4212 *J. Am. Chem. Soc.* **1988**, *110*, 4212-4216

functions to relative energies. At the 6-31G level, the reaction **9** → **12** is 12.9 kcal/mol endothermic and the reaction **9** → **13** is 3.8 kcal/mol exothermic. Clearly, reliable results concerning energetics cannot be determined by using only double-$\zeta$ or minimal basis set calculations.

## Conclusion

Coupled cages have been studied by quantum mechanical methods, which include the effect of both electron correlation and polarization functions. A 1,5-$C_2B_3H_4$ group is nearly equivalent to hydrogen with respect to its interaction as a bridge or terminal group in diborane. This is attributable to the exo/endo separability of the molecular orbitals, which allows the exo orbital on the vacant boron of the $C_2B_3H_4$ group that is outwardly hybridized to interact without significant overlap of the other endo orbitals. In effect these endo orbitals are "folded back" due to their involvement in cluster bonding in the carborane cage.

The terminal hydrogen exchange reaction between $BH_3$ and the carboranes 1,5-$C_2B_3H_5$ and 1,6-$C_2B_4H_6$ was studied. From extrapolation of these results it was estimated that the exchange of terminal hydrogens of 1,5-$C_2B_3H_5$ with $B_2H_6$ takes place with a barrier of about 10 kcal/mol while in 1,6-$C_2B_4H_6$ the barrier is about 35 kcal/mol.

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**Registry No.** **1**, 114467-67-3; **2**, 114467-68-4; **5**, 20693-67-8; **6**, 20693-69-0; **7**, 114467-69-5; **9**, 20693-66-7; **10**, 114467-70-8; **13**, 18972-20-8; $BH_3$, 13283-31-3; $B_2H_6$, 19287-45-7.

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# Cis-Trans Energy Difference for the Peptide Bond in the Gas Phase and in Aqueous Solution

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**Abstract:** Ab initio molecular orbital calculations and Monte Carlo statistical mechanics simulations have been used to study the cis-trans equilibrium for *N*-methylacetamide (NMA) in the gas phase and in dilute aqueous solution. Geometry optimizations were carried out with the 6-31G(d) basis set for the cis and trans forms of NMA. After calculation of the correlation energies with second-order Møller-Plesset theory and the vibrational frequencies, the computed $\Delta H^{298}$ and $\Delta G^{298}$ favor the trans form by 2.1 and 2.5 kcal/mol in the gas phase. Then, in order to proceed to the simulations in water, the predictions of the OPLS potential functions were compared to results of 6-31G(d) ab initio calculations for complexes of *cis*- and *trans*-*N*-methylformamide (NMF) with a single water molecule. This revealed that the OPLS functions erroneously favor the hydrogen bond to the N-H group in the trans form by ca. 1.3 kcal/mol in comparison to the cis. Compensation requires the use of slightly different charge distributions for the cis and trans forms, which are consistent with the order of the ab initio dipole moments ($\mu_{cis} > \mu_{trans}$). Statistical perturbation theory was then applied in Monte Carlo simulations using 216 TIP4P water molecules to compute the difference in free energies of hydration as *trans*-NMA is converted to *cis*-NMA at 298 K. If the OPLS parameters are used for both the cis and trans conformers, the trans isomer is computed to be better hydrated by 2.2 ± 0.3 kcal/mol. However, utilization of the refined charge distributions yields no statistically significant difference in the free energies of hydration (0.1 ± 0.3 kcal/mol favoring trans). These findings emphasize the sensitivity of such results to the details of the intermolecular potential functions. For NMA, the predicted free energy difference of 2.6 ± 0.3 kcal/mol in water is in accord with NMR results (2.5 ± 0.4 kcal/mol), and the negligible solvent effect is consistent with the observed insensitivity of the cis-trans equilibria for NMA and NMF to changes in solvent.

