# Transition Structures of Hydride Transfer Reactions of Protonated Pyridinium Ion with 1,4-Dihydropyridine and Protonated Nicotinamide with 1,4-Dihydronicotinamide

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**Abstract:** Hydride transfer reactions of protonated pyridinium ion with 1,4-dihydropyridine and protonated nicotinamide with 1,4-dihydronicotinamide have been studied with *ab initio* molecular orbital calculations. There is a strong preference for a syn or stacking approach of the two pyridine rings in transition structures. The pyridine rings are slightly puckered into boat conformations in the transition structures. When in a cis conformation, the 3-amide group of nicotinamide slightly increases the activation energy for the hydride transfer. When the group is in a trans conformation, it significantly reduces the activation energy for the hydride transfer. There is a preference for the trans amide group to be out-of-plane, with the carbonyl group directed toward the transferring hydride. The relevance of these findings to enzymatic systems involving $NAD^+$ and NADH is also discussed.

The chemistry of nicotinamide adenine dinucleotide/1,4-dihydronicotinamide adenine dinucleotide ($NAD^+$/NADH) coenzyme-dependent dehydrogenases continues to attract great attention due to its importance in biological systems and due to the fascinating substrate selectivity and stereoselectivity.[1,2] Recent theoretical studies have investigated the mechanism and transition structure features of hydride transfer mediated by $NAD^+$ and NADH.[3−6] The conformations of nicotinamide ribose and amide in both free acid model systems and in the enzymatic environment,[7−11] the role of nicotinamide ring puckering in hydride transfer reactivity,[12,13] and the origin of stereospecificity of the dehydrogenases[7,9,14] have also been studied. The results can be briefly summarized as follows. (1) The 3-amide group prefers a cis rather than a trans conformation by about 1 kcal/mol in both nicotinamide and 1,4-dihydronicotinamide (see **1** for definition), and the barrier for the amide cis−trans conversion is about 4 and 7 kcal/mol for nicotinamide and 1,4-dihydronicotinamide, respectively.[10] (2) Both 1,4-dihydropyridine and 1,4-dihydronicotinamide rings are slightly puckered in boatlike conformations, but the rings are quite flexible with small barriers to inversion.[7,8] Similar boat conformations are also found in hydride transfer transition structures (see **2**).[3] (3) Our previous calculations indicate that hydride transfer transition structures of molecules with extended unsaturation prefer a syn arrangement and a bent C−H−C angle to facilitate overlap between the lowest unoccupied molecular orbitals (LUMOs) of hydride donor and hydride acceptor (see **2**).[3] (4) MINDO/3 and STO-3G by Donkersloot *et al.*[15] and AM1 calculations by Greedy *et al.*[6] suggest that the amide carbonyl oxygen favors the same orientation as the transferring hydrogen in transition structures (see **3**). This is supported by recent experiments of Ohno *et al.* which show that when the amide is forced to be out-of-plane, the hydrogen which is on the same face of the amide carbonyl oxygen reacts faster than the other hydrogen.[16] (5) Our *ab initio* calculations suggest that

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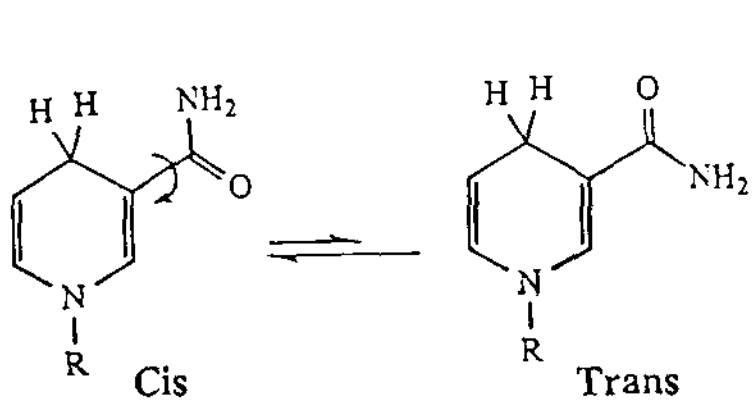
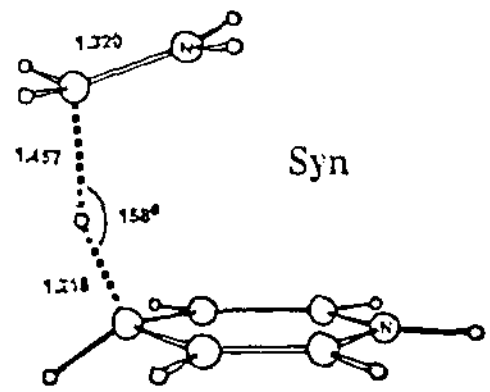
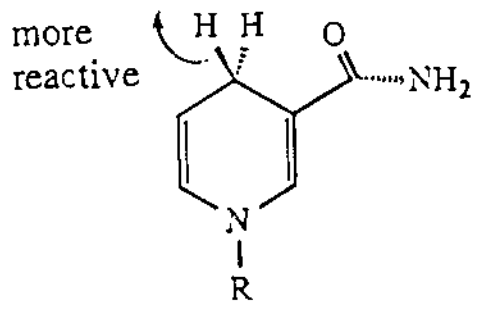
**Chart 1**



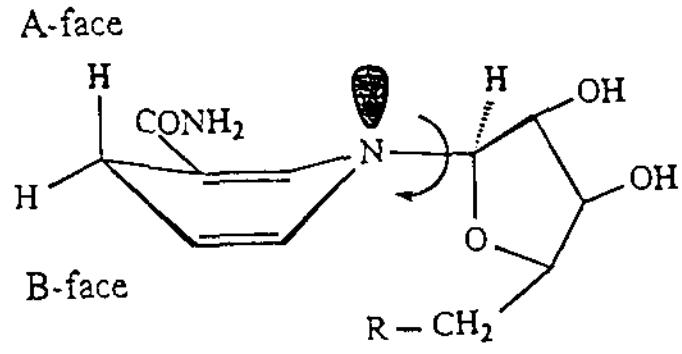

**1**




**2**

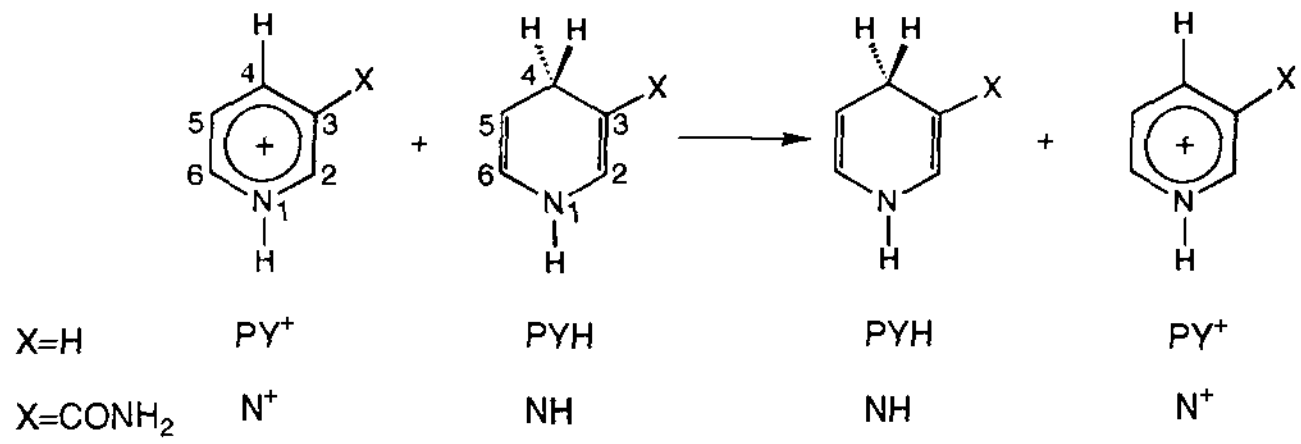



**3**




Anti-NADH **4**

**Scheme 1**




the conformation of nicotinamide with respect to the ribose has an effect on the conformation of nicotinamide ring and therefore can affect the stereospecificity of hydride transfer (see **4**),[7] while Bruice's AM1 simulation shows no such effect.[9]

