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Characterization of the Two Fundamental Conformations of Benzoylureas and Elucidation of the Factors That Facilitate Their Conformational Interchange

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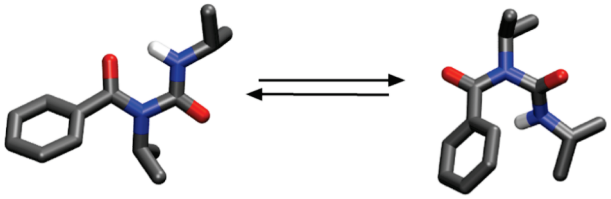

The ability of the benzoylurea core to mimic α helices relies on its ability to form an intramolecular hydrogen bond. The conformational behavior of benzoylureas is investigated in depth in this study via the use of NMR, IR, X-ray, and computational analysis. The results show that the closed conformation maintained by an intramolecular hydrogen bond is favored in most of the cases studied except when steric and electronic effects combined with a solvent possessing a high hydrogen bond accepting ability, such as DMSO, are involved. The study highlights the propensity for benzoylureas to switch conformation depending on the environment of the molecule for a particular set of substituents. We anticipate that our summary of the phenomenon of internal hydrogen bonding and its analysis may further serve as a useful reference source for future workers in this area.

Introduction

The discovery of organic scaffolds that mimic targeted binding epitopes is directly relevant to the development of small molecule inhibitors of protein−protein interactions. We have previously reported on our successful efforts in the design of small molecule structural and functional mimetics of pharmacologically active toxins.[1−6] Our current interest is the design of α-helical mimetics as inhibitors of those protein−protein interactions that comprise a helical epitope in the binding interface.[7,8] We identified the benzoylurea moiety to be particularly suitable for this task as it contains heteroatoms that introduce a certain degree of amphiphilicity within the scaffold without being excessively hydrophobic.[9] Furthermore, we envisaged this system to be synthetically versatile such that, when adequately functionalized, it could act as a α-helical protein mimetic. In particular, we were interested in the topographical similarity between the benzoylphenylurea and the terphenyl system, already shown by Hamilton and co-workers to be able to

(1) Baell, J. B.; Duggan, P. J.; Forsyth, S. A.; Lewis, R. J.; Lok, Y. P.; Schroeder, C. I. *Bioorg. Med. Chem.* **2004**, *12*, 4025–4037.
(2) Baell, J. B.; Duggan, P. J.; Lok, Y. P. *Aust. J. Chem.* **2004**, *57*, 179–185.
(3) Baell, J. B.; Forsyth, S. A.; Gable, R. W.; Norton, R. S.; Mulder, R. J. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 1119–1136.
(4) Baell, J. B.; Harvey, A. J.; Norton, R. S. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 245–262.
(5) Harvey, A. J.; Gable, R. W.; Baell, J. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3193–3196.
(6) Norton, R. S.; Baell, J. B.; Angus, J. A. In *Calcium channel pharmacology*; McDonough, S. I., Ed.; Kluwer/Academic/Plenum Press: New York, 2003; pp 143−179.
(7) Baell, J. B.; Huang, D. C. *Biochem. Pharmacol.* **2002**, *64*, 851–863.
(8) Lessene, G.; Czabotar, P. E.; Colman, P. M. *Nat. Rev. Drug Discovery* **2008**, *7*, 989–1000.
(9) Moreover, benzoylureas have a good track record in marketed pharmaceuticals. For example, see: Ware, G.; Whitcare, D. *The Pesticide Book*, 6th ed.; Meister Media Worldwide, Willoughby, OH, 2004.Okada, H.; Kato, M.; Koyanagi, T.; Mizuno, K. *Chem. Pharm. Bull.* **1999**, *47*, 430–433. Li, J.-N.; Song, D.-Q.; Lin, Y.-H.; Hu, Q.-Y.; Yin, L.; Bekesi, G.; Holland, J. F.; Jiang, J.-D. *Biochem. Pharmacol.* **2003**, *65*, 1691–1699.

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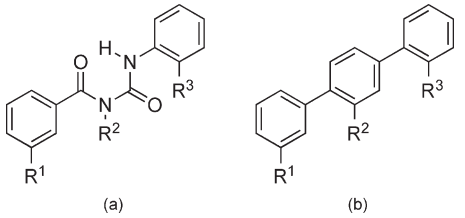


FIGURE 1. Comparison of (a) the benzoylphenylurea core and (b) Hamilton's terphenyl system.

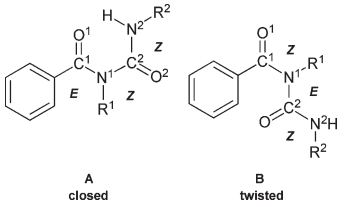


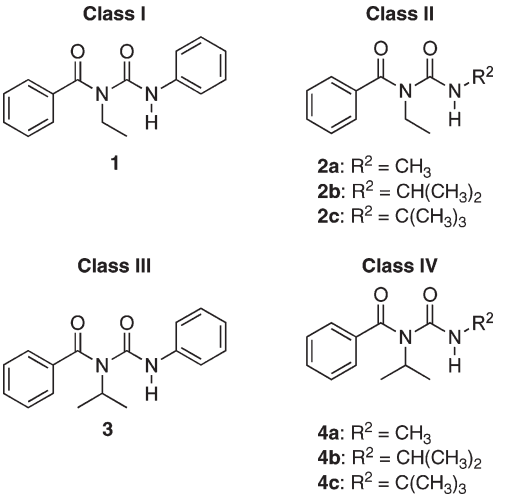
FIGURE 2. Two different benzoylurea conformations represented in the Cambridge Crystallographic Database.

geometrically and functionally mimic biologically active α-helical binding epitopes (Figure 1).[10−13]

The pivotal feature of the benzoylureas is the central acylurea group that is required to exist in the intended "closed" conformation necessary to project functional groups appropriately (**A**, Figure 2), forming a pseudo-6-membered ring with an intramolecular hydrogen bond and a central *Z* amide bond (*E* and *Z* geometry will refer to the position of the R^1 and R^2 groups in relation to their neighboring carbonyl group). Preliminary molecular mechanics modeling used in our design process confirmed this hypothesis. However, alternative conformations can be postulated: formally there exist eight possible conformations through rotation about three bonds.

A search of the Cambridge Crystallographic Database (CCD) confirmed that many benzoylureas exist in this pseudo-6-membered ring conformation. However, there are some benzoylureas for which the C^1-N^1 bond adopts a *Z* conformation[14] as shown by **B** in Figure 2. This conformation is not predicted by molecular mechanics to occupy a global or even local energy minimum.[15] Since appropriate scaffold conformation is clearly critical to mimetic design, we undertook a conformational analysis of model compounds under a variety of experimental conditions and support our findings with selected use of high-level density functional theory computational calculations.

CHART 1. Classification of Benzoylurea Derivatives Considered in the Present Study



We describe here for the first time why benzoylureas can adopt two distinct conformations—the "closed" form **A** or the "twisted" form **B**. We show that the preferred conformation adopted for a given benzoylurea depends on the nature of substituent groups and can be logically explained by a subtle interplay of electrostatic and steric effects.[16] We further elucidate why benzoylureas are better described structurally as bis-amides rather than as acylureas; conformationally, they behave as two distinct amide units without any urea character whatsoever. This structural characteristic enables strengthening of an internal hydrogen bond that stabilizes the closed form, which is otherwise inherently sterically disfavored. This hydrogen bond is not a resonance-assisted hydrogen bond as defined by Gilli et al., but despite this, is shown by gas-phase calculations to remain very strong.[17−19]

Results and Discussion

Compounds Studied. Compounds of interest in this study are shown in Chart 1, divided into four classes characterized by the nature of the substituents R^1 and R^2. A nonbranched R^1 substituent is defined as any alkyl group that comprises a methylene moiety α to the N^1 nitrogen atom, such as methyl or ethyl, whereas we define this substituent as "branched" when the carbon α to the nitrogen is branched, such as for R^1 = isopropyl, *sec*-butyl, *tert*-butyl, etc. Thus, we define

(10) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. *J. Am. Chem. Soc.* **2001**, *123*, 5382–5383.

(11) Kutzki, O.; Park, H. S.; Ernst, J. T.; Orner, B. P.; Yin, H.; Hamilton, A. D. *J. Am. Chem. Soc.* **2002**, *124*, 11838–11839.

(12) Yin, H.; Lee, G.-i.; Sedey, K. A.; Kutzki, O.; Park, H. S.; Orner, B. P.; Ernst, J. T.; Wang, H.-G.; Sebti, S. M.; Hamilton, A. D. *J. Am. Chem. Soc.* **2005**, *127*, 10191–10196.

(13) This work as well as applications to generate biologically active, Bcl-x_L-binding, α helical mimetics was presented in part at the 20th Royal Australian Chemical institute Organic Chemistry Conference, Cairns, Australia, 2004, abstract P(RACIOC) 40 and at Connect 2005, Sydney, Australia, 2005, Abstracts 248 and 364. The worldwide patent disclosing these structures was published in 2006 (Lessene G.; Baell, J. B. α-Helical Mimetics, PCT/AU2005/000968). This system has apparently been "rediscovered" in the interim and published (Rodriguez, J. M.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 8614–8617. Rodriguez, J. M.; Ross, N. T.; Katt, W. P.; Dhar, D.; Lee, G.-i.; Hamilton, A. D. *ChemMedChem* **2009**, *4*, 649–656).

(14) Of 25 structures in version 5.30 of CCD, three were in this alternative conformation.

(15) Maximin2 using the Tripos Forcefield as implemented in Sybyl7.01 (Tripos Associates, MO); a third, extended (*EEZ*) form has been identified as a putative biologically active conformation using the Catalyst/HIPHOP software program but in light of the likely generic inability of molecular mechanics to properly analyse benzoylurea conformation, this finding is questionable (see: Kurogi, Y.; Miyata, K.; Okamura, T.; Hashimoto, K.; Tsutsumi, K.; Nasu, M.; Moriyasu, M. *J. Med. Chem.* **2001**, *44*, 2304−2307).

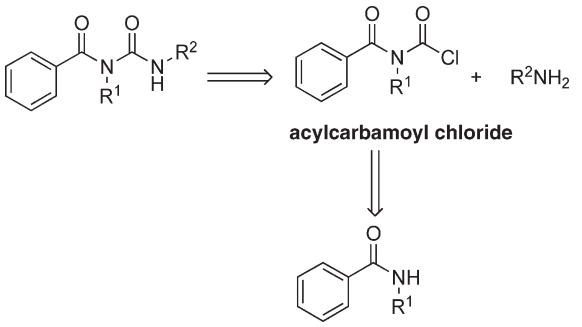
(16) Kohmoto et al. have reported the closed form of a benzoylureas (Kohmoto, S.; Iwasaki, N.; Fukui, D.; Nishio, T.; Iida, I.; Kishikawa, K.; Yamamoto, M.; Yamada, K. *J. Chem. Soc., Perkin Trans. 2* **1996**, 985−988) and analyzed a twisted form of an alkylacylurea (Kohmoto, S.; Kasimura, H.; Nishio, T.; Iida, I.; Kishikawa, K.; Yamamoto, M.; Yamada, K. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1565−1568) but have not extended this to an understanding of conversion between these two forms.

(17) Gilli, G.; Gilli, P. *J. Mol. Struct.* **2000**, *552*, 1–15.

(18) Gilli, P.; Bertolasi, V.; Pretto, L.; Lycka, A.; Gilli, G. *J. Am. Chem. Soc.* **2002**, *124*, 13554–13567.

(19) Gilli, P.; Bertolasi, V.; Ferretti, V.; Gilli, G. *J. Am. Chem. Soc.* **2000**, *122*, 10405–10417.