Amides provide the simplest models for the structure and conformational characteristics of the backbones of proteins. Some well-established features for the peptide bond in both amides and proteins are the coplanarity of the groups attached to it, its partial double-bond character that is reflected in shortened C-N distances (ca. 1.34 Å) and substantial rotational barriers (ca. 20 kcal/mol), and preference for the trans rather than cis configuration.[1] The latter bias is sufficiently strong that no more than a few percent of the cis form is detected in experimental studies of secondary amides, and virtually no cis peptide bonds occur in X-ray structures for proteins except when the nitrogen is tertiary, i.e. in a proline residue.[1-9] The low cis population makes accurate experimental determination of the cis-trans energy difference difficult; consequently, it is unclear how the preference for trans peptide bonds in proteins is partitioned between intrinsic and environmental effects. To help elucidate these fundamental questions, the present study has been undertaken to obtain high-level theoretical estimates for the cis-trans energy difference of *N*-methylacetamide (NMA) both in the gas phase and in dilute aqueous solution. The gas-phase results for the enthalpy, entropy, and free energy change at 298 K come from ab initio molecular orbital calculations, with the 6-31G(d) basis set augmented with correlation energy and vibrational frequency calculations. The difference in free energies of hydration for the two conformers is then obtained from statistical perturbation theory in the context of Monte Carlo simulations for the solutes plus 216 water molecules. Analysis of 6-31G(d) results on complexes of *N*-methylformamide (NMF) with a single water molecule indicates the need to use slightly

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different charge distributions in the intermolecular potential functions for the cis and trans amides. The sensitivity of the aqueous free energy change to this modification provides some indication of the potential difficulties in obtaining reliable conformational predictions from protein simulations. In the following, the computational details are summarized first. The results for NMA in the gas phase, the NMF–water complexes, and NMA in water are then presented along with related findings from previous experimental and theoretical studies.

## Computational Details

**Ab Initio Calculations.** The GAUSSIAN/82 program was used to perform the ab initio molecular orbital calculations on a Gould 32/8750 computer.[10] The 6-31G(d) basis set,[11] which includes d orbitals on all atoms except hydrogen, was used throughout except for computing the vibrational frequencies of *cis*- and *trans*-NMA. In that case, the smaller 3-21G basis set[12] was utilized with 3-21G-optimized geometries since previous results have shown negligible differences in accuracy for 3-21G- and 6-31G(d)-derived frequencies.[13] The vibrational analysis verified that the planar forms of *cis*- and *trans*-NMA with $C_s$ symmetry are true energy minima. Full geometry optimizations were also carried out for both forms of NMA in $C_s$ symmetry with the 6-31G(d) basis set. These calculations were supplemented by computation of the correlation energies via second-order Møller–Plesset perturbation theory.[14] Thus, the cis–trans energy difference was obtained at the MP2/6-31G(d)//6-31G(d) level.[13]

To test the intermolecular potential functions needed in the Monte Carlo simulations, ab initio calculations were also carried out on complexes of *cis*- and *trans*-NMF with a single water molecule. The smaller amide was used for computational efficiency since it was desirable to perform these calculations with the 6-31G(d) basis set as well. This basis set is known to give excellent results for hydrogen-bond geometries and energies of such complexes.[15] Our earlier 6-31G(d) calculations on formamide–water complexes, which used partially optimized 3-21G geometries, left some uncertainty concerning the effect of the shorter hydrogen bonds obtained at the 3-21G level on the energetics.[16] To maintain compatability with the geometries used in the Monte Carlo simulations, standard geometries based on experimental data were adopted for the NMF and water in the ab initio calculations.[16,17] Intermolecular geometrical variables were then optimized for several orientations with the 6-31G(d) calculations.

**Monte Carlo Simulations.** The statistical mechanics calculations followed the same procedures as in earlier studies.[16,18] In particular, Monte Carlo (MC) simulations were previously carried out for pure liquid formamide, NMA, and dimethylformamide in order to develop the OPLS intermolecular force field.[17] Besides giving excellent structural and thermodynamic results for the pure liquids, the OPLS parameters were used in MC simulations for the same three amides in aqueous solution.[16] As in that study, the present simulations employed a single solute plus 216 water molecules in a cubic cell with periodic boundary conditions in the isothermal–isobaric (NPT) ensemble at 25 °C and 1 atm. It may be noted that Metropolis and preferential sampling were used, while the earlier paper can be consulted for additional details.[16] In order to compute the difference in free energies of hydration for *cis*- and *trans*-NMA, a series of MC calculations were required. Statistical perturbation theory[19] was used to calculate the free energy change as the conformers were gradually interconverted. As in an analogous study of the conformational equilibrium for butane in water,[18] the interconversion can be accomplished by perturbing the relevant dihedral angle in increments, $\Delta\Phi$. The free energy change between the reference and perturbed states is then given by eq 1, where $\beta = (k_BT)^{-1}$ and $E(\Phi + \Delta\Phi)$ and $E(\Phi)$

$$G(\Phi + \Delta\Phi) - G(\Phi) = -k_BT \ln \langle \exp[-\beta(E(\Phi + \Delta\Phi) - E(\Phi))] \rangle_\Phi \quad (1)$$

are the *total* energies for the perturbed and reference states, and the average is obtained by running the simulation for the reference system. As in the previous study,[18] the computations were well-behaved for $\Delta\Phi = 15°$. This was implemented by rotating both ends of the solute by 7.5°. Furthermore, 30° could be covered in each simulation by perturbing from $\Phi$ to $\Phi + \Delta\Phi$ and $\Phi - \Delta\Phi$.

