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MOLECULAR MECHANICS PARAMETERIZATION OF THIONUCLEOSIDE DISULFIDES FOR MODELING CROSS-LINKED DUPLEX DNA

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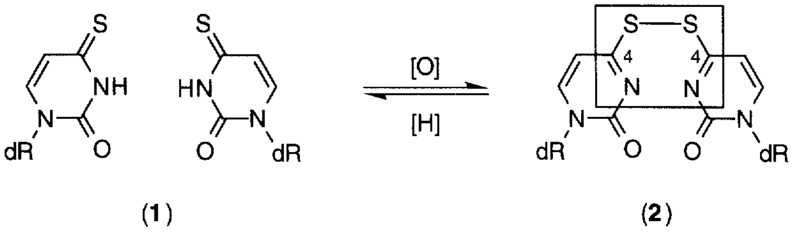
ABSTRACT: We describe a solution to a molecular mechanics parameterization problem involving disulfide bonds between thionucleosides. Key torsional and bending parameters developed from ab initio calculations were incorporated into the AMBER* force-field in order to accurately represent the disulfide linkage in DNA cross-linked via this bond.

INTRODUCTION. In studies on covalently constrained duplex and hairpin DNA using 4-thio-2′-deoxyuridine disulfides (Eq 1),[1] we were faced with the problem of parameterization of stretching, bending, and torsional constants of the interstrand disulfide bond. Because the thioimidate substructure is not effectively parameterized in the AMBER* force field,[2] and because the bond parameters about the disulfide bond critically effect the conformation of the thionucleoside bases relative to the DNA helix, we needed to develop accurate parameters to model these DNA disulfides.



Equation 1

The fundamental issue with which we were faced involved the conformational preferences of the C4–S–S–C4 and N3–C4–S–S dihedral angles of **2** and to a lesser extent the corresponding bond angles and distances. The preferred dihedral angle for a C–S–S–C bond is approximately 90°,[3] and although there is no information in the literature on this point, we anticipated that the N3–C4–S–S minimum energy torsion would occur at 180°, with a higher energy local minimum at 0°, based on an ester or amide model. Because of the manner in which these cross-linked bases will be positioned within a B-DNA double helix, it is feasible for the S–S bond to exist in its preferred conformation. However, the N3–C4–S–S dihedral will necessarily be near 0°, depending upon the exact sequence context in which this covalent base-pair is placed.

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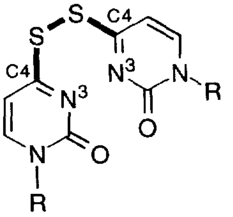
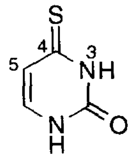


R = H **3**
R = ribose **4**



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RESULTS. The global minimum energy conformation of 4-thiouridine dimer **4** generated by Monte Carlo simulation using the original AMBER* force field (as implemented in MacroModel v 5.5)[4] was in poor agreement with the published structure of **4** determined by X-ray crystallography,[5] particularly around the disulfide linkage, and specifically with respect to C4 geometry and C4–S torsional values. We judged the AMBER* force field to be adequately parameterized for disulfide dihedral and bending angles when compared to ab initio (HF/6-31G*) and disulfide X-ray data.[6] In order to determine the geometry around C4, structural discrepancies between AMBER* and ab initio methods were addressed by calculations on 4-thiouracil disulfide (**3**) and monomeric 4-thiouracil (**5**).

4-Thiouracil (**5**) exhibited a discrepancy about the N3–C4–C5 thiocarbonyl bond angle when the two methods of geometry optimization were compared (Table 1). 4-Thiouracil was constructed in the Spartan (v 4.1) builder using the SYBYL force field and subsequently optimized at the HF/3–21G* and HF/6-31G* levels of theory. The bond angles obtained by ab initio calculations were incorporated into a new substructure section of the AMBER* force-field using standard SMILES notation for the thiouracil ring system. Force constants were adjusted until the geometry around C4 was found to be accurately represented by the modified AMBER* force-field.

Table 1

Comparison of ab initio and AMBER* Geometries of 4-Thiouracil **5**.

Angle	ab initio (6-31G*)	Original AMBER*	Modified AMBER*
N3–C4–S	121°	119°	121°
N3–C4–C5	115°	122°	114°
C5–C4–S	124°	119°	125°

Along with the disulfide bond, the N3–C4–S–S dihedral angle is critical in determining the mutual orientation of the two pyrimidine rings of **3**. In order to establish a more representative N3–C4–S–S geometry, 4-thiouracil disulfide (**3**) was subjected to ab initio calculations at both the HF/3-21G* and HF/6-31G* levels of theory as implemented in Spartan (v 4.1). The correlation of AMBER* with ab initio calculations (Table 2) were within an acceptable deviation only for the N3–C4–S and C4–S–S bending. The non-modified AMBER* force-field resulted in a large deviation about the N3–C4–S–S torsion compared to the crystal structure geometry (Figure 1).

Table 2

Comparison of ab initio and AMBER* Calculated Geometries of 4-Thiouracil Disulfide **3**.