SCHEME 1. Retrosynthesis of Benzoylureas

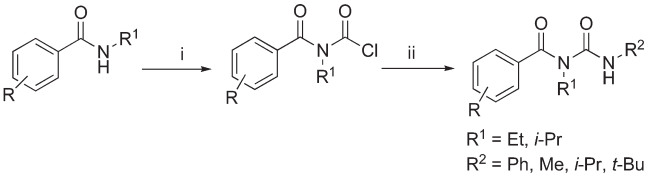


four distinct classes of compounds: class I compounds (**1**) possess a nonbranched N^1 substituent (ethyl) and a phenyl ring on N^2; class II compounds (**2a**−**c**) possess a nonbranched N^1 substituent (ethyl) and a varying alkyl R^2 group; class III compounds (**3**) possess a branched N^1 substituent (isopropyl) and a phenyl ring on N^2; class IV compounds (**4a**−**c**) possess a branched N^1 substituent (isopropyl) and an alkyl R^2 group.[20]

Synthesis of Benzoylureas. Although a large number of benzoylureas are known, the chemistry surrounding this group of compounds is rather disparate, especially when R^1 is not a hydrogen atom. Methods using carbodiimides[21,22] or ureas[23] are known and furnish "symmetrical" benzoylureas ($R^1 = R^2$). Examples where R^1 and R^2 are different alkyl groups have been presented in an Eli Lilly patent and in a study of semisynthetic β-lactam antibiotics.[24,25] The key feature in these two preparations is the formation of an acylcarbamoyl chloride from reaction of a chlorocarbonyl donor with an activated secondary amide. Subsequent reaction with amines furnishes differentially substituted benzoylureas (Scheme 1). In the Eli Lilly report,[24] the amide was activated by generation of the anion via the use of butyllithium, whereas Ohi et al.[25] achieved activation through silylation with trimethylsilyl choride (TMSCl).

We attempted the butyllithium-mediated method first, but this was not successful with amides bearing a branched R^1 substituent and so we turned to the silylation-based approach. We noted that a milder silylation-based route to the synthesis of acylcarbamoyl chlorides than that used by Ohi et al. has also been reported, using trimethylsilyl triflate (TMSOTf) as the silylating agent and phosgene as the chlorocarbonyl donor.[26,27] This synthetic route has not been applied to the synthesis of benzoylureas, but we reasoned that it should be possible to do so by reacting the acylcarbamoyl chloride intermediate with either an aniline using triethylamine as an acid scavenger or with 2 equiv of an alkylamine.

SCHEME 2. Benzoylurea Preparation



Conditions: i) a) TMSOTf 1.1 eq., Et_3N 1.1 eq., Et_2O. b) 20% $COCl_2$ in toluene. ii) a) R_2 = Ph, $PhNH_2$.HCl 1.1 eq., Et_3N 2.2 eq., CH_3CN or b) R^2 = Me, *i*Pr, *t*Bu, RNH_2 2.2 eq., CH_3CN

We applied this approach to the benzoylureas and were pleased to see that this procedure furnished all the desired compounds in high yields and with great ease of purification (Scheme 2).

Spectroscopic Study. A clear feature that distinguishes between the closed and twisted benzoylurea conformations in Figure 2 is the respective presence or absence of an intramolecular hydrogen bond. IR and ^{1}H NMR spectroscopy are classical techniques used to analyze hydrogen-bonding behavior.[28] IR spectroscopy results are relatively straightforward to interpret. Hydrogen bonding can be intermolecular, involving the solvent or another molecule of solute, or can be intramolecular. Assignment of an intramolecular hydrogen bond by IR spectroscopy, therefore, is clearer in a non-hydrogen-bonding solvent (or very weak hydrogen-bonding solvent), such as chloroform, with dilute conditions (to reduce the potential for dimer formation of the solute), and for solutes with only a limited number of amide NH groups (to minimize band overlap).[28−31] The time scale in IR spectroscopy is fast compared with conformational changes and allows the observation of the two characteristic bands of N−H bond vibration. In non-hydrogen-bonding solvents, a broadband at longer wavelengths (usually around 3200−3340 cm^{-1}) corresponds to a hydrogen-bonded NH, while a sharp band at shorter wavelengths (usually around 3400−3450 cm^{-1}) corresponds to a solvent-exposed NH. Caution must be used in the use of IR spectra to estimate conformer ratios since a hydrogen-bonded conformation might have a distinctly different absorption coefficient from a non-hydrogen-bonded conformation. In this study, we used IR spectroscopy to qualitatively assess the presence of hydrogen-bonded versus non-hydrogen-bonded conformations to support quantitative interpretation of data obtained via ^{1}H NMR spectroscopy.

NMR has advantages over IR as a spectroscopic technique since data from experiments in polar solvents as well as nonpolar solvents can be readily and meaningfully interpreted.[32−34] If the benzoylurea interconverts rapidly on an NMR time scale between two different conformations, **A** and **B**, and if the limiting chemical shift values of a given proton signal of each form are known, the equilibrium

(20) All classes are drawn as extended, conformationally "neutral" forms, indicative of neither the closed nor twisted conformations.
(21) Kishikawa, K.; Yamamoto, M.; Kohmoto, S.; Yamada, K. *J. Org. Chem.* **1989**, *54*, 2428–2432.
(22) Lee, W. W.; Martinez, A. P.; Goodman, L. *J. Med. Chem.* **1974**, *17*, 326–330.
(23) Smith, T. D.; Jones, P. G.; Schmutzler, R. *Z. Naturforsch. B* **1992**, *47*, 526–532.
(24) Cooper, R. D. G.; Herron, D. K. Eli Lilly Co.: France, **1975**.
(25) Ohi, N.; Aoki, B.; Shinozaki, T.; Moro, K.; Kuroki, T.; Noto, T.; Nehashi, T.; Matsumoto, M.; Okazaki, H.; Matsunaga, I. *Chem. Pharm. Bull.* **1987**, *35*, 1903–1909.
(26) Horner, J. H.; Musa, O. M.; Bouvier, A.; Newcomb, M. *J. Am. Chem. Soc.* **1998**, *120*, 7738–7748.
(27) Esker, J. L.; Newcomb, M. *Tetrahedron Lett.* **1992**, *33*, 5913–5916.
(28) Aaron, H. S. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Eds.; John Wiley & Sons: New York, 1979; Vol. 11, pp 1−52.
(29) Gellman, S. H.; Dado, G. P.; Liang, G. B.; Adams, B. R. *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173.
(30) Stevens, E. S.; Sugawara, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050.
(31) Analogous arguments apply to OH, SH, and amino NH groups, but our focus here is the amide NH.
(32) Baxter, N. J.; Williamson, M. P. *J. Biomol. NMR* **1997**, *9*, 359–369.
(33) Cierpicki, T.; Otlewski, J. *J. Biomol. NMR* **2001**, *21*, 249–261.
(34) Kessler, H. *Angew. Chem., Int. Ed.* **1982**, *21*, 512–523.

constant can be retrieved providing the ratio of the two conformers (eq 1)[35]

$$\mathbf{A} \rightleftarrows \mathbf{B} \quad K_{eq} = \frac{\boldsymbol{\delta}_{\mathbf{A}} - \boldsymbol{\delta}_{obs}}{\boldsymbol{\delta}_{obs} - \boldsymbol{\delta}_{\mathbf{B}}} \qquad (1)$$

where δ_A is the chemical shift of a given proton signal in conformation **A**, δ_B is the chemical shift of a given proton signal in conformation **B**, and δ_{obs} is the observed chemical shift.

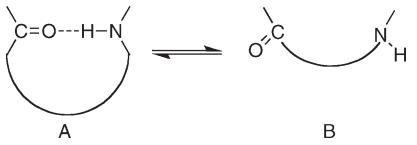
Equation 1 is most useful when δ_A and δ_B are well separated and is highly suited for situations where one conformation (**A**) is hydrogen bonded and the other (**B**) is not (since protons usually undergo a significant downfield shift upon hydrogen bonding).

The temperature dependence of an NH chemical shift has also been used as a tool to study hydrogen bonding.[36] Early experiments involved conformationally rigid cyclic peptides in strong hydrogen-bonding solvents where amide NH protons were either locked in an internal hydrogen bond or hydrogen-bonded to solvent.[37] Whereas the former gave rise to a small negative temperature dependence of the chemical shift (NTDCS), the latter gave rise to large NTDCS values. Hence, a widely used interpretation for polar solvents such as DMSO-d_6 states that an NH NTDCS value of greater than −3 ppb/K is diagnostic of an internally hydrogen-bonded NH proton, whereas a value of less than −4 ppb/K infers a solvent-exposed NH proton.[34,38] While this interpretation has found to have no predictability in the case of less conformationally rigid peptides,[39] we consider it applicable in the context of small molecules.[40]

In nonpolar solvents such as chloroform, the situation is more complex, and equivocal results may be obtained,[39] especially if conformational equilibria are involved. In seminal papers by Gellman et al., a series of di- and triamides were used as tools in IR and NMR studies to develop an understanding of how NTDCS values in nonpolar solvents can be applied to the interpretation of conformationally heterogeneous systems.[29,41−44] The conclusions for studies undertaken in $CDCl_3$ and CD_2Cl_2 are summarized in Table 1. Briefly, a small NTDCS of the NH proton (> −5 ppb/K) can indicate one of three possibilities: (i) an equilibrium between an internally hydrogen-bonded conformation and non-hydrogen-bonded conformation where the two conformations are of similar enthalpy; (ii) a proton mostly engaged in an intramolecular hydrogen bond whose conformational environment is invariant over the course of the experiment; or (iii) a proton mostly not engaged in an intramolecular hydrogen bond whose conformational environment does not change over the course of the experiment. On the other hand, a large NH proton NTDCS (< −6 ppb/K) implies that the hydrogen-bonded and the solvent exposed states are in equilibrium but that neither of these states is largely preferred and **A** is enthalpically favored. The complexity of the interpretation of NMR data underscores the importance for dual consideration of the IR data to unequivocally address the presence of one or more conformers when a solvent such as chloroform is involved. For our study in chloroform, we therefore chose to combine the two experiments and used the rules presented in Table 1 to interpret the data obtained.

NTDCS values can also be useful to study the thermodynamics of systems undergoing rapid conformational equilibrium on an NMR time scale. Provided that the NTDCS

TABLE 1. Interpretation of NMR Spectroscopic Data in Terms of Conformational Behavior (These Rules Are a Synthesis of Findings from Refs 29 and 30 in Chloroform and Ref 34 in DMSO)[a]



Solvent	NMR		Interpretation
$CDCl_3$[b]	Small NTDCS (> -5 ppb/K)	(i)	ΔH° small; A ⇌ B
		(ii)	A ⇌ B
		(iii)	A ⇌ B
	Large NTDCS (< -6 ppb/K)	(iv)[c,d]	ΔH° not small; A ⇌ B
DMSO	Small NTDCS (> -3 ppb/K)	(v)	Internally hydrogen-bonded NH (**A**)
	Large NTDCS (< -4ppb/K)	(vi)	NH intermolecularly hydrogen-bonded to solvent (**B**)

[a]This summary relates mainly to a two-state system, which applies to our case. Other more complex behaviors can be observed with molecules inherently more flexible or having more than one hydrogen bond donor or acceptor. For example, a small positive NTCDS has been observed for a non-hydrogen-bonded NH becoming increasingly hydrogen bonded in an enthalpically less favored conformation with increasing temperature. From our reading it is clear that these rules are still not fully worked out and our interpretations are only intended to be guides.[42] [b]Amounts of water can influence the results of the NMR analysis.[29,54] Dry $CDCl_3$ was used for this study. [c]To put this into context, it has been stated in one particular case that a large enthalpic difference of around 6 kJ/mol in favor of **A** over **B** could correspond to a NTDCS of −10 ppb/K. The proportion of **B** in this case increases slightly with increasing temperature.[29] [d]The degree of enthalpic difference has been related to the how close the geometry of the hydrogen bond in **A** is to the optimum linear geometry, but this can be readily counteracted by steric strain, for example.[45]

(35) Nowick, J. S.; Holmes, D. L.; Mackin, G.; Noronha, G.; Shaka, A. J.; Smith, E. M. *J. Am. Chem. Soc.* **1996**, *118*, 2764–2765.

