of A) Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**), B) Fmoc-TOAC-L-Val-NH*t*Bu (**12**), and C) Fmoc-L-(αMe)Val-TOAC-NH*t*Bu (**14**) in $CDCl_3$ solution (conc. 1 mM).

Abstract in Italian: *L'α-amminoacido achirale TOAC, contenente un gruppo nitrossilico, in combinazione con l'α-amminoacido chirale C$^{\alpha}$-metil-valina [(αMe)Val] è stato utilizzato per sintetizzare peptidi corti (dal di- all'esapeptide) capaci di indurre ossidazione enantioselettiva del racemato dell'1-feniletanolo ad acetofenone. Il miglior catalizzatore è risultato essere un dipeptide alchilammide N$^{\alpha}$-acilato e caratterizzato dalla sequenza –TOAC-(αMe)Val–, che assume una conformazione ripiegata-β stabilizzata da un legame a idrogeno intramolecolare C=O ··· H–N, e da gruppi bloccanti N- e C-terminali largamente lipofilici (idrofobici). Questi risultati sono stati razionalizzati sulla base dei modelli degli intermedi diastereomerici tra l'(R) [e l'(S)]-1-feniletanolo e il catalizzatore Fmoc-TOAC-L-(αMe)Val-NHiPr, elaborati partendo dalla struttura tridimensionale del dipeptide ottenuta dall'analisi della diffrazione dei raggi X.*

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acylated TOAC/(αMe)Val dipeptide amides **1–7** and **11** are of the β-turn type. It is reasonable to assume that the conformation of the longer TOAC/(αMe)Val peptides **8–10** (from tri- to hexapeptides) would evolve into two consecutive β-turns and eventually to the 3_{10}-helix. Indeed, this latter ordered secondary structure has characterized the folding in solution of the penta- and hexapeptides **15** and **16**, respectively.[11]

In addition, we used X-ray diffraction to determine the preferred crystal-state conformation of the dipeptide amide Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and the pentapeptide amide Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**). These molecular structures are given in Figures 3 and 4, and relevant backbone torsion angles are given in Table 2. Table 3 lists the intra- and intermolecular H-bond parameters. Bond lengths and bond angles (deposited) are in good agreement with previously reported values for Fmoc-urethane[27] and NH*t*Bu amide groups,[28] the peptide unit,[29, 30] and the TOAC[7–10, 31, 32] and (αMe)Val[12, 15, 16] residues. In particular, the N^{δ}–O^{δ} bond length of the TOAC nitroxyl group (1.277(3) Å for **1** and 1.278(3) Å for **15**), the external

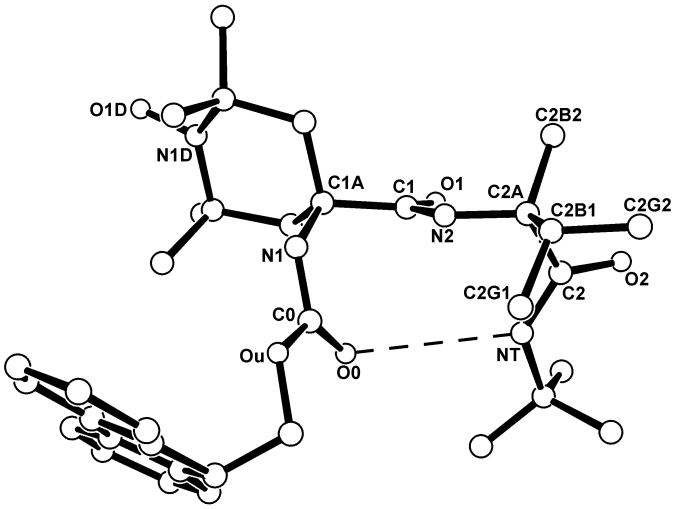


Figure 3. X-ray diffraction structure of Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**). The intramolecular C=O···H–N hydrogen bond is represented by a dashed line.

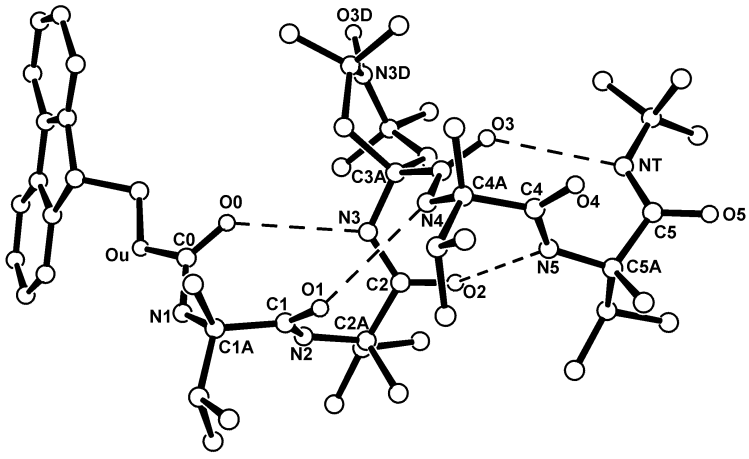


Figure 4. X-ray diffraction structure of Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**). The intramolecular C=O···H–N hydrogen bonds are represented by dashed lines.

