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Postsynthetic Modification of C_3-Symmetric Aza-β^3-Cyclohexapeptides

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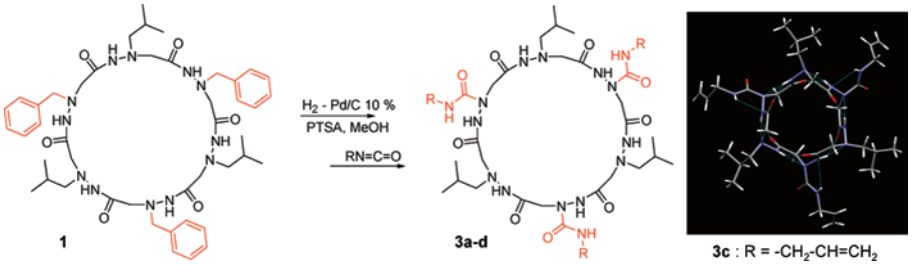

We have synthesized a series of C_3-symmetric aza-β^3-cyclohexapeptides with functionally diverse side chains carrying a good functional diversity. The very simple chemical sequence that we used (debenzylation/acylation) makes it certain that the series synthesized could be easily expanded, leading to a wide family of C_3-symmetric cyclohexapeptides analogues. The macrocyclic backbone of the aza-β^3-cyclohexapeptides shows a highly ordered conformation that is sustained by a dense intramolecular H-bond network where all endocyclic NHs are hydrogen bonded, the side chains being projected in equatorial position around the macrocycle. The resulting internal secondary structure relies on the cooperative alternation of two slightly different C_8-bifidic pseudocycles, which differ mainly by the hybridization of the N^{α} nitrogen atom ($N-N_{sp^3}$-turn and $N-N_{sp^2}$-turn). In both cases, the nitrogen lone pair participates to stabilize the pseudocycle. This has been established by NMR experiments and X-ray diffraction analysis. As in the precursors, the nitrogen stereocenters are characterized by a strikingly slow rate of pyramidal inversion, considering the size of the macrocycle.

Introduction

C_3-symmetric compounds are attractive molecules, both aesthetically and functionally. They have been used to perform asymmetric catalysis and molecular recognition and to develop nanoarchitecture.[1] Among them, C_3-symmetric peptidic or pseudopeptidic macrocycles were recently used for the molecular recognition of carbohydrates,[2] as selective receptors for cations or anions,[3] and to mimic trimeric CD40L, an important ligand of the tumor necrosis factor receptor (TNFR superfamily).[4] De Santis and co-workers were the first to postulate[5] that the synthesis of cyclic hexapeptides should be easier for precursors with syndiotactic chiral sequence (alternation of (L)- and (D)-amino acids), due to a favorable pre-organized conformation. This has been experimentally confirmed by several examples and recently further extended to pseudopeptides.[3d,g,6] In a previous work, we showed that aza-β^3-hexapeptides, where the

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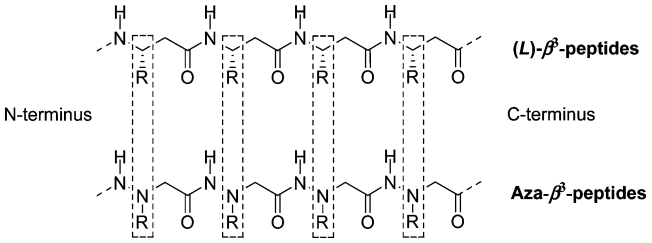

FIGURE 1. Structural relationship between β^3-peptides and aza-β^3-peptides.

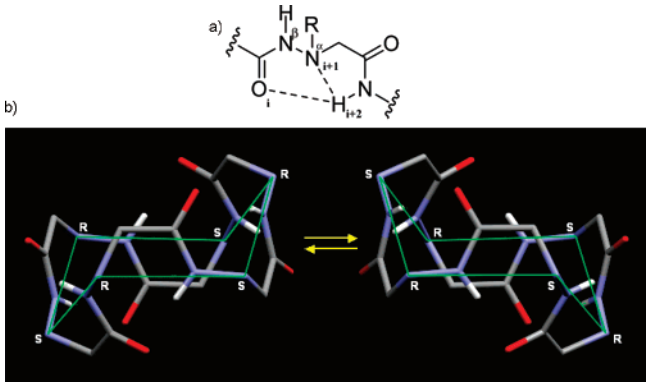

FIGURE 2. (a) Hydrazinoturn or N−N turn, a bifidic hydrogen-bonded C_8 pseudocycle. (b) Equilibrium between the two "chair forms" of aza-β^3-cyclohexapeptides.

