Helical Structures

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Interstrand Interactions between Side Chains in a Double-Helical Foldamer**

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Not so long ago the propensity of a polymeric or oligomeric molecular strand to fold into a well-defined conformation in solution was believed to be mainly a property of biopolymers such as proteins and nucleic acids. Recent studies on foldamers[1]—artificial oligomers that also adopt well-defined folded conformations—have led to a fundamental paradigm shift, and biopolymers are now considered to be one of many classes of molecules that fold. Up to now, studies on foldamers have focused on the prime sources of folding, which are main-chain (backbone) conformational preferences and main-chain/main-chain interactions.[1] In peptides and proteins, side-chain/side-chain interactions within and between helices and β sheets are also key to the stabilization of the folded structures,[2] yet their role in the foldamer structure is largely unexplored. Hydrogen bonding between side chains has been incorporated to stabilize some artificial helices.[3] In aromatic structures, interactions between chiral side chains determine helix handedness[4] (unlike in peptides where handedness is induced by side-chain/main-chain interactions), but their nature is not well defined. Even in β peptides—arguably the most studied foldamer family—the helix propensity of side chains has been studied,[5] but examples of direct side-chain/side-chain interactions are rare.[6]

Here, we describe an original example of attractive interactions between side chains in a synthetic foldamer structure. Specifically, we show that interstrand interactions between aromatic side chains can considerably stabilize the double-helical dimers formed by aromatic oligoamides (AOAs) of 2,6-pyridinedicarboxylic acid and 2,6-diaminopyridine. Like many other AOAs,[7] these oligomers adopt very stable single-helical conformations in solution.[8,9] However, the pyridine-derived helices also possess the remarkable

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ability to extend like springs and intertwine to form double-helical dimers held by direct noncovalent interactions between the strands (Figure 1).[8,10,11] This is a very rare[12] example of a hybridization motif between two organic strands to form a double helix that is neither derived from naturally occurring multiple helices such as DNA, collagen, or gramicidin A, nor assisted by metal-ion coordination.[13]

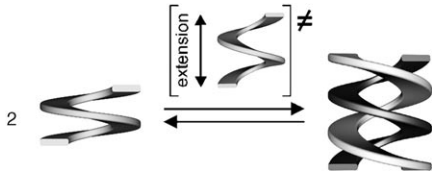
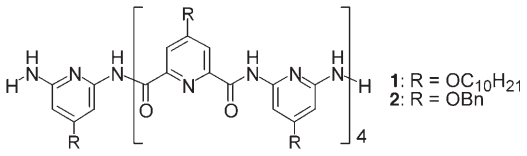


Figure 1. Equilibrium between the single-helical and double-helical structures.

The peculiar hybridization of pyridine carboxamide oligomers to form double helices has been characterized in solution in chlorinated solvents and in the solid state[8,10] and by a computational approach.[11] From these studies, it can be concluded qualitatively that hybridization is driven by intermolecular aromatic stacking, which compensates for the energy cost of extending and doubling the helical pitch of the single-helical monomers. However, numerous aspects of this phenomenon remain unclear. For example, very early on we observed that the presence of 4-decyloxy groups on all pyridine rings leads to a dramatic enhancement of the dimerization constant (K_{dim}). In $CDCl_3$, a heptameric strand comprising three 2,6-pyridinedicarbonyl units and four 2,6-diaminopyridine units shows $K_{dim} = 30\ L\,mol^{-1}$ when it has 4-decyloxy chains on the three 2,6-pyridinedicarbonyl rings only, and $K_{dim} = 6.5 \times 10^4\ L\,mol^{-1}$—a 2000-fold increase—when it has 4-decyloxy chains on all rings![8,10a]

To explain this effect, we proposed that extensive van der Waals interactions between the multiple long side chains of adjacent strands would stabilize the duplex.[8,10a] However this hypothesis could not be verified because 4-decyloxy-substituted oligomers such as nonamer **1** do not crystallize. No



solid-state data about their single- and double-helical conformations are available to compare with the crystal structures of oligomers bearing no side chains[8,10] which show much lower K_{dim} values. We thus undertook the synthesis and structural study of 4-benzyloxy-substituted oligomers such as nonamer **2**. This endeavor was motivated by three assumptions, which all turned out to be valid: 1) the benzyloxy derivatives were expected to be more crystalline; 2) interactions between aromatic benzyloxy chains were expected to be even stronger than those between aliphatic decyloxy chains; and 3) unlike the decyloxy derivatives, the benzyloxy series was expected to be soluble in polar aprotic solvents in which hybridization of pyridinecarboxamide oligomers had not yet been observed.