Table 2. Relevant backbone torsion angles [°] for Fmoc-TOAC-L-(αMe)-Val-NH*t*Bu (**1**) and Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**) with esds in parentheses.

Torsion angle	Dipeptide **1**	Pentapeptide **15**
θ^2	−116.7(3)	−152.8(3)
θ^1	−175.8(2)	178.7(2)
ω_0	−178.4(2)	−157.9(2)
ϕ_1	−59.5(3)	−62.8(3)
ψ_1	−31.7(3)	−32.9(3)
ω_1	−173.6(2)	−173.1(2)
ϕ_2	−57.6(3)	−54.8(3)
ψ_2	−32.4(4)	−28.2(3)
ω_2	−175.5(3)	−174.2(2)
ϕ_3		−50.5(3)
ψ_3		−36.4(3)
ω_3		−168.8(2)
ϕ_4		−55.9(3)
ψ_4		−29.9(3)
ω_4		−175.0(2)
ϕ_5		−61.8(3)
ψ_5		−31.7(4)
ω_5		164.9(4)

O^{δ}-N^{δ}-C^{γ} bond angles (in the range 116.0(2)–119.7(2)°), and the internal bond angle at the N^{δ} atom (124.6(2)° for **1** and 123.1(2)° for **15**) compare well with those published for other TOAC residues.

All L-(αMe)Val and TOAC residues of the two peptides are found in the right-handed helical region of the conformational map. The average φ and ψ values are −57° and −32°, respectively,[33] and are quite close to those typical for a right-handed 3_{10}-helix (−57, −30°).[4] Dipeptide amide **1** forms a regular type-III β-turn.[1, 3] The single $1 \leftarrow 4$ intramolecular C=O···H–N hydrogen bond has the NT–O0 distance at the upper limit of the expected range (2.8–3.2 Å).[34–36] The pentapeptide amide **15** is folded in a right-handed 3_{10}-helical structure stabilized by four consecutive $1 \leftarrow 4$ intramolecular C=O···H–N hydrogen bonds (the N–O distances are in the range 3.049(4)–3.235(3) Å).

All urethane, peptide and amide groups of the two compounds are in the *trans* disposition. Interestingly, in peptide **15** the N-terminal urethane ω_0, the peptideω_3 and the C-terminal amideω_5 torsion angles deviate significantly ($|\Delta\omega| > 11°$) from the *trans* planarity (180°). In both compounds the Fmoc-urethane group (θ^1 and ω_0 torsion angles) is in the usual *trans*,*trans* conformation.[27]

Relevant torsion angles for the TOAC piperidine ring[7–10, 31, 32] of the two peptides are listed in Table 4. Two sets of values are observed for the torsion angles relating the piperidine ring to the peptide chain N-C^{α}-C^{β}-C^{γ} (χ^1) and C′-C^{α}-C^{β}-C^{γ}. In the conformation 60°, −60°/180°,180° adopted by dipeptide **1**, the α-amino group occupies the axial position and the α-carboxyl group occupies the equatorial position.

Table 3. Intra- and intermolecular hydrogen bond parameters for Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**).

	Type of H-bond	Donor (D)	Acceptor (A)	Symmetry operation	Distance [Å] D···A	Distance [Å] H···A	Angle [°] D–H···A
1	intramolecular	NT–H	O0	x, y, z	3.255(3)	2.459(2)	154.1(2)
	intermolecular	N1–H	O2	$x-\frac{1}{2}, \frac{3}{2}-y, 2-z$	3.205(3)	2.610(2)	127.3(2)
15	intramolecular	N3–H	O0	x, y, z	3.151(4)	2.305(4)	168.0(3)
		N4–H	O1	x, y, z	3.235(3)	2.380(3)	173.0(3)
		N5–H	O2	x, y, z	3.163(3)	2.329(3)	163.7(3)
		NT–H	O3	x, y, z	3.049(4)	2.214(4)	163.9(3)
	intermolecular	N1–H	O3D	$1-x, y-\frac{1}{2}, 1-z$	2.976(4)	2.136(4)	165.3(3)

