Organic LETTERS

Letter

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Assessing Stabilization through $\pi-\pi$ Interactions in Aromatic Oligoamide β-Sheet Foldamers

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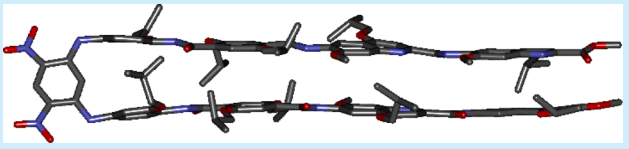
Supporting Information

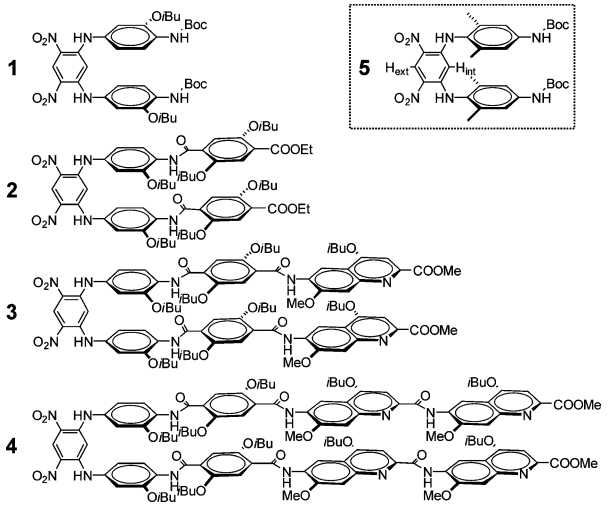
ABSTRACT: We have recently introduced aromatic oligoamide β-sheet foldamers based on rigid turn units and short linear strands that undergo intramolecular $\pi-\pi$ stacking (Sebaoun, L.; Maurizot, V.; Granier, T.; Kauffmann, B.; Huc, I. *J. Am. Chem. Soc.* **2014**, *136*, 2168). We now report that conformational stability in these structures can be reached using less rigid turn units and more extensive $\pi-\pi$ interactions between longer linear strands. For this study, two-stranded sheets of variable length were prepared. Their conformation was assessed in solution by ^{1}H NMR and in the solid state by X-ray crystallography.



In comparison with helical synthetic foldamers, sheet-like structures have much less frequently been observed.[1] A possible reason for this may be that, when removed from the context of a tertiary structure as found in proteins, sheets tend to extensively aggregate and precipitate which makes them difficult to isolate and characterize. In contrast, helices generally are well-behaved discrete and soluble objects that fulfill their potential for noncovalent interactions intramolecularly. Indeed, synthetic foldamer sheets based on hydrogen-bonding are often designed to be dimeric[2] in order to keep aggregation and solubility under control. Thus, the design of a soluble multistranded β-sheet foldamer would entail the orchestration of noncovalent interactions so that they operate preferentially in an intramolecular fashion, thereby preventing extensive aggregation. Following this principle, we recently introduced aromatic oligoamide β-sheet foldamers stabilized by $\pi-\pi$ interactions between short linear segments and rigid turn units such as **5** (Chart 1) that hold them in a face-to-face orientation.[3] A key feature of **5** lies in its four methyl groups *ortho* to the aromatic amine functions holding the two xylyl rings parallel to each other and perpendicular to the dinitrobenzene unit, as reflected by strong ring current effects which cause a large upfield shift of the NMR signal of the proton *meta* to both nitro groups pointing toward the interior of the structure (H_{int}).[3] As an extension to this initial design, we hereby demonstrate that rigidity at the turn units can be loosened and compensated by enhanced $\pi-\pi$ interactions between extended linear aromatic oligoamide segments, without causing aggregation.

