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Crystal Structures of Aza-β^3-peptides, A New Class of Foldamers Relying on a Framework of Hydrazinoturns

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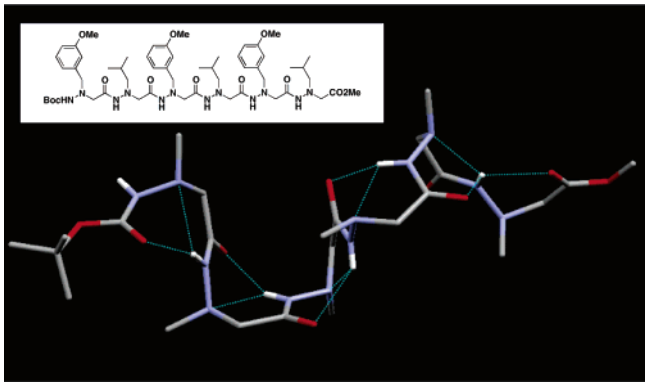

Crystals of aza-β^3-peptides have been obtained. This gives the first opportunity for hydrazino peptides, in the sense of oligomers built exclusively with α-hydrazinoacetic units, to be observed in the solid state. The structures make it clear that the H-bond network developed by aza-β^3-peptides differs radically from those of the corresponding β^3-peptides but strongly resembles that of the α-aminoxy peptides. Our study contributes to the current interest in hydrazino peptides as an extension of the β-peptide concept.

Oligomers of α-amino acid analogues are nowadays the object of intensive synthesis and conformational analysis.[1] The pioneering studies of β-peptides by Seebach and Gellman have stimulated this field of research. They independently demonstrated that these oligomers of β-amino acids could develop numerous stable secondary structures, thus mimicking the behavior of the naturally occurring α-peptides.[2,3] Depending on the sequence, the substitution pattern, and the configuration of the stereocenters, various turn, sheet, and helix types have been observed. From these studies, the general concept of the foldamer,[4] which refers to oligomers with discrete folding propensities, has emerged.

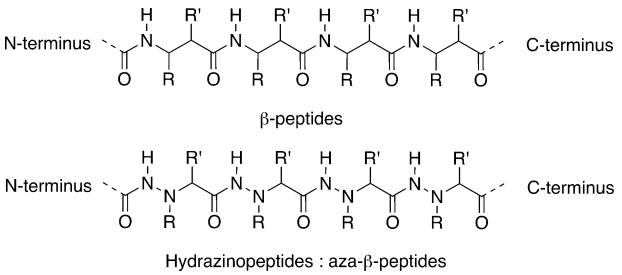

FIGURE 1. Comparison between primary structures of β-peptides and aza-β-peptides.

The ability to design oligomers that will adopt a predictable shape was made increasingly promising in the aim of developing efficient peptidomimetics because these oligomers often reveal resistance to proteolysis.[5] The biological application of β-peptides is currently being investigated.[3,6]

In addition to the β-peptides concept, α-hydrazinopeptides are, in turn, the focus of similar interest. These oligomers, which are aza-analogues of β-peptides (Figure 1), should develop alternative secondary structures owing to the presence of nitrogen atoms that can act both as H-bond donors (R = H) and H-bond acceptors. Using ab initio MO theory and molecular mechanics, Günther and Hofmann[8] thus predicted the most favorable conformations for oligomers of hydrazino acetic acid (R = R′ = H).

Experimental studies are emerging only now as the synthesis of optically pure hydrazinoacids, despite many improvements,[8] remains quiet laborious. Recently, Lelais and Seebach succeeded to prepare a series of hydrazinopeptides of optically pure hydrazinoacids bearing alkyl side chains.[5d] CD curves of some oligomers (R = H) suggest that they could adopt a right-handed helical conformation. However, because of difficulties encountered during NMR analysis, no definitive conclusion could be drawn at the moment.

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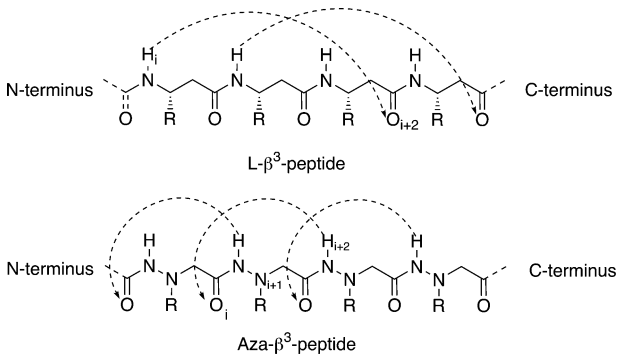

FIGURE 2. Comparison between secondary structures of L-β^3-peptides and aza-β^3-peptides.