CH_β units of β^3-peptides are replaced by nitrogen atoms (Figure 1), are very suitable precursors of macrocycles.[7] In such compounds, the syndiotactic arrangement favorable to cyclization is spontaneously achieved during the chiral tuning that results from the pyramidal inversion at the sp^3 nitrogen stereocenters.

In contrast with the conformational versatility of their precursors, aza-β^3-cyclohexapeptides have a well-defined secondary structure that has been deduced from both solid state and NMR analysis. The ring backbone is organized by an uninterrupted network of reverse-turn, referred to as an hydrazinoturn or N−N-turn,[8] which is a bifidic eight-membered pseudocycle (Figure 2a).

These turns are linked together to confer a C_3-symmetric conformation to the macrocycle. Consequently, the chiral sequence of the nitrogen centers keeps alternating. The macrocycle oscillates slowly (around one time per second based on variable-temperature experiments) between two mirror images (Figure 2b). This represents an exceptionally slow pyramidal inversion considering the size of the ring. By joining the chiral nitrogen centers by virtual bonds (green lines in Figure 2b), a striking analogy appears between the macrocycle reversal and the interconversion of the two chair forms of cyclohexane.

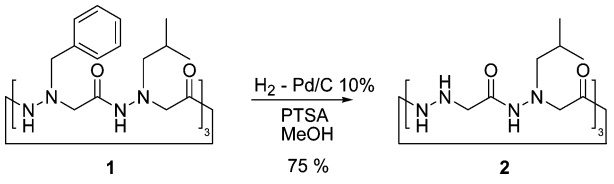

FIGURE 3. Hydrogenolysis of aza-β^3-cyclohexapeptide **1**: synthesis of **2** (aza-β^3-Gly-aza-β^3-Leu)$_3$.

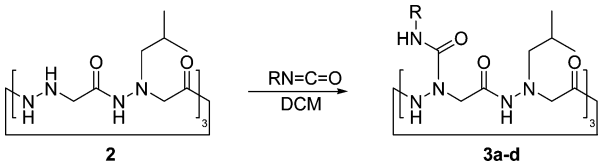

FIGURE 4. Acylation of cyclohexamer **2** (aza-β^3-Gly-aza-β^3-Leu)$_3$. Compounds **3a**−**d**: R = $-C_6H_5$, $-CH_2CH_2Br$, $-CH_2CH{=}CH_2$, and $-CH_2CO_2Et$, respectively.

The potential of C_3-symmetric molecules further improves when they carry functional side chains, likely to take part in host−guest interactions or catalytic processes. Aza-β^3-cyclohexapeptides are built from N^α-substituted hydrazino acetic acids, whose synthesis use aldehydes or ketones,[9] allowing the introduction of a good variety of side chains. This upstream functionalization strategy is currently being developed by our team. We report herewith a very convenient downstream approach, which relies on the linkage of functional arms on a preformed C_3-symmetric macrocycle. The conformation of the new macrocycles is compared with those of the precursors, based on NMR spectroscopic data and X-ray crystallographic structure.

Results and Discussion

Among the side chains that can be regioselectively introduced on aza-β^3-cyclopeptides, the benzylic group is particularly useful as it can easily be removed by hydrogenolysis. This characteristic offers the opportunity to introduce new functionalities by subsequent reactions with electrophiles.

We started the synthesis of modified macrocycles with C_3-symmetric aza-β^3-cyclohexamer **1** (aza-β^3-Phe-aza-β^3-Leu)$_3$ which carried alternating benzyl and isobutyl side chains. The hydrophobic aza-β^3-leucine residue was introduced to retain a good solubility in organic solvents for all new compounds. This was particularly important in order to perform NMR spectra in common solvent ($CDCl_3$). A first attempt to remove the benzyl groups of macrocycle **1** was made using 10% Pd/C in methanol. The cleavage was slow and a mixture of partially deprotected oligomers was obtained. In contrast, the addition of *p*-toluenesulfonic acid induced a complete deprotection with a very low level of side products within 12 h at room temperature (Figure 3).[10]

