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STEREOSELECTIVE HYDRIDE UPTAKE IN MODELSYSTEMS RELATED TO THE REDOX-COUPLE NAD^+/NADH.

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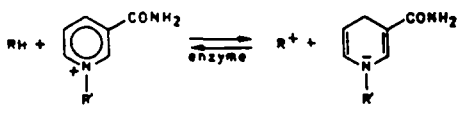
Abstract: The present work deals with the mechanistic investigations of the hydride transfer reactions concerning the redox couple NAD^+/NADH. Based on the theoretical and experimental investigations of NAD(H) model compounds as 3-carbamoyl pyridinium cations (3-carbamoyl-1,4-dihydropyridine) it was found that the out-of-plane rotation of the carbonyl function controls the stereo- and regiospecificity of the introduced hydride anion. It was found that the hydride anion transfered in the reaction, is always syn-positioned with respect to the carbonyl group. The unique stereoselectivity exhibits a strong coherence with the recent crystallographic 3D-data for the ternary complex of NAD bonded horse liver alcohol dehydrogenase. The results show that the amide group is 30° out of the plane with the carbonyl directed toward the A side. There are observations that the absolute configuration of the introduced chirality in the 3-carbamoyl pyridinium cations selects between the hydride uptake corresponding with the enzymatic A or B specificity.

Introduction.

During recent years the coenzyme nicotinamide adenine dinucleotide (NAD^+) has received widespread attention. This coenzyme plays an important role in a large number of enzyme-catalyzed oxidation-reduction reactions.[1] The characteristic event in this reaction as given in Scheme I is always the reversible transfer of a hydride ion from the substrate to the 4 position of the nicotinamide moiety of NAD^+ or vice versa.

Scheme I. Prototype of the enzymatic reduction of NAD^+ to NADH.



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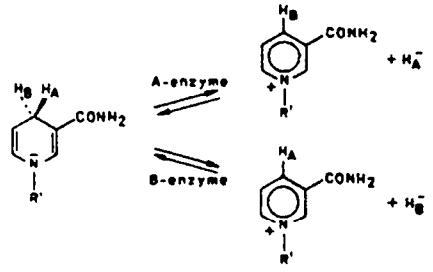
Much work has been done to elucidate the reaction mechanism of this process. In particular, kinetic studies on NAD^+/NADH as well as on the variety of model compounds, with or without enzymatic catalysis, have contributed to our understanding of the hydride transfer reaction. However, up to now it has not been possible to combine all results in one simple overall scheme. An, as yet, not fully understood feature of this equilibrium is the stereospecificity of the hydride transfer. This effect was demonstrated by Vennesland and Westheimer[2] for the oxidation of CH_3CD_2OH by NAD^+, catalyzed by alcohol dehydrogenase. They showed that in this reaction direct transfer of D^- takes place and that this transfer is stereospecific with respect to both coenzyme and substrate. The stereochemical course of the hydride transfer is dictated by the enzyme. With regard to NAD^+/NADH there are two hydrogen atoms available for transfer (H_A and H_B) depending on the type of the enzyme (A or B type). This is illustrated in Scheme II.

Scheme II. Stereospecific hydride transfer by A- and B-type dehydrogenase.



Brändén[5] showed in his crystallographic work on horse liver alcohol dehydrogenase (HLAD; A specific), in detail, the interactions relevant for coenzyme and substrate binding. X-ray data combined with model building techniques revealed that both the substrate and the nicotinamide moiety of the coenzyme are fixed deep inside the binding cleft. Due to steric hindrance as well as to interactions with exactly positioned sites of the enzyme, both substrate and coenzyme take up well-defined positions, in which the B side of the nicotinamide group is shielded by the hydrophobic wall of the cleft whereas the A side (Scheme II) is directed toward the substrate. This feature is essential for the stereochemistry of the enzymatic hydride transfer and it is not surprising to find that the stereospecificity is absent when the reaction is carried out under non-enzymatic conditions. These results indicate that the presence of the amide moiety of the nicotinamide group and its (relative) orientation are essential for the stereochemistry. The stereospecificity results from the positioning of the nicotinamide moiety with respect to the substrate which in turn is largely determined by the hydrogen bonds between the amide group and the enzyme. Until now, no effort has been made to investigate the effect of the orientation of the amide moiety upon the stereochemistry of the process under enzymatic conditions. The relevance of the $CONH_2$ group to the dynamics in the enzyme-catalyzed stereospecific hydride transfer has been suggested by Dutler for LAD.[4] He envisaged the (dihydro)pyridine ring to have enough freedom of motion to change its position during the hydrogen transfer, a movement possibly accompanied by rotation of the $CONH_2$ group out of the plane of the (dihydro)pyridine ring.

We should like to suggest a way in which the stereospecificity of the enzymatic hydride transfer of $NAD^+/NADH$ can arise, that is to say, how the substrate can distinguish between the two hydrogen atoms available for transfer. We expect that, once the coenzyme has formed a reactive complex with a suitable enzyme, the $CONH_2$ group loses the freedom to rotate, for instance, by the formation of a hydrogen bond. When the $CONH_2$ group is fixed, and most likely this will be in an out-of-plane orientation with respect to the dihydropyridine ring, H_A and H_B may migrate to the substrate but with very different rates. This causes the reaction to be effectively stereospecific. The primary goal of our investigations is to provide theoretical background and experimental evidence for the correlation between the stereochemical course of the hydride transfer and the $CONH_2$ out-of-plane orientation, using NAD-model compounds.[5,6]

Results and discussions.

The presence of the $CONH_2$ group enables $NAD^+/NADH$ to react in a stereospecific manner either directly, by permanently breaking the symmetry with respect to the (dihydro)pyridine ring, or indirectly, by breaking the symmetry with respect to the plane perpendicular to the ring, thereby providing an enzyme with the possibility to distinguish between both sides of the molecule. In the first case, it is essential that both NADH and NAD^+ exist in stable chiral configurations, uncontaminated by their enantiomers. In the second case, the transition states in the enzymatic environment must be different. Both possibilities were examined.[6] The possible explanation of the difference in reactivity between H_A and H_B (Scheme II), namely, that one, based on a permanent dissymmetry of both sides of the (dihydro)pyridine ring in both NADH and NAD^+, was investigated as follows. The total standard enthalpy of formation (ΔH_f°) of 3-carbamoyl-1,4-dihydropyridine (CDHP) and 3-carbamoyl pyridinium cation (CP^+) was calculated with MINDO/3 using complete structural optimization, but keeping the torsion angle (ϕ) around the C(3)-$CONH_2$ bond fixed at certain values. Figure 1 shows the resulting ΔH_f° as a function of ϕ (ϕ=0° corresponds to the oxygen of the $CONH_2$ group syn-oriented with respect to C(4); 0°<ϕ<180° corresponds to the oxygen of the $CONH_2$ group being on the same side of the (dihydro)pyridine ring as H_A, etc.). The calculations established that, independent of the value of ϕ, in CDHP as well as CP^+ both the (dihydro)pyridine ring and $CONH_2$ group each preserves a planar configuration at the MINDO/3 level. It can be seen from Figure 1 that MINDO/3 indicates a slight bias toward chirality associated with minimum enthalpy values for perpendicular orientations of the $CONH_2$ group. However, the enthalpy barriers are low enough

