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Pseudopeptide fragments and local structures induced by an α-aminoxy acid in a dipeptide

Laurent Thévenet,[a] Régis Vanderesse,[a,*] Michel Marraud,[a] Claude Didierjean [b] and André Aubry [b]

[a] *Laboratoire de Chimie Physique Macromoléculaire, CNRS-INPL, Groupe ENSIC, BP 451, 54001 Nancy, France*
[b] *Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, associé au CNRS, Université Henri Poincaré, BP 239, 54506 Vandœuvre, France*

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Abstract

An α-aminoxy acid residue has been introduced by liquid-phase procedures in a model dipeptide, by means of the amidoxy (CO–NH–O), oxime (CH=N–O) and hydroxylamine (CH_2–NH–O) pseudopeptide link. The structural properties induced by the three amide surrogates, which are not protonated at the physiological pH, have been studied in organic solution. In all three cases, the α-oxygen interacts with the adjacent amide NH to close a five-membered cycle. The amidoxy link gives rise to a very stable γ-like folded structure and the *cis*-oxime link to a β-like folded structure.

When incorporated in a peptide by N^{β}-acylation, the α-hydrazino acids $N^{\beta}H_2$–$N^{\alpha}H$–CHR–CO_2H and α-aminoxy acid $N^{\beta}H_2$–O^{α}–CHR–CO_2H have been shown to induce quite similar γ-like turns,[1,2] which are more stable than the parent γ-turn in the peptides,[3] due to the presence of a double hydrogen bond involving both the hydrazide or amidoxy carbonyl and the α-nitrogen or oxygen. As the optically active α-aminoxy acids[4] are more easily prepared than the α-hydrazino acids,[5] we have studied the structural properties induced by coupling an α-aminoxy acid residue either to a peptide carboxyl to give the amidoxy Cα–CO–NH–O–Cα link, or to a peptide aldehyde carbonyl to give the oxime Cα–CH=N–O–Cα link which has been further reduced into the hydroxylamine Cα–CH_2–NH–O–Cα link (Scheme 1).[7]

The commercially available α-aminoxyacetic acid was treated with Boc_2O to give **1**, and the *N*-isopropyl-amide **2** was obtained by using the mixed anhydride procedure. The Boc group was eliminated with TFA in CH_2Cl_2. The resulting amino terminus was coupled to Boc-Pro-OH to obtain **3a** in moderate yield due to the weaker nucleophilicity of the hydroxylamine nitrogen compared with the peptide amino terminus, although strong acylating conditions (TBTU/HOBt) were used. The aminoxy group was also coupled to the Boc-Pro-H aldehyde[6] in the presence of AcO^-Na^+ and molecular sieves to create the

* Corresponding author. Tel: +33 (0)3 83 17 52 04; fax: +33 (0)3 83 37 99 77; e-mail: regis.vanderesse@ensic.inpl-nancy.fr (R. Vanderesse)

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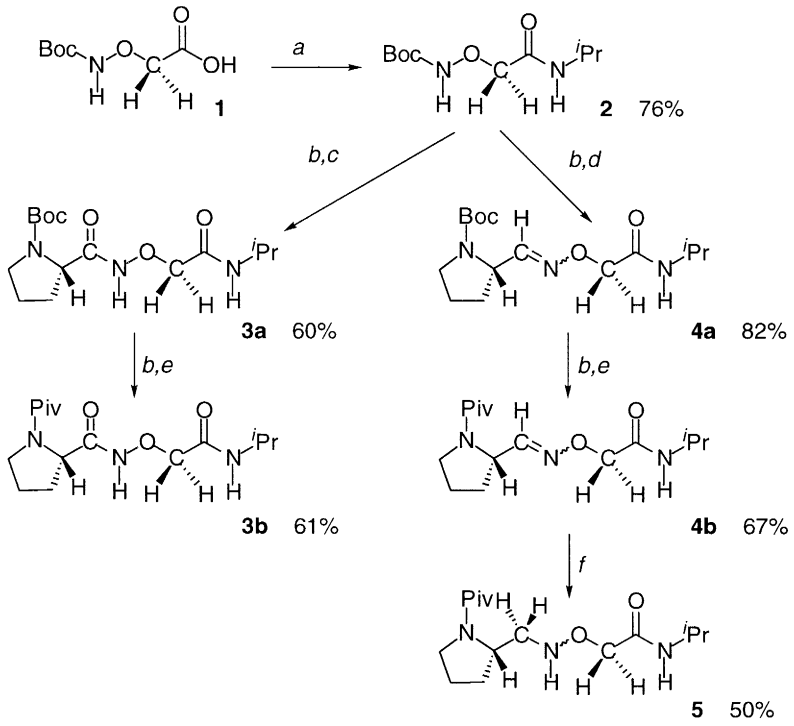

