Conformational Analysis

Dendrimer Folding in Aqueous Media: An Example of Solvent-Mediated Chirality Switching[**]

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Allosteric proteins exhibit highly nonlinear responses to external stimuli that cause small perturbations of a particular parameter to effect very large changes in protein structure and function.[1] This behavior is a direct consequence of the structural cooperativity that correlates the conformational equilibria of multiple subunits within the protein structure.[2] Such allosteric phenomena are commonly observed in biomacromolecules and are critical for their function. There are relatively few synthetic materials, however, that exhibit this conformational cooperativity.[3] The creation of such conformational adaptability in synthetic systems requires the allosteric transmission of local structural or chiral information to the next hierarchical level of structural organization. We have recently developed a dendrimeric system that adopts a compact, helical shape governed by the *syn*,*syn* conformational preference of the pyridine-2,6-dicarboxamide repeat unit.[4] We report herein that water-soluble versions of these dendrons fold in water. Furthermore, the terminal pentaethylene glycol chains and the dendron secondary structure exhibit highly correlated conformational equilibria. Accordingly, solvent-induced conformational fluctuations of the terminal pentaethylene glycol chains are correlated with an inversion in the sense of helical chirality expressed by the dendrons.[5]

Information transfer in folded synthetic systems usually occurs through inter-[6] or intramolecularly[7] self-organized assemblies through an ensemble of noncovalent interactions. Polar protic solvents such as water typically disrupt hydrogen bonding and electrostatic interactions, thereby compromising the stability of such assemblies. For example, elements of peptide secondary structure, independent of the global protein structure, exhibit only marginal stability in pure water.[8] Similarly, many synthetic foldamers exhibit less stable conformations in water.[9] However, any potential biological application of folded synthetic materials requires an ability to adopt conformational order in aqueous media.

The conformational preference of the pyridine-2,6-dicarboxamide repeat unit in our folded dendrons is a result of intramolecular hydrogen bonding present in the *syn*,*syn* conformation, as well as an electrostatic destabilization of

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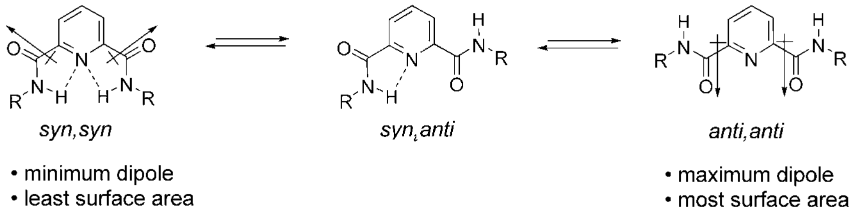
[**] We thank Professor Heather Allen and Dr. Gang Ma for assistance with the IR spectroscopic studies. This work was supported by the National Science Foundation (CHE-0239871).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

DOI: 10.1002/ange.200460943

the alternative *syn*,*anti* and *anti*,*anti* conformations. Previous studies strongly suggest that tighter packing of the dendritic chains in poor solvents plays an important role in the folded conformation of these dendrons.[10] Although water should disrupt the hydrogen bonding interactions of the *syn*,*syn* conformation and decrease the energetic cost of the larger dipole moments of the *syn*,*anti* and *anti*,*anti* conformations, the packing effect should nevertheless dominate, as the propensity of hydrophobic regions to minimize their exposure to solvent ultimately results in a hydrophobic collapse of the dendritic structure.



Chiral pentaethylene glycol **1**, prepared from ethyl L-lactate, was installed at the periphery of the dendrons to impart solubility in water (Figure 1).[6b,9a] Dendrons **2**–**4** exhibited moderate solubility in water, and excellent solubility in organic media. However, millimolar solutions of dendrons in water exhibited a lower critical solution temperature (LCST) that induced clouding and precipitation upon heating to ≈25–30 °C.[9a,11]

