Effect of an Internal Anthranilamide Turn Unit on the Structure and Conformational Stability of Helically Biased Intramolecularly Hydrogen-Bonded Dendrons

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Received December 11, 2000

The synthesis of nonnatural molecules that adopt a specific, compact conformation in solution has been the subject of intense recent interest.[1] Developing dendrimers that fold into stable, ordered conformations remains an elusive goal due to the conformational flexibility of most commonly studied systems.[2−4] Previous work in our group demonstrated that dendrons (with chiral termini, Figure 1, Type I), rigidified through intramolecular hydrogen-bonding interactions, adopt a specific, chiral helical secondary structure at higher generations. However, this secondary structure was only present at the dendron periphery and was extremely sensitive to solvent quality and temperature.[5] In this communication, the stability of helical secondary structure is dramatically enhanced by linking each generational shell through an anthranilamide turn unit (Type II). This modification induces the dendrons to fold into a tightly packed conformation that expresses helical order at each generational shell.[6]

In the folded conformational state, the extremely tight packing of the protein interior is an important determinant of protein stability.[7] Consequently, improvements in packing efficiency usually impart increased stability to the protein.[8] Type I dendrons develop a bias for an M-type helicity, relating a pair of anthranilamide termini at the second and third generations; however, at 60 °C in CH_3CN, this chiral secondary structure is destroyed for I-G2Cl and partially destroyed for I-G3Cl.[5b] We propose that intraterminal group packing interactions engender cooperativity in the conformational equilibria of the peripheral subunits causing small energetic differences between conformational states to be magnified, leading to a more stable folded state.

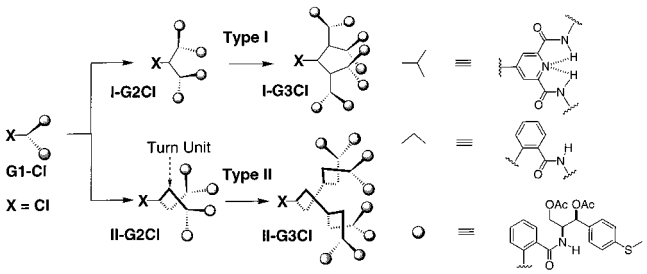

Figure 1. Notional depiction of intramolecularly H-bonded dendrons with internal and peripheral helicity (G = generation).

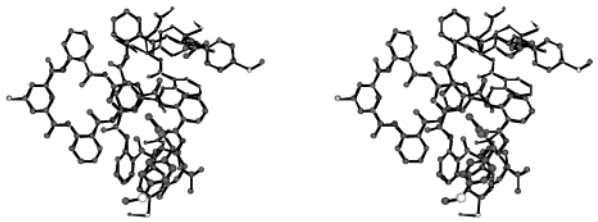

Figure 2. Stereo depiction of lowest energy conformer of II-G2Cl (**2a**) by MM2.[11]

These cooperative effects increasingly favor a single helical sense as the number of terminal groups increase at higher generations.[9]

We reasoned that the stability of the dendron secondary structure would increase if internal helical equilibria were sympathetically correlated with the peripheral helicity. Therefore, to increase packing efficiency and to extend the peripheral helicity to the internal regions of the dendrons, an anthranilamide turn unit was used to link each generational shell of the dendrons. The stable six-membered ring hydrogen bond that forms between adjacent amides linked through this subunit and the *s-trans*-preference of secondary amides induces a turn in the dendron that folds the outer dendritic shells above and below the plane of the branch point (Figure 1, Type II). Monte Carlo conformational analysis at the second generation supports this potential folding model (Figure 2).

A 2-aminobenzamide connector was incorporated at each generational shell as depicted in Scheme 1. Circular dichroic spectra of G1-Cl, I-G2Cl, II-G2Cl (**2a**), and II-G3Cl (**3**) in acetonitrile are compared in Figure 3.[10] The transition that occurs in all the CD spectra in the region of 300−340 nm is exclusively due to a $\pi \rightarrow \pi^*$ transition of the anthranilamide chromophore centered at 316 nm that is polarized along the axis containing C3 and C6 (Figure 4).[12] This transition corresponds to a simple Cotton effect (CE) at the first generation, indicating that the equilibria interconverting two diastereomeric helical conformations (M and P helices) relating a pair of anthranilamide termini is unbiased (Figure 4). However, this transition becomes an exciton couplet (negative chirality) for I-G2Cl and II-G2Cl (**2a**), indicating a preference for the M helical conformation of the anthranilamide chromophores. *Whereas for I-G2Cl this couplet is destroyed upon heating to 60 °C, the couplet remains unchanged up to 60 °C in CH_3CN in the spectra of II-G2Cl (**2a**), and only slight changes are revealed at 110 °C in bis(2-butoxyethyl)ether* (Figure 3). In contrast to the extreme solvent dependence of I-G2Cl,[5b] the spectra of II-G2Cl (**2a**) were insensitive to solvent (see Supporting Information). Furthermore,

(1) For some reviews, see: (a) Barron, A. E.; Zuckermann, R. N. *Curr. Opin. Chem. Biol.* **1999**, *3*, 681. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173.

