Foldamers

DOI: 10.1002/anie.200802648

A Nonpeptidic Reverse Turn that Promotes Parallel Sheet Structure Stabilized by C–H···O Hydrogen Bonds in a Cyclopropane γ-Peptide**

*Christopher R. Jones, M. Khurram N. Qureshi, Fiona R. Truscott, Shang-Te Danny Hsu, Angus J. Morrison, and Martin D. Smith**

It is remarkable that compact folded proteins are constructed from a small number of secondary structural elements such as helices and sheets. Examination of model systems for these structural modules has permitted understanding of the features that regulate their assembly, independent of a tertiary structural context. An extension of these principles has led to the development of non-natural folded oligomers ("foldamers")[1] that populate distinct secondary and tertiary structures (Figure 1).[2]

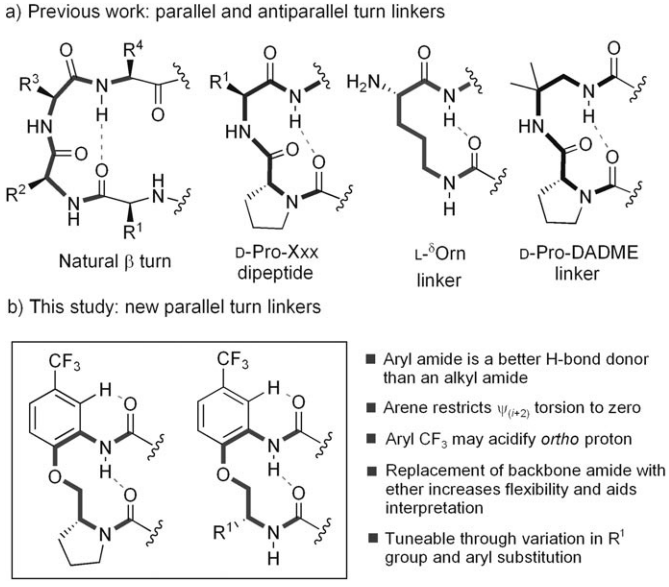


Figure 1. Design of new parallel-turn linkers.

[*] C. R. Jones, M. K. N. Qureshi, F. R. Truscott, Dr. S.-T. D. Hsu, Dr. M. D. Smith
Department of Chemistry, University of Cambridge
Lensfield Road, Cambridge CB2 1EW (UK)
Fax: (+44) 1223-336-362
E-mail: mds44@cam.ac.uk
Dr. A. J. Morrison
Organon Research
Newhouse, Lanarkshire ML1 5SH (Scotland)

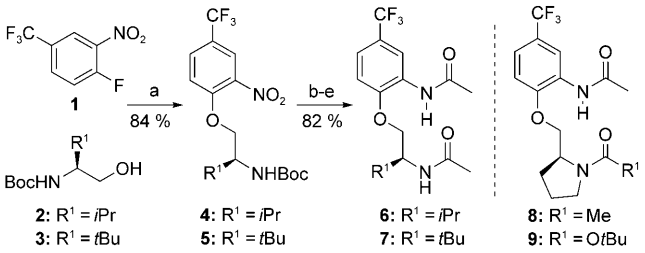
[**] We acknowledge funding from the Royal Society (M.D.S.), Organon/EPSRC (C.R.J.), AstraZeneca (F.R.T.), the Pakistani HEC (M.K.N.Q.), EPSRC National Mass Spectrometry Centre, and an International Human Frontier Science Program Long Term Fellowship LT00798/2005 (S.T.D.H.). We would like to thank Dr. J. Davies for help with X-ray crystallography.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200802648.

Amongst these folding backbones, parallel-[3] and antiparallel-sheet [4] secondary structure has been demonstrated in both β- and γ-peptides,[5] and turn elements as simple as L-$^{\delta}$Orn (L-$^{\delta}$ornithine) have been demonstrated to nucleate hairpin formation in organic and aqueous solutions.[6] A range of parallel-sheet models[7] have been delineated, as exemplified by the D-Pro-DADME (D-proline-1,1-dimethyl-1,2-diaminoethane) reverse turn, an elegant evolution of the naturally inspired D-Pro-Xxx dipeptide (Xxx = any other amino acid).[8] This material has been demonstrated to be effective and versatile in promoting parallel-sheet structure in a wide range of amide-linked strand sequences.[9] Here we demonstrate that a reverse turn comprising an amino acid derived alcohol conjoined with an aromatic amine can promote parallel-sheet structure in a γ-peptide sequence designed to fold with the aid of C–H···O hydrogen bonds.

