Conformational analysis of a covalently cross-linked Watson–Crick base pair model

Erik A. Jensen [a], Benjamin D. Allen [a], Yoshito Kishi [b], Daniel J. O'Leary [a],*

[a] Department of Chemistry, Pomona College, 645 North College Avenue, Claremont, CA 91711, USA
[b] Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA

ABSTRACT

Low-temperature NMR experiments and molecular modeling have been used to characterize the conformational behavior of a covalently cross-linked DNA base pair model. The data suggest that Watson–Crick or reverse Watson–Crick hydrogen bonding geometries have similar energies and can interconvert at low temperatures. This low-temperature process involves rotation about the crosslink CH_2—$C(5')$ (ψ) carbon–carbon bond, which is energetically preferred over the alternate CH_2—$N(3)$ (φ) carbon–nitrogen bond rotation.

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The study of DNA oligomers containing covalent cross-links can illuminate aspects of the structure/function paradigm.[1] The concept of interstrand covalent constructs similar to Watson–Crick hydrogen-bonded base pairs was first described by Leonard in the mid-1980s.[2] Recently, Kishi has reported that CH_2-bridged base pair models can be incorporated into DNA oligomers that exhibit conformational properties close to those of the corresponding native DNA duplexes.[3]
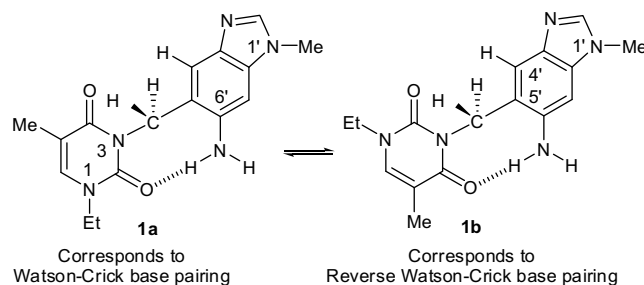
CH_2-bridged base pairs are expected to be conformationally flexible with respect to the covalent linkage and can adopt Watson–Crick or reverse Watson–Crick hydrogen-bonded structures as shown in Figure 1. Compound **1**[3a] is one of several base pair mimics that have been the focus of earlier synthetic and structural studies from the Kishi group. This and the related *N*(3′)-Me isomer (a T–A mimic) had been subject to preliminary low-temperature NMR studies and both were found to exhibit a 2d AB-system for the CH_2 bridge resonances. A single-point analysis at the coalescence temperature identified an approximate 10 kcal/mol barrier associated with the effect. This was suggested as being consistent with the formation of a single conformer which was observed in the solid-state (Watson–Crick mimic **1a**). The present study was undertaken to clarify the conformational behavior of **1**.

To initiate our study, the barrier associated with H(α/β) 2d AB-system formation was first reexamined by the method of complete line shape analysis[4] (Fig. 2) in CD_2Cl_2 and in ethanol-d_6. Eyring analysis of the CD_2Cl_2 data gave $\Delta H^{\ddagger}$ = 7.7 ± 2.2 kcal/mol, $\Delta S^{\ddagger}$ = −5 ± 17 cal mol^{-1} K^{-1}, and $\Delta G^{\ddagger}$ (25 °C) = 9.3 ± 1.3 kcal/mol. Ethanol-d_6 was utilized in order to study the dynamics under polar protic conditions, nominally similar to aqueous solution. The values obtained in ethanol were similar to those obtained in CD_2Cl_2 ($\Delta H^{\ddagger}$ = 7.5 ± 1.0 kcal/mol, $\Delta S^{\ddagger}$ = −5 ± 5.2 mol^{-1} K^{-1}, and $\Delta G^{\ddagger}$ (25 °C) = 10.1 ± 0.59 kcal/mol).

A semi-empirical AM1 φ/ψ[5] grid search (Fig. 3) was used to explore rotational trajectories about the bridging methylene group.[6] Four energy minima were found and used as starting points for

Figure 1. Conformational interchange of covalently cross-linked Watson–Crick base pair models.

