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Aza-β^3-cyclotetrapeptides

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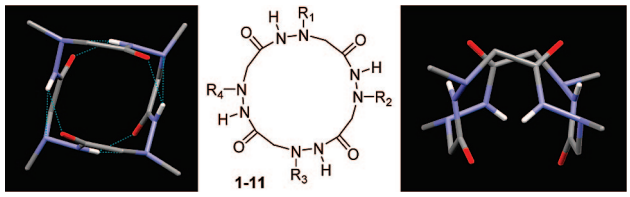

The cyclization of aza-β^3-tetrapeptides gives access to new CTP (cyclotetrapeptide) analogues. These stereocontrolled templates are assembled without any asymmetric synthesis. X-ray crystallographic structure and NMR analysis show that the macrocyclic scaffold is characterized by a fully cooperative intramolecular H-bond network, in sharp contrast with the nanotubular assemblies observed for β^3-cyclotetrapeptides. This folding property reduces considerably the polarity of aza-β^3-tetrapeptides and should be useful in addressing intracellular targets.

Cyclization is a common tool to introduce steric and angular constraints in small peptidic segments, and to probe the conformational requirements for biological activity.[1] Cyclotetrapeptides (CTPs) seem attractive for drug design, due to a molecular weight ranging in the drug-like gap, to an intrinsic resistance to exopeptidases but also to endopeptidases which are mostly active against β-strand conformation,[2] and to the potential side chain diversity that can be introduced by using both proteogenic and nonproteogenic amino acids.[3] Their cyclic nature makes them more rigid than the linear precursors, what is an entropic advantage upon binding, although they can show residual conformational heterogeneity.[4] CTPs represent minimalist β-turns mimetics, the most common reverse turn, comprising four amino acid residues making almost a complete 180° turn in the direction of the peptide chain. β-Turns, generally located on the solvent-exposed surface of proteins, play important roles as recognition and binding sites in numerous biological events.[5] β-Turns are also implied in the receptor affinity of Somatostatin,[6] Bradykinin,[7] and other biologically important peptides.[8,9] Natural CTPs also display interesting biological effects.[10] In particular, Apicidin shows antiprotozoal activities with possible development as antimalarial agents.[11] Yet, all these attractive features are counterbalanced by the fact that the CTP scaffold, with four planar amide groups constrained in a 12-membered ring, remains hardly accessible synthetically.[12] Several groups, both academic and pharmaceutical, have developed new synthetic strategies to obtain easily accessible natural CTP analogues, with high conformational homogeneity. The incorporation of a single β^3-amino acid, which induces a pseudo γ-turn, gives efficient synthesis of rigid 13-membered ring CTP mimetics,[13] also used to develop RGD mimetics.[14] The introduction of a reduced peptide bond proved to be a valuable approach toward the synthesis of Apicidin analogues.[15] Cyclic β-tetrapeptoids also appear as a novel promising scaffold.[16]

Aza-β^3-peptides,[17] built up with N^α-substituted hydrazinoacetic acids[18] (aza-β^3-amino acids) (Figure 1, top), are pseudopeptides which differ mainly from regular peptides by the replacement of amide bonds by hydrazide bonds and the location of the chirality on the N^α nitrogen centers (Figure 1, middle). These pseudopeptidic foldamers are characterized by an intramolecular H-bond network (Figure 1, bottom) based on a repeated bifurcated C_8 pseudocycle (hydrazinoturn) and a phenomenon of chiral scanning along the backbone resulting from nitrogen

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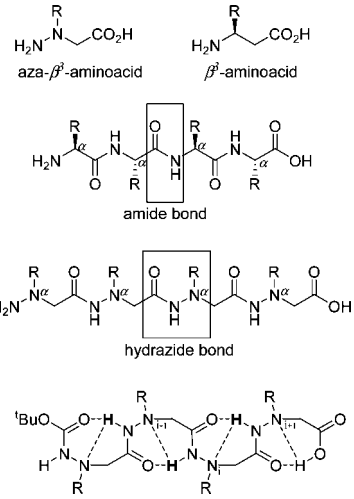

FIGURE 1. Comparative structures between aza-β^3- and β^3-amino acid (top) and between peptides and aza-β^3-peptides (middle). The hydrazinoturn network (bottom).