In this paper, we report our *ab initio* molecular orbital calculations for the hydride transfer reactions of protonated pyridinium ion ($PY^+$) with 1,4-dihydropyridine (PYH) and of protonated nicotinamide ion ($N^+$) with 1,4-dihydronicotinamide (NH), as shown in Scheme 1. In nature, hydride transfer between NADH and $NAD^+$ is catalyzed by transhydrogenases.[17] Similar degenerate reactions have been studied experimentally and theoretically to illustrate the reaction mechanism and the features of transition structures.[18-20] These reactions could also serve as models for oxidation–reduction reactions in $NAD^+$/NADH dependent-dehydrogenases where the substrate is an extended conjugated system.[21,22] Our calculations further substantiate the syn preference of hydride transfer transition structures. We also address the function of the amide conformation in activation of hydride transfer. The relevance of our results to enzymatic systems involving $NAD^+$ and NADH is discussed.

## Methods and Results

All calculations were carried out with GAUSSIAN 92 of Pople *et al.*[23] For the reaction of $PY^+$ with PYH, four structures were optimized with the 3-21G basis set: a $C_{2v}$ structure, a $C_{2h}$

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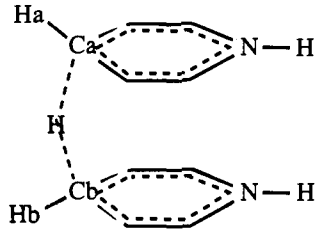
**Chart 2**




| | $C_{2v}$ | $C_2(60°)$ | $C_2(120°)$ | $C_{2h}$ |
|---|---|---|---|---|
| ∠ Ha-Ca-Cb-Hb : | 0° | 60° | 120° | 180° |

**5**

**Table 1.** Total Energies (au) of the Reactants and the Transition Structures of the Hydride Transfer Reaction between 1,4-Dihydropyridine (PYH) and Protonated Pyridinium Cation ($PY^+$) and the Energies of Activation ($\Delta E_a$, kcal/mol)[b]

| | Reactant | | TS | | | | |
|---|---|---|---|---|---|---|---|
| basis set | $E$(PYH) | $E(PY^+)$ | $E(C_{2v})$ | $E(C_2(60°))$ | $E(C_2(120°))$ | $E(C_{2h})$ | $E_a(C_{2v})$[a] |
| AM1 | 0.042 69 | 0.293 32 | 0.357 46 (0.4) | 0.357 39 (0.4) | 0.356 77 (0.0) | 0.357 08 (0.2) | 13.5 |
| 3-21G//3-21G | −246.447 27 | −245.696 41 | −492.114 07 (0.0) | −492.112 38 (1.0) | −492.111 75 (1.4) | −492.109 74 (2.7) | 18.6 |
| 6-31G*//3-21G | | | −494.850 51 (0.0) | −494.849 16 (0.9) | −494.849 16 (0.8) | −494.847 80 (1.7) | |
| 6-31G*//6-31G* | −247.823 17 | −247.070 86 | −494.851 88 (0.0) | | | −494.848 84 (1.9) | 26.4 |
| MP2/6-31G*//3-21G | | | −496.456 23 (0.0) | −496.451 17 (3.2) | −496.450 81 (3.4) | −496.446 67 (6.0) | |
| MP2/6-31G*//6-31G* | −248.612 94 | −247.846 93 | −496.456 02 (0.0) | | | −496.447 65 (5.3) | 2.4 |

[a] $E_a(C_{2v}) = 627.5[E(C_{2v}) - E(\text{PYH}) - E(PY^+)]$, 1 au = 627.5 kcal/mol. [b] Relative energies are in parentheses.

structure, and two $C_2$ structures with 60° and 120° dihedral angle constraints between the two pyridine rings (see **5** for definition). The energies of these structures were then calculated with the MP2/6-31G* method. For the $C_{2v}$ and $C_{2h}$ structures, geometry optimization was also performed with the 6-31G* basis set, which was followed by MP2/6−31G* energy evaluation. The energies are summarized in Table 1.

There is a clear preference for the two pyridine rings to be stacking. Thus, the $C_{2v}$ structure (**6**) is most stable at each level of calculation. The $C_{2h}$ structure (**7**) is least stable. The two $C_2$ structures (which are not shown) have intermediate stabilities with similar energies. The syn preference is moderate at the HF levels but becomes significant when MP2 correlation energy is included.

Semiempirical AM1 calculations for the four structures were also performed to assess the quality of the calculational method in light of several recent AM1 studies on the $NAD^+$/NADH related subjects.[5,6,9] As shown in Table 1, the AM1 calculations failed to show the syn preference. The four structures have similar energies with a shallow well at a dihedral angle between the two pyridine rings of about 120°. A previous MINDO/3 study of the same reaction gave a preference for the $C_{2h}$ structure over the $C_{2v}$ structure.[19] These are qualitatively different from the *ab initio* results.

The 3-21G harmonic vibration frequency calculation was performed for the $C_{2v}$ structure which suggested that it is a true transition structure with one imaginary vibration frequnecy ($1401i$ $cm^{-1}$). The calculated activation energy with respect to the separated $PY^+$ and PYH reactants is 2.4 kcal/mol at the MP2/6-31G* level, lower than the experimentally observed activation energy of 10 kcal/mol for a similar degenerate reaction.[19] It is known that MP2 calculation overestimates the stabilization in a transition structure. When the thermal energy and entropy contributions, which are derived from the 3-21G vibration frequency calculation,[25] are included, we obtain an activation free energy of 16.6 kcal/mol.

The optimized geometries of the $C_{2v}$ and $C_{2h}$ structures are given in Figure 1. The 3-21G and 6-31G* geometries are very similar for both structures. The partially formed C−H bond in the $C_{2v}$ structure is slightly longer than that in the $C_{2h}$ structure. The $C_4$−$C_4$ distance is about 2.6 Å. This distance is similar to those in the transition structures of other model hydride transfer reactions.[3−5] The $C_{2v}$ structure is bent with a C−H−C angle of about 170°. The ring bond lengths and bond angles are close to those of 1,4-dihydropyridine.[10] There is a puckering of the pyridine ring in a boatlike conformation in both structures. This can be described by $N_1$ and $C_4$ out-of-plane angles. The $C_4$ is out-of-plane by about 9°, while the $N_1$ is out-of-plane by 4°. There is also a pyramidalization at $N_1$ as shown by an 8° bend of the N−H bond out of the CNC plane.