Turn unit **1**, which lacks the methyl groups of **5**, was prepared from 4,6-dinitro-1,3-difluorobenzene using previously described procedures.[3] Compound **1** possesses two Boc-protected amine functions meant to attach linear strands and form sheet structures. Two isobutoxy chains were introduced to enhance its solubility in nonprotic organic solvents and to favor a coplanar orientation of each *para*-phenylenediamine units and its appended linear aromatic oligoamide segment, imparted by an intramolecular hydrogen bond between the terminal NH and adjacent isobutoxy oxygen atom, as found in other aromatic oligoamide foldamers.[4]

Chart 1. Two Stranded Aromatic Oligoamide β-Sheets Possessing Linear Segments of Increasing Length



Received: February 18, 2014
Published: April 14, 2014

   dx.doi.org/10.1021/ol500512f | *Org. Lett.* 2014, 16, 2326−2329

Trimeric turn **1** was first elongated using terephthalic acid monomers to form pentamer **2**. In order to substantially enhance face-to-face intramolecular $\pi-\pi$ stacking, it was anticipated that larger units than simple benzene derivatives would be necessary. For this purpose, we introduced a new monomer, 6-amino-4-isobutoxy-7-methoxy-2-quinolinecarboxylic acid. As described in the Supporting Information, its synthesis follows routes developed for other amino-2-quinolinecarboxylic acid monomers.[5] The main feature of the new monomer is that the relative orientation of its 6-amine and 2-acid functions will confer a linear structure to oligoamide segments in which it is inserted.[6] Elongation of pentamer **2** proceeded via the saponification of its terminal esters and the subsequent coupling of two quinoline monomers or two quinoline dimers to yield **3** and **4**, respectively.

The chemical shift values of the H_{int} protons was monitored in $CDCl_3$ as a probe of the conformation of the new oligomers in this solvent (Figure 1), as they were found to undergo

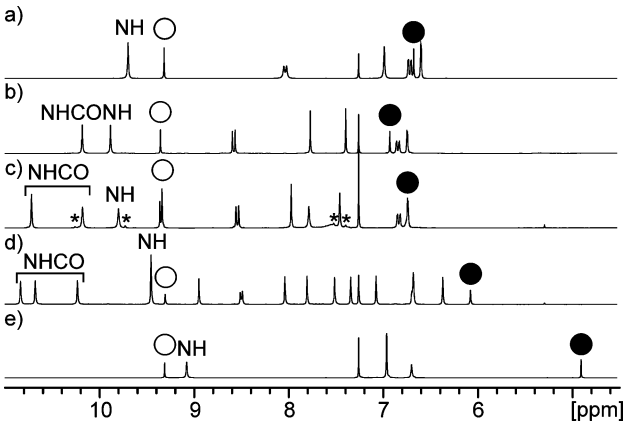


Figure 1. Part of the 300 MHz ^{1}H NMR spectra in $CDCl_3$ at 25 °C of (a) hairpin turn trimer **1**; (b) hairpin turn pentamer **2**; (c) hairpin turn heptamer **3**; (d) hairpin turn nonamer **4**; and (e) hairpin turn trimer **5**. Signals of H_{ext} are marked with white circles. Signals of H_{int} are marked with full black circles. Stars indicate signals belonging to an impurity.

significant variations while δ_{Hext} remained essentially unchanged. In rigid turn **5**, this resonance is strongly upfield shifted (δ_{Hint} = 4.91 ppm), while the absence of a methyl group in **1** makes its structure more flexible and significantly reduces intramolecular ring current effects (δ_{Hint} = 6.68 ppm). Upon elongation of trimer **1** into pentamer **2** and heptamer **3**, no major change is observed; H_{int} resonances actually undergo minor downfield shifts. In contrast, a strong upfield shift is observed between heptamer **3** (δ_{Hint} = 6.80 ppm) and nonamer **4** (δ_{Hint} = 6.08 ppm, $\Delta\delta$ = 0.72 ppm). The effect on δ_{Hint} of the additional quinoline monomers at the end of the linear strands from **3** to **4** is very remote and suggests a better defined conformation of **4** leading to stronger ring current effects upon H_{int}. The nonlinear trend of δ_{Hint} values from **1** to **4** is indicative of cooperative effects.[7]

A series of multidimensional NMR experiments (HSQC, HMBC, TOCSY, and ROESY) allowed us to fully assign the ^{1}H NMR spectrum of **4**. As shown in Figure 2, ROESY experiments revealed contacts that unambiguously establish a folded two-stranded hairpin structure of **4** in solution. In particular interstrand (as opposed to intrastrand) correlations are demonstrated when they occur between protons that are too distant to establish a contact if they would belong to the