Nonamer **1**, which has two terminal amine residues, was prepared by hydrogenolysis of the corresponding bisbenzylcarbamate precursor.[10c] Benzyloxy-substituted oligomers including nonamer **2** were prepared from 4-benzyloxy-2,6-diaminopyridine[14] and 4-benzyloxy-2,6-pyridinedicarboxylic acid following schemes similar to those employed in other series.[10a] The synthesis of these compounds will be reported in a full account about the effects of the side chains on oligomer hybridization.

K_{dim} values can be recorded easily by measuring NMR spectra at different concentrations. The single and double helices are in slow exchange on the NMR time scale and give rise to two sets of signals that can be integrated. The assignment of the signals to the two species can be made unambiguously based on: 1) the variation of their intensity with concentration and temperature; 2) the fact that signals of the double helix are shifted upfield because of the ring-current effects associated with intermolecular aromatic stacking; 3) the lower symmetry of the double helix which gives rise to twice as many signals in $CDCl_3$;[8,10] and 4) diffusion coefficients measured by NMR pulsed-gradient spin-echo experiments (diffusion coefficients are about 35% smaller for the double helix than for the corresponding single helix).

The ^1H NMR spectrum of decyloxy-substituted nonamer **1** in $CDCl_3$ at 1 mM shows that this compound is largely hybridized at this concentration but that signals of the single helix are also clearly visible (Figure 2a). A value of $K_{dim} = 6 \times 10^4\ L\,mol^{-1}$ was calculated. In contrast, the spectrum of benzyloxy-substituted nonamer **2** shows signals of the double helix and no signals of the single helix. Spectra recorded in other weakly polar solvents (CD_2Cl_2, $C_2D_2Cl_4$, [D_8]toluene) did not show any detectable amount of the single helix either, even at a dilution of 0.1 mM. The assignment of the observed signals to a double-helical dimer is supported both by diffusion coefficient measurements and by electrospray ionization mass spectra, in which the monomer is barely detected, the dimer dominates, and no higher aggregate is observed. Thus, the benzyloxy nonamer apparently forms considerably more stable dimers than its decyloxy analogue. In the absence of signals for the single helix, no accurate K_{dim}

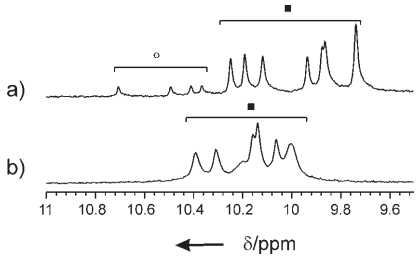


Figure 2. Sections of the ^1H NMR spectra (400 MHz, 25 °C, 1 mM in $CDCl_3$) of a) **1** and b) **2** at showing the amide resonances of the single helix (○) and double helix (■).

value could be obtained, but it is estimated that K_{dim} is at least 100 times larger for **2** than for **1**. Since these two compounds possess identical main chains, the difference in K_{dim} can be attributed to side-chain effects.

Unlike **1**, the benzyloxy-substituted nonamer **2** is soluble in DMSO and hybridizes in this solvent as well. As shown in Figure 3, its hybridization in DMSO is significant (K_{dim} = 2300 L mol^{-1}) though much weaker than in less polar solvents.

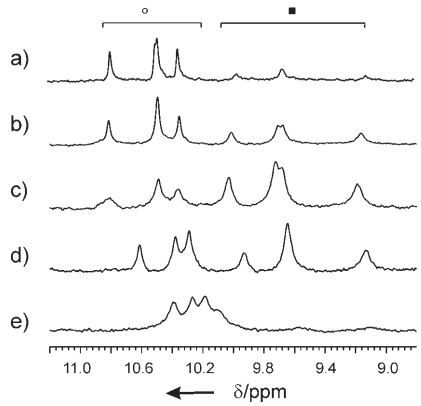


Figure 3. Part of the ^{1}H NMR spectra of nonamer **2** (400 MHz, [D$_6$]DMSO) showing the amide resonances of the single helix (○) and double helix (■): a) 0.12 mM at 25 °C, b) 0.5 mM at 25 °C, c) 4 mM at 25 °C, d) 4 mM at 55 °C, and e) 4 mM at 88 °C.