(36) A slow rate of deuterium exchange is also often used to indicate internal hydrogen bonding. In peptides and proteins, a slow rate of exchange indicates a buried proton, which may or may not be intramolecularly hydrogen bonded (but would usually be expected to be so), or a solvent-exposed proton that is intramolecularly hydrogen bonded. A faster rate of exchange indicates a solvent-exposed and non-hydrogen-bonded proton. In principle, these experiments could be informative for small molecules where solvent exposure is not an issue, and whether an NH proton is hydrogen-bonded or not can be judged by the rate of deuterium exchange. However, these experiments can be tedious to perform and results hard to interpret where rapid equilibria between hydrogen-bonded and non-hydrogen-bonded conformations on an NMR time scale occur, either for a peptide or small molecule.

(37) Llinas, M.; Klein, M. P. *J. Am. Chem. Soc.* **1975**, *97*, 4731–4737.

(38) Martinezmartinez, F. J.; Arizacastolo, A.; Tlahuext, H.; Tlahuextl, M.; Contreras, R. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1481–1485.

(39) Andersen, N. H.; Neidigh, J. W.; Harris, S. M.; Lee, G. M.; Liu, Z. H.; Tong, H. *J. Am. Chem. Soc.* **1997**, *119*, 8547–8561.

(40) This supposition is supported by the fact that the data we obtain for benzoylureas are consistent with interpretation v and vi in Table 1.

(41) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3138–3139.

(42) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 4228–4245.

(43) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054–1062.

(44) Gellman, S. H.; Adams, B. R.; Dado, G. P. *J. Am. Chem. Soc.* **1990**, *112*, 460–461.

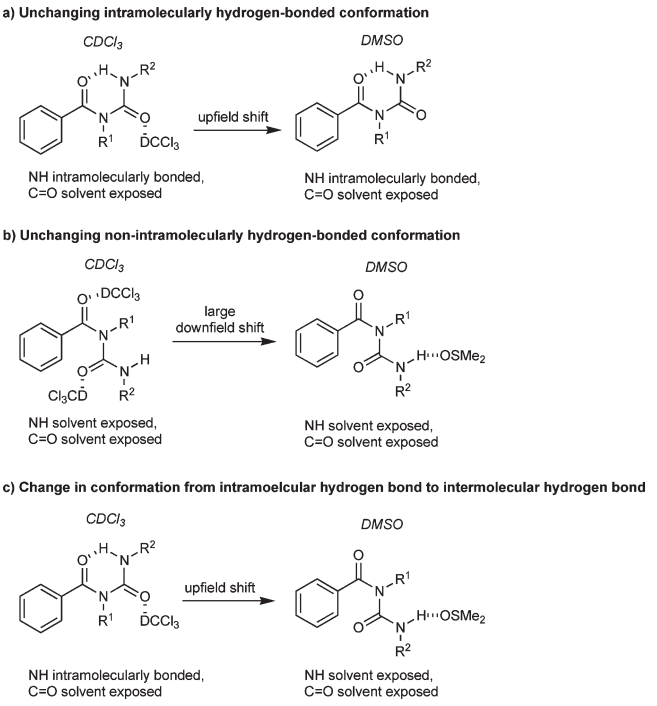


FIGURE 3. Expected changes in an NH chemical shift (shown here using a benzoylurea system) in going from $CDCl_3$ to DMSO-d_6 and when conformational interchange between intramolecularly hydrogen-bonded and nonintramolecularly hydrogen-bonded conformations can occur.

for model compounds representing the fully hydrogen-bonded state and the fully non-hydrogen-bonded state are known,[46] it has been shown that in nonpolar solvents, thermodynamic data can be obtained (eq 2) using K_{eq} obtained at each temperature via eq 1.[43,47,45]

$$\ln K_{eq} = -\frac{\Delta H^\circ}{R}\frac{1}{T} + \frac{\Delta S^\circ}{R} \qquad (2)$$

Hence, while interpretation of NTDCS values in nonpolar solvents such as chloroform is much more complicated than was initially appreciated, these experiments can provide useful qualitative and quantitative information when coupled with another technique such as IR spectroscopy.

Comparison of the values of chemical shift for a given NH proton in $CDCl_3$ or DMSO-d_6 can also provide useful conformational information.[37,47] We illustrate this in Figure 3 using a benzoylurea system rather than a generic structure. Llinas and Klein have shown that if an amide NH proton is intramolecularly hydrogen-bonded and its amide carbonyl is solvent exposed, its chemical shift is further upfield in DMSO-d_6 compared with $CDCl_3$ (Figure 3a).[37] The rationale for this observation is that chloroform can donate a weak hydrogen bond to the carbonyl oxygen atom.

TABLE 2. Spectroscopic Data (See the Experimental Section for Details)

	$CDCl_3$[b]		DMSO-d_6[c]	
compd[a]	δ(NH)[d]	NTDCS[e]	δ(NH)[d]	NTDCS[e]
1	11.33	−4.2	10.04	−1.3
2a	8.97	−3.9	8.11	−2.3
2b	8.83	−5.0	7.93	−2.0
2c	8.90	−5.4	7.84	−1.6
3	10.30	−9.1	9.93	−4.5
4a	7.76	−8.8	7.82	−4.7
4b	6.70	−3.9	7.75	−4.6
4c	6.19	−1.0	7.58	−5.2
5a	7.08	−2.5	9.91	−4.8
5b	5.42	−3.9	7.73	−4.8
5c	5.27	−5.8	7.67	−5.4
5d	5.29	−4.6	7.41	−5.7

[a]Compounds **5a**−**d** are model compounds that cannot form an internal hydrogen bond.[50,51] **5a** is acetanilide, **5b** is *N*-methylacetamide, **5c** is *N*-isopropylacetamide, and **5d** is *N*-*tert*-butylacetamide. [b]Sample concentration: 3.0 mM. [c]Sample concentration: 10.0 mM. [d]ppm. [e]ppb/K.

On the other hand, an exposed amide NH proton in $CDCl_3$ undergoes a significant *downfield* shift when the solvent is changed to DMSO-d_6 due to the strong hydrogen-bond-accepting ability of the latter solvent (Figure 3b).[48] A third situation arises when the conformation differs in the two solvents, in particular where there is a loss of an intramolecularly NH hydrogen-bonded conformation (Figure 3c).[49] In such a situation, the otherwise large upfield chemical shift of an NH proton that would occur on the loss of an intramolecular hydrogen bond would be greatly reduced through intermolecular hydrogen bonding of this proton with DMSO-d_6. Clearly, the observation of an upfield shift of an amide NH proton resonance in changing from an environment of $CDCl_3$ to DMSO-d_6 does not in itself distinguish between cases (a) and (c) but does confirm the presence of an intramolecular hydrogen bond in the former solvent.

We have obtained IR data for the NH protons in the benzoylureas in chloroform and analyzed the chemical shift of the NH proton in each compound and its temperature dependence, both in $CDCl_3$ and DMSO-d_6. We will show how this set of spectral data can be used to define the conformational behavior of this series of compounds, which elucidates a fundamental conformational property common to all benzoylureas. The results of our spectroscopic study are presented in Table 2 for the NMR data, while the IR spectra are shown in Figure 4.

Class I. The IR spectrum of compound **1** showed a diagnostic broad absorption band between 3200 and 3350 cm^{-1}, indicative of NH internal hydrogen bonding at this temperature. This hydrogen-bonded NH proton consequently resonates very far downfield at 11.33 ppm in the NMR spectrum, compared with 7.08 ppm for acetanilide (Table 2), a relative downfield shift of 4.25 ppm. In DMSO-d_6, the NMR signal remained far downfield (10.04 ppm) but upfield compared with the value in $CDCl_3$. The small NTDCS of −1.3 ppb/K and the upfield shift from $CDCl_3$

(45) Gung, B. W.; Zhu, Z. H.; Zou, D.; Everingham, B.; Oyeamalu, A.; Crist, R. M.; Baudlier, J. *J. Org. Chem.* **1998**, *63*, 5750–5761.

(46) Selection of model compounds that accurately represent the two extreme states is not necessarily trivial (see, for example, ref 41).

(47) Gellman, S. H.; Dado, G. P. *Tetrahedron Lett.* **1991**, *32*, 7377–7380.

(48) Where a compound has an amide group with an exposed NH and an exposed carbonyl, a downfield NH chemical shift is still observed in DMSO-d_6 relative to $CDCl_3$ because even though both solvents induce a relative downfield shift, the influence that DMSO-d_6 has by hydrogen bonding to the NH proton is much greater than the influence that $CDCl_3$ has by protonating the carbonyl group.

(49) This is not considered unusual since DMSO is a strong hydrogen bond acceptor and could compete with an intramolecular hydrogen bond in a nonpolar solvent. In principle, if the intramolecular hydrogen bond in $CDCl_3$ is very weak through participation by a poor hydrogen bond acceptor, this situation could give rise to a downfield shift when the solvent is changed to DMSO.

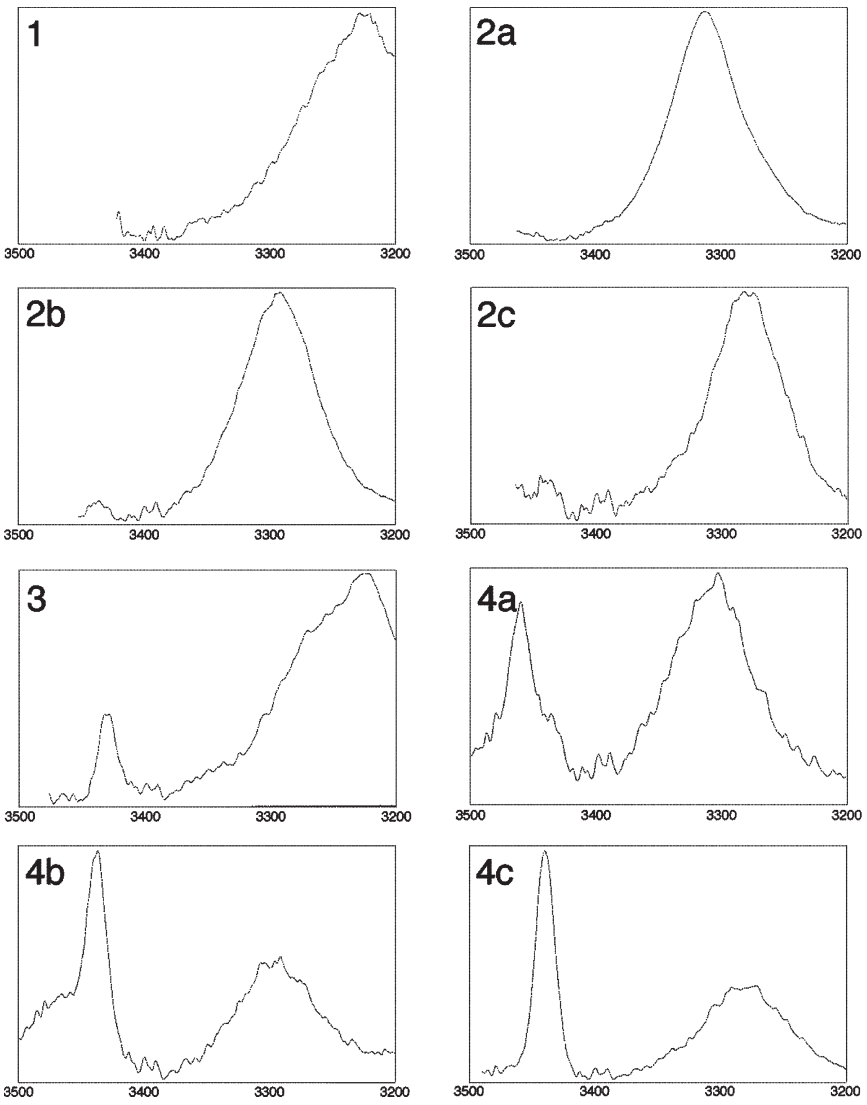


FIGURE 4. Infrared spectra for compounds **1**, **2a**−**c**, **3**, and **4a**−**c**. All samples at 3.0 mM in $CHCl_3$. Spectra shown in absorbance mode.

to DMSO-d_6 are diagnostic of a hydrogen-bonded NH group in $CDCl_3$ (Figure 3a). The thermal stability of the hydrogen-bonded form in the polar, strong hydrogen bond-accepting environment of DMSO would be expected to be even greater in the nonpolar environment of $CDCl_3$. This suggests that the $CDCl_3$ NTDCS value of −4.2 ppb/K equates to interpretation (ii) in Table 1 and that **1** exists in the hydrogen-bonded form in this solvent at all temperatures studied.