The next step was to introduce new side chains by reacting HN^α nucleophilic sites of macrocycle **2** with electrophilic isocyanates RN=C=O (Figure 4). Compounds **3a**−**d** were obtained in quantitative yields starting from crude **2** and the

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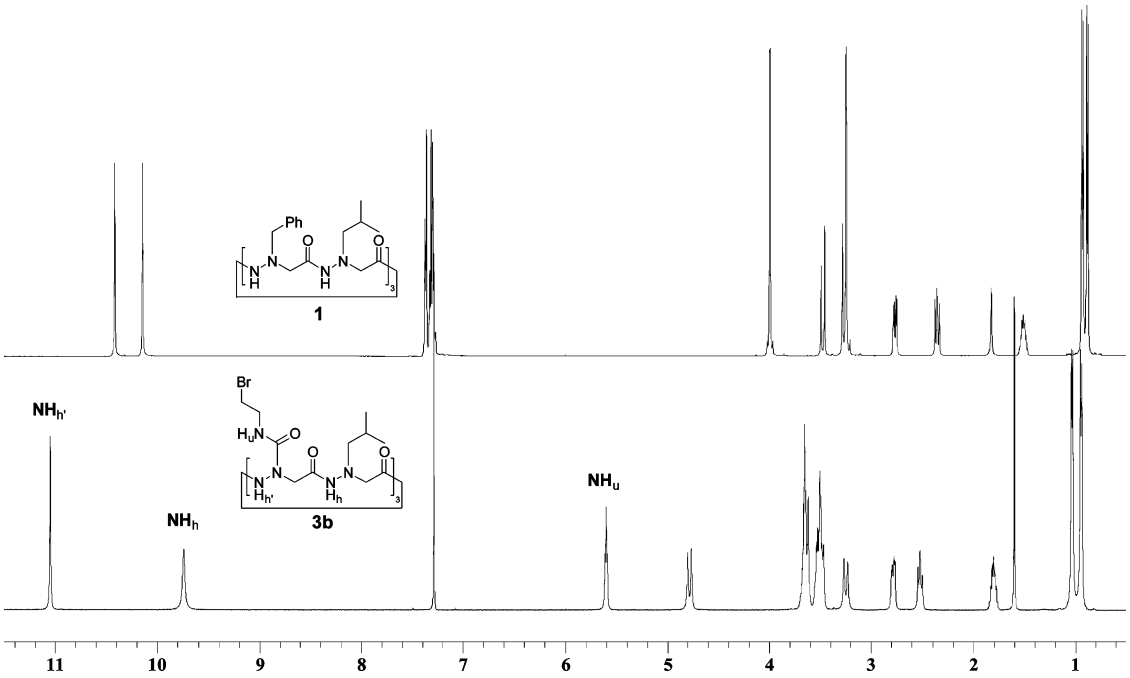

FIGURE 5. ^{1}H NMR (500 MHz) spectra of compounds **1** and **3b** (aza-β^3-*CONHCH$_2$CH$_2$Br*-aza-β^3-Leu)$_3$ ($CDCl_3$, 10 mM, 298 K).

appropriate isocyanate. Our results clearly indicate that this reaction can be easily performed and allows the introduction of a wide variety of functional groups on the aza-β^3-cyclohexapeptidic backbone.

Analysis of the Conformation of Cyclohexamers 3a−d (aza-β^3-*CONHR*-aza-β^3-Leu)$_3$ in $CDCl_3$. Theoretically, the acylation of **2** turns the hybridization of N$^\alpha$ nitrogen atoms from sp^3 to sp^2. This last point raised some interesting questions: How does the alternation of N$^\alpha$sp^3/N$^\alpha$sp^2 nitrogen atoms influence the network of hydrazinoturns? To what extent does this structural modification have an effect upon the conformation of new macrocycle? Is the C_3-symmetrical conformation retained? Interestingly, the following data show that the conformations of macrocycles are very similar, before and after acylation.

The complete assignment of ^{1}H and ^{13}C NMR spectra was made possible using classical 2D-COSY, HMBC, and HMQC sequences. The spectral characteristics of cyclohexamers (aza-β^3-*CONHR*-aza-β^3-Leu)$_3$ present recurrent features. As for precursors **1** and **2**, ^{1}H NMR spectra of **3a−d** show only two sets of signals, as illustrated in Figure 5 for compounds **1** and **3b**. Consequently, macrocycles **3a−d** still adopt a C_3-symmetrical conformation in solution.