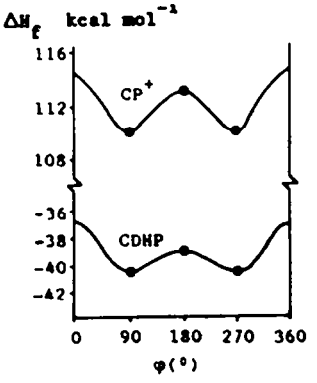
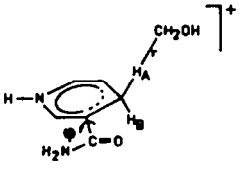


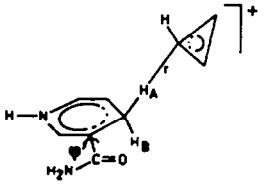
Figure 1. ΔH_f° of CDHP and CP^+ against φ.

for the rotation of the $CONH_2$ group to be almost free: for CDHP there are barriers at φ=0 and 180° of 3.9 and 1.7 kcal mol^{-1}, resp. for CP^+ one finds 4.6 and 3.3 kcal mol^{-1}. Therefore we come to the conclusion that it is unlikely that the experimental stereospecificity of the NAD^+/NADH hydride transfer reaction originates from a permanent chirality of the reactive fragments of NADH and NAD^+. For looking at the kinetic effects, we constructed the configurations as is shown in Schemes III and IV.

Scheme III. Transition state for the transfer of H_A^- from CDHP to CH_2OH^+.



Scheme IV. Transition state for the transfer of H_A^- from CDHP to $C_3H_3^+$.



We calculated the MINDO/3 formation enthalpy of these structures as a function of one parameter r, the distance between the migrating hydrogen and the accepting carbon atom of $C_3H_3^+$ (cyclopropenium cation) and CH_2OH^+ (protonated formaldehyde). All structural parameters except r were optimized with respect to ΔH_f°. The parameter r was varied until a maximum enthalpy on the minimum-gradient reaction path (MGRP) was found. The corresponding structure is the transition state. Similarly calculated transition states but with fixed values of φ were located; that is to say, for each fixed value of φ a local enthalpy maximum on the MGRP was determined with full optimization of all structural parameters but r and φ. The resulting activation enthalpies and values of r locating the transition states are depicted in

Figures 2 and 3, where the curves A and B refer to the choice of H_A or H_B as leaving hydride ion.

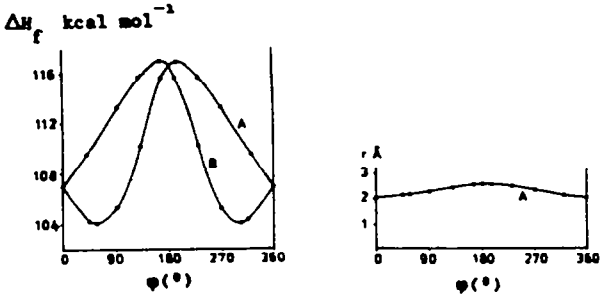


Figure 2. ΔH_f^* and r of the transition state $CP\cdot\cdot H\cdot\cdot CH_2OH^+$ against φ.

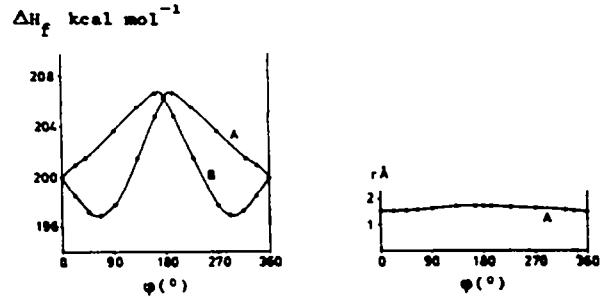


Figure 3. ΔH_f^* and r of the transition state $CP\cdot\cdot H\cdot\cdot C_3H_3^+$ against φ.

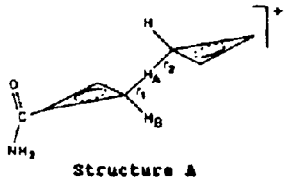
It can be seen that variation of φ has a marked effect on the activation enthalpy. The enthalpy extremes are much wider apart than was found for the free molecules CDHP and CP^+. Moreover, the mutual symmetry of H_A and H_B, essentially preserved in the free molecules, is no longer there: around φ=90 and 270° there is a significant difference in activation enthalpy for the transfer of H_A^- and H_B^-. This difference amounts to 8 kcal mol^{-1} for the combination CDHP + CH_2OH^+ and 6 kcal mol^{-1} for CDHP + $C_3H_3^+$. The origin of this enthalpy difference can easily be denoted.

In the transition state CDHP interacts with a positively charged hydride acceptor, and it is the electrostatic interaction between the acceptor and the $CONH_2$ group which dominates in the interaction enthalpy. Indeed, the $CONH_2$ group, and in particular the carbonyl bond, is highly polarized according to our calculations; the carbon atom carries a (positive) net charge of +0.67, whereas the oxygen atom has a (negative) net charge of -0.61, the NH_2 group approximately making up the balance to render the $CONH_2$ group slightly positive (ca. +0.02; all charges expressed in atomic units of charge). The shape of the curves shown in Figure 2 and 3 can now be understood in terms of interaction between the negatively charged oxygen atom of the $CONH_2$ group and the positive charge of the hydride accepting moiety. A low-enthalpy transition state corresponds to the carbonyl dipole pointing toward

the hydride acceptor and a high-enthalpy transition state to the carbonyl dipole pointing away from the acceptor.

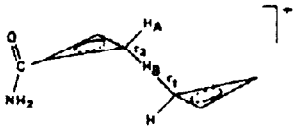
To add additional support we have also performed MINDO/3 and STO-3G calculations on intermediate structures for the reaction of 2-carbamoylcyclopropene (CCP) with the $C_3H_3^+$. In particular, we looked at structures with ϕ=90°, and with H_A or H_B as the leaving hydride moiety (Schemes V and VI). These structures were optimized with MINDO/3, using as constraints ϕ=90° and r_1=r_2. The resulting structures were taken to be representative of the transition states. A more detailed search of the enthalpy surface was not attempted in view of our earlier work, which demonstrated the flatness of the MINDO/3 surface along the reaction coordinate.

Scheme V. Transition state for the transfer H_A^- from CCP to $C_3H_3^+$ with ϕ=90°.



Structure A

Scheme VI. Transition state for the transfer of H_B^- from CCP to $C_3H_3^+$ with ϕ=90°.



Structure B

Table I. Comparison of energies calculated for structures A and B (Schemes V and VI) with MINDO/3 and STO-3G.

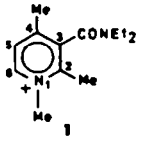
	MINDO/3	STO-3G
Structure A	233.95[a]	-393.54136[b]
Structure B	235.80[a]	-393.53779[b]
Difference	1.85[a]	2.24[a]

[a]In kcal mol^{-1}. [b]In atomic energy units.