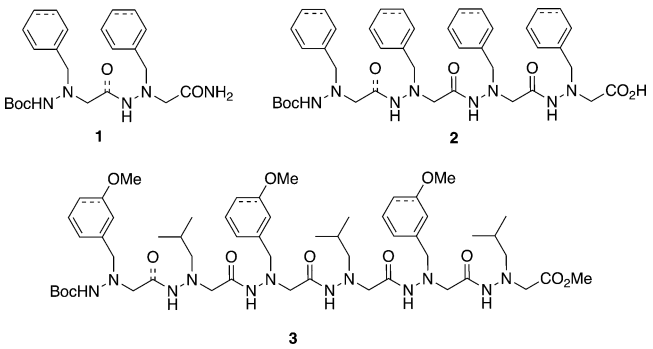

FIGURE 3. Primary structure of dimer **1**, tetramer **2**, and hexamer **3**.

Our team investigated a series of oligomers of N^{α}-substituted hydrazinoacetic acid, which are easily synthesized[9] (R ≠ H, R′ = H). These compounds, referred to as aza-β^3-peptides, possess the peculiarity of including pyramidal nitrogen atoms as stereocenters. From ^{1}H NMR studies, we concluded that they develop an H-bond network that induces the formation of consecutive eight-membered hydrogen-bonded pseudocycles (C_8 pseudocycle). This contrasts with the corresponding homochiral L-β^3-peptides, which adopt a left-handed helical structure relying on 14-membered hydrogen-bonded pseudocycles[10] (Figure 2).

As nitrogen inversion takes place, the conformation induced by the H-bond network, at a given moment, will indeed depend on the relative configurations of the stereocenters. Each diastereoisomer should, therefore, possess a specific conformation. As a consequence, aza-β^3-peptides in solution must behave as a set of secondary structures of unknown relative populations that interchange quickly. All of them, nevertheless, rely on a framework of C_8 pseudocycles.

The crystal structures of the dimer, the tetramer, and the hexamer we present in this work strongly support the conclusions we drew from our previous NMR studies.

Compounds **1**, **2**, and **3** represented in Figure 3 were assembled according to our previous published work.

Compounds **1** and **2** were crystallized from toluene. Compound **2** picked up water molecules from the solvent, which are associated stoechiometrically with the compound in the crystal. Compound **3** was crystallized from ether. All crystals are racemates. The crystal structures represented in Figures 4a−c show that the three oligomers developed as consecutive C_8 pseudocycles as much as was structurally possible (respectively, 2, 4, and 5). A detailed examination of the crystal structures reveals that all of the C_8 pseudocycles actually rely on a bifurcated H-bond where the NH_i interacts with both the carbonyl group of the residue $i - 2$ (average distance of CO···HN around 2.20 Å) and the lone pair of the sp^3 nitrogen atom of residue $i - 1$ (average distance of N···HN around 2.25 Å). This structural feature represented on Figure 4d, which has been observed before only in the case of simple molecular models including a single hydrazino acetic unit, is referred to as a hydrazinoturn.[11]

For all three compounds, the N-terminus hydrazinoturn results from the interaction of a hydrazidic NH with the carbonyl of the carbazidic group. In the case of compound **1**, a second hydrazinoturn relies on the interaction of one of the amidic NHs with the hydrazidic carbonyl. The hydrazinoturns located inside the oligomeric chain of compounds **2** and **3** result from H-bonding between the hydrazidic groups. In compound **2**, the fourth turn (C-terminus hydrazinoturn) is formed by the interaction of the acidic hydrogen with the preceding hydrazidic carbonyl group. We had already depicted this particular case of a hydrazinoturn for a Boc-protected-N^{α}-substituted hydrazino acetic unit,[9] and Seebach more recently observed a similar feature in the crystal structure of an optically pure hydrazinoacid.[5d] The water molecule associated with compound **2** is H-bonded with the carbonyl of the carbazidic group at the N-terminus and also with the carbonyl of the C-terminus acid function. The two ends of the tetramer are held together by this doubly H-bonded water molecule, giving the compound a very compact conformation. In the aza-β^3-peptide ester **3**, a C-terminus hydrazinoturn cannot be formed, but the carbonyl of the ester function makes a H-bond contact with the preceding hydrazidic NH.