The backbones of compounds **3a−d** combine two types of CO−NH bonds: on the one hand, the CO−NH−Nsp^3 hydrazidic linkage of (aza-β^3-Leu) units, and on the other hand, the CO−NH−Nsp^2 acylated fragment of (aza-β^3-*CONHR*) groups. To discriminate both endocyclic NHs, the former were noted NH$_h$ and the latter NH$_{h'}$ (the exocyclic NH is noted NH$_u$ for urea NH) (Figure 5). The downfield signals of NHs inside the ring, combined with their insensitivity to dilution or addition of increasing amounts of DMSO-d_6 (Figure 6),[11] are characteristic of intramolecular H-bonding. These results can be reasonably interpreted by assuming that the NH$_{h'}$s participate to hydrazinoturns and that consequently the NH$_h$s are involved in eight-membered hydrogen-bonded pseudocycles. In contrast, the signal of the urea NH$_u$ is strongly affected by the presence of DMSO, indicating that the exocyclic NHs do not interfere significantly with the intramolecular H-bonding pattern. The downfield chemical shift values observed for NH$_{h'}$s (around 11.0 ppm) compared with the NH$_h$s values in an hydrazinoturn (around 10.2 ppm) could reflect the higher acidity of NH$_{h'}$ induced by the acylation of the N$^\alpha$ nitrogen atom. On the other hand, the chemical shift of a hydrazidic NH$_h$ in **3a−d** (around 9.7 ppm) which are shielded from around 0.5 ppm relative to **1** could be rationalized by the lack of the sp^{3}N$^\alpha$−HN contact. 2D-NOESY spectra of macrocycles **3a−d** were recorded. For all endocyclic NHs, strong NOEs between NH$_i$ and C$_\alpha$H$_i$ but medium NOEs between NH$_i$ and C$_\alpha$H$_{i-1}$ were observed (Figure 7), which are fully consistent with the previous interpretation.

As in compound **1**, N^α-methylene protons of **3a−d** are nonequivalent. AX- ($\Delta\delta \approx 1.50$ ppm) and AB-systems are observed for (aza-β^3-*CONHR*) and (aza-β^3-Leu) units, respectively. In $C_2D_2Cl_4$, these signals coalesce around 378 K, close to the value of 388 K observed for **1**.[7] Clearly, the strong slowing of nitrogen pyramidal inversion that characterizes aza-β^3-cyclohexapeptides still occurs in modified compounds **3a−d**.

After numerous attempts, we succeeded in getting suitable crystals for X-ray diffraction with compound **3c** (dichloromethane/toluene). The high resolution of the structure revealed the presence of disordered solvent molecules but allowed us to resolve the conformation of the macrocycle. The molecule shows a C_3-symmetry axis. Inside the macrocycle, all NH protons are intramolecularly H-bonded. Exocyclic NHs close five-membered pseudocycles whereas endocyclic NHs are alternatively engaged into two different bifurcated eight-membered rings. Bifidic hydrogen-bonded C$_8$ pseudocycles involving the lone pair of the sp^3 N$^\alpha$ nitrogen atoms of (aza-β^3-Leu) groups are typically hydrazinoturns. Corresponding torsional angles ω, ϕ, θ, and ψ, close to average values observed for aza-β^3-cyclohexapeptides[7] ($\omega = 180°$, $\phi = -115°$, $\theta = +90°$, and $\psi = \pm 10°$ for an (*S*)-

