

Macrocycles

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**Efficient Macrocyclization of Preorganized
Palindromic Oligosquaramides*****Carmen Rotger,* M. Neus Piña, Manuel Vega,
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Nature has evolved polymers with highly specific functions. In the last decade, a new generation of natural circular proteins has been found in bacteria, plants, and mammals.^[1] These large cycles show exceptional stability and a wide range of

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biomedical activities, from insecticidal and antimicrobial to anti-HIV properties. Furthermore, they are ideal templates for engineering similar macromolecules of non-natural oligomers for potential biomedical applications.^[2]

The incorporation of turn segments in the linear precursor is frequently the strategy of choice for cyclization reactions.^[3] However, to our knowledge, examples of successful macrocyclization that occur with non-natural hydrogen-bonded self-templated precursors in polar solvents are rare.^[4] Herein, we report on the application of preorganized oligosquaramides of different sizes, for the synthesis of tailor-made macrocycles in methanol.

The most studied families of non-natural oligomers with well-defined conformations in solution are aliphatic,^[5] but several aromatic oligomers based on amide,^[6] urea,^[7] and hydrazide^[8] linkages have also been shown to adopt stable conformations in solution. Such molecules, termed foldamers,^[9,5b] represent a significant step forward to achieving fully synthetic protein analogues. Among those, squaramides, which can be considered as vinylogous amides, are versatile molecules with considerable hydrogen-bonding capabilities and favorable dynamic properties that allow them to adopt secondary structures. Previously, we have shown the ability of some secondary disquaramides, stabilized by an intramolecular hydrogen bond, to fold into a U-turn module in polar and nonpolar solvents.^[10]

The new family of oligomer reported herein is based on squaramide modules and aliphatic linkages, as represented by the general structure shown in Figure 1. These oligomers are palindromically constituted with a flexible backbone and have a related structure to the foldable module.

The modular synthesis of the novel oligosquaramides **1–5** was achieved through standard condensation reactions of the squaramide building blocks in good-to-moderate yields (55–95 %).^[11]

The resulting structural symmetry of **1–5** is readily apparent in the 1D ¹H NMR spectra recorded in CDCl₃ (Figure 1). The peaks are relatively sharp, and the resonance for the squaramide hydrogen atoms are concentration-

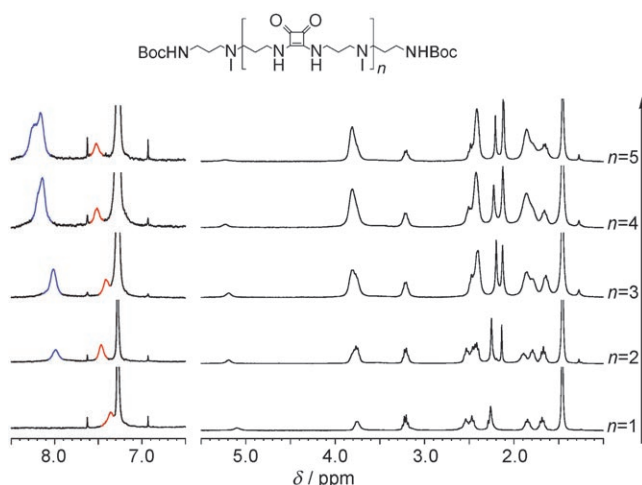


Figure 1. Top: General structure of the oligosquaramides ($n=1-5$ for **1–5**, respectively; Boc = *tert*-butoxycarbonyl). Bottom: Partial ¹H NMR (300 MHz) spectra of **1–5** in CDCl₃.

independent (0.5–20 mM) and shifted downfield relative to the corresponding chemical shift found for squaramide hydrogen atoms ($\delta=6.2-5.4$ ppm), which lack hydrogen-bonding potential.^[10] Compounds **2–5** show two different peaks assignable to the squaramide hydrogen atoms at $\delta=7.5$ and 8.2 ppm, respectively, thus suggesting that they reside in different chemical environments. The downfield area of the squaramide hydrogen peaks varies depending on the number of squaramide modules in the oligomer skeleton, and the integrated area of the upfield peak remains equal to two in all the oligomers.