Thus, the signals of the single helix prevail at low concentration and high temperature. This is the first example of the hybridization of pyridine carboxamide oligomers in this competitive solvent. No hybridization was observed for oligomers having no side chains or with side chains on the pyridinedicarbonyl rings only.[8–10] NMR spectra of **2** recorded in [D$_6$]DMSO at 25 °C, unlike those in CDCl$_3$, show the same number of resonances for the double helix (**2**)$_2$ as for the single helix. This indicates that previously characterized sliding motions within the duplex take place faster in polar solvent and lead to a fast equilibrium between two degenerate duplexes.[8,10]

Clear evidence of interstrand interactions between benzyloxy side chains is found in a crystal structure of nonamer **2**.[15] In view of the very large K_{dim} values of **2** including in the polar solvents (DMSO) that we have often used to grow crystals, we expected the double helix and not the single helix to crystallize. The structure could be solved despite its very large asymmetric unit, which comprise four molecules of **2** entwined in a right-handed and a left-handed double-helical dimer. In fact, the asymmetric unit of **2** appears to be one of the largest all-equal atoms[16] structure solved by ab initio methods.[17]

As shown in Figure 4a,b, the double helix spans two complete helical turns and is one half-turn longer than the longest duplex that we previously described.[8] Since the structure of **2** represents the first X-ray structure of a highly stable duplex with alkoxy substituents on all pyridine rings, we sought for possible structural differences to the structures of oligomers bearing no substituent but could find none. The

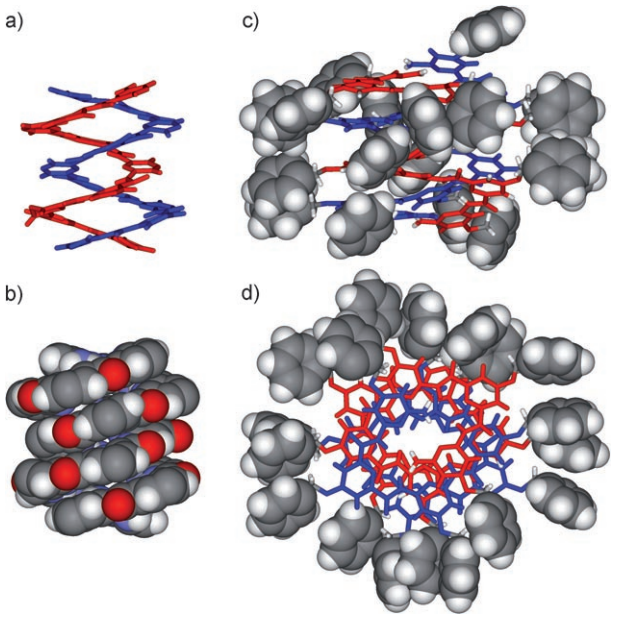


Figure 4. Crystal structure of nonamer **2**. The structures on the left show the double-helical dimer in stick (a) and CPK representations (b). Winding of the two strands produces a three-turn duplex with a pitch of 6.65 Å and with four pyridine–amide units per turn. Benzyloxy groups are omitted for clarity. The stick representation shows that the helical axis of the blue strand is shifted to the right and that of the red strand is shifted to the left. Side view (c) and top view (d) of the dimer showing extensive interstrand aromatic–aromatic interactions between the benzyloxy side chains. The two strands are shown as red and blue sticks and the phenyl rings are represented in CPK. Included solvent molecules are omitted for clarity.

hybridization motif appears in every respect identical to that observed with other oligomers that dimerize more weakly. The two strands are helically shifted by two rings: ring 1 of strand A stacks over ring 3 of strand B and reciprocally. The helix possesses a noncrystallographic C_2 axis along its channel. The helical axes of the two strands are parallel but do not exactly coincide (Figure 4a,b). Contacts between the main chains of the two strands consist essentially of face-to-face aromatic stacking, and of one interstrand bifurcated and presumably weak N–H···N hydrogen bond at one end of the duplex. Some hollows in the helix channel are filled with solvent molecules (H$_2$O, MeOH, or DMSO), but none seems to be engaged in bridging interactions. Other solvent molecules are not involve in direct interactions with the double-helix strands and are simply filling voids in the crystal lattice.