In summary, we conclude that class I compounds are likely to adopt a hydrogen-bonded conformation in both $CDCl_3$ and DMSO-d_6. This conformation remains favored during the variable-temperature experiment.[52]

Class II. The IR spectra for compounds **2a**−**c** showed a classic broad NH absorption band between 3200 and 3400 cm^{-1}, indicative of an intramolecular hydrogen bond. The NH resonances in the NMR spectra at 8.97, 8.83, and 8.90 ppm for **2a**, **2b**, and **2c**, respectively, in $CDCl_3$ are also diagnostic of a hydrogen-bonded conformation, being substantially downfield relative to their respective non-hydrogen-bonded model compounds **5b**, **5c**, and **5d** (5.42, 5.27, and 5.29 ppm, respectively), so that the relative downfield shifts for this NH proton in **2a**, **2b**, and **2c** relative to **5b**, **5c**, and **5d** were 3.55, 3.55, and 3.61 ppm, respectively.[53] These shifts are not quite as great as the downfield shift of 4.25 ppm for class I compounds, which is consistent with the greater hydrogen bond strength through resonance stabilization that an anilide NH proton should be able to provide over an alkylamide NH proton. In DMSO-d_6, the small NTDCS

(50) Hence, **5a** is a model for noninternally hydrogen-bonded conformations of **1** and **3**, **5b** is a model for noninternally hydrogen-bonded conformations of **2a** and **4a**, **5c** is a model for noninternally hydrogen-bonded conformations of **2b** and **4b**, and **5d** is a model for noninternally hydrogen-bonded conformations of **2c** and **4c**.

(51) Several different model systems for internally hydrogen-bonded and noninternally hydrogen-bonded conformations of benzoylureas were investigated before finally choosing the most representative models used herein.

(52) We have made several other class I compounds and have found that all behave similarly (data not shown).

(53) Both **2c** and **5d** exhibit a slight downfield shift of the NH proton resonance relative to their respective counterparts **2b** and **5c**. We propose that this could be due to the effect of the *tert*-butyl group in stabilizing carbonyl protonation by $CDCl_3$. It is possible that the slightly greater relative downfield shift in **2c** is due to steric compression by the *tert*-butyl group pushing the NH proton closer to the C^1 carbonyl.

values of −2.3, −2.0, and −1.6 ppb/K, respectively, clearly indicate a hydrogen-bonded conformation, which is stable over the variable temperature NMR experiment, a situation that is likely to be even more favored in $CDCl_3$. We conclude, therefore, that the $CDCl_3$ NTDCS values for the NH proton of −3.9, −5.0, and −5.4 ppb/K, respectively, align with interpretation (ii) in Table 1, even though the last value lies just outside the conventional range for this interpretation.[54] All compounds exhibited an upfield shift in the resonance of the NH proton in changing solvents from $CDCl_3$ to DMSO-d_6, consistent with the situation in Figure 3a.

In summary, we conclude that class II compounds adopt a hydrogen-bonded conformation in both $CDCl_3$ and DMSO-d_6. This conformation remains favored during the variable-temperature experiment.

Class III. Compound **3** is closely related to **1** (class I) but exhibits a distinctly different conformational behavior. At room temperature, the IR spectrum of compound **3** shows a strong, broad absorption band between 3200 and 3350 cm^{-1}, indicative of hydrogen bonding, and of the closed form. However, there is a small sharp NH band between 3400 and 3450 cm^{-1} indicating the presence of a non-hydrogen-bonded conformation. In chloroform, the NH ^1H NMR signal is far downfield at 10.30 ppm but is still upfield from the corresponding signal in **1**, consistent with a mixture dominated by the hydrogen-bonded form but containing a significant amount of the non-hydrogen-bonded form. Using **1** and acetanilide as models for hydrogen-bonded and non-hydrogen-bonded conformations, respectively, we can deduce from the chemical shift value that the conformer population of **3** comprises 24% of the non-hydrogen-bonded conformation and 76% of the hydrogen-bonded conformation. The large NTDCS of −9.1 ppb/K is supportive of situation (iv) in Table 1.

The upfield shift observed for the chemical shift in DMSO-d_6 at 300 K compared to $CDCl_3$ is also indicative of a mixture dominated by an internally hydrogen-bonded conformation in $CDCl_3$ (Figure 3a). In the more polar solvent, compound **3** adopts a non-hydrogen-bonded conformation: the DMSO-d_6 NTDCS value of −4.5 ppb/K is diagnostic of a solvent-exposed proton. As expected, the NH chemical shift of 9.93 ppm is very close to the value for acetanilide in the same conditions (9.91 ppm). This downfield value is testament to the strong interaction between the NH proton and DMSO for both compounds and is only marginally upfield from the NH chemical shift of the hydrogen-bonded form of **3** in $CDCl_3$. In summary, class III compounds prefer to adopt a hydrogen-bonded form at room temperature in chloroform; however, they are readily perturbed toward a non-hydrogen-bonded form in polar hydrogen-bond-accepting solvents.[55]

Class IV. The IR spectrum of compounds in this class revealed an intriguing but consistent trend in conformational behavior. As the N^2 substituent becomes progressively bulkier, from NHMe (**4a**) to NH-*i*-Pr (**4b**) to NH-*t*-Bu (**4c**), the intensity of the IR absorption band corresponding to a hydrogen-bonded NH becomes progressively smaller and that for a non-hydrogen-bonded NH becomes progressively larger to become the major feature for **4c**. Consistent with this trend, the chemical shift of the respective NH protons are further upfield than those in the corresponding class II compounds **2a**−**c**, but not shifted upfield as much as those in the corresponding respective model compounds **5b**−**d**. Using these NMR chemical shift data, we estimated the respective proportion of the closed form to be 66%, 40%, and 25%, respectively. The hydrogen-bonded form is, therefore, preferred in $CDCl_3$ only for **4a** where R^2 is methyl, and changing to isopropyl and then *tert*-butyl gives rise to an increasing amount of the non-hydrogen-bonded form. The $CDCl_3$ NTDCS data is as follows: compound **4a** displayed a large NTDCS (−8.8 ppb/K), **4b** a moderate NTDCS (−3.9 ppb/K), and **4c** a very small (−1 ppb/K) NTDCS. Considering the IR spectra, these values are consistent with interpretations iv, i, and iii, respectively, in Table 1.

Class IV compounds can adopt a non-hydrogen-bonded conformation in $CDCl_3$, and it is expected that a strong hydrogen-bond-disrupting solvent such as DMSO would completely destabilize the hydrogen-bonded form, analogous with the behavior observed for **3**. This is confirmed by the large DMSO-d_6 NH NTDCS values of −4.7, −4.6, and −5.2 ppb/K, respectively, for **4a**, **4b**, and **4c**. The NH resonance in all class IV compounds is shifted downfield in DMSO-d_6 compared with $CDCl_3$, markedly so for **4c**, less so for **4b**, and only marginally for **4a**. This is consistent with situations b and c in Figure 3, where in the case of **4a**, the situation depicted in Figure 3b is minor but in Figure 3c is major (as **4a** is mostly internally hydrogen-bonded in chloroform). In contrast, for **4c**, the situation depicted in Figure 3b is major and in Figure 3c is minor (as **4c** is mostly not internally hydrogen bonded in chloroform), and so there is a more marked downfield NH chemical shift observed in DMSO-d_6 relative to $CDCl_3$. The situation for compound **4b** lies somewhere between those for **4a** and **4c**.

In conclusion, most of the benzoylureas preferentially adopt hydrogen-bonded conformations in chloroform with the exception of class IV compounds **4b** and **4c**, for which the equilibrium is shifted toward the non-hydrogen-bonded conformer at room temperature. In DMSO, compounds from classes I and II retain a stable hydrogen-bonded conformation, but class III and IV compounds adopt the non-hydrogen-bonded conformation.

Conformation in the Solid State. The inherent good crystallinity that we observed for class IV compounds naturally lent them to X-ray crystallographic analysis. Shown in Figure 5a is the solid-state structure of **4a**, which crystallized from acetonitrile in the twisted conformation. The spectroscopic data for **4a** had indicated that while it preferred to exist as the closed form in $CDCl_3$, the polar environment of DMSO stabilized the twisted form, and it appears the crystal-packing stabilization has done so similarly. In the crystal lattice, this compound formed loose dimers with favorable electrostatic interactions between the N^2−H of one molecule with the C^1=O of another and the N^2−H of the second molecule with the C^1=O of the first. The lack of propensity for this compound to form tight dimers, even when crystallized, was illustrated by the fact that the distance between the respective N^2 atom with its intermolecular O^1 counterpart was long, 3.16 Å.[56] Similar remarks apply to **4c**, which also crystallized from acetonirile in the twisted conformation, as shown in Figure 5c.

(54) Adrian, J. C.; Wilcox, C. S. *J. Am. Chem. Soc.* **1991**, *113*, 678–680.
(55) We have synthesized other class III compounds, and all behave similarly (data not shown).
(56) Steiner, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 48–76.

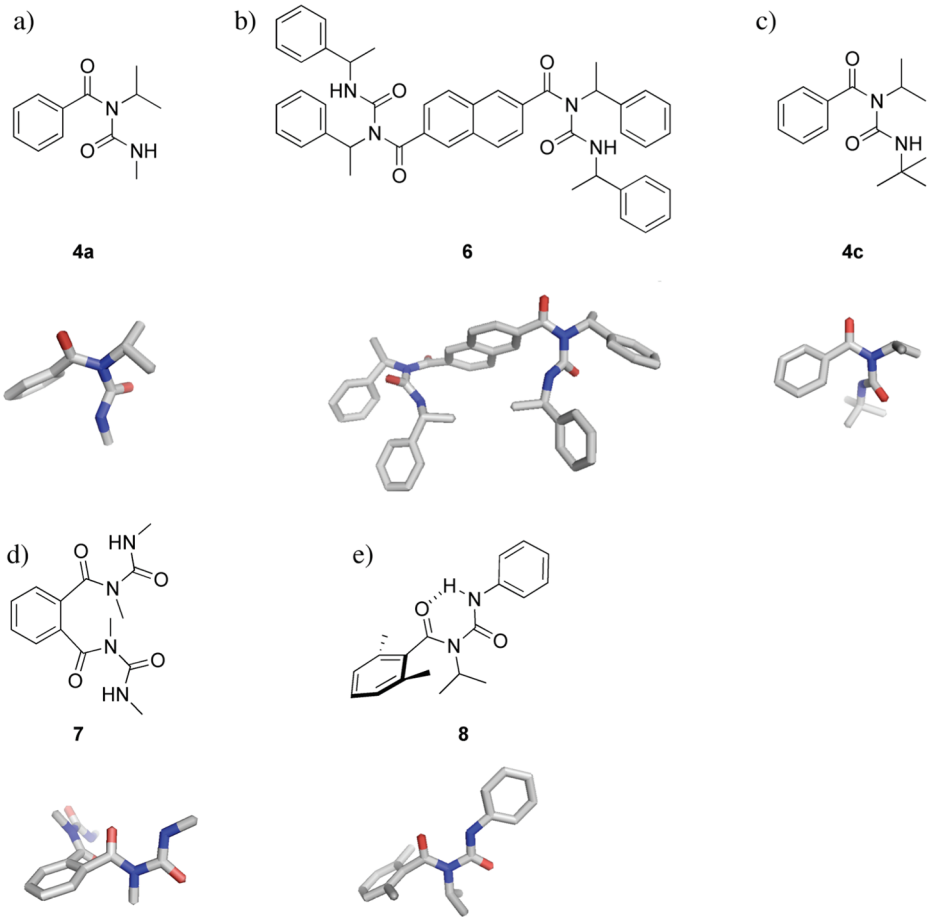


FIGURE 5. Molecular structure and crystal structure of (a) **4a**; (b) compound **6** from Ref 16; (c) **4c**; (d) **7** from Ref 23; and (e) **8**.