For the reaction of protonated nicotinamide with 1,4-dihydronicotinamide, two structures were optimized with a $C_2$ symmetry constraint and with the amide group cis and trans, respectively, as shown in Figure 2. The arrangement of the two amides is such that the steric interactions between the two groups is avoided. Geometry optimizations were carried out with the AM1 semiempirical method and with the *ab initio* 3-21G basis set. The 6-31G* energies were also obtained based on the 3-21G geometries. The calculated energies of these species along with those of the reactants are collected in Table 2.

The AM1 calculations gave almost identical stabilities to the trans structure **11** and the cis structure **10**. The calculated activation energy with structure **10** with respect to the cis $N^+$ and NH reactants is 10.5 kcal/mol, while the activation energy with the structure **11** with respect to the trans reactants is 6.2 kcal/mol. These calculated activation energies are lower than that of the reaction of $PY^+$ with PYH (13.5 kcal/mol), indicating that the addition of the amide group activates the hydride transfer in both cis and trans conformations. The trans structure corresponds to a lower activation energy because the trans reactants are less stable than the cis reactants.[10,11]

The *ab initio* calculations gave qualitatively different results from the AM1 calculations. The trans transition structure **11** is more stable than the cis transition structure **10** by 5.5 and

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(25) Zero-point energy (kcal/mol): $PY^+$, 70.0; PYH, 75.3; TS**6**, 144.4. Overall thermal energy (kcal/mol): $PY^+$, 72.4; PYH, 78.4; TS**6**, 150.2. Entropy (cal/mol·K): $PY^+$, 67.8; PYH, 71.9; TS**6**, 96.7.

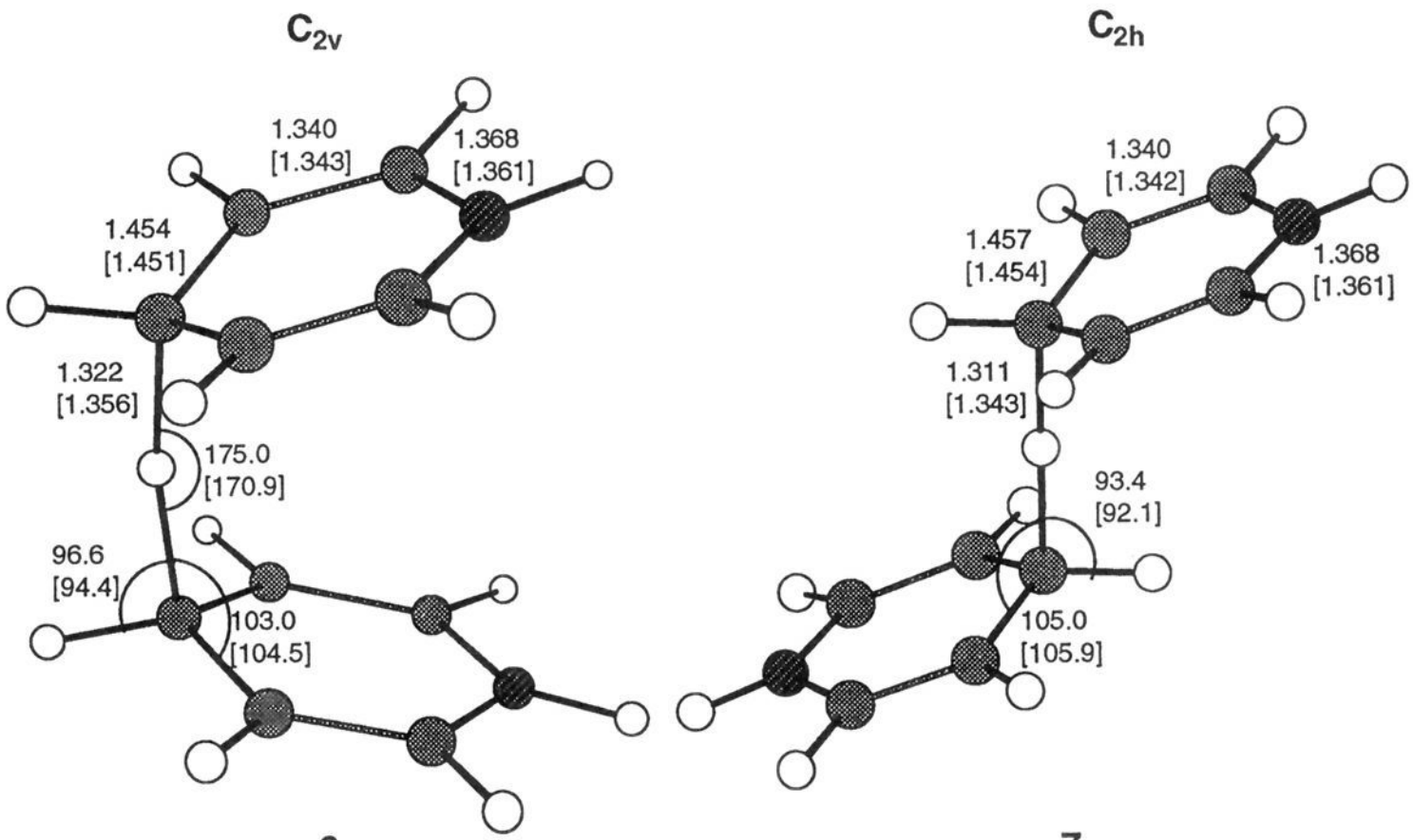



**Figure 1.** The $C_{2v}$ (TS) and $C_{2h}$ structures for the reaction of protonated pyridinium ion with 1,4-dihydropyridine. The 3-21G and 6-31G* (in parentheses) bond lengths (Å) and bond angles as well as 6-31G* natural population charges (with H atom summed in).

4.6 kcal/mol with the 3-21G and 6-31G* basis set (Table 2), respectively. The calculated activation energy with the cis structure **10** with respect to the cis reactants is 19.8 and 28.6 kcal/mol with the 3-21G and 6-31G* basis set, respectively. This is slightly higher than that calculated with the $C_{2v}$ structure for the reaction of $PY^+$ with PYH (18.6 and 26.4 kcal/mol, respectively), indicating that a cis amide group has little effect on the reactivity of hydride transfer. On the other hand, the calculated activation energy with the trans structure **11** with respect to the trans reactants is 7.5 and 19.4 kcal/mol with the 3-21G and 6-31G* basis set, respectively, significantly lower than that of the reaction of $PY^+$ with PYH. We conclude that the amide group significantly activates hydride transfer when it is in a trans conformation.

The amide group has only a minor effect on the geometry of transition structures. As shown in Figure 2, structures **10** and **11** are similar to structure **6**, where the amide is absent. The amide in the cis structure is similar to that in the *cis*-1,4-dihydronicotinamide,[26] with the C=O bond rotated out-of-plane on the opposite face of hydride transfer. In the trans structure, the amide is considerably out-of-plane, with the C=O bond directed toward the transferring hydride. There is a small rotation between the two pyridine rings, as shown by structures **12** and **13**, which have the C−H−C aligned nearly in a line. The two structures have the opposite directions of rotation (−14° and 20°, respectively).