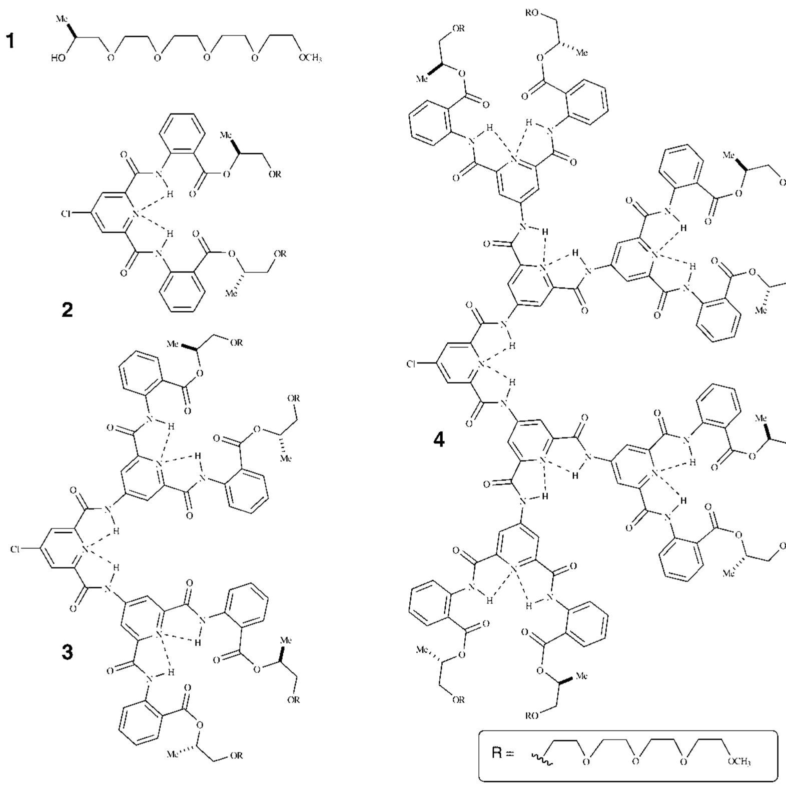


Figure 1. Water-soluble dendrons with chiral, pentaethylene glycol termini.

The circular dichroism (CD) spectra of **2** featured an intense negative couplet centered at ≈316 nm (Figure 2b) in THF and in water, the latter case being slightly lower in magnitude relative to that from the sample in THF. This bisignet signal results exclusively from the excitonic coupling of the $\pi \rightarrow \pi^{*}$ transitions of the anthranilate chromophores, which are polarized along the axis containing C3 and C6 (Figure 2a).[4d] The presence of the negative couplet indicates that the first generation dendron **2** adopts an *M*-type helical conformation, relating the anthranilate chromophores in both THF and in water. This observation highlights the importance of packing interactions in determining the conformational properties of the dendron.

The second generation dendron **3** exhibited a negative couplet in THF comparable in magnitude with that of dendron **2**, which also indicates an *M* helical bias. However, the negative couplet was replaced by an equally intense positive couplet in aqueous media, which indicates that an $M \rightarrow P$ helical transition occurred in water. The third-generation dendron **4** also displayed a similar $M \rightarrow P$ helical transition upon going from THF to water. Varying the THF/H_2O ratio for **3** and **4** at 25 °C revealed $M \rightarrow P$ crossover points in the range of 10–20% THF for **3** and 30–40% THF for **4** (Supporting Information).

The positive components of the CD couplets in water for **3** and **4** were decreased in amplitude relative to the negative branches at shorter wavelengths. The unsymmetrical appearance of these couplets is a consequence of the increased chromophore congestion that occurs in poor, structure-collapsing solvents and is commonly observed for this dendrimer system.[4] Such structural congestion induces further mixing of the couplet transitions with other transitions within the dendron, which results in desymmetrized couplet intensities.[12]

The amphiphilic nature of the dendrons creates a tendency for aggregation to occur in water, and to a lesser degree, in THF. Comparison of the hydrodynamic radii (R_h) of **2**–**4**, measured by DOSY NMR spectroscopy,[13] to the calculated van der Waals radii[14] (R_{vdW}) indicates that significant aggregation occurred in D_2O solution,[15] whereas monomeric species were present in [D_8]THF (Table 1). Aggregation has been shown to play a significant role in the conformational equilibria of chiral molecules[16] and introduces the possibility for intermolecular excitonic coupling[17] in the CD spectra. Therefore, CD and UV/Vis spectra were recorded for **2**–**4** as a function of concentration in THF and H_2O (Supporting Information). The UV/Vis and CD spectra of all dendrons were essentially invariant over a 100-fold concentration range (10^{-4} to 10^{-6} M), thereby ruling out aggregation as a source of or contributing factor to the $M \rightarrow P$ helical inversion.