Angle	ab initio (6-31G*)	Original AMBER*	Crystal Structure	Modified AMBER*
N3–C4–S–S	180°	117°	179°	175°
C4–S–S–C4	87-94°	76°	87°	81°
N3–C4–S	120°	120°	NA	119°
C4–S–S	107°	106°	104°	108°

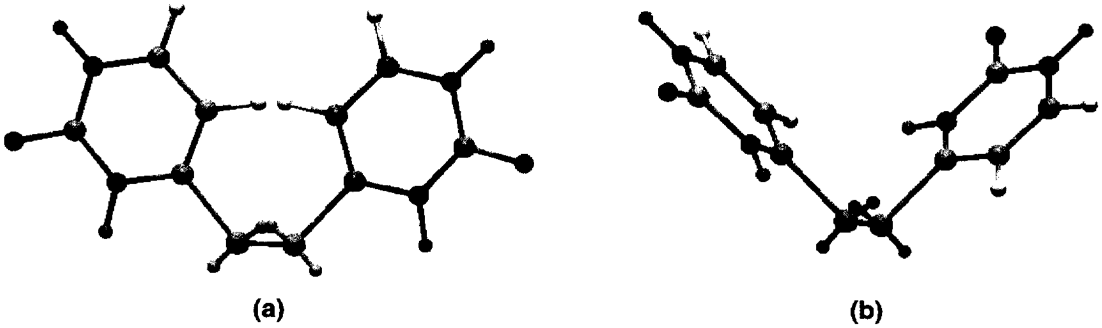

Figure 1. (**a**) Comparison of Original AMBER*, and (**b**) X-ray Crystal Structure Geometry of **3**.

In order to obtain high quality parameters for the torsional interactions around the disulfide bond, we utilized dihedral angle driving and ab initio methods on the thiouracil dimer (**3**).[7] In the original AMBER* torsional profile, there was essentially no barrier to rotation about this torsion. Ab initio calculations were carried out using 4-thiouracil disulfide (**3**). The ring system was built using the Spartan builder and the starting structures were minimized with the SYBYL force field. The dihedral angle about the N3–C4–S–S bond was driven from 0° to 180° while geometry optimizations at the HF/6-31G* level were carried out at 20° intervals. The results of these ab initio calculations exhibited an energy barrier between 0° and 90° of about 6.7 kcal/mol, and an energy preference between 0° and 180° of approximately 0.5 kcal/mol (Figure 2). After subtracting the "torsionless" barrier obtained with MacroModel/AMBER* torsional parameters for the N=C–S–S bond set to 0 kcal/mol, an appropriate torsional curve was obtained by subtracting this torsionless curve from the ab initio curve, and normalizing the resulting data, following accepted protocols.[7] To incorporate these energetic values into AMBER*, a torsional expression for the N3–C4–S–S dihedral was created using these constants, wherein the thioimide bond was specifically defined using the values V1 = –1.0, V2 = 3.06, V3 = 0.0 kcal/mol. As a consequence of this process, the energy minimum about this bond is observed at 0° relative to 180° by about 1.6 kcal/mol (Figure 2).

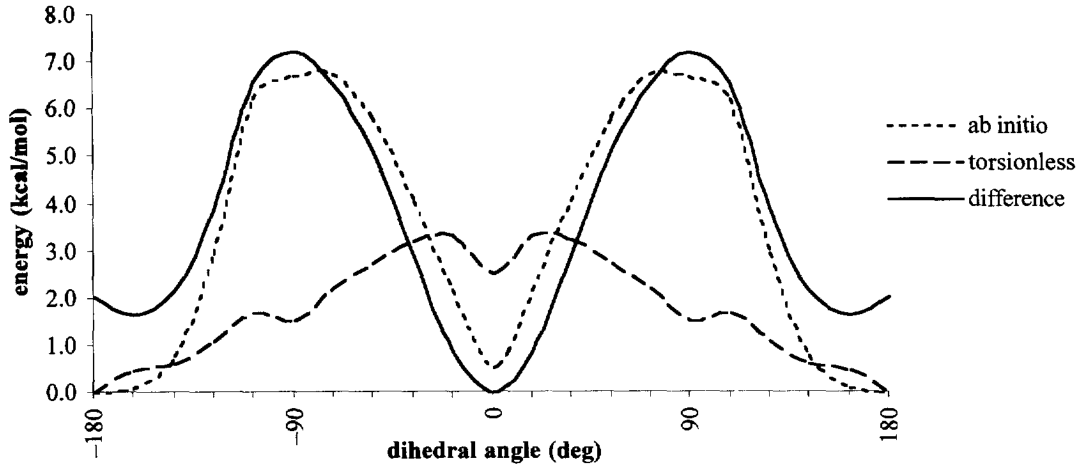

Figure 2. Plot of N3–C4–S–S torsional angle.

CONCLUSION We have developed useful parameters for molecular modeling of thionucleoside disulfides. Parameters for the AMBER* force field were developed by ab initio methods that could be used to model disulfide cross-links in duplex DNA. These torsional parameters will allow a more accurate prediction of the conformation of the disulfide bond and nucleoside bases within cross-linked DNA.

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5. Shefter, E.; Kalman, T. I. *Biochem. Biophys. Res. Comm.* **1968**, *32*, 878. The ribose ring of **4** does not alter the conformation about the internucleoside S–S bond, so we removed the sugar in ab initio and AMBER calculations.
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