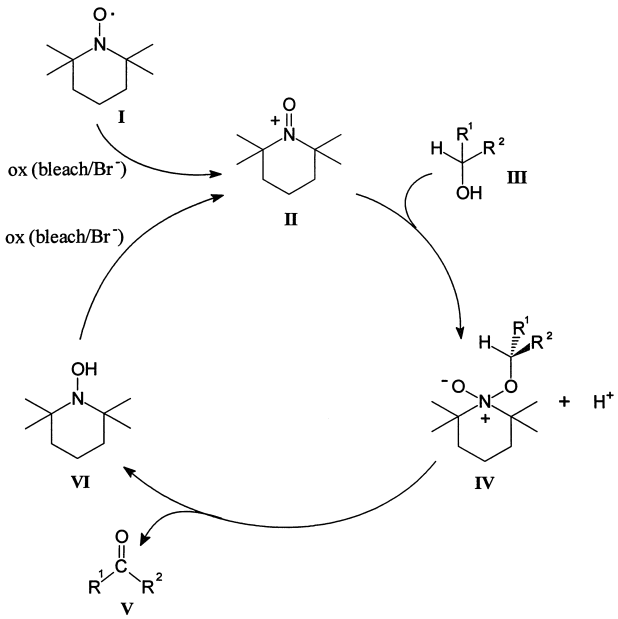
Table 4. Relevant torsion angles [°] for the piperidine ring of the TOAC residue in Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**).

Torsion angle	Dipeptide **1**	Pentapeptide **15**
N-C$^\alpha$-C$^\beta$-C$^\gamma$ (χ^1)	–73.6(3)	–145.3(2)
	69.7(3)	87.0(3)
C′-C$^\alpha$-C$^\beta$-C$^\gamma$	167.6(2)	96.1(3)
	–167.6(2)	–151.2(3)
O$^\delta$-N$^\delta$-C$^\gamma$-C$^\beta$	–162.1(3)	160.8(3)
	160.9(3)	148.1(3)
C$^\gamma$-C$^\beta$-C$^\alpha$-C$^{\beta'}$ (θ^1, $\theta^{1'}$)	51.0(3)	–24.1(3)
	–52.4(3)	–31.9(3)
C$^\alpha$-C$^\beta$-C$^\gamma$-N$^\delta$ (θ^2, $\theta^{2'}$)	–43.6(3)	51.3(3)
	45.8(3)	57.3(3)
C$^\beta$-C$^\gamma$-N$^\delta$-C$^{\gamma'}$ (θ^3, $\theta^{3'}$)	39.0(4)	–23.6(4)
	–40.1(4)	–27.4(4)

In the other conformation 90°, –150°/–150°,90°, exhibited by pentapeptide **15**, the α-amino and α-carboxyl substituents are located at intermediate positions between axial and equatorial, the latter being characterized by the torsion angles 180°,180°/–60°,60°. Except for the θ^1, $\theta^{1'}$, θ^3 and $\theta^{3'}$ torsion angles of pentapeptide **15**, all other endocyclic torsion angles for the two peptides show values in the $\pm 39-58°$ range, close to those of piperidine and cyclohexane. These findings indicate that the piperidine ring of pentapeptide **15** is more flattened than that of dipeptide **1** in both the C$^\alpha$ and N$^\delta$ regions. The piperidine ring of dipeptide **1** adopts an approximate *chair* conformation with the following puckering parameters:[37] $Q_T = 0.46(1)$ Å, $\varphi_2 = 172(2)°$ and $\theta_2 = 13(1)°$, while that of pentapeptide **15** adopts an approximate *twist-boat* conformation, with one C$^\beta$ atom above and the other C$^\beta$ atom below the average plane of the ring, with the following puckering parameters: $Q_T = 0.66(1)$ Å, $\varphi_2 = 91(1)°$ and $\theta_2 = 92(1)°$. The angle between the N$^\delta$–O$^\delta$ bond and the C$^\gamma$-N$^\delta$-C$^{\gamma'}$ plane is 18.8(3)° for dipeptide **1** and 3.9(3)° for pentapeptide **15**. Therefore, the C(C)-N-O group adopts a pyramidal shape in the *chair* form of dipeptide **1** only. The angles between the normals to the average planes of the Fmoc and TOAC rings are 35.4(1)° in dipeptide **1** and 56.8(1)° in pentapeptide **15**.

In the crystals of dipeptide **1**, molecules pack in the unit cell in rows parallel to the *a* direction through weak (urethane) N1–H···O2=C2 (amide) hydrogen bonds. The peptide N2–H moiety does not seem to be involved in the hydrogen bonding array. Along the *b* direction, the helical molecules of pentapeptide **15** are connected by hydrogen bonds between the urethane N1–H donor and the nitroxyl O3D–N3D acceptor groups.