In Table I the energies are given for the structures A and B as calculated with MINDO/3 and STO-3G. It can be seen that MINDO/3 and STO-3G both result in attributing to structure A an enthalpy which is less by ca. 2 kcal mol^{-1} as compared with structure B. Although the effect is here much smaller, the $CONH_2$ group, once fixed in a perpendicular orientation, induces the same stereospecific behaviour as found in the hydride-transfer reaction of CDHP/CP^+. These quantum chemical calculations are in strong agreement with our proposed mechanism concerning the stereospecificity in the redox reactions of NAD^+/NADH. To begin with, the $CONH_2$ group was found to

be almost freely rotating with respect to the ring at normal temperatures. This would count for the observed lack of the stereospecificity under non-enzymatic conditions. On the other hand, the calculations demonstrated that the stereospecificity can come to expression by fixation of the $CONH_2$ group in an out-of-plane orientation originating from interactions with the enzyme. Recently an enzyme-mediated $CONH_2$ out-of-plane orientation was detected in crystallized enzyme/coenzyme/substrate complexes. X-ray diffraction data derived from the ternary complex of NAD-bonded HLAD (A specific), obtained by Eklund et al.[7], showed the amide group rotated 30° out of the plane, with the carbonyl oxygen atom situated at the A side. This orientation of the amide moiety results from interactions with specific sites of the enzyme, i.e. hydrogen bonding of the carbonyl moiety of the $CONH_2$ group with the main chain nitrogen atom of Phe-319 and the NH_2 with the carbonyl groups of Val-292 and Gly-317. Additionally, the B side was adequately shielded, thus making this side inaccessible to the substrate by action of Thr-178. Although the results obtained from crystallized complexes are not to their full extent representative for the dynamic behaviour of the enzyme in solution, we believe the presence of the out-of-plane orientation of the $CONH_2$ group supports our proposed mechanism. In this context it should be noted that the nicotinamide group is situated in the interior of the enzyme, a region in which the influence of the enzyme-surrounding medium will probably be minimized.

Experimental verification of our proposed mechanism of stereochemical hydride uptake, using NAD-model compounds, requires the amide group to be fixed out-of-plane of the pyridinium ring, thereby introducing chirality in the system. Furthermore, it is essential that the conformational chirality is stable, i.e. racemization is excluded. As a model compound we selected 1 in which the non-planar position of the amide group is controlled by two adjacent methyl groups.[8] In order to check the correctness of the out-of-plane orientation of the amide group, our investigations were concentrated on establishing chirality in 1. The separation of both enantiomers of 1 was effectuated by complexation with silver-(+)-α-bromocamphor-π-sulphonate monohydrate. The diastereoisomers could be separated after repeated crystallizations. Treatment of both separated diastereoisomers with Dowex-2 (Cl^- form)



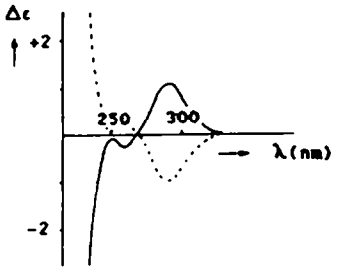
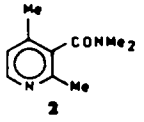


Figure 4. The CD spectra of (-) 1 (——) and (+) 1 (----).

resulted in the free (-) and (+)- N,N-diethyl-1-methyl-2,4-dimethyl-3-carbamoylpyridinium compound, resp. (for (-) 1 $[\alpha]_D^{19.7}$=-5.62 c=1.00 in water, and for (+) 1 $[\alpha]_D^{19.3}$= 4.74 c=1.00 in water). The CD spectra are given in Figure 4. These results lead to the unavoidable conclusion that the amide group is indeed rotated out of the pyridinium plane. In order to determine the rates of racemization, we recorded the CD spectra of enantiomerically pure 1, dissolved in water, at regular intervals. Racemization of optical active 1 did not occur at room temperature but could be observed at 90°C ($t_{\frac{1}{2}}$=7.5 h) and 100°C ($t_{\frac{1}{2}}$=3.1 h). Evaluation of the results, obtained at elevated temperatures, showed a $\Delta H^{\neq}$ for racemization of 22.9 kcal mol^{-1} and a $\Delta S^{\neq}$ of -16.90 cal $mol^{-1}K^{-1}$. Additional studies were performed on racemization of enantiomerically pure N,N-dimethyl-2,4-dimethyl-3-carbamoyl pyridine (2) in solvents of different polarity. Results concerning the chiral stability in several solvents are presented in Figure 5.[9]



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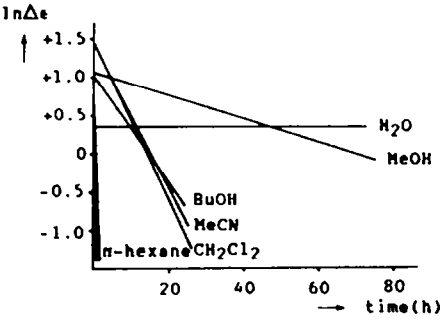
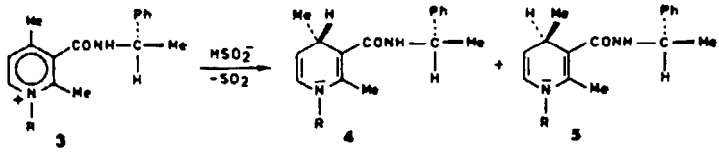


Figure 5. Time-dependent response of the CD for (-) 2 in various solvents at room temperature.

The rather dramatic rate enhancement of racemization, going from a polar solvent to an apolar solvent, can be discussed in a qualitative way by assuming that we are dealing with a combined rotation about the C-C(3) and the C-NMe_2 bonds in the amide group. Increase of the solvent polarity will enhance the contribution of the double bond in C-NMe_2 to the resonance hybrid, thus hampering the rotation around C-NMe_2. The absence of charge migration by conjugation in the transition state for rotation, therefore favours a low barrier in apolar solvents. Model studies of 2 clearly show that rotation about C-C(3) is facilitated when synchronously the rotation about C-NMe_2 occurs to avoid the steric hindrance with the methyl groups on C(2) and C(4). Therefore we suggest that in accordance with the observed rotational barriers in substituted N,N-dimethylbenzamides,[10] with respect to their solvent dependence, the rotation about C-NMe_2 in 2 will be favoured by apolar solvents which results in an increase of the rate of racemization. The pronounced stability in water, undoubtedly due in part to hydrogen bonding, is of major importance to the use of this type of NAD-model compounds in our study of the correlation between the stereochemistry of reduction and the out-of-plane orientation of the amide group. However, in spite of the fact that the chiral stability in water is guaranteed, no reduction of 1 in an aqueous solution of dithionite was observed. The lack of reactivity is probably caused by steric and electronic shielding resulting from the C(2) and C(4) methyl groups. On the other hand, Ohno et al.[11] reported the (9R)-N-α-methylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl pyridinium compound (3) which readily showed reduction by action of dithionite. Reduction

of 3 in aqueous sodium bicarbonate showed an exclusive hydride addition at C(4), resulting in the diastereoisomeric pair (4S,9R)-(+)-N-α-methylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl-1,4-dihydropyridine (4) and the (4R,9R)-(-)isomer (5) (Scheme VII).[12]

Scheme VII. Reduction of the (9R)-N-α-methylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl pyridinium compound (3) reported by Ohno et al.[11]



After crystallization the relevant spectroscopic data were in good agreement with those as previously described. With the help of shift reagents we observed that the position of the proton at C(4) in 4 and 5 is syn-oriented with respect to the carbonyl group, i.e. when $Eu(fod)_3$ is used, a larger down-field shift of the proton at C(4) is found than for the methyl protons at the same position. This demonstrates a syn-orientation of the proton at C(4) and the carbonyl group which is also reflected in the larger down-field shift of the methyl protons at C(2) relative to the protons of the methyl group at C(4). Conclusive evidence for the geometry of 5 has been obtained by determining its X-ray crystal structure as given in Figure 6.