Sheet construction is challenging as it requires the interplay of functional groups that are geometrically far apart in primary structure. Hence, the employment of an appropriate linking segment is of paramount importance. In designing this parallel-turn motif (Figure 1), we reasoned that the incorporation of an *ortho*-amino phenol derivative would restrict the $\psi_{(i+2)}$ torsion to angles consistent with natural β turns.[10] An *N*-aryl amide proton would also possess a greater hydrogen-bond-donor ability than a conventional amide, and the presence of the aryl trifluoromethyl group may offer further conformational control through acidification of the *ortho* proton, facilitating its participation in a hydrogen bond with the adjacent carbonyl group.[11] This additional hydrogen bond has the potential to enhance the donor ability of this amide further, through a cooperative mechanism.[12] We considered that replacement of the amide linkage in the rigid backbone of the Pro-Xxx model with an aryl ether could offer sufficient conformational flexibility to enable favorable hydrogen-bonding geometries to be populated; intramolecular hydrogen bonding offers a moderate driving force for folding in an aprotic solvent. This motif also permits a range of side chains derived from α-amino acids to be incorporated, generating a simple and tunable alternative reverse-turn linker.

In a preliminary investigation to evaluate the turn-forming propensities of this construct, we generated units derived from L-valine (Val), L-*tert*-leucine, and L-proline (Scheme 1). These units were assembled through an S_NAr reaction between the respective amino acid derived potassium alkoxide (from **2** and **3**) and commercially available fluoroarene **1**. Unblocking of the orthogonal N termini (through transfer hydrogenation of the aryl nitro group and subsequent removal of the alkyl *N*-Boc group, Boc = *tert*-



Scheme 1. a) KHMDS, THF, 0 °C; b) NH_4·HCOO, Pd/C, MeOH, 50 °C; c) Ac_2O, pyridine, DMAP; d) TFA/CH_2Cl_2, 0 °C; e) Ac_2O, pyridine, DMAP. KHDMS = potassium hexamethyldisilazide, DMAP = *N*,*N*-dimethyl-4-aminopyridine, TFA = trifluoroacetic acid.

butyloxycarbonyl), and attachment of minimal side chains (in this case, acetyl groups) were accomplished without incident to afford **6**–**8**. The role of these model systems is to investigate the turn-forming ability of this motif in the absence of complicating effects from any side chains, and the conformation of these materials was investigated in both solution and the solid state (Figure 2).[13]

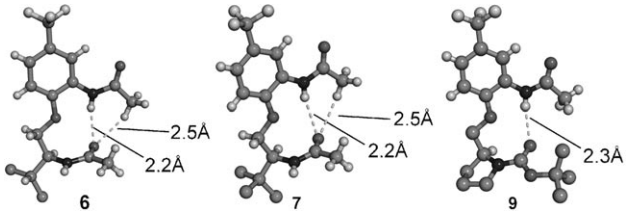


Figure 2. X-ray structures of model turn constructs.

X-ray crystallography of **6** and **7** (recrystallized from CH_2Cl_2) revealed a conformation characterized by both inter- and intramolecular hydrogen bonding. We were unable to obtain suitable crystals of L-Pro *N*-acetate compound **8** but were able to solve the structure of *N*-Boc derivative **9**. For **6**, the intramolecular donor–acceptor distances are favorable (C=O···H–N: 2.2 Å and C=O···C^{α}H: 2.5 Å) though the C=O···H–N angle deviates significantly from planarity (100°). The L-*tert*-leucine-derived unit **7** has a similar geometry in the solid state, and the L-Pro derivative **9** adopts a comparable hydrogen-bonded turn geometry. The properties of these materials were also investigated by FTIR and ^{1}H NMR spectroscopy in nonpolar solvents. Titration experiments (1 mM in $CDCl_3$, 298 K) indicate in both cases that the dependency of the chemical shift of the aryl amide proton on the concentration of DMSO is ten times lower than that of the alkyl amide proton, which is consistent with its involvement in intramolecular hydrogen-bonded conformations. The FTIR spectrum of **6** (1 mM in CH_2Cl_2, 298 K) shows two peaks in the N–H stretch region at 3434 cm^{-1} (sharp) and 3333 cm^{-1} (broad), which we ascribe to non-hydrogen-bonded N–H and internally hydrogen-bonded N–H groups respectively.[13] Similar data were recorded for the L-*tert*-leucine-derived material **7** (N–H stretch region: 3439 cm^{-1} and 3334 cm^{-1}), consistent with an analogous hydrogen-bonded conformation.[14]