Enantioselective chemical oxidation of a secondary alcohol: Efficient methods for the chemical oxidation of alcohols mediated by a nitroxyl derivative have been reported.[38–40] The one-electron oxidation of a nitroxyl compound (**I**) by OBr^- (arising from the reaction of bleach with a bromide ion) affords an *N*-oxoammonium cation (**II**) to which a secondary alcohol, such as 1-phenylethanol (**III**), can add (Scheme 1). The resulting intermediate **IV** undergoes elimination through a cyclic dipolar mechanism, thereby producing the ketone **V** and the hydroxylamine derivative **VI**. Re-oxidation of **VI** to **II** completes the catalytic cycle. Enantioselective chemical oxidations of racemic secondary alcohols, using optically active nitroxyl radicals as catalysts, have recently been performed.[41–43] However, in general, the synthesis of the chiral nitroxyl compound is very laborious and some of these catalysts undergo facile racemization.[44]



Scheme 1. Catalytic cycle for the oxidation of a secondary alcohol using a bulk oxidant and a nitroxyl catalyst.

The results of the enantioselective chemical oxidation of racemic 1-phenylethanol to acetophenone, by means of the chiral, non-racemizable TOAC/(αMe)Val peptides as catalysts, are listed in Table 5. As expected, the enantiomeric dipeptide catalysts **1** and **2** give the same enantiomeric excess of recovered alcohol [the (*R*)-alcohol enantiomer is oxidized

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Table 5. Enantioselective chemical oxidation of racemic 1-phenylethanol with the TOAC/(αMe)Val peptide catalysts.

	Catalyst	*ee* [%]	Configuration[a]	Conversion (*C*) [%]	*S*[b]
1	Fmoc-TOAC-L-(αMe)Val-NH*t*Bu	67	*S*(−)	83	2.3
2	Fmoc-TOAC-D-(αMe)Val-NH*t*Bu	67	*R*(+)	82	2.3
3	Fmoc-TOAC-L-(αMe)Val-NH*i*Pr	69	*S*(−)	82	2.4
4	Fmoc-TOAC-L-(αMe)Val-NHEt	52	*S*(−)	83	1.9
5	Fmoc-TOAC-L-(αMe)Val-NHMe	50	*S*(−)	84	1.8
6	Z-TOAC-L-(αMe)Val-NH*t*Bu	75	*S*(−)	82	2.7
7	Boc-TOAC-L-(αMe)Val-NH*t*Bu	70	*S*(−)	82	2.5
8	Boc-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu	62	*S*(−)	79	2.1
9	Boc-TOAC-[L-(αMe)Val]$_3$-NH*t*Bu	74	*S*(−)	81	2.7
10	Boc-TOAC-[L-(αMe)Val]$_4$-NH*t*Bu	68	*S*(−)	81	2.4
11	Ac-TOAC-L-(αMe)Val-NH*t*Bu	60	*S*(−)	85	2.0
12	Fmoc-TOAC-L-Val-NH*t*Bu	33	*S*(−)	81	1.5
13	Fmoc-TOAC-L-(αMe)Val-OMe	13	*R*(+)	82	1.2
14	Fmoc-L-(αMe)Val-TOAC-NH*t*Bu	10	*R*(+)	83	1.1
15	Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu	36	*S*(−)	80	1.6
16	Fmoc-[L-(αMe)Val]$_3$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu	38	*R*(+)	79	1.6
17	*cyclo*[TOAC-L-(αMe)Val]	18	*S*(−)	81	1.2

[a] Of recovered alcohol. [b] $S = \ln[(1-C)(1-ee)]/\ln[(1-C)(1+ee)]$.

preferentially by the L-(αMe)Val peptide]. Therefore, it is possible to get a mixture enriched in either one of the two enantiomeric alcohols by using the catalyst of the appropriate chirality. The highest enantiomeric excesses (*ee*) and selectivity factors (*S*) were obtained when both N- and C-blocking groups were highly lipophilic (hydrophobic) (compare the catalyst series **1**, **3–5** and **6**, **7**, **11**). Replacement of the turn-inducer (αMe)Val (in catalyst **1**) with Val, which is more prone to adopt partially extended conformations, results in a dramatic decrease in the efficiency of the kinetic resolution (see catalyst **12**). An analogous result is obtained when the alkylamide moiety at the C-terminus (of catalyst **1**) is replaced by an ester functionality, which precludes intramolecularly H-bonded β-turn formation (see catalyst **13**). Interestingly, in this case the final mixture was enriched in the (*R*)-alcohol and not in the (S)-alcohol as was found for catalysts **1** and **3–12**. Also, the β-turn-forming (αMe)Val/TOAC sequence permutation (catalyst **14**) produced a low *R* enantiomeric excess. Lengthening the main-chain of the peptide with two, three and four L-(αMe)Val residues at the C-terminus (catalysts **8–10**), does not improve enantioselectivity with respect to the parent dipeptide **7**. Modest enantioselectivities were observed for the long penta- and hexapeptides with an internal TOAC residue (catalysts **15** and **16**). The TOAC-based cyclic dipeptide **17** was a poor catalyst.