Focusing on **4a**, the phenyl ring is significantly out-of-plane with the adjoining carbonyl group by 35.2°. The C^1-N^1 amide bond is in a *Z* conformation causing R^1 to be *trans* to the benzoyl ring but eclipsed with the C^1 carbonyl group. Conversely, the N^1-C^2 amide bond is neither *E* nor *Z* but twisted so that R^1 is not planar with the C^2-N^2 amide group. The net effect is to cause the plane of the $C^2(=O)-N^2$ amide to face toward the plane of the benzoyl ring. The amide plane can face either way, and indeed, the "acylurea twist" in **4c** is the mirror image of the twist in **4a**, with a dihedral angle for $C^1-N^1-C^2=O^2$ of 121.4° in **4a** and −130.5° in **4c**. The C^2-N^2 amide is in a *Z* conformation according to our definition in Figure 2. For comparison, we have included in Figure 5b the published crystal structure of compound **6**, one of only three other representatives of class IV benzoylureas in CCD. The overall orientation of the acylurea scaffold is very similar in all these compounds, and the twisted conformation in **6** is essentially identical to that in **4a**.

In stark contrast, those benzoylureas in this study that preferred the closed conformation did not yield so readily to X-ray crystallographic analysis. We attributed this to the decreased ability of the closed conformation to form favorable intermolecular electrostatic interactions when $R^1 \neq H$ compared with the twisted form. This is supported by the observation that the crystal structures of benzoylureas that are unsubstituted at N^1, and are consequently able to form favorable electrostatic intermolecular interactions in the solid state, are well represented in the CCD, numbering 21. However, there is only one example of a benzoylurea with an unbranched N^1 alkyl group, this being the class II bis-benzoylurea **7** with an N^1-Me substituent.[57,23] As shown in Figure 5d, **7** adopts a closed conformation, as was expected on the basis of the solution conformational behavior that we observed for class II compounds. Our attempts to crystallize compounds in the closed conformation with an alkyl R^1 group were successful with only one compound made in the course of our research, that being **8** (Figure 5e). This is, therefore, the first report of a class III benzoylurea crystal structure. This particular example shows that the six-membered ring maintained by the hydrogen bond is coplanar with the anilide phenyl ring.

In summary, the X-ray crystallographic results support the notion that the hydrogen-bonded and non-hydrogen-bonded conformation populations observed by NMR and IR spectroscopy represent the closed and twisted conformations, respectively, observed in the solid-state structures of benzoylureas.

Our experimental data show that benzoylureas can adopt two distinct conformations: a closed form stabilized by an intramolecular hydrogen bond and a twisted form that does not contain an intramolecular hydrogen bond. We have shown that conformational preference is determined by the substituents, or indeed both forms can exist in solution,

(57) There are no examples of class III benzoylureas in the Cambridge Crystallographic Database.

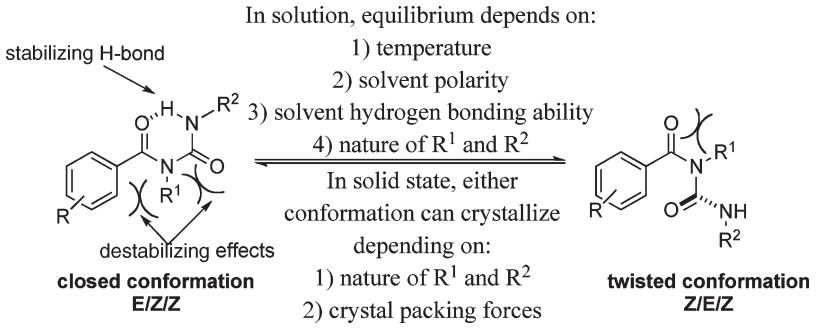


FIGURE 6. Factors influencing the conformation of benzoylureas.

rapidly interconverting on an NMR time scale. Such dynamic conformational behavior was not expected for this hindered and partially conjugated system. There are several features of these compounds that influence their conformational preference as shown schematically in Figure 6. Fundamentally, it is a matter of whether the strength of the intramolecular hydrogen bond stabilizing the closed form, which can be influenced by the nature of the R^2 substituent, can compensate for the opposing hindrance in the closed form between the benzamide ring and R^1 and between R^1 and the C^2 carbonyl group, the magnitude of which clearly depends on the steric bulk of the R^1 substituent. An additional challenge to the intramolecular hydrogen bond is whether it can compete in the environment of a strong hydrogen-bond-accepting solvent such as DMSO. These factors will now be discussed in more detail.

Influence of Substituent R^1. In order to alleviate steric hindrance induced in the closed form, the benzoyl phenyl ring can rotate relative to the plane of the carbonyl group $C^1{=}O^1$. Alternatively, conversion to the *Z* conformation around the $C^1{-}N^1$ bond and rotation about the $N^1{-}C^2$ bond leads to a twisted conformation, as shown by the crystal structures of compounds **4a** and **4c** (Figure 5). This ameliorates the hindered environment of R^1 by replacing the cis $C^2{=}O^2$ carbonyl group in the closed form with an out-of-plane N^2H group in the twisted form. Thus, R^1 has only one steric partner in the twisted form (the C^1 carbonyl group) but two in the closed form (the C^2 carbonyl group and the C^1 phenyl ring). This explains why **3** converts to the twisted conformation more readily than **1**. In both compounds, we expect the C^1 phenyl ring to be rotated significantly out-of-plane relative to the C^1 carbonyl group so that steric hindrance is minimized in the closed form.[58] We propose that this is sufficient to maintain the closed conformation for **1** in both $CDCl_3$ and DMSO-d_6 but not for **3** with its bulkier R^1 group.

In order to test this hypothesis, we prepared compound **8** (Table 3), in which the 2,6-dimethyl substitution forces the plane of the phenyl ring to be roughly perpendicular to the plane of the carbonyl group.[59] Hence, the local steric interaction between the C^1 phenyl ring and R^1 in the closed form should be decreased to the greatest possible extent. We anticipated that this could provide an alternative approach

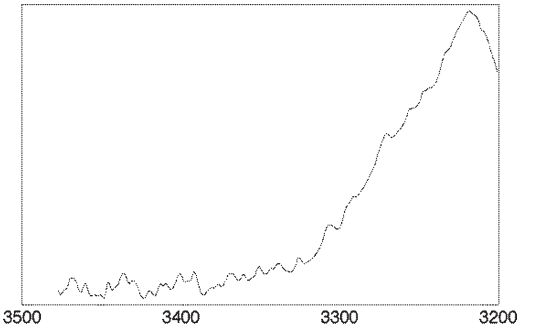
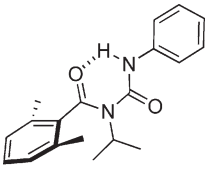


FIGURE 7. IR spectrum of compound **8**.

TABLE 3. Chemical Shift and TDC for Compounds 8



8

	$CDCl_3$[a]		DMSO-d_6[b]	
compd	δ(NH)[c]	NTDCS[d]	δ(NH)[c]	NTDCS[d]
8 (closed)	11.59	−3.0	10.53	~0
8 (twisted)			9.44	−6.0

[a]Sample concentration: 3.0 mM. [b]Sample concentration: 10.0 mM. [c]ppm. [d]ppb/K.

to reduce steric hindrance, rather than through a sterically less demanding R^1 group such as in **1**, and that this subtle effect could be sufficient to stabilize the closed conformation such that **8** would mirror the conformational behavior of **1**. Indeed, unlike **3**, the IR spectrum of **8** in chloroform exhibited only a hydrogen-bonded NH absorption band (Figure 7) and, as shown in Figure 5e, **8** crystallized in the closed conformation, despite R^1 being a bulky isopropyl group.[60]

In $CDCl_3$ the exclusively hydrogen-bonded NH signal in **8** (Table 3) is displaced far downfield at 11.59 ppm, and the small NTDCS value of −3.0 ppb/K therefore corresponds to interpretation ii in Table 1. Also, unlike **3**, **8** maintained some

(58) Tertiary benzamides readily rotate about the phenyl−carbonyl bond to accommodate highly distorted but less sterically hindered structures. A survey of 36 tertiary benzamides in the Cambridge Crystallographic Database revealed deviation from planarity of 35−85°, with a Ph−$CONR^2$ torsion angle of around 75° predominating.

(59) Bowles, P.; Clayden, J.; Helliwell, M.; McCarthy, C.; Tomkinson, M.; Westlund, N. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2607–2616.

(60) It cannot be ruled out that the closed conformation in **8** is not relatively stabilized but that the twisted conformation is relatively destabilized. However, scrutiny of the twisted form of these compounds reveals no reason why 2,6-dimethyl substitution should have such an effect.

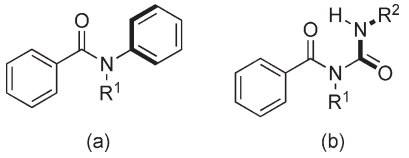


FIGURE 8. Comparison between (a) benzanilide and (b) benzoylurea.

closed conformation even in DMSO, and this could be observed as a separate NH signal downfield at 10.53 ppm in addition to an upfield signal at 9.44 ppm that corresponded to the twisted conformation.[61] This allowed us to measure unequivocally the NH NTDCS values in DMSO-d_6 for the closed and twisted forms. As listed in Table 3, these were ~0 and −6 ppb/K, respectively, confirming that in DMSO-d_6 an NTDCS above −3 ppb/K is diagnostic of an internally bonded NH proton while a value of less than −4 ppb/K is diagnostic of an NH proton hydrogen-bonded to solvent.

Thus, the greater propensity that we observe for benzoylureas with a branched R^1 group to adopt the twisted conformation seems to be driven principally by steric effects and explains why we observe that the closed form of class III and IV compounds (R^1 = branched) is destabilized relative to the closed form of class I and II compounds (R^1 = Et), respectively.

In the appropriate environment, an amide group can be considered a phenyl ring isostere due to its planarity and partial resonance.[62−64] Replacement of the $C^2(=O)N^2R^2$ amide group in benzoylureas with a phenyl ring provides a benzanilide that can be used as model systems for the benzoylureas in which the R^1 steric effects are maintained but the ability to stabilize a closed-like conformation with a hydrogen bond is removed, as shown in Figure 8.

Indeed, while benzanilides favor the "closed" conformation (a) in Figure 8 when R^1 = H, they switch to a twisted-like conformation even when R^1 = Me with the amide group presenting an *E* conformation as observed for the twisted benzoylurea conformation.[62,65] This is clearly shown in Figure 9, where the anilide ring for benzanilide **9** and benzoylurea **4c** assumes the same almost perpendicular

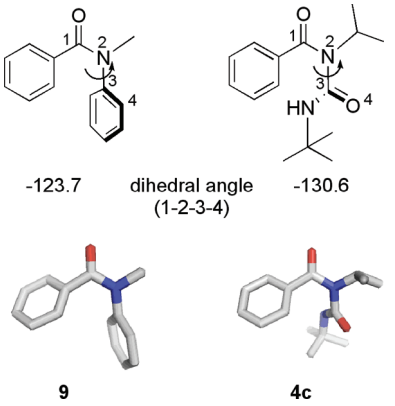


FIGURE 9. Structure comparison between benzanilides **9** (Ref 65) and benzoylurea **4c**.

arrangement to the $C^1(=O)-N^1$ amide group for both compounds.

It is clear from the comparison with the benzanilides that the closed-type conformation in both systems is inherently sterically demanding.[66] This is exacerbated by the steric bulk of R^1 to the point where the twisted conformation, which alleviates some of this steric hindrance, becomes favored. Twisting occurs more readily for benzanilides than benzoylureas because the latter can stabilize the closed conformation through formation of an intramolecular hydrogen bond.