Bodor *et al.* studied the reaction of 1-methyl-1,4-dihydronicotinamide with 1-methylnicotinamide cation with both the MNDO and AM1 methods.[20b,c] They studied four possible

(26) The cis structure calcaulated by AM1 method has the amide significantly out-of-plane with the carbonyl bond directing on the same side of transferring hydrogen ($<C_2-C_3-C=O = 54°$).

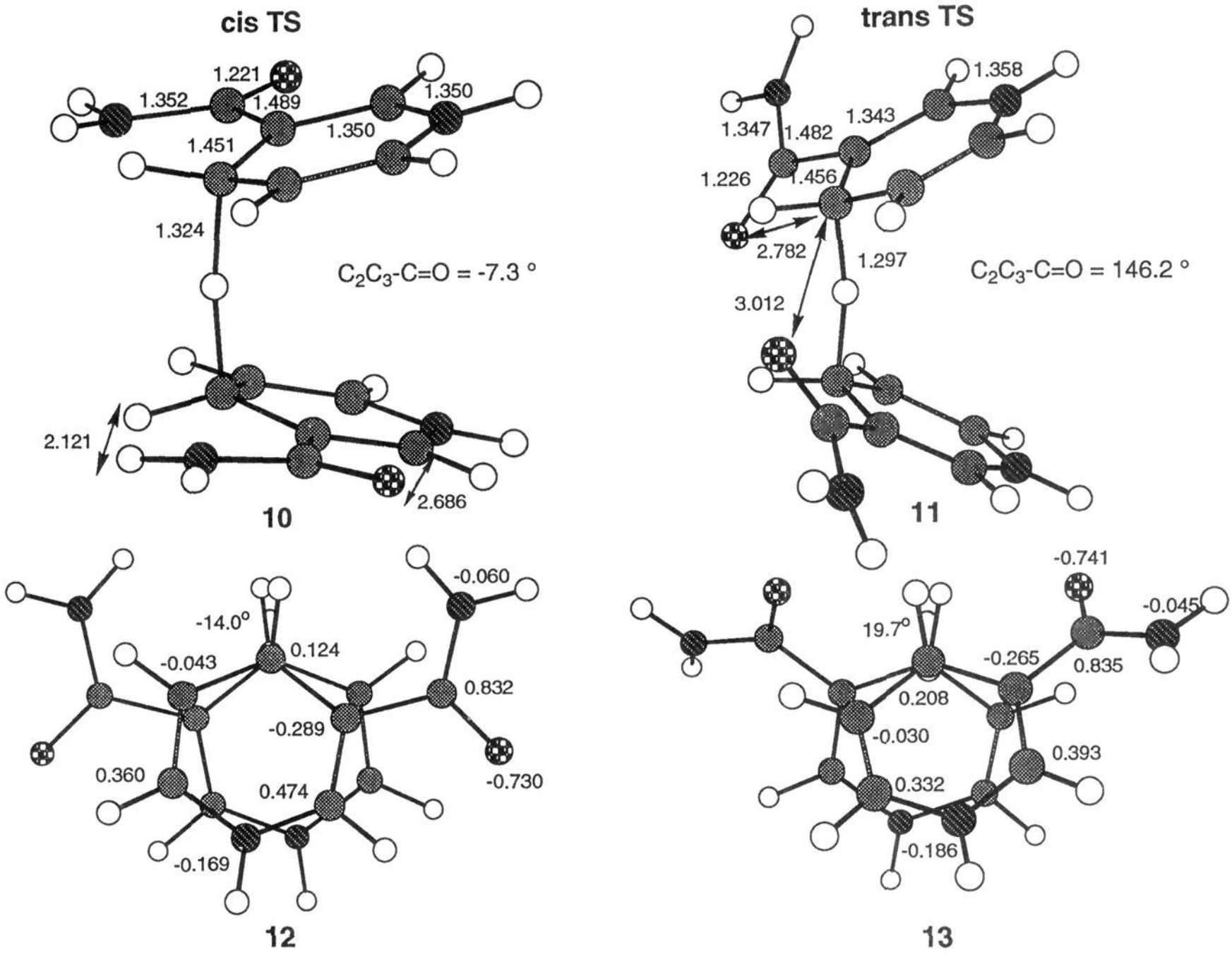



**Figure 2.** The cis and trans transition structures for the reaction of protonated nicotinamide with 1,4-dihydronicotinamide. Calculated with the 3-21G basis set. Selected bond lengths (Å), bond angles (°), and interatomic distances (Å) as well as natural population charges are also shown.

**Table 2.** Total Energies (au) of the Reactants and the Transition Structures of the Hydride Transfer Reaction between 1,4-Dihydronicotinamide (NH) and Protonated Nicotinamide ($N^+$)[c]

| | reactants | | | TS | |
|---|---|---|---|---|---|
| | $E$(NH) | $E(N^+)$ | $E_{rel}$(NH + $N^+$) | $E$(TS)[a] | $E_a$[b] |
| | | | AM1 | | |
| cis | −0.023 42 | 0.239 44 | 0.0 | 0.232 69 (0.3) | 10.5 |
| trans | −0.020 34 | 0.242 62 | 3.9 | 0.232 22 (0.0) | 6.2 |
| | | | HF/3-21G | | |
| cis | −413.305 07 | −412.538 04 | 0.0 | −825.811 55 (5.5) | 19.8 |
| trans | −413.298 41 | −412.533 95 | 6.7 | −825.820 35 (0.0) | 7.5 |
| | | | HF/6-31G*//3-21G | | |
| cis | −415.615 23 | −414.848 51 | 0.0 | −830.418 09 (4.6) | 28.6 |
| trans | −415.610 69 | −414.845 65 | 4.6 | −830.425 40 (0.0) | 19.4 |

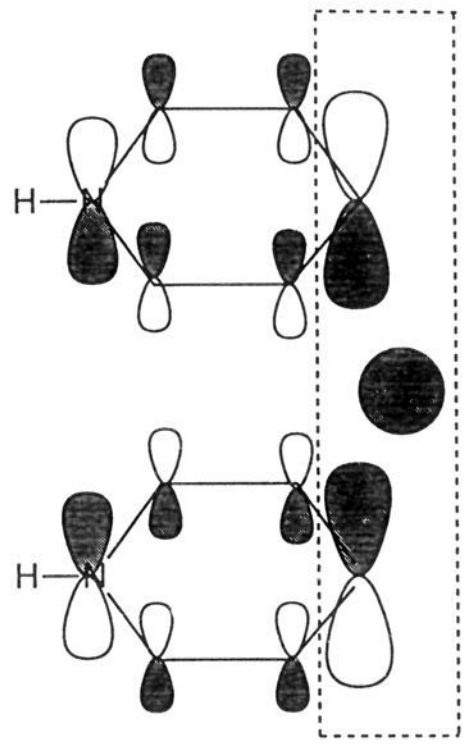
[a] Cis is structure **10** and trans is structure **11**. The relative energies of **10** and **11** are in parentheses. [b] $E_a$ = 627.5 [$E$(TS) − $E$(NH) − $E(N^+)$], 1 au = 627.5 kcal/mol. [c] The activation energy ($E_a$, kcal/mol) is relative to the reactants.

transition structures and found that an endo-cis structure for the hydride transfer is most favorable. The search of this structure started with a syn arrangement between the two pyridine rings and with the two amide groups juxtapositioned, but the transition structure involved a significant rotation between the two pyridine rings to avoid steric interactions between the two amide groups. It is interesting that they found an endo-trans structure, in which steric interaction between the two amide groups is avoided, to be 8 kcal/mol less stable than the endo-cis structure. A clear difference between this endo-trans structure and our AM1 transition structures (refer to structure **11** in Figure 2, and also ref 26) is the orientation of the amide groups. The amide groups in our AM1 transition structures are significantly out-of-plane with the carbonyl bonds directed toward the transferring hydrogen. In the endo-trans structure reported by Bodor *et al.*,[20c] the amide groups are also significantly out-of-plane, but with the carbonyl bond directed away from the transferring hydrogen. As will be discussed later, there is a significant preference for the carbonyl bond to orient on the same side of hydride transfer.