The main conclusion drawn from this investigation is that the best peptide catalysts investigated have large lipophilic (hydrophobic) blocking groups at either terminus, an N-terminal TOAC residue and are folded in a stable, intramolecularly C=O···H–N hydrogen-bonded β-turn conformation.

Enantioselective electrochemical oxidation of a secondary alcohol: Some results on the electrochemical kinetic resolution of racemic secondary alcohols have been reported, either by use of synthetically complex, optically active nitroxyl mediators or nitroxyl-modified electrodes.[45–48] Aprotic media and divided cells are usually employed in these experiments, probably because the *N*-oxoammonium salts are labile in water. Only recently, however, an aqueous/organic, two-phase system has efficiently been exploited in enantioselective electro-oxidations.[49] Such a procedure, in which electrolysis is carried out in an undivided cell under constant current conditions, combines the advantages of practical simplicity and low environmental stress. Indeed, in this case the use of highly concentrated supporting electrolyte solutions (quaternary ammonium salts), which are required to carry out the process in divided cells, is unnecessary. Very recently, a further improvement has been described in which the organic solvent is replaced by an aqueous silica-gel disperse system.[50]

The indirect electro-oxidation in an undivided cell is based on a CH_2Cl_2/H_2O two-phase system employing two mediators. A systematic investigation of the different factors that affect the efficiency of the oxidation under these conditions has been carried out.[51] The reaction conditions have been optimized by using a concentration of NaBr in the aqueous solution >15w%, a pH of 8.6 (saturated $NaHCO_3$ buffer solution), a concentration of the nitroxyl mediator in the organic phase ≥1 mol% with respect to the alcohol and a current density of 10–140 mA cm^{-2} with a Pt electrode. It has been observed, however, that the current efficiency is not unitary and that oxidations are essentially completed after consumption of 2.2–2.6 F mol^{-1} of charge.

In our electrochemical experiments we used a 20 w% NaBr aqueous solution saturated with $NaHCO_3$, a nitroxyl/alcohol ratio of about 0.017 and a current density of 10 mA cm^{-2}. Bromide ions in the aqueous solution undergo electro-oxidation at the Pt anode and subsequently oxidize the nitroxyl in the organic phase; electron transfer takes place at the CH_2Cl_2/H_2O interface. The active form of the mediator can then oxidize the racemic alcohol. The use of a two-phase electrochemical system allowed us to carry out the electrochemical experiments under conditions that better resembled those described above for the chemical oxidations.

The catalytic behaviour of the Boc-TOAC-L-(αMe)Val-NH*t*Bu mediator was analyzed at 0 °C by measuring the *ee* of the alcoholic mixture at different degrees of conversion (*C*). The results (Table 6) display some scatter of the selectivity factor (*S*) values. However, a reasonably good plot of *ee* against *C* was obtained leading to a calculated average *S* value of 1.8. As reported by Tanaka and co-workers,[49] an increase

Table 6. Enantioselective electrochemical oxidation of racemic 1-phenylethanol with the TOAC/(αMe)Val peptide catalysts.

	Catalyst	*ee* [%]	Configuration[a]	Conversion (*C*)[%]	*S*[b]
2	Fmoc-TOAC-D-(αMe)Val-NH*t*Bu	25	*R*(+)	66	1.6
7	Boc-TOAC-L-(αMe)Val-NH*t*Bu	15	*S*(−)	53	1.5
		25	*S*(−)	59	1.8
		52	*S*(−)	76	2.1
		53	*S*(−)	84	1.8
13	Fmoc-TOAC-L-(αMe)Val-OMe	2	*R*(+)	55	1.1

[a] Of recovered alcohol. [b] $S = \ln[(1-C)(1-ee)]/\ln[(1-C)(1+ee)]$.

of selectivity can be induced by decreasing the temperature. Indeed, we obtained an *S* value of 3.1 at −10 °C. The trend of selectivity for the three mediators tested resembles that resulting from the corresponding chemical oxidations, but in general a lower efficiency of the alcohol resolution was observed under the electrochemical conditions.