On Figure 4d, we have also represented the four torsional angles ω, ϕ, θ, and ψ, which characterize a hydrazinoturn. The set of hydrazinoturns in compounds **1**, **2**, and **3** show slight variations of the values taken by these angles but approach the average values listed in Table 1. The two sets of values that appear depend on the absolute configuration of the nitrogen atom involved in the hydrazinoturn. ω results from the fact that the hydrazinoturn imposes the hydrazidic linkage to adopt

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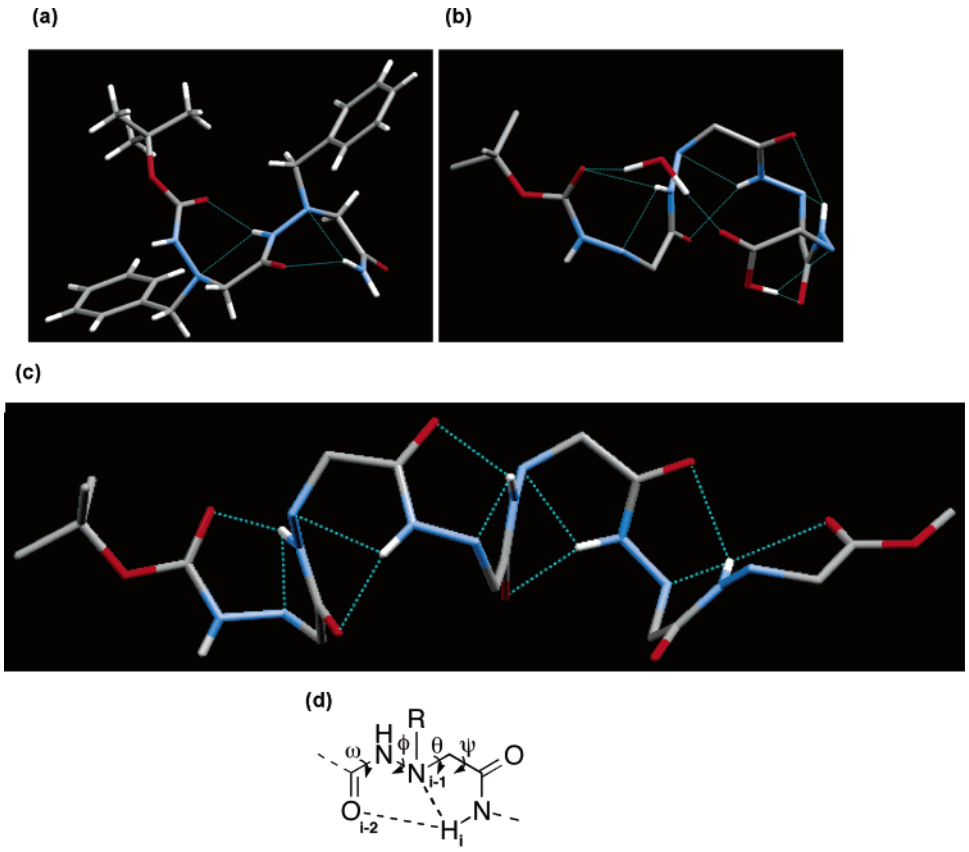

FIGURE 4. (a) Crystal structure of **1**. H-bonds as dotted lines. (b) Crystal structure of **2** H-bonds as dotted lines. Side chains are ommited for clarity. (c) Crystal structure of **3**. H-bonds as dotted lines. Side chains ommited for clarity. (d) Sructural features of a hydrazinoturn.

TABLE 1. Average Torsional Angles (deg) of a *R* and a *S*-Hydrazinoturn

	torsional angle			
	ω	ϕ	θ	ψ
R-hydrazinoturn	180	+120	−75	−15
S-hydrazinoturn	180	−120	+75	+15

the *Z* geometry. The value of the torsional angle ϕ, which approaches +120° or −120°, corresponds to an orthogonal arrangement between the lone pairs of electrons of the two adjacent nitrogen atoms (referred to as N^{β} and N^{α} according to the literature), which minimizes electron repulsion (Figure 5a). The values taken by θ (around −75° or +75°) corresponds to a (−)-synclinal or (+)-synclinal conformation that makes H-bonding between with the hydrazidic NH and the CO possible and further places the R substituent in a free zone of space (Figure 5b). H-bonding between NH_i and N^{α}_{i-1} restricts the rotation around the corresponding bond and forces ψ to adopt a low value (Figure 5c).