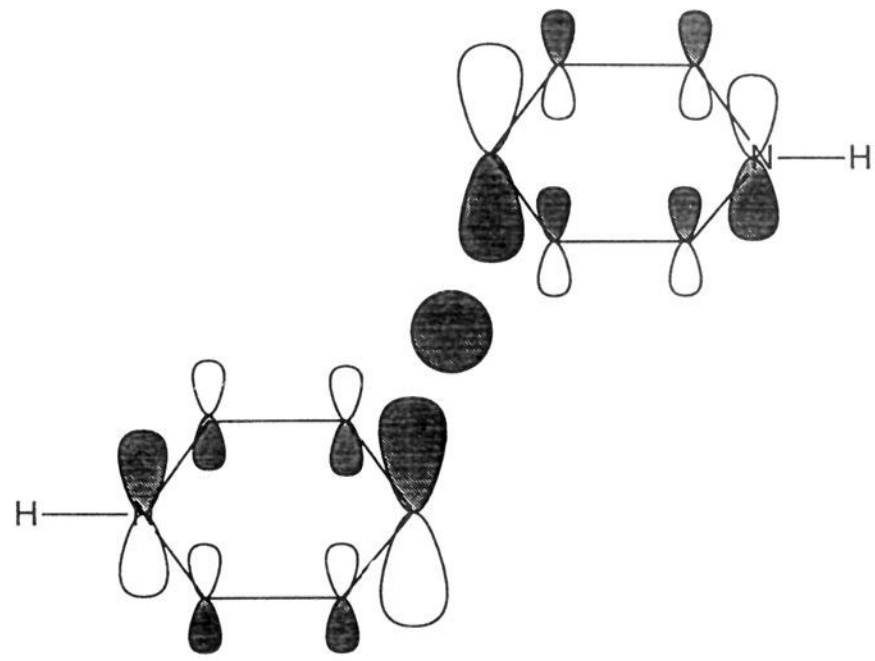
## Discussion

**1. Ring Puckering**. There has been much discussion about the effect of ring puckering on hydride transfer.[9,14,27,28] For example, recently Bruice *et al.* studied hydride transfer transition structures with several constrained pyridine conformations with AM1 calculations and found that when $C_4$ and $N_1$ are allowed to be out-of-plane by 15° and 5°, respectively, there is a 6 kcal/mol decrease in activation energy for hydride transfer compared to a constrained planar pyridine ring. A 5 kcal/mol reduction in activation energy for hydride transfer was calculated if both

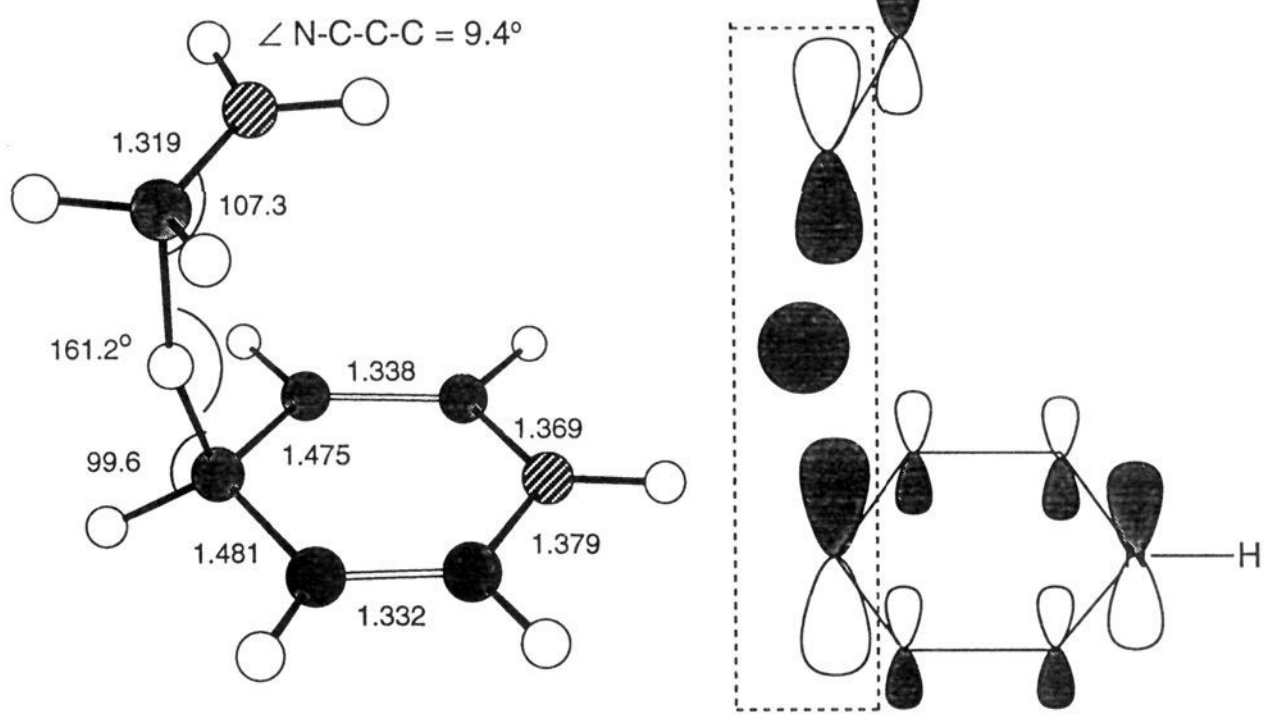
(27) Rotberg, N. S.; Cleland, W. W. *Biochemistry* **1991**, *30*, 4068.
(28) Oppenheimer, N. J.; Arnold, L. J., Jr.; Kaplan, N. O. *Biochemistry* **1978**, *17*, 2613. The ring puckering discussed in this paper may be due to stacking between the adenine and the dihydronicotinamide ring in the folded form.




**14** Better overlap




**15** Poorer overlap




**16**




**17**

**Figure 3.** Molecular orbital illustration for the syn preference of hydride transfer transition structure. Structure **16** is the 3-21G transition structure for hydride transfer from 1,4-dihydropyridine to methyleniminium ion. The favorable LUMO overlap in structure **16** is shown by **17**.