Influence of Substituent R^2. Class IV compounds clearly show that the nature of R^2 influences conformational preference between the closed and twisted forms. In the series **4a**−**c** (R^2 = Me, *i*-Pr, *t*-Bu), the closed form becomes progressively less stable than the twisted form, comprising 66%, 40%, and 25%, respectively, of the conformer population in $CDCl_3$ at room temperature. For these compounds, the R^2 substituent becomes progressively bulkier and more electron-rich while R^1 remains invariant. The R^1 group in this class is *i*-Pr, which we have shown introduces steric hindrance between itself and both the benzoyl phenyl ring and the C^2=O carbonyl group. Thus, there is a propensity for these compounds to adopt the twisted form when the intramolecular hydrogen bond is no longer sufficient to stabilize the closed form. It would be expected that R^2 is decreasingly able to stabilize the internal NH bond in the series R^2 = Me, *i*-Pr, *t*-Bu based on the increasing electron-rich character.

While R^2 can affect the electronic nature of the hydrogen bond and, therefore, its conformation, this does not discount at this point a steric contribution of R^2 to the conformational preference. Shown in Table 4 are the bond angles at the N^2 amide nitrogen atom in selected benzoylureas in the solid state; this angle reflects the steric environment of the amide group since its magnitude affects the distance of R^2 from the eclipsed carbonyl group.

Steric hindrance in an increasingly bulky R^2 group in the series **4a** (R^2 = Me), **4c** (R^2 = *t*-Bu), **8** (R^2 = Ph) is marked by an increase in this bond angle. However, in compounds **4a** and **7**, where R^2 is small, both twisted and closed conformations, respectively, are observed. Moreover, in compounds **4c** and **8**, in which R^2 is bulky, both twisted and closed conformations, respectively, are also observed. Thus, the size of R^2 does not appear to influence conformational

(61) Separation of the NH signals for the two conformations was an unanticipated but useful consequence of the 2,6-dimethyl substitution slowing down the dynamics of conformational interchange. We attribute this to steric hindrance, though a similar phenomenon in certain 2,6-disubstituted tertiary benzamides has been related to an electronic effect on amide resonance. See: Lewin, A. H.; Frucht, M. *Tetrahedron Lett.* **1970**, *11*, 1079–1082.

(62) Stewart, W. E.; Siddall, T. H. *Chem. Rev.* **1970**, *70*, 517.

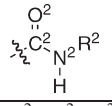
(63) Curran, D. P.; Hale, G. R.; Geib, S. J.; Balog, A.; Cass, Q. B.; Degani, A. L. G.; Hernandes, M. Z.; Freitas, L. C. G. *Tetrahedron: Asymmetry* **1997**, *8*, 3955–3975.

(64) Ahmed, A.; Bragg, R. A.; Clayden, J.; Lai, L. W.; McCarthy, C.; Pink, J. H.; Westlund, N.; Yasin, S. A. *Tetrahedron* **1998**, *54*, 13277–13294.

(65) Itai, A.; Toriumi, Y.; Saito, S.; Kagechika, H.; Shudo, K. *J. Am. Chem. Soc.* **1992**, *114*, 10649–10650.

(66) While $\pi-\pi$ stacking appears as though it could contribute to the stability of the twisted conformation of benzanilides (aryl−aryl stacking) and benzoylureas (aryl−amide stacking) as they are depicted in Figure 8, this is either not the case or is insignificant, since *N*-methylalkylanilides also adopt the twisted conformation (see ref 63). Interestingly, this type of conformational interchange where a twisted form alleviates steric hindrance appears to be a general phenomenon as analogous behavior is observed in *N*-alkyl-*N*′-phenylureas (see: Nowick, J. S.; Abdi, M.; Bellamo, K. A.; Love, J. A.; Martinez, E. J.; Noronha, G.; Smith, E. M.; Ziller, J. W. *J. Am. Chem. Soc.* **1995**, *117*, 89–99) and has been used as a deliberate design strategy to occupy large pockets in protein−protein interactions. See: Park, C.-M.; Oie, T.; Petros, A. M.; Zhang, H.; Nimmer, P. M.; Hensry, R. F.; Elmore, S. W. *J. Am. Chem. Soc.* **2006**, *128*, 16206–16212.

TABLE 4. Bond Angle (deg) around the N^2 Amide Nitrogen Atom in Selected Benzoylureas, Derived from Crystal Structure Data



compd (conformation)	($C^2-N^2-R^2$)	$d(N\cdots O)^a$	R^2
4a (twisted)	121.2	n.a.	Me
4c (twisted)	124.7	n.a.	*t*Bu
7 (closed)	121.2	2.64	Me
8 (closed)	126	2.58	Ph

[a](N−O) distance measured in Å. n.a.: this distance was not measured in the twisted form.

preference. Compound **3** is a useful probe here because R^1, as in **4a**−**c**, is an isopropyl group, but R^2 is a phenyl group. The in-plane R^2 phenyl group in **3** would be expected to exert more steric hindrance than the R^2 *tert*-butyl group in **4c**, and yet, the NMR data indicate that while **4c** in $CDCl_3$ is predominantly in the twisted conformation, **3** is mainly closed. Indeed, the proportion of **3** in the closed conformation (76%) is even greater than that in **4a**, where R^2 is a small methyl group. These data are not in accordance with any steric influence of R^2 on conformational preference. Rather, they are completely in accordance with the trend of increasing hydrogen-bond donor abilities expected along the series R^2 = *t*-Bu, *i*-Pr, Me, Ph.

In summary, the bulkiness of the R^2 group does not affect the conformational preference because the steric effects are similar in both the closed and twisted forms of benzoylureas. It is the electronic effect that the R^2 group has on increasing the hydrogen bond strength in the closed form that stabilizes this conformation. Such stabilization is, however, readily overwhelmed by the nature of the R^1 group because the closed conformation is inherently sterically more demanding. Thus, the hydrogen bond strength in **3** is intrinsically stronger than in class II compounds, and yet it is the latter class with R^1 = ethyl that maintains the closed conformation in both chloroform and DMSO, whereas **3** already exhibits some twisted conformation in chloroform and adopts an entirely twisted conformation in DMSO.

Hydrogen Bond Strength of the Benzoylurea Core in the Closed Conformation. A number of observations suggested to us that the strength of the intramolecular hydrogen bond in the closed form of benzoylureas could be relatively strong.[67] First, some of the compounds such as **1** and **2a**−**c** maintain an intramolecular hydrogen bond even in DMSO-d_6, despite the fact that the NH is not buried but rather is readily accessible to solvent. Second, the downfield shift of the intramolecularly hydrogen-bonded NH proton in the NMR spectrum of several benzoylureas is very significant relative to model compounds for the non-hydrogen-bonded NH proton. In the case of **8**, the NH proton in the NMR spectrum resonates in $CDCl_3$ at 11.59 ppm and the downfield shift relative to acetanilide is 4.51 ppm in the same solvent.

(67) The geometry of the intramolecular hydrogen bond in the closed conformation of the benzoylurea core is not optimal (NH···O nonlinear, see: Peters, D.; Peters, J. *J. Mol. Struct.* **1980**, *68*, 255−270) and comprises a donor and acceptor of different pK_a's (carbonyl C=O and amide NH). It is clear from these two factors that we are not in presence of the strongest type of hydrogen bond, which is linear and comprises a donor and acceptor of equivalent pK_a's (e.g., FHF). The terms "strong" or "weak" are employed as relative characterizations and not in their absolute meaning.

TABLE 5. DFT Calculations of Gas-Phase Energies of Model Acylureas MeC(=O)NMeC(=O)NHR in Twisted Conformation Compared with the Closed Conformation[a]

R	ΔH (kJ mol^{-1})
Me	31.0
i-Pr	29.0
t-Bu	29.1
Ph	30.6

[a]B3LYP/6-311+G(2df,p)//B3LYP/6-31G(d) calculations, reported as the amount by which the enthalpy of the twisted form is higher than that of the more favored closed form. Computational details are provided in the Supporting Information.

Third, the N^2-O^1 distance in a class II benzoylurea such as **7** is 2.64 Å and in a class I benzoylurea such as **8** is 2.58 Å. A value of between 2.48 and 2.65 Å has been used to indicate the presence of a very strong hydrogen bond of around 30−40 kJ mol^{-1}.[19,68]

In order to estimate the strength of the hydrogen bond, we applied density functional theory (DFT) calculations to model acylureas in either the closed or twisted conformations.[69] We reasoned that the use of simple methyl groups for model acyl urea system MeC(=O)NMeC(=O)NHR would minimize the influence of sterics and give a reasonable estimation of hydrogen bond strength in the gas phase. As shown in Table 5, these calculations confirm that in the gas phase, the hydrogen bond strength in acyl ureas is indeed estimated to be very strong, at approximately 30 kJ mol^{-1}.

In carbon tetrachloride, a maximum enthalpic gain obtained from the formation of a geometrically optimal amide−amide intramolecular hydrogen bond has been reported at around 17 kJ mol^{-1}.[29] When this geometry is not optimal this enthalpic gain is readily decreased and not able to compensate for small gauche destabilizing interactions. In our case, we have shown that R^1 induces significant steric constraints on the molecular scaffold. In addition, the benzoylurea core does not place the hydrogen bond partners in an optimal orientation. Despite this, our calculations suggest a solvation effect of approximately 10 kJ mol^{-1} in transferring from the gas phase to chloroform (see the Supporting Information). This places the enthalpic gain from hydrogen-bond formation in benzoylureas greater than that for two amides in the optimum, linear geometry.

It can be observed that the enthalpy decreases slightly on going from R = methyl, to R = isopropyl and *tert*-butyl but increases again for R = Ph. While these differences are small and should not be overinterpreted, this trend supports our earlier proposal that electron-rich R^2 groups destabilize the closed conformation in class IV compounds because of hydrogen-bond weakening and that the steric bulk R^2 does not influence conformational preference. Consideration of the nature of the electronics of the benzoylurea core in the closed conformation can shed light on those factors that contribute to hydrogen bond strength.

Resonance Forms of the Benzoylurea Core in the Closed Conformation. Hydrogen bonds are strengthened by resonance forms that decrease the difference in electronegativity between the hydrogen-bond donor (NH) and hydrogen-bond

(68) Note that it has been stated that firm conclusions on hydrogen bond strength cannot be drawn from spectral and crystallographic data. See: Guthrie, J. P. *Chem. Biol.* **1996**, *3*, 163–170. Perrin, C. L.; Nielson, J. B. *Annu. Rev. Phys. Chem.* **1997**, *48*, 511–544.

(69) See the Supporting Information.

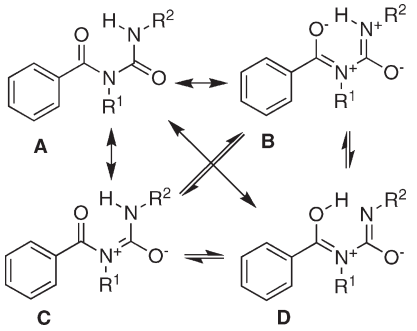


FIGURE 10. Representations of valence bond resonance forms **B** and **C** of the benzoylurea core **A** and tautomeric interconversion involving **D**.

acceptor (C=O). Using simple valence-bond structures of the benzoylurea system, partial resonance can be achieved in two different ways. As shown in Figure 10, the benzoylurea core **A** could plausibly comprise resonance forms **B** and **C**. Here, resonance form **B** and tautomer **D** would confer on each amide group within the core a similarly resonance-shortened C^1-N^1 or C^2-N^2 bond. Conversely, resonance form **C** would lead to a shortening of the N^1-C^2 bond.