Previous vibrational spectroscopic studies showed that poly(oxyethylene) (POE) chains experience an increased preference for the C–C *gauche* conformation, whereas only a small increase in the C–O *anti*

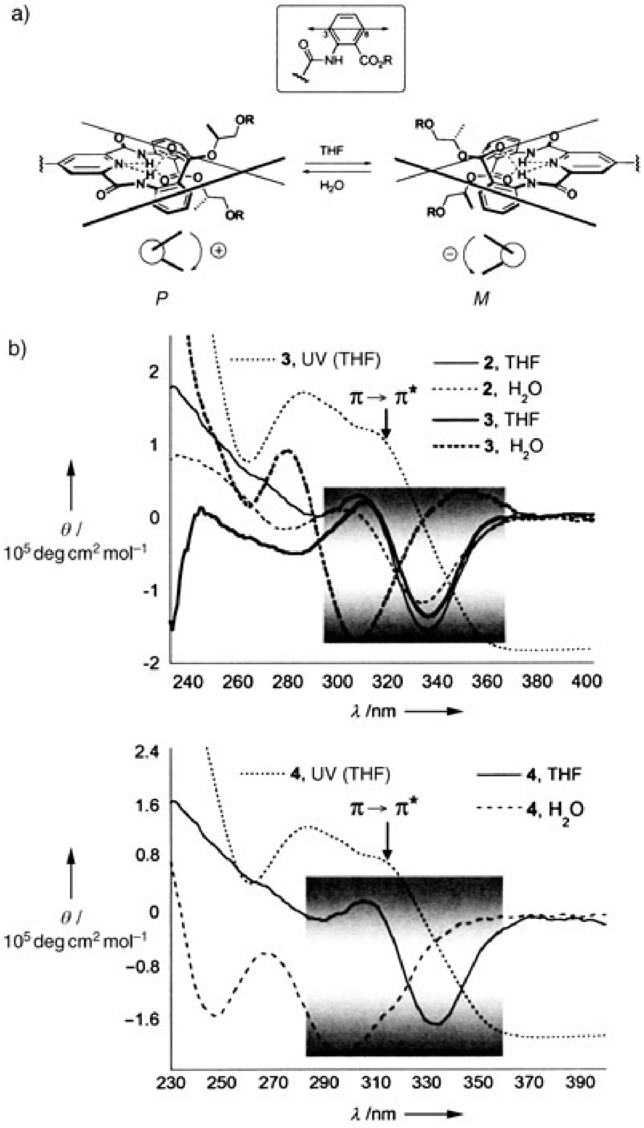


Figure 2. a) Direction of electronic transition dipole moments of the π→π* transition at 316 nm and corresponding sign of CD couplet. b) CD spectra of dendrons **2**–**4** in H_2O and THF. Spectra are normalized with respect to concentration and the number of chiral terminal groups.

Table 1: Experimentally determined (R_h) and calculated (R_{vdW}) hydrodynamic radii [Å]

Compound	R_h ([D$_8$]THF[a])	R_h(D$_2$O[b])	R_{vdW}[c]
2 (3.5 mM)	5.8	19.7	5.80
3 (3.6 mM)	8.4	43.9[d]	7.45
4 (3.4 mM)	8.3	52.8[e]	9.48

[a] DOSY NMR spectroscopy, 27 °C. [b] DOSY NMR spectroscopy, 10 °C. [c] Van der Waals radii, calcd. [d] Measured in [D$_8$]THF/D$_2$O (10%). [d] Measured in [D$_8$]THF/D$_2$O (2%).