* Corresponding author at present address: Department of Chemistry, Bowdoin College, 6600 College Station, Brunswick, ME 04011, USA. Tel.: +1 207 725 3616; fax: +1 207 725 3405.
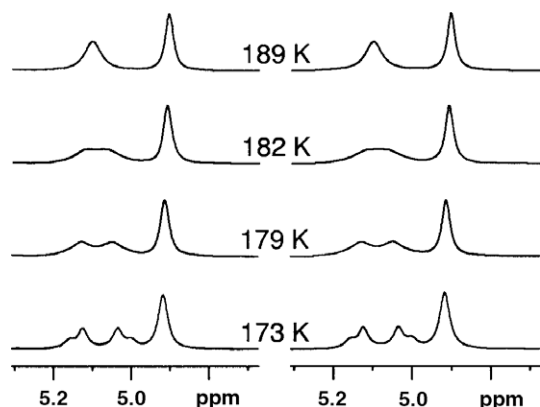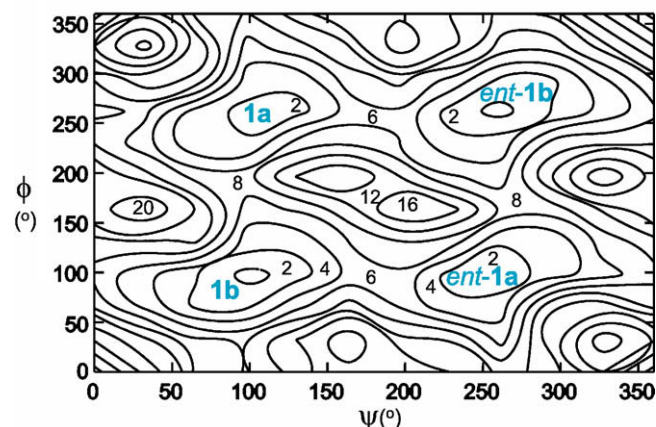*E-mail address:* doleary@bowdoin.edu (D.J. O'Leary).

Figure 2. (Left) Selected variable temperature 400 MHz ^{1}H NMR spectra for **1** in CD$_2$Cl$_2$. The 2d AB-system visible at 173 K arises from H(α) and H(β). The rightmost peak is the 6′-NH$_2$ resonance. (Right) Fit data obtained using the MEXICO iterative procedure.[4]


Figure 3. Relaxed AM1 potential energy surface for **1** as a function of the bridging C—C (ψ) and C—N (φ) torsional angles. The contour levels are spaced by 2 kcal mol^{-1}.

higher-level B3LYP and MP2 calculations which included both exo and endo N-Et orientations (Fig. 4).