Gilli and co-workers have shown that the intramolecular hydrogen bond strength in certain systems is similar to the benzoylureas that also contain an intramolecular hydrogen bond and that form a pseudo-six-membered ring and can be enhanced by the intervention of resonance between the acceptor and the donor.[18,19] They devised a nomenclature to define the nature of these systems, which feature sequences such as $H-N-R_n=O$ where "*R_n ($n = 1, 3, 5,$ etc.) is a resonant spacer of n atoms forming a chain of alternating single and double bonds*". For example, intramolecular resonance-assisted hydrogen bonding (RAHB) in β-enaminone **C′** in Figure 11, for which $n = 3$ and hence the appellation R_3-RAHB (*intra*), helps to stabilize sharing of the intramolecular hydrogen-bonded proton between the NH and carbonyl oxygen atom by way of tautomer **D′**. This leads to a small d(N−O) value of 2.661 Å, which has been associated for this system with a gas-phase hydrogen-bonded energy (E_{HB}) of 29 kJ mol^{-1}.[19] By way of contrast, the nonresonant hydrogen bond in **E′** was estimated to be 12 kJ mol^{-1}.

By analogy with the β-enaminone system, the interconversion between **C** and **D** in Figure 11 would suggest that benzoylureas could in principle exhibit RAHB.

Bond lengths give insight into the electronics underpinning the presence or absence of resonance stabilization and thus can help determine the relative distribution of the individual resonance forms. Shown in Table 6 are selected bond length values for compounds **4a**, **4c**, **7**, and **8**.

The twisted forms of **4a** and **4c** serve as useful controls where intramolecular hydrogen bonding is removed as a factor that could influence acylurea electronics. In these two control systems, the C^1-N^1 and C^2-N^2 bond lengths are 1.36/1.38 Å and 1.32/1.33 Å, respectively. Both are substantially shorter than the N^1-C^2 bond lengths of 1.43 Å, which corresponds to the length of a pure single C(sp2)−N(sp2) bond.[70] This is not unexpected considering the N^1-C^2 torsion angle of 121−137° that would presumably discourage substantial resonance between the amide groups along the N^1-C^2 bond.[71] In these two structures, the amide groups behave as if they are structurally independent of each other. Therefore, it is quite remarkable then, that these respective bonds lengths in **7** and **8** are revealed in Table 6 to be essentially identical to those in **4a** and **4c**. Thus, even in the closed conformation, the acylurea group behaves like two independent amide groups, with substantial resonance within each amide group as represented by resonance structure **B** in Figure 10, but not between the two amide groups, as represented by resonance structure **C** and which would result in a shorter C^1-N^2 bond length. That is, resonance form **C** appears not to contribute to an overall acylurea resonance, and these compounds behave structurally like "bis-amides" without any urea character.[72] This was quite unexpected as the planarity of the closed form should allow for maximum conjugation between the two amide groups.[73]

Conversely, the ^{13}C NMR data reveal that the C^2 carbon in all benzoylureas studied here, irrespective of conformation, experiences an electronic environment of a typical urea, shifted upfield to 151−155 ppm (see the Supporting Information) relative to a typical amide of around 170 ppm. It is instructive that the C^2 carbonyl carbon atom ultimately behaves electronically like a urea carbonyl carbon atom, being flanked by two nitrogen atoms; this is despite the fact that the electron sharing between the carbonyl group and the flanking nitrogen atoms, as witnessed by the differences in the respective N−carbonyl bond lengths, is distinctly different from that in a typical urea, where mutual sharing occurs.[74] This categorically demonstrates that the chemical shift of a given ^{13}C carbon atom cannot be used to infer the degree of partial double bond character with flanking atoms.

Since RAHB requires all bonds in the pseudo-six-membered ring to participate in the π-conjugated system, the absence of **C** as a significant resonance form precludes any RAHB in the benzoylureas, despite their topological similarity with systems in which this effect is observed. The resonant spacers in Gilli's studies strictly comprise double bonds (see **C′**) and therefore favor strong resonance to occur.

(70) Gilli, G.; Bertolasi, V.; Bellucci, F.; Ferretti, V. *J. Am. Chem. Soc.* **1986**, *108*, 2420–2424.

(71) The extent of torsional distortion alone, in the absence of bond length data, has been shown to be an unreliable gauge of the extent of conjugation (see, for example: Chattopadhyay, D.; Mazumdar, S. K. *Z. Kristallogr.* **1986**, *177*, 103−106).

(72) This difference is starkly demonstrated by reference to C−N bond lengths in the CCD. There is only one entry with an *R* factor of < 0.05 for an *N*-alkyl-*N*-phenyl-*N′*-phenylurea. Here, the *N*-carbonyl and *N′*-carbonyl bond lengths are both 1.370 Å, in stark contrast with the values of 1.43 and 1.33 Å, respectively, for the benzoylureas in the current study. This may be a general phenomenon for acylureas, as the more common unsubstituted benzoylureas in the CCD (i.e., with $R^1 = H$) exhibit N^1-C^2 bond lengths of 1.41 Å, only marginally shorter than those of a single bond. We are not aware of this phenomenon having previously been reported.

(73) The two amide groups are by no means equivalent as the C^1-N^1 distance is consistently longer than the C^2-N^2 distance in both closed and twisted forms. This could perhaps be attributable to the electronegativity of the C^2 carbonyl attached to N^1 that might discourage substantial N^1-C^1 resonance. In any case, neither amide group is "typical": the average HN−carbonyl bond length in benzanilides and alkylacylanilides for entries in the CCD is the same, at 1.347 Å, and this is not affected by whether the NH is intramolecularly hydrogen bonded or not. Only 2/40 (5%) of representative entries in the CCD reveal NH-carbonyl bond lengths shorter than 1.335 Å and none are longer than 1.375 Å. Thus, the C^1-N^1 and C^2-N^2 distances are remarkably longer and shorter, respectively, for benzoylureas in the current study than in a comparable amide bond. A full investigation into why this is so is beyond the scope of this paper.

(74) Bharatam, P. V.; Moudgil, R.; Kaur, D. *J. Phys. Chem. A* **2003**, *107*, 1627–1634.

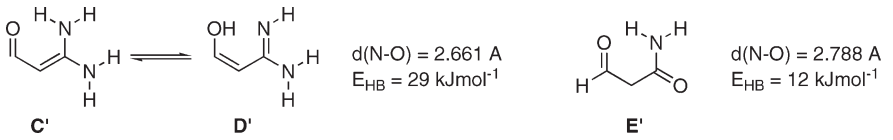


FIGURE 11. Example in a β-enaminone of R_3-RAHB, as defined by Gilli et al.[18,19]

TABLE 6. Bond Distances (Å) in Compounds 4a,c, 7, and 8

compd (conformation)	$d(C^1-O^1)$	$d(C^1-N^1)$	$d(N^1-C^2)$	$d(C^2-O^2)$	$d(C^2-N^2)$	$d(O^1\cdots N^2)$	torsion angle $C^1-N^1-C^2-O^2$ (deg)
4a (twisted)	1.22	1.36	1.43	1.22	1.32	N/A	121.4
4c (twisted)	1.22	1.38	1.43	1.21	1.33	N/A	−130.6
7 (closed)	1.22	1.38	1.43	1.22	1.33	2.64	179.3
8 (closed)	1.23	1.37	1.44	1.21	1.35	2.58	−172

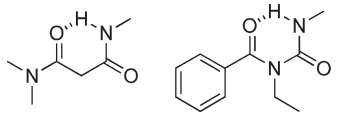


FIGURE 12. Comparison of δ(NH) for compound **10** from ref 29 and compound **2a**.

It appears that in the benzoylurea system the two amides, by being decoupled electronically, occupy a low energy state that also allows for a sufficiently strong hydrogen bond to stabilize the closed form in certain cases.[75] The hydrogen bond does not itself influence the resonance, as the amide bond lengths in Table 6 are the same for closed and twisted forms.

Effect of Conformational Rigidity on the N^2H Chemical Shift in the Closed Formation. The hydrogen-bonded NH proton in benzoylureas resonates significantly further downfield than even some closely related intramolecularly hydrogen-bonded bis amides such as **10** (Figure 12). The chemical shift for the NH proton in this compound has been reported by Gellmann et al. to be 7.7 ppm (in CD_2Cl_2), which is 1.3 ppm further upfield than the corresponding NH signal in **2a**.[29]

The intrinsic hydrogen bond strength in **2a** should be similar to that in **10**, in which amide resonance forms could similarly contribute. The downfield chemical shift of the NH proton in **2a** relative to **10** is consistent with a reduced conformational flexibility in **2a**, caused by the amide C^1-N^1 bond linkage, increasing the probability of the closed form over noninternally hydrogen-bonded conformations relative to **10**.[56,76] However, the N^1-C^2 bond in **2a** is essentially single in character and probably does not confer any extra rigidity relative to the corresponding bond in **10**. The phenyl ring of the benzoyl group does not appear to influence the NH chemical shift, which in alkanoylureas is reported to resonate between 8.75 and 9.32 ppm in $CDCl_3$.[77,78]

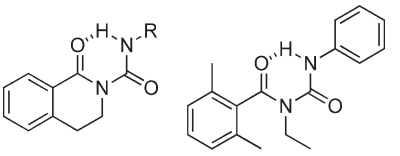
(75) Interestingly, benzoylureas do not appear in Gilli's survey of crystallographic data, although papers by Smith (see ref 23) and Kohmoto (see ref 16) containing the X-ray structure of compound **10** were published before Gilli's study.

(76) Bilton, C.; Allen, F. H.; Shields, G. P.; Howard, J. A. K. *Acta Crystallogr. B* **2000**, *56*, 849–856.

(77) Lin, W. O.; Altoe, A. P. *Monatsh. Chem.* **1982**, *113*, 101–109.

(78) Yogo, M.; Hirota, K.; Senda, S. *Chem. Pharm. Bull.* **1982**, *30*, 1333–1337.

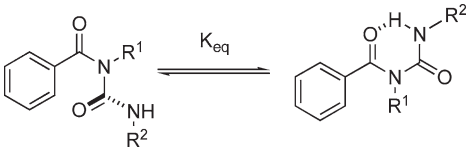
TABLE 7. Conformationally Constrained Compounds



	$CDCl_3$[b]		DMSO-d_6[c]	
compd[a]	δ(NH)[d]	NTDCS[e]	δ(NH)[d]	NTDCS[e]
11a	9.36	−1.9	9.19	−1.8
11b	11.77	−2.2	11.33	−2.1
12	11.69	−2.2	11.0	

[a]These compounds showed a characteristic hydrogen-bonded NH broad IR band (see the Supporting Information). [b]Sample concentration: 3.0 mM. [c]Sample concentration: 10.0 mM. [d]ppm. [e]ppb/K.

TABLE 8. van't Hoff Analysis Performed on Compounds 3 and 4a−c[a]



Parameter	3	4a	4b	4c
ΔG	−2.85	−1.70	1.01	2.74
$\Delta H^{\circ b}$	−5.32	−4.89	1.47	4.39
$\Delta S^{\circ c}$	−8.3	−10.7	1.52	5.52

[a]Compounds **1**, **2a**, **2b**, and **c** were used as references for the hydrogen-bonded conformation of **3**, **4a**, **4b**, and **4c**, respectively, and compounds **5a**, **5b**, **5c**, and **5d** were used as references for the non-hydrogen-bonded conformations of **3**, **4a**, **4b**, and **4c**, respectively, in these calculations. van't Hoff analysis was performed using eqs 1) and 2) at 298.15 K. [b]In kJ mol^{-1}. [c]In J mol^{-1} K^{-1}.

In compounds **11a** and **11b** shown in Table 7, we covalently constrained a benzoylurea to further increase its rigidity to investigate how this may affect the NH chemical shift. This modification directly decreases torsional flexibility about the phenyl−carbonyl, C^1-N^1, and N^1-CH_2 bonds and by pinning back the N^1 substituent also indirectly stabilizes the N^1-C^2 bond by reducing hindrance between the N^1 alkyl group and the C^2 carbonyl group. As shown in Table 7, the NH signals in the NMR spectra of **11a** and **11b** resonate far downfield, more so in both $CDCl_3$ and DMSO than their respective less constrained counterparts **2a** and **1** in Table 2.