**Table I.** Potential Function Parameters for *cis*- and *trans*-*N*-Methylacetamide[a]

| | | | OPLS: | modified | |
|---|---|---|---|---|---|
| group | $\sigma$, Å | $\epsilon$, kcal/mol | $q$, e | $q$(trans) | $q$(cis) |
| $CH_3$(C) | 3.91 | 0.160 | 0.0 | 0.0 | 0.0 |
| C | 3.75 | 0.105 | 0.50 | 0.58 | 0.53 |
| O | 2.96 | 0.210 | −0.50 | −0.53 | −0.53 |
| N | 3.25 | 0.170 | −0.57 | −0.55 | −0.55 |
| H | 0.0 | 0.0 | 0.37 | 0.30 | 0.35 |
| $CH_3$(N) | 3.80 | 0.170 | 0.20 | 0.20 | 0.20 |

[a] Note the parameters for NMF are the same with removal of the $CH_3$(C) group and replacement of C by CH, with $\sigma = 3.80$ and $\epsilon = 0.115$.[17]

Initially, six simulations were run to span the range from *cis*-NMA ($\Phi = 0°$) to *trans*-NMA ($\Phi = 180°$). In this case, the original OPLS potential functions were employed including the TIP4P model for water.[17,20] Consequently, the molecules are represented as collections of interaction sites located on the nuclei, except a united-atom representation is used for methyl groups[17] and the negative charge for water is placed 0.15 Å from the oxygen.[20] The net interaction for two molecules then consists of the Coulomb and Lennard–Jones interactions between all intermolecular pairs of sites (eq 2). Standard combining rules are imposed such that $A_{ij} = (A_{ii}A_{jj})^{1/2}$ and $C_{ij} = (C_{ii}C_{jj})^{1/2}$. Furthermore, the $A$ and $C$ parameters may be expressed as $A_{ii} = 4\epsilon_i\sigma_i^{12}$ and $C_{ii} = 4\epsilon_i\sigma_i^6$ where $\epsilon$ and $\sigma$ are the Lennard–Jones energy and radius terms.

$$\Delta E_{ab} = \sum_i^{\text{on } a} \sum_j^{\text{on } b} (q_iq_je^2/r_{ij} + A_{ij}/r_{ij}^{12} - C_{ij}/r_{ij}^6) \quad (2)$$

Consistent with results from population analyses of quantum mechanical wave functions,[21,22] it is standard practice to use the same charge distributions for cis and trans amides or peptides in force fields. Thus, the original set of six Monte Carlo simulations was carried out by varying the dihedral angle but with no alteration to the OPLS parameters, which are summarized in Table I. However, the results of the ab initio calculations on the NMF–water complexes could only be fit well if some small changes were made such that the charge distributions for the cis and trans isomers are no longer identical. Though the fitting is discussed below, the resultant charges are summarized in the last two columns of Table I; the only difference between cis and trans is a shift of 0.05e from the carbonyl carbon to the hydrogen on nitrogen. Two more MC simulations were then required to obtain the effect of this change on the difference in free energies of hydration. For *cis*-NMA, a calculation was run for a charge distribution halfway between the OPLS and modified alternatives. The perturbations were made to the OPLS and modified charges, which then gives the difference in free energies of hydration for *cis*-NMA with the two charge distributions. A calculation was carried out analogously for the trans form.

The Monte Carlo simulations were run with the BOSS program, which monitors the reference solute and two perturbed forms simultaneously. Each calculation consisted of an equilibration phase of at least $0.5 \times 10^6$ configurations followed by averaging over an additional $2 \times 10^6$ configurations. The computations were all performed on Gould 32/7780 and 32/8750 computers in our laboratory.