population occurs upon progression from nonpolar to aqueous media.[18] The preferred conformation of the *anti,gauche* O–C–C bond pair in water is consistent with the solid-state structure of the POE helix.[19]

The effect of solvent on the conformational preference of the terminal glycol chains of **3** was investigated by IR spectroscopy with the key bands at 1355–1360 cm^{-1} (A), and 1335–1320 cm^{-1} (B) for the respective *gauche* and *anti* C–C conformations; and with those at 1310–1297 cm^{-1} (C), and 1290–1295 cm^{-1} (D) for the *gauche* and *anti* C–O conformations, respectively.[18c] The disappearance of the C–C *anti* band (B) and the slight increase in the C–O *anti* band (D) upon going from [D$_8$]THF[20] to H_2O for glycol chain **1** (Figure 3a) closely mirrors the spectroscopic behavior reported for other POE oligomers.[18] Molecular dynamics simulations estimate that similar observations for POE represent an increase in the C–C *gauche* population from 76% in benzene to 100% in water.[21] However, in contrast to **1**, dendron **3** exhibits a significant increase in the C–O *anti* band (D) as water is added, whereas the C–C *anti* band (B) is missing from the spectra at all H_2O/[D$_8$]THF ratios.

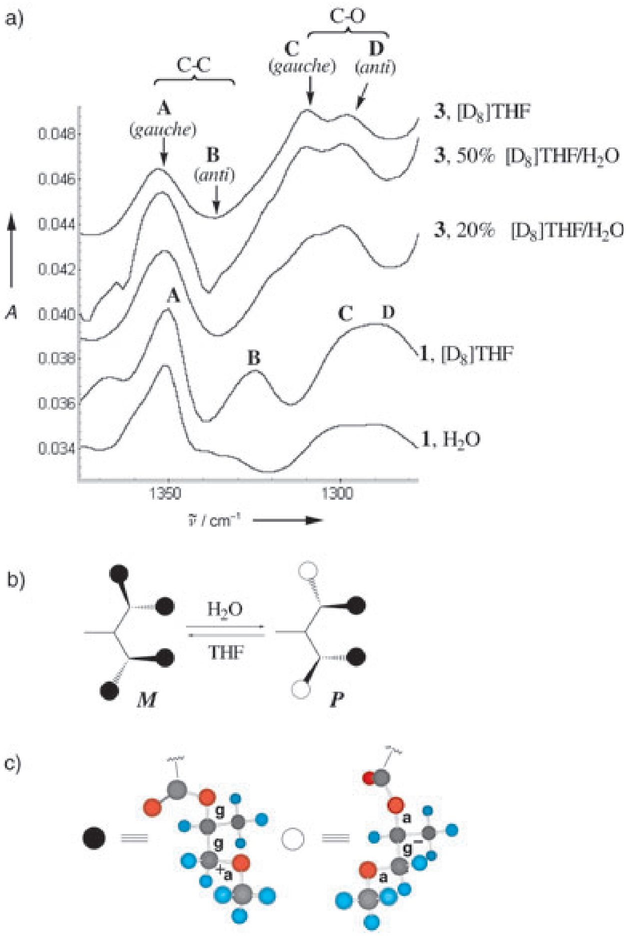


Figure 3. a) IR spectra of **1** and **3** as a function of solvent, measured with a horizontal ZnSe crystal and a 45° single bounce attenuated total reflection (ATR) accessory (20% [D$_8$]THF in H_2O was necessary to attain the 25-mM concentrations of **3** required for IR spectroscopy). b) Schematic depiction of the lowest-energy conformers of **3** (R = Me). c) Conformation of terminal glycol chains in $CHCl_3$ (left) and H_2O (right) as predicted by conformational searching (MM3).