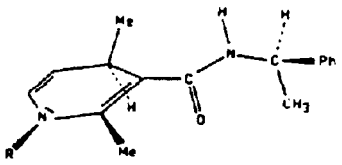
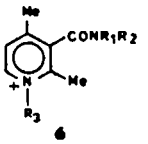


Figure 6. 3D-structure of 5 as elucidated by X-ray analysis.

The out-of-plane orientation of the CONH-group is 65° with respect to the 1,4-dihydro-pyridine moiety. The occurrence of both 4 and 5 in the reaction product is the inevitable result of the two diastereoisomeric structures of 3, due to a combination of the fixed chirality at C(9) and the conformational chirality of the out-of-plane orientation. Fractional crystallization of these diastereoisomers was not successful. In absence of the chirality at C(9) (substitution of the C(9) hydrogen atom by a methyl group), separation of the enantiomers was not feasible. In this context it should be noted that the chiral stability of the carbamoyl pyridinium moiety of 3 will be lower than that of the corresponding cation 1. The presence of the amide hydrogen atom will evoke racemization less difficult compared with interconversion of the conformational isomers in the case of a dialkylated amide group (vide supra). In an attempt to synthesize a 2,4-dimethyl-3-carbamoyl pyridinium cation giving rise to a high degree of chiral stability and showing 1,4

reduction, we tested various cations of the type 6 for their reduction with dithionite. The results obtained in this study are summarized in Table II. As can be seen reduction can be achieved when a phenyl group is present in at least one of the substituents R_1, R_2 or R_3. We tentatively suggest that the hydride or HSO_2^-, generated by dithionite is superficially accommodated by the phenyl group.



6

Table II. Regioselectivity of 3-carbamoyl pyridinium cations of the type 6 in their reduction with dithionite.

R_1	R_2	R_3	Regiosel.
Me,Et	Me,Et	Me	no red.
H	i-Pr	Me	no red.
Me	Me	$(CH_2)_n$Ph, n=1,3,5,12	1,6
Me	Me	$(CH_2)_{11}CH_3$	1,6
CH_2Ph	CH_2Ph,Me	Me	1,6
H	$CH(CH_3)$Ph, $C(CH_3)_2$Ph	n-Pr	1,4
H	$C(CH_3)_2$Ph, CH_2Ph	Me	1,4

In the case of R_3=n-$C_{12}H_{25}$, reduction is probably caused by intermolecular catalysis in associates. Within the category of 2,4-dimethyl-3-carbamoyl pyridinium cations there is a distinct difference in the regioselectivity of hydride uptake. As stated in Table II both 1,4 and 1,6 reductions have been observed. The results mentioned suggest that 1,6 reduction is favourable. The nature of this preference can easily be denoted. Due to steric and electronic shielding, originating from the presence of the 4-methyl substituent, C(4) is deactivated with respect to hydride uptake. 1,4 Reduction could only be achieved in case of R_1=H. This feature may be explained in terms of the proposed suggestion for the phenyl-mediated hydride uptake. Due to the trans-orientation of the carbonyl group with respect to the NH bond in the amide moiety, the phenyl group is directed away from the pyridinium ring, thereby leaving only C(4) sterically available for hydride uptake (Figure 7a). Substitution of the amide hydrogen atom, results in the loss of conformational restrictions of the amide moiety. Therefore, occasionally, both C(6) and C(4) are located within the reach of the phenyl group (Figure 7b). Since C(6) is less deactivated, hydride addition will take place preferably at this position.

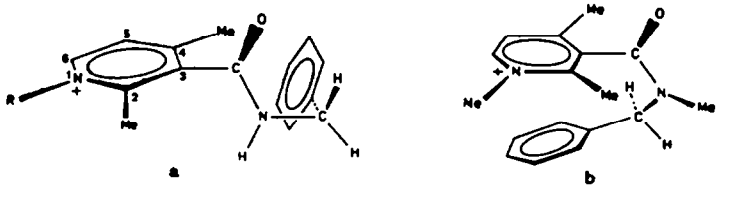
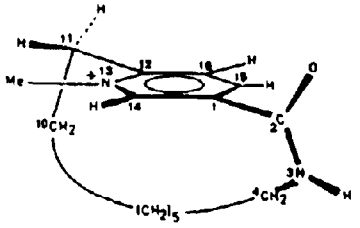


Figure 7. The phenyl group is directed away from C(6) due to the trans orientation of the CONH group (a); when the amide proton is substituted, C(6) becomes in the reach of the phenyl group (b).

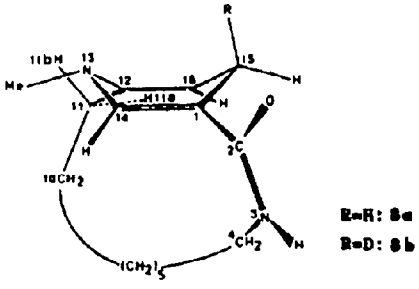
In summary we come to the conclusion that introduction of a phenyl group in the amide moiety can only lead to 1,4 reduction when the amide group is mono-

substituted (presence of CONH), although a high degree of chiral stability requires a dialkylated amide group. The above mentioned results will unavoidably lead to the conclusion that introduction of the phenyl group in one of the substituents R_1, R_2 or R_3 in 6 will not combine adequate chiral stability and 1,4 reducibility within one pyridinium compound. This leaves us two alternatives: (i) removing the two reduction-deactivating methyl groups, which makes the presence and exact positioning of the phenyl group superfluous, and achieving chiral stability by means of an alkyl bridge situated between C(6) and the amide N-atom or (ii) substituting a phenyl group essential in the reduction of 2,4-dimethyl-3-carbamoyl pyridinium cations for one of the C(4) methyl hydrogens. The first option led to the synthesis of the 13-methyl-3-aza-13-azonia-bicyclo(10.2.2)hexadeca-1(14),12,15-trien-2-one cation (7).[13]