Stabilization of a well-defined and stable folded conformation with common structural features to all the oligomers was studied by ¹H NMR and UV/Vis spectroscopy. A diffusion NMR experiment (DOSY) of **1–5** in CDCl₃ completely discards any aggregation at the oligomer level, and a good correlation was found for the logarithms of molecular weight and the diffusion coefficients (Figure 2).

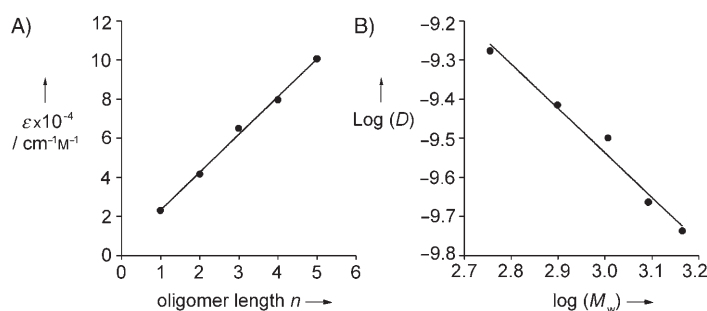


Figure 2. A) The molar extinction coefficients (ϵ at 292 nm) in CHCl₃ versus oligomer length (n). B) Logarithmic plot of the diffusion coefficient (D) versus the molecular weight (M_w) of the oligomer.

Furthermore, variable-temperature (VT) ¹H NMR experiments from 243 to 298 K performed in CDCl₃ of samples of **1–5** (2 mM) revealed a small upfield shift for all the squaramide protons signals. The observed coefficients for the rate of change of the NH squaramide protons with temperature are less than or equal to -6.56 ppb K⁻¹, as expected for intramolecularly hydrogen-bonded protons.^[12] It is also observed that the values of these coefficients decrease when the number of squaramide units in the oligomer skeleton increase. The NH squaramide peak at $\delta=8.2$ ppm in **3–5** splits at 283 K, thus suggesting the existence of a dynamic process involving the hydrogen-bonded protons.

The UV/Vis spectra of **1–5** were also recorded in CDCl₃. The molar extinction coefficient (ϵ) was determined for each oligomer over the range of concentrations 10^{-4} – 10^{-5} M. The Lambert–Beer law was observed for each compound, as expected for monomeric systems. The calculated ϵ values at 292 nm ($\epsilon=23\,088$, $41\,585$, $64\,921$, $79\,544$, and $100\,559$ M⁻¹ cm⁻¹ for **1–5**, respectively) exhibit linear relationships with the number of squaramide residues in the strand, thus implying an absence of stacking for the squaramide chromophore as a result of the secondary structure (Figure 2).

From these data, we obtained preliminary results that support the hypothesis that the oligosquaramides prefer a folded structure in solution, driven by intramolecular hydrogen-bonding interactions that increase with oligomer length.

To further characterize the U-turn module for the folded oligosquaramides, **6** was synthesized and an NMR ROESY spectroscopic experiment was conducted in CDCl_3 (Figure 3). Cross-peaks were observed between the *tert*-butyl protons (f) and the NH (a) and α -methylene (c) protons of the *n*-butyl side chain. The α' -methylene protons (d) give also a cross-peak with the methylene protons (e) of the Boc-protected alkylamino chain. From this data, we propose a folded structure for **6** in which the squaramide proton (b) is hydrogen-bonded to the *N*-methyl donor atom, thus forming a six-membered ring as observed in related systems.^[10] Moreover, an additional hydrogen-bond interaction occurs between the carbamate carbonyl group and the squaramide proton (a), which stabilizes the folded structure.