These observations indicate that the solvation of **3** in water induces a *gauche*→*anti* conformational shift about the

C–O bond. Furthermore, a strong preference for the C–C *gauche* conformation exists for the dendrons at all solvent ratios, in contrast to the parent glycol chain **1** and POE. The fact that the C–C *gauche* preference of dendron **3** is stronger than it is for POE or **1** suggests the occurrence of correlated motions among the terminal glycol chains that shift the conformational equilibria toward the lower energy C–C *gauche* arrangement.[4c]

Monte Carlo conformational searching of **3** (R = Me) with the GB/SA solvation model[22] predicts an *M* helical bias in CHCl$_3$[23] whereas a *P* helix is preferred in water, in accord with the CD studies (Figure 4). Closer inspection of each of the lowest-energy conformers reveals that the $M \rightarrow P$ helical inversion is correlated with a shift of two of the four glycol chains from *gauche*,*gauche*$^{+}$,*anti* to *anti*,*gauche*$^{-}$,*anti* conformations around the respective O–C–C–OMe bonds (Figure 3b). The uniformly *gauche*$^{\pm}$ preference of all the C–C bonds in both water and CHCl$_3$ and the corresponding conformational shift of two O–C bonds from *gauche* to *anti* upon going from CHCl$_3$ to water is consistent with the observed vibrational data.

The results of this study demonstrate that these dendrons not only adopt a stable folded state in aqueous media, but also exhibit correlated chain–chain and dendron–chain conformational equilibria. The correlated chain–chain motions induce a shift in equilibrium of the terminal chains toward the lower-energy *gauche* state, in contrast to the case of an isolated chain (**1**). Similarly, solvent-induced conformational fluctuations of the terminal chains are coupled with dendron helical secondary structure through correlated dendron–chain motions. This work suggests the potential of folded dendrimers to exhibit nonlinear conformational responses to localized structural perturbations.