With the potential of these scaffolds to populate turn conformations established, we decided to explore their utility in a demanding scenario through the generation of a γ-peptide sheet using the valine-derived turn construct **4**. We have previously shown that cyclopropane γ-peptides adopt extended conformations in the solid state[15] and envisaged that these materials could adopt sheet structures when attached to an appropriate turn linker (Figure 3).[16]

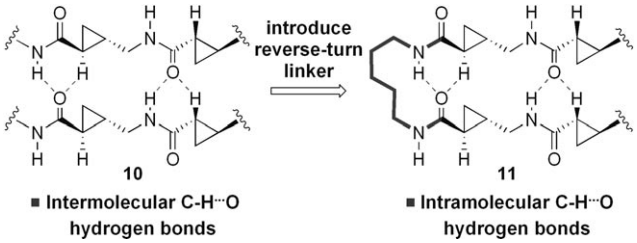


Figure 3. Design rationale: from inter- to intramolecular hydrogen bonds.

We reasoned that exploiting the intermolecular C–H···O hydrogen bonds present in oligomeric cyclopropane γ-amino acids **10** in the solid state [15] as stabilizing interactions in the intramolecular manifold (as exemplified by **11**) would be an interesting test of our turn construct and of the potential role of C–H···O interactions[17] in unnatural sheet formation. High-resolution protein structures have been shown to contain sheet regions with short C–H···O distances,[18] and NMR spectroscopy has provided direct evidence of hydrogen bonding across C–H···O=C in proteins by measuring through-bond $^{h3}J_{C\alpha C'}$ scalar couplings.[19] However, there are few examples of the exploitation of this interaction in the development of novel folding backbones.[20] The absolute configuration of proline in Pro-Xxx reverse turns can have a profound effect on the propensity for sheet formation, and hence we accessed both the D- and L-valine series of our construct in this regard.[21] We were encouraged by the X-ray structure[22] of an intermediate (in the D-Val series) **12** that exhibited a turn structure stabilized by an intramolecular bifurcated hydrogen bond that includes the C^{α}H atom of the cyclopropane group (Figure 4).

The cyclopropane C^{α}H···O separation (2.3 Å) is consistent with similar interactions[23] observed in natural[24] and non-natural sequences.[25] The preference for linearity across the C^{α}H···O interaction clearly influences the conformation adopted by the cyclopropyl side chain. In this hydrogen-

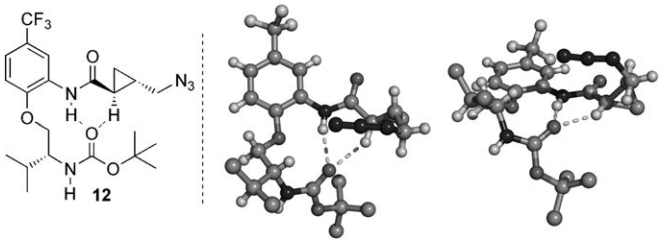


Figure 4. X-ray structure of **12** showing a C–H···O stabilized turn conformation.

bonded conformation, the side chains attached to the reverse turn in **12** are oriented in different directions, which has significant implications for the ability of materials based on this specific diastereoisomeric motif to populate extended parallel-sheet conformations. Close contacts such as these may be regarded as a consequence or a determinant of the observed geometry in molecular crystals, and hence it is important to gain further evidence of their role in determining conformation.[26]

We envisaged that $C^{\alpha}H\cdots O$ interactions such as these could stabilize a parallel sheet in solution, and hence tetrapeptide analogues **13** and **14**, and hexapeptide analogues **15** and **16** were synthesized, and their conformational preferences examined by NMR spectroscopy in organic solvents (Figure 5).