## Results and Discussion

**Ab Initio Results for the Gas Phase.** The key structural parameters computed for *cis*- and *trans*-NMA at the 6-31G(d) level are illustrated in Figure 1. The results for the trans form are in excellent accord with the experimental values from electron diffraction.[8] Specifically, the 6-31G(d) and experimental findings for the experimentally determined bond lengths in angstroms and angles in degrees are the following: $C_1$–$C_2$, 1.515, 1.520; N–$C_3$, 1.447, 1.468; $C_2$–N, 1.353, 1.386; $C_2$–O, 1.200, 1.224; N–$C_2$–O, 122.2, 121.8; $C_2$–N–$C_3$, 121.5, 119.6; $C_1$–$C_2$–N, 115.2, 114.1;

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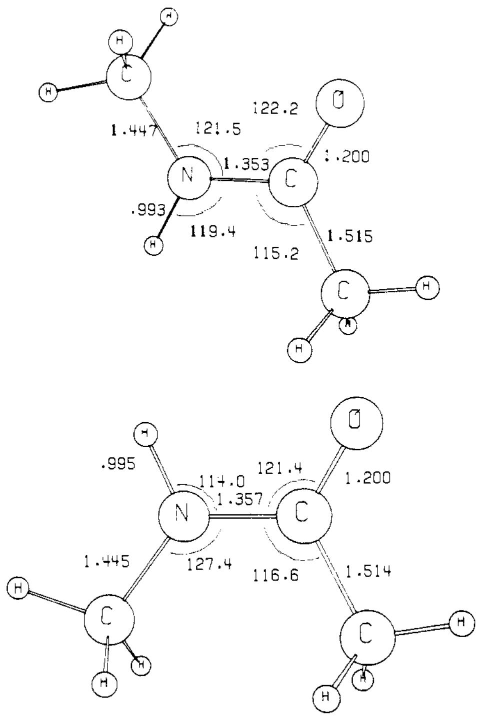

**Figure 1.** Computed structures for (top) *trans-* and (bottom) *cis-N-*methylacetamide with the 6-31G(d) basis set.

**Table II.** Thermodynamic Results for *N*-Methylacetamide in the Gas Phase[a]

| property | cis–trans | property | cis–trans |
|---|---|---|---|
| $\Delta E_e^\circ$ | 2.15 | $\Delta H^{298}$ | 2.07 |
| $\Delta E_v^\circ$ | -0.10 | $\Delta S^{298}$ | -1.43 |
| $\Delta(\Delta E_v)^{298}$ | 0.02 | $\Delta G^{298}$ | 2.50 |

[a] Units are kcal/mol for energies and cal/mol·deg for the entropy change. The electronic energy change, $\Delta E_e^\circ$, is from MP2/6-31G(d)//6-31G(d) calculations, while the vibrational frequencies are from 3-21G//3-21G calculations. The total electronic energy for *trans*-NMA is -247.72662 au at the MP2/6-31G(d)//6-31G(d) level.

$C_2$–N–H, 119.4, 110. The principal changes upon rotation to the cis form appear to help accommodate steric congestion for the methyl groups. Thus, the $C_1$–$C_2$–N and $C_2$–N–$C_3$ angles widen from 115.2° and 121.5° to 116.6° and 127.4°, respectively, while the $C_2$–N–H angle narrows from 119.4° to 114.0°. The largest change for the bond lengths is an increase of only 0.004 Å for the central C–N bond. It may be noted that there is little orientational preference for the methyl groups on nitrogen. Though the illustrated rotamers are lowest in energy, ca. 60° rotation to eclipse a C–H bond with the N–C bond only requires 0.5 and 0.1 kcal/mol for the cis and trans isomers at the 6-31G(d)//6-31G(d) level. In fact, the rotated form is preferred at the 3-21G//3-21G level for *trans*-NMA by 0.3 kcal/mol, so it had to be used in the vibrational analysis to avoid any imaginary frequencies.

The thermodynamic results are summarized in Table II. The electronic energy difference at the Hartree–Fock level (6-31G(d)//6-31G(d)) of 2.42 kcal/mol becomes 2.15 kcal/mol upon inclusion of the MP2 correlation energies. The difference in zero-point vibrational energies, $\Delta E_v^\circ$, and the correction to 298 K, $\Delta(\Delta E_v)^{298}$, were calculated from the frequencies obtained by the 3-21G//3-21G calculations.[23] Naturally, the differences are small; the cis isomer is favored by 0.08 kcal/mol at 298 K. Combination with $\Delta E_e^\circ$ yields a predicted $\Delta H^{298}$ of 2.07 kcal/mol since the translational and rotational energies for the isomers are the same. The 3-21G results also yielded total entropies of 75.8 and 77.2 cal/mol·deg for *cis-* and *trans*-NMA at 298 K and 1 atm in the ideal gas standard state. The translational (38.8) and rotational (25.5) entropies are the same for both isomers, so the small net difference is from the vibrational contributions. Finally, from $\Delta G = \Delta H - T\Delta S$, the computed free energy difference in the gas phase at 298 K is 2.50 kcal/mol.