Scheme 1. (a) (1) *i*BuOCOCl/NMM/THF/−18°C/15 min, (2) NH_2*i*Pr/THF/overnight; (b) TFA/CH_2Cl_2/30 min; (c) Boc-Pro-OH/TBTU/HOBt/DIEA/rt/overnight; (d) Boc-Pro-H[6]/AcO^-Na^+/EtOH/ molecular sieves/rt/overnight; (e) PivCl/DIEA/$CHCl_3$/0°C/2 h; (f) $NaBH_3CN$ (10 equiv. portionwise)/MeOH–AcOH (1–5%)/rt/3 days[8]

oxime link in **4a**. The Piv group in **3b** and **4b** was substituted for the Boc group in order to suppress the *cis–trans* isomerism of the Pro-preceding amide bond. Various catalytic hydrogenation conditions and reducing reagents were tested for obtaining **5** from **4b**, and a large excess of $NaBH_3CN$ was found to give the best yield, without cleavage of the N–O bond, although the reaction proceeded very slowly to a 50% yield in 3 days.

The crystal structure of **2**,[9] where the two independent molecules A and B per asymmetric unit have very similar conformations, confirms the implication of the amidoxy link in a γ-like folded structure[3] where the C-terminal NH interacts both with the Boc-CO carbonyl (N···O=2.90 and 3.14 Å) and the hydroxylamide α-oxygen ($N\cdots O^{\alpha}$=2.76 and 2.75 Å) (Fig. 1a). In CH_2Cl_2, the C-terminal NH in **2** exhibits a major low stretching contribution at 3315 cm^{-1} typical of a strong intramolecular hydrogen bond, also denoted by the very low solvent sensitivity of the NH proton resonance (Table 1). The same observation holds true for **3b** so that the γ-like folded structure seems to be an intrinsic property of the amidoxy peptide fragment, independently of the preceding amino acid residue. The Xaa–NH bond in **3b** also gives rise to two nearly equally intense free (3340 cm^{-1}) and bonded (3215 cm^{-1}) stretching absorptions while the low $\Delta\delta$ value suggests that the Pro residue is γ-folded in a part of the molecules. In DMSO, both NH stretching absorptions are shifted to very low frequencies while the Piv-CO stretching is significantly shifted to high frequencies, indicating that both γ- and γ-like turns in **3b** are disrupted by DMSO solvation.

Due to the absence of the Pro carbonyl in **4b** and **5**, the oxime and hydroxylamine links destabilize the γ-like turn, and the NH(*i*Pr) stretching absorption is thus shifted to higher frequencies at 3421 and 3416 cm^{-1} in CH_2Cl_2, respectively. However, the NH(*i*Pr) solvent sensitivity $\Delta\delta$ is less intense than

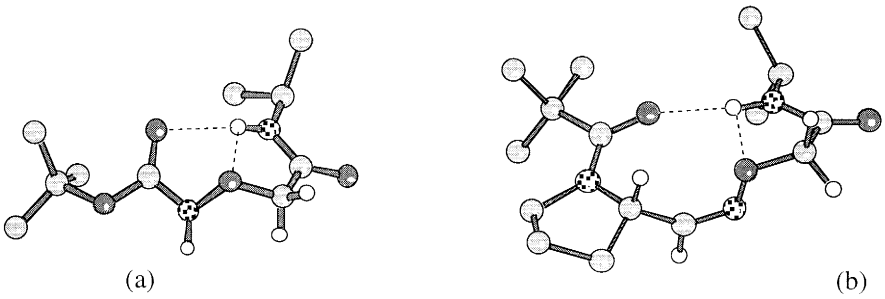

Fig. 1. γ-Like folded crystal structure adopted by **2**, molecule A, (a)[9] and modelled β-like folded structure adopted by **4b** with the *cis*-oxime bond (b)

Table 1
NH and Boc/Piv-CO stretching frequencies (n, cm^{-1}) in CH_2Cl_2 and DMSO,[a] and chemical shift difference (Δδ, ppm) for the NH proton resonances in $CDCl_3$ and DMSO-d_6