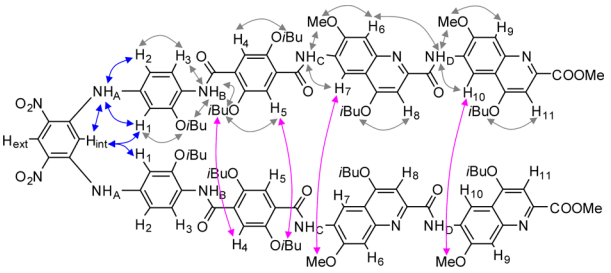


Figure 2. Schematic representation of ^{1}H−^{1}H correlations observed in 400 MHz ROESY-2D spectra of nonamer **4** at 25 °C in $CDCl_3$. Blue arrows represent correlations within the hairpin turn; pink arrows represent interstrand correlations; other correlations are represented by gray arrows.

same aromatic ring such as, for example, 7-methoxy protons and the proton in position 5 of equivalent quinoline rings (OMe/H_7 and OMe/H_{10} correlations in Figure 2). In contrast, no such correlations were observed in the spectra of heptamer **3**. Thus, the greater flexibility of turn **1** as compared to turn **5** can be compensated by extensive intramolecular $\pi-\pi$ interactions between long linear strands. All observed correlations, and in particular OMe/H_7 and OMe/H_{10} (Figure 2), are consistent with an antiparallel orientation of the two strands of **4**, with each ring head-to-tail with respect to the ring on which its stacks, as observed previously with multistranded sheets having shorter linear segments.[3] Yet the existence of conformers with a parallel orientation of the two strands of **4** cannot be completely ruled out from solution studies. Indeed, the interstrand NOE correlations expected in a parallel arrangement may not be distinguished from intrastrand correlations, unless the two strands are strongly offset in which case interstrand correlations may match with those expected in an antiparallel arrangement of the two strands. Spectra recorded over a wide temperature range (−50 to 40 °C) did not reveal any major change (Figure S2), suggesting that the same structure prevails over this range. Even at low temperature, the diastereotopicity of side chain isobutoxy protons does not appear in the spectrum, indicating the fast rotational dynamics of each linear strand with respect to the other. As a final point worth noting, it is unclear whether the H_{int} resonance in the folded conformation of **4** remains at lower field compared to the case of **5** because the latter is still better organized, because the former adopts a conformation at the turn in which ring current effects are weaker, or because ring currents are simply less intense in the former due to the different substitution patterns of its *para*-phenylenediamine rings.

Solid state investigations were undertaken and fully confirmed solution data. Pentamer **2** was crystallized with two independent molecules in the asymmetric unit with both showing divergent linear segments with no apparent stacking interactions between the *para*-phenylenediamine units or the terminal terephthalic acid units (Figure 3a, 3b). In contrast, the crystal structure of **4** (Figure 3c, 3d) revealed a folded structure with extensive face-to-face $\pi-\pi$ overlap between its two linear segments. The dinitrobenzene turn is perpendicular to the linear strands, which are found to be in an antiparallel orientation, presumably favored by local dipole−dipole interactions. Consistent with solution data, quinoline 7-methoxy protons are found to be much closer (3.7 Å) to protons in position 5 of the quinoline stacked above them than