The structural features, which determine the set of angles ω, ϕ, θ, and ψ, clarifies why the hydrazinoturn conformation is so strongly favored. In fact, this particular turn allows the simultaneous minimizing of both steric crowding and electronic repulsion, while satisfying most H-bond donors and H-bond acceptors present in the oligomers.

The values of the torsional angles revealed by our solid-state analysis are very close to the values of the H_8 (1.75_8) helix postulated by Günther and Hofmann to be one of the most stable conformation of hydrazino peptides. This prediction is in good agreement with our observations.

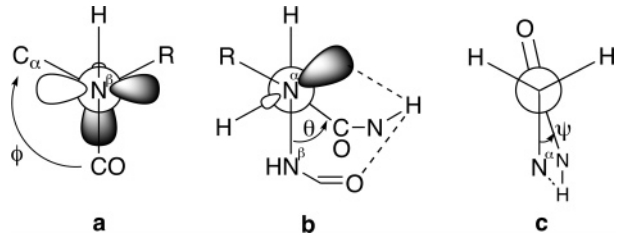

FIGURE 5. Schematic Newman projections showing the structural features of a *R*-hydrazinoturn in relation to the angles ϕ, θ, and ψ.

Wu and Yang calculated that pseudopeptides formed by α-aminoxy acids (oxa-peptides) should develop the same secondary structure with a cooperative effect for the formation of adjacent C_8 structures.[12] It is not surprising that aza-β^3-peptides behave similarly, since the structures of the N^{α}-substituted hydrazino acetic fragment and the α-aminoxy acid fragment are closely related by the presence of the two adjacent heteroatoms (respectively, N−N and N−O), which both induces lone-pair electron repulsion and allows comparable H-bonding. Wu and Yang also predicted that homo-(*S*)-oxa-peptides

(12) Wu Y–D.; Wang, D.-P.; Chan, K. W. K.; Yang, Dan. *J. Am. Chem. Soc.* **1999**, *121*, 11189.

should develop a 1.8_8 left-handed helical structure. Compound **1** crystallizes in a homochiral arrangement that actually resembles a small helical segment (in fact, one pitch of helix) where the side chains alternate on opposite sides of the helix.[13] This is also true for the homochiral segments present in compounds **2** and **3**. For the latter, however, crystallization produced a heterogeneous combination of the stereocenters (respectively, *SSRS*/*RRSR* and *RRSSRR*/*SSRRSS*), which led to more compacted arrangements.

The X-ray structures of the three aza-β^3-peptides we have examined show that the backbone organization invariably relies on a framework of consecutive eight-membered hydrogen-bonded pseudocycles, whatever the length of the oligomer, the nature of the side chains, or the C-terminus functionality. These findings strongly support our previous NMR study.

The pseudocycles observed in the solid state are actually hydrazinoturns, a specific structural feature associated with the hydrazino acetic fragment, that had till now only been observed individually in the crystal structures of simple molecular models. In aza-β^3-peptides, hydrazinoturns are linked together cooperatively.

As a whole, these results demonstrate that aza-β^3-peptides develop a radically different H-bond network in the strict comparison to the corresponding β^3-peptides. However, it should be noted that such C_8 framework has also been observed in the solid state for oligomers of 1-(aminomethyl)cyclopropanecarboxylic acid units,[14] which as $\beta^{2,2}$-peptides are more directly comparable with oxa-peptides.

Our study confirms that the structural relationship between the backbones of aza-β^3-peptides and α-aminoxy peptides can also be found within their intramolecular H-bond networks. Consequently, it helps to establish hydrazino peptides as foldamers.

In the case of aza-β^3-peptides, the molecular contraction induced by the framework of hydrazinoturns does not produce a single secondary structure due to pyramidal inversion of the nitrogen stereocenters.

It is believed that a hydrazinoturn relies on a specific bifurcated H-bond where the H-bond donor interacts with two H-bond acceptors. In fact, the distance between the N^α nitrogen and the hydrazidic NH, observed in the crystal structures, suggesst that it is somewhat H-bonded with the later. Do these interactions really take place in solution and, in this case, to which extent? Günther and Hofmann concluded that such contact should be of lesser importance than the hydrazidic CO to NH interactions. Current studies by our team will attempt to solve this challenging question.