At each level of theory, conformer **1b** was found to be more stable than **1a** by approximately 0.5–0.6 kcal/mol (gas phase) or 0.2–0.4 kcal/mol (CH$_2$Cl$_2$ or CH$_3$CH$_2$OH solvent continuum). The exo/orientation of the N-Et group did not appear to significantly affect the conformational energies. Because the **1a**/**1b** energies are so similar, the calculations suggest that if static non-interconverting conformations are formed upon cooling, then a mixture of **1a** and **1b** would be visible as reasonably populated major and
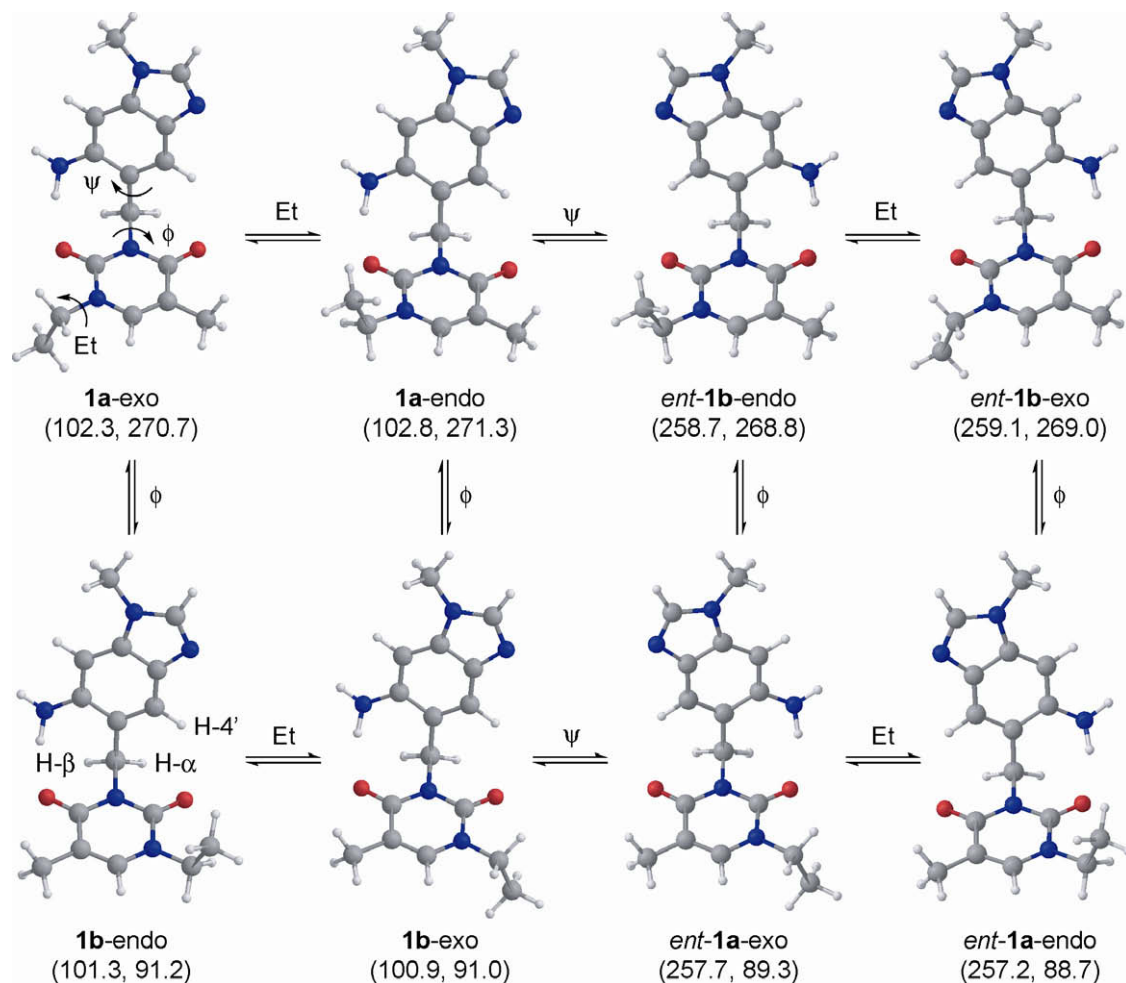
Figure 4. Definition of low-energy conformations of base-pair model **1**. The structures have been computed at the B3LYP/6-31G∗∗ level of theory; their relative energies in the gas phase and solvent continuum (CH$_2$Cl$_2$ and CH$_3$CH$_2$OH) are compiled in Table 1. Exo- and endo- structures interconverted by rotation of the N-Et group are labeled 'Et' over the equilibrium arrows; other rotations are labeled φ (carbon–nitrogen bond rotation) or ψ (carbon–carbon bond rotation). ψ and φ dihedral angles are listed in parentheses below each conformer.
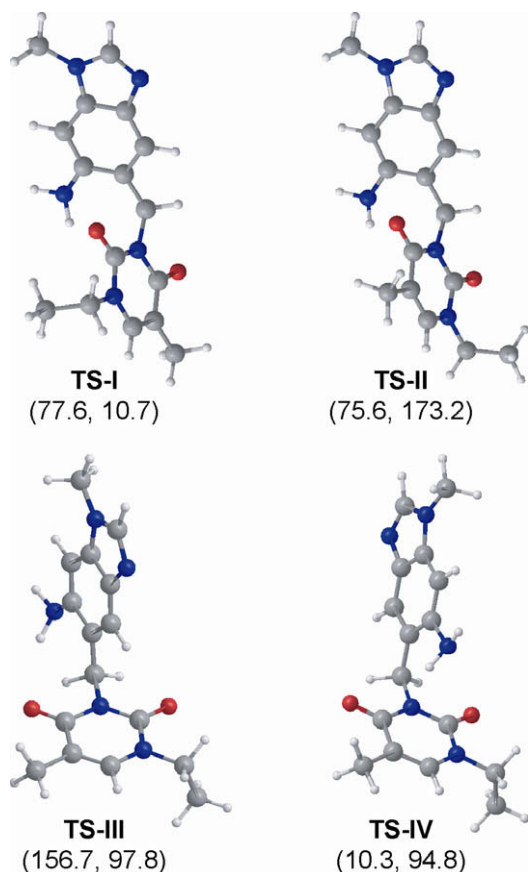
Figure 5. Representative B3LYP 6-31G∗∗ transition state structures for rotation along the φ (**TS-I** and **TS-II**) and the ψ (**TS-III** and **TS-IV**) trajectories. ψ and φ dihedral angles are listed in parentheses.