TABLE 1. Yields of Macrocyclization

compd nos.	R_1	R_2	R_3	R_4	yields (%)
1	Bn	Bn	Bn	Bn	76
2	Bn	Bn	Bn	4-HO_2C-Bn	50
3	3-HO-Bn	3-MeO-Bn	3-HO-Bn	3-MeO-Bn	78
4	3-HO-Bn	Me	Bn	^{i}Bu	71
5	Bn	^{i}Bu	Bn	^{i}Bu	74
6	H	^{i}Bu	H	^{i}Bu	40[a]
7	H	Bn	H	Bn	35
8	3-pyridyl methyl	Bn	3-pyridyl methyl	Bn	65
9	3-pyridyl methyl	^{i}Bu	3-pyridyl methyl	^{i}Bu	59
10	2-pyridyl methyl	^{i}Bu	2-pyridyl methyl	^{i}Bu	15
11	3-HO-Bn	Me	2-pyridyl methyl	^{t}Bu	27

[a] 95% starting from **5**.

pyramidal inversion.[19] These conformational properties are revealed to be very favorable for macrocyclization and allowed us to obtain a series of aza-β^3-cyclohexapeptides in high yields.[20] These macrocycles, as well as their acylated derivatives,[21] are characterized by an internal hydrazinoturn network that gives them a highly ordered conformation and controls the relative configuration of the N^α nitrogen atoms. Thus, it was interesting to check the possibility of obtaining CTPs analogues showing similar properties. We present here a preliminary study bound to show the potential of aza-β^3-CTPs as readily accessible CTP analogues. The structural parameters that govern the efficiency of cyclization are discussed. The conformation of the macrocycle is analyzed on the basis of NMR experiments and X-ray structures.

Tetrameric precursors were assembled following our previous papers.[17,19a] Cyclizations were performed in DCM containing an excess of EDCI/HOBT, by adding slowly a solution of the fully deprotected tetramers to obtain a final concentration of around 1 mM.[20] The yields of macrocyclization are gathered in Table 1. Compounds bearing pyridyl methyl side chains were obtained in very different yields depending on the position of

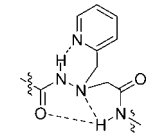

FIGURE 2. C6-conformation possibly disturbing the blue NH to participate to the hydrazinoturn network.

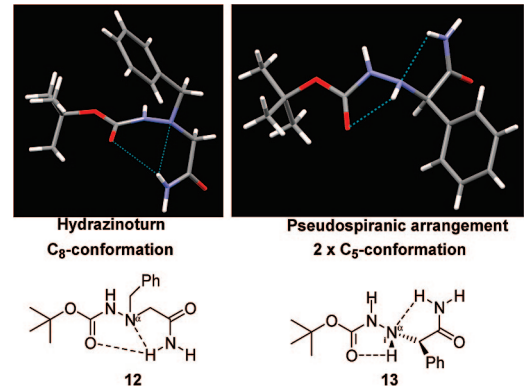

FIGURE 3. Comparative X-ray structures of compounds **12** and **13** showing the divergences between internal-bond networks.

the nitrogen atom inside the aromatic ring (compounds **8**, **9** versus **10**, **11**). We assume that this phenomenon is related to the possible H-bonding of the 2-pyridyl ring nitrogen atom with the adjacent hydrazidic NH, closing a six-membered pseudocycle (C_6-conformation). This interaction could disturb the hydrazinoturn network (C_8-conformation) of the precursor in such a way that the head-to-tail folding should be energetically disfavored (Figure 2).

Compounds **6** and **7**, containing glycine analogues, are also obtained with lower yields. Compound **6** can nevertheless be quantitatively prepared from compound **5** by debenzylation in the presence of Pd/C and a catalytic amount of acetic acid. Compounds containing glycine analogues are synthetically useful as they can be functionalized by reaction with electrophiles.[21] The presence of the unsubstituted $N^\alpha H$ group of the hydrazinoglycine is a competitive reactive site during the coupling steps and thus a possible yield-limiting factor. This point has been previously discussed.[22] The $N^\alpha H$ group could also be involved in alternative conformations. In fact, during the study of small molecular models related to aza-β^3-peptides, we observed interesting divergences between the solid state structures of compounds **12** and **13** (Figure 3). While compound **12** is organized in the classical hydrazinoturn (left), compound **13** adopts an extended conformation, the $N^\alpha H$ being H-bonded to both the amide NH and the N-terminus carbonyle ("pseudospiranic" or 2 × C_5-conformation, right). The two conformations differ by a 180° rotation around the N−N bond, but both respect the orthogonal disposition of the adjacent nitrogen lone pairs, which is energetically favorable.[23]