7

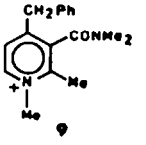
The 300 MHz ^{1}H-NMR spectrum of 7 showed that one of the protons of the methylene bridge has a negative chemical shift value (δ -0.97 to -1.17 ppm), resulting from the pyridinium ring current. This anisotropic shielding corresponds to an out-of-plane orientation of the bridge with respect to the pyridinium system. The introduced rigidity reduces conformational motions of the pyridinium ring to such an extent, that interconversion of the conformational enantiomers is virtually impossible and orients the amide moiety out of the pyridinium plane. The stereoselectivity of the hydride uptake in the reduction of 7, was elucidated by comparing the 300 MHz ^{1}H-NMR spectral data of the resulting 13-methyl-3,13-diazo-bicyclo(10.2.2)hexadeca-1(14),12(16)-dien-2-one (8a) with the data obtained from the corresponding deuterium analogue (8b). These results led to the conclusion that 8 adopts a boat conformation in which the incorporated hydrogen (deuterium) occupies almost exclusively (>95%)



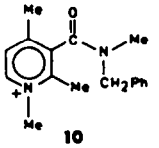
R=H: 8a
R=D: 8b

an axial position at C(15). The non-planarity was deduced from the coupling constants of H(16). This hydrogen appeared in the ^{1}H-NMR spectrum of 8a as an eight-line pattern, due to a vicinal coupling with H_{ax}(15) and H_{eq}(15), and an allylic coupling with H(11a). Reduction of 7 in D_2O, led to 8b. The lack of coupling between H_{ax}(15) and NCH_3, H(14) and H(16), resp., clearly indicates the axial position of the deuteride. In addition, Dreiding models unambiguously showed that the carbonyl group is rotated out of the plane through C(1), C(12), C(14) and C(16) which results in the fact that the incorporated hydride is syn-positioned with respect to the carbonyl group. The observed unique stereoselectivity in the hydride uptake will originate from two major features present in this system, i.e. the out-of-plane orientation of the amide group toward the carbonyl group syn-positioned with the incoming hydrogen donor and the shielding effect of the methylene bridge which prevents equatorial attack for steric reasons.[14] The presence of both effects in this NAD-model system is in complete agreement with the results obtained from the ternary complex of NAD bonded HLAD published by Eklund et al.[7] (vide supra).

The second mentioned alternative suggested to avoid the discrepancy between the regioselectivity in the reduction and the required high degree of chiral stability in 1-alkyl-2,4-dimethyl-3-carbamoyl pyridinium cations (6), will be found in the substitution of 4-methyl by a benzyl group. For this reason, the synthesis of the 1,2-dimethyl-3-carbamoyl-4-benzyl pyridinium cation (9) and its reduction is in progress. If the phenyl group is still required for the dithionite reduction of 9, although substitution of a methyl group into a benzyl group may influence the reducibility, C(4) will more readily be available for hydride uptake than C(6) (vide supra). Accepting that the chirality of 9 is adequately stabilized and the reduction takes place in the predicted manner, i.e. 1,4 reduction, we will be able to give conclusive evidence for our model description concerning the stereoselectivity in the hydride uptake, using CD techniques. The orientation of the carbonyl group in the pyridinium cation and the absolute configuration of C(4) after reduction, can both be determined by means of CD spectroscopy. The relation between CD properties and chirality resulting from the amide out-of-plane orientation, could be clearly demonstrated by the N-benzyl-N-methyl-1,2,4-trimethyl-3-carbamoyl pyridinium cation (10), which is already mentioned in Table II, with respect to its hydride uptake. Individual crystals of 10 appeared to consist of enantiomerically pure pyridinium compounds. One of the single crystals was submitted to an X-ray diffraction study.[15]



9



10

The structural outcome of this analysis is depicted in Figure 8a. These results clearly show that in the enantiomer investigated, the carbonyl group is located at the A side of the pyridinium ring (Scheme II) with an out-of-plane rotation of 67° with respect to the plane of the pyridinium ring. The same crystal, after being used in the X-ray study, was dissolved in both methanol and water in order to obtain CD spectra (Figure 8b and 8c). It may be of interest to note that the similarity of the CD spectra as given in Figure 8c and in Figure 4, then suggests a carbonyl

location at the A side for (-) 1. These observations will enable us to deduce the absolute configuration of 9 with respect to the out-of-plane

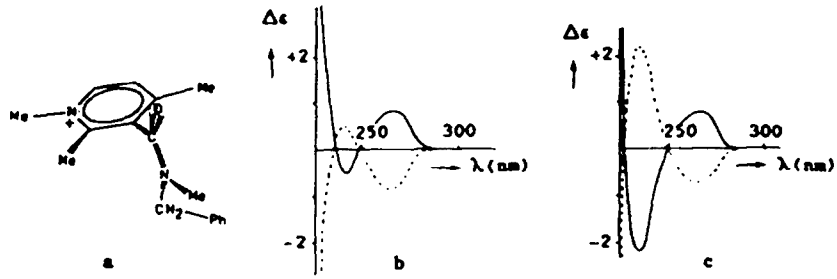


Figure 8. 3D structure of 10 (a) and its CD spectra recorded in methanol (b) and water (c). The broken lines (---) represent the CD spectra of the mirror image of (a).

orientation of the amide group simply from its CD spectrum. On the other hand, the absolute configuration of C(4) of the dihydro derivative of 9 can be elucidated using the CD results of Ohno et al. for the compounds 4 and 5 (vide supra). If, for instance, the carbonyl group is known to be located at the A side of the pyridinium moiety, reduction will have to result in an S configuration of C(4).

Other mechanisms for the stereoselectivity of hydride transfer reactions from nicotinamide cofactors have been recently developed by Cleland et al.[16] and Benner et al.[17] Especially Benner's model based on structural arguments including an anomeric effect is of particular interest. However, the distortion from planarity of the pyridine moiety which is a prerequisite for axial hydride transfer is only compensated by second order MO arguments.

Apart from the synthesis and the reduction of 9, future investigations will also include a more detailed study of the stereo- and regioselectivity in the reduction of the bicyclic pyridinium cation 7. The findings resulting from the observed stereospecific hydride uptake in the latter case, seem to us to be of major importance for a better understanding of the enzymatic NAD-mediated redox reactions, since 7 shows both a selective shielding and an out-of-plane orientation of the amide group, as observed in the ternary complex of HLAD/NAD/substrate.

Experimental Section.

60 MHz ^{1}H NMR spectra were recorded on a Varian EM360A or a Hitachi-Perkin Elmer R-24B NMR spectrometer and 300 MHz ^{1}H NMR data were obtained on a Bruker CXP 300 spectrometer using Me_4Si as internal standard (δ 0.00). A mass spectrum (MS) was recorded on a Finnigan 4000 GC-MS instrument using electron ionization. The CD spectral data were gained from a Jobin Yvon Dichrograph Mark III-S and specific rotations from a polarimeter type AA-10 manufactured by Optical Activity Ltd. A Fisher-Johns apparatus was employed to determine the (uncorrected) melting points. Ethyl-2,4-dimethyl-1,4-dihydronicotinate,[18] ethyl-2,4-dimethylnicotinate[18] and the 2,4-dimethyl-3-carbamoyl pyridine hydro-

chloride[9,12] were prepared using literature procedures. The successive reaction steps are: conversion into the acid-chloride using $SOCl_2$, and then to the amide using the appropriate amine, already detailed outlined in previous literature.[9,11] Prior to reduction with sodium dithionite, the 3-carbamoyl pyridine derivatives were treated with an alkylhalogenide in order to obtain the corresponding cations. General procedures will be mentioned.