Table 1
Relative energy (kcal/mol) as a function of computational approach and basis set for conformers **1a**-*exo*, **1a**-*endo*, **1b**-*exo*, and **1b**-*endo*

Entry	B3LYP/6-31G∗∗	B3LYP/6-311G∗∗	MP2/6-31G∗∗
Gas phase			
1a-*exo*	+0.610	+0.525	+0.570
1a-*endo*	+0.643	+0.559	+0.515
1b-*exo*	+0.041	+0.027	+0.153
1b-*endo*	0.000	0.000	0.000
PCM CH$_2$Cl$_2$			
1a-*exo*	+0.299	+0.203	+0.234
1a-*endo*	+0.385	+0.313	+0.313
1b-*exo*	0.000	0.000	0.000
1b-*endo*	+0.071	+0.006	+0.107
PCM CH$_3$CH$_2$OH			
1a-*exo*	+0.250	+0.196	+0.179
1a-*endo*	+0.391	+0.231	+0.257
1b-*exo*	+0.067	0.000	+0.066
1b-*endo*	0.000	+0.054	+0.066

Computed with Gaussian 03.[4]

minor conformers. This was not observed in the low-temperature ^{1}H NMR spectrum of **1** and the preliminary AM1 calculations suggested that the observed low-temperature dynamic NMR effect is more likely due to differential slowing about the φ/ψ linkage.[7]

The barrier heights were therefore computed at a higher level of theory by calculating transition state structures along each φ/ψ rotational trajectory using the DFT B3LYP/6-31G∗∗ methodology.[8] The barriers were determined to be 11.16 and 11.25 kcal/mol (φ)

Table 2
Relative energy (B3LYP/6-31G∗∗, kcal/mol) of representative transition state structures for φ bond rotation (**TS-I** and **TS-II**) and for ψ bond rotation (**TS-III** and **TS-IV**) as computed in the gas phase and using a polarizable continuum model (PCM, CH$_2$Cl$_2$ or CH$_3$CH$_2$OH)

Entry	Gas phase	PCM CH$_2$Cl$_2$	PCM CH$_3$CH$_2$OH
TS-I	+11.16	+9.82	+9.56
TS-II	+11.25	+9.78	+9.46
TS-III	+5.89	+3.83	+3.45
TS-IV	+10.84	+9.10	+8.77
1a-*exo*	0.00	0.00	0.00

Computed with Gaussian 03.[4]
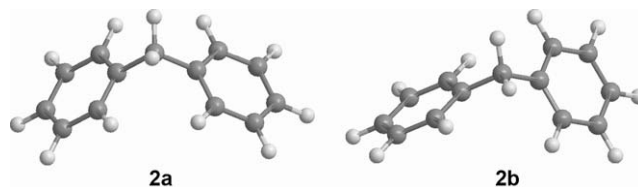
Figure 6. Comparison of C_{2v} gable (**2a**) and C_2 helical (**2b**) conformations of diphenylmethane.