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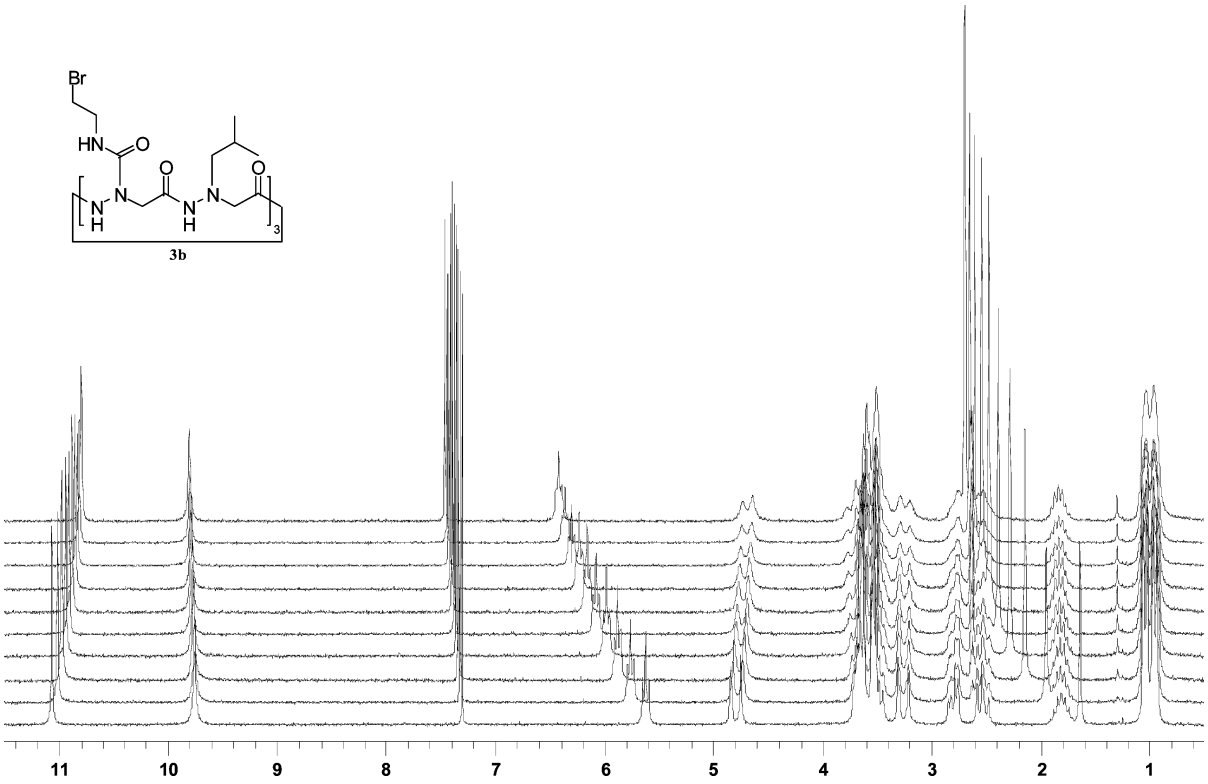

FIGURE 6. Superposition of ^{1}H NMR (200 MHz) spectra in the presence of increasing amounts of DMSO-d_6 (0−9%, from bottom to top) in a 10 mM solution of compound **3b** (1 mL in $CDCl_3$ at room temperature).

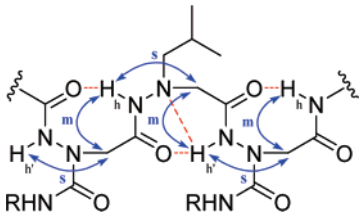

FIGURE 7. Summary of NHs NOEs observed for cyclic backbone of hexamers **3a**−**d** (10 mM in $CDCl_3$) at 288 K. s, strong NOE; m, medium NOE.

TABLE 1. Torsional Angles (deg) of (*S*)-N−N_{sp^3}-Turn and N−N_{sp^2}-Turn in Cyclohexapeptide 3c

	torsional angles			
	ω	ϕ	θ	ψ
(*S*)-N−N_{sp^3}-turn	+178.82	−113.04	+89.83	+7.17
N−N_{sp^2}-turn	−179.21	+100.53	−97.82	+5.21

hydrazinoturn), are listed in Table 1. Unexpectedly, the second intramolecular C_8 pattern also relies on a bifurcated H-bond system where hydrazidic NH_{hi+2} interacts with both the carbonyl group of the residue *i* and the P_z lone pair of the sp^2 N$^\alpha$ nitrogen atom of residue $i + 1$ (aza-β^3-*CONHR*). As illustrated in Table 1, the two sets of dihedral angles are very similar.[12]Therefore, we decided to refer to this new local folded structure as a N−N_{sp^2}-turn (de facto, the foremost "classical hydrazinoturn" will be noted N−N_{sp^3}-turn). The value of the torsional angle $\phi = +100°$ corresponds to an orthogonal arrangement of the lone pairs of electrons of the two adjacent sp^2 nitrogen atoms, which minimizes electronic repulsions and steric hindrance. A slight pyramidalization of the sp^2 N$^\alpha$ nitrogen atom toward hydrazidic HN is observed which contributes to reinforce the H bond closing the five-membered pseudocycle. The N$^\alpha$ bond angles of N−N_{sp^3}-turn and N−N_{sp^2}-turn are given in Table 2. Distances and angles associated with the two bifurcated intramolecular H-bond systems are listed in Table 3. N−N_{sp^3}-turn and N−N_{sp^2}-turn conformations are depicted in Figure 8 (right).