(2) For recent studies relevant to the conformational behavior of dendrimers, see: (a) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665. (b) Wooley, K. L.; Klug, C. A.; Tasaki, K.; Schaefer, J. *J. Am. Chem. Soc.* **1997**, *119*, 53 and references therein.

(3) For some reviews of chiral dendrimers, see: (a) Peerlings, H. W. I.; Meijer, E. W. *Chem. Eur. J.* **1997**, *3*, 1563. (b) Seebach, D.; Rheiner, P. B.; Greiveldinger, G.; Butz, T.; Sellner, H. *Top. Curr. Chem.* **1998**, *197*, 125. (c) Thomas, C. W.; Tor, Y. *Chirality* **1998**, *10*, 53.

(4) Seebach has observed solvent-dependent variations in chiroptical data, see: Murer, P.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 603.

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(6) For oligoanthranilamides related to these dendrons that fold into linear sheet and helical conformations, see: (a) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1997**, *119*, 10587. (b) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1996**, *118*, 7529.

(7) Creighton, T. E. *Proteins: Structures and Molecular Properties;* 2nd ed. W. H. Freeman: New York, 1993. (b) Jaenicke, R. *J. Biotechnol.* **2000**, *79*, 193. (c) Jaenicke, R.; Bohm, G. *Curr. Opin. Struct. Biol.* **1998**, *8*, 738. (d) Levitt, M.; Gerstein, M.; Huang, E.; Subbiah, S.; Tsai, J. *Annu. Rev. Biochem.* **1997**, *66*, 549.

(8) Mutter, M.; Tuchscherer, G. *Cell. Mol. Life Sci.* **1997**, *53*, 851. (b) Ramachandran, S.; Udgaonkar, J. B. *Biochemistry* **1996**, *35*, 8776.

(9) For examples of cooperativity in helical polymers, see: (a) Green, M. M.; Peterson, N. C.; Sato, T.; Teramoto, A.; Cook, R.; Lifson, S. *Science* **1995**, *268*, 1860. (b) Langeveld-Voss, B. M. W.; Waterval, R. J. M.; Janssen, R. A. J.; Meijer, E. W. *Macromolecules* **1999**, *32*, 227. (c) Palmans, A. R. A.; Vekemans, J. A. J. M.; Havinga, E. E.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2648.

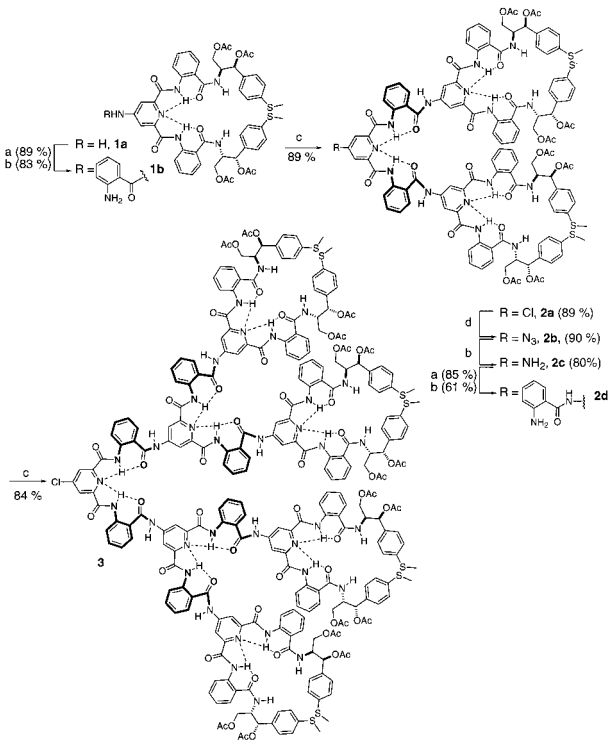
(10) All CD spectra were normalized with respect to concentration and the number of chiral terminal groups.

(11) Generated by employing a Monte Carlo conformational search using the MM2* force field as implemented in Macromodel 6.0.

(12) For a TDDFT study of the electronic transitions of the anthranilamide chromophore, see ref 5b.

10.1021/ja0042229 CCC: $20.00
Published on Web 02/27/2001

Scheme 1. Dendron Synthesis[a]



[a] (a) 2-$NO_2C_6H_4COCl$, pyr., (b) H_2, Pd−C, EtOAc−CH_3OH, (c) 4-chloropyridine-2,6-dicarbonyl chloride, pyr., (d) NaN_3, DMF, 50 °C.