and 10.84 and 5.89 kcal/mol (ψ) in the gas phase (Fig. 5 and Table 2). When a solvent continuum was included in the calculations, the transition state energies diminished somewhat but the general trend was maintained. These results, which agree with the experimentally determined barrier heights, support the proposition that either φ bond rotation is a relatively high energy conformational process and should be accessible by low temperature NMR experiments. On the other hand, ψ bond rotation involves transition states of different energies: a higher energy process comparable with φ rotation in which an NH$_2$ group is rotated past the proximal pyrimidine residue and a lower energy trajectory moving H(4′) moving past the adjacent ring. The calculated barrier for this latter process, 3.45–5.89 kcal/mol, is low enough to preclude it from being accessed by low-temperature NMR experiments.

The nuclear Overhauser effect (NOE) could, in principle, be used to differentiate between static structures **1a** and **1b** and a set of structures exhibiting conformational mobility. For example, a static conformer could be identified by irradiating H(4′) and observing a differential enhancement at the bridging methylene protons. This is due to, for example, in conformer **1b**-*endo*, the closer proximity of H(α) to H(4′). Transient excitation of H(4′) provided equivalent enhancement of the 2d AB-system transitions associated with H(α/β).[4] This behavior was observed for mixing times ranging from 6.25 to 200 ms. Unfortunately, the Overhauser effects in compound **1** at low temperatures are negative, which means the molecule is in the slow tumbling region. Under these conditions, the effect of spin diffusion makes it difficult, if not impossible, to interpret equivalent Overhauser effects between one spin and a geminal pair of protons.[9] Accordingly, this set of experiments did not reveal any conclusive information about the low-temperature behavior of compound **1**.

These findings are discussed in light of earlier conformational studies of structurally related diphenylmethane derivatives (Fig. 6). Empirical force-field and MNDO studies of diphenylmethane **2** revealed a C_{2v} 'gable' conformation that is only ca 0.5 kcal/mol lower in energy than a C_2 helical structure.[10] More recent computational studies using electron correlation[11] and density functional theory[12] have found the C_2 structure to be slightly lower in energy than the gable conformation, which is computed to be a transition state at higher levels of theory.

Our conformational studies suggest that the flexibility of **1** is due to an interconversion among gable conformations, each of which is stabilized by a hydrogen bond. The NMR and computa-

tional data suggest that at low temperatures, the molecule is trapped not as a single conformer but as an enantiomeric pair of rapidly interconverting diastereomers (**1a** ↔ *ent*-**1b** and **1b** ↔ *ent*-**1a**). The conformational process that stays active at low temperatures is very likely a 180° ψ rotation that ruptures the NH_2---O=C hydrogen bond and carries H(4′) across the face of the adjacent ring (**TS-III**, Fig. 5). To the best of our knowledge, this is the first diphenylmethane-like system in which a dynamic stereochemical process has been accessed and quantified with low-temperature NMR spectroscopy.[13]

Base pair model **1** was recently incorporated into DNA oligomers.[3d] Interestingly, spectroscopic and chromatographic evidence suggests that two local conformers, presumably corresponding to the Watson–Crick and reverse Watson–Crick base pairings, are isolable at room temperature. Although the detailed structure of these conformational isomers is not yet known, the general observation is consistent with data presented here.

Acknowledgments

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.113.

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4. Details are provided in the Supplementary data.
5. The C—C (ψ) torsion angle is defined by atoms C(6′), C(5′), C-α/β, and N(3). The C—N (φ) torsion angle is defined by C(2), N(3), C-α/β, and C(5′). A 0° torsion angle is defined by an eclipsed conformation of these atoms, with the sense of rotation defined in Figure 4.
6. The orientation of the *N*-Et group was not changed during the course of scanning the φ/ψ torsion angles.
7. We note that in **1**, full symmetry is not reached until rotation through the perpendicular state for each of the rings is attained. Until that point any CH_2 group in the molecule is chirotopic and the bridge protons share a diastereotopic relationship as shown in Figure 4.
8. Each structure shown in Figure 5 exhibited one imaginary frequency for a vibrational mode associated with φ or ψ bond rotation and thus are identifiable as transition states. We regard these as representative transition structures because two maxima were observed for certain rotations.
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