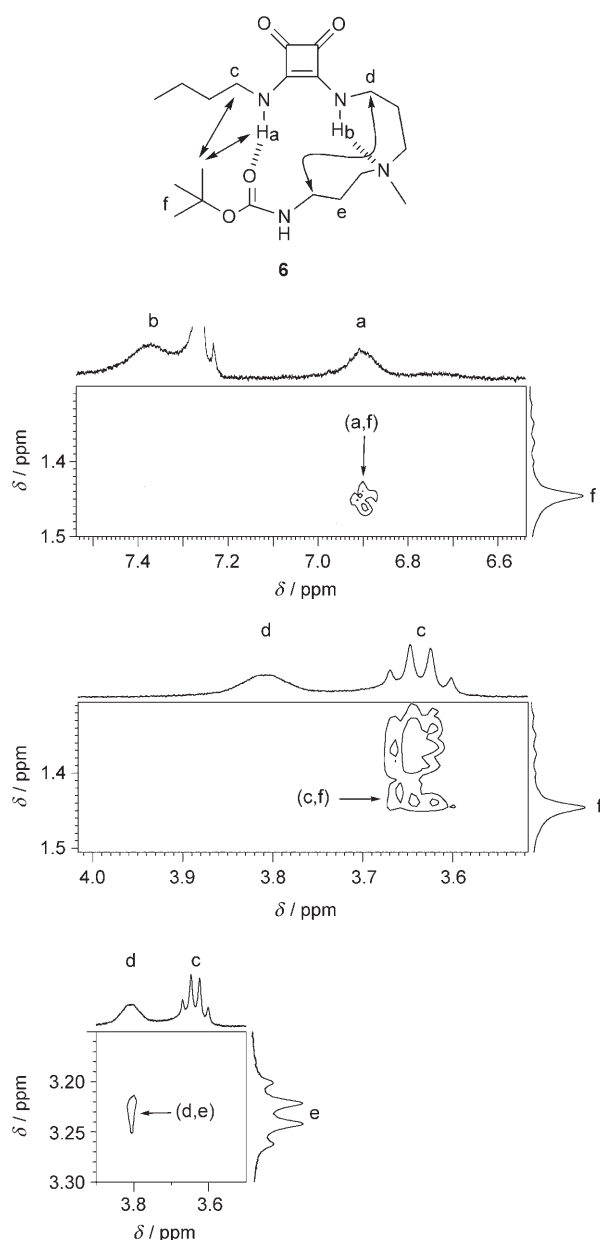


Figure 3. Top: Folded conformation of **6** showing H atom labels and the corresponding NOE interactions. Bottom: NOE interactions as revealed by the NOESY spectrum of **6** in CDCl_3 .

NOE correlations were more difficult to identify for longer oligomers because of the C_2 symmetry of the strand. Under the assumption that all the oligomers have the characterized U-turn in common and all the NH protons are intramolecularly hydrogen-bonded and distributed in two different chemical environments, related folded structures can be anticipated to occur in longer oligosquaramide skeletons in a hairpin-like structure. In these cases, more interactions between the two strands occur and contribute to the stabilization of the folded conformation (Figure 4). As a result of the palindromic nature of these molecules, a dynamic process will occur in which the hydrogen-bonding pattern slips from one end to other, thus yielding conformational and energetically equivalent structures in solution.

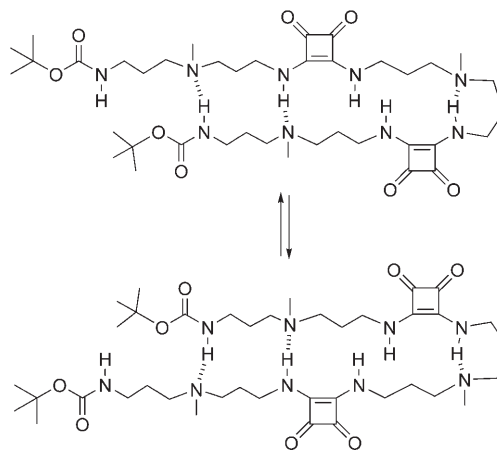


Figure 4. Proposed folded structure for **2** and the slipping equilibrium end-to-end of the hydrogen-bonding pattern.

Typically, the folded structures driven essentially by hydrogen-bonding interactions have been detected in non-protic solvents.^[5] The proposed folded state for the oligosquaramides resists competition from externally added protic solvents, such as MeOH.^[13] The ^1H NMR spectra of **2** and **4** at 285 K (3 mm) in $\text{CDCl}_3/\text{MeOH}$ mixtures containing up to 40% MeOH display little perturbation and persistent deshielded squaramide NH signals, as expected for intramolecular hydrogen bonds; however, the NH carbamate signals move upfield, thus showing solvent exposure.