► N,N-dimethyl-2,4-dimethyl-3-carbamoyl pyridine (2).
Mp 50-51°C. ^1H NMR ($CDCl_3$): δ 2.23 (s,3H,CH_3), 2.44 (s,3H,CH_3), 2.81 (s,3H,NCH_3), 3.15 (s,3H,NCH_3), 6.91 (d,1H,pyrH), 8.28 (d, 1H, pyrH). The diastereoisomers, obtained after complexation of the hydrochloride of 2 with (+) or (-)-α-bromocamphor-π-sulphonate monohydrate could be separately isolated after repeated treatment with acetone. Mp 198.0-199.0°C. Anal. Calcd.: C,49.08; H,5.97; N,5.72. Found (two diastereoisomers resp.): C,48.93, 49.38; H,6.00, 6.02; N,5.88, 5.51. $[\alpha]_D^{21.0}$=+55.00, -55.10 (H_2O). Treatment with NH_3/H_2O yielded the (+) and (-) enantiomers: Mp 52.0-54.0°C. Anal. Calcd.: C,67.38; H,7.92; N,15.72. Found: C,67.14, 67.32; H,7.93, 7.89; N,15.95, 16.19. $[\alpha]_D^{21.0}$=+1.80,-1.72 (H_2O). An alternative route for the enantiomeric separation showed corresponding analytical data.

► N,N-diethyl-2,4-dimethyl-3-carbamoyl pyridine.
^1H NMR ($CDCl_3$): δ 1.05 (t,3H,CH_3), 1.27 (t,3H,CH_3), 2.25 (s,3H, CH_3), 2.47 (s,3H,CH_3), 3.10 (q,2H,CH_2), 3.62 (q,2H,CH_2), 6.93 (d, 1H,pyrH), 8.30 (d,1H,pyrH). Enantiomeric separation can be obtained using the procedure as is outlined for 2.

► Methylation of the 2,4-dimethyl-3-carbamoyl pyridine compounds.
The pyridine derivatives of 2, dissolved in an excess of CH_3I were stirred until the reaction was complete (TLC). The remainder of the CH_3I was evaporated. The residue was repeatedly treated with dry ether in order to obtain a crystalline deposite.

► Alkylation of the 2,4-dimethyl-3-carbamoyl pyridine compounds with n-propyl bromide.
The alkylation, using n-PrBr, was carried out according to the literature[11].

► Alkylation with ω-phenylalkyl bromide.
To a 5 mmol solution of the 3-carbamoyl pyridine derivative in 5-10 ml nitromethane, an excess of the bromide was added (4-7 eq.). This mixture was stirred for 20 hrs. at an appropriate temperature ($PhCH_2Br$ and $Ph(CH_2)_3Br$: room temp.; $Ph(CH_2)_5Br$ and $Ph(CH_2)_{12}Br$: 100°C). Work-up was performed as mentioned in the methylation reaction. The $Ph(CH_2)_5Br$ and the $Ph(CH_2)_{12}Br$ were prepared analogous to the procedure mentioned by Friedman et al.[19]
$Ph(CH_2)_5Br$: Bp 90-105°C/1.5mm. ^1H NMR ($CDCl_3$): δ 1.20 to 2.10(m, 6H,3CH_2), 2.57(t,2H,CH_2), 3.30(t,2H,CH_2), 7.10(s,5H,Ph)
$Ph(CH_2)_{12}Br$: Bp 165-170°C/0.01mm. ^1H NMR ($CDCl_3$): δ 1.10 to 2.00 (m,20H,10CH_2),; 2.57 (t,2H,CH_2), 3.32(t,2H,CH_2), 7.08 (s,5H,Ph).

► Alkylation with dodecyl bromide.
A mixture of 22 mmol of the 3-carbamoyl pyridine compound and 100 mmol dodecylbromide (25 g) was heated for two days at 100°C in absence of a solvent. The separated solid material was collected and treated with dry ether.

►N,N-diethyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide(1).
Mp 177-178°C. ^{1}H NMR ($CDCl_3$): δ 1.00(t,3H,CH_3), 1.45(t,3H,CH_3), 2.50(s,3H,CH_3), 2.78(s,3H,CH_3), 3.35(q,2H,CH_2), 3.60(q,2H,CH_2), 4.47(s,3H,CH_3), 7.78(d,1H,pyrH), 913(d,1H,pyrH). Anal. Calcd. C,44.84; H,6.08; N,8.04. Found C,44.58; H,5.89; N,8.00. Separation of the enantiomers was effectuated by complexation with Ag-(+)-α-bromocamphor-π-sulphonate monohydrate (BKS). The diastereoisomers could be separated after repeated crystallizations from acetone. Treatment of both diastereoisomers with Dowex-2 (Cl^- form) yielded the pure enantiomers (Cl^- form).
(-): Mp 132.0-134.0°C. Anal. Calcd. (monohydrate): C,56.82; H,8.44; N,10.19. Found: C,56.64; H,8.65; N,10.26. $[\alpha]_D^{19.7}$=-5.62 (c=1.00 in water); ^{1}H NMR ($CDCl_3$): δ 0.90(t,3H,CH_3), 1.43 (t,3H,CH_3), 2.48(s,3H,CH_3), 2.78(s,3H,CH_3), 3.23(q,2H,CH_2), 3.57(q,2H,CH_2), 4.52(s,3H, CH_3), 7.82(d,1H,pyrH), 9.36(d,1H, pyrH).
(+): Mp 130.0-132.0°C. Anal. Found: C,56.78; H8.60; N,10.42. $[\alpha]_D^{19.3}$=4.74 (c=1.00 in water);

►(9R)-N-α-methylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl pyridinium bromide (3).
Synthesis was outlined in the work of Ohno et al.[11]. The relevant spectoscopic data were in excellent agreement with those described.