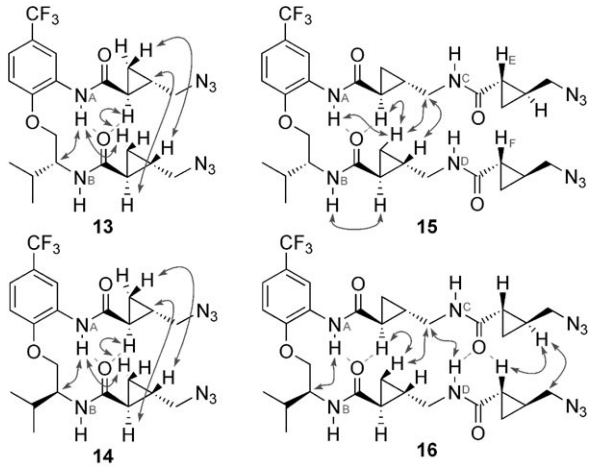


Figure 5. Selected interresidue NOE correlations for valine-derived sheets (700 MHz, 3 mM in $CDCl_3$ (referenced to 7.27 ppm), 298 K).

For these materials, dilution experiments confirmed that no intermolecular aggregation was occurring at concentrations below 3 mM, and a series of 2D experiments permitted assignment of all spin systems. Stereospecific assignment of the cyclopropyl methylene protons was achieved unambiguously for almost all spin systems; this is important as specific nuclear Overhauser effect (NOE) signals from these protons are key in defining turnlike conformers in solution. For the tetrapeptide analogues **13** and **14**, cross-strand NOE correlations are observed between nonadjacent cyclopropane rings, consistent with population of a hairpin conformation (Figure 5). The L-Val-derived hexapeptide **16** possesses key NOE correlations involving the terminal cyclopropane residues, indicative of the population of a sheet conformer whilst in the D-Val-derived analogue **15** these key NOE correlations are absent. ^{1}H NMR chemical shift data for **16** (Table 1) is consistent with the involvement of NH^D and NH^A in hydrogen bonds (both are deshielded), whilst for **15** this effect is exhibited only by NH^A. For **16**, the cyclopropane $C^{\alpha}H$ signal at $C\text{-}2^C$ is deshielded relative to that at $C\text{-}2^D$, as expected in its role as a hydrogen-bond donor.[27] Similar shift patterns are seen for **13**–**15**.

Table 1: Chemical shifts of amide and $C^{\alpha}H$ cyclopropane protons.[a]

	NH^A	NH^B	NH^C	NH^D	$C\text{-}2^C$	$C\text{-}2^D$	$C\text{-}2^E$	$C\text{-}2^F$
13	8.71	5.83	–	–	2.03	1.77	–	–
14	8.71	5.68	–	–	1.98	1.38	–	–
15	8.70	5.96	6.54	6.11	1.89	1.58	1.37	1.37
16	8.75	5.81	6.54	6.97	1.98	1.50	1.68	1.60

[a] Conditions: 700 MHz, 3 mM in $CDCl_3$ (referenced to 7.27 ppm), 298 K.

In order to probe the individual hydrogen-bonding interactions in materials **13**–**16**, we carried out a series of variable-temperature and solvent-dependent experiments.[14] Solvent titration studies demonstrate that only the cyclopropane $C^{\alpha}H$ and amide protons possess a chemical shift dependence on DMSO concentration, which is consistent with their involvement in hydrogen bonding. For tetrapeptide analogue **14**, NH^A has an approximate sevenfold lower chemical shift dependency than NH^B with respect to DMSO concentration, and a similar trend is seen for the $C^{\alpha}H$ at $C\text{-}2^C$ when compared to that at $C\text{-}2^D$. For **16**, these experiments demonstrate that NH^A and NH^D are solvent-protected relative to NH^B and NH^C whilst for **15**, the chemical shift dependency on DMSO concentration of NH^B, NH^C, and NH^D is significantly higher than that of NH^A. This is consistent with the NOE data for **15** that indicate close contacts at the hairpin end of the molecule only.

Temperature coefficients were also determined for **13**–**16**.[13,14,28] Compounds **13** and **14**, which have similar conformational profiles, possess similar temperature coefficient data. In contrast, **15** and **16**, which differ in conformation at the N termini, display significantly different temperature-related data that we believe reflect unfolding and conformational averaging.[29]

When considered together, this evidence suggests that tetrapeptide analogues **13** and **14** populate well-defined turn conformers stabilized by intramolecular hydrogen bonds in which both aromatic amide and the cyclopropane $C^{\alpha}H$ protons are implicated as hydrogen-bond donors. In contrast, **15** and **16** (which differ only in the absolute configuration of the asymmetric center on the reverse-turn) populate significantly different conformations. For **15**, the turn structure is conserved at the hairpin end of the molecule (consistent with the X-ray structure of **12**) but there is little evidence of long-range order, whilst **16** populates an extended sheetlike conformation stabilized by a series of hydrogen bonds.