dx.doi.org/10.1021/ol500512f | *Org. Lett.* 2014, 16, 2326−2329

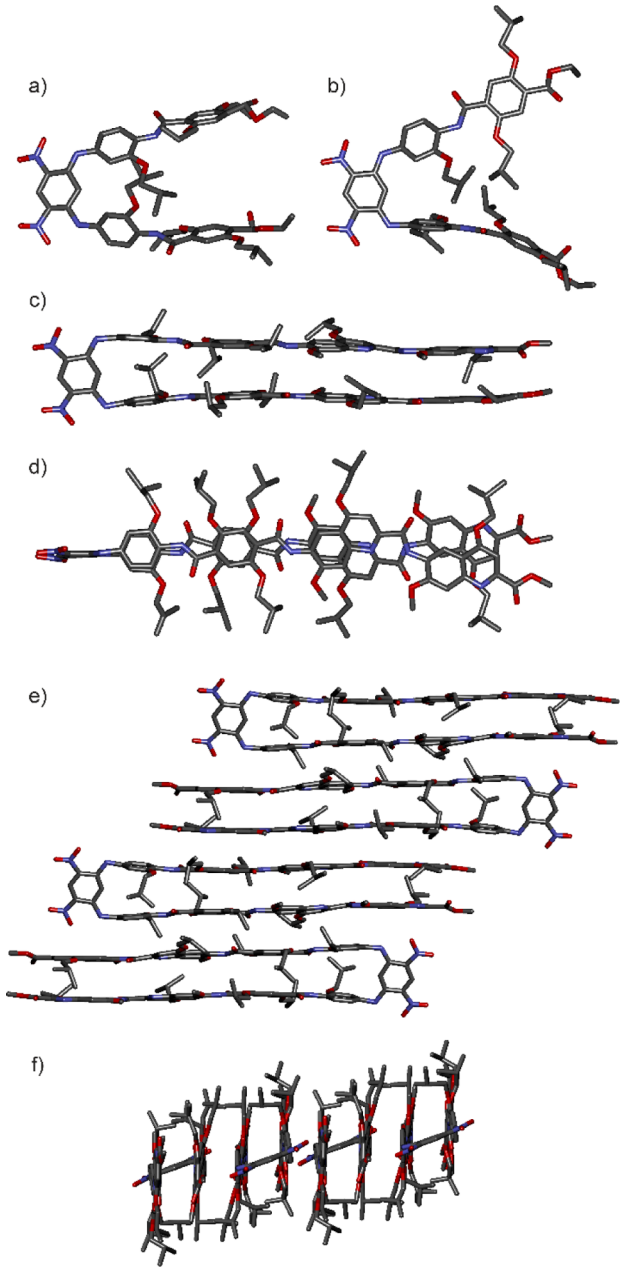


Figure 3. Side views of the crystal structures of (a) form A of hairpin turn pentamer **2**; (b) form B of hairpin turn pentamer **2**; (c) hairpin turn nonamer **4**; and (d) its top view. Packing of **4** in the crystal, (e) side and (f) front views. Protons and included solvent molecules have been removed for clarity.

to the protons in position 5 of the quinoline to which they belong (5.7 Å). It is apparent in the structure of **4** that the interstrand distance imparted by the turn unit (4.7 Å) is too large for tight aromatic stacking to occur in short sequences such as **1** and **2**. Only the long strands of **4** compensate for this impediment, allowing its quinoline rings to stack at a distance of 3.4 Å.

The packing of **4** in the crystal shows extended head-to-tail stacks suggestive of a multistranded sheet-like aggregation mode (Figure 3e). Nevertheless, this organization is restricted to the solid state, and compound **4** shows good solubility (>13 mM in $CDCl_3$) and concentration independent chemical shift values up to 13 mM indicating limited aggregation in solution. Thus, the face-to-face $\pi-\pi$ interactions that stabilize the two-stranded hairpin-turn structure of **4** are strong enough to be effective intramolecularly and weak enough not to prevail intermolecularly.

In summary, we have shown that $\pi-\pi$ stacking may direct the folding of extended two-stranded β-hairpin structures in an organic solvent even when a relatively flexible turn unit is used. The balance between intra- and intermolecular interactions allows folding to occur and aggregation to be prevented. These objects are reminiscent of related foldamer structures including some crescent-like macrocycles,[8] zippers based on oligoanthranilamides,[9] and dimeric, trimeric, and tetrameric aromatic oligoamide β-helices.[10] It is anticipated that folding and aggregation of such β-hairpins will operate differently in protic media due to the very strong solvophobic component that makes $\pi-\pi$ stacking much stronger in these solvents.[11] Research along this line is in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic schemes, experimental procedures, full characterization of new compounds, crystallographic data, detailed NMR investigations including complete ^{1}H NMR assignment and solution structure elucidation of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the French Ministry of Research (predoctoral fellowship to L.S.) and by the European Research Council under the European Union's Seventh Framework Programme (Grant Agreement No. ERC-2012-AdG-320892).

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