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Aza-β^3-cyclopeptides: A New Way of Controlling Nitrogen Chirality

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Abstract: Sixteen and 24 membered aza-β^3-peptidic macrocycles containing a α-hydrazinoacid or a β^3-aminoacid were synthesized. The conformation of these pseudopeptides was determined by using NH chemical shift analysis, NH extinction, VT-NMR experiments, and X-ray diffraction. The study shows that a stable conformation is retained between 223 and 413 K. The latter is characterized by an uninterrupted internal H-bond network and a syndiotactic arrangement of the asymmetric centers. It means that the presence of the optically pure residue acts as a conformational lock to select a single enantiomer through the cyclization by controlling the absolute configuration of all the nitrogen atoms. To our knowledge, this represents the first example of a dynamic enantioselection process involving several centers prone to pyramidal inversion. These results give a new impulsion to the control of nitrogen chirality, which remained limited to small cycles for 60 years.

Introduction

In contrast to its homologues down in the column of the periodic table, the sp^3 nitrogen atom undergoes fast pyramidal inversion which makes challenging to resolve racemic compounds where chirality relies on this element. Very few molecules make exceptions to this fundamental physical rule. As far back as in the 1940s, slow NPI was predicted to occur for a nitrogen atom inside a three-membered ring in relation with the additional angular strain brought by the trigonal transition state.[1] This led, after two decades of efforts, to the demonstration of slow NPI in N-alkyl aziridines.[2] It was later expected that the inversion barrier should be further enhanced by connecting the nitrogen atom to another element bearing an unshared pair, partly due to electronic repulsion during the inversion process.[3] By combining these structural features, the magnitude of the barrier to NPI becomes high enough to allow the isolation of invertomers of N-halogenoaziridines,[4] alkoxyaziridines,[5] diaziridines,[6] oxaziridines,[7] and triaziridine.[8] The concept has been extended to slightly larger rings, mostly cyclic hydrazine like 1,2-diazetidines,[9] 1,3,4-oxadiazolidines[10] and diazabicycloheptane,[11] to some 1,2-oxazolidines,[12] and even acyclic dialkoxyamines[13] and trialkoxyamines.[14] On the whole, slow NPI remained for 60 years the quasi-exclusive prerogative of small cyclic compounds.

Recently, we discovered that aza-β^3-cyclopeptidic macrocycles with 16 or 24 bonds undergo strikingly slow backbone reversal, with energy barriers in the range of 75−80 kJ/mol.[15] The backbone of these pseudopeptides contains exclusively hydrazide linkages. This amide surrogate sustain a cooperative

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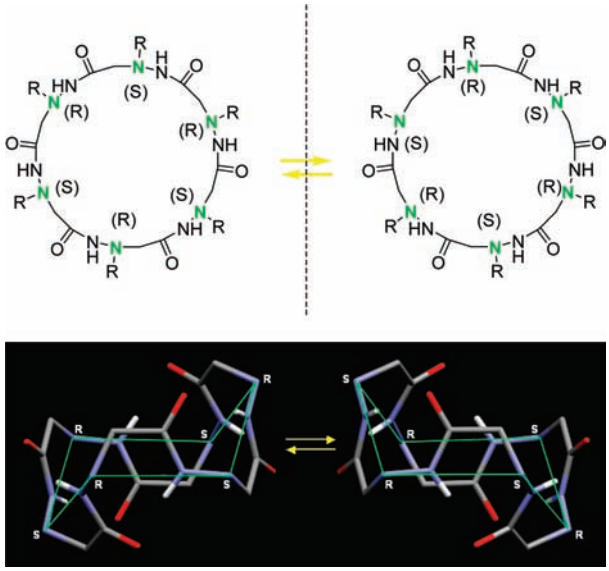

Figure 1. Equilibrium between the two "chair forms" of aza-β^3-cyclohexapeptides. The green lines on the crystal structure represent virtual bonds joining two consecutive chiral nitrogen centers.