In summary, we have generated a new nonpeptidic reverse turn that populates a hairpin conformation in the solid state and in solution. Exploitation of the well-defined conformational preferences of this construct enabled the generation of sheetlike materials that populate hydrogen-bond-stabilized conformations. Both solid-state and solution data indicate significant $C\text{–}H\cdots O$ hydrogen bonding in these materials, which is consistent with the postulated structural role of this interaction in proteins. We are currently exploring the utility of the $C\text{–}H\cdots O$ hydrogen bond as a tool for conformational control in small molecules in aqueous and organic solvents. Our work thus far augurs well for future application of these

scaffolds in the generation of well-defined materials for catalysis and recognition.

Received: June 5, 2008
Published online: July 30, 2008

Keywords: foldamers · hydrogen bonds · parallel turns · peptidomimetics

[1] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; b) R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219; c) D. Seebach, D. F. Hook, A. Glättli, *Biopolymers* **2006**, *84*, 23; d) D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, *Chem. Rev.* **2001**, *101*, 3893; e) N. Rathore, S. H. Gellman, J. J. de Pablo, *Biophys. J.* **2006**, *91*, 3425; f) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252.

[2] a) J. X. Qiu, E. J. Petersson, E. E. Matthews, A. Schepartz, *J. Am. Chem. Soc.* **2006**, *128*, 11338; b) D. S. Daniels, E. J. Petersson, J. X. Qiu, A. Schepartz, *J. Am. Chem. Soc.* **2007**, *129*, 1532; c) J. L. Price, W. S. Horne, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 6376.

[3] a) J. M. Langenhan, I. A. Guzei, S. H. Gellman, *Angew. Chem.* **2003**, *115*, 2504; *Angew. Chem. Int. Ed.* **2003**, *42*, 2402; b) D. Seebach, M. Overhand, F. N. M. Kuhnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* **1996**, *79*, 913.

[4] S. Krauthauser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719.

[5] a) M. G. Woll, J. R. Lai, I. A. Guzei, S. J. C. Taylor, M. E. B. Smith, S. H. Gellman, *J. Am. Chem. Soc.* **2001**, *123*, 11077; b) M. Hagihara, N. J. Anthony, T. J. Stout, J. Clardy, S. L. Schreiber, *J. Am. Chem. Soc.* **1992**, *114*, 6568.

[6] J. S. Nowick, K. S. Lam, T. V. Khasanova, W. E. Kemnitzer, S. Maitra, H. T. Mee, R. Liu, *J. Am. Chem. Soc.* **2002**, *124*, 4972.

[7] a) P. Chitnumsub, W. R. Fiori, H. A. Lashuel, H. Diaz, J. W. Kelly, *Bioorg. Med. Chem.* **1999**, *7*, 39; b) J. S. Nowick, *Acc. Chem. Res.* **1999**, *32*, 287; c) Y. J. Chung, L. A. Christianson, H. E. Stanger, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 10555.

[8] a) T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1994**, *116*, 4105; b) S. K. Awasthi, S. Raghothama, P. Balaram, *Biochem. Biophys. Res. Commun.* **1995**, *216*, 375.

[9] J. D. Fisk, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **2000**, *122*, 5443.

[10] For a related approach see: a) Y. B. Feng, Z. C. Wang, S. Jin, K. Burgess, *J. Am. Chem. Soc.* **1998**, *120*, 10768; b) Z. C. Wang, S. Jin, Y. B. Feng, K. Burgess, *Chem. Eur. J.* **1999**, *5*, 3271.

[11] M. C. Etter, *Acc. Chem. Res.* **1990**, *23*, 120.

[12] a) L. L. Parker, A. R. Houk, J. H. Jensen, *J. Am. Chem. Soc.* **2006**, *128*, 9863; b) H. Guo, D. R. Salahub, *Angew. Chem.* **1998**, *110*, 3155; *Angew. Chem. Int. Ed.* **1998**, *37*, 2985.