Conformational energy calculations: To understand the factors playing a role in the stereoselectivity of 1-phenylethanol oxidation catalyzed by chiral peptides that contain TOAC residues, we performed energy minimization calculations. As a first step, we investigated the conformational behaviour of Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and compared the results with those of the simple dipeptide reference compound Ac-L-Ala-L-Ala-NH*t*Bu. Our data indicated that the minimized Fmoc-TOAC- L-(αMe)Val-NH*t*Bu (**1**) backbone structure ($\varphi_1 = -62.1°$, $\psi_1 = -14.1°$; $\varphi_2 = -45.8°$, $\psi_2 = -38.5°$) is characterized by a type-III β-turn conformation, closely resembling the crystal-state structure described above, with a root-mean-square deviation for all heavy atoms of 0.53 Å. In contrast, the analysis of the minimized structure of the Ac-L-Ala-L-Ala-NH*t*Bu revealed the absence of the β-turn conformation which was instead replaced by a more stable ($\Delta E =$ 1.5 Kcal mol^{-1}) structure with two consecutive γ-turns ($\varphi_1 = 69.9°$, $\psi_1 = -63.9°$; $\varphi_2 = -83.8°$, $\psi_2 = 82.8°$).[2] These findings agree with the known strong preference of the two sterically demanding, C^{α}-tetrasubstituted α-amino acids in the Fmoc-TOAC-L-(αMe)Val-NH*t*Bu dipeptide for the β-turn conformation.

Subsequently, we obtained rigid rotor maps for the torsion angles around the N–O and O–C bonds of the diastereomeric intermediates **IV** (Scheme 1) between (*R*) [and (*S*)]-1-phenylethanol and Fmoc-TOAC-L-(αMe)Val-NHtBu. Models were constructed for the diastereomers considering the two possible directions of addition of alcohol (**III**) to the TOAC *N*-oxoammonium double bond of (**II**). The maps show that the two diastereomeric intermediates **IV**, with addition of the alcohol to the TOAC ring on the side *away* from the Fmoc group, exhibit the same energy (within experimental error). In contrast, the two diastereomeric intermediates (**IV**) from addition to the ring side *close* to the Fmoc group are more stable than those discussed above, and the intermediate from (*R*)-1-phenylethanol presents a conformation with an energy 4.4 Kcal mol^{-1} lower than that computed for the other diastereomer. These findings are confirmed by the minimum-energy conformations obtained after a full minimization procedure of the low-energy conformations for the two models. Figure 5 illustrates the stereoviews for the minimum energy structures of the "*close*" diastereomeric intermediates. Our computational analysis indicates that the phenyl group of (*R*)-1-phenylethanol (Figure 5B) is involved in a favourable, intramolecular, edge-to-face, aromatic–aromatic interaction with the Fmoc moiety of the dipeptide catalyst (the angle between the normals to the planes of the phenyl and fluorenyl systems is 115°).[52] We believe this type of interaction is important for high diastereoselectivity. Interestingly, this finding is in excellent agreement with the conclusions recently put forward to explain the unexpected diastereoselectivity of the Sharpless asymmetric dihydroxylation of allyl D-xylosides,[53] which highlight the essential role of aromatic or, more

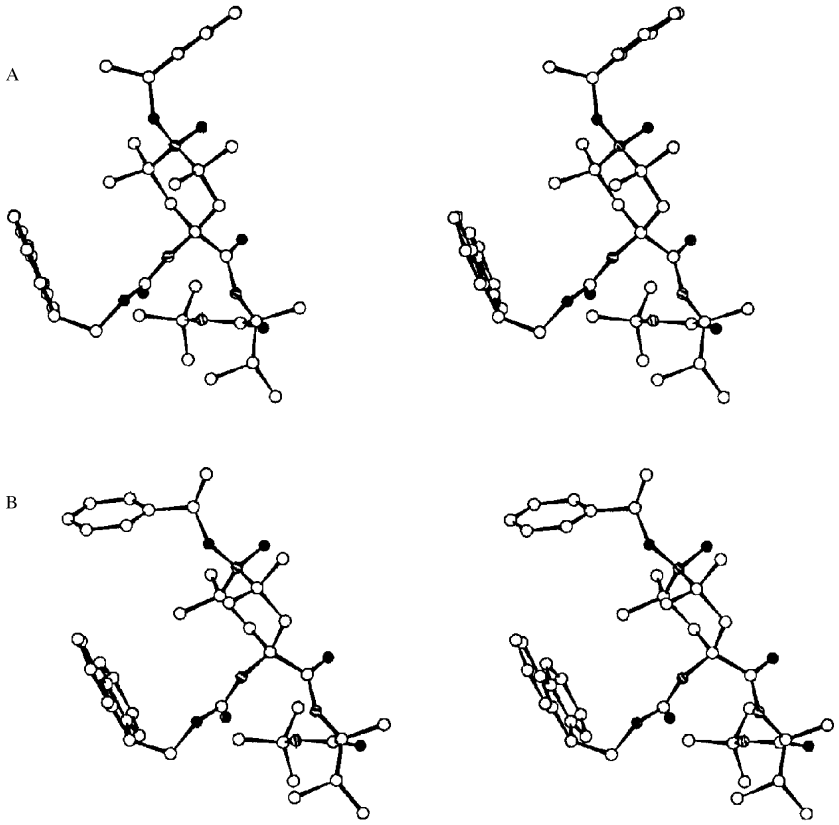

Figure 5. Stereoviews of the minimum energy structures of the diastereomeric intermediates **IV** (Scheme 1) between Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and A) (*S*)-1-phenylethanol and B) (*R*)-1-phenylethanol, with addition of the alcohol on the TOAC ring side *close* to the Fmoc group.