internal hydrogen-bond network based on a recurrent C_8 pseudocycle (hydrazinoturn), a specific folding feature, where the hydrazidic NH*i* makes contacts with the nitrogen atom of the residue *i-1* and the carbonyle of residue *i-2*.[16a,b] This C_8 arrangement was elsewhere predicted by the theoretical investigation of Günther and Hofmann.[16c] The secondary structure that results gives the macrocyclic backbone a wavy form where the hydrazidic bonds are locked in the Z geometry, the NHCO vectors being oriented alternatively up and down relative to the mean plane of the macrocycle. More importantly for our purpose, the shape adopted by the backbone enforces the set of asymmetric nitrogen atoms to adopt a syndiotactic chiral sequence. In solution ($CDCl_3$), two mirror-image conformations interconvert slowly via the reorientation of the NHCO vectors and the reversal of configuration of the full set of pyramidal nitrogen atoms (Figure 1). The later can thus be regarded as undergoing slow NPI, even though the conformational route that underlies the reversal process is certainly complex and requires further investigation. Although the inversion barriers observed with the first aza-β^3-cyclopeptides are not at the level reached by small rings, we are currently engaged in modulating this new molecular family to draw the limit of this unexpected phenomenon. We present here the structural constraints induced by the introduction of a single carbon stereocenter inside the backbone of these compounds.

We reasoned that the introduction of an optically pure residue into the primary sequence of the open chain precursors could be sufficient to select an enantiomeric form by a dynamic process through the cyclization. Considering that the internal H-bonding plays a pivotal role in maintaining the backbone conformation, and subsequently in slowing down the NPI, we chose to use alternative building blocks prone to enter such an intramolecular network. α-Hydrazino acids or a β^3-aminoacids (Figure 2) seemed likely to fulfill this condition. Both of these

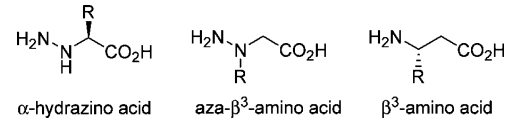

Figure 2. Structural relationship between α-hydrazino acids, aza-β^3-amino acid and β^3-aminoacids building blocks.

monomers, which are closely related to aza-β^3-amino acid, will preserve the ring size and induce very small molecular distortion at the level of the primary sequence. α-Hydrazino acids have been shown to promote hydrazinoturn formation in dipeptide models.[17] If β^3-peptidic oligomers are better known to be the seat of long-range H-bond contacts like in the β-peptidic helices, individual β^3-aminoacid included in peptidic sequence can also sustain a C_8 pseudocycle (pseudo γ-turn) in cyclic structures.[18] Thus, it was reasonably expected that α-hydrazino acids and β^3-aminoacids building blocks could prevent the disruption of the internal H-bond network. The presence of the asymmetric carbon atom immediately adjacent to the chiral nitrogen center in α-hydrazino acids, while remote from the nearest chiral nitrogen atoms in hybrid oligomers containing a β^3-aminoacid, made it further interesting to prepare both types of hybrid compounds.

Results

The synthesis of the precursors and their subsequent macrocyclization were performed following our previously described methodology. Two tetramers including a S-α-hydrazino alanine (compound **1**) and a S-β^3-phenylalanine (compound **2**) and a hexamer including a S-β^3-Leucine (compound **3**) were prepared without particular difficulties (Figure 3).

On the whole, the characteristics of the ^{1}H NMR spectra of the hybrid compounds are very similar to those of the corresponding aza-β^3-cyclopeptides except that the replacement of an aza-β^3-aminoacid unit leads to the loss of symmetry elements and discrimination of the chemical environments (compounds **2** and **3**). The juxtaposition of the spectra of compound **1** and closely related aza-β^3-cyclotetrapeptide **4** illustrate the overall similarity (Figure 4). In all cases, the NHs signals are very well resolved and scattered enough to be individually assigned by using 2D-NMR HMBC and HMQC sequences. This allowed us to compare their chemical shifts, which appear in Figure 3, with those observed in pure aza-β^3-cyclopeptides.

From our first series of compounds, we know that a H-bonded hydrazidic NH engaged in a hydrazinoturn absorbs around 9 and 10 ppm in aza-β^3-cyclotetrapeptides and aza-β^3-cyclohexapeptides, respectively (10 mM, $CDCl_3$). In compound **1**, all hydrazidic NHs respect this empirical rule (Figure 3). The small chemical shift modulation observed can be reasonably attributed to differences in chemical environment. This confirms that the introduction of the α-hydrazino acid unit preserves the cooperative hydrazinoturn framework inside the macrocycle as expected.