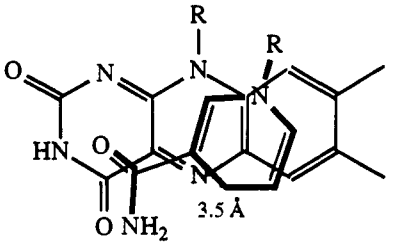
$C_4$ and $N_1$ are allowed to be out-of-plane by 10°.[9] These clearly demonstrate the importance of ring puckering on hydride transfer. Our calculations with full geometric optimizations give boatlike conformations of pyridine rings in all of the hydride transfer transition structures, although the ring puckering does not seem to be very large. The 9° and 4° out-of-plane distortion for $C_4$ and $N_1$ in the calculated transition structures seem to match well with those of enzyme bound NADH.[29] In fact, the 1,4-dihydropyridine ring favors a puckered conformation in the ground state.[10] When it is in solution, there are two nearly equal energy boatlike conformations.[8,12] In an enzyme active site, due to the binding environment, the 1,4-dihydropyridine ring may be forced to pucker in one of the two directions. There is no indication from X-ray structures of dehydrogenase−$NAD^+$ complexes that an enzyme bound $NAD^+$ is nonplanar.[29] It is most likely that $NAD^+$ does not have to be bound in a boatlike conformation in an enzyme active site, but the puckering of the ring in the transition structure can be easily achieved from a nearly planar structure during hydride transfer.[30]

**2. Donor−Acceptor Syn Preference of Hydride Transfer.** The syn preference of hydride transfer can be rationalized by frontier molecular orbital theory (FMO) as we proposed earlier.[3a] In the case of hydride transfer from $PY^+$ to PYH, the transition structure can be divided into three parts: the hydride ion and two protonated pyridinium ions, as shown in Figure 3. Since the reaction is essentially a nucleophilic addition of hydride to pyridinium ion, the activation energy is reduced if the LUMO

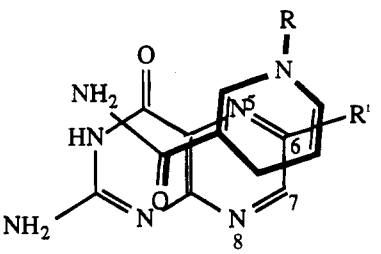
(29) For a nice summary see Table 2 in ref 9.

(30) It has been observed that $NAD^+$ has a weaker binding energy than NADH with several dehydrogenases. For a summary see Table 3 of ref 2d. We previously suggested that this might be due to a destabilization of $NAD^+$ upon enzyme binding because it cannot adopt the optimal conformation for the nicotinamide-ribose (ref 7). Bruice *et al.* suggested the possibility of out-of-plane puckering of $NAD^+$ upon binding by enzymes, which is destabilizing (ref 9). In a molecular dynamics simulation of dihydrofolate reductase-$NADP^+$ (NADPH) binary complex as well as ternary complexes with folate, Cummins *et al.* found that there is a favorable solvation for NADPH over $NADP^+$ (ref 2d).

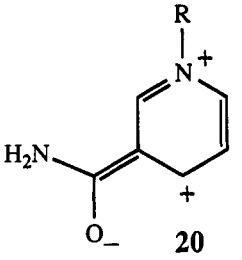
**Chart 3**




**18**




**19**




**20**

of pyridinium ion is reduced, which can be achieved by overlapping between the LUMOs of the two pyridinium ions. Since such overlapping is best in a syn arrangement (see **14**), it results in a favorable syn transition structure with C−H−C bending. It should be emphasized that the most important stabilizing interaction is caused by the overlap as truncated by the dashed box. The other interactions make only minor contribution.

A more realistic model of the $NAD^+$/NADH system would have the $N_1$ alkylated. What would be the effect of the steric interaction between the two alkyl groups on the syn preference? The distance between the two $N_1$ bonded hydrogens in structure **8** is 4.9 Å. When the two hydrogens are replaced by two methyl groups, the shortest interatomic distance between the two methyl groups is 4.2 Å. Therefore, no serious steric interaction is expected, and the syn preference should not be affected by the alkylation.

In the case of hydride transfer from 1,4-dihydropyridine to methylene iminium ion, we previously reported a preference for syn over anti where a $C_s$ symmetry was imposed (see **2**).[3] Full geometry optimization with the 3-21G basis set that we have achieved now gives a transition structure shown by **16**, in which the C=N is nearly above the $C_3-C_4$ bond of the pyridine ring. This is understandable, since it has the best LUMO−LUMO overlap between the iminium and pyridinium moieties, as can be seen in **17**. This arrangement is also found in the X-ray crystal structure of dogfish lactate dehydrogenase−NADH−oxamate ternary complex.[31] The AM1 model calculations by Williams *et al.* on the hydride transfer from 1,4-dihydropyridine to formaldehyde−imidazole complex gave a similar arrangement as found in the X-ray structure.[5]

The syn arrangement of $NAD^+$ or NADH with substrate is also found in other crystal structures of enzyme−$NAD^+$-(NADH)−substrate ternary complexes. For example, Karplus *et al.* reported that in the X-ray crystal structures of ferredoxin-NADP reductase the $NADP^+$ is nearly perfectly stacked with flavin.[21a] This situation is also found in the ternary structure of NADPH-glutathione reductase which was reported by Schulz *et al.*, as schematically shown by **18**.[17b,c] Kraut *et al.* reported a general arrangement of $NADP^+$ with folate or biopterin substrate in which the hydride donor and acceptor are partially stacking, as schematically shown by **19**.[22] This arrangement is found in recombinant dihydrofolate reductase complexes with folate and 5-deazofolate,[22a] in *E. coli.* dihydrofolate reductase−folate−$NADP^+$ complex,[22b] and in chicken liver dihydrofolate reductase ternary complexes with $NADP^+$-biopterin[22c] and thia$NADP^+$-biopterin.[22d] Kraut *et al.* suggested that this partial stacked arrangement allows favorable van der Waals interactions between the hydride donor and acceptor in the transition structure of hydride transfer.[22] The primary function of dihydrofolate reductase is to catalyze the NADPH-linked reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate. We note that the arrangement of the $C_6=N_5$ bond in structure **19** with respect to the nicotinamide ring is similar to that in structure **16**. In a hypothetical transition structure model, Kraut *et al.* noticed that the angle defined by the acceptor−hydride−donor atoms is 154° for hydride transfer to $C_6$ of 7,8-dihydrofolate. Such bent angle is also found in our syn transition structures (see **6** and **16**).[3a]

Bodor *et al.* found a charge−dipole complex during the reaction of 1-methyl-1,4-dihydronicotinamide with 1-methylnicotinamide cation, with the two pyridine rings nearly perpendicular to each other.[20] However, they were not able to locate a $\pi$-complex with the two pyridine rings parallel which could contribute directly to hydride transfer.[32] It is not clear how this charge−dipole complex affects the transition structure of hydride transfer in both nonenzymatic and enzymatic environments, because it is geometrically quite different from the transition structure.

**3. Effect of Amide Group on Hydride Transfer.** The finding that the trans amide significantly reduces the activation energy for hydride transfer may be related to the fact that the $NAD^+$ and NADH coenzymes are bound in most enzyme active sites in a trans conformation and not in a stable cis conformation as is true for the ground state. The origin of this effect remains to be addressed, since a cis conformation of amide does not have this effect.