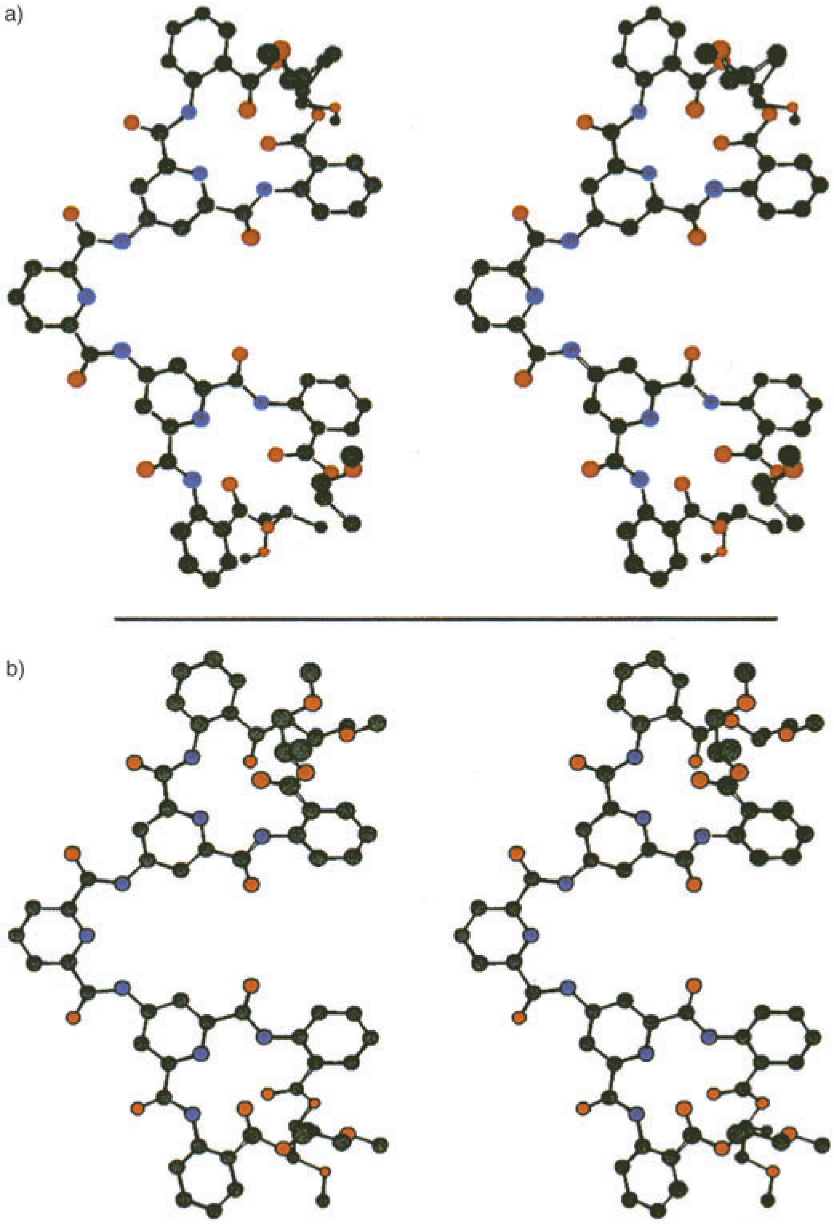


Figure 4. Stereograms of the lowest-energy conformers determined by Monte Carlo conformational searching (MM3) using the GB/SA solvation model for a) CHCl$_3$ (*M* helicity) or b) H$_2$O (*P* helicity) as implemented in Macromodel 8.5.

Received: June 11, 2004
Revised: November 5, 2004

Keywords: amphiphiles · chirality · circular dichroism · dendrimers · IR spectroscopy

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[1] a) J. M. Yon, D. Perahia, C. Ghelis, *Biochimie* **1998**, *80*, 33; b) O. Jardetzky, *Prog. Biophys. Mol. Biol.* **1997**, *65*, 171; c) D. Kern, E. R. P. Zuiderweg, *Curr. Opin. Struct. Biol.* **2003**, *13*, 748.
[2] K. L. Mayer, M. R. Earley, S. Gupta, K. Pichumani, L. Regan, M. J. Stone, *Nat. Struct. Biol.* **2003**, *10*, 962.
[3] For a recent review of conformational cooperativity in helical linear polymers, see: E. Yashima, K. Maeda, T. Nishimura, *Chem. Eur. J.* **2004**, *10*, 42.
[4] a) B. Huang, M. A. Prantil, T. L. Gustafson, J. R. Parquette, *J. Am. Chem. Soc.* **2003**, *125*, 14518; b) B. Huang, J. R. Parquette, *J. Am. Chem. Soc.* **2001**, *123*, 2689; c) J. Recker, D. J. Tomcik, J. R. Parquette, *J. Am. Chem. Soc.* **2000**, *122*, 10298; d) P. Gandhi, B. Huang, J. C. Gallucci, J. R. Parquette, *Org. Lett.* **2001**, *3*, 3129.
[5] For selected examples of solvent-mediated chirality inversion, see: a) V. V. Borovkov, G. A. Hembury, Y. Inoue, *Angew. Chem.* **2003**, *115*, 5568; *Angew. Chem. Int. Ed.* **2003**, *42*, 5310; b) K. Morino, K. Maeda, E. Yashima, *Macromolecules* **2003**, *36*, 1480; c) H. Nakashima, M. Fujiki, J. R. Koe, M. Motonaga, *J. Am. Chem. Soc.* **2001**, *123*, 1963; d) H. Nakako, R. Nomura, T. Masuda, *Macromolecules* **2001**, *34*, 1496; e) S. E. Boiadjiev, D. A. Lightner, *J. Am. Chem. Soc.* **2000**, *122*, 378.
[6] a) L. Brunsveld, E. W. Meijer, A. E. Rowan, R. J. M. Nolte, *Top. Stereochem.* **2003**, *24*, 373; b) L. Brunsveld, H. Zhang, M. Glasbeek, J. A. J. M. Vekemans, E. W. Meijer, *J. Am. Chem. Soc.* **2000**, *122*, 6175.
[7] a) T. Nakano, Y. Okamoto, *Chem. Rev.* **2001**, *101*, 4013; b) M. M. Green, J.-W. Park, T. Sato, A. Teramoto, S. Lifson, R. L. B. Selinger, J. V. Selinger, *Angew. Chem.* **1999**, *111*, 3328; *Angew. Chem. Int. Ed.* **1999**, *38*, 3139.