The differences in the solid state also found expression in solution. Actually, the $\Delta\delta$ value between the two amide NHs of compound **13** is 1.60 ppm (see the Supporting Information, S23), significantly lower than the canonical $\Delta\delta$ value (around 2.60 ppm) associated with the hydrazinoturn like in **12**.[19b] The significant decrease of the $\Delta\delta$ value is very presumably related to the possible competition between the two conformations in

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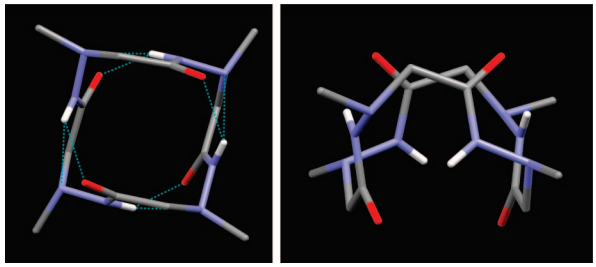

FIGURE 4. Cristal structure of compound **8**. Top and side views. For clarity, original side chains are replaced by methyl groups, and all hydrogen atoms except NHs have been removed. H-bonds appear as dotted lines.

solution in the case of compound **13**. Combined together, the experimental observations and the discussion concerning the lower yields observed for compounds **6**, **7**, **10**, and **11** seem to indicate that the integrity of the hydrazinoturn network is important to achieve efficient cyclization. Besides these less favorable cases, all other macrocycles were obtained in appreciable yields, including precursors bearing unprotected functional groups.

The structural elements that modulate the shape of CTPs are also present in aza-β^3-CTPs. This includes cis/trans isomerism at the level of the hydrazidic bond, and up/down orientation of the hydrazide carbonyle with regard to the mean plane of the macrocycle. The nitrogen pyramidal inversion is an additionnal source of conformational flexibility in our case. Yet, the ^{1}H NMR spectra invariably show a single set of sharp signals. No modification occurs by lowering the temperature of the sample to 213 K (see the Supporting Information). Hydrazide NHs, around 9 ppm, almost insensitive to the concentration or addition of DMSO (see the Supporting Information, S26), clearly take part in intramolecular hydrogen bonds. The geminal protons of methylenic groups adjacent to chiral nitrogen centers give rise to AB spin systems, which coalesce by increasing the temperature (around 398 K for compound **4**), while the NHs signals remain unmodified. The all-trans form can be unambiguously assigned referring to the carbonyle chemical shifts which range roughly between 167 and 170 ppm. Actually, the cis and trans geometry of the hydrazidic bond can be correlated to the carbonyl chemical shift, with values ranging respectively around 174−175 ppm in the cis-isomer, and not exceeding 170 ppm for the trans-isomer.[20]

By analogy with the cyclohexamer series,[20] we assume that the major conformation of aza-β^3-cyclotetrapeptides is an all-trans form in which the hydrazide NHs are implied in hydrazinoturn, enforcing the chiral sequence to be syndiotactic. These conclusions are supported by X-ray structures. All crystals examined are racemates (except the meso compound **1**), with syndiotactic chiral sequence. Only intramolecular H-bonding is observed, each NH participating in a hydrazinoturn. Viewed from the top, the backbone resembles a square, with the chiral nitrogen atoms at the corners, with the atoms between two consecutive chiral centers being almost coplanar (Figure 4). The side view reveals a wavy form that defines a saddle horse shape. The NH and CO vectors point alternatively upside and downside relative to a mean plane that crosses the four N^{β} atoms, making angles of 45° with this plane. The side chains are oriented alternatively upside and downside in pseudoequatorial positions. The molecular contraction that results from internal H-bonding differentiates strongly this structure from the recently published all-cis structure of the alternative 16-membered cyclic β-tetrapeptoid,[16] giving the macrocycle dimensions which approach those of CTPs in spite of the difference in ring sizes (16-membered versus 12-membered). The conformation of aza-β^3-CTPs diverges also radically from that of the closely related isosteric β^3-CTPs (RSRS isomer). The latter shows an all-trans configuration, with the same relative orientation of the NH and CO groups as in our case, but the vectors point almost perpendicularly to the mean plane of the ring, leading to a nanotubular assembly mediated by intermolecular H-bonding both in the solid state and in solution.[24] The intermolecular/intramolecular switch of the H-bond network, elicited by the $C^{\beta}H/N^{\alpha}$ exchange, thus translates into very different solubilities. While aza-β^3-CTPs dissolve in most common organic solvents (except compound **3**), β^3-CTPs are highly insoluble materials.