It may be noted that the sign of $\Delta S$ for the NMA isomerization is the same as that found previously with the ECEPP force field;[24] however, the magnitudes for both $\Delta S$ (4.8 cal/mol·deg) and $\Delta E$ (5.8 kcal/mol) from that study are too large. It appears that previously the most sophisticated calculations for the cis–trans energy difference were by Radom and Riggs.[25] With partial geometry optimizations they obtained $\Delta E_e^\circ$'s of 2.5 and 3.4 kcal/mol with the STO-3G and 4-31G basis sets, respectively. On the experimental side, a "rough" estimate for $\Delta H$ of 2.3 kcal/mol has been made from infrared spectroscopy of NMA in a nitrogen matrix.[9] The most comprehensive study was by Drakenberg and Forsen who used NMR line shape analysis.[7] For NMA in 1,2-dichloroethane, they found $\Delta G = \Delta H = 2.8$ kcal/mol, while in water they obtained $\Delta G = 2.5$ kcal/mol and $\Delta H = 3.4$ kcal/mol. Their estimated errors are ±0.4 kcal/mol for $\Delta G$ and ±2.5 kcal/mol for $\Delta H$. Considering the present gas-phase result and the measured $\Delta G$'s in solution, it would appear that solvent effects on the cis–trans equilibrium are negligible. This position is also supported by recent experiments on NMF and NMA in several solvents.[26] Thus, confirmation from the present simulations in water should be expected if the potential functions are appropriate.

Another notable result from the ab initio 6-31G(d) calculations is that the dipole moment for *cis*-NMA is predicted to be larger than that for *trans*-NMA, 4.21 vs 4.04 D. This order was also obtained in the ab initio study of Radom and Riggs.[25] The order is, of course, interesting because, from classical electrostatics, the isomer with the larger dipole moment is expected to be better solvated, though this theory simplifies the solvent to a structureless dielectric medium.[27] Two other points are worth noting in this regard. First, the computed dipole moments are consistent with recent experimental results for NMA, which range from 3.85 D in benzene to 4.22 D in 1,4-dioxane.[28] Second, the OPLS charge distribution (Table I) and standard geometries[17] translate to dipole moments of 3.86 and 4.23 D for *cis-* and *trans*-NMA. This incorrect order may partly reflect the inadequacies of computing dipole moments from point charge distributions, particularly when atoms are present that formally have lone-pairs of electrons. Nevertheless, it is a concern in the present context. It turns out that the order can be reversed by an all-atom model for the $CH_3$ group on nitrogen. If the carbon is given a charge of -0.25e and each hydrogen is +0.15e, the net charge on the methyl group is still +0.20e and the dipole moments are 4.58 and 4.17 D for *cis-* and *trans*-NMA. Alternatively, the modified charges in Table I give dipole moments of 4.03 and 3.85 D for *cis-* and *trans*-NMA, while still retaining the united-atom methyl groups.

**NMF–Water Complexes.** In development of the OPLS parameters for amides, ab initio results for complexes of formamide and NMA with water were considered.[16] The most sophisticated computations were for formamide and involved 6-31G(d) calculations on geometries that were partially optimized with the 3-21G basis set. However, the 3-21G calculations make the optimal intermolecular distances too short, and differences in hydrogen bonding for cis and trans secondary amides were not addressed. For the present purposes, it is clearly important to examine the latter issue more thoroughly. Consequently, 6-31G(d) calculations were performed on three NMF–water complexes for both the cis and trans isomers. The structures are illustrated in Figure 2 and are analogous to the ones studies previously.[16] In each case, the

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(23) The five frequencies for both *cis-* and *trans*-NMA below 550 cm$^{-1}$ were treated as rotations; i.e., $E_v = RT/2$ for each vibration.