Differential scanning calorimetry (DSC) experiments were run on the oligosquaramides **2–5** to fully characterize the folded structures in protic solvents, ($\text{EtOH}/\text{CHCl}_3$ (95:5, v/v)) by increasing the temperature from 10 to 80 °C at a rate of 10°C h^{-1} (Figure 5). All the tested oligomers undergo a sharp and reversible structural transition as a function of temperature, thus indicating the existence of a well-defined secondary structure. The excess heat capacity peak is complete within a 15–20 °C range, evident of a cooperative nature of the oligomer unfolding.^[14] The ΔH values obtained for the unfolding processes (0.16, 0.32, 0.48, and 0.61 kcal mol^{-1} for **2–5**, respectively) show a clear dependence on the chain length of the oligomers, which is consistent with other observed data.

The melting temperature ($T_m = 63 \pm 2^\circ\text{C}$) was found to be similar for all the tested oligomers and comparable to those

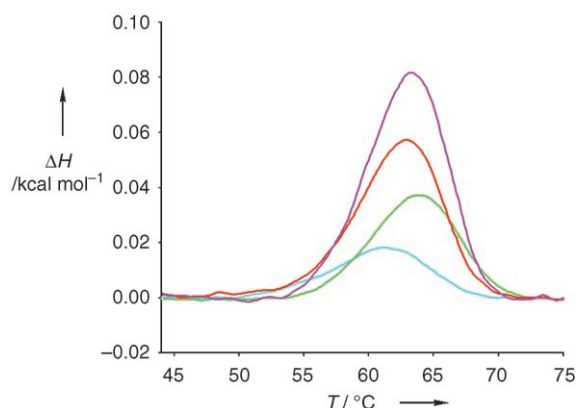


Figure 5. DSC melting curves of **2** (blue), **3** (green), **4** (red), and **5** (purple).

determined for unfolding of the hairpin peptide structure in water.^[15] The ΔH value per residue ($\approx 0.1 \text{ kcal mol}^{-1}$) reflects, mainly, the hydrogen-bond component because the solvophobic effect, as a result of the alkyl chains of the oligoamide skeleton, is assumed to have a minimum contribution on the overall folding–unfolding process.

As we have shown, the proposed folded structures for the oligosquaramides constitute a series of self-organized precursors applicable to one-step macrocyclization reactions in protic solvents without standard high-dilution conditions. Squaramides are particularly well suited to take advantage of the template effect in alcoholic solvents, given that the condensation of amines with squaramide esters is routinely carried out in ethanol or methanol. Cleavage of the Boc groups leaves two reactive amino groups in each oligomer that are close enough to react with a molecule of diethylsquarate in a one-step cyclization reaction to obtain the corresponding macrocycles. Therefore, the corresponding diamines of oligosquaramides **2–5** were condensed in methanol at millimolar concentrations with one equivalent of diethylsquarate. In all the cases, the macrocyclization of the oligosquaramides gave very high and comparable yields (80%) of the corresponding macrocycles **7–10** (Figure 6).^[11] Further oligomerization was not detected by ESI MS studies of the crude product. These results support the dominance of the folded structure for the oligosquaramides under the macrocyclization reaction conditions. The generality of the oligosquaramides macrocyclization is also manifest. In some examples of highly efficient one-step macrocyclizations