The reactivity of a $\pi$ system toward nucleophiles can be influenced by the conformation of electron withdrawing groups. For example, in the case of Diels−Alder reactions, it has been found by *ab initio* calculations that acrolein, which favors an s-trans conformation itself, adopts an s-cis conformation in both endo and exo transition structures.[33,34] The lowest unoccupied

(31) Abad-Zapatero, C.; Griffith, J. P.; Sussman, J. L.; Rossmann, M. G. *J. Mol. Biol.* **1987**, *198*, 445.

(32) (a) Hajdu, J.; Sigman, D. *J. Am. Chem. Soc.* **1976**, *98*, 6060. (b) Creighton, D.; Hajdu, J.; Mooser, G.; Sigman, D. *J. Am. Chem. Soc.* **1973**, *95*, 6855. (c) Powell, M.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 7139.

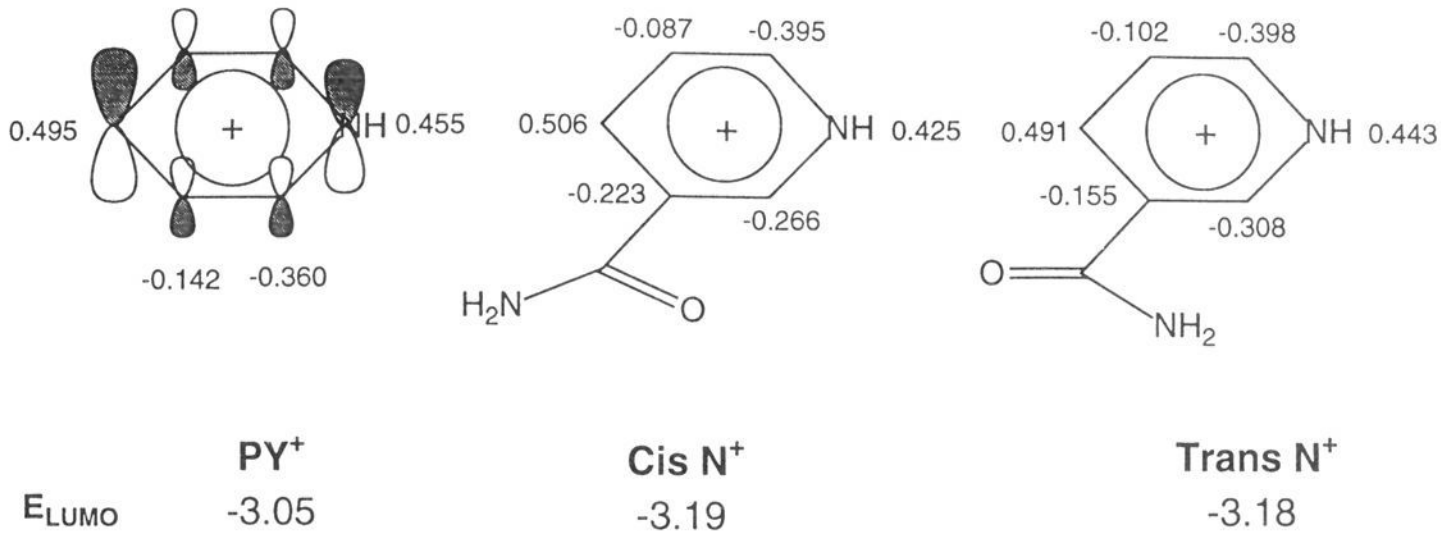



**Figure 4.** The molecular orbital coefficients and energy (eV) of the LUMOs of $PY^+$, cis and trans $N^+$.

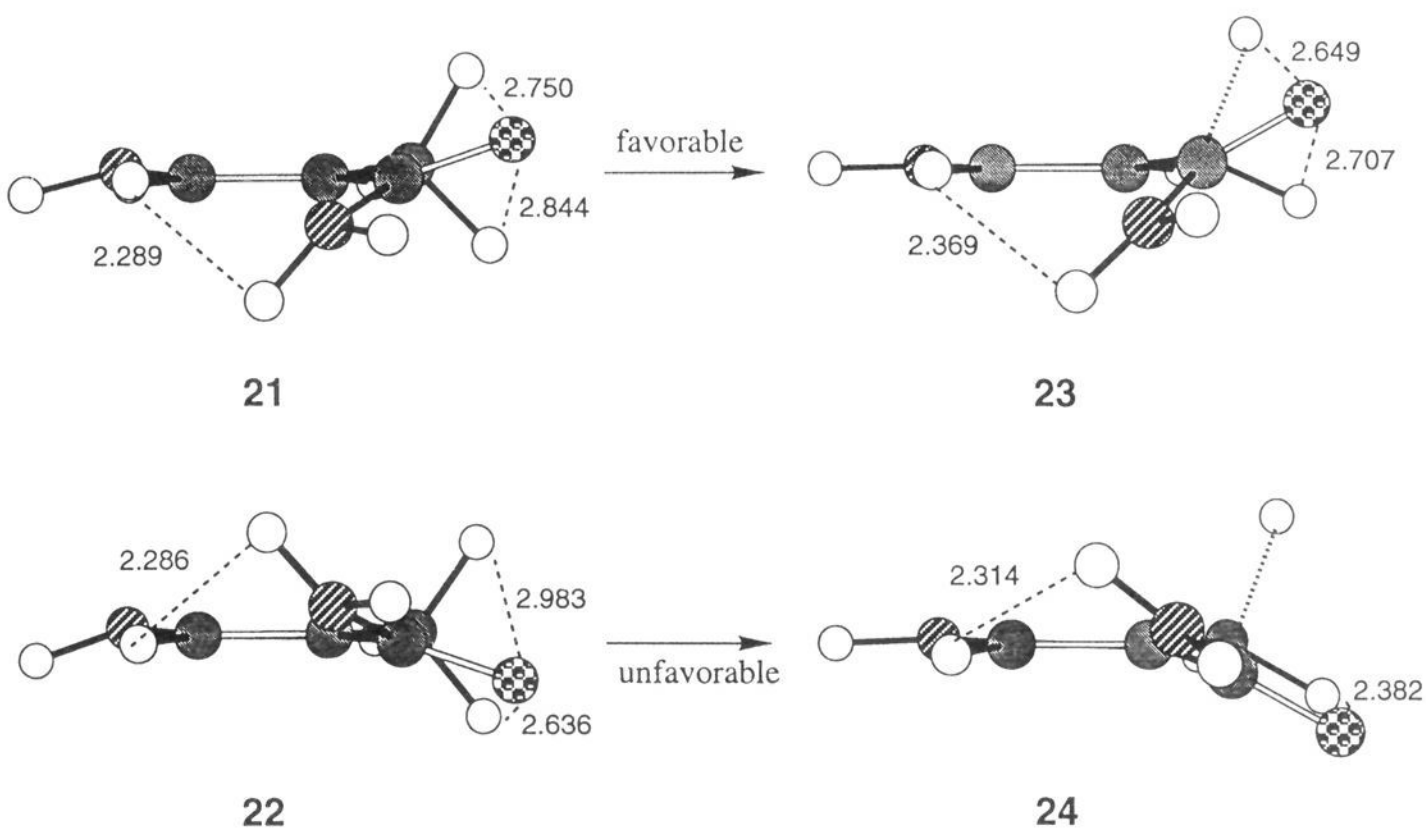



**Figure 5.** Side views of the two trans conformers of the 1,4-dihydronicotinamide (**21** and **22**) and their corresponding geometries in transition structures (**23** and **24**). Selected interatomic distances (Å) are shown. Structure **24** is destabilized by steric interaction between the carbonyl oxygen and the hydrogen on $C_4$.

molecular orbital (LUMO) of the *s-cis*-acrolein is lower-lying than than of the *s-trans*-acrolein, because of the 1,4-overlap. This makes it a better electron acceptor, leading to a favorable transition structure.