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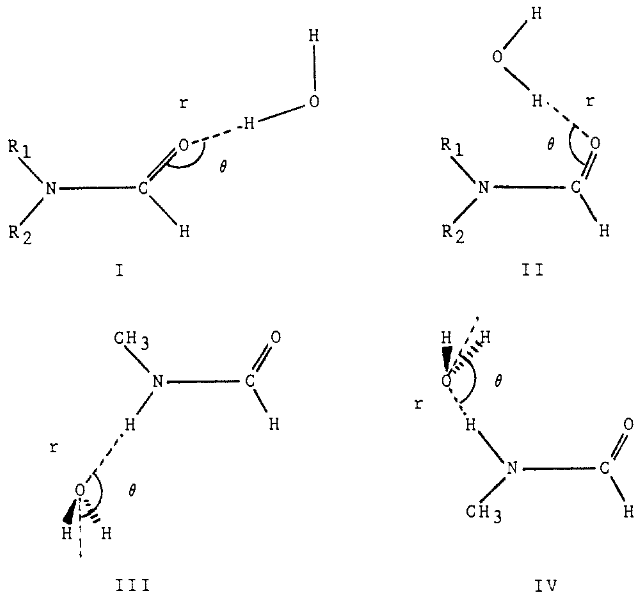

**Figure 2.** Structures considered for the complexes of *cis-* and *trans-N-*methylformamide with a water molecule.

**Table III.** Geometries and Interaction Energies (kcal/mol) for NMF–Water Complexes

| geometry | method[a] | $r_{ox}$,[b] Å | $\theta$, deg | $-\Delta E$ |
|---|---|---|---|---|
| | *trans*-NMF | | | |
| I | 6-31G(d) | 2.94 | 115 | 6.9 |
| | OPLS | 2.73 | 147 | 6.6 |
| | mod | 2.72 | 150 | 6.7 |
| II | 6-31G(d) | 2.94 | 134 | 7.3 |
| | OPLS | 2.73 | 142 | 7.3 |
| | mod | 2.72 | 142 | 7.4 |
| III | 6-31G(d) | 3.08 | 177 | 5.4 |
| | OPLS | 2.86 | 181 | 6.6 |
| | mod | 2.92 | 186 | 5.2 |
| | *cis*-NMF | | | |
| I | 6-31G(d) | 2.94 | 114 | 7.1 |
| | OPLS | 2.73 | 143 | 6.3 |
| | mod | 2.72 | 144 | 6.7 |
| II | 6-31G(d) | 2.90 | 111 | 8.1 |
| | OPLS | 2.73 | 107 | 7.9 |
| | mod | 2.72 | 108 | 8.2 |
| IV | 6-31G(d) | 3.04 | 106 | 6.2 |
| | OPLS | 2.84 | 117 | 6.2 |
| | mod | 2.85 | 114 | 6.0 |

[a] The optimization methods are ab initio 6-31G(d) calculations and computations with the original OPLS force field or with the modified charges in Table I. [b] $r_{ox}$ is the O–O or O–N distance.

hydrogen bonds are linear, the standard geometries for NMF and water were employed,[17,20] and the two intermolecular variables, $r$ and $\theta$, were optimized. Structures I–III were considered for the trans conformer and structures I, II, and IV for the cis form.

The results of the ab initio calculations and of optimizations with the OPLS force field may be compared with reference to Table III. Overall, the accord is basically good; the disagreement for the interaction energies and angles $\theta$ is small, while the separations $r$ are uniformly too short by ca. 0.2 Å from the OPLS calculations. As discussed previously, the latter feature is needed to obtain correct liquid densities from these potential functions.[16,17] Closer inspection, however, reveals some discrepancies that are significant in the present context. The most serious one involves the hydrogen bonds to the NH group, i.e. in structure III for the trans isomer and in structure IV for the cis form. The OPLS $\Delta E$ agrees with the 6-31G(d) value for IV, but the interaction is 1.2 kcal/mol too favorable for III. Furthermore, there is a smaller bias for hydration of the trans form apparent in the results for structure I. For the trans isomer, the OPLS and 6-31G(d) $\Delta E$'s differ by 0.3 kcal/mol; however, they differ by 0.8 kcal/mol for the complex with *cis*-NMF. The bias for structure II is relatively insignificant and favors the trans isomer by 0.2 kcal/mol. The sum of these effects clearly reveals an unintended, artifactual preference for hydration of the trans amide by 1.9 kcal/mol that is embodied in the OPLS parameterization. This bias is expected to be fully transferred to subsequent computation of relative heats of hydration because NMA was previously found to participate in an average of three hydrogen bonds with water molecules in dilute solution, corresponding roughly to structures I–III.[16] Reference 16 should be consulted for additional structural results.