►13-methyl-3-aza-13-azonia-bicyclo[10.2.2]hexadeca-1(14),12,15-trien-2-one iodide (7).
The precursory ketone was prepared according to the procedure of Gerlach et al.[20] (32%). Column chromatography (silica 60, Merck, hexane/50% ethyl acetate). RF (TLC, hexane/50% ethyl acetate)=0.40. Bp 115°C/0.01 mm. Anal. Calcd.: C,77.38; H,8.81; N,6.45. Found: C,77.58; H,9.19; N,6.47. ^{1}H NMR ($CDCl_3$): δ -0.40 to 0.48 (m,1H,Aliphatic H), 0.48 to 2.33(m, 11H,Aliphatic Hs), 2.50 to 3.12(m,4H,$2CH_2$), 7.22(d,1H,pyrH), 7.67(dd, 1H,pyrH), 8.68(d,1H,pyrH). The oxim was prepared according to the method of Reinshagen et al.[21] (80%). Mp 122.8-123.7°C. Anal. Calcd.: C,72.38; H,8.68; N,12.06. Found: C,72.42; H,9.15; N,12.18. RF (TLC, hexane/50% ethyl acetate)=0.26. ^{1}H NMR ($CDCl_3$): δ -0.40 to 1.90(m, 12H,$6CH_2$), 2.22 to 3.12(m,4H,$2CH_2$), 7.15(d,1H,pyrH), 7.52(dd,1H, pyrH), 8.25(d,1H, pyrH), 11.22(br.s,1H,NOH). Beckmann rearrangement in polyphosphoric acid[21] at 100°C (18%). Column chromatography (silica 60, Merck, chloroform/5% ethanol). RF (TLC, chloroform/5% ethanol)=0.26. MS(m/z) 232 ($C_{14}H_{20}N_2O$). IR (KBr) 3220 (ν_{NH}), 1670 ($\nu_{C=O}$) cm^{-1}. ^{1}H NMR (300 MHz,$CDCl_3$): δ -0.38 to -0.04(m,2H,CH_2), 0.73 to 0.92 (m,2H,CH_2), 0.98 to 1.37(m,6H,$3CH_2$), 1.51 to 1.66(m,1H,Aliphatic H), 1.75 to 1.90(m,1H,Aliphatic H), 2.80 to 3.02(m,3H,Aliphatic Hs), 3.07 to 3.23(m,1H,Aliphatic H), 6.43(br.s,1H,NH), 7.30(d,1H,pyrH), 7.71 (dd,1H, pyrH), 8.66(d,1H,pyrH). ^{13}C NMR ($CDCl_3$) δ 24.87 to 28.98 (6t), 39.43(t), 42.53(t), 123.76(d), 128.95(s), 153.55(d), 147.42(d), 164.54(s), 172.97(s). Using $Eu(fod)_3$ shift reagent, we observed an increasing down-field shift going from C(12)CH_2, H(16), NCH_2, H(14), H(15) to NH. Methylation was carried out according to the above mentioned procedure (81%). ^{1}H NMR (CD_3OD): δ -1.17 to -0.97(m,1H,Aliphatic H), 0.40 to 2.15(m,11H,Aliphatic Hs), 2.80 to 3.60(m,4H,NCH_2 and $pyrCH_2$), 4.64(s,3H,NCH_3), 8.21(d,1H,H(16)), 8.67(m,1H,H(15)), 9.88 (d,1H,H(14)).

Compounds of category 6 (Table II):

►N,N-dimethyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide.
Mp 255-256°C (ethanol). Enantiomeric separation with BKS. Anal Calcd.: C,41.26; H,5.35; N,8.75. Found (-): C,41.34; H,5.35; N,8.75. (+): C,41.35; H,5.36; N,8.59. $[\alpha]_D^{20}$=-1.99 and +1.95 resp. (H_2O). ^1H NMR (D_2O): δ 2.60(s,3H,CH_3), 2.78(s,3H,CH_3), 3.05(s,3H, CH_3), 3.28(s,3H,CH_3), 4.30(s,3H,CH_3), 7.73(d,1H,pyrH), 8.70(d,1H, pyrH).

►N-isopropyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide.
Mp 233-235°C. ^1H NMR ($CDCl_3$): δ 1.36(d,6H,2CH_3), 2.53(s,3H, CH_3), 2.76(s,3H,CH_3), 4.26(m,1H,CHMe_2), 4.26(s,3H,NCH_3), 7.63(d, 1H,pyrH), 8.00(d,1H,NH), 8.95(d,1H,pyrH).

►N,N-dimethyl-1-benzyl-2,4-dimethyl-3-carbamoyl pyridinium bromide.
Mp 189.4-190.0°C. ^1H NMR (D_2O): δ 2.47(s,3H,CH_3), 2.57(s,3H, CH_3), 2.87(s,3H,CH_3), 3.10(s,3H,CH_3), 5.73(s,2H,CH_2), 7.30(m, 5H,Ph), 7.80(d,1H,pyrH), 8.63(d,1H,pyrH).

►N,N-dimethyl-1-(3-phenylpropyl)-2,4-dimethyl-3-carbamoyl pyridinium bromide.
^1H NMR (D_2O): δ 2.13(t,2H,CH_2), 2.40(s,3H,CH_3), 2.50(s,3H, CH_3), 2.73(t,2H,CH_2Ph), 2.83(s,3H,NCH_3), 3.13(s,3H,NCH_3), 4.43(t, 2H,NCH_2), 7.13(m,5H,Ph), 7.60(d,1H,pyrH), 8.43(d,1H,pyrH).

►N,N-dimethyl-1-(5-phenylpentyl)-2,4-dimethyl-3-carbamoyl pyridinium bromide.
^1H NMR ($CDCl_3$): δ 1.67(m,6H,3CH_2), 2.50(s,3H,CH_3), 2.80(s,3H, CH_3), 2.97(s,3H,NCH_3), 3.20(s,3H,NCH_3), 3.30(t,2H,CH_2Ph), 4.73(t, 2H,CH_2N), 7.76(d,1H,pyrH), 9.17(d,1H,pyrH).

►N,N-dimethyl-1-(12-phenyldodecyl)-2,4-dimethyl-3-carbamoyl pyridinium bromide.
^1H NMR ($CDCl_3$): δ 1.27(m,20H,10CH_2), 2.47(s,3H,CH_3), 2.57(t,2H, CH_2Ph), 2.80(s,3H,CH_3), 2.97(s,3H,CH_3N), 3.13(s,3H,CH_3N), 4.74(t, 2H,CH_2N), 7.10(m,5H,Ph), 7.74(d,1H,pyrH), 9.13(d,1H,pyrH).

►N,N-dimethyl-1-dodecyl-2,4-dimethyl-3-carbamoyl pyridinium bromide.
^1H NMR ($CDCl_3$): δ 0.90(t,3H,CH_3), 1.25(m,20H,10CH_2), 2.50(s,3H, CH_3), 2.77(s,3H,CH_3), 3.00(s,3H,CH_3), 3.17(s,3H,CH_3), 4.73(t,2H, NCH_2), 7.80(d,1H,pyrH), 9.06(d,1H,pyrH).

►N,N-dibenzyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide.
Mp 153.5-155.5°C. ^1H NMR (D_2O): δ 2.31(s,3H,CH_3), 2.39(s,3H, CH_3), 4.05(s,3H,CH_3N), 4.33(m,2H,CH_2), 4.87(m,2H,CH_2), 6.77 to 7.49(m,10H,2Ph), 7.70(d,1H,pyrH), 8.57(d,1H,pyrH).

►N-benzyl-N-methyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide (10).
Mp 184-185°C. Anal. Calcd.: C,51.52; H,5.34; N,7.07. Found: C,51.28; H,5.39; N,6.95. For detailed ^1H NMR spectroscopic and X-ray diffraction data (vide supra) of the rotamers: See Bastiaansen et al.[15]

►N-α,α-dimethylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl pyridinium iodide.
Mp 220-222°C. Anal. Calcd.: C,54.80; H,6.21; N,6.39. Found: C,54.60; H,6.17; N,6.24. ^1H NMR ($CDCl_3$): δ 1.10(m,5H,Et), 1.84(s,6H, C(CH_3)$_2$), 2.54(s,3H,CH_3), 2.73(s,3H,CH_3), 4.43(t,2H,NCH_2), 7.40(m,5H,Ph), 7.63(d,1H,pyrH), 8.73(d,1H,pyrH), 8.93(s,1H,NH).