At its periphery, the duplex is surrounded by 18 benzyloxy chains. These are engaged in multiple intraduplex interstrand face-to-face and edge-to-face interactions at various angles (Figure 4c,d). No well-defined motif emerges in the arrangement of these chains and not every benzyl group lies in contact with a benzyl group of the same duplex. In fact, the side chains are also interdigitated (and involved in interduplex interactions) with neighboring molecules. The structure of (**2**)$_2$ may be considered to be a snapshot of one of the many possible conformations of the side chains. Considering the flexibility of the benzyl groups and the mobility of the two

strands relative to each other in the duplex, numerous side-chain/side-chain interactions are expected in solution and are presumably responsible for the observed stability of $(\mathbf{2})_2$. The strong effect of aryl–aryl interactions between benzyl side chains in $(\mathbf{2})_2$ contrasts with the reported absence of favorable aromatic interactions between β-sheet peptides.[18]

In summary, solution and solid-state data both suggest that interstrand interactions between phenyl rings in the side chains contribute to the very large hybridization constant of **2**. Similar, weaker, interactions between the decyloxy chains of **1** can also be envisaged, but they remain to be demonstrated. The remarkable increase in the dimerization constant as a result of aromatic interactions between side chains opens a new space for designing oligomers with complementary electron-donor and electron-acceptor groups and/or with positive and negative charges leading to cross-hybridization and sequence-selective recognition. More generally, the effects reported here may foster new studies of the role that side chains play in stabilizing foldamer structures.

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[1] For reviews see: D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893; R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219; I. Huc, *Eur. J. Org. Chem.* **2004**, 17; R. P. Cheng, *Curr. Opin. Struct. Biol.* **2004**, *14*, 512; G. Licini, L. J. Prins, P. Scrimin, *Eur. J. Org. Chem.* **2005**, 969; D. Seebach, A. K. Beck, D. J. Bierbaum, *Chem. Biodiversity* **2004**, *1*, 1111.

[2] For example: F. A. Syud, H. E. Stranger, S. H. Gellman, *J. Am. Chem. Soc.* **2001**, *123*, 8667; S. T. Phillips, G. Piersanti, P. A. Bartlett, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13737; M. Ramirez-Alvarado, T. Kortemme, F. J. Blanco, L. Serrano, *Bioorg. Med. Chem.* **1999**, *7*, 93.

[3] J. M. Cary, J. S. Moore, *Org. Lett.* **2002**, *4*, 4663; X. Yang, L. Yuan, K. Yamato, A. L. Brown, W. Feng, M. Furukawa, X. C. Zeng, B. Gong, *J. Am. Chem. Soc.* **2004**, *126*, 3148; J. J. L. M. Cornelissen, M. Fischer, N. A. J. M. Sommerdijk, R. J. M. Nolte, *Science* **1998**, *280*, 1427.

[4] R. N. Prince, J. S. Moore, L. Brunsveld, E. W. Meijer, *Chem. Eur. J.* **2001**, *7*, 4150; J. J. van Gorp, J. A. J. Vekemans, E. W. Meijer, *Chem. Commun.* **2004**, 60.

[5] J. A. Kritzer, J. Tirado-Rives, S. A. Hart, J. D. Lear, W. L. Jorgensen, A. Schepartz, *J. Am. Chem. Soc.* **2005**, *127*, 167.

[6] P. Chakraborty, U. Diederichsen, *Chem. Eur. J.* **2005**, *11*, 3208.

[7] Y. Hamuro, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1997**, *119*, 10587; H. Jiang, J.-M. Léger, I. Huc, *J. Am. Chem. Soc.* **2003**, *125*, 3448; V. Maurizot, C. Dolain, Y. Leydet, J.-M. Léger, P. Guionneau, I. Huc, *J. Am. Chem. Soc.* **2004**, *126*, 10049; C. Dolain, J.-M. Léger, N. Delsuc, H. Gornitzka, I. Huc, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16146; C. Dolain, H. Jiang, J.-M. Léger, P. Guionneau, I. Huc, *J. Am. Chem. Soc.* **2005**, *127*, 12943; B. Gong, *Chem. Eur. J.* **2001**, *7*, 4337; B. Gong, H. Zeng, J. Zhu, L. Yuan, Y. Han, S. Cheng, M. Furukawa, R. D. Parra, A. Y. Kovalevsky, J. L. Mills, E. Skrzypczak-Jankun, S. Martinovic, R. D. Smith, C. Zheng, T. Szyperski, X. C. Zeng, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11583; C. Li, S.-F. Ren, J.-L. Hou, H.-P. Yi, S.-Z. Zhu, X.-K. Jiang; Z.-T. Li, *Angew. Chem.* **2005**, *117*, 5871; *Angew. Chem. Int. Ed.* **2005**, *44*, 5725; Z.-Q. Hu, H.-Y. Hu, C.-F. Chen, *J. Org. Chem.* **2006**, *71*, 1131.