[13] a) S. H. Gellman, G. P. Dado, G.-B. Liang, B. R. Adams, *J. Am. Chem. Soc.* **1991**, *113*, 1164; b) E. A. Gallo, S. H. Gellman, *J. Am. Chem. Soc.* **1993**, *115*, 9774.

[14] For full details of the FTIR, DMSO titration, and temperature coefficient data see the Supporting Information.

[15] M. K. N. Qureshi, M. D. Smith, *Chem. Commun.* **2006**, 5006.

[16] For a cyclopropyl β-amino acid that populates a helical conformation see: S. De Pol, C. Zorn, C. D. Klein, O. Zerbe, O. Reiser, *Angew. Chem.* **2004**, *116*, 517; *Angew. Chem. Int. Ed.* **2004**, *43*, 511.

[17] a) R. Taylor, O. Kennard, *J. Am. Chem. Soc.* **1982**, *104*, 5063; b) G. R. Desiraju, *Acc. Chem. Res.* **1991**, *24*, 290; G. R. Desiraju, *Chem. Commun.* **2005**, 2995.

[18] Z. S. Derewenda, L. Lee, U. Derewenda, *J. Mol. Biol.* **1995**, *252*, 248.

[19] F. Cordier, M. Barfield, S. Grzesiek, *J. Am. Chem. Soc.* **2003**, *125*, 15750.

[20] a) C. Schmuck, J. Lex, *Eur. J. Org. Chem.* **2001**, 1519; b) A. Sengupta, S. Aravinda, N. Shamala, K. M. P. Raja, P. Balaram, *Org. Biomol. Chem.* **2006**, *4*, 4214.

[21] a) T. S. Haque, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 2303; b) S. Raghothama, S. K. Awasthi, P. Balaram, *J. Chem. Soc. Perkin Trans. 2* **1998**, 137.

[22] CCDC 674635 (**6**), 674637 (**7**), 690077 (**9**) and 674636 (**12**) contain 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.

[23] a) Y. Gu, T. Kar, S. Scheiner, *J. Am. Chem. Soc.* **1999**, *121*, 9411; b) S. Scheiner, *J. Phys. Chem. B* **2006**, *110*, 18670.

[24] G. F. Fabiola, S. Krishnaswamy, V. Nagarajan, V. Pattabhi, *Acta Crystallogr. Sect. D* **1997**, *53*, 316.

[25] a) E. Y. Cheung, E. E. McCabe, K. D. M. Harris, R. L. Johnston, E. Tedesco, K. M. P. Raja, P. Balaram, *Angew. Chem.* **2002**, *114*, 512; *Angew. Chem. Int. Ed.* **2002**, *41*, 494; b) S. Aravinda, N. Shamala, A. Bandyopadhyay, P. Balaram, *J. Am. Chem. Soc.* **2003**, *125*, 15065.

[26] It has been commented that "contact atom pairs that occur in molecular crystals should not necessarily be considered as prime promoters of the (intermolecular) interaction but rather as a result of long range effects". J. D. Dunitz, A. Gavezzotti, *Helv. Chim. Acta* **2002**, *85*, 3949.

[27] a) F. Bernadini, M. Garavelli, M. Scatizzi, C. Tomasini, V. Trigari, M. Crisma, F. Formaggio, C. Peggion, C. Toniolo, *Chem. Eur. J.* **2002**, *8*, 2516; b) P. W. Baures, A. M. Beatty, M. Dhanasekaran, B. A. Helfrich, W. Pérez-Segarra, J. Desper, *J. Am. Chem. Soc.* **2002**, *124*, 11315.

[28] We interpret the larger temperature coefficient (in absolute scales) of a given proton as a reflection of more drastic changes in its microenvironment upon thermoperturbation. With increasing temperature in an aprotic solvent, the population-weighted average reflects a greater contribution from non-hydrogen-bonded conformations, which leads to significant shielding of those protons involved in hydrogen bonds, and hence larger temperature-related upfield shifts. In general, the cyclopropane $C^{\alpha}H$ protons that function as hydrogen-bond donors possess significantly higher temperature coefficients than those that do not. Full details of the temperature coefficient data can be found in the Supporting Information.

[29] a) N. H. Andersen, J. W. Neidigh, S. M. Harris, G. M. Lee, Z. Liu, H. Tong, *J. Am. Chem. Soc.* **1997**, *119*, 8547 b) J. G. Sonicki, P. E. Hansen, *J. Mol. Struct.* **2004**, *700*, 91.