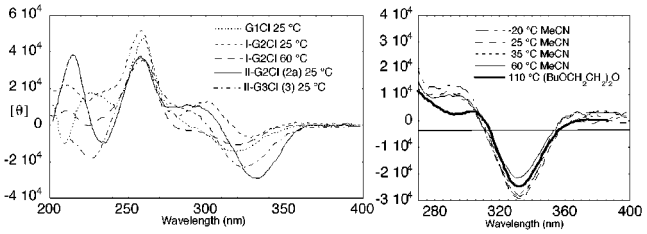


Figure 3. CD Spectra in CH_3CN of Type I and II dendrons (left) and temperature-dependence of exciton couplet of **2a** (right).

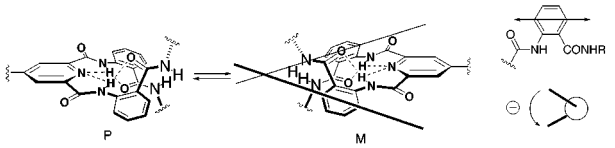


Figure 4. Helical interconversion of anthranilamide chromophores.

the increased intensity of this transition indicates that the conformational equilibrium of the focal anthranilamides is similarly biased toward an M-type helicity and, therefore, constructively contributes to the couplet. The exciton couplet at 316 nm for II-G3Cl (**3**) in acetonitrile at 25 °C (Figure 3) is similar in magnitude to that observed for II-G2Cl (**2a**) and also shows little solvent or temperature dependence (see Supporting Information). This observation indicates that the second and third shells fold into a helical conformation that is biased toward an M-type helicity by the terminal groups as observed for II-G2Cl (**2a**). Accordingly, we can conclude that the first (focal) shell anthranilamide groups of **3** are not contributing significantly to the couplet due to greater conformational freedom that reduces the effect of the chiral terminal groups on the helical bias of this shell.

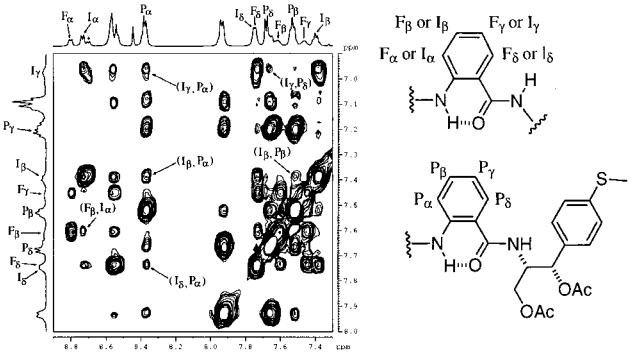


Figure 5. NOESY spectrum (600 MHz, THF-d_8, 27 °C) of II-G3Cl (**3**). F = focal (1st) shell; I = internal (2nd) shell; P = peripheral (3rd) shell.

The conformation of **2a** and **3** were investigated further by 2D (NOESY) ^{1}H NMR to ascertain if close contacts consistent with the folded conformations shown in Figures 1 and 2 were present. Five cross-peaks between protons of the peripheral and the first (for **2a**) or second (for **3**) shell anthranilamides are readily apparent for both **2a** (500 MHz, C_6D_6, 50 °C, Supporting Information) and **3** (Figure 5).[13] The presence of these nOe enhancements provides strong evidence for the folded structure depicted in Figure 2 because protons associated with the first shell (for **2a**) or second shell (for **3**) and the peripheral anthranilamide groups within a single dendritic branch would be too distant for nOe enhancements to be possible (Figure 2).[14] However, the folded conformation places each anthranilamide in close proximity with an anthranilamide present one shell lower in an adjacent branch of the dendron (Figure 2). The fact that these enhancements can be observed at 50 °C for **2a** provides further evidence of the unusually high thermal stability of this conformation. Further, a weak cross-peak (0.8%) between protons of the first (focal) and the second (internal) shells (F_β,I_α) is evident for **3**, confirming the presence of a similar helical fold of the anthranilamides at the focal shell.[15] At 50 °C in THF-d_8 this cross-peak is not evident; however, four cross-peaks between the internal/peripheral anthranilamides are present in the spectrum (see Supporting Information). The temperature dependence of this cross-peak (F_β,I_α) suggests that the helical conformation relating the anthranilamides at the focal shell is less compact than at the internal shell.

In conclusion, we have described dendrons that exhibit a helical secondary structure that occurs over three generational levels. The preliminary evidence described in this paper suggests that molecular packing plays an important role in stabilizing secondary structure in dendrimeric systems.

Acknowledgment. This work was supported by the National Science Foundation CAREER program (CHE-98-75458).

Supporting Information Available: H−H COSY, NOESY, and selective TOCSY NMR spectra and solvent- and temperature-dependent CD spectra for **2a** and **3**; full experimental procedures and analytical detail for dendrons **1b**−**3** (PDF). This material is free of charge via the Internet at http://pubs.acs.org.

JA0042229

(13) Proton assignments for **2a** and **3** based on H−H COSY and selective TOCSY NMR experiments (see Supporting Information).

(14) This structural information is based on crystal structures of closely related structures. See ref 5a and references therein.

(15) See Supporting Information for vertical and horizontal cross-sections of the unsymmetrized NOESY spectrum for this cross-peak.