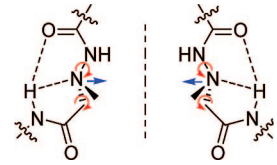

FIGURE 5. Reversal of a hydrazinoturn.

The coalescence, observed at high temperature in $C_2D_2Cl_4$ reflects the fast interconversion between the two mirror images at the NMR time scale (see the Supporting Information, S25). The measured Tc for compound **4** gives an inversion rate between 1 and 0.1 s^{-1}. Despite the less stable conformation the macrocycle must go through during the interconversion process being inherently undetectable, it should be assumed that the reversal of chirality at the level of a hydrazinoturn (Figure 5) requires H-bonds breaking, together with pyramidal inversion at the N^{α} nitrogen atom (blue arrow), and $\pi/2$ rotation around the N−N and N−CH_2 bonds (red arrows). The energy cost associated with the breaking of the cooperative hydrazinoturn network certainly contributes significantly to the short life of the intermediate species.

In summary, during this preliminary work we have performed the synthesis of a series of new CTPs analogues, bearing appreciable side chain diversity on chiral nitrogen atoms. These compounds present a conformation that differ from that of the closely related cyclic β^3-tetrapeptides and cyclic β-tetrapeptoids. The cyclization drives to the diastereoselection of the syndiotactic chiral sequence (*RSRS*/*SRSR*) in an all-trans form stabilized by an internal H-bond network concerning all the hydrazide groups. The macrocyclic backbone undergo a "flip-flop" phenomenon through undetectable transient species. As for the immunosupressive undecapeptide Cyclosporine A, which binds to the cytoplasmic protein cyclophilin,[25] it has been shown recently that internal H-bonding in cyclic peptides enhances their ability to cross cell-membrane models and thus to possibly address intracellular targets.[26] This property should give interesting biological application to aza-β^3-CTPs and aza-β^3-cyclohexapeptides. The abiotic character of these compounds is also of potential pharmacological benefits, as it can confer protection from biological degradation.[27] Compounds **4** and **11** bear side chains which are related to the substitution pattern of the

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neuropeptide Leu-Enkephaline, showing that the extension of the synthesis to biologically relevant targets is conceivable.

Experimental Section

General Macrocyclization Procedure for Compounds 1−11. Typically, a Boc-aza-β^3-OH tetramer (1 mmol) was treated with a mixture of DCM/TFA (6 mL/4 mL) during 12 h. The excess of TFA was then coevaporated under reduced pressure with toluene (3 × 20 mL) then ether (3 × 20 mL) until a white foam appeared. The crude residue was dissolved in 20 mL of DCM and 10 mmol of triethylamine was added. This solution was poured drop by drop into a solution of EDCI (8 mmol) and HOBT (8 mmol) in 1.5 L of DCM. The reaction was stirred vigorously for 72 h (unoptimized). The volume was then reduced to around 100 mL. The addition of 20 mL of 1 N HCl under stirring gave rise to the apparition of a white solid (HOBT, HCl), which was filtrated by succion. The solution was then washed successively with 20 mL of 1 N HCl, twice with 20 mL of water, and twice with 20 mL of 1 N $NaHCO_3$, dried on Na_2SO_4, and evaporated. In the cases of compounds **1**−**5**, **8**, and **9**, the crude reaction product was obtained as an off-white powder that was purified by flash chromatography (DCM/ EtOAc 50/50) and crystallized in EtOAc.

Purification of compounds **6**, **7**, **10**, and **11** was performed on silica gel (DCM, then AcOEt/EtOH 80/20). Monocrystals for **11** were obtained in AcOEt.

Synthesis of 6 starting from 5: Macrocycle **5** (1 g) was dissolved in 20 mL of methanol containing two drops of acetic acid. Pd/C (10%, 50 mg) was added, and the mixture was stirred for 12 h under hydrogen atmosphere. After filtration on celite, methanol was evaporated. The residue was further coevaporated with toluene (2 × 10 mL) then with DCM (2 × 10 mL) to afford **6** quantitatively as a white powder.

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Supporting Information Available: Experimental details, NMR spectra of compounds **1**−**13**, and X-ray crystallography data for compounds **4**, **8**, **11**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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