[8] a) C. A. Rohl, R. L. Baldwin, *Methods Enzymol.* **1998**, *295*, 1; b) M. Goodman, A. S. Verdini, C. Toniolo, W. D. Phillips, F. A. Bovey, *Proc. Natl. Acad. Sci. USA* **1969**, *64*, 444.

[9] For some leading references, see: a) M. T. Stone, J. S. Moore, *Org. Lett.* **2004**, *6*, 469; b) I. Huc, *Eur. J. Org. Chem.* **2004**, 17; c) T. L. Raguse, J. R. Lai, S. H. Gellman, *J. Am. Chem. Soc.* **2003**, *125*, 5592; d) A. J. Zych, B. L. Iverson, *J. Am. Chem. Soc.* **2000**, *122*, 8898; e) Y. Hamuro, J. P. Schneider, W. F. DeGrado, *J. Am. Chem. Soc.* **1999**, *121*, 12200.

[10] J. R. Parquette, *C. R. Chim.* **2003**, *6*, 779.

[11] Clouding was observed at 25–30°C at mM concentrations visually and by UV/Vis spectroscopy, but not at the μM concentrations used for the CD studies. Polyethylene glycol also exhibits a LCST: S. Saeki, N. Kuwahara, M. Nakata, M. Kaneko, *Polymer* **1976**, *17*, 685.

[12] For a discussion, see: G. Gottarelli, S. F. Mason, G. Torre, *J. Chem. Soc. B* **1970**, 1349.

[13] J. K. Young, G. R. Baker, G. R. Newkome, K. F. Morris, C. S. J. Johnson, *Macromolecules* **1994**, *27*, 3464.

[14] Calculated by using increments described in: J. T. Edward, *J. Chem. Educ.* **1970**, *47*, 261.

[15] Small amounts of [D$_8$]THF and lower temperatures were necessary to obtain the mM concentrations required for DOSY NMR spectroscopy in D$_2$O. The degree of aggregation is likely to be significantly lower at the μM concentrations required for the CD studies.

[16] For examples in which CD is enhanced by aggregation, see: a) A. R. A. Palmans, J. A. J. M. Vekemans, E. E. Havinga, E. W. Meijer, *Angew. Chem.* **1997**, *36*, 2763; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2648; b) C. Nuckolls, T. J. Katz, T. Verbiest, S. Van Elshocht, H. G. Kuball, S. Kiesewalter, A. J. Lovinger, A. Persoons, *J. Am. Chem. Soc.* **1998**, *120*, 8656; c) C. Nuckolls, T. J. Katz, L. Castellanos, *J. Am. Chem. Soc.* **1996**, *118*, 3767; d) D. W. Huang, S. Matile, N. Berova, K. Nakanishi, *Heterocycles* **1996**, *42*, 723.

[17] V. V. Borovkov, T. Harada, Y. Inoue, R. Kuroda, *Angew. Chem. Int. Ed.* **2002**, *114*, 1436; *Angew. Chem. Int. Ed.* **2002**, *41*, 1378.

[18] a) S. A. Wahab, H. Matsuura, *Phys. Chem. Chem. Phys.* **2001**, *3*, 4689; b) R. Begum, T. Yonemitsu, H. Matsuura, *J. Mol. Struct.* **1998**, *447*, 111; c) R. Begum, H. Matsuura, *J. Chem. Soc. Faraday Trans.* **1997**, *93*, 3839.

[19] Y. Takahashi, H. Tadokoro, *Macromolecules* **1973**, *6*, 672.

[20] [D$_8$]THF is transparent in this region. Changes in the relative peak intensities represent shifts in population ratios, but not actual population ratios.

[21] K. Tasaki, *Macromolecules* **1996**, *29*, 8922.

[22] As implemented in Macromodel 8.5. For the GB/SA model, see W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127.

[23] Because an *M* helical bias was observed by CD for dendrons **3–4** in all organic solvents studied (i.e., CH$_2$Cl$_2$, CH$_3$CN, THF), we employed the GB/SA model for chloroform to model the conformational behavior in THF.