(13) It should be noted that a homo-(*S*)-aza-β^3-peptide leads to a right-handed helix, whereas the corresponding homo-(*S*)-oxa-peptide leads to a left-handed one.

(14) Abele, S.; Seiler, P.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1559.

Experimental Section

General Experimental Method. The oligomers **1**, **2**, and **3** were assembled from Boc-protected N^α-substituted hydrazinoacetic monomers[9a] following our previous publications.[9b] The only improvement consists of using EDCI/HOBT (1.2 equiv) instead of DCC/DMAP as coupling reagents. We used an unoptimized coupling time of 12 h for each step. After this time the DCM solution was washed successively with 1 N HCl (twice), water (twice), and 1 N $NaHCO_3$, dried on Na_2SO_4, and evaporated. Yields are calculated starting from the monomers.

Dimer 1. The introduction of the C-terminus amid group was quantitatively obtained by simply standing the corresponding ester in a 7 N NH_3/MeOH 40% solution (10 molar equiv relative to the ester) for 24 h. Evaporation gives a crude powder that was directly crystallized from toluene (79%, 3 steps): mp 168°C; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$, 10 mM) δ 1.37 (s, 9H, tBu), 3.34 (s, 2H, CH_2), 3.43 (s, 2H, CH_2), 3.89 (s, 2H, CH_2), 3.98 (s, 2H, CH_2), 5.33 (s, 1H, amidic NH), 5.61 (s, 1H, carbazidic NH), 7.28−7.41 (m, 10H, aromatics), 8.24 (s, 1H, amidic NH), 9,41 (s, 1H, hydrazidic NH); ESI$^+$ HRMS 464.2269, calcd for $C_{23}H_{31}N_5O_4Na$ 464.2274.

Tetramer 2. The crude acid was obtained as an amorphous powder. Crystals grew very slowly from a toluene solution standing at 5 °C (72%, 6 steps): mp 118.5°C; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$, 10 mM) δ 1.31 (s, 9H, tBu), 3.26 (s, 2H, CH_2), 3.29 (s, 2H, CH_2), 3.45 (s, 2H, CH_2), 3.52 (s, 2H, CH_2), 3.85 (s, 2H, CH_2), 3.86 (s, 4H, 2×CH_2), 4.00 (s, 2H, CH_2), 5.57 (s, 1H, carbazidic NH), 7.27−7.34 (m, 20H, aromatics), 9.38 (s, 1H, NH), 9.73 (s, 1H, NH), 9.83 (s, 1H, NH); ESI$^+$ HRMS 789.3705, calcd for $C_{41}H_{50}N_8O_7Na$ 789.3700.

Hexamer 3. The crude ester was precipitated by trituration in a ether/pentane mixture. The powder was dissolved in ether, and crystals appeared by slow evaporation of the solution (46%, 7 steps): mp 130°C; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$, 10 mM) δ 0.87 (d, J = 6.5 Hz, 6H, 2xCH_3), 0.91 (d, J = 6.4 Hz, 6H, 2xCH_3), 0.92 (d, J = 6.35 Hz, 6H, 2xCH_3), 1.36 (s, 9H, tBu), 1.44 (nonuplet, J = 6 Hz, 1H, CH), 1.50 (nonuplet, J = 6 Hz, 1H, CH), 1.57 (nonuplet, J = 6 Hz, 1H, CH), 2.49 (d, J = 7 Hz, 2H, CH_2), 2.51 (d, J = 7 Hz, 2H, CH_2), 2.71 (d, J = 7 Hz, 2H, CH_2), 3.25 (s, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.36 (s, 2H, CH_2), 3.41 (s, 2H, CH_2), 3.42 (s, 2H, CH_2), 3.67 (s, 2H, CH_2), 3.76 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 3.86 (s, 2H, CH_2), 3.88 (s, 2H, CH_2), 5.95 (broad, 1H, carbazidic NH), 6.75−7.28 (m, 12H, aromatics), 9.22 (s, 1H, NH), 9.42 (s, 2H, 2×NH), 9.78 (s, 1H, NH), 9.80 (s, 1H, NH); ESI$^+$ HRMS 1115.6230, calcd for $C_{54}H_{84}N_{12}O_{12}Na$ 1115.6229.

Supporting Information Available: Characterization data for compounds **1**, **2**, and **3**: copies of ^{1}H NMR and ^{13}C NMR spectroscopic data and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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