The exocyclic NHs are intramolecularly H-bonded with the P_z lone pair of the $NH_{h'}$ sp^2 nitrogen atom (N^β−HN = 2.32 Å).

The top view (Figure 8, left) indicates that the 24-membered macrocycle exhibits an hexagonal shape with side chains in radial positions. The side view in Figure 9 shows the wavy conformation of the cyclic covalent backbone. The ring is also

TABLE 2. N$^\alpha$ Bond Angles (deg) of N−N_{sp^3}-Turn and N−N_{sp^2}-Turn in Cyclohexapeptide 3c

	sp^3 N$^\alpha$ bond angles			sum of sp^3 N$^\alpha$ bond angles
	N^β−N$^\alpha$−C_α	N^β−N$^\alpha$−CH_2	CH_2−N$^\alpha$−C_α	
N−N_{sp^3}-turn	110.42	110.01	115.0	335.43
	sp^2 N$^\alpha$ bond angles			sum of sp^2 N$^\alpha$ bond angles
	N^β−N$^\alpha$−C_α	N^β−N$^\alpha$−CO	CO−N$^\alpha$−C_α	
N−N_{sp^2}-turn	116.12	115.93	117.38	349.43

TABLE 3. Distances (Å) and Angles (deg) of Bifurcated Intramolecular H-Bond of N−N_{sp^3}-Turn and N−N_{sp^2}-Turn in Cyclohexapeptide 3c

intra-molecular H bonds	distances (Å)		angles (deg)	
	O_i−HN_{i+2}	N$^\alpha_{i+1}$−HN_{i+2}	O_i−H−N_{i+2}	N$^\alpha_{i+1}$−H−N_{i+2}
N−N_{sp^3}-turn	2.19	2.15	131.02	111.91
N−N_{sp^2}-turn	2.08	2.32	141.13	109.0

(12) As in syndiotactic combination, two sets of dihedral angles with opposite signs are observed.

(13) The N−N_{sp^3}-turn is represented in the *R* configuration in order to facilitate the comparison with the N−N_{sp^2}-turn.

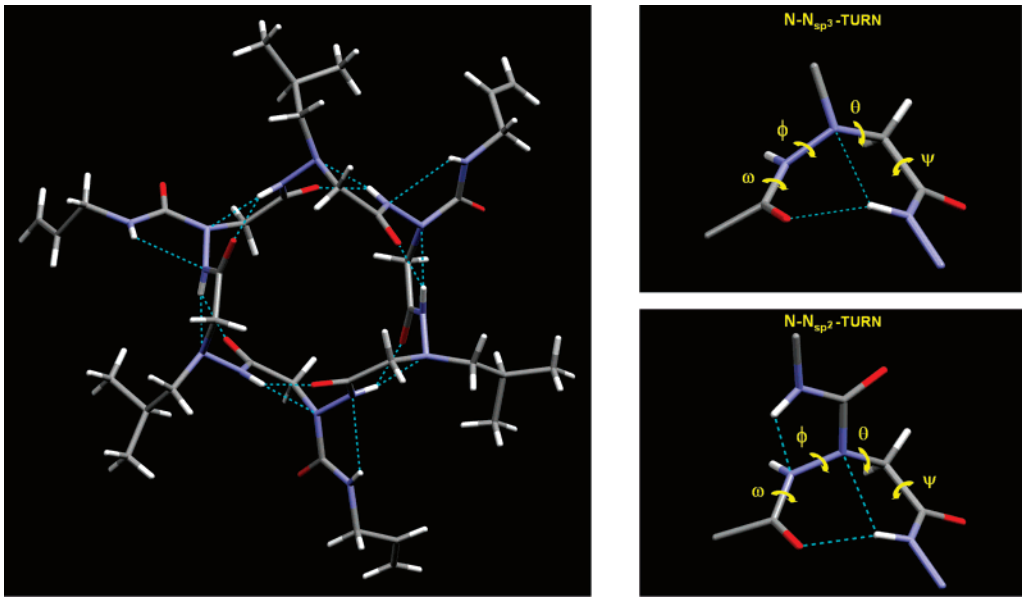

FIGURE 8. (Left) Solid state conformation of cyclohexapeptide **3c**: top view. (Right, top and bottom) (*R*)-N−N$_{sp^3}$-turn and N−N$_{sp^2}$-turn conformations with corresponding torsional angles.[13] Hydrogen bonds are indicated by dotted lines.