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generally, large lipophilic (hydrophobic) moieties of the substrate and catalyst in interactions leading to preferential stabilization of one diastereomeric intermediate.

Conclusion

Considerable recent interest has been focused on the enantioselective oxidation of racemic alcohols with chiral nitroxyl catalysts.[41–51] In this paper we have shown that TOAC-based, conformationally rigid, stable β-turn forming, very short peptides are reasonably valuable catalysts in chemical oxidations and, although less efficient, in electrochemical oxidations as well. Longer helical structures are not required. Large aromatic or aliphatic N- and C-blocking groups and the N-terminal positioning of TOAC have a positive effect on the efficiency. We have explained these experimental findings on the basis of computed models for the intermediate adducts between the chiral dipeptide amide *N*-oxoammonium mediator and the enantiomeric secondary alcohol substrates. The identity of the fast reacting alcohol enantiomer has been correctly predicted.

However, it is clear that the experimental selectivity factors (in the range 2.3–2.7 for our best catalysts, which corresponds to a free-energy difference of only about 0.5 kcal mol^{-1}), although encouraging for a first-generation peptide system, are far from optimal, particularly in view of the high conversion (81–83%) considered in our enantioselective chemical oxidation experiments. It is our contention that these results are related to the only chiral centre of the catalyst, that is, the (αMe)Val α-carbon atom, being too far removed from the TOAC oxidation site. Current attempts in our laboratories are aimed at designing and synthesizing chiral TOAC analogues.

Experimental Section

Materials: The physical properties and analytical data for the new TOAC and (αMe)Val derivatives and peptides are listed in Table 1. The synthesis and characterization of Fmoc-[L-(αMe)Val]$_n$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**: $n=2$; **16**: $n=3$) have already been reported.[11]

FTIR spectroscopy: FTIR absorption spectra were recorded with a Perkin–Elmer 1720X FTIR spectrophotometer, nitrogen flushed, with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with CaF_2 windows and path lengths of 0.1, 1.0 and 10 mm were used. Spectrograde [^{2}H]chloroform (99.8% ^{2}H) was obtained from Fluka.

X-ray diffraction: Crystals of Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**) were grown by slow evaporation from ethyl acetate/petroleum ether and methanol, respectively. Diffraction data were collected on a Philips PW1100 diffractometer. Crystallographic data are summarized in Table 7. Both structures were solved by direct methods with the SHELXS97[54] or SHELXS96[55] programs. Refinements were carried out on F^2 by full-matrix block least-squares, using all data, by application of the SHELXL97[56] and SHELXL93[57] programs, respectively, with all non-H atoms anisotropic and allowing their positional parameters and the anisotropic displacement parameters to refine at alternate cycles.

H atoms of both compounds were calculated at idealized positions. During the refinement they were allowed to ride on their carrying atom with U_{iso} set equal to 1.2 (or 1.5 for methyl groups) times the U_{eq} of the parent atom.

Table 7. Crystallographic data and structure refinement for Fmoc-TOAC-L-(αMe)Val-NH*t*Bu (**1**) and Fmoc-[L-(αMe)Val]$_2$-TOAC-[L-(αMe)Val]$_2$-NH*t*Bu (**15**).

	Peptide **1**	Peptide **15**
formula	$C_{35}H_{49}N_4O_5$	$C_{53}H_{82}N_7O_8$
M_r	605.8	945.3
T [K]	293(2)	293(2)
λ [Å]	1.5418	1.5418
crystal system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_1$
a[Å]	12.120(3)	11.702(2)
b [Å]	14.743(3)	19.410(3)
c [Å]	19.874(4)	12.731(2)
α [°]	90°	90°
β [°]	90°	108.61(4)°
γ [°]	90°	90°
V [Å^3]	3551(1)	2740(1)
Z	4	2
ρ_{calcd} [Mg m^{-3}]	1.13	1.15
μ [mm^{-1}]	0.61	0.62
$F(000)$	1308	1026
crystal size [mm]	0.30 × 0.25 × 0.20	0.40 × 0.30 × 0.20
θ range [°]	3.73–59.99	3.66–59.99
index ranges	$-1 \le h \le 13$	$-13 \le h \le 12$
	$0 \le k \le 16$	$0 \le k \le 21$
	$0 \le l \le 22$	$0 \le l \le 14$
reflections collected	3282	4409
independent reflections	3244 [R(int) = 0.030]	4203 [R(int) = 0.049]
data/restraints/parameters	3244/5/384	4198/9/649
goodness-of-fit (on F^2)	0.94	1.01
final R indices [$I > 2\sigma(I)$]	$R1 = 0.040$, $wR2 = 0.099$	$R1 = 0.038$, $wR2 = 0.101$
R indices (all data)	$R1 = 0.060$, $wR2 = 0.109$	$R1 = 0.047$, $wR2 = 0.106$
largest diff. peak/hole [e Å^{-3}]	0.166/−0.114	0.185/−0.158