**Table IV.** Computed Relative Free Energies of Hydration for the Interconversion of *cis-* and *trans-*NMA Using the OPLS Parameters[a]

| $\Phi_i$ | $\Phi_j$ | $i \rightarrow j$ | $j \rightarrow i$ | $\Delta G_{total}(\Phi_i)$ |
|---|---|---|---|---|
| 0 | 15 | 0.08 ± 0.08 | | 0.0 |
| 15 | 30 | | 0.12 ± 0.10 | 0.08 |
| 30 | 45 | −0.29 ± 0.09 | | −0.04 |
| 45 | 60 | | 0.35 ± 0.11 | −0.33 |
| 60 | 75 | −0.67 ± 0.14 | | −0.68 |
| 75 | 90 | | 0.09 ± 0.06 | −1.35 |
| 90 | 105 | 0.13 ± 0.07 | | −1.44 |
| 105 | 120 | | 0.20 ± 0.07 | −1.31 |
| 120 | 135 | −0.08 ± 0.08 | | −1.51 |
| 135 | 150 | | 0.27 ± 0.05 | −1.59 |
| 150 | 165 | −0.29 ± 0.05 | | −1.86 |
| 165 | 180 | | 0.02 ± 0.04 | −2.15 |
| 180 | | | | −2.18 ± 0.29 |

[a] Angle $\Phi$ is the $C_1$–$C_2$–N–$C_3$ dihedral angle in degrees. Free energies in kcal/mol. [b] Error bars (±1$\sigma$) were obtained from separate averages over blocks of $10^5$ configurations during the Monte Carlo simulations.

In order to compensate for this bias, adjustment of the OPLS charge distribution for secondary amides was attempted. A satisfactory solution could not be found without using different charges for at least the carbonyl carbon and hydrogen on nitrogen as recorded in Table I. The results for the NMF–water complexes with the modified charge distributions are also included in Table III. The fit to the 6-31G(d) $\Delta E$'s is nearly perfect. Moreover, the net bias for hydration of the trans isomer in the three structures is now only 0.2 kcal/mol and comes entirely from structure I.

Optimizations with the original OPLS and modified parameters have also been carried out for the corresponding NMA–water complexes. The results are virtually the same as for NMF and are not detailed here. With the modified charges, the separations $r$ and angles $\theta$ are identical within 0.01 Å and 5°. The interaction energies are 0.06–0.15 kcal/mol more favorable for NMA than NMF. The net bias for hydration of the trans form is seemingly reduced to 0.1 kcal/mol, though this assumes that the same relative $\Delta E$'s for NMF and NMA would be found in 6-31G(d) calculations. Overall, confidence can be expressed in the ability of the modified parameters to faithfully reproduce the trends in the ab initio interaction energies for the cis and trans amides. And, at this time, the present 6-31G(d) results are the best available guide to the true trends.

**Monte Carlo Results in Aqueous Solution.** The series of six MC simulations that interconverted *trans-* and *cis-*NMA was run first with the original OPLS parameters. The results for the relative free energies of hydration are summarized in Table IV and Figure 3. The table includes the twelve incremental free energy changes, $\Delta\Delta G$, from the six simulations, and their total, $\Delta G_{total}(\Phi_i)$. As indicated by the $\Delta\Delta G$ values and Figure 3, the free energy of hydration is found to decrease quite smoothly upon progression from *cis-* to *trans-*NMA with the OPLS parameters. The net difference favors the trans isomer by 2.2 ± 0.3 kcal/mol, a value notably similar to the 1.9 kcal/mol bias found in the analysis of the amide–water complexes above.