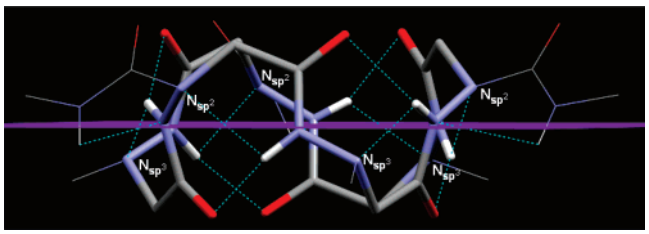

FIGURE 9. Solid state conformation of cyclohexapeptide **3c**: side view. Side chains are partially represented for clarity. Hydrogen bonds are indicated by dotted lines.

characterized by a median plane containing all endocyclic NH nitrogen atoms. The six CO−NH bonds of the backbone are nearly perpendicular to the cycle mean plane and are alternatively orientated in opposite directions. Finally, the secondary structure associated with the alternation of both types of hydrazinoturns induces a very compact conformation superimposable with those of corresponding aza-β^3-cyclohexapeptides precursors.

Conclusions

The preliminary results presented give us confidence in our ability to introduce a wide diversity of functional groups on the aza-β^3-cyclohexapeptidic backbone, leading to numerous structural modulations and applications in the future. The conformational homogeneity of the ring-backbone, which relies on intramolecular H-bond network and exceptionally slow nitrogen pyramidal inversion, is kept in all the series of macrocycles. Most interesting is the ability to achieve a well-defined scaffold without any asymmetric synthesis. Combined with the highly efficient synthesis of the macrocyclic precursors, the robust chemical sequence described here to perform their refunctionalization makes it a very attractive route to a large family of aza-β^3-pseudopeptidic macrocycles. Actually, work in progress by our team shows that the strategy depicted here is limited to neither cyclohexamers nor C_3-symmetric macrocycles.

Experimental Section

General Hydrogenolysis Procedure.

Macrocycle **1** (800 mg, 0,92 mmol) dissolved in 40 mL of methanol, was debenzylated quantitatively under hydrogen atmospheric pressure in the presence of 80 mg of 10% Pd/C with 1 equiv of para-toluenesulfonic acid monohydrate. After 12 h of stirring, the solution was filtrated on Celite and evaporated. The crude residue was dissolved in 100 mL of CH_2Cl_2 and the solution was washed vigorously with 5 mL of 0.5 N $NaHCO_3$. After two extractions (CH_2Cl_2 and EtOAc), drying of the organic phase on Na_2SO_4 and evaporation afforded macrocycle **2** as a white powder (415 mg, 0.69 mmol, 75%). This compound was carried on without further purification.

General Acylation Procedure.

Compounds **3a-d** (0.69 mmol) were obtained from crude **2** (0.69 mmol) and an excess (six equivalents) of the appropriate isocyanate. The reaction is completed at room temperature in dichloromethane in a few hours. Excess of isocyanate was neutralized with ethanol (5 mL). After evaporation, the crude residues gave rise to the apparition of white solids corresponding to the expected macrocycles. The solids were then washed in ether and isolated by filtration in almost quantitative yields.

Acknowledgment. We are grateful for the support provided by ANR (National Research Agency).

Supporting Information Available: Characterization data for compounds **1**, **2**, and **3a**−**d**; copies of ^{1}H NMR and ^{13}C NMR spectroscopic data; graphics showing the variation of the chemical shifts of NHs as a function of the concentration of DMSO-d_6 in $CDCl_3$ (0−9%) for compound **3b**; superposition of ^{1}H NMR spectra at different temperatures in $C_2D_2Cl_4$ for compounds **3a**; portions of the 2D-NOESY spectrum and the summary of NOEs observed for compound **3c** in $CDCl_3$ at 298 K (s, strong NOE; w, weak NOE); and crystallographic data in CIF format for compounds **2** and **3c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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