The aromatic rings of the Fmoc moiety of dipeptide amide (**1**) were constrained to the idealized geometry and restraints were applied to the C–C bond lengths of the C-terminal *tert*-butyl moiety. The atoms of the C-terminal *tert*-butyl group of pentapeptide amide (**15**) were refined on two sets of positions (CT1, CT2, CT3, CT4 and CT1′, CT2′, CT3′, CT4′, respectively) with a population parameter of 0.50 for each set.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 163805 and CCDC 163806. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK CB2 1EZ, (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Chemical oxidation: The experimental procedure was that described by Anelli et al.[38] The oxidation was performed by using the NaOCl/KBr system with 1.5 mol equivalents of the nitroxyl catalyst in a rapidly stirred, two-phase CH_2Cl_2/H_2O (pH 8.6) mixture at 0 °C. The efficiency of the resolution was given by the selectivity factor $S = \ln[(1-C)(1-ee)]/\ln[(1-C)(1+ee)]$, in which *ee* is the fractional enantiomeric excess and C is the conversion.[58]

Quantitative analyses of 1-phenylethanol and acetophenone were carried out with a Varian model 3700 gas chromatographic (GC) apparatus based on a 20-M 15% Carbowax column supported on Chromosorb WAW-DMCS. 1-Phenylethanol *ee* was determined by GC analysis with a C. Erba model HRGC apparatus based on a Chiraldex GTA (30 m × 0.32 mm) column.

Electrochemical oxidation: The instrumentation employed for the electrochemical measurements was an EG&G-PARC 173/179 potentiostat-digital coulometer. Electrochemical measurements were performed in an undivided glass cell, with thermostatic control at the required temperature. The cell was equipped with a thermometer and two Pt electrodes. The working electrode was a Pt plate, having a surface area of 2 cm^2, whereas the counter electrode was a large surface Pt grid. Argon was continuously bubbled into the aqueous electrolytic mixture during the experiments to facilitate the removal of hydrogen evolved at the cathode.

Typically, the experiments were carried out according to the following procedure: A mixture of racemic 1-phenylethanol (0.83 mmol) and the nitroxyl mediator (0.014 mmol) was dissolved in CH_2Cl_2 (3.5 mL) and aqueous NaBr (9 mL, 20w%), saturated with $NaHCO_3$, was added. The two Pt electrodes were immersed in the upper aqueous layer of the resulting two-phase solution, and the mixture was electrolyzed under a constant current flow of 20 mA with moderate magnetic stirring. The electrolysis was interrupted after consumption of 2 F mol^{-1} (usually, after ca. 2 h) and the mixture was stirred for a further hour to allow complete consumption of the reactive species. Reaction mixture work-up, quantitative analysis and *ee*-determination were carried out as described above for the chemical oxidation.

Conformational energy calculations: The conformational spaces for the diastereomeric intermediates **IV** (Scheme 1) between (*R*) [and (*S*)]-1-phenylethanol and Fmoc-TOAC-L-(αMe)Val-NH*t*Bu were mapped by calculating the energy at 5° intervals for the torsion angles around the N−O and O−C bonds. Minimum-energy conformations were obtained in the low-energy regions located in the above search, minimizing the energy with respect to all geometrical parameters, by using the conjugate algorithms. The structure of Fmoc-TOAC-L-(αMe)Val-NH*t*Bu used in the computational analysis was that previously obtained from the X-ray diffraction analysis described in this paper. The geometry of the acetamido group of the reference peptide Ac-L-Ala-L-Ala-NH*t*Bu was that proposed by Scheraga and co-workers.[59] The geometrical parameters for the L-Ala residue were those reported in the InsightII/Biopolymers module.[60] Energy minimization calculations were performed with the Insight/DISCOVER package[60] with the Consistent Valence Force Field (CVFF).[61–63] Minimum-energy conformations were obtained by minimizing the energy with respect to all geometrical parameters using the conjugate algorithms. All conformational energies are expressed as $\Delta E = E - E_0$, in which E_0 is the energy of the most stable conformation. All computations were performed on a Silicon Graphics O2 workstation of the Biocrystallography Research Centre, CNR, at the University of Naples "Federico II".

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