The two simulations that perturbed the OPLS charges to the modified values were then performed. The results can be summarized by the following diagram.

$$\begin{tikzcd}
\textit{cis}\text{-NMA (OPLS)} \arrow[r, "-2.18 \pm 0.29"] \arrow[d, "-0.69 \pm 0.03"'] \arrow[dr, phantom, "\Delta G^{298}(\text{hyd})"] & \textit{trans}\text{-NMA (OPLS)} \arrow[d, "1.36 \pm 0.07"] \\
\textit{cis}\text{-NMA (mod)} \arrow[r, "-0.13 \pm 0.30"] & \textit{trans}\text{-NMA (mod)}
\end{tikzcd}$$

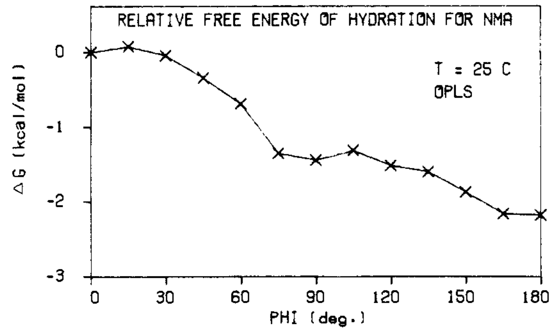

**Figure 3.** Computed change in the free energy of hydration as *cis-N*-methylacetamide is converted to the trans isomer. Results using the original OPLS parameters in the Monte Carlo simulations.

Though the precision obtainable for the free energy differences with the perturbation method is one of its principal strengths,[29] the uncertainties in the $\Delta G$'s for the charge changes are particularly small since the perturbations are modest. The error bars are $\pm 1\sigma$ and were computed in the standard way noted in Table IV. As expected from the findings in Table III, switching to the new charges makes *cis*-NMA better hydrated, while the trans isomer becomes significantly less well hydrated due primarily to the lessened hydrogen-bonding ability for the N–H unit. The combined changes almost exactly cancel the free energy difference from the OPLS parameters. Quantitatively, *trans*-NMA is now found to have a lower free energy of hydration than *cis*-NMA by the statistically insignificant amount of 0.13 ± 0.30 kcal/mol.

Thus, the best estimate of the difference in free energies for *cis*- and *trans*-NMA in water from the present calculations is 2.6 ± 0.3 kcal/mol, which consists of the 2.5 kcal/mol gas-phase preference for the trans isomer (Table II) and the 0.1 ± 0.3 kcal/mol solvent effect. This result is in exact agreement with the finding of 2.5 ± 0.4 kcal/mol from the NMR measurements of Drakenberg and Forsen in water.[7] It is also consistent with the observed insensitivity of the cis–trans equilibrium for secondary amides to changes in solvent.[7,26] Thus, the dominance of trans peptide bonds in proteins can be ascribed entirely to the intrinsic, gas-phase preference.

In closing this section, it should be noted that the present calculations give no information on solvent effects on the activation energies for the cis–trans interconversions. This could be studied with the present methods; however, proper potential functions, particularly the charges, would be needed for the transition state not just the reactant and product. The utilization of the dihedral angle in the present study was just as a convenient, but nonunique, mutation coordinate.[30] An earlier theoretical study of *cis*- and *trans*-NMA in water should also be mentioned. Mezei et al. performed Monte Carlo simulations on just the two conformers in a periodic cell with 215 water molecules.[31] They used two sets of potential functions including OPLS for the amides with TIP4P water. Though they calculated the difference in the *energy* of hydration, the uncertainties in the figure are very large since it was obtained by simply subtracting the two total energies. Their result erroneously favored the cis form by a large amount in water.

## Conclusion

Free energy differences have been computed in the gas phase and in aqueous solution for *cis*- and *trans*-NMA, which represent a fundamental model for the peptide bond in proteins. High-level ab initio calculations find the trans form to have a lower enthalpy and free energy than the cis isomer by 2.1 and 2.5 kcal/mol in the gas phase at 298 K. The difference in free energies of hydration was then determined by Monte Carlo simulations with statistical perturbation theory. Use of the original OPLS parameters favors hydration of *trans*-NMA over *cis*-NMA by 2.2 kcal/mol. However, the preference was traced to intrinsic biases in the potential functions in comparisons with ab initio results for NMF–water complexes. The bias is removed by modifying the OPLS charge distributions for the amides, which also corrects the order of dipole moments for the cis and trans isomer. Use of the new charge distributions in Monte Carlo simulations reveals no statistically significant difference in the free energies of hydration for *cis*- and *trans*-NMA. This result is consistent with experimental observations as is the quantitative result on the free energy difference for *cis*- and *trans*-NMA in water, 2.6 ± 0.3 kcal/mol. The sensitivity of the results in aqueous solution to the details of the charge distributions is particularly notable. In the absence of very extensive bases for the choice of potential function parameters, it is apparent that great care must be exercised in evaluating simulation results for detailed energetic quantities such as those embodied in conformational equilibria, structure prediction, and binding constants.

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