Figure 4 shows the calculated molecular orbital coefficients and energies of the LUMOs of $PY^+$ and the cis and trans conformations of $N^+$. The amide group reduces the LUMO energy by 0.14 and 0.13 eV when it is cis and trans, respectively. The molecular orbital coefficient at $C_4$ is only slightly changed by the presence of the amide group. This suggests that the amide effect on the LUMO of $PY^+$ is the same regardless of whether it is cis or trans, and this effect is too small to account for the variation in reactivity.

The most convenient explanation for the effect of amide group on activation energy seems to be based on a polarization argument.[27] LeReau *et al.* observed a deuterium isotope effect at $C_4$ of the nicotinamide ring upon the binding of $NAD^+$ to lactate dehydrogenase of about 10%.[35] This is explained by the development of carbonium ion character at $C_4$ in the enzyme active site, as illustrated by structure **20**. This effect is further increased by hydrogen bonding involving the amide oxygen in the enzyme active site. Thus, the trans amide group makes the $C_4$ a better hydride acceptor. This effect is absent when the amide is cis.

Our previous calculations for $N^+$ and NH suggest that the positive charge at the $C_4$ increases slightly upon cis to trans conversion for both $N^+$ and NH. Comparing structures **12** and **13** with structure **8**, it is noted that the natural population charge[36] on $C_4$ in structure **12** is almost identical to that in **8**. This suggests that a cis amide has little polarization effect in the transition structure for hydride transfer, which is in agreement with the fact that a cis amide has no effect on activating hydride transfer. The positive charge on $C_4$ of structure **13**, on the other hand, is 0.09 larger than that on $C_4$ of **8**. At the same time the negative charge on the oxygen in structure **13** is about 0.1 units larger than that in structure **12**. Therefore, the trans amide has a significant polarization effect in a hydride transfer transition structure.

How does the cis amide actually increase the activation energy for hydride transfer? In structure **10**, there is considerable steric interaction between the equatorial $C_4$–H and one of the amide hydrogens, as indicated by a 2.12 Å H/H distance. This distance is similar to that in the ground state of protonated nicotinamide

(33) (a) Loncharich, R. J. Brown, F. K.; Houk, K. N. *J. Org. Chem.* **1989**, *54*, 1129. (b) Loncharich, R. J.; Schwartz, T. R.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 14.

(34) Birney, D. M.; Houk, K. N. *J. Am. Chem. Soc.* **1990**, *112*, 4127.

(35) LeReau, R. D.; Wan, W.; Anderson, V. E. *Biochemistry* **1989**, *28*, 3619.

(36) NBO Version 3.1: Glendening, E. D.; Reed, A. E.; Carpenter, J. E.; Weihold. F. University of Wisconsin, Madison.

but is considerably shorter than that in the ground state of 1,4-dihydronicotinamide, where it is 2.30 Å.[10]

As found before, there is a clear preference for the amide carbonyl oxygen to be located on the same face as the transferring hydride when the amide is trans. This conformational preference is called "syn carbonyl" preference. The $C_2-C_3-C{=}O$ dihedral angle in structure **11** is about 146°, about 10° smaller than that in the trans conformation of dihydronicotinamide. We also optimized a structure with the $C_2-C_3-C{=}O$ dihedral angle constrained at −146°. This structure is about 12 kcal/mol less stable than structure **11**. Upon the relief of the dihedral angle constraint, optimization led to structure **11**.

The syn carbonyl preference has been attributed to electrostatic attraction between the amide oxygen and the partially positively charged hydride acceptor.[15] Our calculations clearly indicate that this electrostatic effect is an important factor. The C−H−C bending in structure **11** occurs in such a way that pyridine/pyridine stacking is slightly compromised[37] in order to increase the attractive electrostatic interactions between the carbonyl oxygen and the $C_4$ atom of the other pyridine ring, which is separated by 3.0 Å in structure **11**. Thus, this electrostatic attraction also contributes to the hydride transfer activation by the trans amide.

We note that the syn carbonyl preference in the trans structure (**11**) is also partially attributed to a steric effect. As we reported earlier, there are two nearly equally stable ground state conformations for 1,4-dihydronicotinamide.[10] These are shown by structures **21** and **22**, respectively. The out-of-plane distortion of the amide relieves steric interactions involving the amide $NH_2$ and the hydrogen on $C_2$. Upon hydride transfer, the transferring hydride becomes axial, and the other $C_4-H$ bond becomes equatorial, as shown by structures **23** and **24**, respectively. The amide in structure **23** is in a sterically favorable situation. The carbonyl oxygen in structure **24**, however, is quite close to the equatorial hydrogen on $C_4$, causing a steric repulsion.

Both experiments and calculations suggest that the out-of-plane carbonyl bond will promote the hydride transfer syn to it.[15,16] Whether this conformational feature is necessary for enzymatic reactions is controversial. While Beijer *et al.* used this feature to explain the enzymatic reactivity of horse liver alcohol dehydrogenase based on a molecular mechanics study using AMBER,[13d] the molecular dynamics simulation by Bruice *et al.* for dogfish malate dehydrogenase suggested that the amide swings through an arc of more than 50° such that the carbonyl oxygen can be either on the A-face or on the B-face of the 1,4-dihydropyridine ring (see **4** for definition).[9a]

(37) Compared to structures **6** and **10**, the two pyridine rings in structure **11** are opened up. This can be roughly measured by the $N_1-C_4-C_4'$ angle, which is 106°, 105°, and 117° in structures **6**, **10**, and **11**, respectively.

## Summary

For hydride transfer reactions of the protonated pyridinium ion with 1,4-dihydropyridine and of protonated nicotinamide with 1,4-dihydronicotinamide, there is a strong preference for a syn or stacking arrangement for the two pyridine rings in transition structures. This syn preference maximizes the overlap between the LUMOs of hydride acceptor and hydride donor and can be applied to other systems including enzymatic systems. The pyridine ring is slightly puckered in a boatlike conformation in the transition structures, and the extent of this puckering is very similar to that of 1,4-dihydropyridine itself. The 3-amide group of nicotinamide slightly deactives hydride transfer when it is in a cis conformation due to a steric interaction involving the amide $NH_2$ and the nontransfering hydrogen at $C_4$. When the amide group is in a trans conformation, it significantly reduces the activation energy for the hydride transfer, primarily due to polarization at $C_4$. This explains why the amide is present in the coenzyme and why the coenzyme is bound in a trans conformation by most dehydrogenases. In addition, there is a preference for the trans amide group to be out-of-plane with the carbonyl oxygen directed toward the transferring hydride. This preference is caused both by a favorable steric arrangement and by attractive electrostatic interactions.

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