►N-α,α-dimethylbenzyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide.
Mp 233-235°C. Anal. Calcd.: C,52.69; H,5.65; N,6.83. Found: C,52.54; H,5.71; N,7.15. ^1H NMR (DMSO-d_6): δ 1.70(s,6H,C(CH_3)$_2$), 2.47

(s,3H,CH_3), 2.67(s,3H,CH_3), 4.23(s,3H,CH_3), 7.37(m,5H,Ph), 7.87 (d,1H,pyrH), 8.87(d,1H,pyrH), 8.97(s,1H,NH).

►N-benzyl-1,2,4-trimethyl-3-carbamoyl pyridinium iodide.
Mp 196-198°C. ^{1}H NMR (D_2O): δ 2.30(s,3H,CH_3), 2.43(s,3H,CH_3), 3.87(s,3H,CH_3), 4.22(s,2H,CH_2), 6.84(m,5H,Ph), 7.13(d,1H,pyrH), 7.91(d,1H,pyrH).

►Reductions with sodium dithionite. General procedure.[11]
To a 120 ml. 1M aqueous sodium bicarbonate (saturated with Ar) solution of 1.2 mmol of the pyridinium compound, 120 ml. of CH_2Cl_2 (saturated with Ar) was added. 10 Eq. of sodium dithionite were added in portions to the stirred mixture. The agitation was continued for 3 to 4 hours in an Ar-flushed flask, excluded from light. The dichloromethane layer was separated, dried and evaporated, resulting in the crude dihydro compound.

►N-α-methylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl-1,4-dihydropyridine (4 and 5).
After crystallization the relevant analytical and spectroscopic data were in excellent agreement with those described in the literature.[11] The (4R,9R) diastereoisomer was submitted to an X-ray study. CONH out-of-plane rotation: 65°. Crystal data: Monoclinic, space group $P2_1$, a=12.834(10), b=5.108(6), c=13.166(11)Å, β=92.98(8)°, D_m=1.10 g cm^{-3}, D_c=1.14 g cm^{-3}, Z=2.

►13-methyl-3,13-diaza-bicyclo [10.2.2]hexadeca-1(14),12(16)-dien-2-one (8a).
^{1}H NMR ($CDCl_3$): δ 0.90 to 2.00(m,12H,6CH_2), 2.53(m,2H,CH_2), 2.84(m,1H,H_{eq}(15)), 3.05(m,1H,H_{ax}(15)), 3.06(d,3H,CH_3N), 3.35(m,2H, CH_2N), 4.47(m,1H,H(16)), 5.95(br.t,1H,NH), 6.30(t,1H,H(14)).

►13-methyl-3.13-diaza-15-monodeutero-bicyclo(10.2.2)hexadeca-1(14), 12(16)-dien-2-one (8b).
^{1}H NMR ($CDCl_3$): δ 0.90 to 2.00(m,12H,6CH_2), 2.52(m,2H,CH_2), 2.82(br.d,1H,H_{eq}(15)), 3.06(s,3H,CH_3N), 3.37(m,2H,CH_2N), 4.46(dd, 1H,H(16)), 5.87(br.t,1H,NH), 6.30(t,1H,H(14)).

Dihydro derivatives of the compounds of category 6 (Table II):

►N,N-dimethyl-1-benzyl-2,4-dimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.57(m,3H,CH_3), 1.80(s,3H,CH_3), 2.93(s,3H, CH_3N), 2.98(s,3H,CH_3), 3.74(dd,2H,C(6)H_2), 4.14(s,2H,CH_2N), 4.70 (m,1H,H(5)), 7.17(m,5H,Ph).

►N,N-dimethyl-1-(3-phenylpropyl)-2,4-dimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.28 to 3.25(m,6H,3CH_2), 1.48(m,3H,CH_3), 1.63 (s,3H,CH_3), 2.82(s,3H,CH_3), 2.83(s,3H,CH_3), 3.64(dd,2H,C(6)H_2), 4.57(m,1H,H(5)), 7.00(m,5H,Ph).

►N,N-dimethyl-1-(5-phenylpentyl)-2,4-dimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.20 to 3.12(m,10H,5CH_2), 1.50(m,3H,CH_3), 1.72 (s,3H,CH_3), 2.89(2s,6H,2CH_3), 3.71(dd,2H,C(6)H_2), 4.66(m,1H,H(5)), 7.04(m,5H,Ph).

►N,N-dimethyl-1-(12-phenyldodecyl)-2,4-dimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.20 to 3.12(m,24H,12CH_2), 1.59(m,3H,CH_3), 1.83(s,3H,CH_3), 3.00(2s,6H,2CH_3), 3.77(dd,2H,C(6)H_2), 4.69(m,1H, H(5)), 7.09(m,5H,Ph).

►N,N-dimethyl-1-dodecyl-2,4-dimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 0.81(t,3H,CH_3), 1.20(m,20H,10CH_2), 1.53(m, 3H,CH_3), 1.76(s,3H,CH_3), 2.91(s,6H,2CH_3N), 3.30(t,2H,CH_2N), 3.74 (dd,2H,C(6)H_2), 4.70(m,1H,H(5)).

►N,N-dibenzyl-1,2,4-trimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.63(s,3H,CH_3), 1.81(s,3H,CH_3), 2.67(s,3H, CH_3N), 3.67(dd,2H,C(6)H_2), 6.77 to 7.49(m,10H,2Ph).

►N-benzyl-N-methyl-1,2,4-trimethyl-3-carbamoyl-1,6-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.64(m,3H,CH_3), 1.83(s,3H,CH_3), 2.64(s,3H, CH_3N), 2.89(3,3H,CH_3N), 3.71(dd,2H,CH_2), 4.63(s,2H,CH_2), 4.90(m, 1H,H(5)), 7.21(m,5H,Ph).

►N-α,α-dimethylbenzyl-1-propyl-2,4-dimethyl-3-carbamoyl-1,4-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 0.87(t,3H,CH_3), 1.06(d,3H,CH_3), 1.52(m,2H, CH_2), 1.70(s,3H,CH_3), 1.72(s,3H,CH_3), 2.02(s,3H,CH_3), 3.06(t,2H, CH_2N), 3.22(m,1H,H(4)), 4.51(dd,1H,H(5)), 5.70(d,1H,H(6)), 5.80(s,1H, NH), 7.23(m,5H,Ph).

►N-α,α-dimethylbenzyl-1,2,4-trimethyl-3-carbamoyl-1,4-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.10(d,3H,CH_3), 1.73(s,3H,CH_3), 1.77(s,3H, CH_3), 2.03(s,3H,CH_3), 2.90(s,3H,CH_3N), 3.30(m,1H,H(4)), 4.50(dd,1H, H(5)), 5.67(d,1H,H(6)), 5.77(s,1H,NH), 7.30(m,5H,Ph).

►N-benzyl-1,2,4-trimethyl-3-carbamoyl-1,4-dihydropyridine.
^{1}H NMR ($CDCl_3$): δ 1.02(d,3H,CH_3), 2.06(s,3H,CH_3), 2.92(s,3H, CH_3N), 3.27(m,1H,H(4)), 4.46(d,2H,CH_2N), 4.56(dd,1H,H(5)), 5.73(d,1H, H(6)), 7.22(m,5H,Ph).

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Note.

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