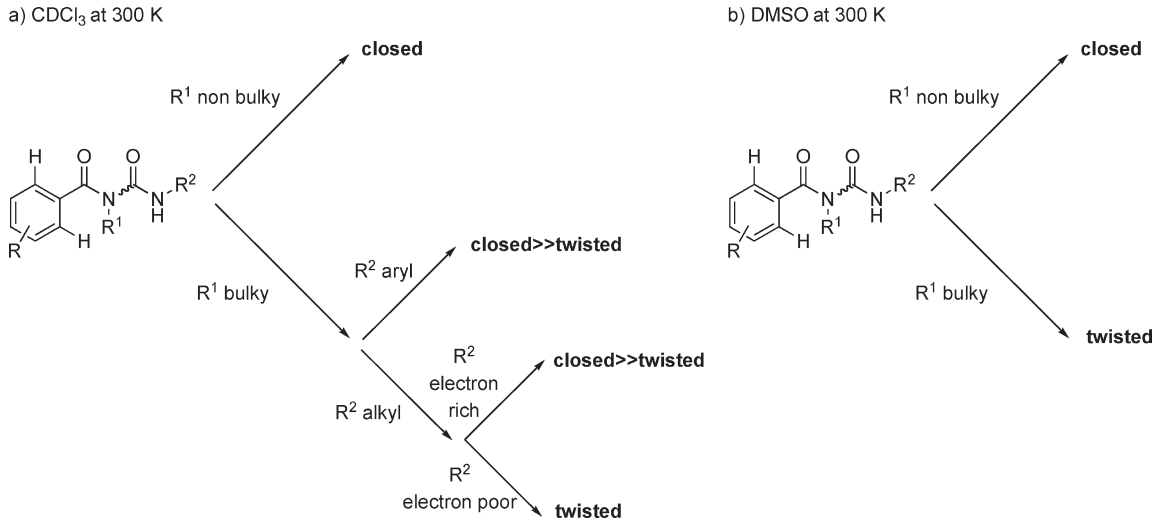


FIGURE 13. Substituent influence on benzoylurea conformation in $CDCl_3$ and DMSO-d_6.

We also prepared **12** as a "rigidified" class I compound, on the basis of our findings in our investigation on the conformational influence of R^1 that the 2,6 dimethyl substitution in compound **8** helped to lock class III compounds into the closed conformation. As shown in Table 7, the NH NMR data for compound **12** are very similar to those for compound **11b**, despite the fact that the C^1 phenyl rings are expected to adopt perpendicular orientations. Results from **11b** and **12** suggest that the torsional aspect of the C^1-phenyl ring has little influence on the electronics of benzoylureas.[79] Rather, the further downfield NH chemical shift in **11a**, **11b**, and **12** relative to their less constrained counterparts **2a**, **1**, and **1**, respectively, is due to increased rigidity leading to a higher probability of the intramolecular hydrogen-bonded conformation. However, this effect on the NH chemical shift within the acylurea series is less marked than the one observed when the bis-amide core **10** (Figure 12) is compared with the next closest analogue acylurea **2a** (Figure 10).

Thermodynamic Analysis of Benzoylurea Conformational Interchange. To obtain further insight into the thermodynamics of benzoylurea conformational change, we used the NMR data to derive energetic information on compounds **3** and **4a**−**c** in chloroform using a van't Hoff analysis (Table 8).

The enthalpy values shown in Table 8 for compounds **3** and **4a** show that for these compounds the hydrogen bond is able to overcome steric repulsion in the closed state and further stabilize this conformation by −5.32 and −4.89 kJ mol^{-1}, respectively. The data in Table 8 confirm that when R^2 is an isopropyl (**4b**) or *tert*-butyl (**4c**) group, the equilibrium enthalpy favors the twisted form due to increasing steric repulsion in the closed form. The values obtained are in agreement with our interpretation of the conformational behavior of class IV compounds and especially of compound **4b** (small NTDCS, compound equilibrating between two conformers of similar enthalpy, i in Table 1), for which we obtain the lowest $\Delta H°$ values in the series (1.47 kJ mol^{-1}). What is more interesting is the fact that the equilibrium entropy favors the twisted conformation for **3** and **4a**, but the closed form for **4b** and **4c**; this suggests that the bulky R^1 and R^2 groups in **4b** and **4c** limit the conformational freedom of these molecules.

Summary. It is now possible to draw a more complete picture of the conformational behavior of the benzoylurea scaffold. The steric interaction between the substituent R^1 and the phenyl ring is the key factor influencing the conformation. Most of the compounds adopt a closed conformation in chloroform at room temperature. In this conformation, the intramolecular hydrogen bond overcomes inherent steric hindrance arising from interactions between the benzoyl phenyl ring and the C^2=O amide carbonyl group and R^1. Thus, the closed conformation of benzoylureas becomes relatively less stable when R^1 substituents are branched and hence bulkier, and if the internal hydrogen bond is weakened due to an electron-rich R^2 substituent. The extreme case is represented by **4c**, which exists predominantly in the twisted form, even in $CDCl_3$. A more polar solvent such as DMSO accentuates this trend, and for those compounds with nonfavorable steric interactions and weaker internal hydrogen bonding, the switch to the twisted conformation occurs more readily because of the competitive hydrogen-bond-acceptor ability of this solvent. Figure 13 summarizes the expected conformational behavior of benzoylureas at 300 K in the two solvents studied.

Conclusion. De novo interactive design, as for many other drug discovery strategies, relies on the innovative use of suitable scaffolds. For our purposes, we have chosen benzoylureas for their ability to form a central six-membered ring induced by an intramolecular hydrogen bond maintaining the molecule in the correct conformation required for mimicry of the α-helix. We have shown that both steric and electronic effects, as well as the relative strength of an internal hydrogen bond, are critical factors influencing the conformation of this scaffold in a given environment. We have also shown that this simple molecular system has interesting conformational properties and that the appellation "benzoylurea" is rather misleading as the structure behaves essentially as two sequential amide groups rather than an acylated urea; a more appropriate appellation would

(79) The influence of increased rigidity on the NH chemical shift is why **1**, for example, is a better model than constrained compounds **12** or **11b** for the closed conformation of **3** in our analysis of conformational equilibria in **3** using the NH chemical shift value.

therefore be "bisamide". These compounds may, therefore, comprise useful probes for investigations of peptide hydrogen bonds as they represent a system where the two amide groups are linked by the shortest possible distance, and so entropic factors, which can be a major consideration in biological systems, are minimized.

In the course of this study we have been also able to confirm conclusions from NMR and IR previously presented by Gellman and Stevens.[29,30] Specifically, the (negative) temperature dependence of an amide NH chemical shift for a small molecule in $CDCl_3$ can furnish useful conformational information when accompanied by IR data, but not on its own. The results shown by us and others indicate that the NTDCS in chloroform may be highly influenced by the internal structure of the molecule and cannot necessarily be anticipated. Conversely, in DMSO the situation is greatly simplified, and there is a marked difference between compounds that are intramolecularly hydrogen bonded with small NH NTDCS values of > -3 ppb/K to those where the NH is not intramolecularly hydrogen bonded and where the NH NTCDS values are relatively larger and all < -4 ppb/K.

Lastly we wish to return to our original problem, which was to know whether or not designed benzoylureas would adopt the desired closed conformation. We now know that when R^1 and R^2 are suitably chosen, the molecules do indeed adopt the closed conformation with an internal hydrogen bond as desired, in both chloroform (hydrophobic environment) and DMSO (polar environment). We are now in a position to rationally functionalize benzoylureas to be α-helix mimetics and will report on this in due course.[80]

Experimental Section

General Experimental Methods. Analytical thin-layer chromatography (TLC) was performed on silica gel 60F254 aluminum-backed plates. Flash chromatography was performed on silica 60 using ACS analytical grade solvents. All reactions were performed in flame-dried Schlenk flasks. Dry diethyl ether, dry triethylamine, and dry acetonitrile were used as received. (**CAUTION!** Phosgene solution must be handled with utmost care in a well-vented fume-cupboard.) *N*-Isopropylacetamide was prepared according to previously reported methods.

General Method of Formation of the Benzoylurea: 1-Benzoyl-1-ethyl-3-phenylurea (1). *N*-Ethylbenzamide (100 mg, 0.67 mmol) was suspended in 1.2 mL dry diethyl ether in an oven-dried Schlenk flask. Triethylamine (103 μL, 0.74 mmol) was added followed by trimethylsilyl trifluoromethanesulfonate (133 μL, 0.74 mmol). The reaction was stirred at room temperature for 16 h. Stirring was stopped to allow the orange oil formed during this first step to settle at the bottom of the flask. The oil was removed via a syringe. Phosgene solution (670 μL of a 20% solution in toluene, CAUTION!) was added dropwise to the clear solution at 0 °C with stirring (133 μL, 0.74 mmol). The reaction was then slowly warmed to room temperature over 4 h. The reaction was then concentrated in vacuo. The residue was dissolved in 1 mL of dry acetonitrile and added at 0 °C to a mixture of triethylamine (224 μL, 1.61 mmol) aniline hydrochloride (104.2 mg, 0.8 mmol) in 1 mL of dry acetonitrile. The reaction was then stirred at room temperature for 1 h after which time the reaction was complete. The reaction was then concentrated. The residue was diluted with dichloromethane and treated with water. The aqueous phase was extracted three times with dichloromethane. The combined organic phases were washed three times with HCl 1 N and three times with saturated $NaHCO_3$, water, and brine. Purification by column chromatography with silica gel (CH_2Cl_2/petroleum ether 5:95 to 60:40) afforded **1** as a colorless film (127 mg, 71%): ^{1}H NMR ($CDCl_3$, ppm) 11.35 (br s, 1H), 7.59 (d, $^3J = 7.53$ Hz, 2H), 7.55−7.46 (m, 5H), 7.37 (t, $^3J = 7.47$ Hz, 2H), 7.14 (t, $^3J = 7.38$ Hz, 1H), 3.85 (q, $^3J = 6.99$ Hz, 2H), 1.19 (t, $^3J = 6.96$ Hz, 3H); ^{13}C NMR ($CDCl_3$, ppm) 175.8, 152.3, 138.1, 136.7, 130.8, 129.3, 129.1, 126.1, 124.5, 120.9, 42.6, 15.3; MS (ESI) 269.1 ($M + H^+$); HRMS (ESI) $C_{16}H_{16}N_2O_2$ calcd M^+ 268.1212, found 268.1216.

Variable-Temperature NMR. All samples for variable-temperature NMR in $CDCl_3$ were dried in vacuo over P_2O_5. $CDCl_3$ was stored on activated sieves and K_2CO_3. NMR samples were prepared in a glovebag with a two-dilution process to obtain a 3 mM solution. For each temperature, samples were equilibrated, and acquisition required 64 scans. The reading chemical shift for NH protons was obtained by applying line broadening (lb = 1) to the original spectrum. Spectra were acquired at 300, 305, 310, 315, and 320 K. A remaining peak of water was observed on all spectra but fell within the range of previously published studies.[29]

All samples for variable-temperature NMR in DMSO-d_6 were dried in vacuo over P_2O_5. NMR samples were prepared without particular precautions to obtain a 10 mM solution. For each temperature, samples were equilibrated, and acquisition required 16 scans. Spectra were acquired at 300, 305, 310, 315, and 320 K.

Graphs for all NMR variable-temperature experiments are available in the Supporting Information section.

Infrared Spectroscopy. All samples for infrared spectroscopy in $CHCl_3$ were dried in vacuo over P_2O_5. $CHCl_3$ was washed with water, dried over K_2CO_3, stirred over $CaCl_2$, and distilled over fresh $CaCl_2$. It was then stored over 4 Å sieves and used quickly afterward. Samples were prepared in a glovebag with a two-dilution process to obtain a 3 mM solution. Spectra were obtained in absorbance mode with a spectral window 3000−3600 cm^{-1} and 128 scans. Background subtraction was applied.

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Supporting Information Available: Detailed procedures for preparation of compounds **2a**−**c**, **3**, **4a**−**c**, **8**, **11a**,**b**, and **12**. Results of NMR temperature experiments, CIF files for new structures presented, and computational details for Table 5. This material is available free of charge via the Internet at http://pubs.acs.org.

(80) We have successfully applied the foundation work here to the development of biologically active helical mimetics that bind to Bcl-x_L and have disclosed this to researchers in a variety of formats (see ref 13).