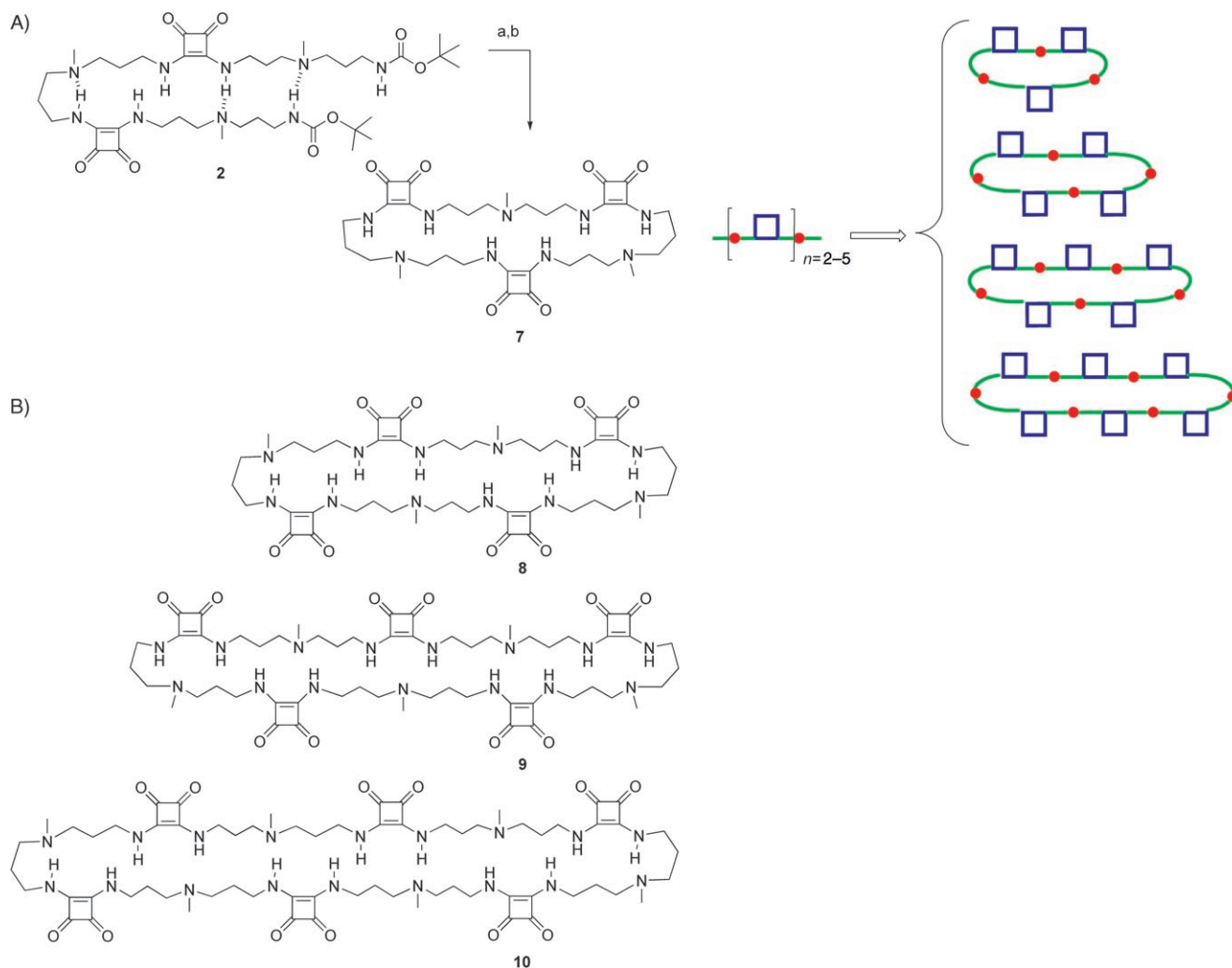


Figure 6. A) Synthesis of macrocycle **7**: a) HCl, 50°C, 3 h; b) diethyl squarate, MeOH, room temperature, 12 h. B) Structures of macrocycles **8–10**.

assisted by folding of precursor oligomers, the macrocycle sizes are limited to a determined length because of the rigidity of their backbones.^[16] The nature of the folded structure adopted by the squaramides permits no size restrictions on the macrocycle synthesis. Therefore, a versatile synthetic strategy to obtain tailor-made macrocycle cavities with squaramide groups, which maintain their considerable hydrogen-bonding capability, has been demonstrated.

In summary, we have demonstrated that oligosquaramides of different length that contain a donor atom (N) in the δ position of the alkyl linker chains, fold to form stable monomeric structures that possess a hairpin-like pattern driven by intramolecular hydrogen-bonding interactions.^[17] The stability of the folded structure increases with oligomer length, but the shortest oligomers also display a well-defined and stable conformation. The characterized folded structures are stable in polar solvents, as indicated by denaturation studies and DSC experiments. Thus, the preorganization shown by the oligosquaramide skeleton make them useful precursors in macrocyclization reactions. The study presented herein is the first example of how unnatural oligomers with designed folded structures driven by hydrogen-bonding interactions can be used to efficiently yield large macrocyclic structures.

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