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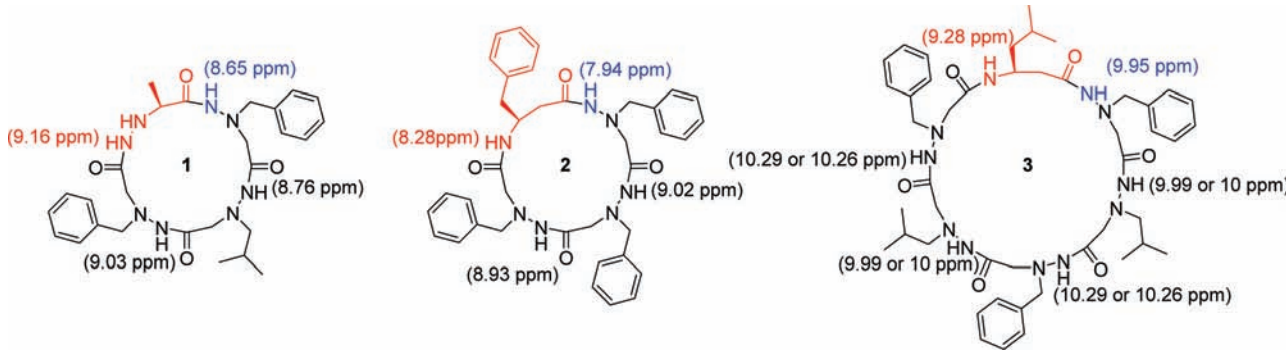

Figure 3. Structures of compounds **1**−**3** with the indexation of the NH's chemical shifts.

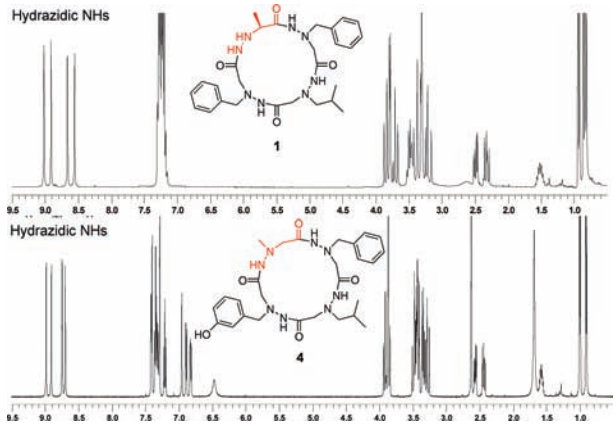

Figure 4. ^{1}H NMR spectra of compounds **1** and **4** (500 MHz, 10 mM, $CDCl_3$, 298 K).

In both compound **2** and **3**, hydrazidic NHs which close hydrazinoturns between two aza-β^3-amino acids fall in the expected window (Figure 3). The hydrazidic NHs following the β^3-aminoacid unit has no partner to close a hydrazinoturn. Their chemical shifts are 7.94 and 9.95 ppm, respectively. These low field values clearly testify for hydrogen-bonded hydrazidic NHs and are compatible with the formation of a C_8 pseudocycle with the carbonyle group of the preceding β^3-amino acid unit. The difference between the values reflects the geometrical difference between C_8 pseudocycles in tetramers and hexamers, as for aza-β^3-cyclopeptides. These values are nevertheless smaller than the corresponding values in a hydrazinoturn (around 9 and 10.3 ppm, respectively), very probably reflecting the difference in nature between a hydrazinoturn (a bifidic H-bonded system) and a more classical C_8 pseudocycle. The low field signals of the amide NH at 8.28 ppm (d, $^3J_{NHCH} = 10.5$ Hz) for compound **2** and 9.28 ppm (d, $^3J_{NHCH} = 8.9$ Hz) in compound **3** reflect their participation in a hydrazinoturn with the preceding aza-β^3-amino acids in the backbone. As for compound **1**, the introduction of a β^3-amino acid unit does not disturb significantly the internal H-bond network.

Discussion

From these first set of observations, it is reasonably likely that, despite the structural modifications introduced, the hybrid macrocycles adopt the same global conformations as their aza-β^3-cyclopeptidic counterparts. This was confirmed by the resolution of the crystal structure of compound **2** and **3** where the backbone organization appears very similar to those of their isosteric aza-β^3-cyclopeptide counterparts (Figure 5). Only small bond length and angular variations of the CO--HN, and N---HN H-bonds occur between the two series, giving the both isosteric macrocycles a very close shape. The major difference between the two solid state structures is that crystals of aza-β^3-cyclopeptides are racemates; they are built from a single enantiomer selected by the S-β^3-aminoacid in the case of **2** and **3**. There is a very good agreement between the HCNH torsion angles of the β^3-aminoacid unit (around 170°) and the corresponding coupling constant[19] observed in NMR spectra (around 10 Hz). This shows that the hydrogen bond network deduced from the NMR analysis gives the macrocyclic backbone a conformation very close to those observed in the solid state.