[8] V. Berl, I. Huc, R. Khoury, M. J. Krische, J.-M. Lehn, *Nature* **2000**, *407*, 720.

[9] V. Berl, I. Huc, R. Khoury, J.-M. Lehn, *Chem. Eur. J.* **2001**, *7*, 2798; I. Huc, V. Maurizot, H. Gornitzka, J.-M. Léger, *Chem. Commun.* **2002**, 578; C. Dolain, A. Grélard, M. Laguerre, H. Jiang, V. Maurizot, I. Huc, *Chem. Eur. J.* **2005**, *11*, 6135.

[10] a) V. Berl, I. Huc, R. Khoury, J.-M. Lehn, *Chem. Eur. J.* **2001**, *7*, 2810; b) V. Maurizot, J.-M. Léger, P. Guionneau, I. Huc, *Russ. Chem. Bull.* **2004**, *53*, 1572; c) H. Jiang, V. Maurizot, I. Huc, *Tetrahedron* **2004**, *60*, 10029; d) C. Dolain, C. Zhan, J.-M. Léger, I. Huc, *J. Am. Chem. Soc.* **2005**, *127*, 2400.

[11] A. Acocella, A. Venturini, F. J. Zerbetto, *J. Am. Chem. Soc.* **2004**, *126*, 2362.

[12] See also: Y. Tanaka, H. Katagiri, Y. Furusho, E. Yashima *Angew. Chem.* **2005**, *117*, 3935; *Angew. Chem. Int. Ed.* **2005**, *44*, 3867.

[13] M. Albrecht, *Chem. Rev.* **2001**, *101*, 3457; C. Piguet, G. Bernardinelli, G. Hopfgartner, *Chem. Rev.* **1997**, *97*, 2005.

[14] T. Braxmeier, M. Demarcus, T. Fessmann, S. McAteer, J. D. Kilburn, *Chem. Eur. J.* **2001**, *7*, 1889.

[15] Crystals were obtained by diffusion of MeOH into a DMSO solution. Data were collected on a Rigaku Rapid diffractometer with a MM07 microfocus rotating-anode generator equipped with confocal optics. $(C_{116}H_{93}N_{19}O_{17})_4(C_2H_6SO)_{11}(CH_4O)_7(H_2O)_9$ $M_r = 9346.22$, triclinic, space group $P\bar{1}$, $a = 21.7200(4)$, $b = 24.4279(7)$, $c = 29.4930(6)$ Å, $\alpha = 70.049(6)$, $\beta = 70.624(7)$, $\gamma = 59.770(5)°$, $V = 12459.8(5)$ Å^3, $\lambda = 1.54180$ Å, $T = 163$ K, $Z = 1$, $\rho_{calcd} = 1.246$ Mg m^{-3}, $\mu(Cu_{K\alpha}) = 1.134$ mm^{-1}. 122432 reflections measured, 44237 unique ($R_{int} = 0.095$), 33118 with $I > 2\sigma(I)$, 3062 parameters in final refinement. The final R indices were R_1 ($I > 2\sigma(I)$) = 0.1983, wR_2 (all data) = 0.5047, residual electron density 0.64 e Å^{-3}. CCDC-299073 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[16] The structure also contains sulfur atoms, but these are highly disordered and did not help solving the phase problem.

[17] G. Bunkóczi, L. Vértesy, G. M. Sheldrick, *Angew. Chem.* **2005**, *117*, 1364; *Angew. Chem. Int. Ed.* **2005**, *44*, 1340.

[18] D. M. Chung, Y. Dou, P. Baldi, J. S. Nowick, *J. Am. Chem. Soc.* **2005**, *127*, 9998.