	"Gly"-*NH*			*NH*(*i*Pr)			Boc/Piv-*CO*	
	Δδ	ν(CH_2Cl_2)	ν(DMSO)	Δδ	ν(CH_2Cl_2)	ν(DMSO)	ν(CH_2Cl_2)	ν(DMSO)
2	2.92	3339	3150[b,br]	-0.06	3418[w] 3315[s]	3289[br]	1739	1723
3b	1.34	3340[m] 3215[m]	3156[b,br]	0.23	3415[w] 3304[s]	3270[br]	1620[w] 1600[s]	1620
4b				1.30[c] 0.16[d]	3421[c,m] 3343[d,m]	3335[d,s] 3275[c,br]	1616	1613
5	0.59	3332	3270[br]	0.66	3416[s]	3270[br]	1608	1611

[a] Weak (w), medium (m), strong (s), broad (br) absorption.
[b] This broad absorption is superimposed on the amide B absorption for the *NH*(*i*Pr) group.
[c] Assigned to the *trans*-conformation of the oxime link.
[d] Assigned to the *cis*-conformation of the oxime link.

expected for a free amide NH as illustrated by the 'Gly'-NH resonance for **2** (Table 1). The Δδ value and the NH(*i*Pr) stretching frequency for **4b** and **5** are rather close to those found for MeO–CH_2–CO–NH*i*Pr where the NH bond interacts with the ether oxygen to close a five-membered pseudocycle.[10] We therefore conclude that the same interaction is retained in the oxime dipeptide **4b** and the hydroxylamine dipeptide **5**. A second, broad absorption at 3343 in **4b** denotes the partial existence of an NH(*i*Pr) to Piv-CO interaction (Table 1).

In fact, the split NH(*i*Pr) stretching absorption and proton resonance illustrate the *cis–trans* equilibrium of the oxime link in **4b**. The *cis*-conformer, characterized by the low field Pro-CaH resonance at 5.28 ppm in $CDCl_3$, gives rise to a very small Δδ value and an AB pattern for the 'Gly'-CH_2 protons, typical of a rigid structure with an NH(*i*Pr) to Piv-CO interaction which is shown by IR to be retained in DMSO. The modelled (MM2 program) β-like folded structure of the *cis*-conformer is depicted in Fig. 1b. The medium Δδ value and A2 pattern for the 'Gly'-CH_2 protons are indicative of a flexible, open structure for the *trans*-conformer.

An α-aminoxy acid residue gives rise to the amidoxy CO–NH–O, oxime CH=N–O and hydroxyl-

amine CH_2–NH–O amide surrogates depending on the carboxylic or aldehydic nature of the partner in the peptide chain. The proton resonances for the three pseudopeptide fragments are not modified in the pH range 3–10 in water, indicating that the above groups are not protonated under the physiological conditions. In all three cases, the α-oxygen is weakly hydrogen bonded to the NH of the following amide group so that to close a five-membered cycle. In the amidoxy peptide, the same NH is involved in an additional hydrogen bond with the preceding amidoxy carbonyl, and the resulting bifurcated hydrogen bond stabilizes a folded structure of the γ-like type, quite similar to that already described for hydrazide peptides.[1] The *cis*-oxime link initiates a very stable β-like folded structure in which the NH is also hydrogen bonded to the preceding peptide carbonyl so that it closes an 11-membered pseudocycle.

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7. The following abbreviations are used: Boc, *tert*-butyloxycarbonyl; *i*Bu, isobutyl; *t*Bu, *tert*-butyl; DIEA, diisopropylethylamine; DMF, dimethylformamide; DMSO-d_6, hexadeuterated dimethylsulfoxide; HOBt, *N*-hydroxybenzotriazol; NMM, *N*-methylmorpholine; Piv, pivaloyl; TBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; THF, tetrahydrofuran.
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9. Compound **2**: $P2_1/n$; a=10.435(1) Å, b=10.731(2) Å, c=24.475(3) Å; Z=8, 2 molecules A and B per asymmetric unit; d_{calcd}=1.128 g cm^{-3}; 4860 reflections; R=0.047. The main torsional angles are (molecule A/molecule B): C–O–CO–NH 165°/177°, O–CO–NH–O 158°/162°, CO–NH–O–CH_2 128°/135°, NH–O–CH_2–CO −89°/−74°, O–CH_2–CO–NH 5°/−18°, CH_2–CO–NH–C 179°/−176°.
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