To evaluate the conformational stability of hybrid macrocycles, two sets of experiments were run. The macrocycle reversal of pure aza-β^3-cyclopeptides can be followed by VT-NMR experiments which drive to the coalescence of diastereotopic signals ($C_2D_2Cl_4$). Although this phenomenon cannot occur with hybrid compounds, as the configuration of the carbon stereocenter cannot invert, no significant modification except small chemical variations was observed when running such experiments between 223 and 413 K. The A and B parts of spin systems move closer or move away while remaining well resolved. No additional signals revealing alternative chiral sequences, namely new diastereoisomeric forms, are observable. As illustrated, Figure 6 shows the VT-NMR profile of both compounds **1** and **4** between T_0 and T_{max} in the methylenic region.

We have also compared the rate of H/D exchange between aza-β^3-cyclopeptides and the hybrid macrocycles depicted here by monitoring the extinction of the NH signals. We ruled out runing the experiments in CD_3OD because the conformation of these macrocycles, which relies on internal H-bonding, should be affected by solvatation effect in a protic medium and thus potentially differ from the conformation that we have resolved in $CDCl_3$. Thus, we choose to work in biphasic conditions by adding a few drops of deuteriated water in a $CDCl_3$ sample. In this way, it was possible to put forward a marked difference in behavior between the two series of macrocycles. In the case of aza-β^3-cyclopeptides **4**, the H/D exchange goes to completion while preparing the sample. In contrast, the intensity of the NH signals of hybrid macrocycles **1**-**3** decreases very slowly along the time, residual pics being still observable after two months. The fast NH extinction observed in the case of aza-β^3-cyclopeptides **4** clearly reflects the reversal of the macrocycle which requires the transient breaking of H-bonds, during which the NHs becomes solvent exposed. In contrast, the conformational stability of the hybrid compounds reduces the solvent

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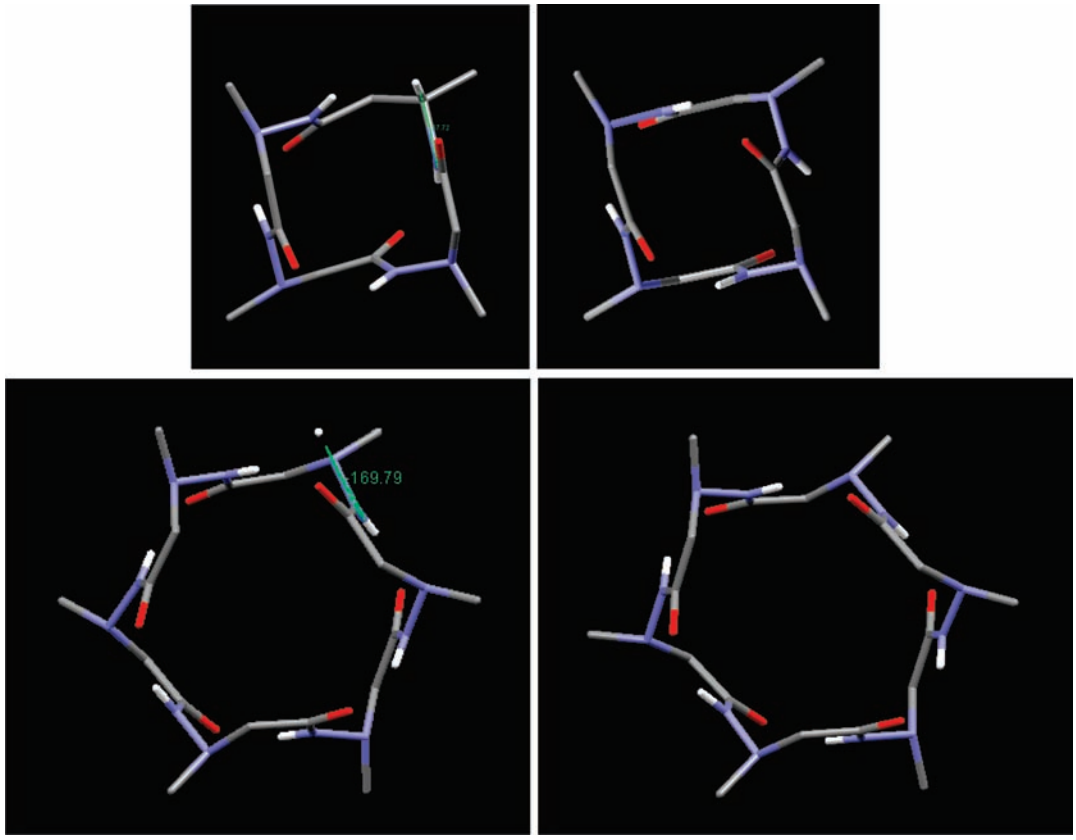

Figure 5. Comparison of solid state structure of hybrid macrocycles **2** and **3** (left) and their aza-β^3-cyclopeptidic analogues (right). For clarity, only NH hydrogen atoms are represented while benzylic and isobutylic side chains are replaced by a single carbon atom. The HCNH dihedral angles are indicated.

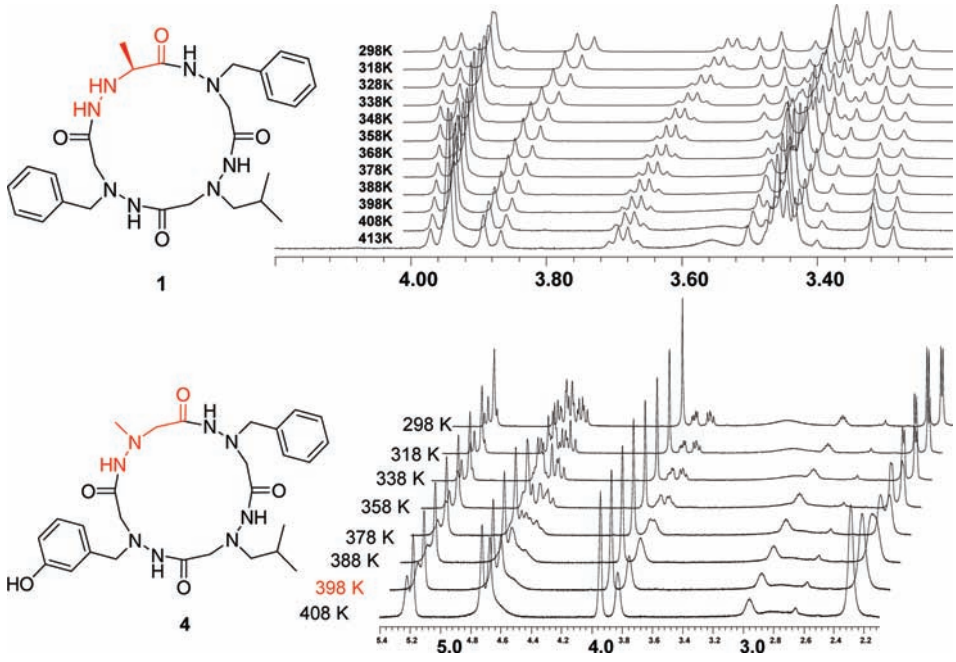

Figure 6. ^{1}H NMR spectra of compound **1** and **4** between 298 and 413 K (500 MHz, 10 mM in $C_2D_2Cl_4$). Methylenic region.

accessibility and thus considerably slows down the exchange phenomenon.

Conclusion

Taken together, these results demonstrate that the carbon chiral center acts as a conformational lock, and that the depicted hybrid aza-β^3-cyclopeptides are conformationally stable compounds where the NPI is therefore fully inhibited. The synthetic strategy takes advantage of the NPI phenomenon by engaging the nitrogen element into a dynamic asymmetric process which combines diastereospecificity as the only syndiotactic sequence is selected, and enantiospecificity as a single enantiomer is

obtained. Even though relying on the most configurationally versatile element, the process leads to macrocycles with perfectly defined shape, while most peptidic macrocycles of similar size often undergo conformational flexibility.[20] The present study thus confirms the real attractivity of aza-β^3-cyclopeptides to investigate nitrogen chirality.

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Supporting Information Available: Preparation and characterization data for compounds **1**, **2**, **3**. Copies of ^{1}H NMR and ^{13}C NMR spectroscopic data. Superposition of ^{1}H NMR spectra at different temperatures in $C_2D_2Cl_4$ and $CDCl_3$. Time dependence of the ^{1}H NMR spectra recorded in the presence of D_2O in $CDCl_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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